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# Primer regarding measurements of chlorophyll fluorescence and the backscattering coefficient with WETLabs FLBB on profiling floats

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This document has been written in response to a request from SOCCOM's director Jorge Sarmiento for the SOCCOM community. It focuses on the use and interpretation of data collected with the WETLabs FLBB sensor, currently the optical sensor most used on profiling floats. Finalized on March, 23rd, 2016.

## Chlorophyll fluorescence

I will start with a quote from John Cullen's 1982 classical paper: "The relationship between chlorophyll a and phytoplankton biomass (organic carbon content) is highly variable as is the yield of in vivo fluorescence per unit chlorophyll. Thus, vertical profiles of chlorophyll or in vivo fluorescence must be interpreted with caution if their ecological significance is to be established. Although the variability of carbon-to-chlorophyll ratios and fluorescence yield is large, much of it can be anticipated, corrected for, and usefully interpreted."

Chlorophyll fluorescence has been measured in-situ since the pioneering work of Lorenzen in the late 60s and early 70s. Cullen (1982) summarized all the complications associated with in-vivo fluorescence measurements. I repeat and expand them here in the context of measurement of profiling floats.

### a. Theoretical/physiological considerations

All phytoplankton have a chlorphyll_a-like pigment (denoted below as Chl).
Chl represent a trace amount of the phytoplankton mass. Carbon (denoted below as C) to Chl ratio (denoted as C/Chl) is highly variable and reflects parameters affecting growth-rate. Nutrient limitation drives this ratio up (less Chl/C), as does reduction in growth irradiance. On a daily basis it can vary by about 25% for sun-synchronized cells, and C/Chl can vary by a factor of 10 or more in the field (Cullen, 1982).

Photons fluoresced are the result of photons absorbed by phytoplankton pigments. Some of the variability in the Fluorescence/Chl ratio is related to pigment packaging, that is the reduction of absorption per Chl with cell size and increase in Chl/cell. Note that phytoplankton have two photosystems, and only one (photosystem 2) is involved in fluorescence. Hence changes associated with the allocation of chlorophyll between the two photosystems can affect the fluorescence yield per chlorophyll.

Only a tiny (0.5-3%) fraction of the photons absorbed result in fluoresced photons. For a given excitation/emission wavelengths the fluorescence yield varies with species composition (e.g. Proctor and Roesler, 2010, and see earlier literature in Cullen, 1982).

Degradation products of Chl do absorb and fluoresce (though less efficiently per mole). There is no consensus regarding their contribution to fluorescence in the environment (if at all).

Non-photochemical quenching (NPQ): Phytoplankton exposed to light above a certain threshold reduce their fluorescence. A curve describing the ratio of measured fluorescence to that if the cells were in the dark (F/F_dark), as function of irradiance as a shape as follows:

1 for E<E_NPQ
E_NPQ/E for E>E_NPQ.

Where E is the ambient irradiance and E_NPQ the irradiance the phytoplankton start quenching at.

**b. Measurement considerations**
Excitation wavelength: 470nm (Full width half max ~20nm).
Emission wavelength 685nm (spectrally wide detector).
Volume of measurement: ~3ml.

If water does not flow in front of the sensor (e.g. because of platform or water motion), the sensor illumination itself can induce NPQ. Absorption at 470nm involves both chlorophyll-a as well as accessory pigments. Hence, in practice, fluorescence provides information on the state of the photosynthetic machinery, not just Chl.

The signal can be spiky due to presence of rare large Chl containing particles. This 'noise' in the signal could be exploited (if sufficient measurements are made such that one can separate the background from the spikes, e.g. Briggs et al., 2011) to provide information pertaining to particle export and provide information on two populations of particles (the background contributing ones and those causing the spikes).

