Development of a chlorophyll sensor based on absorbance and fluorescence measurers

G. L. T. T. de Silva and H. H. E. Jayaweera[*]
Centre for Instrument Development, Department of Physics, University of Colombo, Colombo 03
[*]*hiran@phys.cmb.ac.lk*

ABSTRACT

An optical chlorophyll sensor has been developed by employing its unique light absorption and fluorescence properties. Characteristic absorption peaks of chlorophyll a and b occur at 430 nm and 453 nm in blue region and at 665 nm and 642 nm in red region respectively and fluorescence emission peaks occur at 680 nm and 750 nm. LEDs with peak emission wavelength at 405 nm, 435 nm, 525 nm and 680 nm were used as wavelength selective light sources and Hamamatsu s5972 photodiode together with optical filters (540AESP short pass and 670AF30 band pass filters) were used as detectors. It is found that there is a good linear relationship between the chlorophyll concentration (in arbitrary units) and the natural logarithm of the blue and red absorption intensities (R^2 are 0.99 and 0.93 respectively), when the effect from scattering is removed by dividing red and blue intensities by green light intensity separately. The absorbance measuring sensitivity is about 10.3 % in blue region and 0.4 % in the red region. A non-liner model was developed incorporating absorption as well as fluorescence as the transfer function of the sensor for this arbitrary scale ($R^2 = 0.99$).

1. INTRODUCTION

Water is essential for the survival of living beings. However with the expansion of the human population, growth of industry and other factors, the quality of water is rapidly changing, endangering the lives on earth. Therefore water pollution has become one of the major global crises in today's world. This is basically the result of discharging harmful particles directly or indirectly to water streams. In many places around the world, industry is the largest source of water pollution [1]. A high level of nutrients is common in such water samples. Phytoplankton population is typically high if there are nutrients (mainly Phosphorus and Nitrogen) in water. Therefore Phytoplankton level plays a significant role in measuring the level of pollutants in water sample [2].

Chlorophyll is universally used as an indicator of phytoplankton biomass. Chlorophyll absorbs visible light dominantly in blue and red regions. The absorption peaks for chlorophyll a and b are around 430 nm and 453 nm in blue region and around 665 nm and 642 nm in red region respectively. Chlorophyll also displays a unique fluorescence spectrum with two peaks (around 680 nm and 750 nm) in the red region, due to absorption of light in blue and red regions [3].

Both absorption and fluorescence measurements are widely used for investigating plant physiology. Absorption peak in the blue region can be used as a measure of concentration

while the absorption peak in the red region may not be suitable due to comparably lower level of absorption and the overlapping of the first fluorescence peak [4]. When a system is exposed to blue light source, it will induce the first peak of the fluorescence and that fluoresce will get absorbed and induce a second fluorescence peak.

Spectrophotometers and fluorometers are widely used to detect fluorescence and its intensity and wavelength distribution of emission spectrum, after excitation by a certain spectrum of light [5, 6]. These parameters are used to identify the presence and the amount of chlorophyll in a particular water sample. Modern fluorometers are capable of detecting fluorescent molecule concentrations as low as 1 part per trillion.

The objective of this study is to construct a sensor capable of calculating the chlorophyll concentration in a water sample while they are in particle form. Therefore the samples were not dissolved in a solvent such as acetone or chloroform [7]. The method that has been used is based on the fluorescence and absorption properties of chlorophyll.

