Build Guide:

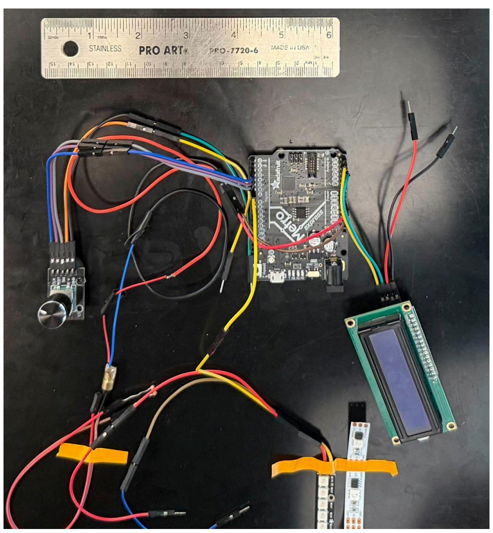
All control hardware for the OptoPACE bioreactor can be built using an Arduino, a breadboard, and basic components detailed in the methods and BOM files. The following guide details how to assemble components in schematic and picture format and assumes you are using a 30-position breadboard. Smaller boards with as few as 20 positions can also be used without repositioning.

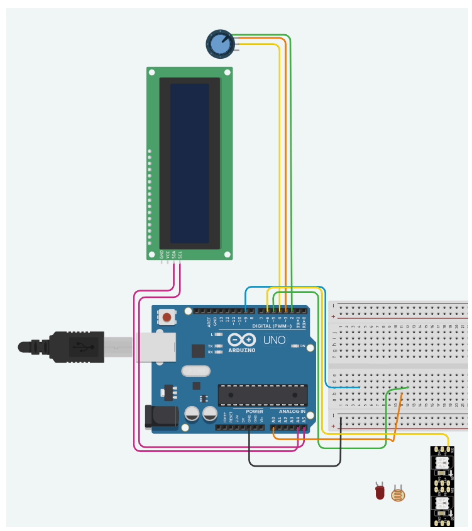
Required materials:

1x Arduino microcontroller, 1x breadboard, 1x 5V laser, 1x photoresistor, 2x DC motors with attached magnet (glued), 1x peristaltic pump, 1x NeoPixel RGB LED strip, 1x NeoPixel UV LED strip, 1x I2C LCD screen, 1x rotary encoder, 2x IRLB8721 MOSFETs, 1x 1000 µF 16V polarized capacitor, 1x 10k Ω resistor, 2x 1k Ω resistor, 2x flyback diodes, 1x 12V power supply, 1x 5V power supply (or 1x 12V/5V integrated power supply).

Step 0: Connect your Arduino components.

Many of the elements in this bioreactor are controlled, powered, or read out from the Arduino. This includes the I2C LCD screen, rotary encoder, peristaltic pump, DC motors, photoresistor, and NeoPixel LED strip controls.

