Volume title:

*Inherent Optical Property Measurements and Protocols:*
Best practices for the collection and processing of ship-based underway flow-through optical data


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1. Introduction
Continuous, flow-through measurements of temperature and salinity have been collected with thermo-salinographs for decades (e.g. Henin and Grelet, 1996), which has led to well-established protocols for quality control, archiving and distribution of such underway data (e.g. http://www.gosud.org/, http://ocean.ices.dk/data/underway/underway.htm). Chlorophyll fluorescence has also been integrated into such systems, with the first underway flow-through fluorometry dating back to the late 1960s (Lorenzen, 1966).

More recently, several research groups have begun collecting additional optical data using the flow-through systems installed on research vessels and ships of opportunity to take advantage of the availability of sea water pumped into the vessel (we do not discuss tethered systems). These “in-line” or “underway” systems are able to provide data at spatial resolutions on the order of 10-100 m. These measurement scales are not accessible with standard hydrographic surveys and enable characterization of sub-pixel variability in satellite ocean color (OC) data. Thus, data collected using this approach are useful for targeted science questions, but also for large-scale calibration/validation of satellite OC products (Werdell et al., 2013).

As the number of research groups making these measurements grows, there is a need to provide coordinated data collection and processing protocols to standardize methodology and data quality. In 2015, a NASA-sponsored workshop was organized to share such knowledge. Here, we discuss the essential issues associated with in-line data collection, provide recommendations on best practices for collection and processing and report on available software.

This report is organized as follows: First, we address the instruments and hardware associated with deploying an in-line system and discuss a number of considerations that can affect data Quality Assurance/Quality Control (QA/QC). Second, we address issues associated with processing of data from specific optical sensors that have been deployed in-line and software that is available for their use.

2. Optical sensors used in in-line systems.
The easiest optical sensors to integrate into underway systems are those designed for flowing or pumped sample (for example, flow-through fluorometers and transmissometers). Other optical sensors can be integrated into underway systems using flow cells available as options from manufacturers, or custom-built. Common sensors included in underway systems are included in the list below.

Table: sensors deployed in flow-through systems

<table>
<thead>
<tr>
<th>Transmissometers (beam attenuation):</th>
<th>Spectrophotometers (measurements of absorption)</th>
<th>Scattering meters</th>
<th>Fluorometers (CDOM, Chlorophyll)</th>
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<tbody>
<tr>
<td>WET Labs C-Star (1)</td>
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<td>WET Labs ac-9</td>
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<td>HOBI Labs HydroScat sensors (2)</td>
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<tr>
<td>WET Labs ac-s</td>
<td>Sequoia LISST 100X (1)</td>
<td>WET Labs ALFA</td>
<td>Seapoint (1)</td>
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</table>

**Notes:**

*WET Labs has been acquired by Seabird Scientific*

(1) Requires manufacturer-supplied flow cell or chamber

(2) Requires custom-built chamber or tank to contain instrument sample volume

It is assumed that a thermo-salinograph (typically a SeaBird SBE 45 or SBE 21) or equivalent is part of the in-line system, as well as a GPS, and that all instruments are synchronized in time. Daily synchronization is necessary to ensure all instrument data is merged appropriately in post processing; a time server can be set to facilitate this. It is also assumed that logging software and computer(s) are available to which data is transferred from the optical sensor(s) for time stamping and storage. Monitoring flow using a flowmeter within the inline system is highly recommended.

### 3. Water system consideration

#### 3.1 Water source

Sample seawater typically enters a vessel from a “sea chest”, a rectangular or cylindrical recess in the hull of the vessel providing an intake reservoir from which seawater is drawn; or directly via a thru-hull fitting. Location at the ship’s bow or keel is preferred (to avoid that the water has had much interaction with the vessel). In the case of a sea chest, a metal grating separates the open ocean from the sea chest, dampening the exchange of water and excluding large debris (centimeters in size) that might clog any downstream pump or plumbing. To measure properties with the in-line system that are as close as possible to those in the water outside the ship, it is critical that the sea chest is clean and not fouled by filter-feeding organisms. We note that this can be difficult to assess without inspection by a diver. Vessels with thru-hull intakes (e.g., R/V Atlantis or schooner Tara) typically pump through a strainer basket (mesh size ~ 3-4 mm) and a vent loop to release accumulated air.

