Cell Culture Incubation Chamber

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Abstract

Dr. John Puccinelli wishes to perform live-imaging of cell cultures during time course experiments that can range from 6 hours to a week. In order to achieve this, the cell cultures must be maintained at the correct environmental conditions throughout the experiment as well as when the cells are being imaged. Our team aims to develop a microscope-compatible cell culture incubation chamber that is able to accurately regulate the internal environment at 37°C, 5% CO_2 and between 90-100% humidity, without interfering with the imaging capability of the microscope. Thus far, we have decided to use a nichrome wire heating element, a bubbler humidifier and an external CO_2 and air mixing chamber that is all regulated through a feedback system. Additionally, the entire microscope housing will be made of acrylic, and it will surround the entire stage of the microscope to prevent the formation of temperature gradients. Next, we plan to model the design in SolidWorks for thermal testing as well as test the fluid dynamics of the humidifier & gas injection system. After thorough testing, our team will build a prototype of our cell culture incubation chamber.

Problem Statement

Dr. John Puccinelli needs a cell culture incubation chamber that is compatible with the Olympus IX71 inverted microscope to analyze various cell cultures over time. He has used similar systems before, but found that these devices often cause temperature gradients, condensation build up and evaporation of culture fluid that disallow proper cellular imaging. Therefore, we plan to develop a cell culture incubation chamber with interchangeable culture plates that is compatible with the Olympus IX71microscope. This incubation chamber must be able to maintain an internal environment of 37˚C, 5% CO_2 and 90-100% over long durations for

time course experiments, without compromising the integrity of the microscopes optics or functionality.

Microscope & Live-Cell Imaging

Live-cell imaging is often used to assess the cellular dynamics of a variety of biological processes. The most common technique used is fluorescent imaging of fluorophores introduced into the cells. These fluorophores can be tagged to a protein and injected into the cell to analyze the function of that protein or how it interacts with other proteins. An alternative method of introducing these fluorophores is to insert a stretch of DNA encoding the fluorophore just downstream of the coding region for the protein of interest; the cell will then generate the fluorescent protein. Additionally, immunofluorescence is can be used to image cellular dynamics. Immunofluorescence uses fluorophore tagged antibodies that interact with their antigen and they can be tracked via their fluorophore; the antibody is chosen so its antigen is the protein of interest [1].

Existing Products

Several products currently exist on the market that perform the same function as the device we are trying to create. These products also come in a few different designs as well. The first type of design is a small incubation

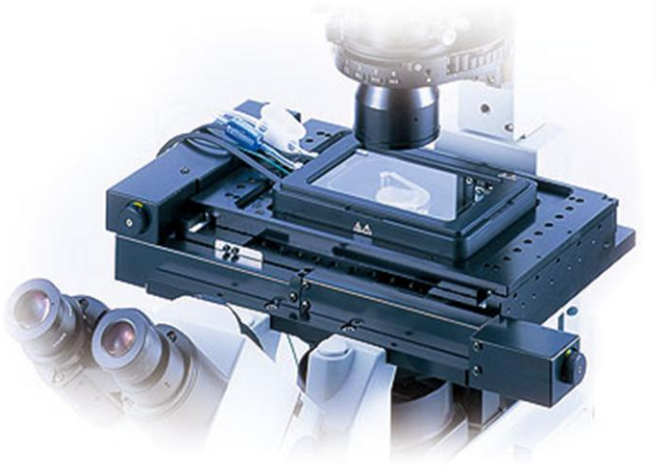

Figure 1: Tokai Hit WELS Chamber [2]

chamber which is fixed to the microscope stage and has tubes connected to provide the correct environmental conditions. One example of this type of product is the Tokai Hit Standard Series Control Unit, shown in Fig. 1. This unit, as well as other incubation chambers, uses an external control unit connected to a CO_2 tank to provide the correct gas mixture. The humidity is provided by a heated water bath surrounding the culture dish. This type of design disallows the use of a variety of culture dishes because the main incubation chamber is small; this unit only allows chamber slides, 35mm and 50/60mm dishes.

The next design type is the box type, which encloses the stage, the lenses, and most other parts of the microscope. Okolab makes this kind of incubator, and it can be seen in Fig. 2. The box is made of a clear plastic and retains the cell culture environmental conditions. There are small doors in the walls of the incubator to allow access to the cell culture. Warm air is blown into the chamber from an external heater.

Figure 2: Okolab Microscope Cage Incubator [3]

Premixed 5% CO_2 air is pumped into the chamber and then into a bubbling chamber to humidify the chamber. The air flows through a column of water and becomes saturated with water vapor, providing the culture with a humid environment. One of the benefits of this type of incubator is the stage is included in the heating environment, meaning it should equilibrate to the same temperature of the rest of the chamber. At the same time, since the box containing the culture is much larger, the potential for temperature gradients is greater. Additionally, the size of this design makes it more difficult to put together and remove every time it is used.

