A portable, low-cost, low-tech and open source fluorometer for the measurement of chlorophyll-a concentration

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1 Introduction

Chlorophyll-a concentration is a key indicator to estimate the phytoplankton biomass in aquatic ecosystems. Measurements of such concentrations can be made with a fluorometer. Its operating principle is to illuminate the water medium with a blue light and to measure the red fluorescence of chlorophyll-a. However, on today's market, commercially available fluorometers are quite expensive (several thousands of euros typically) and bulky. For the scientific community, there is a strong need to develop portable and cost-effective solutions, and a very active research is carried out in this area [1, 2, 3, 4, 5].

We are a team of students in photonics at Enssat, Lannion, France, and we took part in a collaborative project between Enssat and the Institut Universitaire Européen de la Mer (IUEM, *European Institute For Marine Studies*). This project meets IUEM's need to create a portable, low-cost, low-tech, and open source chlorophyll-a concentration sensor. The highly-sensitive device we have successfully developped is affordable (around 200 €) and easily reproducible thanks to the detailed documentaton available on GitHub [6].

The present document aims to provide an overview of some technical aspects of our fluorometer. It first presents the background and specific requirements of the project (defined in collaboration with the IUEM). The design of the fluorometer is then presented, with separate sections in optics, electronics and interfacing. Finally, we present the results of tests carried out with our fluorometer.

2 Needs

The IUEM provided us detailed specifications describing the needs and requirements for the development of the fluorometer. This sensor had to be portable, low-cost, low-tech and open source. The specifications served as the basis for guiding the design and the development of the chlorophyll-a concentration sensor, meeting standard requirements for environmental monitoring.

The technical specifications expected for the sensor in terms of metrology were the following:

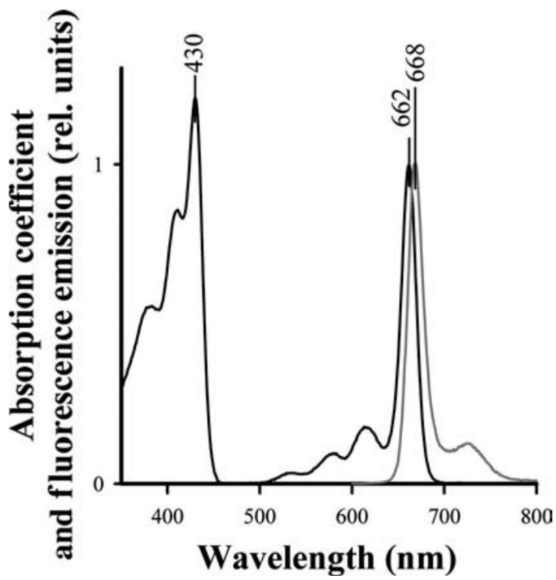
Range	[0.1-50] µg/L
Measurement sensitivity	0.1 µg/L
Measurement error	< 0.2 µg/L

The electronical specifications were:

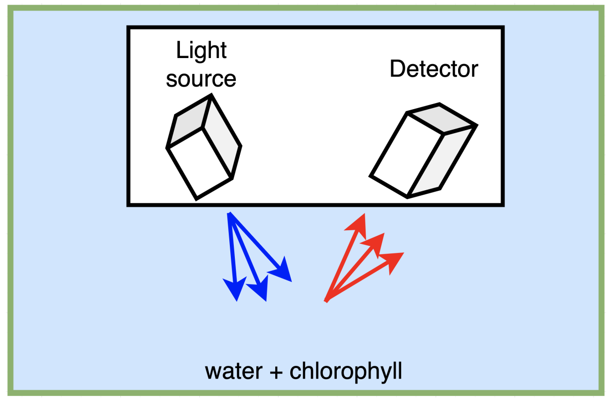
Measurement rate	10 sec
Response time	1 sec
Alimentation	3.3 V, 5 V or 12 V
Data output protocol	I2C, UART or analog

3 General principle

When chlorophyll-a is excited by a blue light source at a wavelength of about 430 nm, it re-emits light in the red spectrum around 670 nm. This red fluorescence is collected by a photodetector, enabling the concentration of chlorophyll-a to be measured. Figure 1(a) illustrates the absorption and emission spectrum of chlorophyll-a, and figure 1(b) shows a schematic diagram of a fluorometer.