#### 3.2 Feeding pump

Impeller pumps are the most common pump used in UNOLS vessels (US academic fleet) and can adversely affect particle assemblages (Cetinic et al., 2016). Diaphragm and peristaltic pumps are recommended to minimize artifacts introduced by the pump (screw pumps may also be good, but we currently have no experience with such pumps). Past comparison (Fig. 3B-C in Westberry et al., 2010), as well as recent comparisons between particle images collected from underway systems and Niskin bottles found very good agreement between the two water sources when using diaphragm pumps (e.g. compare optical properties, and size distribution of particles analyzed from water collected by rosette and from the inline system.)
We have experience with the following pumps (field campaign or R/V shown in parentheses for reference):

1. ARO air-operated diaphragm pumps (SABOR, NAAMES)  
2. Shurflo electric pump (Tara).  
3. Graco Husky 1050E pump (NAAMES).  
4. Tapflo air-operated pump (KORUS, Falkor)  

Note that new or modified installations of feeding pumps and downstream scientific instruments often have initial problems with bubbles in the sample flow, a condition that severely affects the measurements of (mostly) particulate optical properties. Typically, adjustment of the flow such as increasing flow rates through instruments, increased application of debubbler(s), and addition of slight backpressure downstream of instruments solves the bubble problem (though there are sea-states and when it does not). Vigilance is required as ship operations (e.g., maintaining station, bow thrusters) or increase in sea state while underway may also introduce bubbles in the flowing seawater. Note also that the flow from peristaltic, and especially diaphragm pumps, may be pulsed. Semi-rigid and softer tubing tends to damp this pulsation, and we have not found it to adversely affect optical measurements (i.e., we have not observed fluctuations in raw measurements at the pulsation frequency, nor significant particle breakage).

3.3 Plumbing

Plumbing needs to be cleaned prior to cruises, typically by running water with a bleach solution through them followed by water and/or, if possible, by replacing the tubing system. Plumbing that is not bleached and thoroughly flushed has been found to bias $O_2$ and $PCO_2$ and is therefore likely to also bias optical measurements (Juranek et al., 2010). Reducing the amount of contact between the input seawater and plumbing (including sea chest, pump, and plumbing to labs) leads to less opportunity to affect the optical properties of measured particles or introduce dissolved substances to the stream. Larger diameter pipes and avoidance of sharp angles in system are expected to reduce shear and potential consequent particle breakage. Thus, we anticipate better agreement between underway and in situ samples for short and wide pipes compared to long and narrow. While the plumbing of the ship need to be cleaned and can’t be changed in most cases, the end connection to the instrument is often up to the operator. Material of fittings such as valves and bulkheads should be approached with awareness (i.e., could be metals or plastic, but check for degradation). We had good experience with Tygon tubing listed below for ease of installation and no bio-accumulation in the tubing during a five-week long expedition:

- Excelon laboratory tubing from US Plastics  
  o model number: 59062 on [www.usplastics.com](http://www.usplastics.com)
- Tygon R-3603
Do not use E-3603, as they recently removed some plasticizers resulting in them fouling quickly.

- EJ Beverage Ultra Barrier Silver Bexag 4-6
  - harder to bend and cut than other tubing mentioned above.

Attention must be paid to bio-fouling in the line as it can affect the quality of the measurements. Lines, adapters, debubblers, and the filter holder can be cleaned with bleach or RBS and letting them soak in such cleaning solution for a few hours.

4. General considerations

4.1 Flow rate

Flow rate between 2-10 L/min has been found to work well, depending on instruments being deployed in the underway system and how much water is vented in debubbler(s) upstream of the instruments (see below). Flow rate considerations are crucial for assessment of delays between different instruments installed in series along the water path. It is important to monitor the flow rate as well as the pressure within the system as it provides a diagnostic to check (especially when examining data post-deployment) when measurements change with no apparent reason. An example of a schematic of the in-line system installed on the Tara Polar Circle expedition is provided in Fig. 1.

4.2 Bubbles and debubbling

In addition to bubbles introduced by turbulence in the plumbing system, bubble entrainment at ocean surface and intake coming out of water, warming of the water and/or cavitation through the path can cause bubble formation. Further, during rough seas we experience significantly more bubbles in the system, likely due to exposure of the sea chest to hull turbulence.

