MEASUREMENT PROTOCOL OF ABSORPTION BY CHROMOPHORIC DISSOLVED ORGANIC MATTER (CDOM) AND OTHER DISSOLVED MATERIALS

By the CDOM Working Group
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This document is a product of a multi-year effort including a two-and-a-half-day workshop organized by the NASA Ocean Ecology Lab Field Support Group and hosted at NASA Goddard Space Flight Center in Nov. 13-15, 2013 and several CDOM absorption measurement round robins between November 2013 and February 2015 with significant international participation. The resulting protocol document, Measurement Protocol Of Absorption By Chromophoric Dissolved Organic Matter (CDOM) and Other Dissolved Materials, and the associated working group activity were sponsored by the National Aeronautics and Space Administration (NASA) including funding for the Field Support Group (NASA Ocean Biology and Biogeochemistry Program) and a 2012 ROSES proposal to Richard Miller, Norm Nelson, Carlos Del Castillo, Antonio Mannino and Jeremy Werdell under NASA Program Topical Workshops, Symposia, and Conferences with additional support for contributing authors and workshop participants by their respective institutions (see Appendix C for complete list of workshop participants). This document represents an update to the 2003 NASA Technical Memorandum, providing a detailed discussion of the state-of-the-art technologies and protocols for collecting water samples and measuring the absorption coefficients of CDOM. Important contributions by all the authors over many years made the completion of this document possible.

THE AUTHORS RESPECTFULLY DEDICATE THIS PROTOCOL DOCUMENT …
To the memory of Rossana Del Vecchio, a great colleague and better friend. We miss her sorely.

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I. Introduction

Light absorption by chromophoric dissolved organic matter (CDOM) dominates the ultraviolet (UV) and short wavelength blue portions of the absorption spectrum in all aquatic environments, thus exerting primary control on photochemistry, photobiology, and ocean color. In order to produce valid results, ocean color models (e.g., *in situ* or remote sensing-based radiative transfer or bio-optical inversion models) must take into account the absorption spectrum of CDOM. Measuring light absorption by CDOM *in situ* is a necessary condition for developing and validating current and future ocean color algorithms for all applications. We are revising this section of the NASA ocean optics protocols (Mitchell et al. 2003) under the auspices of the International Ocean Colour Coordinating Group to reflect the development of new instrumentation and a greater understanding of the importance of CDOM and its distribution in coastal and open ocean waters over the past twenty years. Our goal here is to develop a set of procedures that will be adhered to by the ocean optics community for making CDOM measurements that can be integrated into NASA's bio-optical database (currently named the SeaBASS) or similar databases, of sufficient quality to develop and validate ocean color algorithms and contribute to CDOM science. An overview of the absorption coefficient of CDOM and particles for pure and natural waters is described in another protocol document (Twardowski et al. 2018).

The relevance of CDOM absorption measurements for satellite and airborne remote sensing applications is constrained to the Earth-surface solar radiation wavelength range (>280 nm). For the purposes of this discussion "CDOM" is operationally defined as material that passes through an approximately 0.2 μm pore-size filter and absorbs light at wavelengths above 250 nm. This material is predominantly composed of organic molecules (hence the CDOM nomenclature), nevertheless, inorganic constituents such as nitrate, bisulfide, dissolved iron, and so-called colloids or nanoparticles of iron oxides and minerals contribute to (and interfere with) absorption measurements of organic molecules (e.g., Zafiriou et al. 1984; Johnson and Coletti 2002; Weishaar et al. 2003). The absorption by inorganic constituents such as nitrate, nitrite, bisulfide, iodide, bromide, etc. becomes pronounced at wavelengths below 250 nm (Johnson and Coletti 2002; Birkmann et al. 2018). Measuring light absorption at shorter wavelengths is useful for characterization of the composition of CDOM and assessment of mineral ion concentrations, but does not play a direct role in ocean color, photochemistry, or photobiology. This operational definition permits colloids and smaller particles (such as some viruses or fragments of organisms) to be considered part of the CDOM, but does not operationally separate out absorption by nitrate and mineral ions particularly at wavelengths below 350 nm.

The main characteristic of typical CDOM absorption spectra is an approximately exponential decline with increasing wavelength. Absorption at 300 nm can be a factor of fifty higher than at 500 nm. Conventional absorption spectroscopy using 1 cm or 10 cm cuvettes can resolve CDOM absorption in the UV, but not the visible, in the oligotrophic ocean and some offshore coastal areas. In order to validate most ocean color products, we must be able to accurately measure CDOM absorption spectra in the visible with

known uncertainties. We have focused our efforts on new technologies now commercially available, in particular liquid waveguide cells, which can have pathlengths up to 200 to 500 cm. These new technologies come with greater capabilities but also have unique problems and considerations. The current edition of these protocols summarizes our current state of knowledge and provides recommendations for how to make accurate and precise measurements going forward.

In most cases our recommendations are based on and reflect the previous protocols (Mitchell et al. 2000; 2003). We have tried to highlight those cases where we have made a significant revision to the protocol.

II. Measurement Protocols

Sample Collection, Filtration, and Storage

When measuring trace organics, it is necessary to minimize organic contamination of the samples during collection. Procedural ultrapure water blanks should be prepared to monitor potential contamination through each step of the sampling, filtration, and storage procedure including from Niskin bottles (or any other water sample collection method), filters, filtration apparatus, handling, sampling and storage containers, and through the shipping and storage process. This is necessary to achieve an end-to-end uncertainty budget.

Pre-cruise preparations

- Sample bottles (amber glass bottles with Teflon-lined caps) used to collect CDOM samples or to store ultrapure standard reference water need to be thoroughly cleaned to remove any potential particulate and organic contaminants. The recommended bottle and cap cleaning procedure[1] entails sequential soaks and rinses in dilute detergent, purified water (deionized Type II), and 10% HCl, followed by final copious rinses with ultrapure water[2] (5 or more rinses). Alternate bottles may be used but should be evaluated prior to use for each specific application (e.g., oligotrophic ocean water, river water, etc.).

Note: The original protocols recommended clear Qorpak® glass bottles. However, this protocol recommends amber glass bottles to mitigate UV exposure and thus potential photooxidation of samples during sample processing, storage, and preparation for analysis. Lab experiments have demonstrated no measurable contribution of CDOM to ultrapure water contained in these types of bottles for over 2 months of exposure (Fig.1).

- Dry bottles and caps in dedicated clean oven at 60°C for 4-12 hours.
- Combust bottles with aluminum foil covers at 450°C for 6 hours.
- Ultrapure water reference materials are prepared by rinsing combusted bottles and caps and filling with fresh ultrapure water directly from the water production unit. Store in the dark.
- These reference water standards can be used to evaluate the quality of the ultrapure water produced at sea[2] or as a replacement if ultrapure water is not available.

Sample Collection

Samples should be collected from clean[1] Niskin or Go-Flo bottles (silicone coated internal springs), using clean, non-contaminating, and non-absorbing/adsorbing high-purity tubing on the bottle outlet such as platinum-cured silicone, certain Tygon® formulations (2275, 2375, 2475) or fluoro-polymers (PFA, PTFE, ETFE). Samplers should wear powder-free non-latex gloves (such as nitrile) while handling samples, though gloves should not come into contact with the sample itself. Before filtration, the sample bottles used to collect whole water from the Niskin or Go-flo bottles should be rinsed three times prior to filling.[1] CDOM measurements on samples collected from an underway flow-through system should be compared with results from Niskin bottle samples. Filtered CDOM samples may be collected directly from the Niskin or Go-Flo bottles using gravity filtration and an appropriate filter after adequate flushing (see section V for discussion on capsule filters).

Triplicate samples, from randomly selected depths, should be collected daily – more frequently if a large number of casts are to be collected each day. The Goddard Space Flight Center (GSFC) High Performance Liquid Chromatography (HPLC) pigment project recommends replication at a rate of 10% of samples for phytoplankton pigments, and this is a worthwhile goal for CDOM absorption measurements.

Samples from Niskin bottles that break the surface at the time of sampling should be distrusted because of potential contamination from a surface film with enhanced CDOM (e.g., Obernosterer et al. 2008; Tilstone et al. 2010). The distance from the pressure

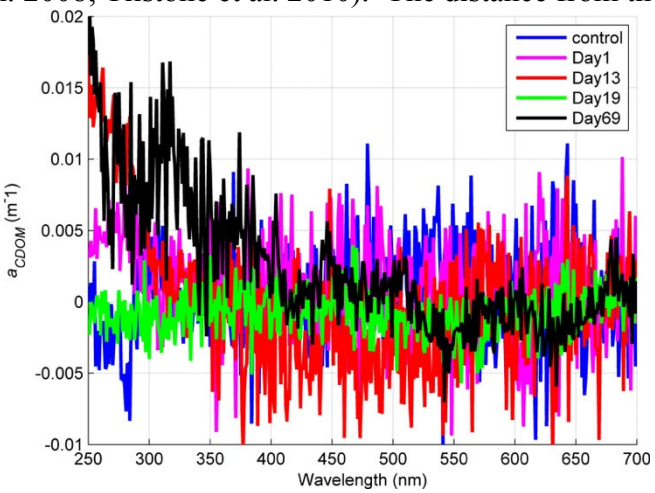

Figure 1. CDOM absorption coefficient spectra ($a_{CDOM}(\lambda)$) of ultrapure water stored in amber glass bottles in the dark at 4°C and analyzed periodically on a double beam spectrophotometer with 10 cm quartz cell to test for leaching of colored material. Note - the Day 19 (light green) spectrum is more representative of typical instrument noise than the other spectra.

[1] For Niskin or Go-Flo bottles, "clean" refers to bottles that have been used multiple times and soaked in de-ionized water prior to the beginning of the cruise. Plasticware and glassware should be cleaned by soaking overnight in alkaline detergent bath (such as RBS™ 35), rinsing with reverse osmosis (Type-II) water, soaking in ~10% hydrochloric acid (HCl; 1.2M) bath overnight followed by copious rinsing (5 times or more) with ultrapure water[2]. Glassware should be baked at 450ºC for at least 6 hours.

sensor to the center of the Niskin bottles should be measured on a CTD rosette package in order to monitor whether the bottles were too close to the sea surface microlayer when they were sealed. It has also been demonstrated that at-sea lubrication of the rosette cable will contaminate CDOM and DOC samples for a considerable time (~10 deep-ocean casts) after the lubricant is applied (Nelson and Carlson unpublished data). Hence, precautions should be taken to prevent or minimize potential contamination of samples.

CDOM samples are prepared by gentle vacuum filtration (<16.9 kPa, which is equivalent to <5 inches of mercury and <127 torr) or gravity filtration or through positive pressure filtration (<69 kPa or <10 psi) (see Figure 2 for example clean sample filtration apparatus). Samples should be filtered immediately following collection of the whole water sample. Note that impacts to CDOM absorption coefficients from delays in filtration are not well documented but could be important. It is preferable to use clean (acid washed and/or combusted) glass filtration apparatus either with stainless steel frits or glass frits. These should be rinsed with ~10% Hydrochloric acid (HCl) and ultrapure water[2] after daily uses and acid soaked (in the case of glass frits) with longer intervals between uses. Sample filtrates should be collected in clean (see above) brown (amber)

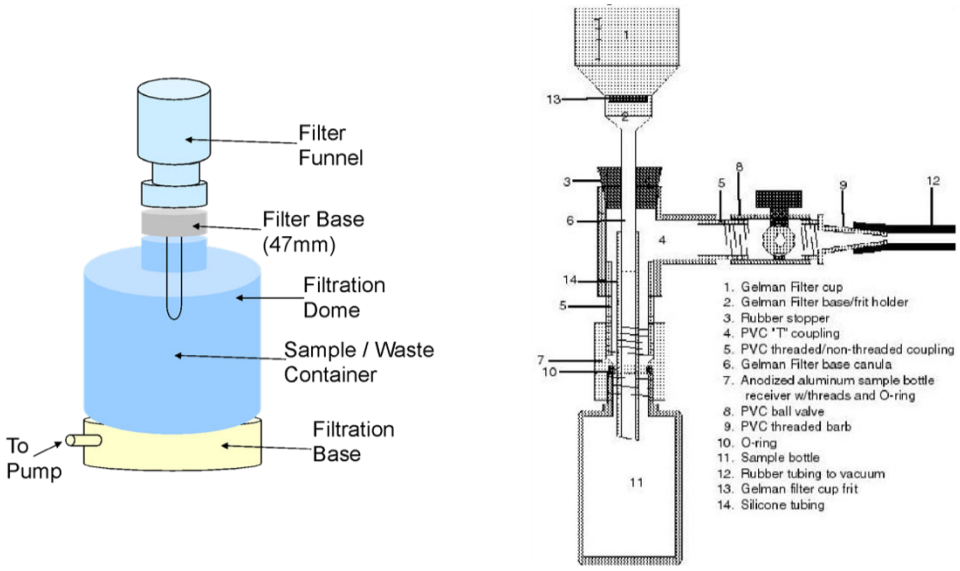

Figure 3. Diagrams of example filtration apparatus for collection of clean sample filtrate directly into sample bottle for measurement of absorption coefficients of the dissolved fraction nominally defined by the filter pore size. (a) Kontes filter dome and common glass filtration apparatus and (b) custom apparatus with Gelman plastic filtration equipment (diagram from Mitchell et al. 2000).

[2] Ultrapure water (Type I; resistivity ≥18.2 MΩ cm, <5 ppt TOC) that is ultraviolet oxidized and 0.2 µm-filtered water with total organic carbon ≤10 µg C L^{-1} (e.g., Milli-Q Gradient, Nanopure Diamond UV, etc.) for preparation of all solutions (acids, consensus reference material), rinsing, cleaning of spectrophotometer cells, and serves as the blank and reference. Water purification systems require diligent system maintenance to ensure high quality laboratory water with low CDOM and total organic carbon (TOC). Several manufacturers equip ultrapure water systems with TOC monitors that provide an indication of the carbon content of the water.

glass bottles or covered clear glass bottles with teflon cap liners. The NASA Ocean Optics Protocols did not recommend amber glass bottles due to a concern of contamination from the tinted glass to soluble absorption (Mitchell et al. 2003). However, testing of cleaned amber glass bottles demonstrate that these bottles do not introduce contaminants that interfere with CDOM absorption (Fig. 1). Sample bottles should be rinsed with filtered water three times before filling. Prior to sample filtration and after setup on the filtration apparatus, filters should be rinsed with ultrapure water and then rinsed with sample that is then discarded before filtration (minimum total volume of 175 to 200 mL is recommended for 47 mm diameter disc filters). If ultrapure water is not available, then sample water alone can be used. Acceptable filter types have effective pore sizes of 0.2 μm for coastal and open ocean waters, whereas 0.45 μm pore size filters are commonly used in freshwater systems and acceptable due to the practicality of working with high particle load samples. Acceptable membrane filter types for disc filters include glass fiber, polycarbonate (PC), polyethersulfone (PES), nylon and hydrophilic polypropylene (GHP) (see Section V for details). Nylon, Versapor and PureFlo SZL PES capsule filters, and GHP syringe filters were evaluated and are also deemed acceptable if adequately flushed with sample or a combination of ultrapure water and sample at the time of sample filtration (see Section V for details). The flushing volume seems to be critical to remove chromophoric and non-chromophoric organic contaminants. In heavy particle load situations, pre-filtration with combusted, rinsed glass fiber filters such as GF/F (manufacturer reported nominal pore size of 0.7 μm) can be used to prevent 0.2 μm filter clogging. Combusted Advantec GF-75 glass fiber filters, which have a manufacturer reported nominal pore-size of 0.3 μm, provide results comparable to the other membrane filters. An ideal filtration protocol to follow would involve pre-filtration of samples with an appropriate pore size filter for the particle concentration of the water sample and final filtration with an acceptable 0.2 μm filter prior to analysis. See section V for discussion on different filter types.

For analysts aiming to quantify absorption spectra of both CDOM and particles, an important consideration is avoiding a gap across the size spectrum. Discrete measurements of particle absorption are performed on GF/F filters, which results in a gap in measurement between <0.2 μm CDOM fraction and the >0.7 μm particles. One possible solution is to utilize the GF-75 filters for particle absorption to reduce and potentially eliminate the possibility of a measurement gap. Comparisons of such discrete measurements with whole water absorbance spectrometers would verify the efficacy of filter choices across all natural waters analyzed.

Samples should be stored in the dark in sealed bottles at ~4°C in a clean environment, and analyzed as soon as possible, preferably within 4 to 24 hours, but no later than within 6 months of collection (see results in Section VI) as it is not always practical to analyze samples at sea. Figure 3 is an example of a low absorbing CDOM sample stored in this manner that does not exhibit a change in absorption. However, this may not always be the case; temporal variability in CDOM absorption is likely dependent on sources and composition of CDOM. Freezing samples is generally not recommended (Fellman et al. 2008). Nevertheless, this storage approach may extend the storage period if loss in CDOM due to flocculation can be avoided.

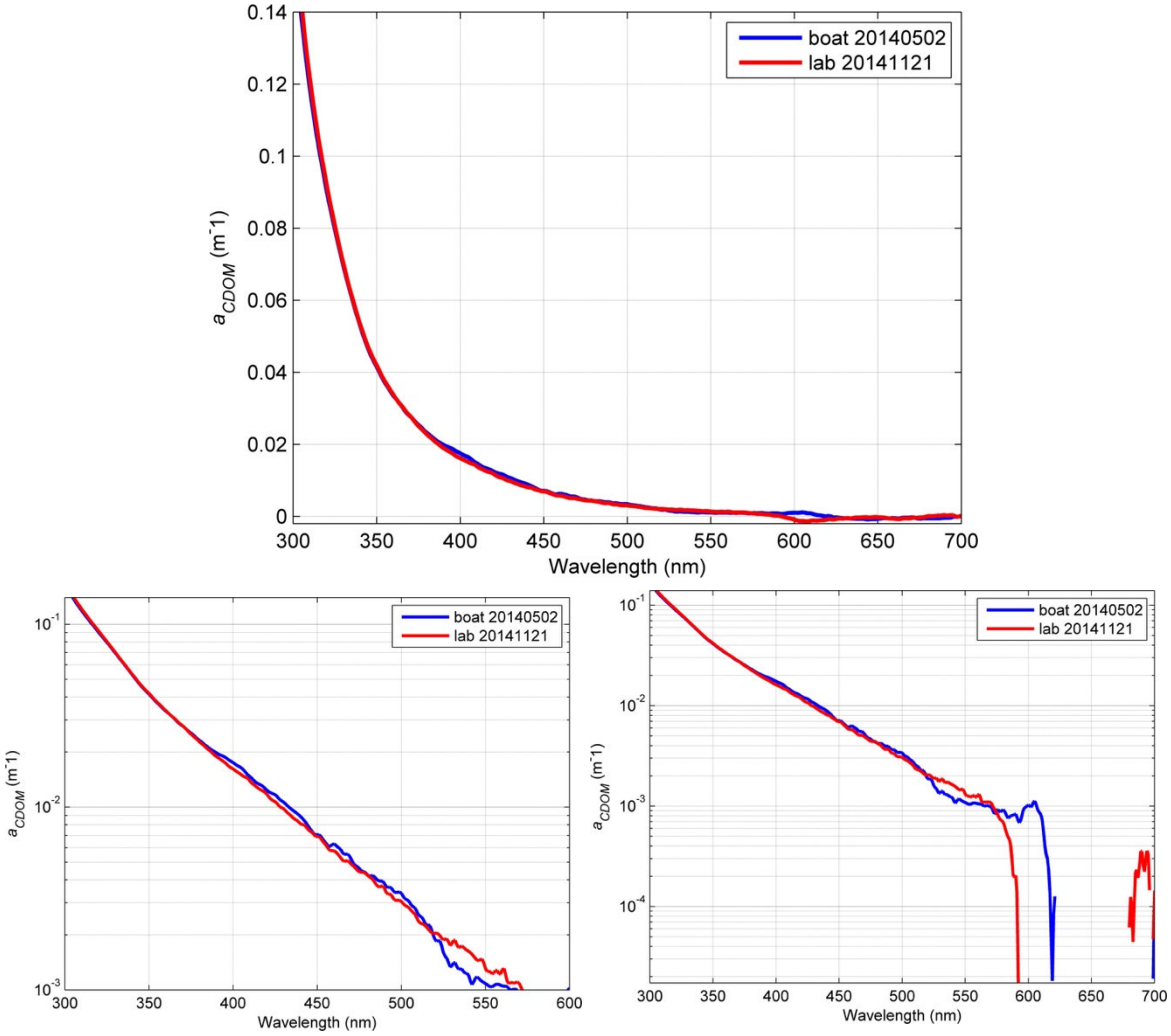

Figure 4. CDOM absorption coefficient spectra for a low absorbing CDOM water sample collected in the south Pacific measured shortly after collection with an UltraPath UPUV system with 200 cm liquid waveguide capillary cell and with the same instrument over 6 months later in the lab plotted on a linear (a) and logarithmic absorption coefficient scales (b) and (c) demonstrating no change in values within uncertainty of the measurement. Dates in the legend are shown in yyyymmdd format.

General Considerations for Spectroscopy

Spectrophotometers should be calibrated prior to analysis of a large batch of samples and annually at a minimum to ensure their accurate photometric performance. If available, the automatic calibration of the spectrophotometer for checking wavelength and slit size should be initiated. Neutral density filters traceable to NIST 930e are recommended for visible spectroscopy and liquid samples traceable to NIST 935a for ultraviolet spectroscopy. For assessing wavelength accuracy, the use of solid-state holmium oxide filters is recommended. NIST-traceable standards are available in various formats that can be immediately used in most double or single beam spectrophotometers. Spectrophotometer performance using NIST-traceable standards is a service that is provided by some spectrometer manufacturers as part of routine maintenance.

Calibration certificates from these exercises should also be provided to the bio-optical databases (e.g., SeaBASS) as part of the documentation.

In addition to photometric calibration, liquid waveguide instruments must have their effective pathlength determined using a solution of known concentration and known extinction coefficient at a particular wavelength. This is typically performed by the manufacturer but must also be performed by individual labs to verify and repeated at least annually.

Reference Materials

Ultrapure water serves as the standard reference water blank in CDOM absorption analysis. The importance of ultrapure water as the reference water blank for CDOM analysis cannot be overstated. Good results have been obtained from ultrapure water systems producing Type I water with low DOC[2]. Filter cartridges in ultrapure systems should be of the "low DOC" variety. Ultrapure water from the system must be filtered (0.2 µm) before use – most systems have this as the final filtration step. Quality of the prepared ultrapure water should be checked periodically by comparing to independent sources or comparison with past measurements. For routine work, Fisher Optima grade water (or comparable) tends to provide consistent results. It can be confirmed that a new cartridge filter set up is clean and working properly by zeroing the spectrophotometer using the Optima water and then measuring the absorption spectrum of ultrapure water from the system. Optima water does yield a small absorption signal in the UV. Routine analysis of DOC concentration of the ultrapure water can also be used to verify the quality of ultrapure water production system (on the order or 5-10 µg C L^{-1}). Some ultrapure water units have built-in total organic carbon (TOC) modules for detecting degradation in water quality. However, it is recommended not to rely solely on readings from TOC units.

