

Fingerprinting Sources of Dissolved Organic Matter (DOM)

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- Study Background
- Brief Fluorescence Theory
- Importance of DOM
- QC of Fluorescence data
- Preliminary Results
- Future work
- Questions?



Horiba Fluoromax 4
Spectrofluorometer



Hach Dr 5000
spectrophotometer

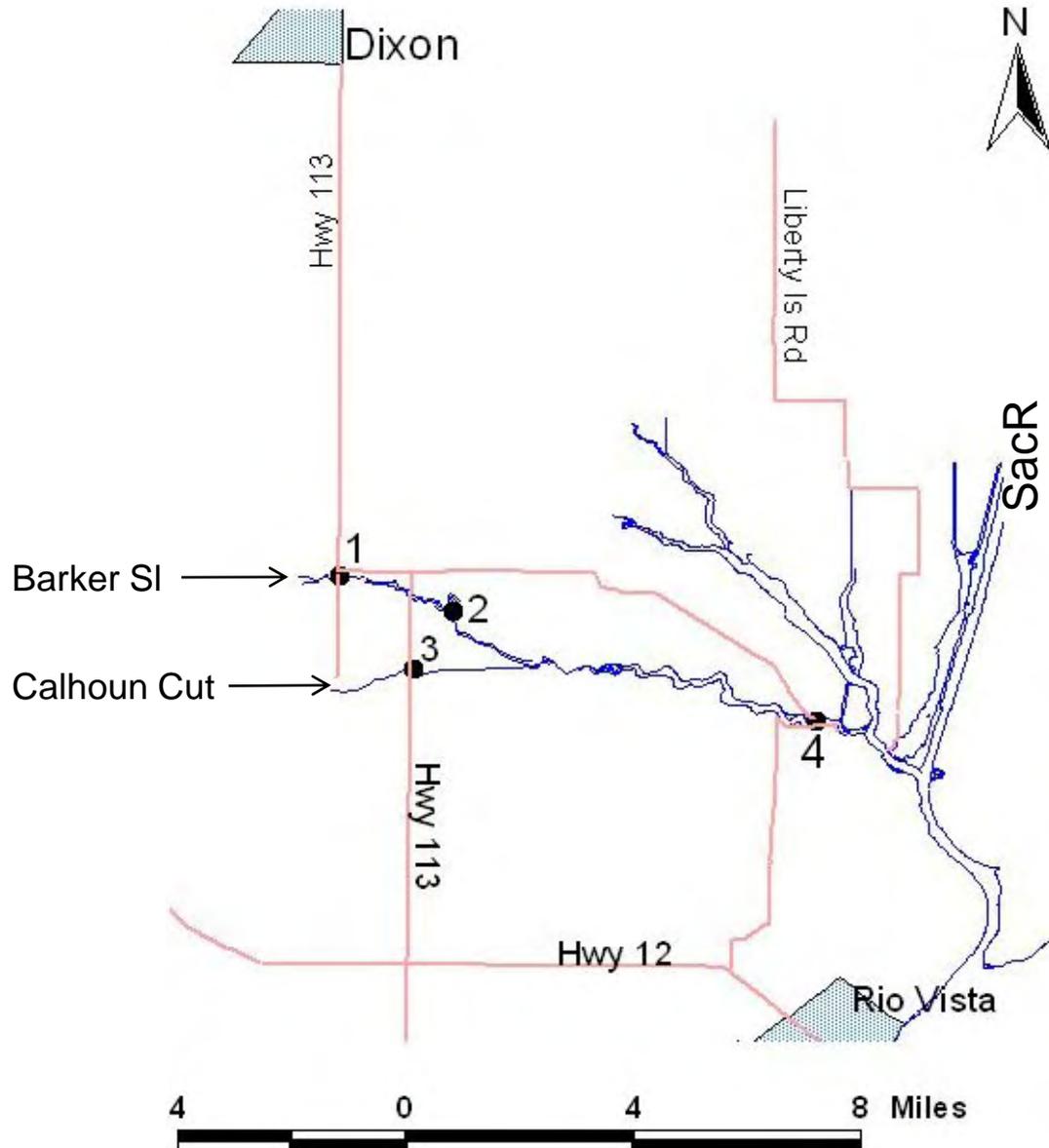
Brief History of Study

- In 1998, Environmental Services Office (now DES) requested QA/QC Program's assistance in developing a Standard Operating Procedure for chlorophyll filtration, algal count sampling & **single point fluorescence**
- Turner **10AU Fluorometer** was used for fluorescence
- North Bay Aqueduct (NBA) was selected for pilot study
- Constituents: phytoplankton, chlorophyll, DOC/TOC, nutrients, THMFP, field single point fluorescence
- QA then developed an SOP for chlorophyll filtration
- Correlation between chlorophyll, algal biovolume and fluorescence was found to be poor

Brief History of Study (cont'd)

- In 2007, the QA/QC program decided to revisit the study to test whether newer fluorometers could provide better correlations between fluorescence, DOM & algal biovolume
- A Fluoromax 4 Spectrofluorometer & a Hach DR 5000 spectrophotometer were acquired
- NBA was still the study area with 3 stations
- Study limited to 2010/11 spring & summer algal growth periods to compare to the 1998/9 data
- Samples: phytoplankton counts, DOC/TOC, nutrients, THMFP, 2D and 3D fluorescence

Sampling Sites



Station Legend

1. Barker Sl @ Cook Rd
2. Barker Sl @ Ag PP*
3. Calhoun Cut @ Hwy 113
4. Lindsey Sl @ Hastings Is Bridge

*Sampled in 1998/9 but not in 2010/11 due to lack of access

Importance of DOM

- DOM reacts with disinfectants such as chlorine to form potentially carcinogenic byproducts
- Interacts with organic/inorganic contaminants & affects their transport, stability and bioavailability. Most pesticide models require DOM as one of the main parameter inputs
- Plays a key role in bacterial and microbial food web processes in coastal ecosystems such as the Sac/SJ Delta
- Involved in carbon sequestration

Why Use Fluorescence for DOM?

