Anonymous Referee #2

For clarity the referees comments are copied in black and our responses are offset in blue.

Overview: In general the manuscript is well written and I believe of great relevance to the bioaerosol scientific community. The use of more complex analysis techniques to glean addition information from online, single particle biological sensors is an important advance. Thus I believe the paper should be published upon the correction of some minor technical/specific issues discussed below.

We thank the reviewer for their careful reading of our manuscript and the helpful comments and recommendations which we address below.

Specific/technical comments:

P7308 L22: The D50 for the WIBS-4 references Gabey et al 2011. Wasn't a WIBS-3 used in that work? Are the WIBS-4 and 3 comparable in this respect?

The WIBS-3 and WIBS-4 used in these studies use a similar optical chamber design and have comparable detection ranges.

P7308: Standardized particles for the calibration of the AF values are talked about. What Standardized particles were used for rod-like particles? or irregular shaped particles?

Corn starch flour was used to represent irregular particles and ellipsoidal haematite particles were used as an analogue for rod-like bacterial particles as described in Kaye et al. (2007). We will clarify this in the text by including a reference to this work.

Does size have any effect on AF values?

Gabey et al. (2010) describes the effect of size on AF which is now briefly described. 1 µm and 3 µm polystyrene latex spheres were sampled with a WIBS-3 where they found the modal values of AF to be 2-3 units greater for the smaller particles. They suggested that the noise in the quadrant PMT causes smaller particles to register slightly greater AF, however the influence is small.

Where would an irregular-shaped particle be placed between 1-100? Between 10-20?

Spheroidal particles typically display AF ~1-20. Irregular and rod-like particles display AF>20.

P7309 L4: Is the excitation used in the FL3 350 or 370nm?

370 nm. We will correct the earlier use of 350 nm earlier in the text and we thank the referee for bring this to our attention.

P7312: How many of the particles saturated the PMT. What percentage of the fluorescent particle count did they make up?

For the BEACHON data presented here of the 913,145 fluorescent particles sampled 97,673 particles saturate the detectors (10.7% of the fluorescent particle population.)

If a particle was fluorescent in all channels but only saturating in one was it removed?

Yes.

Would it be better to keep saturation particles but give them their own Cluster? A saturation cluster? Do you believe these particles are non-biological interferences particles? And are thus more willing to remove them?

We have chosen to remove all particles which cause saturation from the presented ambient analysis as they are fluorescent beyond the detection capability of the instrument which may result in the conflation of different particle types. The use of a saturation cluster could be used in special cases where significant proportions of the fluorescent aerosol population saturate the detectors, however, care must be taken when interpreting the results of such cluster analysis due to the particle fluorescence exceeding the detection range and the presented analysis should be caveated appropriately. In the case of the presented BEACHON-RoMBAS ambient data the large saturating particles are probably highly fluorescent biological particles (e.g. pollen). Non-biological fluorescent interferents are generally considered to be smaller (sub-micron) particles, however more work is needed to evaluate potential interferents.

P7315: The author talk of the WIBS instrument automatically entering forced trigger. Does the pump stop during this process? How low is the chance of coincidentally striking a real particle in this mode? Was there any evidence to suggest this may have happened? If so how was this data dealt with? Was this automatic forced trigger ever compared to the previous method of manually placing the WIBS instrument into the forced trigger mode while the pump was off?

The pump does not stop during forced trigger (FT) sample periods. For the instrument to enter FT sampling mode the concentration must be less than 2 counts s^{-1} for a sustained period where the flash lamps are triggered once per second and all usual single particle data are recorded for each sample. A particle takes 300 µs to traverse the sample volume of the WIBS where a fluorescent particle outside of the sample volume during a flash would not fluoresce with sufficient intensity to interfere with the background measurement. For a concentration of 2 counts s^{-1} the chance of a particle being coincident with the sample volume during a FT sample is therefore 0.06% assuming the particles are well separated. While this probability is low, if this was to occur the contaminated sample can be rejected based on the scattering signal produced by the particle.

The automatic mode has been compared to the manual method where the resultant backgrounds were consistent. FT measurements have also been made with the pump running both with and without a HEPA filter inline with the inlet where both methods yield consistent backgrounds.

How much of the fluorescence data was clustered? Was all the data placed into a cluster or was some of it left unclassified?

All fluorescent particles which meet the criteria detailed in section 3.4 are clustered.

In previous work, the wasp clustering centers and standard deviations about the centers were reported. Could this be done for the clusters here? If so I believe it would be useful.

> We will include the clustering centers for the ambient data in an appendix.

P7316 L19: The text mentions 5 clusters in each of methods used however in figure 5 the Z-Scores normalization only has 4?

> We thank the referee for bringing this typographical error to our attention and we will correct this in the revised manuscript.