One of the factors that enabled researchers studying DOC to have confidence in their measurements in recent years has been the availability of suitable reference material that can be analyzed along with samples. We are requiring as part of the standard protocol, measurement of the absorption spectrum of solutions of Suwanee River Fulvic Acid (SRFA) dissolved in ultrapure water, as part of each session of routine CDOM absorbance measurements. The intent is for CDOM analysts to use SRFA solutions to verify that their measurement process including instrument performance generates absorption coefficient values consistent with values provided in this protocol (Fig. 4). If results do not match, then the analysts should evaluate their instrument, measurement technique, and computations. SRFA-I standards in powder form are available from the International Humic Substances Society, St. Paul, Minnesota (https://humic-substances.org/). Currently, we recommend the use of the SRFA Standard I (SRFA-I; catalog # 1S101F; prepared 2003; US$125 for 100mg)[3] in the following concentrations:

[3] Future protocol revisions will specify an alternate SRFA Standard once IHSS no longer provides SRFA-I or batch 1S101F.

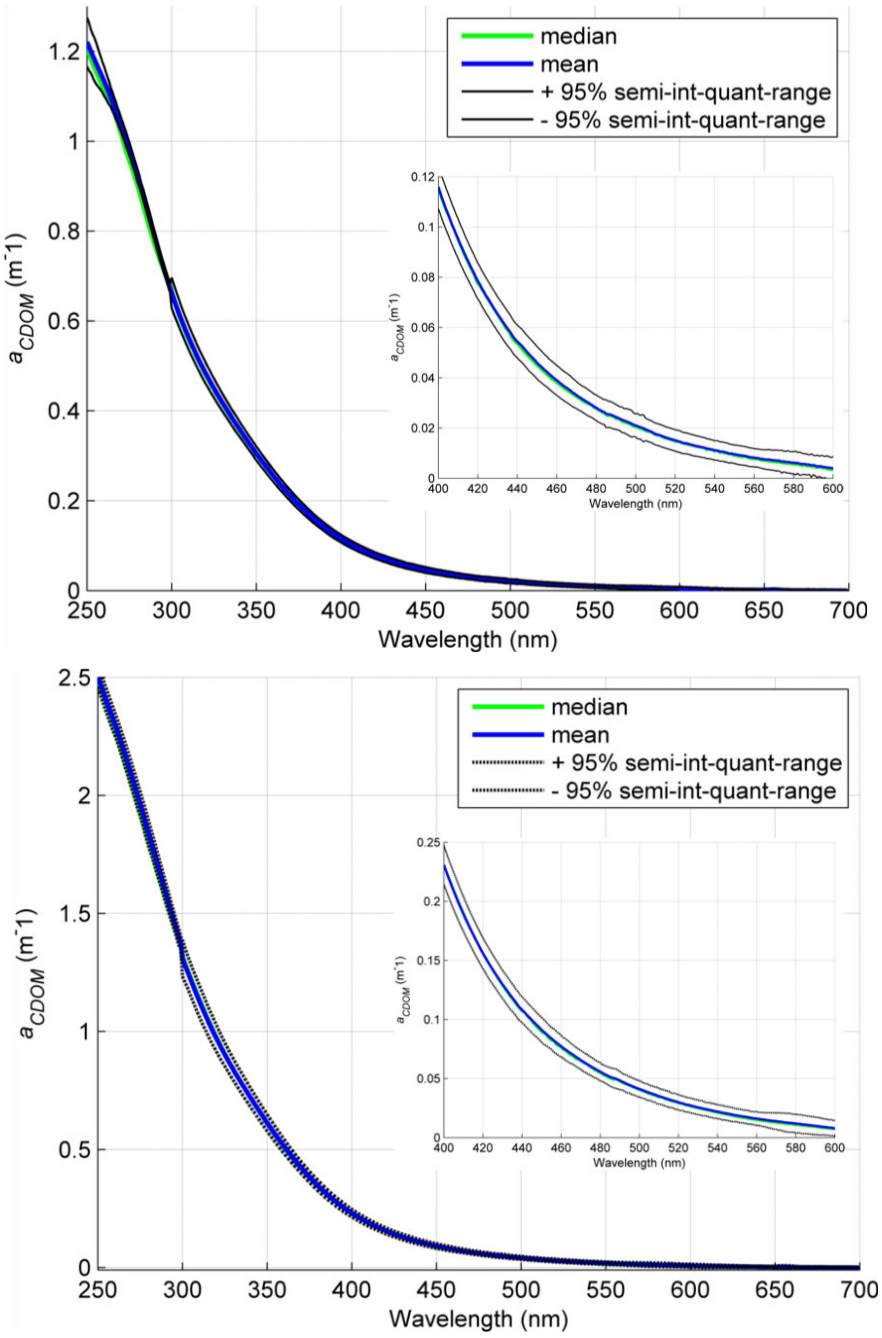

Figure 6. CDOM absorption coefficient spectra of (a) 0.25 mg L^{-1} Suwanee River Fulvic Acid I (SRFA-I) solution and (b) 0. 50 mg L^{-1} SRFA-I analyzed on 12 UltraPath UPUV and Liquid Waveguide Capillary Cell (LWCC) instruments and multiple double beam spectrophotometers from a multi-investigator round robin conducted in February 2015 – see Table 2 for details on instrumenation. UltraPath UPUV and LWCC data extend only between 300-700 nm. Data below 300 nm are from the double beam spectrophotometers. Measurements below 300 nm in the plots were made over time on the Round Robin SRFA-I solution. The 95% semi-interquantile ranges shown in black represent the variability in measurements between instruments and investigators. Tables B1 and B2 in Appendix B contain the tabulated values presented in these two plots.

1 mg/L – for use with 1 cm cuvettes
0.5 mg/L – for use with long pathlength liquid waveguide cells and 10 cm cuvettes
0.25 mg/L – for use with long pathlength liquid waveguide cells and 10 cm cuvettes

SRFA-I solutions should be thoroughly mixed and 0.2 µm filtered before use. Because the absorption response should be linear with concentration, plotting SRFA-I concentration versus absorption coefficient provides a rapid verification that SRFA-I solutions are prepared properly and the instrumentation is functioning properly. Analysts should verify that the SRFA-I solutions measured do not exceed the dynamic range of the double beam spectrophotometer or the liquid waveguide-pathlength combination.

SRFA-I solutions should be stored at 4°C within amber glass bottles in the dark between uses. The powder can be stored long-term in a freezer such as at -20°C to -80°C to minimize degradation. Experiments are still being carried out to determine the useful lifetime of a given solution and for the SRFA-I powder (see Section VI for details). The date of preparation of all solutions should be recorded, and reported with results, so the community can compile data on storage effects, etc. Long-term storage and analysis of SRFA-I solutions reveal minimal changes in the absorption properties of the 0.25 mg L^{-1} SRFA-I solution (Fig. 5). From a stock solution of SRFA-I prepared on February 2, 2015 and periodically re-filtered and measured over 19 months, there was no significant change in absorption throughout the visible and UV spectral regions.

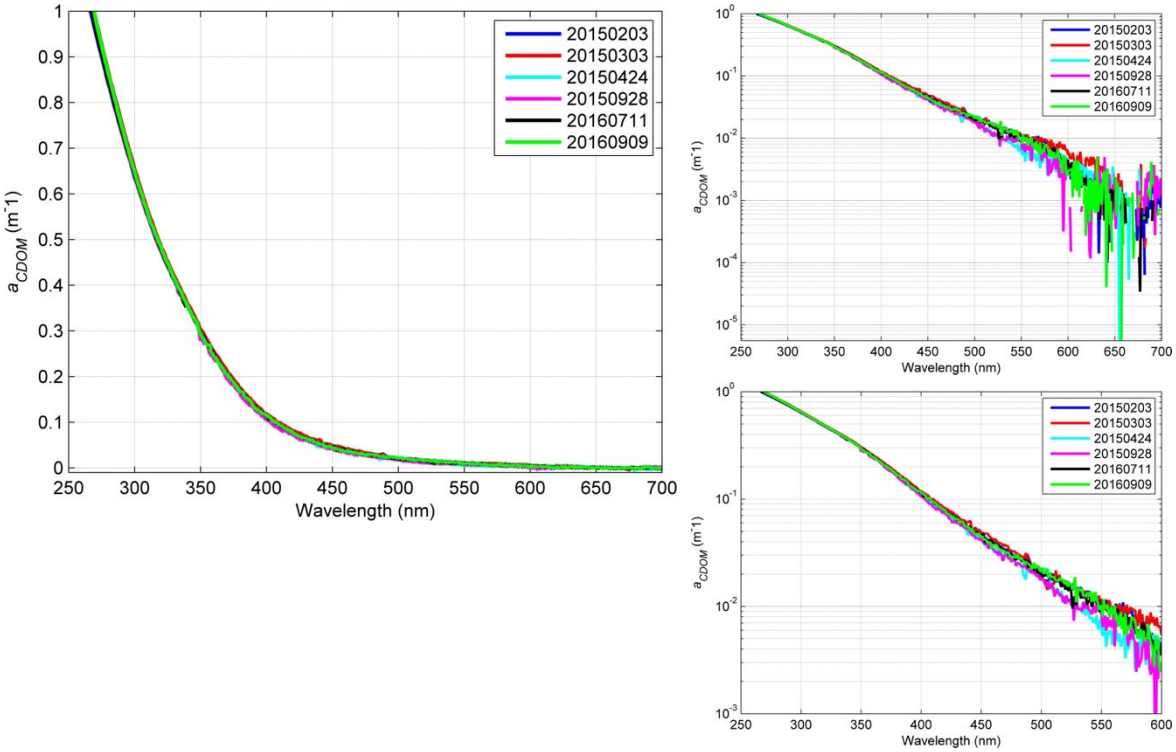

Figure 8. Evaluation of long term storage effects of SRFA-I solution (0.25 mg L^{-1}) on CDOM absorption coefficients measured on a double beam spectrophotometer over a 19-month period plotted on a linear (a) and logarithmic absorption coefficient scales (b) and (c) demonstrating no change in values within uncertainty of the measurement. Dates in the legend are shown in yyyymmdd format.

When submitting data to SeaBASS or similar databases, it is required or recommended that researchers report mass and pathlength normalized decadal absorbance spectra (L g^{-1} cm^{-1}) of SRFA-I solution with each batch of CDOM samples that have been measured as well as the date of preparation (Tables B1 and B2 in Appendix B).

Sample Preparation for Analysis

All samples, blanks, and reference material should be equilibrated to a constant room temperature (±2°C) before analysis, typically standard room temperature, or other appropriate temperature for the sample such as 4°C for polar water samples, but only if the instrumentation is located in a chamber with a comparable temperature as the samples. The stability of the room temperature and the difference in temperature between sample and the reference water blank are the critical factors. Matching the temperature between reference and sample in conventional short pathlength spectrophotometers reduces the depression seen in the near-infrared region of the absorption curve, which is due to the temperature dependence of pure water absorption coefficients in this part of the spectrum (Pegau et al. 1997). This is of particular importance with the long-pathlength liquid waveguide cells, where small refractive index differences between the sample and blank can lead to significant errors. Fluctuating room temperatures can also affect the performance of the instrumentation.

Samples stored for an extended period prior to analysis (more than several hours) should be re-filtered through a 0.2 µm filter as described in the Sample Collection section.

Liquid Waveguide Spectroscopy

The liquid waveguide classes of instruments, which include the UltraPath and liquid waveguide capillary cells (LWCC), are composed of three instrument modules: a light source, a liquid waveguide capillary cell with 2 m or other pathlengths, and a spectrometer to detect the light transmittance through the two ends of the waveguide cell (Fig. 6). The protocol that follows is based on the assumption that CDOM analysts are familiar with operating liquid waveguide instrumentation and have received training from the instrument manufacturer or other expert. The protocol focuses on the use of an UltraPath system and its proprietary software. In case other systems (LWCC) and software are used, the protocol needs to be adapted in an appropriate way.

Preparation of the instrument and samples for analysis

- Equilibrate samples, ultrapure water blanks, and reference materials to a constant room temperature
- Turn on the light source and spectrometer unit at least 30 minutes before collecting measurements. Recent studies have shown that lamps may take two to three hours to stabilize (Cartisano et al. 2018). However, such a long warm-up period may be indicative of aging lamps or unfavorable environmental laboratory conditions.

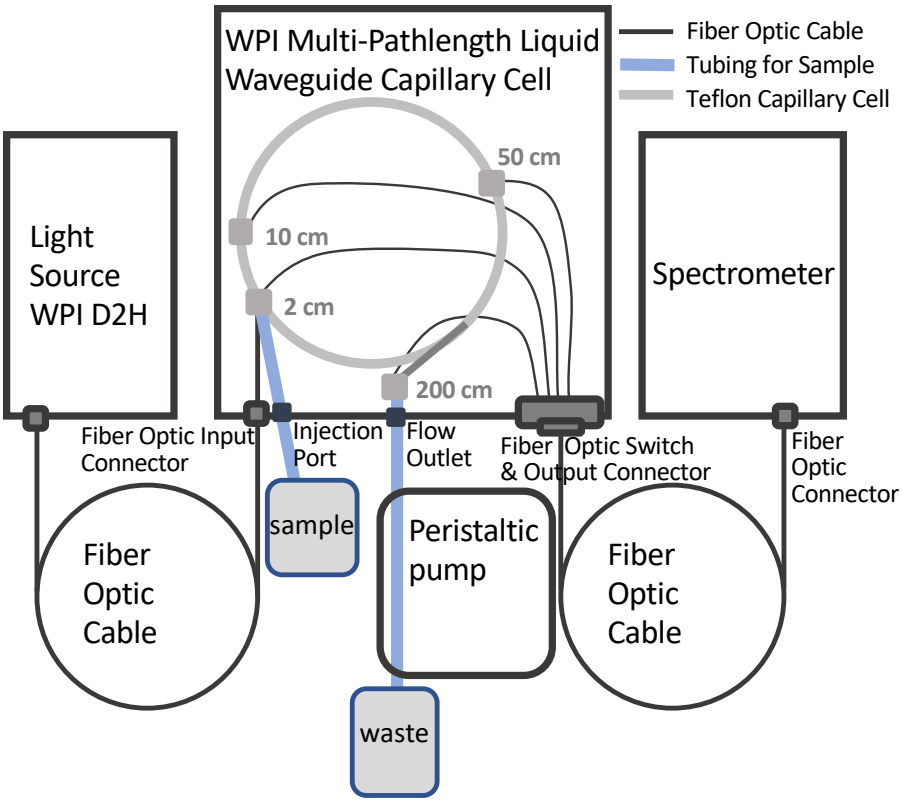

Figure 10. Schematic of the World Precision Instrument (WPI) UltraPath UPUV system, which includes the UltraPath absorbance sample cell (center) with four nominal optical pathlengths (2, 10, 50 and 200 cm), deuterium/tungsten light source (left), photodiode array spectrometer (right), and peristaltic pump.

- The flow rate of the peristaltic pump should be ~10 mL per minute on the UltraPath; the WPI Peri-Star Pro peristaltic pump with 0.5 mm inner diameter tubing set at 30 RPM will achieve this rate. For the LWCC system, a lower flow rate of 1-2 mL per minute would be appropriate. When measuring samples or other solutions, fill the cell until the signal is stable and then stop the flow before collecting multiple scans. Analyst may also collect measurements in continuous flow mode, and preferred for LWCC systems (Lefering et al. 2017).
- A 0.2 µm syringe filter can be added to the injection line may increase measurement stability. Recent work suggests that this may reduce the formation of microbubbles that can cause a bias in measurements. However, the syringe and filter should be thoroughly rinsed by injecting ultrapure water into the instrument until there is no measurable difference between syringe-filtered and non-syringe-filtered ultrapure water (Lefering et al. 2017).
- Clean the waveguide cell according to the following procedure and in the order listed:
    - Load cell with a 10% solution of Contrad NF (or an equivalent alkaline detergent) in ultrapure water – let stand 5 minutes
    - Inject 3 injection volumes (ca. 5 mL each) of HPLC-grade methanol
    - Inject 3 injection volumes of 10% HCl in ultrapure water (~1.2 M HCl)
    - Flush 5 cell volumes with ultrapure water

- On the UltraPath TIDAS spectrometer software, use real-time monitor to confirm an acceptable intensity level of the UV-Vis spectrum with a specific integration time of at least 10,000 counts at the largest peak and an intensity near 70% to assure data quality (Fig. 7). Intensity on the order of 60-70% is acceptable for ultrapure water as a reference.
- Monitor the intensity and confirm that the intensity is stable, not fluctuating more than +/- 1%. If the signal is unstable, follow the advanced cleaning procedure described in Appendix A.

Figure 12. Raw (uncalibrated) intensity spectra of (a) ultrapure water and (b) 40 parts per thousand (ppt) sodium chloride (NaCl) solution from an UltraPath UPUV system with TIDAS diode-array spectrometer in real-time monitoring mode using TidasDAQ software provided by WPI.

Measurement procedure

- After cleaning and flushing the cell with ultrapure water, stop the flow and close the shutter on the light source. Wait a couple of seconds and then collect a dark reference spectrum.
- Open the shutter and wait a couple of seconds before measuring a light reference spectrum.
- Acquire 3 or more scans of ultrapure water to determine instrument noise and make a note of the reference intensity.
- Reload the ultrapure water blank into the waveguide capillary cell and acquire 3 scans to determine the error introduced by sample reinjection. The reference intensity for ultrapure water should be approximately 70% so that when a salty sample is introduced the intensity does not exceed 90%. Adjust integration times to stay within these limits (Fig. 7).

Note: The appropriate integration time varies significantly between instruments. The balancing of the lamps on the light source also affects integration time. As long as the counts are within the recommended range, then the user should be able to determine the appropriate integration time. It is necessary to check that for a given pathlength, the integration time is stable from one measurement series to the other. It should not vary by more than 5-10% over one month. A higher increase may indicate a problem either with the source or with the cleaning of the capillary tubing. Most critical is the stability of the integration time throughout the analysis of a sample set and should not vary by more than 1-2%.

- Load 0.25 mg L^{-1} SRFA-I standard solution into the liquid waveguide cell and scan.
- Reload and rescan if the signal at the long wavelength portion of the spectrum does not reach zero, or the measured values exceed the 95% semi-interquantile range of the reported SRFA-I values (Figs. 4 and 27; Table B1).
    - Refiltration of the SRFA-I solution may be necessary to obtain appropriate values. Ultimately, cleaning of the liquid waveguide capillary cell may be required if the expected values are not achieved.

It is recommended that analysts measure the apparent absorption spectra of sodium chloride (NaCl) solutions with salt concentrations that span the range of seawater salinity expected in their samples (e.g., 30 ppt and 40 ppt for open ocean samples; 15 ppt and 35 ppt for estuarine or coastal samples; etc.; ppt is parts per thousand and analogous to practical salinity units). NaCl is used here as equivalent to sea salt for simple practical reasons, it does not exactly represent sea salt and small differences compared to the effects of sea salt are expected. Analysts should utilize high-purity NaCl (e.g., Sigma-Aldrich Ultra, Acros, etc.) that has been combusted at 450°C for 4 hours. The saltiest solution should be weighed out and dissolved in ultrapure water in a volumetric flask and 0.2 µm filtered (e.g., 40.0 grams NaCl dissolved with ultrapure water up to the 1000 mL mark on a class A volumetric flask). This solution should be scanned in a spectrophotometer to check for contamination after filtration. Class A graduated cylinders can then be used to dilute the highest salt concentration to the required NaCl concentrations. It is good practice to check the salinity using a refractometer or other type of salinometer to confirm that the salinity of each solution is accurate.

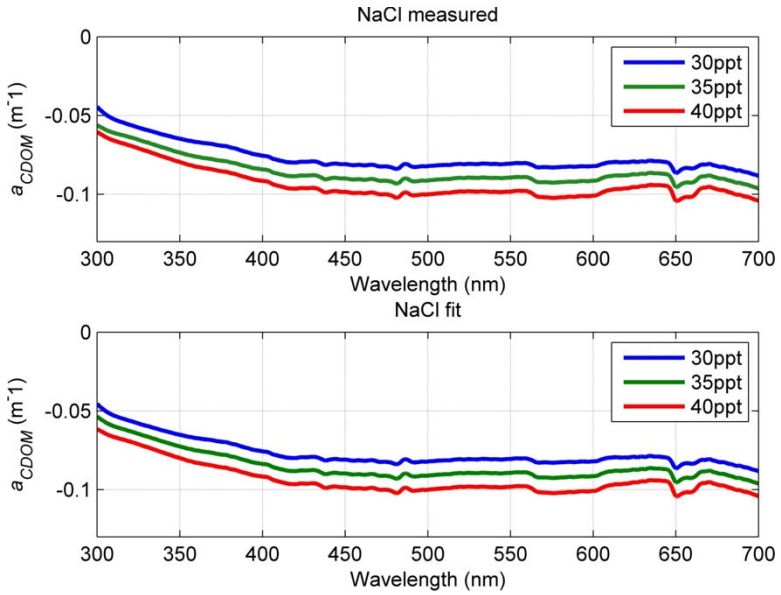

Figure 15. Sodium chloride (NaCl) absorption spectra with negative slope measured on an UltraPath UPUV system (top) and the modeled spectra from the least squares linear fit (bottom).

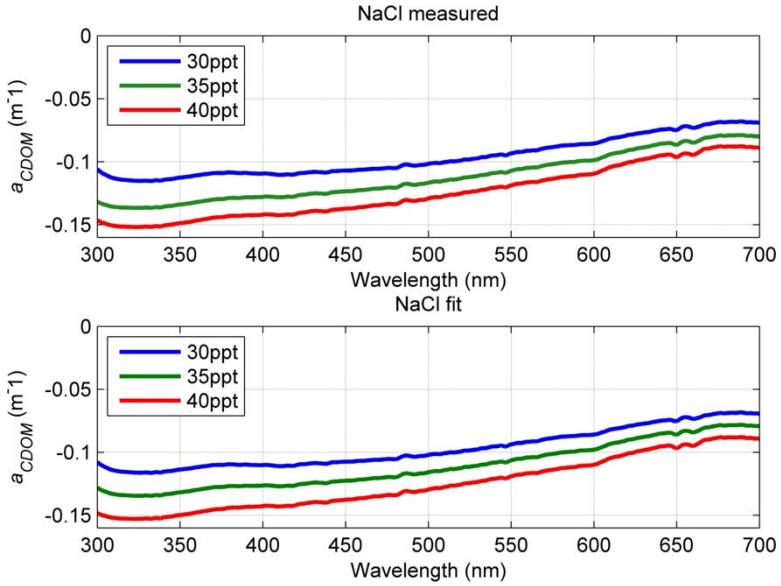

Figure 14. NaCl absorption spectra with positive slope measured on an UltraPath UPUV system (top) and the modeled spectra from the least squares linear fit (bottom).

