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Protocol status: Working
 We use this protocol in our group and it is working.

🌐 Calibrating 10-AU fluorometer to quantify extracted chl-a

Shelby Whitehead¹, Samantha R Coy¹,
 Steven W Wilhelm¹

¹The University of Tennessee, Knoxville

Great Lakes Center for Fresh Waters and Human Health
 Tech. support email: wilhelm@utk.edu

Shelby Whitehead

ABSTRACT

This method is for use for calibrating the 10-AU fluorometer using chl-a extracts. The method also provides guidelines for extracting chl-a and calculating absorbance using a spectrophotometer, making dilutions of extracts that can be used to create different chl-a concentrations

ATTACHMENTS

[Shelby's Notes for Fluorometer.pdf](#)

[Sam's Notes for Fluorometer.pdf](#)

MATERIALS

97% acetone
 spinach or green plant as source for chlorophyll extracts

SAFETY WARNINGS

97% acetone is flammable.

See MSDS safety and hazard warnings.

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BEFORE START INSTRUCTIONS

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fluorometer,
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chlorophyll a, calibrate

- *Fixed Application* can be found under "general tests" on the spectrophotometer.
- Make sure the cuvette is in the correct position for measurements. Misplacement may cause an error such as a negative absorbance measurement.
- Spinach only has chlorophyll a. If using an organism that has more pigments, will have to subtract those from total absorbance (i.e. chlorophyll a - chlorophyll c).

Chlorophyll Extraction

- 1 Extract chlorophyll from 1 g of ground spinach with 5 mL of 90% acetone at -20 °C overnight.

Spectrophotometry

- 2 Use the Biomate 5 spectrophotometer to measure the adsorption of extracted chlorophyll by adding 1 mL to a quartz cuvette.
 - Make sure the cuvette is in the correct position in the machine.
 - Zero the machine by blanking with 1 mL of 90% acetone.
 - Measure "multi λ " with $\lambda_1 = 664 \text{ nm}$ and $\lambda_2 = 647 \text{ nm}$. (Jeffrey 1975)
- 3 Based on the absorbance value from go to step #2 : dilute the chlorophyll extract so that the absorbance is in optimum range for the fluorometer. Optimum range is Abs λ_1 and $\lambda_2 = 0.3\text{--}0.85$.

Example:
Undiluted
Abs $\lambda_1 = 1.88$
Abs $\lambda_2 = 1.324$

20-fold dilution:

Abs λ_1 = 0.736

Abs λ_2 = 0.479

Fluorometry

- 4 Calculate the concentration of diluted chlorophyll extract using the absorbance values from the spectrophotometer and the equation:

$$\text{concentration of chlorophyll a} = 11.93(\lambda_1) - 1.93(\lambda_2)$$

(Jeffrey 1975)

Note

If using an organism that has more pigments, will have to subtract those from total absorbance (i.e. chlorophyll a - chlorophyll c). The spinach we used did not require subtraction of chlorophyll c.

Example:

$$11.93(0.736) - 1.93(0.479) = 7.85601 \text{ ug/ml}$$

Calibrate Fluorometer

- 5 Convert answer from [go to step #4](#) to ug/L, because this is the readout for the fluorometer.
- 6 Turn on the 10-AU Fluorometer and allow the instrument to warm up for at least 30 minutes.
- 7 Create solutions of varying concentrations to test the fluorometer readings. Use $C_1V_1 = C_2V_2$ to make solutions at different concentrations based on expected field concentrations, for example: 20, 10, 5, 1 and 0.5 ug/L. Also, create an acetone blank (with no chlorophyll).

Note

For example:

7,856 ug/L (C1)

For a 5 ug/L concentration (C2) in 7 mls acetone (V2)

$$(7,856 \text{ ug/L})(V1) = (5 \text{ ug/L})(7 \text{ ml})$$

$$V1 = 4.45519 \text{ ug}$$

- 8 Use the concentration most expected in the field to set as the solid standard.

Chlorophyll (ug/l)	Rhodamine WT (ppb)	Range	% FS (+/-5)
240 - 180	100 - 75	High	80%
180 - 160	75 - 65	High	70%
160 - 130	65 - 55	High	60%
130 - 110	55 - 45	High	50%
110 - 80	45 - 35	High	40%
80 - 60	35 - 25	High	30%
60 - 40	25 - 15	High	20%
24 - 18	10 - 7.5	Medium	80%
18 - 16	7.5 - 6.5	Medium	70%
16 - 13	6.5 - 5.5	Medium	60%
13 - 11	5.5 - 4.5	Medium	50%
11 - 8.0	4.5 - 3.5	Medium	40%
8.0 - 6.0	3.5 - 2.5	Medium	30%
6.0 - 4.0	2.5 - 1.5	Medium	20%
2.4 - 1.8	1.0 - 0.75	Low	80%
1.8 - 1.6	0.75 - 0.65	Low	70%
1.6 - 1.3	0.65 - 0.55	Low	60%
1.3 - 1.1	0.55 - 0.45	Low	50%
1.1 - 0.8	0.45 - 0.35	Low	40%
0.8 - 0.6	0.35 - 0.25	Low	30%
0.6 - 0.4	0.25 - 0.15	Low	20%

Table 1 is an example of how to set the scale for a given linear range of the 10-AU.

For this example we are using a 20µg/L standard.

Table 1 is an example of how to set the sensitivity scale for the 10-AU. Use the table to determine the appropriate range and %FS for the calibration standard.

- 9 Clear past calibrations on screen #2.6.

- 10 Access screen #2.43 and set range control to "manual" and range to the appropriate level for the standard chosen. For example, we set range control to "Man" and "Med."
- 11 Access screen #3.2. Loosen the sensitivity locking screw using an allen wrench. (The sensitivity lock is the small screw to the left of the number pad.)
- 12 Insert and cover your selected standard concentration. Use a coin or tool to slowly turn the sensitivity knob (located to the right of the number pad, under the power button) to adjust the %FS to the value appropriate for the standard concentration according to Table 1 in [⇒ go to step #8](#). A deviation of 5% less than or greater than the table is acceptable. When %FS reaches the designated value, tighten the sensitivity lock.

At this point, sensitivity is set.

Note

Example:
For 5 ug/L.
FS = 21%

- 13 Access screen #2.43 again, and set range control to "auto."
- 14 Access screen #2.1. Insert and cover the acetone blank created in [⇒ go to step #7](#). Press "1" to Run Blank. Wait for the reading to stabilize for about 8 seconds, and then press "0." The instrument will take 15 seconds to complete blanking.

At this point, blank is set.
- 15 Calibrate with your standard:

- 15.1** Access screen #2.2 and input the actual concentration of your standard. Return to screen #2.0
- 15.2** Insert and cover the standard.
- 15.3** Run standard by pressing "3." Wait about 8 seconds for reading to stabilize.
- 15.4** Press the "*" to set the calibration point. After about 15 seconds, a message will appear that says "Finished".
- 16** Test the 10-AU fluorometer using the concentrations created in **⇒ go to step #7** to assure appropriate readout.

Note

Example:

Expected	Observed
5 ug/L	5.01 ug/L
1 ug/L	1.11 ug/L
Blank	0.0 ug/L