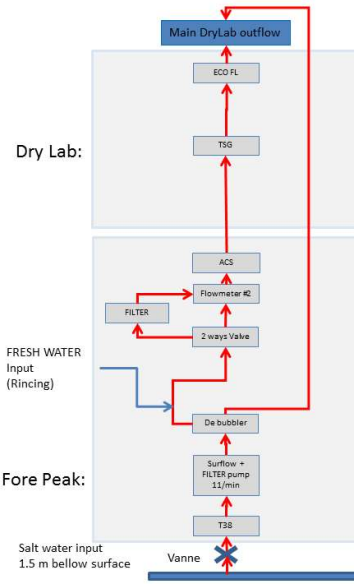
Processing Fluorescence Measurements of the in-line circuit during Tara-Oceans expedition

Vincent Taillandier, October 12, 2015

Context

The in-line system set up for the Tara-Oceans expedition aimed at providing continuously seawater in the dry lab in order to feed the different instrumental platforms. In particular, in order to provide a regular calibration of the optical instrument (AC-S), 0.2µm filtered seawater could be injected for blanking and fresh water could be inserted for cleaning (Fig. 1). In a normal functioning, this blanking operation was automatically conducted every 20 or 50 minutes (less when near the coast), with a mean duration of 10 minutes. The in-line system was instrumented with a CTD and a WetLab's ECO fluorometer (see figure at the right).

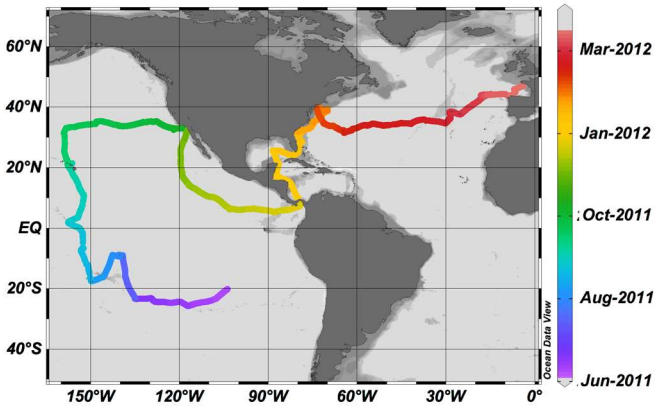


The fluorescence measurement is the concern of this report, which aims at revisiting the current processing based on CTD data flow. More precisely, Fluo measurements are affected by the calibration procedure, by also reducing the signal to clear water measurement. It is thus a requirement to isolate the calibration windows from the Fluo timeseries that are presented as 5 min averages. Moreover, the regular filtered seawater measurements can be exploited for calibration purpose of the sensor (by removing potential contamination of fluorescence of dissolved materials).

This document reports the different steps of the new processing starting from raw data towards separated 5min resolution timeseries for Fluo counts and Background counts. These timeseries are compared to HPLC data in order to convert, as a single regression relation, Fluo counts into total Chlorophyll-a concentrations. Alternatively, since the AC-S data was calibrated with HPLC (Boss et al., Methods in Oceanography 2013) and since their are many more match-ups with AC-S the calibration is performed comparing Fluo and Chlorophyll concentration derived from AC-S.

The dataset

The original dataset from which Fluo measurement are to be reprocessed consists in daily ascii files recording timestamped and georeferenced scans for the following variables: temperature (acquired at the input by SBE38), salinity (derived from CT measures by TSG in the dry lab), and Fluo counts (acquired after filtration by WetLab's ECO). The time resolution of this series is about 10s, it covers the period of June 4, 2011 in South Pacific Ocean until

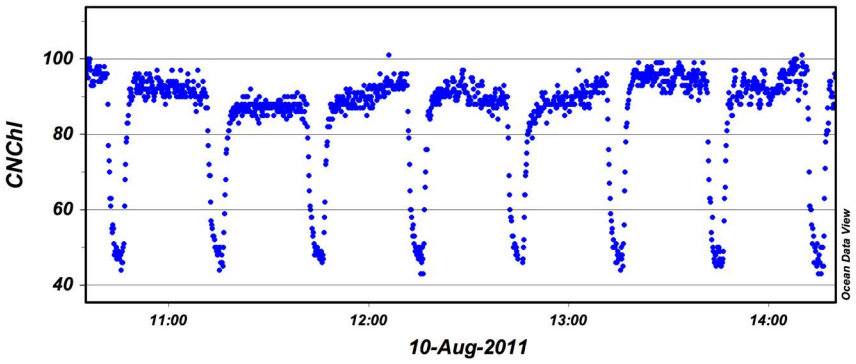


March 30, 2012 in Lorient. The daily files have been concatenated in 5 ascii files of about 30Mb, with shortcuts defined at port calls of the expedition in order to work on continuous timeseries and numerical filtering purposes.

The five periods are: from 2011/06/04 to 2011/08/12, from 2011/08/15 to 2011/09/18, from 2011/09/28 to 2011/10/26, from 2011/11/24 to 2012/01/20, from 2012/01/26 to 2012/03/30.

