Portable Fluorescence Spectrometer for Measuring Water Quality

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***Abstract* — In this project the design and implementation of a portable fluorescence spectrometer to measure the quantity of chlorophyll in a sample is detailed. Chlorophyll is the key photosynthetic molecule present in all plants, algae, and other photosynthetic organisms.[1] This molecule is present in cyanobacteria and thus a large presence of chlorophyll in a body of water can indicate excess cyanotoxins that may be hazardous. When exposed to blue light, chlorophyll fluoresces allowing for it to be quantitatively detected. To this end we will create a compact device that can measure the concentration of chlorophyll in a body of water.**

***Index Terms* — CMOS image sensors, driver circuits, fluorescence, health and safety, lithium batteries, spectral analysis, spectroscopy.**

I. Introduction

In this paper the design and construction of a cheap and portable fluorescence spectrometer to be used for in-house water quality analysis is illustrated. A group of interdisciplinary students, consisting of two Photonic Science & Engineering students, an Electrical Engineering student, and a computer engineering student will be working together to produce the device.

This project is designed with many goals in mind. The device's main function will be to provide consumers with an easy way to test the quality of a body of water by taking a sample and running it through the device. The device should be capable of informing the user about the quality of the body of water from the measurement taken.

Bodies of water can contain many molecules that can be useful in determining the water quality. One such molecule is chlorophyll-a, which will be the focus of this project. The chlorophyll-a content in a body of water can indicate the presence of cyanobacteria, a contaminant which is often found in freshwater sources. This organism can be harmful in large populations, so it is necessary to detect its presence in order to affirm the health of the body of water. If there is a high presence of chlorophyll-a, the device will let the user know so they can take the necessary steps for treatment of the body of water.

The most common way to detect the presence of a material in a solution is through the use of spectrometers. Spectrometers take a signal consisting of many different wavelengths or colors and separates them spatially so the user can determine the presence of a compound in a solution. There are many spectroscopic methods that can be used to identify contaminants within a sample. However, since our sample of interest naturally fluoresces, this project will focus on using fluorescence spectroscopy to detect chlorophyll-a within a sample of water.

Fluorescence spectroscopy is a spectroscopy technique that measures the presence of a molecule in a sample by the fluorescence emitted after exciting the sample with a light source. The excitation source is focused onto a sample using a lens. This light is then absorbed by the sample as energy causing it to excite the electrons in the sample to a higher energy state. While in this higher energy state the electrons undergo vibrational relaxations that cause them to lose energy. After a short period of time the electrons will then naturally fall back to the ground energy state emitting this energy as a photon. As a result of the energy loss due to vibrational relaxation, when the electrons fall back to the ground state, they have less energy than the light used to excite the sample. This results in a photon with less energy and thus lower wavelength being emitted.[2]

II. Sample

The sample of interest in this device is cyanobacteria, a type of algae that is commonly found in freshwater sources. The chlorophyll-a content in a body of water corresponds directly to the cyanobacteria concentration. Chlorophyll-a exhibits an absorption peak at 430 nm and fluoresces light at 665 nm. The sample should be kept under 0.1 OD to ensure accurate results as samples with higher concentrations of chlorophyll may exhibit nonlinearity due to the sample absorbing the fluorescent light. The World Health organization describes a moderate risk level of chlorophyll-a from cyanobacteria in freshwater as 50 micrograms per liter, and a very dangerous level as 50 milligrams per liter. [3]

A. Sample Preparation

The sample to be measured in this device is to be obtained from a body of water where it is necessary to measure the chlorophyll content. A known volume of water should be extracted from the body of water so the final concentration can be determined. Sample preparation consists of separating the chlorophyll from the water using a centrifuge. After this is finished the sample is then decanted. A known amount of spectrophotometric grade acetone must then be added to dissolve the algae. Do this by grinding the algae using a mortar and pestle to break open the cell membranes and release the chlorophyll. The sample should then be placed into a 1 cm optical path length cuvette and sealed to await measurements. It is best to take measurements within one day of sample preparation to avoid evaporation.

