Microscope Cell Culture Incubator

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Client: Dr. John Puccinelli
Date: 02/25/2022

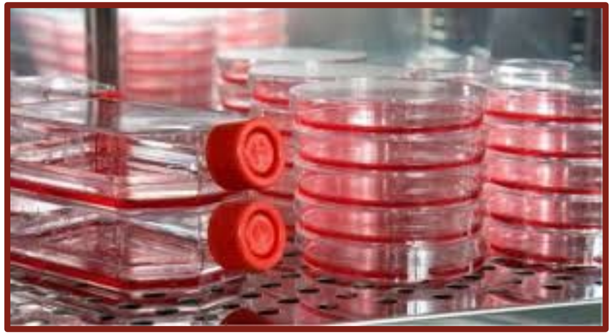

Figure 1: Cell Culture Plates [1]

Background Information

- **Client:** Dr. John Puccinelli; Associate Chair of the Undergraduate Program
- **Cell Cultures**
    - Lab method for the use of studying cell biology, replicating disease mechanisms, and investigating drug compounds [2]
    - Use both primary, transformed, and self-renewing cells
- **Incubators**
    - Replicate cells' natural conditions in order for optimal growth
        - Natural Cell Environment - 37°C, pH = 7.2-7.4, 95% humidity [3]
    - Cost: $500-$40,000 [4]
- **Live Cell Imaging**
    - Allows researchers to continually view cell development
    - Need incubator on a microscope in order to keep cells alive for imaging

Figure 2: On-stage incubator [4]

Problem Statement

- **Purpose*:*** Develop a low cost cell culture incubation chamber that fits on a microscope stand (<310x300x45mm), does not interfere with the lens optics, and is capable of live cell imaging.

- Current commercially available systems
  - Sometimes result in evaporation from low volume cultures
  - Expensive
  - Too large
  - Enclose the entire microscope

Figure 2: Cell Culture Procedure [5]

PDS Summary

Performance requirements:

- Compatible with an inverted microscope in both size and function
- Maintain an internal environment of 37°C, 5% CO_2, and 95-100% humidity

Safety:

- Biosafety Level 1 Standards [6]

Accuracy and Reliability:

- Temperature of 37°C ± 0.5°C, humidity of >95%, and CO_2 levels of 5% ± 0.1%
- Maintain internal environment for at least 1 week

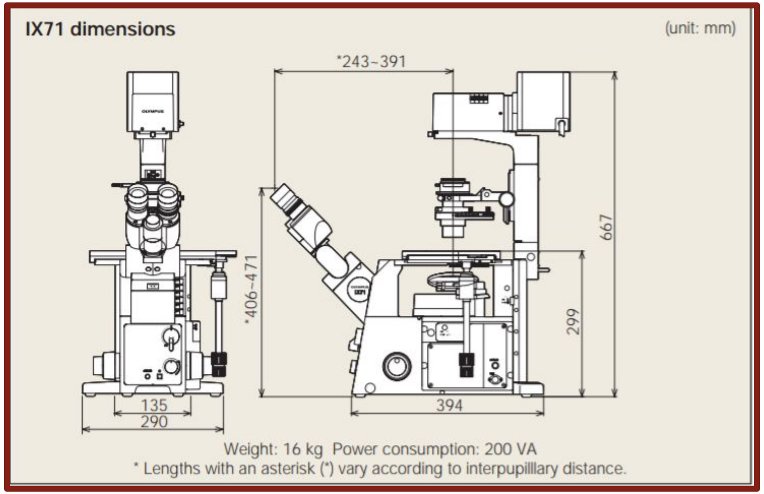

Figure 3: Measurements of Inverted Microscope [7]

PDS Summary cont.