The final design type is the cell incubator with a built in microscope. An example of this design type is shown in Fig. 3, the Nikon BioStation IMQ. This type of product consists of an incubator with a microscope built in below the incubation chamber. The microscope is connected to a CCD camera, which is interfaced with a computer for viewing samples. The air supply comes from an external gas mixer and the gas flows through a bubble humidifier within the system. A heater warms the water and the incubation chamber to the desired temperature with fans. This system works well because the microscope is built around the incubation chamber instead of building the incubation chamber for the microscope. One drawback to this design is the incubator housing doesn't allow for much variation in culture plate size. Also, this device requires you buy the whole system instead of just the incubator.

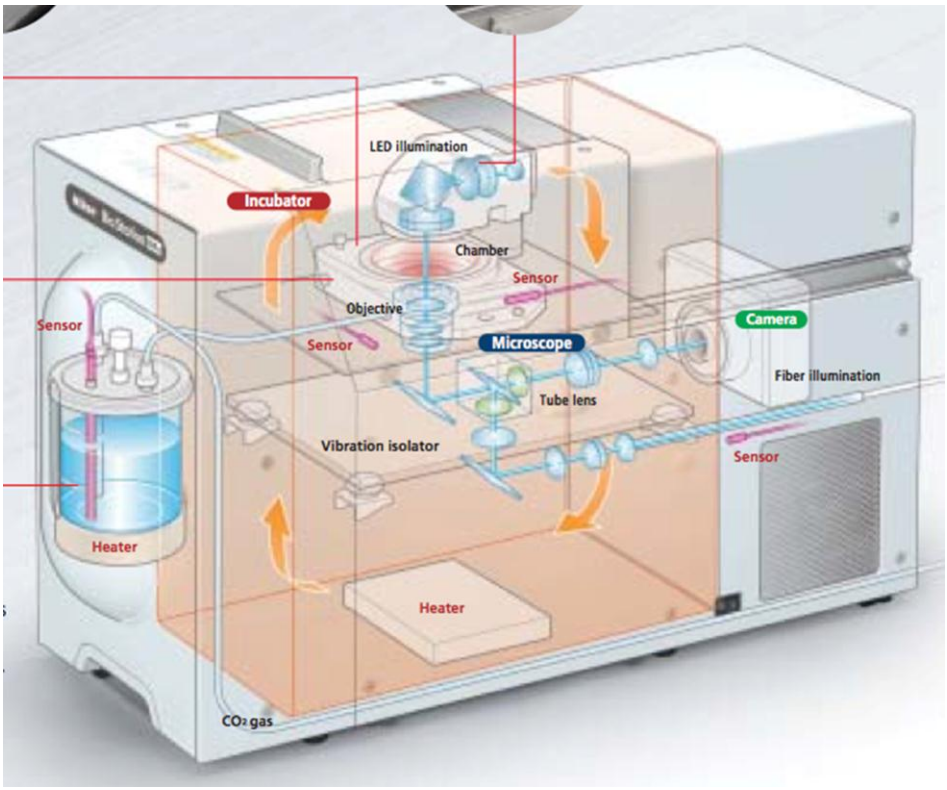

Figure3: Nikon BioStation IMQ Cell Incubator and Microscope[4]

Design Specifications

Our client specified that our design must be able to regulate an internal environment of 37˚C ± 2˚C, 5% CO_2 ± 1%, and 90-100% relative humidity. The entire system must be closed from the external environment to ensure these stable internal conditions. The incubation chamber must be able to properly image cell culture plates of differing size and shape, and cannot experience interference from condensation, temperature gradients or evaporation of culture liquids. Lastly, the incubator housing must be easily assembled and dissembled and it cannot impede the functionality of the microscope once it is set-up.

Components

There are four main components of this cell culture incubation system necessary to achieve the internal conditions laid out by the client. The components include a humidifier, a CO_2 mixer, a heating element, and the housing to enclose the cell culture as well as other system components. The following is a summary of the research conducted for the components as well as the chosen alternative for each part.

Humidifier

As per the design specifications, the humidifier component of the design must be fabricated so that it can release enough water vapor to keep the incubation chamber at a relative humidity of 90-100%. Several design alternatives considered after conducting a patent search and researching commercial products include a “wet blanket model,” an external water-only humidifier, and a bubbler humidifier.

The "wet blanket model" follows a design modeled in a patent for a controlled environment incubator for light microscopy [5]. This device injects gas through a perforated tube that is surrounded by a sponge-like damp material. Depending on the length of the perforated tube, the gas is humidified to a certain relative humidity before it enters the incubator. This method offers a greater degree of control in terms of gas delivered to the incubator. However, this method is fairly expensive in terms of the budget of the project (~$10-$15). Additionally, the sponge material would make the water lost during the humidifying process much more difficult to replace.