P7316 L22: R1,R3 and R5 are similar to R2? Should it read “R1,R3 and R5 are similar to Z2”

> We thank the referee for bringing this typographical error to our attention and we will correct this in the revised manuscript.

P7317 L3 “which was determined to be be representative” "be" is repeated.

> We thank the referee for bringing this typographical error to our attention and we will correct this in the revised manuscript.

The author suggests that a cluster representative either fungal or bacterial. The author in particular suggests that bacteria have a strong positive response to rainfall. How-ever several fungal spore species are known to be positively correlated with rainfall and have sizes and morphological feature similar to those suggested by the data here. This should be discussed as a possibility by the authors. I believe the authors should possibly row back on saying the clusters are representative of bacteria or fungal spores this may be true however without supporting measurements this should described in this way. It feels too strong as other particle types may be contributing. Other WIBS related work has co-located impactors to assess the contribution of fungal for example. With those types of added measurement the current terminology used would be possible.

> The labelling used here was chosen to be consistent with the analysis presented in Crawford et al. (2014) to aid comparison of the methods employed in each study. We agree that supporting measurements are needed to interpret cluster analysis with confidence and such measurements were made as part of this experiment. We agree to include a small discussion on the possibility of such conflations and the need for supporting data.

Also was there any possibility the non-biological particles could have been clustered into the solutions shown here. If so this should be discussed. Of course this is another reason for not suggesting clusters are representative of a single class of particle.

> If non-biological particles of sufficient size and fluorescent yield are present in the sampled aerosol population they would be clustered. When interpreting cluster analysis results it is important to consider the sampling location and potential sources of interferents. At the remote site used during BEACHON access was strictly controlled and the site is not heavily impacted by pollution sources and biofluorescent particles are expected to dominate (Ortega at al. 2014); Contamination from known interferents such as PAHs and light absorbing SOA compounds (Pöhlker et al., 2013) was deemed unlikely at the site as a result and these contaminants are likely to be much smaller than the particles observed in this

study. We agree that when assigning a cluster to a metaclass it is necessary to include suitable caveats where interferent contamination is possible at the sampling site.

Figure 8: was there much difference in diurnal trend for days with and without rainfall for bacterial clusters? Were increased concentrations at 18:00 hours due to rainfall events?

Generally speaking the bacterial clusters display elevated concentrations during rainfall as would be expected. The increased concentrations at around 18:00h were due to rainfall as rainfall events typically occurred in the late afternoon/evening. The figure below demonstrates this for the Z-score Ward cluster solutions. The no rain case is averaged over the period 00:00 22/07 to 00:00 24/07 which features no rainfall events. The rain case is averaged over the period 00:00 24/07 to 00:00 30/07 where each day in this period featured a rainfall event in the afternoon/evening with peak rainfall rates in the range of 0.6 to 14 mm hr^{-1}. It can be seen that the diurnal trends between 00:00 and 12:00 are broadly consistent with a minimum at midday as no rainfall occurred in these times for each case.

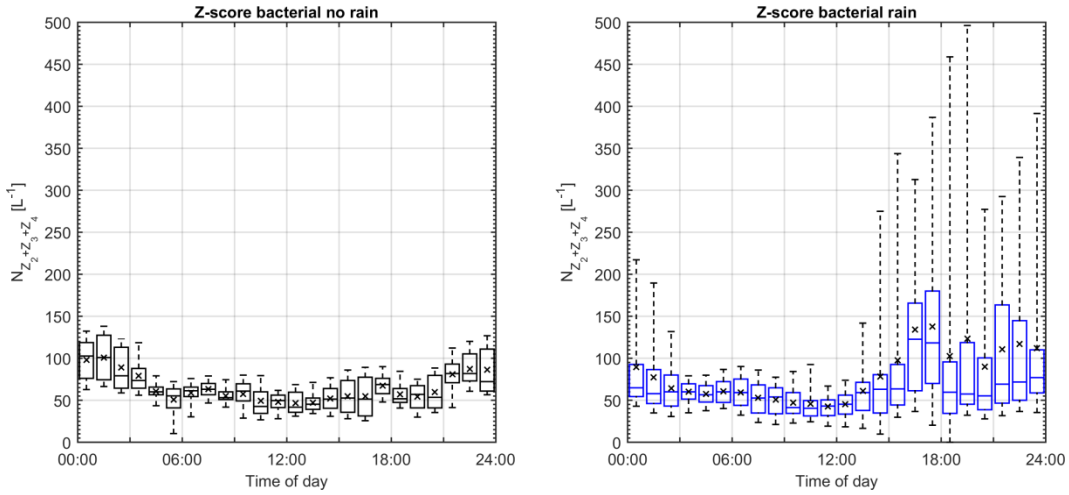

Figure 1. Influence of rainfall on diurnal bacterial cluster concentrations.

References

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