Cell Culture Incubation Chamber

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12/14/2011

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Abstract

Dr. John Puccinelli would like to perform live-imaging of cell cultures during time course experiments that can range from 6 hours to a week. To achieve this, the cell cultures must be maintained at the correct environmental conditions throughout the experiment and cellular imaging. Our team aims to develop a housing chamber compatible with the IX71 Olympus microscope that is able to sustain an internal environment of 37°C, 5% CO_2, and between 90-100% humidity, without interfering with the imaging capability of the microscope. The system will consist of a coiled nichrome wire heating element, a bubbler humidifier and an external CO2 and air mixing chamber that are all regulated through a feedback system. Additionally, the microscope housing will be made of acrylic and it will surround the entire stage of the microscope to prevent the formation of temperature gradients. Also, the gas mixing-humidification system will interface directly with the smaller incubation chamber, which will be contained within this larger housing. Our team has made extensive calculations for the operation of these systems and has begun modeling their dynamic interactions using CAD and finite element analysis.

Problem Statement

Our client, Dr. John Puccinelli needs a cell culture incubation chamber that is compatible with the Olympus IX71 inverted microscope to analyze various cell cultures using live cell

imaging. 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

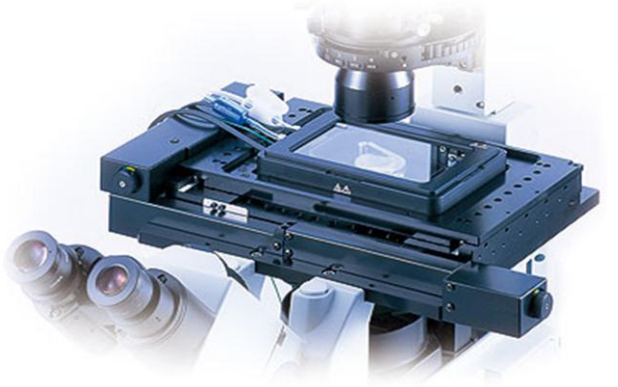


Figure 1: Tokai Hit WELS Chamber [2]

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. The first type of design is a small incubation 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 **figure 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 **figure 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. Premixed 5% CO_2 air is pumped into a bubbling chamber to humidify the interior. 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 that the stage is included in the heating environment, meaning it should equilibrate to the same temperature as the rest of the chamber. At the same time, since the box containing the culture is much larger, the

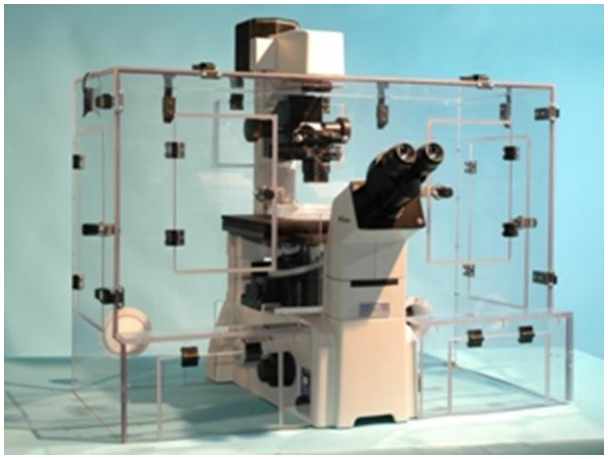


Figure 2: Okolab Microscope Cage Incubator [3]

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.

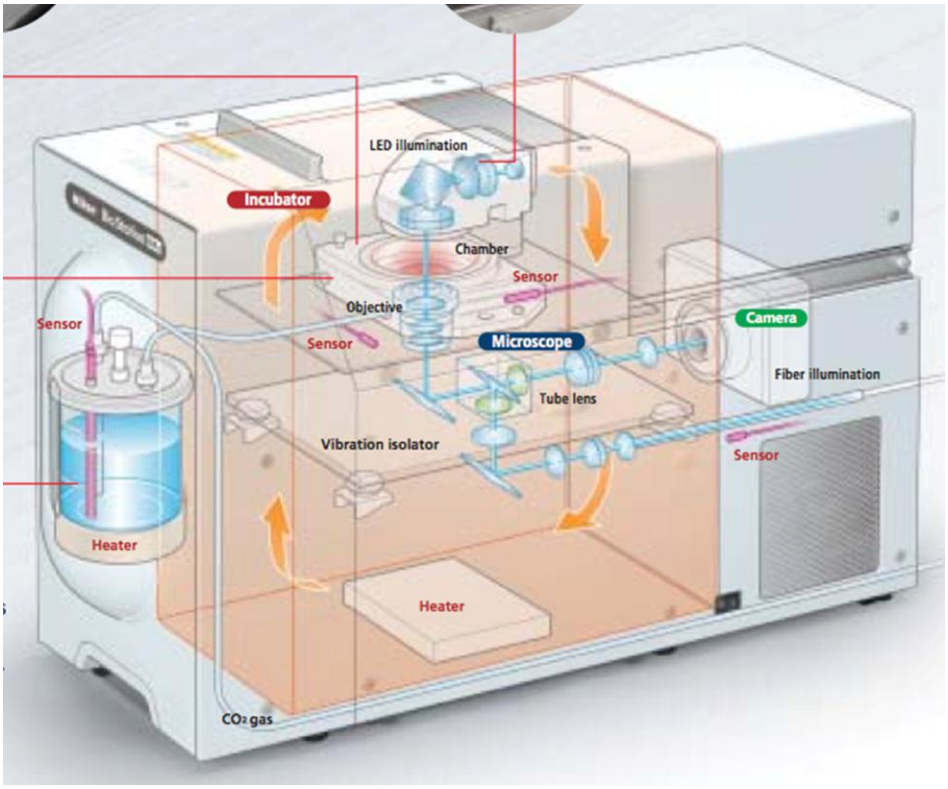


Figure3: Nikon BioStation IMQ Cell Incubator and Microscope[4]

The final design type is the cell incubator with a built in microscope. An example of this design type is shown in **figure 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 the purchase of the whole system instead of just the incubator.

Design Specifications

The microscope housing must be designed specifically for the Olympus IX71 inverted microscope, and it should be able to achieve and maintain the following internal conditions: $37\pm2^{o}C$, 5±1% CO_2, 90-100% humidity. The entire system must be easy to assemble and disassemble and it cannot impede the microscope's functionality or optical path once it is set up. Also, the incubation chamber should have the ability to accommodate various cell culture plates. The system should perform accurately enough to prevent temperature gradients from forming across the stage as well as throughout the rest of the internal environment. Additionally, the system cannot cause evaporation of cell culture liquids or the build-up of condensation on viewing surfaces. All the materials used for the microscope housing and incubation chamber must be biologically compatible and the entire system should cost about $200.

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 preliminary 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 [5] [6] [7].

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, the sponge material would make the water lost during the humidifying process very 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.

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 diffuses, bringing evaporated 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.

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* **(Figure. 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 **(Figure 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.

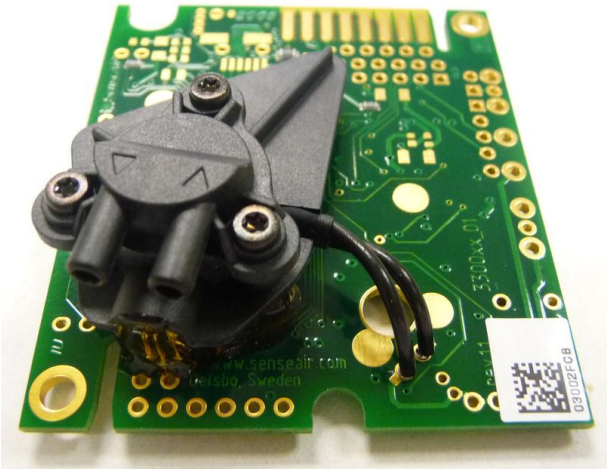


Figure 5: Senseair K-33 BLG 30% CO2/Temp/RH Data Logging Sensor [9]

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 (indium tin oxide) 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 **(Figure 6)** 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 $5.50 for 30 ft [12]. Therefore, nichrome seems to be the best alternative for this design.

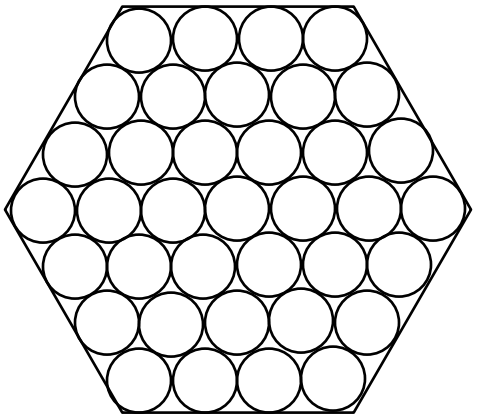


Figure 6. Nichrome heating coil cross-section.

