

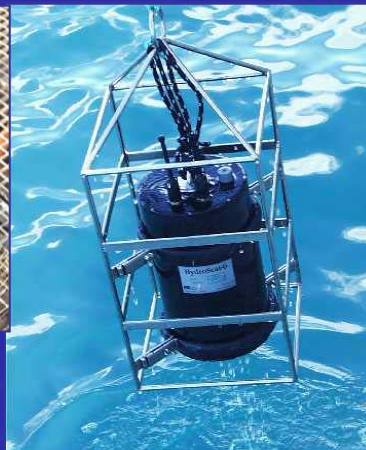
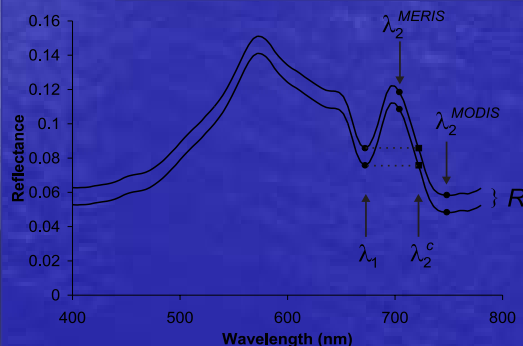
Regional Validation of MERIS Chlorophyll products in North Sea coastal waters.

Proposal: EVG2 – 2001 – 00009

Contract: EVG1 – CT – 2001 – 00049

REVAMP Protocols

G. H. Tilstone, G. F. Moore, K.
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Development of Generic Earth Observation Technologies

REVAMP **Regional Validation of MERIS Chlorophyll products in** **North Sea coastal waters.**

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Protocols Document

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K. G. Ruddick⁴, R. Pasterkamp⁵, P.V. Jørgensen⁶. 2002.**

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Based on NASA and COLORS protocols

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INTRODUCTION TO REVAMP PROTOCOLS

In Case 1 waters Chlorophyll a (Chla) determined from Ocean Color is closely related to the absorption of light by phytoplankton pigments. Algorithms based on blue : blue – green reflectance ratios are reliable for the derivation of Chla in these waters. For Case 2 waters where high suspended particulate material (TSM) and coloured dissolved organic material (CDOM) causes a de-coupling of phytoplankton absorption and the underwater light field, accurate retrieval of Chla is far more complex and as yet remains unresolved. Optical and bio-optical protocols have been well documented for Case 1 waters for validating SeaWiFS data (Mueller & Austin 1992, Fargion & Mueller 2000), but require modification for the more complex Case 2 waters and for validating data from more recent sensors such as MODIS and MERIS. Defining the contribution of CDOM, living and non-living matter to the optical properties of the upper water column and the development of reliable and robust methodologies for Case 2 waters is fundamental for remote sensing research.

The following protocols document draws on the experience of NASA’s SeaWiFS project and the EU Colors project (Coastal region long-term measurements for colour remote sensing development and validation MAS3 – CT97 – 0087; funded by the EU Marine Science and Technology Programme MAST III Startegic Marine Research) and was funded by the EU FP 5 project “Regional validation of MERIS chlorophyll products in North Sea coastal waters” (REAVMP; EVG1-CT-2001-00049). The protocols should be used in parallel with Protocols for the Validation of MERIS water products (Doerffer 2002) which documents MERIS water products, validation strategies and sampling criteria. This document builds on MERIS protocols to give more detailed guide lines for the determination of apparent and inherent optical properties of Case 2 waters.

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In-vivo Absorption Spectra of pigmented and non pigmented Particulate Matter - $a_{pm}(\lambda)$ (m^{-1})

Definition

The light transmission of aquatic particles retained on filter.

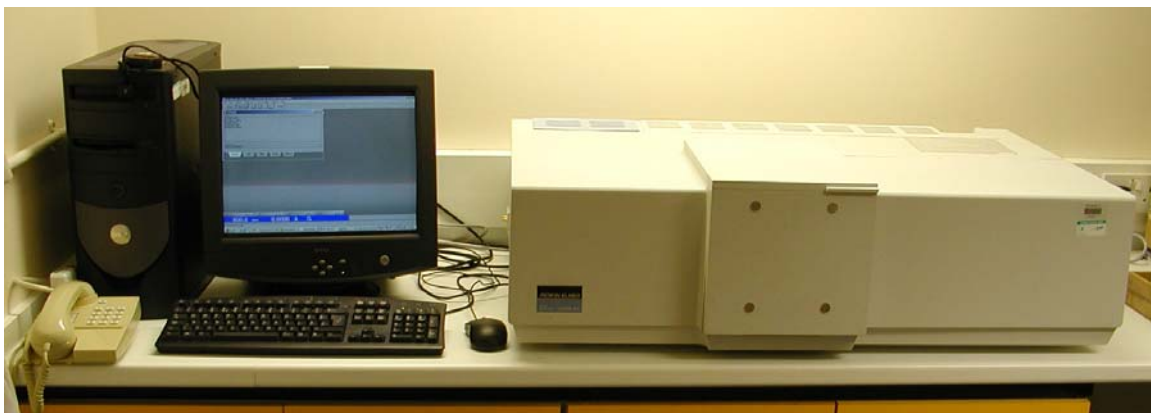
Introduction

The light transmission measurement of aquatic particles retained on a filter is considered a standard method for the determination of the *in vivo* particle absorption. The analysis consists of measuring the fraction of a light beam passing through particles retained on a filter to derive the absorbance $A_{pm}(\lambda)$ on the filter and is then transformed to give the equivalent absorption coefficient $a_{pm}(\lambda)$ [m^{-1}] in suspension.

Instrument description

A dual beam spectrophotometer provided with a Spectralon® coated (barium sulphate degrades with seawater) integrating-sphere attachment, is ideal. In dual beam instruments, the correction for the difference in the beam efficiencies is automatically performed (Tassan and Ferrari, 1995). Single beam instruments are not recommended, as it is difficult to characterize the baseline and spectral performance of the instrument (Mitchell et al. 2000). Before sample measurements are performed, baseline and spectral noise should be well documented using air – air scans to check instrument performance, each time the spectrophotometer is switched on. Measurements are performed in the spectral range 350-800 nm with a 1nm resolution. The instrument photometric accuracy should be at least $\pm 0.003A$ or $\pm 0.08\%T$ at 1A; $\pm 0.002A$ or $\pm 0.05\%T$ at 0.05 A, measured with NBS 930 filters, (Perkin Elmer Lambda specifications). Systems with variable slit widths are preferred from 4 nm to below 4 nm. A NASA workshop recommended the use of Cary 100 (Mitchell et al. 2000). An inter comparison of spectrophotometers carried out at Plymouth Marine Laboratory, showed that the Perkin Elmer range (higher than Lambda 800; Fig 1) also shows quality optical performance comparable to the Cary range (See Technical Annex A).

Figure 1; Perkin Elmer Lambda 800 spectrophotometer.



Recommended baseline noise from 350 to 800 nm for GF/F's is ± 0.005 A and for 10 cm quartz cuvettes with purified water is ± 0.0005 A.

Analytical procedure

- Warm up the spectrophotometer for at least 30 minutes (Check the specific instrument 'warm up' guidelines to meet photometric and baseline accuracy).
- If samples and blank are frozen, place in petri dish on filtered water to ensure hydration and allow to thaw for at least 5 minutes. Store in a refrigerator until analysis.
- Both sample and blank filters will dry out over time and must be re-hydrated regularly after every measurement. If the absorbance signal deviates greater than 0.02 absorbance from zero between 750-800 nm, this indicates a drying of the sample (Mitchell et al. 2000).

Instrument Calibration and Quality Assurance

Spectra should be visually and/or automatically checked, in particular for:

- The presence of a significant peak around 665 nm in $a_{dp}(\lambda)$ spectra, which indicates non complete bleaching of the sample.
- abnormal (< 1) ratio of $a_{ph}(443)/a_{ph}(665)$.

Methodology

Sample collection and filtration

- Filtration volume should be adjusted to keep the samples in the optical density range that is ideal for the path length amplification corrections (see below).
- After collection water samples are transferred to black polyethylene bottles.
- The samples are immediately filtered through 25 mm GF/F filters (nominal pore size $0.7\mu\text{m}$).
- The goals for filtration of particulate samples are to minimize contamination and particle degradation, maximize retention, and concentrate an adequate amount of particles on the filters to permit accurate spectrophotometric measurements (Muller and Austin, 1995). The filtration procedure should therefore be performed as follows:
- Rinse the filtration equipment with distilled water.
- Filter a convenient volume of seawater (500-2000ml). The filtration should be carried under low vacuum pressure (below 120mmHg) to prevent particle breakage and pigment degradation.
- One pair of blank filters for each sample date should be prepared for the subsequent analysis. The blank consists of filters through which $0.22\mu\text{m}$ pre-filtered seawater has been passed. The pre-filtered seawater volume should match or be similar to the sample volume.
- Ensure that for both sample and blank GF/F filters that the same side of the filter is used. For GFF filters there is a striated and smooth side to the filter. The striated shows more scattering than the smooth side and if the sample and blank side are not

equally matched then differences in compensation between sample and blank may arise (See Appendix A; pp. 61 – 63).

Sample storage

- Optical density spectra of the sample filters should be measured as soon after filtering as possible.
- If samples are to be run more than 24 hrs after collection, then samples should be flash frozen and stored in flat containers (e.g. petri dishes, petri slides) in liquid nitrogen. Dry shippers are favored for the transportation of samples but dry ice will suffice for short distances (< 36 hr duration). For further details on sample storage see section on HPLC (p) .

Measurements procedure

The methodology is described in Tassan and Ferrari (1995) with the following modifications:

- The “Autozero” of the instrument should be made with free entrance ports, using high-grade perfectly balanced reflecting plates on the exit ports; these can be replaced by standard spectralon plates for the following measurements. Performing the “Autozero” with filters on the entrance ports is not considered a good practice because of the difference that may occur in filter transmittances. Baseline flatness using integrating sphere should be at least ± 0.004 A units.
- Depigmentation using NaClO is recommended. Bleaching by Methanol is not advised as phycobilins and eukaryotic pigments are not extracted and some loss of the sample can occur. The bleaching concentration of NaClO can be 1 % active chlorine (Tassan & Ferrari 1995) or 0.1% active chlorine (Tassan et al. 2000). The choice of active chlorine solution depends on the dominant particles or species in the sample. If the sample has a high detritus content, 0.1 % active chlorine is recommended since a 1 % active chloride solution may cause excessive bleaching of the detrital fraction which would result in higher phytoplankton absorption coefficients. If a 1 % solution is used the NaClO can be applied to the filter as 4 to 5 drops as described in Tassan and Ferrari (1995) and ensure that the NaClO spreads over the whole of the filtration area. If 0.1% active chlorine NaClO is used, the sample filter should be placed on the filtration port and stood in 5 ml of NaClO for up to 15 mins. Disappearance of the peak at 675 nm in the bleached sample and evidence of a concave shape of the OD spectrum near to 440 nm can be considered evidence of complete filter bleaching (Mitchell et al. 2000). For both 0.1 & 1 % active chlorine treatment, 5 ml of MilliQ should be re-filtered through the treated GFF filter to remove any residual NaClO (Tassan et al. 2000). Blank filters should also be bleached and re-filtered using the same procedure.
- Ensure that both sample and blank filters do not dry out. Dry filters, adversely affect the optical density of the sample.

Data processing

In Case 1 waters a zero offset from the baseline may occur which is presumed to be the product of scattering throughout spectrum. Hence a spectral region is identified where phytoplankton absorption is assumed to be negligible (typically 750 to 800nm) and the scattering observed is due to non-phytoplankton material. However, in Case 2 waters scattering by particles ≥ 750 nm is not negligible since scattering and absorption by detritus increase with decreasing wavelength (Tassan & Ferrari 1995). The experimental and data processing methods of Tassan and Ferrari (1995; equations 11 to 14) are recommended with some modifications to convert the measured absorbance of the filter-retained particles into the equivalent particle suspension absorbance. Four measurements are therefore required for each sample (two transmission and two reflectance). The instrument baseline for the integrating sphere should be recorded. The data is processed by fitting the detrital curve to an exponential with an offset which takes into consideration the baseline. The particulate absorbance spectra is scaled to the exponent of the detrital curve. τ is defined as the ratio of $(1-T_{sd})/(1-T_{sp})$ where T_{sd} is the transmission of diffuse light through the filter and T_{sp} is the transmission of parallel light. The following routine is used to calculate τ :

$$\tau = 1.171 - 0.2615 * \alpha + 0.00013 * \alpha * \alpha \quad (\text{Equation 1})$$

where α is the absorption in transmission mode either of the pigmented or de-pigmented sample given as follows:

$$\alpha = \log_{10} \left(\frac{1}{st} \right) \quad (\text{Equation 2})$$

where st is the sample transmission. The wavelength specific absorption coefficient is calculated from the absorbance of the material in suspension (A_{sus}):

$$a(\lambda) = 2.3 \left(\frac{A_{sus}(\lambda)}{XC} \right) \quad (\text{Equation 3})$$

where X is the ratio of the filtered volume to the filter clearance area and C is the particle concentration. Absorbance of the material retained on the filter is converted to absorbance of the material in suspension using a pathlength wavelength correction factor (see below).

Pathlength Wavelength Correction, β

- The amount of sample filtered should yield an optical density at 675 nm of between 0.05 & 0.25 A and with a blue absorption ≤ 0.4 A. High suspension absorbance leads to increasing errors when applying β (Mitchell et al. 2000).
- Few β values have been reported for Case 2 waters (Tassan & Ferrari 1998). For the purpose of data storage, β is set equal to 2 (Roesler 1998), which is based on the assumption that for GF/F filters the diffuse absorption of a sample is twice the volume of absorption coefficient.
- Specific β correction should be calculated for specific areas and phytoplankton assemblages and the method of β correction should be recorded.

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Backscatter coefficient, $\beta(\theta\lambda)$ (m⁻¹)

Introduction

Few historic data exist on the variation in shape of the volume scattering function $\beta(\theta\lambda)$ in the backward direction. The most widely published data are those of Petzold (1972) and Balch et al. (1994), who used a general angle scattering meter (Mueller et al 2000) to measure $\beta(\theta\lambda)$ for marine hydrosols.

More recently several commercial backscatter meters have been developed and are available from Hobilabs and WETLABS. The HydroScat-6 manufactured by Hobilabs measures scattering at centroid angle of 140° and at many fixed wavelengths. The ECO - VSF 3 manufactured by WETLABS, measures $\beta(\theta\lambda)$ at single wavelengths (450, 530, 650 nm), but at three centroid scattering angles (100, 120, 150°). Both of these instruments will be utilized during the REVAMP contract.

These sensors measure a weighted integral of radiance scattered from a working volume defined by the intersection of illumination source beam and angular field of view of the detector (Mueller et al. 2000). The backscattering coefficient (m⁻¹) is calculated from:

$$b_b(\lambda) = 2\pi \int_{\pi/2}^{\pi} \beta(\theta, \lambda) \sin \theta d\theta$$

The Hobilabs instrument

The following information has been taken from HydroScat 6 Manual 2002.

Instrument description

The Hobilabs HydroScat-6 is a hyperspectral instrument (Fig 2), that measures $\beta(\theta\lambda)$ at six wavelengths and at 140°. It also makes auxiliary measurements of fluorescence.

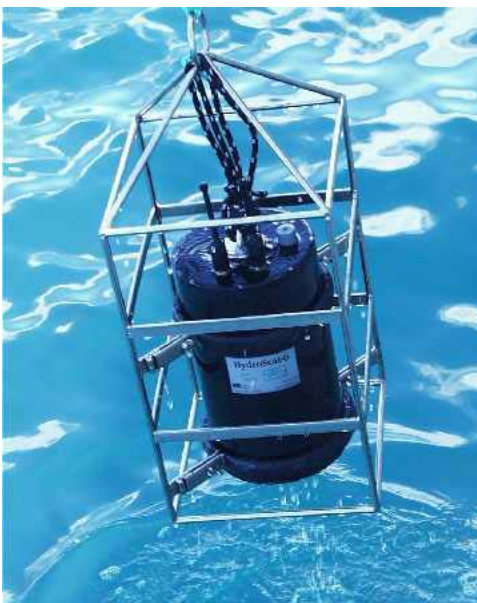


Figure 2; Hobilabs, HydroScat – 6; Multispectral backscattering meter.

