241121_PARAFAC_NMBU

2024-11-21

Here follows an analysis of fluorescence EEM data from NMBU. The data was produced from analysing GF filtered water samples from a lake, covering: - 60 samples from the top - 60 samples from the bottom

Follow this procedure: https://cran.r-project.org/web/packages/staRdom/vignettes/PARAFAC_analysis_of_EEM.html#raman-normalisation

QUESTIONS to NMBU * negative abs-values, likely to influence? - instrumental correction? likely not necessary - there might be some signal in the blanks - influential? - no dilutions performed? - ok to cut the area with the lowest ex wavelengths?

Preparations

Load required packages

```
Packages <- c("staRdom", "dplyr", "tidyr", "data.table", "reshape")
#"libwgeom",
lapply(Packages, library, character.only = TRUE)
```

```
## [[1]]
##  [1] "staRdom"   "parallel"  "eemR"      "ggplot2"   "stats"     "graphics"
##  [7] "grDevices" "utils"     "datasets"  "methods"   "base"
##
## [[2]]
##  [1] "dplyr"     "staRdom"   "parallel"  "eemR"      "ggplot2"   "stats"
##  [7] "graphics"  "grDevices" "utils"     "datasets"  "methods"   "base"
##
## [[3]]
##  [1] "tidyr"     "dplyr"     "staRdom"   "parallel"  "eemR"      "ggplot2"
##  [7] "stats"     "graphics"  "grDevices" "utils"     "datasets"  "methods"
## [13] "base"
##
## [[4]]
##  [1] "data.table" "tidyr"      "dplyr"      "staRdom"    "parallel"
##  [6] "eemR"       "ggplot2"    "stats"      "graphics"   "grDevices"
## [11] "utils"      "datasets"   "methods"    "base"
##
## [[5]]
##  [1] "reshape"    "data.table" "tidyr"      "dplyr"      "staRdom"
##  [6] "parallel"   "eemR"       "ggplot2"    "stats"      "graphics"
## [11] "grDevices"  "utils"      "datasets"   "methods"    "base"
```

```
cores <- detectCores(logical = FALSE)
```

Set directories and load data, and check that the lengts are similar

```
folder = "C:/Users/CBG/OneDrive - NIVA/1 Projects/NMBU_PARAFAC/PARAFAC_filer"
eem_list <- eem_read(folder, import_function = "cary", recursive = TRUE)
eem_overview_plot(eem_list[1:9], spp=9, contour = TRUE)
```

```
## [[1]]
```

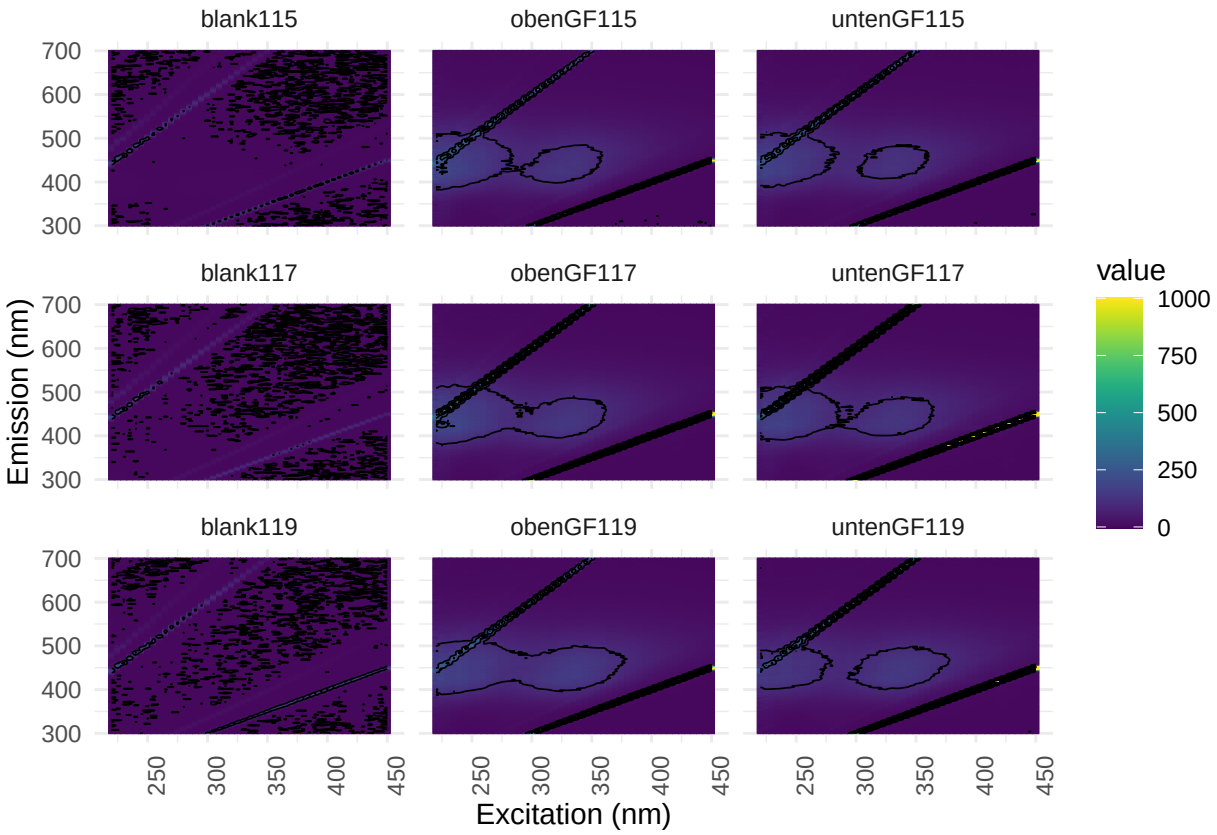

Figure 1: Uncorrected EEMs

```
#Absorbance data
absorbance_dir="C:/Users/CBG/OneDrive - NIVA/1 Projects/NMBU_PARAFAC/Abs_GF_Data_parafac.csv"
absy <- absorbance_read(absorbance_dir, order=TRUE)

metatable = "C:/Users/CBG/OneDrive - NIVA/1 Projects/NMBU_PARAFAC/241121_NMBU_PARAFAC_Metadata.txt"
meta <- read.table(metatable, header = TRUE, sep = "\t", dec = ".", row.names=1)

length(eem_list)
```

```
## [1] 180
```

```r
ncol(absy)
```

```
## [1] 121
```

```r
nrow(meta)
```

```
## [1] 182
```

1) Absorbance wavenegth correction

```r
absorbance <- abs_blcor(absy,wlrange = c(680,700))
```

Check the data for inconsistencies

- seems ok (output from test not plotted here)

```r
#summary(eem_list)
nrow(meta)
```

```
## [1] 182
```

```r
#problem <- eem_checkdata(eem_list,absorbance, metadata=meta)
```

2) Spectral correction with instrument file (not done)

- is this necessary for the instrument at NMBU?
- at NIVA we used, for excorr, measurements from a rhodamineB standard solution at 550nm while nothing for the emcorr

3) Blank subtraction.

```r
eemss <- eem_remove_blank(eem_list)
length(eemss)
```

```
## [1] 180
```

```r
eem_overview_plot(eemss[1:9], spp=9, contour = TRUE)
```

```
## [[1]]
```

