Microscope Compatible Cell-Culture Incubator

BME 400
October 19th, 2016
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Abstract

An on-stage incubator for use with inverted microscopes is desired for time-dependent live cell imaging experiments. Current products are expensive and may not be compatible with multiple microscopes, or are ineffective at controlling the internal environment. The client desires an incubation chamber compatible with cell microscopy that is capable of maintaining desired temperature, CO_2, and humidity evenly throughout the chamber. The device should not alter image quality, and should be accessible for changing media or cell culture dishes. The team developed three preliminary designs, evaluated them using a design matrix, and chose to move forward with the dual chambers design. An initial list of electronics components needed to create the environmental control loop has been ordered, and will be the starting point for design development. The initial prototype will involve a small, cohesive system to regulate temperature, CO_2, and humidity, which will then be expanded into the final design. Creation of a successful design will help to bridge the gap in the market between high-cost, functional incubation systems and cheaper, less effective systems.

Table of Contents

Introduction

Motivation

Many basic science researchers perform live cell imaging studies that require the use of an incubation system compatible with a microscope stage. This type of imaging is useful to evaluate various cell behaviors that may change over time, for example: cell migration, proliferation, apoptosis, and protein expression. Maintaining optimal cell culture conditions for the duration of the imaging study is necessary to view the activity of cells in real time, while preventing unnatural cell death or distress. Many researchers interested in cell behavior invest in an on-stage cell culture system. These systems, however, cost up to $50,000 and not only limit imaging to one type of scope but are also error prone in their environmental control. The client would like to address these issues, and would like the team to create a much cheaper, versatile cell culture system that is compatible with bright field and fluorescence microscopy.

Existing Products

Currently, two distinct markets for microscope-compatible cell culture incubators exist: high-end custom incubation systems, typically sold by the microscope manufacturer, and cheaper systems that allow for less environmental control. The products on both ends of the market fail to meet the the needs of most researchers. One high-end product, the Nikon BioStation IM-Q, houses an entirely automated imaging system in its incubation chamber, seen in Figure 1 [1]. While the system is able to maintain environmental conditions very well, it is only compatible with the microscope enclosed in the chamber, and costs roughly $30,000 [1]. These prices make it difficult for most labs to justify purchasing a system that is used only in specific experiments and is limited to a single microscope type.

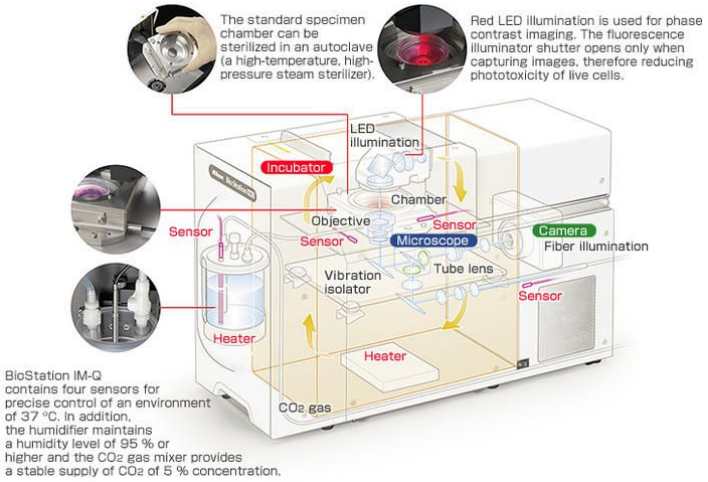


Figure 1: Nikon BioStation IM-Q Incubation system

Figure 2: Bioscience Tools Miniature Microscope Incubator

Other, more cost-effective, products are also available for researchers. An example of this type of product is the Miniature Microscope Incubator by Bioscience Tools. This system is a small, enclosed chamber that allows for inflow and outflow of air to maintain environmental parameters, as seen in Figure 2 [2]. Although the cost is a little over $1000 for the entire system, the client has found that there are typically large

temperature gradients throughout the chamber in these designs. Large temperature gradients are undesirable for cell culture, especially in microfluidic systems where small liquid volumes can evaporate unevenly.

Despite the availability of both high-end and low-end cell culture incubation systems for use with live cell microscopy, none of the products on the market fully meet the client's requirements.

Problem Statement

The client desires an inexpensive incubation chamber for use in live cell imaging on an inverted microscope. The incubator should regulate temperature, CO_2, and humidity levels in the chamber with minimal gradients. The device should also be accessible for changing media and available for use with different types of cell culture dishes. In addition, the device should be able to be moved from the stage of one microscope to another, so that it is not limited to only one imaging system.

