Microscopic Cell Culture Incubator

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UW BME Design

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Abstract

The team was tasked with creating and testing a cell culture incubator that will maintain a specific internal environment while being compatible with an inverted microscope. The internal environment must be 37°C, 95%+ humidity, and contain 5% CO_2 in the air. There are current designs on the market that meet this criteria, but the inverted microscope is encapsulated into the incubator making it bulky and inconvenient to disassemble and is also expensive. The team created a cell culture incubator design that was portable and small enough to fit on the inverted microscope stage, allowing the user to view live cells inside of the incubator. This design utilized a hollow box shape filled with water, containing a heated copper pipe wound around the inside to allow for heating of the water. A heated water and CO_2 pump were located outside the incubator to help maintain an accurate internal environment. The team used a combination of a CO_2, humidity, and temperature sensors to properly record and test the accuracy and effectiveness of the incubator.

Motivation

- Imaging live-cell cultures in real time provides low cost research for drug delivery, vaccine production, and stem cell technology.
- Ability to teach students about microscope functionality while conducting live cell cultures for up to one week at a time
- Current market need for a more affordable, long-term, and smaller-in-size microscope cell culture incubator
- Future marketability for teachers and research labs

Competing Designs

- Previous BME Design Projects
- Okolabs and Elliot Scientific
- Portable Live-cell Imaging Box

- Pros:
  - Relatively reliable
  - Homogenous Internal Environment
- Cons
  - Expensive
  - Encompasses the entire microscope

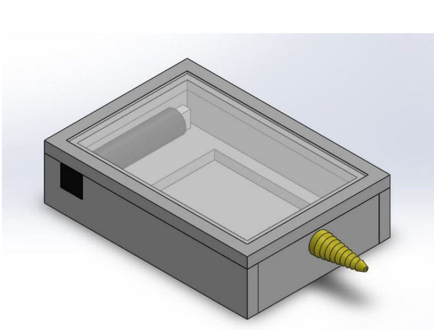

Figure 1: Fall 2020 BME 400 Prototype [1]

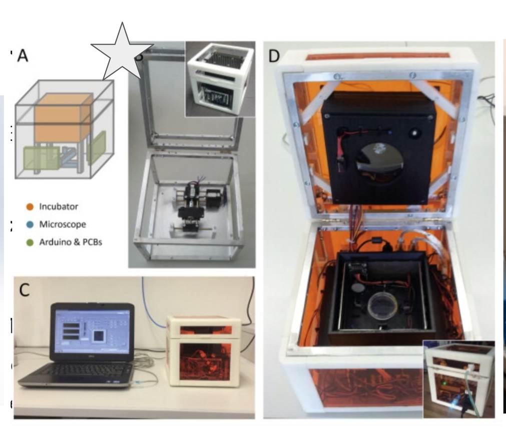

Figure 2: Portable Live-Cell Imaging Platform [2]

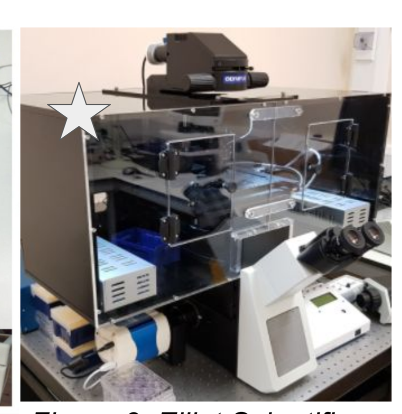

Figure 3: Elliot Scientific Stage-top incubator [3]

Design Criteria

- Ensure compatibility with an inverted microscope
  - Does not inhibit use
  - Custom-fit for stage
- Maintain an internal environment with temperature of 37℃ ± 1℃, humidity of >95%, and CO_2 levels of 5% ± 0.5%
- Support teaching labs for at least 1 week each semester for a minimum of 10 years
- Follow Biosafety Level 2 Standards [4]
- Adhere to a target production cost of < $100
- Consist of transparent top and bottom glasses
- Accommodate size dimensions of < 310x300x45mm and be able to fit a standard well plate with dimensions of 127.55x85.4x22.5mm

