

## **Method: In-line Single Channel Backscattering and Chlorophyll and CDOM Fluorescence, WET Labs ECO BBFL2**

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**Brief description of protocol:** Single-excitation backscattering, and chlorophyll and CDOM fluorescence was obtained from continuous inline observations collected with a WETLabs ECO BBFL2 sensor using the differencing method between unfiltered and filtered observations following Slade et al. (2010). The backscattering sensor was comprised of a 660 nm LED, the Chl fluorometer excites with a 470 nm LED (emission 695 nm), the CDOM fluorometer excites with a 370 nm LED (emission 460 nm). Calibrations were performed just prior to the EXPORTS North Pacific expedition: the backscattering sensor was calibrated by Sunstone Scientific; the CDOM fluorometer was calibrated by WET Labs; the Chl fluorometer was calibrated by WET Labs and also in-house using a monospecific culture of *Thalassiosira pseudonana* (Proctor and Roesler 2010; Roesler et al. 2017). The standard method for estimating in situ chlorophyll *a* concentration is to calibrate in situ observations of chlorophyll fluorescence derived from a 470 nm LED to paired in situ HPLC TChl measurements.

**Deployment methodologies:** A diaphragm pump installed in the sea chest of the R/V Armstrong delivered continuous seawater to the wet lab, with minimal particle disruption. The inline optical system consists of serial flow in the following order: MSRC VDB-1 vortex debubbler, Seabird thermosalinograph, Sequoia Flow Meter, WET Lab acs, WET Labs ECO 3X1M, WET Labs ECO BBFL2 and WET Labs ECO BB3. The ECO BBFL2 was configured in a custom-designed casket (Dall'olmo et al. 2009) that minimizes impacts of scattered light within the flow. The flow meter automatically switched flow from unfiltered to filtered through a large volume 0.2  $\mu\text{m}$  cartridge filter. The automated switch operated continuously through the day such that during each hour 50 minutes is unfiltered and 10 minutes is filtered. Approximately twice per day, an additional filtered configuration was manually triggered such that the hour was parsed into 20 minutes of unfiltered, 15 minutes of 20- $\mu\text{m}$  filtration, 15 minutes of 5- $\mu\text{m}$  filtration and 10 minutes of 0.2- $\mu\text{m}$  filtration. Discrete water samples were collected from each of the size fractions and processed for spectrophotometric particulate absorption, HPLC and POC.

**Data processing:** All flow through data were processed into 1-minute bin median and standard deviation values. Observations compromised by bubbles were removed. Data were time-merged with the ship navigation GPS data streams, also processed into 1-minute bin median values. Data stream was parsed into unfiltered and filtered intervals, with transitional data removed. Filtered observations were interpolated to 1-minute intervals and subtracted from unfiltered data, thus yielding blank-subtracted fluorescence values (Slade et al. 2010).

The particle backscattering coefficient was computed from:

$$b_{bp} (m^{-1}) = M \left( m^{-1} / dc \right) \times \left( DC_{bb_{unfilt}} - DC_{bb_{filt}} \right).$$

Where  $M$  is the calibration slope provided by Sunstone Scientific (with a best-case accuracy of 2.1%) and  $DC$  indicates the 1-minute median digital count values for unfiltered or interpolated filtered observations.

Non-negligible non-photochemical quenching was observed within 2 hours of local midnight. Match-ups between discrete HPLC TChl values and unquenched inline chlorophyll fluorescence values were used to generate an in situ calibration for the 470 nm excitation channel, with  $M \left( \frac{mg}{m^3} dc^{-1} \right)$ , representing the slope of the matchup relationship (with a computed uncertainty in the regression slope of  $\sigma_M$ ):

$$Chl \left( \frac{mg}{m^3} \right) = M \left( \frac{mg}{m^3} / dc \right) \times (F_{unfilt} - F_{filt}).$$

Non-photochemically quenched values are provided and should not be interpreted as chlorophyll concentration.

**Uncertainties and quality control concerns:** Uncertainties associated with natural variations in particle backscattering and chlorophyll fluorescence were determined from the standard deviations of the one-minute bin median data for filtered,  $\sigma_{filt}$ , and unfiltered,  $\sigma_{unfilt}$ , observations. Following the Guide to Uncertainty Measurements (Jcgm 2008), the uncertainty in both backscattering coefficient and chlorophyll concentration are computed as:

$$\sigma_X = X \times \sqrt{\sigma_{unfilt}^2 + \sigma_{filt}^2 + \left( \frac{\sigma_M}{M} \right)^2}$$

where  $X$  is either  $b_{bp}$  or  $Chl$ , and  $\sigma_M$  is the uncertainty in the calibration.

#### Data products originating with this method:

Parameter	Symbol	Units
bbp (660 nm)	$b_{bp}$ (470)	mg m <sup>-3</sup>
Calibrated Fchl (ex470 nm)	Chl	mg m <sup>-3</sup>

#### Key method references:

- Dall'olmo, G., T. K. Westberry, M. J. Behrenfeld, E. Boss, and W. H. Slade. 2009. Significant contribution of large particles to optical backscattering in the open ocean. *Biogeosciences* **6**: 947-967.
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