Spectrophotometer, Fall 2015
Research Report

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Abstract

The goal of this research was to create a spectrophotometer that would efficiently detect red dye concentration in water. Creating a low cost, inline design that would be implemented at each lab station in the AguaClara lab was a priority. Red dye concentration was measured by fabricating a photo sensor that measured the voltage of different standards of red dye concentration. Then, various red dye standards were tested to calibrate the sensor of the system. The goal of calibration was to check in real-time the efficacy of the AguaClara system (or part of the system under study) by integrating the spectrophotometer with the team's Process Controller software.

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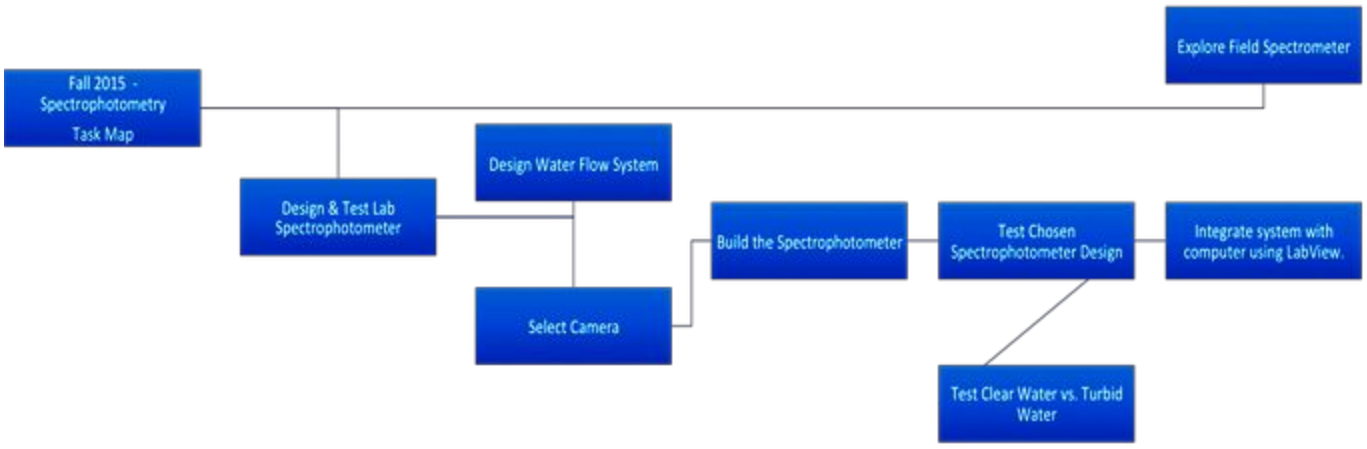

Task Details

1. **Design & Test Lab Spectrophotometer (1st attempt - PublicLab based system):** It is a priority to gain an understanding of how to best detect concentration of red dye in water, and construct a device to this end. The second priority is to discover the best method for constructing this device, whether that be through spectrophotometry, as originally thought, or just through basic color detection. **Complete**
   [Goal: Completed Lab Spectrometer by End of Semester, Bryan and Michael].
   a. **Design Water Flow System:** The first task is to engineer a system that allows dark water to flow through the measurement device, such that the dark water does not get light contamination from the outside [October 1, Bryan]. **Complete**
   b. **Select Camera** (or other measurement apparatus)**:** A small, VIDEW licensed, JDEPC-OV04 camera module was used in tandem with a USB cable to better minimize the space taken up within the enclosed dark box. The research for this clear image camera was based on PublicLab.org data [October 1, Michael] **Complete**
   c. **Build the Spectrophotometer:** The next task was to construct an apparatus that conducted fluorescence spectrometry, in which the material used for diffraction grating (the separation of light by color) was a DVD Disk. [October 15, Bryan]: **Complete**
   d. Test Spectrophotometer Design: The device will be tested after proper calibration with a fluorescent bulb to see its efficacy at accomplishing accurate detection of red dye concentration. [November 1, Michael] **Complete**

2. **Design & Test LED/Photosensor absorbance meter: (**Repeating same process as #1) Due to an increasing demand for our device and uncertain findings from the initial attempts, we have switched our task for this semester to building a red-dye specific photosensor and calibrating it for use by the AguaClara team [November 15th, Michael] **Complete**
3. **Integrate System with Computer Using LabView:** The final step is to integrate the spectrophotometer with LabView by writing computer code and relying on external programming libraries, possibly from PublicLab.org. [December 1, Michael] **Deferred for future exploration.**
4. **Explore Field Spectrometer:** The possibility of expanding spectrophotometry to on-site procedures will be greatly explored [End of semester, Bryan]. **Deferred for future exploration.**

Introduction

The AguaClara mission will be greatly enhanced through the creation of an affordable spectrophotometer. The efficient detection of red dye concentration in samples of turbid and clean water will allow the AguaClara lab - as well as water treatment plant operators - to better determine the efficacy of their filtration and sanitization systems. In other words, the apparatus created will be used to monitor different levels of concentration of red dye in water (which is cheap to use and relatively easy to detect). The spectrophotometer will monitor red dye concentration by identifying the different wavelengths produced by different concentrations of red dye. Through that end, it will also be prioritized to identify the absorbance that different concentrations of red dye standards exhibit when tested using a spectrophotometer. In this case, the red dye serves as pseudo organic matter that, when detected by the spectrophotometer in varying concentrations, allows for an idea of how well the AguaClara system would do in removing organic contaminants from the water.