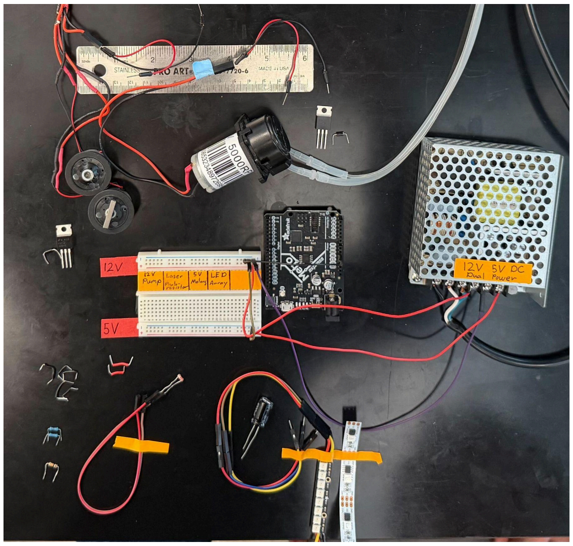


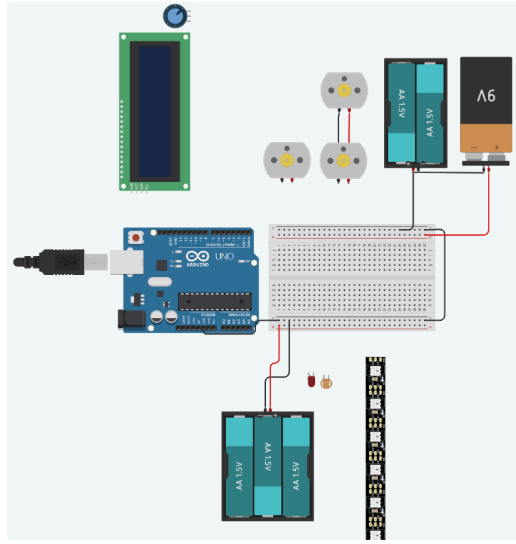


Step 1: Setting up your breadboard.

Before connecting the circuits, establish your power supplies and your grounding. The OptoPACE bioreactor has both 5V and 12V components that need to be controlled from the same board. Your breadboard will have 2 rails to connect power to that are isolated from each other. Connect the grounds of both rails and include the ground pin of the Arduino. If you are using a dual 5-12V power supply both will connect to a common ground anyway. The Arduino can be powered by USB or powered from your 5V power supply.

- Connect 12V (**+30, -25**) and 5V (**+1, -3**) power to opposite rails on the breadboard
- Connect 12V and 5V ground between rails (**-30, -30**)
- Connect Arduino ground to 12V and 5V ground at ground rail (**-1**)

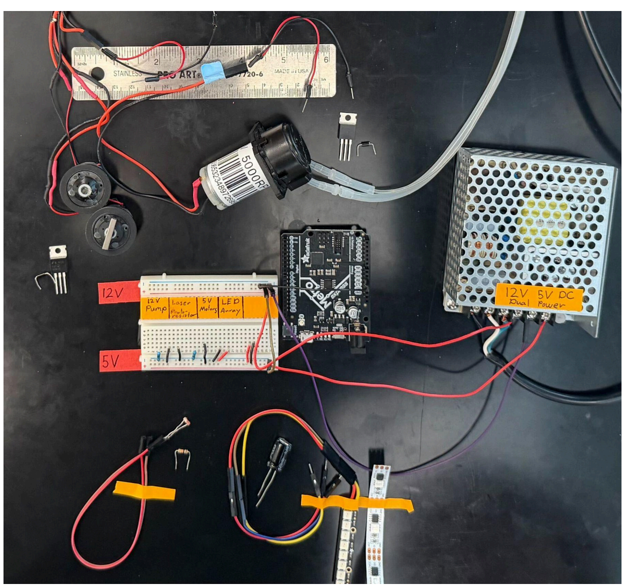


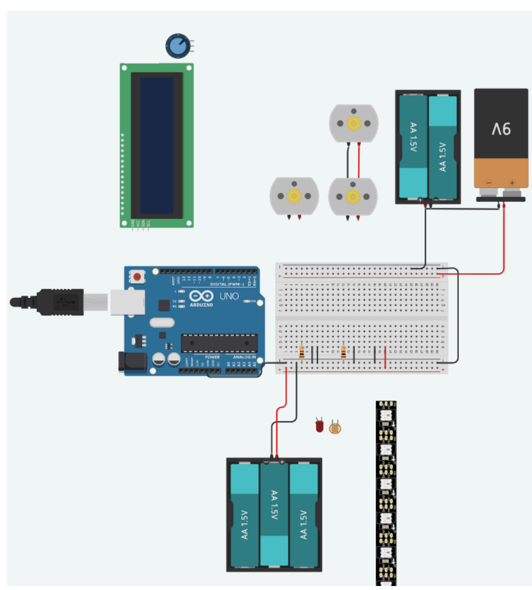


Step 2: Wiring power and initial component connections to your breadboard.

