Brian Gaas:
Ocean Optics 2004 Final Class Project: Fluorescence as a Proxy for Chlorophyll

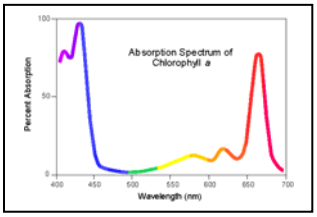
A bet for you: ask 100 random biological oceanographers what the most useful proxy to them is on a research cruise. Accounting for sampling error and the fact I don't actually know the answer, most would say chlorophyll. Now, ask the same people how they measure their chlorophyll. I submit to you that almost all would say "fluorescence." And a couple of them would misspell it, too.

So, what is the big deal about chlorophyll and fluorescence? Glad you asked!

Intro to Chlorophyll

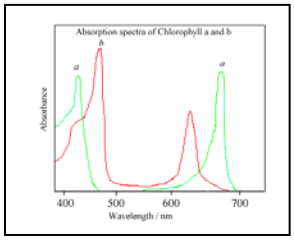
Chlorophyll is one of those amazing molecules that make the world go 'round. Why such a preeminent place in the hierarchy of molecules (and why do I keep asking questions I'm about to answer)? Elementary, my dear reader. Chlorophyll is, in part, responsible for the conversion of light energy into chemical energy via photosynthesis. Thanks to chlorophyll and other pigments, the Earth has oxygen, you are able to breath and hence spend time reading this paper.

Chlorophyll is part of a suit of molecules previously mentioned as pigments. By this I mean they are molecules with a specific wavelength range (in the visible part of the spectrum) at which they do not absorb much light. Conversely, this also means they have a distinct range of wavelengths where there is significant absorption.



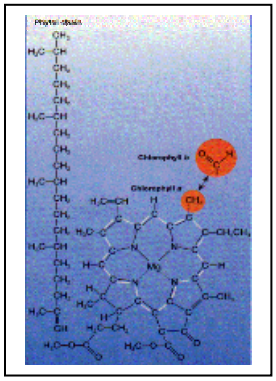
http://www.chm.bris.ac.uk/motm/chlorophyll/chlorophyll_h.htm

The graph shown above is an approximate absorbance spectrum of chlorophyll. As can be seen, the molecule absorbs strongly in the blue (~440nm) and red (~670nm) and weakly in the yellow/green. The low yellow/green absorbance accounts for the green color in most plants and phytoplankton.



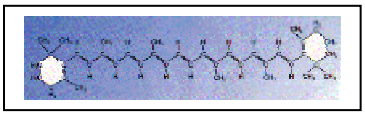
http://www.ch.ic.ac.uk/local/projects/steer/chloro.htm

This graph shows the same shaped chlorophyll curve as above (in green), along with a second curve red-shifted red at small wavelengths and blue-shifted at long wavelengths (in red). Here, the fact that multiple derivates of chlorophyll exist is evident. The green-coloured spectrum with a wider range is chlorophyll a; the red curve is chlorophyll b. The difference between the two, structurally, is shown below. Take special note of the magnesium atom in the center of the ring; it will play a role later in the chlorophyll assay.



http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/Chlorophyll.html

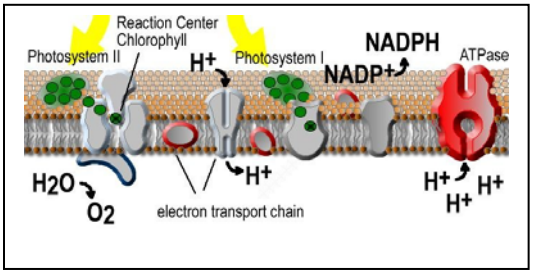
A third type of chlorophyll, chlorophyll c, exists as well with slightly shifted absorption peaks from chlorophyll a and b. To top everything off, accessory pigments like carotenoids (e.g. B-carotene, xanthophyll) are also found in photosynthetic cells:



http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/Chlorophyll.html

Carotenoids and other accessory pigments absorb more yellow/green wavelength light than chlorophyll molecules, broadening the total range of absorbed wavelengths. They also serve a photoprotection purpose, to be detailed later. All plants (and some non-plants, like cyanobacteria) have chlorophyll a, and most either have b or c in addition.

Now that the pigment molecules have been introduced, let's examine how they are used in photosynthesis.

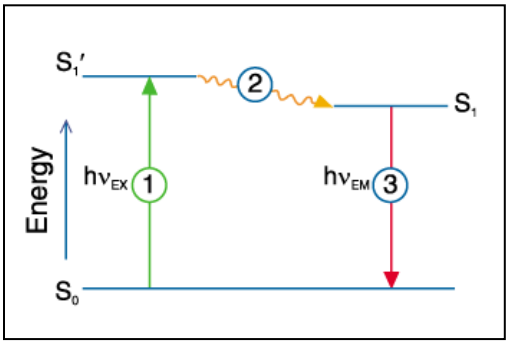


http://bioweb.wku.edu/courses/Biol120/images/Photosynthesis.asp

This diagram is the infamous z-scheme of photosynthesis. In short, photons hit the antenna complex around Photosystem II. Excited electrons from water move from the antenna complex, through lots of other molecules, to Photosystem I. A second set of photons re-excite electrons at Photosystem I where they are transported to an ATPase to produce ATP. Chlorophyll molecules and accessory pigments are bound up in the antenna complex. We can now see that the purpose of chlorophyll (and other pigments) is to absorb photons in the blue and red (as per the absorption spectrum) and transfer that energy through the z-scheme. As a side note, the light energy lost as fluorescence comes primarily from the PSII reaction.

