Dr. Clean Bioreactor

Group 10: Iratxe Astigarraga, Vidal Cruz Ramos, Garrett Doty, Jack Drohan, Vitan Georgievski, Salomon Molko, Denisse Pena-Valerio

Department of Mechanical and Aerospace Engineering, University of Florida

Abstract & Product Functionality

The Doctor Clean Bioreactor grows bacteria cultures with little to no additional work to the user. The sanitation system incorporated in the bioreactor will be able to safely dispose of culture materials and sanitize the equipment used, making the system ready for the next experiment. The well plates and conical tubes, still filled with cell culture media, are placed into a sanitation chamber that will cull the cell cultures and dispose of solid and liquid waste, leaving sanitized well plates and tubes for future use. The Dr. Clean Bioreactor uses a manipulator system to interact with the cell cultures and other system features, including a fluid handling system capable of providing nutrients to cultures, a shaker system capable of linear, orbital, and double orbital motions to aerate cultures, and a climate-controlled storage system. Optical density and fluorescent intensity measurements will be taken frequently to monitor cell growth within the enclosure. The assembly can maintain consistent temperature within the interior to protect electrical components as well as a reasonable temperature on the exterior to allow for movement of the overall system within a laboratory environment. The Doctor Clean Bioreactor was designed for parameters set by the UF Biofoundry and to fulfill customer needs metrics outlined by industry experts. Prioritization of meeting these needs and outperforming previous iterations of bioreactor designs is critical to design evaluation and future performance in the final down-selection stages of the project.

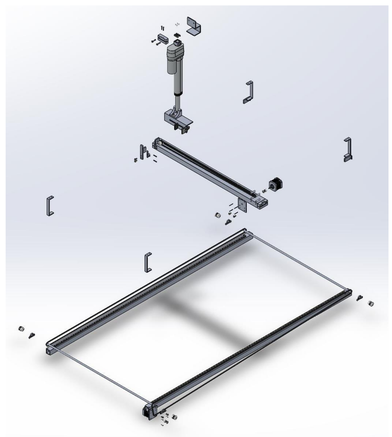
Cost

OTS Parts: $7138.17
Modified OTS Parts: $182.67
Raw Materials: $1001.02

Manufacturing Labor: $834.78
Energy Consumption: $55.44
Assembly Labor: $520.00

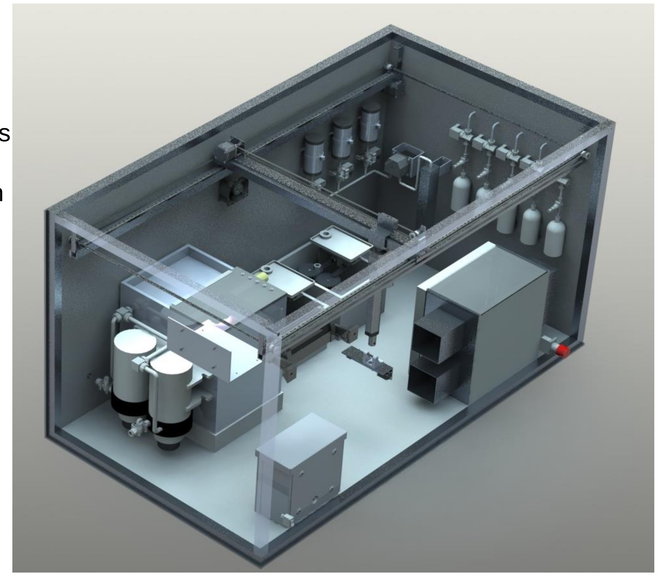
Manipulator

The manipulator subsystem is responsible for transporting well plates and conical test tubes across the entire structure. The system provides precise movement in the X, Y, and Z axes while securely griping the well plates. The movement in the X and Y directions is actuated through Nema-17 stepper motors, which rotate timing belts and are part of a larger pulley system for each drive. To ensure precision, tensioning blocks have been added to each belt. Movement in the Z-direction is controlled by a 6 inch linear actuator which is connected to the gripper and the X drive on opposite ends. The gripper itself operates with a lead screw a moveable jaw, and a fixed jaw.



