DATA PRODUCT SPECIFICATION FOR FLUOROMETRIC CHLOROPHYLL-*a* CONCENTRATION

Version 1-01
Document Control Number 1341-00530
2012-05-18

Consortium for Ocean Leadership
1201 New York Ave NW, 4th Floor, Washington DC 20005
www.OceanLeadership.org

in Cooperation with

University of California, San Diego
University of Washington
Woods Hole Oceanographic Institution
Oregon State University
Scripps Institution of Oceanography
Rutgers University

Document Control Sheet

Version	Date	Description	Author
0-01	2012-03-09	Initial Draft	M. Neely and O. Schofield
0-02	2012-03-19	Sarah Webster/Megan Gibney edits to draft	S. Webster and M. Gibney
0-03	2012-03-29	Sue Banahan edits to draft	S. Banahan
0-04	2012-04-30	1st draft after internal review. Minor updates to formatting and layout.	M. Neely
0-05	2012-05-01	Added scale factor and dark counts to sample input and output table, added reference.	M. Neely
0-06	2012-05-15	Clarified analog is not needed	M. Neely
1-00	2012-05-16	Initial Release	E. Chapman
1-01	2012-05-18	Formatting	E. Griffin

Signature Page

This document has been reviewed and approved for release to Configuration Management.

OOI Chief Systems Engineer:

Date: 2012-05-16

This document has been reviewed and meets the needs of the OOI Cyberinfrastructure for the purpose of coding and implementation.

OOI CI Signing Authority:

Date: 2012-05-16

Table of Contents

1 Abstract ... 1
2 Introduction ... 1
2.1 Author Contact Information ... 1
2.2 Metadata Information ... 1
2.3 Instruments ... 2
2.4 Literature and Reference Documents ... 2
2.5 Terminology ... 3
3 Theory ... 4
3.1 Description ... 4
3.2 OOI pre-deployment field or laboratory calibration and post-recovery data QC considerations: ... 5
3.3 Mathematical Theory ... 6
3.4 Known Theoretical Limitations ... 6
3.5 Revision History ... 7
4 Implementation ... 7
4.1 Overview ... 7
4.2 Inputs ... 7
4.3 Processing Flow ... 7
4.4 Outputs ... 8
4.5 Computational and Numerical Considerations ... 9
4.6 Code Verification and Test Data Set ... 9
Appendix A Example Code ... 1
Appendix B Output Accuracy ... 1
Appendix C Sensor Calibration Effects ... 2
Appendix D Sample Factory Characterization Sheet ... 3

1 Abstract

This document describes the computation used to calculate the OOI Level 1 Fluorometric Chlorophyll-*a* Concentration core data product, which is calculated using data from the WetLabs two and three channel fluorometers (FLORD and FLORT, respectively). This document is intended to be used by OOI programmers to construct appropriate processes to create the OOI Level 1 Fluorometric Chlorophyll-*a* Concentration core data product.

2 Introduction

2.1 Author Contact Information

Please contact Merrie Beth Neely (mneely@oceanleadership.org) for more information concerning the computation and other items in the main document. Contact Oscar Schofield (oscar@rutgers.edu) for more information concerning the sample code and data set appendices, or the Data Product Specification lead (DPS@lists.oceanobservatories.org).

2.2 Metadata Information

2.2.1 Data Product Name

The OOI Core Data Product Name for this product is

- CHLAFLO

The OOI Core Data Product Descriptive Name for this product is

- Fluorometric Chlorophyll-*a* Concentration

2.2.2 Data Product Abstract (for Metadata)

The OOI Level1 Fluorometric Chlorophyll-*a* Concentration core data product is a measure of how much light has been re-emitted after being absorbed by chlorophyll-*a* molecules found in all phytoplankton. By measuring the intensity and nature of this fluorescence, phytoplankton biomass can be estimated. The concentration of chlorophyll-*a* is a proxy for the abundance of phytoplankton in the water column, and thus the amount of primary productivity that can be empirically achieved. Chlorophyll absorbs photons in the visible spectrum (400-700nm) and fluoresces visible blue light.

