Biological Imaging Chamber

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Abstract

The goal of this project is to design an imaging chamber to be used with a high-powered inverted microscope in order to maintain a stable environment for long duration live cell imaging. Some systems are available on the market, but they cost thousands of dollars and would require a new microscope. The motivation for our project is to provide an economical alternative to these designs. Our design features a solenoid valve that opens when CO_2 concentration is low in order to keep the chamber at 5 ± 0.5% [CO_2].

Problem Statement

Construct a live-cell imaging chamber to be used for laser-based confocal and multiphoton imaging. The device needs to keep the concentration of CO_2 at 5 ± 0.5% within the chamber and allow a laser beam to reach cells within the chamber. The chamber acts as a barrier against the outside environment and diminishes cell drift due to air current.

Background Information

Cells are in an aqueous medium, and a small amount of dissolved CO_2 maintains the appropriate pH in the aqueous medium (Swedlow *et al.*, 2007). Therefore, the concentration of CO_2 in the chamber should be between 4.5 and 5.5 percent so a slightly acidic pH is achieved. A slightly acidic pH and temperature around 37 °C is conducive to cell growth. A temperature of 41 °C causes an increased rate of cell degeneration (Lavy *et al.,* 1988) so a tolerance of 37 ± 3 °C is imposed.

Live cell imaging

Live cell imaging is useful for understanding the role of proteins. Interactions between proteins must be examined when cells are alive, and looking at fixed cells does not yield useful information about protein roles (PerkinElmer, Inc., 2007). Confocal laser scanning microscopy (CLSM) is a tool that is used to get a good resolution while imaging cells without killing them (de Leeuw, 2007). Fluorescent staining can be used to tag specimens and image them in 3D on computers as well as allow biologists to understand protein interactions by viewing tagged specimens under a microscope. Limitations to this technique include a relatively slow acquisition speed – too slow to image certain cellular processes – and phototoxicity and photobleaching from the intensity of the laser (PerkinElmer, Inc., 2007).

Perfusion chambers

Perfusion chambers can be used to shield live cells from the external environment. An “open” chamber is similar to a Petri dish and has little control over air flow and gas concentrations. Closed chambers protect cells from evaporation of the medium and make it easier to maintain a constant pH and concentration of carbon dioxide (Dailey *et al.*, 2007). Having a stable environment is a primary concern in order to keep cells alive for imaging. Cells need to be kept at 37 °C and the chamber needs to have an atmosphere of CO_2 at 5 ± 0.5% in order to maintain a slightly acidic pH.

Chambers also protect specimens from airflow and air currents that could move or damage them. Cells are very sensitive to shear forces (Dailey *et al.*, 2007) so a closed chamber allows live cells to be incubated and protected while imaged.

Impact on design requirements

Our client wants to use our device image live cells for up to 4-6 hours. It is essential our device does not damage the cells, so there are various constraints on our design. Dailey *et al.* (2007) make suggestions for considerations needed by imaging chambers, including allowing penetration by a laser, maintenance of the specimen over time, minimal invasion, easy sterilization, sealed, and easy access to cells. Many of these characteristics impact the materials we use. A non-porous substance like plexiglass can be used for easy sterilization. The environment inside the chamber needs to be kept constant (Stephens and Allan, 2003), which will be accomplished by leaking CO_2 in the chamber and using a heated stage on the microscope. Humidity control is not a concern because a sealed chamber itself is an inherent control for humidity (Stephens and Allan, 2003). Plastics should also be avoided when building the chamber because it affects the laser beam – glass should be used instead (Dailey *et al.*, 2007).

Competition

There are several products currently in the field for live cell imaging chambers. However, these products cost thousands of dollars and may or may not be compatible with certain microscopes. We propose to build a product to meet our client's needs for cheaper.

One product is the Focht Chamber System 3 (FCS3, 2007). The Focht Chamber System is a "live cell environmental chamber system for upright microscopes." The temperature of the cell can be controlled up to 50 ˚C with a plus or minus .2 ˚C range. The temperature is also constant across the entire chamber, meaning there is no temperature gradient where one side of the chamber may actually be a couple degrees cooler than the other side. This is ideal for imaging so constant results can be obtained. The chamber also allows for perfusion, or delivery of nutrients to the specimen, to keep the cells alive. And because this is a closed system, CO_2 can be used in the medium. Nonetheless, all these options come at a high price. The Focht Chamber System costs around $2600 (FCS3, 2007).

Figure 1. Incubator 2000 made by 20/20 Technology, Inc. (20/20 Technology, Inc., 2007).

Designed to avoid the disadvantages associated with large, plastic chambers surrounding entire microscopes, the Incubator 2000 is “a miniature chamber that sits on the stage of any upright or inverted microscope.” (20/20 Technology, Inc., 2007) The chamber is small and allows for control of humidity and temperature. Humidity is kept at almost 100% and temperature control is within 0.1 ˚C. Although stability is within 0.1 degrees, temperature accuracy is within 0.2 ˚C (Appl. Sci. Inst., 2007).

20/20 Technology, Inc. (2007) claims the chamber requires “miniscule amounts of a pre-mixed gas,” but Applied Scientific Instrumentation says the gas purge rate of the Incubator 2000 is greater than 0.1 liters per minute.

The chamber feeds air saturated with water into the incubating chamber by first allowing gas to flow through a humidifying chamber as shown in *Fig. 2*.