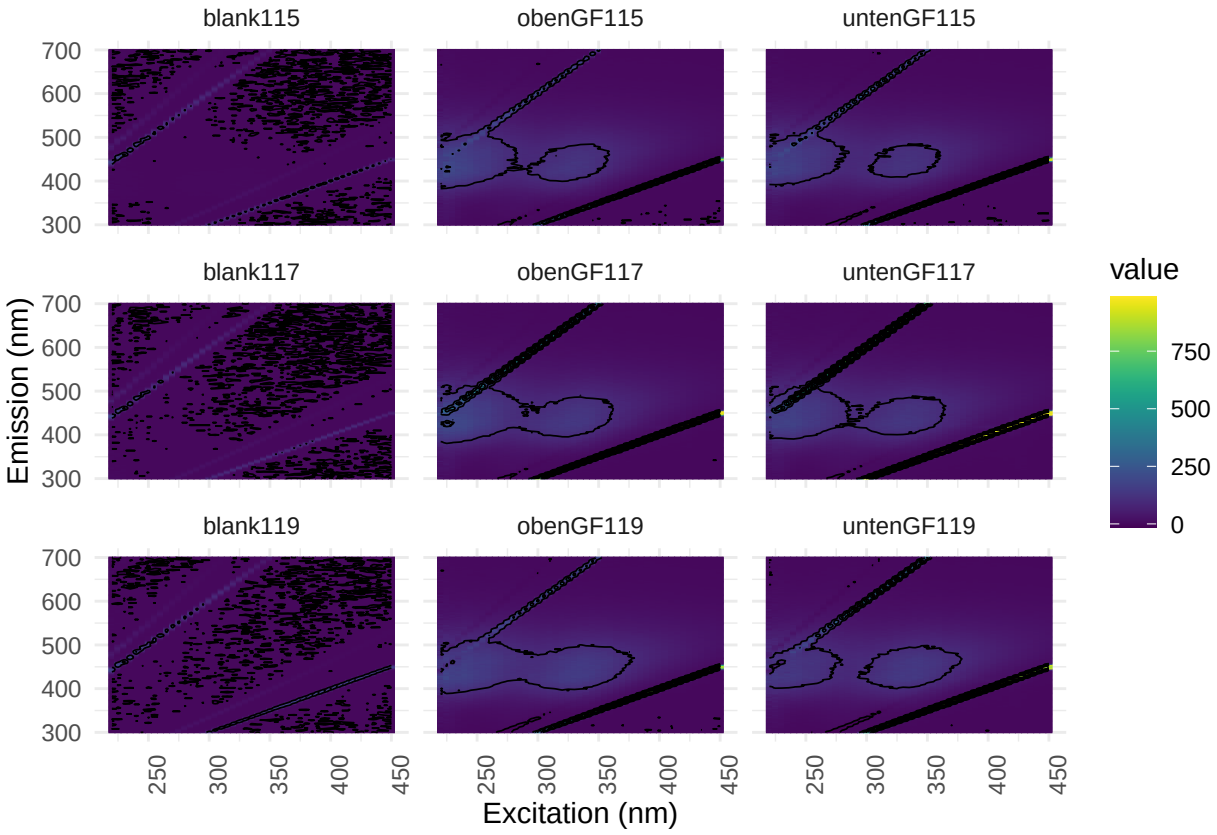

Figure 2: EEMs blank corrected

4) Inner filter effect correction using absorption data

- all abs data must be below 2.0 at any wavelength (else, dilution).
- Several values from the absorbancy data are negative. Will this cause problems???

```
eemsss <- eem_ife_correction(eemss,absorbance, cuvl = 1)
```

```
## obenGF115
## Range of IFE correction factors: 1.0227 2.5463
## Range of total absorbance (Atotal) : 0.0195 0.8118
##
## untenGF115
## Range of IFE correction factors: 1.0254 2.7616
## Range of total absorbance (Atotal) : 0.0218 0.8823

## obenGF117
## Range of IFE correction factors: 1.0263 2.9619
## Range of total absorbance (Atotal) : 0.0225 0.9432
##
## untenGF117
## Range of IFE correction factors: 1.0332 4.2692
## Range of total absorbance (Atotal) : 0.0284 1.2607

## obenGF119
## Range of IFE correction factors: 1.0365 3.993
## Range of total absorbance (Atotal) : 0.0311 1.2026
##
## untenGF119
## Total absorbance is > 1.5 (Atotal = 1.608541)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0535 6.3719
## Range of total absorbance (Atotal) : 0.0453 1.6085

## obenGF128
## Range of IFE correction factors: 1.0383 4.0193
## Range of total absorbance (Atotal) : 0.0327 1.2083
##
## untenGF128
## Total absorbance is > 1.5 (Atotal = 1.507438)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0501 5.6718
## Range of total absorbance (Atotal) : 0.0424 1.5074

## obenGF135
## Range of IFE correction factors: 1.0252 2.4379
## Range of total absorbance (Atotal) : 0.0217 0.774
##
## untenGF135
## Range of IFE correction factors: 1.0266 2.6186
## Range of total absorbance (Atotal) : 0.0228 0.8361
```

```
## obenGF136
## Range of IFE correction factors: 1.0204 2.5637
## Range of total absorbance (Atotal) : 0.0175 0.8177
##
## untenGF136
## Range of IFE correction factors: 1.0219 3.1473
## Range of total absorbance (Atotal) : 0.0188 0.9959

## obenGF139
## Range of IFE correction factors: 1.0364 3.9435
## Range of total absorbance (Atotal) : 0.031 1.1918
##
## untenGF139
## Total absorbance is > 1.5 (Atotal = 1.64384)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0556 6.6362
## Range of total absorbance (Atotal) : 0.047 1.6438

## obenGF148
## Range of IFE correction factors: 1.0354 3.6269
## Range of total absorbance (Atotal) : 0.0302 1.1191
##
## untenGF148
## Total absorbance is > 1.5 (Atotal = 1.582363)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0545 6.1827
## Range of total absorbance (Atotal) : 0.0461 1.5824

## obenGF155
## Range of IFE correction factors: 1.0225 2.5171
## Range of total absorbance (Atotal) : 0.0193 0.8018
##
## untenGF155
## Range of IFE correction factors: 1.0194 2.4586
## Range of total absorbance (Atotal) : 0.0167 0.7814

## obenGF164
## Range of IFE correction factors: 1.0267 3.233
## Range of total absorbance (Atotal) : 0.0228 1.0192
##
## untenGF164
## Range of IFE correction factors: 1.0307 3.4176
## Range of total absorbance (Atotal) : 0.0263 1.0674

## obenGF166
## Range of IFE correction factors: 1.0191 2.5898
## Range of total absorbance (Atotal) : 0.0164 0.8265
##
## untenGF166
## Range of IFE correction factors: 1.0275 3.4491
## Range of total absorbance (Atotal) : 0.0236 1.0754

## obenGF167
```

```
## Range of IFE correction factors: 1.024 2.8109
## Range of total absorbance (Atotal) : 0.0206 0.8977
##
## untenGF167
## Range of IFE correction factors: 1.038 4.5417
## Range of total absorbance (Atotal) : 0.0324 1.3144

## obenGF168
## Range of IFE correction factors: 1.0327 3.436
## Range of total absorbance (Atotal) : 0.028 1.0721
##
## untenGF168
## Total absorbance is > 1.5 (Atotal = 1.521584)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0537 5.7649
## Range of total absorbance (Atotal) : 0.0454 1.5216

## obenGF169
## Range of IFE correction factors: 1.0381 4.3459
## Range of total absorbance (Atotal) : 0.0325 1.2762
##
## untenGF169
## Range of IFE correction factors: 1.0438 4.7315
## Range of total absorbance (Atotal) : 0.0373 1.35

## obenGF185
## Range of IFE correction factors: 1.0257 2.5431
## Range of total absorbance (Atotal) : 0.022 0.8107
##
## untenGF185
## Range of IFE correction factors: 1.0233 2.526
## Range of total absorbance (Atotal) : 0.02 0.8049

## obenGF186
## Range of IFE correction factors: 1.0194 2.5315
## Range of total absorbance (Atotal) : 0.0167 0.8067
##
## untenGF186
## Range of IFE correction factors: 1.0245 4.6377
## Range of total absorbance (Atotal) : 0.021 1.3326

## obenGF189
## Range of IFE correction factors: 1.0354 3.9552
## Range of total absorbance (Atotal) : 0.0302 1.1943
##
## untenGF189
## Range of IFE correction factors: 1.0466 5.0685
## Range of total absorbance (Atotal) : 0.0395 1.4098

## obenGF194
## Range of IFE correction factors: 1.027 3.1176
## Range of total absorbance (Atotal) : 0.0231 0.9876
```

```
##
## untenGF194
## Range of IFE correction factors: 1.0266 3.2096
## Range of total absorbance (Atotal) : 0.0228 1.0129

## obenGF197
## Range of IFE correction factors: 1.0268 3.1904
## Range of total absorbance (Atotal) : 0.023 1.0077
##
## untenGF197
## Range of IFE correction factors: 1.0396 4.538
## Range of total absorbance (Atotal) : 0.0337 1.3137

## obenGF198
## Range of IFE correction factors: 1.0353 3.6572
## Range of total absorbance (Atotal) : 0.0301 1.1263
##
## untenGF198
## Total absorbance is > 1.5 (Atotal = 1.620835)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0567 6.4628
## Range of total absorbance (Atotal) : 0.0479 1.6208

## obenGF209
## Range of IFE correction factors: 1.0338 3.799
## Range of total absorbance (Atotal) : 0.0289 1.1593
##
## untenGF209
## Range of IFE correction factors: 1.0374 4.0933
## Range of total absorbance (Atotal) : 0.0319 1.2242

## obenGF215
## Range of IFE correction factors: 1.021 2.428
## Range of total absorbance (Atotal) : 0.018 0.7705
##
## untenGF215
## Range of IFE correction factors: 1.0272 2.624
## Range of total absorbance (Atotal) : 0.0233 0.8379

## obenGF216
## Range of IFE correction factors: 1.0209 3.0832
## Range of total absorbance (Atotal) : 0.0179 0.978
##
## untenGF216
## Range of IFE correction factors: 1.0251 4.1512
## Range of total absorbance (Atotal) : 0.0215 1.2363

## obenGF217
## Range of IFE correction factors: 1.0253 2.9214
## Range of total absorbance (Atotal) : 0.0217 0.9312
##
## untenGF217
## Range of IFE correction factors: 1.039 4.35
## Range of total absorbance (Atotal) : 0.0332 1.277
```

```
## obenGF218
## Range of IFE correction factors: 1.0353 3.6897
## Range of total absorbance (Atotal) : 0.0301 1.134
##
## untenGF218
## Range of IFE correction factors: 1.0465 4.9397
## Range of total absorbance (Atotal) : 0.0395 1.3874

## obenGF224
## Range of IFE correction factors: 1.0274 3.0756
## Range of total absorbance (Atotal) : 0.0235 0.9759
##
## untenGF224
## Range of IFE correction factors: 1.0261 3.0495
## Range of total absorbance (Atotal) : 0.0224 0.9684

## obenGF236
## Range of IFE correction factors: 1.0242 3.1998
## Range of total absorbance (Atotal) : 0.0208 1.0103
##
## untenGF236
## Range of IFE correction factors: 1.0291 4.2005
## Range of total absorbance (Atotal) : 0.0249 1.2466

## obenGF237
## Range of IFE correction factors: 1.0261 3.0294
## Range of total absorbance (Atotal) : 0.0224 0.9627
##
## untenGF237
## Range of IFE correction factors: 1.0409 4.1546
## Range of total absorbance (Atotal) : 0.0348 1.2371

## obenGF238
## Range of IFE correction factors: 1.0339 3.4836
## Range of total absorbance (Atotal) : 0.0289 1.084
##
## untenGF238
## Total absorbance is > 1.5 (Atotal = 1.686351)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0573 6.9691
## Range of total absorbance (Atotal) : 0.0484 1.6864

## obenGF239
## Range of IFE correction factors: 1.0346 3.7775
## Range of total absorbance (Atotal) : 0.0295 1.1544
##
## untenGF239
## Range of IFE correction factors: 1.0389 4.0901
## Range of total absorbance (Atotal) : 0.0332 1.2235

## obenGF245
## Range of IFE correction factors: 1.0175 2.3484
```

