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Application Note

Chlorophyll a Quantification Using Handheld Fluorometer

Background:

All plant life contains the primary photosynthetic pigment chlorophyll *a*. Microscopic, planktonic plants, or phytoplankton, occupy the lit zone of all water bodies. With over 70% of the surface of the earth covered in water, phytoplankton and photosynthetic bacteria are responsible for almost ½ of the planets primary production while their total biomass comprises less then 1% of the total plant biomass. These extraordinarily efficient plants also act as the single largest CO_2 sink on earth. For these reasons alone it should be clear that there is an interest in measuring concentrations of phytoplankton. Chlorophyll *a* fluorescence is the most versatile, sensitive and easy way to measure the concentrations of phytoplankton in water.

The quantitation, through extracted *In vitro* analysis, or estimation, through *in vivo* analysis, of chlorophyll *a* concentration supplies information on the abundance of phytoplankton present in all aquatic environments. Since chlorophyll-containing organisms are the first step in most food chains, the health and/or abundance of these primary producers will have cascading effects to all higher organisms. Therefore, the determination of chlorophyll concentration is one of the key indices in monitoring the health of any natural system.

Chlorophyll measurements are also used to directly monitor phytoplankton populations. Examples include, but are not limited to, the monitoring of chlorophyll in natural marine and freshwater environments, reservoirs, water and sewage treatment plants, and aquacultural systems.

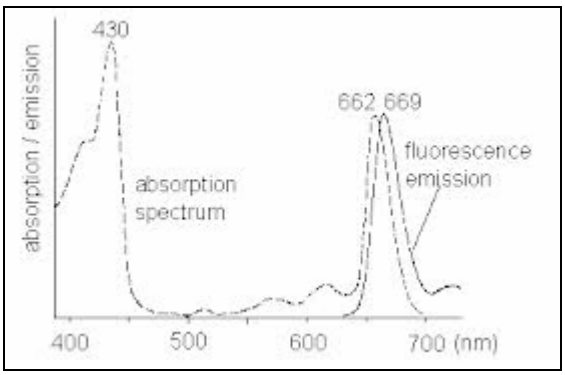
Fluorescence Detection:

Chlorophyll *a* naturally absorbs blue light and emits, or fluoresces, red light. A fluorometer detects chlorophyll *a* by transmitting an excitation beam of light in the blue range (440nm for *In vitro* analysis and 460nm for *in vivo* analysis) and by detecting the light fluoresced by cells or chlorophyll in a sample at 685nm (red). Generally, this fluorescence is directly proportional to the concentration of the material in question.

In vivo chlorophyll analysis is the fluorescent detection of chlorophyll *a* in living algal and cyanobacterial cells in water. In this technique, the excitation light from the fluorometer passes through the untreated sample water and excites chlorophyll within the living cells of the algae present. *In vivo* fluorescence data supplies information on the relative distribution of chlorophyll concentrations and usually correlate well with extracted chlorophyll *a* samples. *In vivo* detection has several very useful applications. An example is the monitoring of general trends in chlorophyll concentrations in real time. It is very easy to obtain large amounts of data using in vivo instrumentation and is an excellent means of following trends and estimating chlorophyll concentration.

In vitro chlorophyll analysis (extracted analysis), on the other hand, entails the concentration of chlorophyll containing cells onto a filter followed by the extraction of the chlorophyll *a* from the cells. This method provides the best quantitation accuracy of chlorophyll concentration, but requires laboratory setup to conduct the analytical procedures.

W2 Optronics's handheld fluorometer (model#: FQ-440/670-A) has been proven to detect low level of chlorophyll *a* in water and in extracted samples. Due to its high portability and low cost, it can be used anywhere in the field to conduct environmental study of natural water resources.



Absorption and emission spectrum of Chlorophyll *a*