Development of underwater camera for phytoplankton monitoring and wireless communication via IoT systems

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1.- Introduction

This report presents a design of how a STS Spectrometer is used to measure chlorophyll levels using fluorescence in water, which then interacts with a Raspberry Pi to send the results via wireless communication using a XBEE Digimesh module. This design was also implemented to measure the fluorescence characteristics of phytoplankton considering its taxonomy - the protein composition of the phytoplankton species (Shapiro H. 2003). As shown in Figure 1, the XBEE module and the STS Spectrometer are connected to a Raspberry Pi. The measurements were taken inside a black box to minimise the amount of light from the exterior so that only the results from the LED with a specific wavelength were used to find a relationship between the amount of chlorophyll and the fluorescence emitted. Additionally, since the Raspberry Pi runs Linux, a GUI program was created on Python 2.7 in order to calibrate the spectrometer and to set the specific parameters for the XBEE module to communicate with the UQ Gatton Digimesh Database.

This project has two main objectives. The first objective was to use a STS Spectrometer to communicate with a Raspberry Pi and later send the collected data via wireless communication using an XBEE module. In addition, a GUI program was created in python in order to modify the parameters for both devices in a simple environment.
The second objective was to find a proper algorithm that uses the readings from the spectrometer to find a relationship between the amount of chlorophyll and its emitted fluorescence. A formula give by Ocean Optics was used in order to find the chlorophyll's fluorescence, the results are explained in a later section.

This report presents the procedures of how the serial communication for both devices were used. Following the procedure, the results obtained when using the information from Ocean Optics website were analysed and explained. Furthermore, conclusions were drawn in order to improve the results obtained after modifying the readings from the spectrometer.

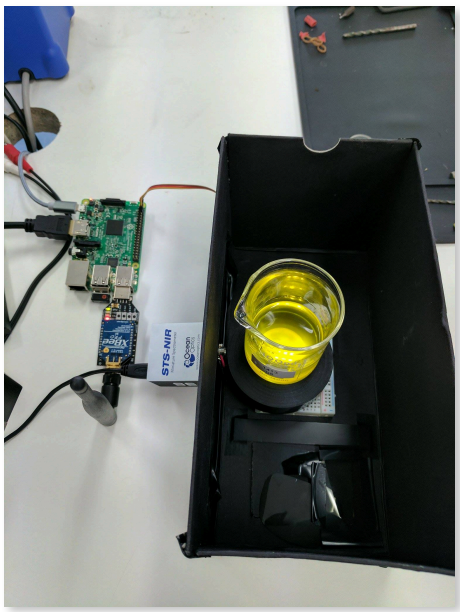

Figure 1. XBEE module and STS spectrometer connection to a Raspberry Pi.

2.- Aim

Phytoplankton monitoring is very important for:

1.- Primary Productivity Quantification
* Concentration of phytoplankton can have a direct effect on all organisms higher up in the food chain.

2.- Eutrophication / Nutrient Status Monitoring
* Eutrophic systems are highly prone to phytoplankton blooms, which leads to dissolved oxygen depletion when phytoplankton cells die.
* Helping to control watershed inputs which may affect nutrient loading with aquatic systems.

3.- Harmful Algal Bloom (HAB) Monitoring
* Cyanobacteria can release toxins that may cause health problems to humans and animals.

4.- Drinking Water Management
* With continuous monitoring of the water, we can know when and where to apply algaecide in the presence of phytoplankton which may produce taste and odour in the water.

In the case of having a rough estimation of phytoplankton's biomass concentration in water, the chlorophyll was the main issue to detect. Measuring chlorophylls' fluorescence is a standard method to estimate algae population. Furthermore, chlorophylls' fluorescence can estimate phytoplankton levels based on chlorophyll concentrations in a water sample. However, it is impossible to identify phytoplankton species using this method.
The method used to measure the chlorophyll's fluorescence is called *In vitro* because results are only used for lab purposes, therefore it is not necessary to consider the variables that may affect the measurements in *In vivo* method. These variables are:

* Water temperature.
* Water quality.
* Light history.
* Phytoplankton Health & Assemblage Dynamics.
* Biological and Particulate fouling in long term deployments.
* Quenching.

