PULSE chlorophyll protocols

<u>Equipment needed for at sea:</u>

- Gast vacuum pump
- Manifold with filter cup holders
- Carboy with nipple top
- Tweezers
- Whatman GF/F glass fiber filters nominal 0.7µm (VWR cat # 1825025) and
- Whatman Nuclepore Track-Etch 8µm (VWR cat # 110614)
- Dewar
- Aluminum foil
- 15 ml centrifuge tubes with screw cap (non-reactive to solvents)
- Dark Nalgene bottles (one for each depth) and carry box

<u>This is a two part process:</u>

AT SEA:

1. Using a dark nalgene bottle that holds at least 600 ml of water- collect water from each Niskin bottle depth. You will try to do two replicates for each depth and each filter, so that's 400 ml total of water filtered per depth. It is important to collect all the water you will be filtering at once so that the cells in the bottle do not have a chance to settle out. A Niskin bottle that has stood around for a while has had cells settling out and concentrating near the tap.
2. At sea and as quickly as possible after collection, filter 100ml of seawater (measure with a graduated cylinder- NOT the filter cup gradations) for each sample by using the Gast pump, a single filter and the manifold. Be sure to not touch the filter with your fingers- always use the tweezers when handling the filters. Be sure to not allow vacuum pressure to exceed 5 on the pumps gauge.
3. Delicately remove the filter by carefully tweezing one edge up and then folding the paper in half with the filtrant in the middle. Do not use your finger to help in the folding process- use another tweezer or something else. Oils from fingers or the deck can alter the extraction results.
   It is recommended to either carefully fold the filter in a labeled piece of aluminum foil or place in a labeled centrifuge tube then place immediately in liquid nitrogen.
4. Repeat the process for the Nuclepore 8µm filter.

Laboratory Chlorophyll and Phaeophytin Extraction and Fluorescence Value Determination

Equipment need in the lab:

Fluorometer (we use the Turner Designs)
Borosilicate tubes
Vortex mixer
Aluminum foil
Kimwipes
Disposable pipettes
Automatic repeater pipette
10% HCL (DDW water)

- If you can't set up the samples to be read 2-24 hours then leave the samples in liquid nitrogen for an indefinite amount of time.

IN THE DARK!! ALWAYS!!:

1. If not in a labeled centrifuge tube, place the filter in a labeled centrifuge tube with 10ml of chilled 90% acetone from an automatic pipettor. The gradations on the centrifuge tube can be inaccurate by a few ml's which would add error to your chl-a calculations. Break up the cells with the vortex mixer then place covered with foil in a standard non self-cleaning freezer 2-24 hours. Chose a time length and stick with it for quality control purposes.

2. Once extraction is complete, turn on your fluorometer 45 minutes before use to let warm up. Remove the extracted samples form the freezer and let them warm up COVERED and IN THE DARK to room temperature at about the same time.

3. Then each tube must go through the centrifuge in Ann Bucklin's closet room next door at 1500 RPM's for 5 minutes.

4. Read a 90% acetone blank before and after 10% acid. Use the same 90% acetone that you used to extract the pigment from your samples. Fill a borosilicate tube to approximately half full with a pipette. Wipe the tube down with a Kim-wipe and then place in the chamber. Cover the chamber with the cap.

5. Once the machine has settled down to within a few tenths of a value, hit the "*" button to get an averaged value. The "*" function will delay for three seconds, average and then produce a "Done!" value that you will record.

6. Once the FL_b (Fluorescence value before acid) value is recorded, remove the sample tube and add TWO drops of 10% HCL to the tube. Cover the opening with a piece of parafilm and invert three times to mix the sample.

7. Place back into the chamber, re-cover, allow to stabilize, and then hit the "*" button. This will be your FL_a value (fluorescence value after acid or phaeophytin pigment).+

8. Read a blank and an acidified blank before and after your run of samples.

9. Dispose of the sample in the acetone waste bottle, tube and all pipettes used and repeat process.

This is also the basic fluorescence "reading" technique as well. Follow these methods for all samples to be read.

On to your sample:

10. Using a fresh pipette for each sample, place extracted sample into a borosilicate tube to just over half full with a disposable pipette and carefully wipe the vial clean of finger prints before placing into the fluorometer chamber. Make sure to recover the chamber.
11. Allow the machine to settle; then have the Turner do its three second reading, average reading and then output. It's a setting on the Turner that can be programmed for the "*" key. Record that value.
12. That's your FL_b. Now add 2 drops of 10% HCL to the extract in the tube that you just read. Mix it with the parafilm method aforementioned and then re-read. This value is the FL_a value. Pheophytin adds spectral interference of chl-a and without correction can bias your true chl-a values. Pheophytin also is a degraded form of chl-a and can tell you a little about the general "health" of the phytoplankton community: when there's more Pheophytin than chl-a then the "health" is of a lesser quality than the inverse.
13. Dispose of the sample in the acetone waste bottle, tube and all pipettes used and repeat process.

Calculations:

Chl-a (μg^{-L} or mg m^{-3})
= $(F_b - F_a - Blk_b + Blk_a)$ * tau/tau-1 * F_r * V_{ext}/V_{filt} * DF

Pheo-a = $\{(F_a - Blk_a)$ tau- $(F_b - Blk_b)\}$* tau/tau-1 * F_r * V_{ext}/V_{filt} * DF

F_b= Fluorometric reading before acidification
F_a= Fluorometric reading after acidification
Blk_b= blank acetone reading before acidification
Blk_a= bland acetone reading after acidification
V_{ext} = volume of the extract (the acetone process; usually 100ml)
V_{filt} = volume of the raw water filtered (usually 100ml)
DF= dilution factor: If the chl-a is so high that the Fluorometer is at it's max then you need to dilute the sample by removing an exact amount of extract and combining it with an exact amount of the same acetone you used for the extraction process. The dilution factor is that ratio of extract to acetone.
Tau and F_r are values determined from each calibration. Calibrations are scheduled for every 6 months.