The HydroScat-6 has six independent channels, each sensitive to a different narrow range of optical wavelengths. Hobilabs will configure the instrument to 3 wavelength pairs. For the REVAMP project the following wave bands have been selected; 420, 442, 488, 550, 671, 850, plus fluorescence excited by 442 and emitted to 671. The source produces a beam of light in the water, and the detector collects a portion of the light that is scattered out of that beam by the water. Each source beam originates from a light-emitting diode (LED) selected to match the desired measurement

The beam from the LED goes through a lens to adjust its divergence, then through a prism that bends the beam before it enters the water.

Methodology and data quality control

Deployment

The HydroScat can be suspended vertically from the metal eye on the connector endcap, or strapped to another support. If mounting it to another structure, the finish on the case should be , protected from direct metal contact. To ensure that the HydroScat does not detect reflections from any other objects, It is best to keep a clear 30° cone in front of the detection windows for at least 1 meter. Even objects that appear very non-reflective, or are well out of the nominal sampling volume, can create substantial offsets in the backscattering measurement. The operator should manually check that readings are not unnecessarily elevated by interference from other reflective objects. The sensor should normally face directly down in the water, to minimize the effect of background illumination. However in shallow water over a reflective bottom and under bright solar illumination, light reflected into the windows may cause high noise levels or, in extreme cases, saturation. In such situations it may be advantageous to mount the sensor horizontally so that the backscattering receivers do not face the bottom.

Windows

HydroScats have acrylic windows that are easily scratched. Minor scratches will not seriously compromise the measurements, but the windows must be treated carefully to avoid abrasion. Do not use acetone or abrasive cleaners. Do not over-clean the windows. Unless the windows become visibly dirty during use, it is usually sufficient to clean them once daily with soap or alcohol and a soft cloth, then rinse them with clean water whenever they are removed from the water.

Precautions and maintenance

- The instrument windows should always be protected. Ensure that the instrument face is covered whenever the instrument is not in use.
- Do not use acetone to clean any part of the instrument.
- Thoroughly clean the HydroScat with fresh water before storing it.
- Avoid letting the sensor sit in direct sunlight on deck.
- If the water temperature is very different from the temperature on deck, let the instrument stabilize in the water for 10 minutes before collecting data.

General cleaning

After deploying the instrument, rinse it thoroughly with fresh water, and rinse the windows with distilled or deionized water. The windows should be periodically inspected for contamination.

Pressure transducer

If your HydroScat-6 is equipped with an oil-filled pressure reservoir and capillary tube, check the tube occasionally to see that it contains oil. It need not be completely full, but the oil meniscus should be visible. For the HydroScat-6 without oil reservoirs, the pressure transducer is located under a black plastic cap, flush with the rear endcap, with four small drain holes. Rinse the sensor with fresh water by gently spraying it into the drain holes.

Data Processing

The HydroScat software HydroSoft allows you to save calibrated data automatically at the time you collect or download data. Raw data files can also be processed by converting raw hexadecimal data to decimal form without calibrating them.

Calibration coefficients

HydroScat data are transmitted in a partially-processed hexadecimal form, which must be converted to calibrated units. The coefficients required for this conversion are unique to each instrument, and may be revised from time to time when the instrument is recalibrated. HydroSoft requires an appropriate calibration to be loaded before it can plot or store calibrated data from an instrument or raw data file.

Calibration

The weighting function can be measured by moving a spectralon reflective target through the working volume (Maffione and Dana 1997).

The WETLABS instrument

Instrument description

The *ECO-VSF 3* measures the optical scattering at three distinct angles: 100, 125, and 150 degrees, at three wavelengths, thus providing the shape of the Volume Scattering Function (VSF) throughout its angular domain. The three-angle measurement allows determination of specific angles of backscattering through interpolation. Conversely, it also can provide the total backscattering coefficient by integration and extrapolation from 90 to 180 degrees using a 3rd order polynomial according to the VSF manual.



Figure 3. The ECO – VSF 3 backscattering meter.

The optics include three sets of three LED-based transmitters that couple to three receivers. The transmitters and receiver are located to establish centroid light scattering angles of approximately 100, 125, and 150 degrees respectively. For each angle the region of intersection encompasses a full width half maximum (FWHM) bandwidth of about 18 degrees.

Each sensor head operates at one wavelength. Presently there are three wavelengths available; 450 nm, 530 nm, and 650 nm.

Instrument Calibration and quality assurance

Calibration of the *ECO-VSF* involves the determination of angular coefficients through direct measurement of suspensions of NIST traceable standard spherical beads, which are serially diluted. The dilutions are extrapolated to zero, hence the VSF calibration does not include the angular scattering of pure water.

Methodology and processing description

Deployment

The *ECO-VSF 3* requires no pumps to assure successful operation. Once power is supplied, the unit is ready for submersion and subsequent measurements. The sensor faces should not be pointed directly into the sun or other bright lights.

Precautions

- When lowering the instrument, ensure that the mounting brackets are not damaging the unit casing.
- Avoid obstructing the sensors' optical paths. The sensor will detect an object directly in front of its optics.

Upkeep and Maintenance

After each cast or exposure of the instrument to natural water, flush the instrument with clean fresh water, paying careful attention to the sensor face. Use soapy water to cut any grease or oil accumulation. Gently wipe clean with a soft cloth. The sensor face is composed of ABS plastic and optical epoxy and can easily be damaged or scratched. Do not use acetone or other solvents to clean the sensor. At the end of an experiment, the instrument should be rinsed thoroughly, air-dried and stored in a cool, dry place.

Data Processing

ECO Host will convert raw data obtained during a deployment to processed data, alternatively the output is in a simple ASCII format that may be processed by a spreadsheet.

Attenuation coupling

Many scattering sensors require a subsequent attenuation correction for pathlength coupling of the transmitted and scattered light. This is typically a function of the propagation distances of the light as well as the magnitude of the water attenuation. Because the *ECO-VSF 3* incorporates very short pathlengths and scattering volumes in its measurements, it is relatively immune to this pathlength coupling (Figure 7). For attenuation coefficients up to approximately 5 m^{-1} no data correction is required. If you are operating the meter in waters with greater turbidity, a different configuration is required.

Determination of primary angular coefficients

The primary angular coefficients for each angle of backscattering can be applied upon raw data downloaded from the instruments. Determination is made by subtracting the clean water offset from the measured value and multiplying the result by the scaling factors provided in the calibration sheet.

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In situ spectral Beam Attenuation coefficient - $c(z,\lambda)$ [m^{-1}],

In situ spectral Absorption Coefficient - $a(z,\lambda)$ [m^{-1}],

Both at wavelengths 412, 440, 488, 510, 555, 630, 650, 676, 715 nm.



Instrument Description

AC9 - Dual Path Absorption and Attenuation Meter (WET Labs Inc., USA).

The ac-9 concurrently determines the spectral beam attenuation and spectral absorption of water over nine wavelengths.

Optical Specifications:

- Bandpass: 10 nm/channel
- Pathlength: 25 cm
- Beam cross section diameter: 8 mm
- Receiver Acceptance Angle: 0.7 deg (in water)

Fig. 4: AC-9 unit (WETlabs Inc, USA)

Instrument Calibration and Quality Assurance

The protocol proposed by Wet Labs (ac9 Protocol document, Revision B) is followed. The salient points regarding deployment and calibration are highlighted below:

Mounting and Deployment of the instrument.

The instrument is deployed, preferably upright, on a frame lowered into the sea by means of a winch. A small pump brings the water through the ac9 flow tubes (flow rate through the tubes should be kept above 1 liter/minute). All tubing is black or covered with black tape (at least the 20 cm at the flow inlet and outlet) to avoid direct light into the tubes.

The lowering speed, for a frequency of acquisition of 6Hz, should be about 0.1-0.2 m s⁻¹.

Air bubbles passing through or even remaining trapped into the flow tubes when the instrument is at surface and/or in the first meters (according to sea state), can affect measurements and induce differences between down- and up-cast values profiles. Assuming that putting the instrument at depth (at least 10 meters) may help purging the system for bubbles only the up-cast profiles are considered here.

Simultaneous profiles of *in situ* temperature and salinity are collected for post-correcting the data (see 4. *Data Post-Processing*).

Field pure water calibration

The instrument must regularly (once per day of measurement, if possible) be calibrated in the field with pure water (milli-Q water is recommended), **in its deployment configuration**, in order to remove the effects of small misalignments of the optical system and/or to track possible long-term drift. See also the air calibration procedure in WetLabs protocol. The calibration is performed by making milli-Q water pass through

the flow tubes (gravimetrically or by pressurizing the tank) and measuring the resulting offsets. Calibration can simultaneously be done for both *a* and *c* (pressurizing the tank is then recommended) or for each one successively.

Milli-Q water is stocked into a clean tank (polycarbonate carboy for example) at least 12h before the measurement to allow for degassing. Water can be checked for particles by pointing a helium-neon laser through a glass beaker (in the dark) and looking for light flashes that indicate particles (big flashes) or air bubbles (small flashes).

Again, all tubing must be black or covered with black tape (at least the 20 cm at the flow inlet and outlet).

The instrument (flow tubes and optical windows) is cleaned using soap water and methanol.

Water temperature must be recorded, several times during the calibration if necessary, for post-correction (see 4. *Data Post-Processing*).

Measurements are taken for about 30 seconds with the WETVIEW software: the measured offset must be stable (within 0.005) for each wavelength. Average a portion of (stable) data. Such a sequence is repeated 2 times (opening and cleaning the instrument each time) and the measured offsets must not differ by more than 0.005. In particular, during the calibration one has to check for bubbles that can induce large spikes in the data recorded.

After correction for temperature effect (see 4. *Data Post-Processing*) the resulting mean offsets are averaged and subtracted from the *in situ* measurements (corrected for temperature, salinity and scattering).

Methodology and processing description

Temperature and salinity corrections.

After collection, raw data must be corrected for the *in situ* temperature and salinity effects (to correct for differences between the absorption coefficient of the optically pure water used as a reference when calibrating the instrument and the absorption coefficient of the water in which the measurements are performed).

These effects are removed by applying to the measured $c_m(\lambda)$ and $a_m(\lambda)$, the following algorithms:

$$c_{m_{ts}}(\lambda) = c_m(\lambda) - [\psi_t(\lambda) * (T - T_{cal}) + \psi_{sc}(\lambda) * (S - S_{cal})] \quad (1)$$

$$a_{m_{ts}}(\lambda) = a_m(\lambda) - [\psi_t(\lambda) * (T - T_{cal}) + \psi_{sa}(\lambda) * (S - S_{cal})] \quad (2)$$

Where *T* and *S* are the temperature and salinity of the water during measurement, respectively, and *T_{cal}* and *S_{cal}* are the temperature and salinity (in principle = 0) of the water during calibration, respectively.

The ψ_t and ψ_s coefficients used are the following (WetLabs ac9 Protocol Document, Revision B, February 2000) for *c* and *a*:

λ	Ψ_t	Ψ_{sa}	Ψ_{sc}
412	0.0000	0.00018	0.00007
440	0.0000	0.00008	-0.00007
488	0.0000	0.00008	-0.00007
510	0.0002	0.00009	-0.00007
555	0.0001	0.00008	-0.00008
630	0.0002	0.000104	-0.000056
650	0.0001	0.00011	-0.00005
676	0.00008	0.00008	-0.00007
715	0.0029	-0.00018	-0.00032

Scattering corrections of the absorption coefficient.

The portion of the scattered light not collected by the reflecting tube absorption meter causes the instrument to overestimate the absorption coefficient. Presently, three methods mainly are available in order to perform a correction of the measured absorption, with methods #2 and #3 implying that $c(\lambda)$ be measured simultaneously with $a(\lambda)$:

$$\#1) a_{m\text{tsb}}(\lambda) = a_{m\text{ts}}(\lambda) - a_{m\text{ts}}(715),$$

(3)

by assuming no absorption at 715 nm and no spectral dependence of scattering.

$$\#2) a_{m\text{tsb}}(\lambda) = a_{m\text{ts}}(\lambda) - \epsilon * [c_{m\text{ts}}(\lambda) - a_{m\text{ts}}(\lambda)],$$

(4)

by assuming the error as a constant proportion of scattering. Typically, $\epsilon=0.14$ but can vary between 0.08 (phytoplankton dominated) and 0.3 (sediment dominated).

$$\#3) a_{m\text{tsb}}(\lambda) = a_{m\text{ts}}(\lambda) - ([c_{m\text{ts}}(\lambda) - a_{m\text{ts}}(\lambda)] * [a_{m\text{ts}}(715)] / [c_{m\text{ts}}(715) - a_{m\text{ts}}(715)])$$

(5)

by using a reference wavelength (715 nm) to determine the proportion of scattering and also assuming no absorption at this wavelength.

Although method #3 is reputed the most accurate and used as default here, the data provider is let free to propose the most appropriate method for his site.

Primary Quality Checks

Quality checks are performed after temperature, salinity and scattering corrections and when depth-binning the data (level 2), results are written in the log file. In particular, the following criteria must be respected:

- $c_{m\text{tsb}}(\lambda) \geq a_{m\text{tsb}}(\lambda) \geq 0$;
- number of points within the binning layer (1 meter per default) > 1 ;
- depth centroid of data comprised within a layer < 25 % of the binning layer nominal central depth.

Calibration coefficients

The calibration coefficients adopted are:

$Coe0=c$

$Coe1=kt$

Limitations

The use of deployment speeds higher than 0.3 m s^{-1} may reduce the possibility of resolving the vertical structures in water. The presence of air bubbles in the measurement “chambers” may irreparably affect measurements.

References

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Zaneveld, J. R. V., J. C. Kitchen, and C. C. Moore. 1994. "Scattering error correction of reflecting tube absorption meters." *Ocean Optics XII*, S.Ackleson, Ed., Proc. SPIE Vol. 2258, 44-55.

Coloured dissolved organic material (m^{-1})

Also known as Yellow substance, Chromophoric dissolved organic material, Gelbstoff.

Definition

Coloured dissolved organic material is defined as the fraction of organic matter which passes through 0.22 μm pore size filter.

Instrumentation

See section on In-vivo Absorption Spectra of pigmented and non pigmented Particulate Matter (p 4).

Instrument Calibration and quality assurance

- Spectra are visually checked for high background such as high absorption values in the red part of the spectra and abnormal slopes.
- Pure water such as Millipor MilliQ, Alpha Q and Barnstead Nanopore is recommended. Ensure when carrying out optical density measurements of CDOM at sea that this water is available otherwise preparation of pure water prior to field work is recommended.
- The response of the spectrophotometer should be verified with Holmium Oxide filters especially at 412 & 443 nm.

Filtration and Storage

It is essential to minimize contamination of the samples by organic materials and the samples should be protected from light to reduce sample degradation.

- Wash hands with soap and water to avoid contamination of samples.
- Use 0.2 μm polycarbonate filters (Whatman Nucleopore are recommended).
- Filtration apparatus all glass (a funnel, flask and borosilicate filter support) and clenching aluminium pliers. Individual vacuum control of each sample and direct filtration to clean bottles is required.
- Mount filters on funnel and filter 100 mls of purified water through filter and discard water.
- Sea water should be collected into all glass brown bottles direct from Niskin bottles or equivalent. Pre-wash dark bottle three times with seawater and collect 200 ml of seawater.
- *Blank preparation.* Filter 75 ml of MilliQ or bi-distilled water into glass storage bottle and discard the filtrate. Filter a further 75 ml of pure water for use as blank.
- *Sample preparation.* Filter 75 ml of sample into clean bottles at a vacuum pressure of 120 mm Hg. Shake bottles and discard water. Repeat. Filter at least 250 ml of seawater into glass bottles. Cap the bottles and store in the dark.
- *Sample Storage.* Samples can be stored for up to 4 hrs at room temperature before being analyzed. Samples can be stored 4 to 24 hrs in a refrigerator (Mitchell et al. 2000). For longer storage, 0.5 ml solution of 10g/l of NaN_3 per

100 ml of sample (Ferrari et al 1996) can be added to prevent degradation of CDOM and sample bottles should be kept upright in a refrigerator (4°C). However, NaN₃ adds to the absorption of the sample. It is recommended that CDOM samples should be run fresh whenever possible. If NaN₃ is added for prolonged storage, the sample should be flagged in the meta data base.