Pre-processing Fluo counts

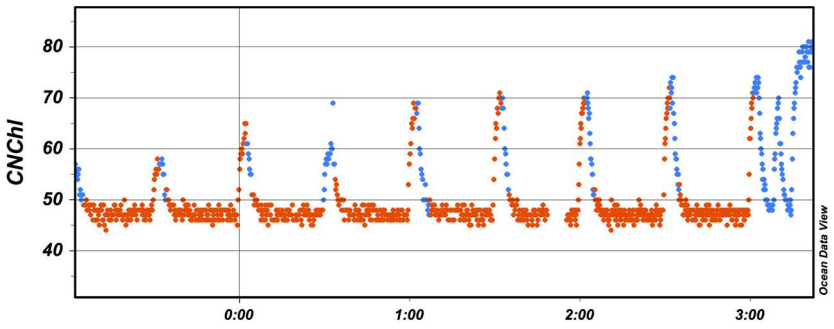
The first step of processing consists in isolating filtered seawater measures from unfiltered seawater results. In normal operating conditions, the original signal looks like the sequence in the figure at the left: cycles of 30min duration of 25min acquisition in unfiltered seawater, 5 minutes acquisition of filtered seawater; During and after each position switching, a transition on the order 3 minutes is observed.



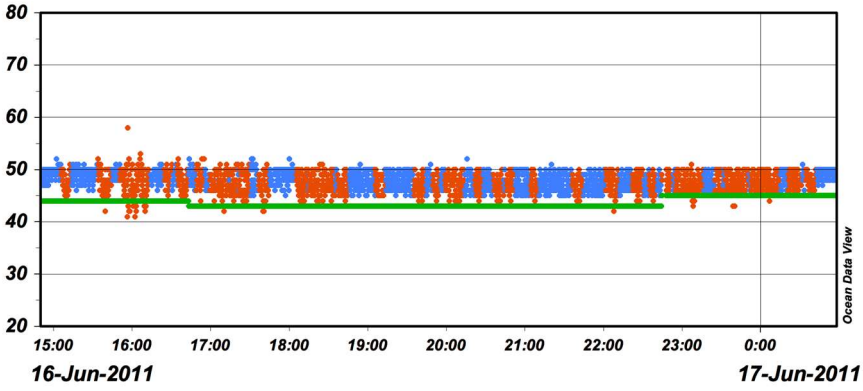
The algorithm set up for this purpose considers time windows of at least 6 hours (2160 scans), extending up to 12 hours in case of discontinuities (larger than 1hour) in the following window. A loop on 6-to-12h time windows is thus processed.

Inside each time window, a histogram of Fluo counts is done and the first percentile is attributed as the Background count for the window. Then a histogram of the residual between Fluo count and Background count is computed. If the difference between the 90th percentile of Fluo counts and the Background counts small (less than 10 counts, in case of ultra-oligotrophic seawaters), the threshold value to isolate unfiltered seawaters is fixed to the first percentile of the residual; otherwise the threshold is equal to the 10th percentile of the residual. The third part of the cycle is isolated considering the relaxation time of 3 min between unfiltered and filtered seawater Fluo measures.

Overall, the determination of the Background (blank) timeseries is performed as well as a mask of Fluo count that isolates unfiltered seawater measurements to the effects filtering operation. Some problems occur in this processing:

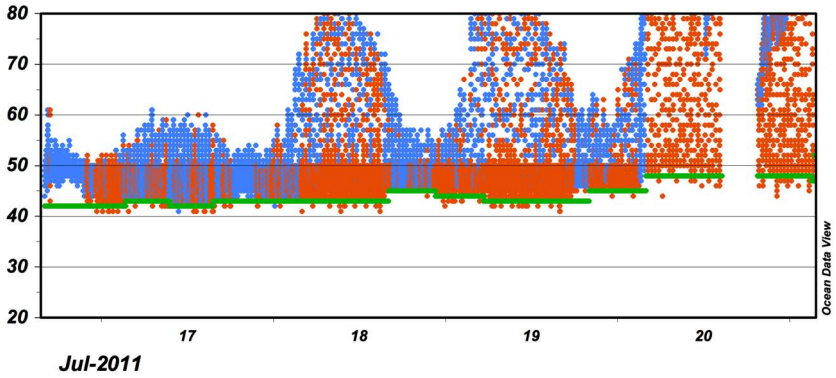


PB1: There are some time windows where the filtering cycling was inverted, with the filtering operation larger than the unfiltered seawater measurement. In this case, the relaxation times are not applicable and the detection algorithm provides several wrong values (see figure above with mask in color: 1 in orange, 0 in blue).



PB2: There are some time windows where Fluo counts stay very close to filtered (background) counts. Even by refining the threshold from the 10th percentile to the first percentile, separation become sensitive when the noise of measurement is larger than the difference between filtered and unfiltered seawater measures.

PB3: Background counts can significantly vary on short time ranges, in the example of 8 counts within 4 days, due to variance in the concentration of dissolved substances (e.g. Rottgers and Koch, 2012[1]) and possibly measurement noise and/or variable efficiency of the filtering operation.



