Microscope Cell Culture Incubator

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Advisor: Dr. Amit Nimunkar
Client: Dr. John Puccinelli
Date: 10/07/2022

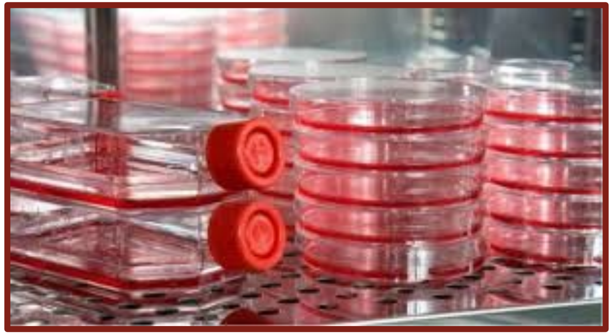


Figure 1: Cell Culture Plates [1]

Background Information

- **Client:** Dr. John Puccinelli; Associate Chair of the Undergraduate Program
- **Cell Cultures**
  - Lab method for the use of studying cell biology, replicating disease mechanisms, and investigating drug compounds [2]
  - Use both primary, transformed, and self-renewing cells
- **Incubators**
  - Replicate cells' natural conditions in order for optimal growth
    - Natural Cell Environment - 37°C, pH = 7.2-7.4, 95% humidity [3]
  - Cost: $500-$40,000 [4]
- **Live Cell Imaging**
  - Allows researchers to continually view cell development
  - Need incubator on a microscope in order to keep cells alive for imaging

Figure 2: On-stage incubator [4]

Problem Statement

- **Purpose:** Develop a low cost cell culture incubation chamber that fits on a microscope stand (<310x300x45mm), does not interfere with the lens optics, and is capable of live cell imaging.

- Current commercially available systems
  - Sometimes result in evaporation from low volume cultures
  - Expensive, too large, Enclose the entire microscope
  - Previous BME 200/300 design projects
  - Portable Live-cell Imaging Box ~ $400 materials
  - Elliot Scientific and OkoLabs Stage Top Incubators[4] ~ $400-$1,000

Figure 3: Cell Culture Procedure [5]

PDS Summary

Performance requirements:

- Compatible with an inverted microscope in both size and function
- Maintain an internal environment of 37°C, 5% CO_2, and 95-100% humidity

Safety:

- Biosafety Level 1 Standards [6]

Accuracy and Reliability:

- Temperature of 37°C ± 1°C, humidity of >95%, and CO_2 levels of 5% ± 1%
- Maintain internal environment for at least 1 week

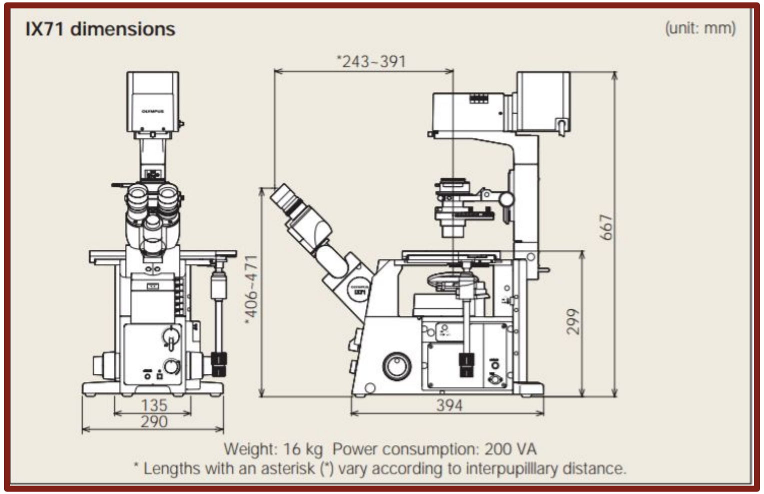


Figure 4: Measurements of Inverted Microscope [7]

PDS Summary cont.

