

Biological Imaging Chamber:

Proposed Designs for Imaging Chamber

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Abstract:

Live cell imaging provides great insight into the behavior of all types of cells. A better understanding of cell behavior can lead to a better understanding of human physiology. Extended cell imaging techniques are currently limited by an inability to create an environment that can promote cell life longevity. The limiting factor is the pH of environment in which the cell is placed for imaging. Through monitoring and control of gas concentrations and humidity, this pH can be regulated extensively, thus prolonging cell life in imaging environments. This project deals with the monitoring and control of carbon dioxide within a controlled imaging environment.

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, DIC, 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 $\sim 37^{\circ}$ 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 effect 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. The chamber must have a port to allow internal access. The design will be smaller than the microscope stage (21cm (l) x 25cm (w) x10cm (h)). The system will consist of a constant 5% CO₂ gas concentration as well as a humid environment. 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 hours. The current method available kills cells long before this time, because the pH changes with a decreasing CO₂ 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 CO₂ 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 similar, reliable system can be constructed for less than a third of that. The purpose of this design is to construct that 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 CO₂ 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 CO₂ concentrations. The system starts by adding gaseous CO₂ to cell media, and then adds this media to the culture. This product is also capable of monitoring the temperature of the culture.

Other products solely create the desired 5% CO₂ mixture. The addition of this mixture to a culture is left for the researcher. These products are capable of receiving pure CO₂ gas and creating a mixture that is the desired 5% CO₂. 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 CO₂ 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% CO₂ is needed (www.wikipedia.com). In

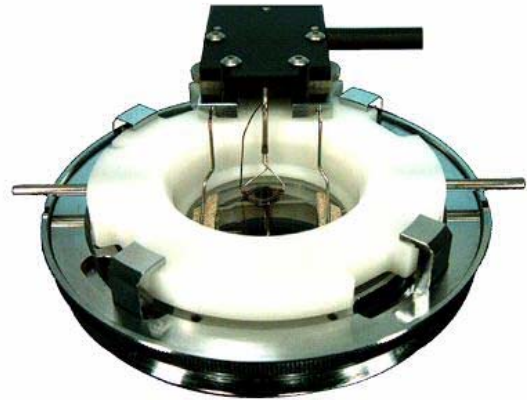


Figure 1: Bioptechs FSC® Imaging Chamber

<http://www.asiimaging.com/bioptechs.html>

normal atmospheric conditions, a .035% concentration of CO₂ is present (www.wikipedia.com). In order to reach the 5% concentration, a way to deliver CO₂ to the culture and monitor the flow is needed. The following design addresses this issue, by monitoring of CO₂ and adjusting to change in CO₂ concentration within the culture.

In order to create such a system, a way of determining the current gaseous concentration of CO₂ is needed. This is accomplished through the addition of a potentiometric CO₂ sensor. These sensors operate using the fundamentals of spectroscopy to measure gaseous concentrations. This concentration is then presented as a voltage output. An example of such a CO₂ sensor is the Edinburgh Instruments GasCheck (<http://www.edinst.com/gascheck.htm>). This voltage would represent the amount of CO₂ in the system. Through simple calibration, the corresponding voltage to a 5% CO₂ 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.

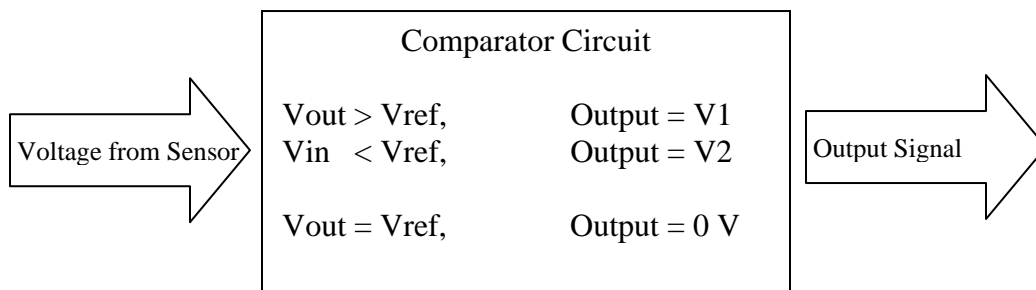


Figure 2: Comparator Circuit Analysis

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 CO₂ into the circuit. If the comparator determines the CO₂ concentration is too small, CO₂ will be added to the circuit as a quick burst. The CO₂ will be added at a controlled rate, so the volume of CO₂ added is proportional to the time the valve is open. It is likely that the amount of CO₂ that will be needed in the system will be very small, so a quick burst of CO₂ is probable.

