Building a modular and robust fluorometer for detecting chlorophyll *a* concentrations in regions surrounding aquaculture operations

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Abstract

Aquaculture has the potential to become the world's most important and sustainable option for protein production, but many of those involved in the industry currently lack the tools, resources, and education to make that happen. The purpose of this paper is to explore the feasibility of creating a robust but low-cost fluorometer that can detect chlorophyll levels and provide offshore aquaculture operations with immediate feedback on how their day-to-day operations impact the nutrient levels in the surrounding waters. The experimental setup involves an LED that emits light at 430 nm, focusing optics, and a photodiode placed perpendicular to the LED that is capable of detecting any light emitted from an algae sample. Preliminary results are promising as they demonstrate that such a setup is sensitive enough to detect the difference between samples containing different concentrations of chlorophyll. Further testing is required to optimize the fluorometer, and more research needs to be done into how to package the sensor and make it suitable for in situ trials.

Introduction

Historically, offshore aquaculture operations haven't been known for being environmentally sustainable. Besides the potential to release hundreds of thousands of invasive species into their surrounding waters (**Bush, 2019)**, marine aquaculture operations have also been criticized for dumping nutrients, chemicals, and antibiotics into the environment and contaminating surrounding areas (**Mavraganis *et al.*, 2020**). Despite all this, aquaculture actually has the potential to be one of the most sustainable and cheapest ways to produce protein for human consumption. Compared to other forms of protein (raising chickens, pigs, cows, etc.), aquaculture species require significantly less feed, energy, and land to produce (**Torrissen *et al*,. 2011 and Froehlich *et al.,* 2018**). However, in order for the benefits of aquaculture to outweigh its potential risks, several important improvements need to be made with how aquaculturists run their operations.

One simple way to help aquaculturists run their farms more sustainably is to equip them with sensing devices that can instantaneously provide data about important water quality parameters such as chlorophyll *a*. The largest source of chlorophyll *a* in water is photosynthetic organisms

such as algae and certain species of bacteria (**Levy, 2017)**. Therefore by tracking chlorophyll levels, one can also assess the risk of toxic algal blooms. The amount of algae in the water is also closely tied to the amount of nutrients present, which is why regulators often measure chlorophyll *a* levels when determining whether or not an aquaculture operation is contaminating its surroundings with excessive nutrient deposition (**Eaton *et al*., 2005**). If aquaculturists were able to track chlorophyll levels in real time, they would have instant feedback on whether their day to day operations were resulting in nutrient levels that exceed regulatory standards and threaten the wellbeing of their stock and wild marine species alike.

Currently, the most accurate and reliable way to measure chlorophyll *a* levels involve taking a water sample to a lab, filtering the sample to concentrate photosynthetic organisms, mechanically rupturing cells containing chlorophyll, extracting chlorophyll from the ruptured cells with acetone, and finally analyzing the extract by spectrophotometric means (**Eaton *et al*., 2005**). This process is both costly and time consuming, therefore not suitable for someone trying to continuously monitor chlorophyll levels.

On the other hand, there are devices suitable for in situ use (**Dauliang *et al*,. 2015**). Unfortunately, the cheapest commercially available hyperspectral imaging devices and fluorometers range from 5-10 thousand dollars, and often require purchasing additional equipment and software to run properly. They also aren't usually designed to be used alongside other instruments, making them difficult to autonomous robots and other platforms. Designing a modular, intuitive, and cost effective fluorometer that can accurately measure chlorophyll levels would therefore prove incredibly useful for aquaculturists looking to improve the sustainability of their operations.

Methods

A blue LED emitting light at 430 nm was the primary light source for this experiment. We used this LED because chlorophyll *a*, the most abundant chlorophyll pigment in most photosynthetic organisms, has a peak absorbance at roughly 430 nm (**Porra *et al.,* 1988**).

Because the LED had a beam angle of 44 degrees, we used two aspheric condenser lenses to focus its light onto our sample. To determine the placing and positioning of our focusing optics, light source, and sample, we used an iPad app called Raylabs to simulate our experiment.

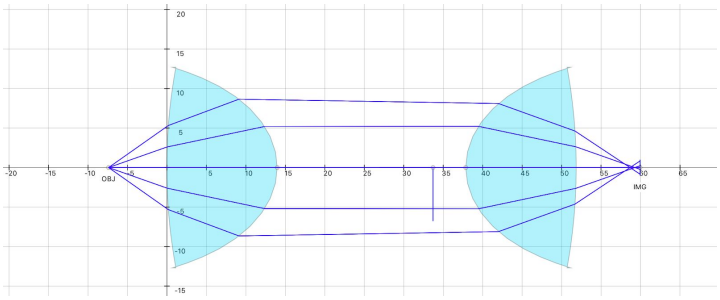

Figure 1. Raylabs simulation of optical setup

Each of the optical components were fitted into a mount of adjustable height, which in turn was attached to an aluminum breadboard, ensuring that everything was securely in place at measurable distances apart. Custom 3D printed mounts were made for the LED, cuvette, and photodiode.

