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Interactive comment on "Composite Catalogues of Optical and Fluorescent Signatures Distinguish Bioaerosol Classes" *by* M. Hernandez et al.

Anonymous Referee #2

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Summary:

The manuscript by Hernandez et al. entitled "Composite Catalogues of Optical and Fluorescent Signatures Distinguish Bioaerosol Classes" presents fluorescence data of a broad selection of standard bacteria, fungal spores, and pollen from laboratory measurements. Their work intends to establish a data base by compiling the fluorescence signatures from various microorganisms based on a unified experimental procedure. The authors claim that this library can serve as a reference basis in future (ambient) bioaerosol studies, using autofluorescence based instruments (i.e., the WIBS).

One of the major challenges in autofluorescence based bioaerosol analysis is the diversity of the airborne organisms as well as the diversity of different fluorescent molecules inside these cells. Therefore, reference fluorescence data/spectra are needed to clas-

sify and interpret the output from online fluorescence detectors (e.g., the WIBS). Accordingly, the overall aim of this study is a very useful one.

However, I think the manuscript suffers from a number of major issues that require more attention. I have listed my major concerns below. Overall, I recommend that the manuscript is appropriate for AMT and will probably receive a lot of attention from the bioaerosol community. However, the manuscript in its current form needs a major revision.

General Comments:

1) It seems that the manuscript was written in a rush. There is a rather high density of typos (some are collected below under minor comments). Moreover, the discussion of the results is rather short and ignores most of the previous studies that have reported related results (see major comment 5 below). A striking example for the improvable quality is the fact that about half of the Latin names for the reference species (a focal point of this study) are misspelled. I am just giving selected examples: "bacillius subtilis" vs. "bacillus subtilis" (#1 in Table 1); "psuedomona aurigenosa" vs. "pseudomonas aeruginosa" (#10 in Table 1); "pennicillium" vs. "penicillium" (#43-49 in Table1); "artemesia" vs. "artemisia" (#18 in Table 1); "fragus" vs. "fagus" (#23 in Table 1).

2) In lines 97-99, the authors express the main objective of the study: "We report a systematic compilation of optical and fluorescence properties, which can be reproduced and expanded as a bioaerosol reference basis for new generations of UVIF instrumentation". I have some objections here.

- First, if the collection of reference organisms is called "systematic", some relevant studies should be cited to underline that the selection of organisms can indeed serve as representatives for the atmospheric bioaerosol population.

- Second, the authors claim that the results can be "reproduced" after stating in line

79 that fluorescence properties strongly depend on the “cultivation history” of the organisms. In its current state, the experimental section is rather vague in terms of “cultivation history”. The following basic information is missing: (i) strain designation for the ATCC and DSMZ cultures, (ii) the basic cultivation protocols for fungi and bacteria, (iii) cultivation times should be specified more precisely, (iv) the term “obvious spore-bearing physiology” should be explained more clearly, (v) the collection procedure and age of the pollen samples should be mentioned.

- Third, I do not really agree that the reported fluorescence data can serve as a “reference basis for new generations of UVIF instrumentation”. My feeling is that this statement is too broad. Given that the “new generations of UVIF instrumentation” have different excitation and/or emission specifications, I wonder if the reported data set, which is defined by the WIBS optical design, is still useful as a reference. I think that this is rather a “reference basis” for future WIBS measurements.

3) In lines 128 and 129, the authors state that pollen samples were aerosolized “with notable fractionation of some grains”. That is a major aspect and important bias. What percentage of pollen grains is “fractionated”? What does it imply for the reported results? The pollen sizing deviates strongly. The equivalent optical diameters (EOD) that have been obtained for all pollen samples in this study are below 7 μm. The physical diameter of most pollen grains is > 10 μm (Despres et al., 2012). For some of the reported species the EOD in this study shows substantial deviation from (optical and physical) diameters in previous reports. For example (i) artemisia tridentata: 3.2 vs. $\sim$21 μm; (ii) betula: 2.5 vs. $\sim$24 μm; (iii) juglans nigra: 1.2 vs. 37 μm; (iv) phleum pratense: 1.5 vs. 34 μm (Healy et al., 2012a;Pohlker et al., 2013). The strong deviation suggests that probably only pollen fragments have been sampled. This strong deviation and the influence of the “fractionation” clearly need a careful discussion. In lines 175-180, the authors provide a brief explanation of the fragmentation. The atmospheric fragmentation of pollen grains is a phenomenon that is by far not fully understood yet. Keeping this in mind, I am not sure the presented pollen samples can serve as a “re-

producible” “reference” compound in this study. Regarding this major bias, it is maybe better to skip the pollen samples in the present study.

4) Line 116: Any comment on the ultra-pure water and the Collison nebulizer, which both are pretty harsh treatments? Do you expect that the bacteria are alive or killed afterwards? Specify the type of microscopy that has been used. Does it allow to confirm cell integrity for cells < 1 μm?

