

**An improved in situ sensing device for freshwater DOM
characterization**

by

Lu Xu

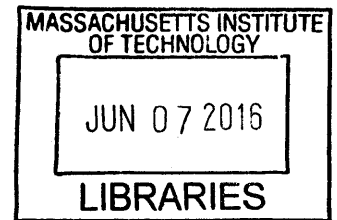
Submitted to the Department of Civil and Environmental Engineering
in partial fulfillment of the requirements for the degree of

Master of Engineering in Civil and Environmental Engineering

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June 2016



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Abstract

DOC characterization of freshwater is poorly understood at areas of harsh or difficult to access, especially areas like peatland rainforest. In order to figure out the level of DOC transport, an in situ instrument was previously designed to measure the DOC concentration at remote locations. However, the data collected by the instrument is not as meaningful as expected due to the complex environment, high DOC concentration and inner shielding. A new instrument is required to be designed to fulfil the objectives. Like the previous instrument, the new instrument still needs to measure the fluorescence and absorbance data to give an estimation of DOC concentration. There are three LEDs to provide fluorescence measurements, a wideband lamp to provide light for absorbance measurement and a spectrometer to record the resulting spectra. Unlike general in situ fluorimeters, the orientation between the excitation source and detector is 90 degrees for the new optical configuration. The new designed optical configuration solves the problem of window obstruction and also successfully prevents the problem of misalignment caused by water turbulence. Furthermore, the instrument also solves the problem of long light path lengths with the new configuration. The instrument was tested to work well in the lab with a range of DOC concentration from 5 ppm to 60 ppm. The recorded data showed a strong positive relationship between fluorescence intensity and concentration of DOC.

Thesis supervisor: Harold F. Hemond

Title: William E. Leonhard Professor of Civil and Environmental Engineering

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Contents

1	Introduction	1
1.1	Purpose	1
2	Literature Review	2
2.1	Absorption of DOC	2
2.2	Fluorescence of DOC	5
2.3	Common Method of Measuring DOC[2]	8
3	Instrument Description	9
3.1	New Optical Configuration	9
3.2	Control Circuit Board[2]	10
3.3	Fluorescence LED Excitation Sources [2]	12
3.4	Wideband Absorbance Lamp [2]	13
3.5	Spectrometer [2]	13
3.6	Batteries [2]	13
4	Light Guide	13
4.1	Coupling an optical fiber to an LED	14
5	Optimize the angle between fluorescence excitation and detection fibers	15
6	Laboratory test with varying concentrations of humic solution with LED 385nm	16
7	Procedures for deploying the instrument	17
8	Calibrate the instrument	20
9	Laboratory test with the instrument	21
10	Conclusion and Future Development	25
	References	27

List of Figures

1	Model structure of humic acid and fulvic acid	2
2	Light Absorbance at 250nm(E250) with concentration of DOC	3
3	The calculated concentration of DOC versus the concentration of observed DOC	4
4	Absorbance at 255, 340, 350, 400,450, and 665 nm in Cottage Hill Sike	4
5	Fluorescence emission spectra at varying pH conditions	6
6	HIX with varying concentration of DOC expressed as transmittance at 254 nm	7
7	Comparisons of the fluorescence of F-L peak versus DOC for RIVER	7
8	Comparison of the fluorescence of H-L peak versus DOC for RIVER	8
9	System overview (Adapted from Schuyler's thesis)	9
10	Isometric view of the optical configuration	10
11	Control Circuit Board	11
12	LED Circuit Board	12
13	Technique for Coupling an Optical Fiber to an LED Lamp	14
14	Angle test with 0.5mA input current	16
15	Angle test with 1mA input current	16
16	Fluorescence peak intensity with varying DOC concentration with LED 385nm	17
17	Battery Compartment	18
18	Overview of the HammerHead	19
19	Fluorescence intensity with varying DOC concentration	19
20	Fluorescence intensity with varying DOC concentration	20
21	Spectrum of 375 nm LED	22
22	Spectrum of 385 nm LED	22
23	Spectrum of 405 nm LED	23
24	Fluorescence Peak Intensity with LED 375nm	24
25	Fluorescence Peak Intensity with LED 385nm	24
26	Fluorescence Peak Intensity with LED 405nm	24
27	Tungsten Light Response	25
28	Absorbance of 60ppm Humic Solution	25

List of Tables

1	LED Coupling efficiency	14
2	Fluorescence peak intensity with varying DOC concentration with LED 385nm	17

1 Introduction

Recent studies show that the high level of DOC transport can be considered as an indicator of climate change and changes in sulfur deposition [1]. With increased awareness of the role of DOC in the global carbon cycle, it is important to understand the fate and transport of DOC. Dissolved organic carbon (DOC) is a mixture of organic matters dissolved in water and is chemically made of humic substances. It could be derived from the decomposition of living creatures, mainly plants and algae; human activities are another possible source of DOC. Human activities could also have a great influence on the fate and transport of DOC in freshwater. A specific example mentioned in Schuyler's thesis was the deforestation in southeast Asia [2]. Due to the land use changes, it led to significant changes in both atmospheric and aquatic carbon fluxes. Quantifying the transport of the DOC in freshwater has caught the attention of climate scientists.

To better understand the DOC flux in peatland, the previous instrument was designed to record fluorescence and absorbance measurements over longer time periods under high levels of DOC. The instrument was successfully deployed underwater in remote locations and was able to record the UV/VIS absorbance and fluorescence spectra of peatland water for several weeks. However, there were few technical issues that limited the instrument.

1.1 Purpose

The purpose of this project is to address and solve the problems that the old instrument faced. The first objective is to solve the problem of a nonlinear relationship between the concentration of DOM and fluorescence intensity data that was provided by the old instrument. There was a positive relationship between the concentration of DOM and fluorescence intensity at low concentration of DOM, but the relationship inverted at high concentration of DOM. The reason behind this might be because of the long light path lengths of the fluorescence excitation sources. The new instrument should be able to shorten the light path lengths of the excitation sources so as to adapt the high DOC concentration.

The second objective is to solve the problem of misalignment between the instrument's absorbance light fiber and the detection fiber. The old instrument had a long thin needle attached to the fiber from wideband lamp. Hence, the light fiber optic was easily shifted by water turbulence. The misalignment between the light source and detector resulted in meaningless absorbance data. The new instrument should be designed to keep the alignment constantly between the absorbance optical fiber and the detector.

The previous instrument also faced the problem that the optical window of the instrument was occasionally obstructed by sediments. The covered optical window would reduce the transparency of the window and then affect the intensity of the excitation sources and fluorescence. The new instrument should avoid using optical window and should be robust enough to face any kind of tough environment.

2 Literature Review

Dissolved organic carbon (DOC) is generally defined as the fraction of dissolved organic matters (DOM) that can pass through a 0.45 μm membrane and is often chemically made of humic and fulvic fractions or acids[3]. Below $\text{PH}=2$, the humic acid is not able to dissolve in water but the fulvic acid still remains in solution[4]. From the sample structure of humic acid and fulvic acid showed below (Figure 1), humic acids generally have higher molecular weight but less oxygen content than that of fulvic acids. Also, humic acid has a higher degree of condensed aromatic character. When dissolved in water, the color of humic acids ranges from brown to black and fulvic acids will appear as light yellow or dark yellow.

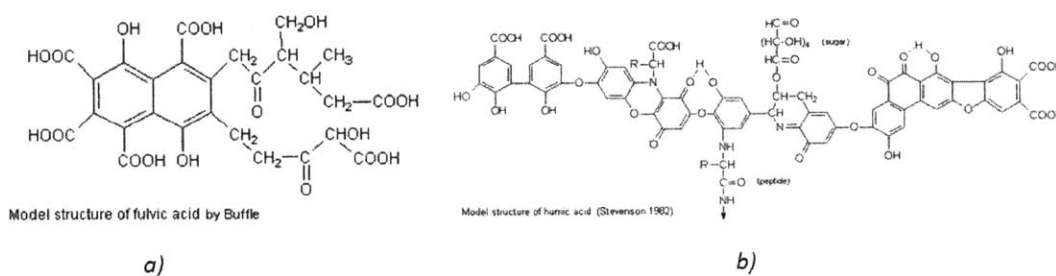


Figure 1: Model structure of humic acid and fulvic acid

2.1 Absorption of DOC

DOC involves in many environmental processes, the character of DOC has been studied intensively. Routine methods for the accurate determination of DOM in different samples are based on the measurement of DOC, usually after conversion to CO_2 [5]. The following part will give a brief review of different methods of using the optical property of DOC to determine the concentration of DOC in freshwater. Optical absorbance at a single wavelength in the UV-visible range usually correlates strongly with concentration of DOC, and is therefore often used to monitor natural DOM[5].

