

Introduction

Cyclops sensors provide an analog output voltage proportional to the sample concentration being measured. This procedure describes how to calibrate Cyclops sensors to approximate chlorophyll $\mu\text{g/L}$ concentrations from Cyclops voltage measurements. For completeness, the procedure includes how to use the Solid Secondary Standard.

After completing this calibration procedure, users can easily convert Cyclops analog output voltage to direct sample concentration estimates in $\mu\text{g/L}$.

Note: *This procedure assumes that the user has already gone through the first time operation procedure in the Cyclops User's Manual and/or the Cyclops Quick Start Guide.*

Note: *It is important to follow good measurement procedures when calibrating and using Cyclops sensors. See Recommended Lab Practices for Measurements (page 5).*

Equipment Required

Only pertains to Chlorophyll *in vivo* measurement procedure. **Note:** *Additional equipment is required for the chlorophyll a extraction procedure.*

- a) Cyclops submersible fluorometer configured for chlorophyll. **Note:** *This sensor will have a letter "C" stamped on the Cyclops' connector.* (Stainless Steel or Titanium or Plastic or 6000 Meter)
- b) Solid Secondary Standard (PN 2100-900 or 2100-908 or 2160-900)
- c) Pigtail cable (PN 2100-750 or 2100-755 or 2100-751 or 2100-752 or 2100-753)
- d) DC Power Supply capable of supplying 3 – 15 VDC
- e) Multimeter capable of reading 0 – 5 VDC
- f) De-ionized water or Artificial Sea Water to be used as a Blank Sample
- g) Sample of water to be measured. To obtain optimum calibration data, the water sample concentration should be in the range of 1 – 100 $\mu\text{g/L}$.

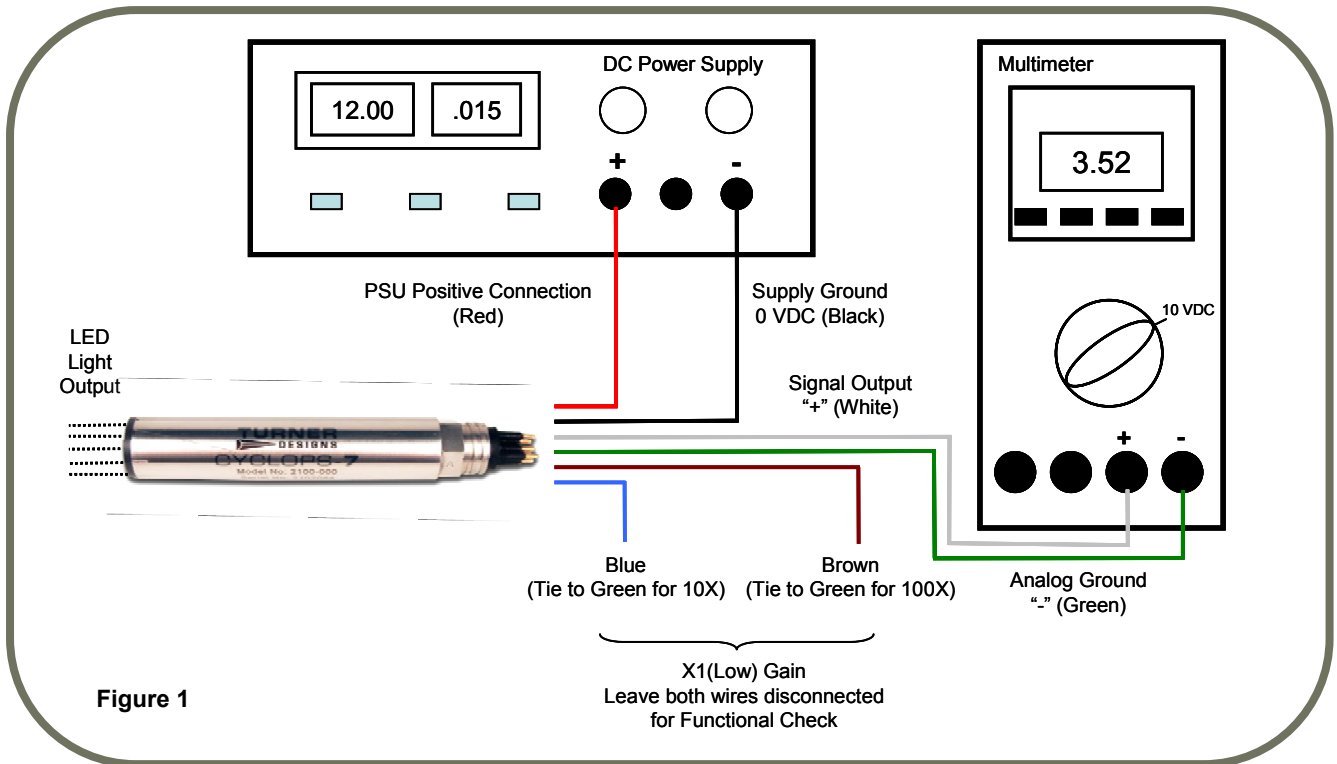
Procedure

A. Blank Measurement

1. Connect the Cyclops fluorometer to the Power Supply and Digital Multimeter as shown in Figure 1.
2. Set the sensor to the X1 Gain Range by not connecting both gain setting wires (blue & brown).
3. Immerse the optical end of the fluorometer in a beaker of de-ionized water (see page 5).
4. Note the output voltage in Table 1 as 'Blank Sample Voltage'.
5. Rewire and repeat steps 1 and 2 for the X10 and X100 gain settings. Note these readings in Table 1 as 'Blank Sample Voltage'.

