

Seeing the light: The effects of particles, dissolved materials, and temperature on in situ measurements of DOM fluorescence in rivers and streams

Bryan D. Downing^{1*}, Brian A. Pellerin¹, Brian A. Bergamaschi¹, John Franco Saraceno¹, and Tamara E.C. Kraus¹

¹U.S. Geological Survey, California Water Science Center, 6000 J Street, Sacramento, CA 95819, USA

Abstract

Field-deployable sensors designed to continuously measure the fluorescence of colored dissolved organic matter (FDOM) in situ are of growing interest. However, the ability to make FDOM measurements that are comparable across sites and over time requires a clear understanding of how instrument characteristics and environmental conditions affect the measurements. In particular, the effects of water temperature and light attenuation by both colored dissolved material and suspended particles may be significant in settings such as rivers and streams. Using natural standard reference materials, we characterized the performance of four commercially-available FDOM sensors under controlled laboratory conditions over ranges of temperature, dissolved organic matter (DOM) concentrations, and turbidity that spanned typical environmental ranges. We also examined field data from several major rivers to assess how often attenuation artifacts or temperature effects might be important. We found that raw (uncorrected) FDOM values were strongly affected by the light attenuation that results from dissolved substances and suspended particles as well as by water temperature. Observed effects of light attenuation and temperature agreed well with theory. Our results show that correction of measured FDOM values to account for these effects is necessary and feasible over much of the range of temperature, DOM concentration, and turbidity commonly encountered in surface waters. In most cases, collecting high-quality FDOM measurements that are comparable through time and between sites will require concurrent measurements of temperature and turbidity, and periodic discrete sample collection for laboratory measurement of DOM.

Advances in field-deployable optical sensor technology over the past 20 years have led to the routine use of compact and relatively inexpensive in situ fluorometers to quantify dissolved organic matter (DOM) concentration and composition in coastal environments (Coble 1998; Vodacek et al. 1995; Chen 1999). In particular, instruments that measure chromophoric DOM (CDOM) fluorescence at wavelengths of ~460 nm in response to excitation at ~370 nm have proven to be a highly sensitive and useful tool for elucidating spatial and temporal DOM variability (e.g., Bergamaschi et al. 2011; Downing et al. 2009). The broader application of field-deployable CDOM fluorometers to freshwater systems is more recent.

Here these instruments have proven equally helpful, providing high-resolution DOM measurements in rivers and streams under a range of hydrologic conditions (e.g., Pellerin et al. 2011; Saraceno et al. 2009).

Continuous in situ measurements of the fraction of CDOM that fluoresces (hereafter referred to as FDOM) in freshwaters provide new opportunities for assessing constituent loads, drinking water quality, terrestrial carbon budgets, and changes in ecosystem food webs (Cole et al. 2007; Findlay and Sinsabaugh 2003; Kraus et al. 2010). However, measuring fluorescence in situ in inland water bodies, which are often highly colored or turbid, can require corrections and accommodating procedures not usually needed for optically clearer coastal waters. The primary issue is light attenuation—of both the light emitted by the instrument to stimulate fluorescence and the light emitted by the fluorescing material. Dissolved constituents in the water column may absorb some of the light, while suspended particles may both absorb and scatter light. A secondary issue is the effect of environmental conditions on the fluorescing material itself. Temperature and pH, for example, may alter the energy state of fluorescing substances and diminish the intensity of their light output (fluorescence

*Corresponding author: E-mail: bdowning@usgs.gov,
phone: (916) 278-3092, fax: (916) 278-3071

Acknowledgments

We thank Angela Hansen, Orlando Schuler, Travis Von Dessonneck, Paul Stumpner, Jon Morrison, and Joe Martin of the U.S. Geological Survey for laboratory and field support. Funding for this study was provided by the USGS Climate Effects Network.

DOI 10.4319/lom.2012.10.767

quenching; Baker 2005; Spencer et al. 2007). However, laboratory studies of organic matter standards and natural samples have shown that pH changes within the range encountered in most natural systems have little impact on fluorescence wavelengths measured by the current generation of FDOM sensors (Patel-Sorrentino et al. 2002; Spencer et al. 2007).

Comparison of in situ FDOM measurements made under different conditions (i.e., in different settings, at different times, or by different instruments or investigators) is possible only if the measurements are properly inter-calibrated. Attenuation and temperature effects must be known and controlled, or corrected for. In the laboratory, these issues are addressed by allowing samples to equilibrate to a constant temperature, filtering, and diluting samples if warranted; results are routinely reported relative to a standard compound or measurement (Henderson et al. 2009; Lakowicz 2006). In the field, however, such procedures are impractical—especially for continuous, high-resolution in situ measurements. Empirical correction offers one avenue for addressing these confounding issues, but at present, such corrections are not routinely applied to field measurements.

This study used environmental standard reference soil and organic materials obtained from the International Humic Substances Society (IHSS) to assess the magnitude of attenuation and temperature effects on fluorescence measurements made by four current-generation, commercially available in situ FDOM instruments. To illustrate the implications of our findings, we then compare uncorrected FDOM data from a large watershed (Connecticut River, USA) to data that has been corrected for the effects of temperature and attenuation using the empirical correction factors determined by the laboratory studies. To assess how often corrections of this type may be needed, we examine discrete and in situ water quality data from several large rivers. Understanding the fundamental influences of water temperature, dissolved materials, and suspended particles on freshwater FDOM measurements and assessing the feasibility of empirical corrections is a critical first step toward the successful and widespread use of FDOM instruments in rivers and streams.

Materials and procedures

Laboratory tests were conducted with four commercially available, field-deployable in situ fluorometers, and three reference materials. The FDOM instruments used in the study were manufactured by Turner Designs, WET Labs, and Sea-point Sensors. Primary design differences (Table 1) included the output of the light-emitting diodes (LEDs), wavelengths of the excitation and emission peaks, spectral width of the band-pass at half the maximum transmission (full-width half-maximum, FWHM) of both the light source and the detector, open path or closed path (flow-through) design, angle between the source beam and detector (acceptance angle), and sample or sensing pathlength or volume (a function of instrument geometry and photodetector size). The instruments were classified as follows: (a) open/90°(1): open-path design with an acceptance angle of 90°, one LED; (b) open/90° (2): combined open- and closed-path capability with an acceptance angle of 90°, two LEDs; (c) open/140°: open path with 140° acceptance angle, three LEDs; and (d) closed/90°: closed path with internal quartz flow tube and 90° acceptance angle, two LEDs.

