

PETRI (Precision Environmental Thermal Regulating Incubator) Complete Microbioreactor is an autonomous microbioreactor device specifically designed with the intent of creating the most versatile and user-friendly product, all without sacrificing cell culturing capability. This is achieved through a combination of automated cell culture processes, an intuitive user interface, and a unique modular tool head system featured in the device. Because the machine performs complex culturing functions autonomously via closed loop control, the need for experienced human intervention is obsolete, making cell culture processes accessible to users of varying familiarity and skill levels. In practice, a user will only be required to simply insert the well plate or tube, fill the feedstock container, and enter the experimental parameters. Additionally, as cell culture conditions are monitored locally on a front panel interactive touchscreen and set of LED status indicators, the user can be kept up to date on the current state of the process and the changes that have incurred. In the event of a malfunction, the device is also enabled to be easily shut down with an immediately accessible and distinguished emergency stop button. Within the machine, actuation and measurement devices are used interchangeably to perform various cell culturing processes and come in the form of separate compact tools, all standardized with the same footprint for system compatibility and exchange. Depending on the desired culturing process, appropriate tools can be selected and mounted by the user on the device's designated tool rack before process initialization. In operation, the tools will be magnetically attached and detached by a lead screw driven moving tool head. Currently available are a fluid handling actuation tools, capable of fluid addition and subtraction, along with OD/FI measurements tool, capable of monitoring cell conditions. With these default tools, any novice can perform the related culture processes involving their use. For more experienced users or researchers, extra tool heads can be designed and added to further expound upon the functionalities if designed for system compatibility and proper reprogramming is performed. By the featured tool head system, the functionalities are virtually endless, equipping **PETRI** with unparalleled versatility to adapt with the ever-advancing field and practices discovered within.

PETRI

Complete Microbioreactor

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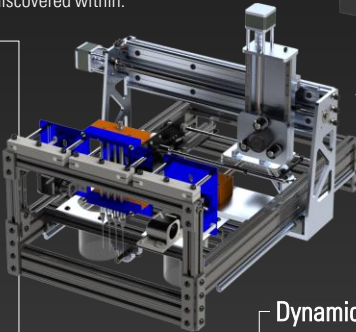
PETRI Major Subsystems & Features

- Modular Tool Head System:
 - Modular Tool Head Exchanger
 - Modular Tool Heads
- Dynamic Conical Tube and Plate Shaker
- Electronics and Controls Subsystem
- Gas and Temperature-Controlled Environmental Chamber



Modular Tool Head Exchanger

Actuation of the modular tool heads is performed by an automatic tool changer. The tool changing mechanism, mounted on a 3-D gantry to align with well plates or conical tubes, consists of a high strength, heat resistant electromagnet along with ball bearings that form a kinematic coupling to mate to the various tools with high repeatability. Through this system, incorporating additional functionality as needed can be performed by simply adding compatible tool head to the tool rack.



Electronics and Controls Subsystem

Supplies all the power and computation/control of the system including
Power Distribution: 500 W Power supply with ability to add more modules for additional power requirements. Supplies power at 12 or 24 V DC.
Controllers: Raspberry Pi 4 and RUMBA control modules are used to control the stepper motors and perform operations based on programmed parameters or user inputs. Linux provides a stable long-term support operating system with full control of user repositories and system contents.
Gas Control: Five high pressure individually controllable solenoids provide a safe way to choose the type of gas supplied to the system. An MKS mass flow controller allows closed-loop control to be implemented for gas control on a real-time and long-term basis.
User Interface: A 7-inch touch screen panel allows for intuitive user interaction with the control components of the system. Expandable interface options are available through additional USB 3.1 ports within the control module. The computer is fully-programmable by default.

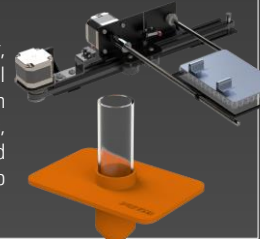
Machine Specifications

- Overall Dimensions: 24" x 42" x 28"
- Energy Consumption: 500 W
- Labor: 45 hrs
- Price: \$7342.08
- MFG: 40 hrs
- OTS Parts: \$5264.86
- Assembly: 5 hrs
- Manufactured Parts: \$953.60
- Labor \$1123.62

Dynamic Tube and Plate Shaker

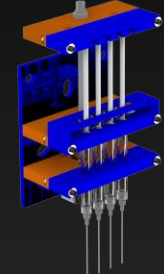
Apart of the motion system within the device is an electronically controlled 2-D plotter that employs a belt and lead screw to enable dynamic shaking of conical tubes or well plates when mounted with their signature holder. By this mechanism, an infinite number of shaking patterns at large amplitudes can be performed to achieve proper cell aeration and distribution of nutrients.

Patterns include, but are not limited to, linear, orbital, and double orbital configurations. And, given the motors' capabilities, various speeds and frequencies are also enabled.



Modular Tool Heads

Optical Measurement Tool Head: OD and FI measurements are performed using a combination of an LED, a focusing lens, a filter cube, and two photodiodes. A vertical and horizontal configurations are available for well plate and conical tube adaptability.



Fluid Handling Tool Heads: A 4-syringe or single-syringe option includes a NEMA 11 Stepper Motor and M5-0.125 lead screw to achieve:

- Dispense Precision: $\pm 0.028 \mu\text{L}$
- Dispense Rate: 302.2 $\mu\text{L/s}$

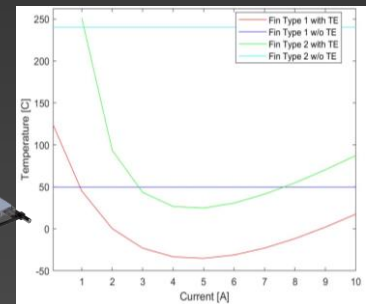
Tool Head Modularity: With footprint and electronic compatibility, actuation and measurement functionalities can be expanded as more tool heads are developed and made available.