Table 1

Gain Range	Blank Sample Voltage	Sample Voltage	Sample Concentration ($\mu\text{g/L}$)
X1			
X10			
X100			



B. Sample Measurement (*in vivo*)

1. Set the sensor to the X1 Gain Range by not connecting both gain setting wires (blue & brown).
2. Immerse the optical end of the fluorometer in a beaker containing the sample of water to be measured.
3. Note the output voltage in Table 1 as 'Sample Voltage'.
4. Rewire and repeat steps 1 and 2 for the X10 and X100 gain settings. Note the readings in Table 1 as 'Sample Voltage' for each gain.
5. Determine the gain to be used (calibrated) such that the sample's response produces an output voltage that is as close as possible to 2.5V.

C. Sample Measurement (extracted)

1. Procedures for doing this are available on the Turner Designs web site as well as the EPA website. We recommend using EPA Method 445.
2. From the Extraction Result(s), note the sample concentration in Table 1 as 'Sample Concentration ($\mu\text{g/L}$)'.

D. Single-Point Calibration Coefficient Calculation

Using values from Table 1, you can create a Calibration Coefficient (K) for the sample collected and used in section B. Use the equations below to calculate your K:

Equation 1 (Sample Voltage) – (Blank Sample Voltage) = Blank Corrected Sample Voltage

Equation 2 (Sample Concentration (µg/L)) / (Blank Corrected Sample Voltage) = K ((µg/L)/Voltage)

You can now convert all voltages at the set gain to (µg/L) concentrations by multiplying the blank corrected voltage for a sample by the K coefficient calculated in Equation 2. **Note: Calibration coefficients will change with respect to season, natural occurrences, algal community changes, and location.**

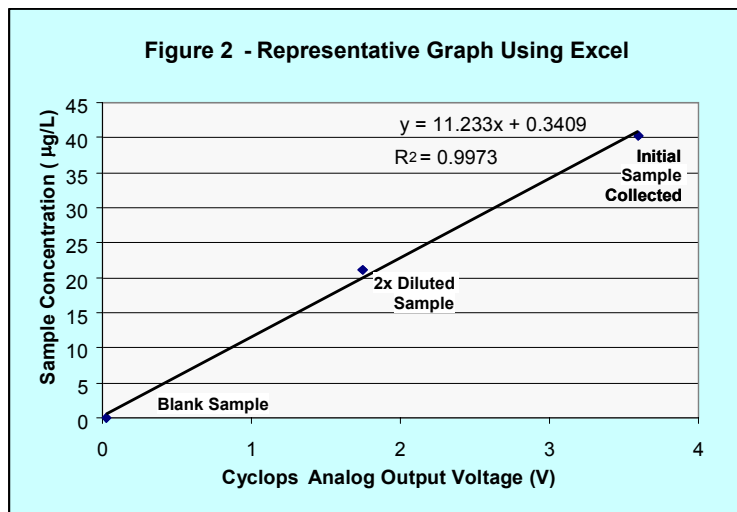
E. Multi-Point Calibration & Linearity Check

1. Set the sensor to the same gain setting used in Section B, step 5.
2. Dilute the sample used in Section B by 2x using either artificial sea water for a marine sample or deionized water for a freshwater sample.
3. Immerse the optical end of the fluorometer in a beaker containing the sample of water to be measured.
4. Note the output voltage in Table 2 as '2x Diluted Sample Voltage'.
5. Measure the concentration of the 2x diluted sample (see section C) and note the sample concentration in Table 2 as '2x Diluted Sample Concentration (µg/L)'.
6. Use a spreadsheet program, such as Microsoft Excel, to generate a graph as shown below using values from Tables 1 & 2.

Table 2

Gain Range	2x Diluted Sample Voltage	2x Diluted Sample Concentration (µg/L)
X1		
X10		
X100		

Figure 2. The plot to the right 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. Excel calculates the R² value and the equation for the best-fit line.



A multi-point calibration curve provides users with a regression equation that can be used to convert voltage responses to concentration ($\mu\text{g/L}$) estimates. Simply substitute the 'x' in the equation with a voltage value and the result will be represented as a concentration ($\mu\text{g/L}$) estimate.

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

1. Dry off the optical end of the Cyclops sensor and attach the Solid Secondary Standard. Adjust the setting screw so that the output voltage is equal to one of the sample output voltages in Table 1 or 2.
2. The Secondary Standard is now set to a sample output voltage that simulates a concentration for a specific gain setting. It can now be used to check for drift in calibration.

Note: *If the sensor drifts by more than $\pm 3\%$ of the value set in step 2 of section F, then a recalibration needs to be made and/or the sensor's optical head needs cleaning.*

Other Factors to Take Into Consideration

There are several other factors which can impact the calibration and measurement accuracy of *in vivo* chlorophyll 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

Recommended Lab Practices for Measurements

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

1. Use a non-fluorescent container for your water samples. (**Note: Plastic may fluoresce and interfere with the sample's fluorescence**)
2. If using a glass container, place the container on a non-reflective black surface.
3. Ensure that the sensor is more than 3 inches above the bottom of the container.
4. Ensure that the sensor is in the center of the container and has more than 2 inches clearance between the circumference of the sensor and the inside surface of the beaker.