B. Concentration Measurements

To determine the concentration of the sample from the fluorescence measurements, a baseline first needs to be established. This will be done using the spectrophotometer made available by CREOL. By measuring the absorbance of the sample, the concentration is found using Beer's Law.

$$A = \varepsilon bc\,. \tag{1}$$

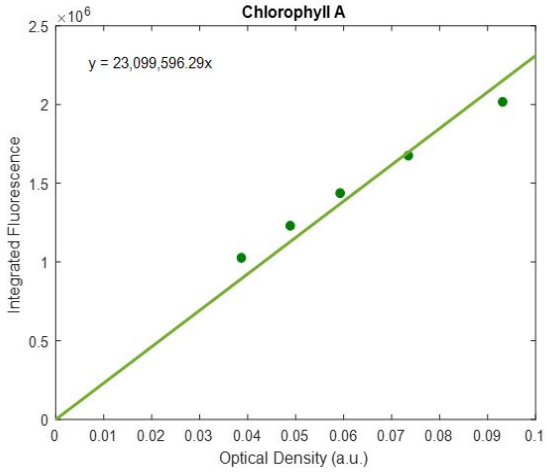

Fig. I. Linear trend of integrated fluorescence vs. optical density of chlorophyll-a.

The optical density of the absorbance spectrum is recorded for a sample. Subsequently, the integrated fluorescence of that sample is calculated using the aforementioned fluorescence spectrometer. This measurement is repeated for a number of samples to determine a linear relation between fluorescence and optical density of which the slope is the quantum yield as displayed in Fig. 1. After this is obtained, any further measurements can be directly converted to concentration using this slope.

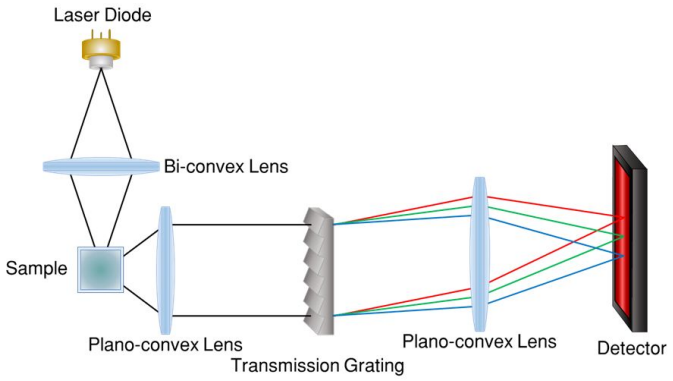

Fig. II. Spectrometer design.

III. Optics

For improved durability we ensure that there are no moving components in the final product. To this end we chose a spectrometer design that is based off of the littrow configuration.[4] Fig. 2 outlines the spectrometer setup we used. The main difference between our design is that we used lenses and a transmission grating. Since our spectral range was not too large and we don't need to worry too much about chromatic aberration, we preferred lenses over mirrors as they are cheaper.

The device has two main sections, an excitation setup and a spectrometer setup. The excitation setup refers to the first portion of the optics in which the laser diode is directed onto the sample to excite the electrons in the sample. The spectrometer setup is the portion of the device that focuses on collecting and spatially separating the fluorescent light by wavelength.

A. Excitation Setup

The device will consist of a laser diode that operates at 405 nm to excite the sample. While chlorophyll-a exhibits a peak of absorbance at 430 nm, a 430 nm laser diode must be custom made and is thus expensive. A 405 nm excitation wavelength was tested and the sample was found to still fluoresce.

The light emitted from the laser diode will be focused onto the sample using a 20 mm bi-convex lens. This lens was chosen due to its short focal length. This not only

allows for a smaller spot size which is useful in enabling the fluorescence from the sample to act as a point source but also increases the compactness of our design.

Next a cuvette holder is used. The sample should be placed in a 1 cm optical path length cuvette which can slide right into the cuvette holder. It is recommended to use optical glass as the cuvette as all measurements were taken using optical glass.