Water absorption itself is changed by the presence of salt ions and dissolving such ions in water reduces the per volume amount of water molecules. As mentioned previously, liquid waveguide cells are sensitive to small changes in refractive index. The refractive index effect dominates in the blue-green portion of the spectrum, and water absorption effects dominate in the red and infrared spectrum. An increase in refractive index leads to higher light throughput (i.e., transmittance), resulting in negative apparent absorbance when a seawater sample is analyzed against ultrapure water as a reference (D'Sa et al. 1999; Miller et al. 2003). This effect has been reported to be spectrally variable (Nelson et al. 2007), to vary over time within a particular instrument (Nelson et al. 2010), and to vary between instruments (Figs. 8 and 9). In UltraPath 200 cm pathlength waveguide

cells, the magnitude of the effect has been reported to range from <0.001 to >0.003 optical density (OD) per ppt NaCl. Small but consistent offsets possibly related to refractive index have been reported for 10 cm cuvettes in spectrophotometers as well (Green and Blough 1994). There are different types of refractive index issues. For example, the refractive index also controls the transmission in the water-glass interface on both sides of a cuvette. The closer the index of refraction between the quartz and water (e.g., the saltier the water) the better the transmission. If ultrapure water is the reference water, differences in the sample salt content affect light transmission, which may or may not be negligible (see Boss et al. 2013).

The UltraPath instruments tend to be stable when collecting multiple scans from the same injection (or loading) of a salty solution; however, reintroducing the same sample possibly produces absorbance spectra that vary significantly in shape and magnitude. Therefore, it is extremely important to "characterize" each UltraPath or LWCC system and create a set of salinity correction curves based on the behavior of each instrument at or near the time of analysis. It is strongly suggested that a minimum of three salt solutions are measured (such as 30, 35, 40 ppt) when generating a salinity correction curve. It is much easier to assess the accuracy of the NaCl solutions when comparing the shape and magnitude of three or more NaCl absorbance spectra (Figs. 8 and 9).

Each salt solution should be injected three times and at least three scans collected per injection. While this may sound excessive, it is recommended to ensure that the salinity correction curves generated from these measurements will not be affected by contamination, bubbles (e.g., Lefering et al. 2017), or other unknown factors. By collecting multiple scans of a solution and also reinjecting the solution, it is less likely that a bad spectrum will be incorporated into the correction curve. Figure 10a shows an example of a 40 ppt NaCl solution that was continuously scanned for several seconds after injection into an UltraPath liquid waveguide cell. The instability is captured over the period of multiple scans that may be missed if only one of these spectra were measured. Figure 10b shows the same solution that was subsequently reinjected, and the variability of the scans was significantly less.

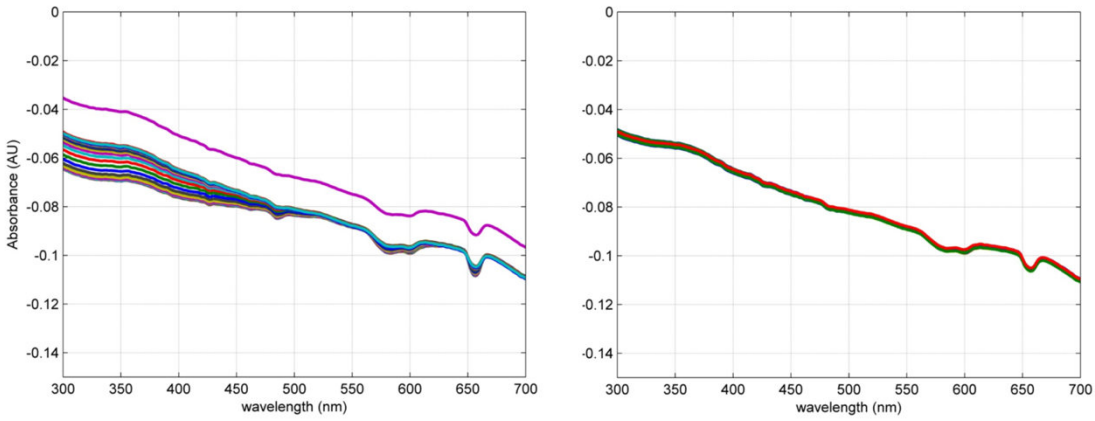

Figure 17. (a) Multiple scans measured in continuous mode collected within several seconds of each after loading a NaCl 40 ppt solution into an UltraPath UPUV 200 cm liquid waveguide system. (b) The same salt solution subsequently re-injected into the waveguide exhibiting significantly less variability between scans over a similar period of time.

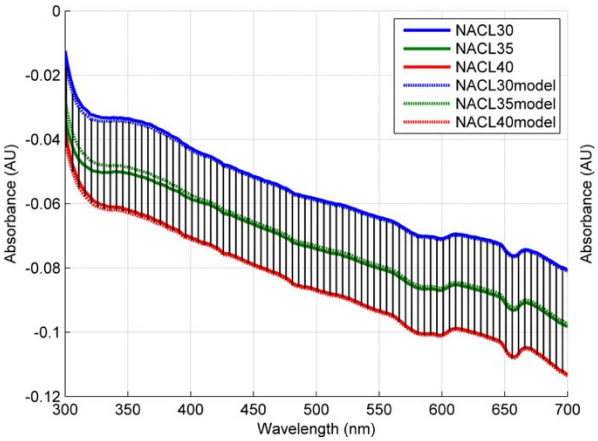

Figure 21. Model of NaCl absorbance spectra using a least squares linear fit to the measured NaCl absorbance spectra. The linear fit is shown here at 5 nm intervals for clarity in the figure; however, the fit should be calculated at 1 nm intervals.

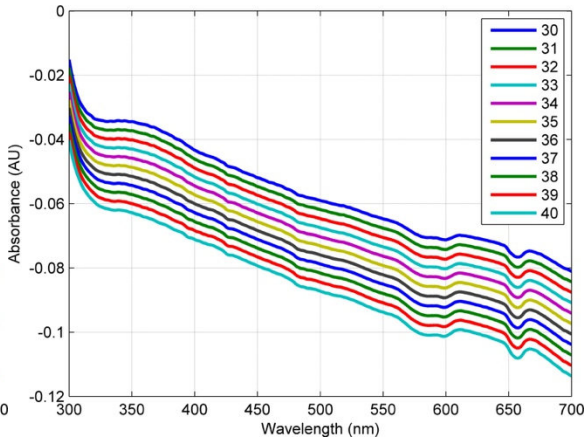

Figure 19. Linear interpolation at 1 ppt increments (for visualization clarity) of the modeled NaCl absorbance spectra. In practice, the interpolation should be carried out at 0.1 ppt increments.

After measuring the salt solutions, calculate the mean and standard deviation of the multiple scans acquired for each injection. The standard deviation at all wavelengths should be less than 0.0007 absorbance units (AU) for the scans from each individual injection. If this criterion is met, then the averages from each injection may be compared. The spectral shape and the magnitude should be similar at all wavelengths. The standard deviation at wavelengths below 700 nm should be less than 0.002 AU and preferably less than 0.001 AU. If there is good agreement between the injections, then calculate their average; otherwise, discard any anomalous injections or repeat the procedure until spectra from at least two injections agree.

The averages of the reinjections for each salt solution should be plotted to evaluate whether the shape of the absorbance spectrum from each salt concentration is similar. The amount of negative (or positive) offset of the NaCl solutions should be directly proportional to the salt content. The general slope of the absorbance spectra may be either positive or negative (higher or lower optical density with increasing wavelength) depending on the composition of the UltraPath liquid waveguide capillary cell as the manufacturer changed the Teflon formulation of the liquid waveguide capillary cell with a Teflon composition yielding a different refractive index (Figs. 8 and 9). If three salt solutions are analyzed, then at each 1 nm wavelength perform a linear fit with respect to salinity using a least squares regression approach (i.e., plot OD vs NaCl ppt at each wavelength and perform a linear regression analysis for each set of three points), the individual linear regression models of the NaCl spectra will account for any small variations in the shape of the measured spectra (Fig. 11).

$A(\lambda) = m(\lambda) * X + b(\lambda)$,

where A = absorbance, X = NaCl salinity value, m = regression slope and b = y-intercept

Finally, absorbance values should be linearly interpolated at 0.1 ppt intervals for each 1 nm wavelength linear regression model (300-730 nm) (Fig. 12). If only two salt solutions are measured (three or more solutions are strongly recommended), perform a linear interpolation to the actual absorbance spectra at 0.1 units. Examples demonstrating the necessity of this procedure to properly attribute the refractive index offset from the sample salinity to the NaCl proxies for each type of waveguide capillary cell material are shown in Figures 13 and 14.

From the results of multiple round robin exercises, it was found that the refractive index offset caused due to the salinity of natural samples tends to be inconsistent among different UltraPath systems and are not entirely due to the UltraPath capillary cell Teflon formulation (Figs. 13 and 14). After testing multiple methods to correct for this, the procedure that produced the strongest agreement between UltraPath systems was the following:

- Measure NaCl solutions across a range of salinities that bounds the salinity of the natural samples.
- Generate a correction curve from these measurements.
- Subtract the NaCl interpolated curve from the CDOM absorbance spectra that produces a value close to zero at 685 nm[4].

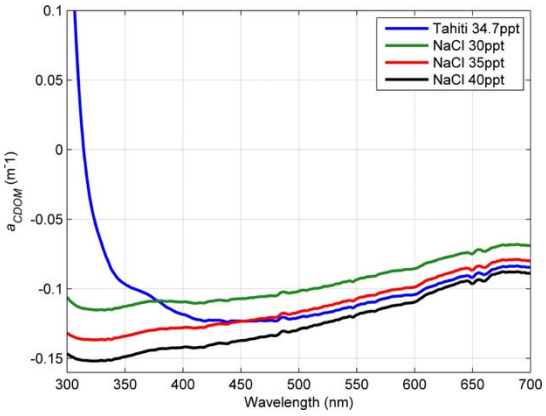

Figure 253. NaCl and "Tahiti seawater" absorption spectra measured on a negative slope UltraPath system. The salinity of the Tahiti seawater was 34.7 ppt, but the offset measured is much closer to the offset from the 40 ppt NaCl solution at the red-end of the spectrum.

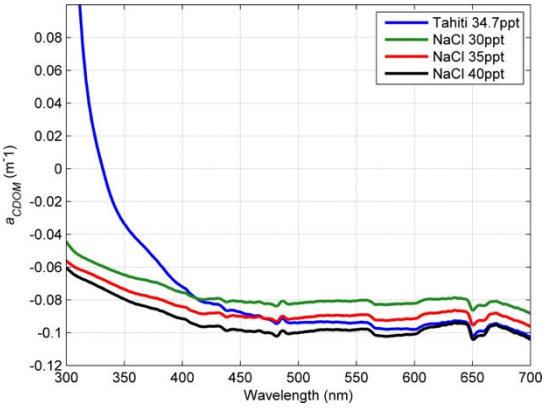

Figure 234. NaCl and "Tahiti seawater" absorption spectra for a positive slope UltraPath system. The salinity of the Tahiti seawater was 34.7 ppt, but the offset at the red-end of the spectrum falls between the 35 and 40 ppt NaCl values.

A simple way to find the optimal correction curve is to compute the absolute value of the difference between the sample absorption at 685 nm and all of the interpolated curve values at 685 nm. The interpolated curve that yields the minimum difference should be used for correction. The optimal NaCl curve may not match the salinity value of the seawater sample because NaCl solutions do not produce an identical refractive index response as seawater. Selecting the interpolated NaCl curve that best matches the null

[4] The specification of this wavelength is justifiable because of the low absorption by CDOM and a plateau in the water absorption at 685 nm, and due to this plateau, the temperature and salinity effects on the water absorption are minimal at 685 nm.

region refractive index response enables a better refractive index correction. The absorbance of all the samples measured between 650-700 nm should fall within the absorption bounds of the high and low NaCl solutions at the same wavelengths. If samples are outside these bounds, it is an indication that there may be a problem within the waveguide flow cell or that the salinity of the sample is not bounded by the salinity of the NaCl solutions.

Once the SRFA-I and salt solutions have been measured and are within acceptable limits, the analysis of samples can begin. Approximately two cell volumes of ultrapure water should be injected and scanned (as a sample) before and after every sample to flush the cell and monitor system performance (at least two cell volumes or ~three minutes). If the reference signal is unstable it may be necessary to flush the cell with greater volumes. When analyzing a low CDOM absorption sample after a high CDOM sample, it is often insufficient to flush the cell only with ultrapure water; a complete cleaning avoids an overestimate of absorption coefficients over the entire spectrum. It is not necessary to conduct multiple injections on each sample; however, it is good practice to reinject at least one to three samples per analysis sequence to assure instrument stability. Analysts should inject sample replicates at this time, including procedural replicates (sampling, handling, filtering, etc.) to quantify the reproducibility of the absorbance spectra.

The practitioner should avoid collecting new reference spectra during the analysis sequence. If there are signs of instability, such as the ultrapure water reference intensity varies more than +/- 2% of the initial value, try flushing the cell again with the cleaning solutions. If the reference intensity of the ultrapure water does not return to its initial value, it may be due to environmental changes in the surroundings. Thus, it may be necessary to collect new dark and light reference spectra. However, the reference intensity must be stable at the new value and new salt solution scans will need to be measured. If there are significant changes in the intensity of ultrapure water between samples, it is an indication that there is a problem within the cell. Most likely it is contamination or clogging within the cell causing bubble formation. If the intensity signal cannot be stabilized with the standard cleaning solutions, follow the advanced cleaning protocol described in Appendix A. When collecting new dark and reference scans, the filename must be changed or the previous scans will be overwritten.

UltraPath and LWCC effective pathlength determination

The effective pathlengths for each pathlength of the UltraPath capillary cell unit and LWCCs should be determined on a regular basis, especially after instrument returns from a field campaign. Several procedures can be employed to determine effective pathlength (Belz et al. 1999; 2006; Cartisano et al. 2018). Here we summarize one approach from Cartisano et al. (2018) using phenol red solutions as opposed to potassium dichromate (NIST 935a) solutions because the latter requires dissolution in perchloric acid[5]. The first step is to prepare a stock of phenol red solution (~16 μM) by dissolving phenol red (ACS

[5] Over time, perchloric acid solutions can form perchlorate crystals such as on the rims of bottles or caps as well as within fume hood exhaust systems. Perchlorate crystals are explosive and can be detonated through friction, heat, fire, or impact with another object.

grade) with 0.05 M Tris-(hydroxymethyl)aminomethane (THAM; molecular biology grade) within a class A volumetric flask. From the stock, at least five separate solutions of phenol red should be prepared per UltraPath capillary cell pathlength to be evaluated and span the linear range of the concentration versus absorbance response, for example ~0.01 to 0.08 µM and ~0.01 to 0.3 µM for use on the 200 cm and 50 cm UltraPath capillary cell pathlengths, respectively (Cartisano et al. 2018). Because of impurities in commercially available phenol red, the actual concentration of phenol red should be determined using the molar absorption coefficient under well characterized temperature and pH conditions (Lai et al. 2016). For example, Cartisano et al. (2018) determined the actual concentration of phenol red at pH of 10.4 and 23°C using a 1 cm cuvette and a double beam spectrophotometer. The effective pathlength can be computed following Beer's Law

$$L_{eff} = A_b(\text{peak } \lambda) / (\varepsilon_{\text{peak } \lambda} * C),$$

where L_{eff} is the effective pathlength, peak λ is the wavelength of maximum absorbance for phenol red (558 nm), A_b is the baseline-corrected absorbance at 558 nm, $\varepsilon_{\text{peak } \lambda}$ is the molar absorptivity (also known as the molar extinction coefficient) of phenol red at the peak wavelength, and C is the actual concentration of phenol red.

Note: In the situation where a spectrophotometer is used, no knowledge of the phenol red concentration is necessary as this can be derived from absorbance measurements and the known molar absorptivity of phenol red.

Double Beam Spectroscopy (1, 5 or 10 cm cuvettes) – Procedure[6]

Absorbance spectra (also referred to as optical density) of CDOM from natural waters can be measured using a double beam ultraviolet-visible scanning spectrophotometer and Suprasil® quartz cells of 1 cm, 5 cm or 10 cm pathlength. The particular pathlength required depends on the raw absorbance signal of the sample compared to detection limit and the linear dynamic range of the spectrophotometer. For oligotrophic waters, obtaining CDOM absorption measurements with sufficient signal-to-noise requires the long pathlengths of the UltraPath and LWCC instruments. Water samples that are visibly colored to the human eye will likely require a 1 cm or 5 cm cuvette for analysis with a scanning spectrophotometer. Marine samples from mesohaline and polyhaline estuarine regions as well as oceanic water samples will require 10 cm pathlength cells. Ultrapure water serves as the blank and reference. Alternatively, single beam instruments may be used but require additional instrument performance characterization and repeated scans of ultrapure water blanks throughout the measurement period to track and correct for fluctuations in instrument performance due to changes in lamp intensity, lab conditions, etc. For the double beam case, typically the water in the reference cuvette heats up as it is illuminated with light and should be exchanged to minimize temperature effects (largely in the red to near-infrared wavelengths between ~690-780 nm) and handling of a

[6] Note that this protocol represents a revision of the previous NASA ocean optics protocols (Mitchell et al. 2000; 2003).

second cuvette requires attention to handling and inspection. Regardless of the instrument used for sample analysis, the specifications of the instrument should be documented and reported. The linear dynamic range (LDR) of the instrument should be known and reported and is not necessarily the photometric dynamic range specified by the manufacturer. Absorbance measurements for the spectral range of interest must not exceed the linear dynamic range of the instrument. Salinity corrections are not necessary for absorbance measurements from double or single beam spectrophotometers using 1 cm to 10 cm pathlength cells (assuming that CDOM absorbance measurements at wavelengths >700 nm are not of interest) but are advised for diode-array detectors (Cartisano et al. 2018 and references therein). The protocol for using a double beam spectrophotometer for CDOM measurements is as follows:

- Turn on the double beam spectrophotometer to warm up for 1 hour.
- Inspect the optical windows in the sample compartment. If necessary, clean light source and detector optical windows inside the sample compartment with lint-free optical lens cleaning tissue slightly moistened with isopropanol, followed by a gentle wipe with dry lens tissue to remove any visible lint on the optical windows. This should be done as needed from daily to weekly depending on the laboratory environment.
- Equilibrate CDOM samples to room temperature in a water bath and filter through pre-rinsed 0.2 µm filters shortly before analysis as described previously (Section II). Reference fluids should also be equilibrated to room temperature.
- Clean quartz cells with the following procedure:
    - For new cells or when more extensive cleaning is required, the cells should be soaked in a basic detergent bath (e.g., RBS™ 35, Thermo Scientific), rinsed with deionized water, then soaked in hydrochloric acid (HCl; ~1.2 M) bath and then rinsed thoroughly with ultrapure water.
    - For routine cleaning of 10 cm cells (adapt to specific cuvette size). The user should wear proper gloves (e.g., powder-free nitrile), safety glasses, and other personal protective equipment.
        - Fill each cell with 5-10 mL of 10% HCl, add caps, shake vigorously, and dispose of HCl as appropriate. Repeat this process two additional times. Clean the exterior of the optical windows of each cell, using a squirt bottle to squirt 10% HCl onto each optical window three times. Wiping with moistened optical lens tissue is also effective as long as precautions are taken to avoid contact of the optical window with gloves or liquid dripping off gloves.
        - Fill each cell with 5-10 mL of HPLC-grade isopropanol, add caps, shake vigorously, and dispose of isopropanol as appropriate. Repeat this process 2 additional times. Clean the exterior of the optical windows of each cell, using a squirt bottle to squirt isopropanol onto each optical window three times. Wiping with moistened optical lens tissue is also effective as long as precautions are taken to avoid contact of the optical window with gloves or liquid dripping off gloves.

        - Rinse each cell by filling with copious amounts of ultrapure water and discard the water. Repeat this process at least five to seven times. Caps should be rinsed as well.
    - Fill each cell with ultrapure water and allow them to equilibrate to room temperature.
- Conduct spectrophotometer instrument performance tests daily, which include wavelength accuracy and reproducibility, photometric noise, and baseline flatness tests. Such performance tests are designed into each instrument's software and hardware, instrument operators should review their instrument's manual for details. Instrument must pass all tests prior to proceeding with analysis. Instrument performance results should be recorded in digital format and archived.
- Furthermore, the National Institute of Standards and Technology (NIST; or other national metrology institution)-traceable calibration standards (for wavelength accuracy, stray light and photometric accuracy) should be conducted routinely to verify instrument performance (available from various commercial sources such as FireflySci, Hellma Analytics, MilliporeSigma, Starna Scientific, etc. and NIST). The required frequency of these tests can range from weekly to monthly depending on instrument usage, whether the instrument is a multi-user facility or dedicated to CDOM measurements. For conventional single beam and diode array spectrophotometers, more frequent calibrations are recommended. Instrument calibration results should be recorded in digital format and archived.
    - Photometric accuracy: use NIST 935a 10 mm cuvette standards (potassium dichromate solutions) and NIST 935e neutral density filters to verify photometric response in the visible range.
    - Stray light: use NIST SRM 2032 (potassium iodide solution) or comparable standards.
    - Wavelength accuracy: use Holmium oxide filter
- Set the instrument for absorbance measurements using the following typical instrument scan settings:
    - Analysts should first review their instrument manual and experiment with the software settings to select the appropriate scan settings for their particular samples and science objectives. The terminology presented here generally refers to Agilent Cary double beam spectrophotometers such as the Cary 100, 300, 4000, etc. Similar instruments from several other manufacturers are capable of providing absorbance measurements of comparable quality.
    - **Wavelength scan range: 250–800 nm** (or other range of interest)
    - **Data interval: 1 nm data interval** (other intervals from <1 nm to 2 nm are also acceptable). Typically, the data interval specifies the spectral step for recording data values and not the data acquisition interval.
    - **Scan rate: 100 nm min^{-1}.** The wavelength scan range and scan rate determine the number of data points acquired. Instrument software may provide the signal averaging time based on scan rate and range. If a faster scan rate is used, the analyst should determine whether such a scan rate provides an adequate number of data points and signal-to-noise. The signal averaging time value should be reported. Instrument software from different manufacturers offer different scan settings. For example, on PerkinElmer

Lambda instruments, the integration time is chosen, and the scan speed is calculated and shown. Therefore, for the Perkin Elmer case, analysts would select an appropriate integration time. Modern spectrophotometer systems offer features that adjust scan settings with wavelength to optimize signal-to-noise or other parameters.