**c. Specific known issues with the fluorometer on the WETLabs FLBB sensor.**
Dark current provided by WETLabs does not match exactly that on the platform on which it is deployed. To get the correct value it is best to obtain it on the platform by covering it with black electrical tape (care needs to be made to remove glue residue using lens paper wet with isopropyl alcohol).

Dissolved organic matter is excited by chlorophyll fluorometers as well, which can cause over estimation of chlorophyll, in particular in the deep waters (below the maximum of mixed-layer and euphotic depth) where fluorescing dissolved organic matter increases in concentration as function of depth and where chlorophyll fluorescence is often negligible (Proctor and Roesler, 2010, Rottgers and Koch, 2012). This issue manifests itself as an increase in chlorophyll fluorescence with depths (at depths where chlorophyll is negligible). If the concentration of fluorescing dissolved organic materials is very high, it *can* affect fluorescence measured with chlorophyll fluorometers even near the surface (e.g. Black Sea).

**d. Links to biogeochemical parameters.**
Chlorophyll fluorescence, when corrected for NPQ, and calibrated with HPLC chlorophyll, provides an estimate of total **chlorophyll-a pigments** distribution in the water.

Chlorophyll_a, being a pigment shared by all phytoplankton, provides an estimate of ***phytoplankton biomass*** (through a conversion factor from chlorophyll to carbon or nitrogen). C/Chl varies from 12-200g/g in phytoplankton cultures. Chlorophyll_a is also a proxy to the **absorption of phytoplankton** (in particular by the photosynthetic machinery). As such it is used in primary production calculations.

The ratio of C/Chl (C being estimated for example from particulate backscattering, see below) at a given light level is an indicator **phytoplankton growth-rate** (Fig. 1 in Cullen, 1982, Geider et al., 1998, Behrenfeld et al., 2005).

**e. Methods to reduce uncertainties in chlorophyll measured with fluorometers.**
**1. NPQ:**
To avoid NPQ, profile at night. If profiling at night is not an option corrections available are:

- Sackmann et al., 2008: correct the NPQ values by extrapolating up to the surface with the chl fluorescence value learned below the mixed layer depth from chl fluorescence and backscattering ratio.
- Xing et al., 2012: if upper layer is relatively well mixed, extrapolate up to the surface with the deepest chl fluorescence value observed in the mixed layer.
- Xing et al., 2011: if the water column is stratified, use radiometry to derive chl independently

**2. Calibration**
WETLAbs provides an equation to obtain Chl from the counts/voltage measured by the sensor of the form:

Chl=slope x (measurement – dark).

Both slope and dark are instrument specific and provided by the manufacturer on a data sheet that comes with the instrument. These sensors have been tested for linearity.
The dark measured in lab on the sensor sometime differs by a significant number of counts from that measured with the sensor on the float. It is recommended that the darks be measured on the float during float construction.
The slope parameter provided by WETLabs has been found to be significantly biased (on average by a factor of two). It is therefore critical to obtain an estimate of this parameter independently if quantitative estimates of chlorophyll are required.

Methods for obtaining slope estimates are as follows:
a. Collection of in-situ samples to be analyzed for pigments by HPLC or extracted fluorometry. HPLC is currently considered the most precise way to determine Chl concentration.

b. Comparison with 676nm absorption line-height estimate of Chl (The latter predicts chlorophyll in the ocean within 40%, e.g. Boss et al., 2013)

c. Comparison of Ocean Color radiometry derived estimates of Chl have been shown to have, on average, an uncertainty on the order of 30% in the world's ocean (Boss et al., 2008). Ocean color derived Chl is not subject to NPQ.

Given that WETLabs fluorometers are all cross-calibrated at the factory (by comparison to a 'golden' sensor), it is possible to use multiple sensor regression to independent measurements to calibrate them. This is very powerful because it decreases the uncertainties on the regression compared to having to calibrate each sensor individually.

