Hypoxic Incubation Chamber

A Senior Project
presented to
the Faculty of the Biomedical Engineering Department
California Polytechnic State University – San Luis Obispo

In Partial Fulfillment
of the Requirements for the Degree
B.S. Biomedical Engineering

By

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Statement of Disclaimer

Since this project is a result of a class assignment, it has been graded and accepted as fulfillment of the course requirements. Acceptance does not imply technical accuracy or reliability. Any use of information in this report is done at the risk of the user. These risks may include catastrophic failure of the device or infringement of patent or copyright laws. California Polytechnic State University at San Luis Obispo and its staff cannot be held liable for any use or misuse of the project.

Executive Summary

In developing a model to test anti-tumor drugs, Dr. Christopher Heylman's lab requires the ability to culture cells in a hypoxic environment. The purpose of this project was to make this possible. Funding for this project comes from the Biomedical Engineering Department, the Hannah-Forbes Fund, and Dr. Heylman's lab. The chamber must be able to reach a user-defined oxygen concentration and hold that concentration for 48 hours while creating an environment conducive to cell culture. Importantly, the chamber must also be sterilizable and cleanable for repeated use. While several products exist to create hypoxic environments for cell culture, they are either too expensive or too simple. The timeline of this project has been largely dictated by the BMED senior project class and its deadlines, culminating in the Final Report and Presentation given on March 9th. Several different ideas were generated to meet the customer requirements in various ways. The final design of the chamber is a rectangular box with a detachable door that is held onto the chamber by 3 latches. The door uses a rubber gasket with neoprene to prevent air from escaping or entering the chamber. Gas is allowed into the chamber through two different tubes- one supplying oxygen as an "up" control and one supplying a nitrogen and carbon dioxide mixture as a "down" control of the oxygen concentration. Flow through the tubes is regulated by two needle valves. A check valve on the side of the chamber allows excess gas to exit if the pressure passes 0.5 psi. An oxygen sensor sits in the top of the chamber that gives a continuous oxygen reading to a connected computer through an Arduino Uno. The only manufacturing required was that of the chamber, but because the chamber is a pressure vessel, the manufacturing had to be quite precise. Tests were to be conducted on the sealability of the chamber, the accuracy of setting the O2 level, the oxygen sensor itself and more. Due to time constraints, the only test that was able to be carried out to any significant extent was a Modified Maintain Oxygen Level test which determined what the best method of sealing the chamber was to maximize the amount of time the chamber was able to hold a given oxygen concentration. More work needs to be done for this project to be useful in a research setting. The chamber needs a way to be heated and held at a temperature conducive to cell proliferation. Additionally, active control of the valves will need to be introduced to hold the oxygen concentration within spec for 48 hours.

Objective

The goal of this project is to develop a device that allows for prolonged culture of mammalian cells at a user-defined oxygen concentration while interfacing with existing culture techniques and mimicking incubators. The scope of this project includes creating a chamber that can maintain a given oxygen concentration for at least 48 and has the ability to emulate existing incubators. An oxygen sensor should be made to accurately read the oxygen concentration within the chamber. It also needs to be both cleanable and sterilizable. It does not include creating a new incubation chamber as a whole, only a component to be used inside an existing incubation chamber. It also does not include a chamber that will change any conditions set in the incubator other than oxygen. For example, the CO_2 content in the hypoxic chamber does not need to be altered from incubator conditions

Project Management

This project will follow a defined, yet fluid process to ensure the most efficient uses of resources, on-time deliverables, and quality of product. The schedule of this project is largely dictated by the schedule of the BMED 455 and 456 classes. Since the project was assigned on September 14th, we have been working on this cumulative report and test plan. The goals of the project are also largely set by the class structure and consist mostly of assignments that need to be turned in and milestones in the form of presentations and reports. These projects were laid out in sequential order with rough time estimates in Microsoft Project. The project time is, of course, determined by the last deliverables. The Senior Design Expo which will happen on March 18th, 2021 marks the end of the project.

Table I. Key deliverables and project timeline.

Deliverable	Date	Significance
Project Start	9/16/20	Start of project
Project Planning Meeting	10/5/20	Present project plan
Conceptual Design Review	10/26/20	Present conceptual design
Critical Design Review	11/18/20	Present detailed design
Functional Prototype Demo	1/26/21	Present prototype and plan for testing
Senior Project Design Report	3/9/21	Summary of entire project
Design Review	3/9/21	Summary of design and process
Expo Poster Presentation	3/18/21	Present design
Project End	3/18/21	End of project

Some of the major deliverables for this project and for the BMED 455 and 456 classes are presented above in Table I.

The network diagram for the project also follows the structure of the BMED 455 and 456 classes closely with the major deliverables described above largely forming the critical path. The

network diagram is depicted in Figures 1 and 2, below. Smaller assignments connect the major deliverables together, with the critical path chain ending in the Senior Design Report, Design Review, and lastly the Expo Poster Presentation.

Figure 1. Project network diagram.

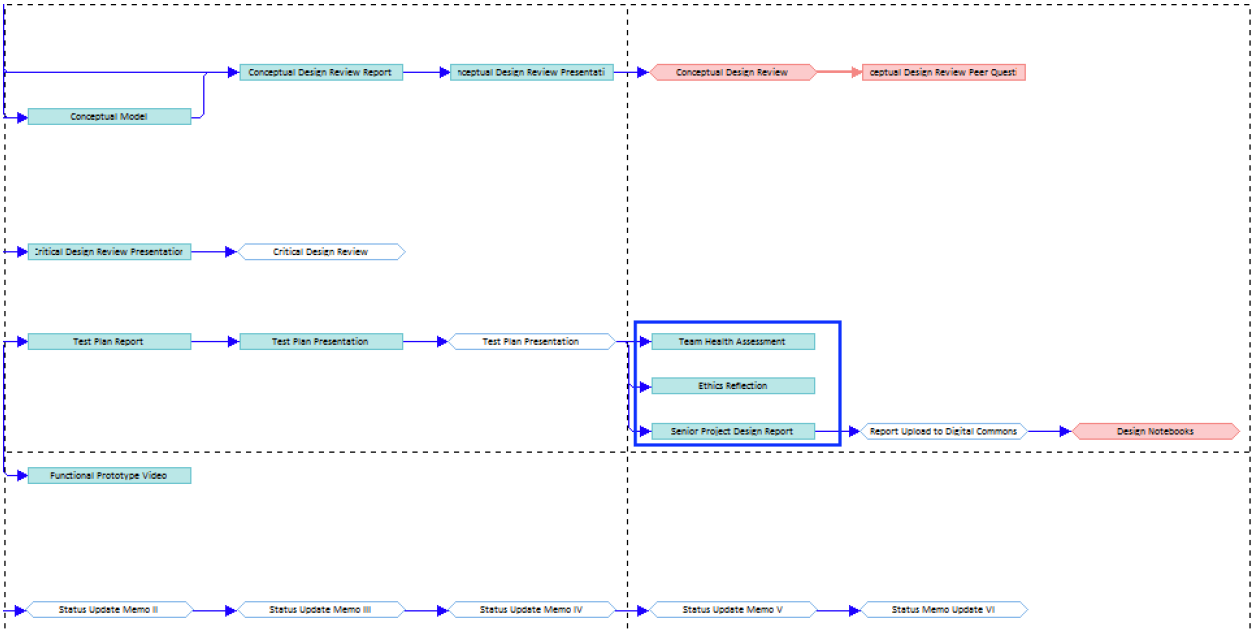


Figure 2. Network diagram zoomed in to end of project.

Indications for Use

The hypoxic incubation chamber is meant to be used by researchers trained in aseptic cell culture techniques with knowledge of using a CO_2 incubator and compressed gas cylinders. The chamber is only to be used in research labs or facilities. The device should not be used by anyone under the age of 18. The chamber can effectively maintain a specified O_2 level within the 0-5% level for at least 48 hours for cells in cultures in a standard CO_2 incubator while maintaining all other incubator environmental conditions.

Summaries of Customer Observations, Meetings, and Interviews

The initial goal of the project was described by Dr. Heylman as creating a device that enabled multi-day cell culture in a user defined O_2 concentration while adapting to and interfacing with the current incubator and cell culture techniques used in his lab.

The first interview with Dr. Heylman was to gauge the scope of the project and discover what he wanted out of the chamber. It was learned that the chamber should fit in an incubator shelf and fit at least 2 T-75 flasks. The chamber should hold its given oxygen concentration until the incubator is opened for media change. The oxygen concentration levels should be below 5% of the total gas with a reading tolerance of 0.1%. It is very important that other than the O_2 concentration, the chamber can conform to the conditions of the incubator, such as CO_2 concentration and humidity. It is also imperative that the chamber is sterilizable, preferably by autoclave. This plays into the fact that ideally, the chamber would be reusable for many experiments. The cost to produce must also be beneath $1200. It quickly became evident that the chamber absolutely must be able to hold a very low (<1 % by volume) O_2 concentration. Without this ability, the device would not be useful in research projects. In a second meeting with Dr. Heylman, he answered many questions surrounding the equipment and processes used by his researchers, the required capabilities of the device, and the budget available for the project. Most recently, Dr. Heylman was also asked to rank and re-rank the customer requirements that were crafted from the project definition and previous conversations with him.

Budget

The budget has undergone several significant revisions. Further materials have been purchased, and other parts were returned. Solenoids were returned to the supplier, as well as some components ordered from Amazon. Additionally, subsequent orders of the same thing were combined into the same category and some components were renamed to more accurately represent their purpose in the project. Shipping costs are not included in this budget but were factored into order forms. Some parts that did not end up being used for the electronics assembly were unable to be returned and are therefore included in the budget even though they are not a

part of the final project. The project budget can be found as Table II. A breakdown of the cost of the final prototype is provided in the Detailed Design section.

Table II. Project budget.

Item Description	Cost
Plastic Chamber (sides)	$216.06
Plastic Chamber (front/back)	$76.41
M4 Screws	$11.83
Latches	$8.27
Heat shrink tubing	$3.06
Wires (22 gauge, silicone + copper)	$16.10
Tubing (adapter)	$6.00
Tubing	$25.79
Handles	$27.95
Incubator Hole Interface	$0.00
Wye fittings	$18.38
Gasket	$17.15
N2/CO2 Tank	$133.45
O2 tank	$11.46
O2 Sensor	$106.43
Arduino Kit	$39.76
Epoxy	$59.00
Pressure Regulator (O2)	$203.72
Pressure Regulator (N2/CO2)	$0.00
Tubing clamps	$6.73
Tube adapter (w/ CO2 regulator)	$17.18
Tube adapter (w/ solenoids)	$19.18
Tube-Tube adapter	$4.91
Tube adapter (w/ chamber)	$9.62
CO2 Regulator-tank Adapter	$22.90
Solenoid Valves	$60.47
Check Valve	$1.04
Adafruit Pro Trinket - 3V 12MHz	$10.65
Adafruit LiIon/LiPoly Backpack Add-On	$5.30
ADS1015 12-Bit ADC	$10.65
Break Away Headers - Straight	$1.50
Nokia 5110/3310 monochrome LCD + extras	$10.70
Breadboard kits	$10.74
Tube adapter (w/ needle valves) v2	$17.29
Tube adapter (w/ O2 regulator)	$2.17
Brass Needle Valves	$14.00
TOTAL	**$1,222.04**

Customer Requirements

For the project, it is required that the chamber be able to integrate with current processes and equipment in their research lab. This includes dimensions of the chamber, the conditions of the incubation chamber it will be placed in, and current cleaning and sterilization techniques. Given the chamber is meant to be hypoxic, it should maintain a given concentration of O_2 and be able to accurately measure the current O_2 concentration. A full list of customer requirements can be found in Attachment A.

These requirements were used to generate the engineering specifications, which can be seen in Table III below, with more in-depth explanations below the table. H, M, L refers to high, medium, and low risks, respectively. The T, S, A, and I letters in the Compliance column refer to the method of determining whether or not the device has met the given specification and stand for test, similarity to existing products, analysis, and inspection respectively.

Engineering Specifications

The development of these specifications was guided by using a House of Quality which has been added as Attachment B. The original intent of specification #9 was to limit the complexity of the design regarding what the researchers had to interact with. The design has far more than 10 parts in total. However, the researchers will only have to account for 4 different parts as the gas cylinders, associated adapters, and Arduino are all self-contained and do not require movement or modification from the researchers. Additionally, the path to achieving each of these specifications has been further developed. It is now clear how the chamber will meet its temperature and pressure specifications, as well as its oxygen level requirements.

Table III. Engineering Specifications

Spec. #	Parameter Description	Requirement of Target (units)	Tolerance	Risk	Compliance
1	Size (max)	18x18x8 in	Max	L	A, T
2	Size (min)	8x3.5x3.5 in	Min	L	A, T
3	Maintain O_2 Concentration	5% [O_2]	Max	M	T
4	Reach O_2 Concentration	3 hours	Max	M	T
5	Maintain input O_2 Concentration	0.1% of input value	Min	H	T
6	Reading Uncertainty of O_2 Concentration	0.1%	Max	H	S
7	Temperature	21 deg C	Min	M	T, S
8	Pressure	16 psig	Min	M	T, S
9	Parts	10 parts	Max	L	I
10	Assembly	10 min	Max	L	T
11	Maintain CO_2 Concentration	0.2% [CO_2]	Max	H	T, S
12	Maintain Temperature	0.5 deg C	Max	H	T, S
13	Electrical Components	48 hours	Min	L	T
14	Longevity	2 years	Min	M	I, T

Specification 1

The first specification pertains to the maximum size of the hypoxic chamber. This specification sets the maximum size of the chamber to that of a shelf size in the incubators that will be used by the research lab of Dr. Heylman. This specification will be measured by analysis of the dimensions of the chamber compared to those of the incubator(s). The chamber will also be placed inside the incubator as a final test to make sure the chamber can function properly in the space of the shelf.

Specification 2

This specification sets the minimum chamber size to be that which two Standard T-75 flasks can fit inside the chamber. This specification will be analyzed by checking the dimensions of the chamber against a selection of T-75 flask specifications as well as placing two flasks in the chamber as a test to check that the flasks fit and can be placed inside, and taken out, of the chamber without disturbing the culturing process.

Specification 3

Maintaining a specified O_2 concentration below 5% for 48 hours will be tested by monitoring several different O_2 concentrations over a specified period of time, at least 72 hours.

Specification 4

The O_2 concentration in the chamber will be monitored from when the input is set, until it is within a specified tolerance of the input and the time it takes the concentration to reach the input will be measured.

Specification 5 - High Risk

Maintaining the input O_2 concentration is very important, but also a complex task to achieve. This will be achieved by gassing the sealed chamber with the desired gas composition at time = 0 and then regasing the chamber after 2 hours. Additionally, it has been requested that the oxygen concentration be kept within 0.1% of the input value which is a fairly small tolerance to achieve and one that most competitors do not provide. This specification will be measured by using an oxygen sensor to monitor the oxygen concentration for specified lengths of time.

Specification 6 - High Risk

Maintaining a reading uncertainty depends on both the monitoring device as well as the precision of the oxygen sensor. The oxygen sensor that will be purchased has a reading specificity of 0.1%.

Specification 7

The maximum temperature that the whole chamber tolerates depends on material selection. The electronics will not be exposed to the autoclave. Finding materials to withstand the elevated temperature in the autoclave will be accomplished by using the manufacturer's described heat tolerance.

Specification 8

The max operational pressure will be tested in the same manner that the max operational temperature will be measured. Information from manufacturers of materials and components will be used.

