

Introduction

The simple technique of in-vivo fluorometry (IVF) for locating and measuring algae has been in use by oceanographers and limnologists for over 30 years (Lorenzen, C.J., 1966) and is based on the direct measurement of the fluorescence of the chlorophyll a in the living algal cells. The same methodology is used to detect the phycobilin pigments of cyanobacteria, phycocyanin and phycoerythrin, in water. Cyanobacterial pigment fluorescence is the only technique available that is sensitive enough to detect cyanobacteria at natural levels without concentration and extraction. Because there is no special sample handling or processing required, IVF is ideal for profiling, moored and real-time data collection using on-line instrument systems. Similar to the IVF chlorophyll detection, the method does not provide quantitative pigment concentration data, but rather supplies relative data on cyanobacterial biomass. However, IVF data can be correlated to quantitative data in order to 'correct' the IVF data to provide concentration estimates. Without correlation, IVF provides a relative cyanobacteria measure that can be used to track trends and trigger more specific tests.

Calibration using Secondary Standards

True calibrations with a primary standard are not practical for IVF applications. IVF measures the relative change in cyanobacterial biomass via pigment fluorescence and the best means of 'calibration' is to use a secondary standard that provides a stable signal that can be correlated to a meaningful cyanobacterial concentration through correlation. The secondary standard is used to check for instrument drift and to re-calibrate if necessary. For example, in the laboratory the fluorescence of a natural water sample or cyanobacteria culture can be read using the fluorometer. Record the reading and then insert or install a secondary standard. If an adjustable secondary standard is being used, adjust until it provides the same signal level as the water sample. Then take the sample and perform a quantitative test for cyanobacteria such as cell count, taste and odor, etc. The result of the quantitative test can then be correlated to that secondary standard.

IVF is the easiest method for collecting large quantities of data but there are variables associated with IVF that may interfere with the fluorescence signal. Light history, temperature, turbidity, dissolved components and cell health can all have an impact on the fluorescence signal, independent of cyanobacteria concentration. For the most accurate data, IVF data should be correlated to quantitative data that can be collected by taking occasional samples to be later analyzed in a laboratory. Unlike the chlorophylls that have relatively easy and well-established extraction methods (Arar, E. J. and Collins, G. B., 1992; Strickland, J.D.H., and Parsons, T.R., 1968; Wright, S. W., et. al., 1991), phycocyanin and phycoerythrin are water soluble pigments which makes extractive methods more challenging. Quantitative methods include high performance liquid chromatography (HPLC) (Wright, S. W., et. al., 1991), cell counting and identification and detection of specific cyanobacterial toxins. Once the cell counts or cyanobacteria pigment concentration is determined, the in vivo fluorescence and quantitative data are compared and a correlation factor is developed (see Figure 1). If samples are taken when there are significant changes in water quality or when the natural assemblage of phytoplankton changes due to changes in location and environment, a tight correlation will be obtained, and accurate data will result. In practice it is unrealistic to obtain a water sample every time there is an environmental change but regular sampling, an awareness of the various factors, and sound sampling and extraction practices will result in strong correlation between in vivo fluorescence and cyanobacteria pigment concentrations.

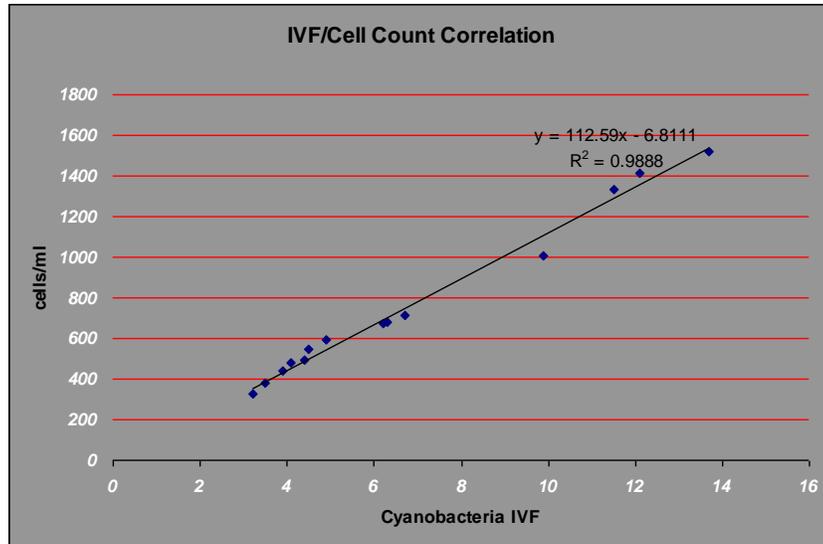


Figure 1: In vivo fluorescence data is compared to cell counts conducted on the same samples. The correlation that develops can then be used to 'calibrate' other data collected at the same or similar site to a quantitative measure, in this case cells/ml.

Secondary standards are available for all Turner Designs fluorometers. Solid, secondary standards are available for submersible (SCUFA & CYCLOPS-7) and discrete sample (TD-700, 10-AU, Aquafluor) instrumentation while liquid, secondary standards are available for flow-through instruments (CyanoWatch).

Correlation of IVF Data to Quantitative Methods

The most common cyanobacterial measure used in the water resource market is cell counts and identification. There are several well-known drawbacks to this approach. First, it is expensive in that it requires many samples to be analyzed to follow trends and a highly trained person. Thus, many water companies send the samples to a water lab, that results in delays and is costly. By the time the results come back, they are frequently just an "after-the-fact" confirmation of a problem, which has already produced clogged filters or a taste and odor problem. Finally, such examination may not even show the problem. Because very thin horizontal layering is common, samples taken without guidance will likely completely miss the "hot" zones. Real-time fluorometric detection can replace cell-counting methods and can be used to trigger more specific and labor intensive analysis such as cell identification, toxin analysis or taste and odor detection tests.

Oceanographers and limnologists use a variety of quantitative methods to detect the presence of cyanobacteria including; pigment detection, biomarkers, and cyanotoxin detection. The benefit of IVF instrumentation is the ability to measure cyanobacteria biomass in real-time. This can be accomplished through horizontal or vertical profiling or attached to an instrumentation buoy. IVF data can then be used to trigger sampling for the more involved and specific methods.

Conclusions

The use of IVF to monitor cyanobacteria biomass is now an accessible technology that is available for use in a wide range of monitoring and research applications. Despite the semi-quantitative nature of IVF methods, the ability to detect cyanobacteria at natural concentrations is a powerful monitoring tool that should be considered for any sensor ecosystem where cyanobacteria plays a role. Turner Designs offers submersible, on-line, handheld and laboratory instrumentation that have been designed specifically for cyanobacteria detection. Please feel free to contact us with your questions or for further information. Or visit us on the web at www.fluorometer.com

Relevant Publications:

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