Binning timeseries

The measured variables are then binned at 5min-resolution as for the original processing of TSG data. Some changes are provided in this operation. The Chlorophyll concentration derived using WetLab's factory calibration is removed and other variables are added to this list:

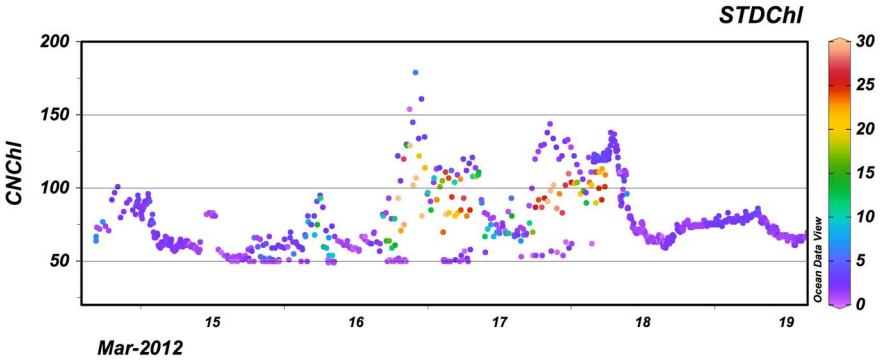
- The Fluo count, which is binned after the mask of pre-processing has been applied (good values are considered and flagged values are not). Note that instead of a mean, a median value has been computed inside each 5min window. When the number of counts is null in the bin, the value is set to -999.
- In the same way, the salinity is affected by the filtering operation; it has been recomputed using the same mask and following the same convention. No significant changes are expected with respect to the original processing, because the salinity measurements are sensitive to changes of flow rates, not of water components.
- The standard deviation of Fluo counts around its median is provided for each 5min bin. In an optional version, the median value for Fluo counts is passed to the maximum value when the number of counts per bin is lower than 5 and the standard deviation is greater than half of the Fluo counts. This option partially solved the encountered problem described hereafter. When the number of counts is null in the bin, the value is set to 0.

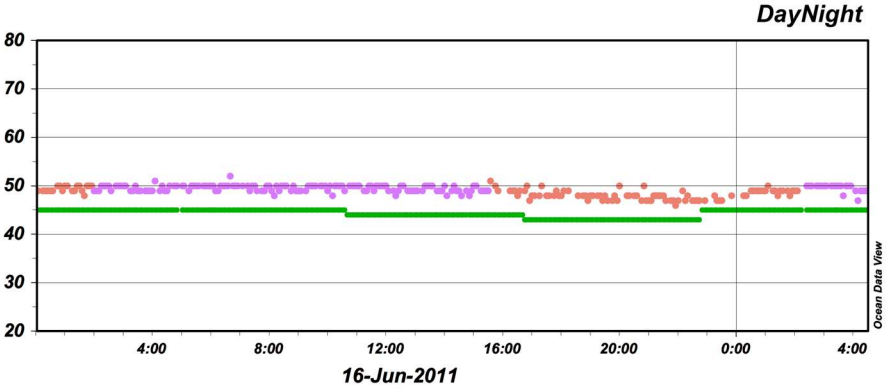
[1] http://www.biogeosciences.net/9/2585/2012/bg-9-2585-2012.html

- The background count consists in the discrete timeseries obtained in the pre-processing step.
- An indication of the light cycle during the day (0: night, 1: day): considering the day of the year and the geolocalisation of the vessel, together with the time of the day (given by GPS NMEA time with is referred to UTC), a day length and a meridian hour (in UTC) can be computed which provides an indication whether the measurement is affected by non photosynthetic quenching.
- The factory calibration consists in a scale factor and a dark count. They have been provided from two calibration sheets (dated on August 16 2010 and June 20 2012), and linearly interpolated with respect to time.

The problems listed in the pre-processing stage directly affect the binning operation.

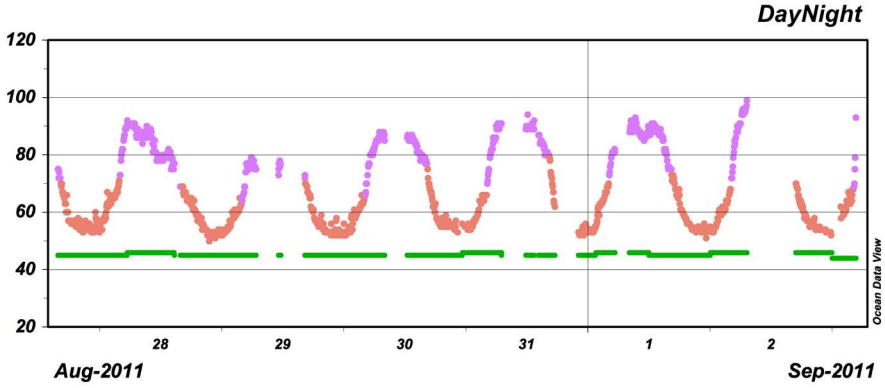
In particular, the PB1 that leaves few Fluo counts with large standard deviation is not completely removed by the threshold operated during the binning. In the figure above, some Fluo counts with high standard deviation remain even if there are underestimated during the binning, and some values belonging to Dark counts are not fully separated by the pre-processing step. After a visual quality control, there are three periods that need attentive care with respect to PB1: (i) from July 16 until July 20 14:30 2011, (ii) December 2 2011, and (iii) from March 15 until March 18 05:00 2012.