2. MATERIALS AND METHODS

2.1 Optical Design

The Fig. 1 illustrates the optical design of the absorbance and fluorescence measuring systems.
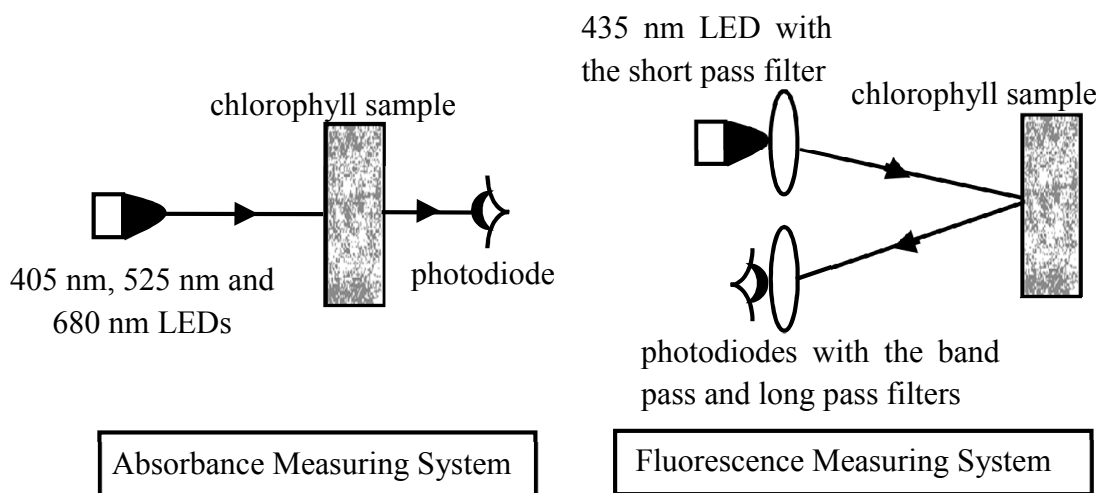

Fig. 1: Optical design of the absorbance and fluorescence measuring systems - arrangement of the light sources, detectors and filters

In the absorbance measuring system 405 nm, 525 nm and 680 nm LEDs were used as light sources. The 525 nm light source was used to emit the effect of back scattering. Once a particular LED is illuminated, the light emitted by the LED passes through the chlorophyll sample and irradiates the photodiode and generates a current.

435 nm LED has been used as the light source in fluorescence measuring system. Light generated by the blue LED is initially passes through the short pass filter 540AESP with a cut off wavelength around 550 nm. And then it propagates through the chlorophyll sample. 0Resulting fluorescence peaks are then measured separately using two photodiodes through 670AF30 band pass and long pass filters. However light source and fluorescence detecting system are in the same side so that photodiodes only measure the fluorescence that scatters back.

Before taking the measurements, the system was placed inside a black box to avoid the unwanted impact from the ambient light. Fig. 2 demonstrates the excitation and emission matrix for both absorbance and fluorescence measuring systems.
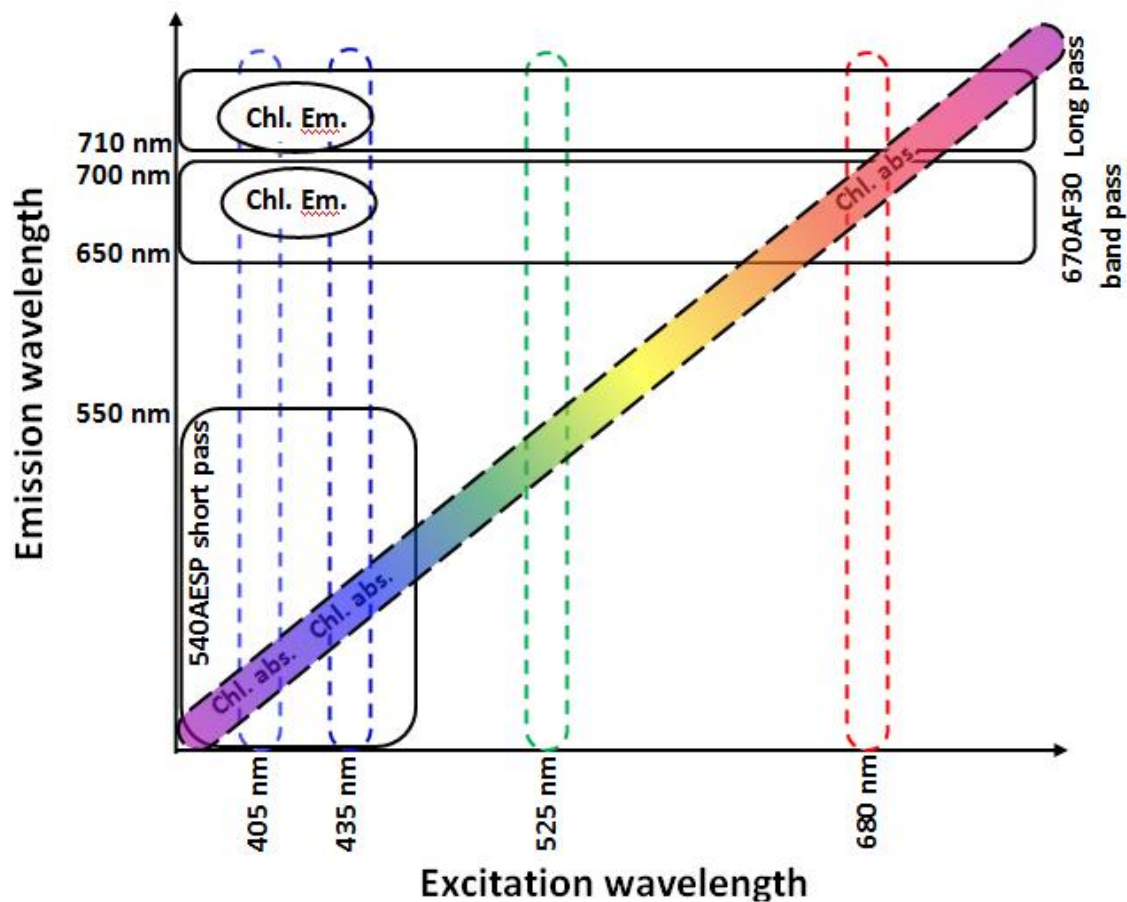

