# Turner Digital 10-AU Fluorometer Calibration

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## Equivalents:

- $mg/m^3$ = µg/L = ppb
- µg/mL = mg/L
- as Span % decreases, FS% increases
- Temp coeff. for Chl*a* = 0.3% / ºC

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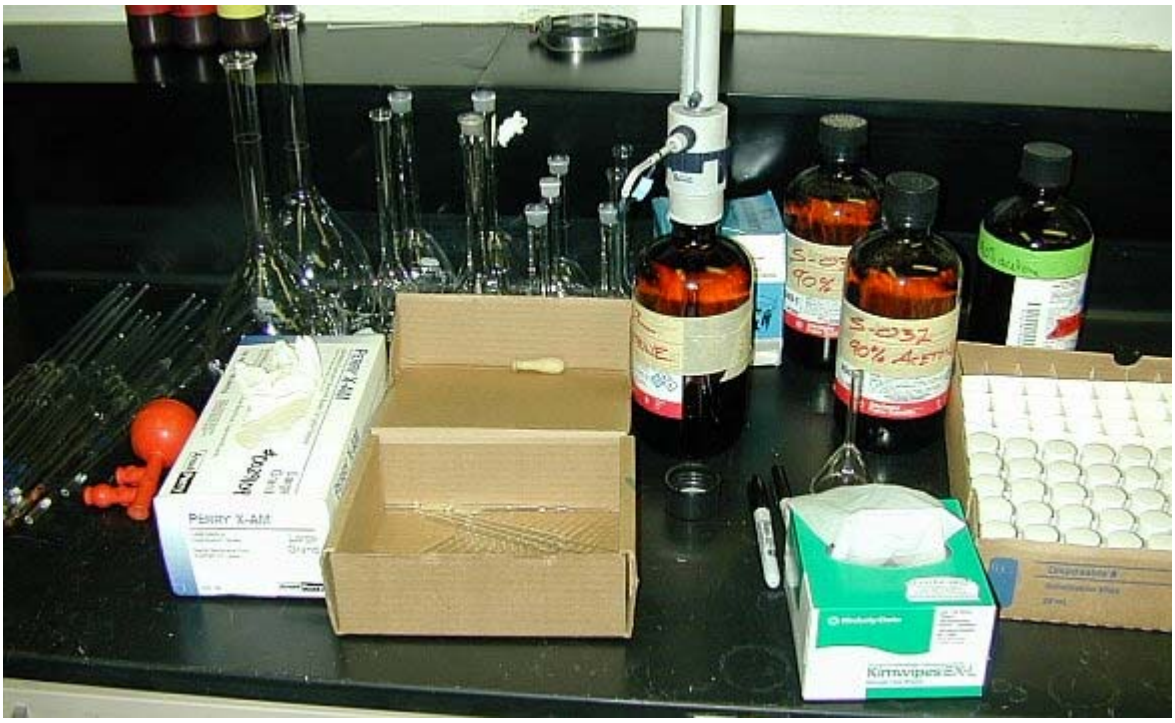
## Stuff Needed

- volumetric pipettes (glass ONLY)
  - 0.5 mL
  - 1 mL
  - 2 ml
  - 5 mL
  - 10 mL
- volumetric flasks (glass ONLY)
  - (3) 200 mL
  - (1) 250 mL
- Glass 100 mL beaker (for holding 90% acetone rinse solution)
- Glass funnel (for filling volumetric flasks)
- Glass 1L beaker (for adding bulk 90% acetone to volumetric flasks)
- Glass 50 mL beaker (for filling volumetric flasks to the line)
- pipette bulbs (lg & sm)
- roll parafilm
- scintillation vials w/ PE tops to freeze remaining standard and dilutions
- disposable glass pasture pipettes
- glass culture (test) tubes
- test tube rack
- at least 4L 90% acetone (use only NANOpure water to dilute from 100%)

- dropper bottle with 10% HCl solution
- waste container
- Sharpie marker
- latex gloves (optnl)
- small box of Kimwipes
- this instruction sheet (chl_calib_howto.html)
- calibration worksheet (_chl_calib_worksheet.xls)
- sample reading howto (chl_read_howto.html)
- spectrophotometer worksheet (chl_spec_protocol.doc)
- Turner Digital 10-AU fluorometer (s/n 5153 and any others to be calibrated)

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## Firstly...