In 1986, Haan mentioned in his paper that light absorbance at 250nm accurately predicted the concentration of DOC in lake Tjeukemeer. The molecular size of the DOM might affect the accuracy of using E250 and F to measure the concentration of DOC, but there still exists a strong linear relationship with middle size DOM(Figure 2). U.V. light was suggested to use for DOC concentration measurements. The following equations are the relation found between E250 and DOC in different lakes[6].

$$\text{DOC} = 18.9 * E_{250} + 5.7 \quad (1)$$

$$\text{DOC} = 24.7 * E_{250} + 2.7 \quad (2)$$

$$\text{DOC} = 30.5 * E_{250} \quad (3)$$

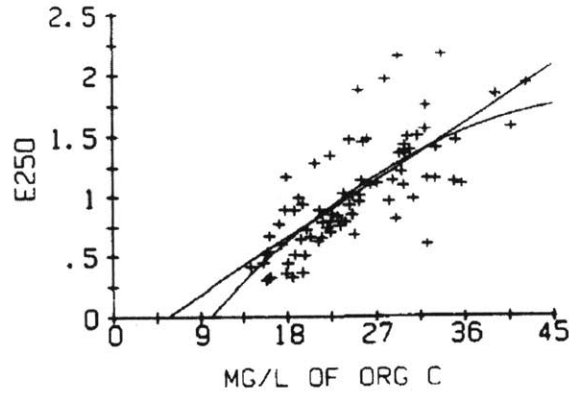


Figure 2: Light Absorbance at 250nm(E250) with concentration of DOC

However, Tipping is not fully agreed with what Hann found in 1986. Tipping claimed that the prediction of [DOC] from absorbance data at a single wavelength was not possible, because of differences in DOM extinction coefficients among the samples. A two-component system was proposed by Tipping's group. The extinction coefficient at any wavelength is given by

$$E_{\lambda} = f_A E_{\lambda,A} + f_B E_{\lambda,B} = f_A E_{\lambda,A} + (1 - f_A) E_{\lambda,B} \quad (4)$$

$$f_A = \frac{E_{\lambda_1,B} - R E_{\lambda_2,B}}{R E_{\lambda_2,A} - R E_{\lambda_2,B} - E_{\lambda_1,A} + E_{\lambda_1,B}} \quad (5)$$

R is the ratio of optical absorbance values ($A_{\lambda_1}/A_{\lambda_2}$) for the sample in question.

The measure of optical properties used here is the extinction coefficient (E) obtained as the ratio of optical absorbance at a given wavelength (λ nm) to [DOC], and with units of $I/(g \cdot cm)$ (Figure 3). The method could be used to predict [DOC] in water samples simply from absorbance values at two wavelengths (we used 254 nm and 340 nm). The downside of this method is that more sets of experiments with a broad scope of DOM and water samples need to be done to improve the parameters, especially to define parameter B [5]. Again, Grayson found that it is perhaps too simplistic to use a single wavelength to characterize DOC in 2011 [7]. Of those wavelengths previously used to characterize DOC concentrations, those below 300 nm exhibit high background values and noisy signals (Figure 4), with little differentiation between wavelengths and rapid fluctuations in absorbance over short periods of time thus limiting their ability to discriminate variations in DOC concentrations. However, at wavelengths above 300 nm (Figure 4) the signal is noticeably clearer suggesting that they have greater ability to discriminate changes in DOC.

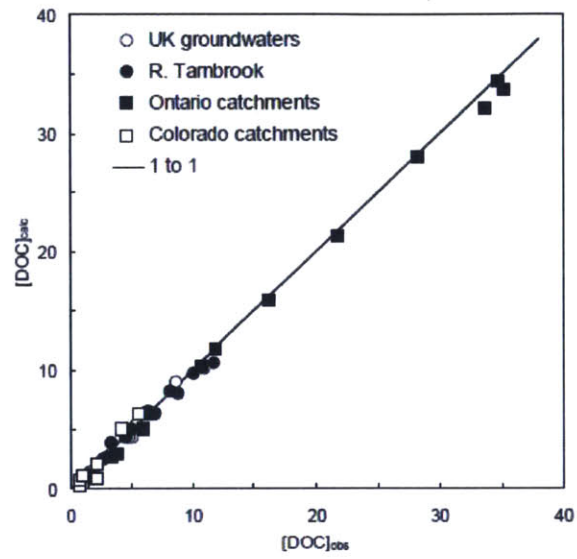


Figure 3: The calculated concentration of DOC versus the concentration of observed DOC

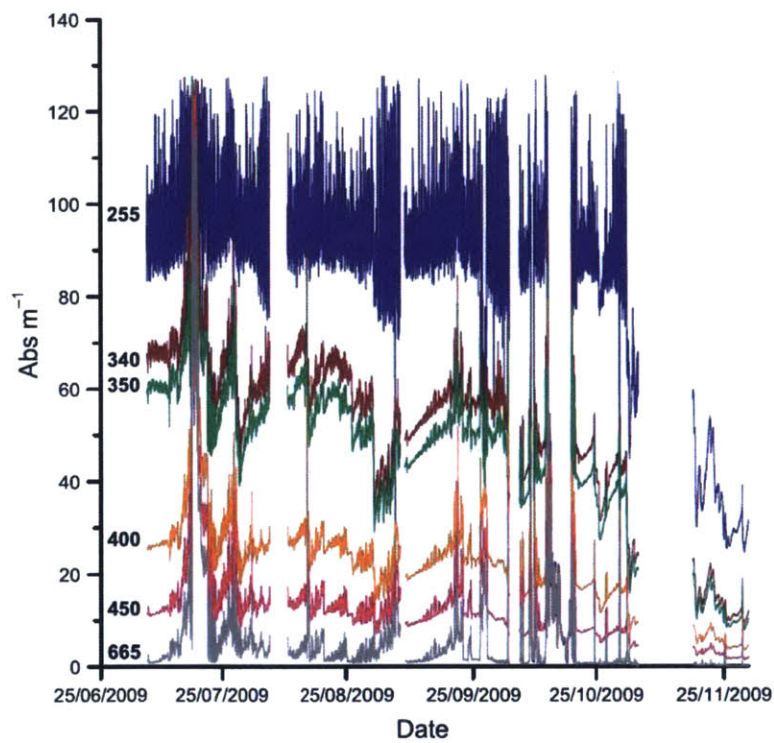


Figure 4: Absorbance at 255, 340, 350, 400,450, and 665 nm in Cottage Hill Sike

Indeed, Tipping et al., (2009) suggest method using absorbance at two wavelengths offers a much-improved estimate of DOC compared with single wavelengths; unfortunately, they use absorbance at both 340 and 254 nm and, as has already been demonstrated, absorbance measurements at less than 300 nm were noisy and so a similar approach for estimating DOC could not be used. Clear decreases in absorbance at 340 and 400 nm coincide with increases in discharge during a large number of the storm events, with decreases in absorbance being witnessed on the rising limb of the hydrograph during storms.

Furthermore, the two dominant components of DOC, humic and fulvic acids, absorb light in different amounts at different wavelengths as their characteristics differ; humic acids are more mature (i.e. better humified) than fulvic acids. As a result, the ratio of absorbance at 465 nm and 665 nm (465/665, or E4/E6 ratio) can be used to measure the proportion of humic and fulvic acids and, hence, the degree of humification or aromaticity (Thurman, 1985). Mature humic acids from soil typically have an E4/E6 ratio of 2 to 5 indicating increased humification, whereas, less mature fulvic acids have a ratio of 8 to 10[4].

2.2 Fluorescence of DOC

Fluorescence is often observed when electrons return from the excited state to ground state. The energy of the emitted light has less energy, therefore, has a longer wavelength as compared with absorbed light. Hence, a red-shift is normally seen on fluorescence emission spectra (Figure 5). The electron donating groups such as hydroxyl and methoxyl groups have also been reported to enhance fluorescence by increasing the transition probability between the singlet and ground state. Also, the exhibited characteristic fluorescence spectra are related to the composition of the structure of DOC. As early as 1971, Datta et al. (1971) indicated that humic substances, regardless of their origin, exhibited characteristic fluorescence spectra which were attributed to the presence of aromatic fluorophores with electron-donating functional groups. Stewart and Wetzel (1980) showed that larger molecular weight aquatic humic fractions had a greater absorbance but lower fluorescence than smaller molecular weight fractions. Hence, we can also use the fluorescence property to determine the concentration of DOC.

Firstly, as the molecular size and aromatic content of DOC affect the fluorescence intensity, the fluorescence intensity could be used for determining the concentration of DOC. The peak emission wavelength of NOM shifted from shorter to longer wavelengths with increased molecular size and aromatic content (i.e., Soil HA > NOM-PP > NOM-CH; Figure 5) at varying PH conditions. An increased fluorescence intensity and red shift in this region may result from an increased content of high molecular weight humic materials. On the other hand, the presence of large amounts of simple, dissociated phenolic and quinone types of organic compounds may result in increased fluorescence in the region of about 380-430 nm [8]. Hence, it is possible to use the extent of redshift to estimate the humic substance.