**f. How SOCCOM data is adjusted to date on our end:**
The equation provided by WETLabs and mentioned above is used to calibrate the fluorometer but the dark counts and the slope coefficients are re-estimated as follow. For every profile, a $dark_{profile}$ is computed which corresponds to the median of the 10 minimum values of the profile. The dark of the float $dark_{dynamic}$ is equal to the median of the 10 minimum $dark_{profile}$ of all the profiles available at the time of computation. This will results in some negative values at great depths, but investigation of these values suggest they are within the instrumental noise.
The slope is estimated with the empirical relationship between the first profile of the SOCCOM floats and HPLC values taken at time of deployment of the float during cruises P16S, PS89, and IN2015v1. To date the regression coefficient between HPLC derive Chl and WETLabs derived Chl is: $1 \div 7.43$ (unitless).
Hence we compute Chl from:

$$Chl = (signal - dark_{dynamic}) \times WETLabs\_slope / 7.43$$

Regarding NPQ correction, floats equipped with PAR sensors will be corrected for NPQ at every depth where $PAR > 80$ Einstein $m^{-2}$ $d^{-1}$ (Claustre et al., unpublished). The average of Sackmann et al. (2008) and Xing et al. (2012) corrections is computed and kept as the corrected value of chl fluorescence. For floats without PAR sensors (all SOCCOM floats to date), the sun elevation $s_e$ is estimated with Reda and Andreas (2003) at the location the float surfaced and at the time it finished its profile. If $s_e > 5$ ° then the profile needs to be unquenched. The depth at which the chl fluorescence profile starts to be corrected is determined by a daily mixed layer depth with a density threshold criterion of 0.005 kg $m^{-3}$. Then the average of Sackmann et al. (2008) and Xing et al. (2012) corrections will be computed and kept as the corrected value.

The global value for this regression coefficient is 2 (Claustre et al., unpublished). Hence it seems that the factor above may be region dependent (indeed two SOCCOM floats had a smaller regression coefficient relative to HPLC during deployment). We will continue to monitor all floats individually as well as clustered, to determine the optimal slope to apply in delayed mode.

MODIS and VIIRS reflectance data and Chl near each float surfacing is archived and will be used, at the end a sensor life, to evaluate drift and whether the regression coefficient needs to be modified.

The algorithms described in this document are available at:
https://github.com/OceanOptics/MISCToolbox

**g. Uncertainties associated with Chl:**
As described above uncertainties in Chl derived from fluorescence on SOCCOM floats arise primarily due to NPQ and uncertainties in the regression with Chl (aggregated HPLC). For most floats to date the latter is smaller than 20% while NPQ (despite correction) can add similar uncertainty to measurements done during the day. Assuming these uncertainties are uncorrelated result in an O(35%) global uncertainty with max(MLD, euphotic depth).

## Backscattering coefficient at 700nm

The backscattering coefficient has been measured with single source/receiver geometries since the late 90s (Maffione and Dana, 1997). It is based on the empirical observations that scattering by marine particles in the backward hemisphere is relatively flat as function of angle and can be estimated within 10% by measurements in a single angle in the back direction. This has been comprehensively reviewed by Stramski et al. (2004) and more recently by Boss et al. (2015). The 700nm was chosen to avoid interaction with dissolved absorbing materials.

**a. Theoretical considerations**
Scattering is affected by particle characteristics such as size, shape, composition (effective index of refraction) and internal structure (e.g. non-homogeneous distribution of material within the particle). Theoretical solutions for populations of particles are available only for idealized particles such as homogeneous or shelled spheres and spheroids. In addition, empirical measurements have been performed on both inorganic sediments as well as phytoplankton. These studies have supported the use of a single angle measurement in the back direction to estimate the backscattering coefficient (integrated over the back hemisphere) suggesting that the uncertainty introduced is on the order of 10%.