After connecting your power supplies to the outside rails, connect 1k Ω resistors and 5V power to inside rails. These will be used in the setup for power and control of the peristaltic pump, DC motors, and NeoPixel LED strip.

- Connect 1x 1k Ω resistor between ground and breadboard rail (**b4**)
- Connect 1x 1k Ω resistor between ground and breadboard rail (**b12**)
- Connect breadboard rails to ground (**a6, a7, a14, a18**)

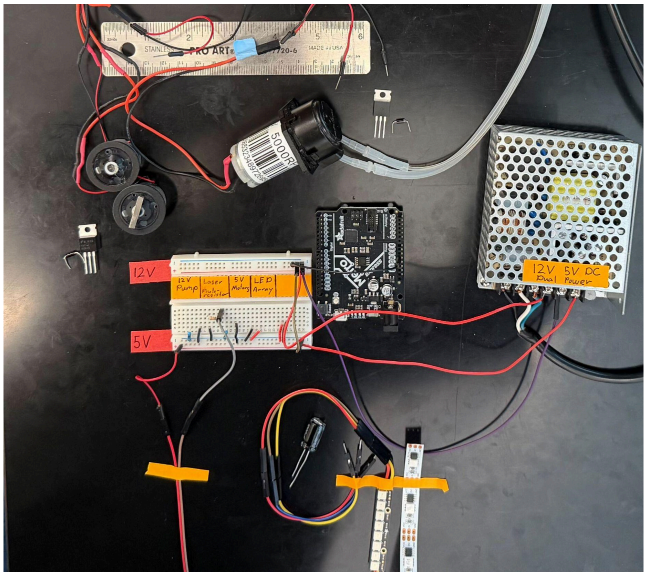


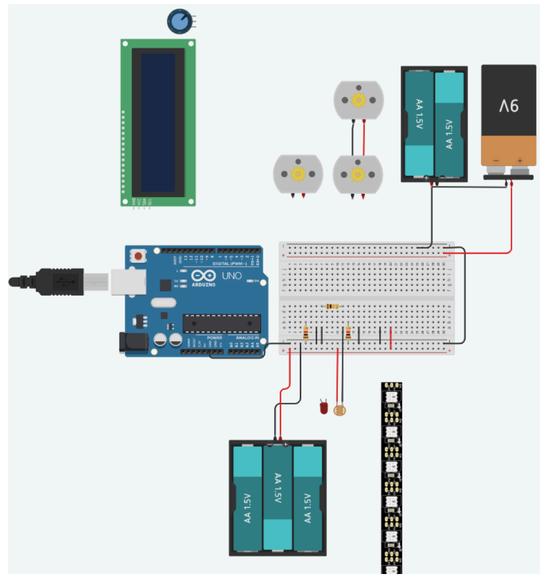


Step 3: Wiring photoresistor to your breadboard.

Powering and collecting data from the photoresistor requires an additional resistor to create a voltage divider, which converts a change in resistance due to light into a measurable voltage difference, which we collect as our measurement of turbidity.

- Connect 1x 10k Ω resistor between breadboard rails (**e7, e11**)
- Connect one end of photoresistor to breadboard rail (**a11**)
- Connect other end of photoresistor to 5V power rail (**+10**)

