Response to referee comments and suggestions on amt-2018-390 by Könemann et al.

Manuscript format description:

Black text shows the original referee comment, blue text shows the authors response, and red text shows quoted manuscript text. Changes to the manuscript text are shown as *italicized and underlined*. We used bracketed comment numbers for referee comments (e.g., [R2.1]) and author's responses (e.g., [A2.1]). Line numbers refer to the discussion/review manuscript.

Referee #2 Dr. Ian Crawford

Received: 4 December 2018

General comment:

This paper examines the technical capabilities of the new SIBS UV-LIF bioaerosol spectrometer and describes several technical corrections and calibrations that are necessary to deliver high quality and accurate data products. As a long term WIBS user it is encouraging to see the next generation of high spectral resolution UV-LIF spectrometers that are coming to market being examined in detail early on in their lifecycle; while there is still undoubtedly still utility in broadband spectrally integrated instruments such as the WIBS for broad bioaerosol detection, it has been clear for some time now that deeper specificity/classification requires greater spectral resolution so these technical developments are timely. The authors present a fair assessment of SIBS capability to resolve key biofluorophores and make a number of suggestions and cautions that apply to the SIBS and also UV-LIF spectrometers generally. Overall the paper is well written and the technical validation experiments are well thought out. The results and methodologies reported here will serve as a useful framework for assessing the performance of other multi-channel high spectral resolution UV-LIF spectrometers which are entering circulation. I recommend publication after the following comments have been addressed.

Author response: We want to thank Dr. Crawford (Referee #2) for his positive assessment and constructive suggestions.

Specific/technical comment:

[R2.1] L98: Can you please check the size range reported for the WIBS-NEO. It is my understanding that the instrument sizes over the range of 0.5-30 µm.

[A2.1] Thanks a lot for pointing that out. The size range we stated for the WIBS-NEO originated from information we had in the beginning of 2017. Since then, DMT seem to have updated related information. The size range, within the manuscript, is now changed from ~0.3 – 100 µm to ~0.5 – 30 µm for the WIBS-NEO, according to: http://www.dropletmeasurement.com/wideband-integrated-bioaerosol-sensor-wibs-neo

[R2.2] L125: I think that a short sentence summarising some of the validation work would round this out while showing some of the limitations of the instrument/approach. A statement on how the Crawford et al. (2015) method was validated by Gosselin et al. (2016) by showing a good correlation between fungal molecular tracers and assumed fungal clusters but poor agreement between bacterial tracers and assumed bacterial clusters would contextualise this. It may also be worth commenting that the relatively high lower size limit of 0.8 µm used in this study due to instrument limitations may have impacted the latter which may potentially be alleviated by an improved lower detection limit.

[A2.2] As suggested, the following sentences have been added to round out the topic of currently used clustering approaches regarding online LIF:

(P4-5, L130-139): *"For example, it was shown for a rural forest study in Colorado that a cluster derived using WIBS-3 data, assigned to fungal spores (Crawford et al., 2015), correlated well with the mass concentration of molecular fungal tracers (e.g., arabitol and mannitol) measured with offline chemical techniques (Gosselin et al., 2016). In contrast, the clusters in the same study that were assigned to bacteria correlated only poorly with endotoxins, used as bacterial molecular tracers (Gosselin et al., 2016). This provides evidence of a limitation to using LIF instrumentation with low spectral resolution to separate or identify some PBAP types. Additionally, the bacterial cluster*

allocation might have also been hampered in that case by the minimum detectable particle size of the WIBS (~0.8 µm), resulting in a lower detection efficiency for bacteria."

[R2.3] L209: Can you comment further on the choice of 1σ thresholding use here. I appreciate that the conventional wisdom used to determine the threshold for WIBS instruments may not carry over here due to the differences in the optical setup but 3σ and 9σ thresholds are used later in the paper when reporting ambient concentrations.

[A2.3] As pointed out by Dr. Crawford, it is currently unknown if thresholding strategies conventionally used for several WIBS models perform similar when applied to the optical setup of the SIBS. For the current manuscript, we decided to use a rather simple 1σ approach, because for the assessment of the spectral accuracy, measuring sets of homogenous particle types (PSLs, biofluorophores), the thresholding plays only a minor role. In contrast, conventional thresholding strategies were applied to a set of ambient data as a first attempt to qualitatively match SIBS results with data derived from established online LIF instruments like the WIBS and UV-APS. In this context, we added the following sentence:

(P7, L223-227): *"Optimization of the thresholding strategy is still an on-going work, for example to investigate whether the often applied 3σ threshold used for the WIBS (e.g., Gabey et al., 2010) also works well with respect to the optical setup of the SIBS. For the assessment of the accuracy of measured fluorescence emissions from reference compounds, a threshold of 1σ was used here."*

[R2.4] L218: This looks like it may be due to coincidence errors arising from multiple particles being present in the sample volume causing odd scattering behaviour. This is a known problem when sampling high concentrations with forward scattering cloud probes, resulting in spectral broadening (e.g., Cooper, 1988).