    - **Slit width (spectral bandwidth of light source): 4 nm.** The broad slit width is typically necessary to provide adequate signal. Since CDOM absorption spectra are generally devoid of any narrow spectral features, there is no known benefit to using a narrower slit width. The analyst may use an alternate slit width such as 2 nm for CDOM absorbance scans.
- Raw absorbance measurements must be recorded and reported to at least four decimal places.
- Conduct a full spectrum baseline with nothing in the sample compartment ("air versus air" baseline scan) to zero the instrument across the full spectral range. The purpose of the air baseline is to balance the reference and sample beam. The reference beam is used internally by the instrument to compensate for variations in the light intensity, monochromator throughput and detector sensitivity. Note that the Agilent Cary instruments use the terminology "baseline" while the PerkinElmer Lambda instruments use the term "autozero".
- Conduct a full spectrum scan with nothing in the sample compartment ("air vs. air" scan)
    - The purpose of this "air vs. air" scan is to evaluate the noise performance of the instrument for the specific instrument settings to be used for CDOM absorbance scans in the absence of cuvettes or solutions within the path of the light beams.
    - Inspect the scan to confirm that this meets the specifications of the instrument, for example ±0.0005 AU throughout the entire spectrum for a typical double beam spectrophotometer (Fig. 15). More advanced instruments have the capability of producing significantly lower noise of ±0.0001. The portion of the UV spectrum (e.g., <350 nm) collected with a deuterium light source has a higher noise level than the portion collected with a tungsten light source (>350 nm). The various light sources overlap in spectral range across the higher wavelength end of the UV spectrum. However, since Tungsten lamp intensity decreases below ~350 nm, spectrophotometers switch light source below ~360 nm to Deuterium lamps due to their stronger UV light source. The actual wavelength in which the switchover occurs varies with instrument and in some instruments can be changed through the software provided by the manufacturer.
- Conduct a full spectrum scan of each cuvette cell pair filled with ultrapure water in the sample compartment (versus air within the reference beam) and confirm that the cells match optically, i.e., the measured absorbance difference is within the noise threshold of the instrument such as within ±0.0005 AU (Fig. 16).

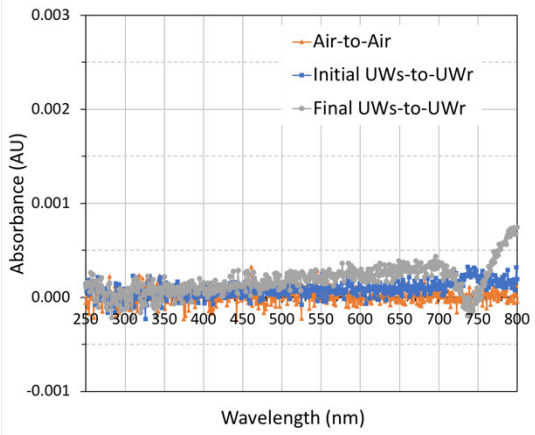

Figure 285. Double beam spectrophotometer absorbance scans of air following air-to-air baseline (nothing in the sample compartment), 10-cm pathlength Suprasil® quartz cylindrical cells with ultrapure water in sample beam and ultrapure water in reference beam following ultrapure water-to-water baseline, and end-of-day (final) ultrapure water-to-water scan. UWr refers to the ultrapure water filled quartz cell used as reference and UWs refers to the ultrapure water filled quartz cell used as sample.

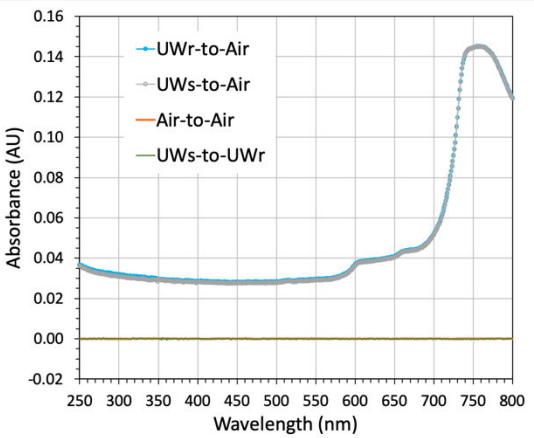

Figure 266. Double beam spectrophotometer absorbance scans of air following air-to-air baseline (nothing in the sample compartment) and each 10-cm pathlength Suprasil® quartz cell filled with ultrapure water in sample beam and air in the reference beam, and Suprasil® quartz cell with ultrapure water in sample beam and ultrapure water in reference beam following ultrapure water-to-water baseline. See Fig. 15 for other details.

- Conduct a baseline with ultrapure water filled cells in the sample (UWs) and reference (UWr) beams (ultrapure water to ultrapure water baseline) to zero the instrument for pure water. By using a matched cuvette filled with ultrapure water, the absorbance contributed by water molecules is accounted for internally by the instrument when measuring absorbance of samples.
- Conduct a full spectrum scan with ultrapure water-filled cells in the sample compartment (UWs to UWr scan).
    - Similar to the air to air scan, inspect the UWs to UWr scan to confirm that this meets the noise specifications of the instrument, for example ±0.0005 AU to ±0.0001 AU throughout the spectrum, depending on the instrument (Figs. 15-16). Note that a slight rise or drop in absorbance in the UV would suggest CDOM contamination in the sample or reference cell, respectively.
- Discard the water in the sample cell, shake gently to remove all the water. Rinse the sample cell with sample water by filling the cell with 5-10 mL of sample water, capping, mixing contents, and rinsing internal surfaces thoroughly. Repeat the rinse process two additional times.
- Fill the sample cell with sample water, rinse the external optical windows of the sample cell with ultrapure water to remove any sample residue, and dry the cell with lint-free tissues (optical lens or Kimwipes®).
- Inspect the contents of the sample cell for particles and bubbles. If particles are observed, then discard the contents, rinse and refill with sample water.
- Inspect the sample cell optical window for any marks (remove with ultrapure water and lint-free tissue) or lint (remove by wiping with lint-free tissues). Inspection is aided by viewing the sample cell against a dark background such as a laboratory counter.
- Place the sample cell inside the sample compartment and initiate a full spectrum scan.

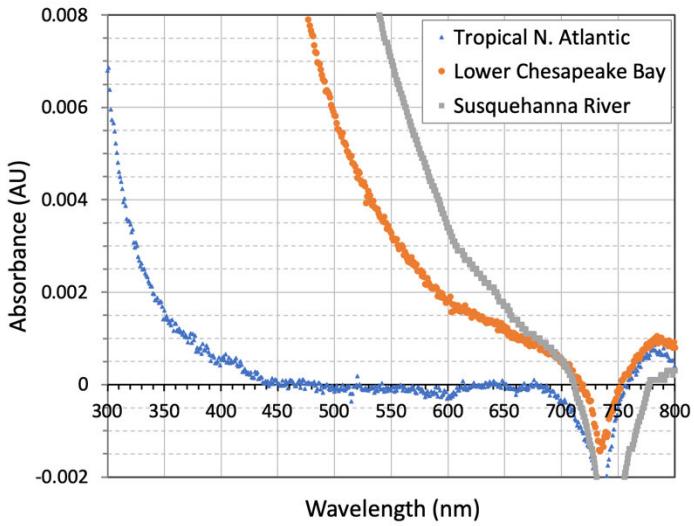

Figure 30. Examples of natural water samples scanned on a double beam spectrophotometer with 10 cm cells that pass the null signal criteria in the red spectral range (Mannino unpublished data).

- Inspect the scan within the 650-700 nm region. The absorbance value should be within the noise threshold of the instrument. If the value does not meet the threshold (typically within ±0.0010 AU), the sample should be prepared again for scanning. There are three approaches (1) a dry wipe or cleaning of the external optical windows, (2) re-loading of the sample into the cell, or (3) re-filtration of the sample. Obtaining a good scan may require re-filtration of the sample. In cases where the sample has fairly high CDOM absorbance (typical of estuarine and river waters), the absorbance values will increase from ~700 to 600 nm rather than expressing a relatively flat signal between ~650 and 700 nm (see Figs. 17 and 18). A scan showing slightly elevated but relatively flat absorbance response within the 650 to 700 nm range, such as 2 to 3 times the instrument noise threshold, may be acceptable and preferred to avoid a null point correction (see null correction notes in section III; Figs. 17 and 18). Null point corrections should be a last resort. Thus, analysts should attempt the recommended approaches to obtain a good scan to avoid the necessity of a null point correction.

If and when practical, procedural CDOM absorption blanks should be prepared in the field using ultrapure water in place of sample water and processed in the same manner as the sample.

Proceed to Data Analysis section.

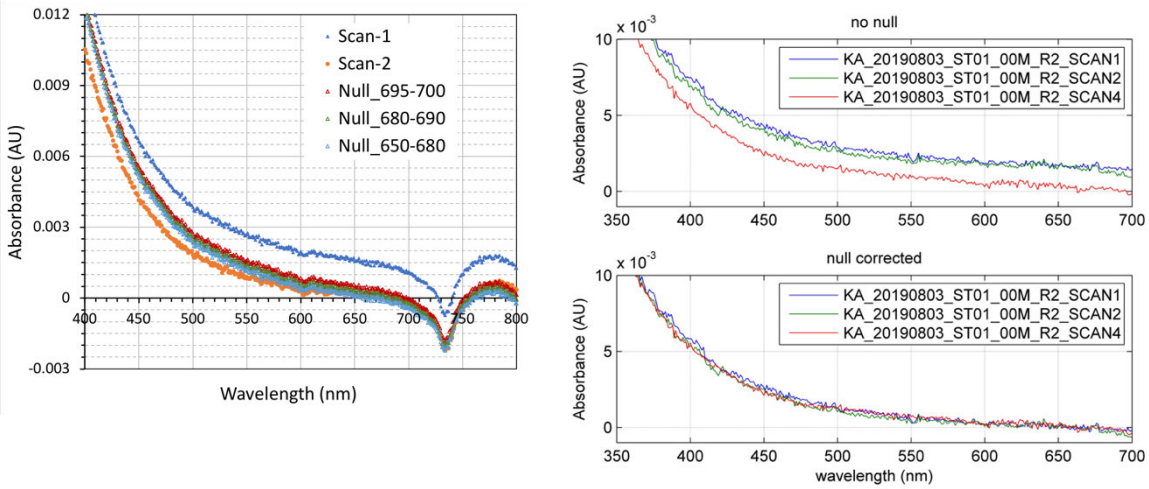

Figure 31. (a) Absorbance spectra of a coastal ocean sample (mid-Atlantic U.S.) scanned on a double beam spectrophotometer with a 10 cm pathlength cell. The first scan is shown without a null correction and with three null corrections implemented by subtracting the average absorbance signal for the wavelength ranges specified in the legend. The sample was reloaded in the 10 cm cell for a second scan to mitigate the null signal criteria failure in the red spectral range of the first scan. (b) and (c) A similar scenario where re-filtering and re-loading of a sample collected in the coastal Beaufort Sea did produce similar results after using the 650-680 nm null region to correct all of the scans. (Mannino and Novak, unpublished data).

Sea-Bird Scientific absorption-attenuation (ac) meters – Laboratory Procedure

The Sea-Bird Scientific (formerly WETLabs) ac-s and ac-9 instruments were not developed with single-sample measurement in mind; rather they were intended for *in situ* measurement of absorption and attenuation. Details on this technology and protocols on calibration and data processing are provided in Twardowski et al. (2018a). With proper attention to flow, pure-water offsets, repeatable measurements, and temperature, these instruments can be used effectively for single-sample measurements. There are several methods available, including slowly filling the tubes while the instrument is horizontal, pouring through a funnel and tubes with the ac-s or ac-9 in vertical position, and using gravity or a pressurized carboy to generate flow, restricting the flow with a valve after the fluid has exited the instrument. The greatest success from the CDOM round robin experiments was attained using the gravity-fed system, which will be described briefly here.

Samples were gravity filtered with a 0.2 µm membrane filter. Two-liter glass carboys with a barbed fitting on the bottom provided the sample water feed to the instrument. A 0.95 cm inner diameter (ID) tube (0.375 inch) was attached and reduced to 0.635 cm ID (0.25 inch) to conserve the sample. Near the intake (bottom of ac-s instrument flow tube), it was expanded to 1.27 cm ID (0.5 inch) to match the size of the ac-s. At the outlet (top of ac-s instrument flow tube), this was reversed. Feeding the sample water from the bottom to the top of the flow tube assists with removal of bubbles from the flow tube. Valves were present near each tubing size change, which were closed when the flow tubes were disassembled and cleaned. Only the absorption tube was used for each

measurement in the round robin, but it is useful to make measurements through both the absorption and attenuation tubes.

A short protocol for single-sample measurements from ac-s or ac-9 follows.

- The carboy with sample water should be positioned approximately 1 m above the inlet to provide sufficient pressure for gravity flow (Fig. 19).
- The flow should be restricted to about 200 ml min^{-1} by partially closing the outlet valve after debubbling the system by tilting, tapping and squeezing the tubing.
- The stability of the measurements can be monitored using the software WETView (Sea-Bird Scientific) in 'Absorption vs Time' mode, choosing 5-6 wavelengths from the full spectrum. Temperature should be monitored with a thermometer in the water flowing out of the outlet. After a minute of stable measurements, the flow and data acquisition are terminated and the data are recorded.
- The measurement is repeated until three sets of measurements match closely (within 0.005 m^{-1} in most wavelengths or 0.01 m^{-1} near 400 nm).
- Prior to making repeat measurements, the instrument is turned off and sample tubes and optical windows are cleaned.
- Pure water offsets are measured before and after analysis of a batch of samples, with the same protocol as the CDOM measurements, but lower tolerance – 0.003 m^{-1} in most wavelengths and 0.005 m^{-1} near 400 nm.

Figure 33. Photo of the instrument setup for the ac-s and ac-9 Sea-Bird Scientific absorption-attenuation (ac) meters used in the CDOM absorption round robins.

Data processing includes taking the mean of the minute-long measurement, subtracting temperature, salinity (for seawater samples), and pure-water offsets based on published tables (Sullivan et al. 2006). Each pure-water offset should be plotted with its standard deviation, and the three ultrapure water scans with values closest to each other selected for use in processing, after eliminating any calibration with high variability. The mean of these three measurements is then subtracted from the sample absorption values.

Discrete Measurements of CDOM Absorption from Integrating Cavity Absorption Instruments

Details on the instrumentation and procedure for absorption measurements from Point Source Integrating Cavity Absorption Meters (PSICAM) are provided in the Absorption Protocol (Röttgers 2018).

Details on the instrumentation and procedure for absorption measurements from an Integrating Cavity Absorption Meter (ICAM) are provided in the Absorption Protocol (Fry 2018).

In Situ Vertical Profiles and Underway Measurement Approaches

For both methods of *in situ* measurement of CDOM absorption by ac meters, it is imperative to have good pure-water calibrations, as the magnitude of CDOM absorption and the errors due to instrument drift are similar. Methods of calibrations are detailed in the Absorption Protocol by Twardowski et al. (2018a), as well as Sullivan et al. (2006) and the WETLabs ac Protocol document (available at the Sea-Bird Scientific website, https://www.seabird.com/asset-get.download.jsa?id=54627862517).

Vertical Profile CDOM Absorption Measurements

Details on the instrumentation and procedure for collecting vertical profile absorption measurements are provided in the Absorption Protocol (Twardowski et al. 2018a).

In general, a 0.2 µm pore-size capsule filter is used to measure only the dissolved fraction of water. It is best to cut away the input part of the filter housing, to increase flow rate and avoid capturing air in the filter housing, and to soak the filter for several hours in ultrapure water before use. When deploying, it is important to have the pumps off until the package is 5-10 m below the surface for several minutes. Once the instrument and software system are turned on, it is good to check that the instrument has completely degassed and values are stable by monitoring the incoming data. If data are suspect, cycle power and try again. When values are stable, bring the package to the surface and begin vertical profile.

The filtered ac-s or ac-9 can be part of a two-ac package or it can be the same instrument either on an alternate cast at the same station, or using an electronic switch to filter only on the upcast (Sequoia Scientific FlowControl Sub). The filtered flow can cause a lag of up to 30 seconds between data acquired from the ac meter and the CTD, which requires attention during data acquisition and data processing. Details are given in the Absorption Protocol (Twardowski et al. 2018a).

Ship-based underway flow-through CDOM Absorption Measurements

Details on the instrumentation and procedure for collecting ship-based underway flow-through absorption measurements are provided in the Flow-through Optical Data Protocol (Boss et al. 2019).

Most underway measurements of CDOM absorption are conducted in conjunction with whole-water (unfiltered) measurements. The whole-water is measured during the majority of the time, with filtered measurement usually during only 10 to 15 minutes per hour. For the filtered measurements, it is best to have a pre-filter (such as a 3 or 5 µm pore size capsule filter) before the final 0.2 µm capsule filter. The initial two minutes (or more) of filtered data should be removed in order to flush the lines of whole water and leftover water remaining in the filters. Further flushing may be required depending on the filter capsule used (see section V).

Bubbles are the main culprit behind poor-quality CDOM absorption data, and all efforts at reducing them (de-bubblers, etc.) described in Boss et al. (2019) should be employed. Spikes in the CDOM absorption data should be removed before averaging.

III. Data Analysis and Error Budgets

Spectrophotometer software typically returns data in decadal absorbance (A) in dimensionless units (AU; also referred to as optical density) defined as:

$$A(\lambda) = \log_{10} ([I(\lambda) - DC(\lambda)] / [I_o(\lambda) - DC(\lambda)])$$

where I and I_o are the light intensity at the detector (in counts or volts, depending on whether the detector is digital or analog) at wavelength λ, and DC is the dark current signal (recorded separately in some systems). The absorbance values must be recorded and reported to at least four and preferably five decimal places.

Dimensionless absorbance is converted to the Napierian absorption coefficient (a) by scaling base 10 logarithms to base e and dividing by the effective pathlength, such that:

$$a(\lambda) = 2.303 * A(\lambda) / l,$$

where $\underline{l}$ is the effective path length (m). See Hu et al. 2002 and references therein for a discussion of and recommendations for optical terminology usage to resolve ambiguities commonly found in publications on the subject. The Napierian absorption coefficient values must be recorded and reported to at least three and preferably four decimal places.

CDOM spectral slope coefficients (S) are determined by fitting a single-exponential nonlinear curve to each a_{CDOM} data set (e.g., 275–295, 350–400, 350–500, 300–600 nm or other wavelength ranges; Helms et al. 2008; Babin et al. 2003; Blough and Del Vecchio 2002):

$a(\lambda) = a(\lambda_o) * e^{-S * (\lambda - \lambda o)}$,

where $a(\lambda)$ and $a(\lambda_o)$ represent the absorption coefficients at wavelength λ and reference wavelength λ_o. The CDOM spectral slopes derived in this manner are insensitive to a_{CDOM} from higher wavelengths; i.e., this approach affords greater weight to a_{CDOM} values in the ultraviolet and blue spectral regions where signal-to-noise is highest. The software applied to compute *S* can impact the *S* value obtained and the non-linear model fit statistics. Such differences are related to how curve fitting software allow the reference wavelength to float versus a fixed wavelength, weighting of the data, etc. Other approaches and non-linear models for computing CDOM spectral slopes have been suggested (e.g., Twardowski et al. 2004). As long as the full spectrum UV-Vis CDOM absorption coefficient measurements (~250 to 700 nm) at 1 nm intervals are reported, all CDOM spectral slopes of interest can be computed. For example, CDOM spectral slope coefficients ($S_{275-295}$) and slope ratios ($S_{275-295}$:$S_{350-400}$) can be used as a tracer of terrestrial DOM, provide a relative measure of molecular weight, aromatic content, and extent of photochemical degradation (Helms et al. 2008; Loiselle et al. 2009; Fichot and Benner 2012). There is no unique slope value that represents the whole spectrum, and the chosen spectral range determines the derived slope values. Furthermore, the error model used in the non-linear fit also influences the spectral slope computed. If no error model is applied, then one basically assumes that the error is constant and the same at all wavelengths. The best approach for non-linear fitting is to weigh the data by their uncertainty distribution, and this way noisy data weigh less than less noisy data. Spectral slope coefficient values are typically reported in units of nm^{-1}. *S* values must be recorded and reported to at least three and preferably four decimal places.

Example error budget

Results from the February 2015 Round Robin suggest that uncertainties in CDOM absorption for UltraPath and LWCC systems are generally on the order of 5-10% and varies with wavelength (Figs. 4, 20 and 28). The absolute uncertainty is lower at higher wavelengths due to comparably low absorption signal. The percent uncertainty is lower at lower wavelengths due to the significantly higher absorption signal in the UV. With the implementation of recommendations described in this protocol document (instrument calibration, pathlength assessment, salinity correction, etc.), the state-of-the-art uncertainties are likely to be the maximum of 0.01 m^{-1} or 10% (Fig. 4). A future update to the CDOM absorption protocols will provide a detailed estimate of UltraPath and LWCC uncertainty.

The uncertainty associated with a_{CDOM} measured from double beam spectrometers with an instrument noise level <0.0046 m^{-1} (e.g., Cary 100) is on the order of 0.023 to 0.039 m^{-1} for 10 cm pathlength cell and based on the summation of the instrument manufacturer's guaranteed specifications for photometric accuracy, stability and noise (Mannino et al. 2008). If measurement uncertainties related to sample collection, filtration, processing and storage in the field and laboratory are also included, then the overall uncertainty is greater. Typical coefficient of variation (CV) for a_{CDOM} for replicate measurements is on the order of 3% to 5% and varies with wavelength, amount

of CDOM and instrument pathlength. Lower CV values in the UV-blue portions of the spectrum and higher in the red wavelengths.

Notes on long wavelength null correction

A widely accepted assumption is that the absorption of CDOM is zero or very close to zero at the long wavelength end of the absorbance spectrum roughly between ~680 to 800 nm. Nevertheless, in practice, this is not always the case for a small number of samples despite heroic efforts to properly prepare a sample for analysis (careful filtration, wiping of cuvettes, re-filtration, re-cleaning of cuvettes, re-loading of sampling, etc.). Hence, it is sometimes difficult to achieve a near zero absorbance (optical density) values between ~680 to 800 nm. This is in part due to temperature and salinity differences between the sample and ultrapure water reference because of their effects on pure water absorption (Pegau et al. 1995). Other factors that contribute to absorbance in the red and near infrared portions of the spectra include scattering by particles that pass through the 0.2 µm filter, high concentration of colloids (<0.2 µm minerals or high molecular weight organic matter), small bubbles, particulate contaminants from the air, filter fibers or material, lint on the optical window, etc. An experimental study on colloid absorption using liquid core capillary waveguides (LWCC; 50 cm pathlength; similar technology to UltraPath) found a significant offset from zero at 700 nm (<0.14 m^{-1} or <0.03 AU) with two molecular weight standards and natural coastal seawater (Floge et al. 2009). They determined that the LWCC with the Teflon AF internal material was more prone to light losses due to scattering than the LWCC that had fused silica as the interior surface between the sample and the Teflon AF, which Floge et al. (2009) attributed to the higher refractive index of the latter (also higher than that of seawater and freshwater). Studies of natural waters ranging from turbid rivers to estuaries to coastal ocean to oligotrophic ocean have not observed a significant scattering impact by colloids at long wavelengths for measurements in 1-10 cm cells and various UltraPath pathlengths (e.g., Mannino et al. 2014; Matsuoka et al. 2017; Tzortziou et al. 2008).

The results from the round robin CDOM absorption experiments indicate that performing a null point correction yields more consistent results among analysts and instrumentation than without performing such a correction (Fig. 20). Therefore, a null point correction is recommended for samples in which the absorbance value exceeds the noise threshold of the instrument (approximately between ±0.0001 to ±0.0005 AU depending on the instrument) in excess of ~±0.001 AU between 650-700 nm after best efforts in attempts to alleviate this. For the UltraPath systems, the recommended salt correction protocol integrates a null point adjustment based on the selection of the salinity curve selected that yields a zero value at 685 nm. No additional null point correction is necessary on the UltraPath when the offset of the sample falls within the range of the salinity curve offset or an alternate salinity (i.e., refractive index) correction approach is applied.

