Microscope Cell Culture Incubator

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Client: Dr. John Puccinelli
Date: 02/10/2023

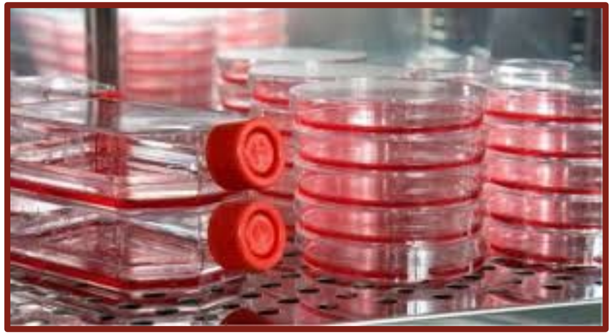


Figure 1: Cell Culture Plates [1]

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

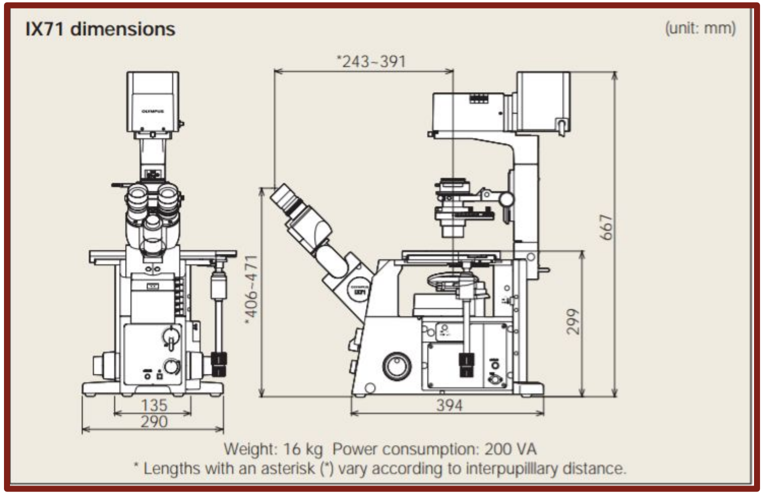


Figure 4: Measurements of Inverted Microscope [7]

PDS Summary cont.

Size:

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

Materials:

- Transparent top and bottom surfaces

Target Production Cost:

- < $100

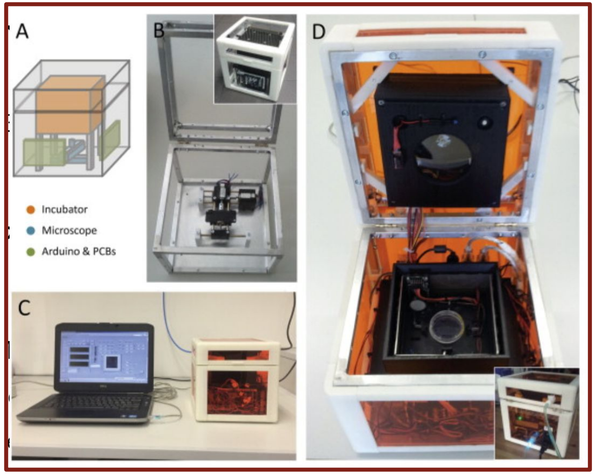


Figure 5: Portable Live-Cell Imaging Platform [8]

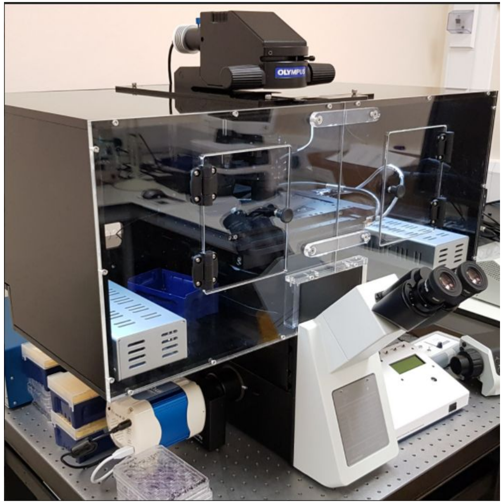


Figure 6: Elliot Scientific Stage Top Incubator [4]