(a) Absorption and emission spectrum of chlorophyll-a.



(b) Schematic diagram of a fluorometer.

Figure 1: Principle of fluorimetry with chlorophyll-a.

4 Fluorometer conception

4.1 Block diagram

Figure 2 illustrates the block diagram of our fluorometer. Blue LEDs illuminate the water medium containing chlorophyll-a. A photodiode detects the red fluorescence of chlorophyll-a through a red filter used to prevent the detection of light at other wavelengths than red. The Arduino board enables modulation and demodulation to eliminate ambient red light.

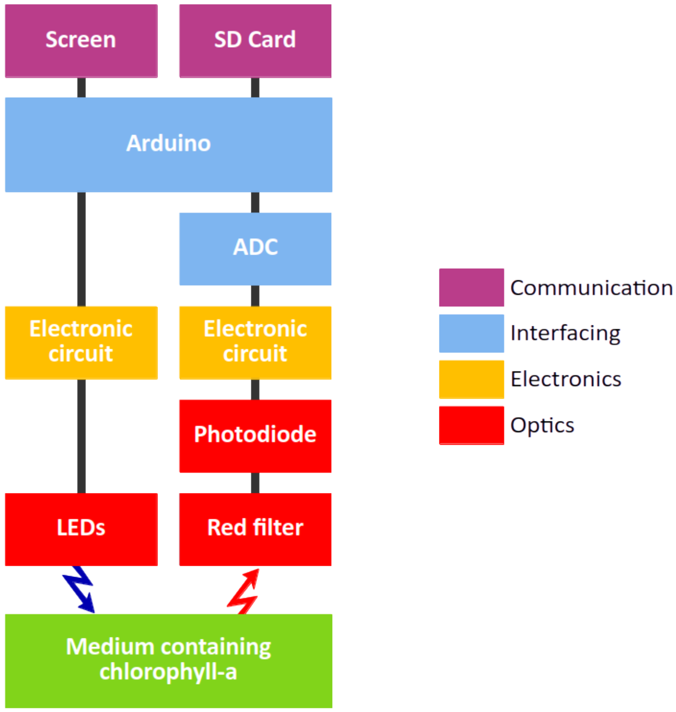


Figure 2: Block diagram.

The different parts shown in the block diagram of figure 2 will now be detailed.

4.2 Optical part

The optical part of our project is divided into two sub-parts : emission and reception.

4.2.1 Emission

As explained previously, a blue LED is needed to make a chlorophyll-a fluoremeter. We actually used two LEDs to double the available optical power. Specifications of the LEDs are given in table 1.

Reference	Luxeon C Color Line - Violet
Peak wavelength	424 nm (measured)
Spectral half-width	20 nm
Typical viewing angle	165°
Typical power	600 mW

Table 1: Specifications of the blue LEDs.

Due to their large viewing angle, we performed measurements to find the optimum angle between the LEDs and the photodiode. Figure 3 shows the measured voltage on the photodiode as a function of the angle between one LED and the photodiode in an experimental setup similar to figure 1(b). It shows that the optimal angle is around 30°. In the following, the photodiode will be positioned parallel to the air-water interface and the two LEDs will be on either side of the photodiode at an angle of 30°, as depicted later in figure 5.