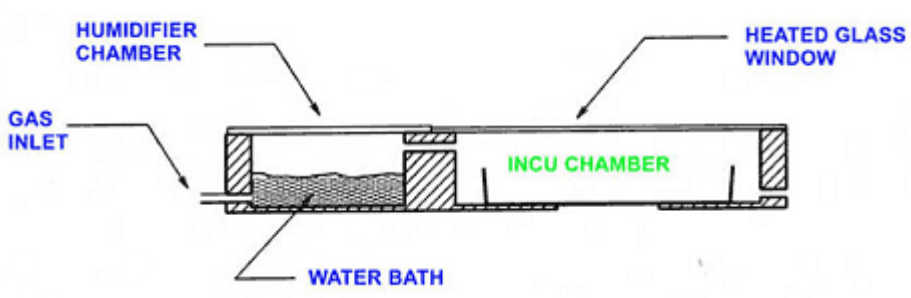

Figure 2. Air humidifier used in the Incubator 2000 (20/20 Technology, Inc.).

The water bath is kept at the same temperature as the incubating chamber, and the glass window above the chamber is kept at a slightly higher temperature to prevent fogging.

The chamber can also accommodate a wide variety of holders including different sized microscope slides and Petri dishes. The inside of the chamber has dimensions of 76 X 56 X 16 mm (20/20 Technology, Inc., 2007).

Figure 3. EMBL Live Cell Observation Chamber by CellBiology Trading (Kern, 2007).

The chamber developed by EMBL as seen in *Fig. 3* features humidity, temperature and CO_2 control, and is an example of a microscope enclosing chamber. The chamber has large doors, but in order to avoid disturbing the inside environment, many components of the chamber are automated. The chamber is accurate to ±0.5 ˚C and is precise to ±0.3 ˚C. CO_2 can be regulated from 0% up to 8% and humidity can be regulated from 0% to 100% (Kern, 2007).

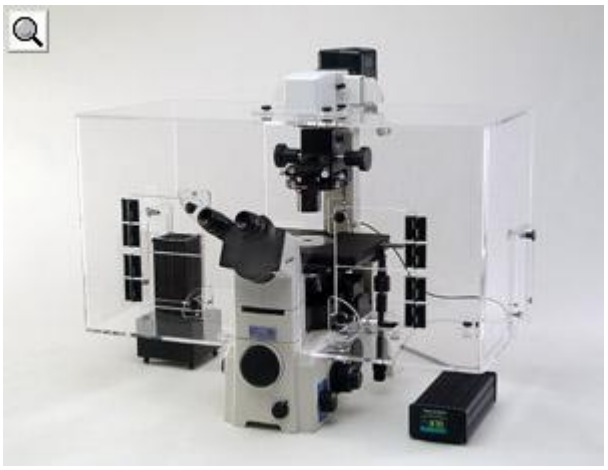

Figure 4. 37° Incubation Chambers by Solent Scientific (Solent Scientific Ltd., 2007).

Another example of a chamber completely enclosing a microscope is the Incubation Chamber by Solent Scientific as shown in *Fig. 4*; this incubating chamber is custom-built by Solent Scientific for their customers. The chamber offers temperature control and CO_2 enrichment. The company also asserts the chamber is easy to disassemble without tools.

Design Solutions

Our design team came up with three alternative solutions to the proposed problem statement. These three designs vary in structure and variety of components used.

Mixed Air Tank Design

This first design is primarily a small, rectangular, Plexiglas chamber resting on a microscope stage. A small chamber would have greater portability than a microscope enclosing design. The specimen lies underneath the chamber, and various tubes carry the gas from a tank to a water bath to warm up the gas, and then finally to the chamber. Many tubes are used because we do not want the specimen to move at all underneath the chamber, and the tubes create a line of symmetry about the specimen. If a line of symmetry is present, equal amounts of gas coming into the chamber at the same pressure and velocity would diminish the effects of the cell specimen moving around in the gas chamber. The gas tank already contains the predetermined mixture of air and CO_2 (95% and 5% respectively). The chamber has a door on the side so the specimen is easily accessible without lifting the entire chamber off the stage as seen in *Fig. 5.* In order to maintain the proper temperature inside the chamber, the pressurized gas will be pumped through hot water, bringing it up to 37 ˚C.

In scenarios involving long term imaging (imaging longer than 6 hours at a time), the gas tank would drain 20 times faster than a pure CO_2 tank (see appendix C). Even if we make our chamber leak proof, there is a small gap of space between the heating disk that the cell specimen rests on and the rest of the microscope. We cannot account for this leak with this design unless we made direct modifications to our client's microscope. Nonetheless, gas flow would be continuous and the tank would drain quickly. A tank of mixed air and CO_2 (95% and 5% respectively) costs approximately $95, while a tank of pure CO_2 costs approximately $15. Since our client and his clients do a lot of long term imaging, using the mixed air tank design will lead to high operating costs.

This design would be highly portable because the chamber would weigh under 10 pounds and have a volume of about 1200 cm^3 (comparable to a small textbook). This design would be relatively cheap because it would not require a CO_2 sensor that can cost upwards of $1000, would be easy to construct and could be used across several different models of microscopes. Some disadvantages are the gas tank mixtures are fairly expensive and different mixture tanks would have to be bought in case imaging needed to be done using a different concentration of CO_2.