Housing

All of the environmental conditions in the incubation chamber were considered when choosing the material to construct the housing. The material needs to be transparent, water resistant and resistant to shape deformation at 37° C. Patent searches show two transparent materials are most commonly

used to construct the incubator housing: acrylic (plexiglas) and polycarbonate (lexan/makroclear) [13].

Both of the materials fulfill the design requirements for transparency but acrylic is more susceptible to yellowing after prolonged exposure to sun or spectrum light, which presents a possible longevity issue [14]. Polycarbonate is also slightly more transparent (96% versus 93%) but the difference is negligible.

Polycarbonate has 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 are not extreme in the incubation chamber, it does not make economic sense to pay for polycarbonate's greater resistance to deformation at high temperatures. Also, polycarbonate has been found to leech bisphenol A, a biologically hazardous material, when heated repeatedly [14].

System Process

To keep the incubation chamber at the correct environmental conditions, a feedback system will be used to adjust the CO_2 concentrations, humidity, and temperature. The K-33 BLG CO_2 sensor monitors the CO_2 concentration in the inner incubation box and sends a value to the mBed microcontroller. The mBed compares the readings to ideal readings and adjusts the voltage at the gate of the MOSFET to either increase or decrease the current flowing through the solenoid valves. The valves are normally closed valves, so increasing the current will increase the amount the valves open to allow airflow. To keep airflow constant through the system, the

valves must be adjusted so their net airflow is unchanged. So, if the CO_2 valve is opened more, the wall air valve must be closed the same amount, and vice versa.

A similar approach is used for the temperature and humidity control. The SH15 temperature and humidity sensor, inside the incubation box, will send a value to the mBed microcontroller. The mBed compares the value to a predetermined ideal value and then adjusts the voltage at the gate of the MOSFET connected to the outer heating element and the humidifier heating element. An increase in current flowing through either heating element will increase the heat output. Since the humidity is dependent on the heat delivered to the humidifier water bath (assuming airflow is constant), the current flowing through the humidifier heating element will be adjusted to change humidity in the incubation chamber. There will be some delay between when the sensors detect a change and the resulting changes in the system, so testing will be needed to determine the delay time. Continuous monitoring of the incubation chamber will ensure acceptable conditions are met to allow cell cultures to grow **(Figure 7)**.

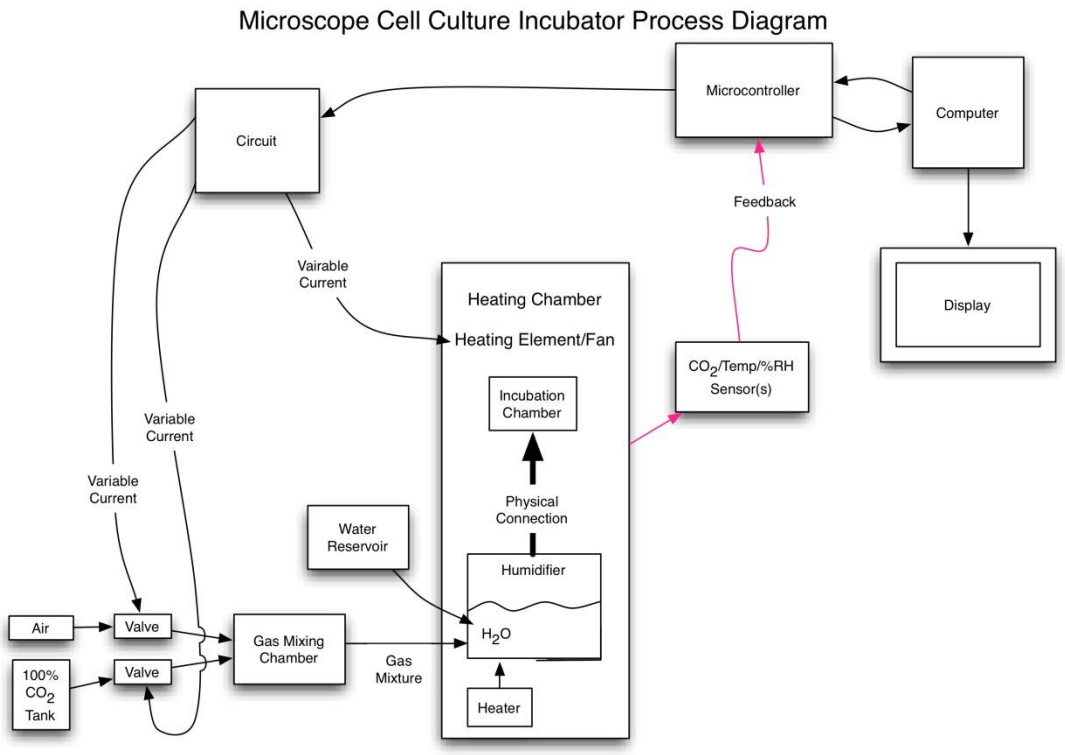
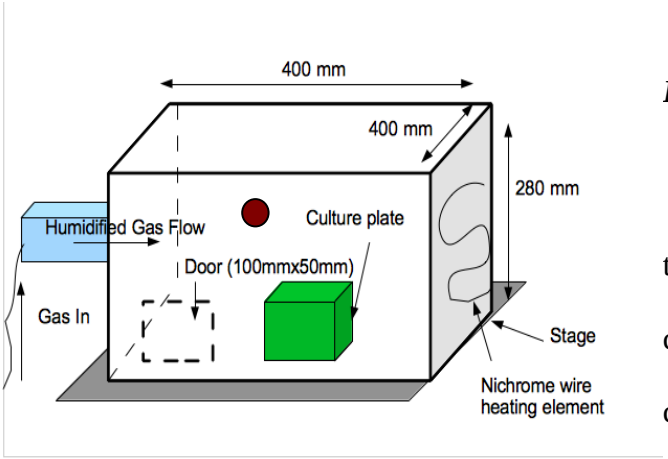


Figure 7: A process diagram of the incubator system. Components with arrows pointing towards them are receiving inputs and outward facing arrows represent outputs from that module.

Design Alternatives



Design 1:

This design features a large incubator that rests on the stage top **(Figure 8).** It is composed of a large plexiglass chamber that is closed to the outside atmosphere. The chamber

Figure (8): Design 1: Weighted stage-top incubator

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 (approximately 100 mL) 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. Here it is represented as only being on one side. There is also a separate but similar current heating the humidifier. There is a one way valve located on the right side of the incubator 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 (small red dot) that detects carbon dioxide levels, temperature, and relative humidity. The slides are pictured here in green and 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 **(Figure 9).** 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.

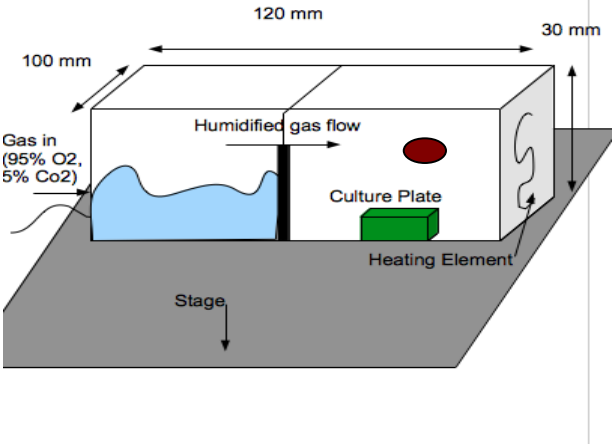


Figure 9: Design 2: Stage-top portable incubator

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 (pictured by a red dot) 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 is 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 function very well. Also, the size of this model limits access to the slides and the humidifier during the course of the observation

Design 3:

This design features a large incubator that surrounds the stage top and sandwiches around the frame of the microscope **(Figure 10).** It is composed of a large plexiglass chamber that is closed to the outside atmosphere. The culture plates are located in their own small, separate chamber located directly over the hole in the center of the stage (the dimensions of all portions of this design are identified in later sections). The humidifier output feeds directly into this chamber which covers the slides. A roughly 2 mm extrusion in the incubation chamber will rest in the hole in the stage. The addition of this chamber helps to prevent humidity and carbon dioxide gradients near the cell culture plate as well as prevent condensation on the surface of the larger chamber. 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 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

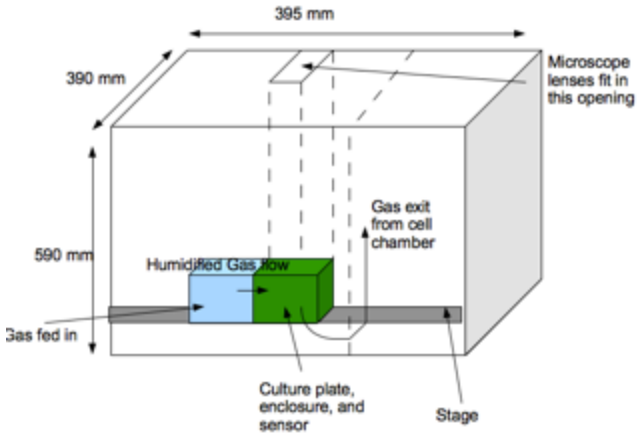


Figure 10: Design 3: Stage-encompassing incubator. Note that a better representation of each portion of this figure can be found in the modeling section.

