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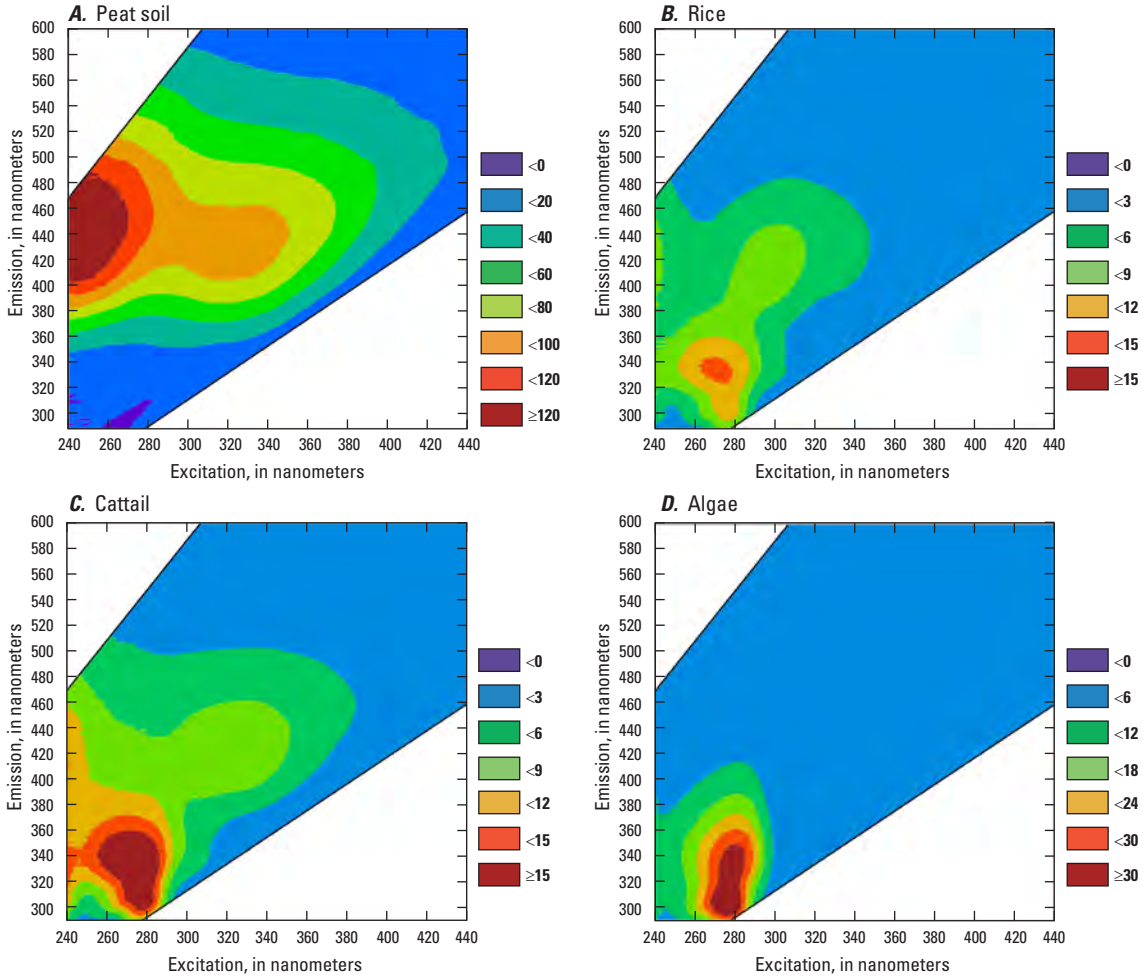
Procedures for Using the Horiba Scientific Aqualog® Fluorometer to Measure Absorbance and Fluorescence from Dissolved Organic Matter



Open File Report 2018–1096

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Cover. Four excitation-emission matrices showing fluorescence from different sources of dissolved organic matter common to the Sacramento–San Joaquin Delta (California, USA): *A*, Peat soil (euic, thermic Typic Medisaprists); *B*, Rice (oryza sativa); *C*, Cattail (typha spp.); and *D*, Algae (thalassiosira weissflogii). Note, the presence or absence of fluorescence peaks, intensity of fluorescence response, changes in the ratios of excitation-emission pairs, and shifts in peak maxima have all been shown to provide information about dissolved organic matter character and origin.

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By Angela M. Hansen, Jacob A. Fleck, Tamara E.C. Kraus, Bryan D. Downing, Travis von Dessonneck, and Brian A. Bergamaschi

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RYAN K. ZINKE, Secretary

U.S. Geological Survey
James F. Reilly II, Director

U.S. Geological Survey, Reston, Virginia: 2018

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Conversion Factors

International System of Units to U.S. customary units

By	Multiply	To obtain
	Length	
nanometer (nm)	25,400,000.0	inch (in.)
micrometer (µm)	25,400.0	inch (in.)
centimeter (cm)	2.54	inch (in.)
meter (m)	0.3048	foot (ft)
	Volume	
microliter (µL)	29,573.5	ounce, fluid (fl oz)
milliliter (mL)	29.5735	ounce, fluid (fl oz)
liter (L)	0.0295735	ounce, fluid (fl oz)
	Flow rate	
meter per second (m/s)	0.3048	foot per second (ft/s)
cubic meter per second (m^3/s)	0.0283168	cubic foot per second (ft^3/s)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32.$$

Supplemental Information

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given in either milligrams per liter (mg/L) or micrograms per liter (µg/L).

Abbreviations

AU	absorbance units
CAWSC	California Water Science Center
CRM	certified reference material
DA	discriminant analysis
DBP	disinfection byproduct
DDL	daily detection limit
DOC	dissolved organic carbon
DOM	dissolved organic matter
EEM	excitation-emission matrix
HNO_3	nitric acid
IFE	inner-filtering effects
IHSS	International Humic Substances Society
LRW	lab reagent water
LT-MDL	long-term method detection limit
MDL	method detection limit
NIST	National Institute of Standards and Technology
NWIS	U.S. Geological Survey National Water Information System database
ORML	Organic Matter Research Laboratory
PARAFAC	parallel factor analysis
PCA	principal component analysis
PMMA	polymethylmethacrylate
POM	particulate organic matter
QA	quality assurance
QC	quality control
RPD	relative percentage difference
RU	Raman-normalized intensity units
SOP	standard operating procedure
SRM	standard reference material
USGS	U.S. Geological Survey
UV	ultraviolet

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Abstract

Advances in spectroscopic techniques have led to an increase in the use of optical measurements (absorbance and fluorescence) to assess dissolved organic matter composition and infer sources and processing. Although optical measurements are easy to make, they can be affected by many variables rendering them less comparable, including by inconsistencies in sample collection (for example, filter pore size, preservation), the application of corrections for interferences (for example, inner-filtering corrections), differences in holding times, and instrument drift (for example, lamp intensity). A documented, standardized procedure to address these variables ensures that the optical (absorbance and fluorescence) measurements collected by U.S. Geological Survey researchers are useful and widely comparable.

Rigorous and quantifiable quality assurance and quality control are essential for making these data comparable, particularly because there is no published guideline for the measurement of dissolved organic matter absorbance and fluorescence, and especially because there is no National Institute of Standards and Technology standard for dissolved organic matter. Validation and quality-control samples are analyzed on a monthly basis to determine laboratory and instrument precision and daily (that is, each day samples are run) to ensure repeatability. Data are not considered acceptable unless they meet laboratory criteria: All standards should be within 10 percent of the target value, laboratory replicates should be within 5 percent relative percent difference, and laboratory blanks (that is, laboratory reagent-grade water) should be less than one-tenth of the long-term method detection limit.

Finally, for data to be useful, they must be accessible to users in a format that can be easily analyzed and interpreted. The Organic Matter Research Laboratory staff has developed a processing routine that extracts a subset of the data, which is made available to the public through the USGS National Water Quality Information System (http://nwis.waterdata.usgs.gov/usa/nwis/qwdata), and organizes the full datasets (that is, complete absorbance spectra and fluorescence excitation-emission matrices) in different forms that allow for these data to be analyzed using multi-parameter and multi-way statistical approaches.

Purpose and Scope

The purpose of this report is to document the procedures developed by the U.S. Geological Survey (USGS) California Water Science Center (CAWSC) Organic Matter Research Laboratory (OMRL) personnel for using the Aqualog® fluorometer to measure the absorbance and fluorescence of dissolved organic matter (DOM). Topics include sample collection and handling, instrument set-up, data quality assurance and quality control, data processing, as well as a brief overview of optical properties and how these data are commonly used. The intended audience is primarily those who will use the Aqualog® fluorometer for research and could benefit from a guide to its specific operation following a set of standard procedures; as such, this report does not address the fundamental principles underlying the measurement technology.

Background

Optical spectroscopy has been used for decades to measure DOM amount and composition, and advances in instrumentation have improved data quality—and the ease of sample analysis—associated with this approach. The optical measurements discussed in this report can be broken up into two different phenomena: (1) absorbance, the measurement of the amount of light absorbed by a water sample over a known distance at a specific wavelength (fig. 1*A*), and (2) fluorescence, the measurement of the amount of light emitted by a water sample at a specific wavelength following absorbance of incident light over a known distance at a specific excitation wavelength (fig. 2*A*). Absorbance scans

are depicted in a two-dimensional array, with wavelength, in nanometers (nm), on one axis and the amount of light absorbed on the second axis (fig. 1*B*). Fluorescence scans comprise sets of emission spectra collected across an array of excitation wavelengths and are typically depicted in an excitation-emission data matrix (EEM) consisting of thousands of excitation-emission pairs from a single water sample, where the excitation wavelength (nm) is on one axis, the emission wavelength (nm) is on the second axis, and the fluorescence intensity is on a third axis (fig. 2*B*).

Advances in commercially available optical spectroscopy instruments and analytical techniques have led to an increase in the use of optical property measurements of absorbance and fluorescence in many research applications across scientific disciplines. Optical spectroscopy increasingly not only is used to provide a proxy for DOM concentration and composition, but also to identify unique parameters that can be used to trace the DOM source, biogeochemical transformations, and roles in ecosystem processes (Jaffé and others, 2008; Hernes and others, 2009; Hansen and others, 2016). These improved techniques are rapid, inexpensive, and allow for tracking DOM both in surface and groundwater. Optical measurements have been used in a range of aquatic systems (reservoirs, streams, estuaries, oceans) as well as to identify DOM associated with plants, soil, phytoplankton, wastewater, oil, and other materials. Optical data can be used in a wide range of applications for natural waters, including organic matter cycling (Coble, 2007; Tranvik and others, 2009), algal production of DOM (Lapierre and Frenette, 2009), and DOM source attribution and fingerprinting (Baker and Spencer, 2004; Carstea and others, 2009; Goldman and others, 2012; Carpenter and others, 2013).

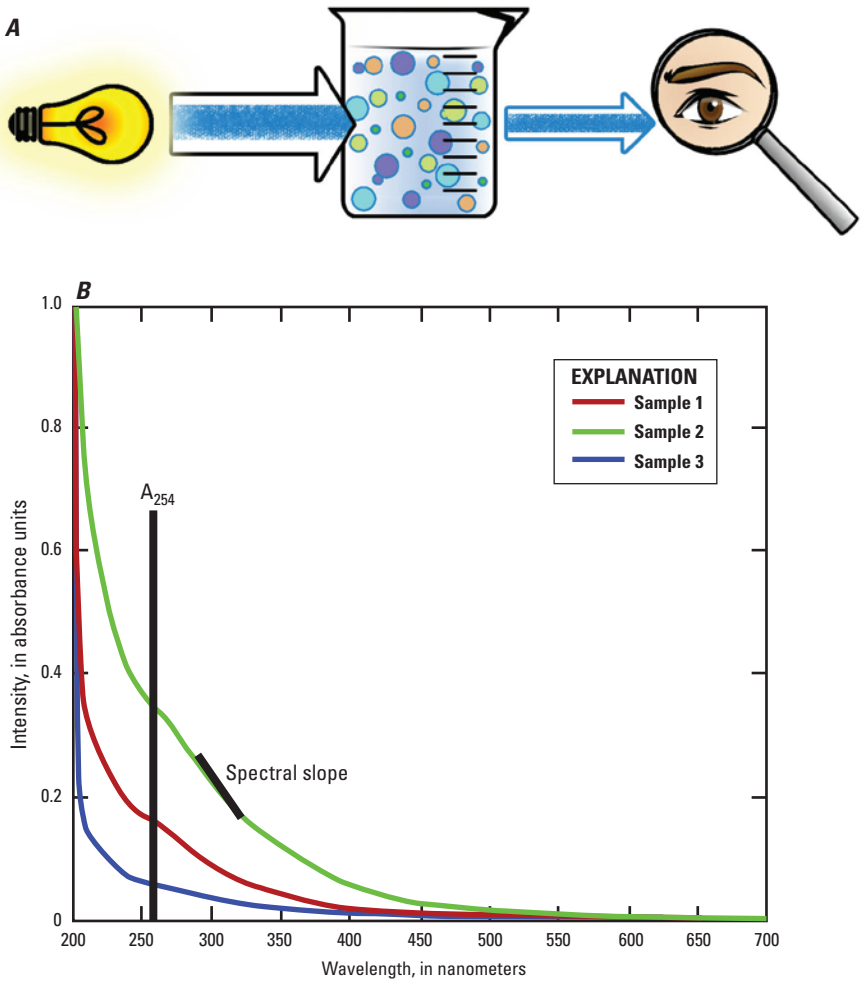


Figure 1. Chromophoric dissolved organic matter (DOM) components: *A*, absorb light thereby decreasing the amount of energy exiting the sample and *B*, the absorbance response at a single wavelength is related to DOM concentration (that is, absorbance at 254 nanometers [A_{254}]), whereas the slope between two wavelengths or the ratios between them provides information about the composition of the DOM.