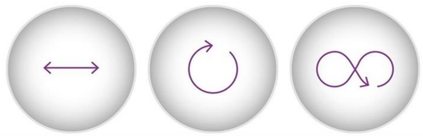
System Design and Key Features

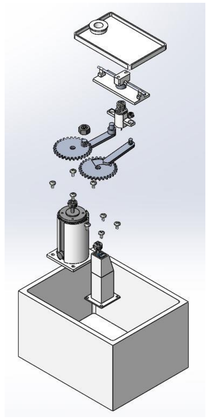
- Self-Sterilizing
- OD/FI Measurements
- 3 Mixing Patterns
- Gas Liquid and Nutrient Injection
- Automated Manipulator
- Insulated with Temperature Control



Shaker

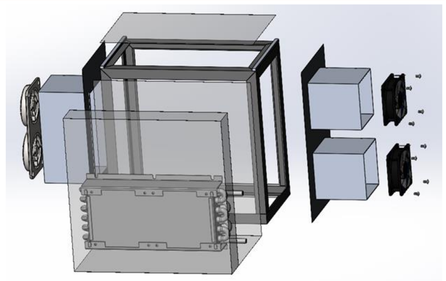
The shaker system is capable of linear, orbital, and double orbital motion patterns to aerate cell cultures. Double orbital motion is created by using two gears with rods connected at a central point. A gear shifter disengages the second gear for single orbital motion, and a scotch yoke mechanism creates linear motion.





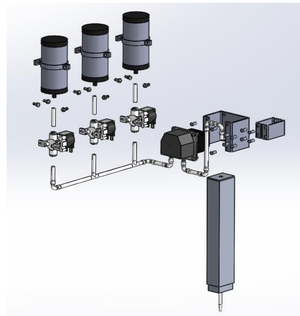
Environmental Management

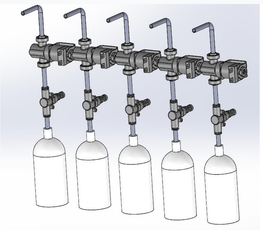
This subsystem is principally responsible for the consistent heating and cooling capabilities exhibited to the bacterial cultures. This includes a controllable heat exchanger which is rated for the temperatures outlined in the customer metrics, with control parameters from user inputted commands. The environment insulates temperature variation from affecting the electrical components of the overall assembly while achieving necessary bacterial culture growth during experimentation



Fluid Handling

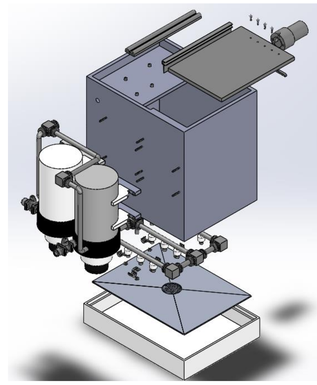
The fluid handling system addresses the needs of satisfactorily moving cultures suspended in liquid between compartments . The system includes three deposits, electromechanically controlled solenoid valves, a pump and a high accuracy dispensing valve, avoiding cross contamination .
The air injection system is completely automated and is capable of injecting, regulating and measuring the composition of different gases. The system also includes a filtering and gas evacuation mechanism .





