Supporting Online Material

Autofluorescence of atmospheric bioaerosols – Fluorescent biomolecules and potential interferences

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Keywords: Autofluorescence, Bioaerosols, Excitation-Emission-Matrix, Fluorescence Spectroscopy, Fluorophores

EEM normalization

The intensity of all raw EEMs of solid state samples shown in this paper has been normalized as described in Section 2.2. Figure S1 shows tails of transmitted light on the left and right side of the Rayleigh scattering bands (1^{st} and 2^{nd} order) due to imperfect monochromators. In particular, this effect was magnified by the settings of the instrument utilized in this study, because the excitation and emission slit widths were fixed at relatively large values of 10 nm, each. This setting allows a higher quantity of light to pass the slits, which provides the advantage of increased sensitivity. However, it also decreases spectral resolution and increases the spurious background light as discussed. Superposition of these spurious light effects leads to the elevated background signal ('plateau') that can be observed between the 1^{st} and 2^{nd} order Rayleigh lines.

Fluorescence spectra of solid state, powder samples in this study were corrected for spurious background light, which was been observed to be significantly stronger for white materials than for materials of other colors. A normalization factor (NF) has been calculated as a function of the emitted light intensity to the left of 1^{st} order Rayleigh scattering within an EEM. This light, by definition, cannot be considered fluorescent , because the wavelength of emission would be shorter than the wavelength of excitation. The NF is represented as the mean of a line parallel to the 1^{st} order Rayleigh signal, but separated by 40 nm vertically (thus in excitation). In Figure S1 the lines for NF-calculation are shown for kaolin, chitin and humic acid (Fig. S1a-c). In Figure S1d the profiles of these lines are shown highlighting that the intensity background light strongly varies with λ_{em}. The highest intensities (e.g. for kaolin) were observed between 375 and 500 nm. Moreover NF shows the highest values for white and highly reflecting materials (i.e. $NF_{kaolin} = 189$, white powder) and significantly lower values for darker and less reflecting materials (i.e. $NF_{humic\ acid} = 29$, dark brown powder).

Due to the wavelength dependence of the spurious light intensity along the normalization line attempts to normalize the EEM matrix based on individual excitation (horizontally) or emission (vertically) wavelengths, respectively, were performed. Two major problems produced by this procedure, however. (I) Significant qualitative changes are reflected into the EEM by the peaking intensity of the normalization line. It has been found that these changes thus influence the characteristic fluorescence pattern in the EEMs ('shadowing effect'). (II). Moreover a certain area of the EEM cannot be normalized because the normalization line is

accessible in vertical and horizontal direction only for a certain wavelength range. Accordingly for horizontal normalization the lower excitation wavelengths and for vertical normalization the upper emission wavelengths are chopped off.

For comparison with normalized EEMs a collection of non-normalized raw EEMs can be found in Figure S4 and S5.

Figure S1. Conceptual illustration of normalization for (a) kaolin, (b) chitin and (c) humic acid. Colored normalization lines for calculation of NF are shown in (d) for comparison.

Figure S2. Additional EEM contour profiles for selected pure biological fluorophores in solid, suspended or solvated state. Color intensity scale has been adjusted to intensity of individual components. All EEMs are normalized as discussed in text (Section 2.2). Normalization factor (NF) is reported for each solid-state sample. Lower NF indicates higher fluorescence intensity.

Figure S3. Additional EEM contour profiles for selected potential interferences in solid or solved state. Intensity color scale has been adjusted to intensity of individual components. All EEMs are normalized as discussed in text (Section 2.2). Normalization factor (NF) is reported for each solid-state sample.

Normalized EEM contour profiles for selected interferences in solid state and/or solution. Intensity color scale has been adjusted to intensity of individual components. EEMs for samples in solid state are normalized.

Figure S4. Raw EEM contour profiles for selected pure biological fluorophores in solid, suspended or solved state. Intensity color scale has been adjusted to intensity of individual components.

Figure S5. Raw EEM contour profiles for selected potential interferences fluorophores in solid, suspended or solved state. Intensity color scale has been adjusted to intensity of individual components.

