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Discussions

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

M. Hernandez et al.

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We thank Dr. Huffman for his helpful comments and criticisms and address them below in the order they appear. The title has been changed to reflect that the work was limited to chamber assessments on a meso-scale (1m3): Chamber based catalogues of optical and fluorescence properties can distinguish bioaerosol classes

Ln 97-99. The authors have chosen to use the term "systematic" in a generic context, as it refers to the method described here. We realize however, this can be confused with microbial systematics in the context of taxonomy. In this context we make no claims nor do we intend to present any inference as such. Thus we have removed the word "systematic" in this context. (Ln 98) We mean to present the perspective of generalized, chamber-based bioaerosol challenge method for UV-LIF, which can be

applied to generate this quality of optical and fluorescence response catalogues. Ln 99, 247 and Figure 4. The authors agree with the reviewer's perspective of figure 4; thus, the discussion has been expanded to highlight this operational example and the fact that different WIBS instrumentation, while configured the same, can generate (somewhat) different aerobiological catalogues as judged by fluorescence distributions of pure culture microbes aerosolized in chambers under the same conditions.

Ln 116. The type of microscopy used was classic phase contrast and fluorescence microscopy (x1000), which the authors believe was appropriately referenced in the original version of the manuscript (citing bioaerosol studies which explicitly include the survivability of many of these same bacterial cultures through nebulization). Using modern microscope equipment, those skilled in this art reliably resolve submicron dimensions of bacterial cells using reticules calibrated on this scale (however, this is a direct optical measurement, and not an Equivalent Optical Diameter (EOD)). Based on previous studies in this and other bioaerosol laboratories (citations added), with many of the same bacterial models used here, the authors acknowledge that some microbes, particularly Gram negative bacteria harvested in their log growth phase, can experience significant viability losses after being refluxed in Collison nebulizers even after 10 minutes. To this end, we purposely harvested bacteria in early stationary phase and held nebulization times to 2 minutes to minimize variance with viability – this was a conscious choice in compiling and executing this method. We did not measure culturability as it was not central to this investigation. The revised version of this manuscript, includes an expanded of detail of (these routine) microscopy and aerosolization procedures.

Ln 128-129. The authors acknowledge(d) that many different types of pollen grains often fractionate in aerosols: this fractionation is well documented to naturally occur in the atmosphere (as well as in the laboratory), and references have been added to support an expanded discussion which include this bioaerosol behavior. Because of this phenomena, the EOD distributions for pollen grains are much wider than their fungal or

bacterial counterparts, and the means of these distributions, as acknowledged, are less than unadulterated pollen grains, which often have true optical diameters larger than 10 um. Direct microscopy was performed on subsets of experiments with each different species aerosolized, and the influence of pollen fractionation was evident; however, pollen image analysis was not central to this study. We expand our discussion to better present the fractionation which was observed in this study. Results and Discussion. As requested, in the revised version of this manuscript, we juxtapose what subset of pure culture bioaerosol (fluorescence) data from other studies can be legitimately and practically compared, including those reported by Hill, Healy, Pan, Pohlker and their coworkers (circa 2001-2014), although many of these previous studies included substantial focus on asymmetry factors, analyzed significantly less bioaerosol (102-3 particles as compared to > 104) and/or did not specifically provide for equilibrium between the chamber humidity and bioaerosol. In some cases (figure 3), fluorescence intensity was notability higher with pollen grains regardless of fractionation.

Ln 181-188. Of four different fungal cultures observed, modest differences in fluorescence distributions (A shifting to AB) were noted between otherwise young (28 d) and aged (180 d) fungal spores: P. crysogenum, P. herbarum, and C. cladosporioides. In response to the prompt by this and other reviewers, the authors make no claims or inferences in the current (or future) form of this manuscript regarding the ability of this UV-LIF equipment configuration to otherwise sub-classify fungal spores based on age (in any channel). While these data beg efforts to specifically (and extensively) study the effects of aging on fluorescence distribution (and intensity), we are cautious to expand discussion beyond this simple observation.