Furthermore, organic materials become more reactive in environmentally relevant reac-

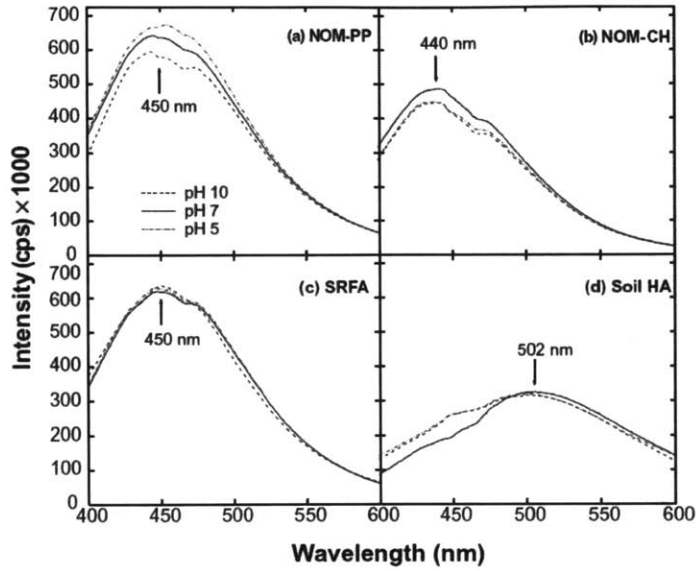


Figure 5: Fluorescence emission spectra at varying pH conditions

tions due to increased functional group density with increased humification. Fluorescence spectroscopy methods have been proposed to determine the extent of humification by quantifying the extent of shifting of the emission spectra toward longer wavelengths with increasing humification. The concentration effects on HIX values are shown in Figure 6 with the HIX value on the y-axis and the concentration of the organic matter expressed as transmittance at 254 nm on the x-axis [9].

$$HIX = \frac{\sum I_{435 \rightarrow 480}}{\sum I_{300 \rightarrow 345}} \quad (6)$$

I is the fluorescence intensity at each wavelength.

Thirdly, the fluorescent properties of dissolved organic matter (DOM) enable comparisons of humic-like (H-L) (Figure 7) and fulvic-like (F-L) (Figure 8) fluorescence intensities with dissolved organic carbon (DOC) in aquatic systems[9].

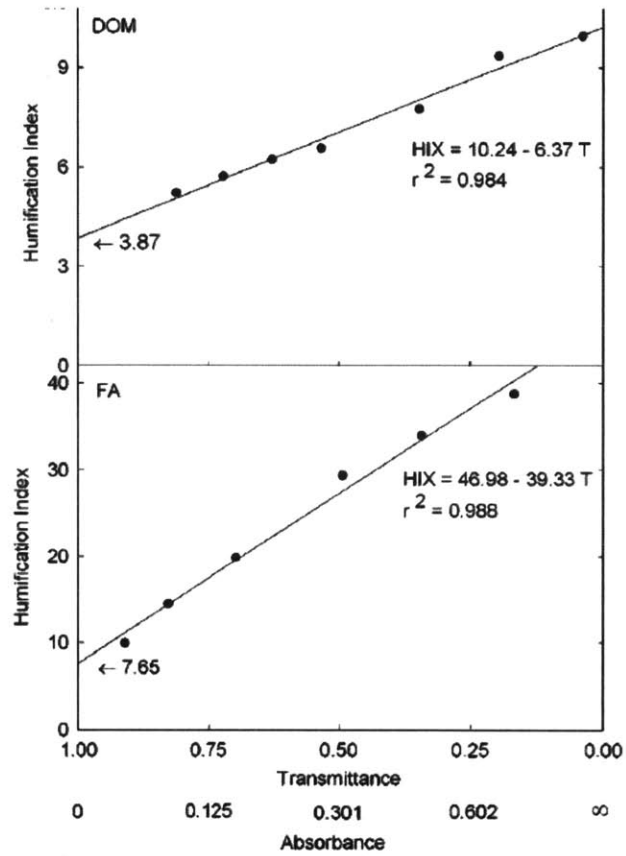


Figure 6: HIX with varying concentration of DOC expressed as transmittance at 254 nm

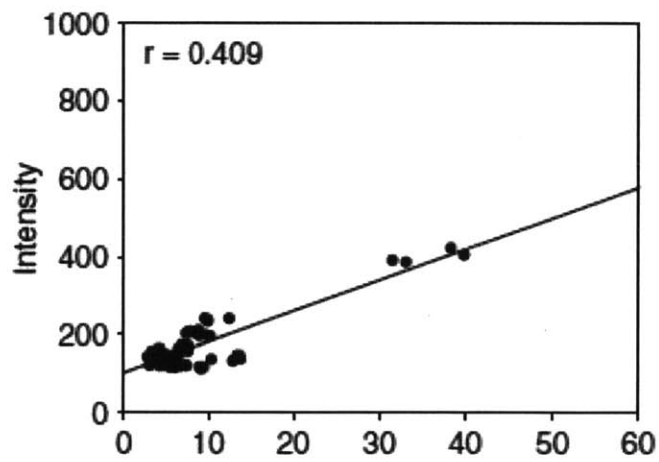


Figure 7: Comparisons of the fluorescence of F-L peak versus DOC for RIVER

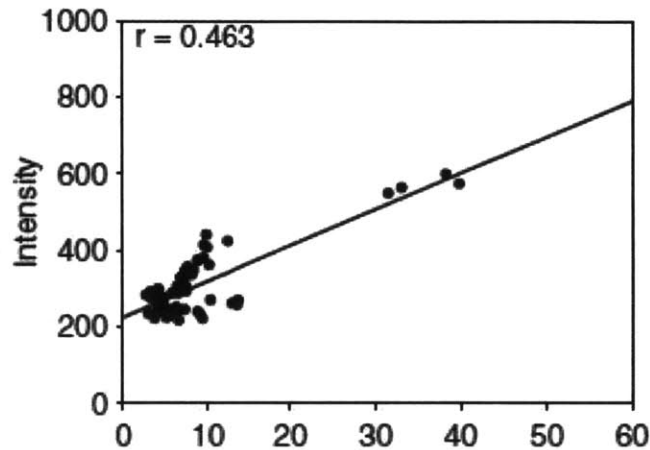


Figure 8: Comparison of the fluorescence of H-L peak versus DOC for RIVER

2.3 Common Method of Measuring DOC[2]

The most common method of measuring DOC in situ relies on the optical properties of water samples. Fluorescence measurements of the water provide estimates of colored dissolved organic matter (CDOM), which is related to the total amount of DOM present. The conversion from CDOM to DOM is specific to the body of water and sources of carbon, but the utility of the method is generally accepted once site-specific conversion curves are developed through more accurate laboratory measurement techniques. Measurements of DOM can be extrapolated to DOC through further laboratory analysis or by estimating the proportion of carbon in organic matter from the literature [10].

There are several commercially available in situ CDOM instruments (e.g. WET Labs ECO, Turner Designs C3). These instruments estimate CDOM from fluorescence by exciting the water with light in the 350 nm range and sensing the level of fluoresced light in the 450 nm range. (The actual excitation/emission wavelengths vary between models.) The orientation between the excitation source and detector in most benchtop fluorimeters is 90 degrees, but for in situ fluorimeters is generally 0 degrees. These sensors typically have a maximum detection limit of approximately 1 mg DOM/L and therefore are not applicable to many highly concentrated water bodies. To the authors' knowledge, there are no commercially available in situ instruments capable of measuring in the range of concentrations often seen in peatland rivers (e.g. 35 mg/L). Estimating DOM at these concentrations becomes difficult because there is no longer a linear or even monotonic relationship between concentration and fluorescence, as high levels of DOM absorb the excitation and fluorescence signals via inner filtering [11].

Additionally, the above-mentioned instruments provide only a single fluorescence value making it impossible to estimate the FI of the sample. Deploying an instrument that can record the entire UV/VIS absorbance and fluorescence spectra allows for corrections of inner filtering and the calculation of FI parameters.

3 Instrument Description

The main objective of this project is to further improve the current instrument that was designed by Schuyler[2]. Since the new instrument still uses the similar components as the old one, some of the descriptions of the components will adapt from Schuyler 's thesis. One requirement of the instrument is that it is capable of measuring high levels of CDOM (up to 60 mg/L) in natural waters, and also that it have capabilities for measuring both absorbance and fluorescence. Additional design constraints are continuous underwater deployment for a minimum of several days, simple data retrieval via a micro secure digital (SD) card, and easy battery replacement in harsh environments (e.g. remote rain-forests).

The instrument is comprised of several subsystems that include: 1) narrow wavelength LED fluorescence excitation sources, 2) wideband absorbance lamp source, 3) spectrometer, 4) battery, 5) controller circuit board and data logger and 6) waterproof pressure case. Figure 9 shows an overview system schematic.

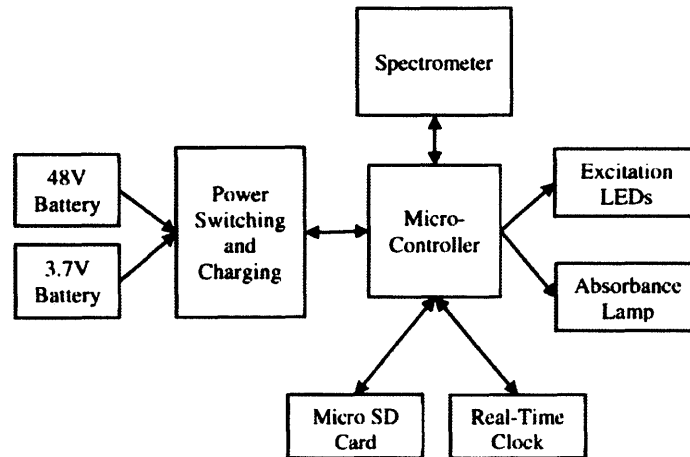


Figure 9: System overview

3.1 New Optical Configuration

The new optical configuration should be able to shorten the light path lengths of the excitation sources to minimize the absorption loss. It should constantly keep the alignment between the fiber connected to wideband light and the fiber connected to the spectrometer. With those objectives, a new optical configuration is designed.