Size:

- Incubator < 310x300 mm with a thickness < 45 mm

Materials:

- Transparent top and bottom surfaces

Target Production Cost:

- < $100

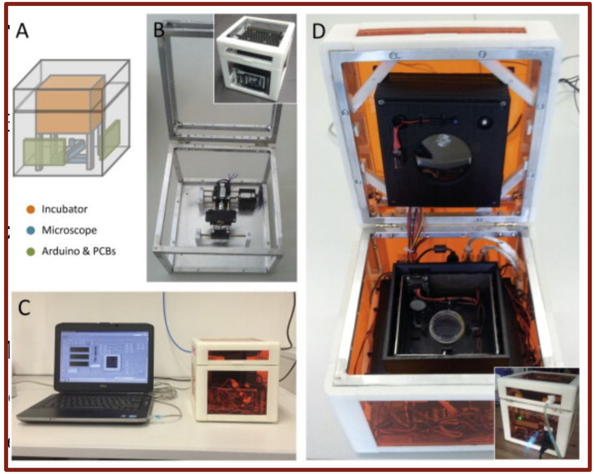


Figure 5: Portable Live-Cell Imaging Platform [8]

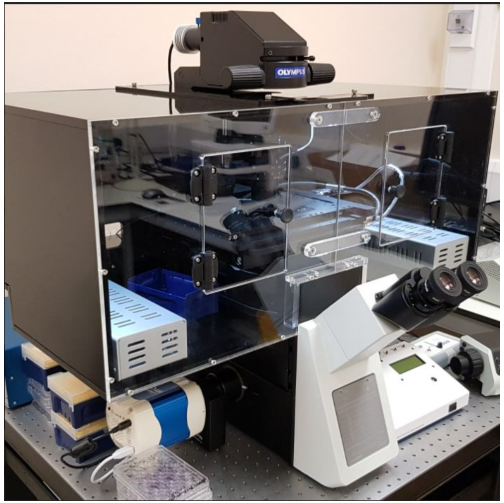


Figure 6: Elliot Scientific Stage Top Incubator [9]

Spring 2022 Work

Fabrication

- Laser cut black acrylic from UW-Makerspace
- Thermistor was used for temperature and humidity
- NDIR CO_2 Sensor used for CO_2 percentage reading

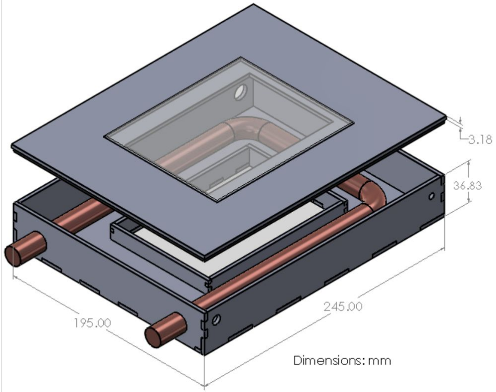


Figure 7: Final Prototype CAD drawing

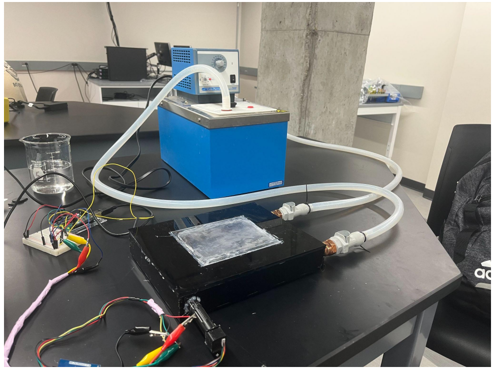


Figure 8: Final Prototype setup

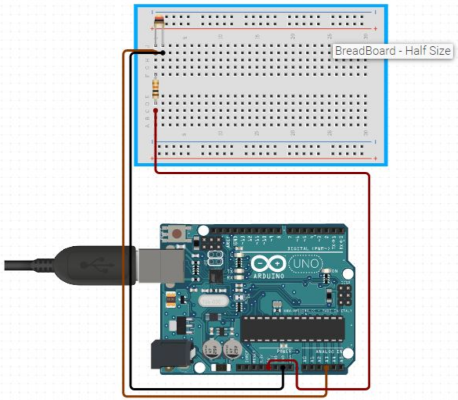


Figure 9: Thermistor Circuit Diagram

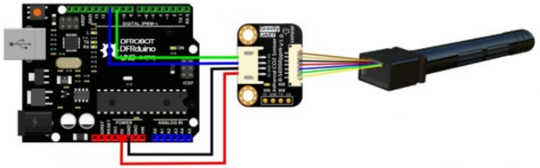


Figure 10: CO_2 Circuit Diagram

Spring 2022 Testing

Results

- Temperature and humidity constant at 37°C and 95+%
- Recovery testing for temperature and humidity were successful
- Optical testing was successful