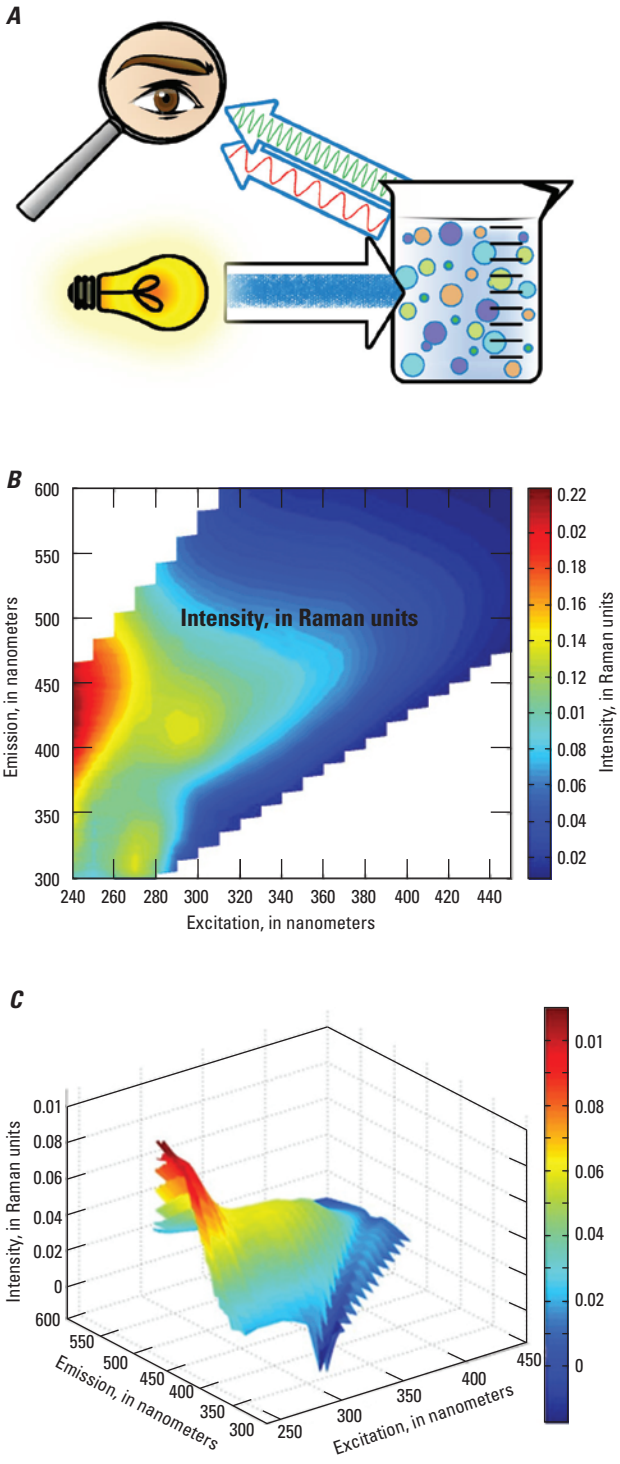


Figure 2. Some chromophoric dissolved organic matter (DOM) components: *A*, absorb light then re-emit it at a longer wavelength and *B*, a two-dimensional representation of fluorescence is referred to as an excitation-emission matrix (EEM) plot. As with absorbance, the response at any given wavelength can be related to concentration; *C*, an EEM is actually a three-dimensional surface with the z-axis indicating intensity.

Measuring DOM absorbance and fluorescence in a filtered-water sample provides information about the concentration of the bulk DOM pool as well as the composition of different types of compounds present and their likely origin. Common parameters and indices derived from optical data include the absorbance at specific wavelengths (for example, absorbance at 254, 280, 370, 412, and 440 nm) and fluorescence at specific excitation-emission pairs (for example, ex260/em450, peak A; ex275/em340, peak T; and ex340/em440, peak C). The response at a specific wavelength or wavelength pair is related to the DOM concentration—the response increases as the amount of the optically active DOM pool in the sample increases. Information about the composition of DOM can be obtained by normalizing the absorbance or fluorescence response to another parameter; normalizing to dissolved organic carbon (DOC) concentration is most commonly reported (Beggs and Summers, 2011; Hansen and others, 2016). In table 1, various indicators of DOM composition are listed, including the examination of ratios of different wavelengths (for example, fluorescence index and humification index) and spectral slopes ($S_{275–295}$, $S_{290–350}$, $S_{350–400}$) across specific regions of the optical spectrum, which can be related to the molecular weight, source, and processing (for example, biodegradation and photolytic exposure) of DOM.

Table 1. Description of commonly used optical properties of absorbance and fluorescence to analyze the composition of dissolved organic matter.

[Adapted from Hansen and others, 2016. **Abbreviations**: cm, centimeter; DOC, dissolved organic carbon; DOM, dissolved organic matter; ex-em, excitation-emission; FDOM, fluorescent dissolved organic matter; L, liter; mg, milligram; nm, nanometer; RU, Raman units; S, slope; SUVA, specific ultraviolet absorbance; UV, ultraviolet; VIS, visibile; >, greater than; —, not applicable]

Measurements	Description		
	Calculation	Purpose	Reference
Absorbance measurements			
SUVA at 254 nm, in liters per milligram of carbon per meter	Absorption coefficient at 254 nm divided by DOC concentration.	Absorbance per unit carbon. Typically a greater number is associated with greater aromatic content.	Weishaar and others (2003); Spencer and others (2012); Chowdhury (2013)
SUVA (280 nm, 350 nm, 370 nm) in liters per milligram of carbon per meter	Absorption coefficient at a given wavelength in the ultraviolet region divided by DOC concentration.	Absorbance per unit carbon. Typically a greater number is associated with greater aromatic content.	Chin and others (1994); Hansen and others (2018)
Specific visible absorbance [SVA (412 nm, 440 nm, 480 nm, 510 nm, 532 nm, 555 nm)] in liters per milligram of carbon per meter	Absorption coefficient at a given wavelength in the visible region divided by DOC concentration.	Absorbance per unit carbon. Typically a greater number is associated with greater aromatic content.	Hansen and others (2016)
Spectral slopes ($S_{275–295}$, $S_{290–350}$, $S_{350–400}$)] (nm^{-1})	Nonlinear fit of an exponential function to the absorption spectrum over the wavelength range.	Typically higher S values indicate low molecular weight material and (or) decreasing aromaticity.	Blough and Del Vecchio (2002); Helms and others (2008)
Spectral slope ratio (SR) $S_{275–295}$ (nm^{-1}):$S_{350–400}$ (nm^{-1})	Spectral slope $S_{275–295}$ divided by spectral slope $S_{350–400}$.	Shown to be negatively correlated to DOM molecular weight and to generally increase upon irradiation.	Helms and others (2008)
Fluorescence measurements			
Specific fluorescence at various peaks [spA, spB, spC, spD, spM, spN, spT, spZ](RU L mg-C^{-1})	Fluorescence at a given ex-em pair divided by DOC concentration.	Fluorescence per unit carbon.	Hansen and others (2016, 2018)
Peak ratio (A:T)	The ratio of Peak A (ex260/em450) to Peak T (ex275/em340) intensity.	An indication of the amount of humic-like (recalcitrant) versus fresh-like (labile) fluorescence in a sample.	Hansen and others (2016, 2018)
Peak ratio (C:A)	The ratio of Peak C (ex340/em440) to Peak A (ex260/em450) intensity.	An indication of the relative amount of photosensitive humic-like DOM fluorescence in a sample.	Moran and others (2000); Hansen and others (2016)
Peak ratio (C:M)	The ratio of Peak C (ex340/em440) to Peak M (ex300/em390) intensity.	An indication of the amount of diagenetically altered (blue-shifted) fluorescence in a sample.	Coble (1996); Burdige and others (2004); Para and others (2010); Helms and others (2013)
Peak ratio (C:T)	The ratio of Peak C (ex340/em440) to Peak T (ex275/em340) intensity.	An indication of the amount of humic-like (recalcitrant) versus fresh-like (labile) fluorescence in a sample.	Baker and others (2008)
Fluorescence index (FI)	The ratio of em wavelengths at 470 nm and 520 nm, obtained at ex 370.	Shown to identify the relative contribution of terrestrial and microbial sources to the DOM pool.	McKnight and others (2001); Cory and others (2010)

Table 1. Description of commonly used optical properties of absorbance and fluorescence to analyze the composition of dissolved organic matter.—Continued

[Adapted from Hansen and others, 2016. **Abbreviations**: cm, centimeter; DOC, dissolved organic carbon; DOM, dissolved organic matter; ex-em, excitation-emission; FDOM, fluorescent dissolved organic matter; L, liter; mg, milligram; nm, nanometer; RU, Raman units; S, slope; SUVA, specific ultraviolet absorbance; UV, ultraviolet; VIS, visibile; >, greater than; —, not applicable]

Measurements	Description		
	Calculation	Purpose	Reference
Fluorescence measurements—Continued			
Humification index (HIX)	The area under the em spectra 435–480 nm divided by the peak area 300–345 nm + 435–480 nm, at ex 254 nm.	An indicator of humic substance content or extent of humification. Higher values indicate an increasing degree of humification.	Ohno (2002)
Freshness index (β:α)	The ratio of emission intensity at 380 nm divided by the maximum emission intensity between 420 and 435 nm at excitation 310 nm.	An indicator of recently produced DOM, with greater values representing a greater proportion of fresh DOM.	Parlanti and others (2000); Wilson and Xenopoulos (2009)
Relative fluorescence efficiency (RFE) (RU cm)	Ratio of fluorescence at ex370/em460 (FDOM) to absorbance at 370 nm.	RFE is an indicator of the relative amount of algal and non-algal DOM.	Downing and others (2009)
Biological index (BIX)	The ratio of emission intensity at 380 nm divided by 430 nm at excitation 310 nm.	An indicator of autotrophic productivity. Greater values (>1) correspond to recently produced DOM of autochthonous origin.	Huguet and others (2009)

Sample Collection and Handling

Samples processed in the OMRL are collected according to the USGS protocols (U.S. Geological Survey, 2012). It should be noted that the use of methanol to rinse field equipment and tubing results in contamination, so its use should be avoided when collecting samples for organic carbon analysis. After collection, samples are filtered immediately in the field or placed on ice and filtered as soon as possible (preferably within 24 hours) in the laboratory through pre-combusted 0.3-micrometer (µm) nominal pore-size glass-fiber filters (Advantec MFS model GF7547mm; Advantec MFS, Dublin, California, USA; also available through the USGS One-Stop Shop, item Q355FLD) or 0.45-µm nominal pore-size syringe, capsule, or disk filters. Following filtration, samples in amber glass should be stored in the dark on ice or refrigerated at less than 4 degrees Celsius (°C) and analyzed within 2 days, preferably. Acid preservation of samples is not recommended. If samples are known to include a large fraction of labile DOM, it is best to filter samples immediately in the field and analyze them within 24 hours to avoid loss of that pool of DOM prior to analysis. Samples are visually inspected just prior to analysis to ensure no colloids or precipitates have formed during storage. In cases where samples become visually cloudy, refiltration may be required. Procedures for handling samples that are known to contain a large amount of volatile organic compounds (for example, oil and gas compounds) are being developed.

Analytical Method

In this section, everything needed to measure absorbance and fluorescence in a water sample is described, from the instrument and software to assessment of quality control and reporting of the data.

Aqualog® Instrument

The method used in the CAWSC OMRL is appropriate for using the Aqualog® instrument to analyze filtered-water samples containing DOM. Procedures for operating the Aqualog® are adapted from the manufacturer's user manual (Horiba Scientific, 2011). This method simultaneously measures the absorbance and fluorescence of a filtered-water sample and produces an absorbance scan of 121 (361 after interpolation) wavelengths and a fluorescence matrix of 11,029 discrete excitation and emission pairs.

Equipment and Materials

The equipment and materials listed in table 2 are for the measurement of absorbance and fluorescence of dissolved organic matter in waters using the Aqualog® instrument. The analyses are done in a lab free of organic solvents to prevent vapor-phase contamination of the samples. Type I (18.2 mega-ohm resistance, organic-free) laboratory reagent-grade water (LRW) is produced onsite with a recirculating water system (Labconco WaterPro Polishing System, Kansas City, Missouri). Routine replacement of water-polishing system cartridges and system sanitization is performed annually. All glassware is acid-washed in 10-percent weight-to-volume (wt/vol) nitric acid $(HNO_3)_{(aq)}$ and water to minimize organic contamination.

The Aqualog® light source is a 150 watt ozone-free xenon arc lamp. Excitation and absorbance spectra are scanned with a double-grating monochrometer from 230 to 600 nm, with a 5-nm bandpass, in 3-nm increments, and emission spectra are collected with a charge-coupled device (CCD) from 250 to 600 nm, with a 5-nm bandpass, in 1.6–3.3-nm (4-pixel) increments. Aqualog® instruments are equipped with different specifications for light sources and CCDs, both of which can affect the matrix size and resolution. Consideration should be given to the specific specifications of the Aqualog® when establishing laboratory-specific methods and tools.

