Final Report for
Sensors for Detection of Harmful Algae Blooms

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Executive Summary

The engineering team has been tasked with developing a sensor to detect harmful algae blooms in Lake George. Like many bodies of water, Lake George is deteriorating and can grow algae which is toxic to wildlife and sometimes humans. One distinct feature about these blooms is their fluorescence to a specific wavelength. The team has continued the design of a more affordable fluorometer.

To establish the proper technical specifications, research of past work and current efforts in fluorometry were assessed. Currently, fluorometers are expensive, so the team is designing an affordable version that can be distributed around Lake George in mass. The scope of this semester's project is the circuitry which can take a sample as an input and output a value proportional to the sample's concentration.

The main components of the design are and LED which shines a light on the sample, a filtering system which blocks undesirable wavelengths at different steps, a photodiode to pick up the fluoresced light, and a circuit to process this output. There is also the inclusion of a microcontroller to time the proper flashes and readings as well as send the output to a CSV file.

The team performed analysis to pick the correct components for the circuit in order to improve on previous results. They used the technical requirements to assist with the concept selection and then evaluated the components before use. The final design features a new LED, photodiode, and filter to increase performance of the circuitry. In addition, the group considered appending an amplification circuit to increase the signal to noise ratio, but found more success in the new components.

Success for the team was measured via rigorous statistical testing, measuring repeatability, accuracy, and ability to detect small concentrations. The final design passed all tests and thus meets the performance specifications. The group did further exploratory testing to aid future work such as investigating the circuit on a higher power, with an amplifier, and with different temperatures and ambient light.

While the group experienced success on their circuit, there are still engineering challenges to solve by future teams. The casing for the circuit itself must be developed and should cycle out water, block ambient light, and prevent biofouling. They must also complete the process for how data is being transmitted and the process data is being collected. Overall, this team has made significant progress on the circuitry and measurement of fluorescence and has laid the groundwork for future teams.

Contents

Executive Summary 2
Contents 3
List of Figures and Tables 4
Revision History 5
Glossary 6
1. Introduction 7
2. Project Objectives & Semester Objectives 8
3. Engineering Tools and Methods 10
4. Technical Background, Assessment of Relevant Existing Technologies and Engineering Standards 10
5. Customer Needs and Engineering Design Requirements 22
6. System Concept Development 24
7. Design Analysis 33
8. Final Design and Engineering Specifications 40
9. System Evaluation 41
10. Significant Accomplishments and Open Issues 49
11. Conclusions and Recommendations 50
References 51
Appendix A: Initial Deliverables and Dates 54
Appendix B: Project Plan 55
Appendix C: System Evaluation Plan 59
Appendix D: Ethical and Professional Responsibilities 62
Appendix E: User Manual 63
Appendix F: Cost Analysis and Manufacturability Analysis 66
Appendix G: Expense Report 67
Appendix H: List of Manuals and Other Documents 68

List of Figures and Tables

Table 1 - Summary of Engineering Technologies and Their Applications 10
Table 3 - Initial Deliverables and Dates Summary 54

Revision History

Version	Date	Name	Reason for Changes
1.0	9/21/21	Statement of Work	Phase 1 Statement of Work changes
2.0	10/12/21	Preliminary Design Review	Phase 2 Preliminary Design Review sections added and updated
3.0	12/10/21	Final Design Report	Phase 3 Final Design Report sections added and updated

Glossary

Alternating Current (AC)
Harmful Algae Blooms (HABs)

1. Introduction

In partnership with Rensselaer Polytechnic Institute, the Darrin Freshwater Institute, and The FUND for Lake George, IBM Global Research is sponsoring the development of an inexpensive, deployable device capable of forecasting and detecting Harmful Algal Blooms (HABs) on a large scale across Lake George. This collaboration, known as the Jefferson Project, broadly aims to study the impact of human activity on freshwater to mitigate its harmful effects by means of an advanced technological approach.

Lake George was once considered an oligotrophic lake which is a lake with low levels of plant nutrients. However, like many bodies of freshwater around the world the water quality of Lake George is deteriorating due to several factors. These include the rise of urbanization, poor land use practices, and climate change [1]. Excessive phosphate and nitrate runoff caused by these surrounding environmental changes has given rise to algal blooms. In the summer of 2020, the first instance of a HAB appeared in Lake George. This is a significant occurrence since HABs have a detrimental impact on the health of the local environment, economy, and human population.

One problem with HAB detection is the unavailability of economical options for widespread autonomous HAB detection. IBM is seeking to develop this kind of device to aid in their ongoing research into HAB development and methods of alleviating its effects. Such an autonomous device needs to detect high concentrations of harmful algae, must be compatible with local Wi-Fi, and use available power sources, cannot cause a negative environmental impact or local disturbance, and should be affordable. Since HABs are becoming increasingly more common across the planet, there is an ever-growing market for HAB mitigation and monitoring [2]. The Jefferson Project is currently monitoring for HABs in Lake George using existing sensing equipment on boats that are expensive and would not be suitable for a large-scale monitoring system with many sensors. Previous teams have worked to design a circuit to detect HABs in water samples, but results were unreliable, and the circuit would be successful in detecting HABs in a non-laboratory environment. A circuit which is more affordable yet still provides reliable results is necessary to monitor HABs on a larger scale. This device would therefore be marketed to freshwater research groups or private dock owners with interests in preserving and monitoring the water quality.

2. Project Objectives & Semester Objectives

2.1 Project Objectives and Customer Payoff

The overall objective for the Sensors for Detection of Harmful Algae Blooms Team is to develop a cost-effective, socially, and environmentally conscious, and accurate device to be deployed into large bodies of water to sample and test water quality while collecting and transmitting comprehensive data to the end-user towards preventing the buildup of harmful algae blooms. The device is intended to work with little to no maintenance over multiple seasons.

A device of this caliber will enhance environmental health by reducing the detection time of HABs providing considerable benefit in the study of the local freshwater ecology. The long-term consequences of the data collection from our device may help safeguard the local economies dependent on consistent water quality by providing insight into growth factors/paths leading to HABs. In general, preventing future overgrowth will ensure safe drinking water for communities and a stable habitat filled with ecologically diverse marine life. The following list outlines the project objectives:

- Reduce the detection time of HABs through widespread data collection
- Reduce ecological and economic impact inherent to HABs
- Develop a device that is easy to use and can be remotely monitored by members of the community
- Design for manufacturability, low maintenance cost, and scalability
- Monitor many attributes of Lake George and use collected data to make informed decisions in the maintenance of ecosystem health

2.2 Current Semester Objectives

During the Fall 2021 Semester the Optical Sensor Detection of Harmful Algae Blooms Team will focus on the following semester objectives:

In Scope:

- Conduct research and evaluate different circuit concepts using fluorescence analysis to detect HABs.
- Design and develop a functional circuit prototype.
- Create a statistically significant testing plan for evaluating the success of the prototype.
- Prepare a circuit prototype operation manual.

The team may need to consider certain aspects of the project when performing research or making design decisions that are considered out of scope for this semester. The following

objectives are out of scope for this semester and will not be critical to the current semester objectives:

Out of Scope:

- Providing a power supply to the device.
    - Assume access to continuous 120v 15A AC power supply
- Developing methods of data transfer and storage over a computer network.
    - Assume a stable connection to a home Spectrum WIFI network.
- Designing a buoyant enclosure for the device to be housed in for data collection.
- Developing means to prevent water fouling on the device.
- Determine the causes of HABs or developing methods to remove or treat HABs.

3. Engineering Tools and Methods

To achieve the proposed long term and current semester objectives, an integrated and comprehensive project should be designed, prototyped, and tested. During this process, many issues and tasks will arise, not limited to: electrical components and circuitry simulation, microcontroller application, calibration, and material selection. The table below states the main technical aspects involved in this project and potential applications to solve the engineering problems and tasks mentioned above. It is assumed that a reliable power source and cloud connection will always be available.

Table 1 - Summary of Engineering Technologies and Their Applications

Project Area	Potential Technologies & Application
Software	Use Programming Language to program Microcontroller used to control the detector circuit
	Use Data Analysis tools or software (Regression, etc.) to analyze data (fluorescence, temperature, and any other parameters) collected from the hardware and determine whether HABs occur or not
Electrical	Use detailed circuit schematics to show how each electrical component is connected
	Use Microcontroller, to control circuits and read voltage output from the light diode
	Use LTspice for circuit simulations
Process	Create Standard Operating Procedure for setting up and calibrating device

4. Technical Background, Assessment of Relevant Existing Technologies and Engineering Standards

The main method of detection of HABs used by our team –along with other teams– is via measuring the fluorescent light emitted by certain pigments within the HABs. A firm understanding of the concept of fluorescence and the external factors that influence the intensity of light emitted are essential to building an accurate and suitable sensor.

4.1 Fluorescence and Rhodamine B

Fluorescence is a material's property of absorbing higher energy electromagnetic radiation (light) and emitting electromagnetic radiation of lower energy, typically in the visible light range [3]. Cyanobacteria –the organism responsible for producing harmful algal blooms (HAB) – obtain their energy through photosynthesis, like plants. Besides chlorophyll, cyanobacteria have other pigments that fluorescence can detect. Cyanobacteria contain the pigments phycocyanin (common in freshwater) and phycoerythrin (common in saltwater) . A fluorometer –a sensor used to detect emitted fluorescent light– can detect concentrations of phycocyanin or phycoerythrin. The pigment phycocyanin absorbs red-orange light around 620 nm and it fluoresces at about 650 nm. Phycoerythrin absorbs light in 495 and 545-566 nm and fluoresces at 575 nm [4].

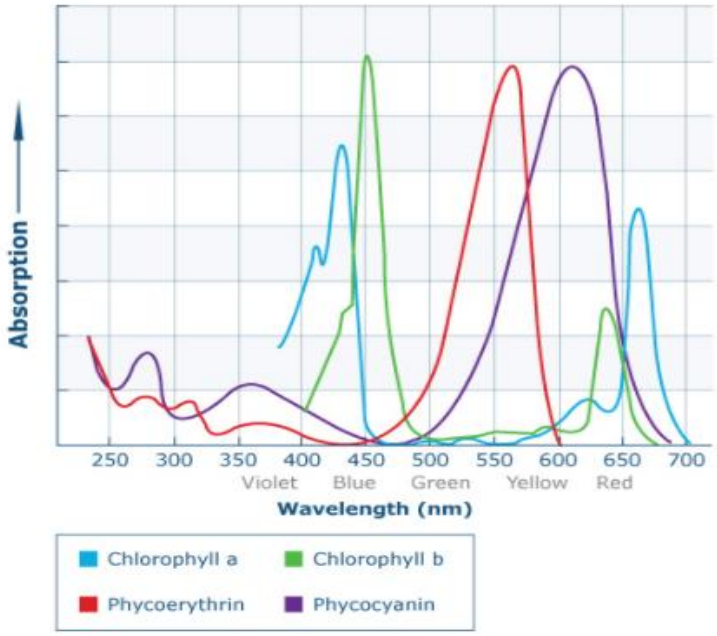


Figure 4.1 Cyanobacteria Wavelength Absorption Chart [4]

In the interest of conducting the proper testing of our sensor to ensure its efficacy, our team has opted to use the substance Rhodamine B – an organic chloride salt and an amphoteric dye commonly used as a fluorochrome (a chemical that fluoresces) [5]. This substance is known for fluorescing; hence, our team's design should expect a linear relationship between the concentration of the substance and fluorescence. The greater the concentration of Rhodamine B, the more fluorescent light will be emitted. Thus, the team can test known concentrations of Rhodamine B for different levels of fluorescence. We can perform experiments to determine how the fluorescence of Rhoadmine B changes at different concentrations by holding other variables constant. These additional variables are factors that can influence the fluorescence of the

Rhodamine and include incident light intensity, molar absorptivity, path length, and quantum yield. In our testing it will be most important to hold the path length and incident light constant so we can observe the changes in light and relate them to changes in concentration. Our team's testing plan should assess the system output using linear regression with the concentration of Rhodamine B, testing with other water samples as needed.

4.2 Existing HAB Detectors

Amongst our team's many goals, one of them is creating a device that can detect the presence of HABs for a significantly reduced cost compared to current technology. Understanding how these existing devices operate via the incorporation of several components is necessary for obtaining a better comprehension of how our project design should be established.

For instance, one device our team discovered is called the "PhycoProbe" made by the company BBE. This device uses seven different LEDs to excite a given sample, ultimately generating a measured spectra graph of the sample [6]. The software uses this graph and compares it against the norm spectra, –a characteristic pattern at defined excitation wavelengths for different kinds of algae– searching for the best fit, which may be a combination of two or more different kinds of algae [7]. This allows the device to determine what kinds of algae exist within the sample.

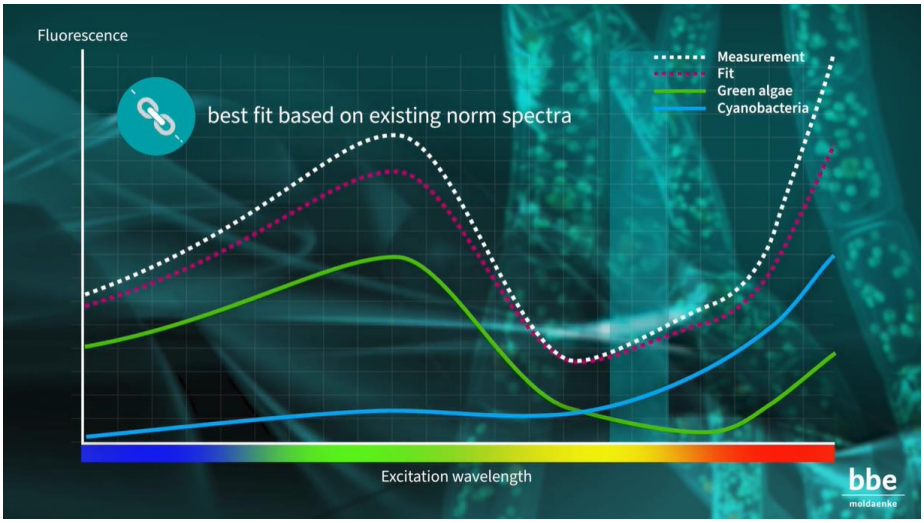


Figure 4.2 Norm spectra graph demonstrating how PhycoProbe software works. [6]

This product demonstrates that it could be beneficial to include multiple different LEDs capable of exciting the sample for better determination of substances within the sample as harmful forms of algae or not. Additionally, it may beneficial for our team to include an algorithm that helps in determining whether the sample contains HAB, a difficult task to

achieve with hardware alone when a sample could contain multiple different types of algae. However, it is not clear how much this device costs, although similar technology available typically ranges within thousands of dollars.

Furthermore, another device our team researched is called the “Aqua TROLL Blue-Green Algae Sensor: Phycocyanin (BGA-PC)”. This device can be used to monitor HABs and provide improved trend analysis [8]. One of the features of this device that supports increased performance is the use of separate algae sensors for chlorophyll and blue-green algae. Each sensor detects a smaller range of the visible light spectrum than combined sensors, reducing interferences from other fluorescence sources [8]. Figure 4.3 shows an image of what this dual sensor deployment looks like.

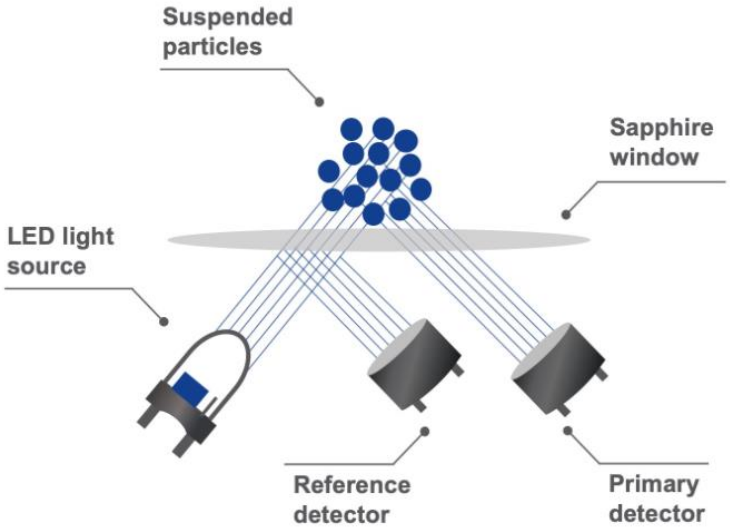


Figure 4.3 Diagram of sensor detection system within In-Situ device using two detectors. [8]

According to their website, each In-Situ optical sensor runs at a unique frequency that creates a digital signature for each light source, allowing the system to separate interference between sensors and improving overall system accuracy [9]. Additionally, the device comes with ambient light rejection technology that blocks external light from affecting the sensor for more consistent monitoring and calibration across different sites [9]. Although these aspects are something a team should consider implementing into our design, a device of this caliber costs almost $2,000. Discovering a way towards implementing similar features at a substantially lower cost is essential. It could potentially be advantageous to consider implementing multiple photodetectors for improving the accuracy in detecting HABs, so long as this does not drive up the cost of the device too much. The team that worked on this project in the spring had trouble detecting the specific frequency response of HABs, so having additional sensors and ambient light reducing technology will certainly help with noise reduction. This device also automatically collects data that can be displayed on a user interface that customers can access via a mobile app.