Sample materials were obtained from the International Humic Substances Society (IHSS, <http://www.humicsubstances.org>). Suwannee River NOM (RO isolation; IHSS 1R101N) is reference aquatic natural organic material collected near the head of the blackwater Suwannee River in southern Georgia, just downstream of the Okefenokee Swamp. Two types of solid-phase bulk source materials were also used: Pahokee Peat Soil (IHSS 2BS103P), a typical agricultural peat soil of the Florida Everglades (freshwater wetlands) collected at the University of Florida Belle Glade Research Station, and Elliott Silt Loam Soil (IHSS 1BS102M), a fertile prairie soil collected near Joliet, IL.

The Suwannee River and Pahokee Soil materials were used to assess light absorption by colored dissolved (filter-passing) substances (i.e., inner filter effects, or IFEs). Stock Suwannee River solutions were prepared by dissolving 200 mg powder in 100 mL organic-free deionized (DI) water, followed by filtra-

Table 1. Manufacturer specifications for FDOM instruments used in this study.

Path type	Excitation (nm) ± bandpass (FWHM, nm)	Emission (nm) ± bandpass (FWHM, nm)	Sensing volume (mm ³)	Emitter detector acceptance angle (°)	Detection limit (ppb QSD)	Dynamic range (ppb QSD)	Gain settings	Signal output	Power requirement (Watts ×10 ⁻³)
Closed	370 ± 10	460 ± 120	250	90	0.1	0-400	fixed	0-5 Vdc or digital (counts)	<600
Open*	350 ± 20	430 ± 30	unknown	90	0.4	0-2000	multiple	0-5 Vdc	<300
Open†	370 ± 12	440 ± 40	340	90	0.05	0-1500	multiple	0-5 Vdc	<300
Open	370 ± 10	460 ± 120	unknown	140	0.1	0-400	fixed	0-5 Vdc or digital (counts)	<600

FWHM: Full width half maximum

*Open/90° (1)

†Open/90° (2)

tion through a 0.45 micron glass fiber capsule filter. Stock Pahokee DOM leachate was prepared by mixing 5 g soil in 300 mL organic-free DI water for 24 hours, then filtering as above. The Elliott Soil sample was used primarily to test for particle interference. Stock solutions containing 4 g soil/L were made by mixing the air-dried standard soil with organic-free DI water, then equilibrating for a period of 24–48 hours. This equilibration period was to preclude release of additional colored substances (CDOM and FDOM) into the dissolved phase during the experimental measurements.

The FDOM open-path instruments were immersed in room temperature ($\sim 22^\circ\text{C}$) test solutions in a 4 L acid-washed black HDPE bucket (previously tested for leaching of substances optically active in the fluorescence region of interest; glass beakers or white plastic containers could not be used due to the reflection of excitation light from those container surfaces). Instruments were suspended at a fixed location in the center of the bucket to avoid edge effects, and care was taken to block ambient light. The closed-path (flow-through) fluorometer was operated using a small submersible pump or manual sample addition. All instruments were pre-cleaned using dilute Liquinox®, ethanol, organic-free DI water, and optical-grade lens wipes. Sensor output (fluorescence intensity) was reported in units of quinine sulfate equivalents (QSE) in ppb using a 5-point calibration curve previously determined for each instrument. The calibration solution was made by dissolving quinine sulfate dihydrate in 0.05 M H_2SO_4 .

Determination of attenuation effects

Light attenuation due to inner filtering by colored dissolved substances was investigated by measuring FDOM over a range of DOM concentrations—i.e., over sequential additions of the Suwanee or Pahokee stock solutions to 2 L organic-free DI water under constant stirring. Discrete samples were collected during each experiment, and samples were analyzed for absorbance of ultraviolet light at 254 nm (A_{254}) and DOC concentration. Loss of FDOM signal was characterized in terms of the ratio (r_d) of signal received to signal expected:

$$r_d = \text{FDOM}_{\text{raw}} / \text{FDOM}_{\text{pred}} \quad (1)$$

where FDOM_{raw} is the fluorescence value (in QSE) measured by the instrument in the test solution, and $\text{FDOM}_{\text{pred}}$ is the predicted or expected fluorescence. These predictions were based on linear extrapolation of FDOM measurements made at low CDOM concentrations, where inner filtering effects should be negligible. FDOM_{raw} was plotted as a function of A_{254} and the linear portion of the curve was identified as those points for which linear regression produced the highest r^2 value. This extrapolation was typically made using data from the first 3–5 samples in the concentration series ($A_{254} < 0.1$ AU).

Light attenuation due to suspended particles (absorption + scattering) was investigated by measuring FDOM over a range of turbidities using sequential filtrations (Saraceno et al. 2009) of ~ 4 L of the Elliott Soil stock solution: sample turbidity was

varied over the course of the experiment by filtering out sediment and returning the filtrate to the test vessel until a zero-turbidity condition existed. This was accomplished by using an in-line filtration system consisting of a 12 Vdc submersible in-line centrifugal pump (LVM105 Amazon; LVM, Inc.) and 0.2-micron membrane filter (Osmonics Memtrex, 0.25 m). The test solution was constantly stirred on a magnetic stir plate, and sediment concentration was held constant for a few minutes at fixed intervals prior to recording the FDOM measurements. Measured FDOM values varied by less than 5% at each concentration step.