In a future where better results are expected, the book by Shapiro (2003) can be referred to. He explained that each phytoplankton species have a set of proteins. Each protein's fluorescence may be emitted if the proper wavelength is applied. Since different algal species may have different amount of various pigments, it is possible to use a multi-station cytometer to measure the fluorescence emitted at different wavelengths and then classify and count phytoplankton population. Refer to Figure 2 to see the fluorescence and source emission wavelengths for proteins-pigments.

On the other hand, the wireless communication is required since all the information are sent to Digimesh Database located at the University of Queensland - Gatton campus. After the data collected are interpreted using the proper algorithm, the systems will be deployed into the water and continue to transmit information using the XBEE Digimesh module. Additionally, the protocol used for wireless communication has a very low power consumption, which is very good when using a portable battery as a power source.

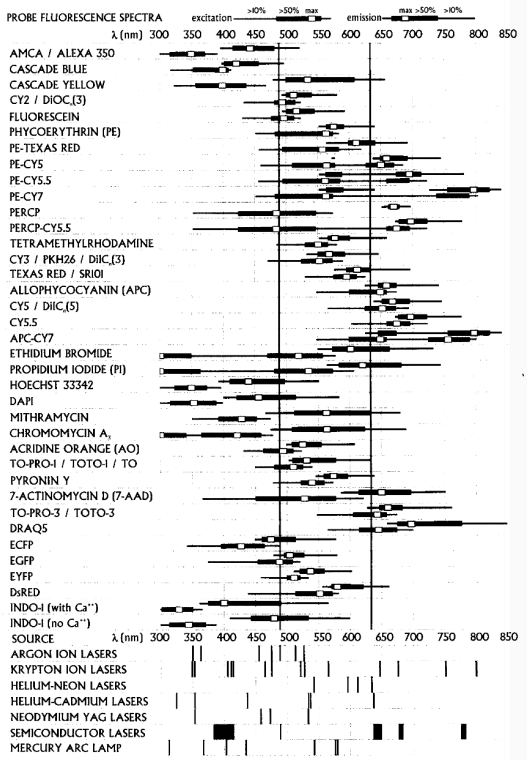

Figure 7-9. Probe fluorescence spectra and source emission wavelengths.

EXCITATION:	UV (325-355 nm)	VIOLET (395-415 nm)	BLUE-GREEN (488 nm)	RED (633-640 nm)
FLUOROCHROME-LABELED ANTIBODIES	AMCA, Alexa 350 (440)	Cascade Blue (420) Cascade Yellow (520)	Fluorescein, Cy2 (520) Cy3 (565) PE (575) PE-Texas Red (610) PE-Cy5 (660) PerCP (670) PerCP-Cy5.5, PE-Cy5.5 (700) PE-Cy7 (780)	APC, Cy5 (660) APC-Cy5.5, Cy5.5 (700) APC-Cy7 (780)
DNA-SELECTIVE DYES	Hoechst dyes (440) DAPI (455)	Hoechst Dyes (440), DAPI (455) Chromomycin, Mithramycin (560) 7-AAD (660)	AO (520) 7-AAD (660) DRAQ5 (700)	DRAQ5 (700)
NONSPECIFIC NUCLEIC ACID DYES			TO-PRO-1, etc. (530) Pyronin Y (575) Ethidium (600) Propidium (615) AO (650)	TO-PRO-3, etc. (660)
REPORTER PROTEINS		ECFP (470)	EGFP (510) EYFP (535) dsRED (575)	
ENZYME SUBSTRATE FLUOROPHORES	7-amino-4-chloro-methylcoumarin (470) ELF 97 (530)	3-cyano-7-hydroxycoumarin (450)	Fluorescein, rhodamine 110 (520) resorufin (585)	
MEMBRANE POTENTIAL PROBES			DiBAC4(3), DiOC2(3), JC-1, Rhodamine 123 (520) JC-1 (585) DiOC2(3) (610)	DiIC18(5) (660)
pH PROBES			BCECF (520) Carboxy SNARF-1 (580) BCECF (620) Carboxy SNARF-1 (640)	
Ca++ PROBES	Indo-1 (405) Indo-1 (480)		fluo-3 (520) Fura Red (660)	

Table 7-2. Fluorescence spectral properties of a selection of reagents usable for common cytometric tasks. Emission maxima are indicated next to names of probes; probes for which two maxima are listed may be usable for ratiometric measurements.