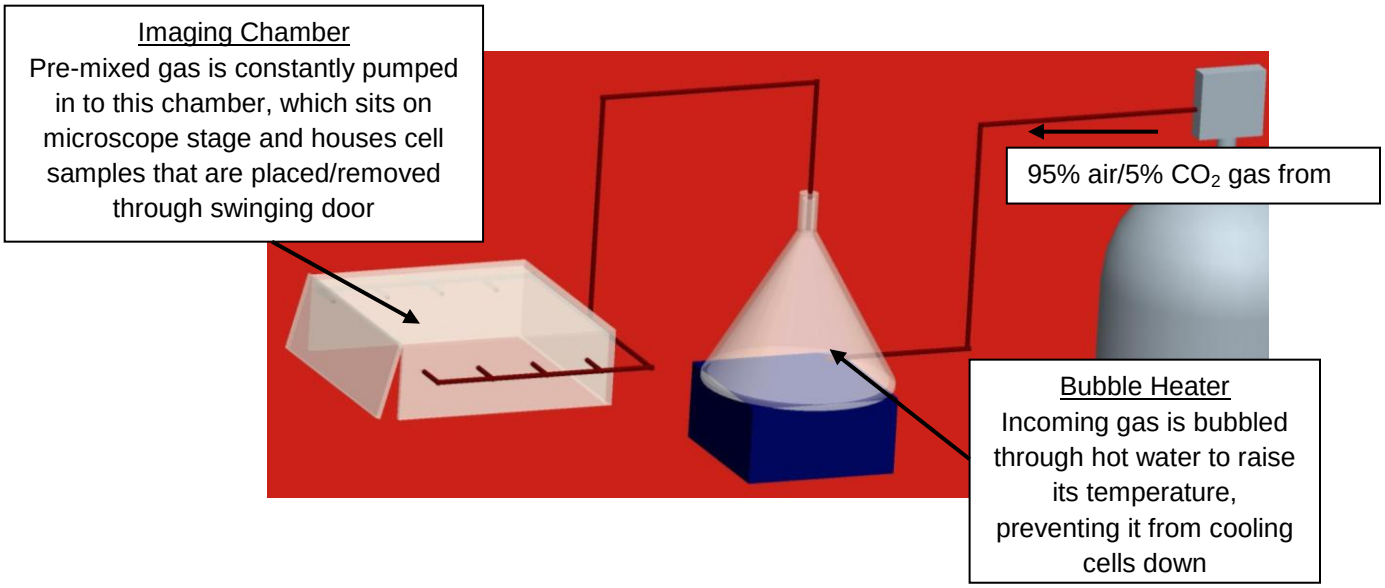

Figure 5. Mixed Air Tank Design. Schematic for mixed air tank design is shown featuring from left to right, chamber, bubble heater, and air supply tank. The cell specimen would be placed in the rectangular chamber, which rests on the microscope stage.

CO_2 Sensor Design

The second design would be similar to the first in that the specimen would be placed in a small chamber resting on the microscope stage with several tubes supplying the gas to the chamber. However, the gas tank would be purely CO_2, not a mixture of air and CO_2. Because of this, a CO_2 sensor would be used to know the concentration of CO_2 inside the chamber. The sensor would be placed outside of the chamber with a probe inserted into the chamber. By having the bulk of the sensor outside of the chamber, the chamber will not be as cluttered. The sensor will have an LCD display for easy monitoring of CO_2 concentration. The circuit will be enclosed in a box made of Plexiglas to protect it from the outside environment. The chamber will be made in an "L" shape to provide ease of access to samples for the user. With this design, the bubble heater would not be needed since only a small amount of gas will be added and the heating plate will be able to keep the temperature at 37 ± 3 °C. Using this method, continuous gas flow would not be needed. The sensor would open flow of CO_2 when the concentration dropped below a set point. This design would save money over time because pure CO_2 tanks are cheaper than mixed air tanks ($15 for pure compared to $95 for mixed) and the CO_2 tank would last longer since a small amount of gas is injected at a time. However, there is a high capital investment due to the CO_2 sensor.

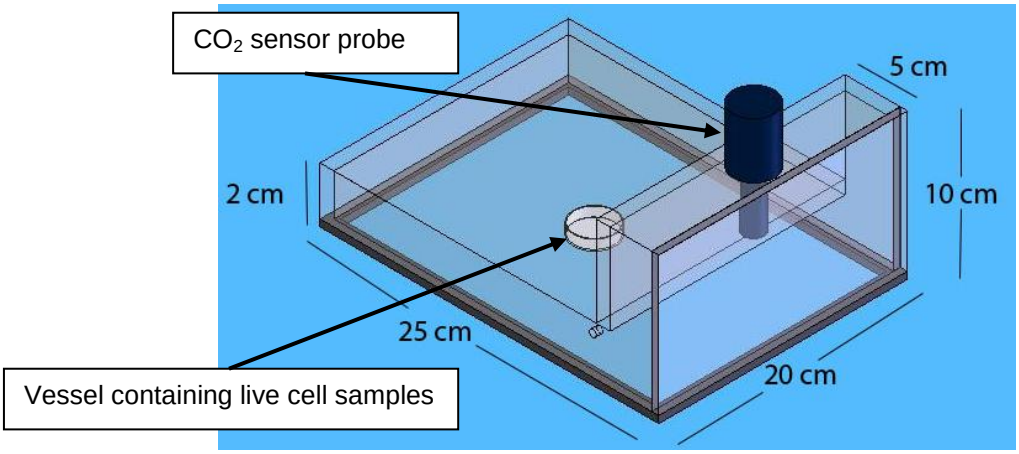

Figure 6. CO_2 Sensor Design. Schematic of Plexiglas chamber with dimensions.