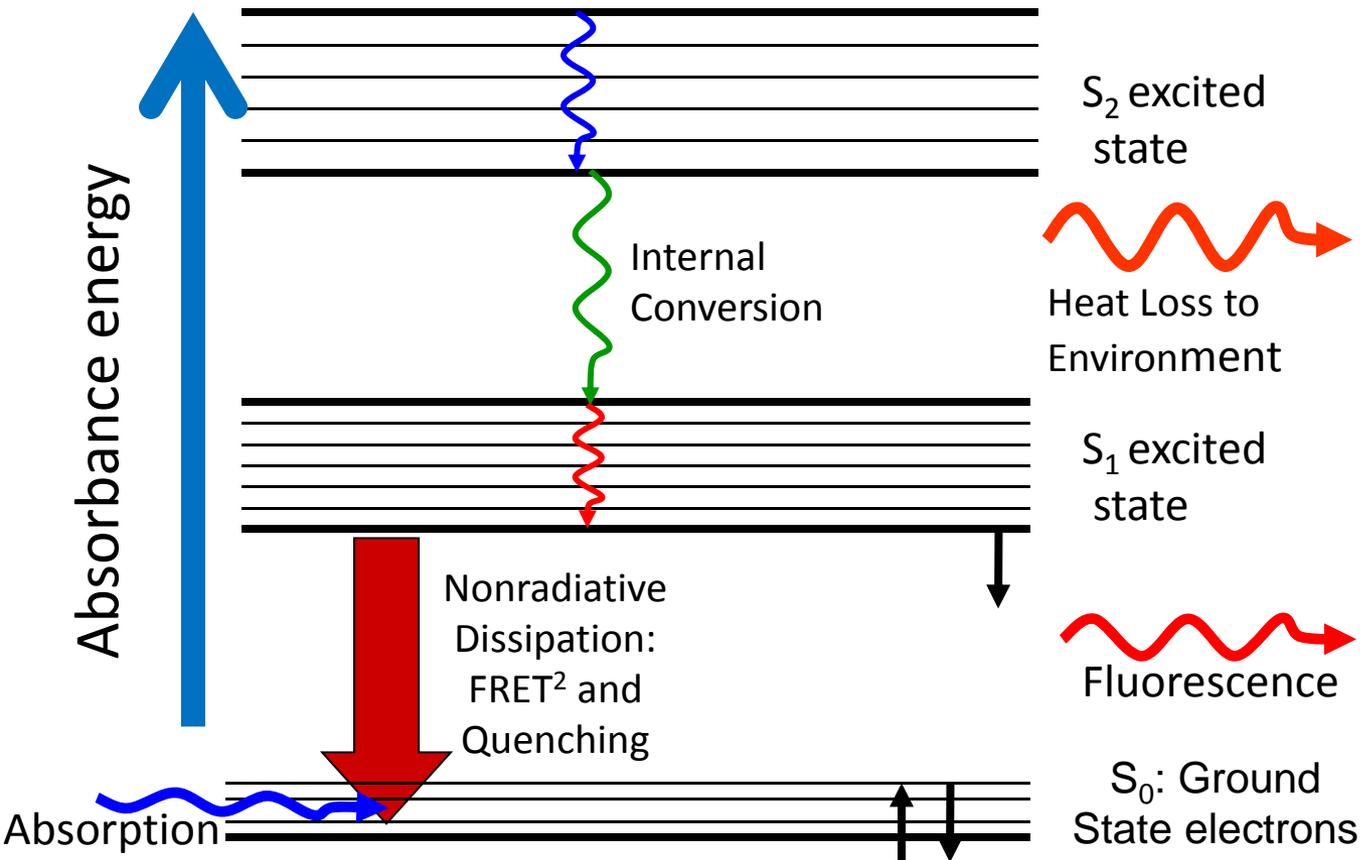
- DOC measurements only give the **quantity** of substances in the sample. DOC is just a gross estimator of DOM
- Multiwavelength fluorescence can provide a lot more information about the **quality** (*composition*) of the sample
- Fluorescence is far more sensitive than UV/VIS absorbance for discriminating 'quality' of substances in solutions

Fluorescence: Jablonski Diagram

- Describes processes which occur between *excitation* & *emission*
- There are different diagrams for different fluorescence phenomena
- Below is one form of a simplified **Jablonski** diagram¹



Aleksander Jabłoński
“Father of Fluorescence Spectroscopy” 1888-1980



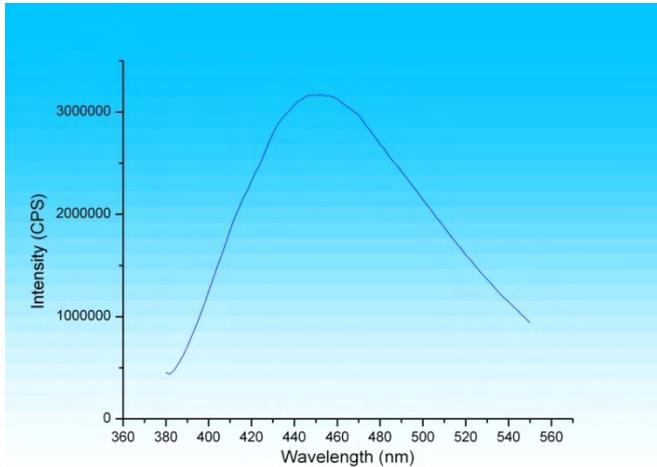
Fluorophore: molecule that fluoresces after absorbing energy . Not all molecules are fluorophores

²FRET: Fluorescence Resonance Energy Transfer

¹Modified from Lakowicz (2006) & Gilmore (Horiba 2010)

