

Frequently Asked Questions About Fluorometric Chlorophyll Analysis

Q: Why measure chlorophyll?

A: 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 planet's primary production while their total biomass comprises less than 1% of the total plant biomass. These extraordinarily efficient plants also act as the single largest CO₂ 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 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.

Q: How do fluorometers detect and quantify chlorophyll *a* in water?

A: Fluorescence is the phenomena of some compounds to absorb specific wavelengths of light and almost instantaneously emit longer wavelengths of light. Chlorophyll *a* naturally absorbs blue light and emits, or fluoresces, red light. Fluorometers detect chlorophyll *a* by transmitting an excitation beam of light in the blue range (440nm for extracted 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.

Q: What is the difference between *in vivo*, *in vitro*, and extracted chlorophyll analysis?

A: *In vitro* (meaning 'in glass' and referring to 'in an artificial environment or outside the living organism') chlorophyll analysis is another term for extracted analysis. It entails the concentration of chlorophyll containing cells onto a filter followed by the extraction of the chlorophyll *a* from the cells. *In vivo* (meaning 'within a living organism') chlorophyll analysis simply refers to the analysis of chlorophyll in the natural environment or, in our case, in the living algal cells.

Q: What is *in vivo* chlorophyll analysis?

A: *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. There are several factors that make *in vivo* analysis a semi-quantitative measure at best. Environmental parameters, physiology, morphology, light history and the presence of interfering compounds all play a role in altering the relationship between fluorescence and the concentrations of chlorophyll *a*. Examples of interfering materials include other plant pigments, degradation products, dissolved organic matter, and turbidity. *In vivo* fluorescence data supplies information on the relative distribution of chlorophyll concentrations and usually correlate well with extracted

Q: Does the E.P.A. approve fluorometric chlorophyll analysis?

A: Yes, the E.P.A. has published [Method 445.0](#) which covers the *in vitro* (extraction) fluorometric analysis of chlorophyll *a*. In the most recent revision (Rev 1.2 Sept., 1997), the E.P.A. also approves the use of the non-acidification method, which is less susceptible to interfering compounds such as chlorophyll *b*. This filter kit supplies only chlorophyll *a* concentrations with no information on pheophytin concentration.

Q: How can I compare chlorophyll data obtained through different measurement techniques?

A: All detection instrumentation used in chlorophyll analysis will result in chlorophyll concentrations that are directly comparable. A side-by-side comparison between a fluorometer and a spectrophotometer is easily done but would require dilution of the chlorophyll sample to put it within the linear range of the fluorometer. A sample that is in range on a spectrophotometer will be over-range on a fluorometer.

Q: Why use a fluorometer over a spectrophotometer for extracted chlorophyll analysis?

A: Benefits of fluorescence over spectrophotometry include the capability of *in vivo* detection, sensitivity, durability, versatility (accepts a wide range of discrete sample cells and flow cells, accepts AC or DC power, and the user can choose and quickly change between many optical kits), ease of use, stability, ease of transport, and a small footprint.

For oceanographic research, the greater sensitivity of fluorescence results in less time and work in the analysis because much less water must be filtered for extracted analysis. The superior sensitivity also enables *in vivo* detection of chlorophyll concentration of < 1µg/L.

Freshwater researchers now have an extremely accurate and easy way to measure chlorophyll *a* even with high chlorophyll *b* concentrations using the non-acidification optical kit. Fluorometers also allow for in-line monitoring to collect data in real time.

Q: What are the chlorophyll detection limits of Turner Designs Fluorometers?

A: The [TD-700 Laboratory Fluorometer](#) and the [10-AU Field Fluorometer](#) with a red sensitive photomultiplier tube (PMT), have extracted chlorophyll detection limits of 0.02µg/L using a 13mm diameter test tube and 0.01µg/L using a 25mm test tube. The [SCUFA[®] Submersible Fluorometer](#) can detect *in vivo* chlorophyll concentrations to 0.02µg/L.