Measurement procedure:

- If samples have been refrigerated allow them to warm up so that sample and blank are at the same temperature before scanning the samples. Temperature differences between reference water and sample can lead to strong spectral absorption features (Pegau & Zaneveld 1993). Temperature of reference and sample should be recorded for each measurement.
- Inspect the cuvettes. Cuvette should be cleaned with MilliQ and lint free wipes. If surface contamination still persists, soak overnight in 10 % HCl and clean with copious amounts of MilliQ.
- Allow the spectrophotometer to warm up for 30 mins. Confirm that the optical windows are clean. If necessary clean with MilliQ, followed by ethanol HPLC grade, and dry thoroughly with a lint free laboratory tissue.
- The instrument scan speed should be 120 and slit width, 4.
- Run an air vs air baseline. Record the baseline. The baseline should be spectrally flat, with < 0.0005 A units.
- Place one empty cuvette in the spectrophotometer and scan relative to air.
- Perform an autozero from 350 to 800nm as follows; place a cuvette filled with MilliQ water in the sample cell and nothing in the reference cell. Record the spectrum.
- Discard the MilliQ from the cuvette and rinse it three times with 5 to 10 ml of the next sample. Then fill the cuvette with the sample and repeat the scan.
- Run a MilliQ scan between every sample to check the stability of the instrument.

Data Processing

The MilliQ spectra is subtracted from the sample spectra. No scattering offset correction should be performed. The spectral absorption coefficient of dissolved organic matter is calculated from the measured absorbance as follows:

$$A_{ys}(\lambda) = 2.303 A_{ys}(\lambda) / l$$

Where l is the cuvette pathlength.

References.

- Mitchell GB, Bricaud A, Carder K, Cleveland J, Ferrari G, Gould R, Kahru M, Kishino M, Maske H, Moisan T, Moore L, Nelson N, Phimney D, Reynolds R, Sosik H, Stramski D, Tassan S, Trees C, Weideman A, Wieland J, Vodacek A. 2000. Determination of spectral absorption coefficients of particles, dissolved material and phytoplankton for discrete water samples. NASA Tech. Memo. 209966. in GS Fargion and JL Mueller Eds. Ocean Optics Protocols for Satellite Ocean Colour Sensor Validation, Revision 2. NASA Goddard Space Flight Center, Greenbelt, Maryland, pp 125 – 153.
- Mueller, J.L., and R.W. Austin. 1995: Ocean Optics Protocols for SeaWiFS Validation, Revision 1. NASA Tech. Memo. 104566, Vol.25, S.B. Hooker and E.R.Firestone, Eds, NASA Goddard Space Flight Center, Greenbelt, Maryland, 67pp.
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Pigments Concentration by High Performance Liquid Chromatography [mg m⁻³ or µg l⁻¹].

The high performance liquid chromatography (HPLC) method described here (JGOFS, 1994), aims at separating the following phytoplankton pigments: chlorophyll a, chlorophyll b, chlorophyll c, chlorophyllide a, fucoxanthin, 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin, zeaxanthin, alloxanthin, peridinin, diadinoxanthin, diatoxanthin, carotene.



Figure 5: Diode array detector (UV6000LP) and pumping system (P4000) for High performance liquid chromatography.

Instrument description

A thermo separations HPLC with diode array detectors is recommended. The equipment could be constituted of:

- diode array detector UV6000LP (190-800nm), pumping system P4000, vacuum degasser; system controller SN4000 (Fig 3).
- reverse phase column Alltech MOS 2 C8 100 x 4.6mmcm
- computer equipped with hardware and software (chromquest);
- 100 µl sample loop (Rheodyne);
- air compressor
- centrifuge

Instrument Calibration and Quality Assurance

Determination of pigment response factors

The HPLC system is calibrated with the pigment standard obtained from VKI¹. Concentrations of the pigment standard are given from VKI but are also checked using a spectrophotometer. The extinction coefficients used are given by VKI.

¹International Agency for ¹⁴C determination VKI Water Quality Institute Agern Allé 11, DK- 2970 Hørsholm, Denmark

Pigments standard concentrations (C_p) are calculated as follows:

$$C_p = [(A_\lambda - A_{750}) / (E_{1\text{cm}} * l)] * 10^6$$

C_p	=	pigment concentration of standard ($\mu\text{g l}^{-1}$)
A_λ	=	absorbance at wavelength λ nm (Table I)
A_{750}	=	absorbance at 750 nm to correct for light scattering
$E_{1\text{cm}}$	=	extinction coefficient $E_{1\text{cm}}$ ($\text{l g}^{-1} \text{cm}^{-1}$) (Table I)
l	=	cuvette pathlength (cm)
10^6	=	conversion factor g to μg

A recalibration of the HPLC with pigments standard is recommended every 3-4 months. The recalibration with respect to internal standard should be performed every day.

Methodology and Processing Description.

Methodology of Sample Processing: *Sampling collection and storage*

For each seawater sample, 1.5 to 2 liters are immediately filtered after collection through a Niskin bottle (or other) using 25 mm GF/F filter. The filter is then folded in half twice and placed into a labeled cryovial and stored in liquid nitrogen until laboratory analysis.

Pigment extraction and sample preparation

For pigment extraction 2 ml of 90 % acetone is added to the filter which is ultrasonicated using an ultrasonic probe for 20 secs. The extracting solvent also has an internal standard (typically Apo-8'-Carotenal (trans)). The concentration of internal standard must be chosen in such a way that pigments and standard peak areas are comparable.

After extraction, the sample is micro centrifuged for 2 minutes. The extract is then injected through a 100 μl loop into the HPLC system.

Analysis program

The solvent systems used are as follows:

- solvent A = 70:30, methanol : 1M ammonium acetate

- solvent B = methanol

-The flow rate is 1-ml min^{-1} with the following gradient:

Time (min.)	% A	% B
0.0	75	25
1.0	50	50
20.0	30	70
25.0	0	100
30.0	0	100
30.1	75	25
39.0	75	25

Processing description

The concentration of each pigment (C_p in $\mu\text{g l}^{-1}$) is computed according to:

$$C_p = (A_p * W_{is} * f_p^{is}) / (A_{is} * v_{filt})$$

A_p = peak pigment area

V_{filt} = volume filtered (l)

W_{is} = internal standard weight (μg)

A_{is} = internal standard area

f_p^{is} = relative response factor for each pigment

Quality Assurance

Use an internal standard, pigment standards are authenticated by VKI, Quasimeme membership.

Sample Storage

If filters are not analyzed immediately, they should be flash frozen and stored in cryovials or petri dishes in liquid nitrogen. Mantoura et al. (1997) found that liquid nitrogen is the best form of sample preservation. The storage of filters in ultra codl freezers (-90°C) also achieves excellent pigment recovery with minimum degradation. Long term storage of samples in -20°C freezers is not recommended, but can suffice for short term (1 wk) storage. Freeze drying causes rapid loss and extensive degradation of chlorophylls and carotenoids and is therefore not recommended.

Limitations

The detection limit of this technique is about $0.001 \mu\text{g l}^{-1}$.

Divinyl-chlorophyll a and b are distinguished using reverse phase C-8 HPLC and the methods described in Barlow et al. (1997).

References

Barlow, R.G., D.G. Cummings and S.W. Gibb. 1997. Improved resolution of mono- and divinyl chlorophylls a and b and zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC. *Mar. Ecol. Prog. Ser.*, **161**: 303-307.

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Surface Downwelling Spectral Irradiance, $E_s(\lambda)$ ($\text{W m}^{-2} \text{nm}^{-1}$)
Subsurface Downwelling Spectral Irradiance, $E_d(z,\lambda)$ ($\text{W m}^{-2} \text{nm}^{-1}$)
Subsurface Upwelling Spectral Radiance, $L_u(z,\lambda)$ ($\text{W m}^{-2} \text{nm}^{-1} \text{sr}^{-1}$)
Surface Downwelling Diffuse Spectral Irradiance over Direct Spectral Irradiance $r(\lambda)$

$E_s(\lambda)$ is normally measured at the nominal MERIS visible bands.

$L_u(z,\lambda)$ measurements are taken to derive the subsurface upwelling radiance $L_u(0^-, \lambda)$. $E_d(z,\lambda)$ measurements are taken in order to derive the diffuse attenuation co-efficient $K_d(z,\lambda)$ and the subsurface downwelling irradiance $E_d(0^-, \lambda)$.

The ratio $r(\lambda)$ between the Surface Downwelling Diffuse Spectral Irradiance and the Direct Spectral Sun Irradiance is computed from $E_{sky}(\lambda)/(E_s(\lambda)-E_{sky}(\lambda))$ where $E_{sky}(\lambda)$ is the Diffuse Sky Irradiance and Direct Sun Irradiance.

Attitude measurement of the $E_s(\lambda)$ sensor is recommended when the instrument is installed on non-stable platforms (i.e. ships). The attitude of the $E_d(z,\lambda)$ and $L_u(z,\lambda)$ sensors must be measured during profiles. Sensor depth must also be determined with high accuracy.

Instrument description

The measurement system consists of a compact seven channel analog sensor capable of 16-bit performance. The analogue signals are digitized by a 16-bit a/d unit (DATA-100). Data is transferred by the DATA-100 as RS232 or RS422. The data acquisition rates are fully programmable, but the normal data stream uses the default of 8 Hz sampling.

Physically the $E_s(\lambda)$ sensor is mounted on a pole clear of any shading structures.

The E_d and L_u sensors are mounted on a profiling rig designed to minimize any shading from close devices.



Figure 6: Satlantic sensor head for $E_s(\lambda)$ measurements.

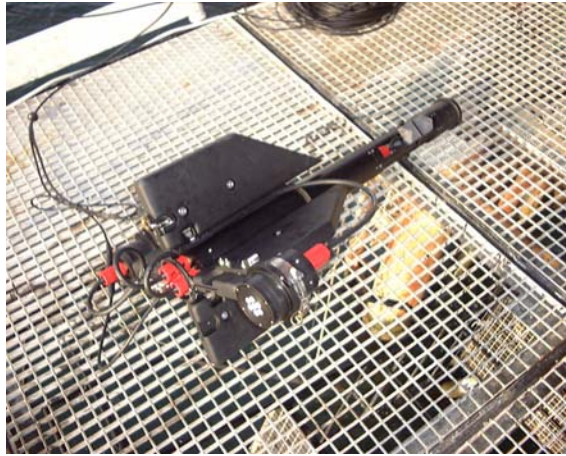


Figure 7: Free-falling profiler unit (Satlantic, Halifax)

Instrument Calibration and Quality Assurance

The calibration methodology is fully described in the NASA SeaWiFS protocols (Mueller & Austin, 1992). In summary the irradiance sensors are calibrated using an FEL 1000W lamp traceable to the NIST scale (Walker et al, 1987), while the radiance sensors can be calibrated with an integrating sphere or with an FEL 1000W lamp and a reference 99% reflectance plaque. The sensors are referenced to the JRC NIST traceable standard lamp through a reference set of sensors maintained by JRC. On each deployment the actual offset is determined by taking a dark reading immediately before deployment.

Methodology and Processing Description.

Deployment of the instrument

The optical measurements should be taken in stable illumination conditions.

The Ed and Lu sensors must be deployed towards the sun or the brightest part of the sky (i.e. the ship or the platform should not shade the instrument). The lowering and raising speed of the in water profiling system (used for Ed and Lu measurements) should be adequate for Case II waters. There should be 100 samples for each optical depth when the $K_d(490)$ is 0.25m^{-1} and for SATLANTIC instruments this corresponds to 0.3 m s^{-1} . Where waters are more turbid a lower speed should be used. The pressure sensor should be checked prior to deployment to remove the effect of on barometric pressure changes.

E_{sky} measurements are taken by shading the direct sun irradiance to the E_s sensor making use of the small disc located at some distance from the instrument (at least 50 cm). It is recommended to take E_{sky} and E_s measurements in sequence.

Description of processing techniques employed

Primary quality control includes data screening for any rapid change in $E_s(\lambda)$, and ensuring that profiles are smooth in log /linear scale.

The data presented for the level 1 archive must be corrected for dark. The most recent calibration factors available should be included in the level 1 file. If any consistent change in calibration is found during field work activities, then the data should be re-submitted to the level 1 archive with a modified calibration date.

Primary Quality Checks

Stability of skylight

Removal of records with bad tilt / roll (higher than five degrees)

Removal of records below instrument noise

Primary Processing

Normalization of $E_d(z,\lambda)$ and $L_u(z,\lambda)$ making use of $E_s(\lambda)$

Calculation of $K_d(\lambda)$ and $K_l(\lambda)$

Calculation of $R_{rs}(0^-, \lambda)$ making use of $L_u(0^-, \lambda)$ and $E_d(0^-, \lambda)$

Spectral consistency of $K_d(\lambda)$ and $K_l(\lambda)$

Calibration coefficients

Calibration and quality assurance as per NIST.

Limitations

Sensor tilt induced by ship roll should produce significant errors on normalized values of L_u and E_d . Surface effects induced by rough sea can induce significant noise in L_u and E_d measurements.

Non stable illumination during the sequential measurements of E_{sky} and E_s could induce erroneous values of r .

References

- Mueller, J. L. & Austin, R. W. (1992) Ocean Optics Protocols for SeaWiFS Validation. SeaWiFS Technical Report Series. NASA Tech. Memo. 104566. 5, 43 pp.
- Walker, J. H., Saunders, R. D., Jackson, J. K. & McSparron, D. A. (1987) NBS Measurement Service: Spectral Irradiance Calibrations. Report NBS/SP-250/20, National Bureau of Standards, Gaithersburg, MD 20899, USA.

Spectral Sky Radiance - $L_{\text{sky}}(\lambda)$
Spectral Direct Sun Irradiance - $E_{\text{sun}}(\lambda)$

Both at wavelengths 440, 670, 870, 940, and 1020nm

Instrument description CIMEL 318 Sun Photometer



Figure 8: CE- 318 Sun photometer

The CIMEL (Paris, France) CE-318 Sun photometer is a radiometer designed to perform atmospheric studies, specifically to determine the optical characteristics of the aerosols.

It is made up of three parts:

- a programmable box that controls the measurement sequences
- a mobile device with two rotational axes (azimuthal and zenithal)
- a sensor head, fixed on the mobile device

The instrument is powered with solar panels and rechargeable batteries.

The optical part of the instrument includes at least five filters: four, to study the aerosols characteristics: 440, 670, 870, 1020 nm (10 nm wide) and one, to determine the water vapour: 940 nm (10 nm wide). The filter wheel includes a dark mask, which is used to determine the dark current. Between the filter wheel and the electronic part, there are two collimators, one used for sky radiance measurements (SKY collimator), the other used for both sky measurements and direct sun measurement (SUN collimator).

Instrument Calibration and Quality Assurance.

The CE-318 calibration for radiance measurements is performed with an integrating sphere. Inter-calibration with a portable radiometer (calibrated with the same integrating sphere) is occasionally performed. Independent calibration is also performed at 440 and 670 nm, using the Rayleigh scattering calibration technique.

The CE-318 calibration for irradiance measurements is performed every two months using the Langley-Bouguer method (weather permitting) applied to data from the measurement sites. Inter-calibration, with the portable radiometer is occasionally performed for the direct sun irradiance measurements.

