

Fluorescence preselection of bioaerosol for single-particle mass spectrometry

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We have designed, constructed, and tested a system that preselects the biological fraction of airborne particles from the overall aerosol. The preselection is based on fluorescence emission excited by a continuous 266 nm laser beam. This beam is one of two cw beams used to measure the aerodynamic particle size of sampled particles. The intention in our system is that single particles, based on size and fluorescence emission, can be selected and further examined for chemical composition by mass spectrometry. © 2006 Optical Society of America

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1. Introduction

One possibility for rapid detection and identification of airborne bacteria is to perform mass spectrometry on ions desorbed from single particles.^{1,2} Laser-based aerosol time-of-flight mass spectrometry functions by generating a beam of particles that pass through the ion source of the mass spectrometer. Particles are located with high precision in time and space (usually by light scattering) and are subsequently intercepted by a short laser pulse generating the characteristic ions. The strength of this approach is that, despite the small size of the particles, the local concentration of material can be quite high. On the other hand, a single-particle technique leads to a tremendous number of mass spectra that must be analyzed.

This limitation inherent in single-particle mass spectrometry is particularly severe in the case where one is looking for a minority species from an overall sample, especially if rapid detection is important. This is the case when sampling for airborne bacteria, where the bacteria can be expected to be a small fraction of the total aerosol. A short example demonstrates the situation. A goal for the sensitivity of a biological point detector is one agent-containing par-

ticle per liter of air detected within five minutes. The particle concentration of ambient aerosol is highly variable, but for this example a value of 30000 particles with diameters between 1 and 10 μm per liter air is used.³ This implies that the expected waiting time between bacteria particles is 30000 samples. Given that the maximum sampling rate of the current system is approximately 10 Hz, this implies 50 minutes of collection between individual bacteria particles. Additionally, each mass spectrum requires about 10 Kbytes of data, implying that 300 Mbytes of data must be collected and analyzed per bacteria-containing particle.

The high sensitivity requirement for point detectors almost certainly requires an aerosol preconcentration step, such as a virtual impactor. These devices are available with an inlet flow rate of 1000 liters per minute. In this case (assuming a 1% transfer efficiency for the particle beam generator (unpublished data)), there are approximately 5000 particles per second passing through the ion source of the instrument. However, within any five-minute sampling period, only 50 bacteria particles would have passed through the system. Further, because the bacteria represent such a minority species, the system is usually processing other particles when the interesting ones pass through the system. It is in an effort to access the relatively few bacteria particles available that is the motivation here.

Considerable work has been done characterizing the fluorescence properties of airborne particles containing bacteria. Commonly, ultraviolet light has been used for excitation because the photon energy is sufficient to excite many of the fluorophores present in significant quantities in bacteria cells. Several ex-

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citation options have been explored: in an early attempt,⁴ particles were passed through the internal cavity of an Ar⁺ laser operating at 488 nm where flavins were excited; another has been to excite aromatic amino acids (primarily tryptophan) using light in the range from 260 to 280 nm⁵⁻⁷; and a third has been to target nicotinamide adenine dinucleotide compounds (NADH) using light in the range from 325 to 360 nm.^{8,9} This last choice implies that the sensor will respond to cells with a high degree of ongoing metabolic activity. In general, none of these options has been shown to provide fluorescence emission that is organism-specific.¹⁰ Also, it is known that other types of particles produce spectra similar to bacteria, especially at the shorter wavelength.¹¹ Under laboratory conditions it has been demonstrated that multiple-wavelength excitation can provide discrimination between some bioaerosol particles, whereas a single excitation wavelength would not. For instance, using 266 nm excitation, certain fungal spores and vegetative *B. subtilis* (BG) cells have nearly identical emission spectra. However, with 355 nm excitation the two types of particles can be distinguished.¹¹ Recently,¹² an effort was made to combine into one system a multiple-wavelength excitation (using 266 nm and 355 nm light) single-particle fluorescence analyzer. This system shows promise in differentiating classes of aerosols, which were composed of pure protein, spores, vegetative cells, and some common confounding types of aerosol.