Literature Review

Spectrophotometry deals with the amount of light a targeted sample absorbs. With respect to water, it is known that the more light a water sample absorbs, the more turbid that sample will generally be.

On a more technical note, it is known that for any sample (chemical compound) exposed to light, it will behave differently from other samples in the sense that its capacity to absorb or transmit light depends on a specific range of wavelengths. Through this measurement, it is also possible to monitor and measure the amount of a known chemical substance in whatever sample is being exposed to light at the time. In short, spectrophotometry seeks to measure how much light (in terms of photons) a chemical compound or substance can absorb or transmit.

The equation of Absorbance is:

$$A = -\log(I_t/I_0)$$

Where A refers to the number of photons absorbed found through the negative logarithm of light intensity after the substance is exposed to light (I_t) divided by (I_0), the light intensity of the substance before it is exposed to light.

Scientists are able to perform quantitative analysis by measuring the amount of photons absorbed after light passes through a sample solution. This measurement is also recorded as the intensity of light (I). Through the intensity of light, one can determine amounts of concentrations of chemical substances present within a water sample. Thus, the intensity of light after photons have been absorbed help us determine specific concentrations of red dye in samples of water.
The equation of Intensity is as follows:

$$I = P/A$$

Where I is the intensity measured in watts per square meter. This conclusion arrives from P (the power), being measured in watts, and A (the area) being measured in square meters.
For all intents and purposes, our apparatus will be constructed as a UV-visible spectrophotometer, which uses light over the visible spectrum (400 - 700 nm) and UV spectrum (185 - 400 nm). A DVD disk will be used as a cost-effective diffraction grating tool. In diffraction grating, the tool used to conduct the diffraction is supposed to separate light by color. In other words, in the visible spectrum, the DVD disk will serve to detect light as color. The goal with our apparatus is to detect light's red component in the visible spectrum by detecting red dye concentration of different water samples.

By using a spectrophotometer with a diffraction grating, we can selectively narrow down our wavelength to about 450 - 550 nm in order to best observe the absorption spectra of red 40 dye in water. In theory, we should then be able to determine the appropriate intensity (I) and thus calculate absorption A through open source software provided by the spectral workbench software created at PublicLab.org.

Previous Work

One approach that was used by a previous subteam was to simply attempt to detect color - i.e. to simply try and determine how "red" the sample was and to use that data as a crude measurement for absorbance. Unfortunately, this did not work because the trials that detected the red color were inaccurate in the sense that lighter and darker hues of red did not match the actual red color of the samples. In other words, by simply detecting color, the spectrophotometer used was not able to efficiently measure concentration of red dye 40 in water samples. Although it is known that the exact results of this subteam cannot be reproduced due to technical difficulties, it was clear that the "red" values found last year were arbitrary and imprecise and did not serve as reliable data.

Since mere color detection was not enough to measure concentration or absorbance of samples, it was necessary to start over at the drawing board with a completely new approach to the challenge by using a UV-visible spectrum spectrophotometer that can detect both wavelength and concentration of the red dye in the water samples. Through these means it is expected that the absorbance of these samples can be calculated based on future data found when the spectrophotometer is integrated into process controller.

