CYCLOPS-7 Calibration Procedure
For Making *In Vivo* Chlorophyll *a* Measurements

Introduction:
CYCLOPS-7 has an analog output that delivers a voltage proportional to the sample concentration being measured. This procedure will describe how to make calibrated readings with the CYCLOPS-7 by converting its output voltage readings to an approximation of chlorophyll concentration readings in µg/L. For completeness, the procedure includes how to use the Solid Secondary Standard.

After completing this calibration procedure for making *in vivo* chlorophyll measurements, the user can easily and accurately convert the CYCLOPS-7 analog output voltage to provide an approximation of sample concentration data in units of µg/L.

(This procedure assumes that the user has already gone though the first time operation procedure in the CYCLOPS-7 User's Manual).

> **Note:** It is important to follow good measurement procedures when calibrating and using CYCLOPS-7. See Appendix A for a summary of Recommended Measurement Practices.

Equipment Required:
This list is for the *in vivo* measurement procedure only. (Additional equipment is required for the chlorophyll *a* extraction procedure).

a) CYCLOPS-7 submersible fluorometer configured for chlorophyll. (Check the letter "C" is stamped on the CYCLOPS-7 connector).
b) Solid Secondary Standard (PN 2100-900)
c) Pig tail cable and connector, (supplied with CYCLOPS-7)
d) DC Power Supply, (Output voltage range 3 – 15 VDC)
e) Multimeter to read 0 – 5 VDC
f) De-ionized water
g) Sample of water to be measured. To obtain optimum calibration data, the water sample concentration should be in the range of 1 –100 µg/L. (Objective is not to use sample concentrations that are at either extreme of the measurement range to be used).

Procedure:

A. Measure the blanking voltage for each of the 3 gain ranges

1. Connect the CYCLOPS-7 fluorometer to the Power Supply and Digital Multimeter as shown in Fig 1.
2. Immerse the optical end of the fluorometer in a beaker of de-ionized water, (see Appendix A), and measure the output voltage of the sensor (equal to the blank voltage) for each of the gain ranges. Note the readings in Table 1 as indicated.

(See next page)

Table 1

Gain Range	Blank Sample Voltage	Sample (1) Voltage	Sample (2) Voltage	Sample (1) Concentration by Extraction	Sample (2) Concentration by Extraction
X1	*Reading 1*				
X10	*Reading 2*				
X100	*Reading 3*				

