Product Design Specifications

Microscope Cell Culture Incubator

BME 400
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Client: Dr. John Puccinelli
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Function: Develop a low cost cell culture incubation chamber that is compatible with an inverted microscope and capable of live cell imaging over the course of one week.

Client requirements:

- Incubation chamber must be able to maintain an internal environment of 37℃, 5% CO_2, and 95-100% humidity
- Microscope's optics and functionality must not be damaged
- Maintain even heating and humidity across the chamber
- Create device that stays within a budget of $100
- Ensure that the device can be easily assembled and removed between uses

Design requirements:

1. Physical and Operational Characteristics

a. ***Performance requirements:*** The device must be able to sit on a microscope stand (less than 310 x 300 x 45mm[1]), be transparent on the top and bottom to allow for optical visualization with an inverted microscope, and maintain an internal environment of 37℃, 5% CO_2, and 95-100% humidity. This device should demonstrate no quantitative difference on the microscope when adding glass compared with solely cells, in order to demonstrate full transparency of the top and bottom slides of the system.

b. ***Safety:*** The incubator and the cell culture environment must be in cooperation with BioSafety Level 1 Standards [2]. Any material and electrical or mechanical machinery must be sterilizable and waterproof.

c. ***Accuracy and Reliability:*** The device must be able to maintain a temperature of 37°C ± 1°C throughout the entire internal environment. The humidity must be kept above 95% humidity. CO_2 levels must be 5% ± 1%. The incubator must be able to maintain these conditions constantly for at least two weeks. The device must also be able to reach these conditions after the incubator has been opened and exposed to the external environment within five minutes of interruption.

d. ***Life in Service:*** The device must be able to be used for two weeks, but optimal usage will occur for one week at a time for teaching purposes in the client's tissue lab.

e. ***Shelf Life:*** The shelf life of this product should be 10 years.

f. ***Operating Environment:*** The operating environment is a clean room. The incubation chamber must be able to maintain an internal environment of 37°C, 5% CO_2, and 95-100% humidity for at least two weeks, without compromising the integrity of the microscope's optics or functionality. Measures must be taken to ensure that the temperature is the same in all areas of the chamber with an error of ± 1° C. The box also must be sealed efficiently to ensure that evaporation does not occur.

g. ***Ergonomics:*** The device should be portable in that one should be able to carry and store the device easily. Wires should not be hanging freely out of the device, and it should be easy to pick up and put away when needed.
h. ***Size:*** The device must be less than 310x300x45mm in order to fit on the microscope stand without interfering with the optics[1].The bottom and top of the incubator will be transparent. Overall, the product must be compatible with an inverted microscope.
i. ***Weight:*** There are no specific weight requirements. However, minimizing weight would be ideal to promote incubator transportability and usability.
j. ***Materials:*** There are no specific materials that are required for development of this device. However, it is important to examine different material properties to determine which materials hold heat effectively, are water tight, and have a transparent appearance.
k. ***Aesthetics, Appearance, and Finish:*** The client does not have a preference in color. Well plates are clear, black (to stop contamination), and white (to increase light). Using materials that would block out external light sources would be ideal, but this is not a requirement for the device. Finish should exclude messy elements, such as long wires, and be transparent on both the top and bottom.

2. Production Characteristics:

a. ***Quantity:*** Only one device is necessary to produce, but ideally, it would have the capacity to be produced on a larger scale to be used repeatedly in the teaching labs.
b. ***Target Product Cost:*** The target product cost for this device is $100. It will be financed via UW BME Departmental teaching funds.

3. Miscellaneous

a. ***Standards and Specifications:*** The incubator would need to adhere to the ISO 13485 regulation which outlines requirements for regulatory purposes of Medical Devices [3]. The incubator would also need to follow the FDA's Code of Federal Regulations Title 21, Volume 8 where it outlines the requirements for Cell and Tissue Culture products [4].
b. ***Customer:*** The client, Dr. John Puccinelli, is an undergraduate advisor in the Biomedical Engineering Department at the University of Wisconsin - Madison. Dr. Puccinelli is asking for the cell culture incubator in order to amplify the teaching curriculum in his classroom environment. Having an incubator that is easy to disassemble and compatible with an inverted microscope would result in efficient classroom lessons.
c. ***Patient-related concerns:*** The accuracy of the temperature, humidity, and CO_2 concentration is of utmost concern for the client. Humidity must be 95-100%,

otherwise cells will begin to dry out. Having a set temperature of 37°C will replicate optimal cellular environments. Lastly, ease of disassembly and disinfecting of the incubator was of concern.

d. ***Competition***: There are currently multiple inverted microscopes and cell culture incubators on the market ranging from $500-$40,000 [4]. Thermo Fisher, NuAire, and New Brunswick all have incubators currently on the market. Thermo Fisher and NuAire are more popular as they have both direct heat and water jacketed incubators. The most popular Thermo Fisher design is the Heracell VIOS 160i CO2 Incubator with Copper Interior Chambers, which has HEPA filtration for ISO Class 5 air quality and an overnight Steri-Run for total sterilization [5]. Others have also attempted to design low-cost live-cell imaging platforms using 3D printed and off the shelf components. Both okolabs and Elliot Scientific have stage-top microscopic incubators available, both of which use the direct heat method, and have had great success in maintaining a homogeneous environment in terms of temperature and CO2 percentage[6,7]. However, these stage top incubators are still extremely expensive ranging from $431-$1000 and are only compatible with XY stage inserts[8]. XY stage inserts are roughly 150x150x36mm[9], slightly smaller dimensions than the stage top the team is currently working on. A team of researchers from Australia were able to successfully design a portable low-cost long-term live-cell imaging platform for biomedical research and education for under $1750 [10]. This low-cost incubator also monitored and regulated temperature, CO2, and humidity as per the parameters for successful mammalian cell culture. A company called ibidi has developed a stage top incubator compatible with an inverting microscope following all of the temperature, humidity, and CO2 requirements as well as producing anti-condensation glass technology. The incubator is currently on the market for $19,000 [11]. Past BME 200/300 design projects have attempted to build incubators for this client, but none have been completely successful.

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