

# Microscope Cell Culture Incubator

BME Design 400  
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## Abstract

Live cell imaging allows researchers to precisely monitor temporal changes in cell morphology and behavior. To image live cells for extended periods of time, the temperature, pH, and concentration of their media must be maintained at optimal levels. Cell culture incubators typically maintain these conditions, but such devices do not fit on microscope stages. Therefore, our client, Dr. Puccinelli, wants the team to create an incubator modified to fit on a Nikon TI-U microscope stage without blocking the path of light from the microscope. This paper describes the team's process in developing a preliminary design for the on-stage incubator. The team plans to fabricate, test, and modify the described device.

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## Introduction

Observation and imaging of cell culture *in vitro* is an important part in understanding *in vivo* cell processes and studies. In order to most effectively represent the conditions of the *in vivo* environment, cell cultures are placed in incubators that maintain a constant homeostasis consisting of 37°C, relative humidity of 95%, and 5% CO<sub>2</sub> concentration [1]. However, when observing and imaging cells, the cells have to be removed from the incubator to be placed under the microscope. While the time outside of the incubator is often short, when imaging cells for long periods of time, the cells are removed from their environment for an extended period of time, which can affect the way the cells live, grow, interact, etc. A microscope stage-top cell culture incubator could be used to eliminate the need to remove the cells from the incubator

There are existing devices on the market including EVOS onstage incubator, Okolab Stage Top incubator, World Precision Instruments Stagetop Environmental Chamber with Controller, and Ibidi Stage Top Incubation system. However, these devices are often very expensive to purchase, for example the World Precision Instruments Stagetop Environmental Chamber with Controller costs \$11,500.00 [2] and the Ibidi Stage Top Incubation system costs \$13,990.00 [3]. The team is looking to design a microscope cell culture incubator that will be a much less expensive alternative to these very expensive devices.



Figure 1: EVOS onstage incubator

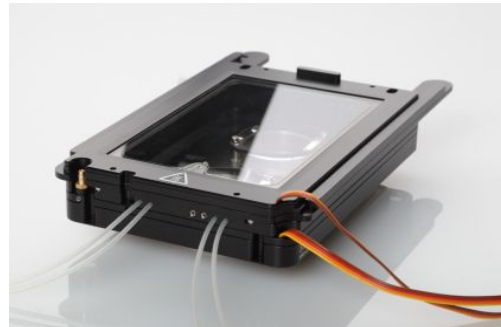


Figure 2: Okolab Stage Top incubator



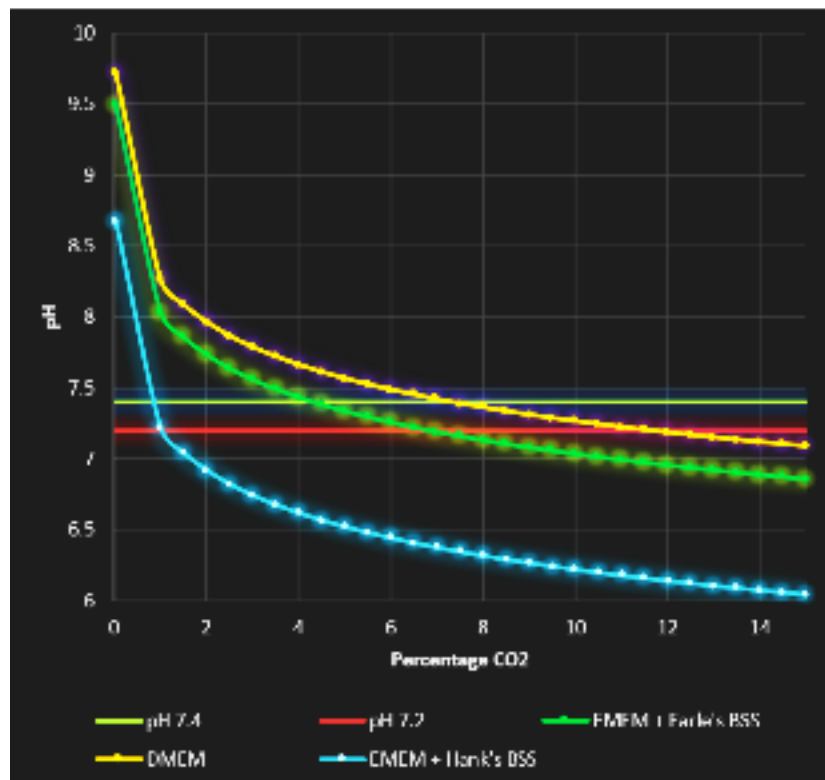
Figure 3: World Precision Instruments Stagetop Environmental Chamber with Controller



