Amiscience Corporation
5500 Stewart Avenue
Fremont, CA 94538
Tel: 510-371-1469
Fax: 510-270-6600
http://www.amiscience.com

Application Note

Chlorophyll a Quantification Using a 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 (436nm for *In vitro* analysis and 460nm for *in vivo* analysis) and by detecting the light fluoresced by cells or *chlorophyll* in a sample at 680nm (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, using narrow band filters to excite and detect the sample fluorescence without acidification of the cells. This method provides the best quantitation accuracy of *chlorophyll* concentration, but requires laboratory setup to conduct the analytical procedures.

Amscience's handheld fluorometers (**FQ-CHL/NAC-C** for extracted non-acidification samples, **FQ-CHL/IV-C** for *in vivo* detection, and **FQD-CHL/IV-CHL/NAC-C** for dual-channel version) have been proven to detect low level of *chlorophyll a*. Due to its high portability and low cost, it can be used anywhere in the field to conduct environmental study of natural water resources.