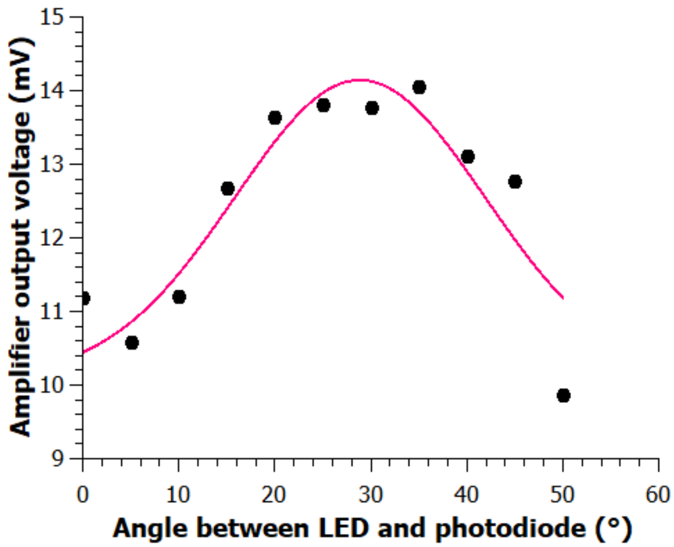


Figure 3: Amplifier output voltage on the photodiode as a function of the angle netween the LED and the photodiode.

4.2.2 Reception

Once the chlorophyll-a is stimulated with blue light, it fluoresces red light. This red light is collected by a photodiode positioned against the transparent window of the fluorometer to capture maximum fluorescence. A photodiode with a large area maximizes the collected light. However, it is also important to take into account the dark current of the photodiode. The dark current must be sufficiently low to detect low chlorophyll concentrations. The specifications of the choosen photodiode are presented in table 2.

Reference	Thorlabs FDS10X10
Dark current	200 pA
Surface	100 mm^2

Table 2: Specifications of the photodiode.

Three layers of a red filter are placed in front of the photodiode to filter out other wavelengths than red. The red filter we use is a light filter used by lighting technicians in the entertainment industry. Its specifications are described in table 3. The transmission spectrum of the filter is shown in figure 4. The red filter of 3D glasses can also be used for example.

Reference	Lee Filters 164 Flame Red
Colour temperature	6774 K
Transmission at 424 nm	3 %
Transmission at 670 nm	79 %

Table 3: Specifications of the red filter.

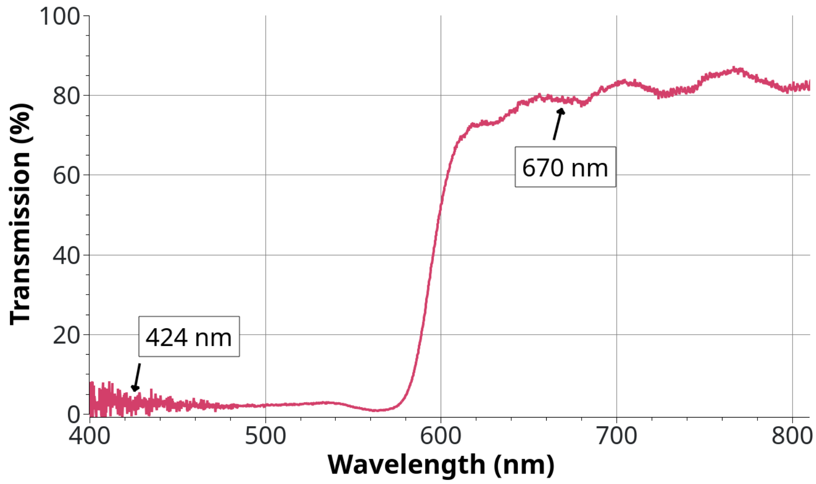


Figure 4: Transmission spectrum of the red filter.

The final setup for the optical components is shown in Figure 5.

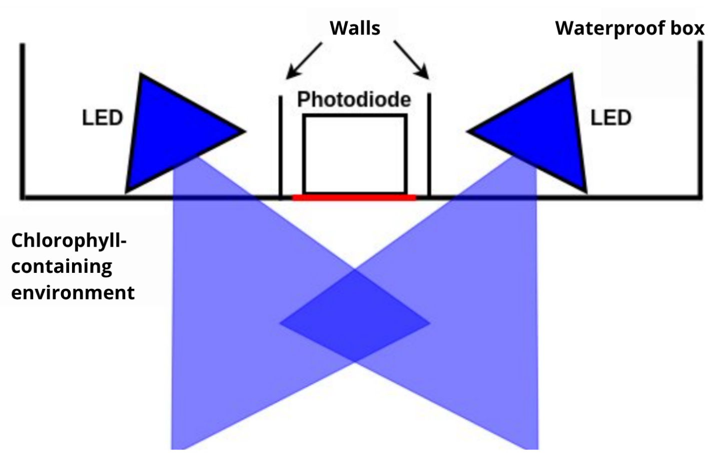


Figure 5: Final configuration of the optical components.