Enclosed Chamber Design

This third design incorporates a giant Plexiglas chamber to enclose the entire system of components, including the microscope. The chamber would be fitted to any protrusions the components or microscope may have, such as wires and cords, and a door would be made to access the specimen on top of the microscope stage. The CO_2 sensor and circuit board would be used. This design would allow for there to be no extra glass between the microscope and the other imaging components, however it would be rather large (approximately 3.335 ft tall) to carry around and it would only be fitted to one model type of microscope. Also, our client maintains primarily fixed cell imaging users, so either setting up then removing this chamber or working around it when it is not needed are both inconvenient for the user.

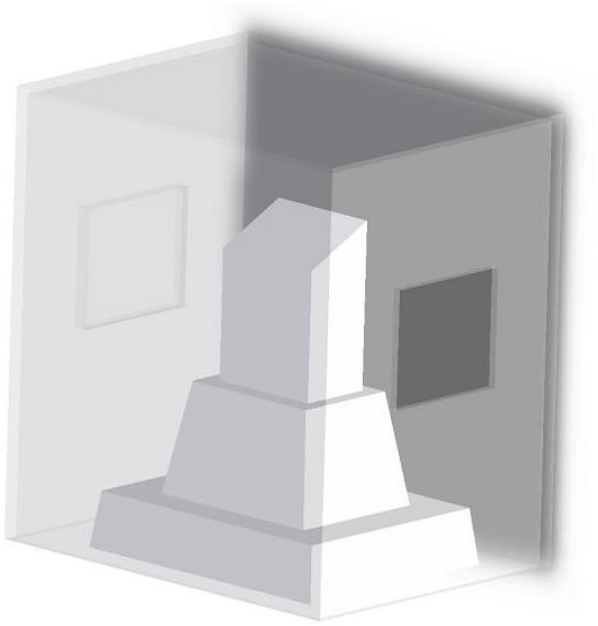

Figure 7. Enclosed Chamber Design. Schematic of the Plexiglas chamber. Microscope is shown sitting inside of chamber, accessible via doors shown on the sides. Cell samples would rest on microscope stage, while premixed air/CO_2 is constantly pumped in from source tank.

Proposed Solution

Table 1. Design matrix which indicates the scoring values of various design possibilities.

Category	Weighting (possible points)	Mixed Air Tank	CO2 Sensor	Enclosed Chamber
Ease of construction	5	5/5	3/5	2/5
Access to samples by user	20	14/20	10/20	17/20
Portability	10	10/10	10/10	0/10
Relative Safety	20	16/20	19/20	18/20
Cost: Capital Investment	15	15/15	5/15	10/15
Cost: Operating	30	12/30	29/30	8/30
Total	100	72/100	76/100	55/100

Our proposed solution is to use the microscope tray sized chamber where pure CO_2 is intermittently pumped inside under direction of a sensor. Since the CO_2 will be delivered at such a small amount, it will not need to be heated by a separate bubble heater. However certain problems arise due to this design choice. These problems are described in the next section. Another drawback of this design is since the sensor has a 20 second response time, the flow rate of CO_2 into the chamber must be calibrated to prevent too much gas from entering the chamber as this could kill the cells. This proposed solution of using the small chamber on top of the stage has the main advantage over the large case because it is portable. The chamber weighs less than two pounds and is 30 x 27.6 x 3 cm which is small enough to fit on other microscope stages. It will be easy to remove from the stage when not needed. As most of our client's users are still using prepared slides for imaging, the chamber would be an encumbrance.

Potential Problems

Precautions need to be taken to prevent the chamber from becoming oversaturated with CO_2. If the concentration gets too high, then the pH inside the medium will drift outside of the desirable range and cause death of cells. Since our circuit opens the valve to let more in when the concentration gets too low, undershooting the range is not an issue. The chamber can become oversaturated if the

flow rate through the valves is too fast. However, a number of different ways exist to remedy this problem should it arise. First, adding a second needle valve or one that restricts flow better could be implemented to lower the flow rate. Second, the circuit could be modified to introduce a 555 timer that would set a specific time that the solenoid valve would be open for allowing control over just how much gas in injected at a time. Finally, the chamber could be made bigger so that the amount of CO_2 added does not have as rapid an effect on the concentration inside.

One of the important design criteria is maintaining a temperature of 37°C ± 3° inside the chamber. However, the gas being injected is not heated. If the volume of gas added is large enough, it can cause the temperature inside the chamber to drop below the defined tolerance. The microscope has a heated stage element that will help to keep within tolerance during imaging, but should that prove insufficient, another method needs to be found to prevent loss of cells. One way to ensure the correct temperature is to add resistance wire inside the chamber that radiates heat. The incoming gas could be passed through an external heating element as well.

Final Design

The chosen design to be finalized and prototyped was the CO_2 sensor design. The presence of a CO_2 sensor and the automated regulation of gas composition made this the best design for an imaging chamber to be usable for years. Also, while the initial cost of construction for this design is much higher than the other alternatives, the CO_2 sensor design will be cheaper to maintain over time because it requires only pure CO_2 tanks, which are significantly cheaper (~$15) than a pre-mixed air/CO_2 tank (~$95).

The CO_2 sensor chosen for this design was the Vaisala GMT221 This sensor reads CO_2 levels from 0-10%, which is sufficiently larger than the desired maximum CO_2 level in the chamber (5.5%) to allow monitoring of any unintended overshoot that may occur when CO_2 is infused. Also, this sensor has a 20 second response time to changes in CO_2 level, which will be important to the system design explained below.