Fall 2022 Work

Fabrication

- Laser cut black acrylic from UW-Makerspace
- Electronics
    - Thermistor = temp and humidity
    - MH-Z16 = CO_2
    - Solenoid valve and Relay circuit = CO_2 tank control
- Squeegee Wiper Blade for anti-fog

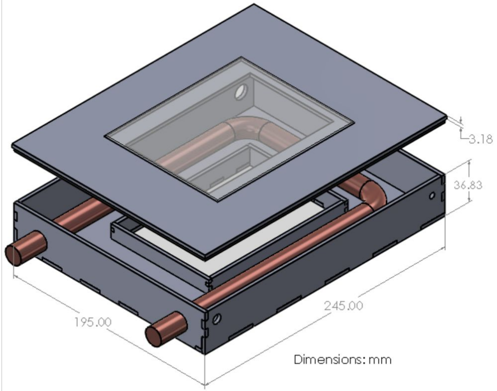


Figure 7: Final Prototype CAD drawing

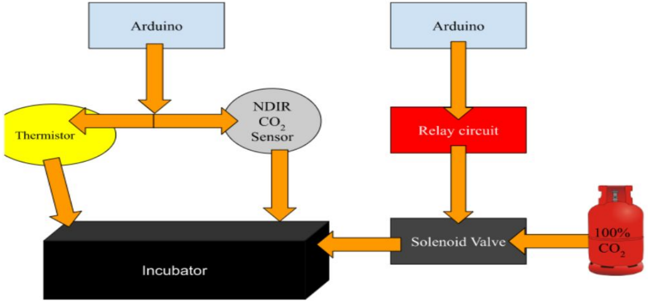


Figure 8: Flow chart of final design

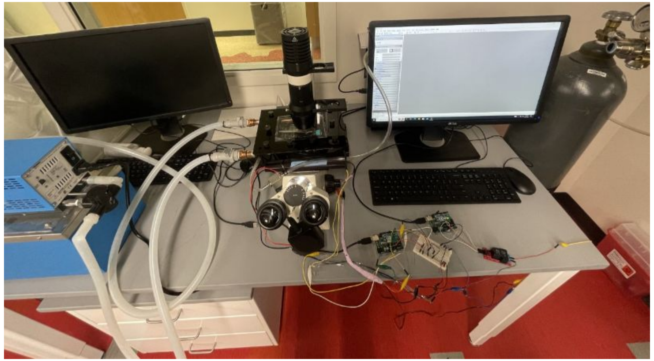


Figure 9: Final Prototype setup

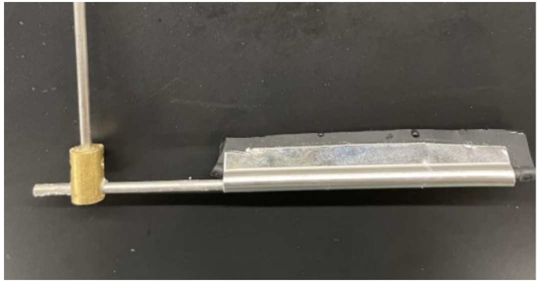


Figure 10: Wiper blade design

Fall 2022 Testing

Results

- Homogeneity testing was successful
- Optical testing was successful
- CO_2 hard-coded input failed along with CO_2 sensor

Conclusions

- Need to find a new CO_2 sensor
- Wiper blade must be better fabricated for anti-fogging methods
- More Live-Cell tests

