

USGSHydroOpt : Tools for Optical Analysis of Water

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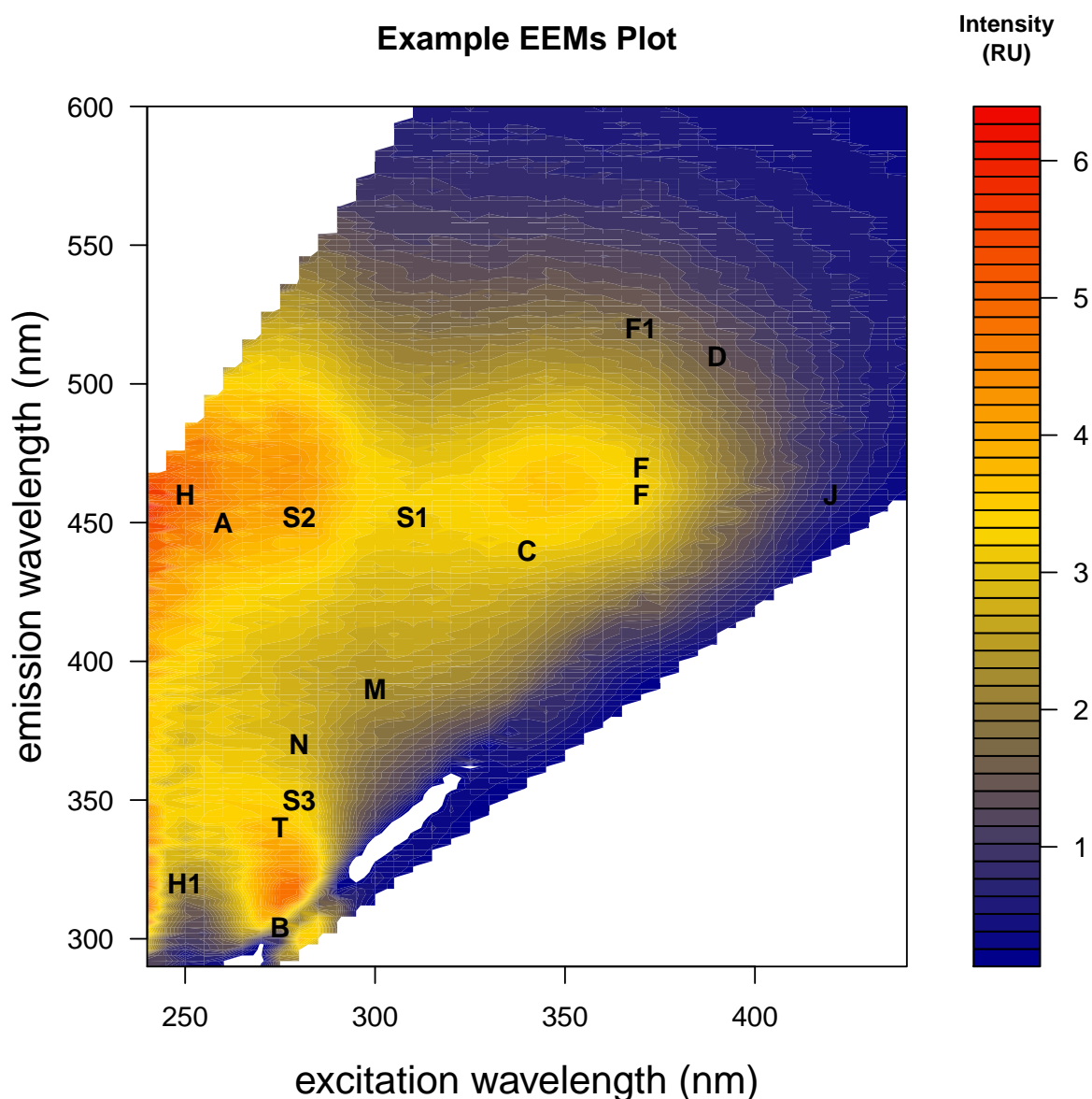
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1 Introduction to USGSHydroOpt

The USGSHydroOpt package was created to streamline the process of creating explanatory optical variables, and excitation-emission (EEMs) plots from absorbance and fluorescence data. Examples of optical summary variables that can be produced with this package include various absorbance peaks, spectral slopes,

fluorescence and humic index variables, and others. The motivation for producing this package is the power of optical variables for explaining biological and chemical phenomena in water. Optical summary variables produced using USSHydroOpt are extremely useful in statistical modeling efforts.