4.3 Electronical part

In this section, we describe the different electronic circuits present in our device.

4.3.1 LEDs alimentation

First, the two LEDs have to be powered with a current of 400 mA each. To do that, we propose the electric circuit of figure 6. The transistor provides the current that powers the LEDs. It is controlled by the desired voltage. The transistor transforms this voltage into a current that is shared equally between the two LEDs. The specifications of the transistor are presented in table 4.

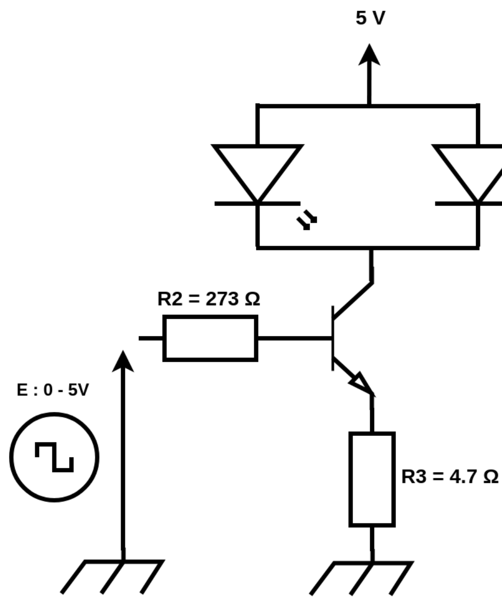


Figure 6: Circuit diagram of the LEDs alimentation.

Reference	PN2222
Type	BJT n-type
V_{CE} (sat)	1 V
V_{BE} (sat)	2 V
h_{FE}	between 100 and 300

Table 4: Specifications of the transistor.

4.3.2 Transimpedance amplifier

For the receiving part of the device, the transimpedance amplifier of figure 7 is proposed. Thanks to this amplifier, the photodiode current is converted into a voltage up to $V_{\max} = 5$ V in order to be read by an analog-to-digital converter (ADC). The amplifier gain corresponds to the value of R_1 and is 10^5 V/A in our case. This indicates that the maximum measurable photocurrent is $I_{\max} = V_{\max}/R_1 = 50$ μA. In the absence of additionnal noise source, the minimum measurable photocurrent is theoretically the dark current of the phototodiode, i.e. 200 pA in our case.

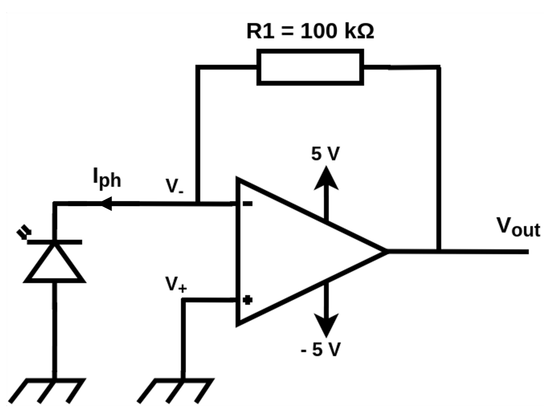


Figure 7: Circuit diagram of the transimpedance amplifier.

4.3.3 Generation of −5/0/+5 V

Self-sufficient power supply (typically a powerbank) can be ideal to power the fluorometer. However, this kind of battery provides only a 0-5 V voltage. To generate the −5/0/+5 V voltage needed by the operational amplifier of figure 7, we use the switched capacitor voltage converter (MAX660) shown in figure 8.