The PB2 doe not significantly degrade the quality of the timeseries. After binning, a consistent Fluo count signal, with little spreading, appears well isolated from background counts.

The diurnal cycle imposed by quenching effects may appear synchronized with with day/night switches. However, it does not look like expected, as soon as the effect of light should match with the Fluo decay or increase (some hours shift).



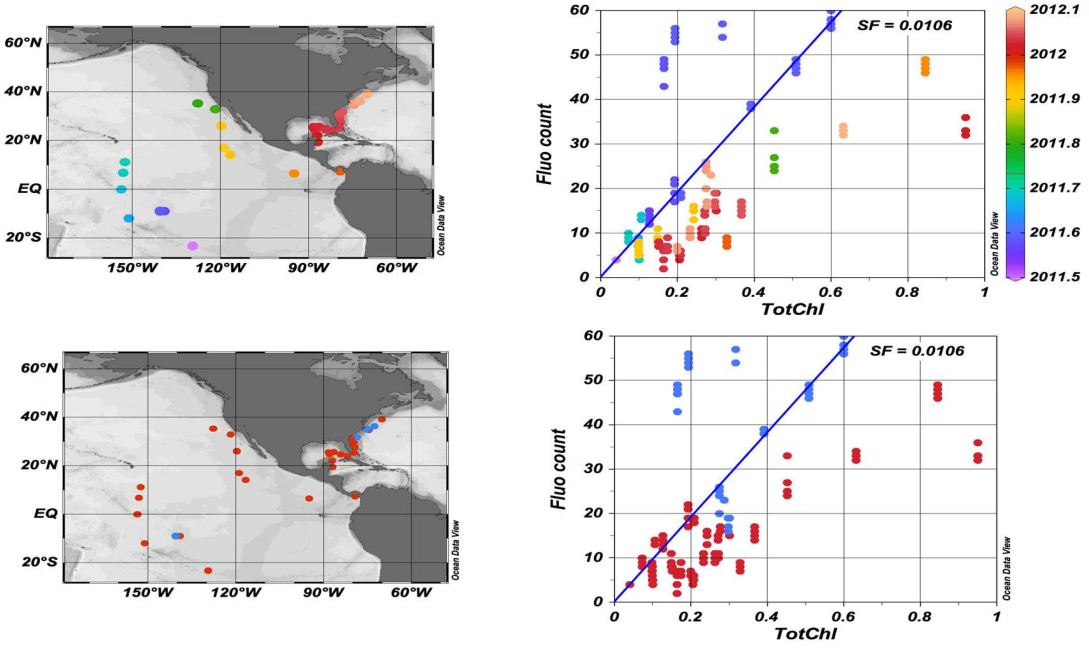
Overall, the Fluo counts timeseries after the binning operation remains quite dynamic with reflects the natural variations of fluorescence on the path of the vessel. One point that will demand some attention at a stage of visual quality control would be to flag the values of high dispersion related to the problem PB1 of default in seawater filtering operation. This quality control can be assisted considering the values of standard deviation that provide a robust indication on the spreading of the values inside each 5min bins. Another way would be to increase the threshold on the number of scans per bin (fixed to 5 scans), with the risk to degrade the signal when it is computed with few scans, such as pointed out by PB2. Another point that needs to be explored is that the effect of quenching is not synchronized with the expected effect of daylight.

Calibration relationship using HPLC measurements

Some surface concentrations of Chlorophyll-a have been measured during the period of interest. Some bottle samples of 2L collected at CTD casts have been filtered on 43mm-diameter HF/F filters and analyzed by chromatographic technique (HPLC). Accounting for bottle samples up to 5m-depth, there are 52 available measurements, reduced to 36 measurements when the matchup with TSG Fluo counts is performed.

A cross-comparison of these 36 measures of total chlorophyll (TChl) is exploited considering a time lag of 30 min around each measure. Up to 5 Fluo counts per TChl are then extracted, retrieving the Dark count given from filtering operation at the same time, for comparison.

The points of comparison are spread in the Pacific Ocean and around the North American coasts (date and position in the upper panel figures). A first guess of quantitative comparison would account for only night casts (blue dots in the lower panel figures, red dots are day casts), when Fluo counts are not affected by quenching effects. Thus the ensemble is drastically reduced, mostly including samples taken at Marquises Island and few offshore New York. Considering the Marquises samples, there are some



