

Microscope Cell Culture Incubator

Product Design Specifications | October 17, 2016

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Function: The device should enable the continuous culture of live cells for up to two weeks on an inverted microscope, without impeding imaging capabilities. The cell culture environment must imitate that of an incubator with precise control and readout of temperature, CO₂ mixture, and humidity all within a sterile environment.

Client Requirements:

- Temperature control and readout
- Humidity control and readout
- CO₂ concentration control and readout
- Incubation container must not impede ability to image
- Accessible for changing cell culture plates and changing media
- Sterilizable with a standard 70% ethanol solution
- Fit securely on an inverted microscope as to ensure imaging of a consistent location

Design Requirements:

1. Physical and Operational Characteristics

- Cell Culture Related Performance Requirements:*** The device should maintain incubator-like conditions for 2 weeks. It must maintain the temperature at 37°C ± 1°C, and reestablish temperature after less than 6 seconds following a 30 second door opening. It must maintain 95-100% humidity within culture chamber. Finally, it should maintain 5% ±0.5% CO₂ concentration and reestablish concentration after less than 6 seconds following a 30 second door opening.
- Incubator Housing Related Performance Requirements:*** Incubator housing and any potential condensation must not disrupt optics during imaging. The housing must not limit ability to navigate the full field of the cell culture plate, and not substantially change the distance between the cell culture plate and the objective. Housing materials must be compatible with culture media and be

sterilizable with 70% ethanol solution. The system should also have adequate insulation to prevent internal temperature fluctuations due to external temperature changes.

- c. **Safety:** Culture environment must be compliant to BioSafety Level 1 standards. All electrical components within the culture environment must be sterile and waterproofed, and all circuitry must be rated to the supplied power and current used.
- d. **Accuracy and Reliability:** The precision of the system components is outlined in the performance requirements listed above. For each of the four environmental parameters we will be controlling (temperature, humidity, CO₂ percentage and air sterility), the combined error of sensor measurement/readout and parameter control must be within the tolerance. The precision measurements taken during system use are as follows:
 - i. Humidity: 95-100% humidity
 - ii. CO₂ concentration: 5% ± .5% of readout value
 - iii. Temperature: 37°C ± 1°C of readout value
- e. **Life in Service:** The incubation chamber should maintain the specified environmental conditions to promote cell life for up to two weeks. The internal portions of the design under these environmental conditions must function without recalibration or repair during this time period.
- f. **Operating Environment:** The internal portion of the incubation chamber must function in conditions of 95% relative humidity or more, temperatures of 37°C and CO₂ levels of 5% during incubator operation. If the system is not in operation, the incubator will be exposed to normal environmental conditions: room temperature, environmental humidity, and low CO₂ concentration. There will be limited dirt exposure inside the incubation chamber, as live cells will be stored in it. Users will be opening and closing the incubation chamber, so the system will also have to adapt to sudden drops in temperature, relative humidity, and CO₂ percentage. It must be possible for the user to change media for cells inside the incubation chamber without changing the location that is being imaged.
- g. **Ergonomics:** The user will have limited interaction with the incubator itself, except to move cell culture dishes in and out of the chamber. The door to the chamber should be easy to open, and allow for enough clearance to fit a cell culture plate, flask, or petri dish inside the incubator.
- h. **Size:** The interior of the incubation chamber should be at minimum 15.4 cm x 9.4 cm x 2.5 cm tall. The incubation chamber should fit securely on a stage with dimensions as small as 16.0 cm x 25.0 cm, with a clearance of 5.3 cm tall for the light source.
- i. **Weight:** Each component of the final product should be no more than 12 kg, such that it is easy to transport between experiments without too much difficulty.
- j. **Materials:** Materials used for the incubation chamber should not have cytotoxic effects on cells inside their culture dishes, and should be sterilizable with ethanol. The materials should be resistant to corrosion from the high humidity levels. We

must use glass for the bottom surface, and the top surface should not deflect light from the light source significantly.

- k. **Aesthetics, Appearance, and Finish:** The surfaces through which imaging will occur should be transparent, and not result in any aberrations or otherwise compromise the quality of imaging. There should also be a mechanism to protect the experiments from light pollution.

2. Production Characteristics

- a. **Quantity:** The client needs a total of one microscope cell culture incubator.
- b. **Target Product Cost:** The target product cost is to be \$200, with an understanding that the product would enter the market for around \$500.

3. Miscellaneous

- a. **Standards and Specifications:** There are no standards and specifications to our knowledge that must be addressed within the design.
- b. **Customer:** Any customer preferences are already addressed above and have been taken into consideration.
- c. **Patient-related concerns:** The product will not have any contact with patients, so patient-related concerns are not applicable.
- d. **Competition:** There are a variety of systems that have been fabricated for similar purposes, but to the knowledge of the team the device we intend to create would be unique in cost, ease of use, and the ability to be used with a number of microscopes. Stage incubators on the market, such as the Pecon Incubation System 2000 fits all functional requirements of the client but is specifically tailored to fit the Olympus IX71/81 microscope. Ideally, we will be able to translocate our final prototype from one imaging system to another and it will be relatively universal. The majority of available systems also enclose the whole microscope, which limits the system's versatility.