Biological Imaging Chamber

Midterm Design Report

October 19, 2007

Team Members

Justin Lundell – Co Leader

Val Maharaj – Co Leader

Jeremy Schaefer – Communicator

Andrew Dias – BWIG

Michael Socie – BSAC

Client

Lance Rodenkirch

W. M. Keck Lab for Biological Imaging

Advisor

William L. Murphy

UW-Madison Biomedical Engineering Department

Table of Contents

Abstract

The goal of this project is to design an imaging chamber to be used with a high-powered inverted microscope in order to maintain a stable environment for long duration live cell imaging. These systems are available on the market but are expensive and would require a new microscope. The motivation for our project is to provide an economical alternative to purchasing a new microscope to be compatible with current retail imaging chambers. The design features a system for heating to correct mixture of gas to the required temperature before dispersing the gas evenly into the chamber.

Problem Statement

Construct a live-cell imaging chamber to be used for laser-based confocal and multiphoton imaging. The device needs to control temperature and gas environment inside the chamber and enable the use of perfusion.

Background Information

Live cell imaging

Live cell imaging is useful for understanding the role of proteins. Interactions between proteins must be examined when cells are alive; looking at fixed cells does not yield useful information about protein roles (PerkinElmer, Inc., 2007). Confocal laser scanning microscopy (CLSM) is a tool that is used to get good resolution while imaging cells without killing them (de Leeuw, 2007). Fluorescent staining can be used to tag specimens and view them in 3D. Software allows biologists to get a good understanding of protein interactions from looking at tagged specimens under a microscope. Limitations to this technique include a relatively slow acquisition speed and phototoxicity and photobleaching from the intensity of the laser (PerkinElmer, Inc., 2007).

Perfusion chambers

Perfusion chambers can be used to shield live cells from the external environment. An “open” chamber is similar to a Petri dish and does not have as much control over the environment as we would like. Closed chambers protect cells from evaporation of the medium and make it easier to maintain a constant pH and concentration of carbon dioxide (Dailey *et al.*, 2007). Having a stable environment is a primary concern in order to keep cells alive for imaging. Chambers also protect specimens from airflow and air currents that could move or damage them. Cells are very sensitive to shear forces (Dailey *et al*., 2007) so a closed chamber adds a layer of protection that would allow live cells to survive for a longer period of time.

Impact on design requirements

Users of our client’s microscope want to image cells over a long period of time. It is essential that our device does not damage the cells, so there are various constraints

on our design. Dailey *et al.* (2007) make suggestions for considerations needed by imaging chambers, including allowing penetration by a laser, maintenance of the specimen over time, minimal invasion, easy sterilization, sealed, and easy access to cells. We also decided to constrain our design so that the chamber can sit on the microscope stage and control carbon dioxide input. Plastics should also be avoided when building the chamber because it affects the laser beam – glass should be used instead (Dailey *et al.,* 2007).

Competition

There are several products currently in the field for live cell imaging chambers. However, these products may cost more than thousands of dollars, and may or may not be compatible with certain microscopes. We believe we can build a product that will meet our client's needs for a fraction of the cost.

One such product is the Focht Chamber System 3 (FCS3, 2007). The Focht Chamber System is a "live cell environmental chamber system for upright microscopes." The temperature of the cell can be controlled up to 50 ˚C with a plus or minus .2 ˚C range. The temperature is also constant across the entire chamber, meaning there is no temperature gradient where one side of the chamber may actually be a couple degrees cooler than the other side. This is ideal for imaging so constant results can be obtained. The chamber also allows for perfusion, or delivery of nutrients to the specimen, to keep the cells alive. And because this is a closed system, carbon dioxide can be used in the medium. Nonetheless, all these options come at a high price. The Focht Chamber System costs around $2600 (FCS3, 2007).

Figure 1. Incubator 2000 made by 20/20 Technology, Inc. (20/20 Technology, Inc., 2007).

Designed to avoid the disadvantages associated with large, plastic chambers surrounding entire microscopes, the Incubator 2000 is "a miniature chamber that sits on

the stage of any upright or inverted microscope." (20/20 Technology, Inc., 2007) The chamber is small and allows for control of humidity and temperature. Humidity is kept at almost 100% and temperature control is within 0.1 ˚C. Although stability is within 0.1 degrees, temperature accuracy is within 0.2 ˚C (Appl. Sci. Inst., 2007).

