***Supplementary Material***

**Quantum yields of natural organic matter and organic compounds: Implications for the fluorescence-based interpretation of organic matter composition**

**Tutorial for the auqaDOM toolbox**

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# Foreword

This document contains a short tutorial on using the aquaDOM toolbox to obtain spectral quantum yields of natural DOM samples or organic compounds. As a prerequisite, fluorescence EEMs should be fully processed and corrected using the drEEM toolbox (Murphy et al., 2013). This tutorial can also be executed by running the code provided in the file “\tutorial\aquaDOM demo.m”

References:

Murphy, K. R., Stedmon, C. a., Graeber, D., and Bro, R. (2013). Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Anal. Methods* 5, 6557. doi:10.1039/c3ay41160e.

# Prerequisites

Prior to using the aquaDOM toolbox, users need a valid installation of MATLAB. To install the toolbox, unyip the contents of the aquaDOM toolbox in a folder (e.g. “C:\Program Files\MATLAB\R2014b\toolbox\aquaDOM”) and add the path to the MATLAB paths (MATLAB, Home, Set Path, Add Folder).

As mentioned before, all EEM data should be processed and corrected using the drEEM toolbox, since variable names are chosen to be fully compatible with the latest release.

# Getting started

To get an overview over the contents of the aquaDOM toolbox, type:

help aquaDOM\_contents

Working with the aquaDOM toolbox requires a daily cross-calibration to be performed to ensure the validity of your quantum yield standards. A recommended standard is quinine sulfate (NIST SRM 936a) and a second standard with a well-known, stable quantum yield.

Depending on the method, a five-point dilution series, or a five-time measurement of these two standards should be carried out. For the dilution series, a 1:1.5 or 1:1.2 dilution is advised. This gentle dilution will enable the user to use the same instrument settings while making sure that the measurement quality is good. For quinine sulfate, the following parameters might be chosen (determined on a HORIBA Jobin Yvon Aqualog):

Start concentration: 1 mg/L

Dilution series: 1:1.2 (five measurements total)

Integration time: 0.1 s

Excitation increment: same as desired sample incerement, e.g. 5 nm

Emission increment: 4 pixel (may be changed to fit sample measurements)

Absorbance pathlength: 1-10 cm, higher pathlength will result in more stable measurements

For salicylic acid, the following parameters might be chosen (determined on a HORIBA Jobin Yvon Aqualog):

Start concentration: 2 mg/L

Dilution series: 1:1.2 (five measurements total)

Integration time: 0.1 s

Excitation increment: same as desired sample incerement, e.g. 5 nm

Emission increment: 4 pixel (may be changed to fit sample measurements)

Absorbance pathlength: 1-10 cm, higher pathlength will result in more stable measurements

Since the determination of the Raman area at an integration time of 0.1 s is difficult, the dataset should instead be normalized to an integration time of 1 s by dividing the signal by the integration time used in every measurement. The drEEM toolbox function *undiluted.m* provides an option to perfom this operation for every sample.

Samples should be named consistently, e.g. for quinine sulfate: QS.1, QS.2, QS.3 ect. This will enable the aquaDOM functions to identify related measurements.

A complete dataset should contain the fluorescence and absorbance measurements of both quantum standards, as well as the desired samples. For this tutorial, a dataset with two standards and one sample is provided ("\aquaDOM\Tutorial\tutorial.mat"). If you want to follow this tutorial, type:

load('\tutorial\tutorial.mat')

# Preparing quantum yield calculations

In order to carry out quantum yield calculations, the methods of the aquaDOM toolbox need supplementary information. Fristly, for standards, the unique filename identifiers, the reference quantum yield, the reference quantum yield wavelength, as well as the emission integration range have to be provided for standards (QS: Quinine sulfate, SA: Salicylic acid, function setting ‘STD’):

% Info for Standard 1: Qunine sulfate (NIST SRM 936a):

% Tag: QS

% Reference quantum yield: 0.51

% Reference wavelength: 350nm

% Number of measurements: 5

% Concentrations: [1.38E-06 1.38E-06 1.38E-06 1.38E-06 1.38E-06]

% Info for Stanard 2: Salicylic acid (CAS: 69-72-7, AppliChem > 99.5% purity)

% Tag: SA

% Reference quantum yield: 0.36

% Reference wavelength: 295nm

% Number of measurements: 5

% %Concentrations: [1.472E-05 1.47E-05 1.47E-05 1.47E-05 1.47E-05]

tagsStandards={'QS' 'SA'};

metadataStandards=assembleMetadata(tagsStandards,[325 600],'STD');

The function *assembleMetadata.m* will ask for the required information. Note that it is not necessary to provide correct concentrations for the standards in order for the toolbox to work. This information is necessary only if the user desires to calculate molar fluorescence and absorbance.

In the same manner, assembleMetadata will gather information about quantum yield samples (Suwannee River XAD-8, function setting ‘SAM’):

tagsSamples={'SwR'}

metadataSamples=assembleMetadata(tagsSamples,[240 600],'SAM');

After the measurement metadata is assembled successfully, the user may split the dataset into 2 subdatasets: One for the cross-calibration, and one for sample quantum yield calculations:

[qy\_R]=splitDS(EEMcor,metadataStandards);

[qy\_X]=splitDS(EEMcor,metadataSamples);

This completes the preparation steps for quantum yield calculations with aquaDOM.

# Calculation of (apparent) quantum yields

First, the cross-calibration will be performed. Type:

[qy\_R]=aqy(qy\_R,1,2);

The function settings specify that the operation to be carried out is a cross-calibration (1), using the dataset qy\_R, and the method to be used is the zero-intercept approach (2). A plot window, along with console output will inform users about the success of the procedure. A warning will be displayed, if the determined quantum yields are more than 10 % out of range compared to the provided reference quantum yields.

After the successful cross-calibtration, the sample quantum yields may be calculated as follows:

[qy\_X]=aqy(qy\_X,2,2,qy\_R,1);

In sequence, the function settings provide the following information: Sample dataset, calculation mode (2, for sample calculations), calculation method (2, for zero-intercept approach (has to match the calibration method)), calibration dataset (qy\_R), and quantum yield standard to be used for calculations (here the first one in qy\_R, quinine sulfate).

The script will produce a result graph and notify the user upon successful completion of the calculations.

# Data analysis

*splitDS.m* modified the datasets for quantum yield calculations: Fluorescence data is stored in a 4D matrix (sample x concentration x emission x excitation), and absorbance data is stored in a 3D matrix (sample x concentration x absorbance). *mergeDS.m* is a function that can merge datasets of different measurement days into a coherent dataset that can be processed further with drEEM, e.g.:

Xout=mergeDS(qy\_X, qy\_R);

Note that this method only takes the first concentration of every series, and ignores the other replicates or dilution series members.

Quantum yields can be viewed with the function aqyview.m, e.g.:

aqyview(EEMcor,[],'SUP');

If desired, molar absorbance, and fluorescence coeffiecients may be determined as follows:

qy\_X1=epsilon(qy\_X)

Note that correct molar concentrations have to be provided during the assembly of the respective measurement metadata!

**A template containing the typical workflow of methods for aquaDOM is provided in *aquaDOM\_workflowExample.m***