Background

A full understanding of many aspects of cell biology requires observation of cell behavior for an extended period of time. Historically, this was not possible because of a lack of a physiological cellular environment during imaging. It wasn't until the 1980's that Sally Temple, a researcher studying neural development in mice at the University of Florida, set out to find a way to observe neural progenitor cells for days at a time [3]. By building an incubator system around an old microscope, Temple was able to obtain time lapsed data over the course of about a week.

Since Temple's makeshift solution, major microscope manufacturers have followed her lead and now offer an array of complex incubator systems for use in conjunction with multiple imaging modalities. However, many of these systems are high-end and costly, which limits their availability to researchers. The client, Dr. John Puccinelli, is in need of a low-cost stage incubator that is able to provide rigid environmental conditions, and is flexible enough to work with different imaging systems. Furthermore, it must work with fluorescence and brightfield imaging modalities. With this design, he will be able to observe proliferation and migration of a variety of cell types, and use fluorescence imaging to examine protein expression over time. Actin and myosin, cytoskeletal proteins typically observed by fluorescence, are shown in Figure 3 along with cell nuclei from a live cell imaging study [4].

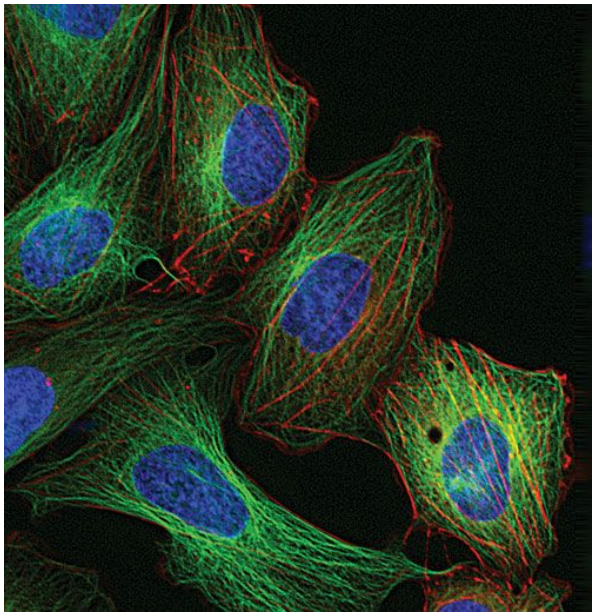


Figure 3: Representative fluorescent image of cells with actin, myosin, and nuclear stains

In order to ensure cell viability, the team will need to maintain temperature, humidity and CO_2 at constant levels, as well as a number of other requirements that can be found in the PDS in Appendix A. The three most important functional demands are regulating temperature at 37 °C ±1°C, humidity at 95% RH ±5% and gaseous CO_2 concentration at 5% ±1%. Ultimately, the designed incubator will provide an effective alternative to costly systems while maintaining healthy cells for long-term imaging.

Preliminary Designs

Given the problem statement and design specifications previously described, the team developed three potential solutions. These three designs are described and compared below.

