Microscope Cell Culture Incubator

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Abstract

The current market for microscope incubators is primarily filled with high cost and large devices that encapsulate the microscope. We created a low-cost cell culture incubator that can maintain a specific internal environment while being compatible with an inverted microscope. The internal environment must be 37 °C, greater than 95% humidity, and contain 5% CO_2 in the incubator. There are current designs on the market meeting this criterion, but these products either integrate the inverted microscope into the incubator, making it bulky and inconvenient to disassemble, or are extremely expensive. The team designed a cost-effective, portable cell culture incubator that allowed users to view live cells inside. With dimensions of 240 mm × 195 mm × 40 mm, it was also small enough to fit on a 310 mm × 300 mm × 45 mm inverted microscope stage. The incubator included heated water and CO_2 pumps in order to maintain viable cell conditions. Condensation, CO_2 input regulation, and live cell testing were conducted to find the optimal working environment for the incubator in order to ensure cellular viability and visibility. Our device succeeded in maintaining homogeneous conditions of 37 °C, 95% humidity, and 5% CO_2. However, we were unable to prevent condensation from forming on the glass cover slide, resulting in compromised optics. The team was also unsuccessful in keeping cells alive due unforeseen issues with the CO_2 sensor when exposed to a humid environment for extended periods of time.

Competing Designs

- ibidi Stage Top Incubator
- Okolabs and Elliot Scientific

➢ Pros:
- Relatively reliable
- Homogenous internal environment

➢ Cons
- Expensive
- Encompasses the entire microscope

Figure 1: ibidi Stage Top Incubator [2]

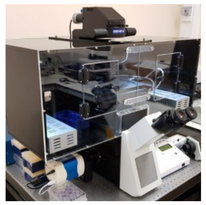

Figure 2: Elliot Scientific Stage-top incubator [3]

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% ± 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 < 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

Final Design

Incubation Chamber:
- Heated water pump used as heating element
- Transparent sheets to view well plates

Circuitry:
- Arduino microcontroller used to receive and send data for collection
- Thermistor coded to calculate temperature and humidity
- MH-Z16 NDIR CO_2 sensor used in conjunction with a beefcake relay and solenoid valve to monitor CO_2 levels and allow 5% CO_2 to be inputted from 100% CO_2 tank

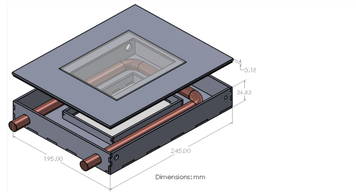

Figure 3: CAD model of incubation chamber with copper tubing to allow for heated water pump to heat water bath and transparent top and bottom to allow for microscope imaging.

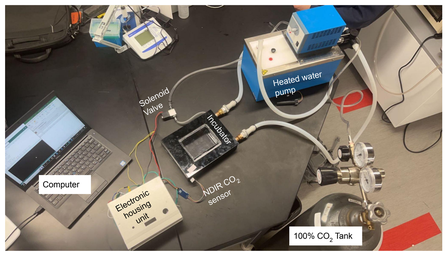

Figure 4: Entire Incubation Set-up consisting of electronic elements to monitor internal conditions (Temperature, Humidity, CO_2) and regulate control of 100% CO_2 from tank, water bath to circulate 37℃ to heat the incubator, and a laptop to record values.

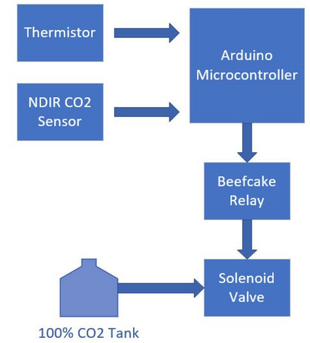

Figure 5: Circuit Block Diagram in which the recorded values from the Thermistor (temperature and humidity) and CO_2 sensor are sent to the Arduino which regulates the opening/closing of the solenoid valve via a beefcake relay.

