

DIYNAFLUOR Build and Validation Instructions

Version 0.5

27/11/2024



Background

This guide provides the necessary instructions to build and validate a Diynafluor fluorometer. It assumes you have already acquired all parts from the complete BOM, including all 3D printed parts.

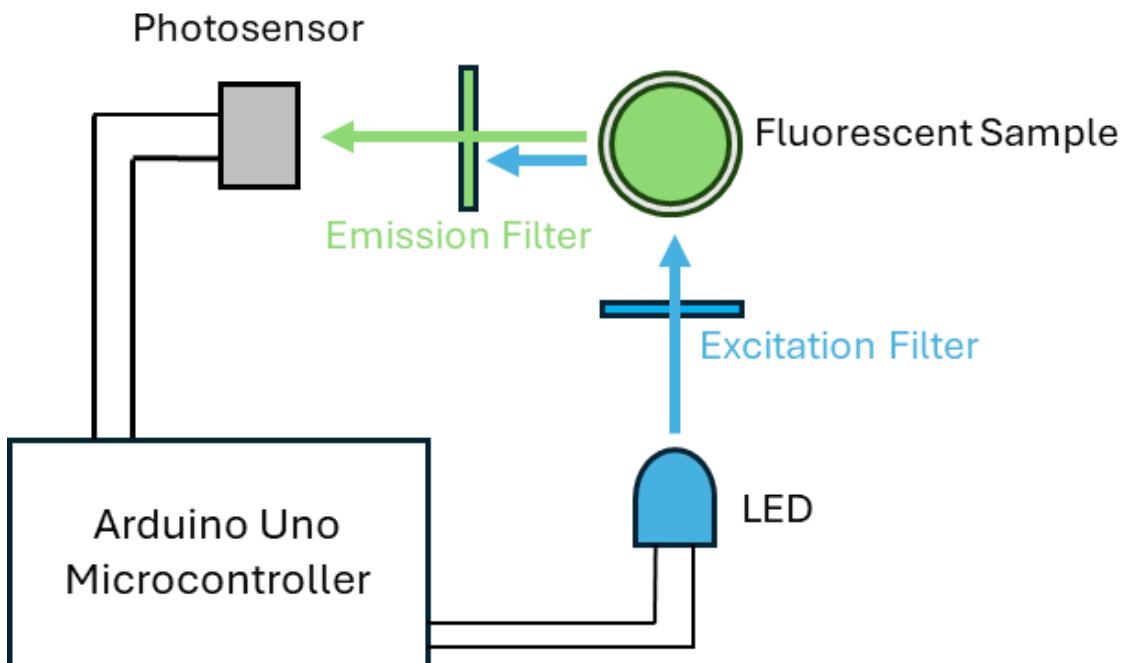
The guide is segmented into 4 sections that can be completed sequentially at the builder's leisure. These are:

1. The Filter Cube assembly.
2. The Body assembly.
3. The software installation instructions.
4. A validation assay to ensure the Diynafluor is working correctly.

Device Operating Theory

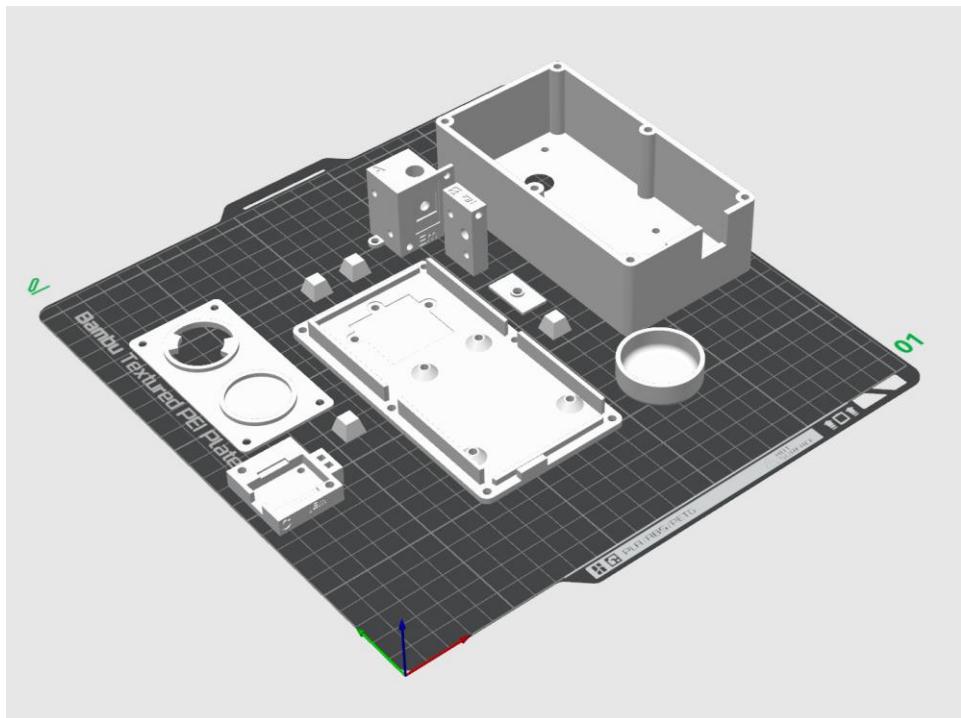
The Diynafluor, at its core, is an extremely simple, single channel fluorometer. It excites a fluorescent sample at ~470 nm using a blue LED and detects emission photons at ~535 nm using a photosensor. It contains an excitation light filter in front of the LED to reduce noise from unwanted wavelengths of light, and an emission filter in front of the photosensor to block the excitation light source from the LED. The LED and the Photosensor are controlled by an Arduino Uno Microcontroller, which simply turns on the LED, and records the fluorescent values obtained by the Photosensor from the sample.

During the Diynafluor build, it can be useful to remember and consider its general operating theory, which is outlined in the below schematic. This can help ensure that your interpretation of the build instructions is correct.

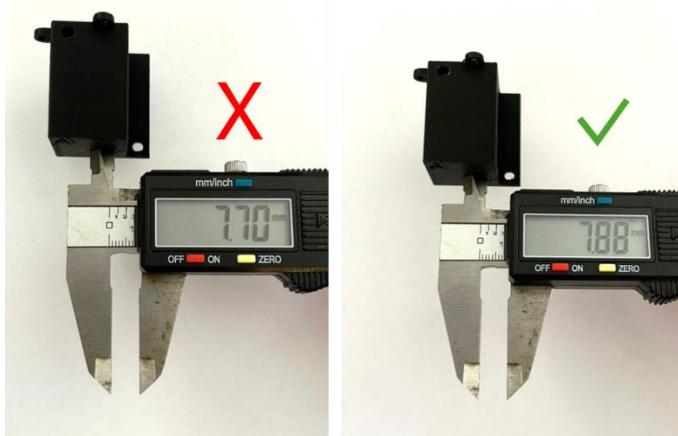


3D Print Guidance

- Print parts with 200 µm layer height on a smooth print plate without supports or rafts.
- Print all core parts with a Matte Black PLA. (Excluding the ‘Vanity Plate’ and ‘DINAFLUOR Logo’ parts, which can be printed if desired in any filament colour – See the appendix section for print details).
- The provided STL files should be orientated in the correct direction by default, however, refer to the below figure to check correct orientation for printing.



- 0.2-0.5 mm tolerances were designed into all connecting parts, which should be easily achieved with a calibrated desktop 3D printer. We have identified the sample well in the ‘Main Body (A)’ part as a good indicator of a calibrated printer, where prints should attempt to achieve an interior diameter of the sample well of 7.8-8, as shown below. Values above or below this indicates under or over-extrusion, or a need to slow print speeds. Poor print tolerances in the filter cube parts can result in difficult assembly and the 0.5 mL thin-wall PCR tubes interfering with the walls of the sample holder.



Filter Cube assembly instructions

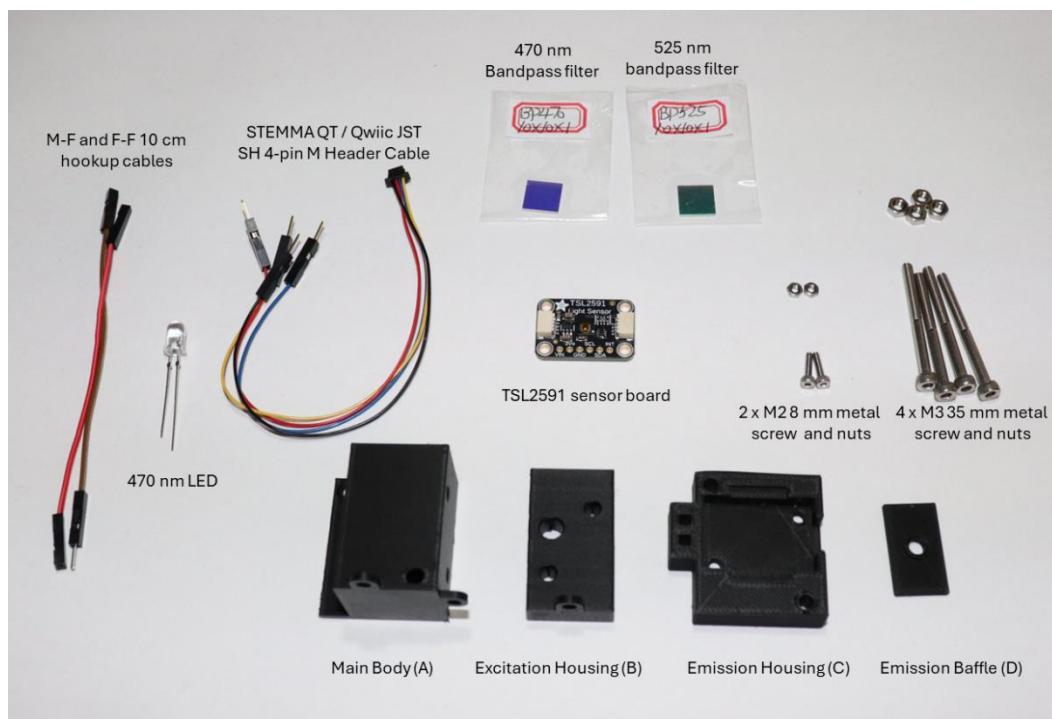
Filter Cube BOM

Commercial parts

- 4 x M3 35 mm metal screw (Torx head)
- 4 x M3 nut
- 2 x M2 8 mm metal screw (Torx head)
- 2 x M2 nut
- 1 x 470 nm LED (C503B-BAS-CZ0A0452)
- 1 x 470 nm Bandpass filter 10x10x1 mm
- 1 x 525 nm bandpass filter 10x10x1 mm
- 1 x TSL2591 sensor board
- 1 x STEMMA QT / Qwiic JST SH 4-pin M Header Cable - 150mm Long (P4209B)
- 1 x M-F hookup cable (10 cm)
- 1 x F-F hookup cable (10 cm)

3D printed parts

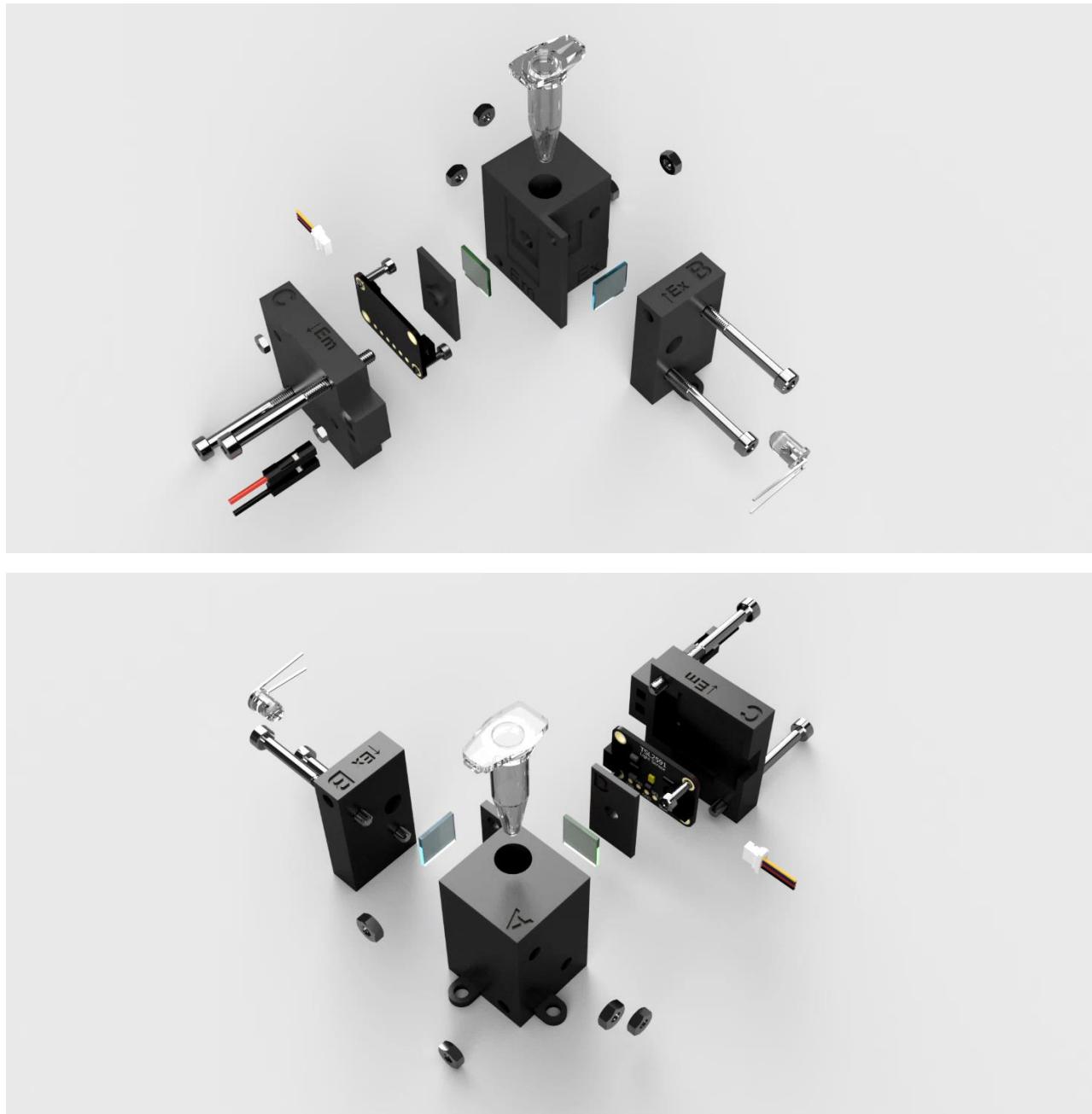
- 1 x Main Body (A)
- 1 x Excitation Housing (B)
- 1 x Emission Housing (C)
- 1 x Emission Baffle (D)



Required Tools

- M3 Torx Driver (or 2.5 mm Allen Key)
- M2 Torx Driver (or 1.5 mm Allen Key)
- Hobby knife or Scalpel
- Side-cutters

Filter Cube Exploded Diagram



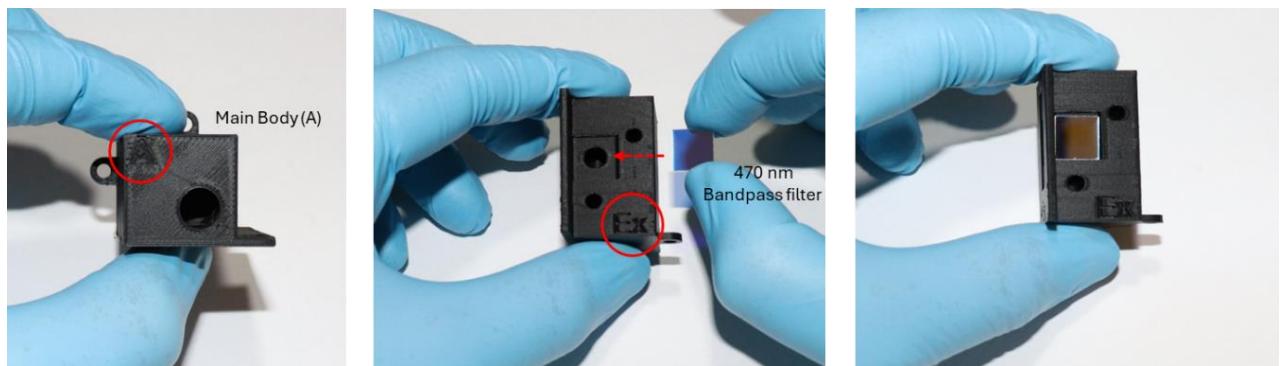
A front and rear exploded diagram of the Filter Cube is provided above to assist with correct assembly during the build.