That should be plenty of biology for now. Let us become chemists and look at how fluorescence works.

Intro to Fluorescence



http://www.probes.com

Part 1: Excitation

A photon hits the ground state fluorophore (a molecule that exhibits fluorescence) (S_0) at its excitation wavelength. This energy is absorbed and raises the compound to an excited state (S_1'). Six different types of energies exist in a molecule; the one responsible for fluorescence is electronic energy, associated with electron-nuclei interactions.

Part 2: Intermission

Some of the energy contained in the fluorophore is released as heat, kinetic energy (conformational changes), and/or through molecular interactions. Regardless of the process, the excited state is reduced to a lower energy state (S_1), though still above the ground state.

Part 3: Fluorescence Emission

A photon with the same energy as the lower energy state (S_1) is emitted, returning the fluorophore to the ground state (S_0). Since some of the initial energy was dissipated, the emitted photon has less energy than the initial excitation photon and therefore a longer wavelength. This difference in wavelength between excitation and emission is called (tada!) Stokes shift.

Fluorescence has some nifty properties making it an enticing method for many experiments. Since the fluorescent signal is dissociated from the excitation light, it can operate with very low backgrounds. Unless something odd happens (like photobleaching), the same compound can be re-excited and caused to fluorescence repeatedly.

<u>Protocol: Chlorophyll Fluorescence</u>

Now that we know everything about chlorophyll and fluorescence, it's time to look at how we use one (fluorescence) to look at the other (chlorophyll concentration). To do this, the following will contain the general protocol for chlorophyll extraction in phytoplankton and running a fluorometer. Interspersed with the steps, I'll detail what the purpose of each step is.

1) Gently filter a known volume of seawater through a small-diameter filter.

-Chlorophyll is contained in chloroplasts in the cells. In order to do the assay, we want all of the chlorophyll (chloroplasts) on the filter. If the cell breaks, the chlorophyll/chloroplasts will be able to pass through the filter, and that would be a Bad Thing. So, we gently filter as to keep the cells intact, and do the filtration through a small pore size to keep as many cells on the filter as possible.

2) Put the filter into 5ml 90%acetone and sonicate for 7 minutes.

-The sonication step breaks open cells to release the chlorophyll. Chlorophyll will dissolve off the filter and enter solution when placed into acetone.

3) Wait 15 minutes, vortex and centrifuge for 5 minutes. Let the fluorometer warm up for 15 minutes.

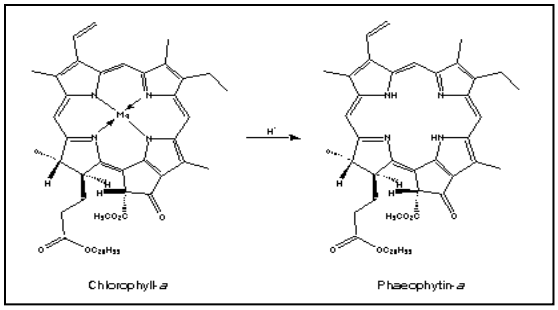
-The 15 minute delay allows the chlorophyll to enter the solution. Vortexing ensures as much chlorophyll originally on the filter is removed as possible. The centrifugation step effectively removes particulate matter by pushing it to the bottom of the tube. Suspended matter could adversely change the reading: some of the excitation light could be absorbed, reducing the amount of excitation light available for fluorescence (lowers apparent chlorophyll concentration); particles could scatter light into the detector (raises apparent chlorophyll concentration); particles could absorb emitted light (lowers apparent chlorophyll concentration). The fluorometer warm-up period allows electronic drift to settle, the lamp to hold a steady temperature, and other "black-box" affects to stabilize.

4) Run a distilled water blank, an acetone blank, and all samples, being careful not to resuspend particles.

-Some water/acetone scattering is expected, as well as electronic "dark" current. The 2 blanks act as both a check for instrument stability as well as a correction for the above effects. The excitation light is provided by a lamp in the fluorometer. Chlorophyll absorbs photons in the range given by the spectrum shown earlier. In acetone, this ends up being approximately 440nm (blue) and 660nm (red). The emission wavelength is approximately 676nm (also red). Approximately 3%-9% of the light energy absorbed by chlorophyll pigments is re-emitted from the excited state.

5) Add 1 drop of 10% HCl to each blank and sample tube and re-measure in fluorometer.

