Microscopic Cell Culture Incubator

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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. 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 water tube wound twice 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 to meet design criteria. 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 culture in real time provides new research possibilities
- Ability to teach students about microscope functionality while conducting live cell cultures
- Current market need for a more affordable and smaller-in-size microscope cell culture incubator
- Future marketability for teachers and labs

Competing Designs

- Previous BME Design Projects
- Thermo Fisher, NuAire, and New Brunswick
  - Direct Heat Incubator
  - Water Jacketed Incubator
- Portable Live-cell Imaging Box

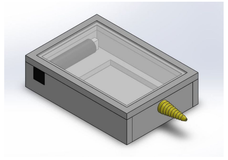

Figure 1: Fall 2020 BME 400 Prototype [1]

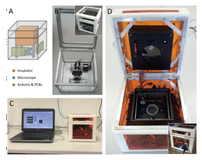

Figure 2: Portable Live-Cell Imaging Platform [2]

Figure 3: Thermo Fisher 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℃ ± 0.5℃, humidity of >95%, and CO_2 levels of 5% ± 0.1%
- 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

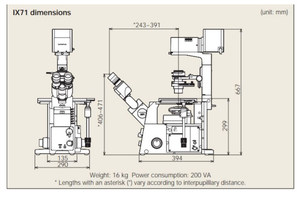

Figure 4: Measurements of Inverted Microscope [5]

Final Design

Incubation Chamber:

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

Materials:

- 3D Printed PLA Casing
- Transparent, Polycarbonate Cover Plates
- ½ x ⅜" Vinyl Tubing
- ⅜ x ⅜" Hose Connectors
- MH-Z16 NDIR CO_2 sensor
- Thermistor Sensor

Table 1: SOLIDWORKS Item List

Item No.	Item Description	Dimensions [mm]	QTY.
1	Glass plates to allow transparent viewing	114.5 x 138.5 x 1.3	2
2	Top removeable crown to allow access to interior	Outside: 189 x 260 x 5 Inside Cut: 85.4 x 127.5 x 2.5	1
3	Well Plate to hold cells	85.4 x 127.5 x 22.5	1
4	Inner heated water pump tubing	Outer Diameter: 12.7 Inner Diameter: 9.525	1
5	Incubator box to maintain a controlled internal environment	Outside: 195 x 245 x 40 Inside Cut: 90 x 132 19	1

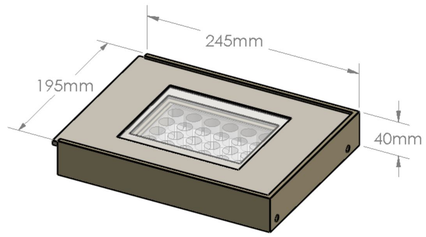

Figure 5: Final SOLIDWORKS Drawing

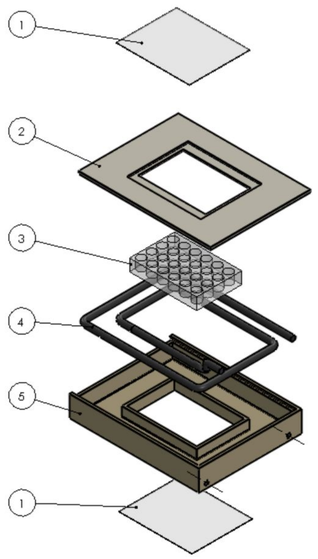

Figure 6: SOLIDWORKS Exploded View

Methods & Testing

Arduino Coding

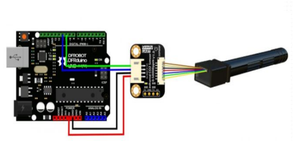

Figure 7: MH-Z16 CO2 Sensor Circuit Diagram [6]