The loss of FDOM signal due to light attenuation by particles was characterized in terms of

$$r_p = \text{FDOM}_{\text{raw}} / \text{FDOM}_{\text{filt}} \quad (2)$$

where FDOM_{raw} is the fluorescence value measured in the solution during the experiment and $\text{FDOM}_{\text{filt}}$ is the value measured in the final filtered solution without particles (i.e., turbidity ~ 0 FNU). Turbidity served as a proxy for particle concentration and was measured using a nephelometric turbidimeter (YSI 6136; YSI Incorporated) with output expressed in units of formazin nephelometric units (FNU).

Determination of temperature effects

The effects of water temperature on FDOM sensor measurements were evaluated over a range of ~ 1 to 25°C using Suwanee River, Pahokee Soil, and quinine sulfide dihydrate (QSD) standards, and using organic-free DI water. The measurements in water-only were conducted to determine the effects of temperature on sensor electronics alone. The test solution was chilled or warmed to the target temperature (measured with a NIST-traceable thermometer) over a 4–8 h period. Fluorometers were equilibrated to the sample temperature by immersion in the test solution. All solutions were continuously stirred during the fluorescence measurements.

FDOM field measurement corrections

To assess the potential impact of attenuation and temperature effects on actual field FDOM measurements we used continuous and discrete data collected from the Connecticut River at Middle Haddam, CT (USGS gage 01193050) from October 2010–October 2011. An open-path, 90° fluorometer (open/ 90° (1); Table 1) measured FDOM in situ continuously, and a co-deployed multi-parameter water quality sonde (YSI 6920, YSI Incorporated) measured turbidity and water temperature. Discrete water samples were collected approximately monthly for dissolved organic carbon (DOC) concentrations and lab absorbance measurements. DOC concentration was measured using high-temperature catalytic oxidation with a Shimadzu TOC-V CSH TOC analyzer (Shimadzu Scientific Instruments) according to the method of Bird et al. (2003). The mean of 3–5 injections was calculated for every sample. Analytical precision, described as a coefficient of variance (CV), was $< 2\%$ for the replicate injections. Spectral light absorbance was measured between 200 and 750 nm at 25°C

using a 1 cm quartz cell in a Cary 300 spectrophotometer (Varian). Organic-free DI water was used as a blank.

To assess the prevalence of conditions needing substantial correction for attenuation, we assessed monthly A_{254} (USGS parameter code 50624) and turbidity (USGS parameter code 68630) for discrete samples collected by the USGS National Stream Quality Accounting Network (NASQAN) in several large rivers ($n = 1898$ samples; collected 1999–2011; http://infotrek.er.usgs.gov/nasqan_query/). For a subset of those rivers, continuous turbidity measurements were also available: the Delaware River at Trenton, NJ (USGS site 01463500, $n = 3789$ for the period of record of continuous measurements), Platte River at Louisville, NE (USGS site 06805500, $n = 668$), Missouri River at Hermann, MO (USGS site 06934500, $n = 1199$), Columbia River at Beaver Terminal near Quincy, OR (USGS site 14246900, $n = 3083$), and Sacramento River at Freeport, CA (USGS site 11447650, $n = 527$).

Results and discussion

Light-attenuation effects on FDOM measurements

Light attenuation is a critical consideration in making fluorescence measurements: it reduces the energy of the excitation signal between the fluorometer and the sample and also the energy of the sample's fluorescent emissions en route to the detector. Attenuation due to dissolved substances (commonly referred to as absorbance) results from interactions between light and conjugated chemical moieties common in natural waters (Weishaar et al. 2003). Attenuation due to particles in suspension results from not only light absorption but also light scattering. Dissolved and suspended particulate materials are common in most environmental samples and can significantly affect optical measurements. In this study, we examined the effects of each separately because fundamental optical theory holds that the effects are simply additive (Lakowicz 2006).

In the test solutions of DOM derived from Suwannee River water and Pahokee soil, appreciable loss of FDOM signal was observed at absorbance (A_{254}) values greater than 0.1 absorbance units (AU; Fig. 1). In typical river systems, this level of absorbance corresponds to a DOC concentration of approximately 4.0 mg L⁻¹. At the highest absorbance tested ($A_{254} = 1.2$ AU; DOC ~ 31 mg/L), attenuation resulted in a measured FDOM signal that was much lower ($r_d = 0.4$ – 0.7) than the values expected in the absence of the inner filter effects that attenuate the fluorescence signal. The closed-path instrument experienced the least signal loss, whereas open path instruments with a longer effective path length exhibited greater signal loss. These differences highlight the need for instrument-specific correction factors that must be applied to account for the effects of light absorption by dissolved materials in the water column. The FDOM signal loss observed in our study generally follows the Beer–Lambert law (Lakowicz 2006), which states that absorbance is directly proportional to the concentration of the absorbing substance. In this case, the

confounding absorbance effects are proportional to concentration when absorbance is less than about 3 AU, corresponding to a DOC concentration ranging from 45 to 65 mg/L. The implication for correction of field FDOM measurements is that for river or stream waters where $A_{254} \leq 3$, instrument-specific correction factors can be applied to $FDOM_{raw}$ to yield a more comparable and accurate measurement.

Light attenuation due to suspended particles can be especially significant (Fig. 2); attenuation was seen even in waters that would be considered very clear in a river or stream setting. The attenuation due to turbidity (r_p) at the highest values tested (~1000 FNU) was ~0.1–0.2, meaning the measured $FDOM_{raw}$ values in highly turbid waters represented only 10% to 20% of the fluorescence of the same water when filtered. Again, open path and closed path instruments responded differently, with the open-faced fluorometers being particularly

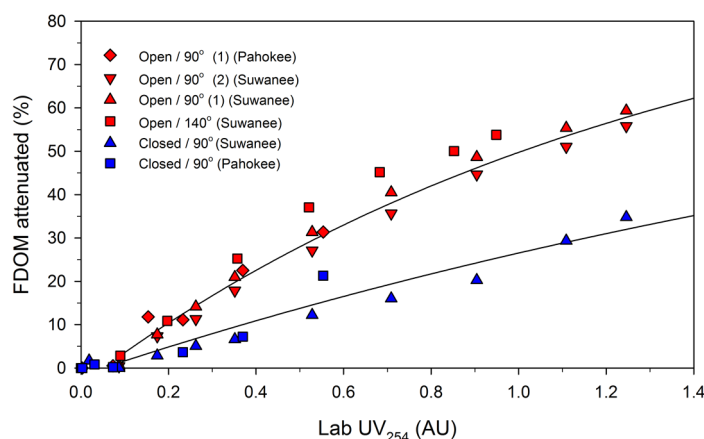


Fig. 1. Attenuation of FDOM signal as a function of absorbance at 254 nm (A_{254}) in test solutions made from IHSS Suwannee River and Pahokee Peat materials. For characteristics of the fluorometers listed in the figure legend, see Table 1.