20/20 Technology, Inc. (2007) claims the chamber requires "miniscule amounts of a pre-mixed gas," but Applied Scientific Instrumentation says the gas purge rate of the Incubator 2000 is greater than 0.1 liters per minute.

The chamber feeds air saturated with water into the incubating chamber by first allowing gas to flow through a humidifying chamber as shown in *Fig. 2*.

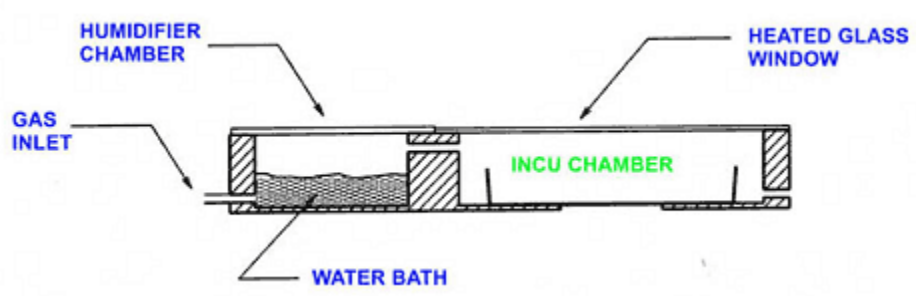

Figure 2. Air humidifier used in the Incubator 2000 (20/20 Technology, Inc.).

The water bath is kept at the same temperature as the incubating chamber, and the glass window above the chamber is kept at a slightly higher temperature to prevent fogging.

The chamber can also accommodate a wide variety of holders including different sized microscope slides and Petri dishes. The inside of the chamber has dimensions of 76 X 56 X 16 mm (20/20 Technology, Inc., 2007).

Figure 3. EMBL Live Cell Observation Chamber by CellBiology Trading (Kern, 2007).

The chamber developed by EMBL as seen in *Fig. 3* features humidity, temperature and CO_2 control, and it is an example of a chamber completely enclosing a microscope. The chamber has large doors, but in order to avoid disturbing the inside environment, many components of the chamber are automated. The chamber is accurate to ±0.5 ˚C and is precise to ±0.3 ˚C. CO_2 can be regulated from 0% up to 8% and humidity can be regulated from 0% to 100% (Kern, 2007).

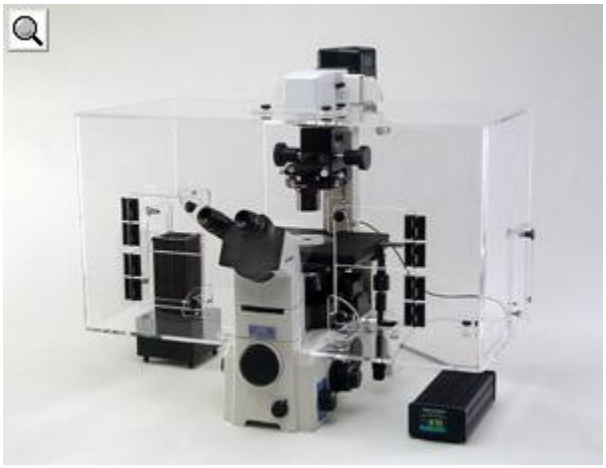

Figure 4. 37° Incubation Chambers by Solent Scientific (Solent Scientific Ltd., 2007).

Another example of a chamber completely enclosing a microscope is the Incubation Chamber by Solent Scientific as shown in *Fig. 4*; this incubating chamber is custom-built by Solent Scientific for their customers. The chamber offers temperature control and CO_2 enrichment. The company also asserts the chamber is easy to disassemble without tools.

Design Solutions

Our design team came up with three alternative solutions to the proposed problem statement. These three designs vary in structure and variety of components used. In order to maintain the proper temperature inside the chamber, in each of our three designs the pressurized gas will be pumped through hot water, bringing it up to 37 ˚C. This “bubble heater” system was requested specifically by our client as the means to heat the gas.

Mixed Air Tank Design

This first design is primarily a small, rectangular, Plexiglas chamber that would rest on the microscope stage. A small chamber would have a greater portability. The specimen would lie underneath the chamber, and various tubes would carry the gas

from a tank to a water bath to warm up the gas, and then finally to the chamber. Many tubes are used because we do not want the specimen to move at all underneath the chamber, and the tubes create a line of symmetry about the specimen. The gas tank would already contain the predetermined mixture of air and CO_2 (95% and 5% respectively). The chamber would have a door on the side so the specimen is easily accessible without lifting the entire chamber off the stage.

