Microscope Cell Culture Incubator

Ian Linsmeier, Tyler Klann, Rebecca Stoebe, Paul Strand

Department of Biomedical Engineering

Advisor: Dr. Randolph Ashton **Client**: Dr. John Puccinelli

Abstract

Dr. John Puccinelli wishes to perform live-imaging of cell cultures during time course experiments that can range from 6 hours to a week. In order to achieve this, the cell cultures must be maintained at the correct environmental conditions throughout the experiment as well as when the cells are being imaged. Our team aims to develop a microscope-compatible cell culture incubation chamber that is able to accurately regulate the internal environment at 37°C, 5% CO_2 and between 90-100% humidity, without interfering with the imaging capability of the microscope.

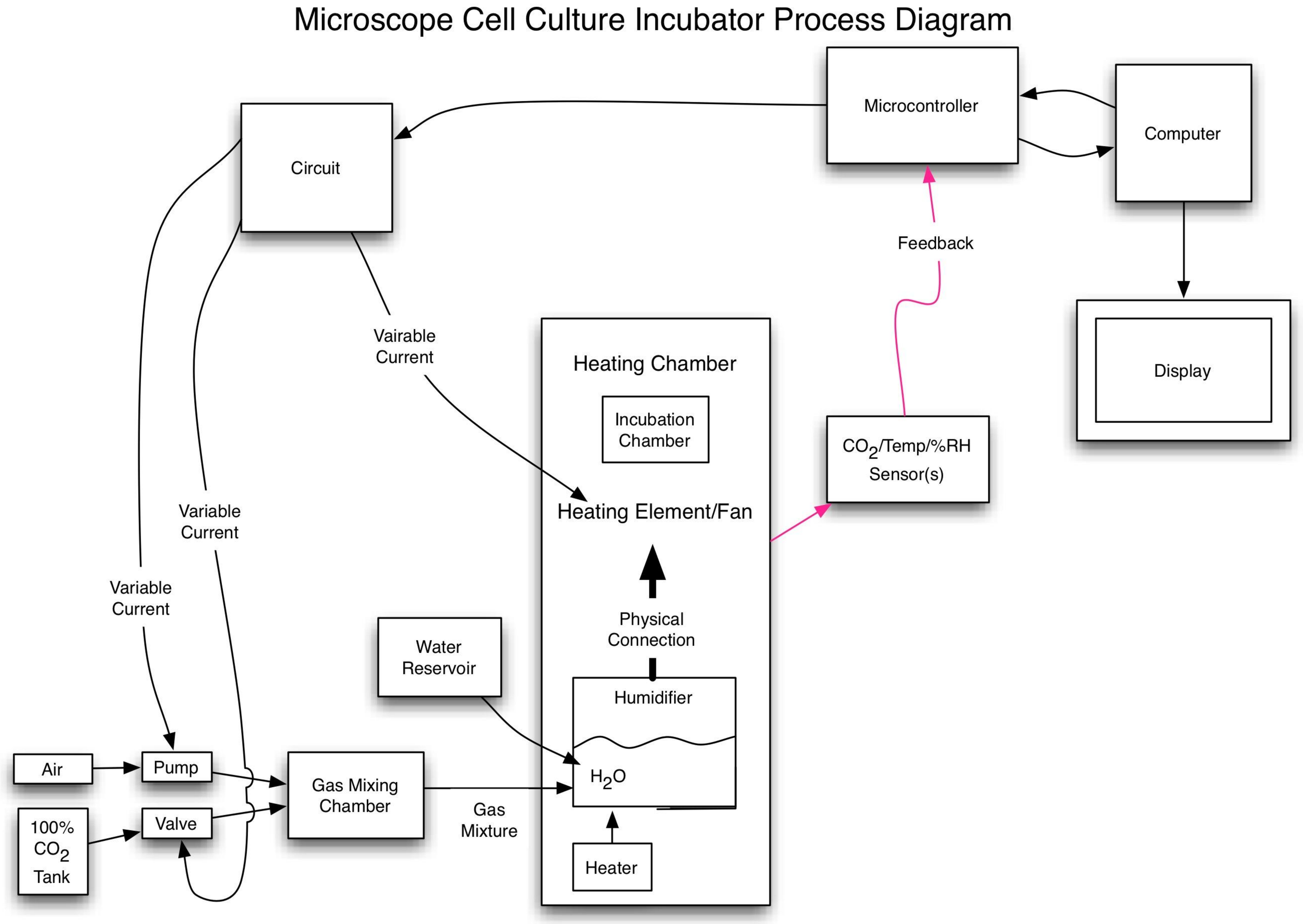
Problem Definition