Precautions and Interferences

Ambient light interferes with measurements. As a result, the instrument is operated with the cover closed to prevent light from entering the chamber during instrument measurement.

All glassware should be cleaned meticulously. The OMRL procedure requires all glassware is washed with detergent and hot tap water. Glassware is then acid-washed by soaking in a 10 percent $HNO_{3(aq)}$ bath for at least 2 hours. Following the acid wash, the glassware is triple-rinsed inside and out with Type I LRW and dried on a laminar-flow table. Clean, dry glassware is then sealed by wrapping with aluminum foil and stored in a dust-free cabinet.

Laboratory water systems have been known to contaminate samples as a result of bacterial breakthrough from resin beds, activated carbon, and filters. Laboratory water systems should be maintained and monitored frequently for background carbon and bacterial growth. The OMRL procedures for maintaining clean LRW were described previously in the "Equipment and Materials" section of this report, and monitoring of this water as part of the daily quality management of an analytical run is explained later in this report.

Table 2. Laboratory equipment and materials used for the measurement of dissolved organic matter by absorbance and fluorescence in the U.S. Geological Survey (USGS) Organic Matter Research Laboratory.

[Equivalent products can be found elsewhere. **Abbreviations**: cm, centimeter; LRW, lab reagent water; Mohm, mega-ohm; mL, milliliter; n/a, not applicable; ppb, parts per billion; W, watt; w/w, weight per weight; U.S.A., United States of America; µL, microliter; µm, micrometer; %, percent; ±, plus or minus; <, less than]

Equipment and materials	Manufacturer	Part number
Acid, nitric (HNO_3, 68–70% w/w)	Fisher Scientific	A200C-212
Aqualog® lamp (xenon, 150 W ozone free XBO)	Horiba Scientific, New Jersey, U.S.A.	1905-OFR
Aqualog® instrument	Horiba Scientific, New Jersey, U.S.A.	n/a
Balance, certified accuracy of 0.050 gram±0.0001	n/a	n/a
Bottles, amber glass, baked (40 mL, 125 mL, 250 mL)	USGS One-Stop Shop	N1560, Q28FLD, Q435FLD
Cuvettes (10-cm pathlength)	Starna Cells, Inc.	3-Q-10
Filter (precombusted 47-mm diameter, 0.3-µm nominal pore-size glass-fiber filters or 0.45-µm syringe filter)	USGS One-Stop Shop	Q355FLD
Lens paper	Fisher Scientific	11-997
Pipette (100–1,000 µL)	Eppendorf	13-690-032
Pipette (1–10 mL)	Eppendorf	13-690-034
Standard reference material (SRM)	See "Validation and Quality-Control Samples" section in report text.	n/a
Type 1 LRW (18.2 Mohm resistance, <10 ppb total organic carbon)	Labconco WaterPro PS	9000500
Workstation installed with Aqualog® software (version 3.6)	n/a	n/a

Blemishes on the cuvette are a regular source of contamination and interference. These include manufacturing defects, scratches, fingerprints, oils, and stains that can cause buildup of optically active material and lead to the misrepresentation of detection limits and measurements. Always rinse the cuvette between each sample with plenty of Type I LRW at the source, always wear gloves, and replace gloves whenever in doubt of cleanliness. Confirm the cuvette is free from blemishes first by visual inspection and then by evaluation of the laboratory blanks (see later) in the analytical run. In the OMRL, lens paper is used to buff and prepare the cuvette for analysis.

Optical measurements are extremely sensitive to light scattering of fine particulates and colloids. Dissolved organic matter is operationally defined as the organic matter fraction that passes through a filter (typically 0.3–0.7 µm), and that which is collected on the filter is defined as particulate organic matter (POM). Although filter pore size typically does not affect DOM concentration, under some conditions, sorption and the formation of colloids and even precipitation can transfer DOM to the POM pool. Baker and others (2007) suggest filter pore size unevenly affects optical measurements and emphasize the need to standardize filter size in individual studies. Consideration of the effects of pore size should be determined prior to interpretation or comparison with published results. Larger or smaller pore sizes may be used in cases where optical measurements are compared to other analyses on the same water sample (for example, biological oxygen demand, chlorophyll-a concentration, disinfection byproduct formation); however, the user should be aware of potential interferences from colloids or particles. The filter pore size should be reported for all samples analyzed.

Several studies have looked at different approaches for storing samples including, for example, freezing or acidifying. In the OMRL, samples are filtered and analyzed as soon as possible, preferably within 2 days of sample collection. Freezing has been used to store organic-rich samples with varying degrees of success that depend on the initial DOC concentrations (Spencer and others, 2007; Fellman and others, 2008; Hudson and others, 2009), with DOC concentrations greater than 5 milligrams per liter (mg/L) and specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) values greater than 3.5 liters per milligram carbon per meter (L mg C^{-1} m^{-1}) exhibiting greater loss of concentration as a result of precipitation and changes in chemical composition than samples with lower concentrations. In certain cases where DOC concentrations are low, freezing can be a practical storage method (Fellman and others, 2008); however, the effects of freezing and thawing or acidifying samples for preservation should be evaluated on a site- and study-specific basis.

Validation and Quality-Control Samples

Validation and quality-control samples are run on a monthly basis to determine laboratory and instrument precision and on a daily basis (that is, each day samples are run) to ensure repeatability. Ideally an official National Institute of Standards and Technology (NIST) certified reference material (CRM) would be used to determine the accuracy and precision of optical data across the full absorbance and fluorescence spectra, but such a standard for the complex composition of natural DOM does not exist. The available CRMs are limited to a specific fluorescence excitation-emission pair or absorbance region (for example, Starna quinine sulfate reference set, RM-4QS00, and Starna 6BF fluorescence reference cells) and are therefore not informative enough to use on a daily basis. Furthermore, although the Starna quinine sulfate reference set (RM-4QS00) is available at a range of concentrations (0.25–1.0 mg/L), even the lowest concentration does not provide an environmentally relevant optical response. To address analytical quality assurance (QA), the OMRL staff measures commonly used optically active compounds monthly in coordination with daily standard reference materials to examine individual wavelengths and wavelength pairs, which we describe in detail in following sections.

Monthly Quality Control

Validation and quality-control samples are analyzed on a monthly basis to determine laboratory and instrument precision.

Aqualog® Validation Scans

Aqualog® validation scans are performed in the OMRL on a monthly basis to validate instrument performance. We perform four validation scans recommended by the manufacturer: (1) excitation validation, (2) water Raman SNR (signal-to-noise ratio) and emission calibration, (3) absorbance photometric accuracy, and (4) quinine sulfate unit. The purpose of the validation scans, the required materials, and criteria for a satisfactory response are summarized in table 3 and detailed further in the user's manual (Horiba Scientific, 2011).

Table 3. Validation scans recommended for Aqualog® calibration.

[cps, counts per second; CCD, charge-coupled device; EEM, excitation-emission matrix; LRW, lab reagent water; mg/L, milligram per liter; nm, nanometer; QS, quinine sulfate; SNR, signal-to-noise ratio; SRM, standard reference material; >, greater than; ±, plus or minus]

Validation scan	Purpose	Required reagent/cell	Satisfactory response
Excitation validation	The purpose of this scan is to verify lamp performance and peak position.	This is a lamp scan. No cell or reagent required. Leave cell chamber empty.	Peak position 467 (±1 nm).
Water Raman SNR and emission calibration	This validation check examines the wavelength calibration of the CCD detector. It is an emission scan of the Raman-scatter band of water performed in right-angle mode.	Standard cuvette with Type 1 LRW.	Raman peak position 397 nm (±1 nm). The results column should read PASS. Select the tab "Raman SNR Calculation." In the B(Y) column below the comments section, there should be a number. This is the lamp intensity, and the value should be >650,000 cps.
Absorbance photometric accuracy	This validation check examines the accuracy of the absorption function of the Aqualog®.	Standard SRM 935a (potassium dichromate blank, potassium dichromate 60 mg/L).	Select the tab "Test Results," the "Pass/Fail" column should display "P" for all four wavelengths measured.
Quinine sulfate unit (QSU)	This function provides a standardized intensity for fluorescence measurements and EEMs.	Quinine sulfate standard kit (RM-QS00) containing blank and standard (1 mg QS/L).	Select the tab "QSU Calculation." The absorbance value at 347.5 nm should be 0.01384. Select the tab "Emission Spectrum Graph." Using the crosshairs icon locate the position of the peak. This value should be 450 nm (±1 nm).

Potassium Dichromate

The use of potassium dichromate ($K_2Cr_2O_7$) dissolved in dilute perchloric acid is a common method for validating the accuracy of the absorbance response (fig. 3) and the linearity of the concentration response (fig. 4) of a spectrophotometer in the ultraviolet (UV) region. In the OMRL, absorbance of potassium dichromate is measured at three wavelengths (that is, 257, 313, and 350 nm) specified by NIST. Figures 3 and 4 show data collected over an annual cycle that, for ease of comparison, are presented in a similar format to those in the Starna Cells (2015) guide for the *NIST traceable UV/Vis/NIR reference sets*. These data are generated using the NIST traceable set (Starna Cells, Inc., Atascadero, Calif., part no. RM-0204060810) that contains five sealed cuvettes of potassium dichromate in a range of concentrations (table 4) and a cuvette containing perchloric acid (0.001 molar) to be used as a blank. Although NIST recommends also measuring absorbance at 235 nm, the Aqualog® method for the simultaneous collection of fluorescence and absorbance data begins scanning at wavelength 240 nm; therefore, we report results at 257, 313, and 350 nm.

Fluorescence Reference Set

Starna 6BF fluorescence reference cells are a set of seven fluorescing materials (anthracene and naphthalene, ovalene, p-terphenyl, tetraphenylbutadiene, compound 610, and rhodamine) set in six polymethylmethacrylate (PMMA) blocks in the dimensions of a standard cuvette. Each block has a unique excitation and emission curve, which allows the user to check the accuracy of the instrument's response across a specified spectrum (fig. 5). Although this set is beneficial for targeting specific peaks (excitation and emission, or ex-em, pairs), it is limited by its inability to simultaneously provide a response both in the regions indicating more humic-like, recalcitrant material (peaks A, C) and the regions indicating the fresher, more labile material (peaks B, T) in a single scan.

Certified Reference Material

Certified reference materials (CRMs) are used to track accuracy and precision among analytical runs; however, there are few certified fluorescence standards available to properly validate instrument performance. The International Humic Substances Society (IHSS) standards are an option; however, preparing these to an exact concentration poses challenges because organic matter is not readily or consistently brought back into solution once fully dried (Mobed and others, 1996). Along with the fact IHSS standards are in limited supply, and no guidance documents exist for their preparation as a fluorescence standard material, they are also costly and, therefore, are not practical for frequent use.

Daily Quality Control

Validation and quality-control samples are analyzed on a daily basis (that is, each day samples are run) to ensure repeatability.

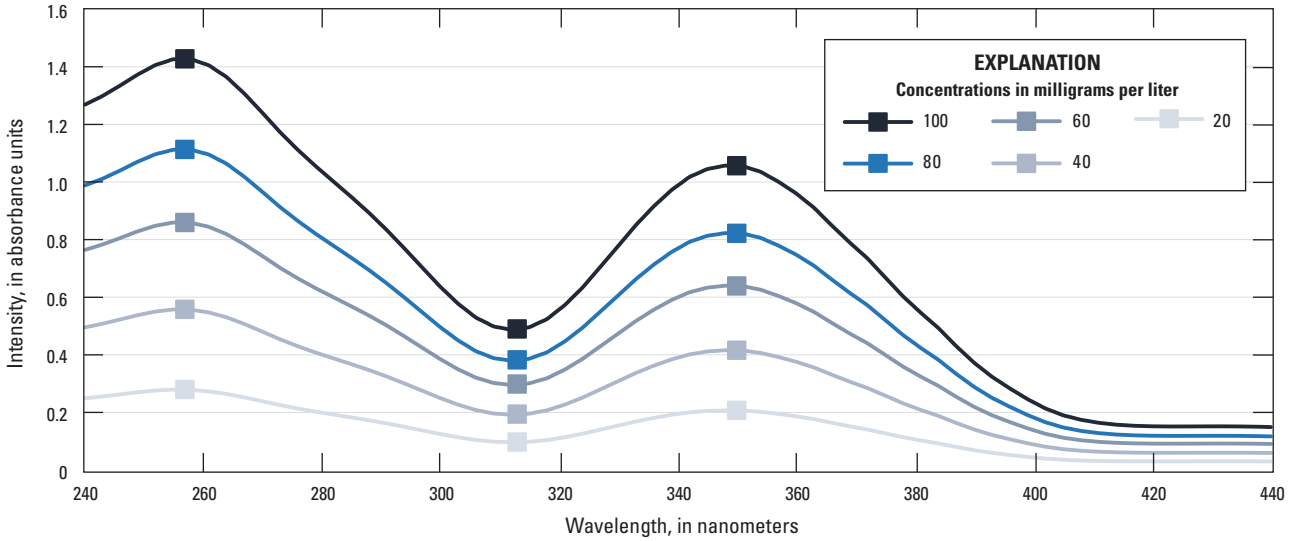


Figure 3. The absorbance response for five concentrations of potassium dichromate solution averaged over an annual cycle (sample count is 11). Peaks and trough are tracked at 257, 313, and 350 nanometer (nm) wavelengths. Error bars depicting standard deviation were less than 0.002 at each point and thus are not visible.

