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Atmospheric Measurement Techniques Discussions
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Interactive comment on "Fluorescence calibration method for single-particle aerosol fluorescence instruments" *by* Ellis Shipley Robinson et al.

Anonymous Referee #1

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With the growth of biological aerosol research in the last decade, commercially-available instruments are now available that utilize fluorescence techniques to quantify bio-aerosol concentrations and properties. This paper provides a framework for calibrating such fluorescence instruments, specifically to provide a method to normalize the fluorescence intensity response over time and between different instruments with different operational settings. This work is well done, well presented, and is a welcomed addition to research in this topic area. I do have some reservations as to the application of normalized fluorescence intensities to ambient data and suggest revising the manuscript to address these hesitations. Otherwise, I support publishing the paper after the authors address the following minor comments:

1. Line-80: Please state which requirements were not fulfilled by NADH and naphthalene here. Were any other materials considered and not used? This information might

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be useful to other groups seeking additional calibration candidates.

2. Section 2.1: One aspect of WIBS operation not discussed is timing. I suggest adding a short paragraph summarizing how the instrument timing was set, so this procedure can be used consistently by the community.

3. Line-118: It is interesting that peak height varies as a function of flow rate and I certainly agree that calibrations should be done at an appropriate flow. Since the data in Figure 1 seem to asymptote toward a constant response at higher flow rates, would you suggest that users operate at a flow rate > 4 LPM so peak height is less sensitive to small variations in flow rate? Users would have to accept limitations of decreased counting efficiency for high concentrations at these higher flow rates.

4. Line-162: What model DMA was used here, and is it able to size select particles greater than 1 micron diameter?

5. Line-438: Larger, philosophical issue. Expressing fluorescence intensity as T- and Q-units is certainly a clean and easy way of comparing the output of different instruments to each other. But, there is a risk of largely over-simplifying the interpretation of ambient results where many complex factors govern fluorescence intensity. This is noted explicitly in the Pohlker review of bioaerosol autofluorescence, "However, fluorescence intensity is a complex function of various parameters such as concentration, extinction coefficient at λex and quantum yield at λem as well as influences by the molecular environment. Accordingly, only semi-quantitative comparison of intensity levels is possible based on the presented results." For example, two ambient populations could result in the same Q-unit fluorescence and have very different actual amounts of fluorescent material because of the properties listed above. Interpreted results that showed similar Q-unit fluorescence intensity would not be at all accurate in this case. By advocating the use of T- and Q-units, are we over-simplifying these systems and risking erroneous interpretation? There is no doubt that using this method to ensure instruments are operating similarly is very beneficial, but I question the ap-

plication to ambient analyses. I suggest adding a caution to users wishing to apply T- and Q-units for ambient applications, potentially including a review of the large range of quantum yields for fluorescent material, and removing this recommendation from the conclusions (Line-453).

Pöhlker, C., J. A. Huffman, and U. Pöschl. "Autofluorescence of atmospheric bioaerosols–fluorescent biomolecules and potential interferences." Atmospheric Measurement Techniques 5.1 (2012): 37-71.

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