- Now would be a good time to turn on the spectrophotometer and the fluorometer. *Each should warm up at least 30 minutes prior to use*.
- **You must prevent Chl *a* breakdown:**
  - Work quickly, but precisely.
  - Keep all Chl *a* solutions limited to light exposure (wrap standard in aluminum foil) and capped (90% acetone is very volatile!).
  - However, all equipment, instruments, and solutions MUST be at room temperature.
  - If a solution/dilution will not be used within an hour or so, it should be placed in a refrigerator and removed approx. 30 min prior to usage.
- Glassware:
  - Be sure to use **ONLY** clean, dry glassware.
  - Do not even think of using plastic tipped pipettes (GLASSWARE ONLY).
  - Use ONLY non-disposable **volumetric** glassware for measurements.
  - If you must reuse glassware on a different solution, ensure it is *at least* triple rinsed with DI water then triple rinsed with 90% acetone first.
  - Use the smallest pipette possible for each dilution.

    - All cuvettes and test tubes need to have the outsides gently wiped down with a Kimwipe prior to insertion into the spectrophotometer or fluorometer chambers.
- Fluorometer calibration should be done at the beginning AND end of the season, and any time you suspect it has grossly deviated from the last calibration (this is checked via the solid standards frozen after each batch analyzed).
- As your samples are being run, enter them into the calibration worksheet. All values will be calculated for you.
- For instructions on making up 90% Acetone or 10% HCl, see chl_sampl_howto.html - Preparing Reagents Section.
- All liquid wastes (even rinses) goes into the waste container (not the drain).

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## Making up the Standard Stock Solution (SSS)

1. Take 1 mg ampoule of Sigma Chl*a* (*Anacystis nidulans*) and, with the lights out, gently snap the top off of the standard vial.
2. Invert vial into the 100ml volumetric flask and tap gently to get the Chl *a* crystals into the flask.
3. Using one of your glass pipettes, fill the standard vial halfway up with 90% acetone, taking care to rinse down the sides of the vial in the process but try not to contaminate the pipette with sample.
4. Using a separate pipette, remove the acetone in the standard vial and put it into the volumetric flask. Repeat the last two steps approximately 5 times or until you are satisfied that allof the standard has been transferred to the Volumetric flask. Do the same process with the snap-off top from the standard vial.
5. Once all of the standard has been transferred to the volumetric flask, fill it the rest of the way up with 90% acetone until the bottom of the meniscus touches the line on the neck of the flask. br>*The exact amount of Chla or acetone is not important, as concentration will be determined below, but this will give a solution of ~10 mg/L.*
6. Stopper the flask and cover it in aluminum foil to minimize light exposure.
7. Record the lot number of *Anacystis nidulans* used on the calibration worksheet.
8. After making up the SSS, hold at least 1 full scint vial (~25 mL) aside for making the dilutions, and put what is left over into scint vials and place in -70 C freezer for future use.

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## Spectrophotometric determination of the SSS concentration:

see spectrophotometer worksheet (chl_spec_protocol.doc)

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## Making Dilutions

- It is *extremely* helpful to have this part done with at least two people.
- Now that we know the concentration of the SSS, we need a series of dilutions (use 90% acetone to dilute):
  (*see also* 'chl_calib_worksheet.xls' for example dilutions)

**Typical Dilutions (given a SSS of ~10 mg/L):**

| Solution used | Total Dilution Factor | mL in mL* |
|---|---|---|

| | | |
|---|---|---|
| **SSS** | **100** | **2 in 200** |
| **SSS** | **133.3** | **1.5 in 200** |
| **SSS** | **200** | **1 in 200** |
| **SSS** | **400** | **0.5 in 200** |
| **SSS** | **625** | **0.4 in 250** |
| **SSS diluted 1:100** | **1000** | **20 in 200** |
| **SSS diluted 1:100** | **2,000** | **10 in 200** |
| **SSS diluted 1:100** | **4000** | **5 in 200** |
| **SSS diluted 1:625** | **6,250** | **20 in 200** |
| **SSS diluted 1:625** | **12,500** | **10 in 200** |
| **SSS diluted 1:625** | **25,000** | **5 in 200** |
| **SSS diluted 1:625** | **62,500** | **2 in 200** |
| **SSS diluted 1:625** | **125,000** | **1 in 200** |
| **SSS diluted 1:625** | **312,500** | **0.4 in 200** |

*'mL in mL' = the first number is the amount of the 'Solution used' placed (via volumetric pipette) into a volumetric flask, then filled to its volume line with 90% acetone.

- If you must reuse glassware on a different solution, ensure it is *at least* triple rinsed with DI water and triple rinsed with 90% acetone first.
- Ensure the volumetric flasks are **well mixed** before placing the dilutions in well capped & labeled (dilution factor, date & initials) scintillation vials - for each dilution; leave 2 full vials out for running on each fluorometer, and place the remaining full vials into the -70 C freezer for potential later use.