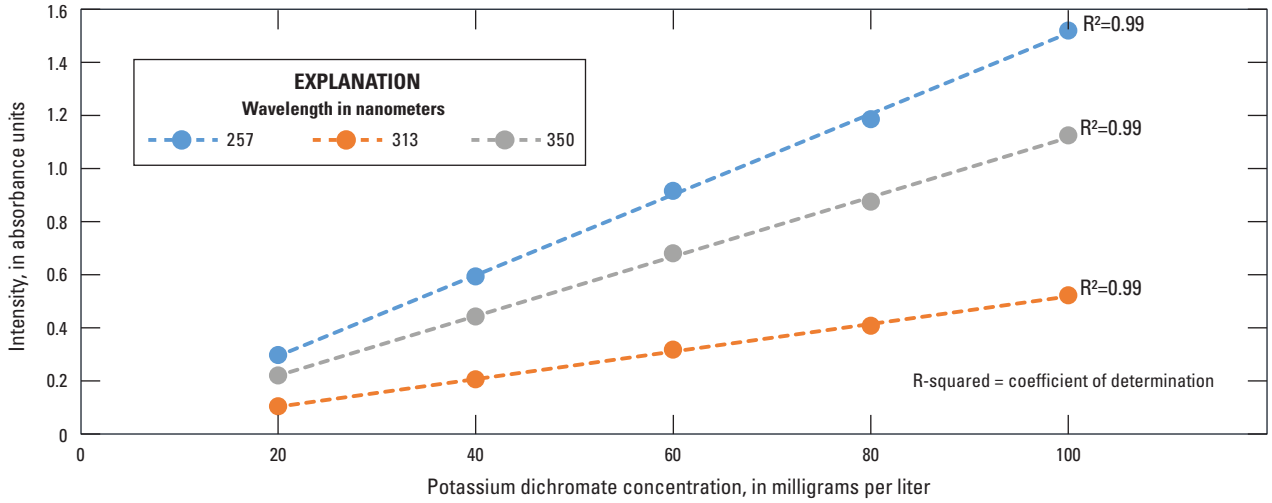


Figure 4. Absorbance response of five concentrations of potassium dichromate at three wavelengths measured over an annual cycle (sample count is 11) tests linearity through the ultraviolet (UV) range. The error bars depicting standard deviation were less than 0.002 absorbance units at each point and thus are not visible.

Table 4. The National Institute of Standards and Technology (NIST) recommended wavelengths and target results for five concentrations of potassium dichromate standard.

[mg/L, milligram per liter; nm, nanometer]

Concentration	257 nm	313 nm	350 nm
20 mg/L	0.281	0.095	0.209
40 mg/L	0.572	0.192	0.426
60 mg/L	0.862	0.289	0.634
80 mg/L	1.159	0.385	0.853
100 mg/L	1.448	0.480	1.069

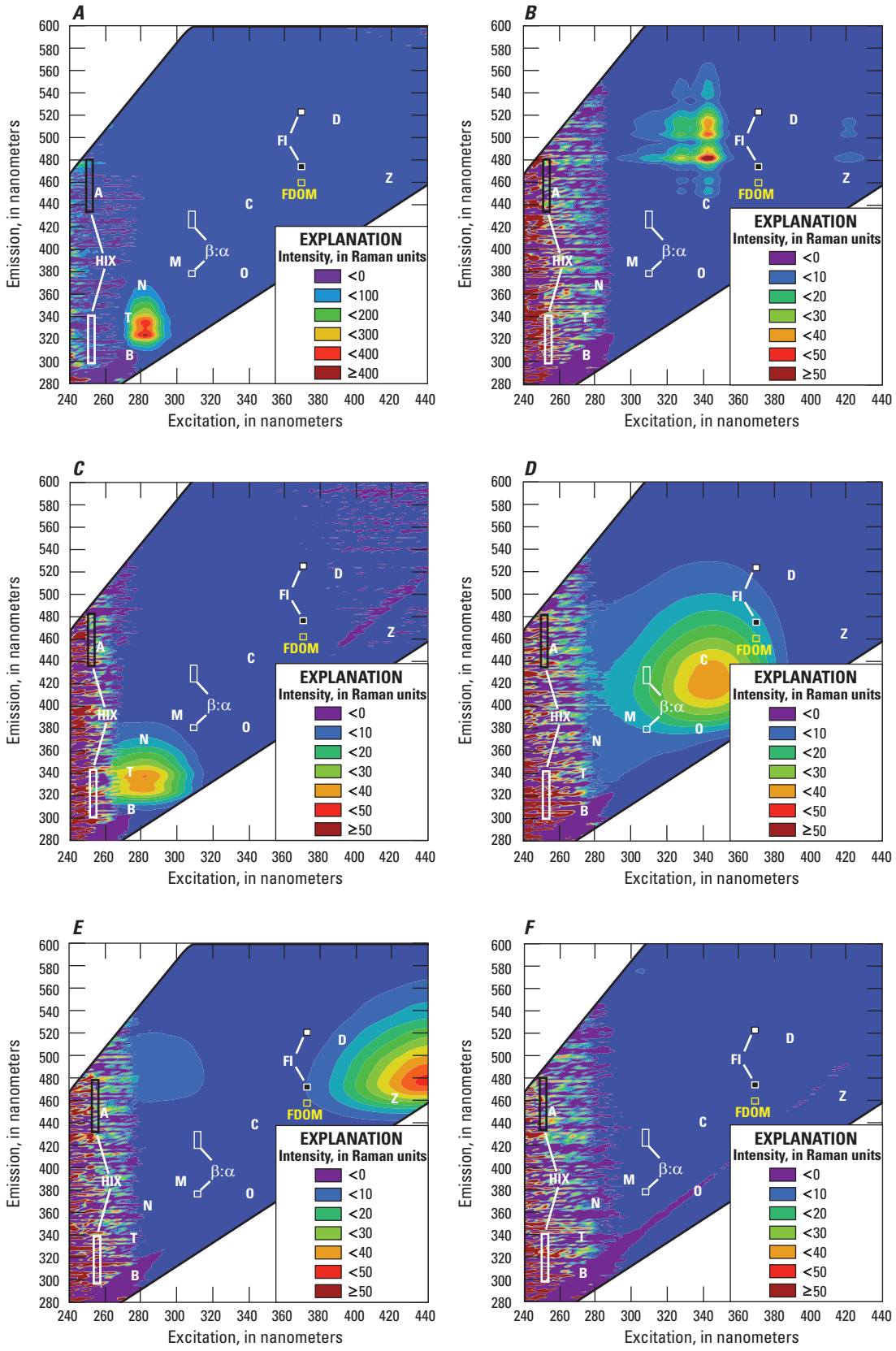


Figure 5. Long-term average excitation-emission matrices (EEMs) for Starna 6BF PMMA (polymethylmethacrylate) blocks: *A*, anthracene and napthalene; *B*, ovalene; *C*, p-terphenyl; *D*, tetraphenyl butadeine; *E*, compound 610; and *F*, rhodamine. Each plot shows the average of 18 scans over a 22-month period. (Labeled regions of the EEM correspond to named peaks or table 1 optical properties; FDOM, fluorescent dissolved organic matter peak.)

Standard Reference Material

The standard reference material (SRM) used in the OMRL for tracking accuracy and precision among analytical runs is a readily available, consumer-grade bottled tea (Pure Leaf®, unsweetened black tea, Purchase, New York), referred to as SRM_{Tea} in this report. Prior to use, the SRM_{Tea} is diluted to 1 percent concentration (1 milliliter of tea to 99 milliliters of Type I LRW); at this concentration, the known standard meets the requirement of inner-filtering corrections. This product has been proven to be stable and internally traceable over a long time (4 years) for absorbance and fluorescence spectra over the full range of measurement (fig. 6). Although minor variability exists among production lots (batches of tea), the different lots have been verified to meet the laboratory quality-assurance and quality-control (QA/QC) criteria across the relevant absorbance spectra and fluorescence EEMs to verify proper lamp function and processing consistency as an internal lab SRM. Users should take into account this is a consumer-grade food product, and although relatively stable, variability in the absorbance spectra and fluorescence EEMs relating to seasonal harvesting and source locations of teas has been observed during the 4-year tracking period.

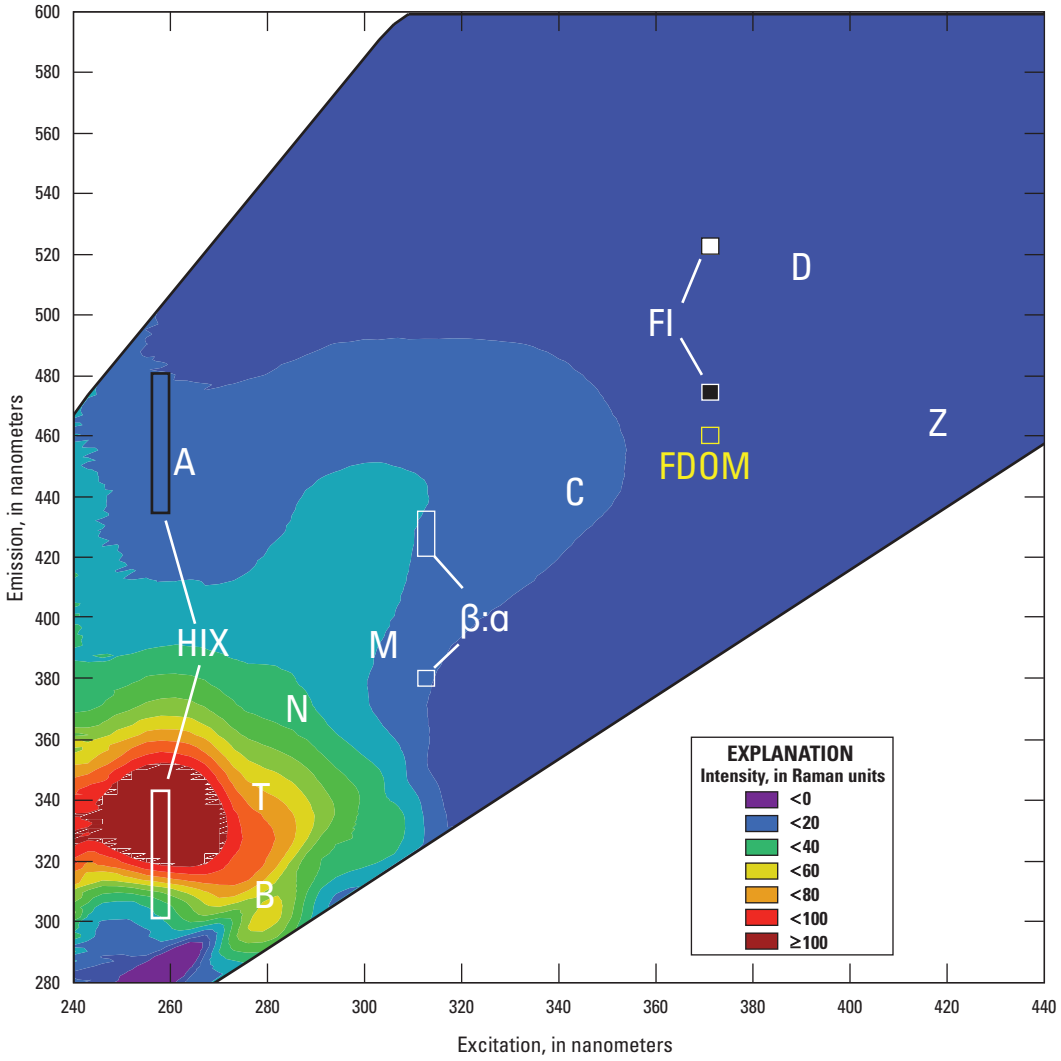


Figure 6. Excitation-emission matrix (EEM) of the standard reference material (Pure Leaf®, unsweetened black tea, Purchase, New York) SRM_{Tea} used in the Organic Matter Research Laboratory. The EEM shows the long-term average for SRM_{Tea}, which includes 189 scans from 22 lot numbers over a 12-month period. (Labeled regions of the EEM correspond to named peaks or table 1 optical properties; FDOM, fluorescent dissolved organic matter peak.)

Although only a small amount of an 8 ounce (oz) bottle of SRM_{Tea} is needed to prepare a standard, each bottle is used only for a maximum of 1 month after opening, with at least 1 week of overlap between old and new bottles. The OMRL lab staff analyzes two different bottles in each analytical run to verify internal consistency before using them as an SRM. Each bottle receives a unique laboratory identifier, and the lot number is recorded for long-term tracking purposes. Although full EEMs are collected for evaluation of performance, internal lab evaluation is tracked for 10 absorbance wavelengths and 8 widely published diagnostic fluorescence ex-em pairs. Acceptance is then based on recovery percentage (plus or minus 20 percent) of the long-term average of the SRM record.

Laboratory Blank Samples

Laboratory blank samples, or blanks, can reveal background levels and possible contamination of the equipment used during the analytical procedure (for example, cuvette blemishes, improper cuvette rinsing, LRW system quality control). A minimum of 5 laboratory blanks are measured in a typical analytical run; 3 blanks are run prior to running samples, and 2 blanks are run following each set of approximately 10 samples (table 5). Analyzing multiple baseline-corrected laboratory blanks daily allows the analyst to track blank-water quality and instrument drift during the course of the day. Individual blank results are acceptable when absorbance and fluorescence are less than one-tenth of the long-term method detection limit (LT-MDL). Calculating the standard deviation of all blanks in a single analytical run confirms the daily detection limit (DDL) is below the LT-MDL. Although the DDL is not a true detection limit, it serves as a diagnostic tool for daily contamination bias.