The external water-only humidifier would be constructed similarly to simple home-use evaporative humidifiers [6]. The bulk of the water is contained in a reservoir and is slowly dispensed into a basin. A wicking filter absorbs the water from the basin and a fan then blows hot hair through the moistened filter, evaporating some of the water within it. This model was seldom found in commercial products or patent applications for microscopy research. This type of humidifier allows for greater degree of control of gas delivered to the incubator, since the gas delivery and humidifying system are separate. Also, this type of humidifier is easy to maintain, but the degree of control over high humidity levels is limited because of the nature of the wick. This design is also more expensive (~$20) than the model decided upon.

The bubbler humidifier model, which is the model used in all three design alternatives, consists of a basin of heated water through which atmospheric air and carbon dioxide is bubbled, and is based off of the design in another incubator for observation by microscope [7]. The humidified gas evaporates, bringing water vapor along with it. This gaseous mixture is then allowed to dissipate into the incubation environment. This model is the most inexpensive out of the three and allows for a strong degree of control over both gas delivery to the incubator and

humidity of the chamber. Also, this design allows easy access to the water basin to replace lost water. Conversely, this humidifier requires its own heating system in order to allow strong control of water evaporation, which negatively affects the ease of construction.

Future work within the humidifier includes calculations to determine how heat delivered to the basin and rate of gas bubbled through relates to water evaporated and thus relative humidity of the incubation chamber. The calculations will be confirmed with testing using modeling software.

CO_2 Sensor

To keep CO_2 levels at 5%, a sensor is needed to monitor the CO_2 content around the cell culture. There are different ways of monitoring CO_2 levels, but the most common and reliable way is using a Non-dispersive Infrared sensor. This type of sensor measures CO_2 concentration by determining how much infrared light is absorbed by a gas sample.

Figure4: Geotech G100 CO2 Incubator Analyzer [8]

A few different sensors were evaluated for monitoring CO_2 around the cell culture. The first sensor considered was the *Geotech G100 CO2 Incubator Analyzer* (Fig. 4). This device detects CO_2 levels between 0 and 20%, measures temperature from 0 to 50° C, and humidity from 0 to 100%. The advantage of this device is the sensor is separate from the probe which can be placed inside the incubator. The main problem with this product, however, is the inability to interface its measurements with a

microcontroller. It can be connected to a computer but only previous logs of measurements can be accessed instead of real-time data acquisition. Also, this device costs $1,412.82, which is outside of the client's proposed budget.

After looking at handheld CO_2 monitors, silicon chip based sensors were considered next. The *K-33 BLG 30% CO2/Temp/RH Data Logging Sensor* made by Senseair (Fig. 5) provides a small NDIR sensor capable of measuring carbon dioxide concentrations up to 30% ± 0.2%. It is also able to measure temperature from -40 to 60° C ± 0.4° C and relative humidity from 0 to 100% ± 3%. This sensor can also be interfaced with a computer or microcontroller for real-time measurement of environmental conditions. Sample air can be injected into the sensor using a tube and pump for remote sampling. This type of product seems most ideal for the type of incubator being designed.

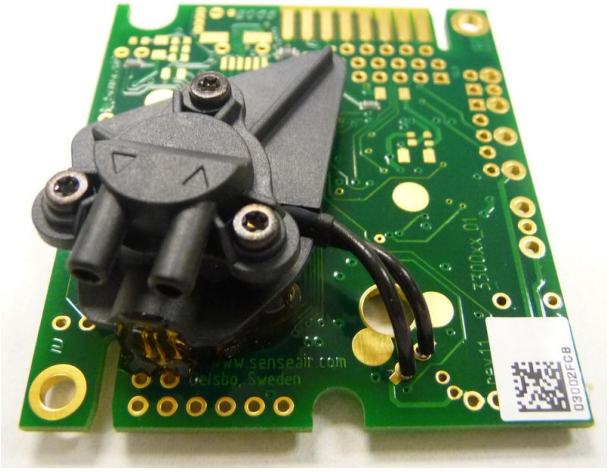

Figure 5: Senseair K-33 BLG 30% CO2/Temp/RH Data Logging Sensor [9]

Heating Element

The heating element of the microscope-compatible incubation chamber must be able to accurately regulate an internal temperature of 37° C without the formation of temperature gradients. Development of such gradients can be damaging to the cell cultures over time due to uneven heating, thereby affecting the reliability of any experimental results obtained using the device. After thorough research of existing patents, two types of materials emerged as the best alternatives for heat generation: transparent conducting oxides or an electrically resistive metal.

Transparent conducting oxides are a group of doped metal oxides that are able of conducting electricity over the material. This type of material is usually surface coated over a substrate because thin films more efficiently conduct electricity. ITO is the best commercially available transparent conducting oxide with a high electrically conductivity and 85% transparency to allow microscope viewing through the chemical. An array of electrodes can be used to run electricity across the entire film, thereby allowing uniform heating [10]. Unfortunately, ITO and other transparent conducting oxides require industrial machines that perform deposition techniques such as sputtering and electron beam evaporation. These methods are prohibitive to this design because they are expensive and require extensive training. Additionally, pretreated ITO surfaces or the chemical itself is relatively expensive and is outside of our client's budget. Therefore, other heating elements were considered.