Basic Box

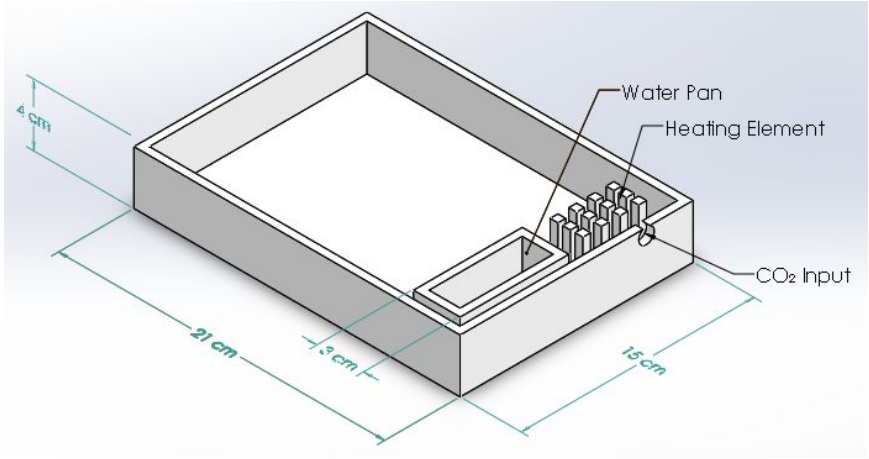


Figure 4: Basic Box Design

The basic box design incorporates all aspects of the control system (Appendix B) into one structure on the microscope stage. The basic box is a natural step in the design iterations following the team's planned initial sensor and component testing, which is described in the testing plan. The heating elements, CO_2 input, and humidifier will all be located within the box. Heat will be introduced using a thermal fin fixed on a heating pad. Convection from this fin will increase by directing airflow over the fins. CO_2 concentration will be maintained using a feedback loop-controlled valve, allowing input of highly concentrated CO_2. Humidity is introduced through an ultrasonic transducer submerged in a water supply. The activity of the humidifier will be controlled by a feedback loop that uses a relative humidity (RH) sensor to detect if the systems meets a desired set point. Homogeneity of these parameters will be reached through rapid mixing of air within the incubation chamber. This mixing will be achieved using the airflow directed from the heat convection fan.

Dual Chamber

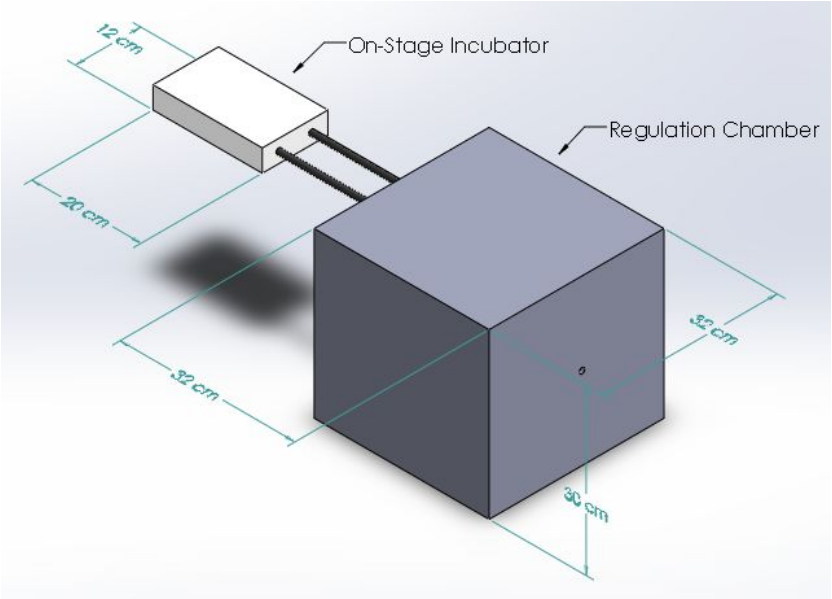


Figure 5: Dual Chamber Design

The second design features two chambers; one containing all environment controllers and the other designed solely as a chamber for cell culture and imaging. Heat, humidity and CO_2 will be introduced to the incubation chamber through connecting insulated tubing. The environment control chamber will contain a heating system similar to that of the basic box. Humidity will be introduced using an ultrasonic transducer immersed within a water source. Finally CO_2 will be controlled through the same methods used in the basic box. While very similar to the basic box, this option will require the design of tubing and tubing-chamber interfaces in order to maintain homogeneity between the two chambers. In addition, the team must evaluate air circulation rates between the two chambers to determine the type of fan and tubing diameter necessary to maintain the environmental conditions.