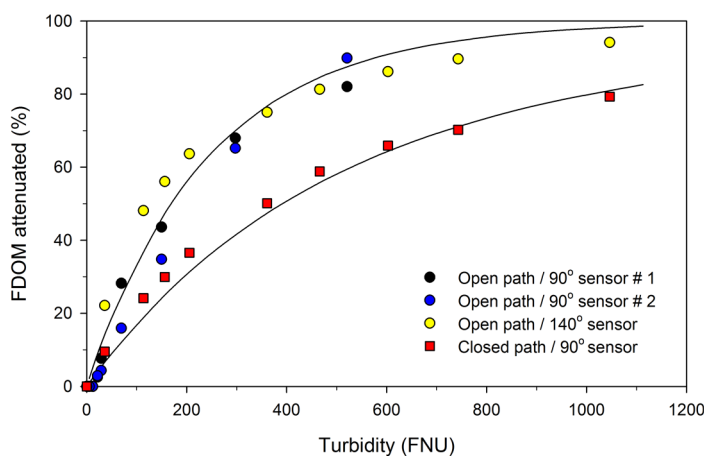


Fig. 2. Attenuation of FDOM signal as a function of turbidity, a proxy for particle concentration, in a test solution containing suspended particles of IHSS Elliot silt loam soil. For characteristics of the fluorometers listed in the figure legend, see Table 1.

sensitive to particle interference effects. For example, at 35 FNU (relatively low turbidity), open faced fluorometers lost 22% of the FDOM signal ($r_p = 0.78$). The implication of these findings is once again that instrument-specific correction factors must be applied to correct for light attenuation by suspended solids.

As with simple absorption, signal loss related to particle attenuation follows the underlying Beer–Lambert law (Lakowicz 2006). For turbidities less than about 600 FNU, signal diminution is directly proportional to the measured turbidity; for more turbid waters, when $r_p < 0.2$, signal is not uniformly related to increasing turbidity. The implication for correction of FDOM field measurements is that for river or stream waters where turbidity ≤ 600 FNU, instrument-specific correction factors can be applied to FDOM_{raw} to provide a more accurate, particulate-corrected assessment of FDOM in the natural water body. Additional studies are needed to assess if corrections are possible for specific sensors above this level.

These laboratory findings are similar to those of a field study (Saraceno et al. 2009) that examined DOM dynamics in a slough draining an agricultural watershed. There, simultaneous measurements by filtered and unfiltered closed-path FDOM instruments deployed during a high-flow storm event indicated a similar loss of optical signal due to particle interference (64% signal loss at turbidity ~ 1200 FNU). As in this study, the soil texture was predominantly silt loam and the same type of side-scatter turbidimeter (YSI) was used as a proxy for particle attenuation.

Temperature effects on FDOM measurements

It is well established that the fluorescence intensity of DOM is inversely related to sample temperature. A higher temperature increases the likelihood that an excited electron will return to its ground state by radiationless decay, thus resulting in reduced fluorescence emission intensity (Lakowicz 2006; Vodacek 1989; Zepp et al. 2004). Laboratory studies have shown that the molecular fluorescence of humic-like organic matter typically decreases $\sim 1\%$ per degree C temperature increase (Henderson et al. 2009). Consistent with this scenario, we found a linear inverse relationship between measured FDOM and water temperature (T). Over the range 1 to 25°C, for all fluorometers and DOM types and concentrations tested, measured FDOM decreased an average of 1.2 (± 0.4)% per 1°C increase in water temperature (range = 0.2 to 1.8%, $n = 16$ tests; data not shown). These results are similar to the 1.5% and 0.8%, respectively, per °C decrease in temperature reported by Watras et al. (2011), who used two open-path field-deployable FDOM sensors in natural waters.

We observed little difference in the slopes of FDOM versus Temperature for different sample types, including quinine sulfate. Other studies suggest that the FDOM–Temperature relationship may be different for some excitation-emission wavelengths, such as the lower-wavelength pairing used to study tryptophan-like Peak T fluorescence (270–280 nm ex, 330–330 nm emission; Baker 2005; Seredyńska-Sobecka et al. 2007).

Our results suggest that it is possible that temperature effects are relatively independent of DOM composition at the excitation-emission wavelengths commonly used by commercial FDOM sensors, but this needs to be verified in a future study.

Accounting for the influence of temperature on the fluorescence properties of DOM—i.e., converting FDOM(T) to FDOM at a reference temperature—is important at sampling sites that experience significant temperature fluctuations, from diel to seasonal time scales. Correction is also required if FDOM measurements from sites in different or changing climatic regimes are to be directly compared. Reporting FDOM data in terms of a reference temperature is important over a wide range of temporal and spatial scales, from short-term studies designed to observe subtle DOM shifts related to biological activity or physical processes (e.g., DOM uptake or photodegradation) to long-term assessments of climate variability and trends.

Application of attenuation and temperature corrections

Based on our results, the first step in applying corrections to continuous FDOM_{raw} field data are to determine the instrument-specific values for $r_d(A_{254})$ and $r_p(\text{FNU})$ (Figs. 1 and 2). Conversion of FDOM_{raw} to a standard temperature (of 25°C in our case) does not appear to be instrument-dependent, at least over the range of instruments and temperatures that we examined. Temperature is easily measured in the field and compensation straightforward. Periodic sampling and establishing the temperature dependence of the material present at the site will provide the best results, but the differences in response among the varieties of natural DOM is likely to be small (Watras et al. 2011). Compensation for light attenuation due to suspended particulates requires that turbidity be measured concurrently with FDOM using an in situ turbidimeter deployed in association with the field fluorometer. However, different turbidimeters behave differently due to variations in instrument design. Therefore, the $r_p(\text{FNU})$ function (Fig. 2) must be determined for not only the particular fluorometer that is to be deployed but also the particular turbidimeter that it is to be paired with in the field.

