Microscope Cell Culture Incubator

Biomedical Engineering 200/300 - Preliminary Report, December 15th, 2023

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Abstract

Cell cultures are a common laboratory practice. To maintain them, cell cultures must be cultivated at specific temperatures, humidity, and carbon dioxide levels. To aid in this, labs will typically use an incubator to maintain a specific climate. Current incubators that are on the market are incredibly expensive, so developing an effective low-cost incubator decreases the economic burden of research in laboratories. Furthermore, the incubators are either too large to use with an inverted microscope, or the incubators specifically designed for inverted microscopes do little to combat condensation buildup. The team improved upon the previous year's incubator by implementing specific anti-condensation features. The team used ITO heating film and copper wires to maintain constant temperature of the top and bottom viewing glasses, effectively removing the condensation problem from previous designs. Testing showed a successful decrease in condensation to zero for all nine zones of the viewing screen except for zone 9 which still decreased by around 45%. The device was successful at keeping temperature, minimizing condensation, and being able to see clearly through the viewing window, but the team was unable to test whether cells could live in the environment because of a broken piece that affected the ability of the CO_2 sensor.

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Introduction

Motivation & Global Impact

As biomedical technology progresses, the need for innovative solutions in research becomes more pronounced. Creating an affordable microscope cell culture incubator is a compelling endeavor, given its potential to democratize access to crucial research tools.

In a landscape where cutting-edge equipment often comes with a hefty price tag, the development of an inexpensive microscope cell culture incubator is a game-changer. Such a device has the potential to empower a broader range of researchers, including those in resource-constrained environments, by providing them with access to essential incubation capabilities.

Cell incubation research is also essential for many different processes, many of which are used in life-saving therapies against bloodborne illnesses and tissue engineering. Creating a more affordable alternative will give access to this research in those resource-constrained environments.

Existing Devices & Current Methods

While there are cell incubators on the market, they are often too large and unportable for use with an inverted microscope. The *Heracell™ Vios 160i CR CO2 Incubator, 165 L* [1], for example, controls for temperature, CO_2, oxygen, and humidity, but its dimensions are 637 x 901 x 881 mm, and weighs 95 kg. This is too large for any microscope even if the vessel was see-through. Designs that aim to specifically work with microscopes, like the patented *Controlled environment incubator for light microscopy* [2], struggle to solve the issue of condensation build-up on the inside of the viewing chamber. Many other solutions are only able to last for short periods of use before needing to be cleaned, like the design shown in the patent *Transparent thermostatic culture vessel for microscope observation* [3]. Many designs like these also pose a cost barrier. As shown in Figure 1, this design has a cost of $13,000 which is much too expensive for the minimal amount of time the client will use it.

Figure 1: Competing design with a cost of $13,000 [3].

Problem Statement

The goal for this team is to create a low-cost cell culture incubation chamber for an inverted microscope that is capable of live cell imaging. An internal environment of 37 C, 5% CO2, and 95-100% humidity must be maintained over a long duration of time, without affecting the microscope's optics or functionality. Maintaining even heating and humidity across the chamber is necessary to prevent gradients that will form condensation on the viewing surfaces. Current commercially available systems are extremely expensive, large, and enclose the entire microscope making it difficult to assemble and remove in between uses. Because of their size, they also hinder the use of the microscope in general.

Background

Anatomy & Physiology

Cell culture is one of the major tools used in cellular and molecular biology. Removing cells from a plant or animal and placing them in a favorable artificial environment causes subsequent growth. Cell culture provides excellent model systems for studying the normal physiology of cells and the effects of drugs and toxic compounds on the cells [3]. Cell cultures involving human cells or genetically manipulated non-human cells fall under Biosafety Level 2 guidelines [4] due to the safety procedures associated with working in a Lab with human diseases. Wild-type, non-human cells are likely BSL 1 unless known to be infected with a virus that may infect humans or unless they express recombinant DNA or proteins. The major types of cells used in culture media are primary cells, transformed cells, and self-renewing cells [5].

Incubators have to maintain very stable environments with regulated temperatures, humidity, light, pressure, CO_2, O_2, and pH levels, and other conditions [5]. Incubators are designed to maintain this environment in order to replicate the cell's natural conditions in the body. These factors are critical for the growth of the cultured cells. CO_2 forms carbonic acid that sets up an equilibrium with a sodium bicarbonate buffer to maintain pH. The medium that the cell culture resides in can be made of either natural or synthetic media. Natural being medium such as plasma, serum, and embryo extract [6]. Synthetics being made of basal medium and supplements, such as serum, growth factors, and hormones [6].

Client Information

Our client is Dr. John Puccinelli, Associate Chair of the Undergraduate Program, BME Design Curriculum Coordinator, and our professor for BME 200/300. Dr. Puccinelli teaches a tissue engineering course in ECB 2001 and plans to use this device for up to two weeks each year for education purposes.

Product Design Specifications

There are 4 major criteria that must be followed during the design process of the product. The first criterion is cost-effectiveness, ideally less than $100. The second criteria is accuracy and reliability. This means that the incubator must be able to maintain internal conditions of 37°C ± 0.5°C, humidity > 95%, and CO_2 levels must be 5% ± 0.1% for up to two weeks at a time. The third of the criteria is durability, which consists of no leaks and an easy sterilization process with ethanol. The last criterion is size. The final incubator must fit in the maximum constraints of 310x300x45mm in order to fit on the stage of the client's microscope. More detailed information can be found in APPENDIX I.

Preliminary Designs

Design 1: Layer of Water Design

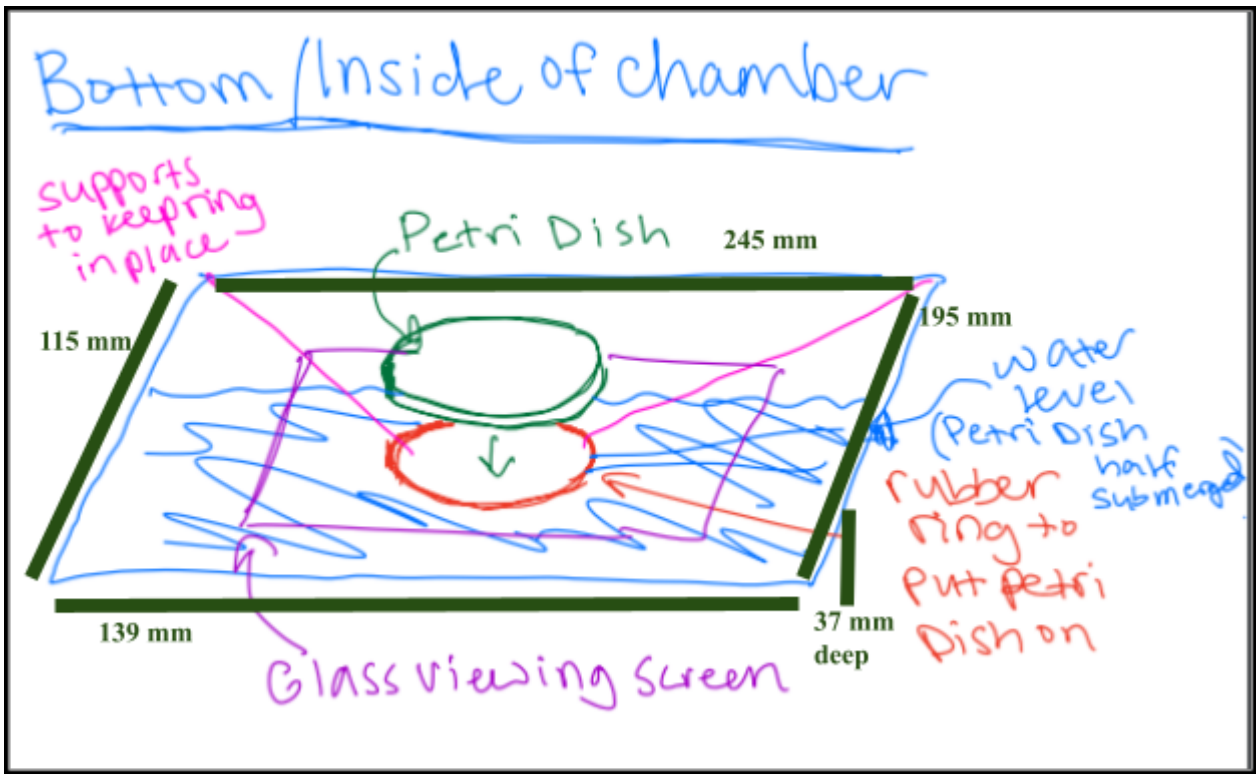

Figure 2. Layer of Water Design

Design one, depicted in Figure 2 consists of a 245x195x37mm black acrylic chassis manufactured by the team from previous semesters. It has two viewing screens made of glass. There will be a thin layer of water at the bottom of the chamber. This layer of water will prevent condensation on the bottom viewing screen because there is a uniform medium. A rubber ring will be secured at the bottom of the chamber and the petri dish will be placed on a rubber ring to

prevent movement. It will also prevent an uneven distribution of water from forming below the petri dish, which would affect the medium the light would travel through. The water level will be as tall as necessary for the bottom of the petri dish to be completely submerged, but not the top of the dish. The setup for this design is relatively simple; the user has to add a layer of distilled water at the bottom of the well, place the cell culture in, and then start the code for the incubator to run. The rubber disk, however, limits the type of petri dish that could be used. Only circular petri dishes that fit the size of the rubber disk could be used. Petri dishes of other sizes and cell culture flasks would not work with this design. Continuing with this design would require a different form of stabilization besides the rubber ring that would incorporate other cell containment forms.