Method

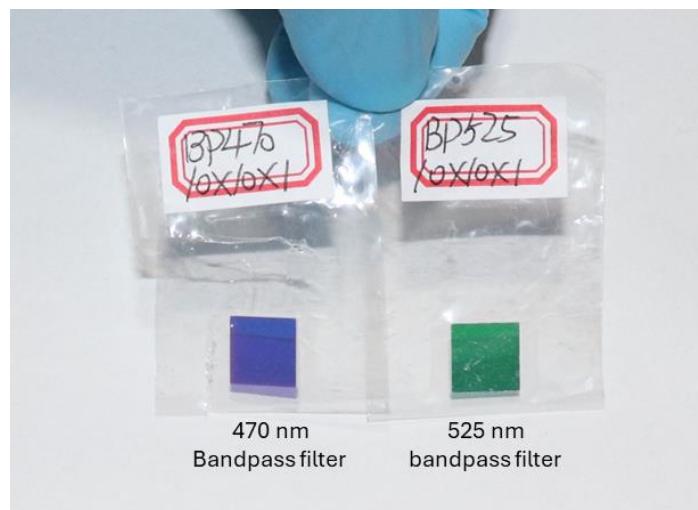
1. Place the 470 nm Bandpass filter into the slot on the Excitation face (labelled Ex) of the Main Body (A) 3d printed piece.

Note: As shown in the image below, the 3d printer parts of the Filter Cube Assembly are labelled A-D for easier identification.

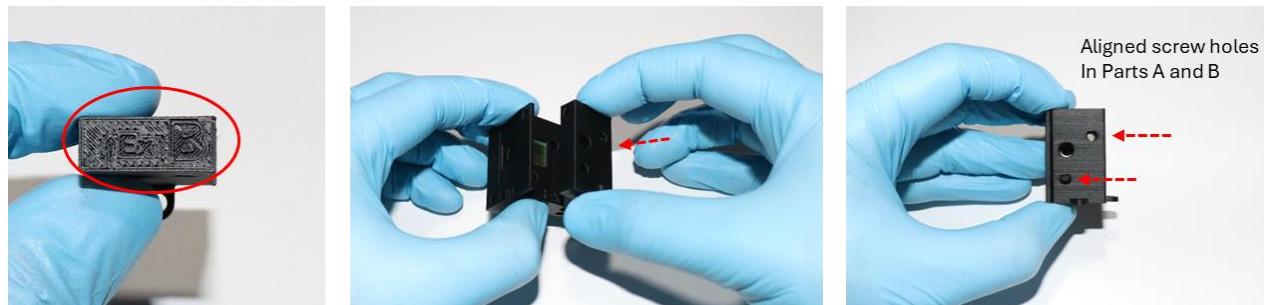
Tip: To avoid dirtying or scratching the Bandpass filters. Hold the filter by its edges or use plastic tweezers. If the filter does get dirty, it can be cleaned with 100% IPA and a cotton tip.



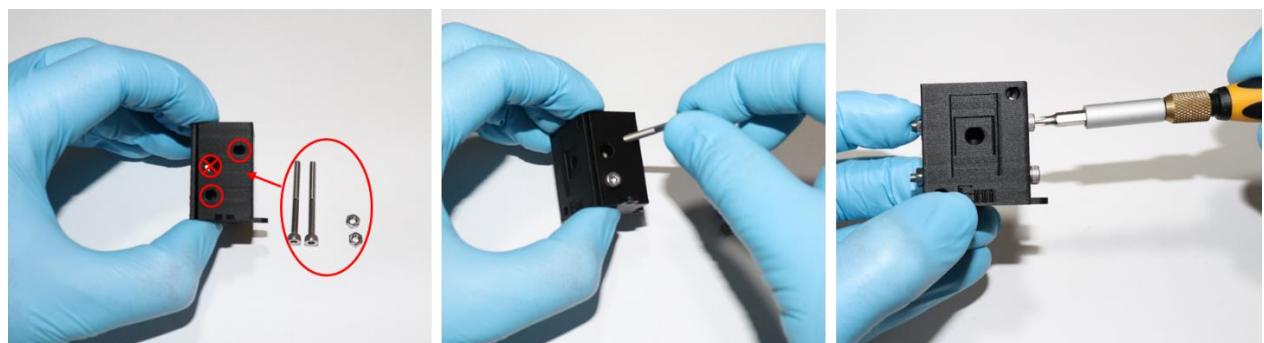
Tip: If you are confused as to which filter is the excitation or emission filter, hold the filters up to a white light source. Only blue light will pass through the 470 nm Bandpass filter and only green light will pass through the 525 nm Bandpass filter.



2. Place the Excitation Housing (B) 3d printed part over the 470 nm Bandpass filter to sandwich it in place, ensuring the LED and screw holes align with those in the Main Body (A) 3d printed part. The arrow on the top of Part B shows the correct orientation for the part.



3. While holding Part A and Part B together, places 2 x M3 35 mm screws through the highlighted holes. Secure Part A and B using 2 x 3M nuts using the M3 Torx Driver.



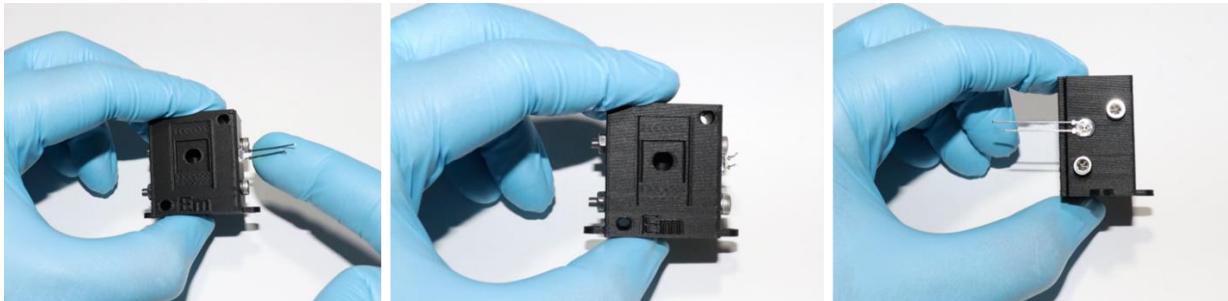
CAUTION: Do not accidentally put an M3 screw through the LED hole where the 470 nm bandpass filter is positioned as you can risk scratching or breaking it.

TIP: All screw/nut joints in this assembly guide can be finger tightened, but the use of a driver is recommended. Additionally, a small amount of lock-tight or cyanoacrylate glue can be used on all metal screws/nuts to prevent loosening.

4. Insert the 470 nm LED into the LED mount located on Part B until the skirt on the LED makes contact with Part B. Ensure that the longer pin (the positive anode pin) is oriented towards the top of the Filter Cube as shown.



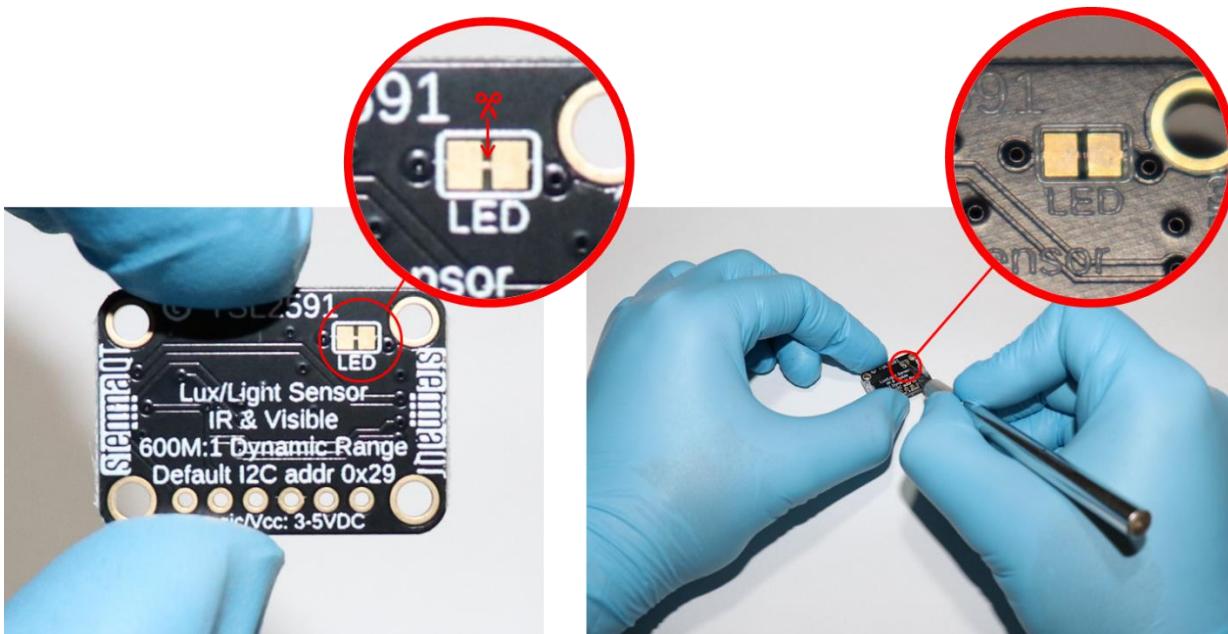
5. Bend the LED pins 90 degrees in a direction towards the emission path as shown. Place the A/B assembly aside.



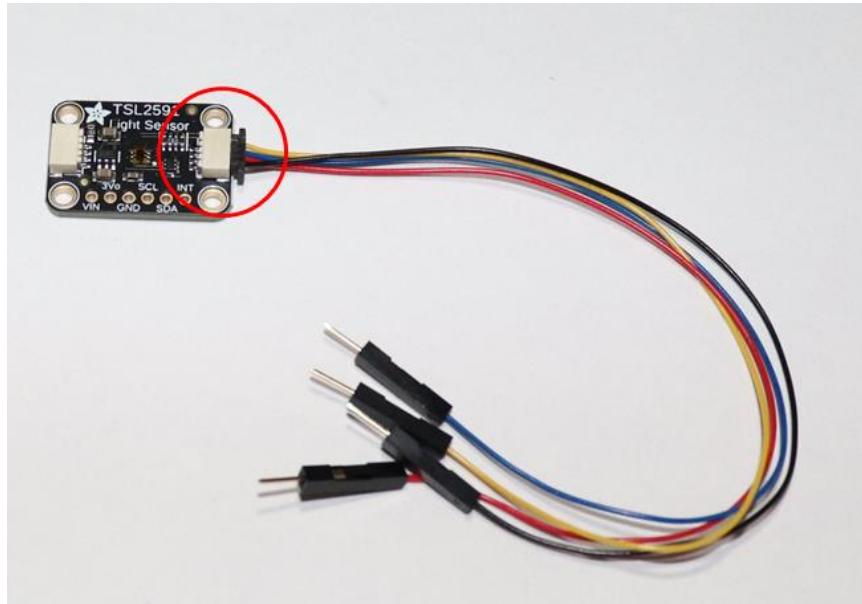
6. Cut the “LED” PCB trace on the back of the TSL2591 sensor board with a hobby knife or scalpel blade.

Note: This disables a green LED indicator on the front of the board that will interfere with fluorescence measurements. The trace is only a few hundred microns thick and can be cut by gently scraping the surface of the trace with the end of a pointed blade. **Do not use excessive force as it may cut other traces on the board.**

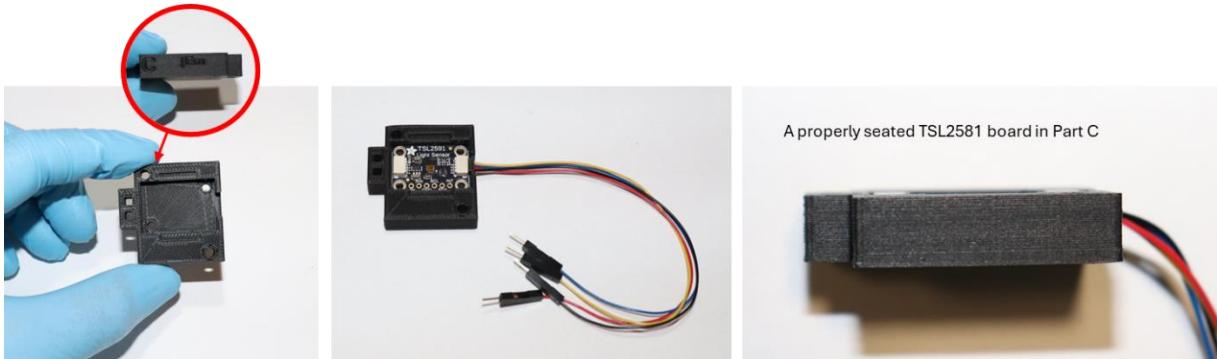
Tip: You can test the trace has been cut using the “continuity mode” of a multimeter.



- With the front of the TSL2591 sensor board facing you (see picture below), install the STEMMA QT cable into the JST port on the **right**.

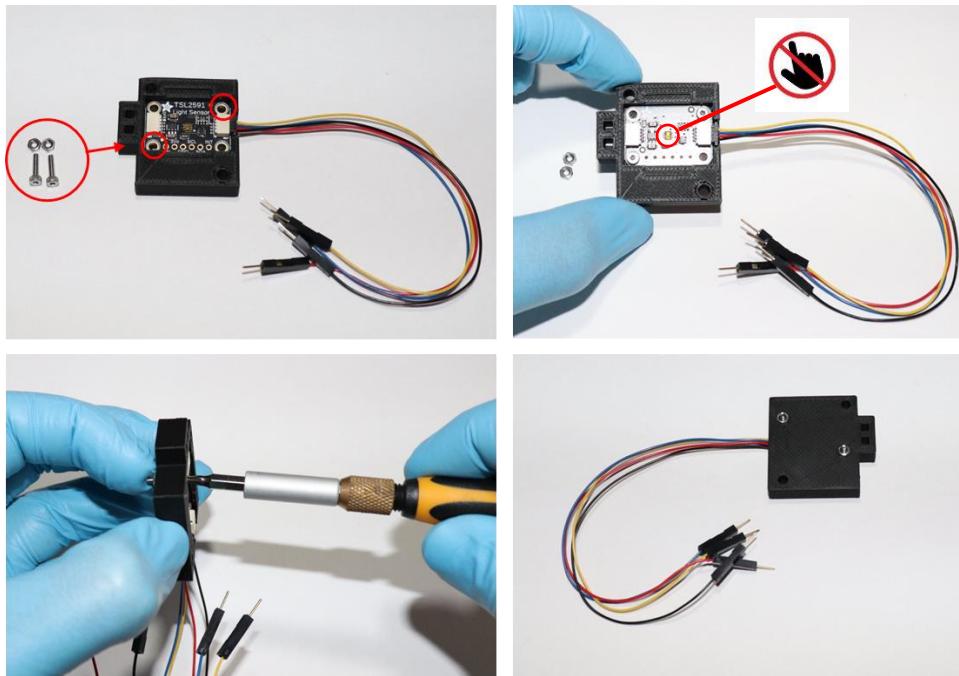


- Place the assembled TSL2591 sensor board into the slot within Emission Housing (C) in the following orientation. Firmly push the board into place so that it sits flat and flush against the back wall of the housing as shown below. The JST ports on the TSL2591 sensor board will not protrude above the walls of the slot within Emission Housing if inserted correctly.