The use of a fluorescence signature as a trigger for other, more specific detection methods is a basic idea behind existing strategies for biological aerosol detection.¹³ Recently,¹⁴ fluorescence emission was used as a trigger for a mechanism to physically separate biological aerosols from nonbiological aerosols by means of a short puff of air. The concentrated biological particles could then be examined by more specific techniques. In an early version of a similar instrument,¹⁵ Chen *et al.* used fluorescence emission generated from dye-doped polystyrene particles passing through a continuous 488 nm laser beam to trigger pulses of 266 nm laser light. Single-particle fluorescence spectra were subsequently collected, where the particles had been differentiated based on the emission from the 488 nm excitation. However, this type of fluorescence trigger system was not further pursued. In this paper we use fluorescence emission, excited with continuous 266 nm laser light, as one of the scattering events in an aerodynamic particle-sizing scheme. In this way, we incorporate a fluorescence trigger for biologically based aerosol particles into a single-particle mass spectrometry system.

2. Instrumentation

The basic aerosol mass spectrometer has been described in detail elsewhere,^{16,17} and we give a brief description here. Aerosol is pulled into the instrument through four differentially pumped chambers, forming a particle beam in the vacuum chamber of the mass spectrometer. Particles between approxi-

mately 0.5 and 10 μm are detected and aerodynamically sized by measuring the time between scattering events generated by particles passing two laser beams. Based on the particle velocity, an ionization laser is triggered intercepting virtually all particles. The ionization laser is an excimer laser operating at 308 nm. Positive ions generated in this manner are accelerated toward multichannel plates for mass identification. This paper explores several versions of the optical configuration and expands the basic detection scheme to include *selection* of the biofraction of the aerosol.

The original optical configuration for our system has been retained and supplemented. In the original configuration a randomly polarized helium–neon laser at 632.8 nm passes through a beam splitter generating two parallel detection laser beams that are vertically displaced by a distance of 2.5 mm. Scattered light from these beams is collected and analyzed at 45° relative to forward scattering. Our triggering circuit can utilize the signals from this single detector to ionize particles regardless of chemical composition. We refer to this arrangement as one-color triggering.

Additionally, we have mounted a lens system and a uv-sensitive photomultiplier tube (PMT) at 90° relative to forward scattering. The collection lens, which is inside the vacuum chamber, is $f/1.6$ and positioned to approximately collimate scattered light from the particles. The second lens, which is external to the vacuum chamber, is $f/6$ and focuses the collected light onto the PMT. This lens system is meant to collect fluorescence emission excited by a continuous-wave 266 nm laser (Coherent MBD-266, which is a Verdi Nd:vanadate with an external doubler). The 266 nm laser spot is approximately 200 μm in diameter and is positioned coincident with one of the helium–neon spots. The approximate spot size of the beams and their relative locations can be estimated by analyzing the PMT output traces (reflecting particle passage) on our oscilloscope. The triggering circuit has been modified to incorporate the output of the second photomultiplier tube in a two-color detection scheme using the fluorescence emission as one of the trigger signals. In this mode of operation, a high wavepass filter (CVI laser, Albuquerque, New Mexico) turning on at 290 nm and a 633 nm rejection filter are placed in the collimated section of the collected light; this is similar to that described by Kaye *et al.*¹⁸ In this way, particles with the fluorescence characteristics of bacteria are selected by the system for further investigation by the mass spectrometer. With a manual switch, the system can be set to detect either all suitably sized particles (elastic scattering only) or suitably sized particles with total fluorescence emission above an adjustable threshold. The particles are resident in the 266 nm beam for about 2 μs , and the laser fluence is approximately 2 mJ/cm^2 . A schematic diagram of the system is shown in Fig. 1.

We have used a slightly different configuration to collect fluorescence spectra from single particles. In

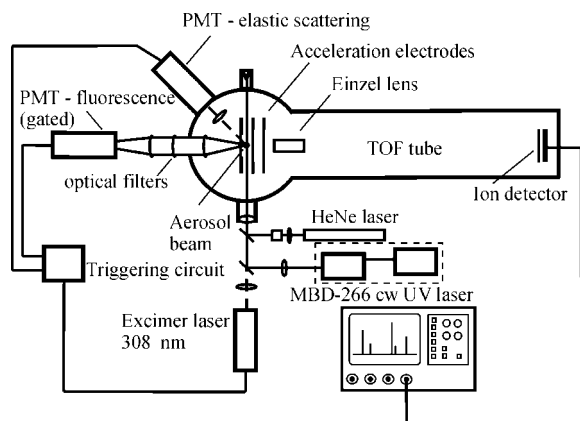


Fig. 1. Schematic diagram of the system.

this configuration the 266-nm laser spot is positioned approximately 1 mm below the second helium–neon laser spot. Light collected at 90° is imaged onto the entrance slit of a 0.3 m spectrograph. On the exit plane of the spectrograph we mounted an intensified CCD camera. Given our choice of diffraction grating, a spectral window of approximately 350 nm could be dispersed along the length of the chip. Based on the measurement of the particle velocity, the intensifier of the detector was timed to coincide with the presence of the particle in the 266-nm beam. Spectra were generally collected for about 1 μ s.

