Research Article

Development, Characterization, and Application of a Versatile Single Particle Detection Apparatus for Time-Integrated and Time-Resolved Fluorescence Measurements—Part II: Experimental Evaluation

Xihong Wu, J. A. Merten, N. Omenetto, B. W. Smith, and J. D. Winefordner

Department of Chemistry, University of Florida, Gainesville, FL 32611, USA

Correspondence should be addressed to N. Omenetto, omenetto@chem.ufl.edu

Received 10 February 2009; Accepted 13 April 2009

Recommended by Savas K. Georgiou

This paper describes the experimental realization and characterization of a versatile single particle detection apparatus. The system utilizes a novel particle beam inlet that can serve as either an on-line particle concentrator (i.e., all diameters confined in a narrow beam) or as a segregator (i.e., selected diameters confined in a narrow beam) and can be operated in a high-speed mode as well as in a low-speed mode, thus allowing different interaction times between the particles and the laser beam. An aerodynamic sizing technique has been incorporated into the system to provide rapid, real-time, and high-resolution sizing. Parameters such as transmission efficiency and size-segregation efficiency have been measured. The performance of the instrument has been demonstrated by on-line detection of spectrally resolved and time resolved fluorescence detection from airborne dyed-particles and aerosolized endogenous fluorophores found in biological agents.

Copyright © 2009 Xihong Wu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Airborne particles, biological or nonbiological in origin, play a crucial role in many health and environmentally related issues [1]. The behavior and impact of aerosols are primarily governed by their physical and chemical properties, such as particle size and chemical composition. Information about the chemical composition of an airborne particle is essential in order to identify its source and ascertain whether it poses an extraordinary hazard to humans or the environment. In fact, in addition to several important studies regarding the generation of particle beams and their focusing properties (see, e.g., [2–10]), in the last 20 years there has been a great interest in the development of methods for real-time detection and identification of individual airborne particles, including bioaerosols and pathogens [11–47]. Various techniques, including Mass Spectrometry (MS) [11–29], Laser Induced Fluorescence (LIF) [30–44] and Laser-Induced Breakdown Spectroscopy (LIBS) [45–47] has been extensively studied, either alone or in combination with

each others (see, e.g., [41, 42]), as means for characterizing both biological and nonbiological particles. It is believed that advances in aerosol instrumentation for the study of individual aerosols on a real-time basis have opened a new avenue for better understanding of the attributes of particles and their interaction with humans and the environment [16, 17].

Modern single particle detection instruments generally consist of three components: an aerosol inlet to transmit aerosols from ambient air into the laser sensing zone, a sizing system to carry out on-line particle sizing; and a characterization system to probe the chemical composition of individual particles [11]. Most systems utilize particle beam inlets to produce a collimated stream of particles. Two types of inlets have achieved widespread use in the on-line particle characterization application: a “segregator” and a “concentrator” [4]. A segregator is used to transmit a narrow particle size range. For example, sharp orifices have been used to tightly focus particles of one size under a specific condition [5]. In contrast, concentrator-type inlets are used

to simultaneously transmit a wide range of particle sizes. The representatives of this type of inlet are aerodynamic lenses. Numerical studies have shown that the focusing characteristics of aerodynamic lenses depend strongly on lens geometry and working pressure [2, 8]. In practice, however, most inlets are designed with a fixed geometry, therefore lacking size focusing range selectivity [8]. Given the fact that the ambient aerosols cover more than five orders of magnitude in size, an ideal particle beam inlet is one whose size focusing range can be adjusted over a broad range to accommodate different applications. For this reason, in our laboratory, it was felt useful and novel to develop a particle beam inlet with an adjustable size focusing range.

The theoretical design and calculations of our inlet were given in a previous paper [48]. The aim of this paper is to describe the experimental realization and characterization of a system that integrates an aerodynamic particle beam inlet with a size measurement and fluorescence analysis system. In contrast to traditional aerodynamic inlets, the new inlet is composed of five *variable* orifices. Consequently, varying the lens diameter and working pressure can change the size focusing range. By choosing the appropriate lens setting, the inlet can function either as a concentrator or as a segregator. Particle size is determined with a dual-laser sizing system (see, e.g., [49, 50]). As with other types of instruments, our apparatus can be used for a variety of applications including air pollution monitoring, contamination control in microelectronics manufacture and industrial hygiene. However, our current research interests are to combine this apparatus with time resolved fluorescence and laser-induced plasma measurements to improve the discrimination of different types of biological particles on a real time basis [43, 51]. An overview of the system design and characterization is provided.

2. System Description

Figure 1 displays the schematic diagram of our apparatus, encompassing three components: a particle beam inlet, a sizing system, and a chemical characterization system. Air is drawn through an aerodynamic inlet into the detection region by differential pumping.

Particles are accelerated and aerodynamically focused into a narrow beam and optically sensed in the sizing region by two continuous wave (CW) laser beams. Size information is obtained from the measurement of the particle time-of-flight between the two CW laser beams. After sizing, laser-induced fluorescence is triggered further downstream in the detection chamber and used for chemical characterization. The corresponding 3D Computer Aided Drawing (CAD) of the system is presented in Figure 2.

2.1. Particle Beam Inlet. In this system, test aerosols are generated by a pneumatic-type medical nebulizer at an air flow rate of 5 L/min. The aerosolized droplets are first directed into the mixing chamber, where they are dried by compressed air at a flow rate of 10 L/min, and then introduced into the first vacuum stage through a sapphire critical orifice. The

distance between the critical orifice and the entrance to the aerodynamic inlet is set to 5 cm: this allows reequilibration of the aerosol flow after expansion from the orifice. As shown in Figure 3, the aerodynamic inlet assembly consists of three elements: entrance, focusing lenses, and focusing nozzle. This inlet differs from traditional aerodynamic inlets in that it consists of five concentric *variable* orifices whose diameters can be continuously changed. Each aerodynamic lens is made of a stainless steel iris diaphragm. A pin located at the top of the iris can be rotated through 90 degrees to allow the iris aperture to be continuously varied from 0.8 mm to 8.5 mm. Motion of the pin is controlled by a high vacuum linear motion feedthrough (K075-BLM-1, MDC Vacuum Products Corporation) *via* a die spring. When turning the micrometer head, its movement is imparted to the shaft that, in turn, moves the pin. As the shaft moves forward and presses against the pin, the aperture opens. When the shaft moves backward, the pin bounces back with the aid of the spring, and the aperture closes accordingly. All five lenses are mounted on flanges at a particular position with respect to the axis of the shaft so that all lenses have the same opening of 8.5 mm if the micrometers read zero. Each linear motion feedthrough has a laser etched black-anodized barrel graduated in 0.001" increments. One revolution of the barrel results in 0.025" linear travel of the shaft. Prior knowledge about the micrometer reading and the aperture size is required so that the aperture size can be controlled by the feedthrough without breaking the vacuum. A series of standard VT gage pins (minus series, Vermont Gage) with different diameters are used to accurately set the iris diaphragm aperture. The corresponding micrometer readings for five lenses at different iris aperture settings are recorded. Figure 4 shows the average of the micrometer readings of five lenses versus the aperture diameters. With one full revolution of the barrelhead, the aperture of the relevant iris is increased/decreased by 0.315 mm. Since the barrelhead is graduated into twenty-five parts, each division represents an opening/closing of 0.0126 mm of the aperture. The design architecture described above is unique, in the sense that the diameters of the focusing lenses can be readily adjusted without breaking of the vacuum conditions. In addition, the inlet is modularly assembled. As a result, the geometry and the number of lenses can be altered easily without changing other parts. This offers high flexibility for mechanical modification at the initial stage of instrument design, optimization, and other fundamental studies.

