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Atmospheric Measurement Techniques Discussions
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3 # Composite Catalogues of Optical and Fluorescent Signatures
4 # Distinguish Bioaerosol Classes
5

6 **Authors & Institutional Affiliations**
7 First Author: M. Hernandez*
8 Department of Civil, Environmental and Architectural Engineering,
9 UCB 428
10 University of Colorado
11 Boulder, CO 80309
12 USA
13 mark.hernandez@colorado.edu
14
15 Second Author: A. Perring
16 National Oceanic and Atmospheric Administration
17 325 Broadway
18 Boulder, CO 80305
19 USA
20
21 Cooperative Institute for Research in Environmental Sciences
22 University of Colorado
23 Boulder, CO 80309
24 USA
25
26 Third Author K. McCabe
27 Department of Sciences
28 Columbia George Community College
29 400 East Scenic Drive
30 The Dalles, OR 97058
31 USA
32
33 Fourth Author: G. Kok
34 Droplet Measurement Technologies
35 2545 Central Ave
36 Boulder, CO 80301
37 USA
38
39 Fifth Author: G. Granger
40 Droplet Measurement Technologies
41
42 Sixth Author: D. Baumgardner
43 Droplet Measurement Technologies
44
45 * Corresponding Author
46

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47 **ABSTRACT**

48 Rapid bioaerosol characterization has immediate applications in the military, environmental and public
49 health sectors. Recent technological advances have facilitated single-particle detection of fluorescent
50 aerosol in near real-time; this leverages controlled exposures with single or multiple ultraviolet
51 wavelengths, followed by the characterization of associated fluorescence. This type of Ultraviolet
52 induced fluorescence has been used to detect some intact airborne microorganisms in laboratory studies,
53 and has been extended to field studies which implicate bioaerosol to compose a substantial fraction of
54 supermicron atmospheric particles. To enhance the information yield which new generation fluorescence
55 instruments provide, we report the compilation of a systematic referential catalogue including more than
56 fifty pure cultures of common airborne bacteria, fungi and pollens. This catalogue juxtaposes intrinsic
57 optical properties and multiple bandwidths of fluorescence spectra, which manifest to clearly distinguish
58 between major classes of airborne microbes and pollen.

59 **KEYWORDS:**

60 Bioaerosol; Fluorescence; Aerosol Cytometry
61
62

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63 **INTRODUCTION**

64 Fluorescence aerosol interrogation is gaining increased attention for its ability to characterize particulate
65 matter suspended in both indoor and outdoor environments (Huffman et al., 2010; Sivaprakasam et al.,
66 2011). When simultaneously reporting across multiple bandwidths, ultraviolet induced fluorescence
67 (UVIF) has successfully detected airborne microbes in bench-scale chamber studies (Healy et al., 2012;
68 Kaye et al., 2005; Toprak and Schnaiter, 2013) and been applied to support large-scale aerosol monitoring
69 campaigns where fluorescent particle cohorts indicated significant bioaerosol contributions to airborne
70 organic carbon pools (Gabey et al., 2010; Gabey et al., 2013; Perring et al., 2015; Poschl et al., 2010).

71 A variety of biogenic fluorophores have evolved in microorganisms and pollen grains, many of which are
72 relevant to UVIF characterization of aerosols (Lakowicz, 2006; Poehlker et al., 2012). These
73 flourophores include metabolic mediators (e.g. NADH) that are widely conserved throughout the
74 microbial and plant worlds. Other fluorescently active biopolymers include a wide variety of structural
75 proteins and pigments; however these microbial compounds are tremendously variable in conformation
76 and intracellular quantity (Hill et al., 2014; Kepner and Pratt, 1994; Madigan, 2011). As such, the
77 distribution of particle fluorescence yields from some microbial bioaerosols has been reported to have
78 sensitivities to age (Kanaani et al., 2007) as well as cultivation history and environmental conditions
79 (Saari et al., 2014; Uk Lee et al., 2010).

80 Sensitivity analyses of bioaerosol spectra from different UVIF instrument configurations are emerging in
81 the literature (Agranovski et al., 2003; Huffman et al., 2010; Poehlker et al., 2012), as are generalized
82 performance indices designated as apparatus–specific detection efficiencies (Saari et al., 2014). These
83 UVIF metrics include, but are not limited to, photomultiplier thresholds, fluorescent particle fraction
84 (FPF) recoveries, and specific quantum yield comparisons. The calibration of modern UVIF instruments
85 has been predominantly executed with thermoplastic spheres that are impregnated with colloidal metals or
86 artificial fluorophores. In this context, conventional UVIF calibrations have relatively short stability
87 windows of specific intensity which are useful for bioaerosol standardization, because of their sensitivity
88 to temperature and light. And while these calibrants are well characterized from a chemical perspective,
89 they are poor biophysical analogues to the bioaerosols they are meant to reference.

90 There is increasing interest in the utility of UVIF for environmental and autecological studies (Despres et
91 al., 2012), yet there remains no unified approach for calibrating and cataloguing the optical signatures
92 associated with primary biological airborne particles (PBAPs). While many PBAPs fluoresce,
93 interpreting UVIF measurements for bioaerosol characterization presents challenges that can only be

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94 addressed using referenced distributions of fluorescence emissions complemented with conventional
95 optical properties. In response, we present a laboratory characterization of aerosolized pure cultures of
96 common airborne bacteria (14), fungi (29) and pollen grains (12) using a portable Wideband-Integrated
97 Bioaerosol Sensor ((WIBS), Droplet Measurement Technologies, Boulder, CO, USA). We report a
98 systematic compilation of optical and fluorescence properties that can be reproduced and expanded as a
99 bioaerosol reference basis for new generations of UVIF instrumentation.