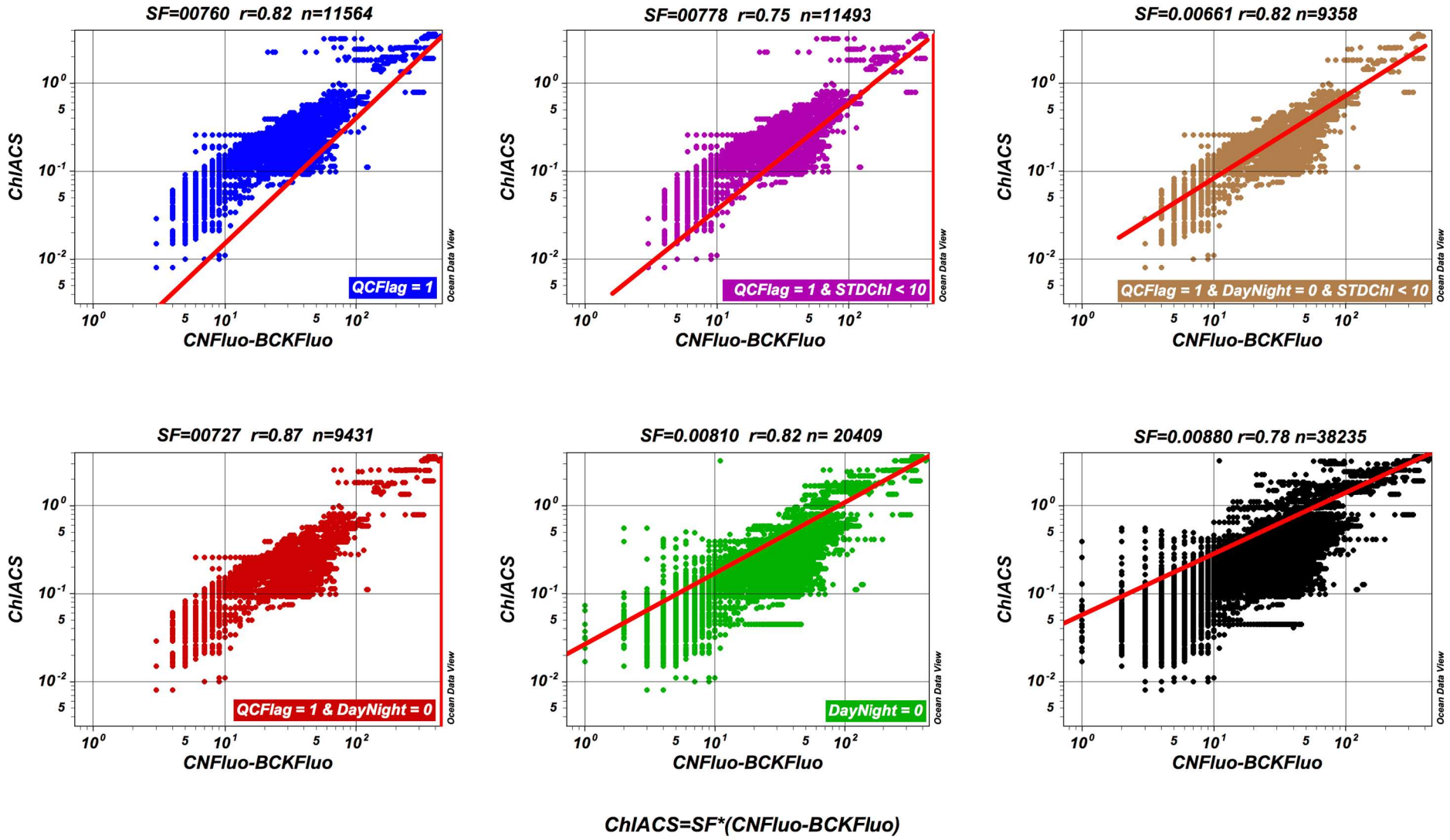
Fluo counts that appear out of range (40-60 counts for 0.2-0.3 mg/m^3 concentrations). The linear regression on the final ensemble (blue points without outliers) provides a scale factor of 0.0106, following the relation: [Chl-a] = SF x (CNFluo – BckFluo).

Calibration relationship using AC-S measurements (1km^2 binned)

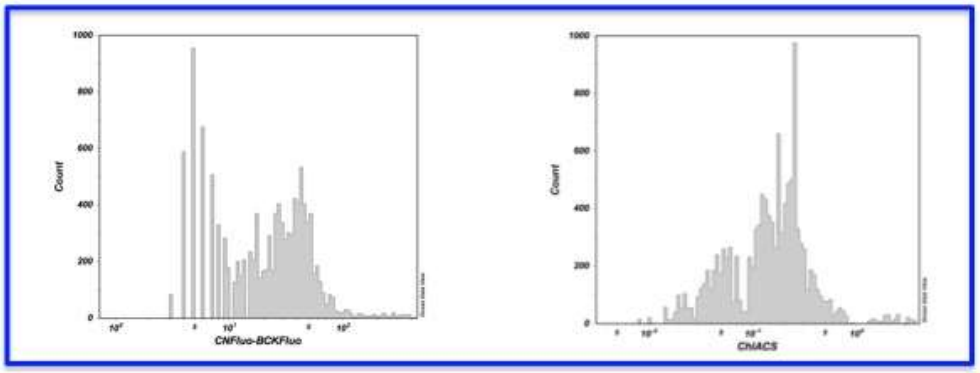
Chlorophyll concentrations have been derived from AC-S measurements along the same cruise track and the same period. In a previous work, HPLC data were used to provide a calibration to AC-S derived concentrations. So it is proposed to use this timeseries, concomitant with fluorescence measurements, in order to fit a calibration scale factor using a more extensive scatter set. The regression has been lead as following:

- A value of AC-S derived concentration (binned at 1 km^2) has been extracted considering the date and time of each fluorescence measurement (binned at 5 minutes),
- The ratio between WETLabs factory calibration data for chlorophyll concentration from Fluo counts, and AC-S derived concentration is computed,
- Considering 2-days time windows, the 7th percentile of the ratio histogram is computed, as high values of ratio should indicate a good correlation between measurements (thus not affected by quenching), and low values of ratio should mark the quenching effect,
- A flag called QCflag is set up in this purpose (QCflag = 1 for good data and QCflag = 2 for data affected by quenching), and added together with AC-S derived Chlorophyll concentration to the final timeseries.