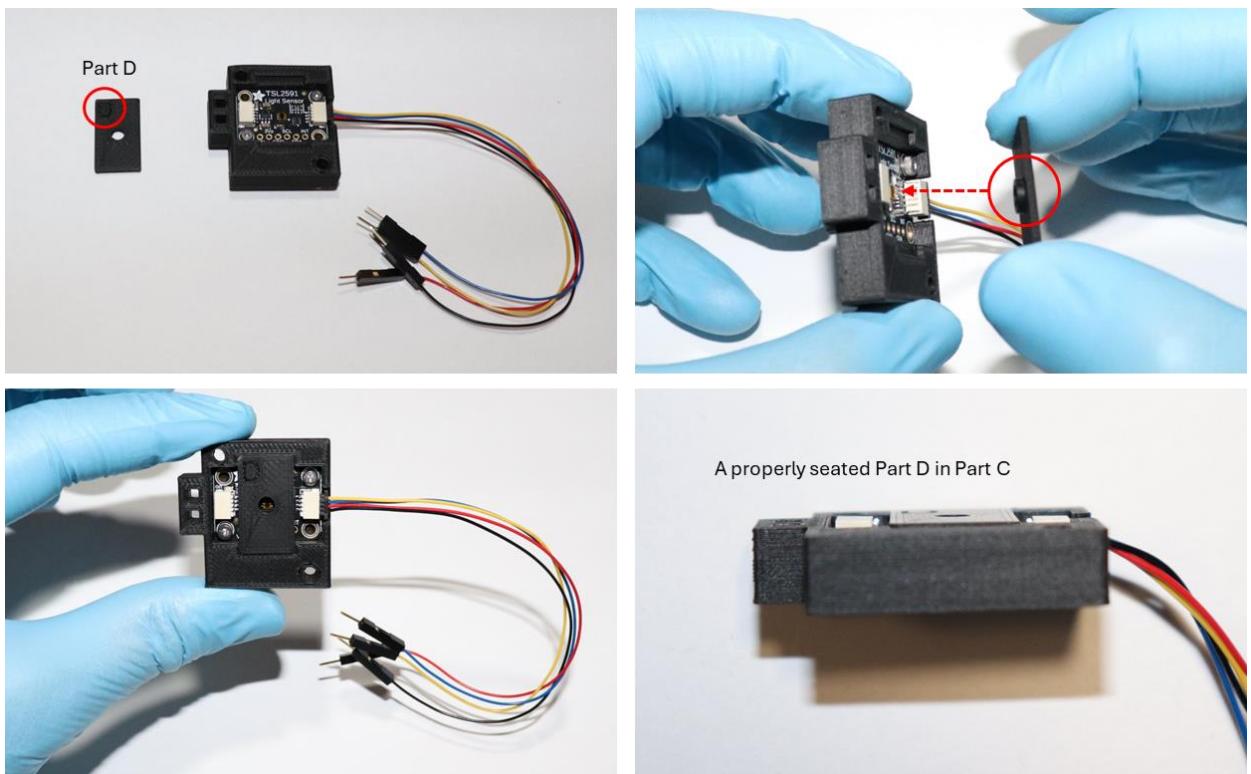
Note: FDM 3D printing artifacts, such as blobs or stringing on the inner walls of the Emission Housing (C) can be removed with a scalpel blade or hobby knife if they prevent the TSL2591 sensor board from sitting flat.

9. Secure the TSL2591 board to Part C using 2 x M2 8 mm screws and 2 x M2 bolts using the designated holes in Part C as shown below. Use a M2 torx bit to tighten in place.



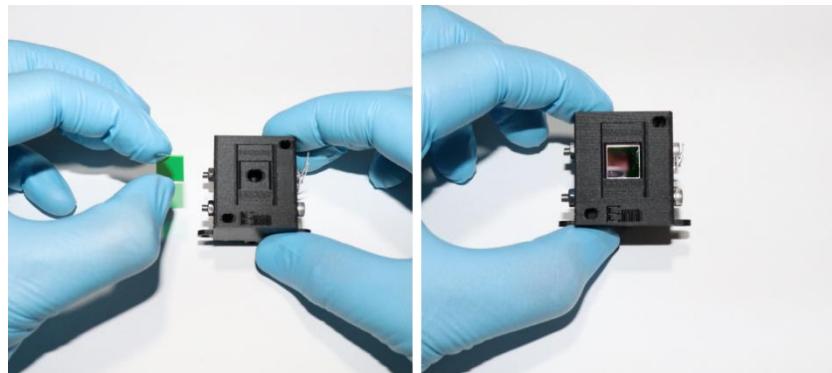
Tip: Try not to touch the optical sensor at the centre of the TSL2591 board or get it dirty, however it can be cleaned using 100% IPA and a cotton tip.

10. Place the Emission Baffle Part D into the assembled Part C so that the baffle tube is facing towards the optical sensor on the TSL2591. Part D should sit flush with the face of Part C as shown below.

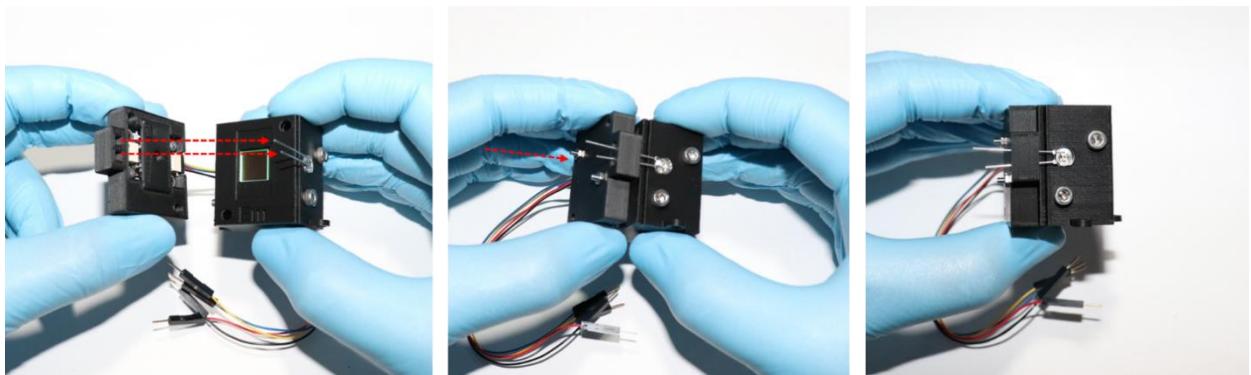


Note: Part D should hold itself in place, but if it does no, see the note on step 12.

11. Place the 525 nm Bandpass filter into the Emission slot (labelled Em) on the assembled A/B Part.



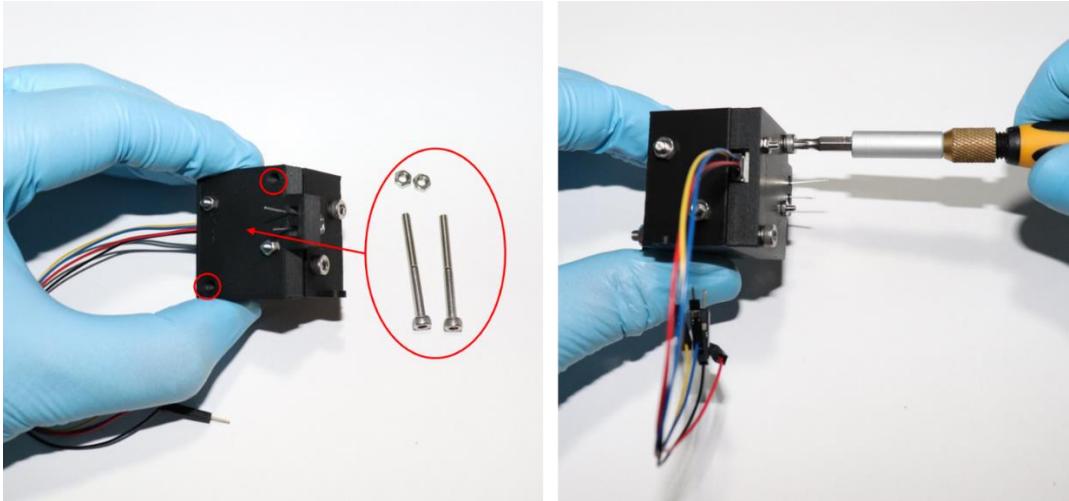
12. Align the assembled Part C with the Assembled Part A/B as shown and bring the two components together, ensuring that the LED pins pass through the two guide holes on Part C as shown. This process will sandwich the Emission Baffle (Part D), against the 520 nm bandpass filter, holding it in place.



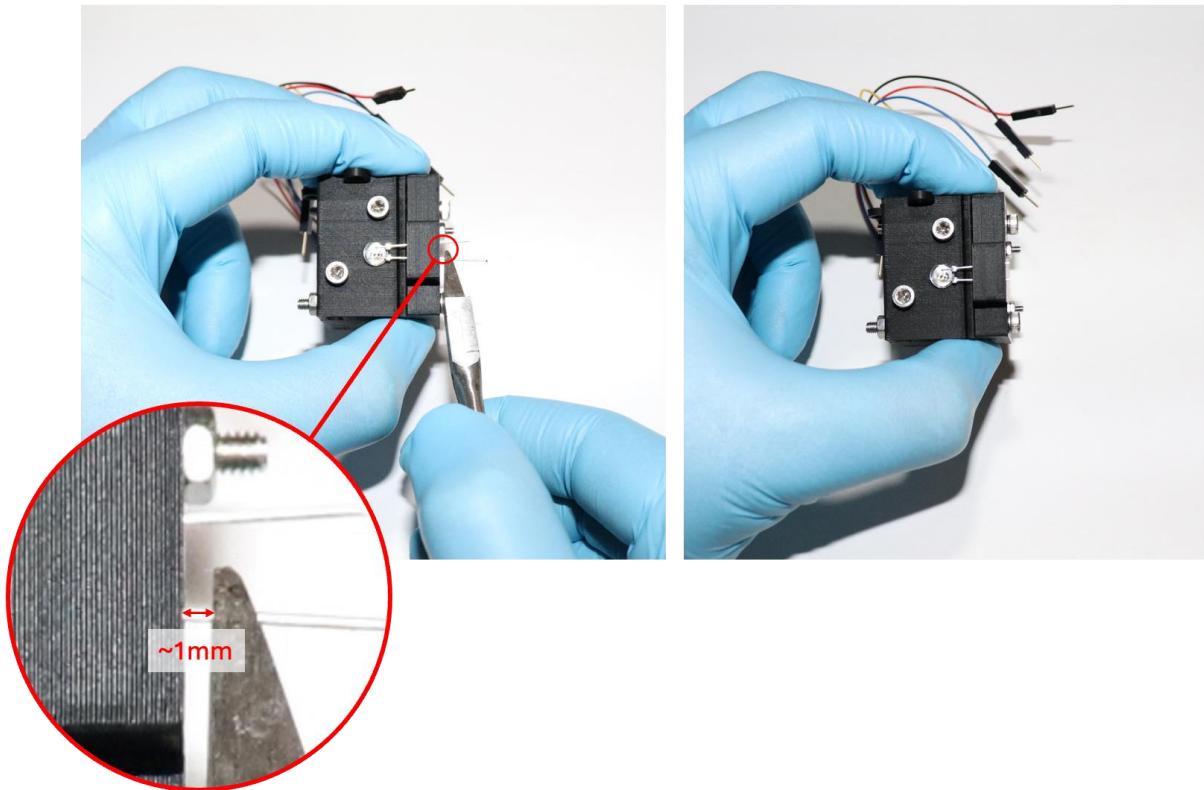
Note: Keep the assembled Part A/B oriented with the Emission Filter facing up while doing this to prevent it from falling out. The emission baffle, Part D is designed to friction fit into Part C and stay in place, however if Part D does fall out, it can be placed on the mounted 525 nm bandpass filter on Part A, taking care to align it with the grooves, and Part C brought down onto it.

Alternatively, a small amount of double-sided tape can be used to hold Part D in place against Part C, but care must be taken to not interfere with the optical path to the sensor.

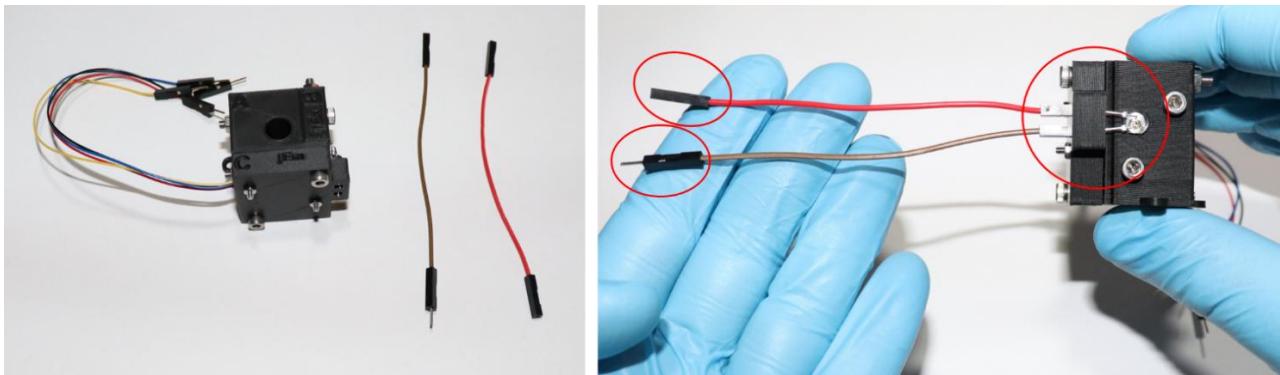
13. Secure the assembled Part C to the Assembled Part A/B using 2 x M3 35 mm screws and 2 x M3 bolts through the designated holes in Part C as shown below. As before, initially finger tighten the screws, then use a M3 Torx bit to tighten them in place.



14. Use a pair of side cutters to cut the LED pins approximately 1 mm away from the guide holes on the assembled Part C.

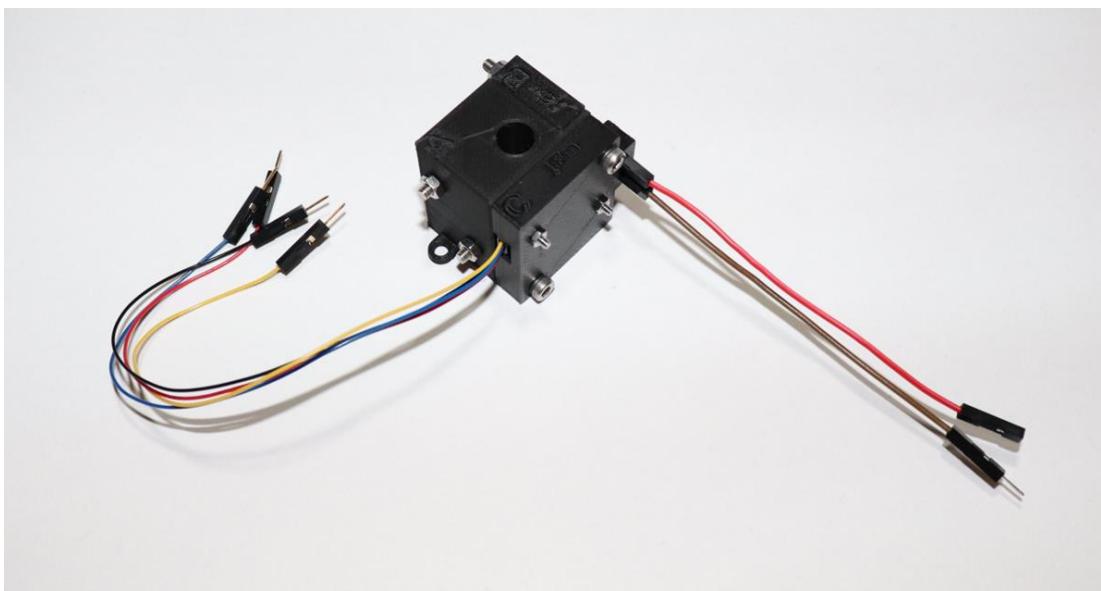


15. Attach the F-F hook-up cable to the top (Anode) pin of the 470 nm LED, ensuring that the LED pins insets into the hookup cable connector, and that the connector is seated firmly into the guide hole on the assembled Part C. Repeat the process with the M-F hook-up cable on the lower anodic pin.



Note: The connectors should go in to about half their length in the guide holes. You may need to “wriggle” them to ensure they are fully inserted. The connectors should sit securely in the guide holes.

The Filter Cube is now complete.