2.2.3 Computation Name

Not required for data products.

2.2.4 Computation Abstract (for Metadata)

This computation computes the OOI Level 1 Fluorometric Chlorophyll-*a* Concentration core data product, which is computed using data from the WetLabs two and three channel fluorometer (FLORD/FLORT).

2.2.5 Instrument-Specific Metadata

See Section 4.4 for instrument-specific metadata fields that must be part of the output data.

2.2.6 Data Product Synonyms

Synonyms for this data product are

- Chlorophyll-*a* fluorescence
- Chlorophyll-*a* concentration

2.2.7 Similar Data Products

A similar product that this data product may be confused with this is CDOMFLO, which yields the same measurement (but at different excitation and emission wavelengths), uses a similar calibration and zeroing principle and essentially the same equation, but processes the Colored Dissolved Organic Matter (CDOM) fluorescence data stream from the instrument instead of the Chlorophyll-*a* fluorescence.

2.3 Instruments

The WETLabs ECO triplet was selected by the OOI to make this measurement on both mobile and fixed platforms. The fixed platform instrument will have a wiper to actively limit biofouling, while those installed on mobile assets (profilers, gliders, and AUVs) will have only passive mitigation of biofouling (coating and copper faceplates). For information on the instruments from which the inputs to OOI Level 1 Fluorometric Chlorophyll-*a* Concentration core data product are obtained, see the FLORT Processing Flow document (DCN 1342-00530). This document contains information on the FLORT and FLORD instrument classes make/models; it also describes the flow of data from the FLORT/FLORD instruments through all of the relevant QC, calibration, and data product computations and procedures.

Note that the raw data from the FLORD/FLORT make/model—the flourometers on board the gliders and autonomous underwater vehicles (AUVs)—are processed onboard the vehicles with proprietary software from the vehicle vendors. These data are presented already in decimal format in appropriate units therefore processing raw hexadecimal data from the FLORD/FLORT is not included in the algorithm described in this document.

Please see the Instrument Application in the SAF for specifics of instrument locations and platforms.

2.4 Literature and Reference Documents

WETlabs ECO FL User's Guide (FL) Revision AM 14 Sept. 2011

WETlabs ECO Triplet-w User's Guide (triplet w) Revision C 28 Sept. 2011

Ivona Cetinić, Gerardo Toro-Farmer, Matthew Ragan, Carl Oberg, and Burton H. Jones, 2009. Calibration procedure for Slocum glider deployed optical instruments. Optics Express, 17(18):15420-15430

Falkowski, P. G. 1981. Light-shade adaptation and assimilation numbers. *Journal of Plankton Research*, **3**, 203-216.

HAWAII OCEAN TIME-SERIES (HOT) AND COUPLED OCEAN-ICE LINKAGES AND DYNAMICS (COLD) FIELD AND LABORATORY PROTOCOLS October 2000 2nd edition

Herbland, A., A. Le Bouteiller, and P. Raimbault. (1985). Size structure of phytoplankton biomass in the equatorial Atlantic Ocean. *Deep-Sea Res.*, **32**: 819-836.

Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes and J. D. H. Strickland. 1965. Fluorometric determination of chlorophyll. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **30**, 3-15.

Holm-Hansen, O. and B. Riemann. 1978. Chlorophyll *a* determination: improvements in methodology. *Oikos*, **30**, 438-447.

JGOFS Protocols Manual 1994 (JGOFS Report Nr. 19) Chapter 14. Measurement of Chlorophyll *a* and Phaeopigments by Fluorometric Analysis (or more recent vers)

Latasa, M., R. R. Bidigare, M. E. Ondrusek and M. C. Kennicutt II. 1996. HPLC analysis of algal pigments: a comparison exercise among laboratories and recommendations for improved analytical performance. *Marine Chemistry*, **51**, 315-324.