Figure 4: Ibidi Stage Top Incubation system

## Background

Cell culture incubators maintain explicit conditions in order to preserve the cells that are being studied. Failure to do so could result in the cells dying or altering of the cells that could inhibit positive research from being completed. These incubators help to create an environment similar to that of the cell's natural one. Mammalian cells grow best at 37 °C. Metabolism slows in colder temperature, and sensitive cells can die from 1 °C change in media temperature. Secondly, deviation in pH can drastically alter cell function and can cause death. 4-6% CO<sub>2</sub> concentration maintains a pH of 7.2-7.4 in growth medium containing 2.2 grams per liter of bicarbonate buffer. Bicarbonate-buffer-free media exists, which eliminates the need for CO<sub>2</sub>, but its uses are limited. Lastly, humid air prevents evaporation of media, which alters the concentration of salts, minerals, growth factors and analytes. Media evaporates from multiwell plates twice as much at 80% humidity than at 95% humidity, so we will try to keep humidity as close to 100% as possible [4][5]. (Humidity is consistently the most difficult parameter to establish, but most cell culture incubators can attain 95% humidity, and humidity is best maintained by keeping the incubator door closed.)



*Figure 5:* Change in pCO<sub>2</sub> results in different pH curves for different media depending on the concentration of bicarbonate buffer in the media. 4-6% pCO<sub>2</sub> maintains a pH of 7.2-7.4 in growth medium containing 2.2 grams per liter of bicarbonate buffer (e.g. EMEM + Earle's BSS). [6]

## Design Specifications

First and foremost, the incubator must maintain a uniform temperature of 37°C, nearly 100% humidity, and 5% CO<sub>2</sub> concentration, and it cannot impede the optical path of the microscope. To fit on top of the microscope stage, it must be smaller than about 190mm x 170mm x 40mm and lighter than 5 kg. Ideally, the incubator should allow a variety of culture plates, including multiwell plates, chamber slides, rectangular dishes, and Petri dishes, with a maximum size of 130mm x 90mm x 20mm. The user should be able to change culture plates and monitor the temperature, humidity, and pCO<sub>2</sub> of the incubator. Additionally, the incubator must be sterilizable to prevent contamination. Finally, the combined budget of the project is \$100.

## Previous Work

Other design groups have worked on this project in 2011, 2016, and 2017. Their progress has informed key decisions for our design. Our design will include a glass top, which McGinnity, et al. (2017) demonstrated to minimize optical impairment and endure many sterilizations while permitting an acceptable amount of heat loss. Additionally, we can reuse the power supply, the PCB, the CO probe, the temperature/relative humidity sensor, and solenoid. The limitations of their design are: the uniformity of the temperature was never validated, and the 3D-printed casing cannot withstand autoclaving and its porous nature limits its applicability as a biomedical device.