As mentioned earlier, this inlet can serve as either a concentrator or a segregator. Numerical simulations with the FLUENT protocol have been carried out to evaluate the capability of the newly designed inlet in focusing particles. Figure 5, which is taken from our previous paper [48], compares the detection efficiencies measured 5 cm downstream of the inlet exit for five different aerodynamic inlet systems, terminated with the same two-stage focusing nozzle under the same working conditions. Here, the *detection efficiency* is defined as the ratio of the number of the particles collected on a 500 μm disc placed 5 cm downstream of the inlet to the total number of particles injected from upstream. Clearly, changing the lens geometry can vary the size focusing range.

FIGURE 1: Schematic diagram of single particle detection system.

FIGURE 2: 3D computer aided drawing of the single particle detection system.

Previous studies [2] have confirmed that no distinct focusing can be observed for a single aerodynamic lens if the lens diameter is larger than or equal to the radius of the tube diameter. Therefore, it can be expected that a multilens inlet can be used as a size segregator *if only one lens is set to the required dimension and the others set to $D/2$ or larger*. Numerical results shown in Figure 6, taken from our previous paper [48], indicate that a five-lens inlet ($d_i = 6, 6, 2.4, 6, 6$ mm) has a focusing capability similar to that of a single lens ($d_i = 2.4$). Only particles with a specific size can be collimated. The focusing efficiency degrades dramatically for other sizes. However, when a two-stage nozzle is coupled to the end of the five-lens inlet system, particles within a relatively broad range can also be focused. Moreover, the numerical results show that when a thin-plate orifice replaces the two-stage nozzle, only 0.02 and $1.0\ \mu\text{m}$ particles can be efficiently collimated. This indicates that if the multilens inlet system is to be used both as concentrator and as segregator, the two-stage nozzle should be removed or replaced by a thin-plate orifice. The major advantage of such an inlet system over conventional aerodynamic lenses is that we can

focus particle of different size range without changing the inlet. These numerical studies [48] have been encouraging for our future experiments.

2.2. Aerodynamic Sizing System. After expansion from the focusing nozzle, aerosol particles are accelerated into the sizing region through a $500\ \mu\text{m}$ diameter skimmer (Ni6750, Thermo Finnigan) located $2\ \text{cm}$ downstream of the nozzle exit. A $6.95\ \text{L/s}$ mechanical pump (E2M-18, Edwards) is used to maintain the vacuum between the nozzle exit and the skimmer. Pressures in the first two-stages are controlled by two butterfly valves and monitored by an Edwards model 1105 controller with Pirani gauge heads (model PRM10K, Edwards).

Particle size and size distribution are basic characteristics for atmospheric aerosols because they largely determine the aerosols behavior in gaseous suspension. In addition, particle composition and properties may correlate with size (see, e.g., [14]). A time-of-flight (TOF) aerodynamic sizing technique [49] has been selected for particle size measurements in this instrument due to its capability of sizing individual particles on the fly with high resolution and its compatibility with further particle characterization. The sizing schematic is illustrated in Figure 7. An individual airborne particle is accelerated through a skimmer into a supersonic expansion flow, after which it reaches a size-dependent terminal velocity. The accelerated particle then travels under vacuum through two continuous-wave (CW) lasers. The particle scattering light generated by the two CW tracking lasers is converted into an electrical signal by two photomultiplier tubes. The electrical signals are then sent to an external timing circuit and recorded. From the transit time of the particle and the distance between two laser beams, the speed of the particle can be determined. The measured velocity is then converted to the corresponding particle aerodynamic size based on a stored calibrated function using particles of known sizes. Moreover, based on the velocity of the particle, a trigger can be generated by the timing circuit to fire the excitation laser as soon as the particle arrives at its focus. As a result, the particle-hit rate can be dramatically increased.

FIGURE 3: Details of the variable orifice aerodynamic inlet system.

FIGURE 4: Variation of micrometer readings versus aperture diameters of five lenses. The data can be well fit to a linear function $M = -0.0794x + 0.6754$ where M is the micrometer reading in inches, and x represents the aperture size in mm. Error bar represents one standard deviation of the micrometer readings for five lenses.

FIGURE 5: Dependence of detection efficiency on orifice geometry for aerodynamic inlets integrated with a two-stage nozzle [7] ($L_t = 10.0 \text{ mm}$; $D_t = 2.0 \text{ mm}$; and $d_n = 1.0 \text{ mm}$) under the same operating condition. Inner tube diameter of the inlet is 12 mm . d_i , P_0 , and P_2 are lens diameter, inlet upstream pressure, and downstream pressure, respectively. Flow rate Q is 60 sccm .

The configuration of the sizing chamber is shown in Figure 8. The main body is a three-way cross equipped with a window port for visual observation of the particle beam and alignment. A multiline CW 300 mw air-cooled argon ion

laser (model 117-G02, Spectra Physics) is used as the source for particle light scattering. The output from the laser is split into two perpendicularly polarized beams via a beam splitter. The split beams are directed into the sizing region in parallel

FIGURE 6: Focusing performance is compared between a thin-plate orifice and several five-lens aerodynamic inlets at $P_0 = 1000$ Pa and $Q = 60$ sccm. Inlet geometry is listed: $D = 12$ mm; single lens ($d_i = 2.4$ mm); five lenses ($d_i = 6, 6, 2.4, 6, 6$ mm); nozzle ($L_t = 10.0$ mm, $D_t = 2.0$ mm, and $d_n = 1.0$ mm); diameter of the orifice is 1.0 mm. Measurement location is 5 cm downstream of the inlet exit.

and focused on two different spots on the particle beam path using Plano-convex lenses with a focal length of 1.5 cm.

