

DWR's Fluoromax 4 Spectrofluorometer

Standard Operating Procedure (SOP) Version 2.0

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Additions by Ted Swift, May 12, 2011

Revision History

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1.0 Scope and Application

This SOP is a guide to using the DWR's QA/QC Horiba Fluoromax 4 located in the DWR's Field Support Unit SMARTS shed in West Sacramento, CA. This SOP supplements (but does not replace) Fluoromax 4 manufacturer's Operating Procedures Manual located next to the instrument. *If an operator decides to deviate from this SOP, it is their responsibility to document those changes.* The Fluoromax 4 is not to be removed from the current location. Please make sure that all the manuals stay with the instrument.

1.1 Known Limitations related to the Scope of this SOP

Application of multiple fluorescence scans in the study of dissolved organic matter (DOM) is not a standard method (although such measurements have been performed for at least the last 30 years). Therefore, there are no widely accepted procedures for instrument setups in fluorescence scans. In this SOP, DOM is defined as materials in water samples filtered in the field with a 0.45 μm filter. (Other filter sizes such as 0.2 μm are found in the literature). Some commonly cited sources of fluorescence information can be found at the end of this SOP.

2.0 Summary of Procedure

This SOP covers fluorescence reading using the standard single sample holder and in its current version specifically excludes the use of the available optional multi-sample holder because there are unresolved issues with the multi-sample holder. This SOP assumes that filtered water samples will be analyzed for one or all of the following:

- a) 3D Excitation Emission Matrices (EEMs)
- b) 2D Fluorescence measurements
- c) Single emission/excitation fluorescence readings

The Fluoromax 4 is capable of making many other measurements when bundled with additional accessories not included in the current DWR configuration.

3.0 Instrument



Fluoromax 4: Front View with optional laptop

Note: Turning the Fluoromax on and off is hard on the Xenon excitation lamp. It is advisable to leave the instrument on for the duration of the analysis (unless you are terminating the analyses for an extended period of time such as overnight).

3.1 Software operation

FluorEssence is the software that controls the Fluoromax 4 and stores the analytical results. You will need to familiarize yourself with the software by reading the FluorEssence User's Guide which is located in the trailer. FluorEssence is a customized version of Origin (by OriginLab, <http://www.originlab.com/>). The FluorEssence currently bundled with the instrument (as of 1/3/2011) is version 3.5.1.2 (the underlying Origin is version 8.1). Fluorescence scans are stored in workbooks (similar to Excel) and saved as project files (.opj extension). The workbooks contain data, graphs and notes about each scan. The default folder for fluorescence scans is:

C:\Program Files\Jobin Yvon\Data

However, you can save your data in a different location of your choice.

3.3 Instrument Start up and Operation

- Make sure that the computer and all the attached peripherals are off
- Make sure the FluorEssence USB software **license dongle** is inserted in the computer
- Turn on the Fluoromax 4 first: the on/off switch is near the back on the right hand side (while facing the front of the instrument).
- Turn on the Hach spectrophotometer (if it is connected to the computer with its USB cable)
- Turn on the computer. User and Password are taped to the front of the computer
- To launch FluorEssence, do one of the following:
 - a) Point to: Start\ All Programs\Jobin Yvon\FluorEssence



- b) Double click the FluorEssence icon on the desktop
- Refer to Fluoromax 4 User's Guide page 3.1 to 5.18
 - You will need a clear glass quartz cuvette for your samples, marked near the top with a letter "Q". All sides should be clear, no ground glass. Do not use the spectrophotometer cuvettes. Quartz is needed to pass the ultraviolet short wavelengths (wavelengths shorter than 400 nm).
 - Always use nitrile gloves when handling cuvettes (the gloves are in one of the drawers to the left of the table). Touching the cuvette with bare fingers will lead to contamination which is difficult to remove. Cuvettes scratch easily. Use Kimwipe tissues to wipe off smudges, water droplets, etc. Using scratched/contaminated cuvettes will result in erroneous scans. Each cuvette costs over \$100 to replace, so treat them with care.