Plastic fibers are used as light guidance for all the excitation sources, which allows arranging the fibers in different degrees. The free end of each fiber is protected with stainless steel needles so that it can be inserted into threaded cajon to make it water tight. There will be 5 pieces of optical fibers in total, 3 for fluorescence excitation sources, one for wideband light and the last one for the spectrometer. Since the spectrometer is able to collect

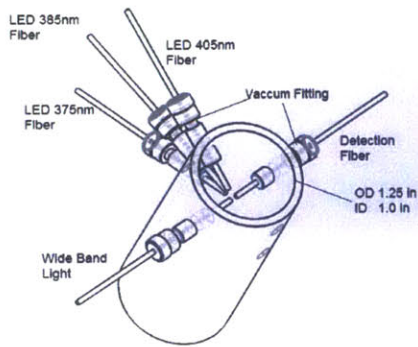


Figure 10: Isometric view of the optical configuration

absorbance and fluorescence data, the other four pieces of fiber should be implemented to focus on the fiber that connected to the spectrometer. The angle between the collection fiber and the fiber of wideband lamp has to be exact 180 degrees for absorbance measurement. The angle between detection fiber and fluorescence excitation fibers is more flexible, which can range from 0 to 90 degree. Zero degree was used for the previous instrument; however, the zero degree configuration collected ambiguous fluorescence data as the light path length is longer than any other degrees. 90 degree is chosen for the new optical configuration for the angle between UV excitation sources and collection fiber as it has the shortest light path lengths. Figure 10 shows the new optical configuration of the absorbance and fluorescence excitation sources and collection. Three LED excitation fibers are on the same side of the pipe and they are all perpendicular to the collection fiber. The absorbance light fiber is put 180 degrees with the collection fiber.

3.2 Control Circuit Board[2]

The new instrument still uses the previous control system that tested by the old instrument. A single printed circuit board (Figure 11) provides a majority of the electronic controls. These include efficiently converting the battery voltage to the required operating voltages, initiating a measurement at set time intervals, communicating with the LED and lamp light sources, communicating with the spectrometer and storing data on the micro SD card.

The circuit uses a microcontroller to control sensor operation. The microcontroller is an Atmel Atmega 328, the same microcontroller used on the Arduino Uno, and is preloaded with the Arduino bootloader so it can be programmed similarly to Arduino circuit boards. This microcontroller was chosen due to its ease of coding and programming, and wide availability of open source Arduino code libraries for communication over a serial UART

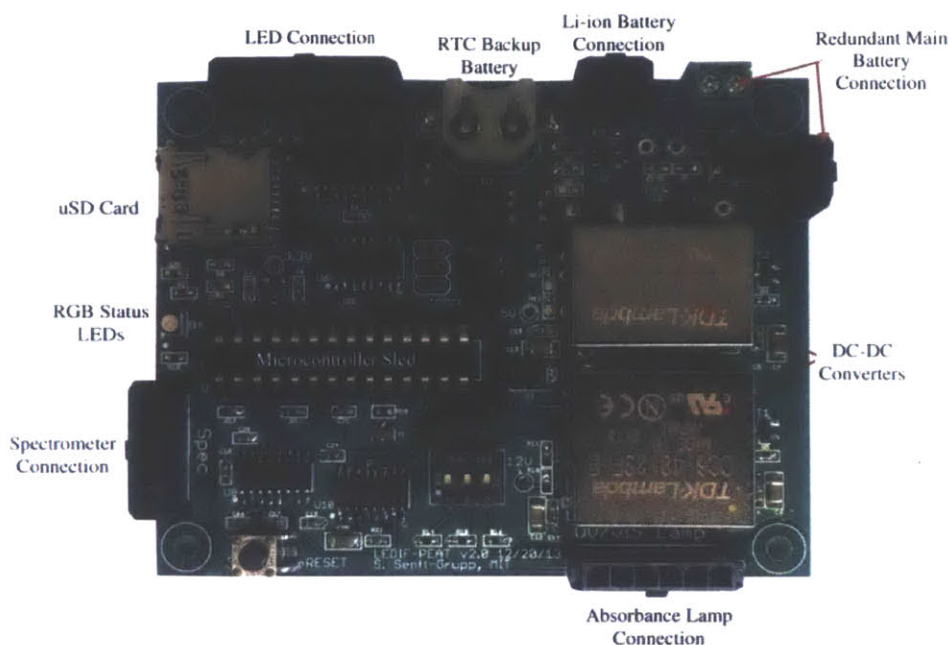


Figure 11: Control Circuit Board

and SPI bus (two different common protocols for communication between integrated circuits), as well as reading and writing to a micro SD card. The microcontroller is a dual inline package (DIP) and can be easily removed from the main circuit board and installed on Arduino Uno board for uploading the code. This provides an easy method to upgrade the instrument firmware in the field - the person servicing the instrument can bring a new microcontroller programmed with updated firmware when she is retrieving the data and changing the battery. The Atmega 328 also has several low-power sleep modes that make it ideal for an application such as this that is largely inactive.

The board maintains time using a real-time clock (RTC). The RTC (Maxim DS3234) is accurate to 2ppm which corresponds to a potential drift of approximately 1 minute/year. Communication with the chip is over SPI and includes the ability to program the current date, time, and two separate alarms. For the alarms, when the current time equals the time stored in the alarm register, an interrupt signal is sent from the RTC to the microcontroller to wake it from sleep mode. A 3V coin cell battery is included on the PCB to provide minimal power to the RTC when no other power is connected. An Arduino library has been written to allow for easy use of the RTC.

Power management is a critical function of the circuit board. The main power comes from a 48V battery source which is converted to 5 and 12V with efficient DC-DC converters. These DC-DC converters can achieve almost 90% efficiency when operating near their power capacity, but are very inefficient at low loads. Therefore, when the instrument is in sleep mode the microcontroller disables both of the DC-DC converters and seamlessly

switches over to a low voltage (3.7V) Li-ion battery. When the instrument is awake, it uses the main battery to recharge the Li-ion battery. The Li-ion battery can stay permanently installed in the sensor and does not need to be replaced or removed for external recharging. Additionally, when the microcontroller initially wakes up, it measures the voltage of both the main battery and backup battery. This information is logged for performance assessment and can also be used by the microcontroller to adjust the instrument behavior (e.g. decreasing the measurement frequency.)

3.3 Fluorescence LED Excitation Sources [2]

Five LEDs were used as the fluorescence excitation sources for the previous instrument. For the new instrument, three LEDs at wavelengths of 375nm, 385nm and 405nm are chosen based on the previous characterization of the organic matter fluorescence and commercially available LED wavelengths [12]. LED 375nm and LED 385nm are used for DOC concentration estimation and LED 405nm is used for Chlorophyll concentration estimation. These LEDs are driven by an integrated circuit (IC) designed to deliver a programmable constant current to up to 8 LEDs (TI TLC5916). The IC communicates over SPI and can be programmed to turn on any arbitrary combination of LEDs with individual currents ranging from 4mA to 100mA. (Only one current is programmed at a time, so if the IC is set to deliver 20mA and 3 of the LEDs are enabled, each LED receives 20mA and in totals the chip sources 60mA.) The IC uses feedback to monitor the current to each LED, and therefore the LEDs do not need to have the same forward voltage, or for that matter, a known forward voltage to maintain constant light intensity. However, the input current for the LEDs is not able to be controlled in the new instrument. The LED driver board only supply rated voltage for each of the LEDs. The excitation sources and LED driver are soldered onto a small PCB (Figure 12). The layout of the PCB precisely positions the LEDs in a circular formation around a central point.

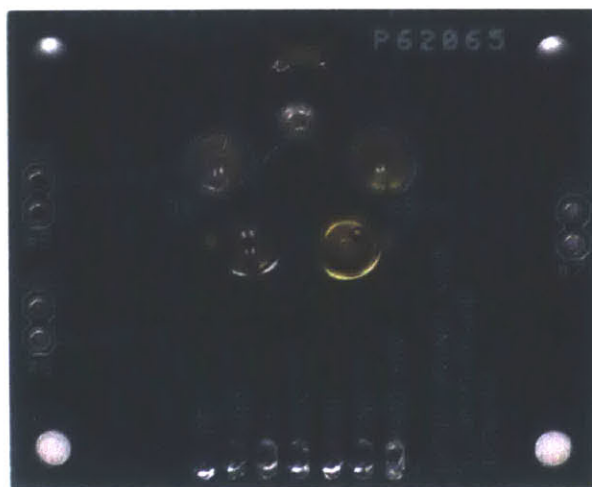


Figure 12: LED Circuit Board

3.4 Wideband Absorbance Lamp [2]

A wideband lamp is chosen for performing absorbance measurements. The lamp is the Heraeus FiberLight® Miniature UV-Vis Light Source (DTM 6/10), a combination tungsten and deuterium lamp with a spectral distribution between 185 and 1100 nm. The lamp is powered with 12V DC and uses 12 W. Each source can be enabled individually or a shutter can block all light output. This lamp is used in other long-term field instruments requiring high reliability and consistent output [13].