These lenses are also used as seals for the vacuum to avoid using additional windows that may introduce reflection and extra loss of the laser beam. A beam dump is placed at the end of the beam line to dissipate excess laser energy. The two foci are separated by 6 cm. Each plano-convex lens is mounted on a welded metal bellow (125-75-1-EE, Standard Bellows Co.) feedthrough assembly and can be steered with four adjusting nuts. Such a steering mechanism provides fine positioning of the plano-convex lenses to ensure that the lasers are focused on the exact particle beam path and that the two foci are 6 cm apart. The scattering radiation is reimaged by two Plano-convex lenses into the PMT detector (R647, Hamamatsu Photonics). The first lens is placed at one focal length ($f_l = 1.5$ cm) away from the particle beam to collimate the scattering light. The second lens ($f_l = 1.2$ cm) is placed 1.2 cm away from the PMT to focus the collimated scattered light onto the cathode of the PMT. A metal sleeve with an opening aperture of 2 mm is inserted between the PMT and the second lens to prevent background stray light from entering the detectors. The first lens also acts as a vacuum seal by fastening the lens into the PMT mount using a threaded retaining ring. A good seal is achieved when the lens compresses against the O-ring. The collection optics are mounted on a bellow alignment apparatus for fine positioning of the lens focus on the particles under vacuum so that maximum scattering signal can be collected by the photomultiplier tubes.

Finally, it should be mentioned that when the system is operated in the aerodynamic sizing mode, pressure in the sizing chamber is maintained at around 10^{-4} mbar by

a 210 L/s turbo-molecular drag pump (TMH 261, Pfeiffer vacuum) backed by a rotary pump (SD-90, Varian). Pressure in this stage is monitored with an MKS DualTransTM transducer.

2.3. Optical Detection. Following passage through a 3 mm skimmer orifice, particles are led into a six-way cross chamber (the fourth vacuum stage) in which vacuum is maintained by a 1.11 L/s mechanical pump (Trivac D4A) in the low-speed particle beam mode or by a 250 L/s turbo pump (V-250, Varian) in the high-speed particle beam mode. Pressure is monitored by either a Pirani (PRM10, $200\text{--}10^{-3}$ mbar, Edwards) gauge or an Edwards CP25 Penning gauge ($10^{-2}\text{--}10^{-7}$ mbar). The Penning gauge is automatically switched on by the Pirani gauge when pressure drops below 10^{-2} mbar. Inside the chamber, particles are directed to the focal point of an elliptical reflector (E214-2, Opti-forms Inc.) through a 0.5 inch hole in the optic. The laser beam is oriented perpendicularly to the particle beam and passes through a 4 mm hole on both sides of the elliptical reflector. The laser beam intercepts the particle at the first focal position of the reflector. The resultant fluorescence and scattering light are reflected toward the reflector's second focal point where the detector is located. The distance between the two foci is 8.4 inches. The transmitted laser beam travels directly into a beam dump, which is machined in aluminum alloy with black anodized finish to inhibit stray light. The elliptical reflector has an aluminum coating with a quartz overcoat. The overcoat is centered at 430 nm and its average reflectance is $\sim 91\%$ from 260 to 600 nm. The ellipsoidal reflector is used to maximize the solid angle of optical collection. The overall collection efficiency is 40% of the 4π sr. In our initial design, a 5 cm plano-convex lens with 10 cm focal length is placed at a right angle to the laser beam to collect laser-induced fluorescence. The solid angle of this collection configuration is only 0.049 sr, corresponding to 0.4% coverage. As a result, no signal was observed. With the use of the elliptical mirror, the collection efficiency has been enhanced about 100 times. In addition, orientation of the elliptical reflector to the particle beam and laser beam shown in Figure 9 allows collection of emission from the particle in the forward and backward directions. Previous studies have proven that fluorescence in the backward direction is two to three times larger than at 90° [52].

To gain maximum optical throughput, it is necessary for the laser beam focus, the particle beam, and the first focus of the elliptical mirror to overlap with one another. The elliptical reflector is housed on a mount that can be rotated 360° around its axis. The reflector and its mount are attached to a compact triple axis XYZ stage (PSM-1502, MDC) via a stainless steel rod. The first step of alignment is to use a collinear CW laser beam to define the axis of the particle beam after pumping down. The excitation laser is oriented perpendicularly to the particle beam axis with the aid of two mirrors mounted on a periscope. Both the laser and the periscope are mounted on a one-dimensional translation stage so that the laser beam can be translated horizontally to intercept the particle beam axis. The second step is to assure that the laser beam traverses the center of two 4 mm holes

FIGURE 7: Schematic diagram of aerodynamic sizing using two CW laser beams.

FIGURE 8: Layout of the sizing chamber: 3D view (top) and 2D section view (bottom).

FIGURE 9: Schematic diagram of an elliptical mirror. The particle beam image is shown in black dot. The mirror is able to be rotated around its centerline and move along with the particle beam in three dimensions.

on the reflector, while the particle beam axis passes through the center of a 0.5 inch hole on the reflector. This is achieved by tilting the reflector mounts and adjusting the compact vacuum XYZ stage. The final fine adjustment is made by checking the image of the actual particle beam at the second focus of the reflector under vacuum.

3. System Characterization

3.1. Operating Modes. As mentioned previously, with regard to the particle velocity, the system can be operated in either a *low-speed* or a *high-speed* mode. Adjusting the pressure in the sizing chamber and detection chamber can alternate these two modes. The low-speed mode is achieved at a medium

FIGURE 10: Time-of-flight spectrum of the $1.093 \mu\text{m}$ particle under four different pressures in the sizing chamber. The zero-time corresponds to the time when the particle arrives at the first CW laser. The time from zero to where the peak appears is the time interval that the particle takes to travel through the distance (6 cm) between the two CW lasers.

pressure and is intended to be used when a high repetition rate excitation laser is used for fluorescence lifetime analysis. Combination of a slow-moving particle beam and a high repetition rate laser allows multiple hits of a single particle during its residence time in the interception zone defined by the spot size of the excitation laser. Therefore, signal averaging techniques can be used to reduce random noise and increase the signal-to-noise ratio. As a result, the reliability of lifetime analysis can be greatly enhanced. In contrast, the high-speed mode operates under high vacuum and can be utilized for both aerodynamic sizing and chemical characterization of individual airborne particles by time of flight mass spectrometry. Figure 10 shows the flight time of a $1.093 \mu\text{m}$ polystyrene particle between two CW lasers under four different sizing chamber pressures. As the pressure decreases from 0.467 to 1.1×10^{-4} mbar, the velocity of the $1.093 \mu\text{m}$ particle increases from 116.4 to 236.4 m/s.

3.1.1. High-Speed Mode: Time of Flight Sizing and Conditional Trigger. For aerodynamic time of flight sizing, the particle should be accelerated rapidly enough to achieve a considerable difference among the terminal velocities of particles of different sizes, and thus to increase the sizing resolution and detection efficiency. In addition, because of the unique feature of the timing circuit, the particle velocity should remain constant across the entire sizing and detection region. Therefore, the pressure inside the sizing chamber has to be kept low enough for the fluid to realize high-speed supersonic expansion after exiting the skimmer and subsequently develop into the free molecular flow where particle-gas molecular collisions are negligible. Experimental

testing has found that a sizing pressure of 1×10^{-4} mbar or lower can meet the above requirements.

