



Blue-Green Algae Calibration with Secondary Standard Video Transcript

Function

The Blue-Green Algae fluorometers use the same principles but different wavelengths of light to detect different chemicals in the sample. The Freshwater fluorometer emits orange light and detects the red light emitted by the pigment phycocyanin. The Marine fluorometer emits green light and detects the orange light emitted by the pigment phycoerythrin.

Monitoring *in vivo*, that is, an active biological condition, is subject to a great deal of variability. The fluorescent properties of a cyanobacterial population in a body of water can change with the health of the organisms, temperature, and exposure to light, even while the concentration remains the same. This makes *in vivo* data useful for trending, or for correlating relative measurements to more quantitative values.

The readings can be reported as a scaled voltage from 0 – 5 Volts or as a concentration from 0 to 2,000,000 cells/mL

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

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 '**Phycocyanin [cells/mL]**' tab or '**Phycoerythrin [cells/L]**' tab. You will see a picture of the fluorometer as well as the current cells/L, 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 blue-green algae, or optionally, clean, fresh water. Wait for one minute for the readings to stabilize. Type '0' into the box and click '**Calibrate**'. A "Calibration Successful" message will appear.





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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 cells/mL 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 cells/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 blue-green algae 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. Record the extracted value and keep it with the standard.

The Blue-green Algae sensor is now calibrated.