Different scenarios of cross-comparison are then drawn, from the complete scatter set of couples (Fluo counts – AC-S Chlorophyll), and a reduction without measurements during daylight, or with respect to the NPQflag. The scale factor evolved from 0.009 until 0.007, given that the factory calibration is equal to 0.008. Two other trials that lead to even lower scale factors consider a reduction of the scatter set without high standard deviations (that mostly affect large values of the scatter set). The comparison of histograms for the scatter set shows low value Chlorophyll concentration decreases and a more equilibrated distribution of measurements in agreement with AC-S measurements (even if the peak of low value remains).



ChlACS=SF*(CNFluo-BCKFluo)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CNFluo-BCKFluo
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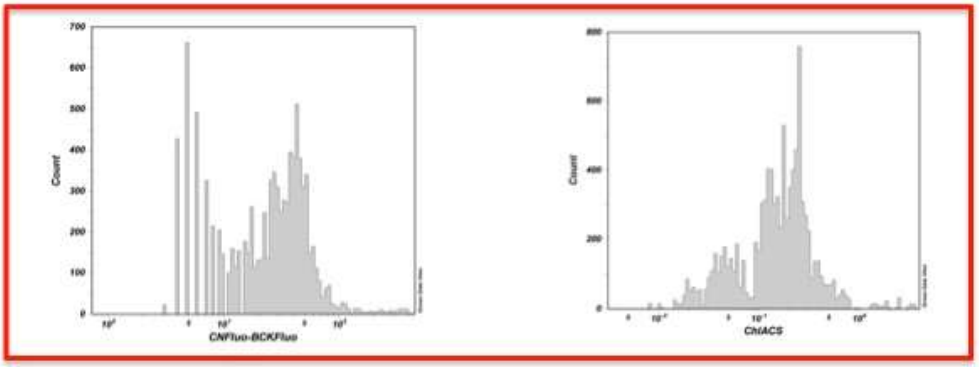
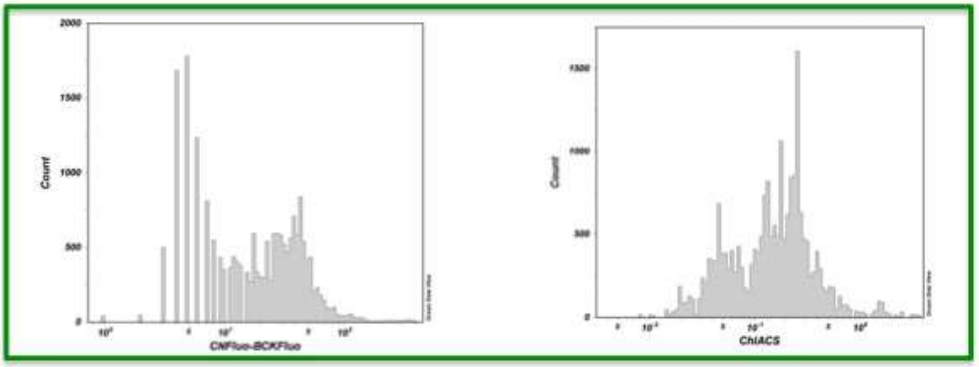
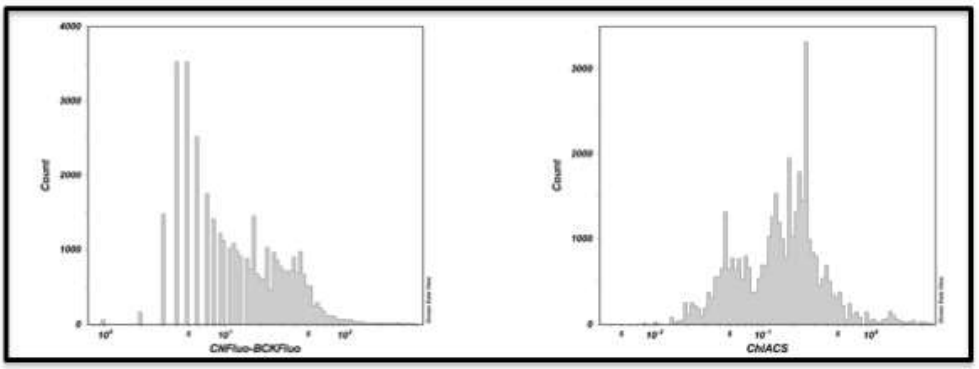
ChlACS


