Review of manuscript acp-2012-724 by Huffman et al., submitted to ACP.

General Comments

The MS presents measurements and analysis of biological aerosol particles in a pristine rainforest in the Amazon. The primary measurement instruments were the UVAPS; a semi-automated SEM that can detect thin organic coatings and analyze with EDX, and which can perform secondary ion mass spectrometry (NanoSIMS) on selected particles; and light microscopy in combination with a stain for chitin. The combination of UVAPS measurements with these powerful additional measurements is an important step forward. The comparison between the UVAPS measurements and the collected particles appears to be simpler at the rainforest location studied because this pristine environment has a relatively small number of anthropogenic particles (e.g., smokes).

I recommend publication. However, I request consideration of the following questions and suggestions.

Specific Comments

A) There seems to be a tendency to either over-generalize regarding LIF, or to not be sufficiently careful in making some LIF related statements.

A1) p. 25183, Abstract: "We provide key support for the suggestion that real-time laser-induce fluorescence (LIF) techniques provide size-resolved concentrations of FBAP as a lower limit for the atmospheric abundance of biological particles."
- - This would be more accurate if changed to:"We provide key support for the suggestion that real-time laser-induce*d* fluorescence (LIF) techniques *using 355-nm excitation in a pristine environment* provide size-resolved concentrations of FBAP as a lower limit for the atmospheric abundance of biological particles."

A2) p. 25202, lines 13-15: ". . . while the UV-APS is able to detect FBAP efficiently during certain periods of the day, a certain fraction of biological material escapes characterization using online autofluorescence."
- - Shouldn't that be, "escapes characterization using *the UVAPS with 355-nm excitation*"?
- - Sivaprakasam et al. (2004) [Multiple UV wavelength excitation and fluorescence of bioaerosols , Sivaprakasam, V; Huston, AL; Scotto, C; et al. OPTICS EXPRESS, Volume: 12, 4457-4466 DOI: 10.1364/OPEX.12.004457, SEP 20, 2004] show fluorescence cross sections they measured using their single particle online LIF system (both at 266 and 355 nm) for a variety of particles such as bacterial spores and vegetative cells prepared under different conditions (see their Table 2). The average particle diameters are 1 micrometer for most of these samples. How could Huffman et al know that their "fraction of biological material" would escape "characterization using online autofluorescence" with Sivaprakasam's instrument operating at 266 nm?

A3) p. 25211, summary, lines 17-19: "FBAP can only be considered a lower-limit of PBAP, however, because some biological particles exhibit fluorescence below the detection limit of the UV-APS and related instruments."

A3.1) Given the totality of this paper (including the citing of the Gabey et al (2010) and its use of the WIBS3, and including the other LIF instruments listed in the introduction) I don't see that the above statement can be taken to exclude 266-nm or 280-nm based LIF systems. It is not clear that the instrument Sivaprakasam et al (2004) used at 266 nm could not see fluorescence from some of these small biological particles that are not detected by the UVAPS or even by the WIBS3.

A3.2) A main finding of the work is that there is a second group of particles, 0.7 to 1.x micrometers, that have negligible or very weak fluorescence as measured by the UVAPS, but which are probably biological because their concentrations have a time dependence similar to that of the fungal spores. That is an interesting observation. I suggest more discussion/explanation/speculation regarding what these weakly or negligibly fluorescent particles might be and whether the fluorescence might be more detectable if the illumination was at wavelengths below 290-nm. Some bioparticles fluoresce negligibly or very little when excited by a 355-nm laser but still fluoresce when excited at 280 or 266 nm. See, e.g., ovalbumin or bovine serum albumin in Fig. 4 in Sivaprakasam et al. (2004), which shows plots of fluorescence excited by 355-nm excitation vs fluorescence excited by 266 nm excitation. The fluorescence of pure proteins is dominated by tryptophan, with contributions from tyrosine. Proteins tend to have weak fluorescence if excited at 355-nm, unless they contain fluorescent impurities. Bacillus spores are reported to have negligible NADH, but may have some fluorescence from flavins. Viruses contain nucleic acids and protein, and in some cases lipids or lipopolysaccharides, but purified viruses typically would not contain fluorophors other than the aromatic amino acids.

