



Chlorophyll a *in Vivo* Calibration with Secondary Standard Video Transcript

Function

The 'Chlorophyll a' fluorometer emits blue light into the water sample, which is absorbed by the 'chlorophyll a' in the algae. The 'chlorophyll a' fluoresces red light in return which is detected by the sensor. Monitoring *in vivo*, that is, an active biological condition, is subject to a great deal of variability. The fluorescent properties of an algal population in a body of water can change with the health of the algae, temperature, and even the time of day. This makes *in vivo* measurements more useful for trending than for exact quantitative values.

The 'chlorophyll a' fluorescence from the sample can be reported as a scaled voltage from 0 – 5 Volts or as a concentration from 0 – 500 µg/L.

The Solid Secondary Standard for the fluorometers can be used to set a calibration point by comparing it to a known sample. It should be noted that a solid standard will give a unique response to each sensor. A given setting is only valid on the sensor it was calibrated on.

Maintenance

The only maintenance required for the fluorometers is to keep them clean. As optical sensors, it is very important to give them a clear field of view into the water sample. Before and after each deployment clean the optical lenses with a cotton swab or lint free towel and soapy water. Rinse with clean fresh water.

Do not use organic solvents such as methanol or acetone to clean the sensor. These chemicals will damage the plastic components of the sensor.

Calibration

Since there is no primary standard available for chlorophyll a, the calibration process requires a sample value to be recorded then an extraction performed to find the true value. When the true value of the sample is known, there are two methods of calibrating the sensor: with a solid standard, and without.

Establish a connection to the sonde with Hydras 3LT. Click the button labeled '**Operate Sonde**'. When the sonde finishes its initialization, click the '**Calibration**' tab, then click the '**Chlorophyll [ug/L]**' tab. You will see a picture of the fluorometer as well as the current value, the date and time, the current temperature, and the voltage reading of the sensor.



Begin with a clean and dry sonde. Attach the calibration cup and fill it to the threads with sample water that has been filtered of all phytoplankton using a 0.45 μm filter, or optionally, de-ionized water. Wait for one minute for the readings to stabilize. Type '0' into the box and click '**Calibrate**'. A "Calibration Successful" message will appear.

Empty the storage cup and rinse the sensors. Dry the sensors and attach the storage cup. Fill the cup to the threads with a fresh sample from the deployment site. Wait one minute for the readings to stabilize. If you are using a solid standard for calibration record the current $\mu\text{g/L}$ value.

Have an extraction performed on the sample to find the true concentration.

If you are using the solid standard, rinse and dry the sensors and place the standard over the top of the fluorometer. Use the small screwdriver provided to adjust the standard until the $\mu\text{g/mL}$ reading displayed in the Hydras window is the same as what was recorded for the sample. When the current value matches the recorded value, the top of the solid standard can be tightened so that it 'locks' the standard to that value.

When the results of the extraction are known, the 'chlorophyll a' sensor is re-calibrated using this value.

If you are using the solid standard, perform the same zero calibration as before. Rinse and dry the sensors. Place the solid standard over the fluorometer and wait one minute for the readings to stabilize. Type the value of the extraction into the box and click '**Calibrate**'. A "Calibration Successful" message will appear.

If the solid standard is not adjusted, it can be used on this specific sensor for future calibrations or to check for drift. Record the extracted value and keep it with the standard.

The Chlorophyll a sensor is now calibrated.