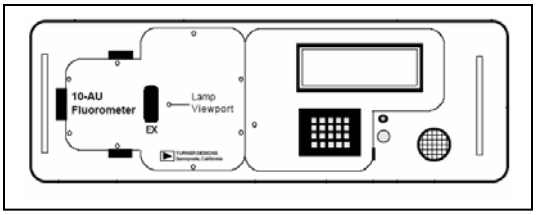
-Remember the magnesium atom in the middle of the chlorophyll molecule? The addition of acid removes the Mg atom and replaces it with two hydrogen atoms. The acidification step changes all of the chlorophyll to a different pigment, phaeophytin-*a.* As would be expected with a modified compound, the excitation wavelength for phaephytin-*a* is different from that of chlorophyll, blue-shifted to approximately 415nm.



http://www.ch.ic.ac.uk/local/projects/steer/chloro.htm

Protocol: Instrument Specifications

Since the details about the fluorometer are crucial to the results, it is worth taking the time to here to describe, what has been in my limited experience, the most common fluorometer used for chlorophyll assays.



http://turnerdesigns.com/t2/doc/manuals/10au_manual.pdf

Unless one is trying to set up the Turner Designs au10 fluorometer, most of the diagram can be ignored. More important are the internal devices providing the signal and sensitivity. First, light is shown onto the sample to induce excitation. The lamp used is wide spectrum (Daylight White). This ensures all fluorophores present in the sample can encounter their appropriate excitation wavelength. However, when only a certain fluorophore is being detected (like chlorophyll and its close relatives), an excitation filter is used. This filter allows a certain (hopefully small) range of wavelengths to enter the sample, blocking all the other light that might be detected as fluorescent signal. For chlorophyll, the excitation wavelength is around 440nm. Hence, the excitation filter used allows wavelengths from 340-500nm. It should be noted that neither the excitation band of chlorophyll, the wavelength of emission, nor window of the filter can be defined as a single wavelength. All usually have a Gaussian curve centered at a point, but allow light of similar wavelengths to interact. Anyway… The detector is set at 90 degrees from the excitation light. The perpendicular orientation is used to reduce the amount of incident light entering the detector. Although scattering can still be a problem, most of the light entering the detector will be from the isotropically-emitting fluorophore. A second filter is usually placed between the sample and detector, known as the emission filter. This filter has a window around the emission wavelength of the fluorophore, >655nm in the case of chlorophyll. Wavelengths around that of the emission are allowed to pass through to the detector, while other wavelengths (such as those used to excite the fluorophore) are blocked. The use of these two sets of filters ensure a low background signal. The detector contains a red-sensitive photomultiplier tube (PMT). By using a voltage cascade, a low signal has its gain increased dramatically. Chlorophyll emits in the red part of the spectrum. Having a PMT tuned to the red will provide higher sensitivity for chlorophyll detection. Apparently PMTs with other tunings do not suffer a lack of accuracy/precision, but do suffer from a lack of sensitivity. With a red-sensitive PMT, detection limits are about 0.02µg/L using a 13mm diameter test tube and 0.01µg/L using a 25mm test tube for extracted chlorophyll.

Signal Interpretation

As described above, the fluorometer only measures voltage. This voltage is related to the fluorescence signal and hence to chlorophyll concentration, but there are some notable steps in between. Even more care must be taken when trying to convert from chlorophyll fluorescence to biomass. The issues contained in signal and proxy conversation are the subject of this section.

The main reason for measuring chlorophyll fluorescence is to arrive at the concentration of chlorophyll in the sample. It follows that, since each chlorophyll molecule presumably has the capability to fluorescence, if the excitation is constant, then the fluorescence will be constant. In equation form, we have something like this (thanks Mary-Jane!): $F = a(\lambda) * E(\lambda) * \Phi_f$, where F equals the fluorescence value, $a(\lambda)$ is the absorption by fluorescent material, $E(\lambda)$ is the excitation energy, and Φ_f is the quantum yield for the fluorescent reaction. In addition, the amount of fluorescence will be directly proportional to the amount of chlorophyll present in the sample. Given optimal situations, this is true. Even under non-optimal circumstances, there is often a linear relationship between fluorescence and chlorophyll. However, because there are many factors controlling energy release, chlorophyll fluorescence quantum yield is not constant. Photosynthesis, antenna energy transfer, heat, and damage to proteins all contribute to varying fluorescence levels.

In order to link machine voltage with fluorescence, a standard curve is needed. The counts on the fluorometer are recorded for a suit of known chlorophyll samples. Given a linear relationship, one can create a regression equation that will allow any given count to be corresponded with chlorophyll concentration. For our particular work, this calibration curve was already inputted into the Excel spreadsheet with the implicit

assumption that the curve is constant over time (and the note that, since each run was prefaced with a high and low chlorophyll sample, machine drift over each day is recorded). A similar standard curve is run after acidification of the standards (where chlorophyll is converted to phaephytin-*a*). The ratio of the two, together with the volume of water filtered, provides the chlorophyll concentration.

Despite the tediousness of the assay, measuring chlorophyll is a pretty straight-forward and quick process. Unfortunately, there are a fair number of issues that might confound results:

1) The presence of humic materials, detritus, or other dissolved fluorescing compounds may or may not change the results, depending on the experiment. Other compounds with similar excitation and emissions would give increased apparent chlorophyll concentrations.

2) Suspended particulate matter. The effects of this were mentioned previously, including absorption of the excitation light (lowers apparent chlorophyll concentration), particles scattering light into the detector (raises apparent chlorophyll concentration), and particles absorbing emitted light (lowers apparent chlorophyll concentration).

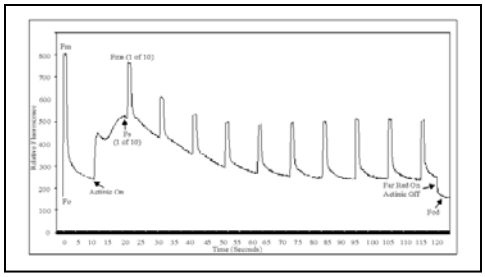
3) The fluorescence efficiency of chlorophyll is species dependent.

