

# Chlorophyll

## Algal Pigments

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### Overview

Phytoplankton, also referred to as algae, are single celled plants which exist in all types of aquatic environments. Microscopic examination and cell counting were the primary techniques used for determining phytoplankton abundances and distribution which enabled researchers, water resource managers, and scientists to better understand phytoplankton dynamics. However, these and similar counting techniques are quite tedious, time consuming, and can be very costly.

After researchers discovered chlorophyll fluorescence measurements could be used to estimate algal biomass, fluorometry became widely accepted as a highly specific, convenient, and sensitive analysis. Fluorometry is simply the measurement of the light emitted from chlorophyll molecules after excitation at a specific wavelength. The sensitivity of this technique is far greater than spectrophotometry, allowing for *in vivo* chlorophyll determinations and minimization of volumes filtered for extractive chlorophyll analyses.

Fluorometric measurements, because of their simplicity, versatility, and economical advantages, are the most commonly used measurements among water treatment engineers, aquatic resource and fisheries scientists, oceanographers, private and government research institutions and facilities.

### Why Measure Chlorophyll?

All phytoplankton groups contain the chlorophyll molecule, which is the primary molecule used for photosynthesis. The optical characteristics of chlorophyll allow for easily detecting and quantifying phytoplankton using fluorometric techniques. Data collected from these studies help water treatment plants, lakes and reservoir management, ocean science and research, aquaculture, and other water related studies to better understand phytoplankton dynamics.

#### *Lake and Reservoir Management*

The most frequent water quality problem in lakes and reservoirs is the excessive growth of phytoplankton due to high concentrations of plant nutrients. Water bodies with high nutrient concentrations and low dissolved oxygen levels are classified as eutrophic waters. Excessive phytoplankton and frequent algal blooms, caused by eutrophication, result in water with an unpleasant taste, odor, and appearance. These problems adversely affect drinking water quality and diminish the water's recreational utility. Also of concern is the production of blue-green algal toxins and clogged drinking water filter systems. Thus, monitoring the algal population and distribution in lakes and reservoirs is extremely important for resource preservation, public health and safety, and overall economics.

## *Water Treatment*

Water resource managers use chlorophyll determinations to monitor drinking water directly at the water source. However, water monitoring just prior to the treatment process holds many economic advantages. Immediate pre-treatment monitoring enables the facility operator to optimize the amount of treatment chemical added and therefore minimizes the downtime and expense of plugged filters. Using a fluorometer, incoming chlorophyll concentrations can be monitored directly on-site at the water treatment facility.

## *Aquaculture*

Fluorometers are used in fish and shellfish hatcheries to estimate changes in the quantity of the phytoplankton food source. Hatchery managers use this information to optimize the amount of phytoplankton present in the larval tanks. A fluorometer is an ideal choice for the simple and rapid estimation of phytoplankton in hatcheries.

## *Ocean Science and Research*

For several decades scientists and engineers have been working hard to facilitate chlorophyll measurements using cutting edge technology to better understand bloom dynamics in open and coastal waters. Recent advances in fluorometry have facilitated this study and have been the popular method for many researchers. Fluorometry increases sensitivity for detecting chlorophyll allowing for measurements such as *in vivo* monitoring thereby minimizing sample handling and providing a larger data set for better resolution of temporal and spatial distribution of phytoplankton. Many research vessels use “along-track” shipboard fluorescence, in conjunction with satellite imagery, to estimate near surface chlorophyll. With increasing technologies fluorometers are becoming smaller, faster, and more versatile finding their way into many aquatic applications.

## **What Methods Exist?**

### *In Vivo Analysis*

*In vivo* chlorophyll *a* analysis is the measurement of chlorophyll in living algal cells, without extraction or chemical treatment. These measurements can be taken using discrete samples or continuous-flow. The obvious advantage of *in vivo* analysis is rapid, on-the-spot measurement eliminating the delays for extraction and laboratory measurement. For qualitative analysis, *in vivo* measurement alone may answer the analyst's questions. For quantitative determinations, *in vivo* data are compared with other measurements, including fluorometric extractive data.

### *Extractive Analysis*

Algal cells are extracted in solvent, releasing the pigments from the cell into solution, which is then analyzed by fluorometric methods to determine absolute concentrations of algal pigments. There is a wide range of solvents that can be used in extractive analyses depending upon the method being used and the pigment of interest. The most commonly used extractive method, using a Turner Designs fluorometer, was developed by Strickland & Parsons in 1972 and has been published by the United States Environmental Protection Agency (EPA) as EPA Method 445.0. A more recently developed method, Welschmeyer (1994), minimizes interferences from chlorophyll *b* and pheopigments, requires one fluorometric measurement, and no acidification. For more details regarding the non-acidification method for measuring extractive chlorophyll, please refer to the Turner Designs' Applications Note, Non-Acidification Technique for Extracted Chlorophyll *a* Analysis.