In addition to the solutions to bubbles described in the pump section, we have found that the installation of a vortex debubbler (Ocean Instrument Laboratory, Stony Brook University, MSRC Vortex Debubbler, Model VDB-1, e.g. http://www.seabird.com/pdf_documents/VortexDebubbler_Oct2012.pdf) upstream of the instruments is necessary to remove bubbles. The debubblers are manufactured in two sizes: either 2- or 3-inch diameter models designed for flow rates of 10 or 20 liters per minute, respectively. Customized debubblers are also possible (e.g. 4H-Jena-Engineering GmbH, Germany, http://www.4h-jena.de/wp-content/uploads/2017/01/4H-Debubbler.pdf). Using multiple debubblers in series does reduce the bubble impact in rough seas; however, it increases residence time in the plumbing system and increases exposure of particles to shear, possibly leading to particle breakage.

In addition, adding a constriction (i.e., valve or section of smaller diameter tubing) at the outlet of the system to create a slight backpressure has been found to help alleviate bubble issues. The backpressure may expose water leaks elsewhere in the system, however such leaks are important to identify as before introduction of the backpressure, they were likely points where air could leak into the system. Additionally, the use of a ‘Y’ or tee fitting placed at a high point in the system with a valve to release trapped air (a “degassing Y”) is useful, especially if positioned between the particle filter and instruments to bleed air introduced into the system when changing the filter.
4.3 In-line filters

It is sometimes of interest to measure the properties of specific size ranges of particles or to use measurements performed with a specific filtered fraction as the blank for larger particles (see calibration below). We have found that industrial filters (similar to ones used for drinking water, but typically with tighter specifications, i.e., “absolute-rated”) work well, providing a large filter surface area which does not excessively constrict the flow (e.g., flow would go down by about 40% between total and filtered). An alternative is to use the industrial filter as pre-filter, then into 0.2-µm capsule filter (e.g. PALL Maxi Capsule 12112). Frequent switching between filtered and non-filtered operation after following a filter change helps reduce bubble problems associated with a new filter. Letting the new filter soak in filtered or DI water overnight before putting it in the flow system also helps alleviate bubble problems. Note that immediately after switching to filtered measurement, there may be a transient in measured optical properties as the slug of water trapped in the filter housing is flushed through the system (for example, scattering and fluorescence measurements may increase due to material that was produced in/on the filter). Such contamination needs to be removed during data processing and one has to be sure to take sufficiently long filter measurements for this artifact to be accounted for. This artifact (in addition to reduction in flow rate during filtered measurements as function of time) should also be used to evaluate the need to replace the in-line filter, such that if it takes several minutes to clear or the flow rate is excessively reduced (e.g., less than 60% of the non-filtered flow) it is time to replace the filter.

It is also very useful to use a valve (either manual or automated) to periodically divert the sample seawater through a particle filter (typically 0.2-µm pore size) in order to measure filtered seawater, and by difference obtain “calibration independent” particulate optical properties. This process assumes that the interpolation between dissolved measurements provides a good estimate of the properties of the dissolved fraction when measurements of unfiltered seawater are made, which could be confirmed/assessed/interpolated using a CDOM fluorometer. In turbid waters, an additional pre-filter with a wider pore-size (e.g. 5-µm) can assist in preventing rapid filter clogging. This method of particulate measurements can provide highly sensitive and high-quality measurements of particulate optical properties (Balch et al., 2004; Slade et al., 2010; Werdell et al., 2013; Liu et al., 2018). Commercial systems for automating filtered seawater measurements are available (FlowControl-Lab, Sequoia Scientific, Inc.) which also include measurements of flow, useful to evaluate when to change filters and QC data post-deployment. Flow meters can also be custom built (e.g. http://www.instructables.com/id/How-to-Use-Water-Flow-Sensor-Arduino-Tutorial/). Similarly, if the backscattering sensor is placed after the valve/filter, measurements of the backscattering by the <0.2-µm fraction in addition to the bulk water can be made. A fluorometer in-line after the switch is also able to assess the contribution of CDOM to the measured chlorophyll (see below) and the validity of the use of interpolation between measurements of the dissolved fraction. It is also beneficial to increase the frequency of filtered measurements if working in regions where dissolved optical properties are expected to be more variable, such as in shelf waters or
along frontal boundaries. Typically, 12-24 filtered measurement intervals per day (10-15 minutes per measurement) are more than sufficient in open-ocean waters. Note that even for the “calibration independent” method, it is critical to send the ac-meter sensor yearly to the manufacturer to insure the temperature LUT in the device file is current.