Aerosol was generated either from solution with a Collison six-jet nebulizer or dispersed with a Devilbiss Model 175 as a dry powder. Typically, a short puff of aerosol into a retention chamber with either device was sufficient for 5 to 10 minutes of sampling by the aerosol mass spectrometer.

3. Results and Discussion

It is known that biological material in general, and whole cell bacteria in particular, exhibit fluorescence under ultraviolet irradiation. The fluorophore primarily responsible for this emission is the amino acid tryptophan, which typically comprises a few weight percent of the cell mass. The emission range for pure tryptophan under 266 nm excitation is from approximately 290 to 380 nm, depending somewhat on the molecular environment, with a maximum at approximately 330 nm.

Figure 2 shows fluorescence spectra obtained from single aerosol particles composed of tryptophan and of three species of bacteria: one from vegetative cells and two from spore samples. The particles resulting in the top three spectra were dispersed from dry powder and had diameters between 2 and 3 μ m in diameter; the *E. herbicola* particles were generated with the Collison nebulizer and were predominately composed of single cells of submicron aerodynamic diameter. The spectra are of reasonable quality considering the small amount of material represented by the sample. It is doubtful, however, whether such spectra could be used for discrimination between species, but it is clear that discrimination between whole cells and

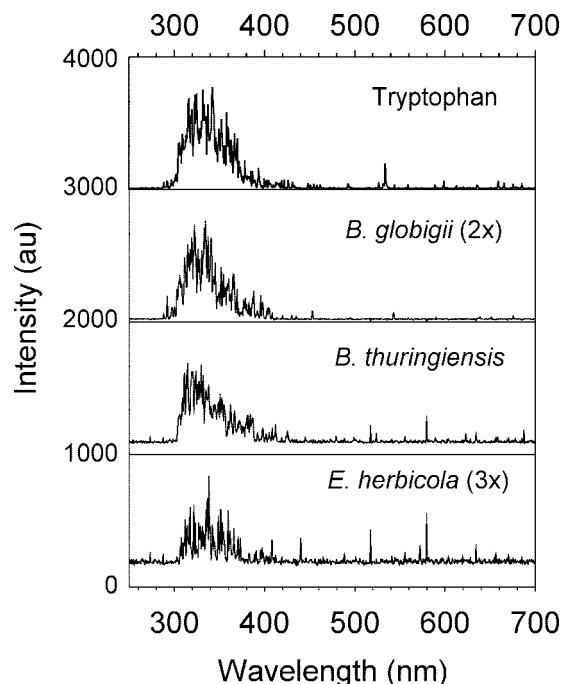


Fig. 2. Single-particle fluorescence spectra obtained from particles containing tryptophan and three species of bacteria cells.

other nonbiologically based material should be possible. The fluence used for this measurement, 2 mJ/cm², is somewhat lower than that reported for published values. For instance, Pan⁴ reports 30 mJ/cm² for his single-particle fluorescence spectrometer; in that case a Nd:YAG laser with 70 ns pulse length is used. We attribute the ability to obtain spectra from single cells to the considerably longer time that the particle is resident in the laser beam.

Figure 3 provides an example of the discriminating power of the two-color triggering scheme with polystyrene latex spheres of five different sizes. Blue fluorescing particles of 1.0 and 2.1 μ m were used, together with undyed particles of 1.3, 1.8, and 2.5 μ m.

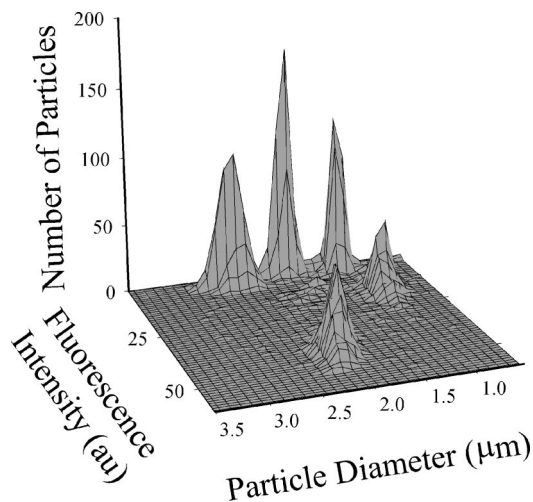


Fig. 3. Particle aerodynamic size/fluorescence intensity histogram for a series of polystyrene latex spheres.