Laws, E. A., D. M. Karl, D. G. Redalje, R. S. Jurick and C. D. Winn. 1983. Variability in ratios of phytoplankton carbon and RNA to ATP and Chl *a* in batch and continuous cultures. *Journal of Phycology*, **19**, 439-445.

Mantoura, R. and C. Llewellyn. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high performance liquid chromatography. *Analytica Chimica Acta*, **151**, 297-314.

Nelson, J. R. 1993. Rates and possible mechanism of light-dependent degradation of pigments in detritus derived from phytoplankton. *Journal of Marine Research*, **51**, 155-179.

Strickland, J. D. H. and T. R. Parsons. 1972. *A Practical Handbook of Seawater Analysis*, Fisheries Research Board of Canada, 167 pp.

See also anything posted:
https://confluence.oceanobservatories.org/display/science/Common+Instrument+ICD

Or

https://confluence.oceanobservatories.org/display/Presentations/OOI+FLOR+Collaboration

2.5 Terminology

2.5.1 Definitions

The following terms are defined here for use throughout this document. Definitions of general OOI terminology are contained in the Level 2 Reference Module in the OOI requirements database (DOORS).

Chlorophyll-*a* fluorescence - is light that has been re-emitted after being absorbed by chlorophyll-*a* molecules found in all phytoplankton. By measuring the intensity and nature of this fluorescence, phytoplankton biomass can be estimated. The FLORD/FLORT instrument delivers the raw counts from chlorophyll-*a* fluorescence contained in a water sample.

CDOM – Colored Dissolved Organic Matter, or Chromophoric Dissolved Organic Matter, either is appropriate.

concentration - is a measure of the amount of something, in this case Chlorophyll-*a* in micrograms per liter.

scale factor – is used to scale the output counts of the instrument to a solution with a known concentration, then subtracting the meter's dark counts. This factor is calculated at the factory for each instrument and provided on the instrument calibration sheet – it is an instrument-specific constant that will change with each factory calibration. The scale factor is then applied to the output signal to provide the direct conversion of the output counts to chlorophyll concentration. For chlorophyll, WET Labs uses the chlorophyll equivalent concentration (XX) as the signal output using a fluorescent proxy approximately equal to 25 microg/l of a *Thalassiosira weissflogii* phytoplankton culture used during factory instrument characterization as shown here.

Scale Factor [microg/l/counts] = 25 microg/l ÷ (XX – dark counts)
Example: 25 ÷ (3198 – 71) = 0.0080.
.

While this constant can be used to obtain approximate values, field calibration is highly recommended (see Cetinic et al for procedures). See appendix D for an example of the factory characterization sheet.

dark counts - The instrument's baseline reading in the absence of source light is the dark count value. A dark count value is determined at the factory for each instrument and provided on the instrument calibration sheet The *factory* dark count is used to calculate the *factory* scale factor. While this constant can be used to obtain approximate values, field calibration is highly recommended. This is determined by measuring the signal output in clean, de-ionized water with black tape over the detector

2.5.2 Acronyms, Abbreviations and Notations

General OOI acronyms, abbreviations and notations are contained in the Level 2 Reference Module in the OOI requirements database (DOORS). The following acronyms and abbreviations are defined here for use throughout this document.

VDC – volts of DC current

2.5.3 Variables and Symbols

The following variables and symbols are defined here for use throughout this document.

XX = concentration of a sample of interest (microgram per liter)
Coutput = raw counts "calibrated" digital data output when measuring a sample of interest
Cdc = dark counts, the measured signal "calibrated" digital data output of meter in clean water with black tape over the detector
scale_factor = multiplier in microgram per liter per volts or ppb per volts

3 Theory

3.1 Description

The WET labs Environmental Characterization Optics (ECO) miniature fluorometer allows the user to measure relative chlorophyll, CDOM, uranine, phycocyanin, or phycoerythrin (the latter three are not currently included as OOI core data products) concentrations by directly measuring the amount of fluorescence emission in a sample volume of water. The ECO miniature fluorometer uses an LED to provide the excitation source. An interference filter is used to reject the small amount of out-of-band light emitted by the LED. The light from the source enters the water volume at an angle of approximately 55–60 degrees with respect to the end face of the unit. Fluoresced light is received by a detector positioned where the acceptance angle forms a 140-degree intersection with the source beam. An interference filter is used to discriminate against the scattered excitation light.