[A2.4] Thanks a lot for this hint. The stated reference might indeed be an explanation for the effects we have observed for asymmetry factor measurements with the SIBS. The following sentence was added:

(P8, L235-237): "*However, one explanation could be optical coincidences caused by high particle concentrations, resulting in multiple particles being simultaneously present within the scattering volume, as reported by Cooper (1988) using forward-scattering signatures of cloud probes.*"

[R2.5] L435: This is a very interesting point that is raised here about the range irradiance imbalance between xenon lamps. This confirms some of my suspicious about the utility of presenting ABC analysis in general terms without appropriate caveats or a calibration standard and I think this is worth further comment. The Hernandez et al. (2016) work showed some of the results of the issues mentioned here when they compared two WIBS-4As where there were some significantly different classifications between the two units for the same test particle. They speculated that the difference between units was due to detector gain but your results suggest that xenon intensity may significantly contribute towards the observed differences. As a follow on comment this also shows the need for a common calibration reference standard to be adopted by the UV-LIF community (e.g., Robinson et al., 2017). This potentially raises a significant challenge for UV-LIF spectrometers with increased spectral resolution as I don't know if there is likely to be a single fluorophore that will adequately cover the whole spectral range?

[A2.5] This observation is indeed a critical point when it comes to the interpretation of fluorescence data derived from online LIF instruments using similar optical setups. Observed differences, between similar instruments as stated in, e.g., Hernandez et al. (2016), are most likely based on the complex interaction of multiple technical components, batch-to-batch variability etc. However, if prospective experiments verify a general imbalance between xenon sources / optical filtering for the WIBS and SIBS, this issue might turn out to be a major contributor to this topic. We agree with Dr. Crawford that it is absolutely necessary to adopt a calibration standard within the online bioaerosol community. However, to the best of our knowledge, there is currently no compound available

that fulfills the requirements (e.g., stability, repeatability, broad spectral range etc.) for being a standard calibrant for multi-channel, multi-excitation LIF-instruments.

Within "5. Summary and conclusions", this existing text passage briefly discuss the data interpretation issue:

(P24, L799-805): "These observations are valid not only for the SIBS, but also for the WIBS-4A and WIBS-NEO and lead to important implications for interpretation of particle data. In particular, a particle that exhibits measurable fluorescence in WIBS channel FL1, but only weak fluorescence in channel FL3 could be assigned as an "A-type" particle in one instrument but an "AC-type" particle in an instrument with slightly stronger xenon 2 irradiance. These differences in classification can be extremely important to interpretation of ambient data (Perring et al., 2015; Savage et al., 2017)."

Additionally, we added the following sentence regarding instrument intercomparisons / calibrant standards:

(P24, L794-799): *"Additionally, alternating irradiance properties might significantly contribute to observed differences in performance of similar instrument types (e.g., Hernandez et al., 2016), expressly underlining the need for a fluorescence calibrant applicable across LIF-instruments (e.g., Robinson et al., 2017). Nevertheless, to the best of our knowledge, there is currently no standard reference available that fulfills the requirements to serve as a calibrant for multi-channel, multi-excitation LIF-instruments."*

[R2.6] L517: In my experience of calibrating forward scattering cloud probes it is often common to find a dip in sizing performance in the lower region of an instruments detection range due to Mie-Lorenz resonances in the applied Mie curve exceeding the bin thresholds or the bin thresholds being relatively narrow. Mis-sizing can also be further exacerbated by the particles position in the sample area as recently demonstrated by Faber et al. (2018), however this is less likely to be an issue with SIBS/WIBS type

instruments as the sample flow jet should be well constrained to the central sampling region. Given that the fit to the calibration has a slope of approximately 1 and a negligible intercept the assumed Mie curve appears to be adequate, however, should there routinely be a dip in the particle size distribution around this size this may explain why.

[A2.6] We considered this possibility, and almost added a comment to the discussion manuscript to this effect. Looking into the Mie curves in more detail, however, we did not find a solid evidence that may serve as an explanation for the effect observed in a size range between 0.6 – 0.8 µm. Because the idea was not strongly supported and to avoid inadvertently leading readers astray, we decided to leave the issue with unknown cause.

[R2.7] Fig. 7: Can you add to the caption what the red line represents. I assume it is the rebinned reference spectra as in Fig. 5.

[A2.7] True, red dashed lines show re-binned reference spectra as stated in Fig. 5 for **c** and **d**. The caption was modified for all corresponding figures (manuscript and supplement) as requested.

[R2.8] Fig. S10: This would be easier to interpret if the two plots were scaled over the same x-axis range.

[A2.8] Within the supplement manuscript, Fig. S10 was modified as requested.

References

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