3.3 Daily Instrument Checks

Lamp Check (excitation)

- The xenon lamp should be checked every day by running a lamp scan and results recorded in that day's project file. Let the lamp warm up for 45 minutes to an hour before running the lamp scan. See the Fluoromax 4 Manual for procedure on how to collect a lamp scan and calibrate if needed (pages 37-42 of the manual). You can run the default factory excitation scan or load a 'lamp' experiment file created by Horiba's Fluorescence engineer in C:\ProgramFiles\Jobyn Yvon.

Cuvette check

- Make sure that the cuvette is clean by washing repeatedly in DI water (use fresh DI water that has not been sitting in the squirt bottle). Open the sample holder cover and slightly pull outwards the spring-loaded clip to insert the cuvette into the holder.
- Run the experiment in S mode at an excitation at 240 nm and record the emission between 300-350 nm. The scan should be relatively flat with no peaks. If you see a peak, clean the cuvette and run the experiment again.

Water Raman Scan (emission)

- You can run the default factory 2D emission scan or load a 'water' experiment file created by Horiba's Fluorescence engineer in C:\ProgramFiles\Jobyn Yvon
- See Manual for procedure and calibration, if needed (pages 43-44 of the manual).

3.4 Running samples and saving results

- In order to correct for (subtract) blank, always run a blank scan with the same experimental settings as your environmental samples. You can automatically

subtract the blank while running the experiment or can subtract the blank manually later after the scan using the HJY tools.

- Individual users will decide the type of fluorescence scan (3D, 2D or single emission) to use depending on the objectives of the study. Individual users will determine whether to use the default instrument settings or their own custom settings depending on the goals and objectives of the study. Settings can be saved and re-loaded.

Note: This SOP recommends running 3D scans in ratio (S/R) mode. Also, correct for 'dark offset' and 'Rayleigh masking'. Make sure that the instrument correction (Mcorrect and Xcorrect) files are also checked (pages 25-28 of the manual).

- **Pay** close attention to how you save the results. Each individual scan generates a series of graphs, data and notes (depending on the configuration of the experiment file). The default filenames are hard to decipher, so it is advisable to rename or put a descriptive title in the '*comment*' section of the experiment file.

3.5 Exporting results from FluorEssence

There are only two export options (formats):

- 1) Galactic Grams (Thermo) **.spc**
- 2) Text (ASCII) formats: **.csv, .dat, .txt**

4.0 Interferences and other limitations

Turbidity, suspended solids, nitrites, nitrates and some metals may interfere with fluorescence for dissolved organic matter (DOM). Using filtered samples will ameliorate the effects of turbidity and suspended solids but there are no hard and fast ways to deal with the other interferences. The xenon lamp has weak output at excitations below 240 nm. So try not to run DOM samples at excitations below 240 nm (the Fluoromax does not perform any corrections below 300 nm according to Horiba's fluorescence engineer). Data can be collected between 240 and 300 nm, but corrections will not be done automatically.

5.0 Sample Handling and Preservation

This SOP for the Fluoromax 4 is primarily to be used for analyzing filtered water samples. Any other type of sample is not covered under this SOP.

- Samples are to be run at room temperature (there is a 'no touch' IR thermometer in one of the top drawers). This means that if samples have been refrigerated, you need to allow them to reach room temperature (20-25°C). Budget sufficient time for your samples to come to room temperature. If you would rather run samples cold, then make sure all of them are run at the cold temperature for comparability reasons.

