Response to referee comment on amt-2016-153 by Huffman et al.

Anonymous Referee #1

Received and published: 25 June 2016

General Comments: This manuscript is interesting, important and well written. I like it. It appears to be a major step forward in developing low cost instrumentation for aerosols, especially biological aerosols. Because of the low cost I suspect that, as the authors suggest, versions of this instrument will be used to study aerosols over a much larger spatial range than possible with presently available instruments. Present instruments are too expensive. The potential for making apps for cellphones to record the spectra and send these to one location for assembling the data from all the sensors is appealing. This is first I remember seeing the suggestion to spectrally disperse the emission from aerosol particles spread randomly in 2D. I recommend publication and do not suggest any mandatory changes.

Author response: We thank the referee for his/her positive assessment and summary. We have indeed not seen an instrument that offers the range of capabilities as the one introduced in our manuscript and we are excited to further the development of the technology.

Note regarding document formatting: black text shows original referee comment, blue text shows author response, and red text shows quoted manuscript text. Changes to manuscript text are shown as *highlighted and underlined*. All line numbers refer to discussion/review manuscript.

Specific Comments (note that referee comments have been labeled by letter and chopped by individual referee-thought so they can be dealt with in a clear sequence): The authors may want to think about, and possibly comment on, the following. **[*a*]** Possibly more could be said about the smaller end of the size range of biological particles that could be detected.

The referee brings up some really good points here. Even though we highlight the positive attributes of the technique we introduced, there are always disadvantages and trade-offs to consider. The points the referee mentions are some of these. Based on the tone and text of the referee comment, we would guess that s/he would agree that a deep analysis of these trade-offs is beyond the scope of this manuscript, but we decided to add a few additional overview statements to the manuscript to make it clear that we acknowledge these important trade-offs. In particular, we added Section 2.3 (before L228) that discusses some practical considerations brought up by the referee and we also added a paragraph to the conclusions (L427) summarizing the novel benefits of the technique. These two additional paragraphs are copied in this document at line 181.

Other responses to specific points raised by the referee:
[*a*] First, the originally submitted manuscript referred to the device investigating "micron-size particles." These statements have been changed to "*approximately super*micron-size particles" in L13 and L71 as also discussed in response to Referee #2 (Point **[*1a*]**).

Second, a rigorous discussion of the lower size limit of detectable particles is complex, because it convolves several instrument parameters. A deeper discussion of this is presented in response to Referee #2 (Point **[*1a,b,c*]**). In short, however, we have investigated particles as small as ~1 µm, and we are confident that the technique will also work for particles smaller than this. The

lower limit will depend strongly on the relative fluorescence intensity of the particle and the exposure time of the camera, among several other factors. We have not yet rigorously probed the interplay of these variables, but will continue to do so as experimental development work continues. In response to the comments from both referees, however, we added supplemental Figure S2 and associated text at L253 discussing micrographs and spectra associated with 1 µm fluorescent polystyrene latex beads interrogated by our benchtop device:

> "This fraction is highly dependent on the threshold one applies to categorize a given particle as fluorescent or not. *Observed* fluorescence intensity is *also* strongly a function of *several factors, including*: particle size, *fluorophore content and quantum yield, intensity of excitation source, instrument optics, and camera exposure time (e.g. Hill et al., 2001; Hill et al., 2013; Hill et al., 2015b; Pöhlker et al., 2012; Sivaprakasam et al., 2011)*. Most fluorescence-based aerosol detectors are faced with the *conceptual* challenge of *how best to define minimum detectable fluorescence*, and the sensitivity of a given detector will significantly influence the comparison of the relative fraction of fluorescent particles detected by any two instruments or types of instruments (*e.g.* Healy et al., 2014; Hernandez et al., 2016; Huffman et al., 2012; Saari et al., 2013). *As mentioned, the particle size contributes significantly to the detectability of fluorescence from individual particles. All particles chosen for discussion here are relatively large (*e.g. >10 µm*) in order to highlight the overall technique and concepts. It should be noted, however, that the instrument is not fundamentally limited to such large particles and can be applied to particles of 1 µm in diameter, or smaller, if higher microscope magnification (e.g. 40x) is utilized and the parameters influencing observed fluorescence are managed appropriately. We have acquired spectra of individual particles as small as 0.96 µm (e.g. supplemental Fig. S2), though this is not intended to be presented as a lower limit. Further limitations will be explored in follow-up studies.*"

References added here:

Hill, S. C., Williamson, C. C., Doughty, D. C., Pan, Y. L., Santarpia, J. L., and Hill, H. H.: Size-dependent fluorescence of bioaerosols: Mathematical model using fluorescing and absorbing molecules in bacteria, Journal of Quantitative Spectroscopy & Radiative Transfer, 157, 54-70, 2015b.