A1. Class 3B laser 405 nm

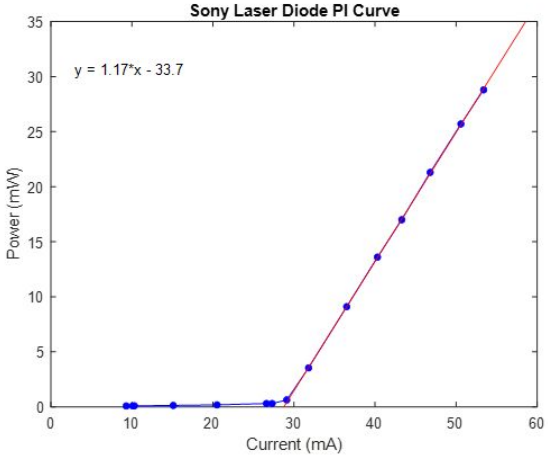

Fig. III. PI-Curve of SLD323VF Sony laser diode.

Some things we had to keep in mind when taking these measurements were the max current and voltage we supplied to the laser diode. According to the specifications on this diode the maximum operating current is 65 milliamps and maximum operating voltage is 5.5 volts. We had to be careful not to exceed these maximum operating values for these as they can damage or even burn out the laser diode.

After testing the spectrum of the laser diode, we found that the laser diode yielded a central wavelength of 409 nanometers [Fig 4]. This is slightly larger than the expected value for the central wavelength, but rather than being an issue, this is actually good for us as it is closer to 430 nanometers our ideal wavelength. The full width half max was also slightly different from specifications, 3 nanometers as opposed to 10 nanometers. This was also a good thing for us as a smaller full width half max ensures that the light is all in the certain part of the spectrum we want.

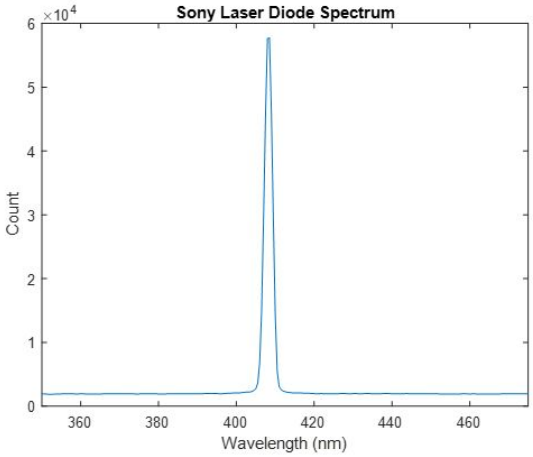

Fig. IV. Spectrum of the SLD3232VF Sony laser diode.

B. Spectrometer Setup

After the cuvette, a plano-convex lens is placed at a 90 degree angle to the incoming light in order to collect the fluorescent light from the sample. This lens is placed at exactly one focal length away from the sample in order to collimate the light. It was chosen such that it has a high NA. This allows for more light to be collected, giving a stronger fluorescence signal.

Following the collecting lens, a transmission grating is placed. This is used to spatially separate the light. A transmission grating of 300 grooves/mm is chosen as that has the highest diffraction efficiency at our wavelength of interest.

Finally, the light is focused onto a detector. For this a Sony IMX219 CMOS sensor with a lens attachment is used. The lens has a small focal length and diameter, allowing for a larger spectral range but at the cost of limiting the resolution.

C. Spectral Range and Resolution

Our goal for this design was to have a spectral resolution of less than 5 nm. We determined that this is enough to distinguish the peak of fluorescence wavelength. As chlorophyll-a fluoresces in the red light range, the spectral range will only need to detect light in the red light range, for this reason we specified a spectral range of 600 nm to 750 nm.

The final design had a resolution of 2.2 nm, and a spectral range encompassing the entire visual light range. This was still within specifications.