Figure S6. Raw fluorescence emission spectra of biofluorophores and potential interferences for selected excitation wavelanghts λ_{ex}; (a) Emission spectra of biological fluorophores at λ_{ex} = 280 nm; (b) Emission spectra of biological fluorophores at λ_{ex} = 355 nm; (c) Emission spectra of potential interferences at λ_{ex} = 280 nm; (d) Emission spectra of potential interferences at λ_{ex} = 355 nm. Dashed lines indicate samples in dry state, solid lines indicate samples in solution.

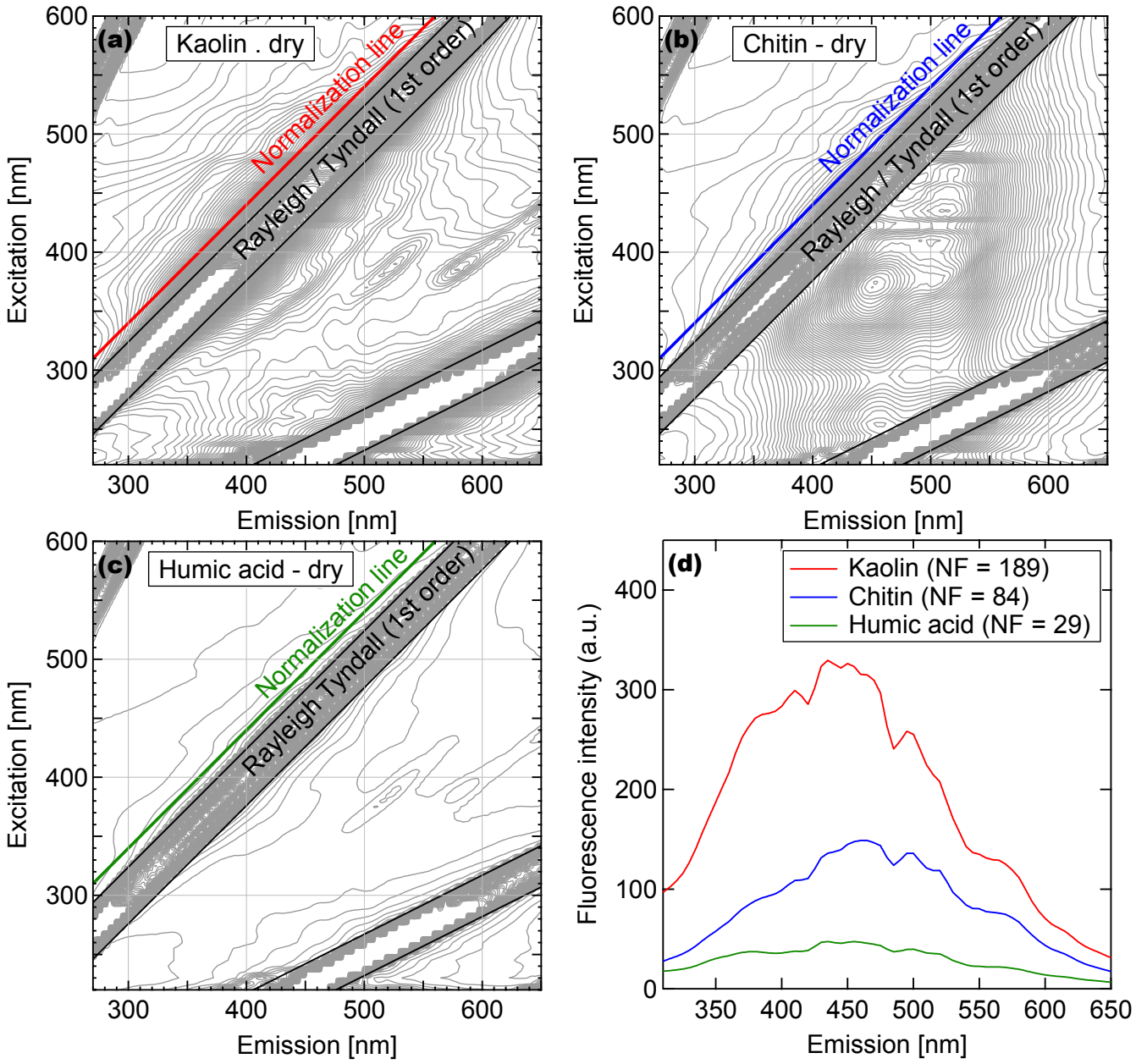
(a)
Kaolin . dry
Normalization line
Rayleigh / Tyndall (1st order)
Excitation [nm]
Emission [nm]
(b)
Chitin - dry
Normalization line
Rayleigh / Tyndall (1st order)
Excitation [nm]
Emission [nm]
(c)
Humic acid - dry
Normalization line
Rayleigh Tyndall (1st order)
Excitation [nm]
Emission [nm]
(d)
Kaolin (NF = 189)
Chitin (NF = 84)
Humic acid (NF = 29)
Fluorescence intensity (a.u.)
Emission [nm]