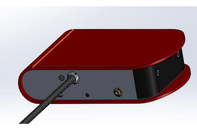
Sanitation

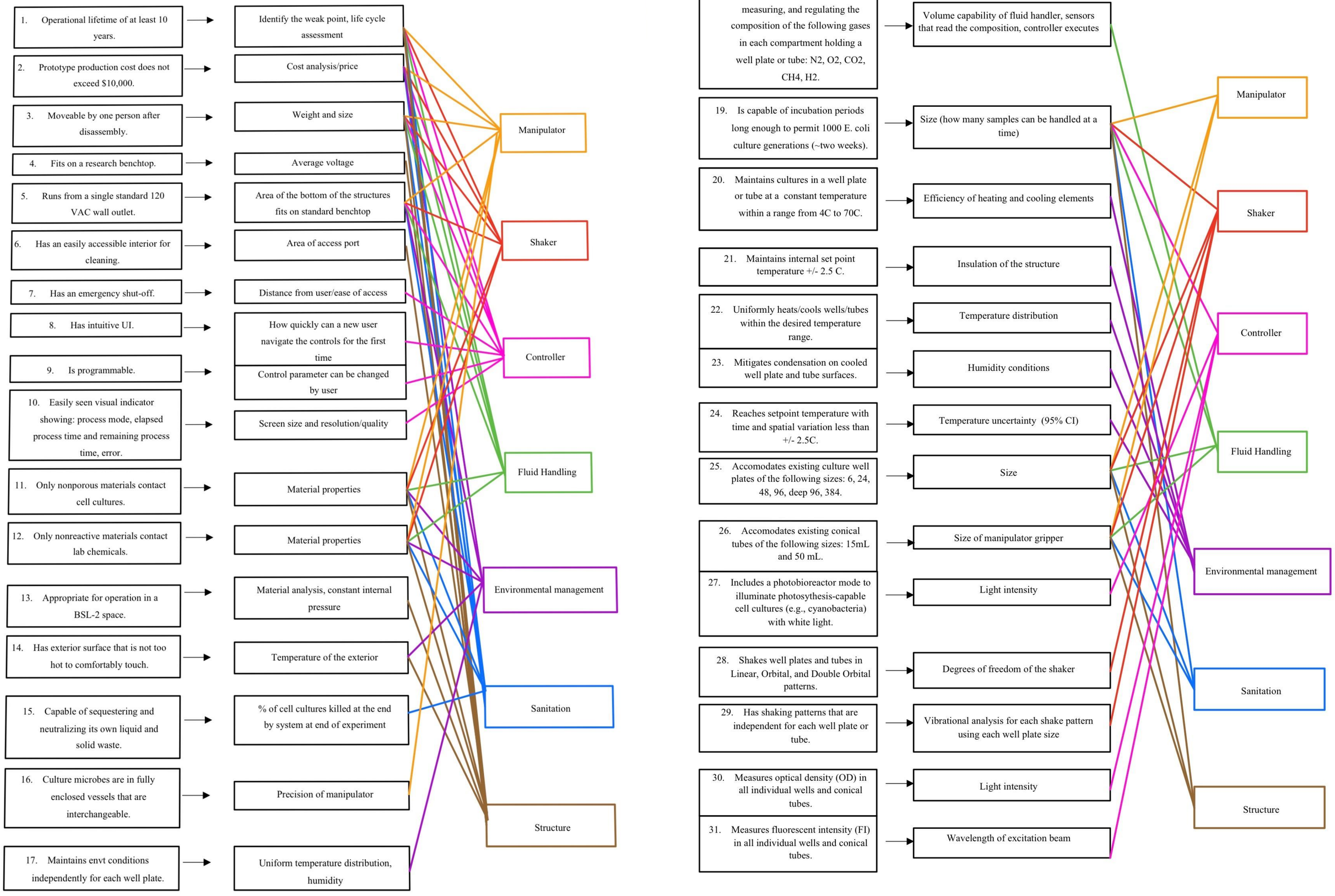
The sanitation subsystem consists of two fluid reservoirs (bleach and DI water) that are pumped into pipe sprayed through a nozzle into an enclosed structure to sterilize the well plates and tubes used while culling and disposing the bacteria after incubation. The base of the structure is 36x27 cm and consists of a drain an waste tray acting as a reservoir.