IV. Hardware

A. Laser Diode

We chose the Sony SLD3232VF Laser Diode because it operates at a wavelength close enough to the absorption peak of chlorophyll-a. We tested the laser diode using a spectrometer and determined that it operates at 409 nm. It has a common cathode arrangement and must be powered by a laser driver to ensure a constant current. Through creating a PI curve and IV curve we determined that the laser diode is best operated at 5.5 V and 55 mA

B. Battery

After reviewing all options, we decided to use a 3.7 volt, 2500 mAH Lithium Polymer battery to power our project. Although it's not as environmentally friendly as NiMH, it's a longer lasting battery and will not get thrown away as frequently. We'll also require only one lithium polymer cell as opposed to three or more NiMH cells. Our battery will be charged via micro USB port on the Adafruit Power Boost using the Raspberry Pi 5V USB power supply.
Due to the pandemic and lack of resources, we also had to add a 9V alkaline battery to power the laser.

C. Indicator LEDs

To make the device user friendly, we decided a simple solution would be to include indicator LEDs to let the user know what part of the process is occuring. The blue LED that indicates the power to the Pi is on. The red LED indicates that the battery power is low so the user knows to plug in the charger. The yellow LED indicates the battery is being charged. The green LED indicates the battery is done charging. The white LED indicates the laser diode is on and the Pi can proceed with collecting the data from the sample.

D. Raspberry Pi

The Raspberry Pi was chosen for the project because it's user friendly with a lot of open source information. The Raspberry Pi satisfies our need to be able to capture an image, process information from our sensor, and translate it into a spectrum. Compared to the MSP430 and the Arduino Uno, the Pi is best suited for image processing that the MSP430 cannot provide in terms of power, and the Arduino Uno cannot provide in terms of memory.

E. Camera Module V2 for Raspberry Pi

We decided to use a Camera Module V2, which is a camera that uses an RGB. Most common sensors used in spectrometers are monochromatic, since they do not filter out wavelengths differently on different pixels. Cameras like the module have a Bayer filter on the sensor which causes pixels to detect only red, green or blue light depending on the location of the pixel automatically correct data.

Raw Bayer Data is data recorded by the camera's sensor before any GPU processing. Bayer data consists of 10-bit values, per the sensitivity used by the IMX219 sensor in our camera's module and is organized in a BGGR pattern. The red values in the bayer data are then separated from blue and green and used as the intensity to find the spectrum.

V. Electrical System

A. Laser Diode Driver

Our original driver was going to be powered by the same Li-Po battery as everything else. We planned to use the same voltage booster as the one in our charging circuit to boost to the necessary voltage for the laser diode but due to the recent delays and restrictions, the laser diode will now be powered from a separate replaceable 9V battery.

The laser diode only requires between 5V and 6 V and 50 mA and 60 mA, so resistors were added in series and parallel to reduce both the voltage drop across the laser diode and the current the laser diode is receiving from the 9V battery. The capacitor in parallel decreases the ripple voltage across the laser diode, keeping it stable.

We added a white indicator LED to notify the user that the laser diode is on. We also added a switch which prolongs the life of the battery therefore making the design as efficient and non wasteful as possible given the circumstances. Using the following circuit, the voltage drop across the laser diode is 5.5V with a current of 55mA and the voltage drop across the white indicator LED is ~3.4V.

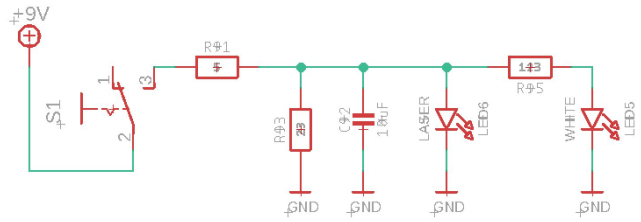

Fig. III. Modified driver circuit.

The user will need to flip the switch to turn on the laser diode before running the program to collect the data. After the data is collected they can return the switch to the off position.

B. Power

There are two major components in the charging circuit related to the lithium polymer battery: the TPS61090RSAR voltage booster and the MPC73781 charging regulator. Due to the recent events, we were not able to use these components because we do not have access to the tools we would require to solder these surface mounted components.

The TPS61090RSAR was chosen because it allows an input voltage range of 1.8V-5.5V, which allows our input voltage of 3.7V, and can boost to a maximum output voltage of 5.5V and the Raspberry Pi only requires 5V.. The circuit below is to boost the voltage in order to power the Raspberry Pi using only the battery. The Pi is what performs the image analysis. The TPS61090RSAR boosts 3.7V from the lithium polymer battery to the 5V required to power the Raspberry Pi via USB A and keeps it regulated.