Another method of generating heat is by running current through an electrically resistive metal. This can be used in combination with a thermally conductive metal to effectively dissipate the heat over a surface. Nichrome wire is often used in general heating elements due to its high electrical resistivity. In addition, this material forms a thin oxide layer after initial heating that serves as a protective coating over the wire that prevents further oxidation of the heating element [11]. Nichrome used in combination with aluminum, a thermally conductive element, creates an effective heat generator and distributor. Nichrome wire is also inexpensive, as it costs about $15 for 25 ft. Therefore, nichrome seemed to be the best alternative for this design.

To incorporate this type of heating element into the cell culture incubation chamber, further calculations are required to determine the current, length and diameter of wire, as well as the electrical resistivity of nichrome at 37°C. The placement of this heating element with respect to the entire housing as well as an aluminum heat sink also needs to be determined.

Housing

All of the environmental conditions in the incubation chamber were considered when choosing the material to construct the housing out of. The material needed to be transparent, water resistant and resistant to shape deformation at 37° C (possibly much greater near the heating element). Patent searches showed two transparent materials were most commonly used to construct the incubator housing: Acrylic (Plexiglas) and Polycarbonate (Lexan/Makroclear) [12].

Both of the materials fulfilled the design requirements for transparency but Acrylic was more susceptible to yellowing after prolonged exposure to sun or spectrum light, a possible longevity issue [13]. Polycarbonate was also slightly more transparent (96% to 93%) but the difference was negligible.

Polycarbonate had two major deficiencies that led to the designs being modeled with Acrylic instead. First, it is much more expensive than Acrylic. Because the environmental conditions aren't extreme in the incubation chamber Polycarbonate's greater resistance to deformation at high temperatures isn't an asset worth paying for. Also, Polycarbonate has been found to leech Bisphenol A, a biologically hazardous material, when heated repeatedly [13].

System Process

A process diagram of our system is shown in Fig. 6. The system starts with the gas mixing, where a 100% carbon dioxide tank will be connected to a current controlled solenoid valve. Atmospheric air will be pumped into the gas mixing chamber with the 100% CO_2 to output a mixture of air that is 5% carbon dioxide. The air will then flow into the bubble humidifier. The

humidifier water will be warmed by a heater to 37° C and the mixed gas moves will become saturated with water vapor as it bubbles through the water. The humidified air will then diffuse or be blown with the assistance of a fan into the plexiglass incubator chamber, where the cell culture would be located. This chamber will be heated to 37° C using nichrome wire as a heat source. The incubating chamber will contain a CO_2 sensor, thermometer, and relative humidity sensor. The outputs of these sensors will interface with a microcontroller that will read all three conditions, and output the data to a computer to display, allowing the operator to view the current conditions. Depending on the conditions inside the incubator, the microcontroller will output electrical signals to a circuit which will control the heating source, the solenoid valve connected to the CO_2 tank, and the pump for the atmospheric air. By monitoring these conditions and adjusting the inputs accordingly, a feedback loop is created, which allows for precise control over the environment of the incubator. A water reservoir for the humidifier might be needed to keep water levels high enough, in which case a water level sensor might be needed to control the addition of water. Finally, an output port for the incubation chamber could either be looped back to the gas-mixing chamber or open to the air. The arrangement of components to this system is discussed in the design alternatives.