Figure 2. Probe fluorescence spectra and source emission wavelengths (protein-pigments). (Shapiro H. 2003).

3.- Design

First of all, Linux need to be installed in the Raspberry Pi. The version used for this project is Raspbian. Both of the devices used communicate with the Raspberry Pi using serial communication. For instance, the faster and easier way of making a stable connection between devices is by using Python 2.7 as the main programming language. C and java may be other options that can be used in the Raspberry Pi. XBEE Digimesh module and STS Spectrometer have their own libraries made by their companies in order to use their products freely.

XBEE Digimesh Library link is https://github.com/nioinnovation/python-xbee.
STS Spectrometer Library link is https://github.com/ap--/python-seabreeze.

For a detailed explanation of how the software was developed, refer to the link (https://uqiot4s-sae@bitbucket.org/uqiot4ssae/phytoplanktonmonitoring.git). Refer to Figure 1 to observe how the devices are connected. For *in vitro* analysis of the chlorophyll's fluorescence, a closed black box was used to reduce the ambient light as much as possible so the results will rely only on the LEDs with fixed wavelength. Once everything is set up, the program OceanView is used to get the fluorescence graph. After a graph is obtained, then the GUI program made in python is used to start testing the algorithm until the results show something similar. Figure 3 and Figure 4 show the algorithm for fluorescence and reflectance which are used in the program OceanView, respectively.

Moreover, an additional push button has been connected to the Raspberry Pi as a calibration method when the project will be deployed in the water. When the push button has been pressed, the first reading to calibrate the fluorescence values was recorded. After that, the STS Spectrometer will continuously read the fluorescence values at that instant of time, use the same algorithm used in the GUI program interface and then send the data using the XBEE module. This is the same process that the GUI program performs, but in an autonomous way.

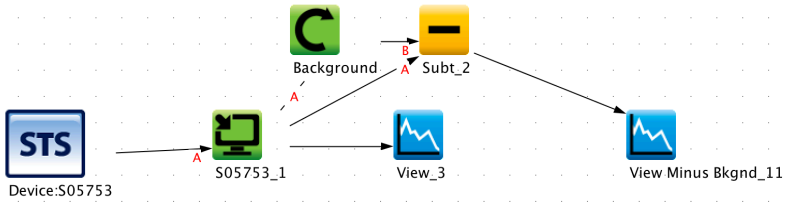

Figure 3. Fluorescence Algorithm used in OceanView

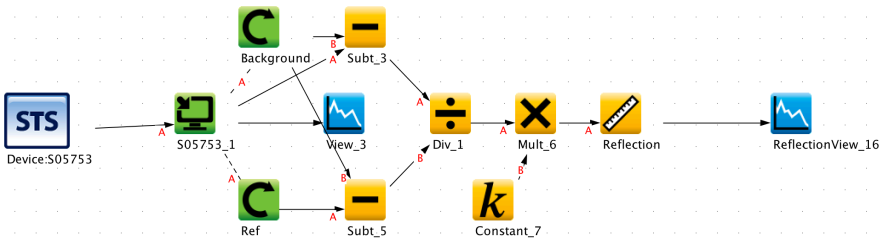

Figure 4. Reflectance Algorithm used in OceanView.

4.- Experiment

According to Kiefer D. (1978) the chlorophyll can be extracted from leaves by using acetone, this process can be observed in Figure 5.

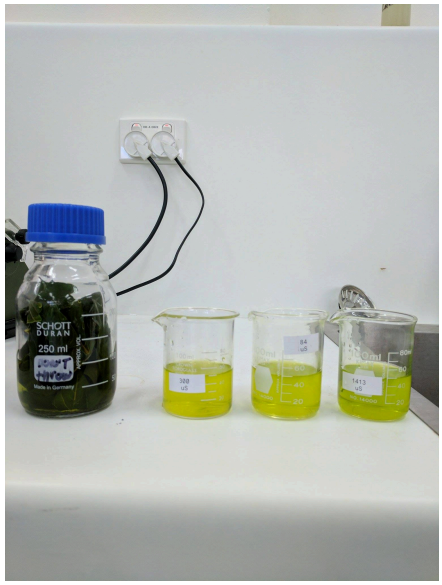

Figure 5. Chlorophyll extraction using acetone.

