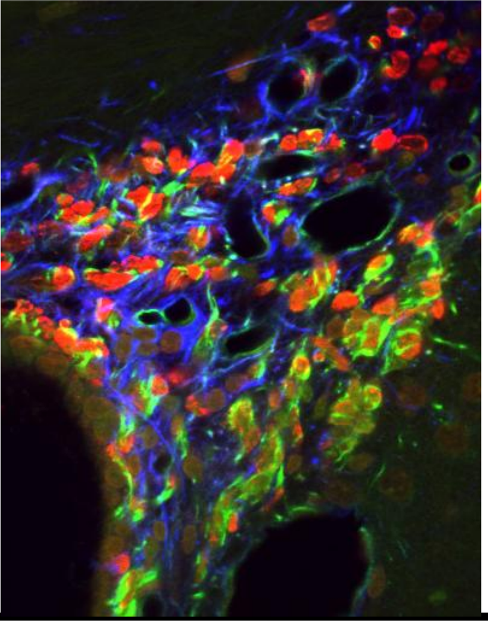
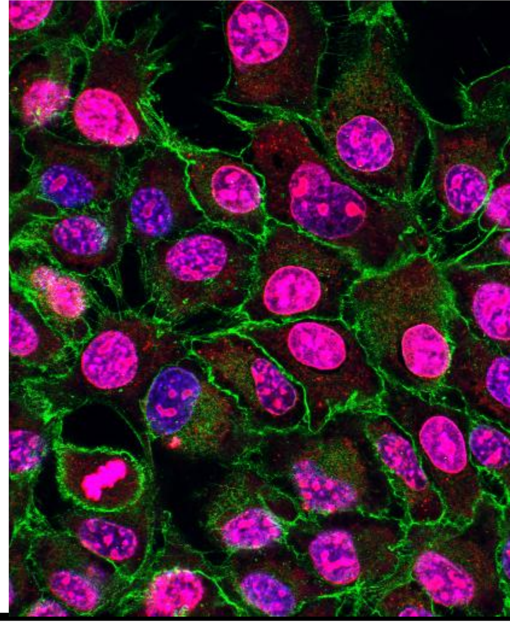


BIOLOGICAL IMAGING CHAMBER



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Client: Lance Rodenkirch, Keck Lab for Medical Imaging, UW-Madison

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-term live cell imaging. These systems are available on the market but are expensive and may not be compatible with the intended microscope. Our device will provide an economical alternative to purchasing a commercially available imaging chamber. Our chamber uses a CO_2 sensor and a feedback circuit to inject CO_2 gas periodically, maintaining CO_2 concentration at 5 ± 0.5% .

Problem Definition

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.

Perfusion chambers can be used to shield live cells from the external environment. An "open" chamber is similar to a Petri dish and has little control over air flow and gas concentrations. Cells are very sensitive to shear forces so a closed chamber allows live cells to be incubated and protected while imaged. Closed chambers protect cells from evaporation of the medium and make it easier to maintain a constant pH and concentration of carbon dioxide. Having a stable environment is a primary concern in order to keep cells alive for imaging.

Existing Devices

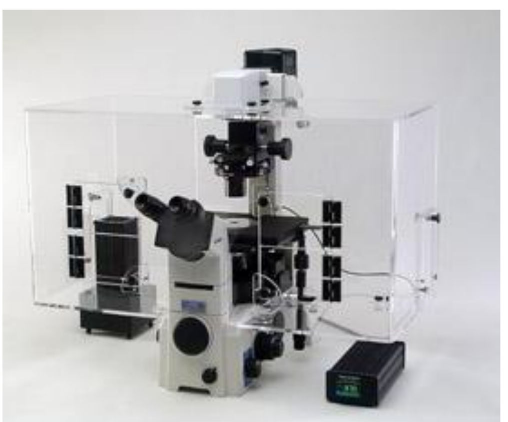

Figure 1. Incubation Chamber by Solent Scientific

Figure 2. EMBL Live Cell Observation Chamber by CellBiology Trading

Problems with existing devices:
- Too expensive ($4,000 - $20,000)
- Not compatible with all microscopes