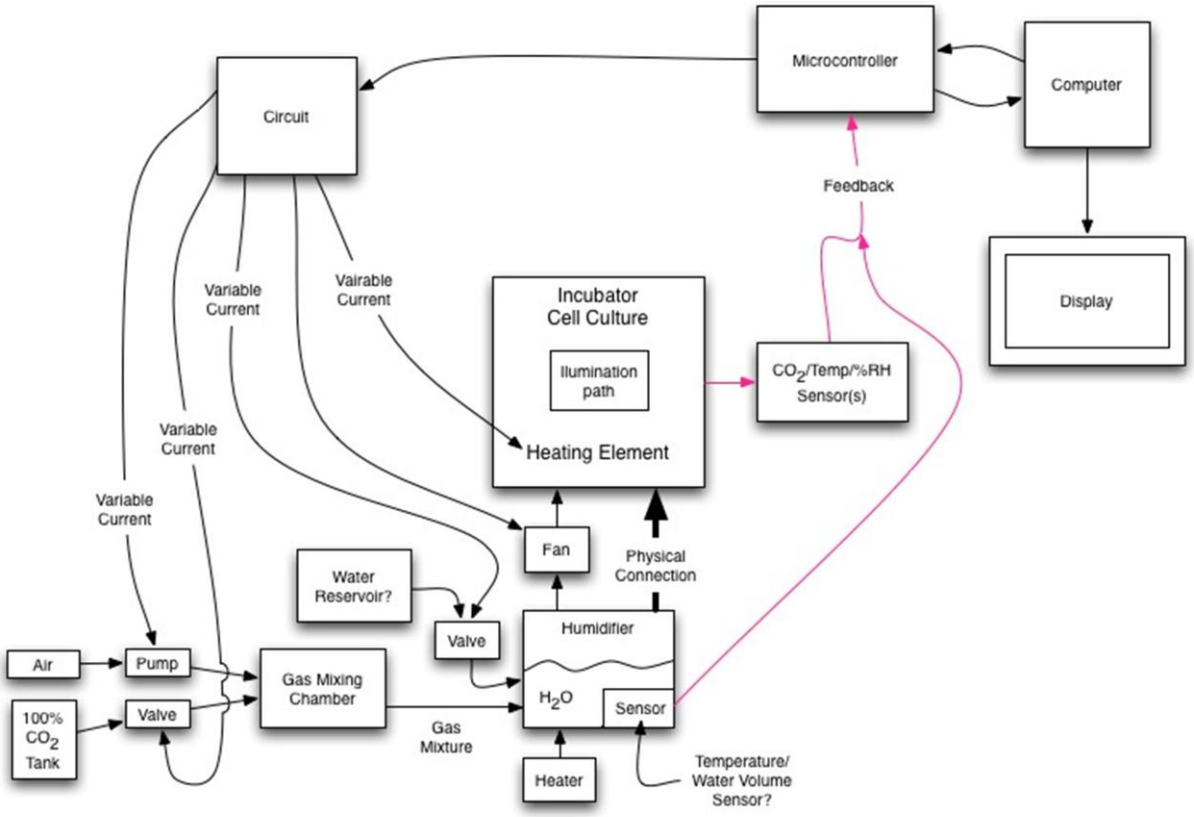

Figure 6: A process diagram of the incubator system. Components with arrows pointing towards them are receiving inputs and outward facing arrows represent outputs from that module.

Design Alternatives

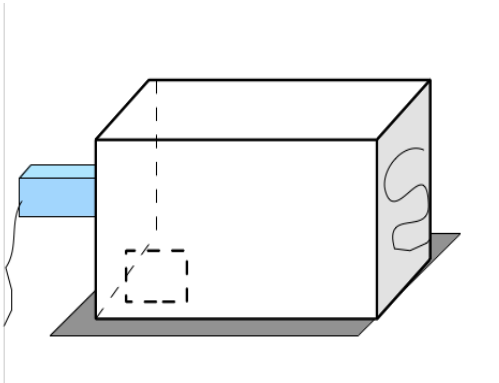

Figure 7: Design 1 - Weighted stage-top incubator

Design 1

This design features a large incubator that rests on the stage top. It is composed of a large plexiglass chamber that is closed to the outside atmosphere. The

chamber encloses the entire stage top and is weighted at each of the four corners to prevent it from tipping or falling. Ambient air and carbon dioxide are fed into the humidifier (pictured here in blue) which contains a small volume of heated water. The humidifier is mounted onto the side of the chamber and air humidified in the humidifier is allowed to dissipate into the chamber. The chamber is heated through the use of current running through an electrically resistant metal. The wire is placed on all four sides of the chamber so that the incubator can be heated evenly. There is also a separate but similar current heating the humidifier. There is a one way valve located on the side of the incubator opposite the humidifier that allows air from inside the incubator to escape into the atmosphere. There is a small door (pictured here as a dotted square) located on the front of the chamber to allow access to the inside. Inside the chamber there is a sensor that detects carbon dioxide levels, temperature, and relative humidity. The slides are not pictured in this design because they are not in their own chamber but rather rest directly on the stage top inside the large chamber. This design is large and encompasses the entire stage top, which allows the (inverted) microscope to focus better. Also, because of the door access entry and a larger overall design, there is a smaller percentage loss of internal environmental conditions when the interior of the incubator is accessed. However, because this chamber is large, it is difficult to maintain a constant temperature without causing temperature gradients which can lead to condensation on chamber walls, poor viewing, and specimen evaporation. Additionally it will be difficult distribute carbon dioxide and water vapor evenly throughout the chamber because of its size.

Design 2

This design features a small incubator that rests directly on the stage-top above the lenses. It is composed of two flushed plexiglass chambers connected by a small aperture located at the top of a dividing barrier. Ambient air and carbon dioxide are fed through a valve into heated water that lies in the first chamber. These gases are humidified and are allowed to dissipate freely through the aperture between the chambers. The second chamber contains the cell culture plate (depicted here in green). This chamber is humidified through the gases dissipating from chamber one. Additionally, it is heated using an electrically resistant metal current that is located on the interior bottom surface of the main incubation chamber and another current on the exterior of the bottom of the humidifier chamber. This allows both chambers to be heated to different temperatures. Inside the second chamber there is a sensor for detecting carbon dioxide levels, temperature, and relative humidity levels. Additionally, there is an aperture at the end of the second chamber that allows air from inside of the chamber to diffuse out to the external environment. This aperture is equipped with a fan to prevent ambient air from re-entering the incubator. The size of these chambers allows humidity, carbon dioxide levels and temperature to be very precisely maintained. Also, the small size of the two chambers discourages temperature gradients, and this model very efficient and not very costly. This model also allows high precision of environmental controls. However, the fact that the slides do not rest directly on the stage top but rather sit inside an incubation chamber does not allow the optics of the microscope to focus very

