

# *Microscopic Cell Culture Incubator*

BME Design Excellence Award

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The goal of the project was to develop a low-cost cell culture incubation chamber compatible with an inverted microscope and capable of live-cell imaging. This incubation chamber must be able to maintain an internal environment of 37°C, 5% CO<sub>2</sub>, and >95% humidity over the course of one week without compromising the integrity of the microscope's optics or functionality.

Current commercially available systems, ranging from about \$500 to \$4,000, are prone to homogeneity issues and are expensive. Available stage-top incubators include those from Okolabs<sup>1</sup> and Elliot scientific<sup>2</sup>, which have had great success in maintaining a homogeneous environment, in terms of temperature and CO<sub>2</sub> percentage. However, these incubators are expensive, require multiple assembly components, and encompass the entirety of the microscope, limiting ease of use, especially for students.

The design process has been broken down into three main parts: fabrication, circuitry, and testing. The resulting final design includes an acrylic box incubator, fabricated via laser cutting black acrylic. The design is a 245×195×40mm black acrylic box with two polycarbonate glass plates on the top and bottom to allow for optical use of an inverted microscope, a top lid, 0.6 meters of copper tubing circling the inside, an internal water bath, and holes for the sensors and polyvinyl tubing that will deliver 5% CO<sub>2</sub> from the 100% tank, controlled by a solenoid valve. The acrylic was chosen as it will provide the best internal environment, reduce most of the leakage, and is cost efficient. The glass was chosen for its low cost and its optical transparency. The 0.6 meters of 0.012 meter copper piping was used for heat transfer and was circled once around the inside of the box. The copper tubing will have heated water pumped through it which will heat the water in the bath. This process will create an internal temperature of 37°C and lead to water bath evaporation creating the >95% humidity needed for cell viability and to meet design requirements. The polyvinyl tubing is the standard tubing that came with the CO<sub>2</sub> tank and was kept to reduce costs. Due to the importance of maintaining a precise internal environment for cell growth, a thermistor, NDIR CO<sub>2</sub> sensor, and a solenoid valve have been implanted for the purpose of monitoring internal conditions and maintaining CO<sub>2</sub> via a feedback loop between the NDIR sensor and solenoid valve. The thermistor and NDIR sensors are waterproof. The thermistor monitors temperature and has been coded to monitor humidity. The NDIR sensor monitors the CO<sub>2</sub> volume and percentage inside the box. The solenoid valve is used to adjust the flow of 100% CO<sub>2</sub> from a tank, so the incubator contains 5% CO<sub>2</sub> at all times.

Testing protocols were created to evaluate sensor accuracy, optical clarity, and the internal environment. All sensors were tested against a commercially available incubator in the teaching lab and were determined to have no statistical significance, proving that sensor accuracy was achieved. Optical clarity tests and image analysis showed that there was no optical difference between slides when under the polycarbonate glass, as compared to without glass. However, condensation remains a problem within the system, affecting the optics over time. The internal environment of the incubator was tested to ensure homogenous conditions within the system, with the average temperature of the incubator being 37.9°C, average humidity being 97.5%, and average CO<sub>2</sub> being 4.97%. Thus, the testing conducted met the main goals of the criteria established by the product design specifications, including accurate sensor readings, optical clarity to allow for imaging, and a homogenous internal environment for cells to thrive.

The total cost of the incubator is \$73, not including gifted items, making it more accessible for research labs, educational institutions, and companies to conduct live cell imaging. Due to its low cost and ability to sit on the microscope stand, instructors will be able to teach more students how to image live cells in a more hands-on approach. With increased education on optical imaging, students will be able to pay forward this investment tenfold to society through meaningful work in the pharmaceuticals, virology, drug development, and genetic engineering sectors.

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<sup>1</sup> Okolab, "Stage Top Chamber," Okolab - stage top - digital gas, 2003. [Online]. Available: <http://www.oko-lab.com/live-cell-imaging/stage-top-digital-gas>. [Accessed: 23-Feb-2022]

<sup>2</sup> "CO<sub>2</sub> incubators," Thermo Fisher Scientific - US. [Online]. Available: <https://www.thermofisher.com/us/en/home/life-science/lab-equipment/co2-incubators.html>. [Accessed: 20-Sep-2021]