Body assembly instructions

Body BOM

Commercial parts

- 6 x M3 45 mm metal screw (Torx head)
- 7 x M3 8 mm metal screw (Torx head)
- 13 x M3 metal nut
- 4 x M3 12 mm nylon screw (Phillips head)
- 4 x M3 nylon nut
- Arduino Uno R3
- 1 x 68 Ohm 2W through-hole resistor (PR02000201000JR500)
- 1 x USB A to USB B (M-M) cable
- Double-sided tape (10 mm wide)

3D printed parts

- 1 x Base
- 1 x Top Housing
- 1 x Light Baffle Holder
- 1 x Light Baffle
- 4 x Foot

Pre-assembled components

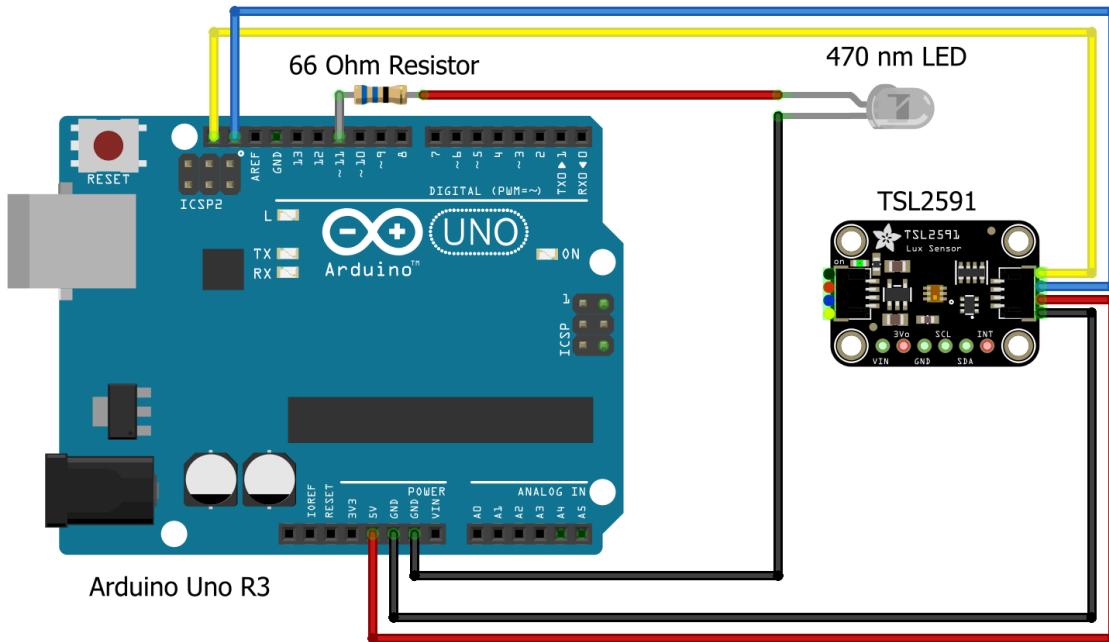
- Filter cube



Required Tools

1. M3 Torx Driver (or 2.5 mm Allen Key)
2. PH1 Phillips Driver
3. Side-cutters
4. Scissors

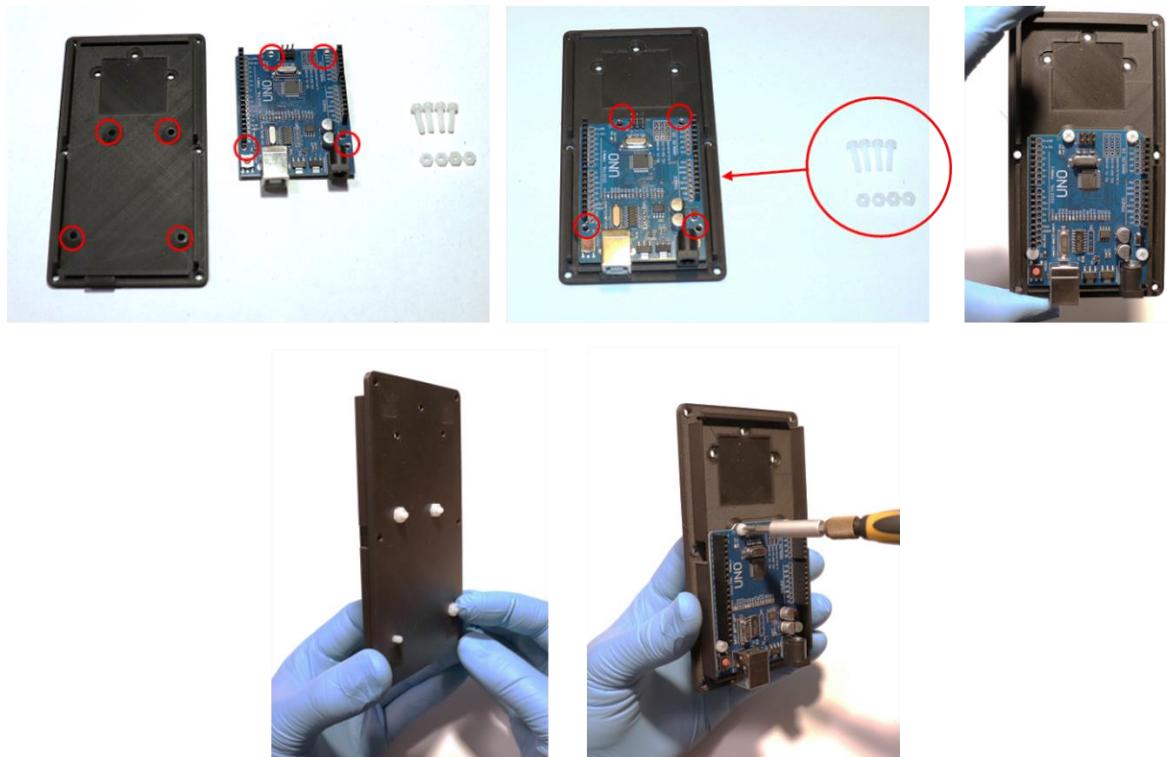
Electronic Circuit



The electronic circuit of the Diynafluor is provided above to assist with correct wiring during this section of the build.

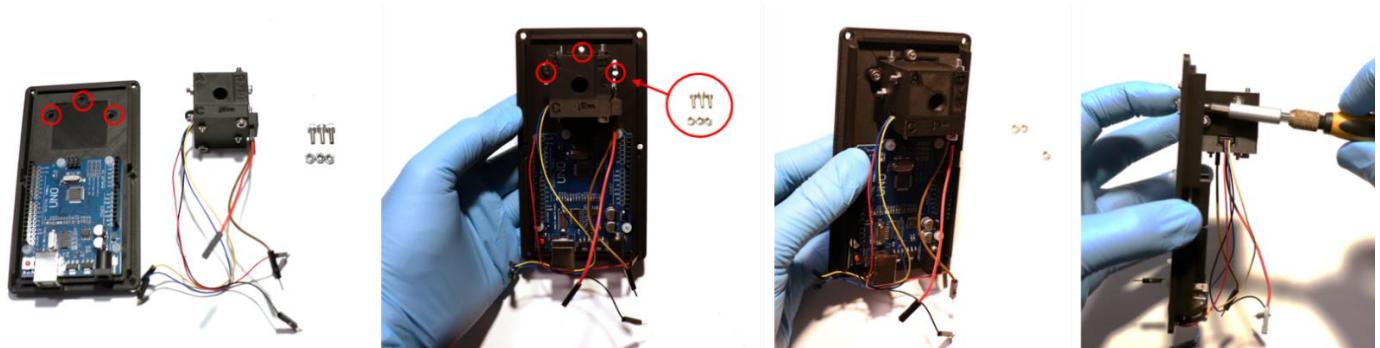
Method

1. Align the Arduino Uno R3 over the mounting holes in the 3D printed Base and secure it with 4 x M3 12 mm nylon screws and nuts using the PH1 Phillips driver.



Note: The mounting hole near the SCL pin on the Arduino R3 is close to the connector headers and may interfere with the head of the nylon screw. It is safe to use a small amount of force to slightly bend the nylon screen as it is screwed in.

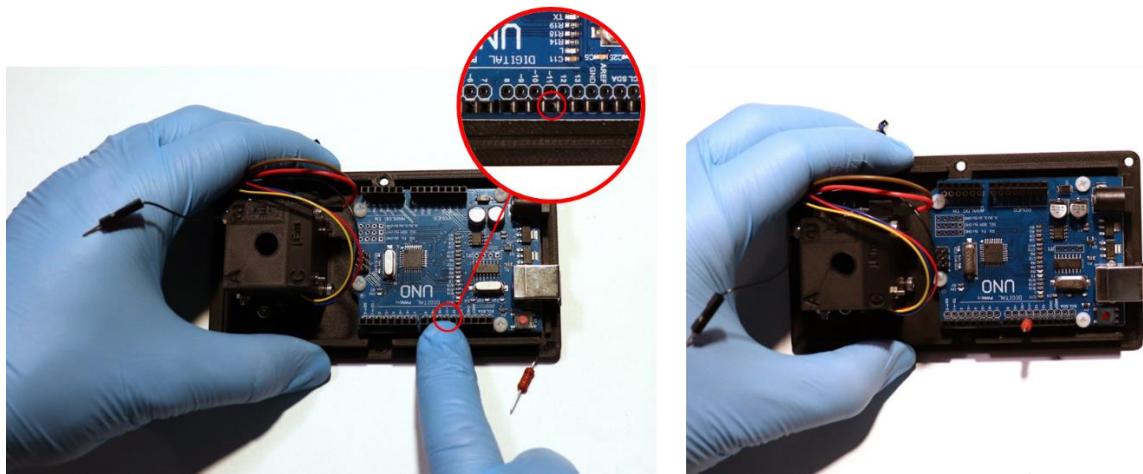
2. Align the pre-assembled Filter cube over the mounting holes in the 3D printed Base and secure it using 3 x M3 8 mm metal screw (Torx head) and nuts using the M3 Torx Driver.



3. Using a pair of wire cutters, cut each end of the 68 Ohm 2W through-hole resistor to leave behind ~10 mm of wire on each end of the resistor.

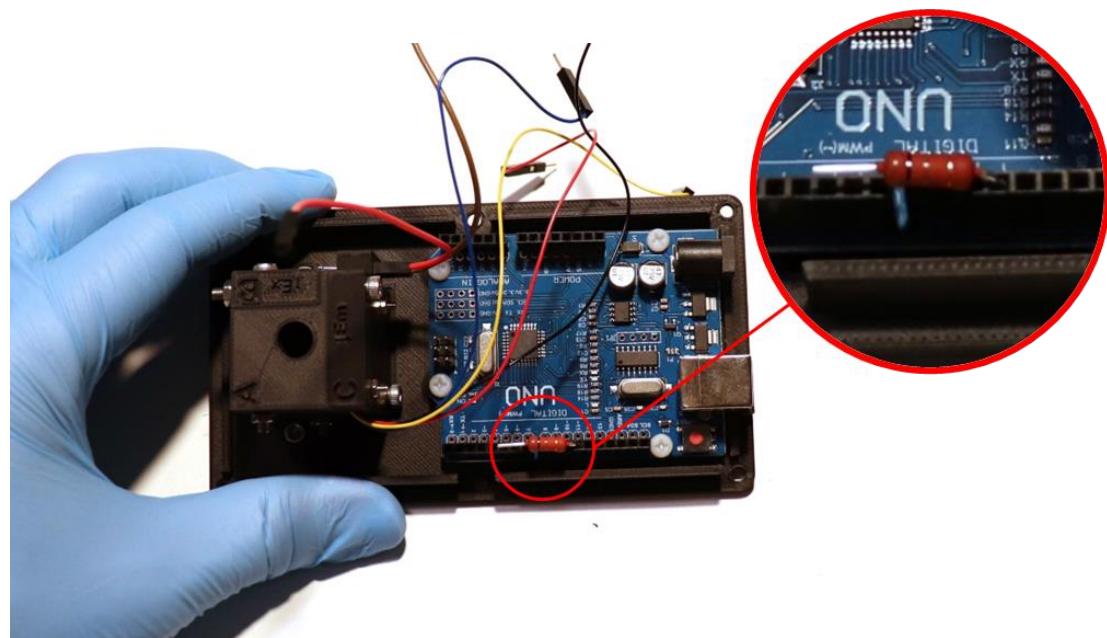


4. Insert one end of the resistor into pin-hole 11 on the Arduino R3.

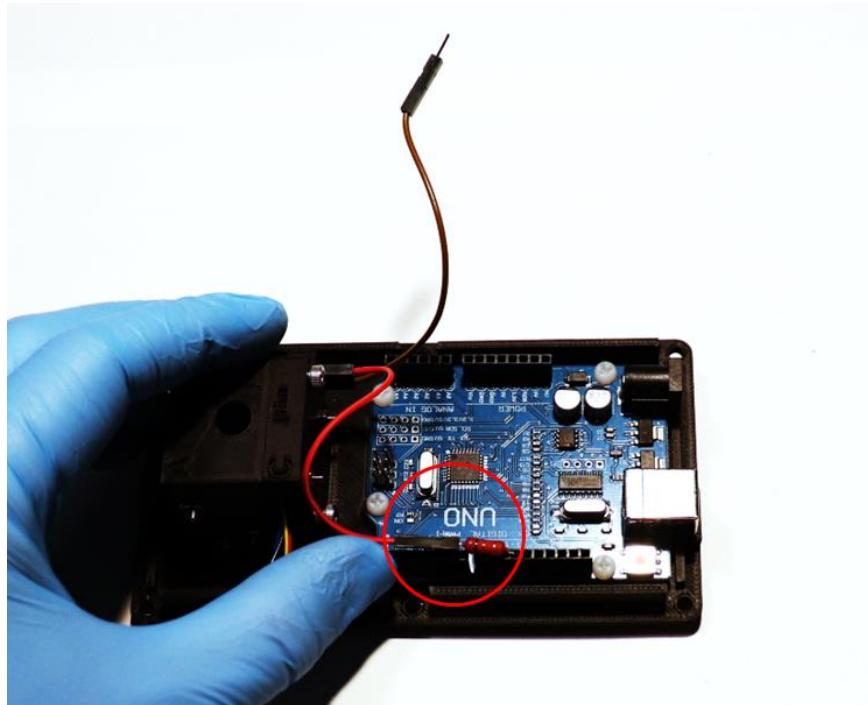


Note: The direction of the resistor does not matter.

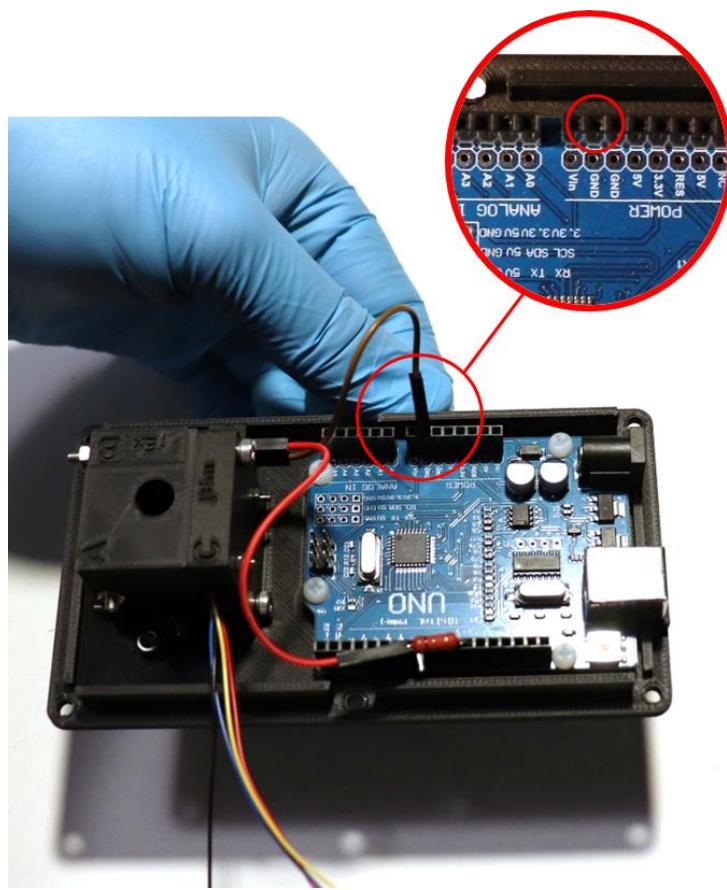
5. Bend the Resistor so it sits flat along the row of connector pins.



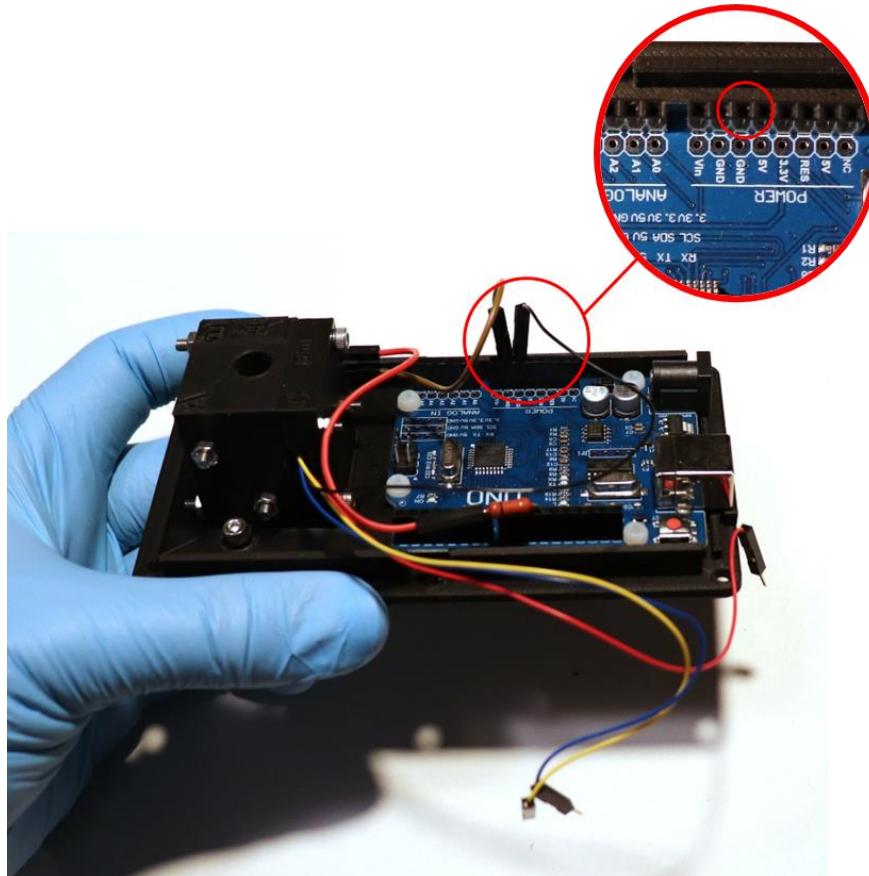
6. Attach the F-F hook-up cable from the LED on the filter cube (The one connected to the top Anode pin on the LED) to the free end of the resistor wire.