100 **MATERIALS & METHODS**

101 A cohort of conventional and fluorescent bioaerosol spectra were obtained from consecutively
102 aerosolizing pure cultures of bacteria, fungi and pollens in an environmentally controlled chamber. The
103 chamber into which all bioaerosol cultures were introduced was cubic, and constructed of grounded 1.25
104 cm (thick) Lucite; chamber air was continuously mixed by two 1.5W fans (**Fig. 1**). Comprehensive
105 details of chamber construction and its operation for pure culture bioaerosol characterization have been
106 previously described (Peccia et al., 2000). A WIBS continuously drew chamber air at 1.2 L/min through
107 0.25m of conductive neoprene tubing for these tests. The chamber was vented to a hood at ambient
108 pressure (c.a. 88 kPa); RH was held between 25 and 40%; temperature was held between 20-22C; and,
109 bioaerosol was allowed to equilibrate to these conditions for 5 minutes prior to UVIF sampling.

110

111 **Bacteria.** Fifteen pure bacterial cultures were grown into early stationary phase using standard protocols
112 described by the American Type and German Culture Collections (ATCC and DSMZ). These cultures,
113 listed with their accompanying fluorescence distributions in **Fig. 2**, have been used as models for the
114 environmental behavior of airborne bacteria with public health relevance (CDC, 2013). Upon entering
115 stationary phase, they were washed by sequential centrifugations in cold, ultrapure deionized water, and
116 immediately aerosolized into a chamber using a six-jet Collison nebulizer. Direct microscopy showed
117 these bacteria remained intact after inspecting nebulizer reflux immediately following their aerosolization.

118 **Fungi**. Twenty-nine pure cultures of commonly occurring indoor fungi (Vesper et al., 2007) were
119 cultured until they presented an obvious spore-bearing physiology after being inoculated on MEA agar
120 and held at 20C and 25% RH. As judged by direct microscopy, copious fungal spores were presented
121 between 18 and 28 days, depending on the species. These fungal cultures, also presented in **Fig. 2,** were
122 dry aerosolized directly off their host agar into the chamber using dry ultrapure nitrogen guided through
123 sterile glass containments specifically designed for this purpose (modified fungal spore source strength

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124 tester (FSSST)(Sivasubramani et al., 2004)). Direct microscopy showed these fungal spores dominated
125 the airborne biomass with less than 1% of the airborne volume as associated hyphae.

126 **Pollen.** Thirteen pure stocks of common temperate tree and grass pollens (O'Connor et al., 2013) were
127 obtained from a collection at the Denver Botanic Garden. These pollen stocks, also listed in **Fig. 2,** were
128 aerosolized into the chamber with the same FSSST configuration used for fungi, with notable
129 fractionation of some grains as judged by microscopy.

130 **Optical spectra acquisition.** The WIBS (v 4.0), previously described by Healy and coworkers (2012)
131 uses dual-wavelength excitation and fluorescence detection, while simultaneously measuring
132 characteristic dimensions from scattered light. With the portable WIBS variant used here, fluorescence
133 was induced by sequential exposure to UV irradiation from a flashlamp filtered at 280 nm and 370 nm.
134 Fluorescence emitted due to 280 nm excitation was detected in two wavebands, 310-400 nm, and 420-
135 650 nm, using dedicated photomultipliers. Fluorescence emitted due to 370 nm excitation was detected
136 between 420-650 nm. Optical diameter was determined by light scattered from exposure to a 635nm
137 laser; it is reported here as an equivalent optical diameter (EOD), defined as the diameter of a spherical
138 particle, with a fixed refractive index (relative to that calibrated with 2.0 and 2.8 µm latex beads in air
139 between 25-45% RH), scattering the same light intensity as the measured bioaerosol. These optical
140 properties were recovered from a minimum of between 10^3 and 10^4 bioaerosol particles for each pure
141 culture aerosolized over at least a 5 min period. Between each aerosolization challenge, the chamber
142 was evacuated using a high volume HEPA filter such that total particle counts were below 10^2 m^{-3}, and
143 the chamber subsequently purged with ethanol vapor and/or ozone while illuminated with UV light.
144
145 Following the annotation introduced by Perring and coworkers (2015), particle fluorescence was
146 categorized as one of seven types, which considers each of three fluorescence bandwidths individually,
147 as well as in all possible combinations. Here the subscripts denote excitation wavelengths, and
148 parentheticals indicate the emission bandwidths observed:
149
150
151 TYPE $\mathbf{A_{280}}$ =(310-420); TYPE $\mathbf{B_{280}}$=(420-650); TYPE $\mathbf{C_{370}}$ = (420-650)

152

153 Leveraging these measured quantities, the following metrics were analyzed and compiled for each
154 bioaerosol: (i) the frequency of particles that could be segregated by fluorescence signal (bandwidth) into

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155 any of the seven types; (ii) the average fluorescence intensity within each bandwidth (if any); and (iii) the
156 average EOD of each particle type.