Size:

- Incubator < 310x300 mm with a thickness < 45 mm

Materials:

- Transparent top and bottom surfaces

Target Production Cost:

- < $100

***Competition*:**

- Previous BME 200/300 design projects
- Portable Live-cell Imaging Box ~ $400 materials
- Elliot Scientific and OkoLabs Stage Top Incubators[4] ~ $400-$1,000

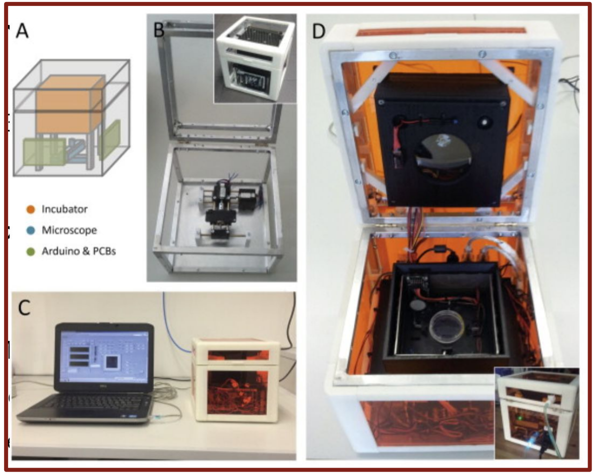

Figure 4: Portable Live-Cell Imaging Platform [8]

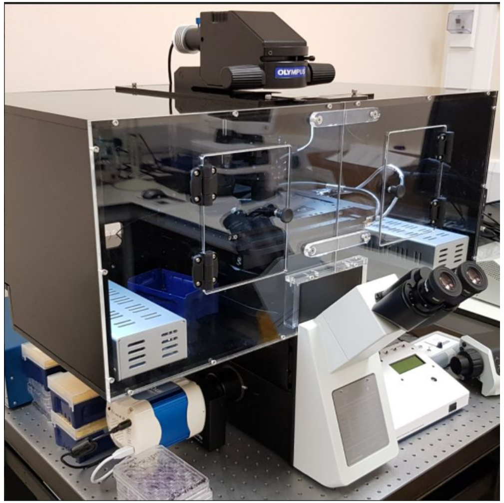

Figure 5: Elliot Scientific Stage Top Incubator [4]

Fall 2021 Work

Fabrication

- 3D printed PLA plastic via UW-Makerspace
- Thermistor was used for temperature and humidity
- NDIR CO_2 Sensor used for CO_2 percentage reading