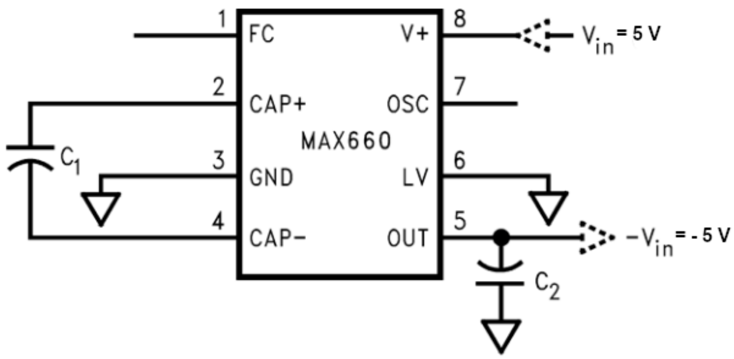


Figure 8: Switched capacitor voltage converter (MAX660) for −5/0/+5 V generation.

4.4 Interfacing part

4.4.1 Modulation of the LEDs

The blue light emitted by the LEDs is on-off modulated by the Arduino at a frequency of 100 Hz and a duty cycle of 1/2. This modulation is maintained during 1 s, then the LEDs are switched off for 9 s. The time diagram of the modulation scheme is shown in figure 9. The modulation frequency of the LEDs is limited by the execution time of the Arduino program.

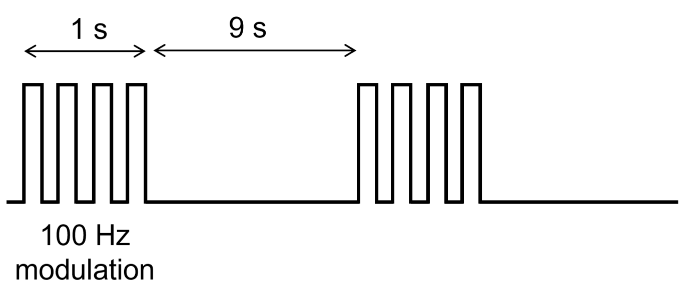


Figure 9: Modulation time diagram.

4.4.2 Analog-to-digital conversion

The Arduino board we use is the UNO R3. Its internal ADC is a 12-bit ADC. To increase the resolution of our fluorometer, we prefer to use an external ADC (ADS 1115) with a 16-bit sampling. Thus, the resolution of the fluorometer is 76 μV per bit, corresponding to 0,76 nA per bit.

4.4.3 Demodulation

To demodulate the signal, the program reads the photodiode voltage during both the on-state and the off-state of the LEDs during the flashing phase of 1 s. Then it substracts the off-state voltage V_{off} from the on-state voltage V_{on} in order to eliminate the ambiant red light. This ensures to measure only the fluorescence of the chlorophyll due to the blue excitation. Figure 10 summaries the demodulation process.

Then the programme averages the 100 values calculated during the 100 periods of the flashing phase and returns one single value of photocurrent every 10 s.

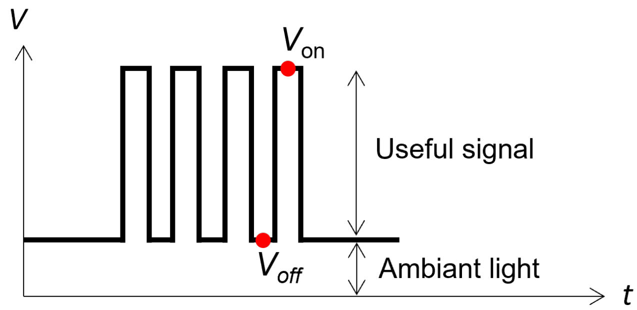


Figure 10: Demodulation of the received signal.

4.4.4 Display and storage

A OLED screen is added to facilitate interactions with the users. It displays the values of the measured photocurrent in nA.