Figure S1.

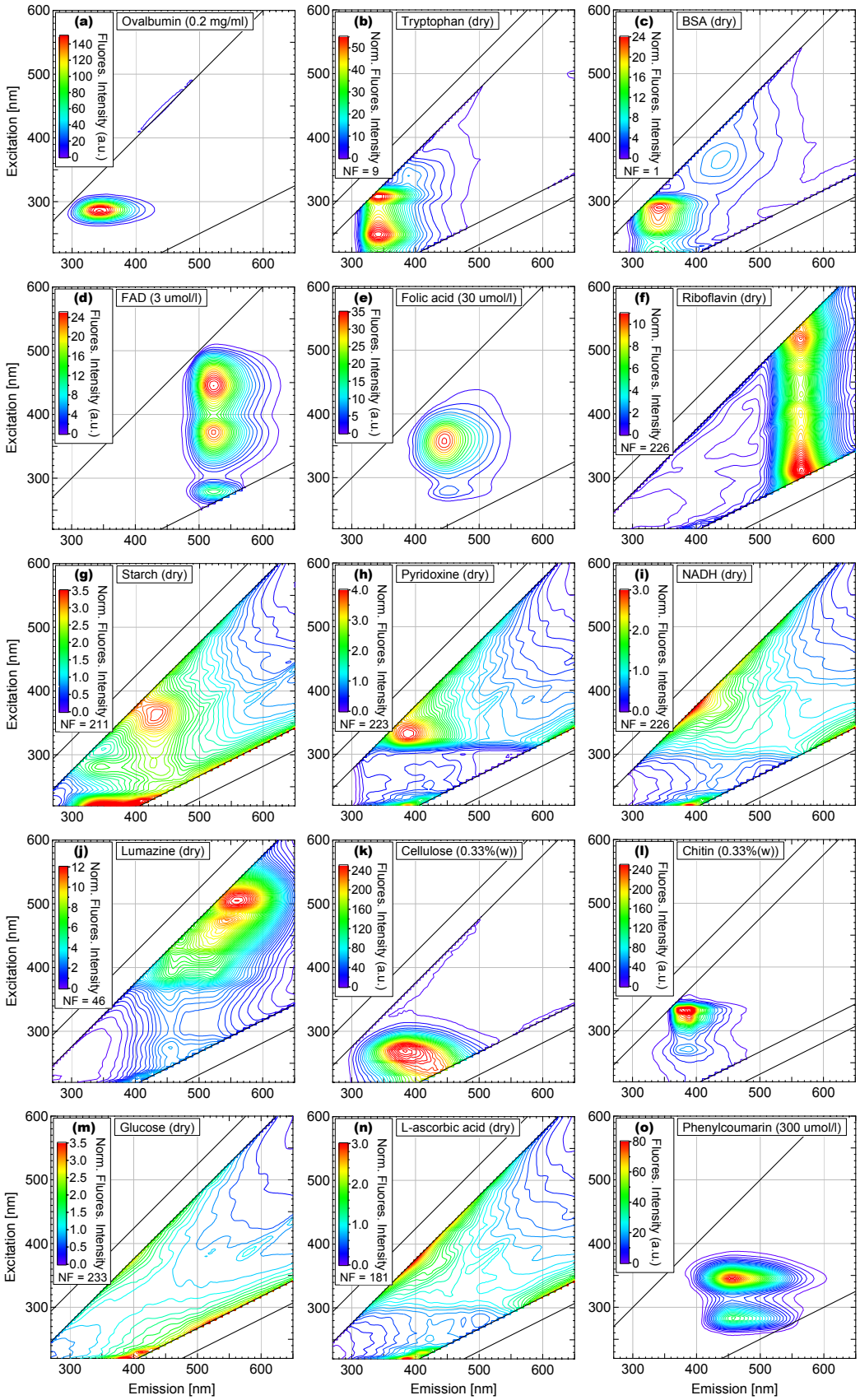
(a) Ovalbumin (0.2 mg/ml)
(b) Tryptophan (dry)
(c) BSA (dry)
(d) FAD (3 umol/l)
(e) Folic acid (30 umol/l)
(f) Riboflavin (dry)
(g) Starch (dry)
(h) Pyridoxine (dry)
(i) NADH (dry)
(j) Lumazine (dry)
(k) Cellulose (0.33%(w))
(l) Chitin (0.33%(w))
(m) Glucose (dry)
(n) L-ascorbic acid (dry)
(o) Phenylcoumarin (300 umol/l)
Fluores. Intensity (a.u.)
Norm. Fluores. Intensity
NF = 1
NF = 226
NF = 211
NF = 223
NF = 226
NF = 46
NF = 233
NF = 181
Excitation [nm]
Emission [nm]