The functions in this package were designed to operate on dataframes with standardized formats. This package is not amenable to dataframes or arrays that do not fit the defined formats. The example dataframes and array in this package illustrate exactly how dataframes and arrays should be formatted, and the examples illustrate how to use the functions. USSHydroOpt contains a very useful function for creating contour plots of EEMs spectra. Depicted below is an example of an EEMs plot produced with this package. The user is encouraged to step through each piece of R code as it is introduced in this document. In many instances a sample of the function output is provided via plots and tables, but not in all cases.



2 Required Data Formats

The functions contained in USGSHydroOpt operate on dataframes with standardized schemas. Users interested in using USGSHydroOpt should take care to ensure that dataframes and arrays are formatted according to the schemas described in this section.

2.1 Absorbance Data

Absorbance dataframes used by functions in USGSHydroOpt should be formatted such that each sample occupies a column, and one column contains the wavelength (nm) for which the absorbance measurement was made (*See example Table 2.1 below*). The example absorbance dataframe included in this package is called **dfabs**. The column with the wavelengths in Table 2.1 does not need to be called "wavelengths," as it is in Table 2.1. Since this package was developed primarily for USGS activities, the default for naming samples is "gr" followed by the sample number. This convention was started by the USGS California Water Sciences Center (CA WSC), and the USGS Wisconsin Water Science Center (WI WSC) follows the same naming convention to minimize heterogeneity.

	gr13307	gr13351	gr13353	gr13357	wavelengths
1	0.0001296	-0.0003505	-0.0003480	-0.0002695	750
2	-0.0002367	0.0000305	-0.0000915	-0.0001407	749
3	-0.0001582	-0.0004900	-0.0006325	-0.0001534	748
4	-0.0004642	-0.0000105	-0.0000932	-0.0000245	747
5	-0.0002551	-0.0000653	0.0000841	-0.0001615	746
6	-0.0001842	-0.0002135	0.0002082	0.0001429	745

2.2 Fluorescence Data

Fluorescence dataframes used by functions in USGSHydroOpt should also be formatted such that each sample occupies a column, and one column contains the excitation-emission wavelength pairs (nm) for which the fluorescence measurement was made (*See example Table 2.2 below*). The example fluorescence dataframe included in this package is called **dfFluor**. The column with the excitation-emission pairs in Table 2.2 does not need to be called "Wavelength.Pairs," as it is in Table 2.2. Again since this package was developed for USGS activities, the default sample naming convention is "gr" followed by the sample number.

	Wavelength.Pairs	gr13307	gr13308	gr13351	gr13352
1	240/290	0.09457	0.05358	-0.07268	-0.19368
2	240/292	0.07386	0.09194	0.45433	0.39722
3	240/294	0.08116	0.09930	0.49135	0.37943
4	240/296	0.12013	0.09138	-0.12248	0.09239
5	240/298	0.15264	0.15428	0.54694	0.14980
6	240/300	0.16467	0.13010	0.43319	0.33064

2.3 Spectral Slopes Data

Information on the upper and lower wavelength (nm) for which a spectral slope should be calculated needs to be stored in a dataframe. The example spectral slopes dataframe included in this package is called **dfsags**. The dataframe should contain exactly three columns. The first column should contain the upper wavelength (nm), the second column should contain the lower wavelength (nm), and the third column should contain the name of the spectral slope being calculated (*See example Table 2.3 below*). The columns in Table 2.3 need to be in this exact order, although the names of the columns may be different. The data types for each column are integer, integer, and character, respectively. The user can add additional spectral slopes to Table 2.3.

	wvlength1	wvlength2	Name
1	275	290	Sag275_295
2	290	350	Sag290_350
3	350	400	Sag350_400
4	412	676	Sag412_676

2.4 Optical Summary Data

This dataframe contains many of the summary optical variables that can be produced using functions in USGSHydroOpt (*See example Table 2.4a below*). The example summary optical dataframe included in this package is called **dfsummary**. The functions in USGSHydroOpt calculate summary optical variables and add to a dataframe formatted according to Table 2.4a. Table 2.4a is exemplary of how the WI WSC stores optical summary variables. Note that this dataframe can contain other columns with metadata, for instance, the sample date and time, the sample ID, or whether or not the sample went through QA/QC.

	GRnumber	B	T	A	J	FI_2005	A254
1	gr13307	0.050997	0.1826	0.4553	0.03705	1.639	0.05228
2	gr13351	-0.245602	0.4085	3.0783	0.19110	1.456	0.43531
3	gr13353	-0.175220	0.7794	6.8624	0.56309	1.523	0.68274
4	gr13357	0.111561	0.2691	0.9451	0.06969	1.572	0.08698
5	gr13360	-0.001569	0.4593	2.8254	0.37231	1.563	0.28605
6	gr13363	0.052137	0.4892	1.9518	0.19098	1.583	0.19283

However, also note that the naming of the summary optical variables in Table 2.4a must be identical to those specified in Table 2.4b below. For example, the user cannot name the variable Optical Brightener 1 ("OB1") in Table 2.4a as "OptBright1."

