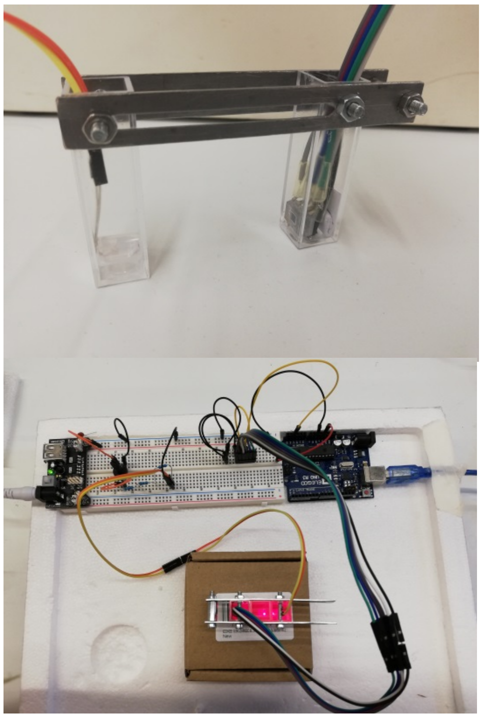
Introduction

To perform our needed functions, we build a small low-cost DIY photometer which can measure the optical density (OD) at 680 nm or any other wavelength in the visible spectrum. The photometer is designed to not only work inside our cultivation setup, but can also be used as a stand-alone solution for OD measurement.

Photometer

This is a small do it yourself photometer, using the light sensor Opt101 from Texas Instruments. It measures the absorption at 680nm of a given solution or cell culture as optical density (OD).

Parts List:



- Monolithic linear ambient light sensor Opt101
- LED 680nm (or else in visible light)
- 2 standard cuvettes
- Arduino with USB cable
- 2x 1kohm, 2x 10ohm,1x 5kohm resistors (optional potentiometer)
- Cables
- Two steel plates 80x10x1mm with two 3mm holes and a 50mm slit.
- 3x 3mm screws and nuts.
- optional 1-2 spacer (12mm long steel pipe)
- Breadboard
- Cardboard box

Software:

- Arduino IDE
- Putty (for serial monitor to csv file)
- Code for the photometer

Cost list and links:

LED680-02AU, 680 nm, 25 mW/sr, 4 mW at 20 mA, ±7°, 5 mm clear epoxy
http://www.roithner-laser.com/datasheets/led_div/led680-series.pdf 3,98 Euro

OPT101 Lightsensor linear VIS IR Photodiode 300nm-1100nm 2,7-36V Sensor Modul
https://www.amazon.de/gp/product/B07WHTF4S9/ref=ppx_yo_dt_b_asin_title_o00_s00?ie=UTF8&psc=1 4,97 Euro

ARDUINO UNO REV 3

https://www.amazon.de/Arduino-Uno-Rev-3-Mikrocontroller-Board/dp/B008GRTSV6/ref=sr_1_4?__mk_de_DE=%C3%85M%C3%85%C5%BD%C3%95%C3%91&keywords=Arduino&qid=1570469452&s=industrial&sr=1-4 17,89 Euro

Total: ~ 27 Euro

We would recommend buying an Arduino set with a lot of other fun parts and everything you need like cables and resistors.

ELEGOO UNO R3 Ultimate Starter Kit

https://www.amazon.de/Elegoo-Vollst%C3%A4ndige-Ultimate-Tutorial-Mikrocontroller/dp/B01IHCCKKK?ref_=ast_sto_dp 46,99 Euro

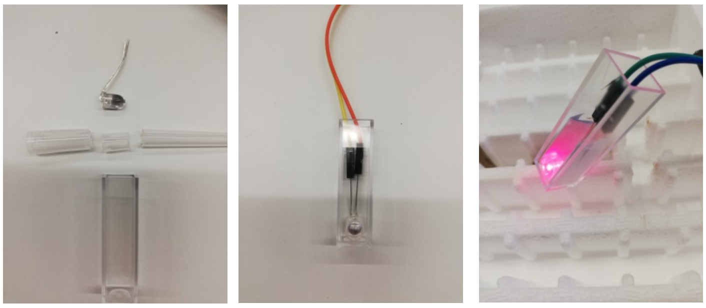
Tools:

- soldering iron
- Knife
- Tongs
- Hot glue (optional)

Assembling of the parts.

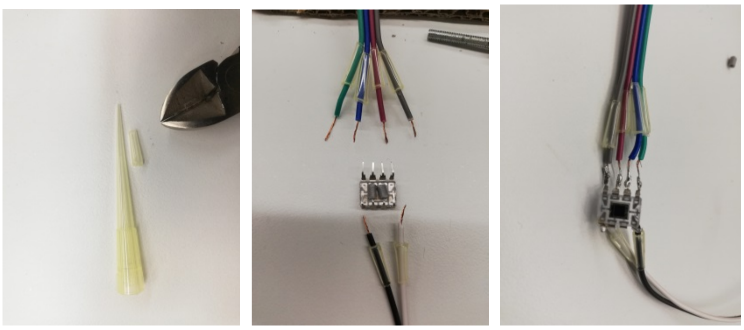
1.Prepare the LED attachment with a 1ml Pipet tip.

