Microscope Compatible Cell Culture Incubator

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ABSTRACT

Live cell imaging experiments are difficult to perform over long periods of time on normal lab microscopes. The client desires an inexpensive on-stage incubation chamber that is capable of maintaining temperature, CO_2, and humidity evenly throughout the chamber at a physiological set point. An initial prototype has been developed that involves a small, cohesive system to regulate these parameters through a feedback systems. Further development of the design will further test and refine the hardware and feedback systems, ultimately bridging the gap in the market between high-cost, functional systems and cheaper, less effective systems.

BACKGROUND

- Real time imaging offers researchers the opportunity to perform new assays
- Current market in need of affordable and more robust system for real time imaging in cell culture
- Mimicking the physiological environment requires control of temperature, humidity and CO_2 concentration
- Optical compatibility: desired magnification (focal length) and size limitations

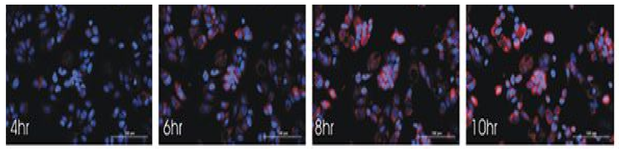

Figure 1. Sample time-lapse imaging using cell fluorescence.

DESIGN CRITERIA

- Environmental Controls for Physiological Maintenance:
  - Temperature: 37℃ ± 1℃
  - Relative Humidity: 95% ± 5%
  - CO2: 5% ± 0.5%
- Recovery: Temperature and CO2 recovery in 6 seconds after 30 second chamber opening, comparable to current products
- Compatible with various microscopes

MARKET DEMAND

- Limitations of current market
  - High end systems
    - Expensive & limited to one microscope
  - Low end systems
    - Offer poor environmental control
- Our Design Attributes
  - Simplicity
  - Affordability

Figure 2: Low end system (4).

Figure 3: High end system (5).

DESIGN SOLUTION

- Control systems independently validated
  - integrated to regulate CO2, RH, and temperature effectively
  - LCD display of environmental conditions
- 3/32” glass below cell culture to optimize imaging
- Removable lid for fast plate removal

Design Costs

Design Area	Product names	Category Cost
Environment Sensors	DHT-22, MHZ -16	$80.05
Environment Control Components	Fluid valve, Immeserion Heater	$50
Structural Materials /PCB	ABS filament, acrylic, glass	$76.79
Testing Materials	CO_2 and media	$13.94

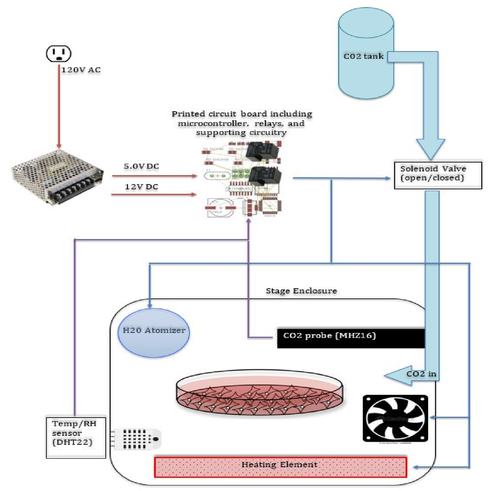

Figure 4. System diagram of final incubation system

METHODS & TESTING

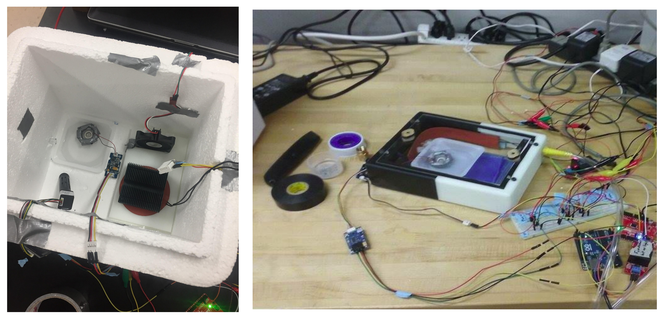

Figure 5. First iteration of control system validation (left) and second generation validation in fabricated enclosure(right)

