Portable Water Quality Sensor

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Senior Design I
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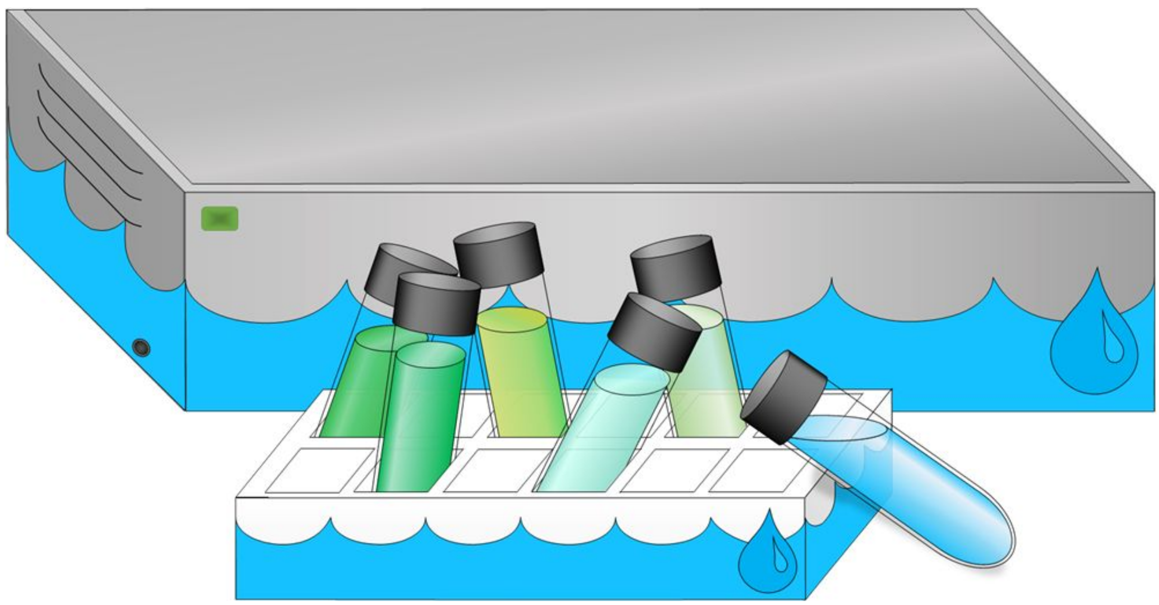
Group 3



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1. Executive Summary

In this paper the design and construction of a cheap and portable 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. The 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 able to tell the user about the quality of the body of water from the measurement taken.

Bodies of water can contain many contaminants that can be useful in determining the water quality. One such contaminant 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, which is often found in freshwater sources. This organism can be harmful in large populations, so it can be 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 our 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. The design and construction of the fluorescence spectrometer is described in this paper.

The device will consist of a laser diode that operates in the blue light range to excite a sample. The fluorescence from the sample will be collected and pass through a monochromator to spatially separate the light and then focused onto a detector. As chlorophyll-a fluoresces in the red light range, the spectral range will only need to detect light in the red light range and display a spectrum of the fluorescence intensity of the sample vs. wavelength. For improved durability we will ensure that there are no moving components in the final product.

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.

2. Project Description

Spectrometers are essentially devices used to measure colors. These colors are represented as different wavelengths. Spectrometers by separating a signal containing many different wavelengths to measure each wavelength individually. This yields a spectrum of the signal vs. wavelength. This spectrum can be used to identify certain compounds within a sample.

The device we intend to construct is a portable fluorescence spectrometer. It consists of an excitation setup to excite the sample causing it to fluoresce and spectrometer to detect the fluorescent light from the sample. The sample we have in mind for this project is chlorophyll-a in a solution of water as chlorophyll-a fluoresces strongly without the aid of any fluorescent reagents, which will allow us to obtain a good fluorescence signal.

The device will be capable of taking the spectrum of chlorophyll-a, such that it can distinguish the presence of chlorophyll-a from that of any other possible contaminants in the sample, and from the intensity of the spectrum find the concentration. While there may be other contaminants in a body of water, our research has not yielded the possibility of anything fluorescing at the same wavelength as chlorophyll. This is important because it means we can determine that our signal is that of chlorophyll-a.

The device will be portable, so a battery is needed to power it as well as a method to recharge the device. The output data from the device will be processed and must be able to be exported onto a USB for further analysis if necessary. The final goal of this product is to determine whether or not a body of water is safe for recreational use or even local wildlife.

2.1. Motivation

Chlorophyll is the key photosynthetic molecule present in all plants, algae, and other photosynthetic organisms. It is a component of these organisms' cells that break down water and carbon dioxide into energy.[1] This molecule makes it unique compared to cells of most other species as it fluoresces naturally when exposed to blue light, which allows us to quantitatively detect it. These organisms may be in areas that may cause ecological harm or damage our freshwater supply raising concerns on how to detect such plants or algae.

Cyanobacteria, one of the most common algae found in freshwater. It uses chlorophyll-a to create energy for itself via photosynthesis. It is dangerous in high concentrations, especially to a fresh-water ecosystem. The presence of excess colonies of cyanobacteria in water sources are referred to as algal blooms. Algal blooms can release an unsafe amount of toxins into the water supply affecting humans and other animals in the ecosystem.[2] Such toxins can cause a variety of negative health effects such as gastroenteritis or liver and kidney damage.

The main toxins released in this case are cyanotoxins, which can contaminate when introduced into a freshwater and drinking supply of water. Common cyanotoxins include Cylindrospermopsin, and Microcystins. The EPA has created a guideline for these cyanotoxins such that there can be a limited tolerance of contaminants in our drinking water. The concentration at which Cylindrospermopsin is considered harmful in a source of drinking water is 3,000 micrograms per milliliter of chlorophyll-a for school age children and adults, and 700 micrograms per milliliter for preschool aged children and bottle fed infants. For Microcystins concentrations above 1,600 micrograms per milliliter are considered dangerous for school age children and adults, and 300 micrograms per milliliter for preschool aged children and bottle fed infants.[3] While these are not regulations, and are thus not legally enforceable, they should still be followed for public safety reasons.

2.2. Goals and Objectives

To ensure that a body of water is safe for drinking water, ecosystems and/or recreational purposes, we aim to detect the presence of chlorophyll-a in a body of water. Creating a spectrometer that can detect the fluorescence of chlorophyll-a from a sample of water will allow users to identify if a volume of water contains any algae, or other harmful photosynthetic organisms that contain chlorophyll-a. This enables the users to prevent adverse health effects by determining what steps are necessary for the treatment of the body of water if at all necessary. For this reason, it is in our interest to quantitatively describe the amount of algae in our volume of water. Finding the concentration of this algae will be our main objective for this project as it will allow us to determine how contaminated the sample of water is.

Techniques used to determine the health of water sources often use light-based methods to quantitatively describe their presence, and concentration in a separate volume. Commercialized instruments that provide the quantitative analysis for these organisms all require a spectrophotometric instrument that can measure contaminants in a sample. Such properties include the absorbance and fluorescence of the material, through which both can be measured independently to find the same concentration or identify what the substance is. We aim to replicate the functions of these instruments to determine the properties of such substances, but most likely not to the full extent of these instruments.

We will be creating our own fluorescence spectrometer to test solutions with chlorophyll-a containing organisms present. We aim to obtain a fluorescence spectrum from the device to allow us to differentiate the presence of chlorophyll-a from other possible contaminants. The integrated fluorescence of the spectrum must then be able to be used to calculate the concentration of chlorophyll-a in the sample. Our spectrometer also needs a high enough spectral resolution that it can resolve between different fluorescent peaks such as chlorophyll-a and chlorophyll-b.

Most fluorometers or spectrophotometers include a wide range of excitation sources, that is, a broader spectral region of light sources. This is useful for the case of laboratory purposes such that it is required for a broader spectral range to determine the fingerprinting region of each compound. We however aim to specialize it for the general purpose of detecting chlorophyll-a in a body of water. Thus, our fluorometer should only contain a single excitation source in the blue/violet light range to avoid exciting other compounds as much as possible.

Our proposed fluorometer must be able to store a sample such that it can be excited by the light source. After this occurs, the sample will fluoresce. We plan for our device to collect and run the fluorescent light through a monochromator. The monochromator should be able to spatially separate different wavelengths of light thus allowing the user to differentiate between chlorophyll-a and other potential contaminants.

Going from this approach, we can prepare a device that is cheaper than what is on the market for most fluorometers, because those are too general for a purpose. The fluorometer we propose will be lighter and more portable than most spectrometers. Our device should be meant for those that are environmentally conscious of what is in marine life and the quality of it. And because of this, our parameters will not have to be as flexible as other commercial fluorometers.

2.3. Functions

This product is a spectrometer. Due to the intended nature of the device, it falls under the classification of a fluorometer. To operate the device we must create a list of the intended functions of the device so as to create methods of implementing these functions into our design. These functions are listed below.

- The product will allow for a sample of water to be collected into a sample container and inserted into the device.
- The device will activate and shine light from a laser diode onto the sample, causing it to fluoresce.
- The fluorescence will be collected and run through a monochromator to spatially separate different wavelengths of light.
- The spatially separated wavelengths of light will be detected by a camera and used to find its intensity and wavelength.
- A spectrum containing the Intensity vs. Wavelength will be displayed on a separate device.
- The spectrum can be used to find the integrated fluorescence of chlorophyll inside the solution.
- The final output will help the user to be able to determine whether or not a source of water is safe.

2.4. Requirements and Specifications

To ensure that our product is a viable market option it is necessary to set some requirements and specifications to be met. These are used to create goals in the design phase of our product that can be referenced as we begin construction of the device. To ensure that the quality of our device is sufficient for marketing purposes we will aim to achieve each requirement and specification we set. The overall goal of the spectrometer will be to measure chlorophyll-a concentration to determine whether or not a body of water poses a threat to local wildlife and as such must at the very minimum must be sensitive enough to be capable of measuring 50,000 micrograms per milliliter of chlorophyll-a. Both physical and technical specifications will be set for the device to make sure we achieve this.

2.4.1. Physical Requirements and Operating Conditions

- To ensure that the device is portable, the physical dimensions of the device must not exceed 20x20x15 cubic centimeters.
- Similarly, the device must weigh no more than 10 pounds.
- The device needs to be durable enough that minor movement of the device will not affect its measurement capabilities.
- All optics must be completely enclosed to eliminate stray light that may affect the signal to noise ratio.
- A sample insertion slot with a closable lid that can fit a 1x1x5 centimeter cuvette must be included in the device.
- The device must be able to operate at temperatures between 20 degrees celsius and 30 degrees celsius and still provide reliable measurements.
- The device must be able to be used between 20% and 85% humidity without significant effects on measurements.

2.4.2. Electrical

- The battery must be able to provide the Raspberry Pi 3 board with a 5 volt power supply.
- A recharging circuit for the battery must be constructed.
- The sensor that detects fluorescence will be powered by the Pi board.
- The diode requires 5.4 volts and 55 milliamps to begin lasing. A driver must be constructed to supply this constant current to the diode.
- To ensure that the sensor is capable of taking the whole range of data without interruptions the device needs to last at least 15 minutes before a recharge is needed.

2.4.3. Spectrometer

- The optical system to be used for this device will be a transmission grating based spectrometer.
- The light source must be a laser diode or LED with a central wavelength between 405 and 430 nanometers with a FWHM less than 15 nanometers.
- The NA of the collecting lens will be at least 0.4 and the size of the lens must be able to cover the entire area of the grating.
- The grating used will have at least 300 grooves per millimeter.
- The spectral resolution shall be less than 5 nanometers to allow us to create a spectrum with high enough resolution to distinguish the peak fluorescence of chlorophyll-a.
- The spectral range will be between 600 nanometers and 750 nanometers.

- A high enough signal to noise ratio to distinguish between fluorescence and stray light must be achieved.
- The spectrometer must be able to read the spectrum of chlorophyll-a within different solvents, including but not limited to water and acetone.
- The fluorescent light must reach a detector with separated wavelengths to record the spectrum.
- The device must have high linearity, or be capable of repeatable measurements with minimum error.
- The device must be able to detect concentrations of chlorophyll-a as low as 50,000 micrograms per milliliter.

2.4.5. Software Requirements

- The program will convert the fluorescent signal given by the spectrometer into an electrical signal for calculation.
- The program must be able to detect the peak fluorescence value of a spectrum the specific wavelength in order to determine the concentration of chlorophyll is in the sample.
- The program will be able to calibrate itself to ensure that the user does not have to readjust the sample more than twice.
- The program will be able to differentiate chlorophyll from at least two different samples.
- The program will inform the user if there is algae in the water, and give its concentration with an accuracy of 10%.
- The program will calculate the wavelength of the sample to an accuracy of 10%.
- Data can be exported onto a USB device for further analysis.
- The last 5 spectrometer readings will be recorded until it is overwritten.

2.5. House of Quality

The house of quality is a useful tool to develop the idea for the main goals of the project. It is a well known method in product development used to show the intended customer desires and determine the necessary tradeoffs between marketing and engineering requirements. This allows us to develop realistic goals for our project that can be met. We will use our house of quality to determine the engineering requirements using our knowledge of our capabilities and resources.

The house of quality developed for this device is seen in Figure 1 which details the necessary engineering requirements and their correlation with marketing requirements. In making the house of quality we determine what the customer wants and the engineering requirements necessary to satisfy the customer. The relationship between the two requirements are described qualitatively as a correlation between the two. We will use the house of quality as a guideline to make sure that the goals of our device are met.

House of Quality

Marketing Requirements		Weight	Dimensions	Power Output	Energy Efficiency	Spectral Range	Spectral Resolution	Signal Accuracy	Cost
		-	-	+	+	+	+	+	-
Durable	+	↓↓	↓↓					↓	↑
Battery Life	+			↓↓	↑↑	↑		↓	↑
Detection Sensitivity (mg/mL)	+	↓				↑	↑	↑↑	↑
Portable	+	↑↑	↑↑		↑		↑	↓	↑
Easy to Use	+	↑	↑		↑				
Cost	-	↑	↑	↑	↓	↑	↑		↑↑
Targets for Engineering Requirements		Less than 10 pounds	Less than 20x20x15 cm^3	Between 5 ~ 12 V	Will last for at least 15 mins	600 ~ 750 nm	At least 5 nm		Less than $1000

Legend			
+	Positive Polarity	↑	Positive Correlation
		↓	Negative Correlation
-	Negative Polarity	↑↑	Strong Positive Correlation
		↓↓	Strong Negative Correlation

Figure 1: House of quality

2.6. Product Operation

We aim for the product to be easy to operate such that it can be used without prior knowledge of the device. The main concerns we have with regards to this is sample preparation which is discussed later in this document. Here we will describe the necessary steps to be taken in order to successfully operate the device.

A sample should be collected, placed into the sample cuvette and inserted into the sample compartment prior to operation of the device. Then the device can be turned on and the measurements can be taken. An on switch will be used to activate the device, which will start the Raspberry Pi power source and prepare the device for measurements. When the user is ready to begin taking measurements, they will need to activate a separate separate switch to turn on the laser diode then start the program from the laptop. This will activate the excitation source. After the excitation source of the device is activated, the optical portion of the device will operate independently and measurements will automatically be taken from the detector. In practice, the optics of the device should remain fixed at all times and will thus be considered active at any time during the active acquisition of the information. Unless the excitation signal is blocked or the fluorescence signal from the cuvette, the optics will be considered active.

After the measurements are complete, the excitation source will automatically turn off and measurements can be analyzed. The device will then transmit the data to a user friendly interface such as a computer or phone using either Bluetooth or Wi-Fi. From there the user can read the data as a spectrum of fluorescence intensity vs. wavelength on their respective devices such they choose. A better understanding of the device operation can be seen in the overall block diagram in figure 2 below.

We want this to be a user-friendly device so we will reduce the amount of steps the user needs to take to as little as possible. The user should only be required to turn on the excitation source of the laser and commit their software commands on their phones or on their computers. The excitation source can also be turned on through software commands, but will potentially be easier to use a switch. Signal acquisition must be done through the software portion to the users desired parameters. If the user desires to observe the spectrum at a smaller or larger span, it must be done through software. It will be harder to arrange the physical components of the device rather than deciding from the full image where the user would like to observe the signal.

Some parameters of the device will however remain fixed, such as the intensity of the excitation source and the tilt of our surface grating such that we observe only the spectrum that the fingerprint of chlorophyll-a is located. These parameters must be fixed such that the accuracy of the devices results will be consistent for multiple samples with different levels of contamination.

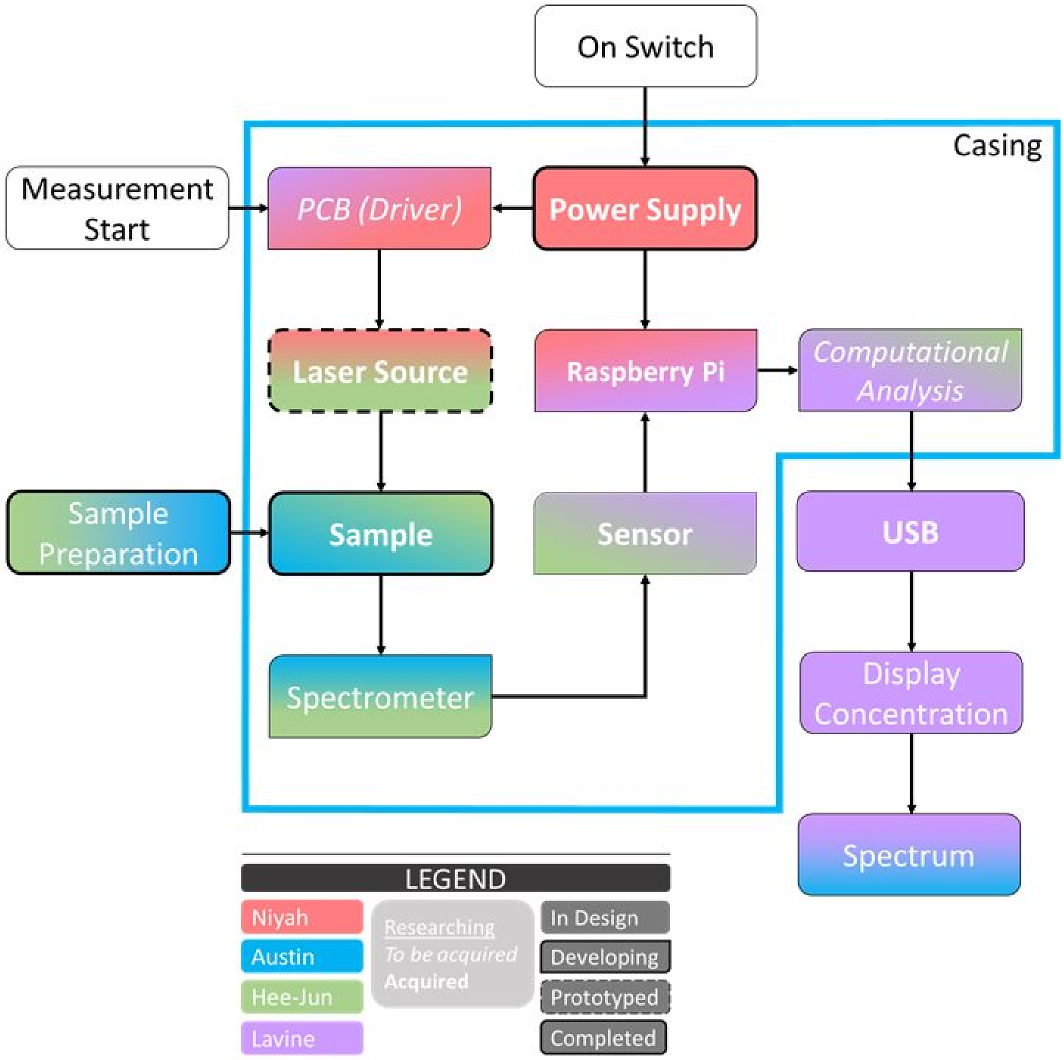

Figure 2: Overall block diagram.

The above diagram visualizes the responsibilities of each team member. The blocks with solid colors are the responsibility of only one of the team members. If the block contains more than one color it is the responsibility of at least two team members. The status of each block is also expressed using the type of border it has. While we will attempt to split the workload evenly by dividing the optical portion to the photonics majors and the electronics portion to the ECE majors, it will be highly likely that all members of the team will focus on some portions. The optoelectronics in our device, such as our preferred excitation source, will most likely be worked upon by all members of the group. With most likely guidance from the optics majors describing the main working principles of these excitation sources to the other members.

3. Research and Background Information

There are many things to take into consideration when building a product. Whether or not there is a market for it, whether or not it is cost-efficient are just two examples. To this end we research similar products and compare with our intended design. We also research the necessary components for the design as well as alternative options. Ultimately we decided to create a fluorescence spectrometer as that will produce the best results for our purpose.

For our research, we must first come up with a design. Fluorescence spectrometers all have a similar basic design, but a few modifications can be applied for different intended uses. We have not been able to find similar designs for a portable fluorescence spectrometer but other types of portable spectrometers were researched. The design of these devices is for the most part the same. As such our design will be similar to that of most portable spectrometers, but since our design is targeted at a specific task, we will modify it slightly to improve cost effectiveness.

The device requires a lot of background in electronics, software, optics and chemistry based knowledge. As the group is only composed of engineering students, the chemistry especially needs to be researched in detail to make sure that we can successfully implement the design. The main limitation on the specs, primarily the resolution and sensitivity, will come from the optics and the camera used. To this end we will have to carefully consider each component before we decide on the final design.

In determining the necessary components for a device that meets our specifications we must consider the cost and functions of each component. For example, we will compare different options to each other so as to tell if a part is too expensive. The functions will also be compared so that we can see the tradeoffs between the uses of each component and be aware of whether or not they will meet our specifications.

3.1. Similar Products

The market for spectrometers has become popular and many different designs have been implemented to include more excitation wavelengths to collect a wider range of emission wavelengths. In addition to the spectral capabilities for all fluorometers, resolution is another topic of interest for these spectrometers as the more they can distinguish between separate wavelengths the better. The spectral range for the cheaper and more accessible fluorometers can go from 200 nanometers to over 1000 nanometers. That is, from ultraviolet to near infrared light. Spectrometers more recently have been meant to become more accessible and readily used for any buyer. To this end portable spectrometers have become more popular in recent times.

Designs of such portable spectrometers include an already built cuvette holder for the sample that are meant for most spectrophotometric cuvettes; ones that include four flat sides to ensure the light that enters or exits the cuvette does not bend, to be able to collimate the fluorescence and focus the excitation source to the sample.

Some spectrometers, in order to become more compact and be able to be somewhat more adjustable, utilize optical fibers. The optical fiber is meant to collimate both excitation and emission signals such that they propagate in a direction that is appropriate to the environment of the user so they adjust the housing of their spectrometer. These fibers are typically multimode fibers which have a typically large numerical aperture, so they collimate as much light entering the fiber as possible. These allow for measurements of a sample outside of the device to be taken. We briefly considered this but ultimately decided on using an enclosed sample area that can be opened and closed as this may be more compact and still suits our intended functions.

Laboratory Grade Spectrometers include any large spectrophotometers or spectrofluorometers that are too large to be moved. These spectrometers are usually made with a specific function in mind such a fluorescence or transmission. While it may seem that these are more cumbersome than portable spectrometers, they tend to have much better accuracy and more features. A big advantage of these is that most laboratory grade spectrometers can selectively choose wavelengths of input light, an option that portable spectrometers don't have. They can perform more different types of measurements as well such as taking full spectra of a sample.[4]

Portable Spectrometers as stated have become especially popular lately as they provide easy access to quality control methods. They have especially progressed in the past decade as the limitations in spectral resolution and range of portable spectrometers have been increased to specs almost comparable with laboratory spectrometers. They have also become much more accessible and easy to use for common users. Compared to laboratory spectrometers, they are much more accessible in terms of price and ease of use. One problem with portable spectrometers however is that oftentimes an optical setup is still needed before measurements can be taken. For the purpose of our product research we will compare portable spectrometers as they are more similar to the product we plan to make

3.1.1. OceanOptics

OceanOptics tends to sell modular spectrometers to their customers, each one being specific for a certain wavelength or including better electronic or optical features. They vary their price of their modular spectrometers based on their spectral response range, size, slit width and excitation source that they place. These prices may jump from hundreds to thousands of dollars because of these main parameters and may be unaffordable for some labs that require a variety of purposes. All of their modular devices, however, are meant to be compact and easy to use as seen in figure 3, especially for an instrument that is meant to hold a monochromator setup inside of their product.

Figure 3: HDX Miniature Spectrometer, (Courtesy of OceanOptics).[5]

The external portion of their product is meant to hold their sample and fluoresce it from the outside excitation source. The main port that you are able to see on the front of the modular miniature spectrometer is the port for which the fluorescence signal shall enter through. The signal is to be transmitted through the means of an optical fiber, most often a multimode fiber for its better light collecting ability. Inside the modular spectrometer, or what is the monochromator, is the diffraction grating that separates the wavelengths of the signal spatially. The setup is similar to that of the Czerny-Turner monochromator described later in section 3.3.2. The only separation from that and the main design is that theirs does not include the entrance or exit slit to narrow the bandwidth.

The preconfigured models include a spectral range that goes from 200 nanometers to 800 nanometers, which will be from the ultraviolet to visible spectrum for their general purpose fluorometers. Other models include a spectral range from 350 nanometers to 950 nanometers, which range from the visible spectrum to the near infrared. These are both separate models, and must be bought separately for each consumer's purpose.

Their modular devices are meant to be able to be rearranged to whatever cuvette sizes they are able to support. For all their models of spectrometers, mainly the monochromator portion is the main focus and sold separately for each design. They include their universal, Square One Cuvette Holder that come with their fiber connectors and various filters. The overall design of their products, while useful, are not cost friendly. Even just the cuvette holder is worth over 1000 dollars alone, mainly because of the accessories that come with it. The design does also come with more features other than retrieving the fluorescence data, as it can also come with absorbance, transmission, reflectance, and transmittance.

3.1.2. StellarNet

The Blue Wave, Black Comet, Green Wave, Silver Nova, and high resolution miniature spectrometers from Stellarnet can all be easily comparable to the previous spectrometers provided by Ocean Optics. It also has the same feature as being the most portable it can be, while housing enough space to fit a monochromator optical setup inside. Inside the blue wave miniature spectrometers include the diffraction grating, mirrors, and charge coupled device. This spectrometer, as can be seen in figure 4, also utilizes optical fiber to transmit the signal from the fluorescence into the monochromator, and the excitation source onto the cuvette.

Figure 4: Blue Wave Spectrometer, (Courtesy of StellarNet).[6]

Similar to the price scheme of the Ocean Optics variety of spectrometers that is mentioned in the last section, the trade offs include either the spatial resolution, wavelength range that it can detect, and the excitation sources that come with. Each series and variety of these miniature spectrometers come with their own purpose for the demographics of their buyers.

For educational purposes or possibly even home use, the Green Wave series may be more suitable for those consumers as they will not require such a broad spectral range or high resolution, just to observe the peak of the emission signal. For those in a high research intense setting, the Back Comet series may be more appropriate, for its high spatial resolution and broader spectral range. Obviously, the more sensitive the equipment, the higher the cost as it will include more optics or better detectors.

The fiber connectors for these devices are also meant to support multimode fibers for their larger numerical apertures as compared to single mode fibers. The signal may decrease in orders of magnitude for both the excitation source and the fluorescence spectrum. For this reason, we consider the use of free space optics to collimate as much of the signal as we can onto our detector, to avoid significant losses.

3.2. Hardware Research

This section will cover the background information used as consideration for the project's hardware components. Some components will present various options as well as its benefits and risks in design choice. Comparisons will be provided in a table to help visualize each option's strengths and weaknesses. For each section we will provide our considerations for the component we intend to buy and the reasons building up to this choice.

3.2.1. Power Supply

To power this project, we can either use a wall adapter, single use batteries, or rechargeable batteries. The main electrical components we will consider are the microcontroller, the laser diode, and the camera. We expect not to supply the camera or any other potential detector with energy directly from the power source, so we will neglect the electrical requirements of the detector when comparing it to the whole of the design.

A wall adapter eliminates the need to replace a battery and the need to build a separate charging circuit. We also would not need to worry about how many amps our project will pull since residential outlets supply can supply up to about 15 amps indefinitely or at least as long as the electric bill is paid. Batteries are mandatory in order for this project to be absolutely portable. Single use batteries are inconvenient for the user and can get extremely wasteful and harmful for the environment. If we were to consider using the rechargeable battery, it will need to be small, light, and supply enough power for all of the components.

All of the components combined should not pull any more than 2000 mA, as to not destroy or damage any electrical components. Therefore, in order to achieve our goal of at least 15 minutes of operation, our battery must supply no less than 500 maH. If we were to consider the battery, we would be more concerned with the size, environmental impacts, and longevity of the battery. How much power the battery supplies will be dependent on the material it will be made from, and we are hoping to avoid batteries that have cathodes and anodes made of toxic materials.

3.2.1.1. Lead Acid Rechargeable Battery

Created by Gaston Plante in 1859, the first ever made and most popular rechargeable battery is Lead Acid. It operates using the chemical reaction due to the potential difference between lead and sulphuric acid. The positive electrode is made of lead dioxide and the negative electrode is made of lead oxide.

This battery is very large and heavy and therefore not portable. It is typically used when a high amount of power is required, for example to power a car. Not only is the battery too large and too high powered for this project, but the chemicals are extremely toxic for the environment especially when disposed of incorrectly. If lead is absorbed into the body in any way, it causes long term health risks. Lead poisoning can cause chronic pain, memory loss, changes in personality, and weakness. It can also go as far as causing reproductive complications. We do not want to contribute so heavily to the contamination to which we are trying to bring awareness. For this reason we decided not to use this type of battery.

3.2.1.2. Nickel Cadmium (NiCd) Rechargeable Battery

Created by Waldemar Jungner in 1899, Nickel Cadmium batteries followed lead acid batteries. NiCd batteries are small, lightweight cylinders which are highly portable. They are more expensive than lead-acid but still relatively low price. This battery operates due to the chemical reaction between nickel oxide at the positive electrode and iron and cadmium at the negative electrode.

A huge advantage of this battery is the high efficiency of the discharge rate. Each cell carries about 1.2 volts so we would need at least 3 cells. They were commonly used in things like power tools and emergency lighting. A huge disadvantage of this battery is that nickel oxide hydroxide and metallic cadmium are toxic for the environment if not disposed correctly. Cadmium is particularly harmful. Depending on the way cadmium is introduced to the body, it can cause anywhere from cold-like symptoms to pneumonia. It can also cause anywhere from joint pain to loss of bone density.

3.2.1.3. Nickel-Metal Hydride (NiMH) Rechargeable Battery

NiMH made NiCd nearly obsolete because one NiMH cell does the work of almost three NiCd cells. It's more expensive than NiCd but still relatively cheap, small, and portable. It also has a low internal resistance which works well with components that draw a lot of current. For that reason, they are commonly used to power things like digital cameras.

A huge advantage of this battery is that it is environmentally friendly because it does not contain chemicals that are toxic for the environment. This battery operates due to the chemical reaction between the positive electrode which is made of nickel hydroxide and the negative electrode which is made of hydrogen ions

3.2.1.4. Lithium Ion (Li-ion) Rechargeable Battery

Commercialized in the early 90s, lithium ion batteries are small but powerful with a very low self discharge rate. Due to their portability, and low size to power ratio, they are used to power a wide variety of things from electric vehicles to medical equipment.

This battery is more sensitive than the previous batteries. It is prone to ageing due to high temperature and requires a protection circuit. This battery operates due to the potential difference between positive electrode, which is made of a lithium compound, and the negative electrode, which is made of a carbon material.

3.2.1.5. Lithium Ion Polymer (Li-Poly) Rechargeable Battery

Commercialized in the late 90s, lithium polymer batteries are similar to lithium ion batteries but lighter, cheaper, and more versatile in size. Unlike lithium ion batteries, the casing is light and flexible allowing us more freedom of space. It does not require a protection circuit although it is possible, it is not likely to overheat or expand. It also has an even lower self discharge rate which will allow more time in between recharges.

We weighed our options using table 1 shown below and we chose what we felt best suited our project. Our final choice was the lithium ion polymer battery as it is quite durable, portable, and cost effective. More on this choice is discussed in section 5.1.2.

Table 1: Battery comparison.

	Lead Acid	Nickel Cadmium	Nickel-Metal Hydride	Lithium Ion	Lithium Ion Polymer
Weight (lbs)	2	0.54	0.9	0.3125	0.07
Size (in^3)	21.28	7.29	6	5.6	1.09
Environmentally friendly	✘	✘	✔	✘	✘
Discharge rate/week	4%	5%	2.5% (after initial 20%)	1% (after initial 3%)	1%
Price	$8	$22	$30	$34	$12

3.2.2. Charging methods

As we have decided to use a rechargeable battery to enable our spectrometer to be portable, we need to decide on a method to recharge the battery. There are two main options that we are considering to recharge the battery, an AC to DC wall adapter and a solar charger.

A wall adapter is very easy to obtain. We could even use the wall adapter that comes with the Raspberry Pi. The only other component that is required is a charging board to join the wall adapter and lithium polymer battery. As long as the user has access to electricity, they can recharge the battery quickly and easily. This adapter is plugged into any household outlet and the AC source is converted to DC which is then able to be used to charge the battery.