- For every sample, you need to run a corresponding multiple UVA scan (MUVAs) on the Hach DR 5000 Spectrophotometer (200-750 at 1 nm intervals). These MUVAs will be used to correct (post-processing) the fluorescence scans. For inner filter effects.
- (Specifics: Jim Sickman (pers. comm. with Ted Swift) has suggested that DOC concentration be kept below 6 mg/L. This is sufficiently high that MOST Delta and tributary samples will not require dilution during MOST seasons. Ted Swift has developed a regression relating DOC concentration to light absorption at 254 nm using WDL data from Barker Slough and Banks pumping plants as an approximate test. The regression found that 6 mg DOC/L corresponds to a UVA₂₅₄ absorbance of 0.20 /cm. Thus, use the Hach spectrophotometer to measure and record an absorption spectrum of the sample, pick off the absorbance value at 254 nm, and compare it to 0.20 /cm. If it is more than 0.20 /cm, dilute the sample with DI water before measuring it on the Fluoromax. Important: Record the dilution factor in the FluorEssence sample description and in the Excel sample spreadsheet.
- This procedure assumes that DOM samples have been preserved in the same way as regular MWQI DOC samples i.e. using phosphoric acid at pH <2. If you are using a different preservation method, your results may not be comparable to results collected under this SOP. Note: Even with phosphoric acid, you may want to run some scans un-acidified to find out the effect of acid.
- This SOP assumes a DOM holding time of 28 days, with samples refrigerated at 4°C in dark glass bottles during the holding time. In your own sample-tracking system, record the sample time and date, analysis time and date, sampling personnel, and analysis personnel.

6.0 Quality Control

Quinine sulfate (QS) is the commonly used standard for comparing fluorescence readings. An often cited information source is Velapoldi & Mielenz (Velapoldi and Mielenz 1980). QS is available at the prep trailer. Velapoldi and Mielenz call for perchloric acid in the QS solvent, which can be hazardous. An alternative solution using sulfuric acid is often used (e.g., DePaul Univ. lab procedure). Frequency of standards measurement will depend on the individual study. Other standards such as humic and fulvic acids (HA and FA) from the International Humic Substances Society have also been used. The area under the pure water Raman curve can also be used for day-to-day calibration.

7.0 Running Samples

- Always run a DI water blank first. This is milliQ blank water run in the same excitation/emission range as the regular sample. MilliQ water is available from the MilliQ filtering station in Bryte Laboratory. MilliQ water should be transported in clean glass bottles, not plastic. The blank is to be subtracted from the emission data.
- You need to collect an absorbance scan either using the Hach spectrophotometer (or Bryte Lab's Perkin-Elmer). The absorbance scan is over the same range as the emission scan.
- Rinse with sample water before filling the cuvette with next sample, to prevent dilution from DI water droplets or offsets from the previous sample.

8.0 Between samples and Finishing up

- Be sure to rinse the cuvette with DI water between samples.
- When finished collecting scans, exit the FluorEssence software. Review your stored data files and confirm that all data files are stored in the desired folder (FluorEssence defaults to storing data in a designated "home" folder, and project data can end up in other places than intended. Copy sample data to a USB flash drive or similar. If absorbance scans were collected on the Hach spectrophotometer, transfer them to a USB flash drive. (The Hach requires an unencrypted flash drive; plan accordingly). Turn off the computer and monitor. Turn off the Fluoromax. Turn off the Hach, if used.
- Make sure to clean the cuvette(s) and store it (them) in the appropriate container.

9.0 Cuvette Cleaning

1. Regular cleaning: Use milliQ only or milliQ with dilute acid, e.g. HCl
2. If step 1 does not clean the cuvette, use Starna cuvette cleaning solution (if available)
3. If step 2 does not clean the cuvette, rinse the cuvette with methanol and then thoroughly rinse with milliQ.
4. If steps 1-3 do not clean the cuvette, dissolve 1-2 pellets of NaOH in 200 mL of methanol. Soak cuvette in this solution for 1-3 hours or up to no more than 6 hours. After this treatment, rinse cuvette many times (at least 20x inside and out) with milliQ water.

Acknowledgements

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References

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Appendix 1. DWR Fluoromax 4 Vital Statistics

Purchase Date: 2007

DWR Asset #: 208888

Manufacture Date:

Serial No: 0227C-3507

First Repair: January 2, 2011. PMT replaced by JY repair engineer

FluorEssence Version 3.5.1.2: May 2011