[1]	"OB1"	"OB2"	"OB3"	"S1.50"
[5]	"S2.50"	"S3.50"	"S1.25"	"S2.25"
[9]	"S3.25"	"Mrange.25"	"Mrange.50"	"B"
[13]	"T"	"M"	"A"	"C"
[17]	"N"	"D"	"F"	"J"
[21]	"S1"	"S2"	"S3"	"H1"
[25]	"H2"	"F1"	"F2"	"W"
[29]	"LT1"	"LT2"	"LT3"	"LA"
[33]	"HIX_2002"	"FI_2005"	"FI_2001"	"FreshI"
[37]	"A254"	"A275"	"A280"	"A290"
[41]	"A295"	"A350"	"A370"	"A400"

```
[45] "A412"      "A440"      "A488"      "A510"
[49] "A532"      "A555"      "A650"      "A676"
[53] "A715"      "Sag275_290" "Sag290_350" "Sag350_400"
[57] "Sag412_676" "Aresids"
```

2.5 Excitation-Emission (EEMs) Peak Data

The EEMs peak data contains three columns listing the name of a characterized EEM peak along with the corresponding excitation-emission wavelengths (nm) (*See example Table 2.5 below*). The example EEMs peak dataframe included in this package is called **ex_ems**. The first column, in Table 2.5, "Peak," contains the name of the characterized EEM peak. The next two columns in Table 2.5 contain the excitation and emission wavelengths (nm) at which a given peak occurs. The column names must be identical to those displayed in the example below, although the order of the columns can be different.

	Peak	ExCA	EmCA
1	B	275	304
2	T	275	340
3	M	300	390
4	A	260	450
5	C	340	440
6	N	280	370
7	D	390	510
8	F	370	460
9	J	420	460
10	S1	310	452
11	S2	280	452
12	S3	280	350
13	H	250	460
14	H1	250	320
15	F	370	470
16	F1	370	520

2.6 Optical Ratio and Signals Data

This dataframe contains one column called "ratioSignals," which contains all of the summary optical variables currently identified by the WI WSC (*See example Table 2.6 below*). The example of Table 2.6 included in USGSHydroOpt is called **ratioSignals**. Note that these are the same variables as those listed in Table 2.4b. The first column must contain the various "ratioSignals" that the user desires, although the column name need not be "ratioSignals."

	ratioSignals	keep
1	OB1	NA
2	OB2	NA
3	OB3	NA
4	S1.50	NA
5	S2.50	NA
6	S3.50	NA

7	S1.25	NA
8	S2.25	NA
9	S3.25	NA
10	Mrange.25	NA
11	Mrange.50	NA
12	B	NA
13	T	NA
14	M	NA
15	A	NA
16	C	NA
17	N	NA
18	D	NA
19	F	NA
20	J	NA
21	S1	NA
22	S2	NA
23	S3	NA
24	H1	NA
25	H2	NA
26	F1	NA
27	F2	NA
28	W	NA
29	LT1	NA
30	LT2	NA
31	LT3	NA
32	LA	NA
33	HIX_2002	NA
34	FI_2005	NA
35	FI_2001	NA
36	FreshI	NA
37	A254	1
38	A275	1
39	A280	1
40	A290	1
41	A295	1
42	A350	1
43	A370	1
44	A400	1
45	A412	NA
46	A440	NA
47	A488	NA
48	A510	NA
49	A532	NA
50	A555	NA
51	A650	NA
52	A676	NA
53	A715	NA
54	Sag275_290	1

55	Sag290_350	1
56	Sag350_400	1
57	Sag412_676	1
58	Aresids	NA

2.7 Optical Signals Data

This optical signals dataframe is similar to "ratioSignals" except it provides more metadata about important peaks that have been characterized for EEMs plots. The example optical signals dataframe included in this package is called **signals**. The dataframe should contain six columns (*See example Table 2.7 below*). The first column in Table 2.7, "Peak," contains the name of the characterized EEM peak. The next two columns, "Ex1" and "Ex2," contain the excitation wavelength range (nm) for a given peak. "Ex1" is the lower wavelength and "Ex2" is the upper wavelength for the excitation wavelength range (nm) of a given peak. Similarly, "Em1" and "Em2" contain the emission wavelength range (nm) for a given peak. The final column, "Source," lists the source that characterized the peak. The last column, "Source," is recommended, but not required. The user must exactly replicate the column names in Table 2.7 before using functions in USGSHydroOpt.