Solar charging has recently become more accessible to the general public. Solar charging eliminates the need for electricity and allows the user to charge the device anywhere there is sunlight which maximizes the portability of the device. Unfortunately, solar power has very low efficiency. The maximum efficiency we can find within our budget is still under 25%. This option is also obviously very dependent on the sun and not ideal for cloudy regions. Even in sunny conditions the solar charger could take days to fully charge. This would greatly inconvenience the user. Solar charging can also affect the durability of the product, as a solar charger would have to be placed on the outside of the device. This would inconvenience the user as this device should be as waterproof as possible.

For convenience, efficiency, and budgeting reasons, we have decided to use an AC to DC wall adapter to recharge our lithium polymer battery rather than a solar charger. We plan on using the wall adapter that came with the Raspberry Pi unless we run into unexpected issues.

3.2.3. Voltage Booster

The light source in this project will require a higher voltage than the other components. Therefore, a voltage booster will be required in order to keep the input voltage low for the laser diode driver circuit. In this section we look at the available boosters and compare in table 2 them to find the best available option for us.

Table 2: Booster comparison.

	XL6009 Boost Converter	Onyehn LTC1871	Yeeco Buck Booster
Price	$12 (5)	$12 (2)	$10
Weight (lbs)	0.12	0.1	0.04
Size (in^3)	0.864	2.11	0.8
Input	3V-30V	3V-35V	5V-32V
Output	5V-35V	3.5V-35V	1.2V-35V
Function	Step Up	Step Up	Step Up & Down

While the Yeeco buck booster is the most versatile and has the smallest footprint, the input range is not low enough. Our input voltage is coming from the 3.7 volt battery which is lower than 5 volts. The Onyehn booster was a great option because it included an LED display of the output voltage for convenience. The LED display made the board a bit more bulky, which slightly hinders our goal of portability, but we were willing to overlook this. Unfortunately after we tried testing two of the Onyehn booster boards, they were both faulty and almost immediately failed. This left us with the XL6009.

3.2.4. Sensors

While the sensor doesn't have to be sophisticated, one that can capture a high enough resolution for analysis while keeping costs low is ideal. A photodiode will be used to help the sensor read the spectrum of a sample. Two types of sensor technologies were considered, CMOS and CCD, both based on metal–oxide–semiconductor technology will be discussed, as well as cameras that were considered for the project.

One important thing to keep in mind when using sensors is the quantum efficiency. Sensors are not able to detect all wavelengths at the same efficiency. For example, a camera sensing a wavelength in the blue range may give a higher intensity than the same camera detecting a wavelength in the red range. To fix this, we must use a correction file that multiplies each wavelength's corresponding intensity by a value obtained from the quantum efficiency of the camera in order to achieve the real intensity. Other things to keep in mind for the quantum efficiency is whether or not the detector will produce a readable signal in our spectral range. If the quantum efficiency of the detector is too low, our values may become inaccurate.

Originally, we had plans to build our own sensor to save money. However, building a sensor would be a project on its one on top of the one we have to develop. As advised by a professor in the photonics department, we could simply use any camera rather than spent extra time to create one for our device. For this reason, we chose to buy the component instead of making one ourselves. Discussed in this section we will talk about the components of a sensor, and the features of both CMOS and CCD that will lead us to choosing a camera.

3.2.4.1. Photodiode

The photodiode is a semiconductor device composed of a p-type semiconductor and an n-type semiconductor that are joined together to act as a diode. They receive the incoming power from incident light to convert this into electrical energy where we can record this as voltage. From the depletion region of the photodiode, where the electrons and holes recombine to generate a current, the energy supplied to generate these electron hole pairs are provided from the incident light. Modes that the photodiode operates include a photovoltaic mode and photoconductive mode.

The photovoltaic mode is the simplest mode to observe the input power versus the output current. There is no other exciting energy source other than the input power from the light. This method of conductivity does not show a linear relationship between the input power in watts vs the output current in amperes.

The photoconductive method, not only includes the incident light hitting the diode, but also applies a reverse voltage to the diode. This will increase the width of the depletion region, which will decrease the diodes ability to hold the carriers, then increase the electric field such that the electrons and holes can be faster recombined. This mode of operation allows us to visualize the responsivity as a linear function such that we can observe the effects of the incident light more accurately.

In the event that we do not expect to see a full spectrum so that we are no longer able to scan with a device that will act as our linear detector, we may consider a photodiode to be used to record our fluorescence signal such that it only records the intensity. A single photodiode will be cheaper compared to imaging devices to record our signal. This will be an easier method to try and record the electrical energy from the photodiode rather than to reconstruct the whole spectrum.

However, depending on the size of the photodiode, it may be far more expensive to use rather than a camera. But, the price is also driven by the response time of the signal for each photodiode. For our consideration, the response time will be irrelevant as we only consider the amplitude of the signal. Response times are all short enough for our needs anyway, with the highest typically being tenths of a microsecond. The only thing limiting the selection of photodiodes for our design will be its size and the spectra it is responsive to, 660 nanometers.

3.2.4.2. CCD

A CCD, in principle, is a semiconductor device meant to create incident photons into electrons. A charge coupled device as an imaging tool is built as an array of metal oxide semiconductor capacitors that are used as pixels. When incident photons hit these capacitors, which are grouped as the photoactive region, each capacitor gathers enough electrical energy scaled to the amount of energy from each photon. Quantum efficiency, the amount of incident photons converted into electrons, in wavelength or energy is dependent on materials.

Using a CCD will require us to include an analog to digital converter in our circuit. CCD are more commonly used than CMOS detectors in spectrometers. If we are to use a CCD we would have to make the circuitry for the PCB and software from scratch and that would be too difficult for the scope of this project. Otherwise a CCD would cost upwards of 500 dollars for an already constructed one. As such we are currently leaning towards using a detector with a CMOS already constructed and coded on a PCB as described below.

3.2.4.3. CMOS

Complementary Metal Oxide Semiconductor (CMOS) is a combination of p-type and n-type transistors. CMOS has an array of light capturing cells that pick up the photons at their various wavelengths and translates them into electrons for digital processing. The wavelengths are picked up by these cells focusing on a lens and cause them to act like a tiny solar cell. The cells are surrounded by transistors that amplify the charge of these electrons that are gathered by the cell. A digital-to-analog converter reads the electrons and translates the charges of the individual cells into pixels of various colors to produce an image.

As an image sensor, CMOS has an amplifier for each pixel, while the CCD has fewer. As a result, a CMOS captures less area than a CCD. CMOS consumes less power than a CCD and due to the low demand for CCDs, CMOS are less expensive. CMOS generally have a square array of pixels, but for our purpose we only need a line array. To resolve this, approximately 3 rows of pixels will be binned using software. A summary of the tradeoffs between the two camera types is shown in table 3. We ultimately choose to go with CMOS

Table 3: Camera type tradeoffs.

Factor	CCD	CMOS
Power	High Consumption	Low Consumption
Noise	Sensitive	Less sensitive
Quantum Efficiency	High	Low

Image Quality	Superior	Inferior
Speed	Slow	Fast
Cost	High	Low

3.2.4.4. Camera Module V2 for Raspberry Pi

If the Raspberry Pi is the choice for the microcontroller, the Camera Module V2 will be ideal. For the project. The Camera Module is a custom designed add-on board for the Raspberry Pi based on Sony's IMX219 8-megapixel CMOS sensor. It is a cheap camera easy to use for beginners, yet has features that have plenty to offer for more advanced users. The camera's module offers libraries bundled with the camera to create effects such as producing a 3-dimensional RGB array from raw Bayer data, which may pose difficulties reading data as discussed later.

Raspberry Pi also features a similar camera to the Camera Module V2 called the Pi NoIR. The NoIR offers everything the Camera Module V2 can do except it does not employ an infrared filter. As the project does not need this feature, the V2 was chosen over the Pi NoIR camera. The Camera Module V2 is not a monochrome camera, but as we will only be working in the red spectral range, the red filter can be applied to detect the wavelengths in our area of interest. The Camera Module V2 comes equipped with a focusing lens, but as the specifications for this lens are not clearly stated we plan to remove the lens and mount our own focusing lens as described later.

The camera includes a 3280 x 2464 pixel count with each pixel having a width of 1.12 micrometers, making the whole detector size 3.7 millimeters by 2.8 millimeters. We expect this to be wide enough as opposed to other linear detectors as our spectral range will be required to be less than 150 nanometers. We have also performed calculations confirming this which are described in section 3.3.5. We will be using the full 3280 pixels on the long side of the sensor for our measurements but on the other side we only need to group together around two or three lines of pixels as we expect a linear output signal with only a slight width.

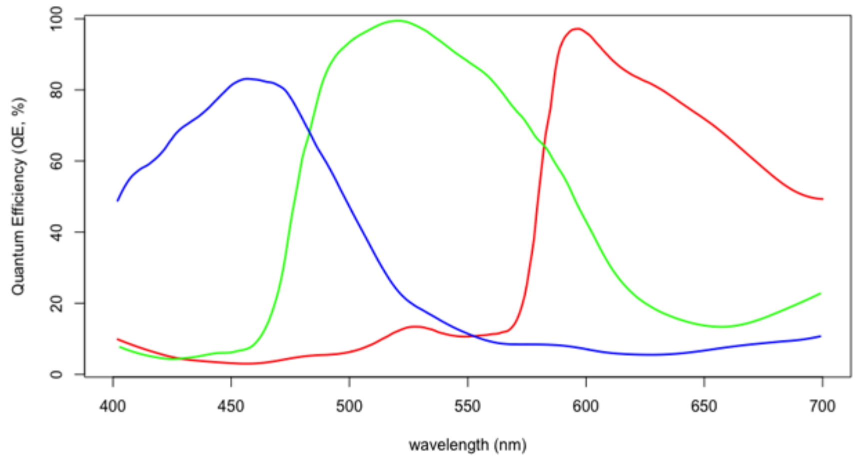

Figure 5: Quantum efficiency of Sony IMX219 camera.

We see from figure 5 the quantum efficiency of the Sony IMX219 sensor used in the Camera Module V2. This camera is a color filtered camera, and as such a monochrome quantum efficiency was not available for this, but we are specifically working in the red wavelength range, so the quantum efficiency with the red filter is sufficient. The main concern with this though is that this light can only be detected by certain pixels on the sensor due to the bayer pattern of the filter and extra programming may be necessary to determine which pixels are measured. We see that the quantum efficiency in our wavelength of interest, 600 nanometers to 750 nanometers is very good, between 40% and 70%. This lets us know that the camera is suited for our purposes.

3.2.4.5. Arducam MT9J001 Monochrome Camera Module

The Arducam MT9J001 is a high resolution, and highly sensitive camera. While a bit more expensive, the MT9J001 Camera features low-noise CMOS imaging technology that achieves near-CCD image quality while keeping the cost at a CMOS level. The MT9J001 has application as a medical camera and in microscopy, making it ideal for the project. Though it uses the monochrome version of the MT9J001 sensor on board, color isn't necessary for the project, and it can be used with both an Arduino and Raspberry Pi. This feature actually makes it preferable as all of the pixels will be able to read and there is no extra coding such as with the color filtered camera.

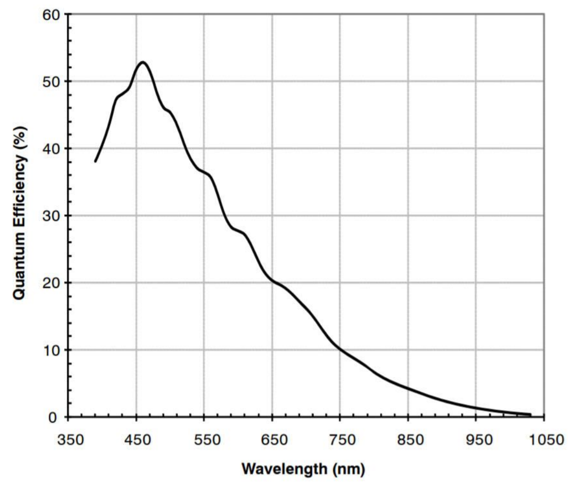

Figure 6: Quantum efficiency of the Arducam MT9J001 sensor.[5]

In figure 6 the monochrome quantum efficiency of the Arducam MT9J001 sensor is displayed. The quantum efficiency in our spectral range, 600 nanometers to 750 nanometers is relatively low, between 30% and 10%. This raises concerns on whether or not this camera will be able to distinguish our signal from noise as we expect a low intensity signal. For this reason, amongst others, we decided not to use the arducam MT9J001 camera.

In table 4 we outline the name differences between the two types of sensors before ultimately deciding on the Raspberry Pi's Camera Module V2.

Table 4: Camera comparison.

	Raspberry Pi's Camera Module V2	CCD Line Camera	Arducam MT9J001
Type	CMOS	CCD	CMOS
Sensor	Sony IMX219 8-megapixel Sensor	2048 Pixel Line Array	10MP 3856 x 2764 MT9J001 CMOS sensor
Pixels	3280 x 2464 pixel static images	2048 x 1	3856 x 2764
Size	25 mm x 23 mm x 9 mm	20mm x 115 mm	40 x 40 mm
Weight	3 g	2.74 lbs	10g
Price	$25	$1384.03	$40

3.2.4.6. LC100 - Smart Line Camera

The LC100 Smart line camera is the general design for the detectors for most spectrofluorometers for its high pixel count in one direction. The pixel count is one of the driving factors for us to buy our camera, because if it's higher, then we will obtain a better resolution. This may not be as much of a concern for us if the spectral response we wish to observe only lies in the 600 nanometers to 700 nanometers range.

For a span of 100 nanometers, we strongly consider a camera that is significantly cheaper but includes a broad enough detection region as well. Trying to spread our signal across a full 20 millimeters may not be as effective with the effect of dispersion once our signal has passed through the monochromator. We strongly consider avoiding the line camera for its high price and unnecessary component of including a long detection region.

3.2.5. Development Kit

The development kit would not only need to be able to process the information given by the sensor, it must be able to translate it for the user to understand as well as to control the instrument. How much manipulation the sensor needs to do the image as well as the complexity of the calculations will be the biggest determinants of the board we're going to use. If the calculations are simple, a microcontroller such as the MPS430G2 or Arduino will be preferred, whereas if more complex applications are necessary, a microcontroller with a stronger processor, or a microprocessor/computer is ideal. There are two types of development kits we can choose from:

Microcontroller-based. Microcontrollers are designed to perform specific tasks where you would need to control multiple I/O devices and sensors. Since these tasks are very specific, they only need small resources and thus, can be embedded in a specific chip. The reduction in size is what makes them cheap. If our water quality sensor is simply taking a reading, a microcontroller is sufficient. Two boards we will be discussing that fit into this category are the MSP430G2 LaunchPad and the Arduino Uno as we initially consider both.

Microprocessor-based. A microprocessor on the other hand is typically when you want processing power. Microprocessors are typically used for applications such as video games and websites where tasks aren't as specific. Because it focuses on processing power, they need a high amount of resources in comparison to the microcontroller, but tend to be much faster.

- Microcomputers and microprocessors tend to be used interchangeably, but they actually are separate from each other. A microcomputer is a small computer with a microprocessor as its central processing unit (CPU) with limited I/O devices, circuitry, and memory. The board that we will be discussing that fits into this category is the Raspberry Pi 3.

Table 5: Microcontroller and microprocessor tradeoffs.

	Microcontroller-based	Microprocessor-based
General	- single-purpose + cheap cost + smaller size	+ general purpose - expensive cost - larger size

Performance	+ low power consumption - low processing power - lower clock speed	- high power consumption + high processing power + higher clock speed
Applications	+ great at applications that perform a simple task - cannot be used for tasks with intensive processing	- inefficient for applications that perform a simple task + used for tasks that require intensive processing
Examples	Discussed in the Project: ➔ MSP430G2 LaunchPad ➔ Arduino Uno	Discussed in the Project: ➔ Raspberry Pi 3

In table 5 we consider the tradeoffs between both a microcontroller-based and microprocessor-based development kit. After careful consideration, we will ultimately decide on the microprocessor-based Raspberry Pi 3 discussed further in this section. On top of the characteristics between a microcontroller and microprocessor to consider, the following factors will be emphasized when considering in our decision to choose the appropriate development kit:

- Cost: the goal of the project is to make the device as cheap as possible.
- Performance: the development kit needs to be powerful enough to perform the analysis to create the spectrograph. It's possible that we may need to trade off a cheaper cost for a higher performing development kit.
- Feasibility: a development kit is easy to work with could potentially save us a large amount of time in implementation.

3.2.5.1. MSP430G2 LaunchPad

Texas Instruments's MSP430 family features ultra-low-power microcontrollers with the MSP430G2 in the voltage range of 1.8 volts to 3.6 volts. As an aid, the MSP430G2 features EnergyTrace technology for ultra-low-power debugging as well as on board buttons. Below are some of the features the MSP430 provides that make it an ideal choice for our project.

Cost. What separates the MSP430 from other options is its cheap price, at only 10 dollars. Because TI is such a large company, they're able to price their development board for a lot less compared to the other options. If this board were to break during testing, replacing it won't cost us as much as the other options.

Low Power. Another feature that sets the MSP430 from the other options is it's low power. Low power consumption will allow our device to last longer and reduce the amount of charging/battery replacement.

Feasibility. The MSP430G2 Launchpad was the first choice of consideration due to its use within UCF's curriculum. As each member of the team has taken a course with this microcontroller, not only would it alleviate the cost, it will reduce or eliminate the barrier between design and photonics theory.

Cons. Due to its low power, it may be necessary for us to use more than one MSP430 to not only perform both the reading, but to interface the camera. Additionally, trying to add more complex programs will be difficult due to the MSP430's limited functionality as a microcontroller.

3.2.5.2. Arduino Uno

The Arduino Uno is the most used and documented board of the entire Arduino family, making it a great board for someone getting started with electronics and programming. The board is based on the ATmega328P processor. The Uno can either be powered by the USB cable or by a voltage between 7 volts and 20 volts. Each of its 14 pins on the can be used as an input or output and operate at 5 volts. The Arduino can perform the same as the MSP430 at the cost of higher power, here are the following features that draw attention to this microcontroller:

Programming. Unlike the MSP430, the Arduino uses a high-leveled programming language that makes it not only easier for a beginner to pick up, but to work with in general. Compared to the MSP430's use of bitwise operations, reading and understanding another's code will be easier and more intuitive.

Open-source. There are plenty of resources and examples are available for the Arduino including a forum that will allow us to request assistance in implementing this design. For the MSP430 however, finding resources is a bit more difficult.

3.2.5.3. Raspberry Pi 3

If a standard microcontroller isn't enough to perform the analysis, the Raspberry Pi is a standalone computer, and reduces the need of a mobile or computer application to assist with the task. The Raspberry Pi is a Linux based microcomputer capable of running multiple distributions of embedded Linux releases. Below are some of the features the Raspberry Pi 3 supports that makes it an ideal candidate for the project.

Bluetooth/Wi-Fi Support. Unlike its predecessors, the Raspberry Pi 3 has built in 4.2 Bluetooth and dual-band Wi-Fi, reducing the need to implement the feature ourselves. This feature opens a door to allow our device to be operated remotely, and with W-Fi, access a database that can assist the average user into understanding the readings conducted by the device.

Processor. Unlike the MSP430 and the Arduino, Raspberry Pi's 1.2 GHz clock speed and 1 GB RAM allows the Raspberry Pi to perform advanced functions such as a cloud server or gaming console that the other two options cannot.

Camera Port. The Raspberry Pi has a built in Camera Port, so a camera can be connected without any issues.

Cons. Though the Raspberry Pi seems to be the ideal choice, it is not without its flaws. The difficulty with using this microcontroller is that the processor on the Raspberry Pi is not available outside of it, which will make the PCB Board design a challenge for the project if we wish to integrate it as a part of it. This is further elaborated in section 4.1.7.1. below. Nevertheless, the features that the Raspberry Pi is able to provide us makes it a desirable choice for the project. In table 6 we summarize the features of the different development kits we consider and highlight our final choice.

Table 6: Development kit comparison.

	MSP430G2 LaunchPad	Arduino Uno	Raspberry Pi 3
Languages	Registers and Bitwise Operations	Based on C/C++	Python, C/C++, Ruby
Processor	MSP430	ATmega328P	Quad Core 1.2GHz Broadcom BCM2837 64bit CPU
RAM	512 B	2 KB SRAM	1 GB
USB Ports	1	1	4
Size	6.3 x 6.1 x 1.7 inches	68.6 x 53.4 mm	4.9 x 3 x 1.3 inches
Weight	4.8 ounces	2.88 ounces	2.4 ounces
Pricing	$15	$22	$30

3.2.6. Communication Mediums

If the microcontroller selected isn't suitable to process the information on its own, the design of the hardware will shift from an all around portable device to exclusively a sensor that requires a medium to process the data. To be able to use that medium, various forms of information sharing were discussed.

The communication medium played a small part in our choice in development kit as one option may not be innately compatible with it and would require to implement that feature. While the chosen development kit supports all the mediums discussed in this chapter, there will still be some form of set-up or implementation needed. Both the medium's influence on the development kit as well as the decision leading to what form of communication we will use will be discussed in their respective sub-sections below.

3.2.6.1. Bluetooth

Bluetooth is a wireless technology standard that is used to exchange data over short distances. Using a bluetooth will open a way for a feature to the device to regularly check the water's quality without having to manually bring and check the device each time. A bluetooth will allow the application to be accessible without the need of the internet. However, if the microcontroller used does not have this feature built in, it would be necessary to implement that in our design. Additionally, if the project were to use Bluetooth however, a mobile application will be the choice of design, or an additional feature we can add to the device.

Fortunately, bluetooth is built into the chosen development kit, and so, we have the option of using bluetooth for optimization to be discussed in a later section. The software needed to get it officially working on the Raspberry Pi is easy to install, but setting it up is a bit more difficult, especially if it's the user trying to pair up the device. To set it up, a series of commands in the line is required, but it is relatively easy once you understand the process.

3.2.6.2. Wi-Fi

On top of Wi-Fi has all the features of Bluetooth, while allowing the user to have the option of using either a mobile phone or a browser as an application. However, because Wi-Fi can extend farther than Bluetooth, a feature that can store information into a database and connect to other water quality sensors has room in the future. If Wi-Fi is to be considered, a website would be ideal. The constraint with WiFi however is that the user must have internet access, and if our development kit does not have built in Wi-Fi, we would have to add that to our hardware design.

Just like for bluetooth, the development kit we have chosen features Wi-Fi compatibility, and if Wi-Fi isn't available, an ethernet cable can be connected to the Raspberry Pi. This built in feature will allow us to optimize our programs at a later point to connect to a database and back up data in the event something were to happen to our device. Discussed in chapter 5 are some of the additional features or programs we could use that would benefit from this feature.

3.2.6.3. USB

USB (Universal Serial Bus) was designed to connect peripherals to a personal computer. Not only can it be used to communicate, it has been increasingly used to supply electrical power. Though it does not have the range and mobility Bluetooth and Wi-Fi has to offer, its strengths come from its ease of use for the user. At the same time, using a USB will force the user to have a more manual process to extract the data.

Table 7: Communication medium tradeoffs.

	Bluetooth	Wi-Fi	USB
Power Consumption	Low	High	Low, can also save power by charging device
Bit Rate	2.1 Mbps	600 Mbps	5 Gbps
Range	10 meters	100 meters	Limited

Ease of Use	Simple	Requires configuration of hardware and software	Simple

In table 7 above we summarize the tradeoffs of each communication medium. Ultimately we chose to use a USB. While it's simpler to use in the beginning, it also may be a bit more of a hassle in the long run compared to the other options. However, because of how easy a USB is to set up, it will be used for testing out our project's prototype. Further discussed in a future section below, we will consider and discuss the option of optimizing the software to utilize the Raspberry Pi 3's built in Bluetooth and Wi-Fi capabilities to provide the user other options of data transfer.

3.2.7. SD Card

The SD card is a key part of the Raspberry Pi. For the Raspberry Pi to run, it will require a micro SD. Because the Raspberry Pi does not have a flash memory chip, an SD card is needed to store the programs for storage of items such as OS, libraries and user programs. The image captured by the camera will also be stored in the SD card. When the Raspberry Pi starts up, a piece of code called the bootloader is executed. The bootloader reads code from the SD card that is used to start up the Raspberry Pi. If there is no SD card inserted, it will not start.

The Raspberry Pi should work with any compatible SD card, although there are some guidelines that limit the choice. The SD card must at least be 8 GBs. SD cards have a limited life, and each time they're read or written, their lifespan decreases before it needs to be replaced. If a larger card is selected, such as 32 GBs, the frequency of replacement will be shorter as there's more room for reading or writing options in addition to there being additional storage space for various files and media on the card.

Higher Capacity Cards. While larger SD cards may be used, the Pi only supports up to 32 GBs. During set up, we found that despite setting up everything correctly, the Raspberry Pi was not booting up. Rather than the Raspberry Pi being unable to handle a larger size, it is simply a formatting issue. Fortunately, higher sized cards can be formatted from exFAT to FAT32 to make them usable for the Pi.

During initial testing, we will start with an SD card of 8 GBs, then progress to a larger size if necessary as the program goes through its development. As numerous changes will be made throughout, starting with 8 GBs saves us money in the development process, but also allows us to observe the effects of wear.

3.2.8. Display

To make the sensor as portable as possible, a display that is simple, yet will tell the user enough information is the goal. There are a couple types of displays that can be used for the project to display the information. At the bare minimum we want the display to have the following characteristics:

- Cheap: the goal of the project is to create a spectrometer cheaper than the market counterparts. It is important that the display is cheap, but also can fulfill the requirements.
- Size: the sensor is meant to be portable, as to not hinder the user. The display should be both small and light enough to carry, however, it cannot be too small that its difficult for the user to read.
- Low Power: we want the display to consume as little power as possible to limit the frequency of charging it needs.

Depending on the design of the project, there are also other characteristics to consider when selecting the display. If the design requires that the device is independent on its own and can display graphs, then a high-resolution screen will be considered. If no graphs are needed, or we want to use another interface to create the spectrum, a simple LCD would suffice to describe the status of the spectrometer. Below are the types of displays considered for the project and the three options are discussed and summarized in table 8 at the end of this section.

3.2.8.1. Light Emitting Diode Display (LED)

As the name suggests, an LED uses light emitting diodes function as if they are pixels. Each of the pixels are simulated by a cluster of colored diodes that enables them to produce a bright, high quality image. These diodes are energy efficient and have lesser heat dissipation than an LCD. Because of their brightness and the high quality images that can be displayed, LEDs are ideal for the outdoors and are commonly used for traffic signals and television.

3.2.8.2. Liquid Crystal Display (LCD)

LCDs are made of liquid crystals arranged in front of a light source to produce images. Liquid crystals do not emit light directly and relies on the light source to produce an image. Though the image is not as brilliant as an LED, if a high resolution image is not what is needed for the project, then the LED loses their appeal. For this reason, LCD screens are more common in smaller electrical appliances such as calculators and watches because they are much cheaper to use than LEDs.

3.2.8.3. Generic 16x2 LCD Display Module

A 16x2 display is best used to display essential data when a display too large or expensive is not needed. At only 6$, this display is sufficient enough to support the goals of our project. While it would be nice to have a touchscreen or a high quality display, the scope of our project does not require those features as they’re more cosmetic rather l than functional. For this reason, we will start with specifically the Arducam 1602 Display Module, and then consider expanding to the following options discussed in this section.

Adafruit Blue&White 16x2 LCD+Keypad Kit. The Adafruit kit is designed to make it for people to get LCDs into their project. This kit is essentially the Arducam 16x2 LCD module along with extra components that could be beneficial to the design. Included with the 16x2 LCD is a PCB with buttons and a header built into it. Assembly can be completed within 30 minutes which makes it ideal for a person getting into the Raspberry Pi for the first time. If we didn't already have the Arducam 1602 16x2 LCD Display Module ahead of time, this would've been the to-go kit to help us get started with implementing our device.

3.2.8.3. HyperPixel 4.0 - Hi-Res Display for Raspberry Pi

The HyperPixel is a high speed, high resolution display for the Raspberry Pi. The display features 18-bit color and a 60 FPS frame rate to match its high-speed DPI Interface. The display comes in already pre-assembled with no soldering required which makes a matter of simply connecting it to the Pi to get it started. The HyperPixel has both a touch screen and non-touch screen version both at the size of 800x480 pixels. If we choose to upgrade our 16x2 Display Screen, this would be an ideal choice to display graphs without the user needing to upload the data into a USB to another device.

3.2.8.4. Adafruit PiTFT

The PiTFT is a 320x240 pixel 16-bit touch screen display that also comes fully assembled and can be easily plugged into the Raspberry Pi. Though it is not as detailed as the HyperPixel 4.0, its size makes it a bit more portable by comparison, and it's slightly cheaper than the HyperPixel 4.0's non-touchscreen version.

Table 8: Display comparison.

Display	Generic 16x2 Display	HyperPixel 4.0 Non-touchscreen	Adafruit PiTFT
Price	6$	37$	35$
Dimensions	80x35x11mm	58.5x97x12mm	56x85x11mm
Weight	35 g	49.9 g	42 g
Touchscreen	No	No, but has a touchscreen version	yes

3.3. Optical Research

There are a variety of spectroscopic methods that can be used to find the concentration of substances inside of a solution. The following describes the different spectroscopy techniques and why we choose fluorescence spectroscopy as our main focus. We also research in more detail the necessary components and compare between different types that can be used to build this spectrometer so as to decide on what components we need to build the device.

3.3.1. Spectroscopy

Spectroscopy is the measure of the intensity of light as a function of its wavelength. Since the goal of this product is to detect the content of chlorophyll-a in a sample volume, and since chlorophyll-a fluoresces only at specific wavelengths, spectroscopy was determined to be the best option. A spectrometer separates wavelengths in a signal of interest spatially and measures the intensity of light at each wavelength in a specified spectral range. There are many types of spectrometers that can be used to detect concentration. The pros and cons of the many spectrometers considered are listed below.

3.3.1.1. Raman Spectroscopy

There are three types of scattering that takes place in raman spectroscopy, Rayleigh scattering, Stokes Raman scattering, and Anti-Stokes Raman scattering. Raman spectrometers function by exciting a sample with a light source. The electrons of the molecules in the sample jump to a higher 'virtual' energy state for a short period of time before scattering light.

For three cases of scattering, different effects are produced. For the case of Rayleigh scattering, the electrons fall back to the initial ground state with the same energy as the excitation source, scattering a photon of equal wavelength. For Stokes Raman scattering, the electrons fall down to a higher vibrational state than that of the initial electron state, this results in the scattering a photon with higher wavelength than the excitation source. Anti-Stokes Raman scattering occurs when the electron is initially in a higher energy state and falls down to a lower energy state releasing a photon with smaller wavelength than the excitation source[7].

The scattering is detected to produce a spectrum of the intensity of Raman scattering vs. wavelength. Raman spectroscopy generates a very weak signal and while methods may be taken to improve the signal[8], the cost of the device would increase too much. A Raman spectrometer was considered but as Raman spectroscopy typically yields low intensities of Raman scattering, it was ultimately decided to not be sensitive enough for production.

3.3.1.2. Fluorescence Spectroscopy

In fluorescence spectroscopy, whenever light is incident on a sample the molecules in that sample will absorb energy from the incident photons and jump to a higher energy and vibrational state. The molecules in the higher energy states will interact with other molecules, causing them to lose energy and fall to a lower vibrational state. After a short period of time (nanoseconds to femtoseconds), the molecule will then fall to one of the vibrational states of the ground energy level.

This energy drop releases energy in the form of a photon. As the energy released is less than that of the energy absorbed, the wavelength of the photon emitted by this release of energy must be greater than the wavelength of the excitation source[9]. The emitted light is collected by a detector and the intensity at different wavelengths is measured and collected by the detector to yield a fluorescence spectrum. The fluorescence is easily detected which resulted in this technique being the best candidate for the product.

3.3.1.3. UV-Vis Spectroscopy

Ultraviolet-visible (UV-Vis) spectroscopy utilizes either absorption, transmittance, or reflectance spectroscopy to gather information from a portion of the ultraviolet spectrum and the entirety of the visible spectrum. We have discussed from the previous section the nature of fluorescence spectroscopy and how it is manipulated. Bonded and non bonded electrons that are included in molecules can absorb energy from wavelengths in the ultraviolet range and visible spectrum.

How much energy these electrons can absorb to be excited to a higher energy level depends on where these electrons are located on the energy band diagram. The higher the electrons are on the energy band diagram, the less energy that is required to move them to an excited state, therefore, the lower the wavelength of the light required. The spectrometer measures the reflected/transmitted light to gather the necessary spectra. UV-Vis spectrometers are in general hard to make as they take a lot of time and effort to eliminate interference from conditions such as stray light. They are also very sensitive to small vibrations, a big worry for a portable spectrometer.[10]

3.3.1.4. IR Spectroscopy

Infrared spectroscopy deals with light from the infrared region (700 nanometers to 1000 nanometers) and matter interaction with these energies. This could be used in principle for our design, to measure the concentration of our sample. Infrared spectroscopy operates based on the excited molecules vibrational frequency such that it matches the frequency of the absorbed energy. The bond of the molecule can only absorb the energy of the radiation if it matches the frequency of the excitation source. Once the incident light has been passed through the sample, the objective is to measure the transmitted light. The energy lost from the incident power compared to the transmitted power is what will be measured.

Infrared spectroscopy may not be the easiest method to consider for the characterization of chlorophyll molecules. Because of the many derivatives of chlorophyll that we will be unable to, or otherwise very difficult to characterize, infrared spectroscopy will pose many challenges. Different solvents would most likely be required to find particular derivatives for peak signal response and that wouldn’t be entirely feasible for our application as our solutions will not be examined that closely. Infrared spectroscopy may be too unique for our case such that we will be required to find a more general approach, one that is not specific to particular molecules of the same group. Infrared, as well as the other spectroscopy techniques available to us have been tabulated as seen in table 9 and as a result we ultimately decided to use fluorescence spectroscopy.

