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 that is compatible with an inverted microscope and capable of live-cell imaging. This incubation chamber must be able to maintain an internal environment of 37℃, 5% CO_2, and 95-100% humidity over the course of one week without compromising the integrity of the microscope's optics or functionality.

Current commercially available systems are prone to homogeneity issues and are expensive. Available stage-top incubators include those from Okolabs and Elliot scientific, which have had great success in maintaining a homogeneous environment, in terms of temperature and CO2 percentage [1,2]. 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 a latch-top acrylic box incubator, fabricated via laser cutting black acrylic. The design is a 245x195x40mm 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 with a rubber lining, 2 feet of copper tubing circling the inside, an internal water bath, and holes for the sensors and polyvinyl tubing that will deliver 5% CO_2 from the 100% tank, as controlled by a DC motor. The acrylic was chosen as it will provide the best internal environment, reduce the majority of leakage, and is cost efficient. The glass was chosen for its low cost and its optical transparency. The rubber lining was added to the top lid in order to provide a better fit for the box so that humidity, temperature, and CO_2 levels would be internally maintained over the course of a week. The 2ft of ½ inch copper tubing is used for heat transfer and was circled once around the inside of the box because it was too large to wrap around twice. The group was provided with copper tubing by Dr. Puccinelli and chose to proceed with the tubing in order to minimize cost. Copper has high thermal transfer properties and will sit in the water bath. 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, meeting design requirements. The water bath that is heated will also lead to evaporation creating the 95%+ humidity which is needed for cell viability. The polyvinyl tubing is the standard tubing that came with the CO_2 tank so it was decided to keep that tubing to reduce the total cost of the project. Due to the importance of maintaining a precise internal environment for cell growth, a thermistor, NDIR CO_2 sensor, and a DC motor have been implanted for the purpose of monitoring and inputting temperature, humidity, and CO_2. The thermistor is waterproof, automatically monitors temperature, and additionally has been coded to monitor humidity. The NDIR sensor monitors the CO_2 volume and percentage inside the box. The DC motor, which is paired with a 3D printed attachment, is used to adjust the flow of 100% CO_2 from a tank so the incubator contains 5% CO_2 at all times.

Testing protocols were written and conducted on 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. Optical clarity tests showed that there was no optical difference between slides when under the polycarbonate glass, as compared to without glass. The internal environment of the incubator was tested to ensure that the internal conditions were met, with the average temperature of the incubator being 37.6℃ and the average humidity being 93.8%, which although low can be attributed to small gaps in the junction between the box and the glass.

The total cost of the incubator is $51.45, 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 as the device is easy to use and assemble, as long as there is a basic understanding of how to use an inverted microscope. 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.

Works Cited

1. "CO2 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].
2. 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].