An issue due to human error could be unintentional movement of the incubator, causing movement of the layer of water and possibly leakage into the petri dish; this could potentially cause issues with cell proliferation or reliable data collection.

Design 2: ITO Film Design

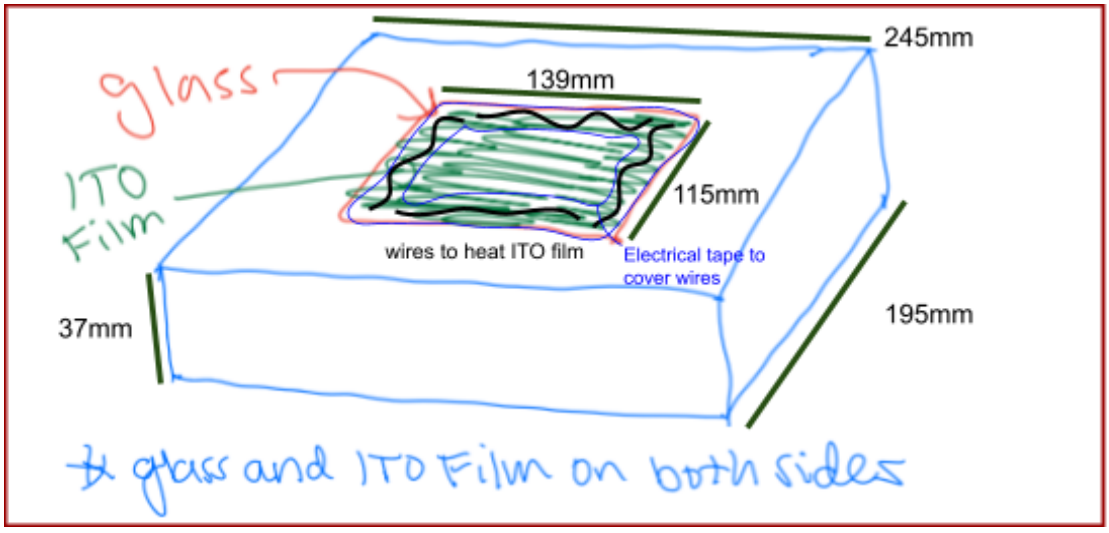

Figure 3. ITO Film Design

Design two consists of the same base chassis used in Design 1, as shown in Figure 3. Both viewing screens will be made out of glass but with an added layer of indium-tin-oxide (ITO). ITO is a transparent and conductive material. Both layers will be connected to an Arduino that will run charge through the copper wires connected to the ITO. This will heat the ITO and as a result, heat the glass underneath it. The heated glass will then no longer be able to form condensation due to uniform heating. Electrical tape will cover the wires to prevent shock from accidentally touching the wires.

This method suffers from being the most expensive solution; although, its connection to the Arduino and evenly distributed heating make it hypothetically the most reliable. The cost of

this solution might make other additions to the design much more difficult to fit into the team budget.

Design 3: Heated Wire Design

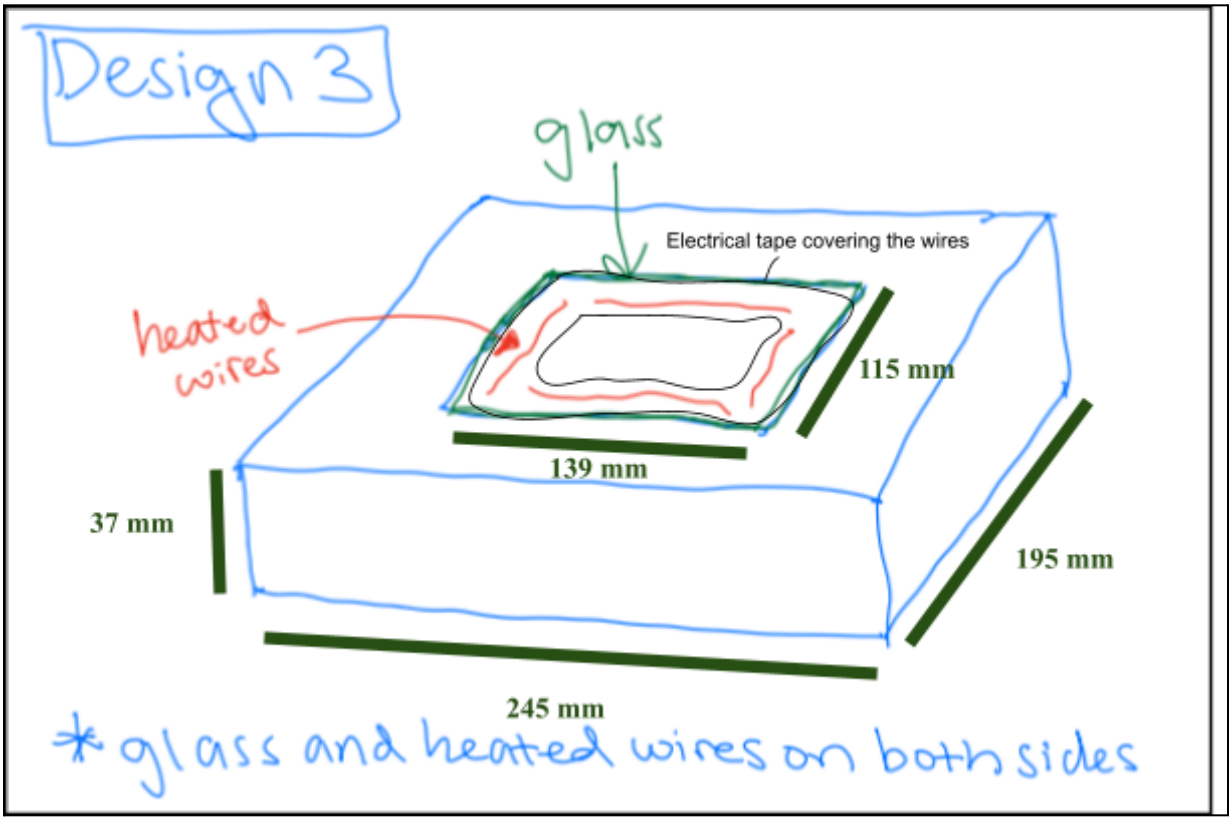

Figure 4. Heated Wire Design

Design three, shown in Figure 4, will have the same chassis and glass viewing screens as designs one and two. On the viewing screens, there will be copper wires around the edges. These wires will be connected to an Arduino that will run current through them and monitor the temperature inside the chamber. This design's benefits come primarily in cost, as it does not include the ITO on either side of the chamber. The glass and copper wire upgrade to the existing design will be very inexpensive and should fix the condensation problem. Electrical tape will cover the wires to prevent shock from accidentally touching the wires.