Table 9: Tradeoffs between different spectroscopy techniques.

	IR	UV-Vis	Fluorescence	Raman
Pros	More than 1 technique of gathering information	Multiple techniques can be applied, Large spectral range	Wide range of wavelengths that can be used and recorded	Unique signal that can be recorded from each molecule
Cons	Requires Reference Sample, Limited spectral range	Sensitive to stray light	Sensitive to stray light	Raman signal can be very weak

3.3.2. Spectrometer

The spectrometer is the portion of the device that will ensure that the detector is only reading one wavelength from the fluorescence signal at a certain point. It is an optical device that transmits or reflects light, depending on the type of grating used, from an input signal with a range of wavelengths as spatially separated wavelengths. The wavelength of interest is isolated and detected. Spectrometers commonly use monochromators for this purpose. Common monochromators use diffraction gratings or prisms such as an optical prism, or dispersive prism to separate wavelengths. Prisms are usually shaped as a right triangular so that two ends are not faced parallel from each other and beam deflection can occur. Because of the chromatic dispersion of the material, the angle of which wavelength becomes angle dependent.

Most spectrometers used the Czerny-Turner monochromator setup to spatially separate light. This consists of two mirrors and a diffraction grating. The first mirror collimates the signal onto the diffraction grating. The diffraction grating then separates the light to its different chromatic components. Finally as the light dispersed from the grating is spread out over a much larger area than the detector, the separated light must be focused on the detector with the second mirror.[11] This is seen in figure 7 below. The Czerny-Turner monochromator is commonly used in compact spectrometers such as the one we plan on making.

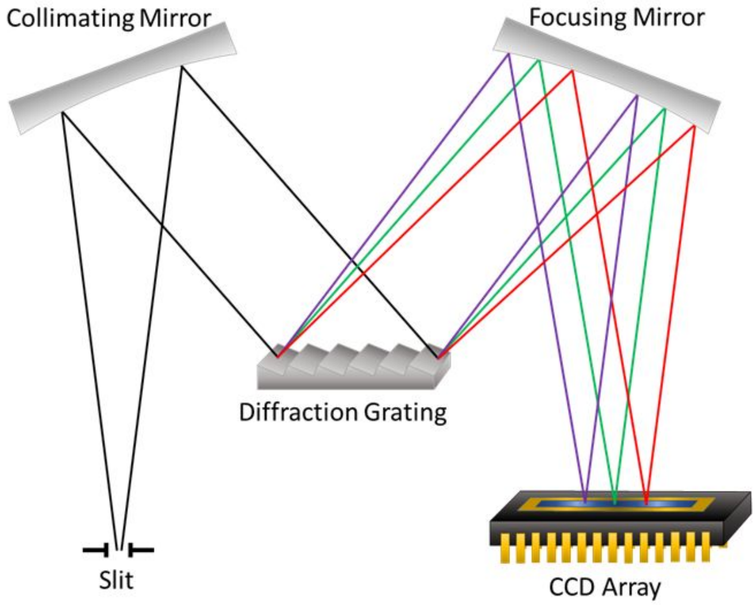

Figure 7: Czerny-Turner Monochromator.

Likewise for this project, a diffraction grating is preferred over the prism in the spectrometer. The diffraction grating is an optical element that is built as a periodic structure to exploit the diffraction of light. Different types of optical gratings include, reflective gratings, transmission gratings, and volume bragg gratings. Unlike the conventional monochromator setup, we will be using a single lens placed at a focal length away from the sample to collect light and will collimate it directly onto the grating. This eliminates the need to buy a collimating mirror as mirrors can be expensive. It is for this reason that we will also use a lens instead of the second mirror. This causes our setup to look more that shown in figure 8. While not technically a monochromator due to the fact that it does not isolate a single wavelength, each pixel will read a separate wavelength. Thus, for the purpose of this project, we will be using this in place of a monochromator.

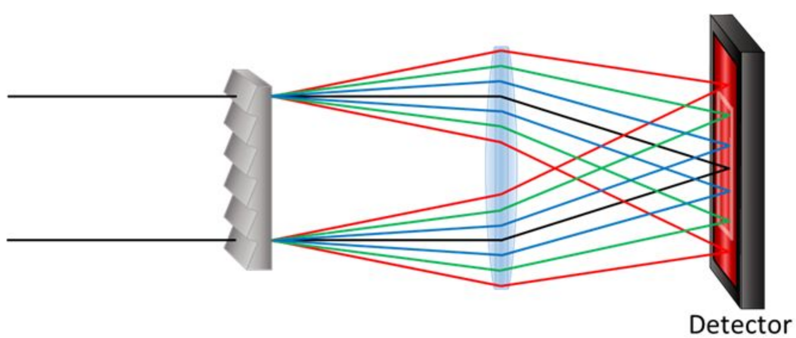

Figure 8: Our spectrometer setup.

As we can see in figure 8, the rays of light are separated by wavelength after exiting the transmission grating. They are then focused to different spots on the detector. There are many diffraction orders, or points on the line with the same spectral profile. We will be using the first order in our detection.

3.3.3. Light Source

As we have decided on fluorescence spectroscopy as the technique, we need to choose an excitation source with a wavelength that the sample has high absorbance at. We have chosen chlorophyll-a as our sample as it has a strong fluorescence signal and is abundant in cyanobacteria. The peak absorption for chlorophyll-a is 430 nm, as will be discussed later on in section 3.4, so we will be choosing a light source in the blue/violet range. It is also necessary to choose a light source with sufficient power to create a fluorescence signal but also a narrow enough FWHM that it does not affect the spectral resolution.

The most common light sources used in spectrometers are lamps. However, these are expensive and mainly useful when an excitation monochromator or filter is available to isolate a specific excitation wavelength.[12] For our product, we do not need the option to choose between different wavelengths, and can use a cheaper light source such as a laser diode or LED that operates at a specific central wavelength.

To properly excite the sample, a light source with a wavelength between 400 nanometers and 440 nanometers will suffice. It is actually better to have the light source further away from 450 nanometers as that avoids exciting chlorophyll-b. The energy for each photon can be found using equation 1. Since our sample has a relatively high quantum yield (0.26) and the number of photons calculated from equation 2 is very high, we can conclude that the sample fluoresces very strongly and the power of the excitation source does not need to be overly high. We aim to excite the sample between 20 milliwatts and 30 milliwatts of power, which we expect will be enough to excite enough sample molecules to yield a strong signal.

$$E_{ph} = hf \qquad Equation\ (1)$$

$$\#\ photons = \frac{P}{E_{ph}} \qquad Equation\ (2)$$

3.3.3.1. Laser Diodes

The emission wavelength of laser diodes can be determined from the material its made from. For our purpose, we would use a laser diode that has a wavelength of around 400 nanometers, which will be made of either indium gallium nitride or gallium nitride. The advantage to laser diodes over most light sources is that they have a narrow bandwidth due to lasing by stimulated emission, typically lower than ten nanometers, but the central wavelength and FWHM can also be temperature sensitive. For this reason, some laser diodes with a narrow bandwidth, can be tuned by regulating the temperature of the junction of the diode. We may need to do this if our laser diode needs a higher wavelength as laser diodes typically don’t come at specific wavelengths of operation unless custom ordered. A disadvantage to using the laser diode is that we will be required to construct or buy our own current and voltage driver.

Aside from technical characteristics of the laser diode, they happen to be usually more expensive than light emitting diodes. And, it is harder to find a laser diode centered at our preferred excitation wavelength, 430 nanometers. Most common blue/violet laser diodes are either centered at 405 nanometers or 450 nanometers. For laser diodes at 430 nanometers it is harder to find one because they have to be custom ordered. This is due to the difficulty in making these laser diodes and the unpopularity. This causes such laser diodes to be very expensive.

We were originally concerned that exciting the sample at wavelengths too far off from the peak absorption wavelength, 430 nanometers, would not properly excite the sample. However we have since found that exciting the sample at 405 nanometers produces a relatively strong fluorescence signal and at 430 nanometers the signal is weak and includes chlorophyll-b. With this in mind, we decided that the most appropriate laser diode would be a laser diode centered at 405 nanometers.

3.3.3.2. LED

Compared to laser diodes, LEDs are cheaper, more powerful, and different wavelengths are more available. LEDs operate by spontaneous emission, this results in an emission spectra with a larger FWHM. This could be useful if we are unable to find an LED centered at 430 nanometers as it may still include 430 nanometers in the emission spectral range. However, if the emission spectra is too large it may excite other substances which absorb strongly at wavelengths near the intended. This could result in unwanted fluorescence in our fluorescence signal, so we have to avoid this. The emission spectra of the LED is also determined by the material from which the semiconductors are made out of.

For our purpose, we are considering using a light emitting diode of central wavelength in the 430 nanometers, which will probably consist of the material being made out of indium gallium nitride. The light emitting from these diodes are not spatially coherent, it can be assumed that the light emitted from them propagate in all directions. This can be mitigated by placing a lens with a short focal length in front of the diode that also has a large diameter, to collimate as much of the signal we can from the diode.

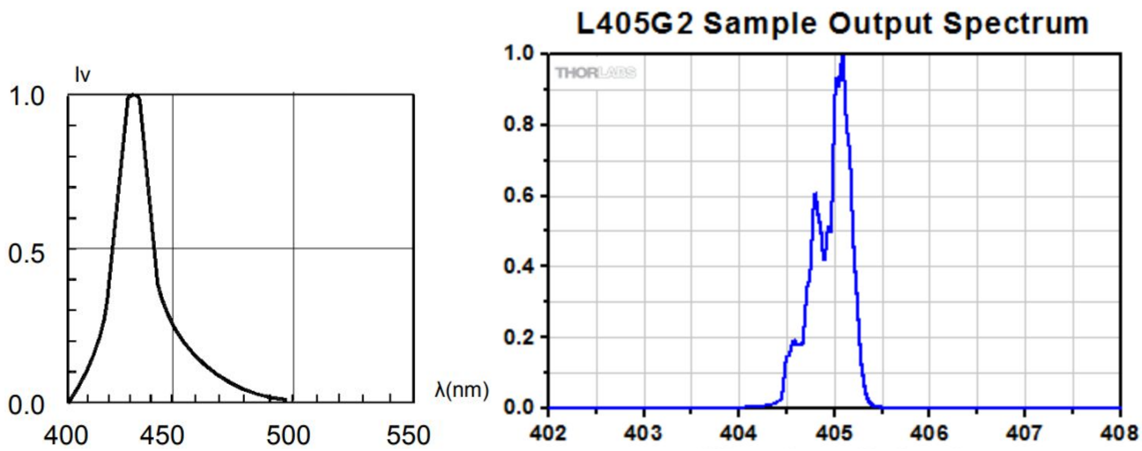

Figure 9: Spectra of LED (left) and laser diode (right).

As shown in figure 9, the full width at half maximum for light emitting diodes is broad compared to other light sources; we expect our diode to have a full width at half maximum of 40 nanometers or above. For our consideration, we will not consider the quantum efficiency, the lifetime, or other electrical characteristics of the light emitting diode. Our main focus will be the emission spectra, required power to make our sample fluoresce, and bandwidth of the light emitting diode.

To operate light emitting diodes, we must apply a forward bias across the diode. However, since this is a diode, even the smallest amount of excess current applied to the diode can cause it to burn out and be destroyed, which is why when building our circuit for the diode, we must consider a resistor to be placed in series with it. We will consider the operating voltage and current for our diode and other electronics for this project based off of their specifications.

What draws us to purchase the light emitting diode is that it is cheaper than other light sources and the wavelength can be more available. A laser diode custom made to have a central wavelength of 430 nanometers costs upwards of 300 dollars so we did not consider ordering one. However, after sample testing, we realized that a laser diode with a 405 nanometer central wavelength is sufficient to excite the sample and can be found fairly cheaply.

While an LED was also considered, the main worry is that due to the large full width at half maximum, the chlorophyll-b in the sample might also fluorescence which will interfere with our spectra. The different laser sources considered are listed in table 10 below. We can see that the laser diode at 405 nanometers is relatively cheap and has a narrow enough full width at half maximum that we do not have to worry about exciting chlorophyll-b. For this reason, we decided to use the Sony 405 nanometer laser diode that is highlighted in table 10 below.

Table 10: Light source comparison.

Company	Sony	ThorLabs	Bivar	ThorLabs

Type	Laser Diode	LED	LED	Laser Diode
Part #	SLD3232VF	M430D2	3BWC-F	L405G2
Peak Wavelength	405 nm	430 nm	430 nm	405 nm
FWHM	10 nm	15 nm	Large > 40 nm	
Operating Power	50 mW	600 mW	150 mW	30 mW
Operating Current	55 mA	500mA	70mA	50 mA
Price	$12.99	$58.35	$1.76	$95.58

3.3.4. Lens & Mirrors

Lenses can either be convex or concave. Convex lenses have a positive focal length and are commonly used in spectroscopy. We are considering three different types of lenses for this product, a lens to focus light from the excitation source onto the sample, a lens to collect the fluorescent light from the sample and collimate it onto a diffraction grating, and a lens to focus the light from the grating onto the detector.

When choosing a lens, we also have to keep in mind how well it transmits the light. Both 405 nanometers and 665 nanometers, our wavelengths of interest are transmitted near perfectly for all optical glass, so this shouldn't be a problem. We also keep in mind that the refractive index is wavelength dependent. Having a broad spectral range passing through the lens can cause chromatic aberration. In this case we consider that we must potentially use an achromatic lens to correct for this phenomena. We will discuss in detail the effects this may have on our device and our decision in the following sections.

3.3.4.1. Singlet Lens

A singlet lens is just a lens consisting of a single material. There are many different types of singlet lenses, but we will only be considering bi-convex and plano-convex in this project. In choosing between bi-convex and plano-convex lenses, it is necessary to compare the relative distance of the two conjugate points. If one conjugate point is less than five times the second point, a bi-convex lens should be used for decreased aberrations.[13]

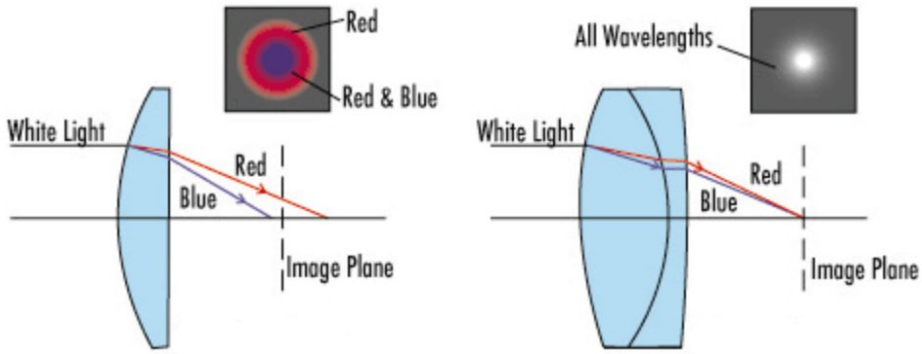

Figure 10: Effects of chromatic aberration in a singlet and correction of an achromatic doublet.[14]

3.3.4.2. Achromatic Lens

The refractive index of materials changes with wavelength, this causes light passing through a lens to spatially separate. For measurements that require the detection of specific wavelengths such as the one to be used in this design, it is necessary to choose an achromatic lens that corrects this aberration. Achromatic lenses consist of two or more lenses that are usually cemented together. The combination of these two lenses bring different wavelengths to the same focus.[14] Figure 10 depicts a demonstration of this concept by comparing the relative focus of different wavelengths using a singlet and achromatic doublet.

3.3.4.3. Mirror

Two mirrors are commonly used in spectrometers, for the Czerny-Turner monochromator. The advantage of mirrors is that they do not have as much chromatic aberration as lenses. If we were to use a mirror in this deviceThis mirror will focus the wavelengths from the grating onto our detector to collect the information of the wavelengths of each spatially separated wavelength.

The mirrors can be of different quality and materials. Depending on the material of the reflective surface, some mirrors have a reflection coefficient at specific spectral ranges thus making them reflective to only that range of wavelengths, while other wavelengths would be almost completely absorbed. This is unnecessary for our design and can even increase the cost of the mirror and as such, if necessary we will choose the metallic coated mirror type as they are good for general-purpose and are cheaper than other mirror types such as dielectric coated mirrors. When using this mirror, the main thing to keep in mind is to be careful when handling it as it is more susceptible to damage than other mirror types.[15]

For all lenses, the shorter the focal length of the lens, the more expensive the lenses tend to be. While achromatic lenses may improve performance of our spectrometer, they are more expensive than singlet lenses and as such, choosing an achromat that is cost effective and has a great enough NA poses a difficulty. Table 11 describes the tradeoffs between singlet and achromatic lenses. With our final decisions between the biconvex and achromatic lenses highlighted.

Table 11: Tradeoffs between optic types.

	Singlet Lens	Achromatic Lens	Mirror
Chromatic Aberration	Yes	Little	None
Cost	Low	High	High

3.3.4.4. Lens 1

For the first lens, the light coming from the light source diverges quickly, thus a positive focal length lens is necessary to focus this light from this source onto the sample. In order to make efficient use of space within the device, we will not use a collimation setup and will directly focus the diverging light from the light source onto the sample. A biconvex lens will be used rather than a plano-convex lens because the distance from the light source to the lens is less than five times the distance from the lens to the sample.

The dimensions of the lens such as focal length or diameter can be calculated using ray tracing. Figure 11 demonstrates the ray tracing diagram of the focusing lens. When calculating the dimensions of the lens necessary there are several factors to take into account such as the distance from the sample, divergence angle and distance of the incoming light, or beam waist at focus. The effects of divergence angle and distance from the laser source to the lens can be calculated using equation 3. To calculate the focal length of the lens necessary to focus the excitation source onto the sample equation 4 is used. After some preliminary calculations, we realized that due to the compact nature of the device, a lens lens with a shorter focal length is required.

Using the gaussian equation and the paraxial equations, we can estimate the magnification of the image and the focal length required to find the proper lens for our design. Equation 3 is for the paraxial approximation which we will most likely use to estimate the height of the image that we will take from the diffraction grating. Using the size of the diffraction grating and comparing the height of the image, we can then find our magnification and compare if it will be enough to fill the whole array onto our detector.

$$y_{i+1} = y_i + t_i u_i \qquad \textit{Equation}\ (3)$$

$$\frac{1}{t_0} + \frac{1}{t_1} = \frac{1}{f} \qquad \textit{Equation}\ (4)$$

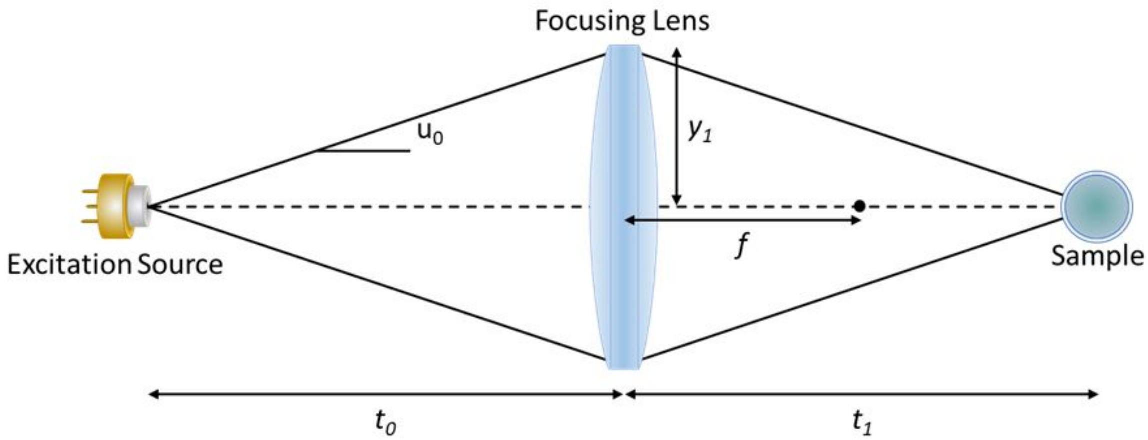

Figure 11: Ray tracing diagram of the focusing lens.

The beam waist at the sample is also affected by the lens focal length. One thing to take into account when choosing the focusing lens is to make the beam waist as small as possible. The beam spot should be as small as possible for best fluorescence divergence effects. The impact of the effect of focal length of the lens is shown in equation 5. The considered lenses and their specs are described in table 12 below.

$$2\omega_0 = \frac{4\lambda f}{\pi d} \qquad \textit{Equation (5)}$$

Table 12: Lens 1 comparison.

Company	Newport	ThorLabs	Edmund Optics
Part #	KBX043	LB1092	#63-550
Lens Type	Bi-Convex	Bi-Convex	Bi-Convex
Substrate	N-BK7	N-BK7	N-SF11
Diameter	25.4 mm	12.7 mm	20.0 mm
Focal Length	19.0 mm	15.0 mm	20.0 mm
ARC	Uncoated	Uncoated	Uncoated
Price	$41.00	$26.18	$25.50

3.3.4.5. Lens 2

The second lens will collimate the fluorescent light from the sample onto the detector. This means that the second conjugate point of this lens is infinity. As this is greater than 5 times the focal length, we will be using a plano-convex lens for decreased aberrations. We also consider a coating on this lens that may block out scattered light from the excitation source.

Generally, in spectrometers an achromatic lens is used as the spectral range can be large. We originally intended on purchasing an achromatic lens for our collecting lens but eventually realized that our spectral range is narrow. Since we only intend to test one sample, chlorophyll-a an achromatic lens is unnecessary and will increase the expense of the total design. For this reason We decided to use a singlet lens.

The amount of fluorescent light collected is determined by the lens placed after the sample. For this reason, when choosing the lens to collect the fluorescent light from the sample a high NA should be used to collect the most light possible. The area of light collected can be approximated using equation 6. Since the lens will be placed at focus with the sample, the light will be perfectly collimated. This eliminates the need for a second lens or a collimating mirror in the monochromator. It is also necessary for the diameter of the lens to match the grating size for increased resolution.

$$A = 2\pi r^2[1 - cos(\frac{\theta}{2})] \qquad Equation\ (6)$$

Other than having a large NA, another consideration for this lens is that the lens size should be large enough that the fluorescent signal is collimated to cover the complete area of the grating. This increases the resolution of the device and will be discussed in more detail in section 3.3.7.

Table 13: Lens 2 comparison

Company	Edmund Optics	ThorLabs	Edmund Optics	Edmund Optics
Part #	#49-321	LA-1951-B	#38-578	#37-785
Lens Type	Achromat	Plano-Convex	Plano-Convex	Plano-Convex
Substrate	N-BAF10/N-SF 5	N-BK7	N-SF11	N-SF11
Diameter	12.5 mm	12.7 mm	12.5 mm	12.5 mm
Focal Length	14.0 mm	15.0 mm	12.5 mm	12.5 mm
AR-Coating	400 nm - 1000 nm	Uncoated	500 nm - 1100 nm	Uncoated
Price	$72.50	$22.94	$42.00	$27.00

As we see from table 13, we decide on a lens from edmund optics with a focal length of 12.5 millimeters and a diameter of 12.5 millimeters. This lens has an NA of 0.50, which is relatively large. We also choose for this lens to have a YAG-BBAR coating, which is an antireflective coating in the range of 500 nanometers to 1100 nanometers. This blocks out any excess blue light from our excitation source that may have scattered onto the spectrometer setup.

3.3.4.6. Lens 3/Mirror

The final optic we consider is a lens that is used to focus light from the grating onto the sample. Normally when light diffracts from a grating, the light disperses over a wide line. The detector we use however, is very small (order of millimeters). For this reason we must focus the light from the grating onto the pixels. To maximize resolution we will choose a lens with a large focal length but not large enough that it makes our device too big. We are also considering a mirror for this part as they are more commonly used.

The calculations to find the focal length of this lens depend on the grating. As discussed later in section 3.3.5.3., the linear dispersion of the grating affects the necessary focal length of the lens. The exit slit width, in this case the detector width, L_{Det}, constrains the focal length as we need the detector to contain the full spectrum. We also need the optic to be large enough to collect the whole signal from the grating, so we choose a lens greater in size than the grating. From the linear dispersion equation in section 3.3.5.3. we find that for a grating of groove length d, the focal length should not exceed 79.94 millimeters in order to not exceed the grating size.

Table 14: Lens 3/mirror comparison

Company	ThorLabs	Edmund Optics	Edmund Optics	Edmund Optics
Part #	LA1608-A	#45-360	#68-034	#46-239
Optic Type	Plano-Convex Lens	Plano-Convex Lens	Plano-Convex Lens (Cylindrical)	Concave Mirror
Substrate	N-BK7	N-BK7	N-BK7	N-BK7
Diameter	25.0 mm	15.0 mm	12.5 mm x 25 mm	20.0 mm
Focal Length	75.0 mm	75.0 mm	75.0 mm	80 mm
Coating	Uncoated	Uncoated	Uncoated	400 nm - 2000 nm
Price	$23.27	$26.00	$49.00	$38.50

After performing these calculations we can determine the necessary dimensions for the third lens. We then compared several different lenses that would work under these conditions from different suppliers based off of price and usability in our design. This is shown in table 14. In the end we decided to go with a 15.0 millimeter diameter and 75.0 focal length lens purchased from Edmund Optics for this part of the design. It is highlighted in the table.

3.3.5. Grating

There are two types of gratings being considered for this project, surface gratings and bragg gratings. These include different forms of gratings that operate through different concepts. For example, the surface grating diffracts light from a broadband source and results in a signal with spatially separated wavelengths of light. On the other hand bragg gratings have to satisfy a certain condition to let light pass through.

3.3.5.1. Surface Gratings

Different types of surface gratings include reflective or transmissive. Most commonly used in spectrometers are the reflective gratings, due to the popular Czerny-Turner design. These gratings consist of either many grooves or slits, depending on the type. The signal is originally a superimposition of many different wavelengths. When it reaches these gratings will diffract causing the signal to disperse. This can be predicted, as the different wavelengths will disperse at different angles. This allows us to take a spectrum measuring the intensity at each wavelength rather than of many of the same wavelengths superimposed on each other.[16]

Surface gratings are considered more easy to work with. They are easier to align and calculations for them are rather straightforward. On the other hand there are more considerations to take into account for Bragg gratings.

3.3.5.2. Bragg Gratings

Unlike the surface grating, the bragg grating is not only angle dependent but also wavelength dependent. The bragg grating can be manufactured depending if it is reflective or transmissive, on how it can either reflect or transmit a certain wavelength. The internal structure of the bragg grating consists of periodic structures of varying refractive indices, so that certain wavelengths are able to pass through or be reflected at a separate angle.

For each angle of incident light that hits the surface of the bragg grating, it correlates to a specific wavelength that is a portion of the incident light, and will either be the only wavelength transmitted or reflected. Depending on the angle that it is incident on the surface of the grating, it can also vary in the diffraction efficiency of the grating. The diffraction efficiency of the grating is measured as the amount of energy that is reflected or transmitted as a ratio of the amount of energy that is incident to the grating.

This poses a problem to our design as we want to make a spectrometer without any moving components for improved durability. The fact that a bragg grating only allows one wavelength to pass under a certain angle means that it requires a rotating stage. This would negatively impact our design. The bragg grating is also more expensive and harder to work with than a surface grating. For these reasons, we have decided to use a surface grating.

3.3.5.3. Grating Calculations

The diffraction efficiency can be explained as how much of the incident signal is transmitted or reflected depending on the type of grating. It is labeled as a ratio of how much power that is transmitted or reflected, versus the incident power on the surface of the grating. The diffraction efficiency of our grating will be a major priority of our setup, we do not expect to collect a large signal from our samples so it will be crucial that we collect as much power we can.

When choosing our grating, we will need to take into account the resolving power, R, and spectral resolution, Δλ, of the grating. This depends on the width of the grating, the angle of incidence and diffraction of the grating, the groove density and the average wavelength. Equation 7 shows how to find the resolving power. We will attempt to maximize this, allowing us to more easily distinguish between each average wavelength. From this equation we can see that a larger width of the grating will have better resolving power. However, this is still dependent on the width of the light incident on the grating, W_G. So there is no point obtaining a grating larger than our collimating lens.

We will also attempt to obtain a spectral resolution of at least 5 nm. The resolution is also dependent on the groove density, G. We find from equation 7 that the resolving power of a grating with 300 grooves per millimeter and size 12.7 millimeters is 3,810 and the spectral resolution is 0.1745 nanometers at our central wavelength. This is enough to satisfy our specs.

$$R = \frac{\lambda}{\Delta\lambda} = W_G \cdot G \qquad \textit{Equation } (7)$$

We also have to make sure the grating has a high enough diffraction efficiency at the wavelengths of interest. Gratings with 300 grooves per millimeter tend to have greater than 70% diffraction efficiency in the red wavelength range while also providing high enough resolution, so we decide to go with a 300 groove per millimeter grating. Our grating selection is highlighted in table 15 on the following page.

Table 15: Grating comparison

Company	Edmund Optics	Thorlabs	Optigrate
Part #	#49-575	GT13-03	Custom
Grating Type	Surface - Transmission	Surface - Transmission	Volume Bragg - Transmissive
Groove Density	300/mm	300/mm	Custom

Size	12.7 mm	12.7 mm	Custom
Diffraction Efficiency	>70%	~70%	~90%
Price	$87.50	$82.78	~$600

Once we determine the groove density, *d*, of our grating, we must calculate the dispersion angle, *β*, of the signal. As our signal will have a range of wavelengths we determine from equation 8 that our dispersion angle is 10.37 degrees at our minimum wavelength, 600 nanometers and 13.00 degrees at our maximum wavelength, 750 nanometers. This enables us to determine how large and how far away the third lens must be from the grating in order to collect the whole signal. We attempt to place it as close as possible for this reason.

$$\beta = sin^{-1}(m\lambda/d) \qquad Equation\ (8)$$

The dispersion angle is also necessary to calculate the linear dispersion. We also need to determine the linear dispersion of the grating in order to detect the spectral range of our signal on the detector. This is the rate that the light diverges from the grating and allows us to determine what size focal length we need for our final lens. It is calculated using equation 9 below. The linear dispersion for the 75.0 millimeter focal length lens and 300 grooves per millimeter grating we plan on using at our central wavelength is found to be 43.52 micrometers.

$$P = \frac{d \cdot cos(\beta)}{m \cdot f} \qquad Equation\ (9)$$

The bandpass, B, is the spectral range of the signal on the detector. It is calculated from equation 10. Using the linear dispersion found earlier, we calculate the bandwidth of our signal to be 159.89 nanometers. This is sufficient to cover the entire spectral range. Thus we can conclude that these optics satisfy our requirements and specifications.

$$B = L_{Det} \cdot P \qquad Equation\ (10)$$

3.3.6. Bandpass Filter

In the event that our monochromator cannot be used to spatially separate the signal wavelength regions, we can use the bandpass filter to isolate the fluorescent signal that we send onto our detector to collect the energy that is sent. Spectrometers generally have two modes to collect the fluorescence signal or transmittance, using a monochromator to spatially separate the desired wavelengths of the signal, or a filter to collect the desired spectral range and its intensity.

In terms of using the bandpass filter for the purpose of electronics and testing, The bandpass filter is used to select the range of frequencies from the middle of the signal. By the middle of the signal from comparing frequencies, it begins by selecting the range of frequencies such that the energy climbs to $\frac{\sqrt{2}}{2}$ of the peak energy, and cuts off the frequency at the point where the energy has decreased to $\frac{\sqrt{2}}{2}$ of the peak energy. This would be considered analogous to the notch filter, where such range of frequencies would instead be filtered out.

For the optical bandpass filter, this would effectively be the same function as compared to a circuit filter. The difference being, is that it would change by selecting the wavelength, as opposed to frequency, and the energy would then be considered to be in units of intensity of the signal. The optical bandpass filter can be manufactured to be wavelength selectable between the peak wavelength to either allow the spectral range to be larger or smaller. For our consideration, even if the bandpass filter were large enough to gather the whole spectral range of the fluorescence, this would still not improve our signal by orders of magnitude. Most likely, the signal intensity would only be improved by a factor of single digits.

In this case, the output of the device would end up being a single value for the total fluorescence intensity. This would be acceptable as we are only concerned with measuring the fluorescence of one sample and are not trying to identify a specific contaminant. Measuring the total fluorescence intensity of chlorophyll-a would still allow us to make assumptions as to the health of a certain body of water.

For the event that we decide to use a filter, the CCD Array that we have already bought or a CMOS detector, both devices meant to be used for imaging are still applicable, but using the filter also allows us to utilize instead the photoconductor, which will be cheaper than both imaging devices. Instead we will only read the output voltage and current rather than looking for and drawing the whole spectrum.

Our final purchases are highlighted in each section. Figure 12 shows these components combined. On the left side of figure 12 is the sony laser diode and the lenses and on the right side is the transmission grating.