Raw data from the FLORD/FLORT instrument is output in counts from the sensor, ranging from 0 to approximately 16000. The scale factor is factory-calculated by obtaining a consistent output in a solution with a known concentration, then subtracting the meter's dark counts. The scale factor, dark counts, and other characterization values are given on the instrument's characterization sheet shipped by the vendor with each meter. The scale factor is then applied to the output signal

to provide the direct conversion of the output to chlorophyll *a* concentration. While scale factor can be used to obtain approximate values, field calibration is highly recommended. It is important to perform calibrations using similar seawater and blanking with 0.2micron filtered seawater (or suitable replacements for both – see Cetinic et al) as you expect to encounter *in situ.* This will provide a refined dark count, an accurate blank, equivalent phytoplankton types and similar physiological conditions for further refining the scale factor, thereby providing an accurate and meaningful calibration. The instrument response is linear over the measurement range provided (0 to 16000 counts, or 0-5 Volts). Once a zero point has been determined and a refined scale factor established from the field/shipboard calibration prior to deployment, the conversion of dark count volts to chlorophyll *a* concentration is straightforward using the equation:

XX = (Coutput – Cdc) * scale_factor

Where:

XX = concentration of a sample of interest (microgram per liter)
Coutput = calibrated voltage output when measuring a sample of interest
Cdc = dark counts, the calibrated measured signal output (in VDC) of meter in 0.2micron filtered seawater with black tape over the detector
scale_factor = multiplier in microgram per liter per volts

Optical Specifications for Fluorescence Meters

Specifications given below are typical. Linearity for all is 99% R2. Other ranges are available on Request from WETlabs.

Parameter	EX/EM	Sensitivity	Range
Chlorophyll-*a* (Chl)	470/695 nm	0.02 µg/l	0–125 µg/l
Colored Dissolved Organic Matter (CDOM)	370/460 nm	0.09 ppb	0–500 ppb

3.2 OOI pre-deployment field or laboratory calibration and post-recovery data QC considerations:

Scale factor: WET Labs supplies a scale factor that can be found on the instrument-specific calibration sheet that ships with each meter. In the field the Scale factor is refined by obtaining a consistent output in a solution with a fluorescent proxy approximately equal to 25 microg/l of a *Thalassiosira weissflogii* phytoplankton culture (XX), then subtracting the meter's dark counts. Land-based or shipboard laboratory determination of chlorophyll *a* fluorescence (both **pre-and post-deployment**) should be made to further refine the scale factor calculation for data QC use when processing real-time L1b data. This measurement should be repeated multiple times, using replicates of serial dilution cultures. Field proxy measurements from *in situ* instruments and filtered water samples for chlorophyll *a* extraction (see various references above for example protocols – the one implemented will eventually be found in OOI 1102-00300: PROTOCOLS AND PROCEDURES FOR OOI DATA PRODUCTS: QA, QC, CALIBRATION, PHYSICAL SAMPLES) are necessary for comparison at the time of deployment and recovery of both fixed instruments and instruments on mobile platforms. This is especially important for instruments deployed in the hulls of gliders and AUVs. Instrument-specific drift attributable to biofouling vs. inherent drift can be determined **post-recovery** by taking replicate chlorophyll *a* measurements in filtered seawater both before and after the biofouling has been thoroughly chemically and mechanically removed from the optical window (see Cetinic et al). The latter measurement represents inherent drift and the difference between the initial measurements and the inherent drift measurements represents that from biofouling that affect the scale factor calculation applied (see also dark counts below). These post-recovery measurements require minimal human in the loop time and effort and are used to determine the linear application of automated QC for both types of drift in L1c data.