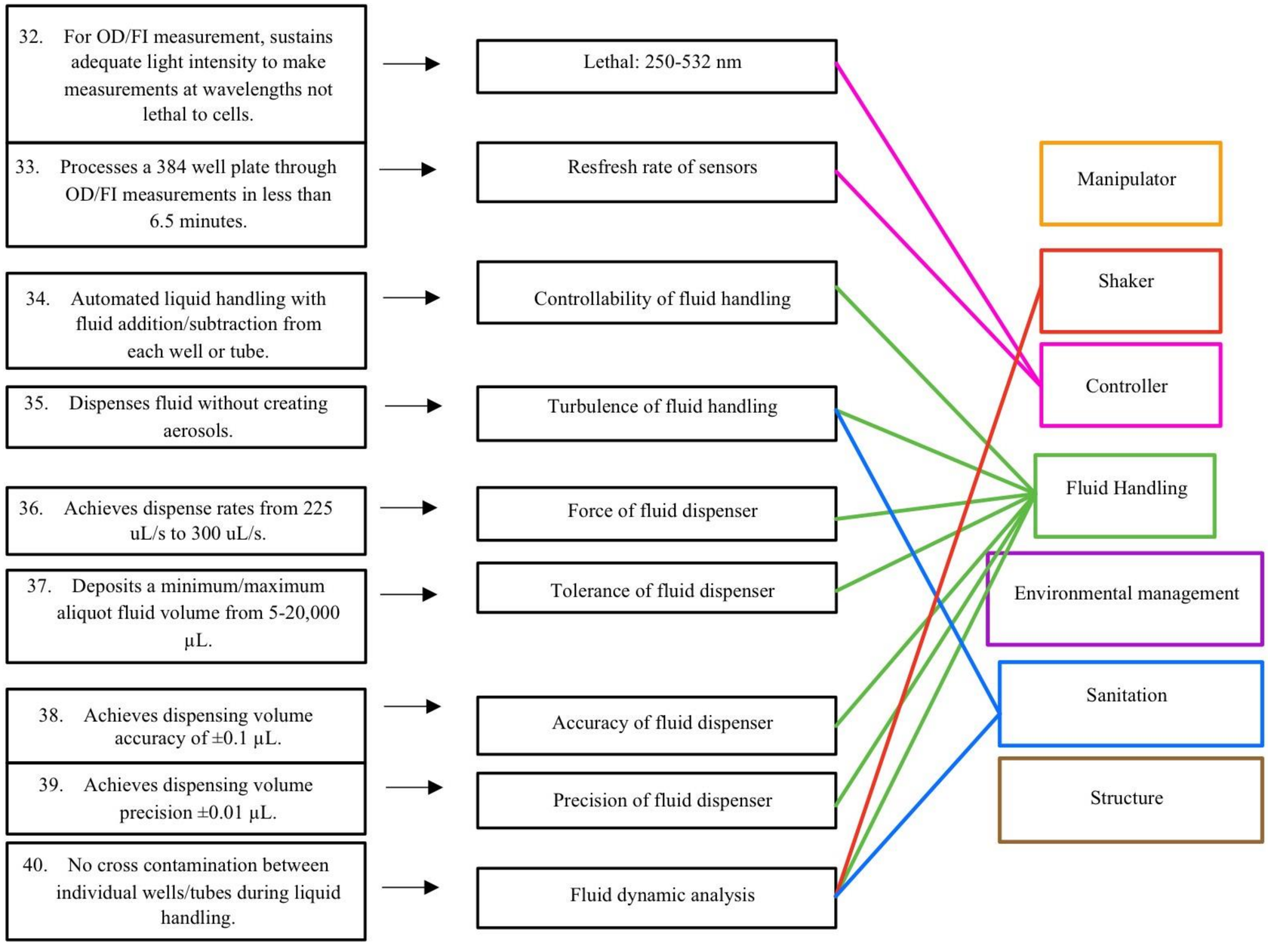


Controller

This subsystem uses 3 Arduino Uno Rev3s which handle the interaction between the processor and the motors, pumps, and anything that would require an on/off switch. The bioreactor mode is accomplished by directing the modified light bar onto the well plates. This light bar will have the standard yellow LEDs swapped out for RGB LEDs that can change the color to either red, green, or blue. The spectrometer probe will be mounted so that it takes measurements above the well plates or tubes using the manipulator arm as the guide.