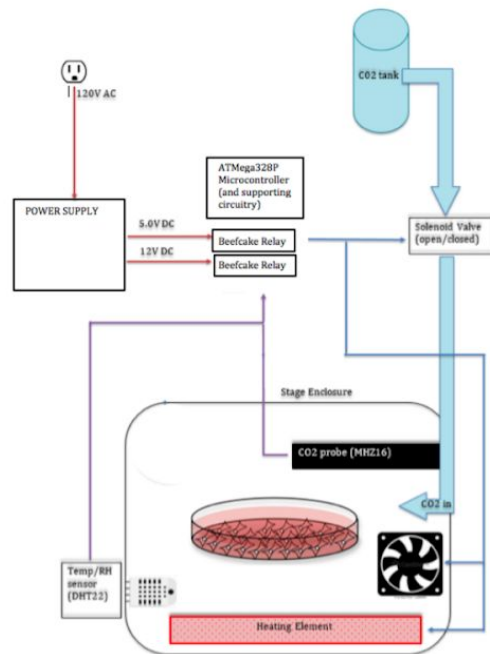
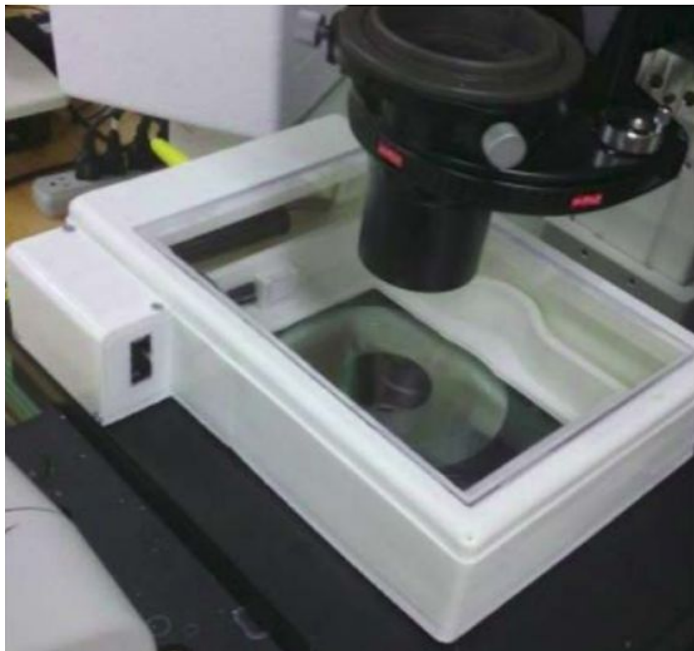
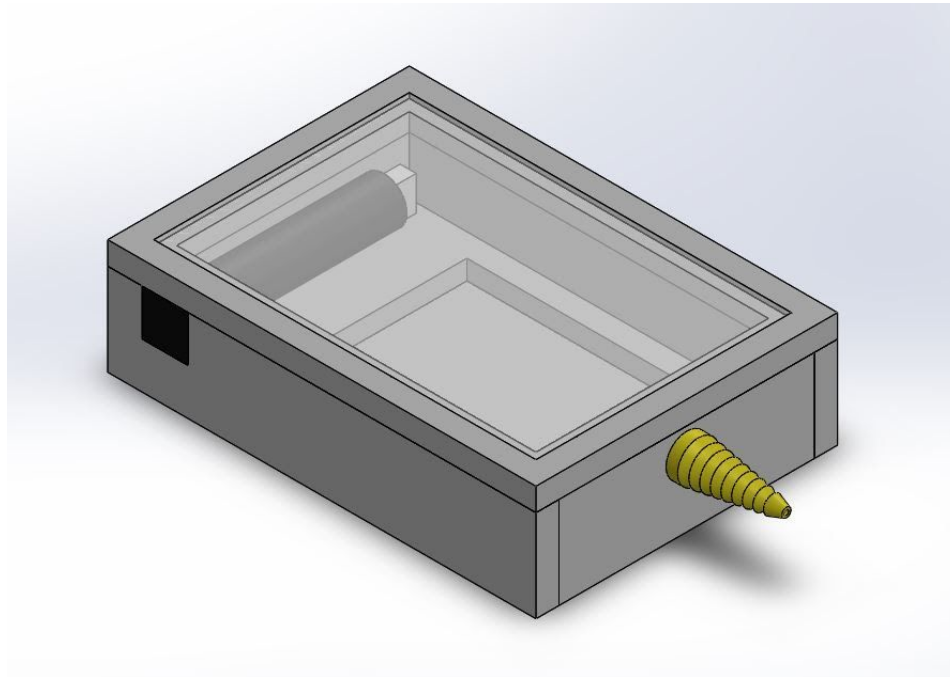


Figure 6: i) The final prototype created by McGinnity, et al. (2017). ii) A system diagram of the associated electronics. Red traces indicate the voltages coming from the power supply, blue wires indicate components that the microcontroller is influencing, and purple wires show feedback from the two sensors [7][8].

## Preliminary Designs



*Figure 7: View of a simple case that will be made of the different materials discussed*

The overall design includes a glass cover on top, a transparent heating element on the bottom, and a case shown in Figure 7. The top and bottom are optically transparent so that light from the microscope can pass through to the sample unobstructed. The case has holes in the side to fit a CO<sub>2</sub> input tube, a thermistor, a humidity sensor, a CO<sub>2</sub> sensor, and the leads for the transparent heating element. The case must be easy to fabricate, cheap, insulative, sterilizable, and durable. The potential case materials and the conditions are discussed below.

## Preliminary Design Evaluation

### **Design Matrix 1 Criteria:**

#### Heat Insulation

Heat Insulation describes the ability of the device to maintain the proper heating and humidity conditions required by the design specifications. This category is weighted at 30, the highest of all the categories as it is the most critical component of the design as it controls the conditions within the incubator.

#### Cost

The client has imposed a very modest budget, so cost is a limiting factor for all elements of the design. This is why cost is given a weight of 25 as it is one of our most important aspects to this project.

### Sterilizable

The appropriate and available methods of sterilization are autoclaving and EtO treatment, although autoclaving is preferred due to its cost and ease of use. This is weighted at 20 and is one of the highest categories because without sterile conditions, the cells will not be good for testing.

### Ease of Fabrication

The Ease of Fabrication design constraint describes how hard it would be to make the final design. This criteria was given a weight of 15 because, due to restrictions within ECB, all final designs will most likely have to be outsourced. In order to save money, the team can also create the incubator on their own, if COVID-19 guidelines allow.