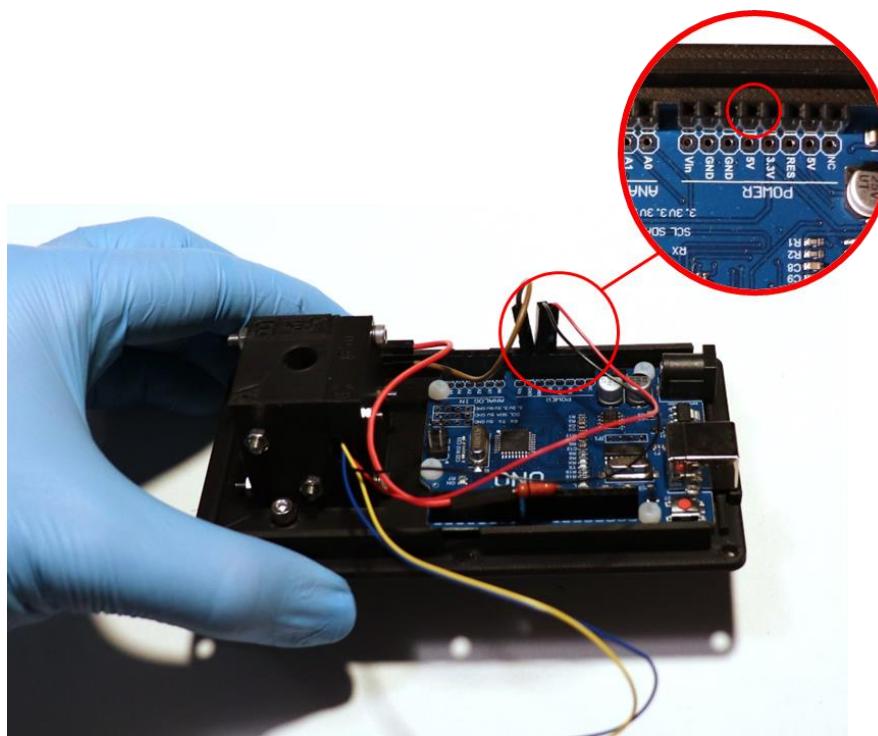
7. Connect the F-M hook-up cable from the LED on the filter cube (The one connected to the bottom cathode pin on the LED) to the highlighted Ground (GND) pin on the Arduino R3.



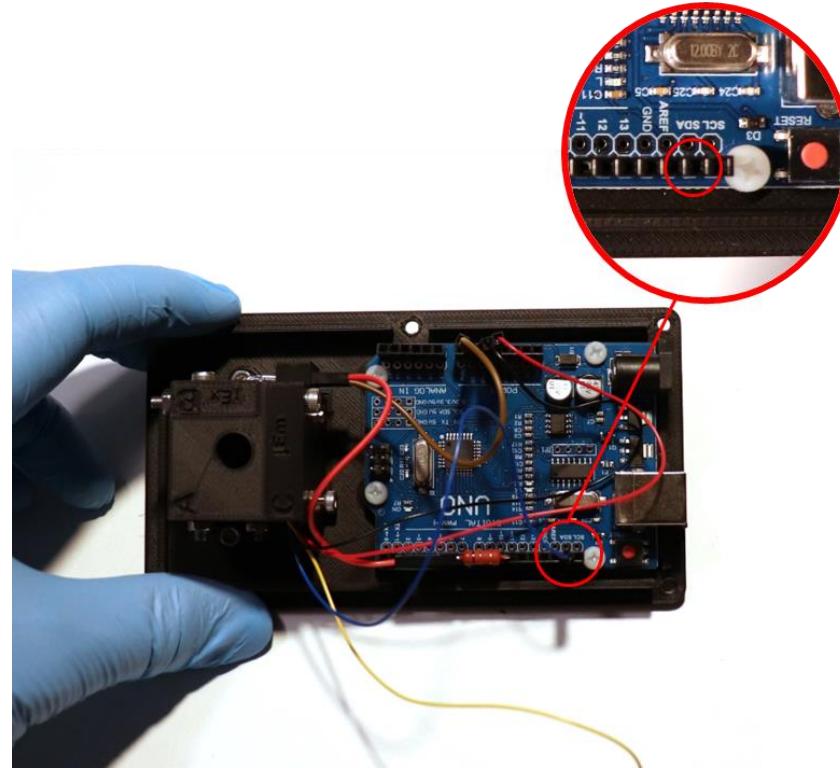
8. Connect the Black (ground) wire from the TSL2591 sensor board in the Filter cube to the highlighted Ground (GND) pin on the Arduino R3.



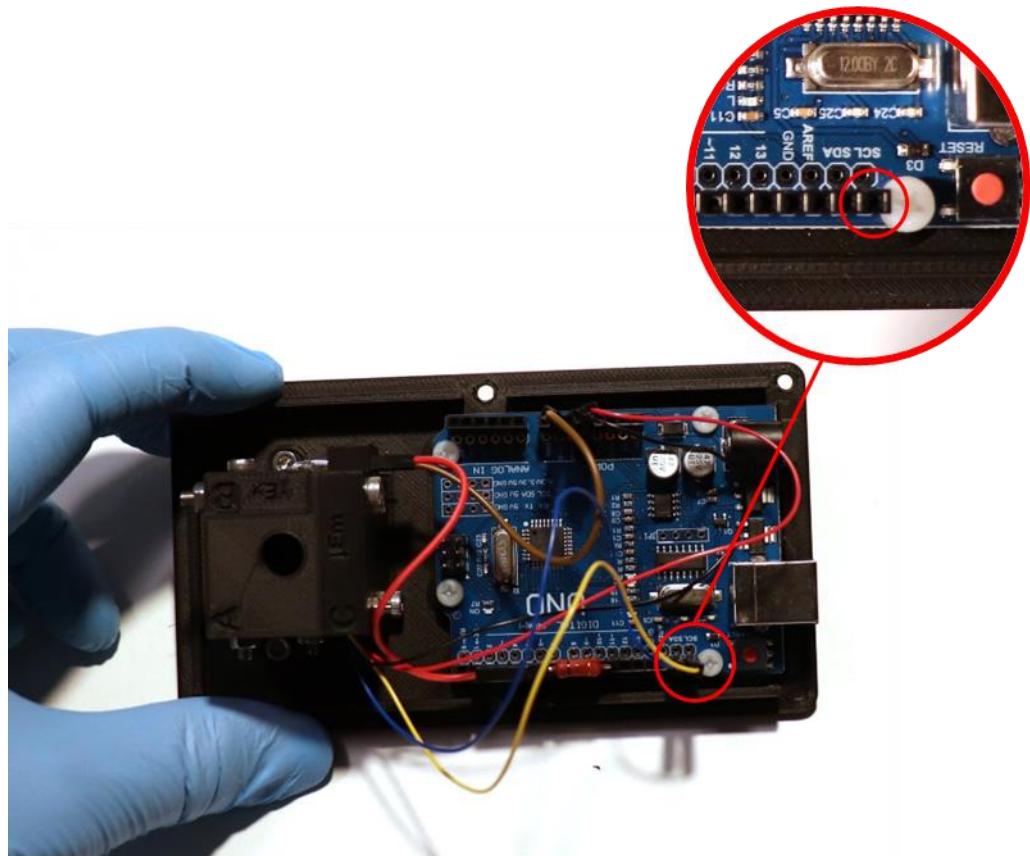
9. Connect the Red (VCC) wire from the TSL2591 sensor board in the Filter cube to the highlighted 5V pin on the Arduino R3.



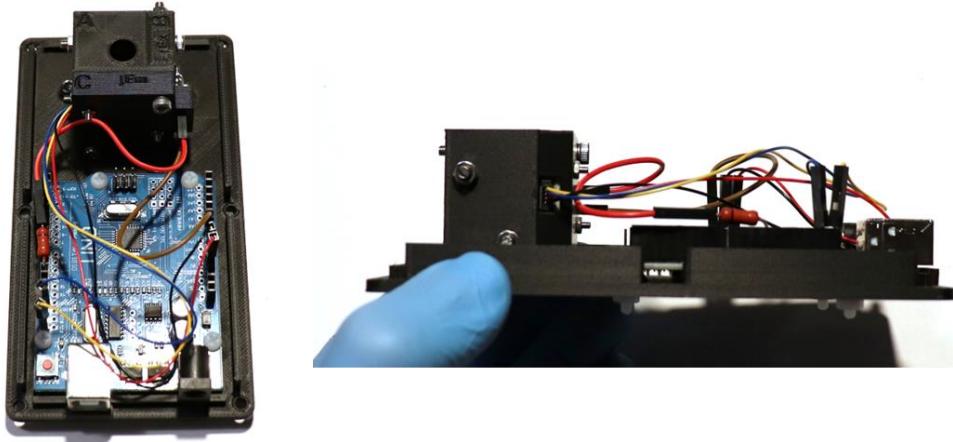
10. Connect the Blue (SDA) wire from the TSL2591 sensor board in the Filter cube to the highlighted SDA pin on the Arduino R3.



11. Connect the Yellow (SCL) wire from the TSL2591 sensor board in the Filter cube to the highlighted SCL pin on the Arduino R3.



12. Manage the wires so that they are all within the guide brackets (as shown) and below the height of the filter cube. This will help ensure the wires aren't pinched when attaching the 3D printed Top Housing piece.

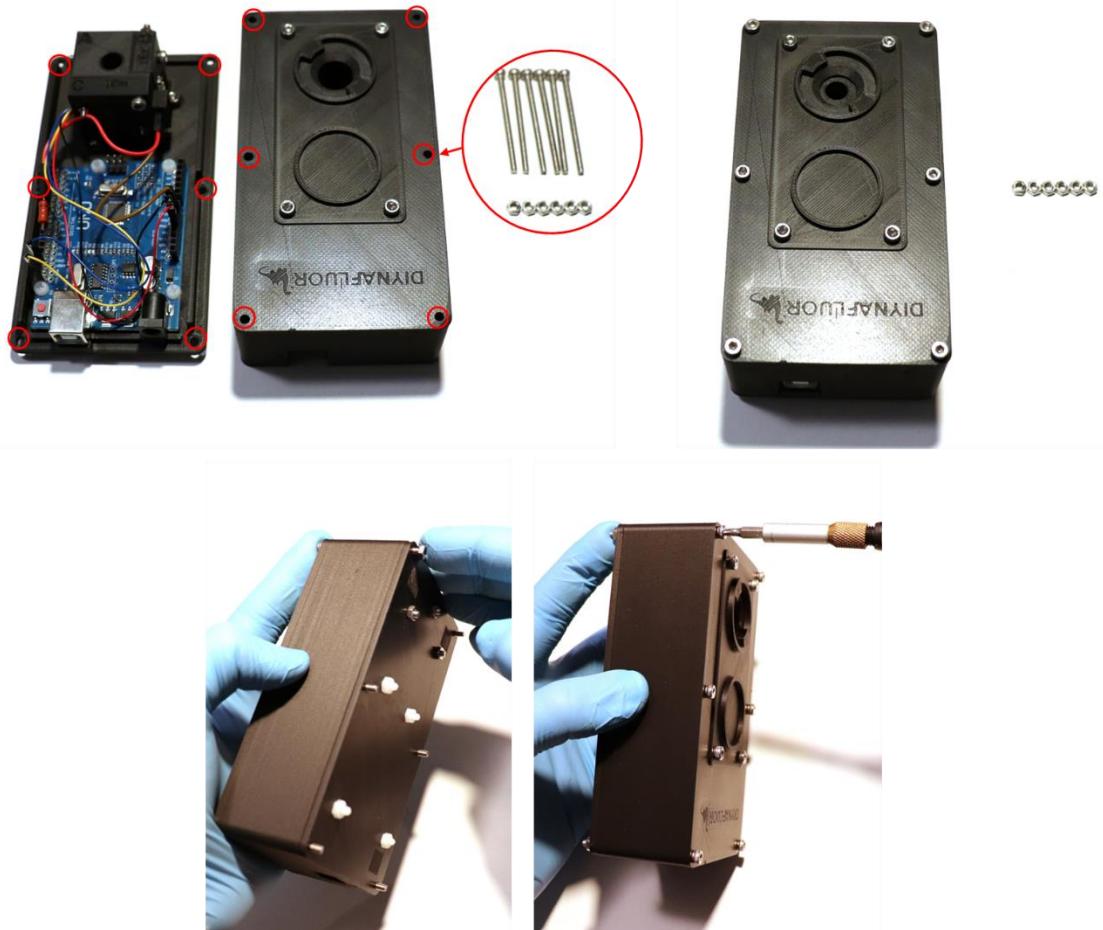


13. Attach the 3D printed Light Baffle Holder to the 3D printed Top Housing piece using 4 x M3 8 mm metal screw (Torx head) and nuts using a M3 Torx driver.

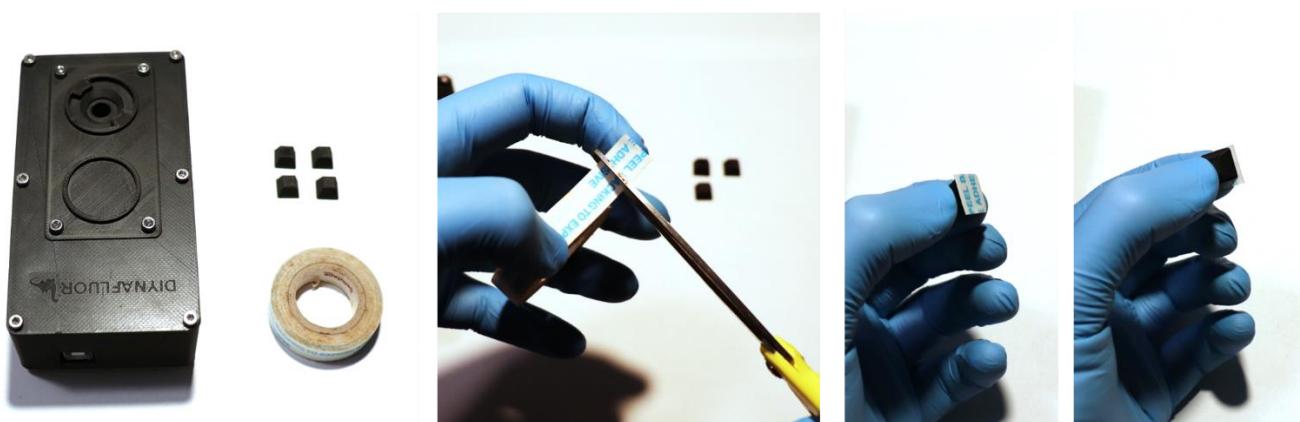


Note: Ensure the Light Baffle Holder is attached in the correct orientation so that the tube well is accessible.

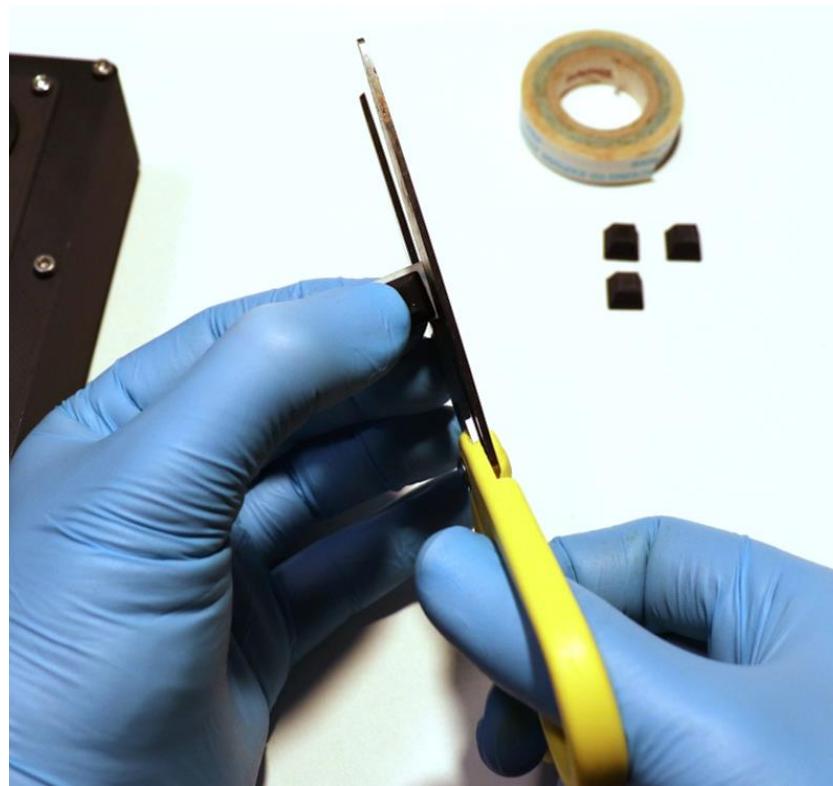
14. Attach the assembled 3D printed Top Housing to the assembled Base with 6 x M3 45 mm metal screw (Torx head) and nuts using the M3 Torx Driver.