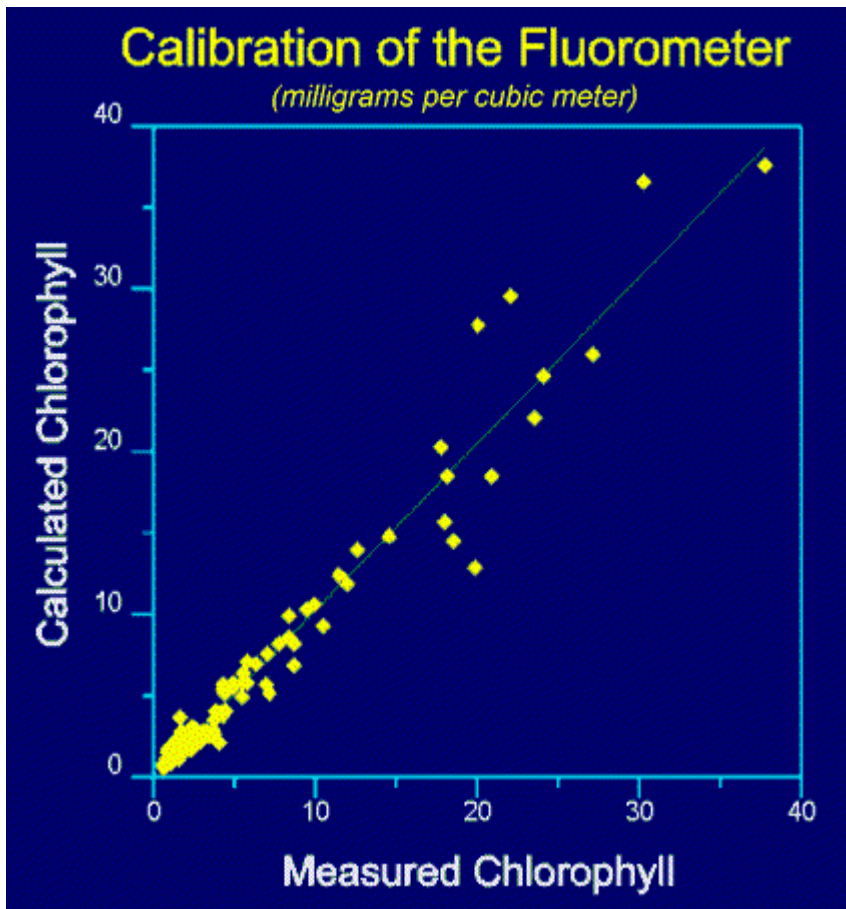


FIG.
1 -
USGS,
1998

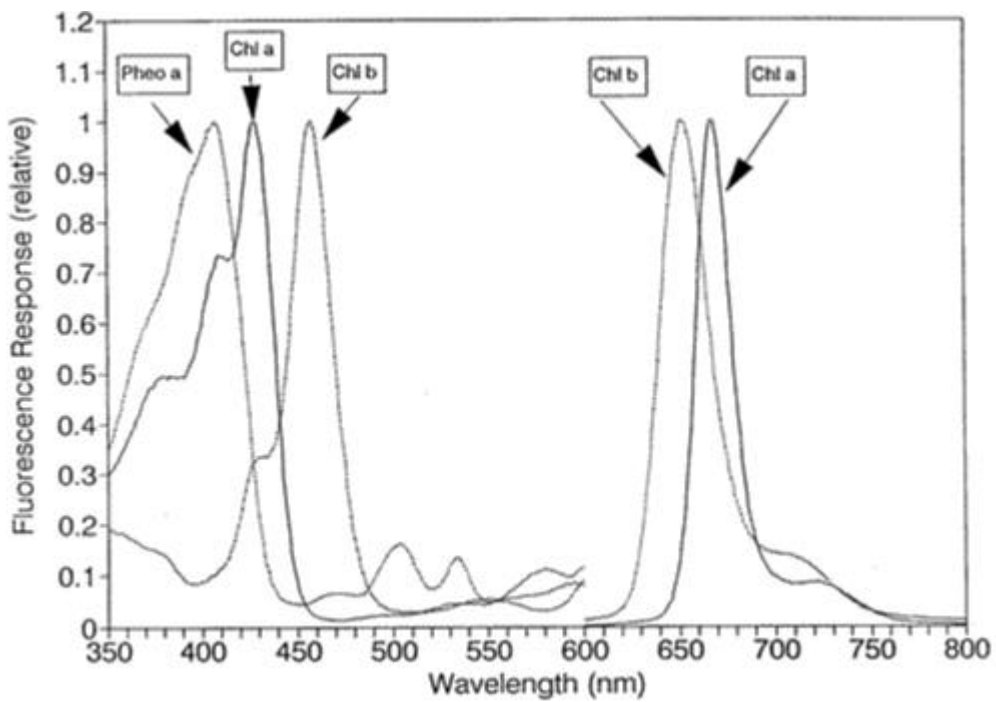
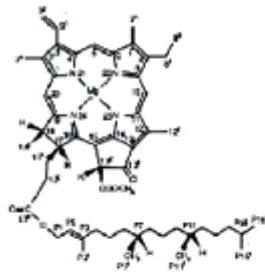


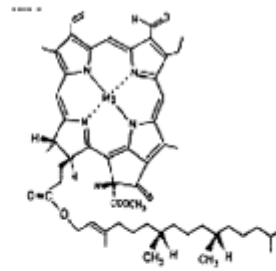
FIG.
2

chlorophylls and derivatives

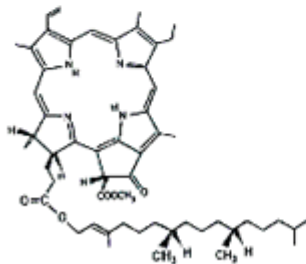
FIG.
3



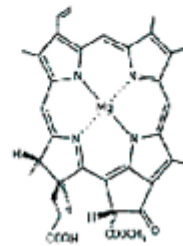
chlorophyll a



chlorophyll b



phaeophytin a



chlorophyllide a