Preliminary Design Evaluation

Criteria	Weight	Design 1 Layer of water + glass on bottom, heating element on top		Design 2 ITO Film + Glass (maintain constant temp)		Design 3 Heated Wire Design + glass on both sides	
Reliability	40	4/5	32	5/5	40	2/5	16
Cost	25	4/5	20	2/5	10	5/5	25
Durability	10	3/5	6	3/5	6	2/5	4
Ease Of Fabrication	10	3/5	6	3/5	6	5/5	10
Ease of Use	10	3/5	6	4/5	8	5/5	10
Safety	5	5/5	5	4/5	4	3/5	3
Total	100	75		74		68	

Table 1: Preliminary Design Matrix

Summary of the Design Matrix

In order to effectively evaluate each of the preliminary designs, a comprehensive design matrix was created. This matrix analyzes several factors related to the application of designs through the use of each criteria. Each design is scored on a scale from 1 to 5 with each criteria having a different weightedness. A score of 1 is unsatisfactory and a 5 is very satisfactory. Six different criteria were defined as the following:

- *Durability*: Considers how long the parts of the design could be expected to last with consistent use. A higher score represents a design that would not be expected to break, even with long-term use. A low score means that the design would likely be prone to breaking down.
- *Reliability*: Considers how consistently and to what standard the design would fulfill its intended purpose of defogging the glass. A higher score means that the design is expected to consistently and totally solve the fogging issue. A low score means that the design is expected to infrequently or poorly solve the fogging issue.

- *Ease of Fabrication*: Considers which designs would require the least amount of effort and strenuousness to fabricate correctly. A higher score indicates a design that can be fabricated with less effort, while lower scores represent higher effort.
- *Cost*: Considers the amount of money needed to fabricate and maintain each design. Low scores indicate a higher cost and higher scores indicate a lower cost.
- *Safety:* Consider how safe each design is to use. Low scores indicate a less safe design and higher scores indicate a safer design.
- *Ease of Use*: Considers how easily the client will be able to use each design. Low scores indicate a design that will be harder to use (involves more moving parts) and higher scores indicate a design that will be easier to use.

Proposed Final Design

Our proposed final design was initially a combination of Design 1 and Design 2. It uses Design 1's layer of water solution as shown in Figure 2 for the bottom viewing port and Design 2's ITO heating element for the top light window, shown in Figure 3. After further discussion, design 2 was chosen, applying ITO film to both the top and the bottom of the viewing windows and the team plans to keep all other designs and materials from the predecessor's designs including the copper tubing with a heated water pump, NDIR sensor to detect CO_2 levels, thermistor to detect temperature, and CO_2 pump. The previous team's final design is shown in Figure 5 and Figure 6.

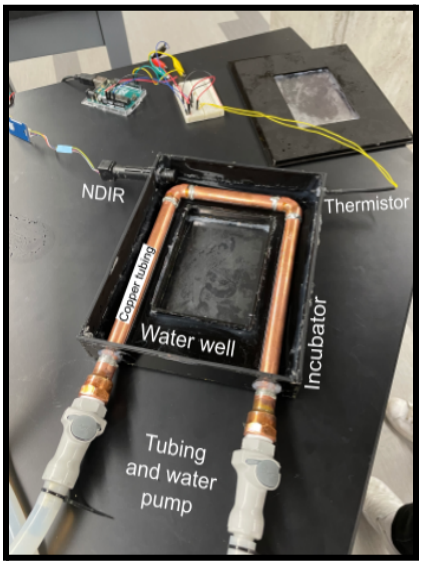

Figure 5: Previous Team's Final Design - Chamber

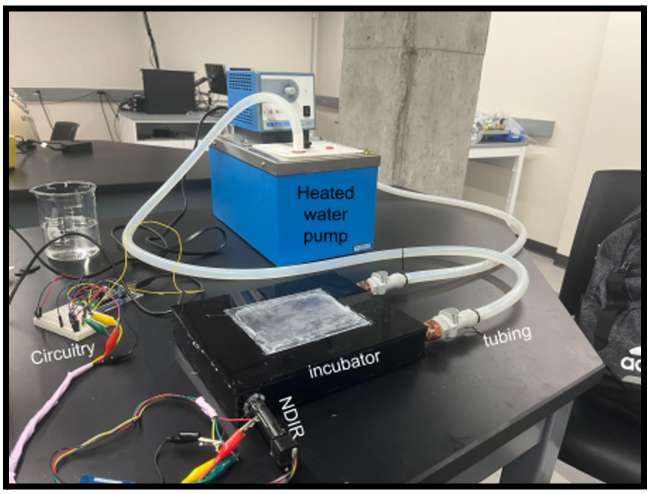

Figure 6: Previous Team's Final Design - Full View

Fabrication and Development Process

Materials

For the viewport, we will be using glass, distilled water, and a rubber ring to hold the petri dish in place above the distilled water, and polycarbonate to hold the water in a basin below the dish. We will use a layer of Indium Tin Oxide on the glass and use an Arduino to run a variable current through the layer and warm the glass depending on the internal temperature of the incubator. We will be using a solenoid valve and a NDIR CO_2 sensor to regulate the CO_2 in the environment. For heat control in the incubator, we will use a copper pipe that is connected to a heated water pump. Lastly, the frame of the device will be acrylic and all parts that connect from outside of the device will be sealed using silicone.

Methods

CO_2 Control

Previous work has determined that the MH-Z16 NDIR sensor part number SEN-000030 from Sandbox Electronics accurately measures CO_2 levels [7] and that a 100% CO_2 tank along with a relay circuit system with a solenoid valve connected to the incubator can be used to regulate carbon dioxide levels [7]. A gas permeable RKI Sensor Cover, part number 33-0172RK from RKI will cover the sensor to make it water-proof in the high-humidity environment of the incubator. Both the sensor and the solenoid valve will be attached to an Arduino, which will only permit the solenoid valve to release CO_2 when the sensor detects CO_2 levels within the incubator are below 5%. Tests by the previous team have shown this to be effective over their testing period of 9 hours [7].

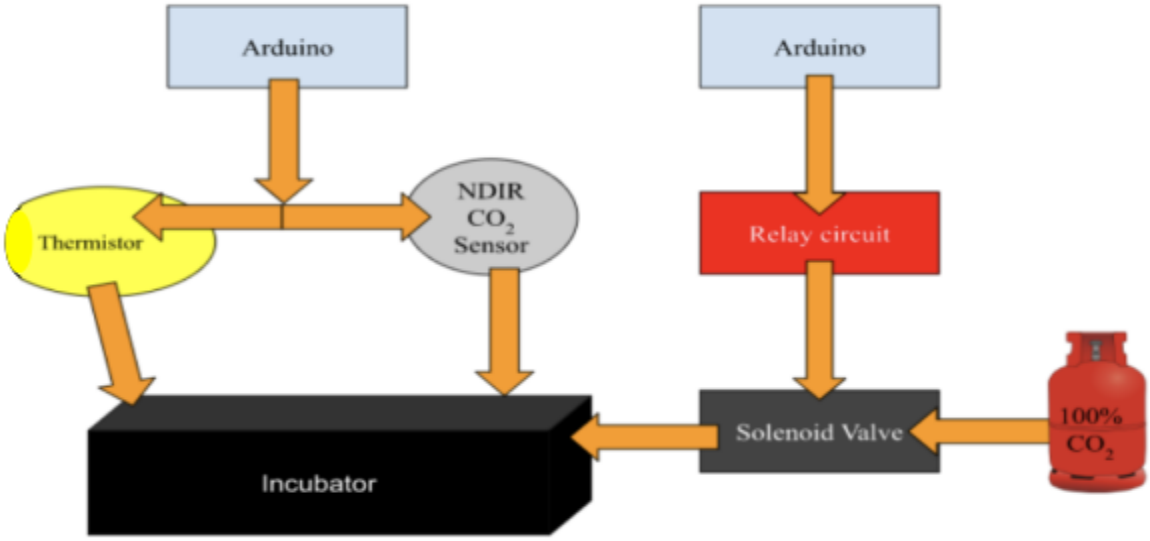

Figure 7. Diagram of CO_2 Maintenance Method by Previous Team

Temperature Control

A thermistor will continue to be used to measure temperature and humidity levels as previous work found through a two-sample t-test assuming equal variances that its accuracy is comparable to that of the commercially available DHT22 humidity and temperature sensor [7].

Condensation Control

The methods of the previous team to combat condensation were ineffective as microscope visibility was still significantly impaired. Previously used methods include anti-fogging spray, wipers, and mini-fans, which were either ineffective or caused issues such as requiring manual intervention. The team will utilize two sheets of ITO on the top and bottom viewing panes.

Due to the laws of refraction ($n_1 \sin\theta_1 = n_2 \sin\theta_2$), light from our eyes that enter droplets refract off the spherical in a multitude of directions. This causes vision through the droplets to be unclear. However, a uniform film on both viewing ports would cause all light to refract in the same direction, causing vision to be clear past it. With no condensation with the ITO, a uniform medium for light to pass through, phase contrast is ideally maintained.

