**Protocol: Spectrophotometric Particulate Absorption Analysis – EXPORTS**

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**Brief Overview:** Spectral absorption coefficients of particulate matter is determined from spectrophotometric analysis of particles filtered onto glass fiber filters. The particulate matter is partitioned chemically into in vivo pigments (phytoplankton absorption) and non-extractable particulate matter (non-algal particles). The conversion of absorbance to absorption required quantification of the geometric pathlength (m), accounted for by the ratio of the volume filtered (m3) and the effective filter area (m2), and the ratio of the geometric to optical pathlengths, accounted for by experimental comparisons between particulate samples measured in suspension to those on filter pads.

**Water sample collection:** Discrete water samples were collected from a Niskin bottle rosette sampler from approximately 8 depths, 1-3 times per day. In order to ensure compatibility between pigment, carbon and optical samples, the entirety of each Niskin bottle (from each depth) was collected into a large volume carboy. Carboys were be kept cold and dark until subsampling, within one hour of CTD sampling. Carboys were kept well mixed, by multiple-directional changes in swirling, during subsampling into analysis-specific sampling bottles. Discrete water samples were also collected from an inline water system, pumped from the sea chest with a diaphragm pump, that was coupled to the flow-through optical sensor suite. Samples were collected once or twice a day, in conjunction with a CTD cast. The intake depth was approximately 5m, coincident with the surface Niskin bottle depth. The inline samples were size fractionated with 5 m and 20 m cartridge filters (Pall®), in addition to the unfiltered samples. Real time optical data display was used to identify when the outflow water was completely size fractionated (approximately 7 minutes for complete sample flow through and reservoir turn over).

**Water sample preparation:** Discrete water sub-samples were collected in 2-Liter Nalgene sample bottles and filtered through Whatman® glass fiber filters (25 mm diameter, nominal pore size GF/F) under low pressure (< 5 mm Hg) vacuum with an aspirator pump. The exact volume and volume uncertainty of each sample was recorded. A set of 3-5 blank filter pads for baseline and blank reference scans were prepared in the similar manner but with comparable volumes of MilliQ® purified water filtered for each run of samples. Filter pads were transferred to taped glass petri dishes and laid, in order, on top of moist Kim Wipes® to maintain uniform moisture. Samples were measured immediately after filtration.

**Absorbance measurements:** Absorbance scans were measured spectrophotometrically in a Cary 300 dual beam spectrophotometer configured with a Labsphere® integrating sphere with center-mounted Plexiglas sample holder. The integrating sphere was aligned before the ship departed and the alignment was checked throughout the cruise. The wavelength range was 350 nm to 800 nm, the slit band width was 2 nm, the wavelength interval was 1 nm, the integration time was 0.2 s for a scan rate of 300 nm/s. One of the set of blank filter pads was selected as the baseline filter. After the baseline scan, the filter, untouched, was run as a sample. This is referred to as the *zero* absorbance scan. This represents the uncertainty due to instrument noise. The remaining blank filters were scanned relative to the baseline scan, these are referred to as *blank* scans. These represent the variability in absorbance due to filter-to-filter variability. Each sample filter was scanned relative to the baseline. The initial scan is referred to as the *particulate* absorbance scan. The filter is then place back into the filter cup and extracted with approximately 10 ml of hot methanol for 15 minutes. After the methanol was filtered through, samples were rinsed with approximately 10 ml of MilliQ water and examined for residual color. If necessary, extraction was repeated. The extracted filter was scanned; this is referred to as the *non-algal particle* (or nap) scan (Kishino et al. 1985). Most filters were extracted twice. By the end of the cruise, there was a shortage of methanol and only a single extraction was performed. Phycobilipigments are not extracted with methanol and those samples were extracted with hot water (Roesler and Perry 1995). In some cases residual pigment absorption was observed and the fully extracted absorbance was estimated by exponential fit (see data processing).