The Quality Assurance of CE-318 data is mostly addressed to remove contamination by cirrus following the methodology used in AERONET (Holben et al., 1998).

Methodology and Processing Description.

Deployment of the instrument

The sky measurements are made using two different procedures: I) the Almuquantar procedure; and ii) the Principal Plane procedure. The sun measurements are made using the Sun procedure.

During the Almuquantar procedure, the CE-318 points at the sun and then takes measurements with fixed sun zenith angle at different azimuth angles over 360 degrees. During the Principal Plane procedure the CE-318 points at the sun and takes measurements at different zenith angles in the sun plane.

During the Sun procedure, the CE-318 points at the sun and takes irradiance measurements for each wavelength (these measurements are repeated 3 times to check the stability).

Methodology for sample collection

CE-318 data are regularly transmitted to the AERONET server at NASA-GSFC through a satellite link (Holben et al., 1998). Data are then downloaded twice a month by ftp to the LISE/ULCO laboratory to produce calibrated data. Several aerosol high-level products (i.e. scattering phase function, aerosol downward fluxes) are generated (ULCO, 1998).

Primary quality checks before submission of Level 1 data

- Screening data for rapid variability (temporal and angular) of measurements taken in the principal plane (off solar views).
- Screening data for rapid variability (temporal and angular) of measurements taken in the almuquantar.
- Checking the symmetry of the almuquantar (versus the solar plane)
- Checking the variability of the triplet of sun irradiance measurements
- Screening data for very rapid temporal variability of the optical thickness
- Thresholding of the sun irradiances with boundary values
- Checking the spectral dependency of the optical thickness

Calibration coefficients

The calibration coefficients adopted are as follows:

Coe1=sun_exoatmospheric_irradiance

Coe2=sun_radiances_cal

Coe3=sky_radiances_cal

Limitations

Cloudless conditions are required.

References

University du Littoral Côte d'Opale, March 1998, "Ground- based atmospheric measurements during the COLORS experiment" Report, Version 1.0.

Holben et al., 1999, "AERONET-A Federated Instrument Network and Data Archive for Aerosol Characterization", Remote Sensing of Environment, 66: 1-16, 1998

Total Suspended Matter - TSM (g m^{-3})

Also known as Suspended particulate material.

Instrument description

Electro-balance.

Definition

The net weight of material collected on a GF/F by sea water filtration.

Units: mg l^{-1} , g m^{-3} .

Instrument calibration and quality assurance

The electrobalance should be accurate to at least 10^{-4} g. The electro balance zero should be checked before weighing.

Methodology

Filter preparation

- GF/F filters ($0.7 \mu\text{m}$) are pre-ashed at 450°C for 1 hr.
- Filters are then pre-washed in MilliQ to remove friable fractions that can be dislodged during filtration. Soak not more than 20 filters at a time together for 5 mins in 0.5 l of MilliQ.
- Place the filters on the shiny surface of clean aluminium foil.
- Dry the filters in a hot air oven at 75°C for 1 hr.
- Store filters in a dessicator with dry silica gel.
- Pre- weigh dry filters to 5 significant figures noting the temperature and humidity in the weighing chamber.

Filtration

- A volume of seawater should be filtered through pre-washed, pre-ashed, pre-weighed $0.7 \mu\text{m}$ filters. The volume of seawater filtered is dependent on the amount of material present in the water and should be sufficient to detect weights to 5 significant figures.
- Water samples should be filtered immediately on collection. If this is not possible, it is recommended that 1 ml of 4 % formalin per litre of sea water is added to the water sample. Multiple replicates should be taken to quantify sample variability. A blank filter should be used for each sample, to calculate the handling error of the sample.
- After filtration leave the filter on the glass frit and the filtration apparatus standing. Filter at least 50 mls of distilled water through the filtration apparatus to remove any salt. Repeat this procedure three times. With the vacuum pressure still on, carefully remove the filtration cup and using a wash bottle gently wash the outer edge (unfiltered area) of the filter. The filters should then be dried in an

oven at 75°C for 24 hrs after which they are stored in a dessicator before weighing (See Van der Linde 1998).

TSM concentration

is deduced from the difference between original filter weight minus sample filter weight divided by filtration volume.

Limitations

Non accurate washing of filters could induce very large errors in the derived TSM values.

References

- J.D.H. Strickland and T.R. Parsons, 1972. A practical Handbook of seawater Analysis, 8, 181-184.
- D. Van der Linde, Protocol for Total Suspended Matter estimate. JRC Technical Note. June 1998.

Above-water Water Leaving Radiance, L_w ($\text{Wm}^{-2}\text{nm}^{-1}\text{sr}^{-1}$) and Downwelling Irradiance, E_s ($\text{W m}^{-2}\text{nm}^{-1}$)

Instrument Description

The PR-650/640 is a hand-held portable, battery powered spectroradiometer manufactured by Photo Research. The instrument measures radiance within a 1° aperture angle in 101 wavelength bands from 380-780nm in 4 nm steps. Full-width-half-mean is 8 nm. The detector integration time is varied automatically to provide the necessary dynamic range.

(Fig 9).



Figure 9 : PR650 instrument PR®-650/640 SpectraColorimeter™ System.

A 1° field of view is used with the PR650 for measuring $L_t(\lambda, \theta, \phi)$, the radiance emanating from the water surface, and the sky radiance $L_{sky}(\lambda, \theta, \phi)$. The downwelling irradiance is measured from a calibrated Lambertian reflectance panel. Alternatively, a cosine collector can be used with the PR640 to measure the incident spectral irradiance $E_s(\lambda)$. Photometric and colorimetric accuracy is assured by virtue of the fact that the PR-650/640 measures sources spectrally by diffracting the visible simultaneously over the 128

Regardless of the spectral distribution of the source, be it a CRT or an incandescent lamp, the correct luminance and color values displayed without special calibration. The operating program, calibration factors, and the capacity for storing over measurement files reside on the standard 256 Kbyte PCMCIA card (Personal Computer Memory Card International Association). The PR-650 incorporates Automatic Adaptive Sensitivity that optimizes the detector signal to noise for accurate measurement regardless of the signal level.

QA and data processing details

The radiance measurement of the reflectance standard is used to calculate above-water downwelling irradiance $E_s = \pi \frac{L_p}{\rho_{panel}}$, where ρ_{panel} is the reflectance of the reflectance standard (~99%). The standard is measured under an angle of 45 degrees.

The MERIS reflectance can then be calculated as $\rho_w = \frac{\pi L_w}{E_{ad}}$, where L_w , the water leaving radiance is calculated as $L_w = L_t - \rho_{sky} L_{sky}$, with ρ_{sky} is the effective Fresnel reflection coefficient for the wind-roughened sea surface.

Instrument Calibration and Quality Assurance

The absolute radiometric response for each radiometer is determined at the start and end of the project using an NIST standard 1000W lamp. A Photo Research near-Lambertian calibrated spectralon reflectance standard (\varnothing 5 cm) of about 99% reflectance is used as a reflective standard to calibrate the instrument. Because reflectance is a relative quantity, the absolute radiometric calibration has no influence on the accuracy of the derived water leaving reflectance, provided that L_t , L_{sky} and E_s are measured with the same instrument.

Methodology and Processing Description

The PR650, from an altitude of 2-4 m above the sea, is pointed towards the sea surface 135 degrees azimuth away from the sun with a viewing angle of 35-40 degrees. The downwelling irradiance is measured from a calibrated Lambertian reflectance panel, or E_s is simultaneously measured with a PR640 looking straight upwards (cosine). At each station, reflectance is measured at least three times as quick as possible to reduce effects of changing water masses and illumination conditions. Preferable position on the ship is on the bow, to minimize surface wave effects and shading and/or reflectance from the ship's superstructure.

In general each reflectance measurement consists of four radiance measurements

1. radiance emanating from the water surface L_t
2. radiance from the sky L_{sky}
3. radiance from the reflectance standard L_p , or simultaneously with a PR640
4. (optionally) radiance from the shaded reflectance standard L_{pr}

Each radiance measurement is an average of five readings, internally averaged by the radiometer. The sky radiance is measured to correct the total surface radiance for sky radiance reflected at the sea surface to yield water-leaving radiance $L_w = L_t - \rho_{sky} L_{sky}$, where ρ_{sky} is the effective Fresnel reflection coefficient for the wind-roughened sea surface (Fargion and Mueller 2000). The measurement of the shaded reflectance panel is not required for calculating MERIS reflectance, but can be used to derive the fraction diffuse/total downwelling irradiance, which serves as input in numerical radiative transfer code such as Hydrolight.

Limitations

Foam caused by waves. Low sun heights can cause high contributions of sun-glint. The PR650 cannot be operated under rainy conditions because the instrument is not water proof.

References

Fargion, G.S., J.L. Mueller, (2000) *Ocean Optics Protocols For Satellite Ocean Color Sensor Validation, Revision 2*, NASA/TM-2000-209966, Goddard Space Flight Space Center, Greenbelt, Maryland, USA, 184 p.

Mobley,C.D., (1999) Estimation of the remote-sensing reflectance from above-surface measurements. *Appl. Opt.*, Vol. 38, No. 36, p. 7442-7455.
Mueller and Austin 1995 Volume 25, SeaWiFs Techn. Rep. Ser. Chapter 6.2

Above-water MERIS reflectance, $\rho_w(\lambda)$ (dimensionless) –

TriOS method

The MERIS reflectance, $\rho_w(\lambda)$, as defined by:

$$\rho_w = \pi \frac{L_w(\lambda)}{E_s(\lambda)}$$

is calculated from simultaneous above-water measurements of downwelling irradiance, $E_s(\lambda)$, radiance from the water surface, $L_t(\lambda)$ and sky radiance, $L_{sky}(\lambda)$. The latter two measurements are used to calculate the intermediate parameter, $L_w(\lambda)$, the water-leaving radiance (after removal of air-sea interface reflection). This method corresponds to “Method 1” of (Mueller et al. 2000). Results of the method as used for MERIS Validation are presented in (Ruddick et al. 2002).



Figure 10. (left) System of two radiance and one irradiance sensor installed on steel frame. (right) As installed at prow of ship with irradiance sensor mounted separately to reduce optical interference from mast.

Instrument description

The measurement system consists of three TriOS-RAMSES hyperspectral spectroradiometers, two measuring radiance and one measuring downwelling irradiance with a cosine collector.

The sensors measure over the wavelength range 350-950nm with sampling approximately every 3.3nm with spectral width of about 10nm. The sensors are based on the Carl Zeiss Monolithic Miniature Spectrometer (MMS) incorporating a 256 channel silicon

photodiode array. Integration time varies from 4ms to 8s and is automatically adjusted to measured light intensity. The data stream from all three instruments is integrated by a IPS-104 power supply and interface unit and logged on a PC via a RS232 connection. The radiance sensors have a field of view of 7°. A two-axis tilt sensor is incorporated inside the downwelling irradiance sensor. The instruments are mounted on a steel frame, similar in concept to that used by (Hooker and Lazin 2000). The frame is fixed to the prow of the ship, facing forwards to minimise ship shadow and reflection and 1-8m above the water surface. Where necessary to avoid optical interference the downwelling irradiance sensor is mounted separately elsewhere on the ship.

Instrument Calibration and Quality Assurance

The instruments are calibrated twice per year at NIST-traceable facilities in the framework of MERIS Validation Team workshops.

Methodology and Processing Description.

Deployment of the instrument

Before measurements the frame is levelled horizontally and the sea and sky-viewing angles are fixed at 40° with respect to zenith and viewing in the same azimuth angle. In this way the sky is viewed in the direction from which light will enter the sea-viewing sensor after reflection at a flat sea surface. The radiance sensor lenses and the irradiance sensor collector are inspected manually before each measurement and are cleaned of spray and dust when necessary. The ship is manoeuvred on station to point the radiance sensors at a relative azimuth angle of 135° with respect to sun. When the correct position and angle are achieved measurements are started and continue for 10 minutes, taking a scan of the three instruments every 10s. During measurements wind speed is recorded and sea, sun and sky state conditions are noted, especially if variable because of cloud movement or floating matter. The ship position and orientation are monitored for drift. Lens caps are used to protect all three sensors except during the 10 minute measurement sequence.

Measurements can also be made underway for a ship heading of 135° relative to sun, providing a transect of reflectance spectra. For such measurements the lenses are inspected at the end of the transect and any spray droplets are noted. During such measurements visual checks are made of the sea surface for variability such as fronts or floating material and the ship heading is monitored.

Description of processing techniques employed

Data is acquired with the MSDA software (v1.94 in 2001-2002) using the file recorder function and calibrated radiometrically using nominal calibration constants. Dark values are removed with the “dynamic offset” function, which uses blocked photodiode array channels. Calibrated data for $E_s(\lambda)$, $L_t(\lambda)$ and $L_{sky}(\lambda)$ is interpolated to 2.5nm intervals and exported to Excel for recalibration to the MERIS Validation Team standard and for further processing.

Preprocessing Quality Checks

The multitemporal dataset is screened to:

- Remove dropout (incomplete spectra)
 - Avoid measurements during temporal fluctuations of $E_s(\lambda)$, arising mainly from clouds or haze passing in front of the sun
 - Avoid measurements during strong temporal fluctuations of $L_{sky}(\lambda)$, arising mainly from variable cloudiness in the sky-viewing direction
 - Avoid outliers of $L_t(\lambda)$
 - Avoid measurements with high tilt or roll (greater than five degrees)
- Five scans of $E_s(\lambda)$, $L_{sky}(\lambda)$ and $L_t(\lambda)$ are used for further processing.

Data Processing

The water-leaving radiance is calculated by,

$$L_w = L_t - \rho_{sky} L_{sky}$$

where ρ_{sky} , the air-sea interface reflection coefficient, is estimated for sunny conditions from Figure 9 of (Mobley 1999) as function of wind speed in m/s, W :

$$\rho_{sky} = 0.0256 + 0.00039 * W + 0.000034 * W^2$$

The reflectance, $\rho_w(\lambda)$, is then calculated for each scan and the mean and standard deviation over the five scans are calculated and plotted.

Postprocessing Quality Checks

Reflectance spectra are inspected subjectively to ensure:

- limited variability over scans (comparing standard deviation with mean)
 - internal consistency of spectra in red and near infrared (positive reflectances with reflectance ratios given approximately by the inverse ratio of pure water absorption)
- Measurements outside the range 400-900nm are not used for scientific analysis because of high uncertainty and instrument noise.

Limitations

- Measurement uncertainties associated with the air-sea interface reflection correction become significant in conditions of cloudy sun (and to a lesser extent cloudy sky in the sky-viewing direction) and high wind. Such uncertainties are relatively more important for clearer waters.
- Measurement uncertainties increase for underway measurements because of increased tilt/roll and possible contamination of lenses by spray.
- Underway measurements from small ships, e.g. Rigid Inflatable Boats, are limited to calm sea state (e.g. $Bf \leq 3$) to avoid excessive tilt and roll.

References

Hooker, S. B. and G. Lazin (2000). The SeaBOARR-99 Field Campaign. Greenbelt, Maryland, NASA: 46.

- Mobley, C. D. (1999). "Estimation of the remote-sensing reflectance from above-surface measurements." *Applied Optics* **38**: 7442-7455.
- Mueller, J. L., C. Davis, R. Arnone, R. Frouin, K. Carder, Z. P. Lee, R. G. Steward, S. Hooker, C. D. Mobley and S. McLean (2000). Above-water radiance and remote sensing reflectance measurements and analysis protocols. *Ocean Optics protocols for satellite ocean color sensor validation Revision 2*. Greenbelt, Maryland, National Aeronautical and Space Administration: 98-107.
- Ruddick, K., V. De Cauwer, Y. Park, G. Becu, J.-P. De Blauwe, E. D. Vreker, P.-Y. Deschamps, M. Knockaert, B. Nechad, A. Pollentier, P. Roose, D. Saudemont and D. v. Tuyckom (2002). *Preliminary validation of MERIS water products for Belgian coastal waters*. Envisat Validation workshop, 9-13th December 2002, Frascati, European Space Agency.