Figure 11 is an example of the particle time-of-flight spectrum from a mixture containing three different sizes of polystyrene microspheres. It reveals that different sizes of particles can be well differentiated by the TOF sizing technique. In order to determine the unknown particle size, a calibration curve is constructed based on the velocity of particles with known sizes and density. The result is shown in Figure 12 where each data point is the average of three measurements carried out on three different days, and the corresponding one standard deviation is represented by the error bar. Each measurement is obtained by averaging 250 traces. By checking the calibration curve within a single run, it was found that the shift in TOF is negligible. The data can be well fitted to the following function (cf. the solid line in Figure 12):

$$V_p = 241.663 - 53.768 \ln D_p, \quad (1)$$

where V_p is the velocity of the particle derived from the particle's time-of-flight, and D_p is the particle aerodynamic size, verified by an (Amherst Process Instruments.) APIs Aerosizer.

Another well-known feature of the high-speed mode is that the excitation laser can be conditionally triggered. Only those particles that have a defined correlation between two CW lasers can be detected by the excitation laser in the detection zone. A comparison of the excitation laser hit rate between conditional trigger mode and free running mode is given in Figure 13. Clearly, the conditional trigger mode is superior to the free running mode in terms of the increased hit rate. It is also noted that, in the free running mode, the hit rate increases monotonically with the particle concentration. In contrast, the hit rate decreases as the particle concentration increases in the conditional trigger mode, which is a strong indication of the false coincidence of the TOF sizing [53].

As with other similar systems, the sample rate has to be indeed limited. This is however due more to the possibility of coincidence effects than to multiple start pulses due to a short particle-to-particle pulses period. In fact, due to the unique design of the timing circuit, a multiple start pulse occurring after any active start pulse, but before receiving a stop pulse, will be inhibited. This guarantees the accuracy of TOF measurements unless a coincidence occurs (multiple particles in the probing area at the same time). Theoretically, the probability of having more than one particle contributing to the signal was estimated in our previous paper [43] where an approach was described that allowed calculating the upper limit of particle suspension concentration to satisfy single particle detection. These results, converted into particle fluxes, gave a maximum value to avoid coincidence effects of approximately 14 particles/s.

3.1.2. Low-Speed Mode: Particle Beam Characteristics. In the low-speed mode, increasing the pressure of the detection chamber can increase the particle transit time through the laser excitation zone. We have experimentally determined

FIGURE 11: Time-of-flight spectrum of a mixture containing three different aerodynamic sizes of particles. The spectrum is obtained from averaging 250 oscilloscope traces. Sizing chamber pressure: 1.1×10^{-4} mbar.

FIGURE 12: Particle velocities as function of particle sizes: data obtained at a sizing chamber pressure of 1.1×10^{-4} mbar can be fit to the function: $V_p = 241.663 - 53.768 \ln D_p$ ($R_2 = 0.9938$).

that the transit time of a $1.093 \mu\text{m}$ polystyrene latex spheres through a 532 nm CW laser with 1 mm spot size is about 10 milliseconds at a detection chamber pressure of 2 mbar. Currently, the aerodynamic lenses are set to focus particles in the range of $0.5\text{--}10 \mu\text{m}$. Particles are introduced into the entrance of the aerodynamic inlet through a $400 \mu\text{m}$ critical orifice. The pressure between the orifice and the inlet entrance is maintained at 10 mbar. The particle beam inlet consists of five aerodynamic lenses with opening diameters of 6, 5, 4, 3, and 2 mm, followed by a two-stage acceleration nozzle (see Figure 5) with $L_t = 10$ mm, $D_t = 3$ mm, and

FIGURE 13: Particle hit rate as a function of fluorescent particle ($1 \mu\text{m}$) nebulizer suspension concentration compared between the conditional trigger mode and the free firing mode.

$d_n = 1.5$ mm. The aerodynamic lenses are equally separated with spacers that are 63 mm long and have an inner diameter of 12 mm. After exiting the nozzle, the carrier gas (air) expands through a $500 \mu\text{m}$ skimmer into the detection chamber. The stage between the acceleration nozzle and the skimmer is evacuated to a pressure of 5.8 mbar. The detection region is located 13.5 cm from the skimmer exit throat. Several important parameters are experimentally characterized in this mode, namely, the particle beam spatial collimation and the overall transmission efficiency of the inlet. The same characterization methodologies can be applied to the high-speed mode of operation at different lens geometry and working conditions.

To obtain a scattering image of the particle beam, a Powerchip laser (JDS Uniphase, repetition rate: 1 kHz, pulse energy: $20 \mu\text{J}$) was aligned perpendicularly to the particle beam. The resultant scattering image of the particle beam was captured with a charge-coupled device (CCD) camera (Penguin 150 CL; Pixera Co.) placed at a right angle to the particle beam axis. Figure 14 displays particle beam images captured 13.5 cm downstream of the skimmer. Clearly, beams for 0.11 and $0.356 \mu\text{m}$ particles are significantly divergent. The beam sizes gradually decrease for particles from 0.11 to $4.902 \mu\text{m}$, where it levels off at a beam width that is approximately 10% of the value for the $0.356 \mu\text{m}$ particles.

We also collected monodispersed blue and red dyed microspheres (Polysciences Inc.) of known sizes on Vaseline-coated glass slides [6] at the same observation location. After collection, the glass slide was removed from the chamber, and the particle deposition pattern was visualized with a microscope. The results are displayed in Figure 15. It can be seen that the particle beam size measured from the scattering image is consistent with that obtained from the greased glass slide collection, with the exception that the beam width of

FIGURE 14: Scattering images of particle beams with different diameters. The laser is aligned perpendicularly to the particle beam axis at 13 cm downstream of the skimmer.

$0.528\ \mu\text{m}$ particles estimated from the scattering light image is smaller than that with greased slide deposition. This may be due to disturbance of the free jet by the glass slide, which causes the broadening of small particles due to their small inertia.

In order to experimentally evaluate the transmission efficiency of the inlet system in the low-speed mode, a simple experiment is designed here for the first time to measure the transmission efficiency with good reproducibility and accuracy. Monodispersed red fluorescent particles (Duke Scientific) within the $0.3\text{--}10\ \mu\text{m}$ size range are aerosolized. These particles yield maximum emission at $575\ \text{nm}$ under excitation at $532\ \text{nm}$. The aerosol stream then enters a sampling manifold where it is split into three portions. One portion is sent to the house air exhaust. To assure equal loss of particles on the wall of the tubing, the other two portions pass through two parallel flows with the same length of tubing before crossing a $400\ \mu\text{m}$ critical orifice. Pressure at the downstream of the critical orifice is set below the critical pressure (approximately 50% of the upstream pressure); hence the flow through the orifice is choked to guarantee the same mass flow rate for both streams. After being drawn through the critical orifice, one of the aerosol streams travels through a series of aerodynamic lenses that confine the particles into a narrow and low-divergence beam. The collimated particle beam then enters the detection chamber via a two-stage acceleration nozzle and a skimmer. A glass vial ($5.7\ \text{cm}$ height and $1.8\ \text{cm}$ diameter mouth) located $13\ \text{cm}$ downstream of the skimmer is used to collect the particles. The bottom of the vial is coated with a thin layer of silicone oil to prevent particle bounce. The silicone oil (30200 centipoise viscosity standard, Brookfield Eng. Lab.