Design Criteria

Product Design Specifications
- Maintain 5 ± 0.5% carbon dioxide in chamber
- Cell medium maintained at 37 ± 3⁰C
- Chamber must fit on 30 × 27.6 cm stage
- Must fit between lens and base of microscope (3 cm maximum height)
- Top face of chamber must be glass
- Allow for easy access to samples
- Compatible with Nikon TE2000U microscope

Final Design

Our final design consists of an acrylic and glass chamber with a CO_2 sensor. The CO_2 sensor is connected to a feedback circuit controlling infuse of CO_2 gas to maintain a 5 ± 0.5% level. Cell medium is kept at 37 ± 3⁰C by plate heater, set into microscope stage.

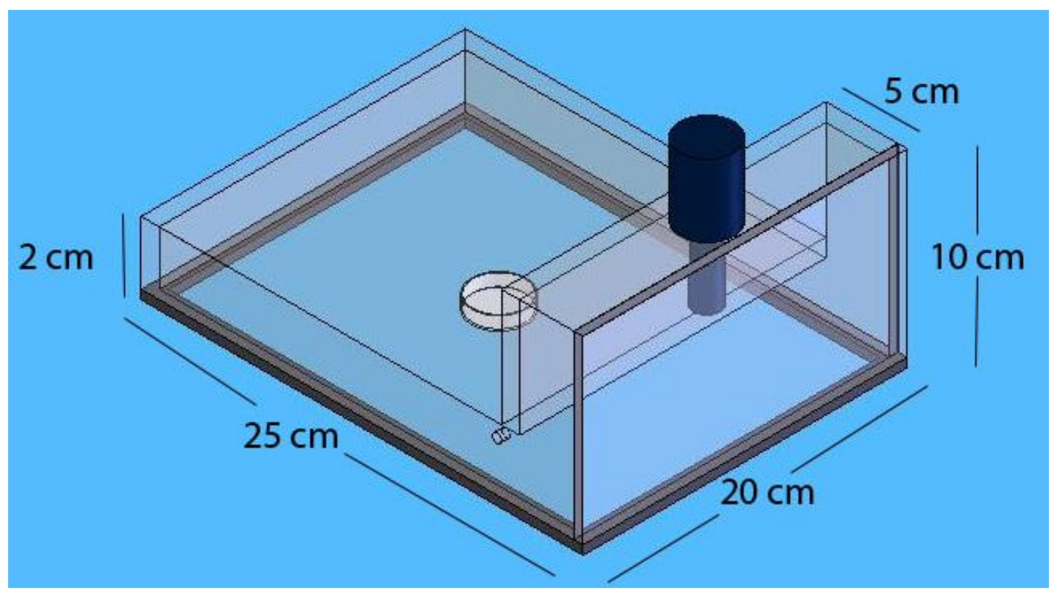

Figure 3. Imaging chamber. White disc shows location of Petri dish with cells. Blue rod represents CO_2 sensor probe.