In scenarios involving long term imaging, we do not want to continuously flow the gas through the chamber, because this would drain the tank quickly. If we were to make our chamber leak-proof, then we can pump the right amount of gas inside the chamber, seal the chamber, and just hold the gas inside the chamber. That way the flow of gas would have stopped, increasing the life of the gas tank. A caulking material can be used around the edges, and a rubber seal can be placed at the door and the bottom of the chamber.

This design would be highly portable, relatively cheap and easy to construct, and could be used across several different models of microscopes. Some disadvantages are the gas tank mixtures are fairly expensive and different mixture tanks would have to be bought in case imaging needed to be done using a different concentration of CO_2.

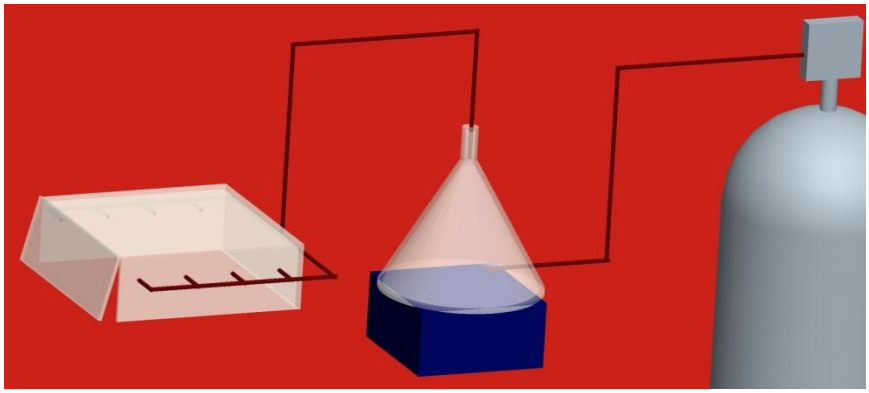

Figure 5. Mixed Air Tank Design. Schematic for mixed air tank design is shown featuring from left to right, chamber, “bubble heater,” and air supply tank.

CO_2 Sensor Design

The second design would be similar to the first in that the specimen would be placed in a small chamber resting on the microscope stage with several tubes supplying the gas to the chamber. However, the gas tank would be purely CO_2, not a mixture of air and CO_2. Because of this, a CO_2 sensor would have to be used so we know the concentration of the CO_2 inside the chamber. The sensor would be placed into the chamber, and to accommodate for the sensor, the chamber would be “L-shaped” rather than rectangular. This extra space could house both the sensor and the circuit board used to control the flow valve on the gas tank. Using this method, continuous gas flow

would not be needed at all. The sensor would open and close the valve on the gas tank when it senses more or less CO_2 is needed. This design would be ideal because while there is a high initial cost because of the CO_2 sensor, the gas tank would have a longer cycle, and therefore decrease the cost of constantly refilling a new tank.

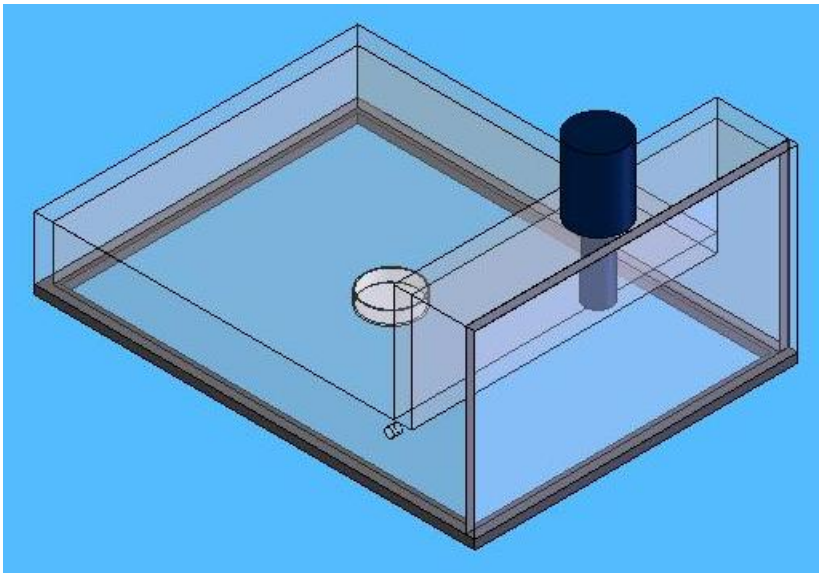

Figure 6. CO_2 Sensor Design. Schematic of Plexiglas chamber showing CO2 sensor in dark blue and sample Petri dish in white.

