Live Cell Imaging Gas Control

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
Bachelor of Science

by

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March, 2022

Statement of Disclaimer

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Executive Summary

The following document outlines the entire process for designing and manufacturing a Live Cell Imaging Gas Control subsystem in tandem with the Heated Stage subsystem to make a CO_2 and temperature-controlled chamber for use with the Olympus IX73P2F Microscope. It highlights the current literature and patents surrounding live cell imaging and touches on the existing live cell imaging setups currently being used by other entities. Following this, it defines the scope of the project, key customer requirements, and engineering specifications to ensure the design meets all the needs of its users. This document includes project planning steps to establish important deadlines and a budget for project completion. As the project has progressed, additional documentation has been added such as morphologies of possible design functions, conjoint analyses, conceptual evaluations, and conceptual models for development of the final design. To note, these initial designs highlighting a drawer like chamber were soon dismissed as it would not allow imaging within the device due to objective focal length. The design evolved into the final chamber with a removable lid of a polysulfone chamber with aluminum base. The manufacturing process instructions and design history file show the exact steps taken to manufacture the chamber prototype. Similarly, circuitry diagrams and instructions on the Arduino controlled CO_2 system are outlined. In depth test plans were outlined, including testing for the materials, chamber itself, CO_2 control system, and fully combined system with the Heated Stage subsystem. Material testing showed that polysulfone, glass, and aluminum withstand autoclave sterilization and laboratory cleaning agents. Chamber testing highlighted the compact size and usability of the device with a multitude of culture vessels. CO_2 control system testing showed that the control system effectively keeps CO_2 concentration between 4.5% and 5.5% during imaging, with the system being able to re-establish CO_2 concentration within 2 minutes of the lid removal, and the system establishes required CO_2 concentration about 5 minutes after setup. The entire Live Cell Imaging Device was shown to keep cells alive compared to an ambient air flask for a period of 3 hours. Finally, this document outlines the next steps for this device, including finer tuning of the CO_2 control system and increasing cellular viability in the chamber for longer periods of time with the addition of humidity control.

Introduction

The imaging capabilities of microscopes are critical for the conduction of microbiological research, which may range from simpler studies of cell metabolism to tissue-on-a-chip investigations of drug candidates. Traditional microscopic imaging techniques involve culturing cells, then taking a sample and staining it for inspection under the scope. As the staining process results in cell death, this method effectively fixes the cells at a snapshot in time. A complimentary imaging technique is to pursue live cell imaging – that is, investigating the microscopic behavior of cells which are still alive. This permits the collection of valuable data, which is otherwise inaccessible, but comes with a host of challenges. Chief among these is finding a method to keep the cells in a life-sustaining environment while being imaged (reminiscent of the interior of a cell incubator).

The goal of this project is to develop a device which maintains a microscope-observable chamber at a set CO_2 concentration which, in turn, maintains cell culture media at the necessary pH. In combination with a heated stage for the chamber, this device will permit extended, biocompatible imaging sessions of live cell cultures. This will enable the BMED Microscopy

Core to pursue novel microbiological research projects and obtain high-quality, meaningful images which support the development of new technologies.
In the remainder of this document, the reader will find a survey of the current technological and regulatory landscape related to live cell imaging devices. Following this, we provide a detailed description of the technical objectives of this project and the project management infrastructure that will guide its completion. As needed, in-depth appendices are included for reference.

Background

Customer Information

Our team has conducted various interviews and surveys to gather information from customers, particularly regarding who may use this device and what features they would like to see in a gas control device for live cell imaging. Currently, it appears such a device would be used by undergraduate and graduate students working within the BMED Department's various research labs. All labs use primarily mammalian cell cultures which are maintained at the necessary pH via a sodium bicarbonate buffer. Students who use these microscopes must receive faculty training and guidance, and faculty may, from time to time, use these microscopes as well. These labs use many cell culture vessels, including simple glass slides, comparably sized microfluidic devices, and larger well plates. The dimensions of any imaging enclosure or chamber must accommodate each of these cell culture vessels as well as permit full range-of-motion of all microscope components (e.g., the overhead light source). Fortunately, there is also significant lab bench space for any necessary auxiliary control systems and CO_2 tanks. Maintaining sterility of the enclosure is crucial to the integrity of any studies which involve its use. On the other hand, the system should be removable to reflect the fact that not every imaging task at this microscope will involve live cells. Hence, the processes of removing and reinstalling the device need to allow for sterilization of any relevant components.

Existing Designs

Table 1: Summary of Existing Live Cell Imaging Devices

Product Name	Product Image	Overview/Special Features
Biotek Lionheart FX Automated Microscope (*Lionheart FX Automated Microscope - Overview*| *BioTek*, n.d.)		All-in-one microscope design to compactly offer fluorescence, brightfield, color brightfield, and phase contrast imaging of live cells. The environmental control cover provides incubation up to 40°C and CO_2/O_2 control.
Leica Microsystems Live Cell Imaging System DMi8 (*Live Cell Imaging | Solutions | Leica Microsystems*, n.d.)		Large enclosure intended for use with Leica microscopes, primarily for fluorescence microscopy. Modular and removable. Provides temperature and CO_2 regulation.
PerkinElmer MuviCyte Live-Cell Imaging Kit (*Muvicyte Live-Cell Imaging Kit | PerkinElmer*, n.d.)		Small imaging device intended for use inside of a separate cell incubator. Does not itself regulate temperature, CO_2, or humidity. 4x, 10x, and 20x objective lenses included.

Okolab H301-K-Frame, Stage Top Chamber (*Stage Top Chamber | Microscope Incubators | Okolab*, n.d.)		This stage top chamber connects to Okolab temperature, gas, and humidity controllers to maintain a proper environment for live-cell imaging. Magnetic inserts allow it to hold petri, slides, and multiwell plates.
Olympus cellVivo Incubation System (*CellVivo | Modular Incubation System | Olympus LS*, n.d.)		Large, modular system for use with Olympus microscopes. Allows control of temperature, CO_2, and O_2 levels. Cell culture vessels are placed inside a small case for the regulation of CO_2.

Intellectual Property Identification

Most patents related to live cell imaging devices are primarily associated with dedicated cell incubators, rather than specialized devices intended for cell incubation on the stage of a microscope. A summary of these patents (and patent applications) is provided in Table 2. Because we have no intention of marketing or selling the live cell imaging system requested by the BMED Microscopy Core, these documents serve primarily as sources for design ideas rather than introducing the risk of infringement on intellectual property.

Table 2: Summary of Patents Related to Live Cell Imaging

Patent/Patent Application Number, Title	Key Aspects
US Patent 6,265,210: Controlled atmosphere equipment (Silley & Annable, 1999)	• Controls gas composition inside a cabinet to permit testing, manipulation of microbiological samples. • Pressure inside this cabinet is monitored as gas is injected or released to maintain biocompatible atmosphere. • Interior of the enclosure is fully visible from outside the enclosure.
US Patent 11,034,927: Cell culture incubators with integrated imaging systems (Blanchard, 2016)	• Cell culture incubator including an imager (intended to image cells within the incubation chamber). • Produced images are processed and used to produce control signals to alter the incubator environment.
US Patent 10,190,085: Micro-incubation systems for microfluidic cell culture and methods (Lee et al., 2016)	• Microfluidic device anchored on a well plate. • Incubator manifold, once attached to the culture unit, encloses a volume of gas and modulates the interior temperature. • Incubator manifold has a transparent window above the culture chamber.
US Patent Application 20210054331: Method and system for use in monitoring biological material (Axelrod et al., 2020)	• Infrared light sensor designed to determine the concentration of various metabolic gases. • The infrared light source is tunable to different wavelengths to allow study of many different gases.

US Patent Application 20200291345: Dynamic incubator system and method (Katakowski, 2020)	• Cell incubator which maintains a programmed temperature and gas concentration in its interior. • Involves a purging mechanism, connected to the programming device, which permits rapid changes in temperature and the levels of various gases inside the incubator.

Technical Literature

Automated cell incubators have proliferated over the past few decades, providing efficient and precise control of temperature, relative humidity, and CO_2 concentration to facilitate culturing of cells. These devices commonly maintain their interiors at 5% CO_2 through use of a gas injection protocol that counterbalances any losses in CO_2 (Triaud et al., 2003). The quality of an automated incubator can be partially assessed by how much time is required to reestablish the desired CO_2 concentration after the interior environment is disturbed (e.g., by opening the chamber door). Figure 1 shows an expected recovery curve of the CO_2 concentration inside an incubator after such a disturbance.

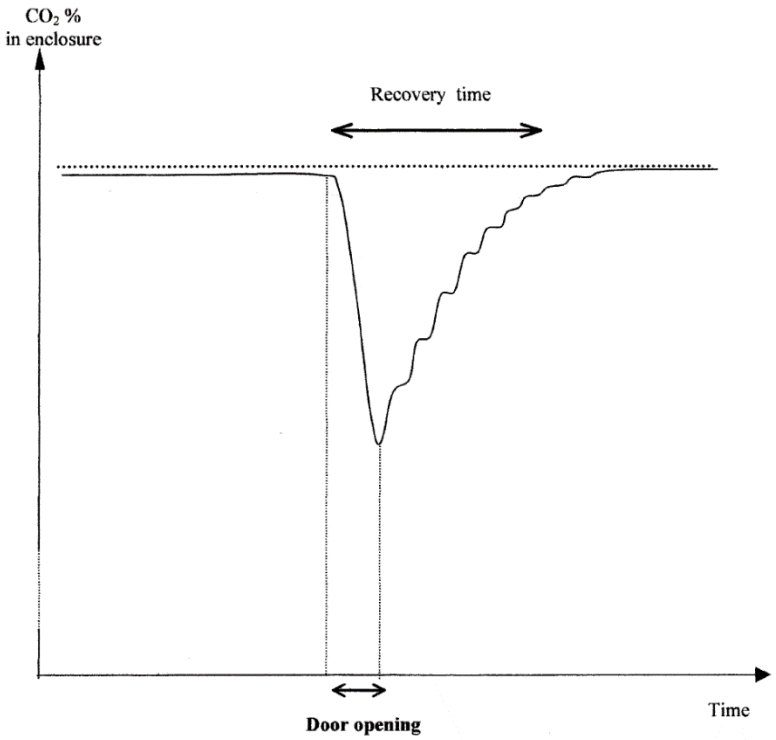

Figure 1: Recovery of desired CO_2 concentration inside an automated cell incubator subjected to a door opening disturbance (Triaud et al., 2003).

In the past decade, as miniaturization of cell culture devices has grown in popularity, alternative methods of maintaining cells at a proper CO_2 concentration have emerged. One such approach is an on-chip CO_2 incubation system based on mass and heat transfer between a bicarbonate buffer

and cell culture media through a semipermeable membrane (Takano et al., 2012). This system maintains standard cell culture media at physiological pH levels without any external CO_2 supply or feedback control, which drastically increases its portability and even allows cell culture in the atmosphere, which enables live cell imaging with only a conventional microscope. Though the potential of this technology is impressive, it is also highly specialized to a particular type of cell culture vessel, thus reducing its versatility.

Other areas of growth in the field of live cell imaging involve developing new fluorescent reporter molecules. Certain new classes of reporter molecules (a.k.a. biosensors) which are genetically encodable have been developed which enable super-resolution visualization of dynamic cellular biochemistry (Mo et al., 2017). These biosensors are genetically encodable in the sense that many are derived from cellular proteins, which enables them to be incredibly substrate-specific (Ni et al., 2018). This, in turn, enables in-depth studies of cell signaling pathways such as G protein-coupled receptor (GPCR) signaling. One drawback is that these protein-derived biosensors often require extended, costly protein maturation programs. Certain methods have emerged to develop simpler, brighter biosensors that function well at 37°C and still exhibit high substrate specificity (Ai et al., 2014; Ren et al., 2020). The overall effect of growth in this area relative to our specific goal is to reinforce the importance of maintaining physiological temperature and pH levels not just to support cell life but to allow these biosensors to function properly.

Industry Codes, Standards, and Regulations

Code of Federal Regulations, Title 21, Chapter I, Subchapter H, Part 864: Hematology and Pathology Devices, Subpart D, Sec. 864.3600: Microscopes and accessories.

Sec. 864.3600 Microscopes and accessories.

(a) Identification. Microscopes and accessories are optical instruments used to enlarge images of specimens, preparations, and cultures for medical purposes. Variations of microscopes and accessories (through a change in the light source) used for medical purposes include the following:

(1) Phase contrast microscopes, which permit visualization of unstained preparations by altering the phase relationship of light that passes around the object and through the object.

(2) Fluorescence microscopes, which permit examination of specimens stained with fluorochromes that fluoresce under ultraviolet light.

(3) Inverted stage microscopes, which permit examination of tissue cultures or other biological specimens contained in bottles or tubes with the light source mounted above the specimen.

(b) Classification. Class I (general controls). These devices are exempt from the premarket notification procedures in subpart E of part 807 of this chapter subject to the limitations in § 864.9. If the device is not labeled or otherwise represented as sterile, it is exempt from the current good

manufacturing practice requirements of the quality system regulation in part 820 of this chapter, with the exception of § 820.180, with respect to general requirements concerning records, and § 820.198, with respect to complaint files.

[45 FR 60590, Sept. 12, 1980, as amended at 54 FR 25044, June 12, 1989; 66 FR 38789, July 25, 2001]

(Code of Federal Regulations Title 21, 2020).

Objectives

Problem Statement

Our customer has an Olympus IX73P2F Microscope equipped with a color camera used for bright field and fluorescent microscopy. The current stage setup accommodates microscope slides, multi-well plates, flasks, and petri plates for live cell imaging. This setup lacks a gas controlled and heated environment, meaning that live mammalian cells cannot be imaged while in physiological conditions (37°C and 5% CO_2).

The Live Cell Imaging Gas Controller, together with the Live Cell Imaging Heated Stage, is indicated for modular use with the Olympus IX73P2F Microscope by undergraduate and graduate researchers. It will maintain an environment suitable for live cell imaging of mammalian cells for up to 6 hours by regulating CO_2 concentration inside its chamber in parallel with the Heated Stage, which regulates the temperature of the sample.

Boundary Definition

The Live Cell Imaging Gas Controller includes the development of a chamber system that will enclose the culture vessel(s). The chamber system will be able to maintain physiological conditions throughout the live cell imaging process. To reach physiological conditions, the setup will include a CO_2 gas canister and pump that will pump CO_2 into the enclosed chamber. To ensure that physiological conditions are met, the setup will contain a monitor that displays CO_2 concentrations. To maintain correct CO_2 concentrations, the system will also have a pump to insert room air to equalize the chamber environment if the monitor detects that the CO_2 concentration is too high. This chamber system will be modular and able to be easily removed when live cell imaging is not being performed using the microscope. This system will work in unison with the Live Cell Imaging Heated Stage to ensure that correct CO_2 concentration and temperature are established while imaging.

Indications for Use

The Live Cell Imaging Gas Control and Heated Stage system is indicated for modular use with the Olympus IX73P2F Microscope by undergraduate and graduate researcher technicians. It will maintain an environment suitable for live cell imaging of mammalian cells for up to 8 hours by regulating CO_2 concentration to 5% and within a ± 0.5 °C of user specified temperature.

Customer Requirements

After consulting with the customer and other users of the Live Cell Imaging Gas Controller, a list of customer requirements has been drafted. These requirements cover all aspects of the design including types of imaging performed, types and sizes of culture vessels used, and appropriate CO_2 concentrations for pH modulation. Other important aspects of the design that were discussed with the customer include the amount of time the device will be used for, vital material properties, and chamber modularity. A full list of these customer requirements can be found in *Appendix A*.

Specification Development (House of Quality)

Using the list of customer requirements, a list of specifications was developed in the House of Quality document (*Appendix B*). One of our customer requirements was modularity and ease of use, which was translated into weight and total chamber volume specifications. These specifications ensure that the chamber may be easily picked up and stored by the users. Optical clarity of the material at the top and base of the chamber is a vital aspect of this project which was translated into a measurement of the percentage of relative focus. This specification will ensure that the material chosen is optically clear. Durability of the chamber was another concern brought to us by our sponsors, which is why we decided to test the material properties of the bulk material. Performing a 3-point bend test will ensure that the material will not deform or crack when exposed to physiological conditions. Imaging time and initialization time were two aspects that we considered as well. To define these specifications, we determined the maximum amount of time that users would need to image for as well as the maximum amount of time for the chamber to reach physiological conditions. The customer requirement of sterility was turned into an engineering specification by using chemical resistance charts to determine which materials are resistant to the solvents that will be used to sterilize the chamber. Accuracy of the CO_2 concentrations was translated to an engineering specification by defining the frequency that the sensor will update the CO_2 readings on a display. Another customer requirement was that this system can account for imaging of culture vessels of various sizes. This requirement was translated into an engineering requirement by measuring the volume of the largest culture vessels that could be imaged and ensuring that the internal volume of the chamber is larger than that measurement. Lastly, the customers specified that the device should be able to be used for both the bright field and fluorescent microscopy. To ensure that both imaging capabilities are possible, we created a specification that ensures that the condenser will not hit the chamber and obstruct the imaging process.

These specifications can be observed in Table 3. This table also outlines the parameter descriptions, requirements that these specifications must meet, the tolerances of those requirements, the risk associated with each parameter, and the compliance.

Table 3: Engineering Specifications

Spec. #	Parameter Description	Requirement or Target (units)	Tolerance	Risk	Compliance
1	Weight	20 lbs.	Max	L	A, T, S
2	Optical clarity	15%	+/- 5%	H	A, T
3	Material properties	100%	+/- 1.00	M	I, T
4	Imaging time	6 hrs.	Min	L	T
5	Signal display	1 sec	+/- 1.00	M	T
6	Sterility	E	Min	H	A, T, I
7	Initialization time	5 mins	Max	H	T
8	Internal volume	17.5 in^3	Min	L	A, I
9	Range of motion	73 mm	+/- 2.00	M	T
10	Transport	3 ft^3	Max	M	A, I

Specification Measurement

- Spec. # 1 Weight: The weight includes the weight of the chamber and not any tubing, monitors, regulators, or any other equipment that may be stored on the bench external to the chamber itself. The weight of the chamber will be measured in pounds. The requirement for this specification is that the weight of the chamber is less than 20 lbs. This specification helps ensure that the system is modular and can be easily setup by the user. Failure of this design aspect will mean that the chamber system weighs more than 20 lbs.
- Spec. #2 Transmittance: Optical clarity of the material is an important specification because this quantity determines the amount of light that will pass through the material and allow for imaging of the live cells. This measurement will be determined by imaging with and without the chosen material under the culture vessel, and then comparing the relative image focus percentages to confirm that the material does not blur the image. The requirement for this specification is that the material has a percent of relative image focus that is within 15% +/- 5% of the control image. Failure of this specification means that the image focus with material fails to be within 20% of the control image focus.
- Spec. #3 Material strength: The material strength is used to ensure the material will not crack or deform when exposed to heat. The material strength will be tested using a 3-point bending test. The requirement for this specification is that the material be able to withstand 100% ± 1% of a force that is applied to it prior to being exposed to 50°C for 6 hours. Failure to meet this material property means that the material shows signs of cracking or deformation after being exposed to the heated environment.
- Spec. #4 Imaging time: Imaging time includes the entire time taken to add the sample to the chamber, initialize the desired environment, image the sample, and then repeat this process until all the desired samples are imaged. The requirement for this specification is that the device may be used for up to 6 hours consecutively. This specification ensures that the system will be able to be used for the entire imaging process for longer imaging

sessions. For future use, this specification also opens the door for time-lapse videos and imaging to be performed using this setup. Failure of this specification means that the system fails to maintain the desired environment for any time less than 6 hours.

- Spec. #5 Signal display: The signal display will indicate the CO_2 concentration throughout the entire imaging process. The requirement for this specification is that the CO_2 concentration be updated every 1 second with a tolerance of ± 1 second. This condition will be tested by counting the frequency of concentration updates on the monitor display. Failure to achieve this requirement means that the CO_2 concentration display is updated every 2 seconds or more.
- Spec. #6 Sterility: The sterility of the material is important to ensure that the live cells and the environment that they are housed in are not contaminated by external microbials. This performance specification will not be tested in house, but materials will be chosen based off their chemical resistance chart results. The chemical resistance chart is rated on a scale from E to N. E indicates that the material was exposed to the reagent for 30 days of constant exposure with no damage, indicating that the material may even tolerate the chemical for years. G indicates that there was little or no damage after 30 days of constant exposure to the reagent. F indicates that there was some effect after 7 days of constant exposure, resulting in softening or swelling of the material. N indicates that the reagent is not recommended for continuous use, resulting in severe crazing, cracking, or permeation losses. The requirement for this specification is that the material receives an E at conditions between 20 C and 50 C for the chosen reagent used to clean the surfaces of the chamber. Failure to achieve this standard means that the chemical resistance rating was any score other than E.
- Spec. #7 Initialization time: The initialization time includes the amount of time it takes to reach physiological or desired conditions after placing the sample in the chamber. The requirement for this specification is that chamber will reach physiological conditions and be ready to image in 5 minutes or less. Failure of this specification requirement means that the chamber takes more than 5 minutes to reach physiological conditions.
- Spec. #8 Internal volume: The internal volume includes the volume of the chamber that houses the various culture vessels. This specification is important because the volume of the largest culture vessels that will be imaged is 17.5 in^3. Therefore, the requirement for this specification is that the internal volume be greater than 17.5 in^3. Failure to achieve this specification is that the internal volume of the chamber is less than 17.5 in^3 and the well plates will be too large to be appropriately imaged.
- Spec. #9 Range of motion: The range of motion includes the working distance that adjusts how close the lens is to the stage. Without the full range of motion, it would be difficult if not impossible to perform bright field microscopy on any given sample. The full working distance on this microscope is 73 mm. Therefore, the requirement for this specification is that the chamber allows for a working distance of 73 mm ± 2 mm. Failure to achieve this requirement means that the chamber inhibits the working distance to less than 71 mm.
- Spec. #10 Transport: The transport requirement includes the maximum external volume of the chamber setup. This requirement ensures that the chamber setup will be small enough to be easily broken down and stored while it is not being used for imaging. The requirement for this specification is that the external volume of the chamber is a

maximum of 3 ft^3. Failure to achieve this requirement means that the external volume of the chamber is greater than 3 ft^3.

Conjoint Analysis

The conjoint analysis was used to determine what device characteristics, including chamber size and material and gas control are most important aspects to our design. The factors, the device characteristics, and levels, the alternatives for each factor, are listed in Table 4.

Table 4: Factors and levels for conjoint analysis.

Factor	**Level 1**	**Level 2**
Chamber size	Just the sample	The entire scope
Material	Opaque	Clear
Gas Control	Dial	Button

Factors and levels were analyzed using the Taguchi method combining different combinations of the levels which then is used to determine which factors are most important to the customer. The specific conjoint combinations are described in Table 5.

Table 5: Conjoint cards for gas control factors and levels using the Taguchi method.

Taguchi method numbering	**Gas control characteristics**
1,1,1	The enclosure surrounds just the sample, the enclosure is clear on the sides, dialed CO_2 control
1,2,2	The enclosure surrounds just the sample, the enclosure is opaque on the sides, button CO_2 control
2,1,2	The enclosure surrounds the entire scope, the enclosure is clear on the sides, button CO_2 control
2,2,1	The enclosure surrounds the entire scope, the enclosure is opaque on the sides, dialed CO_2 control

The raw data from 18 respondents and multivariant regression output can be seen in *Appendix C*. Overall, this analysis showed that gas control is not a significant factor in the design of the chamber and controller while the chamber size and material is significant ($p<0.01$). As a result, the following equation can be used to determine customer attraction based on chamber size and material.

$$Customer\ Attraction = 6.106 * 10^{-16} + 0.667\ X_{SIZE} + 0.833\ X_{MATERIAL}$$

Percent contribution of the significant factors was determined by dividing the coefficients in the customer attraction equation divided by the sum of the factor coefficients. Overall, chamber material contributes 55.56% and chamber size contributes 44.47% to customer attraction. As both factors have positive coefficients, they are both positively correlated with customer attraction. This results in a lower numbered ranking (1) to be correlated with the lower number level (1). Therefore, a fully clear chamber that encompasses just the sample is preferred.

Project Management

Key Deliverables

The BMED Microscopy Core will be updated on the status of their live cell imaging apparatus based on the following deliverables:

Table 6: Expected deliverables to BMED Microscopy Core between September 2021 and March 2022.

Deliverable	**Date**
Statement of Work	Monday, October 11, 2021
Project Planning Meeting	Monday, October 11, 2021
Status Update Memo #1	Monday, October 25, 2021
Conceptual Design Review Report and Presentation	Monday, November 1, 2021
Status Update Memo #2	Monday, November 8, 2021
Status Update Memo #3	Monday, November 15, 2021
Critical Design Review Report and Presentation	Monday, November 29, 2021
Winter Quarter Project Plan	Monday, December 6, 2021
Updated Project Plan	Thursday, January 6, 2022
Status Update Memo I	Tuesday, January 11, 2022
Status Update Memo II	Thursday, January 20, 2022
Test Plan Report and Presentation	Tuesday, January 25, 2022
Functional Prototype Video	Tuesday, January 25, 2022
Status Update Memo III	Tuesday, February 8, 2022
Status Update Memo IV	Tuesday, February 15, 2022
Status Update Memo V	Tuesday, February 22, 2022
Status Update Memo VI	Tuesday, March 1, 2022
Design Review Report and Presentation	Tuesday, March 8, 2022

Network Diagram

Figures 2 and 3 are overview Network Diagrams of tasks to complete the design and building of the Live Cell Imaging Gas Control microscope compatible device. The red boxes designate the critical path, each box being a milestone or task that if moved could impede the progress of the entire process. Subtasks and critical deliverables and milestones are highlighted in Table 7.

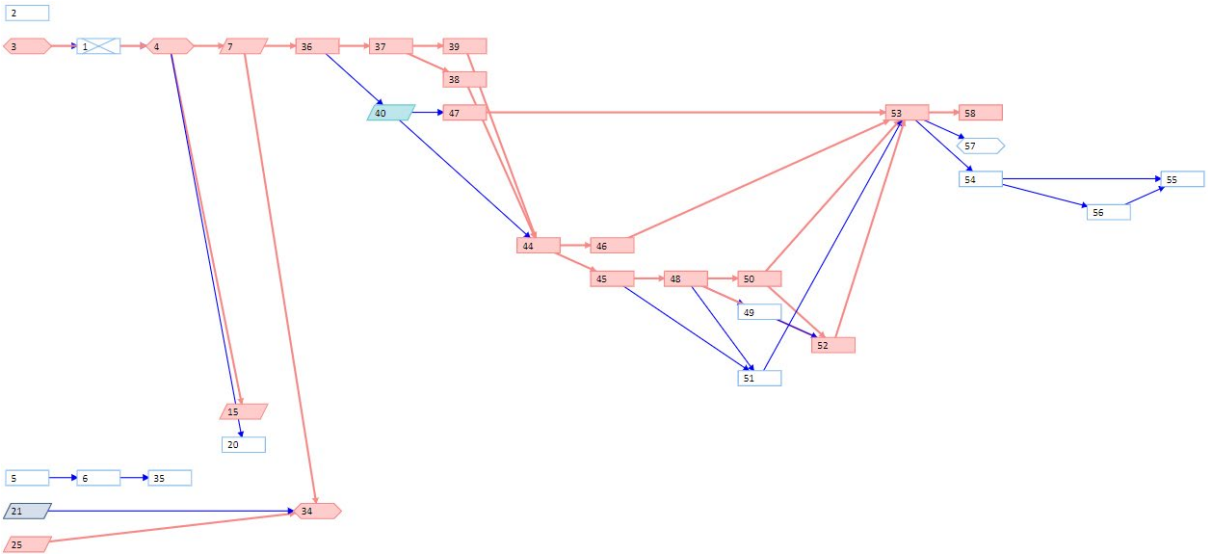

Figure 2. Network diagram of the design process of the Live Cell Imaging Gas Control from September to December 2021. The green box surrounds current tasks regarding the conceptual design and the red boxes and arrows designate the critical path. Tasks are numbered for clarity and their detailed descriptions can be found in Table 7.

Prototyping and testing were completed from January through March of 2022 (Figure 4).

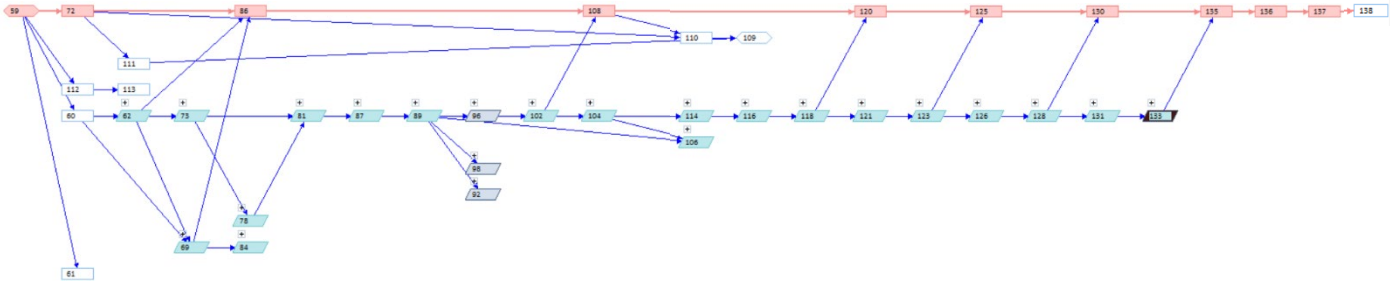

Figure 3. Winter Quarter 2022 Network Diagram. All subtasks within summary tasks are removed from the diagram for clarity.

All completed deliverables and milestones for September 2021 through March 2022 are listed in Table 7. Critical tasks are italicized, and subtasks are indented. Subtasks listed here are not shown on the Network Diagrams above for clarity.

Table 7. Network diagram task legend for Fall Quarter 2021 (Figure 2). Critical tasks are italicized, and subtasks are indented.

Task Number	Deliverable/Milestone	Task Number	Deliverable/Milestone
1	Introduction to Sponsor	30	Conclusion
2	Team contract	31	Executive Summary
3	*Receive Project Information*	32	References
4	*Meeting with Sponsor*	33	Appendices
5	Conjoint Form	34	Project Planning Meeting
6	Conjoint Surveys	35	Conjoint Analysis ANOVA
7	*House of Quality*	36	*Morphology and concept sketches*
8	*Customer Survey*	37	*Pugh Chart*
9	*Customer Requirements*	38	*Status update Memo #1*
10	*Determine Customer Requirement Ranking*	39	*FMEA*
11	*Engineering Specifications*	40	Conceptual Models
12	*Relate Customer Requirements to Engineering Specifications*	41	Cardboard Chamber Model
13	*Engineering Targets*	42	Gas Diffusion Mathematical Model
14	*Engineering Specification Relationships*	43	*Gas Movement Diagram Model*
15	*Network Diagram*	44	Conceptual Design Report and Presentation
16	BMED 455 Deadlines	45	*Status Update Memo #2*
17	House of Quality Subsections	46	*Chamber Top/Bottom Pugh Matrix*
18	Statement of Work Subsections	47	*Status Update Memo #3*
19	Budget Subsections	48	*Hazard and Risk Assessment*
20	Indications for Use	49	*Finalized CAD Chamber Model*
21	Budget	50	Analyze Manufacturability at Mustang 60
22	CO_2 Controller Electronics	51	*Detailed Drawings*
23	Enclosure materials	52	Detailed Budget
24	Gas exchange materials	53	*Manufacturing Process Instructions*
25	*Statement of Work and Meeting Slides*	54	*Critical Design Review Report*
26	Introduction	55	McMaster Order
27	Background	56	Amazon Order
28	Objectives	57	Other Orders
29	Project Management	58	Winter Quarter Project Plan

Table 8. Network diagram task legend for Winter Quarter 2022 (Figure 3). Critical tasks are italicized, and subtasks are indented. See Testing for more details on testing resources and timing.