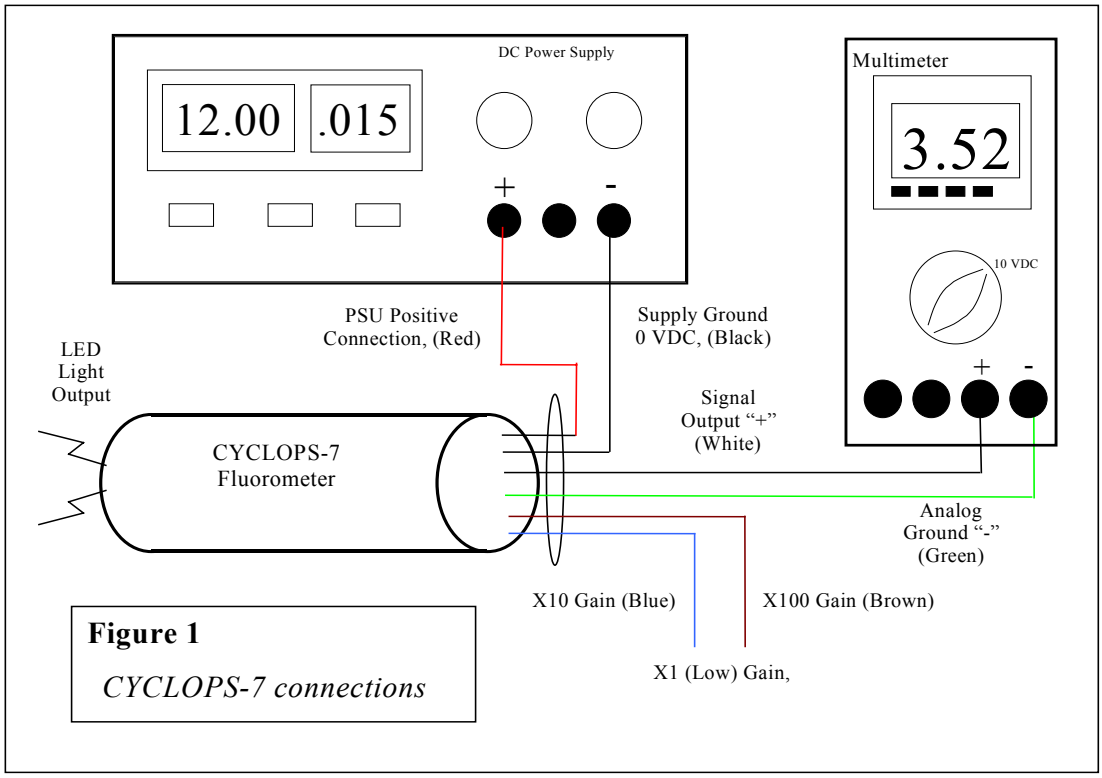


Figure 1

CYCLOPS-7 connections

B. Measure the Output Voltage for the Sample on each of the Gain ranges

1. Set the sensor to the X1 Gain Range, (both gain setting wires disconnected)
2. Immerse the optical end of the fluorometer in a beaker containing the sample of water to be measured and note the output voltage.
3. Repeat steps 1 and 2 for the X10 and X100 gain settings. Note the readings in Table 1 in the Sample (1) Voltage column.
4. Determine the range to be used (calibrated) such that the sample concentration level produces an output voltage that provides the best possible measurement range. Select the range that results in an output voltage greater than 0.5V. Typically, if the concentration level can increase/decrease equally, the ideal would be to choose the range that puts the sample as close to possible to producing an output voltage of 2.5V.
5. For a 2-Point (or more) calibration, use a second sample taken from a different part of the same body of water. Repeat (2) and (3) for the second sample, using the Sample (2) Voltage column.