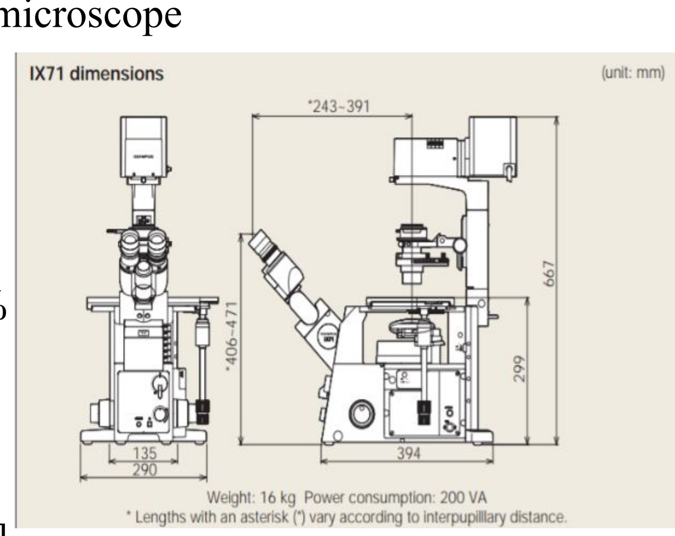

Figure 4: Measurements of Inverted Microscope [5]

Final Design

Incubation Chamber:
- Dimensions: 195mm x 245mm x 40mm
- Heated Water Pump Used as Heating Element
- Transparent Sheets to view Well Plates

DC Motor Regulator
- Dimensions:
  - Shaft: 101.6 mm
  - Inner Diameter: 34.62 mm

Materials:
- Laser cut black acrylic
- Transparent, Polycarbonate Cover Plates
- 2 ft Copper piping
- Rubber lining
- Piping to Hose Adaptors
- MH-Z16 NDIR CO_2 sensor
- Thermistor Sensor
- DC Motor
- 3D printed PLA CO_2 valve attachment