Methods

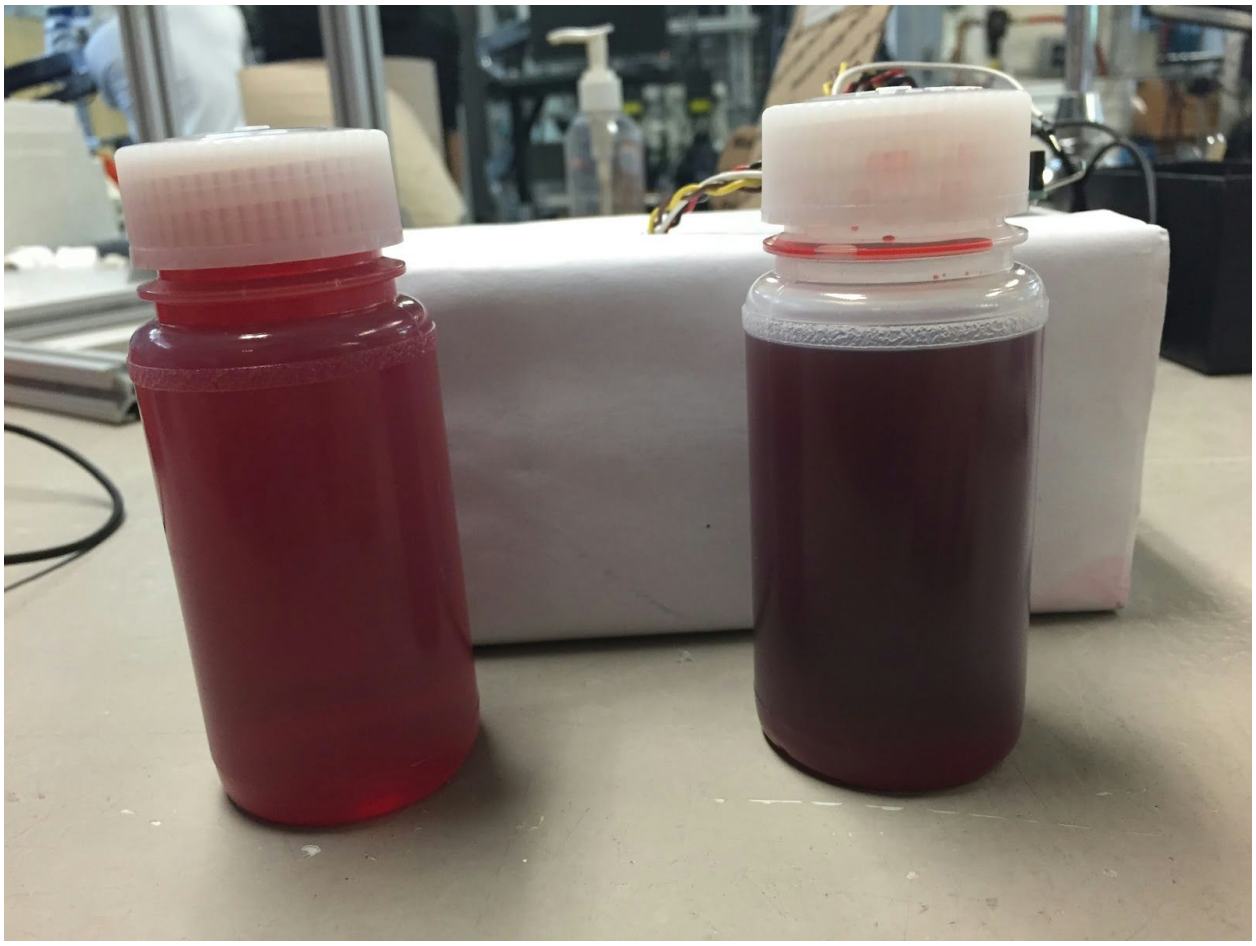

Figure 1: Samples of Red Dye 40 Water

Figure 1 above shows two samples of different hues of red dye 40. Our mini-experiment was to shine an LED light upon each sample and compare each intensity on a laptop screen as one last attempt at using color detection to measure intensity and absorbance of the sample.

The concentrations of each dye above were calculated using the principles of molarity and moles:

$$Molarity = mols/liters$$

$$mols = mass(g)/molar\ mass(g/mol)$$

For Sample 1 (lighter colored water):
Standard Molar Mass for Red Dye No. 40: 496.42 g/mol & Concentration = 0.05 M Red Dye 40.
For Sample 2 (darker colored water):
Standard Molar Mass for Red Dye No. 40: 496.42 g/mol & Concentration = 0.1 M Red Dye 40.

Figure 2: Crude Spectrophotometer Model

While awaiting the spectrophotometer kit from PublicLab.org, several webcams were tested in an effort to measure intensity and color of light using two different concentrations of Red Dye No. 40 water. The cardboard above shows the basic, crude setup that modeled in the simplest way the idea behind color measurement without a diffraction grating and UV-visible spectrophotometer.

After the UV-visible spectrophotometer kit arrived, it was assembled by first building the two-piece box included in the kit's contents. After the box was built, the JDEPC-OV04 camera module included in the kit was attached to the ash bench using the double-sided tape purchased from PublicLab. Afterwards, the USB cable was hooked through the opening within

the assembled box so that it could connect to the camera module snugly. From there a quarter of the included disk was cut out and attached as a diffraction grating at an angle near the camera module. Lastly, the collimation slit card included in the kit was inserted into an opening at the end of the assembled box since the 200mm thick line lined up with the slit would be where light would strike. After that process, the diffraction grating would separate light by its colors. The figure below represents the complete kit after assembly:

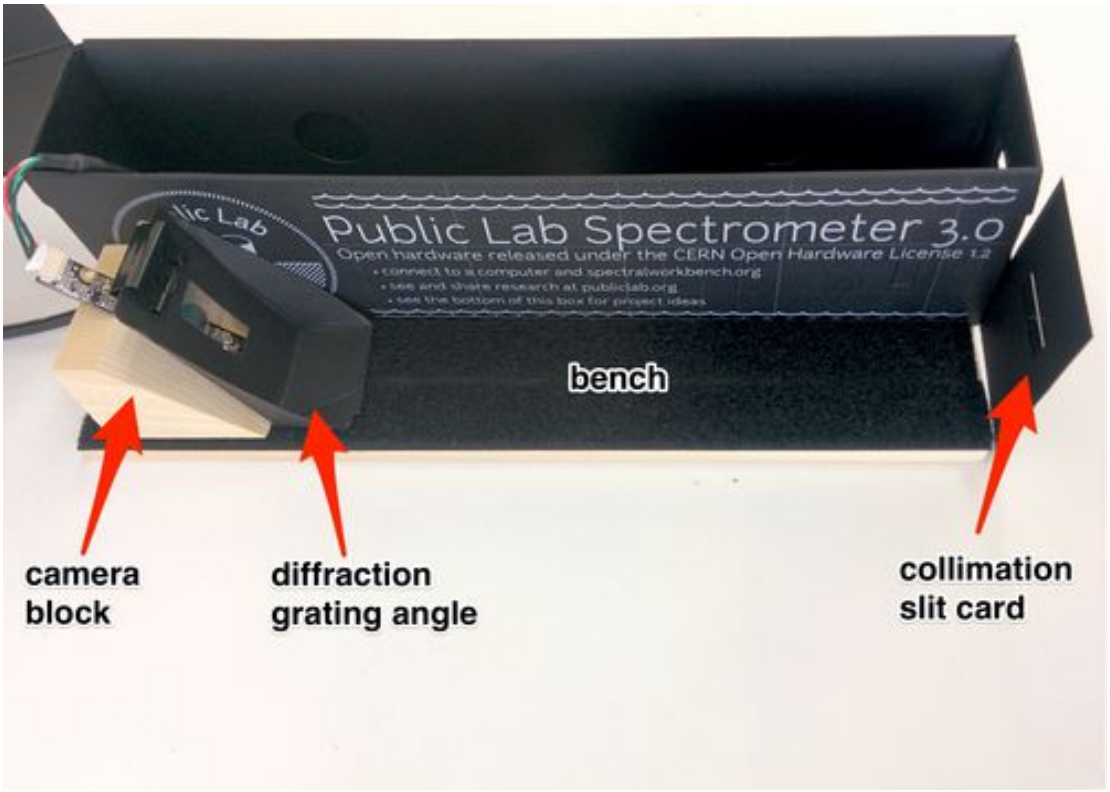

Figure 3: UV-visible Spectrometer Model

Although the public lab spectrometer that was assembled was able to detect wavelengths of light using its diffraction grating through the assistance of Spectral Workbench, an open-source software that displays emission and absorption spectra from the webcam image provided by this kit, the project and its findings were eventually replaced in favor of a photosensor. Shortly after running tests with the assembled spectrometer pictured above, Paul Charles discovered a light intensity detector that could be wired up and plugged into the ProCoDA (Process Control) box in order to directly find the absorbance of different samples of red dye concentrations in water.

It was decided that a light intensity sensor would perform the acquisition of data much more reliably than a camera simply because direct measurement of light intensity would make finding absorbance very easy. Absorbance is measured as the negative logarithm of light intensity penetrating a given substance divided by the light intensity penetrating a sample without the substance of interest. Gathering light intensity data was difficult due to the issue of variable exposure webcams, which led to the use of a light intensity detector that proved to be a better

option than a camera when attempting to find absorbance of given concentrations of red dye. On the other hand, this is not a true spectrophotometer, and is limited to, essentially, a “red dye concentration meter.” Because of this divergence, both options will continue to be researched going forward.

Below are images of the fabricated photosensor that Paul Charles assembled. It comes equipped with an Ambient Light Sensor, a blue LED, and the capability to be plugged into the ProCoDA to power the LED and measure the voltage output of the sensor. This voltage reading is a measure of light intensity, allowing us to calculate the concentration.

