Atmos. Meas. Tech. Discuss.,
doi:10.5194/amt-2015-372-AC2, 2016

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

M. Hernandez et al.

anne.perring@noaa.gov

Received and published: 27 April 2016

We thank this anonymous reviewer for his/her helpful comments and criticisms. We address them below in the order they appear.

General Comments

We apologize for any oversights and editorial inconsistencies between the tables, text and figures in regard to the formal Latin names of the cultures used in this study. We have reviewed the Latin spellings for all the source cultures used in this investigation, and cross-checked them against each other. Those that were misspelled were corrected.

Ln 97-99. The authors have chosen to use the term "systematic" in a generic context,

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Discussion paper

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.

Justification and citations for the selection of microorganisms used in this study: The microbial inventory of the atmosphere remains a topic of intense study, which has recently been accelerated by high throughput DNA sequencing. While genotypic characterization is advancing our understanding of the relative abundance atmospheric microbes, classical culture and microscopy still represent the majority of aerobiology investigations published to date. Optical properties, whether fluorescent or not, are phenotypic properties. Thus, to demonstrate this library approach, we chose a subset of pure-cultures of bacteria, fungi and pollens which have been (repeatedly) recovered and identified from aerosols in both indoor and outdoor environments by culturing a microscopy. Of the 14 bacterial cultures used here, all but two cultures are medically relevant to public health or bioterrorism, and eight cultures have (commonly) been used as bioaerosol models to their persistence under different atmospheric / disinfection conditions (e.g. UV). The three bacterial cultures used here that have not been previously recovered from the atmospheric environment, or otherwise used in prior bioaerosol studies (Thiobacillus sp., Vibrio sp. and Enterobacter sp.) were simply chosen to broaden the range of bacterial phenotypes used for these fluorescence challenges such that all the major bacterial physiologies were represented: Gram Positive, Gram Negative, bacterial spores, cocci, bacilli, and vibrio (filamentous bacteria were purposely not included).

With respect to the fungi and pollens chosen, the same logic applied in choosing which cultures to use for this demonstrative library (challenge): all members of the 26 fungal cultures used and all 13 pollen grain stocks have been recovered from different atmospheric environments. While certainly not exhaustive, these cultures cover a broad range of fungal and pollen physiologies commonly recovered from the atmospheric

environment (both indoors and out), and reported on a phenotypic basis. This discussion has been summarized and supporting references added to the manuscript as this reviewer has requested.

Response to reviewer's comments regarding cultivation history: The authors are in agreement that this manuscript (re)states that "cultivation history" can be a factor in observing fluorescence properties of airborne microorganism with UVIF; however, we present this statement through a carefully qualified citation [Saari et al, 2014]. As such, we believe that this statement is appropriately cited and presented. Because of the significant length it would add the manuscript, the authors purposely chose to minimize the (routine) level of microbial culturing detail this reviewer is asking forâĂŤsince it can be easily accessed from their source collections (ATCC and DSMZ) or classic microbiology lab manuals. We believe that providing culture details, beyond where the cells were harvested in their growth cycle (e.g. early stationary phase) and their immediate preparation prior to aerosolization, is considered so routine (and protocols so easily attained) that this information should at most be included in supplementary information (if at all). We believe the length that generic culturing protocol material would add to the manuscript would distract from the main point of the work, and respectfully decline to do so with exception to separate supplementary files. We have however, expanded the manuscript to include more culturing details with respect to harvesting (cultivation time) and immediate cell preparation. In response to the reviewer's request, we have added ATCC and DSMZ strain designations to table 1, where appropriate.

Response to reviewer's comment with respect to determining "spore bearing physiology": Each culture was observed with phase contrast microscopy and classical spore staining to ensure that spores we present and dominant. These classic methods were cited in an expanded version of this manuscript. Response to reviewer's comment with respect to determining "spore bearing physiology: The pollens were collected directly from their sources at the Botanic Garden cited, and stored dry (between 20-40% RH). Unlike bacteria and fungi however, pollen granules are not cultured, but acquired from

their botanical source; pollen grains do not have classical microbial growth cycles is not practically possible to determine their age.