It is assumed that the cells will be consuming CO₂, making it unlikely that there will be a case indicating that CO₂ concentrations are too high. This design is more concerned with CO₂ concentrations below the 5% mark. The entire design is summed in Figure 3.

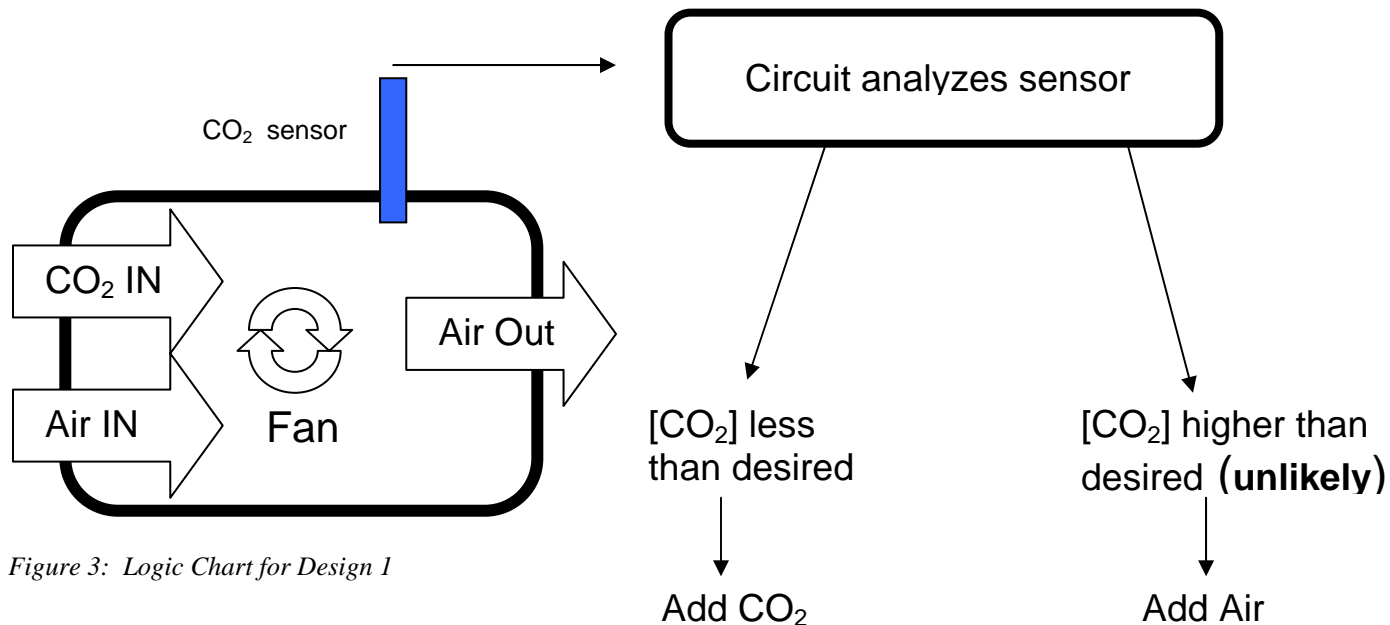


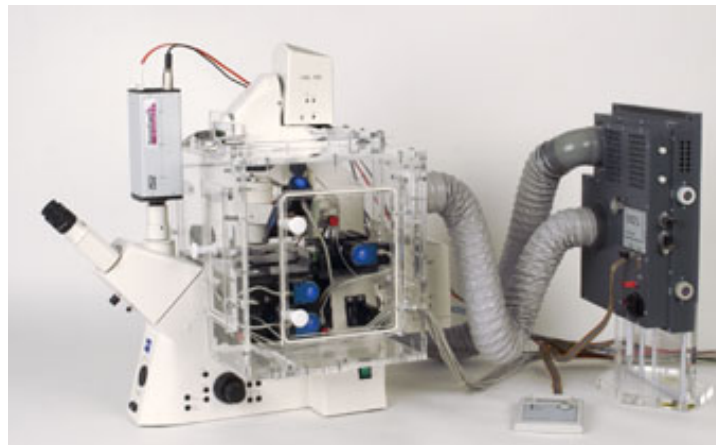
Figure 3: Logic Chart for Design 1

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 incorporate the diffusion of gas into an environment as well as a media perfusion system. Shown is a product available from “Cell Biology Trading” (<http://cellbiology-trading.com/>)