### Durability

Durability describes how long this material will last over repeated uses while maintaining its original properties. Durability is weighted at 10 because it will be important for each material to not break down over time due to the incubator's conditions.

**Table 1: Design Matrix 1**

Categories	Weight	Design Element		
		Plastic with Insulation	Metal with Insulation	Acrylic Glass
Heat Insulation	30	4/5 (24)	5/5 (30)	3/5 (18)
Cost	25	5/5 (25)	3/5 (15)	4/5 (20)
Sterilizable	20	5/5 (20)	4/5 (16)	1/5 (4)
Ease of Fabrication	15	5/5 (15)	5/5 (15)	5/5 (15)
Durability	10	3/5 (6)	2/5 (4)	4/5 (8)
Total	100	90	80	65

### Plastic

Plastic material was chosen because it can withstand high temperatures for a sustained period of time, is cheap, can be 3D printed, and can be sterilized. As a result the plastic received a perfect score for ease of fabrication, cost, and sterilizability. The inner casing of the incubator will be made of plastic and then insulation with an outer casing of styrofoam will provide additional heat insulation. Plastic is also very cheap to manufacture, so the team's budget will not be affected much. Finally, most plastics can be sterilized easily through autoclaving. The plastics also did receive a decent score for heat insulation because an outer box with insulation will be used also.



Plastics received a low score for durability because compared to the other materials, they are not as strong.

### Metal with Insulation

The metal that the team would use for the incubator would be stainless steel. Stainless steel provides strong insulation and can easily be sterilized from an autoclave. The metal box would be easy to fabricate because the making of the box would be outsourced to another company. The metal box with insulation also provides strong heat resistance because of the inherent properties of the steel and the insulation. Stainless steel is an expensive material that would cost the team a decent amount of money to produce. The metal received a low score on durability because over time the metal might accumulate rust, which would affect the sterility of the incubator.

### Acrylic Glass

A glass case would be autoclavable, durable, relatively inexpensive, and easy to fabricate, but glass has poor thermal insulation. The glass we would choose to use would be acrylic or PMMA. PMMA can be easily fabricated for similar reasons above as we would outsource this aspect. PMMA, however, tends to be more expensive and would cut more into our budget so it did not win the cost category. PMMA is also not known for its heat insulation capabilities, so it scored the least of the three materials within this category. It can not be sterilized easily so it did the poorest in this category. Lastly, PMMA won the durability category because it has more durable qualities than the other two designs.

## **Design Matrix 2 Criteria:**

### Cost

The cost design constraint expresses how much each of the plastics will cost. The cost constraint is weighted as one of the highest constraints with a value of 25. This criteria describes how much each plastic will cost to purchase. The money saved in the cost of the sheets of plastics can be used in other aspects of the project.

### Heat of Insulation

Heat of insulation describes how effective the plastics are in keeping the heat within the incubator box. Due to its importance, heat of insulation was also given a weight of 25 because in order to build an effective incubator, constant heat needs to be maintained within the device.

### Sterilizable

To be sterilizable in this matrix means that the material can be autoclaved, or sterilized in another way without breaking down. Sterilizability was given a weight of 20 because the casing of the incubator must be sterile to not contaminate the cells it may be housing.

### Ease of Fabrication

Ease of fabrication describes how easy it will be for the team to make the incubator's casing. Due to this, ease of fabrication was given a weight of 15 because compared to the other constraints it is not as important. If the team cannot fabricate the incubator box, then the production of the box will be outsourced. All of the plastics in the matrix have similar properties so fabrication will be similar between them.

### Durability

The design constraint of durability describes how strong the plastic is. Durability was given a weight of 15 because compared to other design constraints it is not as important.

**Table 2: Design Matrix 2**

		Design Elements		
Categories	Weight	Acrylonitrile butadiene styrene (ABS)	High Density Polyethylene (HDPE)	Polypropylene (PP)
Cost	25	2/5 (10)	4/5 (20)	5/5 (25)
Heat Insulation	25	4/5 (25)	5/5 (25)	3/5 (12)
Sterilizable	20	5/5 (20)	2/5 (8)	5/5 (20)
Ease of Fabrication	15	4/5 (12)	5/5 (15)	5/5 (15)
Durability	15	5/5 (15)	3/5 (9)	4/5 (12)
Total	100	82	77	84

### Acrylonitrile butadiene styrene (ABS)

ABS was given a low score for cost because compared to the other plastics, it is the most expensive. It has very good electrical and thermal insulation properties, which is part of the reason it is expensive. It was given a perfect score on sterilizability because ABS can be autoclaved. It was only given a % score for ease of fabrication because it is most often welded into the final design, something that is not needed in the other plastics. Finally, it was given the highest durability score because it is the strongest of the materials.