157 To assess the potential effect of aging, fluorescence emissions from airborne spores of *Phoma spp.*,
158 *Penicillum chrysogenum*, *Cladosporium cladosporides*, and *Aspergillus versicolor* that were less than 28
159 days old, were compared to the spectra of spores from these same cultures, which were allowed to age
160 180 days (raised and maintained under identical environmental conditions).

161 **RESULTS & DISCUSSION**

162 **Composite optical recognition patterns of bioaerosol classes:** The seven fluorescence categories,
163 the fluorescence intensities and the relative size expressed by the EOD provide a
164 multidimensional matrix from which composite signatures can be constructed for the three
165 bioaerosol classes tested here. Figure 2 shows the EOD and the distribution of fluorescence type for
166 each of the cultures studied while Figure 3 shows the normalized fluorescence intensity they emitted. The
167 pure culture bioaerosols used to challenge this WIBS distinctly clustered into respective physiologic
168 groups. All of the bacterial cultures aerosolized, clustered below an EOD of 1.5 µm while presenting the
169 weakest fluorescence intensities of the bioaerosols observed. With the exception of spore forming
170 *Bacillus subtilis*, bacterial bioaerosol was dominated by a single fluorescence type (A). The fungal spores
171 aerosolized were in a markedly higher range with respect to their equivalent optical diameters (2-9 µm),
172 but presented several fluorescence types: A, AB, BC and ABC, with a prevalence of A and AB. While
173 the pollens aerosolized were overlapping in equivalent optical diameter ranges with some of the fungal
174 spores observed, they presented significantly different fluorescence type distributions, which were
175 dominated by combinations of fluorescent types BC and ABC. We note that many of the pollen EODs
176 presented here are considerably smaller than the true diameter of their nascent intact-grain size, which we
177 attribute to pollen fragmentation during collection, storage or aerosolization. Observational studies
178 indicate that pollen grain fragmentation happens in the atmosphere as well (Miguel et al., 2006; Taylor et
179 al., 2007). The pollens also clustered based on their markedly higher fluorescence intensities relative the
180 other bioaerosol classes observed (**Fig. 3**).

181 **Fluorescence response to spore aging:** Fluorescence spectra from four pairs of young and aged fungal
182 spores were compared by these analyses (aged 28 vs. 180 days). Of these, two presented no discernable
183 change in the EOD and fluorescence signals used as cataloguing metrics here: *Cladosporium*
184 *cladosporiodes* and *Aspergillis niger*. There was however, a significant shift in the fluorescence

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185 distribution, from younger to older spores, of the species *Pennicillium chrysogenum* and *Phoma*
186 *herbarium* (**Fig. 2** (**bottom**)). More than 90% of the spores of these species presented their fluorescence
187 as Type A when aerosolized at an age of 28 days, but this decreased to near 70% after 180 days of culture
188 aging under identical conditions; the balance becoming type AB.

189 **Instrumental variability and gain considerations:** We present here the response of a single WIBS
190 instrument to pure culture bioaerosols grown, collected and aerosolized under carefully controlled
191 conditions. The instrument manufacturer (Droplet Measurement Technologies) typically sets detector
192 gains based on photomultiplier fluorescence detection from monodispursed challenges with commercially
193 available microspheres (polystyrene latex (PSL)), the intensity of which is known to vary from batch to
194 batch. The fluorescence of PSLs however, significantly degrades on the timescale of months (even with
195 proper storage). While this strategy provides general consistency across different instruments, the lack of
196 an absolute calibration means that there can be appreciable variability in fluorescence signal recovery of a
197 particular bioaerosol between one UVIF instrument and another. In response, we aerosolized a subset of
198 test bioaerosol cultures to challenge two WIBS in parallel: one owned by the manufacturer, which was
199 used for the larger set of measurements presented here, and another owned by the Chemical Sciences
200 Division of the National Oceanic and Atmospheric Sciences Administration (NOAA). We find
201 differences in spectral classification (Figure 4), likely attributable to differing gain settings, with the
202 NOAA WIBS recording a larger fraction of a given particle population as having signal above threshold
203 in the B and C channels (both of which are detected using the same photomultiplier), than does the
204 manufacture's market issue WIBS.

205 **Implications for real-time detection of atmospheric bioaerosol:** Given the potential variability
206 introduced with culture age and between instruments, genera- or species-type classifications for different
207 bioaerosols should be treated not as absolute "signatures" but, rather, as likely groupings that, when
208 considered in conjunction with EOD, allow for discrimination between broad bioaerosol categories. There
209 is also particle-to-particle variability in the type manifestation of a given sample (i.e. not all particles of a
210 given species present as the same spectral type) that makes reliable identification on a single particle basis
211 unlikely and requires, instead, a statistical treatment. For example, bacterial populations tend to present
212 as mostly type A at small (<1 μm) EOD. Fungi present a mixture of type A, AB and ABC fluorescence at
213 larger EOD sizes (2 - 9μm), although the exact distribution between the dominant types may differ
214 substantially from that presented here depending on instrumental parameters, culture conditions and
215 environmental (aerosol) conditions. Finally, pollen tends to present as a mixture of C, BC and ABC and is
216 the only class of bioaerosol for which types C or BC are significant contributors. This facilitates

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217 discrimination between fungi and pollen despite the fact that the likelihood of pollen fragmentation
218 reduces the utility of EOD as an identifier. Another distinguishing feature of pollens is their relatively
219 high fluorescence intensity although we note that very little is known about the temporal evolution of
220 fluorescent intensity over atmospherically relevant timescales of photochemical processing.