4) The fluorescence efficiency of chlorophyll is nutrient dependent. A higher degree of nutrient limitation corresponds to a higher level of fluorescence per cell. The same hold true with toxicity issues.

5) The fluorescence of chlorophyll varies inversely with temperature. According to Turner Designs, *in vivo* chlorophyll fluorescence has a temperature coefficient of -1.4% per ° C, while that of extracted chlorophyll in 90% acetone is -0.3% per °C. While this is certainly an issue between samples of different temperatures, the effect can usually be ignored for temperature increases occurring during sample measurement. Taken from Turner Design's notes, samples at or near room temperature may be left in the instrument for about one half minute before a drift in reading caused by sample warming will be noted, a period at least three times the amount of time required to take an accurate reading. Interestingly, the continual decrease in fluorometer counts some of our groups experienced when taking readings might be explained by this; many easily exceeded a 30 second period.

All of these issues and more exist when trying to use chlorophyll concentration as a proxy for phytoplankton biomass. Some extra issues include:

6) The amount of organic matter (carbon) associated with a given quantity of chlorophyll pigment (carbon : chlorophyll ratio) varies widely, depending on the type of organism.



www.optisci.com/ tutorial1.htm

7) Kautsky effects (demonstrated on the graph above) from a dark-adapted sample moving towards a lit sample chamber. When dark-adapted chloroplasts are illuminated, there is a rapid rise in fluorescence from PSII fluorescence followed by a series of slow oscillations trending downward. As far as the Turner fluorometer goes (so they claim), the illumination level (about 2 W/m^2) is low enough that this effect is not seen. However, samples that are kept in the dark would be expected to show different apparent concentrations if stored in the light.

8) Packaging effects. This is a huge one and a source of confusion for many years for people measuring the subsurface maxima for chlorophyll and biomass. Packing refers to the fact that there is a finite volume available in the cell to store chlorophyll, and a finite surface area that chlorophyll is exposed to light. For spherical particles, the available volume changes as a function of cell radius cubed, whereas surface area

changes as a function of cell radius squared. This means that a small cell can have more pigment exposed per volume than a large cell (like a diatom). Unexpected results occur because of packaging effects. A cell under high light conditions does not need to worry about getting enough light. Since chlorophyll is an expensive molecule to make, it will only want to make enough chlorophyll to meet the light demands comparable to other nutrients, i.e. nutrients other than light are likely to limit phytoplankton in high light conditions. As a chlorophyll profile versus depth is examined, chlorophyll concentration usually increases as the irradiance decreases. More chlorophyll is needed to capture the same amount of light found at the surface. This trend continues until packaging effects take over. At some point lower in the water column, the energy required to make more chlorophyll outweighs the energy gained by increasing the chlorophyll concentration. More chlorophyll molecules can be made, but they will get "shaded out" by molecules above; remember, the area available for light-harvesting increases with the square of the diameter, while the volume increases with the cube. Packaging effects describe the increasing inefficiency of chlorophyll molecules as the surface area is covered and excess molecules are "stacked" under others. In acetone solutions (such as the one used in the assay described above), chlorophyll is unpackaged, and so is not a concern. *In situ*, though, chlorophyll is packaged and fluorescence does not necessarily scale directly with chlorophyll concentration.

9) Quantum yield varies with light, nutrients, photoquenching, photoadaptation, and system damage. The relationship with light was detailed in the packaging section. In general, higher light fields correspond to reduced chlorophyll concentration. High light conditions also induce photoquenching, a reduction in fluorescence. Low nutrient conditions are sometimes reflected by increased fluorescence values. System damage corresponds to interruptions in electron/energy transport due to free radicals, high irradiation, photoinactivation (by light), toxins (DCMU, for example, which shuts off Photosystem II), and other things not helpful for living organisms. These generally increase the amount of fluorescence, as energy is no longer able to be dissipated through the photosynthetic pathway.

Fluorescence Experiment

Given the wide range of variable effects and a result determined by the accuracy of a standard curve, just how well can fluorescence predict chlorophyll concentration? In addition, how strong is the correlation between fluorescence measurements at an observatory and those taken in the field on a ship? Although the first is the more fundamental question, the second is of more interest to me. As such, the rest of this discussion will be an analysis on the Darling Marine Center dock Bb2F fluorescence meter and the one used on consecutive research cruises in the Damariscotta River.

The Darling Marine Center uses a WetLabs Bb2F sensor for continuous measurement of scattering and fluorescence. The sensor is arranged with two perpendicular sets of opposing sources/sensors (forming a cloverleaf), measures backscattering at 117 degrees at both 470 and 700 nm and fluorescence at 700 through use of a blue and red/IR detector. The advantages of the Bb2F are a small size and multifunctional design. Scattering detection at 117 degrees is a convergence point of most phase functions (for varying particle compositions), keeping water scattering low, and the blue source/detector and red source/detector allow measurements of chlorophyll fluorescence. This said, some technical details lower the efficiency and "oh...ah" value of the Bb2F. Scattering at 117 degrees has very little water scattering; however, it is standard practice to subtract Ramen scattering as a matter of principle, allowing for a wider range of efficient detector angles. The blue scattering source is off-set from the main pigment peak of ~440nm. In the case of some organisms (like cyanobacteria), the excitation light is sufficiently off-peak to lower fluorescence below detection limits. In addition, the red scattering source emits at the same wavelength of chlorophyll fluorescence, increasing the probability of fluorescence signal distortion.