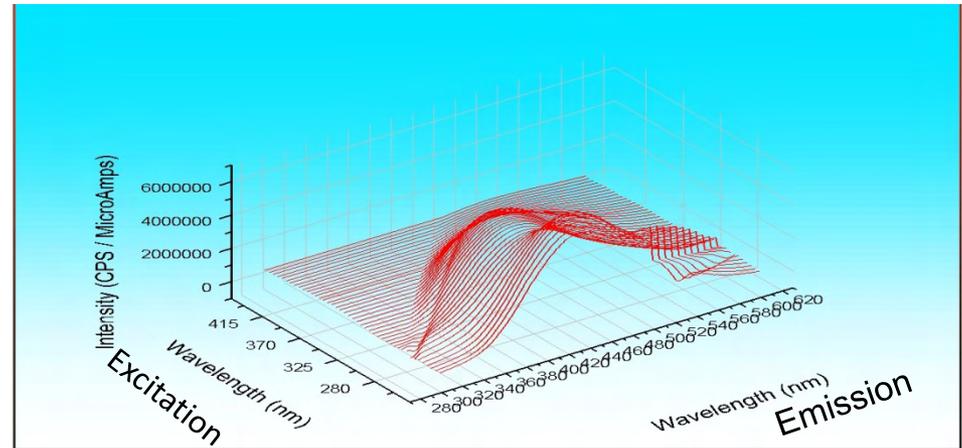
2D & 3D Fluorescence

2D



- Fixed (constant) Excitation wavelength used
- Emissions measured at several wavelengths of interest
- Example above has **86** data points

3D (EEMs)



- Several Excitation wavelength utilized
- Several Emissions measurements made at each excitation creating an EEM matrix
- A lot of data are generated
- Example above has **6342** data points!

There are many other types of fluorescence measurements generally not used for DOM studies such as: phosphorescence, anisotropy, lifetimes, etc.