dark counts: Land-based or shipboard laboratory determination of dark counts (pre-deployment) should be made to further refine the scale factor calculation for data QC use when processing real-time L1b data. This is determined by measuring the signal output in filtered seawater with black tape over the detector in a darkened room or chamber devoid of ambient light. This measurement should be repeated multiple times, using different placements of tape to maximize detector coverage. This is especially important for instruments deployed in the hulls of gliders and AUVs. Instrument-specific drift attributable to biofouling vs. inherent drift can be determined **post-recovery** by taking repeating the pre-deployment dark count procedures – making repeated measurements in fluorescence free water both before and after the biofouling has been thoroughly chemically and mechanically removed from the optical window (see Cetinic et al). The latter measurement represents inherent drift and the difference between the initial measurements and the inherent drift measurements represents that from biofouling that affect the dark count value applied to determine the corrected post-recovery scale factor. These post-recovery measurements require minimal human in the loop time and effort and are used to determine the linear application of automated QC for both types of drift in L1c data.

3.3 Mathematical Theory

It is possible that the instrument may be set up to operate in either analog or digital format which are both measured in volts, but the OOI will only support digital. The driver and transforms do not need to support analog. The equations are essentially the same and the OOI will use the digital version provided below.

Digital data:
Scaling is linear. Once a zero or blank has been determined and a refined scale factor established from field/shipboard calibration prior to deployment, the conversion of volts to chlorophyll *a* concentration is straightforward using the equation below. Obtaining the concentration (XX) simply involves subtracting a digital dark counts value and multiplying the difference by the instrument scaling factor. Use the equation:

XX = (Coutput – Cdc) * scale_factor

3.4 Known Theoretical Limitations

Failure to account for fluorescence from degraded chlorophyll-*a*, phaeopigments and other refractory pigments found in CDOM can lead to overestimates of the chlorophyll-*a* concentration, phytoplankton biomass and primary productivity in an ecosystem. If both constituents are measured coincidentally (i.e. three channel fluorometry is enabled, vs. only two channel) then a correction factor can be applied to the chlorophyll-*a* data stream. This is not possible unless CDOM is coincidentally measured, so the inherent error in chlorophyll-*a* must be reported where CDOM is not coincidentally measured.

Additionally, the quantum yield of chlorophyll fluorescence is variable when light values are saturating. Light intensities that saturate the photosynthetic machinery will lead to a decrease in the fluorescence quantum yield (fluorescence/unit chlorophyll-*a*), therefore the fluorescence will decrease even though the chlorophyll concentrations have remained constant. Therefore caution should be used in interpreting the chlorophyll fluorescence as an absolute measure of chlorophyll concentrations in the well-lit surface waters during periods of peak light intensity.

Also chlorophyll-*a* concentration is a proxy for phytoplankton biomass and is not a direct measure of it. The same chlorophyll-*a* concentration can be reported for a water mass with low abundance of large phytoplankton or high abundance of small phytoplankton. In addition, only the light harvesting pigment of chlorophyll-*a* fluorescence is measured by this method, and no estimate of the presence or abundance of accessory pigments is included, which can be diagnostic of phytoplankton community groups; and the photosystem ‘health’ of the phytoplankton assemblage is also not assessed with this method, as may be possible with other types of fluorometry.

No other known theoretical limitations to date.

3.5 Revision History

No revisions to date.

4 Implementation

4.1 Overview

Level 0 (raw data) from the FLORD/FLORT instrument is output in counts from the sensor, ranging from 0 to approximately 16000. The conversion from L0 to the L1 Fluorometric Chlorophyll-*a* Concentration data product is implemented using either the analog or digital version of a linear scaling equation.