```
## Range of total absorbance (Atotal) : 0.0151 0.7415
##
## untenGF245
## Range of IFE correction factors: 1.0214 2.5531
## Range of total absorbance (Atotal) : 0.0184 0.8141

## obenGF254
## Range of IFE correction factors: 1.0258 2.9847
## Range of total absorbance (Atotal) : 0.0221 0.9498
##
## untenGF254
## Range of IFE correction factors: 1.0259 2.9249
## Range of total absorbance (Atotal) : 0.0222 0.9322

## obenGF257
## Range of IFE correction factors: 1.0297 3.3125
## Range of total absorbance (Atotal) : 0.0254 1.0403
##
## untenGF257
## Range of IFE correction factors: 1.042 4.5305
## Range of total absorbance (Atotal) : 0.0357 1.3123

## obenGF268
## Range of IFE correction factors: 1.0328 3.6901
## Range of total absorbance (Atotal) : 0.0281 1.1341
##
## untenGF268
## Total absorbance is > 1.5 (Atotal = 1.69006)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0577 6.9989
## Range of total absorbance (Atotal) : 0.0487 1.6901

## obenGF27
## Range of IFE correction factors: 1.023 2.7022
## Range of total absorbance (Atotal) : 0.0197 0.8634
##
## untenGF27
## Range of IFE correction factors: 1.0258 3.3108
## Range of total absorbance (Atotal) : 0.0221 1.0399

## obenGF276
## Range of IFE correction factors: 1.0251 2.972
## Range of total absorbance (Atotal) : 0.0216 0.9461
##
## untenGF276
## Range of IFE correction factors: 1.0284 4.1536
## Range of total absorbance (Atotal) : 0.0243 1.2368

## obenGF277
## Range of IFE correction factors: 1.0291 3.0901
## Range of total absorbance (Atotal) : 0.0249 0.9799
##
```

```
## untenGF277
## Range of IFE correction factors: 1.046 4.7143
## Range of total absorbance (Atotal) : 0.039 1.3468

## obenGF28
## Range of IFE correction factors: 1.0309 3.2615
## Range of total absorbance (Atotal) : 0.0264 1.0268
##
## untenGF28
## Total absorbance is > 1.5 (Atotal = 1.656022)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0609 6.7299
## Range of total absorbance (Atotal) : 0.0513 1.656

## obenGF285
## Range of IFE correction factors: 1.0175 2.3549
## Range of total absorbance (Atotal) : 0.0151 0.7439
##
## untenGF285
## Range of IFE correction factors: 1.023 2.6704
## Range of total absorbance (Atotal) : 0.0197 0.8532

## obenGF288
## Range of IFE correction factors: 1.0298 3.3112
## Range of total absorbance (Atotal) : 0.0255 1.04
##
## untenGF288
## Total absorbance is > 1.5 (Atotal = 1.587833)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0524 6.2218
## Range of total absorbance (Atotal) : 0.0444 1.5878

## obenGF29
## Range of IFE correction factors: 1.0332 3.5435
## Range of total absorbance (Atotal) : 0.0284 1.0989
##
## untenGF29
## Total absorbance is > 1.5 (Atotal = 1.69642)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0593 7.0503
## Range of total absorbance (Atotal) : 0.05 1.6964

## obenGF294
## Range of IFE correction factors: 1.0259 2.9539
## Range of total absorbance (Atotal) : 0.0222 0.9408
##
## untenGF294
## Range of IFE correction factors: 1.0264 2.9324
## Range of total absorbance (Atotal) : 0.0227 0.9344

## obenGF297
## Range of IFE correction factors: 1.0292 3.0251
```

```
## Range of total absorbance (Atotal) : 0.025 0.9615
##
## untenGF297
## Range of IFE correction factors: 1.0477 5.083
## Range of total absorbance (Atotal) : 0.0405 1.4122

## obenGF306
## Range of IFE correction factors: 1.0224 2.7523
## Range of total absorbance (Atotal) : 0.0192 0.8794
##
## untenGF306
## Range of IFE correction factors: 1.0299 4.2174
## Range of total absorbance (Atotal) : 0.0256 1.2501

## obenGF308
## Range of IFE correction factors: 1.031 3.2812
## Range of total absorbance (Atotal) : 0.0266 1.0321
##
## untenGF308
## Total absorbance is > 1.5 (Atotal = 1.605173)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0546 6.3473
## Range of total absorbance (Atotal) : 0.0462 1.6052

## obenGF315
## Range of IFE correction factors: 1.0177 2.4291
## Range of total absorbance (Atotal) : 0.0152 0.7709
##
## untenGF315
## Range of IFE correction factors: 1.0244 2.8585
## Range of total absorbance (Atotal) : 0.0209 0.9123

## obenGF317
## Range of IFE correction factors: 1.0346 3.4756
## Range of total absorbance (Atotal) : 0.0296 1.082
##
## untenGF317
## Total absorbance is > 1.5 (Atotal = 1.564761)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0566 6.0587
## Range of total absorbance (Atotal) : 0.0478 1.5648

## obenGF35
## Range of IFE correction factors: 1.0249 2.8964
## Range of total absorbance (Atotal) : 0.0213 0.9237
##
## untenGF35
## Range of IFE correction factors: 1.0266 2.9115
## Range of total absorbance (Atotal) : 0.0228 0.9282

## obenGF46
## Range of IFE correction factors: 1.0167 2.287
```

```
## Range of total absorbance (Atotal) : 0.0144 0.7185
##
## untenGF46
## Range of IFE correction factors: 1.0291 3.0518
## Range of total absorbance (Atotal) : 0.0249 0.9691

## obenGF48
## Range of IFE correction factors: 1.0308 3.2513
## Range of total absorbance (Atotal) : 0.0263 1.0241
##
## untenGF48
## Range of IFE correction factors: 1.0513 5.3572
## Range of total absorbance (Atotal) : 0.0435 1.4579

## obenGF49
## Range of IFE correction factors: 1.0358 3.7257
## Range of total absorbance (Atotal) : 0.0306 1.1424
##
## untenGF49
## Total absorbance is > 1.5 (Atotal = 1.693917)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.061 7.03
## Range of total absorbance (Atotal) : 0.0514 1.6939

## obenGF65
## Range of IFE correction factors: 1.0235 2.7518
## Range of total absorbance (Atotal) : 0.0202 0.8792
##
## untenGF65
## Range of IFE correction factors: 1.0254 3.0469
## Range of total absorbance (Atotal) : 0.0218 0.9677

## obenGF68
## Range of IFE correction factors: 1.0329 3.4879
## Range of total absorbance (Atotal) : 0.0281 1.0851
##
## untenGF68
## Total absorbance is > 1.5 (Atotal = 1.568013)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0557 6.0814
## Range of total absorbance (Atotal) : 0.047 1.568

## obenGF69
## Range of IFE correction factors: 1.0298 3.3352
## Range of total absorbance (Atotal) : 0.0255 1.0462
##
## untenGF69
## Total absorbance is > 1.5 (Atotal = 1.563688)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0517 6.0512
## Range of total absorbance (Atotal) : 0.0438 1.5637

## obenGF76
```

```
## Range of IFE correction factors: 1.0162 2.4221
## Range of total absorbance (Atotal) : 0.014 0.7684
##
## untenGF76
## Range of IFE correction factors: 1.0212 2.7761
## Range of total absorbance (Atotal) : 0.0182 0.8869

## obenGF88
## Range of IFE correction factors: 1.0333 3.5659
## Range of total absorbance (Atotal) : 0.0285 1.1043
##
## untenGF88
## Total absorbance is > 1.5 (Atotal = 1.619685)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0577 6.4542
## Range of total absorbance (Atotal) : 0.0487 1.6197

## obenGF95
## Range of IFE correction factors: 1.0249 2.6496
## Range of total absorbance (Atotal) : 0.0213 0.8464
##
## untenGF95
## Range of IFE correction factors: 1.0268 2.8095
## Range of total absorbance (Atotal) : 0.023 0.8973

## obenGF96
## Range of IFE correction factors: 1.0221 2.5998
## Range of total absorbance (Atotal) : 0.019 0.8299
##
## untenGF96
## Range of IFE correction factors: 1.0323 3.6427
## Range of total absorbance (Atotal) : 0.0276 1.1228

## obenGF97
## Range of IFE correction factors: 1.026 2.8798
## Range of total absorbance (Atotal) : 0.0223 0.9187
##
## untenGF97
## Range of IFE correction factors: 1.0337 3.7367
## Range of total absorbance (Atotal) : 0.0288 1.145

## obenGF99
## Range of IFE correction factors: 1.0325 3.632
## Range of total absorbance (Atotal) : 0.0278 1.1203
##
## untenGF99
## Total absorbance is > 1.5 (Atotal = 1.554391)
## A 2-fold dilution is recommended. See ?eem_inner_filter_effect.
## Range of IFE correction factors: 1.0533 5.9868
## Range of total absorbance (Atotal) : 0.0451 1.5544
```

```
eem_overview_plot(eemsss[1:9], spp=9, contour = TRUE)
```

```
## [[1]]
```