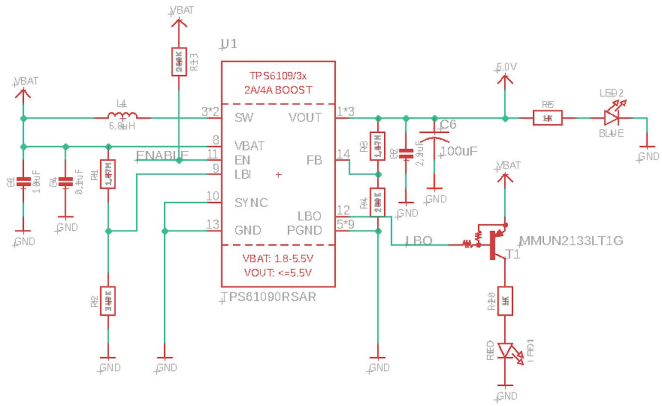

Fig. IV. Voltage boost circuit.

The MPC73781 was chosen because it was specifically made for our situation. It was created to be used to charge Lithium Ion and Lithium Polymer batteries using an AC-DC wall adapter via USB. The circuit shown below is used to perform and regulate the charging process of the 3.7V Li-Po battery. The MPC73781 takes power from the Raspberry Pi's 5V wall adapter, via Micro USB, and allows it to charge the 3.7V lithium polymer battery. VBUS refers to the 5V "rail" from the wall adapter.

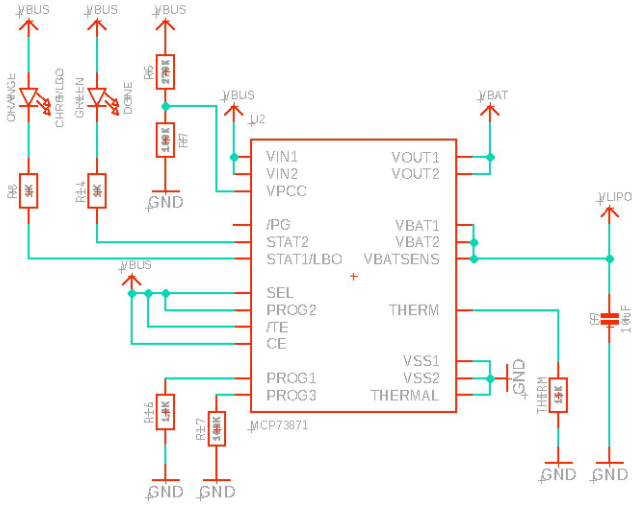

Fig. V. Charging circuit.

VI. Software

The main application of the Portable Water Quality Sensor captures the Bayer data of the light fluorescence from the sample after it is excited using a laser diode, and analyzes it to produce a spectrum. The device will store the latest recording and will allow the user to download the data onto a personal USB flash drive. Figure VI details the process of the analysis.

A. Calibration

The system has to be calibrated to ensure that nothing is misaligned within the system. The user would only have to run the calibration program once during initial start up, and any instance where the interior of the box could be compromised.

The Calibration program takes a reading of the device when the diode is on, and when the diode is off as

prompted to the user. After the reading is obtained by the sensor, the intensities are mapped to specific wavelengths whose intensities are already known. For this purpose we use the blue laser diode peak wavelength of 409 nm and the red fluorescent peak of 665 nm that we obtained using measurements taken with a commercial spectrometer. It would be more ideal to match more wavelengths, but due to time constraints we did not have this option and it did not make it to our final demo.

The first step of this program was to read the spectrum of a test sample containing chlorophyll-a. A numpy array containing the same size as the spectrum was created. If the wavelength is not in range of the spectrometer, it will be read to the array as a zero. The index for the peak of the blue bayer data was mapped to 409 nm and the peak of red bayer data was set to 665 nm. We then extrapolate all the wavelengths in the range between 400 nm and 750 nm. This gives the coordinates needed to plot the graph of the spectrum. The plot is created using the matplotlib library and saved as an .csv file for the main program to use.