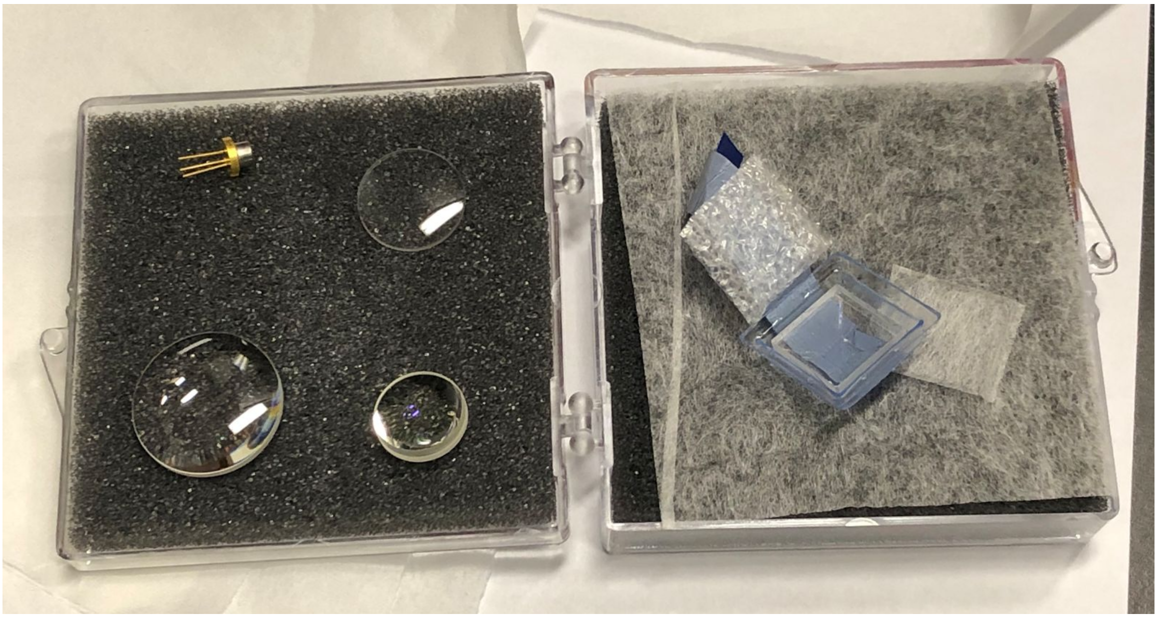

Figure 12. Purchased optical components.

3.4. Sample

Several samples were considered but ultimately the test sample decided on was chlorophyll-a. The reasons this sample was chosen is that it is easily obtained, strongly absorbs blue light around 430 nanometers, and naturally fluoresces red light at around 665 nanometers.[17] Such wavelengths as these are all domained under the visible spectrum of light, which we can determine quantitatively as well as qualitatively for all future experiments.

The sample we plan to use for initial testing is a leaf of the Forestiera Segregata tree, more commonly known as the Florida Privet. It is a common type of tree found in the Southeastern United States and in the Carribeans. We have determined that this sample would be an optimal sample for testing out chlorophyll-a concentration as this was not very excitable for an energy with a wavelength of 450 nanometers, the excitation wavelength for chlorophyll-b or an other potential fluorophores.

Further into our research, we will consider other organisms that could be considered a harm to our environment in larger numbers, specifically algae containing cyanobacteria. We will not initially use cyanobacteria as good cultures can cost a lot of money and be hard to store for long periods of time. We conclude that the spectral response of the samples should not vary too much.

The main difference is that cyanobacteria does not contain high concentrations of chlorophyll-b but the Forestiera Segregata leaf does. We expect to see differences in the intensity at which they fluoresce. The cultures considered for cyanobacteria are anabaena, eucapsis, fischerella, spirulina, merismopedia, and tolypothrix. These are all common cyanobacteria present in harmful blue-green algae. Cultures are obtained from *Carolina Biological Supply Company* and cost 8.30 dollars per vial.

3.4.1. Chlorophyll Background

The desire to measure chlorophyll-a comes from its presence in mostly all photosynthetic organisms. Chlorophyll-a is able to be excited at a wavelength of 430 nanometers, through which it is then able to emit its fluorescence at around 660 nanometers in wavelength. The issue for trying to excite the chlorophyll-a is that there are other potential fluorophores that we will not expect to see. Chlorophyll-b could be one of these potential fluorophores that we will not expect to see. Most photosynthetic organisms will also utilize chlorophyll-b to create the energy to sustain itself. Chlorophyll-b absorbs energy in a wavelength of 450 nanometers, which may be an issue for our design such that we are able to pick the right light source for our spectrofluorometer.

The main issue of chlorophyll-b is that it is typically higher in concentration inside land plants than chlorophyll-a is. We must ensure that we prepare a sample that includes a higher amount of chlorophyll-a than it does for chlorophyll-b, a rather large amount to be safe.

The quantum yield of chlorophyll-a experimentally obtained is typically between 0.25 and 0.3 depending on the solvent it is dissolved in for the spectrophotometric measurement.[18] In general, the quantum yield will not differ greatly, even in the same order of magnitude for which the substance is dissolved in. This could not be said the same for chlorophyll-b, which makes chlorophyll-a an even more desirable compound to quantitatively analyze. Using chlorophyll-a, we will not be limited to which solvent we may use as the peak quantum yield for chlorophyll-a in most solvents are relatively equal to each other.

3.4.2. Quantum Yield

The quantum yield of the sample is important in determining how well the sample fluoresces. To this purpose, a spectrophotometer and fluorometer must be used to calculate the optical density, OD, at the excitation wavelength and the integrated fluorescence signal, <F>, for both the sample and reference solvent. These values can be used in equation 11 to calculate the fluorescence quantum yield of the sample[19] where η is the fluorescence quantum yield, and n is the refractive index.

$$\eta_{sample} = \eta_{ref} \times \frac{<F>_{sample} \cdot n^2_{sample} \cdot OD^{ref}_{exc}}{<F>_{ref} \cdot n^2_{ref} \cdot OD^{sample}_{exc}} \qquad Equation\ (11)$$

For the reference of the fluorescence and optical density, it is unnecessary to consider both sample and reference of our chlorophyll solutions. The reference of the fluorescence can be any solution, and for our convenience, we will most likely consider Rhodamine-6G. The Rhodamine-6G would be a good consideration for our reference for its purity in the solution as a whole, and because we can already find the quantum yield of the solution itself.

3.4.3. Concentration

A principal part of the spectrometer is its ability to detect the fluorescence of a sample of chlorophyll-a and convert that to concentration. The integrated fluorescence will be measured and from the slope of predetermined data of the integrated fluorescence vs. absorbance graph we will make we can find the absorbance of the sample. Using this value we can find the content of chlorophyll-a using beer's law (equation 12) and compare it to our standards for chlorophyll-a. This enables the device to tell the user information about the quality of water.

$$A = \varepsilon bc \qquad \textit{Equation}\ (12)$$

Beer's law is a widely used equation for finding the concentration of a sample. The absorbance, A, is a logarithmic ratio of the intensities of the input and output light of the sample. This value is usually obtained using a spectrophotometer. ε is the molar extinction coefficient, a constant for each sample. For chlorophyll-a in acetone the molar extinction coefficient is 78.75×10^3 per mol per centimeter.[20] The term b is the optical path length of the cuvette, 10 millimeters in our case. Finally, c is the concentration of the sample in mols.

3.4.4. Sample Container

There are three common types of sample containers used in spectrometers, quartz cuvettes, optical glass cuvettes, and plastic cuvettes. Such cuvettes usually have between 10 millimeters and 1 millimeters optical path length. There are two different types of cuvettes specific to different applications, two clear sided cuvettes or four clear sided cuvettes. For fluorometers, four clear sided cuvettes are required. The different materials are quartz, optical glass, and disposable plastic cuvettes. Each have different spectral properties:

Quartz cuvettes are useful when taking spectra in the UV to IR spectral range, but optical glass cuvettes absorb UV light and is more efficient when taking spectra in the visible to IR light spectral range. Quartz cuvettes are also more expensive than optical glass cuvettes.[21] Quartz is also resistant to acetone for the consideration of our solution, which will be one of the biggest factors aside from cost.

Optical glass cuvettes have an optimal wavelength range of 340 to 2500 nanometers, except that their chemical tolerance is less than the amount of chemicals that can be handled by quartz of fused quartz materials. This transmission is bordering near the ultraviolet spectrum, but not optimally. Glass will also be a suitable material considering its resilience to acetone, which will also be very high in concentration, the same for the quartz material.

Disposable plastic cuvettes are the cheapest but are difficult to be used for this application as they are not resistant to organic solvents such as acetone. These cuvettes are typical for use with solutions that are non-corrosive or damaging to most materials,

Table 16: Cuvette comparison

Company	Science Outlet	Science Outlet
Part #	B0081LZGRC	B00GYL4XBG
Type	Quartz	Optical Glass
Quantity	1	2
Optical Path Length	10 mm	10 mm
Spectral Range	190-2500 nm	350-2000 nm
Price	$49.95	$39.99

When choosing a cuvette it is important to take into account the quality of the manufacturer as low quality cuvettes can result in inaccurate light paths, fluorescence impurities, leakage, and short cuvette lifetimes. The tradeoffs of both types of cuvettes considered is shown in table 16 on the previous page.

The cuvette we decided to use is a 10 millimeters optical path length optical glass cuvette, highlighted from the table above. The 10 millimeter optical path length cuvette is more standard for spectrometers as a large enough sample volume should be measured to minimize the errors. As we are not measuring any UV light, it is a better option to use the optical glass cuvettes as they are cheaper. We thus decided on the optical glass 10 millimeter optical path length cuvettes from Science Outlet shown in figure 13 below.

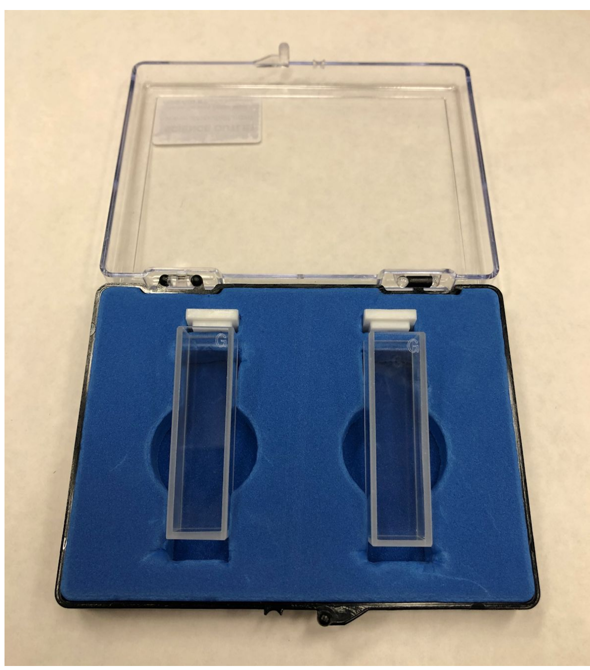

Figure 13: Sample cuvettes made of optical glass purchased from ScienceOutlet.

One thing to keep in mind when using a 10 millimeter optical path length cuvette is that some of the fluorescence may be reabsorbed by the sample before it passes through the cuvette. This can result in a lower signal and cause inaccuracies in the measurement. To ensure that this does not happen, it is important to choose samples with optical densities below 0.1. We also need to ensure that when preparing the samples that we put the lids on in an airtight seal. This prevents excess evaporation of the sample due to the excitation source.

Sample cuvettes in fluorometers are usually placed in a 4 sided cuvette holder. These can however be expensive. For this reason we are considering 3D printing our own cuvette holder.

3.5. Software

The application's interface should be straightforward for the user to operate. Functionally, it will be able to process the data from the sensor and translate it for the user to understand. The direction and design of the software will also be primarily dependent on the capability and skill set of the programmer, but also the microcontroller used. Discussed in this chapter are the decisions needing to be made for the features, as well as options for additional features to be implemented.

3.5.1. Raspberry Pi's Main Language

For this section, we will discuss some of the default languages the Raspberry Pi supports and it's features that led us to choosing the standard language. With the Raspberry Pi chosen for our project, we are given a large option of languages to choose from. By default, the Raspberry Pi has following languages built in: the GCC compilation software which supports C, C++, and C#, as well as the Java Development Kit, Ruby, Scratch, and Python. At the same time, it also has the capability to support hundreds of languages as a Linux computer by simply downloading other compilers and interpreters to support the desired language. With all the languages available to select from, we want to focus on one language as a standard convention to better assist in keeping the members of the project in the loop on software development. As such, when choosing the language, we want to emphasize feasibility and readability on top of practicality:

Ease of Use. While the Computer Engineer member of the team has experience with many of the languages the Raspberry Pi has by default, the wish for the Electrical Engineer to also have hands on experience in programming with a development board is also being considered. Certain languages have features that can optimize the project's software, but unfortunately we would have to trade off performance in favor of a language that is easy to intuitively understand. For this reason, the language has to at least be beginner friendly that wouldn't make it too strenuous of a challenge for the Electrical Engineering member of our group to pick up.

Readability. The language not only has to be easy to implement with, we also want it to be readable to the members that might not have an in depth knowledge of programming to follow along. The readability is necessary to the project as one of the challenges our project faces is for the Computer Engineering student in charge of the software development to be able to translate the optics behind the project from the Photonics side. By using a language that the Photonics members of our group can intuitively understand, it will be much easier for them to describe to the developer what they need fixed or added on in the program. Python's conventions will be further elaborated in the following chapter when discussing coding standards.

Practicality. While feasibility is important, we cannot ignore a programming language that may be beneficial to the project. Because the Raspberry Pi can support additional languages, it may be necessary for us to have a file or program outside of the main language we wanted to use for the software. If another language besides the chosen convention is necessary for the program, it will be discussed in the in its respective section in this chapter.

From the numerous languages the Raspberry Pi can support, we narrowed down the options to two selections. In this section, we will discuss between C and Python as the languages to use as the standard, as well as additional languages that may be helpful for the project.

3.5.1.1. C Language

In UCF's curriculum for all of our respective majors, the first programming language we're introduced to is C from EGN 3211 - Engineering Analysis and Computation. C is a powerful and efficient language that is considered to be a fundamental language for anyone studying programming. As such everyone in our group has been exposed to C, it satisfies our condition on the language being feasible and readable. However, EGN 3211 only has given a surface level of the program, and doesn't necessarily go in depth on the language's strengths and limitations:

Strengths. C is a machine independent language that could extend itself and to make the code simpler. C is also structure-based and procedural which makes it easy to identify code structure and solve issues with the program. It also features dynamic memory allocation, so if we are not sure how much memory is needed, the program could still be run as the memory will be assigned at the same time.

Limitations. Though C so far seems to be a great choice, the fact that it is procedural means that it does not support the concepts of OOP. C also lacks in exception handling, a crucial feature in programming languages that allow us to catch errors when a bug or anomaly can occur during compilation. C also does not have any constructor or destructor, which would require the programmer to take caution on how memory is allocated to prevent a memory leak. C also has a low level of abstraction that causes it to have little data hiding and security.

While C is relatively easy to pick up on a surface level, utilizing it to its full potential is not as intuitive compared to an object oriented language. Though each of our members have experience with C, learning it at a deeper level would be too time-consuming for anyone in this project trying to get more hands-on with programming. For this reason, the selection of choice will be narrowed to Java or Python.

3.5.1.2. Python

Python, compared to C is a relatively new programming language that emphasizes code readability. On top of emphasizing the code's syntax and grammar to be simple and uncomplicated, an important goal for Python's developers was to keep the language fun to use. For this reason, Python is considered to be the most beginner friendly language to many, and is perhaps used most often in projects that uses the Raspberry Pi. Because of how feasible and readable this language is, it has become our choice as the standard for the project. Though it’s great in readability, we must also take into consideration its weaknesses not only in choice, but implementing with this language as a whole.

Strengths. Python is an open source language that has an extensive support library scripted into it that limits the length of code needed to be written into it. This includes string operations, operating system interfaces, and protocols that allows programmers to be productive as less writing needs to be done. Python is also extensible, which allows us to use other languages with it in tandem, and it’s also embeddable which allows us to put the code onto another language that allows us to script in other languages should it be necessary to. Python also supports numerous databases such as MySQL, MicrosoftSQL Server, and Oracle which opens up a feature to store data for record keeping as well as helping us to analyze the different substances in the sample. Additionally, one of the photonics members of our group has experience with Python, which will make translating the optics simpler, if not more than C.

Limitations. With Python’s numerous strengths that make it seem like it's the perfect choice, there are unfortunately downsides to be aware of. Python consumes a lot of memory, which may pose an issue if we have memory limitation in our project. Python also has a slow execution time due to it being an interpreted language that executes code line by line. Fortunately, speed isn’t as huge of a concern to our project, especially when the benefits outweigh the consequences. The most important thing to consider is that Python's simplicity may also be its downfall. Run-time errors can emerge due to the fact that we don't need to mention data types in the program.

3.5.2. Interface Mediums

To be able to handle all of the calculations and processing needed for the signal, the option of using an application in conjunction with the microcontroller was considered. While the use of an outside application will reduce the load on hardware design, it may require that our device uses a method of transferring data either through Bluetooth, Wi-Fi, or USB. Fortunately, the Raspberry Pi supports all three methods, but for this section, we will describe the thought process behind the software design that influenced us to pick the Raspberry Pi over the other microcontrollers. The various options to host our device’s information or applications are listed below, and summarized in table 17 at the end of this section.

3.5.2.1. Website

If the analysis program was hosted on the website, the benefit of using a website opens its doors to numerous features that can improve the project's usability and performance. If we have our application web-based it can host the database discussed in an earlier chapter, and allow room for the project to be able to connect to other devices. A website will also allow the user to keep the device right by the body of water being tested, and allows a future implementation of automatic reporting for the user to utilize. Additionally, using a website can allow a community to look at the water quality and work together to address issues that might be causing it.` Below are the following skills needed to create the application:

Hosting. The most important step to having a website is finding a suitable host to contain our content. The host not only has to be for the website, but also a possible database to store information. Hosting these days is fortunately easier and cheaper, but finding a host is only half the battle. Setting up the website and database together is necessary to overcome to see the full functionality of the website as discussed in scripting languages below.

Scripting Languages. Implementing this feature would require knowledge of scripting languages such as Javascript for not only the website, but to connect to a database to host the data such as .php. Python is fortunately able compatible to connect with numerous databases, but due to the website being separate from the Raspberry Pi, we can go off our standard language as the Electrical Engineer also working on the microcontroller would most likely not touch it.

Data Protection. While not a key feature, some level of security must be implemented for the website to ensure data isn't lost or corrupted. It is crucial that data is properly managed to prevent our project's progress being damaged.

Content Creation. For the scope of the project, it isn't necessary to develop a website that looks professional and business-like, but knowing the basics of CSS and HTML is necessary to be able to navigate and access the application. The website, while not needing to be fancy, has to be simple and straightforward enough to navigate.

Drawbacks. The drawback to this medium is that the device must be dependent on either being Wi-Fi compatible to send data, or be able to manually update its files online through a USB. For both cases, the user must have Internet access. Fortunately, in the U.S, it's easier to access internet virtually anywhere, however, it would not see much use in countries where the internet is not as accessible.

3.5.2.2. Mobile Application

In an era where most users with cell phones have smartphones, a mobile application will make the device easily accessible. A mobile application would eliminate the need for internet access, which would allow the device to be travel farther than if we used a web-based application and to perform the analysis on the fly. However, unlike a web-based application, the feature to leave the device and to access it remotely is not an option.

Drawbacks. Using a mobile application however would make the device dependent on Bluetooth, and like a website, would only see use in areas where everyone had access to a smartphone. Additionally, as the chosen standard language is Python, implementing a mobile application would be difficult unless we switched languages due to Python being made for server-side coding.

3.5.2.3. Development Kit

If possible, the analysis program can be coded within the development kit, reducing the need of using either a mobile or web based application. The biggest benefit to this is that there is no dependency, however, the development used would need a large enough processing power to handle the information. In addition to that, the hardware design of the device would increase in complexity. Fortunately, because we have chosen the Raspberry Pi as the standalone computer, this would not be an issue and the requirement to handle all of these programs is why it was chosen.

3.5.2.4. Existing Software

Due to the focus of the project, designing an interface for the data might not be necessary. Not only will it add extra time to the project, but there are already several programs that can graph the data the sensor will provide. The software at the bare minimum should produce graphable data for the user to observe. Programs such as Microsoft EXCEL can use the data generated by the program to produce a graph.

Table 17: Interface pros and cons.

Web-based	Mobile	Development Kit	Existing Software
+ Multiple devices supported + Has room for a database. + More room for complexity - Device must support Wi-Fi - Reliant on internet access for both devices.	+ Can be used without internet + No internet connection necessary. - Requires a smartphone or tablet. - Device must be bluetooth compatible or Wi-Fi compatible	+ No dependency on another device. + Most portable option. - Must use a Development kit with strong enough processing power - More complex hardware design	+ Time saved on development. + Simpler for user to use. - Design is constrained to be compatible with the software. - Missed opportunity to develop current skill set.

3.5.4 Additional Features

Several features that can be implemented to optimize the design and functionality of the project has been discussed in passing throughout this chapter. In the following section, these additional features will be discussed on how it can improve the project, yet why it wasn't included in our core requirements.

3.5.2.1. Database

The option of creating a database was one of the first features discussed for the project. Originally, the water sensor was to detect and identify various substances in water rather than the concentration of chlorophyll in water. The respective spectras of the substances would've been stored onto a database that the software would use to compare to the results generated from the sensor. Though the project's focus has shifted, the use of a database could still see potential use. Implementing a database opens a tracking feature to the project that allows the device to monitor the quality of water overtime and automate the process rather than having to manually go to the site and record the data. Additionally, comparing the databases used by the device could be used to help identify a more widespread issue by comparing data to areas nearby. Though a database is very useful, it will be more of an optimization rather than a requirement.

3.5.2.1. Remote Access

The possibility of implementing a feature to have remote access to the device was also considered. The biggest benefit to this feature would be that the device can be left at a site and monitored over a distance, reducing the amount of time traveled. Additionally, the device having a remote access functionality will allow several users to monitor the water quality and can potentially identify trends within the area.

The biggest constraint to this feature however is an additional feature needing to be added to the hardware side: the ability for the device to collect the water sample on its own. This feature in turn would require the device to have some form of self maintenance including having the ability of movement to readjust its position in the site, a gps to know its location, alerting the user if there is an issue, security measures to prevent theft, and a camera for the user to monitor the device. On top of that, a bigger focus on casing would need to be emphasized to protect the device from weather, and abuse. As useful as this feature may be, the hassle to implement and manage it is more trouble than is worth and will most likely not be considered when optimizing our design.

3.6. Housing

The design of the housing not only has to consider how it will hold all the components, it has to be able to create a controlled environment for the sample to increase the accuracy of the spectrometer's readings. Something we also need to take into consideration is protecting the device both from external forces, but also the sample from accidentally spilling onto the device. Several options have been explored to create the casing for the design.

3.6.1. 3D Printing

3D Printing is a relatively new invention that creates an object by adding thin layers of material on top of each other. The material is usually a form of plastic, which is inserted into the printer to be melted and used like ink would be on a printer. 3D printing begins with a modeling software such as AutoCAD to design the objects to be printed. If we decide to 3D print our materials, one of the members in our team would have to learn AutoCAD as none of us had experience before the beginning of the project.

Pros. 3D printing is both on demand and low cost which makes it ideal to use when creating a project. It’s great for rapid prototyping because it can quickly advance from the design phase. The process of 3D printing is also sustainable and there’s minimal waste produced from the product.

Cons. 3D printing isn’t user friendly so trying to learn how to use one in the beginning may take a while. Because 3D printing uses additive construction, or prints layer by layer, creating the object can take up to an entire day, so we must be meticulous with our planning to ensure time isn’t wasted. Trying to own a 3D printer is not only expensive to purchase, it’s expensive to maintain as printing consumes a lot of energy and buying fiber in bulk.

3.6.2. Manufacturing

Manufacturing will follow a similar process to 3-D printing, except that after a design has been created, a company will be contacted to manufacture. Seeking out a company to create a casing will give our project a sleek and more professional design, and also provide us help to optimize the design. The design and material suggested by the manufacturer could increase our device’s durability that 3-D printing may not be able to provide. While ideal, seeking a manufacturer will us cost money, and we would have to wait for them to finish their part before we can use it if we decide to have something custom made.

3.6.3. Manually Built

If the previous two options aren’t possible, creating a casing from scratch is a highly possible solution. This option can not only provide a durable casing to the device, it can also be cheap as it creates an option of using recycled materials for the project. This option can also be optimized with 3-D printing as an aid to the design. As CREOL students, we have access to CREOL’s machine shop to aid in this process. Two of the students working on this project have experience taking a machine shop workshop and will be able to construct the casing.

Table 18: Tradeoffs of different construction processes.

	3-D Printed	Manufactured	Manually Built
Cost	$	$$	$
Design	Needs to be designed in a 3-D Modeling software.	Professionals could optimize the design for us.	Possible lots of trial and error if not careful.
Protection	Low, but can vary depending on the material used	High as it is professionally done.	Medium, depends on parts used and how its built.
Time	High	Medium	Low

In table 18 above, we compare the pros and cons of each construction process possible for creating the casing. As our dimensions of this device do not need to be extremely precise, we decide to manually build the casing using CREOL's machine shop. This way we can potentially even customize the design after initial testing if needed such as adding openings to the casing for USB ports for the Raspberry Pi or for the cuvette holder insertion area of the spectrometer.

3.6.3.1. Materials

There are many materials that can be used to construct the casing. Such materials include but are not limited to aluminum, stainless steel, acrylics, and teflon. As of this stage in the planning of our project, we are strongly considering acrylics as they are easy to work with. They can be easily attached using an acrylic adhesive, that takes effect almost immediately and only takes a single day to cure.

Other options such as aluminum or stainless steel are either harder to machine or are not as customizable. Materials that are made from metal are stronger than materials that are carbon based, or other plastics. The tensile strength is also higher for metal materials than non-metal materials. This drives us to the obvious choice that metal would be in application the best material for our housing. We may expect the least amount of changes in our device over time, using metal holders, but these are obviously more expensive compared to most other choices.

Other metals, such as copper, are even more difficult to machine that we may be unable to create the holder from this material if the threads are not exact. For our design, we will need to consider the quality of all the lens holders especially. We will consider using a lens holder that is meant to screw on the holder such that it will be fixed in one position, ideally for a long period of time. Using this type of holder, the threading will be crucial to the holder, so using a material that will be the easiest to machine will also be a crucial factor.

Table 19. Cost vs. machinability

Materials	Aluminum 7075	Acrylics	Copper	Wax

Prone to Defects	Little	Moderate	High	Moderate
Dimensions	10''x5''x5''	2''x6''x6'	1.25''x11''x1.25''	3''x3''x7''
Cost	~$174.14	~$68.12 (4)	~$159.99	~$21.95

Comparing the materials from table 19, we can see that our obvious choice will be to buy the acrylic as compared to any of the metal substitutes. While considered to be more brittle, it is significantly cheaper and able to be worked with by a skilled machinist. As far as the design of the holding components, 25 cubic inches may be enough to satisfy the material requirements.

4. Constraints and Standards

Our project pushes heavily on us to acquire items and devices that are similarly implemented onto other commercial spectrometers. Considering that our design will most likely be extremely objectionable compared to other commercial spectrometers, we may be more prone to other defects that major companies have already resolved. On top of these constraints limiting our project, we have to be aware of standards as well to ensure our project is a quality design. Discussed in this chapter, we will go over both constraints and standards and their role in our project.

4.1. Constraints

A major constraint for our project would be is how strong the signal of our fluorescence can potentially be. As a rule of thumb, the higher the concentration of our chlorophyll in our sample is, the more it should fluoresce. However, our sample, chlorophyll a, is also seen to absorb the same wavelength that it fluoresces when excited with light of 430 nanometers. Due to this effect, we can only add a concentration high enough for it to fluoresce but also mitigate the effect of reabsorption. If our concentration were too high, i.e., an optical density greater than 0.1, we could experience nonlinearities from inside our cuvette, such as the substance absorbing the fluorescence energy too much and disrupting the actual quantum yield of chlorophyll a. We are also confined to making sure that our optical density will be less than 1 due to the property of our cuvette.

The spectral resolution of our camera will be another factor to consider when analyzing our signal. Ideally, we would like a camera that includes the smallest size pixel, and the most amount of pixels on the array. The spectral resolution can be calculated as in equation 10 where $\delta\lambda$ represents the spectral resolution, RF is the resolution factor, $\Delta\lambda$ is the FWHM of the peak, W_s and W_p are the width of the slit and pixels respectively, and n is the number of pixels.[21]

$$\delta\lambda = \frac{RF \cdot \Delta\lambda \cdot W_s}{n \cdot W_p} \qquad \textit{Equation}\ (13)$$

Our goal is to acquire a resolution of at least 5 nanometers, even though we will still be limited by the wavelength range that we will set ourselves (600 nanometers to 750 nanometers). For our consideration, we will remove the parameters set by the slits, as there are no other peak wavelengths that we have detected in our samples. This means that since our signal is only available at 665 nanometers, we can fix our monochromator onto one position such that all of the diffracted light will cover the entire detector. Slits are used in general spectrometers to cover a certain spectral region, but we will only consider our results to be from 600 nanometers to 750 nanometers.

A non-technical constraint would include the accessibility of our device. The samples used for our fluorescence spectrometer were prepared in a laboratory with an extensive array of lab equipment. The main device that would be crucial for our sample preparation would be a centrifuge. The mortar and pestle, beakers, graduated cylinders, and cuvettes are all relatively cheap compared to the centrifuge.

4.1.1. Economic

One of the goals the project is hoping to achieve is to maintain a low enough cost that would see commercial use in this application rather than mostly industrial use. Water Quality sensors can cost up to thousands of dollars, and if we could keep our implementation under a budget of 1000 dollars, the project would be considered a success. Thus, it is necessary to be willing to sacrifice performance in favor of price. The system is reliant on a camera to perform the task, so one of the biggest challenges in its design is to balance a high quality camera at a reasonable cost.

We will ultimately avoid trying to obtain the general spectrometer cameras as they cost too much. General spectrometer cameras, while being essentially the same device as other CCD or CMOS cameras, are considered specialized which make them more expensive to other square cameras. They're only feature is that they include several pixels in the vertical direction compared to many pixels in the vertical direction. They are lower in demand, so they generally have to be custom ordered. This drives the price of these cameras upwards of 400 dollars, which we will actively try to avoid.

Different materials of cuvettes will also be a driving force of the costs. The cheapest rectangular cuvettes will be made of glass, then quartz, while glass will fit into our design with ease. The electrical components, not including the camera, will not be comparable to the optics, with the most expensive portion being the Raspberry Pi, at roughly 30 dollars.

4.1.2. Environmental

The device is expected to be taken to various testing sites and could potentially be damaged on the way there. In the event the device is mishandled, the device should be able to withstand at the very least, mild cases of misuse. The ability to protect the device is highly important to ensure long use for the user.

In addition to the environment affecting the hardware, it could potentially affect the accuracy of the water quality sensor. It's crucial that the device is able to perform in various locations and weather to test the water quality. The Water Quality sensor will be used outdoors to conveniently sample data. It's necessary that the device can handle up temperatures up to 95 degrees Fahrenheit for testing.

4.1.3. Social

There are no major social impacts of this product outside of the group. The device will be privately used and not affect other people very much. Perhaps if the product is used to collect data for a research paper or the like it can cause a concern on whether the device is accurate enough to be considered a valid source of data. However, at this point we do nor expect this to be a problem. On the other hand, a social barrier that has impacted the group is the CREOL members translating the optics part of the project in a way the CECS members can understand and implement the theory.

4.1.3.1 Cross-Disciplinary Challenges

While the curriculum for UCF's Photonics, Electrical Engineering, and Computer Engineering overlap in classes, the study of optics is a relatively new field. The overlapping classes allows both Photonics and Electrical Engineers to understand on a surface level on programming and computer architecture Computer Engineer will specialize in, and for Photonics and Computer Engineers to learn the basics of Circuit Theory that an Electrical Engineer will specialize in. However, outside of extracurricular classes, there is little an Electrical or Computer Engineering student will be exposed to regarding Photonics.

To counter this barrier, research was done by the CECS members on optics to gain a better understanding for designing the project. Frequent meetings to discuss the project will be conducted to help the CECS members of the project translate the techniques and information to the design. While this adds on to time, crossing this barrier is crucial for the success of this project.

4.1.4. Political & Ethical

There are no major political concerns to our project. This device will not be listed on any major federal organization to be listed for improper use. As long as we exhibit proper copyright material and provide credit for all resources used during this project. We will not consider selling this device so that it will not be inspected by health organizations such as the Environmental Protection Agency or the United States Department of Agriculture.

There are no major concerns to the ethical concerns of our project. This device is not meant to act as a weapon, be invasive towards another person or being, but purely for quantitative purposes. The device cannot be distributed for sale without a proper license, and we are also using many already integrated products.

4.1.6. Health and Safety

As is often the case with projects or designs that utilize chemistry, lasers, or electronics there are significant health and safety concerns for this project. For example, the chemicals used in preparing the sample of chlorophyll can pose potential health hazards. Laser safety must be considered when constructing this device, such as keeping the light source completely enclosed.

Batteries can also contain toxic chemicals and pose health problems. Knowledge of these risks will help keep our members safe should an accident occur while developing. Discussed in this section are some of the specific health risks due to the nature of our project.

4.1.6.1. Chlorophyll

While natural chlorophyll isn't toxic, possible side effects due to overexposure include digestive issues, gastrointestinal cramping, and diarrhea. Chlorophyll may also give a person a rash from overexposure. Though extreme means are not necessary when handling chlorophyll, caution should be taken when handling this substance. The chlorophyll may not be the toxic portion to human beings, but the toxins produced by the bacteria must be handled with care. We cannot separate the toxins from the algae, so they will most likely be together in the same solution.

Because of the potential sensitivity of the device, we will aim to find the most amount of chlorophyll in our sample, 50milligrams per liter. This is also considered to be the least amount of the most dangerous concentration of cyanobacteria in a volume of water. It is crucial that we aim to not ingest any of these photosynthetic organisms for our health, and if this device were to be replicated, other users will have to consider the health risks of handling a high concentration of these microorganisms and the toxins that they are actively producing and secreting.