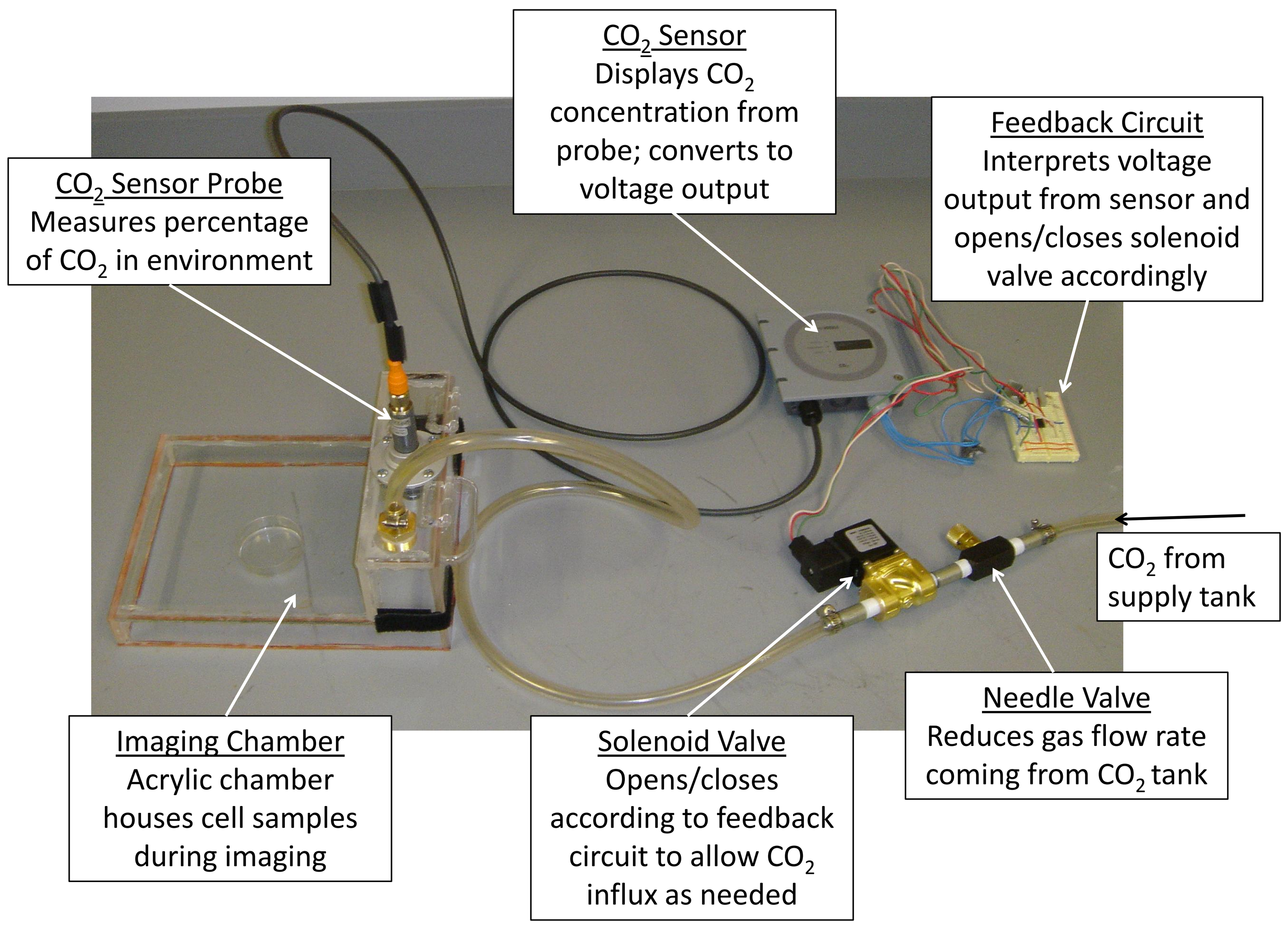

Figure 4. Imaging chamber prototype.