SIMBADA method

The MERIS reflectance, $\rho_w(\lambda)$, as defined by:

$$\rho_w = \pi \frac{L_w(\lambda)}{E_s(\lambda)}$$

is calculated from sequential above-water measurements of the vertically polarised component of radiance from the water surface, $L_t^{pol}(\lambda)$ and sun radiance, $L_{sun}(\lambda)$. $L_w(\lambda)$

is calculated from $L_t^{pol}(\lambda)$ after correction for residual air-sea interface reflection and downwelling irradiance, $E_s(\lambda)$, is calculated from $L_{sun}(\lambda)$ using an atmospheric model.

This method corresponds to “Method 3” of (Mueller et al. 2000). Full details of the method and processing can be obtained from the Laboratoire d'Optique Atmosphérique of the University of Lille, France. Results of the method as used for MERIS Validation are presented in (Ruddick et al. 2002).



Figure 11. (left) View of SIMBADA showing foreoptics. (right) As used in sun-viewing mode.

Instrument description

As described in the SIMBADA User’s Guide (http://www-loa.univ-lille1.fr/recherche/ocean_color/src/) the SimbadA instrument is an above-water radiometer designed and manufactured by the Laboratoire d'Optique Atmosphérique of the University of Lille, France. It measures water-leaving radiance and aerosol optical thickness in 11 spectral bands (each bandwidth of 10nm), centered at 350, 380, 410, 443, 490, 510, 565, 620, 670, 750, 870nm, by viewing the sun and the ocean surface sequentially.

The same optics, with a field-of-view of about 3°, the same interference filters, and the same detectors are used in both ocean-viewing and sun-viewing mode. A different electronic gain, high and low, is used for each mode, respectively. The optics are fitted with a vertical polarizer, to reduce reflected skylight when the instrument is

operated in ocean-viewing mode. Pressure, temperature, and viewing angles are also acquired automatically.

An integrated GPS antenna acquires automatically the geographic location at the time of measurement and a display indicates various information.

Instrument Calibration and Quality Assurance

The instruments are calibrated at the Université de Lille – detailed calibration histories for each instrument can be found on the SIMBADA web site.

Methodology and Processing Description.

Deployment of the instrument

The instrument is operated from the deck of a ship using the measurement sequence: Dark (with lens cap on), 3*Sun, 3*Sea, 3*Sun, Dark. The ship is manoeuvred on station to point to a ship heading of 135° with respect to sun. Sun measurements are made from anywhere offering a clear view of the sun. Sea measurements are made from the prow of the ship, pointing forwards at relative azimuth angle of 135° with respect to sun and zenith angle of approximately 40° . The complete measurement sequence lasts approximately 5 minutes.

During measurements wind speed, atmospheric pressure, cloud cover and type, and sea and sky state conditions are noted. Any variability in illumination (e.g. clouds passing near sun) requires a restart of the measurement sequence.

Measurements can also be made underway for a ship heading of 135° relative to sun. During such measurements visual checks are made of the sea surface for variability such as fronts or floating material and the ship heading is monitored.

Description of processing techniques and quality checks

Data is processed at the Université de Lille. Reflectances are given for 10 wavelengths, excluding the 870nm band. The processing method is outlined on the SIMBADA web site. The use of a polarizer to reduce air-sea interface reflection is discussed in (Fougnie et al. 1999).

Limitations

- Measurements can only be in clear sun and clear sky (cloud cover $\leq 2/8$) conditions to ensure accurate calculation of $E_s(\lambda)$. Cloud cover is estimated subjectively.
- Measurements from small ships, e.g. Rigid Inflatable Boats, are limited to calm sea state (e.g. $Bf \leq 3$) to ensure accurate sun-pointing.

References

- Fougnie, B., R. Frouin, P. Lecomte and P.-Y. Deschamps (1999). "Reduction of skylight reflection effects in the above-water measurement of diffuse marine reflectance." *Applied Optics* **38**(18): 3844-3856.
- Mueller, J. L., C. Davis, R. Arnone, R. Frouin, K. Carder, Z. P. Lee, R. G. Steward, S. Hooker, C. D. Mobley and S. McLean (2000). Above-water radiance and remote

sensing reflectance measurements and analysis protocols. Ocean Optics protocols for satellite ocean color sensor validation Revision 2. Greenbelt, Maryland, National Aeronautical and Space Administration: 98-107.

Ruddick, K., V. De Cauwer, Y. Park, G. Becu, J.-P. De Blauwe, E. D. Vreker, P.-Y. Deschamps, M. Knockaert, B. Nechad, A. Pollentier, P. Roose, D. Saudemont and D. v. Tuyckom (2002). Preliminary validation of MERIS water products for Belgian coastal waters. Envisat Validation workshop, 9-13th December 2002, Frascati, European Space Agency.

APPENDIX A

Experiments on In-vivo Absorption Spectra of pigmented and non pigmented Particulate Matter - $a_{pm}(\lambda)$ (m^{-1}) protocols.

Gavin Tilstone, David Blondeau, Gerald Moore & Rüdiger Röttgers.

Introduction

Laboratory experiments were conducted to assess the following aspects of in-vivo particulate matter absorption protocols:

- *Instrument performance and accuracy*
- *De-pigmentation agent*
- *Blank Filter*
- *Data processing*

INSTRUMENT PERFORMANCE AND ACCURACY

Comparison of Spectrophotometers.

A high precision instrument using latest double beam, double monochromator optics technology with stable baseline, low stray light & signal:noise ratio, high photometric accuracy is required for satellite data / algorithm validation. The instrument should have the following accessories; long path length cell holder to 100 mm & thermo regulator (for determination of optical density of CDOM), integrating sphere for transmission – reflectance measurements (for absorption coefficient). A NASA SeaWiFS protocols workshop showed that Cary spectrophotometers are suitable for accurate determination of inherent optical properties.

Instruments considered

During the REVAMP intercalibration held at Plymouth Marine Laboratory we compared the following instruments: Beckman DU 800, Hitachi U-3310, Perkin Elmer Lambda 800, UVIKON XL.

Table A1. Instrument characteristics; Out standing optical characteristics are highlighted in red.

	Perkin Elmer L800	Hitachi U3310	UVIKON XL	Beckman DU 800
Operating principle	Double beam UV / Vis	Double beam UV / Vis	Double beam UV / Vis	Single beam UV / Vis
Optical system	True double mono chromator	Single and pre – mono chromator	Single mono chromator	?? Not given
Wavelength range (nm)	190 – 900	190 – 900	180 – 900	190 - 1100
Spectral bandpass (nm)	Selectable 0.05 to 5 in 0.01 increments	Selectable 0.1 to 5 fixed	0.2, 1, 2, 4	0.2, 1, 2, 4
Stray light Reduction	<0.00008 %	<0.0003 %	< 0.015 %	< 0.05 %
Wavelength accuracy (nm)	± 0.08	± 0.30	± 0.25	± 0.20

Photometric Accuracy	$\pm 0.0006A$	$\pm 0.002 A$	$\pm 0.003 A$	$\pm 0.005 A$
Baseline flatness	$\pm 0.001 A$	$\pm 0.001 A$	$\pm 0.001 A$	$\pm 0.001 A$
Photometric Noise RMS	$\pm 0.0002 A$?? not listed	$\pm 0.0004 A$	$\pm 0.0002 A$

Instrument performance and suitability

Beckman DU 800

Consumer market; molecular biology, nucleic acid, protein assay, enzyme analysis.

- **Suitability**

Optics unsuitable; single beam rather than double. NASA only recommend dual beam instruments for bio-optics and satellite data calibration. Stray light reduction, wavelength and photometric accuracy low. Integrating sphere and long path cell holder to 100 mm not commercially available.

- **Instrument performance**

Comments; baseline noise high, electrical connections unstable especially at sea.

UVIKON XL

Consumer market; medical science.

- **Suitability**

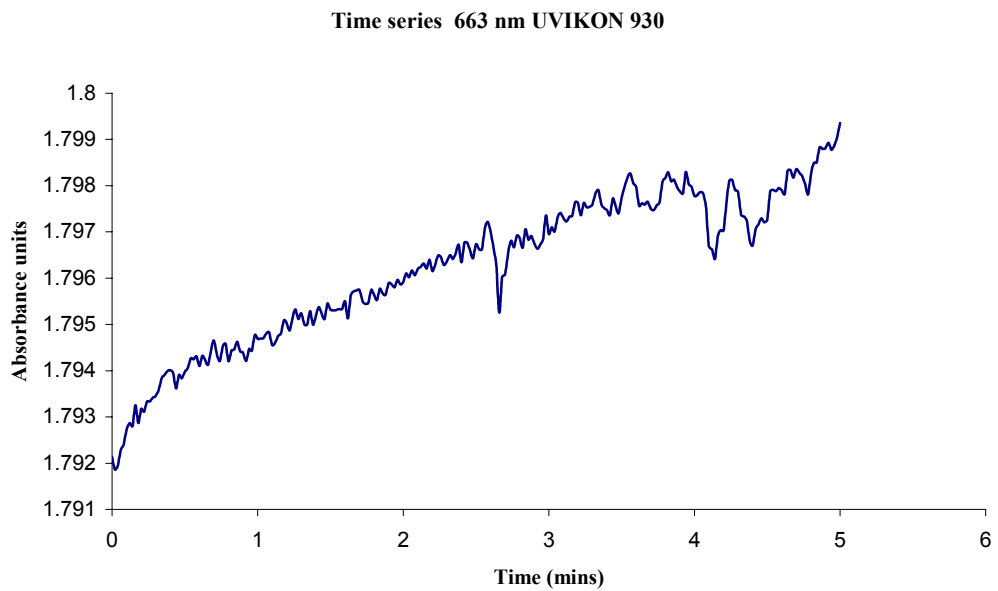
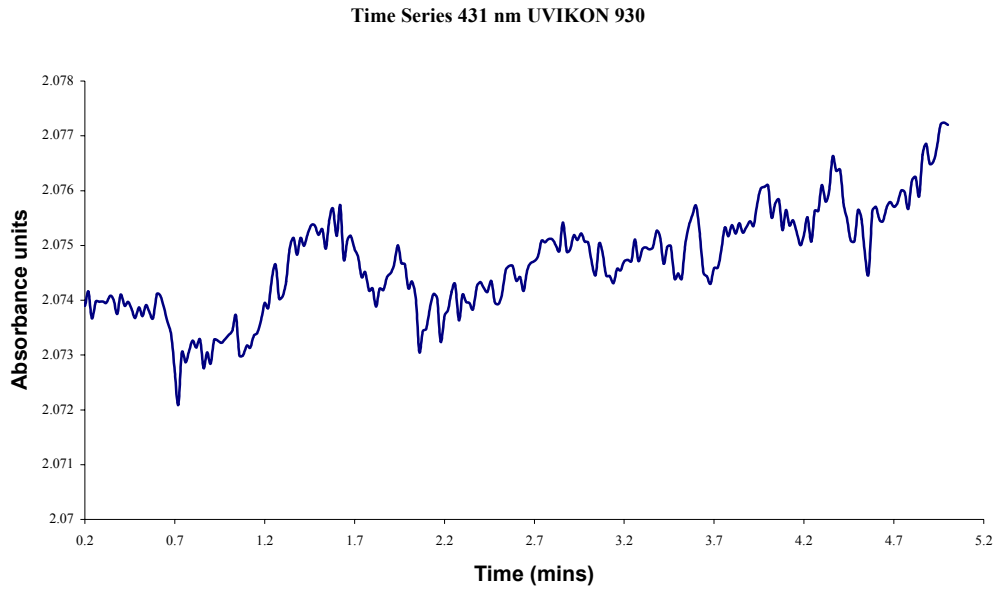
Optics unsuitable; single monochromator, stray light reduction, wavelength accuracy and photometric accuracy poor.

- **Instrument performance**

UVIKON 930 (equivalent to UVIKON XL) used for determining inherent optical properties of sea water during OSTC contract T4/36/34 on project MULTICOLOR at Universite Libre de Bruxelles, BELGIUM by GHT

Comments; baseline flatness and stray light high, instrument noise high (see Fig 1), sample compartment too small to accommodate filter holders, spectralon plugs and light traps for integrating sphere.

Fig A1. Time series at 431 & 663 nm for UVIKON 930 spectrophotometer.



Variation @ 431 nm – 0.0068 A (\equiv 6.82 mA)

Variation @ 663 nm – 0.0075 A (\equiv 7.49 mA).

Hitachi U-3310

Consumer market; precision spectrometry.

- **Suitability**

Optics suitable; pre monochromator / single monochromator system, stray light reduction, wavelength accuracy and photometric accuracy comparatively low.

- **Instrument performance**

Comments; photometric accuracy and stray light good (Table 1). Sample compartment sufficient to accommodate filter holders, spectralon plugs and light traps for integrating sphere.

Perkin Elmer Lambda 800

Consumer market; precision spectrometry.

- **Suitability**

Optics suitable; true double monochromator, stray light reduction, wavelength & photometric accuracy, noise reduction very good.

- **Instrument performance**

Comments; Very versatile - programmable slit width, band pass and scan speeds. Software very comprehensive. Integrating sphere and cuvette cell holder can be operated simultaneously. A lot of space around sphere for T-R components, although transmission reference cell very close to sphere mirror. Many optical accessories (e.g. peg board for optical calibration).

Instrument comparison - Hitachi U-3310 versus Perkin Elmer Lambda 800

Baseline flatness, photometric accuracy and instrument stability were compared

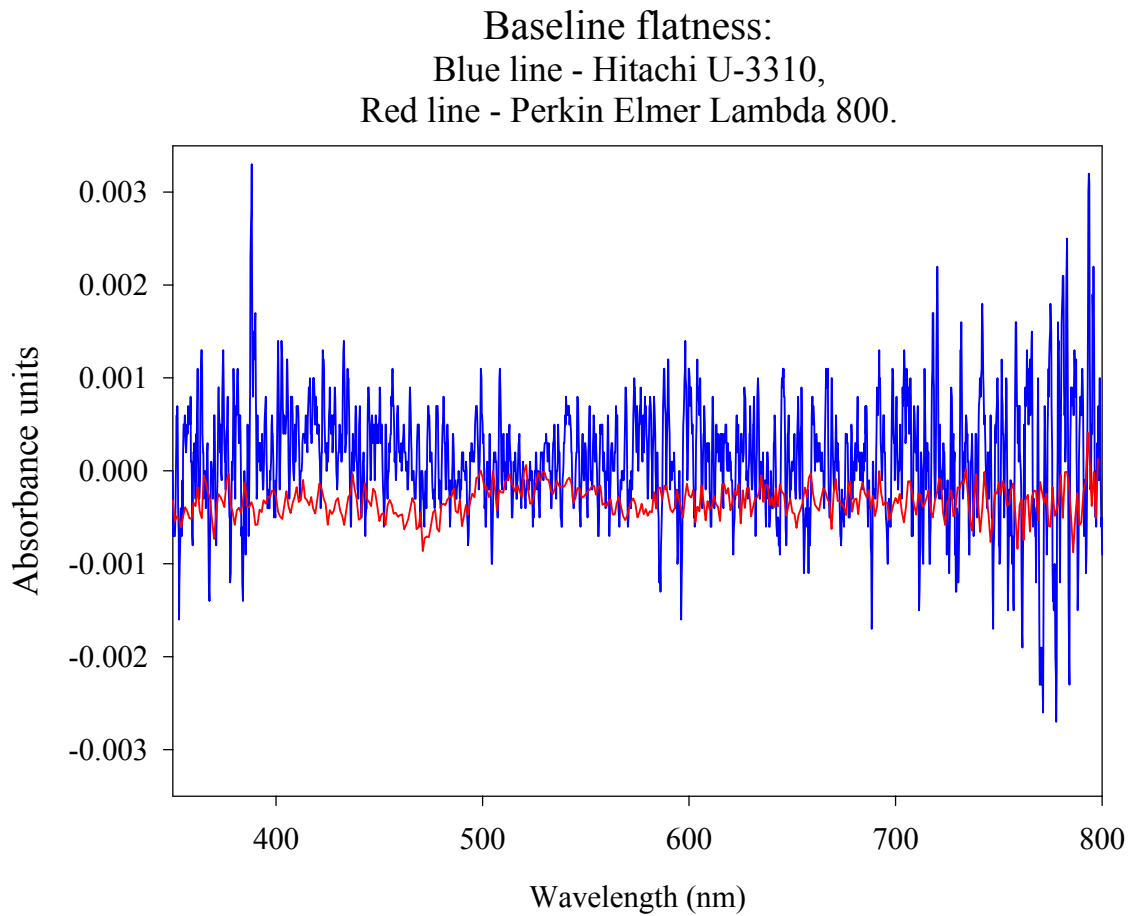
RESULTS

Summary of instrument comparison given in Figs 2 – 4.

