Microscopic Cell Culture Incubator

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Advisor: Dr. Amit Nimunkar - UW-Madison Department of Biomedical Engineering
Date: December 9th 2022

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

Background

- Cell cultures are mainly used in the study of cell biology due to their ability to easily manipulate genes, molecular pathways, and culture systems to remove interfering genetic and environmental variables
- Incubators used in cell cultures have to maintain a stable microenvironment and can achieve this via regulated temperature, humidity, CO_2, O_2, and pH levels
- The client is Dr. John Puccinelli, an undergraduate advisor and professor in the BME department at the UW-Madison, who plans to use the incubator in a teaching lab
- This is the team's third consecutive semester working on this project

Competing Designs

- Previous BME Design Projects
- ibidi Stage Top Incubator
- Okolabs and Elliot Scientific
  - ➢ Pros:
    - Relatively reliable
    - Homogenous internal environment
  - ➢ Cons
    - Expensive
    - Encompasses the entire microscope

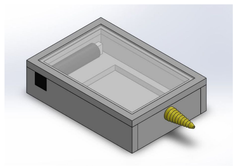

Figure 1: Fall 2020 BME 400 Prototype [1]

Figure 2: ibidi Stage Top Incubator [2]

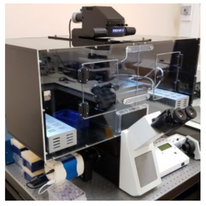

Figure 3: Elliot Scientific Stage-top incubator [3]

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 >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 < 310 mm × 300 mm × 45 mm with ability to fit a standard well plate with dimensions of 127.55 mm × 85.4 mm × 22.5 mm

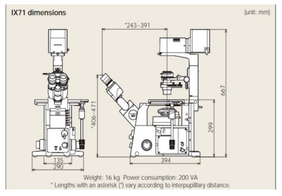

Figure 4: Measurements of Inverted Microscope [5]

Final Design

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

Wiper Blade:
- Total: Width: 138 mm
  Height: 52 mm
- Blade: 86 mm × 20 mm

Circuitry:
- 2x Arduino Uno Microcontroller
- Thermistor [6]
- MH-Z16 NDIR CO_2 sensor [7]
- Beefcake Relay [8]
- Solenoid Valve [9]

Materials:
- Laser cut black acrylic
- Transparent, Polycarbonate Cover Plates
- 2 ft Copper piping
- Rubber lining
- Piping to Hose Adaptors
- Squeegee Rubber Blade