A3.3) p. 25201, lines 7-10. "Gabey et al. (2010) also reported a mode of non-fluorescent particles associated with the FBAP peaks, suggesting weakly fluorescent particles [could?, should?] not be characterized as biological by online aerosol LIF instrumentation."

- - Gabey et al (2010) wrote, "an unknown number of fluorescent particles inevitably go undetected through instrument sensitivity limitations." As far as I can tell, Gabey et al (2010) operated their WIBS-3 with settings that allowed them to measure fluorescent particles as large as 20 micron diameter, maybe higher. It seems that if a UVLIF system is operated with parameters optimized to see the fluorescence of 1 or sub-1 micron particles, it will have a much greater chance of detecting these smaller, maybe more weakly fluorescent, particles. The fluorescence cross section increases roughly as the square of the diameter for tryptophan-containing bioparticles greater than about 1 micron diameter excited at 266 or 280 nm. The ratio of fluorescence cross sections for a 20-micron diameter particle relative to a 0.8-micron particle composed of the same material is roughly 625. A system optimized for the 0.8 to 20 um diameter range is not optimized for the 0.5 to 2.0 micron diameter range. Optimizing for that smaller range might entail: using more sensitive detectors or increasing the gain on the detectors; increasing the output of the lamp or laser; replacing the lamp/laser with one that emits more

energy; focusing the excitation light more tightly. Also, it may entail using some type of particle filter (e.g., similar to the tubing used in Huffman et al study under consideration which limited particle sizes to roughly 7.5 micron diameter) in order to reduce the particle sizes that reach the LIF system so that fluorescence from large fluorescent particles does not damage detectors setup for high sensitivity measurements.

A3.4) Of course the statement, "some biological particles exhibit fluorescence below the detection limit of the UV-APS and related instruments," is probably true if very small PBAP (e.g., a 3-nm or 50-nm bit of cellulose blow off of some dying plant) are included. Such a small particle would be far more difficult to detect with LIF than would any of the particles mentioned in this paper. However, in the context of the sizes of the particles studied in this paper, the authors do not show that this statement is true for "related instruments", i.e., other single particle LIF instruments.

A3.5) Whether or not the FBAP is a "lower limit of PBAP" depends upon the concentration of fluorescent nonbiological particles in the air at the particular location and time. I believe that FBAP is (or is likely to be) a lower limit of PBAP at the AMAZE study site. I don't know enough to say that it is a lower limit in the case of some smoky, urban or industrial environments.

B) p. 25211, lines beginning at 26, regarding detection of, "relatively transparent basidiospores whose relatively thin cell walls allow the interrogating laser pulse to excite fluorescence without significant absorption or quenching."

Why is quenching relevant here? Please cite a study or two which suggest how it would be.

C) Having been influenced by some papers and talks by this group, I sometimes try to use "online" in the way that this group does. But I tend to receive questions about the meaning of the word, and the resulting discussions aren't necessarily productive. Typically in discussions about meanings of words I like to find the word in a dictionary so I can say, e.g., "the definition I mean is similar to definition 3.a in whatever dictionary is relevant." I'm not sure what all is in the meaning of online intended by Huffman et al. Continuous sampling is part of it. What else? If there is nothing else, how about using "continuous." Real-time? Connected to a computer? I suggest that the authors: i) add to this paper the definition of online they are using/assuming; and, ii) indicate which dictionary (if any) uses that definition of online. Unfortunately, I know of more than one case where older literature does not appear in computer searches because the terminology for some phenomenon has changed. I can easily imagine that happening with a word like "online."

D) Please mark sunrise and sunset times on the figures. The diurnal analysis is a central part of this MS. It is simpler to think of diurnal variations in local times. Please change all UTC times to local times.