Laboratory Sample Replicates

Analysis of laboratory sample replicates (aliquots of the same sample) are a demonstration of precision as measured by the relative percent difference (RPD) between the measurements of the original sample and the replicate sample, calculated as 100 times the absolute difference between the two measurements divided by their average. Laboratory replicates not only check instrument precision, but also reflect the homogeneity of the sample and the precision of the analyst. A minimum of 1 laboratory replicate is measured in a typical analytical run (table 5), where 1 laboratory replicate is measured approximately every 10 samples. Laboratory replicates are considered acceptable when the RPD is less than 5 percent.

Table 5. The scan order for a typical analysis run includes the water Raman, baseline, and analytical set.

[ba, baseline; bl, blank; MMDDYY, month day year; OMRL, Organic Matter Research Laboratory; SRM, standard reference material; —, not applicable]

Scan order	Sample type	File-naming convention	Comments
1	Water Raman	wrMMDDYY	—
2	Baseline	baMMDDYY	—
3	Lab blank	blMMDDYYa	—
4	Lab blank	blMMDDYYb	—
5	Lab blank	blMMDDYYc	—
6	Sample	OMRL 1	Sample names should be unique to the analyzing lab.
7	Sample	OMRL 2	—
8	Sample	OMRL 3	—
9	Sample	OMRL 4	—
10	Sample	OMRL 5	—
11	Sample	OMRL 6	—
12	Sample	OMRL 7	—
13	Sample	OMRL 8	—
14	Sample	OMRL 9	—
15	Sample	OMRL 10	—
16	SRM_{Tea}	SRMs01	Tea at 1-percent concentration. Analyze two SRMs once per day.
17	SRM_{Tea}	SRMs01	Tea at 1-percent concentration. Analyze two SRMs once per day.
18	Lab replicate	OMRL 1d	Sample suffix “d” indicates lab replicate.
19	Lab blank	blMMDDYYd	—
20	Lab blank	blMMDDYYe	A minimum of five blanks should be run per day.
Continue analyzing samples, repeating above sequence from rows 6–20 (do not repeat the SRMs). Always complete the run with a replicate followed by two blanks to ensure quality assurance.			

Analysis Procedure

General operational procedures and instrumental parameters essential to the proper execution of the method for analyzing absorbance and fluorescence on the Aqualog® are described here. A detailed description of the CAWSC OMRL standard operating procedure (SOP) is provided in appendix 1.

Initial preparations for sample analysis include warming up the instrument for 1 hour prior to the first scan. Lamp hours are recorded when the instrument is switched on because the lamp degrades over time, and the manufacturer recommends the lamp be replaced either at 1000 hours of use or after 1 year of installation. The OMRL staff replaces the lamp annually regardless of hours of lamp use. Always wear gloves when handling cuvettes and samples for the Aqualog®. All blanks, dilutions, and standards should be prepared using Type I LRW.

A typical analytical run includes three unique experiments: (1) Raman, (2) baseline, and (3) analytical set. The purpose of these experiments, the reagents used, and the Aqualog® experiment parameters used for each are described in table 6. Aqualog® software enables the analyst to create experiment files that contain specific information for the instrument parameters, including the experiment set-up and acquisition type (Horiba Scientific, 2011, page 13-4). Software setup should include creating these files for each of the three unique experiments (that is, water Raman, baseline, and analytical set) to improve run-time efficiency and to avoid inconsistent sample analysis. Further details regarding the Raman, baseline, and analytical set are given in the following paragraphs.

1. Raman scan. It should be emphasized that long-term stability of the instrument is one of the chief concerns of the manufacturer and the user, and one of the best known methods to achieve this is by performing the daily Raman scan using LRW. Routine examination of the water Raman spectrum serves as an early indicator of the instrument's integrity. The Raman peak area is used to normalize fluorescence signals, thus producing data in units that can be compared across instruments. This scan should be performed at the start of each analytical run using LRW to verify that the peak location of the water Raman is 397 plus or minus 1 nm (fig. 7). The Raman area is calculated using the baseline-corrected peak boundary definition (Murphy and others, 2011) outside of the Aqualog® user interface and is determined after collecting a baseline scan (see experiment 2) using a processing routine developed by the OMRL staff. In the OMRL, the Raman area is expected to be within 75 percent of the value obtained after the last lamp change. If the Raman peak location and area do not meet acceptance criteria, allow the instrument to warm up for another 30 minutes and repeat the scan. If acceptance criteria continue not to be met, examine common sources of interference or contamination (for example, cuvette blemishes, LRW contamination, lamp alignment) before contacting the manufacturer for technical support.

Table 6. Aqualog® experiments used in a typical analytical run, along with method, parameters, and reagents.

[CCD, charge-coupled device; Em, emission; Ex, excitation; LRW, lab reagent water; NY, New York; SRM, standard reference material; 3D, three-dimensional; =, equal to]

Scan type	Aqualog® method	Experiment parameters	Purpose	Reagent
Raman	Spectra. Two-dimensional emission spectra	Integration time=10 Accumulation=1 Ex=350 Em=1.64 (should be equivalent to 3D scan) CCD gain=medium Sample only Cuvette position=1	Used to normalize baseline, blanks and samples to the daily LRW.	Type 1 LRW
Baseline	3D. Three-dimensional emission spectra plus absorbance.	Integration time=1 Ex=600-240-3 Em=1.64 CCD gain=medium Blank/sample setup=blank only Cuvette position=1	Establishes a daily baseline for the instrument and is used to baseline correct scans (blanks, samples, and standards).	Type 1 LRW
Analytical set	3D. Three-dimensional emission spectra plus absorbance.	Integration time=1 Ex=600-240-3 Em=1.64 CCD gain=medium Blank/sample setup=blank from file Cuvette position=1	The analytical set includes samples, verification samples (SRM_{Tea} or knowns), sample duplicates, and laboratory blanks.	Blank reagent is organic-free Type 1 LRW. Verification sample (SRM_{Tea}) reagent is Pure Leaf®, unsweetened black tea, Purchase, N.Y.

2. Baseline scan. The baseline scan must be collected prior to the analysis of samples to obtain the signal associated with the instrument, cuvette, and LRW in the absence of fluorescent DOM. The daily baseline scan is subtracted from the analytical set collected afterward (see next item). The baseline scan must be quality checked to determine whether it meets laboratory objectives. Quality check the baseline by confirming the response is less than the LT-MDL in the regions of interest. The OMRL staff has developed a processing routine that Raman-normalizes the baseline scan, calculates water Raman area and peak location, and generates three graphs for visual determination of baseline quality (fig. 8). The first graph shows the current baseline scan, the second graph shows the average of the long-term baseline subtracted from the daily baseline, and the third graph shows the difference between the standard deviation of the long-term average baseline and the daily baseline. If the baseline does not pass quality-assurance standards (for example, it exceeds the LT-MDL), allow the instrument to warm up for another 30 minutes and repeat along with the water Raman scan. If acceptance criteria continue not to be met, examine common sources of interference or contamination (for example, cuvette blemishes, LRW contamination) before contacting the manufacturer for technical support.

3. Analytical set. Only after the water Raman and baseline scans have met laboratory-defined objectives should the analytical set begin. An analytical set includes samples, verification samples (that is, SRM_{Tea} or knowns), laboratory replicates, and laboratory blanks. A typical analytical set scan order is presented in table 5.

Examination of QA/QC in the analytical set should be done to determine sample reruns and dilution requirements. Fluorescence measurements of highly concentrated samples are subject to measurement errors like detector saturation and inner-filtering effects (IFE). Previous studies indicate the IFE is linear and correctable for most natural samples when A_{254} is between 0.03–0.3 absorbance units (AU) when measured in a 1 centimeter (cm) cuvette (for example, Ohno, 2002; Lakowicz, 2006, Miller and others, 2010). Most undiluted natural samples from riverine, lake, and estuarine systems far exceed that level, however, as do samples associated with high concentrations of DOM inputs, such as those of wastewater effluent or with oil and gas contamination. To standardize the correction procedure, all samples initially are analyzed at full concentration, and the A_{255} value is noted. If the A_{255} exceeds 0.3 AU, the sample is diluted with LRW to a concentration at which the A_{255} is in the range of 0.03 to 0.3 AU. Note that use of A_{255} reflects the output of the Aqualog® method for simultaneous collection of fluorescence and absorbance data, where absorbance data are collected in 3-nm increments from 240 to 600 nm.

It should be emphasized that this value of 0.3 AU at A_{255} was developed for samples containing a "typical" array of DOM compounds (fig. 6); however, samples that have particularly high absorbance and fluorescence in other regions of EEM space may require further dilution to avoid interferences due to IFE or instrument detector saturation in those regions. For example, a sample containing very high fluorescence at low-UV wavelengths could require additional dilution so that the absorbance and fluorescence response falls within a linear range of the instrument across the entire EEMs space.

Data Reporting and Limits

Given there is not a CRM for optical measurements from which to calculate a minimum detection limit (MDL) across the absorbance spectrum or EEM space, the OMRL staff has developed reporting limits by modifying the approach recommended by Childress and others (1999) and the U.S. Environmental Protection Agency (2000). The LT-MDL is reported as three times the standard deviation plus the average of baseline-corrected blanks collected over a 2-year period. Three times the standard deviation is a commonly used estimate of the measured response corresponding to the critical Student's t value at a significance level of 99 percent, as referenced in Childress and others (1999).

Absorbance LT-MDL was calculated across the spectrum (240–600 nm) from 1200 baseline-corrected instrument blanks analyzed over a 2-year period (January 2013–December 2014). Absorbance is reported in absorbance units (AU) obtained directly from the instrument. Absorbance LT-MDLs vary by wavelength, ranging from 0.01 AU at A_{240} to 0.004 AU at A_{600} (fig. 9).

Fluorescence LT-MDL was calculated at each ex-em pair determined from 1200 baseline-corrected, water Raman-normalized blanks collected during the same 2-year period as absorbance (January 2013–December 2014). Fluorescence data are expressed in Raman-normalized intensity units (RU; Murphy and others, 2010). Fluorescence LT-MDLs vary by excitation-emission pairs, ranging from 0.004 RU throughout much of the EEM spectra to 0.1 RU in the region of peak B (ex275, em304; fig. 10).

