Microscope Compatible Cell Culture Incubator

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UW BME Design

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/Motivation

- Imaging cell culture in real time provides researchers with flexibility to perform a variety of unique experiments
- 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

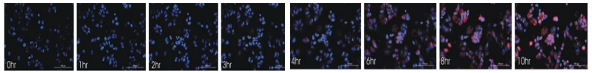

Figure 1: Sample time-lapse imaging using cell fluorescence.

Design Specifications

- 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
- Demonstrate stable system for at least 2 weeks: desired imaging study length
- Compatible with various microscopes

Final Design

- Control systems independently validated, integrated to regulate CO2, RH, and temperature effectively
- .08" plexiglass above and below cell culture to optimize imaging
- Removable Plexiglass for media changes

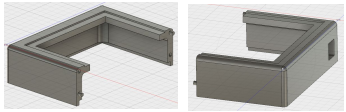

Figure 2: CAD diagram showing the fabrications of the stage enclosure in two components

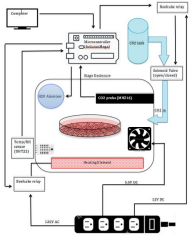

Figure 3: Systems diagram of final design.

Methods and Testing

Material testing for imaging
- Quantified with % of relative image focus
- MATLAB used with Brenner's Law

Environmental feedback systems
- Sensors and control circuitry for each parameter
- Integration into one system

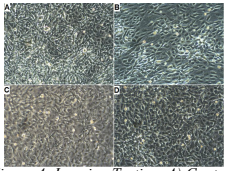

Figure 4: Imaging Testing. A) Control. B) Glass. C) Polystyrene. D) Plexiglass.

Material Type and Thickness	% of Relative Image Focus (Brenner's Focus Law)
Control, t = 0mm	37.44%
Glass, t = 2.2 mm	23.27%
Plexiglass, t = 2.3 mm	22.14%
Polystyrene, t = 1.15 mm	16.56%

Table 1: Summary of Image Focus, 20X

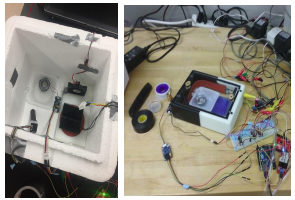

Figure 5: First iteration of control system validation (left) and second generation validation in fabricated enclosure(right)

Results and Discussion

Design evaluation:
- Plexiglass allows for optimal imaging capability
- Design regulates temperature, CO2, humidity

Design Limitations:
- System Recovery
- Cost

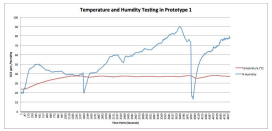

Figure 6: Temperature and Humidity Testing Data over time

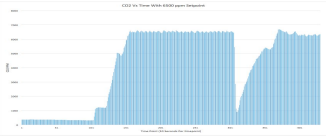

Figure 7. CO2 Stability and recovery over time

Future Work

- Optimize control loops and electrical circuit
- Chamber recovery testing,
- Long-term cell survival and imaging tests
- Tests between various microscopes

Acknowledgements

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References

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