Our water samples were placed in quartz cuvettes, and the photodetector was placed perpendicular to the light source and parallel to one of the cuvette's sides in order to minimize the amount of light that reached the photodetector from the LED. The photodetector was also encased in a small blackbox and placed behind an aperture to further ensure that any light detected by the photodiode was from the sample's fluorescence, and not from the LED. When collecting data, the entire setup was placed inside of a blackbox to eliminate ambient light.

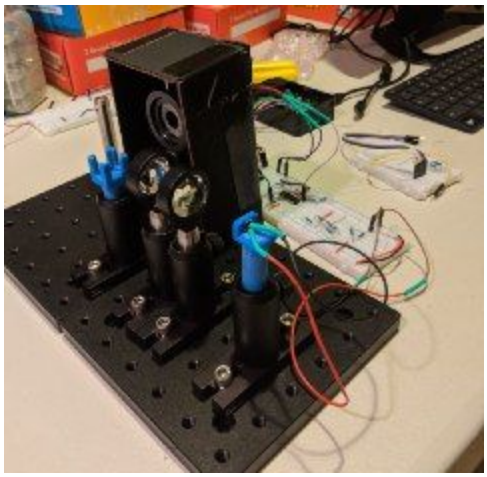

Figure 2. Completed setup with the photodiode encased in a black box, and blocked by an aperture

The photodetector we used was sensitive to light in the 350-1100 nm range, which suits our purposes well since chlorophyll *a* fluoresces at around 673 nm. In order to read data from our photodetector, we connected the diode to an ADC, which in turn fed data to a raspberry pi. We

used a simple low pass filter to generate a cleaner signal, and amplified the voltage output by a factor of 100.

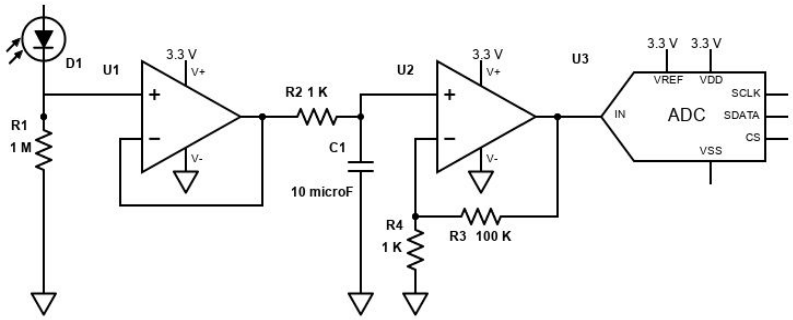

Figure 3. The circuit diagram of the photodiode signal processor

Our experiment involved two different chlorophyll samples. Sample A was made by cutting up 2 grams of grass and soaking it in 8 oz of 70% isopropyl alcohol for 2 hours. After that, the mixture was strained through tissue paper to remove all grass particles, leaving all the chlorophyll that was extracted in the alcohol solution. Sample B consisted of a colony of chlorella vulgaris, a species of green algae, that was allowed to grow in a saline nutrient solution for 2 weeks.

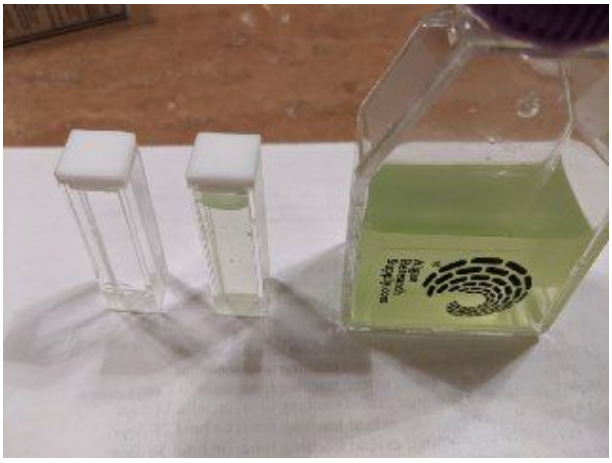

Figure 4. The colony of chlorella vulgaris (on the right), next to a sample at 50% dilution (center cuvette) and a sample of plain water (left most cuvette)

Results and Discussion

After sample A was prepared, it was diluted with isopropyl alcohol at consistent intervals. Each diluted sample was placed into a clean cuvette, and then placed in the fluorometer. For each sample, the fluorometer ran for 50 seconds, collecting data at every half second. The final result was an average of those 100 points. As seen in figure 6 (below), the fluorescence increased with increasing concentration up to around 25% concentration, at which point fluorescence remained consistent. This is likely due to the fact that emitted light begins to get reabsorbed by ground state chlorophyll molecules past a certain concentration.