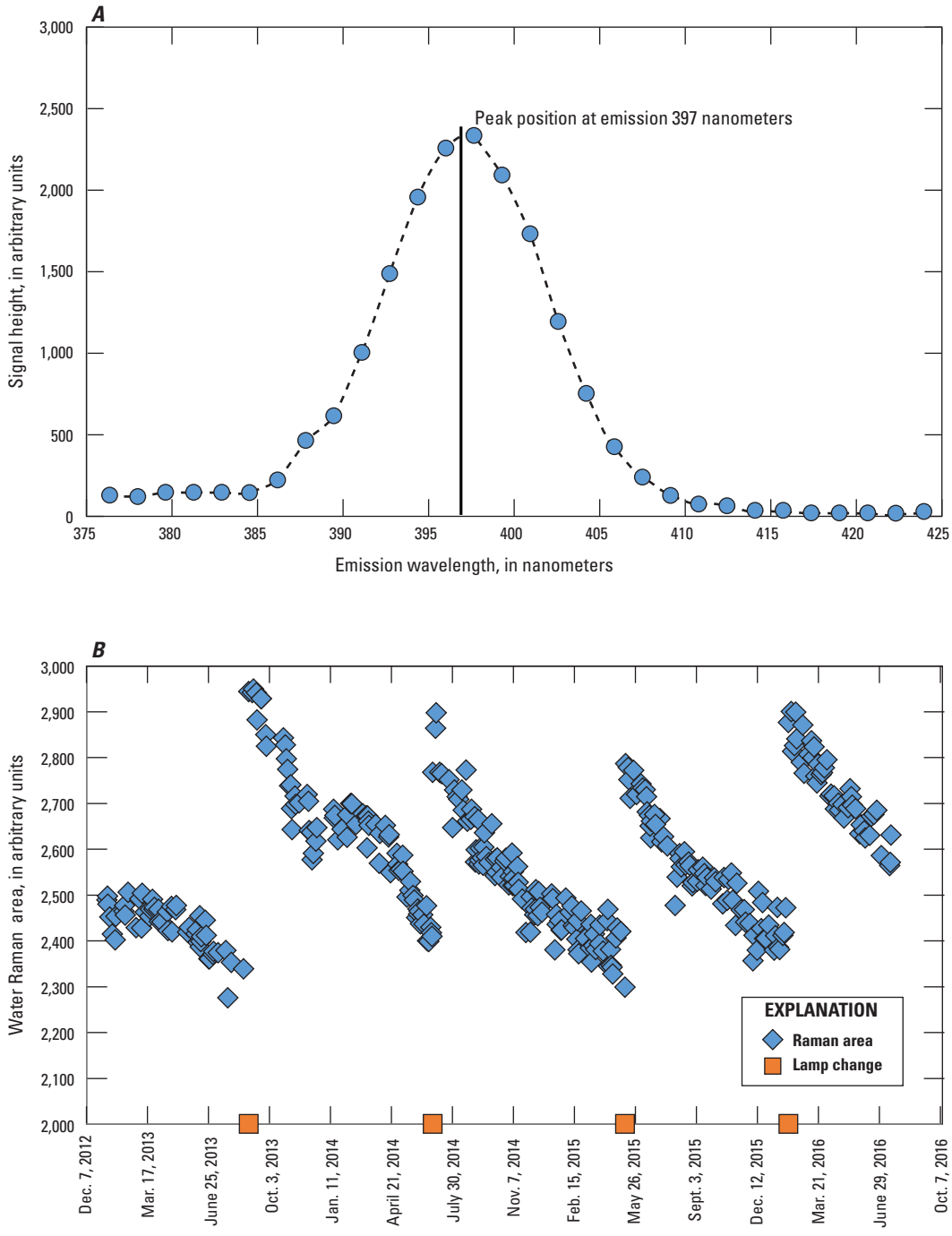
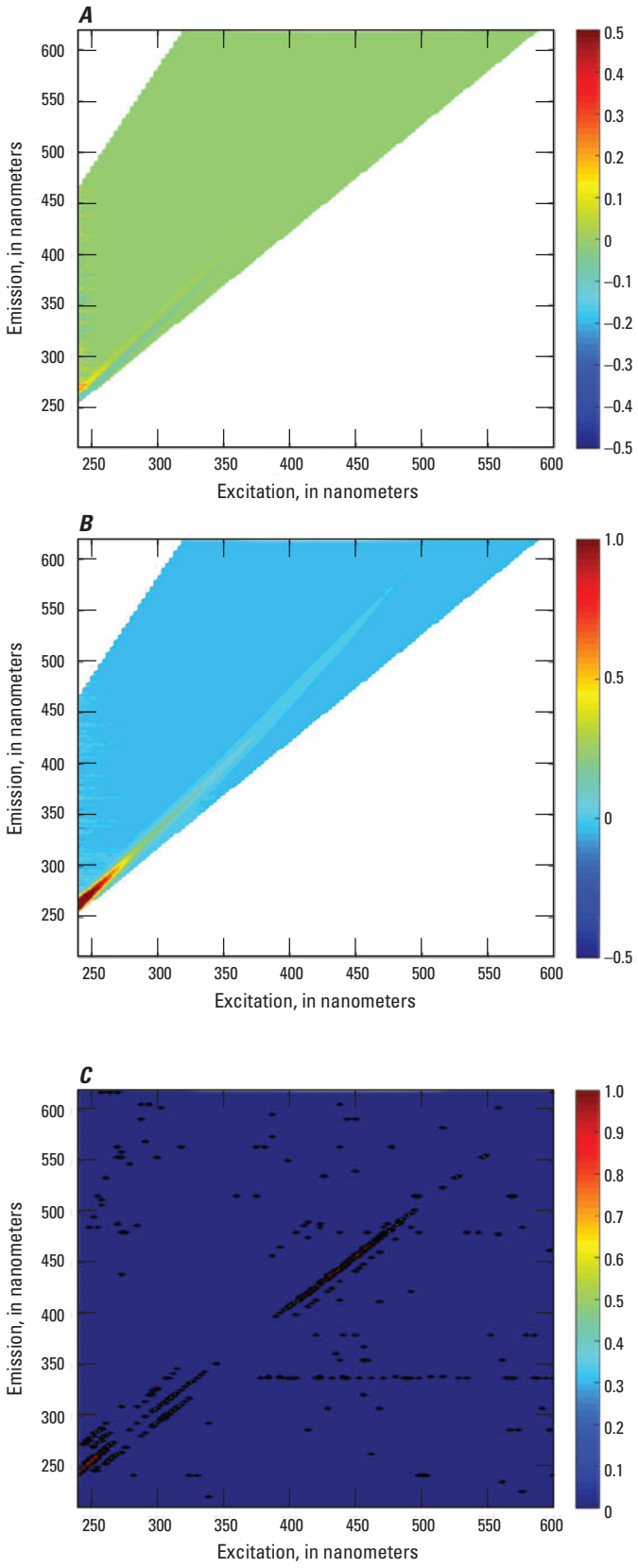


Figure 7. Aqualog® water Raman criteria: *A*, peak position should be 397 nanometers plus or minus 1 nanometer; and *B*, the area should be at least 75 percent of the value obtained after the last lamp change.



Acceptance of Data

Optical properties are measured within quality-control thresholds, as indicated by laboratory standards, which for the OMRL include monthly measurements of potassium dichromate and quinine sulfate, daily measurement of a standard reference material (Pure Leaf®, unsweetened black tea, Purchase, New York), and laboratory replicates measured approximately every 10 samples. Results for analytical sets are considered acceptable if all standards are within 20 percent of the target value, laboratory replicates are within 5 percent RPD, and laboratory blanks (that is, LRW) are less than one-tenth of the LT-MDL. Analytical runs are evaluated daily by the analyst to ensure all data-quality indicators meet laboratory objectives and are reviewed by the quality-assurance manager. If standards, replicates, and blanks are not within acceptable thresholds, all analyses in that set must be repeated. Because the recommended sample hold time for optical analysis is 2 days following sample collection, sample reruns should be done as soon as possible.

Figure 8. Visual inspection of the daily baseline excitation-emission matrix (EEM): *A*, the daily baseline; *B*, the average of the long-term average baseline subtracted from the daily baseline; and *C*, the standard deviation of the long-term average baseline subtracted from the daily baseline. The EEM plots should be free of a response in area of interest.

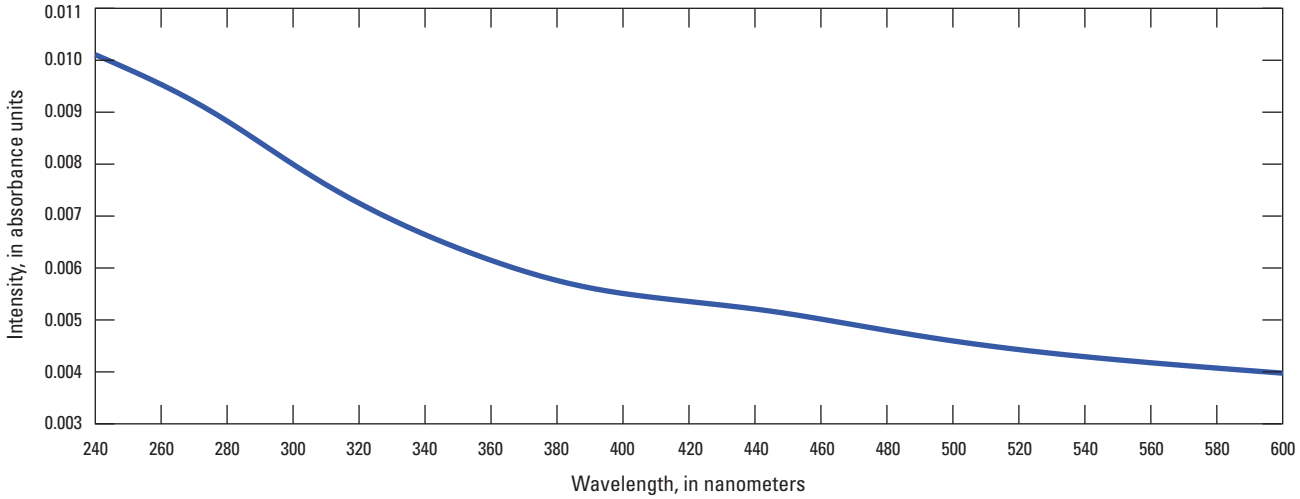


Figure 9. Absorbance spectra measured between 240 and 600 nanometers indicating the long-term method detection limit calculated using 1200 baseline-corrected blanks analyzed during January 2013–December 2014.

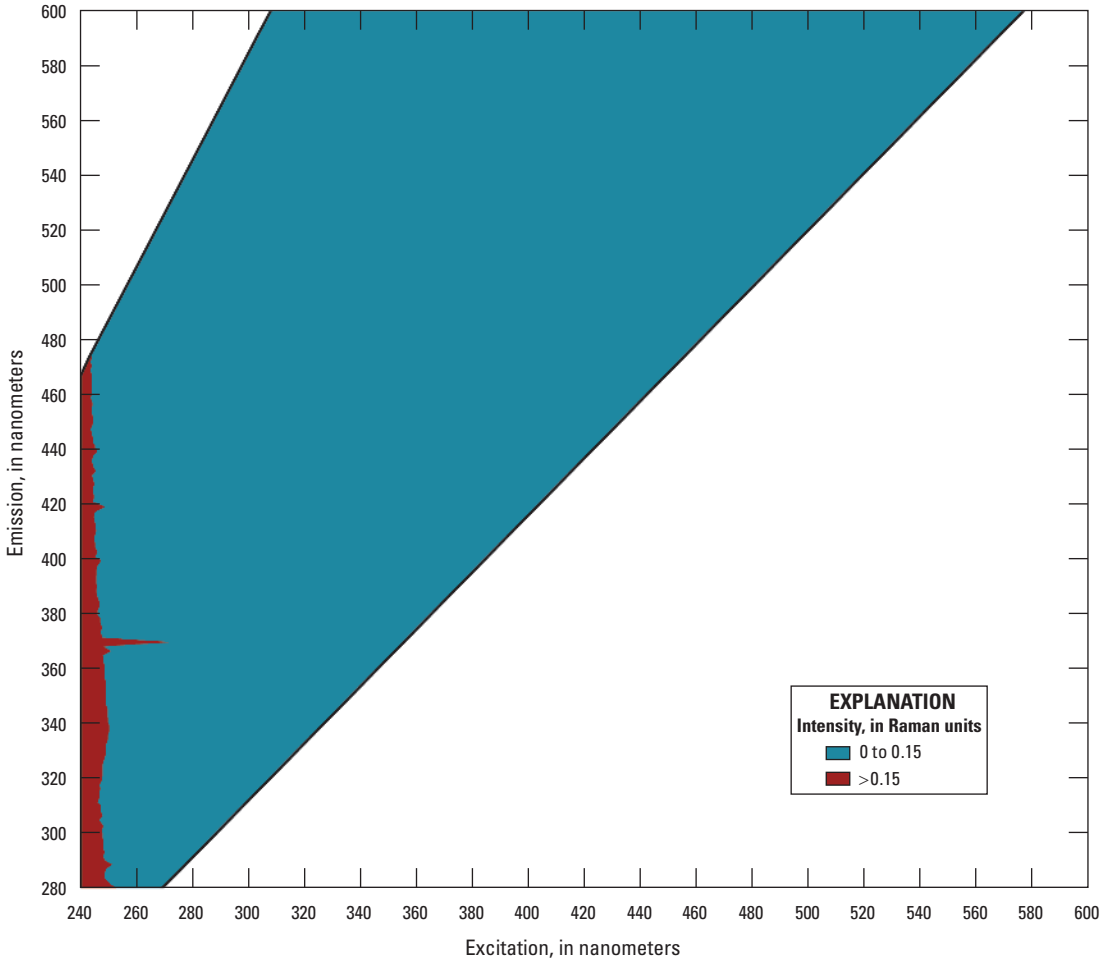


Figure 10. Fluorescence excitation-emission matrix indicating the long-term method detection limit calculated using 1200 baseline-corrected blanks analyzed January 2013–December 2014.

Data Processing and Corrections

Data processing is performed using processing routines developed by the OMRL staff. These processing routines are laboratory specific because they are dependent on the sample- and analytical batch-naming conventions and data-storage structure established by the OMRL staff, as well as on the instrument setup and lab-defined QA/QC measurements. A description of the processing and correction of absorbance and fluorescence measurements is given here.

Data organization is paramount for effective processing, retrieval, compilation, and analyses; thus, establishing a consistent file-storage system and standardized sample-naming convention is highly recommended. For reference, an example showing the OMRL folders and files created from one analytical run date (May 18, 2016) is shown in table 7.

All data produced by the Aqualog® using the analysis procedure in this report are instrument- and baseline-corrected. The instrument correction factors account for wavelength-dependent components of the system, like the xenon lamp, gratings, and signal detectors. A set of instrument correction factors—instrument specific and provided by the manufacturer—for excitation (that is, xcorrect.spc) and emission (that is, mcorrect.spc) are automatically applied by the software to each scan (Horiba Scientific, 2011, page 13-2). Baseline correction is applied by the software during analysis, as described previously.

All other data corrections are completed in MATLAB v8.5 R2015a. Performing remaining data treatment outside of the Aqualog® software environment ensures standardization of data and better comparability among datasets and across instruments.

Upon completion of an analytical run, baseline-corrected and instrument-corrected data are exported to the desired storage location using the Aqualog® HJY batch export feature (appendix 1). A MATLAB data processing script developed by OMRL staff then is used to perform corrections (for example, water-Raman normalization, correct for IFE), calculations (for example, multiply diluted samples to the appropriate concentration, calculate designated slopes and indices), data manipulation (for example, Rayleigh trims, vectorize fluorescence data), compile summary data (for example, extract a subset of widely published absorbance wavelengths, fluorescence ex-em pairs, and indices), evaluate QA data, assign data qualifiers (for example, processed summary reports for absorbance and fluorescence; appendixes 4 and 5, respectively), and manage data structure (for example, add samples to MATLAB structure array where related data types are stored in fields). The OMRL structure array stores complete absorbance spectra and full excitation-emission matrices for all samples analyzed in the laboratory, including all QA/QC samples (for example, daily laboratory blanks, replicates, and SRM_{Tea} and monthly Starna potassium dichromate and reference-cell scans). Storage in this type of array allows for easy data retrieval for modeling and reporting.