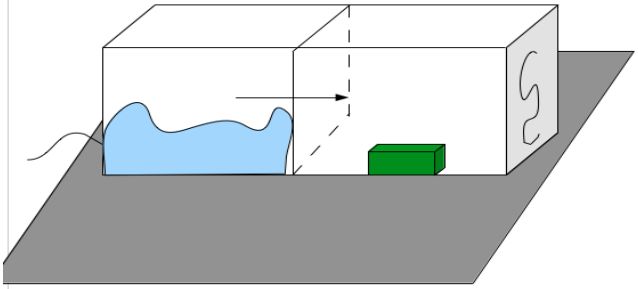

Figure 8: Design 2 - Stage-top portable incubator

well. Also, the size of this model limits access to the slides and the humidifier during the course of the observation.

Design 3

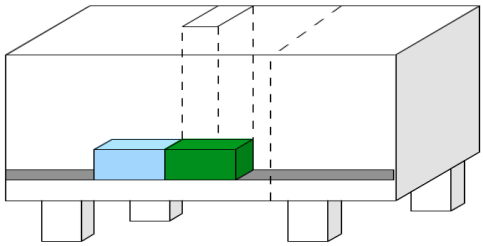

Figure 9: Design 3 - Encompassed stage incubator

This design features a large incubator that surrounds the stage top and rests on the laboratory bench top. It is composed of a large plexiglass chamber that is closed to the outside atmosphere. The chamber surrounds the entirety of the stage top while the four legs rest on the lab bench top around the perimeter of the microscope. There is enough room beneath the incubator for the optics system of the microscope. Additionally, the plexiglass chamber is not a perfect rectangular prism but has a rectangular prism-shaped notch on the side so lenses can approach the slide incubation chamber (represented here in green). This notch is represented by the dotted lines above the slide. The slides are located in their own small, separate chamber located directly over the hole in the center of the stage. This chamber covers the top of the slides and it is into this chamber that the humidified carbon dioxide, air, and water vapor are fed. However, this chamber is also open to the larger incubation environment. The addition of this chamber helps to prevent humidity and carbon dioxide gradients as well as prevent condensation. The humidifier in this model lies directly adjacent to the slide incubation chamber and is open to the slide chamber. Ambient air and carbon dioxide are fed into a warm water bath within the humidifier through a tube system. The humidified gases are then allowed to dissipate into the incubation environment. This incubator is heated through the use of a current running through an electrically resistant nichrome wire. This wire is placed on all four sides of the chamber on both the interior and the

exterior and is primarily placed near the stage. This heating situation utilizes the idea that the stage acts as a heat sink and therefore can aid in avoiding temperature gradients. Inside the incubator there is a sensor to monitor carbon dioxide levels as well as temperature and humidity. There is a one way pressure sensitive valve on the side of the incubator to allow the gases in the incubation environment to escape and these are then recycled and fed back into the humidifier. Finally, the entire chamber is hinged to allow access to the inside when necessary. This is represented by the dotted line near the incubator leg. In contrast to design one the slides in this design rest directly on the stage top. This, along with the notch that is created in the side of this chamber allows the microscope to focus much better than in the other two designs. Additionally, though the chamber is large, which can create temperature and carbon dioxide gradients, the use of the second, smaller chamber surrounding the slides helps prevent condensation on the incubator and evaporation of the sample. The placement of the humidifier, and thus the gas entry point, in the middle of the chamber and the use of the heating system described also helps to minimize these effects. However, this system is the most complex out of the three and will be the most difficult to construct.

Design Matrix

Prototype	Weight	Design #1	Design #2	Design #3
Cost	15/100	10	12	**9**
Heating	20/100	12	16	**15**
Microscope Functionality	15/100	13	10	**14**
CO_2	20/100	5	17	**16**
Humidity	10/100	5	7	**6**
Ease of Use	20/100	5	0	**18**
Total	100	50	62	**78**

Table 1: Design Matrix: Each design is scored according to the quantitatively weighted elements in the left most column.

As seen from Table 1 the three designs were evaluated according to Cost, Heating, Ease of Use, CO_2, Humidity, and Microscope Functionality. Of these six categories, CO_2, Heating, and Microscope Functionality were rated highest because they had the most direct impact on the functionality of the prototype. CO_2 was considered pertinent because it is a primary function of the culture environment. The design specifications gave a relatively small range for acceptable CO_2 levels. Heating was weighted highly because of the emphasis our client placed on it in our design specifications; he articulated multiple times that a major flaw in current designs is they create temperature gradients that alter the environmental conditions in the culture chamber. Microscope Functionality was also weighted highly because it was critical to our design being operable.

In the next tier was Cost and Ease of Use. Cost was specified as a major factor by our client but because all three of the prototypes used many of the same components the total cost didn't vary a drastic amount. Ease of Use was also more consistent among the design alternatives. None of the prototypes gave an exceptionally superior user experience and none of them were impossible to manage either.

