

## Project P16082: Microscope Environmental Chamber



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### **Abstract:**

The focus of this project is to design, construct, and test a self-contained chamber for use in microscope time-lapse imaging (ideally for up to four days). The final product will be capable of maintaining an environment suitable for cell survival on two specific microscopes, and will be used by Biomedical Engineering faculty to teach students concepts about time-dependent cellular functions. The device will provide several adjustable parameters, and will be interchangeable both in terms of which microscope it is mounted on and which culture vessel the specimen is contained within.

## **Introduction:**

Cell culture is a common occurrence in many aspects of the biomedical engineering discipline. One particular event which is of interest to observe is the multiplication and motility of mammalian cells. As cells grow to confluence in a given culture vessel, they will reproduce and move in certain patterns which can give opportunity for study and for education. However, standard culture involves the use of large, opaque incubators to allow growth of the cell lines. In this situation, the only option to capture motion over a period of time greater than around a half hour would be to continually come into a lab, remove the cells from the incubator and take an image on the microscope. This, however, requires a large degree of time investment (and some time points may be in the middle of the night), along with the need for high degrees of precision to find the same location each time the vessel is placed on the microscope. Also, while the cells are on the microscope, they are exposed to ambient conditions which are non-ideal for cell survivability and could also affect their biological responses. By utilizing a small chamber which can create an incubator-equivalent environment, the cells can remain on the microscope and be imaged without further time investment from the experimenter besides setup and teardown. This enables better capture of cell motility and growth phenomena, and grants more precise locational repeatability since the chamber can be left in place, focused on an area of interest.

## **Design Process**

The chamber is required to be a mobile, self-contained device that can be operable by 3<sup>rd</sup> year Biomedical Engineering students. In designing the chamber, dimensional constraints of 6.3" by 8.7" were established for the base to allow the device to be used on two different microscopes. In order to keep the device mobile, all the components that aid the chamber lie on the table on either side of the microscope when the chamber is in use.

In the process of coming up with the final concept in the design stages of this project, Schemes A-E were developed as shown in Figure 1. Schemes A and C were deemed unreasonable or impractical due to having more complicated setups for displaying data and unorthodox temperature controls. Scheme D appeared to be the simplest and most practical out of the remaining 3. This is the concept that the project was ultimately built off of. Some features like using an Aluminum and Plastic frame with see-through windows were taken from Scheme E rather than constructing a Clear Box which was originally proposed to due to simplicity.

Function	A	B	C	D	E
Control CO2	Input Pure CO2 into Void Space	Bubble Pure CO2 Through Liquid	Bubble Pure CO2 Through Liquid	Bubble Pure CO2 Through Liquid	Bubble Pure CO2 Through Liquid
Control Temp.	External Heating Coil & External Fan	Peltier Cell in Bottom	Peltier Cell in Bottom	Peltier Cell in Bottom	Peltier Cell in Bottom
Transmit Information	Low Voltage Wire & DAQ	USB Cable Interface	Low Voltage Wire & DAQ	Low Voltage Wire & DAQ	Low Voltage Wire & DAQ
Provide Source of H2O	Valve and Tube to House Water	Small Trough/ Pool in Chamber	Small Trough/ Pool in Chamber	Small Trough/ Pool in Chamber	Small Trough/ Pool in Chamber
Secure Chamber to Stage	Slots Around Microscope Base	External Clamps	External Clamps	External Clamps	External Clamps
Secure Sample in Chamber	Rest on Supporting Structure (rails)	Clamping	Slide into Fitted Hole	Clamping	Slide into Fitted Hole
Seal Chamber	Drawer that Seals When Pressed Shut	Gasket and Buckles	Gasket and Buckles	Direct Screw-down	Direct Screw-down
Display Set/ Control Points	Independent LCD Display (~3"x1")	Only Shown on Computer Screen	Only Shown on Computer Screen	Only Shown on Computer Screen	Only Shown on Computer Screen
Alarm Function	Popup on Computer Display	Email Update to Experimenter	Email Update to Experimenter	Email Update to Experimenter and have status lights on device	Email Update to Experimenter
Allow Light Infiltration	Redirected Microscope Light Source	Direct Microscope Light Source	Direct Microscope Light Source	Direct Microscope Light Source	Direct Microscope Light Source
Observe Cells	Non-Clear Box with Windows	Clear Box	Non-Clear Box with Windows	Clear Box	Aluminum & Plastic Frame with Windows
Access Cells	Drawer Style Design	Removable Top	Sides Open	Removable Top	Removable Top