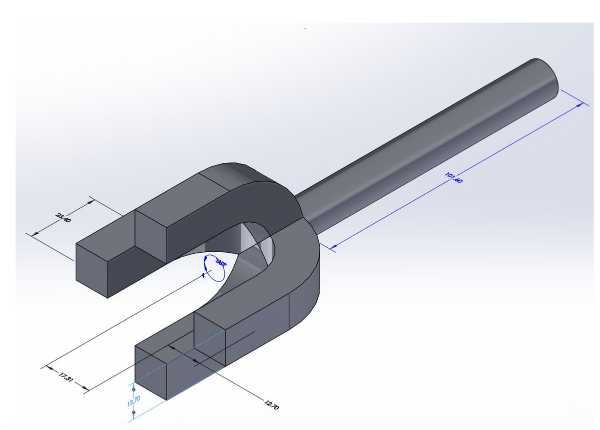

Figure 7: SOLIDWORKS DC Motor Attachment Drawing

Arduino Coding

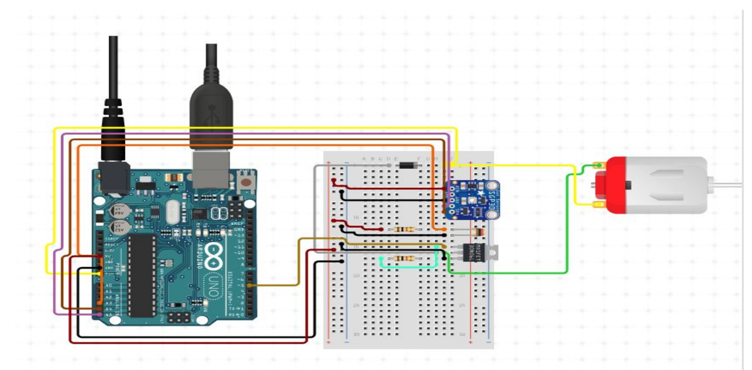

Figure 5: Circuit Diagram for Integrated Sensing/Regulation Elements

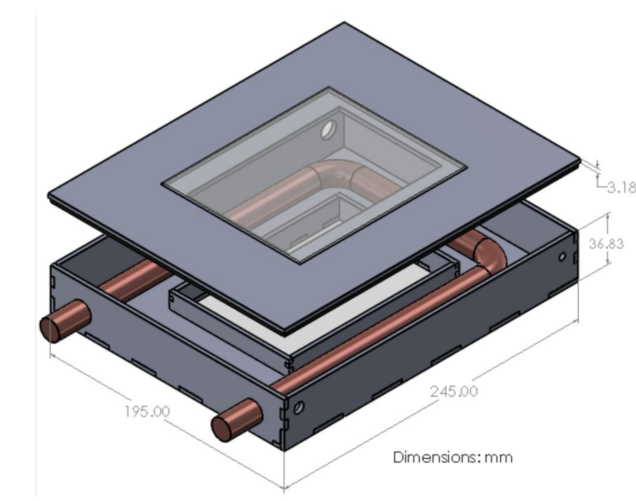

Figure 6: Final SOLIDWORKS Drawing

Figure 8: Motor Attachment connected to DC motor and circuit

Prototype Fabrication

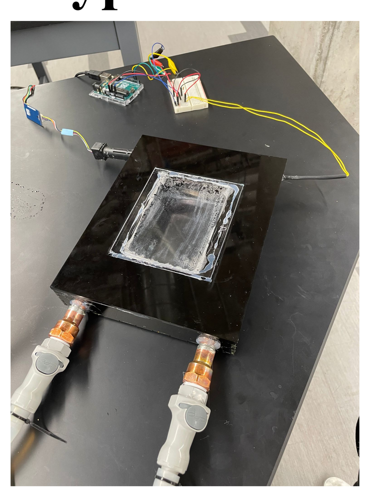

Figure 9: Final Prototype Exterior

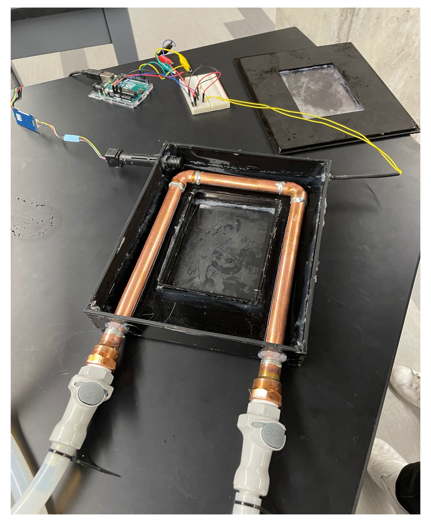

Figure 10: Final Prototype Interior

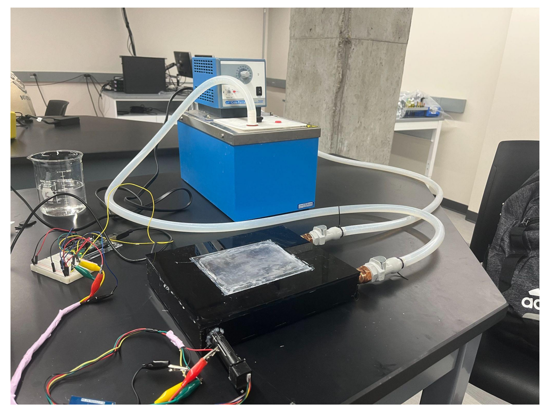

Figure 11: Whole Incubation Set-up

Results

Optical Testing

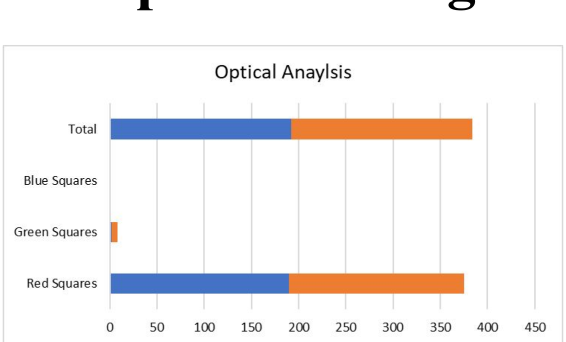

Figure 12: Optical Analysis

- Microscope focus quality was similar with and without the glass

CO_2 Testing

- DC Motor broke upon testing due to lack of torque.