4.2 Inputs

Inputs are:

- L0 counts ranging from 0 to 16000 (corresponds to a direct current volt range of 0 to 5).
- Scale factor from at-sea, pre-deployment calibration (or factory-supplied instrument calibration sheet in the absence of a field calibration), saved as part of the instrument metadata
- Cdc from pre-deployment bench test, saved as part of the instrument metadata
- Preset emission (470nm) and excitation wavelengths (695nm) for chlorophyll *a*

Input Data Format

- The L0 Fluorometric Chlorophyll-*a* Concentration data product is a 6 digit floating decimal string.

Range checks on the inputs are as part of the global range check (GLBLRNG, DCN 1341-10004) specified in the FLORD/FLORT Processing Flow documents (DCN 1342-00531/DCN 1342-00530). A separate range check on the inputs does not need to be applied.

4.3 Processing Flow

Pre-processing and Product-specific calibration: *The instrument-specific information (such as the scale factor and factory calibration method) must be captured from the most-recent factory calibration sheet, the instrument must be appropriately field calibrated using in situ water and also ‘zeroed’ or a blank determined using water free of fluorescent matter.*

The dark count and scale factor values from the factory documentation can be used as a default setup for the concentration calculation, but the refined values for both obtained during field/shipboard calibration pre-deployment must be transmitted to CI to ‘update’ the automated pre-deployment (L1b) data QC equations applied to the real-time data.

Product-specific quality control: *As described above under* ***OOI pre-deployment field or laboratory calibration and post-recovery data QC considerations****, a linear or curvilinear optical biofouling correction can be applied based upon at-recovery on-board testing or ship of opportunity comparisons that are available. This too must be transmitted to CI to ‘update’ the automated post-recovery (L1c) data QC equations that will be applied to the data.*

Error handling: *none.*

Post-processing: *The appropriate automated QC tests are Global range, spike, stuck value, and with some future local knowledge applied a local range and temporal spatial gradient tests may be applied. However, the nature of phytoplankton abundance is patchy and blooms can be both transient and dynamic – characteristics which make it difficult to set the parameters for all these tests with certainty. See also* ***OOI pre-deployment field or laboratory calibration and post-recovery data QC considerations*** *above.*

The specific steps necessary to create all calibrated and quality controlled data products for each OOI core instrument are described in the instrument-specific Processing Flow documents (DCN 1342-00530). These processing flow documents contain flow diagrams detailing all of the specific procedures (data product and QC) necessary to compute all levels of data products from the instrument and the order in which these procedures are to be applied.

The processing flow for the Fluorometric Chlorophyll-*a* Concentration computation is as follows:

Step 1:
The marine operator must perform the field/shipboard determination of dark counts to serve as a zero or blank procedure using *in situ* field water filtered through 0.2 uM membrane filters. The dark count from field/shipboard calibration is saved as instrument metadata to be provided to CI immediately post-deployment as an update from the factory provided dark count used as a default.

Step 2:
The marine operator must perform a field calibration using similar seawater as you expect to encounter *in situ*. This provides equivalent phytoplankton types and similar physiological conditions for calculating the scale factor, thereby providing an accurate and meaningful calibration. The scale factor from field calibration is saved as instrument metadata to be provided to CI immediately post-deployment as an update from the factory provided scale factor used as a default. The instrument can then be deployed.

Step 3 (digital mode):
For digital mode, the conversion of counts from the instrument to chlorophyll concentration is straightforward using the equation:

XX = (Coutput – Cdc) * scale_factor

Where:
XX = concentration of a sample of interest (microgram per liter)
Coutput = output in counts when measuring a sample of interest
Cdc = dark counts, the measured signal output of the fluormeter in clean water with black tape over the detector
scale_factor = multiplier in microgram per liter per volts

4.4 Outputs

The output of the Chlorophyll-*a* concentration computation is

- Chlorophyll-*a* concentration in micrograms per liter as a 6 digit floating decimal string.

There is no OOI Level 2 science program requirement for accuracy, precision or drift of chlorophyll-*a* estimates.