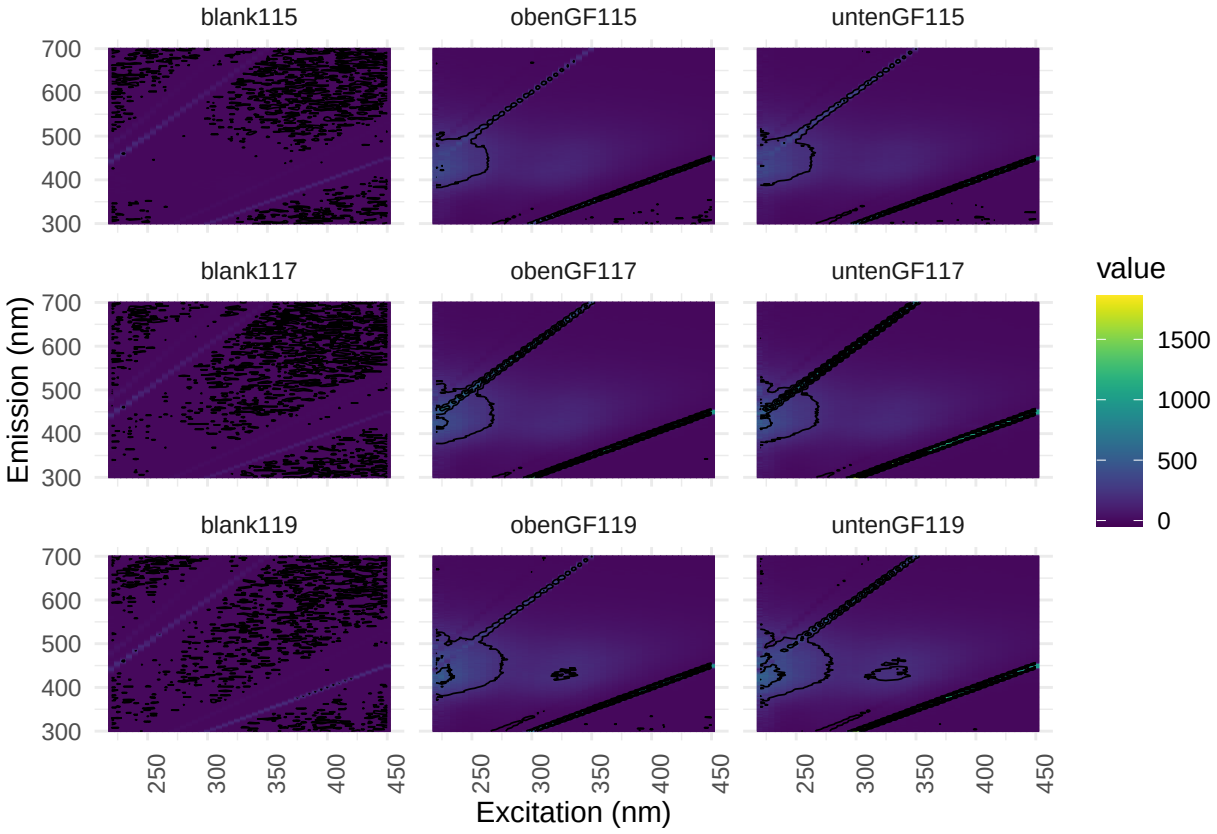

Figure 3: EEMs corrected for blanks and inner filter effects

5) Raman normalization

- could the negative abosrbance data cause problems here?

```
eemsssy <- eem_raman_normalisation2(eemsss, blank = "blank")
```

```
## Raman area: 214.8339
## Raman area: 214.8339

## Raman area: 170.342
## Raman area: 170.342

## Raman area: 170.0362
## Raman area: 170.0362
```

```
## Raman area: 209.9491
## Raman area: 209.9491

## Raman area: 179.2403
## Raman area: 179.2403

## Raman area: 177.8661
## Raman area: 177.8661

## Raman area: 180.432
## Raman area: 180.432

## Raman area: 177.4727
## Raman area: 177.4727

## Raman area: 197.6292
## Raman area: 197.6292

## Raman area: 195.9439
## Raman area: 195.9439

## Raman area: 218.296
## Raman area: 218.296

## Raman area: 165.6076
## Raman area: 165.6076

## Raman area: 181.0322
## Raman area: 181.0322

## Raman area: 184.0585
## Raman area: 184.0585

## Raman area: 228.2809
## Raman area: 228.2809

## Raman area: 250.5522
## Raman area: 250.5522

## Raman area: 182.8733
## Raman area: 182.8733

## Raman area: 213.794
## Raman area: 213.794

## Raman area: 188.0717
## Raman area: 188.0717

## Raman area: 221.4777
## Raman area: 221.4777
```

```
## Raman area: 220.8581
## Raman area: 220.8581

## Raman area: 179.587
## Raman area: 179.587

## Raman area: 253.4779
## Raman area: 253.4779

## Raman area: 203.78
## Raman area: 203.78

## Raman area: 192.5584
## Raman area: 192.5584

## Raman area: 201.8313
## Raman area: 201.8313

## Raman area: 172.5745
## Raman area: 172.5745

## Raman area: 203.9234
## Raman area: 203.9234

## Raman area: 313.3893
## Raman area: 313.3893

## Raman area: 171.0698
## Raman area: 171.0698

## Raman area: 187.2296
## Raman area: 187.2296

## Raman area: 198.3333
## Raman area: 198.3333

## Raman area: 171.7966
## Raman area: 171.7966

## Raman area: 185.6056
## Raman area: 185.6056

## Raman area: 182.9471
## Raman area: 182.9471

## Raman area: 194.8612
## Raman area: 194.8612

## Raman area: 175.3832
## Raman area: 175.3832
```

```
## Raman area: 184.2973
## Raman area: 184.2973

## Raman area: 248.3666
## Raman area: 248.3666

## Raman area: 197.4343
## Raman area: 197.4343

## Raman area: 279.647
## Raman area: 279.647

## Raman area: 176.1771
## Raman area: 176.1771

## Raman area: 174.7216
## Raman area: 174.7216

## Raman area: 171.7322
## Raman area: 171.7322

## Raman area: 273.9418
## Raman area: 273.9418

## Raman area: 199.7596
## Raman area: 199.7596

## Raman area: 168.4033
## Raman area: 168.4033

## Raman area: 183.6553
## Raman area: 183.6553

## Raman area: 220.3338
## Raman area: 220.3338

## Raman area: 187.7203
## Raman area: 187.7203

## Raman area: 239.3028
## Raman area: 239.3028

## Raman area: 166.8044
## Raman area: 166.8044

## Raman area: 165.7617
## Raman area: 165.7617

## Raman area: 165.7616
## Raman area: 165.7616
```

```
## Raman area: 224.9515
## Raman area: 224.9515

## Raman area: 220.1393
## Raman area: 220.1393

## Raman area: 208.8049
## Raman area: 208.8049

## Raman area: 173.0365
## Raman area: 173.0365

## Raman area: 175.9874
## Raman area: 175.9874

## Raman area: 185.2848
## Raman area: 185.2848
```

```
eem_overview_plot(eemsssy[1:9], spp=9, contour = TRUE)
```

```
## [[1]]
```

```
# ALTENRATIVE Raman normalization
# because of negative values. Will try alternative-manual way
#raman1 = "C:/Users/CBG/OneDrive - NIVA/1 Projects/1000 Lakes fEEM Data/DOM-1000-lake-PARAFAC/201216_Ra
#raman2 <- read.table(raman1, header = TRUE, sep = "\t", dec = ".", row.names=1) # load data
#eemsss2 <- eem_raman_normalisation2(eemsss, blank = raman2)
```

