Biological Imaging Chamber:

Proposed Designs for Imaging Chamber

BME 200/300

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Abstract:

This project was designed to create a glass-topped, acrylic imaging chamber that will fit on the stage of a confocal microscope with dimensions 21 cm (l) x 25 cm (w) x 4 cm (h). The chamber is engineered to fit Petri dishes as well as cell plates. The chamber will regulate the internal carbon dioxide to 5% +/- 0.25%, sustain a humid environment and maintain 37° C in the cell culture. The goal of this chamber is to imitate the conditions of cells *in vivo*.

I. Introduction

Live cell imaging is a very important part of biological research. There are two main reasons for live cell imaging. The first is to determine the natural behavior of cells in a developmental study. The second is derived from inducing a controlled change in the environment and studying the effects. The latter is very important in tissue engineering and pharmaceutical studies. Live cell imaging provides a means for studying cells *in vitro* in order to gain a better prediction of how they will interact *in vivo*. Cells can be imaged in light field, dark field, differential interference contrast, confocal, phase, reflection interference, 2-Photon, and fluorescence. Images can also be processed using two and three-dimensional analysis. Three-dimensional analysis is being used more regularly due to the increased processing capacity of computers. Fluorescent imaging is also becoming increasingly important in microscopy because of the developing technologies to measure cell viability, transcription rates, and gene therapy efficiencies.

There are several challenges with maintaining mammalian cells in the microscope field. The primary challenge is keeping the cells healthy and viable. Chances of recording meaningful data are greatly decreased if cells cannot be kept alive. To ensure cells are kept alive, many steps are taken to regulate the environment of cells while during imaging. First, cells are kept in nutrient rich serum through the duration of the imaging process. The temperature is also held constant at ~37° C. The pH of the media is also a very important factor in keeping cells alive and is regulated by the exchange of CO_2 gas. A saturated, humid environment is also necessary. While cells are not being imaged, incubators do a phenomenal job of maintaining this environment.

Two types of systems (open and closed) are used for live cell imaging. Variables that affect open chamber imaging are: the volume of the container, clear aperture, material, geometry of evaporation and condensation, view angle, and ambient light. Open system is generally used for short-term experiments and very versatile. It is often useful in multi-user facilities because of this. Closed systems present more specific applications than open chambers because of the greater opportunities for manipulation of the cells. However, closed systems are limited by a trade-off between the functionality and compatibility of the microscope being used. Closed systems are generally used for long-term, induced change or developmental assays, where shear and flow control are imperative. Variables affecting closed chamber imaging are: fixed versus variable volume, perfusion-flow characteristics, volume rate exchange, laminarity, shear stress, and flow geometry. The variables for each system must be considered when choosing between systems.

Imaging chambers are often necessary because of the flat, transparent surface they provide for imaging. These chambers also offer a means of fluid containment, a characteristic

lacking in traditional microscope slides. Chambers are available in various shapes and sizes ranging from single glass-bottom Petri dishes to 96-well plates. Chamber material also varies depending on the type of experiment. These materials may also be coated with different laminates depending on the cell lines.

Design Constraints

The system created will be designed around a 35mm glass bottom dish and a cell plate. The chamber must have a port to allow internal access. The design will be smaller than the microscope stage (21 cm (l) x 25 cm (w) x 3 cm (h)). The system will consist of a constant 5% CO_2 gas concentration as well as a humid environment. The top of the chamber must be made of glass or have a glass inset to enhance imaging. Finally, the overall cost of the project will be under $1000.

II. Client Information

Our client, Lance Rodenkirch, is the laboratory manager for the W.M. Keck Laboratory for Biological Imaging. His research involves imaging progenitor cells and associated research. The laboratory contains two different microscopes, the Bio-Rad MRC-1024 and the Bio-Rad Radiance 2100 MP Rainbow. The primary purpose of the design is to maintain continuous imaging on a Petri dish for 72 h. The current method available kills cells long before this time, because the pH changes with a decreasing CO_2 concentration. Another important aspect, thermal control, is provided.

III. Motivation

This projects aims at solving the problem of maintaining cell viability during prolonged live cell imaging. This is accomplished through perfusion of media, pH control via the regulation of CO_2 gas concentration, a heating element, and humidity. Systems currently on the market cost upwards of $10,000 and are often unreliable. The client feels that a similar, reliable system can be constructed for less than a third of that. The purpose of this project is to construct this system.

IV. Previous Work

Live cell imaging has been the subject of much research in the past. As one might assume, the technology has advanced with the science. This fosters the creation of a market containing many products capable of aiding in such research. Because the current project aims to monitor and adjust CO_2 concentrations, only these products will be examined. Bioptechs (www.bioptechs.com) is a company devoted to the production of live cell imaging chambers. One of their current products, the FSC2® (Figure 1), is capable of controlling CO_2 concentrations. The system starts by adding gaseous CO_2 to cell media, and then adds this media to the culture. This product is also capable of monitoring the temperature of the culture.

Figure 1: Bioptechs FSC® Imaging Chamber
http://www.asiimaging.com/bioptechs.html

Other products solely create the desired 5% CO_2 mixture. The addition of this mixture to a culture is left for the researcher. These products are capable of receiving pure CO_2 gas and creating a mixture that is the desired 5% CO_2. By keeping a steady flow of this mixture into and

out of the culture, the necessary imaging environment is created. This system, however, is not efficient in its CO_2 consumption.

V. Design Alternatives

Design 1: CO2 Monitoring and Feedback System

The first design option addresses the problem of creating an environment with hyper-atmospheric carbon dioxide concentrations. To recreate an environment suitable to support living cells, an atmospheric concentration of 5% CO_2 is needed (www.wikipedia.com). In normal atmospheric conditions, a .035% concentration of CO_2 is present (www.wikipedia.com). In order to reach the 5% concentration, a way to deliver CO_2 to the culture and monitor the flow is needed. The following design addresses this issue, by monitoring of CO_2 and adjusting to change in CO_2 concentration within the culture.

In order to create such a system, a way of determining the current gaseous concentration of CO_2 is needed. This is accomplished through the addition of a potentiometric CO_2 sensor. These sensors measure gaseous concentrations and then prensent the concentration as a voltage output. An example of such a CO_2 sensor is the Edinburgh Instruments GasCheck (http://www.edinst.com/gascheck.htm). This voltage would represent the amount of CO_2 in the system. Through simple calibration, the corresponding voltage to a 5% CO_2 concentration mixture can be determined. The next part of the system deals with interpreting the output voltage presented by the sensor.

The output voltage presented by the sensor provides the means for a circuit to analyze this voltage and act accordingly. In its most basic form, this would be done through the use of a comparator operational amplifier circuit. In a comparator circuit a reference voltage is

designated, and if the incoming voltage is smaller than the reference, a corresponding output voltage is sent from the comparator. If the incoming voltage is greater than that voltage, a different output voltage is sent out. If the value is the same, no output voltage is produced.