Task #	Task Description	Duration
59	*Beginning of Winter Quarter*	1 day
60	*Discussion with Shop Techs about timing for machinery (Water Jet, Mill)*	1 day
61	Complete compressed gas training	1 day
62	*1/13/22 Polysulfone and Aluminum Manufacturing*	1 day
63	Water Jet Polysulfone and Aluminum (MPI Steps 1,2)	2 hrs
64	Measure CO2 sensor hole and update sensor housing CAD model (MPI Step 23)	5 mins
65	Mill Polysulfone and Aluminum (MPI Steps 3-6)	1 hr
66	Drill and tap holes in Polysulfone and Aluminum (MPI Steps 7-12) - Drill the pieces not being milled (Including item 2.2, 2.3)	1 hr
67	Drill holes for M4 plastic heat set inserts on excess scrap of polysulfone, practice using soldering iron to press in heat sets	1 hr
68	Print CO2 Sensor housing (personal printer)	3 hrs
69	*1/6/22 Gas Control Manufacturing*	1 day
70	Build circuitry with breadboard to test the CO2 sensor, solenoid, arduino etc	2 hrs
71	Try test code for relay, CO2 sensor, solenoid	2 hrs
72	*Updated Project Plan*	2 days
73	*1/18/22 Polysulfone and Aluminum Manufacturing*	4 hrs
74	Drill and tap holes in Polysulfone and Aluminum (MPI Steps 7-12)	1 hr
75	Assemble chamber walls and sand to flatten (MPI Steps 13,14)	1 hr
76	Drill holes in the top and bottom of Chamber Wall subassembly (MPI Step 15)	1 hr
77	Heat press the M4 Heat Set Inserts into chamber walls (MPI Step 16)	1 hr
78	*1/18/22 Glass and Silicone Manufacturing*	2 hrs
79	Cut glass inserts for aluminum and polysulfone (MPI Step 17)	1 hr
80	Cut the silicone sheets to make gasket and bumper (MPI Step 19)	1 hr
81	*1/18/22 Assembly*	1 day
82	Glue cut glass to chamber top and heat plate, let cure for 24 hours (MPI Step 18)	1 day
83	Glue excess piece of cut glass to polysulfone piece for material testing (x2)	1 day
84	1/11/22 Gas Control Coding/Manufacturing	4 hrs
85	Working on Arduino code and circuitry	4 hrs
86	*Status Update Memo I*	4 days
87	*1/20/22 Assembly*	1 day
88	Glue silicone gasket to the chamber top (MPI Step 20)	1 day
89	*1/20/22 Assembly*	0.13 days
90	Assemble chamber walls, chamber top, and heat plate (MPI Steps 21-22)	30 mins
91	Assemble chamber with CO2 sensor and sensor housing (MPI Step 24)	30 mins
92	1/20/22 Testing	0.13 days
93	Measure resulting internal volume	30 mins
94	Weight of system	30 mins
95	Compressor motion restriction	1 hour
96	*1/20/22 Gas Control*	0.38 days
97	Test CO2 sensor with compressed CO2 cylinder with the manufactured chamber.	3 hrs
98	1/22/22 Material Testing	7 days

99	Cleaning test with submerged silicone sealant, polysulfone, glass in IPA for 1 week	7 days
100	Toaster oven durability test with silicone sealant, polysulfone, and glass	1 day
101	Autoclave durability test with silicone sealant, polysulfone, glass, silicone	1 day
102	1/18/22 CO2 Sensor Setup	0.5 days
103	Continue CO2 sensor code manipulation	4 hrs
104	1/20/22 CO2 Sensor Setup	0.5 days
105	Continue CO2 sensor code manipulation and testing with compressed cylinder	4 hrs
106	1/28/22 User Testing	0.5 days
107	Relative Focus Testing	4 hrs
108	*Status Update Memo II*	7 days
109	Test Plan Presentation	1 day
110	Test Plan Report	2 days
111	Functional Prototype Video	5 days
112	Peer Evaluation	1 day
113	Team Health Assessment	1 day
114	2/1/22 Testing	0.5 days
115	System reestablishes 5% within 2 minutes of top removal	4 hrs
116	2/3/22 Testing	0.5 days
117	TRIAL 1: Initialization test, CO2 maintenance after gas input is stopped, CO2 output update for 10 seconds (3 different tests)	4 hrs
118	*2/8/22 Testing*	0.5 days
119	TRIAL 2: Initialization test, CO2 maintenance after gas input is stopped, CO2 output update for 10 seconds (3 different tests)	4 hrs
120	*Status Update Memo III*	13 days
121	2/10/22 Testing	0.5 days
122	TRIAL 3: Initialization test, CO2 maintenance after gas input is stopped, CO2 output update for 10 seconds (3 different tests)	4 hrs
123	*Status Update Memo IV*	5 days
124	2/15/22 Testing	0.5 days
125	Continued Testing (user testing, external volume)	4 hrs
126	2/17/22 Testing	0.5 days
127	Continued Testing (user testing, external volume)	4 hrs
128	*Status Update Memo V*	5 days
129	2/22/22 Testing	0.5 days
130	Continued Testing (user testing, external volume)	4 hrs
131	2/24/22 Testing	0.5 days
132	Continued Testing (user testing, external volume)	4 hrs
133	*Status Update Memo VI*	5 days
134	*Senior Project Design Report*	5 days
135	*Upload Project to Digital Commons*	1 day
136	*Design Review Presentation*	1 day

Budget

The final budget can be seen in Table 9, with each item associated with its specific design aspect (Chamber, CO_2 Gas Control, and Heated Stage).

Table 9: Current budget for Live Cell Imaging Gas Control chamber, gas regulation, and electronics. Final total includes shipping and taxes calculated in.

Chamber

Glass Sheets	$15.98
Glass Cutter	$4.97
Silicone Sheet	$11.85
Sand Paper	$7.99
15psi Pressure Release Valve	$12.88
Silicone sealant	$10.46
M4 Heat Set Inserts	$11.02
Polysulfone Sheet	$268.81
M4 25mm Screws	$8.91
M4 25mm Thumb Screws	$51.30
Other (shipping, taxes)	$73.93
Subtotal:	$478.10

Gas Control

Gas Input (Male 1/4" NPT Adapter and Barbed Coupler)	$5.32
Transistors	$6.99
Diodes	$4.23
12V Relays	$10.49
12V Power Supply	$6.99
5V Power Cord Wires	$5.99
Arduino Uno R3	$16.07
CO_2 Sensor	$262.80
Solenoid Valve	$38.10
Inert Tubing	$15.00
Teflon Tape	$3.09
Hose Clamps	$7.57
1/4" NPT Barbed Tubing Adaptor	$4.94
CO2 Compressed Gas Cylinder	$46.00
Printed Circuit Board	$22.85
5V/10 Amp Relays	$10.85
26 Gauge Wire	$1.99
Jumper Wire Kit	$9.95
Gas Regulator	--
PETG Filament	--
Subtotal:	$479.22

Heated Stage	
Subtotal:	$368.23
TOTAL	$1,302.76

Concept Generation and Evaluation

Morphology

The following morphologies were developed to compare the compatibility of various functionalities of the design including drawer slides, drawer closures, drawer clasps, and CO_2 concentration displays. The full list of each morphological design input and the corresponding concept ideas can be found in ***Appendix D.*** The three different morphological combinations are displayed below in Figures 4, 5, and 6.

The first morphology, as pictured in Figure 4**,** has the following functions: metal slides, flush drawer with gasket, buckle clasp, and an RGB output. This concept builds on a standard model for drawers (e.g., kitchen drawers) to create an airtight version that would permit the regulation of CO_2 levels inside the cabin. Metal drawer slides will hold the drawer firmly and allow direct access to the drawer platform. The gasket around the drawer front minimizes gas leakage in collaboration with a buckle clasp, and the CO_2 levels inside the cabinet are reported on an RGB screen that uses color to reinforce the status of the device (blue = initializing, green = physiological conditions, red = outside physiological conditions).

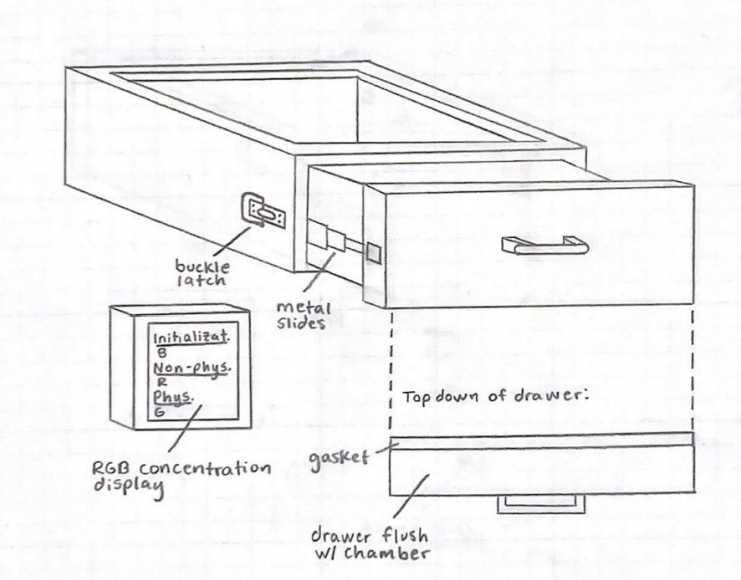

Figure 4: Morphology detailing the drawer slides, drawer closure, drawer clasp, and CO_2 concentration display for Concept #1.

The second concept, as illustrated in Figure 5, has the following functions: side mount slides, T-shape drawer with gasket, roller catch clasp, and a digital output. This concept explores

variations on each of the form aspects of concept 1, namely changing how the drawer slides in and out of the cabin and what mechanisms attempt to minimize gas leakage. Side-mounting the drawer eliminates the weight associated with metal drawer slides, and a T-shaped drawer with a gasket should also minimize gas leakage (whether this results in a significant functional difference is presently unknown). Lastly, a fully digital screen provides consistent readouts of the CO_2 levels inside the chamber and simulates an analog readout for quick perception of the data.

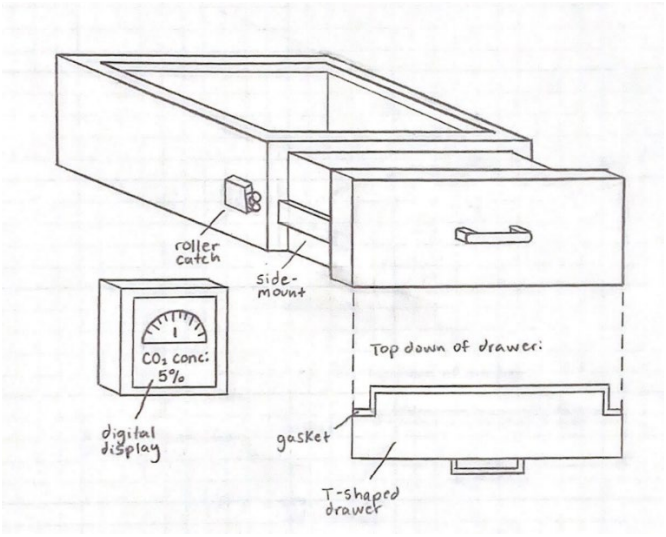

Figure 5: Morphology detailing the drawer slides, drawer closure, drawer clasp, and CO_2 concentration display for Concept #2.

The third concept, pictured in Figure 6, has the following functions: roller slides, flush drawer without gasket, elastic clasps, and an analog output. In this concept, we explored a more bare-bones option for the form of our device. The roller slides maintain basic drawer functionality, while the flush drawer design without a gasket provides a simpler form overall. Elastic clasps (essentially amounting to rubber bands) hold the drawer face tightly against the cabin body, and the analog CO_2 display provides continuous, basic information about gas levels inside the chamber.

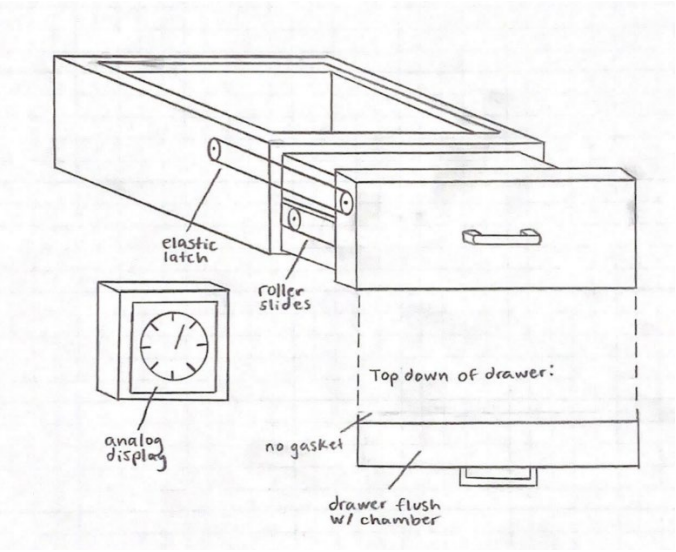

Figure 6: Morphology detailing the drawer slides, drawer closure, drawer clasp, and CO_2 concentration display for Concept #3.

Pugh Charts

Our Pugh chart helped to determine the front-runner concept for our design. The relative rankings of the three concepts are as follows: 2>1>3. The specifications that we considered for the Pugh chart included maintaining a sterile environment, rapid environment initialization, supports multiple culture vessel sizes, CO_2 concentration is updated every 1 second, and material does not crack or deform at temperatures reaching 50 °C. These specifications were chosen using the customer requirements and the higher risk specifications from the Engineering Specification table (Table 10). The results from each Pugh chart iteration for the group are listed in Tables 10.1, 10.2, and 10.3. The rest of the Pugh charts, prepared individually by each group member, are listed in *Appendix E*.

Table 10.1: Pugh chart with Concept 1 as the baseline. Relative ranking is 2>1>3.

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Metal slides, flush drawer with gasket, buckle clasp, RGB output	Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	Roller slides, flush drawer without gasket, elastic clasps, analog output
Maintaining a sterile environment	25	DATUM	1	-1
Rapid environment initialization	16.6666		0	-1
Supports different culture vessels sizes	21.6666		0	0
CO2 concentration is updated every 1 second	15		0	-1
Material does not deform/fog at temperatures reaching 50C	21.6666		0	-1
	Total		1	-4
	Weighted Total		25	-78.3332

Table 10.2: Pugh chart with Concept 2 as the baseline. Relative ranking is 2>1>3.

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	Metal slides, flush drawer with gasket, buckle clasp, RGB output	Roller slides, flush drawer without gasket, elastic clasps, analog output
Maintaining a sterile environment	25	DATUM	-1	-1
Rapid environment initialization	16.6666		0	-1
Supports different culture vessels sizes	21.6666		0	0
CO2 concentration is updated every 1 second	15		0	-1
Material does not deform/fog at temperatures reaching 50C	21.6666		0	-1
	Total		-1	-4
	Weighted Total		-25	-78.3332

Table 10.3: Pugh chart with Concept 2 as the baseline. Relative ranking is 2>1>3.

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Roller slides, flush drawer without gasket, elastic clasps, analog output	Metal slides, flush drawer with gasket, buckle clasp, RGB output	Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output
Maintaining a sterile environment	25	DATUM	-1	0
Rapid environment initialization	16.6666		1	1
Supports different culture vessels sizes	21.6666		0	0
CO2 concentration is updated every 1 second	15		1	1
Material does not deform/fog at temperatures reaching 50C	21.6666		1	1
	Total		2	3
	Weighted Total		28.3332	53.3332

The most important specification for our Pugh chart was sterility, which makes up 25% of the weight of the chart. We ranked Concept 2 the highest for the sterility specification because it will be easier to clean than the metal or roller slides. Concepts 1 and 2 were given the same relative ranking for the rapid environment initialization because both concepts have gaskets, which will help to maintain CO_2 levels better than the design without a gasket. All three concepts were given the same ranking for supporting different vessel sizes because all three concepts were designed with different vessel sizes in mind. Concepts 1 and 2 were both given the same relative ranking for the specification regarding updating CO_2 concentrations. This is because the digital displays will be constantly updated and easily update the user about CO_2 concentrations. Concept 3 was ranked below Concepts 1 and 2 in this area because the analog display will not alert the user about CO_2 concentrations as easily. The last requirement was that the material does not fog when exposed to heat. Concepts 1 and 2 were given the same ranking for this specification because they both have gaskets, which will prevent any cold air from entering the chamber. For this reason, Concept 3 was ranked below Concepts 1 and 2 for this specification.

All in all, the main differentiator between Concepts 1 and 2 is the drawer slides. The main reason that Concept 2 came out on top was side-mount slides will be easiest to clean and maintain a sterile environment, as sterility was our most important, high-risk specification.

Conceptual Model

Functional Decomposition Diagram

The functional decomposition diagram defines the different components our team is designing and how they will interact with each other and the user. Specifically, this diagram was created to help our team understand the components that would be directly attaching to our physical model discussed below as well as what interactions the user will perform

From this model we were able to determine that we will have external connections for gas injection and sampling directly into our chamber (Figure 7). Having established this, we will use our physical model and a MATLAB gas diffusion model to determine the appropriate placement of the tubing as it pertains to both gas sensing, ergonomics, and other space-related restraints. Additionally, this model details the ways in which the user will interact directly with the chamber. As we analyze the physical model we will interact with the chamber as the user would define in this diagram. This will highlight if we need to manipulate the physical model to better meet user needs and are within human ergonomic capabilities. Knowing explicitly where user interaction is required will also aid in our eventual development of an instruction manual or a set of guidelines for use and maintenance of the system.

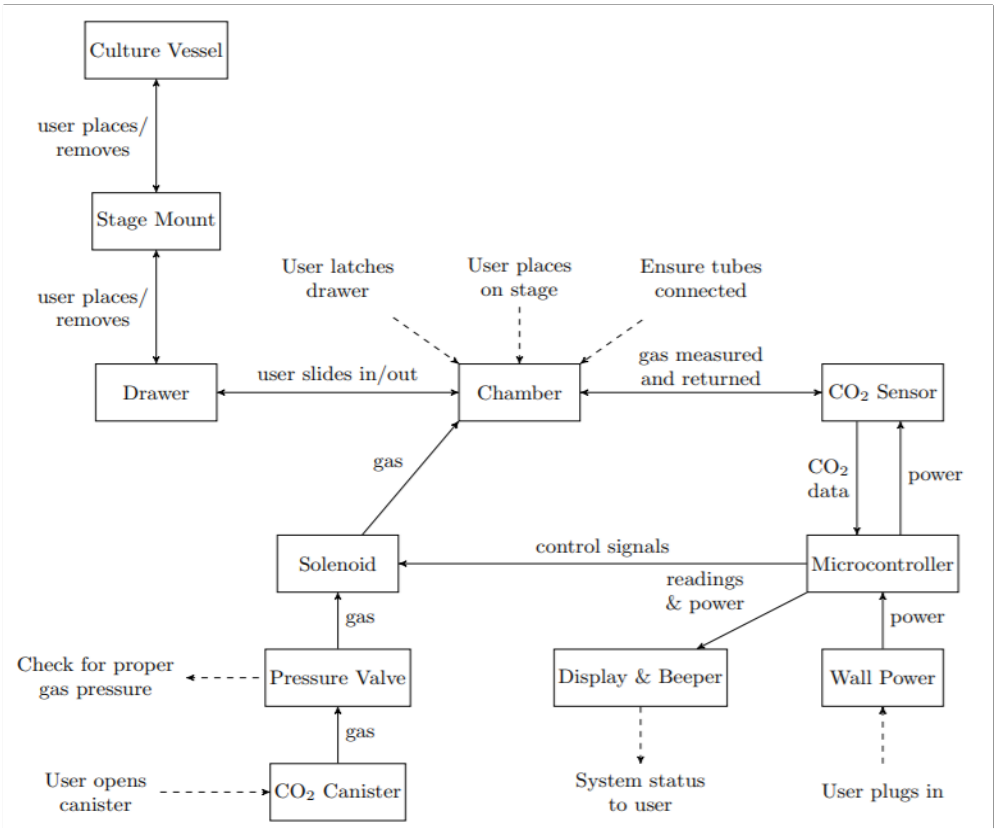

Figure 7: Flow diagram highlighting the interaction between each component of our design (chamber, microcontroller, and gas control) and with the user.

Our team determined that insertion of the correct stage insert and sample is currently difficult for the user with the current drawer model. Specifically, we have not yet designed into our model an

ability for the drawer to stay parallel with the microscope stage, so when the stage insert and sample are put into place, the drawer may fall off of the microscope stage. We should manipulate our design such that the drawer maintains rigidity and stays parallel with the stage, potentially by adding rails above the drawer insert as well as below.

For optimal use of the entire system, we recommend that the CO_2 gas cylinder be placed behind the user in the microscope directly next to the sink in 38-134. This will minimize the amount of inert tubing used that the user must check before each use. Similarly, this will allow for easier access to turn on and off the gas cylinder and verify that the pressure values are in the proper range.

Physical Model

The physical model of our chamber was made using cardboard and hot glue. It was designed to test functionality based on the size of the model. For example, to determine if the size of the drawer (Figure 8B) fits the stage inserts (Figure 8C) that are already compatible with Olympus IX73P2F Microscope and all customer required culture vessels. Also, it was used to determine if the size of the chamber is compatible with the Olympus IX73P2F Microscope (including both fluorescent and widefield functionalities) and where the inert tubing connections should be placed on the chamber.

Figure 8: Different views of the physical cardboard model of the chamber. **A**, overall view of gas control chamber. **B**, visualization of the drawer that fits the Olympus IX73P2F Microscope inserts already owned by the BMED Microscopy Core. **C**, bottom view of chamber, highlighting the placement of legs to fit into the microscope stage to ensure the chamber stays stationary.

To analyze the functionality of the drawer and overall chamber size, we brought our physical model to the BMED Microscopy Core's Olympus IX73P2F Microscope. We placed the chamber on the microscope stage without any of the inserts (Figure 9A, 9B). From there, we tested the full capacity of the stage to move in the Y, X, and Z direction. Then, we tested the capacity for the chamber to hold the stage insert (Figure 9C). Finally, our team discussed different routings for inert tubing and gas cylinder placement within the room, specifically as it pertained to the physical chamber model and the scope environment. Also, we took more measurements of the microscope stages and stage inserts.

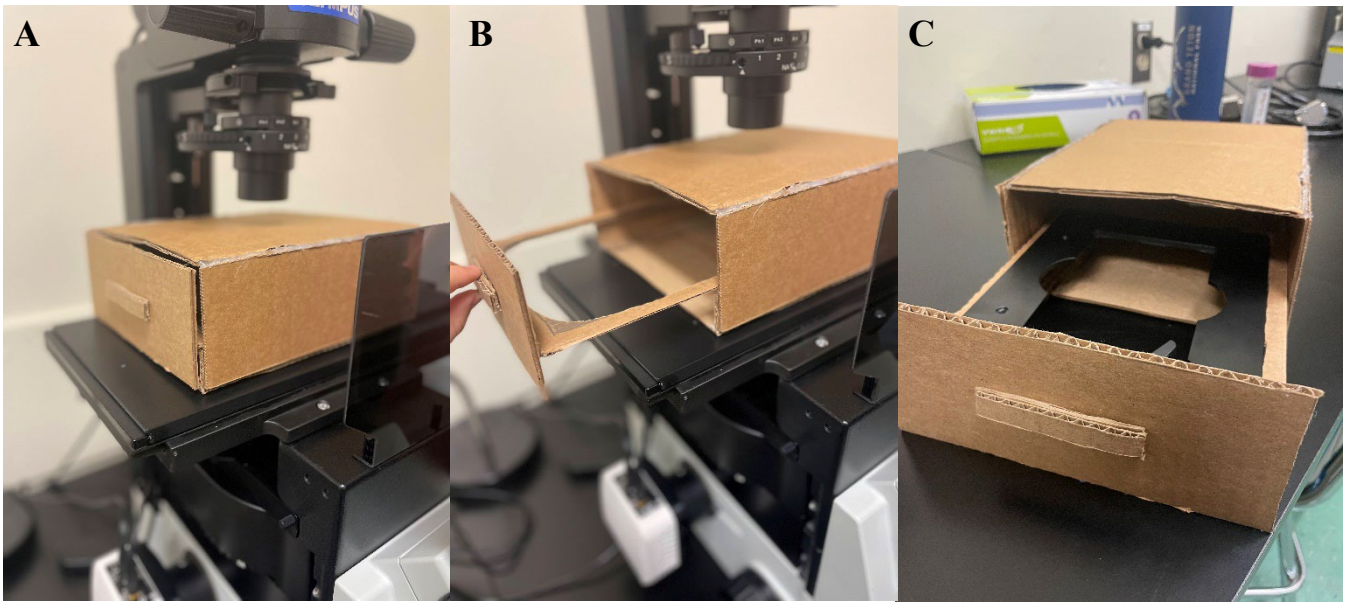

Figure 9: Physical model analysis on the Olympus IX73P2F Microscope.

Through this analysis we saw that when the drawer is open, there will be no support as it extends past the microscope stage. Therefore, we must create a drawer slide system that diminishes the ability for the drawer to slide out fully and allows the drawer to maintain the parallel position with the microscope stage so that the sample does not spill. Shown in Figure 9, the drawer is positioned to the left side of the microscope, this is the only direction in which a drawer can be placed due to physical obstruction on the other side. Therefore, it will be best to position our gas input on the opposite side of the drawer face.

Our current physical model impacts the y-axis movement of the brightfield. It only maintains about 30% of its range. Overall, any device we create will not allow for use of 100% of the range, but we would like to maximize the distance that can be used. In future iterations of the design, we may consider constructing a removable top for the enclosure. When the user is therefore working with the top off this would need to be done without gas control, as the gas would dissipate with removal of top.

Further, when considering the overall live cell imaging system (i.e., combined with the heating components), we noticed this would be too large to fit on the stage and maintain full x-axis movement. To minimize this the drawer system can be manipulated. For example, rather than

having the stage insert set into the drawer, the stage insert could be set on top of the drawer. This would be designed to fit exactly into the bottom of the stage insert such that it stays secure within the drawer.

Gas Diffusion Model

The MATLAB gas diffusion model will be used to represent the CO_2 gas diffusion in a chamber with dimensions that matched our chamber design. In conjunction with analysis of the physical location of gas injection, this model will allow us to determine the best placement for CO_2 injection tubing and sensor placement.

The diffusion model assumes that an initial amount of CO_2 has been injected into the chamber at one end and that ambient air contains negligible CO_2. This is expressed as an elevated concentration in a particular region (see Figure 10), which then diffuses across the chamber over time. Note that the plots generated by MATLAB show two spatial dimensions in the horizontal plane, and the vertical axis represents CO_2 levels. As a solution was calculated with respect to all 3 spatial dimensions, a particular level curve (i.e., a fixed height) must be selected to visualize the solution.

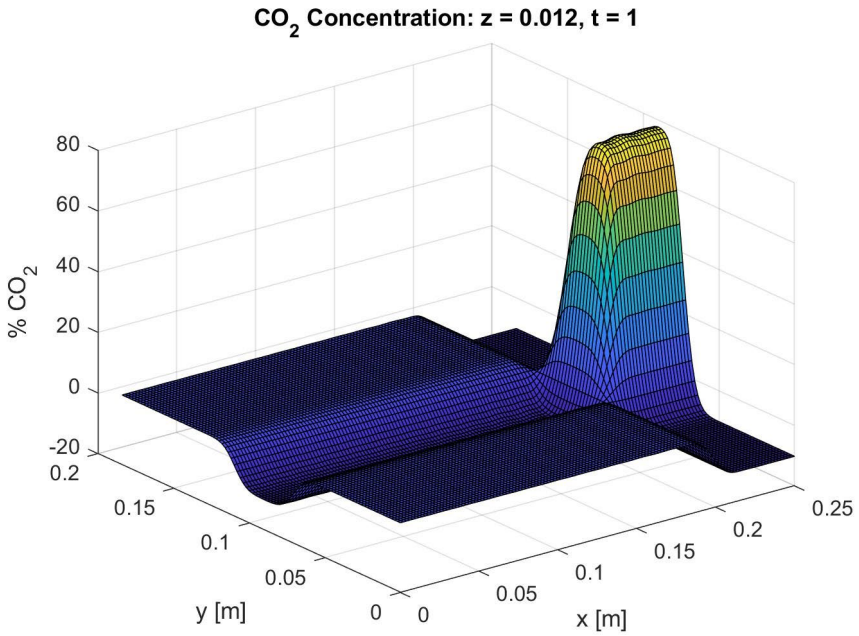

Figure 10: A snapshot of the MATLAB gas diffusion model after one second has elapsed. The chosen height plane is $z = 12\text{mm}$.

This model supports the placement of the CO_2 sensor at the opposite end of any gas injection, as placement too close to the gas inlet could result in vastly skewed readings. Furthermore, since the CO_2 is predicted to diffuse smoothly, a reading of 5% CO_2 at the opposite end of the gas inlet is strongly associated with a reading of 5% CO_2 throughout the chamber and, in particular, at the location of the cell culture vessel. Future uses of this model may include assisting in evaluating system function regarding the amount of CO_2 that is injected into the chamber. If more CO_2 than

predicted by this model is consistently necessary to maintain a 5% CO_2 output reading, this may indicate a nontrivial leak in the enclosure or some interference with the integrated gas sensor.

Detailed Design

Changes to Conceptual Model

After using the conceptual model to analyze functionality of the drawer and overall chamber size, we determined that the height of the chamber prototype was too tall. This dimension is a concern for two reasons: for one, the minimum distance between the condenser and the microscope stage is 25 mm, and secondly, the focal length of the objective determines how far away the sample may be from the objective while remaining in focus. These two factors, which are critical to the functionality of the chamber for imaging purposes, meant that we had to decrease the height of the chamber. The chamber prototype that we tested on the microscope was 38.1 mm in height. After calculating all our critical dimensions, we determined that the minimum height of the chamber is 32 mm. Although decreasing the height of the chamber is critical to the functionality of the project, another critical dimension is the distance between the bottom of the sample and the objective lens. The previous design included a drawer insert that the sample would rest inside, but the cutouts required for placing the drawers into added additional height between the sample and the objective lens. Therefore, we determined that the drawer slides were not an integral part of design and that the inserts could be placed in by hand and rest on the bottom of the chamber. This eliminated the need for a drawer pullout that we tested in our prototype. The final design consists of a box whose lid screws on and off through which the inserts can be placed in by hand. This design also allows the user to take the top off the chamber and locate the cells using the bright field microscopy and then place the top back on before performing fluorescent microscopy. This functionality allows the user to have a greater range of motion with the condenser to help locate the cells that are desired for imaging.

To determine the maximum height that the chamber can be, we measured the distance between the bottom of the condenser when located as low as possible and the top of the stage. This distance was determined to be 25 mm. Although we determined this distance to be 25 mm, there is no feasible way for our current design to accommodate for this dimension. Therefore, we will alert all users that a limitation of this design is that the condenser may not be any closer than 25 mm from the top of the stage. As previously stated, the top of the chamber is modular and can be removed while performing bright field microscopy, but the drawback to this is that the cells will not be contained within physiological conditions while the top of the chamber is removed. To calculate the focal distance of the objectives and determine the maximum distance that the cells may be from the objective, we imaged cells at varying distances from the objectives. The cells were initially seated 3 mm from the 4x and 10x objectives and images of the cells were captured. The distance from the objective was increased in increments of 1 mm until the cells were no longer in focus. The cells were in focus when placed 5 mm away from both the 4x and 10x objective lenses, but the image became blurred and unclear when the cells were placed 6 mm from the objectives. Therefore, the focal distance is somewhere between 5 and 6 mm for both the 4x and 10x objectives. This is an aspect of the design that we considered and must ensure that the bottom of the sample remains within 5 mm from the objectives to allow for clear imaging.

Material Selection

The material selections for the final design were chosen using Pugh matrixes for all three chamber components. The factors that we considered for these Pugh charts included ability to be sterilized, durability, cost, manufacturability, and ability to withstand heat. Ability to withstand heat and be sterilized are critical requirements expressed to us by our users, which is why these two factors were most heavily weighted. Manufacturability is also a main concern for the scope of our project, so that was a key factor that was considered while making material choices. The first component of the chamber that we determined was the bulk material of the chamber. This decision was between glass, acrylic, and polysulfone. Polysulfone is a thermoplastic known for its toughness and stability at high temperatures, which is why we wanted to consider this material for the bulk of the chamber. The results from each Pugh chart iteration for the bulk material of the chamber are listed in Tables 11, 12, and 13. The relative rankings for this aspect of the chamber are as follows: polysulfone, glass, and acrylic.

Table 11. Pugh matrix with glass as the baseline. Relative ranking: polysulfone>glass>acrylic.

Purpose: To determine the best material for the bulk of the chamber		BASELINE: Glass	Polysulfone	Acrylic
Ability to be sterilized	25	DATUM	0	-1
Durability	15		1	1
Cost	15		-1	0
Manufacturability	20		1	1
Ability to withstand heat	25		0	-1
	Total			
	Weighted Total		20	-15

Table 12. Pugh matrix with polysulfone as the baseline. Relative ranking: polysulfone>glass>acrylic.

Purpose: To determine the best material for the bulk of the chamber		BASELINE: Polysulfone	Glass	Acrylic
Ability to be sterilized	25	DATUM	0	-1
Durability	15		-1	0
Cost	15		1	1
Manufacturability	20		-1	0
Ability to withstand heat	25		0	-1
	Total			
	Weighted Total		-20	-35

Table 13. Pugh matrix with acrylic as the baseline. Relative ranking: polysulfone>glass>acrylic.