### High Density Polyethylene (HDPE)

HDPE was given a % ranking for price because it is more expensive than PP and less expensive than ABS. Despite the price, HDPE has the highest insulation properties compared to the other plastics. Unlike the other plastics, HDPE cannot be autoclaved, forcing the team to find other

ways to sterilize the plastic. HDPE scores high in the ease of fabrication category because it is a versatile plastic that can be molded into almost any shape. Finally, HDPE scores moderately high for durability because it is a durable plastic that is abrasion and scratch resistant.

### Polypropylene (PP)

PP was given the highest score for cost because it is the cheapest material in the matrix. The plastic was also given a decent score of heat insulation because it has similar thermal insulation than ABS, but lower heat insulation than HDPE. PP can also be autoclaved, which is necessary for the final design. This material can also be molded into many different shapes, allowing for easy fabrication for the team. Finally, PP received a % score for durability because it is a strong material, but not as strong as ABS.

### **Proposed Final Design:**

The team's proposed final design will be made out of PP plastic with a glass cover top and bottom, with a heating element. The box will also have inlet holes for tubes, sensors, and leads that will be necessary in keeping the conditions of the incubator consistent.

## Fabrication

### **Current Materials:**

The MH-Z16 CO<sub>2</sub> sensor measures the CO<sub>2</sub> concentration through infrared strength received. It claims a +/- 50 ppm +5 % reading value. [9] The DHT-22 Temperature/Humidity sensor detects temperatures within the range -40 to 80 degrees Celsius +/- 0.5 degrees Celsius accuracy, and 0-100% humidity with a 2-5% accuracy [10]. The (1/4") Gas Solenoid Valve is used to control the CO<sub>2</sub> intake within the incubator. It has a power rating of 5W and takes 12V DC [11]. The Grove Water Atomizer is helpful in creating a humidifier that heats its surroundings using ultrasound. Operating voltage is 5V DC and frequency is 105 +/- 5kHz [12]. CO<sub>2</sub> and H<sub>2</sub>O are available within the teaching lab where it will be in use. Other electronics include wiring, an arduino microcontroller, breadboard, and two beefcake relays.

### **Methods:**

The team will be using a table saw to cut the PP plastic and a power drill to screw the screws in, forming the box. The sensors and heating elements will be placed on the inside of the box, while the circuit and Arduino microcontroller will be external to the box. The team will model the temperature distribution in COMSOL before testing.

### **Testing:**

For testing, the team will be testing the environmental conditions within the box. Graphs will depict the spatiotemporal variability of temperature, pCO<sub>2</sub>/pH and humidity/evaporation.

Additionally, the team will test the sterilizability of the plastic casing and the viability of multiple cell types grown in the incubator. Standard deviation and mean values of these conditions will be calculated. A student's T test will be run to determine statistical significance between the mean temperature of the experiment and the mean of the sensor's expected output, taking the sensor's variability into consideration.

A simulation was run to predict the distribution of temperature readings of the DHT-22 Temperature/Humidity sensor at 500 different time points. The expected error reading of the temperature sensor based on the data sheet was  $0.5\text{ }^{\circ}\text{C}$  and the error of the humidity sensor was  $2.5\%$ . The mean of the simulated temperature values can be calculated and then compared to the temperature recording inside the box. This same type of simulation can be run on the  $\text{CO}_2$  sensor which has an expected variability of  $5\%$ .

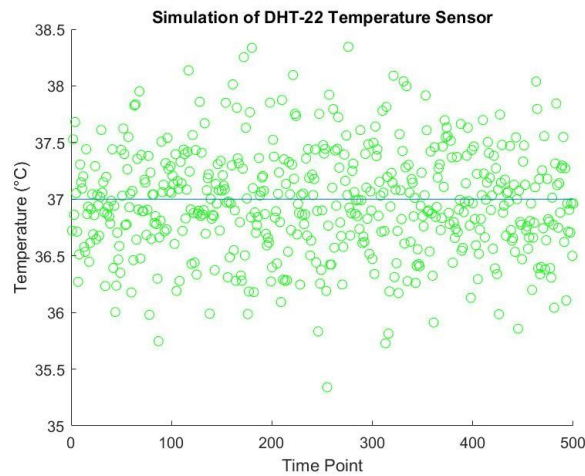


Figure 8 : Simulation of the DHT-22 Temperature Sensor based on 500 different time points