Humidity was given the lowest rank because all of the prototypes would theoretically achieve the desired environmental condition.

The third design scored the highest, due primarily to its high score in microscope functionality. By encompassing the entire stage and allowing the condenser move freely in a boxed-in channel, the microscope will have full range of focus and function. Additionally, by enclosing the aluminum stage in the incubator housing it will help to prevent temperature gradients rather than causing them. The larger chamber size will also allow for easier access by lab workers and a smaller percentage of the environmental conditions will diffuse when accessed.

Due to its low microscope functionality score the second design ranked second. Placing a sheet of acrylic under the culture plate on the stage removed it from the focusing range of the microscope. The smaller and completely enclosed incubation chamber would have been easier to humidify and CO_2 sensing would have given a more accurate representation of the entire chamber. Furthermore, the stage wouldn't have been incorporated and would have acted as a minimal heat sink due to the low thermo conductivity of acrylic [12]. Because of these characteristics the second design scored the highest of our three designs in Humidity, CO_2, and Heating.

The first design scored third overall and didn't excel in any categories. The housing didn't incorporate the entire stage so temperature gradients would have formed both through the stage and across its surface. Also, because the humidifying chamber was so far from the culture plates the entire housing would need to be humidified. Coupled with the gradients from the stage, condensation would likely form on the cover of the culture plate, walls of the housing, and condenser lens.

Final Design

For our final design we chose Design #3. It scored the highest average in our three highest weighted categories (16.3) and the highest overall. The design is estimated to cost roughly $450, exceeding the $100-200 budget, but will use a 100% CO_2 tank to reduce costs long term. The final design is appropriately cost efficient compared to current models on the market. Moving forward we will work to model and fabricate the components of Design #3.

Future Work

First, the current final design must be modeled to collect data and precisely quantify characteristics such as: 1) Flow rate of gasses needed to maintain constant humidity; 2) mass, length, and current required for the Nichrome wire to heat the chamber; 3) placement of the heating element to reduce temperature gradients; 4) delivery of heat to humidify the basin. Additionally, the microcontroller will need to be programmed to be capable of performing functions including but not limited to: operating the CO_2 sensor, regulating the mixing chamber for gasses, and displaying real-time data. Furthermore, the exact placement, design, and size of gas input valves, fans and diffusion channels will also need to be determined. After all of these

components have been modeled, materials will need to be ordered through our client Dr. Puccinelli and fabricating a prototype may begin.

Acknowledgments

Our team would like to acknowledge Dr. Randolph Ashton, our advisor, Dr. John Puccinelli, our client, and lastly Dr. Amit Nimunkar for their help on this design.

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Appendix

Product Design Specifications

Project Title: Cell Culture Incubation Housing

Team Members: Ian Linsmeier, Tyler Klann, Becca Stoebe, Paul Strand

Date: 9/14/11

Function: To develop a cell culture incubation chamber with interchangeable culture plates that is compatible with the Olympus IX71microscope. This incubation chamber must be able to maintain an internal environment of 37^{o}C, 5% CO_2 and 95-100% over long durations for time course experiments, without compromising the integrity of the microscopes optics or functionality.

Client Requirements

- Internal Environment: 37^{o}C, 5% CO_2, 95-100% humidity
- Little to no temperature gradient
- Easy to assemble and remove
- Housing cannot interfere with optics (transparent)
- Housing doesn't impede microscope function
- Compatible with different cell culture plates
- Cannot allow evaporation

- Condensation cannot interfere with optics

Design Requirements:

1.) Physical and Operational Characteristics

a. ***Performance Requirements*:** The incubation chamber must be able to maintain 37^{o}C, 5% CO_2, 95-100% humidity for hours or days for time course experiments, without the formation of a temperature gradient; these environmental conditions cannot damage the optics of the microscope. When the housing is attached, the microscope must remain fully functional. Also, the housing must prevent condensation build-up on a wide variety of cell culture plates as well as the microscopes optics.

b. ***Safety:*** The incubation chamber will contain a warming device that will heat the cultures to 37^{o}C, but a malfunction in the heating regulation system could lead to much higher temperatures that could damage the cells, microscope, or even the lab technician. Our microscope housing may be used to image pathogenic strains of cells.