To control the CO_2 level inside the chamber, the GMT221 is connected to a feedback circuit. The output from the sensor is in volts and is linearly related to the percentage of CO_2 the sensor detects. The control circuit (see Appendix B) is based around a comparator, which determines if the voltage from the sensor is above or below a certain threshold value. In our case, the comparator decides if the voltage coming from the sensor is above or below the minimum CO_2 level, 4.5% (corresponding to 4.5 volts). If the voltage is above the minimum, the level of CO_2 is in the proper range, so no additional CO_2 is added. However, if the voltage from the sensor is below 4.5 volts, the control circuit opens a solenoid valve, allowing CO_2 into the chamber at a very slow rate. Because of the 20 second response time of the sensor, once the solenoid valve is

opened, it will stay open for at least that long. After 20 seconds has passed, the sensor will produce a new voltage corresponding to a new level of CO_2 in the chamber. If that voltage is still below the threshold, the valve remains open for an additional 20 seconds as more CO_2 is infused. If that voltage is above the threshold, the valve is closed.

Since the solenoid valve is opened for at least 20 seconds at a time, there needed to be a way to prevent CO_2 from rushing in too fast and going beyond the maximum CO_2 level of 5.5%. The solution to this problem was to insert a needle valve in between the solenoid valve and the CO_2 tank. By opening the needle valve only a small amount, CO_2 is allowed to pass through it, through the solenoid, and into the chamber at a very slow rate such that in 20 seconds, only enough CO_2 to raise the overall composition by 1% (from 4.5 to 5.5) is delivered.

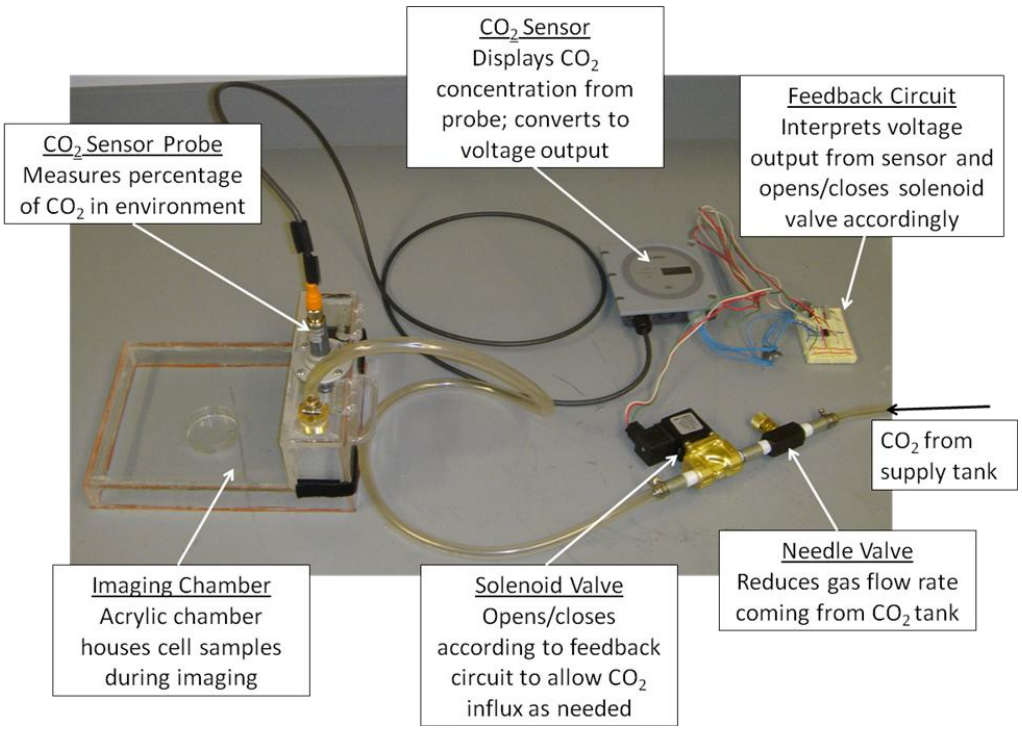

Figure 8. Final prototype.

Testing and Results

Testing

Testing of our prototype system consisted of two phases. The first phase was theoretical testing of the circuit and solenoid valves. The second phase of testing included the carbon dioxide sensor when our carbon dioxide tank arrived.

First, we tested the circuit and the solenoid valves in the BME instrumentation lab. The primary goal here was to see if the circuit opened and closed the solenoid valve at the correct voltage. Because we wanted the voltage to correspond with the amount of carbon dioxide in the imaging chamber, we set the circuit to open the solenoid valve when 4.5 volts are input into the circuit. This would correspond to the carbon dioxide sensor sensing 4.5% carbon dioxide in the chamber. To do this, we connected a power supply to the circuit and adjusted the input voltage. When the voltage was below 4.5 volts, the solenoid valve was open, and when the voltage was above 4.5 volts, the solenoid valve closed. This proved that the circuit would open and close the solenoid valve at the correct voltages.

Next, we connected the carbon dioxide sensor to the circuit. The goal of this test was to see if the carbon dioxide sensor would send a correct voltage to the sensor to open and close the solenoid valve. Because our gas tank had not arrived yet, we tried inhaling and exhaling into a plastic bag. Over time, this would increase the carbon dioxide concentration in the bag. We placed the sensor into the bag and sealed it up. The LCD display from the carbon dioxide sensor displays the concentration of the gas in the chamber. When the concentration of the gas finally increased above 4.5%, we heard the solenoid valve close, indicating a successful test.