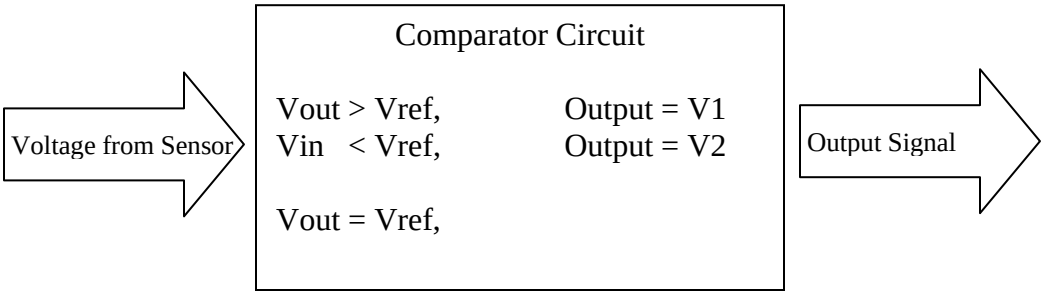


Figure 2: Comparator Circuit Analysis

This comparator circuit essentially creates a digital signal. The output signal can only be one of two voltages, and nothing in between. This signal is then sent to the next part of the circuit, which has mechanical control over a valve governing the entrance of CO_2 into the circuit. If the comparator determines the CO_2 concentration is too small, CO_2 will be added to the circuit as a quick burst. The CO_2 will be added at a controlled rate, so the volume of CO_2 added is proportional to the time the valve is open. It is likely that the amount of CO_2 that will be needed in the system will be very small, so a quick burst of CO_2 is probable.

It is assumed that the cells will be consuming O_2 during cellular respiration and will produce CO_2 as a byproduct. The CO_2 concentration in the media needs to remain a constant 5%. CO_2 will move down its concentration gradient into the atmostphere if the internal environment inside the box drops below 5% CO_2. This will result in a pH change fatal to the cells. This design is more concerned with CO_2 concentrations below the 5% mark because it is the nature of CO_2 to leak out of the box into the atmosphere due to the partial pressure gradient between the atmosphere and the internal environment of the CO_2 infused box. Also, we will be

able to regulate the CO_2 flow into the box and be able to control it from becoming too high. The entire design is summed in Figure 3.

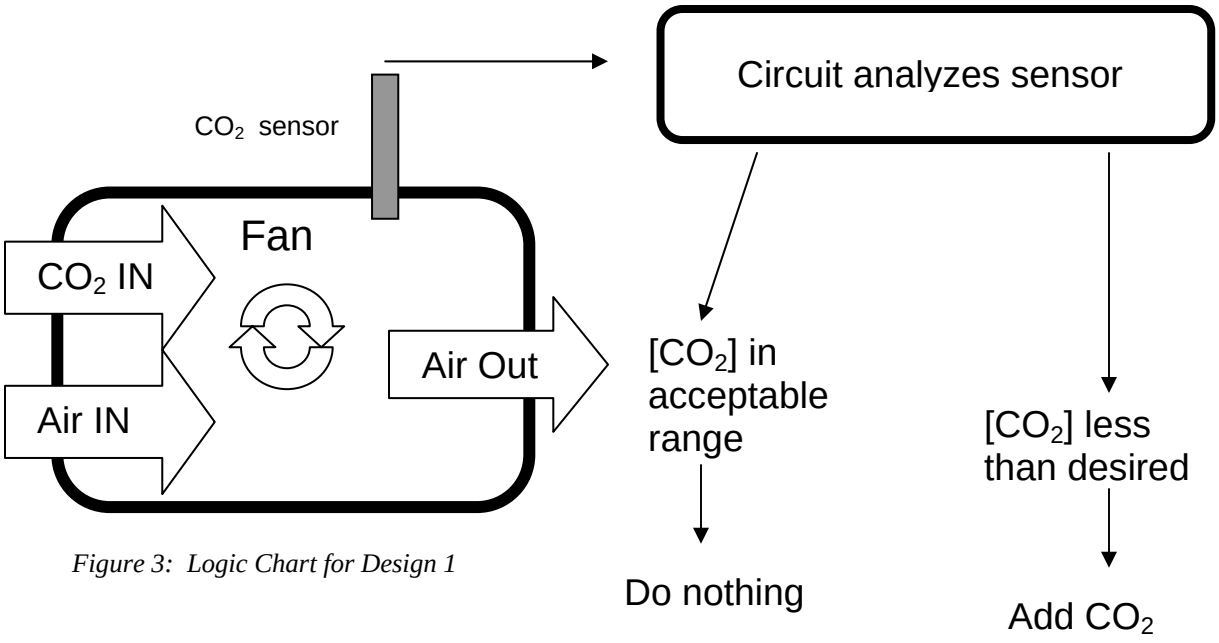


Figure 3: Logic Chart for Design 1

Another concern is the diffusion of gases within the chamber. This is addressed through the addition of a small fan to the system. It is important to ensure that the power of this fan is not great enough to disturb satisfactory imaging conditions

Design 2: Modified Petri Dish

The second option available for the solution of our problem is to modify a Petri dish, in order to allow for delivery of carbon dioxide gas, as well as the removal of waste gas. This option, the most easily assembled, requires no outside assembly of an apparatus, as it is simply the modification of currently used parts.

Similar products exist in this market, mainly consisting of Petri dish inserts or complete microscope enclosures, allowing for a completely controlled environment. Many designs

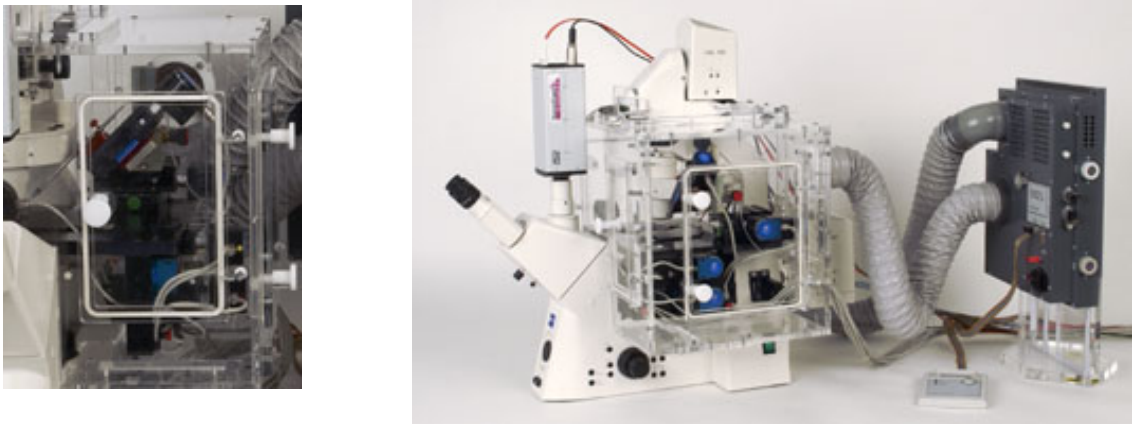


Figure 4: Current Market Designs of Microscope Chambers
www.cellbiology-trading.com

incorporate the diffusion of gas into an environment as well as a media perfusion system. Figure 4 shows a product available from "Cell Biology Trading" (http://cellbiology-trading.com/)

In this Petri dish modification, two small holes will be placed into the lid of an existing Petri dish, and two tubes will be inserted. These tubes will be connected to a pump (tan cylinder in image), which will aid in the circulation of the air throughout the dish. Connected to the pump will be a CO_2 tank (green cylinder in image), which will be used as the input for the system. A rough outline of the system is shown in Figure 5. If an optional pump is not desired for the system, the high pressure in the gas tank will provide sufficient flow into the dish. A regulator will need to be installed to prevent an overfeed of gas in to the system. A tube aiding in the release of excess gas is also not necessary, as waste gas can escape through the area of contact between the dish and lid.