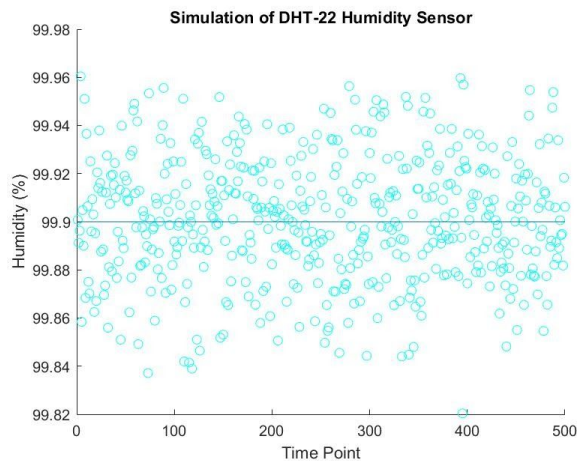


Figure 9: Simulation of DHT-22 Humidity Sensor based on 500 different time points

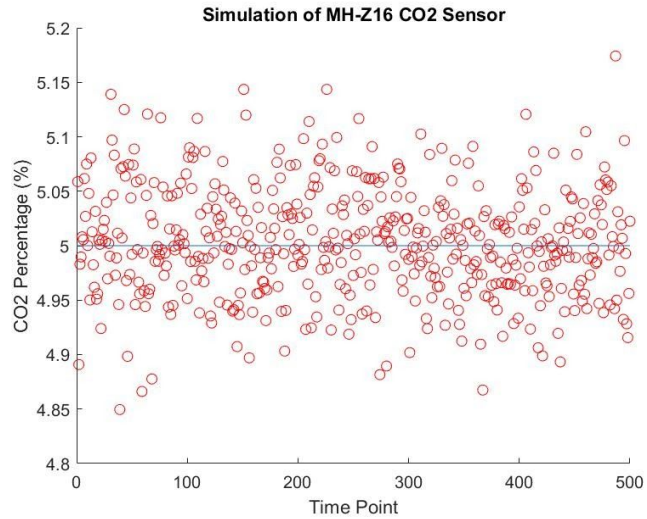


Figure 10: Simulation of the MH-Z16 CO<sub>2</sub> sensor based on 500 different time points

ANOVA tests will also be conducted by the team to compare the variability of the conditions within the box at different time points. Measurements within the box will be taken at different time points and the variability of the results will be analyzed. The code will be adjusted depending on the results of the testing.

The team also ran simulations using COMSOL simulation software to observe the differences in heat distribution using a water immersion heating element versus a transparent heating element beneath the cell plate.

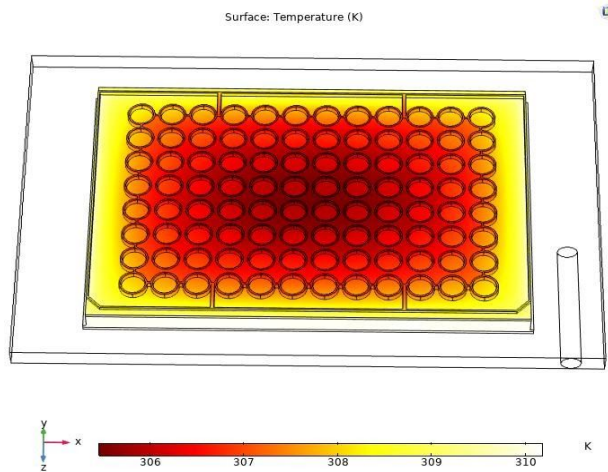
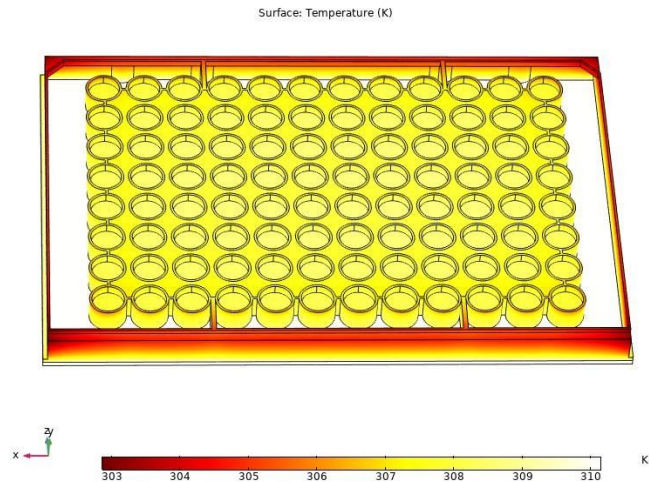


Figure 11: Simulated heat distribution using water immersion heating element



**Figure 12:** Simulated heat distribution using transparent heating element under plate

## Results

Graphs of the different environmental conditions and their variability will be displayed in this section. In addition, p-values of the different statistical tests run will be displayed here.