Once we received our carbon dioxide tank for further testing, we were able to start phase two. However, our ordered power supply had not come in yet. Thus, for the testing, we decided to make our own power supply. Then, if our ordered power supply had not come in the mail yet, we could still turn our system on during the presentation. The problem is that our makeshift power supply, which came from a modified laptop charger, was only 19 volts. Our circuit is designed to open and close the solenoid valve at approximately 19% of the input power supply voltage. When a 24 volt supply is used, the valve is opened and closed at 4.5 volts. But when a 19 volt supply is used, the solenoid valve is opened and closed at 3.6 volts, which is 19% of 19 volts. This fact is inherent in all our testing. We can still achieve useful data from the testing if we just extrapolate our results to 24 volts.

The first test is our long term testing. We turned our whole system on and ran it for 76 minutes. The carbon dioxide concentration was recorded every 30 seconds, except when the solenoid valve opened and the concentration was increasing. During that time, the concentration was recorded every 5 seconds. This change was made to see how fast the concentration of the carbon dioxide would increase in the chamber.

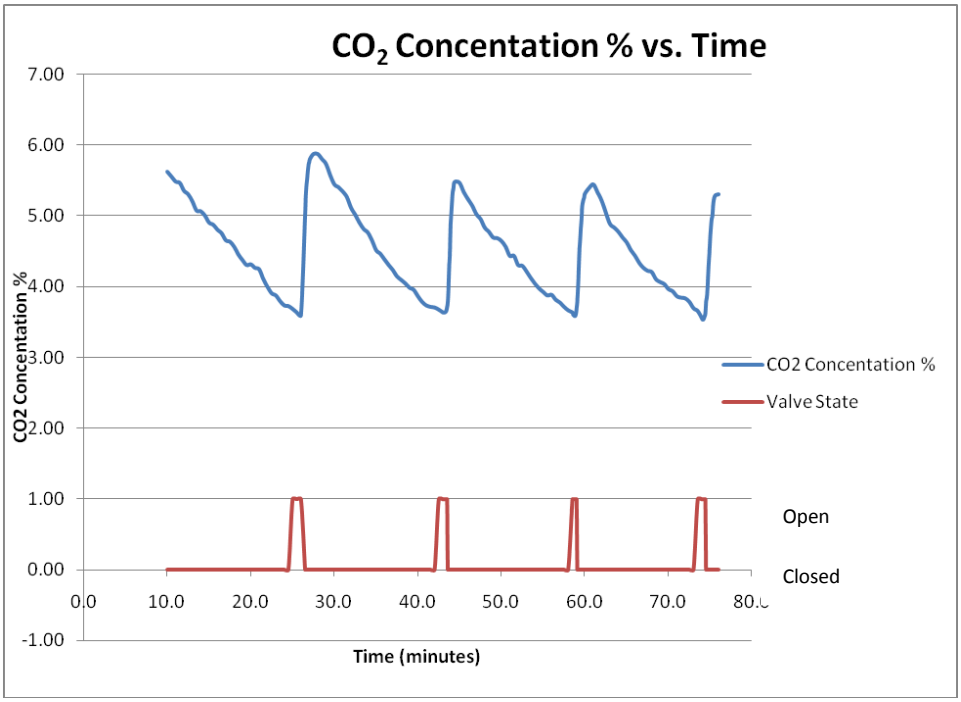

Figure 9. CO_2 Concentration % vs. Time. This graph shows the CO2 level inside the imaging chamber during a 1.5 hour test. Also, solenoid position (open or closed) is shown over the course of the test.

The second test was to measure the warm up time of the system. Warm up time is defined as the period of time when the system is first turned on, to when the carbon dioxide concentration gets to its maximum level before it begins to slowly decrease.

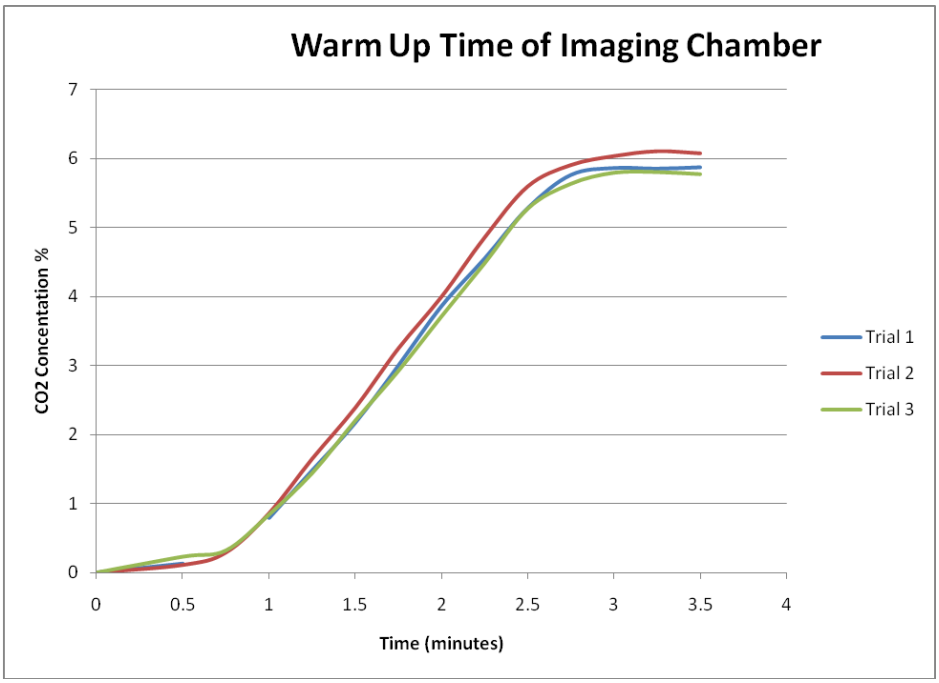

Figure 10. Warm Up Time of Imaging Chamber. This graph shows the time (in min.) it takes for the imaging chamber to go from normal gas composition in the room, corresponding to when the door is opened to change samples or to when the system is first turned on, to its peak value.