Create an incubation chamber that is compatible with the Olympus IX71 inverted microscope that has the ability to accommodate a variety of cell culture plates in a fixed internal environmental state, without compromising the integrity of the instrument optics or functionality. This will allow the microscope to be used for live cell imaging to assess the cellular dynamics of various biological process through fluorescent imaging. The result will produce a complete analysis of cellular behaviors over time.

Design Criteria

- Internal environment of 37˚C ± 2˚C, 5% CO2 ± 1%, and 90-100% relative humidity
- Interchangeable with different cell culture plates
- Cannot experience interference from condensation, temperature gradients or evaporation of culture liquids
- Easy to assemble and disassemble
- Budget is ~$200
- All materials used must be biologically compatible

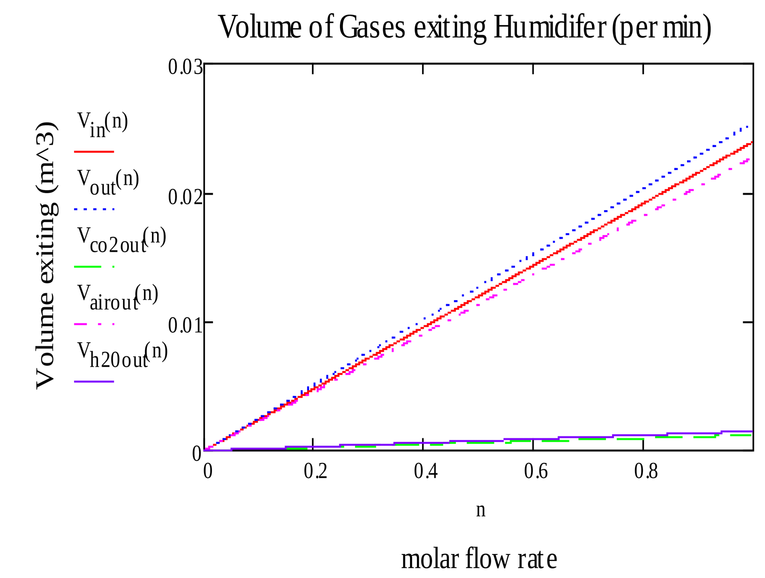
Microscope Cell Culture Incubator Process Diagram