For 0.2-µm filtration Sequoia and UMaine use:
1. Filter housing, Cole Parmer part EW-01508-24
2. Spacer “sump extension adapter” for filter, Cole Parmer part EW-01508-96
3. Filters, Cole Parmer part EW-06479-18
Other filters used (with appropriate housing) are: PALL AcroPak Supor Membrane and the GE Osmonics Memtrex NY.

4.4 In situ vs. instrument temperature
Differences between the in situ water temperature and the instrument temperature can affect optical measurements in several ways. For example, ac-meter calibration tables in the device file rely on the instrument temperature being within a predetermined range of temperatures to apply the correct calibration coefficients (this range is found in the device file). We have also found that ac-meters that have not been properly purged for humidity can develop condensation on the interior of the instrument windows further contaminating the measurements when cold waters flow through them. To avoid these problems, immerse all or part of the instrument (especially detector-end pressure housing) in a bucket or other enclosure with flowing water (e.g., outflow from the instrument).

4.5 Contamination by ambient light
Instruments such as the LISST and the ac-meter measurements are sensitive to ambient light. One needs to make sure that no ambient light can reach an instrument detector, e.g. by putting opaque black electric tape on plumbing entering/exiting the instrument (about 20cm), using black opaque tubing, and/or covering the setup with blackout material. One can check for contamination by turning on/off the lights in the lab and looking for a change in signal in the real-time display (note that there may be a delay in the display due to issues with the software, particularly WETLabs’ COMPASS).

4.6 Enclosures for flat-faced instruments
Commercial backscattering meters and some fluorometers perform open path measurements. Therefore, they require an enclosure of known (and minimal) effect on the measurement in order to deploy them in-line. To determine accurate measurements it is also critical to assess (and later remove) the impact of reflections from the internal walls of the flow-through chamber on the measured signals. This can be accomplished by computing the difference between a measurement of pure water at a given temperature (obtained by filling the enclosure with high quality DIW water\(^1\)). A large curved PVC elbow (septic clean-out), with the interior painted flat black, also can be used to minimize

\(^1\) Note that by high quality DIW we mean deionized water that has a resistance of 18.2MΩ and has been radiated with a UV lamp to photo oxidize organics.
internal wall reflectance for backscattering measurements and is relatively inexpensive to fabricate (e.g. Fig. 2). Special attention must be paid to large particles accumulating in these boxes, it’s recommended to clean them at least once per day even in the open ocean.

4.7 Cleaning
Periodic cleaning is required to remove bacterial films from instrument windows and particles that may not get flushed out of the flat-faced instrument enclosure. We have found, for typical oligotrophic open-ocean conditions, that weekly cleaning is sufficient while in meso- and eutrophic conditions, more frequent cleaning is needed. If, following cleaning, a significant change (drop) in signal is observed, fouling has likely degraded the previous data, which should be flagged accordingly and, if possible, corrected (for example by removing a trend). It is still unclear, however, whether it is better to assume a linear trend or an exponential trend, given that fouling organisms typically grow exponentially (Manov et al., 2004). For cleaning details (e.g., suggested solvents and/or detergents to be used) for specific sensors, refer to manufacturer protocols. We recommend using lens paper on all optical surfaces (e.g., windows, flow sleeves) to ensure that their properties do not change in time due to scraping with harsher materials. More careful procedures are warranted when cleaning heavily fouled instruments, as optical surfaces can be damaged if grit is scraped across them. More frequent cleaning (e.g. daily) is recommended for the enclosures of flat-faced sensors (such as employed with WET Labs ECO-type sensors) as the slower flow within the chamber allows for particles to more easily settle onto the instrument optical surface.

4.8 Calibration
Pre- and post-cruise calibration of optical instruments is highly recommended to help establish measurement uncertainty. For example, some optical instruments - in particular, the ac-9, ac-s, backscattering and transmissometers with 660 nm red LEDs - are known to drift significantly throughout a single cruise. It is therefore recommended, if high quality DIW is available and conditions are adequate, to calibrate these instruments throughout the cruise (e.g. Dall’Olmo et al., 2017). Taking discrete water samples to measure CDOM absorption/attenuation on the vessel or back in shore, if following correct protocol, can be used to vicariously calibrate the in-line ac-meter, as long as it is sufficiently close in time (as reported in Matsuoka et al., 2017), to provide hourly CDOM estimates (in this mode the ac-meter is used to interpolate between the discrete samples).