EEMs have a lot of data

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	Wavelength	240	245	250	255	260	265	270	275	280	285	290	295	300	305	310	315	320	325	330	335	340	345
2	300	112973.8	92297.51	97487.33	114562.7	145992.6	177749	51563.19	24051.17	267144.8	232248.8	161675.7	127313.4	114593.8	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
3	302	122722.2	102472.4	109561.5	130269.1	160494.9	212943.5	171971.4	8311.652	245001.4	260162.3	169508.7	127313.4	114593.8	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
4	304	135294.5	117998.7	124191.1	146721.6	180077.3	234414.1	271328.1	72229.23	204780.3	279995.2	179019.9	127313.4	114593.8	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
5	306	155581.9	127470.5	133652.5	157354.2	194587.5	258477.1	321667.4	178552.5	135577.3	295856.8	200103.9	127313.4	114593.8	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
6	308	165734.2	137578.4	150127.2	173040.5	217083.2	273987.2	357984.2	308258.7	122009.4	276431.8	222726.9	128529.4	114593.8	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
7	310	179272	156014.8	163473.6	186894.1	228052.6	299216.7	384387.9	400613.2	168218.3	228066.8	240233	139278.9	114593.8	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
8	312	191019.7	161459.4	175017.4	203890.2	248788.4	315485.2	398805.3	454719.2	279249.1	177023.9	251978.7	155671.9	101976.9	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
9	314	204600.2	182776.4	194253.9	220462.6	264164.6	334868.5	426841.1	492992.9	390868.2	157549.8	241719.2	176341.8	105285	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
10	316	224159.2	199808.6	205461.6	240703.7	289750.1	356171.4	450087.2	515409.9	479655.6	198904	213768.9	196147.6	119039.3	106371.7	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
11	318	246137.1	210436.8	219453.6	257071.9	305522.6	381793.7	469690.1	544671.7	544290.8	284308.3	166253.3	211622.2	136615	101408.9	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
12	320	254881.9	221987.5	247707.2	273468	331700.1	403120.8	497143.3	576144.1	591371.8	412322.3	157828.3	208919.8	156493.1	109099.4	122340.8	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
13	322	272796.7	250703.2	257798	301036.3	355790.4	426305	531525.6	609774.7	623719.8	507807.6	183983.9	186298.1	183045.2	123681.3	103437.3	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
14	324	306074.5	278014	283231.4	326230.4	383633.4	459143.1	553792.1	637212.6	658804.7	575001.2	281267.6	165072.8	202501.1	145050.9	106585.3	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
15	326	336244.9	297747.1	306032.6	347067	413937.2	490399	597445.5	670328.6	697420.5	631855.1	394941.3	165119.2	213061.6	171288.1	124135.7	122437	159903	180987.1	214668.8	236440.5	284907.2	32601
16	328	360141.7	321557.8	338009.7	374308.7	436866	524782.8	617501.1	710383	737422.3	680393.9	499334.7	196942	199623.4	196975.7	142736.6	119556.8	159903	180987.1	214668.8	236440.5	284907.2	32601
17	330	402553.1	352939	368936.5	406089.8	466167.7	558683.6	658453.9	739050.3	775221.8	728754.5	583459.4	288738.2	182830.4	225304.5	171055.7	132959	159903	180987.1	214668.8	236440.5	284907.2	32601
18	332	430011.9	396590.6	391016	436775.6	499645.7	592110.2	697270.1	779413.4	815883.5	773081.5	645676.4	39010.9	185286.5	242994	202958	152826.5	137271.7	180987.1	214668.8	236440.5	284907.2	32601
19	334	481089.4	436583.3	432286	471039.6	545185.6	626984.2	736410.1	822059.3	862818.8	815426.8	704028.5	503340.5	234218.1	248576.4	238497	184315.8	146671.5	180987.1	214668.8	236440.5	284907.2	32601
