PARTICLE ABSORPTION: SAMPLE FILTER PREPARATION AND ANALYSIS USING AN INTEGRATING SPHERE

Supplies Needed
- Combusted 25mm Whatman Glass fiber GF/F filters (0.7 μm nominal pore size)
- Glass filter cups and stems
- Gloves
- Forceps
- Histoprep tissue capsules (Fisherbrand 29x6 mm, Cat no: 15-182-219; white)
- Kim wipes
- Liquid nitrogen (LN) dewar

Prior to field campaign
- Combust the appropriate amount of 25mm GFF filters at 450°C for 6 hours. Note the lot number. During analysis you will use the same lot of filters.
- Combust the glass filter cups. Do not combust the stems; they will be damaged.

Sample Collection and Storage
- Set up glass filter cups/stems with combusted GF/F filters.
- Measure the required seawater volume with a graduated cylinder and pour into appropriate-sized amber bottle. Record filter volume (Vf) in log sheet.
- Filter samples under low vacuum (5-7 psi).
- Repeat steps 1 through 3 until appropriate amount of color can be seen on the filter. For particulate absorption, LIGHTER IS BETTER. Only filter enough to see light color, otherwise you may either overload the sample or make extraction of chlorophyll more difficult.
- When the appropriate volume has been filtered, rinse the filter cup with 0.2 um filtered seawater. Do not let filter run dry under vacuum. Close valve when last few milliliters (ml) are running through the filter.
- Remove filter from stem with forceps. Place filter into a CLEARLY pre-labeled histoprep and store in LN. Samples can be stored for a longer term at -80°C.

Sample Analysis

NAME: Cary 4000 UV-Visible scanning Spectrophotometer
S/N MY14180001

1) Introduction
The Cary 4000 UV-Visible scanning spectrophotometer is equipped with an integrating sphere (Labsphere DRA-CA-900, sold by Agilent Technologies).

2) Calibration/Maintenance
Instrument performance tests (wavelength accuracy and reproducibility, photometric noise, and baseline flatness) were conducted each day prior to analysis. Furthermore, National Institute of Standards and Technology (NIST)-traceable calibration standards (Holmium oxide filter for wavelength accuracy
and Spectronics standards, Thermo Electron Corporation, to evaluate stray light, wavelength accuracy, and photometric performance) were also used to verify instrument performance.

3) **Measurement**

Scans were performed between 290-850nm with a 2nm Slit Band Width (SBW), 0.2nm data interval and 120nm per minute scan speed. The calculation for $\beta$ was made using the equation from Rottgers and Gehnke (2012).

a. Alignment of the beam and mirrors was checked.
b. The system is baselined using air. An air scan was performed to assess the stability of the system. Scan should measure 0.000 absorbance units ±0.005. If not, system was baselined again.
c. A moistened blank GF/F filter was placed inside the integrating sphere chamber and scanned. The instrument was not baselined with the blank. The blank scan was manually subtracted during data processing.
d. Moistened blank filters (3-4) were scanned periodically throughout the day to monitor instrument drift.
e. For samples, three to four drops of artificial seawater were placed in a petri dish. The sample filter was placed biomass up onto the water droplet. The sample filter was allowed to thaw for ~5 minutes before measurement. The petri dish was covered with the lid and foil to protect from the light.
f. The sample filter is placed on a plexiglass holder and jaw mount inside the integrating sphere chamber and measured two times at 0 and 90 degrees.
g. The diameter of the biomass was measured using calipers (Fisher Scientific Digital Caliper, model # 14-648-17)
h. The extraction protocol was based on Kishino et al. (1985). Briefly, the sample filter was placed in glass filter cup and stem. Approximately 10-20ml of 95% methanol/5% ultrapure water was gently added to the filter cup and immediately filtered at 5 psi. After the first 10-20 ml are filter through, the valve was closed and another 20 ml were added to the filter cup. The samples were allowed to soak for 20 minutes then filtered again. Another 20 ml methanol were added to the cup and the sample was allowed to soak for at least another 20 minutes. Filter cups were covered to prevent debris from contaminating the sample.
i. After extraction, the last 20 ml of methanol were filtered through, and the filter was rinsed with 20 ml of ASW. The filter was not allowed to dry.
j. The moistened, extracted filter was scanned again using the protocol described above.

4) **Data processing**

a. Aircans (closest in time) were subtracted individually from each filter blank scan. The mean of all blank scans were calculated.
b. Mean of $a_p$ and $a_d$ rotation scans was calculated
c. Aircans (closest in time) were subtracted individually from averaged sample filter scan.
d. Mean blank scan was subtracted from the airscan-corrected sample filter scan (apcorr or adcorr).
e. Absorption coefficient was calculated using the following equations:
Pathlength = volume filtered (cm$^3$)/area of filter (cm$^2$)
Area of filter = $\pi r^2$
$\beta = 3.096\cdot(apcorr \text{ or } adcorr)^{-0.0867}$
$a_p = apcorr\cdot[2.303\cdot100/\beta\cdot\text{pathlength}]$
$a_d = adcorr\cdot[2.303\cdot100/\beta\cdot\text{pathlength}]$
$a_{ph} = a_p - a_d$

5) Data reporting
Each SeaBASS submission of $a_p$ scans will include the following:

a. Blank-corrected raw absorbance of particles on the filter pad (OD$_f$) of both $a_p$ and $a_d$ (abs$_{ap}$ and abs$_{ad}$, respectively).
b. Standard deviation of rotation scans for both $a_p$ and $a_d$ (abs$_{ap}$sd and abs$_{ad}$sd, respectively)
c. Blank absorbance (abs_blank) and standard deviation of blank scans throughout the day (abs_blank_sd)
d. Absorption coefficient calculations for one replicate (where applicable) for $a_p$, $a_d$ and $a_{ph}$. Replicates should be submitted as separate files.
e. Standard deviation of absorbance of all blank filters measured throughout the analysis period

Note: files that contain both replicates and more than one column of blank error indicates that replicates were analyzed on different days.

6) Reporting Notation

abs$_{ap}$ = raw $a_p$ absorbance with blank subtracted
abs$_{ap}$sd = standard deviation of 2 filter rotations
abs$_{ad}$ = raw $a_d$ absorbance with blank subtracted
abs$_{ad}$sd = standard deviation of 2 filter rotations
abs_blank = mean blank scans
abs_blank_sd = standard deviation of blank scans
ap = absorption coefficient
ad = absorption coefficient
aph = absorption coefficient ($a_{ph} = a_p - a_d$)
