**Calibration and data processing for the Turner Designs 10-AU fluorometer for the 2013 field season**


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| Instrument: | Turner Designs 10-AU fluorometer |
| Model/SN: | 10-AU-005-CE/1100246 |
| Purchase Date: | 2013-02-07 |
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| Document: | Version 1, 2017-01-17 |


## I. Description

The Turner Designs 10-AU fluorometer was used to measure extracted Chl *a* and pheopigments in the laboratory following the NASA Ocean Optics Protocols, Revision 5, Volume V (Trees *et al.* 2003) for both instrument calibration and sample analysis. The fluorometer was equipped with Turner Designs Optical Kit 10-037R, which consists of a daylight white lamp (Turner Designs 10-045), an excitation band-pass filter from 340-500nm (Turner Designs 10-050R), a long-pass emission filter from 665nm (Turner Designs 10-051R) and a reference filter (Turner Designs 10-032). The 10-AU fluorometer has three gain settings (high, medium and low) and auto-corrects for gain in the sample readout. For example, a sample with a fluorescence of 9 FSU on a gain of "low" will also have a fluorescence of 9 FSU on a gain of "medium". Although the fluorometer allows the user to create and store blanks/calibration values to directly calculate chl at the time of measurement, we made all measurements in FSU and retrieved chl concentration during post-processing.

## II. Calibration and Maintenance

The instrument was calibrated on 2014-01-16 with pure Chl *a* (Sigma-Aldrich C6144) extracted in 90% acetone for 24 hours. Optical surfaces in the fluorometer were cleaned at the time of calibration. The concentration of chl for the primary standard was determined from absorption spectra measured in a Lambda 35 UV/VIS spectrophotometer (Perkin-Elmer) in a 1-cm quartz cuvette using 90% acetone as a reference. Chl concentration was retrieved as described in Trees *et al.* 2003 via peak absorption in the red and the extinction coefficient of 87.67 L $g^{-1}$ $cm^{-1}$ (Jeffrey *et al.* 1997). The concentration of the primary standard was 631 µg chl $L^{-1}$. A dilution series ranging between 3 and 40µg chl $L^{-1}$ was created using 50mL volumetric flasks and chilled 90% acetone. Instrument sensitivity was adjusted prior to calibration such that a 10µg chl $L^{-1}$ standard was mid-range on the "medium" scale following the instructions in the user's manual (Turner Designs 1999). Fluorescence was measured for each dilution in triplicate before ($F_b$, FSU) and after ($F_a$, FSU) the addition of a 50µL of 10% HCl. A blank of 90% acetone was also measured. Blank fluorescence was not different before and after acid addition.

A linear regression, forced through the origin, of blank corrected $F_b$ vs. Chl for the dilution series yielded a slope ($F_R$, FSU (µg chl $L^{-1}$)$^{-1}$) and 95% confidence interval of 2.253 ± 0.027 FSU (µg chl $L^{-1}$)$^{-1}$. The mean acid ratio ($\tau$, unitless) was 2.04 ± 0.03 (standard deviation).

## III. Sample collection and processing

All samples from 2014 were analyzed within 6 months of calibration following the procedure of Trees *et al.* 2003. Briefly, water samples were retrieved from the field and filtered within 6 hours of collection onto GF/F filters (Whatman, 25mm, 0.7µm pore size) in triplicate. Filters were immediately frozen in liquid nitrogen and then stored at -80°C until analysis. Working in the dark, filters were placed in pre-chilled 90% acetone and sonicated for 20 to 30s to break cells open. Samples were placed in the freezer (-20°C) and allowed to extract for a minimum of 24 hours prior to analysis. Samples were brought to room temperature and fluorescence measured before and after the addition of 50µL of 10% HCl. Blanks of 90% acetone were measured for each corresponding gain setting.

Chl *a* concentration (µg $L^{-1}$) was retrieved from blank-corrected fluorescence as:

$$[Chl] = \frac{(F_b - F_a)}{F_R} \cdot \frac{\tau}{\tau - 1} \cdot \frac{V_s}{V_F}$$

where $V_S$ and $V_F$ are the volume of solvent (mL) and volume of filtered water sample (mL), respectively. Pheopigment concentration (µg $L^{-1}$) was retrieved from blank-corrected fluorescence as:

$$[Pheo] = \frac{(F_a\tau - F_b)}{F_R} \cdot \frac{\tau}{\tau - 1} \cdot \frac{V_s}{V_F}$$

A solid standard (Turner Designs 10-AU-904) was also measured each day on both medium and high gain. Results indicated no change in instrument response over the course of sample analysis.

## IV. References

Jeffrey, S.W., R.F.C. Mantoura and S.W. Wright, eds. (1997) *Phytoplankton Pigments in Oceanography, Monographs on Oceanographic Methodology*. UNESCO, 661 pp.

Trees, C.C., R.R. Bidigare, D.M. Karl, L.V. Heukelem and J. Dore (2003) Fluorometeric chlorophyll *a*: sampling, laboratory methods and data analysis protocols, In: Mueller, J.L., G.S. Fargion, and C.R. McClain [Eds.] *Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Revision 5, Volumne V: Biogeochemical and Bio-optical measurements and data analysis protocols.* NASA/TM-2003-211621, NASA Goddard Space Flight Center, Greenbelt, MD, Chapter 3, pp 15-25.

Turner Designs (1999) *Model 10-AU-005-CE Fluorometer User's Manual*.