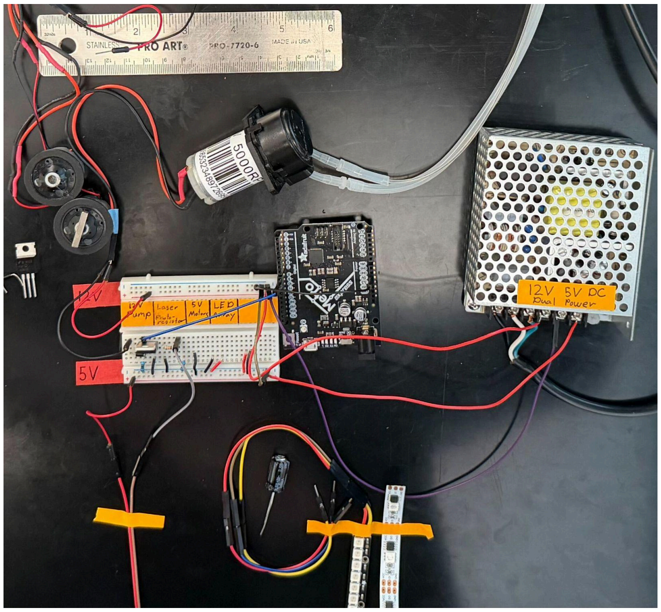


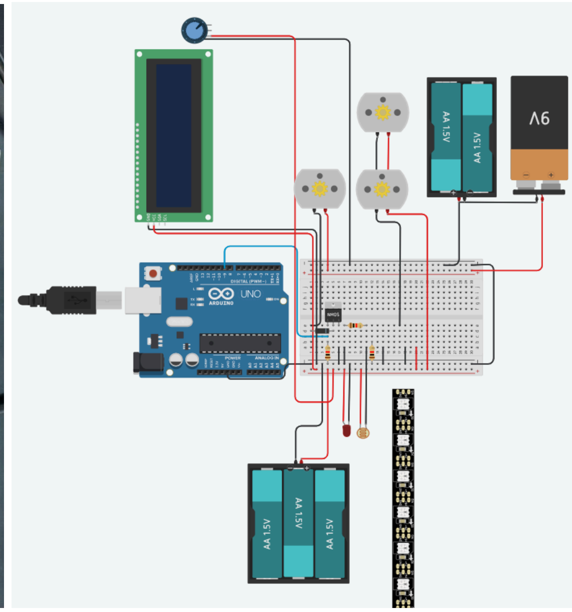


Step 4: Powering peristaltic pump and various other components.

To control the peristaltic pump, a MOSFET is required for high-speed voltage-controlled switching, and so a MOSFET should be installed on the breadboard. The IRLB8721 MOSFET is proficient in high switching loads with low voltages (3V/5V). Further, it is important to note that the peristaltic pump should be powered with 12V, in contrast to the various other components, which use 5V power.

- Connect 1x IRLB8721 MOSFET to breadboard rails (**e4, e5, and e6**) (gate to e4, drain to e5, and source to e6)
- Connect 1x flyback diode between breadboard rails (**d1 and 5**)
- Connect Arduino pin 9 to breadboard rail (**c4**) (gate)
- Connect peristaltic pump power to 12V power rail (**+4**)
- Connect peristaltic pump negative terminal to breadboard rail (**e1**)
- Connect laser to 5V power rail (**+7**)
- Connect laser ground to universal ground (**-8**)
- Connect I2C LED screen power to 5V power rail
- Connect I2C LED screen ground to universal ground (**-2**)
- Connect rotary encoder power to 5V power rail (**+5**)
- Connect rotary encoder ground to universal ground (**-8,-9**)