221 Type A was dominant in all bacterial and most fungal populations aerosolized in this study (at least for
222 certain gain settings, like those in the DMT WIBS) and thus EOD is an important consideration in
223 distinguishing bacteria from fungi. In this work, under laboratory conditions, we typically sampled single,
224 intact, bacterial cells. Atmospheric observations of bacteria and DNA in aerosol, however, indicate that
225 bacteria are frequently found associated with particle sizes larger than that of a single cell. (Burrows et al.,
226 2009; Shaffer and Lighthart, 1997; Tong and Lighthart, 2000; Wang et al., 2008). It is hypothesized that
227 this is due to the presence of either multi-cell bacterial agglomerates or particle mixtures including
228 bacteria and dust or leaf fragments (Bovallius et al., 1978; Lighthart, 1997) and we acknowledge that such
229 agglomerates could be easily confused with intact fungal spores given their spectral similarities as
230 detected by the WIBS. Another notable observation of the ensemble of experiments presented here, is the
231 lack of an appreciable fraction of type B particles associated with these pure culture bioaerosol
232 challenges. Type B particles are detected as a minor fraction (up to 15%) in only a few fungal and pollen
233 species and, therefore, any atmospheric sampling in which type B is a dominant fluorescent type should
234 be examined carefully for potential (non-biological) interferents. Type AC was also strikingly absent from
235 the measurements presented here.

236

237 **CONCLUSIONS**

238 Recent advances in UVIF instrumentation have led to a new generation of atmospheric particle sensors
239 with promising utility for rapid characterization of fluorescent bioaerosols, both indoors and out. Here we
240 have demonstrated the ability of this technique to characterize whole, airborne microbial cells in a
241 sensitive cytometric capacity.

242 Where conventional light scattering analyses is coupled with the spectral analyses of fluorescence
243 emission(s), a novel and powerful combination of phenotypic information becomes available on a high-
244 throughput, single-particle basis—the broader the excitation and emission spectra observed, the higher the
245 potential for the fundamental characterization of (bio)aerosol *in-situ*. As has been previously described,
246 the WIBS technology employed here demonstrates the potential of multi-channel fluorescence analyses in
247 a laboratory setting; however, this work describes a novel approach for compiling a primary optical

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248 biological particle catalogue through a simple, unified reference method created under defined conditions.
249 Such a library can be reproduced, expanded and shared by users for inter-laboratory comparisons and/or
250 associations to field observations.

251 In the course of this analysis, the composite evaluation of optical diameter and fluorescent spectra
252 associated with airborne fungal spores, pollens, bacteria revealed that these primary physiologies can be
253 unambiguously differentiated from each other when airborne under defined conditions (culture condition,
254 RH and Temperature). While deeper cluster analyses were beyond the scope of this demonstrative report,
255 within the fungi and pollens there emerged optically defined phenotypes, which also formed distinct sub-
256 groupings—an exciting cataloguing outcome with implications for future algorithmic developments and
257 the potential for confident identification of "interferences" which may complicate bioaerosol quantitation
258 in the field (Despres et al., 2012; O'Connor et al., 2013; Poehlker et al., 2012).

259 The identity of fluorescent aerosol, and the fraction thereof composed of primary biological materials
260 remains unknown in many different environments (Despres et al., 2012; Heald and Spracklen, 2009). To
261 this end, these findings suggest that a composite analyses of wide-band UVIF, as referenced to robust
262 bioaerosol libraries, offers a promising approach to better characterize airborne particulate matter in the
263 laboratory as well as during wide area environmental surveillance.

264

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265 **APPENDICES**

266 A table containing discrete EOD and intrinsic fluorescence values observed for each culture
267 aerosolized (used to construct Fig. 2 and 3)

268

269 **TABLE. 1 Optical Statistical Summary of Airborne Bacteria, Fungi and Pollens Observed**