A heated ITO film will be attached to the top and bottom viewing glass with an adhesive and powered by an Arduino. The formula for dew point for relative humidity levels above 50% is shown in Figure 8: Td = dew point temperature ℃, T = observed temperature ℃, RH = relative humidity (in percent) [8]. Based on the levels of temperature and humidity of the incubator, the Arduino will set the temperature of the ITO film to be higher than that of the dew point temperature, preventing condensation from forming. Through testing, the team has realized that 20V at 0.2 Amps is enough to heat both sheets to 38℃.

$$T_{Dew\ Point} = T - \frac{(5100 - RH)}{5}$$

$$T_{Dew\ Point} = 38 - \frac{(5100 - 95)}{5} = 38℃$$

Figure 8. Humidity Calculation Equation

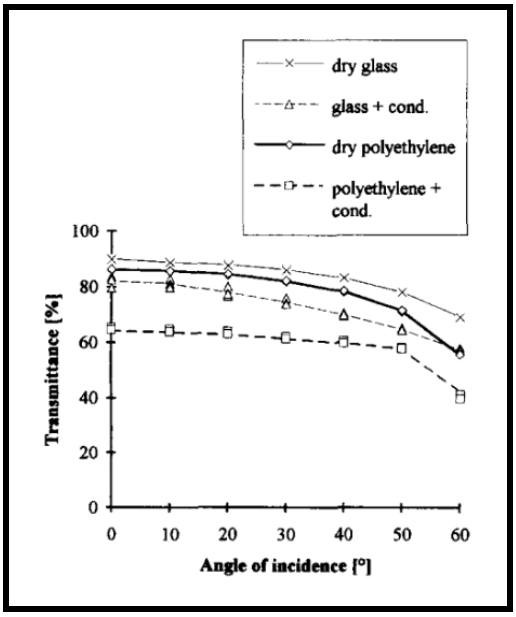

Figure 9. Light Transmission Scatter Plot of Glass and Polyethylene

Final Prototype

Our final product as shown in Figure 12, involves the ITO and heated wires on both sides of the chamber. The water pump heats the inside of the chamber by pushing warm water through the inner-pipes. There will be a water tray placed at the bottom of the incubator to maintain high humidity. A thermistor will be used to measure temperature and humidity. We kept the polycarbonate for our transparent surfaces to minimize cost and time. ITO is on the top and bottom viewing screens and the Arduino is connected to the wires attached to the ITO, which heats the top and bottom viewing screens creating a more uniform heat distribution across the chamber and reducing condensation on the screens. A MH-Z16 NDIR CO_2 sensor, 100% CO_2 tank with a relay circuit system, and a solenoid valve connected to the incubator will maintain necessary CO_2 conditions. An Arduino will be connected to each of the sensors along with the CO_2 valve and ITO film to maintain the required conditions.

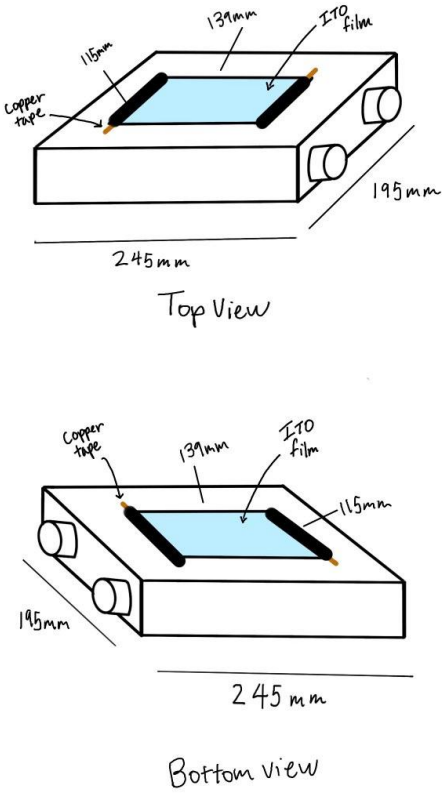

Figure 10: A drawn top and bottom view of the final design

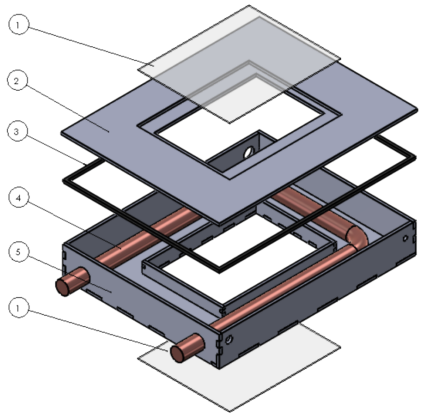

Figure 11. Exploded CAD model of final product

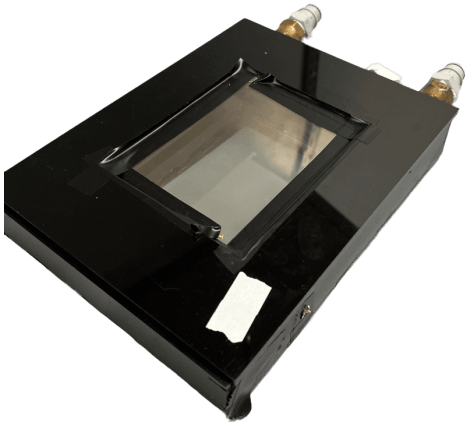

Figure 12. Final product

Results

Testing

Condensation Testing

For the condensation testing, we followed the protocol listed in the Appendix V. This test will evaluate how well the ITO film will meet the criteria of having no condensation as indicated in the PDS. The testing primarily consisted of running the water pump with the ITO film off first as a control and then with the film activated as the independent variable. Pictures of both test scenarios were taken under a microscope and then ImageJ was used to calculate the number of water droplets and the percent area coverage of the water droplets in 9 different zones in the viewing windows of the incubator. Statistical analysis was run using MATLAB. The results from

this test will qualitatively and quantitatively show us how well the ITO film removes condensation from the incubator viewing ports in normal use.

Condensation Testing Results

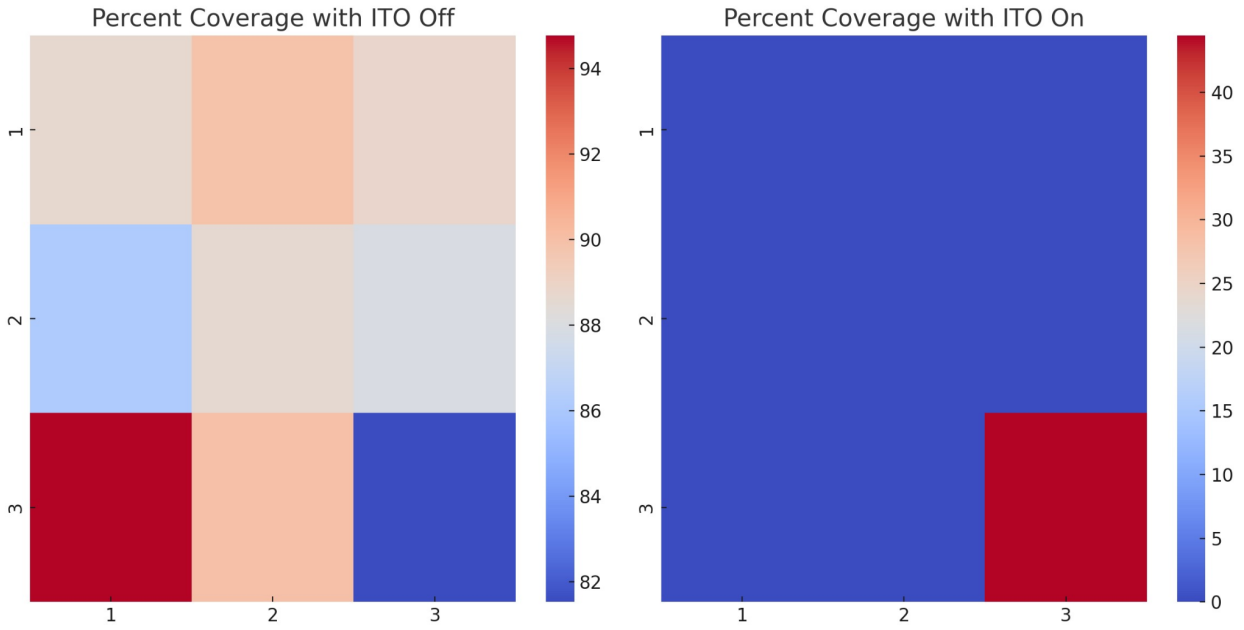

Figure 13. Heat maps visualized the effect of using ITO film.