Fig. 2: Excitation and emission matrix – light sources, absorption and fluorescence
     signatures of chlorophyll pigments

2.2 Data Model

According to Beer-Lambert's law [8] the following relationships were expected to be observed between the chlorophyll concentration (C) and the natural logarithm of the absorbed intensities. In Eq. 1 and Eq. 2, I_V^0, I_G^0 and I_R^0 are incident blue, green and red light

intensities respectively. I_V, I_G and I_R are the intensities of absorbed blue, green and red light respectively. L is the path length.

$$\ln\left(\frac{I_V}{I_G}\right) = -(\alpha_V L - \alpha_G L)C + \ln\left(\frac{I_V^0}{I_G^0}\right) \qquad \text{………………(1)}$$

$$\ln\left(\frac{I_R}{I_G}\right) = -(\alpha_R L - \alpha_R L)C + \ln\left(\frac{I_R^0}{I_G^0}\right) \qquad \text{……………(2)}$$

Fluorescence intensity (F) is expected to vary with the chlorophyll concentration (C) according to the relationship mentioned in the Eq. 3.

$$F = \beta I^0 (1 - e^{-\propto CL}) \qquad \text{……(3)}$$

Where β is the probability that the absorbed light is converted to fluorescence, I^0 is the intensity of incident light, α is the absorption coefficient.

10 samples of chlorophyll were prepared and numbered in such a way that its concentration reduces by half the amount from the previous sample. Therefore the analysis was undertaken for the arbitrary units of chlorophyll concentration.

3. RESULTE AND DISCUSSION

For each of the 10 samples readings were taken for the on state and off state of the LEDs. Concentrations of the 10 samples were indicated in an arbitrary scale from 0 to 512.

3.1 Absorbance Measuring System

Measured intensities for blue, red and green LEDs were fit according to the Eq. 2 and Eq. 3. However it was observed that the ratio of I_R/I_G did not vary with the concentration as expected for the samples with low chlorophyll concentration. In fact the ratio appeared to be a constant (around 0.83) when the concentration is low. It was observed that sample no 1 and sample no 2 were too thick to obtain valid measurements for the intensity of the blue LED. Therefore the readings corresponding to the sample no 1 and 2 were ignored as outliers. Data points were plotted to see whether they follow a linear fit as expected. The Fig. 3 illustrates the final plot for both red side and blue side absorption. The R^2 is calculated to be 0.995 and 0.929 respectively.