#6) Remove blanks from sample set

```
eemb <- eem_extract(eemsssy, c("nano", "miliq", "milliq", "mq", "blank", "Blank"),ignore_case = TRUE)
```

```
## Removed sample(s): blank115 blank117 blank119 blank128 blank135 blank136 blank139 blank148 blank155 b
```

```
absorbance <- dplyr::select(absorbance, -matches("nano|miliq|milliq|mq|blank|Blank", ignore.case = TRUE)
```

7) Remove and interpolate scattering

- seems ok with regards to the Raman and Rayleigh

```
remove_scatter <- c(TRUE, TRUE, TRUE, TRUE)
remove_scatter_width <- c(13,12,12,13) #is either a number or a vector of wavelength width in nm
eemsc <- eem_rem_scat(eemb, remove_scatter = remove_scatter, remove_scatter_width = remove_scatter_widt

#interpolate the removed datapoints
#different methods are available for interpretting .recommends to start with no 1
eemint <- eem_interp(eemsc, type = 1, extend = FALSE)
eem_overview_plot(eemint[1:9], spp=9, contour = TRUE)
```

```
## [[1]]
```

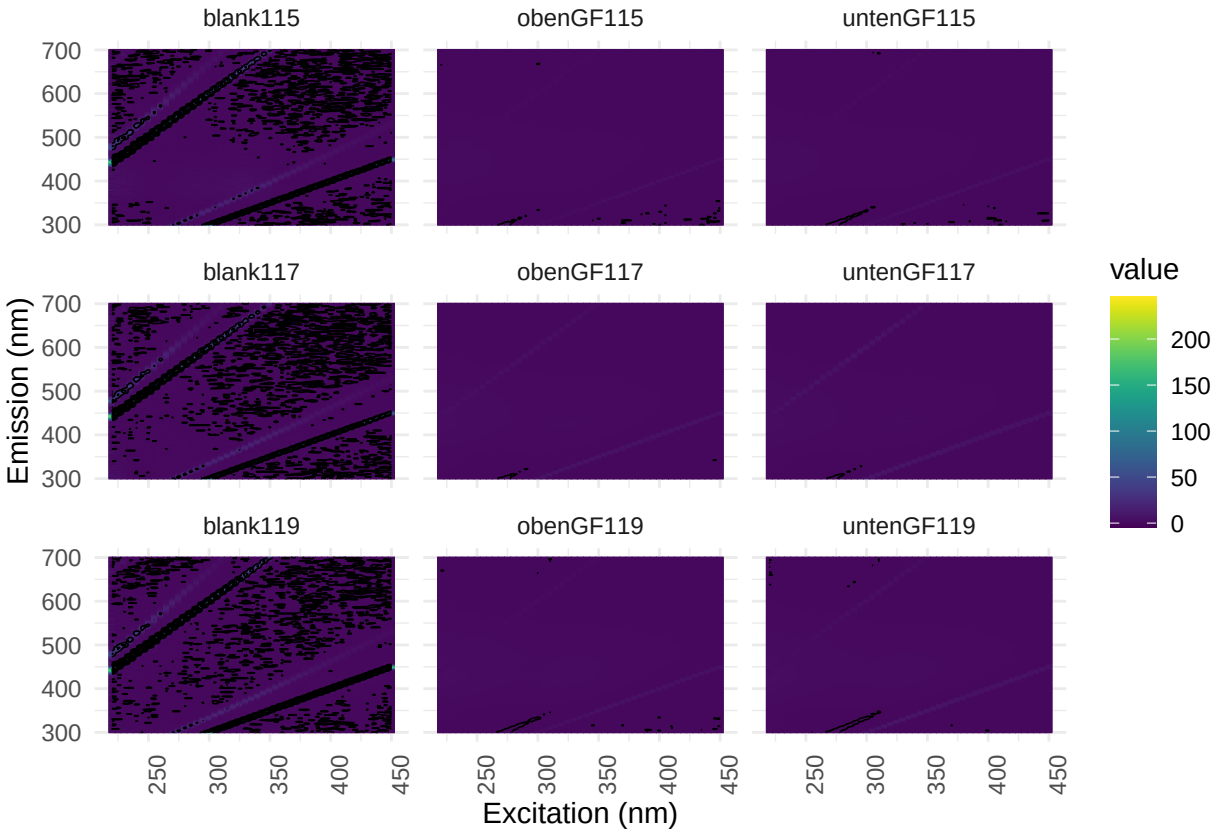

Figure 4: EEMs corrected for blanks and inner filter effects, and Raman normalised

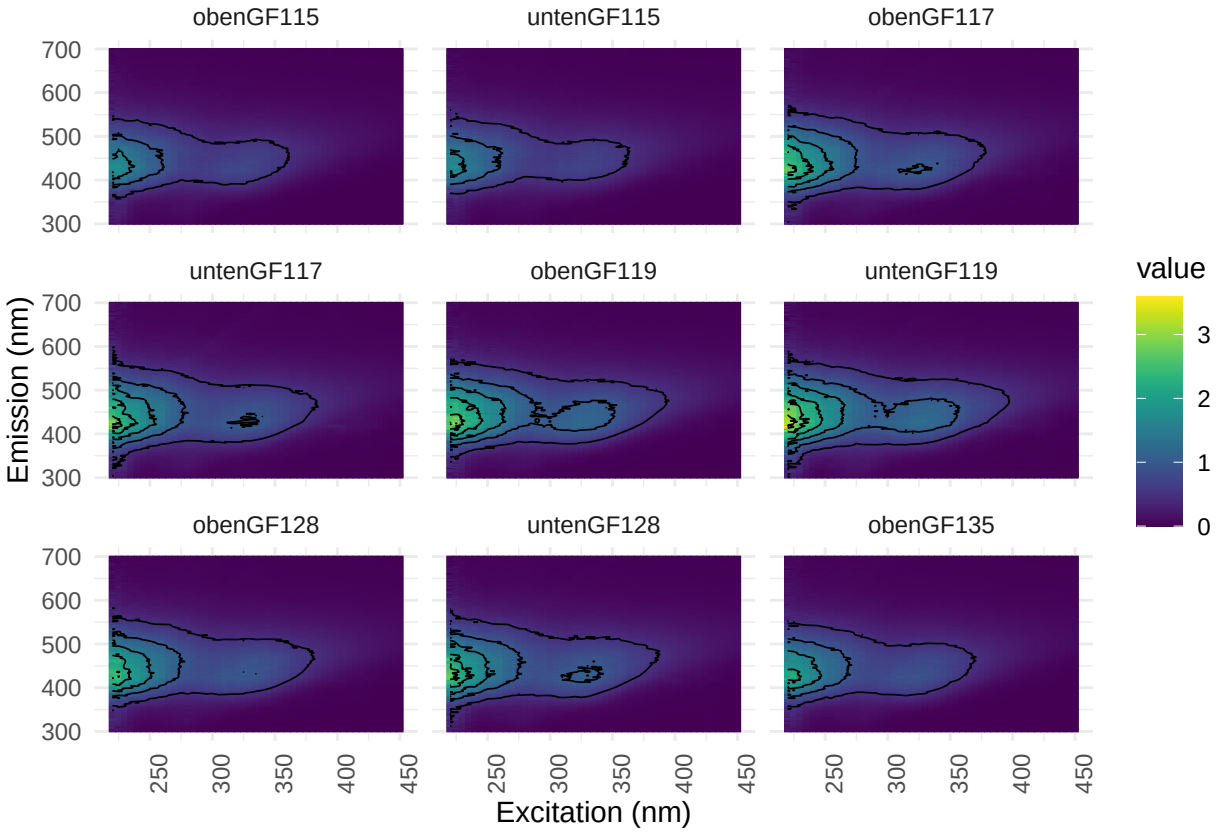

Figure 5: EEMs corrected for blanks and inner filter effects, Raman normalised and scattering removed and interpolated