3.5 Spectrometer [2]

The instrument's spectrometer is an Ocean Optics USB4000. This spectrometer was chosen for its small size, performance, ease of use and has been demonstrated to work in similar instruments [14]. The spectrometer communicates over serial RS-232 with the microcontroller. A microcontroller code library has been written to control the spectrometer's various settings and capture spectrum data.

3.6 Batteries [2]

The instrument operates off of a 48 VDC source generated from four 12 V batteries wired in series. The sealed lead acid batteries were chosen for availability (they are widely available around the world), convenience (sealed lead acid batteries can be carried in checked airplane luggage), and cost. Lead acid batteries also come in a variety of sizes that fit into standard 5 inch PVC pipe. Additionally, at this operating voltage we were able to reuse components and circuits that had already been designed and tested for previous research projects utilizing 48 V [14]. The capacity of the battery was chosen to be 4.5Ah, compared with 7.5Ah for the previous instrument.

4 Light Guide

A light guide is a tool that is able to transport light from a source to another point. A good light guide means small amount of light loss due to the internal reflection and connection. There are a lot of different ways to couple an optical fiber to an LED lamp. However, the most effective way is to directly insert the end of optical fiber to LED package (Figure 13)[15]. The fiber should insert as close to the emitter as possible to minimize the losses at the fiber input and maximize output power. The coupling efficiency strongly depends on core material, core diameter and the numerical aperture (NA) of the connected fiber.

For this project, the three fluorescence excitation LEDs is coupled with plastic optical fibers. Plastic fiber is used as it is cheap and installation is much simpler, compared with quartz fiber. The plastic fiber can also be easily bent to a small radius with only small amount of light loss. In practice, only a small portion of the light from LED can be captured by the fiber. The coupling efficiency is also strongly depends on the fabrication

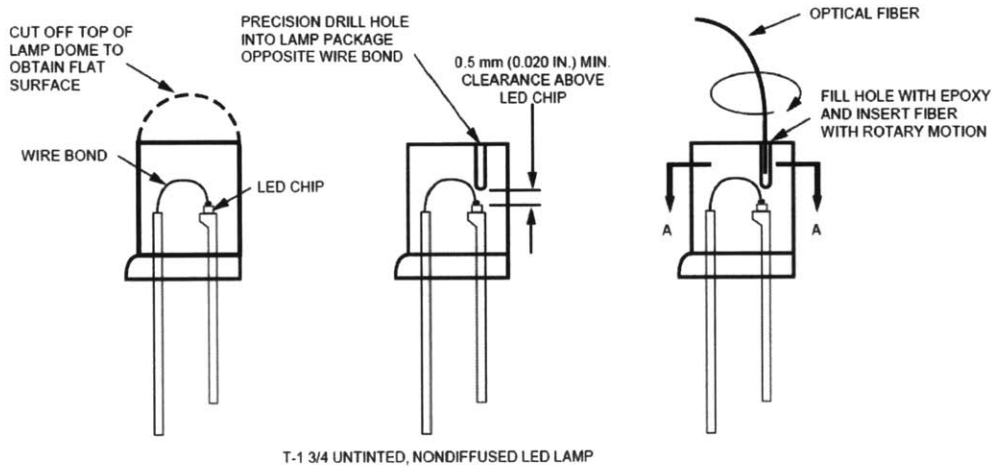


Figure 13: Technique for Coupling an Optical Fiber to an LED Lamp

works of butt-coupling. This is because it is hard to drill an insertion hole down to the middle of the reflector cup and stop at a distance of 0.5mm. The table below shows the efficiency of coupling of two different LEDs. It can be found that the efficiency of coupling an optical fiber to an LED is relatively low.

LED Wave-length	Rated Voltage	Rated Current	Radiation Power	Coupled Fiber Length	Radiation Power From Fiber	Coupling Efficiency
380nm	3.29V	20mA	163mW	51cm	19.2mW	11.78%
375nm	3.54V	20mA	14.2mW	38cm	2.37mW	16.69%

Table 1: LED Coupling efficiency

4.1 Coupling an optical fiber to an LED

Here are some detailed steps about how to couple an LED with plastic fiber[15]:

1. Instead of cut off the top of the dome package to obtain a flat surface, the top of the LED was polished to obtain a flat surface by using a file.
2. Check the position of the reflector cup, which is normally located in the center of the LED.
3. Precision drill an insertion hole down to the middle of the reflector cup and stop at a

distance of 0.5mm.

4. Insert clear, room temperature curing epoxy into the hole by using a syringe with a thin needle.
5. Immediately insert the optical fiber (well-polished) into the hole with a steady rotary motion to assure no air bubbles.
6. Let the epoxy cure for the required length of time.
7. Check the radiation power by using optical power meter to test how good is the coupling.

5 Optimize the angle between fluorescence excitation and detection fibers

The angle between fluorescence excitation fibers and the fiber connected to spectrometer is more flexible, which can range from 0 to 90 degree. As mentioned before, zero degree was used for the previous instrument; however, the 0 degree configuration collected ambiguous fluorescence data as the light path length is longer than any other degrees. Hence, laboratory tests need to be done to find the best angle between them.

The concentration of DOC in freshwater can range from 0 ppm to 40 ppm or even higher. In an attempt to qualify the instrument's ability, 60ppm humic solution is used for lab testing to make sure the instrument is able to work under high DOC concentration environment. Several sets of experiment were performed with angles range from 20 degrees to 90 degrees. The tips of the fibers should be kept as close as possible to each other to minimize the light path length during the tests. The intensity of fluorescence is strongly related to the concentration of DOC, the higher the DOC concentration the stronger the fluorescence intensity. Since 60ppm humic acid solution is used for experiments, the required current for the excitation LEDs need not to be very high. Two input currents for LED, 0.5mA and 1 mA, are selected for experiments. Figure 14 and Figure 15 showed the intensity of the fluorescence spectrum with angles range from 20-90 degrees with current input 0.5mA and 1mA.

According to Figure 14 and Figure 15, it can be found that the fluorescence intensity is sensitive to the input current of the LED. When the input current doubled, the intensity of fluorescence is almost four times higher for all different degrees. It can also be observed that 90 degrees always have the highest fluorescence intensity and 20 degrees is always the lowest. Hence, 90 degrees should be the best angle for fluorescence measurement.

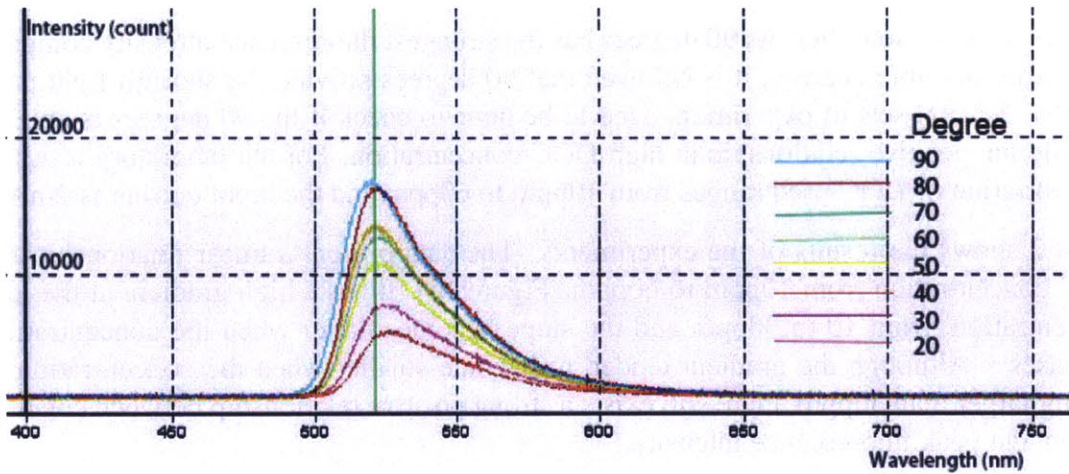


Figure 14: Angle test with 0.5mA input current

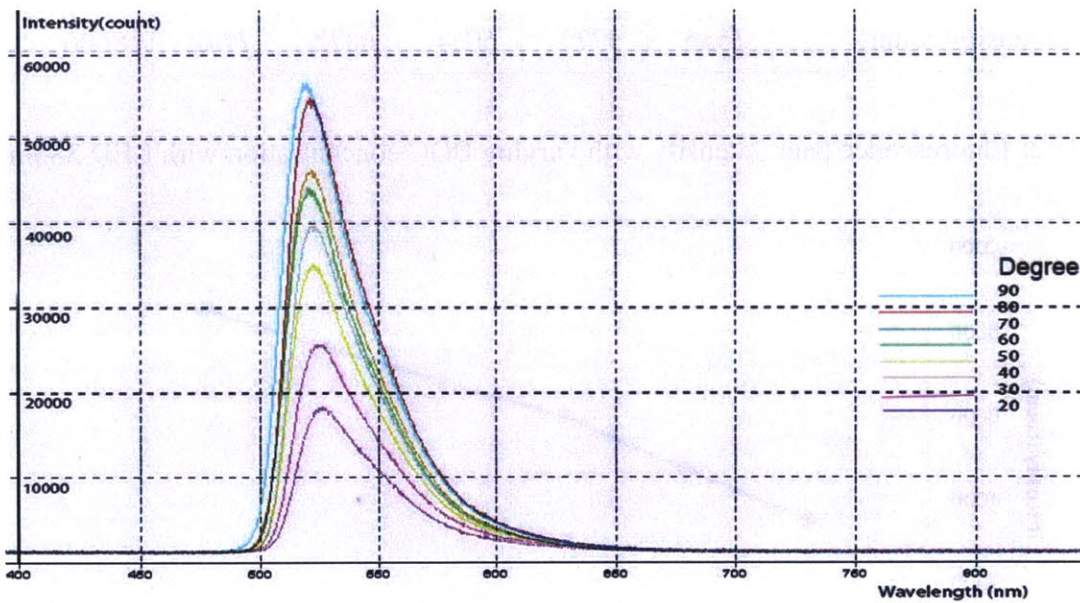


Figure 15: Angle test with 1mA input current

6 Laboratory test with varying concentrations of humic solution with LED 385nm

A major problem that the old instrument faced was the relationship between the concentration of DOC and peak fluorescence intensity. There was a positive relationship between concentration and peak fluorescence intensity at low concentration of DOC, but a negative relationship at the high level of concentration. The objective of this laboratory test is to check if the new optical configuration is able to keep the relationship linearly. The negative relationship was possibly due to the long light path lengths from excitation

