UWT Chlorophyll Procedure

(1) Turn on fluorometer 30 minutes prior to running any samples.
(2) Make sure to check the big glass filtration water jug prior to sampling & empty if getting full. If it fills, it will flood the pump with seawater. This is bad.
(3) Keep sample bottles cold & in the dark until filtration to minimize degradation due to heat & light exposure. (Best to filter immediately – do not keep for more than 6 hrs.)
(4) After putting filters in filtration cups squirt them with $MgCO_3$ solution. (Changes pH so cells don't burst before you want them to) <$MgCO_3$ is 1 gm/100ml; Whatman GF/F 25mm glass fiber filters>
(5) Put on cups and make sure screwed on.
(6) Put 145 ml (125/250 ml) water in filtration cups (measure with graduated cylinder – rinse between samples), open manifold valves, turn on pump to between 5-7 psi.
(7) Filter 145ml (125/250 ml) of sample through filter without allowing it to run dry in between sample additions. After adding all 125ml (250 ml), allow to run dry and then close valve on manifold.
(8) Carefully remove filter with tweezers and place in **LABELED** 15 ml centrifuge tube.
(9) Add 10 ml of 90% acetone to each tube, submerging the filter.
(10) Place tubes in icewater bath in sonicator & sonicate for 7 min. at an amp. of 50%.
(11) After it is done let it sit for an additional 10 min. in the dark.
(12) Remove and invert tubes several times to mix.
(13) Place in centrifuge and run 5 min. at 3700 rpm. Make sure centrifuge is balanced prior to turning on.
(14) Remove the tubes carefully from the centrifuge and decant (using Pasteur pipette) into labeled clean glass culture tubes (or can pipette in), filling them to within ~1 inch of the top or about 2/3 full. Be careful not to transfer any of the solid material at the bottom of the centrifuge tube. <13x100mm pyrex disposable glass culture tubes>
(15) Put solid standard in fluorometer with L facing left as you face the machine. (You will have to remove cuvette holder to put in solid standard.) Push * and take a reading & record in logbook. (It will read DLY for 7 seconds, AVE for 12 seconds, and then END for 5 seconds. Record the END reading quickly.) Then put solid standard in so H is facing left and do the same thing. (Do this at the beginning of each sample run. It helps determine if colored lenses are clouding & need to be cleaned. If numbers recorded start going down, need to take out and clean lenses with lens paper and acetone. Then can stick back in & continue to operate.)
(16) Prepare a blank by filling another culture tube with straight 90% acetone.
(17) Wipe blank tube with kimwipe and place the blank in the fluorometer and push "0" and then "1 - YES". Wait for it to read zero.
(18) Next, wipe sample tube with kimwipe and place sample in the fluorometer and push *. It will read DLY for 7 seconds, AVE for 12 seconds, and then END for 5 seconds. Record the END reading quickly – this is F_0. (Make sure reading in fsu.) NOTE: IF SAMPLE READS OFFSCALE DILUTE THE SAMPLE, WRITE DILUTION DOWN IN LOG SO YOU CAN TAKE INTO ACCOUNT IN YOUR CALCULATION AND RUN SAMPLE AGAIN. **DO NOT ADJUST THE FLUOROMETER (WILL MESS UP MEASUREMENTS)!!!**

(19) Now open the fluorometer, take out sample, carefully add 2 drops of 10% HCl into the tube, wipe with kimwipe and put back in fluorometer. DON'T DRIP ACID IN THE FLUOROMETER!
(20) Press * again and record this number as F_a.
(21) Take sample and dump into chlorophyll waste container.
(22) Do next sample.
(23) Run a blank every 10-15 samples (minutes).
(24) At the end of sample run record another set of solid standard readings.
(25) Use the Excel spreadsheet to calculate mg chlorophyll/m^3 (see attached equation - Strickland, J.D.H. and T.R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada.)

Calculation of Chlorophyll a Concentration:

Equations (Lorenzen, 1966):

$$Chl\ a\left(\frac{\mu g}{L}\ or\ \frac{mg}{m^3}\right) = \frac{\frac{F_0 / F_a\ \max}{F_0 / F_a\ \max - 1} \cdot K_x (F_0 - F_a) * V_{acetone}}{V_{filtered} * \text{Dilution Factor}}$$

F_o = reading before acid
F_a = reading after adding acid
$V_{acetone}$ = volume of acetone added (should be 10 ml)
$V_{filtered}$ = volume of sample filtered
K_x = slope of calibration line for your fluorometer
F_0/F_a max = calibration ratio for your fluorometer

Phaeopigment Concentration

$$\text{Phaeo}\left(\frac{\mu g}{L} \text{ or } \frac{mg}{m^3}\right) = \frac{\frac{F_0/F_a \text{ max}}{F_0/F_a \text{ max} - 1} \cdot Kx\left((F_0/F_a \text{ max})(F_a) - (F_0)\right)}{\text{volume of water filtered (in liters)}}$$

where: F_0/F_a max = the ratio of F_0 to F_a for a sample which contains only chlorophyll and no phaeopigments, around 1.8 to 2.2 (variability is due to different instruments)

K_x = calibration factor, to be determined for each fluorometer. Usually K_{1x} is determined, then

$$K_x = \frac{K_{1x}}{\text{door factor for scale used}}$$

This equation assumes you extracted in 10 mL acetone volume. If you used a different extract volume, you will have to adjust the phaeo concentration by a factor of (volume used (mL)/10 mL).

If the sample extract was diluted, you will have to adjust by the dilution factor. For instance, if you used 1 mL of extract to 4 mL of acetone for a total volume of 5 mL, this is called a dilution of 1 in 5. Phaeo concentrations are calculated as above and then the results are multiplied by the inverse of the dilution ratio, or, in this case, 5.