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Spectroscopic Sorting of Aerosols by a Compact Sensor Employing UV LEDs

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A compact sensor for physically sorting bioaerosols based on fluorescence spectra from single particles excited using arrays of ultraviolet light emitting diodes (UV LEDs) is presented. The optical system integrates electronics for real-time processing of spectral data and a miniaturized aerodynamic deflector for particle separation. Fluorescent polystyrene latex spheres are used to demonstrate fluorescence collection on-the-fly, operation of a real-time spectral algorithm, and physical separation of individual particles. This sensor illustrates the utility of recently developed UV LEDs, in conjunction with novel optical design and custom electronics, to shrink the size of aerosol fluorescence detection systems.

INTRODUCTION

Ultraviolet laser-induced fluorescence (LIF) is a technique that has long been used in bioaerosol particle detectors; see for example Kaye et al. (2005), Pan et al. (2003), and Hairston et al. (1997). Recent advances in device performance have prompted interest in ultraviolet light-emitting diodes (UV LEDs) at 290 nm and 340 nm, key wavelengths of excitation for intrinsic biofluorescence, as potential alternative sources of UV excitation over solid-state lasers. Jeys et al. (2003) have investigated the use of dual-wavelength, single-element LEDs, and Davitt et al. (2005) have demonstrated a compact system operating with a linear array of UV LEDs. In keeping with miniaturization of the UV source and design of a compact spectrometer for on-the-fly fluorescence analysis of single airborne particles in the latter demonstration, we present an optical system with an integrated miniature aerodynamic deflector to separate and concentrate particles with interesting or suspicious fluorescence spectra. Custom electronics have been developed to manage a standalone system and accomplish real-time processing of optical data. Together this represents a practical step toward a portable, semiconductor UV source-based, bioaerosol sensor that has the same functionality as traditional tabletop systems, which have also benefited from particle concentration techniques but employ bulkier deflection technologies such as those described by Pan et al. (2004, 2005). We note that our system has functional similarities to schemes employed in flow cytometry; see for example the extensive review by Davey and Kell (1996) and a more recent review emphasizing particle sorting by Mattanovich and Borth (2006). Although the demonstration presented here uses aerosolized particles, the LED-array excitation source, compact spectrometer, real-time electronics, and particle deflector in particular are portable to aqueous environments in general.

Here the aerosol particle sensor is designed with the primary objectives of making a real-time and compact front-end sensor able to classify particles by fluorescence spectra and physically sort a subset of particles from the background. In this demonstration we use fluorescent polystyrene latex spheres (PSL) as test particles for sorting purposes. In the bioaerosol detection application, a front-end sensor is one that can rapidly sort a sample of particles from the ambient and provide an early warning signal if potentially suspicious particles are present. Thus, it need not have microbiological-level specificity in identifying threats but, instead, serve as a trigger to subsequent analyses that take significantly longer, up to hours, but have better identification capabilities. In general, hazardous aerosols are present as a tiny minority of particles in the air, a condition that renders many biochemical analysis methods totally ineffective or too slow to serve as useful warning systems to people in the affected area. Our compact front-end system has the additional ability to physically sort sampled particles. This improves the prospects for

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many second-stage analysis methods, such as biochemical assays or Raman spectroscopy, by reducing the background particle burden and providing a suspicious-particle enriched sample. In addition, physical particle sorting allows a direct measure of the success of the optical and air-handling systems and fluorescence algorithm in identifying classes of particles.

SYSTEM DESIGN

Optical Apparatus

Figure 1 is a schematic illustration of the optical system and location of the mini-aerodynamic deflector, all of which is contained within a footprint of 25 × 35 cm. The proof-of-concept optical apparatus and UV LED light source design have been described in detail previously in Davitt et al. (2005). Briefly, particles ejected from the nozzle travel in a laminar jetstream for approximately 1 cm and travel at a velocity of approximately 2 m/s. Immediately upon exiting the nozzle, particles pass through the focused spot of a 635 nm laser diode (LD). The scattering signal, detected by a photomultiplier tube (PMT) equipped with a red bandpass filter, is used as the trigger signal to control the timing for the subsequent events as described below. Each particle passes through the focal plane of the UV LED array during which time the fluorescence spectrum is collected by a compact spectrometer consisting of a collection lens, transmission grating, filter, and 32-anode PMT; scatter intensity is collected simultaneously. This arrangement collects spectra spanning 325–700 nm in 32 equally spaced bins. The collection lens (focal length f) is placed a distance between f and $2f$ away from the particle stream. This produces a magnified image of the fluorescence line at the PMT, and the light passing through the transmission grating is not well collimated. Due to the large spectral width (∼10–15 nm) of each channel of the PMT, this arrangement does not contribute to any significant error and is employed because of its simplicity. The UV illumination source is a linear array of 32 independently addressable LEDs oriented vertically such that sequential flashing of the LED elements at a rate matched the average particle velocity in the jetstream results in tracking the particle over a vertical distance of approximately 3 mm. The 290 nm– or 340 nm–emitting LEDs are fabricated from MOCVD-grown AlGaN and AlGaInN quantum-well p-n junction heterostructures whose growth represents significant challenges in device science. Details of epitaxial growth, structure, process conditions, and device characterization are given in Ren et al. (2005); Adivarahan et al. (2004) and references therein illustrate state-of-the-art UV LED development. Currently, semiconductor UV emitters have lower output powers than other UV sources; however, their geometry can be tailored specifically to the task and have greatly reduced size and cost advantages over traditional LIF systems employing solid-state lasers.