According to the Fig. 3 it is clear that absorption variation of chlorophyll with concentration in blue region is more acute than in red region. Therefore absorption in blue region is a more accurate measurement to calculate the concentration. However it is also important to verify that the calculated concentration by the device is in fact the chlorophyll concentration and not some other compound that absorbs light from blue region. Therefore it is important to consider the absorption in red region as well.
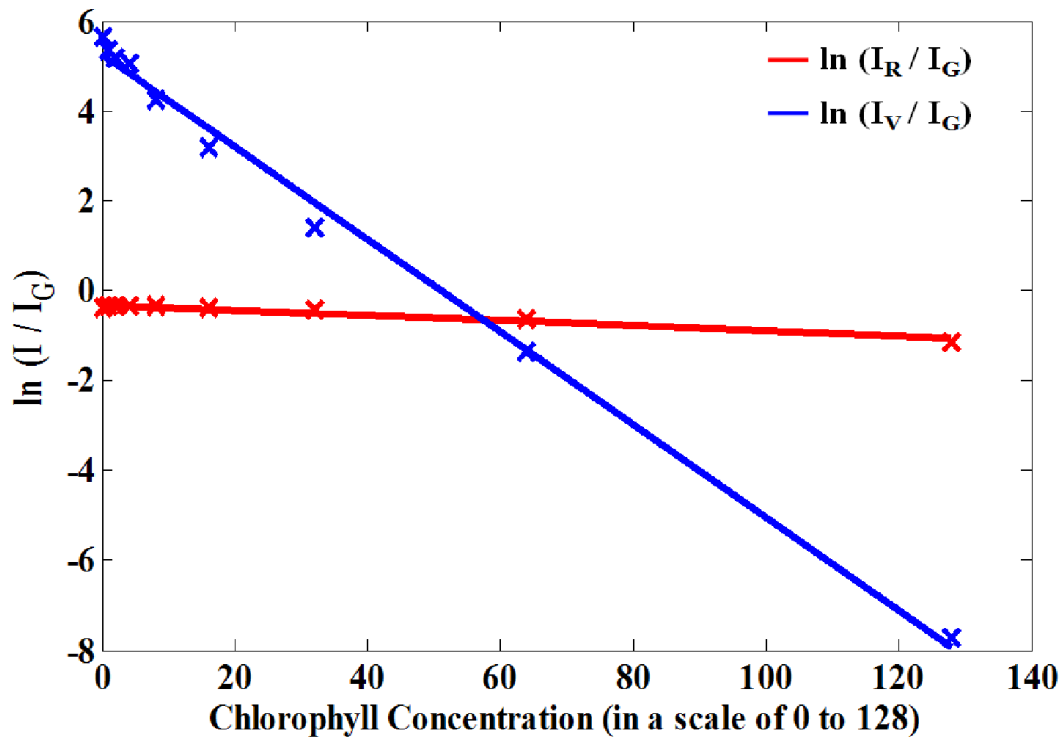
Fig. 3: Sensitivity difference of the variation of $\ln(I_V/I_G)$ and $\ln(I_R/I_G)$ against chlorophyll concentration separately

A surface can be plotted by fitting both blue region and red region absorption with the chlorophyll concentration. Once the values for $\ln(I_V/I_G)$ and $\ln(I_R/I_G)$ are known for a particular sample, it is possible to calculate the chlorophyll concentration using the plotted surface.

3.2 Fluorescence Measuring System

When measuring the fluorescence intensities it was observed that the second fluorescence peak (around 750 nm) is quite low that it could not be detected. One obvious reason for such result is the fact that the intensity of the second absorption peak is much lower compared to the first absorption peak. Also when observing the absorption and florescence spectrum of chlorophyll, it can be noted that the second absorption peak in the red region overlaps with the first peak of the fluorescence spectrum [4]. Therefore maybe the reduction of the transmitted light intensity, due to absorbance was compensated by the increment of the emission of fluorescence so that total absorbance was not detectable.