4.1.6.2. Laser Exposure

Though the laser exposure for the device isn't hazardous, it's important that the laser is properly handled correctly to minimize accidents. The biggest concern when dealing with lasers is the risk of eye injury and even small amounts of laser light can lead to permanent eye injuries. The maximum output power emitted from our laser is considered enough to be damaging to our eye without the use of focusing optics, so we will be careful to ensure that both the user and ourselves when making this shall not be able to be in direct field of view of the laser.

4.1.6.3. Battery

The battery choice for the project is a lithium-polymer, which is considered a dangerous chemical material, classified as a Class 9 miscellaneous hazardous materials under United State regulations (40 CFR173.21 ©). Precaution must be taken in consideration to prevent any fire or leakage due to overheating or under discharge. Lithium-polymer also has environmental concerns as its difficult to recycle. Rechargeable lithium batteries include highly flammable electrolytes and may burn. Causes of lithium battery failure include mechanical/physical defects to the battery such as puncturing or scratching, overcharging the battery more than it can handle, which in turn will cause it to overheat, short-circuiting, and damage to the cells. We consider overcharging the battery to be the biggest concern to us as it is the most likely. We will consider that the battery will be charged using the manufacturers given charging supplies, in case we encounter the battery to be able to also be rechargeable with a universal serial bus.

The lithium ion batteries undergo thermal runaway, which is a process that occurs when too much heat and pressure are created than is lost instead. If one cell in the battery were to undergo this process, thermal runaway, the adjacent cells next to it will also undergo this process, similar to an avalanche reaction. The cells will release their contents and possibly combust. These fires are not similar to regular fires, but are also not considered to be any more dangerous either. Still, one must be properly trained to handle battery fires and know certain extinguishing techniques, none of which any members of our group would know. It is crucial that we avoid this form of fire hazard as well as possible, also as an economic benefit to not purchase more batteries.

4.1.6.4 Soldering

Soldering involves toxic materials and is done at extremely high temperatures which can be dangerous if done carelessly. Our soldering work station will always need to be clean, organized, and well lit so we can easily identify any hazards. When soldering a circuit, we must use a soldering iron to heat solder wire. The solder wire contains lead which is toxic for our bodies (see section 3.2.1.1). When the iron touches the wire, it typically releases smoke. To avoid inhaling this smoke, we place a fan next to us to sweep the fumes to the side and away from our faces. There is also the possibility of the solder splashing up at us when it turns into its liquid form due to the rapid temperature change. To protect our eyes, we wear goggles

4.1.7. Manufacturability

It can be hard to align optical components for such accurate measurements as the ones required for spectrometers. The alignment alone can take days. This can cause manufacturability of the device to drop as experienced personnel are required for such alignments. This may pose a significant constraint on how accessible the device may be to potential consumers or on product maintenance.

4.1.8. Sustainability

The device may need regular maintenance to operate if heavily used. Over time the optical components may become misaligned causing the measurements to vary. To this end we plan to make the optical components as stable as possible so there is minimum chance of this occurring. Problems with maintenance though can be that a background knowledge of optics is required for safely maintaining the device while also keeping the accuracy of the device.

The booster for the battery will most likely fluctuate with the amount of energy that it is feeding to the circuit, it will need to be calibrated almost daily potentially to ensure that it does not feed too many carriers to the laser diode. The laser diode will potentially need to be checked on frequently as well. The responsivity of the device will change over time, not in a matter of weeks or months, but if were to consider the longevity of the device it will matter. The responsivity of the camera will also be of concern, which will degrade over time. We may consider an easy way to remove these devices such that it will be easy to calibrate individually so that the whole spectrometer will still be functional.

4.1.8.1 Water and Other Solvents

Due to the variation of environments the device may be exposed to, a durable structure is necessary to protect the contents of the device. Water could potentially damage components in the device, so it is crucial that the components are waterproofed or at the very least, water resistant in case spillage should occur. We will ensure to use the same safety features for the concern of the device as we have for the concern of the spectrofluorometer and the spectrophotometer that we have been using. The biggest concern of water shall be from the cuvettes. Spilling will be the most obvious concern from the device, so it will be imperative to create a holder that fits the cuvette in the same exact dimensions such that the cuvette will be immobile. The holder should be clearly visible such that we can observe where to put the cuvette.

While water is also a concern to the device, it is also meant for other solvents which are also expected to be used for our device, such as ethanol, dimethylamine, and very pure acetone. For our consideration, all of these solvents will be extremely pure and barely diluted. Because of this, evaporation will also be a major concern to the device. We are unable to observe this effect happening, so our only solution is to try and stop it from evaporating inside the device. The cuvette containing whatever solution, most likely highly evaporative, will have to be sealed from the tip. A plastic will have to be wrapped around the tip such that it will also prevent spilling, and gaseous molecules. This should ensure the longevity of the device, and the accuracy of our results for this project and practice. Any gaseous molecules may potentially contaminate electronics or optics inside the device.

4.1.3. Time

As the members of the group are all working students, working around schedules to make time to work on the project will challenge both the speed and the quality of the development. With the schedules at the time of writing, a time where all four of us are free is sparse, without accounting for the time we possibly may need to work on other schoolwork. To offset some of the issue with being able to meet, both the software and the electronics engineers have separately purchased a microcontroller for individual testing.

On top of all that, unfortunate circumstances can also delay the project such as sickness, or natural disaster. Because of how sensitive and important time is to the success of the project, we need to take careful consideration on the scope of our project. If our project is too large to implement, time becomes more crucial, and any lost time can greatly delay the project. One particular roadblock that affected the project was Hurricane Dorian. The hurricane took a week of class time that not only significantly impacted the productivity of our project, it also shortened the amount of free time we had as not only did we have to catch up on Senior Design I, but our other classes as well.

4.1.4. Testing

To ensure that the device works consistently and accurately, tests need to be conducted and repeated. The sample testing may lead to different results, which is why repetition is important to observe the results. This can be a result of the different samples, their preparation methods, alignment or optical component problems, and even detector quantum efficiency. For these reasons it is necessary to test the product and each component under different conditions on top of repetition to get the information we need for the product. Each component as well as the final product should meet the specifications set earlier on in this document before it can be considered a finished product ready for manufacturing and marketing purposes. These tests will also eat into our time, but several methods to offset that such as scripting to automate the results have been planned, shown in a later chapter.

4.1.4.1 Laser Wavelength

The device will operate with a 405 nanometer laser diode. The energy at this wavelength is absorbed by chlorophyll, not as well compared to a wavelength of 430 nanometer, but still viable. However, other samples may not absorb strongly at this wavelength. This means that a different light source must be used depending on which sample is used. The sample preparation may be considered more strongly using the 405 nanometer for our chlorophyll-a solution. This will actually be a benefit to us as whatever chlorophyll-b may or may not be in the solution, will absorb even less of the energy emitted from the excitation source.

The sample we tested was chlorophyll. This sample is hard to detect in small concentrations and thus may not give off a strong signal. To get a better signal, the sample must be prepared. It may be necessary to use a laboratory environment to do this preparation. Our method to preparing the sample is to find the concentration of Chlorophyll-a in our glass cuvette from the spectrophotometer. The reading from the spectrophotometer will tell us our absorbance where we will already know the molar extinction coefficient and the length that the distance light propagates. Using Beer's law, our target concentration will be a concentration of 50,000 milligrams per milliliter. We will iteratively find the concentration by placing some concentration into our cuvette that is above our target concentration. We will then dilute the sample until we reach our target concentration.

4.2. Standards

To ensure our device works, has a meaningful impact on the market, and is safe to use we must set some standards. There are many different considerations in this aspect, such as sample accuracy, safety features, and technical aspects. The sample accuracy will be dependent on the concentration of chlorophyll-a in our water. The device will not be able to distinguish what the photosynthetic organism will be, rather knowing if the photosynthetic organism will be there.

We hope to achieve from our absorption and fluorescence fit that we may be able to achieve within 20% of accuracy as compared to the other spectrofluorometer that we have been using.

4.2.1. Sample Detection

Our major standards will include that we are able to measure the concentration of chlorophyll-a with an optical density of less than 0.1. We would like for our design to be at least 20% accurate to the right concentration of chlorophyll in our solution. As far as aiming to find the concentration of chlorophyll in the correct amount of concentration, without being refined and tested with laboratory equipment, we will not attempt to achieve this as we require a separate solvent other than water. Therefore, our current objective will be to find a concentration of chlorophyll in our preferred solvent, acetone (>99.8%), also spectrophotometric grade.

There is a certain limit in concentration that health regulatory and sanitation organizations deem to be safe for certain compounds in an area of freshwater. Our goal will be to find at least the highest amount of concentration of our water that we test based on the standards of these health organizations. Health and sanitary organizations include the Center for Disease Control, domestic organizations limited to the United States, the Environmental Protection Agency, and the World Health Organization. There are different levels of risk that are associated with certain cyanobacteria in a body of water, depending on how high of a concentration they are. They can either be classified as low risk, high risk, or very high risk. Increasing levels of risk include a higher degree of damage that can be done unto our bodies.

The environmental protection agency (EPA) is currently developing a draft of safe conditions for recreational use of water concerning the concentrations of cyanotoxins.[22] We will attempt to follow the guidelines outlined in this draft as a determination of how safe the water is.

The World Health Organizations (WHO) has a guideline of the relative risk of different ranges of concentrations of cyanotoxins in recreational water[23]. WHO has created a guideline for the concentration of these algal cells in different categories, labeled as moderate-risk, high-risk, and very high-risk. For their low-risk category, they consider on average that 50 micrograms per liter is acceptable. For their moderate to high risk category in freshwater, they consider on average that this concentration is 5,000,000 micrograms per milliliter. For the very high risk category in freshwater, they consider that this concentration on average is 50,000,000 micrograms per milliliter. For these scaled concentrations, scaled by a factor of 10 for each level, they are considered to be 100,000 cells per milliliter, 10,000,000 cells per milliliter, and 100,000,000 cells per milliliter. These concentrations and weight were all considered using chlorophyll-a. Extraction of chlorophyll-a will be necessary in this regard to ensure that this is purely the fluorophore that we are trying to excite in our solution.

For our project, we aim to find a concentration and extract a signal from at least the most dangerous amounts of these cyanobacteria in the water. We also aim to find these certain cyanobacteria that include excreting these cyanotoxins, as some species are more harmful than others. The World Health organization has concluded that the cyanobacteria species, Anabaena is one of the species in the genus cyanobacteria that secrete microcystins and other cyanotoxins. For a more specific goal, we aim to try to find at least 50,000 micrograms worth of Anabaena in one liters worth of freshwater, or other conditions where Anabaena can live and reproduce. We will not limit ourselves to find only the concentration of Anabaena in a volume of water, but also other cyanobacteria that are known to secrete these toxins into a freshwater supply.Other species of cyanobacteria that are available to us that we can commercially acquire are Fischerella or Oscillatoria.

4.2.2. Laser Standards

The laser diode we plan to use is classified as class 3B of IEC60825-1 and 21 CFR part 1040.10 safety standards. These lasers are categorized to have a power between 5 milliwatts to 500 milliwatts. According to the American National Standards Institute (ANSI), their classification of laser classes start from Class 1, a separate class 1M, Class 2, a separate Class 2M, Class 3 which has two subclasses which include class 3R and 3B, and Class 4. In the following we outline the characteristics of each class laser and in table 20 we outline the necessary requirements of each laser type.

Label requirements that include the secondary (1), are meant to indicate that is still not required under the exception for conditions of intentional intrabeam exposure applications. Label requirements that include the secondary (2) are meant for lasers that are class 1M or 2M and exceed the use of laser classes 1 and 2, may require hazard evaluation and potentially the manufacturer's information.

Table 20. Requirements by laser classification ANSI.

Class	Procedural and Admin. Controls	Training	Medical Surveillance	Laser Safety Officer
1	Not Required	Not Required	Not Required	Not Required
1M	Required (1)	Application Dependent (2)	Application Dependent (2)	Application Dependent (2)
2	Not Required (1)	Not Required (1)	Not Required	Not Required
2M	Required	Application Dependent (2)	Application Dependent (2)	Application Dependent (2)
3R	Not Required (1)	Not Required	Not Required (1)	Not Required (1)
3B	Required	Required	Suggested	Required
4	Required	Required	Suggested	Required

Training must be provided by the employers or management of these laser systems. The employer is responsible for ensuring a safe work environment while the employee is responsible for complying with laser safety training. These standards were set to prevent a hazard that could cause damage such as blindness, or a fire. The ANSI classified lasers by the type of risk they pose as detailed below:

Class 1. A class 1 laser system is considered the weakest levels of the laser classes such that it cannot produce radiation levels that are harming during operation. It does not require any control measures and overseeing operators or other methods of surveillance. The Class 1M Laser by itself is considered to be incapable of producing dangerous or harmful levels of radiation. It does not require any form of supervision for any reason other that the potential event of improper viewing. It is however considered dangerous to the eye if viewed with a viewing instrument such as a loupe or a lens.

Class 2. The Class 2 laser is categorized for lasers that emit in the visible spectrum of light. Anyone around or working with the laser is normally recommended to wear protective eye wear. The class 2M laser includes a hazard warning for any viewer that is viewing the laser with an optical aid.

Class 3. A class 3 laser is also categorized for lasers that emit in the visible spectrum, but also categorized for power which is above class 1 and 2. Class 2 lasers are classified with power that is categorized with less than 1 milliwatt. Class 3 lasers are categorized from power that is 5 milliwatts to 500 milliwatts. Class 3 lasers are hazardous under direct viewing and reflective viewing. A class 3R laser is categorized for any laser with an output power of less than 1 milliwatt, but is still considered a potential hazard if the eye is focused on the laser in appropriate conditions. It is not considered a fire hazard. A class 3B laser is for any laser that has an output power between 5 milliwatts and 500 milliwatts. There is a high probability of eye damage if viewing the laser appropriately. Exposure to the beam at all is not recommended for class 3B lasers.

Class 4. The class 4 laser is the most dangerous level of laser classes. The laser is considered to be a hazard to both the eyes and skin, can pose as diffuse reflection and become a fire hazard, and may produce laser generated air contaminants and dangerous plasma radiation. These lasers have an output power of over 500 milliwatts in the visible spectrum.

4.2.3. Power Supply Standards

According to the National Electrical Manufacturers Association (NEMA), ANSI C18.2M, Part 2-2014, American National Standard for Portable Rechargeable Cells and Batteries, are standards set to ensure the safe operation of portable lithium-ion, nickel cadmium, and nickel metal hydride rechargeable cells and batteries. This standard describes all safety tests and requirements on the portable rechargeable battery that is used for the project. These standards are written not only for normal use, but potential misuse contains information to avoid any potential hazards. This is a general standard for all batteries

ANSI C18.2M, Part 2-2014 is the general standard for all batteries but ANSI C18.3M Part 2-2017 outlines the tests and requirements specifically for lithium batteries.

4.2.4. USB Standards

USB (Universal Serial Bus) is an industry standard that defines the cables, connectors, and communications protocol used in a bus between computers and electronic devices. USB is used for communication, and a power supply between these devices and used to standardize the connection of computer peripherals such as keyboards, mouses, and digital cameras. USBs are designed to connect the peripherals to the same tabletop and not between rooms or buildings, so the cables are limited in length. The speed of data transfer is increased with each update.

The development of the USB standard was due to the combined efforts of the seven companies Compaq, Intel, Microsoft, IBM, Nortel, NEC, and DEC. Their work began in 1994. These companies wanted to unify several ports that were included in the back of personal computers. This would simplify the connection of external components to the personal computer. Over time, the data speed of a USB went from a mere 12 MBits per second to a whopping 40 GBits per second. The USB went through the following specifications, shown in table 21, from 1996 until its most recent that was introduced in 2019 as detailed below:

USB 1.x. The original 1.0 USB standard was written in January of 1996. There were many issues with this version which hindered the spread of its popularity. The 1.1 update that came out in August of 1998 solidified the USB standard as the industry standard. During this time, Apple kind of led the herd in including USB ports in their products as a standard inclusion.

USB 2.0. The 2.0 USB standard introduced different sized connectors: Mini-A, Mini-B, and Micro-USB. There was also an increase in the maximum amperage for charging which was 1.5 A.

USB 3.x. We are currently using the USB standard 3.0 which was released in September of 2007. This standard is backwards compatible with 2.0 and introduced the SuperSpeed bus. This version eliminated the Mini-B connector and introduced the Mini-C connector.

USB4. The USB4 standard was very recently released in August of 2019 and it is backwards compatible. It uses only the USB-C connector. This version is based off of the Thunderbolt 3 standard which was developed by Intel.

Table 21: USB standards.

Version	Date of Release	Data Speed	Noteworthy changes
1.0	January 1996	12 Mbit/s	Mass standardization

2.0	April 2000	480 Mbit/s	Mini-A, Micro-B, Micro
3.0	November 2008	5 Gbit/s	SuperSpeed Bus, USB-C
USB4	August 2019	40 Gbit/s	Thunderbolt 3

4.2.5. Coding Standards

As code is driven by logic, it will always perform what the writer has asked it to and never question why. While an experienced programmer can follow the logic of the program, figuring out why it was implemented a certain way isn't as intuitive, and it's even more difficult for a junior programmer to figure out the reasoning. In other words, without specific instructions or guidelines that talk about the program itself, it's easy to get lost when looking at a program. Coding standards are essentially guidelines for programmers to help in the development of the program and reduce errors. It's important to have these standards in place as it can not only affect the end user and product, but impact development as well.

While practicing good coding standards isn't necessary to create software that can perform a specific task, leaning to implement good code will make it easier in the long run for not only the programmer, but anyone else who will be involved in the program. For a cross-disciplinary project, it is essential that everyone can be able to read and understand its code not only to ensure that we are correctly implementing a spectrometer, but also to assist in debugging by identifying if the error is internal and external. To summarize, no one wants to look at a program and spend hours going through the code and figure out its logic when instructions can be written and clarify without the physical need of the programmer to do so. Quite often, that person trying to figure out how the code was written was the same person that wrote it in the first place. f coding standards are followed, the code becomes not only nicer to look at, but also easier to maintain due consistency. The most important reason that coding standards will be emphasized is shown below:

Coding standards help collaboration. While it is certainly likely that another person may look at your code written in the future, it's possible that for the project you're working on, multiple people will be working on the same application concurrently. If the coding styles differ too much from each of the collaborators, spending time to try and explain the intricacies of how your method was deployed would eat away time into development. Each of the members of the project will at some point be directly involved with a program during development, so it is important that the program is as simple as possible to make as much use of development as possible.

It’s necessary that we utilize coding standards to ensure a smooth development of the project's software. In this chapter, we will discuss the coding standards we will have in place and their benefit to the project

4.2.5.1 Python’s Standards

Clean code is code that is easy to understand (intuitive) and work with (simple). To maintain clean code, it’s crucial that the program follows a coding convention. For the project, we will be following Python Enhancement Proposals’ (PEP) *Style Guide for Python Code*[25], which gives coding conventions that was adapted from both Guido's original Python Style Guide essay and Barry's style guide. Python emphasizes its readability, and in the guide, it emphasizes consistency and mentions that code is read more often than it is written. Here are some of the following standards that were discussed in the guide:

Indention. Indenting helps clarify the structure of a program to human readers, but in Python, it's also used to determine the structure instead of braces or keywords. Python prefers spaces over tab as the use of indentation method.

Line Length. Lines should be limited to a maximum of 79 characters, and for comments or docstrings, 72 characters. Some teams however may strongly prefer a longer line length.

Line Breaks. In Python code, you can break before or after a binary operator, as long as the convention is consistent throughout the program.

Naming Conventions. As there’s a variety of naming conventions out there, there is no set standard for naming conventions other than the one we decide on. For our project, we will be using Capitalized Words for our naming.

Project over Standards. While these standards were written, it also made an important note that if the project follows their own coding style guidelines, project-specific guides will take precedence. If in any case a certain way of writing code makes it easier for the other members of the team to understand, then slight modifications will be made accordingly and documented in the code.

4.2.5.2 Code Documentation

The practice of documenting code is not only important for the end product, it is a powerful tool for the development of the new project as well. To aid with code documentation, we will use GitHub to host all of the information: both the programs as well as its documentation. GitHub is an open-source version control system that allows you to work with other programmers on a project. Going through the process of code documentation serves as a mental save point during for the development, making it easier for those working on the project to pick up where they, or code developer might’ve left off. While more writing is needed, the benefits of code documentation listed below outweigh the cons to where it’s better to have it than without.

Planning. Documents allow the developers to forecast even the smallest parts of code before it is implemented. It's important that a function of a feature is analyzed and that the team understand it's impact before it is added.

Makes Life Easier. Documentation helps explain the why of programming. For the next developer, or even the same developer, documentation that explains how the code does it job will save a lot of time without having numerous meetings to go over the same piece of code over and over again.

Preserve Data. In the event that the program files become corrupted, we want to make sure that we have a back up so we do not lose progress in the design. If an issue were to arise during development, we could look at documentation to backtrack and easier identify the issue rather than scanning all the files and figuring out where in our program is giving us trouble.

Caution. While documenting code is an effective tool, we must be careful not to write too much for things that are easily explained, or be too redundant with our information. Documentation doesn't necessarily have to be a handbook, but could simply be simple, explanatory comments above functions and blocks.

4.2.6. Housing Standards

Housing Standards for electronics are decided by the IEC. The International Electrotechnical Commission (IEC) published the IP rating system to unify everything and create a standard. The Ingress Protection (IP) rating that a device has defines the degree of protection against possible solid hazards and possible liquid hazards. The rating has the format IP##. The first number is the rating against liquids and the second number is the rating against solids. These ratings are tabulated in table 22.

Ideally, we would like our device to be at least IP31 rated. This means it is protected from solid items the size of fingers and bigger and also protected from vertically dripping fluids. This rating should suffice since the device will be frequently handled and adult finger tips are generally larger than 2.5mm. The device does not need to be immersed in water to take the measurement so it should only need to withstand drops of water simply because the user will be near water and could possibly transfer some droplet during handling of the device. We will make sure to emphasize that immersion of the device in water is absolutely not necessary.

Table 22: IP rating chart

IP rating	Protection against solids	Protection against liquids
0	None	None
1	>50mm	Vertical dripping water
2	>25mm	Dripping water at 15 degrees
3	>2.5mm	Sprayed Water
4	>1mm	Splashing Water
5	Dust Protected	Regular Water Jets
6	Dust Tight	Powerful Water Jets
7	X	Immersion in 1m deep water
8	X	Immersion in >1m deep water

4.2.6. Optics Standards

One of the leading organizations for engineering and manufacturing standards come from the International Standards Organization. The ISO is a conglomerate of committees and subcommittees from many different countries that lead in technology and advancements. These teams are working to ensure that all designs reach their parameter goals such that failure will not be met and safety is considered. In particular, ISO committee number 172 is the committee that focuses on optics and electronic-optical applications. ISO TC/172 creates the standards for terminology, testing, requirements, interfaces, and test methods for optics and photonics. This includes complete systems, devices and other accessories that use these principals, including materials. Optics and photonics, in general, is the field generating optical radiation, or handling and detecting them for whatever purposes. ISO TC/172 does not entirely cover the full use of optics and photonics such as fiber optic communication, but will create the standards for our use of optics.

There are ten subcommittee groups that are under ISO TC/172, with over 290 published ISO standards. We observe only the references of 3 subcommittees under ISO TC/172, which include fundamental standards, optical materials and components, and laser and electro-optical systems. These standards while published, are too expensive for us to purchase, including the electrical engineering requirements. We resort to the optical standards that are made available by Photonics, of which they describe the new optical standards written for free. For example, our diffraction grating that we intend to use is not included in any publication by ISO TC/172, but was intended to be included for ISO CD 10110-16, and never made it. For laser damage, which we will most likely never encounter, we will most likely refer back to ISO10110-17. In the event that we will resort to using a reflective surface grating instead of our transmission grating, the anti-reflective coating that we will use will most likely be standardized to OP1.9211-3 and ISO 9211-3 for durability. This can also be said if we were to use the achromatic lens or any other optic that includes a special coating. For testing of optics that are coated, standards can be referred to OP1.9211-4. For any coated optic that is not being measured for durability or testing, it will only be required to be referred in text as a note[26].

We can also refer to the American standards that are adopted from the International standards. For drawing and formatting, both America and the ISO use the same standards anyway, which include OP1.0110-1 and OP1.0110-10. These include the tolerances most importantly. Scratch and dig is covered by section OP1.002 for America and ISO10110-7 for international standards. This section will provide standards for how “clean” the optic is and how suitable it will be for use. Including how far apart the dirt and contaminant concentrations are. The coatings are under review for the American standards, but can be referred to ISO10110-9 for the international version. The surface roughness is still under development for the American standards, but can still be referred by section OP1.0010-8 and ISO10110-8. This section describes the different depth levels of the optics such that it can still be considered flat and uniform more or less[27].

These are several standards that we can consider for our design during and after it is finished. When the device is completed, it can be inspected to make sure that all our components and systems are matched and operated within guidelines by American and international standards. Not all devices are inspected thoroughly and are correct, but we will try to uphold these conditions to the best of our abilities.

5. Product Design

With the components chosen from our research and the standards and constraints to keep in mind, it's important to implement a design plan to ensure a smooth transition in development. In this section, we will elaborate more on the design choices and how they will be used to develop our device. The section will map out the design path for hardware, optics, and the software's individual roles, and how they will work together to attain the goal of a spectrometer.

5.1. Hardware Design

The role of the electrical engineer in charge of the design is to piece together the equipment needed for the respective optics and software parts of the project. There is a flow of information between all of the components of the device to make it function properly. This will combine both electrical and optical components which includes the PCB, power supply, microcontroller, and sensors. Each piece is constructed and then tested separately before they are all put together to form the complete device. In this section, we will cover the individual components chosen that will operate both the optical and software side of the project, and any necessary modifications needing to be made. The details of the testing process are discussed further in chapter 6.3. Figure 14 outlays a brief overview of the hardware design.

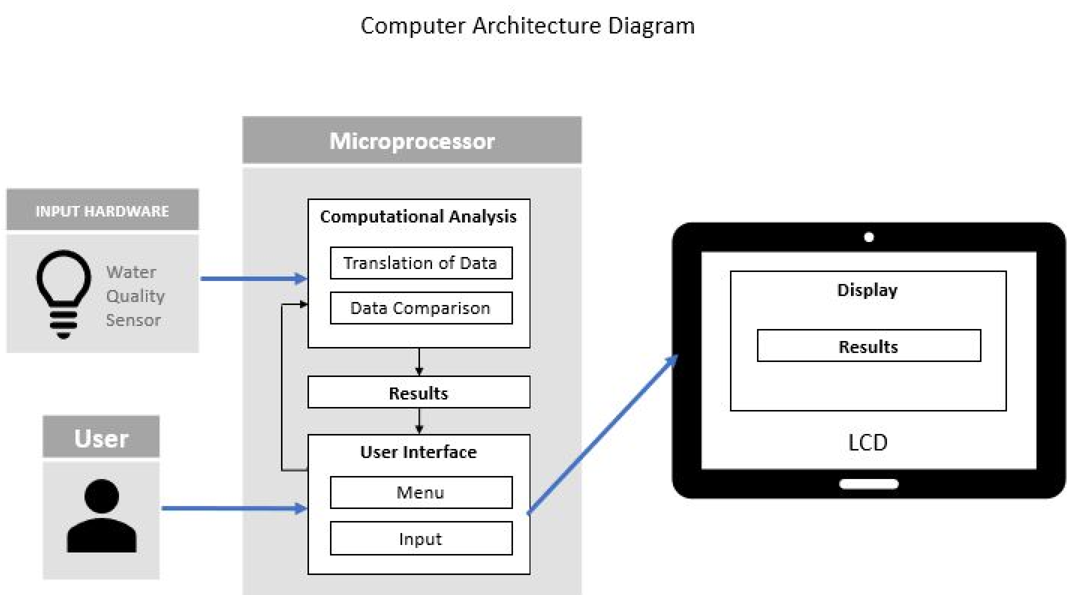

Figure 14: Hardware diagram

5.1.1. Power Supply

After reviewing all options, we have 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 5 volt USB power supply

5.1.1.1 Making the Raspberry Pi Rechargeable

A power booster is required to power the 5 volt microcontroller using a 3.7 volt rechargeable battery. We'll be using the Adafruit Power Boost 1000c. The Raspberry Pi board will plug into the USB port and the power boost will charge via the micro USB port. The Li-Po battery will attach to the JST connector. An SPDT switch will be attached to the power booster to prolong the life of the charge. It will be a through-hole solder to the VS, EN, and GND holes.

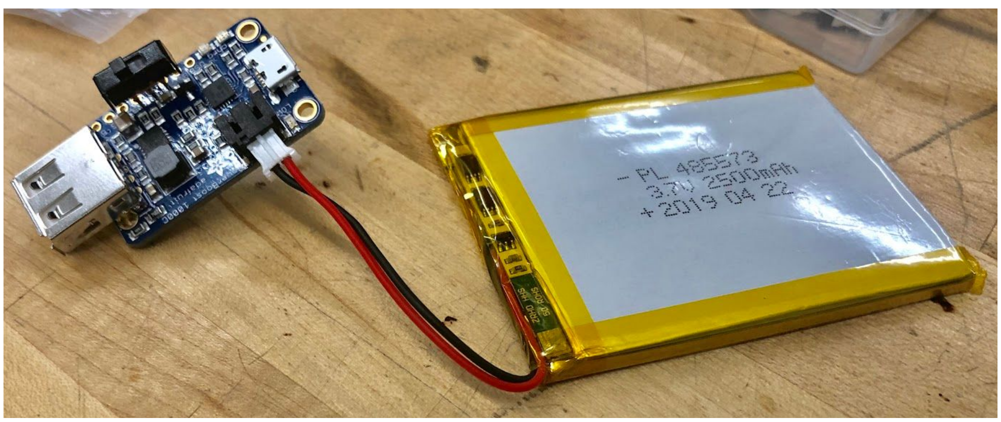

Figure 15: Charging board assembled and connected with the battery.

Figure 15 shows the battery connected to the JST connector the way it was shipped to us. After the first charging of the battery, we tried to see if the battery alone would power the Raspberry Pi without the wall adapter being plugged in. After we turned on the switch, nothing happened. Upon further testing and inspection, we found the battery was assembled with the incorrect polarity. We then used the breadboard to switch the polarity before connecting it to the Power Boost board and it powered the Raspberry Pi without a problem.

We designed our own charging board but due to shipping delays and closures in the global pandemic, there was no way to get the PCB and populate it in time. Below is the schematic for what would have been our charging circuit.

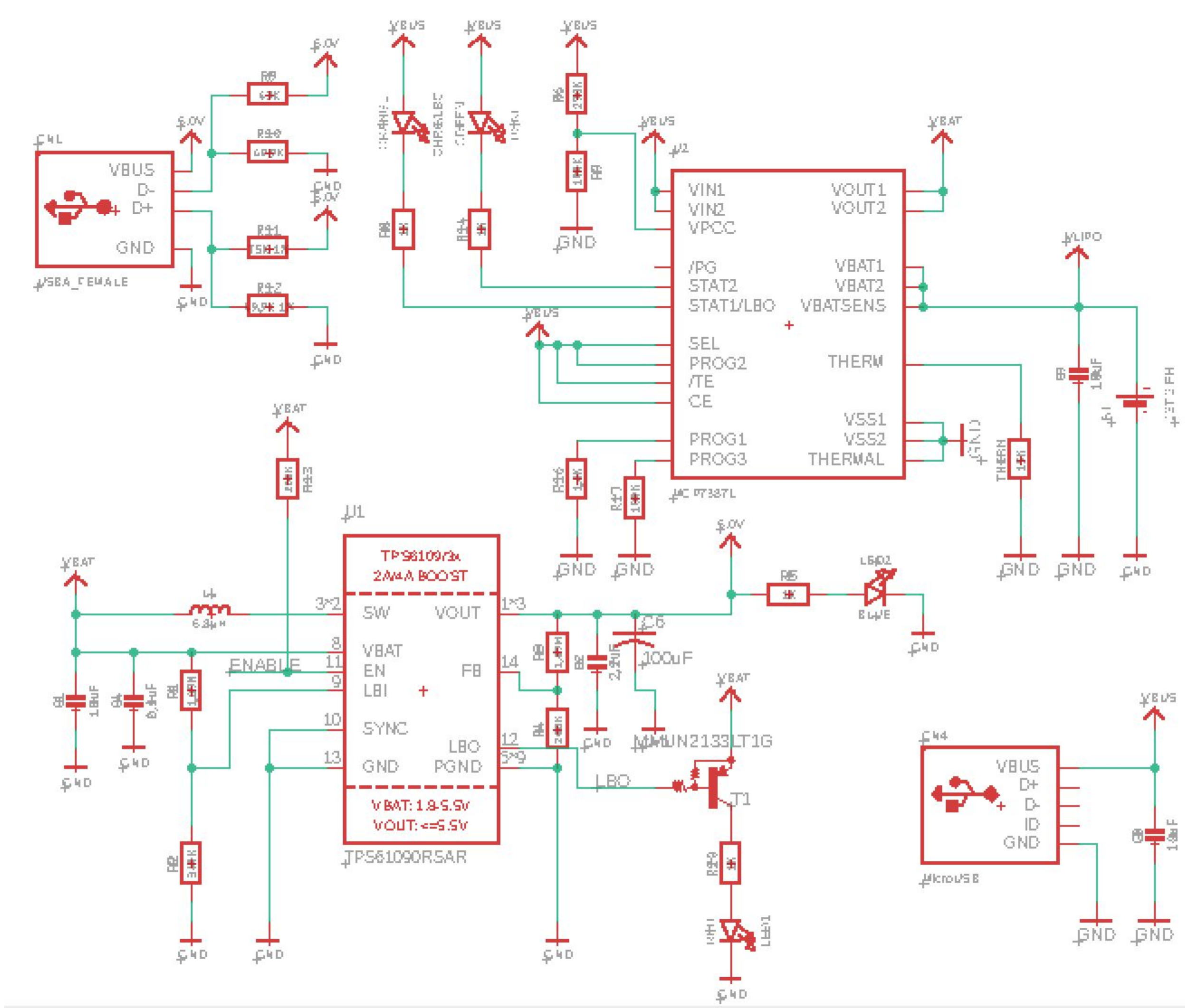

Figure: Complete charging schematic

5.1.2. Raspberry Pi

An appropriate microcontroller to handle the analysis of a given sample is crucial to the success of the project. Because the Raspberry Pi is essentially a stand-alone computer, it is the ideal choice for our project, eliminating the need of a mobile or web-based application. The Raspberry Pi 3 will be used to handle everything related to processing the information received from the sensor.