The metadata that must be included with the output are

- factory or field/shipboard refined scale factor used in this calculation
- factory or field/shipboard refined dark counts (in volts or counts) used in this calculation
- zero or blank (in volts or founts) set on the instrument during field/shipboard calibration

- Any corresponding post-deployment or at-recovery field chlorophyll-*a* extraction, *in situ* measurements, or post-recovery drift measurements used to refine the above field calibration factor for L1b or L1c data.

4.5 Computational and Numerical Considerations

4.5.1 Numerical Programming Considerations

There are no numerical programming considerations for this computation. No special numerical methods are used.

4.5.2 Computational Requirements

- Assuming we are reprocessing the data upon recovery of the various assets, and that one sample is a single data point from any FLOR, and example number of samples are as follows.
- For an RSN or global mooring riser or global flanking mooring: 1 FLORs each mooring * 12 samples/hour * 24 hours *365 days= 105120 samples.
- For an endurance or Pioneer mooring: 3 FLORs each mooring * 12 samples/hour*24 hours*365 days = 315360 samples
- For a coastal glider: 1 sample/second * 6 months = 1.6 *10^7 samples.
- For an AUV: 1 sample/second * 6 months = 1.6 *10^7 samples.
- For a deep profiler on RSN: 1 sample/second for a 1000m profile with a profiler moving at 0.5 m/s operating 48 times per day (assumes that a CTD profile is taken on both down and up casts and profiler is operating continuously) for 365 days = 3.5 *10^7 samples. NOTE these instruments are also located on global, endurance, pioneer and RSN surface piercing profilers, deep profilers, and wire following profilers.

4.6 Code Verification and Test Data Set

The code will be verified using the test data set provided, which contains inputs and their associated correct outputs. CI will verify that the code is correct by checking that the output, generated using the test data inputs, is identical to the test data density output.

Input: factory dark count 45, factory scale factor 0.0121 micrograms chl/count, factory temp at time of characterization 21.5˚C. Red highlighted text below indicates L0 data input.

date (RT)	time (RT)	CHL (em)	CHL raw
99/99/99	99:99:99	695	54
99/99/99	99:99:99	695	52
99/99/99	99:99:99	695	51
99/99/99	99:99:99	695	52
99/99/99	99:99:99	695	52
99/99/99	99:99:99	695	51
99/99/99	99:99:99	695	51
99/99/99	99:99:99	695	52
99/99/99	99:99:99	695	52
99/99/99	99:99:99	695	54
99/99/99	99:99:99	695	51
99/99/99	99:99:99	695	51

99/99/99	99:99:99	695	51
99/99/99	99:99:99	695	50
99/99/99	99:99:99	695	50
99/99/99	99:99:99	695	52

Output:

Count	Date	Time	CHL(ug/l)
1	999999	999999	0.1089
2	999999	999999	0.0847
3	999999	999999	0.0726
4	999999	999999	0.0847
5	999999	999999	0.0847
6	999999	999999	0.0726
7	999999	999999	0.0726
8	999999	999999	0.0847
9	999999	999999	0.0847
10	999999	999999	0.1089
11	999999	999999	0.0726
12	999999	999999	0.0726
13	999999	999999	0.0726
14	999999	999999	0.0605
15	999999	999999	0.0605
16	999999	999999	0.0847

Appendix A Example Code

There is no example code for CHLAFLO, but see example code in CDOMFLO which can be used as a framework for building code.

Appendix B Output Accuracy

There is no accuracy requirement for chlorophyll or CDOM fluorescence in the OOI requirements database (DOORS). There is no statement of accuracy by the manufacturer.

Appendix C Sensor Calibration Effects

Failure to account for fluorescence from degraded chlorophyll-*a*, phaeopigments and other refractory pigments found in CDOM during the predeployment calibration process can lead to overestimates of the chlorophyll-*a* concentration in a discrete water sample, and consequently the phytoplankton biomass and primary productivity in an ecosystem. If both chlorophyll-*a* and CDOM concentration are measured coincidentally (i.e. three channel fluorometry is enabled, vs. only two channel) then a correction factor can be applied to the chlorophyll-*a* data stream. This is not possible unless CDOM is coincidentally measured, so the inherent error in chlorophyll-*a* must be reported where CDOM is not coincidentally measured.