**b. Measurement considerations**

Most often the wavelength measured on floats is at 700nm. The reason for that choice was to avoid absorption effects by pigments and dissolved materials on the measurement. The backscattering spectra in the ocean is not spectrally flat and to compare with measurements performed at other wavelengths a power-law spectral dependence is most often assumed:

$$b_{bp}(\lambda) = b_{bp}(700nm)\left(\frac{\lambda}{700nm}\right)^{-\gamma}$$

A guess at a global value of $\gamma$ is 0.78 (Boss et al., 2013), based on global measurements of the scattering spectrum and assuming the ratio of scattering to backscattering to be spectrally flat (e.g. Boss et al., 2015).

The signal can be spiky due to the presence of rare large particles. This ‘noise’ in the signal could be exploited (if sufficient measurements are made such that one can separate

the background from the spikes, e.g. Briggs et al., 2013) to provide information pertaining to particle export and to provide information on two populations of particles (i.e. those contributing to the background and those causing the spikes).

**c. Specific known issues with the WETLabs FLBB sensor.**
A review of the WETLabs sensor performance and issues was recently published (Sullivan et al., 2013). The sensor has no reference detector. LEDs emit more light at low temperatures. This effect has not been observed to be significant. LED output drifts in times (a few percent per year). This effect has not been observed to be significant for the FLBB sensors (no drift of values at depths as been observed).
Dark current values are different than those provided by the manufacturer – measure them on the float by covering with black tape, immersing in water, and taking a reading.

**d. Links to biogeochemical parameters.**
The backscattering coefficient has been empirically linearly related to suspended particulate mass and particulate organic carbon concentration (see reviews by Cetinic et al., 2012 and by Boss et al., 2015). More recently it has been linearly related to phytoplankton carbon ($C_{phyto}$, Martinez-Vincente et al., 2014, Graff et al., 2015). These relationship should be avoided when suspended inorganic sediment may be in the waters as well as below the MLD. Together with the chlorophyll measurements the ratio of $Chl/C_{phyto}$ can be estimate. This ratio at a given light-level is directly linked to phytoplankton growth rate (e.g. Geider et al., 1998, Westberry et al., 2008), and if it can be measured with sufficient accuracy, could be an important constraint on primary productivity estimates.

**e. Methods to reduce uncertainties in the backscattering measured with EcoBB sensors.**
WETLAbs provides an equation to obtain the volume scattering function at 700nm from the counts/voltage measured by the sensor of the form:

$$\beta(700)=\text{slope\_b} \times (\text{measurement} - \text{dark\_b}). \; [m^{-1} \; sr^{-1}]$$

Both slope and dark are instrument specific and provided by the manufacturer on a data sheet that comes with the instrument To obtain the backscattering coefficient we follow Boss and Pegau (2001):

$$b_{bp}(700)=\chi(\theta) \times 2 \times \pi \times \{\beta(700) - \beta\text{water+salts}(700)\}, \; [m^{-1}]$$

where $\beta$water+salts(700) is computed using Zhang et al (2009, code available at http://www.und.edu/instruct/zhang/programs/betasw_ZHH2009.m) and $\chi(\theta)$ is based on Sullivan et al. (2013) for the appropriate centroid angle of the instrument.

The dark measured in lab on the sensor sometimes differs by a significant number of counts from that measured with the sensor on the float. It is recommended that the darks be measured on the float during float construction (we have found for SOCCOM floats

that while the difference in darks result in an average of 3% between estimated $b_{bp}$ near the surface while 11% near 2000m).

The slope parameter of WETLabs is assumed to be correct.

Methods to check that measurements made are consistent:

1. Comparison with 'golden' sensors measured on CTD rosette during deployments (and additional optical sensors on the rosette such as beam transmissometers). These are also useful to check that the dark values used are consistent.

2. Comparison with backscattering estimated from Ocean Color remote sensing all are useful as checks of sensors deployed on profiling floats. Ocean Color and trends of deep values are useful to assess *drift* in sensor output (e.g. Boss et al., 2008), though to date no drift has been observed in float-mounted backscattering sensors.