This extension is somewhat out of our scope but is good to keep in mind as a feature of some of the existing forms of technology that can detect HABs.

Our design for a device to successfully detect HABs should try to incorporate some of the aspects of these high-end devices using cheaper components. Important items to consider include the incorporation of multiple LEDs and multiple photodetectors for improving accuracy and reducing noise. This will allow us to better determine if a sample truly contains harmful forms of algae or not. It will also be useful to generate software that can further help to reduce the errors seen on the hardware side, reducing costs further.

4.3 Methods for Measuring the Presence of Light

In the design of our team's fluorometer circuit that will be used to detect HAB, our circuit will need to utilize a component that can measure light. A fluorometer is a device used to measure the fluorescence of an object once it has been excited and begins emitting a specific frequency of light. A light sensor is necessary to measure the fluorescence of an object once it has been excited and convert the measured light into a signal that would be useful for recording purposes. Most light sensors are photoelectric devices –converting the measured light's energy into an electrical signal [10]. However, there is more than one photoelectric device that could be utilized in the fluorometer circuit design, each with their own tradeoffs.

A light sensor commonly used in fluorometers is a photodiode –a semiconductor device that harnesses the energy of light –presents as photons– and converts them into electrons that are emitted as an electrical signal. Amongst the several properties of a photodiode, the key property to observe for this project is the responsivity of a photodiode. The responsivity is the level of current the photodiode produces relative to the unit of optical power it is receiving. The responsivity is dependent on the wavelength of light it is measuring [11].

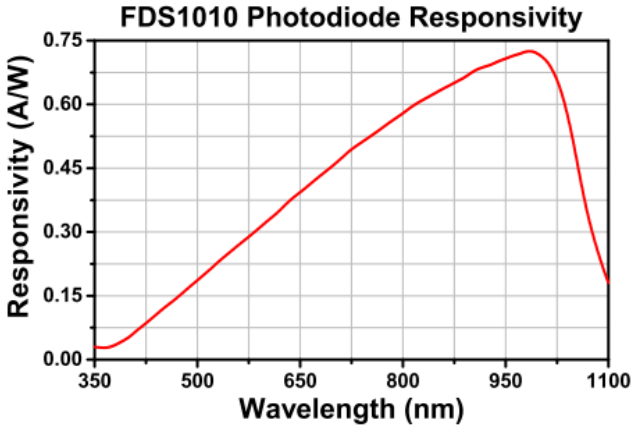


Figure 4.4 Example Photodiode Responsivity Graph [11]

Accurately detecting HAB requires the fluorometer to measure specific wavelengths of light that are emitted by HAB when excited. Each photodiode has a range of responsivity that

depends on what wavelength and intensity of light it is receiving. If a photodiode is the desired method, the team uses to detect light it will be important to select a photodiode that will provide a measurable output for the desired wavelength of light being measured.

In situations when the incident light source is less intense, a photodiode may not be able to output a strong enough signal to be useful. This could be an issue upon measuring the fluorescence of HAB in water where various light sources (i.e. ambient light) can decrease the ability of a photodiode to produce a strong output. This problem can be solved via an amplification circuit- amplifying the current or voltage being delivered from the photodiode. However, another issue with the photodiode would be ambient light interfering with our circuit's data collection, producing unreliable data. To limit the light the photodiode senses, a fluorescent bandpass filter can be utilized for filtering specific ranges of light wavelengths [12].

Additionally, a phototransistor is another photoelectric device that can be used for light sensing purposes. A phototransistor behaves similarly to a photodiode but has an internal amplification. This internal amplification results from the design of a phototransistor being based on the concept of a bipolar transistor, which are often used in amplification circuits [13]. This internal amplification removes the need for an external amplification circuit alongside the light sensor, which would be the case if the incident light source is less intense and the output from the light sensor is small. With internal amplification, phototransistors can produce higher currents when detecting fluorescent light, making them more sensitive than photodiodes.

A photoelectric device is crucial in measuring the fluorescence of water samples in detecting the presence of HAB. Photodiodes and phototransistors are two more commonly used photoelectric devices, each with its benefits and tradeoffs within our circuit's design. Our team will likely need to include an amplifier in the fluorometer circuit design for amplifying the output from the light sensor so the data can be comprehensible. A phototransistor with internal amplification properties sounds like a solution, however, it can be less reactive and slower to deliver results compared to photodiodes, potentially hindering the fluorometer's ability to read samples at a specific sampling rate [13].

4.4 Electronic signal processing

Once the reduction of sensor noise has been implemented, our team can begin further manipulation of our sensor's signal through amplification- increasing the intensity of a signal by a given factor. This stage is necessary for the operation of our product because the amplitude of the excitation light detected by our sensor must be kept low to avoid bleaching effects, thus producing fluorescence light with low intensity [14].

Due to the low amplitude of the incident fluorescence light, a low-noise amplifier placed next to a photodiode -minimizing the distance between the devices and reducing parasitic noise- can process the current generated by a photodiode [14].

One potentially efficient way of achieving this function would be through the usage of a *"lock-in amplifier"*.

The "*lock-in*" amplifier is a common instrument used in solving signal-to-noise problems in research laboratories. It is a very powerful instrument where signals of interest can be detected- even if they are smaller than the noise signals they accompany. Lock-in amplifiers use a technique known as phase-sensitive detection to single out the component of the signal at a specific frequency and phase. Once it does this, noise signals at other frequencies or random phases are rejected through filtering [15].

Circuit designs by other teams have been implemented towards solving a similar problem to our own team's issue.

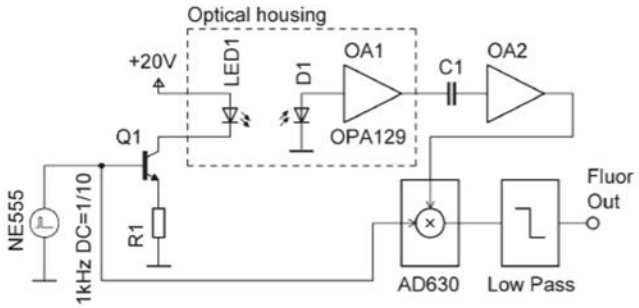


Figure 4.5 Electronic circuitry for the detector [16]

Figure 4.5 demonstrates the circuitry used by the "Fluorescence-based Fiber-optic Chemical Sensors" team. They created a detector system for fiber-optic sensors relying on a light-emitting diode (LED) and a photodiode. The team found that wavelength separation was unnecessary and lock-in amplification alone provided excellent discrimination against fluctuations in ambient light intensity, resulting in reduced complexity and cost [16].

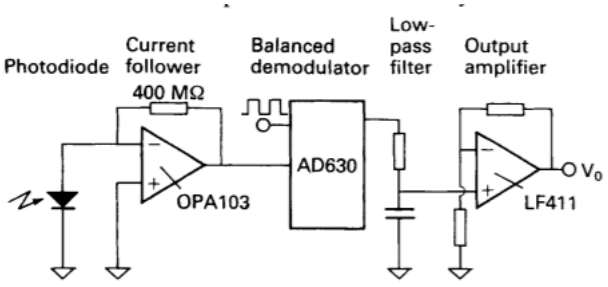


Figure 4.6 Simplified schematic of the electronic circuit for optical excitation and detection.

The LED is powered by current pulses, generated by a pulse voltage generator NE555, and converted into current by transistor Q1. The fluorescence light, detected by a photodiode D1, is

converted into a voltage by OA1. Its output voltage is then amplified by OA2, filtered by a demodulator AD630, followed by a low pass filter

Figure 4.6 shows the "Integrated fluorescence detection system for lab-on-a-chip" team's circuitry implementation for the "lock-in" amplifier. This configuration worked as a filter with a bandwidth of 1.5 Hz, around the frequency of the LED reference signal. Due to the narrow bandpass, the system was insensitive to ambient light and other noise contributors [14].

Adapting and applying the principles of the other team's circuitry for our own team's objectives would prove extremely beneficial. Those specific designs have not only been tested and proven to work with the desired outcome for the other team; additionally, it is relatively cheap, efficient, and has a compact design- perfect for upscaling. The "lock-in" amplifier functions as an additional filter circuit, further reducing the probability of producing an unreliable reading.

4.5 Signal processing on the software side

One of the most difficult parts of building a HAB sensor is that regardless of the circuit design, the signal received from the hardware will still be either too weak or too noisy to be analyzed. Therefore, the primary focus software-wise is to correct the corrupted data and make the signal easier to analyze.

Signal processing can be important when the data is hardly readable due to corrupted data. This is especially true for the current project, since the team is building a low-cost fluorometer and the hardware is less sensitive and might generate more uncertainty, compared to other expensive devices. Processing the signal with software can be a great way to save the budget.

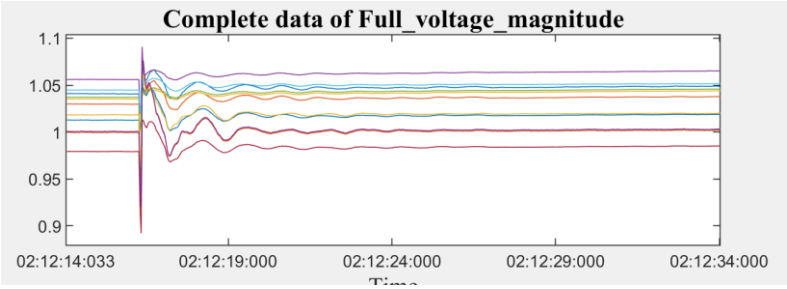


Figure 4.7 Clean voltage data from EPRI

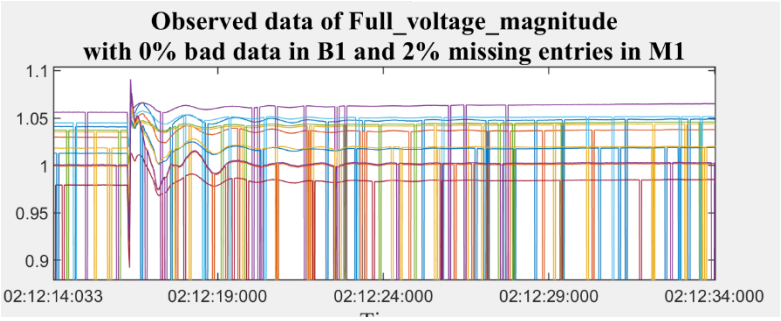


Figure 4.8 voltage data with 2% missing data added

Corrupted data can be divided into two types –missing data and bad data. Missing data occurs when no data value is stored in an observation. This might be due to hardware malfunction at that specific time instant or a certain data point fails to be passed to the storage place. One example from EPRI (Zhenyu Lu's previous research) is shown in Fig. 4.7 and Fig. 4.8 When only 2 percent of missing data was added, the curve becomes much less understandable.

To deal with the missing data, the simplest way is to delete those entries, if the curve is still readable without those missing data points. However, if removing the missing data largely affects how the pattern looks and, especially when the missing data happens not at random (MNAR), and simply removing them might produce a bias in the model. Data imputation could be used to assign values to the missing data. For the current project, time-series specific methods are more suitable, including last Observation carried forward(LOCF), linear interpolation, etc. [17].

Bad data occurs when the computer receives the data point but the data point is inaccurate, inconsistent, or unstructured. This is especially tricky for the current project, since the signal fluctuates heavily even with a high resolution fluorometer (shown in Figure 4.8) and this brings greater difficulty to differentiate real data and bad data. There is no good way to totally separate clean data and bad data because some data points might look weird, but they do record the true level of fluorescence at that time.

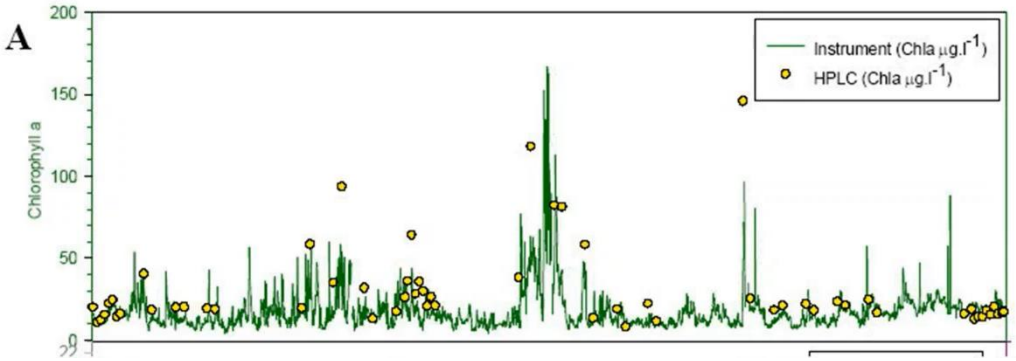


Figure 4.9 Signal of chlorophyll from a typical fluorometer

Therefore, the proposed solution is that when deciding the occurrence of an event (HAB), the program checks whether one or more values from the dataset without any processing are higher than the HAB threshold. Furthermore, signal smoothing can be an add-on function for users to better monitor the trend of fluorescence levels. There are many algorithms that exist for smoothing, including Moving Average, Extracting Average Differences, etc., and algorithm selection depend on the data shape received from hardware [18]. As for now, a moving average filter should be enough for the current project and one example run is shown in figure 4.9 where the blue line represents the original curve and the red line represents the newly generated smoothed line.

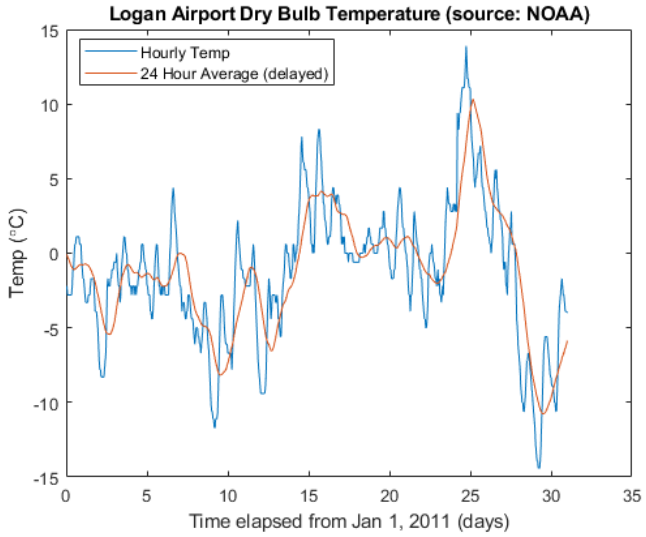


Figure 4.10 Smoothed curve generated by moving average filter [18]

the device from previous group works only under complete darkness and is largely affected by other light sources. This problem can be mostly solved on the hardware side by building a better enclosure (out of scope) and designing a more sensitive circuit. However, the effect of solar radiation, which is proven to be correlated with chlorophyll fluorescence [19] as

shown in Figure 4.11, is not easily modulated on the electrical side. Therefore, software-based solutions are needed to mediate the effect on sunlight.

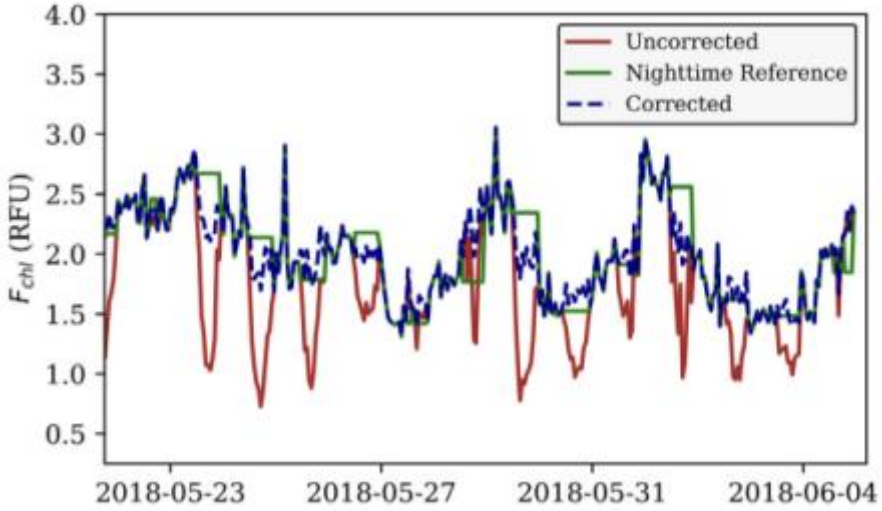


Figure 4.11 the influence of solar radiation over a 24-hour period (Top) on Fchi [19]

Lucius et al. from RPI Jefferson Project (2020) have proposed one possible algorithm. They used random forest regression incorporating chlorophyll, solar radiation, water temperature, depth, and dissolved oxygen saturation together to build the analysis model. For the current project, it does not have to be that complicated, but what can be learned from their study is that they used the data during nighttime when sunlight doesn't exist, as a reference dataset to correct the data during the daytime, and Fig. 4.12. shows a great illustration of their result.