Statistic	ITO Off	ITO On
Mean Droplets	565.72	18.06
Median Droplets	614.00	0.00
Std Dev Droplets	208.45	51.07
Mean Coverage (%)	88.48%	4.94%
Median Coverage (%)	88.62%	0.00%

Table 2. Descriptive statistics of the condensation testing.

For the statistical analysis, common descriptive statistics were calculated, as shown in Table 2, paired t-tests were run for the quantity of droplets and percent coverage of droplets on the viewing windows. The results from the paired t-test for the number of water droplets with the

ITO film were as follows: t-value: 8.219 , p-value: 3.594e-05. The results for the percent coverage of water droplets was: t-value: 14.246, p-value: 5.742e-07. These results indicate that the addition of ITO film was extremely statistically significant.

Temperature Testing

Temperature testing consisted of 2 aspects, exact details in Appendix V but instead of 14 days of testing, 15 minutes per trial was done due to time constraints. For both tests, the first aspect was testing if the internal temperature of the incubator matched the PDS criteria of 37℃ average. The second temperature testing aspect was analyzing the correlation between different levels of voltage and the temperature of the ITO film. This test allows us to understand and get a better idea of how much power we need to apply to get the ideal temperature to prevent dew to furthermore condensation from forming. For both testing scenarios, the heated water pump was run at a setting of 38℃ and the ITO film was applied with designated voltage. Descriptive statistics were calculated in MATLAB.

Internal Temperature Testing Results

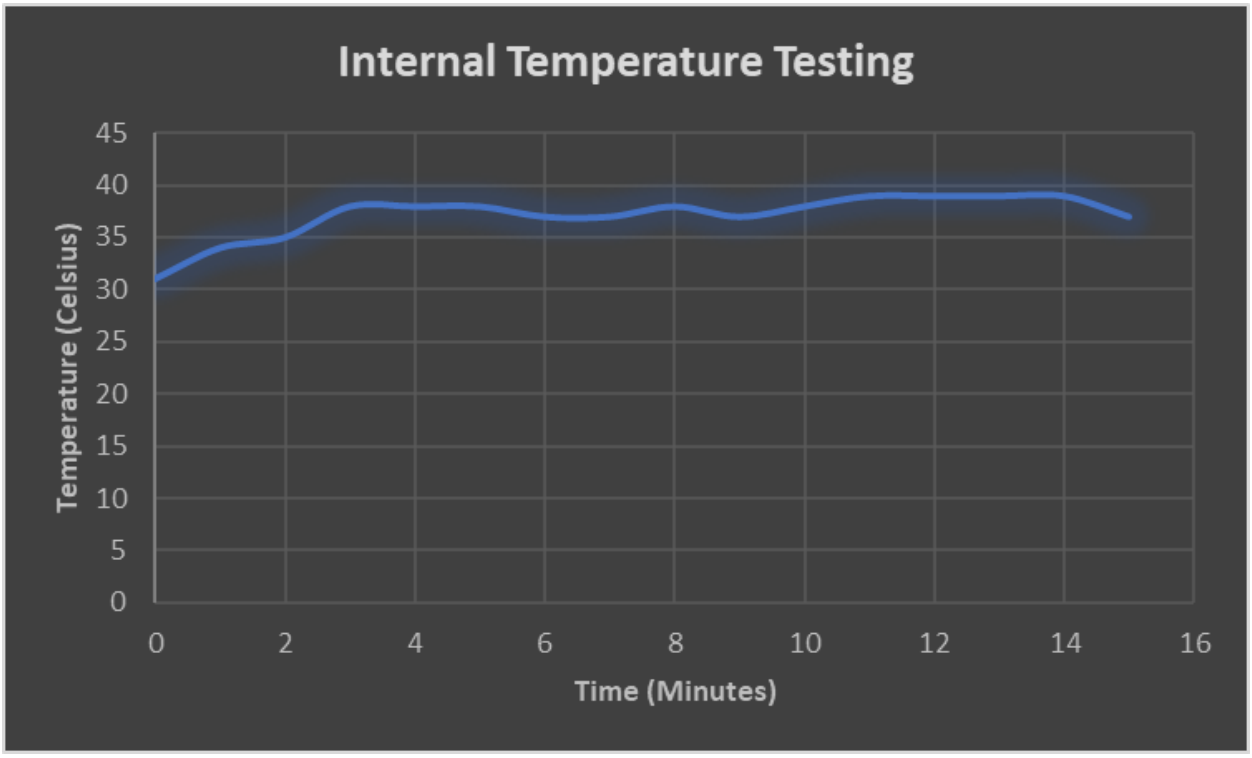

Figure 14. Plot of Temperature vs Time in the internal environment of the incubator

Statistic	Value
Values	16
Mean (°C)	37.13
Standard Deviation (°C)	2.16
Minimum (°C)	31.00
25th Percentile (°C)	37.00
Median (50th Percentile) (°C)	38.00
75th Percentile (°C)	38.25
Maximum (°C)	39.00

Table 3. Descriptive statistics of internal temperature testing.

These results from the internal temperature testing indicate how the use of the heated water pump and 20v with ITO film was successful in keeping the temperature at an average of around 37°C. Although the standard deviation is somewhat high, as shown in Table 3, the results successfully pass the criteria required by the PDS.

ITO Film Temperature Testing Results

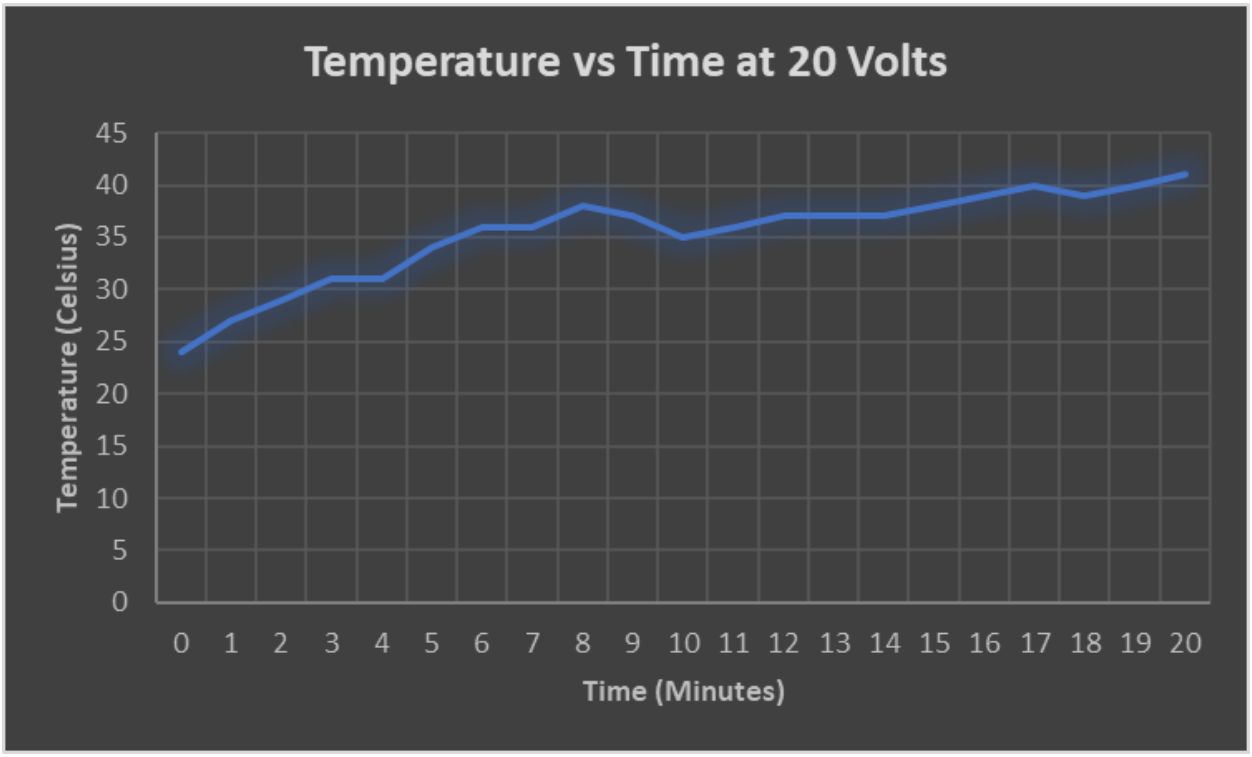

Figure 15. Temperature results of ITO film at 20V.