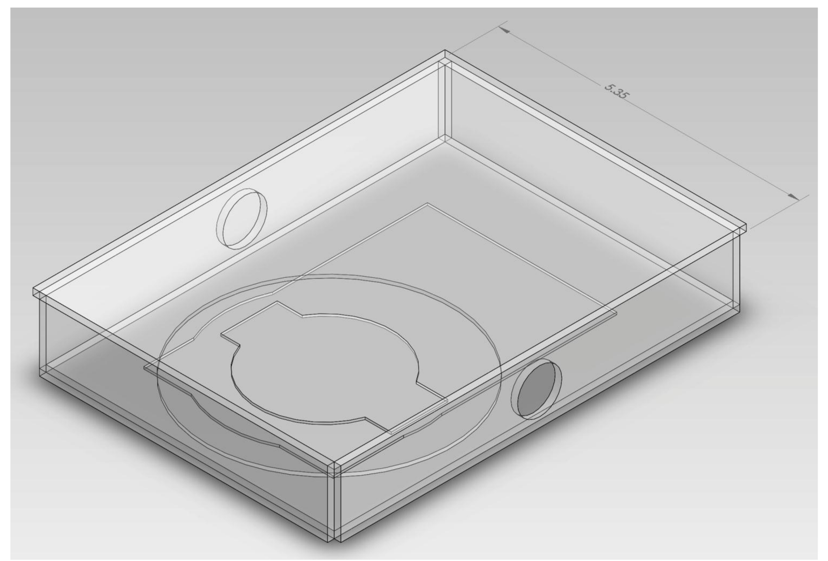


Final Design

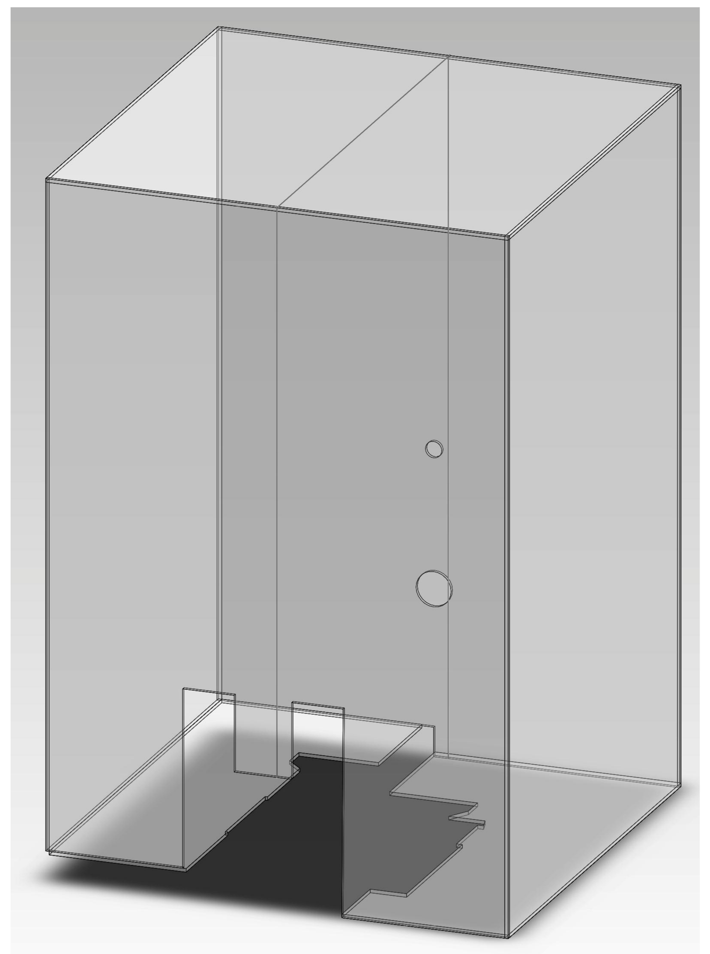
Humidifier

- 5x5x6 (cm) hollow box with 3 circular inlets for fresh gas, recycled air, and fresh water and circular outlet for gas diffusion into incubation chamber.
- Inner basin for water fits inside (4.5x4.5x2 cm). Exterior made of Plexiglas. Interior water basin is made of aluminum coated with ceramic.
- Allow for approximately 0.5 molar flow/min [1]
- Humidifies a mixture of 95% air/5% CO_2 to 95% humidity using heat (approx. 19 W) delivered [2] from nichrome wires situated between the interior basin and the outer chamber.
- Exterior water reservoir (not shown) inputs 0.5 mL of water per minute to compensate for evaporation