Inc.) is dissolved at 10% w/v in toluene, and $4\ \text{mL}$ of the mixture are allowed to evaporate in the bottom of each vial. Meanwhile, in the reference arm, air passes through an identical critical orifice and is then drawn through a $4.7\ \text{cm}$, $0.22\ \mu\text{m}$ pore GS membrane filter with (Millipore, Bedford, MA). After the required vacuum is achieved in the system, particles are introduced into the gas line and collection begins. The particle number density in the nebulizer is kept low enough to prevent coagulation. After several hours of collection, the filter and the glass vial are removed from the chamber for further analysis. The transmission efficiency of the inlet system, which is defined as the percentage of the particles entering the aerodynamic inlet that are collected downstream of the skimmer, is thus calculated from the ratio of the number of the particles collected by the glass vial to the number of the particles deposited on the filter. After the removal of the vial from the chamber, $10\ \text{mL}$ toluene (99+% Spectrophotometric Grade, Acros Organics) is added to dissolve the silicone oil and extract the dye from the collected particles. Similarly, fluorescent particles accumulated on the filter are immersed in $10\ \text{mL}$ toluene to extract the dye for quantitative analysis.

For each particle size, two matrix-matched assays are performed. In order to quantitate the particles collected in the reference arm, standard filters with different numbers of fluorescent polystyrene microspheres are prepared by pipetting known amounts of stock fluorescent microsphere suspension onto membrane filters. After drying, $10\ \text{mL}$ toluene is added to each filter sample, and the fluorescence intensity of each solution is measured at $575\ \text{nm}$. Second, to determine the number of fluorescent particles collected downstream of skimmer, different amounts of stock fluorescent microsphere

FIGURE 15: Deposition patterns of different size particles collected 13 cm downstream of the skimmer. The deposit of $0.528\ \mu\text{m}$ is too big to be examined under the microscope even with the minimum magnification of four. Its beam size is estimated to be about 3.5 mm in diameter.

suspension are added to a series of standard glass vials. The vials are then dried at room temperature, leaving behind only the particles. 4 mL of silicone oil solution is then added to each standard vial and allowed to evaporate. Finally, 10 mL of toluene is used to release the dye from the particles. For both assays, fluorescence intensity at 575 nm versus the number of microspheres is plotted to construct a standard curve for each size microspheres. Fluorescence is excited by the 532 nm emission of the Powerchip laser. Fluorescence emitted by the particles passes through a monochromator (*MicroHR*, Horiba Jobin Yvon) to a PMT detector (R928, Hamamatsu) with an amplifier. The fluorescence intensity at 575 nm is obtained by averaging 500 laser shots. Each measurement is made in triplicate.

Particle transmission efficiency as a function of the particle size is shown in Figure 16. Nearly 100% efficiency is achieved for $1\ \mu\text{m}$ and $2\ \mu\text{m}$ particles, while transmission efficiency for larger or smaller particles is lower. Nonetheless, more than 35% of the particles entering the critical orifice can still be collected even for $0.49\ \mu\text{m}$ and $9.91\ \mu\text{m}$ microspheres. For particles smaller than $0.49\ \mu\text{m}$, the efficiency falls off sharply.

The transmission efficiency predicted by numerical simulation for particles from 0.5 to $10\ \mu\text{m}$ is very close to unity. The low transmission efficiency obtained experimentally for small and large particles might be accounted for the following reasons: (1) numerical simulations do not take into account nonideal forces such as Brownian motion, which have stronger influence on small particles; (2) divergence for smaller particles (e.g., $0.3\ \mu\text{m}$) is more serious and the skimmer acts as a limiting aperture; therefore only a portion of the particles beam can reach the collection vial; (3) after expanding from the critical orifice, small

FIGURE 16: Transmission efficiency as a function of the particle size. The error bar refers to the one standard deviation from three measurements.

particles may diffuse or pump away in the first stage due to their small inertia, whereas larger particles with larger inertia may impact on the wall or the entrance of aerodynamic lenses. Therefore, the number of particles that enter the aerodynamic lenses is overestimated by the filter collection; finally (4) misalignment between the skimmer and aerodynamic lenses may cause deviation of the jet from the axis of the skimmer and result in the lower transmission efficiency of large and small particles through the skimmer.

4. Real Time Measurement of Single Airborne Particles by Spectrally-Resolved and Time-Resolved Fluorescence

The performance of the system has been demonstrated by measuring spectrally-resolved and time-resolved fluorescence of single dye-doped fluorescent particles and biological simulants such as tryptophan and NADH aerosols. For these measurements, the system is operated in the low-speed mode. Particles are illuminated by a pulsed laser when passing through the sampling volume located at 13.5 cm downstream of the 500 μm skimmer. Laser-induced fluorescence (LIF) is collected by an elliptical mirror and reimaged on a spectrometer (SpectraPro-2150i, Acton Research Corp.) using two short focal length lenses. The spectrometer is a Czerny-Turner design with two F/4 aspheric mirrors and a 300 groove/mm grating blazed at 500 nm. Fluorescence spectra are recorded by a thermoelectrically cooled intensified CCD (ICCD) camera (Princeton Instruments, Trenton, NJ, USA) mounted on the exit slit of the spectrometer. The signal from the CCD is transferred to a personal computer via the WinSpec/32 software package (Roper Scientific). All spectra are collected in a single-shot mode without averaging. For fluorescence lifetime analysis, fluorescence emission collected by the elliptical mirror is reimaged at its second focal point and then collimated by a plano-convex lens (1'' focal length). The collimated beam then traverses a bandpass filter (299–400 nm) before being detected by a fast photomultiplier (R3091, Hamamatsu) mounted at a right angle to the laser beam. This PMT is selected because of its nominal 0.4 nanosecond rise-time and high gain (10^6). The single shot total fluorescence decay pulse is acquired by an oscilloscope (TD S6604, Tektronix) with a sampling rate of 20 Gs/s.