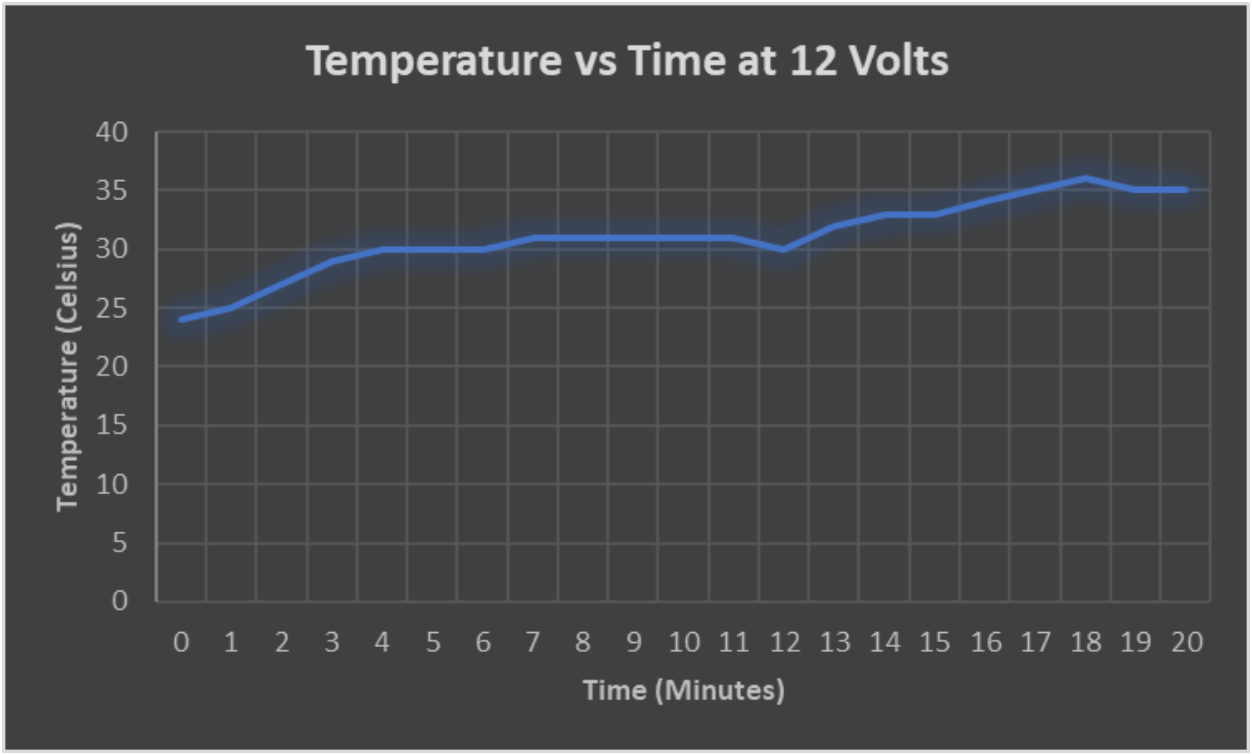

Figure 16. Temperature results of ITO film at 12V.

Voltage	Statistic	Count	Mean (°C)	Standard Deviation (°C)	Minimum (°C)	25th Percentile (°C)	Median (50th Percentile) (°C)	75th Percentile (°C)	Maximum (°C)
12V	Temp (°C)	21	31.10	3.16	24.00	30.00	31.00	33.00	36.00
20V	Temp (°C)	21	35.33	4.51	24.00	34.00	37.00	38.00	41.00

Table 4. Descriptive statistics of ITO film temperature testing

These results communicate to us that 12V is not enough to reach the dew point, due to the average temperature of 31°C and only a max temperature of 36°C, which is still not high enough to prevent condensation. The 20V however heats up very quickly and easily maintains a temperature close to 40°C after 15 minutes, which based on the dew point calculations found prior, should prevent any and all condensation from forming. These results from the 20V test show that ITO can successfully heat to ideal temperatures when applied with 20V of DC power.

CO_2 and Humidity Testing

During the fabrication process, our CO2 sensor detached from its interface board which meant we couldn't conduct any CO_2 testing. The testing would have followed the protocol outlined in Appendix V. One key aspect that was changed was the Arduino code controlling the solenoid. Instead of hardcoding a set time for the solenoid to be open, the valve will fluidly open and close based on the readings from the NDIR CO2 sensor.

The team was not able to conduct any humidity testing as well due to the CO_2 Sensor being non-operable. Previous teams have had success with placing a water tray at the bottom of the incubator.

Cell Visibility Testing

Cell and visibility testing was not completed due to time constraints, but we hypothesize that cell cultures will be visible due to the use of uniform mediums for light to pass through, which ideally will maintain phase contrast. Due to the lack of definitive results, this aspect of the incubator is not confirmed and is something that we encourage to be tested in the future.

Discussion

The application of Indium Tin Oxide (ITO) film in our study has shown remarkable effectiveness in mitigating condensation within the cell culture incubator environment. This

aligns with the inherent properties of ITO, notably its high electrical conductivity and optical transparency, which have been exploited in various fields ranging from optoelectronics to energy-saving windows [3]. The results indicate a robust anti-condensation effect, as evidenced by the near-elimination of droplets in most observed zones.

Our investigation into the efficacy of ITO film in controlling condensation within cell culture incubators has yielded compelling results. The significant reduction in condensation, particularly in zones 1 through 8, suggests that the ITO film creates a more stable microenvironment conducive to cell culture maintenance. The issue with zone 9 having condensation was most likely due to inadequate contact of the ITO film to the polycarbonate viewing window. Our statistical analysis underscored the film's efficacy, where a significant reduction in droplet counts and percent area coverage was observed. The p-values obtained from our paired t-tests, which were orders of magnitude below the conventional alpha level of 0.05, reject the null hypothesis, indicating that the differences in condensation with and without the ITO film are extremely statistically significant.

The implications of these findings are substantial, considering the pivotal role of controlled environments in cell culture studies. The ITO film's ability to maintain clear observation windows without compromising the incubator's internal conditions could be revolutionary, improving both the accuracy and reliability of experimental outcomes [1]. Comparative analysis with literature indicates that while the use of anti-fogging technologies is prevalent in various applications, their implementation in incubator design is novel. Our approach offers a low-cost alternative to more complex humidity control systems, potentially increasing accessibility for under-resourced laboratories [2][3].

Acknowledging the limitations of our study, such as the short observational period, the lack of CO_2 measurements, and the lack of live-cell culture data, we recognize the need for a longitudinal approach to fully understand the long-term impacts. External environmental factors, such as ambient temperature fluctuations and airflow dynamics within the lab, may also affect the internal conditions of the incubator, presenting variables that were not controlled in this initial study.

Future research directions include a comprehensive evaluation of cell viability over extended periods within the modified incubator environment, the assessment of ITO film longevity, and the exploration of scalable integration methods for larger incubator models. Investigations into the environmental impact of widespread ITO film adoption in lab settings could also provide insights into the sustainability of this innovation.

In conclusion, our findings suggest that the integration of ITO film into cell culture incubators could represent a significant advancement in laboratory equipment design. By providing a clearer and more controlled environment, this technology has the potential to enhance experimental reproducibility and reliability, ultimately contributing to the advancement of biological research and therapeutic development.

Conclusion

Conclusion

With this project, the team set out to develop a low-cost cell culture incubator that would function with an inverted microscope. The final design incorporates ITO films, a water pump, and multiple sensors to maintain internal conditions. The final design has multiple strong aspects including the reduction of condensation, the maintenance of the conditions necessary for the growth of the cells in the incubator, and the success of keeping the design within budget. The main weaknesses of the design include the excessive power it draws due to multiple parts used in aiding the machine's functionality and the lack of cable control that creates desk clutter due to these same parts.

Future Work

Multiple alterations and additions can be done to increase the ease, durability, and functionality of the incubator. Finding an alternative way to heat the viewing screens besides copper wires could prove to be more durable, more uniform for heat distribution across the screens, and slightly safer from accidental wire shocks. Changing the viewing screens from plastic to glass allows for easier heat transfer from ITO and maintenance of the incubator. Minimizing the clutter of tubes and wires will make the incubator easier to store and move around the workspace. Running more trials with all components of the incubator, including a functional CO_2 sensor, will be important for determining if the incubator can successfully keep cells alive.