1. Operational lifetime of at least 10 years.
2. Prototype production cost does not exceed $10,000.
3. Moveable by one person after disassembly.
4. Fits on a research benchtop.
5. Runs from a single standard 120 VAC wall outlet.
6. Has an easily accessible interior for cleaning.
7. Has an emergency shut-off.
8. Has intuitive UI.
9. Is programmable.
10. Easily seen visual indicator showing: process mode, elapsed process time and remaining process time, error.
11. Only nonporous materials contact cell cultures.
12. Only nonreactive materials contact lab chemicals.
13. Appropriate for operation in a BSL-2 space.
14. Has exterior surface that is not too hot to comfortably touch.
15. Capable of sequestering and neutralizing its own liquid and solid waste.
16. Culture microbes are in fully enclosed vessels that are interchangeable.
17. Maintains envt conditions independently for each well plate.
18. Is capable of safely injecting, measuring, and regulating the composition of the following gases in each compartment holding a well plate or tube: N2, O2, CO2, CH4, H2.
19. Is capable of incubation periods long enough to permit 1000 E. coli culture generations (~two weeks).
20. Maintains cultures in a well plate or tube at a constant temperature within a range from 4C to 70C.
21. Maintains internal set point temperature +/- 2.5 C.
22. Uniformly heats/cools wells/tubes within the desired temperature range.
23. Mitigates condensation on cooled well plate and tube surfaces.
24. Reaches setpoint temperature with time and spatial variation less than +/- 2.5C.
25. Accomodates existing culture well plates of the following sizes: 6, 24, 48, 96, deep 96, 384.
26. Accomodates existing conical tubes of the following sizes: 15mL and 50 mL.
27. Includes a photobioreactor mode to illuminate photosythesis-capable cell cultures (e.g., cyanobacteria) with white light.
28. Shakes well plates and tubes in Linear, Orbital, and Double Orbital patterns.
29. Has shaking patterns that are independent for each well plate or tube.
30. Measures optical density (OD) in all individual wells and conical tubes.
31. Measures fluorescent intensity (FI) in all individual wells and conical tubes.
Identify the weak point, life cycle assessment
Cost analysis/price
Weight and size
Average voltage
Area of the bottom of the structures fits on standard benchtop
Area of access port
Distance from user/ease of access
How quickly can a new user navigate the controls for the first time
Control parameter can be changed by user
Screen size and resolution/quality
Material properties
Material properties
Material analysis, constant internal pressure
Temperature of the exterior
% of cell cultures killed at the end by system at end of experiment
Precision of manipulator
Uniform temperature distribution, humidity
Volume capability of fluid handler, sensors that read the composition, controller executes
Size (how many samples can be handled at a time)
Efficiency of heating and cooling elements
Insulation of the structure
Temperature distribution
Humidity conditions
Temperature uncertainty (95% CI)
Size
Size of manipulator gripper
Light intensity
Degrees of freedom of the shaker
Vibrational analysis for each shake pattern using each well plate size
Light intensity
Wavelength of excitation beam
Manipulator
Shaker
Controller
Fluid Handling
Environmental management
Sanitation
Structure


32. For OD/FI measurement, sustains adequate light intensity to make measurements at wavelengths not lethal to cells.
33. Processes a 384 well plate through OD/FI measurements in less than 6.5 minutes.
34. Automated liquid handling with fluid addition/subtraction from each well or tube.
35. Dispenses fluid without creating aerosols.
36. Achieves dispense rates from 225 uL/s to 300 uL/s.
37. Deposits a minimum/maximum aliquot fluid volume from 5-20,000 µL.
38. Achieves dispensing volume accuracy of ±0.1 µL.
39. Achieves dispensing volume precision ±0.01 µL.
40. No cross contamination between individual wells/tubes during liquid handling.
Lethal: 250-532 nm
Resfresh rate of sensors
Controllability of fluid handling
Turbulence of fluid handling
Force of fluid dispenser
Tolerance of fluid dispenser
Accuracy of fluid dispenser
Precision of fluid dispenser
Fluid dynamic analysis
Manipulator
Shaker
Controller
Fluid Handling
Environmental management
Sanitation
Structure