270

Figure 1 Position (#), *Genus species*	Samples	EOD	Intensity	Fluorescence Type Frequency						
				A	B	C	AB	AC	BC	ABC
Bacteria										
(1) *Bacillius subtilis*	19786	0.7	656	11	2		87	0	0	0
(2) *Vibrio fisherii*	15073	0.7	581	97	0	0	1	2	0	0
(3) *Bordetella pertussis*	12834	0.5	84	93	0	1	1	3	0	1
(4) *Psuedomonas aurigenosa*	28190	0.4	248	94	0	0	1	3	0	2
(5) *Acinetobacter baumanii*	30006	1.1	556	96	0	0	0	3	0	1
(6) *Micrococcus luteus*	28197	0.9	502	97	0	0	1	2	0	0
(7) *Mycobacterium parafortuitum*	18197	0.4	265	90	0	0	8	0	0	2
(8) *Staphylococcus aureus*	30015	0.7	283	96	0	0	2	1	0	1
(9) *Burkholderia cepacia*	30003	0.5	399	97	0	0	2	1	0	0
(10) *Psuedomona GFP aurigenosa*	16015	0.6	112	97	0	0	1	1	0	1
(11) *Serratia marcesens*	30027	0.6	393	96	0	0	3	1	0	0
(12) *Enterobacter faceialis*	17401	0.8	327	96	0	0	1	1	0	2
Fungi										
(28) *Aspergillus niger (28 d)*	30772	4.4	157	79	2	7	4	2	2	5
(29) *Aspergillus niger (180 d)*	23086	4.4	181	78	2	5	5	2	2	5
(30) *Aspergillis versicolor*	32620	4	304	67	2	0	9	0	8	14
(31) *Aspergillis flavus.*	15847	4	364	87	0	0	3	1	2	4
(32) *Aspergillus pinenceus*	33085	3.6	206	90	0	0	5	0	0	3
(33) *Aspergillus syndowii*	5098	3.5	515	90	0	0	5	0	0	3
(34) *Aspergillus tubinigenisis*	28801	2.5	172	41	7	7	25	0	5	12
(35) *Botretious spp.*	7024	8	294	21	6	0	55	0	0	16
(36) *Cheatomium*	19264	4.9	258	77	0	0	16	0	1	3
(37) *Cladosporium spp*	39294	3.3	710	80	0	0	16	0	0	2
(38) *Cladosporium cladosporiodes (28 d)*	26474	3	570	72	0	0	24	0	0	3
(39) *Cladosporium cladosporiodes (180 d)*	34085	3.3	702	70	0	0	23	0	0	4
(40) *Cladosporium herbarium*	2962	3.2	651	80	0	0	12	0	0	6
(41) *Fusarium spp*	1188	3.3	638	81	0	0	12	0	0	5
(42) *Paecilmyces variotii*	11188	4.2	772	0	8	0	74	0	3	13
(43) *Pennicillium chrysogenum (28 d)*	14577	3.5	867	92	0	0	4	0	0	2
(44) *Pennicillium chrysogenum (180 d)*	33452	3.2	733	73	0	0	23	0	0	2
(45) *Pennicillium canescens*	7840	3.1	659	90	0	0	5	0	0	4
(46) *Pennicillium citreonigrum*	8470	2.8	596	68	0	0	27	0	0	4
(47) *Pennicillium commune*	9255	3.3	762	79	0	0	3	2	0	14
(48) *Pennicillium corylophium*	12654	2.6	687	90	0	0	6	0	0	3
(49) *Pennicillium cecumbens*	13676	2.4	420	89	0	0	8	0	0	2

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(50) *Pennicillium thomii*	5739	3.1	721	91	0	0	4	0	0	3
(51) *Phoma herbarium (28 days)*	5518	2.9	607	91	0	0	5	0	0	2
(52) *Phoma herbarium (180 days)*	2648	3.5	688	79	0	0	14	0	0	5
(53) *Stachybotrius spp.*	12697	2.8	137	65	7	2	14	1	1	6
(54) *Syncephalstrum racemosom*	6753	2.8	618	75	0	0	12	0	0	10
(55) *Triterachium spp.`*	5595	2.2	227	95	0	0	3	0	0	0
(56) *Ulocladium*	4513	3.8	767	80	0	0	15	0	0	4
Pollens										
(15) *Acer saccharum*	800	3.3	753	6	1	3	1	1	17	68
(16) *Alnus Rubra and Rugosa spp.mix*	389	4.7	387	6	4	11	1	0	28	46
(17) *Ambrosia trifida*	1219	3.2	876	11	2	2	6	1	12	62
(18) *Artemesia tridentata*	1703	2.5	666	6	2	2	3	0	19	66
(19) *Betula Lenta, Nigra & Populifolia*	2208	2.5	990	4	1	3	2	0	54	33
(20) *Carya lacinosa*	1942	1.3	1043	5	0	5	2	0	53	33
(21) *Eucalyptus*	1095	6.1	481	2	1	13	0	0	47	34
(22) *Fern (unknown source)*	1571	6.8	373	1	13	17	1	0	38	28
(23) *Fragus americana*	1991	1.8	1155	6	2	6	3	0	60	20
(24) *Juglands nigra*	1968	1.2	1197	3	1	4	1	0	62	26
(25) *Juniper ashei*	2536	3	766	0	1	1	0	0	16	79
(26) *Morus rubra*	4232	2.8	1229	0	0	0	0	0	1	96
(27) *Phelum pratense*	3390	1.5	936	2	0	1	0	0	11	82