Purpose: To determine the best material for the bulk of the chamber		BASELINE: Acrylic	Glass	Polysulfone
Ability to be sterilized	25	DATUM	1	1
Durability	15		-1	0
Cost	15		0	-1
Manufacturability	20		-1	0
Ability to withstand heat	25		1	1
	Total		0	1
	Weighted Total		15	35

Pugh charts were prepared to determine the best material for the optically clear material through which imaging will be performed as well as the best material for the conductive base of the chamber. The factors that went into choosing these materials remained the same as for the bulk material of the chamber. The materials that were considered for the optically clear material included glass, acrylic, and polypropylene. The Pugh charts revealed the best material to use for the optically clear portion of the chamber is glass, followed by polypropylene and then acrylic. The Pugh charts for choosing an optically clear material are listed in *Appendix F*. The materials that were considered when determining what to use for the conductive base of the chamber included aluminum, stainless steel, and Indium-coated glass. The Pugh charts revealed that the best material to use for the conductive base of the chamber is aluminum, followed by stainless steel and then Indium-coated glass. The Pugh charts used to select the material for the conductive base of the chamber are listed in *Appendix G*. Therefore, the final design includes a polysulfone bulk material with a conductive base made from aluminum along with glass windows through which imaging will be performed.

CAD Model

A model of the final design was developed using SolidWorks and can be seen in Figure 10. This model indicates the dimensions, material choices, and inputs that will be used in the final design. This model gives us an idea of how all the inputs will interact with each other, which has proven helpful as several of the inputs were moved around once the model was developed. This model will be used in tandem with COMSOL to test the CO_2 gas diffusion throughout the chamber once CO_2 is injected, which will give us insight into the rate at which physiological conditions take to initialize as well as how well the gas disperses throughout the chamber.

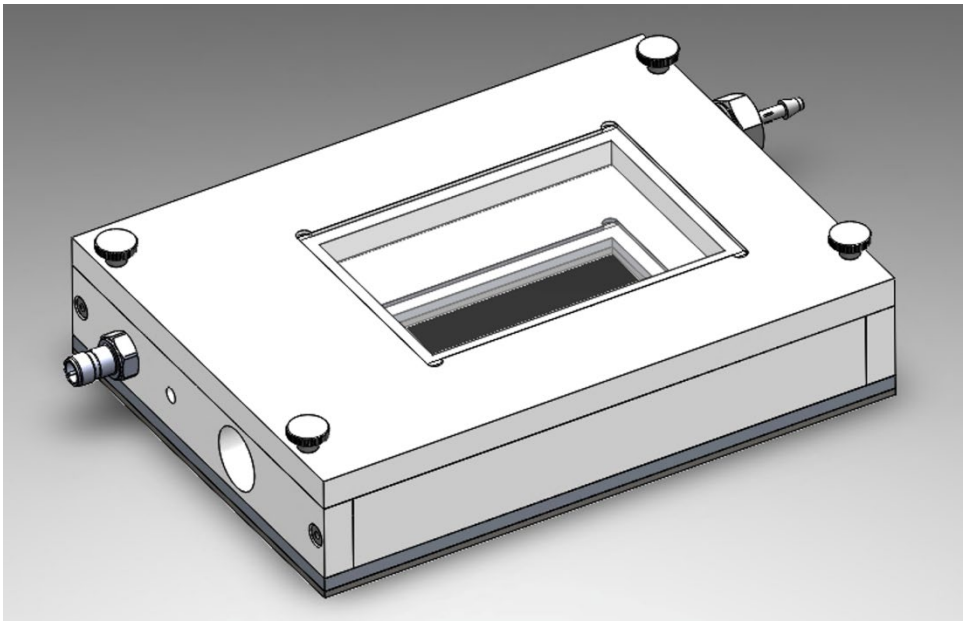

Figure 11: SolidWorks model of final chamber design.

Detailed Drawings

Using the SolidWorks model of the final design, detailed drawings were developed for each component of the chamber. These drawings indicate the dimensions, material choices, and locations of each of the inputs that will be used in the final design. Drawings were developed for the front, back, left side, right side, top, and bottom of the polysulfone as well as for the aluminum bottom, silicone bottom, glass top, and glass bottom pieces of the design. A detailed drawing of the right polysulfone piece of the chamber can be seen in Figure 11. The rest of the detailed drawings can be found in *Appendix H*. These drawings will be brought to the shop technicians on campus and used to manufacture the chamber.

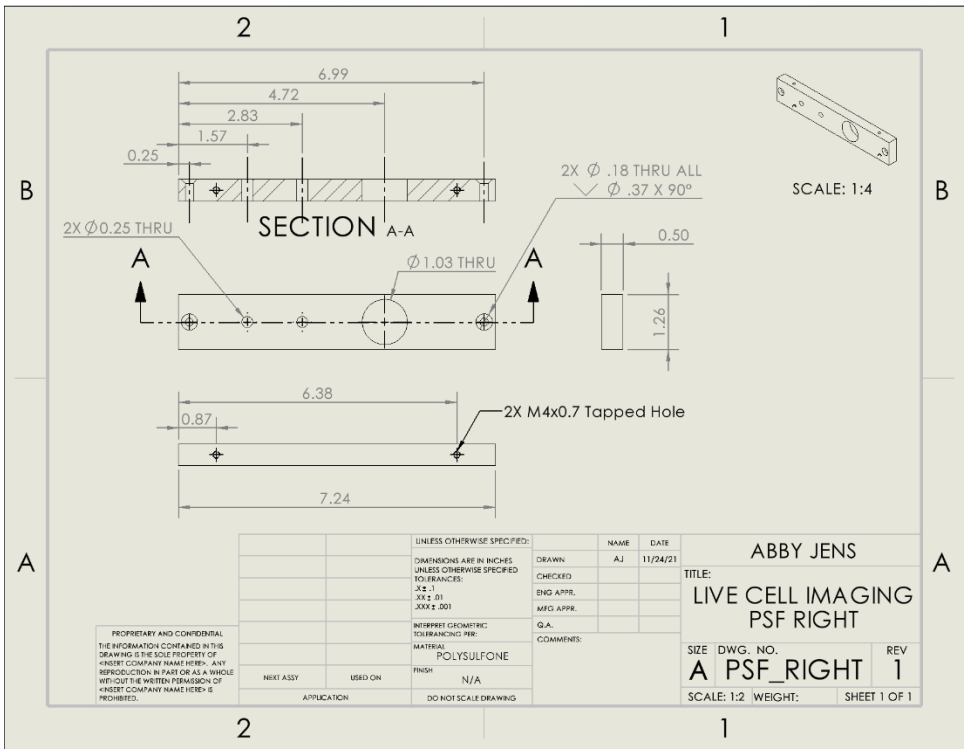

Figure 12. Detailed drawing of the right polysulfone chamber side.

Prototype Manufacturing Plans

This section provides an in-depth description of the manufacturing practices to be implemented to create a functional prototype of the Live Cell Imaging Device.

Bill of Materials

Table 14 is a detailed table of the materials required for the manufacturing of the Live Cell Imaging Device Chamber. Table 15 describes the subsystems that will be referenced during the Manufacturing Process Instructions (Table 17). Table 16 details the materials required for the manufacturing of the associated gas control system.

Table 14. Detailed Bill of Materials for the Live Cell Imaging Chamber used for the Manufacturing Process Instructions.

Item Number	Part Number	Quantity	Name	Material	Source
1	89155K11	1	Heat plate	6061 Aluminum	McMaster Carr
2	CS381-00500	1	Polysulfone sheet	Polysulfone	Boedeker Plastics Inc.
2.1		1	Chamber top	Polysulfone	
2.2		1	Chamber left side	Polysulfone	
2.3		1	Chamber right side	Polysulfone	
2.4		2	Chamber side	Polysulfone	
3	93395A266	8	25 mm M4 Screws	316 Stainless Steel	McMaster Carr
4	97163A152	4	M4 Tapered Heat Set Inserts	303 Stainless Steel	McMaster Carr
5	B08974SQ74	2	Glass Inserts	Tempered Glass	Amazon (Langdon House)
6	6937T92	2	Silicone Sealant	Silicone	McMaster Carr
7	5S-062-12	2	Silicone Sheet	Silicone	Amazon (Small Parts)
7.1			Silicone Bottom	Silicone	
7.2			Silicone Gasket	Silicone	
8	92558A370	4	25 mm M4 Thumb Screws	18-8 Stainless Steel	McMaster Carr
9	23MG87	1	APC Inline Insert, Male 1/4" NPT	Acetal	Grainger
10	403325	1	1/4" NPT Temperature Probe	316L Stainless Steel	Automation24
11	CAS25015	1	15 psi 1/4" NPT Pressure Relief Valve	Brass	Compressor Source
12	6802K22	1	Teflon Tape	PTFE	McMaster Carr
13	GC-0029	1	SprintIR®-6S 20% CO2 Sensor	NDIR LED	CO_2 Meter
14		1	PETG Filament	PETG	Hatchbox
14.1		1	CO_2 Sensor Housing	PETG	

Table 15. Table of subassemblies referenced in the Manufacturing Process Instructions for the Live Cell Imaging Chamber.

Subassembly Identifier	Subassembly Name	Item #	Item Name
SA1	Chamber Walls	2.2	Chamber Left Side
		2.3	Chamber Right Side
		2.4	Chamber Sides
		3	25 mm M4 Screws
SA2	Chamber	1	Heat Plate
		2.1	Chamber Top
		2.2	Chamber Left Side
		2.3	Chamber Right Side
		2.4	Chamber Sides
		3	25 mm M4 Screws
		8	25 mm M4 Thumb Screws

Table 16. Detailed Bill of Materials for the Live Cell Imaging Gas Control System used for the Manufacturing Process Instructions.

Item Number	Part Number	Quantity	Name	Material	Source
15	N/A	1	Relay PCB	Various	Osh Park, LLC
16	14-426-1K14	18 ft.	26 Gauge Hook-up wire	Copper, insulation	Coast Electronics, Inc.
17	FBA_12200001P	1	DC12V 2A Power Supply Adapter	Various	Amazon.com (Sansun)
18	LM2596	1	DC-DC Buck Converter/Voltage Regulator	Various	Amazon.com (Maxmoral)
19	B01AXIEDX8	1	9V Battery Clip with 2.1mm X 5.5mm Male DC Plug	Various	Amazon.com (Corpco)
20	EL-CB-001	1	Elegoo Uno R3 microcontroller	Various	Elegoo
21*	N/A	2	1 kΩ through-hole resistor	Various	Elegoo
22*	N/A	1	Red through-hole LED	Various	Elegoo
23	2N2222	1	TO-92 Transistor	Various	Amazon.com (C YUMU)
24	1N4007	1	DO-41 Axial Standard Rectifier Diode	Various	Amazon.com (HityTech)
25	RW-SH-105D	1	10 Amp relay, SPDT, Coil: 5VDC/73mA	Various	Coast Electronics, Inc.
26	N/A	1	Control system housing	PETG filament	California Filament
27	RA1113112R	1	Rocker Switch SPST 10A (AC) 125 V Panel Mount, Snap-In	Various	DigiKey Electronics
28*	N/A	1	Breadboard expansion board	Various	Elegoo
29*	N/A	1	LCD1602 Module (with pin header)	Various	Elegoo
30	800-00064	1	Jumper Wire M-F 40 Piece	Various	Coast Electronics, Inc.
31*	N/A	1	10 kΩ Potentiometer	Various	Elegoo
32*	N/A	1	220 Ω through-hole resistor	Various	Elegoo
33*	N/A	12	Jumper Wire M-M 1 Piece	Various	Elegoo
34	RSSM-3-12VDC	1	1/4" 12V DC Electric Brass Solenoid Valve	Various	electricsolenoidvalves.com
35	FIS160	2	Nylon Spade Connector, M/F pair	Nylon, Brass	Amazon.com (Amlits)
36	GC-0029	1	SPRINTIR - 6S 20% CO2 Sensor	Various	co2meter.com

*Obtained as part of an Elegoo Uno R3 Super Starter Kit (Product number B01D8KOZF4).

Summary of Required Machinery

To perform the machining described in the Manufacturing Process Instructions (Table 16) our team requires access to the Cal Poly Machine Shops. Figure 13 shows multiple machines necessary. Figure 13A shows the metric drill and tap set required for creating the holes necessary for our M4 screws; Figure 12B is the drill press; Figure 13C is the hand saw which may be necessary for recutting polysulfone pieces; Figure 13D is the manual mill; Figure 13E is the water jet. As none of the members of the group currently have a yellow tag, we require a shop technician to perform steps on the mill. Similarly, a shop technician is required to operate the water jet.

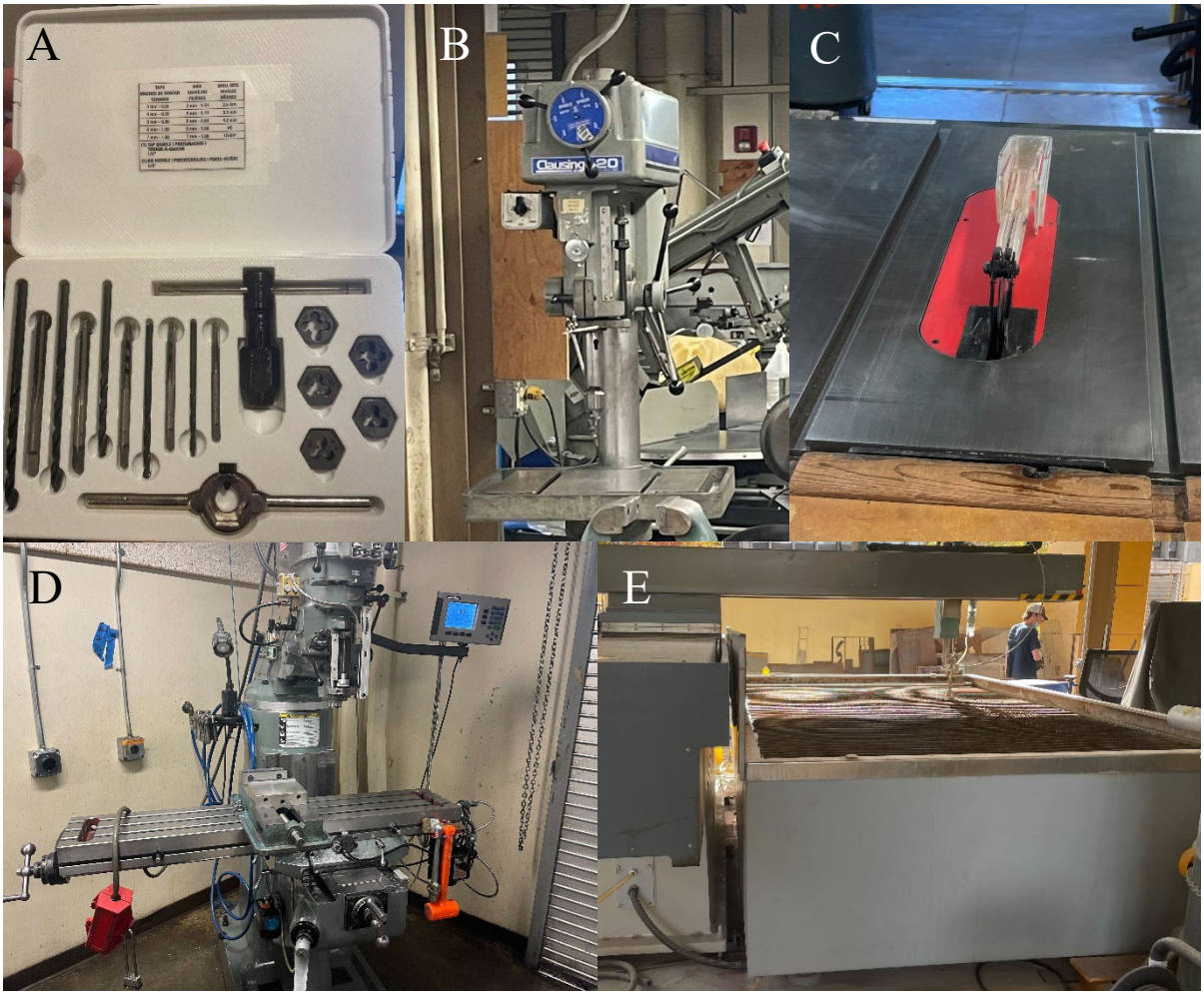

Figure 13. Required machinery to perform manufacturing of the stage top incubator. A, metric tap and drill set. B, drill press. C, hand saw. D, manual mill. E, water jet.

Other tools and machinery not shown include a soldering iron and tips, ¼" NPT tap, and 2.5 mm hex are all available through the Cal Poly Machine Shops. Similarly, PLA filament and 3D printers are available through the Cal Poly Innovation Sandbox. Other tools such as calipers and Exacto knife are available in 192-330 and sandpaper and glass scorer have been purchased for this project.

Manufacturing Process Instructions: Chamber

Detailed Manufacturing Process Instructions (MPI) for the system chamber can be found in Table 17. All item numbers referenced are detailed in the Bill of Materials (Table 14). The MPI describes the manufacturing required to produce the final chamber made of polysulfone chamber sides and top, the aluminum bottom, glass sheets placed into the top and bottom, and silicone gasket and bumper. Finished manufactured parts can be seen in Appendix I.

Table 17. Manufacturing Process Instructions for Chamber

Step #	Item #	Name	Tools	Description
1	1	Heat Plate	Water jet	Cut aluminum into 184mm x 250mm, cut a 83mm x 128mm rectangle as shown in Figure A. Holes will be piloted by the water jet.
2	2	Polysulfone Sheet	Water jet	Cut polysulfone out of the 12" by 24" sheet according to Figure B. Smaller holes will be piloted by the water jet.
3	1	Heat Plate	Mill	Use a 1/2" end mill to create a 1.8 mm recess around the center cut rectangle to a 6 mm width.
4	1	Heat Plate	Mill	Use a 1/4" end mill to cut into the corner of the 1.8 mm cut in order to create a dogbone shape shown in Figure A.
5	2.1	Chamber Top	Mill	Use a 1/2" end mill to create a 1.8 mm recess around the center cut rectangle to a 6mm width.
6	2.1	Chamber Top	Mill	Use a 1/4" end mill to cut into the corner of the 1.8 mm cut in order to create a dogbone shape shown in Figure A (Top and bottom pieces, although different materials maintain the same dimensions).
7	1	Heat Plate	Drill Press	Use an No. 19 drill bit (.1660 in, 4.21 mm) and drill four clearance holes centered on the four pilot holes created by the water jet.

8	1	2	Drill Press	Use a 90 degree counter sink bit and counter sink all four holes to a depth of 3 mm.
9	2.1	Chamber Top	Drill Press	Drill four 3.3 mm holes using a 3.3 mm drill bit on the four pilot holes created by the water jet.
10	2.2	Chamber Left Side	Drill Press, Tap Set	Use an No. 19 drill bit (.1660 in, 4.21 mm) and drill four clearance holes centered on the two outer most pilot holes created by the water jet. Use a 7/16" drill bit and drill holes centered on the final two pilot holes created by the water jet. Use a 1/4" NPT tap to tap both holes previously drilled (7/16").
11	2.3	Chamber Right Side	Drill Press, Tap Set	Use an No. 19 drill bit (.1660 in, 4.21 mm) and drill four clearance holes centered on the two outer most pilot holes created by the water jet. Use a 7/16" drill bit and drill a hole centered on the final pilot hole created by the water jet. Use a 1/4" NPT tap to tap the previously drilled hole (7/16").
12	2.2 2.3 2.4	Chamber Left Side Chamber Right Side Chamber Sides	Flat Work Surface, Drill Press	Temporarily align the front/back sides with the left side and right side. Ensure all surfaces are flush with each other then mark the front/back pieces through the M4 screw holes. Take the marked front/back sides and drill a 3.3 mm hole using a 3.3 mm drill bit to a depth of 14 mm (four holes total).
13	2.2 2.3 2.4	Chamber Left Side Chamber Right Side Chamber Sides	2.5 mm Allen wrench	Assemble chamber front/back sides and the left and right sides together using the M4 screws and 2.5 mm

	3	18 mm M4 Screws		hex to create the chamber walls subassembly. Disassemble and reassemble chamber walls with the front/back sides switched to verify interchangeability, if not interchangeable, label the parts.
14	1	Heat Plate	Mill	Use a 7/16" endmill to deepen the existing recess an additional 2 mm.
15	SA1 1 2.1	Chamber Walls Heat Plate Chamber Top	Drill Press, Metric Tap Set	Temporarily align the chamber wall subassembly with the heat plate such that all sides are flush. Mark the chamber wall subassembly through the four drilled clearance holes. Repeat by turning over the walls subassembly and using the chamber top. Use a No. 2 drill bit and drill four holes on the marked spots to a depth of 6.5 mm. Turn over the wall subassembly and use a 3.3 mm drill bit to drill four holes on the marked spots to a depth of 14 mm. Tap each 3.3 mm holes using the M4 x 0.7 mm tap.
16	SA1 4	Chamber Walls M4 Tapered Heat Set Inserts	Soldering iron	Heat the soldering iron to 650 -750 F. Ensure the chamber walls subassembly has the No. 2 drill bit holes facing upwards (the top). Place a heat-set insert on top of a hole, place the soldering iron within the heat set hole and slowly press the insert into the hole. Quickly remove the soldering iron once the insert is flush with the top. Repeat for all four holes.
17	5	Glass Inserts	Diamond glass cutter, Flat Work Surface	Score the 5" x 7" glass to create a 140mm x 95mm rectangle. Place the cuts parallel to a work surface, hold down one end firmly on the table and with firm downward movement on the other end break off the glass.

18	1 2.1 5 6	Heat Plate Chamber Top Glass Inserts Silicone Sealant	Weight	Apply a thin layer of silicone sealant to the recess in the chamber top and place the glass top within the recess. Place a weight on top of the entire surface and allow the chamber top with glass to cure for 24 hours. Repeat with the heat plate and glass bottom.
19	7	Silicone Sheet	Exacto Knife	Cut the 12" x 12" silicone sheet into a 184 mm x 250 mm rectangle. Cut out a 83 mm x 128 mm rectangle 50.5 mm from the 250mm edge and 44.0 mm from the 184 mm edge (7.1 Silicone Bottom). Then cut two 184 mm by 9.525 mm and two 224.6 mm by 9.525 mm . Use the chamber top to mark where the M4 screws will pass through the silicone then punch out 4 mm holes using a 4mm biopsy punch (Silicone Gasket)
20	1 6 7.2	Heat Plate Silicone Sealant Silicone Gasket	Weight	Apply a thin layer of silicone sealant to the chamber top and place the cut four silicone rectangles, which make up the silicone gasket on top. Place a surface weight over the entire surface and leave to cure for 24 hours.
21	SA1 1 2.1 3 8	Chamber Walls Heat Plate Chamber Top 18 mm M4 Screws 16 mm M4 Thumb Screws	2.5 mm Allen wrench	Assemble the heat plate to the chamber walls subassembly using the 2.5 mm hex. Turn over the subassembly and using the M4 thumb screws tighten the pieces together.
22	SA2 9 10 11	Chamber APC Inline Insert, Male 1/4" NPT 1/4" NPT Temperature Probe 15 psi 1/4" NPT Pressure Relief Valve	--	Apply teflon tape to APC inline insert and screw into the chamber right side 1/4" NPT tapped hole. Apply teflon tape to the temperature probe insert threads and screw into the chamber left side 1/4" NPT tapped hole closest to the M4 screw. Apply teflon tape to the pressure

	12	Teflon Tape		relief valve thread and screw into the chamber left side 1/4" NPT tapped hole next to the temperature probe insert.
23	SA2 13 14	Chamber SprintIR®-6S 20% CO2 Sensor PLA Filament	Calipers , 3D Printer and PLA	Measure the inner diameter of the final open hole and measure the diameter of the CO2 sensor. Determine the difference between the two and edit the CO2 sensor housing CAD to ensure the correct thickness. Slice the .stl file of the CO2 sensor housing and print the file.
24	SA2	Chamber	--	Place the CO2 sensor in the CO2 sensor housing and press into the hole in the chamber subassembly.

Manufacturing Process Instructions: Gas Control

Detailed MPI for the gas control system can be found in Table 18. Step numbering continues from the MPI in Table 17 to avoid ambiguity. All item numbers referenced are detailed in the Bill of Materials (Table 16). Finished manufactured parts can be seen in Appendix K.

Table 18. Manufacturing Process Instructions for Gas Control System

Step #	Item #	Name	Tools	Description
25	15 21 24	Relay PCB 1 kΩ through-hole resistor DO-41 Axial standard rectifier diode	Soldering iron	Solder resistors into R1 and R2 positions marked on item 15. Solder diode into position marked D1, being mindful of polarity.
26	15 22 23	Relay PCB Red through-hole LED TO-92 transistor (2N2222)	Soldering iron	Solder LED into position on item 15, orienting shorter lead toward blunt end of silkscreen mark. Solder transistor into position Q1, aligning flat ends of package and silkscreen mark.
27	15 25	Relay PCB 10 Amp relay, SPDT	Soldering iron	Solder relay to position K1 on PCB.
28	15 16	Relay PCB 26 Gauge Hook-up wire	Soldering iron, wire stripper, screwdriver	Solder about 6 in. of wire each to V_IN and GND wire pads on relay PCB. Using a screwdriver, secure

	17	DC12V Power Supply Adapter		other ends to DC power adapter: V_IN wire to (+) terminal, GND wire to (-) terminal.
29	15 16 18	Relay PCB 26 Gauge Hook-up wire DC-DC Buck Converter/Voltage Regulator	Soldering iron, wire stripper, screwdriver, multimeter	Solder 3-4 in. of wire each to VR_12+ and VR_12- wire pads on relay PCB. Solder other end of VR_12+ wire to IN+ terminal on buck converter, and solder VR_12- wire to IN- terminal on buck converter. Power the circuit and adjust the potentiometer on the buck converter until the output voltage reads 5V on a digital multimeter. Using 3-4 in. of wire, solder OUT+ terminal on buck converter to VR_5+ wire pad on relay PCB. Repeat to connect OUT- terminal on buck converter to VR_5- wire pad.
30	15 16 19 20	Relay PCB 26 Gauge Hook-up wire 9V Battery Clip with Male DC Plug Elegoo Uno R3 microcontroller	Soldering iron, wire stripper, wire cutter	Cut the battery clip end off of item 19, leaving a male DC plug with a red and black wire attached. Solder red wire to V_UNO+ wire pad, and solder black wire to V_UNO- wire pad. Connect the male DC plug to the DC power input on the microcontroller.
31	15 16 34 35	Relay PCB 26 Gauge Hook-up wire 12V DC Brass Solenoid Valve Nylon spade connector, M/F pair	Soldering iron, wire crimping tool, wire cutter	Cut two 10 in. lengths of wire, crimping F spade connectors onto one end of each. Solder other end of one wire to V_SOL+ wire pad. Repeat with other end of remaining wire and V_SOL- wire pad. Crimp matching M spade connectors onto solenoid valve wires.
32	15 33	Relay PCB Jumper Wire M-M 1 Piece	Soldering iron	Solder one end of jumper wire to CTRL wire pad on relay PCB.
33	20	Elegoo Uno R3 microcontroller	Laptop, USB cable, Arduino IDE	Upload control program (Appendix L) to microcontroller.
34	26	Control system housing	3D printer	Print box to house electrical components.
35	15 26	Relay PCB Control system housing	Wire cutter, soldering iron	Press switch into control system housing. Cut wire connecting (+) terminal on power adapter to V_IN

	27	Rocker switch SPST 10A		wire pad on relay PCB. Solder one end of the cut wire to one terminal on the switch. Solder other end of the cut wire to the other terminal on the switch.
36	16 20 33 36	26 Gauge Hook-up wire Elegoo Uno R3 microcontroller Jumper Wire M-M 1 Piece SPRINTIR-6S 20% CO_2 Sensor	Soldering iron, wire cutter, electrical tape	Solder wires (about 3 ft. each) to each of the 4 pins on the CO_2 sensor. Solder other ends of these wires to M-M jumper wires and wrap these junctions in electrical tape to protect against short circuits. Connect R_x in pin to digital I/O pin 6 on microcontroller. Connect T_x out pin to digital I/O pin 7 on microcontroller. Connect GND pin to a GND pin on microcontroller and connect V_{in} pin to 3.3 V power from the microcontroller.
37	20 28 29 30 31 32 33	Elegoo Uno R3 microcontroller Breadboard expansion board LCD1602 Module (with pin header) Jumper Wire M-F 40 Piece 10 kΩ Potentiometer 220 Ω through-hole resistor Jumper Wire M-M 1 Piece	None	Make the following connections between the LCD module and the microcontroller: LCD RS pin to digital pin 12, LCD Enable pin to digital pin 11, LCD D4 pin to digital pin 5, LCD D5 pin to digital pin 4, LCD D6 pin to digital pin 3, LCD D7 pin to digital pin 2, LCD R/W pin to GND, LCD VSS pin to GND, LCD VCC pin to 5V, LCD LED+ to 5V through a 220 Ω resistor, LCD LED- to GND. Also, wire the potentiometer inputs to 5V and GND, and wire its output to LCD VO pin.

Table 19. Design History File for Chamber Manufacturing

MPI Steps	Deviations from MPI	Completed By	Initials	Date
7	No 19 holes were still drilled without pilot holes	Abby Jens	AJ	1/12/2022
9	No 30 holes were still drilled without pilot holes	Abby Jens	AJ	1/12/2022
8		Abby Jens	AJ	1/12/2022
9		Abby Jens	AJ	1/12/2022
1-2	Pilot holes were no longer water jetted as it caused cracking in the polysulfone.	Abby Jens	AJ	1/15/2022
3-4	Milled down recess in aluminum sheet 2mm with a 7/16" end mill.	Abby Jens	AJ	1/15/2022
5-6	A 1/2" end mill was used for all milling.	Elsa Bean	EB	1/18/2022
19		Elsa Bean	EB	1/18/2022
12		Abby Jens	AJ	1/18/2022
13		Abby Jens	AJ	1/18/2022
15		Abby Jens	AJ	1/18/2022
3-4	Depression was milled to 12 mm compared to written 6 mm width. A 1/2" end mill was used for all milling.	Elsa Bean	EB	1/20/2022
17	Aluminum glass insert was cut to fit the new 12mm width such that the insert was 152mm x 107mm.	Elsa Bean	EB	1/22/2022
10-11		Abby Jens	AJ	1/24/2022
20		Abby Jens	AJ	1/24/2022
16		Abby Jens	AJ	2/2/2022
18		Abby Jens	AJ	2/2/2022
23-24	PETG filament was used instead of PLA.	Elsa Bean	EB	2/8/2022
21		Abby Jens	AJ	2/8/2022
14		Abby Jens	AJ	2/8/2022
22		Elsa Bean	EB	2/11/2022

Table 20. Design History File for Gas Control System Manufacturing

MPI Steps	Deviations from MPI	Completed By	Initials	Date
36		Elsa Bean	EB	2/10/2022
34		Elsa Bean	EB	2/28/2022
25		Brady Berg	BB	3/5/2022
26	Change R1 to 10 Ω through-hole resistor and replace LED with an electrical short (use M-M jumper wire) to supply sufficient voltage to solenoid valve.	Brady Berg	BB	3/5/2022
27		Brady Berg	BB	3/5/2022
28		Brady Berg	BB	3/5/2022
30		Brady Berg	BB	3/5/2022
31		Brady Berg	BB	3/5/2022
32		Brady Berg	BB	3/5/2022
33		Brady Berg	BB	3/5/2022
35		Brady Berg	BB	3/5/2022
36		Brady Berg	BB	3/5/2022
37		Brady Berg	BB	3/6/2022
29	Change output of buck converter from 5V to 9V to supply sufficient power to LCD screen via the microcontroller.	Brady Berg	BB	3/6/2022

Test Plans

In this section, we detail all tests planned to evaluate the function of our prototype. Note that any test which involves CO_2 tanks and pressure regulators will require additional safety training before being undertaken. We expect that most, if not all, intended end users are already trained in how to safely interact with these tanks due to their use with standard cell incubators, and thus assume a good number of end users will be ready to interact with and rate the function of our prototype as soon as it is manufactured.

General Performance Tests

Specification: Material allows for captured images to be within 20% of the maximum relative degree of focus obtained without use of the enclosure.

Test Procedure:

1. Have a trained lab researcher prepare cell culture sample for imaging on the Olympus IX73P2F scope as normal (i.e., without the incubation chamber).
2. Capture 10 distinct images of the sample using a 4x objective lens.
3. Capture 10 distinct images of the sample using a 10x objective lens.

4. Reconfigure the microscope stage using the same cell culture sample, this time placed inside the incubation chamber.
5. Repeat steps 2 and 3 for this setup.
6. Use MATLAB to obtain numerical values for relative degree of focus for all images captured.
7. Determine, at each magnification level, the maximum relative degree of focus for the images captured in steps 2 and 3.
8. Determine whether the relative degree of focus for images captured at 4x magnification while using the incubation chamber falls within 20% of the maximum determined for 4x magnification in step 7. Repeat for 10x magnification.

Materials Required:

- Olympus IX73P2F microscope, including 4x and 10x objective lenses and the connected computer.
- Existing cell culture sample.
- Prototype incubation chamber.
- MATLAB, including various auxiliary toolboxes.

Specification: System establishes desired 5% concentration of CO_2 within 5 minutes of powering on.