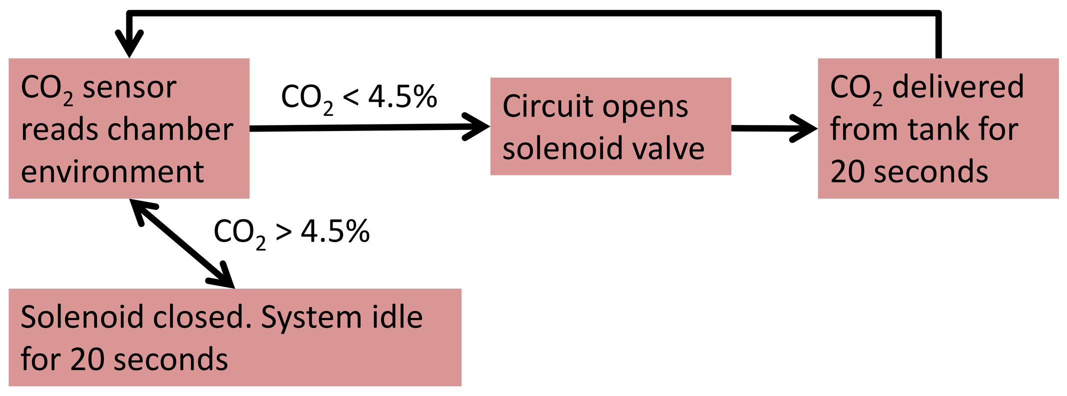

Figure 5. Flow chart showing operation of CO_2 sensor feedback. 20 seconds is the delay between sensor readings. Needle valve can be adjusted such that 20 seconds of flow raises the overall CO_2 level to no more than 5.5%.

Budget

Item	Cost
Vaisala CO_2 Transmitter and accessories	$1030.00
Solenoid Valve	$62.60
Needle Valve	$33.36
100% CO_2 Tank	$15.00
Hardware (rubber sealer, nuts, bolts, etc)	$30.00
Circuit Elements (wires, resistors, etc)	$10.00
24 V DC Power Supply	$22.50
TOTAL	$1203.46

Testing

Testing of the chamber was performed with a 19 volt power supply instead of a 24 volt power supply. We expect an average concentration of 5% CO2 when the correct power supply is used.

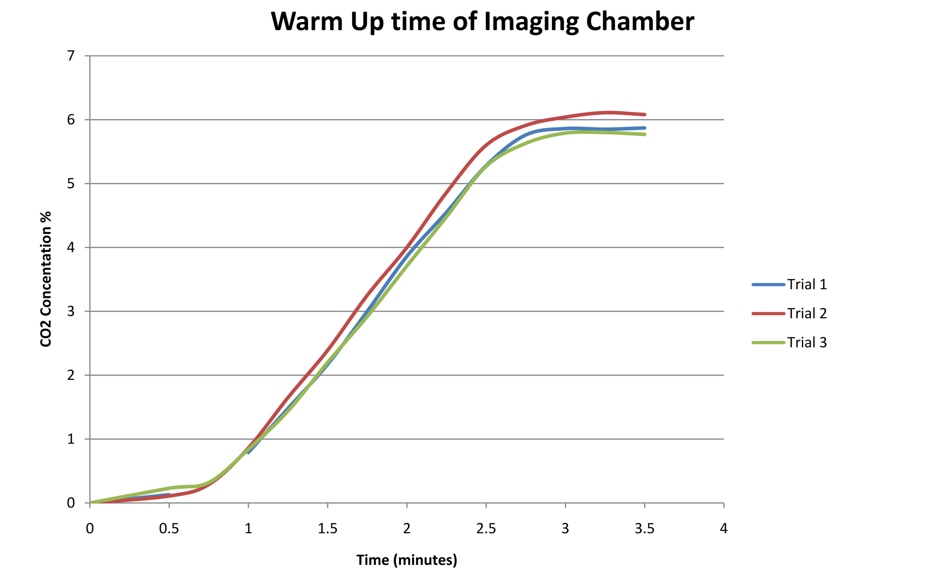

Figure 6. Warm up time of imaging chamber. This graph shows the time it takes for CO_2 to go from room concentration up to peak, which would occur every time the system is turned on or the chamber door is opened to change samples.