Real-Time Electronics

Control of the LED-based fluorescence sensor, which requires a sequence of timed events, is entirely accomplished with 3 electronics boards designed for this purpose. The central PhotoniQ-3G iQSP480 board is a commercial product (Vtech Engineering Corp.), the primary function of which is to acquire and process the real-time fluorescence data from the 32 anode PMT and trigger the aerodynamic deflector based on a spectral algorithm applied to the fluorescence data. The functionality of the iQSP480 has been expanded by the integration of two

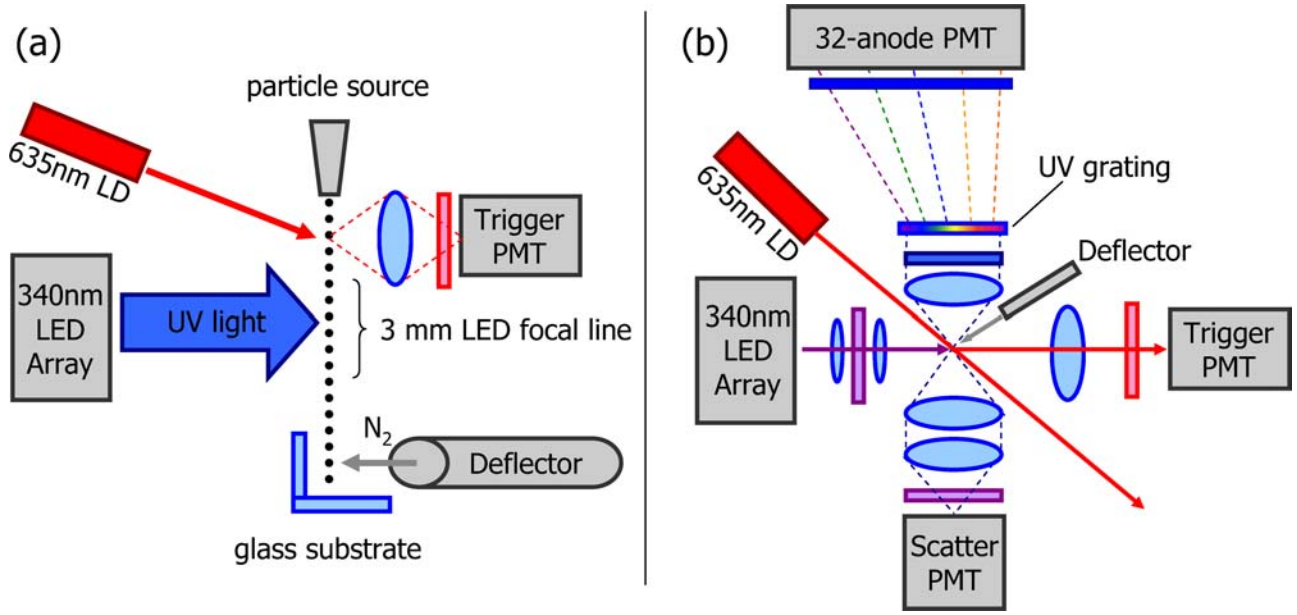

FIG. 1. Schematic of core optical system showing placement of aerodynamic deflector and substrate for sample collection. (a) Front view and (b) top view.