Test Procedure:

1. Connect the incubation chamber with CO2 sensor, pressure release valve, and 3D printed 1/4" NPT bolt.
2. Open the CO2 gas canister and observe the pressure on the regulator. Ensure there is no leakage.
3. Open up processing with data recording code (**Appendix**).
4. Flush ambient air through the incubation chamber.
5. Install the chamber lid.
6. Ensure the file output name is correct. Press the "play" button to begin recording data on processing while holding the CO2 input tubing.
7. Once the CO2 has flushed the tubing for 1 second, attach the input to the chamber.
8. Monitor the CO_2 concentration readings closely for attainment of 5% CO_2.
9. After 10 minutes stop recording data.
10. Graph results and record when 5% was reached and the highest CO_2 concentration recorded.
11. Repeat steps 4-10 for a total of 10 trials.

Materials Required:

- Complete prototype, including control systems, gas tubing, and power sources.
- CO_2 gas tank and pressure regulator.

Specification: After suspension of CO_2 regulation, the 5% CO_2 levels inside the chamber do not return to standard atmospheric values within 3 hours.

Test Procedure:

1. Follow the procedure the procedure for "System establishes desired 5% concentration of CO_2 within 5 minutes of powering on." for steps 1-7.
2. Once the system has completed to CO2 injections and the concentration has reached about 5% CO_2 unplug the solenoid from the breadboard (green wire attached to the solenoid).
3. Close the CO_2 gas tank.
4. Monitor the data collection on the processing console every 10 minutes (600,000 ms) .
5. If you observe CO2 concentrations below 0.3% stop data collection and record the time elapsed.
6. Repeat steps 1-5 for a total of 3 trials.

Materials Required:

- Complete prototype, including control systems, gas tubing, and power sources.
- CO_2 gas tank and pressure regulator.

Specification: System reestablishes 5% CO_2 concentration within 2 minutes after removal and replacement of enclosure lid.

Note: continued readings until they appeared stable above 5%

Test Procedure:

1. Follow the procedure the procedure for "System establishes desired 5% concentration of CO_2 within 5 minutes of powering on." for steps 1-7.
2. Once 5% has been established for a minimum of 2 minutes continue to step 3.
3. Remove the chamber lid, move in a sample, then close the lid again.
4. Monitor the CO_2 concentration readings closely for attainment of 5% CO_2.
5. Record time at which 5% CO_2 is first attained. If CO_2 levels do not reach 5% by the time 2 minutes have elapsed, record the CO_2 level at the 2-minute mark and continue monitoring until up to 5 minutes have elapsed total.
6. Repeat steps 3-7 for a total of 10 trials.

Materials Required:

- Stopwatch.
- Complete prototype, including control systems, gas tubing, and power sources.
- CO_2 gas tank and pressure regulator.

Durability Tests

Specification: Material does not crack, deform, or fog after 6 hours of exposure to temperatures up to 50°C.

Test Procedure:

1. Obtain a scrap/excess rectangular piece of polysulfone and an analogous piece of glass from the manufacturing of the prototype.
2. Seal scrap pieces together in an L-shape with the epoxy used to connect polysulfone and glass for the incubation chamber. Allow to set as needed.
3. Place assembly in toaster oven set to approximately 50°C. Start a stopwatch.
4. After an hour has elapsed, open toaster oven and briefly inspect assembly for cracks, degradation, and other signs of damage. Record any that are found.
5. Replace assembly in toaster oven.
6. Repeat steps 4 and 5 until 6 total hours have elapsed.

Materials Required:

- Scrap pieces of polysulfone and glass (from prototype manufacturing).
- Epoxy (from prototype manufacturing).
- Stopwatch.
- Toaster oven.

Specification: Body of enclosure withstands regular cleaning with 70% isopropyl alcohol solution and periodic sterilization in an autoclave.

Test Procedure:

1. Obtain a scrap/excess rectangular piece of polysulfone and an analogous piece of glass from the manufacturing of the prototype.
2. Seal scrap pieces together in an L-shape with the epoxy used to connect polysulfone and glass for the incubation chamber. Allow to set as needed.
3. Submerge assembly in 70% isopropyl alcohol solution for 1 week.
4. Remove assembly from solution and dry.
5. Visually inspect for cracks, degradation, and other signs of damage. Record any that are found.
6. If none are found, place the assembly in an autoclave and cycle once.
7. Repeat step 5.

Materials Required:

- Scrap pieces of polysulfone and glass (from prototype manufacturing).
- Epoxy (from prototype manufacturing).
- 70% isopropyl alcohol solution.
- Autoclave

Usability Tests

Specification: System weighs no more than 20 lbs.

Test Procedure:

1. Use digital scale to weigh assembled incubation chamber (enclosure only, no control system wiring or gas tubing attached).

Materials Required:

- Digital scale.
- Assembled prototype enclosure.

Specification: Internal volume of enclosure exceeds 17.5 in^3.

Test Procedure:

1. Use tape measure to measure internal length, width, and depth of incubation chamber. Record resulting volume.

Materials Required:

- Tape measure.
- Assembled prototype enclosure.

Specification: Total external volume of system (excluding CO_2 tank) does not exceed 3 ft^3.

Test Procedure:

1. Locate a cabinet shelf (or subsection of a shelf) whose internal volume is at most 3 ft^3.
2. Attempt to place all components of system (excluding CO_2 tank) into cabinet shelf.

Materials Required:

- Storage cabinet.
- Complete prototype, including control systems, gas tubing, and power sources.

Specification: Users ask at most 5 separate questions of designers when attempting to set up system for the first time.

Test Procedure:

1. Present user with instruction manual for use of system.
2. Request that the user attempt to set up system using their knowledge and the information available in the instruction manual, and that if they have questions (ambiguity or difficulties implementing the setup protocols) to please ask for clarification.
3. Record all questions asked by a specific user during their attempt, and answer questions as thoroughly as possible.

4. Evaluate the finished effort of the user for any noticeable errors. Record these errors and tabulate the total number of questions and errors for the user.
5. Repeat steps 1-4 for a total of at least 2 separate users.

Materials Required:

- Complete prototype, including control systems, gas tubing, and power sources.
- CO_2 gas tank and pressure regulator.
- Instruction manual.
- Olympus IX73P2F microscope.

Summary of Tests

All tests are described in Table 21, 22, and 23 along with required resources including personnel, equipment, materials and where and when the test will happen. The final testing days are to encompass if other tests are not passed, allowing time for manipulation of CO_2 control code or circuitry and repeating testing protocols as well as time to work in tandem with the Heated Stage group.

Table 21. Overview of testing for the chamber including material testing, relative focus testing, user testing, and system specifications.

Task #	Task	Start Date	End Date	Location	Peronnel	Equipment	Materials	Time
1	Cleaning test with submerged silicone sealant, polysulfone, glass in IPA for 1 week	1/25/2022	2/1/2022	192-330	Abby	Container	70% IPA, polysulfone, silicone, glass, silicone sealant combined test scraps	N/A
2	Toaster oven durability test with silicone sealant, polysulfone, and glass	1/25/2022	1/25/2022	Brady's Home	Brady	Toaster Oven	polysulfone, silicone, glass, silicone sealant combined test scraps	2-8PM
3	Autoclave durability test with silicone sealant, polysulfone, glass, silicone	1/25/2022	1/25/2022	192-328	Elsa	Autoclave	polysulfone, silicone, glass, silicone sealant combined test scraps	8-11AM
4	Relative Focus Testing and Compressor Restriction Testing	1/26/2022	1/26/2022	38-134	Elsa	Olympus Microscope, Computer, Matlab, Calipers	Completed assembled chamber	4-8PM
18	System weight, internal volume, external volume,	2/8/2022	2/8/2022	192-330		Digital scale, tape measure	Completed chamber	12-1PM
19	User Testing: Number of questions and number of actions to initialize the system	2/8/2022	2/8/2022	38-134		Olympus Microscope, CO2 Gas Cylinder	Chamber, gas control, and heat control	1-4PM
25	Continued Testing (user testing, repetition of tests)	2/22/2022	2/22/2022	38-134		Olympus Microscope, CO2 Gas Cylinder	Chamber, gas control, and heat control	12-4PM
26	Continued Testing (user testing, repetition of tests)	2/24/2022	2/24/2022	38-134		Olympus Microscope, CO2 Gas Cylinder	Chamber, gas control, and heat control	12-4PM

Table 22. Overview of testing for gas control group to determine the ability of the control system to establish or re-establish 5% CO_2 during system initialization and sample manipulation.

Task #	Task	Start Date	End Date	Location	Peronnel	Equipment	Materials	Time
7	System establishes 5% within 5 minutes of powering on	1/27/2022	1/27/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12-12:15PM
8	System reestablishes 5% within 2 minutes of top removal	1/27/2022	1/27/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12:15-12:30PM
9	TRIAL 1: Initialization test, CO2 maintanence after gas input is stopped, CO2 output update for 10 seconds	1/27/2022	1/27/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12:30-4PM
11	System establishes 5% within 5 minutes of powering on	2/1/2022	2/1/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12-12:15PM
12	System reestablishes 5% within 2 minutes of top removal	2/1/2022	2/1/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12:15-12:30PM
13	TRIAL 2: Initialization test, CO2 maintanence after gas input is stopped, CO2 output update for 10 seconds	2/1/2022	2/1/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12:30-4PM
14	System establishes 5% within 5 minutes of powering on	2/3/2022	2/3/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12-12:15PM
15	System reestablishes 5% within 2 minutes of top removal	2/3/2022	2/3/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12:15-12:30PM
16	TRIAL 3: Initialization test, CO2 maintanence after gas input is stopped, CO2 output update for 10 seconds	2/3/2022	2/3/2022	38-134	Brady, Elsa, Abby	CO2 Gas Cylinder	Assembled chamber and gas control	12:30-4PM
22	System established 5% within 5 minutes of power on (7) and System reestablished 5% within 2 minutes of top removal (7)	2/15/2022	2/15/2022	38-134	Brady, Elsa, Abby	Olympus Microscope, CO2 Gas Cylinder	Chamber, gas control, and heat control	12-4PM

Table 23. Overview of testing for the heated stage group including COMSOL model validation, sensor accuracy, and the maintenance of a set temperature during initialization and sample manipulation.

Task #	Task	Start Date	End Date	Location	Peronnel	Equipment	Materials	Time
5	COMSOL heating simulation	1/26/2022	2/8/2022	192-329	Simon, Anna	Computer, COMSOL	N/A	
6	Test accuracy of RTD sensor	1/27/2022	1/27/2022	192-329	Simon, Anna, Kerri	RTD sensor, heating controll system, temp radar gun	ice water, hot water, beakers	12-3PM
10	Determine optimal heating cycle time with PID control	2/1/2022	2/1/2022	192-330	Anna, Simon, Kerri	Temperature radar gun	Assembled chamber and heat control	12-3PM
17	Test heaters for 3-4 hours to ensure proper function over long usage	2/8/2022	2/8/2022	192-329	Anna, Simon, Kerri	Temperature radar gun	Assembled chamber and heat control	12-4PM
20	Determine accuracy of COMSOL model	2/10/2022	2/10/2022	192-329	Anna, Simon, Kerri	Temperature radar gun	Assembled chamber and heat control	9AM-12PM
21	Initialization testing	2/15/2022	2/15/2022	192-329	Anna, Simon, Kerri	Temperature radar gun	Assembled chamber and heat control	11AM-12PM
23	Test that temperature variation at the sample does not differ beyond tolerance from setpoint	2/17/2022	2/17/2022	192-329	Anna, Simon, Kerri	Temperature radar gun	Assembled chamber and heat control	12-3PM
24	Repetition of temperature variation testing	2/22/2022	2/22/2022	192-329	Anna, Simon, Kerri	Temperature radar gun	Assembled chamber and heat control	12-3PM
27	Testing: time to reheat up after perturbation	2/24/2022	2/24/2022	192-329	Anna, Simon, Kerri	Temperature rada	Assembled chambe	12-3PM

Testing Results

Material Testing

Heat Exposure

After following protocols to test the material for 6 hours in temperatures up to 50˚C, there was no change in the material. There were no cracks, deformations, or fog on the material. When held, the polysulfone was not hot, but the aluminum and glass were warm. See Figure 14 for samples after they have been removed from the toaster oven. The material durability testing of heat over time met our specifications. Our team defined passing as the material resisting all cracking, deformation, and fogging after 6 hours of exposure to temperatures up to 50˚C which the material did.

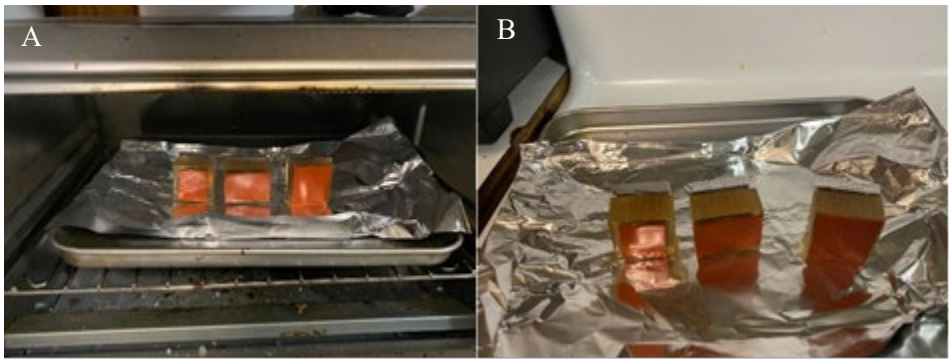

Figure 14. A, Material testing samples in the toaster oven. B, Material testing samples after they have been removed from the oven showing no deformation

Laboratory Cleaner (70% Isopropyl Alcohol) Exposure

After following protocols to test if the material could withstand regular cleaning with a 70% isopropyl alcohol solution, we found that the silicone, aluminum, and polysulfone kept their physical properties. There was a slight reaction on the aluminum as seen in Figure 15. The silicone sealant was broken down enough to have the pieces come apart by the isopropyl after being submerged for 7 days, but the silicone sealant did not break down completely. The material testing of 70% isopropyl alcohol solution passed our specifications. Even though there was slight corrosion and silicone glue degradation when submerged in the isopropyl, it was not drastic enough to affect the functionality of the device. If there is degradation over the many years of isopropyl exposure equivalence, users would be able to apply a new coat of silicone glue.

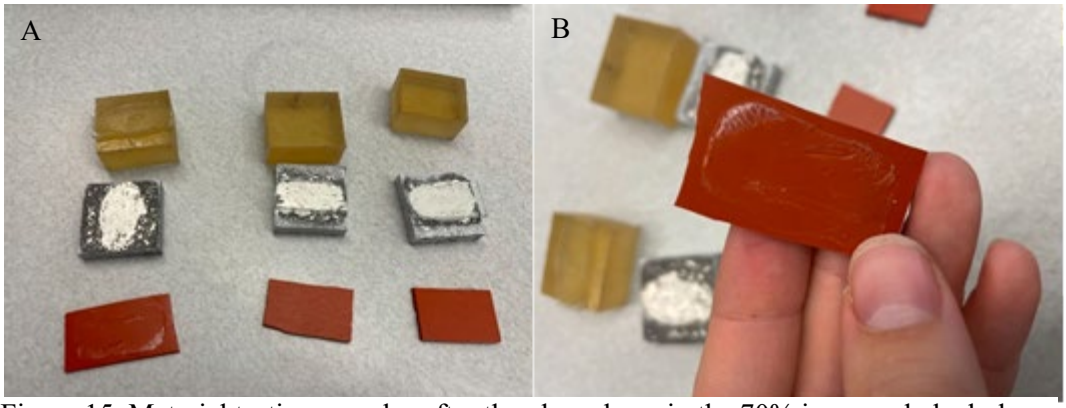

Figure 15. Material testing samples after they have been in the 70% isopropyl alcohol solution for 7 days. A, Slight corrosion of aluminum after material testing. B, Silicone glue came apart from the glass and the aluminum but stuck to the silicone.

Autoclave Exposure

Autoclave sterilization, which will be done periodically, showed that the silicone, aluminum, and polysulfone did not deform during the autoclave process. Immediately after the samples had been removed from the autoclave the silicone glue was hot enough to pull apart the silicone pad from the polysulfone, see Figure 16. After letting the samples sit and cool for a few minutes, the silicone was no longer able to be pulled apart from the other materials because the glue had regained its mechanical strength. The material testing of autoclave sterilization passed our specifications. The outcome of the autoclave has actually been noted as a good way to potentially modify the device in the future. For instance, if the glass needs to be replaced because it has been scratched, the device could be put in the autoclave so that the old glass could be removed easily.

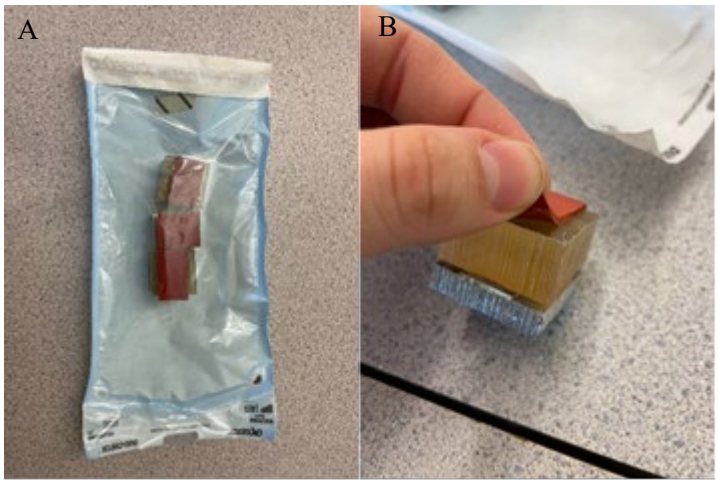

Figure 16. Material testing samples after they had undergone the autoclave sterilization process. A, Samples in sterile packaging. B, Sample demonstrating that when at high temperatures, the silicone glue can be affected.

General Chamber Assessment

For usability of the chamber, specifications such as internal area, external area, and imaging area were defined. Overall, the chamber meets the size specifications. Table 24 shows the dimensions of the chamber.

Table 24. Comprehensive measurements of the Live Cell Imaging Chamber.

	Dimension	Length (mm)
Chamber External	Height	60
	Length	250
	Width	183
Chamber Internal	Height	30
	Length	219
	Width	154
Imaging Area	Length	125
	Width	80
Glass Area	Length	152
	Width	106

Data was extrapolated from Table 24 measurements to calculate internal and external volumes. The entire system, including the gas control (without the compressed gas cylinder) and the heated stage control systems, fits within Cabinet 17 in 38-134 where the device will be used (Table 25).

Table 25. Overall measured and desired specifications for the Live Cell Imaging Chamber.

	System Weight (lb)	Internal Volume (in^3)	External Volume (ft^3)
Measured Value	7.2	61.74	< 3
Desired Value	< 20	> 17.3	3

CO_2 Initialization

Statistical significance between the target specification (under 5 minutes) and the data from the testing stage was determined through a one-sample t-test. The initialization testing included 10 tests where the chamber was initialized, and the amount of time for the system to reach 5% CO_2 concentration was recorded. The initialization times for these tests are displayed in Table 26. The null hypothesis for this test was $\mu = 300$ s and the alternate hypothesis was $\mu > 300$ s. There was a statistically significant difference between the test results and the target specification ($p < 0.05$). This indicated that the null hypothesis should be rejected and that the amount of time to initialize the chamber is greater than 5 minutes. In order to meet this specification, the line pressure and injection duration have been altered to minimize initialization time.

Table 26. Amount of time (s) after beginning injections for the chamber to reach a 5% CO_2 concentration. A series of 10 tests were performed.

Test #	Time to reach 5% (s)
1	308.9
2	375.5
3	379.1
4	294.6
5	275.8
6	350.2
7	316.8
8	310.1
9	364.5
10	424.1

CO_2 Re-initialization

Statistical significance between the target specification (under 2 minutes) and the data from the stage was determined through a one-sample t-test. The reinitialization testing included 10 tests where the chamber lid was removed, culture vessels were replaced, and the lid was replaced and secured. The reinitialization times for these tests are displayed in Table 27. The null hypothesis for this test was $\mu = 120$ s and the alternate hypothesis $\mu > 120$ s. There was not a significant difference between the test results and the target specification ($p = 0.838$). This indicated that the null hypothesis should not be rejected and that the reinitialization times are within the target.

Table 27. Amount of time (s) between opening the chamber lid and reinitialization of the 5% CO_2 environment. A series of 10 tests were performed.

Test #	Time between lid removal and 5% (s)
1	124.5
2	53
3	108
4	144
5	136
6	96
7	109.3
8	123.2
9	95
10	125.1

CO_2 Leakage

Statistical significance between the target specification (greater than 0.045% CO_2) and the data from the stage was determined through a one sample t-test. The leakage testing included 3 tests where the system was initialized, and the injection mechanism was removed from the chamber. The chamber was allowed to sit for 3 hours while the sensor recorded measurements. The final CO_2 concentrations were recorded for all tests. The leakage testing times are displayed in Table 28. The null hypothesis for this test was $\mu = 0.045$ and the alternate hypothesis was $\mu > 120$. There was a statistically significant difference between the test results and the target specification ($p < 0.05$). This indicated that the null hypothesis should be rejected and that the chamber does not reach environmental CO_2 levels over the course of the test.

Table 28. CO_2 concentration (%) at the end of the 3-hour leakage test. A series of 3 tests were performed.

Test #	CO_2 concentration at 3 hours (%)
1	0.857
2	0.955
3	1.026

Relative Degree of Focus

Statistical significance between chamber and control groups for each magnification was determined through a two-sample t-test. There was not a significant difference between images taken with and without the chamber at 10x magnification ($p = 0.127$). However, the images taken with and without the chamber at 4x magnification was statistically significant ($p < 0.05$). The mean value for all groups is displayed in Table 29.

Table 29. Relative degree of focus presented as a mean ± SE. Images were taken with and without the chamber apparatus at both 4x and 10x magnifications. Each photo taken and the relative degree of focus calculated for each can be seen in Appendix 1.

Image Type	Clarity (%)
4x Control	18.82 ± 0.17
4x Chamber	21.06 ± 0.25
10x Control	18.24 ± 0.092
10x Chamber	17.75 ± 0.29

Although, significance was detected, the images seen in Figure 17 show the clarity of an image without the chamber in A and with the chamber in B. When visually observed, the user could not tell a difference between the focus, specifically as the user used coarse and fine focus before obtaining each photo. Overall, this test shows us that the glass in the chamber does not cause inhibition of user define in-focus images as there was no difference seen at 10x.

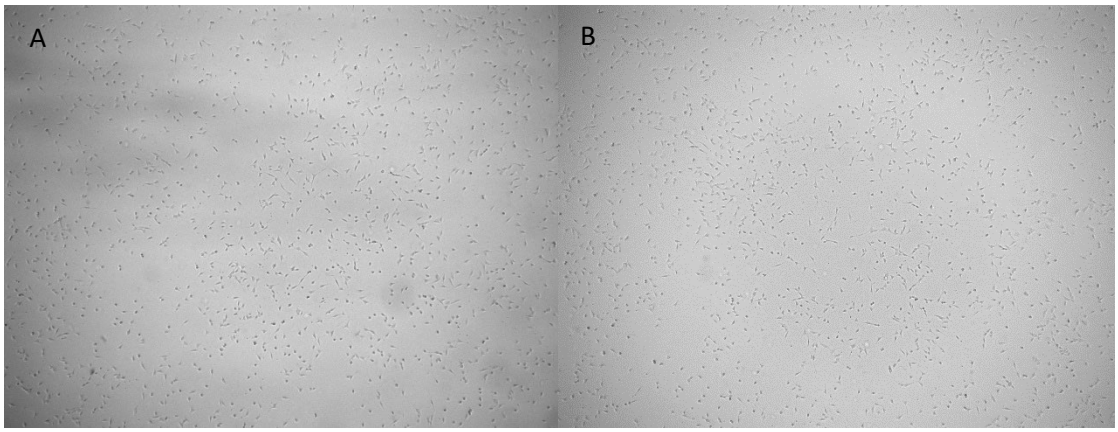

Figure 17. Images of 3T3-GFPs taken at 4x. A, cells without the chamber at 18.43% focus. B, cells with the chamber at 22.69% focus. Percent focus determined by fmeasure() in Matlab, code seen in Appendix P.

User Testing

User testing was performed on February 24th, 2022 with two users that were already trained on the Olympus inverted microscope.

After User 1 completed the procedures, the User Operations Manual was updated and given to User 2. Overall, our user testing did not meet our specification of only 5 questions as the users asked 13 and 6 questions taking 29.57 minutes and 13.46 minutes to complete the procedures, respectively. However, the Operations Manual has been extensively updated to include images of all steps that confused the users as well as clarify wording. Questions asked of the Live Cell Imaging Group can be seen in Appendix Q.

Control System Manipulations After Initial Testing

The testing described above was collected when the line pressure was 20 psi. This resulted in higher bound of 6% CO_2 and we had to inject CO_2 at 4% as the smallest solenoid opening time of 15ms caused about a 2% CO_2 increase. After all of the testing was performed, in order to meet the specification of ±0.5% CO_2 the line pressure was decreased to 5 psi. This changed the control system as the diffusion of injected CO_2 was slower than before and more bursts of CO_2 were required to reach 5% during initialization and re-initialization. This resulted in a new initialization protocol that has four 15 ms bursts of CO_2 every 20 seconds, then waits four minutes for diffusion. Similarly, the re-initialization protocol causes a 15 ms burst when the CO_2 reading is below 4.5% then waits 30 seconds, if the CO_2 continues to drop, specifically below 3.75%, there is a 30 ms burst then it waits 45 seconds. In recent testing this procedure took between 4 and 6 minutes. The Arduino then decides which type of injection to perform next based on the CO_2 reading. Generally, in recent testing this process was completed between 2 and 3 minutes, potentially meeting the specification. Overall, the general range of CO_2 readings was between 4.5% and 5.8%. The modifications to our system to meet our specifications have not been confirmed through the testing procedures listed above, similarly it has not undergone statistical analysis.

Live Cell Testing

The combined subsystems of Gas Control and Heated Stage were tested together with a live sample of 3T3 fibroblasts in the chamber. During the 3-hour period, another flask of cells was outside of the

chamber in ambient air conditions. Flasks were imaged before and after imaging to determine if cell death occurred outside or inside the chamber (Figure 18).

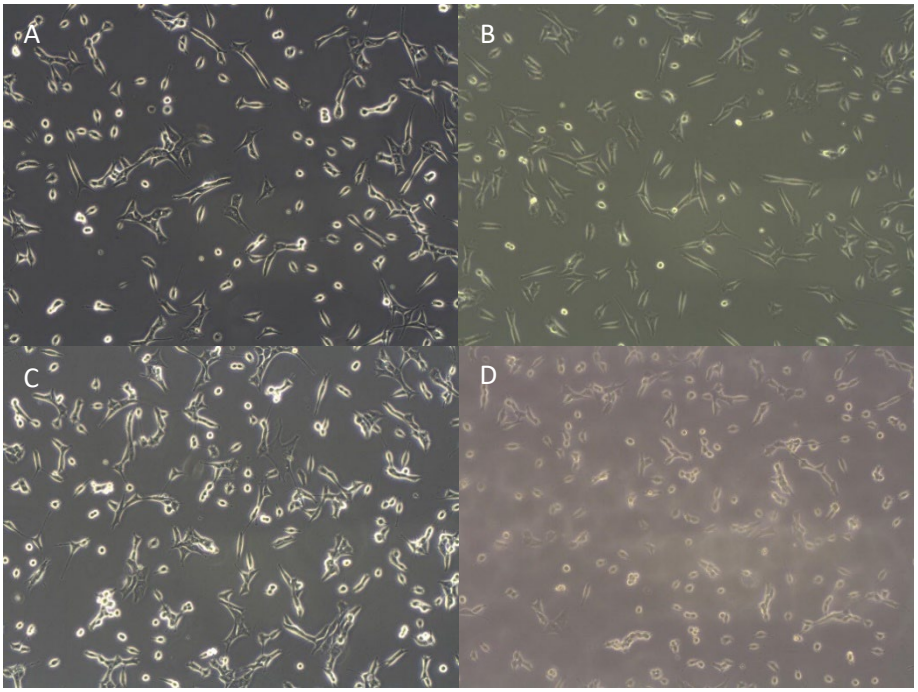

Figure 18. Images were taken at 10X of both the flask in the chamber and flask outside of the chamber with a phase contrast microscope. A, Chamber flask before addition to the chamber. B, Chamber flask after being in the operating Live Cell Imaging device for 3 hours. C, Control flask before the testing. D, Control flask after testing.

Visually, the control flask after imaging appears to have more circular cells. This is indicative of cell death, where the cells become unadhered to the flask and float in the cell media. An analysis using ImageJ shows that there were more circular cells with circularity 0.75-1.0 in the control after testing compared to the chamber after (Table 30).

Table 30. Number of circular cells counted before and after a three-hour test of the combined Live Cell Imaging device with two flasks, one in the device and one outside of the device.

	Circular Cells Before	**Circular Cells After**
Chamber	194	211
Control	219	361

The control flask without CO_2 and heat regulation had 361 circular cells compared to the chamber flask within the device with 211 circular cells. Overall, this test showed that the Live Cell Imaging Device does keep cells in an optimal condition for survival in comparison to the ambient room such that cells are able to stay alive.

Other Specifications

Some specifications did not require a test to ensure the user needs were met. Two specifications, User-Facing Output Readings and Ease of Use through the number of system set-up steps were evaluated below.

User-Facing Output Readings

A specification for the system display was that updates of CO_2 concentration readings were at the slowest every 1 second. This metric is determined by the output speed defined in the Arduino microcontroller code. Here we have data being recorded every 0.25 seconds and the display updating every 0.25 seconds. Further, it was shown in the CO_2 Initialization, CO_2 Re-Initialization, and CO_2 Leakage tests that CO_2 data can be recorded and reported every 0.1 seconds.

Ease of Use: System Set-up

For the gas control set-up, a specification regarding the number of steps was defined as 20 steps maximum. This can be determined through the Operations Manual number of steps defined for the gas control set up. With set-up and take-down the gas control operations has a maximum of 14 steps, if something does not load properly during set up, and a minimum of 10 steps.

Conclusion

Results showed that the Live Cell Imaging Device was suitable for maintaining the cellular environment required to keep cells alive during imaging. The device met all usability specifications. The chamber can hold customer required culture vessels such as chamber slides, petri dishes, well plates, and culture flasks. Similarly, the system is easily movable, with the chamber weighting 7.2 pounds, the control systems and chamber fitting into a laboratory cabinet for ease of storage. Material testing showed that the polysulfone, aluminum, silicone and glass that were used to manufacture the chamber withstand laboratory cleaning procedures such sterilization with an autoclave and consistent cleaning with 70% isopropyl alcohol. Autoclaving was identified as a way to manipulate the silicone sealant seal between the silicone and polysulfone as well as the glass and polysulfone or aluminum to remove adhered surfaces when warm, however once the system cools the sealant is cured again and unable to be manipulated. This is optimal for device maintenance is the glass become cracked or silicone become dried and can no longer maintain the gasket-like seal. The device was able to maintain sufficient properties such as 5% CO2 and 37°C over a 3-hour period with a sample of fibroblasts in a T25 culture vessel. A second culture vessel was left outside of the chamber during the testing period. Both flasks were compared before and after testing the flask outside of the chamber had more circular cells, or cells starting to become unadhered from the flask and die, than the flask within the chamber. This shows that the Live Cell Imaging Device maintains the environment necessary for cellular survival. This device can be used for imaging with 4x and 10x objectives without distortion of the image clarity. Testing showed that clarity had no significant difference when imaged with and without the chamber at 10X ($p<0.05$), however there was a significant difference between 4X pictures ($p=0.127$), this discrepancy is noted in the discussion. Similarly, during the User Testing, one user asked 16 questions of the protocol and the other 6. Although this did not meet our specification, the knowledge gained was used to improve the User Operations Manual and increase clarity of the device use through images. The Live Cell Imaging Gas Control is able to maintain CO_2 concentrations consistently between 4.0% and 6.0%. This range did not meet the ± 0.5% specification, and resulted in a line pressure manipulation from 20 psi to 5 psi. This resulted in a 4.5% to 5.8% CO_2 range still outside of specifications, however there are mechanical limitations to manipulation of the current control system noted in the discussion. When the chamber lid is removed, the CO_2 concentration

drops significantly but the CO_2 control system is able to re-establish CO_2 at an average of 111.14 seconds, which is within the 120 seconds (2 minutes) of opening. Initialization of the system occurs in an average of 339.96 seconds, longer than the specified 300 seconds (5 minutes). Although longer than specified, the Heated Stage control takes about 15 minutes for initialization and occurs in tandem with gas initialization, so user set-up time is not extended by not meeting this specification. The chamber can maintain CO_2 concentrations higher than atmospheric for 3 hours, meeting the leakage specification. This shows that the device will not use a significant amount of CO_2 from the gas canister resulting in high user expenses. Testing mentioned above was at a CO_2 line pressure of 20 psi, statistical significance cannot be determined for the updated system with line pressure of 5 psi. Overall, the entire Live Cell Imaging Device can be used for time periods up to 3 hours while successfully maintaining 37°C heat and 5% CO_2 concentration and therefore a suitable and aseptic environment for live cell imaging.