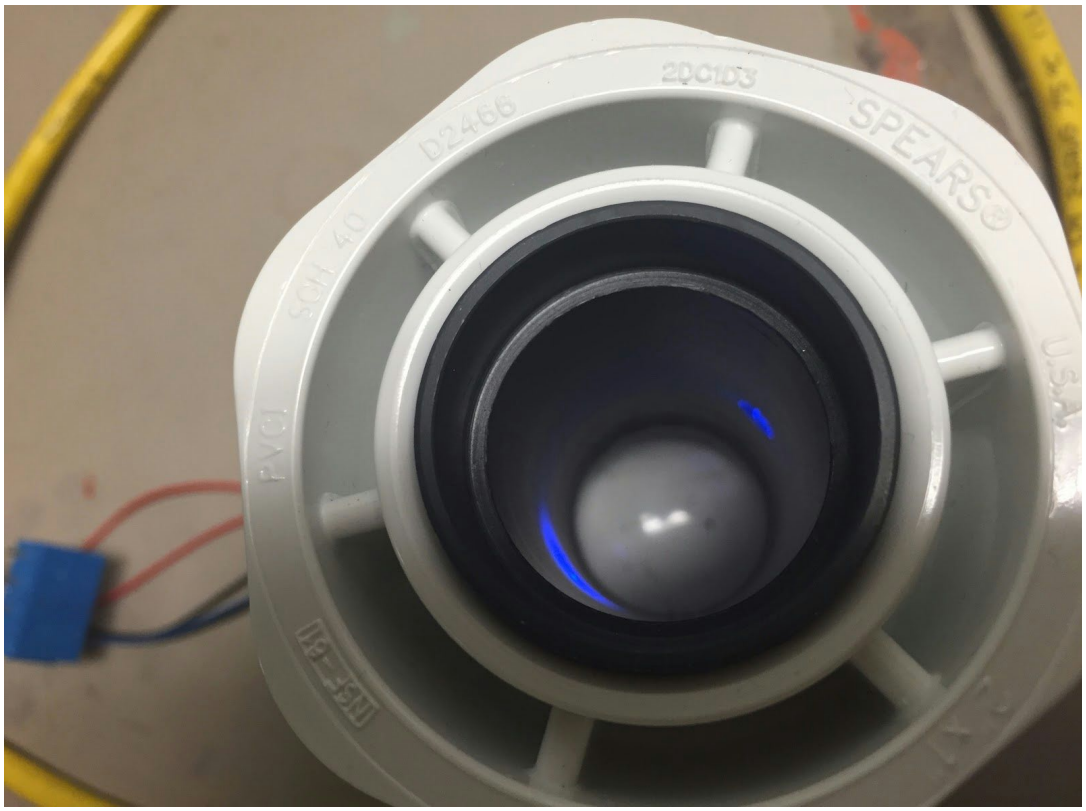

Figure 4: Ambient Light Photosensor (Zoom-in)

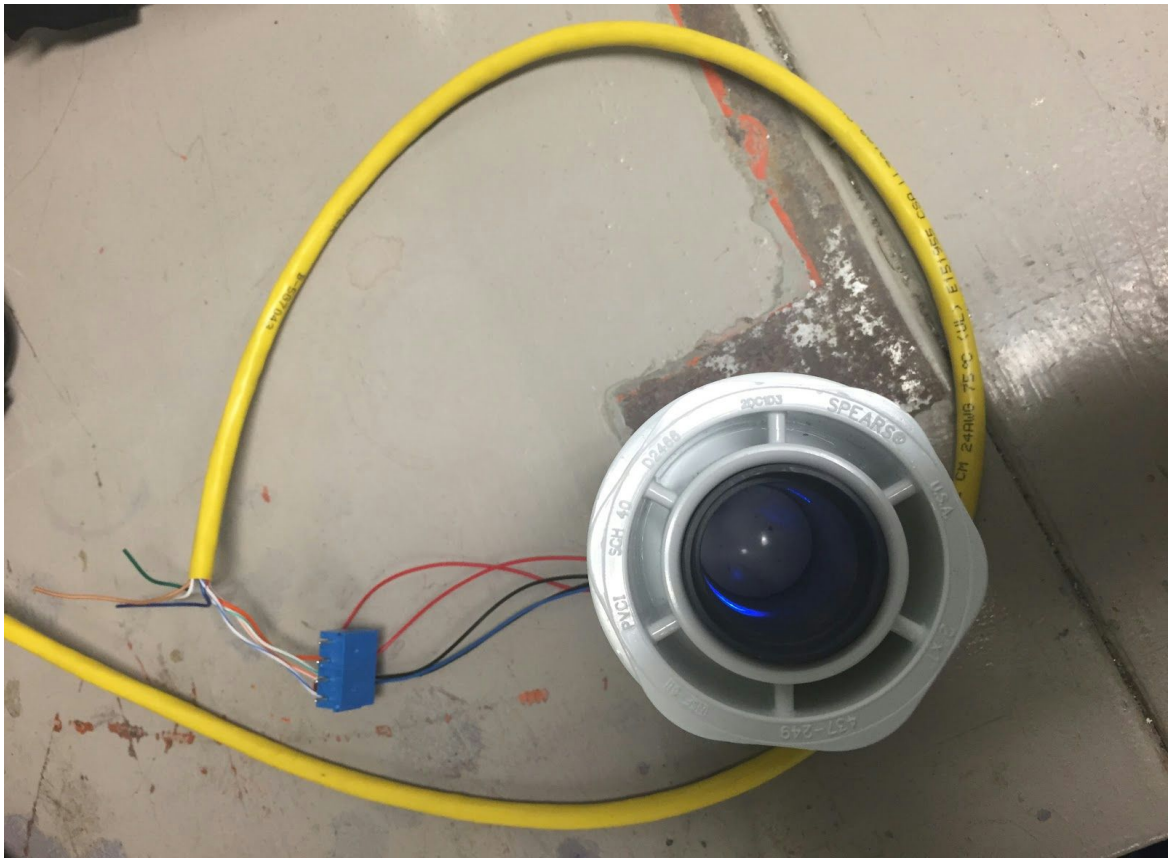

Figure 5: Ambient Light Photosensor (Top-view)