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. An access door on a side panel of the chamber will also be fabricated. The door will be large enough to easily allow lab workers to access and manipulate the incubation chamber as well as exchange culture plates. The access door will be sealed with the silicone gasket and clipped shut, similarly to the top of the incubation chamber. 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 crucial impact on the functionality of the prototype. CO_2 was considered pertinent because it is of primary importance to the culture environment. Additionally, 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.

The next highest weighted elements were cost and ease of use. Cost was specified as a major factor by our client but, he also told us that his budget was slightly flexible for this prototype. Ease of use was also given a middle rank because, while it is necessary to be able to access the culture plates, this function is not crucial to the functioning of the prototype.

Humidity was given the lowest rank because of the large range in the allowable humidity level as specified by the client. However, it is still important to maintain high humidity levels because humidity is crucial to the functions of the cells.

The third design scored the highest, due primarily to its high score in microscope functionality. By encompassing the entire stage, the microscope will have full range of focus and function. Additionally, enclosing the aluminum stage in the incubator housing will help to prevent temperature gradients. The larger chamber size will also allow for easier access by lab workers. However, the incorporation of a smaller, partially enclosed incubation chamber within the larger chamber ensures that a smaller percentage of the environmental conditions will diffuse out when the incubator is accessed.

Due to its low microscope compatibility, the second design ranked second. Placing a sheet of acrylic under the culture plate on the stage removed the culture plate from the focusing range of the microscope. The smaller and completely enclosed incubation chamber would have been easier to humidify and in this case CO_2 sensing would have given a more accurate representation of the entire chamber. Furthermore, the stage would not have been incorporated and would have acted as a minimal heat sink due to the low thermo conductivity of acrylic [13]. 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 did not excel in any categories. The housing did not incorporate the entire stage so the prototype would have been more susceptible to temperature gradients. 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 the final design, design #3 was chosen. It scored the highest average in our three highest weighted categories (16.3) and the highest overall. This design features the use of the nichrome wire heating element, the use of acrylic material for housing, a bubble respiratory humidifier, a gas mixer, a chip-based sensor for monitoring, and an integrated circuitry system. The design is estimated to cost roughly $700, exceeding the $100-200 budget, but will use a 100% CO_2 tank instead of a pre-mixed air and CO_2 tank to reduce costs long term. The final design is appropriately cost efficient compared to current models on the market [2][3][4].

Modeling

Each of the design components that will ultimately be used in the final prototype was designed and modeled individually. These models show how each component will interact with an entire system. The following is a summary of the modeling and design conducted for the housing and incubation apparatus, the mixing chamber, the humidifier, the heating system, and

the circuitry. Each section presents the research or models, as well as the final design and/or a process flow.

Heat Housing and Incubation Chamber

The outer heating chamber is made of acrylic measuring 59 cm. tall on the stage control side, 57.5 cm. tall on the opposite side, 39 cm. deep and 39.5 cm. wide. The housing has a bisecting cut down the center (highlighted in aqua on **figure 11**) that is sealed on each side with a silicone gasket material. To place the chamber around the microscope the two halves are separated and then rejoined, sandwiching around the base of the microscope. The bisecting cut is held together with clips placed along perimeter of the chamber. All parts of the chamber in direct contact with the microscope (highlighted in red in **figure 11**) will be covered in a silicone gasket material. The red highlighted areas are dimensioned to fit snuggly along the frame of the microscope. The combination of pressure from the clips and sealant properties of the silicone gasket will minimize leaks and exterior atmospheric intrusion. All other plexiglass seals not highlighted in **figure 11** will be made using Weld- On 4, an acrylic cement typically used in fish tanks. The base of the chamber does not interfere with the optical path of the microscope and has cut-out with a gasket sleeve through which the stage control arm can translate.

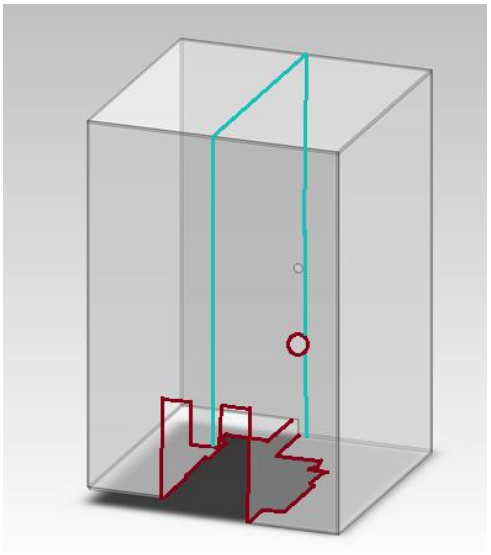


Figure 11: Outer Heating Chamber

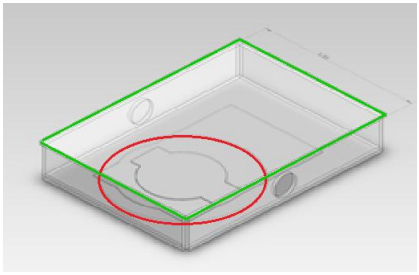


Figure 12: Incubation Chamber

Lying on the stage inside the heating chamber is a smaller incubation chamber. It measures 13 cm. wide, 18 cm. deep and 3 cm. tall which is large enough a variety of cell culture plates and small

enough to minimize the volume of the atmospheric conditions required. The entire chamber is made of acrylic and the base has an extrusion 2 mm. deep (highlighted red in **Figure 12**) that sits down in the opening of stage. This extrusion allows the plates to sit on a layer of acrylic and seal the chamber but also does not remove them from the range of focus of the optics. Additionally, the base layer of the incubation chamber outside of the extruded cylinder will be fabricated to be only .5 mm. thick . Two separate extrusions into the base plate of .2 mm. will be made with surface dimensions representing 5 typical cell culture plates. The top of the incubation chamber (highlighted as green in **Figure 12**) is removable and has a strip of silicone gasket around the bottom of its edge. Clips will hold the top plate on and seal the environment when culture plates are not being accessed.

CO_2 Mixing Chamber Design and Calculations

The final system design incorporates an ante-chamber to the entire system that allows gases that are input to the system to diffuse together before they enter the humidification chamber and the incubation chamber. The incorporation of this mixer into our design has various advantages. First, allowing the gases to directly diffuse together before incorporation into the humidifier, grants greater control over injection pressures of the individual gases; that is, if needed, the addition of an antechamber permits the addition of another valve that can ensure that the pressure of the gases that are entering the humidifier is of a desirable level. Similarly, the addition of this chamber creates another location where conditions can be monitored to ensure the integrity of the system. Therefore, with the addition of this chamber it is possible, if desired, to monitor the CO_2 content of our input gas mixture even before it reaches the cells.

It was decided that the flow rate through the system should be approximately 0.5 moles per minute. This rate was selected for various reasons. First, in using this flow rate, in contrast

to a very low flow rate, the cell culture readily receives an ample supply of fresh air and CO_2. This rate actually exceeds the normal rate of alveolar ventilation in the human body (which is approximately 0.1 molar flow) [15] however, the greater flow rate ensures that the cells are receiving an ample air supply regardless of their metabolic activity. Additionally, this flow rate ensures that CO_2 is not used overly rapidly, which would increase the cost of maintaining the system. Also, this rate, coupled with the design of the system, ensures that a need for more or less CO_2 in the incubation chamber causes only a 1-2% change (**Figure 13**) in the flow rate to correct the change. The reasoning mentioned here is also furthered in the following paragraphs.