8) Taking a look at the blanks

- since the blanks are used both for subtraction and making Raman area, it is worth looking into.
- There is a signal at the shorter ex wavelengths and em 300-400 nm

```
#looking at the blanks
#Finding LOQ
blanks <- eem_extract(eem_list, c("nano", "miliq", "milliq", "mq", "blank", "Blank"),keep = TRUE)
```

```
## Extracted sample(s): blank115 blank117 blank119 blank128 blank135 blank136 blank139 blank148 blank155
```

```
blanks2 <- eem_rem_scat(blanks, remove_scatter = remove_scatter, remove_scatter_width = remove_scatter_w
blanks3<- eem_interp(blanks2, type = 1, extend = FALSE)
blanks4 <- blanks3 %>%
  eem_range(ex = c(250,Inf), em = c(0,580))

eem_overview_plot(blanks4[1:9], spp=9, contour = TRUE)
```

```
## [[1]]
```

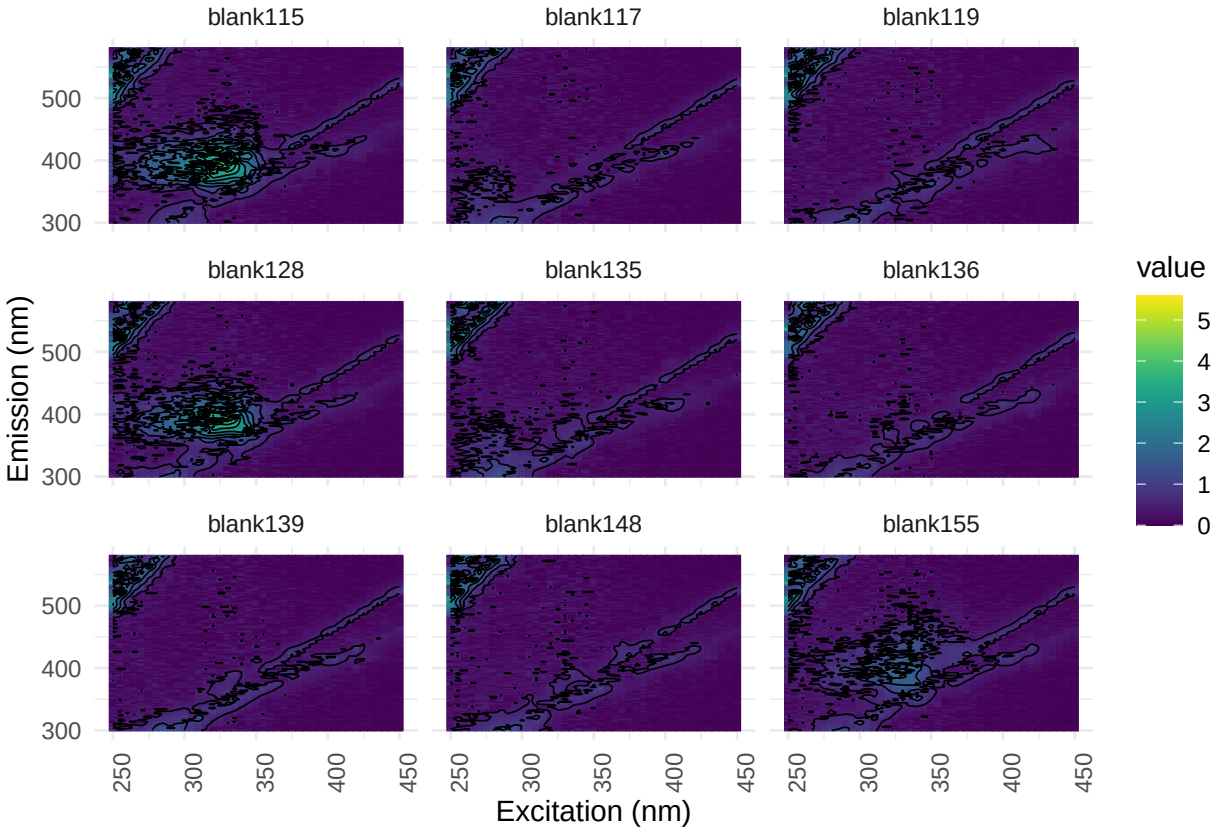

Figure 6: EEMs of the blanks with scattering removed and interpolated

```
#could be considered if LOQ is needed
#bix <- eem_biological_index(blanks)
#coble_peaks <- eem_coble_peaks(blanks)
#fi <- eem_fluorescence_index(blanks)
#hix <- eem_humification_index(blanks, scale = TRUE)

#indices_peaks <- bix %>%
#  full_join(coble_peaks, by = "sample") %>%
#  full_join(fi, by = "sample") %>%
#  full_join(hix, by = "sample")

#indices_peaks

#barplot(indices_peaks$a)

#LOQ <- 10*sd(indices_peaks$a[1:10])
#LOQ
```