This system has many advantages in its design. Primarily, as the complexity and number of parts in this design are both relatively low, this system has a small probability of encountering mechanical failure, and also would assemble rapidly, allowing for imaging to begin quickly. Also, because the Petri dish is the only space requiring a maintained concentration of CO_2, a

smaller amount of gas will be used over the life of the system, as compared to a large chamber. This will result in a prolonged life of the tank, resulting in a lower cost. Finally, this system will provide a very cost efficient method in solving this problem, and in case of system failure, could be replaced inexpensively.

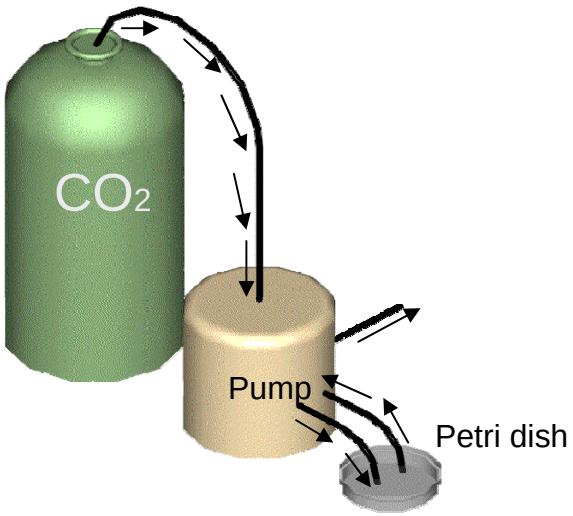


Figure 5: Modified Petri Dish Assembly

This option also has many negative aspects in its design. The area being worked with is very small, and causes problems in trying to add components and modify pieces. As Petri dishes are small and fragile, adding holes to the lid of the dish may prove itself a problem. A second disadvantage in utilizing this setup is the fact that the dish lid is in very close proximity to the cells and their medium and would require frequent cleaning in order to preserve sterility in the system. As this would take time, it would detract from time available to image cells. Finally, an important disadvantage to the use of this system is the fact that a carbon dioxide sensor cannot be mounted inside of the chamber or into the serum through the lid. The reasoning behind the latter is possible contamination problems and the process necessary for sterilization between uses. An additional component is necessary to monitor carbon dioxide concentrations. This component

would entail an outside system wherein a carbon dioxide monitor would have to be remotely placed, and then connected to the system through a wire insert into the dish lid. While causing some concern for the accuracy of this method, it also begins to create clutter in the laboratory workspace, and requires more caution to ensure that it is not damaged or moved, requiring a recalibration.

Design 3: Micro-Perfusion

The final design option, micro-perfusion, involves the pumping of CO_2 infused medium into the Petri dish to maintain an appropriate level of CO_2. This system involves the use of a micro-perfusion pump; a peristaltic pump designed specifically for low flow rates. In addition, a flask (or other storage container) is necessary for storing the medium and allowing for a net influx of CO_2 gas, to saturate the solution. This enriched medium would then be pumped to the Petri dish, while excess medium is removed and deposited in a waste container.

There are currently available commercial solutions that accomplish this. One such option by Bioptech, the Delta T Perfusion Configuration, has a flask containing fresh media with CO_2 bubbled in. Using a peristaltic pump, it is then pumped into the cell culture; where there is another tube driven by the same pump removing excess medium from of the dish (Figure 6).

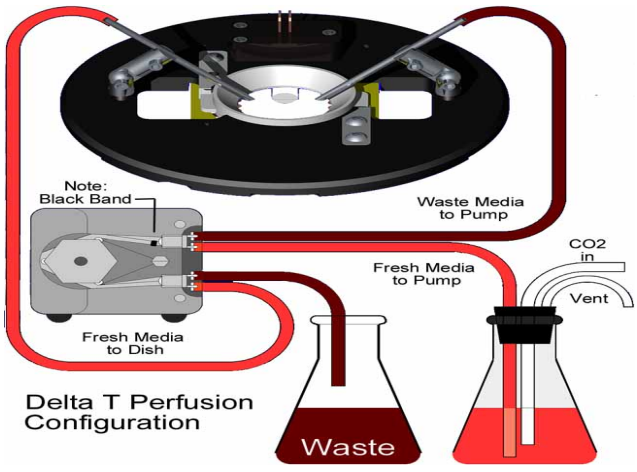


Figure 6: Delta T Perfusion Configuration
http://www.bioptechs.com/Products/Delta T/D Perf/d perf.html

The main component of this design is the peristaltic pump, which is $995 from Bioptech. The main drawback to this system is its cost as well as the necessity of maintaining stock of additional tubing. The preferred tube sets by Bioptech are autoclavable, which increases price to $2 per foot, but allows reuse. As the client is more interested in a cheap, disposable solution this system shows probable disadvantage because of the time necessary for cleaning.

Bioptech has another configuration based on the same pump. By having a constant supply of fresh media, it is possible to inject dyes, growth factors, or inhibitors and view the effect on the cells microscopically. This increased capability is desirable, and only requires a few modifications to the previous system, namely the addition of a T-adapter to allow the connection of a syringe. This simple addition greatly enhances the capabilities of the system, but would require the changing of tubing between each different additive to prevent contamination. This part could be very tedious, and if not reusing the tubing, expensive as well.