Two Bb2Fs were compared over the same day, time, and depth, the first attached to the dock and the second on a CTD. Dock data was downloaded from the Darling Marine Center site (http://optics2004.dmc.maine.edu/data/dmc_realtime/ecobb2f_0604.html) and converted into Excel spreadsheet files. The files are also available in an easily-readable Matlab file, but my ignorance with the program prevented analysis through that technology. Bb2F data is formatted to include date, time, scattering data at both wavelengths, their reference values, fluorescence counts, and temperature. For this comparison, only the date, time, and fluorescence counts were deeply examined. The Marine Center Bb2F resides on the pier side of the

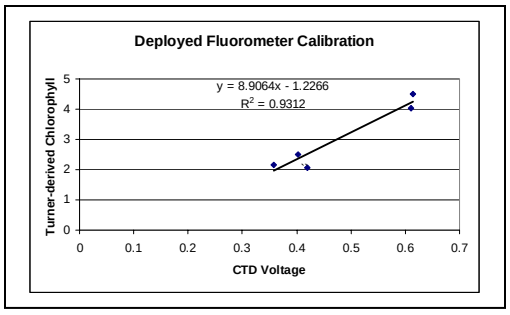
floating dock at approximately 1 meter depth.

The cruise Bb2F was attached to a CTD cage with other instrumentation. The device was oriented away from the cage and in parallel with other instruments. A deployable datalogger saved the data in the same format as that of the dock. However, the time measurement was in relative time from powering on, opposed to actual time. Unfortunately, this restricted the accuracy of the CTD measurements to time points written down with general cruise notes. Measurements were taken at 5 different stations over two different days. Day 1, July 8th, two unfiltered casts were done. The first cast was at the Damariscotta bridge (43'48"N 69'33"W), and the second at the "hole" downstream (44'2"N 69'32"W). Bottle samples were taken at ~4m at both stations for calibration. Cruise2-Stn 1-3 were taken at the mouth of the Damariscotta River (43'49"N 69'34"W).

The previous discussion on fluorometric assays were specifically relating to the Turned Designs fluorometer. The Wetlabs Bb2F is designed completely differently, yet operates on the same principles. Instrument counts need to be changed into chlorophyll values; this is again done via a calibration curve. The Bb2F is an *in situ* instrument (not easily capable of measuring a standard chlorophyll solution) so an additional step to convert from counts to fluorescence is necessary. Identical (as possible) aliquots of a sample are run through the Bb2F and a Turner Designs fluorometer. The Turner instrument will determine actual chlorophyll concentration as described above, which can be plotted against Bb2F counts to form a second calibration curve. The slope of the Bb2F vs chlorophyll curve is then used to calculate the chlorophyll concentration of the profiles/dock series.

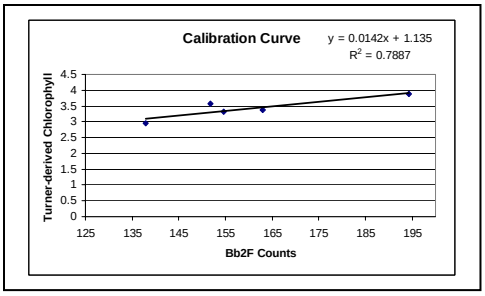
For this experiment, water samples were collected by niskin bottle around 4m depth. The actual depth is quite imprecise at the first station due to a very strong current pulling the CTD and niskin bottle tangentially towards the water surface. The samples were then stored on ice for the duration of the cruise, around 1-2hrs.

Two different calibration curves were needed to correlate the dock data with the cruise data. The first was conducted on July 8th after returning from the first cruise. Well-mixed bottles, following strict R-R-L swirling procedure, were assayed according to the guidelines detailed for Turner-style chlorophyll determinations.



Turner Designs calibration curve

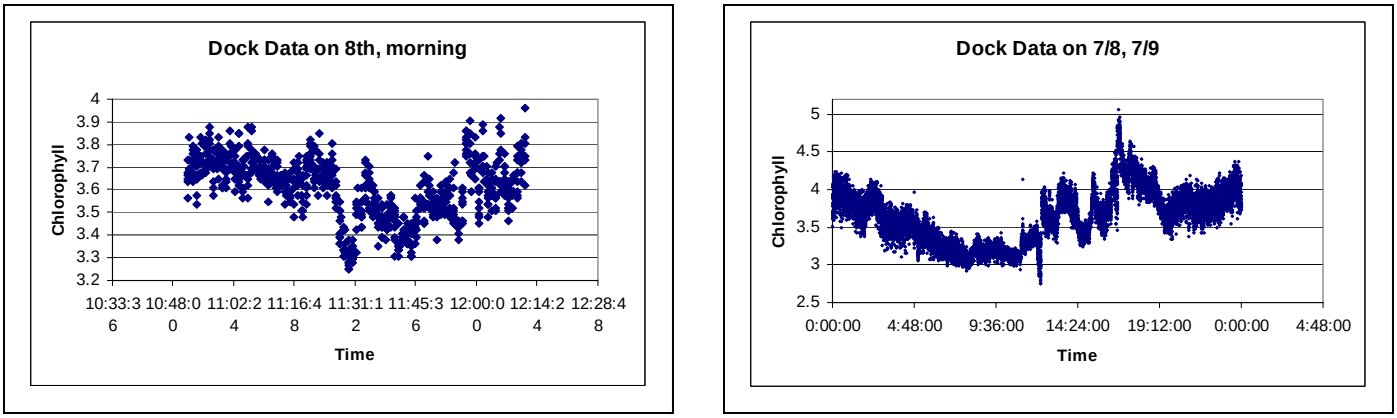
As we have learned, regression lines aren't the greatest method of determining data strength. However, an r^2 of .93 over a factor of 2 difference is fairly good. As expected, there was a strongly positive trend. The least-squares fit gave an equation of $y = 8.91x - 1.23$ for the Turner counts-to-chlorophyll conversion. The second calibration between Bb2F and chlorophyll also exhibited a strong positive trend, although not as much as with the Turner instrument ($r^2 = 0.79$)