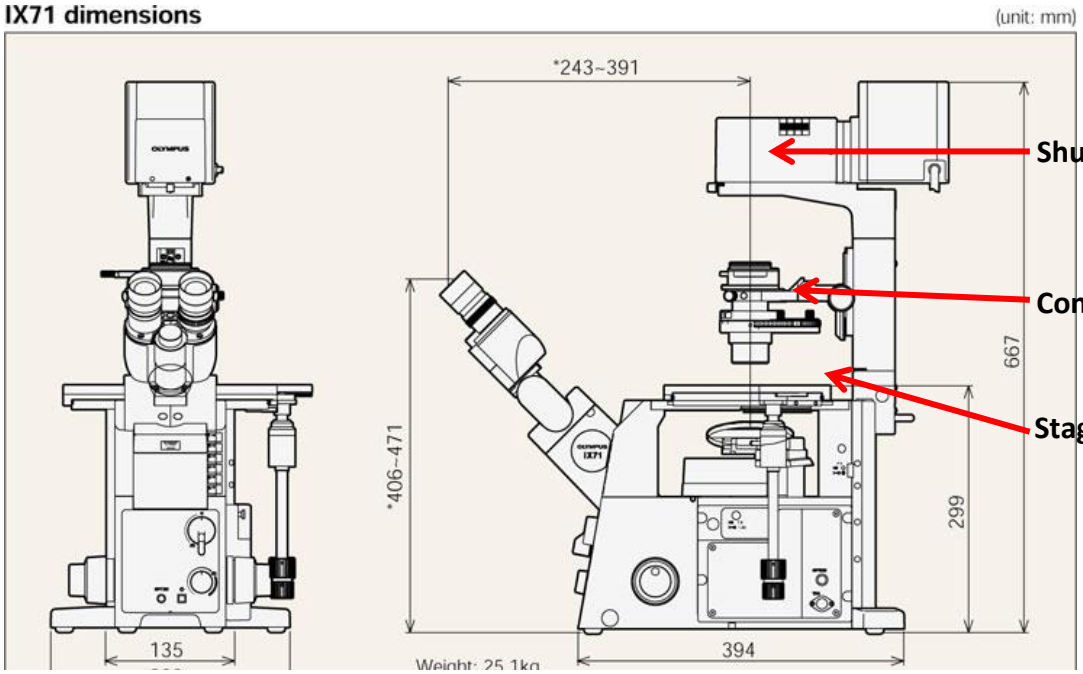
c. ***Accuracy and Reliability:*** The cell culture housing must maintain an internal temperature of 37±1^{o}C, a CO_2 concentration of 5±.5%, and humidity between 95-100%. The housing should allow for accurate and reproducible measurements of the microscope that are close or identical to the accuracy and reliability achieved without the housing.

d. ***Life in Service:*** The device must be able to be used for up to 1 week while maintaining the environmental constraints previously discussed. The microscope housing should be reusable for different cell culture experiments.

e. ***Operating Environment:*** This device will be used by a skilled lab technician and should only experience the environmental conditions previously discussed as well as the environment of the lab, which will be well controlled.

f. ***Ergonomics:*** The microscope housing must be easy to assemble, disassemble and store.

g. ***Size:***



IX71 Inverted Microscope overall dimensions http://www.olympusamerica.com/seg_section/product.asp?product=1023&p=72

Stage

- Fixed Bottom Plate: 240mm(X) x 232mm(Y)
- Middle Plate: 260mm(X) x 180mm(Y), Translates 25mm forward and 25mm backwards from the center point of the stage
- Top Plate: 260mm(X) x 200mm(Y)
    - This excludes the irregularity that juts out at the upper right corner of the stage; this extension measures 42mm(X) x 85mm(Y)
    - Also has a 110mm diameter hole in the center for the stage insert plate
    - Translates to the left 25mm and to the right 25mm from the center of the stage

Working Space

- The distance from the bottom of the shutter to the top of the stage is 232mm
- The condenser extends out 210mm from the back of the neck of the microscope; it is 107.5mm tall and 110mm wide (measuring from the front)
- Assuming the effective working space is the area of the top plate of the stage all the way up to the bottom of the shutter, then the dimensions of this area are 260mm(X) x 200mm(Y) x 232mm(Z).
    - These measurements exclude the volume of the condenser as it will most likely be contained within our housing.

h. ***Weight:*** Excluding external humidifiers, heat sources or CO_2 tanks, our device should be light enough to carry.

i. ***Materials:*** The materials used must be biologically compatible, transparent, water resistant and non-corrosive. The materials should also be able to withstand the expected temperatures range.

2.) Production Characteristics

a. ***Quantity:*** 1

b. ***Target Product Cost:*** Between $100-$200

3.) Miscellaneous

a. ***Customer:*** Our client wants a cost-effective design and would prefer that our product cost about $100 dollars, but we are allowed to spend up to $200; however, if we came up with a superior design that is outside of this price range, he would consider funding that design. Our client would also prefer if we designed a system that is compatible with non-mixed (100%) CO_2 tanks. The design must be compatible with different cell culture plate shapes and sizes. One of our main goals our client had for this project is to prevent condensation from interfering with microscope readings. Ideally, our microscope housing should be compatible with an automatic stage.

b. ***Competition:*** There are many other designs similar to ours on the market. The following is a list of website links that correspond to another companies competing product:

- http://spectraservices.com/Merchant2/merchant.mvc?Screen=PROD&Product_Code=THSS&Category_Code=EC
- http://www.autom8.com/brain_slice_bsc1.html
- http://www.20-20tech.com/inc-2000.html
- http://www.olympusconfocal.com/resources/specimenchambers.html
- http://biosciencetools.com/catalog/Closed.htm