20	336	526048.3	473741.9	474776.2	512554	575473.1	670636.5	774933.1	866907.3	907333.1	867912.2	760983.6	591665.3	328349	250603.1	276006.2	218502.9	170332.1	180987.1	214668.8	236440.5	284907.2	32601
21	338	574806.5	521329.4	524441.5	559652.3	623897.7	717767.8	825433.2	910953.8	954941.2	923423	821688.6	668386.7	445839.3	269672.5	314449.9	259864.6	201546.7	178169.4	214668.8	236440.5	284907.2	32601
22	340	640660.3	589722.1	576421	609730.4	661042.2	759899.6	877751.6	963437	1015876	986094.1	884005.1	737422	560695.9	324460.7	329069.2	313261.7	243856.9	202500.1	214668.8	236440.5	284907.2	32601
23	342	709977	655833.5	646084.7	666870.9	721106.2	813240.6	920902.6	1016066	1061069	1044979	944085.1	811348.6	659089.2	424126.3	352340.2	364995.6	293070.1	238586.1	199953	236440.5	284907.2	32601
24	344	785955.7	736140.1	712709.3	732342.1	778865.7	871728.5	984991.7	1077551	1124169	1106346	1022618	885889.2	753610.7	552454.9	387327.1	412352.3	355753.8	287114	224478.4	236440.5	284907.2	32601
25	346	866173.8	801732.1	791996	804241.6	843486.4	932139.9	1053513	1145994	1192977	1180942	1099512	967925.1	840752.1	684433.7	460113.5	457688.1	421344.1	344238.9	269847.9	236440.5	284907.2	32601
26	348	954036.8	901909.1	893635	886049.3	926625.9	1007496	1114694	1211729	1263906	1253997	1186475	1059586	929888.1	811939.7	586250.2	500483.5	493034	412999.4	322031.1	259154	284907.2	32601
27	350	1047680	1006924	987142.2	1083002	11014808	1083002	1189178	1283011	1345908	1331843	1265176	1146013	1029352	917327.2	731084	563868.8	569592.8	492991.7	389055.2	303849.6	284907.2	32601
28	352	1166067	1124772	1083465	1086660	1105440	1167980	1282000	1375008	1425379	1420137	1364486	1245225	1135043	1024479	874169.5	655519.4	639488.4	583647.4	468432.1	363295.1	291028.4	32601
29	354	1274838	1231909	1203153	1198249	1191665	1264817	1369939	1456581	1520681	1515617	1455645	1242402	1139767	1010804	786021.4	708692.6	617988.4	553034.1	435093.2	335263.3	32601	
30	356	1398006	1388827	1341391	1316115	1307130	1371600	1463073	1552067	1605130	1609948	1562403	1460765	1356471	1264086	1156519	960404.5	784677.7	765037.1	652739.8	518831.9	394320.1	32601
31	358	1540541	1510592	1465174	1440165	1434733	1480912	1563483	1653817	1696552	1706541	1660268	1568777	1475745	1381746	1273995	1117995	885910.5	849177.4	752944.5	609897.2	469424.6	35768
32	360	1654747	1668106	1628391	1583889	1557032	1586238	1675837	1751016	1807612	1817797	1710164	1674728	1593320	1504797	1402151	1275085	1031312	932467.1	855969.8	705069.9	548164.9	40995
33	362	1801610	1789284	1764234	1724937	1695746	1724609	1784835	1861618	1912037	1915055	1879386	1791412	1705478	1625986	1531150	1412453	1192963	1025342	968983	814035.2	640681.8	48072
34	364	1929957	1949594	1928638	1875793	1837585	1833995	1912554	1983445	2039352	1994287	1915822	1834245	1763572	1663909	1548109	1360240	1137906	1060980	917642.2	735104.3	55871	
35	366	2089197	2097168	2085974	2033106	1986875	1979565	2035692	2095363	2136623	2143005	2108258	2032781	1970150	1892309	1810012	1696326	1534781	1284555	1159853	1039047	844055	64517
36	368	2233180	2258583	2237385	2212454	2133920	2112922	2175757	2220707	2254867	2253366	2237759	2151426	2088028	2031377	1946031	1836163	1687197	1451764	1255627	1157993	950787.7	73951
37	370	2402505	2427592	2422702	2365951	2285057	2259487	2297932	2338725	2373427	2380440	2345039	2278288	2258004	2173332	2084747	1977796	1830657	1618431	1365725	1261657	1068929	8418
38	372	2562926	2596391	2579769	2525525	2453501	2409224	2434710	2471108	2494716	2500475	2466529	2408695	2362087	2300611	2227944	2121790	1975186	1794598	1502942	1360504	1187661	94687