3. Measurements of POC on samples collected near the time of deployment provides for constraints on the $b_{bp}$-POC relationship, particularly for a region where few data have been collected, mostly in a few sectors, such as the SO.

**Note:** WETLabs manufacture a similar sensor to the FLBB called MCOMS which is used on three SOCCOM NAVIS floats. The only difference is the angle of scattering in the back-direction (140 degrees on FLBB rather than 150 degrees on NAVIS). Everything else discussed here pertains to that sensor as well.

**f. How SOCCOM data is adjusted to date at UMaine:**
The equation provided by WETLabs and mentioned above is used to obtain the volume scattering function with the manufacturer slope coefficient but the dark counts are replaced by the one measured before deployment of the float if they are available (otherwise we use the manufacturer's dark).

**1. Particulate backscattering $b_{bp}$**
The backscattering coefficient of particles $b_{bp}$ is commonly estimated from measurement of scattering at a single angle in the backward hemisphere $\beta(\theta)$. Sensors embedded have: $\lambda = 700$ nm and $\theta = 140^{\circ}$ (ECO-FLBB) and $\theta = 150^{\circ}$(MCOMS).

$$\beta_p(\theta) = \beta(\theta) - \beta_{sw}(\theta)$$

$$b_{bp} = 2\times\pi\times\chi(\theta)\times\beta_p(\theta)$$

$\beta_{sw}(\theta,\lambda)$ is the angular scatterance of sea water and is estimated with Zhang et al. (2009) and $\chi(\theta)$ is a conversion coefficient from Sullivan et al. (2013).

**2. Particulate organic carbon (POC)**

Particulate organic carbon is linearly proportional to particulate backscattering, an

empirical relationship built for the SOCCOM floats based on the relationship between the first profile of the floats and in-situ measurements taken during deployment (cruises: PS89, P16S and IN2015v1). Note that the results are similar to previous studies listed in Cetinic et al. (2012).

$$POC = 3.23 \times 10^4 \times b_{bp}(700) + 2.76 \ [mg\ m^{-3}]$$

**g. Uncertainties associated with $b_{bp}$:**
Uncertainties associated with $b_{bp}$ are due to methodological uncertainties (10%, Boss and Pegau, 2001) and those due to the darks (3% near the surface and 11% at depth). Assuming no correlation between them we estimate the uncertainty as 11% in max(MLD, euphotic depth) and 15% near park depth.

## 3. Phytoplankton carbon biomass, $C_{phyto}$

Phytoplankton carbon biomass $C_{phyto}$ is estimated from with an empirical relationship described in Graff et al. (2015) derived for backscattering at 470nm.

$$b_{bp}(470) = b_{bp}(700)\left(\frac{470}{700}\right)^{-\gamma} \ [m^{-1}]$$

$$C_{phyto} = 12128 \times b_{bp}(470) + 0.59 \ [mg\ m^{-3}]$$

with $\gamma = 0.78$ from Boss et al. (2013), which assumes that the backscattering spectra of particles is spectrally flat. Uncertainties in this relationship are on the order of 50%. This relationship was derived for surface samples and is likely to be biased at depth > max(MLD, euphotic depth) and/or if inorganic sediment are in the water (e.g. river inflow, bottom resuspension).

## 4. Particulate organic carbon, C

To date using SOCCOM floats and POC samples collected during deployment we find:

$$C = 3.23 \times 10^4 \times b_{bp}(700) + 2.76 \ [mg\ m^{-3}]$$

Which is consistent with relationships linking backscattering to POC in the literature for samples throughout the world's ocean (see table 2 in Cetinic et al., 2012). Uncertainties in this relationship are on the order of 20% (in comparison to other published ones using a similar sensor). This relationship was derived for surface samples and is likely to be biased at depth > max(MLD, euphotic depth) and/or if inorganic sediment are in the water (e.g. river inflow, bottom resuspension).

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