Put the LED in the middle part of the pipet tip that should be around 10mm long and stick in to the cuvette. Paper on the sides helps to center the LED.

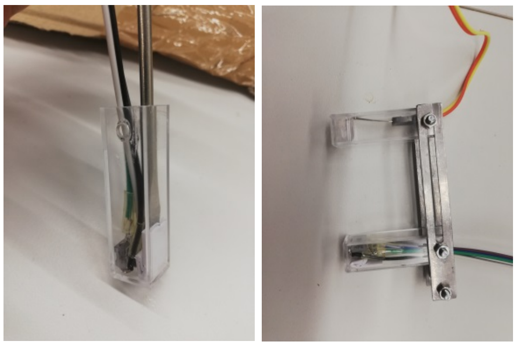


2. Assemble the cuvette holding and burn holes in the cuvettes with the soldering iron. Put the screw to the holes and fix them with the nuts.

3. Prepare cables and something to protect the contacts for example 200µl pipet tips. Solder the cables to the Opt101.



4. Insert the Opt 101 in to its cuvette and screw it in the cuvette holder. Add some paper so it stays in place and fits tight.



7. Cut out a hole in a cardboard box and put the photometer in.

Wiring of the Opt101 to the Arduino.

Connect the cables to the Opt101 as following:

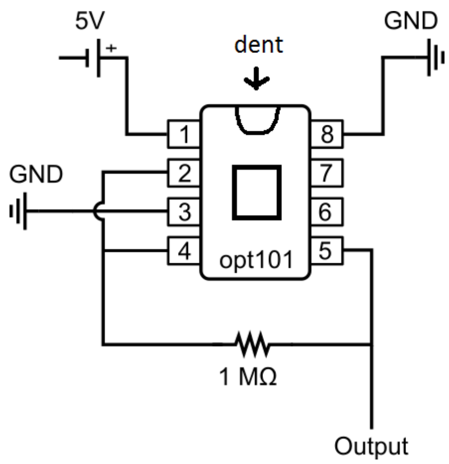
1: green 5/3,3V (VCC)

3: black to GND

2: blue, 4: grey, 5: white over a 1M ohm resistor to Output

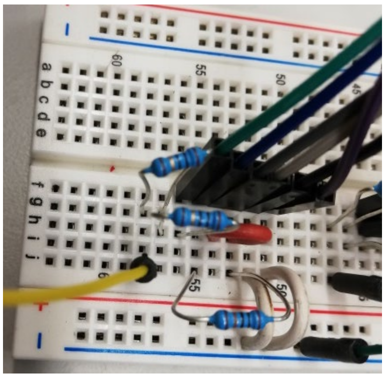
8: violet to GND

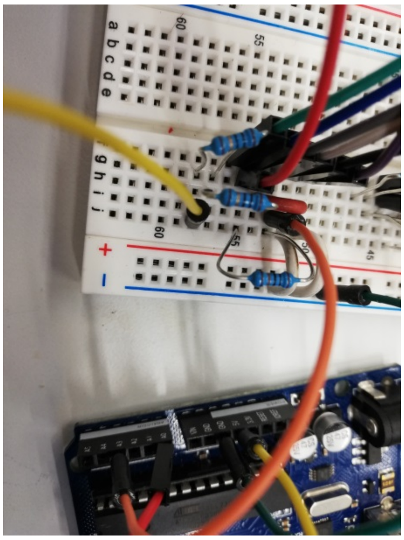
6 and 7 to nothing.



The sensor has a dent on one side this shows the orientation.
It should look like this:

- Connect the Output (orange) and a cable to monitor the current (red) to an analog pin (A0, A3) as well as the 5V (yellow) to the Arduino. Connect a cable (black) to GND.

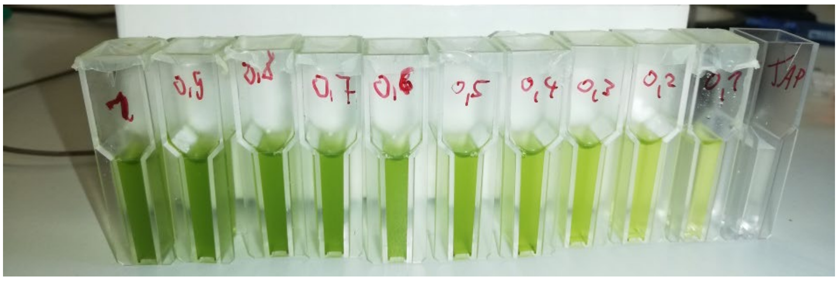




- For the 680nm LED connect a cable to 5V and put one 1 kohm and a 5 kohm resistor in a row to generate ~1.4 kohm resistance. This depends on the distance between LED and sensor as to the LED itself so it will differ from setup to setup. You can also put a Potentiometer instead of the resistor to find out which resistance fits your setup. But don't give the LED 5V, OR IT WILL BURST!