Specification 9

This specification will be measured by counting the parts relevant to the researchers. The relevant parts refer to the number of parts that the researcher must interact with every time the incubator is used. Therefore, the valve and vast majority of the electrical components will not be relevant.

Specification 10

Assembling and disassembling will both separately be performed by people familiar with the chamber and all its parts as well as with the assembly and disassembly procedure itself. Both procedures will be timed, and multiple trials will be averaged to get final assembly and disassembly times.

Specification 11 - High Risk

The incubator CO_2 concentration will be measured with a separate sensor periodically through the life of the chamber. This specification has a high risk because of the small tolerance on the concentration and the precision it will require from monitoring equipment, sensors, and the method of controlling the CO_2 levels in the chamber.

Specification 12

Maintaining the temperature in the chamber is intended to be a passive process relying on convection to maintain the same temperature inside and outside of the chamber. This specification will be measured by measuring the temperature inside and outside the chamber while it is in the incubator and taking the difference between these two measurements.

Specification 13

Electrical components will be tested by running the chamber for up to 72 hours multiple times and inspecting the electrical components for corrosion or decay.

Specification 14

All components as well as the interface between components and between components and incubator will thoroughly be inspected for any flaws that might become issues over time and fatigue tested if possible or applicable. Manufacturer's specification from off-the-shelf components will also be used to determine expected lifetime.

Intellectual Property Assessment

Because this device will only be used academically, patent infringement is not relevant. Therefore, this section is only relevant with respect to idea generation and improvement. The team is in a position to combine the optimal parts from any design on the market in order to make the chamber.

Table IV. Patent Search Summary

Title	Number	Claim Summary	Address of Claim
Cell culture incubators with integrated cell manipulation systems	US10696937B2	A cell culture incubator that controls temperature and gas mixture	Incubator in and of itself; ours will be placed within and existing incubator
Cell culture system	US10647955B2	A cell culture having many operation isolators connected to many incubators	The device uses a conveyer to move the incubation chambers, which ours will not have
Highly sensitive oxygen sensor for cell culture	US8398922B2	An oxygen sensor that has a layer permeable to gas, a second layer permeable to gas but not to oxygen sensing material, and a third layer permeable to gas and to facilitate cell attachment	We must ensure that the oxygen sensor does not have the same layer configuration
Mesenchymal Stem Cells Grown Under Hypoxic Conditions: Compositions, Methods and Uses Thereof	US20100330047A1	Ex vivo cell culturing where MSCs are in hypoxic conditions comprising about 0.2% to 7% oxygen	We could use a different cell type than MSCs
Microfluidic device and method for modulating a gas environment of cell cultures and tissues	US20130295551A1	A method for cell culturing in low oxygen conditions on a microfluidic device with differing oxygen concentrations in a chamber separated by a porous membrane	We could avoid using our device with a microfluidic device
Methods and uses of hypoxic compartment cells	US20120045419A1	Cell culturing in a hypoxic environment with an oxygen concentration between 1.5% and 10% and between 2% and 5%	We could have a different range for oxygen concentration

As a part of the background research, patents that could relate to the project were analyzed. In Table IV above, the title and patent number are listed along with a summary of a claim that could be infringed upon during the course of this project and how that could be addressed and/or avoided. However, because this project is purely academic and not for commercial production, patent infringement is not a concern. The patent search is now useful in creating new ideas. For instance, the fourth and last patents shown above gave several ideas on chamber geometry and methods for inducing hypoxia. Links to the patents discussed in the table may be found in Attachment C.

Conjoint Analysis ANOVA

The conjoint analysis was used to determine which device characteristics were considered the most important by potential consumers. The factors and levels chosen for the conjoint analysis are shown in Table V below.

Table V. Factors and levels for conjoint analysis.

Factor	Level 1	Level 2
Flask Organization	Stacked on top of each other	Side by side
Size	Fills entire incubator shelf	Holds a max of 2 T-75 flaks
O_2 Concentration	Accurately maintains O_2 concentration	Accurately monitors the O_2 concentration

The conjoint cards were based on the Taguchi method with 111, 122, 212, and 221 combinations of levels. The cards were therefore 1; stacked, fills entire shelf, and maintains O_2 concentration, 2; stacked, max 2 T-75 flasks, and monitors O_2 concentration, 3; side by side, fills entire shelf, and monitors O_2 concentration, and lastly 4; side by side, max 2 T-75 flasks, and maintains O_2 concentration as choice 1, 2, 3, and 4 respectively. The raw data from the survey is shown below in Table VI.

Table VI. Raw data from survey.

Name	Group #	Group Name	Option 1 Rank	Option 2 Rank	Option 3 Rank	Option 4 Rank
Sam Borda	1	EMMT	2	4	3	1
Hailey Casino	1	EMMT	3	4	1	2
Jake Javier	1	EMMT	1	2	4	3
Charlotte Anderson	1	EMMT	2	3	4	1
Jamison	3	Knee Heating	4	3	1	2
Jaden Frazier	3	Knee Heating	2	3	4	1
Natalia Robles	3	Knee Heating	3	2	1	4
Troy Uysal	4	Sperm Separation	1	3	4	2
Evan Yap	4	Sperm Separation	2	1	3	4
Courtnee	5	PEFAM	1	2	4	3
Tyler Arias	5	PEFAM	4	1	3	2
Cami Dozois	5	PEFAM	1	3	4	2
Jaylynn Markey	6	S4	1	2	3	4
Marissa Martinez	6	S4	3	2	1	4
Joseph Gruchacz	6	S4	2	4	1	3
Gabriel	7	Resistance Training	1	4	3	2
Abby Youngblood	7	Resistance Training	4	2	1	3
Michael Hansen	7	Resistance Training	2	4	3	1
Spencer Ross	8	Thigh Support	1	2	4	3
Yael	8	Thigh Support	4	2	3	1
Randy Hau	8	Thigh Support	2	4	1	3

In Figure 3 below, the output from the regression statistical analysis is shown.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.18687064
R Square	0.03492063
Adjusted R S	-0.0012698
Standard Erro	1.12546287
Observations	84

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	3.66666667	1.22222222	0.96491228	0.41352046
Residual	80	101.333333	1.26666667		
Total	83	105			

	Coefficients	*Standard Erro*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.57142857	0.64978629	2.41837754	0.01786148	0.27831264	2.8645445	0.27831264	2.8645445
Flask Organiz	0.0952381	0.24559613	0.38778337	0.69920624	-0.3935138	0.58398998	-0.3935138	0.58398998
Size	0.14285714	0.24559613	0.58167505	0.56242108	-0.3458947	0.63160902	-0.3458947	0.63160902
O2 Concentra	0.38095238	0.24559613	1.55113347	0.12481695	-0.1077995	0.86970426	-0.1077995	0.86970426

Figure 3. Regression output from Microsoft Excel.

From the data shown in Figure 3, it can be seen that none of the factors have a p-value below 0.05, with the lowest value being 0.125, well above the threshold for significance. This suggests that the sample size was not large enough or that the factors chosen were of equal importance to the survey takers.

The "percent contribution" was found by dividing each coefficient by the sum total of the three coefficients. Although none of the factors were statistically significant some information can still be derived. The results of the contributions were flask organization with 15%, size with 23%, and O_2 concentration with 62%. In conclusion, the focus going forward with the design should be on the O_2 concentration handling, while flask organization and size should be considered less important for the conceptual design.

Morphology

Several functions necessary to the incubation chamber were analyzed using the table shown below. Multiple concepts for each function were generated, as seen in Table VII.

Table VII. Morphology table.

Morphology						
Product: Hypoxic Incubation Chamber		Organization Name: California Polytechnic University, San Luis Obispo (Cal Poly)				
Function	Concept 1	Concept 2	Concept 3	Concept 4	Concept 5	Concept 6
O_2 Sensor	Digital sensor inside of chamber	Analog sensor inside of chamber	Digital sensor interfacing with chamber	Analog sensor interfacing with chamber	Digital sensor in separate chamber connected to main	Analog sensor in separate chamber connected to main
Hold Flasks	Fits T-75 flasks side-by-side	Stacked T-75 flasks	Fits T-75 flasks stacked and side-by-side	Flasks sit in normal incubator	Cylindrical incubation chamber	
Modify O2 Level	Arduino-controlled valves	In-house built circuit-controlled valves	O2 buffer reaction in chamber	Not modify, rely on chamber seal		
Closing/ Locking Mechanism	Hinged door with latches	Removable door with latches	Ring latch (like with STEMCELL)			
Sealing Mechanism	Silicone ring on both interfacing pieces	Pressure fit the plastic pieces	Silicone ring on one interfacing piece			
Setting O2 Concentration	Gas chamber with N2 and CO2	Gas chamber with N2, CO2, and O2	O2 consuming reaction			
User Interface for Modification	Display and modify on chamber	Display and modify on computer	Display and modify outside chamber (not on computer)	Display on chamber, modify outside (not on computer)	Display on chamber, modify outside (on computer)	
Team member: Henry Elsner	Team member: Andrea Skattenborg	Prepared by: Makenzie J.				
Team member: Makenzie Jones	Team member: -	Checked by: Andrea Skattenborg	Approved by: Henry Elsner			
The Mechanical Design Process David G. Ullman Copyright 2008, McGraw Hill			Designed by Professor Form # 15.0			

From the morphology table, Table VII, three concepts were generated. The first concept, shown in Figure 4 below, was based on the idea of keeping as many electronics as possible outside of the chamber. It uses Bluetooth to communicate readings and instructions between the sensors on the chamber and the computer which runs the code. Sensors are on a removable insert to allow for ease of repair/replacement. This concept allows for continuous monitoring of gas concentrations since the sensors are continuously transmitting over Bluetooth. The front side of the chamber is removable with handles that can also be used to pull the chamber out of the incubator. Latches keep the front door secured to body of the chamber. Gaskets are placed on the chamber and the front door for an airtight seal. Gas supply lines are on the side of the chamber for ease of connection without being in the way of the removable door.

Table VIII. Concept 1 functions.

Function	Concept
O_2 Sensor	Digital display inside chamber
Hold Flasks	Stacked and side-by-side
Modify O2 Level	Arduino-controlled valves
Closing/Locking Mechanism	Removable door
Sealing mechanism	Seal on both interfaces
Setting O2 Concentration	Gas with N2, CO2, and O2
User Interface for Modification	Display and modify on computer

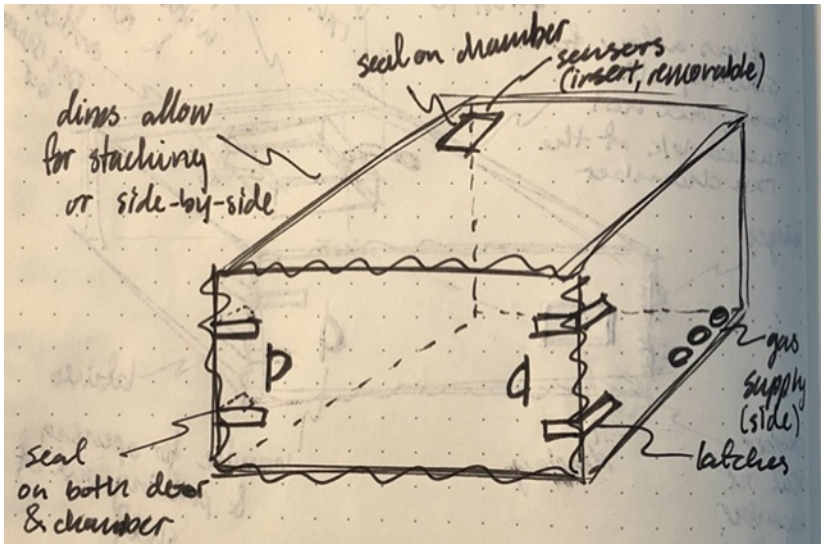


Figure 4. Concept 1 Sketch.

The second concept, shown in Figure 5, has a removable door with a silicone ring and clamps for an airtight seal. The sensor and display are on the side of the chamber with battery

access as well. These will be made using an Arduino kit. There are four holes in the chamber: one each for N_2, CO_2, O_2 and an outlet. It can fit flasks side-by-side, stacked, or both.

Table IX. Concept 2 functions.

Function	Concept
O_2 Sensor	Digital display inside chamber with accessible battery port
Hold Flasks	Stacked and/or side-by-side
Modify O2 Level	Arduino-controlled valves
Closing/Locking Mechanism	Removable door with clasps
Sealing mechanism	Seal on door
Setting O2 Concentration	Gas with N2, CO2, and O2
User Interface for Modification	Display and modify on chamber

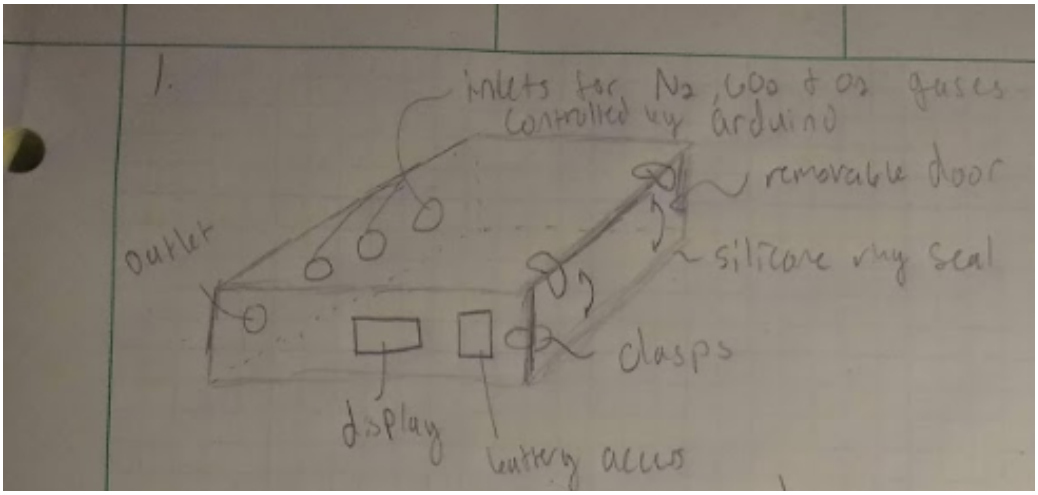


Figure 5. Sketch of concept 2.

The third concept, shown in Figure 6, uses a digital sensor that interfaces with the wall of the chamber to measure the O2 level. The chamber fits stacked T-75 flasks with a latched, hinged door that seals down onto a silicone lining on one of the interfacing pieces. The O2 level is initially set by introducing a reagent that begins an oxygen-consuming reaction and maintained with an oxygen buffer in a liquid media beneath the chamber. The display is housed on top of the chamber and is connected to the Arduino that utilizes the O2 sensor.

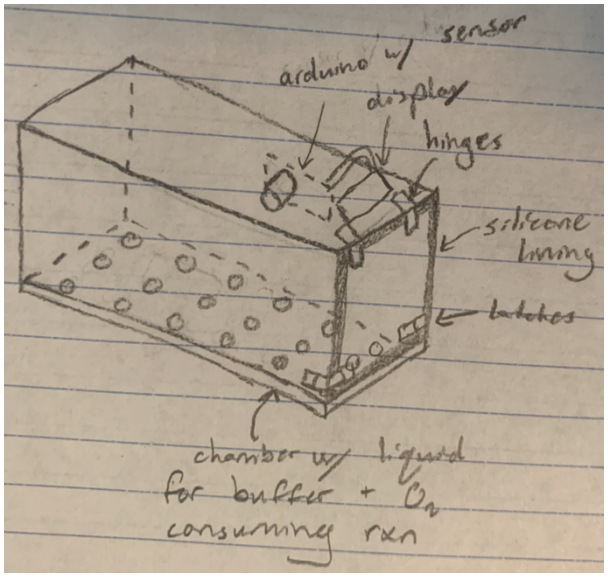


Figure 6. Sketch of concept 3.

