

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

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Manuscript amt-2015-372 submitted by Hernandez et al. presents an overview of laboratory measurements performed using a recently commercialized optical particle counter applied to the detection and characterization of biological aerosol particles. The authors aerosolized representatives from three key classes of bioparticles (i.e. bacteria, fungal spores, and pollen) and present a summary of optical size and fluorescent properties observed. The authors also present a brief comparison of data from two WIBS instruments and a few fungal spores aged for different time periods in order to introduce complexities of applying the WIBS instrument (and other UVIF instruments) to be considered when analyzing field measurements. The manuscript presents a nice introduction of the response of this instrument to these bioparticle types and will be very useful to the UVIF/LIF research community. As someone who has been engaged

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in similar lab characterization projects for several years, I hope that the publication of this manuscript can motivate further research in this area. I am confident that AMT is the correct choice for this manuscript and I anticipate that it will be well cited in the near future. In the present form, I suggest some manuscript improvements before publication to improve readability and communication of project scope. After these suggestions have been addressed I happily endorse its publication. (Review comments by Alex Huffman)

General suggestions for improvement: 1. My main overall comment is that I think the scope of the manuscript is somewhat less than may be implied to the reader by the title and portions of the text. While the work is undoubtedly worthwhile, I think some of the wording suggests a broader characterization than was presented here. For example, the title implies to me a relatively comprehensive study that is ready for use as a “library” for the user community. The work is a great step in that direction, but the authors even admit in the text that there are significant challenges to using the WIBS in a standard way for fine-level discrimination. I would suggest changing the title somewhat and also editing a few sections of text to make sure the reader understands the scope of the measurements and conclusions.

a. For example, I would suggest changing the title to something like: “Lab Characterization of Optical Size and Fluorescence Properties of Key Bioaerosol Classes by Wideband Bioaerosol Sensor (or WIBS)”. This would clearly communicate the point that the manuscript deals with WIBS data and removes some possible misinterpretations by the reader. Specifically, the clause “composite catalogue” seems too much for the title, “signatures” may be a bit of a stretch for this instrument (as discussed in L207 of the manuscript), and “optical signatures” in contrast to the “fluorescent signatures” to me implies more addition and deeper information than just acquiring and presenting the equivalent optical diameter. b. I would also suggest changing the wording in L97-99, specifically the wording of “systematic compilation of ...”. I think something along the lines of “fluorescent properties and optical size from selective bioparticle

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types/species” would be a little clearer. c. The end of the last sentence of the introduction (L98) highlights that the properties “can be reproduced and expanded.” Is this sentence meant to imply that these data could be used as a foundation on which other researchers could use to synergize into a “bioaerosol reference basis for a new generation of UVIF instrumentation?” Or do you mean the results are reproducible from instrument-to-instrument? I think clarifying here a bit would help. d. More importantly related to the last comment is that I think that the nice results of the paper actually show that the WIBS instrument, at this stage, is not yet reproducible instrument-to-instrument enough to create a general database useful as a library for unrelated users. For example, Figure 4 suggests strongly to me that there are significant differences between the two instruments utilized for this part of the study. Looking at the category break-down for some of the species you can see that there can be significant differences in the way instrument interpret the same particles. So without some ability to standardize between instruments, the reproducibility may be difficult. e. L91: “cataloguing the optical signatures ...”. Fits in the same set of comments to be somewhat revised. f. Introduction and aspects of the conclusions are somewhat disconnected. For example, the introduction states that one motivation of the work is to acquire and provide a bioaerosol reference basis (L99) and the conclusions state again that the “library” (L249) could be reproduced and expanded. This is certainly a worthwhile goal, but Figure 4 suggests to me that the goal is not yet realized. I would suggest changing the statement in L247, for example, that “this work describes a novel approach for compiling ...” to something like “an initial approach to compiling ...”. I think this paper is a great first step in this direction, but may motivate continuing work by the authors and others to understand how differences in instruments may improve the reproducibility and ability to draw conclusions from WIBS and other UV-LIF instruments. g. Importantly, however, most of these suggestions amount to a slight re-wording of text and title that shouldn’t detract from the importance of the work, but that may help reframe the expectation of readers only able to invest a casual glance. For the community of researchers that may not know much about the WIBS or who have one, but are not as deeply involved with

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characterization work, I think this manuscript is a good opportunity to point out some of the important things that should be taken into consideration (i.e. variability between instruments) when interpreting WIBS data.