sources to collection fiber. As 90 degrees has the strongest fluorescence intensity compare with other possible degrees, it is believed that 90 degrees provide the shortest light path lengths. Several sets of experiment need to be done to check if the 90 degrees is able to provide the positive relationship at high DOC concentration. For the laboratory test, the concentration of DOC used ranges from 10ppm to 60ppm and the input current is 2mA.

Table 2 shows the results of the experiments. The data present a linear relationship for DOC concentration from 10ppm to 60ppm (Figure 16). It has a high gradient at the low concentration, from 10 to 30ppm and the slope becomes flatter when the concentration increases . Although the gradient tended to become smaller when the concentration is getting larger than 30ppm, there still exists a strong positive relationship between concentration and peak fluorescence intensity.

Concentration(ppm)	10	20	30	40	50	60
Intensity(count)	3536	4771	6064	6872	7742	8606

Table 2: Fluorescence peak intensity with varying DOC concentration with LED 385nm

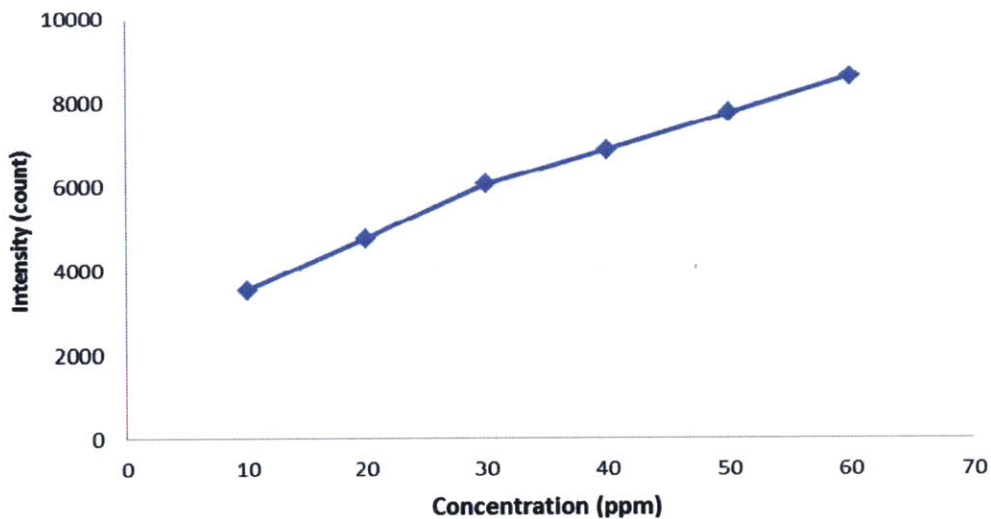


Figure 16: Fluorescence peak intensity with varying DOC concentration with LED 385nm

7 Procedures for deploying the instrument

1. Connect the four 12 V Lead Acid batteries in series to supply 48 V to the system. Always remember to fully charge the batteries before putting them into the battery compartment.

2. Put the battery packs into the battery compartment and press down the union to the top of the pipe. The union will help to confine the battery from moving inside the compartment (Figure 17).

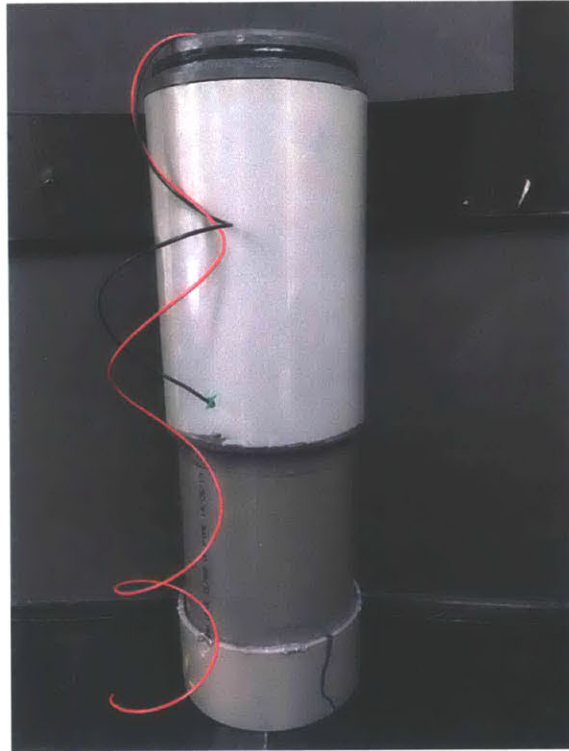


Figure 17: Battery Compartment

3. Check the configuration file (Appendix 1) inside the SD card before inserting to the main circuit board.
4. Insert fibers into the threaded cajons and tighten the screws.
5. Connect the 48 V battery to the main circuit board and connect the 3.7 V lithium battery to the main circuit board as well.
6. Press the reset button to check if the system is running successfully based on the sequence of LED lights (Appendix 2). Continue to assembly if the program runs normally.
7. Firmly press the sensing compartment to the battery compartment and press the cap to the battery compartment (Figure 18).
8. Perform a vacuum test with a pressure of 25 inch mercury. The reading on the pressure gauge should be the same after 12 hours (Figure 19).

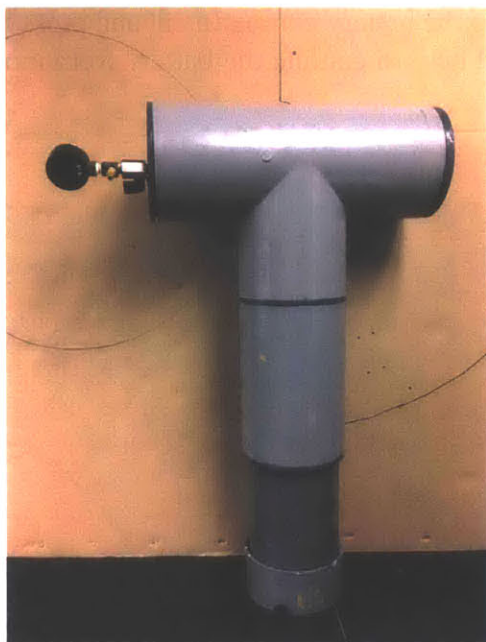


Figure 18: Overview of the HammerHead

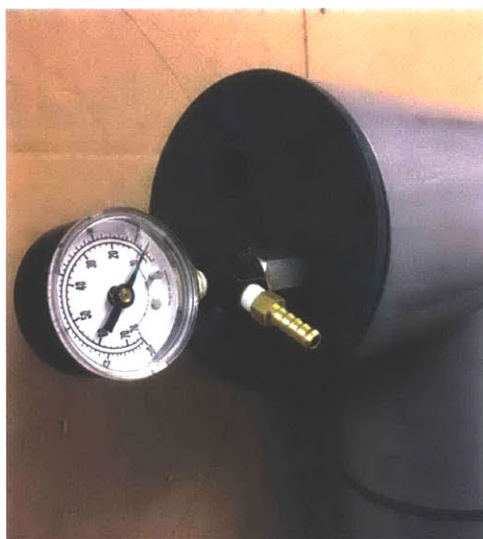


Figure 19: Fluorescence intensity with varying DOC concentration

9. Remove the pressure gauge to let the vacuum come out and screw plugs to the two holes of the T-fitting.
10. Time to deploy!

8 Calibrate the instrument

Before deploying the instrument, three parameters need to be adjusted to fit the environment of the deploying place. The three parameters are LED integration time, LED light-up time and the distance between each fiber. For this project, the expected environment contains relative high DOC concentration. The parameters were calibrated to fit DOC concentration range from 0 to 60 ppm in freshwater.

By coupling the fibers to the excitation LEDs, it helped to change the light path and direction between each fiber. The fiber used to couple the LEDs have a relative large numerical aperture (NA), which characteristics the range of angles that emitted from the end of the fiber (Figure 20). The larger the numerical aperture is the fiber, the larger the emitted angle is. The detection fiber could easily receive the original excitation signals, which might cover the fluorescence signal, especially for high DOC concentration solution. Calibration the LED integration time and distance between each fiber are the key to making the instrument be able to detect a wide range of DOC concentration.