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272

273

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282 **6. REFERENCES**

283 Agranovski, V., Ristovski, Z., Hargreaves, M., J. Blackall, P., and Morawska, L.: Performance
284 evaluation of the UVAPS: influence of physiological age of airborne bacteria and bacterial
285 stress, Journal of Aerosol Science, 34, 1711-1727, 2003.
286
287 Bovallius, A., Bucht, B., Roffey, R., and Anas, P.: Long-Range Air Transmission of Bacteria,
288 Applied and Environmental Microbiology, 35, 1231-1232, 1978.
289
290 Burrows, S. M., Elbert, W., Lawrence, M. G., and Poschl, U.: Bacteria in the global atmosphere -
291 Part 1: Review and synthesis of literature data for different ecosystems, Atmospheric Chemistry
292 and Physics, 9, 9263-9280, 2009.
293
294 CDC: Antibiotic Resistance Threats in the United States. Department of Health and Human
295 Service (Ed.), Atlanta, GA, 2013.
296
297 Despres, V. R., Huffman, J. A., Burrows, S. M., Hoose, C., Safatov, A. S., Buryak, G.,
298 Froehlich-Nowoisky, J., Elbert, W., Andreae, M. O., Poeschl, U., and Jaenicke, R.: Primary
299 biological aerosol particles in the atmosphere: a review, Tellus Series B-Chemical and Physical
300 Meteorology, 64, 2012.
301
302 Gabey, A. M., Gallagher, M. W., Whitehead, J., Dorsey, J. R., Kaye, P. H., and Stanley, W. R.:
303 Measurements and comparison of primary biological aerosol above and below a tropical forest
304 canopy using a dual channel fluorescence spectrometer, Atmospheric Chemistry and Physics, 10,
305 4453-4466, 2010.
306
307 Gabey, A. M., Vaitilingom, M., Freney, E., Boulon, J., Sellegri, K., Gallagher, M. W., Crawford,
308 I. P., Robinson, N. H., Stanley, W. R., and Kaye, P. H.: Observations of fluorescent and
309 biological aerosol at a high-altitude site in central France, Atmospheric Chemistry and Physics,
310 13, 7415-7428, 2013.
311
312 Heald, C. L. and Spracklen, D. V.: Atmospheric budget of primary biological aerosol particles
313 from fungal spores, Geophysical Research Letters, 36, L09806, 2009.
314
315 Healy, D. A., O'Connor, D. J., Burke, A. M., and Sodeau, J. R.: A laboratory assessment of the
316 Waveband Integrated Bioaerosol Sensor (WIBS-4) using individual samples of pollen and fungal
317 spore material, Atmospheric Environment, 60, 534-543, 2012.
318
319 Hill, T. C. J., Moffett, B. F., DeMott, P. J., Georgakopoulos, D. G., Stump, W. L., and Franc, G.
320 D.: Measurement of Ice Nucleation-Active Bacteria on Plants and in Precipitation by
321 Quantitative PCR, Applied and Environmental Microbiology, 80, 1256-1267, 2014.
322
323 Huffman, J. A., Treutlein, B., and Poeschl, U.: Fluorescent biological aerosol particle
324 concentrations and size distributions measured with an Ultraviolet Aerodynamic Particle Sizer
325 (UV-APS) in Central Europe, Atmospheric Chemistry and Physics, 10, 3215-3233, 2010.
326

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327 Kanaani, H., Hargreaves, M., Ristovski, Z., and Morawska, L.: Performance assessment of
328 UVAPS: Influence of fungal spore age and air exposure, Journal of Aerosol Science, 38, 83-96,
329 2007.
330
331 Kaye, P. H., Stanley, W. R., Hirst, E., Foot, E. V., Baxter, K. L., and Barrington, S. J.: Single
332 particle multichannel bio-aerosol fluorescence sensor, Optics Express, 13, 3583-3593, 2005.
333
334 Kepner, R. L. and Pratt, J. R.: Use of fluorochromes for direct enumeration of total bacteria in
335 environmental samples: past and present, Microbiological Reviews, 58, 603-615, 1994.
336
337 Lakowicz, E. R.: Principles of Fluorescence Spectroscopy. Springer Science and Buisness
338 Media, Berlin, 2006.
339
340 Lighthart, B.: The ecology of bacteria in the alfresco atmosphere, Fems Microbiology Ecology,
341 23, 263-274, 1997.
342
343 Madigan, M. T., Clark D. P., Martinko, J.M.: Brock Biology of Microorganisms. New York,
344 New York, USA, 2011.
345
346 Miguel, A. G., Taylor, P. E., House, J., Glovsky, M. M., and Flagan, R. C.: Meteorological
347 influences on respirable fragment release from Chinese elm pollen, Aerosol Science and
348 Technology, 40, 690-696, 2006.
349
350 O'Connor, D. J., Healy, D. A., Hellebust, S., Buters, J. T. M., and Sodeau, J. R.: Using the
351 WIBS-4 (Waveband Integrated Bioaerosol Sensor) Technique for the On-Line Detection of
352 Pollen Grains, Aerosol Science and Technology, 48, 341-349, 2013.
353
354 Peccia, J., H., W., Miller, S., and Hernandez, M.: Effects of relative humidity on the ultraviolet
355 induced inactivation of airborne bacteria, Journal of Aersol Science and Technology, Submitted
356 for review, Feb. 2000, 2000.
357
358 Perring, A. E., Schwarz, J. P., Baumgardner, D., Hernandez, M. T., Spracklen, D. V., Heald, C.
359 L., Gao, R. S., Kok, G., McMeeking, G. R., McQuaid, J. B., and Fahey, D. W.: Airborne
360 observations of regional variation in fluorescent aerosol across the United States, Journal of
361 Geophysical Research: Atmospheres, 120, 1153-1170, 2015.
362
363 Poehlker, C., Huffman, J. A., and Poeschl, U.: Autofluorescence of atmospheric bioaerosols -
364 fluorescent biomolecules and potential interferences, Atmospheric Measurement Techniques, 5,
365 37-71, 2012.
366
367 Poschl, U., Martin, S. T., Sinha, B., Chen, Q., Gunthe, S. S., Huffman, J. A., Borrmann, S.,
368 Farmer, D. K., Garland, R. M., Helas, G., Jimenez, J. L., King, S. M., Manzi, A., Mikhailov, E.,
369 Pauliquevis, T., Petters, M. D., Prenni, A. J., Roldin, P., Rose, D., Schneider, J., Su, H., Zorn, S.
370 R., Artaxo, P., and Andreae, M. O.: Rainforest Aerosols as Biogenic Nuclei of Clouds and
371 Precipitation in the Amazon, Science, 329, 1513-1516, 2010.