Enclosed Chamber Design

This third design incorporates a giant Plexiglas chamber to enclose the entire system of components, including the microscope. The chamber would be fitted to any protrusions the components or microscope may have, such as wires and cords, and a door would be made to access the specimen on top of the microscope stage. The CO_2 sensor and circuit board would be used. This design would allow for there to be no extra glass between the microscope and the other imaging components, however it would be rather bulky to carry around and it would only be fitted to one model type of microscope. Also, our client still does a lot of non-live cell imaging, so either setting up then removing this chamber or working around it when it is not needed are both inconvenient for the client.

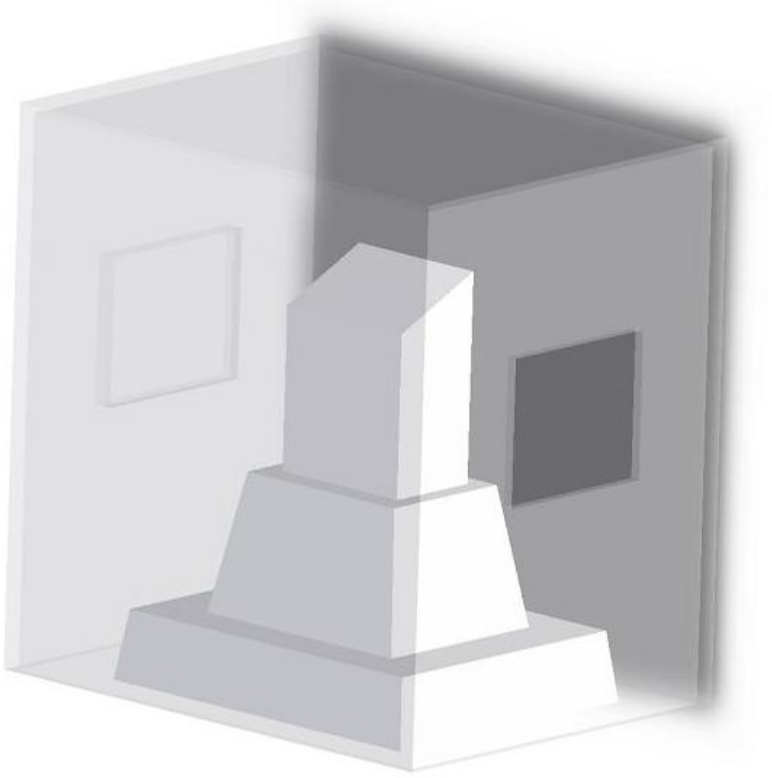

Figure 7. Enclosed Chamber Design. Schematic of the Plexiglas chamber. Microscope is shown sitting inside of chamber, accessible via doors shown on the sides.

Proposed solution

Table 1. Design matrix which indicates the scoring values of various design possibilities.

	Possible Points	**Mixed Air Tank**	CO2 Sensor	Enclosed Chamber
Ease of construction	10	**10**	4	7
Access to samples by user	10	**7**	5	8
Portability	5	**5**	5	0
Relative Safety	10	**8**	10	9
Cost: Capital Investment	10	**10**	2	9
Cost: Operating	15	**9**	15	4
Total	60	**49**	41	37

Our proposed solution is to use the microscope tray sized chamber where a premixed tank of 95% air and 5% CO_2 is continuously pumped inside. Since the CO_2 will be delivered at an appropriate amount, more time can be allotted to the heating of the gas, and assuring the proper flow of gas inside the chamber. We have decided to build our own chamber for this reason, to create the best flow of gas as well as increase accessibility. The benefit of bubbling it through water is the simplicity of the design. However certain problems arise due to this design choice. These problems are described in the next section. Another drawback of this design is since the gas will be constantly leaking out of the tank, we may go through gas tanks at a high rate causing an increase in running costs, even if the capital investment would be lower (than in the case of investing in a CO_2 sensor.) This proposed solution of using the small chamber on top of the stage has the main advantage over the large case because it is portable. Most of our client's users are still using prepared slides for imaging, where the extra live cell imaging chamber would be added encumbrance.