Though it is not necessary for the basic goals of the project, we plan to make use of some of the Raspberry Pi's built-in features such as its Wi-Fi and Bluetooth compatibility if we find time to optimize the programs for the project. As the electrical engineer wishes to dabble more in programming, we have purchased two of these development kits for both the computer engineer and electrical engineer to work together on implementing these additional features.

5.1.3. Camera

The amount of pixels and the size of each pixel will be a consideration for the overall spectral resolution of the design. The more pixels along the array will allow us to include a larger spectral range from our signal, and the smaller the size of each pixel will allow us to achieve a higher resolution.

For our consideration, we do not expect to achieve a spectral resolution that is comparable to most commercial fluorometers (typically less than 1 nanometer), so about any camera will suffice. However, it's important to test that the camera module is compatible with the Raspberry Pi to ensure that it will be able to properly receive the image for testing. While the chosen component is the Raspberry Pi Camera Module V2, that is built specifically for the chosen microcontroller, it's important that the following test plan is followed should we choose to upgrade or downgrade the camera for the project.

5.2. Spectrometer Design

There are two different types of commonly used fluorometers, filter fluorometers and spectrofluorometers. These select the excitation wavelength by isolating it from a broadband source such as a lamp. As we are only using one sample, chlorophyll-a, it only needs to be excited at one wavelength. For this case we choose a light source with a single wavelength and we do not need the option to select a wavelength.

The optics to go into constructing the fluorometer include a light source, two lenses, and a monochromator. Generally, monochromators consist of a collimating mirror, a diffraction grating, and a focusing mirror, however this design can be expensive and take up a lot of space. Instead, we will set the collecting lens exactly one focal length away from the sample to collimate the fluorescent light and direct that light straight onto the diffraction grating. This eliminates the need for the collimating mirror.

The first lens will be a biconvex lens that focuses light from the light source onto the sample. The sample will be placed in a 10 millimeter path length optical glass cuvette which will be inserted into a 3D printed cuvette holder. The second lens will be an achromatic lens that will be placed at a 90 degree angle to the side of the sample being excited to avoid detection of light from the excitation source. This lens direct collimated light onto the diffraction grating as explained above. This diffraction grating will then spatially separate different wavelengths and send them onto a focusing mirror. The light will then focus at different positions of the sensor depending on the wavelength, enabling us to detect different wavelengths and produce a spectrum. The purpose of each component will be elaborated on in detail in the following sections. The setup can be better visualized as in figure 16 below.

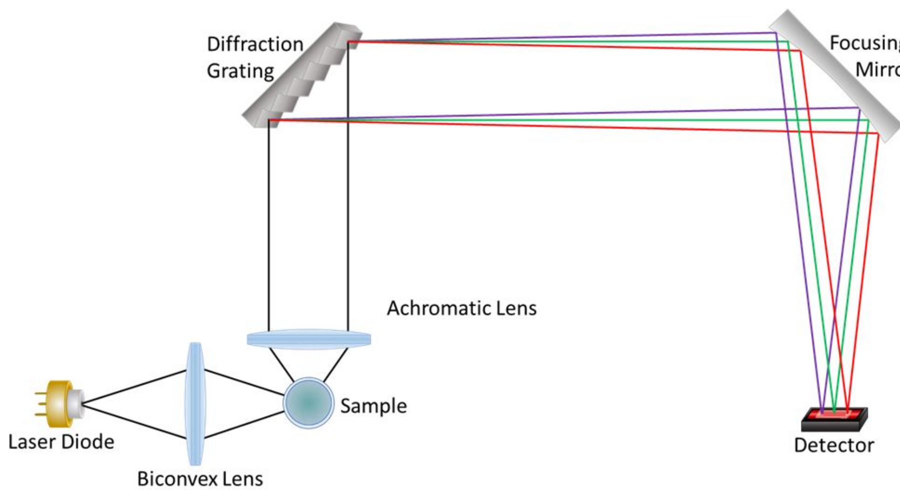

Figure 16: Optical design of the proposed spectrometer.

5.2.1. Optical Mounting

Optical mounts will be designed using CAD software to get a 3D design. We decided to create the lens mounts in the machine shop in order to save on expenses. As the material that we have decided on will be the acrylic plastic, we will consider using CAD designs that are based off of those that are supplied by ThorLabs and slightly modifying them so that can be used for our purposes. The retaining rings will be included with the lens holder, the size is relatively standard. We will also create a CAD file for the laser diode mount. All mounts need to be able to be screwed onto an optical post for mounting.

5.2.3. Laser Diode

We'll be placing the laser diode in front of a lens which will focus it so the beam size is smaller at the sample so it can excite the sample. The Sony SLD3232VF Laser Diode has a common cathode arrangement. It is appropriate for our case to limit the width of the excitation beam to be the smallest it can be. We expect our beam size to be on the order of hundreds of microns. This should be an adequate size in the area of the beam considering how many molecules we expect to excite, which will be comparable to Avogadro's number.

5.2.3.1 Laser Diode Driver Circuit

A driver circuit will be required to stabilize the current and voltage across the laser diode. The laser diode will, and must include a current driver and temperature controller, in order to keep the temperature at working conditions and so we can control how powerful we can make the signal. This will consist of resistors, capacitors, a voltage regulator, and a heatsink. The voltage regulator will need to output 5.4 volts or slightly higher to power the diode The temperature regulator will be a function of resistance and current for the laser diode. The method of temperature regulation for the diode is by placing a resistor that also has a current running through it to offset the voltage of the whole system. Calculations must consider the condition of 25 degrees celsius.

The driver circuit for this diode will need to be built and tested on the breadboard. It is so far assumed we will power the laser diode from the Raspberry Pi. V_{in} will be connected to the 5 volts power supply via pin 2 or 4 on the Raspberry Pi board. V_{out} will be connected to the laser diode.

For the initial test we used the LM317 adjustable voltage regulator. Voltage regulators supply a constant and stable voltage regardless of the resistance of the load that is attached. The value of our resistors were found using the following equation

$$R2 = \frac{(V_{out} - V_{in})\,R1}{V_{in}} \qquad \textit{Equation}\ (14)$$

The power supply that was connected to this test circuit was supplying 5.2 volts which is what the pi board would have been supplying for the actual product. Once we received the laser diode, the plan was to add a resistor between the positive pin of the capacitor and anode of the laser diode to taper the current.

After performing the test on this initial circuit, we first discovered that the GPIO pins from the Raspberry Pi can only supply a maximum of 50 milliamps and we need at least 55 milliamps to turn on the laser diode. We also realized that the LM317 we are using cannot reach the required voltage 5.4 volts which is the operating voltage of the laser diode. We could add a simple booster circuit before this driver circuit which would raise the voltage then allow the LM317 bring it back down and keep it regulated or we could consolidate everything by switching out the LM317 completely for a DC to DC step up voltage regulator.

We are going to test connecting a step up voltage regulator directly to the battery in parallel with the Power Boost board and then use resistors at the output of the step up voltage regulator to reduce the voltage and/or current within the operating parameters of the laser diode. The laser diode needs a stable input in order to remain functioning without any damage.

A step up voltage regulator (or boost converter) is a circuit that increases the voltage while decreasing the current in order to maintain the conservation of energy. In an ideal step up transformer, the output power (P_{out}) must equal the input power (P_{in}). According to Ohm's Law, Power = Voltage * Current
In order to comply with the conservation of energy,

$$P_{in} = P_{out}$$

$$\therefore\ V_{in} I_{in} = V_{out}\, I_{out} \qquad \textit{Equation}\ (15)$$

After testing the XL6009 Step-up Power Module, we found that it pulls about 120 milliamps on average with the 3.7 volt, 2500 milliamp-hours lithium polymer battery input. Using equation 15,

$$P_{out} = P_{in} = V_{in} * I_{in} = 3.7V * 120\ mA = 0.444\ W$$

To simplify the output of the step up voltage regulator, we can set it to either the desired voltage or the desired current.
If we set the output to the desired 5.4 volts, the output current (I_{out}) will be 444mW / 5.4V = 822 milliamps. We will need to place a resistor in parallel with the laser diode to pull 82.2-55 = 27.2mA in order to decrease the current going through the laser diode to 55 milliamps. The value of this resistor will be 5.4V / 27.2mA = 198.53 ohms which can be rounded up to the available 200 ohms.

Conversely, if we want the output current to be the desired 55 milliamps, we will need to set the output voltage to 8.073 volts and place a resistor in series with the laser diode. In order to decrease the voltage across the laser diode to 5.4 volts, the voltage drop across the resistor must be 8.073V-5.4V = 2.673V. The resistor in series must be 2.67V / 55mA = 48.6 ohms which can be rounded up to 50 ohms.

It is also worth noting that when using a step up voltage regulator, it is ideal to have the output voltage at least two volts higher than the input voltage. This is to avoid unpredictable behavior of the step up voltage regulator. Our input voltage will be no less than 3.7 volts. Therefore, our output voltage must be at least 5.7 volts.

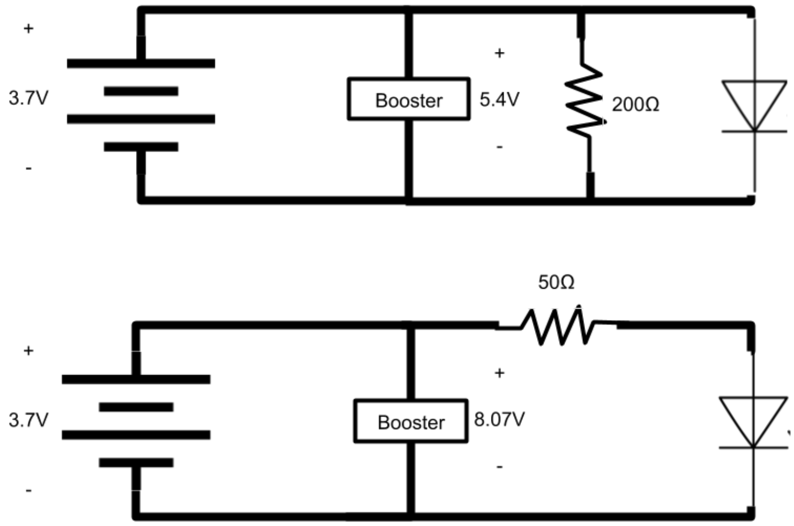

Figure 17: Options for driver circuit configurations.

We opted to use the second circuit with the 50 ohm resistor in series with the laser diode. To allow at least 2 volts between the input and output voltages. Due to the limited availability of resistors, we had to use two 100 ohm resistors in parallel to create a 50 ohm resistor. This didn't noticeably affect our testing and we were able to successfully turn on the laser diode. This is shown in figure 18.

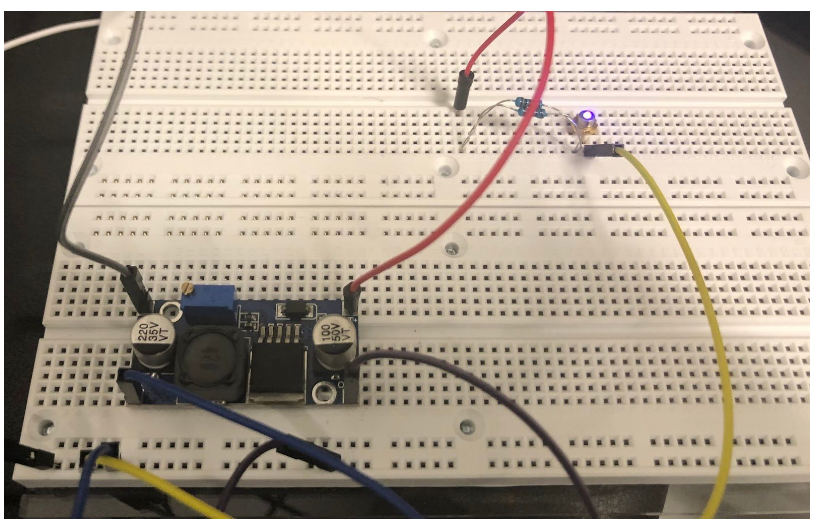

Figure 18: Successful test of driver circuit.

A possible modification we are considering is adding a fuse between the resistor and the LD anode of the laser diode. This will prevent the current that's going through from exceeding the operating current and damaging, or even possibly destroying, the laser diode. The SLD3232VF datasheet states the operating current is between 55 milliamps and 65 milliamps, but after testing (shown in section 6.4.1, Figure 27) we found the lower bound to be closer to 40mA. This suggests the upper bound will also be lower. For this reason, we believe a 60mA fuse should suffice in both not limiting the laser diode and also protecting it from overloading. The fuse will be included in series with the laser diode. We suspect the difference in measured capabilities and calculated capabilities is due to the different standards that change internationally. The laser diode we ordered is from China and their standards are not the same as the United States.

Due to the global pandemic, the components we needed would not arrive on time. Therefore we needed to improvise. We revised the driver circuit to be independent of the charging circuit and powered by a regular 9V battery you would commonly find in the household. We took some parts from old items lying around the house to possibly use in the new design. Our final design is powered by a 9V battery and toggled using a switch as shown below.

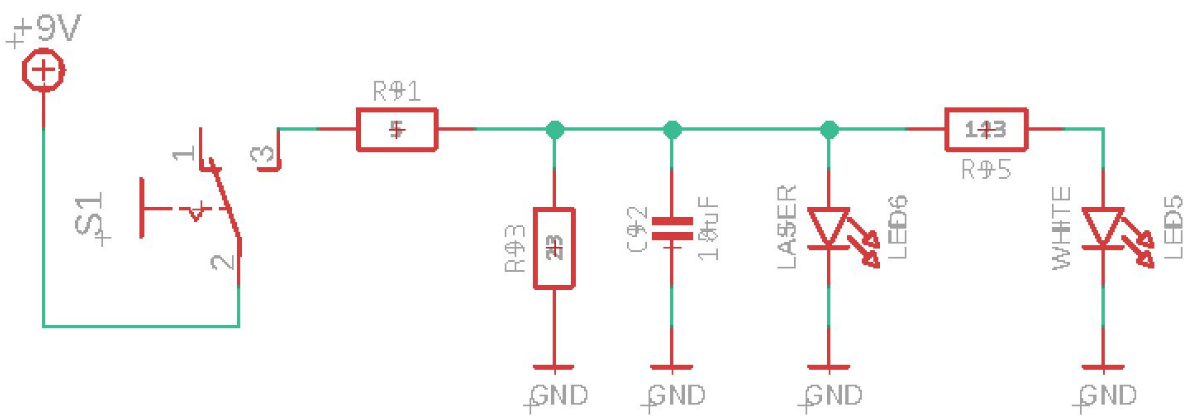

Figure: Modified driver circuit

5.2.2. Collimation Setup

The setup of our collimation is fairly straight forward. Rather than an actual collimation setup where the first lens is place a focal length away from the source and, we plan to focus the excitation signal to a point onto our sample, which is in the cuvette. If we were to consider the glass of the cuvette also as an optic for our design, we will consider that the cuvette has four sides so that all faces are flat. This will ensure that the excitation source still collimates to a point onto our sample, without refracting into different angles

if we were to use a cylinder cuvette. This will be similar to the regular 4f system in terms of the quantity of lenses and with respect to where the lenses are placed.

Once the sample is excited with our 430 nanometers light source, it will begin to fluoresce a signal at 630 nanometers. Because we have focused the excitation signal into a point at our cuvette, it can then be assumed that the fluorescence signal should emit as a point source. Or, illuminating 4*Pi steradians, essentially in all directions with the same intensity. The collecting lens will be high in numerical aperture and thus short focal length to gather as much of the signal it can. As it passes through the diffraction grating, it will then separate the wavelength that will be visible to us.

5.2.3. Monochromator

The signal as it passes through the grating will be used in place of the monochromator portion of the spectrometer. The transmission grating we chose will spatially separate the signal to an amount that we decide. As per our specifications, we expect our grating to have less than 5 nanometer resolution. For our consideration, we expect our grating to spatially separate the peak wavelength of 600 nanometers by the longest distance of our camera, which includes 3280 pixels and each pixel has a width of 1.12 micrometers. So we would like our signal to be close to 3.67 millimeters wide. We would like our signal to have similar width to the long portion of our camera to have the highest resolution.

As we are simply collimating the light collected by the lens, we do not need a collimating mirror. Instead the light will directly reach the transmission grating. Depending on the grating's angle dependence, we aim for it to be a planar shape to reduce the amount of optics that will be required in the system. If it were to be smaller, we could also focus the signal down onto the detector as well.

The light exiting the transmission grating will still be collimated, however the wavelengths will transmit at different angles. This allows us to choose a lens to focus onto the detector exactly at the focal length.

This section of the optical design must be completely enclosed and separated from the excitation source setup. This will isolate the red light as much as we can from the excitation signal. It is also for this reason we plan on using an AR coated lens that blocks blue light.

5.3. Software Design

The design of the software, shown in figure 19, will start out with a simple implementation of the spectrometer, then gradually add more features as well as optimize its performance on memory and run time. The program will cycle through various states as shown in the diagram above, and the output of the software will return numerical data to be graphed through an outside software. To reduce the dependency on another device, we have decided the functions to analyze the sample will be developed within the Raspberry Pi, and an LCD will be used to communicate to the user. The various subsections in this section will discuss the basic implementation needed, and plans to optimize it. Additionally, a subsection on additional, optional features we would like to add to the project if time enables it for the programmer will be discussed at the end.

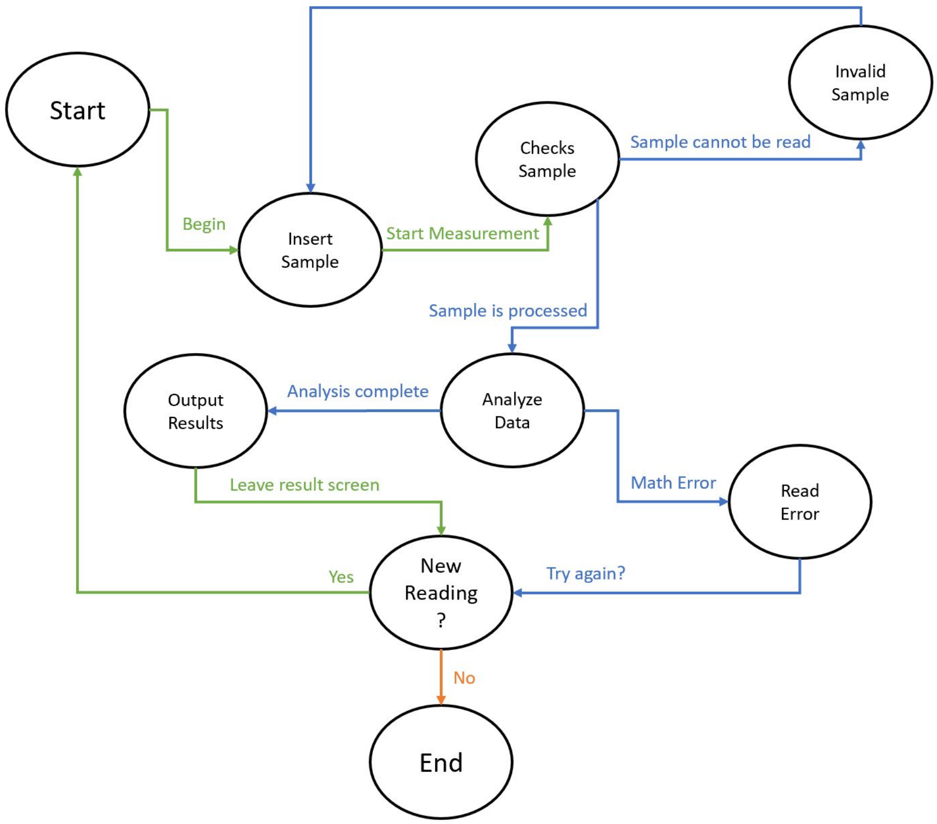

Figure 19: Software state machine.

5.3.1. Spectrometer

The most important part of the project is to implement a spectrometer to detect the water sample. To ensure that our project sees completion, we will focus on getting a spectrometer working at a basic level before we expand on the application. We are approaching this method to ensure that we have everything we need before enhancing anything as time may be wasted from working on its extra features rather than its core features. By focusing on the primary goal of our project, we can guarantee that

To get a basic, working implementation of a spectrometer, we would need the program to get an image of the sample, read each pixel, and process the information into data that we can graph onto a program. For this reason, we have chosen for the software to focus on its basic features before we work on more advanced features to be discussed in the following sections while the necessary functions needed to implement a spectrometer will be discussed in this section.

5.3.2.1. Sample Detection - DetectSample

The analysis can only begin if it detects that there is a sample within the device. Once the sample has been placed into the device, the program will first check if the sample has been properly placed and aligned to the spectrometer. If the sample is properly placed into the device, the user will be informed that it will be ready for analysis. This check is to reduce the likelihood of error to ensure that the results obtained are as accurate as possible and to reduce any possible noise generated from the environment. DetectSample will then take a command from the user as an input that tells the program the user has put in a sample into the device for testing. The input will also act as a signal for the diode to turn on so that the sensor can get a reading.

5.3.2.2. Sample Capture - CaptureImage

After the program gives the signal that the sample is ready, it will capture an image for processing. The image will be taken and cropped as the entire image isn't necessary and only a specific portion of it is going to be analyzed. The program will check if the image is valid by checking the image's pixels to see if a signal is being read. If the image is valid, it will be ready to be analyzed for data and a signal. If the program deems that the image is not valid, it will try to identify the error and alert the user the possible issue with the reading to better assist them in readjusting it for the next reading. Possible errors that can arise from the analysis that this program will try to detect are looking for obtrusions to the reading, misalignment, or a low signal.

Optimization. If possible, the device will attempt to address these errors itself. We plan to have the device be able to calibrate or diagnose itself to reduce the amount of times the user would need to adjust the sample or possibly the camera. If the image is misaligned, it will try to reorient the image to the best of its ability, and run the test again. If the image taken is of poor quality, it will attempt to take a second picture and use that instead to measure the sample. If it fails the test a second time, it will alert the user to take manual measures to fix it.

5.3.2.3. Signal Processing - ConvertImage

Once the program finds there's nothing off with the image, it will then look at each pixel of the area in the image and translate it into numerical data. GetSpectrum will take the image from Capture image as an input. Each pixel from the image will have its brightness read to be used as its intensity. The information will initially be stored in an array for testing purposes, but has room for optimization as described below.

Optimization. The basic implementation of the program will only be able to take one recording at a time. If we want the program to save multiple recordings, we would have to use a different or additional data structure. While a database can be used to store information, it would require that our device has access to Wi-Fi and will not be considered an option for optimization. Python fortunately has access to a number of data structures including the *pickle* module.

5.3.2.2. Signal Analysis - GetSpectrum

We plan to take the information from the detector to read it as an intensity profile across different wavelengths in order to provide the full spectra of the fluorescence. Once the program has received the data, it will begin the calculations and translate the data into information the user can understand. The signal analysis will take the array generated in the signal processing function, similar to the *improfile* command on matlab.

Upon completion, the result will be displaced on the LCD, and it will prompt the user if they wish to take another reading. If there is an error with the calculation, an error will be displaced and will take them to the same prompt. If the user does not want another reading, the program will end and turn off the device to save power.

5.3.2. User Interface

Because the focus is on being able to test water quality, a fancy User Interface is not necessary, but has room for optimization. The interface at the very least must tell the user enough information while being simple to navigate around. For this reason, a simple LCD that can display text and a series of buttons will suffice for the basic implementation of our project. Once the basic implementation has been achieved, a more expanded interface will be used that will be more. Discussed in this section is the design for the basic implementation, ways to improve it, and additional features to be added to further enhance the device.

5.3.2.1. Visuals

The initial display screen planned to be used for the device will be a 16x2 LCD screen. The LCD screen is small, but will be sufficient enough to display messages that describe the status of the analysis such as “Measurement in process”, “Error”, or “Measurement complete”. These basic messages will also be represented by the Indicator LEDs described in the following section. For the purposes of a basic menu, detailed messages would not be necessary as it’s assumed that the user would have a basic understanding of what the data is for.

Optimization. Once the basic implementation has been completed, the software will be able to provide more information to the user in the form of graphs, detailed messages, and even a visual of how the data is being translated. Along with upgrading the menu, the display screen also be upgraded to better display these new features. Several additional programs can be implemented to further guide the user on using our product with a larger display.

5.3.2.2. Indicator LEDs

It’s good practice to have indicators for the different stages of the process. In addition to the display for the user interface, we will include LEDs to indicate the following stages from the state diagram:

- Device on
- Laser diode on
- Low battery
- Charging
- Charged

These LED signals are similar to the display messages discussed in the earlier section, but simpler and less prone to issues. These LEDs can also be color coded to even further assist the user in understanding. In the event that the display is damaged and no longer works, we want the user to still be able to use the device and navigate through the various stages until they can get the screen fixed or replaced.

5.3.2.3. Buttons

Three buttons will be used: one to select, one to submit a response, and once to cancel the process. The select and submit will help the user navigate through various options. In the event the program needs to be stopped due to an endless loop or process taking too long, the cancel button will force the program out of that state without having to shut the device off. As buttons will be used for our project, we will have no plans to upgrade specifically to a display screen that is touch compatible and can go with or without one.

5.3.3. Data

The device must be able to hold the history of the last 5 recordings conducted by the device. Upon the spectrometer completing the reading, it will automatically save the information for record keeping. These past recordings will be stored locally in the device and will give users the option to export their files, or delete an entry. The exported files can be downloaded into a USB and viewed on a desktop. Initially, we will have the exported files be numbers to be graphed in an outside application such as Excel. If time permits, we hope to have a graphing application locally on our device to be able to print the spectrum without having to export the data.
Optimization. Once a working spectrometer is in place, we want our program to be able to produce various data formats for the user to look at and keep some of these records around for a certain amount of time. These records may be used to compare the water quality at various points in time to check if there’s an underlying issue with a water source.

5.3.4. Optional: Additional Features

As our goal is to make this device as user friendly as possible, we would like to add programs and features that provide more information and resources for them to utilize. These features discussed in this section, while optional, have plans to be incorporated into the final prototype if time permits. With code documentation to help us keep track of the program, we will be able to add these features without causing too much drastic changes that would mess up the project.

Graphing Application. We eventually want the user to be able to see the spectrum without having the user to manually disconnect the device and upload the information on another platform unless they want to preserve their data. Fortunately, the Raspberry Pi has access to several programs that can be used to graph data on the device. One particular program we were considering is using gnuplot, which is perfect if we were to use a more detailed display of a low resolution. The graph produced is simple, yet functional enough for us to understand what is being produced.

6. Prototype Construction and Testing

Before beginning construction of the device, we need to test each individual component to confirm they work to specs. After that, we need to construct the project before we can combine everything into a singular device. Discussed in this chapter is the processes we will go through to test that each individual part of our project is in working order, and how some parts will be tested together.

6.1. Project Housing

Housing is necessary to physically combine all elements of the device into a single product that can be portable. Waterproofing is not only too expensive for our budget but out of scope for the goals of the project. However, because our device will be used around water it needs to be at least water resistant. We consider to use a material that is not easily damaged by water, such as wood or other organic and hard materials.

The enclosure for the whole device, cuvette, and placements of the printed circuit boards along with the detector and the raspberry pi must be considered with extreme caution. Our aim for the device was to create it as compact as possible, such that it can be considered portable. To do this, both our cuvette holder and lens holders must be the smallest they can be to allow for more room in the device. The optical path length before and after the fluorescence of the sample will be small considered to the size of our raspberry pi, so we will design our optical layout in a way such that the signal will travel around the computational board.

6.1.1. Project Enclosure

A 3D printer may be used to design the casing through AutoCAD. Other options being considered is a metal enclosure constructed in CREOL's machine shop. The enclosure will house the main components necessary to make the device work such as the optics, PCB and detector. We need to make sure that the excitation and detection of the optics are separated by a wall. In case we are not able to collect all of the energy from the diode such that it diffracts over too large of an area and is occupying the same space as the fluorescence signal, it will be necessary to separate the signals from each other. The excitation signal may also somehow make its way to the detector unless it is blocked from actually hitting the detector. The material for the wall should also be considered to be non-reflective so there is no contamination of signals. This will help in mostly a small portion, due to the fact that we have placed the signals orthogonally from each other to avoid interference.

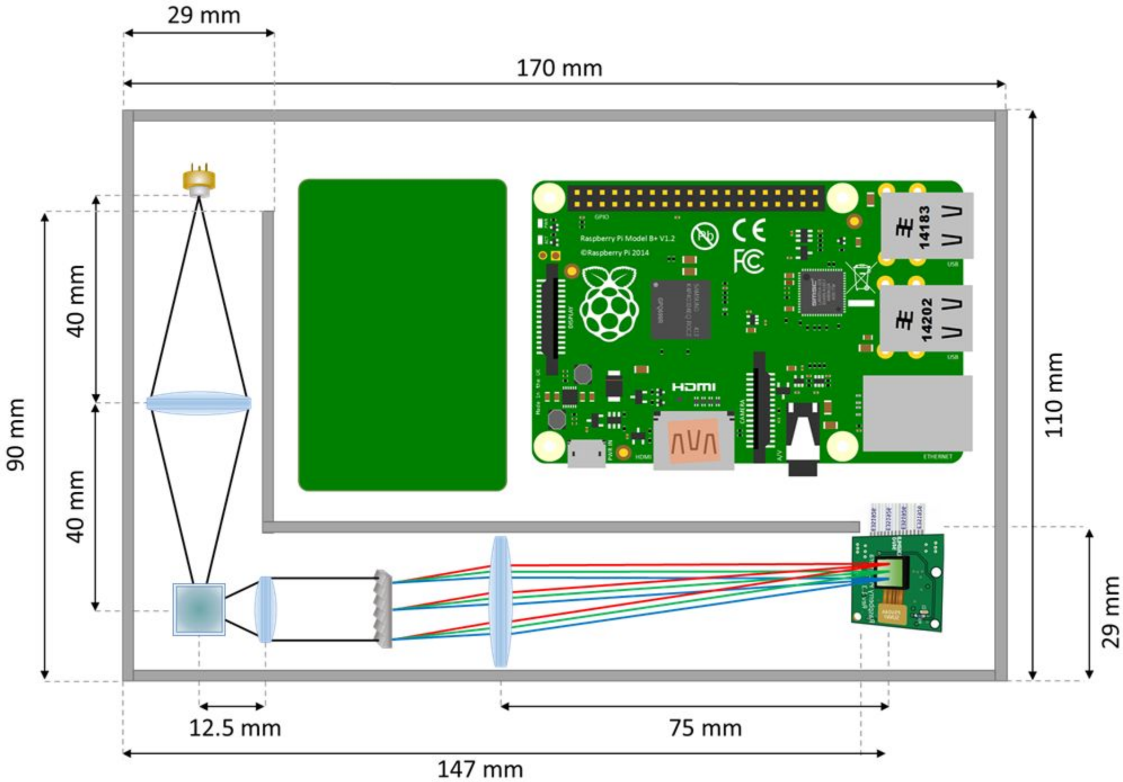

Figure 20: Proposed enclosed design.

Our initial estimates on the casing size are obtained by measuring the optics distances via ray tracing as well as taking into consideration the size of the Raspberry Pi and PCB. The optics are actually very condensed, so the spectrometer may in fact be more compact than our specified. We do plan to make the dimensions slightly bigger than necessary though, in case we need to have extra space later on. But the device will still be within our specified dimensions as it still needs to be portable.

The enclosure will be made in the machine shop using acrylic. We will create each side and connect them via acrylic adhesive. Sections for the LED indicators and USB outputs will be added. We will use a side panel that can easily be removed to edit the cuts on this panel to customize the design later if necessary such as to add more slots for USB ports. The enclosure will also have a section for the switches to turn on the device and measurements to be accessed from the outside.

6.1.2. Cuvette Holder

AutoCAD will also be used to create the housing for the cuvette, as we will most likely not be able to get our holder to be made specific for our spectrometer setup. The design for the cuvette can be seen in figure 21.