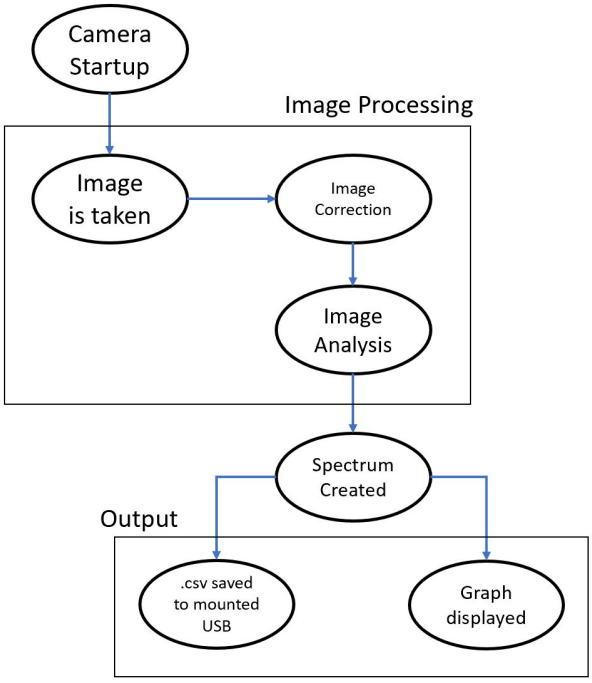

Fig. VI. Software Process

B. Image Processing

The image processing step is used to convert the image taken by the sensor into readable data. After the diode turns on, the program starts up the camera and waits a couple of seconds to allow the camera to warm up. The camera module takes the raw bayer data of the program and stores the results in a 2D numpy array. The red values of the bayer are separated and used as the intensity values for the spectrum.

The program uses the results from the calibration set up to map the intensity values to a wavelength. After the intensities have been obtained, the values must be adjusted to the quantum efficiencies of the sensor to gain the proper results we need. Once adjusted, the program has the results to produce a spectrum.

C. Quantum Efficiency

To find the spectral response curves of the Sony IMX219, sensor's spec images were used to digitize the quantum efficiency of the range of 400 nm to 700 nm. The data used was provided by Koen Hufkens[5] using this method. For the remaining near infrared spectrum, an estimation was used based off of similar camera modules. These measurements are not exactly the same as our sensor, since we were note able to take our own measurements due to constraints, however it is a safe estimation as the Sony IMX219 sensor quantum efficiency is relatively constant for different sensors.

The program takes in the results obtained from the analysis and corrects the intensities based on the wavelength's quantum efficiency by dividing the measured intensity value at a certain wavelength by the quantum efficiency value at that same wavelength then multiplying by 100.

Both the .csv file containing the results obtained by the spectrometer and the data of the quantum efficiencies is read into a 2D array. The columns containing the wavelengths in each array are separated, then the wavelengths of the results are searched through the wavelengths of the quantum efficiencies to find their index. Once the index has been obtained, the quantum efficiency can be used to adjust the intensity for a more accurate reading.

D. Output

After the program has adjusted the values for its quantum efficiency, the concentration of the sample can be deduced. The program will then indicate to the user if the concentration contained in the sample is at a concerning level. Due to time constraints however, we were not able to put research into finding the baseline for a safe concentration of the sample. As a result, the current output of our time of writing is a graph of the spectrum, and a .csv file that can be saved to a mounted USB.

E. Standards

Due to the multidisciplinary nature of our group, a standard was set to use a programming language that can easily be understood by all members of the group. The programs were written in Python due to its easy readability for non-programmers. Each of the core programs were to be based on Python whenever possible. Because python is a scripting language, it's best suited for web-based applications over a desktop-based application. Due to time-constraint, we were not able to provide an application, but intended to use a desktop-based application with Java as the language.

VII. Enclosure

So as to make taking the measurements more simple, the device should be compact. This improves the portability of the product, and enables potential users to take the spectrometer to field sites for testing water quality. This will be done by making a simplified optical setup. The entire optical setup, as well as the power source and detector will be enclosed in a single container. To compete with similar products already available on the market, the device needs to have a realistic production cost, less than 1000 dollars is our goal.