Table X. Concept 3 functions.

Function	Concept
O_2 Sensor	Interfaces with chamber
Hold Flasks	Stacked
Modify O2 Level	Buffer in liquid
Closing/Locking Mechanism	Removable door
Sealing mechanism	Seal on one interface
Setting O2 Concentration	$CoCl_2$ in liquid
User Interface for Modification	Display and modify on chamber

Concept Evaluation

Concept 1 from the morphology was the only concept from that evaluation to be assessed. Concept 2 was modified to be a circular chamber for better airflow. Concept 3 was omitted entirely after learning that reaction-based control of O_2 would not be possible. The initial idea relied on $CoCl_2$ to participate in an oxygen consuming reaction. However, it was later discovered that $CoCl_2$ does not induce hypoxia in cells by reducing the ambient O_2 level. Instead, it simply acts as a stimulus for the Hypoxia Inducible Factor – 1 (HIF-1) pathway that, under physiological conditions, is the cell's response to hypoxia. Instead, an inflatable chamber was examined.

The first concept used for this analysis is centered around the chamber being inflatable. It would be constructed out of two sheets of plastic sealed together on three sides with a Ziplock-

type opening on the fourth side. The chamber would have an inlet tube and an off gassing one-way valve that opens into the incubator. Before inflating the chamber, it would be vacuumed to remove as much air as possible. The electronics would all be housed in a self-contained sub-chamber. Sensors would poke through a hole in the electronics sub-chamber wall to measure oxygen and carbon dioxide while the rest of the electronics would remain sealed off in the separate sub-chamber. The electronics would interface with a computer through a cable only while the incubator door is open. In Figure 7. below, an example of an inflatable chamber from Wang et al. is shown (Wang et al.). The electronics chamber would be placed inside the bag with the culture flasks.

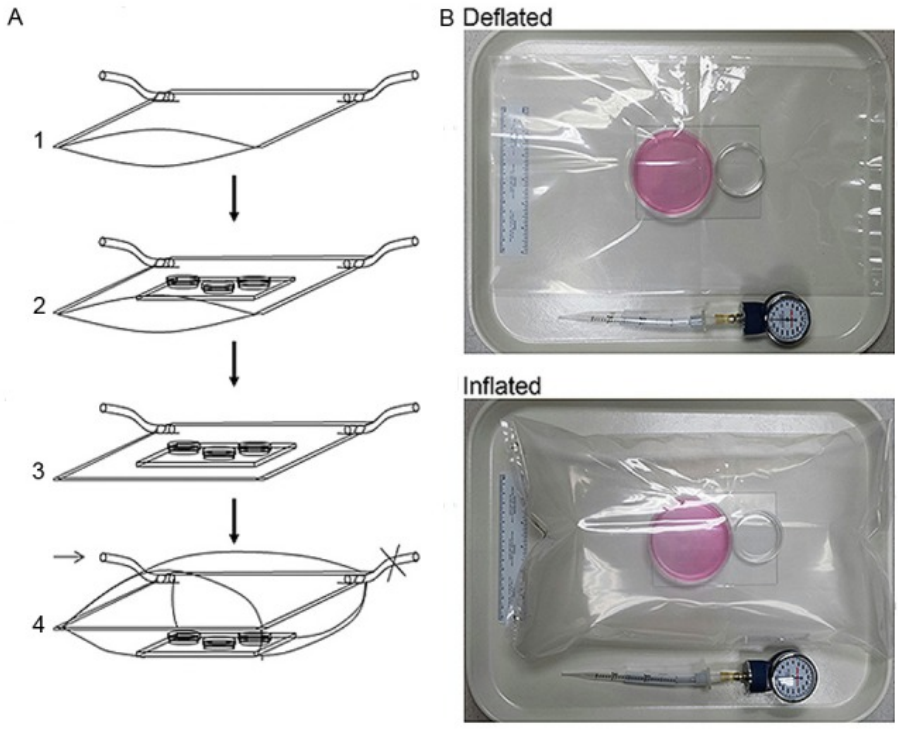


Figure 7. Illustrative picture of concept 1 without electronic chamber (Wang et al.).

The second concept is a rigid, rectangular chamber. This chamber would have one inlet and one one-way valve outlet. To access the interior, there would be a removable door on the side or front of the chamber with pressure latches and a silicone ring for an airtight seal. Oxygen and carbon dioxide sensors would be placed inside the chamber and would be connected to a wire which passes out of the incubator. The wire would directly connect to a circuit and then to a computer where oxygen and carbon dioxide levels could be monitored and changed. This chamber would be sterilizable by autoclave. This concept is illustrated in Figure 8. below.

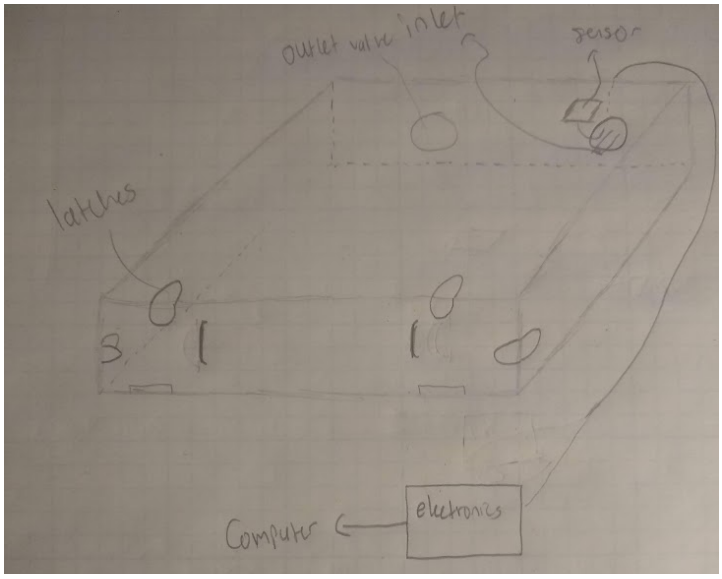


Figure 8. Concept 2 sketch.

The third concept is based on a cylindrical form factor for potentially better air circulation compared to concept 2. The cylindrical chamber would have one inlet for the desired gas mixture coming from outside the incubator and one one-way valve outlet from the chamber to the incubator for off gassing. The top of the chamber would function as a removable lid for access to the chamber. The lid would be secured with latches. A smaller chamber containing all the electronics with oxygen and carbon dioxide sensors sticking out from the bottom would be placed on top of an opening in the lid and secured tightly to the main chamber. Access to interfacing with the electronics would be limited to when the chamber is open, and a USB connection can be made directly from the electronics chamber to a computer. Concept 3 is illustrated in Figure 9. with the cylindrical chamber and the electronics chamber drawn separately and not to scale.

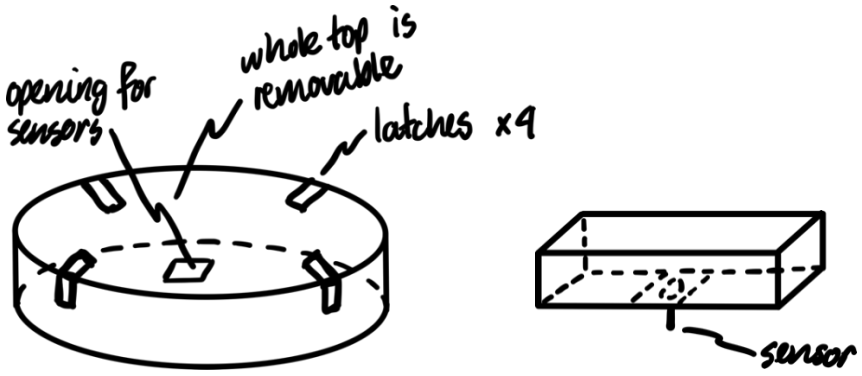


Figure 9. Concept 3 sketch.

These concepts were compared in a Pugh chart based off their durability, the ability to achieve a specified O2 concentration, its ease of use, the simplicity of data gathering, its space efficiency, its manufacturability, and how easy it would be to sterilize.

Issue		Concept 1	Concept 2	Concept 3
Durability	17	Datum	1	1
Achieve [O2]	26		-1	-1
Ease of Use	12		1	1
Data Gathering	3		1	0
Space Efficiency	8		0	-1
Manufacturability	8		-1	-1
Sterilizable	26		1	1
		Total	2	0
		Weighted Total	24	13

Figure 10. Pugh chart with concept 1 as datum, scoring identical for all team members.

Issue		Concept 2	Concept 1	Concept 3
Durability	17		-1	0
Achieve [O2]	26		1	1
Ease of Use	12		-1	-1
Data Gathering	3	**Datum**	-1	-1
Space Efficiency	8		0	-1
Manufacturability	8		1	-1
Sterilizable	26		-1	0
		Total	-2	-3
		Weighted Total	-24	-5

Figure 11. Pugh chart with concept 2 as datum, scoring identical for all team members.

Issue		Concept 3	Concept 1	Concept 2
Durability	17		-1	0
Achieve [O2]	26		1	-1
Ease of Use	12		-1	1
Data Gathering	3	**Datum**	0	1
Space Efficiency	8		1	1
Manufacturability	8		1	1
Sterilizable	26		-1	0
	Total		0	3
	Weighted Total		-13	5

Figure 12. Pugh chart with concept 3 as datum, scoring identical for all team members.

Concept 2 was chosen as the front runner concept after analyzing Pugh charts of the concepts compared to each other. All three team members filled out Pugh charts separately and then came together to discuss the differences in scoring. All differences were discussed, and a final score was agreed on. The three final Pugh charts used are shown in this document as Figures 10, 11, and 12. In Figure 10, it can be seen that both concept 2 and 3 scored higher than concept 1 as seen by the weighted total scores. From Figure 11, using the same score, it can be seen that both concepts 1 and 3 scored lower than concept 2. In Figure 12, concept 1 scored below and concept 2 scored above concept 3. Concept 2 is more durable than concept 1 because of its inflexible material. One concern with concept 1 was susceptibility to puncture and thus the durability of the chamber itself. Concept 2 will likely have the worst air flow and thus the least ability to achieve the desired oxygen concentration due to the sharp corners, but analysis of both the rigid chambers will be performed in COMSOL. Concepts 2 and 3 would be easier to use than concept 1 due to the rigidity of the chambers. Chamber 2 was determined to have a better ease of use than concept 3 due to not having to open the incubator in order to interact with the electronics. The electronics configuration of concept 2 is the only configuration that would allow for constant, real time monitoring of oxygen and carbon dioxide levels. Concept 2 is more space efficient than concept 3 because the T-75 flasks that the chamber will contain are rectangular which would result in substantial wasted space in a cylindrical chamber. Concept 1 would have wasted space simply because it is inflated, but it would also be able to conform to the space that it is in better, essentially equalizing its space efficiency with concept 2. Concept 1 would easier to manufacture, especially considering the concept is illustrated and explained in Wang et al. The curved geometry of concept 3 would be quite difficult to manufacture, making concept 2 a middle ground in manufacturability. Concept 2 and 3 can be autoclaved, making sterilization quite simple. Concept 1, however, cannot be autoclaved and would have to be sterilized using

alcohol. The ability to achieve the desired oxygen concentration and sterilizability are the most important characteristics of the chamber. Concept 2 and 3 were quite close, but concept 2 overtook concept 3 in the Ease of Use, Data Gathering, Space Efficiency, and Manufacturability categories.

Conceptual Model

The frontrunner model from a Pugh chart analysis was further studied in this conceptual model analysis. This concept consisted of a boxed design which could fit four T-75 flasks, two stacks of two flasks. A CAD model was drawn up in Fusion 360 to get a perspective on the size of the chamber. This model is shown in Figures 13. through 15. below. This model only includes components that would affect the internal size of the chamber. The components used in the model are only representative components of potential fittings and valves and the sensor insert is a simplified model of the potential electrical chamber component.

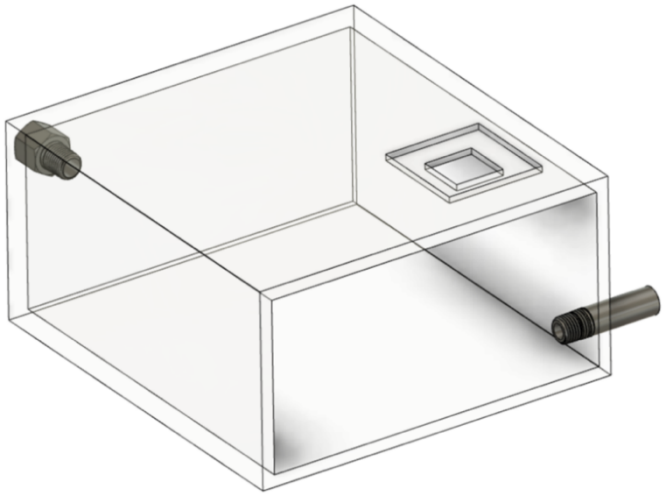


Figure 13. Hypoxic incubation chamber CAD model without front door and sensor insert.

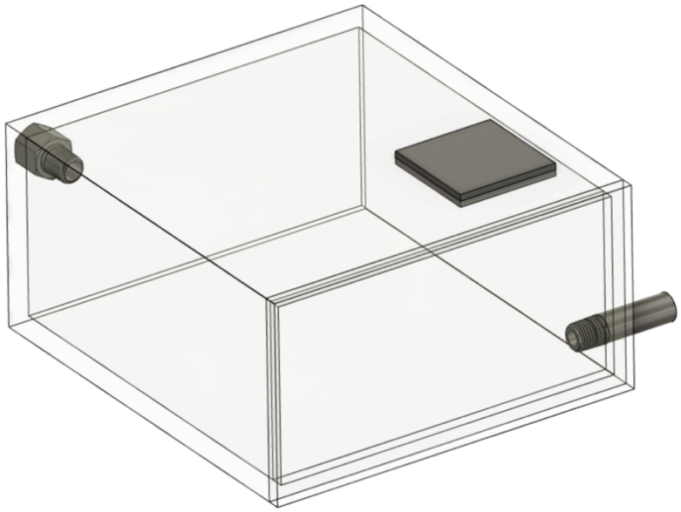


Figure 14. Hypoxic incubation chamber CAD model with front door and sensor insert.

Figure 15. below, shows that four T-75 flasks can fit inside the chamber which has dimensions of 7.5 x 8 x 4 inches externally with all walls having a wall thickness of 0.25 inches. There is little room to spare with four flasks in the chamber but depending on the method use to induce hypoxia in the chamber, it will not have a significant effect on the process.