Data Storage

The Aqualog® collects high-resolution scans that include thousands of individual datum points for each sample; thus, data storage is not trivial. The OMRL staff archives the raw-data files and processed-data files on an internal network server. These files are available upon request and include the Aqualog® software project file; original exported data files including baseline, water Raman, sample and blank scans; and processed files containing full-absorbance scans, fluorescence EEMs, and laboratory QA data.

Only a subset of these data from selected projects and samples for which sites have been established are available to the public through the USGS National Water Information System (NWIS); these are limited to the commonly published diagnostic parameters and indices listed in table 8. The diagnostic wavelengths and EEMs pairs are transferred to the OMRL internal database when all QA/QC have been evaluated and accepted.

Table 7. The Organic Matter Research Laboratory data-storage structure for example analysis date of May 18, 2016.

[Files are in alpha-numeric order. Sample suffix "s" followed by numbers denotes diluted sample percent concentration. Sample suffix "d" indicates a laboratory replicate scan. File extension .csv indicates a comma-separated file and .dat indicates a generic data file created upon export by Aqualog software. **Abbreviation**: DOM, dissolved organic matter]

Aqualog - DOM
– Aqua DOM 2016
– q2016-05-18
– Exported Aqualog Files
– qa2016-05-18
– bl051816a.csv
bl051816b.csv
bl051816c.csv
bl051816d.csv
bl051816e.csv
gr20329.csv
gr20329d.csv
gr20330.csv
gr20331.csv
gr20332.csv
gr20332s50.csv
gr20334.csv
gr20335.csv
gr20335s50.csv
gr20336.csv
gr20336s10.csv
gr20337.csv
gr20338.csv
gr20339.csv
grSRMHTs01.csv
grSRMHUs01.csv

Aqualog - DOM
– Aqua DOM 2016—Continued
– q2016-05-18—Continued
– Exported Aqualog Files—Continued
– qf2016-05-18
bl051816a.dat
bl051816b.dat
bl051816c.dat
bl051816d.dat
bl051816e.dat
gr20329.dat
gr20329d.dat
gr20330.dat
gr20331.dat
gr20332.dat
gr20332s50.dat
gr20334.dat
gr20335.dat
gr20335s50.dat
gr20336.dat
gr20336s10.dat
gr20337.dat
gr20338.dat
gr20339.dat
grSRMHTs01.dat
grSRMHUs01.dat
wr051816.dat

Table 8. National Water Information System (NWIS) codes for commonly published diagnostic parameters and indices.

[cm, centimeter; em, emission; ex, excitation; FDOM, fluorescent dissolved organic matter; nm, nanometer; RU, Raman units; UV, ultraviolet; wf, filtered water]

Method tool	Analyte	Result name	Parameter definition	Method code	Parameter code
Horiba Scientific Aqualog®	Absorbance	A254	Absorbance, 254 nm, water, filtered, absorbance units per centimeter.	UV008	50624
Horiba Scientific Aqualog®	Absorbance	$SUVA_{254}$	Specific UV absorbance, 254 nm, water, filtered, 1-cm path length, calculated, liter per milligram of dissolved organic carbon per meter.	UV003	63162
Horiba Scientific Aqualog®	Absorbance	A280	Absorbance at 280 nm, water, filtered, absorbance units per centimeter.	ABS01	32296
Horiba Scientific Aqualog®	Absorbance	A370	Absorbance at 370 nm, water, filtered, absorbance units per centimeter.	ABS01	32297
Horiba Scientific Aqualog®	Absorbance	A412	Absorbance at 412 nm, water, filtered, absorbance units per centimeter.	ABS01	32298
Horiba Scientific Aqualog®	Absorbance	A440	Absorbance at 440 nm, water, filtered, absorbance units per centimeter.	ABS01	32299
Horiba Scientific Aqualog®	Absorbance	$S_{275–295}$	Absorption spectral slope, wavelengths 275–295 nm, unitless.	ABS02	32300
Horiba Scientific Aqualog®	Absorbance	$S_{290–350}$	Absorption spectral slope, wavelengths 290–350 nm, unitless.	ABS02	32301
Horiba Scientific Aqualog®	Absorbance	$S_{350–400}$	Absorption spectral slope, wavelengths 350–400 nm, unitless.	ABS02	32302
Horiba Scientific Aqualog®	Absorbance	$S_{412–600}$	Absorption spectral slope, wavelengths 412–600 nm, unitless.	ABS02	32331
Horiba Scientific Aqualog®	Fluorescence	Peak A	Fluorescence, wf, ex260 em450 RU.	FL020	32304
Horiba Scientific Aqualog®	Fluorescence	Peak B	Fluorescence, wf, ex275 em304 RU.	FL020	32305
Horiba Scientific Aqualog®	Fluorescence	Peak C	Fluorescence, wf, ex340 em440 RU.	FL020	52901
Horiba Scientific Aqualog®	Fluorescence	Peak D	Fluorescence, wf, ex390 em510 RU.	FL020	32307
Horiba Scientific Aqualog®	Fluorescence	FDOM	Fluorescence, wf, ex370 em460 RU.	FL020	52902
Horiba Scientific Aqualog®	Fluorescence	Peak M	Fluorescence, wf, ex300 em390 RU.	FL020	32309
Horiba Scientific Aqualog®	Fluorescence	Peak N	Fluorescence, wf, ex280 em370 RU.	FL020	32310
Horiba Scientific Aqualog®	Fluorescence	Peak T	Fluorescence, wf, ex275 em340 RU.	FL020	32311
Horiba Scientific Aqualog®	Fluorescence	FI	Fluorescence index—ratio of emission intensities that characterizes the slope of the emission curve at an excitation of 370 nm.	FL020	32312
Horiba Scientific Aqualog®	Fluorescence	HIX	Humification index—calculated by dividing the fluorescence intensity in the 435–480 nm region by the total intensities in the 300–345 and 435–480 nm regions.	FL020	32313

Data Analysis

The approach to optical data analysis depends on the objective of the study and the scale of measurement (that is, quantitative or qualitative). As mentioned before and described in table 1, discrete diagnostic wavelengths and indices can be extracted from the absorbance and fluorescence scans. In addition, multi-parameter techniques can be used that incorporate all the data (that is, complete absorbance spectra and fluorescence matrices). A brief overview of some of the ways optical measurements of DOM have been used, with a focus on approaches used by the OMRL staff, is provided here. For additional information, the reader is referred to an extensive body of literature on this topic (Coble, 1996; McKnight and others, 2001; Stedmon and others, 2003; Fellman and others, 2010; Coble and others, 2014).

The measurement of ultraviolet absorbance has been used by the drinking-water industry for decades and has been shown to be a useful proxy for DOC concentration (that is, A_{254}; Edzwald and others, 1985; Rathbun, 1996; Korshin and others, 1997; Sadiq and Rodriguez, 2004) and disinfection byproduct (DBP) precursor concentrations (that is, A_{272}; Korshin and others, 2002; Li and others, 2002; Roccaro and others, 2008). In addition to concentration, absorbance data can provide insight into the chemical composition of the DOM pool. Carbon normalization of the intensity of the response at a given absorbance wavelength (that is, $SUVA_{254}$ calculated as A_{254} value divided by DOC concentration, reported in L/mg C/m) has been shown to be correlated with the hydrophobic organic acid fraction of DOM (Spencer and others, 2012) and is a useful proxy for DOM aromatic content (Weishaar and others, 2003) and molecular weight (Chowdhury, 2013). Similarly, spectral slopes and slope ratios have been related to the relative molecular weight and aromaticity of DOM (Chin and others, 1994; Helms and others, 2008); lower values are generally indicative of higher molecular weight DOM, which can change upon photolytic exposure (Helms and others, 2008; Spencer and others, 2009; Hansen and others, 2016).

Fluorescence intensity at a given ex-em pair also can be related to DOC concentration, DBP precursor concentrations (Kraus and others, 2010; Beggs and Summers, 2011; Carpenter and others, 2013), biological oxygen demand (Baker and Curry, 2004; Hudson and others, 2008), mercury concentrations (Fleck and others, 2014), and wastewater content (Goldman and others, 2012). Like absorbance data, fluorescence data also are widely used to gain insight into DOM composition, source, and reactivity. Information about DOM composition can be inferred from the presence or absence of fluorophores, shifts in peak maxima, ratios of peaks, and calculated indices, as well as by normalizing each ex-em pair by DOC concentration (Beggs and Summers, 2011). Commonly reported DOM compositional indicators include the fluorescence index, humification index, freshness index, and biological index (table 1).

Although these diagnostic wavelengths and indices can be very informative, they often miss information that can be gleaned by using the full absorbance and fluorescence dataset generated by instruments like the Aqualog®. Other approaches to data interpretation involve the use of multivariate and multi-way modeling techniques (Stedmon and others, 2003; Fellman and others, 2008). In the following paragraphs, we discuss three commonly used approaches—principal component analysis (PCA), discriminant analysis (DA), and parallel factor analysis (PARAFAC)—and then provide more detailed information about tools the OMRL staff have developed to run PARAFAC.

Principal component analysis (PCA) is one of the most frequently used multivariate techniques to explore optical data (for example, Stepanauskas and others, 2003; Baker and others, 2008; Jaffé and others, 2008; Kraus and others, 2008; Dalzell and others, 2009; Miller and McKnight, 2010; Goldman and others, 2012, 2014). The primary aim of PCA is dimension reduction, in which uncorrelated linear combinations called principal components are identified. The first component explains the most variability in a dataset, the second component explains the second-most, and so on. Model output displays scores (clustering and/or separation of objects and their relationship to the principal components) and loadings (the original loadings projected onto the principal axes; fig. 11).

Discriminant analysis (DA) is a predictive method that classifies a sample to a user-designated group on the basis of known responses. Although DA is performed more rarely than PCA, it has potential to improve our understanding of DOM biogeochemical processes because of its ability to analyze complex spatial relationships, and it allows for the identification of the most important discriminating variables that lead to proper assignment of a sample to a designated group (for example, Spencer and others, 2007; Hansen and others, 2016; fig. 12).

Parallel factor analysis (PARAFAC), a multi-way statistical approach for detecting underlying structures in complex datasets like fluorescence EEMs, has proven to be a powerful tool for studying DOM (Bro, 1997) and has been widely applied in combination with other measurements to rapidly quantify and characterize DOM across a range of environments (for example, Stedmon and Markager, 2005; Fellman and others, 2008; Kowalczuk and others, 2009). The aim of PARAFAC is to distill fluorescence signatures into distinct fluorescence components (fluorophores) that are not restricted to a single excitation-emission pair.

Unlike PCA or DA, which are common statistical methods available in many software packages (for instance, OMRL uses SAS JMP v.12.2) PARAFAC requires the use of MATLAB, or a similar high-performance programming language (for example, *Python or R*), that allows users to perform matrix calculations, develop and run algorithms, and create data visualization products and user interfaces.

In order to run PARAFAC in MATLAB, the data structure must be arranged in a specific format according to the procedures in Stedmon and Bro (2008).

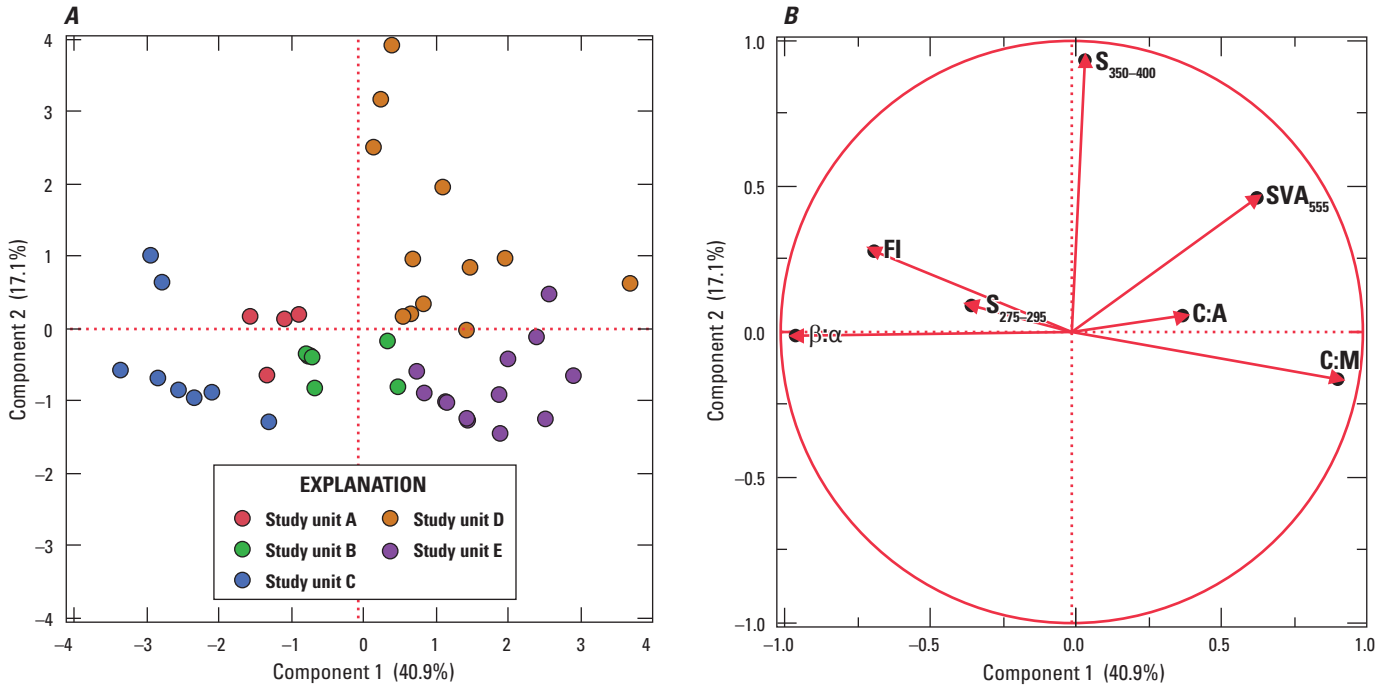


Figure 11. Example of model output from principal component analysis: *A*, the score plot displays clustering or separation of individual samples and their relationship to the principal components; and *B*, the loadings plot graphs the original loadings projected onto the principal axes. (Original loadings correspond to selected optical measurements, labels abbreviated as in table 1.)