Figure S2.

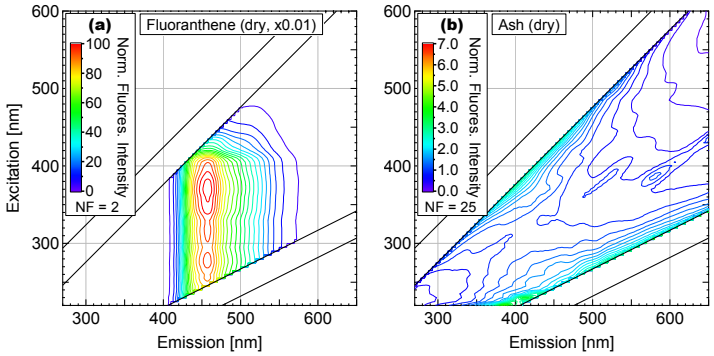
(a)
Fluoranthene (dry, x0.01)
Norm. Fluores. Intensity
100
80
60
40
20
0
NF = 2
Excitation [nm]
Emission [nm]
600
500
400
300
(b)
Ash (dry)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
NF = 25

Figure S3.

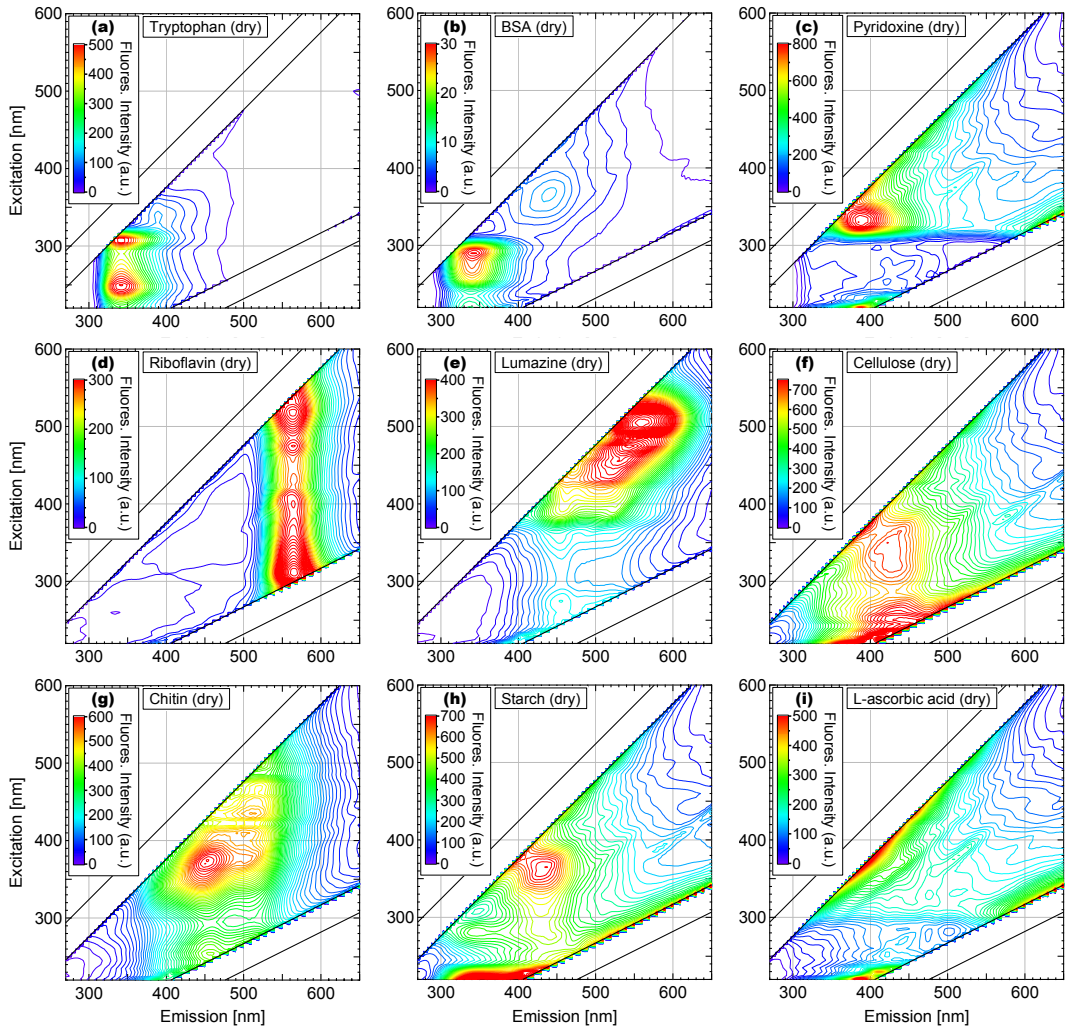
(a) Tryptophan (dry)
(b) BSA (dry)
(c) Pyridoxine (dry)
(d) Riboflavin (dry)
(e) Lumazine (dry)
(f) Cellulose (dry)
(g) Chitin (dry)
(h) Starch (dry)
(i) L-ascorbic acid (dry)
Fluores. Intensity (a.u.)
Excitation [nm]
Emission [nm]

Figure S4.