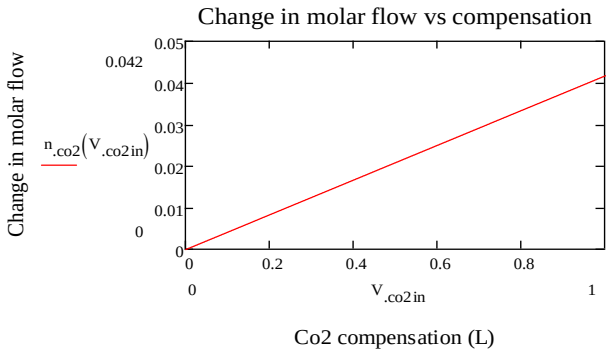


Figure 13: Change in volume versus molar flow rate through the system

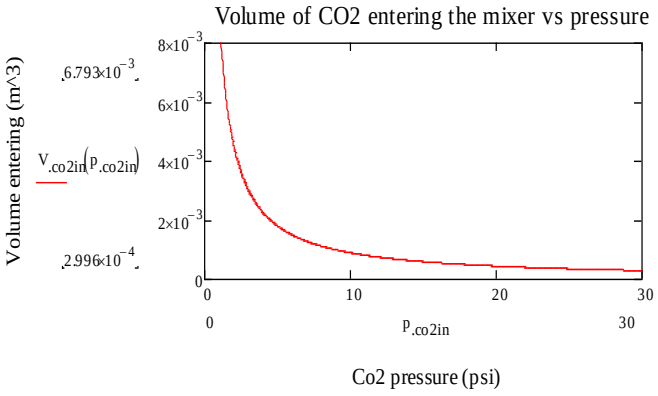


Figure 14: Injection pressure versus volume injected into the mixer for 5% CO_2 injected

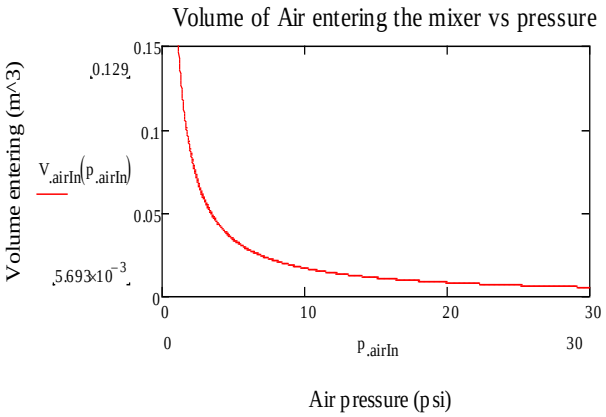


Figure 15: Injection pressure versus volume injected into the mixer for 95% air injected

For the design of the mixing chamber various factors needed to be taken into account. First, the gases need to enter individually at a rate such that the outgoing mixture is composed of 95% ambient air and 5% CO_2. Second, these rates are dependent upon the pressures at which the gases are being injected into the mixer (**figures 14 and 15**). Also, commercial valves have rate limits for how great of a volume of gas can be released for a certain injection pressure.

Using the ideal gas law, it was calculated that, at 15 psi, approximately 0.6 L CO2 and 11.4 L air would need to be injected into the system per minute as shown in **eq. set 1**: (where n_{co2in}=0.025 mole, n_{airIn}=0.475, T_{in} =25°C):

$$V_{co2in}(p_{co2in}) := \frac{n_{co2in} \cdot R \cdot T_{in}}{p_{co2in} \cdot psi} \qquad V_{airIn}(p_{airIn}) := \frac{(n_{airIn} \cdot R \cdot T_{in})}{p_{airIn} \cdot psi}$$

$$V_{co2in}(15) = 0.599L \qquad V_{airIn}(15) = 11.386L$$

Equation Set 1:

From this data, the Parker pneumatic control valve miniature solenoid valve was selected because of its ability to release 0-30 L/min at psi from 0-50 [16]. From this it was calculated that the gas exiting

the chamber would feed into the humidifier at a pressure of approximately 103.4 kPa (a pressure slightly above atmospheric) as shown in **eq. set 2** (where n_{out}=0.5 mole, T_{out}=25^{o} C):

$$p_{out}(V_{out}) := \frac{n_{out} \cdot R \cdot T_{out}}{V_{out}} \qquad p_{out}(V_{co2in}(15) + V_{airIn}(15)) = 1.034 \times 10^{5}\,Pa$$

Equation set 2:

Based on the flow rate data, the mixing chamber was designed to be a hollow cubical box with sides of 25 cm. This box is composed of acrylic and has the same sealant as the incubation chamber: Weld- On 4. The mixing chamber also contains an inlet for CO_2, an inlet for air, and an outlet for the gas mixture to diffuse into the humidifier. The mixing chamber is located outside of the large incubator encompassing the stage. All inlets and outlets are 1 cm diameter in this design but this is subject to change based on the piping used. In this device, the calculated amount of air and CO_2 is injected into the box and, because of the volume of the box and the placement of the outlet, diffuse together before flowing into the humidifier **(Figure 16).**

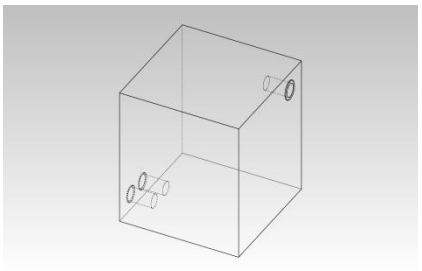


Figure 16: Model of mixing chamber design.

Humidifier Design and Calculations

In accordance with the client specifications, the humidifier for this system needs to be designed in order to maintain the incubation chamber at a relative humidity of 90-100%. For reasons discussed above, the chamber was selected to be designed of a "bubbling humidifier" style in which gases are humidified by being bubbled through a water bath before diffusing into the incubation chamber.

Using Roult's Law [17], the partial pressure of water vapor in 95% humidity was calculated to be 0.059 atm. This means that the molar fraction of water in the gas mixture that ultimately enters the incubation chamber must be 0.059. Using the half molar flow rate values calculated for the mixing chamber, approximately .75 L of water vapor must be exiting the humidifier per minute (**eq. set 3**) (where P_{out}=1 atm and T_{out} = 37° C) :

$$V_{out}(n) := \frac{n \cdot mol \cdot R \cdot T_{out}}{P_{out}}$$

$V_{co2out}(n) := V_{out}(n) \cdot y_{co2out}$ $\quad V_{co2out}(0.5) = 0.599L$

$V_{airout}(n) := V_{out}(n) \cdot y_{airout}$ $\quad V_{airout}(0.5) = 11.377L$

$V_{h20out}(n) := V_{out}(n) \cdot y_{h20out}$ $\quad V_{h20out}(0.5) = 0.749L$

$p_{H20} = 0.059$ atm

$y_{h20out} := p_{H20}$

$y_{co2out} := 0.05(1 - p_{H20})$

$y_{airout} := 0.95(1 - p_{H20})$

Equation Set 3:

(For other molar flow rates, see **figure 17**). This represents approximately 0.0254 moles of water. Using specific enthalpy tables [18] the amount of power that must be delivered to the system in order to evaporate this much water is 39.019 watts. This yields an evaporation of about 0.5 mL of liquid water per minute (**eq. set 4**):

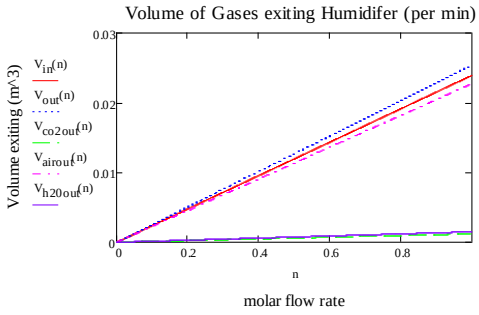


Figure 17: Exiting volumes from the humidifier of various gases

$\Delta H_{vap} := 39.68 10^3 \frac{J}{mol}$ $\quad H_D(n_{h20}) := \frac{\Delta H_{vap} \cdot n_{h20} \cdot mol}{60s}$ $\quad H_D(0.029) = 19.179W$

Equation Set 4:

If a drop or rise in CO_2 levels causes a need for increased or decreased CO_2 delivery, CO_2 delivery can be altered in the mixer which slightly alters the molar flow rate in the system.

