# PROCESSING CHLOROPHYLL SAMPLES – DIGITAL FLUOROMETER USE

## PREPARATION:

Put about 1.5" of room temperature Nano water in the square white styrofoam cooler
Take a batch of samples from the freezer, sit them in the racks inside the cooler to let the temperature equilibrate (approx. 30minutes), keep lid on cooler to keep samples in the dark
The fluorometer should be left on at all times, but if it has been turned off, let it warm up for 30 minutes prior to use
Ent = Main Menu, HOME = Data Display
Check Fluorometer settings:

| | |
|---|---|
| Pre-average delay (Ent, 1, 6, 3, 10 sec.) | 10seconds |
| Averaging period (Ent, 1, 6, 3, 15 sec.) | 15seconds |
| Blank subtract (Ent, 2, 1, 2, YES | YES |
| Autorange (Ent, 2, 4, 3, AUTO) | YES |

## BLANK:

If you forgot to prepare a filter blank during filtration, SHAME ON YOU! (preparing the blank during filtration is desirable because it ensures that the blank undergoes the same treatment as the sample)
You can make one now by placing a filter into a scintillation vial and adding 10ml of 90% acetone, shake until filter dissolves and sit in cooler with samples
Blank the fluorometer using a 90% acetone blank – fill a borosilicate glass tube with 90% acetone and cover tightly with parafilm
Wipe down the tube with a kimwipe to remove all fingerprints and smudges, place tube in sample chamber on fluorometer and cap
Blank as follows:
- from screen 2.0 (Calibration)
- press <1> to access screen 2.1 (Blanking) then
- press <1> to bring up 2.11 (Run Blank)
- insert 90% acetone blank
  - press <0>when reading stable and time count (TC) is stable at 8 seconds.
  - value of blank has now been entered
  - note %value in comments column on log sheet
  - fluorescence value of blank can be read on screen <3.2> (Diagnostic Info) or HOME screen.
  - obtain value for blank
    - access screen 2.12 (Subtract Blank)and choose NOT to subtract blank
    - go to HOME screen and press <*> to start average
    - record value of blank in the Rb column
    - return to 2.12 and choose YES to subtract blank

Place acetone blank in correct orientation (so that you can return it to the fluorometer facing the same way) in the cooler while running the samples – the blank will be re-read at the end of sampling

## FLUORESCENCE READINGS:

Decant sample from scintillation vial into glass tube being careful to leave any particulate matter in the vial
Wipe the tube with a kimwipe to ensure there are no fingerprints or smudges
Return the fluorometer display to the HOME screen
- insert sample
  - if >999 won't read, go to 3.2
  - when <999, press <*> to start pre-average delay and beep gives average
  - if 3.2 doesn't allow reading, need to dilute: 5ml sample, add 5ml 90% acetone
    - note dilution on log sheet – record volumes used for dilution
  - read fluor readout (Rb) and write <u>scale and reading</u> in log
  - add 2 drops 10% HCl
  - take Ra reading (approx. 0.5 of Rb), write <u>scale and reading</u> in log

Pour dregs into waste container, place scintillation vial and test tube in glass waste box, place vial cap in garbage.

## FINAL BLANK:

When all samples have been run, place the tube containing the acetone blank back into the fluorometer in its proper orientation and re-read the blank value as follows:
- access screen 2.12 and choose NOT to subtract blank
- go to HOME screen and press <*> to start sample averaging
- record value of blank in the Ra column

return to 2.12 and choose YES to subtract blank