Perimeter Moat

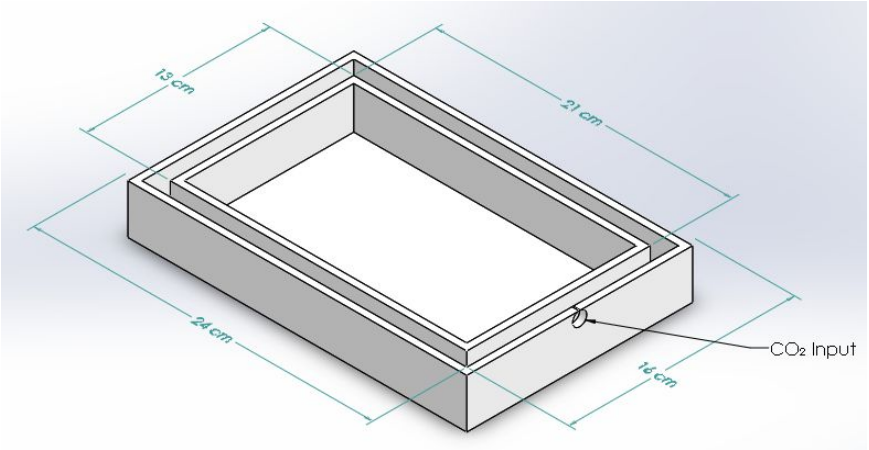


Figure 6: Moat Design

The final proposed design utilizes a unique method of temperature control as well as an alternative method of maintaining high humidity within the incubation chamber. Similar to the basic box, the perimeter moat combines the incubation chamber and environmental controls into one structure. This structure contains a double wall, inside of which heating pads are used to directly heat air within the chamber. Unlike the Basic Box and Dual Chamber designs, the perimeter moat replaces the water atomizer with a large surface area moat of water. With the high temperature, the water should evaporate at a rate high enough to sustain the 95-100% humidity desired.

Preliminary Design Evaluation

The three primary system integration solutions were compared using a number of weighted criteria. The values earned for each criterion were summarized in a design matrix (Appendix C). The cross design consistency of the weighting allowed for one design, in this case the two chamber solution, to earn the most scores towards the design criteria. The allotted scores for each criteria are discussed below to allow for design review. The final scores from the design matrix comparison of these designs indicate shortcomings and strengths in all three designs. These close results indicate that a final design should include aspects of all three designs in order to increase the overall score according to the design priority weightings.

Heat Regulation

The first and highest weighted category in the design matrix is heat regulation. This environmental parameter is the most susceptible to losing uniformity throughout the system and therefore the most difficult to design for. Additionally, relative humidity will also be stabilized by the maintenance of uniform temperature in the culture environment. Successful cell culture relies heavily on the uniformity of these parameters in the incubator and therefore heat regulation is a priority in our design considerations.

The first design, the Basic Box, was scored 2/5 in heat regulation due to high potential for temperature gradients. Close heater/culture dish proximity and low air volume both present risks of overshooting temperatures locally during opening recovery. Opening recovery refers to the re-stabilization of an environmental parameter following the exposure of the system (for > 30 seconds) to the outside environment.

The second design, Dual Chambers, scored 3/5 in this category. While the second chamber allows for a more aggressive air heating method before transfer to the incubation chamber, this transfer presents potential for a temperature drop making feedback loops unpredictable. The positive aspects to this system are that the large air volume will make opening recovery much faster and that separation between incubation chamber and heater minimize large temperature gradients.

The final design, the perimeter moat, scored highest in this category, 4/5. This high score was due to the simplicity of using heating pads without additional air distribution components. The negative to this design is the potential long temperature recovery time. Given

that the heating pads will have to be at the desired temperature (37°C), uniform recovery after an opening will rely on natural convection.

CO_2 Regulation

As the first line of defense in pH buffering, CO_2 levels must also be constantly maintained during incubation. While all of the proposed designs utilize the same CO_2 control system; structure and air mixing will influence uniformity and opening recovery of this parameter.

Due to lower airflow rates, the moat will have the slowest “opening recovery”. However, the low volumes of both the moat and basic box will allow for more rapid diffusion of CO_2 within the system. For this reason these two designs tied for the high score of 4 out of 5. It follows that the most difficult system for CO_2 maintainance is the dual chambers. The large CO_2 mixed air volume will have to be rebalanced during opening recovery. As the CO_2 valve is binary (open or closed), the ability to accurately rebalance in a short amount of time will require high air mixing rates and high valve release resolution.

Humidity

High humidity is vital to the design’s accommodation of long-term culture studies. Evaporation of liquid (and resulting pH and growth media changes) will occur rapidly within the system without high relative humidity. Both the basic box and dual chamber designs include the use of a water atomizer to introduce water vapor into the environment. The disparity in scoring between these two designs lies in the problems that arise from humidifying in close proximity to the viewing area in a small air volume like the basic box. Condensation in the this system could interfere with the ability to effectively image during incubation. The dual chamber system avoids this risk by introducing water vapor to the air mix in the environmental control chamber. While the less aggressive humidifying technique of the moat reduces condensation likelihood, the natural evaporation method will have much slower recovery time following an opening. Additionally, the perimeter wall heating could create a conduction heating through structural components; potentially inducing condensation.

Ease of Fabrication

This category was scored based off of the similarity of the design to our early testing protocol structures and the design’s accommodation for parallel iteration of the system components and controls. Due to the simple system integration of the basic box, this design was given the highest score. This was followed by the dual chamber design. Despite the complex tubing feature, this design allows for independent work on the incubation chamber and environment control chamber. Finally, the moat design was determined the most difficult to fabricate. The moat not only introduces new fabrication complexity during the construction of a dual walled perimeter heating system but is also lacks the heating component flexibility of both other designs.