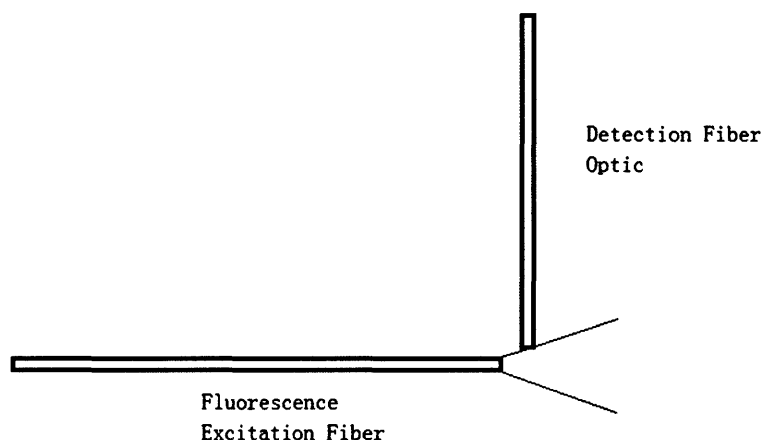


Figure 20: Fluorescence intensity with varying DOC concentration

Since 0 ppm to 60 ppm of DOC is the interested range, the first step of the experiment is to test the parameters with both 5 ppm and 60 ppm humic solutions. 60 ppm humic is suggested to test first as it could help to identify the light path and emitted angle. Try not to place the detection fiber within the range of the emitted angle of excitation sources. This is because if the detection is so close to the excitation sources, it would result in a high intensity of excitation signal on the spectrum. However, if the excitation source is placed far enough, the detected signal would be weaker. Finding the appropriate distance is the first challenge. The second step is to calibrate the LED integration time. Longer integration time would give a higher intensity spectrum. If the LED integration time is too long, the light intensity of the excitation source will cover the fluorescence signal as well. On the contrary, the instrument will not be able to detect meaningful signals if

the LED integration time is too short, especially when the DOC concentration is small. The LED light up time should be better to keep longer than LED integration time. After this, the instrument should be tested with 5 ppm humic solutions. Although the calibrated parameters might give a good response with 60 ppm concentration, the instrument might not work well with low concentration due to the high signal from the excitation sources. Only when the instrument is successfully able to sense the fluorescence signal with both 5 ppm and 60 ppm humic solutions, the instrument is done with calibration.

In general, a high input current will give a stronger fluorescence intensity. However, the LED input current is not able to be controlled for this instrument. LED input current is not one of the controlled parameters in this case. If the LED driver code will be fixed in the future, the magnitude of the LED current will be another controlled parameter to decide the spectrum.

9 Laboratory test with the instrument

Different concentration of humic solutions are used to test the performance of the instrument. The different concentration of the humic solution, from 5 ppm to 60 ppm, are filled to the cavity of the top cap separately. The received intensity from the spectrometer is strongly dependent on the LED output power, the distance between excitation fiber optic and detection fiber and the LED integration time. The higher the output power, the shorter light path and longer integration time will give the higher intensity. The integration time is needed to be calibrated to be able to measure the intensity from the wide range concentration of humic solution. The LED integration time used for laboratory test of LED 375nm, LED 385nm, LED 405nm, absorbance and turbidity are 200ms, 200ms, 800ms, 100ms and 200ms respectively.

As the ambient environment is not always absolute dark, the spectrometer would record the background signal together with the fluorescence signal. The instrument would also record the dark spectrum when the LED integration time is different with the previous value. A Matlab program was set up by Schuyler to analysis the recorded data from the SD card [2]. The code helps to extract the raw data from the instrument and process the data to the format that is easier to visualize. The Matlab code also helps to subtract the background light noise. There are few parameters in the Matlab code that are needed to adjust before using it. They are wavelength calibration values and non-linearity coefficient values of the USB 4000. Each individual USB 4000 have different values, so it is better to check the values before using the code. Lastly, always remember to change the file path before running the code as you need the raw .txt file as the input and .csv files as the output. Figure 21 to Figure 28 are plotted from the output data from the Matlab code.

Figure 21, Figure 22 and Figure 23 show the fluorescence response to 375nm, 385nm and 405nm excitation for DOC tests. The rectangle shape located at the left of each graphs show the intensity of the excitation sources. The peak located at the right side of the figures show the fluorescence response. The three different LEDs gave the same fluo-

rescence peak, which located around 395nm. By looking at the three figures, high DOC concentration generally have strong signals than the low concentration. The difference of intensity count are almost uniform for LED 375nm, LED 385 and LED 405nm. However, a weak fluorescence signal appeared on these three graph at the origin, this is because tap water are used as the solution, which contains certain amount of DOC. The three figures also show fluorescence peaks does not vary with concentration of DOC.

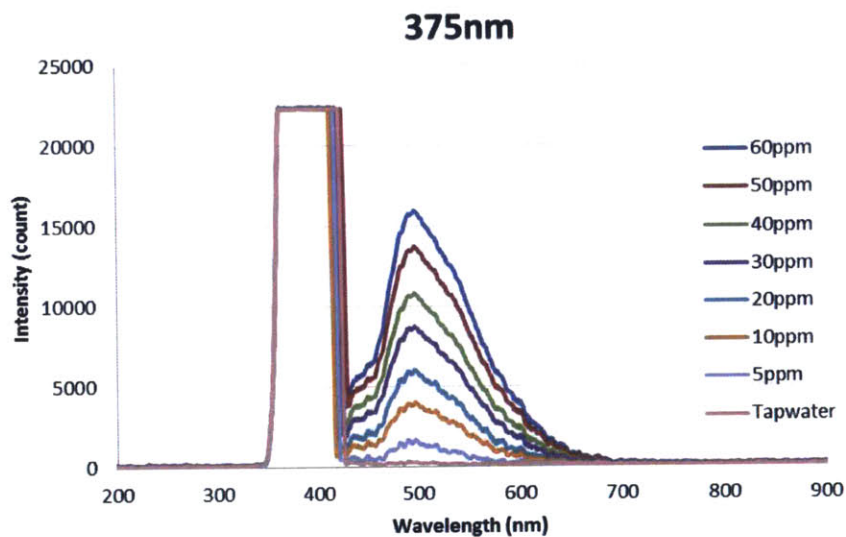


Figure 21: Spectrum of 375 nm LED

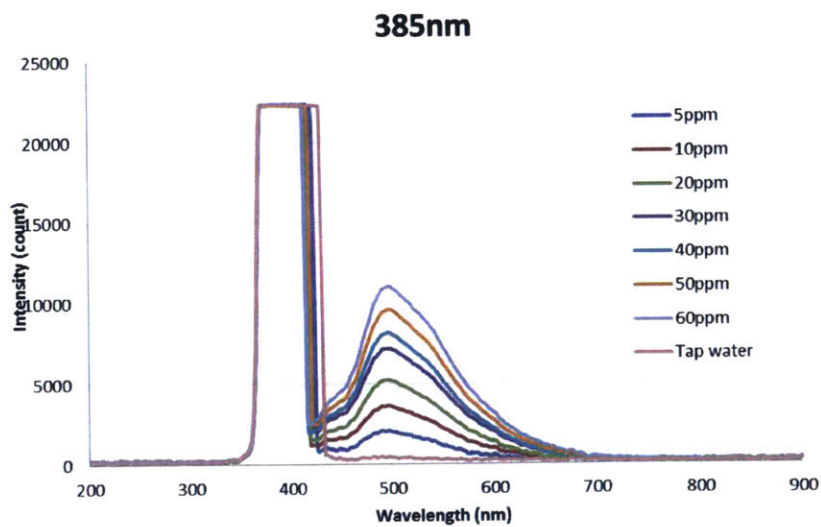


Figure 22: Spectrum of 385 nm LED

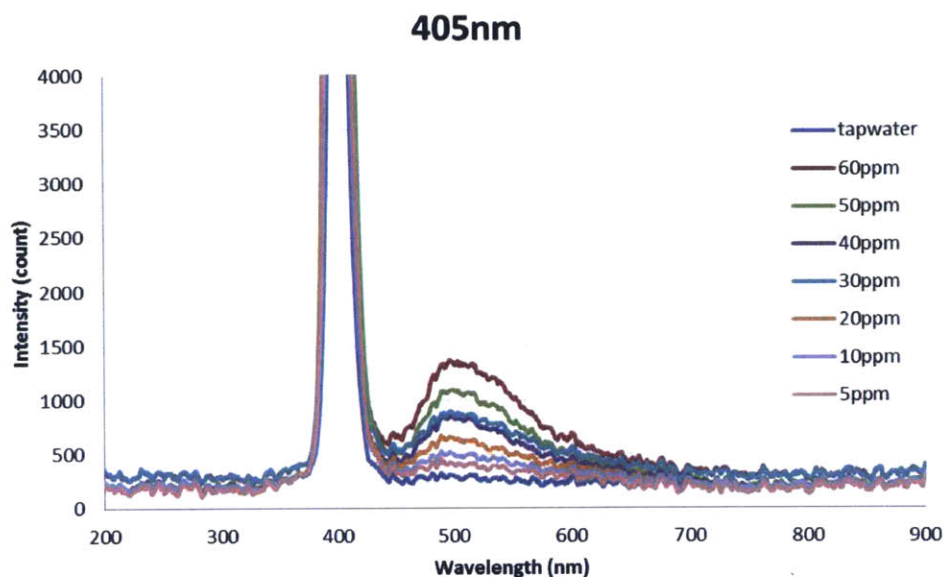


Figure 23: Spectrum of 405 nm LED

Figure 24, Figure 25 and Figure 26 show the relationship between fluorescence peak intensity (at 495nm) and concentration of DOC. Both LED 375nm and LED 385nm gave a strong linear relationship between fluorescence peak intensity and concentration of DOC. With increasing DOC concentration, the fluorescence intensity is increased linearly. Furthermore, the lines do not exactly start from zero, which might due to the small amount of DOC in tap water. LED 405nm does not provide a strong linear relationship between concentration and fluorescence. This might because LED 405nm is mainly used for chlorophyll detection. As there is not much chlorophyll in the tap water, the long integration time might cause a high noise.

