Response to referee comments and suggestions on amt-2017-407 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., [R1.1]) and author's responses (e.g., [A1.1]). Line numbers refer to the discussion/review manuscript.

Anonymous Referee #1

Received: 10 April 2018

General comment:

This paper provides the most comprehensive and detailed analysis of steady-state fluorescence properties of PSL particles. The manuscript was well written and easy to follow. I believe this manuscript should be accepted for publication.

Author response: We want to thank Referee #1 for his/her overall positive assessment.

Specific/technical comment:

[R1.1] It is interesting that particle size influences fluorescence intensity value on a single-particle scale but not for bulk solutions. I think a short discussion would be beneficial.

[A1.1] The size-dependence of fluorescence intensity on single particle scale is crucially important for LIF-based PBAP detection (e.g., Sivaprakasam et al., 2011; Hill et al., 2015; Swanson et al., 2018). In bulk measurements presented here, the fluorescence intensity depends on PSL particle size and number concentration in the cuvette light path. Accordingly, the fluorescence intensities in EEMs, which are based on bulk measurements, are not really comparable among the different PSL samples since PSL particle concentrations are only roughly documented by the manufactures (i.e.,

~1 % mass mixing ratio). The primary aim of measuring bulk solutions of PSLs with the same fluorophore embedded, but with different sizes, was to verify that no spectral alterations occur as a function of particle size. The topic of fluorescence intensity was, in this case, disregarded and we even tried to avoid potential fluorescence intensity shifts by adjusting PSL concentrations based on their size for each measurement individually. The following existing text passages address this issue:

(P6, L10-13): "The aqueous mass mixing ratio (mass PSL in mass water) of PSL particles in the stock suspensions is stated by the manufacturer as ~1 % (see corresponding information from manufacturer websites). Accordingly, for PSLs of different size, the number concentration of suspended PSL particles decreases steeply with increasing diameter ($N \sim 1/d^3$ based on the relationship between diameter and volume of an individual spherical particle)."

(P6, L16-19): "Table 1 specifies the adjusted mixing ratios (volume of PSL stock suspension in volume of ultrapure water) for the individual PSL samples. Larger quantities (6 and 9 µl) of the PSL stock suspension were used for particles with larger diameters (≥ 1.9 µm) to partially compensate for decreasing PSL particle number concentrations."

In P11, L26-30: "To investigate the relationship of particle size to fluorescence, Figure 2 shows a comparison of PSLs of different sizes, but consistent fluorophore. For wet PSLs, fluorescence emission spectra are qualitatively consistent between the two sizes analyzed for each particle dye. Slightly different intensity distributions between 0.53 and 2.07 µm Plum Purple PSLs (Fig. 2A, B) and between 3.10 and 10.0 µm Yellow Green PSLs (Fig. 2C, D) originates from different concentrations of solids in aqueous solution as described above."

has been changed to (P11, L30 / P12, L1-8): *"Figure 2 verifies that PSLs of different sizes, but with consistent fluorophore, show the same spectral fluorescence signatures. Generally, fluorescence emission spectra are qualitatively consistent between the two sizes analyzed for each particle dye. Note that for PSL bulk measurements, the fluorescence mode intensities are a function of the excited*

amounts of fluorophore in the light path inside the cuvette, which in turn depends on the size of the PSLs and their number concentration in suspension. Accordingly, slightly different mode intensities between 0.53 and 2.07 µm Plum Purple PSLs (Fig. 2A, B) and between 3.10 and 10.0 µm Yellow Green PSLs (Fig. 2C, D) originates from different concentrations of solids in aqueous solution as described above. Due to the uncertainty of the PSL number concentrations, the absolute intensities in the EEMs in Figure 2 are not particularly informative here."

In P4, L10-17, we further added the following statement for clarification: "*Since the size-dependence of fluorescence intensity on single particle scale is crucially important for LIF-based PBAP detection (Hill et al., 2015; Sivaprakasam et al., 2011; Swanson & Huffman, 2018), we further address selected aspects of the PSL size-intensity relationship. However, it is important to note that a comparison of fluorescence intensities from different instruments (e.g., offline spectroscopy and microscopy as well as online WIBS-4A measurements) is not trivial as it depends on the properties of the fluorescent particles, on one hand, and on the optical design and detector settings of the instruments, on the other hand. Therefore, we discuss certain intensity-related aspects here semi-quantitatively, whereas an in-depth analysis of single particle fluorescence intensities is beyond the scope of this work.*"