Three excitation wavelengths are used in this experiment. A nitrogen laser operated at 18 Hz (VSL-337ND-S, Thermo science laser) provides 3.5 nanosecond pulses at 337 nm with 183.6 μJ energy per pulse. This laser is used for excitation of XPR-801, Fluorophore, and NADH. The other is a 20 μJ powerchip laser (532 nm, PNP-002025-140- JDS Uniphase Powerchip Nanolaser) operating at 1 kHz. It has a relatively short pulse width of about 500 ps. The wavelength emitted by this laser is well matched to the excitation peak of R0100 red fluorescent microspheres. Excitation at 266 nm is generated by frequency doubling the 532 nm output using a BBO crystal (type I, Photox Optical Systems Ltd.). The conversion efficiency is about 10%, providing a UV pulse energy of 2 μJ . The laser is triggered at 1 kHz by a DG535 (Stanford Research Corp.) pulse delay generator. A UV pass filter is placed in the laser beam path after the BBO crystal to block radiation at 532 nm and transmit 266 nm.

Figure 17(a) shows the fluorescence spectra for 1 : 1 mixtures of XPR-801 (2 μm , Duke Scientific Corporation) and Fluorosphere (2 μm , Molecular Probes, Inc.) at a suspension concentration of 2.27×10^5 particles/mL. As can be seen, the laser "hits" mostly one color of particle and coincidence rarely occurs [43]. Representative fluorescence emission spectra from NADH-doped particles excited at 337 nm are displayed in Figure 17(b). Figure 17(c) is the typical emission

FIGURE 17: (a) Fluorescence spectra of 1 : 1 mixtures of different color fluorescent particles (XPR-801 and Fluorosphere). Total concentration of particles in the nebulizer reservoir is 2.27×10^5 particles/mL (337 nm excitation). (b) Fluorescence emission spectra of NADH aerosols produced from a 1000 $\mu\text{g/mL}$ aqueous solutions (337 nm excitation). (c) Fluorescence emission spectra of R0100 microsphere (532 nm excitation).

FIGURE 18: Time-resolved fluorescence decay profile from polystyrene particles and tryptophan aerosols (266 nm excitation).

spectra of R0100 red fluorescent microspheres under 532 nm excitation. All spectra are obtained with single shot excitation. Since these spectra were taken with the laser in the free-running mode, significant shot-to-shot intensity fluctuations are expected. In the next generation of this instrument, data acquisition will be triggered by the entry of a particle into the illumination volume. This will allow recording of the data only when a particle passes through the excitation laser beam.

It is worth noting here that indicating that the shape of the fluorescence spectra obtained in this work, in view of the fact that no achromatic lenses were available, are likely to be affected by chromatic aberration: as a consequence, their spectral shape should therefore not be regarded as true fluorescence spectra.

The fluorescence decay profiles of monodisperse polystyrene particles and tryptophan aerosols are displayed in Figure 18. The scatter of the laser light, which is representative of the instrumental temporal profile of our detector, is also shown in this Figure. It is clear that the waveforms obtained (normalized to the tryptophan signal) possess adequate signal-to-noise ratio even for single-shot operation. Many microorganisms contain intrinsic fluorophores that can be used as characteristic markers of their biological nature. The use of fluorescence spectra to differentiate bioaerosols has the inherent advantages of being fast, in situ and nondestructive, thus allowing further analysis of the same sample [54]. However, its discrimination ability is often hampered by the featureless fluorescence spectrum as well as by possible interference from nonbiological particles and/or natural bioaerosols such as pollen and fungi. It is well known that not only is the lifetime of fluorophores dependent on the chemical structure of molecules, but it is also dramatically affected by their interaction with environment or biomolecules such as proteins, peptides,

nucleic acids, or the cellular membrane [54]. For instance, NADH has a lifetime of ~ 0.4 nanosecond in water but can be as long as 9 nanoseconds when bound to dehydrogenases [55]. The lifetime of tryptophan in proteins can range from ~ 0.1 nanosecond up to ~ 8 nanoseconds [56] and could be used as an additional parameter to enhance the system's discrimination ability. In a study that will be reported later, we have measure fluorescence decay profiles of suspensions of five bacteria at different emission wavelengths. Preliminary results have shown that even though these five bacteria have similar fluorescence spectra under 266 nm excitation, their fluorescence decay times at different emission wavelengths can vary, indicating that fluorescence lifetime could provide an additional means for distinguishing bioaerosols [51, 57].

5. Conclusions

A real-time single particle detection apparatus that can provide size and composition of individual particles has been designed and built. The system is equipped with a variable-orifice aerodynamic inlet, which is used to form a narrow particle beam and introduce individual particles to the excitation/detection zone defined by the laser beam spot. Theoretically, we have proved that this variable-orifice inlet has the ability to cope with particles over a wide size range [48]. Its focusing size range can be altered dynamically by varying the lens diameter and the working pressure. The inlet can serve as either a concentrator or a segregator, depending upon the choice of different operating conditions. Time-of-flight aerodynamic sizing has been successfully employed to determine the particle's aerodynamic size, while chemical composition analysis is carried out using laser-induced fluorescence spectroscopy. The design of the system is modular and versatile, which allows for the implementation of different detection schemes (such as mass spectrometry and laser-induced breakdown spectroscopy for the characterization of airborne particles).

Another important feature of our system is that the speed of particle in the detection region can be manipulated by changing the pressure in the detection chamber. Particles can travel at high speed for aerodynamic time of flight sizing, or at low-speed, which allows multiple hits on a given particle during its transit time across the detection zone.

Based on the initial success of steady-state and time-resolved fluorescence analysis of single dye-doped particle and biological simulants, we suggest that a combination of the two approaches in a double-discrimination experiment may represent a viable step forward in improving the discrimination among different bioaerosols.

Acknowledgments

This work was supported by DOE Grant no. DOE-DE-F602-99ER 14960. The authors would like to thank Dr. Chang-Yu Wu (Environmental Engineering Sciences, University of Florida) for useful discussions regarding the experimental evaluation of the transmission efficiency of their apparatus.