Inner Incubation Chamber



Outer Heating Chamber

Incubation Chamber

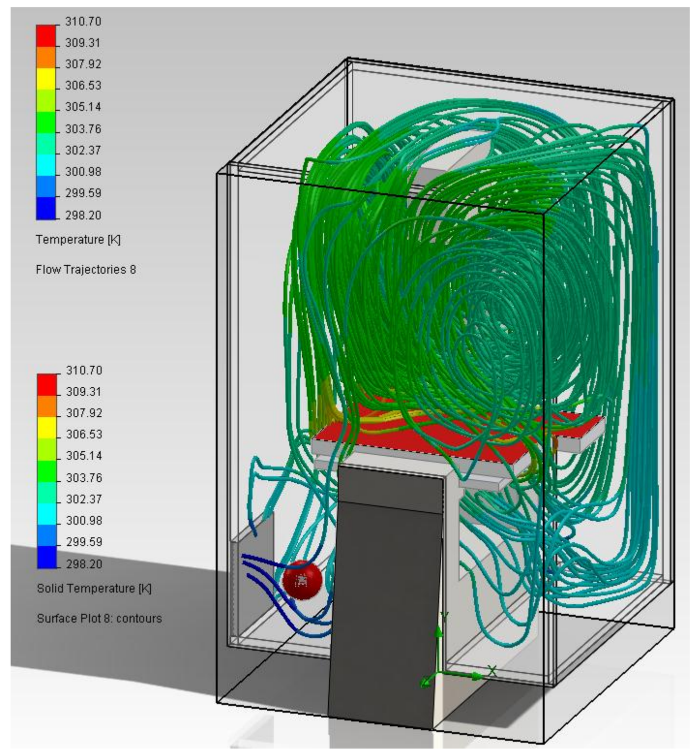
- Made of medium impact acrylic .117 in. thick.
- Allows humidified air and CO_2 to enter.
- Secures multiple types of culture plates for accurate viewing.
- Creates an isolated environment that is easier to regulate and quicker to start up.

Heating Housing

- Made of medium impact acrylic .117 in. thick.
- Fits around microscope and allows full microscope functionality.
- Separates into halves for removal and is sealed with a silicone gasket.
- Edges are sealed with Weld-On 4 acrylic adhesive.
- Allows incorporation of aluminum stage as a heat sink.

Heating Element

- 30 ft. coil of 30 AWG (D=0.010 in.) nichrome 60 wire
- Fan distributes heat
- Supplied with 189 mA of current to heat it to 37ºC
- Modulated based on temperature readings
- Coated in a thin ceramic with high thermal conductivity and small electrical conductivity
- Possible ceramics include: AlN, SiC, Al_2O_3, Si_3N_4, ZrO_2
- Same heating element design for the humidifier
- 306.3 mA of current → 19 Watts of power to H_2O



Ideal Heat Flow Model

$$N_{Nu} = \frac{h_{conv}D}{k_{air}} = a(N_{Gr}N_{Pr})^m = a\left(\frac{D^3\rho^2 g\beta\Delta T}{\mu^2}\frac{c_p\mu}{k}\right)^m \Rightarrow h_{conv} = \frac{N_{Nu}k_{air}}{D}$$

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

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

$$P = I^2R = I^2\rho L$$

$$q_{total} = P \Rightarrow h_{total}\pi DL\Delta T = I^2\rho L \Rightarrow I = \sqrt{\frac{h_{total}\pi D\Delta T}{\rho}}$$

Heating Element Calculations [3]

Temperature and Air Flow Control

- Two current controlled solenoid valves control airflow into mixing chamber
- Pressurized CO_2 comes from tank and air comes from pressurized wall source
- Regulators control set pressure to 15 PSI feeding into valves
- K-33 BLG sensor detects CO_2 concentration and microcontroller opens or closes valves to correct concentration
- LPC1768 mbed microcontroller to be used, C++ code
- N-Type MOSFET used to control current through valves and through heating element
- Microcontroller detects output from Sensirion SH15 to find incubator temp and adjusts current to nichrome accordingly

Future Work

- Integration of the entire design for flow modeling
- Fine tune design of individual components to incorporate results of total flow models
- Development of a control system to allow for recycling humidified air
- Extensive testing of entire system to compensate for sources of error
- Order materials and build physical prototype

References

References

[1] "Roult's Law." Chemical Education at University of Wisconsin. UW-Madison, 2009. Web. 08 Dec. 2011. <http://chemed.chem.wisc.edu/chempaths/GenChem-Textbook/Ideal-Solutions-Raoult-s-Law-850.html>.

[2] "Standard Enthalpies of Formation." Chemical Education at University of Wisconsin. UW-Madison, 2009. Web. 08 Dec. 2011. <http://chemed.chem.wisc.edu/chempaths/GenChem-Textbook/Standard-Enthalpies-of-Formation-551.html>.

[3] Geankoplis, C. (1978). Principles of steady-state heat transfer. In *Transport processes and unit operations* (pp. 141-201). Boston: Allyn and Bacon.

Acknowledgements

- Dr. John Puccinelli
- Dr. Randolph Ashton
- Pete Klomberg
- Dr. Amit Nimunkar