2. The text is a bit overly concise to convey specific meaning in places. For example: a. L116: The discussion of “direct microscopy showed ...” is nice, but I would have liked a bit more detail here. Were images of aerosolized bacteria analyzed after/during every experiment, or only once? What kind of microscopy, and at what magnification and resolution? In addition to adding more experimental information here I would suggest adding a few images to the supplement. Consider adding some estimated statistical information (e.g. roughly 1% of bacteria showed ...; similar to statement made about fungi in L125). b. Also with this concept of aerosolized bacteria, I’m a bit surprised that the process was as simple to collect systematically intact bacterial cells via Collision nebulization and impaction. From (limited) personal experience I have found this to be challenging, and there seems to be reasonably good evidence in the literature that Collision nebulization is violent and can impart significant damage to bacterial cells. Without presenting a summary here, a quick Google scholar search with “Collision nebulizer bacteria damage viability” gives some good examples. I think it would be good to put in one or two references about this topic here and the possible implications it might have on the bacterial part of the study here. c. L125: How was the 1% estimated here and how often? d. L128-129: Similar to the comment above about bacterial aerosolization, what does “notable fractionation of some grains” mean? How was it quantified, and how often was the microscopy performed? I also suggest adding a couple images to the supplement here. e. I would suggest adding some text about the relationship between fluorescence and particle size in the first section of the Results and Discussion. There is a clear increase (~cubic relationship) between size and observed fluorescence intensity, and so it is not surprising that the pollen show markedly increased fluorescence. The text need not include exhaustive discussion of this topic, but brief mention of the idea (1-2 sentences) would help round out the discussion. There are several references by Pan/Pinnick/Hill et al. that discuss this, as

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well as several others. f. L188 suggests that “B Channel” fluorescence increased with spore aging, but doesn’t give any suggestion why. Can the authors suggest a possible reason for this?

3. Was the asymmetry factor (AF) measured as a part of this study? I understand that it is an unreliable tool at present, but I think it would be worthwhile to actively state something in the text on this topic, even if it is to say that the AF is not reproducible and will need to be investigated further, etc. Many WIBS users will look to this manuscript for guidance when interpreting bioaerosol data, and a brief statement here would be very useful.

4. The nomenclature suggested in L151 feels subtly different than what Perring et al. 2014 and others have recently used for WIBS data. In the Perring et al. paper (i.e. footnote under Table 1) there was a clear mention of the difference between “Channel A”, meaning any particle fluorescing in the FL1 channel, and “Type A” meaning a particle that fluoresces in Channel 1 / FL1 but NOT in either of the other two channels. This is maybe a subtle difference, but probably a useful one for the WIBS community to become/stay consistent with. I personally like the term e.g. FL1 to refer to the channel and the e.g. Type A to refer to the active statement of the combination of channels exhibiting fluorescence for a given particle. However, I’m also happy with the terminology utilized by Perring et. al, 2014, which I think is a bit clearer than using “Type A” to mean any particle fluorescing in A (even if also in B and C) as implied in L151 here.

5. I suggest expanding the caption from Table 1 to include details about what the numbers mean. For example, it appears that the fluorescence type frequency is normalized to a sum of 100, but this is not explicitly mentioned. There are no units on EOD or Intensity, and ‘samples’ is not clearly defined as ‘observed particles’ here. I would also suggest adding a standard deviation for the EOD and intensity measurements here. The text makes mention of a range of observed properties within a particle type, and this range should be reported in some form here.

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6. L136-138: This was a confusing sentence to me. What does the 2.0 and 2.8 um mean? This suggests to me that a two-point size calibration was done, but I doubt I’m interpreting this correctly. Can you say briefly, but specifically more detail about the size calibrations performed (by PSLs over X-Y range, etc.)

