**Method: Determination of chlorophyll a concentrations**

**1-Measure directly with the small blue Turner fluorometer (Calibration is done!)**

1. Calibration of the fluorometer
2. Switch on at least 20 min. before use
3. Calculate the concentration of chlorophyll a in the ethanol extract that was measured using a spectrofluorometer, as follows:

 $C\_{water}=\frac{C\_{extract}× v}{V}$

$C\_{water}$=concentration of chlorophyll a in water (µg l-1)

$C\_{extract}$=concentration of chlorophyll a in the extract (µg l-1) (measured in fluorometer)

$v$ = volume of ethanol (l)

$V$ = filtered volume of water (l)

Measure a blank with only ethanol (ask your instructor, or read the lazy-dog).

Place the extract in the fluorometer and set the standard value to the extract concentration. If the extract is too concentrate (value > 350), dilute the extract and start again. The best is to calibrate the fluorometer with an extract of similar concentration range as your samples.

**2- Fluorometric determination of chlorophyll a**

* Filter onto glassfiber filters (make sure that you are in a low light environment, and that you note the EXACT volume you filter- use a graduated pipette)
* Filter carefully a known volume (10-50 ml) with low vacuum (< 0.3 bar)
* Let the filter dry
* Insert filter in labeled glass tubes (pencil and paper tape…)
* Add 4 ml Ethanol
* Add caps and store in dark (room temp) for extraction (6-8h)
* Fluorometer ON (20-30 min before measurements)
* Use the appropriate calibration program (see with your instructor)
* Use the round vial and appropriate holder (ask instructor)
* Measure a blank sample with only ethanol (ask your instructor, or read the lazy-dog).
* Transfer extract into the 2 ml glass vial
* Dry carefully with paper
* Measure the fluorescence/ chlorophyll a concentration in the extract (important to remember while calculating the chla concentrations in the cultures)

Volume extract = 4 ml

Volume filtered = X ml

Concentration in extract = known chla µg l-1

Calculate concentration in culture.