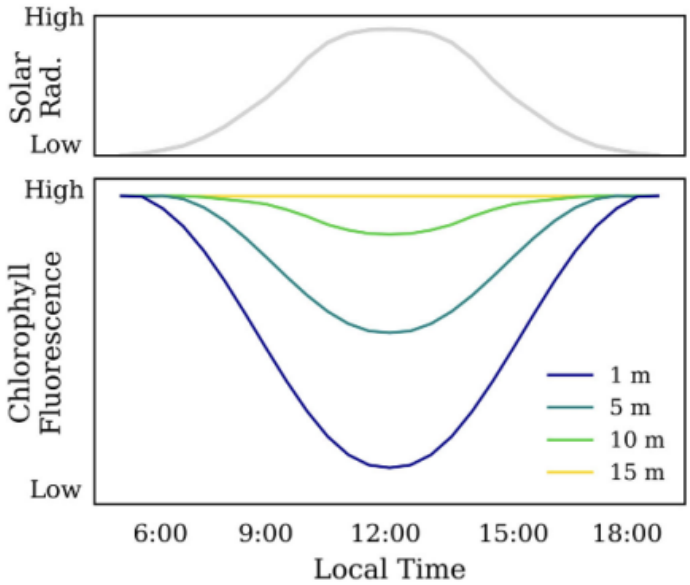


Figure 4.12 Time series plot of uncorrected Fchi, static nighttime reference values, and corrected Fchi [19]

A signal-processing program needs to be constructed to receive the raw data from the hardware. Software-based signal processing techniques will be built into the program to better visualize the data. Matlab is selected as the primary coding tool for its powerful built-in functions and easy testing.

The multi-parameter monitor is widely used in the water quality industry. To detect whether HAB occurs, monitoring other parameters, other than chlorophyll and phycocyanin, can also be useful. Currently, the team focuses on monitoring fluorescence and temperature and monitoring other indicators that might be a little bit out of scope. However, if more indicators are included for analysis, machine-learning techniques, like random forest regression, neural networks, can be trained to make decisions.

5. Customer Needs and Engineering Design Requirements

Workbook Reference: Needs and Requirements Revision 224

The customer needs and project requirements shown in the workbook are organized in this section. Needs and requirements have been sorted into two categories, Overall project needs and requirements and specific needs and requirements. Both categories are shown in Table 2 and Table 3.

Table 2 - Customer Needs and Requirements for Overall Project

Customer Need	Requirement Number	Description	Translated Engineering Requirement
Detect Harmful Algae Blooms	1.1	The device should provide data on the presence of HABs	Device shall be able to detect the presence of HABs
Inexpensive	2	Cost should be low to enable reproduction of the device	The final cost of the device should be less than $500

Table 3 – Specific Customer Needs and Requirements

Customer Need	Requirement Number	Description	Translated Engineering Requirement
Detect Harmful Algae Blooms	1.2	The device should excite a water sample to measure for the presence of HABs.	Choose a light source to excite cyanobacteria pigments (phycocyanin). Light source should emit light at 580-620nm.
Detect Harmful Algae Blooms	1.3	The device should measure for light wavelengths of excited cyanobacteria pigments (phycocyanin) to measure for the presence of HABs.	Choose a photoreceptor to measure for light emitted from excited cyanobacteria pigments (phycocyanin). Photoreceptor should detect 640-650nm light.
Detect Harmful Algae Blooms	1.4	The device should obtain consistent, reliable readings.	The device should have a percent error of less than 5% when detecting samples.
Accuracy	4	The device shall detect varying concentrations of HABs (or representative synthetic pigment Rhodamine B)	The device shall be able to detect samples with HAB or Rhodamine B concentration of at least 0.03 mL/L.

Consistent Sampling Frequency	6	The device will measure results at a specific frequency.	The device shall measure results at no more than 4.0Hz (Sponsor supplied measurement)
Delivery of Results	7.1	The device will deliver results through connection to a home Wi-Fi network.	The device shall prepare a CSV file of results and wirelessly transmit data.
Delivery of Results	7.2	The device will deliver water temperature with the measured results in the CSV file.	The device shall measure the water temperature and output it alongside the other data in a CSV file format.
Delivery of Results	7.3	The device will deliver a timestamp with the measured results in the CSV file.	The device shall output time based on the microcontrollers internal clock and output it alongside the other data in a CSV file format.

6. System Concept Development

A fluorometer or fluorimeter, is a device capable of emitting a prescribed light wavelength exciting a sample in this case that may be suspect for HABs, measuring the light intensity that is fluoresced from a sample, the reduction of ambient light is done through design and the use of filters.

Concept generation was conducted and using decision matrixes, systematic decisions for each major component was employed in a device that is capable of detecting algal blooms. Criteria pertinent to each concept will be realized and a scoring system assessed by the team ranging in values from 1 (worst) to 5 (best) will be used to make selections in an unbiased methodical way.

To understand how subsystems will be utilized together to meet the needs and requirements of the customer and ensure project criteria is realized, Figure 6.1 showcases how different components work together to achieve autonomy of the whole system. The microcontroller will signal the excitation light sources on/off through a defined frequency. And the light source at a specific frequency irradiates the samples, causing it to fluoresce. The fluorescence emitted from the samples are captured by the photodiode in the form of electrical signal and the electrical signals are processed in the signal processing unit and saved in the microcontroller. The measurement is then recorded in a CSV file and is no longer held locally.

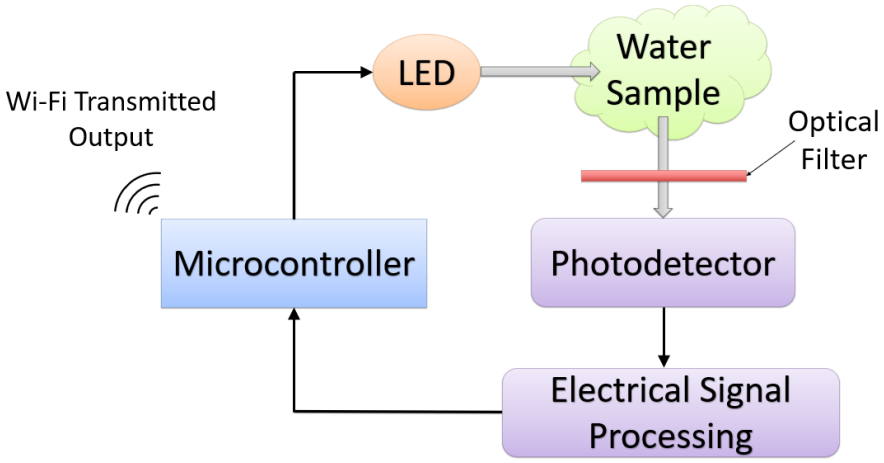


Figure 6.1 – Basic System logic

The whole system was composed of three major subsystems including Microcontroller unit, Fluorescence detection and signal processing.

Fluorescence detection

There are generally two types of fluorescence detection designs -- Filter Fluorometer and Spectro-fluorometer.

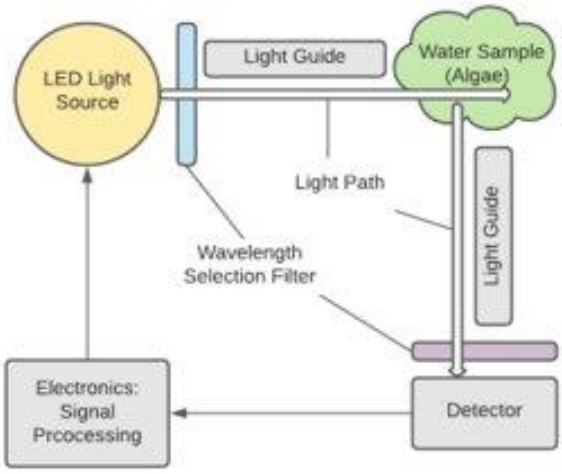


Figure 6.2 – Filter Fluorometer Block Diagram

Figure 6.1 displays the block diagram of the components that comprise a functioning filter fluorometer. To begin, a light source is signaled on, passing through a wavelength selection filter, following a light path illuminating by excitation the water sample. The water sample will then emit or fluoresce through another light guide and a secondary wavelength selection filter and into a detector that is able to take a measurement of light intensity.

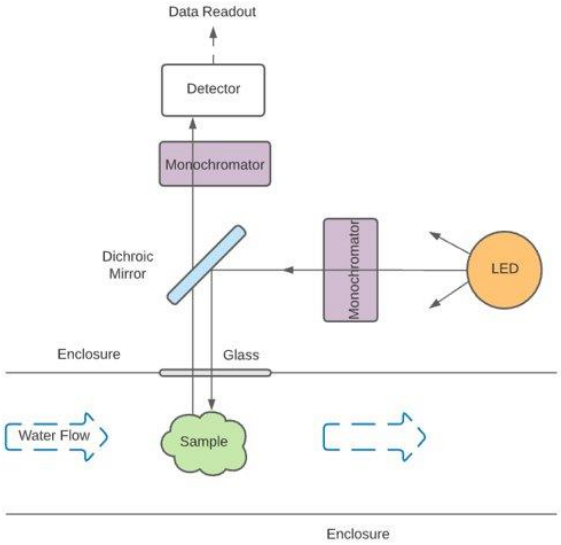


Figure 6.3 – Spectro-Fluorometer Block Diagram

The Spectro-fluorometer as exhibited in Figure 6.2 displays a light emission source that passes through a monochromator to refine the signal to narrower bands meeting a dichroic mirror or beam splitter, where the light received will either be passed through the mirror or reflected, by design. The reflected light will pass into the sample and flow back through the mirror,

again by design and onto a second monochromator where the light received will be filtered to refine the response and strengthen what is measured on the detector.

Table 6.1 compares these two designs that may be incorporated into a field deployable device that meet the ability of the customer's needs. The criteria considered include: the number of components, as this may have some correlation to the complexity of the design. The cost, as the team is subject to budgetary constraints. The amount of space each concept may require, and whether each are able to be placed within waterproof enclosures, and how well the level of technology within are able to detect light intensity.

Table 6.1 - Detection Method Decision Matrix

Criteria	Filter Fluorometer (Figure 6.1)	Value	Spectro-fluorometer (Figure 6.2)	Value	Weight
Components (#)	~ 6	3	~ 6	3	0.1
Cost	Less [1]	4	More [1]	2	0.2
Footprint Required	Less [2]	5	More [2]	3	0.2
Weatherproof Capable	Yes	5	Yes	5	0.3
Accuracy	Decent resolution	2	Better resolution	5	0.2
Total:		19		18	
Weighted Total:		4.0		3.8	

1 - A less or more statement is valid here as each concept price requires specific components, that may only be obtained through written interest to the manufacturer. However, it is known that the price of LEDs and photodetectors as compared to the price of monochromators and photomultipliers is substantially lower, relative to one another.

2 – Based on the geometry of the components as aforementioned in clause 1, LEDs and photodetectors as compared to the size of monochromators and photomultipliers is substantially less.

The filter fluorometer was given the higher score, due to the cost and having a relative smaller footprint than that of which would be required for the Spectro-fluorometer. Although it is known that the filter fluorometer is not as accurate as the Spectro-fluorometer, to satisfy the customer's needs and requirements as outlined in Section 5, due to budget constraints and the number required a filter fluorometer meets the customer's criteria.

Signal processing

Learned from the experience from previous group, one of the major challenges that they faced was that the signal received from the detector was too noisy to be analyzed. The primary focus of current stage is electrical signal processing, and if needed, numerical signal processing algorithm might be incorporated into the design.

Microcontroller Unit

Microcontroller plays an important role of coordinating and controlling different components. Some of its major functions include saving and sending data collected, converting analog signals to digital signals, controlling the on/off of LEDs, etc.

Two types of microcontrollers are present in Table 6.2: Arduino Nano and Raspberry Pi. The criteria that were sought: Cost, WIFI capable, data storage, general purpose input/outputs (GPIO), clock speed, and the power consumption requirement.

Table 6.2 - Microcontroller Decision Matrix

Criteria	Arduino Nano	Value	Raspberry Pi Zero	Value	Weight
Total Cost	$10.5 (purchase 5) $26 (purchase 1)	4	$19	5	0.2
WIFI capability	Yes, can be added externally	2	Yes, internally	3	0.1
Data Storage	Yes, can be added externally	2	Yes, has internal data storage	3	0.1
Size (in)	1.7 x 0.7 x 0.4	5	2.56 x 1.18 x 0.2	4	0.2
GPIO	20pins, 5V tolerance with ADC Pin	5	40 pins, 3.3V tolerance. No ADC Pin	2	0.5
Clock Speed	16 MHz	3	1.4 GHz	5	0.1
Power Consumption	175 mW	5	700 mW	2	0.2
Total:		26		24	
Weighted Total:		6		4.3	

The microcontroller chosen is the Arduino Nano as the size, cost and ports selection aligned with producing a cheap, small deployable device. Although the capabilities of the Raspberry Pi Zero are higher than the Arduino Nano, it does not have analog to digital pins and each pin only has 3.3V tolerance. These two factors make using a temperature sensor and light diode inconvenient. Therefore, Arduino Nano is chosen over Raspberry Pi Zero. Also, with the extensive library Arduino has, it will be easier for development of code.

Photodetector and Light Source Concepts

Two key components in the fluorometer circuit are the light source which is used to excite the sample and the photodetector which is used to measure the emission of the sample. Both components were selected based on the project requirements developed from customer needs. The source LED was selected based on requirement 1.2 which states that the light source needs to excite cyanobacteria pigments (phycocyanin) which occurs with light wavelengths of 580nm to 620nm. The photodetector was selected based on requirement 1.3 which states that the photodetector needs to measure light emitted by the phycocyanin which is light at wavelengths 640nm to 650nm [20]. A visualization of this relationship can be seen in Figure 6.6.

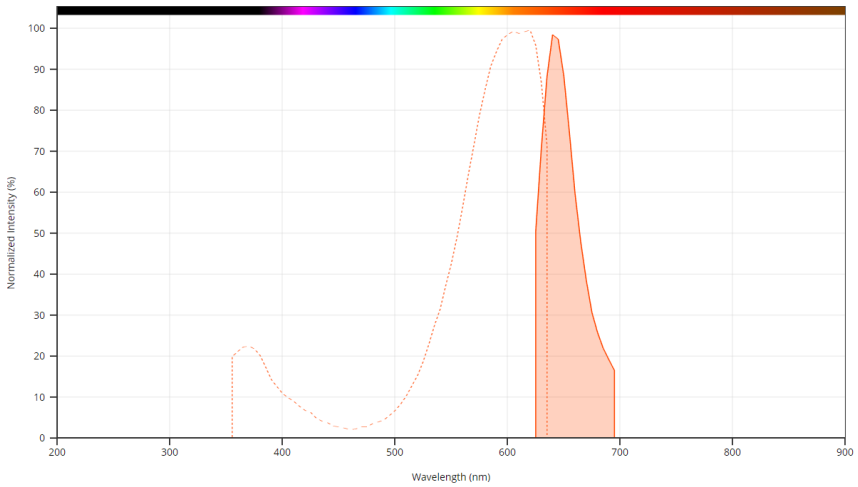
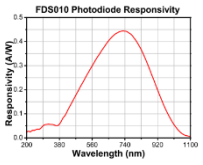
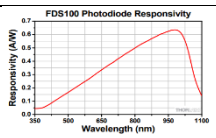
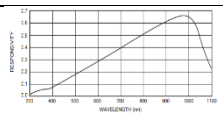


Figure 6.4 –Excitation and Emission Spectrum of Phycocyanin [20]

The unshaded region to the left is the excitation range and the shaded region to the right is the emission range. The light source responsible for exciting the sample was selected based on several criteria with a focus on selecting a high intensity light that delivered light in the 580-620nm wavelength range.

The photodetector responsible measuring the light emitted by the sample was also selected based on several criteria with a focus on selecting a component that had a high responsivity to the emitted light in the 640-650nm range. Several photodetector options are present in Table 6.4: FDS010 Photodiode, FDS100 Photodiode, and ODD-15W Photodiode

Table 6.4 – Photoreceptor Decision Matrix [21] [22] [23]

Criteria	FDS010	Value	FDS100	Value	ODD-15W	Value	Weight
Wavelength Range	200-1100 nm	1	350-1100 nm	2	400-1100 nm	3	0.1
Peak Wavelength	730 nm	4	980 nm	2	940 nm	3	0.2
Price	$48.15	1	$14.94	3	$13.48	4	0.2
Response Time	1 ns	5	10 ns	3	20 ns	2	0.1
Responsivity		5		3		1	0.4
Total:		16		13		13	
Weighted Total:		3.6		2.7		2.3	

The photodetector chosen is the FDS010 as the peak wavelength and Responsivity curve are desirable for the range of light that needs to be measured. This photodiode has a responsivity peak closer to the desired range than the other photodiodes which will result in a stronger output for the wavelengths the device is designed to measure.

Optical Filtering Method

To limit the amount of additional light that the photodiode could be detecting when the water sample is fluorescing an optical filtering stage will be used. There are two primary methods that can be used to filter light before it reaches the photodiode. Illustrated in Figure 6.6 one filtering method is using an excitation filter which involves placing a filter just after the source LED and filtering the light coming from the LED. The other method is using a emission filter which involves placing a filter just before the photodiode and filtering the light coming from the sample and LED. Both methods can help focus the light towards the desired specific range of light wavelengths needed to both fluoresce the sample and be measured by our photodetector.