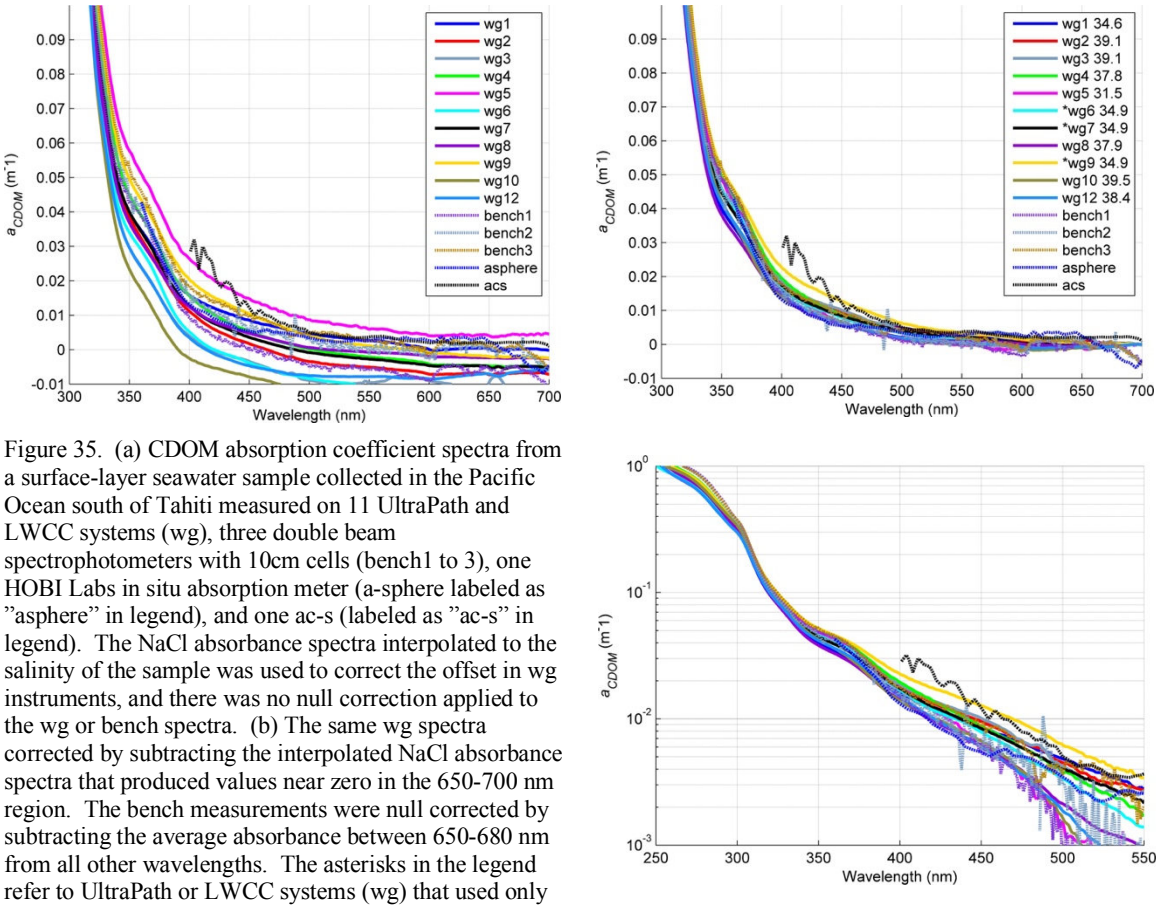

Figure 35. (a) CDOM absorption coefficient spectra from a surface-layer seawater sample collected in the Pacific Ocean south of Tahiti measured on 11 UltraPath and LWCC systems (wg), three double beam spectrophotometers with 10cm cells (bench1 to 3), one HOBI Labs in situ absorption meter (a-sphere labeled as "asphere" in legend), and one ac-s (labeled as "ac-s" in legend). The NaCl absorbance spectra interpolated to the salinity of the sample was used to correct the offset in wg instruments, and there was no null correction applied to the wg or bench spectra. (b) The same wg spectra corrected by subtracting the interpolated NaCl absorbance spectra that produced values near zero in the 650-700 nm region. The bench measurements were null corrected by subtracting the average absorbance between 650-680 nm from all other wavelengths. The asterisks in the legend refer to UltraPath or LWCC systems (wg) that used only two NaCl solutions for the refractive index ("salinity") correction, as opposed to three NaCl solutions (wg with no asterisk), and required a null correction even after applying the salinity correction. The legend lists the matched salinity value for each UltraPath and LWCC system derived from the NaCl spectra. (c) Values from plot b with log scale y-axis.

In the final revisions of the NASA Ocean Optics Protocols describing double beam spectrophotometer measurements of soluble absorption, it was recommended that the long wavelength absorption value – average over a discrete wavelength range of 590-600 nm – be subtracted from the entire spectrum (Mitchell et al. 2003). This was recommended to avoid misapplication of a null point correction due to the effects of temperature and salinity on the observed absorbance spectra near and above 700 nm. Prior editions of that protocol also recommended a null point correction near 600 nm and acknowledged that while this may be appropriate for oligotrophic ocean samples, it would not be appropriate for samples with high levels of CDOM such as from rivers or estuaries due to non-zero CDOM absorption at those wavelengths (e.g., Fig. 17). However, a much stricter quality control procedure is recommended in the current edition of the protocols when implementing a null point correction. For double beam spectroscopy, the null point correction recommended is the average absorbance between 650-680 nm and should generally not exceed ~0.0007 to ~0.0015 AU for oligotrophic to coastal and inland waters, respectively. This null value should then be subtracted from the entire spectrum prior to computing the absorption coefficient. In some instances,

using 10 cm cells in a double beam spectrophotometer, CDOM absorbance scans were observed with a positive or negative offset greater than +/- 0.0015 AU (Mannino et al. 2014). After wiping the optical windows, reloading of the sample, cleaning the cell and reloading the sample, or re-filtering the sample, the scans were repeated and found to have an offset much closer to zero. However, offsetting the first scans to zero using the long wavelength null point correction did not reproduce the second spectra (Fig. 18). Alternatively, a similar scenario of re-filtering and null correcting did produce almost identical spectra (Fig. 18). Therefore, absorbance scans with significant offsets in the null point region should be rejected based on this uncertainty and the sample reloaded and scanned again. The null correction assumes that the error is spectrally neutral, which is generally not true, particularly when the residual absorption is due to scattering by small particles. Only after significant efforts to reduce the offset measured by applying these procedures should the null correction be applied. The region between 650-680 nm is being suggested to avoid artifacts in the spectra from differences in sample and reference temperatures and to avoid true CDOM absorption at shorter wavelengths. The null correction value for double beam measurements or the interpolated salinity value used to correct the UltraPath spectra should be reported in the comments section of the file submitted to SeaBASS or similar databases.

IV. Data Reporting

Data reported to bio-optical databases (including the NASA SeaBASS) should include the final data converted to Napierian absorption coefficient in units of m^{-1}. For each sample measurement session, the SRFA-I standard absorption spectrum should be reported in L g^{-1} cm^{-1} units (equivalent to dividing $a_{CDOM}(\lambda)$ [m^{-1}] by SRFA-I [mg L^{-1}] concentration). For instruments that output absorbance values, raw data in absorbance units should also be reported for each measurement scan including samples, ultrapure water, air and ultrapure water baseline scans (if applicable), and for the sodium chloride solution standards (if applicable). The null correction value for double beam spectroscopy or the interpolated salinity value used to correct the UltraPath spectra should be reported in the comments section of the SeaBASS file. The results of the NIST traceability and instrument performance tests should be included in the documentation files along with the effective pathlength for the UltraPath. Spectra of electronic noise floor (rescans of the same blank) and reinjection error (reinjections of the same blank) should be included (root-mean-square deviation, RMSD) with each batch of samples. A complete description of the water sampling, processing, filtration (filter type, pore size, and diameter, rinse volume, etc.), and analysis should be submitted with the data to bio-optical databases and archives.

V. Evaluation of Filter Materials for Contamination

Background

A variety of 0.2 µm disc filter materials and manufacturers were evaluated to ascertain if any colored material or dissolved organic carbon leached from the filter during filtration. The filter types and manufacturers are listed in Table 1. Each filter was tested in triplicate, and the mean was reported for each analysis. In addition, syringe and capsule filter types were examined with different filter membrane and housing compositions. These were only measured in duplicate, and the averages were reported.

Methods

For evaluation of disc filters, a vacuum filtration bell jar with acid washed 47 mm glass frit and filter funnel was used in these experiments (Fig. 2a). Each disc filter was pre-rinsed by filtering 175 mL of ultrapure water into a waste cup which was discarded. Another 175 mL of ultrapure water was filtered into clean combusted amber glass bottles and analyzed on a Cary100 benchtop spectrophotometer with 10 cm Suprasil® quartz cells. The filtrate from select filters was also analyzed for DOC content on a Shimadzu TOC-V instrument (Mannino et al. 2014). Syringe filters and sterile disposable plastic syringes were rinsed three times with 40 mL of ultrapure water before filtering into the sample bottle. Each disc filter was tested in triplicate and the averages of the three scans are presented.

The capsule filters were flushed with ultrapure water by directly attaching the filters to the final 0.2 µm filter of a Milli-Q trigger using acid washed and Milli-Q-rinsed Tygon® tubing formulation 2375.

- CDOM and DOC sample bottles were filled without stopping the flow at incremental volumes (1L, 5L, and 10L).
- After 10 Liters was filtered, the flow was stopped and the ultrapure water within the capsule was allowed to stand for ten minutes. Without flushing the filter again, sample bottles were filled.
- Ten more liters of ultrapure water were flushed through the filter and sample bottles were filled again without stopping flow – a total of 20 L rinse.
- After the bottle was filled, water was allowed to stand inside the capsule for an additional ten minutes, then the water was collected without flushing representing the 20L rinse and retentate samples.

Each capsule filter type was measured in duplicate and the average of the scans are depicted in figures. Samples at 1 and 5 liters were not collected for the Whatman Polycap filters and the second retention sample was collected after filtering a total of 30 Liters through the filter.

Pall Supor polyethersulfone (PES) disc filters were soaked in ACS-grade 10% HCL for 20 minutes and tested for contamination after rinsing with different volumes of ultrapure

water (175 and 300 mL rinses). The absorption measurement results were compared to PES filters that were not soaked in HCl and rinsed with 175 mL of ultrapure water.

Nylon and PES filters were rinsed with several different volumes of ultrapure water up to one liter. The absorbance spectra were measured on the filtrates from each volume level evaluated.

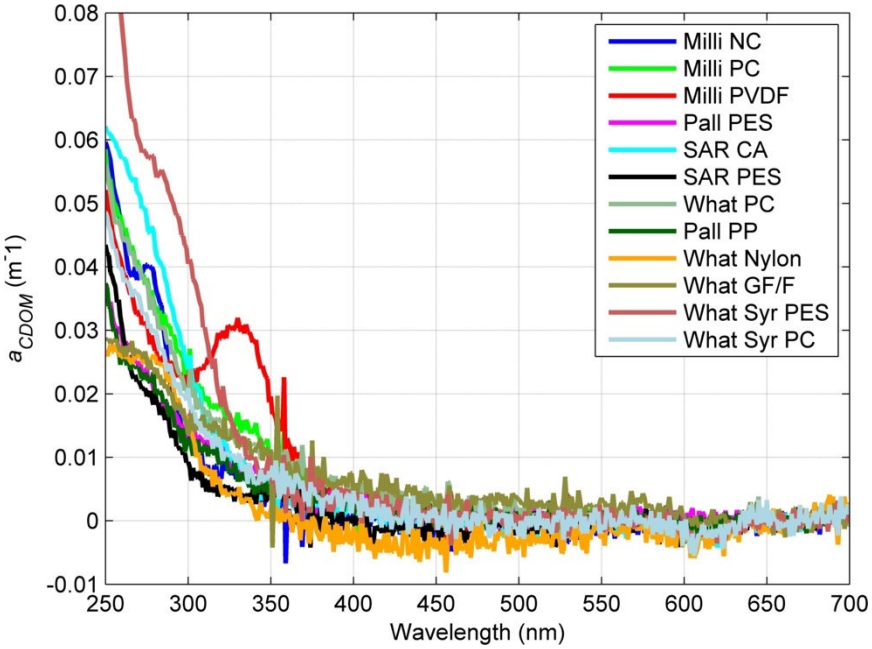

Figure 37. CDOM absorption spectra of ultrapure water (Milli-Q) collected after rinsing flat disc filters with 175 ml of ultrapure water measured on a double beam spectrophotometer. See Table 1 for details on filter material and manufacturers. (Novak et al. unpublished data).

Results

None of the flat disc filter material tested produced any measurable amounts of CDOM in the visible spectrum. However, in the ultraviolet region of the spectrum, all filter materials introduced a small amount of CDOM contamination. PES, Nylon, Hydrophilic Polypropylene (GHP), and polycarbonate disc filters tended to leach lower amounts of CDOM than the other filter types (Fig. 21). The DOC values measured on the filtrate for these filters were all less than 50 µg L^{-1} of carbon (Table 1). The average of all ultrapure water blanks measured during this analysis was 46.19 +/- 6.14 µg L^{-1} of carbon (n=46). The syringe filters yielded higher CDOM absorption at the lower UV wavelengths. Based on this evaluation, the PES, GHP, nylon, GF/F and polycarbonate disc filters are appropriate materials for natural water sample filtration for CDOM absorption analysis. Of these filters, the polycarbonate disc filters will require the longest amount of time for filtration of a water sample due to their inherent design – flat sheet with pores versus membrane filters, which have a greater surface area.

Capsule filters are used for various water sampling applications and can facilitate the efficient collection of many types of samples that require filtration such as nutrients,

DOC, CDOM absorption and DOM molecular analyses. These larger volume capacity filters are also used to filter water for optical measurements of DOM (or <0.2 µm material) such as the Sea-Bird Scientific ac-9 and ac-s meters. Hence, several types of capsule filters were evaluated for potential interference or contamination of CDOM absorption measurements.

The results from the experiments were extensive and elucidated two types of contamination responses over the flushing and retention periods. For brevity, not all filter types results will be shown here, only an example from each case. In the first case, a significant amount of CDOM and DOC was released after minimal flushing (<10 L; Fig. 22). However, the colored material produced did not significantly absorb in the visible spectrum. After 10 L of flushing, the colored material and DOC produced were significantly less. However, when water was allowed to sit in the filter for ten minutes, there was a significant amount of CDOM and DOC leached from the filters (Fig. 22). In the second case, some colored material and DOC were produced at lower volumes of flushing (<10 L; Fig. 23). However, once 10 L were flushed through the filter, there was significantly less CDOM and DOC produced from the filters, even after the liquid was allowed to sit in the filters for ten minutes (Fig. 23).

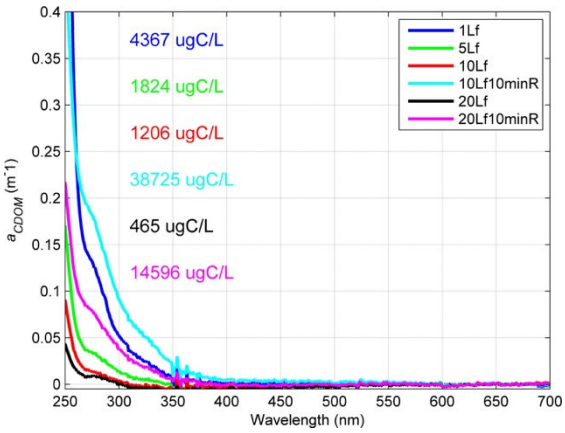

Figure 39. CDOM absorption spectra of ultrapure water collected from Pall capsules with Tuffryn membrane filter after rinses with different volumes of ultrapure water measured on a double beam spectrophotometer. The corresponding values for the measured dissolved organic carbon (DOC) concentration are listed as text on the plot with colors to match the lines specified in the legend. The 10minR designation refers to the analysis of water retained inside the capsule filter after a 10 minute hold period, which was preceded by rinsing with the specified volume of ultrapure water. See Table 1 for further details. (Novak et al. unpublished data).

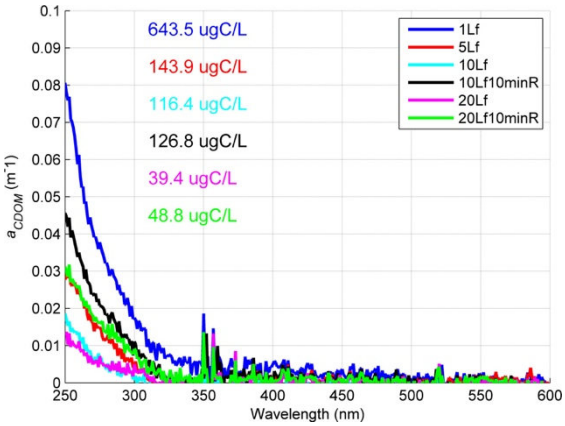

Figure 41. CDOM absorption spectra and DOC values of ultrapure water collected from PureFlo SZL capsules with polyethersulfone (PES) membrane filter after rinses with different volumes of ultrapure water. See Table 1 and Figure 22 for further details.

The filters that fell into case 1 were Whatman Polycap PES, Pall Versapor (acrylic co-polymer), Pall AcroPak Supor, and HT Tuffryn (polysulfone). The filters that fit into case 2, were Pureflo SZL, Whatman MAPP, and Geotech Versapor blue filters. For all filters tested, the amount of CDOM leached was negligible in the UV and visible spectra after 20 liters of flushing as long as the water was not allowed to sit inside the filters (Fig.

24). DOC measured after 20 liters of flushing was also minimal in all filters with the exception of the HT Tuffryn which still produced a significant amount of DOC (Figs. 22-24). These experiments highlight the importance of significantly flushing any type of filter (20 or more Liters) with pure water or sample water before making measurements or collecting samples for the purposes of UV absorption measurements and DOC studies. Ultrapure water process blanks from the field, consistent with sample processing, are recommended to verify whether any appreciable level of contamination occurred.

Previous protocols suggested rinsing or soaking filters with HCl to remove contaminants. However, this is no longer recommended. Disc filters soaked in ACS grade HCl for 20 minutes and then flushed with 175 mL of ultrapure water did not seem to reduce contamination and in fact significantly added to the absorption signal below 300 nm. When flushed with 300 ml after the HCl soak, the HCl contamination was mostly rinsed off; however, CDOM absorption was still lower for filters not treated with acid (Fig. 25).

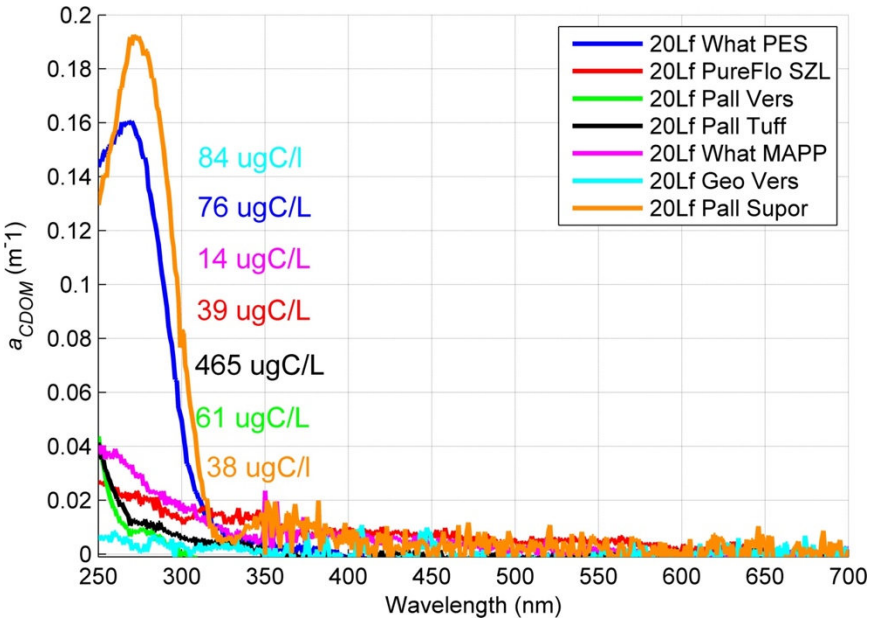

Figure 42. Comparison of the CDOM absorption spectra and DOC of ultrapure water collected after 20 Liters were flushed through various filter types. See Table 1 and Figure 21 for further details.

The best method to remove contaminants from filters is to rinse with copious amounts of ultrapure water or sample. Experiments with nylon disc filters showed that the magnitude of the contamination signal was closely related to the rinsing volume (Fig. 26). When the filter was rinsed with 1 L of ultrapure water, there was no measurable signal of contamination present. Other filter types were tested with similar results. In general, the contamination signal with low rinsing volumes (175-200 mL ultrapure water) is less than 3% of typical CDOM absorption in the UV and can be considered negligible. For samples with very low signal in the UV, then high volume pre-rinses are required but will suffice to mitigate contamination of CDOM.

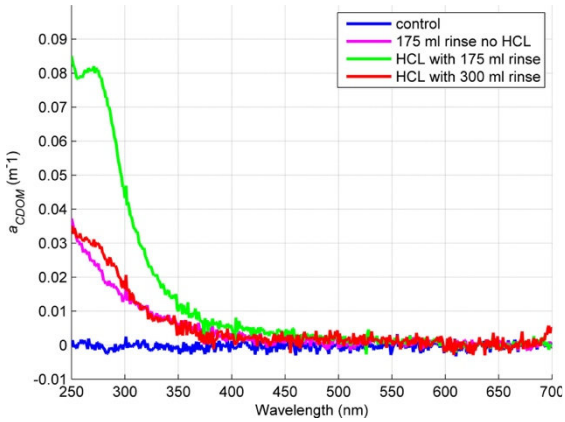

Figure 455. CDOM absorption of ultrapure water filtrates measured from Pall Supor PES disc filters soaked in acid and rinsed with different volumes of ultrapure water compared to a filter not soaked in acid and only rinsed once with 175 ml of ultrapure water. Control represents ultrapure collected directly from the Milli-Q water system without re-filtration. See Table 1 and Fig. 21 for further details.

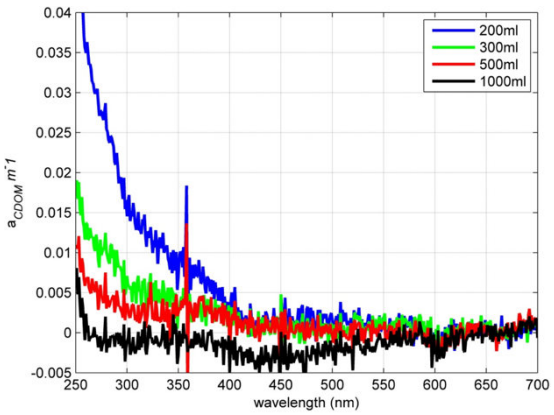

Figure 45. CDOM absorption spectra of ultrapure water collected after multiple rinsing volumes through Nylon disc filters. See Table 1 and Fig. 21 for further details.