*Figure 4: Current Market Designs of Microscope Chambers
www.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 CO₂ 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 a pump is not desired for the system, it will not be considered necessary, as the high pressure in the gas tank will provide sufficient flow into the dish. A regulator will need to be installed to prevent an over feed 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 CO₂, a smaller amount of gas will be consumed 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 cheaply.

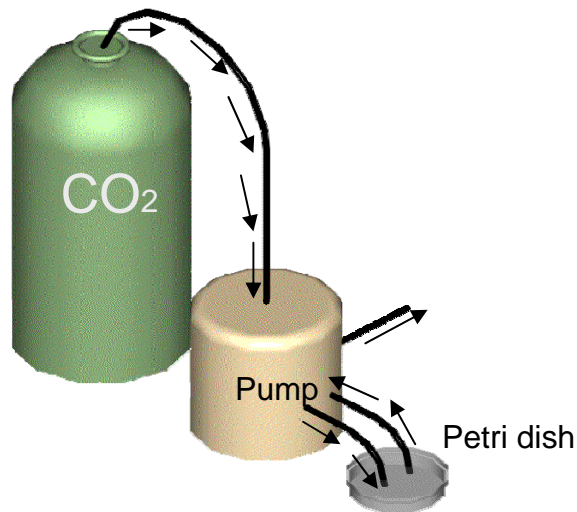


Figure 5: Modified Petri Dish Assembly

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. As this is an important component to maintaining a specified concentration of the gas, an additional component is necessary. 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 CO₂ infused medium into the Petri dish to maintain an appropriate level of CO₂. 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 CO₂ 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 CO₂ 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).

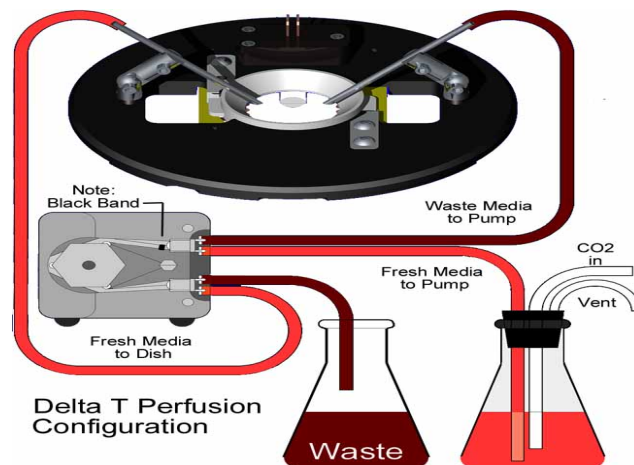


Figure 6: Delta T Perfusion Configuration
http://www.bioptechs.com/Products/Delta_T/D_Perf/d_perf.html

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.