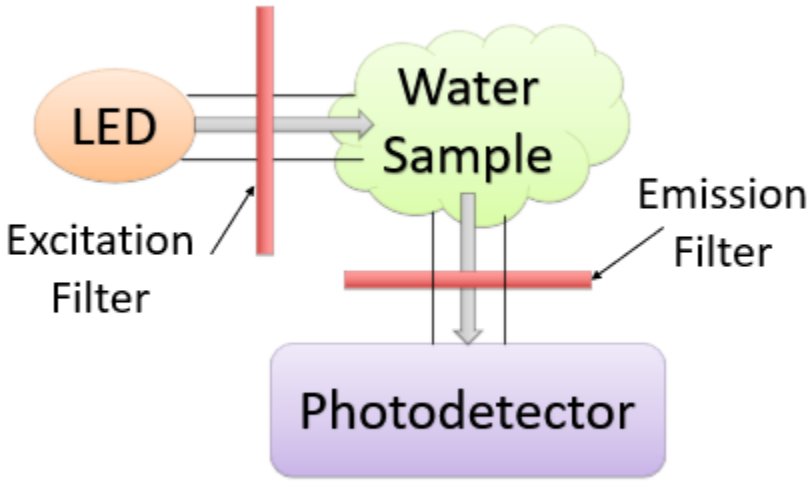


Figure 6.5 – Optical Filtering Concept Block Diagram

Based on our overall fluorometer design the main source of ambient light we wanted to limit was additional wavelengths coming from our source LED. Our light source is the MTE6000L-HP an amber LED with a peak wavelength of 600nm, the spectrum of light provided by the LED is shown in Figure 6.7.

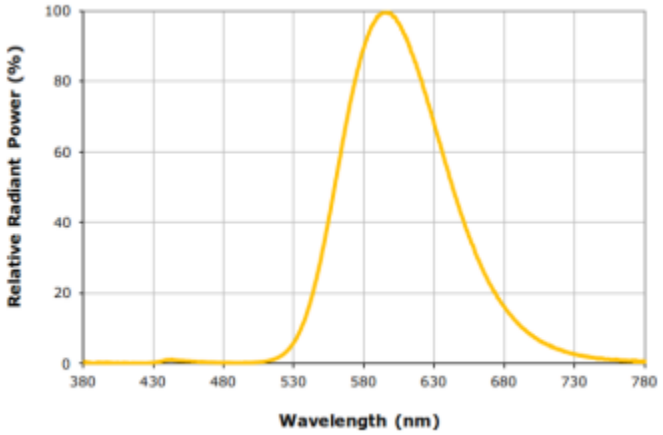
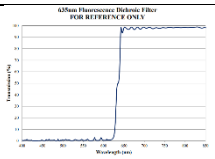
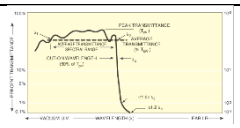
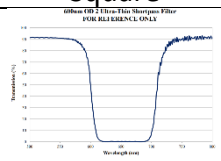


Figure 6.6 – MTE6000L-HP Light Spectrum [24]

The source LED will provide the wavelengths needed to excite our sample but it will also emit wavelengths greater than 620 which could be measured by the photodetector. To filter out these wavelengths we need to use either an excitation filter that can filter out light at wavelengths greater than 620 or use an emission filter that can filter out light at wavelengths less than 640. Using an emission filter will ensure that the light reaching the sample is light that will excite the sample but will not add to the light being emitted by the sample at the same wavelengths of 64-650nm. Using an excitation filter will ensure that the light reaching the photodiode is only light greater than 640 which is the light being emitted by the sample and is the only light we are interested in measuring. A combination of both of these filters would provide the greatest results but using more than one filter greatly increases the cost of manufacturing this design. An analysis of which filter was used in the final design is shown in Table 6.3.

Several filter options are present in Table 6.3: 635nm Dichroic Filter, 600nm Shortpass Filter, and Everix Ultra-Thin OD 2 Shortpass Filter.

Table 6.3 – Optical Filter Decision Matrix [25] [26] [27]

Criteria	635nm Dichroic Filter	Value	600nm Shortpass Filter	Value	Everix Ultra-Thin OD 2 Shortpass Filter	Value	Weight
Cut-On/Off Wavelength	635nm	3	600nm	3	600nm	3	0.2
Filter Method	Emission Filter	1	Excitation Filter	1	Excitation Filter	1	0.1
Price	$108.50	3	$135	1	$110	2	0.2
Size	12.5mm Diameter	5	25.4mm Diameter	3	12.5mm Square	5	0.1
Filter Response		3		4		4	0.4
Total:		15		12		15	
Weighted Total:		3.0		2.8		3.2	

The optical filter chosen was the Everix Ultra-Thin OD 2 Shortpass Filter as the filter response was very desirable because it would block all light greater than 600nm and less than 700nm coming from the LED. This response is desirable because there is very little light coming from the LED that is greater than 700nm so the only light reaching the sample are wavelengths less than 600nm. This means any wavelengths measured by the photodetector that were greater than roughly 600nm were coming only from the sample. This filter was similar in price to the dichroic emission filter but this response as an excitation filter removed more of light in the 600-650nm range that could be coming from the LED. This optical filter was used as an excitation filter and performs the desired function of filtering out light greater than 620nm.

Temperature sensor

Temperature sensor is a standalone subsystem that is separated from the fluorometer. However, it is very necessary to continuously monitor the temperature because water temperature is directly related to the growth and persistence of HAB. Therefore, a digital temperature sensor is incorporated into the design. Current design doesn't require a high-precision temperature sensor and the only special requirement is its waterproof ability.

Table 6.4 – Temperature Sensor Selection Decision Matrix

Criteria	Adafruit 381	Value	LM335Z	Value	Weight
Size (Diameter)	4 mm	5	5.2 mm	4	0.1
Price	$9.95	2	$0.48	5	0.3
Temperature Range	(-55 to 125) °C	4	(-40 to 100) °C	3	0.2
Accuracy	± 0.5 °C	5	± 1 °C	4	0.1
Waterproof	Yes	5	No	1	0.3
Total:		21		17	
Weighted Total:		7.7		4.2	

The Adafruit 381 is clearly the choice due to its design being waterproof versus the cheaper alternative, additionally this is a more accurate device, and can operate over a broader range of temperatures.

7. Design Analysis

7.1 Circuit Design

The prototype circuit design is shown in Figure 7.1. The photodiode acts as a current source when it is exposed to light. The intensity of light is proportional to output current produced by the photodiode. The magnitude of the current is in nano-amps. For the Arduino to be able to read this voltage signal, the circuit principles of a transimpedance amplifier circuit (Figure 7.2) were implemented in order to convert and amplify the photodiode current to voltage. The section on the left is a voltage follower circuit that converts the current from the photodiode to a voltage via the bias resistor connected in series with the photodiode. The current produced by the photodiode flows through the bias resistor, creating a voltage drop across it. This voltage across the resistor is expressed in the output of the circuit. The section on the right is a non-inverting amplifier; this amplifies the voltage of the bias resistor by a factor before the output of this section flows into the Arduino. Choosing values for R_f and R_{in} to both be to 1kiloohm, a gain of 2 is achieved from the non-inverting amplifier based on the equation: $Gain = 1 + \frac{R_f}{R_{in}}$

The benefit of this circuit configuration, as opposed to a standard transimpedance amplifier circuit, is that the signal quality can be increased via a reduction in the output signal noise from the voltage follower stage of the circuit. Although the response time of this circuit configuration is lower than that of a transimpedance amplifier, sampling rate at which we record data makes this problem negligible.

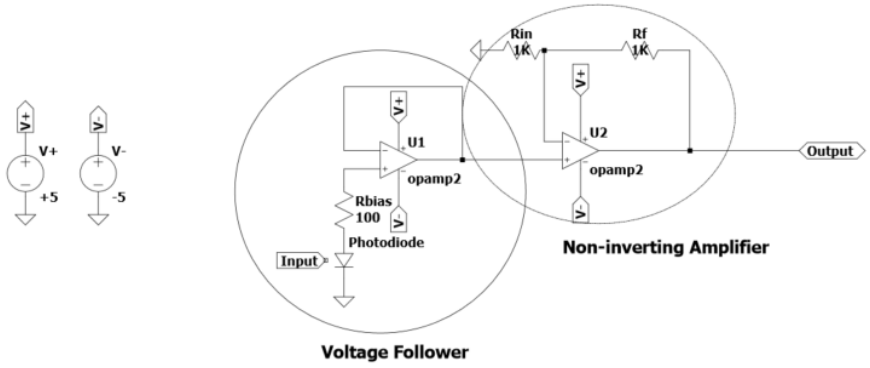


Figure 7.1 – Prototype circuit design

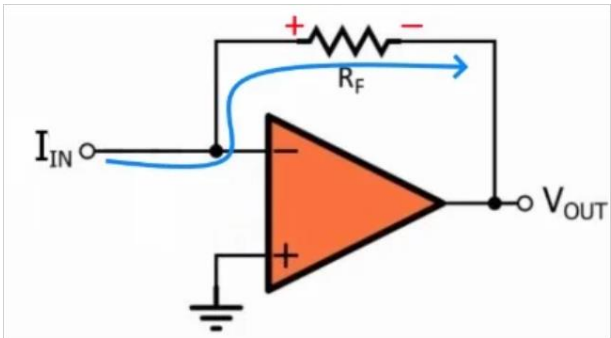


Figure 7.2 – Transimpedance Amplifier (TIA) Circuit Principles

From this prototype circuit, experimentation was performed to determine which circuit components would meet the necessary photodetector amplification requirements for the Arduino to be readily able to read and record. An amber LED was utilized to mimic the wavelength of that would be utilized in exciting the sample, causing the fluorescent light to hit our photodetector. The data from our prototype is shown in Figure 7.3.

Figure 7.3 – Prototype Circuit Data

7.2 Optical Filter Evaluation

To quantitatively compare the filters being examined, a metric called the Added Extra Light (AEL) was developed. An ideal excitation filter would filter out all light in the emission period and higher, thus all emission period light reaching the photodiode would be from the sample's fluorescence. The AEL was developed by calculating the difference between the ideal filter and the tested filter at every relevant wavelength. Then a root mean squared average is taken of these difference terms shown in the equations below. I_i is the ideal intensity and A_i is the actual experimental intensity.

$$AEL = \sqrt{\frac{1}{160}\sum_{i=640nm}^{800nm}(I_i - A_i)^2}$$

Each filter was evaluated with this score to compare. 4 different colored films were evaluated against the Everix Shortpass Filter. Without a filter, the amber light alone has an AEL value of 22.9%. Hence, the Red and Blue Films perform worse than no filter. The Yellow and Green Films perform slightly better than no filter. The Everix Shortpass Filter performs the best with a score of 13.8%

Filter	AEL
Ideal Filter	0%
No Filter	22.9%
Red Film	23.1%
Yellow Film	22.1%
Green Film	21.9%
Blue Film	69.3%
Everix Shortpass Filter	13.8%

7.3 Temperature sensor

The implementation of the temperature sensor is straightforward. The adafruit 381 sensor is a digital sensor that can connect to the microcontroller directly with a pull-up resistor of 4.7k ohm wired between the data pin and the source pin as shown in Figure 7.4. Although calibration wasn't conducted so far, it did produce reasonable values when measuring the room temperature and it is also acceptable for current project even if minor errors exist.

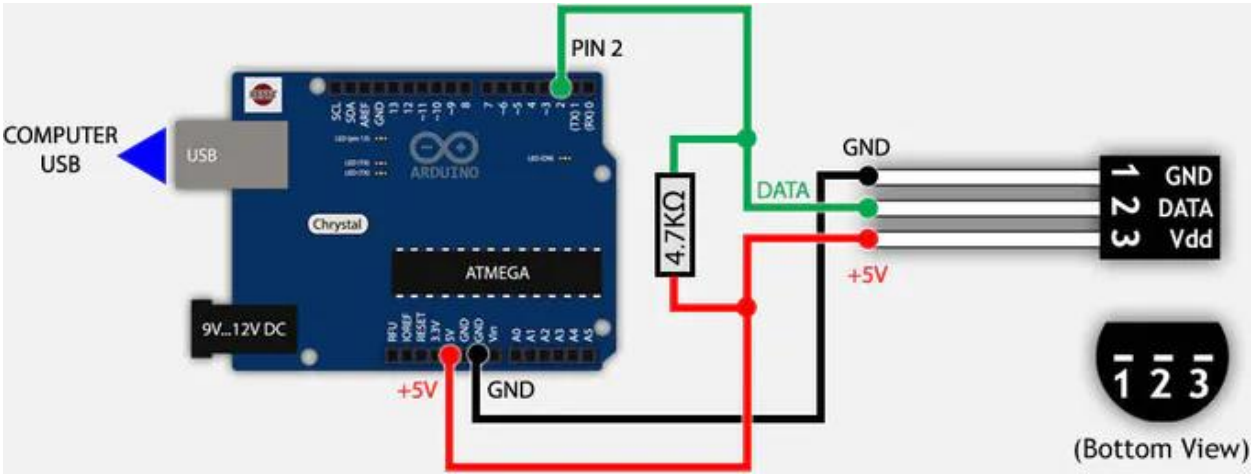


Figure 7.4 – Temperature sensor circuit

7.4 Microcontroller Design

Microcontroller unit (MCU) plays an important role of linking all the subsystems together as a whole. Figure 7.5 shows how it brings all the subsystems together. The primary function is to assign orders to each subsystem. It can control the ON/OFF period of the light source used to excite the sample and receive the signal from the photodiode after processing. Since the signal transmitted in is analog, the built-in ADC unit of the microcontroller can convert it to a digital one. The signal from the temperature sensor is already in the digital form and, therefore, there is no need to convert. However, the DallasTemperature function within the Arduino libraries is needed to control the sensor to take measurements and it also performs the role of converting the received digital signal into a Celsius value. All the collected data is then stored in the SD card module.

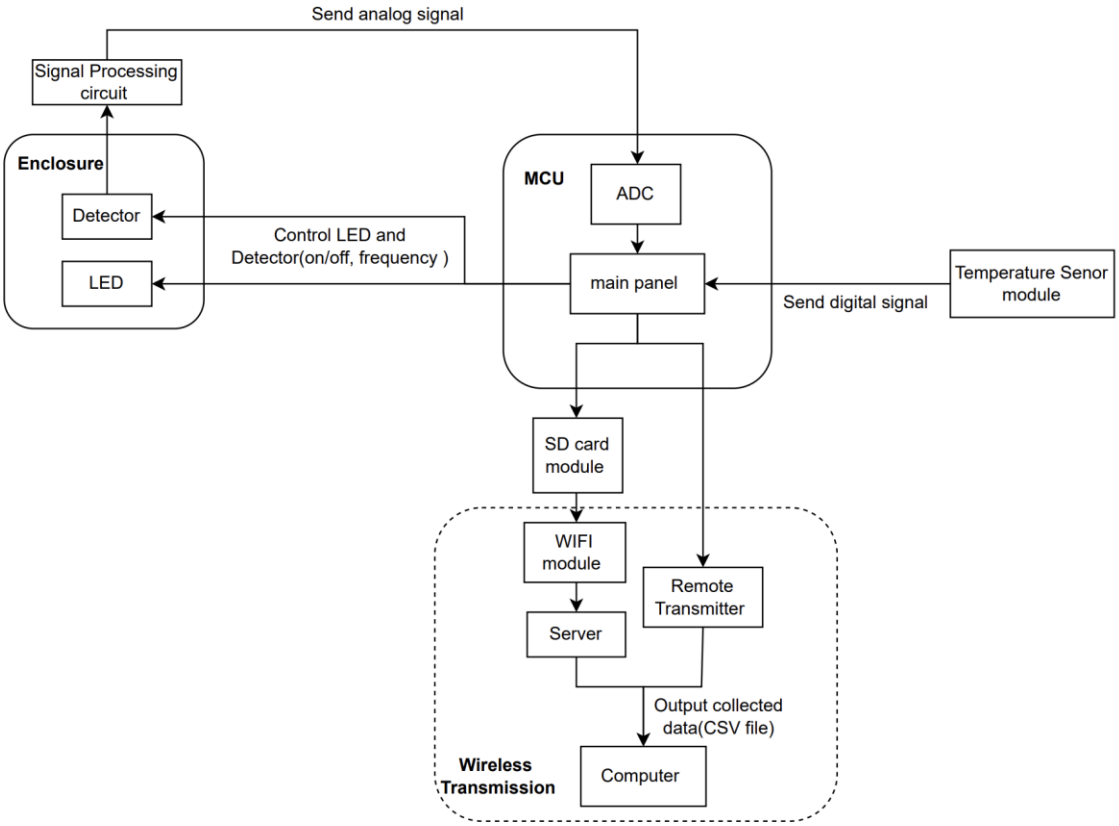


Figure 7.5 – Microcontroller flow diagram

The program operation of the MCU is illustrated in Figure 7.6. The program enters a loop which switches between ON and OFF states of the LED. Three measurements are collected for each iteration, including ON-time fluorescence, OFF-time fluorescence and temperature.