C. Do an extraction to determine the sample concentration

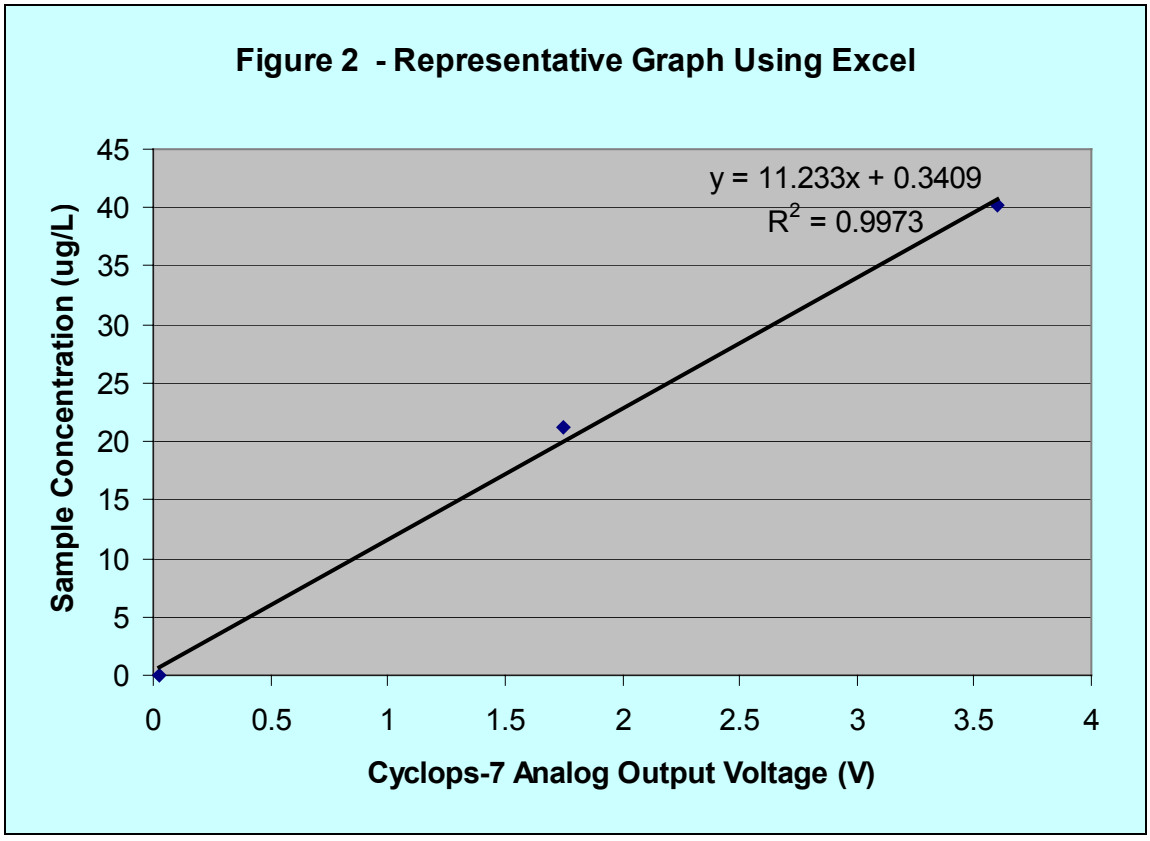
1. Procedures for doing this are available on the Turner Designs web site. An example is **"A Procedure For Measuring Extracted Chlorophyll a Free From The Errors Associated With Chlorophyll b And Pheopigments** (Without Acidification - Using 13mm Test Tubes). (The procedure uses EPA Method 445). See the following URL:

   http://www.turnerdesigns.com/t2/doc/appnotes/998_9000.html

2. From the Extraction Result(s), insert the sample concentration levels obtained in Table 1.

D. Construct the straight-line graph for the optimum gain range for the sample concentration.

A spreadsheet program such as Microsoft Excel is a convenient way to generate a graph as shown below. The graph below was created using the Excel "XY Scatter" chart type, then right clicking on a data point, and selecting "Add a Trendline" to generate the best fit line in the chart below. (Excel calculates the R^2 value, and the equation for the best-fit line).



E. Using the "Trend Line" to calculate the concentration of samples under test.

1. Assume the sample was measured on the X10 Gain Range, and that the measured values were as shown in Table 2:

(See next page)

Table 2

Gain Range	Blank Sample Voltage	Sample (1) Voltage	Sample (2) Voltage	Sample (1) Concentration by Extraction	Sample (2) Concentration by Extraction
X1					
X10	25 mV	3.6V	1.75V	40.2 µg/L	21.2 µg/L
X100					

2. From the Excel spreadsheet in Fig 2, the equation for the response of CYCLOPS-7 is:

   $y = 11.233x + 0.3409$, (Equation (1)

   (where y = sample concentration, and x = sensor output voltage).

3. To calculate the concentration level of subsequent samples, in µg/L, insert the corresponding output voltage for the sample under test in equation (1). For example, if the output voltage = 1.25V, then the sample under test concentration equals:

   y (µg/L) = (11.233 x 1.25) + 0.3409 = 14.38 µg/L

F. Setting the Solid Secondary Standard for *in vivo* measurements

1. Dry off the optical end of the CYCLOPS-7 sensor, and attach the Solid Secondary Standard. Adjust the setting screw so that the output voltage is equal to one of the output voltages in Table 2.
2. The Secondary Standard is now set to simulate a concentration level equivalent to the output voltage used. For example, if the Secondary Standard is set to produce an output voltage of 3.6V, then it is equivalent to a concentration level of 40.2 µg/L
3. The Secondary Standard can now be used to check/calibrate the readings from the graph at a subsequent date.

