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Atmospheric Measurement Techniques Discussions

Interactive comment on "Technical note: Characterization of steady-state fluorescence properties of polystyrene latex spheres using off- and on-line spectroscopic methods" *by* Tobias Könemann et al.

Anonymous Referee #2

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The detailed analysis of steady-state fluorescence properties of PSL particles presented in this article will be useful to researchers using LIF-based measurement technologies. This article does make a useful practical contribution to the field. However, the discussion has the opportunity to go further in presenting and discussing results on single-particle fluorescence, which is likely to be what the majority of researchers reading the article will be interested in.

The Excitation-emission matrices obtained by the bulk analyses and the associated discussion are informative with respect to PSL characteristics and excitation/emission

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bands and as a guide for selection of optimal PSL spheres for a particular instrument/experiment. It is also important to highlight the influence of the detergents and additives as has been done in this work.

However, the reported results are difficult to translate into practically useful information about how the PSL spheres would appear in a LIF-based single-particle instrument like the WIBS-4A, which is the one used in this study. The extensive measurements of bulk solutions that are discussed in the article are accompanied by measurements carried out with the WIBS-4A on a select sample of PSL spheres, but the article in its current form has missed the opportunity to extend the discussion on how the intensities of PSL spheres measured by a single particle LIF instrument compare to those of real-world bioaerosols.

One issue that is important in this respect is the fluorescence intensities. As stated on page 9, line 5, the fluorescence intensity derived from individual particles is a function of size (because bigger particles contain a greater volume of fluorophore) and the fluorescent quantum yield of the fluorophores within the particle. On page 9, line 9 the authors refer to saturation of the detector in the WIBS-4A. Saturation occurs when the amount of fluorophore in the PSL sphere produces a signal that is outside the range of the detector. In other words, saturation of the detector can occur both due to particle size, amount of fluorophore in the particle and the quantum yield of the fluorophore. Naturally, the amount of fluorophore in a particle depends on both the volume of the particle and the concentration of fluorophore in that volume.

Saturation of the intensity signal is a non-trivial problem. The fluorescence intensities presented in Table 3 indicate that PSL spheres of diameter 4.52 micrometrres that are ostensibly non-fluorescent according to the manufacturer nevertheless causes saturation of the detector in the FL1 channel of the WIBS-4A. Saturation also occurs in channel FL2 with 3.1 micrometres PSL spheres and in channel FL3 with 2.1 micrometres PSL spheres. These observations deserve a lot more comment than what they receive in the article. The implication of the saturation in FL1 is that even non-fluorescent par-

ticles are recorded as fluorescent by the WIBS-4A if they are above a certain size and contain even a negligible amount of fluorophore. This suggests that either the PSL spheres were contaminated or the authors have identified an important issue with the WIBS-4A related to the detector calibration settings. This needs some further comment. In the case of the other two channels, the PSL spheres did contain fluorophores, but the particles were an order of magnitude smaller than many important bioaerosols.

This observed saturation suggests the question of whether the WIBS-4A is too sensitive for real bioaerosols of size 20-30 micrometres when a 2-micrometre PSL sphere saturates the detector or a 4.5 micrometre particle saturates it even without fluorophore content. When supposedly non-fluorescent particles that are not even big compared to many bioaerosols can saturate the detector, it becomes impossible to distinguish between biological and non-biological aerosols on the basis of fluorescence intensities in real-world environments.

Therefore, the discussion on fluorescence intensity per sphere could be extended, the article has the opportunity to start a discussion on fluorescence intensities and particle size with respect to both PSL spheres and real bioaerosol, which would be useful technical information for researchers employing LIF-based measurements in the study of bioaerosols. Instruments that are calibrated using PSL spheres (that may not even be in the relevant size range) are probably not appropriate for real bioaerosols in all cases.

Dependence on particle size in bulk solution is not particularly instructive in this context, because the measurements in that situation relates to the total amount of fluorophore in the cuvette. The work also involved fluorescence microscopy measurements that do report fluorescence intensity per sphere. However, the intensities in the fluorescence microscopy experiments cannot be compared to the detector responses in a single particle instrument like the WIBS-4A for the same spheres, as units are arbitrary and recorded intensities are dependent on detector settings in the individual instruments.

As shown in figure 5B, the fluorescence intensity of a fluorescent particle increases with size of the particle, and the dependency appears to be linear. If fluorescence intensity in single particles shows a linear dependence on size within a given type of PSL sphere or bioaerosol, then there is valuable information in the size-normalised fluorescence intensity that can aid bioaerosol identification. If, however, the detector is saturated, then the information value in the size-normalised fluorescence intensity is lost. Based on the figures reported in Table 3, it is likely that the majority of bioaerosols would saturate the WIBS-4A detector and the information would be lost.

This may not be the case, but the article has an opportunity to address this question based on the experiments carried out; it would increase the relevance of the article if it included a table or estimate of fluorescence intensities or fluorophore concentrations and comparing to bioaerosols or at least discussing whether the fluorophore content in the PSL spheres is appropriate for real bioaerosols, also with respect to size.

This would enable other researchers in the field to use the data to estimate optimal detector settings in their instruments in order to measure real bioaerosols without saturating. Of course, the fluorophores in the PSL spheres would have different concentrations and fluorescence quantum yields from biological fluorophores like tryptophan/NADH in real bioaerosols, but having the information would nevertheless enable researchers to make a more informed decision than is currently possible, and the article would have greater potential interest than it does in its current form.

Table 3 also reports standard deviations of the intensities in each channel for different spheres. Presumably, this is related to the Gaussian distribution that is referred to on page 9, line 8, but this should be clarified. Furthermore, standard deviations for the intensities are reported also at saturation levels, but once the detector saturates, one cannot know by how much the signal exceeds saturation and consequently it makes no sense to give a range above the saturation point.

The article is recommended for publication with some further discussion as suggested

above.

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