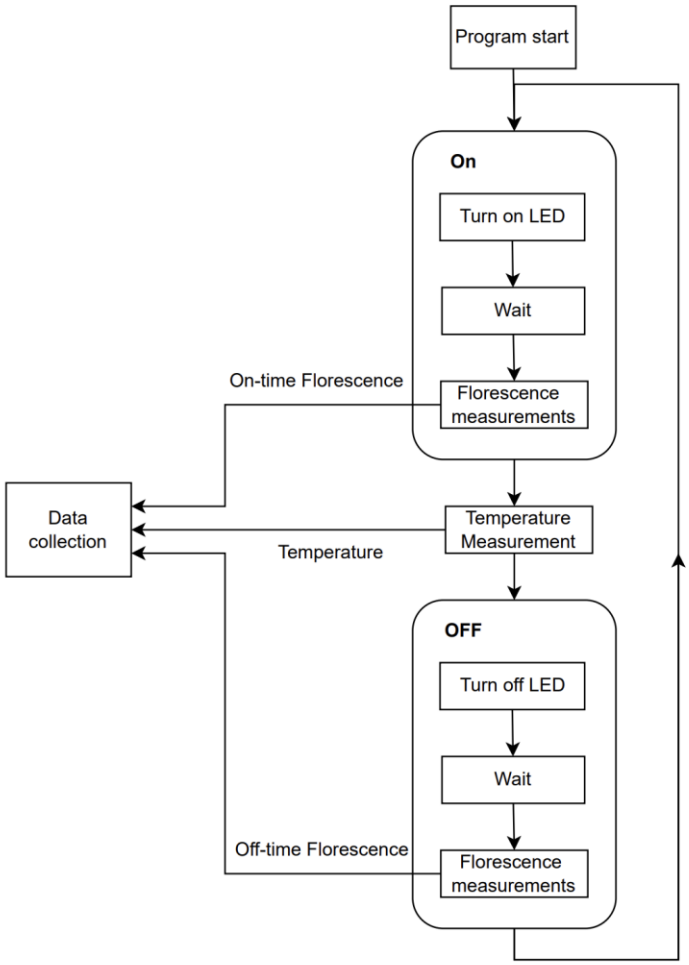


Figure 7.6 – Program operation

The mechanism explaining how it works in extensive detail is shown in Fig 7.7. The ON state of the light source is shaded in blue, and the OFF state is shaped in red. The period length is all adjustable. Through experiments, we found that it takes time for the sample to get excited or get back to normal state after the light source turns on or off. Therefore, the fluorescence measurement does not start until the florescence becomes stable. Multiple measurements are also taken for each measurement period but only the average for each measurement period is recorded. Temperature is measured only once for each iteration and happens right after the end of ON state.

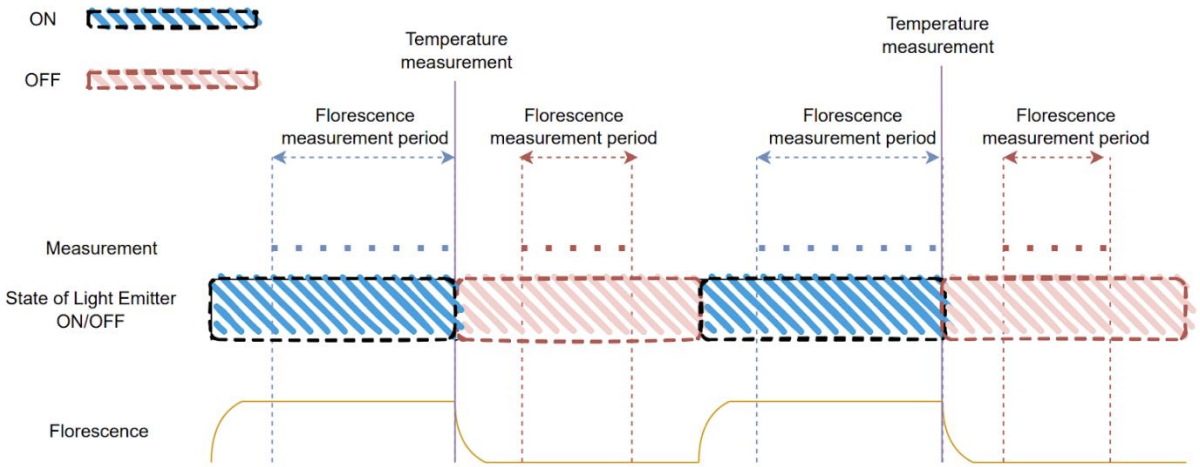


Figure 7.7 – System timing diagram

8. Final Design and Engineering Specifications

The development of the final design for the device was separated into three subsystems, the circuitry, microcontroller integration and programming, and physical components such as the optical filter and temperature sensor. Each of these subsystems were designed based on the concepts generated in Section 6, the final presentation of each part and the device is shown in this section. A high-level overview of the final design is shown below in Figure 8.1.

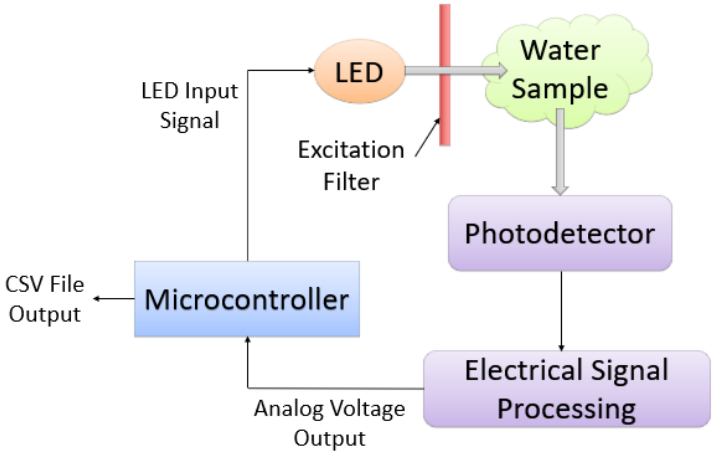


Figure 8.1 – Final Design Block Diagram

Our final design for our fluorometer device follows the filter fluorometer concept selected in Section 6 which utilizes a light source to excite a sample and a photodetector to measure the emission of a sample both separated by a right angle with the water sample in between. The microcontroller controls the frequency at which the light source operates at and measures the results output by the photodetector while the light source is both on and off. This data is collected and averaged over several readings and output to a CSV file which can be transmitted wirelessly. Rather than using two unique excitation and emission filters which would greatly increase the cost of manufacturing the device the team opted to use one excitation filter. The function of this filter is to block light at wavelength greater than 600nm from reaching the sample. This ensures that any light greater than 600nm measured by the photodetector has come from the sample and not the source LED. The final design was tested both with and without the electrical signal processing subsystem and results were not significantly different which was not what was expected. These results are described in Section 9.

The final design of the circuitry is shown in Figure 8.2. After some experimentation with the prototype, the bias resistor in the voltage follower stage was removed in favor of a shunt resistor. This shunting creates a voltage drop across the resistor that is equal to the voltage drop across the photodiode. Although the photodiode ultimately functions as a current source, its equivalent circuitry (Figure 8.3) generates a small voltage drop across it. However, the internal junction capacitance of the photodiode causes the output voltage to oscillate. This oscillation is reduced via the high input impedance of the voltage follower stage, making this configuration

more favorable as the linearity of the circuit output increased. Unfortunately, the previous gain of 2 was not enough for proper amplification. This issue was easily fixed via changing the Rf (feedback resistor) to a value of 100 kiloohms, increasing the gain to 101. This increase in output linearity and circuit stability proved enough to be utilized as the final design.

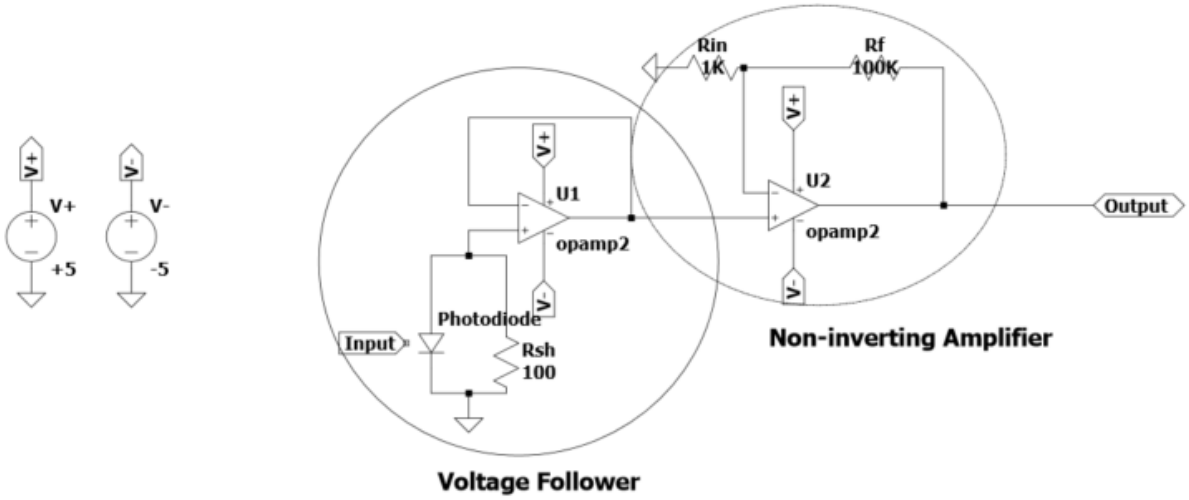


Figure 8.2 – Final Circuit Design

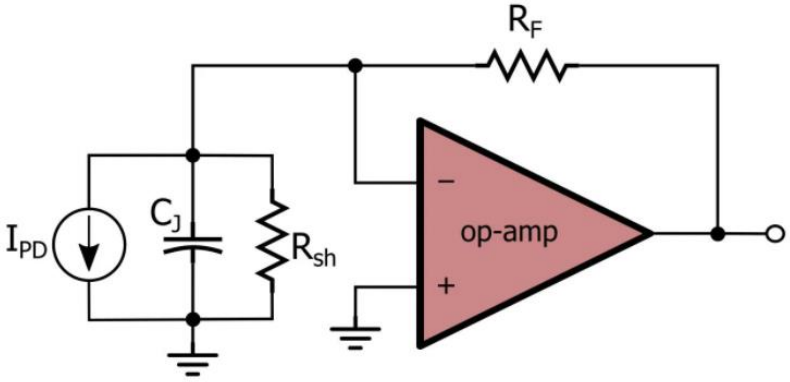


Figure 8.3 – Equivalent Photodiode Transimpedance Amplifier Circuit

The detailed wiring diagram for the Arduino microcontroller is shown in Figure 8.4. D5 is the pin used to receive digital signals from the temperature sensor and D2 is the pin used to control the state of the light emitter. A9 is responsible for receiving analog signals from the photodiode after processing and the specific wiring for SD card module is also shown in Figure 8.4.

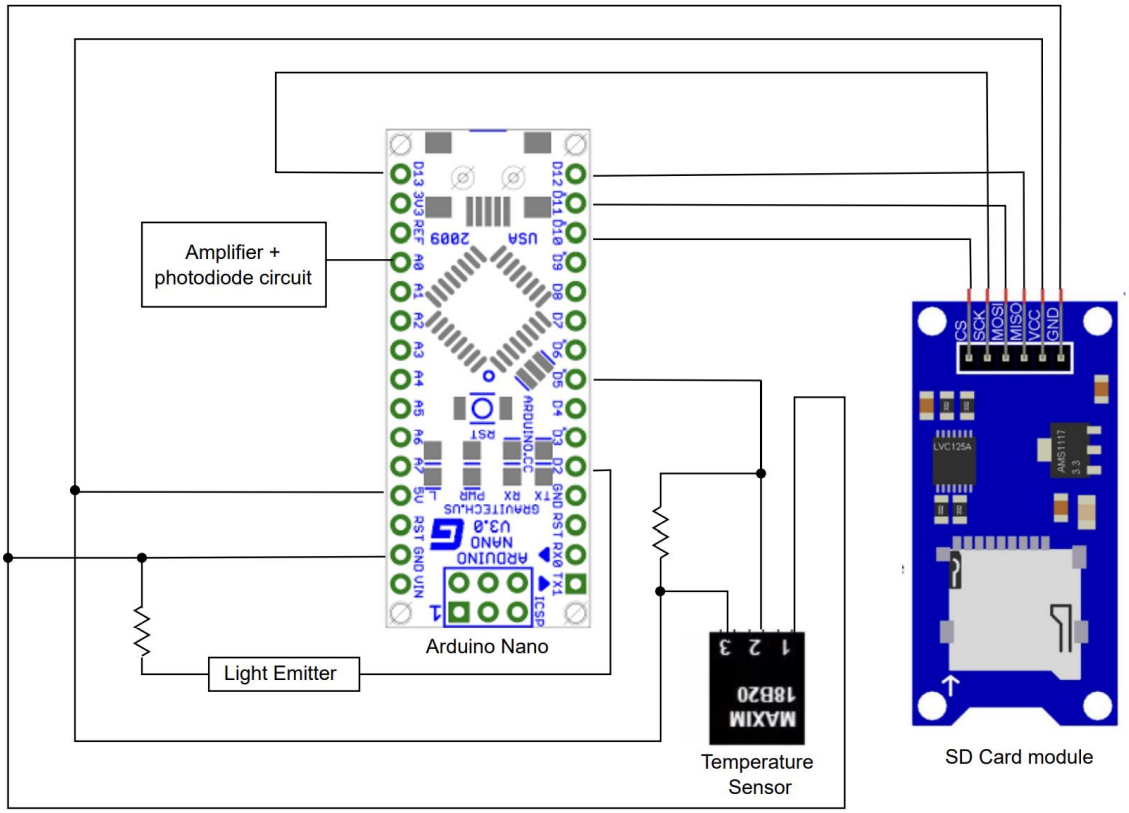


Figure 8.4 – detailed circuit schematic

Parameter setting for the program includes:

1. The length for each iteration is set to 1.1 second with 0.55 second of ON state period and 0.55 second of OFF state period.
2. Based on experience learnt from experiments, the delay time to ensure the fluorescence of the sample stable is set to 0.05 second.
3. 100 measurements are taken for each measurement period and the interval between two measurements is 1 millisecond.

The current parameter setting is for experiment purpose only and since there is a requirement that sampling frequency should be more than 4 Hz, the program should be adjusted (set a shorter period) in accordance with the required frequency, when implemented.

The wireless data transmission module is not included in the current design, but two possible implementation suggestions are offered as shown in Figure 8.5. Firstly, since the data is stored in a CSV file in the SD module, the user may choose to add a WIFI module to the system, such as ESP8266 microchip, and transmit the saved data to a web server. Then the user can download the data to a local computer from the server. The second option is to install a remote transmitter. For instance, nRF24LU1 2.4GHz 1mW USB Wireless Data Transceiver is a module that can be inserted into a computer with the USB port. This method can directly link the device to a local computer via a Python program (already included) and is able to update the collected data synchronously if needed. The powerful Python library is also a huge benefit for further numerical data processing if needed.

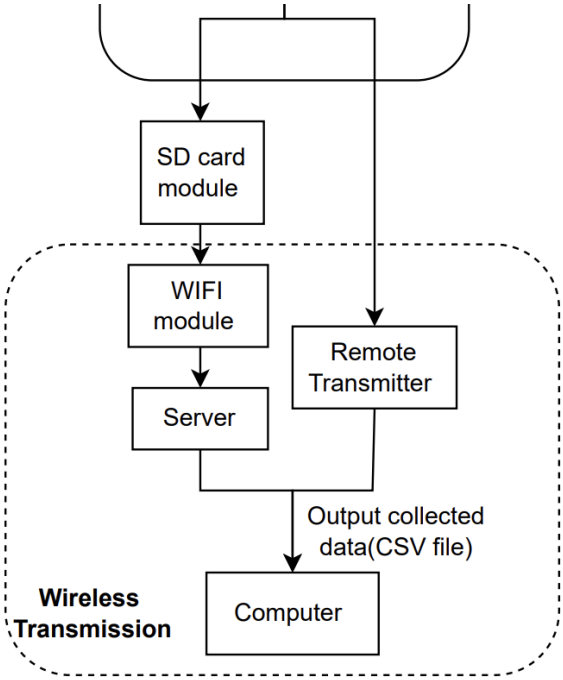


Figure 8.5 – wireless data transmission

The final design of the optical filter used in our fluorometer follows the concept selection shown in Section 6 and utilizes the filter analysis described in Section 7. The selected filter shown in Table 6.3 was the Everix Ultra-Thin OD 2 Shortpass Filter. This filter was chosen based on its cut off frequency and its filter response which can be seen in Figure 8.6.