A SD card shield is also added for long-term recording. The photocurrent values, as well as the time of measurement, are recorded on the SD card in the form of a text file, as shown in figure 11.

```
Time (ms) Current (nA)
5744 4.28
8744 4.20
11745 4.19
14744 4.53
17744 15.34
20745 18.23
23745 20.77
26744 23.01
29745 26.20
32745 28.18
35744 42.23
38745 50.27
41745 50.62
44744 70.00
```

Figure 11: Text file recorded on the SD card.

4.5 Final diagram

The global circuit diagram of the fluorometer is shown in figure 12.

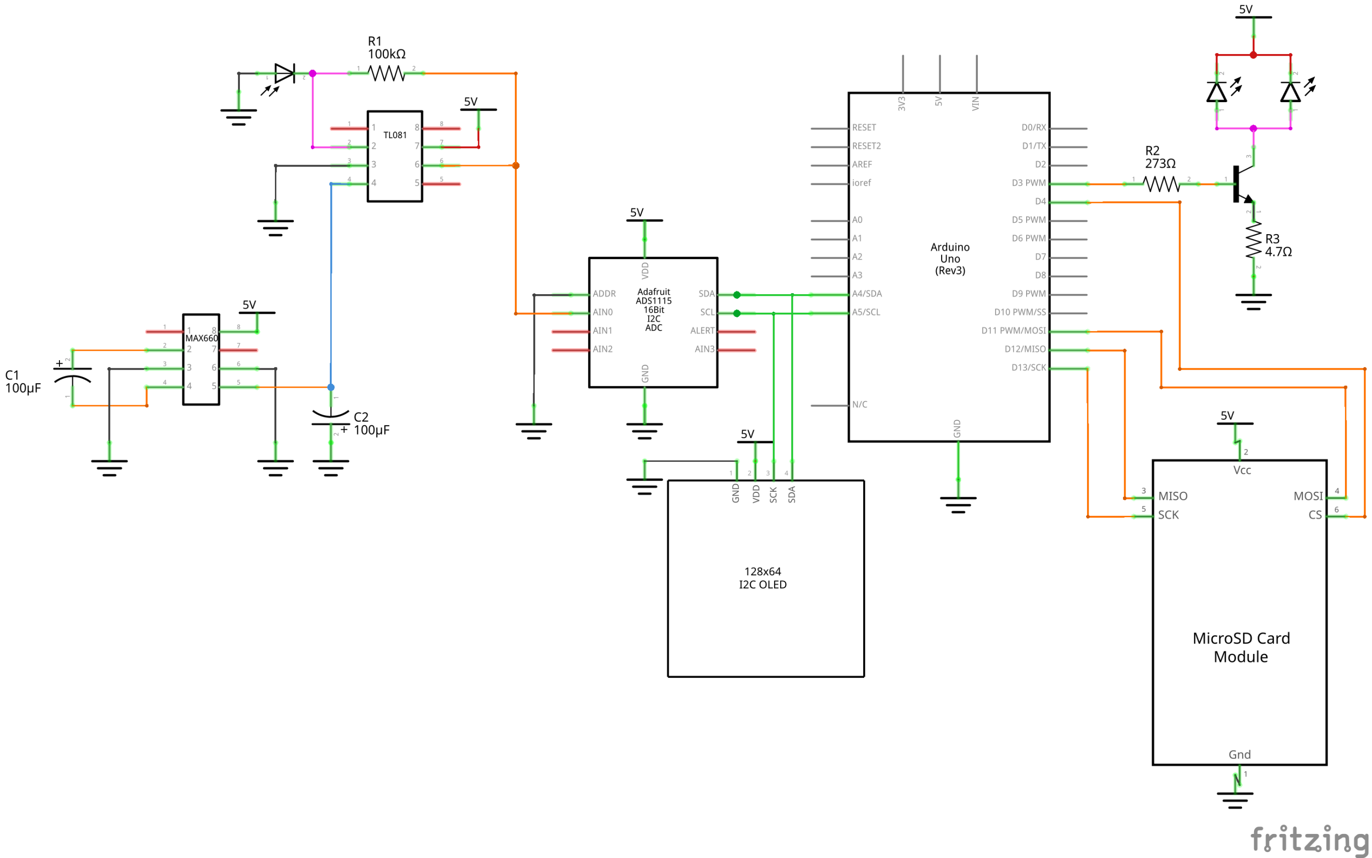


Figure 12: Fluorometer's global circuit diagram.