Conclusions

- Need to reduce condensation on glass
- Conduct live cell testing
- Develop best way to regulate CO_2 input

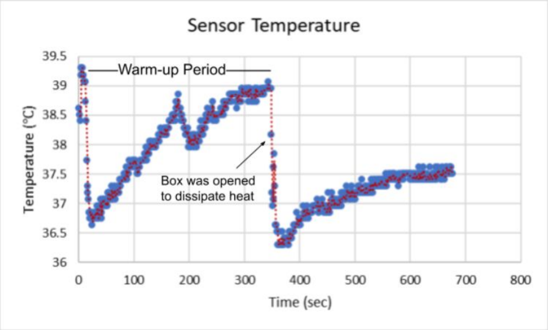


Figure 11: Temperature sensing results

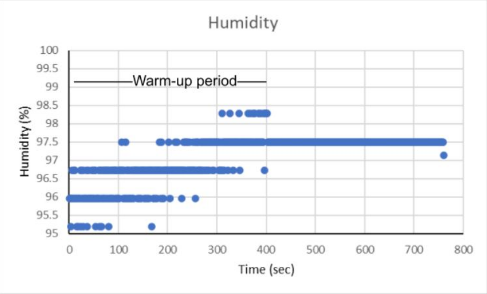


Figure 12: Humidity sensor results

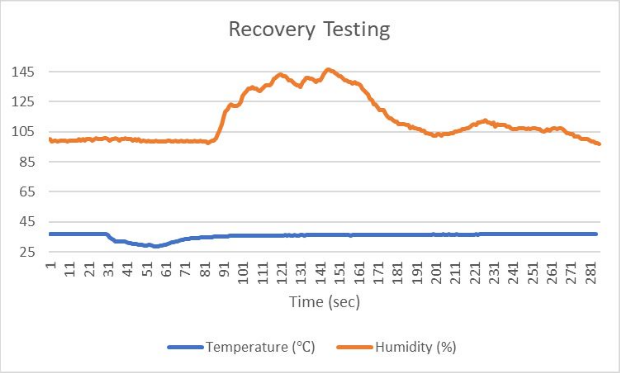


Figure 13: Recovery testing results.

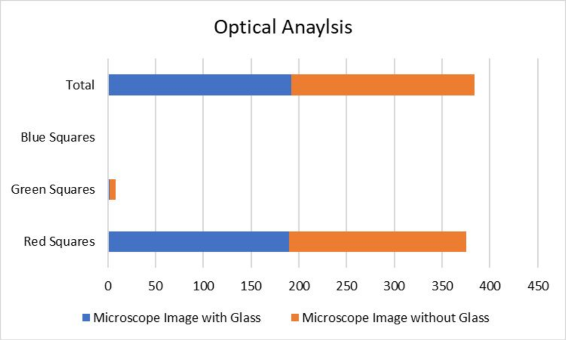


Figure 14: Optical testing results

Preliminary Design #1

Solenoid Valve

Strengths:

- No Fabrication
- Electrically controlled
- No leaks

Weaknesses:

- Costs
- Power source

Figure 15: Image of the solenoid valve [10]

Preliminary Design #2

Threaded Pin Valve

Strengths:

- Costs
- Safety

Weaknesses:

- Leakage
- Accuracy

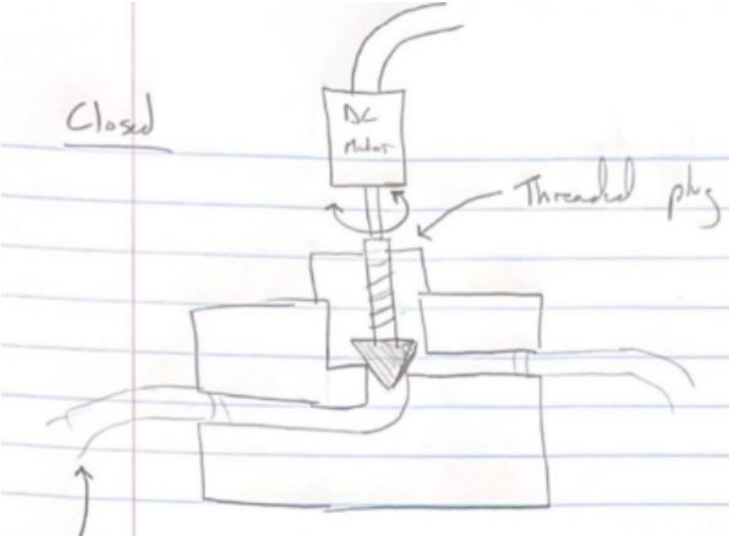


Figure 16: Drawing of the threaded pin valve design

Preliminary Design #3

Spring Pin Valve

Strengths:

- Fast closing response time
- Homemade

Weaknesses:

- Complex
- Leakage

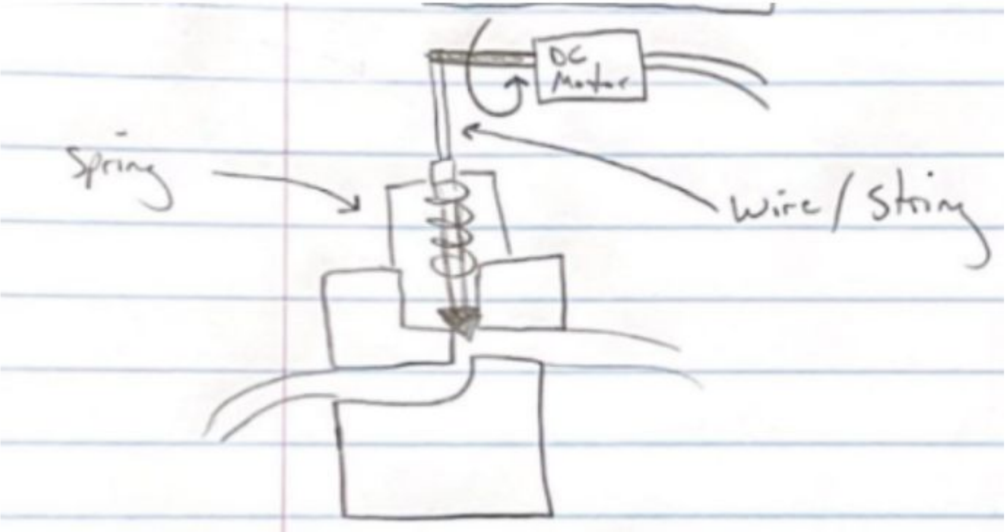


Figure 17: Drawing of the spring pin valve design

Design Matrix Criteria

- **Accuracy and Reliability:** Accuracy of CO_2 input control
- Cost: What is the cheapest, but most reliable design?
- Ease of Use: Circuitry and coding control
- Fabrication: How easy is it to build?
- Life in Service: How long until device is not reliable?
- Safety

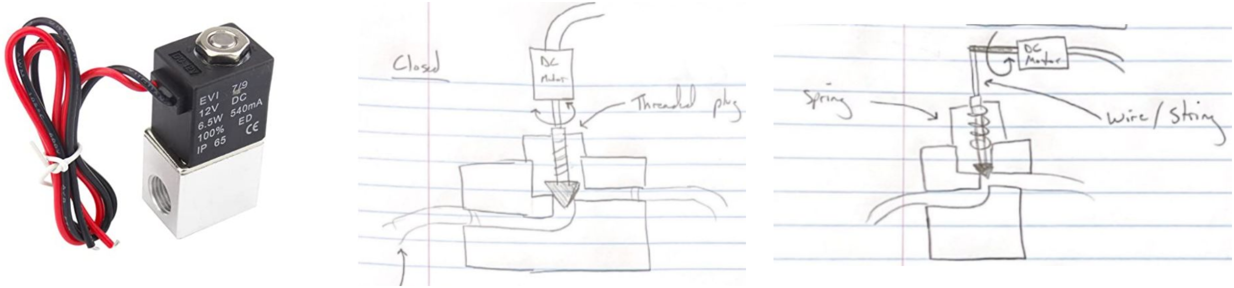
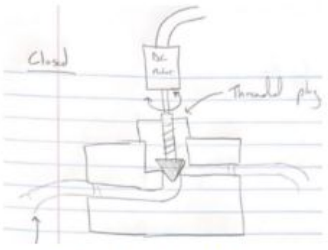


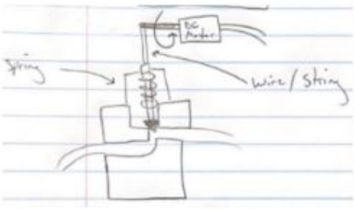
Figure 18: Images of Preliminary Designs #1-3