Fig. 4 illustrates the non-linear relationship between the chlorophyll concentration and the fluorescence intensity of the first peak. Data points could be fitted according to the derived relationship in the Eq. 3 and the R^2 is 0.9957 for the following fit.
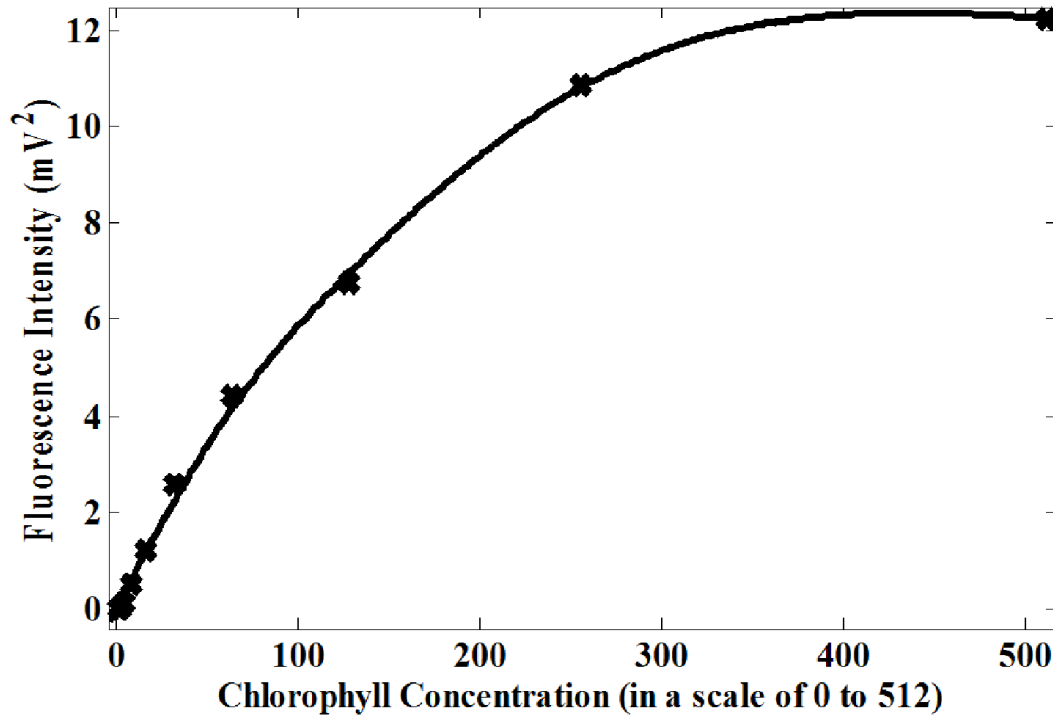
Fig. 4: Graph of the variation of first fluorescence peak intensity against chlorophyll concentration with 435 nm LED as the light source

Another data set was obtained to observe the relationship between the chlorophyll concentration and the intensity of the first fluorescence peak (Fig. 5), using a laser diode as the light source. It was observed that the readings obtained for the fluorescence intensities were much higher comparatively. The non linear relationship illustrated by Eq. 3 is more evident in this data set. However R^2 which was calculated to be 0.98, is slightly lower.

Currently, the popular method to measure the chlorophyll concentration is to dissolve chlorophyll in an acetone, methanol or chloroform medium and use a spectrophotometer to observe the two absorption peaks and their intensities. Other method is to measure fluorescence. Measuring chlorophyll concentration in a water sample (when they are in particle form) is a bit challenging compared to chlorophyll in a dissolvable medium because light must penetrate through the cell and reach chlorophyll pigments to excite fluorescence. This reduces the efficiency of fluorescence greatly. Better results can be obtained by using a laser diode instead of a LED to excite fluorescence.
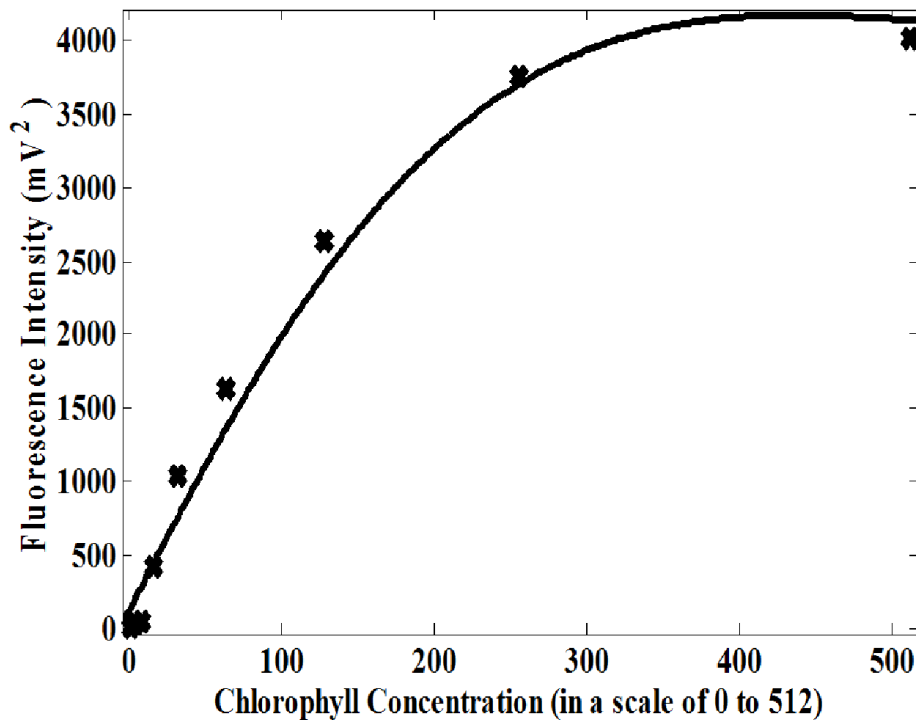
Fig. 5: Graph of the variation of first fluorescence peak intensity against chlorophyll
concentration with a laser diode as the light source