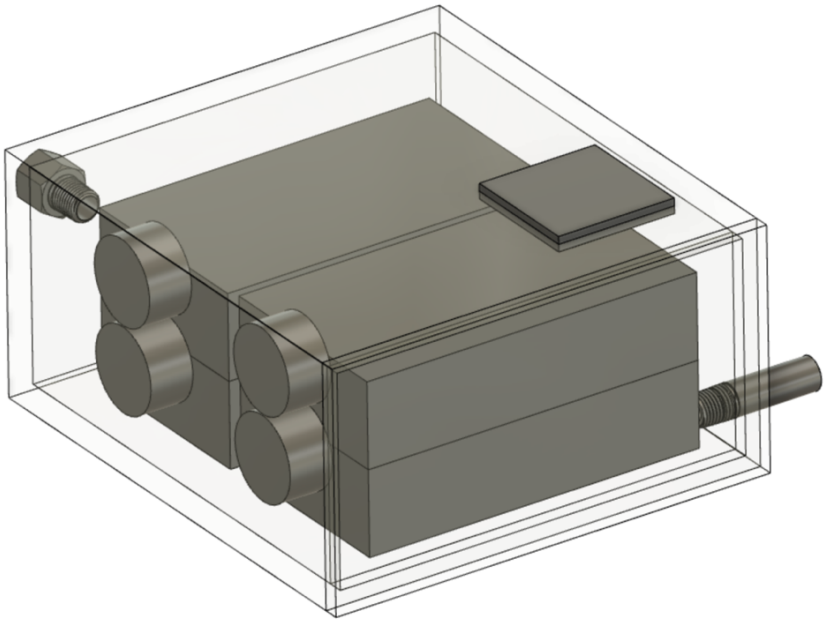


Figure 15. Hypoxic incubation chamber CAD model with four simplified T-75 flask models.

A COMSOL simulation was used to obtain information about the velocity field inside the conceptual model and thus the distribution of gas in the chamber. The parameters provided in Table XI. were used in the simulation along with the dimensions found from the CAD model. The parameters were approximated and will be refined at a later point to exhibit a more accurate simulation output. A time-dependent study can also be performed at a later point and the inlet and outlet can be moved around to determine if their positions change the air distribution significantly. A slice plot of the velocity magnitude from the COMSOL simulation is shown below in Figure 16.

Table XI. Parameters used for the COMSOL simulation.

Parameter	Value
Density	$1.205\ kg/m^3$
Viscosity	$1.825 \cdot 10^{-5}\ Pa \cdot s$
Inlet/Outlet diameter	$0.5\ in$
Inlet velocity	$8\ m/s$

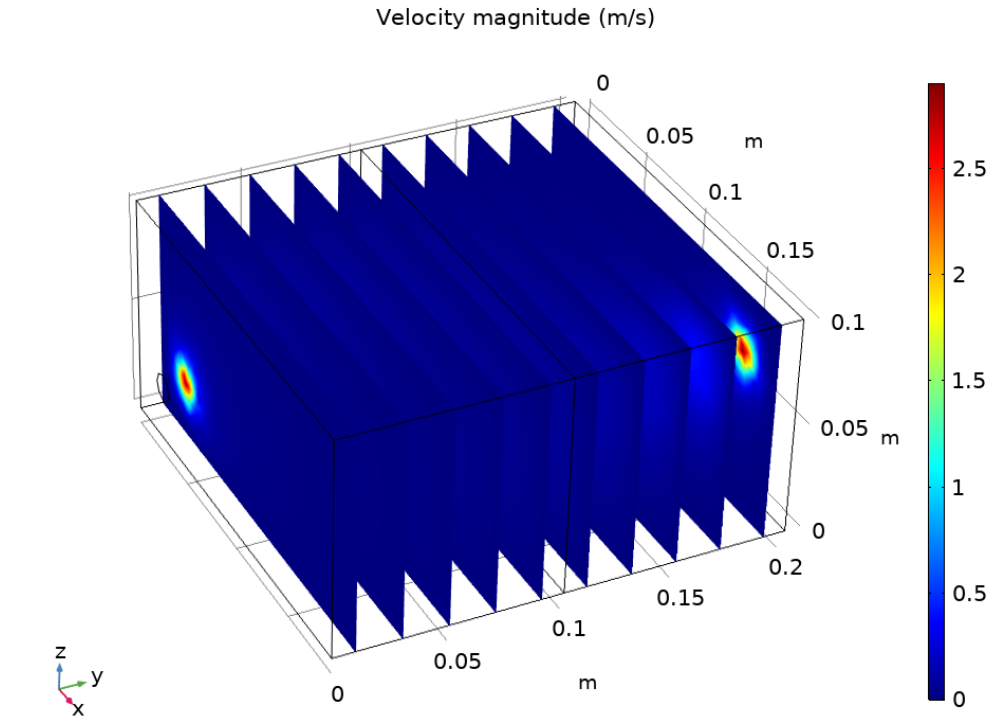


Figure 16. Velocity magnitude output from a COMSOL study.

The geometric model showed that previously, there was not enough room in the chamber to fit 4 flasks. The inlet port, CO2 sensor, and O2 sensor would all collide with T-75 flasks inside the chamber. In the last iteration of the model, the dimensions were sufficiently large to accommodate four flasks. A different route would be to make a chamber to fit only two stacked flasks. Applying what we learned from this geometric model, the two-chamber flask would have to have larger dimensions than previously thought to make space for the protrusions into the chamber. Alternatively, we can test different inlet/outlet/sensor configurations and placements to try to optimize spatial efficiency and keep the chamber dimensions the same.

The COMSOL model illustrated the need for increased air circulation. The incoming gas does not circulate around the chamber sufficiently. Especially considering the scenario in which the chamber is full of T-75 flasks which will hinder air movement further, we need to make sure that the hypoxic conditions are well dispersed. One possibility to alleviate this concern is the increase the inlet velocity. The cells are significantly protected from perturbation by the flask walls and will therefore not be negatively affected by the increase in velocity. Another option that can be used in conjunction with the first is to test out different inlet/outlet locations to determine which location pairing leads to the greatest amount of air movement. Finally, a fan could be installed to circulate the air as it is entering and for a set amount of time after flushing the chamber.

Detailed Design

The detailed design is based entirely on the conceptual design, with only a few modifications. The first and most obvious change to the eye is the size of the chamber itself. Initially, the conceptual design had enough space for four T-75 flasks, but that has been changed to a holding capacity of two flasks. This change was made mostly based on price concerns as the material for the chamber itself is a major factor of the budget. The sensor insert described for the conceptual design remains the same in function, but the actual sensor plate is not included in the detailed design. The oxygen sensor will be secured at the top of the chamber using a gasket and a pressure fit. The electrical wires required for the sensor will run directly from the sensor to the Arduino outside of the chamber. Heat shrink tubing will be used to cover the connectors on the sensor from any humidity or other undesirable conditions.

The final design consists of three main parts, the chamber, the electronics, and the gas cylinders and tubing. Pictures of the design can be found as Figure 17, below. Dimensioned drawings of the chamber body and door are provided in Attachment D. The full Bill of Materials (BOM) which lays out all the components needed for the design is provided in the subsection below.

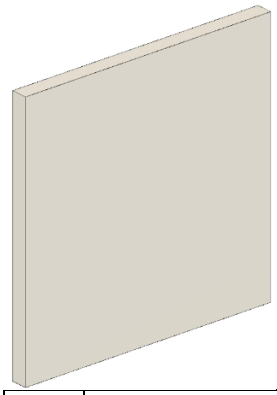
Figure 17. Pictures of the final design.

Bill of Materials

The BOM is split into four sections determined by the larger parts of the design; Chamber, Electrical, Gas/Tubing, and Sterilization/Cleaning. The BOMs are provided as Tables XII through XV, below. The detailed BOMs are provided as Attachment E.

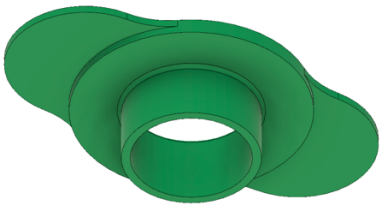
Table XII. Pictures of Chamber Bill of Materials Items (Item #9 Epoxy, not shown).

# 1	Chamber Sides

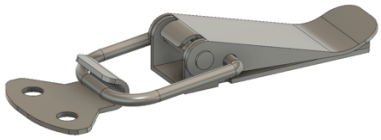


# 2	Chamber Back/Door (x2)

# 3	Check Valve



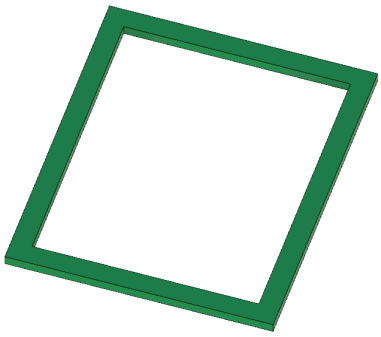
# 4	Sensor Gasket



# 5	Latch (x3)

# 6	M4 Screw (x14)

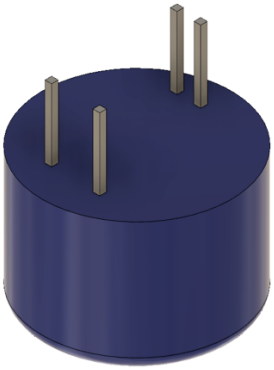
# 7	Handle



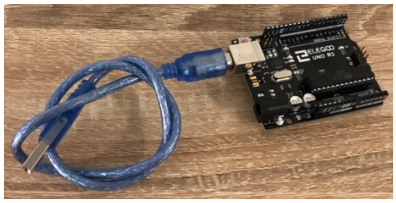
# 8	Door Gasket

# 10	Neoprene

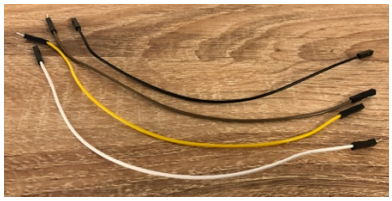
Table XIII. Pictures of Electrical Bill of Materials Items.



# 11	Oxygen Sensor



# 12	Uno R3/USB Cable



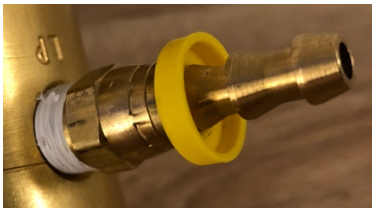
# 13	Wires

Table XIV. Pictures of Gas/Tubing Bill of Materials Items (Item #14/15 Gas Cylinders, not shown).

# 16	O2 Regulator

# 17	N2/CO2 Regulator

# 18	O2 Regulator Tube Fitting

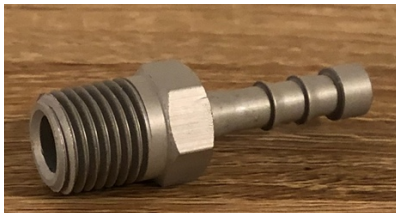


# 19	N2/CO2 Regulator Tube Fitting

# 20	N2/CO2 Regulator Gas Cylinder Fitting

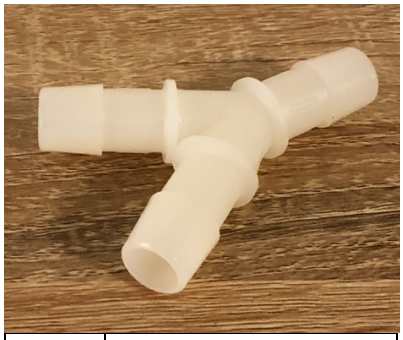
# 21	Needle Valve (x2)

Table XIV. Pictures of Gas/Tubing Bill of Materials Items (Item #14/15 Gas Cylinders, not shown) cont.

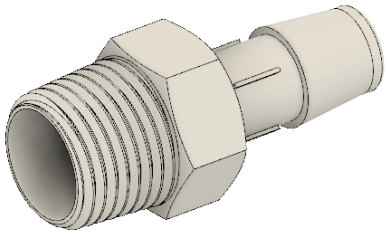


# 22	Valve Fitting (x4)

# 23	Reducer (x2)



# 24	Wye Fitting



# 25	Inlet Fitting

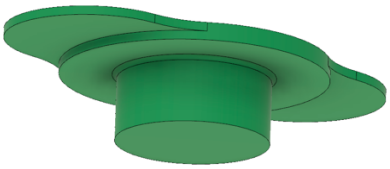
# 26	1/2” Tubing (10ft)

# 27	1/4” Tubing (25 ft)

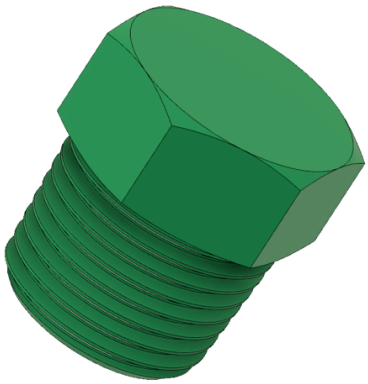
# 28	Teflon Tape

# 29	Tube Clamp (x4)

Table XV. Pictures of Sterilization/Cleaning Bill of Materials Items.



# 30	Sensor Hole Stopper



# 31	Chamber Inlet Stopper

# 32	Inlet Fitting Stopper

# 33	Neoprene Bath

Manufacturing Process Instructions

Detailed Manufacturing Process Instructions (MPI) for the chamber and chamber door are provided in Table XVI, below. All other parts of project do not require manufacturing, only assembly. Detailed instruction on how to assemble all components can be found in the Operation Manual which is provided as an attachment to this document. The code used to run the oxygen sensor can be found in Attachment F.

Table XVI. MPI for Chamber.

Step #	Item #	Name	Tools	Description
1	1	Chamber Sides	Table Saw	Cut into two 6” x 8” and two 5” x 8” pieces
2	2	Chamber Back/Door	Table Saw	Cut each sheet into a 5” x 6” piece
3	1	Chamber Sides	Table Saw	For each piece from 1, miter the two long sides and one short to 45°
4	2	Chamber Back/Door	Table Saw	For one of the pieces from 2, miter all sides to 45°
5	2	Chamber Back/Door	Mill	For the other piece from 2, mill a little over the thickness of the material on all sides to a thickness of the material
6	2	Chamber Back/Door	Drill Press, Drill Bit, Tap, Tap Handle, Clamp	Piece from 4, drill a hole appropriate for tapping 1/2“ – 14 NTP threads centered and about 1.5” from one bottom (5” side), tap 1/2“ – 14 NTP threads from the side without miters
7	1	Chamber Sides	Drill Press, Drill Bit, Clamp	For one of the 5” x 8” pieces from 3, drill a hole at about 0.1” larger than the diameter of the oxygen sensor about 1.5” from one of the corners with miters all around
8	3	Check Valve	File, Saw/Knife	File off the threads on the inlet stem and cut off the outlet stem
9	1	Chamber Sides	Drill Press, Drill Bit, Clamp	For one of the 6” x 8” pieces from 3, with the short side miter on the right side and the miters facing down, drill a hole as close to the diameter of the check valve inlet stem (but not smaller) as possible in the top left corner, about 1.5” in from both sides
10	2 6 7	Chamber Back/Door M4 Screw Handle	Drill Press, M4 Tap Set, Clamp, Screwdriver	For the piece from 5, drill and tap two M4 sized holes to a depth of 4 mm in line (parallel to 5” sides) at the vertical center (3” from top/bottom), distance apart determined by handle geometry, attach handle using M4 screws
11	1 3 9	Chamber Sides Check Valve Epoxy	-	For piece from 9, epoxy the check valve to the chamber side
12	1 2 9	Chamber Sides Chamber Body/Door Epoxy	-	Epoxy all mitered pieces together starting with the long sides, then epoxy the back to the sides (with tapped hole towards bottom)
13	1 2 5 6 8 10	Chamber Sides Chamber Body/Door Latch M4 Screw Door Gasket Neoprene	Drill Press, M4 Tap Set, Clamp, Screwdriver	Assemble the door with door gasket and neoprene, slot into the chamber inlet, line up and draw out screw hole locations for all three latches (top – centered, sides – 2” from bottom), drill all 12 (6 for latch plates, 6 for latch body) M4 holes as described previously, attach latch plates and bodies

Device History Record

Table XVII. Device History Record.