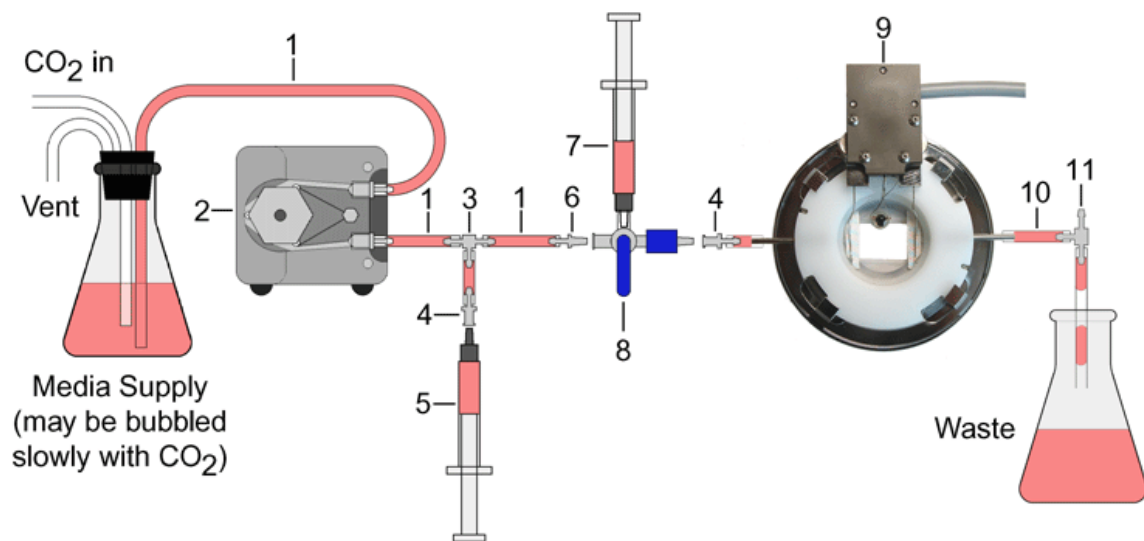


Figure 7: FCS2 Perfusion Configuration
http://www.bioptechs.com/Products/FCS2/F_Perff_perf.html

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 CO₂ 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 CO₂ 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 micro-perfusion 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.

In summary, micro-perfusion allows an increased variety of experiments to be run, but is more costly to implement and creates more waste. With the resources available and customer specifications for this project, this design is not feasible.

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 CO₂ 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. Future Work

Choosing a design from the three proposed designs was a large step proceeding with the project. The design matrix helped accomplish this goal, but there are many other components of project still unfinished, that need to be completed before a prototype can be produced. Before our design is implemented into a prototype, a meeting with the client is necessary to ensure his approval of the design and satisfaction that it will fulfill expectations for this project. This meeting minimizes chances of creating a prototype that has missing elements or has unsatisfactory performance. In order to build a prototype, a number of design elements must be ordered. In-depth research of these elements will need to be done to ensure that they perform to sufficient standards and cost a reasonable price. A large factor in bringing this design to a working prototype will be integration of all elements properly. Research will help in

accomplishing this goal, but modifications may be made to each element before combining together into a working prototype.

Upon completion of the prototype, it will need to be tested repeatedly in its new environment to ensure proper function. These tests will be conducted either by the client or under his directed supervision, as he is most qualified to work with the design in its environment. Results of these tests will determine the amount of modification necessary to the project, before it is considered complete. Completion of these modifications will lead us to our final step of implementing our design into the laboratory environment. The implementation will be final, meaning that the completed project must be set-up so that it can be used for an extended period of time. This may require a number of attempts at implementation and a necessary meeting between the team members, client and all persons using the product in the future. Communicating vital knowledge about the project to those using it in the future is an important final step toward ensuring project completion, and client satisfaction.

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.

Appendix A:

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:

Live cell imaging is crucial in understanding the behavior of many types of cells, including stem cells. Through the observation and research of the behavior of these cells under controlled environments, much useful information can be gained. This information could possibly lead to great advancements in healthcare, including the treatment of diseases once though untreatable. Currently, live cells can only be kept under the microscope for no more than 3 hours. Research could be far more useful if this length were to be increase to at least 48 hours. It would result in far more useful investigations, and could aid in the quality and effectiveness of current live cell research.

Problem Statement:

Design a live cell imaging chamber that gives the observer control over variables such as temperature, gas content, and cell media concentrations.

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%. The system should have a gas sensor built in as to provide feedback to a system that monitors and controls the carbon dioxide levels. A perfusion system should be able to deliver an aqueous drug to the culture at a rate near 1 mL/min.
- 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. The dishes are made of plastic with a glass slip inserted in the bottom for better focus during microscopy. The solution should contain parts that are easily sterilized between uses, or contain cheap parts that may be replaced with each use.

- 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.
- 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 10cm.
- h. Size and Weight:* The device should be no larger than the platform on which the imaging takes place.
- 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.
- 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 B: References

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