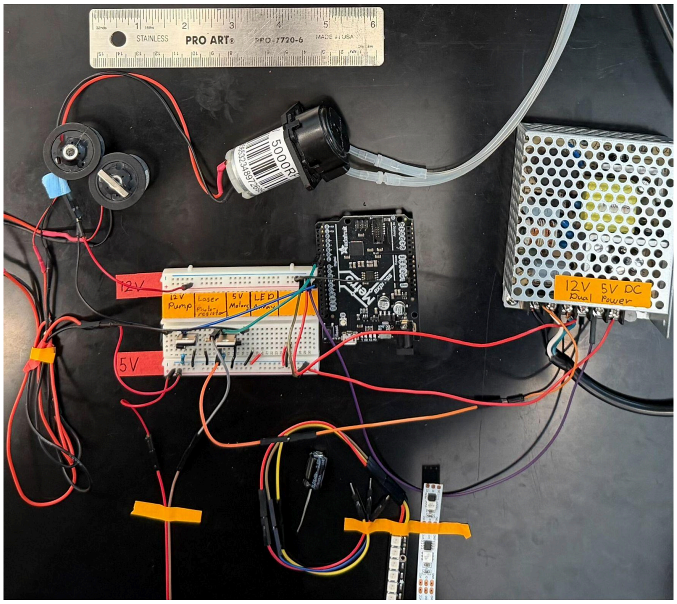


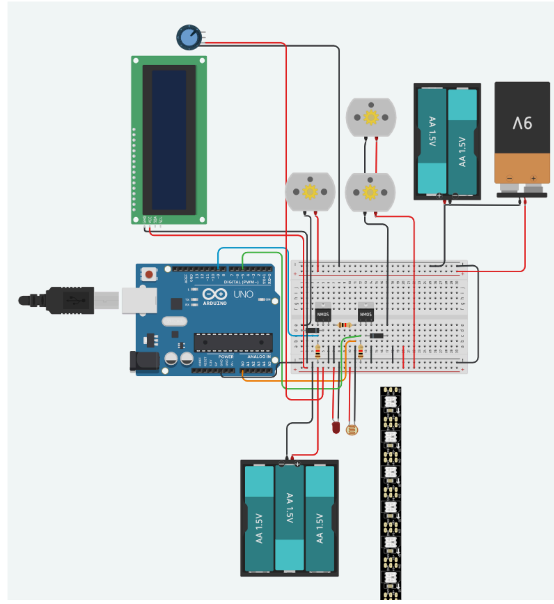


Step 5: Powering DC motors.

Like the peristaltic pump, DC motors require a MOSFET for high-speed voltage-controlled switching, and so a second MOSFET should be installed on the breadboard to control the DC motors. The 2x DC motors should be connected in parallel with one another so that they are easily controlled with one Arduino function and spin at the same speed. The DC motor should be powered with 5V, not 12V like the peristaltic pump is.

- Connect 1x IRLB8721 MOSFET to breadboard rails (**e12**, **e13**, and **e14**) (gate to e12, drain to e13, source to e14)
- Connect 1x flyback diode between breadboard rails (**c13 and c17**)
- Connect Arduino pin 5 to breadboard rail (**c12**) (gate)
- Connect 2x DC motors in parallel with one another
- Connect DC motor power to 5V power (**+22**)
- Connect DC motor ground to breadboard rail (**a17**)