Discussion

Clarity testing revealed that there was a significant difference between the images captured with and without the chamber at 4X ($p=0.127$). Although there was a statistically significant difference in the level of clarity at this level of magnification, this result is not a concern in the context of this project. The user responsible for imaging determined the images were in focus and were appropriate for the cell quantification techniques necessary for data analysis. Additionally, the test performed at 10X had no significant difference in image clarity. Larger magnifications are more difficult to bring into focus because the depth of field of these objectives is smaller, meaning that fewer cells will be in focus at the same time. Therefore, the lack of difference in image clarity at a magnification of 10X indicates that users should not have any difficulty focusing the images with the 4X objective. Testing also revealed that the chamber CO_2 concentration ranged between 4.5% and 5.8%, which was outside of our specification that the system maintains a concentration of 5% ± 0.5%. This is a limitation of the device that cannot be resolved given the current solenoid that we have access to. The solenoid valve that is responsible for injecting CO_2 into the chamber can currently only open for a minimum of 15 milliseconds, which allows too much CO_2 to enter the chamber at once. To account for this, the line pressure on the regulator was decreased from 22 psi to 5 psi. When the line pressure was set at 22 psi, a single injection increased the concentration inside the chamber by approximately 2%. After decreasing the line pressure to 5 psi, a single injection increased the concentration by approximately 1%. This adjustment gave us greater control over the CO_2 concentration modulation inside of the chamber. The other factor that could be adjusted to create tighter controls over the CO_2 concentration is the amount of time the solenoid valve opens for. The control system is currently set to open the solenoid valve for 15 milliseconds, which is the shortest period that is feasible with the current system. To allow for shorter injection bursts, a higher quality solenoid must be installed, but these types of valves are far too expensive for the scope of this project. The base of the chamber presents limitations for the use of this device. The added distance between the sample and the objective created by the aluminum and silicone base makes imaging at a magnification higher than 10X not possible. Another limitation of this system is that none of the testing was performed for longer than 3 hours. We do not have any reason to believe that the system is incapable of being used for longer periods than this, but we do not have any data to validate that the system can be used for up to 8 hours. Additionally, this system currently does not have any humidity control. A heated environment containing solution may create condensation on the glass. This may prove to prevent the user from properly imaging samples if the system is used for extended periods of time. In the future, a humidity control system could be implemented to help combat this factor. Other future directions for this project include introducing an improved solenoid into the system. This would allow for shorter injection bursts, which would consequently allow for tighter control over the CO_2 concentration inside of the chamber. Another way to

tighten the control over the concentration inside of the chamber would be to purchase a regulator that is rated for lower pressures. The current regulator can go as low as 5 psi, but injecting CO_2 at an even lower pressure would allow bursts to increase the concentration by smaller amounts. The system currently does not have a way to alert the user that the concentration is out of the specified range other than the user voluntarily looking at the screen. Installing an alarm that sounds when the concentration falls above or below the desired range would improve the user's ability to be confident in the control system. The system also currently only operates in physiological CO_2 conditions. In the future, the system could be designed to be set to a user specified concentration but doing so would require a different control system than is currently being used.

Operations Manual

Revision: 2

Date: February 24, 2022

Revisions:

1-January 11, 2022-Original Operations Manual-Byrne/Jens
2-February 24, 2022 - Updated Operations Manual-Bean/Byrne

Manual Purpose

The purpose of this manual is to outline instructions for the live cell imaging heated stage and gas control. This manual will walk through the placement of the samples within the device, the heat control set up, and the gas control set up.

Contact Information

Any questions regarding this user manual can be directed to:

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Procedure 1

Summary

This procedure is used for setting up the physical device and loading the samples into the device.

Revision

1-January 11, 2022-Original Operations Manual - Byrne
2-February 24, 2022 - Update setup procedure – Bean/Byrne

Procedure Steps

1. Spray a paper towel with 70% isopropyl alcohol and wipe down all surfaces inside the chamber. Specifically, ensure that the glass at the top of the chamber and bottom of the chamber do not have fingerprints.
    1. Before moving on to the next step, ensure the chamber top is screwed securely to the base of the chamber.
2. Attach the pressure release valve. See Figure 1.
3. Attach the CO2 sensor (press fit) on the left side of the chamber. See Figure 1.

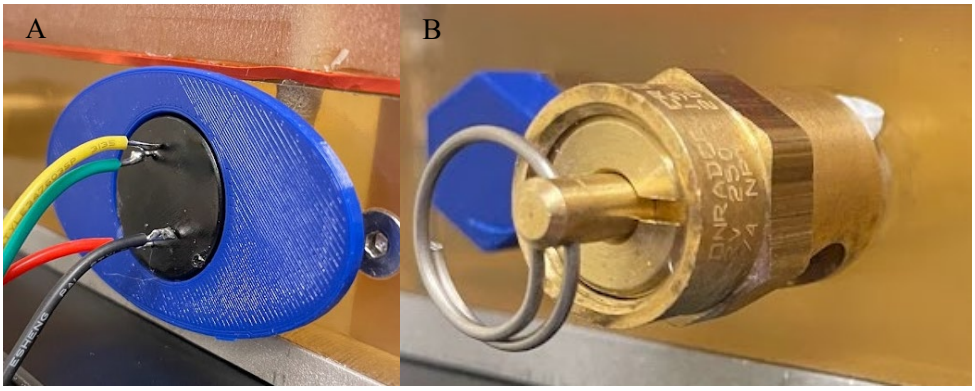

Figure 1. **A.** CO2 sensor. **B.** Pressure release valve.

4. Attach the temperature probe by screwing in until finger-tight. See Figure 2.
5. Attach the temperature probe cord (orange top) to the temperature probe by aligning the half-circle in the appropriate indent and then twisting the metal until finger tight. See Figure 2.

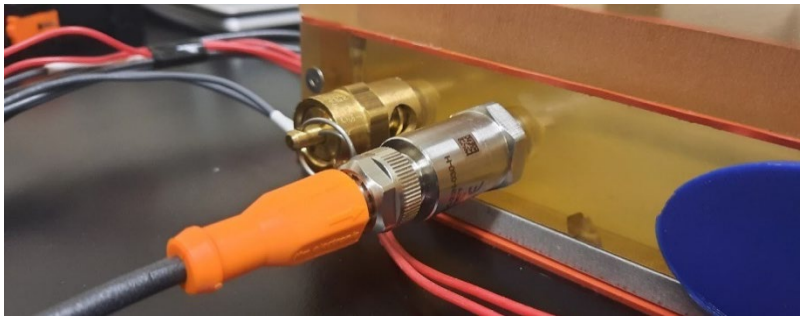

Figure 2. Temperature probe (silver) and cord (orange)

6. Move to Procedure 2 and complete Steps #1-6.
7. Unscrew all four thumb screws at the top of the device by twisting to the left.
8. Remove the lid of the device and set aside.
9. Carefully place the sample directly on the glass, being careful not to slide it around as this might scratch the glass.
10. Place the device lid back on the top of the device. Place rear screws first and then front screws. Begin tightening the back two screws then tightening the front two screws. Tighten by twisting to the right until finger tight.
    WARNING: *Be careful when placing the lid back on the device that you do not pinch your fingers between the lid and the device base.*
11. Move to Procedure 3 and complete Steps #1-5
12. Move to Procedure 4 and complete all steps.
13. When imaging is complete, see Procedure 2, Step #7-10, and Procedure 3, Step #7-10.

Procedure 2

Summary

This procedure is used for setting up the heat control aspect of the device.

Revision

1-January 11, 2022-Original Operations Manual – Byrne
2-February 25, 2022-Update setup procedure – Byrne

Procedure Steps

1. Place the silicone heating mat on the stage, as far to the right as possible, with the heaters facing up and cords coming off of the left side. See Figure 3.

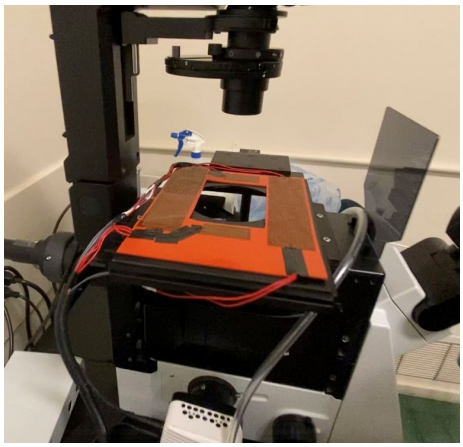

Figure 3. Silicone heating mat on stage with wires coming off the left side.

2. Place the device on top of the silicone layer with the aluminum base in contact with the silicone and heaters. The glass should be aligned with the silicone cutout with two inputs on the left and one on the right. See Figure 4.
   WARNING*: Ensure the chamber does not sit on top of any wires.*

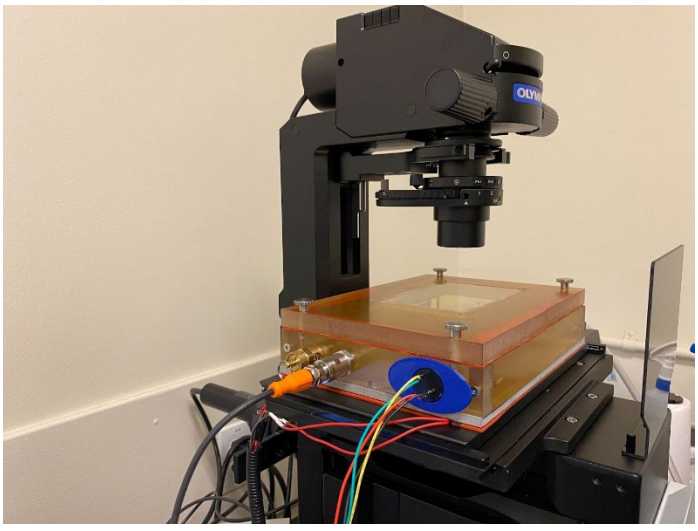

Figure 4. Device on top of the silicone heating mat on the stage.

3. Plug in both power cords, labeled “heater” and “controller,” to the extension cords behind the counter. The controller screen should turn on.

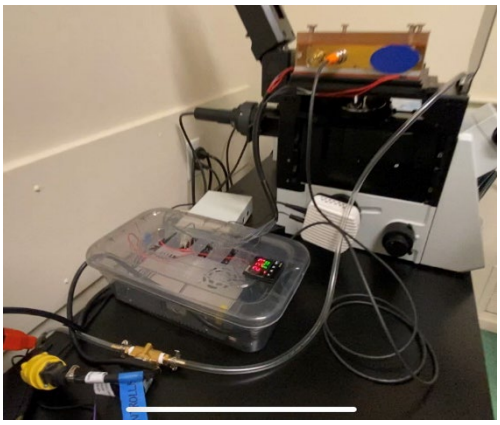

Figure 5. Heat control box with heater and controller plugged into extension cords.

4. Wait about 10 seconds for the controller screen to display a temperature reading on the top line in large red font. The set-point temperature is on the bottom line in small green font. The heaters will also turn on at this point, indicated by a green light on the relay (see figure 5).

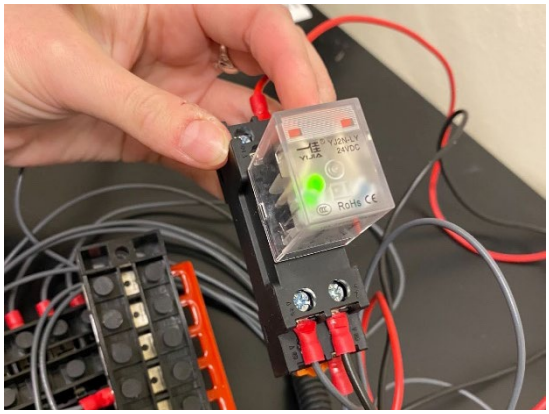

Figure 5. Relay with green light on, indicating the heaters are on.

5. Allow the chamber to heat to the set-point temperature. The red temperature on the controller screen will match the green set-point when it has heated up. This will take about 15 minutes.
   WARNING: *The base of the device will be hot once the heating system is turned on. Do not touch the aluminum base while the heating system is on.*
6. Return to Procedure 1, steps #6-10.
7. When imaging is complete, unplug both power cords. The top can be unscrewed and the sample removed.
8. Wait 5 minutes for the device to cool down before removing the chamber and heaters from the stage.

9. Allow the device to cool down completely on the benchtop until they are no longer hot to the touch.
10. Move device and all heating elements (silicone heating pad, controller, and power source) to Bench Cabinet #17.

Procedure 3

Summary

This procedure is used for setting up the gas control aspect of the device.

Revision

1-January 11, 2022-Original Operations Manual – Jens
2-February 24, 2022-Update setup and cleanup procedures – Bean

Procedure Steps

1. Make sure that the attachments on the gas canister are secured. Then open the valve to the CO_2 canister. Ensure that there is pressure reading on both pressure gauges. See Figure 7.
    1. NOTE: The output pressure needs to read 5 psi.

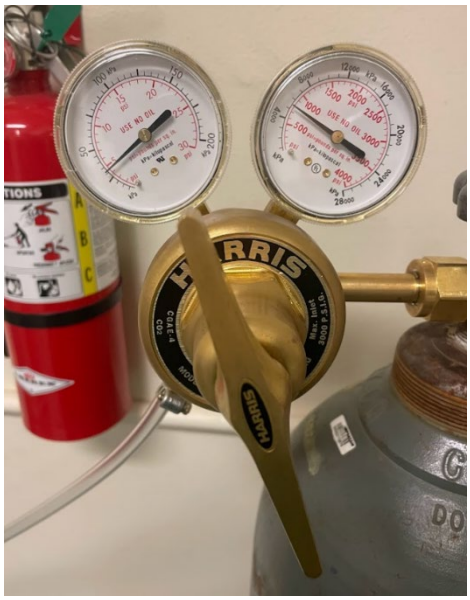

Figure 7. Pressure regulator shown with appropriate input and output pressures. The handle shown in this photo is for changing the output regulation. DO NOT rotate this handle, the control system established for this chamber is for an output pressure of 5 psi only.

WARNING: *Ensure there that the gas regulator is securely fastened onto the CO_2 canister if not the gas could quickly leak from the canister filling the room with CO_2 and could cause asphyxiation.*
WARNING: *If there is no pressure reading on one or both gauges, close the valve and determine if there is a leak or if there is no CO_2 gas left in the canister.*

2. Plug in the power cord, labeled “12V” into the power strip adjacent to the heating control box.
3. Grab the CO_2 input tubing, turn on the switch next to the LCD screen of the gas control box, then wait for the CO_2 valve to open for 1 second. Once the CO_2 has been flushed through the tubing, connect the adaptor to the outlet immediately.
WARNING: *A loud sound will occur when the CO_2 is being flushed through the tubing.*

4. Observe the CO_2 output readings and listen for solenoid valve 'clicks' to determine if CO_2 gas is being injected into the chamber. If the CO2 reading is not visible, unplug the power cord and restart from Step 1 of Procedure 3.
5. Once the desired CO_2 concentration no gas will be injected until the system reaches 4.5% or the lid is opened causing a decrease in CO_2 concentration in the chamber.
    a. If during use you replace the sample, the CO_2 concentration will decrease and cause intermittent CO_2 injection indicated by the solenoid clicking noise.

        WARNING: *If at any point there is no CO_2 reading output immediately close the CO_2 canister valve. Then unplug all cords and restart the system in Step 2 of Procedure 3.*
6. Return to Procedure 1, Step #12 (directs you to begin Procedure 4).
7. When imaging is complete, turn off the pressure from the compressed gas cylinder. And remove the gas input tubing from the chamber.
    a. To release pressure from the solenoid valve, use the switch to turn off the gas control then turn it back on to flush the remaining CO_2 from the system.
    b. Visualize the pressure regulator to see the output pressure reach 0 psi and turn off the gas control system. If it has not reached 0 psi, repeat Step 7a.
8. Unplug the "12V" power cord.
9. Remove the CO_2 sensor by the blue tabs from the chamber and replace the sensor protectors.
10. Return the entire CO_2 gas control system to Bench Cabinet #17.

Procedure 4

Summary

This procedure is used for using the device while imaging.

Revision

1-January 11, 2022-Original Operations Manual - Bean
2-February 24, 2022 -Update procedure - Bean

Procedure Steps

1. Turn on the Olympus light source.
2. While watching the objective you will use to visualize the samples, move the objective up towards the stage. Move it as close as possible to the device, then use the lock function of the stage adjustment knob to ensure the objective does not go above this desired height. See Figure 8.

Figure 8. The stage adjustment knob unlocked (left) and locked (right).

3. For bright field imaging
    a. Turn the bright field light on
    b. Ensure that the filter cube is on an empty space and the shutter is closed
4. For fluorescent imaging
    a. Turn the burner on. See Figure 9.
    b. Ensure the bright field light is off
    c. Choose the appropriate filter cube
    d. Open the shutter

Figure 9. Once turned on, the burner light will be blue. In this image, the burner is currently on and at an intensity of 3.

5. Use the coarse focus to visualize sample
    1. May require the removal of the top of the chamber (see Procedure 1) if using the compressor
6. Using the desired objective visualize your sample with the correct filter cube
    WARNING: Ensure that when you use the coarse and fine focus that it does not hit the glass.
    WARNING: Ensure that when moving the X-Y stage controls that the glass top of the objective does not hit the edge of the aluminum and silicone bottom
    Reminder: This device is designed to work with 4x and 10x objectives
7. Power down the microscope
    a. If using bright field turn of the light
    b. Ensure the shutter is closed
    c. Lower the stage all the way
    d. Turn off the burner
    e. Turn off the Olympus light source
8. Return to Procedure 1, Step #13 for Live Cell Imaging Heated Stage and Gas Control clean up.

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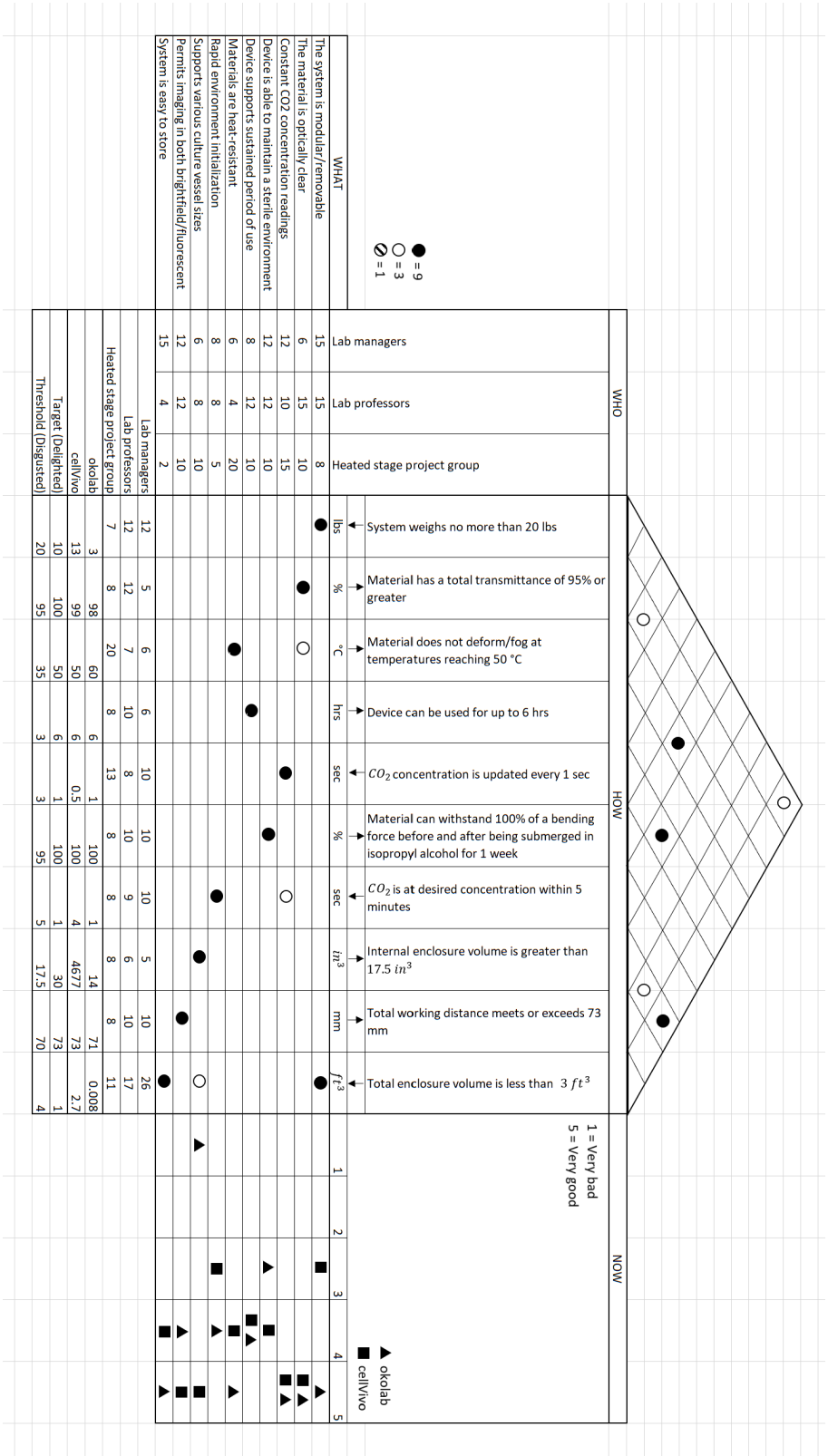
Appendices

Appendix A: Customer Requirements

- Type of imaging:
  - Bright field
  - Fluorescent
- Culture vessel sizes:
  - 6, 24, and 96 well plates
  - Glass microscope slides
  - Microfluidic devices
- CO_2 modulation:
  - 5% CO_2
  - Control CO_2 levels through injection of CO_2 or room air
- Imaging time:
  - Between 0 and 6 hours
- Material properties:
  - Completely clear for visibility of sample
  - Must not crack/deform when in temperatures up to 50°C
- Chamber usability:
  - Modular and removable
  - Light enough to be easily removed and stored

Appendix B: House of Quality



WHAT	Lab managers	Lab professors	Heated stage project group	lbs	%	°C	hrs	sec	%	sec	in^3	mm	ft^3
The system is modular/removable	15	15	8	●									●
The material is optically clear	6	15	10		●	○							
Constant CO2 concentration readings	12	10	15					●		○			
Device is able to maintain a sterile environment	12	12	10				●		●				
Device supports sustained period of use	8	12	10				●						
Materials are heat-resistant	6	4	20			●							
Rapid environment initialization	8	8	5							●			
Supports various culture vessel sizes	6	8	10								●		○
Permits imaging in both brightfield/fluorescent	12	12	10									●	
System is easy to store	15	4	2										●
		Lab managers		12	5	6	6	10	10	10	5	10	26
		Lab professors		12	12	7	10	8	10	9	6	10	17
	Heated stage project group			7	8	20	8	13	8	8	8	8	11
		okolab		3	98	60	6	1	100	1	14	71	0.008
		cellVivo		13	99	50	6	0.5	100	4	4677	73	2.7
		Target (Delighted)		10	100	50	6	1	100	1	30	73	1
		Threshold (Disgusted)		20	95	35	3	3	95	5	17.5	70	4

Raw Conjoint Survey Results for Live Cell Imaging Gas Control

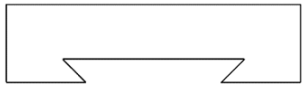
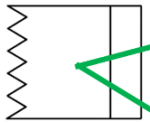
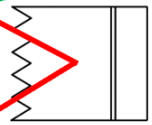
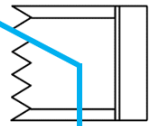
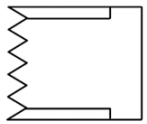
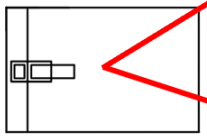
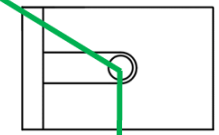
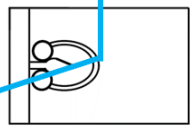
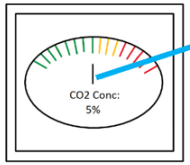
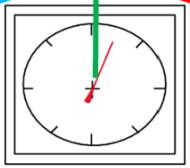
What is your name?	**What is your Group #?**	**What is your Group Name?**	**1,1,1**	**1,2,2**	**2,1,2**	**2,2,1**
Kameryn Laureano	4	FeverDots	1	2	3	4
Simon Park	6	Live Cell Imaging Heated Stage	1	3	2	4
Anna Frauenheim	6	Live Cell Imaging Heated Stage	1	4	2	3
Nick Martinez	2	Syringe Pump	3	1	2	4
Sophie Moffatt	2	Syringe Pump	2	4	3	1
Lauren Martin	2	Syringe Pump	1	2	3	4
Bryce Sakata	3	Semi-Automated Membrane Characterization	1	2	3	4
Rita Grigorian	4	Fever Dots	1	3	2	4
Andrea ng	3	Semi-Automated Membrane Characterization	1	2	4	3
Melanie Mitton	3	Semi-Automated Membrane Characterization	2	4	3	1
Heidi Silk	4	FeverDots	3	1	4	2
Kerri Byrne	6	Live Cell Imaging Heated Stage	1	2	3	4
Kendall Gentzen	5	Heart Pump Impeller Research and Development	3	2	1	4
Thomasina Hinkle	1	Bone and Cartilage Cyclic Loading	1	3	2	4
Amit Sharir	5	Heart Pump Impeller Research and Development	3	4	1	2
Karoline Wucherer	1	Bone and Cartilage Cyclic Loading	1	3	2	4
Ben Parmentier	1	Bone and Cartilage Cyclic Loading	3	4	2	1
Sophia James	5	Heart Pump Impeller Research and Development	1	2	3	4

Multivariant Regression Statistical Analysis Output

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.483045892							
R Square	0.233333333							
Adjusted R Square	0.199509804							
Standard Error	1.007326105							
Observations	72							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	3	21	7	6.898550725	0.000400572			
Residual	68	69	1.014705882					
Total	71	90						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	6.10623E-16	0.628178194	9.72053E-16	1	-1.253510069	1.253510069	-1.253510069	1.253510069
Size	0.666666667	0.23742904	2.807856473	0.006502906	0.192884394	1.140448939	0.192884394	1.140448939
Material	0.833333333	0.23742904	3.509820591	0.000800541	0.359551061	1.307115606	0.359551061	1.307115606
Control	0.166666667	0.23742904	0.701964118	0.485097339	-0.307115606	0.640448939	-0.307115606	0.640448939

Appendix D. Morphology Combinations

Morphology

Product: Live Cell Imaging Gas Control	Organization Name: Group 7			
Function	Concept 1	Concept 2	Concept 3	Concept 4
Drawer slides	Metal side-mount 	Side-mount 	Rollers 	Center-mount 
Drawer closure	Flush without gasket 	Flush with gasket 	T-shaped with gasket 	T-shaped without gasket 
Drawer clasp	Buckle-latch 	Elastic 	Roller-catch 	Magnetic 
CO2 conc. display	Digital 	Analog 	RGB screen 	

Team member: Abby Jens	Team member: Elsa Bean	Prepared by: Abby Jens	
Team member: Brady Berg	Team member:	Checked by: Elsa Bean	Approved by:

The Mechanical Design Process Designed by
Professor David G. Ullman
 Form # 15.0

Appendix E: Individual Team Member Pugh Charts

Pugh Charts Prepared by Brady Berg

Purpose: To determine the best conceptual design of the Gas Control Chamber			BASELINE: Metal slides, flush drawer with gasket, buckle clasp, RGB output	Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	Roller slides, flush drawer without gasket, elastic clasps, analog output	
						Check Ranks = 100
Maintaining a sterile environment	20	DATUM		1	-1	100
Rapid environment initialization	15			0	-1	
Supports different culture vessels sizes	30			0	0	
CO2 concentration is updated every 1 second	15			0	-1	
Material does not deform/fog at temperatures reaching 50C	20			0	-1	
	Total			1	-4	
	Weighted Total			20	-70	2>1>3

Purpose: To determine the best conceptual design of the Gas Control Chamber			BASELINE: Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	Metal slides, flush drawer with gasket, buckle clasp, RGB output	Roller slides, flush drawer without gasket, elastic clasps, analog output	
						Check Ranks = 100
Maintaining a sterile environment	20	DATUM		-1	-1	100
Rapid environment initialization	15			0	-1	
Supports different culture vessels sizes	30			0	0	
CO2 concentration is updated every 1 second	15			0	-1	
Material does not deform/fog at temperatures reaching 50C	20			0	-1	
	Total			-1	-4	
	Weighted Total			-20	-70	2>1>3

Purpose: To determine the best conceptual design of the Gas Control Chamber			BASELINE: Roller slides, flush drawer without gasket, elastic clasps, analog output	Metal slides, flush drawer with gasket, buckle clasp, RGB output	Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	
						Check Ranks = 100
Maintaining a sterile environment	20	DATUM		-1	0	100
Rapid environment initialization	15			1	1	
Supports different culture vessels sizes	30			0	0	
CO2 concentration is updated every 1 second	15			1	1	
Material does not deform/fog at temperatures reaching 50C	20			1	1	
	Total			2	3	
	Weighted Total			30	50	2>1>3

Pugh Charts Prepared by Elsa Bean

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Metal slides, flush drawer with gasket, buckle clasp, RGB output	Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	Roller slides, flush drawer without gasket, elastic clasps, analog output	
Maintaining a sterile environment	25	DATUM	0	1	
Rapid environment initialization	25		0	-1	
Supports different culture vessels sizes	10		0	0	
CO2 concentration is updated every 1 second	15		0	1	
Material does not deform/fog at temperatures reaching 50C	25		-1	-1	
	Total		-1	0	Ranking: 1>3>2
	Weighted Total		-25	-10	

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	Metal slides, flush drawer with gasket, buckle clasp, RGB output	Roller slides, flush drawer without gasket, elastic clasps, analog output	
Maintaining a sterile environment	25	DATUM	0	1	
Rapid environment initialization	25		0	-1	
Supports different culture vessels sizes	10		0	0	
CO2 concentration is updated every 1 second	15		0	1	
Material does not deform/fog at temperatures reaching 50C	25		1	-1	
	Total		1	0	
	Weighted Total		25	-10	Ranking: 1>2>3

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Roller slides, flush drawer without gasket, elastic clasps, analog output	Metal slides, flush drawer with gasket, buckle clasp, RGB output	Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	
Maintaining a sterile environment	25	DATUM	-1	-1	
Rapid environment initialization	25		1	1	
Supports different culture vessels sizes	10		0	0	
CO2 concentration is updated every 1 second	15		-1	-1	
Material does not deform/fog at temperatures reaching 50C	25		1	0	
	Total		0	-1	
	Weighted Total		10	-15	Ranking: 1>3>2

Pugh Charts Prepared by Abby Jens

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Metal slides, flush drawer with gasket, buckle clasp, RGB output	Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	Roller slides, flush drawer without gasket, elastic clasps, analog output	
					Check Ranks = 100
Maintaining a sterile environment	30	DATUM	1	0	100
Rapid environment initialization	10		0	-1	
Supports different culture vessels sizes	25		0	0	
CO2 concentration is updated every 1 second	15		0	-1	
Material does not deform/fog at temperatures reaching 50C	20		0	0	
	Total		1	-2	
	Weighted Total		30	-25	2>1>3

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	Metal slides, flush drawer with gasket, buckle clasp, RGB output	Roller slides, flush drawer without gasket, elastic clasps, analog output	
					Check Ranks = 100
Maintaining a sterile environment	30	DATUM	-1	-1	100
Rapid environment initialization	10		0	-1	
Supports different culture vessels sizes	25		0	0	
CO2 concentration is updated every 1 second	15		0	-1	
Material does not deform/fog at temperatures reaching 50C	20		0	0	
	Total		-1	-3	
	Weighted Total		-30	-55	2>1>3

Purpose: To determine the best conceptual design of the Gas Control Chamber		BASELINE: Roller slides, flush drawer without gasket, elastic clasps, analog output	Metal slides, flush drawer with gasket, buckle clasp, RGB output	Sidemount slides, T-shape drawer with gasket, roller catch clasp, digital output	
					Check Ranks = 100
Maintaining a sterile environment	30	DATUM	0	1	100
Rapid environment initialization	10		1	1	
Supports different culture vessels sizes	25		0	0	
CO2 concentration is updated every 1 second	15		1	1	
Material does not deform/fog at temperatures reaching 50C	20		0	0	
	Total		2	3	
	Weighted Total		25	55	2>1>3

Appendix F: Pugh charts for optically clear material selection

Purpose: To determine the best material for the optically clear material through which imaging will be performed		BASELINE: Glass	Polypropylene	Acrylic
Ability to be sterilized	25		-1	-1
Durability	15		1	1
Cost	15	DATUM	-1	-1
Manufacturability	20		1	1
Ability to withstand heat	25		-1	-1
	Total		-1	-1
	Weighted Total		-30	-30