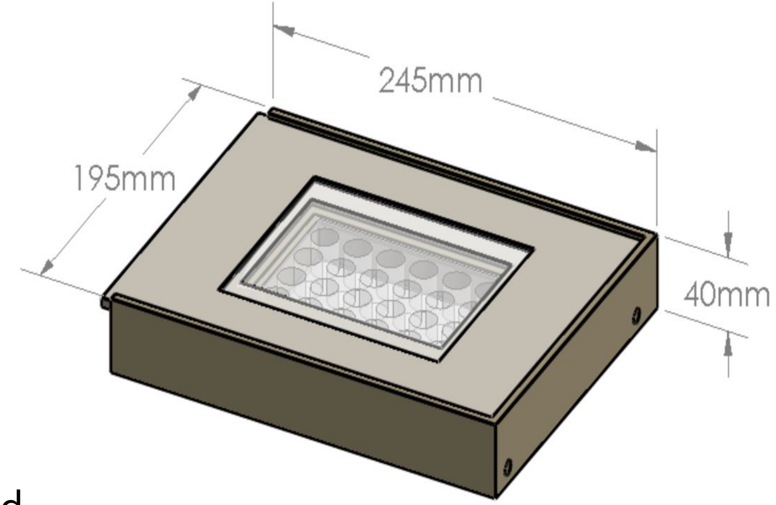

Figure 6: Final Prototype CAD drawing

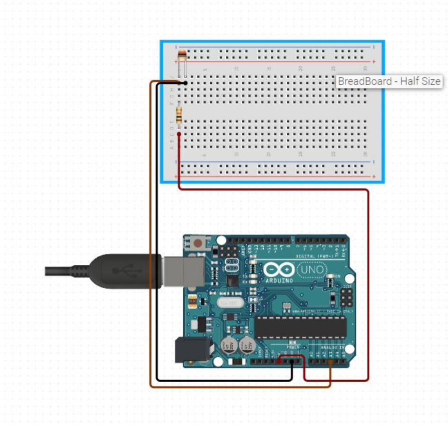

Figure 7: Thermistor Circuit Diagram

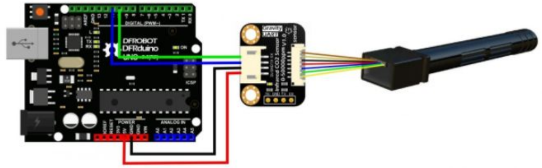

Figure 8: CO_2 Circuit Diagram

Fall 2021 Work

Results

- Temperature constant at 20°C
- Optical Analysis showed minimal difference in the optical clarity of the microscope with and without the glass plate covering

Conclusions

- Materials were not producing desired results
- Glass is usable
- Humidity calculations were not accurate

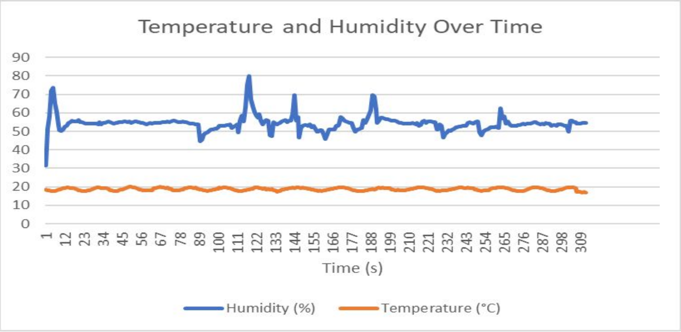

Figure 9: Temperature and Humidity Results

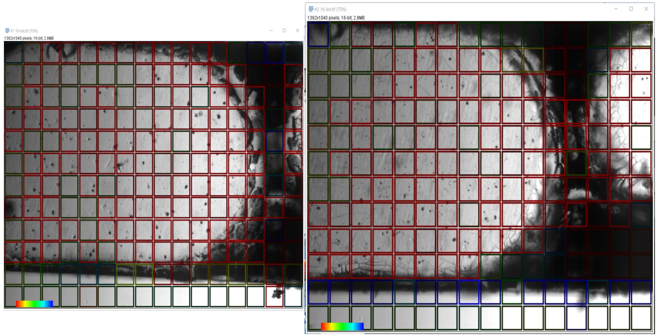

Figure 10: Optical analysis from ImageJ of microscopic cells with glass (left) and without glass (right)

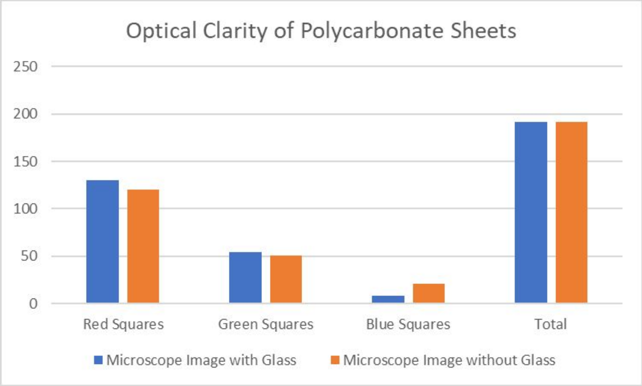

Figure 11: Optical Analysis of Polycarbonate Sheet Results

Preliminary Design #1

Hinge Top Acrylic Incubator

Strengths:

- Tightly sealed
- Lowest in Cost
- Allows for copper tubing and 1L water bed for thermal conductivity

Weaknesses:

- More sources for problems
- Most fabrication

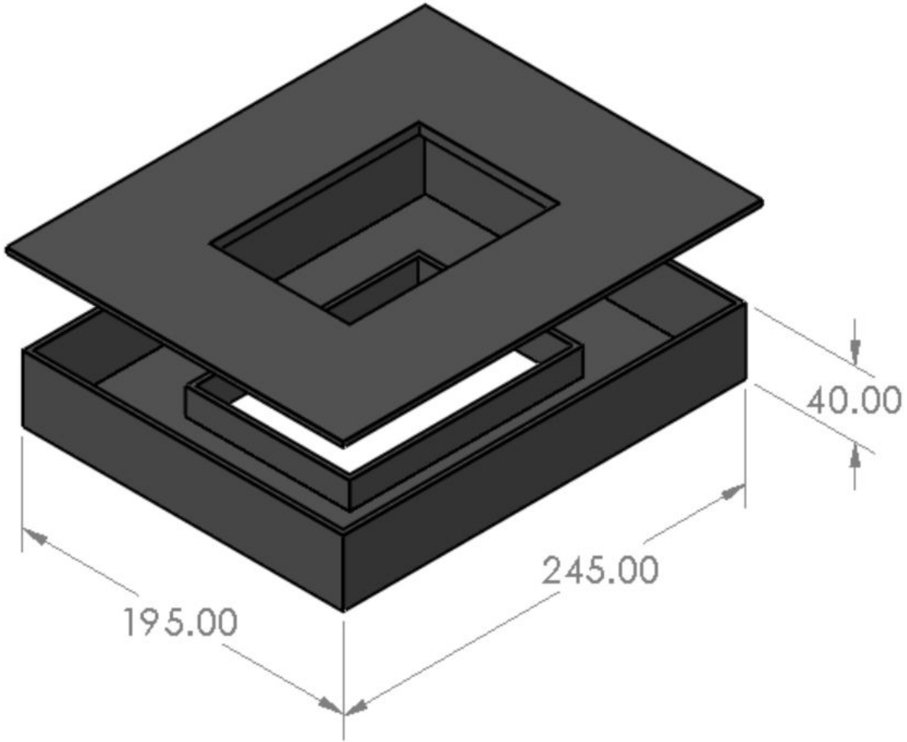

Figure 12: Solidworks Image of Preliminary Design #1 (all units in mm)

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Preliminary Design #2

Slide Top Acrylic Incubator

Strengths:

- Similar concept as last semester design
- Less internal environment lost if someone had to check the inside
- Allows for copper tubing and 1L water bed for thermal conductivity

Weaknesses:

- Not completely sealed

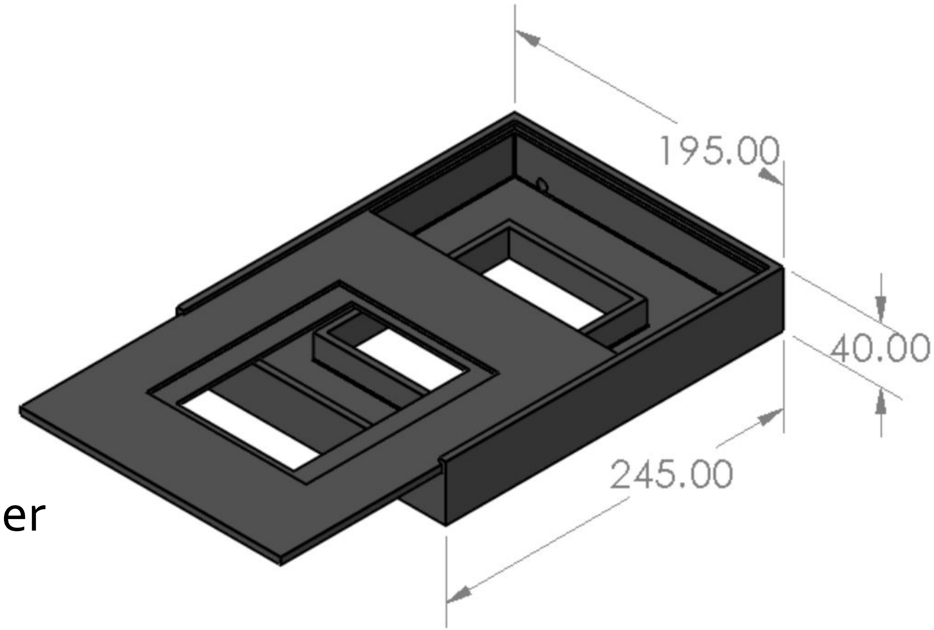


Figure 13: Solidworks Image of Preliminary Design #2 (all units in mm)