Temperature and Humidity Testing

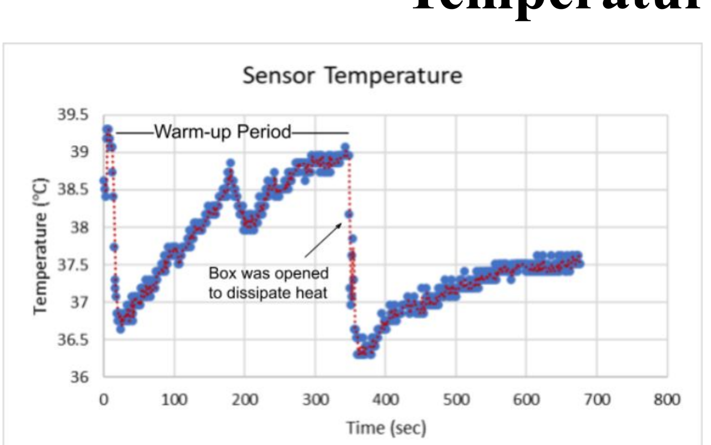

Figure 13: Sensor Temperature Results

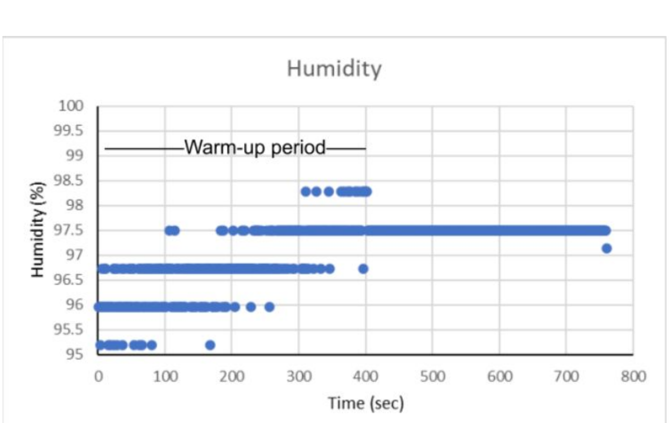

Figure 14: Sensor Humidity Results

- Temperature had an average of 37.6℃. The dip in the graph represents turning the heated water pump down from it's warm up temperature of 40℃ to slightly below 34℃ (Fig 13).
- Humidity testing was successful on the second try, after the formula was calibrated in the Arduino code. The results showed an average of 97.1% over the tested time interval (Fig 14).
- Recovery testing took approximately 3 minutes to recover after being opened for 0.5 min (Fig 15).

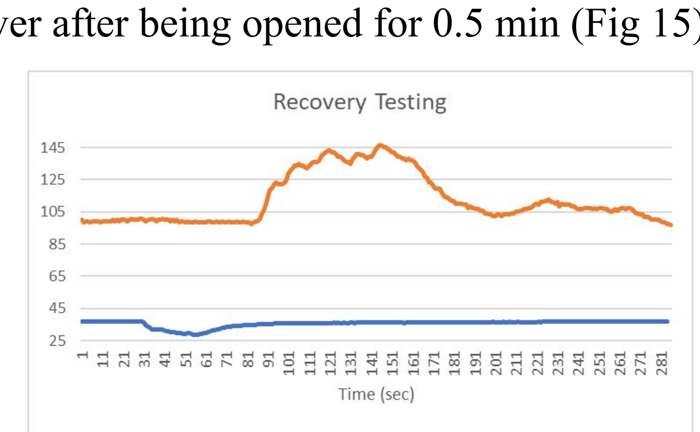

Figure 15: Recovery Testing Results

Methods and Testing

- Temperature and Humidity Testing
  - Evaluated precision in a dynamic range and accuracy over a 10 minute time interval