Figure 27 shows the resulted spectrum of the different humic solution under Tungsten light. The tap water has the highest intensity because tap water does not absorb much of the light. In general, much light will be absorbed when the concentration of the humic solution is higher. From Beer-Lambert Law,

$$A = \log(I_0/I) = \epsilon bc \quad (7)$$

A is the absorbance and I refers to the intensity of the light that passed through the solution. ϵ is the molar absorptivity, b is the light path length and c is the concentration of the compound in solution. Figure 28 shows the absorbance of 60 ppm humic solution. Once the data is collected by the instrument, Beer-Lambert Law can be used to estimate the concentration of DOC in freshwater.

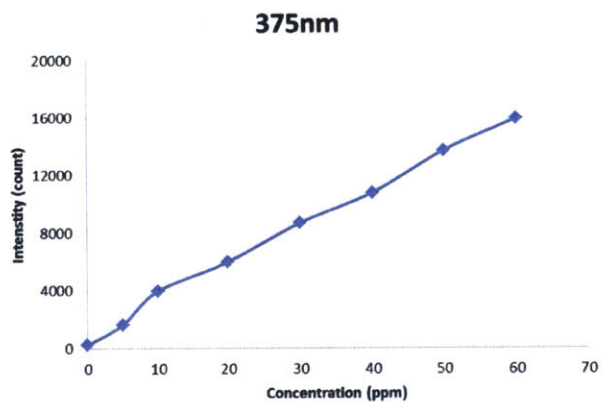


Figure 24: Fluorescence Peak Intensity with LED 375nm

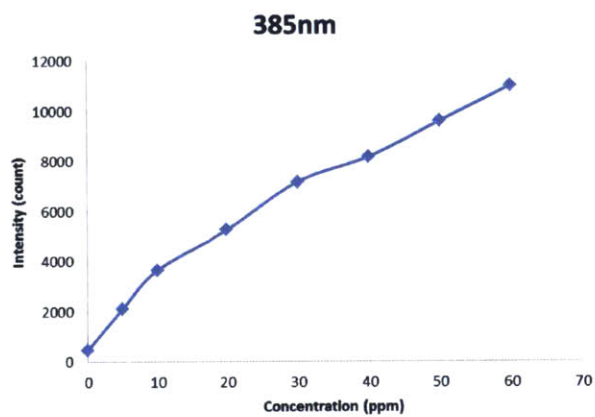


Figure 25: Fluorescence Peak Intensity with LED 385nm

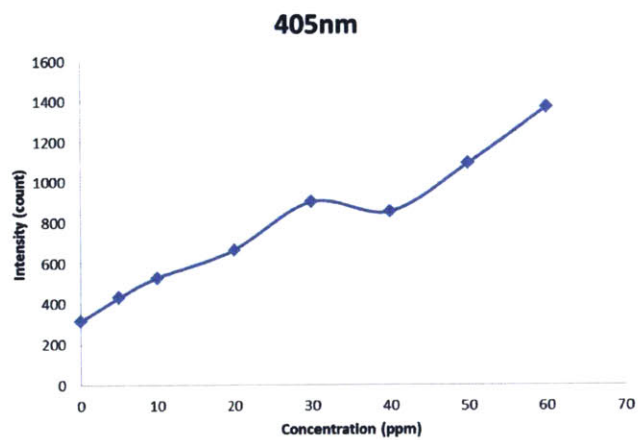


Figure 26: Fluorescence Peak Intensity with LED 405nm

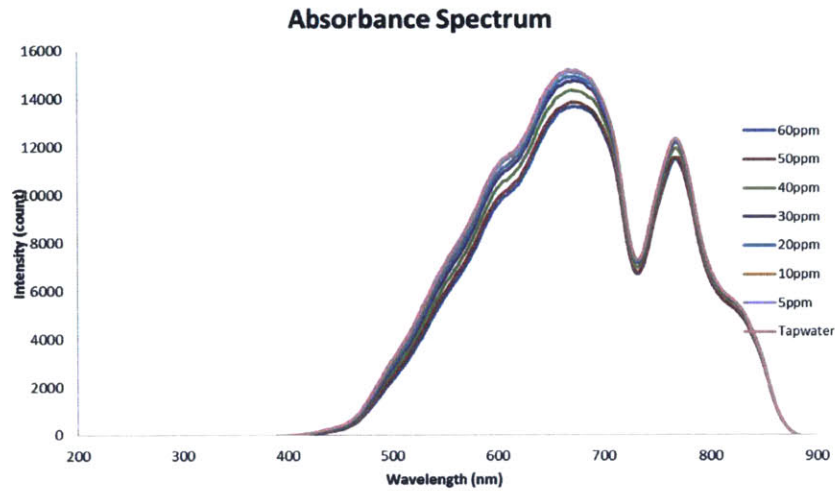


Figure 27: Tungsten Light Response

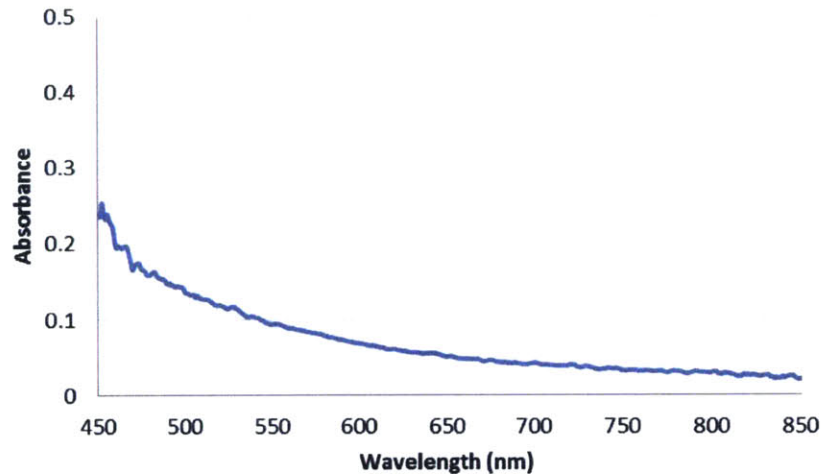


Figure 28: Absorbance of 60ppm Humic Solution

10 Conclusion and Future Development

An improved instrument with the ability to measure high levels of DOC in freshwater is designed and tested at the laboratory. The new instrument successfully minimizes adverse effects of inner shielding by using fibers that are coupled to LEDs and minimize the path lengths between excitation and detection devices. The fibers also help to minimize the light path lengths between fluorescence excitation sources and detection fiber. Stainless steel needles are used to protect the end of the fiber to make sure the alignment is good even under strong water turbulence. Furthermore, the new instrument does not really require a perfect alignment between Tungsten light and detection fiber, as a larger diameter fiber is used for transmitting the light.

The instrument demonstrates the ability to sense the fluorescence intensity with a wide range of DOC concentration. Integration time and distance between each fiber are needed to carefully calibrate to obtain optimal signals. The laboratory test successfully shows the peak fluorescence intensity is located at 495nm and it also presents a linear relationship between peak fluorescence intensity and DOC concentration, which is the main objective of this project.

For future development, the switch on the main board can be replaced with either coding switch or redesign the main circuit board to allow the wideband lamp switch between Deuterium and Tungsten light. The instrument only uses Tungsten light as the excitation source now as the circuit board was designed with a mechanical switch for switching between them. The instrument can get more meaningful data if both Deuterium and Tungsten light are used. Furthermore, more LEDs could be implemented on the instruments to sense other materials in water as the LED circuit board was designed for five LEDs.

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Appendix 1 - Configuration File Explanation

B=5	Boxcar Width
C=0	Wait for Full Charge
H=0	Data Log Hours
L=100	LED Warm Up Time
M=3	Data Log Minutes
P=5	Pixel Transfer
S=0	Data Log Seconds
W=3000	Lamp Warm Up Time
a=2000	The First LED Light Up Time
b=2000	The Second LED Light Up Time
c=2000	The Third LED Light Up Time
d=2000	The Fourth LED Light Up Time
e=2000	The Fifth LED Light Up Time
f=50	Wide Band lamp light up time
g=500	Turbidity LED light Up time

Appendix 2 - LED Color Code

Main Board LED Signal

If a series of colorful light - two green - Six time Blue - three times green was observed, that means everything is fine.

The different LED colors have different meanings:

Red - Main board power or back up battery

Blue - Check the spectrometer connection

Purple - Configuration file wasn't read

Green - Pass