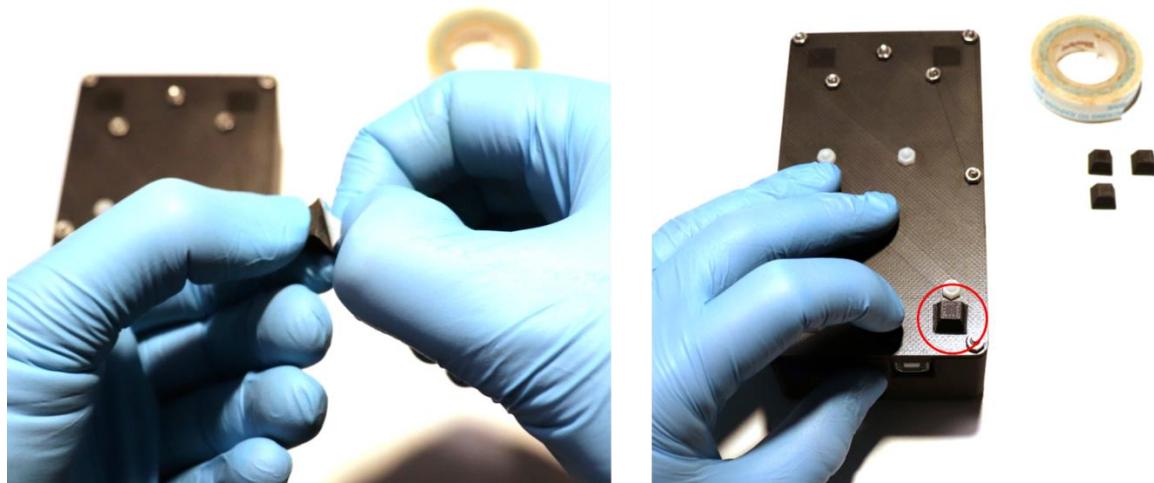
15. Cut approximately 10 mm of the double sided tape with scissors and attach it to one of the 3D printed 'Foot' pieces.



16. Cut away any excess tape around the edges of the 3D printed Foot with scissors.

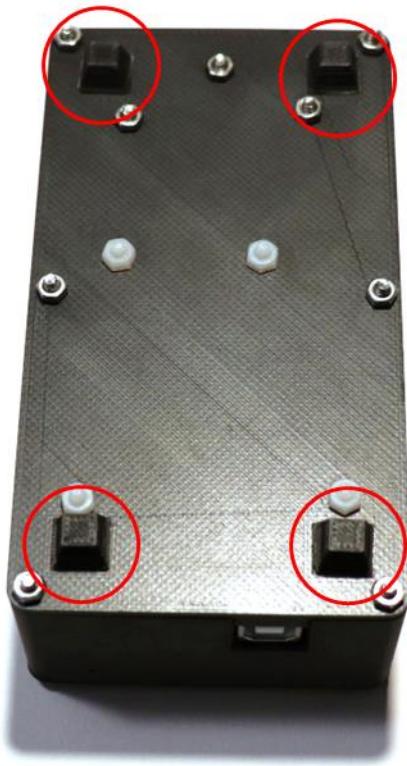


17. Remove the tape backing. Turn the assembled Dynafluor over and attach the 3D printed Foot to one of the four square markings at the corners of the bottom of the assembled Dynafluor body.



Note: If a more permanent method of attaching the Dynafluor feet is desired, superglue or araldite epoxy can be used instead of double sided tape.

18. Repeat this process for the other 3 feet.



19. Turn the assembled Diynafluor back over so that it is now sitting on its attached feet.
Attach the USB cable and place the 3D printed Light Baffle part on the Dynafluor.



The Diynafluor is now complete.



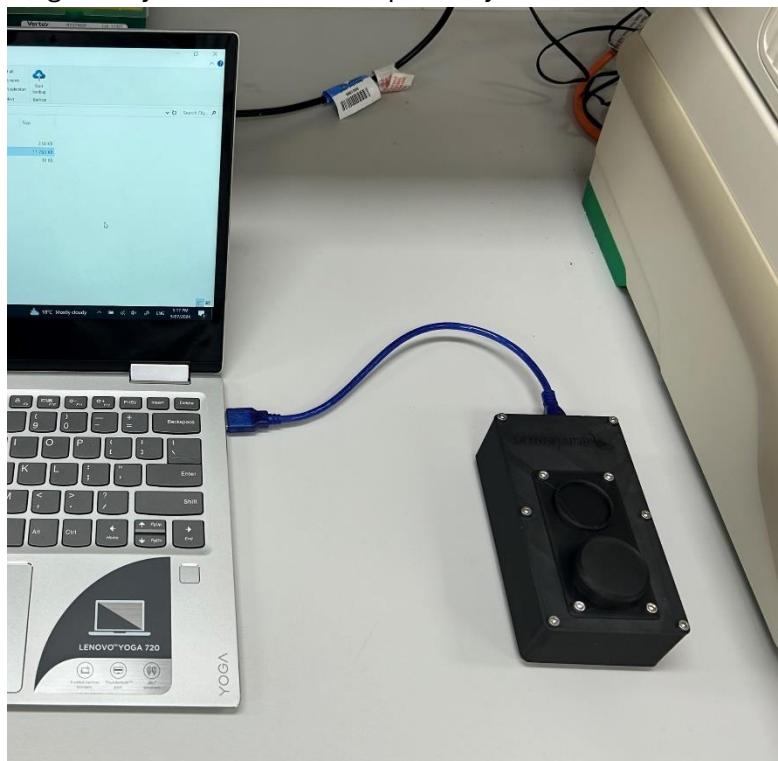
Software Installation Instructions

Required Equipment and Software

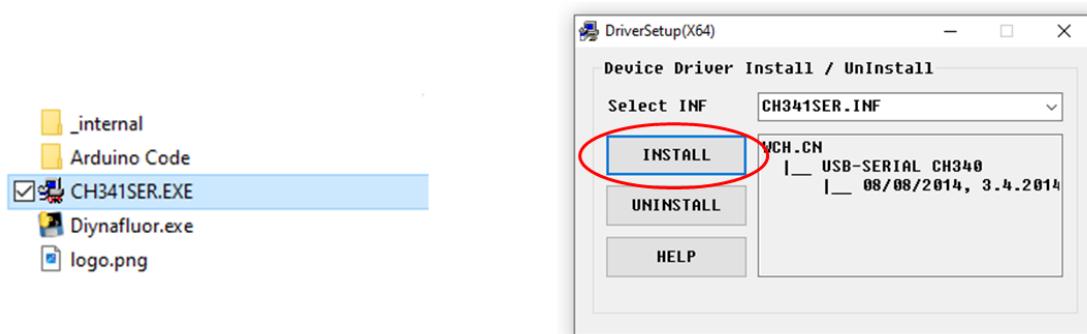
- A PC with Windows 10 or 11
- The Assembled Diynafluor
- The “Diynafluor.zip” file

Method

1. Plug the Diynafluor into a USB port on your PC.



2. Extract Diynafluor.zip to a location on your computer (such as the desktop).
3. Open the extracted Diynafluor folder and install the CH341SER USB to Serial port driver.



Note: The CH341SER driver does not need to be installed if you are using a genuine Arduino Uno.

Note: You will need Admin permissions to install the CH341SER USB to Serial port driver.

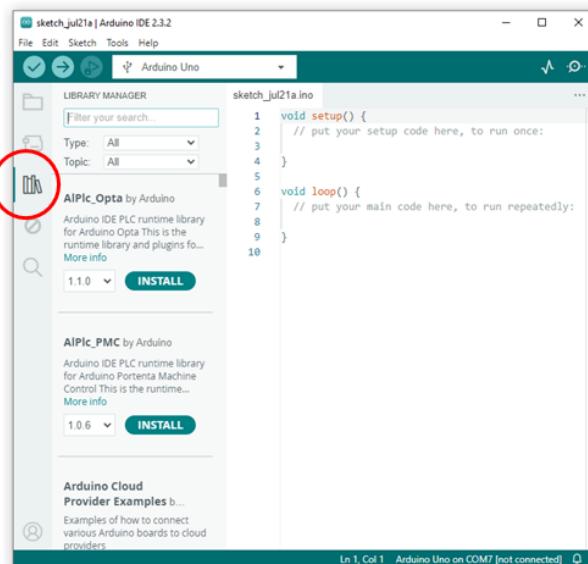
4. Install Arduino IDE:

- Download and install the current version (at the time of writing, this 2.3.2) of the “Windows 10” Arduino IDE from: <https://www.arduino.cc/en/software>

Note: You will need Admin permissions to install the Arduino IDE.

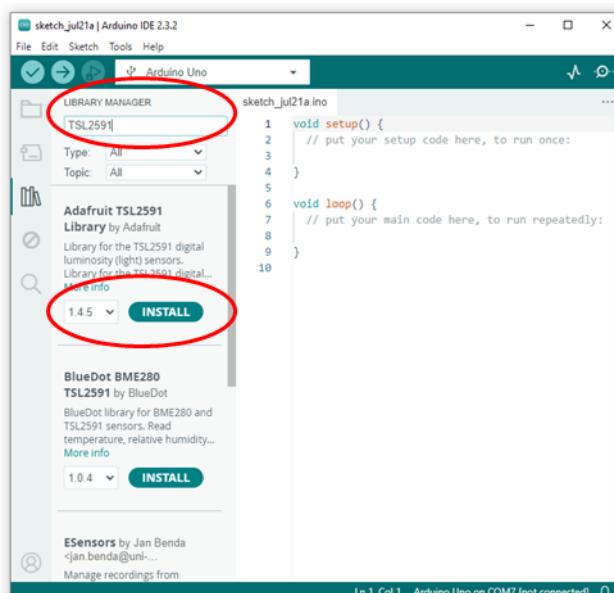
5. Install Adafruit TSL2591 Arduino Library

- Open the Arduino IDE from its installation location.
- Click the “Library manager” icon on the left-hand side of the Arduino IDE.

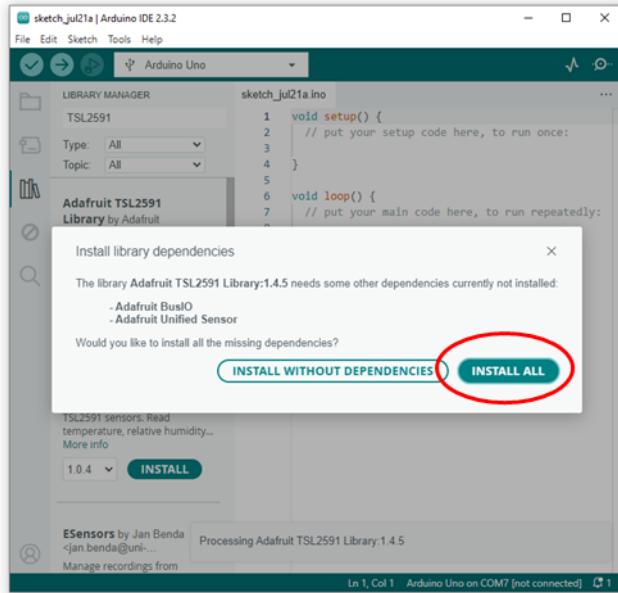


Note: You may need to restart the Arduino IDE to see the library page open if this is the first time it is being run.

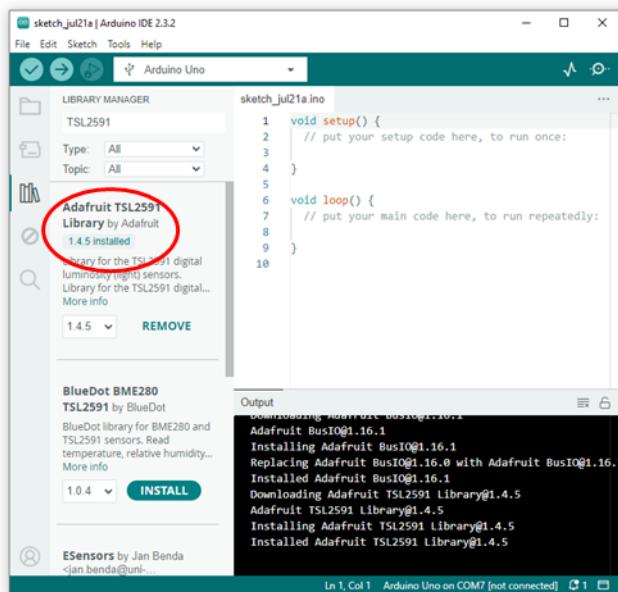
- Type “TSL2591” into the Library Search bar. The “Adafruit TSL2591 Library” should appear. Click the Install button.



- d. When Prompted select the “Install All” option.

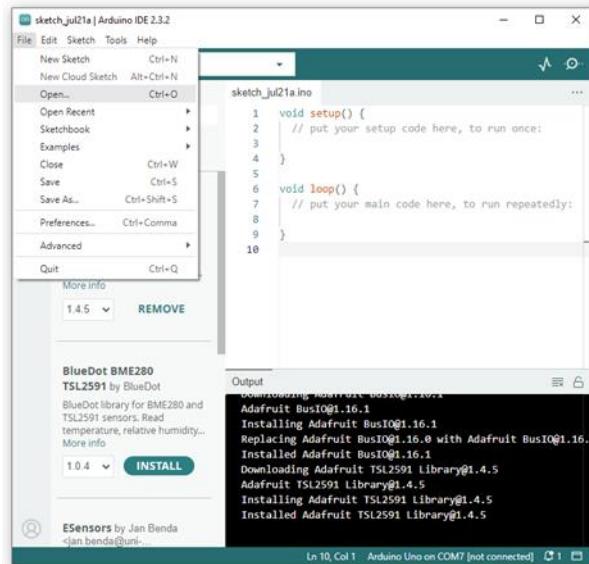


- e. When Installed the Library Manager will indicate that the “Adafruit TSL2591 Library” is installed.