MPI Step(s)	Deviations from MPI	Completed By	Signature	Date
1 2 3 4 8	-	Andrea Skattenborg	A.S.	01/19/21
6 7 9	-	Andrea Skattenborg	A.S.	01/21/21
11 12	-	Andrea Skattenborg	A.S.	01/22/21
5	Used table saw with dado stack instead of mill	Andrea Skattenborg	A.S.	01/25/21 01/26/21 01/30/21
10	-	Andrea Skattenborg	A.S.	02/09/21
13	-	Andrea Skattenborg	A.S.	02/23/21

Test Plan

Oxygen Sensor in Ambient Oxygen Test

It is essential to ensure the oxygen sensor works in room air before installing it in the hypoxic chamber. This is to test the capabilities of the sensor and verify the circuit and script work in a relatively known concentration of oxygen. The testing protocol is rather simple, just place the sensor in a room and run the script to measure the oxygen concentration. The ambient concentration of oxygen in completely dry air is about 21%. However, with humidity, this value can change rather dramatically.

1. Plug the oxygen sensor into the Arduino
2. Plug the Arduino into the computer and open the Arduino IDE
3. Bring the oxygen sensor set up either outside or into a ventilated room
4. Run the oxygen sensor code and open the Serial Monitor
5. Wait 5 minutes, then record the current oxygen reading
6. Take at least 4 more measurements at least 5 minutes apart

No special facilities are required to run this test and the materials required are the constructed circuit for the oxygen sensor and a computer. There is also no training or certification involved to run this test. Every value should lie between 20 and 21 percent. The sensor passes the test if every value is between 20 and 21 percent. If the oxygen sensor does not

pass the test, the environment of the test will be changed to prevent stagnant air from affecting the results. If the sensor again fails, the manufacturers will be contacted. This test relates to engineering spec 6.

Oxygen Sensor in Hypoxic Environment Test

The oxygen sensor will then need to be tested in hypoxic conditions. The goal of this next test is to verify the sensor's accuracy in very low oxygen conditions.

1. Connect the chamber to the mixed gas line. The oxygen gas line can be connected as well, but only the mixed gas is required
2. Fully flush the chamber with mixed gas by opening the needle valve in the mixed gas line
3. Close the needle valve and allow the sensor reading to stabilize
4. Repeatedly flush the chamber up to 5 times
5. If the oxygen level is read as below 0.5% within 5 flushes, the test is considered a success.

This test requires use of a facility that can properly store gas cylinders (likely Dr. Heylman's lab). It follows that before testing, proper use of gas cylinders should be reviewed for safety. This test is measured on a simple pass or fail scale. If the oxygen reading does not drop below 0.5%, the test will be redone using a CO2 sensor to determine if the problem is the flushing technique or the sensor itself. This test relates to engineering spec 5 and 6.

Reach Desired Oxygen Level Test

Possibly the most important and most challenging specification to meet is achieving an O2 level within 0.1% of the desired level. To test this, we will conduct a full test of the system.

1. The entire project must be assembled from the gas cylinders to the oxygen sensor. Both gas tanks will be used and attached in this step
2. Flush the chamber with mixed gas by partially opening the needle valve on the mixed gas line. Close it after 5 seconds
3. If the sensor reading stabilizes below the desired oxygen level, move one. If not, repeat the flushing procedure
4. Slightly and briefly open the needle valve in the oxygen gas line
5. After closing the valve, give the oxygen sensor time to reflect the change in oxygen level
6. Repeat steps 4 and 5 until the oxygen reading matches the desired oxygen level

To perform this test, the chamber needs to be built and fully functional. Facilities that have proper storage for gas cylinders are required, and knowledge of how to connect, disconnect, and safely use gas cylinders is required as well. The test relates to engineering specs 4 and 5.

Simple Maintain Oxygen Level Test

An integral part of the chamber is holding its oxygen concentration for a prolonged period of time. The specifications dictate that the chamber should hold a given oxygen level to within 0.1% for 48 hours. A simple test to determine the chamber's ability to hold a given oxygen level can be conducted without the gas cylinders or relevant tubing.

1. Close the chamber door and insert the oxygen sensor into the relevant hole with the sensor gasket
2. Apply electrician's or plumber's tape to the chamber inlet adapter (a few layers) and screw the adapter into the chamber
3. Hook the oxygen sensor up to the Arduino and a computer
4. Open the Arduino IDE and run the oxygen sensor code then open the serial monitor
5. Exhale into the inlet of the chamber inlet adapter for at least a full 60 seconds
6. Immediately upon finished, tape of the chamber inlet adapter opening to seal it
7. Wait a couple of minutes to allow the sensor reading to stabilize
8. Once the reading stabilizes, record the oxygen level and record what time the reading was taken
9. Continue to take measurements and record the time after the initial measurement until the measurements fall out of spec

Once the reading is no longer within 0.1% of the initial value, the test can be halted. Modifications to the door seal, the chamber inlet adapter seal, the oxygen sensor and gasket seal, and the chamber itself can be made to lengthen the time that the chamber remains within spec. This test relates to engineering specs 3 and 5.

CO_2 is important in maintaining the pH of the culture. Verification is needed that the CO_2 concentration will not need to be monitored or actively changed during culturing. This test requires that the hypoxic chamber be fully set up and culture samples in T-75 flasks for half of the tests, since they would be altering the CO_2 concentration. This also requires an external CO_2 sensor. The test protocol will be similar to the protocol for holding oxygen concentration. The only difference will be that instead of using the oxygen sensor, a difference gasket will be used to interface with the CO_2 monitor. Again, a facility that can properly store gas cylinders is required and the person testing should be familiar with proper handling of gas cylinders. A one-sample T-test will be used to analyze the data. This test relates to engineering spec 11.

Testing Data and Analysis

Due to time constraints, the only tests that we were able to collect data for were the Oxygen Sensor in Ambient Oxygen and the Simple Maintain Oxygen Level tests. While other

tests were conducted like a soapy water test for leaks, these tests did not provide quantifiable or meaningfully qualitative data.

The Oxygen Sensor in Ambient Oxygen test was used early in the testing process to verify our sensor. Initial readings were outside of the range of acceptable ambient oxygen levels, reaching as low as 19.4% for ambient oxygen concentration. This value was not significantly changed by conducting the test outside. Further research was conducted to determine what the oxygen sensor "should" read in our environment and the manufacturer was contact. With help from the manufacturer, we decided that sensor readings between 19 and 20 percent were still within spec due to the humidity of the air. The manufacturer guarantees an accuracy of 2% of full scale, which reduces to 0.5%. Humidity can reduce the relative concentration of oxygen in air and reduce our reading to the levels seen.

The entirety of the rest of the testing was focused on the chamber's ability to maintain a given oxygen concentration. Before the gas cylinders were accessible, testing of this capability was conducted through the Simple Maintain Oxygen Level test. The protocol for this test is laid out above. Before conducting any testing, our group thought the seal would perform much better than initial testing indicated. We expected the chamber to be able to hold the initial oxygen concentration for at least 24 hours. As shown in Table XVIII below, that was not the case. The first tests using the silicone gasket proved that the chamber was very leaky. Later iterations used a 3D printed gasket instead of the purchased silicone gasket. The 3D printed gasket was more deformable and produced a better seal. Possibly the most significant difference between the early and later tests was attaching the latches more firmly to the chamber. By screwing the latches into the plastic instead of gluing them on with epoxy, the latches were able to apply much more pressure to the door-chamber body interface. Furthermore, we started using a thin (1/16 inch) layer of neoprene between the 3D printed gasket and the chamber body to improve the seal and that appeared to work as well.

Table XVIII. Data from the Simple Modified Maintain Oxygen Concentration. The Settings column is in the form "door gasket used, sensor gasket used". The Slope column describes the change in oxygen percentage per minute and the Time in Spec calculates how long the oxygen concentration remained within 0.1% of the initial value.

Date	Settings	Slope (%/min)	Time in Spec (min)
2/19	Silicone + sensor v1	0.0085	11.8
2/21	silicone + fabric, sensor v1, top latch undone	0.0187	5.4
2/22	silicone + fabric, sensor v1	0.0193	5.2
2/25	3Dv2 + sensor v2	0.0034	29.4
2/25	3Dv1 + neoprene, sensor v2	0.0024	41.7
2/28	3Dv2 + neoprene, sensor v2	0.0025	40.0
3/2	3Dv3, sensor pull tabs	0.0037	27.0
3/4	3Dv2 + 2neoprene, sensor pull tabs	0.0022	45.5
3/4	3Dv3 + sensor pull tabs	0.0061	16.4
3/5	3Dv2 + neoprene, pull tabs	0.0039	25.6
3/5	3Dv1 + 2neoprene, pull tabs	0.0075	13.3
3/6	3Dv3 + neoprene, pull tabs	0.0053	18.9
3/6	3Dv2 + neoprene, pull tabs	0.0055	18.2
3/8	3Dv4 + 2neoprene, pull tabs	0.0029	34.5
3/8	3Dv4 + 2neoprene, pull tabs	0.0042	23.8

No clear conclusions are able to be drawn from this data. It is, however, clear that the neoprene is beneficial in producing a seal. Furthermore, the thickness of the v3 gasket leads to visible bending of the polysulfone. The v4 gasket appears to be thick enough to produce sufficient pressure from the latches while being thin enough to not lead to too dramatic bending in the door. With the current project configuration, the chamber's inability to hold the oxygen concentration in spec for longer than 1 hour is not feasible. A researcher would have to manually open the mixed gas line whenever the chamber fell out of spec. But in later renditions of the chamber, the needle valves would ideally be switched out for electronically controlled solenoid valves. The oxygen concentration could be continually modified by an Arduino that is capable of taking in data from the oxygen sensor and opening the appropriate solenoid valve in either the mixed or oxygen gas lines to correct the oxygen level.

Testing with the gas cylinders was not able to be conducted for several reasons. First, the cylinders were not accessible until about 2 weeks before the project finished. Then, the connection between the mixed gas cylinder and the CO2 regulator was different than initially understood. This problem was rectified quickly. Finally, it was discovered 2 days before the end of the project that the mixed gas cylinder delivered was the wrong combination of N2 and CO2, so we were unable to test using it.

A critical additional test to run is the Reach Desired Oxygen Level Test. Currently, we do not know how accurate the release of gas from the needle valve is and if we will be able to achieve an oxygen concentration within 0.1% of the goal value.

Conclusion

Over the course of 6 months, our group worked to create a chamber that would enable Dr. Heylman's research lab to culture cells in a hypoxic environment. Through a long process of ideation and iteration, our group developed and manufactured the design described in this report. The chamber should theoretically be able to achieve any oxygen concentration because the oxygen and non-oxygen gasses are controlled through different lines, allowing different amounts of each gas into the chamber. When plugged into a computer, the oxygen sensor is able to continuously read the oxygen concentration of the chamber and deliver that reading to the researcher.

Discussion

A critical additional test to run is the Reach Desired Oxygen Level Test. Currently, we do not know how accurate the release of gas from the needle valve is and if we will be able to achieve an oxygen concentration within 0.1% of the goal value.

A lot can and needs to be done to improve this project before it can be used in a research setting. One immediate step to take is thermoregulation of the chamber. Originally, the chamber was designed to be inside of an incubation chamber that would control the temperature surrounding the chamber and therefore hold the inside temperature stable as well. However, Dr. Heylman's incubator was not capable of interfacing with the chamber in the way originally intended, so an external thermoregulation method is required. Furthermore, the chamber has not yet been tested with the gas cylinders. A mistake on the delivery side of the mixed gas cylinder and safety concerns with the oxygen gas cylinder as well as time constraints prevented us from tested our gas line connections. This would have been the next step in our process. Additionally, two rails need to be epoxied onto the floor of the inside of the chamber to allow a shallow pan filled with water to slide between and rest beneath the cell culture flasks. Finally, long term, repeated use of this chamber in experiments will require frequent adjusting of the internal oxygen concentration to keep it from rising more than 0.1% above the initial value. This will require external current and voltage sources, solenoid valves, and many code iterations that were outside the scope of this project.

Looking back on the progress of the project, it would have been helpful to start with a smaller scope, tackling fewer new and complex design regions. Without any Electrical Engineering focused group members, attempting to include a solenoid system that operated on continuous feedback from the oxygen sensor was overzealous. Similarly, because our group came into this project having only low level knowledge of pressure vessels and electronics, it would have been helpful to confer with subject matter experts available to us earlier and more frequently. While we used the knowledge our professors and local experts had during the second part of our project heavily, we did not use them often enough during the design phase of our project. This would have helped steer us in the right direction earlier when changes to the design were easily done and cheap. As the project entered its later phase of testing and iteration,

professors also reminded us to keep our eyes on the big picture. We got lost in the details of figuring out one aspect of the chamber at the expense of a different, more important component. This took precious time from testing the chamber's ability to hold a given oxygen concentration. With a perpetual constraint on time, it is important to always be sure to delegate time according to task priority, being capable of pivoting when necessary.

In summary, this project represents the first several major steps in creating a hypoxic incubation chamber capable of delivering a wide range of oxygen concentrations, something not currently available on the market for less than many thousands of dollars. Significant modifications are required to make this a feasible instrument for research, but the foundation for future work has been laid. It is our hope that this project will be taken up by a future group to see this chamber to completion and enable its use in Dr. Heylman's research lab.

References

Wang, Ruoxiang, et al. “A Novel Experimental Hypoxia Chamber for Cell Culture.” *American Journal of Cancer Research*, vol. 4, no. 1, 2014, pp. 53–60, http://www.ncbi.nlm.nih.gov/pubmed/24482738%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3902232.