	Peak	Ex1	Ex2	Em1	Em2	Source
1	OB1	360	NA	410	598	Hartel Turner
2	OB2	360	NA	436	436	Hartel Turner
3	OB3	365	NA	400	550	Hagedorn Turner
4	S1.50	310	NA	402	502	Sniffer
5	S2.50	280	NA	402	502	Sniffer
6	S3.50	280	NA	310	390	Sniffer
7	S1.25	310	NA	427	477	Sniffer
8	S2.25	280	NA	427	477	Sniffer
9	S3.25	280	NA	330	370	Sniffer
10	Mrange.25	300	NA	365	415	test
11	Mrange.50	300	NA	340	440	test
12	B	275	NA	304	NA	CA
13	T	275	NA	340	NA	CA
14	M	300	NA	390	NA	CA
15	A	260	NA	450	NA	CA
16	C	340	NA	440	NA	CA
17	N	280	NA	370	NA	CA
18	D	390	NA	510	NA	CA
19	F	370	NA	460	NA	CA
20	J	420	NA	460	NA	CA
21	S1	310	NA	452	NA	CA
22	S2	280	NA	452	NA	CA
23	S3	280	NA	350	NA	CA
24	H1	250	NA	460	NA	Ohno2002
25	H2	250	NA	320	NA	Ohno2002
26	F1	370	NA	470	NA	Cory and McKnight, 2005
27	F2	370	NA	520	NA	Cory and McKnight, 2005
28	W	255	290	302	350	
29	LT1	250	NA	340	NA	

30	LT2	260	NA	340	NA
31	LT3	240	NA	340	NA
32	LA	240	NA	440	NA

2.8 3-Dimensional Excitation-Emission Array

A 3-D array with fluorescence data is used by many of the functions in USGSHydroOpt. The first dimension contains the excitation wavelengths (nm) as data type character, for which a given fluorescence measurement was made. The second dimension contains the emission wavelengths (nm) as data type character, for which a given fluorescence measurement was made. The third dimension contains the sample numbers as data type character for a given observation. The user must ensure that the third dimension of the array contains the sample numbers. Again, in this example the default "gr" followed by the sample number is used as a naming convention for samples. The example 3-D array with EEMs data included in this package is called **a**.

To view the categories for each dimension, use the following commands along with the example 3-D EEM array included in USGS HydroOpt:

```
# this command shows the excitation wavelengths (nm)
colnames(a)

[1] "290" "292" "294" "296" "298" "300" "302" "304" "306"
[10] "308" "310" "312" "314" "316" "318" "320" "322" "324"
[19] "326" "328" "330" "332" "334" "336" "338" "340" "342"
[28] "344" "346" "348" "350" "352" "354" "356" "358" "360"
[37] "362" "364" "366" "368" "370" "372" "374" "376" "378"
[46] "380" "382" "384" "386" "388" "390" "392" "394" "396"
[55] "398" "400" "402" "404" "406" "408" "410" "412" "414"
[64] "416" "418" "420" "422" "424" "426" "428" "430" "432"
[73] "434" "436" "438" "440" "442" "444" "446" "448" "450"
[82] "452" "454" "456" "458" "460" "462" "464" "466" "468"
[91] "470" "472" "474" "476" "478" "480" "482" "484" "486"
[100] "488" "490" "492" "494" "496" "498" "500" "502" "504"
[109] "506" "508" "510" "512" "514" "516" "518" "520" "522"
[118] "524" "526" "528" "530" "532" "534" "536" "538" "540"
[127] "542" "544" "546" "548" "550" "552" "554" "556" "558"
[136] "560" "562" "564" "566" "568" "570" "572" "574" "576"
[145] "578" "580" "582" "584" "586" "588" "590" "592" "594"
[154] "596" "598" "600"

# this command shows the emission wavelengths (nm)
rownames(a)

[1] "240" "245" "250" "255" "260" "265" "270" "275" "280"
[10] "285" "290" "295" "300" "305" "310" "315" "320" "325"
[19] "330" "335" "340" "345" "350" "355" "360" "365" "370"
[28] "375" "380" "385" "390" "395" "400" "405" "410" "415"
[37] "420" "425" "430" "435" "440"
```



```
# this command shows the emission wavelengths (nm), only the
# first 20 shown for simplicity
names(a[1, 1, ])[1:20]

[1] "gr13307" "gr13308" "gr13351" "gr13352" "gr13353"
[6] "gr13354" "gr13357" "gr13358" "gr13360" "gr13361"
[11] "gr13362" "gr13363" "gr13364" "gr13365" "gr13374"
[16] "gr13375" "gr13433" "gr13434" "gr13435" "gr13439"
```

The user should be aware of **two important caveats**: (1) There should rarely be emission wavelengths (nm) below the excitation wavelength for a given fluorescence reading. Where this occurs an NA will be found in the 3-D EEMs array. (2) Intensities at an emission wavelength that is two times the excitation wavelength will be influenced by second order Rayleigh scatter. Thuse user should keep these in mind when using USGSHydroOpt.