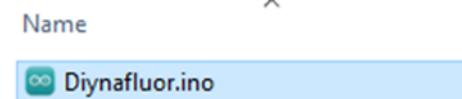


6. Upload the Diynafluor microcontroller code to the Arduino board

- In the Arduino IDE Select File -> Open



- Navigate to the unzipped Diynafluor folder from step 1 and open Diynafluor.ino in the “Arduino Code/Diynafluor/” subfolder



- A new window will open with the Diynafluor Arduino code.

```

#include <Wire.h>
#include <Adafruit_Sensor.h>
#include <Adafruit_TSL2591.h>

#define LED_PIN 11 // Pin connected to LED

Adafruit_TSL2591 tsl = Adafruit_TSL2591(2591); // Initialize TSL2591 sensor
bool executeLoop = false; // Flag to indicate whether to execute the loop or not
int ledPower = 255;

void setup() {
    Serial.begin(9600); // Initialize serial communication
    pinMode(LED_PIN, OUTPUT); // Set LED pin as output
    Serial.println("Ready!"); // Print message to serial monitor
}

void loop() {
    if (executeLoop) {
        float totalVisible = 0.0;
        const int numReadings = 3; // Number of readings to take
        for (int i = 0; i < numReadings; i++) {
            analogWrite(LED_PIN, ledPower); // Turn on LED
            delay(100); // Wait for LED to stabilize
            tsl.setTiming(TSL2591_INTEGRATIONTIME_600MS);
            tsl.setGain(TSL2591_GAIN_MAX);
            uint16_t visible = tsl.getLuminosity(TSL2591_VISIBLE); // Read visible light only
            totalVisible += visible;
        }
        delay(100);
        analogWrite(LED_PIN, 0); // Turn off LED
        delay(100); // Wait before taking the next reading
    }

    // Calculate the average of the readings
    float averageVisible = totalVisible / numReadings;
}

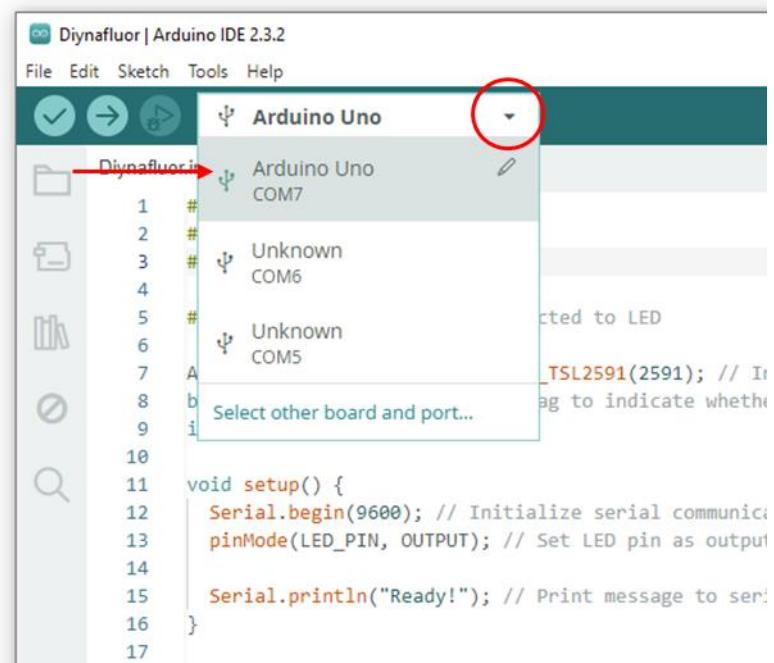
// Print average sensor reading
Serial.println(averageVisible);

// If you want to turn the LED on/off based on the average
// executeLoop = !executeLoop; // Invert the loop flag

```

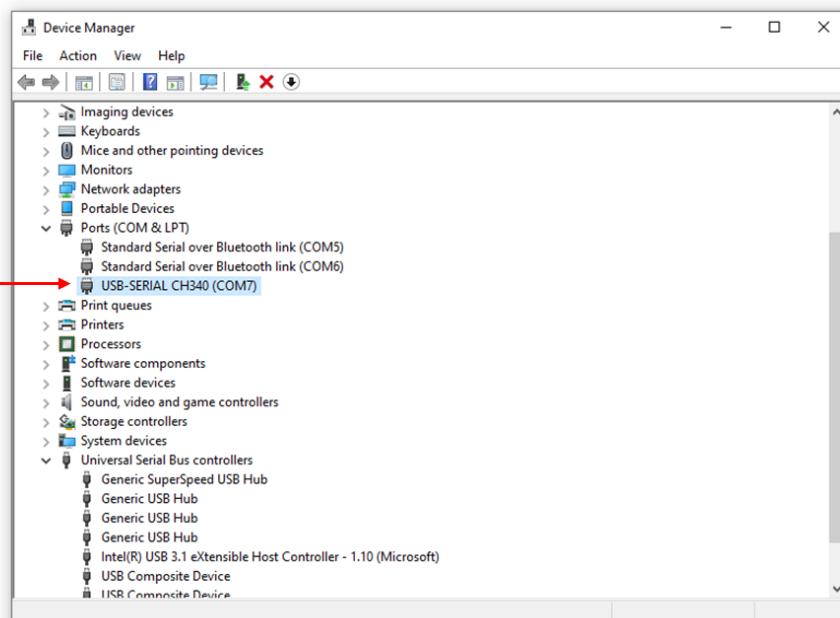
Note: **DO NOT EDIT THE CODE IN ANY WAY.**

- d. In the Arduino IDE for the window showing the Diynafluor code, click the expand triangle for the board select menu and select the COM port for the “Arduino Uno”.



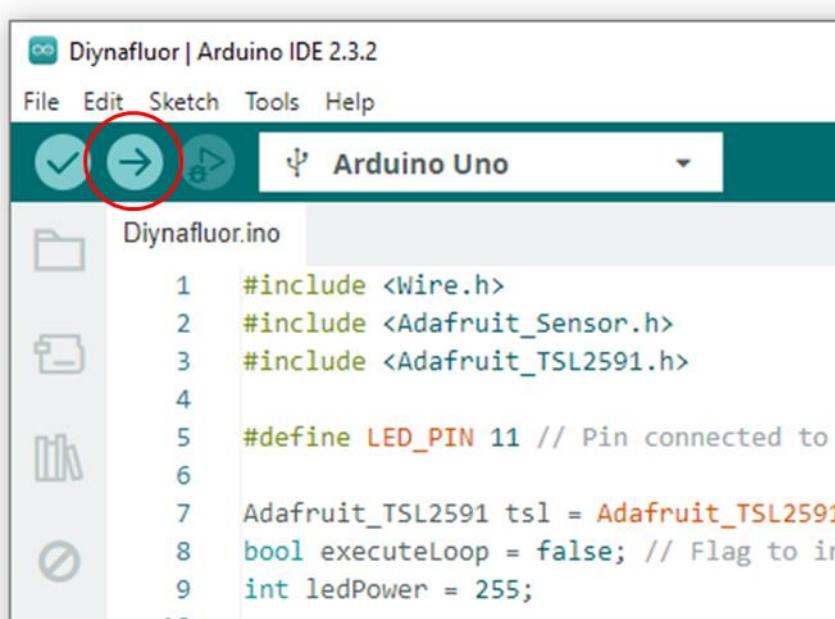
Note: If you do not see “Arduino Uno” assigned to a COM port as shown in the drop-down menu in the above image, you will need to manually select ‘Arduino Uno’ and assign it to its COM port from the “Select other board and port...” option.

Note: The COM port number for your “Arduino Uno” will most likely be different than what is shown here and depends on how many COM ports have been assigned by Windows. If you are using an Arduino R3, you can check it’s assigned COM port in the Windows Device manager under “USB-SERIAL CH340”.



If the device is not listed in Device Manager or is listed as an “Unknown Device” then the CH341SER driver was not installed correctly in step 2.

- e. With the Arduino Uno device selected, click the “Upload” button in the Arduino IDE.



Note: The Arduino IDE will indicate that the code is being compiled and uploaded to the board through prompts at the bottom right of the program:



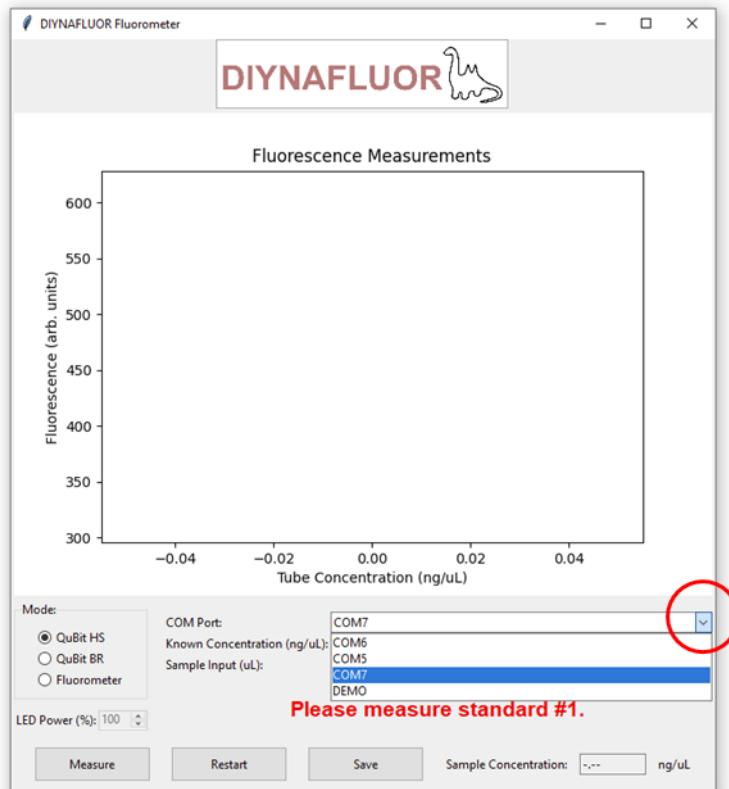
Note: If the correct COM port and “Arduino Uno” are selected in the Arduin IDE fails to compile and upload the code with a “No connection established” error, restart the Arduino IDE, reopening the code, check that the right COM port and “Arduino Uno” board are selected and attempt to upload the code.

7. Test the Diynafluor system.

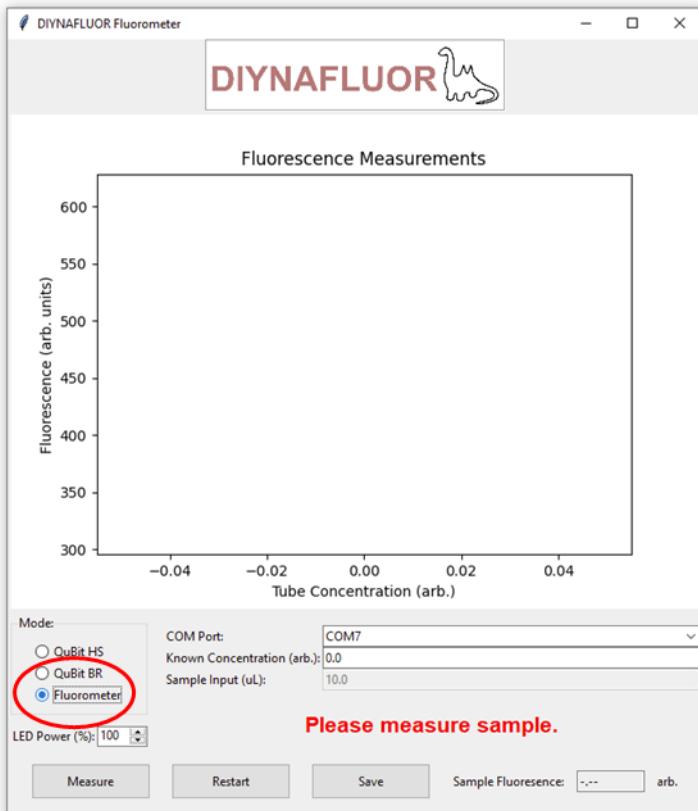
- Navigate to the unzipped Diynafluor folder and open the “Diynafluor.exe” GUI.

Note: If this is the first time opening the Diynafluor GUI, it may take a short time to open as it is ‘interpreted’ for the first time.

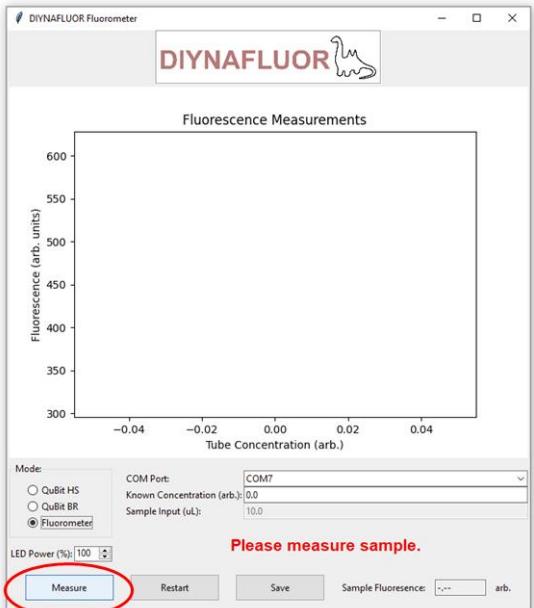
- Select the COM port for the Diynafluor (The same as the Arduino IDE Comport from step 5.)



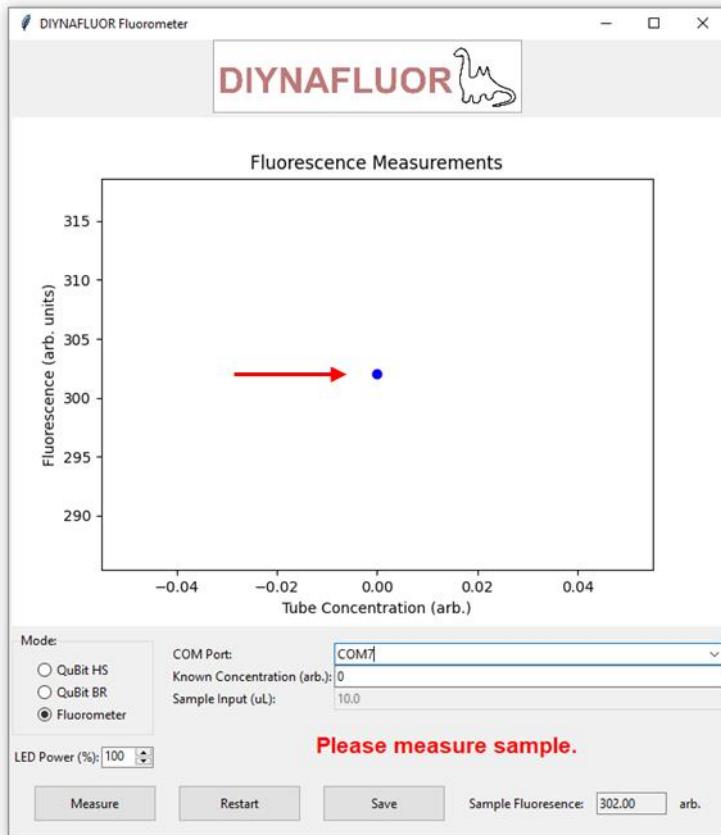
- c. Select the “Fluorometer” mode from the radial button



- d. Press the measure button. The Sample well in the Diynaflour should emit 3, 1 second long, flashes of blue light as the LED is turned on and a measurement is made.



- e. Check the Diynaflour GUI. The measured fluorescence should between 0 and 1000 units. If so, the Diynaflour is communicating correctly with the GUI and you can proceed to the Validation Assay.



Note: The following conditions can be used to troubleshoot issues if the Dynafluor fails to make the expected measurement at this step.

- The LED does not turn on: Check the LED is wired in the correct polarity and that the wires to the pins on the Arduino board are correct.
- The LED turns on but no measurement is reported: Check the wires from the TSL2591 sensor are connected to the correct pins on the Arduino, (Especially the SDC/SCL wires).
- The LED turns on but measured value is 0 or well above 1500 units:
This can be caused by several issues:
 - Incident green light is entering the sample well: Cover the sample well with the Light Baffle and take another measurement to check. This isn't a problem for future measurements if the Light Baffle is used.
 - There is fluorescent dirt/dust in the sample well: Check the sample chamber for contaminants. The sample chamber can be cleaned with a lint free tissue and a pipetting tip.
 - The “LED” trace on the TSL2591 board was probably not cut and the green LED on the front of the board is still active: Disassemble the filter cube and check the trace is cut.
 - The Excitation and Emission Filters are missing or in backwards: Disassemble the filter cube and check the filters are present and in the correct orientation.

Validation Assay Instructions

This assay is a QA check that the Diynafluor has been assembled correctly and is operating as expected. The assay should generate a linear curve of arbitrary fluorescence units across a 2-fold dilution series of measured DNA input concentrations using the Qubit 1X dsDNA High Sensitivity (HS) Kit.

The Validation Assay assumes the user is proficient in analytical pipetting techniques, standard calibration curves, Qubit assay procedures and Microsoft Excel.

Reagents and Consumables

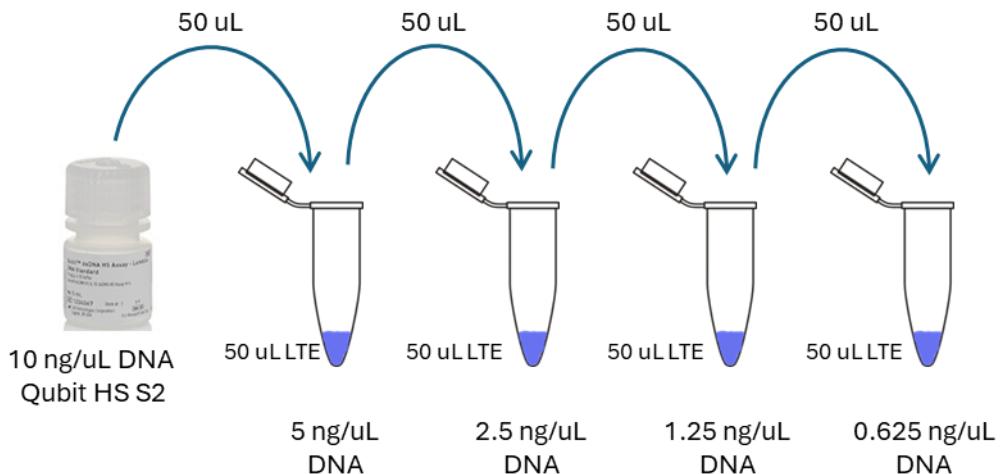
- Qubit 1X dsDNA High Sensitivity (HS) Kit (Thermofisher)
- LTE Buffer (10 mM TE, 0.1 mM EDTA, pH 8.0)
- 1.5 mL Eppendorf Tubes
- 500 ul Qubit Tubes.