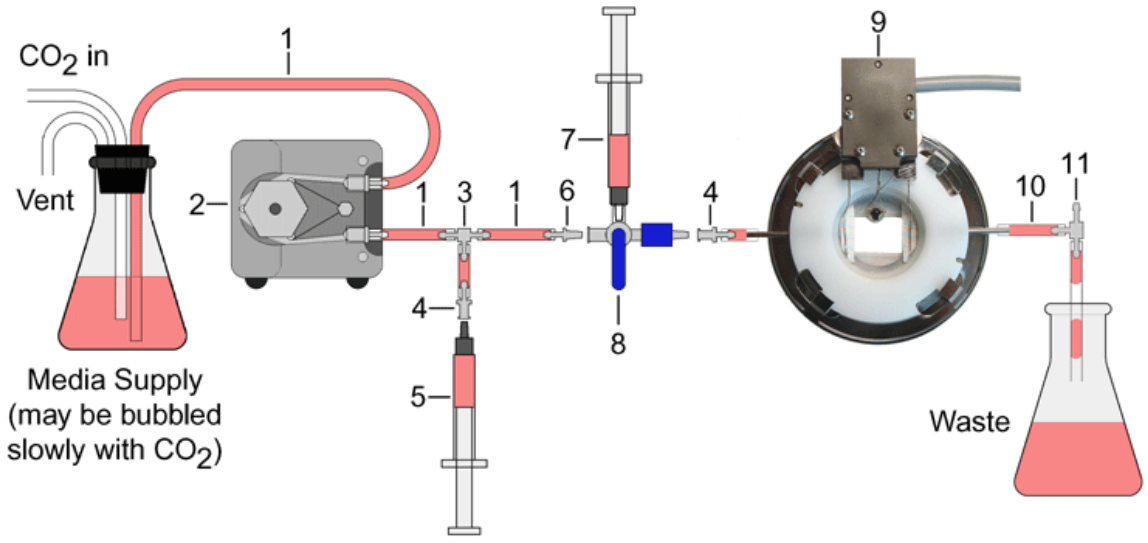


Figure 7: FCS2 Perfusion Configuration
http://www.bioptechs.com/Products/FCS2/F_Perf/f_perf.html

There are several main drawbacks of these two designs. As previously mentioned, the tubing will often be in contact with the medium and will need to be replaced for each new culture analyzed, in order to maintain sterility. This will generate solid waste and requires the stocking of additional tubing. This system also generates more wasted medium, especially in comparison with a gaseous system (which requires no medium).

In addition, since there is no liquid carbon dioxide sensor in this design, the concentration of CO_2 in the medium will not be known, and could be a source of variation from experiment to experiment. Since a primary specification of this design is to control CO_2 concentration, this design would fail.

The most significant problem that could arise involves a change in flow rate. If the flow rate is not constant, small changes in pressure will occur, causing the cover slip to flex out of focus. Because of this, it is necessary to have a very accurate pump, which would deplete the entire budget for this project. Cheaper options are available, however if a loss of focus occurs, imaging will cease, and the experiment may need to be restarted.

Despite all of these negative aspects, a correctly constructed microperfusion system allows a greater diversity of experiments to be run, making the system more effective than merely a gaseous one. It also doesn't require an external enclosure, making it easier to work with the cell culture and equipment without worrying about escaping gas.

VI. Design Matrix

To analyze the three proposed designs, a design matrix was created that took into account multiple characteristics of the designs. The four different aspects chosen to rate the designs were: practicality, ease of use, durability, and cost efficiency. These design aspects attempted to

minimize the number of categories while maximizing the specific design characteristics taken into account. Additional aspects including the number of components in each design could have been taken into account separately, as well as complexity of product, but all aspects have been taken into account by these four categories.

Another important aspect in developing the design matrix was choosing different scales for each of the criteria and deciding how to weigh them differently. The categories of practicality and cost efficiency were given heavier weighting scales of 1-10, as the actual possibility of turning a design into a working prototype is primarily measured by practicality. The category of cost efficiency has equal importance because of our client's focus on price. Ease of use received a scale from 1-5 since this project is to be integrated into an environment that is already very complex. Similarly, the personnel involved with these devices are advanced in the field of study, and will be perfectly capable of operating the proposed designs. The last ranked criterion, durability, was given a scale of 1-5, because the environment of project implementation is not considered extreme, and wear and tear the environment will impose on the product will be minimal.

The completed design matrix is a measure of each design's pros and cons. This matrix was used to decide which of the three designs will be most suitable for turning into a working project best meeting the client's design specifications. The design matrix is illustrated in Figure 8, and CO_2 monitoring design rated best in among all options, and will be the focus of work in the future.

DESIGN	Practicality (1-10)	Ease of use (1-5)	Durability (1-5)	Cost Efficiency (1-10)	TOTAL (4-30)
CO_2 Monitor	8	4	5	7	**24**
Modified Petri Dish	6	5	2	6	**19**
CO_2 Infused Medium	7	3	5	4	**19**

Figure 8: Design Matrix

VII. Final Design

The final design chosen integrates a CO_2 monitoring sensor into the chamber that will send a voltage input to a circuit that controls valves regulating CO_2 flow into the chamber. This process is explained in Figure 9 below. Further development of each component of this system is explained in the following sections.