- Past semester testing:
  - Environmental feedback systems
    - Sensors and control circuitry for each parameter
    - Integration into one system
- Current semester testing:
  - Testing of integrated power supply and PCB
  - longer-term integrated testing

- Cell Imaging Tests
  - Captured cell images in updated prototype
  - Focus achieved similar to control
- Cell Culture Tests
  - Scratch assay
    - test for migration
    - healthy cells migrate
  - Standard incubator
  - Final incubator prototype
  - Ambient conditions

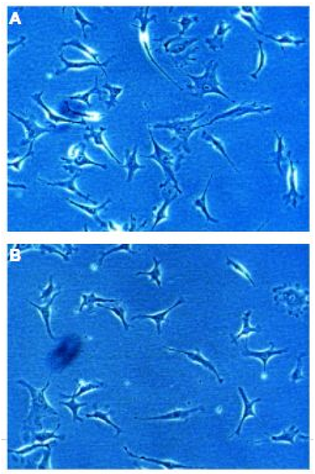

Figure 6. Images of cells in A) incubator, and B) on TCPS control at 20X magnification. Focus measure A = 31.8641% B = 31.0734%.

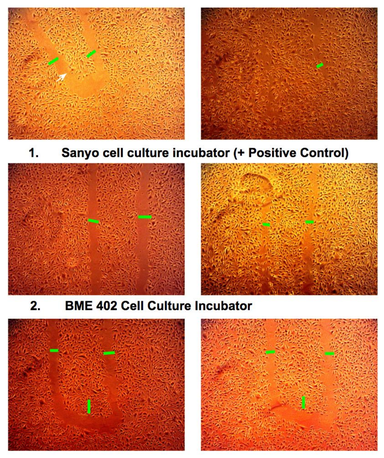

Figure 7. Images of cells migration in A) standard incubator, B) final prototype, and C) in ambient conditions. Lines added to show scratch “healing”.

RESULTS

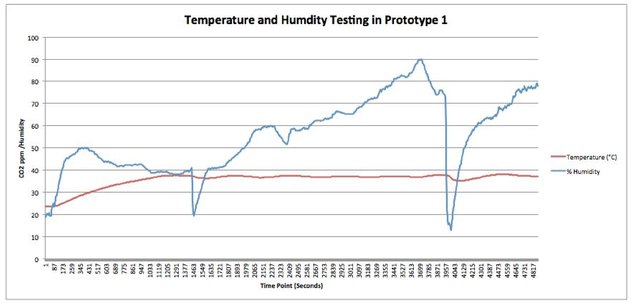

Figure 8. Humidity and Temperature data from first semester prototype.

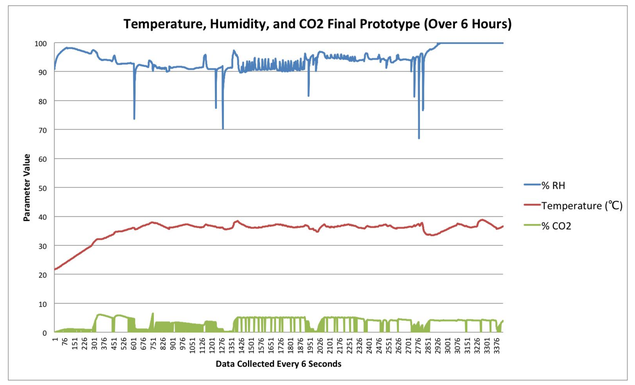

Figure 9. Humidity, temperature, and CO2 data from final prototype.

IMPACT & FUTURE WORK

- Extended testing duration with variety of cell types
- Further optimization of environmental control
- Selection of sensors/components for moving to larger volume production
- Design changes for manufacturing
  - Injection molded outer casing
  - Robust materials for sterilization

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