Cost

The clear winner of this category is the basic box due to the absence of features present in the moat and dual chamber designs. The dual chamber system with tubing/tubing interfaces and the moat with a double wall/heating pads both require components that will substantially increase the design cost.

Accessibility

Defined as user ease of access to the culture plate, accessibility results in opening recovery and must therefore be designed as to minimize environmental disruption during opening. Additionally, access to the plate must not require the user to remove the system from the microscope stage.

This parameter pointed to the moat as an outlier due to the significant difficulty in providing user access across the heated, double wall. Although not to the same extreme, the dual chamber design also limits user access due to the inlet and outlet ports connecting the incubation and environmental control chambers. Thus the basic box scored highest in this category due to the numerous areas on the design that can be access points.

Fabrication and Development

Materials

The design selected for the project is the dual chamber design. The key issues to be addressed with the design are temperature, CO_2, and humidity regulation. In order to maintain temperature, a heating pad, along with a heat exchanger will be used in the design. Humidity will be introduced into the system through utilizing the Grove 101020090 water atomizer. CO_2 regulation will be performed using a CO_2 tank and the JFSV00005 gas solenoid valve. A fan will be included in the larger chamber with these elements, separate from the imaging chamber to circulate the heat, humidity, and CO_2 through tubing into the imaging chamber, and back through additional tubing into the larger chamber, to be recirculated.

Measuring these elements' levels will be critical to the success of the design. Each sensor discussed will incorporate a feedback loop which will monitor the respective levels of heat, humidity, and CO_2. The DHT-22 humidity and temperature sensor will allow measuring of the humidity and temperature with a humidity accuracy of ±2% RH, and a temperature accuracy of ±0.5°C. For measuring CO_2, the MH-Z16 CO_2 sensor will be used, providing an accuracy of ±50ppm. Plexiglas will be used for the exterior of the design, as it provides adequate optical properties for imaging, as well as good insulation to maintain temperature, humidity, and CO_2 levels inside of the design.

Fabrication Plan

The first steps in fabrication involve constructing a rudimentary initial prototype. This platform will enable the team to easily test temperature, humidity, and CO_2 feedback systems in a setting similar to the larger regulation box of the Dual Chamber design. As the project develops more complexity can be added, such as the second chamber with tubing connecting the two pieces. Eventually, permanent materials will be used to replace the temporary parts of the prototype, giving rise to a more professional final product.

Testing Plan

The testing plan within the scope of this semester is broken up into separate phases and components, as listed below. For simplicity, the team will first test the electronics components functions and calibration, optical compatibility of plexiglass, and the fully integrated design.

Electronics Function and Calibration

Electronic regulatory systems and feedback loops will first be tested in a single insulated system that will likely consist of a styrofoam box. Once the team is satisfied with these results, a second chamber with tubing and circulation between the systems will be added. Testing will commence on this rudimentary two-box system, and then finally on the final design with the proper materials included in place of the prototype materials.

Optical Compatibility Tests

To test the compatibility of using plexiglass with an inverted brightfield and fluorescent microscope, the team will place plexiglass on top of the imaging platform, and then image cells through this plexiglass as well as a standard cell culture dish. Varying thicknesses of plexiglass can be used to ensure proper focusing ability. Images taken of live or fixed cells with this system will be analyzed using ImageJ to ensure that the membrane resolution is not significantly altered.

Fully integrated design testing

Once the plexiglass incubation chamber and insulated environmental control chamber have been built, put together, and integrated with the electronics control systems, the team will be able to perform testing on the final design. This will involve long-term tests of the system to ensure it maintains environmental conditions for at least two weeks, and can sustain culture of live cells. The system will also be tested to ensure that it recovers quickly after opening the system, to the specifications listed in the PDS (Appendix A).

Discussion

This system, when completed, will meet a functional need that fills a niche in the current market. All researchers who work with migratory and proliferative cell types can benefit from

long term imaging capabilities, especially at a low cost. While a prototype has not been constructed or tested yet, design of the circuitry will be consistent regardless of the box design. The sensors and regulatory elements that the team has ordered should fulfill the design requirements given by the client.

The device itself poses very little ethical dilemma, however the cell types that are used with our incubator could. It is an important responsibility of any researcher using the incubator to adhere to the appropriate cell culture guidelines and regulations.