To test the photosensor, various concentrations of Red Dye 40 samples were created - 100, 50, 25, and 10 mg/L concentrations. In order to maximize efficiency, a precise pipette that was accurate to the nearest microliter was used to extract the exact amounts necessary from the 1000 mg/L stock in order to try and get the closest approximation to the actual concentration of the red dye in the water samples. The voltage of water with 0 mg/L concentration of red dye was also tested as a constant that reportedly had a voltage of 0.09 V. This discrepancy was later subtracted from each resulting voltage found for the other tested concentrations in order to make the calibration of the sensor more accurate. Each of these red dye water samples were then placed one-by-one in a turbidimeter vial - the sample container for this device - and the vial was placed in the photo sensor fabricated by Paul. The voltage provided by the sensor for each sample was then recorded.

Analysis

Based on the first set of experiments using only a webcam, it became clear that the use of a camera to measure light intensity, and ultimately, absorbance of different concentrations of red dye was going to be very difficult. An initial camera experiment shown below demonstrates two different sets of concentrations of red dye (0.05 and 0.1 M) images taken by the same webcam. Although the images below are distinguishable, they are not significantly different from each

other in the sense that one cannot accurately tell which one is which in terms of concentration. In other words, the differences between the two images is not nearly as distinguishable as the concentrations of red dye displayed in figure 1 of the methods section of this report. To illustrate this point, images of the experiment were included below:

Figure 6: Trial 1 (shows 0.05 M sample from Figure 1)

Figure 7: Trial 2 (shows 0.1 M sample of water from Figure 1)

Although the quality of the camera above is blurred, the hue of the red dye in each image is practically the same. Thus, it was concluded that directly using webcams would not help in detecting nuances in the concentrations of red 40 dye in samples of water.

As can be seen from the small experiment, it will be necessary to gather a more accurate measurement device that will satisfy the physics and mathematics of calculating absorption and getting reliable, useful data that can be effectively utilized as part of the larger AguaClara process.

To this end, we decided to use a more “true” spectrophotometry approach to the problem, and thus once the spectrophotometer kit arrived from PublicLab.org, this approach was tested. After the Public Lab Spectrometer 3.0 (the name of the assembled spectrometer) was assembled, a simple test was ran to see whether or not the spectrometer could monitor changes in wavelength in real time according to a specific color. In the case below in Figure 5, the spectrometer was able to monitor the changes in wavelength the diffraction grating captured through the lens of a small, VIDEW licensed, JDEPC-OV04 camera module. The photo below shows differences in hues for the actual red color of the wavelength captured by the diffraction grating. The wavelengths of red light below range from about 590 to 630 nm depending on how light or dark the red color of the wavelength is. In the case below, the lighter hues of red are close to 590 nm, while the darker portions of the red color indicate a wavelength approaching or at 630 nm.

Figure 8: Evidence of red wavelength detection

As can be seen in the figure, there was a significant difference in wavelength for the two samples, but in consultation with Monroe, it was decided that it would be overly complicated to try and manipulate this data to yield a usable concentration value. As a result, this approach was shelved, but is perhaps worth looking at in the future for a case more general than measuring only Red Dye 40.

Red Dye 40 Photosensor

For our next set of experiments, the completed photosensor that measures voltage output and light intensity of samples was used in order to calibrate the ranges of concentration it could determine absorbance for. Following the procedure outlined in Methods, samples with varying concentrations of Red Dye 40 were prepared, and the voltage produced for each sample by the photosensor was recorded.

Based on the data below, the photo sensor was slightly off in calculating the exact concentrations of tested red dye samples. For this reason, it was questioned whether or not the sensor system was linear, thus logarithmic calculations were performed to analyze whether or not the system would yield more accurate results through logarithmic manipulation. However, both linear and logarithmic manipulation of data both differed slightly from the actual concentrations of the red dye when tested. The table below shows the results of calculations on some sensor data calibrated using the 10 mg/L sample, assuming a logarithmic sensor device, for sample concentrations of red dye samples.