Since compensation for light attenuation due to dissolved substances is concentration dependent, periodic collection of water samples and measurement of DOC or A_{254} in the laboratory on filtered samples will be necessary to assess the appropriate magnitude of the correction. Care must be taken to ensure samples are collected across the full range of DOM concentrations. These less intensive discrete measurements will be sparse compared with the in situ FDOM_{raw} measurements and some form of temporal interpolation will be required to fill in the gaps between the infrequent water samplings.

Our results, therefore, show that continuous raw field measurements of FDOM can be corrected according to:

$$\text{FDOM}_{\text{corr}} = \text{FDOM}_{\text{raw}} + \rho(T_{\text{meas}} - 25) / r_p(\text{FNU}) \times r_d(A_{254}) \quad (3)$$

where T_{meas} is the continuously measured water temperature in

$^{\circ}\text{C}$ and (ρ) is the temperature-specific fluorescence coefficient (Watras et al. 2011); $r_d(A_{254})$ and $r_p(\text{FNU})$ are the instrument-specific correction functions for dissolved substances and suspended particulates, respectively; FNU is the continuously measured turbidity in formazin nephelometric units, and A_{254} is the interpolated absorbance value. Functions for $r_d(A_{254})$ and $r_p(\text{FNU})$ are unique, meaning that functions for each will be both instrument and site dependent. These corrections are generally robust for $0 \leq A_{254} \leq 3$ AU and $0 \leq \text{FNU} \leq 600$ FNU. Highly colored or highly turbid waters may not be amenable to the correction approaches explored in this study and may not be good candidate sites for long-term field deployment of the types FDOM sensors we examined.

Implications for in situ FDOM measurements in rivers and streams

We applied temperature and attenuation corrections to an example continuous FDOM data set to assess if application of this correction approach resulted in a significant change in the trends and magnitudes of the measured FDOM values. The site chosen, the Connecticut River, drains one of the largest watersheds of the eastern U.S. (31,000 km²) and provides about 70% of the freshwater input to Long Island Sound (Balcom et al. 2004). At Middle Haddam, on the lower reaches of this temperate river, twelve different water quality parameters are continuously measured in situ (USGS gage 01193050). We examined FDOM data collected over the course of a year (October 2010 to 2011) with an open-face, 90° sensor [open/90° (1); Table 1]. Temperature and turbidity (Fig. 3b) were measured concurrently in situ using a YSI model 6560 conductivity/temperature probe and YSI model 6136 turbidity probe, respectively. Discrete water samples were collected monthly for measurement of DOC and absorbance, which

were used to correct the FDOM_{raw} values reported by the FDOM sensor (Fig. 3c).

Temperature varied seasonally, with a broad minimum observed in late winter and a maximum in late summer. Our reference temperature of 25°C was characteristic of mid-summer and late fall. Temperature-correction effects were greatest during the winter months, when river waters were much colder than the reference temperature. Corrections of up to 25% were required to depress the wintertime FDOM_{raw} values to fluorescence values that would have been observed at 25°C.

Attenuation of light by dissolved colored substances proved to be minimal, but turbidity effects were significant, especially during some strong episodic events (Figs. 3c and 4). Tropical Storm Irene struck the state on August 27, 2011, and the remnants of Tropical Storm Lee arrived in early September. Strong light attenuation due to elevated turbidity resulted in very low FDOM_{raw} values. At the time of peak turbidity (595 FNU), the uncorrected FDOM_{raw} measurements suggested that FDOM had been diluted by high discharge to ~0.3 times its pre-event value. Corrected FDOM values, however, indicate that FDOM actually increased to ~1.6 times its pre-event level. Flux calculations based on the uncorrected FDOM_{raw} values would have therefore underestimated FDOM flux over the period of elevated flow (28 Aug - 14 Sep) by 35%. DOC concentrations measured in discrete water samples collected over the course of the year (Fig. 4) lend support to the validity of the turbidity correction. DOC concentrations and corrected FDOM values are highly correlated ($r^2 = 0.93$). This relationship would have been largely obscured by particle attenuation effects had the raw sensor output been used uncorrected.

To further examine the implications of our findings on in situ FDOM measurements, we assessed the extent to which

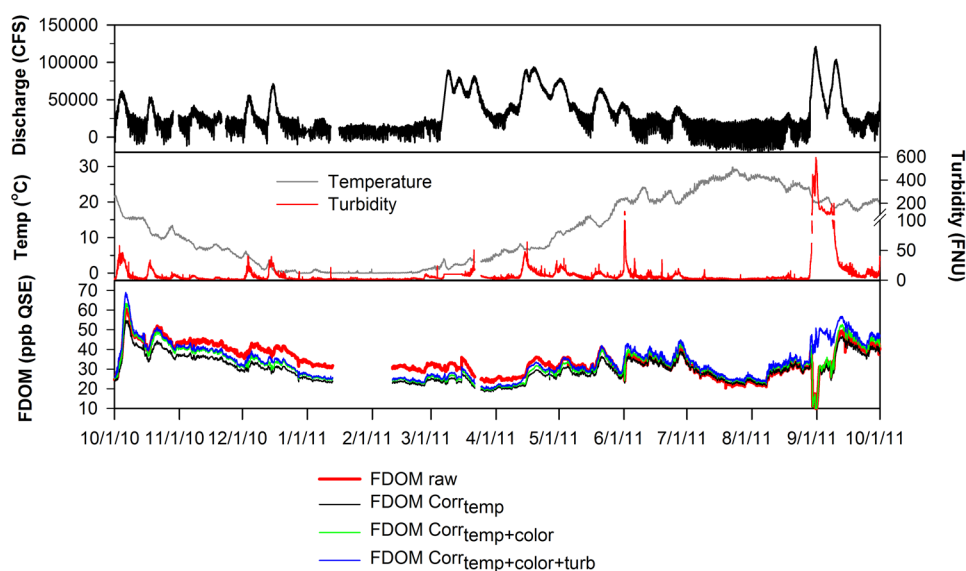


Fig. 3. Continuous in situ data from the Connecticut River at Middle Haddam, CT (USGS 01193050) from October 2010 to October 2011: a) discharge, b) water temperature and turbidity, c) Raw FDOM and FDOM corrected for the effects of temperature and light attenuation by colored dissolved substances and turbidity. The FDOM measurements were obtained using an open-path, 90° fluorometer (1).