VI. CDOM Absorption Measurement Round Robin Results

A CDOM absorption measurement round robin activity was conducted in February 2015 to evaluate (1) the recommended refractive index correction for the long pathlength UltraPath and Liquid Waveguide Capillary Cell (LWCC), (2) the measurement uncertainty of CDOM absorption across multiple investigators, 13 UltraPath UPUV and LWCC, and several other different types of instrument that measure absorbance, and (3) verify the utility of SRFA-I material as a consensus reference material. The GSFC field support group personnel prepared CDOM sample material for distribution to the round robin participants and included the following:

- SRFA-I Standard at a concentration of 0.25 mg L^{-1}
- SRFA-I Standard at a concentration of 0.5 mg L^{-1}
- High purity sodium chloride (NaCl) solutions of 30, 35 and 40 ppt salinity
- Natural seawater sample with low CDOM absorption, which was collected from the Pacific Ocean at a location south of Tahiti (Latitude: -25.66; Longitude: -150.00 on 28 April 2014 at 19:55:00 UTC) referred to as "Tahiti seawater"

Fourteen analysts participated in this round robin using 13 liquid capillary waveguide systems (UltraPath UPUVand LWCC; notated as wg in the figure legends), three double beam spectrophotometers (notated as "bench" in the figure legends), three ac-s, one ac-9, and one a-sphere. Each analyst was asked to conduct the CDOM absorbance measurements on the same specific day. The summary results for the SRFA-I solutions are presented and discussed in section II (Figs. 4 and 27). Examples of the liquid waveguide absorption coefficient measurements of the NaCl solutions were shown and

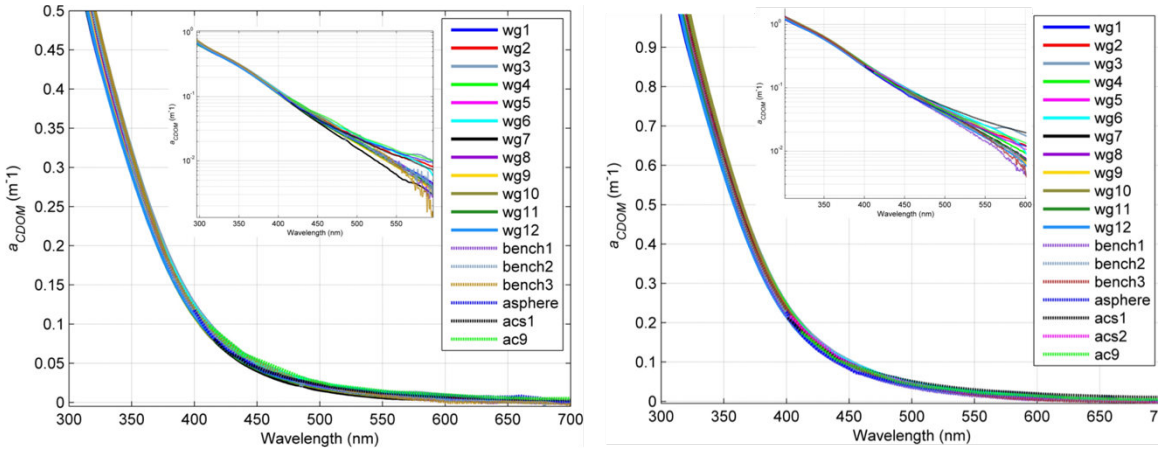

Figure 46. (a) CDOM absorption coefficient spectra of 0.25 mg L^{-1} SRFA-I solution analyzed on 12 UltraPath UPUV and LWCC instruments, 3 double beam spectrophotometers, one a-sphere, one ac-9 and two ac-s from a multi-investigator round robin conducted in February 2015. (b) CDOM absorption coefficient spectra of 0.50 mg L^{-1} SRFA-I solution measured on the same instruments on the same day with an additional ac-s. Inset plots present the a_{CDOM} values on a log-scaled axis.

described (Figs. 8-12). The CDOM absorption spectra of the Tahiti seawater computed for the various instruments with the interpolated refractive index correction described previously shows greater variability (Fig. 28) than observed for the SRFA-I materials (Fig. 27). The root mean square error (RMSE) computed from the mean of all the measurements (one a-sphere, three benchtop double beam spectrophotometers, and 11 UltraPath) after removal of outlier spectra ranged from 0 to 0.01 m^{-1}. The RMSE for visible wavelengths was <0.003 m^{-1} with exception of one UltraPath and the a-sphere (Fig. 28). The RMSE increases in the UV for many of the instruments. Figure 29 shows the Tahiti seawater measurements from different ac instruments in comparison to the overall mean among all instruments. Figure 30 includes the standard deviation of the scans averaged to produce the absorption spectra for each instrument. The uncertainty when measuring absorption at this low range on ac meters is quite high and should be taken into consideration when making field measurements. The importance of producing consistent and multiple ultrapure water calibrations for data processing becomes very apparent when working with these types of water. The measurements of the SRFA-I 0.25 mg L^{-1} from the ac instruments were in closer agreement to each other and the overall mean than to the Tahiti seawater. This was most likely due to the much higher CDOM (stronger signal) in SRFA-I than that for the Tahiti seawater.

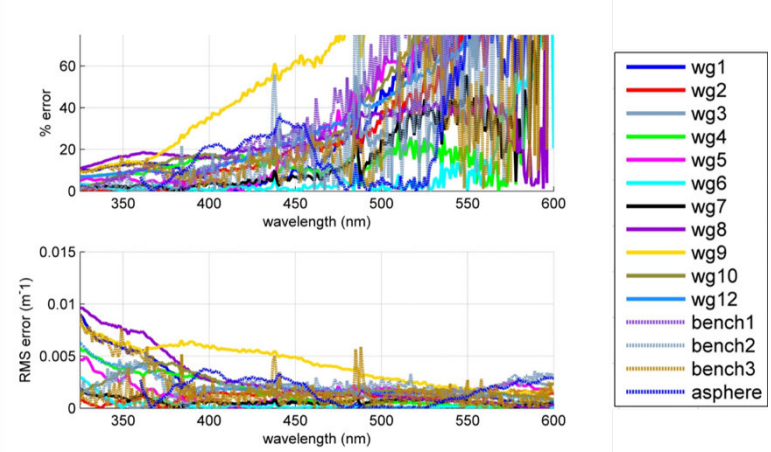

Figure 2850. Mean absolute percent error and root mean square error (RMSE) of Tahiti water sample with respect to the mean of all instruments shown in Figure 20. The ac-s instrument shown in Fig. 20 was not included in this analysis.

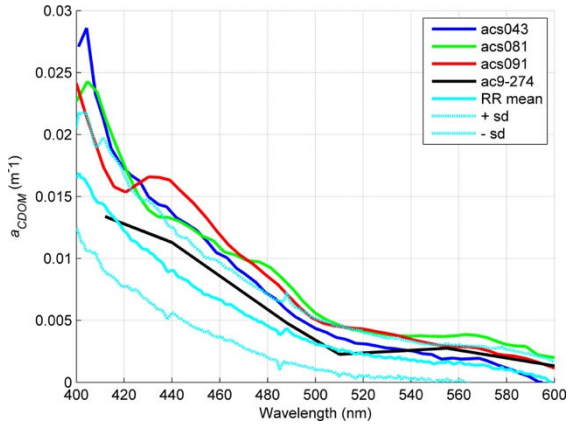

Figure 48. CDOM absorption spectra of Tahiti Water measured on various ac-s and ac-9 meters compared to the overall Round Robin group average and 1 standard deviation. Note the ac instrument analysis was conducted 2 months after the initial round robin.

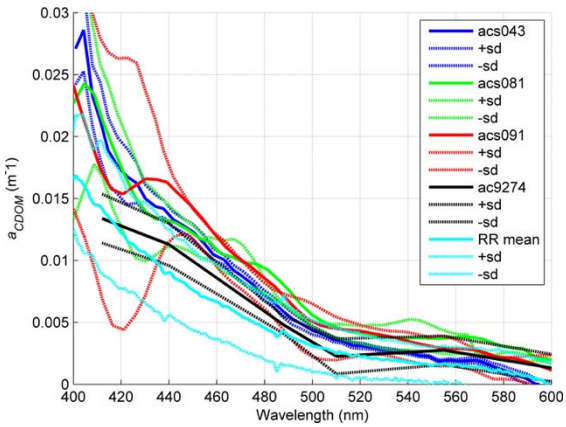

Figure 30. CDOM absorption spectra of Tahiti Water measured on various ac-s and ac-9 meters depicting the first standard deviation (sd) of replicate scans compared to the overall Round Robin group average.

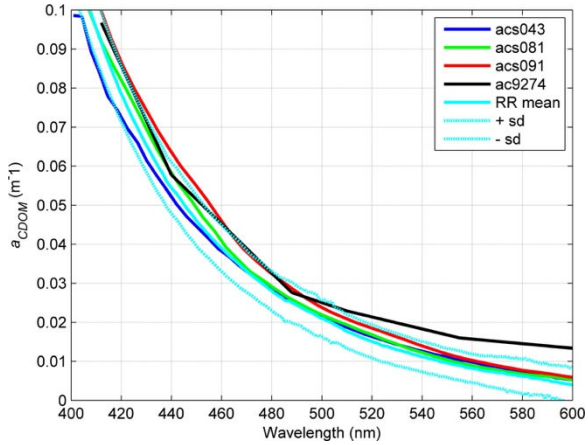

Figure 52. SRFA-I 0.25 mg L^{-1} measured on various ac-s and ac-9 meters compared to the overall Round Robin group average.

Table 1. Filter type and manufacturer tested for contaminants. Table lists the filter abbreviations used in the figures and Dissolved Organic Carbon (DOC) contamination measured.

Manufacturer	Membrane Type and Pore Size	Abbreviation	DOC Measured
Sartorius	Disc Polyethersulfone 0.2 µm	SAR PES	9.5 ±6.3 µgC/l
Sartorius	Disc Cellulose Acetate 0.2 µm	SAR CA	N/A
Pall	Disc Polyethersulfone 0.2 µm	Pall PES	43.3 ±13.3 µgC/l
Pall	Disc Hydrophillic Polypropylene 0.2 µm (GHP)	Pall PP	12.7 ±5.3 µgC/l
Millipore	Disc Nitrocellulose 0.2 µm	Milli NC	138.2 ±2.9 µgC/l
Millipore	Disc Polyvinylidene fluoride 0.2 µm	Milli PVDF	N/A
Millipore	Disc Polycarbonate 0.2 µm	Milli PC	20.6 ±15.5 µgC/l
Whatman	Disc Polycarbonate 0.2 µm	What PC	7.9 ±12.0 µgC/l
Whatman	Disc Nylon 0.2 µm	What Nylon	28.7±14.6 µgC/l
Whatman	Disc Glass Fiber Filter 0.7 µm	What GF/F	N/A
Whatman	Syringe Polyethersulfone 0.2 µm	What Syr PES	27.7±5.3 µgC/l
Whatman	Syringe Polypropylene 0.2 µm	What Syr PP	N/A
Whatman	Capsule Polyethersulfone 0.2 µm	What Cap PES	20Lf 26 µgC/l
Whatman	Capsule Polycap Monofilament Anisotropic Polypropylene 5 µm	What Cap MAPP	20Lf 14 µgC/l
Pall	Capsule Versapor 0.2 µm	Pall Cap Vers	20Lf 61 µgC/l
Pall	Capsule HT Tuffryn 0.2 µm	Pall Cap Tuff	20Lf 465 µgC/l
Pall	Akrodisc Supor (Polyethersulfone) 0.2 µm	Pall Cap Supor	20Lf 38 µgC/l
Saint-Gobain	Capsule PureFlo SZL Polyethersulfone 0.2 µm	Pureflo SZL	20Lf 39 µgC/l
Geotech	Blue Capsule filter Versapor 0.45 µm	Geo Vers	20Lf 84 µgC/l

Note: Pall has discontinued the GHP filters and replaced them with hydrophilic teflon filters.

Table 2. CDOM absorbance Round Robin participants and the instrumentation used in the comparison.

Researcher	Institution	Instruments	Pathlength	Slope
Mathias Belz	WPI Germany GmbH, Friedberg, Germany	two UltraPath UPUV	1.10 m, 1.82 m	negative positive
Jean-Francois Berthon	European Commission, Joint Research Center (JRC), Ispra (Va), Italy	UltraPath UPUV	2.01 m	negative
Annick Bricaud	Laboratoire d'Océanographie de Villefranche (LOV), France	UltraPath UPUV	2.00 m	negative
Emmanuel Boss	University of Maine Orono, ME, USA	acs and ac9	0.25 m	N/A
Joaquin Chaves	NASA Goddard Space Flight Center Greenbelt, MD, USA	UltraPath UPUV	2.02 m	negative
Rosanna Del Vecchio	Earth System Science Interdisciplinary Center University of Maryland College Park, MD, USA	UltraPath UPUV Double Beam Spectrophotometer Shimadzu/UVPC 2401	2.08 m 0.1 m	positive
Eurico D'Sa	Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA, USA	UltraPath UPUV PerkinElmer Lambda 850 Double Beam Spectrophotometer	1.98 m 0.1 m	Positive N/A
Scott Freeman	NASA Goddard Space Flight Center Greenbelt, MD, USA	ac-s ac-9	0.25 m 0.25 m	N/A
Atsushi Matsuoka	Departement d biologie Univertsite Laval, Quebec, Canada	UltraPath UPUV	1.91 m	Negative
Richard Miller	Department of Geological Sciences and the Institute for Coastal Sciences and Policy East Carolina University Greenville, NC, USA	UltraPath UPUV Double Beam Spectrophotometer Perkin Elmer Lambda 850	2.06 m 0.1 m	Negative
Norm Nelson	Earth Research Center University of California, Santa Barbara, CA, USA	2 UltraPath UPUV	2.02 m 1.94 m	Negative Positive
Aimee Neeley	NASA Goddard Space Flight Center Greenbelt, MD, USA	Agilent Cary 4000 Double Beam Spectrophotometer	0.10 m	N/A
Michael G. Novak	NASA Goddard Space Flight Center Greenbelt, MD, USA	UltraPath UPUV Cary 100 and 4000 Double Beam Spectrophotometers	1.975 m	Negative N/A
Rüdiger Röttgers	Institute for coastal research, Center for Materials and Coastal Research Geesthacht, Germany	LWCC Perkin Elmer Lambda 950 double beam spectrophotometer PSICAM	2.503 m	Positive N/A N/A

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Appendix A: WPI Advanced Flowcell Cleaning

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08/14/2013

Advanced Flowcell Cleaning

Purpose of Document:

The purpose of this document is to describe a new cleaning procedure for aggressive cleaning of WPI flowcells. This includes LWCC units, as well as UltraPath flowcells.

Preparation of Chemicals:

All chemical reagents should be of at least ACS-Grade, preferably HPLC-Grade. This procedure involves the use of caustic and flammable reagents. Consult the manufacturer's MSDS for necessary safety precautions.

Cleaning Solution #1:
0.5M Potassium Hydroxide in 100% Ethanol (briefly, 7.013g KOH in 250mL EtOH). After thorough mixing, the solution should be filtered through a 20µm pore size filter.

Cleaning Solution #2:
100% Methanol.

Cleaning Solution #3:
Ultrapure water, Type I per ASTM D1193-99 or equivalent.
Note: Grade 1 ultrapure water per ISO 3696 differs significantly from the above classification.

Cleaning Procedure:

The preferred method of cleaning involves the use of a spectrophotometer in "monitor mode" throughout the entire cleaning process. This allows the technician to observe the extent of performance improvement as a function of time.

The simplest cleaning method involves using a peristaltic pump to flow each cleaning solution through the sample cell in numerical order. It is recommended the pump is configured to "pull" through the cell to avoid possible contamination from degraded peristaltic pump tubing. Each solution is cycled for approximately 3-4 minutes, with a bolus of air introduced between each solution. This procedure is repeated until there is no noticeable improvement in sample cell performance. The flow direction can be reversed between cycles to ensure thorough cleaning.

To lessen the time required for the above cleaning method, large bubbles of air can be introduced into the sample cell alternately with the cleaning solutions. This method uses a laminar flow profile and radial diffusion to effectively "scrub" the inside of the sample cell.

It is imperative that Solution #2 immediately follow Solution #1 to remove residue remaining on the optical components. Failure to do so will result in poor flowcell performance.

Another possible cleaning method involves the use of two syringes of appropriate volume connected to the liquid ports of the sample cell. In numerical order, each cleaning solution is introduced into the sample cell and flushed back and forth between the syringes 10-12 times. This procedure is repeated until there is no noticeable improvement in sample cell performance.

Final Rinsing Procedure:

Once the technician identifies the point where subsequent cleaning cycles no longer improve the performance of the flowcell, the unit should be flushed with ultrapure water for a period of at least 15 minutes to ensure all cleaning solutions have been completely removed and there are no persistent residues that might affect flowcell performance or stability.

Appendix B: Consensus CDOM absorption coefficient values of Suwannee River Fulvic Acid I (SRFA-I) solutions.

Table B1. CDOM absorption coefficient values for SRFA-I 0.25 mg L^{-1} solution from the February 2015 Round Robin mean, median and 2.5% and 97.5% quantile values.

wavelength (nm)	mean	median	2.5% Quantile	97.5% Quantile
250	1.2343	1.2340	1.1860	1.2949
251	1.2204	1.2189	1.1729	1.2804
252	1.2074	1.2079	1.1614	1.2599
253	1.1934	1.1927	1.1513	1.2459
254	1.1830	1.1810	1.1420	1.2387
255	1.1704	1.1675	1.1313	1.2256
256	1.1575	1.1566	1.1189	1.2075
257	1.1438	1.1436	1.1085	1.1889
258	1.1315	1.1308	1.0976	1.1729
259	1.1185	1.1169	1.0880	1.1560
260	1.1058	1.1036	1.0761	1.1422
261	1.0938	1.0927	1.0667	1.1264
262	1.0798	1.0783	1.0541	1.1093
263	1.0671	1.0660	1.0420	1.0931
264	1.0542	1.0524	1.0317	1.0738
265	1.0410	1.0387	1.0197	1.0617
266	1.0280	1.0254	1.0082	1.0450
267	1.0146	1.0129	0.9948	1.0329
268	1.0014	0.9988	0.9838	1.0161
269	0.9888	0.9869	0.9701	1.0043
270	0.9759	0.9735	0.9598	0.9899
271	0.9629	0.9601	0.9472	0.9780
272	0.9506	0.9485	0.9356	0.9647
273	0.9385	0.9355	0.9235	0.9548
274	0.9266	0.9243	0.9127	0.9405
275	0.9147	0.9130	0.9002	0.9291
276	0.9022	0.9001	0.8884	0.9155
277	0.8907	0.8886	0.8779	0.9049
278	0.8779	0.8753	0.8669	0.8922
279	0.8666	0.8636	0.8539	0.8814
280	0.8541	0.8514	0.8414	0.8695
281	0.8434	0.8413	0.8320	0.8575
282	0.8329	0.8314	0.8220	0.8446
283	0.8218	0.8200	0.8106	0.8354

284	0.8094	0.8080	0.7984	0.8225
285	0.7991	0.7980	0.7880	0.8115
286	0.7882	0.7854	0.7786	0.8025
287	0.7767	0.7748	0.7677	0.7917
288	0.7668	0.7650	0.7570	0.7802
289	0.7553	0.7532	0.7469	0.7682
290	0.7447	0.7435	0.7362	0.7577
291	0.7344	0.7322	0.7268	0.7476
292	0.7241	0.7232	0.7152	0.7358
293	0.7136	0.7129	0.7047	0.7255
294	0.7030	0.7016	0.6961	0.7145
295	0.6938	0.6925	0.6851	0.7053
296	0.6839	0.6835	0.6761	0.6950
297	0.6749	0.6744	0.6677	0.6868
298	0.6650	0.6648	0.6568	0.6758
299	0.6546	0.6530	0.6478	0.6665
300	0.6513	0.6502	0.6191	0.6874
301	0.6412	0.6385	0.6100	0.6762
302	0.6318	0.6285	0.6010	0.6652
303	0.6219	0.6192	0.5920	0.6545
304	0.6126	0.6111	0.5833	0.6442
305	0.6035	0.6024	0.5747	0.6340
306	0.5943	0.5938	0.5663	0.6240
307	0.5849	0.5824	0.5576	0.6142
308	0.5763	0.5747	0.5489	0.6047
309	0.5678	0.5664	0.5403	0.5955
310	0.5588	0.5556	0.5320	0.5865
311	0.5508	0.5479	0.5240	0.5776
312	0.5423	0.5393	0.5160	0.5689
313	0.5345	0.5327	0.5082	0.5603
314	0.5264	0.5246	0.5007	0.5519
315	0.5187	0.5165	0.4933	0.5437
316	0.5103	0.5086	0.4862	0.5357
317	0.5033	0.5009	0.4791	0.5279
318	0.4959	0.4941	0.4723	0.5203
319	0.4889	0.4875	0.4656	0.5130
320	0.4818	0.4804	0.4591	0.5059
321	0.4748	0.4733	0.4526	0.4989
322	0.4679	0.4659	0.4462	0.4918
323	0.4610	0.4582	0.4401	0.4847
324	0.4542	0.4523	0.4342	0.4778
325	0.4476	0.4442	0.4282	0.4709
326	0.4417	0.4395	0.4222	0.4643

327	0.4350	0.4331	0.4164	0.4577
328	0.4285	0.4257	0.4107	0.4512
329	0.4224	0.4209	0.4051	0.4447
330	0.4161	0.4135	0.3993	0.4383
331	0.4102	0.4081	0.3935	0.4321
332	0.4040	0.4013	0.3880	0.4258
333	0.3977	0.3962	0.3825	0.4195
334	0.3920	0.3907	0.3768	0.4132
335	0.3861	0.3845	0.3711	0.4069
336	0.3803	0.3781	0.3656	0.4012
337	0.3741	0.3713	0.3601	0.3963
338	0.3688	0.3667	0.3547	0.3909
339	0.3628	0.3604	0.3493	0.3845
340	0.3578	0.3552	0.3440	0.3778
341	0.3522	0.3503	0.3388	0.3718
342	0.3474	0.3456	0.3336	0.3662
343	0.3416	0.3389	0.3283	0.3606
344	0.3359	0.3332	0.3231	0.3550
345	0.3306	0.3291	0.3179	0.3495
346	0.3253	0.3230	0.3127	0.3440
347	0.3202	0.3187	0.3077	0.3385
348	0.3147	0.3114	0.3026	0.3332
349	0.3105	0.3085	0.2974	0.3278
350	0.3053	0.3035	0.2922	0.3225
351	0.3002	0.2984	0.2872	0.3172
352	0.2951	0.2934	0.2823	0.3120
353	0.2900	0.2882	0.2774	0.3067
354	0.2849	0.2829	0.2725	0.3015
355	0.2799	0.2779	0.2677	0.2963
356	0.2750	0.2730	0.2630	0.2912
357	0.2702	0.2682	0.2582	0.2862
358	0.2653	0.2635	0.2536	0.2813
359	0.2606	0.2588	0.2489	0.2764
360	0.2558	0.2540	0.2444	0.2716
361	0.2512	0.2492	0.2398	0.2669
362	0.2466	0.2447	0.2353	0.2621
363	0.2420	0.2401	0.2308	0.2572
364	0.2375	0.2358	0.2267	0.2526
365	0.2331	0.2314	0.2225	0.2481
366	0.2288	0.2271	0.2184	0.2437
367	0.2245	0.2227	0.2143	0.2393
368	0.2202	0.2184	0.2102	0.2349
369	0.2161	0.2140	0.2063	0.2307