Design Matrix for CO_2 Input Regulation

Solenoid Valve



Threaded Pin Valve



Spring Pin Valve

			Solenoid Valve		Threaded Pin Valve		Spring Pin Valve	
Rank	Criteria	Weight	Score (5 max)	Weighted Score	Score (5 max)	Weighted Score	Score (5 max)	Weighted Score
1	Accuracy and Reliability	30	5	30	3	18	4	24
2	Cost	20	3	12	4	16	4	16
3	Ease of use	20	5	20	3	12	2	8
4	Fabrication	15	5	15	3	9	2	6
5	Life in Service	10	4	8	1	2	2	4
6	Safety	5	4	4	5	5	5	5
	Sum	**100**	**Sum**	**89**	**Sum**	**62**	**Sum**	**63**

Proposed Final Design

- Design #1
- Easiest setup
- No fabrication of the valve
- Most reliable CO_2 input regulation via Arduino

Figure 19: Image of the Solenoid valve that will be used

Current Work

- Anti Fog Testing
- Cell Confluency Testing
- Solenoid Circuitry

Future Work

- CO_2 input testing
- Incubator Homogeneity Testing
- Live cell testing

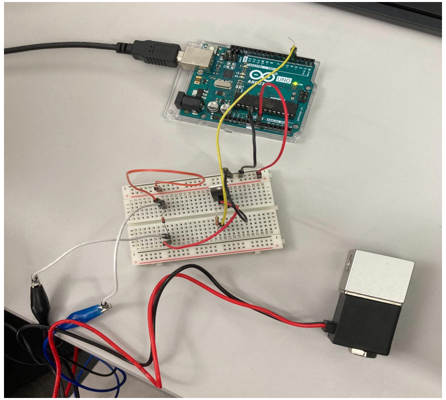


Figure 20: Solenoid Circuit Setup

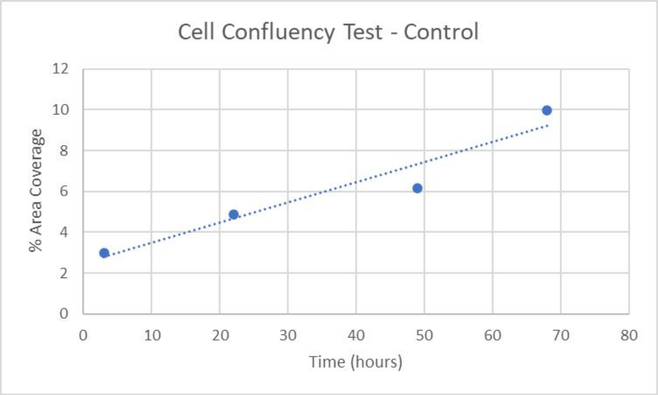


Figure 21: Cell Confluency Test - Control

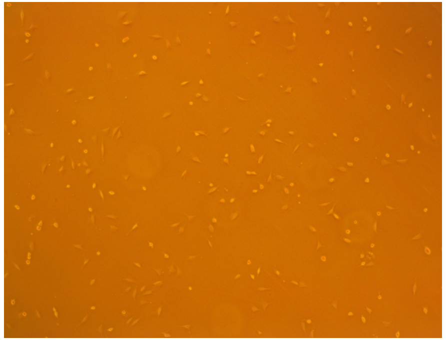


Figure 22: Control Cells Day 0

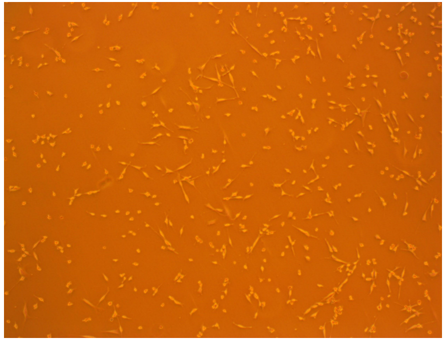


Figure 23: Control Cells Day 3

Upcoming Project Goals

Purchase Materials
Present

Test Materials
Wednesday, October 12th

Begin Prototyping
Wednesday, November 2nd

Test Prototype
Wednesday, November 23rd

Final Deliverables
Wednesday, December 14th

Special Thanks

Dr. John Puccinelli

Dr. Amit Nimunkar

BME Department

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Questions?