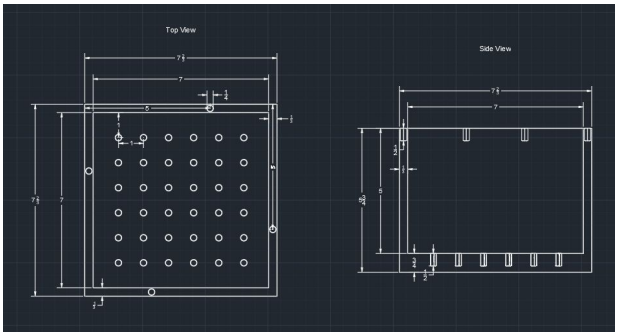

Fig. VII. Casing design.

To ensure that our signals from our fluorescence and excitation will not be interfered with, the walls, ceiling, and base will all be covered by an absorptive material with low reflection. The acrylic material walls are opaque and extremely reflective, so we have decided to cover them with black cardboard paper along with the lid. We have decided to go with acrylic for the housing of this material due to its high level of durability. Each optic in the portable container must be held fixed in their constant position at all times, such that our data will remain consistent. The acrylic is suitable as a base to hold our optics for this purpose.

VII. Conclusion

Fluorescence spectrometers are advantageous in their ability to record a concentration of a particulate in some solution. It is a less involved method compared to other biochemical approaches to find a certain concentration inside a mixture. Commercial spectrofluorometers are rather expensive, and worth upwards of $1000 or more. We created ours far below the cost, only including the price of materials.

Our portable water quality spectrometer is functional and does show the emission spectra of chlorophyll when properly excited. Due to recent complications, we could not compare our fluorescence signals that we have measured to different concentrations of a similar solution. It is highly expected that our fluorescence spectrometer can still provide different intensity values based on their respective concentration reliably. We only lack the dataset of the intensity to relative concentration for our Raspberry Pi. While it will not tell us the exact concentration of chlorophyll in our cuvette, it is still possible to do repeated measurements with different samples. Our device has the capability to warn its users about the quality of their available freshwater supply. This can potentially be used to prevent a user from consuming too many toxins and injure themselves.

Acknowledgement

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Biography

Lavine Von is studying at UCF's College of Engineering and Computer Science to earn a Bachelor's in Computer Engineering. She has experience with application development from working with video games and developing websites. She currently works in FARO Technologies as a ServiceNow Administrator and is working her way to becoming a certified Developer for the platform. For the project, she wanted to put her skills to the test in implementing an application for a cross-disciplinary project as well as picking up new skills to further her interests such as Python and learning to develop with the Raspberry Pi.

Niyah Lowell is studying Electrical Engineering, pursuing a Bachelor's of Science in Electrical Engineering. She is currently working as a Circuit Board Technician at Levil Aviation which is a small company that produces aviation instruments for small planes. This has provided extensive experience with soldering, reworking, and repairing circuit boards She also works as a freelance Audio Visual Technician with a focus on lighting which provides hands on experience with power balancing. For the project, she wanted to develop the skills she learned from her prior experience as well as branch out into learning Python to program a microcomputer.

Austin Dziewior Austin Dziewior is currently studying at CREOL, pursuing a Bachelor's Degree in Photonic Science and Engineering. He has experience in a research environment from his time working with the Nonlinear Optics Research Group at CREOL. It is actually due to this experience that many of the part testing came easier for the group. He plans on continuing onto a job after graduation with the intent to eventually go back to school and work on his Master's Degree.

Hee Jun Jang is also studying at CREOL to obtain a Bachelor's in Photonic Science and Engineering. At the time of this report he currently works as an intern at Optigrate, a company that manufactures gratings and filters. He has experience in a research environment from his time working with the Microstructured Fibers and Devices group at CREOL. His experience with optical devices and handling has allowed him to aid in the physical testing of components. He plans to enter industry after graduation with the intent of returning to CREOL's graduate student program to obtain his Master's Degree in Optics.