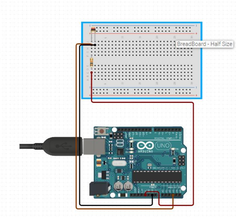

Figure 8: Thermistor Circuit Diagram

Prototype Fabrication

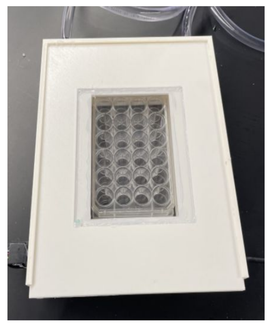

Figure 9: External View of Incubator

Figure 10: Internal View of Incubator

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

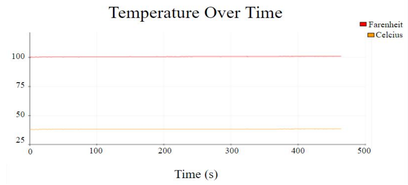

Figure 11: Graph of Thermistor Readings in Incubator Over 10 min Time Interval

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

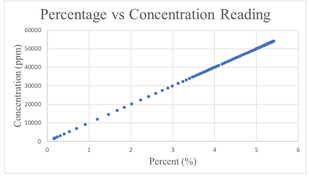

Figure 12: Sensor Percent vs Concentration Reading

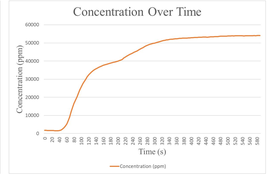

Figure 13: Concentration of CO_2 in Incubator over Time

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

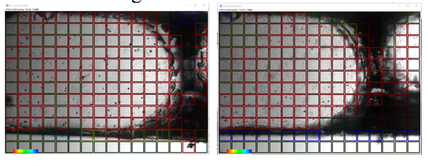

Figure 14: Optical analysis from ImageJ of microscopic cells with glass (left) and without glass (right)

Results

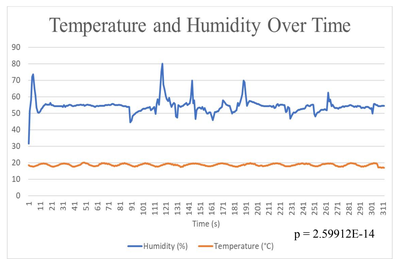

Figure 15: Temp and Humidity Over 10 min Interval

Table 2: Table displaying the number of red (in focus), green (mid focus), and blue (out of focus) squares shown in the optical testing section.

	Microscope Image with Glass	Microscope Image without Glass
Red Squares	130	120
Green Squares	54	51
Blue Squares	8	21
Total	192	192

- PDS specifications for internal conditions not met
- T-test conducted on the accuracy of the humidity formula used in the thermistor code to the outputted values on the DHT22 sensor showed statistical significance ($p < 0.05$).
- The polyethylene tubing does not allow for sufficient conduction of thermal heat to produce optimal temperature
- PLA plastic causes leakage and should not be used as the incubator casing material
- 100% of randomly selected subjects expressed no difference in clarity between the optical photos
- The image with glass had a slightly higher, yet very similar focus quality compared to the image without glass present

Discussion

- Thicker casing material is needed to prevent leakage
- Better conductive tubing is needed to reach temperature specifications
- Code for humidity sensor must be revisited so that the outputted value is not statistically significant to the DHT22 sensor.
- Transparent, polycarbonate sheets approved for use in incubator

Future Work

- More sealed incubator box
- Conduct more testing on the box as a whole
- Do live cell testing
- Use better insulating or conducting materials
- Improve CO_2 input

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

References

1. N. Pauly, B. Meuler, T. Madigan, and K. Koesser, *SolidWorks rendering of Final Design*. University of Wisconsin-Madison, 2020.
2. M. P. Walzika, V. Vollmar, T. Lachnit, H. Dietz, S. Haug, H. Bachmann, M. Fath, D. Aschenbrennera, S. A. Mofradab, O. Friedrich, and D. F. Gilbert, "A portable low-cost long-term live-cell imaging platform for biomedical research and education," *Biosensors and Bioelectronics*, vol. 64, pp. 639–649, Feb. 2015.
3. "Forma™ series II 3110 water-jacketed co2 incubator, 184 L, polished stainless steel," *Thermo Fisher Scientific - US*. [Online]. Available: https://www.thermofisher.com/order/catalog/product/3110#/3110. [Accessed: 11-Oct-2021].
4. A. Trapotsis, "Biosafety levels 1, 2, 3 & 4: What's the difference?," Consolidated Sterilizer Systems, 01-Apr-2020. [Online]. Available: https://consteril.com/biosafety-levels-difference/. [Accessed: 20-Sep-2021].
5. "Research IX71/IX81 - olympus america." [Online]. Available: https://www.olympusamerica.com/files/seg_bio/IX71IX81%20brochure.pdf. [Accessed: 11-Oct-2021].
6. "Infrared CO2 Sensor ," *DFRobot*. [Online]. Available: https://wiki.dfrobot.com/Infrared_CO2_Sensor_0-50000ppm_SKU__SEN0220. [Accessed: 01-Dec-2021].