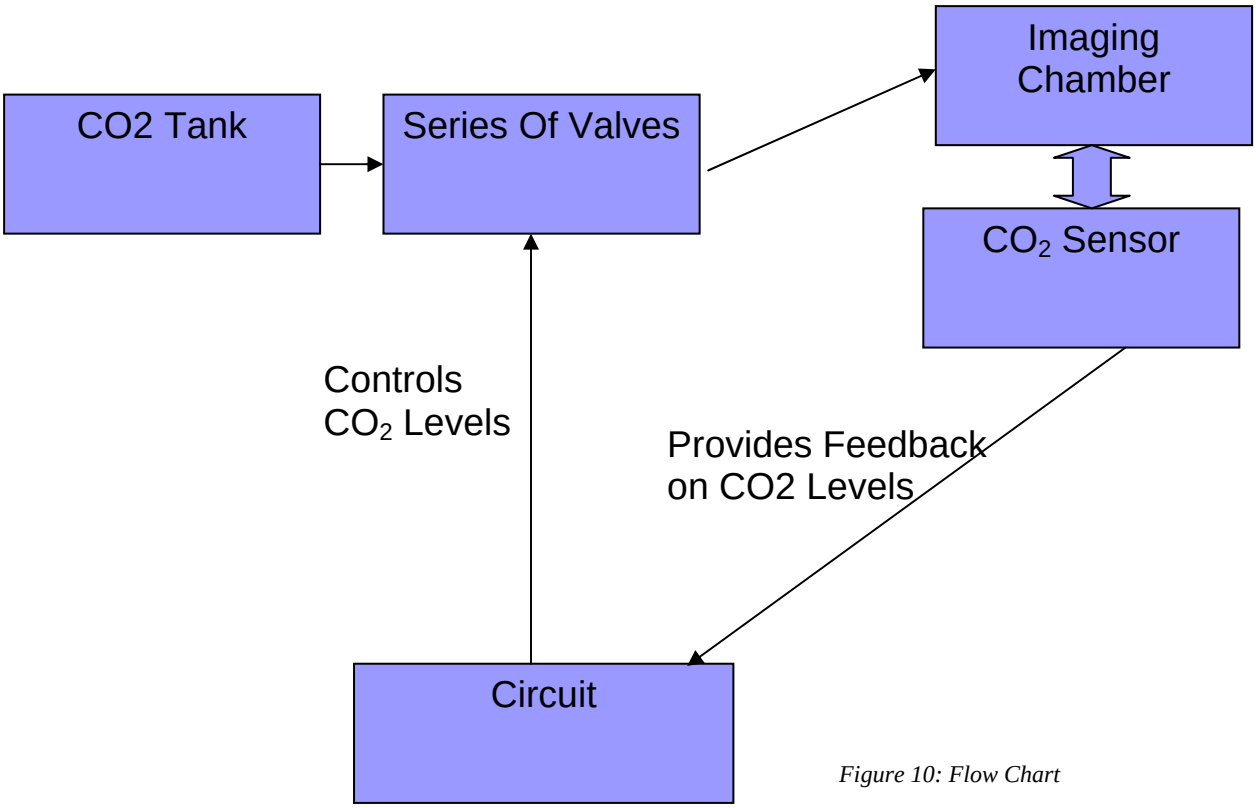


Figure 10: Flow Chart

Chamber

Primarily, the client's main concern was to be able to allow the condenser to be as close to the imaged cells as possible. The condenser's minimum distance to the stage was 3.0 cm. It was also necessary to allow ample room for a hand to be inserted into the box comfortably in order to allow an individual using the microscope the ability to move the object being imaged as necessary. A door also needed to be included in the design, to be able to access into the box without having to remove it from the stage. To accommodate both needs, a box was designed in the shape of a three dimensional "L" (see Figure 11.) The lower portion was designed to be only 3.0 cm high to accommodate the condenser, while the rest of the box was designed to be 10 cm tall, to allow a hand to move comfortably in the box.

To allow maximum light to efficiently pass into the box from the condenser, a glass top was placed on the box in the area planned to sit under the condenser. Glass was used because it

has few aberrations than the acrylic used for the rest of the box. Also, an inverted microscope is used allowing the Petri dish to sit directly on the microscope stage preventing the box from interfering with imaging except with the condenser issue.

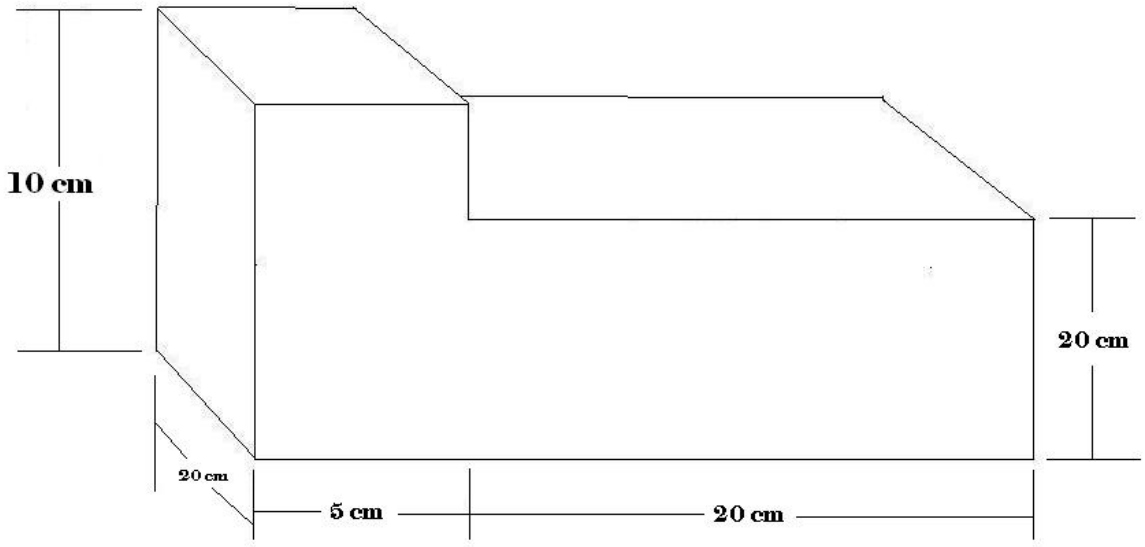
The box also needed to be designed to allow any well of a multi-well plate to be imaged. Since the imaging components of the microscope are fixed, the box needed to be designed large enough to be able to adjust a multi well-plate to accommodate this.

The final product also needed to provide a relatively airtight environment, so that as CO_2 is placed into the system, it is not lost to the outside environment.

The box was fabricated using ¼" (.6cm) thick acrylic sheet. This thickness was chosen to give the product sturdiness without adding a lot of bulk. The glass used for the area below the condenser was standard 1/8" (.3cm) window glass. Additional items included in the final product were a set of hinges and a door handle. Velcro straps were attached to the door to allow it to close the door tighter and create a better seal with the box. The box was assembled using polycarbonate cement, and was made airtight using silicone caulk.

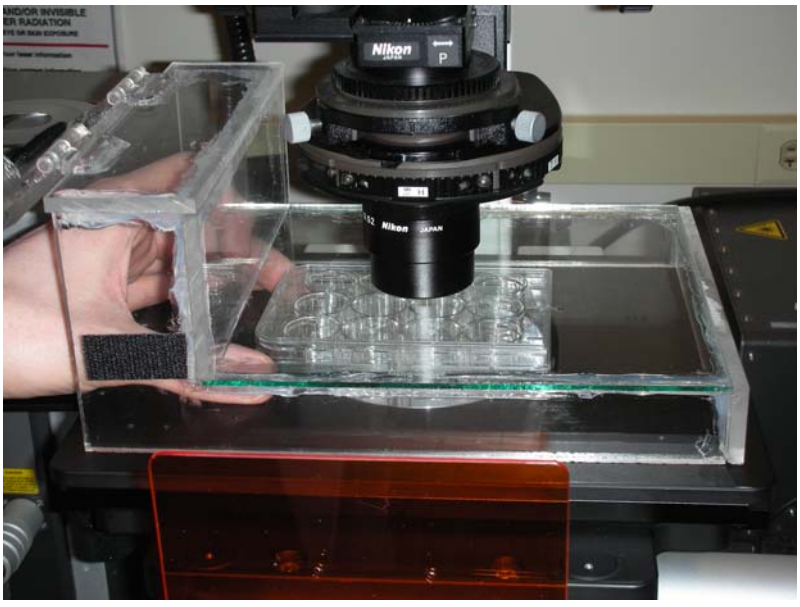
In the future, a rubber seal will be placed along the lower boundary of the box to ensure a tight seal with the microscope stage. Also, the valve and CO_2 sensor will be installed in the tall area of the box, to not interfere with the movement of a Petri dish in the lower area. The design of the box is shown in Figure 11.

Figure 11: Flow Chart



Total Volume: = 9000 cubic cm.

The box is also shown under the microscope, in Figure 12.