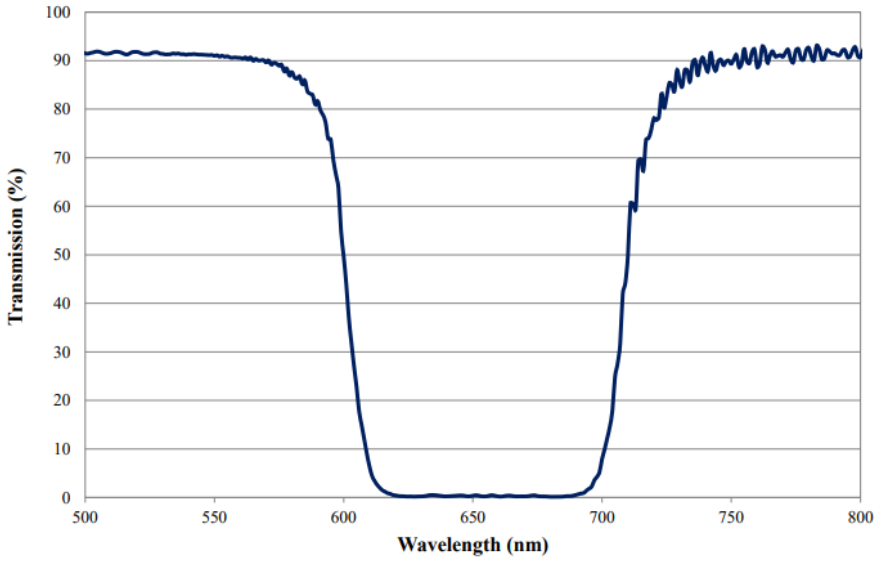


Figure 8.6 – Everix 600nm Shortpass Filter Response

The selected filter is used as an emission filter and blocks all light coming from the LED that is greater than 600 nm and less than roughly 700 nm. Our target wavelength of light to measure for is 640 to 650 nm which is ideal for this filter because no light coming from the LED should reach the photodiode at these wavelengths. This means any light measured in the target range of wavelengths should be coming from the water sample assuming there is no other ambient light present.

Additionally, our final design includes a digital temperature sensor that reads the temperature of our water sample when the sample is to be measured. The adafruit 381 digital temperature sensor was selected to be used in the final design as an additional recording sensor because of its waterproof specifications and ability to cover a wide range of temperatures with greater accuracy. The adafruit temperature sensor can measure across the range of -55 °C to 125 °C with an accuracy of ± 0.5 °C. The temperature of the samples the fluorometer will be testing will not reach the extreme ranges of this senser but the greater accuracy and ability to function submerged in water make this sensor strong choice for use in the final design.

9. System Evaluation

The goal of testing is split into 2 phases. First, the system was evaluated on how well it can perform against the engineering specifications. The device should be able to input a sample with a concentration of fluorescent material and output a digital value proportional to the sample concentration. The first phase of testing measures this directly, evaluating the accuracy and trend, the repeatability and consistency, and the ability to detect the threshold concentration of 0.30µg/L. A success in accuracy requires testing of multiple different concentrations, achieving an R^2 of 90% or higher for the linear regression. The measure of precision requires that 95% of points is within 3 standard deviations of the mean value. The detectability of the threshold concentration is evaluated by a hypothesis test measuring whether 0.30µg/L and 0µg/L have statistically significantly different output value.

Rhodamine B is synthetic biological sensor with fluorescent properties like blue-green algae with a peak wavelength emission of 637nm. It can be easily diluted in water but requires more care to handle – see chemical information. The Rhodamine content can be used until the labeled expiration date if the bottle is stored in 5 °C to 30 °C and the bottle is tightly sealed. A concentration of 0.30µg/L Rhodamine B correlates to the average amount of algae in needed to reproduce significantly. Smaller concentrations are not large enough and have a high likelihood of dying out. Rhodamine B is measured at 5 different concentrations ranging from 0.30µg/L to the concentration of the stock 9.60µg/L.

9.1 Phase 1 Testing

The following table shows a dilution guide of how to make the 6 designated concentrations. The smaller 2 concentrations were made at higher batches to lower the

uncertainty and due to equipment constraints. Solutions were made using a 9.60µg/L stock solution.

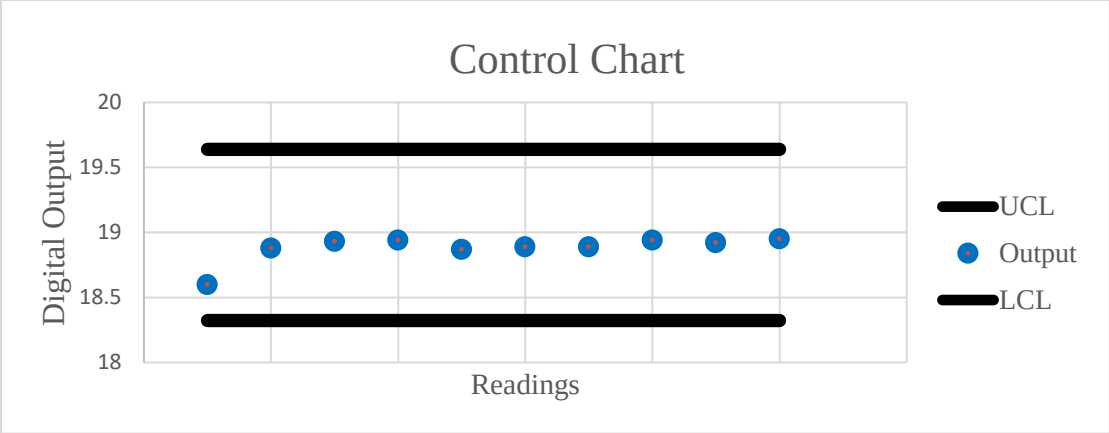
Table 9.1 – Rhodamine B Sample Dilution Guide

Concentration µg/L	Total Volume mL	Stock Volume mL	Water Volume mL	Uncertainty µg/L
9.60	4.00	4.00	0.00	0.34
5.00	4.00	2.08	1.92	0.27
3.00	4.00	1.25	2.75	0.25
1.00	8.00	0.83	7.17	0.12
0.30	8.00	0.25	7.75	0.12

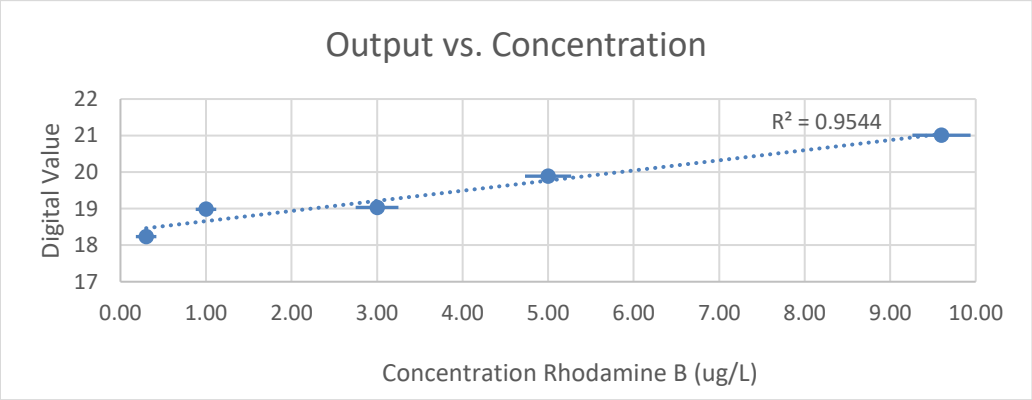
Each concentration was measured 10 times, with each reading being an average of 100 readings. The following data was collected with the selected filter and LED but without the amplifier circuit attached. The procedure follows:

1. Measure out proper stock volume using a graduated cylinder
2. Fill graduated cylinder to the desired total volume
3. Agitate the sample to properly mix
4. Transfer solution to a cuvette and place in device
5. Cover device with lid and run the program
6. Collect outputted data

The system exceled in measures of repeatability. Below is a control chart outlining the readings taken with 1.00µg/L. All points lie within the control limits for all samples, so the system passes the test of repeatability.



In terms of accuracy, the linear trend of the system was measured with a linear regression. Below, the trendline is plotted over the collected data. Overall, the plot has an R^2 which exceeds the 90% benchmark. Thus, the device successfully outputs a linear trend.



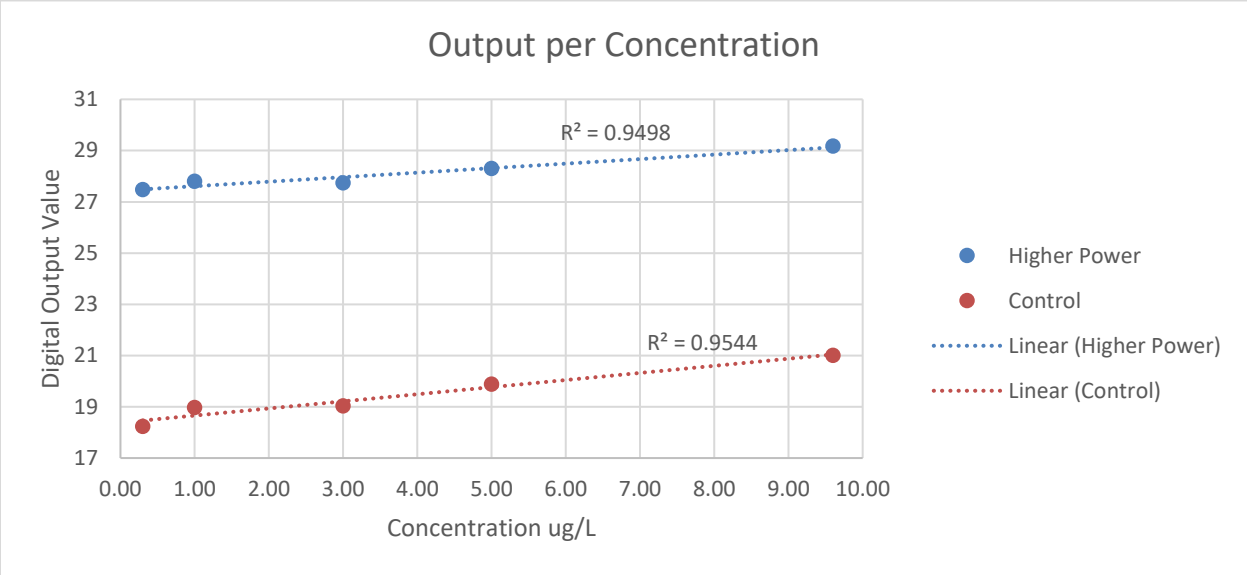
Finally, the data collected was evaluated at the 0.30µg/L using a hypothesis test. The test concluded that the average fluorescence at 0.30µg/L was larger than the fluorescence of distilled water.

$$H_0 = \mu_{0.30} \leq \mu_{0.00}$$
$$H_a = \mu_{0.30} > \mu_{0.00}$$

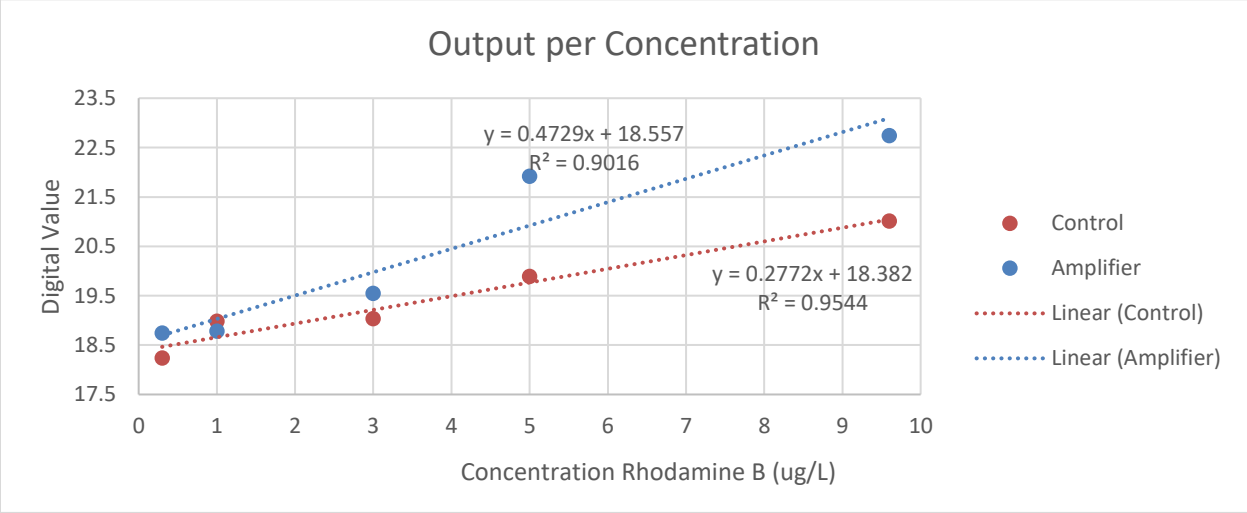
The hypothesis test was run with 10 samples from each group and returned a p-value of $3.2 \cdot 10^{-7}$. Since this is less than 5%, the 0.30µg/L has a statistically significant output, and the system passes the test of detecting the threshold volume. Overall, the new circuit design satisfies all the statistical tests and technical specifications set by the team.

Further testing was done in Phase 1 to evaluate the possibility of specific modifications to the circuit. The system was tested at a higher power; it was also tested with the amplifier circuit connected. The higher power seemed to have little effect on the trend itself, only

translating the data to a higher value. This indicates that power can be adjusted slightly without a significant effect on the accuracy of the design.



Testing with the amplifier was less successful. The addition of the amplifier did magnify the scale of larger concentrations but had little effect on smaller concentrations. The R^2 also became worse overall. The recommendation is that the amplifier is not used without further modifications.

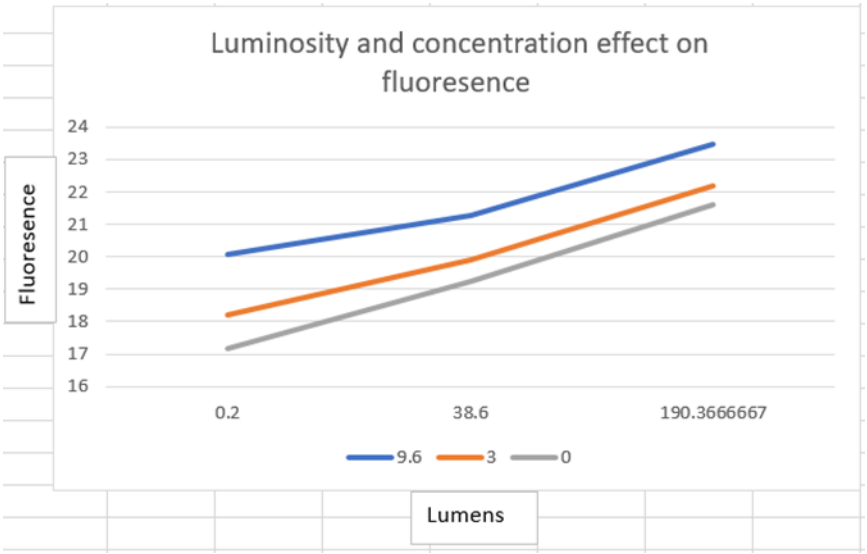


9.2 Phase 2 Testing

Phase 2 consisted of a series of tests to identify specifications for future teams designing the casing for the spectrometer. The two factors tested were temperature and ambient light. Temperature was measured by heating or cooling the samples before measuring. Readings with

a temperature probe was used to detect the temperature of the sample. Overall, the results of the temperature test were inconclusive and insignificant. Further research should be done to see how the circuit reacts to temperature. The expected trend was that fluorescence would increase slightly with temperature, but that was not observed.

The other aspect changed was ambient light. The circuit was run in 3 levels of light: complete darkness ~0 lumens, shaded ~40 lumens, open ~200 lumens. This test, like temperature, was run on 3 different concentrations of 9.6, 3, and 0 µg/L. The luminosity had a strong positive linear relationship with the fluorescence output for each different concentration (each color on the graph is one of the different concentrations).



	Lumens			
ug/L		0.2	38.6	190.3667
	9.6	20.078	21.259	23.466
	3	18.214	19.922	22.183
	0	17.15	19.228	21.616

The R^2 that resulted from the experiment was 0.98. These are considered statistically significant results. Future teams should remain aware of this relationship going forward in their testing, and not allow large fluctuations of ambient light to skew their data and make it less reliable.

10. Significant Accomplishments and Open Issues

In this project, our team was able to make several improvements to the previous teams design in order to be able to collect a significant amount of data regarding the devices ability to detect the presence of harmful algae blooms. We were first able to determine that our device was consistent in outputting repeatable data for one concentration. We were then able to determine that the device had a linear trend in outputting data for varying concentrations. This showed us that our device was accurately reading and reporting varying concentrations of Rhodamine in a linear trend as the concentration increased. Whereas the previous team was unable to get a reliable high signal output, we were able to observe a linear trend from our output with consistent and accurate data. With the success of our Rhodamine trials, we knew that the device would then also be able to detect the presence of harmful algae blooms.

With this success, we were then able to perform more tests to determine what could make our device work even better, or what caused our device to output less than desirable results. We were able to determine that giving the LED higher power had a minimal impact and adding an amplifier circuit shifted our results to make the linear trend worse.

Some of our unsuccessful results from our testing include the results from our temperature tests which we were unable to get consistent results from. This may have been due to the original photodiode breaking and needing to be replaced. It may also be due to the fact that the temperature sensor was not very accurate and did not give good readings. Further testing could be done using a better temperature sensor to be able to conclusively determine the effect of temperature on the detection of HABs.