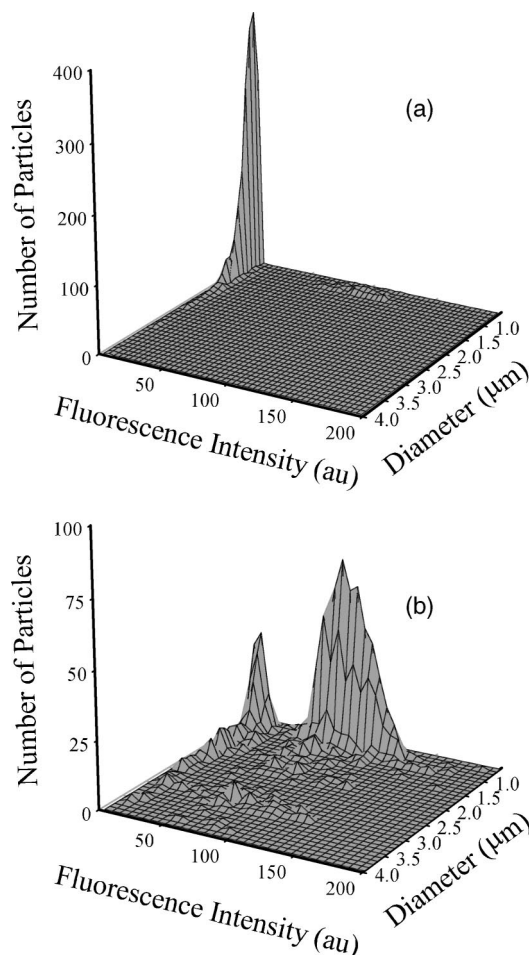


Fig. 4. The aerodynamic size/fluorescence intensity histograms generated by sampling an aerosol produced from unwashed *B. globigii* spores using (a) one-color detection and (b) two-color detection.

The diagram consists of approximately 1000 particles of each type. In this set of measurements, the aerodynamic size is measured from 633 nm scattering around 45° and the fluorescence emission is recorded from light emitted at 90°, for all particles. The fluorescence intensity reported here is integrated over the time window for particles resident in the 266 nm laser spot. The full width at half-maximum of the particle size measurement for these monodisperse spheres is approximately 0.2 μm over the range of the diameters. Based on this value, the particles are just discriminated based on aerodynamic diameter. However, the particles are clearly separated once the fluorescence intensity is incorporated. The undyed particles do show some fluorescence, but an amount that is much less than the dye-doped particles.

Figure 4 demonstrates the selective capability of the two-color triggering. Aerosol is generated from a sample of unwashed BG spores in a Collision nebulizer. In Fig. 4(a), one-color particle detection is employed. The result is that the predominance of particles has aerodynamic diameters less than one micron, with very little fluorescence emission. The

size distribution of these particles is consistent with that formed by evaporating solvent from a nonvolatile solute. In this case, the nonvolatile material could have been stabilizing salts commonly used in bacterial preparations or from organic material extracted from the cells into the fluid. However, there is another population of particles within the sample. These are particles with aerodynamic diameter between approximately 0.7 and 0.9 μm and have significant fluorescence. These particles appear to contain a single spore. Figure 4(b) shows the selected particle distribution when the triggering circuit is switched to two-color triggering. In practice, this means moving a switch, so that the data of Fig. 4(b) were collected immediately after the data presented in Fig. 4(a). The result is that the particle distribution shifts dramatically, emphasizing the particles that were only present as an extreme minority in the one-color detection scheme. In principle, the trigger threshold could have been increased somewhat further to eliminate particles with little fluorescence (below the arbitrary value of about 35) and do not seem to be the particles of interest in a bacteria analyzer. This example demonstrates the selectivity of the two-color triggering method. Using this configuration, the mass spectrometer, which is the limiting component in terms of speed, can be utilized more effectively by limiting its analysis to particles that are likely to contain the bacteria cells of interest.