Valves

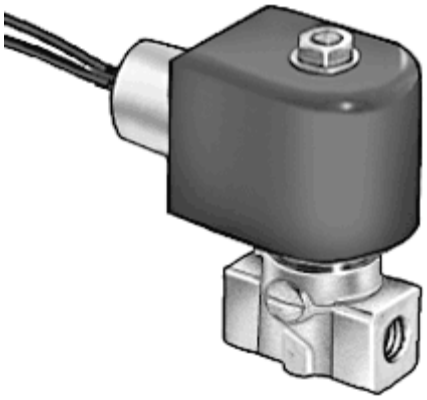
The valves are necessary to control the release of carbon dioxide gas into the imaging chamber at an acceptable flow rate. The system we will employ consists of two different valves connected in series: a needle valve and a solenoid valve.

The first valve, a needle valve, is adjustable to reduce the flow rate directly from the carbon dioxide Tank. This is important due to the small volume of our chamber and the high pressure and flow of gas that would come directly from a carbon dioxide tank. By constricting the opening, a needle valve effectively reduces the flow rate of gas that can flow into the system. The valve we recommend after extensive research is model number 46425K22 from McMaster-Carr, constructed of Carbon Steel and suitable up to 5000 psi at 400° F. These characteristics will be able to withstand the conditions of the lab of its intended use. This particular valve also has a color coded valve stem that provides a visual reference to assist with the calibration of the flow rate, and for verification that it is always set to the same level.

Figure 12: Needle Valve

The solenoid valve comes is the intermediary between the needle valve and the chamber that controls the volumetric addition of carbon dioxide gas into the chamber. The solenoid is directly connected to the circuit, which will process the information from the sensor and send a voltage output opening the valve if the carbon dioxide gas concentration is too low. The solenoid valve is model number 8077K42 from McMaster-Carr. One of the main requirements for this component is direct circuit compatibility to avoid the

Figure 13: Solenoid Valve



necessity of another power connection – which means either a 12 or 24 DC Voltage capability. This valve is 24 volts, and it requires 0.40 amps of current for activation. The valve is normally closed inhibiting gas flow into the chamber until a voltage is applied. As a result, any failure in the circuit or power source will result in the valve remaining closed to conserve gas.

Between the two valves is a section of tubing that is also important for the flow into the imaging chamber. When the solenoid valve is closed, the pressure in this section will rise to that in the carbon dioxide tank. When the valve is opened, this bolus will rapidly flow into the chamber, followed by a constant flow rate determined by the needle valve. This system will initially release the bolus, along with a few seconds of pre-determined flow before it closes and waits for the carbon dioxide sensor to equilibrate. As the pressure decreases in the carbon dioxide tank, the volume of the bolus will decrease, and it will take more cycles for the chamber to reach the desired carbon dioxide concentration of 5%. In order to minimize the bolus, the length of tubing between the two valves should be kept to a minimum.

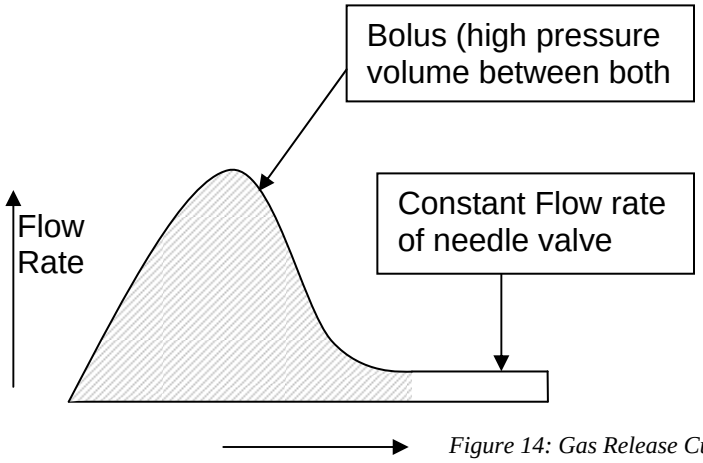
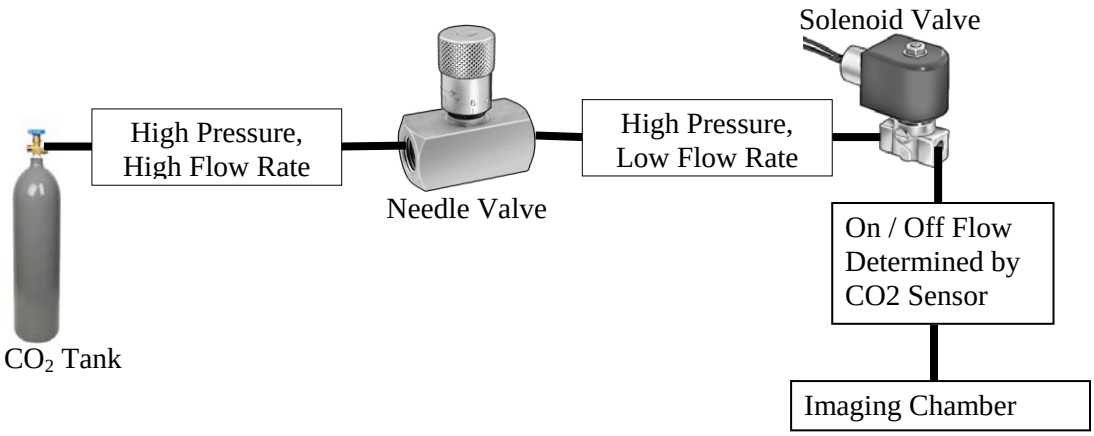


Figure 14: Gas Release Curve

The following diagram demonstrates the system. The gas begins in the carbon dioxide tank pressurized with a high flow rate and then travels through the needle valve at a reduced flow rate. Between the needle valve and solenoid valve, the pressure will build to form the bolus, which is released through the solenoid valve. The gas then travels through the rest of the system to arrive in the imaging chamber, where it will diffuse.

Figure 15: Valve System



Circuit

Purpose of the circuit: The circuit should be capable of deciding whether or not CO_2 gas should be delivered to the chamber. The circuit should then be able to deliver the correct amount of CO_2 gas to the chamber in order to maintain 5% CO_2 levels. The logic flow for the circuit is seen in Figure 16.

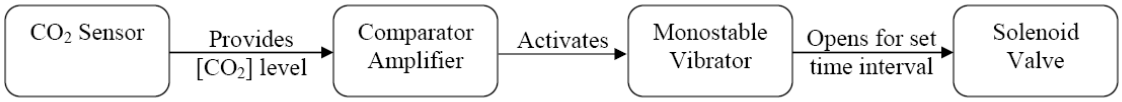


Figure 16: Logic for circuit development

The logic flow is achieved through the following components:

1. *Vaisala CARBOCAP® GM221 Carbon Dioxide Module:* The sensor consists of a probe and a circuit that relays the measured CO_2 concentration as a voltage. The voltage output is linear and proportional to the CO_2 concentration. The sensor arrives calibrated.