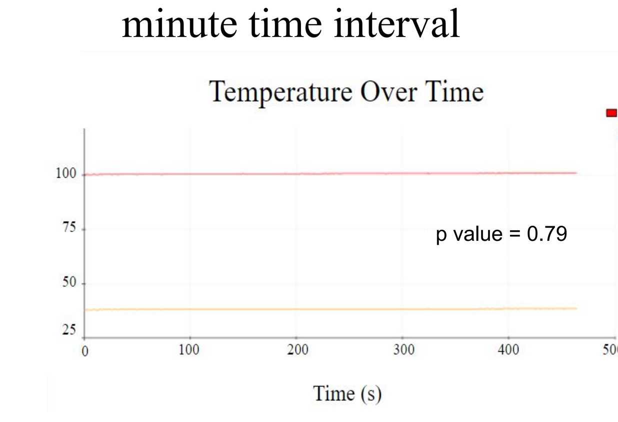

Figure 16: Graph of Thermistor Readings in Incubator Over 10 min Time Interval

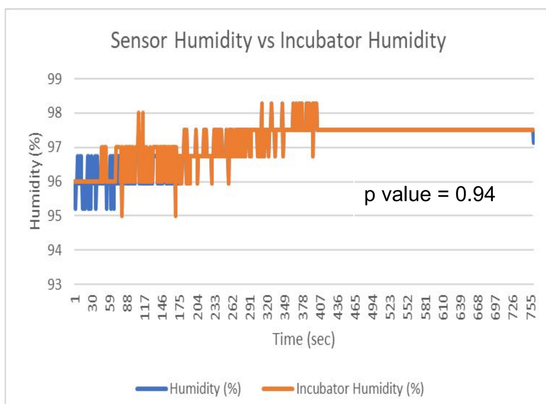

Figure 17: Graph of Humidity Readings in Incubator Over 10 min Time Interval

- CO_2 Testing
  - Evaluated accuracy of percentage reading and precision of concentration output over incubation period

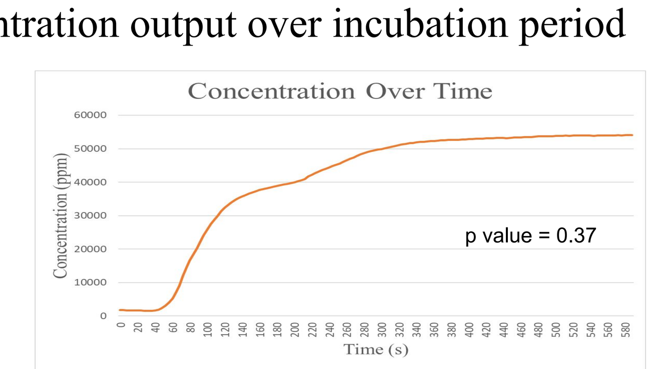

Figure 18: Concentration of CO_2 in Incubator over Time

- Optical Testing
  - Evaluated the focus quality of the microscope with and without glass

Discussion

- Significant improvement with leakage prevention from last semester
- Statistical analysis indicated no significant difference between the sensors in the team's final design and a commercially available incubator
- More powerful DC motor for torque or use a solenoid valve in order to achieve CO_2 input
- Determine why humidity values went over 100% during humidity testing
- Met budget requirement. Total cost was $51.45.

Future Work

- Run a live cell culture in the incubator for 1 week to ensure that the design keeps the cells alive
- Continue troubleshooting CO_2 regulation and output
- Improve visual components of the system

Acknowledgements

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- Dr. John Puccinelli - UW-Madison, Department of Biomedical Engineering
- Dr. Amit Nimunkar - UW-Madison, Department of Biomedical Engineering
- UW TeamLab & Makerspace

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