Bb2F calibration curve

As seen in the regression equation (y = .0142x + 1.135), an increase in variation is expected and seen. The Bb2F calibration, by relying on a second instrument to determine chlorophyll concentration, inherits the uncertainty in the former. The propagation of error through sub-sampling and multi-instrument calibration is a problem with not being able to calibrate within a single instrument.

The high sampling rate of the dock system and relatively immobile position provides a very stable system for tracking small effects over long time scales.



Dock observatory time series data.

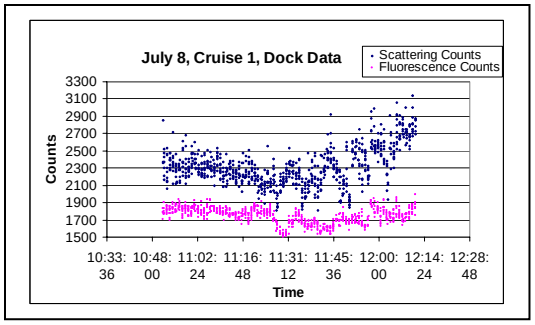
The left graph is dock data at the time of cruise 1 sampling. The right graph shows continuous data from noon on July 8th until noon on July 9th, covering the last station of cruise 1, and all of cruise 2. Note the time scale on the right graph is incorrect- it should go from 12:00-12:00hr, not 00:00-00:00hr.

The first set of graphs are a time series of dock data the days of the two cruises. The first graph's data begins at ~10:50am and continues until 12:12am. This almost covers the entire first cruise sampling period. The logged data times for the first cruise have the first sample taken at 10:30am, and the second at 11am. As mentioned before, the lack of a "truthed" time stamp (as opposed to one just measuring elapsed time) makes an accurate time determination of fluorescence profiling difficult. The values of Bb2F fluorescence are relatively stable over the 2 hrs shown, and within the usual range of chlorophyll values from discrete dock sampling.

25-Jul-03 Filtr. Date	Analysis Date	Vol. Filt. [L]	Station ID	Dilution Factor	Fo [FSU]	Fa [FSU]	Chl [ug/L]	Phaeo [ug/L]
9/02/0042	9/02/0042	0.066	Example	1	435	230	6.38	6.00
7/6/04	7/6/04	0.2	dock	1	436.00	277.00	3.24	1.74
7/6/04	7/6/04	0.2	dock	1	447.00	283.00	3.34	1.74
7/6/04	7/6/04	0.2	dock	1	429.00	263.00	3.39	1.34
7/6/04	7/6/04	0.2	DI blank	1	2.14	1.98	0.00	0.03
7/6/04	7/6/04	0.2	acetone blank	1	0.15	0.16	0.00	0.00
7/7/04	7/7/04	0.2	Chl blank	1	1.42	1.00	0.01	0.0125
7/7/04	7/7/04	0.2	Chl 1	1	455.00	277.00	3.62	1.3499
7/7/04	7/7/04	0.2	Chl 2	1	456.00	286.00	3.46	1.6743
7/7/04	7/7/04	0.2	Chl 3	1	452.00	275.00	3.60	1.3384
7/7/04	7/7/04	0.05	Acetone	1	0.16	0.21	0.00	0.0199
7/8/04	7/8/04	0.22	dock	1	546.00	328.00	4.03	1.33
7/8/04	7/8/04	0.18	dock	1	484.00	320.00	3.71	2.67
7/8/04	7/8/04	0.21	dock	1	528.00	327.00	3.90	1.69
7/8/04	7/8/04	0.19	DI blank	1	2.67	1.97	0.02	0.02
7/8/04	7/8/04	0.2	acetone blank	1	0.20	0.23	0.00	0.00
7/9/04	7/9/04	0.2	acetone blank	1	0.54	0.54	0.00	0.01
7/9/04	7/9/04	0.2	dock	1	518.00	337.00	3.68	2.37
7/9/04	7/9/04	0.2	dock	1	476.00	313.00	3.36	2.26
7/9/04	7/9/04	0.2	dock	1	440.00	288.00	3.09	2.09
7/1 2/04	7/1 2/04	0.2	acetone blank	1	0.28	0.28	0.00	0.01
7/1 2/04	7/1 2/04	0.2	dock	1	376	231	2.95	1.20
7/1 2/04	7/1 2/04	0.2	dock	1	381	235	2.97	1.25
7/1 2/04	7/1 2/04	0.2	dock	1	376	231	2.95	1.20
7/1 2/04	7/1 2/04	0.2	DI blank	1	1.73	1.38	0.01	0.02