2.9 Creating a 3-Dimensional Excitation-Emission Array

In Section 2.8 the format of 3-D arrays of fluorescence data that can be used with USGSHydroOpt is discussed. USGSHydroOpt can also produce such 3-D EEMs arrays given the appropriate fluorescence dataframe. Below is an example of how USGSHydroOpt is used to accomplish this task:

```
# set an arbitrary data frame (df) as dfFluor (the example
# fluorescence dataframe in USGSHydroOpt)
df <- dfFluor

# define the column in dfFluor
ExEm <- "Wavelength.Pairs"

# run the VectorizedTo3DArray function from USGSHydroOpt that
# creates a 3-D EEMs array given a vectorized fluorescence
# dataframe
aTest <- VectorizedTo3DArray(df, ExEm)
```

In the example above, dfFluor is formatted according to the fluorescence dataframe discussed in Section 2.2.

3 Creating Summary Optical Variables

Most of the functions included in USGSHydroOpt are for creating variables that summarize optical data for water samples. Such variables can then be used in statistical modeling efforts aimed at describing various phenomena in aquatic ecosystems. This sections steps through the six functions that USGSHydroOpt offers for creating summary optical variables.

3.1 Creating Absorbance Coefficients

The function getAbs operates on a dataframe formatted according to Section 2.1. Specifically, it picks out absorbance coefficients for a defined set of wavelengths (nm) and adds those coefficients to an optical

summary data frame formatted according to Section 2.4 (for each sample). Shown below is an example of how the function is used.

```
# assign a variable dataAbs to the absorbance dataframe
# included in USGSHydroOpt
dataAbs <- dfabs

# define the column with the wavelengths in dataAbs
waveCol <- "wavelengths"

# define the wavelengths for which absorbance coefficients
# should be defined
wavs <- c(430, 530, 630, 730)

# define which columns contain the absorbance data, by
# default per WI WSC and CA WSC samples start with 'gr'
colSubsetString <- "gr"

# assign a variable dataSummary to the dfsummary dataframe
# included in USGSHydroOpt
dataSummary <- dfsummary

# assign a variable grnum to the column in dataSummary with
# sample numbers
grnum <- "GRnumber"

# use getAbs to produce absorbance coefficients
testAbs <- getAbs(dataAbs, waveCol, wavs, colSubsetString, dataSummary,
  grnum)

# note that the absorbance coefficients as defined by wavs
# have been added to dataSummary
colnames(testAbs)[69:72]

[1] "A430" "A530" "A630" "A730"
```

3.2 Spectral Slopes by Linear Regression

The function `getExpResid` computes spectral slopes by a first order decay function determined by linear regression (Helms et al. 2008). The residual at a specified wavelength (nm) is then calculated based on the spectral slope. The residual at a given wavelength and spectral slope is then added to a summary dataframe formatted according to Section 2.4. Displayed below is an example of how the function can be used. The function produces a plot with the absorbance data for each sample. On the plot the red indicates the spectral slope fit by linear regression, the blue is the absorbance data in `rangeGap`, and the black is the data in `rangeReg` but not in `rangeGap`. The dataframe `dataAbs` is formatted according to Section 2.1, but is shortened for simplicity. If the user calls `"pdf(genericName.pdf)"`, *See ?pdf*, then the function plots will be printed to a Portable Document Format in the working directory (one plot or sample per page).

```

# absorbance wavelength (nm) for which residual is calculated
wavelength <- 267

# the absorbance wavelength range (nm) to be considered as a
# numeric string
rangeReg <- c(240, 340)

# the absorbance wavelength range (nm) to be considered as a
# numeric string
rangeGap <- c(255, 300)

# assign a variable dataAbs to a shortened version of the
# absorbance dataframe included in USGSHydroOpt
dataAbs <- dfabs

# define the column with the wavelengths in dataAbs
waveCol <- "wavelengths"

# assign a variable grnum to the column in dataSummary with
# sample numbers
colSubsetString <- "gr"

# assign a variable dataSummary to the dfsummary dataframe
# included in USGSHydroOpt column 68 or 'Aresids' is removed
# because we are computing this summary optical variable with
# this function and then adding it to dataSummary
dataSummary <- dfsummary[, -c(68)]

# assign a variable grnum to the column in dataSummary with
# sample numbers
grnum <- "GRnumber"

# use getExpResid to calculate the residual at a given
# wavelength given a spectral slope calculated per Helms et
# al. 2008.
testdfOpt <- getExpResid(wavelength, rangeReg, rangeGap, dataAbs,
  waveCol, colSubsetString, dataSummary, grnum)

# notice that the variable 'Aresids' has been added to
# dataSummary
colnames(testdfOpt)