The third and final test we conducted was to see how much carbon dioxide gas the chamber loses when the door is open. The door will have to open to place a cell culture or to move the culture to a different position. For this test, the door was to be opened for 30 seconds; an amount of time we thought was adequate enough to move a Petri dish or well plate. The amount of concentration lost was recorded, as well as the minimum value the concentration reached.

Discussion of Testing Results

The results from the long term testing show that while our system works, the carbon dioxide concentration still oscillates too much. Ideally, we wanted the concentration to oscillate +/- .5% from our average value of 5%. These results showed that the average value was about 4.5% +/- .8%. Even if this test were done with the normal 24 volt power supply, the average value would be the correct 5%, but the range would still be the +/- .8%, and not the +/- .5% our design requires. This is a problem inherent in the circuit design and/or possibly the needle and solenoid valves. There is a solution to this problem in either obtaining a different needle valve or instituting a “555” timer in the circuit design and this will be gone over further in the future work section of this report.

The results from the second test measuring the warm up time of the system showed that the system takes an average of 3.25 minutes to get to the desired carbon dioxide concentration. While this is true, our group recommends at least one full cycle before imaging. A cycle is defined as the period of time between the two maximum carbon dioxide levels. For example, if the concentration starts at 5.4%, then goes all the way down to 4.3 before the concentration begins increasing again to a maximum of 5.5%, that is considered one cycle. Allowing at least one cycle before imaging permits a better range of carbon dioxide levels for imaging. This observance is also apparent in test 1, the long term testing. As the test went on, the maximum carbon dioxide concentration kept decreasing with each concurrent cycle.

The results from the third test measuring the carbon dioxide gas loss when the door was opened showed that the average percentage lost was 2.38% while the door was open. However, because of the inherent lag in our sensor, the actual value decreased all the way to about .83% average. This was not surprising to us. If the door is opened for longer than 30 seconds, the concentration may decrease all the way down to 0%. However, we can be confident that the chamber will be ready for imaging in about 3 minutes, since that was the average warm up time of the system we tested in test 2. Our group also concludes that this 3 minute time constraint will not be so bad, considering a researcher may be imaging the same cell culture for more than 2 hours, a break will be more than welcome.

Future Work

Moving forward, there are two main phases of work that need to be completed. First of all, the range of CO_2 concentration that was determined from prototype testing was too broad. The target range of fluctuation was a total of 1%, 0.5% above and below 5% CO_2. Through testing, a range of ~1.6% was discovered during prototype activity. One of the three possible solutions to this problem that were listed previously (see Potential Problems) needs to be implemented.

After that problem is solved, the imaging chamber will be set up in the laboratory to be used for live cell imaging. The results of the first live cell imaging sessions need to be analyzed to look at image quality with the chamber vs. image quality without it. Also, problems such as having too much air movement that creates shear forces on the cells could be identified through actual live cell imaging tests. Any problems that arise will be addressed at that point.

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Appendix A

Product Design Specifications

Function

Construct a live-cell imaging chamber to be used for laser-based confocal and multiphoton imaging. Device needs to control temperature and gas-environment inside as well as enable the use of perfusion.

Client Requirements

- Maintain environment with 95% air, 5% CO_2
- Device must allow control of temperature
- Allow perfusion to sample
- Optional mechanism for control of X, Y, theta of cell culture
- Temperature at 37 ± 3 °C

Design Requirements

1. Physical and Operational Characteristics

a. *Performance Requirements:* Imaging chamber should allow live cell imaging to occur in a controlled environment. Gas make-up, temperature, and perfusion need to be controlled for.

b. *Safety:* Chamber must not damage microscope or surrounding equipment in the client's lab. Use of pressurized gas tanks including CO_2 needs to be done in a safe manner.

c. *Accuracy & Reliability*: pH level must be maintained between 6-8 in culture media. CO_2 level must remain close enough to 5% (+/- 0.5%) to maintain cell life.

d. *Life in Service:* Approximately five years.

e. *Shelf Life:* The CO_2 sensor will need to be recalibrate anunally.

f. *Operating Environment:* Chamber will be used with an Inverted Nikon Eclipse TE2000U microscope in the W.M. Keck Laboratory on the UW campus.

g. *Ergonomics:* Chamber must allow for easy-access to place and remove samples.

h. *Size:* Must fit on mobile XY stage in between lens and base of microscope – 30 x 27.6 x 3 cm.

i. *Weight:* Must not damage microscope stage.

j. *Materials:* No plastic in microscope image field.

k. *Aesthetics, Appearance, & Finish:* Chamber must be easy to sterilize. Good organization of peripheral tubes, etc.

2. Production Characteristics

a. *Quantity:*1

b. *Target Production Cost:* $1250

3. Miscellaneous

a. Standards & Specifications:

Recommended procedures for CO_2 tank handling:

1. Do not drop
2. Designate an area for storage
3. Protect tank against weather
4. Do not drag or slide
5. Do not use cylinders as rollers for other objects
6. Close valve before return shipment of empty containers
7. Do not subject to temperatures above 125 deg F (52 deg C)
8. Use regulators, pressure release devices, check valves, and a safety release valve
9. Open cylinder valve slowly
10. Only qualified producers of compressed gasses should refill

b. *Customer:* N.A.