Log of daily discrete dock sample

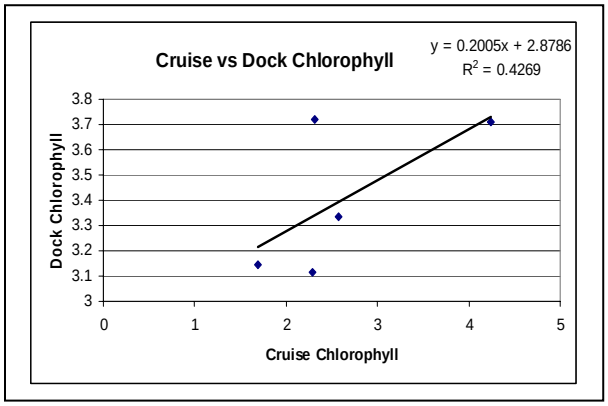
Since the Bb2F is attached to the dock, many factors could contribute to the noise. Previous experiments have shown drastic changes (increases) in fluorescence when the excitation light is in close proximity to kelp or other plants. Unless one gets caught on the sensor, though, an hour-long trend is unlikely to be seen. Boat wake and tidal influences, if at all visible, would both operate on time scales other than the one seen here (shorter and longer, respectively). Of course, it could just be "random" variations without an easy explanation, or junk getting in between the source and detector. Fortunately, one can look at the scattering data to help determine the cause of variation.



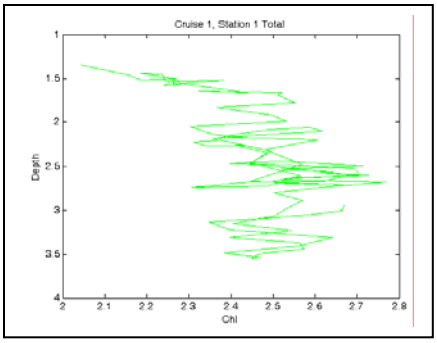
Dock Bb2F Scatter vs Fluorescence

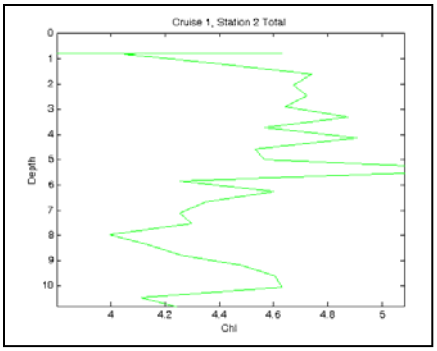
The graph shows a general tendency of increasing scattering counts (blue detector) with decreased fluorescence counts. The magnitude of the fluorescence count was multiplied by 10 for the comparison, but this modification would not change the shape of the curve. The blue detector was used to avoid concurrent measurements of fluorescence and red scattering that might cause signal coincidence. Diel variations can cause apparent chlorophyll concentrations to change, but this is unlikely to be present in the 2hr time series. Although impedance by stuff floating in the water could mask fluorescence and increase scattering, I would not expect to see multiple minute trends as is present in the data, barring a massive exodus of zooplankton out to the ocean. Instead, I propose changes in irradiance causing the decrease in scattering and increase in fluorescence. Both cruise days were totally overcast, but varied with rain. Irradiance is a function of the amount of sunlight hitting the surface, which will be less under rainy conditions even under cloud cover. When rain starts, the irradiance on phytoplankton is less, lowering the total amount of light available for scattering and decreasing the effects of photoquenching. The rain was not constant, giving rise to the variation over minute scales.

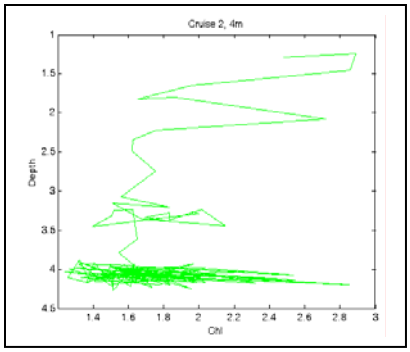
Let's now look at a comparison between dock-sampled data and measurements taken on the cruise. Five comparisons were made to generate the scatter plot. Two stations were evaluated from the first cruise, and three from the second. Counts were converted to chlorophyll using the two regressions mentioned above (one for the dock, one for the cruise). Averages were made of all cruise chlorophyll values corresponding to the integer depth of the dock Bb2F. For example, all values falling at a depth between 0.00m and 1.99m were averaged. Due to sampling technique, only three of the stations had values at 1m: Cruise 1-Stn 1 and 2, and Cruise 2-Stn 2. Cruise 2-Stn 1 and 3 were evaluated at 4m, the shallowest depth recorded for those locations.



Bb2F data was averaged over a 10minute interval starting at the time recorded on the cruise log sheets. For instance, Cruise2-Stn2 started their cast at 1050hr. The comparable dock Bb2F data was the average value from 1050-1100hrs. The expected regression line of 1:1 is not seen. Rather, the dock chlorophyll was consistently measured as being higher than that measured on the cruise. Some of the chlorophyll variability may be due to changes in chlorophyll with depth, especially on the first cruise. The strong current kept the CTD with the fluorometer at a large angle. As the ship was moved in the current, the actual depth of the fluorometer might have changed a significant amount. The depth-dependence of chlorophyll in the upper 4 meters of station one is demonstrated by the following graphs. Chlorophyll values change between .28 and .41ug/mL over a 4 meter depth. In some cases, the bulk of this change occurs right at the surface.