All tests were made with the instrument fitted with the integrating sphere. Significant results are highlighted after each of the figures.

Baseline flatness

Fig A2. Comparison of baseline flatness Hitachi U-3310 and Perkin Elmer L-800.



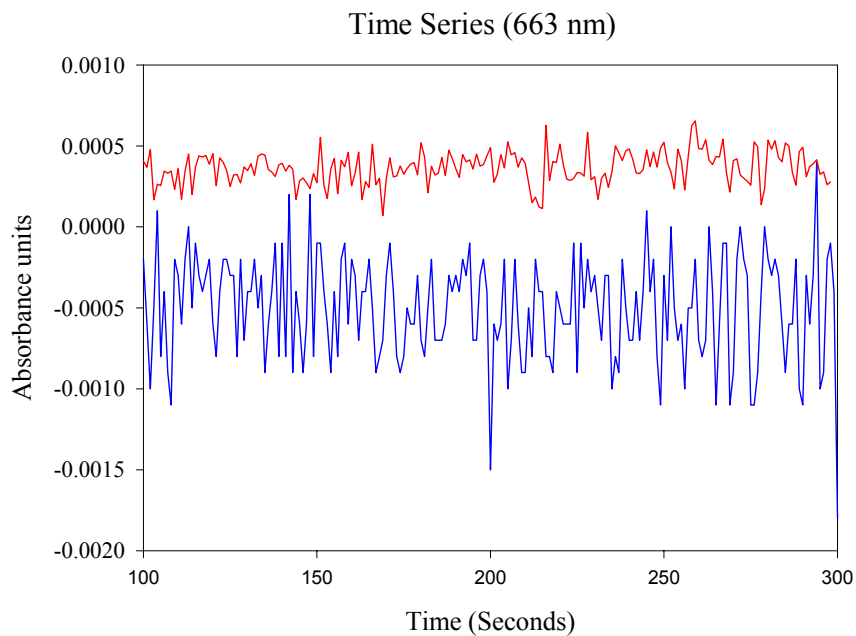
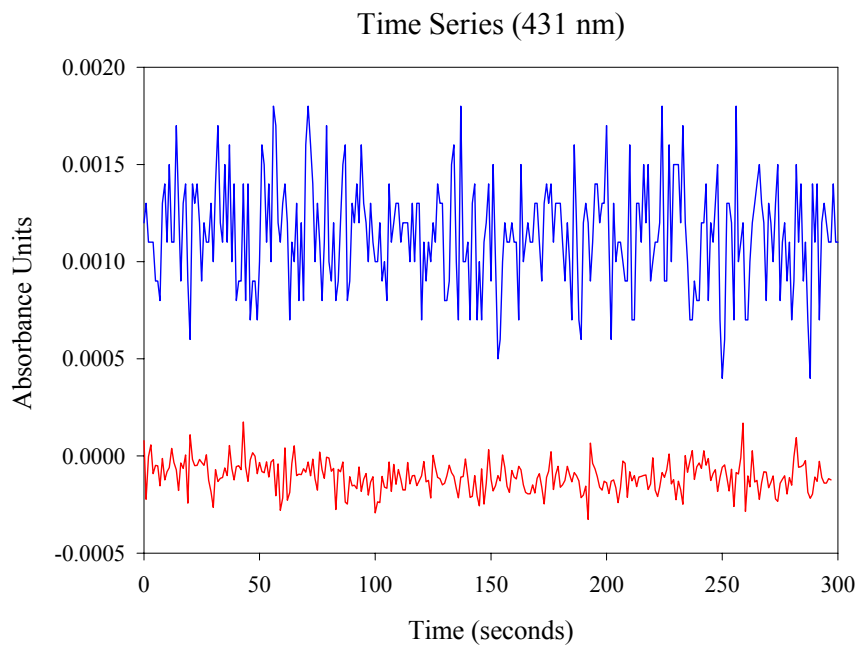
Baseline variation

Hitachi U-3310 – 0.006 A

Perkin Elmer Lambda 800 – 0.0006 A

There was a factor of 10 difference in baseline flatness between the Perkin Elmer and Hitachi instruments. Perkin Elmer L800 performed best.

Fig A3. Comparison of instrument stability and wavelength accuracy.
Blue line - Hitachi U-3310; Red line - Perkin Elmer L-800.



Time series

Variation @ 431 nm

Hitachi U-3310 – 0.0019 A

Perkin Elmer Lambda 800 – 0.0005 A

Variation @ 663 nm

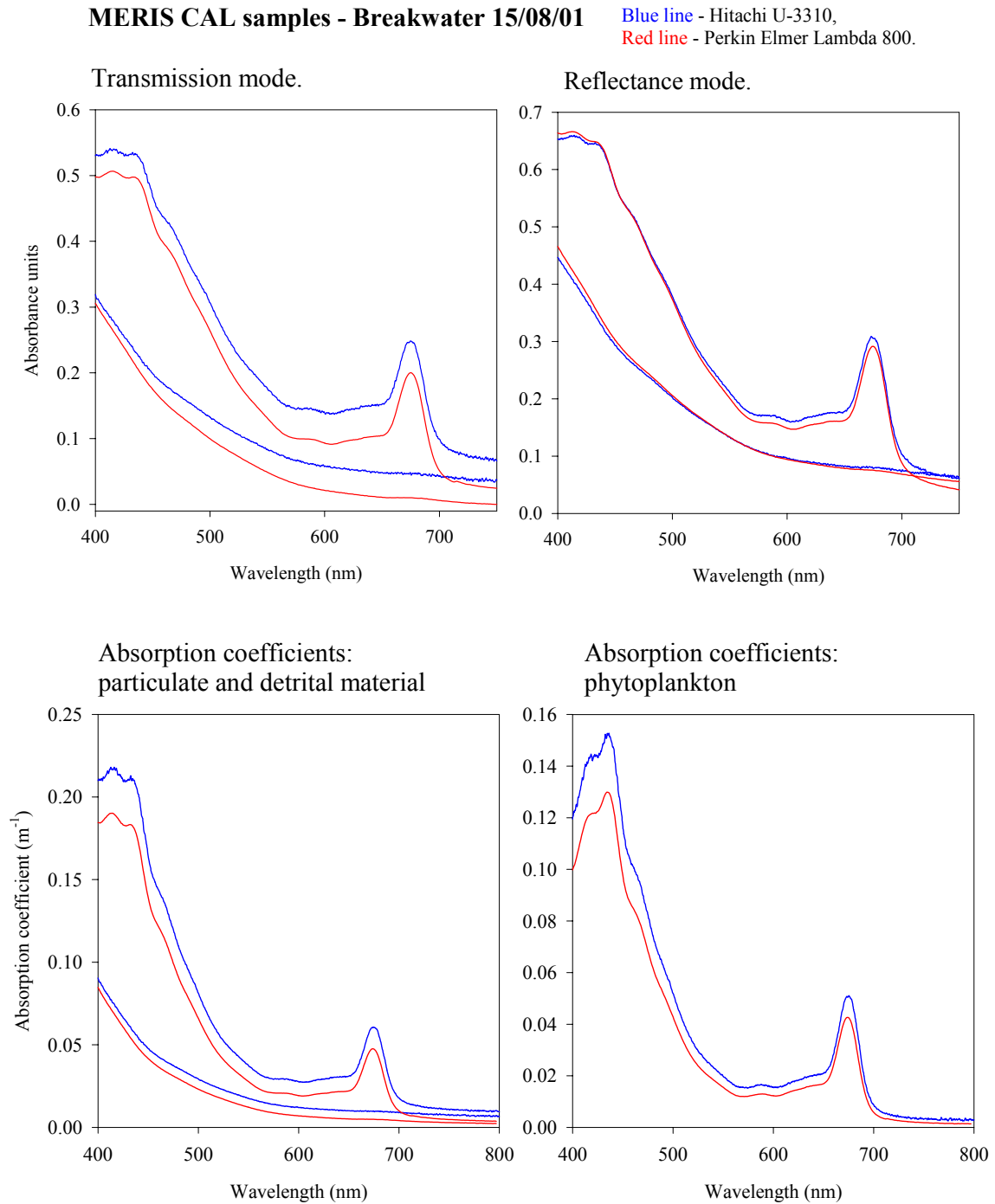
Hitachi U-3310 – 0.0024 A

Perkin Elmer Lambda 800 – 0.0012 A.

% difference

16 %

Fig A4. Absorption coefficient comparison;



Detrital and particulate absorption coefficients reduce closer to zero at 800 nm for the Perkin Elmer instrument. A large variation was observed ($\pm 0.021 \text{ m}^{-1}$) in phytoplankton absorption coefficient between instruments at 443 nm.

Photometric accuracy

Table A2. Comparison of photometric accuracy for Perkin Elmer Lambda 800 and Hitachi U3310.

Two commercial standards with known absorbance were tested. F3 is a dilute sample similar to concentrations of phytoplankton in oligotrophic regions. F4 is a more concentrated sample similar to levels typically found at the Plymouth time series station L4. Data are from the mean of three replicates.

	Perkin Elmer L800				Hitachi U3310			
Std	F3		F4		F3		F4	
	Mean of 3 reps	Variation from std	Mean of 3 reps	Variation from std	Mean of 3 reps	Variatio n from std	Mean of 3 reps	Variatio n from std
440	0.497	-0.001	0.9691	0.0021	0.4989	0.0009	0.9727	0.0057
465	0.4565	-0.0005	0.8922	0.0012	0.4563	0.0004	0.8941	0.0031
546	0.4647	0.0001	0.8992	-0.0008	0.4651	0.0001	0.9009	0.0009
590	0.5126	-0.0004	0.9639	-0.0021	0.5112	-0.0018	0.9640	-0.002
635	0.5135	-0.0005	0.9338	-0.0012	0.5147	0.0007	0.9365	0.00153

Significant results are highlighted in red; for std F3 Hitachi showed a greater photometric accuracy at wavelengths in the blue region of the spectrum, whereas the Perkin Elmer instrument performed better in the green – red. For the std F4 the Perkin Elmer instrument performed better at all wavebands.

Over all, the Perkin Elmer Lambda 800 showed a better photometric accuracy.

Conclusions

Perkin Elmer Lambda 800 showed a more suitable baseline, instrument stability and photometric accuracy than the other spectrophotometers. The difference in baseline caused an 16 % difference in phytoplankton absorption coefficients at 442 nm.

DEPIGMENTATION AGENT

Introduction

The filter pad method has become the standard method of determining phytoplankton absorption coefficients and over the past thirty years methodologies have diverged. A number of different de-pigmentation agents have been proposed for the extraction of phytoplankton pigments to enable the spectrophotometric determination of particulate and detrital material and from these the estimation of phytoplankton absorption coefficients. Early research was centred on the use of acetone as an extraction solvent since it was effective in removing Chlorophyllides, but it was ineffective in extracting pigments from some species (Jeffrey et al. 1997). In 1985, Kishino et al. developed an extraction method using a series of heated methanol baths. The method was applicable to a range of marine phytoplankton (Marker 1972, Stauffer et al. 1979) but was ineffective in extracting certain pigments (phycocyanin, phycoerythrin) from some phytoplankton especially the freshwater groups; Chlorophyceae and Cyanobacteria (Bowles et al. 1995). This method can also lead to a disturbance and loss of the material retained on filter (Tassan & Ferrari 1995). The use of 15 % NaClO (1 % active Cl⁻) was proposed by Tassan & Ferrari (1995) since it is effective in extracting pigments from these more problematic phytoplankton, it is also applicable to Case 2 waters with heavy sediment loads since the loss of material retained on the filter can be minimised and the technique is simple and efficient (approximately 4 mins for pigment extraction). Since the publication of their original method, Tassan & Ferrari have refined their protocol; a 1 % active Cl⁻ solution of NaClO is an aggressive bleaching agent and may also bleach some of the organic detrital material within the sample thus producing higher phytoplankton absorption coefficients than expected. Subsequently an active Cl⁻ concentration of 0.1 % (Ferrari & Tassan 1996, Tassan & Ferrari 1998) has been recommended. The application of NaClO to a blank GFF filter leaves a residual yellow colouration on the filter which may absorb light. The re-filtering of 5 ml of MilliQ through the treated GFF filter to remove any residual NaClO was also recommended (Tassan et al. 2000, Mitchell et al. 2000).

We conducted a series of experiments to compare different de-pigmentation solvents and different applications of a single solvent to assess which is the most suitable for use in Case 2 waters.

Methods

Seawater was sampled using 10l Niskin bottles at three stations off Plymouth Sound and transferred to 5 litre dark carboys. Three replicate samples from each station were filtered onto Whatman GF/F filters (25 mm ø, 0.7 µm pore size) with between 300 ml and 2 litres of seawater. Each filter was flash frozen and stored in Liquid Nitrogen. The following treatments were compared:

Table A3- Different treatments used for pigment extraction

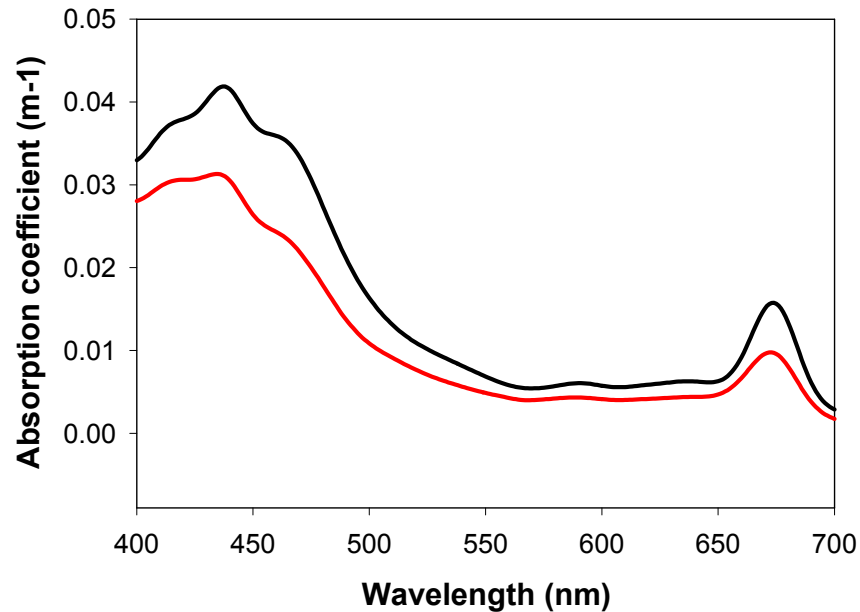
Solvent	Treatment (blank + sample)
Acetone	Bleached for 30 mins
Methanol (5 ml)	Filtration and wetted with MilliQ
NaClO 0.1 % active chloride	Bleached for 30 mins, no washing with MilliQ
NaClO 1 % active chloride	Bleached for 30 mins + washed with MilliQ
NaClO 0.1 % active chloride	Bleached for 30 mins + washed with MilliQ

Results

Fig A5 shows the comparative extraction using MeOH and NaClO (active Cl⁻ 0.1%). There was a 29 % difference between the spectra and MeOH exhibited lower phytoplankton absorption coefficients throughout the spectrum. The detrital MeOH spectra suggests that the extraction was not fully de-pigmented. Fig A6 compares NaClO treatments. The difference between 1 and 0.1 % active Cl⁻ was 15 % at 442 nm and 20 % over MERIS wavebands. No washing resulted in higher absorption coefficients than washing with MilliQ due to absorption by residual bleach on the filter. The difference

between washing with MilliQ and no washing was 26 % at 442 nm and 27 % at MERIS bands.