For ac-9 & ac-s yearly calibration at the manufacturer is necessary as it provide a look-up table (which is part of the device file) that is necessary to deal with instrumental drift due to the instrument’s temperature changes. It is critical that this table match temperature that are likely to be found in the environment in which the sensor is deployed (make sure to request an ‘extended’ table if you plan to work in low (warm waters) or high (cold water) latitudes.

If absolute calibration is not feasible, a valve can be used to measure “calibration independent” particulate optical properties, as discussed above (Section 4.3). Long term changes in the 0.2-µm filtered water fraction can also provide a diagnostic of drift due to
accumulation of material on the instrument’s face, and those measurements can be used to correct for it (though the best strategy is to clean sufficiently often to avoid the drift). Passage of DIW throughout the whole system also provides means to estimate the enclosure-effect on flat-faced sensors. When doing so, attention must be paid to large particles detaching from the plumbing due to the difference in temperature and salinity of the DIW water, which could result in biased dissolved calibrations.

Dark counts of instruments such as ECO-BB3 and fluorometers should be periodically measured using black electrical tape on the face (and immersed in water) for flat faced sensors and using DIW for flowthrough instruments. The difference with manufacturer value may be significant (~10% of signal) in open ocean conditions.

For the LISST we have found that the 0.2-µm provides for a more consistent and lower calibration (termed zscat), than using that derived from DIW water (Boss et al., 2018). This is because the salinity driven change in index of refraction between window and water create a significant bias in instrument with short path-length (Boss et al., 2013b). Since the instrument measures at 670nm the contribution of CDOM to the transmission measurement can usually be neglected.

4.9 Ancillary data
In many instances, optical measurements are used as proxies for biogeochemical parameters (e.g. Chlorophyll a, POC, TSM, DOC, pigments, particle size distribution). Often, the proxies are of much wider utility than the IOPs. While global proxy relationships exist, it is highly desirable that biogeochemical measurements are made periodically along the cruise to establish the cruise-specific or regional relationship. Training of operators taking discrete samples of water directly on the inline system must be provided to avoid having them collect during periods of filtered sea-water. Moreover, as the variability in the signal can be important in 5 minutes (e.g. 1 order of magnitude of chlorophyll a in the North Atlantic when the boat is cruising at 12 knots), accurate time of the discrete sample must be recorded.

4.10 QA/QC
To assess that the in-line system does not bias the measurements, it is critical to make measurements on both in-line as well as surface waters from discrete Niskin bottle samples and contrast the two. These may include different IOP measurements as well as biogeochemical measurements (to check for consistency). In addition, we expect certain relationships between parameters measured by different instruments. For example: transmissometers should agree within a consistent difference due to their design differences (e.g., acceptance angle); beam-attenuation, backscattering and chlorophyll are all related in the surface ocean and relationships between them have been derived (e.g., Westberry et al., 2010); and crossing of oceanic fronts is generally observed in both physical and optical measurements. Significant deviations from these relationships may point out a problem in the data. In general, measurements should evolve smoothly with the exception of spikes due to large particles (or bubbles). Instability in the signal might reveal that bubbles, light, or other unrealistic elements are perturbing the observations. Ancillary measurements such as underway system flow rate and pressure, changes in
ship’s course or speed, and sea state can also be used to flag regions of data requiring more detailed examination. A more general QA/QC manual for optical data is the QARTOD manual (https://ioos.noaa.gov/project/qartod/).

5 Acquisition software, logging data
A general recommendation for data logging software is that it should be stable and write data frequently to the hard drive of the computer instead of buffering large amounts of data in memory. Small digestible files that are simple to read will ease data processing. For example, for the ac-9 and ac-s, a custom version of Compass (r2.1) was provided by the manufacturer to write hourly files, avoiding the generation of gigabyte-sized files that are hard to open and process. Note that compass r2.1 will timestamp files at the beginning or the end of the hour depending on how data is recorded, it might significantly slow down your computer, and the last hour of data is kept in memory and is lost if the software is not stopped properly. Some groups (AWI) elect to generate 10min files. Other sensors can be logged with software such as Haëntjens’ Inlinino (http://inlinino.readthedocs.io/) a simple data logger and visualizer built specifically for acquisition of underway system data; alternatively, Wet Labs’ host program (WLHost, with or without their DH-4 data-logger) is useful to record hourly files, with data data from individual instruments connected to their native software using virtual serial ports for real-time data visualization. Avoid using DH4 during extended period of times (more than a day) as its internal clock drift with time and result in poor timestamping of the data on month long expeditions. Also, Terminal software such as TeraTerm (version >1.9.5) can be used to save data from any serial sensor, timestamped robustly, however it does not provide a plot of the data. When possible, visualization of the data in real-time will help to monitor the inline system and to troubleshoot issues.