The ranges (after final sensitivity adjustments) will be as follows:
low: 0-2 µg/L
med: 2-20 µg/L
high: 20-200 µg/L

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## FLUOROMETER CALIBRATING

### Setting Course Sensitivity

If the course sensitivity of the 10-AU has already been adjusted it will not be necessary to readjust the course sensitivity unless you are changing cuvette size, lamps or filters. This is what they used to think! We believe that unless you've actually had the fluorometer that you are using in your hot little hands since the initial course adjustment setting, you may have to reset it!

On the fluorometer, screen numbers refer to the numbers you'd punch on the keypad to access that screen (e.g. to access screen 2.42, starting from HOME, press 'ENT', then the numbers 2, 4, 2).

1. Go to screen **2.12** and set subtract blank to NO.
2. Go to screen **2.43** and set the concentration range control to MAN.

3. Go to screen **2.42**, set the concentration range to MED.
4. Go to screen **2.6**, reset the calibration setting to their default values.
5. Go to screen **3.2**.
6. Insert a test tube of the 1:1000 SSS dilution (i.e. ~10 µg/L).
7. **If necessary**, adjust the sensitivity adjustment knob (first unlocking with an Allen wrench) with a screwdriver until the percentage of the full scale on the MED range is 25%. It should read "FS: 25% of 90.000"
   *What this does is set our standard of ~10.0 µg/L at 25% of the medium range. This sets the maximum concentration that can be read on the MED range to about 40.0 (10.0 / 0.25). In doing this, we set the ranges as follows (in terms of µg/L of solution):*
8.
9. 10.0 µg/L=25%
10. ^
11. |_____|____|_____|_____|
12. 0 4.0 40.0 400.0(µg/L)
13. low medium high
14. Ensure to re-lock the sensitivity adjustment lock with the Allen wrench, turning it clockwise.

**Running SSS**

1. Go to screen **2.2**, enter 90.0 for the concentration of your standard.
   *This is done to give us more readable decimal places when analyzing samples much more dilute than the standard. (Don't confuse it with the default value for FS MED, which also happens to be 90.0).*
2. Run Blank
   a. Go to screen **2.11**
   b. Insert your blank (90% acetone solution)
   c. Press <0> when the reading is stable
   d. Note the percent value of the blank.
      *The value of the blank has now been entered. The fluorescence signal for the blank is stored as 'blank' and can be accessed on screen **3.2**. (This is not the same as the fluorescence readout, which is what you will be recording when you run samples).*
3. Go to screen **2.12**, choose YES to 'subtract blank?'
4. Go to screen **2.3**.
5. Insert the test tube of the 1:1000 SSS dilution (i.e. ~10 µg/L).
   This is the FULL SCALE (FS) value at that range. The FS value represents the maximum concentration (in arbitrary units) that can be read on that range. You will notice that the FS values for all ranges differ by about a factor of ten.
6. Write down the FS value for the MED scale.
7. Increase the SPAN (sensitivity) by pressing the up or down arrows on the keyboard until the FS value is approximately one-half the value from the previous step.
8. When the reading is stable, press <*>.
   *The fluorescence signal for the standard is stored as 'Cal. St. Val.' and can be accessed via screen 3.2.*

   The reason for increasing the sensitivity to 50% of FS using the SPAN instead of doing it during the course calibration with the sensitivity adjustment knob is that the sensitivity adjustment knob increases the voltage to the PMT, which will increase the noise to the system in a nonlinear fashion. The SPAN, on the other hand, increases the amplification of the signal after it comes from the PMT so there is no relative increase in noise. The dilution for the standard was chosen to give the best range of full scale values for the chlorophyll concentrations expected in the field.
   In this case we end up with:

9.
10. 10.0µg/L=50%
11. ^
12. |_____|____| SPAN|_____|_____|
13. 0 2.0 20.0 200.0(µg/L)
14. low medium high

15. Go to screen **3.2**, the %FS of MED should be ~50%.
    - If it isn't, either your standard is not correct, the course sensitivity is off, or you did something wrong in this section. You can check the course sensitivity and your standard by setting the instrument settings to default (screen 2.6) and accessing screen 3.2. Insert your standard of 10.0 µg/L. If the sensitivity has been adjusted correctly and your sample is indeed 10.0 µg/L then the %FS MED should read 25%. Make sure you are on the MED range! If the %FS is nowhere near 25%, then either your standard is nothing close to 10.0 µg/L or the course sensitivity will have to be readjusted.

1. On the bottom right of the worksheet , be sure to record the fluorometer values.

**Running the Dilution Series through the fluorometer:**

1. Start with the most concentrated working to most dilute.
2. Follow all directions in chl_read_howto.html, with each dilution being run in *at least* duplicate.
3. Record dilution values on calibration worksheet (chl_calib_worksheet.xls), and calculations will be made automatically.
   The 'tau' and 'door factor' values will be transferred to the season's chlorophyll logsheet to properly calculate pigment concentrations based on the fluorometer values.

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