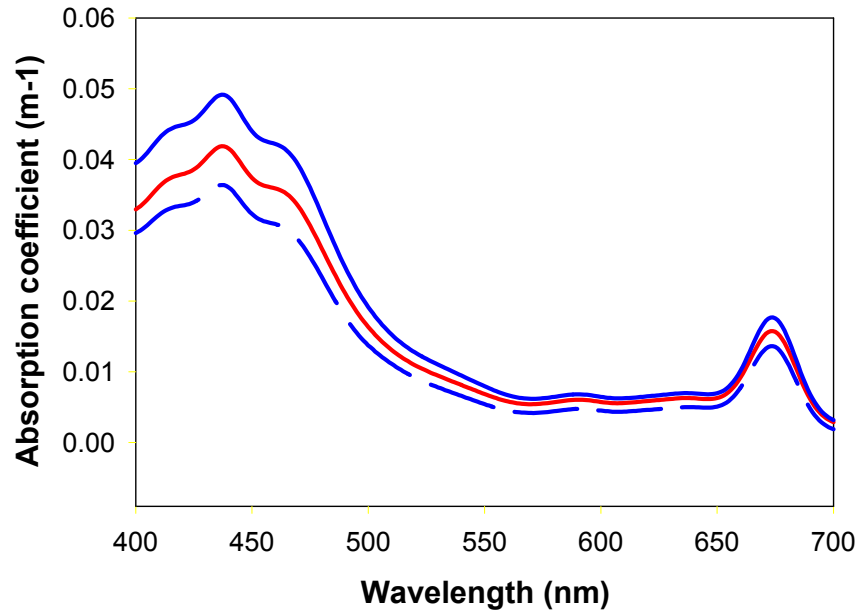
Fig. A5 - Comparisons between MeOH and 15 % NaClO (0.1% active Cl-) extraction treatments. (Red line – pigment extraction using MeOH; Black line - NaClO)



Conclusions and Recommendations.

The use of NaClO has been proposed as an effective de-pigmentation agent for Case 2 water samples. The difference between washing and not washing the filter with MilliQ after application of NaClO was greater than the difference between active Cl⁻ concentrations. The use of 0.1 % active Cl⁻ is recommended, since 1 % Cl⁻ causes bleaching of the detrital material and produces higher phytoplankton absorption coefficients. Washing the filter with 5ml is also recommended since residual NaClO can also increase the absorption coefficient.

Fig A6. Comparison of pigment extraction using different application of NaClO.
(Solid blue line – 1 % active Cl⁻ with no washing; dashed blue line - 1 % active Cl⁻ with washing; Red line – 0.1 % active Cl⁻ with no washing)



BLANK FILTER PREPARATION

Introduction

In the NASA protocols (Mueller & Fargion 2000), Mitchell et al. (2000) and Tassan & Ferrari (1998) noted that dry filters adversely affects the optical density of the sample and that care should be taken that sample and blank filter are at the same humidity when increasing the absorption spectrum.

Method

A series of experiments were conducted using the Tassan and Ferrari (1995) protocol to compare the quality of the signal with blank filters in different states: dry, wetted, soaked or filtered either with MilliQ, or with sea water. The absorbance of three replicate filters was compared

Results

The results are presented in Figures A8 to A10. The humidity of the filter significantly influences the glass fibre filter (GFF) reference filter. Measurements made with dry filters were higher (O.D >1.5) than using wet filters (O.D <1). The difference between the dry filter and wetted or filtered GFFs was > 50 % (Fig A8). GFF blanks wetted or filtered with MilliQ, gave higher optical densities than filters wetted or filtered with seawater. The difference in optical density between using MilliQ and seawater was not significant (~3 %). The difference between wetting and filtering had a larger affect (~9 %). Wetted filters always gave a higher optical density than filtered GFFs. Filtering opens the pore aperture of the GFF and allows a higher transmission of light through the filter (Fig9). GFF filters have a smooth and a striated side and the manufactures recommend that samples are concentration on to the striated side. The filter side may however affect the absorbance and reflectance measurements since the striated side may cause more scattering of light than the smooth side of the filter. We found that the side of the filter did not affect the accuracy of the measurement significantly and differences between using the smooth or striated side were only 4 % (Fig. A10). Care should however be taken when carrying out the measurement and it is recommended that the same side of the filter is used for sample and reference filters to ensure the highest measurement precision.

Fig. A8 – Optical density of blank filters. (Blue line – dry filter, red line – filter wetted with MilliQ; green line - filter wetted with seawater; pink line – GFF filtered with seawater).

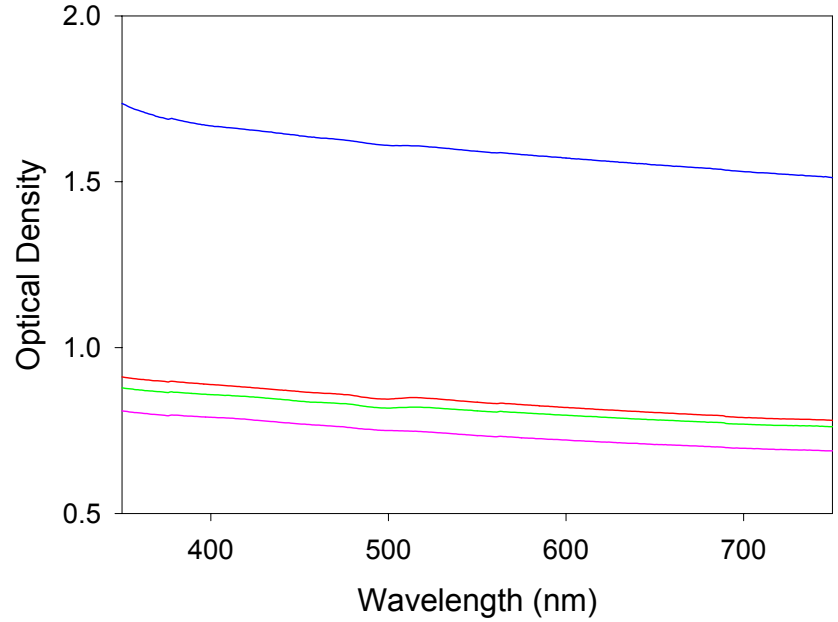


Fig. A9 – Comparison of the optical density of wet and filtered blank GFFs . (Blue line – MilliQ, red line –seawater; solid line – wetted; dashed line - filtered).

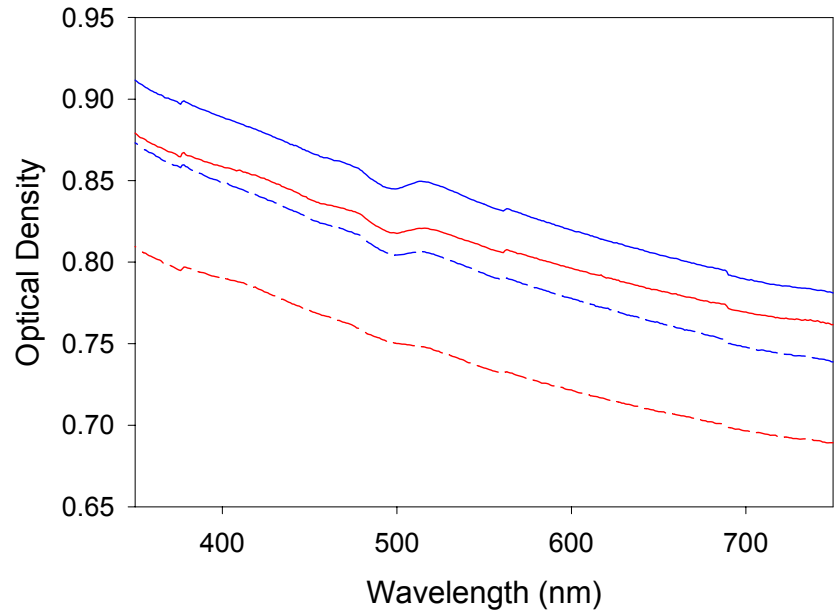
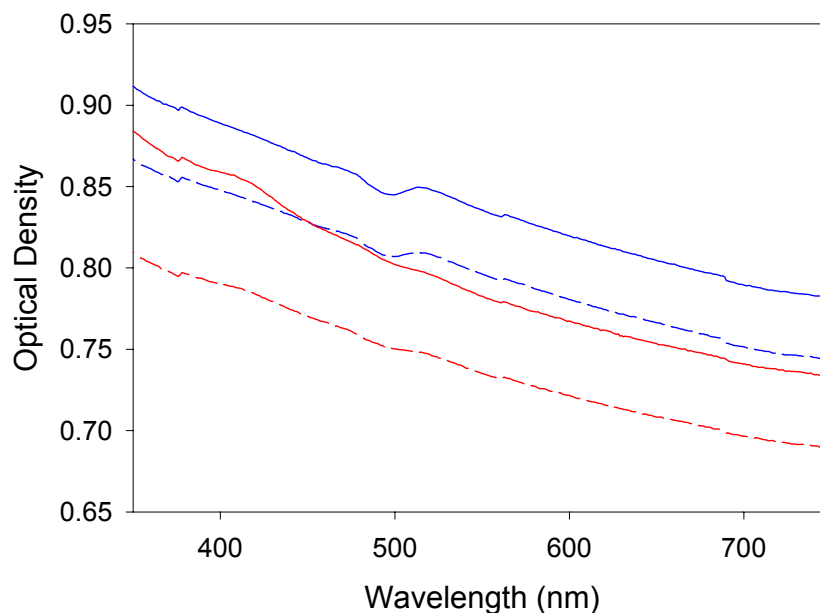


Fig. A10 – Comparison of the optical density of striated and smooth filter side of blank GF/Fs . (Blue line – filtered MilliQ, red line – filtered seawater; solid line – smooth side; dashed line – striated side).



Conclusions and Recommendations

Dryness of filters significantly affects GFF blank reference filters. A deviation of >0.02 OD in the NIR can be used as an indicator of adverse filter dryness. It is strongly recommended that filters are hydrated with 0.2μ pre-filtered seawater before each scan. A few drops of seawater should be spotted into a petri dish and the underside of filter should be placed onto the seawater to prevent disturbance of the material on the filter. There was a significant difference between the absorption of filtered GFF and those that have been wetted with seawater. Since the striated side of the filter results in more scattering and less absorption than the smooth side, care should be taken to use the same filter side for both blank and sample filter when running measurements in spectrophotometer.

DATA PROCESSING

Introduction

Roesler (1998) defined two main sources of error for the determination of particulate absorption coefficients using this technique. The first is methodological, which includes variability in optical density of blank filters, filter moisture content and blank filter preparation as outlined in the previous sections. The second is due to differences in data processing and analysis; Roesler (1998) observed that the pathlength wavelength correction (β) produced the highest source of error. Different filter pad loadings lead to large variations in phytoplankton absorption coefficients Pegau & Zaneveld (1993). In Case 1 waters a zero offset from the baseline may occur which is presumed to be the product of scattering throughout spectrum. Hence a spectral region is identified where phytoplankton absorption is assumed to be negligible (typically 750 to 800nm) and the scattering observed is due to non-phytoplankton material. In Case 2 waters scattering by particles ≥ 750 nm is not negligible since scattering and absorption by detritus increases with decreasing wavelength Tassan & Ferrari (1995).

Methods

The experimental and data processing methods of Tassan and Ferrari (1995; equations 11 to 14) were used to convert the measured absorbance of the filter-retained particles into the equivalent particle suspension absorption. τ is defined as the ratio of $(1-T_{sd})/(1-T_{sp})$ where T_{sd} is the transmission of diffuse light through the filter and T_{sp} is the transmission of parallel light. The following routine was used to calculate τ :

$$\tau = 1.171 - 0.2615 * \alpha + 0.00013 * \alpha * \alpha \quad (\text{Equation 1})$$

where α is the absorption in transmission mode either of the pigmented or de-pigmented sample. Four measurements were made for each pigmented and de-pigmented sample (two transmission and two reflectance Tassan & Ferrari [1995]). The instrument baseline for the integrating sphere was recorded every four samples. An exponential curve was fitted to the detrital absorbance which was then used to offset the particulate absorbance to baseline. High suspension absorbance leads to increasing errors when applying

pathlength wavelength corrections - β (Mitchell et al. 2000) and few β values have been reported for Case 2 waters (Tassan & Ferrari 1998). β was set to 2, following Roesler (1998) and MERIS protocols (Doerffer 2002), which is based on the assumption that for GF/F filters the diffuse absorption of a sample is twice the volume of the absorption coefficient.

Results and Discussion

In this study errors due to the β factor were minimized by using the same filtration volume and setting the β value to 2. Other differences in data processing could include the derivation of τ . In the PlymCal 2 inter-comparison all laboratories calculated τ using Equation 1. However, some laboratories defined α as the absorption in transmission mode either of the pigmented or de-pigmented sample as follows:

$$\alpha = \log_{10}\left(\frac{1}{st}\right) - 0.5 * \log_{10}\left(\frac{1}{st_{750nm}}\right) \quad (\text{Equation 2})$$

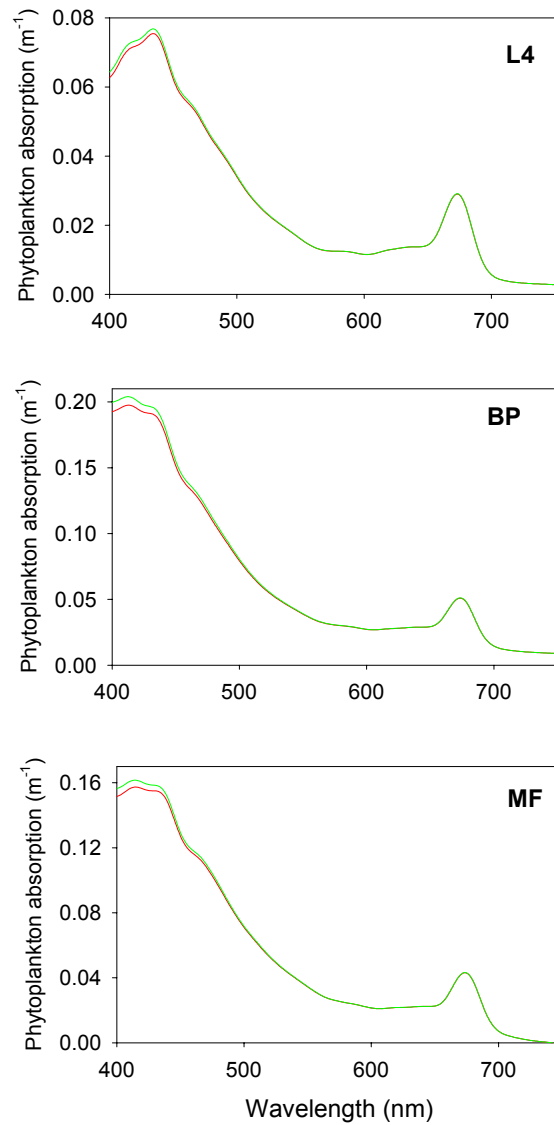
where st is the sample transmission and st_{750nm} is the sample transmission at 750 nm. Using data from one laboratory only, we compared absorption coefficients derived from the two methods of calculating α but the difference was not significant (Fig A11).

Conclusions and Recommendations

No difference was found between different ways of calculating τ . However to reduce methodological cumulative errors it is recommended that τ is calculated using Equation 1 and α is calculated from:

$$\alpha = \log_{10}\left(\frac{1}{st}\right).$$

Figure A11. Differences in Phytoplankton absorption using $\alpha = \log_{10}(1/st) - 0.5 * \log_{10}(1/st750)$ (red line) and $\alpha =$ sample absorption in transmission mode (green line).