Chlorophyll vs Depth: Cruise 1, Station 1 Cruise 1, Station 2 Cruise 2, Station 2

Chlorophyll values change between .28 and .41ug/L over a 4 meter depth. In some cases, the bulk of this change occurs right at the surface.

Conclusions

Since it's a lot easier to read what I write than to interpret the graphs, I'll put down my ideas on what this all means. First, though, some things to note. Since the Bb2F is on a fixed platform, on the CTD needed to be sent to the same depth as that on the dock to make an appropriate comparison. The dock-cruise comparison wasn't formulated until well after the cruise was over, so the depths were not measured as close as they optimally should be. As already mentioned, the actual depth of the calibration sample is uncertain due to the high flow on Cruise 1-Stn 1. Similar uncertainty existed in the time of the CTD-deployed fluorometer data. I was just now informed that the datalogger has a time file saved outside of the scattering and fluorescence data. This would have proved immensely useful for pulling dock data at the same time. However, I am currently out of time to figure out how to addend this and replot the data. Fortunately, I think accurate conclusions can be reached even if the time points plotted differ by 10s of minutes (the estimated maximum error of the data used). The derived chlorophyll values from the dock and from the cruises matched up very poorly. This means either one or both of the Bb2Fs weren't functioning well, the calibration of one or both instruments were inaccurate, or there physically isn't much similarity between the cruise stations and dock observatory. The correlation coefficients of both the cruise and dock were quite high, lending credibility to that portion. The calculated chlorophyll values were not unreasonable, although did vary widely by station, day, and depth. This given, it would seem that significant differences actually exist between the chlorophyll measured by the dock. Direction away from the dock did not seem relevant; chlorophyll values were lower going both upriver towards Damariscotta (Cruise 1, both stations) as well as to the river mouth (Cruise 2, all stations). This would seem to refute the idea that chlorophyll at the limited depths and spatial scales measured vary with proximity to the river source.

The Damariscotta River has a strong tidal influence, with a tidal range of ~5m. Salinity is almost that of open ocean, between 29-33 psu on average, with dock sampling consistently producing 33 psu samples during the week of the cruises. Given limited freshwater input and strong haline circulation, I am skeptical that nutrient sequestration is common in the area. However, the dock sampling site has one particular feature about it that can explain the high variability with the cruise data: it is a dock. As most layperson can attest to, fish and other organisms tend to congregate around such objects in the water. They offer food, shelter, protection, and other amenities that can be hard to come by in the ocean. A high concentration of biology in the vicinity of the dock could easily raise the concentration of nutrients in the area, and could persist despite tides. High nutrient concentrations in a non-light limited area (which I presume the dock is not) would explain the persistently higher chlorophyll signal.

This mini-project demonstrates a couple of general ideas. Time courses can be very important, and one needs to have good resolution to make sense of data. The dock averages and bins data every minute, providing ample data for almost any type of analysis. The cruise data was quite the opposite, offering only one averaged data point for a single location and meter-wide depth. Granted, these are artifacts of the comparison (cruise Bb2F data was also sampled every second), but does highlight the limitations. The low-resolution temporal

cruise data made a good comparison difficult. The difference might not be as bad if I had access to time data for the cast, but that will forever remain a mystery. The fast sampling rate of the dock observatory presented its own problems. I managed to crash Matlab twice trying to read in a weeks worth of data at once. The cruises examined horizontal chlorophyll concentrations at a maximum resolution of 30 minutes (closer to 50 minutes in practice). Managing such huge (dock) data files in order to condense the output into (cruise) comparable 1hr averages was quite a task. Small trends were difficult to differentiate from noise at the single-minute scale. Since the CTS casts were performed over small time scales (although there were large time intervals between casts), small time intervals were needed in the dock data. Fortunately, the dock Bb2F signal was stable over the 10 minute intervals considered and no large variations were seen.

Perhaps the most useful part of this exercise was showing the effect of observatories on the observations they make. If my conclusion was correct, then the measurement of water near the pier is a measurement of its own mesocosm and does not accurately reflect conditions of the river. Instrument placement is apparently very important. It is unfortunate that I did not have the chance to look at the placement of the dock Bb2F and see what orientation it has compared to the dock, pier, sky, and depth underwater with tide. Variables such as these could also have profound effects on chlorophyll fluorescence, measuring changes in physical ocean conditions rather than biological ones. As with the discipline of oceanography, the measurements of ocean parameters are also interdisciplinary. Fluorescence varies with chlorophyll, but also with irradiance, temperature, and non-biological parameters. Likewise, physical measurements can be affected by biology in instances like beam attenuation and, in more extreme cases, biofouling. One of the main difficulties in fluorescent measurements is separating the physical (light, temperature) effects from biological ones (packaging, health). In my opinion, this project did not adequately distinguish between physical and biological effects on dock/cruise variability. It did, however, highlight some of the difficulties and variables involved with trying to reach conclusions based on fluorescence data. Since the point of this class and project was to learn something worthwhile, I suppose it was a success after all!