9) print a summary table of the samples

- wavelength ranges, and corrections that have been done

```
summary(eemint)
```

```
##         sample ex_min ex_max em_min em_max is_blank_corrected
## 1    obenGF115    220    450    300    700               TRUE
## 2   untenGF115    220    450    300    700               TRUE
## 3    obenGF117    220    450    300    700               TRUE
## 4   untenGF117    220    450    300    700               TRUE
## 5    obenGF119    220    450    300    700               TRUE
## 6   untenGF119    220    450    300    700               TRUE
## 7    obenGF128    220    450    300    700               TRUE
## 8   untenGF128    220    450    300    700               TRUE
## 9    obenGF135    220    450    300    700               TRUE
## 10  untenGF135    220    450    300    700               TRUE
## 11   obenGF136    220    450    300    700               TRUE
## 12  untenGF136    220    450    300    700               TRUE
## 13   obenGF139    220    450    300    700               TRUE
## 14  untenGF139    220    450    300    700               TRUE
## 15   obenGF148    220    450    300    700               TRUE
## 16  untenGF148    220    450    300    700               TRUE
## 17   obenGF155    220    450    300    700               TRUE
## 18  untenGF155    220    450    300    700               TRUE
## 19   obenGF164    220    450    300    700               TRUE
## 20  untenGF164    220    450    300    700               TRUE
## 21   obenGF166    220    450    300    700               TRUE
## 22  untenGF166    220    450    300    700               TRUE
## 23   obenGF167    220    450    300    700               TRUE
## 24  untenGF167    220    450    300    700               TRUE
## 25   obenGF168    220    450    300    700               TRUE
## 26  untenGF168    220    450    300    700               TRUE
```

```
## 27   obenGF169    220    450    300    700              TRUE
## 28  untenGF169    220    450    300    700              TRUE
## 29   obenGF185    220    450    300    700              TRUE
## 30  untenGF185    220    450    300    700              TRUE
## 31   obenGF186    220    450    300    700              TRUE
## 32  untenGF186    220    450    300    700              TRUE
## 33   obenGF189    220    450    300    700              TRUE
## 34  untenGF189    220    450    300    700              TRUE
## 35   obenGF194    220    450    300    700              TRUE
## 36  untenGF194    220    450    300    700              TRUE
## 37   obenGF197    220    450    300    700              TRUE
## 38  untenGF197    220    450    300    700              TRUE
## 39   obenGF198    220    450    300    700              TRUE
## 40  untenGF198    220    450    300    700              TRUE
## 41   obenGF209    220    450    300    700              TRUE
## 42  untenGF209    220    450    300    700              TRUE
## 43   obenGF215    220    450    300    700              TRUE
## 44  untenGF215    220    450    300    700              TRUE
## 45   obenGF216    220    450    300    700              TRUE
## 46  untenGF216    220    450    300    700              TRUE
## 47   obenGF217    220    450    300    700              TRUE
## 48  untenGF217    220    450    300    700              TRUE
## 49   obenGF218    220    450    300    700              TRUE
## 50  untenGF218    220    450    300    700              TRUE
## 51   obenGF224    220    450    300    700              TRUE
## 52  untenGF224    220    450    300    700              TRUE
## 53   obenGF236    220    450    300    700              TRUE
## 54  untenGF236    220    450    300    700              TRUE
## 55   obenGF237    220    450    300    700              TRUE
## 56  untenGF237    220    450    300    700              TRUE
## 57   obenGF238    220    450    300    700              TRUE
## 58  untenGF238    220    450    300    700              TRUE
## 59   obenGF239    220    450    300    700              TRUE
## 60  untenGF239    220    450    300    700              TRUE
## 61   obenGF245    220    450    300    700              TRUE
## 62  untenGF245    220    450    300    700              TRUE
## 63   obenGF254    220    450    300    700              TRUE
## 64  untenGF254    220    450    300    700              TRUE
## 65   obenGF257    220    450    300    700              TRUE
## 66  untenGF257    220    450    300    700              TRUE
## 67   obenGF268    220    450    300    700              TRUE
## 68  untenGF268    220    450    300    700              TRUE
## 69    obenGF27    220    450    300    700              TRUE
## 70   untenGF27    220    450    300    700              TRUE
## 71   obenGF276    220    450    300    700              TRUE
## 72  untenGF276    220    450    300    700              TRUE
## 73   obenGF277    220    450    300    700              TRUE
## 74  untenGF277    220    450    300    700              TRUE
## 75    obenGF28    220    450    300    700              TRUE
## 76   untenGF28    220    450    300    700              TRUE
## 77   obenGF285    220    450    300    700              TRUE
## 78  untenGF285    220    450    300    700              TRUE
## 79   obenGF288    220    450    300    700              TRUE
## 80  untenGF288    220    450    300    700              TRUE
```

```
## 81    obenGF29    220    450    300    700                TRUE
## 82   untenGF29    220    450    300    700                TRUE
## 83   obenGF294    220    450    300    700                TRUE
## 84  untenGF294    220    450    300    700                TRUE
## 85   obenGF297    220    450    300    700                TRUE
## 86  untenGF297    220    450    300    700                TRUE
## 87   obenGF306    220    450    300    700                TRUE
## 88  untenGF306    220    450    300    700                TRUE
## 89   obenGF308    220    450    300    700                TRUE
## 90  untenGF308    220    450    300    700                TRUE
## 91   obenGF315    220    450    300    700                TRUE
## 92  untenGF315    220    450    300    700                TRUE
## 93   obenGF317    220    450    300    700                TRUE
## 94  untenGF317    220    450    300    700                TRUE
## 95    obenGF35    220    450    300    700                TRUE
## 96   untenGF35    220    450    300    700                TRUE
## 97    obenGF46    220    450    300    700                TRUE
## 98   untenGF46    220    450    300    700                TRUE
## 99    obenGF48    220    450    300    700                TRUE
## 100  untenGF48    220    450    300    700                TRUE
## 101   obenGF49    220    450    300    700                TRUE
## 102  untenGF49    220    450    300    700                TRUE
## 103   obenGF65    220    450    300    700                TRUE
## 104  untenGF65    220    450    300    700                TRUE
## 105   obenGF68    220    450    300    700                TRUE
## 106  untenGF68    220    450    300    700                TRUE
## 107   obenGF69    220    450    300    700                TRUE
## 108  untenGF69    220    450    300    700                TRUE
## 109   obenGF76    220    450    300    700                TRUE
## 110  untenGF76    220    450    300    700                TRUE
## 111   obenGF88    220    450    300    700                TRUE
## 112  untenGF88    220    450    300    700                TRUE
## 113   obenGF95    220    450    300    700                TRUE
## 114  untenGF95    220    450    300    700                TRUE
## 115   obenGF96    220    450    300    700                TRUE
## 116  untenGF96    220    450    300    700                TRUE
## 117   obenGF97    220    450    300    700                TRUE
## 118  untenGF97    220    450    300    700                TRUE
## 119   obenGF99    220    450    300    700                TRUE
## 120  untenGF99    220    450    300    700                TRUE
##     is_scatter_corrected is_ife_corrected is_raman_normalized
## 1                  TRUE             TRUE                TRUE
## 2                  TRUE             TRUE                TRUE
## 3                  TRUE             TRUE                TRUE
## 4                  TRUE             TRUE                TRUE
## 5                  TRUE             TRUE                TRUE
## 6                  TRUE             TRUE                TRUE
## 7                  TRUE             TRUE                TRUE
## 8                  TRUE             TRUE                TRUE
## 9                  TRUE             TRUE                TRUE
## 10                 TRUE             TRUE                TRUE
## 11                 TRUE             TRUE                TRUE
## 12                 TRUE             TRUE                TRUE
## 13                 TRUE             TRUE                TRUE
```

```
## 14                TRUE             TRUE             TRUE
## 15                TRUE             TRUE             TRUE
## 16                TRUE             TRUE             TRUE
## 17                TRUE             TRUE             TRUE
## 18                TRUE             TRUE             TRUE
## 19                TRUE             TRUE             TRUE
## 20                TRUE             TRUE             TRUE
## 21                TRUE             TRUE             TRUE
## 22                TRUE             TRUE             TRUE
## 23                TRUE             TRUE             TRUE
## 24                TRUE             TRUE             TRUE
## 25                TRUE             TRUE             TRUE
## 26                TRUE             TRUE             TRUE
## 27                TRUE             TRUE             TRUE
## 28                TRUE             TRUE             TRUE
## 29                TRUE             TRUE             TRUE
## 30                TRUE             TRUE             TRUE
## 31                TRUE             TRUE             TRUE
## 32                TRUE             TRUE             TRUE
## 33                TRUE             TRUE             TRUE
## 34                TRUE             TRUE             TRUE
## 35                TRUE             TRUE             TRUE
## 36                TRUE             TRUE             TRUE
## 37                TRUE             TRUE             TRUE
## 38                TRUE             TRUE             TRUE
## 39                TRUE             TRUE             TRUE
## 40                TRUE             TRUE             TRUE
## 41                TRUE             TRUE             TRUE
## 42                TRUE             TRUE             TRUE
## 43                TRUE             TRUE             TRUE
## 44                TRUE             TRUE             TRUE
## 45                TRUE             TRUE             TRUE
## 46                TRUE             TRUE             TRUE
## 47                TRUE             TRUE             TRUE
## 48                TRUE             TRUE             TRUE
## 49                TRUE             TRUE             TRUE
## 50                TRUE             TRUE             TRUE
## 51                TRUE             TRUE             TRUE
## 52                TRUE             TRUE             TRUE
## 53                TRUE             TRUE             TRUE
## 54                TRUE             TRUE             TRUE
## 55                TRUE             TRUE             TRUE
## 56                TRUE             TRUE             TRUE
## 57                TRUE             TRUE             TRUE
## 58                TRUE             TRUE             TRUE
## 59                TRUE             TRUE             TRUE
## 60                TRUE             TRUE             TRUE
## 61                TRUE             TRUE             TRUE
## 62                TRUE             TRUE             TRUE
## 63                TRUE             TRUE             TRUE
## 64                TRUE             TRUE             TRUE
## 65                TRUE             TRUE             TRUE
## 66                TRUE             TRUE             TRUE
## 67                TRUE             TRUE             TRUE
```

```
## 68                TRUE            TRUE            TRUE
## 69                TRUE            TRUE            TRUE
## 70                TRUE            TRUE            TRUE
## 71                TRUE            TRUE            TRUE
## 72                TRUE            TRUE            TRUE
## 73                TRUE            TRUE            TRUE
## 74                TRUE            TRUE            TRUE
## 75                TRUE            TRUE            TRUE
## 76                TRUE            TRUE            TRUE
## 77                TRUE            TRUE            TRUE
## 78                TRUE            TRUE            TRUE
## 79                TRUE            TRUE            TRUE
## 80                TRUE            TRUE            TRUE
## 81                TRUE            TRUE            TRUE
## 82                TRUE            TRUE            TRUE
## 83                TRUE            TRUE            TRUE
## 84                TRUE            TRUE            TRUE
## 85                TRUE            TRUE            TRUE
## 86                TRUE            TRUE            TRUE
## 87                TRUE            TRUE            TRUE
## 88                TRUE            TRUE            TRUE
## 89                TRUE            TRUE            TRUE
## 90                TRUE            TRUE            TRUE
## 91                TRUE            TRUE            TRUE
## 92                TRUE            TRUE            TRUE
## 93                TRUE            TRUE            TRUE
## 94                TRUE            TRUE            TRUE
## 95                TRUE            TRUE            TRUE
## 96                TRUE            TRUE            TRUE
## 97                TRUE            TRUE            TRUE
## 98                TRUE            TRUE            TRUE
## 99                TRUE            TRUE            TRUE
## 100               TRUE            TRUE            TRUE
## 101               TRUE            TRUE            TRUE
## 102               TRUE            TRUE            TRUE
## 103               TRUE            TRUE            TRUE
## 104               TRUE            TRUE            TRUE
## 105               TRUE            TRUE            TRUE
## 106               TRUE            TRUE            TRUE
## 107               TRUE            TRUE            TRUE
## 108               TRUE            TRUE            TRUE
## 109               TRUE            TRUE            TRUE
## 110               TRUE            TRUE            TRUE
## 111               TRUE            TRUE            TRUE
## 112               TRUE            TRUE            TRUE
## 113               TRUE            TRUE            TRUE
## 114               TRUE            TRUE            TRUE
## 115               TRUE            TRUE            TRUE
## 116               TRUE            TRUE            TRUE
## 117               TRUE            TRUE            TRUE
## 118               TRUE            TRUE            TRUE
## 119               TRUE            TRUE            TRUE
## 120               TRUE            TRUE            TRUE
```