Automated backup, clock synchronization across instruments and computers used for data logging should be set up at the beginning of the cruise. If possible, it’s also helpful to log GPS data directly onto the computer. It is preferable that multiple copies of the data are located in different rooms of the ship and are synchronized frequently (every few hours). Many software options exist to backup data; we have had good experiences with SyncToy from Microsoft that we run every 4 hours using the Task Scheduler of Windows. It is recommended that the data logging software not only save processed raw ASCII files (in engineering units), but also saves the raw binary files to the computer. This is critical in case raw data processing is done wrongly, e.g. using the wrong device file for the ac-meters.

6. Considerations for specific instruments/measurements
6.1 Chlorophyll fluorescence and non-photochemical quenching
Phytoplankton decrease their fluorescence within seconds of exposure to high light. Hence, measurements of chlorophyll fluorescence will be dependent upon the short-term light-acclimation state of the phytoplankton, which in turn will be affected by the residence time of the water within the dark plumbing system, clear tubing, or within an illuminating instrument. Look (and if possible, correct) for differences between day and night as well as effects of lights within the ship/lab.
6.2 Chlorophyll fluorescence measurement and CDOM
It is also known that fluorescence by CDOM, if significant in the water, contaminates the measurements by chlorophyll fluorometers (e.g., Proctor and Roesler, 2010). To assess this problem, one may do the measurement periodically through a 0.2 µm filter to provide a baseline (see Sections 2.3 and 2.5 on In-line filters and Calibration).

6.3 Absorption and attenuation
Instruments commonly-used to measure absorption and attenuation in ocean optics are designed for in situ deployment but can be adapted to underway systems (the WET Labs ac-s and ac-9, and C-Star are built with flow cells, and the Sequoia Scientific LISST-100X has a flow chamber accessory that allows for flow-through measurements). As indicated above, regular calibrations (every ~half a year) of the ac-meter by the manufacturer are important for the stability of the collected data. Every time after manufacturer’s calibration, a new device file recording the electronic responses of the ac-meter to instrument temperature will be generated. Remember to backup all the device files and always note to use the latest device file for new data collection. Furthermore, calibration is key to obtain quality measurements; or, if particulate measurements are primarily of interest, periodic filtration with a 0.2 µm filter can be used to provide “calibration independent” particulate measurements by difference of total and dissolved measurements. This is particularly important when calibration (and other instrumental) uncertainties become a significant part of the signal, such as the LISST-100X or LISST-200X whose pathlengths are short (5 or 2.5 cm) and therefore less sensitive in very clear water (meaning calibration uncertainties become a large part of the signal); or even when measuring particulate absorption in extremely clear open ocean water (Slade et al., 2010). If dissolved or total absorption/attenuation are of interest, at least a daily pass of DIW through the system (Dall’Olmo et al., 2017), or a daily sample of CDOM absorption (Matsuoka et al., 2017), is required.

7. Processing flow-through data
7.1 Binning
The high temporal resolution of in-line data allows one to bin, a process that increases signal to noise. Using a median bin or a specific percentile helps in reducing contamination by spikes due to bubbles or rare large particles. The longer the bin, however, the more smeared the resulting spatial signal and hence we do not advise for binning beyond a minute (providing a spatial scale of ~300 m for a vessel moving at 10 kn), unless increased signal/noise is required and the lower spatial resolution is acceptable.

7.2 Removal of data contaminated by bubbles
Periods with enhanced bubble contamination are easy to visualize and need to be removed from the data. These periods are characterized by increased variance and “spiky” data, and in general should be flagged or discarded. Note that zooplankton trapped in the bb casket can cause similar contamination (Burt and Tortell, 2018).

7.3 Merging
Quantifying the delays between instruments (if significant) is important when merging data from multiple sensors. This may be more important for some measurements, such as
temperature and salinity correction of absorption. Generally, it is advised to merge prior to processing.

### 7.4 Interpolating
When using periodic calibrations and/or periodic filtered periods for particulate measurements, it is important to view all of them to assess their consistency and remove obvious outliers (e.g., large change in values not associated with fronts or change in total measurements). We have found that application of interpolated blanks derived from linearly interpolating ‘good’ blanks provide for consistent data (e.g., data that transition to similar values of particulate properties from before and after a filter change).