Response to reviewer's comment with respect to referential basis for UVIF: We respect to this anonymous reviewer's opinion that THIS particular cohort of fluorescence data can serve as reference basis for new generations of UVIF instrumentation – we agree this statement is too broad. We also believe this reviewer is simply pointing out a semantic issue around the word "can". We thus amend this statement in an updated version of the manuscript to generically state that this type of referential approach may (colloquial equivalent to "can") add value to the bioaerosol characterization field. We amend the text as the reviewer requests to explicitly present this work as a reference method (not about the absolute value of these reference data).

Ln 128-129 and Ln 175-180. 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. We expand our discussion to better present the fractionation which was observed in this study, but are reticent to remove our reporting of pollen grain fluorescence for the reason that their fluorescence type distributions are so markedly different than fungal spores or bacterial cells – this is a noteworthy finding and the authors believe this juxtaposition is worth presenting in this methodological context.

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. Thus, 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 microscopy and aerosolization procedures.

Response to reviewer's comment with respect to previous UVIF studies including similar culures: We appreciate this reviewer's perspective and his/her request to juxtapose the results reported here, to those previously reported from other studies, which isolated UVIF response of similar, if not identical cultures (as catalogued by ATCC or DSMZ). Since this is an archival journal of Measurement Techniques, our point was to report the method in a concise manner as this reviewer suggests, which a respectable cohort of demonstrative data. 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.

Ln 162-176 and Ln 179-180. The authors agree with the reviewer's suggestion that we state how these results show clear differences in fluorescence (distribution) patterns

between pollen(s) and pure cultures of fungi (spores) and bacteria. As suggested above, we (will) acknowledge (and cite) that Pan, Pohlker and their coworkers also observed differences in UVIF signatures between certain pollens and other cultures of airborne microbes – although those cultures and aerosol conditions were different than those reported here.

Ln 169-170. The reviewer reiterates the authors' statement referring to the fact that the only culture of sporulated bacteria aerosolized in this study (B. subtilis), was markedly different from the other bacteria cultures aerosolized in terms of its fluorescence distribution. This is a straightforward observation. In response to the prompt by this reviewer, the authors make no claims or inferences in the current (or future) form of this manuscript regarding the ability of this UVIF equipment configuration to otherwise sub-classify bacterial cultures. Since only one type of bacterial spore was used, we are reticent to expand discussion beyond this simple observation.

Ln 181-188. The reviewer reiterates the authors' statement referring to the fact that 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 reviewer, the authors make no claims or inferences in the current (or future) form of this manuscript regarding the ability of this UVIF equipment configuration to otherwise sub-classify fungal spores based on age. While this does, however, beg efforts to specifically study the effects of aging on fluorescence distribution (and intensity); we are reticent to expand discussion beyond this simple observation.

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

The term "aerosol cytometry" has been dropped from the text to avoid any potential confusion. Keywords: WIBS has been added to the keyword list.

Ln 64: intrinsic fluorescence will replace "fluorescence" where appropriate in context

throughout the text.

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

Ln 73. The first instance of Nicotinamide Adenine Dinucleotide Hydrogenase will be introduced and thereafter referred to by acronym, NADH and its phosphorylated derivative NADPH.

Ln 92. The first instance of Primary Biological Aerosol Particle will be introduced as consistent with the recent literature and thereafter referred to by acronym- PBAP.

Ln 101. Conventional (light) will be delineated from fluorescent spectra.

Ln 102. The culture of fungi (pl.) will be delineated from fungal spores throughout the text.

Ln 108. In all instances, the reporting of temperature within ranges in the Centigrade scale will be reported with the degree symbol preceding a capital C (e.g. Xo C).

Ln 119. MEA is malt extract agar, which is commonly used for the cultivation of fungi. Its first instance will be introduced, and thereafter referred to as MEA.

Ln 130. The WIBS was operated in low gain mode; this operational setting will be added to the methods section. Ln 134. The typographical error for the channel specification in the range between 310-400 nm has been corrected throughout the text.

Table 1. Standard deviations for EOD have been added to Table 1.

Figures 2 and 3. Fluorescence intensity in Figure 3 is associated with the range between 310-400 nm “Fungi” has been replaced with “Fungal Spores” in Figures 2 and 3.

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