Hill, S. C., Pan, Y. L., Williamson, C., Santarpia, J. L., and Hill, H. H.: Fluorescence of bioaerosols: mathematical model including primary fluorescing and absorbing molecules in bacteria, Optics Express, 21, 22285-22313, 2013.

Hill, S. C., Pinnick, R. G., Niles, S., Fell, N. F., Pan, Y. L., Bottiger, J., Bronk, B. V., Holler, S., and Chang, R. K.: Fluorescence from airborne microparticles: dependence on size, concentration of fluorophores, and illumination intensity, Applied Optics, 40, 3005-3013, 2001.

Sivaprakasam, V., Lin, H.-B., Huston, A. L., and Eversole, J. D.: Spectral characterization of biological aerosol particles using two-wavelength excited laser-induced fluorescence and elastic scattering measurements, Optics Express, 19, 6191-6208, 2011.

[*b*] What is the large dimension of the smallest particles measured?

[*b*] We are not quite sure what this question is asking, but provide here response that we think addresses the question. Using Figure 4 as an example, the vertical extent of the elastic (e.g Fig. 4b) and inelastic/fluorescence (e.g. Fig. 4c) spectra shown vary as a function of particle size. For example, if a particle is large in the vertical (*y*) dimension, the height of its spectral swath will be approximately equal to the height of the particle itself.

We added the following text at L108 of the manuscript:

"*For example, if a particle is large in the vertical (y) dimension, the height of its spectral swath will be approximately equal to the vertical dimensions of the particle itself.*"

[*c*] Could a 1 micron bit of a fungal spore be detected?

[*c*] Yes, a 1 µm fungal spore could be detected, as long as it is "sufficiently" fluorescent and the exposure time of the camera is set appropriately. See response to Point **[*a*]**.

[*d*] As compared to illuminating with a line source that must be stepped in one direction over the image, this approach needs no moving parts. What is given up for this advantage?

[*d*] One technical disadvantage of the method described here is that spectral resolution in the 'x-direction' (i.e. the dimension into which the spectrum is dispersed) is reduced when analyzing a large particle. The reason for this is as follows. Assume an illumination source is a line of infinitesimal width, shining across the whole field of view in the y-direction (i.e. top-to-bottom on Fig. 2), and scanning slowly from left to right. As it scans, the source will hit the left side of a given particle and disperse fluorescence emitted from that small portion of material (dx) into the x-direction. As the scan line moves to the right it will excite a fluorescence spectrum from a different small piece (dx) of material. The angle of dispersion (Θ) for a given wavelength (color) of light emitted is a constant, however. Thus, fluorescence emitted from the first point at one emission wavelength will be convolved into the emission spectrum from a second physical point of excitation, but at a different emission wavelength. This will blur the fluorescence spectrum in wavelength space increasingly as a function of particle size. Additionally, if a given particle is homogeneous in composition, the fluorescence spectrum will not vary as the illuminating line traverses the width of the particle. If a particle is inhomogeneous, however, the fluorescence spectrum may change as the illumination point moves, further smearing the fluorescence spectrum. Fortunately, as the referee points out, the emission bands for fluorescence spectra are broad, and the extent of this smearing is small for particles e.g. < 50 µm.

[*e*] Is the maximum number of particles per area that could be analyzed lower? I think yes.

[*e*] The short answer here is yes, the maximum number of particles analyzed by the technique as presented is theoretically lower than a hypothetical technique that utilizes stepped-line illumination. This is because, when all particles in a field of view are illuminated at the same time, the emission spectrum from one particle may be projected onto a location that overlaps with another particle. Illuminating particles individually would reduce this issue. The point of this concept, however, is to create a simple and inexpensive device to produce information about fluorescence of individual particles. As the referee points out, adding a stepping

illumination line would introduce either moving parts or more complicated components and would also increase analysis complexity.

[*f*] Is the spectral range less? Again, I think yes.