### 7.5 Products used for biogeochemical analysis
As mentioned previously in section 4.9, optical measurements are often used to estimate products used in biogeochemical analysis. Following thorough QA/QC of optical measurements, products can be estimated using previously determined relationships between optical measurements and independent ancillary data products. For example, several phytoplankton pigments can be estimated from particulate absorption spectra measured in-line (Chase et al., 2013; Liu et al., 2019), and the derived slope of particulate attenuation spectra (denoted $\gamma$) is linked to bulk particle size (Slade and Boss, 2015, Boss et al., 2018). Other products are POC and phytoplankton carbon for which proxy relations have been derived from beam attenuation and backscattering (Graff et al., 2015). It should be noted that datasets used to derive empirical relationships between optical measurements and biogeochemical parameters may not always be appropriate for application to an independent dataset/water type, and tuning to regional conditions may be preferable.
8. References and additional papers on in-line systems with optical sensors


Cetinic, I., N. Poulton and W. Slade, 2016. Characterizing the phytoplankton soup: pump and plumbing effects on the particle assemblage in underway optical seawater systems. Optics Expresss, 24(18), http://dx.doi.org/10.1364/OE.24.020703.


9. Figures and picture gallery

Figure 1. Schematic of the in-line system installed on the Tara during Tara Polar Circle expedition. Figure provided by Marc Picheral.
Figure 2. In-line set-up of B. Balch in a container.

Figure 3. Moon pool aka Straza Tower (center and left), custom intake (center) and the compressed air driven diaphragm pump and hose installed for the flow through system on the Atlantic Explorer by Norm Nelson.
Figure 4. In-line set-up of W. Slade in a UNOLS vessel lab.

Figure 5. Flow through installed in the bilge of the S/V Tara. Flowing seawater enters system at (a) a Vortex debubbler, before (b) the three-way electrically actuated valve, which sends flow directly to (c) the ac-meter instrument, or diverts it through (d) a 0.2 µm cartridge filter, before entering the ac-s instrument. Seawater flow is measured using (e) a paddle-wheel flow sensor. The valve controller and logging computer are located in dry laboratory space (From Slade et al., 2010).
Figure 6. Intake scoop on the bottom of the vessel (left), intake pipe (center) and sea strainer debubbling and venting loop (right) of the R/V Atlantis.

Figure 7. Underway instrument loop and pump on the R/V Atlantis during NAAMES03.

Figure 8. Front (left) and top (right) views of the sink on the R/V Atlantis where the inline system was set up. The water carboy on the left side was used for daily calibrations. In the sink there are a bb-box and the ac-s sitting in a plastic bin with ambient waters. The LISST is on the edge of the sink. The long white tube from the roof is the source of seawater which is routed through the debubbler. From there water flow into the automated switch (connected with yellow power/control cable) and from there to the instrument either through the 0.2μm filter or unfiltered.
Figure 9. Underway ac-s flow-through system on R/V Polarstern. To avoid the condensation on the interior of the ac-s windows when cold waters flow through them, the ac-s detector windows were immersed in a blue bucket with the instrument outflow.
Appendix I: Pre-cruise checklist
- Contact the ship regarding pump and cleaning of in-line pipes.
- Contact the ship regarding adequate DIW source (UV lamp, 18.2MΩ, sufficient quantity) and replacement filters for it.
- Contact ship regarding possibility to visit or get pictures of the lab + sink where you will install your system. Know in advance how you will connect to the intake pump and bring several possible adapters.
- Make sure the ship’s personnel know how much water (from instruments and debubbler) will go into the sink – some sinks empty directly into the sea, some empty into a hold.
- Check about access to GPS data for your logging computer (typically a serial or Ethernet feed).
- Check all instruments and cables are packed including spares. Bring spare power supplies, serial to USB converters and required drivers. Plan for each electronic element to be splashed with seawater; think about what would need replacing?
- Check you have sufficient filters to last the whole expedition (pack for extras in case you encounter productive waters).
- Sufficient tubing + replacement tubing. Hose clamps, connectors, valves.
- Tool box.
- Cleaning supplies (soap, isopropyl alcohol, wipes, sponges).