Important Statistics for GM221:

- Measurement Range: 0-10% CO_2 concentrations
- Accuracy: +/- .02% CO_2

- Output: 0-5V
- Response Time: 20 s

2. Comparator Amplifier: This amplifier compares the input voltage from the CO_2 sensor to a set threshold value. If the input drops below this set value, the output of the amplifier drops to 0 volts. This threshold is controllable to a range from 4-6% CO_2 and is adjusted by a potentiometer in the circuit. A 3240 Op Amp was used as the component because of its ability to be referenced to the ground rather than a negative voltage.

3. 555 Timer: A 555 timer as a monostable vibrator, or one shot, responds to the drop in voltage by creating a 10 V output for a set amount of time. This time interval is determined by a capacitor and a potentiometer in the circuit. Experimentation and calibration in conjunction with the solenoid valve will determine the length of this time interval. This voltage is then delivered to a FET transistor which provides a high enough current to activate the solenoid valve. The valve is then opened, delivering a predetermined volume of pure CO_2 gas to the chamber.

Because of the 20 second delay in the sensor, it is important to make sure that the valve remain open at least 20 sec, so that the sensor has enough time to recover without falsely tripping the comparator again. A hypothetical response to a given CO_2 input is seen in Figure15. Note that the sensor does not read a change in CO_2 concentrations at the same time the timer opens.

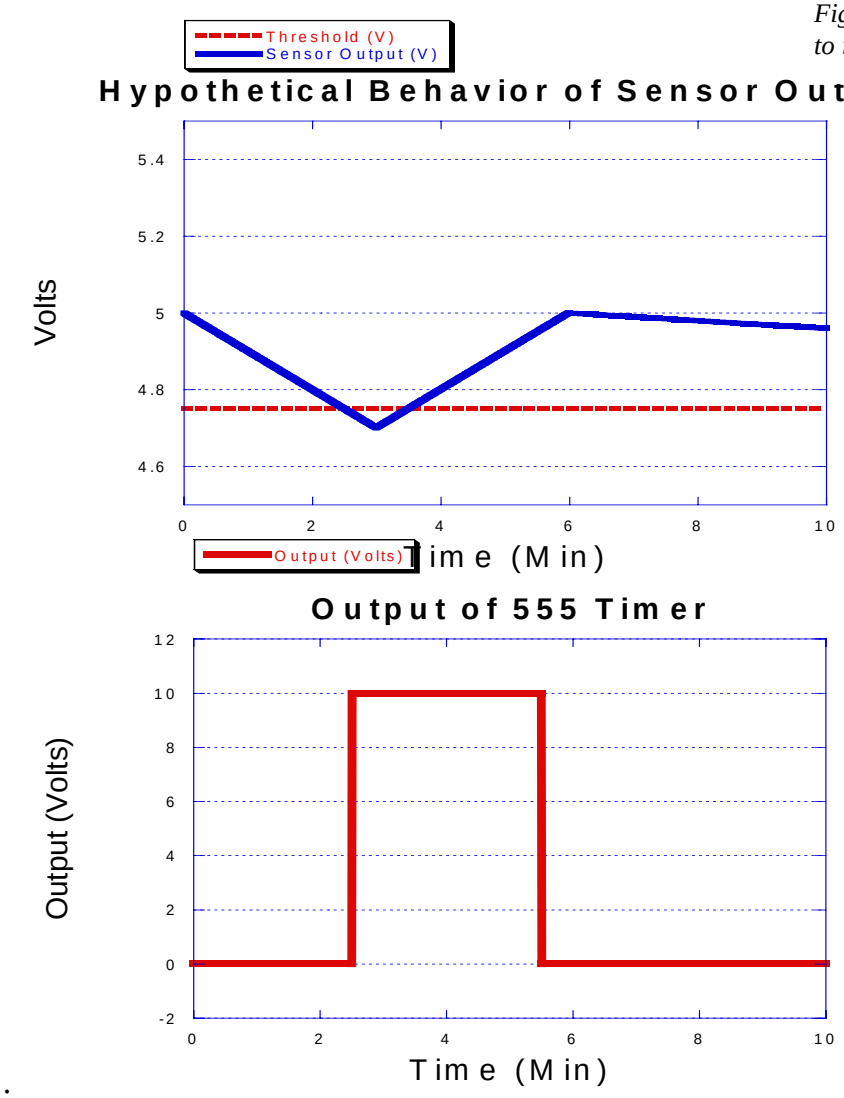


Figure 15: Response of 555 timer to input from sensor.

The circuit schematic is seen in Appendix A.

VIII. Ethics

Team members are very aware of existing products on the market used for the same purposes as the proposed product. Whenever such products exist, it is of the utmost importance to have an original design that does not violate any laws associated with protecting others' product designs. In the proposed design, a number of elements will be used that have not been created by the team. Implementing these elements into our design will require that we give

proper credit to the developer of these products, and their purpose in the proposed design. Although the ethical issues may be of a limited number, they are one of the most critical components to the design project and will serve as an important guide to the completion of a successful design.

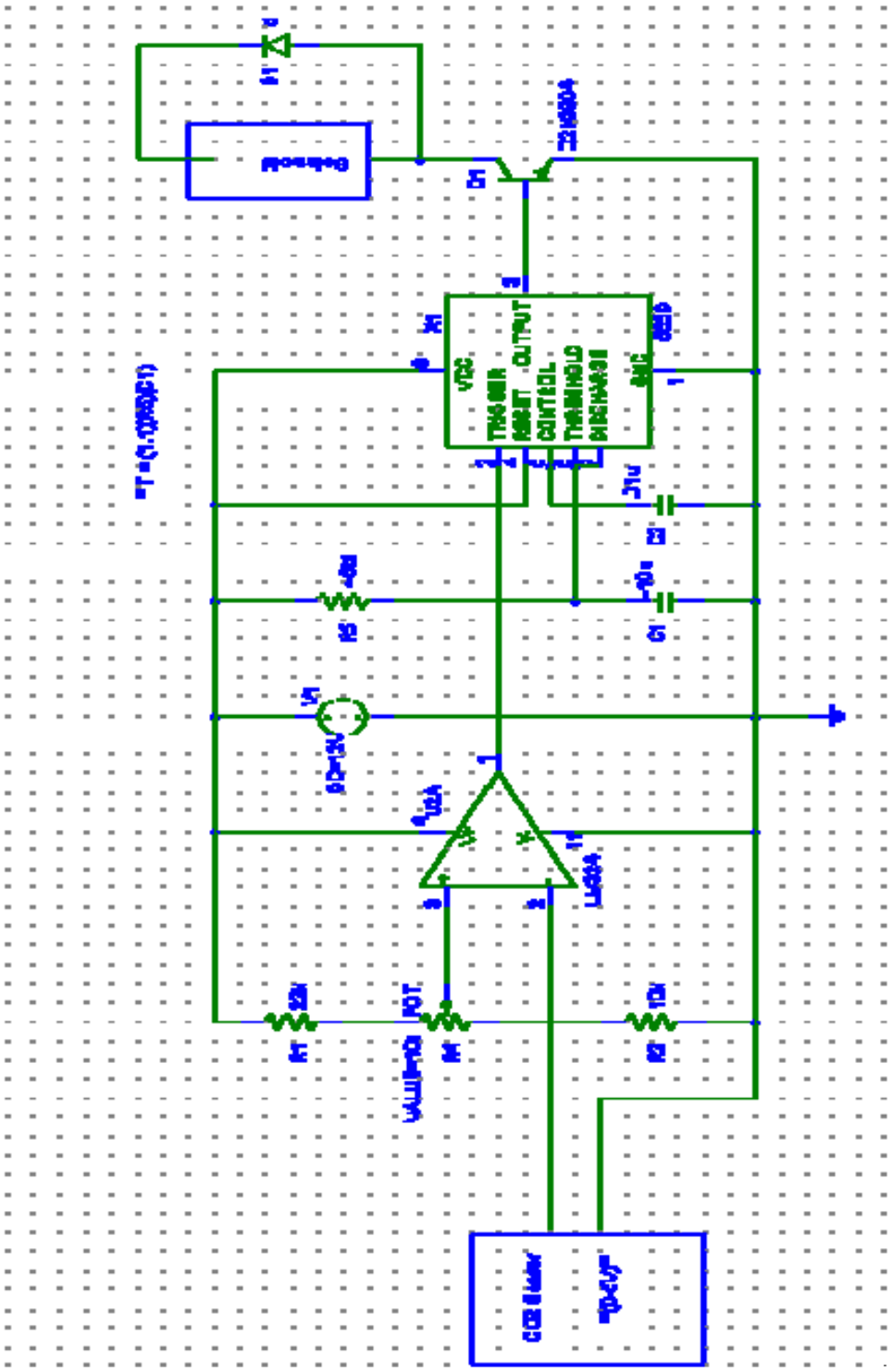
Future Work:

Moving forward with this project requires a number of additions to the existing prototype as well as a good amount of additional research and testing. In order to achieve accurate regulation of a 5% carbon dioxide environment inside the chamber, a carbon dioxide sensor must be purchased. It is the most essential component as well as most expensive piece of the design. Extensive research has been done in looking for appropriate carbon dioxide sensors and a decision to purchase a final model must be approved by the client. After purchasing the sensor, it must be calibrated appropriately and integrated to work with the existing circuit and chamber. Placing a sensor that works inside the chamber will require small modifications to be made to the prototype so that the sensor is in an area where it can most accurately measure the carbon dioxide levels. The second element of the project that remains to be completed is a valve system used to distribute carbon dioxide to the chamber. A design for this system has been created and must simply be converted into a working prototype. Once again this addition to the project will need to be integrated with the existing elements of the project.

Testing that the project works effectively will be accomplished as specific elements are added. The circuit has proven effective through a number of voltages tests, but it must be re-tested once connected with the valves carrying the carbon dioxide and the carbon dioxide monitor. Also, the effectiveness of the chamber in achieving and maintaining a constant 5% carbon dioxide will only seen once the monitoring apparatus has been calibrated and

implemented into the chamber. With a functioning, integrated prototype many more tests should be conducted in the laboratory environment by laboratory personnel. The length of time that one can image live cells with the prototype must be tested and evaluated. Results of these tests will reveal further improvements that need to be made along with working to incorporate the prototype into the environment for which it was designed to be used. A new project that would enhance the prototype's capabilities is the creation of a profusion system to effectively supply cell media to the living cells being imaged in the chamber. There are a number of different problems to consider in engineering such a system. First off, the media is a liquid that cannot be distributed throughout the entire chamber, but instead must be localized to the element holding the cells such as a petri dish or cell plate. This also means that the amount of media supplied must be closely regulated so that it will not interfere with the images being produced. The remaining work to create such a system is considerable and would be accomplished most efficiently by a continuation of the project into next semester where further research, testing, and engineering can take place.

Appendix A:



Appendix B:

Product Design Specifications:

Creation of a live cell-imaging device for use with laser-based confocal microscopy.

Team Members/ Roles :

Jon Sass/ Team Leader
David Schurter/ Team Leader
Bryan Fondrie/ Team Communicator
Matt Kudek/ BSAC
Dan Jonovic/ BWIG

Abstract:

This project was designed to create a glass-topped, acrylic imaging chamber that will fit on the stage of a confocal microscope with dimensions 21 cm (l) x 25 cm (w) x 4 cm (h). The chamber is engineered to fit Petri dishes as well as cell plates. The chamber will regulate the internal carbon dioxide to 5% +/- 0.25%, sustain a humid environment and maintain 37° C in the cell culture. The goal of this chamber is to imitate the conditions of cells *in vivo*.

Problem Statement:

Engineer a cell imaging chamber that maintains an internal 5% carbon dioxide concentration and is adaptable for a future perfusion system.

1. Client Requirements:

a. Performance Requirements: The client has suggested two improvements be made to the current cell imaging system. Firstly, the client would like to be able to control the level of atmospheric carbon dioxide. Secondly, a perfusion system, which allows the client to administer different drugs in a controlled manner to the culture, is desired. The client leaves the choice of which system to build to the team, although a system which combines both would be exceptional.

b. Accuracy and Reliability: The atmospheric carbon dioxide should be kept at 5%+/- 0.25%. The system should have a gas sensor built in as to provide feedback to a system that monitors and controls the carbon dioxide levels.

c. Life in Service: Unknown at this point.

d. Materials: The device will be designed as to suit current 35 mm circular glass bottom petri-dishes and cell plates (12, 24, 48, and 96 wells). The dishes are made of plastic with a glass slip inserted in the bottom for better focus during microscopy.

e. Aesthetics, Appearance, and Finish: The device will be used in a laboratory situation, so aesthetics are not crucial to the success of the final product. The product should look professional though.

f. Shelf Life: Ideally, the product would not wear down until initially used.

g. Operating Environment: The device will be placed on the platform above the objective of the microscope. The platform measure 25cm by 20 cm, and has a limiting height of 3 cm. The height is limited by the condenser.

h. Size and Weight: The device should be no larger than the platform on which the imaging takes place. The materials used should be as thin as economically and practically possible.

i. Ergonomics: Should be easy to use and operate.

2. Production Characteristics

j. Quantity: Preferably, one reusable device should be constructed.

k. Target Product Cost: Under $1,000

3. Miscellaneous

l. Standards and Specifications: Must be able to fit in space above confocal microscope, and bottom of petri dish or other housing design for the samples must be exactly on the stage so that imaging microscope can image clearly from below the chamber. The top of the chamber should be made of glass as to not interfere with imaging.

m. Patient Related Concerns: Diffusion and concentration of gas with the media in the petri-dish can affect proficiency of the media in sustaining sample. Also there must be easy distribution of both these supplies throughout the chamber.

n. Competition: There are existing models of this device that fulfill requirements. Devices are currently very complicated and very expensive. For the purpose of this project, the device would not need to be integrated as these existing products are.

Appendix C: References

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