Attachments

Attachment A – Full List of Customer Requirements

Attachment B – House of Quality

Attachment C – Table of Links to Patents

Attachment D – Detailed Design Drawings

Attachment E – Detailed BOM

Attachment F – Oxygen Sensor Code

Operation Manual

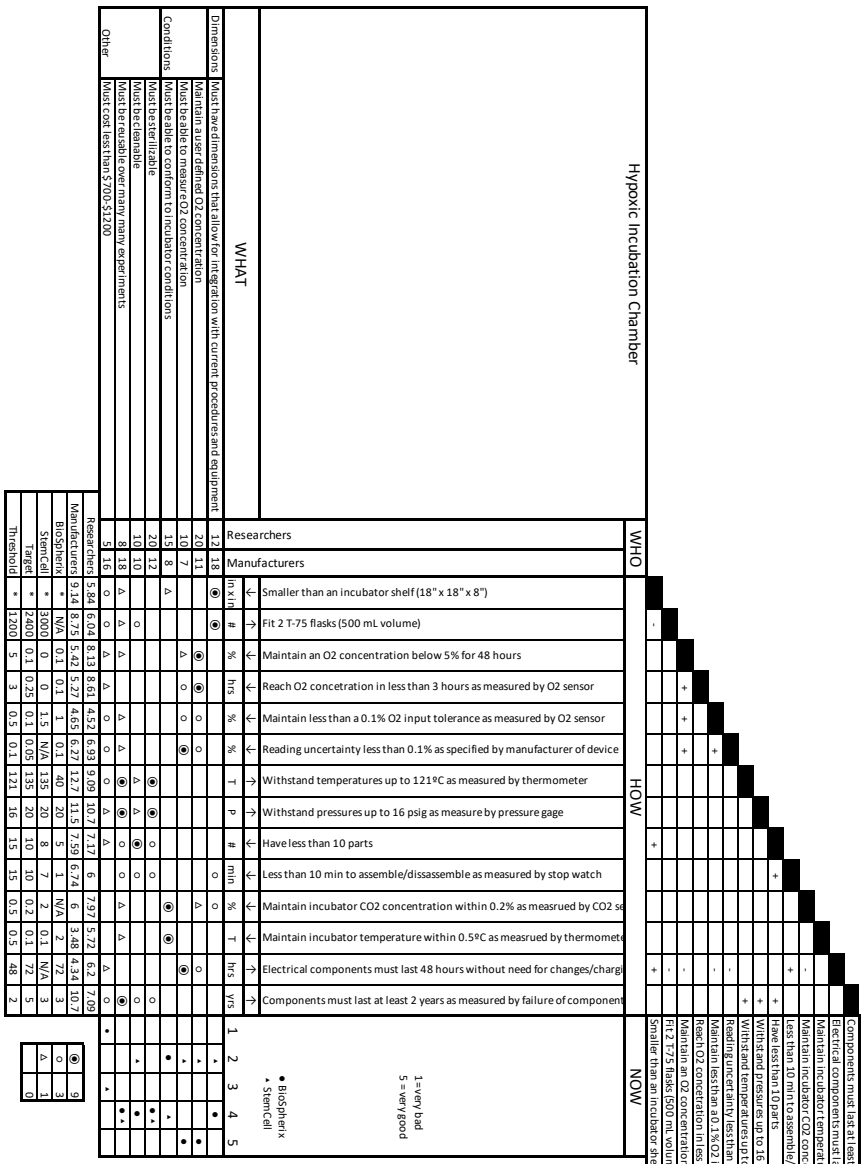
Attachment A – Full List of Customer Requirements

List of customer requirements

- Have dimensions that allow for integration with current procedures and equipment.
- Maintain a user defined O_2 concentration.
- Must be able to measure O_2 concentration.
- Must be sterilizable.
- Must be cleanable.
- Must be able to conform to incubator conditions.
- Must be reusable over many, many experiments.
- Must cost less than $700-1200.

Attachment B – House of Quality

Figure B.1. House of Quality diagram.

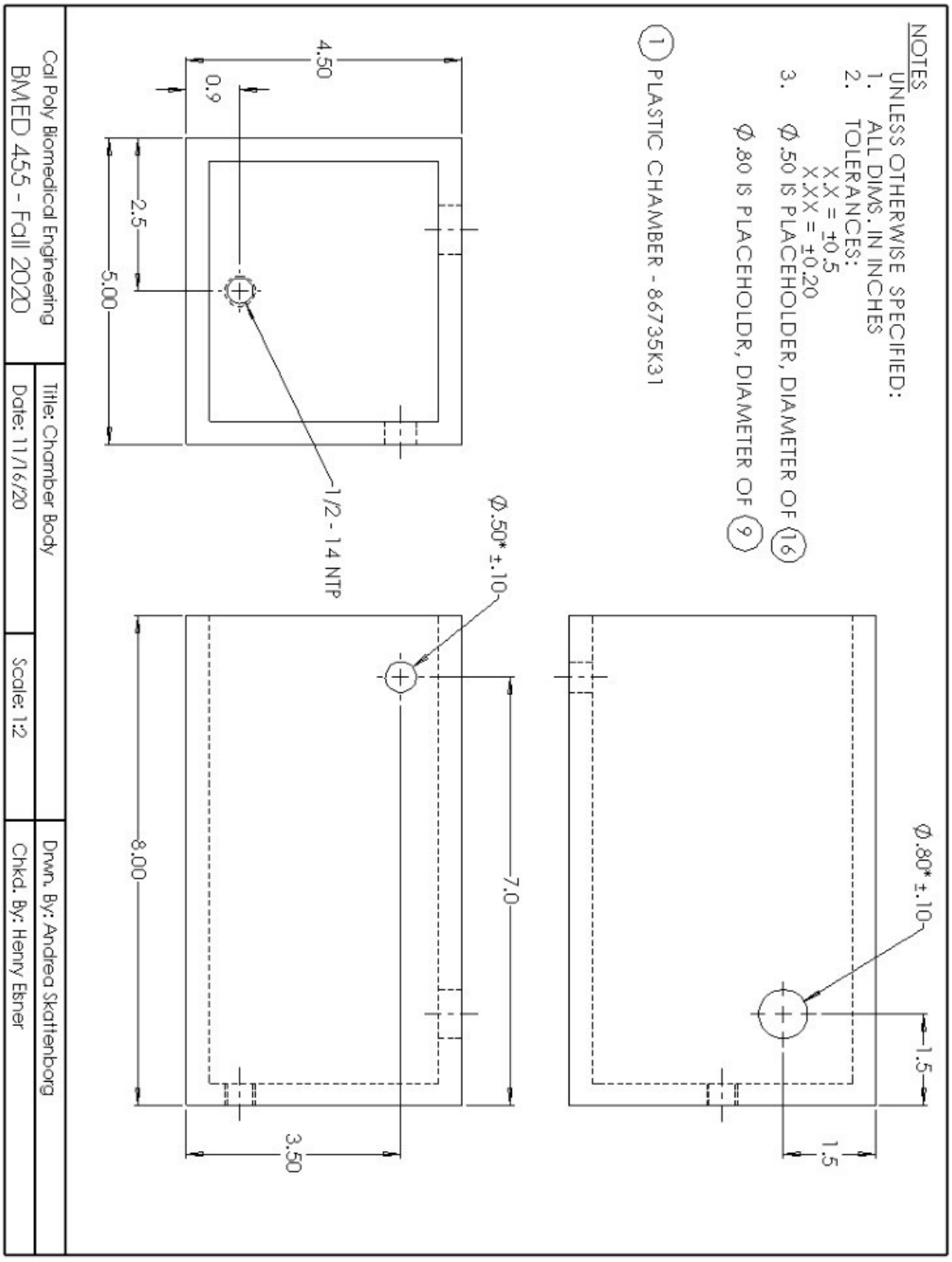


Attachment C – Table of Links to Patents

Table C.1. Links to patents

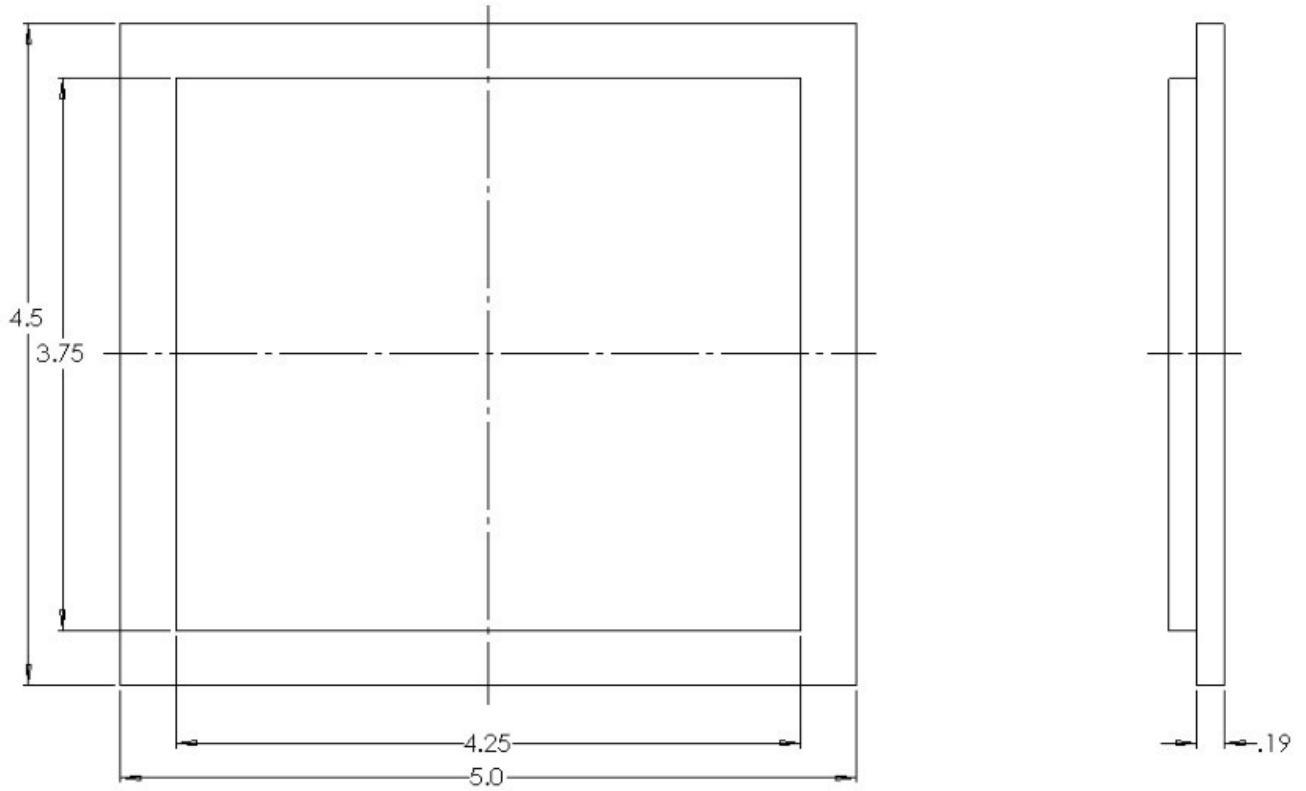
Patent	USPTO link
Cell culture incubators with integrated cell manipulation systems	http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=10696937.PN.
Cell culture system	http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=10647955.PN.
Highly sensitive oxygen sensor for cell culture	http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=8398922.PN.
Mesenchymal Stem Cells Grown Under Hypoxic Conditions: Compositions, Methods and Uses Therefor	http://appft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PG01&s1=20100330047.PGNR.
Microfluidic device and method for modulating a gas environment of cell cultures and tissues	http://appft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PG01&s1=20130295551.PGNR.
Methods and uses of hypoxic compartment cells	http://appft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PG01&s1=20120045419.PGNR.

Attachment D – Detailed Design Drawings



NOTES
1. ALL DIMS. IIN INCHES
2. TOLERANCES:
X.X = ±0.5
x.xx = ±0.10

(1) PLASTIC CHAMBER - 86735K31



Cal Poly Biomedical Engineering BMED 455 - Fall 2020	Title: Chamber Door		Drwn. By: Andrea Skattenborg
	Date: 11/16/20	Scale: 1:1	Chkd. By: Makenzie Jones

Attachment E – Detailed BOM

Table E.1. Chamber Bill of Materials.

Item #	Part #	Qty	Name	Material	Source
1	86735K31	1	Chamber Sides	Polysulfone	McMaster-Carr
2	86735K71	2	Chamber Back/Door	Polysulfone	McMaster-Carr
3	2141N4	1	Check Valve	Polycarbonate Plastic	McMaster-Carr
4	-	1	Sensor Gasket	TPU	NinjaTek
5	-	3	Latch	Stainless Steel	PIXNOR
6	90116A202	14	M4 Screw	316 Stainless Steel	McMaster-Carr
7	11255A13	1	Handle	304 Stainless Steel	McMaster-Carr
8	-	1	Door Gasket	TPU	NinjaTek
9	7467A95	1	Epoxy	-	McMaster-Carr
10	-	2	Neoprene	Neoprene	-

Table E.2. Electrical Bill of Materials.

Item #	Part #	Qty	Name	Material	Source
11	OX-0052	1	Oxygen Sensor	Various	GasLab
12	-	1	Uno R3/USB Cable	Various	Elegoo
13	-	1	Wires	Various	-

Table E.3. Gas/Tubing Bill of Materials.

Item #	Part #	Qty	Name	Material	Source
14	OX K	1	Oxygen Gas Cylinder	Steel	Praxair
15	NI CD5P-A3	1	N2/CO2 Gas Cylinder	Aluminum	Praxair
16	3687N114	1	O2 Regulator	Brass	McMaster-Carr
17	3302276	1	N2/CO2 Regulator	Brass	The Harris Products Group
18	1520409K	1	O2 Regulator Tube Fitting	Brass	Dixon Valve & Coupling
19	-	1	N2/CO2 Regulator Tube Fitting	Brass	Contractors Maintenance Service
20	7923A32	1	N2/CO2 Regulator Gas Cylinder Fitting	Brass	McMaster-Carr
21	T9FB454153	2	Needle Valve	Brass	Global Industrial
22	5357K32	4	Valve Fitting	Aluminum	McMaster-Carr
23	5372K519	2	Reducer	Nylon Plastic	McMaster-Carr
24	5463K727	1	Wye Fitting	Nylon Plastic	McMaster-Carr
25	5463K469	1	Inlet Fitting	Nylon Plastic	McMaster-Carr
26	6516T29	10	1/2" Tubing	PVC Plastic	McMaster-Carr
27	5233K56	25	1/4" Tubing	PVC Plastic	McMaster-Carr
28	-	1	Teflon Tape	Teflon	-
29	5388K14	4	Tube Clamp	301 Stainless Steel	McMaster-Carr

Table E.4. Sterilization/Cleaning of Materials.