Count
CNFluo-BCKFluo
Count
ChlACS


Count
CNFluo-BCKFluo
Count
ChlACS

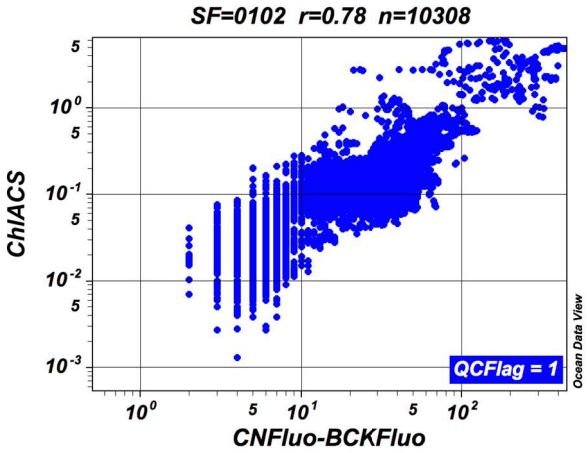

Count
CNFluo-BCKFluo
Count
ChlACS

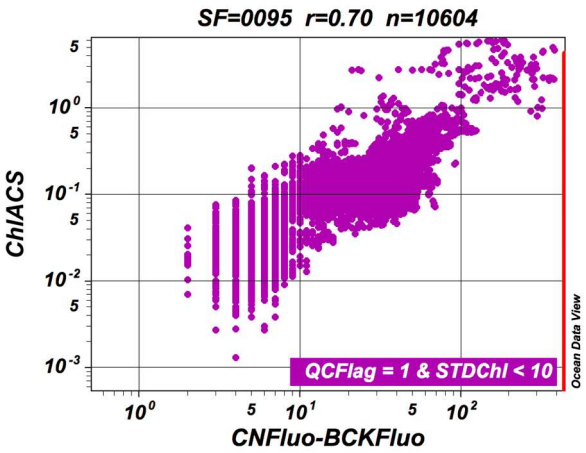
Calibration relationship using AC-S measurements (1min binned)

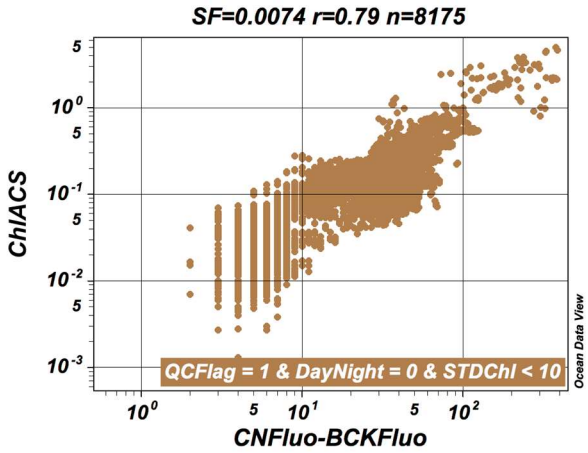
A similar approach has been lead using the same parameter as calibration. Chlorophyll concentrations have been derived from AC-S measurements along the same cruise track and the same period, but with a resolution of 1minute instead of spatial resolution of 1 km^2. Changes are expected at stations that were lasting some hours to some days. The following processing has been performed:

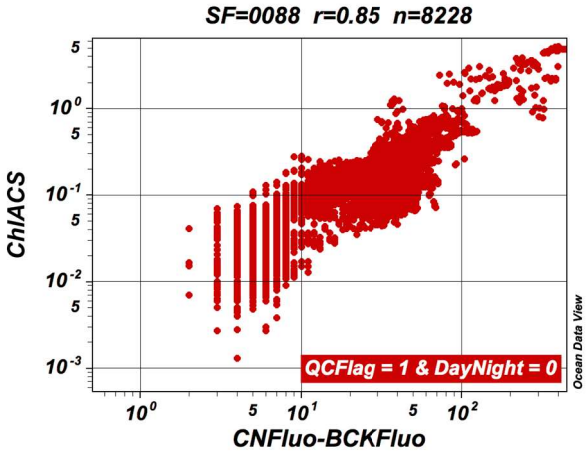
- A mean value of AC-S derived concentration has been calculated every 5 minutes in order to fit with the frame of the timeseries of fluorescence measurement,
- The ratio between WETLabs factory calibration data for chlorophyll concentration from Fluo counts, and AC-S derived concentration is computed,
- Considering 2-days time windows, the 7^{th} percentile of the ratio histogram is computed, as high values of ratio should indicate a good correlation between measurements (thus not affected by quenching), and low values of ratio should mark the quenching effect,
- A flag called QCflag is set up in this purpose (QCflag = 1 corresponding to good data, QCflag = 2 corresponding to data probably affected by quenching, QCflag = 3 corresponding to questionable data specially with respect to PB1 stressed before), and added together with AC-S derived Chlorophyll concentration to the final timeseries.

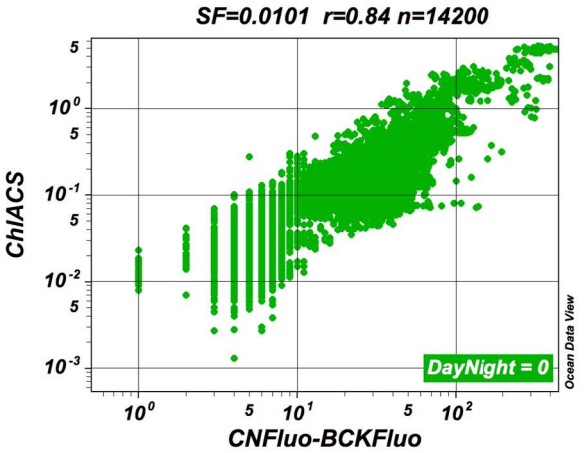
As for the previous calibration exercise, different scenarios of cross-comparison are then drawn, from the complete scatter set of couples (Fluo counts – AC-S Chlorophyll), and a reduction without measurements during daylight, or with respect to the QCflag. The scale factor evolved from 0.007 until 0.011 with a scale factor of 0.0088 in the case “red”, given that the factory calibration is equal to 0.0079. This alternative approach tends to overestimate the previous calibration approach that was closer to factory calibrations. However, higher scale factors are in better agreement with the direct calibration with HPLC measurements.