370	0.2119	0.2098	0.2023	0.2265
371	0.2078	0.2057	0.1983	0.2223
372	0.2037	0.2017	0.1944	0.2181
373	0.1997	0.1977	0.1905	0.2140
374	0.1957	0.1937	0.1867	0.2100
375	0.1918	0.1896	0.1830	0.2060
376	0.1880	0.1856	0.1794	0.2019
377	0.1843	0.1821	0.1758	0.1981
378	0.1807	0.1786	0.1722	0.1944
379	0.1771	0.1750	0.1688	0.1907
380	0.1736	0.1714	0.1655	0.1869
381	0.1701	0.1682	0.1623	0.1833
382	0.1668	0.1650	0.1591	0.1798
383	0.1635	0.1615	0.1558	0.1764
384	0.1602	0.1581	0.1526	0.1730
385	0.1569	0.1548	0.1495	0.1695
386	0.1538	0.1517	0.1464	0.1661
387	0.1507	0.1487	0.1435	0.1628
388	0.1476	0.1458	0.1405	0.1597
389	0.1447	0.1429	0.1376	0.1566
390	0.1417	0.1400	0.1346	0.1534
391	0.1388	0.1372	0.1318	0.1504
392	0.1360	0.1345	0.1290	0.1476
393	0.1333	0.1319	0.1263	0.1450
394	0.1306	0.1293	0.1235	0.1422
395	0.1280	0.1268	0.1210	0.1393
396	0.1254	0.1243	0.1186	0.1365
397	0.1229	0.1220	0.1161	0.1338
398	0.1205	0.1196	0.1137	0.1313
399	0.1181	0.1173	0.1114	0.1289
400	0.1158	0.1150	0.1092	0.1265
401	0.1135	0.1128	0.1070	0.1240
402	0.1111	0.1104	0.1047	0.1215
403	0.1090	0.1082	0.1025	0.1192
404	0.1069	0.1061	0.1004	0.1170
405	0.1050	0.1047	0.0984	0.1148
406	0.1029	0.1027	0.0964	0.1127
407	0.1008	0.1004	0.0945	0.1106
408	0.0990	0.0987	0.0926	0.1086
409	0.0970	0.0966	0.0907	0.1065
410	0.0954	0.0947	0.0888	0.1045
411	0.0933	0.0929	0.0870	0.1026
412	0.0917	0.0910	0.0853	0.1008

413	0.0898	0.0891	0.0836	0.0990
414	0.0881	0.0873	0.0821	0.0972
415	0.0865	0.0857	0.0805	0.0953
416	0.0848	0.0841	0.0789	0.0935
417	0.0832	0.0825	0.0773	0.0918
418	0.0817	0.0809	0.0757	0.0902
419	0.0799	0.0789	0.0741	0.0886
420	0.0785	0.0773	0.0726	0.0869
421	0.0772	0.0766	0.0712	0.0853
422	0.0756	0.0750	0.0699	0.0839
423	0.0743	0.0739	0.0685	0.0826
424	0.0729	0.0724	0.0671	0.0812
425	0.0717	0.0712	0.0658	0.0798
426	0.0704	0.0699	0.0645	0.0784
427	0.0691	0.0687	0.0632	0.0771
428	0.0678	0.0674	0.0619	0.0757
429	0.0666	0.0662	0.0606	0.0745
430	0.0653	0.0650	0.0595	0.0732
431	0.0642	0.0637	0.0583	0.0720
432	0.0631	0.0625	0.0571	0.0709
433	0.0619	0.0612	0.0560	0.0698
434	0.0608	0.0598	0.0548	0.0686
435	0.0596	0.0589	0.0537	0.0674
436	0.0586	0.0579	0.0526	0.0662
437	0.0575	0.0561	0.0516	0.0650
438	0.0562	0.0551	0.0506	0.0638
439	0.0553	0.0541	0.0496	0.0627
440	0.0544	0.0535	0.0486	0.0617
441	0.0536	0.0526	0.0477	0.0608
442	0.0530	0.0516	0.0468	0.0600
443	0.0521	0.0507	0.0460	0.0591
444	0.0512	0.0497	0.0451	0.0582
445	0.0502	0.0489	0.0442	0.0574
446	0.0493	0.0480	0.0433	0.0565
447	0.0485	0.0473	0.0425	0.0556
448	0.0477	0.0465	0.0417	0.0547
449	0.0469	0.0456	0.0409	0.0539
450	0.0460	0.0449	0.0402	0.0531
451	0.0452	0.0441	0.0394	0.0523
452	0.0445	0.0434	0.0387	0.0514
453	0.0438	0.0426	0.0380	0.0505
454	0.0430	0.0419	0.0373	0.0496
455	0.0424	0.0412	0.0366	0.0487

456	0.0416	0.0405	0.0359	0.0479
457	0.0409	0.0398	0.0352	0.0472
458	0.0403	0.0392	0.0346	0.0466
459	0.0396	0.0386	0.0340	0.0460
460	0.0390	0.0380	0.0333	0.0453
461	0.0384	0.0374	0.0327	0.0445
462	0.0376	0.0367	0.0320	0.0438
463	0.0371	0.0362	0.0313	0.0431
464	0.0365	0.0356	0.0307	0.0424
465	0.0360	0.0351	0.0302	0.0418
466	0.0355	0.0345	0.0296	0.0412
467	0.0349	0.0339	0.0290	0.0405
468	0.0341	0.0334	0.0285	0.0398
469	0.0337	0.0329	0.0280	0.0390
470	0.0331	0.0324	0.0274	0.0382
471	0.0325	0.0319	0.0268	0.0374
472	0.0321	0.0313	0.0264	0.0368
473	0.0315	0.0308	0.0259	0.0363
474	0.0310	0.0303	0.0254	0.0358
475	0.0305	0.0299	0.0249	0.0355
476	0.0300	0.0296	0.0245	0.0350
477	0.0295	0.0292	0.0241	0.0343
478	0.0291	0.0288	0.0238	0.0338
479	0.0285	0.0278	0.0233	0.0333
480	0.0281	0.0279	0.0229	0.0330
481	0.0275	0.0270	0.0224	0.0326
482	0.0271	0.0269	0.0221	0.0323
483	0.0266	0.0261	0.0220	0.0320
484	0.0264	0.0262	0.0218	0.0317
485	0.0257	0.0250	0.0205	0.0316
486	0.0255	0.0248	0.0210	0.0314
487	0.0254	0.0250	0.0208	0.0312
488	0.0250	0.0247	0.0204	0.0308
489	0.0247	0.0245	0.0199	0.0301
490	0.0244	0.0235	0.0194	0.0292
491	0.0241	0.0237	0.0188	0.0285
492	0.0237	0.0233	0.0184	0.0279
493	0.0233	0.0226	0.0180	0.0282
494	0.0229	0.0222	0.0176	0.0272
495	0.0227	0.0222	0.0172	0.0272
496	0.0224	0.0217	0.0169	0.0273
497	0.0221	0.0216	0.0166	0.0272
498	0.0217	0.0210	0.0163	0.0268

499	0.0213	0.0206	0.0160	0.0252
500	0.0210	0.0204	0.0158	0.0250
501	0.0208	0.0201	0.0155	0.0257
502	0.0204	0.0198	0.0152	0.0246
503	0.0202	0.0197	0.0150	0.0251
504	0.0199	0.0191	0.0148	0.0255
505	0.0194	0.0188	0.0146	0.0234
506	0.0191	0.0185	0.0143	0.0231
507	0.0188	0.0186	0.0140	0.0228
508	0.0185	0.0183	0.0137	0.0224
509	0.0180	0.0176	0.0134	0.0221
510	0.0177	0.0173	0.0132	0.0218
511	0.0174	0.0170	0.0129	0.0216
512	0.0172	0.0171	0.0127	0.0214
513	0.0169	0.0166	0.0125	0.0212
514	0.0166	0.0163	0.0122	0.0208
515	0.0163	0.0158	0.0120	0.0205
516	0.0160	0.0158	0.0118	0.0203
517	0.0158	0.0155	0.0116	0.0201
518	0.0156	0.0152	0.0113	0.0199
519	0.0153	0.0149	0.0111	0.0196
520	0.0151	0.0146	0.0109	0.0194
521	0.0148	0.0145	0.0107	0.0192
522	0.0147	0.0146	0.0104	0.0190
523	0.0144	0.0142	0.0102	0.0188
524	0.0140	0.0139	0.0101	0.0185
525	0.0140	0.0136	0.0100	0.0183
526	0.0138	0.0134	0.0098	0.0181
527	0.0136	0.0133	0.0096	0.0178
528	0.0134	0.0132	0.0093	0.0176
529	0.0132	0.0129	0.0091	0.0174
530	0.0130	0.0127	0.0090	0.0173
531	0.0128	0.0125	0.0089	0.0171
532	0.0127	0.0124	0.0087	0.0169
533	0.0124	0.0122	0.0086	0.0166
534	0.0123	0.0122	0.0084	0.0164
535	0.0122	0.0120	0.0082	0.0162
536	0.0118	0.0111	0.0081	0.0160
537	0.0117	0.0116	0.0080	0.0158
538	0.0115	0.0113	0.0078	0.0156
539	0.0114	0.0112	0.0077	0.0154
540	0.0111	0.0106	0.0075	0.0153
541	0.0111	0.0109	0.0073	0.0151

542	0.0109	0.0106	0.0071	0.0149
543	0.0106	0.0101	0.0070	0.0147
544	0.0106	0.0104	0.0068	0.0145
545	0.0104	0.0098	0.0067	0.0144
546	0.0103	0.0100	0.0066	0.0143
547	0.0099	0.0095	0.0064	0.0140
548	0.0098	0.0096	0.0063	0.0138
549	0.0098	0.0093	0.0063	0.0136
550	0.0095	0.0089	0.0061	0.0135
551	0.0095	0.0089	0.0060	0.0135
552	0.0093	0.0087	0.0058	0.0134
553	0.0092	0.0086	0.0057	0.0132
554	0.0090	0.0084	0.0055	0.0131
555	0.0089	0.0085	0.0055	0.0129
556	0.0088	0.0086	0.0053	0.0128
557	0.0085	0.0080	0.0052	0.0127
558	0.0084	0.0079	0.0051	0.0126
559	0.0083	0.0077	0.0051	0.0124
560	0.0082	0.0077	0.0051	0.0124
561	0.0081	0.0075	0.0050	0.0123
562	0.0080	0.0077	0.0050	0.0123
563	0.0078	0.0072	0.0049	0.0123
564	0.0077	0.0071	0.0048	0.0123
565	0.0076	0.0070	0.0047	0.0123
566	0.0075	0.0069	0.0046	0.0124
567	0.0074	0.0068	0.0046	0.0126
568	0.0074	0.0067	0.0046	0.0126
569	0.0072	0.0066	0.0046	0.0125
570	0.0072	0.0065	0.0045	0.0126
571	0.0071	0.0064	0.0044	0.0127
572	0.0071	0.0063	0.0045	0.0127
573	0.0069	0.0063	0.0043	0.0126
574	0.0066	0.0060	0.0035	0.0126
575	0.0067	0.0061	0.0044	0.0126
576	0.0065	0.0057	0.0043	0.0127
577	0.0065	0.0059	0.0037	0.0126
578	0.0064	0.0058	0.0039	0.0125
579	0.0063	0.0056	0.0037	0.0124
580	0.0062	0.0056	0.0035	0.0124
581	0.0061	0.0055	0.0029	0.0124
582	0.0061	0.0054	0.0040	0.0123
583	0.0058	0.0053	0.0027	0.0120
584	0.0057	0.0050	0.0031	0.0118

585	0.0057	0.0051	0.0035	0.0117
586	0.0056	0.0050	0.0033	0.0116
587	0.0054	0.0047	0.0032	0.0114
588	0.0055	0.0049	0.0034	0.0112
589	0.0051	0.0045	0.0016	0.0109
590	0.0053	0.0047	0.0031	0.0108
591	0.0050	0.0042	0.0027	0.0107
592	0.0047	0.0042	0.0018	0.0107
593	0.0048	0.0043	0.0025	0.0106
594	0.0047	0.0043	0.0016	0.0104
595	0.0045	0.0038	0.0013	0.0102
596	0.0045	0.0041	0.0017	0.0101
597	0.0043	0.0036	0.0007	0.0100
598	0.0042	0.0035	0.0011	0.0100
599	0.0041	0.0034	0.0016	0.0099
600	0.0040	0.0034	0.0004	0.0097
601	0.0038	0.0032	-0.0003	0.0096
602	0.0036	0.0031	0.0001	0.0095
603	0.0036	0.0030	0.0004	0.0094
604	0.0035	0.0030	-0.0004	0.0093
605	0.0033	0.0029	-0.0004	0.0091
606	0.0034	0.0028	0.0001	0.0089
607	0.0033	0.0027	-0.0003	0.0089
608	0.0033	0.0029	0.0001	0.0088
609	0.0031	0.0026	-0.0001	0.0086
610	0.0031	0.0028	-0.0010	0.0082
611	0.0031	0.0026	0.0002	0.0080
612	0.0029	0.0026	-0.0003	0.0080
613	0.0029	0.0026	-0.0007	0.0078
614	0.0029	0.0025	0.0006	0.0073
615	0.0028	0.0027	0.0001	0.0070
616	0.0028	0.0026	-0.0008	0.0071
617	0.0028	0.0025	-0.0003	0.0071
618	0.0028	0.0025	0.0005	0.0067
619	0.0027	0.0026	0.0004	0.0064
620	0.0026	0.0023	0.0003	0.0064
621	0.0026	0.0024	-0.0001	0.0064
622	0.0025	0.0024	-0.0009	0.0062
623	0.0025	0.0023	0.0001	0.0058
624	0.0025	0.0022	0.0001	0.0055
625	0.0024	0.0023	-0.0008	0.0053
626	0.0023	0.0022	-0.0002	0.0052
627	0.0022	0.0021	0.0003	0.0051

628	0.0022	0.0022	-0.0003	0.0049
629	0.0020	0.0020	-0.0001	0.0047
630	0.0021	0.0021	-0.0004	0.0045
631	0.0019	0.0020	-0.0006	0.0044
632	0.0020	0.0020	0.0006	0.0044
633	0.0018	0.0020	-0.0008	0.0044
634	0.0018	0.0020	-0.0002	0.0042
635	0.0018	0.0019	-0.0005	0.0039
636	0.0017	0.0018	-0.0002	0.0038
637	0.0018	0.0019	-0.0003	0.0037
638	0.0017	0.0018	-0.0005	0.0036
639	0.0018	0.0019	-0.0004	0.0034
640	0.0016	0.0018	-0.0004	0.0033
641	0.0017	0.0018	0.0003	0.0032
642	0.0016	0.0017	-0.0002	0.0031
643	0.0017	0.0017	0.0001	0.0029
644	0.0016	0.0016	0.0006	0.0028
645	0.0016	0.0015	0.0006	0.0029
646	0.0014	0.0015	-0.0001	0.0029
647	0.0016	0.0015	0.0006	0.0029
648	0.0017	0.0015	0.0006	0.0031
649	0.0015	0.0013	0.0004	0.0037
650	0.0015	0.0011	0.0000	0.0045
651	0.0016	0.0011	0.0002	0.0052
652	0.0016	0.0011	0.0001	0.0058
653	0.0017	0.0012	0.0001	0.0061
654	0.0016	0.0012	0.0000	0.0063
655	0.0017	0.0011	0.0003	0.0064
656	0.0016	0.0011	-0.0006	0.0064
657	0.0016	0.0010	0.0004	0.0064
658	0.0015	0.0009	-0.0001	0.0063
659	0.0014	0.0009	-0.0002	0.0060
660	0.0013	0.0009	-0.0003	0.0055
661	0.0012	0.0009	-0.0001	0.0047
662	0.0011	0.0008	0.0000	0.0039
663	0.0008	0.0007	-0.0011	0.0032
664	0.0008	0.0007	-0.0003	0.0024
665	0.0007	0.0007	-0.0002	0.0019
666	0.0006	0.0007	-0.0005	0.0016
667	0.0006	0.0006	-0.0005	0.0013
668	0.0005	0.0006	-0.0010	0.0015
669	0.0006	0.0006	-0.0008	0.0016
670	0.0006	0.0005	-0.0007	0.0018

671	0.0005	0.0005	-0.0007	0.0017
672	0.0004	0.0005	-0.0013	0.0013
673	0.0004	0.0005	-0.0008	0.0013
674	0.0005	0.0005	-0.0005	0.0015
675	0.0003	0.0005	-0.0009	0.0015
676	0.0006	0.0005	-0.0006	0.0037
677	0.0005	0.0005	-0.0006	0.0013
678	0.0005	0.0005	-0.0009	0.0013
679	0.0001	0.0004	-0.0049	0.0013
680	0.0003	0.0003	-0.0013	0.0013
681	0.0002	0.0003	-0.0017	0.0012
682	0.0002	0.0003	-0.0012	0.0012
683	0.0002	0.0004	-0.0009	0.0010
684	0.0002	0.0003	-0.0011	0.0010
685	0.0002	0.0003	-0.0010	0.0010
686	0.0002	0.0003	-0.0011	0.0013
687	0.0001	0.0003	-0.0014	0.0013
688	0.0001	0.0002	-0.0011	0.0011
689	0.0002	0.0002	-0.0009	0.0010
690	0.0000	0.0002	-0.0019	0.0006
691	0.0001	0.0002	-0.0013	0.0005
692	0.0000	0.0002	-0.0015	0.0006
693	0.0000	0.0001	-0.0014	0.0007
694	-0.0001	0.0001	-0.0018	0.0006
695	-0.0001	0.0000	-0.0020	0.0004
696	-0.0001	0.0001	-0.0016	0.0003
697	-0.0004	0.0000	-0.0022	0.0002
698	-0.0004	-0.0001	-0.0029	0.0000
699	-0.0005	-0.0001	-0.0027	0.0000
700	-0.0006	-0.0001	-0.0027	0.0000

Table B2. CDOM absorption coefficient values for SRFA-I 0.50 mg L^{-1} solution from the February 2015 Round Robin mean, median and 2.5% and 97.5% quantile values.

wavelength (nm)	mean	median	2.5% Quantile	97.5% Quantile
250	2.5075	2.4996	2.4620	2.5609
251	2.4855	2.4749	2.4414	2.5402
252	2.4630	2.4518	2.4182	2.5189
253	2.4425	2.4288	2.3996	2.4991
254	2.4216	2.4063	2.3789	2.4798
255	2.4020	2.3851	2.3608	2.4602
256	2.3820	2.3629	2.3404	2.4426
257	2.3616	2.3414	2.3213	2.4222
258	2.3419	2.3209	2.3018	2.4032
259	2.3230	2.2998	2.2846	2.3845
260	2.3036	2.2782	2.2677	2.3649
261	2.2842	2.2567	2.2505	2.3453
262	2.2634	2.2352	2.2284	2.3266
263	2.2413	2.2137	2.2057	2.3044
264	2.2189	2.1910	2.1836	2.2822
265	2.1968	2.1676	2.1616	2.2611
266	2.1738	2.1444	2.1371	2.2399
267	2.1518	2.1209	2.1164	2.2182
268	2.1285	2.0972	2.0928	2.1955
269	2.1046	2.0731	2.0679	2.1728
270	2.0820	2.0494	2.0457	2.1510
271	2.0584	2.0250	2.0230	2.1273
272	2.0355	2.0010	1.9998	2.1058
273	2.0109	1.9776	1.9741	2.0810
274	1.9870	1.9535	1.9515	2.0561
275	1.9634	1.9288	1.9283	2.0331
276	1.9387	1.9042	1.9031	2.0087
277	1.9142	1.8798	1.8791	1.9838
278	1.8875	1.8549	1.8482	1.9593
279	1.8624	1.8306	1.8229	1.9338
280	1.8373	1.8064	1.7974	1.9080
281	1.8135	1.7821	1.7755	1.8829
282	1.7901	1.7586	1.7554	1.8564
283	1.7655	1.7343	1.7313	1.8309
284	1.7408	1.7100	1.7060	1.8065
285	1.7147	1.6865	1.6803	1.7773
286	1.6898	1.6623	1.6559	1.7513
287	1.6652	1.6377	1.6337	1.7240

288	1.6402	1.6146	1.6083	1.6976
289	1.6139	1.5908	1.5818	1.6690
290	1.5893	1.5667	1.5588	1.6424
291	1.5647	1.5439	1.5339	1.6164
292	1.5403	1.5205	1.5108	1.5895
293	1.5159	1.4983	1.4874	1.5620
294	1.4914	1.4756	1.4629	1.5357
295	1.4683	1.4530	1.4415	1.5103
296	1.4444	1.4309	1.4193	1.4829
297	1.4194	1.4088	1.3921	1.4572
298	1.3953	1.3872	1.3675	1.4312
299	1.3748	1.3659	1.3528	1.4057
300	1.3100	1.3195	1.2259	1.3809
301	1.2904	1.3001	1.2076	1.3551
302	1.2716	1.2810	1.1898	1.3314
303	1.2531	1.2619	1.1720	1.3091
304	1.2347	1.2435	1.1541	1.2916
305	1.2161	1.2252	1.1366	1.2737
306	1.1979	1.2071	1.1198	1.2552
307	1.1801	1.1892	1.1033	1.2380
308	1.1624	1.1713	1.0869	1.2214
309	1.1452	1.1535	1.0706	1.2047
310	1.1279	1.1361	1.0547	1.1877
311	1.1112	1.1189	1.0392	1.1710
312	1.0946	1.1018	1.0244	1.1548
313	1.0783	1.0847	1.0099	1.1384
314	1.0620	1.0680	0.9956	1.1209
315	1.0460	1.0522	0.9812	1.1035
316	1.0308	1.0364	0.9672	1.0890
317	1.0154	1.0206	0.9536	1.0745
318	1.0005	1.0048	0.9402	1.0582
319	0.9858	0.9902	0.9271	1.0417
320	0.9713	0.9756	0.9142	1.0265
321	0.9571	0.9615	0.9015	1.0117
322	0.9432	0.9475	0.8892	0.9969
323	0.9293	0.9319	0.8772	0.9821
324	0.9160	0.9192	0.8654	0.9676
325	0.9028	0.9041	0.8537	0.9535
326	0.8896	0.8908	0.8419	0.9396
327	0.8766	0.8769	0.8303	0.9262
328	0.8639	0.8637	0.8188	0.9129
329	0.8515	0.8516	0.8075	0.8996
330	0.8389	0.8382	0.7957	0.8864