There are a number of potential pitfalls and obstacles which we will need to circumvent. For instance, our team will need to analyze how fast the incubator is able to stabilize once changes are introduced into the system. If it takes too long, we could consider decreasing the volume of the second chamber. Another possible issue is condensation on the plexiglass that impedes the ability to image. If this occurs when testing, we can explore alternate materials or research hydrophobic coatings that could be applied. Finally, there is the ever-present dilemma of reducing temperature and humidity gradients to a minimum. Other problems could certainly arise, but these two are predictable given the nature of the project, and can be remedied with more testing, analyzing, and slight alterations of the design or processes.

Conclusion & Future Work

To meet the client's specifications of a portable, environmentally stable, and cost-effective cell culture incubator, the team will move forward with the dual chambers design for the rest of the semester. This design was chosen as it will allow for improved humidity, heat, and CO_2 distribution throughout the incubation chamber, as well as easier integration of the environmental regulation components.

In order to create a working prototype, the team must follow the fabrication and testing plans, as described above, and consider potential design modifications that can be made in the following semester. Once a robust prototype has been developed, and hardware and software testing of the design is complete, the team will test the incubator with live cells. Cells must be obtained from the client, and cultured for periods of up to two weeks, to show the product's viability for typical live-imaging studies. Other design considerations may also be incorporated once a working prototype has been created. These considerations include an automated method of changing cell culture media and an improved method of maintaining culture dish position. Depending on the design's success, the team may pursue making this a marketable product, and may consider using high-volume manufacturing techniques.

The success of this device will help to fill the gap in the market between very high-end, fully integrated cell culture microscopy systems and low-cost, poorly functioning culture systems. Researchers will be able to perform live cell imaging studies without having to spend $50,000 on a high-end incubation system, or compromise the health of their cells in a low-performing system.

References

[1] N. Inc., "BioStation IM-Q | Live Cell Screening Systems | Products | Nikon Instruments - Microscopes and Imaging Systems", *Nikoninstruments.com*, 2016. [Online]. Available: https://www.nikoninstruments.com/Products/Live-Cell-Screening-Systems/BioStation-IM-Q. [Accessed: 17- Oct- 2016].

[2]"Miniature Incubator for slides and petri dishes", *Biosciencetools.com*, 2016. [Online]. Available: http://www.biosciencetools.com/catalog/Incubator_Universal.htm. [Accessed: 17- Oct- 2016].

[3]Baker, M (2010). "Cellular imaging: Taking a long, hard look". *Nature*. 466 (26): 1137–1140. doi:10.1038/4661137a.

[4] BioTek Imaging, "BioTek Imaging and Microscopy," *BioTek Imaging and Microscopy*, 2016. [Online]. Available: http://www.cellimager.com/. [Accessed: 19- Oct- 2016].

Appendices

Appendix A: Product Design Specifications

Microscope Cell Culture Incubator

Product Design Specifications | October 17, 2016

Client: Professor John Puccinelli
Advisor: Professor Mitchell Tyler
Team: Trevor Zarecki, *Leader*
Jenny Westlund, *Communicator*
Steve Gock, *BSAC*
Jack McGinnity, *BWIG*
Peter Hartig, *BPAG*

Function: The device should enable the continuous culture of live cells for up to two weeks on an inverted microscope, without impeding imaging capabilities. The cell culture environment must imitate that of an incubator with precise control and readout of temperature, CO_2 mixture, and humidity all within a sterile environment.

Client Requirements:

- Temperature control and readout
- Humidity control and readout
- CO_2 concentration control and readout
- Incubation container must not impede ability to image
- Accessible for changing cell culture plates and changing media
- Sterilizable with a standard 70% ethanol solution
- Fit securely on an inverted microscope as to ensure imaging of a consistent location

Design Requirements:

1. **Physical and Operational Characteristics**
    a. ***Cell Culture Related Performance Requirements:*** The device should maintain incubator-like conditions for 2 weeks. It must maintain the temperature at 37℃ ± 1℃, and reestablish temperature after less than 6 seconds following a 30 second door opening. It must maintain 95-100% humidity within culture chamber. Finally,

it should maintain 5% ±0.5% CO_2 concentration and reestablish concentration after less than 6 seconds following a 30 second door opening.

b. ***Incubator Housing Related Performance Requirements:*** Incubator housing and any potential condensation must not disrupt optics during imaging. The housing must not limit ability to navigate the full field of the cell culture plate, and not substantially change the distance between the cell culture plate and the objective. Housing materials must be compatible with culture media and be sterilizable with 70% ethanol solution. The system should also have adequate insulation to prevent internal temperature fluctuations due to external temperature changes.