Other Factors To Take Into Consideration

There are several other factors which can impact the calibration and measurement accuracy of *in vivo* chlorophyll *a* results. Examples of these variations are:

1. Variation between sites
2. Seasonal changes, (summer, winter, etc)
3. Water Quality, (such as effect of high turbidity and dissolved organic matter)
4. Physiological state of the algal cells, ("health" of the cells).

For more information on how these factor affect the calibration and use of the CYCOPS-7 sensor, please refer to the following web site:

http://www.turnerdesigns.com/t2/esupport/understanding.html

Appendix A

Recommended Measurement Practices For Using your CYCLOPS-7 Fluorometer in the Lab

The following steps will improve the accuracy and repeatability of your measurements, especially at low concentration levels:

1. Use a **Glass Beaker** for your water samples. (Avoid plastic beakers – plastic fluoresces, and will interfere with the sample fluorescence)
2. Place the glass beaker on a **Non-Reflective Surface**, preferably black.
3. Ensure that the sensor is **more than 3 inches above the bottom** of the glass beaker.
4. Ensure that the sensor is in the center of the glass beaker, and has **more than 2 inches clearance** between the cirumference of the sensor and the inside surface of the beaker. Turner Designs recommends using a 1L Glass Beaker for measurements with the CYCLOPS-7 fluorometers.
5. Check that the optical surface of the sensor is **free of air bubbles**.
6. Be sure your **sensor is calibrated**, (see User's Manual for Calibration Procedure).
7. To maximize consistency between measurements, place sensor **at exactly the same height** for each sample. This is most easily done using a Lab Stand.

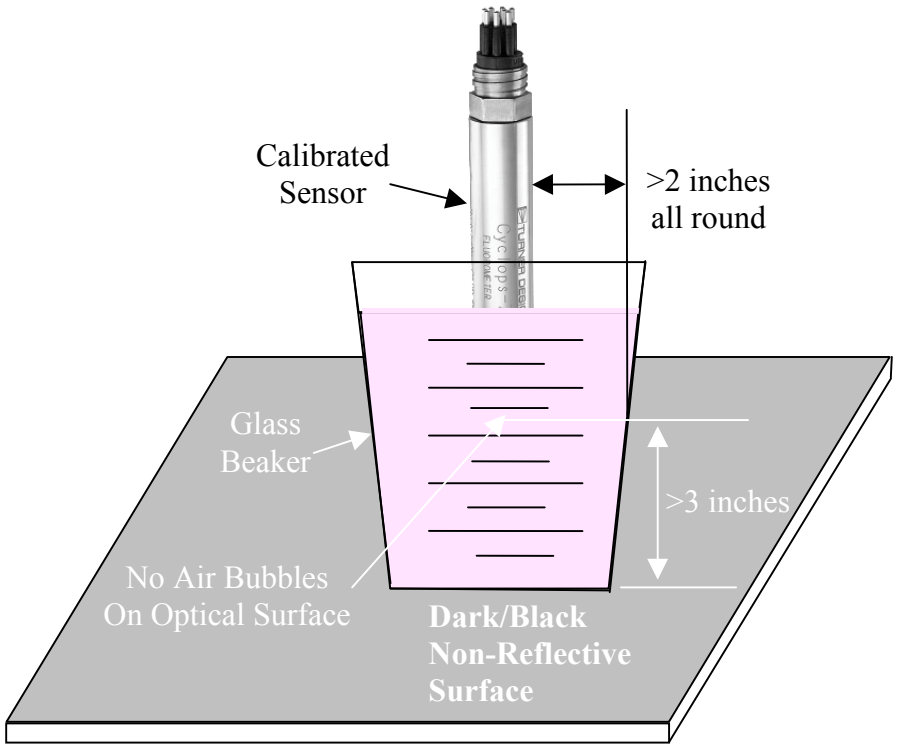


Figure 3