NAME: Cary 100 UV-Visible scanning Spectrophotometer
S/N EL03127292

1) Introduction
   The Cary 100 UV-Visible scanning spectrophotometer (250-800 nm) is equipped with an integrating sphere (Labsphere DRA-CA-30, sold by Agilent Technologies: PN 190022900).

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 290-800nm with a 2nm Slit Band Width (SBW), 0.2nm data interval and 120nm per minute scan speed. The calculation for β 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 5-7 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. The average and standard deviation of the $a_p$ and $a_d$ scans were calculated.
   b. The mean of the blanks scans were subtracted across spectra from the mean $a_p$ and $a_d$ scans ($OD_f$).
   c. Absorption coefficient was calculated using the following equation
      $a_p(\lambda) = [2.303*100/\beta*Pathlength]$

      Pathlength = $V_f$ ($cm^3$)/area of filter ($cm^2$)
      Area of filter = $\pi*((D_f/10)/2))^2 = \pi r^2$
      Diameter was divided by **10 to convert mm to cm and by 2 to get radius**
      $\beta = 6.475*( OD_f \text{ ^2})-6.474*( OD_f)+4.765$ (Rottgers and Gehnke, 2012)

      To calculate spectral absorption of phytoplankton:
      $a_{ph}=a_p - a_d$
5) Data reporting
      Each SeaBASS submission of $a_p$ scans will include the following:
   a. Blank-corrected raw absorbance of both $a_p$ and $a_d$
   b. Standard deviation of rotation scans for both $a_p$ and $a_d$
   c. Absorption coefficient calculations for each replicate (where applicable) for $a_p$, $a_d$ and $a_{ph}$
   d. Standard deviation of absorbance of all blank filters measured throughout the analysis period
   e. Standard deviation of all air scans 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 total absorbance with blank subtracted
   abs_ap_sd= standard deviation of filter rotations
   abs_ad = raw $a_d$ absorbance with blank subtracted
   abs_ad_sd = standard deviation of filter rotations
   ap = absorption coefficient
   ad = absorption coefficient
   aph = absorption coefficient ($a_{ph}=a_p - a_d$)
   abs_blank_sd = standard deviation of filter blanks
   abs_air_sd = standard deviation of air scans

Kishino, M.N., Takahashi, N., Okami, N., and S. Ichimura, 1985. Estimation of the spectral absorption coefficients of phytoplankton in the sea. Bulletin of Marine Science. 37, 634-642.

Rottgers, R. and S. Gehnke, 2012. Measurement of light absorption by aquatic particles: omprovement of the quantitative filter technique by use of an integrating sphere. Applied Optics. 51: 1336-1351.