Purpose: To determine the best material for the optically clear material through which imaging will be performed		BASELINE: Polypropylene	Glass	Acrylic
Ability to be sterilized	25		1	-1
Durability	15		-1	0
Cost	15	DATUM	1	0
Manufacturability	20		-1	0
Ability to withstand heat	25		1	0
	Total		1	-1
	Weighted Total		30	-25

Purpose: To determine the best material for the optically clear material through which imaging will be performed		BASELINE: Acrylic	Glass	Polypropylene
Ability to be sterilized	25		1	0
Durability	15		-1	0
Cost	15	DATUM	1	0
Manufacturability	20		-1	1
Ability to withstand heat	25		1	0
	Total		1	1
	Weighted Total		30	20

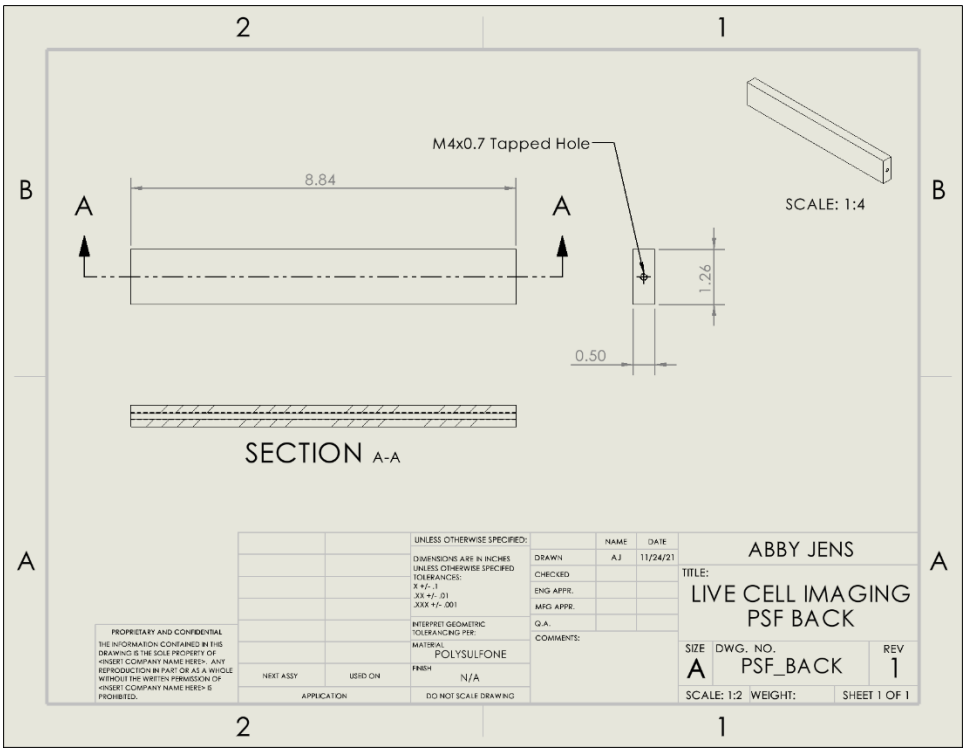
Appendix G: Pugh charts for conductive base material selection

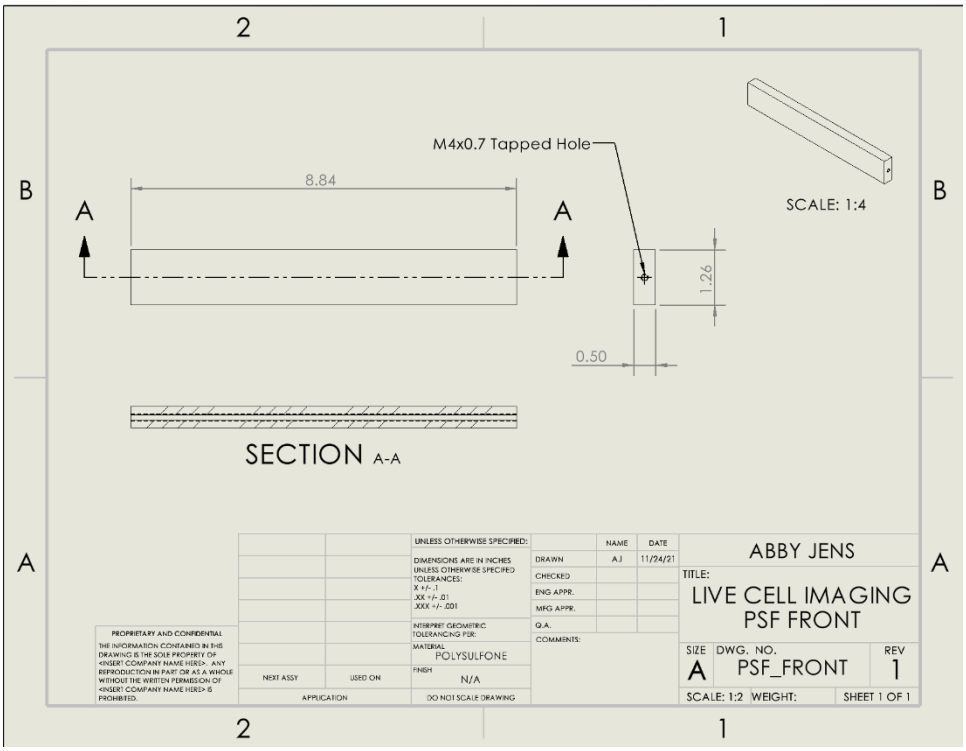
Purpose: To determine the best material for the conductive base of the chamber		BASELINE: Aluminum	Stainless steel	Indium coated glass
Ability to be sterilized	25		0	-1
Durability	15		1	-1
Cost	15	DATUM	-1	-1
Manufacturability	20		-1	-1
Ability to withstand heat	25		0	0
	Total		-1	-4
	Weighted Total		-20	-75

Purpose: To determine the best material for the conductive base of the chamber		BASELINE: Stainless steel	Indium coated glass	Aluminum
Ability to be sterilized	25		-1	0
Durability	15		-1	-1
Cost	15	DATUM	0	1
Manufacturability	20		0	1
Ability to withstand heat	25		0	0
	Total			
	Weighted Total		-40	20

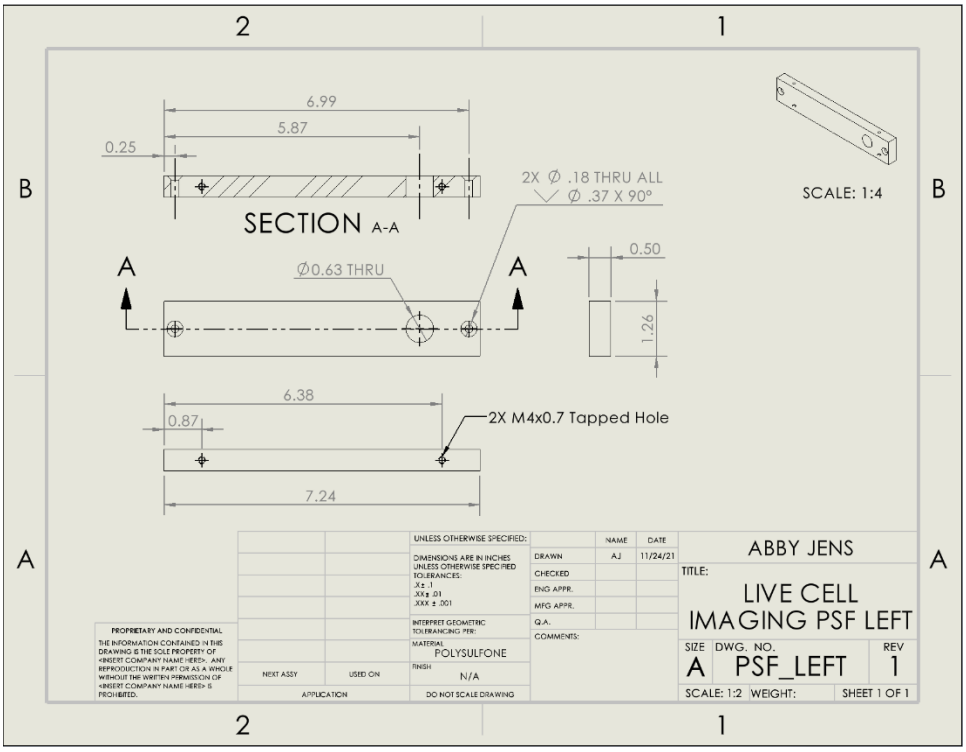
Purpose: To determine the best material for the conductive base of the chamber		BASELINE: Indium coated glass	Stainless steel	Aluminum
Ability to be sterilized	25		1	1
Durability	15		1	1
Cost	15	DATUM	0	1
Manufacturability	20		0	1
Ability to withstand heat	25		0	0
	Total		2	4
	Weighted Total		40	75

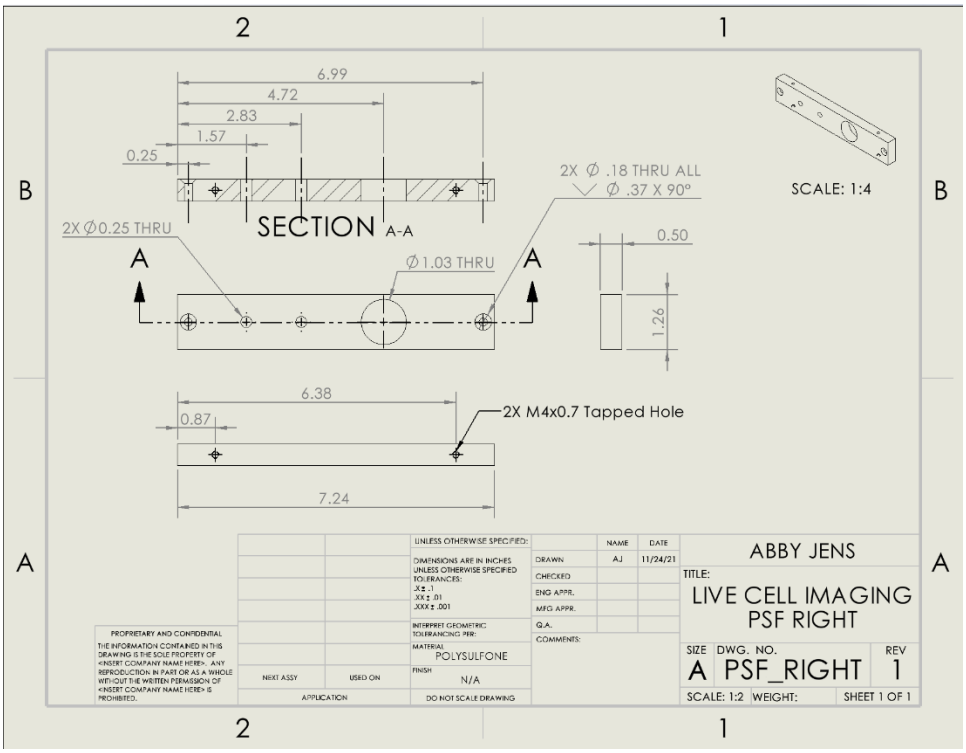
Appendix H: SolidWorks drawings for chamber



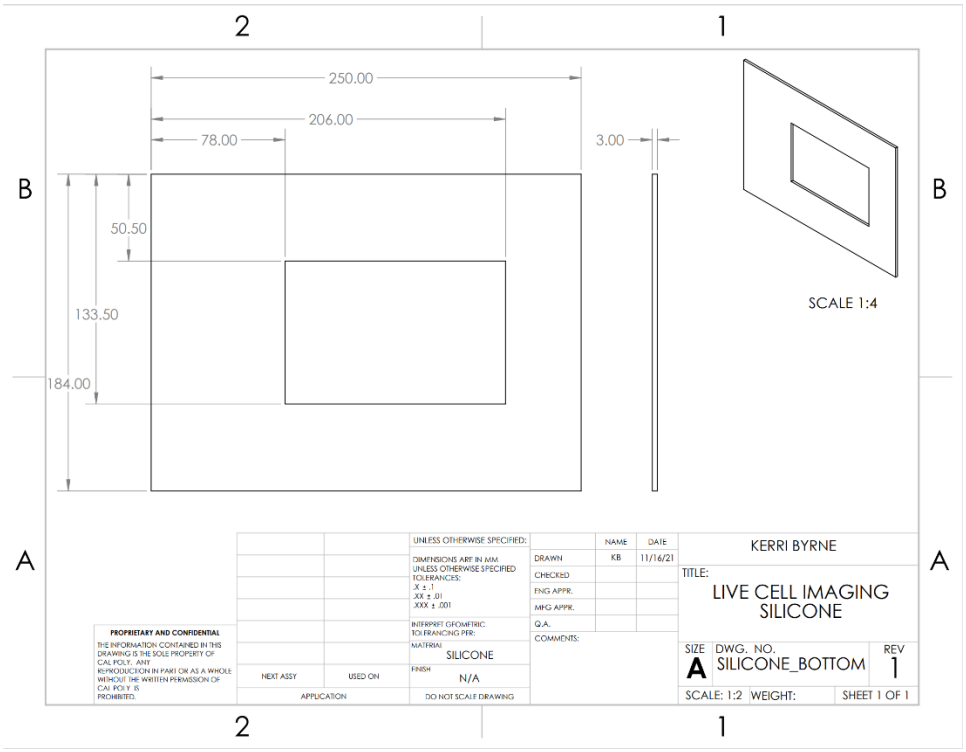


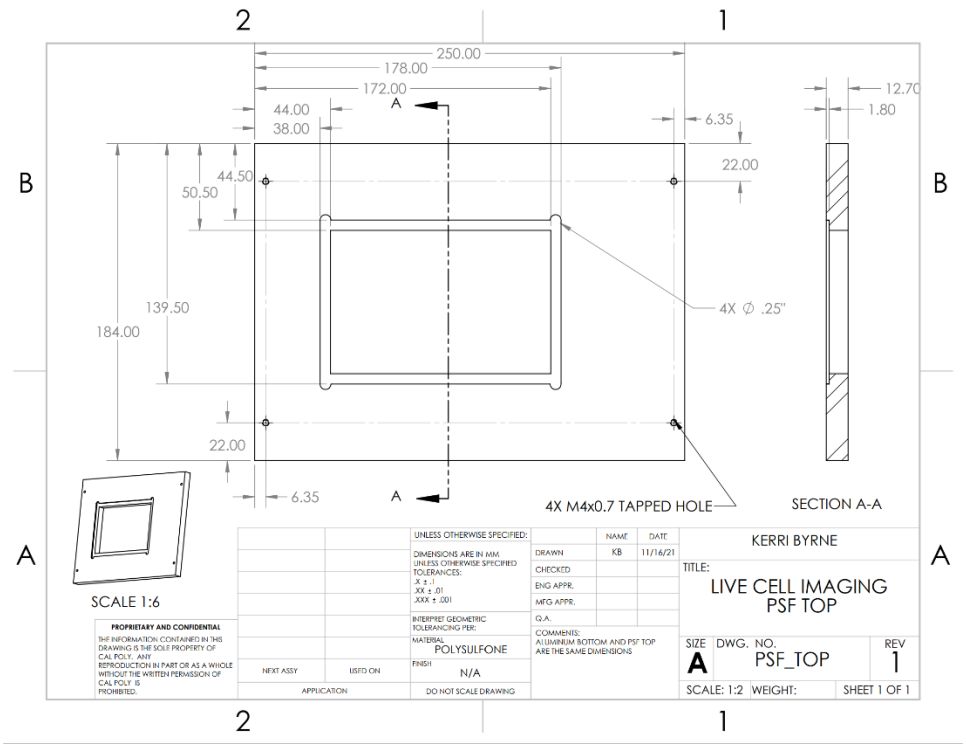
Detailed drawings of left and right polysulfone pieces



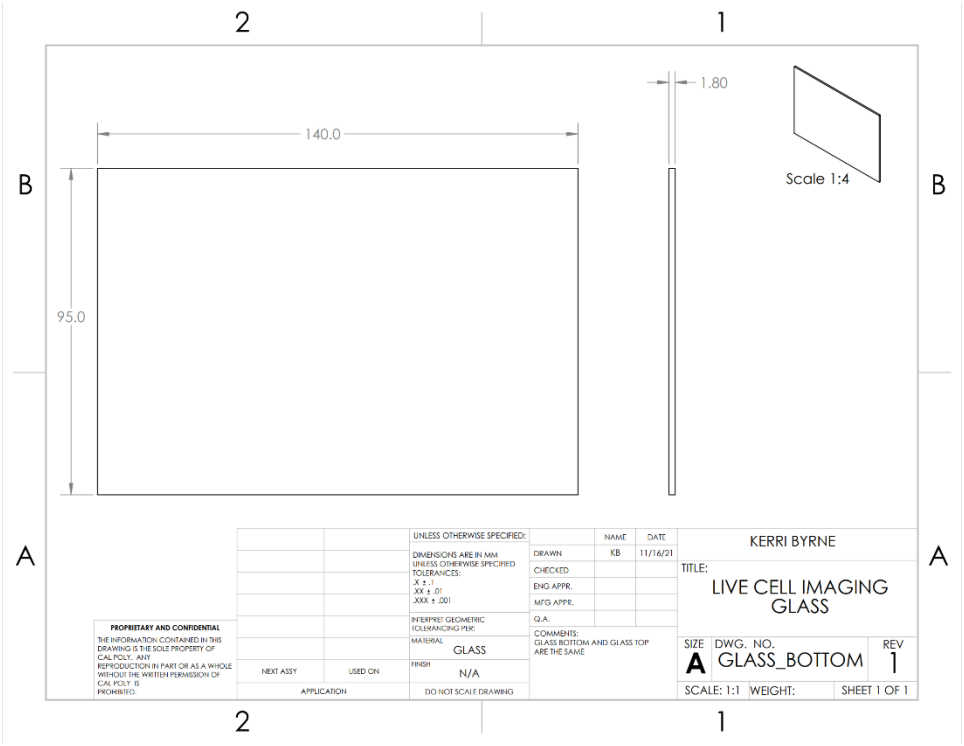


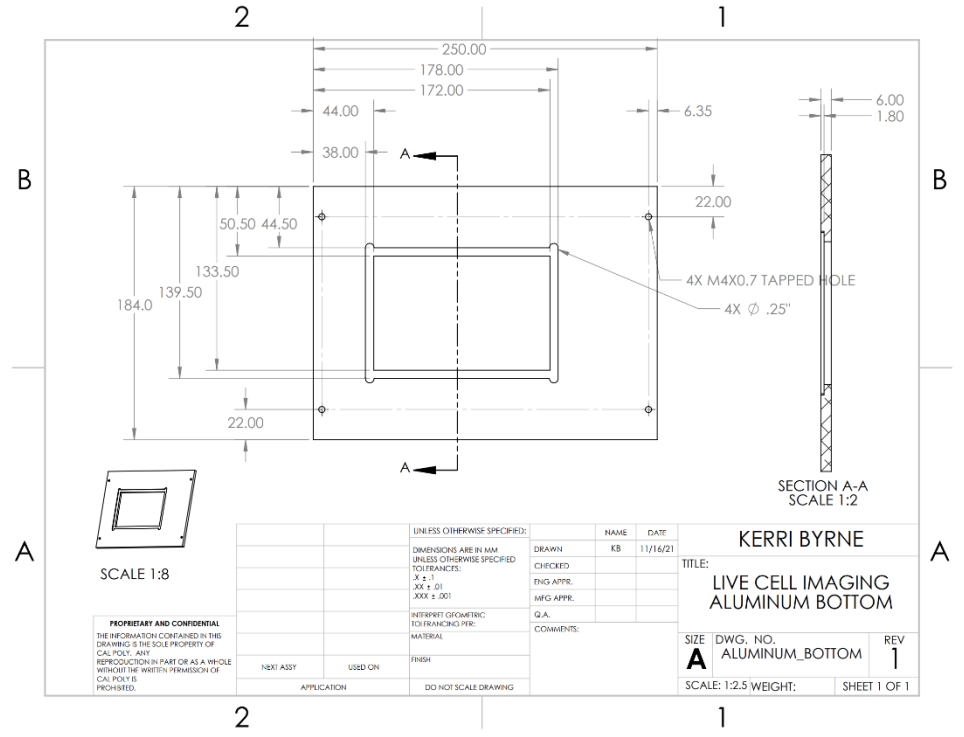
Detailed drawings of silicone bottom and polysulfone top





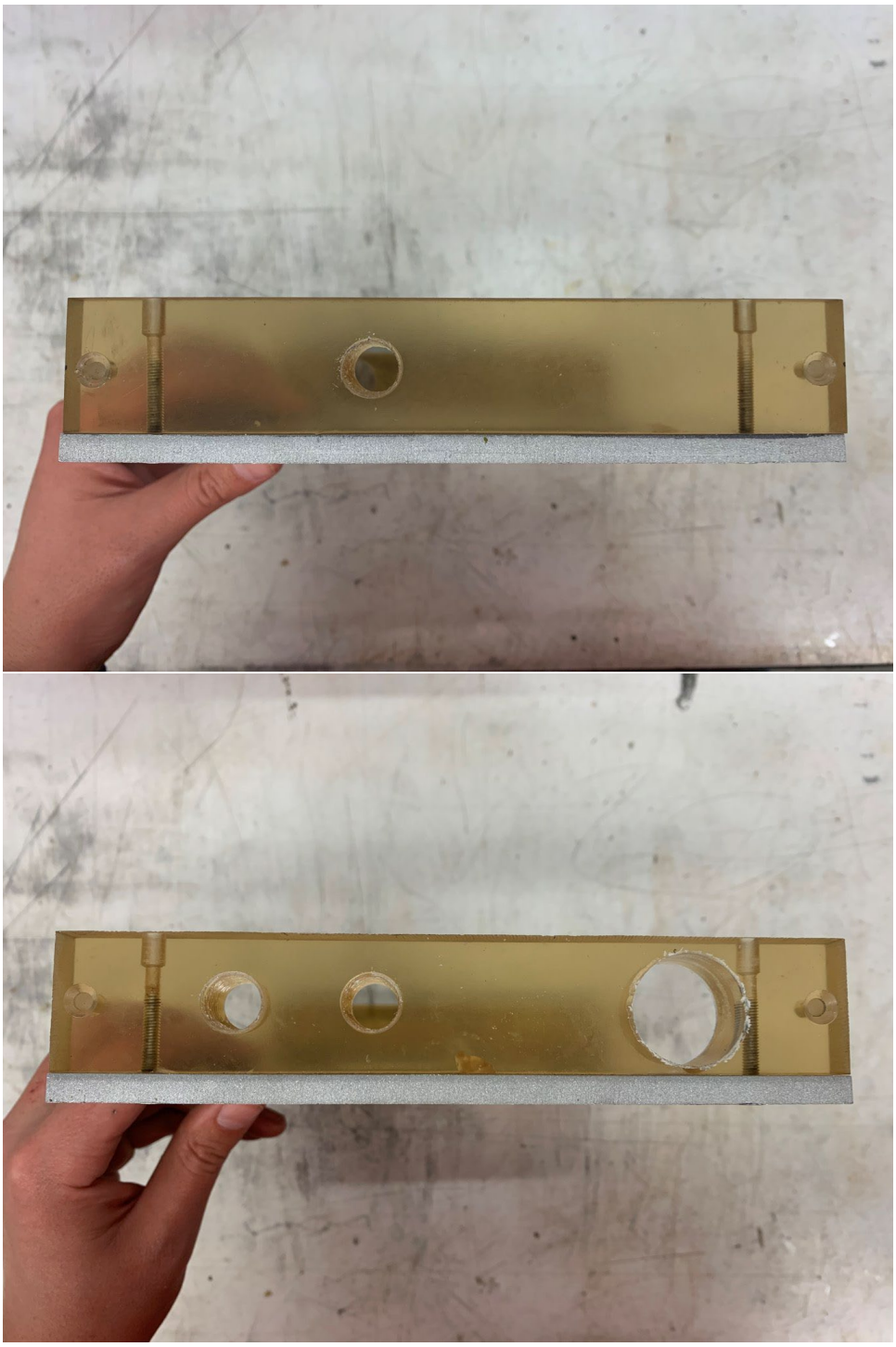
Detailed drawings of glass and aluminum bottom



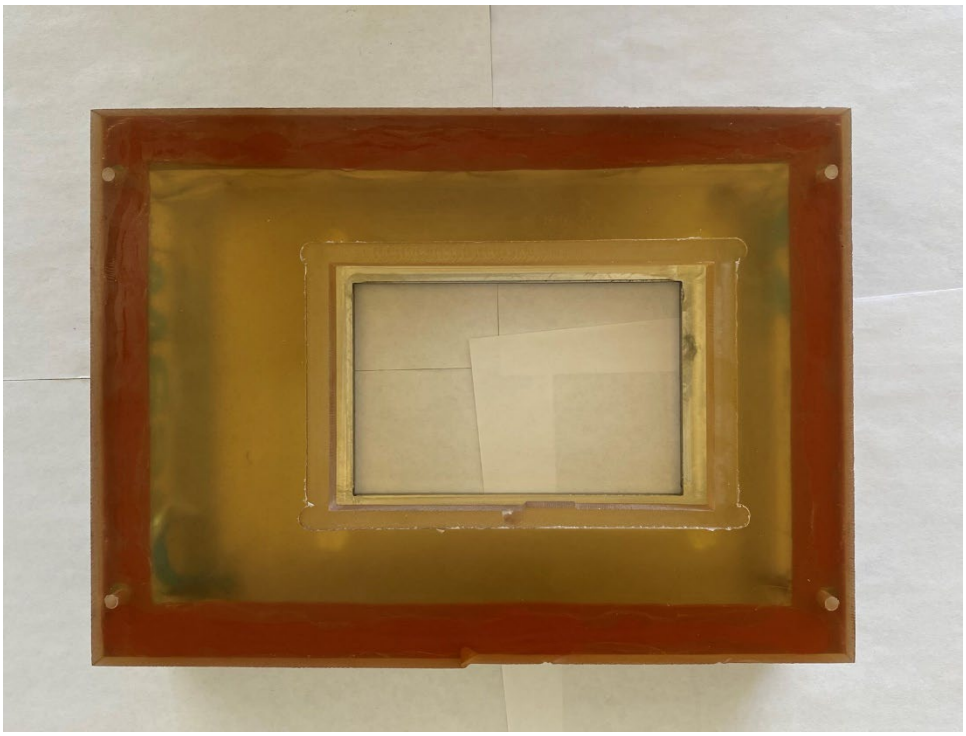


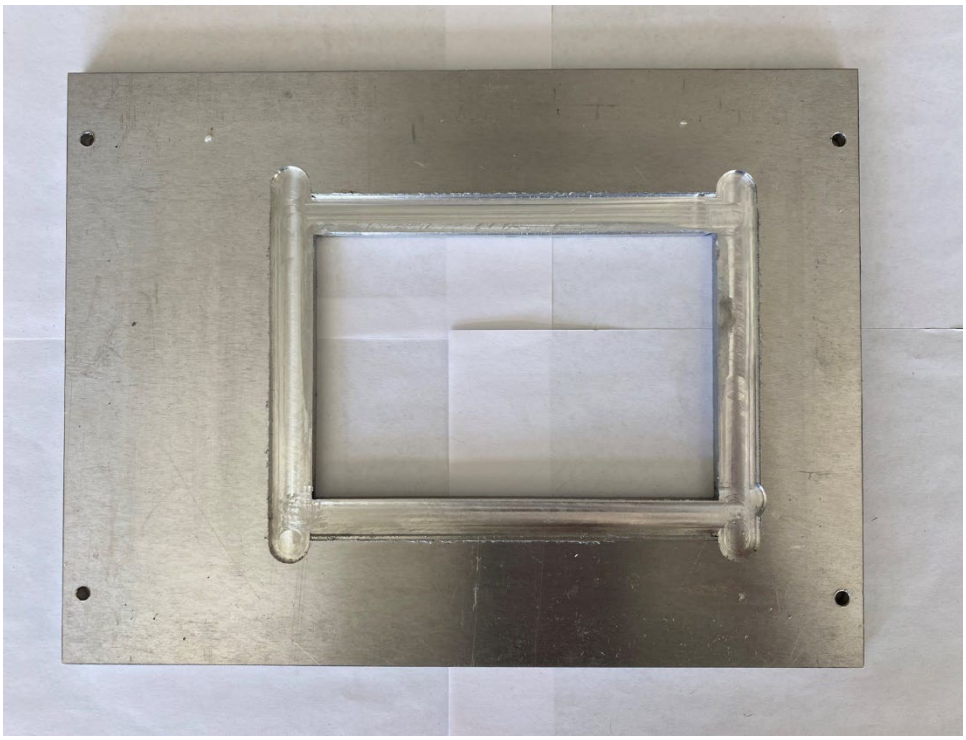
Appendix I: Manufactured chamber front and back (Items #2.4 from Table 15)

Manufactured chamber right and left side (Item #2.2 and #2.3 from Table 15)

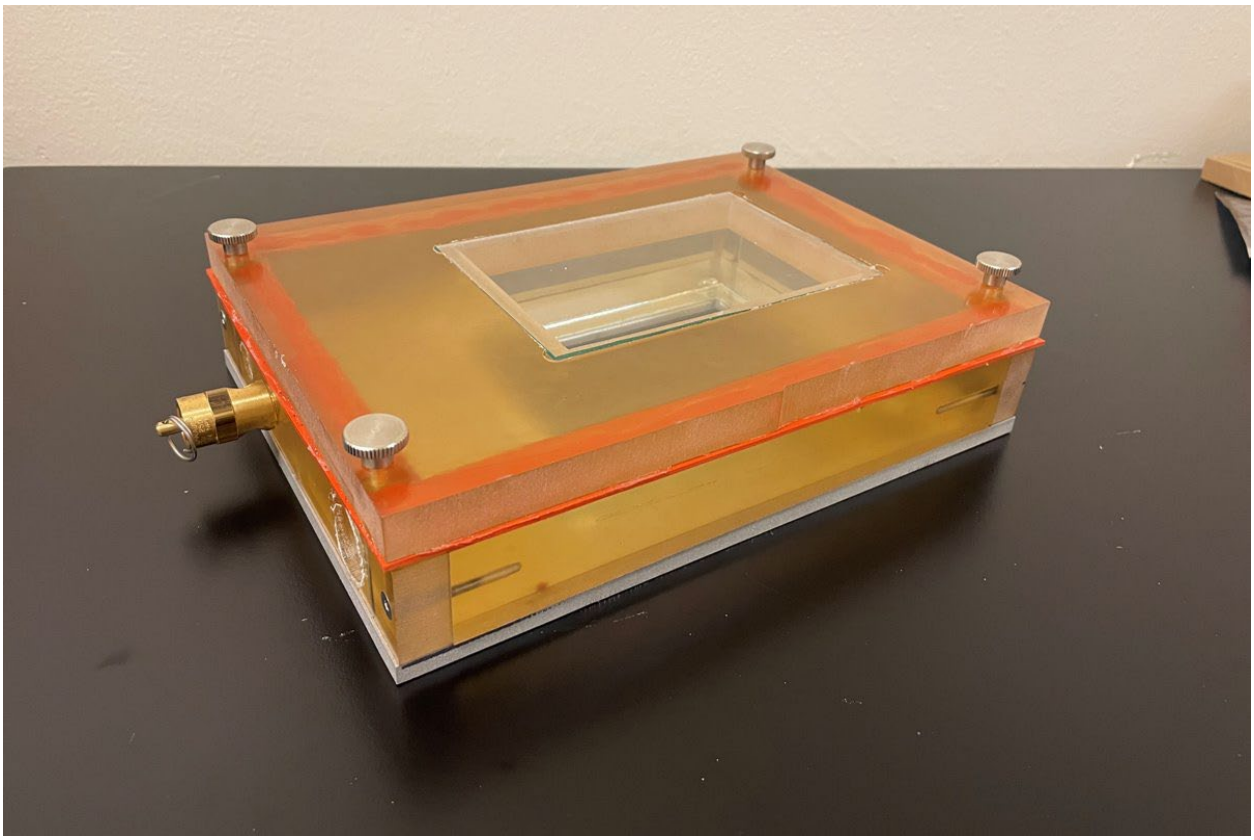


Manufactured chamber top and heat plate (Item #2.1 and #1 from Table 15)