```

3.3 Humification and Fluorescence Indices

Humification and fluorescence index summary variables can be computed using the function `getIndex`. The humification index summary variable called "HIX_2002" is calculated according to Ohno (2002). The first fluorescence index summary variable "FI_2005" is computed according to Cory and McKnight (2005).

The second fluorescence index summary variable, "FI_2001" is computed according to McKnight et al. (2001). The final summary variable produced by this function is the freshness index, "FreshI," is computed according to Parlanti et al. (2000). Each of these four summary variables are computed and added to the optical summary dataframe, formatted according to Section 2.4. In the example below, `getIndexes` is used to compute these four optical variables, which are subsequently added to an optical summary dataframe. A 3-D excitation-emission dataframe with fluorescence data formatted according to Sections 2.8-2.9 is used in computing these summary variables.

```
# set a variable called a as the example 3-D excitation
# emission array included with the package
a <- a

# assign a variable dataSummary to the dfsummary dataframe
# included in USGSHydroOpt
dataSummary <- dfsummary

# remove those columns with the fluorescence and humic
# indices that we are computing here
dataSummary <- dataSummary[, -c(43:46)]

# assign a variable grnum to the column in dataSummary with
# sample numbers
grnum <- "GRnumber"

# use getIndexes to compute the four humification and
# fluorescence indices
testIndexes <- getIndexes(a, dataSummary, grnum)

# note that the four indices have been added to dataSummary
colnames(testIndexes) [65:68]

[1] "HIX_2002" "FI_2005" "FI_2001" "FreshI"
```

3.4 Optical Ratios

The function `getRatios` uses absorbance peaks and spectral slopes in an existing summary optical data frame (e.g., `dfsummary`) and creates ratios between the different peaks and ratios. These ratios can be useful as predictor variables in statistical modeling efforts. In the example below, 65 different ratios are computed using an optical summary dataframe formatted per Section 2.4, and a ratio signals dataframe formatted per Section 2.6. The names of the calculated ratios added to the optical summary dataframe begin with "r" to signify "ratio" followed by the absorbance peak and/or spectral slope used to calculate the ratio. For example, the ratio of the absorbance peak at 254nm (A254) and the spectral slope between 350 and 400 nm (Sag350-400) is called "rA254_Sag350_400."

```
# assign a variable dataSummary to the dfsummary dataframe
# included in USGSHydroOpt
dataSummary <- dfsummary

# note the number of variables in dataSummary
```

```

length(colnames(dataSummary))

[1] 68

# pick out the absorbance peaks and spectral slopes to be
# used for calculating ratios these correspond to those with
# a 1 in the 'keep' column.
sigs <- ratioSignals[which(ratioSignals[2] > 0), 1]

# assign a variable grnum to the column in dataSummary with
# sample numbers
grnum <- "GRnumber"

# use getRatios to calculate 65 different ratios of
# absorbance peaks and spectral slopes
test <- getRatios(dataSummary, sigs, grnum)

# notice that 65 ratios have been added to dataSummary
length(colnames(test))

[1] 134

# example of some ratios added
colnames(test)[69:75]

[1] "rA254_A275" "rA254_A280" "rA254_A290" "rA254_A295"
[5] "rA254_A350" "rA254_A370" "rA254_A400"

```

3.5 EEM Peaks Computed by Average Wavelength

Various excitation-emission (EEMs) peaks can be used to predict the presence of different chemical constituents in water. An EEMs peak is always characterized at a specific excitation and emission wavelength (nm), or within specific excitation and emission wavelength ranges. It has become standard practice to extract these peaks from fluorescence data, and identify them on EEMs plots. When given a fluorescence dataframe formatted according to section 2.2, the function `getMeanFl` computes the various peaks based on a dataframe of signals formatted according to section 2.7. Many of these peaks can occur within an excitation wavelength (nm) range, and also an emission wavelength (nm) range. For peaks where this is true, the function computes the average of the excitation and/or emission wavelength (nm) range. The resultant mean excitation and/or emission wavelength (nm) is then used to compute the EEMs peak from the fluorescence data. An example of how this function can be used is illustrated below.

```

# set a variable a as the example 3-D excitation emission
# array included with the package
a <- a

# set a variable signals as the example signals dataframe
signals <- signals

# note the length of the EEMs peaks to be computed