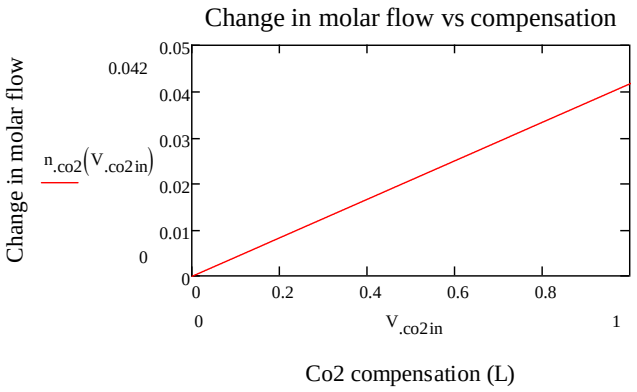


Figure 18: Change in molar flow (for CO2) versus liters of CO2 needed to be compensated for

However, because of the small size of the incubation chamber, even a 2% drop in CO_2 concentration (ie from 5% to 7%) causes a need for a change in molar flow rate by only about 0.0058 moles per min. This is because, a difference in 2% CO_2 corresponds to a change of 0.014 L of CO_2 in the smaller chamber at any given point. Therefore, the input of CO_2 must be altered by this rate, which corresponds to a difference of 0.0058 moles per minute (**eq. set 5**) (**Figure 18**):

$$n_{co2}(V_{co2in}) := \frac{p_{co2in} \cdot psi \cdot V_{co2in} \cdot L}{R \cdot T_{in}} \qquad n_{co2}(0.014) = 5.841 \times 10^{-4}\,mol$$

Equation Set 5:

If this does occur, the circuitry of the system is designed to alter current so that relative humidity is not altered as a result. For example, if CO_2 levels fall to 3%, increasing the current by 0.234 W changes the amount of water evaporated by enough of an amount that humidity is not altered (**eq set 6**):

$$V_{h20out}(0.5) = 0.749L \qquad n_{h20out}(0.758L - 0.749L) = 3.536 \times 10^{-4}\,mol \qquad H_D\left(3.536 \times 10^{-4}\right) = 0.234W$$

$$V_{h20out}(0.5058) = 0.758L$$

Equation Set 6:

If a fall in humidity levels in the incubation chamber causes a need for more humidified air, the current to the water bath can be temporarily altered to increase the rate at which the water is evaporated. The calculations for this are nearly identical to those for the change in humidity to compensate for CO_2 changes and show that changing the humidity in the incubation chamber by

even 5% changes the power needed to be delivered by only about 1 watt (p_{h20} was calculated using Roult's law and is not shown. Additionally, V_{h20out} represents the change in volume needed to compensate for a 5% drop in humidity with a 0.5 molar gas flow rate) **eq. set 7**:

$$p_{h20} = 3.099\times 10^{-3} \qquad V_{h20out}(n) := V_{out}(n)\cdot p_{h20} \qquad n_{h20out}(0.039L) = 1.532\times 10^{-3}\,mol$$

$$V_{h20out}(0.5) = 0.039L \qquad H_D\left(1.532\times 10^{-3}\right) = 1.013W$$

Equation Set 7:

Additionally, the gas flow and heating should be returned to normal values after any change has been compensated for in order to ensure integrity of the system and also to ensure that a need for changes beyond the capability of the system do not arise.

Based on the above data, the humidifier was designed to be a 5x5x6 cm hollow box with a circular inlet for fresh gas, a circular inlet for recycled air, a circular inlet for replacement of water, and a circular outlet for diffusion of humidified gases into the incubation chamber. Inside of this hollow box there is a small basin (4.5x4.5x2 cm) that is designed to hold approximately 25 mL of water. The exterior box is made of plexiglass and is sealed with the same sealant as the outer chamber: Weld- On 4. The inlets are all currently designed to be 1 cm in diameter but can easily be redesigned to fit different piping sizes. The interior water basin is made of aluminum coated with ceramic to allow heat transfer. Nichrome wires coated with ceramic are used as a heat delivery method and are situated between the bottom of the inner

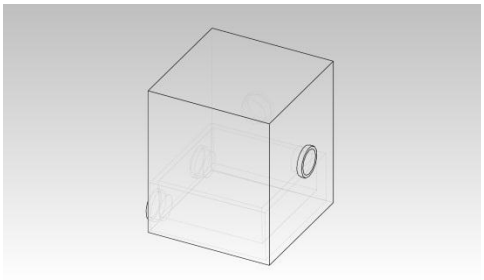


Figure 19: Humidifier design

water basin and the base of the humidification chamber. Upon entering the humidifier from the mixer, the gas is injected directly into the water bath. The gas (after fully saturating the water bath) diffuses out of the water along with evaporated steam. This gas mixture then exits through the outlet valve into the incubation chamber (**Figure 19**).

The design of this humidification system for the incubation chamber has several advantages. The use of humidification of the gases (instead of direct injection of evaporated water) helps avoid the creation of temperature gradients due to the fact that the gases are heated before entering the incubation chamber. Additionally, the use of this system creates consistency in the pressures of elements entering the incubation chamber: if the three elements were injected separately, there could be greater variation in pressure due to the increased or decreased need for the individual gases and thus a greater inconsistency of input. The utilization of a bubbler humidifier ensures that the input will be more consistent and thus not impede the integrity of the system.

Heating Element Design

The following are the design parameters for the heating element; however, many of the chosen values were arbitrarily selected to provide a good basis for calculations and modeling. The heating element will be 9.144 m (30ft.) of nichrome 60 wire (60% nickel, 16% chromium, 24% iron) coiled into a doughnut shape or annular ring with an outer diameter of 8 cm. The wire itself will be 30 American Wire Gauge (AWG), which means it has a diameter of .000254 m (.01 in), and has an electrical resistivity of 22.1457 Ω/m (6.750 Ω/ft.). To achieve the geometry of the annular ring, the wires will be coiled so that their cross-section resembles a regular hexagonal

array, which is shown in **Figure 6** (pg. 12); each side of the hexagon will be four lengths of the wires diameter to achieve the most symmetrical geometry of the heating element. In total, there will be 37 turns of the wire through this cross section, which will achieve the aforementioned geometry and incorporate the entire length of the wire. Using this geometry, the approximate inner diameter of the annular ring will be 7.648 cm. This coil of nichrome will be heated to 37^{o}C and positioned in front of a fan that will help distribute the heated air around the heating chamber at a rate of 4.00 m/s.

The heating element will be coated with a thin ceramic that has a high thermal conductivity, but a very low electrical conductivity to ensure that the current passes along the entire length of the wire for maximal heat generation. Some possible ceramics that could be used are AlN, SiC, Al_2O_3, Si_3N_4, ZrO_2. The specific ceramic used should be chosen by the future design team based on the ease of application of the ceramic and the cost of this material [19].

The humidifier will using the same heating element design to create humidified air from the water bath; however, instead of a fan, the ceramic coated nichrome coil will be positioned underneath an aluminum heat sink to distribute the heat across the entire bottom of the humidification chamber.

Calculations

Although the nichrome heating element is designed as a coil, all of the subsequent calculations and modeling assume a straight nichrome wire for simplicity. The nichrome wire calculation constraints and well as other pertinent values are listed in **Table 2**.

Nichrome Wire Calculation Values	
Wire Length (L)	9.144 m
Wire Diameter & Characteristic Diameter (*D*)	0.000254 m
Temperature of Wire Surface (T_s)	37°C
Ambient Temperature (T_a)	25°C
Fan Flow Rate (*v*)	4.00 m/s
Nichrome emissivity (ε)	0.72
Stefan-Boltzmann constant (σ)	5.67e-08 $W/m^2 \cdot K^4$

Table 2. This table lists the geometric and thermal properties of the nichrome wire used in the calculations. Additionally, the fan flow rate and the Stefan-Boltzmann constant are listed.

Also, the film temperature (T_f), which is the average between the temperature of the wire surface and the temperature of the ambient air, is 31°C for the above thermal constraints; the film temperature equation is shown in **Equation Set 8.** The properties of air can then be determined at this film temperature and are listed in **Table 3**.

Air Properties at Film Temperature of 31°C	
Prandtl number (N_{Pr})	0.712
Conductivity (k)	0.0264 W/m
Density (ρ)	1.1644 kg/m^3
Viscosity (μ)	16.04e-06 m^2/s

Table 3. This table lists the properties of air that are dependent upon the film temperature of 31°C.

To determine the total amount of heat transferred from the nichrome wire to the surrounding air, the coefficient of convective (forced) heat transfer as well as the coefficient of radiative heat transfer must first be calculated. For this heating element design, the fan forces the fluid to flow perpendicularly to the cylindrical nichrome wire as well as completely immerse the solid body in flowing fluid; therefore, the forced convection heat transfer coefficient can be found using **Equation Set 8**; where N_{Nu} is the dimensionless Nusselt number, N_{Re} is the

dimensionless Reynolds number, h_{conv} is the coefficient of convective heat transfer (W/m^2·K), and the coefficients *C* and *m* in the Nusselt equation are determined by the magnitude of the Reynolds numbers; *C* = 0.683 and *m* = 0.446 for this calculation. Substituting the appropriate values into **Equation Set 8** and solving for the coefficient of forced convection yields h_{conv} = 470.336 (W/m^2·K).

$$T_f = \frac{T_s+T_a}{2} \qquad N_{Nu} = CN_{Re}^m N_{Pr}^{1/3} = \frac{h_{conv}D}{k} \qquad N_{Re,L} = \frac{Dv\rho}{\mu}$$

Equation Set 8:

Next, the coefficient of radiative heat transfer can be calculated using **Equation Set 9**. Applying the aforementioned conditions to this equation yields h_{rad} = 4.5895 (W/m^2·K^4).

$$h_{rad} = \frac{\varepsilon\sigma\,(T_s^4 - T_a^4)}{(T_s - T_a)}$$

Equation Set 9:

With the two heat transfer coefficients calculated, the overall heat transfer to the surroundings can be calculated using **Equation set 10.** Plugging in the calculated values and solving for the total heat transfer yields q_{total} = 13.2366 Watts [20].

$$q_{total} = q_{conv} + q_{rad} = (h_{conv} + h_{rad})A(T_w - T_a) = h_{total}\pi DL\Delta T$$

Equation Set 10:

To calculate the current required to heat the nichrome wire to the desired temperature of 37^{o}C, a numerical relationship was established between these two variables. This was

accomplished by graphing tabulated temperature and current values for a 30 AWG straight nichrome wire; the data is displayed in **Table 4** and the resulting graph is shown in **Figure 20**.