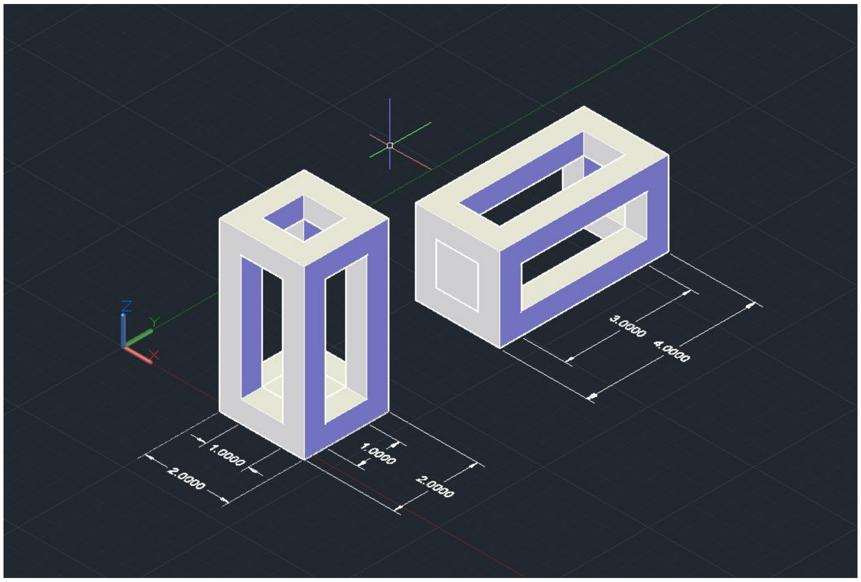

Figure 21: CAD design of cuvette holder.

The material for the 3D printing can either be made of resin, PLA, nylon, or other carbon based materials. We would like a material for both the inner and outer housing to be structurally stable such that there will be minimum movement in our housing due to vibrations from other sources. It will be crucial that the signal is placed as perfect as can be onto our detector.

6.2. Printed Circuit Board (PCB)

The PCB unifies all parts of the project. It will be used to control the laser diode driver and the microcontroller. The PCB will be housed within the device enclosure with the laser diode, prior to the optics.
Will be elaborated...

6.2.1. PCB Design

Before the PCB board can be created, the individual components need to be tested, to ensure each part is working correctly on the breadboard. It's important to have a working prototype as the PCB cannot be changed once manufactured. The board will be designed using the free student version of Eagle PCB design software which is a program in the Autodesk family. EAGLE stands for Easily Applicable Graphical Layout Editor. This program allows us to design schematics, create a PCB layout with all wire paths, and create a 3D model of the PCB to prepare it for fabrication. The main part of the PCB will contain the driver circuit for the laser diode and the charging board for the Raspberry Pi. Certain things cannot be mounted on the PCB and need to be attached by wire because it must be positioned a certain way. For example, the micro usb port and the SPDT switch must be accessible from outside of the casing. Therefore, they must be separate from the PCB.

6.2.2. PCB Fabrication

After testing using breadboard and designing using the software, we'll send it to a fabrication company. There are many companies to choose from and this is a decision we will make later on when it's clear how large our circuit will be and how many layers we will need. The design will be printed onto a resist and bonded to a copper plate to prepare for the etching process. The resist protects the copper in the areas there won't paths. It also helps with the soldering process because the solder will not stick to the resist. This prevents shorts from happening. Shown in figure 22 is the cross section of before etching. The two different methods used for PCB etching are dry etching and wet etching.

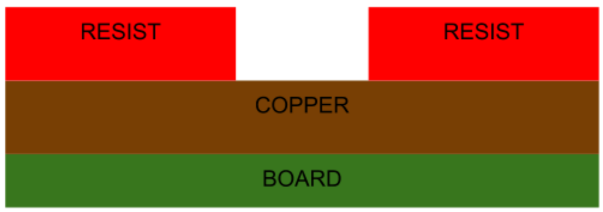

Figure 22: Cross section of resist bonded to copper plate before etching.

6.2.2.1 Wet Etching

Wet etching is the older, cheaper, and simpler of the two methods. This process uses either alkaline solutions or strong acids to cause a chemical reaction to essentially eat away at the copper plate, leaving only the paths you need. The resist protects the desired path but there's still a slight undercut under the resist using this method as shown in figure 23 since it is being soaked in the liquid which leaves somewhat messy paths. This method can also be risky. The soaking process is a very volatile and delicate process. If soaked for too long, the undercut under the mask can get way too big. This can cause shorts all over the circuit and completely destroy the board. It is possible to do the wet etching method at home by ourselves and this could save a lot of money, Although, this could cost us much more in time and money in materials if it takes us multiple tries to get it correct. After the paths are etched, the board needs to be cleaned. The board is again soaked in a different solution to remove any remaining excess copper. Using this method, all chemicals that are harmful to the environment are controlled and disposed of properly.

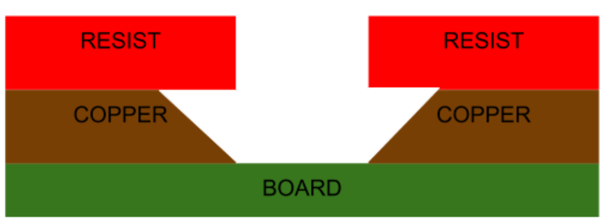

Figure 23: Cross section of wet etching.

6.2.2.2 Dry Etching

Dry etching, also called plasma etching, was introduced in the late 80s and is the newer, more popular method of the two. It is commonly used for the steady manufacturing of PCBs. Plasma, which is positively ionized gas particles, is used instead of acids in order to etch the paths. This is a much more expensive method because it requires a separate machine dedicated to performing the task. The machine must take in gas, positively ionize the gas particles, then use a radio frequency to excite those particles creating plasma. The machine must then shoot the plasma at the board at very high speeds in pulses which then collides with the board causing the chemical reaction that etches the paths. This method allows for much cleaner, sharper paths than wet etching as shown in figure 24 and the board does not require any further cleaning. Unfortunately, the chemical reaction caused in this method creates by-products that are not safe for the environment and then shoots them into the air due to the impact to the board.

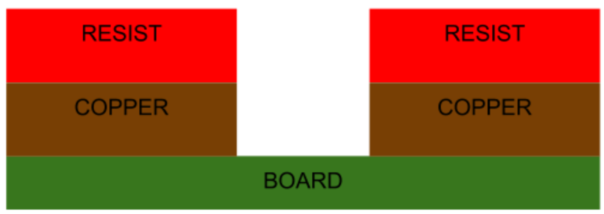

Figure 24: Cross section of dry etching.

After reviewing both processes, we found our standards for environmental impact and our limited budget align more with the wet etching process. Therefore, we will attempt to seek a manufacturer that uses that method.

6.2.3 PCB Population

The PCB will be populated by hand using a soldering iron and solder wire. Both the surface mounted and through-hole components will be soldered by hand. Any necessary reworking will be done using a heat gun at no more than 340 degrees celsius in order to not damage the components.

6.3. Hardware Testing

Considering the optical components in our design as well with the electrical components, it will be necessary to test each component individually and further integrate them for future testing. The main devices in our design which we will need to consider testing is our power supply, laser diode, camera or other detection devices which are still under consideration, microcontroller, and the optical components in the design. Particularly, the power supply and the laser diode will most likely go hand-in-hand together as it is crucial the energy supplied to the diode must be matched to the standards on the specifications sheet of the diode. The energy derived from the circuit must not exceed the energy that the diode is meant to handle.

If we wish to consider the longevity of the device, we will consider that all devices will be in tolerance of their energy demands, especially. The camera and the microcontroller will together be considered to go hand-in-hand with each other for our device. A feature of the microcontroller that we are also willing to examine would be the clock speed of its central processing unit. The feature of the device is not meant to be extraordinarily fast, but would still like to consider how fast it may do its image processing and other computations. The sensitivity of our camera will be the main concern for our detector, in this design. Although our camera will only need to recognize a relatively narrow spectra (~100 nanometers) and includes enough pixels and is a decent physical length, the responsivity of the device will be of importance. We speculate that our device being silicon based will be of higher responsivity, should be enough to capture the signal from the fluorescence.

6.3.1. Power Supply Testing

The power supply will first be built on a breadboard. The voltage will be checked across each component to ensure it's receiving what it needs to operate properly. We initially need to ensure the Power Boost 1000C is actually charging the rechargeable lithium polymer battery. We will leave it plugged in to the wall adapter to charge and make sure that neither the battery nor the charging board are overheating. We will then use a multimeter to first check the battery is 3.7 volts and secondly check that the Power Boost is outputting about 5.2 volts.

The green and orange LEDs respectively indicate the wall adapter is supplying power and the Power Boost board is successfully charging the battery. After unplugging the wall adapter and turning the switch on, a blue LED should turn on indicating the board is supplying power to the Pi board. Once we confirm the battery can power the Pi board, we need to ensure it can also power the the driver circuit for the laser diode if it's connected in parallel.

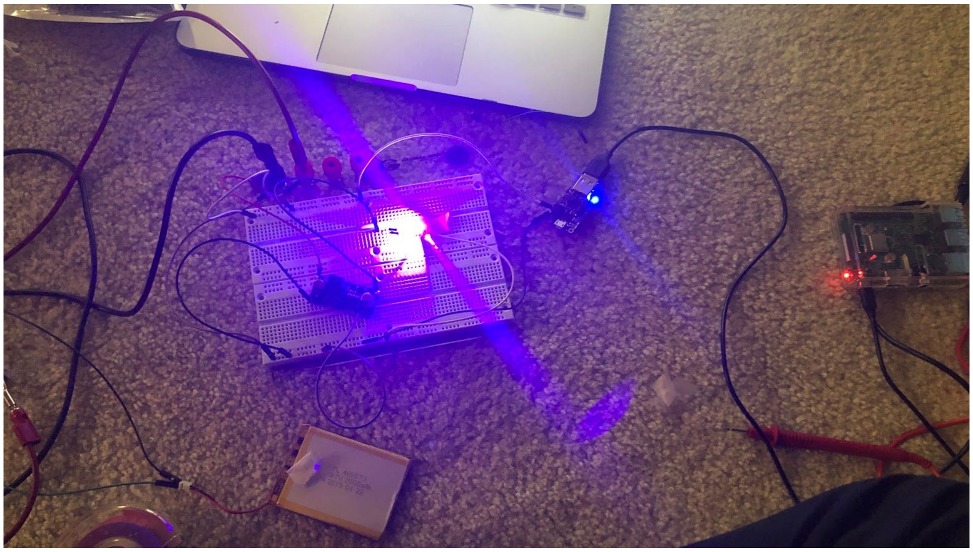
Figure 25: Battery powering laser diode driver circuit and Raspberry Pi.

After setting up the circuit we are able to power both the laser diode driver and raspberry pi using our battery. As such, our initial test is successful and we are sure the battery is able to power the complete circuit. This is proven as shown in figure 25.

6.3.1.1 Power Supply Modifications

There are modifications we will need to make to the design for functionality. The first modification we will need to make is to relocate the SPDT switch so it controls the entire circuit and not just the Power Boost board. Another modification is the inclusion of the indicator LEDs that were discussed in section 5.3.2.3.

6.3.2. Development Board Testing

The first step to testing the microcontroller is to run a test program to see if the microcontroller is working properly by checking its LEDs and Multimeters. If the device fails this check, the microcontroller might be damaged, and a new one needs to be used. For that reason, two Raspberry Pi's have been purchased should one become damaged. Setting up the Raspberry Pi is straightforward, and simply requires that Rasbian operating system is set up on the microSD card. If the Raspberry Pi isn't booting up, the LEDs on the board can indicate what the precise issue is.

During our testing for the Raspberry Pi, we found that it sensed an SD card, but could not boot up the operating system in it. After investigating, we found the issue to be that the SD card was too big and needed to be reformatted. Once the SD card was reformatted, we were able to operate it and conduct a test with the Camera as shown in the section below.

6.3.3. Sensor Testing

Our ultimate goal is to compare the intensity of the signal for different concentrations, expecting them to be higher or lower for different concentrations. While any camera/sensor will suffice for the purposes of testing, the type of camera we will use for our final design may be different than what is used for the initial design. Essentially, we want to ensure that our camera/sensor will be able to capture an image that will be processed into data. Our initial testing will begin with the Raspberry Pi Camera V2 Camera Module for the device.

6.3.3.1 Raspberry Pi V2 Camera Module

Setting up the Raspberry Pi Camera Module is a simple process as it is an official product of the Raspberry Pi Foundation. Once it is connected to the slot the module is connected to the cable slots between the Ethernet and HDMI ports, with the silver connectors facing the HDMI port, the Pi simply has to be booted up, the camera module has to be enabled either through using *sudo raspi-config* on the command line or if a monitor, keyboard, and mouse is set up, it can be configured in the desktop options by opening up Raspberry Pi Configuration under Preferences and enabling it under the Interface tab. Once enabled, the camera can take a picture by running *rapsistill -o [path]/[imagename.file]* on the command line, and will do a preview for about 5 seconds before it captures the image. The file will be created with *[imagename]* as the file, and if you run the command again after capturing the image, it will overwrite the previous value. Shown in figure 26 on the following page is the test's output we did for the camera module.

The image below was taken with the Raspberry Pi camera as itself, further testing will be required for it to see the image without the optics attached to the camera. The lens will have to be removed from the camera such that there is no focus to the camera and the intensity profile will not be skewed. To test this without our fluorescence signal, we will test this further using our own excitation source, the 405 nanometer. We will create an optical setup such that the laser will be placed in front of the visible transmission grating so the signal from the grating will be placed directly onto the detector. The detector has a longest side that is 3.2 millimeters, the signal coming from the grating will be focused to that size onto the detector. We should expect to see the same spectra that we have recovered from the Stellar spectrometer for our excitation source. We can also compare the responsivity of the device and find the quantum efficiency of the device, which we expect to be at least 70% and should be doable as a detector.

The lens from the camera can be removed using the included screw that has arrived with the camera. If not doable with the screw because the holder for the lens does occupy some space in front of the detector, it may be necessary to force the holder off from the board or to polish it away.

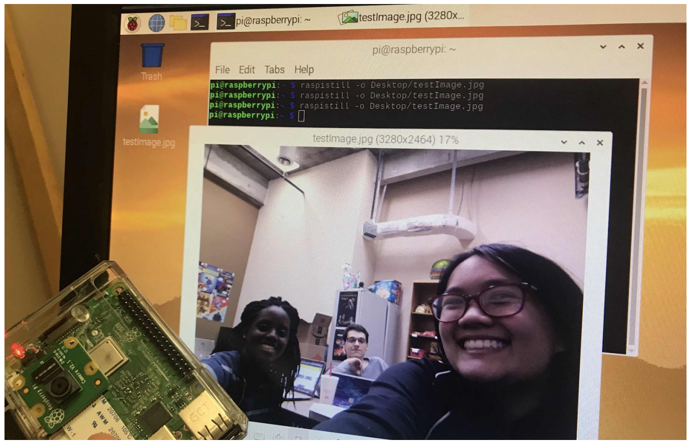

Figure 26: Picture captured with the Camera Module V2.

6.4. Optical Testing

There are many optical components that make up the spectrometer and each has their own specifications that need to be met for optimal performance. The device can potentially be used in many different areas that may have different environmental conditions that can affect the functionality. It is thus necessary to test each part under different conditions to ensure that they will function correctly when used in the product such as temperature or power. The lenses need to be placed in a fixed position as well as the grating to ensure that our results stay the same for each trial

6.4.1. Laser Diode Testing

The laser diode will be set up with its driver circuit (discussed prior in section 5.2.3.1) on a breadboard for testing. Two of the most common tests to see if the working ability of a laser diode is up to specs is to find the threshold current and threshold voltage. To do this we measure the power as a function of current and the current as a function of voltage. Before threshold voltage a laser diode only produces spontaneous emission. After threshold, the laser diode starts ‘lasing’ and emits stimulated emission, which will cause a sudden increase in the power emitted by the laser diode. As such laser diodes can operate at different currents to give a linear representation between itself and the intensity that it emits after threshold.

Because we will require to change the current as a function of voltage, we will require a DC source to change the amount of energy we will put into the system. The initial graph that we will need to consider is a voltage vs current (IV curve).

The IV curve will be obtained by changing the input voltage across the laser diode. A multimeter is placed in parallel across the laser diode to measure the voltage difference across the laser diode. The corresponding currents are measured simultaneously by placing a second multimeter in series with the laser diode. We take several measurements, enough to see the curve. One issue we had with this testing however was that due to our step-up voltage, the voltage difference across the laser diode would not be able to go below 4 volts. This however was okay because we were still able to see the vague trend of the IV curve we expected as in figure 27.

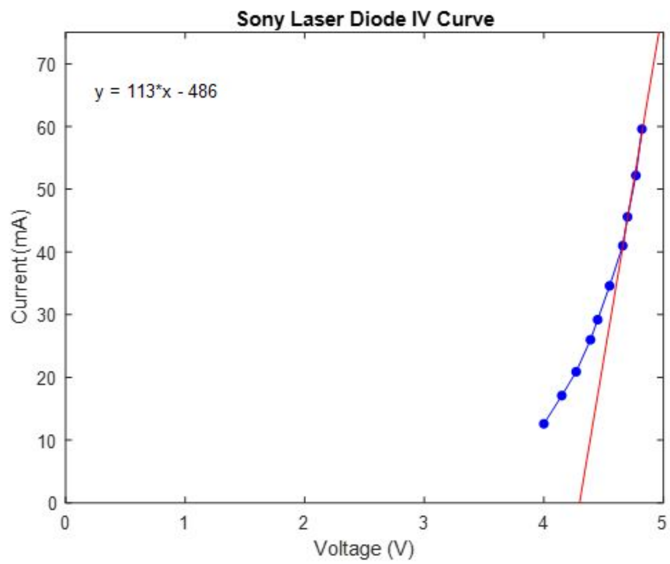

Figure 27: IV-Curve of the SLD3232VF Sony laser diode.

We see that at a certain point the data points in our IV curve tend to spike in a linear trend. By taking the linear fit of these data points after threshold we can obtain the threshold voltage by calculating the voltage value for 0 amps. The value we obtained for the threshold voltage was 4.3 volts. What this tells us is that our laser diode will have to operate at above 4.3 volts.

Next, the power vs current (PI curve) is obtained by changing the current through the diode and measuring the corresponding power at different currents. A power meter will be used to record the output power of the laser diode while a multimeter will remain in series with the laser diode to measure the current. As we change current, we will measure the corresponding power. The plot of these values will yield a PI curve where, similar to the IV curve, the location that the linear fit of the data after threshold crosses the x-axis will enable us to determine the threshold current. We show this PI-Curve in figure 28 on the following page.

The current is also a function of voltage. To change the current, we must operate the diode at different voltages, which will not be linear until after the threshold voltage has been achieved. From here, we can observe clearly that our semiconductor is lasing, as seen from the exponential increase after 30 milliamps. We did not see the maximum output power as described by the specifications sheet for the sony laser diode, but under these conditions it should still be intense enough to fluoresce our solution and provide a good enough fluorescence signal for us to read. By taking the linear fit of the laser diode after threshold, we find the threshold current to be 28.8 mA.

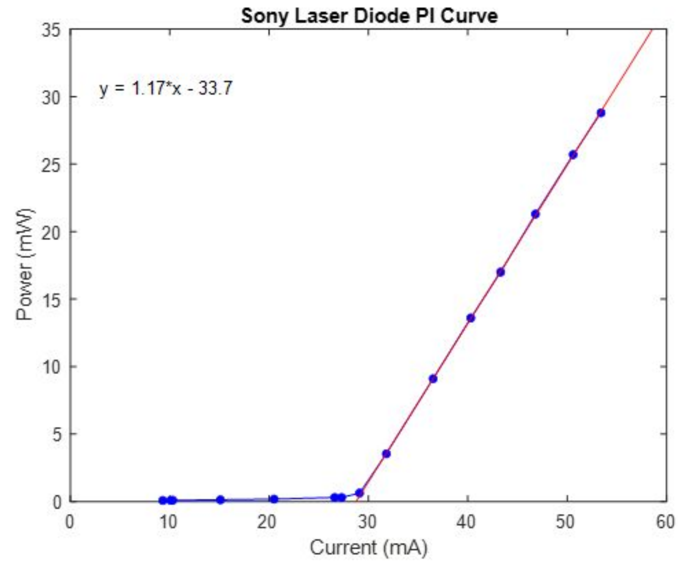

Figure 28: PI-Curve of the SLD3232VF Sony laser diode.

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.

Other than the PI-curve and IV-curve, we also use a spectrometer to determine the full width at half max and central wavelength of the laser diode. These values will be measured when the laser diode is at room temperature to ensure that the most common environmental conditions do not affect the excitation source of the device. A StellarNet blue wave portable spectrometer borrowed from CREOL is used to take these measurements. The output signal of the laser diode was detected using a multimode fiber from free space to be collected inside the spectrometer. No focusing was needed as all we required was a high enough intensity onto the spectrometer such that it displayed our spectra.

Testing our laser diode, we conclude that this will be a suitable excitation source for this project for its high output power, minimal electrical requirements, and narrow bandwidth.

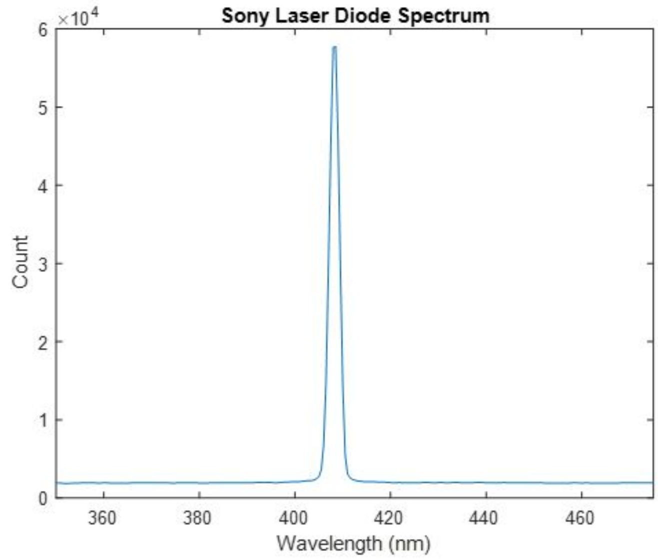

Figure 29: Spectrum of the SLD3232VF Sony laser diode.

After testing the spectrum of the laser diode, we found that the laser diode yielded a central wavelength of 409 nanometers. 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 than 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.

6.4.2. Lens Testing

The testing for the lenses will be simple. First the focal length of the lenses will be confirmed. A collimated light source will be directed at the lens and the focal spot will be detected by finding the distance from the lens to where the beam waist is at its minimum width. This distance is the focal length, and it should match up with the values provided by the manufacturer. The diode can also be potentially be used to test these optics to ensure our lenses are up to standard. The focal length of our lenses can be tested from any light source, but may be easier to execute using an already collimated light source. But, with our tools on hand, it may be necessary for us to do the optical testing with the laser diodes that we have already purchased.

To test the transmission of the lenses we need to run them through a spectrometer. This will enable a spectrum of the transmission of the light through the lens to be gathered. Comparing this spectrum with our expected spectrum provided by the suppliers will allow us to confirm the material used in the lens and the AR coating if any.

6.4.3. Diffraction Grating Testing

The diffraction grating can be tested for it’s diffraction angle, to observe the maximum efficiency of the grating at the angle for which it will diffract. In the event that we utilize a grating from optigrate, the testing for the diffraction angle can be done on their site. There will be an incoming beam that will surface of the grating, and the beam is parallel to the optical table. From there, the grating being perpendicular to the optical table, will then be rotated to observe the Bragg angle. This must be done twice as to observe the Bragg angle from rotations of the grating from both directions.

In the event that we utilize the surface transmission grating, we can test for the fixed angle of the grating by utilizing a supercontinuum source that has a broad spectral range. With this grating, we would only be required to observe the fixed angle it is specified for. It is also in our consideration to find the diffraction efficiency of the diffraction grating. Again, we may still use the supercontinuum source or even our laser diode, but also the actual signal that we capture from the fluorescence of the cuvette. Optimistically, we expect to see at least 70% for the diffraction efficiency of the grating as was described from its specifications sheet. It should be 70% as that is the peak efficiency for the wavelength that it was designed for, 660 nanometers.

6.4.3.1. Grating Configuration

We also need to configure our grating to our sensor. The most common way to do this is to use different laser diodes in the spectrometer to detect a singular wavelength and find the position where it lands on the detector. Doing this around two or three times with two or three different wavelength sources allows us to approximate the location of each wavelength on the detector using equation 16 and equation 17.

$$m\lambda = a \cdot sin \qquad Equation\ (16)$$

$$= tan^{-1}(\frac{\Delta x}{d}) \qquad Equation\ (17)$$

The main problem with this though, is that our device is only taking a narrow spectral range, between 600 nanometers and 750 nanometers at most. In this spectral range, there are very few laser sources with a singular wavelength that can be used to optimize the device. If we are unable to find appropriate sources, it might not be possible to do it this way.

In this case we may try to configure our spectrum using the expected spectra that we obtained from the spectrofluorometer used in sample testing. The problem with this though is that we may need to use identical optics and more research is needed on the implementation of this in general.

6.4.5. Cuvette Testing

As cuvettes are specified based on their absorbance spectrum, it is necessary in making sure the cuvettes we order are up to specifications to test the absorbance across the specified wavelength range. We have two optical glass cuvettes to test. The specifications for the cuvettes is that it transmits light from 350 nanometers to 2000 nanometers. We are only interested in the visible range, so an absorbance spectrum from 300 nanometers to 800 nanometers was obtained. From figure 30, we see that the cuvette has little absorbance from 350 nanometers onwards. This matches our specified range, so we can conclude that these optical glass cuvettes will be acceptable for transmitting the excitation source to our sample and the emission wavelength.

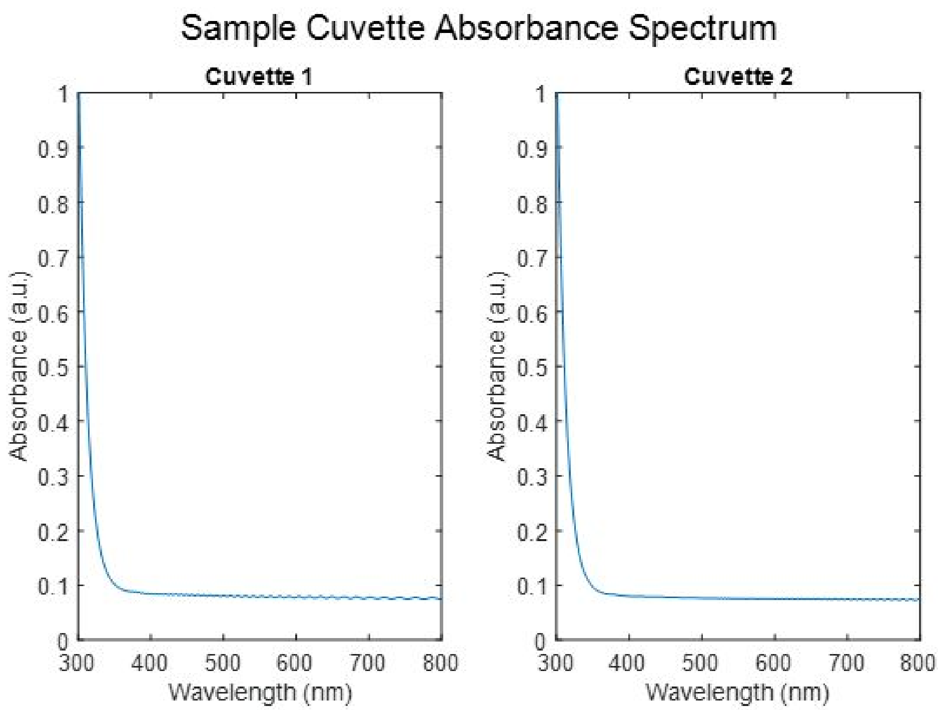

Figure 30: Absorbance spectrum of the purchased optical glass cuvettes.

The cuvettes are also specified to have a 10 millimeters optical path length. We confirmed this using a caliper. Measuring the outside width of the cuvette (12.4 mm) and then measuring the width of the glass on each side of the cuvette (1.2 mm) and then subtracting from the outside width, we obtained a measurement of 10 millimeters. From this we gather that the cuvettes will be sufficiently wide enough to excite the sample at low concentrations.

6.5. Sample Testing

We will test the sample using CREOL's Cary 500 spectrophotometer and PTI Quantamaster fluorometer. The goal of testing the sample is to ensure that we can determine the concentration of the sample experimentally. Sample testing will be done in two parts, we will test our sample of chlorophyll-a and a standard reference sample of Rhodamine-6G with a known fluorescence quantum yield. The reference sample is necessary to draw conclusions on the quantum yield of the chlorophyll sample.

The spectrophotometer is used to determine the optical density of the samples and the fluorometer is used to determine the integrated fluorescence. We will take measurements of several different concentrations of our sample to ensure the sample follows a linear trend at low concentrations. The optical density for all measurements below 0.1 to avoid nonlinearity in the fluorescence due to reabsorption of fluorescence by the samples. These measurements are crucial to drawing conclusions on the validity of our sample.

We first test the chlorophyll content in a Forestiera Segregata leaf to determine if chlorophyll-a will emit high enough intensities of fluorescence to be considered as a sample. We will then investigate several different species of cyanobacteria such as anabaena, fischerella, and oscillatoria.

Methods that include preparing the right concentration for our samples include either finding the concentration from our spectrophotometer, or using a different device, a cell counter, to ensure we have the right concentration we desire to fill our cuvettes. Using the spectrophotometer will be the quickest solution for us as we can utilize Beer's law to find the concentration after it has given us the absorbance.

The extinction coefficient may vary as there is more than just water in the solution. It would include the solution that our algae has been cultured in, which is mostly water, and acetone. But, even with these varying solutions, the difference in the molar extinction coefficient would vary slightly, and would still equal somewhere between 80-100, which will not change our concentration by orders of magnitude. Using this method, we will have to iteratively prepare samples until we find the right concentration between the volume of acetone, water, and ensuring that we do not pass the limit of surpassing our target optical density, which is around 0.1.

Using the cell counter would be another option for our case to prepare the right solution for our sample. We would instead grow our bacteria on a petri dish until enough cells have been replicated so that we may quantitatively identify how many cells there are instead of looking for the concentration per volume. Our standard is to find at least 100,000,000 cells per liter for the species of cyanobacteria, so this will be another approach that we consider.

6.5.1. Chlorophyll in Forestiera Segregata Leaf

The sample is prepared by extracting chlorophyll from a Forestiera Segregata leaf. One square mm of the leaf is cut out and weighed. The mass of this section of the leaf is 0.0248 g. The sample is then mixed with 5 milliliters of acetone. The sample is then ground using a mortar and pestle. Up to 10 milliliters of acetone was added because the acetone was getting absorbed by the container or evaporating. After grinding the sample for around five minutes, the sample was completely dissolved.

The solution was collected into a sample vial. The solution was a murky light green color, likely contributed to the marble from the mortar and pestle as well as the non-chlorophyll components of the leaf. To isolate the sample, it was transferred to several small centrifugal cuvettes and centrifuged for 3 minutes. At this point, the sample was a clear, light green color with a white solid precipitate at the bottom of the cuvette. The liquid was collected into a one cm optical path length cuvette and prepared for testing. To first determine whether or not the solution would fluoresce, a more concentrated solution was also taken, and the fluorescence was detected using a UV light. This is shown in figure 31 below.

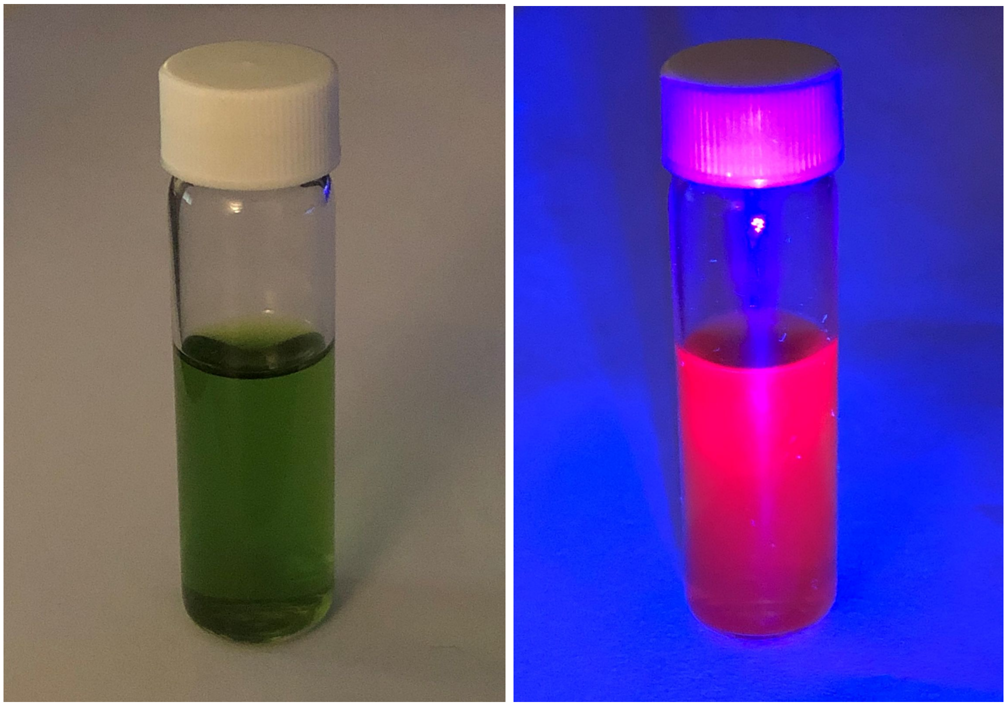

Figure 31: Concentrated solution of chlorophyll from forestiera segregata leaf without fluorescence (left) and with fluorescence (right).

Due to the method of sample preparation, it is hard to know the concentration without spectroscopic analysis. To this extent, we measure the sample using the Cary 500 spectrophotometer to find the absorbance of our sample. All samples measurements were recorded the same day of sample preparation. This machine requires the preparation of both a cuvette of the sample and reference solvent. The average time is set to 0.500 seconds and the data interval is 1 nanometer. All measurements on the spectrophotometer were taken in the 200 nanometer to 800 nanometer range. First, a baseline is taken without any sample in the machine. Next, the sample and reference solvent were placed into the machine and the absorbance was recorded.