5) This study presents the fluorescence properties from a variety of different organisms. However, most of the scientifically interesting aspects are not discussed in the results and discussion section. Moreover, several previous studies have reported fluorescence properties from bacteria, fungal spores, and pollen already. None of them is cited and used in the discussion to examine if the reported results agree with what is known. I understand that this is a technical paper. Moreover, I appreciate if papers are kept short and concise. However, a certain extent of discussion is still desirable in technical papers to put the results in the context of the existing knowledge. I think that the following aspects require (at least some) more explanation/discussion:

- In lines 162-176, the authors summarize the observed properties of the major classes (bacteria, fungal spores, pollen). The intensities of the observed fluorescence are lowest for the bacteria and highest for the pollen grains and, thus, correlate with size. It is well known that bioaerosol fluorescence intensity strongly depends on particle size (Hill et al., 2001;Hill et al., 2015;Healy et al., 2012b). I think that the fluorescence-size dependence is important information to explain the reported results. Cite some previous studies here.

- In line 169-170, the authors state that “with the exception of spore forming bacillus subtilis, bacterial bioaerosol was limited to a single fluorescence type (A)”. Does this sentence imply that the formation of spores is responsible for the different fluorescence signatures? If essentially all bacteria show very similar fluorescence signatures, is there any hope to sub-classify bacteria based on WIBS measurements? Several stud-

ies have reported fluorescence signature from bacterial species (Pan et al., 2010;Pan et al., 2007). Some of them should be cited here to check the agreement of fluorescence spectra and WIBS-related “fluorescence type frequencies”.

- In line 179-180, the authors state that pollen samples show a “significantly different fluorescence type distribution” (I am ignoring the sizing issue here). This corresponds with published results (Pan et al., 2011;Pohlker et al., 2013). A short statement would be helpful to embed this observation into the existing knowledge.

- In lines 181-188, the authors summarize the “fluorescence response to spore aging”. An aging effect is observed for some species. What does this imply for the use of the reported WIBS “reference basis” for ambient measurements? Any chance to visualize this shift in Fig. 3?

Minor and Specific Comments:

- Keywords: I think the use of “aerosol cytometry” is confusing here. There are several studies that apply ‘classical’ cytometry to bioaerosol analysis (Chen and Li, 2007;Prigione et al., 2004). By using the same term, the different analytical approaches (airborne particles vs. particles suspended in water) may be mixed up.

- Keywords: Probably “WIBS” is another good keyword for this study.

- Line 64: I strongly suggest using the term “intrinsic fluorescence” instead of “fluorescence” here and throughout the entire text. The discrimination between intrinsic and extrinsic fluorescence is a fundamental one and should be very clear from the beginning.

- Line 67: The majority of related studies uses the terms light induced fluorescence (LIF) or ultraviolet light induced fluorescence (UV-LIF)(Toprak and Schnaiter, 2013;Robinson et al., 2013;Healy et al., 2012a;Huffman et al., 2012). Do the authors see any strong reasons to establish another terms, namely “ultraviolet induced fluorescence (UVIF)” in addition? My feeling is that the community should try to avoid using

different terms for the key aspects since this fosters confusion.

- Line 69: “the” is missing in “contribution to airborne carbon pool”.

- Line 73: NADH is not defined. Moreover, the biologically correct abbreviation would be NAD(P)H.

- Line 92: In the majority of bioaerosol studies, PBAP refers to primary biological aerosol particles (Despres et al., 2012;Robinson et al., 2013;Perring et al., 2015). Here, it refers to “primary biological airborne particles”. I suggest replacing “airborne” by “aerosol”.

- Line 101: What is the difference between the “conventional and fluorescent bioaerosol spectra” in this context?

- Line 102: The terms fungi vs. fungal spores should be used more precisely throughout the text: The fungi are cultivated, but the fungal spores are aerosolized.

- Line 108: Replace “20-22C” by “20-22°C”.

- Line 112: This sentence refers to “fifteen pure bacterial cultures”. In Table 1, 12 cultures are listed, while in Figure 2, 14 cultures are shown. Make sure that these numbers are consistent.

- Line 119: Define “MEA”.

- Line 130: Was the WIBS operated in high or low gain mode (Healy et al., 2012a)?

- Line 134: The channel specification here “310-400 nm” does not agree with line 151 “310-420 nm”.

- Table 1: It would be very helpful to add information about the scattering of EOD and intensity (e.g., +/- one standard deviation) to get a feeling for the width of these distributions. Moreover, it is not clear where the intensity is derived from. Is it the total fluorescence intensity of the intensity of a specific WIBS channel?

- Figure 2: Replace “fungi” by “fungal spores” in the figure.

- Figure 3: Replace “fungi” by “fungal spores” in the figure. The unit of the x axis is missing.

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