4.6 Final Assembly

The fluorometer is assembled in a PVC tube. Access plugs are glued to the ends of a sleeve. The caps are cut out and a transparent plate of PMMA plastic is glued on to let the light going through. The inner structure that holds the Arduino and the circuit board is made in black PMMA and cut with a laser cutter. Spacers are used to strengthen the structure. The holder for the LEDs and the photodiode is 3D-printed and screwed to the structure. A powerbank is added in the box for the autonomy of the system.

The bottom cap is glued and cannot be opened. The top cap can be opened and the structure is easy to remove in order to recharge the powerbank, take the SD card, load a program on the Arduino or replace a component. The complete box is waterproof. Yet, PVC box may not withstand high pressure underwater.

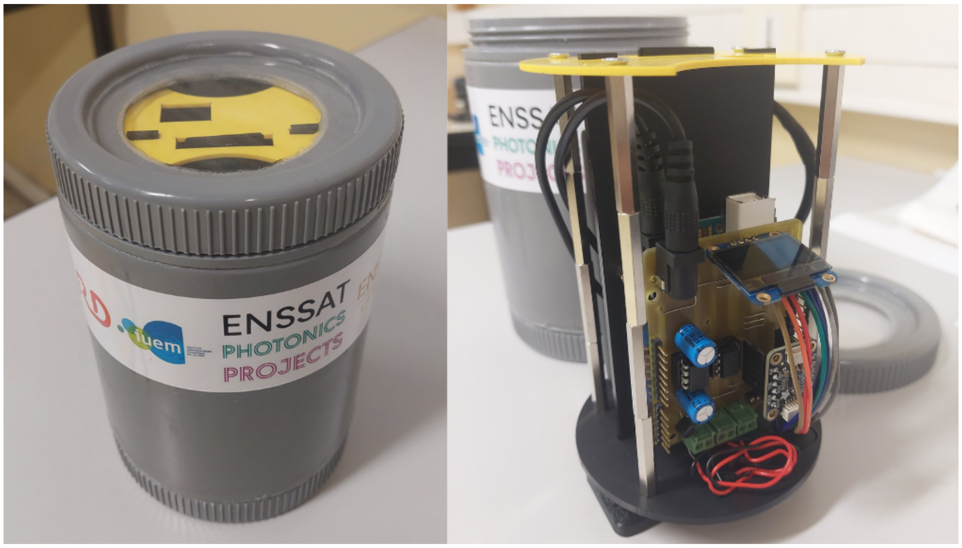


Figure 13: Photos of the prototype.

5 Tests and results

A series of tests were carried out to caracterize and calibrate the fluorometer.

5.1 Measurements with spinach chlorophyll-a

Firstly, measurements of solutions of chlorophyll-a extracted from spinach leaves have been made. The spinach chlorophyll-a was extracted following this method : spinach leaves have been shredded and the chlorophyll-a filtered using 96% ethanol. Then the concentration has been measured with a spectrometer thanks to the formula demonstrated in [7]. These measurements were done in a dark room. The results are shown in figure 14.