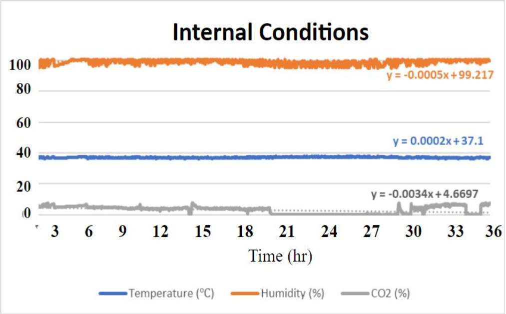


Figure 11: Internal Incubator Conditions Over Time

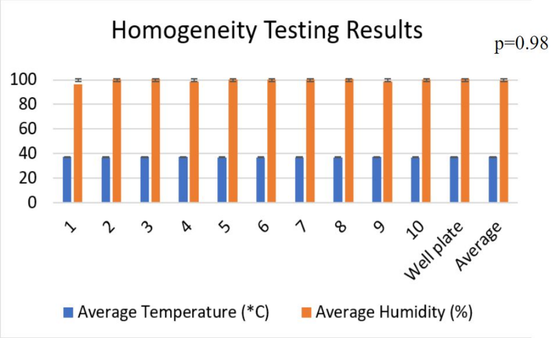


Figure 12: Homogeneity Testing Results

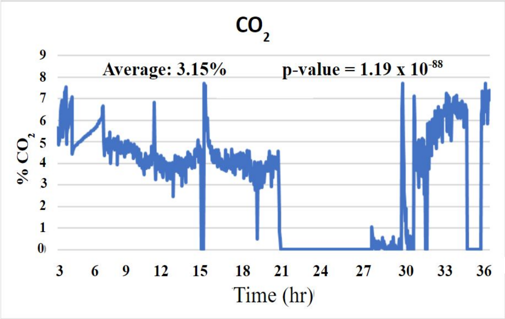


Figure 13: CO_2 Monitoring Over Time

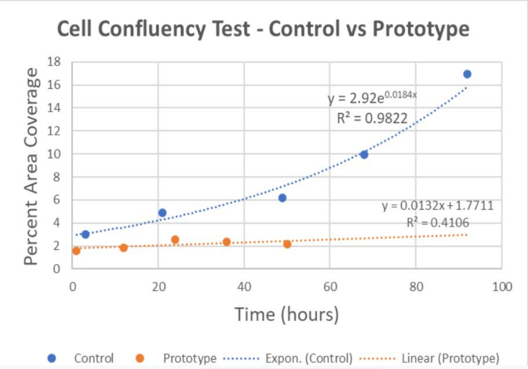


Figure 14: Cell Confluency Testing

CO_2 Sensors

Preliminary Design #1

MH-Z16 CO_2 Sensor

Strengths:

- Previous Use
- Compatibility with Arduino and Solenoid Valve
- Water resistant

Weaknesses:

- Costs
- Shorter Lifespan

Figure 15: MH-Z16 CO_2 Sensor [9]

Preliminary Design #2

BME 680

Strengths:

- Reads Temperature, humidity, and CO_2
- Arduino Compatible
- Low Cost

Weaknesses:

- Requires I2C
- Not waterproof
- Reads CO_2 in kOhm not ppm

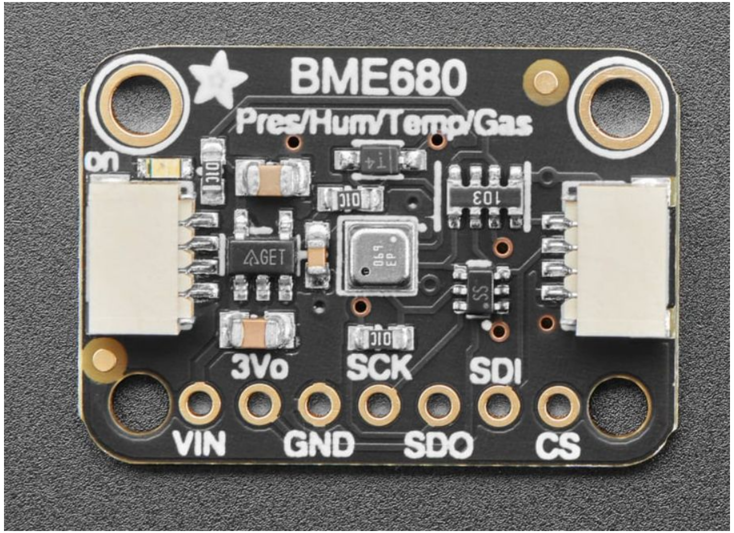


Figure 16: BME 680 Temperature, Humidity, and CO_2 Sensor [10]