References

- [1] W. C. Hinds, *Aerosol Technology: Properties, Behavior and Measurement of Airborne Particles*, John Wiley & Sons, New York, NY, USA, 2nd edition, 1999.
- [2] P. Liu, P. J. Ziemann, D. B. Kittelson, and P. H. McMurry, "Generating particle beams of controlled dimensions and divergence—I: theory of particle motion in aerodynamic lenses and nozzle expansions," *Aerosol Science and Technology*, vol. 22, no. 3, pp. 293–313, 1995.
- [3] P. Liu, P. J. Ziemann, D. B. Kittelson, and P. H. McMurry, "Generating particle beams of controlled dimensions and divergence—II: experimental evaluation of particle motion in aerodynamic lenses and nozzle expansions," *Aerosol Science and Technology*, vol. 22, no. 3, pp. 314–324, 1995.
- [4] B. E. Dahneke and Y. S. Cheng, "Properties of continuum source particle beams. I. Calculation methods and results," *Journal of Aerosol Science*, vol. 10, no. 3, pp. 257–274, 1979.
- [5] Y. S. Cheng and B. E. Dahneke, "Properties of continuum source particle beams. II. Beams generated in capillary expansions," *Journal of Aerosol Science*, vol. 10, no. 4, pp. 363–368, 1979.
- [6] T. J. Estes, V. L. Vilker, and S. K. Friedlander, "Characteristics of a capillary-generated particle beam," *Journal of Colloid and Interface Science*, vol. 93, no. 1, pp. 84–94, 1983.
- [7] R. V. Mallina, A. S. Wexler, and M. V. Johnston, "High-speed particle beam generation: simple focusing mechanisms," *Journal of Aerosol Science*, vol. 30, no. 6, pp. 719–738, 1999.
- [8] J. Schreiner, U. Schild, C. Voigt, and K. Mauersberger, "Focusing of aerosols into a particle beam at pressures from 10 to 150 Torr," *Aerosol Science and Technology*, vol. 31, no. 5, pp. 373–382, 1999.
- [9] X. Zhang, K. A. Smith, D. R. Worsnop, J. Jimenez, J. T. Jayne, and C. E. Kolb, "A numerical characterization of particle beam collimation by an aerodynamic lens-nozzle system—part I: an individual lens or nozzle," *Aerosol Science and Technology*, vol. 36, no. 5, pp. 617–631, 2002.
- [10] X. Zhang, K. A. Smith, D. R. Worsnop, et al., "Numerical characterization of particle beam collimation—part II: integrated aerodynamic-lens-nozzle system," *Aerosol Science and Technology*, vol. 38, no. 6, pp. 619–638, 2004.
- [11] J. Marijnissen, B. Scarlett, and P. Verheijen, "Proposed on-line aerosol analysis combining size determination, laser-induced fragmentation and time-of-flight mass spectroscopy," *Journal of Aerosol Science*, vol. 19, no. 7, pp. 1307–1310, 1988.
- [12] M. V. Johnston and A. S. Wexler, "Mass spectrometry of individual aerosol particles," *Analytical Chemistry*, vol. 67, pp. 721A–726A, 1995.
- [13] M. Weiss, P. J. T. Verheijen, J. C. M. Marijnissen, and B. Scarlett, "On the performance of an on-line time-of-flight mass spectrometer for aerosols," *Journal of Aerosol Science*, vol. 28, no. 1, pp. 159–171, 1997.
- [14] C. A. Noble and K. A. Prather, "Real-time measurement of correlated size and composition profiles of individual atmospheric aerosol particles," *Environmental Science and Technology*, vol. 30, no. 9, pp. 2667–2680, 1996.
- [15] C. A. Noble and K. A. Prather, "Aerosol time-of-flight mass spectrometry," in *Analytical Chemistry of Aerosols*, K. R. Spurny, Ed., pp. 353–376, Lewis, Boca Raton, Fla, USA, 1999.
- [16] D. T. Suess and K. A. Prather, "Mass spectrometry of aerosols," *Chemical Reviews*, vol. 99, no. 10, pp. 3007–3035, 1999.
- [17] M. V. Johnston, "Sampling and analysis of individual particles by aerosol mass spectrometry," *Journal of Mass Spectrometry*, vol. 35, no. 5, pp. 585–595, 2000.
- [18] M. A. Stowers, A. L. van Wuijckhuijse, J. C. M. Marijnissen, B. Scarlett, B. L. M. Van Baar, and Ch. E. Kientz, "Application of matrix-assisted laser desorption/ionization to on-line aerosol time-of-flight mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 14, no. 10, pp. 829–833, 2000.
- [19] D. B. Kane, B. Oktem, and M. V. Johnston, "Nanoparticle detection by aerosol mass spectrometry," *Aerosol Science and Technology*, vol. 34, no. 6, pp. 520–527, 2001.
- [20] S. N. Jackson, S. Mishra, and K. K. Murray, "On-line laser desorption/ionization mass spectrometry of matrix-coated aerosols," *Rapid Communications in Mass Spectrometry*, vol. 18, no. 18, pp. 2041–2045, 2004.
- [21] N. Erdmann, A. Dell'Acqua, P. Cavalli, et al., "Instrument characterization and first application of the single particle analysis and sizing system (SPASS) for atmospheric aerosols," *Aerosol Science and Technology*, vol. 39, no. 5, pp. 377–393, 2005.
- [22] D. G. Nash, T. Baer, and M. V. Johnston, "Aerosol mass spectrometry: an introductory review," *International Journal of Mass Spectrometry*, vol. 258, no. 1–3, pp. 2–12, 2006.
- [23] M. Tolocka, M. Reinard, D. Lake, J. Ondov, A. Wexler, and M. Johnston, "Characterization of short-term particulate matter events by real-time single particle mass spectrometry," in *Aerosol Science and Technology*, vol. 40, no. 10, pp. 873–882, 2006.
- [24] M. S. Reinard, K. Adou, J. M. Martini, and M. V. Johnston, "Source characterization and identification by real-time single particle mass spectrometry," *Atmospheric Environment*, vol. 41, no. 40, pp. 9397–9409, 2007.
- [25] C. A. Zordan, S. Wang, and M. V. Johnston, "Time-resolved chemical composition of individual nanoparticles in urban air," *Environmental Science and Technology*, vol. 42, no. 17, pp. 6631–6636, 2008.
- [26] M. S. Reinard and M. V. Johnston, "Ion formation mechanism in laser desorption ionization of individual nanoparticles," *Journal of the American Society for Mass Spectrometry*, vol. 19, no. 3, pp. 389–399, 2008.
- [27] K. R. Spurny, "On the chemical detection of bioaerosols," *Journal of Aerosol Science*, vol. 25, no. 8, pp. 1533–1547, 1994.
- [28] A. L. van Wuijckhuijse, M. A. Stowers, W. A. Kleefsman, B. L. M. van Baar, Ch. E. Kientz, and J. C. M. Marijnissen, "Matrix-assisted laser desorption/ionisation aerosol time-of-flight mass spectrometry for the analysis of bioaerosols: development of a fast detector for airborne biological pathogens," *Journal of Aerosol Science*, vol. 36, no. 5–6, pp. 677–687, 2005.
- [29] I. Kleefsman, M. A. Stowers, P. J. T. Verheijen, A. L. van Wuijckhuijse, Ch. E. Kientz, and J. C. M. Marijnissen, "Bioaerosol analysis by single particle mass spectrometry," *Particle and Particle Systems Characterization*, vol. 24, no. 2, pp. 85–90, 2007.
- [30] P. P. Hairston, J. Ho, and F. R. Quant, "Design of an instrument for real-time detection of bioaerosols using simultaneous measurement of particle aerodynamic size and intrinsic fluorescence," *Journal of Aerosol Science*, vol. 28, no. 3, pp. 471–482, 1997.
- [31] A. P. Snyder, Ed., "Chemical and Biological aerosol detection and identification with field analytical instrumentation," *Field Analytical Chemistry and Technology*, vol. 3, pp. 219–326, 1999.
- [32] S. C. Hill, R. G. Pinnick, S. Niles, et al., "Real-time measurement of fluorescence spectra from single airborne biological particles," *Field Analytical Chemistry and Technology*, vol. 3, no. 4–5, pp. 221–239, 1999.
- [33] Y. L. Pan, R. G. Pinnick, S. C. Hill, R. K. Chang, et al., "Particle fluorescence spectrometer for real-time single particle