Arduino Coding

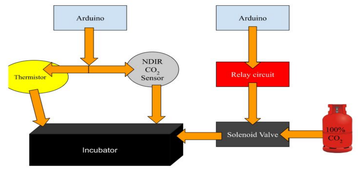

Figure 5: Circuit Block Diagram

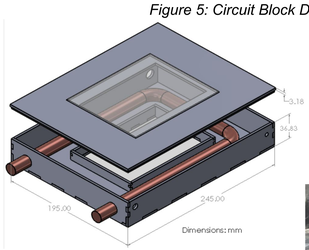

Figure 6: CAD model of completed device

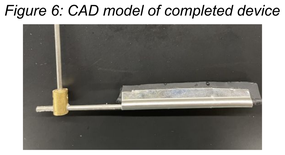

Figure 7: Fabricated preliminary wiper blade

Figure 8: Manual wiper blade attached to incubator lid

Prototype Fabrication

Figure 9: Prototype Homogeneity Testing Lid

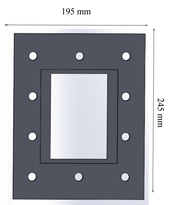

Figure 10: CAD model of homogeneity testing lid

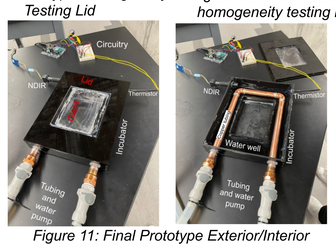

Figure 11: Final Prototype Exterior/Interior

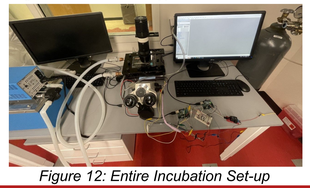

Figure 12: Entire Incubation Set-up

Results

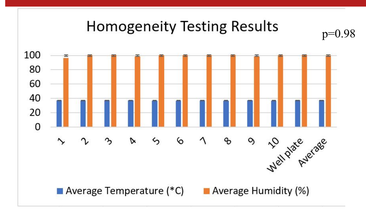

Figure 13: Temperature and Humidity Homogeneity Testing Results

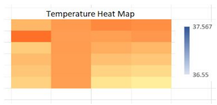

Figure 14: Temperature Heat Map

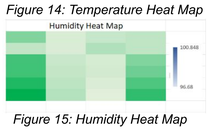

Figure 15: Humidity Heat Map

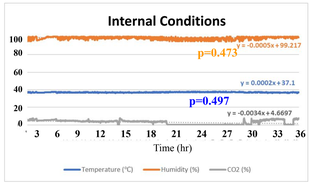

Figure 16: Internal Conditions over 36 hours

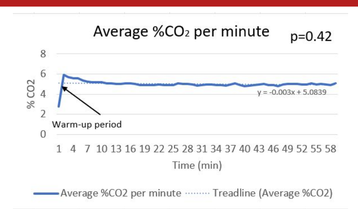

Figure 17: Concentration of CO_2 in Incubator

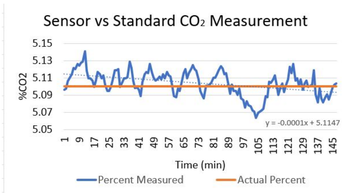

Figure 18: CO_2 Testing Results

Solenoid valve wiring was disrupted

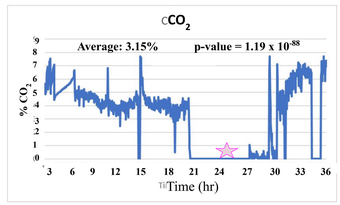

Figure 19: % CO_2 over 36 hours

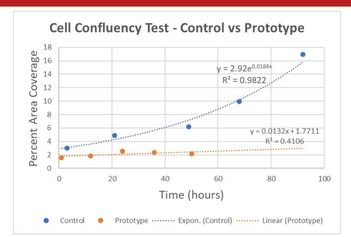

Figure 20: Cell confluency test comparison results

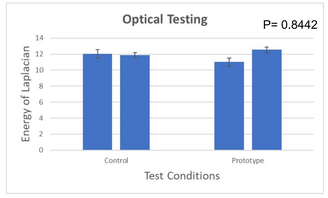

Figure 21: Comparison of optics with and without glass slides

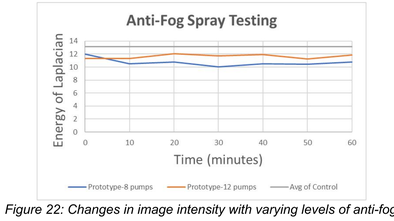

Figure 22: Changes in image intensity with varying levels of anti-fog spray

Methods and Testing

- CO_2 Testing
  - ➢ Evaluated accuracy of sensor percent reading and precision of concentration output over incubation period
  - ➢ Evaluated accuracy of coded solenoid valve over a one hour period to determine if it would be able to keep cells alive during live-cell testing
- Wiper Testing
  - ➢ Qualitatively evaluated effectiveness of wiper blade by heating internal environment above standard incubator temperature/humidity and assessing wiper usability
- Homogeneity Testing
  - ➢ Evaluated the homogeneity of the system by obtaining measurements from different areas over a five period interval and averaging the values to create an idea of the humidity and temperature in the system
- Live-Cell Testing
  - ➢ Cells were tested over a two day period in which the cell death, temperature, humidity, and CO_2 were measured
  - ➢ Cell death was measured every 12 hours, while internal conditions were measured every 10 minutes
- Optical Testing
  - ➢ Evaluated if there was a difference in optics between two images of cells just in a T25 flask and two images of cells in a T25 flask inside the prototype
- Anti-Fog Testing
  - ➢ Evaluated how effective the purchased anti fog solution was in preventing condensation and maintaining visibility of the system

Discussion

- Statistical analysis indicated no significant difference between the sensors in the team's final design and a commercial incubator
- CO_2 proved accurate in the lab incubator and the hard-code proved viable during initial testing, however during live-cell testing it became apparent that with changing conditions it will not be a viable option in the future
- Temperature and humidity corroded the hot glue holding the glass plates onto the incubator
- Live cell testing indicated that pH of the cell's environment was not up to standard and therefore died
- Met budget requirement - Total cost was $42.11

Future Work

- Fabricate slider wiper blade to remove condensation droplets
- Fabricate a new, more streamlined/presentable box with updated fabrication dimensions and designs
- I2C coding to pair NDIR sensor and solenoid for CO_2 regulation
- Conduct prolonged live-cell testing

Acknowledgements

Thank you to our client, Dr. John Puccinelli, and advisor, Dr. Amit Nimunkar, as well as Dr. Aviad Hai for guidance and mentorship throughout the semester. We would also like to acknowledge the UW TeamLab & Makerspace for assisting with laser cutting and fabrication methods used for the project.

References

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