In order to attain a relationship between fluorescence and chlorophyll concentration, then different amounts of chlorophyll must be separated in different recipients, as Figure 5 shows starting from the left, there are 20ml, 30ml, and 40ml of chlorophyll extraction and adding water up to 50ml for each chlorophyll extraction as marked on each beaker.

Subsequently a small breadboard inside the black box must be added in order to connect 3 LEDs in parallel as shown in Figure 6.

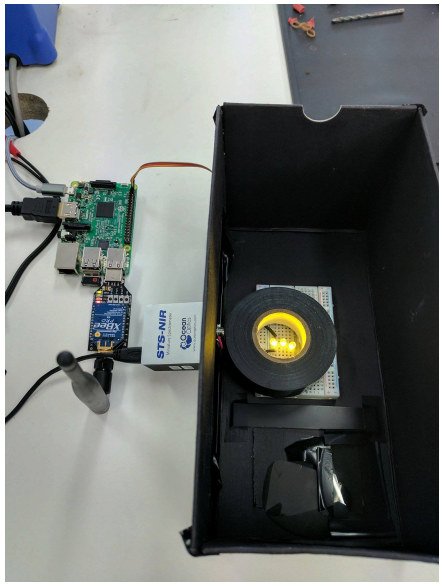

Figure 6. Blackbox setup.

This entire process was also used by Adam in 2016 in his project called “PULSE: Profiling underwater light sensor”.
After everything was set up, a baker was put inside the box on top of the LEDs as shown in Figure 7 and the fluorescence readings were recorded. Note that the top of the box must be covered with a black surface, this is not shown in the pictures.

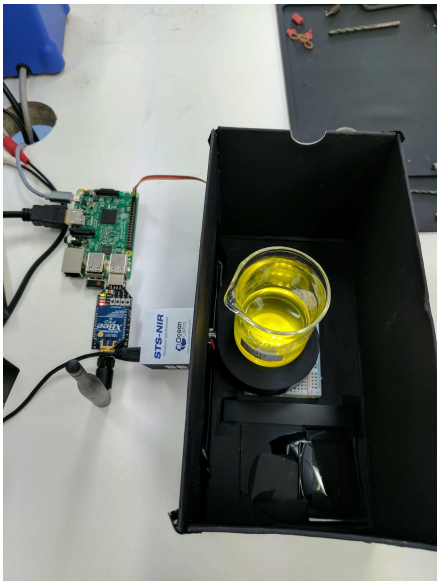

Figure 7. Measuring Fluorescence from 20ml Chlorophyl extraction.

Several types of LEDs were used in order to observe which wavelength produced a better fluorescence response. Figure 8 shows the fluorescence response of the chlorophyll extraction when excited with LEDs in the UV region.

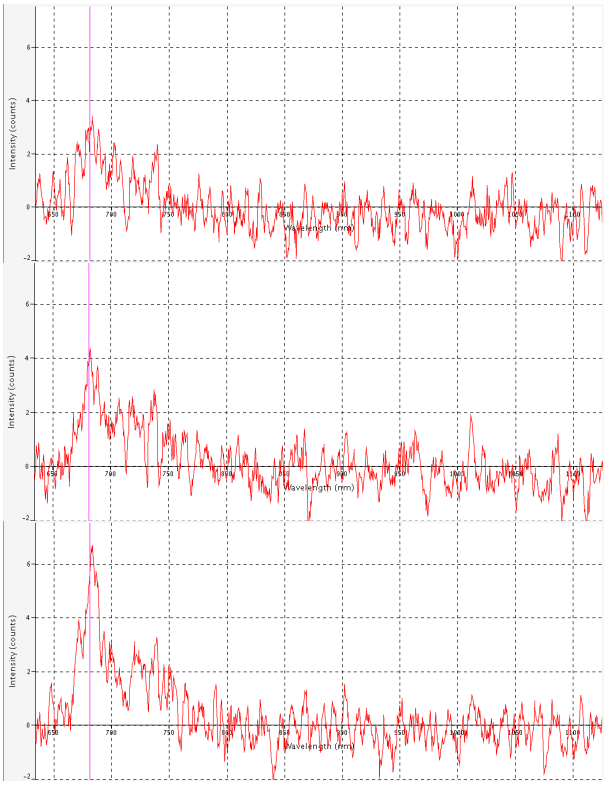

Figure 8. Fluorescence response with UV wavelength excitation (starting from the top, 20ml, 30ml and 40ml chlorophyll extraction (OceanView Application))