A further experiment attempted to link the fluorescence triggering with the mass analysis. For this purpose, combining particles of lead chloride with particles of tryptophan formed an externally mixed aerosol. The lead chloride aerosol was generated from aqueous solution, and the tryptophan aerosol was dispersed as a dry powder. In addition to the fact that only the tryptophan particles will fluoresce under 266 nm light, the ionization energy of these two materials is comparable. Therefore good quality mass spectra are obtained from either material under the same conditions. Particles of the respective materials yield distinct mass spectra. The mass spectra from the lead particles are dominated by lead-containing ions, including the Pb^+ ion at $m/z = 207$ Da and those containing two lead atoms with m/z greater than 400 Da. The spectra of the tryptophan particles are dominated by decomposition products, with the most intense peak frequently being the residual indole ring structure with $m/z = 130$ Da.

Figure 5(a) shows 20 consecutive mass spectra obtained from the mixed aerosol using one-color detection. The particular sequence was chosen to make a point, but was typical of the data collected during the experiment. The first particle in the series is generated from a lead chloride particle and has the characteristic ion markers at above 200 Da and then again at above 400 Da. The second particle results from the ionization of tryptophan, with the characteristic mass peak at 130 Da. The next few particle spectra contain the characteristics of lead chloride, but the eighth particle in the sequence indicates a

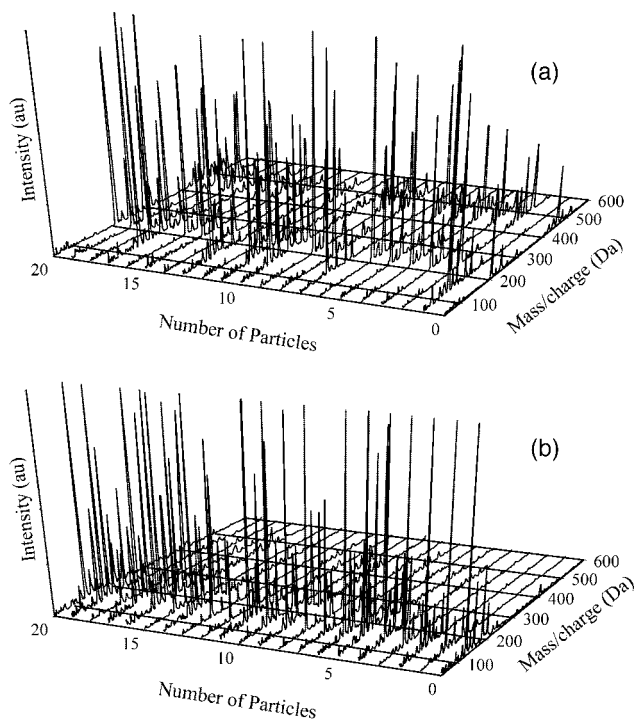


Fig. 5. Consecutive single-particle mass spectra collected from an externally mixed aerosol containing lead chloride particles and tryptophan particles using (a) one-color detection and (b) two-color detection.

tryptophan particle. The series continues in this apparently random sequence. However, when the triggering circuit is switched to two-color detection, particles of nonfluorescing lead chloride are ignored, and only mass spectra of tryptophan are recorded. This is evident in Fig. 5(b), where all spectra exhibit the mass peak at 130 Da.

4. Conclusions

We have developed a system for selecting the fraction of aerosol that exhibit the fluorescence properties of bacterial cells for further examination by time of flight mass spectrometry. Fluorescence emitted under 266 nm excitation is used as one of the light scattering events in an aerodynamic sizing mechanism and differentiates bacteria particles from the bulk of other particles likely to be present in the atmosphere. Our system can generate an aerodynamic particle size/fluorescence intensity histogram and mass spectra for selected particles entering the system. The result is that the mass spectrometer is reserved for the relatively few particles likely to contain bacteria that are of interest for our application.

Pinnick¹⁹ presents fluorescence spectra from atmospheric particles in the size range of 3 to 10 μm . Approximately, 8% has significant fluorescence, and about 1/2 of the fluorescing particles show spectra peaking in the 317–341 nm range, indicating the possible presence of bacteria and spores. The authors conclude that, in general, these spectra cannot be interpreted unambiguously. We propose that the flu-

orescence emission be used to trigger a mass spectrometric analysis of the particles to obtain more detailed chemical information.

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