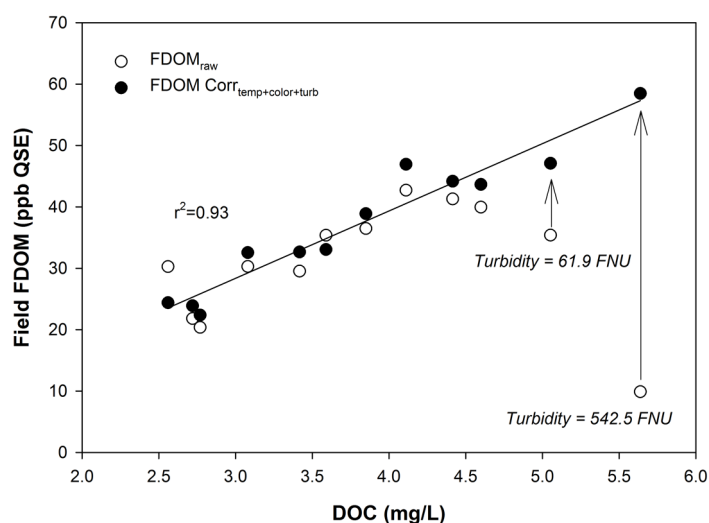


Fig. 4. Field FDOM (raw and corrected) versus DOC concentration in the Connecticut River at Middle Haddam (USGS 01193050), October 2010–October 2011.

corrections to in situ FDOM measurements would be necessary using historic data from a variety of rivers spanning a broad range of DOC concentration, turbidity, and temperature conditions. These historic water quality data also help to identify sites where and when in situ sensor deployments may be problematic due to very large attenuation effects.

To assess the effects of DOM on in situ FDOM measurements, we examined A_{254} measurements for 1000 discrete samples from thirteen subnetwork rivers of the USGS NASQAN program (<http://water.usgs.gov/nasqan>). The range of corrections needed based on the reported A_{254} and the results of our laboratory study (Fig. 1) indicates that in most of these rivers, FDOM-signal attenuation by colored dissolved substances is minor, with corrections typically ranging from < 1 to 10% (Fig. 5). More than 50% of the samples had values of $A_{254} < 0.1$ AU—i.e., CDOM concentrations were below the level at which $FDOM_{raw}$ would be affected by DOM attenuation. These results highlight the general applicability of field FDOM instruments under DOM concentrations commonly observed in rivers and streams.

To similarly assess the effects of particle attenuation on in situ FDOM measurements, we examined field data from 31 large rivers in the USGS NASQAN network plus the Connecticut River (Fig. 6). For these rivers, 98% of the daily maximum turbidity values were below 500 FNU—i.e., within the range for which in situ $FDOM_{raw}$ measurements can be corrected for particle attenuation. The cumulative frequency distribution for continuous turbidity measurements at several rivers showed that maximum daily values were frequently higher in agriculturally influenced rivers such as the Platte River and the Missouri River, suggesting that these types of sites may not be amenable to unfiltered FDOM measurements made by the types of sensors tested in this study. In general, particle atten-

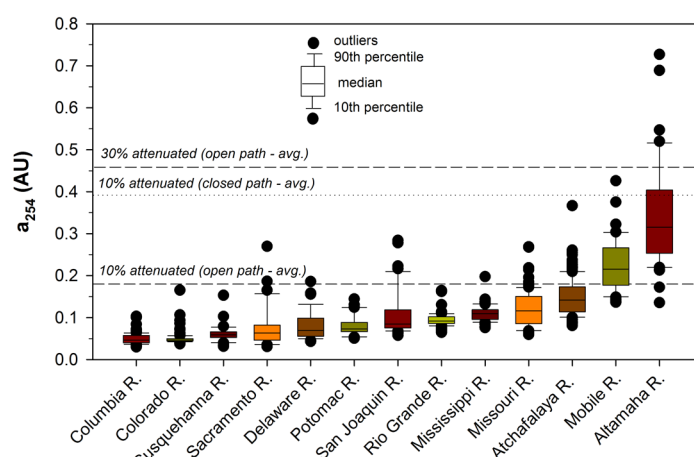


Fig. 5. Box plots of absorbance at 254 nm (A_{254}) data for thirteen large rivers in the USGS NASQAN Coastal Subnetwork. The horizontal dashed lines mark the A_{254} levels at which our tested open- and closed-path sensors would be expected to experience the indicated levels of FDOM signal loss.

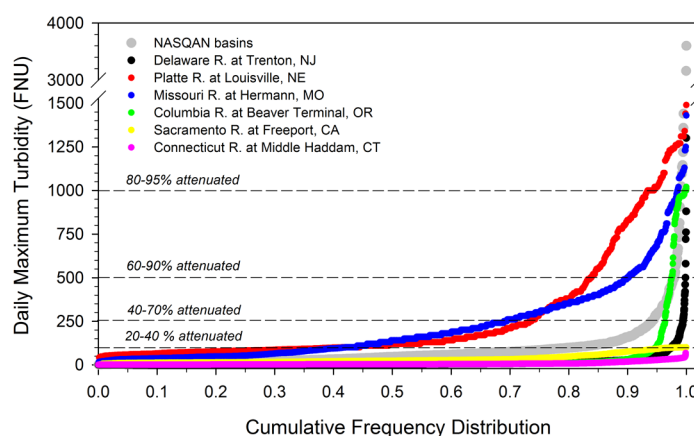


Fig. 6. Cumulative frequency distribution of maximum daily turbidity values for selected USGS NASQAN rivers, plus the Connecticut River. The horizontal dashed lines mark the turbidity levels at which our tested open- and closed-path sensors would be expected to experience the indicated levels of FDOM signal loss.

uation of the FDOM signal typically appears to be low in many large rivers, but high turbidity during short-duration, high-flow periods has the potential to result in significant underestimates of DOM concentrations, fluxes, and loads if FDOM measurements are not properly corrected for particle attenuation.