Figure 1: Concept Selection

The device must replicate the environment of the human body, maintaining a temperature of 37°C and a CO<sub>2</sub> concentration 5%. A series of sensors working in conjunction with a LabVIEW program and a DAQ are used to maintain the internal environment of the chamber within these bounds, since the cells will only remain alive for 20 minutes when taken out of the incubation chamber. The delivered LabVIEW program must be user friendly so that students can modify sampling rates for control purposes and for logging. Lastly, the device needs to provide an unobstructed optical pathway for imaging via inverted microscopes. Every component in contact with water within the chamber must be completely sealed to prevent leaks, and the other components must be sealed reasonably well to contain the controlled atmosphere. The device will be used to conduct experiments autonomously for a period of up to four days, so the setup and teardown needs to be accomplished in under 3 hours to accommodate class schedules. Additionally, the chamber must be able to be cleaned with ethanol after use to sterilize its interior.

## Physical Chamber

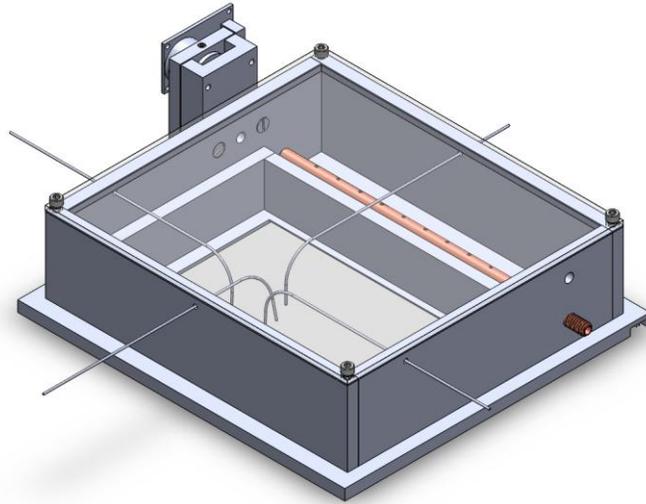


Figure 2: CAD Chamber Assembly

The chamber itself is designed with a large see-through portion for imaging on an inverted microscope. The baseplate of the chamber is approximately 7.5 inches wide by 7.0 inches deep. The outer walls of the chamber are approximately 6.9 inches wide by 6.4 inches deep. This creates a 1/2 inch lip around the entire perimeter of the chamber to be used for securing the chamber in place once on the microscope. All of the aluminum structural components are 0.25 inches thick. The majority of the chamber is made from individual 6061 Aluminum components machined to 0.010 inch tolerances. The upper window is made from plexiglass, while the lower window is made from borosilicate glass to limit scratching as it may be a contact surface. Openings in the chamber walls allow for the insertion of thermocouples to measure temperature and a vent to pass atmosphere over the CO<sub>2</sub> sensor. In the lower part of the chamber, a stainless steel pipe is inserted to percolate incoming atmosphere through a trough of water. The entirety of the device is assembled using either socket cap or pan head 6-32 screws with the exception of the plastic set screw used to secure the CO<sub>2</sub> sensor permanently in place.

### **Temperature Control**

The chamber was designed with 3 holes through which thermocouples are inserted. This allows temperature measurements to be taken at various locations within the chamber, cutting down on the impact of hot and cold zones within the chamber. On the bottom of the chamber, 4 peltier thermoelectric coolers are secured using a thermally conductive epoxy.

These four cells operate on 0-10V at 5.8A each. The peltier cells obtain their power from a power supply which delivers power to the cells that are wired in parallel through a 40A relay.

### **Carbon Dioxide Control**

Carbon dioxide concentration is measured as a ppm value and then extrapolated to a percentage value to compare to experimental requirements. The atmosphere leaving the chamber passes through hole in the right side of the chamber, through a desiccant chamber to remove excess humidity, and then over the carbon dioxide sensor.