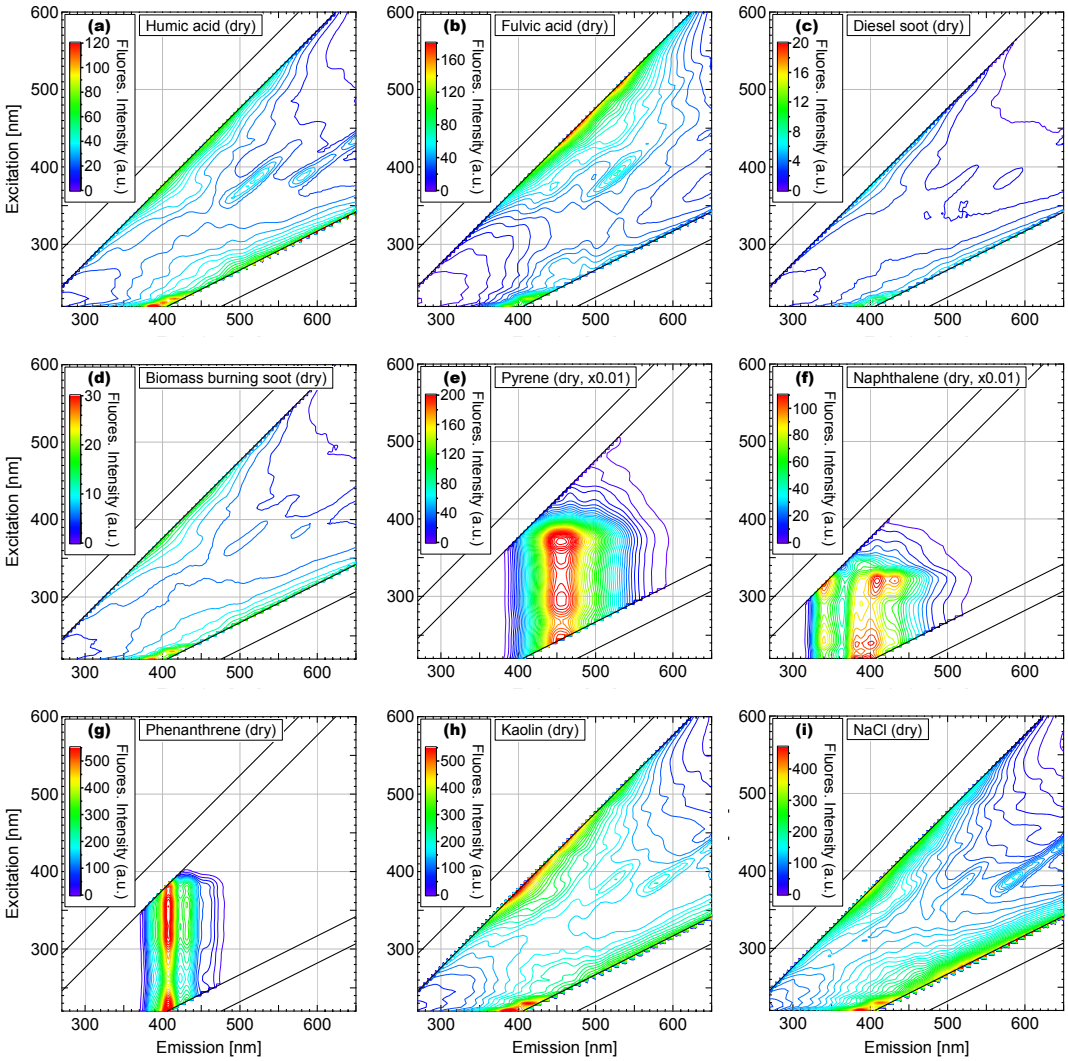
(a)
Humic acid (dry)
(b)
Fulvic acid (dry)
(c)
Diesel soot (dry)
(d)
Biomass burning soot (dry)
(e)
Pyrene (dry, x0.01)
(f)
Naphthalene (dry, x0.01)
(g)
Phenanthrene (dry)
(h)
Kaolin (dry)
(i)
NaCl (dry)
Fluores. Intensity (a.u.)
Excitation [nm]
Emission [nm]

Figure S5.