This matrix has 6342 data points!

DOM Source Fingerprinting

Approach in this Study

- 1 Collected 2D data to develop source fingerprint indices at 3 NBA (and several Delta & watershed) stations
 - Experimented with several indices but **only 2** from NBA will be presented today
- 2 Collected 3D data to model possible DOM components. Modeling not started yet

Major data acquisition problem was instrument failure that took a long time to repair due to state budget issues

Example 2D Fingerprint Indices

Fluorescence Index (FI)

- Ratio of 470/520 (for spectrally corrected data)
- FI can 'distinguish' algal & microbial DOM produced within the aquatic system (**autochthonous**) from outside terrestrially produced (**allochthonous**) DOM.
- **High FI & low SUVA**: microbial; **Low FI & high SUVA**: terrestrial

Humification Index (HIX) [used more in soils]

- Humification: conversion of lower molecular weight plant and animal breakdown products to higher molecular weight substances mainly through microbial activity
- Conceptually: **a**) active pool (<1 yr); **b**) Intermediate pool (years to centuries old); **c**) passive stable pool (thousands of years old)

$$HIX = \sum I_{435 \rightarrow 480} / (\sum I_{300 \rightarrow 345} + \sum I_{435 \rightarrow 480})$$

(I = fluorescence)

One 3D Data Decomposition Method

- **Parallel Factor (PARAFAC)** analysis is a multi-way decomposition method (a higher order PCA)
- It models the EEMs to find the distinct 'components' in the data
- Originated in psychometrics but has gained wide use in chemical and DOM analyses
- Quite complex to implement

The PARAFAC guru



Rasmus Bro -- Prof, Chemometrics Group, Dept. of Food Science, Univ of Copenhagen

PARAFAC Analysis

Number of components

Concentration (moles)

Fluorescence quantum yield

Molar absorptivity

Fluorescence intensity

Residual

$$\mathbf{x}_{ijk} = \sum_{f=1}^F a_{if} b_{if} c_{kf} + \varepsilon_{ijk}$$

The diagram illustrates the PARAFAC model equation. It features five descriptive labels with arrows pointing to specific parts of the equation: 'Number of components' points to the summation limit F ; 'Concentration (moles)' points to the coefficient a_{if} ; 'Fluorescence quantum yield' points to the coefficient b_{if} ; 'Molar absorptivity' points to the coefficient c_{kf} ; and 'Residual' points to the error term ε_{ijk} . The variable \mathbf{x}_{ijk} is labeled as 'Fluorescence intensity'.