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APPENDIX B

Experiments on Coloured dissolved organic material (m^{-1}) protocols.

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The following experiments were conducted to assess the following aspects of the measurement protocol of CDOM absorption:

- *Reference baseline*
- *Filter preparation*
- *Sample storage*

REFERENCE BASELINE

Introduction

The greatest difference in measurement protocols between partner laboratories in the EU project REVAMP, was the use of reference blanks. Some laboratories autozero the spectrophotometer using MilliQ in the sample cell and air in the reference cell. The sample is then measured in the sample cell with nothing in the reference cell. The two spectra are then subtracted to give the absorbance of the sample. Other laboratories autozero the instrument using MilliQ in both sample and reference cells and then run the sample against MilliQ to give the absorbance of the sample directly.

Methods

During the inter-calibration workshop held at Plymouth Marine Laboratory from 11 to 14 June 2002, an experiment was designed to investigate the effects of differences in reference blank autozero on CDOM absorption. Natural seawater samples were taken from two sites close to Plymouth Sound, with varying CDOM concentrations (L4 and Mayflower) and both autozero methods were compared.

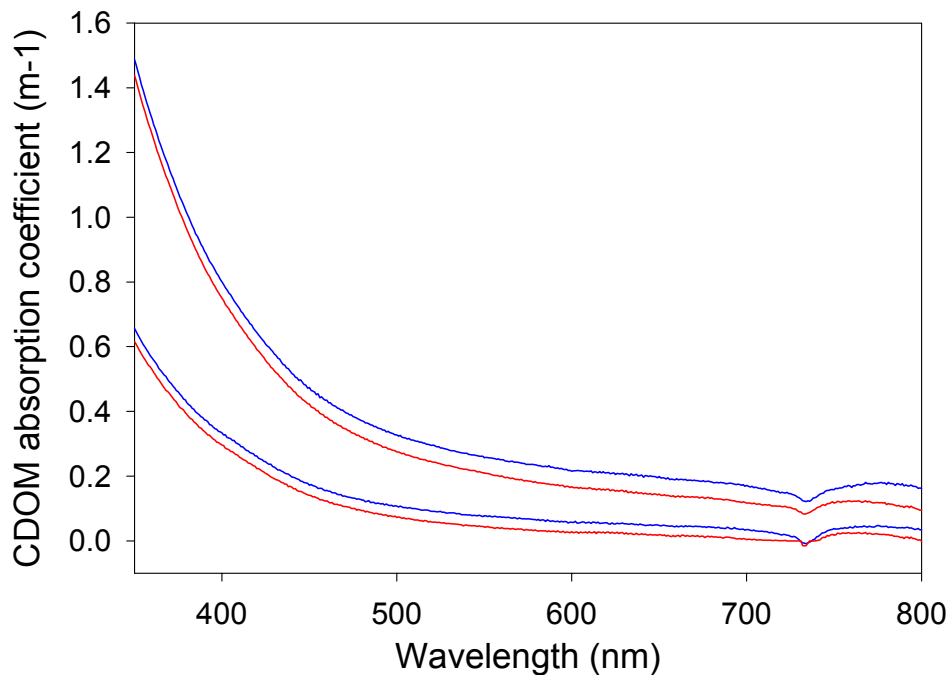
Table B1. YS Inter-comparison; air versus MilliQ reference blank.

MERIS Band	Mean L4 Ref - air	Std dev L4	Mean L4 Ref - MQ	Std dev L4	Mean MF Ref - Air	Std dev MF	Mean MF Ref - MQ	Std dev MF
412	0.252	0.016	0.290	0.020	0.652	0.085	0.703	0.091
442	0.161	0.014	0.194	0.019	0.459	0.079	0.506	0.084
490	0.082	0.015	0.115	0.019	0.296	0.071	0.347	0.075
510	0.064	0.014	0.100	0.017	0.258	0.067	0.310	0.071
560	0.041	0.012	0.072	0.016	0.201	0.060	0.251	0.064
620	0.027	0.013	0.056	0.013	0.159	0.054	0.210	0.055
680	0.014	0.008	0.042	0.012	0.133	0.051	0.182	0.050
708	0.002	0.004	0.028	0.008	0.112	0.048	0.162	0.047

Results

Fig B1 illustrates the difference between samples using MilliQ against air autozero (red line) and MilliQ against MilliQ autozero (blue line). Over the whole spectrum there was a 21 % difference between samples. The difference was greater at lower CDOM absorption coefficients (e.g. L4 – 29 %) compared to higher CDOM concentrations (e.g. MF – 14 %). For the 442 nm MERIS bands there was a 20 % difference in CDOM absorption for the L4 sample and a 10% difference for Mayflower and the standard deviation for both samples was low indicating little difference between replicates. The percentage difference over blue to green MERIS wavebands (412 to 620 nm) increased (Table 1) to 48 % for L4 and 19 % for Mayflower and showed that at longer wavelengths the effect of the reference blank on CDOM absorption increased.

Fig B1. CDOM absorption coefficient of samples from L4 and Mayflower using different reference blanks. Red line – sample run against air; Blue line – sample run against MilliQ.



Conclusions and Recommendations

Autozeroing the spectrophotometer against air gives less temperature and salinity dependence, greater measurement stability and a better reduction to baseline.

FILTER PREPARATION

Introduction

A number of methods have been proposed for pre-treating 0.2 μ m filters. NASA recommend the pre-soaking of Nucleopore membrane filters in 10 % HCl followed by rinsing in MilliQ (Fragion & Mueller 2000) to remove any color from the filter. COLORS protocols recommend soaking the filters in MilliQ.

Methods

The CDOM absorption spectra of filtrate that had been passed through pre-soaked Millipore polycarbonate filters in 10 % HCl and MilliQ were compared using replicate samples at 7 stations visited on a transect from Oostende, Belgium to Harwich, UK during REVAMP cruise Belgica 2002-10.

Results

Pre-soaking in 10% HCL significantly increased the absorption of the filtrate (Fig. B2).

Recommendation

It is recommended that Nucleopore membrane filters are rinsed in MilliQ before use.

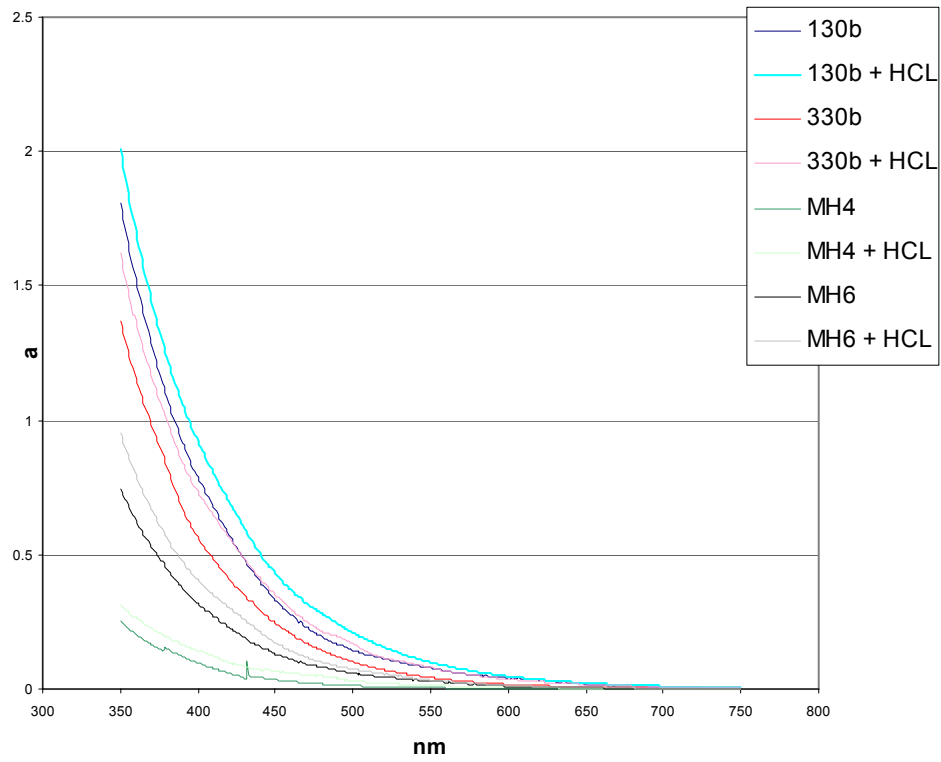


Fig. B2 – Absorption spectra of CDOM filtrate from four stations during cruise Belgica 02-10 using filters pre-soaked in 10% HCl or MilliQ.

SAMPLE STORAGE

Introduction

Coloured Dissolved Organic Material (CDOM) degrades at room temperature and different protocols have been recommended for the analysis of live samples to prevent the degradation of CDOM. NASA recommend that the analysis of live samples is conducted within 4 hours of collection (Fargion & Mueller 2000). Samples can be stored in a refrigerator for 4 to 24 hrs with no adverse affects on CDOM absorption (Mitchell et al. 2000). For longer term storage, the addition of 0.5 ml solution of 10g/l of NaN_3 per 100 ml of sample has been suggested, to prevent the degradation of CDOM (Ferrari et al 1996), but this may affect the absorption properties of the sample. We conducted a series of experiments to investigate the effects of NaN_3 on the absorption properties and the long term (> 24 hrs) storage of samples.

Methods

Replicate samples from the time series station L4, off Plymouth Sound (50 15 °N, 04 12.5 °W) were spiked with and without NaN_3 , and the absorption spectra of CDOM were determined every three days for 9 days.

Results

In the fresh samples after two days, the material began to degrade and the absorbance of the sample decreased by 12 % (Fig. B3). After 9 days the sample had degraded by 45 %. In the NaN_3 spiked sample even after 9 days there was no significant reduction in CDOM absorption coefficient. Table B2 shows the effect of different treatments on CDOM slope. The mean slope of replicate samples stored at room temperature over a period of 9 days caused an 8 % variation in the CDOM slope. For samples stored in the refrigerator this was reduced to 6 % and for samples spiked with NaN_3 the variation in CDOM was only 2 %.

Fig B3 – The effect of NaN₃ on CDOM degradation in L4 seawater over 9 days.
 (Yellow line – samples spiked with NaN₃; Red line – fresh samples, no NaN₃).

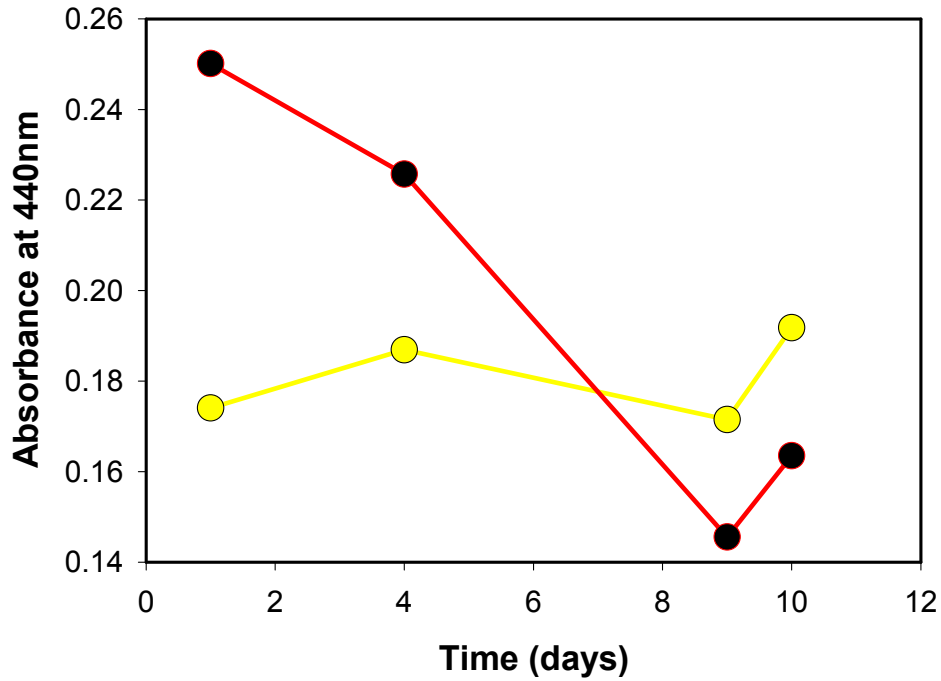


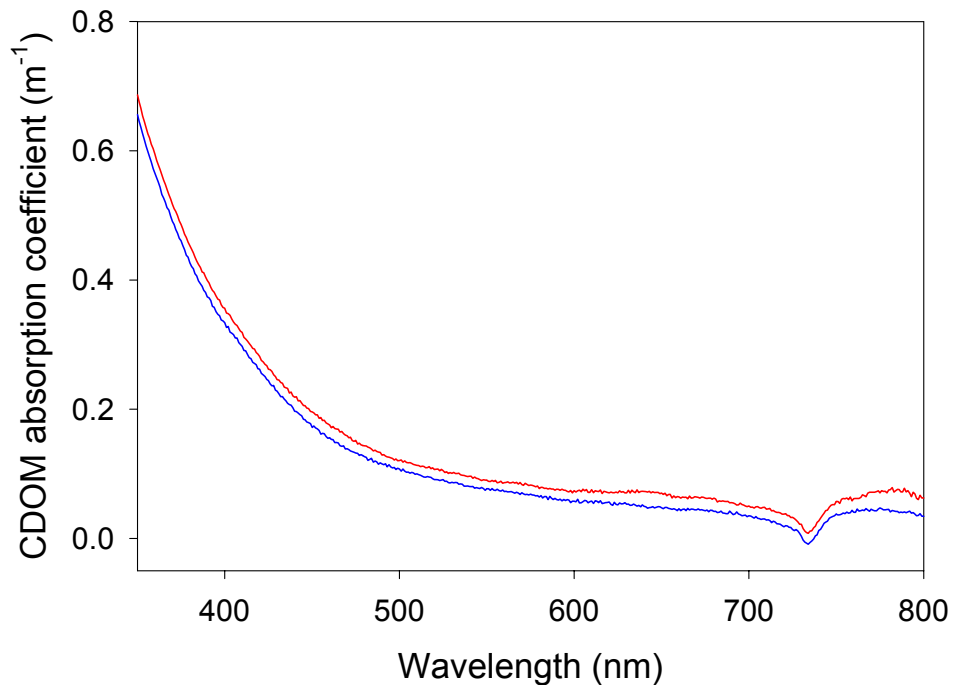
Table B2. The effect of sample storage on CDOM slope.

Treatment	Time (days)	Slope	% difference
Fridge + NaN ₃	1	-0.0141	4
	4	-0.01479	0
	9	-0.01528	4
Mean		-0.01472	
Fridge	1	-0.01472	6
	4	-0.01422	3
	9	-0.01254	9
Mean		-0.01383	
Room Temp	1	-0.01308	9
	4	-0.01391	3
	9	-0.016	12
Mean		-0.01433	

The effects of sodium Azide on CDOM absorption

To check the effects of NaN_3 on the resulting absorption coefficient fresh seawater samples from L4 were spiked with NaN_3 and were compared with fresh samples that had not been spiked with NaN_3 . There was a 10 % difference between CDOM absorption coefficients (Fig B4) at 442 nm and a 14 % difference over MERIS wavebands. Overall the difference between samples spiked and non-spiked was relatively low, however to reduce analytical errors in MERIS data validation, it is recommended that CDOM samples should be run fresh whenever possible.

Fig B4. The effect of sodium azide on CDOM absorption. Red line – mean of three replicate natural seawater samples with sodium azide. Blue line - three replicate natural seawater samples without sodium azide



Conclusions and Recommendations

NaN_3 arrests the degradation of CDOM in samples and reduces the variability in the spectral slope during long term storage. It does however increase the absorption coefficient of natural samples and it is therefore recommended that the sample is run fresh (within 4 hrs of collection).

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