Item #	Part #	Qty	Name	Material	Source
30	-	1	Sensor Hole Stopper	TPU	NinjaTek
31	-	1	Chamber Inlet Stopper	TPU	
32	-	1	Inlet Fitting Stopper	TPU	
33	-	1	Neoprene Bath	TPU	

Attachment F – Oxygen Sensor Code

```
// leftmost drop down menu in Serial Monitor window MUST be set to
"Newline" #include <SoftwareSerial.h>
SoftwareSerial output(10, 11); // data input pins: 10 and 11 const
byte numChars = 40;
const byte numO2Chars = 6;
char allData[numChars];
char o2Data[numO2Chars]; boolean newData = false;
void setup() { Serial.begin(9600); output.begin(9600);
}
void loop () { recvData(); dispO2Data();
}
// receive all data from oxygen sensor void recvData() {
static byte index = 0; static byte ind = 0; char endMarker = '\n';
char reading;
while (output.available() > 0 && newData == false) { reading =
output.read();
// if current reading is not a newline character (endMarker) // then
add reading to allData char
if (reading != endMarker) {
allData[index] = reading;
if (index >= 27 && index <= 31) { o2Data[ind] = reading;
ind++;
} index++;
// if index reaches length of if (index >= numChars) {
index = numChars - 1;
} }
else {
allData[index] = '\0'; // terminate string index = 0;
ind = 0;
newData = true;
} }
}
// display data to Serial Monitor void dispO2Data() {
if (newData == true) { Serial.print("Oxygen Concentration: ");
Serial.print(o2Data); Serial.println("%");
newData = false;
} }
```

Hypoxic Incubation Chamber: Operation Manual

By:

Makenzie Jones

Andrea Skattenborg

Henry Elsner

California Polytechnic State University

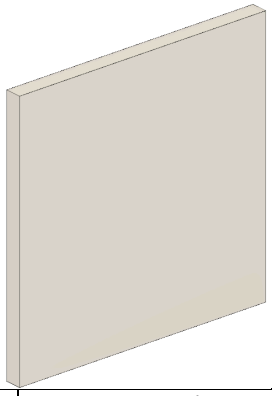
BMED 455/456

March 9th, 2021

Table of Contents

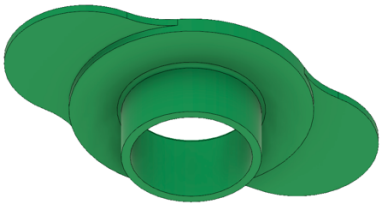
Components

# 1	Chamber Sides

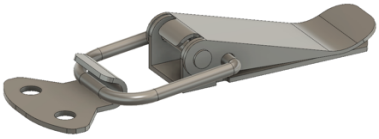


# 2	Chamber Back/Door (x2)

# 3	Check Valve



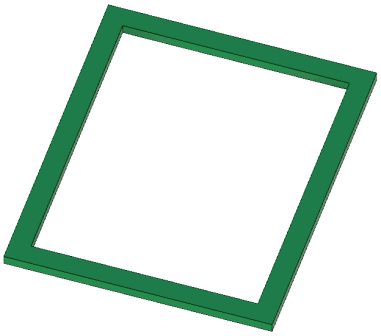
# 4	Sensor Gasket



# 5	Latch (x3)

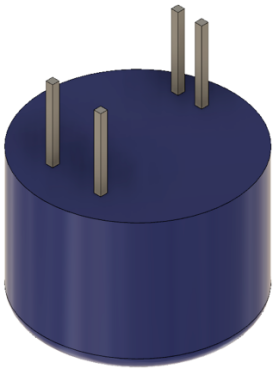
# 6	M4 Screw (x14)

# 7	Handle

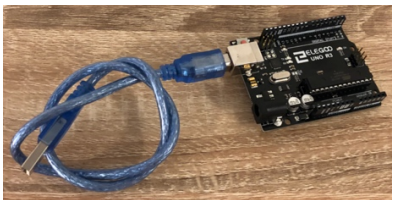


# 8	Door Gasket

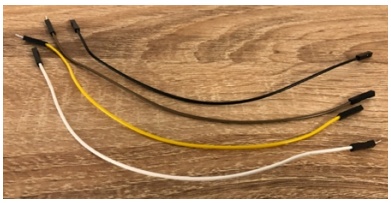
# 10	Neoprene



# 11	Oxygen Sensor



# 12	Uno R3/USB Cable

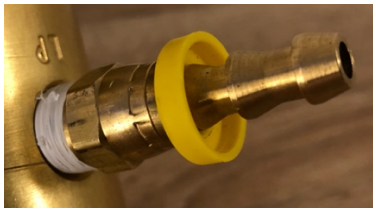


# 13	Wires

# 16	O2 Regulator

# 17	N2/CO2 Regulator

# 18	O2 Regulator Tube Fitting



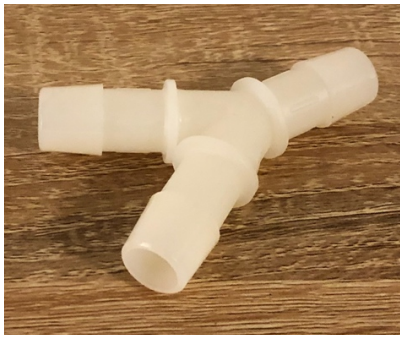
# 19	N2/CO2 Regulator Tube Fitting

# 20	N2/CO2 Regulator Gas Cylinder Fitting

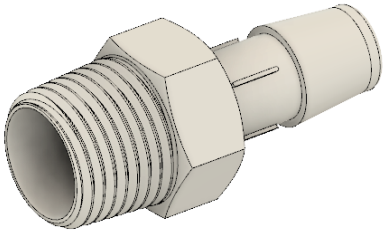
# 21	Needle Valve (x2)

# 22	Valve Fitting (x4)

# 23	Reducer (x2)



# 24	Wye Fitting



# 25	Inlet Fitting

# 26	1/2” Tubing (10ft)

# 27	1/4” Tubing (25 ft)

# 28	Teflon Tape

# 29	Tube Clamp (x4)

Set-Up

This section will illustrate how to set up the different components of the Hypoxic Incubation Chamber to get ready for an experiment. The Chamber Set Up section should be attempted after the chamber and various gaskets have been sterilized.

Software Installation and Preparation

Any computer to be used with the Arduino and oxygen sensor needs to download the Arduino IDE from:

https://www.arduino.cc/en/Main.Software

Follow the instructions in the installer to download the Mac or Windows version. Once the IDE has downloaded, open the IDE and type out the lines of code seen in Figures 3 and 4 at the end of this section.

After getting the code ready plug the Arduino Uno board into the computer using the USB cable provided. The green “on” light should be lit as well as the orange “L” light. Then, go to “Tools” -> “Programmer:” -> “ArduinoISP” as seen in Figure 1. The location of the “Tools” tab will vary between Mac and Windows. For Mac, the “Tools” tab will appear in the ribbon at the top of the screen when the Arduino IDE window is selected.

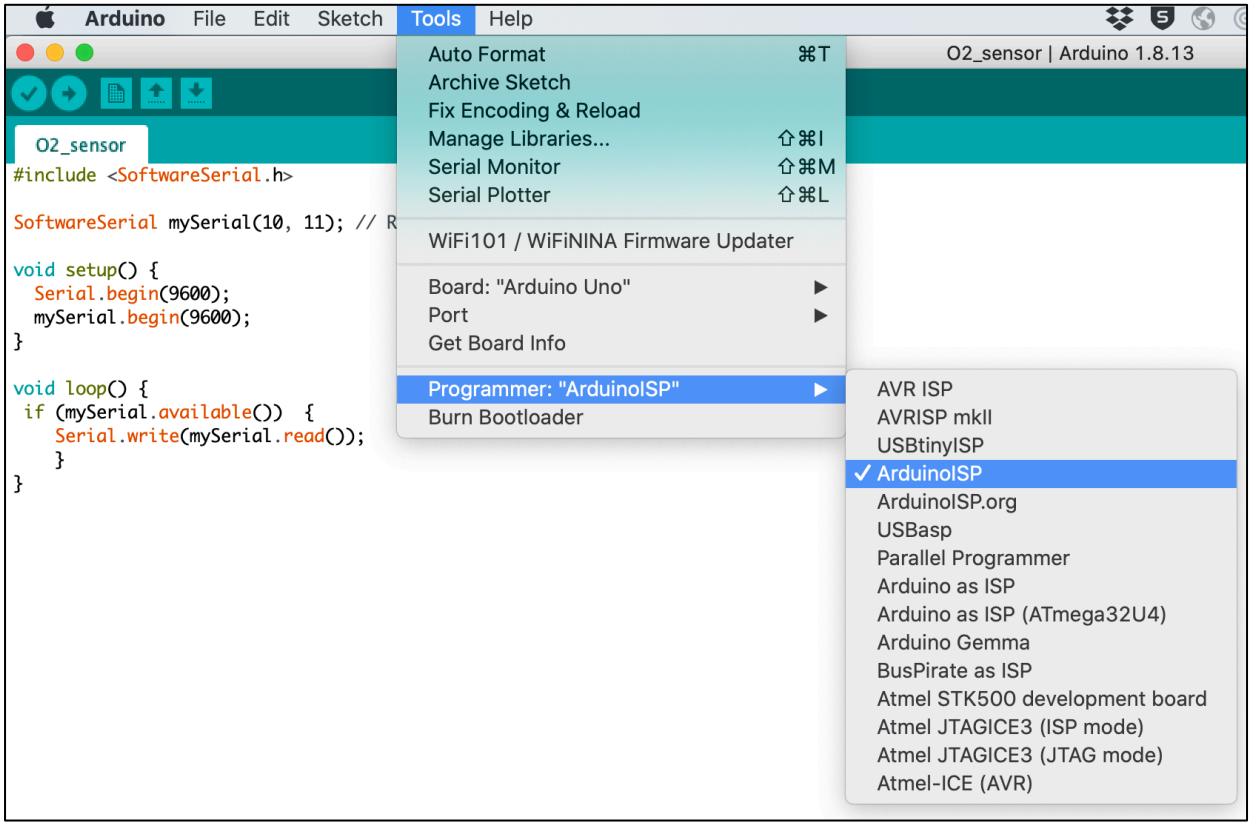


Figure 1: Selecting the correct Programmer

Next go to “Tools” -> “Port” -> “/dev/cu.usbmodemXXXXX (Arduino Uno)”. This will be the serial port through which the computer and Arduino communicate. The port name may vary, but the correct name will only come up after the Arduino has been plugged in. Additionally, ensure that the “Board” selected under the same “Tools” tab is “Arduino Uno”. This should be the default.

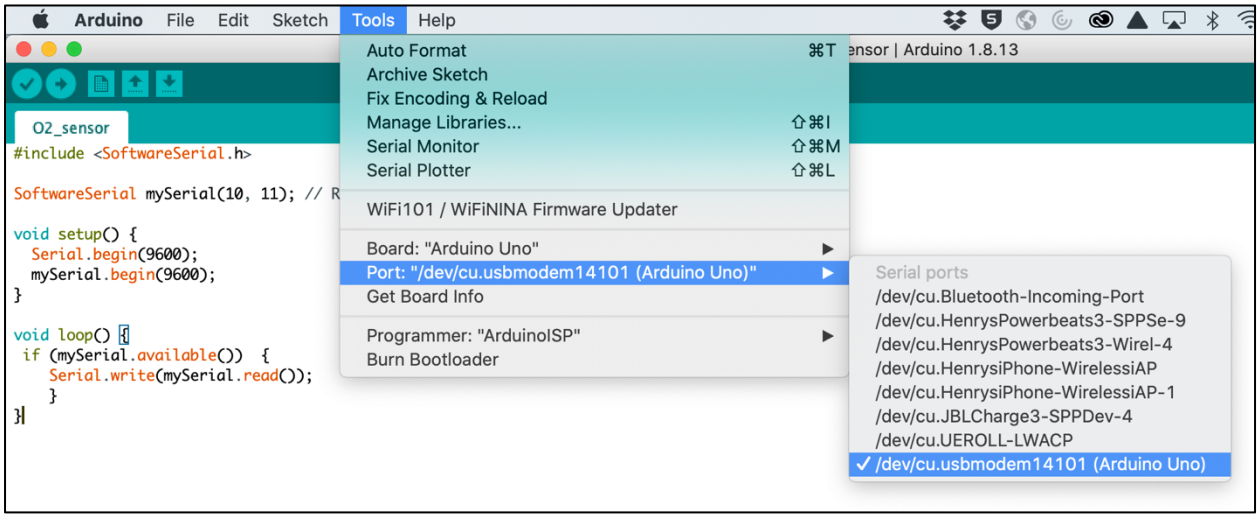


Figure 2: Selecting the correct Port

The computer is now ready to send and receive information to and from the Arduino. To use the Arduino to collect O2 level data, continue through the operation manual.

O2_Sensor_Current

```
// leftmost drop down menu in Serial Monitor window MUST be set to "Newline"

#include <SoftwareSerial.h>

SoftwareSerial output(10, 11); // data input pins: 10 and 11
const byte numChars = 40;
const byte numO2Chars = 6;
char allData[numChars];
char o2Data[numO2Chars];
boolean newData = false;

void setup() {
  Serial.begin(9600);
  output.begin(9600);
}

void loop () {
  recvData();
  dispO2Data(); // comment this line out if you do not want to see the oxygen concentration
}

// receive all data from oxygen sensor
void recvData() {
  static byte index = 0;
  static byte ind = 0;
  char endMarker = '\n';
  char reading;
```

Figure 3: The first half of the code used to operate the oxygen sensor and Arduino

```
  while (output.available() > 0 && newData == false) {
    reading = output.read();

    // if current reading is not a newline character (endMarker)
    // then add reading to allData char
    if (reading != endMarker) {
      allData[index] = reading;

      if (index >= 27 && index <= 31) {
        o2Data[ind] = reading;
        ind++;
      }

      index++;

      // if index reaches length of
      if (index >= numChars) {
        index = numChars - 1;
      }
    }

    else {
      allData[index] = '\0'; // terminate string
      index = 0;
      ind = 0;
      newData = true;
    }
  }
}

// display data to Serial Monitor
void dispO2Data() {
  if (newData == true) {
    Serial.print("Oxygen Concentration: ");
    Serial.print(o2Data);
    Serial.println("%");
    newData = false;
  }
}
```

Figure 4: The second half of the code used to operate the oxygen sensor and Arduino

Arduino Hardware Set-up

The Arduino needs to be connected to the computer and the oxygen sensor. This connection should be done after the chamber has been connected to the gas lines before an experiment. The Arduino-computer connection is simple and completed through the blue USB cable provided. This will supply power to the Arduino as well as allow data to flow between the Arduino and computer. The cable can plug into the USB ports in the computer and the silver box on the Arduino.

Four wires are required to connect the Arduino to the oxygen sensor. Connect the “5V” pin of the Arduino power strip to pin 1 on the oxygen sensor. Connect the “GND” pin of the Arduino power strip to pin 2 on the oxygen sensor. Connect pin 10 of the Arduino Digital strip to pin 3 on the oxygen sensor. Connect pin 11 of the Arduino Digital strip to pin 4 on the oxygen sensor. A picture of the connections can be seen in Figure 5 and a schematic of the oxygen sensor’s pins can be seen in Figure 6.

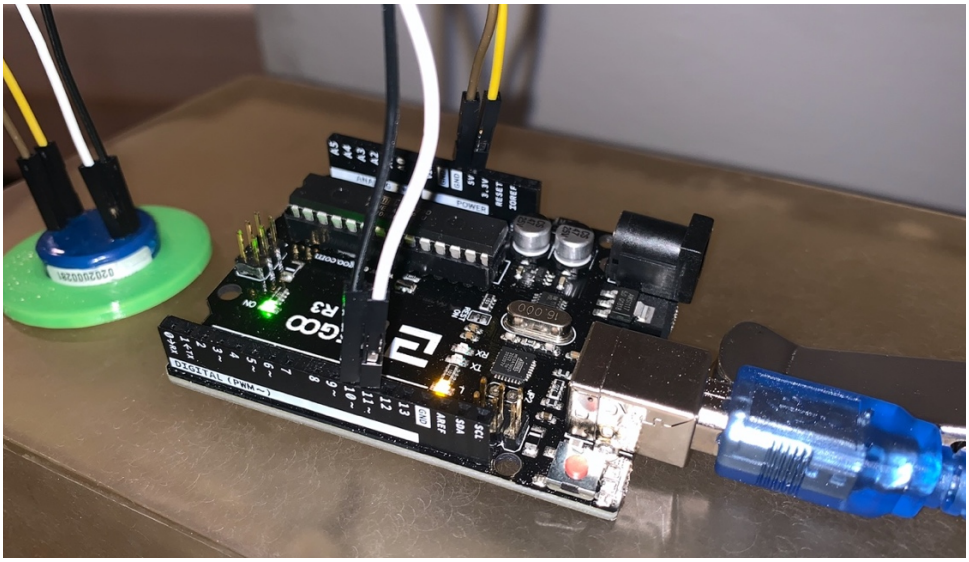
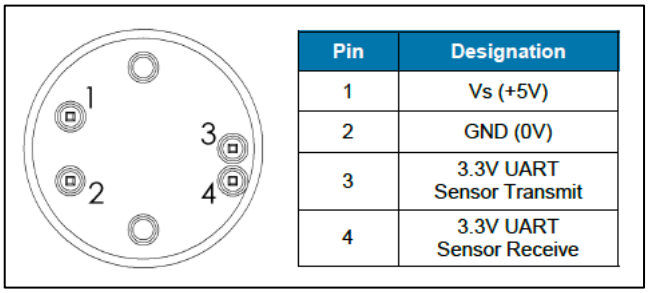


Figure 5: The Arduino connections to the oxygen sensor (yellow, brown, white, and black wires) and computer (blue cable)



Pin	Designation
1	Vs (+5V)
2	GND (0V)
3	3.3V UART Sensor Transmit
4	3.3V UART Sensor Receive

Figure 6: Schematic of oxygen sensor pins

Gas Cylinders Connection

Each of the gas cylinders will be equipped with a regulator. Tubing will connect these regulators to needle valves, a wye connector, and ultimately the chamber. Before setting up the tubing and adapters, determine how far away the chamber will rest from the gas cylinders, this length will be the total length.