QC of Fluorescence Measurements

There are no universally accepted procedures!

- Use correct, clean cuvettes without scratches
- Calibrate lamp, and emission monochromator
- Use high quality double distilled blank water
- Can use quinine sulfate solution standard (confusing methodology) or NIST solid standards (expensive)
- Can use **reference** fulvics and humics from the International Humics Substances Society (**IHSS**)
- Must make spectral corrections (Raman and Rayleigh scatter, inner filter effects, etc) of data

IHSS Reference Substances Utilized for QC Purposes in this Study:

1. Pony Lake Fulvic Acid (FA)

Pony L (*Antarctica*) is an IHSS's aquatic microbial FA 'end-member'



2. Suwannee River Fulvic Acid

Suwannee R (*originates in Georgia*) is an IHSS's terrestrial FA 'end-member'



Preliminary Results: Indices

Station	Sample Date	Fluorescence Index	DOC (mg/L)	UVA ₂₅₄	Humification Index
Barker	9/9/2010	1.83	14.5	0.36	
	7/6/2011	1.85	12.2	0.35	0.944
Calhoun	9/9/2010	1.75	6.5	0.19	
	7/6/2011	1.79	5.6	0.20	0.929
Lindsey	9/9/2010	1.82	2.4	0.07	
	7/6/2011	1.88	2.6	0.08	0.895

FI suggests that at NBA stations, most DOC is **microbial autochthonous** but Barker and Lindsey are closer together than to Calhoun. FI values are out of suggested published ranges and need further research why this is so!

HIX suggests Lindsey's DOM's age is a little different from the other stations. More data are needed!

QC Results

Reference FA	Analysis Date	DOC (mg/L)	FI from this Study	FI from literature*
Pony Lake	2/15/2011	4.2	1.46	1.46
Suwannee R	2/15/2011	4.5	1.23	1.21