Nichrome Wire Properties 30 AWG			
Current (A)	Temperature (°C)	Resistance Increase (%)	Resistance (Ω/m)
N/A	20	0.0	22.145
N/A	93	1.7	22.522
0.92	205	3.5	22.920
1.19	315	5.2	23.297
1.47	427	6.9	23.673
1.78	538	9.2	24.183
2.14	649	9.2	24.183
2.52	760	9.8	24.315
2.9	871	10.2	24.404
3.3	982	10.5	24.470
3.7	1093	N/A	N/A

Table 4. 30 AWG nichrome wire technical data for current, temperature and percent increase in resistance [21].

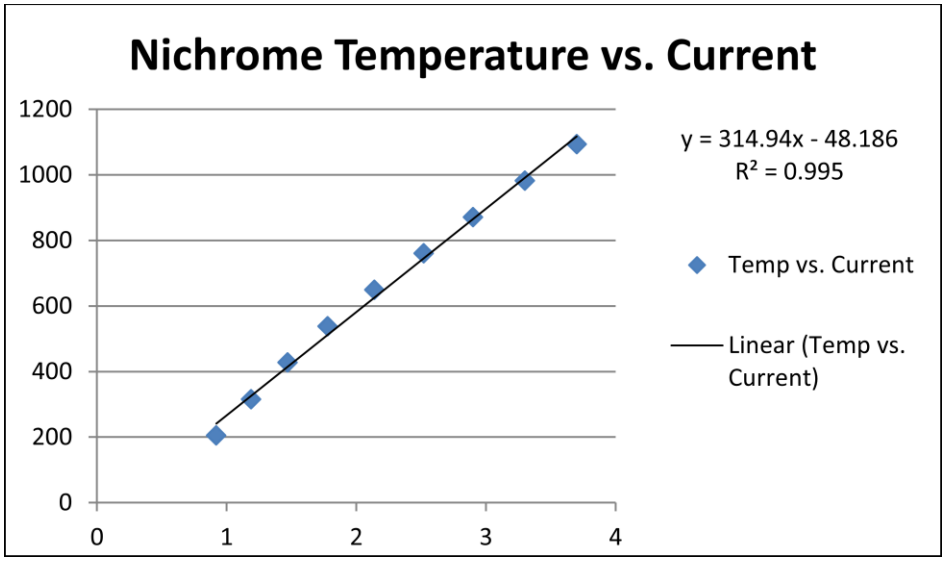


Figure 20. This graph depicts the linear relationship between the temperature of nichrome wire and the supplied current.

Adding a trend line to the graph in **Figure 20** shows that there is a fairly linear relationship between the current supplied to nichrome wire and its resulting temperature; this is

supported by an R^2 value of .995. The resulting trend line equation was found to be T=314.94*I–48.186, where T is the temperature in degrees Celsius and I is the current supplied to the wire in amps. By plugging in 37^{o}C into the left side of the equation and solving for I, it can be found that it takes about 270 mA of current to heat the nichrome wire to 37^{o}C. The total power supplied to the nichrome wire can be found using the equation $P=I^2R$. Plugging in the appropriate values for current and resistance yields a power rating equal to 14.76 Watts. Comparing this value to the 13.2366 Watts of transferred heat means that most of the supplied power is being transferred to the surroundings via forced convection and radiation.

Lastly, the total amount of energy required to heat the microscope chamber to 37^{o}C as well as the time to reach this temperature were calculated. First an approximate volume of air was calculated using the overall dimensions of the microscope housing: .39 x .395 x .595 m^3 = .09166 m^3. Assuming the initial ambient air temperature within the housing to be 25^{o}C, then air properties are the following: density = 1.1839 kg/m^3 and specific heat (c_p) = 1.005 kJ/kg·K. **Equation Set 11** can then be used to calculate that the total energy input required to reach 37^{o}C is Q=1.3087 kJ, and with a heat transfer rate of q = 13.2366 W, this can be accomplished in 98.9 seconds [20].

$$Q = c_p m \Delta T \qquad m = Density \times Volume \qquad time = Q/q$$

Equation Set 11:

Modeling Results

The previous calculations were then transferred into FloWorks, a SolidWorks fluid modeling add-on, to determine the most effective method of heating element the microscope housing. First, an approximate CAD model of the microscope housing surrounding the Olympus IX71 microscope was created for the thermal analyses; this CAD design is shown in **Figure 21**.

Also, included in the model was a spherical heating element approximation with an equivalent surface area to the straight nichrome wire for more accurate meshing of the model. In addition, a fan was simulated in the program to pass air over this heating element at the same flow velocity used in the calculations.

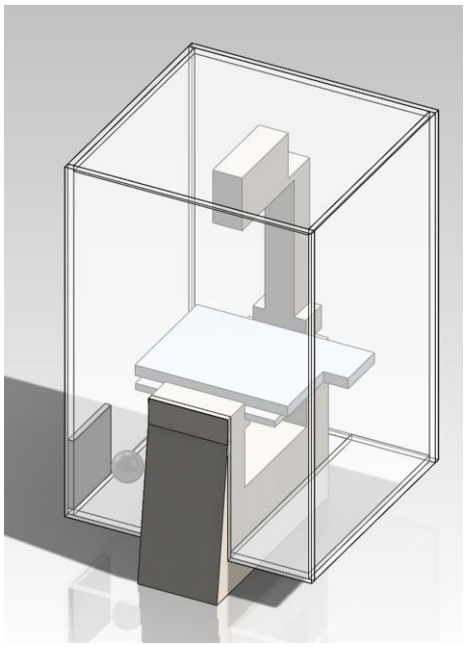


Figure 21. CAD model used for thermal fluid modeling.

The microscope housing was modeled as acrylic; it was assumed that there was a negligible amount of heat loss through this material due to its low thermal conductivity and was therefore modeled as a perfect insulator. The microscope itself was modeled as aluminum with a high thermal conductivity, whereas the heating element approximation was modeled as nichrome

The heating element was constrained to a certain surface temperature and was assigned accurate thermal radiation properties. Due to the placement of the heating element next to the fan, there was no need to assign forced convection properties because the model would automatically account for this. The remaining surfaces were all assigned initial temperature values of 25^{o}C; consequently, the program would calculate each surface's coefficient of natural convection, which is dependent on the instantaneous temperature of the surrounding fluid medium.

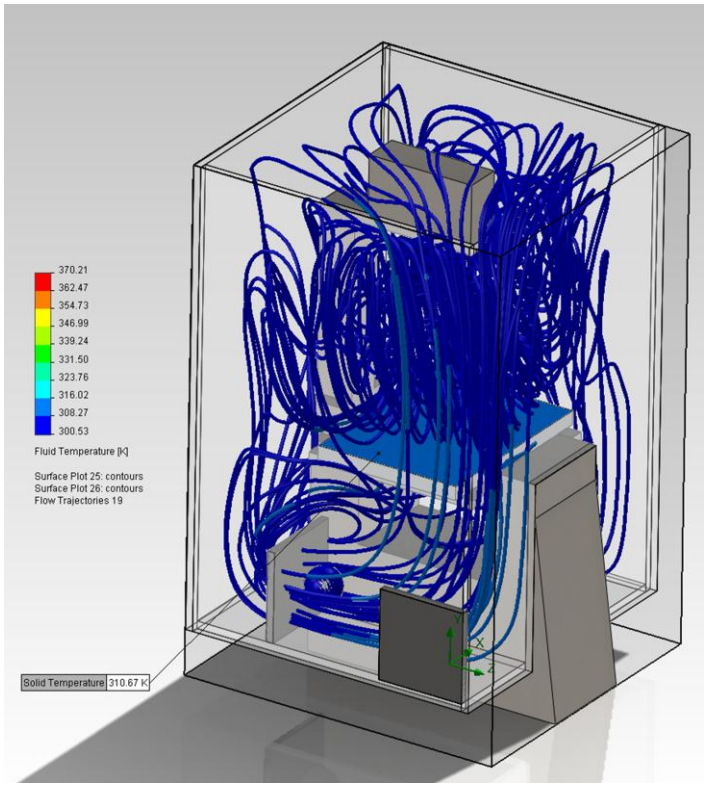


Figure 22. Thermal fluid modeling results with a stage surface temperature of 37°C.