c. ***Safety:*** Culture environment must be compliant to BioSafety Level 1 standards. All electrical components within the culture environment must be sterile and waterproofed, and all circuitry must be rated to the supplied power and current used.

d. ***Accuracy and Reliability:*** The precision of the system components is outlined in the performance requirements listed above. For each of the four environmental parameters we will be controlling (temperature, humidity, CO_2 percentage and air sterility), the combined error of sensor measurement/readout and parameter control must be within the tolerance. The precision measurements taken during system use are as follows:
    i. Humidity: 95-100% humidity
    ii. CO_2 concentration: 5% ± .5% of readout value
    iii. Temperature: 37℃ ± 1℃ of readout value

e. ***Life in Service:*** The incubation chamber should maintain the specified environmental conditions to promote cell life for up to two weeks. The internal portions of the design under these environmental conditions must function without recalibration or repair during this time period.

f. ***Operating Environment:*** The internal portion of the incubation chamber must function in conditions of 95% relative humidity or more, temperatures of 37℃ and CO_2 levels of 5% during incubator operation. If the system is not in operation, the incubator will be exposed to normal environmental conditions: room temperature, environmental humidity, and low CO_2 concentration. There will be limited dirt exposure inside the incubation chamber, as live cells will be stored in it. Users will be opening and closing the incubation chamber, so the system will also have to adapt to sudden drops in temperature, relative humidity, and CO_2 percentage. It must be possible for the user to change media for cells inside the incubation chamber without changing the location that is being imaged.

g. ***Ergonomics:*** The user will have limited interaction with the incubator itself, except to move cell culture dishes in and out of the chamber. The door to the chamber should be easy to open, and allow for enough clearance to fit a cell culture plate, flask, or petri dish inside the incubator.

h. ***Size:*** The interior of the incubation chamber should be at minimum 15.4 cm x 9.4 cm x 2.5 cm tall. The incubation chamber should fit securely on a stage with

dimensions as small as 16.0 cm x 25.0 cm, with a clearance of 5.3 cm tall for the light source.

i. ***Weight:*** Each component of the final product should be no more than 12 kg, such that it is easy to transport between experiments without too much difficulty.
j. ***Materials:*** Materials used for the incubation chamber should not have cytotoxic effects on cells inside their culture dishes, and should be sterilizable with ethanol. The materials should be resistant to corrosion from the high humidity levels. We must use glass for the bottom surface, and the top surface should not defract light from the light source significantly.
k. ***Aesthetics, Appearance, and Finish:*** The surfaces through which imaging will occur should be transparent, and not result in any aberrations or otherwise compromise the quality of imaging. There should also be a mechanism to protect the experiments from light pollution.