Since the objective of the project is to measure these values in the lake at the University of Queensland - Gatton campus, a water sample was obtained to see the fluorescence response at the same UV wavelength excitation. This can be observed in Figure 9.

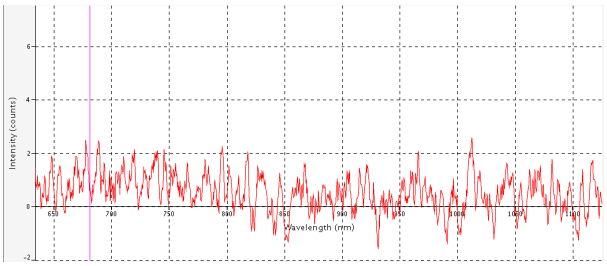

Figure 9. Fluorescence response with UV excitation into university's lake (OceanView Application).

From the results from Figure 9, there was not a clear fluorescence response, for this reason another wavelength was used. From Figure 10, LEDs with wavelength in the red region of the spectrum (650nm) were used and smoother results were obtained for 20ml, 30ml and 40ml chlorophyll extraction.

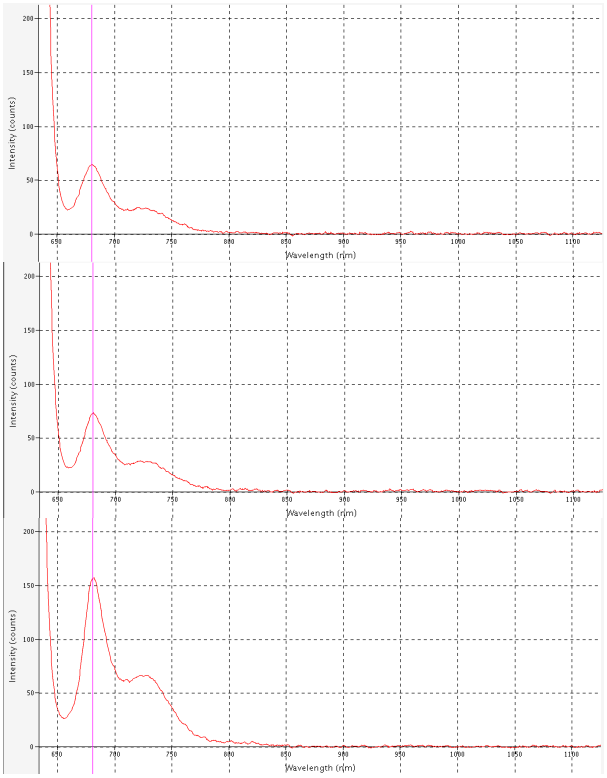

Figure 10. Fluorescence response with 650nm wavelength excitation (starting from the top, 20ml, 30ml and 40ml chlorophyll extraction (OceanView Application)).

However, the same thing happened. From Figure 11, there was no clear reading from the lake water even when using a 650nm LED.

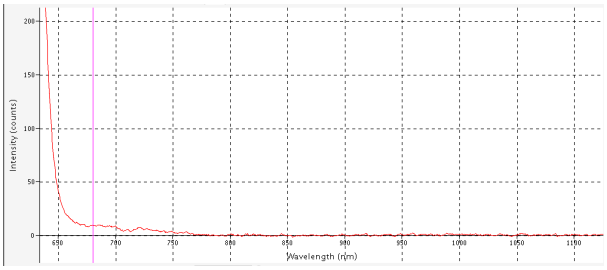

Figure 11. Fluorescence response with 680nm excitation into university's lake (OceanView Application).

Consequently, another LED must be used until better results are collected. After using 3 yellow LEDs (575nm) in parallel, the results are shown in Figure 12.