Because optical surfaces can be colonized by phytoplankton, and the chlorophyll-*a* in such colonial phytoplankton would be measured as a consequence of being within the optical path of the instrument and likely to contribute to the fluorescence signal, biofouling must be corrected for in the post-deployment signal. Such colonization is likely to be nil at the time of deployment, but rapidly colonize the optical surfaces to quickly reach a carrying capacity, and can be corrected with a linear or logarithmic curve applied to the data. However, unless a reliable estimate of the period of time when the carrying capacity is reach *in situ* can be determined, it is more reasonable to assume a linear or logarithmic correction applied across the entire deployment time period, which assumes no biofouling at deployment and maximum biofouling at recovery.

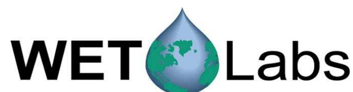
The post-recovery handling and testing procedures for all optical instruments should be well documented in the field procedures because even slight adjustments to the biofouling community will result in dramatic changes to the optical properties reported by the instruments. Thus care should be taken to not disturb, desiccate, or alter the temperature and salinity under which the instruments were held *in situ*. The post-recovery handling methodology shall be informed by the oceanographic community and the vendor's recommendations – as collected and adopted by the OOI program through QC workshops, whitepapers, webinars, and other outreach and community validation efforts.

Additionally, the quantum yield of chlorophyll fluorescence is variable when light values are saturating. Light intensities that saturate the photosynthetic machinery will lead to a decrease in the fluorescence quantum yield (fluorescence/unit chlorophyll-*a*), therefore the fluorescence will decrease even though the chlorophyll concentrations have remained constant. Therefore caution should be used in interpreting the chlorophyll fluorescence as an absolute measure of chlorophyll concentrations in the well-lit surface waters during periods of peak light intensity.

Also chlorophyll-*a* concentration is a proxy for phytoplankton biomass and is not a direct measure of it. The same chlorophyll-*a* concentration can be reported for a water mass with low abundance of large phytoplankton or high abundance of small phytoplankton. Using algorithms and ancillary measurements, some passes at particle size can be obtained through intense "human-in-the-loop" post-processing; however, this level of effort is beyond the scope of the OOI program. In addition, only the light harvesting pigment of chlorophyll-*a* fluorescence is measured by this method, and no estimate of the presence or abundance of accessory pigments is included, which can be diagnostic of phytoplankton community groups; and the photosystem 'health' of the phytoplankton assemblage is also not assessed with this method, as may be possible with other types of fluorometry.

Appendix D Sample Factory Characterization Sheet

PO Box 518
620 Applegate St.
Philomath, OR 97370



(541) 929-5650
Fax (541) 929-5277
www.wetlabs.com

ECO Chlorophyll Fluorometer Characterization Sheet

Date: 1/27/2011 S/N: FLBBCDSLK-2110

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

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

	Digital
Dark counts	45 counts
Scale Factor (SF)	0.0121 μg/l/count
Maximum Output	4130 counts
Resolution	1.1 counts
Ambient temperature during characterization	21.5 ℃

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.

The relationship between fluorescence and chlorophyll-a concentrations in-situ is highly variable. The scale factor listed on this document was determined using a mono-culture of phytoplankton (Thalassiosira weissflogii). The population was assumed to be reasonably healthy and the concentration was determined by using the absorption method. To accurately determine chlorophyll concentration using a fluorometer, you must perform secondary measurements on the populations of interest. This is typically done using extraction-based measurement techniques on discrete samples. For additional information on determining chlorophyll concentration see "Standard Methods for the Examination of Water and Wastewater" part 10200 H, published jointly by the American Public Health Association, American Water Works Association, and the Water Environment Federation.

FLBBCDSLK-2110.xls Revision S 10/4/07