Preliminary Design #3

MH-Z14A

Strengths:

- No I2C
- 10000 ppm
- Same company as current NDIR (design #1)

Weaknesses:

- Water droplet resistance
- May fail with high humidity

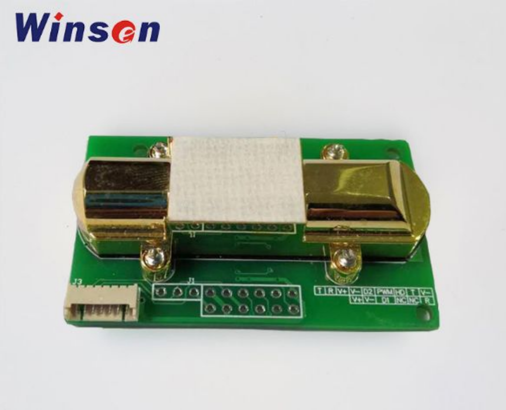
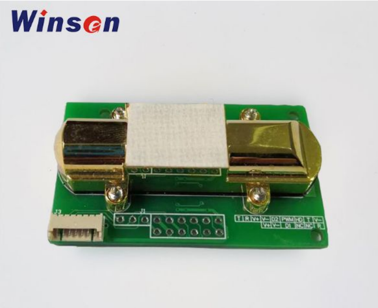


Figure 17: MH-Z14A CO_2 Sensor [11]

Design Matrix Criteria

- Performance - How well the sensor is able to read CO_2 in our environment?
- Water resistance - Will the sensor be able to withstand the water bath and condensation?
- Ease of use - ease of circuitry, ease of coding, ease of integration with existing electronics
- Cost - How expensive is the sensor?
- Life in service - How long will it be reliable?
- Safety - Will the sensor harm cells?



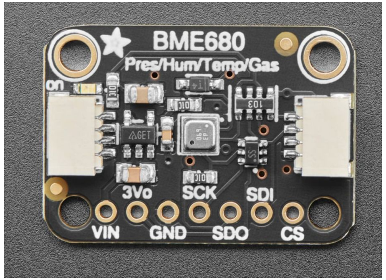


Figure 18: Images of Preliminary Designs #1-3 [9][10][11]

Design Matrix for CO_2 Sensor

			MH-Z16		**BME 680**		**MH-Z14A**	
Rank	Criteria	Weight	Score (5 max)	Weighted Score	Score (5 max)	Weighted Score	Score (5 max)	Weighted Score
1	Performance	30	3	18	5	30	3	18
2	Water Resistance	20	5	20	0	0	4	16
3	Ease of use	20	5	20	1	4	2	8
4	Cost	15	1	3	5	15	3	9
5	Life in Service	10	3	6	3	6	5	10
6	Safety	5	5	5	4	4	5	5
	Sum	**100**	**Sum**	**72**	**Sum**	**59**	**Sum**	**66**

Table 1: Design Matrix for CO_2 Sensor

Proposed Final Design

Figure 19: Proposed Final Design [9]

Anti-Fog/Condensation

Preliminary Design #1

ITO (Indium Tin Oxide) Coated Glass

Strengths:

- Excellent electrical conductivity and optical transparency
- Coating uniformity
- Pre-fabricated

Weaknesses:

- Expensive
- Difficult to purchase/acquire

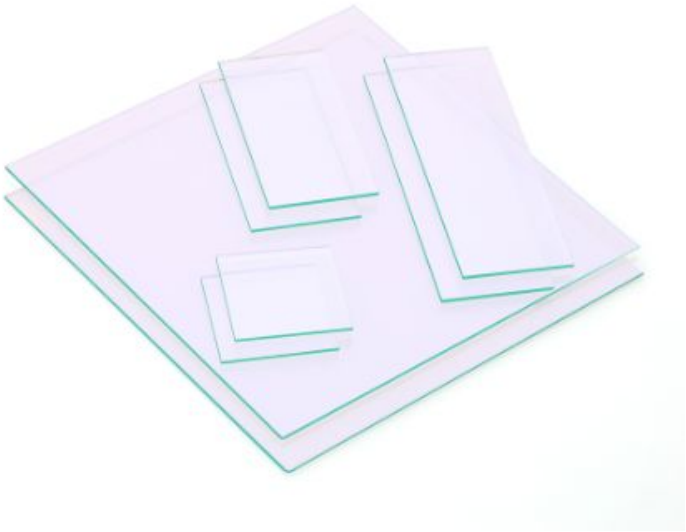


Figure 20: ITO Conductive Glass [12]

Preliminary Design #2

Copper Grid Tape

Strengths:

- Easy to fabricate
- More surface area than copper wire

Weaknesses:

- Limited heating area
- Blocks optics in some areas
- Requires a lot of current

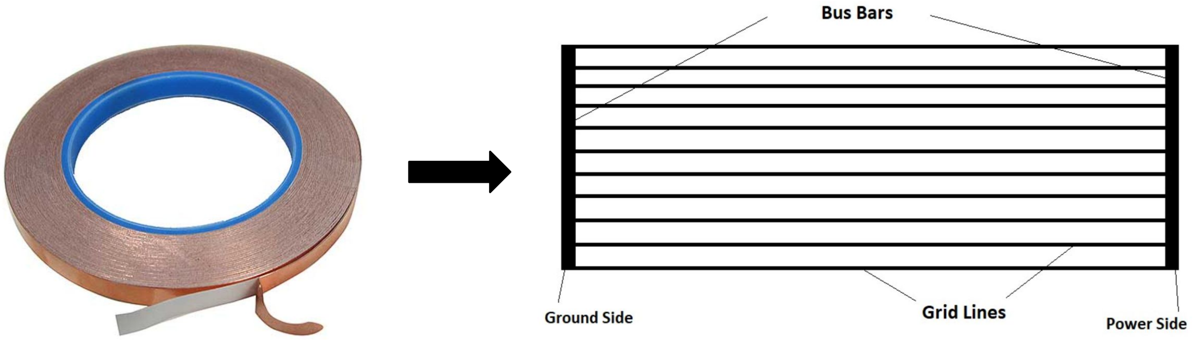


Figure 21: Copper tape for condensation prevention grid [13][14]

Preliminary Design #3

Micro Fan

Strengths:

- Easy to obtain
- Easy fabrication

Weaknesses:

- Not waterproof
- Hard to fit in the incubator box

25×25×10 mm

Figure 22: Micro Fan for condensation prevention[15]

Design Matrix for Anti-Fog/Condensation Method

			 ITO Coating		 Copper Grid Tape		 Microfan	
Rank	Criteria	Weight	Score (5 max)	Weighted Score	Score (5 max)	Weighted Score	Score (5 max)	Weighted Score
1	Ease of Fabrication	25	3	15	4	20	5	25
2	Performance	25	5	25	3	15	4	20
3	Cost	20	1	4	4	16	5	20
4	Safety	15	5	15	4	12	4	12
5	Life in Service	15	5	15	3	9	2	6
	Sum	**100**	**Sum**	**74**	**Sum**	**72**	**Sum**	**83**

Table 2: Design Matrix for Anti-Fog/Condensation

Proposed Final Design

Figure 23: Micro Fan for condensation prevention [15]

Current Work

- Box fabrication ✓
- Condensation prevention design development
- Standard- Live cell testing

Future Work

- CO_2 Sensor coding
- CO_2 Sensor testing
- Condensation testing
- Prototype- Live cell testing

Figure 24: Top view of the new incubator box showing the caulking and copper piping

Upcoming Project Goals

Purchase Materials
Present

Test Materials
Friday. February 24th

Begin Prototyping
Friday, March 10th

Test Prototype
Friday, March 31st

Final Deliverables
Wednesday, May 3rd

Special Thanks

Dr. John Puccinelli

Dr. Amit Nimunkar

BME Department

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Questions?