Concentration (mg/L)	Voltage	Measured Concentration
0	4.48	0
10	2.405	10
25	0.247	23.24
50	0.122	24.13
100	0.104	24.26

Table 1: Calibrated Data for Sample Concentrations of Red Dye No. 40

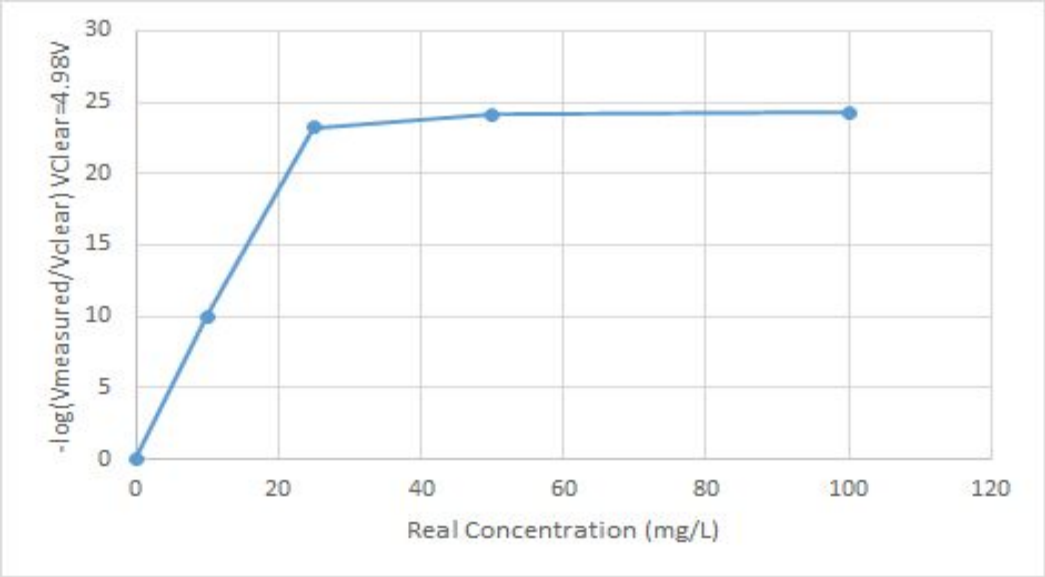

Figure 10: Real Concentration vs. Measured Concentration

The above figure displays that the maximum range for the concentration is at 25 mg/L. This means that the photo sensor can only reliably record concentrations and absorbances of constituents (or tested samples) at a maximum of 25 mg/L. From this data, another experiment was done with new samples of concentration in order to take closer advantage of the sensor's range. The results from forming 5, 15, 20, and 25 mg/L samples were as follows:

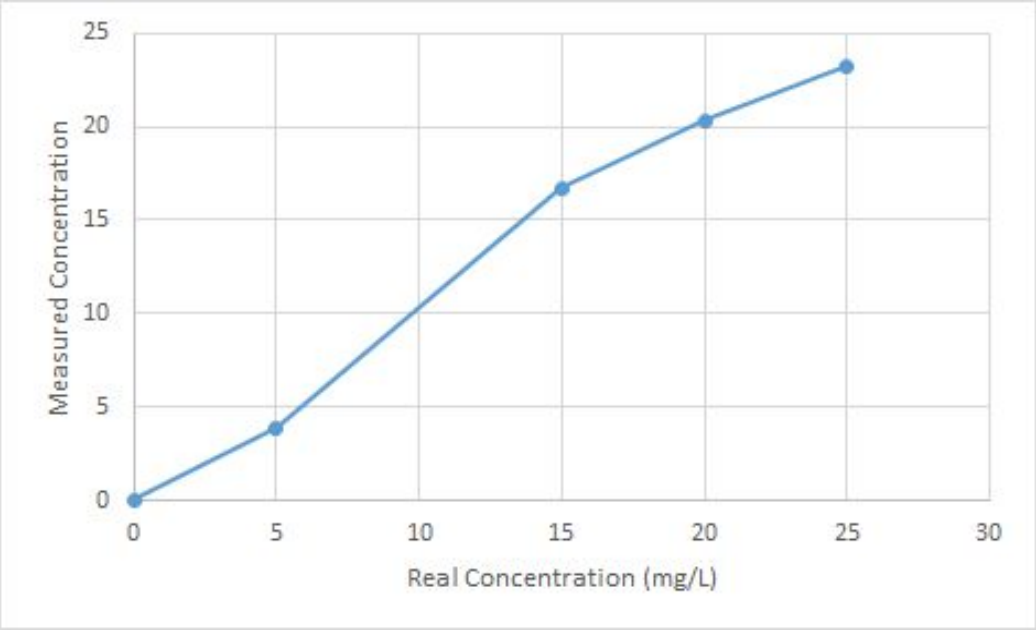

Figure 11: Real Concentration vs. Measured Concentration (up to 25 mg/L)

This new gathered data fit a more linear curve, however, the photo sensor continued to be slightly inaccurate since actual concentrations were not measured correctly. Below is the data corresponding to the above graph:

Table 2: Calibrated Data for Sample Concentrations up to 25 mg/L

Concentration (mg/L)	Voltage	Measured Concentration
0	4.48	0
5	3.62	3.9
15	1.24	16.7
20	0.673	20.3
25	0.241	23.3

The above data shows that although the sensor records up to concentrations of 25 mg/L, the measured concentration that the sensor outputs continues to not match the actual concentrations of the tested samples. From there, another experiment was conducted in which the voltage output was lowered for the photo sensor, which changed the results minimally.

