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

Anonymous Referee #2

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This manuscript describes the development of a new instrument to obtain scattering and fluorescence spectra from individual aerosol particles collected on a microscope slide. The new technology will certainly be of interest to the atmospheric science community and the manuscript is generally well structured however I think it could say more about certain aspects of the technology and the implementation. Therefore I recommend publication after the following comments have been addressed:

> Author response: We thank the referee for his/her positive assessment and recommendation for publication. The comments that s/he has brought up below are good ones, and the changes we have processed as a result have improved the quality and clarity of the manuscript. In preparation of the originally submitted manuscript, our goal had been to provide an introduction to the concept of the aerosol analysis technique, attempting to balance brevity and sufficient detail. In some case the balance may have been overly concise, lacking a few details that could help understand some of the process and trade-offs. We have added text in association with each of the points discussed below.
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> Note regarding document formatting: black text shows original referee comment, which have been chopped into individual thoughts, 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.

1. **[*1a*]** It seems that the size range of particles detectable by this instrument is a critical piece of information that is currently not addressed quantitatively. The authors state that they are targeting "micron-sized" particles, however, all of the known particles that they look at are pollen species which are significantly larger than a micron.

> **[*1a*]** It is true that the size range of particles detectable by this technique is important to introduce. First, the referee points out that we introduce the technique as detecting "micron-size particles," but only show particles > 10 µm in diameter. To be somewhat clearer we changed the wording in both instances it appears to refer to "supermicron-size particles" as described here:
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> > L13 (in Abstract): "We describe a novel, low-cost instrument to acquire both elastic and inelastic (fluorescent) scattering spectra from individual *super*micron-size particles …"
> > L71: "…where a minority of *approximately super*micron-sized particles …"
>
> To a deeper level, a discussion of the lower size limit of detectable particles brings in a much more complex discussion that we tried to avoid for this initial overview of the detection technique. The answer here is also inextricably linked to the question posed below, **[*1b*]** and **[*3a*]**.

[*1b*] Can the authors show what it looks like when this technology is applied to samples of smaller particles such as bacteria, spores or man-made size-selected particles such as polystyrene latex spheres?

Since detection of spores seems to be one of the main motivations it would be nice to show that this instrument can work with something other than pollen.

> **[*1b*]** The main point of the manuscript was to introduce the general concept and to provide a proof-of-concept, but not to explore all physical relationships. As a result we had originally chosen to show images and spectra representative of certain pollen. The request to show the response of the instrument to smaller particles is certainly a reasonable request, however, and we agree that adding this information would improve the manuscript. In response to the referee's comment we added Supplemental Figure S2 showing images and spectra of 0.96 µm polystyrene latex beads and we added the following text at L253:
>
> > "This fraction is also 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.*"

[*1c*] Along similar lines, I believe the authors state that the height of the swath is related to the particle size. More explicit discussion of this relationship would be helpful.

> **[*1c*]** We addressed a similar question posed by Referee #1 in Point **[*b*]**. The following text was added at L108:
>
> > "*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.*"

2. In general, it would be nice if all of the graphics could be accompanied with a quantitative statement of what is "found" in the view graph. **[*2a*]** For example, in figure 2, what percentage of the particles appearing in panel a result in a spectrum in panel d? Clearly it is most of them but it would be nice to know if it's 100% or something less than that.

> **[*2a*]** The statement requesting quantitative statements about the images is also understandable. In the case of Figure 2, we previously addressed this idea in the submitted manuscript by stating the following (L207): "By comparing Figures 2c and 2d one can see that the relative fraction of pollen particles fluorescent in this sample is nearly 100%, since this

particulate sample is made up of a single kind of pollen." To be clearer we changed the text in the manuscript as follows:

"By comparing Figures 2c and 2d one can see that the relative fraction of pollen particles fluorescent in this sample is nearly 100%, since this particulate sample is made up of a single kind of *relatively highly fluorescent particles. A particle-by-particle comparison is somewhat more difficult, because each spectral swath (Figs. 2b-d) extends to the left from the non-dispersed particle location (Fig. 2a). In some cases the swaths of multiple particles overlap, and in other cases the spectrum is dispersed to a point out of the field of view. A more detailed analysis of all individual particles in Figure 2 is described in the online supplemental information (Fig. S1).*"

We also added Figure S1 and associated supplemental text to unambiguously discuss how the spectrum from each individual particle is presented in the dispersed and undispersed panels of Figure 2.