Preliminary Design #3

3D Printed Incubator

Strengths:

- Easy fabrication
- Reusable SOLIDWORKS file
- Allows for copper tubing and 1L water bed for thermal conductivity

Weaknesses:

- Cost
- Potential for leaking
- Brittle Material

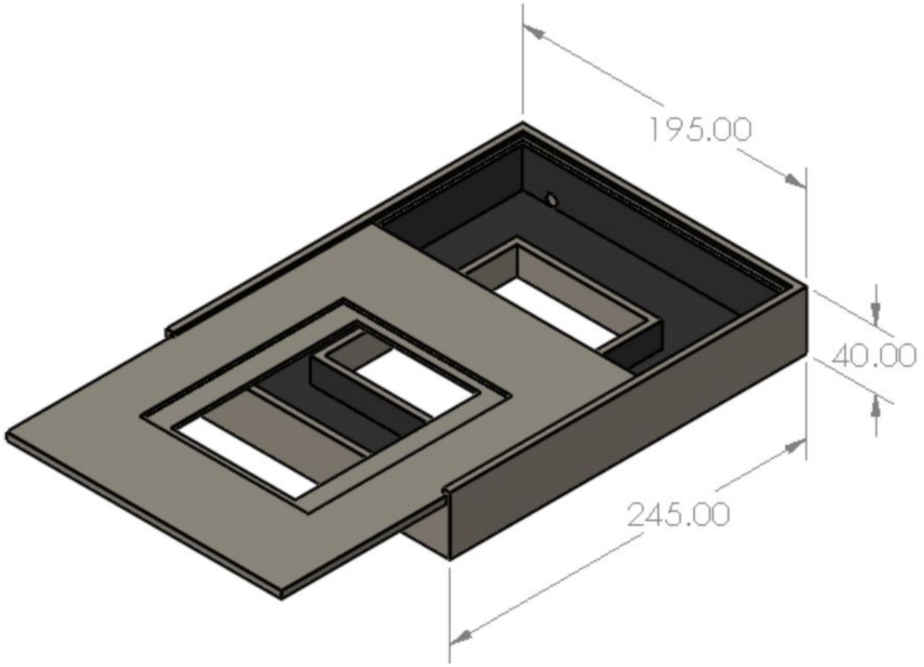


Figure 14: Solidworks Image of Preliminary Design #3 (all units in mm)

Design Matrix

- **Internal Environment:** 37°C, 5% CO_2, and 95-100% humidity
- Microscope compatibility: product < 310x300x45mm
- Accuracy and Reliability
- Ergonomics
- Cost: <$100
- Life in service: up to one week
- Safety

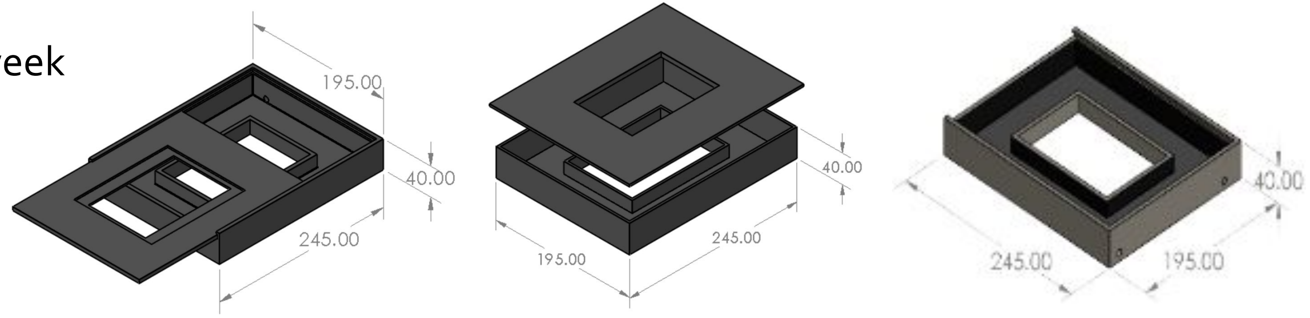
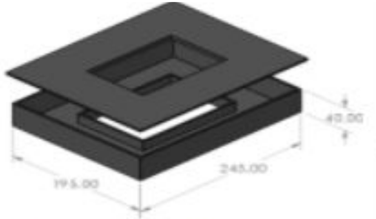
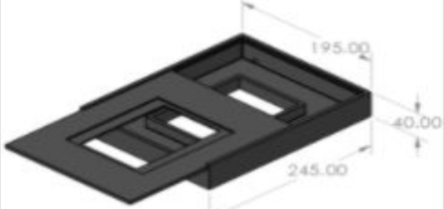
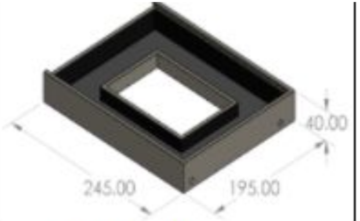