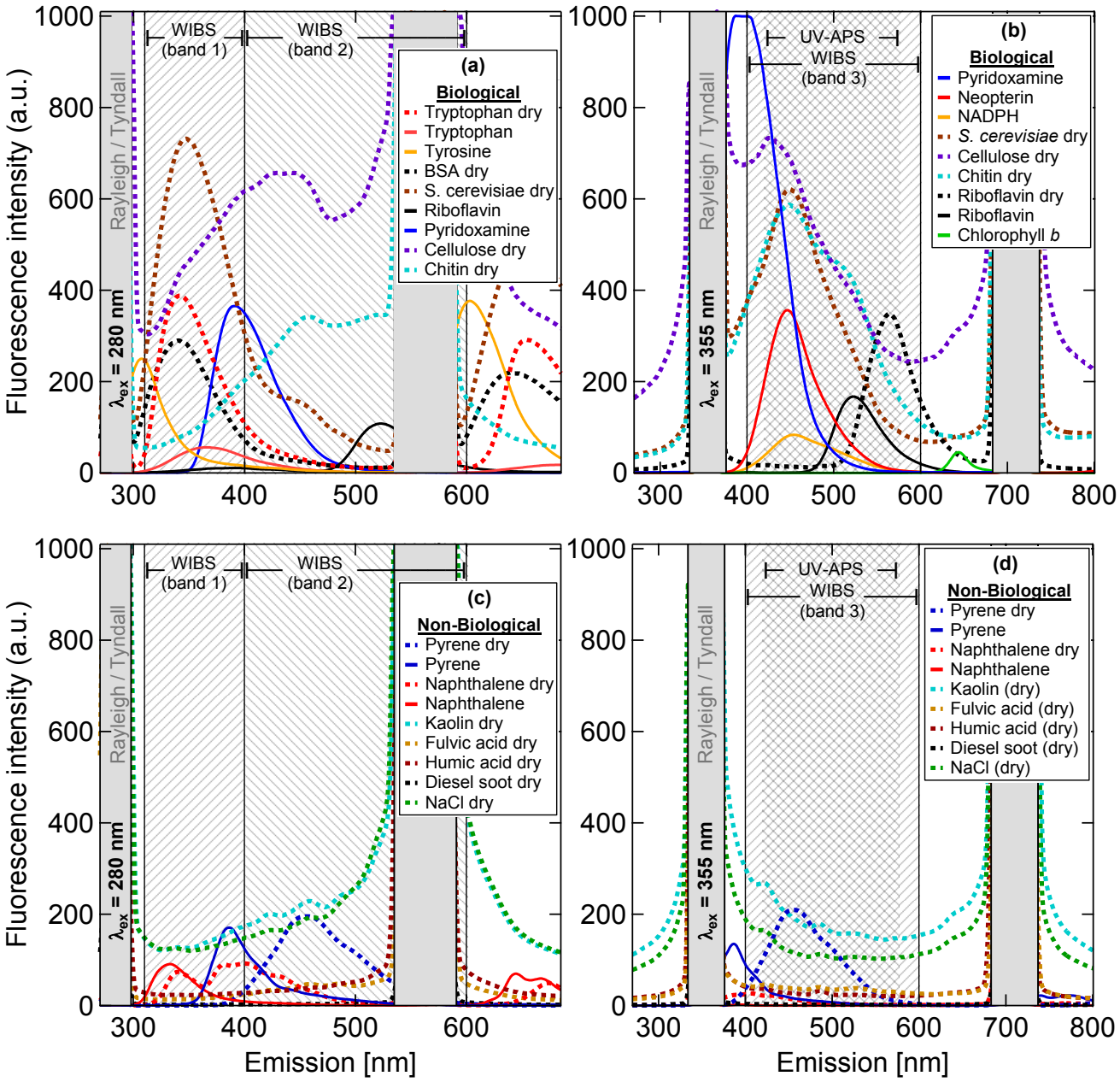
Fluorescence intensity (a.u.)
Emission [nm]
WIBS (band 1)
WIBS (band 2)
UV-APS
WIBS (band 3)
Rayleigh / Tyndall
λex = 280 nm
λex = 355 nm
(a)
Biological
Tryptophan dry
Tryptophan
Tyrosine
BSA dry
S. cerevisiae dry
Riboflavin
Pyridoxamine
Cellulose dry
Chitin dry
(b)
Biological
Pyridoxamine
Neopterin
NADPH
S. cerevisiae dry
Cellulose dry
Chitin dry
Riboflavin dry
Riboflavin
Chlorophyll b
(c)
Non-Biological
Pyrene dry
Pyrene
Naphthalene dry
Naphthalene
Kaolin dry
Fulvic acid dry
Humic acid dry
Diesel soot dry
NaCl dry
(d)
Non-Biological
Pyrene dry
Pyrene
Naphthalene dry
Naphthalene
Kaolin (dry)
Fulvic acid (dry)
Humic acid (dry)
Diesel soot (dry)
NaCl (dry)

Figure S6.