To assess the accuracy of the system several concentrations were back calculated using
the resulted graphs. It was noted that there is a difference between the calculated and the
actual concentration values. Especially for the graph that has been plotted based on
$\ln(I_V/I_G)$, the difference was calculated to be around 16%. This may be due the
assumption that the path length L is a constant for all the samples. In the real scenario
L tends to increase slightly with the concentration as the number of particles increase in
the sample.

4. CONCLUSIONS

In this study, the project scope was to measure the chlorophyll concentration in a water
sample using an optical method. Both fluorescence and absorbance measuring techniques
were tried out to develop the sensor. It has been demonstrated that it is possible to detect
chlorophyll using photodiodes and LEDs with the relevant filters using absorption and
fluorescence [9]. In the developed sensor absorbance measuring sensitivity is about
10.3% in blue region and is about 0.4% in the red region, when the LEDs 405 nm and
680 nm were used as light sources. The sensor was able to detect the first fluorescence
peak at 680 nm with the LED 435 nm as the light source, but was unable to detect the
second fluorescence peak at 720 nm. A laser diode would be more preferable as a light
source to excite fluorescence. However a further study should be carried out about its
suitability.

REFERENCES

1. World Nature Organization, *Water Pollution,* online: WNO, (2010), http://www.wno.org/water-pollution

2. Moritsch, M., Szendrenyi, A., Leger, C. and Frossard, B., *Associations among Plankton abundance, water quality and sediment quality in the San Francisco bay: Nitrogen and Phosphorus*, Berkeley Sci. J., 14, (2010) 46-54

3. Taiz, L. and Zeiger, E., *Plant Physiology,* MA: Sinauer Associates, 5th edition, (2010) 163-166

4. Bergevin, C., *Choloplast Translocation,* online: physwiki http://www.yorku.ca/planters/photosynthesis/2014_08_15_lab_manual_static_html/index.html

5. Allen, D., Cooksey, C. and Tsai, B., *Spectrophotometry,*online:NIST, (2009), http://www.nist.gov/pml/div685/grp03/spectrophotometry.cfm

6. So, P. T. C. and DONG, C. Y., *Fluorescence Spectrophotometry,* online:Ency. of Life Sci., (2002),http://web.mit.edu/

7. Carlson, R. E. and Simpson, J., *A Coordinator's Guide to Volunteer Lake Monitoring Methods*, North Am. Lake Manage. Soc., 96, (1996)

8. Kazakevich, Y. *Molecular Spectroscopy.* Online: Anal. Chem. http://hplc.chem.shu.edu/NEW/Undergrad/Molec_Spectr/Lambert.html

9. Sinclair, A., *Photodetection System for the Measurement of Chlorophyll Concentration in Water*, Photodetection & Photodetectors, (2009)