[*2b*] Then in figure 4, quantitative information is given for the top panels but not for the bottom. Here it would be nice to know how many quartz particles are identified in the viewgraph and what fraction of that number the "fluorescent needle in the haystack" contributes. If only 10% of all particles are identified as fluorescent in an ambient sample, then a "false positive" rate of even a few percent could be significant.

[*2b*] First, we added a quantitative assessment to the statement in the text, as copied here: L266: "Figure 4f, however, shows one unexpected, strongly fluorescent particle and approximately three *to five* other weakly fluorescent particles *out of the 200-250 particles in the image (e.g. ~2%)*."

More importantly, however, the comment by the referee reveals a misunderstanding that we realized we need to clarify. It is true that when applied to ambient aerosol sampling, a "false positive" of a few percent could add significantly to the uncertainty of the overall measurement. In the case described here using ground quartz, however, the few particles that fluoresce are introduced as anomalous not because they show erroneous fluorescence as an instrumental error, but rather the fluorescence exhibited is real and reveals that a small, but non-zero, fraction of particles exhibit unexpected fluorescence. The point of this example was indeed to show that one application of the technique may be to probe for fluorescent contaminant particles in a matrix of predominantly non-fluorescent particles (i.e. "haystack"). So the quantitative percentage (i.e. ~2% in Fig. 4f) is not the point as much as that individual particles can be detected despite the fact that there are so many others in the image. Of course, the quantitative assessment of the fluorescent fraction may be useful, but this will depend on the threshold at which 'fluorescent particle' is determined, as discussed above.

To clear up some of this we changed the following sentence at L273:

"*This example* illustrates how *fluorescent* impurities might easily be detected *at a glance* with our apparatus even in the presence of a large majority of non-biological, or otherwise non-fluorescent particles, *and without needing to go through the extra step to extract the actual spectra. Analyzing images in this way also removes the restriction of limiting spectral swaths from overlapping, and enables a user to collect a rather large number of particles in one field of view (e.g. hundreds) compared to the far smaller number limiting the analysis if determination of individual spectra is desired. The collection and analysis of particles in this*

case can be very rapid and yet can provide a powerful diagnostic tool by positively identifying the approximate fraction of fluorescent impurities in a collection of particles."

3. **[*3a*]** Related to comment 1 above, the functional minimum size for fluorescent detection may also not be a limitation purely of how small a particle can be imaged through the microscope optics but, rather, how much fluorophore a particle must contain to yield a detectable spectrum given the hardware.

[*3a*] In short, the referee is absolutely correct. However, in addition, the concept of detectability here is actually somewhat more complicated even than the referee points out, because it is inextricably related to at least five physical parameters: (1) particle size, (2) fluorophore amount and quantum yield, (3) intensity of excitation source, (4) instrument optics, and (5) detector exposure time. This has been dealt with at length in various other publications and was hinted at in the originally submitted manuscript, however, we have added brief discussion of this concept more directly, including five associated references.

Based on this, the functional minimum size for fluorescence detection is not a trivial parameter to rigorously define, but it is at least < 1 µm. We have added a new figure to the online supplement that shows images and spectra for 0.96 µm fluorescent polystyrene latex beads supporting the idea that the lower limit for particle detection is at least smaller than this size. We feel that a rigorous exploration of these four inter-related variables as it relates to particle size would be well beyond the scope of the present manuscript. See point **[*1b*]** for text that was inserted along with the new supplemental Figure S2.

[*3b*] What is the primary limitation to detection of "less bright" fluorescent things? For example, in Figure 4c, I can see the 7 spectra discussed in the paper but I can also see 5 or 6 other, more faint spectra that could also be fluorescent particles.

[*3b*] See response to **[*3a*]**.

[*3c*] How have the authors determined the intensity threshold required to call a particle fluorescent?

[*3c*] Essentially any fluorescence threshold applied to this style of instrument (including the commercial single-particle instruments such as the UV-APS and WIBS instruments commonly applied to atmospheric aerosol analysis) rely on what is essentially an arbitrary fluorescence threshold limit. WIBS users, for example, typically use a somewhat more rigorously defined lower limit of fluorescence based on the baseline plus three sigma (standard deviations) of observed fluorescence intensity as the lower limit. This is strongly dependent on the voltage gain applied to the PMT detector, however, and this value is rarely monitored or reported, so the threshold becomes somewhat arbitrary. Laboratory work is being done to understand and find suitable calibration routines to make these procedures more standardized for such instruments, and we plan to do similar laboratory work to develop a rigorous calibration procedure to define thresholds for our instrument. This could be done, for example, by measuring fluorescence spectra of a standard particle with a known fluorescence intensity. This in itself is a very challenging task, however, because fluorescence intensity for most particles varies as a function of age and chemical environment, not to mention particle size. So the development of routine procedures are planned for future developments, but are excluded from the present manuscript due to the high level of complication and complexity.