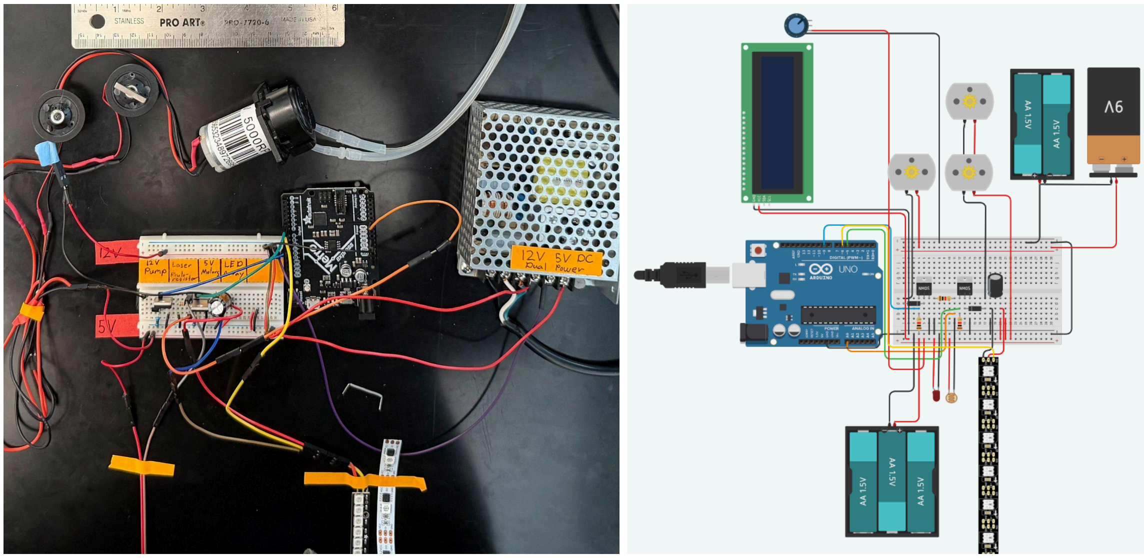




Step 6: Powering the NeoPixel LED strip.

After connecting your peristaltic pump and DC motors with MOSFET, the final major component that requires a large portion of the breadboard is the NeoPixel LED strip, which requires a capacitor with high capacitance to absorb power spikes, stabilize voltage, and buffer sudden current draws, particularly when first powering the component in order to protect it. It is important to take note that this capacitor is polarized, meaning the direction in which it is connected matters. The positive terminal of the capacitor (longer side) should be connected to breadboard rail 20, and the negative terminal of the capacitor (shorter side) should be connected to breadboard rail 18. The NeoPixel RGB LED strip and NeoPixel UV LED strip are connected in series so they can be controlled as a single strip of lights.

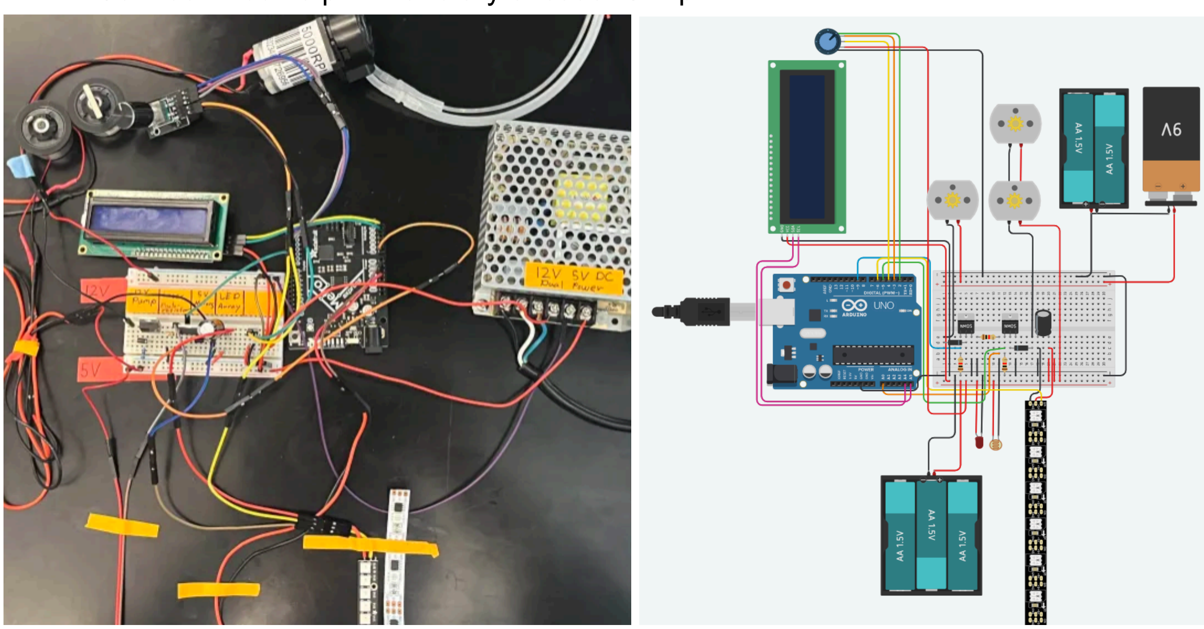
- Connect 1x 1000 µF 16V polarized capacitor to breadboard rails (**e18** and **e20**) (negative to e18, positive to e20)
- Connect 1x NeoPixel RGB LED strip and 1x NeoPixel UV LED strip in series
- Connect NeoPixel LED strip power to breadboard rail (**c20**)
- Connect NeoPixel LED strip ground to breadboard rail (**c18**)
- Connect breadboard rail c20 to 5V power (**c20**, **+20**)
- Connect breadboard rail c18 to universal ground (**c18**, **-18**)
- Connect Arduino pin 6 to NeoPixel In pin