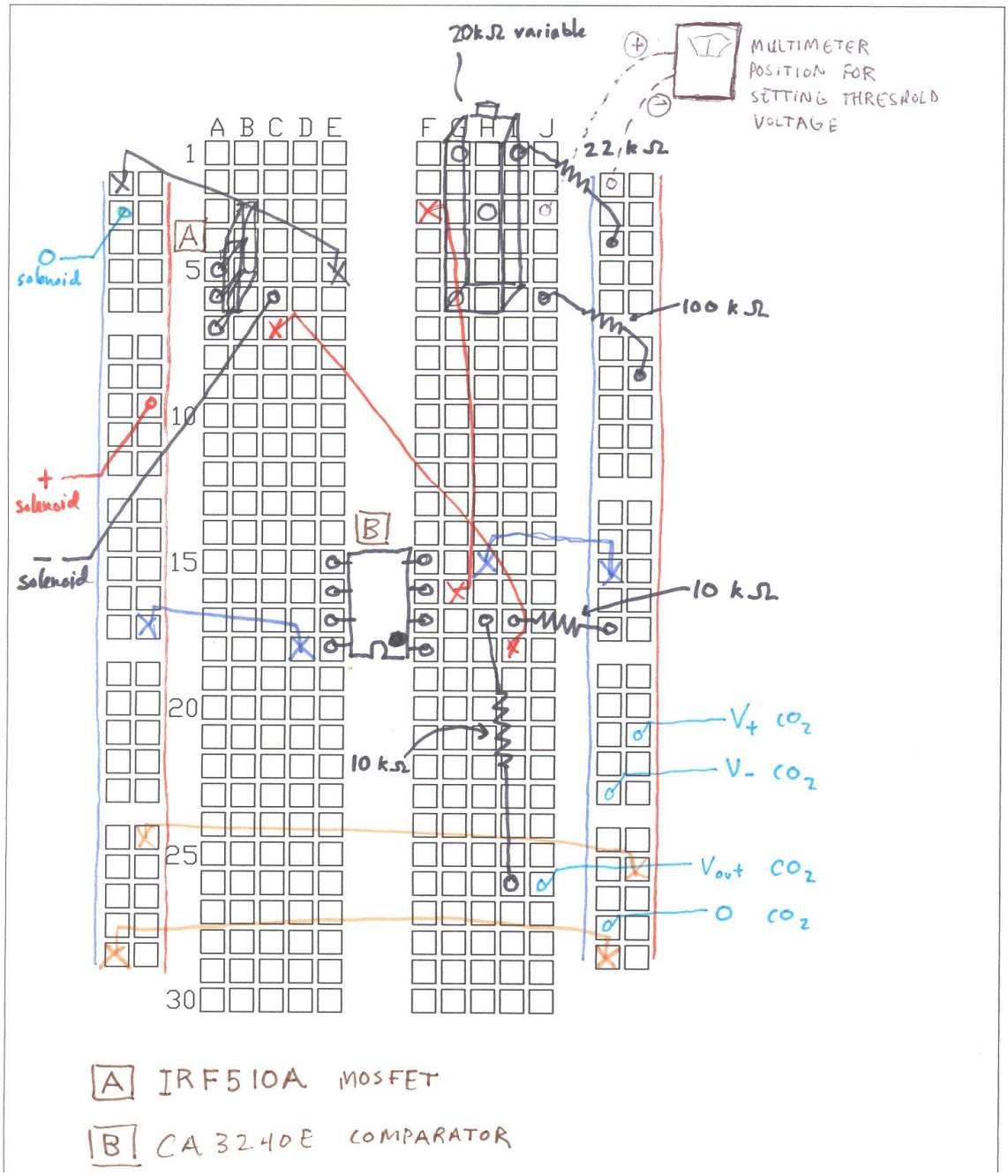
c. *Patient-related Concerns:* N.A.

d. *Competition:* Incubator 2000, Focht Chamber System 3, Solent Scientific Incuabation Chamber

Appendix B

Decision Circuit Schematic

To adjust threshold voltage, connect volt meter (or digital multi-meter) with positive lead connected to 3F:J and negative lead to ground. Then, turn screw on 20 k-ohm variable resistor.



Appendix C

Tank Life Calculations

We performed four trials during which carbon dioxide concentrations decreased over a period of time. We graphed the trends in Microsoft Excel and obtained the following equations for each line:

Trial 1	$y = -0.1563x + 5.7468$ $R^2 = 0.9804$
Trial 2	$y = -0.1303x + 5.5932$ $R^2 = 0.996$
Trial 3	$y = -0.122x + 5.2482$ $R^2 = 0.9744$
Trial 4	$y = -0.1181x + 5.1296$ $R^2 = 0.9444$

The units of the y axis is % concentration of CO^2.
The units of the x axis is time in minutes.
Average slope: -0.131675
Standard deviation: 0.017186889

The slope corresponds to a loss of 263.35 mL/min of 5% CO_2 air.
When we use a 100% CO_2 tank, 5% * 263.35 = 13.17 mL/min of 100% CO_2 is used.

A 20 liter 100% CO_2 tank will last 25.310 hours. A 50 liter 100% CO_2 tank will last 63.275 hours.

A 20 liter 5% CO_2 / 95% air tank will last 1.267 hours. A 50 liter 5% CO_2 / 95% air tank will last 3.167 hours.

The 100% CO_2 tank lasts about 20 times as long as a mixed tank.

All calculations assume an average consumption of CO_2 in mL/min and a linear decrease in carbon dioxide concentration over time.