331	0.8268	0.8262	0.7839	0.8734
332	0.8148	0.8142	0.7723	0.8604
333	0.8028	0.8022	0.7607	0.8482
334	0.7909	0.7903	0.7492	0.8360
335	0.7791	0.7785	0.7378	0.8238
336	0.7677	0.7671	0.7269	0.8116
337	0.7561	0.7559	0.7162	0.7993
338	0.7448	0.7446	0.7053	0.7870
339	0.7334	0.7329	0.6944	0.7750
340	0.7221	0.7213	0.6835	0.7635
341	0.7109	0.7101	0.6727	0.7519
342	0.6998	0.6993	0.6620	0.7399
343	0.6890	0.6884	0.6516	0.7280
344	0.6779	0.6773	0.6412	0.7168
345	0.6672	0.6665	0.6309	0.7055
346	0.6563	0.6558	0.6206	0.6945
347	0.6457	0.6452	0.6102	0.6834
348	0.6352	0.6347	0.5998	0.6724
349	0.6237	0.6207	0.5897	0.6613
350	0.6128	0.6107	0.5796	0.6501
351	0.6028	0.6004	0.5696	0.6388
352	0.5922	0.5902	0.5597	0.6281
353	0.5823	0.5799	0.5500	0.6177
354	0.5720	0.5695	0.5403	0.6071
355	0.5618	0.5594	0.5308	0.5963
356	0.5521	0.5496	0.5213	0.5856
357	0.5424	0.5399	0.5119	0.5755
358	0.5326	0.5304	0.5027	0.5654
359	0.5230	0.5209	0.4935	0.5551
360	0.5135	0.5111	0.4844	0.5449
361	0.5040	0.5014	0.4753	0.5350
362	0.4948	0.4938	0.4666	0.5251
363	0.4854	0.4831	0.4579	0.5151
364	0.4762	0.4744	0.4492	0.5052
365	0.4676	0.4656	0.4405	0.4956
366	0.4588	0.4566	0.4323	0.4861
367	0.4498	0.4476	0.4240	0.4767
368	0.4413	0.4389	0.4159	0.4674
369	0.4328	0.4303	0.4077	0.4584
370	0.4246	0.4219	0.3998	0.4500
371	0.4161	0.4137	0.3920	0.4415
372	0.4080	0.4055	0.3842	0.4331
373	0.3998	0.3974	0.3764	0.4248

374	0.3919	0.3893	0.3687	0.4167
375	0.3841	0.3812	0.3612	0.4085
376	0.3763	0.3733	0.3538	0.4004
377	0.3688	0.3660	0.3466	0.3927
378	0.3616	0.3587	0.3396	0.3853
379	0.3544	0.3516	0.3327	0.3779
380	0.3472	0.3447	0.3259	0.3704
381	0.3403	0.3381	0.3194	0.3632
382	0.3335	0.3315	0.3128	0.3561
383	0.3265	0.3250	0.3065	0.3491
384	0.3197	0.3186	0.3001	0.3421
385	0.3137	0.3122	0.2938	0.3351
386	0.3072	0.3059	0.2876	0.3284
387	0.3008	0.2998	0.2818	0.3218
388	0.2947	0.2939	0.2761	0.3154
389	0.2888	0.2881	0.2703	0.3091
390	0.2829	0.2824	0.2645	0.3029
391	0.2770	0.2768	0.2589	0.2968
392	0.2714	0.2712	0.2536	0.2910
393	0.2658	0.2658	0.2483	0.2855
394	0.2606	0.2607	0.2433	0.2799
395	0.2554	0.2555	0.2383	0.2743
396	0.2502	0.2504	0.2334	0.2688
397	0.2452	0.2454	0.2286	0.2635
398	0.2404	0.2407	0.2242	0.2584
399	0.2356	0.2360	0.2197	0.2535
400	0.2309	0.2313	0.2154	0.2486
401	0.2262	0.2265	0.2111	0.2436
402	0.2216	0.2223	0.2067	0.2387
403	0.2173	0.2181	0.2022	0.2341
404	0.2130	0.2139	0.1980	0.2296
405	0.2089	0.2096	0.1938	0.2253
406	0.2048	0.2056	0.1900	0.2211
407	0.2008	0.2017	0.1862	0.2170
408	0.1969	0.1978	0.1827	0.2130
409	0.1930	0.1940	0.1793	0.2091
410	0.1894	0.1903	0.1759	0.2052
411	0.1856	0.1865	0.1724	0.2014
412	0.1821	0.1828	0.1690	0.1976
413	0.1785	0.1792	0.1660	0.1939
414	0.1752	0.1757	0.1629	0.1904
415	0.1718	0.1723	0.1596	0.1869
416	0.1685	0.1690	0.1562	0.1834

417	0.1653	0.1656	0.1533	0.1800
418	0.1622	0.1622	0.1501	0.1767
419	0.1590	0.1591	0.1472	0.1735
420	0.1560	0.1559	0.1441	0.1704
421	0.1532	0.1530	0.1419	0.1673
422	0.1502	0.1501	0.1387	0.1646
423	0.1476	0.1473	0.1364	0.1620
424	0.1449	0.1445	0.1336	0.1592
425	0.1423	0.1418	0.1317	0.1565
426	0.1397	0.1391	0.1291	0.1538
427	0.1372	0.1365	0.1266	0.1512
428	0.1347	0.1339	0.1241	0.1486
429	0.1323	0.1314	0.1220	0.1461
430	0.1297	0.1289	0.1198	0.1436
431	0.1275	0.1265	0.1174	0.1412
432	0.1252	0.1241	0.1148	0.1390
433	0.1230	0.1217	0.1129	0.1367
434	0.1206	0.1195	0.1106	0.1345
435	0.1185	0.1173	0.1090	0.1321
436	0.1164	0.1150	0.1064	0.1297
437	0.1142	0.1128	0.1054	0.1274
438	0.1118	0.1108	0.1016	0.1252
439	0.1099	0.1087	0.1012	0.1231
440	0.1082	0.1068	0.0991	0.1212
441	0.1066	0.1068	0.0977	0.1193
442	0.1047	0.1045	0.0958	0.1176
443	0.1029	0.1018	0.0941	0.1158
444	0.1013	0.1007	0.0926	0.1140
445	0.0995	0.0986	0.0908	0.1123
446	0.0978	0.0962	0.0891	0.1107
447	0.0962	0.0945	0.0881	0.1089
448	0.0946	0.0929	0.0865	0.1071
449	0.0930	0.0912	0.0847	0.1054
450	0.0913	0.0896	0.0829	0.1038
451	0.0897	0.0881	0.0811	0.1021
452	0.0884	0.0881	0.0801	0.1004
453	0.0868	0.0851	0.0793	0.0986
454	0.0853	0.0836	0.0780	0.0969
455	0.0840	0.0822	0.0767	0.0953
456	0.0826	0.0821	0.0743	0.0939
457	0.0812	0.0799	0.0729	0.0925
458	0.0798	0.0781	0.0723	0.0911
459	0.0786	0.0773	0.0706	0.0897

460	0.0774	0.0763	0.0702	0.0884
461	0.0760	0.0743	0.0694	0.0869
462	0.0748	0.0738	0.0673	0.0854
463	0.0737	0.0729	0.0661	0.0839
464	0.0724	0.0706	0.0650	0.0827
465	0.0713	0.0695	0.0644	0.0815
466	0.0703	0.0694	0.0639	0.0802
467	0.0691	0.0673	0.0626	0.0789
468	0.0679	0.0663	0.0613	0.0775
469	0.0669	0.0662	0.0600	0.0761
470	0.0657	0.0648	0.0592	0.0750
471	0.0646	0.0640	0.0573	0.0738
472	0.0636	0.0627	0.0571	0.0727
473	0.0626	0.0624	0.0550	0.0716
474	0.0615	0.0609	0.0546	0.0705
475	0.0605	0.0595	0.0539	0.0695
476	0.0596	0.0590	0.0529	0.0685
477	0.0585	0.0573	0.0521	0.0675
478	0.0577	0.0567	0.0516	0.0664
479	0.0567	0.0554	0.0505	0.0654
480	0.0558	0.0552	0.0489	0.0644
481	0.0548	0.0536	0.0492	0.0634
482	0.0539	0.0527	0.0481	0.0626
483	0.0531	0.0522	0.0475	0.0622
484	0.0523	0.0510	0.0467	0.0618
485	0.0514	0.0503	0.0457	0.0614
486	0.0508	0.0497	0.0450	0.0609
487	0.0504	0.0490	0.0442	0.0603
488	0.0499	0.0495	0.0427	0.0595
489	0.0492	0.0487	0.0423	0.0582
490	0.0484	0.0477	0.0412	0.0567
491	0.0475	0.0468	0.0414	0.0558
492	0.0468	0.0460	0.0409	0.0550
493	0.0460	0.0453	0.0395	0.0541
494	0.0452	0.0448	0.0386	0.0533
495	0.0445	0.0441	0.0390	0.0526
496	0.0438	0.0433	0.0374	0.0519
497	0.0431	0.0425	0.0372	0.0511
498	0.0425	0.0418	0.0363	0.0503
499	0.0418	0.0411	0.0359	0.0496
500	0.0412	0.0405	0.0350	0.0490
501	0.0406	0.0398	0.0347	0.0483
502	0.0399	0.0392	0.0344	0.0475

503	0.0393	0.0387	0.0338	0.0469
504	0.0386	0.0380	0.0327	0.0464
505	0.0381	0.0368	0.0327	0.0458
506	0.0375	0.0369	0.0325	0.0452
507	0.0369	0.0358	0.0313	0.0446
508	0.0364	0.0359	0.0311	0.0440
509	0.0357	0.0347	0.0294	0.0434
510	0.0351	0.0342	0.0294	0.0428
511	0.0346	0.0342	0.0295	0.0423
512	0.0341	0.0338	0.0286	0.0417
513	0.0335	0.0326	0.0277	0.0412
514	0.0330	0.0322	0.0276	0.0406
515	0.0324	0.0317	0.0263	0.0400
516	0.0319	0.0311	0.0268	0.0395
517	0.0315	0.0306	0.0270	0.0390
518	0.0309	0.0305	0.0248	0.0384
519	0.0305	0.0299	0.0252	0.0379
520	0.0299	0.0293	0.0244	0.0374
521	0.0294	0.0288	0.0242	0.0370
522	0.0291	0.0286	0.0243	0.0365
523	0.0285	0.0279	0.0233	0.0360
524	0.0280	0.0275	0.0230	0.0355
525	0.0277	0.0271	0.0230	0.0351
526	0.0274	0.0266	0.0223	0.0347
527	0.0269	0.0262	0.0214	0.0342
528	0.0265	0.0258	0.0218	0.0337
529	0.0260	0.0254	0.0208	0.0334
530	0.0256	0.0250	0.0206	0.0330
531	0.0253	0.0246	0.0206	0.0326
532	0.0249	0.0242	0.0193	0.0321
533	0.0245	0.0238	0.0198	0.0317
534	0.0241	0.0235	0.0201	0.0314
535	0.0238	0.0231	0.0194	0.0310
536	0.0234	0.0228	0.0179	0.0306
537	0.0231	0.0223	0.0188	0.0302
538	0.0227	0.0219	0.0171	0.0298
539	0.0224	0.0216	0.0179	0.0294
540	0.0220	0.0212	0.0173	0.0291
541	0.0218	0.0209	0.0170	0.0288
542	0.0213	0.0201	0.0165	0.0284
543	0.0209	0.0202	0.0156	0.0280
544	0.0207	0.0197	0.0165	0.0276
545	0.0204	0.0194	0.0154	0.0273

546	0.0201	0.0192	0.0151	0.0269
547	0.0197	0.0187	0.0146	0.0265
548	0.0193	0.0183	0.0145	0.0261
549	0.0191	0.0180	0.0146	0.0258
550	0.0188	0.0177	0.0149	0.0256
551	0.0185	0.0174	0.0138	0.0254
552	0.0182	0.0171	0.0139	0.0252
553	0.0180	0.0168	0.0141	0.0249
554	0.0177	0.0168	0.0133	0.0246
555	0.0175	0.0164	0.0131	0.0243
556	0.0171	0.0160	0.0125	0.0241
557	0.0169	0.0157	0.0134	0.0239
558	0.0165	0.0154	0.0125	0.0237
559	0.0164	0.0152	0.0125	0.0236
560	0.0162	0.0152	0.0117	0.0234
561	0.0159	0.0147	0.0121	0.0232
562	0.0157	0.0144	0.0117	0.0231
563	0.0154	0.0142	0.0110	0.0230
564	0.0152	0.0140	0.0113	0.0231
565	0.0149	0.0138	0.0109	0.0232
566	0.0147	0.0135	0.0103	0.0233
567	0.0145	0.0133	0.0105	0.0235
568	0.0143	0.0131	0.0101	0.0236
569	0.0141	0.0129	0.0096	0.0235
570	0.0140	0.0127	0.0099	0.0236
571	0.0137	0.0125	0.0089	0.0237
572	0.0136	0.0123	0.0092	0.0237
573	0.0133	0.0122	0.0087	0.0236
574	0.0130	0.0120	0.0080	0.0236
575	0.0128	0.0118	0.0079	0.0235
576	0.0127	0.0115	0.0077	0.0235
577	0.0125	0.0114	0.0074	0.0234
578	0.0124	0.0113	0.0078	0.0232
579	0.0121	0.0110	0.0078	0.0230
580	0.0119	0.0108	0.0069	0.0229
581	0.0117	0.0106	0.0075	0.0228
582	0.0117	0.0105	0.0070	0.0226
583	0.0113	0.0103	0.0065	0.0223
584	0.0111	0.0102	0.0064	0.0219
585	0.0110	0.0100	0.0063	0.0216
586	0.0108	0.0098	0.0064	0.0214
587	0.0106	0.0096	0.0058	0.0211
588	0.0104	0.0094	0.0060	0.0207

589	0.0101	0.0093	0.0049	0.0203
590	0.0101	0.0090	0.0056	0.0200
591	0.0097	0.0089	0.0049	0.0198
592	0.0096	0.0087	0.0050	0.0196
593	0.0093	0.0084	0.0048	0.0193
594	0.0092	0.0083	0.0053	0.0190
595	0.0089	0.0081	0.0051	0.0187
596	0.0088	0.0079	0.0048	0.0185
597	0.0085	0.0078	0.0051	0.0183
598	0.0084	0.0076	0.0043	0.0181
599	0.0083	0.0074	0.0048	0.0178
600	0.0078	0.0072	0.0042	0.0175
601	0.0077	0.0071	0.0040	0.0173
602	0.0076	0.0069	0.0039	0.0171
603	0.0075	0.0068	0.0041	0.0169
604	0.0073	0.0066	0.0038	0.0165
605	0.0070	0.0064	0.0037	0.0162
606	0.0070	0.0063	0.0037	0.0159
607	0.0067	0.0062	0.0029	0.0157
608	0.0067	0.0061	0.0037	0.0155
609	0.0065	0.0060	0.0027	0.0152
610	0.0063	0.0059	0.0025	0.0148
611	0.0062	0.0058	0.0033	0.0144
612	0.0061	0.0057	0.0024	0.0142
613	0.0060	0.0056	0.0026	0.0138
614	0.0060	0.0055	0.0033	0.0132
615	0.0058	0.0054	0.0029	0.0128
616	0.0056	0.0053	0.0022	0.0127
617	0.0056	0.0051	0.0029	0.0126
618	0.0056	0.0051	0.0026	0.0121
619	0.0055	0.0050	0.0027	0.0116
620	0.0054	0.0049	0.0029	0.0114
621	0.0052	0.0048	0.0022	0.0113
622	0.0051	0.0047	0.0014	0.0109
623	0.0050	0.0046	0.0022	0.0104
624	0.0049	0.0045	0.0020	0.0100
625	0.0047	0.0044	0.0009	0.0098
626	0.0046	0.0044	0.0015	0.0097
627	0.0046	0.0043	0.0018	0.0094
628	0.0045	0.0042	0.0012	0.0090
629	0.0044	0.0041	0.0017	0.0085
630	0.0043	0.0041	0.0020	0.0081
631	0.0042	0.0040	0.0009	0.0079

632	0.0042	0.0039	0.0019	0.0078
633	0.0041	0.0038	0.0017	0.0077
634	0.0040	0.0037	0.0010	0.0074
635	0.0038	0.0037	0.0012	0.0070
636	0.0037	0.0036	0.0012	0.0068
637	0.0037	0.0035	0.0010	0.0068
638	0.0036	0.0035	0.0011	0.0066
639	0.0036	0.0034	0.0009	0.0062
640	0.0035	0.0033	0.0006	0.0061
641	0.0034	0.0032	0.0011	0.0060
642	0.0031	0.0031	-0.0013	0.0058
643	0.0032	0.0030	0.0008	0.0054
644	0.0031	0.0030	0.0009	0.0052
645	0.0031	0.0029	0.0005	0.0053
646	0.0029	0.0028	0.0003	0.0054
647	0.0030	0.0027	0.0012	0.0054
648	0.0030	0.0028	0.0011	0.0058
649	0.0029	0.0026	0.0006	0.0070
650	0.0029	0.0025	-0.0002	0.0083
651	0.0029	0.0023	-0.0003	0.0095
652	0.0029	0.0022	0.0002	0.0105
653	0.0029	0.0022	0.0003	0.0111
654	0.0028	0.0021	0.0002	0.0115
655	0.0028	0.0021	0.0002	0.0116
656	0.0027	0.0020	-0.0005	0.0116
657	0.0027	0.0020	0.0002	0.0116
658	0.0026	0.0019	-0.0001	0.0113
659	0.0025	0.0018	0.0001	0.0107
660	0.0023	0.0018	-0.0001	0.0097
661	0.0021	0.0017	-0.0002	0.0084
662	0.0019	0.0017	-0.0003	0.0070
663	0.0017	0.0016	-0.0007	0.0056
664	0.0015	0.0015	-0.0004	0.0044
665	0.0014	0.0014	-0.0010	0.0033
666	0.0012	0.0014	-0.0010	0.0026
667	0.0011	0.0013	-0.0010	0.0025
668	0.0011	0.0012	-0.0007	0.0027
669	0.0011	0.0012	-0.0010	0.0027
670	0.0011	0.0012	-0.0004	0.0027
671	0.0011	0.0012	-0.0003	0.0025
672	0.0009	0.0011	-0.0011	0.0024
673	0.0010	0.0010	-0.0007	0.0025
674	0.0009	0.0010	-0.0006	0.0026

675	0.0008	0.0010	-0.0010	0.0027
676	0.0008	0.0009	-0.0008	0.0026
677	0.0011	0.0009	-0.0003	0.0031
678	0.0009	0.0009	-0.0008	0.0023
679	0.0005	0.0008	-0.0051	0.0024
680	0.0006	0.0007	-0.0015	0.0025
681	0.0005	0.0007	-0.0025	0.0024
682	0.0005	0.0007	-0.0015	0.0022
683	0.0005	0.0007	-0.0014	0.0020
684	0.0004	0.0006	-0.0016	0.0019
685	0.0004	0.0005	-0.0015	0.0020
686	0.0005	0.0005	-0.0016	0.0022
687	0.0002	0.0005	-0.0023	0.0023
688	0.0003	0.0004	-0.0015	0.0021
689	0.0004	0.0004	-0.0008	0.0017
690	0.0002	0.0004	-0.0025	0.0013
691	0.0001	0.0003	-0.0016	0.0011
692	0.0001	0.0003	-0.0017	0.0010
693	0.0000	0.0002	-0.0020	0.0009
694	-0.0001	0.0002	-0.0024	0.0008
695	-0.0001	0.0001	-0.0020	0.0006
696	-0.0001	0.0001	-0.0016	0.0004
697	-0.0004	0.0000	-0.0024	0.0002
698	-0.0004	-0.0000	-0.0029	0.0000
699	-0.0006	-0.0001	-0.0031	-0.0000
700	-0.0006	-0.0002	-0.0028	-0.0000

Appendix C. November 2013 CDOM Working Group Workshop

List of Participants for November 13-15, 2013 CDOM Working Group Workshop

Mathias Belz, World Precision Instruments Germany, GmbH, Friedberg (Hessen), Germany
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Rüdiger Röttgers, Institute of Coastal Research, Center for Materials and Coastal Research Geesthacht, Germany
Maria Tzortziou, City College of New York (CCNY), City University of New York, NY, USA
Jeremy Werdell, NASA Goddard Space Flight Center Greenbelt, MD, USA

Agenda of November 13-15, 2013 CDOM Working Group Workshop

Day 1 - Nov. 13, 2013
8:15-8:30am – Visitors arrive at NASA Goddard main gate for badging

Morning Session

8:30-8:45 am	Welcome, Logistics, Workshop Goals (Antonio Mannino)
8:45am-11:30 am	Discussions with introductory presentations (~10 min) describing the technical issues with Ultrapath CDOM measurements
8:45-9:15 am	Introduction and possible solutions to the Ultrapath salinity correction (Rick Miller)
9:15-9:45 am	Trials and tribulations of the Ultrapath in high salinity/low CDOM waters (HSLC) (Norm Nelson)
9:45-10:15 am	Comparison of Ultrapath measurements with double beam UV-Vis spectrophotometer in coastal systems (Mike Novak); Discussion on differences in Ultrapath protocols (Antonio Mannino)
10:15-10:30 am	Break
10:30-11:30 am	Comparison and discussion of results from pre-workshop sample analysis (TBD)
11:30-noon	Instrument setup in the lab
noon-12:45 pm	Lunch

Afternoon Session in the Lab

12:45-4:00 pm	Laboratory measurements of NaCl solutions, CDOM samples, dilution series of CDOM samples; data analysis;
4:00-5:00 pm	Discussion of results

6 pm ***Group dinner***

Day 2 - Nov. 14, 2013

Morning Session

8:45 am	Review individual protocols
9:30-9:45 am	Presentation on PSICAM capabilities for CDOM (Rüdiger Röttgers)
9:45-10:00 am	Presentation and discussion on Ultrapath results from BIOSOPE

Lab

10:00-12:30 pm	2nd round of laboratory measurements; data analysis
12:30-1:15 pm	Lunch

Afternoon Session

1:15-2:15 pm	Discussion of instrument comparison results ● Document measurement variability between different Ultrapaths and different instruments. ● Define the causes of any revealed differences. ● Document precision and accuracy of CDOM measurements for each type of instrument
2:15-2:45 pm	Discussion on salinity correction protocol to recommend
PM	Discussion on procedures and NIST-traceable materials to verify instrument performance ((wavelength accuracy, stray light and photometric accuracy) for Ultrapath, bench spectrophotomers, ac-s, and PSICAM/OSCAR.
PM	Begin drafting protocols ● Sample collection, processing & storage ● Sample analysis by spectrophotometry, ultrapath, etc. ● Salinity correction for Ultrapath; cell cleaning ● Data analysis from raw OD to naperian absorption coefficients and spectral slope computations
3:15-3:30 pm	Break
5:00-5:30 pm	Discuss approaches to developing NIST-traceable CDOM standard reference material or community reference material
6 pm	***Group dinner***

Day 3 - Nov. 15, 2013

Morning Session

8:30-8:45 am	Recent advances by WPI on Ultrapath cells (Mathias Belz)
8:45-10:30 am	Continue discussion on protocols; other instruments to consider (per proposal review comments)
10:30-10:45 am	Break
10:45-noon	Discussion on future plans for protocols, dissemination to community, etc.

noon End of Workshop