Microscope Cell Culture Incubator

BME 400

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Client: Dr. John Puccinelli, PhD **Advisor:** Dr. Paul Campagnola, PhD

Abstract

Live cell imaging allows researchers to precisely monitor temporal changes in cell morphology and behavior. To image live cells for extended periods of time, the temperature, pH, and concentration of their media must be maintained at optimal levels. Cell culture incubators typically maintain these conditions, but such devices do not fit on microscope stages. Therefore, our client, Dr. Puccinelli, wants the team to create an incubator modified to fit on a Nikon TI-U microscope stage without blocking the path of light from the microscope. This paper describes the team's process in developing a preliminary design for the on-stage incubator. The team plans to fabricate, test, and modify the described device.

Introduction

- Microscope cell culture incubator is important for cellular imaging over extended periods of time
- Eliminates need to remove cells from incubator to microscope and vice versa
- Better preserves cells
- Existing devices cost upwards of $10,000

Figure 1: The Ibidi Stagetop Incubation System costs $13,990

- Importance of incubator parameters:
  - Temperature (37°C)
    - Maintains viability and healthy metabolic rate
  - pH (7.2-7.4)
    - Maintains cell viability and function
    - Corresponds to 5±1% CO_2 concentration
  - Relative Humidity (over 95%)
    - Prevents media evaporation
      - Maintain concentration of salts and analytes in media
    - Primarily dependent on number of times incubator is opened

Design Criteria

Client Requirements:

- 37°C, 100% Humidity, and 5% CO2 concentration
- Does not impede optical path
- Fit cell plates with maximum size of 130mm x 90mm x 20mm
- Uniform heating throughout
- Easy readout of conditions
- Ability to change out cell cultures
- Ability to be sterilized
- Combined budget: $100

Figure 2: Ti-U Fluorescence microscope

Fabrication and Final Design

Materials:

- 2 Polypropylene sheets 12in x 12in x 1in
- Screws
- 2 pieces acrylic glass
- MH-Z16 CO2 sensor
- DHT-22 Temperature/Humidity sensor
- (¼") Gas Solenoid Valve
- Heating Element
- Arduino

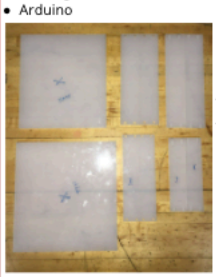


Figure 3: Cut, drilled, and tapped pieces of polypropylene plastic

Figure 4: Assembled box with top removed

Final Design:

- Inner and outer box chamber
  - Inner: 14.9 x 13.65 cm,
  - Outer: 20.32 x 18.10 cm
- Insulation between chambers
- Top and bottom both include glass to view cells

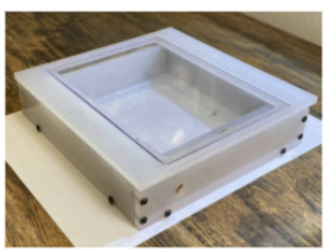


Figure 5: Fully constructed box with top on and entry ports drilled

Coding Circuitry and Difficulties

Arduino Code:

- Temperature control
- Relative humidity
  - To reduce vapor pressure of liquids in culture
- CO_2 sensor read in and control

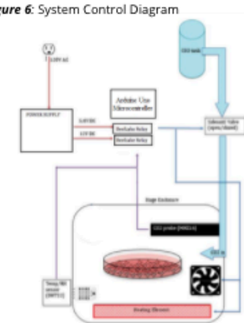
Circuit and Other Electronics:

- DHT temperature and humidity sensor
- MH-Z16 CO_2 Sensor
- Heating Element

Circuit Difficulties

- PCB short circuited multiple times
  - Had to re-solder
- Previous semester's circuit diagram did not exist
- Multiple heating elements were defective

Figure 6: System Control Diagram



Testing

Testing Plans:

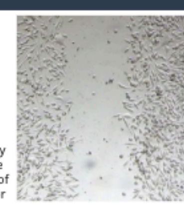
- Collect data on CO_2 Pressure, Temperature, and Humidity
  - Temperature and humidity experiments will be conducted first
  - CO_2 experiments will be conducted second
  - Trials will be 5 min, 15 min, 1 hour, 8 hours, 24 hours, 72 hours
- Run T Tests and ANOVA

Future Work

- Testing with temperature and CO_2 sensors
- Cut a hole for the solenoid valve
- Test with live canine kidney cells

Figure 7: MDCK cells that will be tested within the incubator

Figure 8: Scratch assay to determine the viability of the incubator



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Sources

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[2] Y. D. Sie, Y.-C. Li, N.-S. Chang, P. J. Campagnola, and S.-J. Chen, "Fabrication of three-dimensional multi-protein microstructures for cell migration and adhesion enhancement," Biomedical Optics Express, vol. 6, no. 2, p. 480, Dec. 2015.