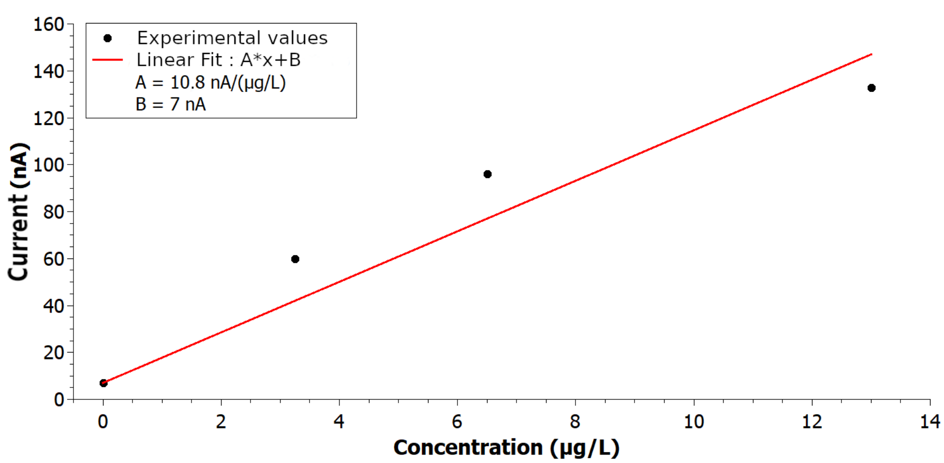


Figure 14: Calibration of the fluorometer with spinach chlorophyll-a.

This calibration shows that the fluorometer can measure low concentrations of chlorophyll. The first point, corresponding to 7 nA, is a measurement in clear water. This gives the detection threshold of our prototype. This value is not the 200 pA dark current of the photodiode because of other noise sources (due do electronics for example).

5.2 Measurements with phytoplankton

Then, measurements of solutions of phytoplankton have been run with our fluorometer. A highly concentrated phytoplankton solution has been diluted. Values of concentration were measured with a commercial fluorometer (the RBR*tridente*) and the corresponding current was read on our low-cost fluorometer. Figure 15 shows the results.

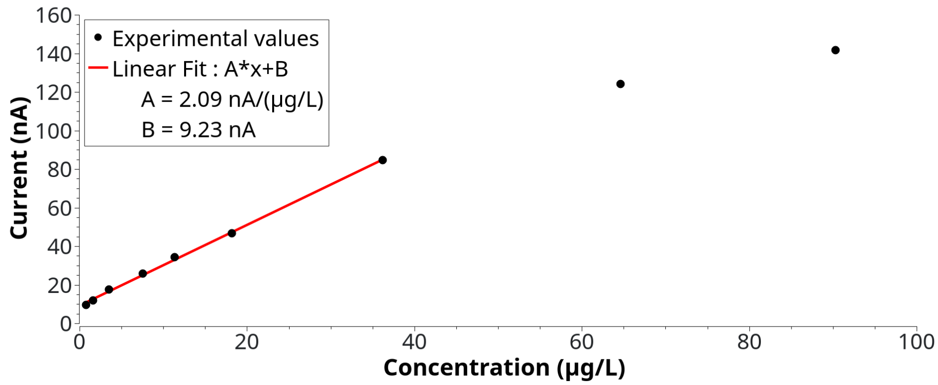


Figure 15: Calibration of the fluorometer with phytoplankton.

The fluorometer operates linearly over a range from 1 to 50 µg/L. The interesting point is that our fluorometer can measure chlorophyll-a concentrations as low as 1 µg/L under experimental laboratory conditions. The prototype is limited to indoor use, as the photodiode saturates outdoors in bright light.

6 Conclusion

During this project, we have realised a fluorometer for chlorophyll-a concentration measurements. This fluorometer is portable, low-cost and low-tech. It can be completely reproductible thanks to a public GitHub repository [6].

Our fluorometer can measure chlorophyll-a concentrations as low as 1 μg/L. This performance is achieved thanks to the use of two blue LEDs illuminating a large volume of water and a large-area photodiode with a low dark current.

Our fluorometer is simple, low-cost (around 200 €) and easy to make thanks to the use of standard components.

In the context of global warming, the need to measure phytoplankton is crucial. Portable and low-cost solutions might play a very important role in the future for diagnosing the ecological status of aquatic environments. We sincerely hope that our solution can help the scientific community, and would be delighted to make a small contribution to environmental protection through the study of coastal zones.

Acknowledgments

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Hence, we would like to thank Maël Guibré (student at IUT de Lannion) for the time he took to make some videos of the project.

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