Screw adapter 1 (part 20) into the inlet port of the CO2 regulator. Then, screw adapter 2 (part 19) into the outlet port of the CO2 regulator. To this adapter, attach ¼" ID tubing (part 27) to this using a worm drive clamp (part 29) to secure the connection. Use enough ¼" tubing so that the tubing is able to reach approximately 1' less than the total length.

Figure 7: CO2 regulator with adapters, tubing, worm drive clamps, and needle valve

To the end of the tubing, attach adapter 3 (part 22), one of the brass needle valves (part 21), and another adapter 3 in sequence. For each adapter-tubing interface, use a worm drive clamp. Ensure the arrow on the brass needle valve is facing in the direction of gas flow (from cylinder to chamber).

Connect 2” of ¼” tubing to this exposed end of the distal adapter 3, continuing to use worm drive clamps. Use a ½ - ¼” reducer (part 23) to increase the tubing ID to ½”. Attach 2” of this ½” tubing (part 26) to one of the pair of prongs of a wye connector (part 24). Use another 2” of ½” tubing to connect the single end of the wye connector to the chamber inlet adapter (part 25).

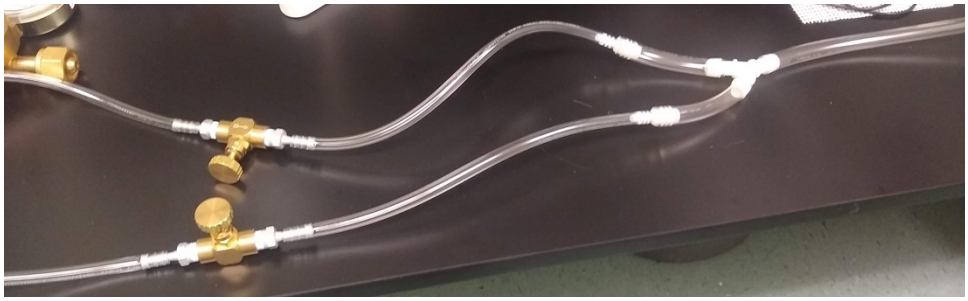


Figure 8: Picture of the brass needle valves with their adapters, tubing, reducers, and wye connector

For the O2 cylinder, begin by screwing adapter 4 (part 18) into the outlet port of the O2 regulator.

Figure 9: O2 gas cylinder with the regulator and adapter with tubing attached

Then, attach the ¼” ID tubing to the distal end of the adapter. As for the other gas line, every tubing-adapter interface should be secured using a worm drive clamp. The other end of this tubing will attach to another brass needle valve book-ended by two adapter 3s, similar to the gas line from the other tank. Use enough ¼” tubing to get the brass needle valves to be the same distance from their respective gas cylinders. From here, connect the distal end of the adapter 3 to the only exposed prong on the wye connector that is already a part of the N2/CO2 gas line. Use the same lengths of tubing and ½ - ¼” reducer placement as in the other line.

Chamber Set Up

Bring the sterilized chamber, sterilized water tray, DI water, sterilized door gasket, and sterilized sensor gasket into the hood. DO NOT TOUCH THE WHITE MEMBRANE OF THE SENSOR WITH ANYTHING. Push the oxygen sensor (part 11) into the sensor gasket (part 4) so that the pins of the sensor face towards to end of the gasket with the wide ring and pull tab. Remove the sensor gap filler and replace it with the sensor and gasket combo. The white membrane of the sensor should face into the chamber. Push down on the oxygen sensor without applying force to the pins of the sensor to get a good snug fit between the sensor, gasket, and chamber. If the connection is a little loose, remove the oxygen sensor then grab the sensor pull tabs with both hands and rotate the gasket back and forth while applying downward pressure. Then, push the oxygen sensor into the gasket again.

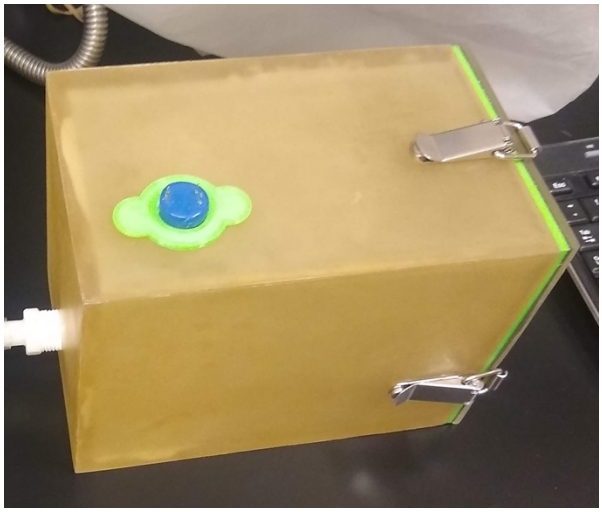


Figure 10: Chamber with the oxygen sensor inserted

Pour enough DI water into the water pan to cover the bottom with about 5 mm of water. Undo the latches of the door and remove the door. Place the door gasket on the door so that the neoprene layer is facing towards to chamber. Place another neoprene layer on the exposed side of the gasket. Line the door up with the chamber on a flat surface and hook all latches onto their latch plates without actually latching them. Latch the side latches, then the top latch. Slide the water tray into the chamber between the rails on the base of the chamber. Return the door to the chamber and close the side latches.

Operation

This section of the Operation Manual will provide instructions on how to run an experiment by putting cells in the chamber, attaching the chamber to the gas, getting the Arduino up and running, and gassing the chamber.

Introducing Cells to the Chamber

The cells to be used in the experiment should be in 1 or 2 T75 flasks. Bring the flasks into the hood with the chamber. Open the latches of the chamber and remove the door. Stack the flasks on top of one another and slide them onto the rails in the chamber. They should sit above the water tray. With the flasks inside the chamber, replace the door to the chamber and close all three latches.

Attaching the Chamber to the Gas Lines

Carefully move the chamber from the hood to where it will rest for the experiment. This needs to be close enough to the gas cylinders for the tubing to be able to reach the chamber. Prepare a 3” long piece of electrical or plumbing tape. Wrap this piece of tape around the threads of the chamber inlet adapter in a clockwise direction when the threads are facing you. Then, remove the plug from the end of the chamber inlet adapter, remove the plug from the chamber inlet, and screw the adapter into the inlet to connect the tubing to the chamber. When screwing the adapter in, first twist the adapter and tubing counterclockwise 360-720 degrees, then begin screwing. This will prevent twisting and kinks in the tubing.

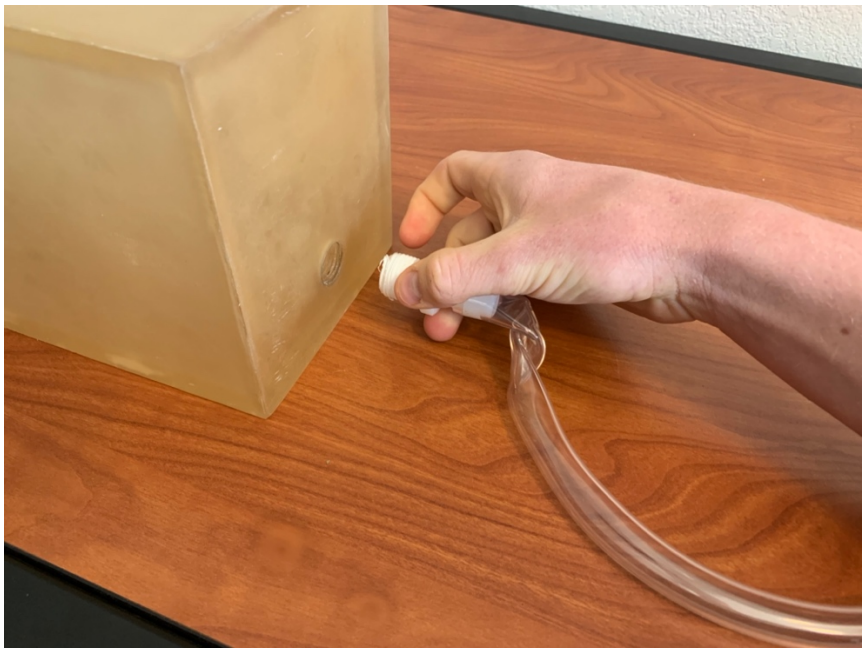


Figure 11: Screwing in the chamber inlet adapter with tubing

Arduino Operation

Connect the Arduino to the oxygen sensor and computer as described in Arduino Hardware Set Up. On the computer that will be used to collect oxygen level data, open the Arduino IDE. Open the code for the oxygen sensor and in the IDE press the right-facing arrow in the top left corner of the IDE called “Upload”. Once the code has been uploaded, the window at the bottom of the IDE should say “Done uploading”. Then, go to “Tools” -> “Serial Monitor” to pull up the serial monitor window that displays the O2 level as a percentage in the rightmost column.

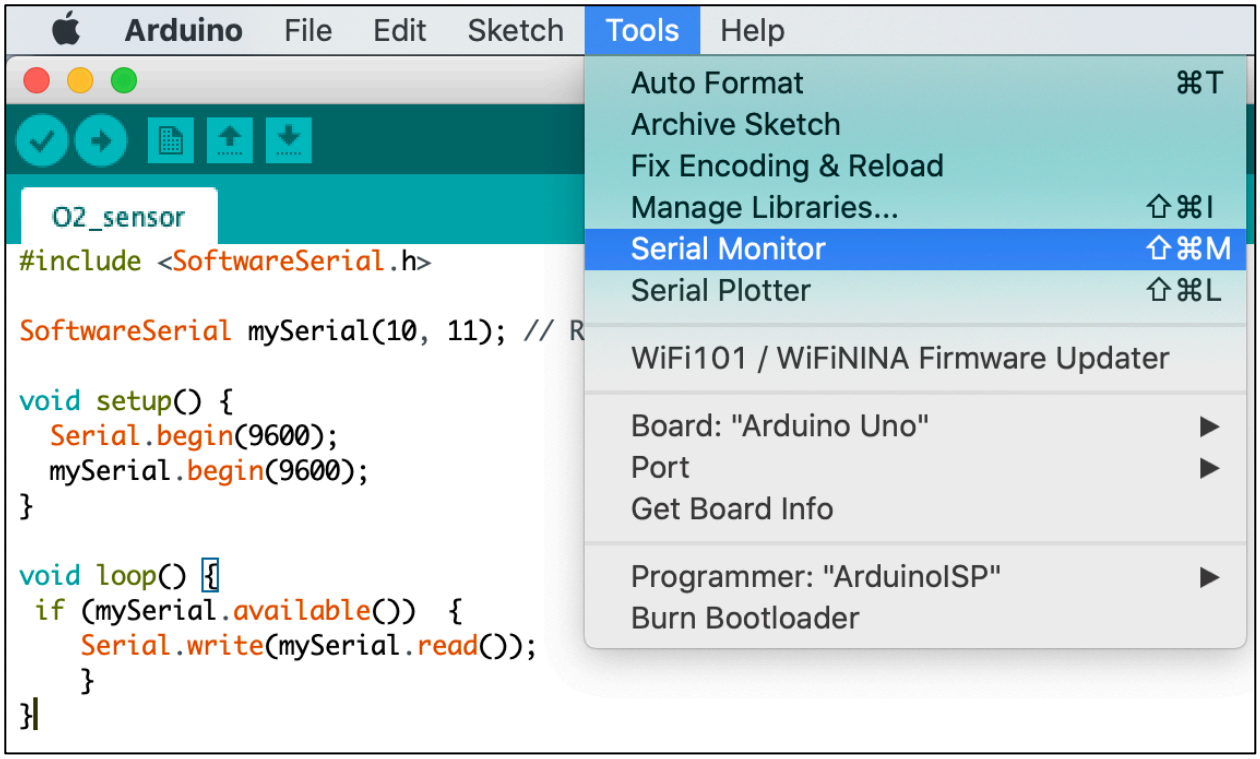


Figure 12: Opening the Serial Monitor to observe the oxygen level

Setting the O2 level

With the entire chamber and Arduino set up, the chamber can now be gassed. Open the valves that are on the gas cylinders for both gases. Then, open the brass needle valve connected to the N2/CO2 tank a full turn. Wait 5 seconds, then close the valve for 5 seconds. Repeat this opening and closing once more. Wait for the oxygen reading to stabilize on the serial monitor, this will take several minutes. If the oxygen reading stabilizes above the desired oxygen level, repeat the gassing procedure with the mixed gas. Once the oxygen reading stabilized below the oxygen level desired for the experiment, close the mixed gas needle valve and move to the oxygen gas needle valve.

Achieving the desired oxygen level is tricky and takes patience. Slightly open the brass needle valve in the oxygen gas line and close it quickly. Watch the oxygen reading on the serial monitor as it changes to meet the oxygen level. Once the reading stabilizes, determine how much to open the brass needle valve on the next iteration. Compare the amount that the oxygen level increased on the last valve opening with how much more the oxygen value has to increase to determine how significantly to open the brass needle valve. Continue to open and close the brass needle valve on the oxygen line, allowing sufficient time for the oxygen sensor to adjust, until the desired oxygen level is reached. If the oxygen concentration gets too high, then simply conduct the same procedure with the needle valve in the mixed gas line.

Cleaning and Sterilization

Cleaning of the chamber, tubing, plastic adapters, and gaskets can be completed with DI water, ethanol, and/or soap as desired. Be careful to not let soap enter the check valve on the side of the chamber. Never attempt to clean the Arduino or oxygen sensor with liquid. The oxygen sensor blue body can be lightly cleaned, but do not let any liquid or cleaning solution get onto the white membrane of the sensor. And any water on the pins of the sensor must be quickly dried.

Gasket Sterilization

In the hood, prepare two baths of sterilized ethanol. Use the 3D printed baths provided as part of this project. Fill each bath with enough ethanol or IPA to fully submerged the parts. In one of these baths, place the door gasket with neoprene and the sensor gasket. In the other bath, place the chamber inlet and sensor plugs. Allow the gaskets and plugs to sit in the liquid for 15-20 minutes. When sterilizing the chamber, transfer the bath with the chamber inlet and sensor plugs next to the autoclave immediately before removing the chamber from the autoclave.

Chamber Sterilization

To sterilize the chamber, first detach the chamber inlet adapter from the chamber by unscrewing it. Place a piece of tape over the exposed opening of the adapter to reduce contamination potential. Remove the door gasket as well as the oxygen sensor and sensor gasket. The plastic chamber, check valve, metal latch components, and chamber inlet adapter can all be autoclaved, everything else cannot. Close the door of the chamber using the latches. This will not create a good seal around the door because of the lack of a gasket. Place the chamber in the autoclave with the back of the chamber facing towards the opening of the autoclave.

After the autoclave has finished, open the door of the autoclave and immediately plug the inlet and the oxygen sensor holes with sterilized plugs. Transfer the chamber to the hood to conduct the rest of the experiment.