PARAFAC model validation is determined by examining a variety of methods in combination (for example, analysis of spectral properties, split-half analysis, residual analysis, and random initialization). Although there is no quantitative measure of "best fit" (Murphy and others, 2013), subjectivity in determining the final modeled components can be minimized by evaluating each dataset carefully using the diverse validation methods explained by Stedmon and Bro (2008).

When validated, component loadings are interpreted to represent individual fluorophores and can be examined by the absolute value (that is, component loadings), which is a reflection of the intensity of fluorescence, and by the relative contribution to total fluorescence (that is, component percentage loadings), which is a reflection of composition (fig. 13). Model component loadings can be used for further analysis of the individual observations like "new parameters" in other multivariate approaches discussed previously, such as PCA (for example, Kraus and others, 2008; Miller and McKnight 2010; Fleck and others, 2014) or DA (Spencer and others, 2007; Hansen and others, 2016), to gain insight into DOM composition.

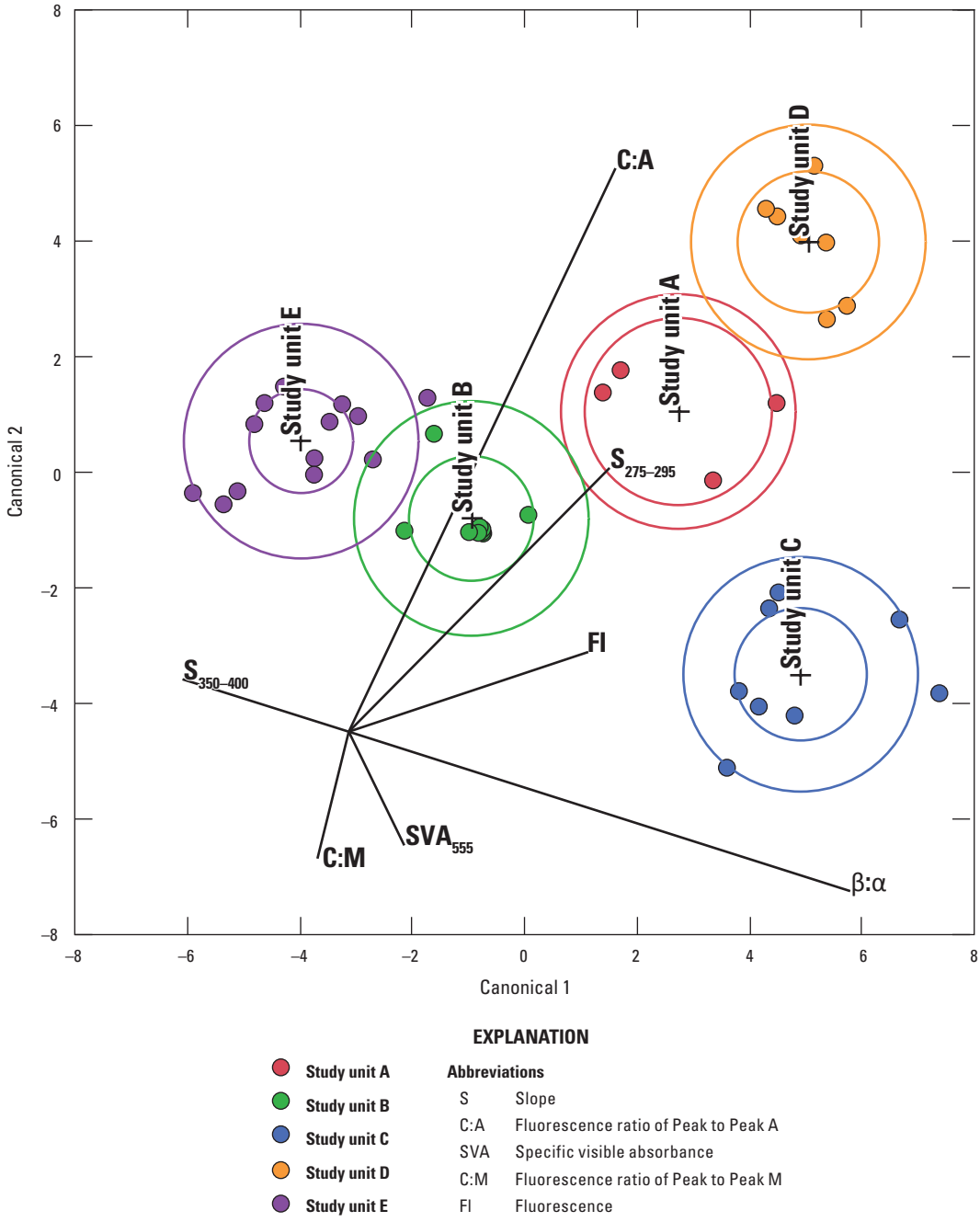


Figure 12. Example of model output from discriminant analysis. The canonical plot shows the two dimensions (canonicals 1 and 2) that provide the best separation among user-defined groups. Points are individual samples; inner ellipses represent the 95-percent confidence interval for group means and outer ellipses represent the region estimated to contain 50 percent of the population of each group; and the biplot ray represents the covariates and the degree of association of the covariates with the two canonical variables.

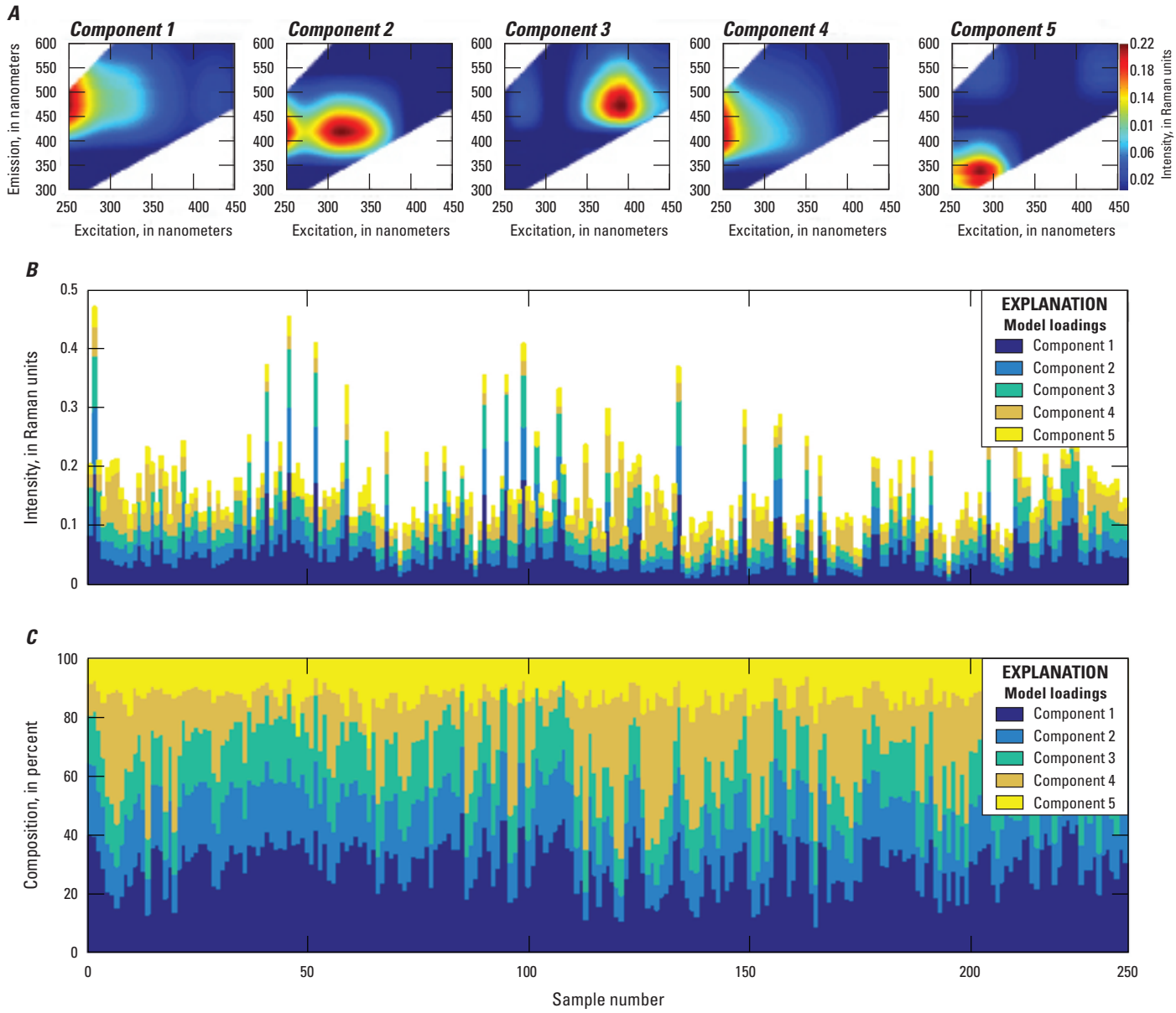


Figure 13. Example of model output from parallel factor analysis (PARAFAC): *A*, component loadings are interpreted to represent individual fluorophores and can be examined; *B*, by absolute value, which is a reflection of the intensity of fluorescence; and *C*, by the relative contribution to total fluorescence, which is a reflection of composition.

Summary

The purpose of this document is to provide a standardized method for the collection, correction, and interpretation of optical data produced by the Aqualog®. Optical measurements such as absorbance and fluorescence are easy to take; however, they can be affected by many variables rendering them less comparable, including such things as inconsistencies in sample collection (for example, filter pore size, preservation), the application of corrections for interferences (for example, inner-filtering corrections), differences in holding times, and instrument drift (for example, lamp intensity). A documented, standardized procedure to address these variables ensures that the optical (absorbance and fluorescence) measurements collected by U.S. Geological Survey (USGS) researchers are useful and widely comparable.

Rigorous and quantifiable quality assurance and quality control are essential for making these data comparable, particularly because there is no published guideline for the measurement of dissolved organic matter (DOM) absorbance and fluorescence, and especially because there is no National Institute of Standards and Technology standard for DOM. Validation and quality-control samples are analyzed on a monthly basis to determine laboratory and instrument precision and daily (that is, each day samples are run) to ensure repeatability. Data are not considered acceptable unless they meet laboratory criteria: All standards should be within 20 percent of the target value, laboratory replicates should be within 5 percent relative percent difference, and laboratory blanks (that is, laboratory reagent-grade water) should be less than one-tenth of the long-term method detection limit.

Finally, for data to be useful, they must be accessible to users in a format that can be easily analyzed and interpreted. The Organic Matter Research Laboratory staff has developed a processing routine that extracts a subset of the data, which is made available to the public through the USGS National Water Quality Information System (http://nwis.waterdata.usgs.gov/usa/nwis/qwdata), and organizes the full datasets (that is, complete absorbance spectra and fluorescence excitation-emission matrices) in different forms that allow for these data to be analyzed using multi-parameter and multi-way statistical approaches.

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Appendix 1. Aqualog® Standard Operating Procedure Walkthrough

The Aqualog® Standard Operating Procedure (SOP) Walkthrough provides detailed operational procedures and instrumental parameters essential to the proper execution of the method for using the Horiba Scientific Aqualog® fluorometer to measure absorbance and fluorescence from dissolved organic matter. Click here to view.

Appendix 2. Processed Summary Report for Absorbance Data

A processed summary report for absorbance data is created by a MATLAB processing routine developed by the Organic Matter Research Laboratory staff. This summary report is intended to provide users of the Aqualog® fluorometer with a standardized approach by which to evaluate and validate data produced by the Aqualog® fluorometer. The processed summary report for absorbance data includes calculations (for example, multiply diluted samples to appropriate concentration, calculate designated slopes and indices), data manipulation (for example, compile summary data, extract a subset of widely published absorbance wavelengths and indices), evaluation of quality-assurance data, and assignment of data qualifiers. Click here to view.

Appendix 3. Processed Summary Report for Fluorescence Data

A processed summary report for fluorescence data is created by a MATLAB processing routine developed by the Organic Matter Research Laboratory staff. This summary report is intended to provide users of the Aqualog® fluorometer with a standardized approach by which to evaluate and validate data produced by the Aqualog® fluorometer. The processed summary report for fluorescence data includes corrections (for example, water Raman normalization, correct for inner-filtering effects), calculations (for example, multiply diluted samples to appropriate concentration, calculate designated slopes and indices), data manipulation (for example, Rayleigh trims, vectorize fluorescence data, compile summary data; for example, extract a subset of widely published fluorescence excitation-emission pairs, indices), evaluation of quality-assurance data, and assignment of data qualifiers. Click here to view.

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U.S. Geological Survey
6000 J Street, Placer Hall
Sacramento, California 95819
http://ca.water.usgs.gov

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