The previous design team used a heating element that was immersed in water, which dispersed heat more evenly and caused less media evaporation than a fan heater. As shown in Figure 11, the immersed heater still creates a mild temperature gradient across the cell culture plate. The temperature range is not large enough to kill most cells – all the wells are between 36 and 38 degrees Celsius, as indicated by the green box, but this gradient adds a confounding variable to the experiment. Not to mention, since there are wells in which the media temperature is exactly 36 and 38 degrees (the limits of the acceptable range, see Appendix A) the temperature of the water needs to be very precisely controlled to avoid harming the cells.

This semester the team considered coating the glass on the bottom of the incubator in Indium Tin Oxide, which is transparent and heats up when current runs through it. As shown in Figure 12, it provides a uniform temperature throughout the 96-well plate. Unfortunately, this setup would rely solely on the water atomizer for humidity since the transparent heating element cannot withstand constant exposure to standing water. Plus, a sufficiently sized transparent heater would exceed the project budget, whether sourced commercially or from the UW-Madison Nanoscale Fabrication Center. Therefore, we will use the immersion heater and increase the sampling rate of the temperature sensor to more precisely keep the wells between 36 and 38 degrees. It is also worth noting that the temperature gradient is less pronounced in plates with fewer wells.

Once we finish fabricating the device, we will measure the CO<sub>2</sub> concentration, temperature, and relative humidity at multiple points in the incubator for up to 7 days and determine whether

significant gradients exist (using a student's t test) and – qualitatively - whether the parameters remain in the appropriate ranges. If needed, we will improve the code or purchase more precise sensors. Then we will perform the same tests and determine if we significantly improved the performance of the incubator by comparing the number of deviations from the appropriate ranges and applying a Mann-Whitney U test.

## Discussion

Once testing has been done, discussion regarding the results and what went right and wrong will be here. Sources of error will be presented here also. Ideally, the student's t tests will indicate no statistical differences in pCO<sub>2</sub>, relative humidity, and temperature between multiple sensors at individual time points (i.e. no significant gradients exist) or between a single sensor and the expected mean (see Figs. 8-10) across multiple time points (i.e. conditions do not significantly fluctuate over time). In addition, the team hopes there is no statistical difference in the ANOVA test showing that the conditions within the box are the same at different time points. If the tests indicate that the incubator is unsatisfactory, we will purchase more precise sensors or modify the code or design before testing live cells.

## Conclusions

The goal of this project is to create a stage top cell incubator that still allows imaging of cells. The incubator box will be fabricated in early 2021, allowing the team to test the system and make improvements. Amendments to the code will be made based on the calculated quantitative results and the graphs generated from the time point measurements. Once the incubator box is deemed to keep a constant temperature, humidity percentage, and CO<sub>2</sub> concentration, culture plates seeded with live cells will be placed within the incubator to determine if the incubator can successfully keep cells alive. Images regarding proliferation rates of cells will be taken to show if cell growth is successful within the team's incubator.

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# Appendix

## Appendix A: PDS

### Microscope Cell Culture Incubator Preliminary Product Design Specifications

**Team:** Nick Pauly - *Team Leader*  
Bob Meuler - *BWIG & BPAG*  
Tim Madigan - *Communicator*  
Kevin Koesser - *BSAC*

**Client:** Dr. John Puccinelli

**Advisor:** Dr. Paul Campagnola

**Date:** Friday, September 18, 2020

#### **Function:**

To support live cell imaging in the BME teaching lab, the client needs a cell culture incubator that fits on the stage of a Nikon TI-U Inverted Fluorescence Microscope. The device must maintain constant environmental conditions (37°C, 100% Humidity, and 5% CO<sub>2</sub> concentration) for up to one week. Moreover, the incubator must have an optically clear top and bottom that do not interfere with fluorescence imaging. The device should accommodate different types of cell culture containers including multiwell plates, chamber slides, rectangular dishes, and Petri dishes. The client would like the device to display the environmental conditions and notify the user if conditions deviate from an acceptable range. Commercially available devices satisfy these design requirements, although they are expensive; the client's budget is \$100 per device.