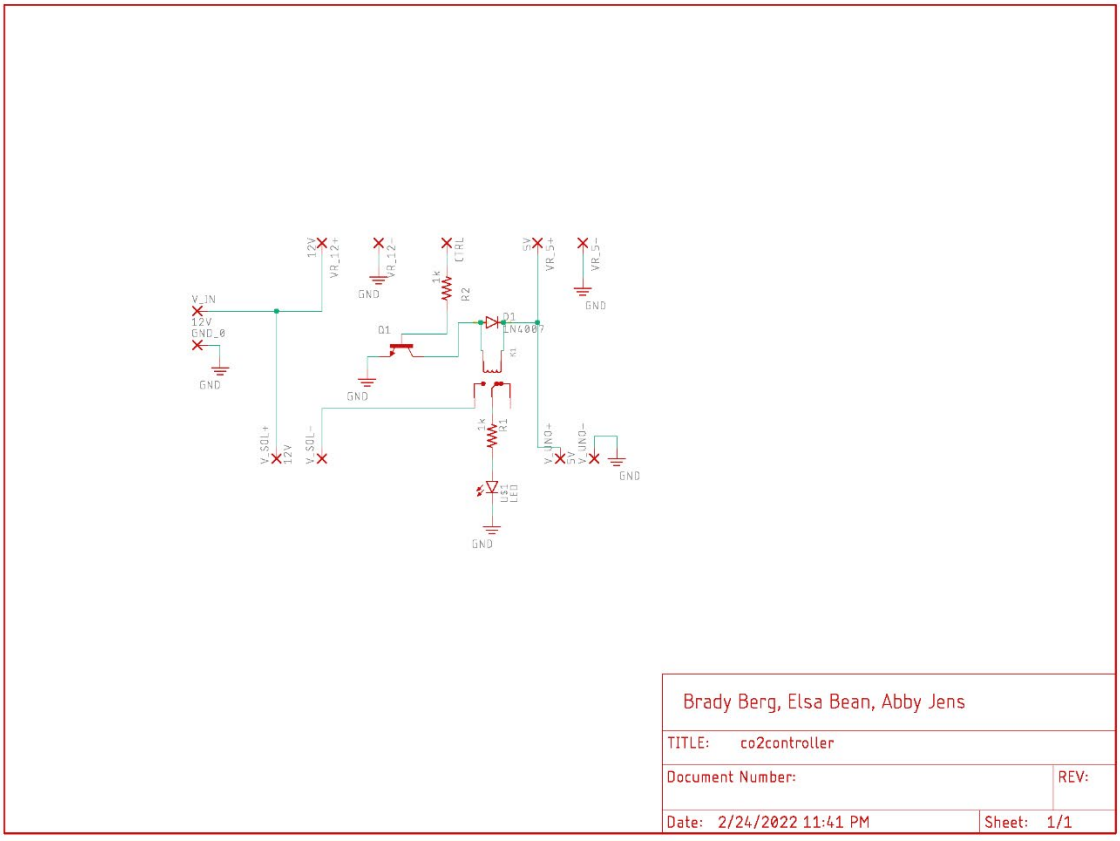


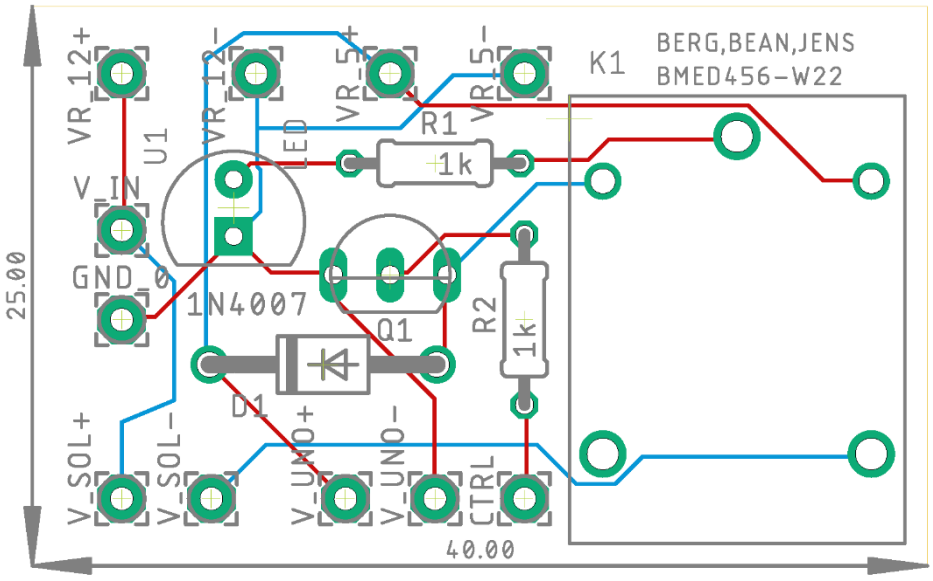


Assembled Chamber Prototype (without CO_2 or temperature sensor inputs)

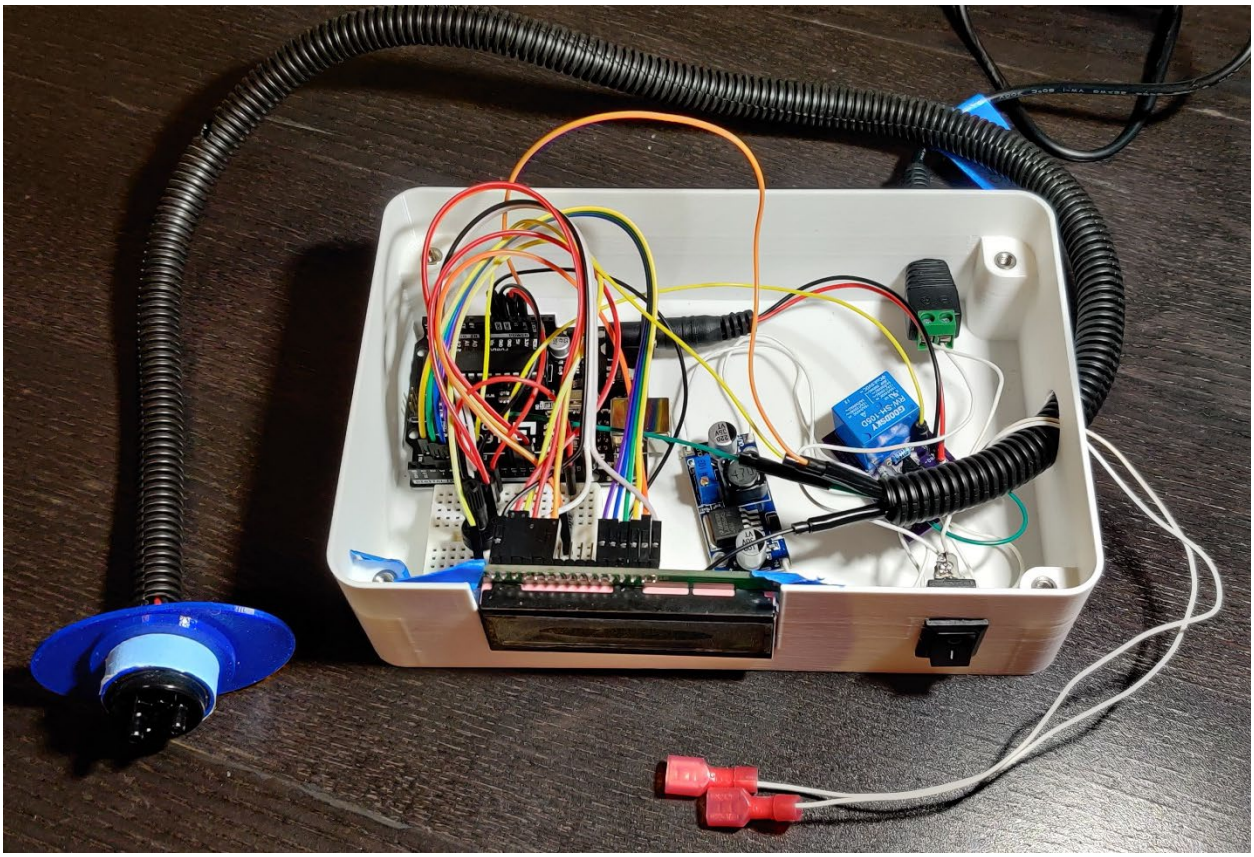


Appendix J: Relay PCB Schematic and Board Layout





Appendix K: Assembled CO_2 Control System



Appendix L: Arduino Program for Gas Control System

```
/*
 * CO2 control system - live cell imaging, gas control subsystem
 * BMED 455/456 W2022, Brady Berg, Elsa Bean, Abby Jens
 *
 * LCD Screen info
 *   The circuit:
 * LCD RS pin to digital pin 12
 * LCD Enable pin to digital pin 11
 * LCD D4 pin to digital pin 5
 * LCD D5 pin to digital pin 4
 * LCD D6 pin to digital pin 3
 * LCD D7 pin to digital pin 2
 * LCD R/W pin to ground
 * LCD VSS pin to ground
 * LCD VCC pin to 5V
 * 10K resistor:
 * ends to +5V and ground
 * wiper to LCD VO pin (pin 3)
 *
 */

#include <cozir.h>
#include <SoftwareSerial.h>
#include <LiquidCrystal.h>

// initialize the library by associating any needed LCD interface pin
// with the arduino pin number it is connected to
const int rs = 12, en = 11, d4 = 5, d5 = 4, d6 = 3, d7 = 2;
LiquidCrystal lcd(rs, en, d4, d5, d6, d7);

SoftwareSerial nss(7, 6); // Rx, Tx from the sensor to Pins 12, 13 on Arduino
COZIR czr(&nss);

float c, reading = 0;
float multiplier = 0.001;  // 0.001 = 10/10000 (Hardware multiplier/ppm conversion)
```

```
                        // For more details see sensor specificaiton sheet
float setpoint = 5; // desired CO2%
int relayPin = 10; // control pin # for relay
int pulseDur = 17; // in ms
int delayDur = 250; // in ms
bool startup = true;
bool showCO2 = false;

float takeReading() {
    uint32_t c = czr.CO2(); // read the sensor, values output as ppm
    reading = c*multiplier; // convert ppm reading to percent
    return reading;
}

void giveReading(unsigned long msec, float reading) {
    Serial.print(msec);
    Serial.print(",");
    Serial.println(reading, 4);
}

void displayReading(float reading) {
    char buff[7];
    dtostrf(reading, 4, 3, buff);
    lcd.setCursor(0,0);
    lcd.print("CO2 (%): ");
    lcd.print(buff);
    lcd.setCursor(15,0);
    lcd.print(".");
    delay(delayDur/2);
    lcd.setCursor(15,0);
    lcd.print(" ");
    delay(delayDur/2);
}

void wait(unsigned long seconds, bool showCO2) {
    unsigned long curTime = millis();
```

```
    unsigned long endTime = curTime + seconds * 1000UL; // wait number of seconds
    while (millis() <= endTime) {
      reading = takeReading();
      if (showCO2) {
        displayReading(reading);
      }
      else if (!showCO2) {
        delay(delayDur);
        // giveReading(millis(),reading);
      }
    }
}

void startup_protocol() {
  lcd.setCursor(0,0);
  lcd.print("Initializing CO2");
  lcd.setCursor(0,1);
  lcd.print("Flushing line...");
  // flush the line
  digitalWrite(relayPin, LOW);
  delay(1000);
  digitalWrite(relayPin, HIGH);
  lcd.clear();
  lcd.print("Attach tubing");
  wait(30UL, showCO2);

  // fill the chamber
  lcd.clear();
  showCO2 = true;
  int idx = 0;
  while (idx < 4) {
    idx += 1;
    lcd.setCursor(0,1);
    lcd.print("Injection ");
    lcd.print(idx);
    lcd.print("/4");
```

```
    digitalWrite(relayPin, LOW);
    delay(pulseDur);
    digitalWrite(relayPin, HIGH);
    wait(20UL, showCO2);
  }
  lcd.setCursor(0,1);
  lcd.print("Gas mixing...");
  wait(240UL, showCO2);
}

void maintain_protocol(float reading) {
  lcd.clear();
  displayReading(reading);
  char buff[5];
  dtostrf(setpoint, 4, 2, buff);
  lcd.setCursor(0,1);
  lcd.print("Target: ");
  lcd.print(buff);
  lcd.print("%");
  if (reading >= 0.75 * setpoint && reading < 0.9 * setpoint) {
    digitalWrite(relayPin, LOW);
    delay(pulseDur);
    digitalWrite(relayPin, HIGH);
    wait(30UL, showCO2);
  }
  else if (reading < 0.75 * setpoint) {
    digitalWrite(relayPin, LOW);
    delay(2 * pulseDur); // this is an extra-long injection to help speed reinitialization
    digitalWrite(relayPin, HIGH);
    wait(45UL, showCO2);
  }
  else {
    digitalWrite(relayPin, HIGH);
    delay(delayDur);
  }
}
```

```
void setup() {
  // setup code runs once
  // set up the LCD's number of columns and rows:
  lcd.begin(16, 2);
  pinMode(relayPin, OUTPUT);  // Set pin for output to control relay
  nss.begin(9600);
  czr.init();
  czr.setOperatingMode(CZR_POLLING);
  lcd.print("Initializing CO2");
  lcd.setCursor(0,1);
  lcd.print("Flushing line...");
  delay(1000);
  // Serial.begin(9600);   // start serial port
  // Serial.println("Time (ms),CO2 (%)");
}

void loop() {
  // put your main code here, to run repeatedly:
  reading = takeReading();
  if (reading >= 0.01) {
    // only proceed into protocols if reading is not identically 0 (due to sensor error)
    unsigned long msec = millis();
    // giveReading(msec, reading);
    if (startup) {
      // bool startup is set to true in setup function, set to false after startup protocol runs
      startup_protocol();
      startup = false;
    }
    else if (!startup) {
      // after startup protocol runs, follow maintenance protocol
      maintain_protocol(reading);
    }
  }
}
```

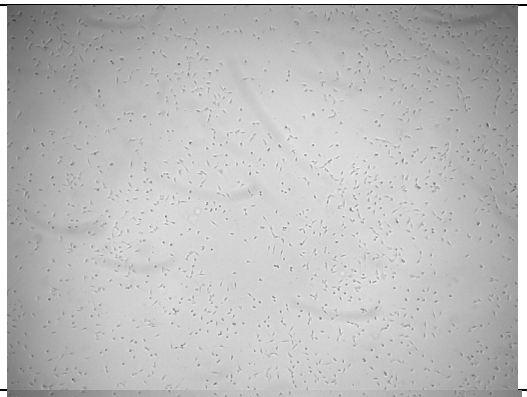
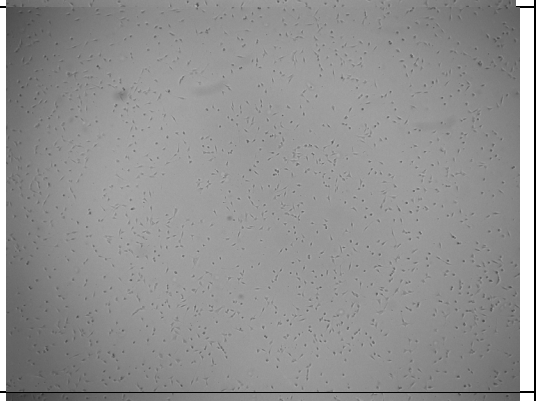
Appendix M: Processing Code Used for Collecting Gas Control Data (Processing 4.0b5)

```
// Clicking "Run" above will initialize the Arduino and begin recording data
// Data will appear in the console
// File will automatically be saved in the location of the co2DataRecord.pde file
// e.g., C:\Users\bcber\Documents\Processing\co2DataRecord
// BE SURE TO CHANGE FILENAME ABOVE BufferedWriter() FUNCTION BEFORE STARTING A NEW TEST

import processing.serial.*;
import java.io.FileWriter;
import java.io.BufferedWriter;

Serial COMPort;  // Create object from Serial class

//String[] lines = new String[0];
BufferedWriter output;

void setup()
{
  frameRate(5);
  String portName = Serial.list()[0];
  COMPort = new Serial(this, portName, 9600);
}

void draw()
{
  if (COMPort.available() > 0) {  // If data is available,
    String read = COMPort.readStringUntil('\n');  // read and store it to string read
    if (read != null) {
      read = trim(read);
      println(read);
      try {
        // CHANGE FILENAME BELOW BEFORE RUNNING TEST
        String filename =
"C:\\Users\\bcber\\Documents\\Processing\\co2DataRecord\\zero_noise_fix.txt";
        output = new BufferedWriter(new FileWriter(filename, true)); //the true will append the
new data
        output.write(read);
        output.write("\n");
      }
      catch (IOException e) {
        println("It Broke");
        e.printStackTrace();
      }
      catch (Exception e) {
        println("Other error");
        e.printStackTrace();
      }
      finally {
        try {
            if (output != null) {
              output.close();
            }
        }
        catch (IOException e) {
            println("Error while closing the writer");
            e.printStackTrace();
        }
      }
    }
  }
}
```

Appendix N: Relative Degree of Focus for each image in Appendix O

Image Type	Clarity (%)	Image Type	Clarity (%)
Control 4x1	18.4341	Control 10x1	18.0236
Control 4x2	18.9788	Control 10x2	18.0128
Control 4x3	18.2739	Control 10x3	18.1357
Control 4x4	19.6676	Control 10x4	17.7205
Control 4x5	18.8460	Control 10x5	17.2631
Control 4x6	17.9239	Control 10x6	17.9071
Control 4x7	18.6844	Control 10x7	17.5814
Control 4x8	19.1743	Control 10x8	17.5079
Control 4x9	18.7488	Control 10x9	17.9165
Control 4x10	19.4476	Control 10x10	17.4574
Chamber 4x1	21.0231	Chamber 10x1	18.8334
Chamber 4x2	21.5743	Chamber 10x2	19.3646
Chamber 4x3	21.2226	Chamber 10x3	18.1246
Chamber 4x4	22.6921	Chamber 10x4	18.4637
Chamber 4x5	20.6378	Chamber 10x5	17.7172
Chamber 4x6	19.8643	Chamber 10x6	19.8297
Chamber 4x7	21.4559	Chamber 10x7	17.5966
Chamber 4x8	20.7677	Chamber 10x8	18.1862
Chamber 4x9	20.1253	Chamber 10x9	17.2293
Chamber 4x10	21.2348	Chamber 10x10	17.0104

Appendix O: Photos Taken for Relative Focus Measurements

Image Number	Chamber 4x	Control 4x
1		
2		
3		
4		

5		
6		
7		
8		

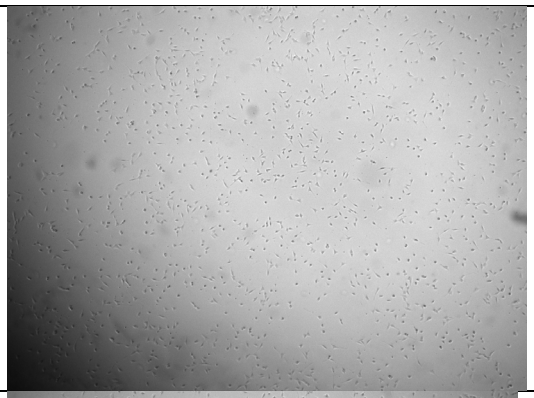
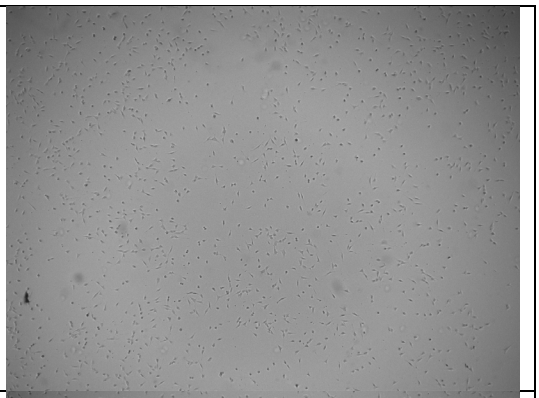
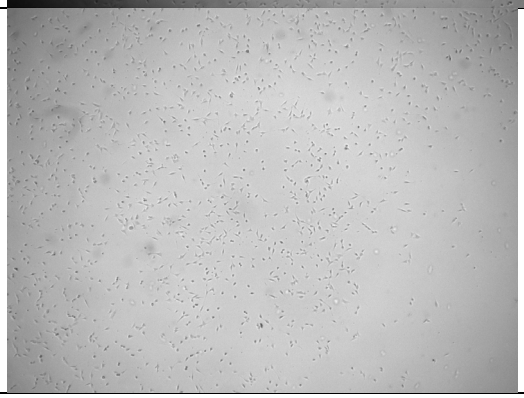
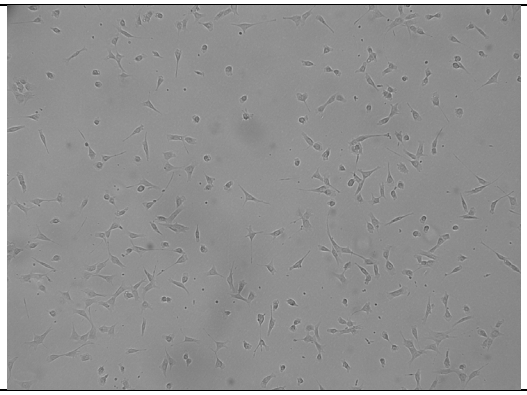
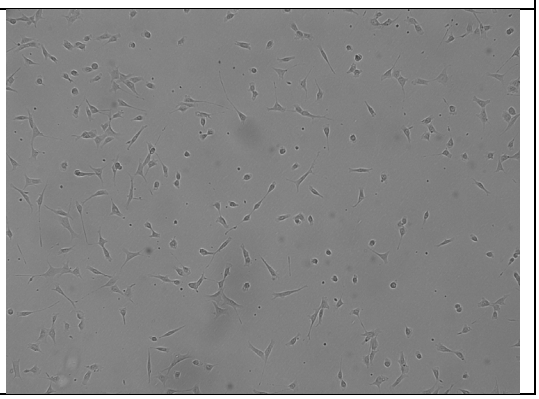
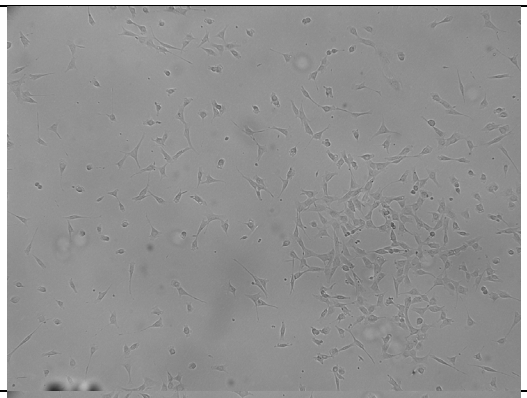
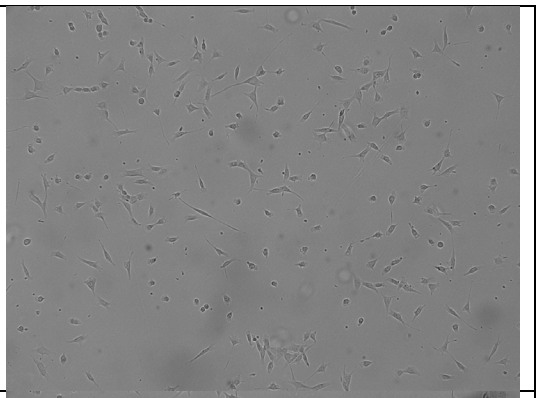
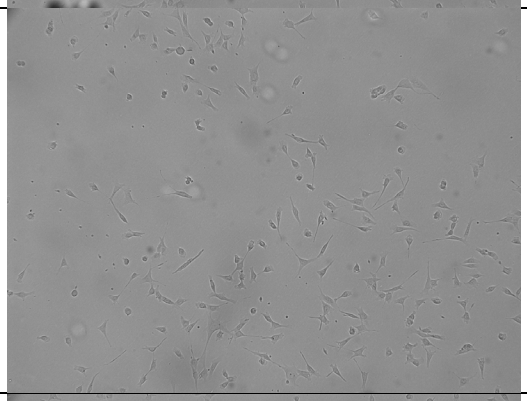
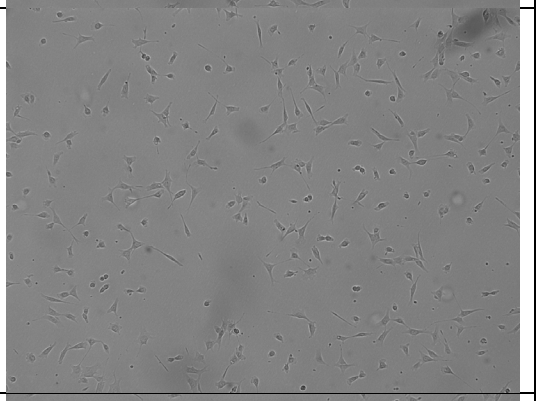
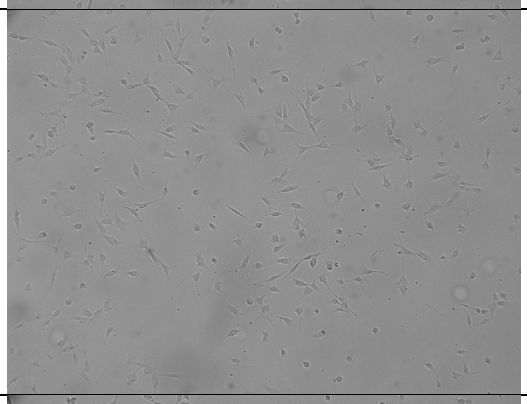
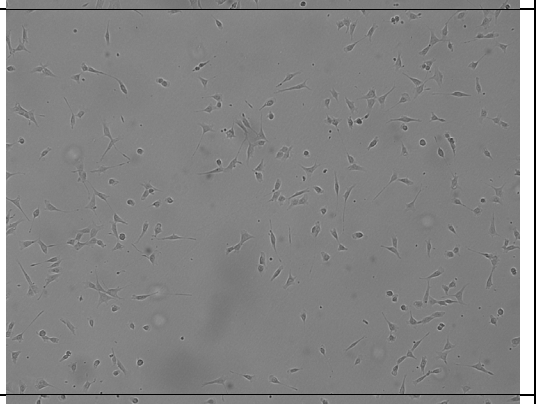
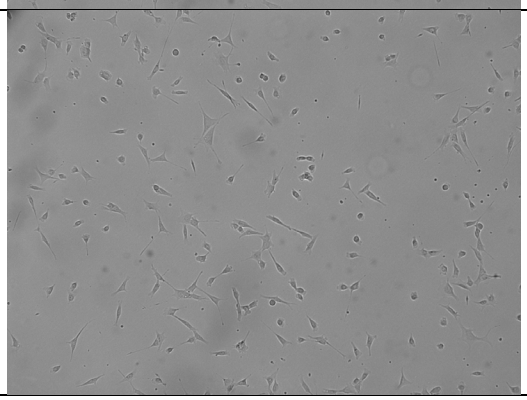
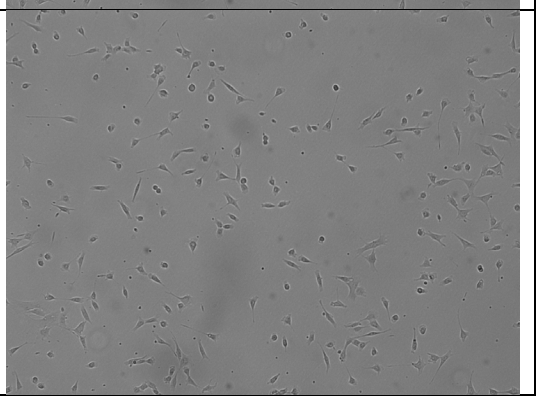
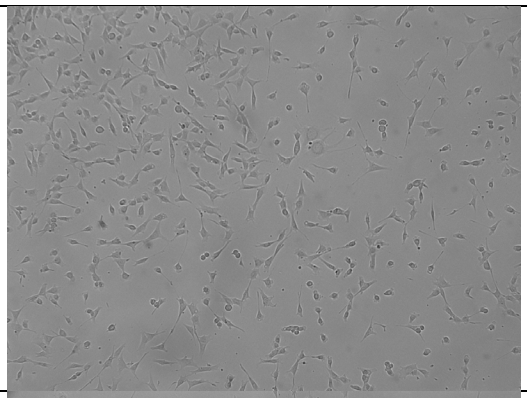
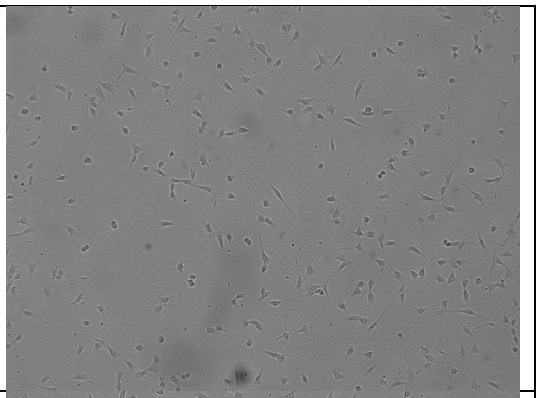
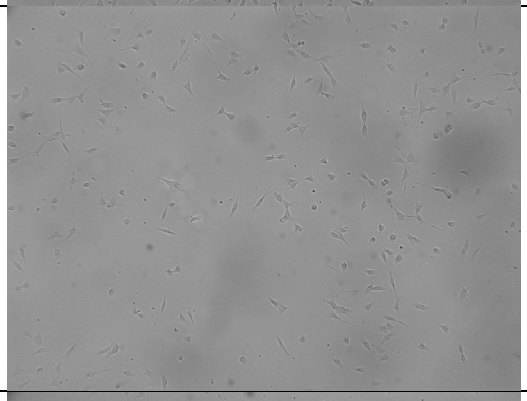
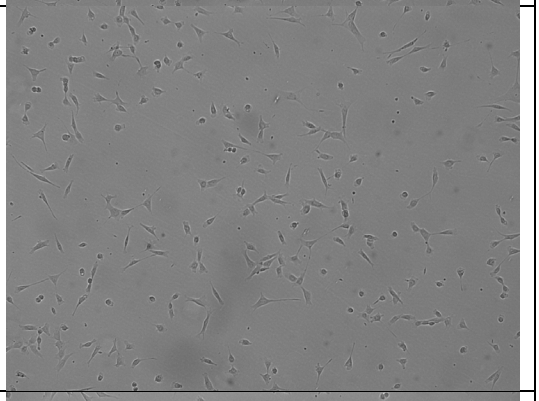
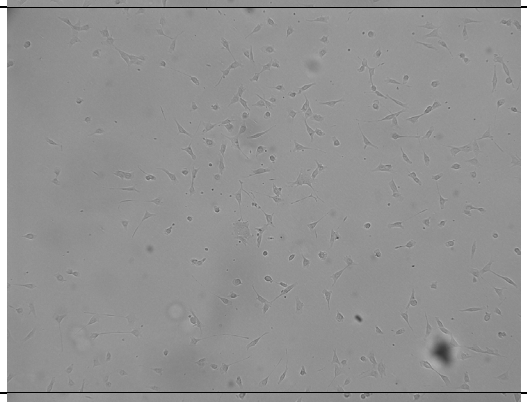
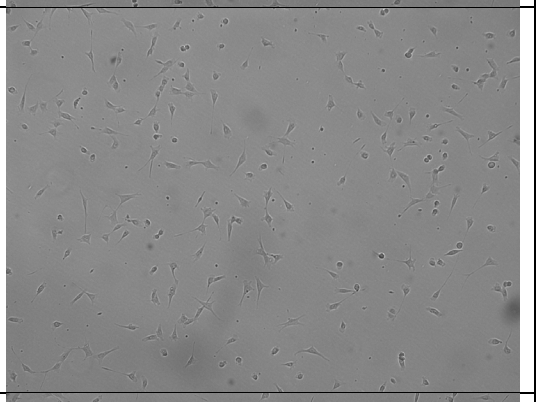
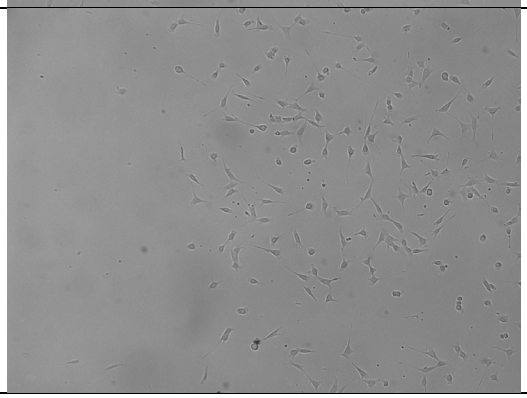
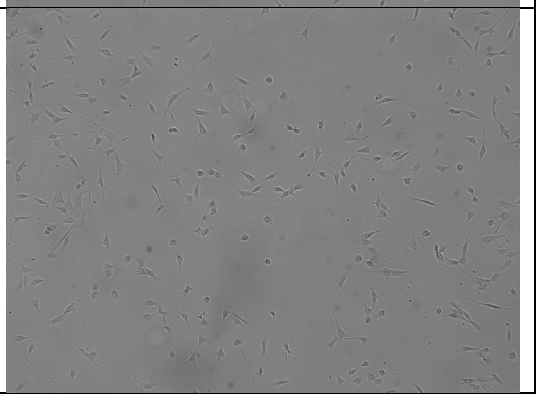
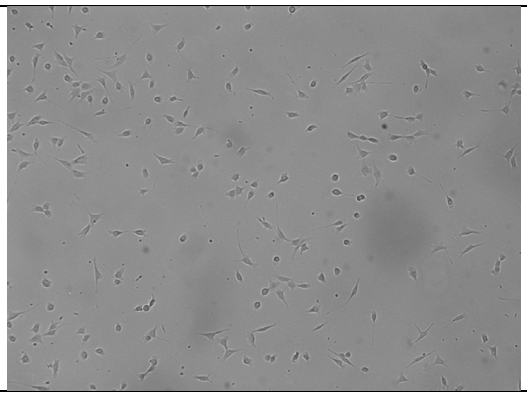
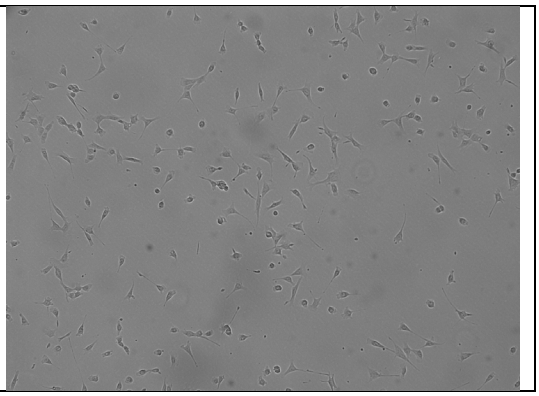
9		
10		