Questioning the accuracy of our samples at such low concentrations, a new stock solution was created that would be used to create more accurate samples at these low concentrations. Below are data for what was gathered so far of the new testing of the new low-concentration samples, again calibrating using the 10 mg/L sample:

Expected Concentration (mg/L)	Voltage	Voltage-Offset (Offset = 0.09V)	Vsamp /Vclear	LOG10	Calculated Concentration
0	3.42	3.33	1	0	0
5	1.69	1.6	2.081	0.318	10
10	0.871	0.781	4.264	0..630	19.785
15	0.503	0.413	8.063	0.906	28.477
20	0.288	0.198	16.818	1.226	38.507
25	0.182	0.092	36.196	1.559	48.964
30	0.124	0.034	97.941	1.991	62.545
35	0.102	0.012	277.500	2.443	76.754

Table 3: Data for Latest Measured Concentrations

As one can see, the difference between 19.94 and 20 is negligible, so this is considered to be a successful measurement.

Further trials with more samples of varying concentrations were performed and the sensor did admirably in measuring the concentration of Red Dye 40 at all concentrations up to about 25 mg/L.

Conclusions

Through the camera module and diffraction grating provided by the assembled spectrophotometer, it has become viable to detect concentrations of Red Dye No. 40 because our new simple experiment proved that wavelengths for specific colors of light can be indeed detected. Thus, the use of webcams to observe the depictions of different Red Dye No. 40 samples of water will be disregarded for now in favor of the aforementioned diffraction grating option. Moving forward with this discovery, it will be important to calibrate the spectrometer using a fluorescent bulb so that the detection of wavelengths and their nuances can be

measured real-time in nanometers. Through calibration, the ability to have access to scaled data will allow for measured results in the lab. By expanding on software called Spectral Workbench, (provided by PublicLab.org) the next goal in our project is to capture and create a single-value data stream representing the sample's absorption.

Despite the further exploration needed on the spectrophotometer, an immediate concern for now will be the photosensor device. The team has continued to validate its findings and mathematical processes for this device, and has been working with Monroe to incorporate the device as an additional measurement apparatus in the ProCoDA II software.

Future Work

Firstly, a detailed procedure to calibrate the sensor will be documented in order that others can use it accurately. Following that, the prospect of designing and building a DOM, or Dissolved Organic Matter sensor, will be researched in the last days of this term, building on our experience with Red Dye 40. Currently, we are working to replicate the success of the photosensor we have built this semester, but at 254nm - a wavelength of light suitable for use in a photosensor for Dissolved Orgainic matter. We are exploring a variety of 254nm light sources, from LEDs (costly, but perhaps easier to build with) to fluorescent bulbs (which can surprisingly be found for very inexpensive prices). We must also explore further the detector device, as well as a flow cell that will work for 254nm light (the current glass flow cell from the turbidimeter will not work for this wavelength).

The following is a table that the team has prepared from our research of possible parts to use in building the 254nm spectrophotometer:

Part	Estimated Cost ($)	Use
254nm Fluorescent Light (Generic Brand)	$25	Light Source
254nm LED	Around $236-274	Light Source
OSI Optoelectronics UV Photodiode	Unsure, found one model for sale in the UK for 90 GBP (~$135)	Main sensor device
HF Scientific UV Flowcell (Quartz) Part # 24232S	List price is $318.00- Cornell receives 50% off , cost is $159.00	Flowcell

Quartz Tube	Found one for $25; unsure if adequate	Flowcell

Table 4: 254nm Photosensor Parts

Lastly, one of the other AguaClara subteams who used the prototype photosensor found a need to switch to blue dye - Red Dye 40 was inadequate for use in their experiment. The LED was changed to match the absorbance spectra of the particular blue dye in use, and it is unclear if the photosensor was as effective in this configuration. This is another area for further exploration of the spectrophotometer team. Perhaps it would be useful to research the appropriateness of various dyes in various experiments, and targeting the work of the team towards those dyes. This semester's team assumed that Red Dye 40 could act as a universal tool in the AguaClara lab, but this other subteam's work shows that Red Dye 40 is perhaps not a universal tool.

References

"Spectrophotometry." - Chemwiki. UC Davis, 02 Oct. 2013. Web. 25 Sept. 2015.

Warren, Jeffrey. (2012, February 24). "Spectral Workbench Calibration". - *Public Lab*. Web. 9 Oct. 2015.