References

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Appendix

Appendix I - Project PDS

Product Design Specifications: Microscope cell culture incubator

Client:	Dr. John Puccinelli
Advisor:	Dr. Joshua Brockman
Team:	Rishi Mereddy *(Leader)*
	Cherry Qui *(Communicator)*
	Presley Hansen *(BSAC)*
	Edward Han *(BWIG)*
	Roshan Patel *(BWIG)*
	Keleous Lange *(BPAG)*
Date:	September 22nd, 2023

Problem Statement

This goal for this team is to create a low cost cell culture incubation chamber for an inverted microscope that is capable of live cell imaging. An internal environment of 37 C, 5% CO2, and 95-100% humidity must be maintained over a long duration of time, without affecting the microscope's optics or functionality. Maintaining even heating and humidity across the chamber is necessary to prevent gradients that will form condensation on the viewing surfaces. Current commercially available systems are extremely expensive, large, and enclose the entire microscope making it difficult to assemble and remove in between uses. Because of their size, they also hinder use of the microscope in general.

Client Requirements

- No condensation can form on the viewing screen of the incubator
- Cannot hinder the optics of the microscope
- Must be able to be cleaned with ethanol
- Make the temperature across the incubator and cell culture as uniform as possible
- Limit the amount of wires if possible

Design requirements

1. **Physical and Operational Characteristics**

a. *Performance requirements*: The device must fit on the inverting microscope used by the client, have a window that allows the user to see the cell culture as well as if there was no incubating chamber there. The device must maintain an internal environment of 37℃, 5% CO_2, and 95-100% humidity. Condensation build up due to humidity must be removed in order to preserve clarity for the user.

b. *Safety*: The device should operate without harming the user in any way. Some issues that we may encounter include the heat of the object surface coming into contact with the user, improper gas connections leaving CO_2 gas to fill the room, and breaking of glass due to improper usage or drastic changes in temperature.

c. *Accuracy and Reliability*: The device must be able to maintain a temperature of 37°C ± 0.5°C [1] throughout the entire internal environment. The humidity must be kept above 95% humidity [2]. CO_2 levels must be 5% ± 0.1%. 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 should be able to sustain two weeks of active use with a viable cell culture. This includes humidity, CO_2 levels, and temperature that are all within the optimal range. Optimal use will be for one week at a time as part of a tissue engineering lab.

e. *Shelf Life*: The shelf life of this device will be ten years.

f. *Operating Environment*: The device will be used in a clean room and there are no adverse effects caused by this environment. The incubation chamber will contain internal conditions of 37 °C, 5% CO_2, and 95-100% humidity over a long duration of time.

g. *Ergonomics*: This device is being reinvented in part due to the lack of ergonomics for devices that are currently on the market. In the other devices, our client described difficulties in setting up the microscope to work with the cell cultures despite being experienced with microscope usage. Therefore, our device must function in such a way that it is easy to take on and off of the microscope while still maintaining its primary function. The device must also be easy to set up and start the process so that it can be done by a user with minimal experience [3].

h. *Size*: The device must have a maximum size of 310x300x45mm [4]. This size constraint will allow the device to effectively interface with the current set up used by the client. In general, this device needs to fit with all inverting microscopes.

i. *Weight*: The stage of an inverted microscope can usually handle a maximum weight of 30kg [4]. Although the stage can hold anything weighing below 30kg, the incubator will and should be much lighter, so it can be easily moved and stored.

j. *Materials*: The device may use stainless steel as its core and incorporate copper wirings as a variety of sensors. The device may use a water heater as its heating source and may thus also use a water pump.

k. *Aesthetics, Appearance, and Finish*: The device may look rectangular in shape, with a water heater pipe running along its perimeter. The device may be filled with water, with a glass window for the microscope to see through.

2. Production Characteristics

a. *Quantity*: Only one device is necessary. However, two would be preferable for the tissue engineering class it is designed for. The device should ideally be able to be produced at a larger scale if required.

b. *Target Product Cost*: The target cost for the whole project is under $100. The goal is to utilize other models, previous prototypes, and used sensors to minimize cost so that we can effectively accomplish the tasks we set out to accomplish. It is currently estimated by the client that most of the budget for this project will be allocated to sensors and the devices needed to make use of the sensor functions.

3. Miscellaneous

a. *Standards and Specifications*:

i. Product should follow the standards and regulations set by the FDA in CFR Title 21

1. The incubator must have multiple chambers or compartments filled with water in which controlled environmental conditions, particularly temperature, are maintained [5].
2. As a class one medical device, the device is exempt from premarket notification procedures and good manufacturing practice requirements [5].

b. *Customer*: The primary customer is John Puccinelli, professor at UW-Madison using this for the education of future BMEs. It may also be used by other professors and researchers that are looking for a more affordable option for a microscope cell culture incubator.

c. *Patient-related concerns*: N/A

d. *Competition*:

i. Current inverted microscope incubators and standard incubators are priced from around $500-$40000. These prices are costly in comparison to the client's demands. [4]

ii. Previous UW-Madison BME design team designed an incubator with a copper tube heating element that had water flowing through the copper tube. The design also had a solenoid valve to manage the CO_2 levels. One main issue with the design was the humidity impaired visibility of the microscope.

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Appendix II – Materials and Expenses

Final Cost Table:

Item	Description	Manufacturer	Part Number	Quantity	Cost Each	Total
Category 1: Incubator						
3D Printed Casing	Sides of Incubator	Makerspace		1	$20.00	$20.00
Plastic Latches	Secure Lid to Incubator	Cambro	Cambro 60264	4	$4.69	$18.76
ITO film	Heats Glass/Polycarbonate layer to prevent fogging	KDJ Electronics		1	$12.50	$12.5
Rubber Lining Tape	Lines the 3D printed sides of incubator	Makerspace		1	$0.00	$0.00
Lower ITO film	ITO (Indium Tin Oxide) Coated PET Plastic - 100mm x 200mm	Adafruit		1	$14.32	$14.32
Category 2: Components						
3/8x12 Stainless Steel Tube	Heated water will flow through	K & S Precision Metals	87119	1	$6.00	$6.00
3.8 in. Compression Brass Coupler	To connect stainless steel tube to water pump	Everbuilt	20715323	2	$3.65	$7.30
1.5mm Tube Connector	Connection between CO2 Tank and Incubator	Fisher Scientific	35031	1	$14.96	$14.96
Project Total:	$93.84					

Appendix III – CO2 Feedback Loop Code

CO2 Feedback Loop Code (From previous team):

```
#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);
int relayPin = 13;
float x;

void setup(){
```

```
 Serial.begin(9600);
 pinMode(relayPin, OUTPUT);
 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);
 }
}

 //concentration
  if (mySensor.measure()) {
    Serial.println();
  } else {
    Serial.println("Sensor communication error.");
  }
  x = (mySensor.ppm*5.0)/10000;
    if( x <= 4.5){
      digitalWrite(relayPin, HIGH); //switch relay on
      delay(50); //short input of CO2
      digitalWrite(relayPin, LOW);
      delay(5000);
    }else{
     digitalWrite(relayPin, LOW); //switch relay off
    }
Serial.println(x);
delay(600000);

int const CS; // input CO2 solenoid
int const VA; // CO2 valve
int const TS; // temperature value or voltage
int const IF; // ITO film

int CO2_floor;
int temp_floor
int DR; // duty ratio

void setup() {
 pinMode(CS, INPUT);
 pinMode(VA, OUTPUT);
 pinMode(TS, INPUT);
 pinMode(IF, OUTPUT);
 analogWrite(IF, DR);
```

```
}

void loop() {
 if(analogRead(CS) < CO2_floor){
   digitalWrite(VA, HIGH);
 }
 else{
   digitalWrite(VA, LOW);
 }
```