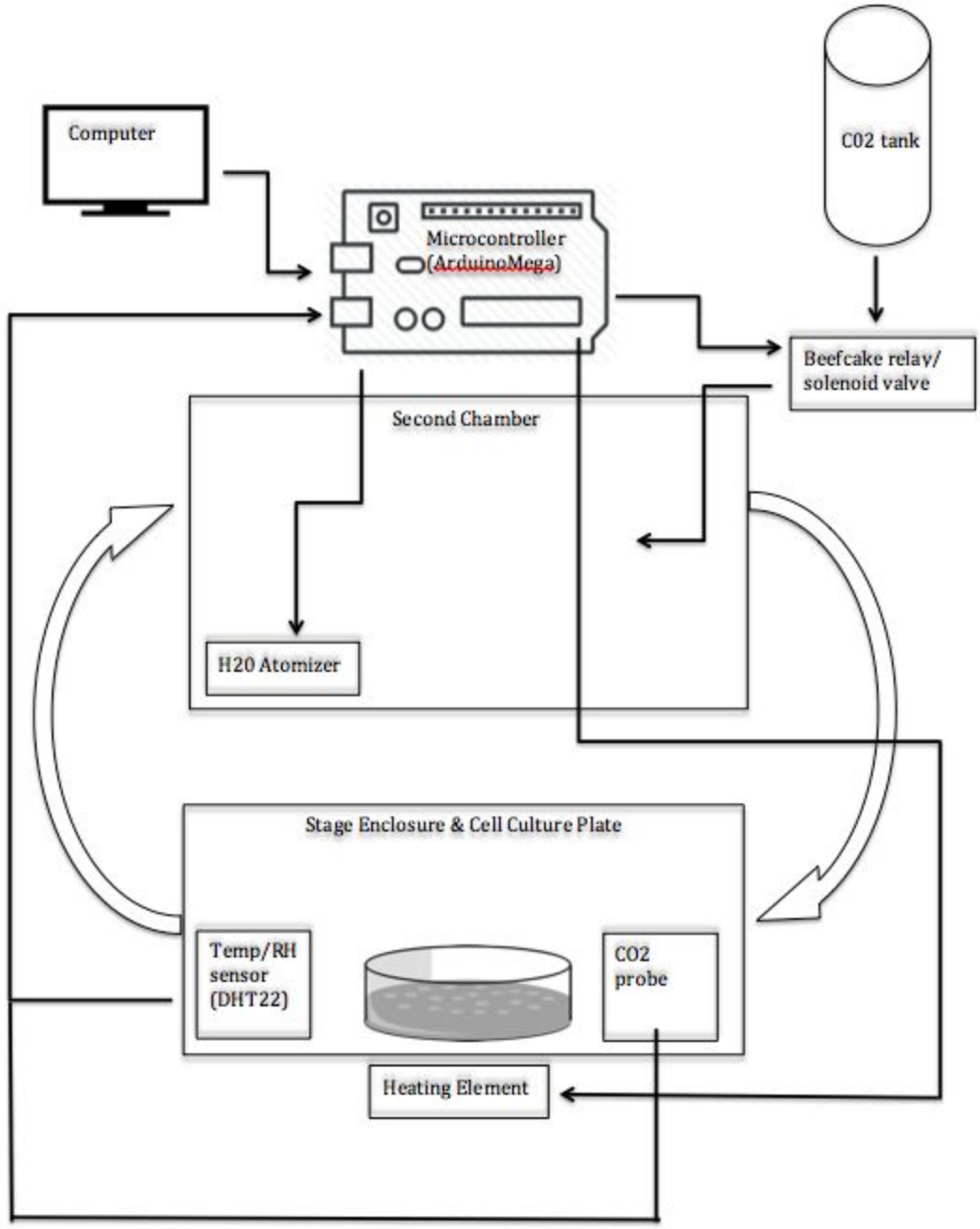
2. Production Characteristics

a. ***Quantity:*** The client needs a total of one microscope cell culture incubator.
b. ***Target Product Cost:*** The target product cost is to be $200, with an understanding that the product would enter the market for around $500.

3. Miscellaneous

a. ***Standards and Specifications:*** There are no standards and specifications to our knowledge that must be addressed within the design.
b. ***Customer:*** Any customer preferences are already addressed above and have been taken into consideration.
c. ***Patient-related concerns:*** The product will not have any contact with patients, so patient-related concerns are not applicable.
d. ***Competition:*** There are a variety of systems that have been fabricated for similar purposes, but to the knowledge of the team the device we intend to create would be unique in cost, ease of use, and the ability to be used with a number of microscopes. Stage incubators on the market, such as the Pecon Incubation System 2000 fits all functional requirements of the client but is specifically tailored to fit the Olympus IX71/81 microscope. Ideally, we will be able to translocate our final prototype from one imaging system to another and it will be relatively universal. The majority of available systems also enclose the whole microscope, which limits the system's versatility.

Appendix B: System Control Diagram



Appendix C: Design Matrix

Criteria	**Weight**	**Design 1:** Basic Box		**Design 2:** Dual Chambers		**Design 3:** The Moat	
Heat Regulation	25	2/5	10	3/5	15	4/5	20
CO2 Regulation	20	4/5	16	3/5	12	4/5	16
Humidity	20	2/5	8	4/5	16	3/5	12
Ease of fabrication	15	4/5	12	3/5	9	2/5	6
Cost	10	4/5	8	3/5	6	3/5	6
Accessibility	5	4/5	4	3/5	3	1/5	1
Safety	5	4/5	4	4/5	4	3/5	3
Total	100	62		65		64	

Appendix D: Materials List and Budget

Material:	Cost:
MH-Z16 CO2 Sensor	$67.95
JFSV00005(1/4") Solenoid Valve	$11.99
DHT-22 Temperature/Humidity Sensor	$9.95
Grove 101020090 Water Atomizer	$9.90
Fan	N/A
Beefcake Relay Control	N/A
Tubing	N/A
Tubing Adapters	N/A
Heating Elements (TBD)	TBD
Heat Exchanger (TBD)	TBD
CO2 Tank (TBD)	TBD
Arduino Mega microcontroller	N/A
Sparkfun Redboard	N/A
Total:	$99.79