**Data Processing**: The absorbance scans from the EXPORTS NE Pacific cruise were at the low end of the optimal absorbance range, approximately 0.1 to 0.2 in the blue to green spectral band. However, they were much noisier than is typically found for coastal water samples with similar sample loading on the filter. This was true for samples collected on the Revelle process ship, stored in liquid nitrogen, and processed in the same spectrophotometer in the lab. Thus it is not an instrumental artifact nor a filter loading artifact, but likely a result of the size and composition of the natural particles. The sample absorbance scans were thus smoothed with a 9 nm moving box average filter. They were smoothed twice.

The sample absorbance, $A\_{sample}$, was corrected for pathlength amplification following the empirical relationship of Stramski et al. (2015):

$A= 0.323×\left(A\_{sample}^{1.0867}\right)$.

Spectral absorption coefficients, $a\left(λ\right) (m^{-1})$, for both the *particulate*  and *non-algal particles* were computed from the corrected absorbance scans using the following equation:

$$a\left(λ\right)=2.303 ×100 \frac{A}{\left(^{V\_{filt}}/\_{πr^{2}}\right)}$$

where $2.303 $converts the log10 absorbance to loge absorption, $A$ is the measured absorbance corrected for pathlength amplification, $V\_{filt}$is the volume filtered (ml or cm3), and $πr^{2}$is the effective area of the filter (cm2) and the factor of $100$converts cm to m. Absorption by the *phytoplankton* component was computed by difference between particulate and non-algal particle absorption coefficients. Uncertainty values were computed by mathematical propagation of instrumental and sample uncertainty terms following NASA protocols (2018).

In the case of incomplete extraction, an exponential fit to the *non-algal particle*  absorption spectrum was estimated over the wavelength range 375 nm to 750 nm, the endpoints representing wavelengths for which extractable algal pigment absorption is minimal. The spectral slope of the exponential was assumed to be 0.01 (nm-1), based upon the least-square best fit to well-extracted samples. The revised *phytoplankton* absorption, $\hat{a}\_{phyt}$, was computed from the difference between the *particulate* absorption and the modeled *non-algal particle* absorption, $\hat{a}\_{nap}$.

**SeaBASS data file structure:** The file structure for the SeaBASS data submission is:

wavelength, $A\_{zero}$, median$A\_{p\\_blank}$, median$A\_{nap\\_blank}$ $A\_{p}$, $A\_{nap}$,$ a\_{p}$, $a\_{nap}$,$ a\_{phyt}$, $\hat{a}\_{nap}$,$ \hat{a}\_{phyt}$

where size fractions are ordered *total*, *<20m* and *<5 m* within each data type.

**References:**

Roesler, C. S., D. Stramski, E. J. D’Sa, R. Röttgers, and R. A. Reynolds. 2018. Chapter 5: Spectrophotometric Measurements of Particulate Absorption Using Filter Pads. *In*  IOCCG Protocol Series (2018). Inherent Optical Property Measurements and Protocols: Absorption Coefficient, Neeley, A. R. and Mannino, A. (eds.), IOCCG Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation, Volume 1.0, IOCCG, Dartmouth, NS, Canada. <http://dx.doi.org/10.25607/OBP-119>

Kishino, M., M. Takahashi, N. Okami, and S. Ichimura, 1985: Estimation of the spectral absorption coefficients of phytoplankton in the sea. *Bull. Mar. Sci.*, **37(2)**: 634-642.

Roesler, C. S. and M. J. Perry. 1995. In situ phytoplankton absorption, fluorescence emission, and particulate backscattering spectra determined from reflectance. J. Geophys. Res. 100(C7): 13,279-13,294.

Stramski, D., R. A. Reynolds, S. Kaczmarek, J. Uitz, and G. Zheng, 2015: Correction of path length amplification in the filter-pad technique for measurements of particulate absorption coefficient in the visible spectral region. *Appl. Opt.*, **54**: 6763-6782.