Response to reviewer's comment with respect to asymmetry factor: Because of its variance, we purposely avoided including asymmetry factors in this cataloging demonstration study – in fact we did not collect this data during these experiments. We cannot formulate a consensus about how to incorporate a generalized statement about asymmetry factors given we did not collect these data.

Line 151. We have cited Perring et al, 2015 as generalized guidance for cataloging fluorescence distribution response(s) of the pure cultures aerosolized here, and report our results in this format as such. As requested, we have expanded our explanation of Perring reporting format (and legends) in a revised version of this manuscript.

Table 1. We agree with this reviewer's observations and will explicitly present the fluorescence distributions on a percent (%) basis. Standard deviations for EOD have been added to Table 1.

Line 136-138. An expanded and detailed discussion of the two-point (PSL) instrument calibration has been added to a revised version of the manuscript.

Line 182 – 186. We acknowledge the density of figure 2; however, we have considered pulling out young (28d) and aged (180d) comparisons in a separate plot. This would unfortunately add significant graphical redundancy to the manuscript. We have however, added detail in the discussion of what differences have been noted between the fluorescence distributions of three cultures young and ages spores (see comment above Ln 181-188) in a revised version of the manuscript.

Ln 252-253. As a point of clarification in this context, primary physiology refers to that defining the major bioaerosol classes based on their differences in structural phenotype: virus, bacteria, fungal spores and pollen grains. Figure 2 juxtaposes fluorescence distribution and EOD of these major classes (not including virus) where distribution is represented across (all) seven possible fluorescence combinations for a given (pure culture) population.

We have addressed the following "minor" comments as requested by this reviewer:

Ln 67. UV-LIF will be used as the acronym to describe all instance of ultraviolet light induced fluorescence and any variant thereof.

Request and Clarification: We don't understand this reviewer's request for "standard" history. Given this is a methods presentation, the authors believe the motivation and

historical context are adequate.

Request and Clarification: The WIBS was not “inside” the chamber, and the methods section has been amended to better reflect the apparatus configuration.

Ln 103. The tense describing the state of the chamber was changed from was to is (present).

Ln 143. Resuspension was unlikely given the evacuation and cleaning protocol and confirmed at the beginning of each test by particle monitoring. As judged by particle monitoring, evacuation and cleaning never failed to remove large particles.

Ln 192. Spelling of monodispersed corrected.

Ln 254. Cluster analyses have been acknowledged and citations added in accordance with reviewer 1.

Table 1. The numbers correspond to cultures as grouped by their major physiologic class, but have been removed for clarity.

Request and Clarification: Both instruments were calibrated with PSLs obtained from the same source, but they were not calibrated at the same time.

L221. Gain settings were not “standardized” between instruments, but both instruments were operated in their lowest gain range. We have added this statement to the methods section of a revised manuscript.

Figure 2. The legend for this figure includes a horizontal bar, which represents the scale for EOD. It is approximate simply because it cannot be directly calibrated with pixels in the graphical program used to create it (Igor v 6.0).

Figure 3. Like Dr. Crawford, Dr. Huffman asked for clarification and amendment to figure three. We apologize for this graphical oversight and have corrected the symmetry and labels on the ordinate axis and expanded this figure’s caption.

Ln 52. The words “some intact” have been removed from this sentence.

Ln 86. The statement about the “short stability window” has been expanding to include a time frame on the order of “days”.

Ln 92 and Ln 132. The term “portable” has been removed from describing the WIBS, and the serial numbers for the WIBS used in this study, are included in the methods section of a revised manuscript

Ln, 104, 107, 108 and 120. Spaces removed.

Ln 136-138. Sentences shortened.

Ln 167. The term “clustered” was changed to “related assemblages”

Ln 179. Pollens is the correct generic plural of pollen, as referred to different physiological types or species.

Ln 208, 211, and 219. The citations requested have been added.

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