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

This Application Note applies to the following WET Labs fluorometers and turbidity meters:
- ECO-AFL fluorometer - older model, not in current production by WET Labs
- ECO-FL fluorometer - FL(RT), FLD, FL, FLS, FLB, or FLSB
- ECO-NTU turbidity meter – NTU(RT), NTU(RT)D, or NTUS
- 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** (older model, not in current production) - The offshore version had a measurement range of approximately 0.02 - 100 µg/l, while the coastal version had 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.

| | | | |
|---|---|---|---|
| **Chlorophyll *a* Fluorometer:** Nominal Range (µg/l) | 0 - 30 | 0 - 50 | 0 - 125 |
| **Rhodamine Fluorometer:** Nominal Range (ppb) | 0 - 55 | 0 - 110 | 0 - 230 |
| **Phycocyanin Fluorometer:** Nominal Range (ppb) | 0 – 100 | 0 - 200 | 0 - 400 |
| **Analog Scaling Value / Range** (enter in ECOView Host) | 1 | 2 | 4 (factory default) |

- **ECO-FL-NTU** – The measurement ranges for these combination fluorometer and turbidity meters are factory-configured, and cannot be adjusted in the field. For the fluorometer channel, the ranges are approximately 0 to 30, 50, 75, or 125 µg/l (see *Turbidity Meter Calibration Coefficients* below for the turbidity meter channel).

In our SEASOFT V2 suite of programs, edit the CTD configuration (.con or .xmlcon) file using the Configure Inputs menu in Seasave V7 (real-time data acquisition software) or the Configure menu in SBE Data Processing (data processing software). Select the ECO-AFL/FL fluorometer when editing the .con or .xmlcon file; the software prompts for Vblank and Scale Factor and calculates concentration as:

concentration (µg/l or ppb, as applicable) = (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 or ppb/volts, as applicable) = 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 or .xmlcon) file.

*Example Chlorophyll a 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 (.xmlcon or con) file.

## Turbidity Meter Calibration Coefficients

The turbidity meter supplied with your Sea-Bird CTD has a response that is linear over the measurement range provided.

- **ECO-NTU**- The measurement range can be adjusted with the analog scaling value. To change the range, connect the ECO-NTU directly to the computer and use WET Labs' ECOView Host software to enter a new analog scaling value.

| | | | |
|---|---|---|---|
| **Sea-Bird PN 24345, 24346, and 24348:** Nominal Range (NTU) | 0 - 30 | 0 - 60 | 0 - 125 |
| **Sea-Bird PN 24367:** Nominal Range (NTU) | 0 - 250 | 0 - 500 | 0 - 1000 |
| **Analog Scaling Value / Range** (enter in ECOView Host) | 1 | 2 | 4 (factory default) |

- **ECO-FL-NTU** – The measurement ranges for these combination fluorometer and turbidity meters are factory-configured, and cannot be adjusted in the field. For the turbidity meter channel, the ranges are approximately 0 to 10, 25, 100, 200, or 1000 NTU (see *Fluorometer Calibration Coefficients* above for the fluorometer channel).

***Note:*** If you require $m^{-1}$ $sr^{-1}$ output, WET Labs can provide a secondary calibration for these instruments in $m^{-1}$ $sr^{-1}$ at an additional cost. Alternatively, you can purchase the ECO-BB, which comes calibrated to $m^{-1}$ $sr^{-1}$. See *Application Note 87* for details on how to use $m^{-1}$ $sr^{-1}$ output with Sea-Bird CTDs and software.

The ECO-NTU turbidity meter and the turbidity meter channel in the ECO-FL-NTU is not directly supported in Sea-Bird software. However, you can set up the turbidity channel as a User Polynomial in the configuration (.con or .xmlcon) 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-NTU and the ECO-FL-NTU come with a calibration sheet that lists values for Vblank and Scale Factor for the turbidity meter. If you changed the analog scaling value (ECO-NTU series only), change the Scale Factor to correspond.

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:

$$(- Scale\ Factor * Vblank) + (Scale\ Factor * V) = a0 + (a1 * V) + (a2 * V^2) + (a3 * V^3)$$

Solving: **a0 = - Scale Factor * Vblank** **a1 = Scale Factor** **a2 = a3 = 0**

In our SEASOFT V2 suite of programs, edit the CTD configuration (.con or .xmlcon) file using the Configure Inputs menu in Seasave V7 (real-time data acquisition software) or the Configure menu in SBE Data Processing (data processing software). Select the User Polynomial for the turbidity channel when editing the configuration file; the software prompts for a0, a1, a2, and a3 as well as a sensor name (which will appear in the data file header).

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.**