Step 7: Making final connections to your Arduino for the full setup.

The final step is to make the final connections (photoresistor, I2C LCD screen, and rotary encoder) to the Arduino microcontroller. Refer to step 0 for a clear schematic of the Arduino connections.

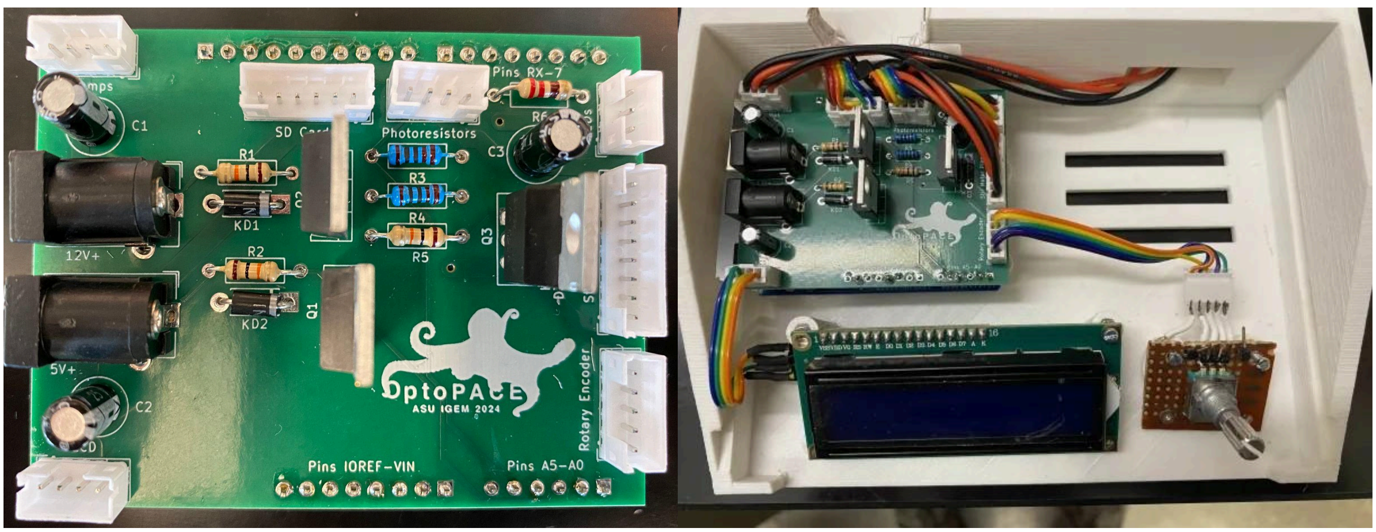
- Connect Arduino pin A0 to breadboard rail (**b11**)
- Connect Arduino pin A4 to I2C LED screen SDA pin
- Connect Arduino pin A5 to I2C LED screen SCL pin
- Connect Arduino pin 2 to rotary encoder CLK pin
- Connect Arduino pin 3 to rotary encoder DT pin
- Connect Arduino pin 4 to rotary encoder SW pin



Extended Build with PCB.

Given the simple control circuit, ordering a PCB is not economical, unless multiple bioreactors are being built. However, to make our design even more accessible for kit-based builds or educational projects, we designed a printed circuit board for our system and simplified the wiring of components with an Arduino shield design.