Required Equipment

- The Assembled Diynafluor
- A PC with Windows 10 or 11 with the prerequisite Arduino Drivers and Diynafluor GUI installed
- Microsoft Excel
- 200 uL pipette
- 20 uL pipette
- Pipette tips
- Vortex
- Benchtop minicentrifuge
- Appropriate tube racks
- 1 Felt pen

Method

1. Prepare a 2-fold dilution series from the 10 ng/ μ L S2 HS qubit standard to the following concentrations - 10, 5, 2.5, 1.25, 0.625 ng/ μ L.



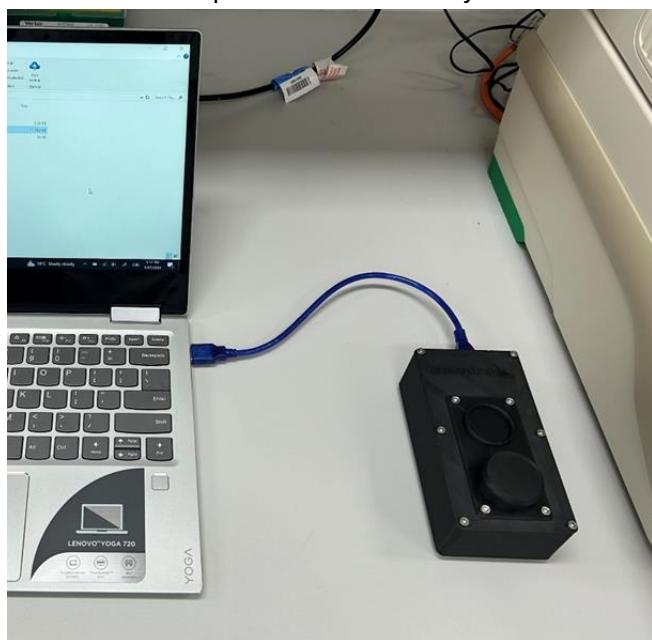
- a. Pipette 50 μ L of LTE buffer (10 mM TE, 0.1 mM EDTA, pH 8.0) into 4 x 1.5 mL Eppendorf Tubes labelled 5, 2.5, 1.25 and 0.625 ng/ μ L
- b. Add 50 μ L of the 10 ng/ μ L HS DNA S2 standard from the “Qubit 1X dsDNA High Sensitivity (HS) Kit” to the tube labelled 5 ng/ μ L.
Note: We recommend pipetting a minimum of 50 μ L for this dilution series to reduce pipetting errors, however this can be reduced to 15 μ L for skilled laboratory workers.
- c. Vortex to mix and spin down with a benchtop centrifuge.
- d. Pipette 50 μ L from the tube labelled 5 ng/ μ L into the tube labelled 2.5 ng/ μ L.
- e. Vortex to mix and spin down with a benchtop centrifuge.
- f. Pipette 50 μ L from the tube labelled 2.5 ng/ μ L into the tube labelled 1.25 ng/ μ L.
- g. Vortex to mix and spin down with a benchtop centrifuge.
- h. Pipette 50 μ L from the tube labelled 1.25 ng/ μ L into the tube labelled 0.625 ng/ μ L.
- i. Vortex to mix and spin down with a benchtop centrifuge.

2. Prepare the 1 x HS quit reagents.

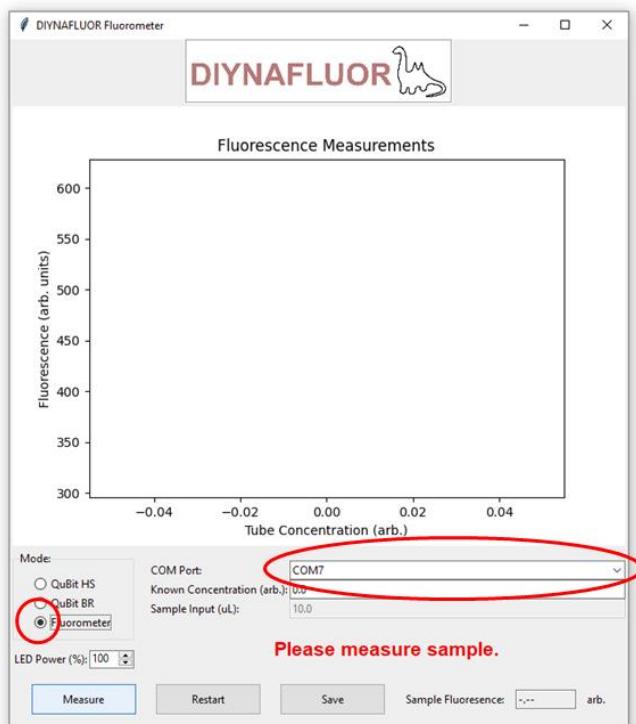
- a. Label the lids of 6 x 500 μ L Qubit tubes from 10, 5, 2.5, 1.25, 0.625 and 0 ng/ μ L, respectively.
- b. Add 190 μ L of the 1x HS Working Solution from the “Qubit 1X dsDNA High Sensitivity (HS) Kit” to each Qubit tube.
- c. Add 10 μ L of the S2 standard from the “Qubit 1X dsDNA High Sensitivity (HS) Kit” to the Qubit tube labelled 10 ng/ μ L.
- d. Add 10 μ L of the 5 ng/ μ L dilution from step 1 to the Qubit tube labelled 5 ng/ μ L.
- e. Add 10 μ L of the 2.5 ng/ μ L dilution from step 1 to the Qubit tube labelled 2.5 ng/ μ L.
- f. Add 10 μ L of the 1.25 ng/ μ L dilution from step 1 to the Qubit tube labelled 1.25 ng/ μ L.
- g. Add 10 μ L of the 0.625 ng/ μ L dilution from step 1 to the Qubit tube labelled 0.625 ng/ μ L.
- h. Add 10 μ L of LTE buffer to the Qubit tube labelled 0 ng/ μ L.
- i. Vortex to mix and spin down with a benchtop centrifuge.

3. Measure the standard curve.

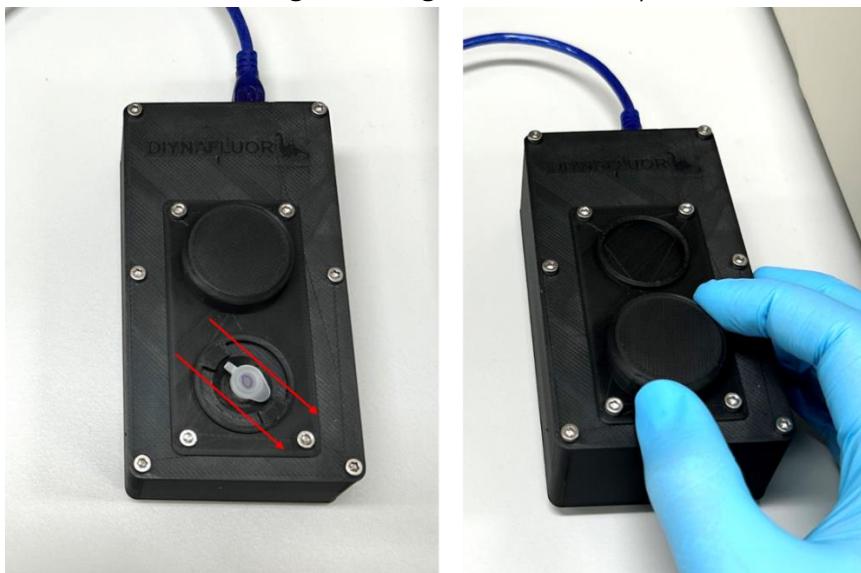
- a. Plug in the Diynafluor to a USB port and start the Diynafluor GUI.



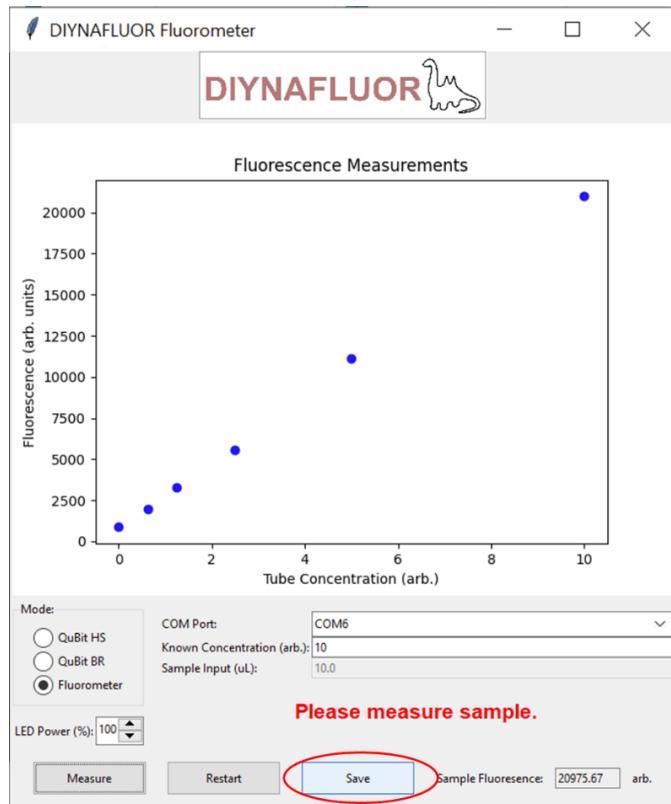
- b. Select the correct COM port for the Diynafluor and select the Fluorometer mode.
(Leave the LED power at 100%)



- c. Insert the 0 ng/ μ l into the Diynafluor, ensuring the tube is fully inserted, and in the orientation show in the below image, i.e., the hinge and front of the lid should sit within the guides-lines on the Light Baffle Holder. Cover the tube with the Light Baffle. (Note: We have since identified that tube orientation most likely plays an insignificant role tube-to-tube variation, so aligning the tube to the DIYNAFLUOUR markings can be ignored if desired.)

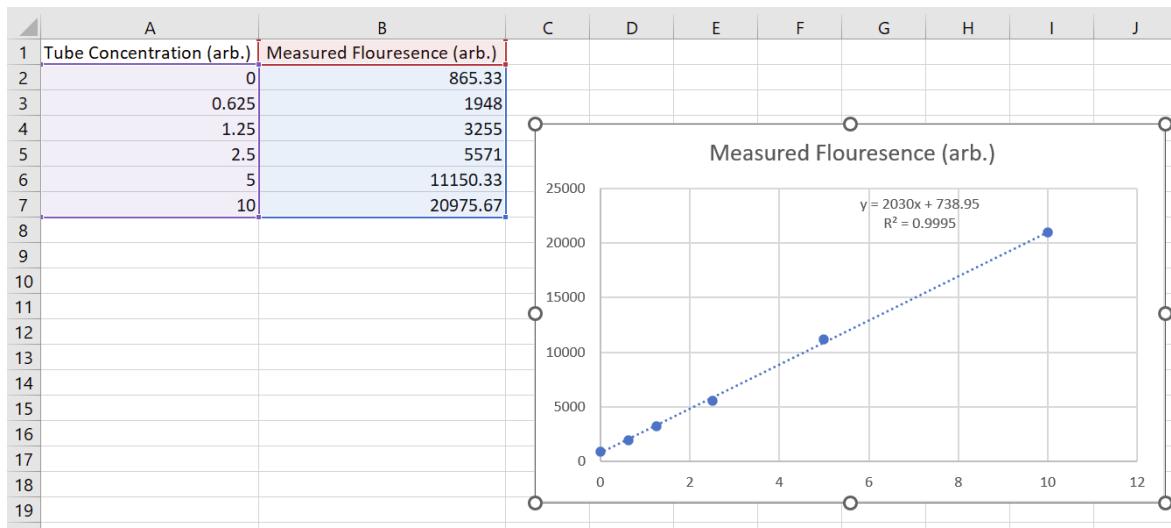


- d. Enter 0 into the “known concentration” value in the GUI.
e. Press the “Measure” button in the GUI.
f. Remove the Qubit tube from the Diynafluor and repeat this process for all the qubit tubes in the 2-fold dilution series, making sure to enter there “known concentration” in the GUI. A linear trend should be observed.
g. When the measurements are complete, press the “Save” button to save the file as a .csv to the computer.



4. Analyse the data.

- Open the CSV file in Microsoft Excel and plot the fluorescence measured vs the known concentration (in ng/μl).
- Perform a linear regression on the data. **Do not** force the y-intercept through 0. The gradient of the fitted line should be ~1800-2500 and the y-intercept ~1000. The R² should be greater than 0.98.



- Save the file as an excel worksheet for future reference.

- If a highly linear trend is not observed, then consult the trouble shooting steps outlined at the of step 6 of the “Software Installation Instructions” section of this document. Otherwise, please enjoy your new Diynaflouor unit and go measure some DNA samples!



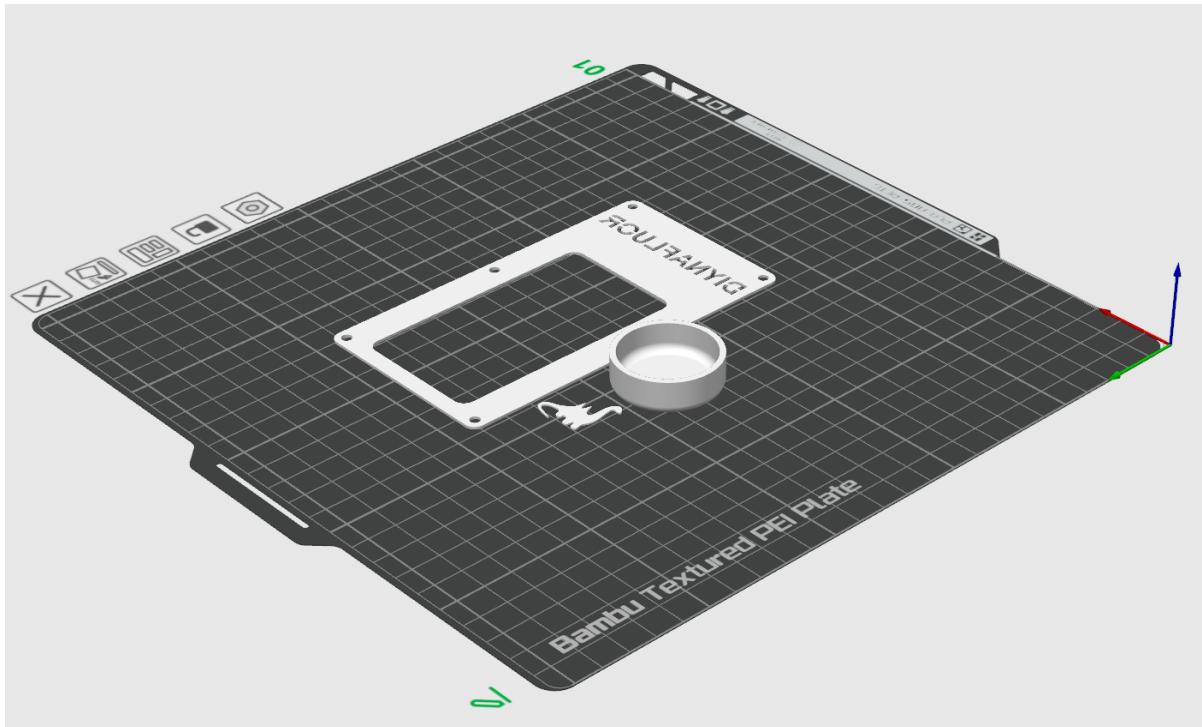
Appendix

Optional Parts

To add some colour and variety to a DIYNAFLUOR, the additional ‘Vanity Plate’, ‘DIYNAFLUOR Logo’ and ‘Light Baffle DIYNAFLUOR Logo Inset’ parts can be printed. Note: A DIYNAFLUOR does not require these parts to function.

The ‘Vanity Plate’ and ‘DIYNAFLUOR Logo’ parts can be printed in any colour or material desired by the user. The ‘Light Baffle DIYNAFLUOR Logo Inset’ part, which contains an inset depression for the ‘DIYNAFLUOR Logo’ part to sit in, should still be printed in Matte Black PLA.

Orient the parts for printing as shown below.



Glue the ‘DIYNAFLUOR Logo’ part into the ‘Light Baffle DIYNAFLUOR Logo Inset’ part with a small amount of superglue, as shown below. The ‘Vanity Plate’s’ mounting holes align with the mounting holes on ‘Top Housing’ partt. Simply align the two parts and assemble with the M3 45 mm metal screws, as shown below.