[*f*] Again, the short answer is yes, the spectra range of the device discussed here is theoretically reduced by illuminating all particles at once. This is because the emission spectrum of one particle can be projected in the x-dimension such that it can overlap with the emission spectrum of another particle. The wider the spectral range of interest, the further individual particles must be separated to be able to illuminate them simultaneously.

[*g*] That probably isn't so important for fluorescence because the bands are not sharp so 20 wavelength bands may be adequate. Raman was mentioned. In Raman spectroscopy the light from 0 to 4000 cm-1 might be spread over 1000 pixels or so when illuminating with a line source. That requires significant distance on the camera. I wonder if the problem of overlapping spectra would make this multi-particle spectrometer approach unworkable for Raman in cases where a large wavenumber range is desired.

[*g*] Again, yes. As mentioned above, the fact that fluorescence bands are naturally broad reduces the requirements for high resolution. In concept, the device could be applied to the acquisition and analysis of Raman spectra, though there are a whole host of practical challenges associated with this extension of the idea. One of these challenges is that Raman spectra are fundamentally much narrower than fluorescence spectra, and thus, to acquire a Raman spectra with any reasonable level of resolution would require much higher resolution than would be required to achieve the fluorescence spectra discussed here. So it is possible that this technique could not practically be applied to Raman spectra. We very briefly introduced the idea as a tantalizing future possibility, but tried to do so in a way that did not promise that it would work. Based on the referee's valid comment we amended the statements in the manuscript (mostly in the final paragraph of Section 6: A vision for broad scale use) as follows:

(Starting L402): "The technique of acquiring spectra from individual particles *could* perhaps also be applied to the acquisition of Raman scattering spectra, though this *would* introduce additional technical challenges *such as the need for relatively high spectral resolution, which is compromised in our slitless spectrometer technique*. Recently an instrument for real-time detection of single particles in air by Raman spectroscopy has been made commercially available (Hill et al., 2015*a*; Ronningen et al., 2014). The instrument described here could be developed in the future to provide Raman spectroscopy of individual atmospheric particles, with reduced resolution or signal-to-noise, but also with significantly reduced cost. *The development of a Raman-oriented instrument would require significant future development, however.*"

Text added to before L228 as new Section 2.3:

"As a practical matter, the density of particles distributed on the slide should be sufficiently sparse that the spectral swaths do not overlap if individual particle spectra are to be determined. This requirement arises as a result of the fact that the entire field of view is illuminated at once, ideally exciting many, e.g. 5-30, particles. The wider the spectral range desired, the more this effect is enhanced. This particle density limitation is diminished, however, if one is only interested primarily in the relative fraction of particles that fluoresce at a given excitation wavelength.

The technique introduced here also presents fundamental limitations in spectral resolution influenced, in part, by particle size and homogeneity. For example, fluorescence emitted from the near side of a large particle at a given wavelength and Θ angle will be dispersed at the same Θ angle to a dissimilar point in the color swath from the far side of the same particle. This will blur the fluorescence spectrum in wavelength space, increasingly as a function of particle size. Additionally, if a given particle is inhomogeneous in composition, the fluorescence spectrum emitted by two points on the particle will be dissimilar, and thus the resultant spectrum will be smeared somewhat. Fluorescence emission bands are fundamentally broad and smooth, however, and so the extent of the associated smearing due to particle size or inhomogeneity does not practically impact the observed spectra for particles that are smaller than many tens of microns."

Text added to conclusions (at L428) summarizing advantages and disadvantages of the technique and to address many of the comments introduced by the referee:

"*The strong benefits of the described technique include that many particles can be analyzed simultaneously and that fluorescence spectra can be rapidly acquired for individual particles, each at multiple wavelengths, and at a cost potentially orders of magnitude lower than existing techniques. Further, the technique provides the possibility to probe at a glance for contamination of fluorescent particles that could contaminate a collection of non-fluorescent material, even without needing to analyze spectra."*

Technical Corrections 586, 592, 602 "fluorescent spectra", should be changed to "fluorescence spectra" as in every other time it occurs in the paper.

All changed.

425 "grass-type pollens (i.e. Ambrosia or ragweed)"? Ambrosia is not a grass. It is in Compositae (Aster family). If ragweed is in a grass-type pollen group, I suggest a citation for "grass-type pollen."

This is a good catch by the referee and a mistake on our part. We changed the statement in this case to say "grass-type pollens (i.e. *Dactylis glomerata* or *Orchard grass*) …". As re-written the existing citations are sufficient.