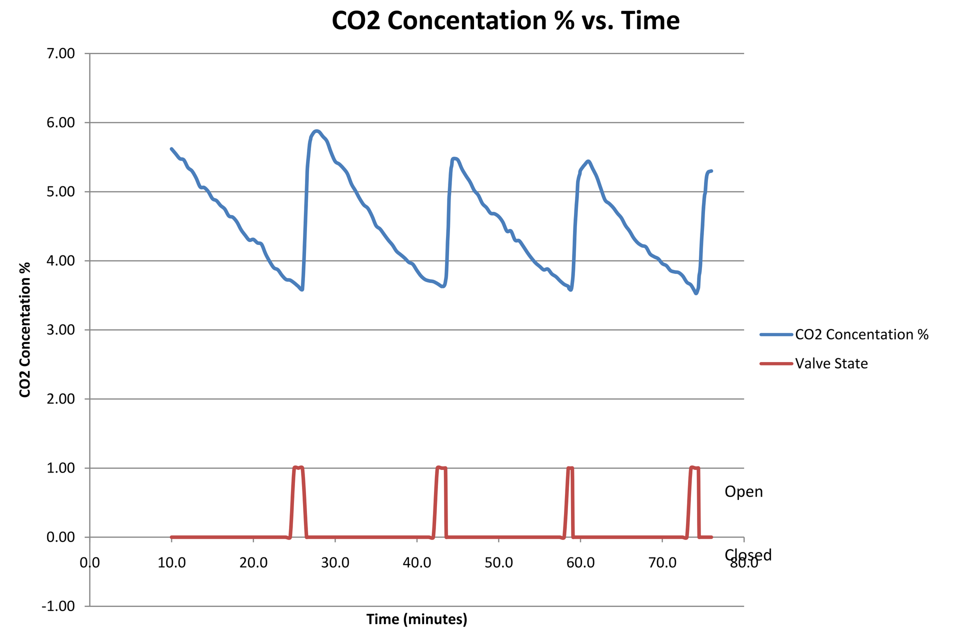

Figure 7. CO_2 concentration (%) vs. time. This graph shows the % of CO_2 inside the chamber over a 1.5 hour test. Also, the activity of the solenoid valve over time (open or closed) is indicated.

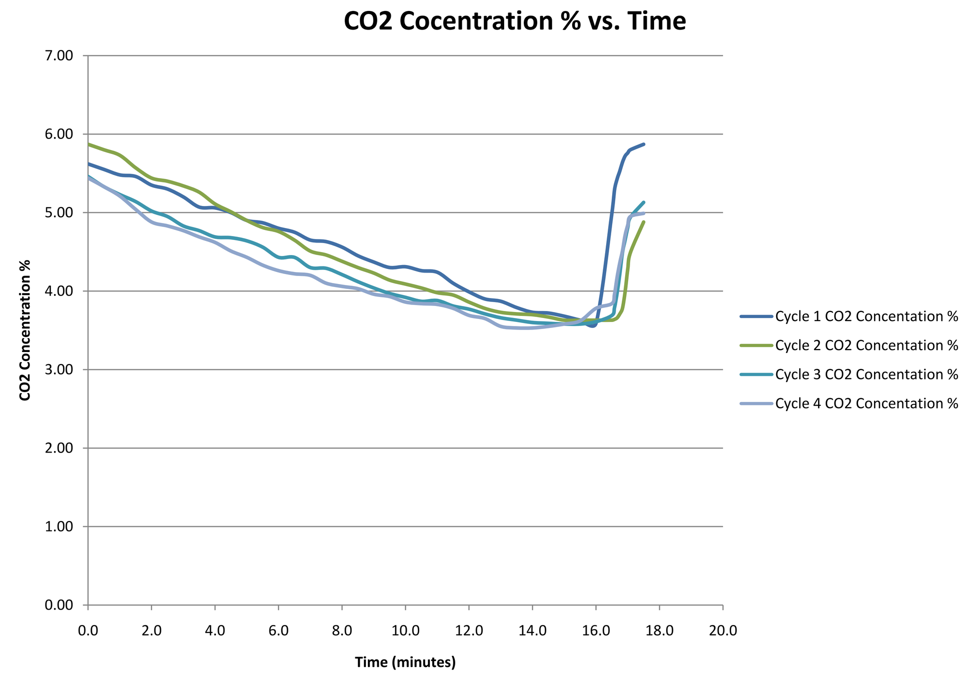

Figure 8. CO_2 concentration (%) vs. time. This graph shows the lower peak of CO_2 concentration over 4 trials. Lower peak was very repeatable, peaking down to ~3.6%.

Future Work

- Fix range of CO_2 fluctuation
- Set up prototype in client's lab
- Use chamber for live cell imaging
- Evaluate quality of live images and identify any problems

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