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APPLICATION NOTE NO. 62 **revised December 2006**

Calculating Calibration Coefficients for the WET Labs ECO-AFL or ECO-FL Fluorometer, or ECO-FL-NTU Fluorometer/Turbidity Meter

This Application Note applies to the following WET Labs fluorometers:
- ECO-AFL fluorometer - older model, not in current production by WET Labs
- ECO-FL fluorometer - FL(RT), FLD, FL, FLS, FLB, or FLSB
- ECO-FL-NTU - combines a fluorometer and turbidity meter, and requires two channels in your CTD

Fluorometer Calibration Coefficients

The fluorometer supplied with your Sea-Bird CTD has a response that is linear over the measurement range provided.
- **ECO-AFL** - The offshore version has a measurement range of approximately 0.02 - 100 µg/l, while the coastal version has a measurement range of 0.04 - 200 µg/l.
- **ECO-FL** – The measurement range can be adjusted with the analog scaling value. To change the range, connect the ECO-FL directly to the computer and use WET Labs' ECOView Host software to enter a new analog scaling value.

Nominal Range (µg/l)	0.01 - 30	0.01 - 60	0.01 - 120
Analog Scaling Value / Range (enter in ECOView Host)	1	2 (factory default)	4

- **ECO-FL-NTU** – The measurement range is approximately 0.02 to 50 µg/l.

Select the ECO-AFL/FL fluorometer when setting up the configuration (.con) file in Sea-Bird software (SBE Data Processing or SEASAVE in SEASOFT-Win32 or SEACON in SEASOFT-DOS). The software prompts for Vblank and Scale Factor and calculates chlorophyll concentration as:

concentration (µg/l) = (Vsample - Vblank) * Scale Factor (see Note)

where:

Vsample (volts) = in situ output of the fluorometer

Vblank (volts) = measured output for a seawater blank (pure, de-ionized water) (see Note)

Scale factor (µg/l-volts) = multiplier

The fluorometer comes with a calibration sheet that lists values for Vblank and Scale Factor (see Note). If you changed the analog scaling value (ECO-FL series only), change the Scale Factor to correspond.

Note: Calibration sheets from WET Labs for newer fluorometers may list ***Dark Counts*** instead of ***Vblank***. Use the Dark Counts value in place of Vblank when setting up the configuration (.con) file.

Example Concentration Calculation in Sea-Bird Software:
Vblank = 0.05 volts and Scale Factor = 12.35 µg/l-volts (from calibration sheet)
Measured voltage from fluorometer = Vsample = 4.65 volts
Calculated concentration (µg/l) = (Vsample - Vblank) * Scale Factor = (4.65 - 0.05) * 12.35 = 56.8 µg/l

While the factory-supplied Scale Factor can be used to obtain approximate values, field calibration is highly recommended. The relationship between fluorescence and chlorophyll-*a* is highly variable, and is not easy to determine in the laboratory. Species distribution, ambient light level, and health of the stock are just some of the factors that affect the relationship. To accurately measure chlorophyll-*a* concentration with a fluorometer, perform calibrations on seawater samples with concentrations of plankton populations that are similar to what is expected in situ. Determine chlorophyll-*a* concentrations independently, and use those concentrations, as well as readings from the fluorometer, to determine the correct Scale Factor. **The Scale Factor is correct as long as the condition of the plankton population does not change; the condition does change with season and geographic location.**

Example Calculation of Scale Factor from field calibration:
Seawater sample analysis shows chlorophyll-*a* is 50 µg/l when fluorometer reads 3.2 volts; measured signal for seawater blank is 0.05 volts.
concentration (µg/l) = (Vsample - Vblank) * Scale Factor → 50 = (3.2 - 0.05) * Scale Factor
Solving: Scale Factor = (50) / (3.2 - 0.05) = 15.87 µg/l → Enter new Scale Factor in configuration [.con] file.

Turbidity Meter Calibration Coefficients

The ECO-FL-NTU's turbidity meter has a response that is linear over the measurement range provided: 0 to 25 NTU. The turbidity channel in the ECO-FL-NTU is not currently directly supported in Sea-Bird software. However, you can set up the turbidity channel as a User Polynomial in the configuration (.con) file, which allows you to define an equation to relate the sensor output voltage to calculated engineering units:

$$Value = a0 + (a1 * V) + (a2 * V^2) + (a3 * V^3)$$

Wet Labs defines turbidity as:

turbidity (NTU) = (Vsample - Vblank) * Scale Factor

where:
Vsample (volts) = in situ output of the turbidity meter
Vblank (volts) = measured output for a seawater blank (pure, de-ionized water)
Scale factor (NTU/volts) = multiplier
The ECO-AFL-NTU comes with a calibration sheet that lists values for Vblank and Scale Factor for the turbidity meter.

Setting the Wet Labs equation equal to the user polynomial equation and calculating a0, a1, a2, and a3:

$$(Vsample - Vblank) * scale\ factor = a0 + (a1 * V) + (a2 * V^2) + (a3 * V^3)$$

Expanding the left side of the equation and using consistent notation (Vsample = V),
$$scale\ factor * V - scale\ factor * Vblank = a0 + (a1 * V) + (a2 * V^2) + (a3 * V^3)$$

Rearranging:
$$(-\ scale\ factor * Vblank) + (scale\ factor * V) = a0 + (a1 * V) + (a2 * V^2) + (a3 * V^3)$$

$$a0 = -\ scale\ factor * Vblank \qquad a1 = scale\ factor \qquad a2 = a3 = 0$$

Select the User Polynomial for the turbidity channel when setting up the configuration (.con) file in Sea-Bird software (SBE Data Processing or SEASAVE in SEASOFT-Win32 or SEACON in SEASOFT-DOS). The software prompts for a0, a1, a2, and a3.

While the factory-supplied Scale Factor can be used to obtain approximate values, field calibration is highly recommended. The relationship between turbidity and NTU is highly variable, and is not easy to determine in the laboratory. Particle shape and size are some of the factors that affect the relationship. To accurately measure NTU with a turbidity meter, perform calibrations on seawater samples with distributions of particles that are similar to what is expected in situ. Determine NTU independently, and use those values, as well as readings from the turbidity meter, to determine the correct Scale Factor. **The Scale Factor is correct as long as the distribution of particle sizes and shapes does not change; the condition does change with season and geographic location.**