Figure 15: Solidworks Images for Preliminary Designs #1-3 (all units in mm)

Design Matrix for Fabrication







			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

Proposed Final Design

- Design #1
- Use of copper pipe for thermal conductivity
- 1L waterbed
- Compatible with Thermistor, NDIR CO_2 Sensor

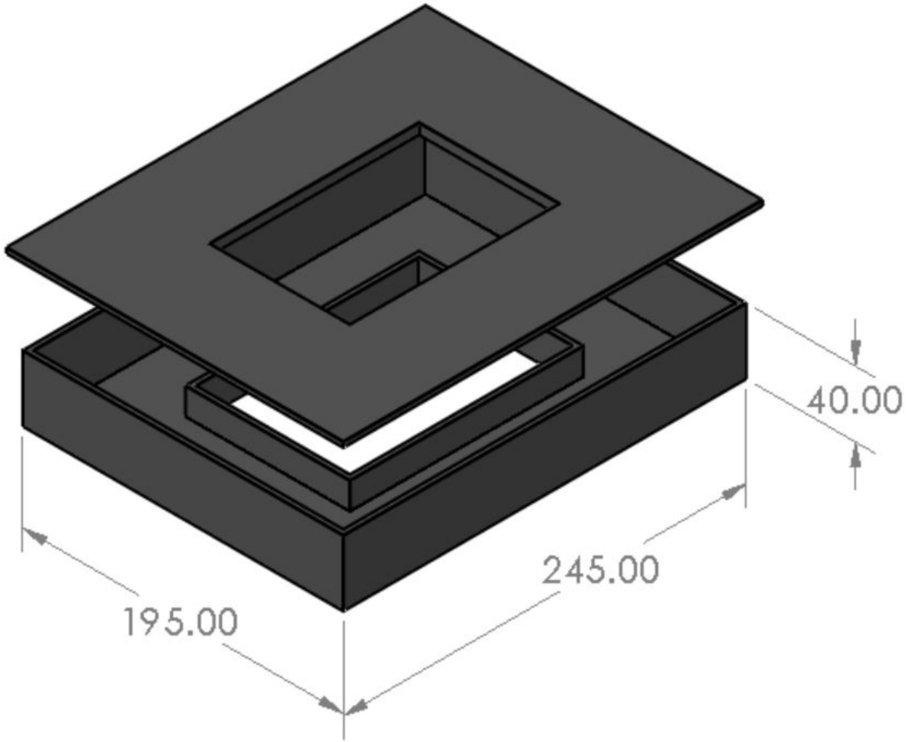


Figure 12: Solidworks Image of Preliminary Design #1 (all units in mm)

Future Work

1. Laser cut acrylic to fabricate the box
2. Order materials
3. Copper Tubing
4. Develop CO_2 input
5. Conduct thorough testing

Figure 16: UW MakerSpace Logo [10]

Upcoming Project Goals

Purchase Materials
Friday, March 4th

Testing Materials
Tuesday, March 8th

Begin Prototyping
Tuesday, March 22nd

Testing Prototype
Tuesday, April 12th

Final Deliverables
Wednesday, May 4th

Special Thanks

Dr. John Puccinelli

Melissa Kinney

BME Department

References

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Questions ?