Comments and recommendations

Our laboratory investigations show that the effects of temperature and light attenuation on field FDOM measurements can be significant, but, given that they generally exhibit the behavior expected from optical theory, compensation strategies are relatively straightforward – up to a point. Examination

of water-quality data from major rivers across the U.S. indicates that FDOM_{raw} measurements made under field conditions typical of most rivers and streams will require some correction or adjustment to account for these effects.

We recommend that users of in situ FDOM instruments

(a) simultaneously deploy turbidity and temperature sensors, and incorporate regular sample collection for DOC and absorbance measurements into field protocols

(b) adopt a systematic approach to instrument characterization and development of instrument-specific correction procedures that provide comparable reported values independent of site conditions.

(c) use standard reference materials for instrument characterization (e.g., Figs. 1 and 2) to ensure repeatability and permit future testing of instruments under identical conditions.

Certainly additional information and tests are needed to ensure high quality FDOM measurements are reported. Our study did not address, for example, the question of interference effects or instrument stability over time or instrument variability within a given model line. Given the rapid evolution of optical sensor LEDs and electronics, future FDOM instruments may well include instrument-specific correction factors developed by manufacturers and verified by users, all according standard methods. Correction procedures may also turn out to be specific to field conditions. For now, concurrent measurement of FDOM_{raw} , turbidity, A_{254} or CDOM, and temperature are critical. As correction algorithms improve, so will the accuracy of the in situ FDOM measurements, thus facilitating direct comparison of measurements acquired over long periods of time and wide ranges of environmental conditions.

Additional testing is also needed to characterize FDOM interference effects over a broader range of DOM composition and particle types. Such tests would include additional standard and reference materials as well as site-specific environmental samples. In particular, the influence of particle size on the attenuation of light emitted and measured by FDOM instruments (Boss et al. 2001) may need to be incorporated into more refined turbidity corrections.

Accurate measurements of turbidity concurrent with FDOM_{raw} are critical for applying appropriate corrections to FDOM data at many sites. However, use of turbidity as the sole basis to correct for particle interference may be problematic. Many different methods and instruments are currently used to measure turbidity. Modern turbidimeters, for example, use different wavelengths, detection angles, and beam configurations (Gray and Glysson 2003). In addition, measured turbidity is a function of not only particle concentration but also particle size and character (Baker and Lavelle 1984; Foster et al. 1992; Schoellhamer and Wright 2003). These dependencies and their implications for FDOM corrections need to be further investigated. If either the fluorometer or the turbidimeter is replaced by another instrument, particle attenuation effects and the correction function (e.g., r_p , Fig. 2) should be re-characterized for the new pairing.

Highly colored or highly turbid waters represent a particular challenge. Under these conditions, the excitation and emitted light are strongly attenuated and large measurement corrections will be required. For our particular set of instruments, correction via data processing for turbidity > 600 FNU or A_{254} > 3 AU are subject to very high uncertainty given that the vast majority of emitted light is attenuated and the measured values no longer behave according to optical theory. Some high-absorbance, high-turbidity environments may require special attention in terms of instrument selection or design. For example, for the open-path instruments we tested, > 90% of the FDOM signal was lost when turbidity was > 1000 FNU (Fig. 2). In such cases, other types of instruments (e.g., closed-path instruments with smaller turbidity-related effects) might be appropriate, or alternative approaches (e.g., pumping and pre-filtering the water) may be required. High attenuation due to dissolved or colloidal material is more difficult to address with a mechanical solution (such as real-time sample dilution). Modifications to FDOM instrument design, such as smaller sensing volumes or brighter light sources, might prove helpful. For some sites, in situ optical sensors may simply be impractical for the foreseeable future.

References

- Baker, A. 2005. Thermal fluorescence quenching properties of dissolved organic matter. *Water Res.* 39(18):4405-4412 [[doi:10.1016/j.watres.2005.08.023](https://doi.org/10.1016/j.watres.2005.08.023)].
- Baker, E. T., and J. W. Lavelle. 1984. Effect of particle size on the light attenuation coefficient of natural suspensions. *J. Geophys. Res.* 89(C5):8197-8203 [[doi:10.1029/JC089iC05p08197](https://doi.org/10.1029/JC089iC05p08197)].
- Balcom, P.H., W.F. Fitzgerald, G.M. Vandal, C.H. Lamborg, K.R. Rolfhus, C.S. Langer, and C.R. Hammerschmidt. 2004. Mercury sources and cycling in the Connecticut River and Long Island Sound. *Mar. Chem.* 90: 53-74.
- Bergamaschi, B. A., J. A. Fleck, B. D. Downing, E. Boss, B. Pellerin, N. K. Ganju, and R. Fujii. 2011. Methyl mercury dynamics in a tidal wetland quantified using in situ optical measurements. *Limnol. Oceanogr.* 56(4):1355-1371 [[doi:10.4319/lo.2011.56.4.1355](https://doi.org/10.4319/lo.2011.56.4.1355)].
- Bird, S. M., M. S. Fram, and K. L. Crepeau. 2003. Method of analysis by the U.S. Geological Survey California District Sacramento Laboratory—Determination of dissolved organic carbon in water by high temperature catalytic oxidation, method validation, and quality-control practices. *In* U.S.G. Survey [ed.], Open-file report 03-366. U.S. Geological Survey. <<http://pubs.usgs.gov/of/2003/ofr03366/text.html>>
- Boss, E., W. S. Pegau, W. D. Gardner, J. R. V. Zaneveld, A. H. Barnard, M. S. Twardowski, and T. D. Dickey. 2001. Spectral particulate attenuation and particle size distribution in the bottom boundary layer of a continental shelf. *J. Geophys. Res. C Oceans* 106(C5):9509-9516 [[doi:10.1029/2000JC900077](https://doi.org/10.1029/2000JC900077)].