Potential Problems

Precautions will need to be made to protect the equipment and operators from the boiling water. While an Erlenmeyer flask sitting on a hot plate would be a simple solution, there is a risk it could be bumped and burn someone or damage the microscope. To address this issue we hope to firmly anchor and attach the whole heater/flask so there is no chance it could tip over and damage the equipment. Another potential problem is some of the CO_2 bubbled through the water becoming dissolved in it and as a result reducing the concentration of the delivered gas. To address this issue we will allow a warm up time so the water becomes saturated with CO_2 before a sample is analyzed in the chamber. Since the air coming out of the water bath will be saturated with water vapor, precautions need to be made so that water does not condense on the microscope. We hope to accomplish this by warming the stage with a heating ring.

References

20/20 Technology, Inc. 2007. "INC-2000 INCUBATOR SYSTEM."

Applied Scientific Instrumentation. 2007. "20/20 Technology Heating/Cooling Chambers and Incubators." <http://www.asiimaging.com/20-20tech.html>

Dailey, Michael E. *et al*. 2007. Nikon Microscopy. <http://www.microscopyu.com/articles/livecellimaging/culturechambers.html>

de Leeuw, Wim. 2007. "Computer at the Microscope: Visualization and Analysis of Three-Dimensional Microscopy Data." Ercim News Magazine. <http://www.ercim.org/publication/Ercim_News/enw60/de_leeuw.html>

"FCS3- Focht Chamber System 3." Bioptechs. 2007. <http://www.bioptechs.com/Products/FCS3/fcs3.html>

Kern, Rudolf. 2007. “Live Cell Imaging: Integrated Cell Observation and Injection System.” CellBiology Trading. <http://www.ais2.com/Pages/Incubator1.php>

Nicolas, George. 2003. “Confocal Microscope Systems – A Comparison of Technologies” *Bioscience Technologies*, 11: 12-14.

PerkinElmer, Inc. “About Live Cell Imaging.” 2007. http://las.perkinelmer.com/content/livecellimaging/about.asp

“Price List.” Bioptechs. 2007. <http://www.bioptechs.com/Products/Prices/prices.html>

Solent Scientific Ltd. “37° Incubation Chambers.” 2007. <http://www.solentsci.com/UK/nikon/micro_inc.htm>

W.M. Keck Laboratory for Biological Imaging. 2007. <http://keck.bioimaging.wisc.edu>

Appendix

Product Design Specification

Function

Construct a live cell imaging chamber to be used for laser-based confocal and multiphoton imaging. Device needs to control temperature and gas-environment inside as well as enable the use of perfusion.

Client Requirements

- Maintain environment with 95% air, 5% CO_2
- Device must allow control of air temperature
- Allow perfusion to sample
- Mechanism for control of X, Y, theta of cell culture

Design Requirements

1. **Physical and Operational Characteristics**

a. *Performance Requirements:* Imaging chamber should allow live cell imaging to occur in a controlled environment. Gas make-up, gas temperature, and perfusion need to be controlled.

b. *Safety:* Chamber must not damage microscope or surrounding equipment in the client’s lab. Use of pressurized gas tanks including CO_2 needs to be done in a safe manner.

c. *Accuracy & Reliability:* pH level must be maintained between 6-8 in culture media. CO2 level must remain close enough to 5% to maintain cell life. Testing will show what level is too high.

d. *Life in Service:*
e. *Shelf Life:* Chamber itself will not degrade with time. Shelf life of whole system will depend on size of input gas tank and flow rates which will be determined by testing.
f. *Operating Environment:* Chamber will be used with an Inverted Nikon TE2000 U microscope in W.M. Keck Laboratory on the UW campus.
g. *Ergonomics:* Chamber must allow for easy-access to put in and remove samples.
h. *Size:* Must fit on moveable XY stage in between lens and base of microscope. 30 x 27.6 x 3 cm.
i. *Weight:* Must not damage microscope stage.
j. *Materials:* No plastic in microscope image field.
k. *Aesthetics, Appearance, & Finish:* Chamber must be easy to sterilize. Good organization of peripheral tubes, etc.

2. Production Characteristics

a. *Quantity:*1
b. *Target Production Cost:* $500

3. Miscellaneous

a. *Standards & Specifications:* N.A.
b. *Customer:* N.A.
c. *Patient-related Concerns:* N.A.
d. *Competition:*

Incubator 2000; 20/20 Technology, Inc. Incubator 2000 is a miniature imaging chamber with control for temperature, humidity, and atmosphere. PRICE

Focht Chamber System 3: Bioptechs, Inc. FCS3 is a live cell imaging chamber with control for gas flow speed, temperature, and gas make-up as and is perfusion compatible. The FCS3 system starts at $4000.