7. L182-186: This statement is a bit confusing to me. Looking at Figure 2, there are some differences in the young/old spores (e.g. ratio of A and AB) as mentioned. The cladosporium difference (considered indistinguishable) doesn’t look much difference than the phoma herbarium difference (considered a significant shift). I would consider defining or tightening up how these statements were made. Along these lines, I would suggest considering to pull out the aging effect of the spores into a separate plot. It is really hard to see this difference in the current Figure 2, and it would show the point clearly by putting only the young/old together.

8. One conclusion (L252-253) states that “. . .primary physiologies can be unambiguously differentiated from each other . . .”. This is true, with respect to Figure 3, but the only clearly discernable difference between most of the fungal spores and bacteria is the size. The fluorescence properties (i.e. breakdown of categories, FL1 intensity) are generally within range of one another. So assuming the bacteria are aerosolized individually in the lab, this may be possible, but I think an important conclusion that is nicely shown here is that extrapolating this technique to the atmosphere may make differentiation between bacteria and spores exceedingly difficult. Would it make the differentiation easier to see in Figure 2 to use FL3 as the color scale rather than FL1?

Minor / specific comments: • I suggest adding section numbering to help organize and guide the reader. • I’m not familiar with the term UVIF, but have seen UV-LIF for laser and light-induced fluorescence. Does UVIF have a standard history in some area of scientific literature? If so I suggest adding a reference in the second paragraph to this history, simply because it may be that the atmospheric measurement audience may be more familiar with the other. If not, I would suggest using the UV-LIF terminology. • Was the WIBS inside the chamber? I had understood that previously,

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but the text implied otherwise.   L70: Indeed, fluorescence-based instrumentation has been utilized frequently in recent years as a part of many measurement studies. I would suggest adding one by Pan et al. (e.g. JGR, 2007) and also one by Huffman et al. (e.g. ACP, 2013 or ACP, 2012) to round out some of the groups that have published a number of studies in this area.   L103: chamber . . . “is” cubic (instead of was) ?   L136: space between 635 and “nm”   L143: How likely was re-suspension of large particles in a subsequent test? Was physical cleaning ever performed in the case that UV light, ozone, ethanol vapor did not physically wash out large particles?   L192: monodispersed is misspelled   L216: Cite Pohlker et al., AMT, 2013 regarding fluorescence spectra of pollen.   L254: The text brings up the mention of “deeper cluster analysis,” which I agree is beyond the necessary scope of this text. I still think at this mention it would be useful to cite the Manchester team who has been working on this area, e.g. Robinson et al. 2013 or Crawford et al. 2014 or 2015.   L254: Is there a reason why Table 1 lists 1-12, then skips to 28-56 before returning to 15-27?   L254: Were two instrument “calibrated” with any of the same particles at same time (with all four channels)? I realize this is a difficult task and one of on-going research efforts. However, if no standardization of signals was attempted, it is hard to know how much to trust Figure 4, or not. E.g. L221-222 mentions gain settings, etc. Were these standardized?   L254: Figures 2, 4: The bar width scale is confusing. Why is the scale approximate?   L254: Figure 3 has no units   L52: remove “some intact” from sentence   L52: Add UV-LIF (or equivalent) as keyword   L51: remove capitalization from ultraviolet   L86: Statement about “short stability window” needs to explicitly say time somewhere in the sentence to be less ambiguous. Also, this statement needs a reference of some kind, even if to say “personal experience.”   L96 and 132: I’m confused by the addition of the word “portable.” Aren’t all WIBS instruments portable? Is the “portable variant” the Introscope (?) version? If so, I think this should be mentioned in the methods section to highlight the differences between the WIBS and whatever the portable “variant” is.   L104 space between 1.5 and W   L107, L108, L120 space between number and unit: m, C, C   L136-138

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sentence is long and should be shortened and simplified   L167, L179: avoid using “distinctly clustered” to avoid confusion with cluster analysis   L179: Is the correct term “pollens” here? I would suggest species or pollen types to disambiguate.   L194: comma before however   L208: Add citation to Perring et al. and Manchester clustering efforts (this is already being done)   L211: Cite Pan et al. here. Also, briefly explain what you mean by “statistical treatment”. If you mean clustering, then state specifically and cite. If something else, I would add another few descriptive words.   L219-220: Cite Pohlker et al. 2013

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Interactive comment on Atmos. Meas. Tech. Discuss., doi:10.5194/amt-2015-372, 2016.

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