```

```

length(rownames(signals))

[1] 32

# identify the name of the column with the EEMs Peak names
Peak <- "Peak"

# identify column with the lower excitation wavelength in the
# excitation wavelength range
Ex1 <- "Ex1"

# identify column with the upper excitation wavelength in the
# excitation wavelength range
Ex2 <- "Ex2"

# identify column with the lower emission wavelength in the
# emission wavelength range
Em1 <- "Em1"

# identify column with the upper emission wavelength in the
# emission wavelength range
Em2 <- "Em2"

# assign a variable dataSummary to the dfsummary dataframe
# included in USGSHydroOpt
dataSummary <- dfsummary

# remove the variables in dataSummary that are going to be
# computed with testMeanFl
rm <- which(colnames(dataSummary) %in% signals$Peak)
dataSummary <- dataSummary[, -c(rm)]

# note the number of variables in dataSummary
length(colnames(dataSummary))

[1] 36

# assign a variable grnum to the column in dataSummary with
# sample numbers
grnum <- "GRnumber"

# use getMeanFl to compute the different EEMs signals and add
# them to the optical summary data frame
testMeanFl <- getMeanFl(a, signals, Peak, Ex1, Ex2, Em1, Em2,
  dataSummary, grnum)

# notice that signals have been added to dataSummary
length(colnames(testMeanFl))

[1] 68

```

```
length(rownames(signals)) + length(colnames(dataSummary))
[1] 68
```

3.6 Log transformation of Summary Optical Variables

Often times it is useful to log 10 transform summary optical variables to help stabilize variance. This is especially true when using optical variables as predictors for biological response variables such as bacteria and viruses. The function `getLog10` log10 transforms user defined optical summary variables to a log10 scale.

```
# select those variables from ratioSignals which should be
# log 10 transformed in dataSummary
signals <- ratioSignals[which(ratioSignals[2] > 0), 1]

# note the number of variables to be log 10 transformed
length(signals)

[1] 12

# assign a variable dataSummary to the dfsummary dataframe
# included in USGSHydroOpt
dataSummary <- dfsummary

# note the number of variables in dataSummary
length(colnames(dataSummary))

[1] 68

# assign a variable grnum to the column in dataSummary with
# sample numbers
grnum <- "GRnumber"

# use getLog10 to log 10 transform signals in dataSummary
testLog10 <- getLog10(dataSummary, signals, grnum)

# note that the log 10 transformed signals have been added to
# dataSummary
length(colnames(testLog10))

[1] 80

length(signals) + length(colnames(dataSummary))

[1] 80
```