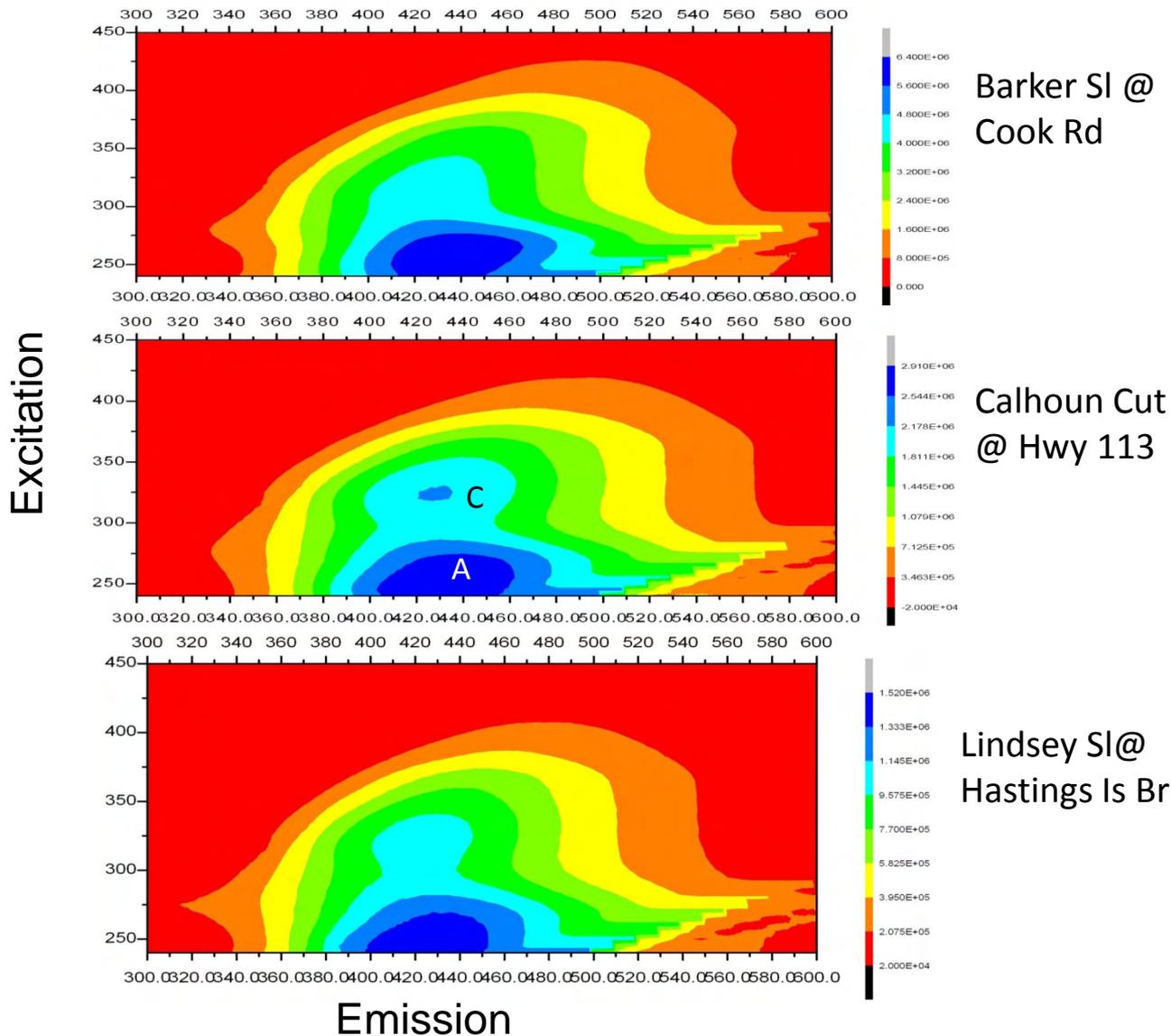
*Cory et al (2010) using the older Fluoromax 3. This indicates that correction procedures (QC) in this study were operating well but does not explain why environmental samples' FI are out of range.

Phytoplankton Counts

Station	CDate	Diatoms		Soft bodies	
		Cell counts	Dominant taxa	Cell counts	Dominant taxa
Barker	7/28/10	8	<u>Nitzschia sp.</u>	403	<u>Oscillatoria sp.,</u> <u>Phormidium sp</u>
Barker	9/9/10	26	<u>Cyclotella sp.</u>	409	<u>Phormidium sp.</u>
Calhoun	7/28/10	397	<u>Cocconeis sp.</u>	8	<u>Chlorococcum</u>
Calhoun	9/9/10	383	<u>Cocconeis sp.,</u> <u>Nitzschia sp.</u>	23	<u>Oscillatoria sp.</u>
Lindsey	7/28/10	396	<u>Fragilaria sp</u>	39	<u>Chlorococcum</u>
Lindsey	9/9/10	320	<u>Fragilaria sp.</u>	95	<u>Sphaerocystis sp.</u>

- During the two sampling events, diatoms dominated at Calhoun Cut and Lindsey SI
- Non-diatom species dominated at Barker

3D Contour Plots¹



2Peaks in literature

Ex/Em

- A: Fulvic 260/400-600
(humic, terrestrial)
- B: Tyrosine 275/305
- C: Humic 320-360/420-460
(anthropogenic, Ag)

¹(Sample Date: 7/6/2011)

²(Cobble, 2007)

Summary

- Instrument failure in 2010 prevented full year collection of fluorescence measurements
- Fingerprinting indices need more work . **NBA is not a good test area (water is of too low quality)**
- Correlation between FI and algal counts not as good as expected (but too few data points)
- Will continue indices in Delta & watershed stations
- Spectral corrections with software still a challenge
- For 3D data, next step is to find a way to run the PARAFAC model.

Acknowledgements

- Thanks to the DWR's QA/QC Program for supporting this project
- Thanks to the Field Support Unit for field sample collection
- Thanks to Bryte Lab for chemical analyses

Questions?