Methods and Testing

- Anti-Fog Testing
  - ➢ Copper tape, mini-fans, antibacterial solution, and PDMS
- 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 day period to determine if it would be able to keep cells alive during live-cell testing
  - ➢ Evaluated accuracy of solenoid valve with gas permeable water cover for one day period
- Live-Cell Testing
  - ➢ Cells were tested over a four day period in which the cell proliferation, temperature, humidity, and CO_2 were measured
  - ➢ Cell death was measured every 24 hours, while internal conditions were measured every 10 minutes
- pH Testing
  - ➢ Increased the CO_2 release time opened for CO_2 flow and increased pressure of CO_2 tank from 103.4 to 137.9 kPa
  - ➢ Attempted to obtain right release time that would allow for a pH of 7

Results

Mini-fan Anti Condensation Testing

Figure 6: Same-side mini fan anti-fog testing after 45 minutes

Figure 7: Antibacterial solution anti-fog testing

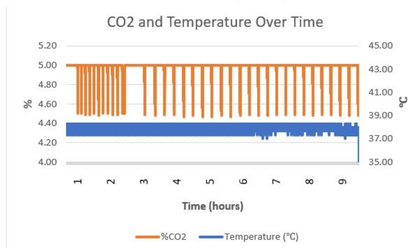

Figure 8: Co_2 and Temperature Initial Trial

Live Cell Testing

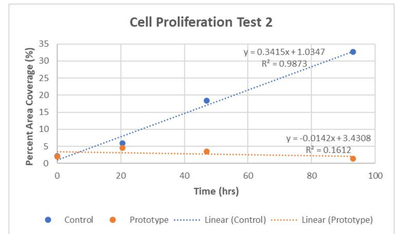

Figure 9: Cell Proliferation Trial 2

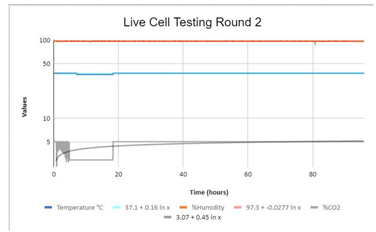

Figure 10: Internal Conditions graphed on log scale

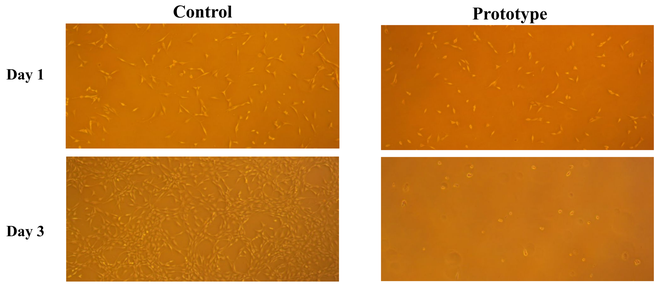

Figure 11: Cell Proliferation Results for 1.75x CO_2 Over 3 Day Period

Table 1: pH testing trial

CO_2 release time change from original amount (50ms)	pH	Average %CO_2
3x	5	10%
2x	6	6.5%
1.5x	8	3%
1.75x	7-9	Sensor Failure

Discussion

- T-test showed there was no statistically significant difference between the thermistor's temperature and humidity calculations and the CO_2 sensors %CO_2 from the standard incubator as the p-value was >0.05
- Mini-fan method was the best anti-condensation method, but was not sufficient enough to allow for optical clarity
- The NDIR sensor used could not withstand the significant amount of humidity in the incubator and lead to eventual CO_2 failure
- Basic and acidic pH conditions within the incubator lead to cell death during the variable CO_2 input testing. Sensor failure halted precision experimentation
- Lid warping occured after running the experiment for approximately 1.5 weeks

Future Work

- Low cost anti-condensation prevention
- More sealed incubator box design
- Metal lid instead of acrylic to prevent warping
- Research a waterproof CO_2 sensor or other form of pH control
- Switch to a direct heat design and eliminate water/condensation all together

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