To control the carbon dioxide concentration within the chamber, two solenoid valves are used. Both valves, one for compressed air and one for CO<sub>2</sub>, are controlled using 2A relays. Gas from the CO<sub>2</sub> cylinder is regulated down to 2.5 psi at the tank and delivered to the valve using flexible 1/8" I.D. tubing. Compressed air is taken from the source within the laboratory and run through an air filter to remove contaminants before being regulated by an in-line pressure regulator down to 2.5 psi also. Both sides of the gas assembly utilize check valves to eliminate possible backflow. The combined gas streams are then input into the chamber through another piece of flexible tubing connected directly to the stainless steel percolator, allowing the gas to bubble slowly through the water.

### **Software Design & Software-Hardware Interaction:**

Due to customer requirements and hardware being used, National Instruments LabVIEW was selected as the platform for software development. The initial plan was to use the inputs of a standard National Instruments USB DAQ-6215 (data acquisition unit) to take in the necessary sensor data (temperature and %CO<sub>2</sub>), and to use the DAQ's outputs to actuate solenoid valves and peltier cells. The inputs have no issue for these sensors, and there is actually inherent compatibility with K-type thermocouples which removes the need for any extra components beyond the thermocouples themselves. However, the DAQ outputs cannot produce the required current (despite having the properly rated voltage). Therefore, the DAQ outputs were connected instead to relays, allowing for actuation of a pathway connected to a power supply.

The code itself was split into three segments. The first segment sets up the parameters to control against - desired temperature and CO<sub>2</sub> percentage. This section also sets up some parameters automatically, including the graphs' y-scales and locating of the desktop directory pathway. The second segment is the primary section, operating to take in the sensor data and output control commands as needed. It includes comparators to evaluate if temperature is too low, and if %CO<sub>2</sub> is too low or high. Due to limitations in polarity of voltage across the peltier cells, along with the lower probability of experiencing too high a temperature (as the standard desired temperatures will be above ambient temperature), the functionality to actively cool the chamber was not included. This section also creates the arrays of data to actively show graphically, as well as store as a plain text spreadsheet file. The final section deals with more formal data output, generating an excel file and shutting down any remaining actuated output values.

The code was designed to be able to be user-friendly in operation and does not require knowledge of LabVIEW. However, it can be freely edited and adjusted as desired if certain programmed parameters are needed to be changed, or functionality changed/added/removed.

### **Results and Discussion**

The system has been tested and verified to be able to sustain temperature and %CO<sub>2</sub> for an indefinite period of time. The graphs below show the chamber being controlled over a period of approximately 4.5 hours. The temperature, due to its more precise control, stays fairly tight to the set point value. %CO<sub>2</sub> experiences more of a sinusoidal value due to the less precise method of control (gas pulses). The sharp jolts observed in Figure 4 are due to sensor movement while handling the chamber and components. However, both remain within the spec range.