In the future, different design changes can be made in order to update the enclosure itself in order to better be able to get samples in and out of the device and test their contents throughout the day. We were able to determine that ambient light has a significant impact on the output that is seen, so it would be important in future designs to be able to keep this at a minimum. One potential solution would be to have some sort of mechanism to be able to reload the enclosure with new water at certain time intervals. The device would do this automatically and seal itself back up each time to ensure that no ambient light would get in. Additionally, corrections could be made to the data within the Arduino code based on the measurements taken when LED is not exciting the sample to eliminate the effect of ambient light. It would be advisable to also consider a different material or color for the enclosure in order to block out more ambient light, perhaps starting by painting the current enclosure black.

Future improvements to the device would also need to be made in order to wirelessly transfer data from the device out to a lab to be analyzed. The device could also potentially have a light system on it in order to indicate when HABs have been detected and reflect how dangerous the conditions are based on the concentration detected. Consideration for the power supply to the device would also have to be factored in, but given a typical 120 V supply these configurations for the circuitry we have made should not be overly complicated.

11. Conclusions and Recommendations

Our main goal for this project was to be able to take what the last team started with and be able to significantly improve upon their work to be able to create functioning circuitry for the detection of harmful algae blooms. We started with their original design and advice to improve the circuit by adding amplification technology to the back end of the circuit and sought to improve some of the components they used. We discovered that by utilizing a higher power LED with more concentrated light, we were able to get the samples to fluoresce with higher intensity. By using a photodiode with a peak operating wavelength that matched that of the fluorescence of cyanobacteria, we were able to confirm that the photodiode was in fact conducting when the fluorescence was observed. Lastly, by adding a high-tech filter, we were able to confirm that the device was in fact detecting cyanobacteria and not getting false readings from the light of the LED. Additionally, the code within the microcontroller used to control the LED and the measurements received from the photodiode was improved to determine the best sampling rate and when to record the measurements from the photodiode for the best results. With all these improvements made to the circuitry, we found that the amplifier circuitry did not end up being necessary.

These improvements to the previous team's work allowed us to run enough tests on the device itself to determine what worked the best for detecting different concentrations of Rhodamine. We were able to determine the effectiveness of the device, as well as test different parts that could affect the devices operation. Overall, the device passed the testing requirements in repeatability, accuracy, and its ability to detect small concentrations of Rhodamine B.

The future for this project mostly includes putting the circuit into a usable state so it can be tested in the lake. Creating the casing for the circuit has a multitude of challenges that need to be addressed. First, the device needs to stay watertight around the components to protect them while also letting water into a chamber for observation. Water needs to be circulated through the device and cycled to get as much data as possible. This will directly conflict with the need to limit ambient light as much as possible to yield the best possible results. Additionally, more research and testing should be done to determine the effect of temperature on the readings for HABs and how best to measure the temperature of the water. Bacteria and algae can build up on the walls of device and prevent light from traveling through. This process, biofouling, needs to be prevented in the new casing. Finally, the next team must address how this device connects and sends data. The data is currently in an SD care via CSV, but how the data is communicated, how often, and when are all questions that must be addressed.

12. References

[1] Lake George Association, "Arrival of Harmful Algal Blooms Calls For Unprecedented Protective Actions," Lake George Association, [Online]. Available: https://lakegeorgeassociation.org/science-protection/harmful-algal-blooms. [Accessed 10 September 2021].

[2] T. Leeuw, E. S. Boss and D. L. Wright, "In situ measurements of phytoplankton fluorescence using low cost electronics," *Sensors (Basel),* 19 June 2013.

[3] E. o. Enyclopaedia, "Fluorescence," 24 March 2017. [Online]. Available: https://www.britannica.com/science/fluorescence.

[4] K. Rose, "Biological Sensors," Fondriest Enviornmental Inc., 2020. [Online]. Available: https://www.lakescientist.com/biological-sensors/. [Accessed September 2021].

[5] "PubChem Compound Summary for CID 6694, Rhodamine B," 2021. [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/Rhodamine-B.

[6] T. Johengen, "PERFORMANCE VERIFICATION STATEMENT," 2021. [Online]. Available: https://www.act-us.info/Download/Evaluations/Fluorometer22/BBE_Fluorometer_Verification_Report_Final.pdf. [Accessed 28 September 2021].

[7] Youtube, "BBE Unique Method of Fluorometry," 2021. [Online]. Available: https://youtu.be/QCbh7_prjIU. [Accessed 28 September 2021].

[8] In-Situ, "Chlorophyll a, Phycocyanin (BGA-PC), Phycoerythrin," In-Situ Inc., 2018. [Online]. Available: https://in-situ.com/pub/media/support/documents/In-Situ_FluorSensors_SpecSheet.pdf. [Accessed September 28 2021].

[9] In-Situ, "Aqua TROLL Blue Green Algae Sensor: Phycocyanin (BGA-PC)," In-Situ Inc., 2020. [Online]. Available: https://in-situ.com/us/blue-green-algae-sensor-phycocyanin- bga-pc. [Accessed 28 September 2021].

[10] Electronics Tutorials, "Light Sensors," AspenCore Inc., [Online]. Available: https://www.electronics-tutorials.ws/io/io_4.html. [Accessed 26 September 2021].

[11] Thor Labs, "Si Photodiode FDS1010," 24 February 2017. [Online]. Available: https://www.thorlabs.com/drawings/a9151145e4edb459-5C3C5BE1-B42E-D1D9-D5E5E941260D147C/FDS1010-SpecSheet.pdf. [Accessed 26 September 2021].

[12] C. A. L. Ebbing P. de Jong, "Spectral filtering of light-emitting diodes for fluorescence detection," *Analytica Chimica Acta,* vol. 546, no. 1, pp. 37-45, 2005.

[13] Elprocus, "What is a Phototransistor : Circuit Diagram & Its Working," Elprocus, [Online]. Available: https://www.elprocus.com/phototransistor-basics-circuit-diagram-advantages-applications/. [Accessed 26 September 2021].

[14] P. N. J. P. Y. Z. a. S. L. Lukas Novak, "An integrated fluorescence detection system for lab-on-a-chip," *The Royal Society of Chemistry ,* 2006.

[15] J. Petricka, "An Introduction to the Lock-In Amplifier and a Mie Scattering Experiment 3 Week Sequence, for Gustavus Adolphus College PHY-305 (Junior advanced lab course)," Gustavus Adolphus College, 2014.

[16] P. C. H. a. S. S. S. Tan, "All-solid-state Instrument for Fluorescence-based Fibre-optic," *Analyst,* vol. 118, 1993.

[17] M. A. Lucius, K. E. Johnston, L. W. Eichler, J. L. Farrekk, V. W. Moriarty and R. A. Relyea, "Using machine learning to correct for nonphotochemical quenching in high-frequency, in vivo fluorometer data," Association for the Sciences of Limnology and Oceanography, 2021. [Online]. Available: https://aslopubs.onlinelibrary.wiley.com/doi/10.1002/lom3.10378. [Accessed September 2021].

[18] MathWorks, "Signal Smoothing," MathWorks Inc., 2021. [Online]. Available: https://www.mathworks.com/help/signal/ug/signal-smoothing.html. [Accessed September 2021].

[19] A. Swalin, "How to Handle Missing Data," towards data science, 30 January 2018. [Online]. Available: https://towardsdatascience.com/how-to-handle-missing-data-8646b18db0d4. [Accessed September 2021].

[20] AAT Bioquest, "Spectrum [C-Phycocyanin]," AAT Bioquest Inc., 2019. [Online]. Available: https://www.aatbio.com/fluorescence-excitation-emission-spectrum-graph-viewer/C_Phycocyanin. [Accessed October 2021].

[21] ThorLabs, "FDS010 - Si Photodiode, 1 ns Rise Time, 200 - 1100 nm, Ø1 mm Active Area," Thorlabs Inc., 2021. [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=FDS010. [Accessed October 2021].

[22] ThorLabs, "FDS100 - Si Photodiode, 10 ns Rise Time, 350 - 1100 nm, 3.6 mm x 3.6 mm Active Area," ThorLabs Inc., 2021. [Online]. Available: https://www.thorlabs.com/thorproduct.cfm?partnumber=FDS100. [Accessed October 2021].

[23] Digi-Key Electronics, "ODD-15W," Digi-Key Electronics, 2021. [Online]. Available: https://www.digikey.com/en/products/detail/opto-diode-corp/ODD-15W/2397147. [Accessed October 2021].

[24] Marktech Optoelectronics, "MTE6000L-HP," 2021. [Online]. Available: https://www.marktechopto.com/pdf/products/datasheet/MTE6000L-HP.pdf. [Accessed October 2021].

[25] Edmund Optics, "635nm, 12.5mm Diameter, Dichroic Filter," Edmund Optics, 2021. [Online]. Available: https://www.edmundoptics.com/p/635nm-125mm-diameter-dichroic-filter/29461/?tab=RS. [Accessed October 2021].

[26] Newport, "Optical Filter, Shortpass, 25.4 mm, 600 nm Cut-off, 415-590 nm Trans.," Newport Corporation, 2021. [Online]. Available: https://www.newport.com/p/10SWF-600-B. [Accessed October 2021].

[27] Edmund Optics, "Everix Ultra-Thin OD 2 Shortpass Filter, 600nm, 12.5mm Square," Edmund Optics Inc., 2021. [Online]. Available: https://www.edmundoptics.com/p/600nm-125mm-square-od-2-ultra-thin-shortpass-filter/41327/. [Accessed October 2021].

Appendix A: Initial Deliverables and Dates

Table 4 - Initial Deliverables and Dates Summary

Deliverables	Details	Date
Deliverable Planning Phase	General planning for project deadlines in developing accurate sensor detection of harmful algae blooms.	September 21st
Design of Circuitry	Developing general schematics and completing research on component concepts	October 8th
Acquisition of Materials	Have all materials acquired to begin constructing circuitry, building off of previous teams work.	October 19th
Testing Plans Created for Testing Circuits Functionality	A specific plan shall be laid out describing a procedure that tests the circuit's functionality and checks its operating requirements to ensure safety.	October 26th
Testing Plan Created for Measuring Accuracy	A test plan for determining usability and accuracy of the circuit in detecting HABs shall be created and well documented.	October 26th
Build Prototype Circuit	The initial circuit design shall be fully constructed and functional and ready to be tested on.	October 26th
Initial Circuitry Testing Phase	An initial round of testing on the original design shall be completed and next steps for improving performance will be determined.	November 9th
Build Final Design Prototype Circuit	The final circuit design shall be fully constructed and functional and ready to be tested on.	November 16th
Final Design Phase Testing of Circuitry	Finalize results of testing on improved and final circuitry implementation for successful detection of harmful algae blooms.	November 23rd
Documentation of Results and Final Report	Create user manual for proper usage of device and fully document development and testing process along with results achieved.	December 10th

Appendix B: Project Plan

Our project will have five main sub-plans to be accomplished throughout the rest of the semester. These main sub-plans include the preliminary circuit design, the creation of testing plans, the circuit prototype build phase, a revision phase, and a testing phase. Each of these milestones have general due dates and sub tasks assigned to different individuals as described in this section.

Preliminary Circuit Design – 10/19

This phase of the project involves initial design creation and research of certain concepts in order to best determine what the best design will be for our circuit.

1. *Evaluate Photoreceptor Concepts*: Cameron will evaluate different photoreceptor concepts to select a component for use in the prototype.
2. *Evaluate Microcontroller Concepts*: Scott will evaluate different microcontroller concepts and evaluate current microcontroller to determine best component.
3. *Circuit Design Simulation*: Papa-Sam will create initial schematics of basic outline of circuitry to be built.
4. *Evaluate and Choose LEDs*: Kara will evaluate LEDs of different wavelengths, intensity, and sizing for use in fluorometer.
5. *Understand Previous Teams Circuit + Testers Apparatus:* Team will review previous team's circuitry and apparatus for rebuilding and developing new concepts from there.
6. *Research Electrical Signal Amplification Methods:* Papa-Sam will choose a signal amplification method and begin design process of circuitry.
7. *Filtering Circuitry:* Begin simulation of filtering circuitry.

Testing Plan – 10/26

This phase involves the creation of testing plans in order to perform and document the necessary tests on the circuitry to determine the functionality and accuracy of our design.

1. *Plan DoE:* Parker will set up a plan for the design of experiments to answer questions like "which conditions" and "which variable" do we need to hold constant and which should we change?
2. *Create Statistical Methods Plan:* Jason will create a "copy and paste" ready worksheet for when we start collecting data.
3. *Create Intermediate Testing Documentation:* Parker will create a skeleton for intermediate testing.

Circuit Prototype Build Phase – 10/28

This phase involves the ordering and acquiring of materials in order to begin building the initial design of our circuitry and will happen in conjunction with the creation of the testing plans.

1. *Rebuild Foundations of Previous Teams Circuitry:* Team will work together to rebuild the circuit that the team created in Spring 2021 to the best of our ability to attempt to re-create their results and be able to work on improvements from there.
2. *Initial Circuit Creation:* Electrical engineers of team will finish design simulation and begin wiring of a tangible circuit.
3. *Determine Lead Time on Materials:* Cameron will determine the lead time of any critical materials that will need to be ordered and discuss with team for final decisions.
4. *Order Materials:* Cameron will order any necessary materials that will be needed for the prototype.
5. *Prepare Purchase Orders:* Cameron will prepare purchase orders for any materials that need to be purchased in order for team to be reimbursed.
6. *Adding Filter Circuitry to Product:* Papa-Sam will add in filtering components to the circuit to ensure the signal being amplified is the right one.

Revision of Circuitry Design Phase – 12/3

This phase involves the budgeting of time for any revisions we want to incorporate to our original design.

1. *Create Schematics for Redesign:* Kara and Papa-Sam will create new schematics and the team will evaluate a basic plan for any improvements that can be made to the original design.
2. *Construct New Circuitry:* Electrical engineers will build and rewire any new components or reworks to the original design.
3. *Debug Circuitry:* Electrical engineers will debug improved circuitry design.
4. *Perform Testing Procedures:* See testing phase for more detailed testing plans, parts of which may be repeated on improved circuitry for testing accuracy and functionality.

Testing Phase – 12/10

This phase is the most elaborate and important part of our project as our goal is to make the device as accurate as possible. This phase includes performing functionality and accuracy tests on our initial and final circuitry.

1. *Safety Training for Rhodamine:* Team members will each review MSDS to learn of important safety hazards to know for when using Rhodamine.
2. *Evaluate Program from Previous Team:* Scott will evaluate the previous team's program to collect data and process data.
3. *Create a CSV Processing Program:* Scott will create a program that should be able to read a CSV file and process it.
4. *Debug Circuitry and Code:* Kara will assist team in optimizing original design by debugging circuitry and refining code.
5. *Perform Photoreceptor Testing:* Cameron will perform testing on the photoreceptor to ensure it is functioning properly and can be used in the prototype.
6. *Testing the Light Source:* Kara will ensure that the LED light(s) are working properly and having the desired effect on the circuitry to create an effective fluorometer.
7. *Test Circuit Functionality:* Papa-Sam and EEs will test circuit to determine whether or not it functions as desired with tests on individual components and tests for overall functionality.
8. *Collect Data from Experiments:* Parker and Jason will run tests using the Rhodamine B samples in order to gather data from the circuitry regarding its capability to accurately detect HABs.
9. *Analyze & Synthesize Data from Testing:* After collecting raw data, Parker and Jason will use the data to determine accuracy of the device.
10. *Further Circuitry Testing:* Papa-Sam and team will continue further testing on iterative designs of circuitry in order to ensure the desired outcome.
11. *Examine Customer Requirements:* Papa-Sam and team will determine if the circuitry meets the customer requirements and accomplishes what we set out to achieve.
12. *Document Testing in Final Report:* Team will work collectively to well document testing procedures throughout the process and different iterations.

The Gantt chart included below includes all of the above sub-plans and sub-tasks as well as the due dates that we aim to reach for each of them. The Gantt chart also includes the documentation work that will need to be completed by the team for the final documentation.