4. **[*4a*]** Can the authors provide the dimensions (distance to camera and angle theta for collection) of their two instrumental set-ups along with the imaging area and pixel size of the cameras and phones used?

[*4a*] Some of these details were introduced previously. For example, horizontal dimension of benchtop instrument field-of-view was presented in L178. We have extended specifics of instrument, as requested and as outlined below, and have added Table S1 to the supplemental information, copied below:

Camera								
Manufacturer	Model	Type	Color / Monochrome	Detector type	Number of pixels (x 10^6, Mp)	Pixel matrix (L x H)	Pixel size (L x H) µm	Citation
Canon	Powershot A2300 HD	Point-and-Shoot	Color	CCD	15.9	4608 x 3456	1.3 x 1.3	[1]
Lumenera	Infinity 2-1R	Research microscopy	Monochrome	CCD	1.45	1392 x 1040	4.6 x 4.6	[2]
Apple	iPhone 5s	Smartphone	Color	CMOS	8.0	3264 x 2448	1.5 x 1.5	[3]

In addition, the following text was added or amended:

L169: "*See supplemental Table S1 for details regarding specifications of cameras discussed here.*"

L172: "At the approximate angle of first order diffraction (*e.g. approx. 11° for red, 7° for blue light as defined by Eq. 1*)"

L178: "field of view (of the order of *1.0* mm *wide by 0.7 mm high under 10x magnification*)"

L580: "*For standard bench-top set-up approx. distances are as follows: objective lens to grating (20.5 cm), grating to camera (11.4 cm).*"

L590: *"Canon Powershot A2300 HD camera utilized offers 4608 x 3456 square pixels 1.3 µm in size."*

L625: "*As shown, field of view is a 2 mm circle. Approx. distance from objective lens to camera is 6 cm.*"

Additionally, the magnification of the objective lens used is an important instrumental parameter, as was introduced to the revised text at L253 and as was discussed in response to Point **[*1b*]**. 10x objective lens used for all images shown, and these details were added to each corresponding figure caption, i.e. at Lines L584, L602, L619, and L623.

[*4b*] I believe the combination of these choices is what determines the spectral resolution achieved and it would be nice to walk the reader through these relationships.

[*4b*] The referee is partially correct in his/her statement about the factors that determine spectral resolution. The physical set-up does, indeed, influence the spectral resolution. The most important physical parameters involved, however, are the dispersion angle, as defined by the

grating, and the distance from the grating to the detector (camera). Because the *Θ* angle is fixed for a given grating according to Equation (1), increasing this distance the between the grating and camera results in a longer spectral swath. The longer spectral swath projected onto a CCD with fixed number and density of pixels per unit area or distance thus provides more spectral resolution. If the instrument is otherwise adjusted such that the image is in focus, these parameters influence spectral resolution most directly. As discussed in response to Referee #1 under Point **[*d*]**, the size and homogeneity of the particle interrogated will also influence the limit of spectral resolution.

5. In section 4, I don't follow why a 3000 k blackbody spectrum is used to approximate a theoretical scattering curve for NaCl. Is that supposed to read 300 k? If so the same type-o occurs in the legend of Figure 5.

This is a simple misunderstanding, and we altered the text a bit to make sure it is less likely to happen for other readers. The caption text of Figure 5 states: "Reference spectrum (dashed blue) shows calculated blackbody radiator at 3000 K multiplied by CCD sensitivity curve …", and the text (L293) states similar text. We clarified the manuscript text as follows:

(L293) "For comparison, Figure 5 also shows the emission spectrum from a 3000 Kelvin blackbody, *as an approximation of the emission from the heated tungsten filament source used for white light,* multiplied by the theoretical sensitivity curve of the CCD used in the monochrome camera. The theoretical ~~*blackbody*~~ curve *represents the spectrum that the CCD should detect assuming the particle does not introduce any wavelength-dependent scattering features. In this case* the measured elastic scattering curve *(black line)* matches closely *with the theoretical curve (blue, dashed line)*, suggesting that the monochrome camera introduces very little aberration as a function of wavelength. In contrast, the color camera shows a spectrum with pronounced peaks that are *introduced by* the different color pixels."