

An *In-vivo* Chlorophyll Procedure for the 10-AU Field Fluorometer



The 10-AU Field Fluorometer can be set up for mapping or monitoring of chlorophyll on site in the field. Please keep in mind that fluorescence is a relative measurement. The fluorescence intensity is directly proportional to the concentration of the sample. But the **actual concentration** of the sample in $\mu\text{g/L}$ or ppb must be determined by an accepted laboratory method such as extractive analysis using a fluorometer (U.S.E.P.A. Method 445.0) or spectrophotometer.

Note also that the number of discrete sample comparisons that are run should be determined by the level of accuracy your study requires. If you are simply mapping an area for high or low concentrations of chlorophyll, fewer discrete sample comparisons are required. If, however, you are interested in actual concentrations and you must be precise, more discrete samples are required. This is true because there are many factors that affect the fluorescence intensity of chlorophyll: temperature, species, diurnal cycles, nutrients, etc. Refer to Appendix 6A in the 10-AU operating manual for a discussion of temperature, linearity, etc.

Please note that the extracted chlorophyll standards and the purified chlorophyll *a* standards cannot be used as direct standards *in vivo*. With *in vivo* measurements you are measuring chlorophyll inside the cells mixed in water. Extracted chlorophyll (outside of the cells) does not fluoresce at the same rate— whether in acetone or water.

When using the fluorometer for *in vivo* analysis, it is not necessary to actually “calibrate” the instrument. What is required is a basic adjustment of the operating level of the instrument so that sensitivity is adequate for your study. After you set the basic operating level, you may, however, calibrate if you like; i.e., run a blank and a standard as described in Section 3 of the operation manual. But the standard concentration

is only an “estimate” until an actual extraction has been made of the sample water used for the Run Standard phase (on Screen 2.3 of the 10-AU).

Fluorometer set up:

- 25 mm Continuous Flow Cuvette System
- 10-037R *In-vivo* Chlorophyll Optical Kit or in cases where high concentrations of dissolved organic matter or chlorophyll *b* are present, the 10-096R Chlorophyll *a* Optical Kit.

Procedure:

See the operating manual or the Model 10-AU Quick Start Instructions for locations of various controls or for more details on the fluorometer software screens and calibration.

1. Turn on the fluorometer and allow it to warm up for at least 10 minutes.
2. Access screen 2.43 and set the concentration range control to MAN.
3. On screen 2.42, set the instrument to the MED concentration range.
4. On screen 2.6, reset the calibration values to their default position. I.e., Blank will be zero; Cal std val will be 50.000; Standard solution concentration will be 15.000; Span will be 48%; and maximum for the Full Scale Value Table will be 900 for High, 90 for MED, and 9 for Low.
5. Access screen 3.2.
6. Unlock the Sensitivity Adjustment Lock with an Allen wrench.
7. Start sample water flowing through the fluorometer. It is recommended that the process be started as close as possible to the location where you will do the primary mapping. For example, levels of chlorophyll close to shore can be much higher than in the open ocean; if sensitivity is set close to

shore, readings may not be optimum at low levels of chlorophyll.

8. Adjust the Sensitivity Adjustment Knob until the FS% (third line from the bottom) reads approximately 30-80%; i.e., "FS%: 45% of 90.000 units at MED." It is not necessary to be exact; you are simply after an adequate sensitivity level.
 - a) Turning the Sensitivity Knob clockwise increases sensitivity; turning it counterclockwise decreases sensitivity.
 - b) The Sensitivity Adjustment Knob is very responsive and even a small adjustment can cause a great change in readings. Thus, the proper way to adjust using the Sensitivity Adjustment Knob is to turn it very slightly (using a coin), then pause until the reading reaches equilibrium, then adjust, pause, etc., until the desired reading is obtained.
 - c) Setting the Time Constant to 1 or 2 seconds on screen 2.5 will provide a faster response time. There is a clutch mechanism that prevents damage if the control is turned beyond its maximum and minimum ranges. When you have reached the maximum or minimum, the FS% will not change, even though you turn the control.
 - d) If the FS% is less than 10% and barely changes no matter which way you adjust the Sensitivity Knob, first make sure the Sensitivity Adjustment Lock is unlocked. If it is and sensitivity is still less than 10%, go to screen 2.42 and change to the LOW range. Return to screen 3.2 and adjust the Knob until the FS% reads 30-80% of LOW.
 - e) If you cannot obtain adequate sensitivity in the LOW range, refer to Appendix 6A, for methods for further increasing sensitivity.
 - f) If the FS% on the MED range reads greater than 100% and will not go below 90% with the Sensitivity Knob fully counter-clockwise, first make sure the Sensitivity Adjustment Lock

is unlocked. If it is and sensitivity is still greater than 90%, go to screen 2.42 and change to the HIGH range. Return to screen 3.2 and adjust the Knob until the FS% reads 30-80% of HIGH. If FS% on HIGH is still too high, see Appendix 6A for ways of further decreasing sensitivity.

9. When the desired reading is obtained, LOCK the Sensitivity Adjustment Lock by turning it clockwise with the Allen wrench.
10. Press <HOME> to return to the HOME screen.
11. While sample water is flowing through the fluorometer, take a grab sample for later laboratory analysis and note the reading of the sample on the HOME screen.
12. Carry out your study by logging readings of sample water at various sites. Take grab samples and note the reading on the fluorometer as often as necessary for the level of accuracy required by your study.
13. After laboratory analysis of the grab samples, plot readings of samples against the fluorometer readings and calculate the actual concentration of chlorophyll a in your sample waters.

For example:

When sample water is running through the fluorometer, the HOME screen reads "100". Later, the laboratory reports the actual concentration of chlorophyll a in the sample water is 25 µg/L. An unknown sample of water read "88" on the fluorometer.

Calculation of unknowns is a simple ratio:

$$25/100 = X/88 \quad \text{or} \quad X = 22 \mu\text{g/L}$$

