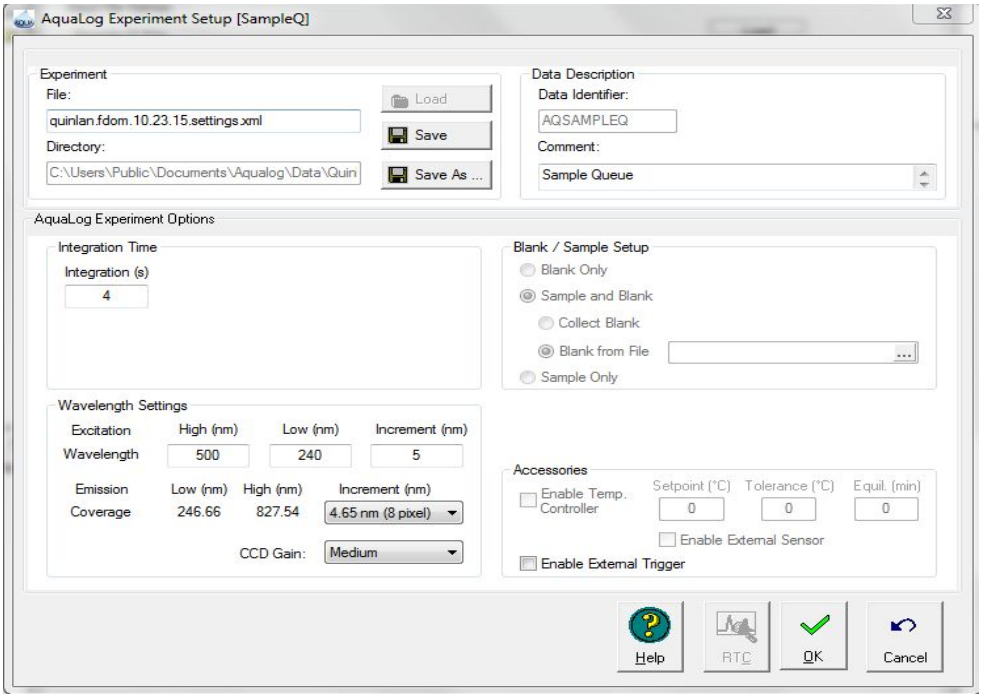
**Written by Zach Quinlan 2017**

***Collection***

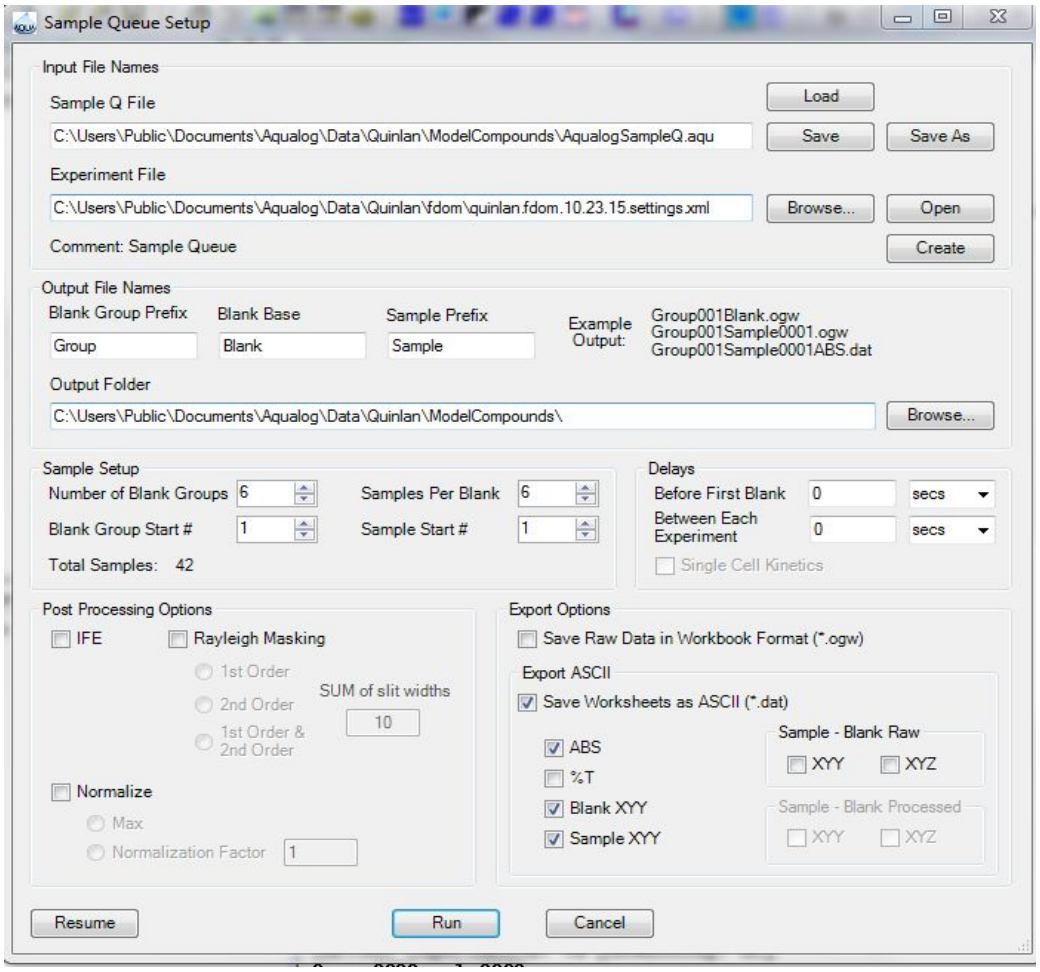
1) Collect samples by filtering water through 0.2µm polyethersulfone filter into acid washed and combusted amber vials.
2) Store filtrate at 4˚C
3) Analyze on aqualog spectrometer within a month

***Aqualog setup***

1) Take samples out of fridge and let warm to room temperature
2) Turn on the Aqualog spectrometer and let it warm up (0.5-2 hours)
3) Open Aqualog V3.6 software on desktop of Lenovo laptop
4) Make a new Q file by clicking on the Q in the Aqualog quickbar 
    a) The Aqualog will make a series of loud noises. After the secondary screen opens. Close the entire application.(There has been some communication error where the Q needs to be activated twice otherwise the internal lazy susan won't rotate).
    b) After re-opening and new file tool (Q) continue with set up
    c) First create or load experiment file (settings)
        i) New: press create and name the file Yourname.Experiment.SampleQ.Settings.xml
        ii) Change integration time 4-seconds
        iii) Excitation wavelength - change to high=500 nm, Low=240 nm
        iv) Increment - change to 5nm
        v) Emission - select 8 pixel
        vi) CCD gain - medium
        vii) Blank and accessories is greyed out
        viii) Save as - deposit in folder wtih your name
        ix) Press okay



d) Back at the sample Queue setup window
e) Make a sample Q file in folder of choice (Your named folder)
    i) Make sure output is into the same file C:\Users\Public\Documents\Aqualog\Data\yourname\experiment
f) Adjust sampling settings for sets and blanks
    i) Divide your samples in sets (blank groups), usually 6-8 samples per set. At the beginning of each set you will run another blank of Q-water
        (1) Number of blank groups == number of sets
        (2) Samples per blank == samples per set
    ii) Blank group sample start # == 1
    iii) Start Sample # == 1
g) Do not select for any delays because it automatically runs samples in machine after delay resulting in possible reruns of samples
h) Post processing options: uncheck all post processing options
    i) All standardization will be done using the matlab script
i) Export options: Uncheck “save raw data”
j) Export ASCII: Check “save worksheets as ASCII”
    i) Check ABS
    ii) Check blank XYY
    iii) Check sample XYY