Taking the circuit diagrams from TinkerCAD, we created a schematic and then completed the PCB layout for our custom circuit board in KiCad. While designing our circuit we added labels for where wires were supposed to connect and redesigned how our system used capacitors. We included a current-regulating capacitor for each power bus (5V and 12V) and an additional capacitor next to our stir motors to absorb voltage spikes. Additionally, we added additional pins for the system to be expanded upon while still using the same circuit board. Additional pins remain available for extra stir motors, photoresistors, lasers, and pump.

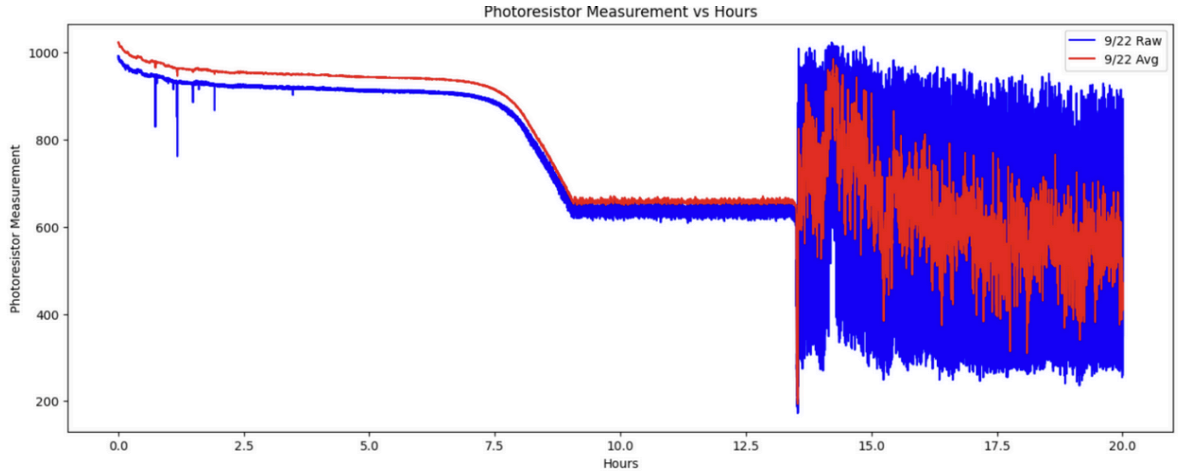


Calibration and Stress Testing:

Running PACE and Calibration Function: Running the “Run PACE” and “Calibration” submenus, should activate the stir motors, photoresistor, and in the case of “Run PACE,” the pump. These can not be stopped from the interface and require the user to unplug the power sources from the system to reset the hardware. Cleaning Function: allows the user to input a set time for cleaning. This function halts other functions such as “Run PACE” but the previous program will resume after cleaning ends.

Photoresistors have high tolerance; however, laser alignment will change the initial measurements every time it is set up or adjusted. A test culture must be run with the stir motors to calibrate mid log growth phase measurements. Alternatively a culture can be grown to a desired OD600 measurement and re-measured in the turbidostat to determine the correct voltage reading for desired conditions.

Once a set point has been entered into the turbidostat firmware, we strongly recommend a stress test, leaving the turbidostat to run to the point of failure, either by a faulty component or by running out of media. Once air is introduced into the culture or media falls below the laser level, turbidostat readings will become overwhelmed by noise.



Stress Test Figure: Turbidity measurements from a 20 hour continuous run. Early measurements required an hour to stabilize before giving consistent readings. Turbidity set point was 650, which was achieved after 9 hours of culture. The turbidostat was maintained for the next 4 hours before the media bottle ran out, giving high noise readings as air bubbles were introduced from the empty line.

PCB Files:
https://github.com/BioProteanLabs/OptoPACE_Bioreactor/tree/main/Gerber_Files

Bill Of Materials:
https://github.com/BioProteanLabs/OptoPACE_Bioreactor/blob/main/OPB_BillOfMaterials.pdf