

**Anonymous Referee #1**

For clarity, the referee's comments are copied in black and our responses are offset in blue.

We thank the reviewer for their helpful comments and recommendations which we address below.

Important contribution, worthy of publication.

1. Calibration and classification of bio-particles is required (minimum theoretically, but possible practically) 'Bio-particles' misleading as measuring fluorescence, most of which may be down to biological origin, but by no means all Title: Real time detection of fluorescent particles in Antarctica

We do not feel that the title is misleading. We clearly state our conservative estimates of bioparticle concentrations and how they were classified.

P4 L22 Needs calibrating for different bio-particles as 2.35 L min-1 is a low flow rate

The sheath flow is filtered using a HEPA filter which will remove all particles at this flow rate.

P4 L30 What about non fluorescent bio-particles?

PBAP of interest (e.g., pollen, bacteria & fungal spores) have been demonstrated to show a detectable autofluorescent response with the WIBS (Hernandez et al., 2016, Savage et al., 2017). Non-fluorescent particles will exhibit fluorescent signal below the instrument fluorescence threshold, thus the fluorescent signal will be clipped at zero in the processed data as described in Crawford et al., (2015), however, this information and the particle size is still recorded and used to define the non-fluorescent particle population.

P5 L16-17 This sentence strongly suggests that UV-LIF needs proper calibration for bio-particles

We assume that the referee is referring to the requirement for a training library of autofluorescent signatures for comparative attribution, rather than a calibration for fluorescent intensity in our answer.

The laboratory categorisation of bioaerosols of interest is an ongoing area of research. To date there have been two significant systematic laboratory characterisation studies published using a similar instrument (WIBS-4A); Hernandez et al., (2016) and Savage et al., (2017). We have also performed our own characterisation for the purpose of validating machine learning algorithms experiments (e.g., Ruske et al., 2017 & Crawford et al., 2015).

The Hernandez et al., (2016) study characterised the autofluorescence of 14 bacterial, 13 pollen and 29 fungal spore samples. The Savage et al., (2017) study characterised 3 bacterial, 5 fungal, 14 pollen, 12 pure biofluorophore, 13 mineral dust, 6 HULIS, 3 PAH, 7 combustion soot and smoke, 3 brown carbon and 3 miscellaneous non-biological particle samples. These studies showed that each particle type demonstrated a broad characteristic autofluorescence, size and asymmetry factor that can be used to interpret and classify ambient measurements. We use such libraries to aide interpretation of our results, along with our own laboratory measurements, such as those provided in the supplementary material.

2. P4 L1 'near-sterile' is not appropriate as it cannot be substantiated, use 'low biomass'

We will revise this as suggested.

3. Further methodological detail required. P4 L16 'The instrument was designed to identify common fluorophores' detail needed here as fundamental to what is being measured

This is elucidated on P4 L34, briefly; FL1 is optimal for the detection of tryptophan and proteins; FL3 is optimal for NADH detection as described in Kaye et al. (2005).

P4 L22 Filtered – how, what proportion of bio-particles is removed by filtration?

This refers to the filtration of the sheath flow. This is filtered with a HEPA filter to remove all particles, such that the 0.23 L/min sample flow is sheathed in particle free air to constrain the aerosol into a controlled jet and to minimise contamination of the optics. As such, none of the sample flow aerosol is removed.

P5 L3 Many more bacteria are common aerosols, a diverse range of examples could be tested

The laboratory categorisation of bioaerosols of interest is an ongoing area of research. To date there have been two significant systematic laboratory characterisation studies published using a similar instrument (WIBS-4A); Hernandez et al., (2016) and Savage et al., (2017, under review). These studies cumulatively sampled 16 different bacterial samples and found that each predominantly fluoresces in channel FL1 and were generally under 2.5  $\mu\text{m}$  in diameter. While these studies are not exhaustive, the authors note that the fluorescent spectra observed should hold as a broad trend for each particle type.

P5 L1 This needs more detail in order for the reader to be able to repeat the approach

The details are provided in Gabey (2011) and Toprak & Schnaiter (2013). We will include a reference to the latter and update the text to the following:

"Whilst there have been no previous measurements of bioaerosol in the Antarctic using the UV-LIF technique, expected bacteria, such as the common *Pseudomonas* spp. (Antarctica), have been shown to fluoresce strongly in these wavebands, e.g. the laboratory studies reported by Gabey (2011) as part of the BIO-05 series of experiments where PBAP samples were wet sprayed into the 3.7  $\text{m}^3$  NAUA aerosol chamber to be characterised prior to their injection into the 84  $\text{m}^3$  AIDA cloud simulation chamber to assess their efficiency as atmospheric ice nuclei (Toprak & Schnaiter, 2013)."

P16 L1 What was the rationale for these pollen types?

The pollens selected are common allergens in the UK are readily available from commercial suppliers.

4. Further contextual detail helpful P5 L16 Specify what these 'many advantages' are?

We will update the sentence to the following to include the requested details:

UV-LIF spectrometers such as the WIBS have many advantages over traditional bioaerosol sampling methods (e.g., on-line single particle detection & high time resolution)...

5. Minor issues and typos P5 L3 Genus and species names in italics P5 L3 Capital A for Antarctica

We will correct this in the revised manuscript.

## References

Crawford, I., Ruske, S., Topping, D. O., and Gallagher, M. W.: Evaluation of hierarchical agglomerative cluster analysis methods for discrimination of primary biological aerosol, *Atmos. Meas. Tech.*, 8, 4979-4991, <https://doi.org/10.5194/amt-8-4979-2015>, 2015.

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Savage, N., Krentz, C., Könemann, T., Han, T. T., Mainelis, G., Pöhlker, C., and Huffman, J. A.: Systematic Characterization and Fluorescence Threshold Strategies for the Wideband Integrated Bioaerosol Sensor (WIBS) Using Size-Resolved Biological and Interfering Particles, *Atmos. Meas. Tech. Discuss.*, <https://doi.org/10.5194/amt-2017-170>, in review, 2017.

Toprak, E. and Schnaiter, M.: Fluorescent biological aerosol particles measured with the Waveband Integrated Bioaerosol Sensor WIBS-4: laboratory tests combined with a one year field study, *Atmos. Chem. Phys.*, 13, 225-243, <https://doi.org/10.5194/acp-13-225-2013>, 2013.