Calibration of the Opt101

Prepare a dilution series of your Algae in this case *C. reinhardtii.* And measure it with a at 680 nm photometer.



1. To calibrate the sensors set up a decadal dilution series of a given OD (from a reference photometer) from OD 2.5 down do OD 0.1.

2. Set the interval in the openPBR_control to 30 seconds

3. Start PuTTY and measure a single data point for each step of the dilution.

4. Include the data in to a excel file.

In order to get a net voltage, you need to subtract the output value from the input value. Subtract the V_{out} values from the V_{in} values and plot them against the reference OD from your photometer. Then perform a logarithmic fit (Fig. 1)

Your equation should look something like this: $\Delta V = a * \log(OD) + b$

In our example we got *a = 0.496* and *b = 4.389*.

In this case, y is the voltage ΔV and x the OD.

To get the OD from your measured values, solve your equation for x:

$$x = e^{\frac{\Delta V - b}{a}}$$

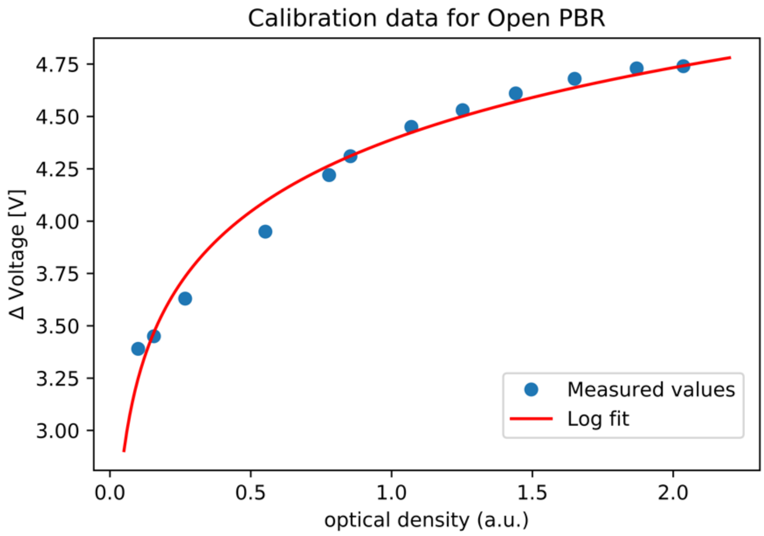


Image 1:Measured OD with reference photometer against measured ΔV with openPBR

After the calibration, we measured other random samples both with our reference photometer (Expected OD) and with openPBR (Figure 2).

We also plotted the “ideal correlation”, meaning expected = measured, in order to get an idea of how good the openPBR measurements are.

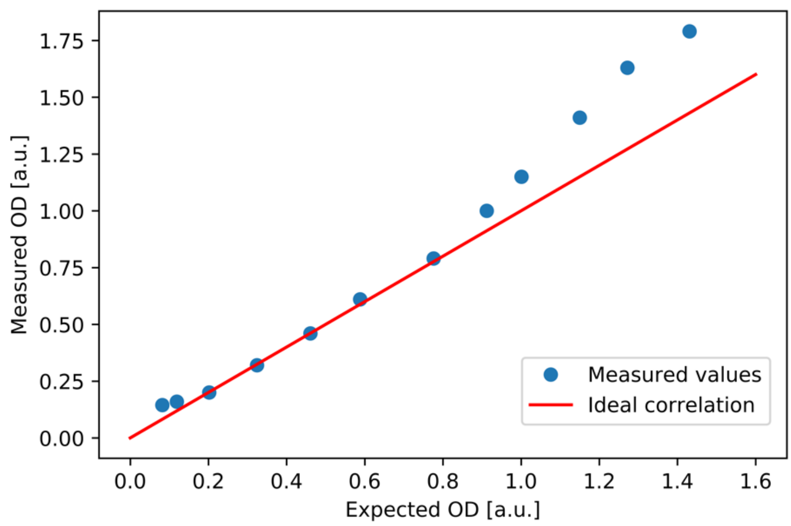


Figure 2: Expected OD values from reference photometer plotted against openPBR measurements with an “ideal correlation” of x = y

From this we can conclude that, while the openPBR measurements between OD = 0 and OD = 1 are quite reliable, further calibrating points are needed in order to improve the range beyond OD = 1.