

Background

Dissolved, or Particulate, Organic Matter (DOM/POM) is ubiquitous in aquatic systems. These materials maximally absorb UV and fluoresce blue light thereby greatly interfering with fluorescence detection that uses UV excitation wavelengths. Other fluorescence applications are also affected by the presence of these materials, specifically those that use blue wavelengths for excitation energy. The overall effect is dictated by 1) the DOM/POM type and 2) the optical configuration of the fluorometer.

Introduction

Fluorescence detection of algae is commonly used in all aquatic habitats and the fluorometers used for this detection are typically configured with blue excitation light sources. Because algae maximally absorb blue light, this optical configuration allows for the best sensitivity for fluorescence detection of algae. However, the presence of DOM/POM decreases instrument sensitivity hindering the primary benefit of having a blue excitation fluorometer. Although, correction algorithms are used to correct for DOM/POM effects, to increase measurement accuracy when estimating algal abundance, each DOM/POM type requires a new set of correction coefficients. This, coupled with the innumerable DOM/POM types that potentially exist, makes it difficult to accurately estimate or even relatively assess algal abundance in the presence of these interference materials.

We can resolve this problem, effectively eliminating interference effects from DOM/POM, by using a specific set of optics. Because these materials don't absorb red excitation energy, but algae do, we can use red excitation fluorometry to detect and estimate algal abundance free from DOM/POM interference. Although this optical setup is less sensitive for detecting eukaryotic algae, the errors associated with these interference materials may outweigh the increased sensitivity of blue excitation fluorometry. Also, red excitation fluorometry is significantly more sensitive to prokaryotic algae (blue-green or cyanobacteria), making it a favorable instrument for freshwater systems which are typically rich in DOM and blue-green algae.

In Vivo Fluorescence Using Red Excitation

The Red Excitation Chlorophyll sensor is configured with optics that maximize accuracy of algal abundance estimates in the presence of DOM/POM. This sensor has increased sensitivity to prokaryotic algae but is less sensitive to eukaryotic algae. The table below lists some minimum detection limits (MDL) for various algal cultures for both blue and red excitation sensors. Calculated MDL values are well below 0.5 µg/L chlorophyll for all cultures analyzed. The Red Excitation Chlorophyll sensor is ideal for estuarine, coastal, and freshwater systems, which typically have algal concentration >0.5 µg/L.

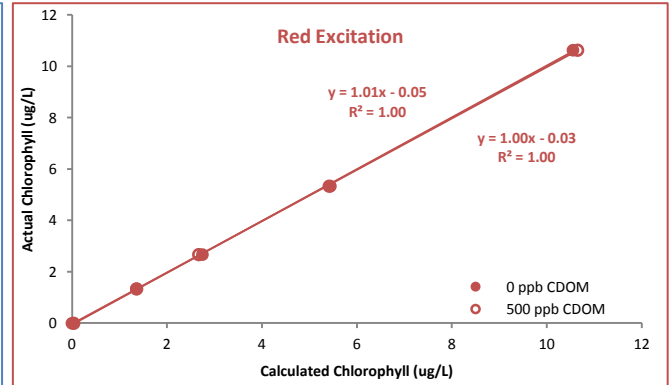
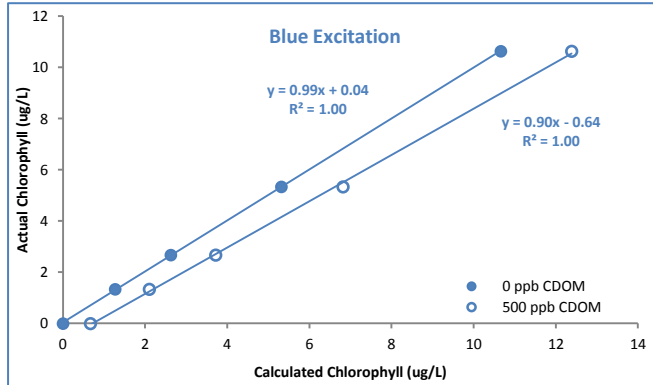
Blue Sensor	MDL	Red Sensor	MDL
<i>Dunaliella</i>	0.23	<i>Dunaliella</i>	0.47
<i>Cyanothece</i>	0.35	<i>Cyanothece</i>	0.09
<i>Isochrysis</i>	0.06	<i>Isochrysis</i>	0.32
<i>Rhodomonas</i>	0.05	<i>Rhodomonas</i>	0.40
<i>Prorocentrum</i>	0.04	<i>Prorocentrum</i>	0.18

Lab Data

Rejection of DOM interference was tested by serially diluting algae using DOM-free and relatively high DOM water. When there is no DOM interference with fluorescence detection, the calculated slope would be 1, accurately estimating the actual chlorophyll in the system. The blue excitation plot below shows an offset for

fluorescence detected from algae mixed into a high DOM solution. In the presence of DOM the blue excitation sensor overestimated the actual chlorophyll concentrations by 16%, no correction was applied. The red excitation sensor measured the same solution accurately estimating the actual concentration of chlorophyll in the dilutions.

Although a correction can be applied to increase accuracy in the blue excitation sensor's response, this is an example using one DOM type in a controlled environment. Correction algorithms can quickly become complicated when measuring algal fluorescence in natural waters containing DOM because of the various DOM types that may exist and change over time.



Field Data

In situ data were collected in an effort to show how the Red Excitation Chlorophyll functions in natural DOM rich systems compared to blue excitation fluorometry. The following data are part of a long term deployment (~1 month) at a location in the San Francisco Bay. A three sensor instrument (C3 Submersible Fluorometer) was deployed to simultaneously monitor DOM and chlorophyll using both blue and red excitation. A negative correlation between depth and DOM indicates the flood tide is diluting materials in this system. A corresponding drop in the algal standing stock is to be expected as they would also be diluted by the incoming tide if bay water was relatively low in algal abundance. There is very little to no change in fluorescence detected by the blue excitation fluorometer over this tidal change, whereas the red excitation fluorometer shows the expected drop in algal fluorescence. The incoming water that dilutes the DOM seems to cause the blue excitation sensor's response to drop slightly but remain at a baseline above the actual concentration as detected by the red excitation sensor. In this case, the blue excitation sensor overestimates the algal abundance by about 20%; the more accurate estimate is obtained using red excitation fluorometry.