Appendix II: At sea checklist
Throughout the day
- Note logged flow rate and compare to previous day (ideally 3 to 5 L/min).
- Look for bubbles by viewing the output of the AC-S and bb3 and noticing the variance in the signal (in AC-S bubbles will result in noticeable disruption in the middle of the spectra).
- Make sure filtration periods occur when scheduled and are long enough for value to stabilize. If high variability area such as costal water, increase frequency of filtered periods (e.g every 30 min instead of every hour)
- Check that data are backed up.
- Check that all software is recording data.
- Check date and time of computer (must be UTC).
- Check power supply (e.g 12-13.5 V for ACS, LISST, ECO)
- If using Compass r2.1 to log data, check that the number of records lost on Compass r2.1 is not too high (>20). If it is reboot software, consider defragmenting hard drive and restarting logging computer.

Once a day
- Clean casket for back-scatter measurements and LISST flow cell.
- Run DIW through the whole system until you reach a steady-state value in all instrument. Record at least 1 min of these conditions.
- Analyze some data to make sure it is reasonable.

Weekly (more frequent in eutrophic waters or if you notice a significant jump in the data following the cleaning)
- Clean ac-s.
- Replace 0.2\(\mu\)m filter.
- Once filter replaced, run the system switching back and forth between filtered and unfiltered mode until no more bubbles get into the system from the filter housing (this can be accelerated using the purging valve in the filter housing).

**Appendix III: Processing Software for ac-meters in flow-through**

The UMaine group has posted several processing codes for in-line optical data in the public domain:

These codes contain processing, QC modules and modules to generate Seabass files. The AWI Phytooptics group has posted processing codes for in-line ac-s data in the public domain:
https://github.com/phytooptics/acs_flowthrough

Example processing steps associated with the calibration independent AC-S inline:

![AC Data Processing Software Overview](image)

*Figure A1. Example schematics of the in-line ac-meter processing software.*
**Ingest**
The software stores data processing parameters from the user in a text-based .ini file, allowing customization of the processing. All the necessary data files (ac-meter data files, device files, etc.) to process ac-meter data are ingested, allowing for differences in file formats or file availability. It is flexible enough to allow for new file types to be added, such as those for post-processing (F_CDOM, HPLC, etc.)

**Pre-Processing**
The data is processed by first differencing the filtered from total data. The software automatically identifies the start and end of each filtered/unfiltered period. Users decide to either use the valve on/off data or detects the transitions automatically within the data itself. The transitions between filtered/unfiltered can also be manually edited and saved. Once the transitions have been identified and checked, the data is ready for separation into ‘total’ and ‘filtered’ data sets and for “Processing”.

**Processing**
Both attenuation and absorption data are median minute-binned. Data values between 2.5% and 97.5% percentiles are used (to remove rare outliers). It is also possible to use the mean for binning.

Dissolved values needed to obtain the particulate values are linearly interpolated to the time of particulate measurements. Wavelengths over 750nm are then removed and a 750 wavelength is linearly interpolated. Spectral band of absorption are interpolated to those of attenuation.

The 3rd method of Zaneveld et al., 1994 is typically used to correct for scattering with 730nm as the null wavelengths simultaneously performing a residual temperature correction (see Slade et al., 2010). Attenuation is also corrected for residual temperature. Other types of scattering corrections are also possible to choose from (e.g. Rottgers et al., 2013).

Then, a spectral unsmoothing based on the method in Chase, A., et al., 2013 is used. Finally, data is filtered out (deleted) based on two criteria:

1. If the bin fails the check:

\[
\text{abs(TSW\_bin\_median - TSW\_bin\_mean))/(TSW\_bin\_median-FSW\_interp\_median) > max(0.3 , 0.001/(TSW\_bin\_median-FSW\_interp\_median))}
\]

2. If the bin fails the check:

\[
\text{TSW\_bin\_std > stdThreshold}
\]

where the stdThreshold is .015 for a, and .030 for c.

**Output**
SeaBASS-compatible files are the standard output of the software, for both spectral particulate absorption \( (a_p) \) and attenuation \( (c_p) \). Date, time, latitude, longitude,
temperature, salinity, standard deviation and bin counts (number of good data in each bin) are included in each file, along with either the absorption or attenuation data. Spectra with negative absorption in the blue regions are kept, as these values are not significantly different from zero. In extreme cases, bad values are replaced with -9999.

To facilitate human-in-the-loop QA/QC, many plots are also produced as the software runs, with final plots of the SeaBASS data produced at the end.

References