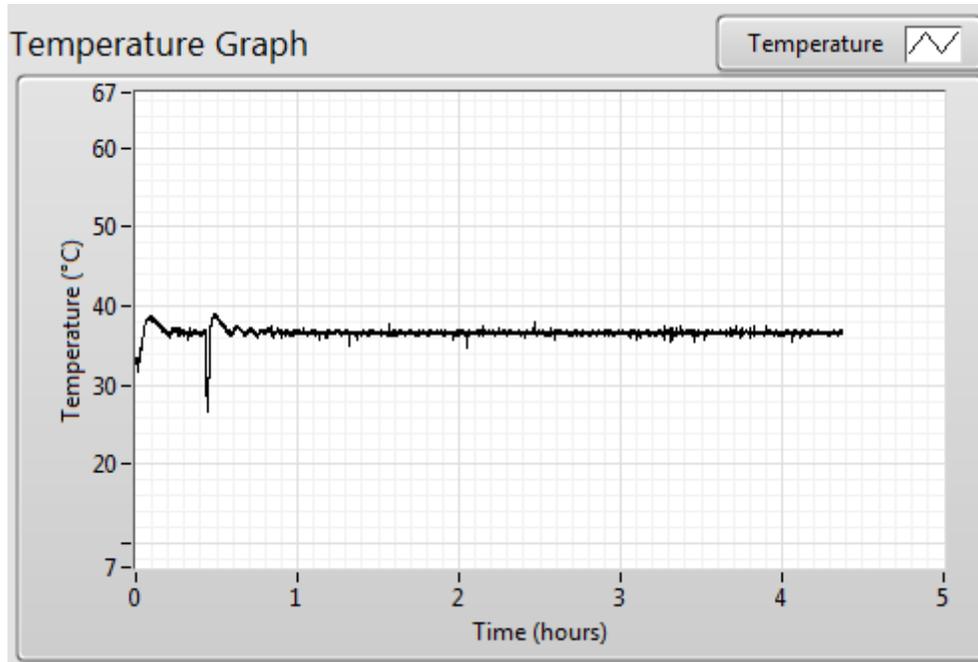


Figure 3: Temperature Results - ~4.5 hours

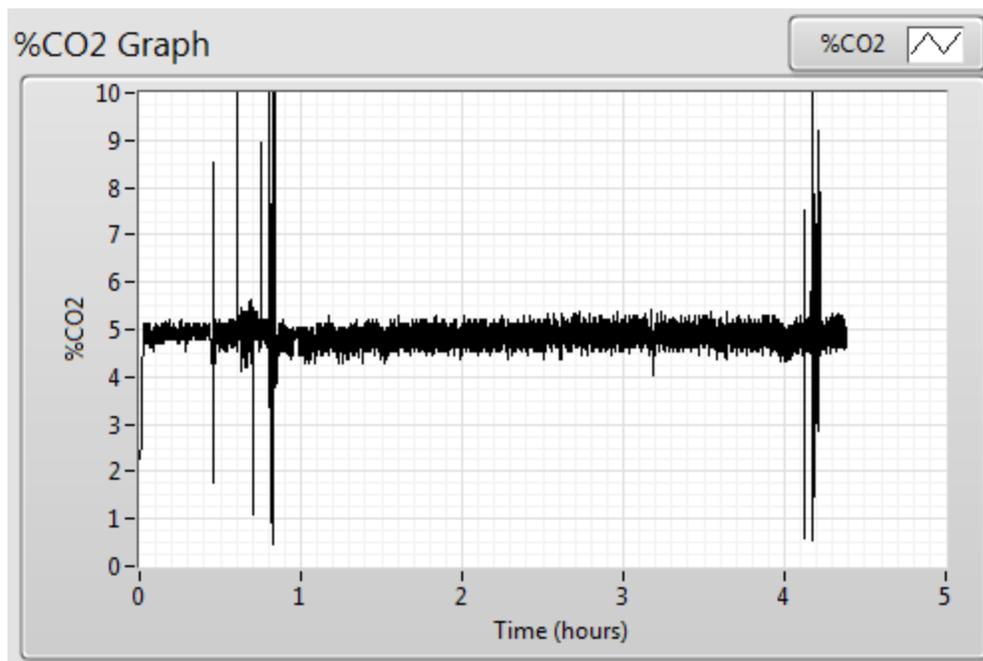


Figure 4: CO<sub>2</sub> Results - ~4.5 hours

While a full four-day test has not been run, the control should continue until user ending unless power disconnects or the CO<sub>2</sub> tank depletes, both unlikely. A test with live cells is pending.

## **Conclusion**

\_\_\_\_\_ This project was intended to assist with the BME departments teaching of class 470. The chamber would allow students to view cell motility over lengthier periods of time compared what was available prior to this project.

This project extensive use of coding languages including labview, the version of labview used was 2013, since drivers, for the 2014 version, were not present and the IT were not able to install the drivers in time for our use. It is recommended, since the only reason the code can't be run on 2014 is the drivers, that labview 2014 be updated with drivers to interface with the DAQ that the BME department uses.

## **Areas of Improvement**

The power used to run the heaters for the water is from a pc power supply. This power supply provides a high amperage direct current output for the Peltier cell to run off of. This method of power output allowed for different voltages to be supplied based on testing. It is recommended that a power supply that provides the optimal voltage to the peltier cells be acquired.

There are delays in the CO<sub>2</sub> signals being received by the sensors and the response time of the control valves. Both of these delays are due to the long path that the CO<sub>2</sub> needs to travel between the control valve and the CO<sub>2</sub> sensor. There is a 10ft line from the tank to the chamber which has to fill up with CO<sub>2</sub> prior to the chamber and a desiccant chamber immediately after the chamber which needs to process the humidity before the sensor. Each component increase resistance along the path, upon startup the device takes about 5 minutes to get any reading above 0%.

## **Acknowledgements:**

The team worked alongside their Gerald Garavuso who supported and questioned all the proposed ideas to help solidify a fully functional and dependable product. The team remained in contact with the customer, Professor Jennifer Bailey from the Biomedical Engineering Department to finalize functional requirements and monitor project progress.