5) Press Run and the system will prompt you to insert your first two samples

***Running the samples***

1) Before proceeding with your run, make a sample list worksheet
    a) Or fill in sample names on “matlab setup” worksheet (located in google drive)
2) Before pipetting samples into cuvettes, rinse the cuvettes three times and shake empty
    a) Shake vigorously but not hard enough that you throw the cuvettes and shatter them
3) Fill quartz cuvettes with ~3ml of samples
    a) First sample of set will be the first blank and then your first sample of set 1
    b) Pop up will read
        i) SC position 1: Blank 1 (Group001Blank)
        ii) SC position 2: Sample 1 (Blank 1) [Group001Sample0001]
4) Wipe quartz cuvettes with Kimwipe to clean smudges and fingerprints
5) Press continue to run your samples once they are loaded and lid is shut
6) It takes around 8 minutes to run two samples. You can watch the progress of each samples on the “Intermediate Display” which pops up.
7) The sample holder will automatically advance to position 2 when position 1 has been run.
8) ~1minute before samples are done running pipette next samples into rinsed cuvettes and wipe down sides
    a) Use the same pipette tip and Kimwipe(change both each blank)
9) Once the two samples have run, the system, will pause and a window will open listing the expected next samples for positions 1 and 2. Removed the old cuvettes and inset the new ones (note that the sample holder has shifted positions from the first two) and press continue. Keep in mind that if you are running an even number of samples per blank you will be placing the final sample of a set and the next blank in the same holder. This can be a tad confusing.
10) After each non-blank sample the “script window” will report that the files have been exported. Check the directory to verify they have been exported there in .dat format. Files ending in BEM are blanks, SEM are samples, and ABS are absorbance scans.

***Matlab Script and runlog***

1) On Google Drive in the Nelson Lab folder access the “fDOM basic Matlab input sheet DO NOT EDIT. COPY/PASTE ONLY”
    a) Page one are step by step guide to editing
    b) Page two is the actual matlab input spreadsheet
2) n= number of samples per blank group

| | |
|---|---|
| Step 1. | Delete extra blank groups and samples |
| Step 2. | Delete the last (8-n) samples of each blank group. This should make it so there are n samples in between each "BLANK" |

| | |
|---|---|
| Step 3. | In the the E collumn Copy and paste "C:\Users\Public\Documents\Aqualog\Data\yourfilepathhere\Group001Sample 0001BEM.dat" into the bottom (1-n) |
| Step 4. | Put in your own sample names. These are the names that will be put into the final summary sheet. NO SPACES EVER! |
| Step 5. | "Find and replace" "yourfilepathhere" with your file location if it is within the .\Aqualog\Data\ folder |
| Step 6. | Download as .tsv and name file something that starts with a letter (matlab is dumb). |
| Step 7. | Put name of .tsv into line 112 of .m script |
| | |

3) Download the latest version of the script. Found at https://github.com/Zquinlan/fDOMmatlab/edit/master/Script.md

4) Edit the matlab script as need-be
    a) Lines 1-112 explain file
    b) Line 113 is where you input the matlab input worksheet file name (E.G. “fDOM.tsv”)
    c) Lines 118-254 have to do with settings of ran samples
        i) Should not have to be edited if above steps were taken

d) Lines 255-288 are for specific peaks to acquire values and input into final spreadsheet
    i) Edit only if you want to add new peaks (NOTE: if added need to adjust lines 335-367)
e) Line 289-334 are for split half validation, and PARAFAC modeling
    i) This is where you change how many PARAFAC components you would like to input. These have to be modified with each experiment because there should be a different number of components in each experiment.
f) Lines 335-367 make the Summary Data Matrix
    i) Edit for PARAFAC models and new peaks from above
g) Remaining lines have to do with other spreadsheets which are created such as EEMain
    i) These should not have to be edited