#### **Client Requirements:**

- Maintain 37°C, 100% Humidity, and 5% CO<sub>2</sub> concentration
- Does not impede the optical path
- Allow cell plates with a maximum size of 130mm x 90mm x 20mm
- Uniform heating throughout the incubator
- Easy readout of conditions
- Ability to change out cell cultures
- Ability to be sterilized
- Combined budget: \$100

#### **Design Requirements:**

##### *Performance Requirements*

- Must be able to maintain uniform heat distribution, CO<sub>2</sub> concentration, and humidity within the incubator
- Limit the condensation within the incubator as to not visually impair the microscope

##### *Safety*

- Compliant with standards set by Biosafety Level 1
- Can controllably deliver CO<sub>2</sub> from a tank regulator maintained at 15 psi

##### *Accuracy and Reliability*

- Maintain a temperature of  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- $\text{CO}_2$  concentration  $5\% \pm 0.5\%$
- 95%-100% humidity

#### *Life and Service*

- The incubator must maintain these conditions for one week
- The electronics should not have to be adjusted or fixed during this time to preserve sterility

#### *Shelf Life*

- 3 years

#### *Operating Environment*

- Used within a Biosafety Level 1 environment
- All electronics must be sterile and water resistant
- System will have to adjust to changing environmental conditions due to opening and closing of the incubator

#### *Ergonomics*

- Device should be easily accessible for user
- Should be big enough for user to easily place and remove cell culture plate, flask, or petri dish
- Incubator door should be easy to open

#### *Size*

- Allow cell plates with a maximum size of 130mm x 90mm x 20mm
  - Slightly bigger than the size of a 96 well plate

#### *Weight*

- Lightweight as to allow the device to be easily movable
- Cannot be more than 5kg

#### *Materials*

- Transparent on the top and bottom for the microscope. Other incubator materials need to be heat resistant from the high humidity levels and sterilized

#### *Aesthetics, Appearance, and Finish*

- The materials through which imaging occurs needs to be transparent so as to not interfere with the image quality.
- There should be a user interface to display the current conditions of the incubator

### **Production Characteristics:**

- *Quantity:* 1 design
- *Target Production Cost:* less than \$100

### **Miscellaneous:**

#### *Standards and Specifications*

- None

#### *End User Concerns*

- Easy enough for undergraduate students to use

#### *Competition*

- EVOS Onstage Incubator [1]
  - Fully integrated environmental chamber for live cell time-lapse imaging
  - Easily maintain physiological conditions

- Sleek design
- Wide range of vessel holders
- Okolab Stage Top Incubator [2]
  - Temperature Accuracy:  $\pm 0.1^{\circ}\text{C}$  in sample feedback mode,  $\pm 0.3^{\circ}\text{C}$  in chamber feedback mode
  - Compatible with: Bold Line T, UNO Combined and H401-T Controllers
  - Embedded temperature sensor in heated glass lid and in chamber body
  - Interchangeable magnetic inserts allow to host Petri, Slides and MW Plates
  - Magnetic locks hold the Petri, the Slides and the MW Plates in the correct position inside the chamber
  - Perfusion holes available for inlet and outlet of tubes
  - Sliding lid allows easy access to the sample
- World Precision Instruments Stagetop Environmental Chamber with Controller [3]
  - Four programmable digital control loops:
    - Independent incubator base temperature PID control with  $\pm 0.1^{\circ}\text{C}$  precision
    - Independent incubator lid temperature PID control with  $\pm 0.1^{\circ}\text{C}$  precision
    - Airflow digital PID control from 0–900 SCCM
  - USB-based remote control and data logging
  - Electronic flow meter
  - Programmable alarm for out of tolerance condition on all four channels
  - Compact and lightweight
  - Monitor and control the flow and temperature in a microscope chamber or stagetop environment
  - \$11,500.00
- Ibidi Stage Top Incubation System [4]
  - Precise control of temperature, humidity, CO<sub>2</sub>, and O<sub>2</sub>
  - Compatible with all inverted microscopes that have a K-Frame stage (160 mm x 110 mm)
  - Prevents condensation by heating culture dish lid
  - \$13,990.00

## Appendix B: MATLAB Code for Sensor Simulations

```
%% For CO2 Sensor

rng(20)

x = 5+ 0.05*randn(500,1);

figure(1)

scatter(1:1:500,x, "r")

refline(0,5)

xlabel('Time Point')
```

```
ylabel ('CO2 Percentage (%)')  
  
title("Simulation of MH-Z16 CO2 Sensor")  
  
%% For Temperature Sensor  
  
rng(1000)  
  
figure(2)  
  
temp = 37 + 0.5*randn(500,1)  
  
scatter(1:1:500, temp, "g")  
  
refline(0,37)  
  
xlabel("Time Point")  
  
ylabel("Temperature (°C)")  
  
title("Simulation of DHT-22 Temperature Sensor")  
  
%% For the Humidity Sensor  
  
rng(2000)  
  
figure(3)  
  
humidity = 99.9 + 0.025*randn(500,1)  
  
scatter(1:1:500, humidity, "c")  
  
refline(0,99.9)  
  
xlabel("Time Point")  
  
ylabel("Humidity (%)")  
  
title("Simulation of DHT-22 Humidity Sensor")
```

## Sources

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