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372
373 Saari, S., Mensah-Attipoe, J., Reponen, T., Veijalainen, A. M., Salmela, A., Pasanen, P., and
374 Keskinen, J.: Effects of fungal species, cultivation time, growth substrate, and air exposure
375 velocity on the fluorescence properties of airborne fungal spores, Indoor Air, doi:
376 10.1111/ina.12166, 2014. 2014.
377
378 Shaffer, B. T. and Lighthart, B.: Survey of culturable airborne bacteria at four diverse locations
379 in Oregon: Urban, rural, forest, and coastal, Microbial Ecology, 34, 167-177, 1997.
380
381 Sivaprakasam, V., Lin, H.-B., Huston, A. L., and Eversole, J. D.: Spectral characterization of
382 biological aerosol particles using two-wavelength excited laser-induced fluorescence and elastic
383 scattering measurements, Optics Express, 19, 6191-6208, 2011.
384
385 Sivasubramani, S., K., Niemeier, R., T., Reponen, T., and Grinshpun, S., A.: Fungal spore source
386 strength tester: laboratory evaluation of a new concept, Science of the Total Environment, 329,
387 75-86, 2004.
388
389 Taylor, P. E., Jacobson, K. W., House, J. M., and Glovsky, M. M.: Links between pollen, atopy
390 and the asthma epidemic, International Archives of Allergy and Immunology, 144, 162-170,
391 2007.
392
393 Tong, Y. Y. and Lighthart, B.: The annual bacterial particle concentration and size distribution in
394 the ambient atmosphere in a rural area of the Willamette valley, Oregon, Aerosol Science and
395 Technology, 32, 393-403, 2000.
396
397 Toprak, E. and Schnaiter, M.: Fluorescent biological aerosol particles measured with the
398 Waveband Integrated Bioaerosol Sensor WIBS-4: laboratory tests combined with a one year
399 field study, Atmospheric Chemistry and Physics, 13, 225-243, 2013.
400
401 Uk Lee, B., Jung, J. H., Yun, S. H., Hwang, G. B., and Bae, G. N.: Application of UVAPS to
402 real-time detection of inactivation of fungal bioaerosols due to thermal energy, Journal of
403 Aerosol Science, 41, 694-701, 2010.
404
405 Vesper, S., McKinstry, C., Haugland, R., Wymer, L., Bradham, K., Ashley, P., Cox, D., Dewalt,
406 G., and Friedman, W.: Development of an Environmental Relative Moldiness index for US
407 homes, J Occup Environ Med, 49, 829-833, 2007.
408
409 Wang, C.-C., Fang, G.-C., and Lee, L.-Y.: The study of ambient air bioaerosols during summer
410 daytime and nighttime periods in Taichung, Central Taiwan, Environmental Forensics, 9, 6-14,
411 2008.

412

413

414

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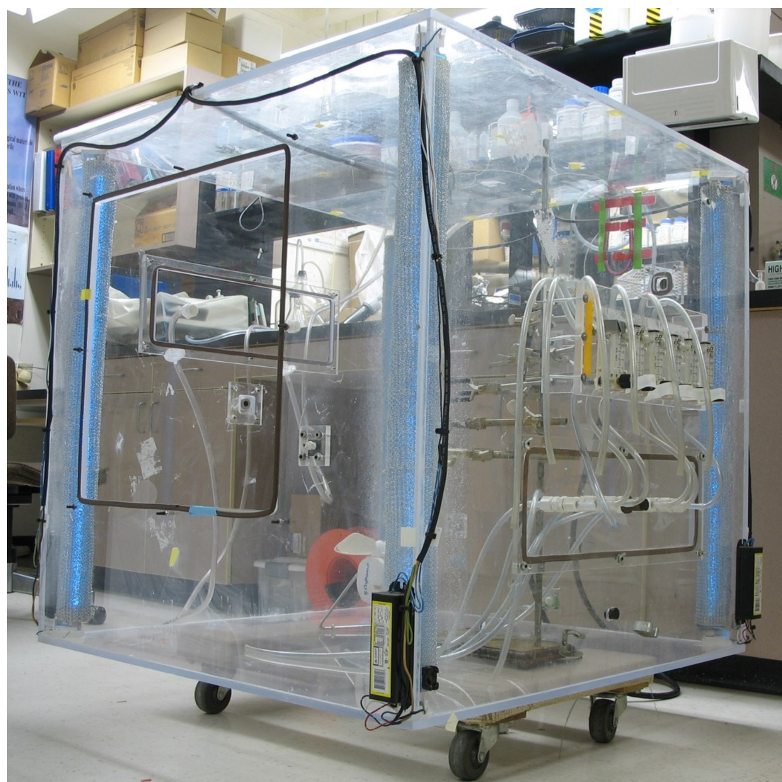
415 **TABLES AND FIGURES**



416

417 **Figure 1.** Photograph of bioaerosol test chamber. In independent trials, dry ultrapure nitrogen aerosolized and
418 diluted different pure cultures of bacteria, fungi and pollens into a temperature (20C) and RH (25-40%) controlled
419 chamber (890L). Bioaerosol delivery vessels were fitted with steel ports, and all gas streams were contained in 0.4
420 cm conductive neoprene tubing, carrying 151kPa at flow rate of 12 l/min into the chamber.