Environmental Chamber



Temperature Control: Peltier elements and finned Aluminum 1100 heat sinks comprise the thermoelectric (TE) cooling and heating system within the inner chamber. Optimal culturing temperatures within the 4 - 70°C range can be achieved and regulated via closed-loop control by an internal temperature sensor.



Temperature distribution for various heat sink designs. Fin Type 1 (pictured above) demonstrated optimal performance and is used in PETRI's TE cooling system.

Gas Control: PETRI's exterior features 5 intake valves to allow simultaneous intake of Hydrogen, Carbon Dioxide, Methane, Nitrogen, and Oxygen. High-pressure solenoids and check valves ensure redundancy and precision for controller-injection of individual or mixed gases within the chamber to create optimal cell culturing conditions. Gas exhaust is ejected through the chamber's ceiling valve for subsequent plumbing to a laboratory's ventilation system.

Customer Needs

- 1 Fit inside a research facility on a benchtop
- 2 120V AC electric powered from standard wall outlet with 15-amp breaker
- 3 Easily accessible to a user of average height and build
- 4 Can be assembled/disassembled with minimal know-how
- 5 1.15 factor of safety
- 6 Fail-safe system and software alert that informs user of culture condition data
- 7 Emergency shut-off actuated by user
- 8 Automatic force-based safety limit shutoff
- 9 Visual Indicator
- 10 Minimum operational lifetime of 10 years
- 11 Cost less than \$10,000
- 12 Does not require permanent external fixtures or support apparatus
- 13 Programmable
- 14 Intuitive user interface
- 15 Nonporous and nonreactive materials
- 16 Meet BSL-2 lab space requirements
- 17 Exterior surface does not exceed 55°C
- 18 Incubation/hibernation periods of up to 2 weeks
- 19 Perform culture condition closed-loop control
- 20 Culture at a temperature range from 4°C to 70°C
- 21 Culture microbes in enclosed and interchangeable vessels that allow for media exchange
- 22 Accommodate culture plates of sizes 6 ,24 ,48, 96, deep 96, and 384
- 23 Accommodate conical tubes of sizes 15mL and 50 mL
- 24 Uniformly heats wells and tubes to prevent condensation
- 25 Automated liquid handling that permits new fluid addition and subtraction from each well and tube
- 26 Capture effluent gas
- 27 Gas controller that injects and regulates nitrogen, oxygen, carbon dioxide, methane, and hydrogen
- 28 Measure OD and FI in individual wells and tubes
- 29 Designed with respect to trade-offs in using monochromators vs. filter cubes for FI readings
- 30 Achieve a light intensity of 1 kW/cm² for several minutes
- 31 Has linear, orbital, and double orbital shaking patterns
- 32 Shaking patterns and atmospheric conditions are independent for each plate/tube
- 33 Achieve a dispense rate of 300uL/second and a minimum of 225 uL/second for well plates
- 34 Deposit a minimum and maximum volume of 5-20,000 uL in 1 uL increments with an accuracy of ± 1 uL or 2%, and a dispensing precision of less than 2% at 50-200 uL
- 35 Dispose and neutralize waste

Fluid Handling

- 22 Well plate carriage that fits standard well plate sizes
- 23 Tube carriage that fits standard conical tube sizes
- 25 Fluid handling tool with syringes (1.12 mm diameter tip)
- 33 Dispense rate of 302.2 uL/second
- 34 Minimum volume of 0.028 uL
- 35 Beakers for disposal/waste

Machine Enclosure

- 1 24" x 48" x 23" dimensions
- 3 Touchscreen panel height of 46.7" from floor
- 4 80/20 Frame
- 5 Frame of system has a factor of safety of 175
- 7 Red stop push button
- 9 4 LED status indicator lights
- 10 Aluminum sheet metal with 80/20 frame
- 17 Polyurethane spray foam insulated wall panels

Environmental Management

- 12 Removable gas input nozzles, high pressure solenoids, exhaust tubing that connects to fume hood
- 16 Gas control/ventilation with gas aerosol prevention
- 18 Radwell Mass flow controller
- 20 12V TEC1-12710 Thermoelectric Peltier heating element with 5V DC cooling fans
- 24 Heating/cooling element on enclosure's back wall
- 26 Outlet on top of enclosure that connects effluent gas to fume hood
- 27 5 high pressure solenoids connected to a single gas manifold connected to a mass flow controller

Culture Processing

- 15 Polypropylene syringes, borosilicate glass waste beakers, glass well plates and conical tubes
- 21 Well plates have removable top covering
- 28 OD / FI measurement tools for well plates and tubes
- 29 Uses filter cubes
- 30 Cree Xlamp XM-12 High Power LED
- 31 Lead screw/belt driven system capable of movement in x and y axes
- 32 Fluid handling/culture processing system holds one well plate or tube at a time

Electronics and Controls

- 2 24V 500W Power Supply
- 6 End stops and limit switches with touchscreen alert
- 8 Raspberry Pi motor force readings
- 11 Inexpensive controllers and motor
- 13 Raspberry Pi 4 controller with Linux OS
- 14 Computer uses Linux, a common OS
- 19 Raspberry Pi, temperature sensors, OD/FI readings