10) Remove noisy area at low wavelength excitation

```
eemint4 <- eemint %>% eem_range(ex = c(250,Inf), em = c(300,580))
eem_overview_plot(eemint4[1:9], spp=9, contour = TRUE)
```

```
## [[1]]
```

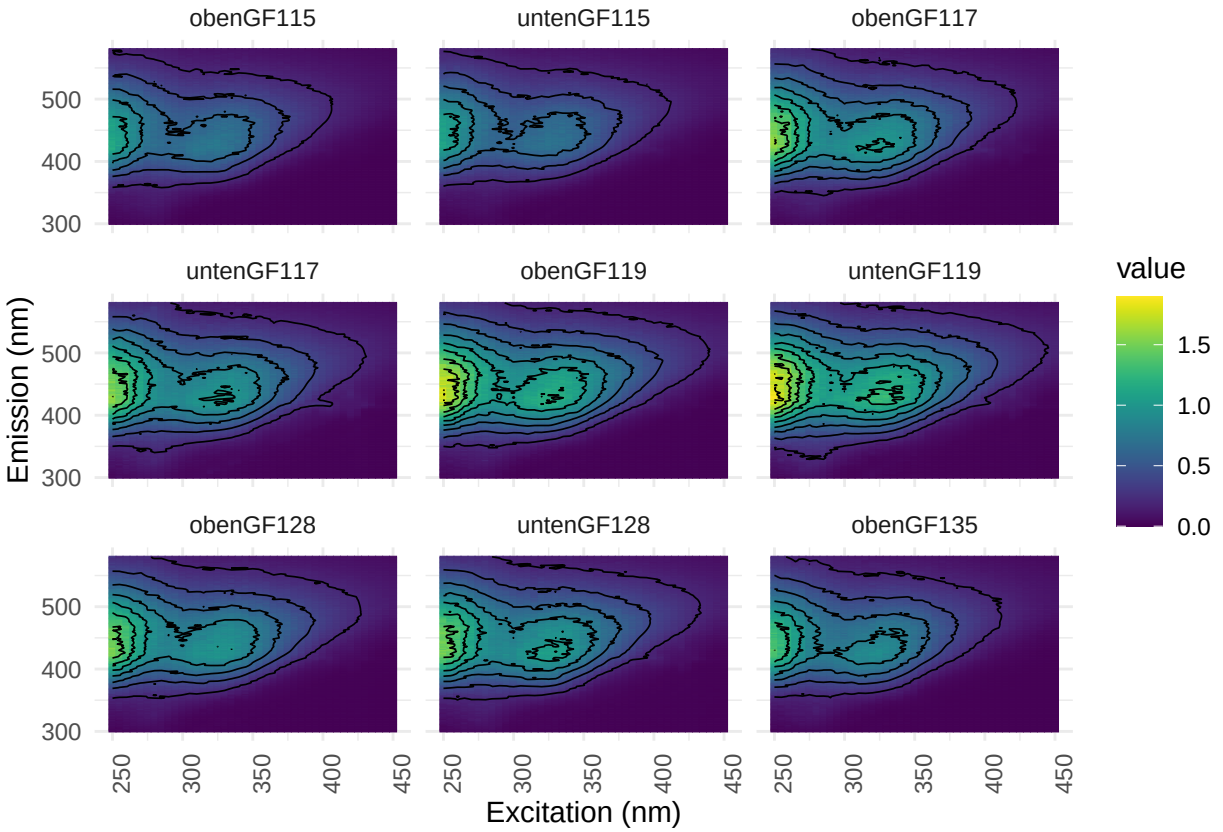

Figure 7: EEMS corrected and noisy area removed

11) Peak picking

- smoothing can be performed prior to peak picking (not donw here)
  - from a quick look at BIX there are no big differences between the smoothed and the non-smoothed

```
#WITHOUT SMOOTHING
bix <- eem_biological_index(eemint4)
coble_peaks <- eem_coble_peaks(eemint4)
fi <- eem_fluorescence_index(eemint4)
hix <- eem_humification_index(eemint4, scale = TRUE)
```

```r
indices_peaks <- bix %>%
  full_join(coble_peaks, by = "sample") %>%
  full_join(fi, by = "sample") %>%
  full_join(hix, by = "sample")

#combine with data and depth column from metadata
met <- data.table::fread("C:/Users/CBG/OneDrive - NIVA/1 Projects/NMBU_PARAFAC/241121_NMBU_PARAFAC_Meta

#merging with date from other dataset
df_merge <- merge(indices_peaks, met, by = "sample",
                  all.x = TRUE)

write.csv(df_merge,"C:/Users/CBG/OneDrive - NIVA/1 Projects/NMBU_PARAFAC/Output/NMBU_indices and peaks_
```

```r
df_merge$date_analysis<-as.Date(df_merge$date_analysis,format="%d.%m.%Y")

class(df_merge$date_analysis)
```

```
## [1] "Date"
```

```r
ggplot(df_merge, aes(x=date_analysis, y=bix, fill=lake_layer))+
  geom_col(position = position_dodge2(width = 0.4, reverse=TRUE))+
  theme(axis.text.x = element_text(angle = 45, hjust = 1))+
  coord_cartesian(ylim = c(0.3, 0.7))
```

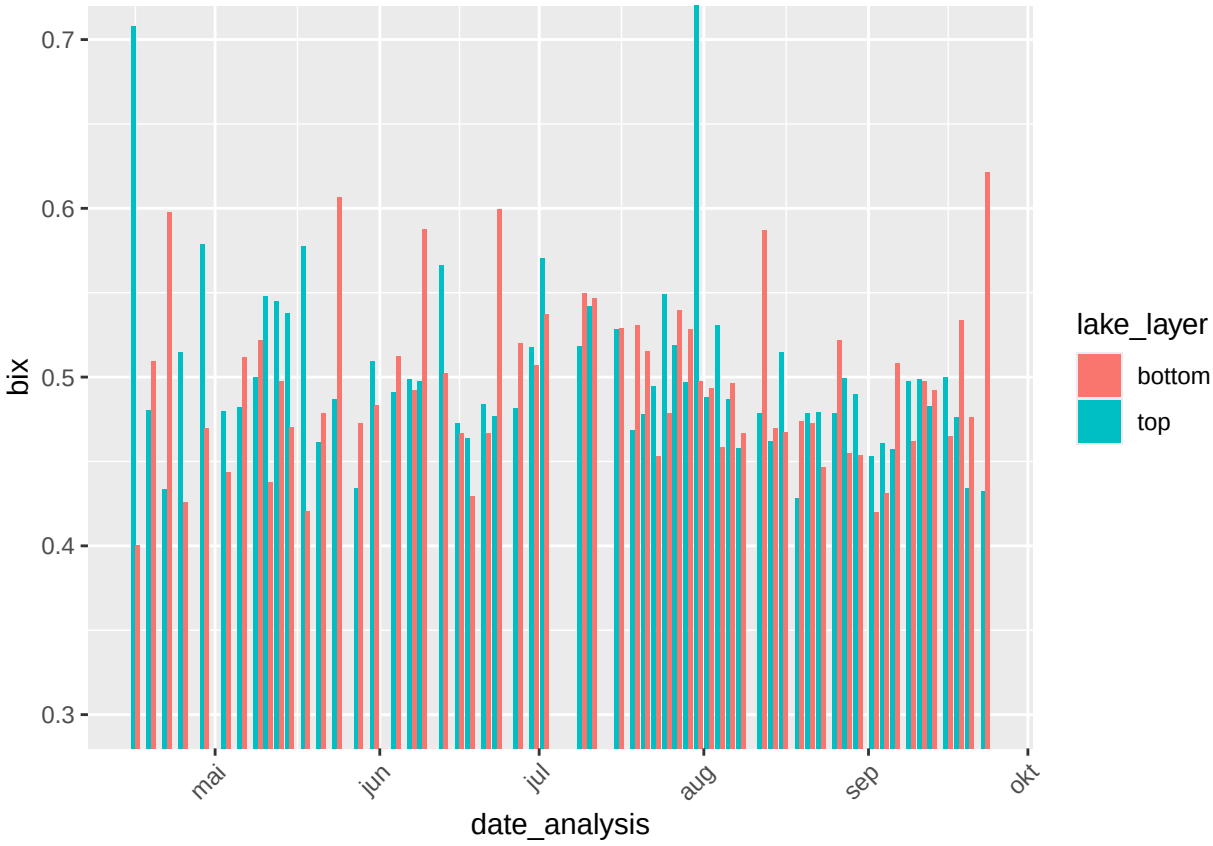
0.7
0.6
0.5
0.4
0.3
bix
mai
jun
jul
aug
sep
okt
date_analysis
lake_layer
bottom
top

Figure 8: BIX