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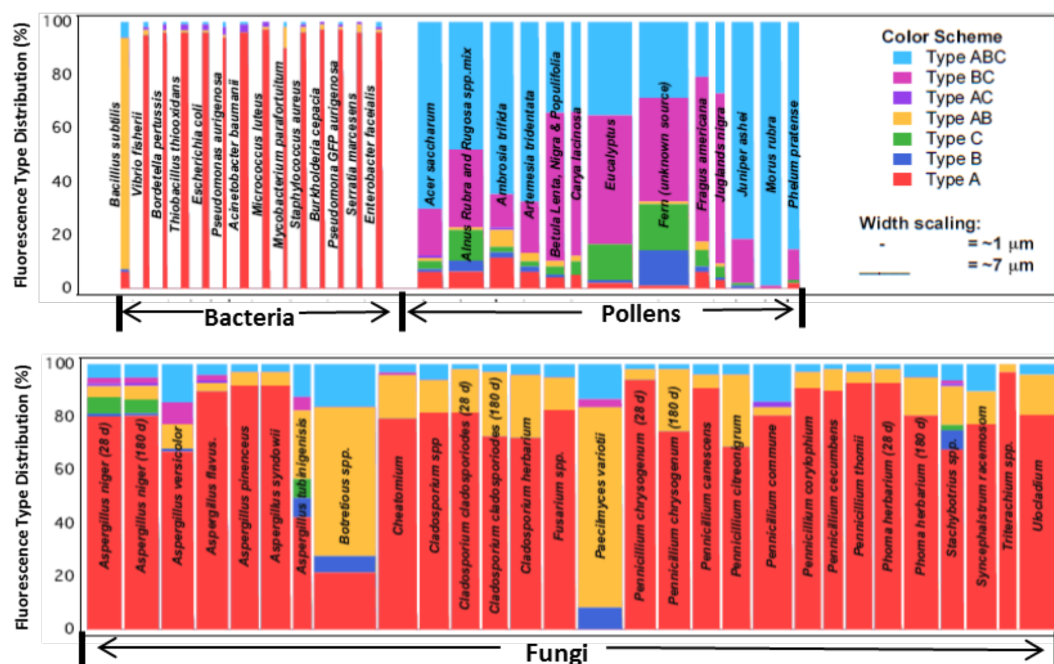
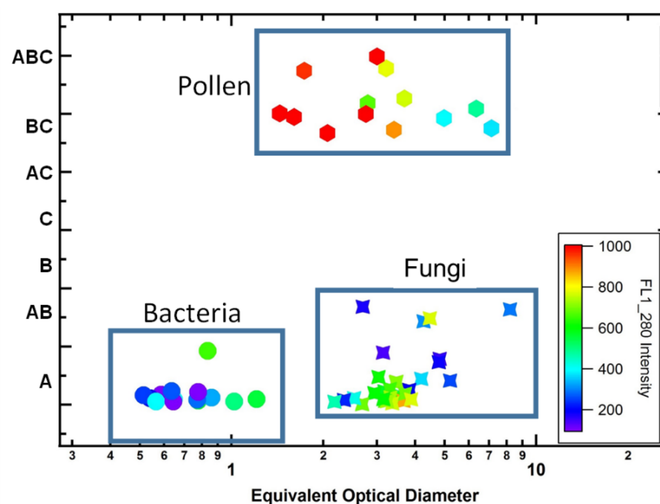


421
422 **Figure 2.** Juxtaposition of optical and fluorescence properties of aerosolized pure cultures of bacteria and pollens
423 (**top**), and fungal spores (**bottom**). Fluorescence type distribution is defined by excitation and emission from any of
424 three possible channels alone (A, B or C), or in any combination. Bar width is proportional to equivalent optical
425 diameter.



426

427 **Figure 3.** Juxtaposition of optical and fluorescence properties of aerosolized pure cultures of bacteria (●), fungi
428 spores (**X**), and pollen grains (♦). Fluorescence type is the mode associated with each culture aerosolized.
429 Fluorescence intensity is a relative scale.

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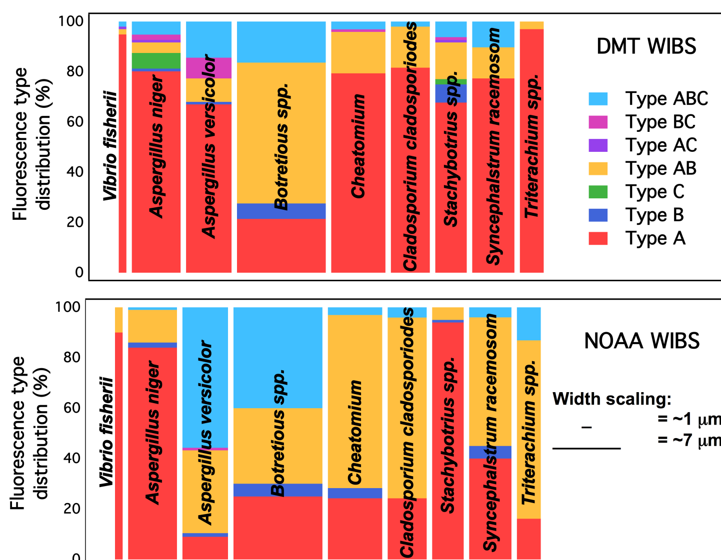

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433 Figure 4. Comparison of type classifications for two different WIBS instruments over a subset of the pure
434 bioaerosol cultures used in this analysis. The top panel shows the type classification breakdown in the DMT WIBS
435 which was used for all of the experimental trials presented here. The bottom panel shows the type classification
436 breakdown in the NOAA WIBS, used in parallel for a subset of the species used in the larger work.

437