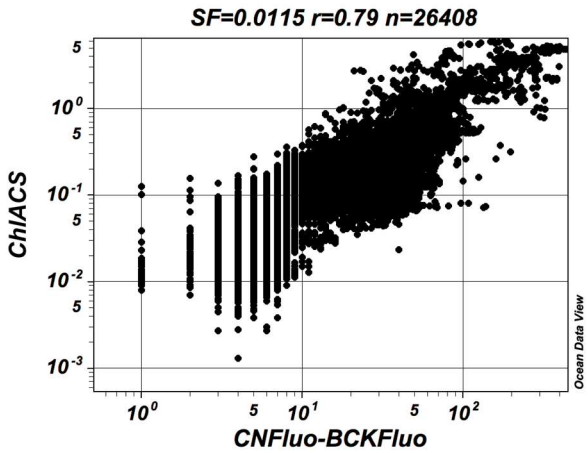












ChlACS=SF*(CNFluo-BCKFluo)

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ECO Chlorophyll Fluorometer Characterization Sheet

Date: 6/25/2009 S/N: FLRTD-1537

Chlorophyll concentration expressed in µg/l can be derived using the equation:

CHL (µg/l) = Scale Factor * (Output - Dark Counts)

	Analog Range 1	Analog Range 2	Analog Range 4 (default)		Digital	
Dark Counts	0.056	0.026	0.010	V	49	counts
Scale Factor (SF)	6	13	25	µg/l/V	0.0076	µg/l/count
Maximum Output	4.96	4.96	4.96	V	16396	counts
Resolution	0.6	0.6	0.6	mV	1.0	counts

Ambient temperature during characterization 22.3 °C

Analog Range: 1 (most sensitive, 0–4,000 counts), 2 (midrange, 0–8,000 counts), 4 (entire range, 0–16,000 counts).

Dark Counts: Signal output of the meter in clean water with black tape over detector.

SF: Determined using the following equation: SF = x ÷ (output - dark counts), where x is the concentration of the solution used during instrument characterization. SF is used to derive instrument output concentration from the raw signal output of the fluorometer.

Maximum Output: Maximum signal output the fluorometer is capable of.

Resolution: Standard deviation of 1 minute of collected data.

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ECO Chlorophyll Fluorometer Characterization Sheet

Date: 8/16/2010 S/N: FLRTD-1537

Chlorophyll concentration expressed in µg/l can be derived using the equation:

CHL (µg/l) = Scale Factor * (Output - Dark Counts)

	Analog Range 1	Analog Range 2	Analog Range 4 (default)		Digital	
Dark Counts	0.056	0.026	0.011	V	49	counts
Scale Factor (SF)	6	13	26	µg/l/V	0.0077	µg/l/count
Maximum Output	4.96	4.96	4.96	V	16396	counts
Resolution	0.9	0.9	0.9	mV	1.0	counts

Ambient temperature during characterization 22.3 ℃

Analog Range: 1 (most sensitive, 0–4,000 counts), 2 (midrange, 0–8,000 counts), 4 (entire range, 0–16,000 counts).

Dark Counts: Signal output of the meter in clean water with black tape over detector.

SF: Determined using the following equation: SF = x ÷ (output - dark counts), where x is the concentration of the solution used during instrument characterization. SF is used to derive instrument output concentration from the raw signal output of the fluorometer.

Maximum Output: Maximum signal output the fluorometer is capable of.

Resolution: Standard deviation of 1 minute of collected data.

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ECO Chlorophyll Fluorometer Characterization Sheet

Date: 6/20/2012 S/N: FLRTD-1537

Chlorophyll concentration expressed in µg/l can be derived using the equation:

CHL (µg/l) = Scale Factor * (Output - Dark Counts)

	Analog Range 1	Analog Range 2	Analog Range 4 (default)		Digital	
Dark Counts	0.054	0.025	0.011	V	46	counts
Scale Factor (SF)	6	13	25	µg/l/V	0.0076	µg/l/count
Maximum Output	4.96	4.96	4.96	V	16396	counts
Resolution	0.7	0.7	0.7	mV	0.8	counts

Ambient temperature during characterization 22.2 °C

Analog Range: 1 (most sensitive, 0–4,000 counts), 2 (midrange, 0–8,000 counts), 4 (entire range, 0–16,000 counts).

Dark Counts: Signal output of the meter in clean water with black tape over detector.

SF: Determined using the following equation: SF = x ÷ (output - dark counts), where x is the concentration of the solution used during instrument characterization. SF is used to derive instrument output concentration from the raw signal output of the fluorometer.

Maximum Output: Maximum signal output the fluorometer is capable of.

Resolution: Standard deviation of 1 minute of collected data.