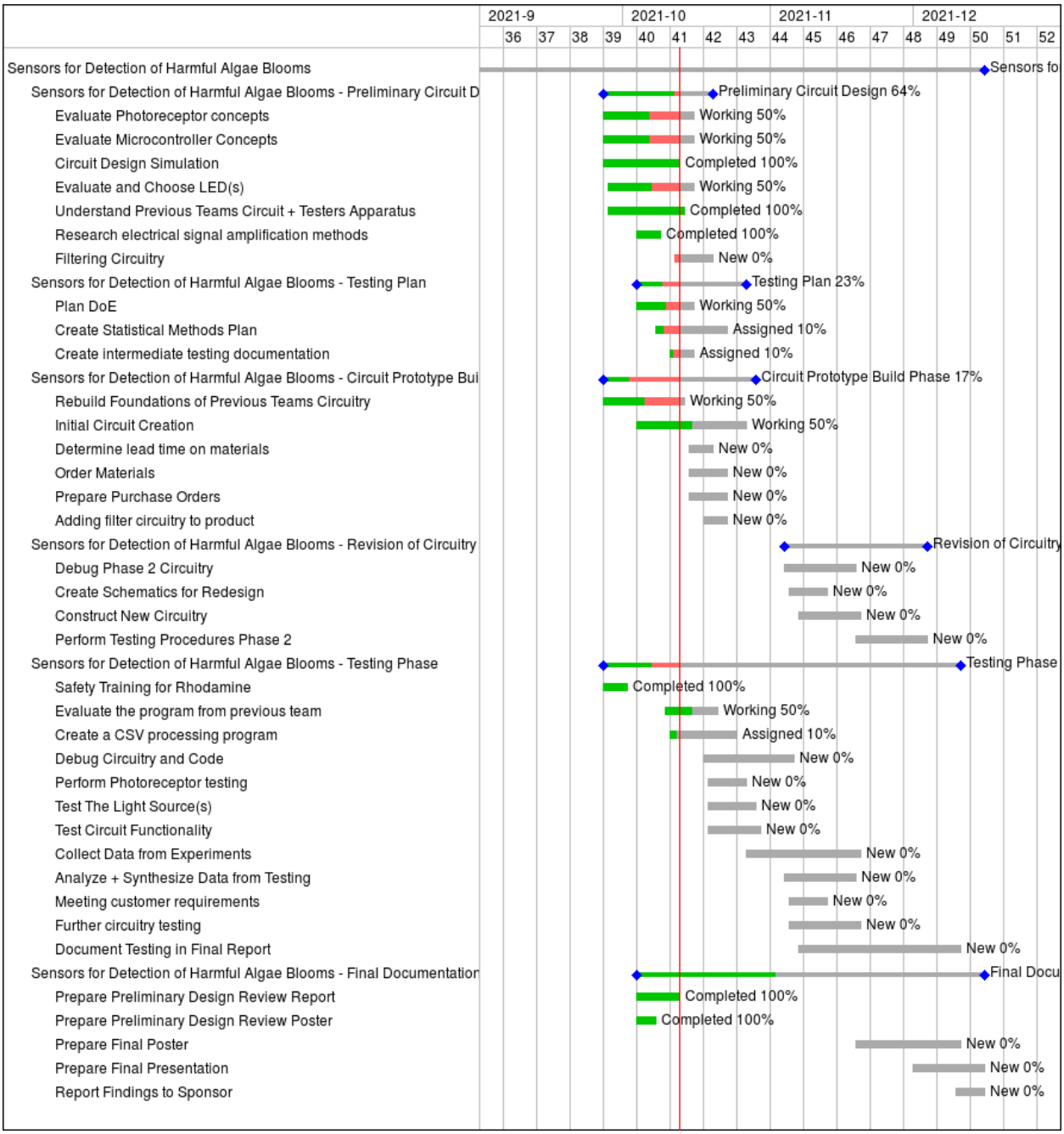


Figure 7: Preliminary Gantt Chart

Appendix C: System Evaluation Plan

The project prototype will be evaluated in two phases: fluorometer circuit output testing and analysis of sensitivity to outside factors. The fluorometer prototype will be subjected to an experiment designed to test its ability to detect harmful algae blooms, or its synthetic variations, and relative concentrations. The main goal of phase one is to provide feedback to the team to iterate on and improve the design. Phase 1 testing can be performed again after revisions to share with sponsor. Phase 2 testing is designed to provide future teams with the details needed to create specifications for casing and implementation.

Fluorometer Prototype Test Plan – Phase 1

To test if our fluorometer prototype can detect the distinct wavelength peaks from the emitted fluorescence, the following procedure will be used. Multiple samples of water are taken and are tested with a synthetic fluorescent compound. The samples help evaluate the circuit on a variety of standards. First, the accuracy, or correctness, of the system is evaluated by it's ability to detect the linear trend of concentration and fluorescence for small concentrations. Precision is tracked using multiple samplings of the same concentration, and the creation of a control chart to evaluate deviation of the system. Third, the system needs to detect small concentrations, so a hypothesis test is constructed to evaluate whether the system provides significant output at low concentrations. Finally, the effective range of the system is important information for the sponsor, which is analyzed by slowly increasing the concentration until failure.

Rhodamine B Samples

Rhodamine B is synthetic biological sensor with fluorescent properties similar to blue-green algae with a peak wavelength emission of 637nm. It can be easily diluted in water, but requires more care to handle – see chemical information. The Rhodamine content can be used until the labeled expiration date if the bottle is stored in 5 °C to +30 °C and the bottle is tightly sealed. A concentration of 0.3% Rhodamine B correlates to the average amount of algae in typical freshwater lakes. Therefore, this test should be conducted after acceptable results are achieved with the food coloring to validate the ability of the instrument to detect a typical concentration of algae in freshwater.

Equipment

1. Glass or quartz cuvettes
2. 10 mL vial
3. Rhodamine B
4. 2 Pipettes
5. Distilled Water
6. Beaker or graduated cylinder

7. Scale
8. Fluorometer Prototype
9. Proper waste bin
10. Nylon gloves
11. Googles or safety glasses

Procedure

1. Take a control reading of the fluorometer in complete darkness no water
2. Take a control reading of just the cuvette and again for 3 mL of distilled water
3. Measure out samples using 1.2% Rhodamine B Stock according to the table below
4. Fill the samples to 10mL with distilled water
5. Cover and shake to properly dissolve the sample

Table B2-Vial Sample Concentrations for Rhodamine B

Concentration µg/L	Total Volume mL	Stock Volume mL	Water Volume mL	Uncertainty µg/L
9.60	4.00	4.00	0.00	0.34
5.00	4.00	2.08	1.92	0.27
3.00	4.00	1.25	2.75	0.25
1.00	8.00	0.83	7.17	0.12
0.30	8.00	0.25	7.75	0.12

6. Take readings with fluorometer
    a. Collect a CSV file with 100 samples taken (25 seconds of sampling)
7. Discard samples and gloves in appropriate bin when finished

Pass and Fail Criteria

The experimental outcome can be deemed a success in accuracy if all the following criteria are met:

1. Voltage output for control samples is 90% lower than the most concentrated sample
    a. This guarantees that there is an appropriate resolution
2. As percent concentration increases, voltage output also increases
3. A good linear trend is observed between voltage output and concentration, quantifiable with a R^2 value of at least 0.9
4. The relationship passes the hypothesis test:

H_0: $\beta = 0$ (No linear relationship)

H_A: $\beta \neq 0$ (There is a linear relationship

Significance level: $\alpha = 0.05$ (95% confidence)

DF = (n – 2)

The experimental outcome can be deemed a success in precision the following criteria is met. For each grouping of samples, the process remains in control. This means that 90% of data falls within 3 standard deviations of the mean. An out of control process dictates that there is an error in the system which causes erradic results.

The outcome is a success in terms of significance if the voltage for any non-control sample is higher than that of the control sample. This fact is evaluated with a hypothesis test and is a success if the corresponding p-values are less than or equal to 5%.

Fluorometer Prototype Test Plan – Phase 2

The next steps in the fluorometer testing plan is to evaluate sensitivity in temperature and ambient light. Both of these are factors that are subject to change in the system when monitoring water quality at Lake George. 2 concentrations of Rhodamine will be evaluated at varying conditions. The ambient light will be tested at 5 different light levels and 5 different temperature ranges. The output voltage will be recorded accordingly. Light level will be simulated by testing in rooms of different brightness and blocking out light using a cover over the circuit. A temperature probe will be used to verify the proper measurements

Light Levels (lux)
~0
~50
~100
~1,000
~10,000

Temperatures (°C)
0.0
4.5
10.0
15.6
21.1

Appendix D: Ethical and Professional Responsibilities

Table D1 – Ethical and Professional Responsibilities

Issues	Impact, 1(low) – 5(high)	Description of Impact and Related Project Decisions
Public Health, Safety, and Welfare	2	The team did not prioritize any public health safety issues in the design of the circuit as this part of the device will be enclosed in the future. The circuit will be placed in an enclosure that has yet to be designed but Public Health and Safety issues should be considered by future groups working on this project. The device will be present in public spaces such as Lake George so it will be important to consider potential safety hazards that could be created by having this object in the water.
Global	3	The fluorometer design produced by the team can have some future global impact. The fluorometer is designed to detect HABs in freshwater which in this case is specifically Lake George but the design itself has the potential to be used in many other freshwater ecosystems throughout the world. There are plenty of other freshwater ecosystems that could benefit from HAB monitoring so people who are responsible for maintaining its health can be alerted of a potential HAB threat and take action. Decisions made to reduce overall cost of the fluorometer design were made so the device could eventually be mass produced and used in many locations.
Cultural	1	Our design currently tests water samples from bodies of water and will deliver results remotely so there will not be much human contact with the device at all. For this reason, the team feels the cultural impact of our design is very low and we did not consider them in our design.
Social	2	Our internal circuit design will likely not be seen by the public and its only purpose is monitoring water samples which the team believes will not produce any major social issues. The final design of this device will eventually be present in public bodies of water so it could be important for future teams to understand how the device will look in the water and how people perceive it.

Issues	Impact, 1(low) – 5(high)	Description of Impact and Related Project Decisions
Environmental	5	The purpose of our design is to prevent HABs from negatively impacting the health of freshwater ecosystems, so our device is directly impacting the environment. The team did not make any major design decisions with environmental issues in mind because the circuit will eventually be held in a watertight enclosure. It will be important for future teams to design a secure enclosure that prevent any of the electronic components from negatively effecting the environment.
Economic	5	A major requirement of the project and design was to keep the overall cost low so mass production of the design was possible in the future. The team kept the economic impact of the device in mind when selecting hardware and cost was included in many of the component selection decisions. Future teams can revisit cost of materials and components used if the overall cost becomes too high, but our design does its best to find an even ground between functionality and cost.

The main issues that were considered by the team when creating our design include Environmental and Economic issues. These two categories are most directly related to our design and the purpose of this project which is to develop a low-cost method of detecting HABs which can negatively impact the environment. Major environmental concerns will likely need to be addressed by future teams when designing parts of the device such as the enclosure which will have direct contact with the environment. Our design mainly focuses on limiting cost of components so it can be eventually mass produced and limiting size, so the overall size of the device does not impact the environment too greatly. It is also important to note that if this device can be mass produced and deliver successful results the design can be used globally to help other ecosystems and not just Lake George giving this project some global impact potential.

Appendix E: User Manual

This appendix serves as a User Manual for use of the fluorometer circuit by future teams. The basic operation of the circuit includes setting up the prototype by inserting the LED and photodiode into both ends of the 3D printed testing apparatus and connecting both components to the proper pins on the Arduino Nano.

General Arduino Connections

1. Wire Connections
    a. LED Resistor Network to Arduino D2 (Pin 5)

b. LED Anode to Resistor Network
c. LED Cathode to Arduino GND (Pin 29)
d. Photodiode Cathode to Arduino GND (Pin 29)
e. Photodiode Anode to Arduino A0 (Pin 19)
f. Temperature Sensor Pin 1 to Arduino GND (Pin 29)
g. Temperature Sensor Pin 2 to Arduino D5 (Pin 8)
h. Temperature Sensor Pin 3 to Arduino 5V (Pin 27)
i. SD Card Module CS Pin to Arduino D10 (Pin 13)
j. SD Card Module SCK Pin to Arduino D13 (Pin 16)
k. SD Card Module MOSI Pin to Arduino D11 (Pin 14)
l. SD Card Module MISO Pin to Arduino D12 (Pin 15)
m. SD Card Module VCC to Arduino 5V (Pin 27)
n. SD Card Module GND to Arduino GND (Pin 29)
o. Arduino connected to computer through USB

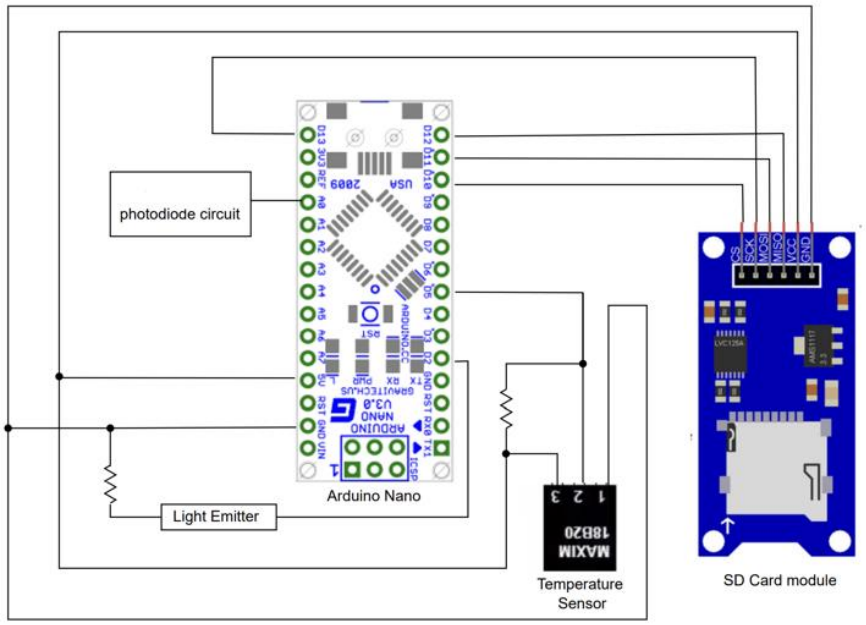


Figure E1 – Arduino Circuit Connections

2. Software Operation
    a. Open Arduino IDE, select “Tools” and Board: “Arduino Nano”
    b. Select “Tools” and “Port”, then choose the port matching the USB port used on the computer (COM 3/5/7 etc.)
    c. Open the selected program file and select “Upload”
    d. To view results on your computer, select “Tools” and “Serial Monitor” to open a window where results will be displayed

3D Printed Testing Apparatus Use

1. Testing a Sample

a. Once the prototype is set up and the program is uploaded to the Arduino the program should run automatically and either display results to the serial plotter or to the SD card if the module is connected
b. Collect a sample in one of the cuvettes and place the cuvette into the square chamber in the middle of the prototype
c. Insert the desired filter into the excitation slot in between the LED and the sample
d. Place both the lid on the filter chambers and the sample chamber
e. Reupload the program to the Arduino and results will display on your computer or be saved to the SD card

Appendix F: Cost Analysis and Manufacturability Analysis

For this project, we started off with a design that had been proven to work scientifically, but needed updates in order to actually perform as desired. Many of the initial components that were used were basic components with broad applications, and we updated these items with components that had the specific requirements we were looking for in order to have a successfully operating fluorometer. Such components include the LED, photodiode, and optical filter that was used. These were critical system elements that we decided needed to be specially selected to fit our design requirements and purchased from approved vendors instead of using the supplies given by the school or making our own versions of.

The previous group used LEDs that were provided by the school that are your typical small LED indicator lights that can be used for a wide range of applications. It was decided that it would be more beneficial to buy a higher powered LED that is designed for applications requiring high output optical alignment. This was crucial to our design in seeing improved readings from the photodiode.

Additionally, a photodiode was selected with a responsivity that much more closely matched the wavelength of light that we were trying to capture. This was also crucial in being able to determine that a signal from the photodiode was actually due to the fluorescence of HABs being detected.

Finally, our team also decided to purchase an expensive optical filter that was designed for cutting off the wavelengths of our LED that we did not want to detect rather than using the thin film filters that the previous team was making use of. We decided that it would be worth it to ensure that if we are using a filter, that it is the most beneficial to the final design. Our final design needed to be able to filter out the light from the LED so that we knew the fluorescence detected was from the presence of bacteria and not a faulty reading coming from the wavelength of the LED. We found that a precision filter would accomplish this the best without muting the emission wavelength of the LED needed to get the sample to fluoresce.

Given the expense report seen in the Appendix G below, the total cost of the items required to build the circuitry can be estimated to be around $90, and around $100 with an added temperature sensor. With the optical filter and the enclosure added, the final projected cost to recreate the device we have made for this project would be $200.

Appendix G: Expense Report

This table shows the main items that would need to be purchased to reconstruct this project. We had to buy a second photodiode because the first ones legs fell off during testing phases. The last 3 items are things that we either had on hand or in the case of the Arduino NANO, the previous group had used this so we did not need to purchase it, but felt it should still be covered in the expense report.

The only other items used for this project that are not included here are the pipettes used for holding the sample and the physical enclosure that the previous team had 3D-printed. It does not seem that they left any indication of what the cost of the material was that they used to print the enclosure.

Item	Quantity	Unit Price	Subtotal	Vendor
FDS010 Si Photodiode	2	48.15	96.3	Thor Labs
MTE6000L-HP Emitter 5mm 590nm flat lens LED	1	17.86	17.86	Digi-Key
Everix Ultra-Thin OD 2 Shortpass Filter, 600nm, 12.mm Square	1	110.00	110.0	Edmund Optics
Glass Vials, 2 Dram, Pack of 12	1	4.79	4.79	Fantastic Place
Resistors	5	0.10	0.50	Digi-Key
Arduino NANO/ ABX00033	1	14.24	14.24	Digi-Key
OP482GPZ Op Amp	1	4.87	4.87	Analog Devices
Total		**200.41**	**248.56**	

Appendix H: List of Manuals and Other Documents

Arduino Nano Datasheet:
https://media.digikey.com/pdf/Data%20Sheets/Arduino%20PDFs/A000005.pdf

3D Printed Testing Apparatus CAD and Guide:
https://pubs.acs.org/doi/suppl/10.1021/acs.jchemed.6b00495/suppl_file/ed6b00495_si_006.pdf