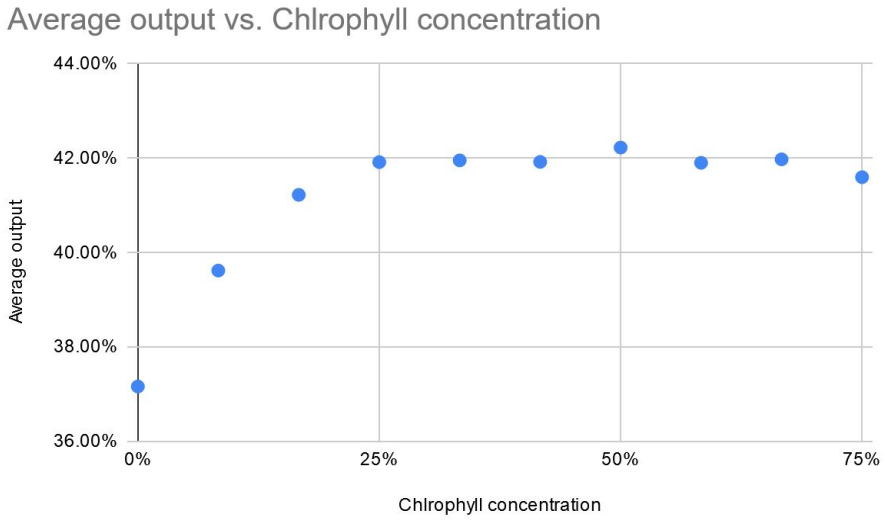

Figure 5. Average output of the fluorometer as a function of chlorophyll concentration. The y axis was scaled between 0 - 100%, where 0% represents a voltage output of 0 V, and 100% represents a voltage output of 3.3 V (max voltage).

A similar process was taken when collecting data for sample B, except the algae sample was diluted with bottled water rather than isopropyl alcohol. Similar to sample A, one can see that fluorescence increases with increasing concentration, and then past a certain concentration, the fluorometer begins to lose sensitivity as emitted light begins to get reabsorbed by ground state molecules. However, this may have also been due to human error as very few data points were taken.

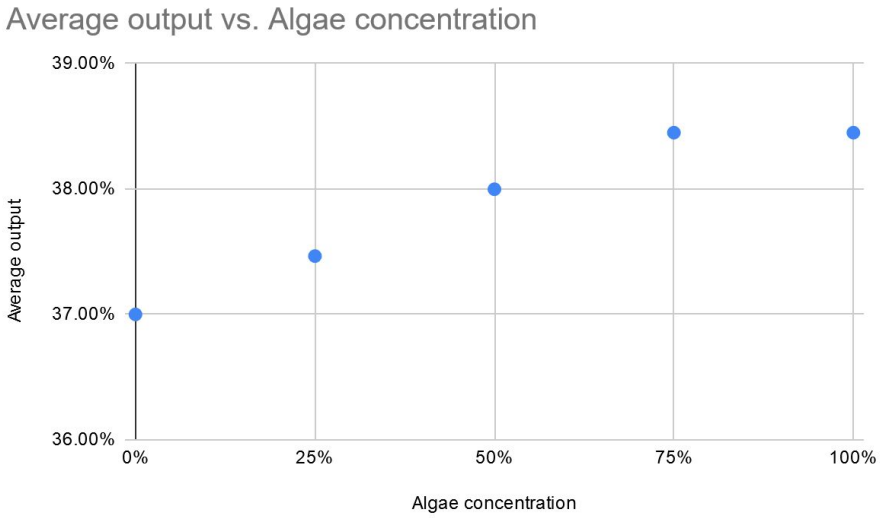

Figure 6. Average output of the fluorometer as a function of the algae concentration.

Conclusion

Preliminary results from this experiment demonstrate the feasibility of creating a robust and relatively simple fluorometer capable of discerning between small increments of chlorophyll. Due to the limitations of remote work and the lack of proper lab equipment, this experiment may have been impacted by small inaccuracies throughout. Regardless, the results clearly demonstrate that the fluorometer will detect more fluorescence at greater concentrations of chlorophyll, proving its potential as a proof of concept for future fluorometers. Besides collecting more data, our short term plans involve trying out different light sources and increasing the amplification of the voltage signal to hopefully increase the sensitivity of the sensor. In the future, we also plan on integrating this device with a moored profiler and using it for in situ trials.

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