- Chen, R.F., 1999. In situ fluorescence measurements in coastal waters. *Org. Geochem.* 30, 397–409.
- Coble P.G., C.E. Del Castillo, and B. Avril. 1998. Distribution and optical properties of CDOM in the Arabian Sea during the 1995 Southwest Monsoon. *Deep Sea Res. II*, 45: 2195–2223.
- Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, and J. Melack. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* 10(1):171–184 [doi:10.1007/s10021-006-9013-8].
- Downing, B. D., E. Boss, B. A. Bergamaschi, J. A. Fleck, M. A. Lionberger, N. K. Ganju, and R. Fujii. 2009. Quantifying fluxes and characterizing compositional changes of dissolved organic matter in aquatic systems in situ using combined acoustic and optical measurements. *Limnol. Oceanogr. Methods* 7:119–131 [doi:10.4319/lom.2009.7.119].
- Findlay, S. E. G., and R. L. Sinsabaugh. 2003. *Aquatic ecosystems*. Academic Press/Elsevier Science.
- Foster, I. D. L., R. Millington, and R. G. Grew. 1992. The impact of particle size controls on stream turbidity measurement; some implications for suspended sediment yield estimation. Erosion and sediment monitoring programmes in river basins, p. 51–62. *In* Proc. international symposium, Oslo.
- Gray, J. R., and G. D. Glysson. 2003. Proceedings of the federal interagency workshop on turbidity and other sediment surrogates. US Geological Survey Circular (1250):1–4.
- Henderson, R. K., A. Baker, K. R. Murphy, A. Hambly, R. M. Stuetz, and S. J. Khan. 2009. Fluorescence as a potential monitoring tool for recycled water systems: A review. *Water Res.* 43(4):863–881 [doi:10.1016/j.watres.2008.11.027].
- Kraus, T. E. C., C. A. Anderson, K. Morgenstern, B. D. Downing, B. A. Pellerin, and B. A. Bergamaschi. 2010. Determining sources of dissolved organic carbon and disinfection byproduct precursors to the McKenzie River, Oregon. *J. Environ. Qual.* 39(6):2100–2112 [doi:10.2134/jeq2010.0030].
- Lakowicz, J. R. 2006. *Principles of fluorescence spectroscopy* (3rd ed.). Springer Science and Business Media [doi:10.1007/978-0-387-46312-4].
- Patel-Sorrentino, N., S. Mounier, and J. Y. Benaim. 2002. Excitation-emission fluorescence matrix to study pH influence on organic matter fluorescence in the Amazon basin rivers. *Water Res.* 36(10):2571–2581 [doi:10.1016/S0043-1354(01)00469-9].
- Pellerin, B. A., J. F. Saraceno, J. B. Shanley, S. D. Sebestyen, G. R. Aiken, W. M. Wollheim, and B. A. Bergamaschi. 2011. Taking the pulse of snowmelt: in situ sensors reveal seasonal, event and diurnal patterns of nitrate and dissolved organic matter variability in an upland forest stream. *Bio-geochemistry* 108:1–16.
- Saraceno, J. F., B. A. Pellerin, B. D. Downing, E. Boss, P. A. M. Bachand, and B. A. Bergamaschi. 2009. High-frequency in situ optical measurements during a storm event: Assessing relationships between dissolved organic matter, sediment concentrations, and hydrologic processes. *J. Geophys. Res.* G 114(4):G00F09.
- Schoellhamer, D. H., and S. A. Wright. 2003. Continuous measurement of suspended-sediment discharge in rivers by use of optical backscatterance sensors. *IAHS-AISH Publication* (283):28–36.
- Seredyńska-Sobecka, B., A. Baker, and J. R. Lead. 2007. Characterisation of colloidal and particulate organic carbon in freshwaters by thermal fluorescence quenching. *Water Res.* 41(14):3069–3076 [doi:10.1016/j.watres.2007.04.017].
- Spencer, R. G. M., L. Bolton, and A. Baker. 2007. Freeze/thaw and pH effects on freshwater dissolved organic matter fluorescence and absorbance properties from a number of UK locations. *Water Res.* 41(13):2941–2950 [doi:10.1016/j.watres.2007.04.012].
- Vodacek, A., F.E. Hoge, R.N. Swift, J.K. Yungel, E.T. Peltzer, and N.V. Blough. 1995. The use of in situ and airborne fluorescence measurements to determine UV absorption coefficients and DOC concentrations in surface waters. *Limnol. Oceanogr.* 40:411–415.
- . 1989. Synchronous fluorescence spectroscopy of dissolved organic matter in surface waters: Application to airborne remote sensing. *Rem. Sens. Environ.* 30(3):239–247 [doi:10.1016/0034-4257(89)90065-5].
- Watras, C. J., P. C. Hanson, T. L. Stacy, K. M. Morrison, J. Mather, Y. H. Hu, and P. Milewski. 2011. A temperature compensation method for CDOM fluorescence sensors in freshwater. *Limnol. Oceanogr. Methods* 9:296–301.
- Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* (37):4702–4708 [doi:10.1021/es030360x].
- Zepp, R. G., W. M. Sheldon, and M. A. Moran. 2004. Dissolved organic fluorophores in southeastern US coastal waters: Correction method for eliminating Rayleigh and Raman scattering peaks in excitation-emission matrices. *Mar. Chem.* 89(1–4):15–36 [doi:10.1016/j.marchem.2004.02.006].

Submitted 30 January 2012

Revised 5 June 2012

Accepted 19 June 2012