The overall goal of the thermal fluid simulation was to hold the microscope stage surface temperature at 37°C without any temperature gradients. This fluid simulation was optimized to meet these criteria, and after numerous iterations of the simulation, this goal was finally achieved; a picture depicting the resulting fluid and surface temperature plots is shown in **Figure 22**. Unfortunately, these results are far from ideal for a few reasons. It is evident from the fluid plot in **Figure 22** that temperature gradients still exist in the surrounding fluid as well as between the fluid-solid interfaces. However the major flaw of this result is that the heating element had to be constrained to a surface temperature of 527°C, which is far beyond the reasonable temperature ranges that a biologically sensitive environment is willing to tolerate. Using the trend line equation from **Figure 20** and solving for the necessary current to achieve this temperature, yields a result of over 1 amp; this is more current than the circuit can provide and therefore this result is impossible to achieve. Due to the unattainable modeling results, further fluid simulation optimization or physical testing will be necessary to obtain a result that would feasible to implement into the final design.

Circuit Design

To monitor the environment and control airflow and temperature, a microcontroller capable of multiple inputs and outputs is nessessary. Also, sensors which monitor CO_2

concentrations, temperature, and relative humidity are required. For the microcontroller, the mBed NXP LPC 1768 [22] was selected based on it's relative ease to code and multiple inputs and outputs. For CO_2 concetration monitoring, the K-33 CO_2Engine – BLG [23] sensor was selected for it's ability to monitor CO_2 conecntrations from 0-30% CO_2 and it's ability to communicate with the microcontroller digitally. The Sensirion SH15 [24] sensor was selected to monitor both temperature and relative humidity. This chip is small enough to be placed inside the incubation chamber directly and can communicate with the microcontroller through serial input and output. An external filter can be purchased to protect the sensor from the internal environment of the incubator [25]. The K-33 BLG will be placed outside of the inner incubation chamber to prevent the environmental conditions from damaging the chip. In order to sample the CO_2 concentration within the chamber, then, an external pump is needed to pull a sample through the sensor. A pump can either be designed or an existing pump can be purchased. The response time for the K-33 BLG sensor is less than 25 seconds for gas diffusion, which makes the data acquisition near real-time. The valves that control airflow into the system will also be controlled by the mBed microcontroller. Since both valves are solenoid valves, they are controlled by a current. Using an N-channel MOSFET, the mBed can control the current flowing through the valves, thus controlling how much the valves are open. Depending on the voltage at the gate of the MOSFET, the amount of current that flows through the transistor and the valve is varied: the correlation between gate voltage, drain current, and drain source voltage can be found on the data sheet for the 2N7000 datasheet [26]. To vary the voltage to the gate of the MOSFET, either the analog_out pin or the PWM (pulse width modulation) pins can be used on the mBed microcontroller. The heating element control consists of the same setup as the valve control. The current flowing through the heating element will be controlled by the same N-channel MOSFET,

allowing the microcontroller to adjust current and thus temperature as needed. A diagram of the entire circuit system can be seen in **Figure 23**

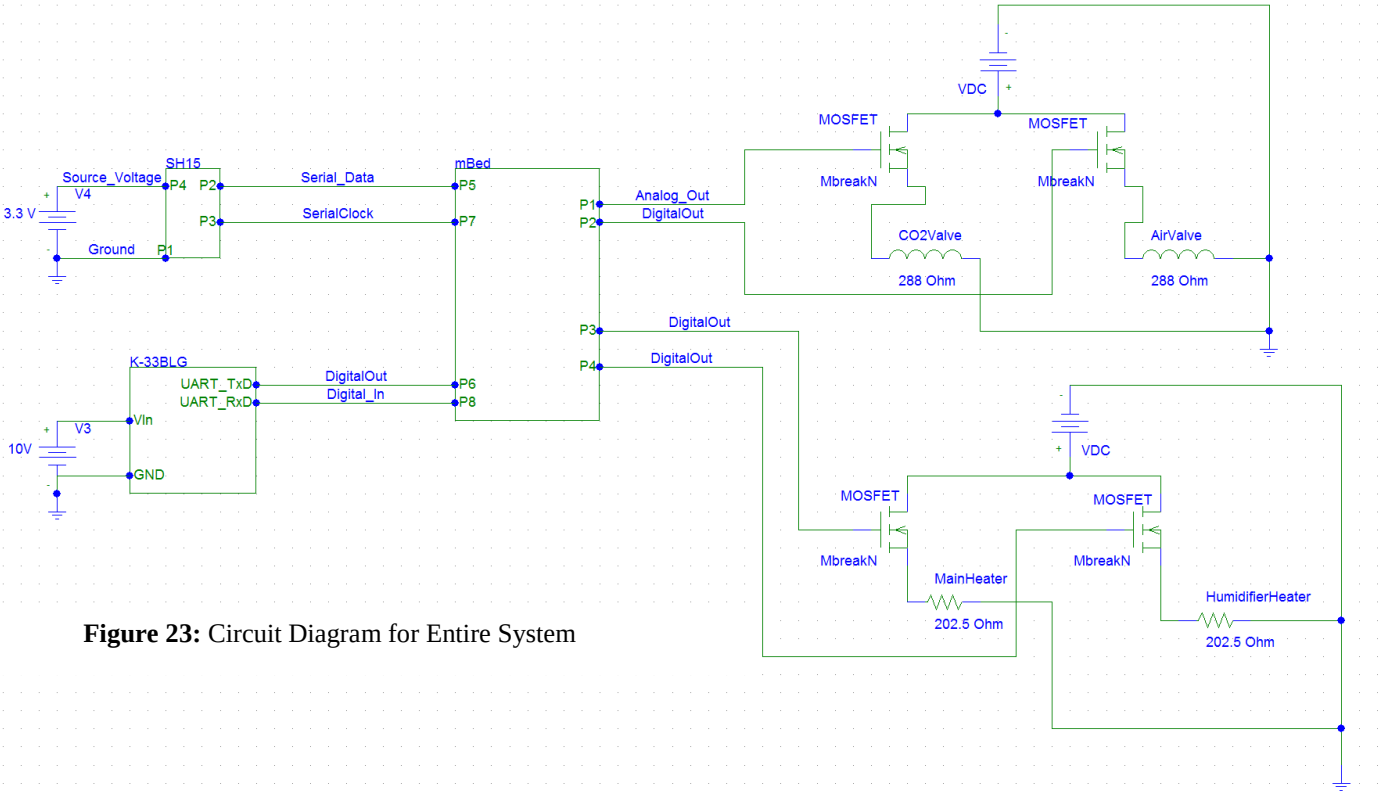


Figure 23: Circuit Diagram for Entire System

Full System Integration

Upon start-up of the system the internal environment will need to reach the aforementioned environmental conditions. The heating element will draw current from the circuit and heat up to 37^{o}C or higher and the fan will distribute this heat about the outer heating chamber. Simultaneously, the heating element for the water bath will heat up the surrounding water to create water vapor and thereby humidify the incubation chamber. Additionally, the air and CO_2 will mix in the correct ratio within the mixing chamber and bubble through the

humidifier causing the flow of both 5% CO_2 and between 90-100% humidified air to flow into the incubation chamber; the humidifier and incubation chamber are physically connected via a small port. To prevent the build-up of pressure within the incubation chamber there is an outlet that feeds into a recycling duct, which has a bleed-off opening that releases pressure into the outer heating chamber; the recycling duct is connected to the humidifier to allow a continuous flow of fluids throughout these chambers. The entire system will be regulated through a feedback control system that obtains input from the various sensors in our incubation chamber. The incubation chamber will contain a temperature sensor as well as another sensor that detects both the relative humidity and CO_2 concentration. Based on the readings of these devices the supplied current will be increased or decreased to the two heating elements as well as the valves controlling the influx of CO_2 and air. Increasing the current to the heating elements will increase their heat generation and decreasing the current will do the opposite; an increase in current to the external heating element will cause an increase in the temperature within the chamber, whereas an increase in current to the humidification element will cause an increase in the relative humidity. Depending on the valves used in the system, increasing the current may open or close the valves more; the valves proposed previously are initially closed and providing them with current opens them. Continuous regulation using these sensors will ensure that the internal environment remains within the acceptable ranges for all three conditions.