Our sample exhibited several peaks, corresponding with the expected peaks of chlorophyll-a and chlorophyll-b. The optical density was found to be 0.181777. This is too high of an optical density due to the size of the cuvette. 5 mL of the sample was thus diluted with 5 milliliter of acetone. The optical density was once again recorded, this time the value was 0.093127. This is within our tolerated optical density of 0.100, and thus this sample is labeled sample 1 and used for later calculations. The wavelength of peak absorbance was 432 nanometers which is close to our expected 430 nanometers.

After the absorbance was within our acceptable range, the fluorescence spectrum of sample 1 was taken with the PTI Quantamaster fluorometer. The integration time was set to 0.100

seconds, the step size was 1 nm, and five averages were taken. The sample was excited at 405 nanometers as that is the wavelength of the laser diode we plan to use. The spectrum was also taken when excited at 430 nanometers and 450 nanometers, the peak excitation wavelengths for chlorophyll-a and chlorophyll-b respectively. By demonstrating both chlorophyll-a and chlorophyll-b measurements we were able to see that as long as our excitation source does not have too high of a FWHM being able to distinguish between chlorophyll-a and chlorophyll-b should not be a problem as they excite at two distinct wavelengths.

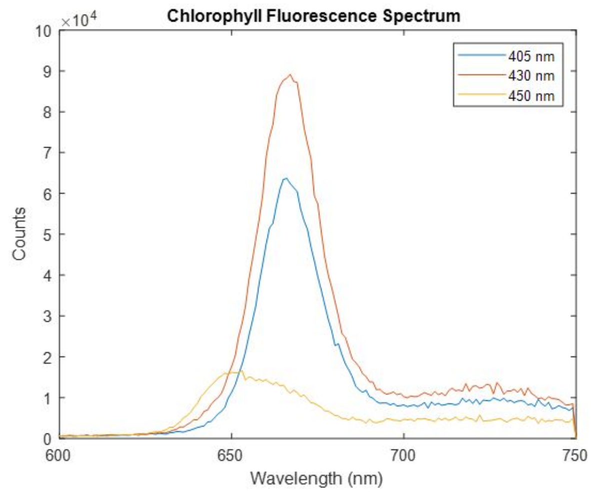

Figure 32: Fluorescence of sample 1 at excitation wavelengths of 405 nm, 430 nm, and 450 nm.

The resultant spectra can be seen in figure 32. We observe that at 405 nanometers, the spectrum closely resembles the spectrum when excited at 430 nanometers. The wavelength of peak emission was 666 nanometers when excited at 405 nanometers and 667 nanometers when excited at 430 nanometers. This is close to our expected peak absorbance of 665 nanometers and both spectra correspond to the expected spectrum of chlorophyll-a, confirming our sample content. When excited at 450 nanometers, the wavelength of peak emission was 650 nanometers, similar to the expected 652 nanometers, and the fluorescence spectrum corresponded with the expected spectrum of chlorophyll-b. From this we determine that our laser diode is an acceptable wavelength for properly exciting the sample.

Next 5 mL of the sample was separated four times and diluted with acetone to 80%, 64%, 51.2%, and 41% concentrations. These samples were labeled samples 2 through 5 respectively. The solutions were then prepared in cuvettes for testing. The absorbance and fluorescence spectra are taken for each sample and the optical density and integrated fluorescence were recorded. The spectra can be seen in figure 33.

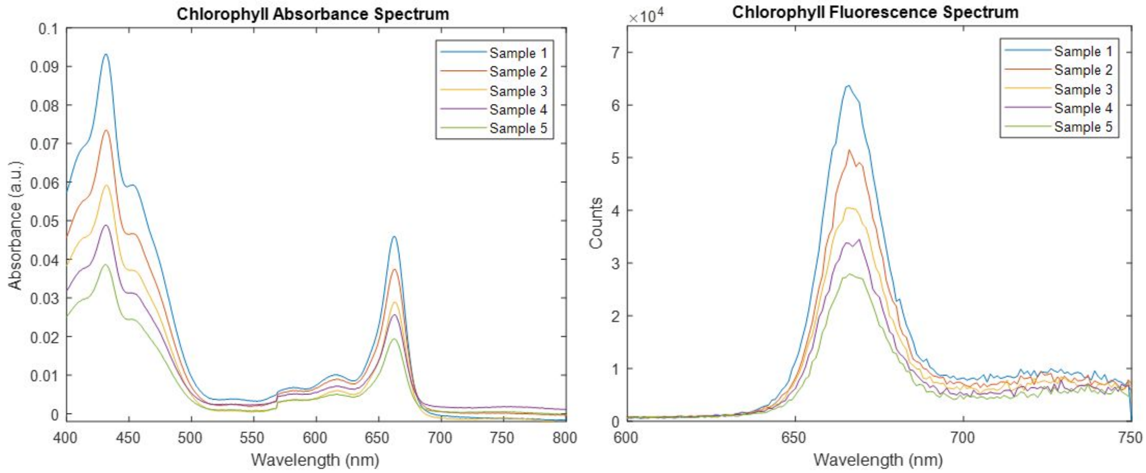

Figure 33: Absorbance and fluorescence spectra of chlorophyll-a in acetone solution at five different concentrations, excited at 405 nm.

To confirm the linearity of our samples, the integrated fluorescence was plotted as a function of absorbance as seen in figure 34. This yielded a linear trend for samples with an optical density below 0.10 as expected. The slope of this trend will be used when calculating concentration by Beer's law (equation 12) described in section 3.4.3. For the sample with an optical density above 0.10, the same trend was not applicable. This is attributed to the fluorescence signal being reabsorbed by the sample before it is collected to the spectrometer.

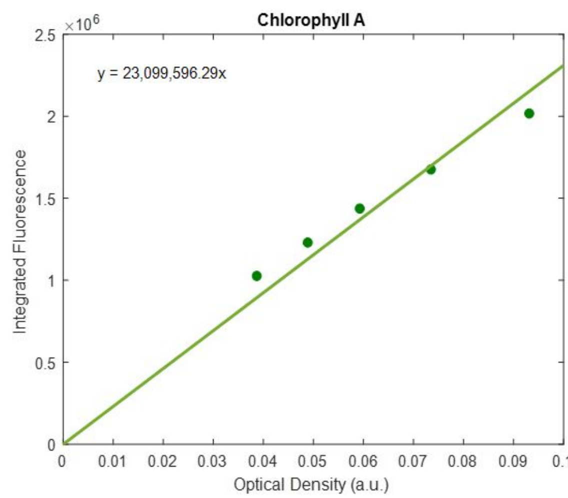

Figure 34: Linear trend of integrated fluorescence vs. optical density.

6.5.2. Reference Testing

Rhodamine-6G is a standard reference for finding the quantum yield of a sample. A reference sample of Rhodamine-6G was prepared in a solution of ethanol and the absorbance and fluorescence spectrum was taken as seen in figure 35. The due to the wavelength of the peak absorbance being close to the wavelength of peak emission, the fluorescence spectrum was taken when the reference sample was excited at 500 nm. The reference sample exhibited peak absorbance at 530 nm and peak fluorescence at 550 nm. The optical density of the reference sample was 0.10239, a value within reason of our required optical density, 0.100.

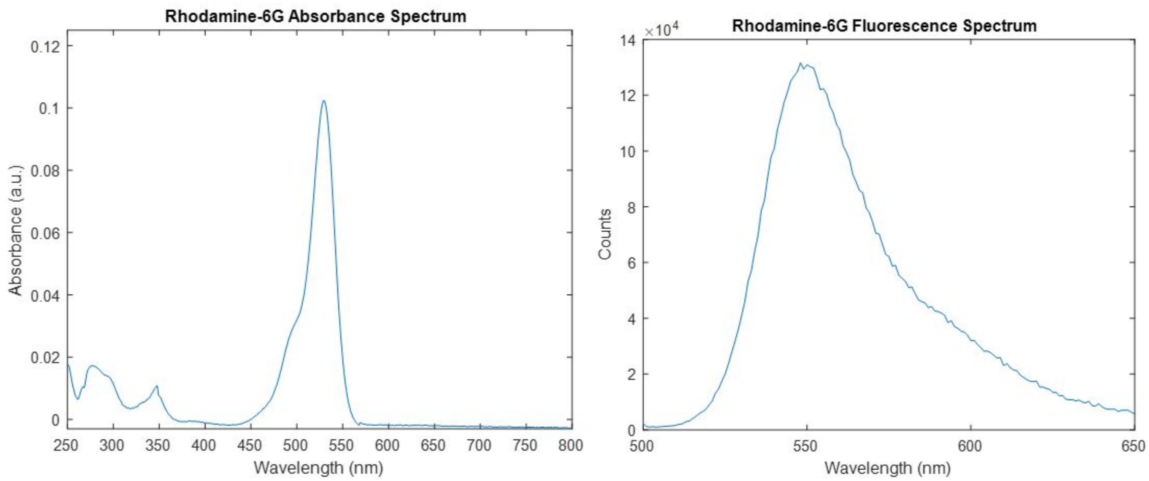

Figure 35: Absorbance and fluorescence spectrum of the reference sample, rhodamine-6G in ethanol solution, excitation wavelength of 500 nm.

6.5.3. Quantum Yield Calculations

After sample and reference testing is done, it is necessary to find the quantum yield of the sample. Using equation 9 from section 3.4.2., along with our calculated and known values for both the reference and sample solutions, the quantum yield of our sample was found to be 0.264755. This is within our expected range of quantum yield for chlorophyll-a, between 0.25 and 0.30.

It is important to find the quantum yield because this allows us to determine if our sample will fluoresce strong enough. Using equations 1 and 2, we can determine whether or not our excitation source will have enough power to generate a signal strong enough to be measured.The sample has a high enough quantum yield that the photons that fluoresce will be enough to generate a detectable signal. From this, we can determine that our power source is more than enough to generate fluorescence, and we may even have to use a voltage regulator to control the power so as to not oversaturate the sample.

The excitation source is expected to have a maximum output power of 50 milliwatts, but in reality what we see is closer to 30 to 40 milliwatts. Judging from these averages, we can expect our fluorescence signal to be at most 8 milliwatts in total, but this energy will be distributed in every radial direction, or 4pi. The actual amount of power from the signal may be significantly lower than we expect, possibly on the order of microwatts, but may still be enough to charge the pixels in our detector.

6.5.4. Chlorophyll in Cyanobacteria

After determining that chlorophyll-a has a strong enough fluorescence signal to be considered for our sample, we can move onto testing different cultures of cyanobacteria. This testing has to be completed quickly so that the samples do not degrade and skew the results. The first sample tested was anabaena. As of the time of writing the report, we have only tested the fluorescence and concentration of a single concentration, approximately 0.1. When compared to our previous chlorophyll sample, the had significantly less fluorescence intensity, over a factor of 2. We still believe that the chlorophyll presence in our cyanobacteria culture may be enough to detect the fluorescence, as we can observe qualitatively that there is a signal when excited with a 405 nm.

Another reason with the change in our intensity from the two different samples is the solvents that they were contained in. From the Forestiera Segregata leaf, we only used the leaf and spectrophotometric grade of acetone for making our solution. This effectively means that there is only the contents of the leaf, which would include the rest of the plant cell organelles, chlorophyll, and 99.5% acetone. From our cyanobacteria culture, we also include the use of the culture medium that the cyanobacteria has arrived in. The culture medium that it has arrived in is labeled as Alga-Gro® Freshwater Medium. As trade secret purposes, the company, Carolina, does not disclose what this solution is, only providing its pH level and notifying that it is sterile. The pH level is buffered at 7.8, which we can infer that it is a water solution added with minerals which will slightly raise the pH.

Trying to find the main substances in this solution would be out of our capabilities, so the only way for us to create a reference solution would be to purchase this freshwater medium such that we can create an even distribution with the spectrophotometric grade acetone. Creating this reference solution would only be useful for finding the absorption, initially our testing was to add as much acetone into the anabaena solution and placing it in a centrifuge to extract the chlorophyll and ensuring that we found an absorbance of 0.1. The baseline of the Cary 500 spectrophotometer was only based on a pure spectrophotometric grade of acetone. With just the spectrophotometric grade acetone, the baseline of the graph may have been raised to an inaccurate level. Further research is needed to determine the reason behind this.

We conclude that it will be required to make multiple samples with exact distributions of spectrophotometric grade acetone and the freshwater medium, compared to the reference solution in the spectrophotometer. Further purchasing of this medium will be required, but only in the smallest volume that Carolina will sell, which is 1 quartz.

6.6. Spectrometer Testing

The spectrometer will be tested by comparing the fluorescence spectrum of the sample obtained using the product with a fluorometer known to work. A comparison between the results obtained by the two devices will enable us to verify that the spectrometer is working. We don't expect to see the same values for the intensity of the fluorescence as the optics of our device is different than the laboratory device but we do expect the same profile of our chlorophyll-a spectrum and a similar trend in the intensity value of our samples.

6.6.1. Spectral Range

To test the spectral range of our device, we must first configure the sensor as described in section 6.4.3.1. After this is complete, we will have mapped each pixel to a specific wavelength. The spectral range will extend from the first pixel to the last pixel. After each pixel is mapped to a wavelength, we can take the wavelength corresponding to the first and last elements of the array and the difference between these two values will be our spectral range. To aid in testing the spectral range, the basic implementation as discussed in 5.3.1 will use an array to store data. Using an array will allow us to look at the individual pixels and allow us to identify that these pixels are properly being mapped, and the wavelengths are properly being found.

The spectral resolution will be the difference in wavelength of each data point collected. As our pixels will technically be considered our exit slits, the difference in wavelength between each pixel is also determined by the configuration of the grating to the sensor. We can just take the spectral range and divide it by the amount of elements in the array (pixels).

6.6.2. Linearity Testing

The final testing to be done on the spectrometer is linearity testing. This means to test the device multiple times to ensure we get the same results. Testing will be straightforward for this part. We will simply reproduce the steps taken for earlier testing of chlorophyll-a using our built spectrometer multiple times and compare the results when using the same spectrometer. Results will be compared as a margin of error between different measurements. If this testing passes within a certain margin of error, we can be considered to have built a working spectrometer that is ready for consumer use. This test can be automated by using a script that compares the several readings conducted discussed in the next section under software in section 6.7.5.

6.7. Software Testing

Software tests will be used to ensure that the hardware is efficiently interacting with one another. The software first and foremost must be able to translate the data from the camera for analysis. This feature will be tested by first observing if the microcontroller is receiving an input signal from the sensor and can read the file. The program will then be tested if it can use the file and properly translate it into numerical data for graphing. Each of the tests discussed in this section has the official steps to reproduce it shown in a table for the other members to replicate the test in the event the computer engineer is unable to do so. This section will also cover how some tests from the discussed in previous sections can be automated by using a script.

6.7.1. Signal Processing

The most important step is to ensure that the camera is properly configured and working before a spectrograph can be created. The test described below will check that a proper image is being produced and cropped through the camera module, the program being able to trigger its capture, and that it senses there's a sample present in the device in the first place. As noted, the camera is incredibly sensitive, and thus, it is important that the user is grounded to ensure the camera does not break.

Table 23: Signal processing testing procedure.

Purpose	To test if the Raspberry Pi can read the data from the sensor and create a file of the image.
Prerequisites	Materials: An image generated from the Camera module. Program: IPResults.py
Procedure	1. Before handling the camera, ensure that the user is properly grounded. 2. Power up Raspberry Pi 3. Take a picture using the camera using the program *TestImage.py*.
Expected Results	The Raspberry Pi will return a captured image in the form of a .png file, its cropped version, and give a message that the sample is present in the first place.

6.7.2. Image Processing

Before the image can be used to detect substances in water, the program has to be able to properly digitize an image's pixels to a function of wavelength. While the program is analyzing the pixels in the image, a script called IPResults.py will be made to evaluate if each pixel of the image is properly translating the data into numerical values. The script will return an image of a raw spectrograph, the number of pixels that were analyzed, and list the amount of pixels that fall into various wavelengths.

This test will be ran constantly throughout the project's development to check to see if the program needs any adjustments to its calibration process as well as the sample's environment to reduce or eliminate the need for manual adjustment of the user. Additionally, the test will allow us to observe how much noise is present to adjust the program's filtering that will detect if a substance is in the water.

Table 24: Image processing testing procedure.

Purpose	To evaluate if the image is being properly digitized.
Prerequisites	Materials: An image generated from the Camera module.

	Program: IPResults.py
Procedure	4. If an image isn't present, generate one through the Camera module. 5. Run *$ IPResults.py [imagefilename]* on the command line.
Expected Results	The script will generate the following: 1. An image of the spectrograph generated from the image. 2. A report of the pixels that fall into their respective wavelengths.

6.7.3. Accuracy Check

The analysis computed by the program's accuracy will be tested by comparing it to the theory. A script to compare the data will be written to automate and speed up the testing process. The script, ConfirmData.py will generate a report to give us a better visualization including how accurate it is to the theoretical resulted without having to compare the spectrographs unless necessary. The script will run the analysis function from the program and take its output to compare the results.

Table 25: Accuracy check testing procedure.

Purpose	To check if the program's results match the theoretical data.
Prerequisites	Materials: Raspberry Pi, Camera Module Program: ConfirmData.py
Procedure	1. Power up Raspberry Pi 2. Run the program through ConfirmData.py 3. Validate results
Expected Results	The script will return the following: 1. Result of the analysis 2. The expected result. 3. Percentage of Error

6.7.4. User Interface

The interface will be tested by observing if the display properly displays instructions and messages, and that the buttons are able to navigate the user to each state. To test any automatic transitions, each state will be hardcoded and observed on the screen. This test will be conducted multiple times as the functions are added to the program to ensure that it is still working, and also if we decide to upgrade the display to the device. This test will be important to do each time to make sure that the software is properly communicating with the hardware components of our device, but can also be used to help us debug additionally if there's issues with reaching a state or checking to see why an error isn't being read.

Table 26: User interface testing procedure.

Purpose	To check if the program's results match the theoretical data.
Prerequisites	Materials: Raspberry Pi connected to either a display, or Indicator LEDs. Program: SMCheck.py
Procedure	1. Turn on the Raspberry Pi. 2. Run the debugging program, SMCheck.py 3. Use the commands to trigger an error
Expected Results	The program will be able to cycle through each state and run the functions associated with each.

6.7.5. Spectrometer: Linearity Testing

As discussed in the previous section, testing can be automated by using a script to validate its consistency. The script *ConsistencyCheck.py* will take the ideal results as its argument, the percentile of error, the number of times we want the scan to run, and run the script. The script will trigger the spectrometer to read the sample as indicated in the argument. The result of each reading will be compared to the ideal number and see if it's within the margin of error. The script will record the number of passes and number of fails and output the results of that to give an idea of how consistent the device is. Any fails the test finds, it will save a file of the failures for us to individually look at. Running this script will save us a tremendous amount of time and allow us to look at a large amount of readings without having to individually filter through each unless an error is found present.

Table 27: Linearity testing procedure.

Purpose	To validate the consistency of our device.
Prerequisites	Materials: Completed Spectrometer
Procedure	1. Turn on the device. 2. Place the sample onto the device for reading. 3. Run *ConsistencyCheck.py* on the commandline with its arguments.
Expected Results	The program will run the program a number of times as indicated in the argument, and output the amount of passes and fails the reading was close to the ideal result.

6.8 PCB Testing

After creating the PCB design and receiving it from the manufacturer, we will need to test it as to not damage the components we are attaching. We will connect a power supply and use a multimeter to test the voltage of all parallel connections. As we attach each component we will test the voltage drop and current going through it to make sure there are no errors. This also helps with troubleshooting because if anything is wrong, we know exactly where the problem is. As discussed in the previous chapter, we want to emphasize the design process of the PCB to prevent a situation where we would have to redesign and purchase a new board, especially when time is sensitive.

7. Administrative Content

Here we summarize the project, including details on the team members, their responsibilities to the project, and the project content. We also include the goals for the project milestones as a means to keep us on track for completing the device on time, and the overall budget to keep us in a realizable cost scope.

7.1. Personnel

This project is the result of a four person interdisciplinary group consisting of two photonics science and engineering students, a computer engineer, and an electrical engineer. As the device as a whole utilizes knowledge from different areas of study, a wide range of skills are needed to successfully implement the design. To this purpose, each member will be assigned with different tasks. The individual responsibilities are described in more detail in the section below. The group members must also make sure to communicate with each other on how to combine each part of the device into a collective whole.

7.1.1. Team Members

Our team is composed evenly with two optics and photonics students, one electrical engineer, and one computer engineer.

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

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 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.

Lavine Von

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

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.

7.1.2. Individual Responsibilities

To assist each other during development, we decided to have as much involvement with each other as possible to better understand how this project will come together and close the gap between our respective majors. In the event a member has difficulty or an emergency has come up that would prevent them from working on their responsibility, we can rely on another member to continue its development and management. While each member is accountable for their own part, we want to emphasize to each other that this is a team project, and that we will support each other through hardship and motivate each other to see its completion.

With such a multidisciplinary project as this, it is important for each team member to not only be responsible for completing tasks suited for their major but also integrating their parts into the whole device. Much of the features that are needing to be developed will affect another part of the design and knowing how and why will improve the project's development. For this reason, some of the responsibilities feature additional members of the group assisting with its implementation as shown in the diagram below.

Table 28: Overview of responsibilities.

Responsibility	Primary	Secondary(s)
Power Supply	Niyah Lowell	Hee-Jun Jang
PCB	Niyah Lowell	Lavine Von

Laser Source	Hee-Jun Jang	Niyah Lowell
Sample	Austin Dziewior	Hee-Jun Jang
Sensor	Lavine Von	Austin Dziewior
Spectrometer	Austin Dziewior	Hee-JunJang
Casing	Austin Dziewior	Niyah Lowell
Raspberry Pi	Lavine Von	Niyah Lowell
User Interface	Lavine Von	Niyah Lowell
Computational Analysis	Lavine Von	Hee-Jun Jang Austin Dziewior
Spectrum	Austin Dziewior	Lavine Von Hee-Jun Jang

7.2. Project Milestones

In order to hold ourselves accountable, we've created a schedule to plan the progression of our project. This outlines the dates we need to get to certain points in our project. We will work to stay ahead of this schedule and if we are in danger of falling behind, we will combine our efforts to stay on track. In Senior Design I, the semester was focused on documentation, researching, and purchasing equipment. Senior Design II will focus primarily on development, testing and implementation. We have separated the tables into the two semesters for Senior Design One and Two as shown in the diagrams below.

Senior Design I. This semester prioritized the process of documentation and research for the project. As discussed in the previous chapters, it's important that we spend a lot of time planning to see the success of our project. During this period, we also purchased parts and individually tested them to ensure its compatibility with each other. Though it wasn't necessary to be able to have and physically test each part for this semester, we want to try to stay on top to have as much time as possible for Senior Design II. Having all the parts ready and tested for PCB design before Senior Design II occurs guarantees we'll be ahead at the start of the semester.

Table 29: Initial Senior Design I milestones.

Milestone	Target Week	Description
1	9/20/2019	Divide and Conquer

2	9/23/2019	Update Divide and Conquer
3	9/30/2019	Research Design & Specs
4	10/14/2019	Begin Report
5	10/21/2019	30 Page Draft
6	11/1/2019	60 Page Draft
7	11/15/2019	100 Page Draft
9	11/22/2019	Finish Ordering & Testing Parts
8	12/4/2019	Final Document

Senior Design II. Senior Design II will be more hands on than Senior Design I because we're focusing on development and construction to get a prototype ready for the Senior Design showcase. As the product is being developed, we will have to implement, test, document, and repeat until the project achieves its goals and requirements. For this reason we must finish all the milestones in Senior Design I to ensure that we have plenty of time for development and testing. As shown in the table below, Senior Design II emphasizes that we build the essentials to get a functioning project before we can optimize and improve its performance.

Table 30: Initial Senior Design II milestones.

Milestone	Target Week	Description
10	12/31/2019	Finish Power Supply Design
11	1/21/2020	Build Spectrometer
12	1/31/2020	PCB
13	2/14/2020	CCD
14	3/1/2020	Build Prototype
15	3/14/2020	Begin Testing & Optimization
16	3/31/2020	Final Prototype
17	TBD	Peer Presentation
18	TBD	Final Report
19	TBD	Final Presentation

7.2.1. Project Management

For the first half of the Fall 2019 semester, staying on top of the project and meeting on a weekly basis had no challenges. Towards the middle of the semester where midterms picked up, it became increasingly difficult to meet due to the workload, and for our third milestone, we were behind by several pages in our documentation. Proper scheduling is essential to the success of the project to ensure that it can be completed within the given time. Though this project isn't too difficult in theory, different technologies as well as it being a cross-disciplinary project makes it essential that the project plan is realistic and attainable. In addition to code documentation discussed in Chapter 4, we will be using the Scrum methodology for project management.

7.2.1.1. Scrum

While our teamwork and individual accountability was strong, time management was our biggest downfall for Senior Design I. To not let this mistake happen, we decided to implement the Scrum methodology for Senior Design II. Agile is a practice that helps in continuous iteration of development and testing commonly in software. Agile breaks the product into smaller builds so that it can be divided and conquered, and Scrum is an agile process that focuses on delivering the project in the shortest time. As implied in previous chapters, we want to start with a simple implementation before improving on its performance which makes Scrum an ideal methodology to follow. Scrum's principals emphasizes accountability, teamwork, and iterative progress towards a well-defined goal and also has recurring sprint meetings for review and feedback, while also emphasizing that time is a limiting constraint.

For this project, we will have weekly team meetings as well as a progress report to fill out so that everyone is aware on individual contributions towards the project. Scrum's biggest advantage is its flexibility, so in the event that an individual isn’t able to meet with our goals, this will be conveyed as early as possible so the other members can work around and with the person.

7.2.2. Project Construction

The project first began with the intention of testing for multiple substances in a water supply using Raman Spectroscopy. However, this initial idea, seemingly simple, would actually be a project on a much larger scale compared to the one discussed in this report.

7.3. Budget and Financing

The budget for this project has to be cheap as the students will be funding most of the project. While we have a sponsor for the project, optigrate, they will only be providing a grating and filter. The project also has to be cost efficient to ensure that the device is comparable to other devices. Having a product that is more expensive than all other device eliminates the marketing purposes of the device and must be avoided.

7.3.1 Suppliers

There are quite a few suppliers considered for this project, especially for those of optical components. It is important to choose a supplier with a good reputation as that can ensure the specifications of the component is up to standards.

7.3.1.1. Optigrate

While Optigrate has generously agreed to supply the group with any necessary gratings or filters we may need for the construction of this device, at the time of writing this report we do not plan on using any of their products. We list them here as we may eventually need to change our design if testing falls through. However, at this point in time we will be using a transmission surface grating and Optigrate mainly supplies bragg gratings. But we may decide to use a filter later on depending on how our research and testing progresses.

7.3.1.2. Thorlabs

Located in New Jersey, Thorlabs is a major supplier of optical components such as lenses or lasers. As a relatively new company, Thorlabs has done well in a business setting. Their reputation for dedication to excellent customer service has greatly assisted their rise to popularity. At the time of writing we only need the transmission grating from Thorlabs.

7.3.1.3. Edmund Optics

Edmund Optics, formerly known as Edmund Scientific, was created in 1942 and is also located in New Jersey. The company is another well known supplier for all kinds of optical components internationally. The lenses we decided on for this device will be obtained from Edmund Optics.

7.3.1.4. Adafruit

Adafruit is well known hardware company based in New York. They gained popularity mainly because their hardware is open-source. They provide an abundance of help (including videos) and documentation for their products. Both the Raspberry Pi and the Power Boost 1000C are from Adafruit. We will also be considering some displays produced by them should we choose to upgrade our interface.

7.3.1.5 Levil Aviation

Levil Aviation is a small, family-owned company that creates instruments for small planes along with desktop CNC machines. They're located in Oviedo, Florida and have been in business since 1999. They've been very generous in allowing us to use their reworking station. This will give us access to their more advanced tools which will lessen the difficulty of reworking our circuit board after population.

7.3.1.6 Senior Design Lab

The UCF Senior Design Lab has supplied us with all of our testing and reworking equipment so far. We have rented a breadboard, probes, and alligator clips from them. We also have access to breadboard-friendly components like resistors and capacitors. A triple power supply and digital multimeters are always available to us for all testing. There is also a soldering station available to us for building our circuits and whiteboards which provide a better means of visualizing ideas in groups.

Table 31: Estimated project budget.

Item	Estimated Price	Notes
Laser Diode (5)	$11.99	*405 nm*
Lenses (3)	$93.50	
Glass Cuvettes (2)	$39.99	*10 mm OPL*
Algae Culture	$48.25	
Diffraction Grating	$82.78	*Thorlabs*
Development Kit (2)	$80	*Raspberry Pi*
SD Card	$7	*8 GB MicroSD Card*
Sensor	$20	*Camera Module V2*
LCD Display	$10	*Arducam 16x2 1602 Display Module*
PCB manufacturing	$150	
Rechargeable battery	$12	*3.7V 2500maH Li-Po*
Unit Housing	$50	*3D printing*
Charging board	$20	*Power Boost 1000c*
Boosters (5)	$12	
Solder wire	$10	
Components (Resistors, Capacitors, switches, wires, etc.)	$11	*So far...*
Total	**$658.51**	*Estimated budget is less than $800*

8. Appendices

8.1. Index of Figures

8.2. Index of Tables

8.3. References

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8.5. Data-Sheets

https://www.arducam.com/downloads/modules/industrial/1Inch2.3_10_Megapixel_MT9J001_CMOS_Camera_Module_DS.pdf
http://www.ti.com/lit/ug/slau772/slau772.pdf
https://img1.tongtool.com/v/trtuAvvyrvtuAqtwr01uxCtBuuAAyCxCwsMSHJT.jpeg

8.6. Pin Diagrams

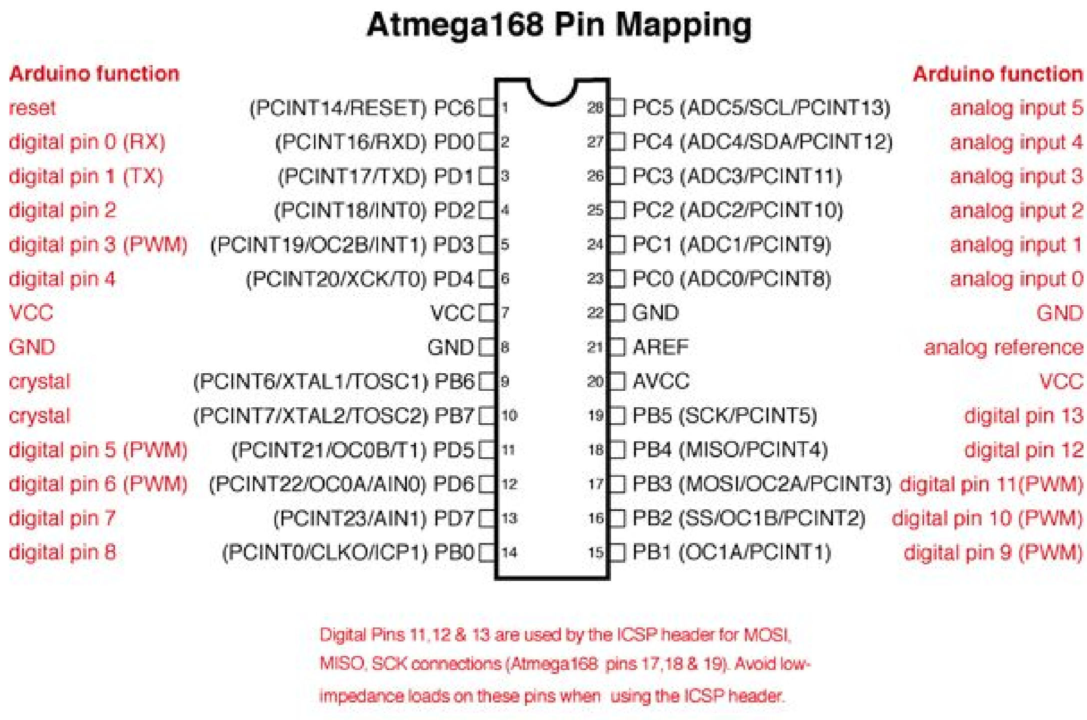

Figure 8: Arduino pinout

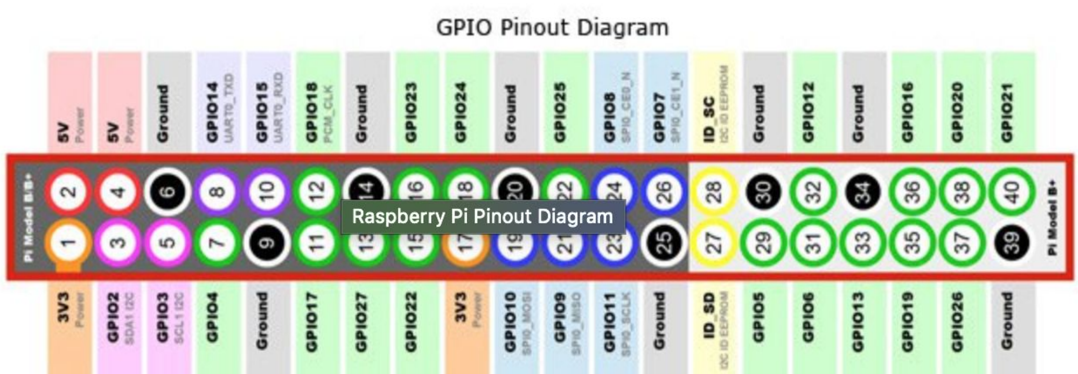

Figure 18: Raspberry Pi 3 Pinout