Image Number	Chamber 10x	Control 10x
1		

2		
3		
4		
5		

6		
7		
8		
9		

10		

Appendix P: Relative Degree of Focus MATLAB Code

```
clear all;
close all;
%Image clarity code for Live Cell Imaging Gas and Heat Control

% Read in image files

Control4x1 = imread('4x_Control_Image1.tif');
Control4x2 = imread('4x_Control_Image2.tif');
Control4x3 = imread('4x_Control_Image3.tif');
Control4x4 = imread('4x_Control_Image4.tif');
Control4x5 = imread('4x_Control_Image5.tif');
Control4x6 = imread('4x_Control_Image6.tif');
Control4x7 = imread('4x_Control_Image7.tif');
Control4x8 = imread('4x_Control_Image8.tif');
Control4x9 = imread('4x_Control_Image9.tif');
Control4x10 = imread('4x_Control_Image10.tif');

Chamber4x1 = imread('4x_Chamber_Image1.tif');
Chamber4x2 = imread('4x_Chamber_Image2.tif');
Chamber4x3 = imread('4x_Chamber_Image3.tif');
Chamber4x4 = imread('4x_Chamber_Image4.tif');
Chamber4x5 = imread('4x_Chamber_Image5.tif');
Chamber4x6 = imread('4x_Chamber_Image6.tif');
Chamber4x7 = imread('4x_Chamber_Image7.tif');
Chamber4x8 = imread('4x_Chamber_Image8.tif');
Chamber4x9 = imread('4x_Chamber_Image9.tif');
Chamber4x10 = imread('4x_Chamber_Image10.tif');

Control10x1 = imread('10x_Control_Image1.tif');
Control10x2 = imread('10x_Control_Image2.tif');
Control10x3 = imread('10x_Control_Image3.tif');
Control10x4 = imread('10x_Control_Image4.tif');
Control10x5 = imread('10x_Control_Image5.tif');
Control10x6 = imread('10x_Control_Image6.tif');
Control10x7 = imread('10x_Control_Image7.tif');
Control10x8 = imread('10x_Control_Image8.tif');
Control10x9 = imread('10x_Control_Image9.tif');
Control10x10 = imread('10x_Control_Image10.tif');

Chamber10x1 = imread('10x_Chamber_Image1.tif');
Chamber10x2 = imread('10x_Chamber_Image2.tif');
Chamber10x3 = imread('10x_Chamber_Image3.tif');
Chamber10x4 = imread('10x_Chamber_Image4.tif');
Chamber10x5 = imread('10x_Chamber_Image5.tif');
Chamber10x6 = imread('10x_Chamber_Image6.tif');
Chamber10x7 = imread('10x_Chamber_Image7.tif');
Chamber10x8 = imread('10x_Chamber_Image8.tif');
Chamber10x9 = imread('10x_Chamber_Image9.tif');
Chamber10x10 = imread('10x_Chamber_Image10.tif');


% Analyze Image focus based on Brenner's focus measure operator, outputs %
relative degree of focus percent of the image ?

FM_Control4x1 = fmeasure(Control4x1, 'CONT');
```

```
FM_Control4x2 = fmeasure(Control4x2, 'CONT');
FM_Control4x3 = fmeasure(Control4x3, 'CONT');
FM_Control4x4 = fmeasure(Control4x4, 'CONT');
FM_Control4x5 = fmeasure(Control4x5, 'CONT');
FM_Control4x6 = fmeasure(Control4x6, 'CONT');
FM_Control4x7 = fmeasure(Control4x7, 'CONT');
FM_Control4x8 = fmeasure(Control4x8, 'CONT');
FM_Control4x9 = fmeasure(Control4x9, 'CONT');
FM_Control4x10 = fmeasure(Control4x10, 'CONT');

FM_Control4x_Avg = (FM_Control4x1 + FM_Control4x2 + FM_Control4x3 ...
                    + FM_Control4x4 + FM_Control4x5 + FM_Control4x6 ...
                    + FM_Control4x7 + FM_Control4x8 + FM_Control4x9 ...
                    + FM_Control4x10)/10;

FM_Chamber4x1 = fmeasure(Chamber4x1, 'CONT');
FM_Chamber4x2 = fmeasure(Chamber4x2, 'CONT');
FM_Chamber4x3 = fmeasure(Chamber4x3, 'CONT');
FM_Chamber4x4 = fmeasure(Chamber4x4, 'CONT');
FM_Chamber4x5 = fmeasure(Chamber4x5, 'CONT');
FM_Chamber4x6 = fmeasure(Chamber4x6, 'CONT');
FM_Chamber4x7 = fmeasure(Chamber4x7, 'CONT');
FM_Chamber4x8 = fmeasure(Chamber4x8, 'CONT');
FM_Chamber4x9 = fmeasure(Chamber4x9, 'CONT');
FM_Chamber4x10 = fmeasure(Chamber4x10, 'CONT');

FM_Chamber4x_Avg = (FM_Chamber4x1 + FM_Chamber4x2 + FM_Chamber4x3 ...
                    + FM_Chamber4x4 + FM_Chamber4x5 + FM_Chamber4x6 ...
                    + FM_Chamber4x7 + FM_Chamber4x8 + FM_Chamber4x9 ...
                    + FM_Chamber4x10)/10;

FM_Control10x1 = fmeasure(Control10x1, 'CONT');
FM_Control10x2 = fmeasure(Control10x2, 'CONT');
FM_Control10x3 = fmeasure(Control10x3, 'CONT');
FM_Control10x4 = fmeasure(Control10x4, 'CONT');
FM_Control10x5 = fmeasure(Control10x5, 'CONT');
FM_Control10x6 = fmeasure(Control10x6, 'CONT');
FM_Control10x7 = fmeasure(Control10x7, 'CONT');
FM_Control10x8 = fmeasure(Control10x8, 'CONT');
FM_Control10x9 = fmeasure(Control10x9, 'CONT');
FM_Control10x10 = fmeasure(Control10x10, 'CONT');

FM_Control10x_Avg = (FM_Control10x1 + FM_Control10x2 + FM_Control10x3 ...
                    + FM_Control10x4 + FM_Control10x5 + FM_Control10x6 ...
                    + FM_Control10x7 + FM_Control10x8 + FM_Control10x9 ...
                    + FM_Control10x10)/10;

FM_Chamber10x1 = fmeasure(Chamber10x1, 'CONT');
FM_Chamber10x2 = fmeasure(Chamber10x2, 'CONT');
FM_Chamber10x3 = fmeasure(Chamber10x3, 'CONT');
FM_Chamber10x4 = fmeasure(Chamber10x4, 'CONT');
FM_Chamber10x5 = fmeasure(Chamber10x5, 'CONT');
FM_Chamber10x6 = fmeasure(Chamber10x6, 'CONT');
FM_Chamber10x7 = fmeasure(Chamber10x7, 'CONT');
FM_Chamber10x8 = fmeasure(Chamber10x8, 'CONT');
FM_Chamber10x9 = fmeasure(Chamber10x9, 'CONT');
FM_Chamber10x10 = fmeasure(Chamber10x10, 'CONT');
```

```
FM_Chamber10x_Avg = (FM_Chamber10x1 + FM_Chamber10x2 + FM_Chamber10x3 ...
                     + FM_Chamber10x4 + FM_Chamber10x5 + FM_Chamber10x6 ...
                     + FM_Chamber10x7 + FM_Chamber10x8 + FM_Chamber10x9 ...
                     + FM_Chamber10x10)/10;

disp(FM_Control4x_Avg);
disp(FM_Chamber4x_Avg);
disp(FM_Chamber10x_Avg);
disp(FM_Control10x_Avg);



function FM = fmeasure(Image, Measure, ROI)
%This function measures the relative degree of focus of
%an image. It may be invoked as:
%
%   FM = fmeasure(IMAGE, METHOD, ROI)
%
%Where
%   IMAGE,  is a grayscale image and FM is the computed
%           focus value.
%   METHOD, is the focus measure algorithm as a string.
%           see 'operators.txt' for a list of focus
%           measure methods.
%   ROI,    Image ROI as a rectangle [xo yo width heigth].
%           if an empty argument is passed, the whole
%           image is processed.
%
%  Said Pertuz
%  Jan/2016
if nargin>2 && ~isempty(ROI)
    Image = imcrop(Image, ROI);
end
WSize = 15; % Size of local window (only some operators)
switch upper(Measure)
    case 'ACMO' % Absolute Central Moment (Shirvaikar2004)
        if ~isinteger(Image), Image = im2uint8(Image);
        end
        FM = AcMomentum(Image);

    case 'BREN' % Brenner's (Santos97)
        [M, N] = size(Image);
        DH = zeros(M, N);
        DV = zeros(M, N);
        DV(1:M-2,:) = Image(3:end,:)-Image(1:end-2,:);
        DH(:,1:N-2) = Image(:,3:end)-Image(:,1:end-2);
        FM = max(DH, DV);
        FM = FM.^2;
        FM = mean2(FM);

    case 'CONT' % Image contrast (Nanda2001)
        ImContrast = @(x) sum(abs(x(:)-x(5)));
        FM = nlfilter(Image, [3 3], ImContrast);
        FM = mean2(FM);

    case 'CURV' % Image Curvature (Helmli2001)
```

```
    if ~isinteger(Image), Image = im2uint8(Image);
    end
    M1 = [-1 0 1;-1 0 1;-1 0 1];
    M2 = [1 0 1;1 0 1;1 0 1];
    P0 = imfilter(Image, M1, 'replicate', 'conv')/6;
    P1 = imfilter(Image, M1', 'replicate', 'conv')/6;
    P2 = 3*imfilter(Image, M2, 'replicate', 'conv')/10 ...
        -imfilter(Image, M2', 'replicate', 'conv')/5;
    P3 = -imfilter(Image, M2, 'replicate', 'conv')/5 ...
        +3*imfilter(Image, M2, 'replicate', 'conv')/10;
    FM = abs(P0) + abs(P1) + abs(P2) + abs(P3);
    FM = mean2(FM);

case 'DCTE' % DCT energy ratio (Shen2006)
    FM = nlfilter(Image, [8 8], @DctRatio);
    FM = mean2(FM);

case 'DCTR' % DCT reduced energy ratio (Lee2009)
    FM = nlfilter(Image, [8 8], @ReRatio);
    FM = mean2(FM);

case 'GDER' % Gaussian derivative (Geusebroek2000)
    N = floor(WSize/2);
    sig = N/2.5;
    [x,y] = meshgrid(-N:N, -N:N);
    G = exp(-(x.^2+y.^2)/(2*sig^2))/(2*pi*sig);
    Gx = -x.*G/(sig^2);
    Gx = Gx/sum(abs(Gx(:)));
    Gy = -y.*G/(sig^2);
    Gy = Gy/sum(abs(Gy(:)));
    Rx = imfilter(double(Image), Gx, 'conv', 'replicate');
    Ry = imfilter(double(Image), Gy, 'conv', 'replicate');
    FM = Rx.^2+Ry.^2;
    FM = mean2(FM);

case 'GLVA' % Graylevel variance (Krotkov86)
    FM = std2(Image);

case 'GLLV' %Graylevel local variance (Pech2000)
    LVar = stdfilt(Image, ones(WSize,WSize)).^2;
    FM = std2(LVar)^2;

case 'GLVN' % Normalized GLV (Santos97)
    FM = std2(Image)^2/mean2(Image);

case 'GRAE' % Energy of gradient (Subbarao92a)
    Ix = Image;
    Iy = Image;
    Iy(1:end-1,:) = diff(Image, 1, 1);
    Ix(:,1:end-1) = diff(Image, 1, 2);
    FM = Ix.^2 + Iy.^2;
    FM = mean2(FM);

case 'GRAT' % Thresholded gradient (Snatos97)
    Th = 0; %Threshold
    Ix = Image;
    Iy = Image;
```

```
    Iy(1:end-1,:) = diff(Image, 1, 1);
    Ix(:,1:end-1) = diff(Image, 1, 2);
    FM = max(abs(Ix), abs(Iy));
    FM(FM<Th)=0;
    FM = sum(FM(:))/sum(sum(FM~=0));

case 'GRAS' % Squared gradient (Eskicioglu95)
    Ix = diff(Image, 1, 2);
    FM = Ix.^2;
    FM = mean2(FM);

case 'HELM' %Helmli's mean method (Helmli2001)
    MEANF = fspecial('average',[WSize WSize]);
    U = imfilter(Image, MEANF, 'replicate');
    R1 = U./Image;
    R1(Image==0)=1;
    index = (U>Image);
    FM = 1./R1;
    FM(index) = R1(index);
    FM = mean2(FM);

case 'HISE' % Histogram entropy (Krotkov86)
    FM = entropy(Image);

case 'HISR' % Histogram range (Firestone91)
    FM = max(Image(:))-min(Image(:));


case 'LAPE' % Energy of laplacian (Subbarao92a)
    LAP = fspecial('laplacian');
    FM = imfilter(Image, LAP, 'replicate', 'conv');
    FM = mean2(FM.^2);

case 'LAPM' % Modified Laplacian (Nayar89)
    M = [-1 2 -1];
    Lx = imfilter(Image, M, 'replicate', 'conv');
    Ly = imfilter(Image, M', 'replicate', 'conv');
    FM = abs(Lx) + abs(Ly);
    FM = mean2(FM);

case 'LAPV' % Variance of laplacian (Pech2000)
    LAP = fspecial('laplacian');
    ILAP = imfilter(Image, LAP, 'replicate', 'conv');
    FM = std2(ILAP)^2;

case 'LAPD' % Diagonal laplacian (Thelen2009)
    M1 = [-1 2 -1];
    M2 = [0 0 -1;0 2 0;-1 0 0]/sqrt(2);
    M3 = [-1 0 0;0 2 0;0 0 -1]/sqrt(2);
    F1 = imfilter(Image, M1, 'replicate', 'conv');
    F2 = imfilter(Image, M2, 'replicate', 'conv');
    F3 = imfilter(Image, M3, 'replicate', 'conv');
    F4 = imfilter(Image, M1', 'replicate', 'conv');
    FM = abs(F1) + abs(F2) + abs(F3) + abs(F4);
    FM = mean2(FM);

case 'SFIL' %Steerable filters (Minhas2009)
```

```
    % Angles = [0 45 90 135 180 225 270 315];
    N = floor(WSize/2);
    sig = N/2.5;
    [x,y] = meshgrid(-N:N, -N:N);
    G = exp(-(x.^2+y.^2)/(2*sig^2))/(2*pi*sig);
    Gx = -x.*G/(sig^2);Gx = Gx/sum(Gx(:));
    Gy = -y.*G/(sig^2);Gy = Gy/sum(Gy(:));
    R(:,:,1) = imfilter(double(Image), Gx, 'conv', 'replicate');
    R(:,:,2) = imfilter(double(Image), Gy, 'conv', 'replicate');
    R(:,:,3) = cosd(45)*R(:,:,1)+sind(45)*R(:,:,2);
    R(:,:,4) = cosd(135)*R(:,:,1)+sind(135)*R(:,:,2);
    R(:,:,5) = cosd(180)*R(:,:,1)+sind(180)*R(:,:,2);
    R(:,:,6) = cosd(225)*R(:,:,1)+sind(225)*R(:,:,2);
    R(:,:,7) = cosd(270)*R(:,:,1)+sind(270)*R(:,:,2);
    R(:,:,8) = cosd(315)*R(:,:,1)+sind(315)*R(:,:,2);
    FM = max(R,[],3);
    FM = mean2(FM);

case 'SFRQ' % Spatial frequency (Eskicioglu95)
    Ix = Image;
    Iy = Image;
    Ix(:,1:end-1) = diff(Image, 1, 2);
    Iy(1:end-1,:) = diff(Image, 1, 1);
    FM = mean2(sqrt(double(Iy.^2+Ix.^2)));

case 'TENG'% Tenengrad (Krotkov86)
    Sx = fspecial('sobel');
    Gx = imfilter(double(Image), Sx, 'replicate', 'conv');
    Gy = imfilter(double(Image), Sx', 'replicate', 'conv');
    FM = Gx.^2 + Gy.^2;
    FM = mean2(FM);

case 'TENV' % Tenengrad variance (Pech2000)
    Sx = fspecial('sobel');
    Gx = imfilter(double(Image), Sx, 'replicate', 'conv');
    Gy = imfilter(double(Image), Sx', 'replicate', 'conv');
    G = Gx.^2 + Gy.^2;
    FM = std2(G)^2;

case 'VOLA' % Vollath's correlation (Santos97)
    Image = double(Image);
    I1 = Image; I1(1:end-1,:) = Image(2:end,:);
    I2 = Image; I2(1:end-2,:) = Image(3:end,:);
    Image = Image.*(I1-I2);
    FM = mean2(Image);

case 'WAVS' %Sum of Wavelet coeffs (Yang2003)
    [C,S] = wavedec2(Image, 1, 'db6');
    H = wrcoef2('h', C, S, 'db6', 1);
    V = wrcoef2('v', C, S, 'db6', 1);
    D = wrcoef2('d', C, S, 'db6', 1);
    FM = abs(H) + abs(V) + abs(D);
    FM = mean2(FM);

case 'WAVV' %Variance of  Wav...(Yang2003)
    [C,S] = wavedec2(Image, 1, 'db6');
    H = abs(wrcoef2('h', C, S, 'db6', 1));
```

```
        V = abs(wrcoef2('v', C, S, 'db6', 1));
        D = abs(wrcoef2('d', C, S, 'db6', 1));
        FM = std2(H)^2+std2(V)+std2(D);

    case 'WAVR'
        [C,S] = wavedec2(Image, 3, 'db6');
        H = abs(wrcoef2('h', C, S, 'db6', 1));
        V = abs(wrcoef2('v', C, S, 'db6', 1));
        D = abs(wrcoef2('d', C, S, 'db6', 1));
        A1 = abs(wrcoef2('a', C, S, 'db6', 1));
        A2 = abs(wrcoef2('a', C, S, 'db6', 2));
        A3 = abs(wrcoef2('a', C, S, 'db6', 3));
        A = A1 + A2 + A3;
        WH = H.^2 + V.^2 + D.^2;
        WH = mean2(WH);
        WL = mean2(A);
        FM = WH/WL;
    otherwise
        error('Unknown measure %s',upper(Measure))
end
 end
%************************************************************************
function fm = AcMomentum(Image)
[M, N] = size(Image);
Hist = imhist(Image)/(M*N);
Hist = abs((0:255)-mean2(Image))'.*Hist;
fm = sum(Hist);
end
%************************************************************************
function fm = DctRatio(M)
MT = dct2(M).^2;
fm = (sum(MT(:))-MT(1,1))/MT(1,1);
end
%************************************************************************
function fm = ReRatio(M)
M = dct2(M);
fm = (M(1,2)^2+M(1,3)^2+M(2,1)^2+M(2,2)^2+M(3,1)^2)/(M(1,1)^2);
end
%************************************************************************
```

Appendix Q: User Testing Questions Asked

User 1

1. Do I need to wear gloves?
2. Do you have a picture/diagram with the components labeled?
3. Is this [the CO_2 sensor hole] on the other side?
4. Where is the power cord [for the heater power supply]?
5. What's a condenser?
6. Am I taking the heating pad with me?
7. Should I start moving things? [while trying to put the pad and the chamber on the stage, in reference to the wires and cables]
8. What does "replace" mean [in the heated stage procedure]?
9. The gas canister is referring to what component, exactly?
10. Where is the 12V power cord [for the gas control system]?
11. What does "next to the heating power strip" mean?
12. How do I know the CO_2 gas has been flushed through the tubing?
13. Do I need to turn off the CO_2 gas canister at any point?

User 2

1. Are steps [attach temp probe] and [attach temp probe cord] the same?
2. The gas canister is where?
3. Which knob do I turn [CO_2 gas canister and regulator]?
4. How much do I turn the knob [CO_2 gas canister and regulator]?
5. And there's another power cord [for the gas control system]?
6. Did something happen? [in reference to whether the gas has flushed through the chamber]

Appendix R: MATLAB Code for Plotting Testing Data

```
clear clear;
close all;

load IT1
load IT2
load IT3
load IT4
load IT5
load IT6.txt
load IT7

load LT1
load LT2
load LT3

load MT1

% Initialization test 1
t1_ms=IT1(:,1);
t1_s=t1_ms.*0.001;
conc1=IT1(:,2);
%ind=find(conc1>=5);
%t1_s(ind);

figure(1)
plot(t1_s,conc1);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Initialization Test #1');
%xlim([0 600]);

% Initialization test 2
t2_ms=IT2(:,1);
t2_s=t2_ms.*0.001;
conc2=IT2(:,2);
%ind=find(conc2>=5);
%t2_s(ind)

figure(2)
plot(t2_s,conc2);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Initialization Test #2');
%xlim([0 600]);

% Initialization test 3
t3_ms=IT3(:,1);
t3_s=t3_ms.*0.001;
conc3=IT3(:,2);
%ind=find(conc3>=5);
%t3_s(ind)

figure(3)
plot(t3_s,conc3);
xlabel('Time (s)');
```

```
ylabel('CO2 Concentration (%)');
title('Initialization Test #3');
%xlim([500 900]);

% Initialization test 4
t4_ms=IT4(:,1);
t4_s=t4_ms.*0.001;
conc4=IT4(:,2);
%ind=find(conc4>=5);
%t4_s(ind)

figure(4)
plot(t4_s,conc4);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Initialization Test #4');
%xlim([1200 2000]);

% Initialization test 5
t5_ms=IT5(:,1);
t5_s=t5_ms.*0.001;
conc5=IT5(:,2);
%ind=find(conc5>=5);
%t5_s(ind)

figure(5)
plot(t5_s,conc5);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Initialization Test #5');
%xlim([0 600]);

% Initialization test 6
t6_ms=IT6(:,1);
t6_s=t6_ms.*0.001;
conc6=IT6(:,2);
%ind=find(conc6>=5);
%t6_s(ind)

figure(6)
plot(t6_s,conc6);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Initialization Test #6');
%xlim([600 1200]);

% Leakage test 1
t1l_ms=LT1(:,1);
t1l_s=t1l_ms.*0.001;
conc1l=LT1(:,2);
%ind=find(conc1l>=5);
%t1l_s(ind)

figure(7)
plot(t1l_s,conc1l);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
```

```
title('Leakage Test #1');
%xlim([0 600]);

% Leakage test 2
t2l_ms=LT2(:,1);
t2l_s=t2l_ms.*0.001;
conc2l=LT2(:,2);
%ind=find(conc2l>=5);
%t2l_s(ind)

figure(8)
plot(t2l_s,conc2l);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Leakage Test #2');
%xlim([0 600]);

% Maintenance test 1
t1m_ms=MT1(:,1);
t1m_s=t1m_ms.*0.001;
conc1m=MT1(:,2);

figure(9)
plot(t1m_s,conc1m);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Maintenance Test #1');

% Initialization test 7
t7_ms=IT7(:,1);
t7_s=t7_ms.*0.001;
conc7=IT7(:,2);
%ind=find(conc7>=5);
%t7_s(ind)

figure(10)
plot(t7_s,conc7);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Reinitialization Test #10');
%xlim([3000 3500]);

% Leakage test 3
t3l_ms=LT3(:,1);
t3l_s=t3l_ms.*0.001;
conc3l=LT3(:,2);
ind=find(conc3l>=5);
t3l_s(ind)

figure(11)
plot(t3l_s,conc3l);
xlabel('Time (s)');
ylabel('CO2 Concentration (%)');
title('Leakage Test #3');
%xlim([0 600]);
```

Appendix S: Budget with Source and Part Number

Part #	Qty	Name	Material	Source	Price/qty	Total Price
GC-0029	1	R - 6S 20% CO2 Sensor (Sensor Only)	-	CO2meter.com	$ 249.00	$ 249.00
					Shipping/tax	$ 13.80
RSSM-3-12VDC	1	1/4" 12V DC Electric Brass Solenoid Valve	Brass	electricsolenoidvalves.com	$ 29.95	$ 29.95
					Shipping/tax	$ 8.15
CAS25015	1	15 PSI 1/4" Male NPT Air Compressor Pressure Relief Safety Pop Off Valve	Brass	Compressor Source	$ 7.29	$ 7.29
					Shipping/tax	$ 5.59
5233K56	1	Clear Masterkleer Soft PVC Plastic Tubing for Air and Water, 1/4" ID, 3/8" OD, 50ft. Length	PVC Plastic	McMaster Carr	$ 15.00	$ 15.00
6802K22	1	High-Density Thread Sealant Tape PTFE, 0.0032" Thick, 1/4" Wide, 14 Yard Long, White	PTFE	McMaster Carr	$ 3.09	$ 3.09
70355K107	2	Power Cord NEMA 5-15 Plug, 16 Wire Gauge, 3 Feet Long, SJTW	--	McMaster Carr	$ 6.80	$ 13.60
5388K14	1	Worm-Drive Clamps for Firm Hose and Tube Steel Screw, 5/16" Wide Band, 7/32" to 5/8" Clamp ID	301 Stainless Steel	McMaster Carr	$ 7.57	$ 7.57
6937T92	2	UV-Resistant Glass Silicone Sealant, Clear	Silicone	McMaster Carr	$ 5.23	$ 10.46
97163A152	2	Tapered Heat-Set Inserts for Plastic M4 x 0.7mm Thread Size, 5.7 mm Installed Length	303 Stainless Steel	McMaster Carr	$ 5.51	$ 11.02
2838N15	2	Barbed Hose Fitting for Drinking Water Straight Adapter for 1/4" Hose ID x 1/4 NPT Male	Brass	McMaster Carr	$ 2.47	$ 4.94
8054T15	1	Stranded Wire, 300V AC, 18 Gauge, Gray Outer Insulation, 25 Feet Long	--	McMaster Carr	$ 5.66	$ 5.66
89155K11	1	Oversized Multipurpose 6061 Aluminum Sheet	6061 Aluminum	McMaster Carr	$ 54.48	$ 54.48
					Shipping	$ 27.69
					Tax	$ 30.59
RTD1-25N-030-H	1	Pt100 RTD, 30mm length, 6mm diameter, 1/4in male NPT, 4-pin M12 quick-disconnect	Stainless Steel	Automation Direct	$ 63.28	$ 63.28
CS381-00500	1	Polysulfone Natural Sheet .500, 0.500 THK X 12.000 WID X 24.000 LEN	Polysulfone	Boedeker Plastics Inc.	$ 234.75	$ 234.75
					Shipping	$ 34.06
93395A266	1	316 Stainless Steel Hex Drive Flat Head Screw, 90 Degree Countersink, M4 x 0.70mm Thread, 25mm Long, Packs of 25	316 Stainless Steel	McMaster Carr	$ 8.91	$ 8.91
92558A370	6	Stainless Steel Raised Knurled-Head Thumb, Screw, M4 x 0.70 mm Thread Size, 25 mm Long	Stainless Steel	McMaster Carr	$ 8.55	$ 51.30
					Shipping	$ 4.37
					Tax	$ 8.56
RW-SH-105D	2	10 amp relay, SPDT, Coil: 5VDC/73mA	--	Coast Electronics, SLO	$ 4.99	$ 9.98
					Tax	$ 0.87
N/A	3	co2controller_core Custom PCB	--	Osh Park	$ 15.50	$ 15.50
					Shipping	$ 7.35
14-426-1K14	1	26 Gauge Solid Hook-up & Lead wire, misc. bundle	Copper	Coast Electronics, SLO	$ 1.99	$ 1.99
800-00064	1	Jumper Wire 40 M-F 40 Piece	Copper	Coast Electronics, SLO	$ 8.99	$ 8.99
					Tax	$ 0.96
23MG87	1	APC Inline Insert, Male 1/4" NPT (into chamber)	Acetal	Grainger		1.82
23MG77	1	APC Inline Coupler, barbed	Acetal	Grainger		3.07
					Shipping/taxes	0.43
100160	1	M12 Sensor cable ifm elector EVC001	Nickle-plated brass	Automation24		8.8
404734	1	PID temperature controller NOVUS N1040-PRR USB - 8104211200	--	Automation24	85.5	85.5
					Shipping/taxes	12.23
N/A	1	Maxmoral 2pcs DC-DC Buck Converter Power Module 3A Adjustable Step-Down LM2596 Voltage Regulator 24V to 12V 5V 3V	--	Amazon	$7.99	$7.99
N/A	2	Langdon House Picture Frame Glass Replacements (Crystal Clear, 5x7, 3 Pack) High-Definition Glass Sheet	Glass	Amazon	$7.99	$15.98
N/A	1	General Tools 8501 Glass Cutter, Perfect for plate glass, mirrors, window panes, custom picture frames, shelves and stained glass	--	Amazon	$4.97	$4.97
N/A	2	Small Parts - SQ-R-SIL-DSP60-006-1200X1200 "Silicone Sheet Gasket, Red, 1/16" Thick, 12"" x 12"" (Pack of 1)"(SS-0062-12)	Silicone	Amazon	$11.85	$23.70
N/A	1	TigerShark 9 inch by 11 inch Sanding Sheets Grit 80/100/120/150/180/220/320/400 8pcs Pack Paper Gold Line Special Anti Clog Coating	--	Amazon	$7.99	$7.99
		ALITOVE DC 24V 15A 360W Power Supply Universal Regulated Switching Transformer Adapter LED Driver 110V/220V AC Input for LED Strip				
N/A	1	CCTV Radio	--	Amazon	$24.99	$24.99
		Electromagnetic Power Relay, 8-Pin 10 AMP 24V DC Relay Coil with Socket Base, LED Indicator, DPDT 2NO 2NC - LY2NJ [Applicable for DIN Rail				
N/A	1	System]	--	Amazon	$9.99	$9.99
N/A	1	Icstation 12V 7W Flexible Polyimide Heater Plate Adhesive PI Heating Film 25mmx50mm (Pack of 4)	--	Amazon	$14.21	$14.21
N/A	1	Icstation 24V 30W Flexible Polyimide Heater Plate Adhesive PI Heating Film 45mmx100mm (Pack of 4)	--	Amazon	$15.99	$15.99
		12 pcs (4 Sets) Terminal Block - 4 pcs 8 Positions 600V 25A Dual Row Screw Terminals Strip with Cover + 8 pcs 400V 25A 8 Positions Pre-Insulated				
N/A	1	Terminal Barrier Jumper Strips Black & Red by MILAPEAK	--	Amazon	$15.89	$15.89
N/A	1	uxcell 22-16 AWG Wire Cable Connector Fork Spade Terminal #8 with 200 Piece, Red/Blue	--	Amazon	$10.51	$10.51
N/A	1	(Pack of 200 Pieces) 2N2222 Transistor, 2N2222 to-92 Transistor NPN 40V 600mA 300MHz 625mW Through Hole 2N2222A	--	Amazon	$6.99	$6.99
N/A	1	Cafurty 1N4007 DO-41 Axial Silastic Guard Junction Standard Rectifier Diode (Pack of 20)	--	Amazon	$4.23	$4.23
N/A	1	uxcell 5 Pcs JQC-3FF-S-Z DC 12V Coil SPDT 5Pin PCB Electromagnetic Power Relay	--	Amazon	$10.49	$10.49
		DC12V 2A Power Supply Adapter, SANSUN AC100-240V to DC12V Transformers, Switching Power Supply for 12V LED Strip Lights, 12 Volt 2 Amp				
N/A	1	Power Adaptor, 2.1mm X 5.5mm US Plug (1pcs)	--	Amazon	$6.99	$6.99
N/A	1	5pack 9v Battery Clip with 2.1mm X 5.5mm Male DC Plug for Arduino by Corpco	--	Amazon	$5.99	$5.99
					Shipping/taxes	$2.72
N/A	1	ELEGOO UNO R3 Board ATmega328P with USB Cable(Arduino-Compatible) for Arduino		Amazon	$ 14.98	$ 14.98
					Shipping/taxes	$ 1.09
	1	CO2 tanks		Linde/Praxair		$ 46.00
					Total	$1,325.59