Appendix III - MATLAB Code

```
% Averaged data for Control tests
droplets_off = [614, 351, 343, 700, 823.5, 651, 227.5, 524, 857.5];
percent_coverage_off = [88.6185, 89.8915, 88.816, 86.1845, 88.6075, 87.9135,
94.7765, 90.0085, 81.527];
% Averaged data for ITO tests
droplets_on = [0, 0, 0, 0, 0, 0, 0, 0, 162.5];
percent_coverage_on = [0, 0, 0, 0, 0, 0, 0, 0, 44.483];
% Reshape the data into 3x3 matrices
droplets_off_matrix = reshape(droplets_off, [3, 3]);
percent_coverage_off_matrix = reshape(percent_coverage_off, [3, 3]);
droplets_on_matrix = reshape(droplets_on, [3, 3]);
percent_coverage_on_matrix = reshape(percent_coverage_on, [3, 3]);
% Create a custom colormap
cmap = [linspace(1, 1, 50)', linspace(1, 0, 50)', linspace(1, 0, 50)';
linspace(1, 0.5, 50)', zeros(50, 1), zeros(50, 1)];
% Create heatmaps for percent coverage
figure;
subplot(2, 2, 1);
heatmap(percent_coverage_off_matrix, 'Colormap',
cmap, 'ColorbarVisible', 'on');
title('Percent Coverage with ITO Off');
subplot(2, 2, 2);
heatmap(percent_coverage_on_matrix, 'Colormap',
cmap, 'ColorbarVisible', 'on');
title('Percent Coverage with ITO On');
% Create bar graphs for number of droplets
subplot(2, 2, [3, 4]);
bar([1:9; 1:9]', [droplets_off; droplets_on]', 'grouped');
set(gca, 'XTickLabel', {'Zone 1', 'Zone 2', 'Zone 3', 'Zone 4', 'Zone
```

```
5', 'Zone 6', 'Zone 7', 'Zone 8', 'Zone 9'});
ylabel('Number of Droplets');
legend('ITO Off', 'ITO On');
title('Comparison of Number of Droplets');
% Paired t-test for droplets
[h_droplets, p_droplets, ci_droplets, stats_droplets] = ttest(droplets_off,
droplets_on);
% Paired t-test for percent coverage
[h_coverage, p_coverage, ci_coverage, stats_coverage] =
ttest(percent_coverage_off, percent_coverage_on);
% Display results
fprintf('Droplets Test: t(%d) = %0.3f, p = %0.3e\n', stats_droplets.df,
stats_droplets.tstat, p_droplets);
1
fprintf('Coverage Test: t(%d) = %0.3f, p = %0.3e\n', stats_coverage.df,
stats_coverage.tstat, p_coverage);
Droplets Test: t(8) = 8.219, p = 3.594e-05
Coverage Test: t(8) = 14.246, p = 5.742e-07
```

Appendix V – Test Protocols

Protocol for Condensation Testing in Cell Culture Incubator

Objective

To assess the effectiveness of ITO (Indium Tin Oxide) film in reducing condensation within a cell culture incubator by comparing the number of water droplets and the percent area covered in control versus experimental conditions.

Equipment and Materials

- Cell culture incubator with viewing screen

- ITO film capable of being run at 20V

- Microscope with camera attachment

- ImageJ software for image analysis

- Standardized grid for defining zones on the viewing screen

Procedure

Setup

1. *Preparation of the Incubator*:

- Ensure that the incubator is clean and the viewing screen is clear of any obstructions.

- Mark the viewing screen into 9 equal zones for consistent image capturing.

2. *Equipment Calibration*:

- Calibrate the microscope and camera setup to ensure consistent magnification and focus across all zones.

- Set the ITO film to operate at 20V and verify the voltage with a multimeter.

Data Collection

Control Test

3. *Incubation for Condensation Formation*:

- Run the cell culture incubator for 45 minutes without the ITO film activated to allow for natural condensation build-up.

4. *Image Capturing*:

- Using the microscope with the camera attachment, capture images from each of the 9 zones on the viewing screen.

- Ensure that each image is taken at the same magnification and focus level.

5. *Image Analysis (Control)*:

- Open the captured images in ImageJ software.

- Use the counting feature to enumerate the number of water droplets in each image.

- Utilize the software to calculate the percent area covered by condensation in each zone.

Experimental Test

6. *ITO Film Activation*:

- Activate the ITO film and maintain the incubator at an internal temperature of 38 degrees Celsius.

- Run the incubator for another 45 minutes with the ITO film activated.

7. *Image Capturing (Post-ITO Film Activation)*:

- Repeat the image capturing process for each of the 9 zones as performed in the control test.

8. *Image Analysis (Experimental)*:

- Analyze the new set of images using ImageJ to determine the number of water droplets and the percent area covered post-ITO film activation.

Data Recording

9. *Documentation*:

- Record all measurements in a structured data sheet.

- Ensure that the data for control and experimental conditions are clearly labeled.

Data Analysis

10. *Statistical Analysis*:

- Compare the number of droplets and percent area covered between the control and experimental images.

- Use appropriate statistical tests to determine the significance of any observed differences.

Notes

- Ensure that all images are taken in a consistent manner to reduce variability in your results.

- Maintain the internal temperature of the incubator at 38 degrees Celsius throughout the experiment.

- Document any deviations from the protocol or any issues encountered during the experiment.

Cell Proliferation Testing Protocol

Experimental Design:

1. Cell Culture Setup:

- Initiate cell cultures of the same type in both the team's designed incubator and the control traditional incubator.

- Ensure consistent culture conditions, including media, seeding density, and environmental parameters.

2. Proliferation Period:

- Allow cells to proliferate for a designated period (e.g., 5 days).
- Perform daily inspections using an inverting microscope in the tissue engineering lab.

3. Image Capture:

- Capture images of each cell culture at 24-hour intervals using the inverting microscope.
- Ensure consistent image capture settings for both incubators.

4. Image Analysis:

- Import all captured images into ImageJ for quantitative analysis.
- Utilize ImageJ's measurement tools to quantify the following:
- Cell count per image at each time point.
- Proliferation rate based on the increase in cell count over time.

5. Success Criteria:

- A successful test will demonstrate comparable proliferation between the team's incubator and the traditional incubator.
- Quantitative analysis should reveal similar trends in cell count and proliferation rate over the 5-day period.

Additional testing:

1. Statistical Analysis:

- Apply statistical tests to assess the significance of any observed differences.
- Use appropriate statistical methods (e.g., t-tests or ANOVA) to determine if variations in cell proliferation are statistically significant.

2. Control Parameters:

- Ensure that both incubators are well-calibrated and maintained throughout the experiment.
- Monitor and record environmental parameters (temperature, CO2 levels, etc.) to account for any potential factors influencing cell proliferation.

3. Documentation:

- Maintain a detailed record of the experimental setup, including any deviations or unexpected events.
- Document any observations made during the inspections that may impact cell proliferation.

Temperature Testing Protocol

Thermistor Calibration:

1. Procedure:

- Calibrate the thermistor using the resistance values provided on the Arduino website.
- Ensure accurate calibration to establish a reliable baseline for temperature measurements.

2. Calibration Verification:

- Confirm the accuracy of the calibrated thermistor by comparing its readings with a reference thermometer.

Accuracy Testing:

1. Experimental Setup:

- Operate the incubator under normal conditions, with the thermistor recording temperature through the Arduino IDE.
- Place a secondary digital thermometer within the incubator for comparative purposes.

2. Recording Duration:

- 14 consecutive days, considering the intended usage period of the incubator.

3. Sampling Interval Adjustment:

- Sampling interval to record temperature every 10 minutes to balance data granularity and practicality.

4. Data Analysis:

- Analyze the recorded data to assess the thermistor's ability to maintain temperatures within 37°C ± 0.5°C throughout the extended recording period.
- Compare the thermistor readings with those from the secondary digital thermometer.

5. Success Criteria:

- The thermistor should accurately maintain temperatures within 37°C ± 0.5°C over the 14-day testing period.
- Record temperatures must remain within a 1°C deviation from the secondary digital thermometer.

Extended Testing Considerations:

1. Real-world Conditions:

- Mimic real-world conditions as closely as possible during the extended testing period.
- Monitor and document any variations in environmental factors that may influence temperature readings.

CO2 Testing Protocol

Calibration:

1. Procedure:
- Calibrate CO2 sensor to room conditions
- Increase and decrease the CO2 to check if the sensor could measure broad changes in CO2 level.

Accuracy Testing

1. Experimental Setup:
- Connect the incubator to the heated water pump and turn on the pump.

- Connect the incubator to the CO_2 tank and insert the CO_2 sensor and solenoid valve.

2. Recording Duration:
   - 14 consecutive days, when considering the intended usage of the incubator.

3. Sampling Interval Adjustment:
   - The sampling interval will be set to 10 minutes to balance data quality and practicality.

4. Data Analysis:
   - Analyze the recorded data to assess the solenoid's ability to maintain CO_2 levels within 5% CO_2 ± 0.5% throughout the extended recording period.
   - Compare readings with a secondary CO_2 Sensor

5. Success Criteria:
   - The solenoid should accurately maintain CO_2 levels within 5% CO_2 ± 0.5% over the 14-day testing period.
   - The recorded level of CO_2 must be within 0.5% of the secondary sensor.

Extended Testing Considerations:

1. Real-world Conditions:
   - Mimic real-world conditions as closely as possible during the extended testing period.
   - Monitor and document any variations in environmental factors that may influence CO_2 readings.