- measurements of atmospheric organic carbon and biological aerosols," *Environmental Science and Technology*, vol. 43, no. 2, pp. 429–434, 2009.
- [34] M. Seaver, J. D. Eversole, J. J. Hardgrove, W. K. Cary Jr., and D. C. Roselle, "Size and fluorescence measurements for field detection of biological aerosols," *Aerosol Science and Technology*, vol. 30, no. 2, pp. 174–185, 1999.
- [35] V. Sivaprakasam, A. L. Huston, C. Scotto, and J. D. Eversole, "Multiple UV wavelength excitation and fluorescence of bioaerosols," *Optics Express*, vol. 12, no. 19, pp. 4457–4466, 2004.
- [36] Y.-L. Pan, P. Cobler, S. Rhodes, et al., "High-speed, high-sensitivity aerosol fluorescence spectrum detection using a 32-anode photomultiplier tube detector," *Review of Scientific Instruments*, vol. 72, no. 3, pp. 1831–1836, 2001.
- [37] P. H. Kaye, W. R. Stanley, E. Hirst, E. V. Foot, K. L. Baxter, and S. J. Barrington, "Single particle multichannel bio-aerosol fluorescence sensor," *Optics Express*, vol. 13, no. 10, pp. 3583–3593, 2005.
- [38] Y.-L. Pan, J. Hartings, R. G. Pinnick, S. C. Hill, J. Halverson, and R. K. Chang, "Single-particle fluorescence spectrometer for ambient aerosols," *Aerosol Science and Technology*, vol. 37, no. 8, pp. 628–639, 2003.
- [39] K. Davitt, Y.-K. Song, W. R. Patterson III, et al., "290 and 340 nm UV LED arrays for fluorescence detection from single airborne particles," *Optics Express*, vol. 13, no. 23, pp. 9548–9555, 2005.
- [40] K. Davitt, Y.-K. Song, W. R. Patterson III, et al., "Spectroscopic sorting of aerosols by a compact sensor employing UV LEDs," *Aerosol Science and Technology*, vol. 40, no. 12, pp. 1047–1051, 2006.
- [41] D. P. Fergenson, M. E. Pitesky, H. J. Tobias, et al., "Reagentless detection and classification of individual bioaerosol particles in seconds," *Analytical Chemistry*, vol. 76, no. 2, pp. 373–378, 2004.
- [42] M. A. Stowers, A. L. van Wuijckhuijse, J. C. M. Marijnissen, Ch. E. Kientz, and T. Ciach, "Fluorescence preselection of bioaerosol for single-particle mass spectrometry," *Applied Optics*, vol. 45, no. 33, pp. 8531–8536, 2006.
- [43] X. Wu, N. Omenetto, B. W. Smith, and J. D. Winefordner, "Single particle fluorescence: a simple experimental approach to evaluate coincidence effects," *Applied Spectroscopy*, vol. 61, no. 7, pp. 711–718, 2007.
- [44] H. C. Huang, Y.-L. Pan, S. C. Hill, R. G. Pinnick, and R. K. Chang, "Real-time measurement of dual-wavelength laser-induced fluorescence spectra of individual aerosol particles," *Optics Express*, vol. 16, no. 21, pp. 16523–16528, 2008.
- [45] J. D. Hybl, G. A. Lithgow, and S. G. Buckley, "Laser-induced breakdown spectroscopy detection and classification of biological aerosols," *Applied Spectroscopy*, vol. 57, no. 10, pp. 1207–1215, 2003.
- [46] P. B. Dixon and D. W. Hahn, "Feasibility of detection and identification of individual bioaerosols using laser-induced breakdown spectroscopy," *Analytical Chemistry*, vol. 77, no. 2, pp. 631–638, 2005.
- [47] J. D. Hybl, S. M. Tysk, S. R. Berry, and M. P. Jordan, "Laser-induced fluorescence-cued, laser-induced breakdown spectroscopy biological-agent detection," *Applied Optics*, vol. 45, no. 34, pp. 8806–8814, 2006.
- [48] X. Wu, N. Omenetto, and J. D. Winefordner, "Development, characterization and application of a versatile single particle detection apparatus for time-integrated and timeresolved fluorescence measurements—part 1: theoretical considerations," *Laser Chemistry*. In press.
- [49] K. Salt, C. A. Noble, and K. A. Prather, "Aerodynamic particle sizing versus light scattering intensity measurement as methods for real-time particle sizing coupled with time-of-flight mass spectrometry," *Analytical Chemistry*, vol. 68, no. 1, pp. 230–234, 1996.
- [50] G. A. Petrucci, P. B. Farnsworth, P. Cavalli, and N. Omenetto, "A differentially pumped particle inlet for sampling of atmospheric aerosols into a time-of-flight mass spectrometer: optical characterization of the particle beam," *Aerosol Science and Technology*, vol. 33, no. 1–2, pp. 105–121, 2000.
- [51] J. A. Merten, N. Omenetto, X. Wu, B. W. Smith, and J. D. Winefordner, "Time and wavelength resolved laser-induced amino acid fluorescence of bacteria," to be submitted to *Applied Optics*.
- [52] Y.-L. Pan, S. C. Hill, J.-P. Wolf, S. Holler, R. K. Chang, and J. R. Bottiger, "Backward-enhanced fluorescence from clusters of microspheres and particles of tryptophan," *Applied Optics*, vol. 41, no. 15, pp. 2994–2999, 2002.
- [53] K. Willeke and P. A. Baron, *Aerosol Measurement: Principles, Techniques and Applications*, Van Nostrand Reynolds, New York, NY, USA, 1993.
- [54] A. R. Holzwarth, "Time-resolved fluorescence spectroscopy," in *Biochemical Spectroscopy*, vol. 246, pp. 334–362, 1995.
- [55] S. R. Piersma, A. J. W. G. Visser, S. De Vries, and J. A. Duine, "Optical spectroscopy of nicotinoprotein alcohol dehydrogenase from *Amycolatopsis methanolica*: a comparison with horse liver alcohol dehydrogenase and UDP-galactose epimerase," *Biochemistry*, vol. 37, no. 9, pp. 3068–3077, 1998.
- [56] J. M. Beechem and L. Brand, "Time-resolved fluorescence of proteins," *Annual Review of Biochemistry*, vol. 54, pp. 43–71, 1985.
- [57] A. Thomas, D. Sands, D. Baum, L. To, and G. O. Rubel, "Emission wavelength dependence of fluorescence lifetimes of bacteriological spores and pollens," *Applied Optics*, vol. 45, no. 25, pp. 6634–6639, 2006.