4 Plotting Excitation-Emission (EEMs) Spectra

One of the most useful visualizations of fluorescence data is creating a contour plot that includes the intensities of all excitation-emission wavelength (nm) pairs for a given sample. The function `plotEEMs2` creates contour plots of EEMs spectra and also labels important EEM peaks. Displayed below is an example of how `plotEEMs2` can be used to plot EEMs spectra. The 3-D Excitation-Emission array discussed in Sections 2.8-2.9 is used to produce the EEMs plot.

```
# choose a sample of interest from the 3-D EEMs array
GRnum <- c("gr14500")

# create a matrix from the 3-D EEMs array with only data for
# sample GRnum
mat <- a[, , GRnum]

# define the excitation wavelengths (nm) as Ex data type
# numeric
Ex <- as.numeric(names(a[, 1, 1]))

# define the emission wavelengths (nm) as Em data type
# numeric
Em <- as.numeric(names(a[1, , 1]))

# define the number of numeric color levels for contour plot
nlevels <- 50

# set a variable Peaks as the example dataframe ex_ems
Peaks <- ex_ems

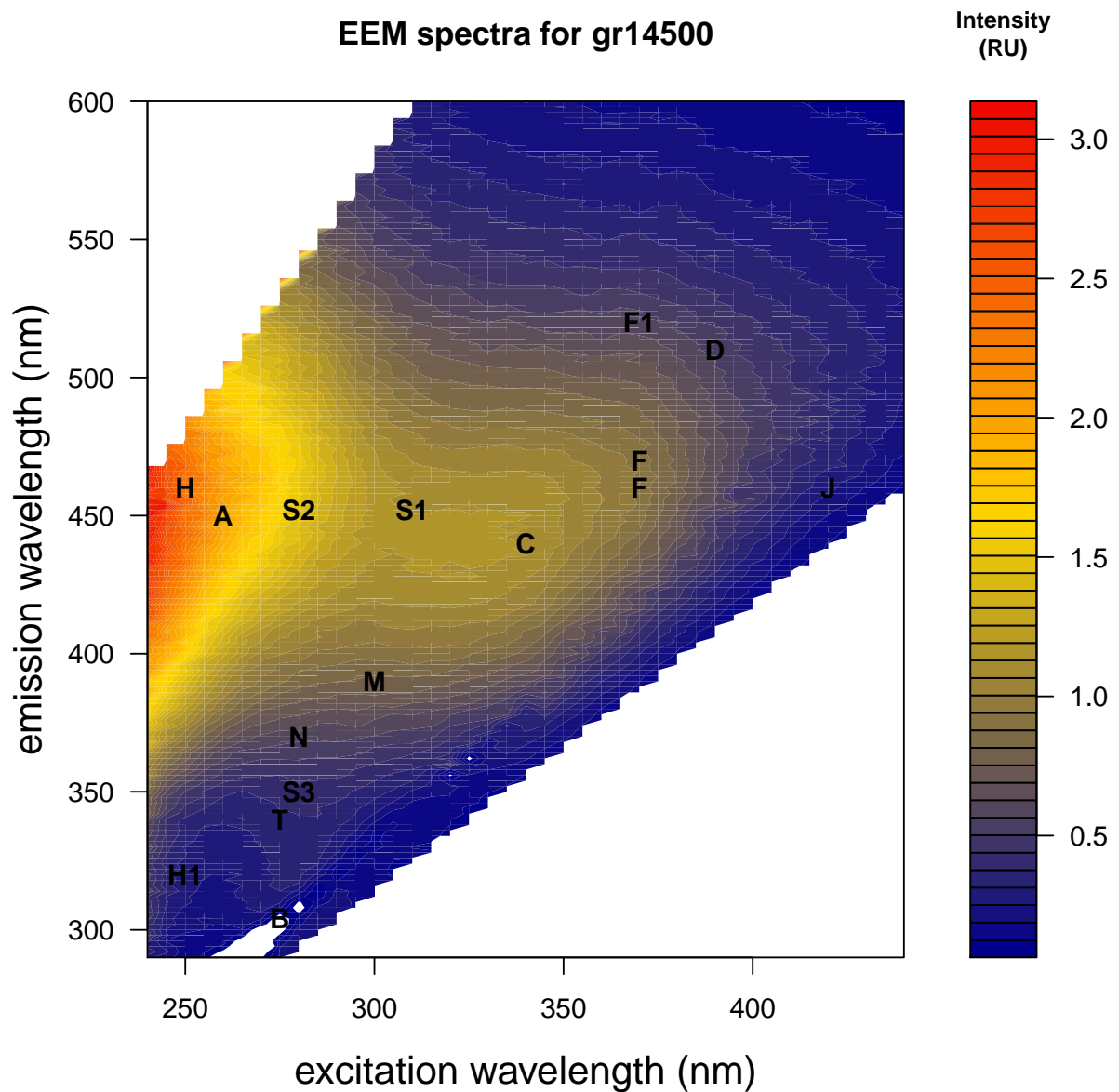
# define the column in Peaks which contains the name of EEMs
# peaks
peakCol <- "Peak"

# define the column in Peaks which contains the excitation
# wavelength for a given Peak
peakEx <- "ExCA"

# define the column in Peaks whcih contains the emission
# wavelength for a given Peak
peakEm <- "EmCA"

# assign the main plot title
mainTitle <- paste("EEM spectra for", GRnum)

# use plotEEMs2 to create a contour plot of EEM spectra
exampleEEMs2 <- plotEEMs2(mat = mat, Ex = Ex, Em = Em, nlevels = nlevels,
  Peaks = Peaks, peakCol = peakCol, peakEx = peakEx, peakEm = peakEm,
  mainTitle = mainTitle)
```

5 An Example Workflow Leveraging USGSHydroOpt

6 Conclusion

USGSHydroOpt is a useful R package for creating optical summary variables for absorbance and fluorescence spectra. These optical predictor variables are useful for explaining biological and chemical phenomena in water samples. This package also contains a useful function for visualizing fluorescence spectra by creating EEMs contour plots. A downfall of the package is that it is designed for dataframes and arrays that adhere to standardized formats, and therefore it is not ammenable to schemas that deviate from the prescribed formats.

7 References

- Cory RM, McKnight DM. 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environmental Science Technology* 39: 8142-8149.
- Helms JR, Stubbins A, Ritchie JD, Minor EC, Kieber DJ, Mopper K. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography* 53: 955-969.
- McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Andersen DT. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography* 46: 38-48.
- Ohno T. 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science Technology* 36: 742-746.
- Parlanti E, Worz K, Geoffroy L, Lamotte M. 2000. Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. *Organic Geochemistry* 31: 1765-1781.