Estimated Cost

Item	Quantity	Estimated Cost
Acrylic	9 sq. ft.	$50
Aluminium	1 sq. ft.	$5
Gasket	1	$8
Bonding Cement	1	$7
Nichrome Wire	60 ft.	$11
Parker Solenoid Valves	2	$94
mBed Microcontroller	1	$60
2N7000 MOSFETs	10	$3
Sensirion SH15 (Temp/RH Sensor)	1	$30
Sensirion SF1 Filter Cap	1	$4.26
K-33 BLG (CO2 Sensor)	1	$250
Sample Draw Pump Kit	1	$170
Total Cost		**$692**

Table 5: Estimated cost of the product by component

Future Work

Currently, each aspect of the design exists as a separate model. These models need to be incorporated and modeled as a system to precisely quantify gas flow rates and heat flow throughout the entirety of the system. After the creation of this whole-system model, each

individual design will need to be modified to incorporate the results of the whole-design model. Additionally, the exact specifications for valves, tubing connecting elements, and fans must also be determined.

Aside from further modeling, there are various elements that were not designed for but should be included in future versions of this product. First, due to lack of time constraints, several minor elements still need to be designed. One of these elements is a simple reservoir that is programmed to drip 0.5 mL of water per minute into the water reservoir located in the humidifier. The other of these elements is a small door-like hinged piece of plexiglass located in the side of the larger external incubation chamber that allows for access to the cultures inside without the need to disassemble the entire apparatus. Second, the team expressed a desire to design a system that allows for recycling of the air that is delivered to the smaller incubation chamber. This system would take the output from the incubation chamber and deliver it back to the humidifier where it could be re-humidified if necessary and sent back to the incubation chamber. The design of a system such as this causes a greater variation in air flow through the system and thus requires greater monitoring and precision in circuitry. The inclusion of this recycling system in the final design will greatly decrease the need for constant CO_2 input (as much of the CO_2 will be recycled through the system) and thus decrease the cost of operation of the system.

Additionally, specific work is needed in the modeling of the heating system. In the future, there is a need to continue with the fluid modeling of the heat transfer from the heating element throughout the outer chamber. Also, it will be necessary to determine the optimal placement for this heating element as well as to determine the optimal heating temperature. If temperature

gradients form, it may become necessary to redesign the system using two heating elements, one of which could be placed within the incubation chamber. Due to the use of a fan, it will become important to decide if it is feasible to draw air from the environment at 25^{o}C or if recycled air from the outer heating chamber needs to be used to create a closed system. Additionally, there is a need to determine if another temperature sensor in the external heating chamber is necessary to accurately regulate the temperature within the area. The future work will also involve choosing the best ceramic coating for the nichrome heating element. Finally, calculations and modeling with preliminary tests should be confirmed before implementing the heating elements into the system.

Finally, after thorough modeling and refining of all systems, material will need to be ordered and building of a physical prototype may begin. After the physical prototype is built, physical tests will also need to be conducted to ensure that the models accurately reflect the actual processes throughout the system.

Acknowledgments

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

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Appendix

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 for the Olympus IX71microscope that is compatible with various culture plates. This incubation chamber must be able to maintain an internal environment of 37°C, 5% CO_2 and 95-100% for time course experiments ranging from 6 hours to a week without compromising the integrity of the microscopes optics or functionality.

Client Requirements

- Sustain an internal environment of 37±2°C, 5±1%, 90-100% humidity
- Able to accommodate cell culture plates of differing size
- Apparatus is easy to assemble and remove
- Housing cannot interfere with optics (transparent)
- Housing doesn't impede microscope function
- Internal environment cannot develop temperature gradients, condensation buildup or cause the evaporation of cell culture liquids
- Materials must be biologically compatible
- Budget is about $200

Design Requirements:

1.) Physical and Operational Characteristics

a. ***Performance Requirements*:** The incubation chamber must be able to maintain 37°C, 5% CO_2, 90-100% humidity for time course experiments lasting hours or days. When the housing is attached, the microscope must remain fully functional. Also, the housing must prevent the formation of temperature gradients across the stage, condensation build-up on viewing surfaces and the evaporation of culture fluids that are important to the viability of the cells.

b. ***Safety:*** The incubation chamber will contain a heating element that will heat the cultures to 37°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±2°C, a CO_2 concentration of 5±1%, and humidity between 90-100%.

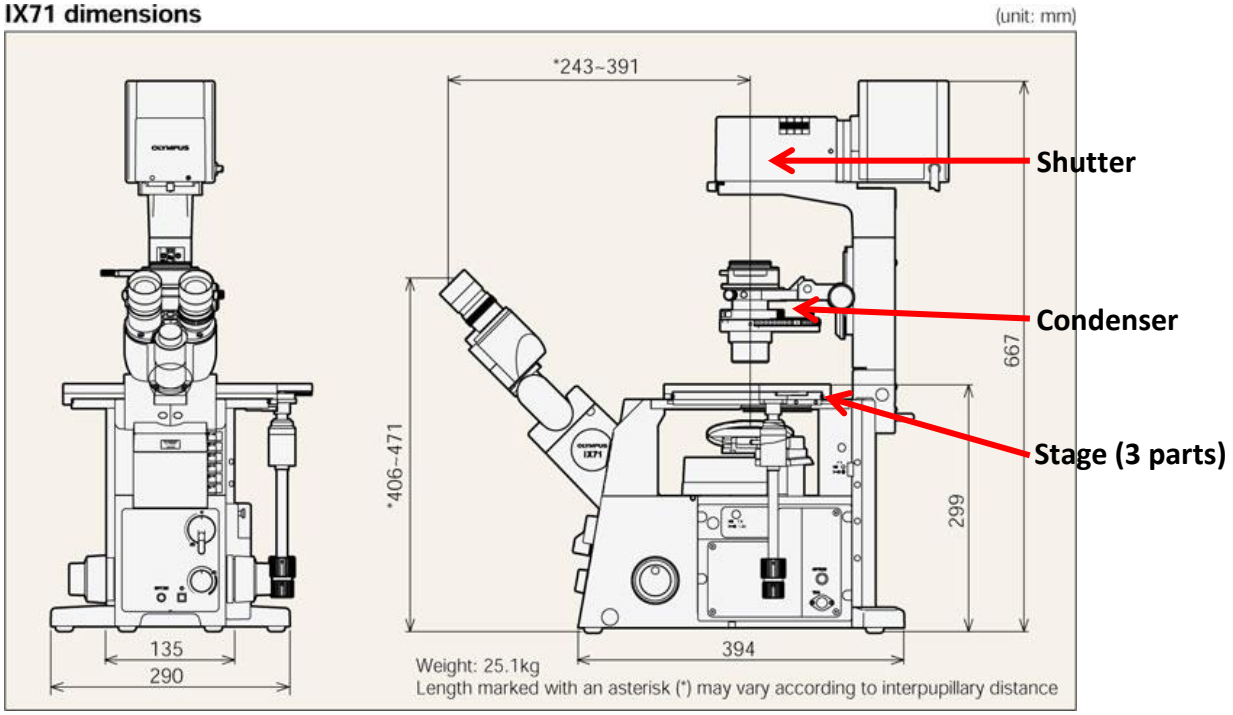
The housing should allow for accurate and reproducible measurements of the microscope that are close or identical to the accuracy and reliability achieved without the housing.

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. The technician must be able to access the cell cultures within the incubation chamber through a port in the outer housing.

g. ***Size:***



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

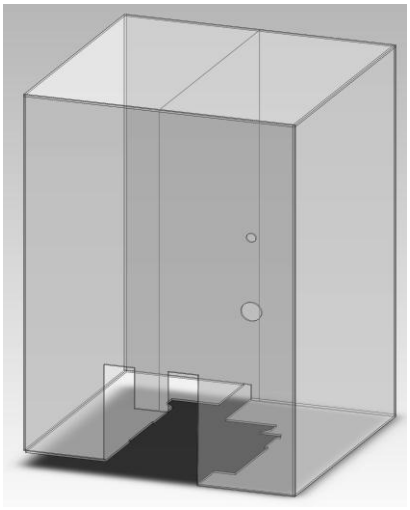
Stage

- 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

Housing

- The external housing chamber will encompass the stage as well as the entire neck of the microscope.
- The overall dimensions of the housing from the front view of the microscope are as follows: 39.5 cm wide, 59.5 cm tall and 39 cm deep.
  - The right side of the chamber (59.5 cm) is taller than the left side of the housing (57 cm) so it can accommodate moving parts of the microscope without obstructing their functionality.
  - The housing has a seam along the middle that splits it into two halves for easy assembly onto the microscope



Outer housing chamber compatible with the Olympus IX71 microscope

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:*** $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..

b. ***Competition:*** There are many other designs similar to ours on the market, but they are very expensive and most aren't compatible with the Olympus IX71.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