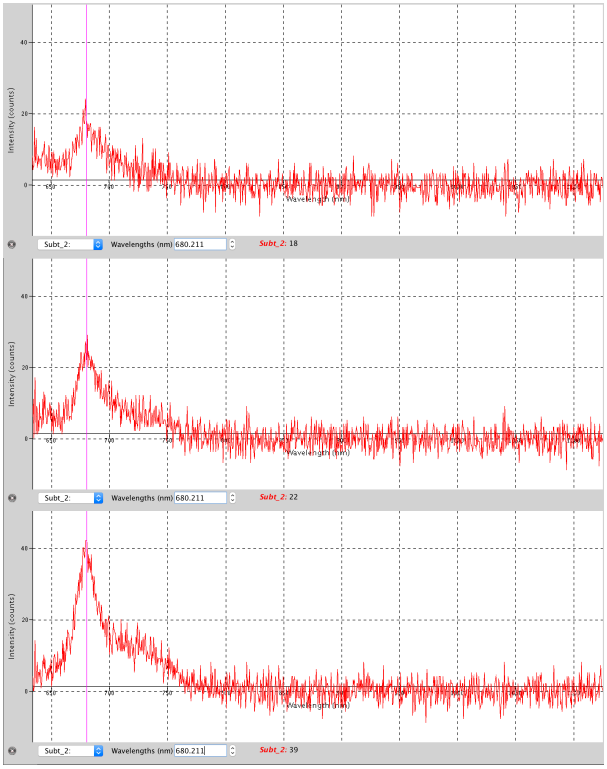

Figure 12. Fluorescence response with 575nm wavelength excitation (starting from the top, 20ml, 30ml and 40ml chlorophyll extraction (OceanView Application)).

Better results compared with the other tests were obtained from the lake water. This is shown in Figure 13.

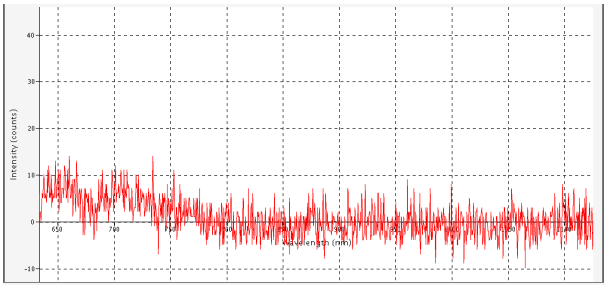

Figure 13. Fluorescence response with 680nm excitation into university's lake (OceanView Application).

Although the results for fluorescence obtained when using yellow LEDs are better than other wavelengths, fluorescence from the lake still could not be clearly noted.

Laney S. et al (2001) and Poryvkina L. et al (2000) stated in their respective researches that chlorophyll has a fluorescence of around 680nm at its peak. This is clearly shown in Figure 8, Figure 10, and Figure 12 that the fluorescence of the chlorophyll when emitted at any wavelength (less than 680nm) has its peak at around 680nm.

5.- Analysis and Conclusion

This report has explained how a Raspberry Pi can be used to connect 2 serial devices (XBee module and STS Spectrometer) to measure chlorophylls' florescence and send the results through wireless communication. The first objective of this project was to connect both devices and use them to work consecutively, which means reading fluorescence then processing data and finally sending the results to the Digimesh Database. This objective was accomplish by using python 2.7 as the main programming language. This program has great documentation for serial communication. A GUI program was also included in order to calibrate both serial modules.

On the other hand, the second objective of this project did not work as expected since the calibration algorithm for fluorescence readings from OceanView (Figure 3) were not producing the expected results that could be observed in the application "OceanView". Additionally, the water from the lake did not present a measurable fluorescence compared to the chlorophyll extraction. An additional algorithm to calculate reflectance of light was added to the project and could be tested in the GUI application. This was not needed since the main focus of this project was fluorescence, but in the future if reflectance is needed, then researchers can be confident that the results are consistent with the application "OceanView".

To improve the results, lasers or lamps can be used to induce fluorescence in chlorophyll (Fernandez A. et all 2012). Fernandez A. et al (2012) also suggested another approach to process the readings from the spectrometer and get the value for the chlorophyll's fluorescence.

6.- References

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