additional boards; one to drive the LED array current-pulsing and the other to control and monitor more than a dozen other sensor signals. A tailored PC-based graphical user interface (GUI) provides user control and programmability, particularly useful for testing, although the electronics can stand alone in a portable system. Upon a particle entering the optical path, a sequence of timed events occurs. The presence of the particle is detected from elastic scattering from a 635 nm LD. Timing of all subsequent events is tied to this trigger. Elements of the linear array of LEDs are fired sequentially, with 2 adjacent devices on at any time. The block of 2 LEDs as opposed to a single LED increases the overall light delivered to the particle—hence the resulting fluorescence—by reducing the in-between time. It also tolerates slight mismatching of the LED firing rate to the particle velocity. The fluorescence collected by the 32-anode PMT is evaluated by a spectral algorithm running on the DSP (digital signal processor) in the iQSP480. If the fluorescence spectrum is suspicious according to the algorithm, the electronics records a flag event and sends a trigger to the aerodynamic deflector in order to remove the particle from the jetstream. Owing to the finite time (5 msec in the experiments presented here) that the particle is in the jetstream, signal collection and evaluation of the algorithm must be done in real time. This is possible because the decision circuitry resides in the DSP and not in the PC. Furthermore, the DSP may be easily reprogrammed through the USB 2.0 interface to accommodate different spectral algorithms. The electronics support many more I/O channels than described here, for example, additional red LDs and PMTs for monitoring the particle before and after a deflection event and photodiodes for tracking the output of the UV LEDs.

Particle Deflection

A solenoid-based aerodynamic deflector is triggered to give a burst of nitrogen gas lasting for approximately 250 usec. When energized by a current pulse an electromagnetic plunger opens the valve, causing a burst of gas. The response time of the valve, which is returned to the closed position by a spring, means that the deflector may be fired at a rate of up to 1,200 Hz. The physical size of the cylindrical deflector, about 0.25″ in diameter and 1.5″ long, is much smaller than the aerodynamic deflectors that have been demonstrated in functioning LIF systems by Pan et al. (2004, 2005), and is thus more appropriate for integration into a compact optical and electronic system presented here. Particles are collected onto a glass substrate made from microscope coverslips, which is placed 2 mm below the deflector nozzle as illustrated in Figure 1. Deflected particles are deposited onto the vertical portion of the substrate while the undeflected jetstream accumulates on the horizontal glass.

DEMONSTRATION RESULTS

For demonstration, dye-filled polystyrene latex spheres (PSL) were used as simulants of biofluorescence. Two types of PSL were used, 1 um diameter blue fluorescing (Molecular Probes, F8815) and 2 um green fluorescing (Duke Scientific, XPR-801). Both are readily excited by 340 nm light, hence all experimental data shown in this report use a 340 nm linear array of LEDs as the excitation source. This is also a key wavelength of excitation for intrinsic biofluorescence, attributed broadly to NADH. The spheres are diluted to known concentrations in water and the solution is then ejected in 70 um diameter droplets at a rate of 10 Hz by a piezoelectric generator (MicroDrop GmbH). The concentrations are chosen such that the particles consist of water with statistically less than 1 PSL per drop. PSL spheres, as opposed to amino-acid mixtures such as tryptophan, tyrosine, and NADH that have been previously tested with our core optical system in Davitt et al. (2005), are useful characterization tools as they are present in discrete quantities (i.e., 0, 1, or 2 PSL per drop) and can be counted on the substrate to verify experimental results.

For the data shown in Figure 2, the drive current pulse per element was a modest 20 mA for a duration of 53 usec corresponding to 0.3 mW output power per element at the source. Figure 2(a) shows a portion of the PC display that shows the UV scatter intensity detected per element of the LED array. Intensity differences over the 32 LEDs are due to imaging optics, which contribute to center elements being better focused, and to element-to-element variation in device characteristics. For example, element 25 of this particular array was not functional, however, due to the 2-block sequential pulsing of the LED elements described above, a finite intensity is measured while the adjacent elements are on. The precise fraction of this scatter intensity depends on the synchrony of the sequential flashing and the particle fly-by. Figure 2(b) summarizes a series of demonstrations. Either blue-doped or green-doped PSL were separately passed through the system and a simple one-spectral-band algorithm was used to evaluate whether a particle was present or not. In this case, a flag to the deflector was generated upon the condition that the average charge collected over a 35 nm wide spectral range centered about the peak fluorescence (525 nm and 435 nm for the green and blue PSL respectively) was greater than a threshold level of 1 pC. The threshold was chosen to lie above the background level—due to detector noise and stray LD light—of roughly 10 photons per PMT channel accumulated during the total 1.6 msec fluorescence collection time, the time during which the particle traverses the LED array focal line. Shown in Figure 2(b) are the average fluorescence spectra of flagged events during a test of 5,000 particles; commensurate with the PSL concentration, nearly 200 particles were flagged to create each plot. A solution containing a mixture of both types of PSL was tested, statistically approximately 0.1 green and 0.05 blue PSL spheres per drop, and a one-band algorithm to isolate the green particles was applied. In addition, a two-band algorithm where the same wavelength bands were used but where a flag was generated only upon the boolean condition (green = true) AND (blue = false) was used in order to eliminate the possibility of highly fluorescent blue particles, or clusters, from tripping the green flag. The system collects not only fluorescence

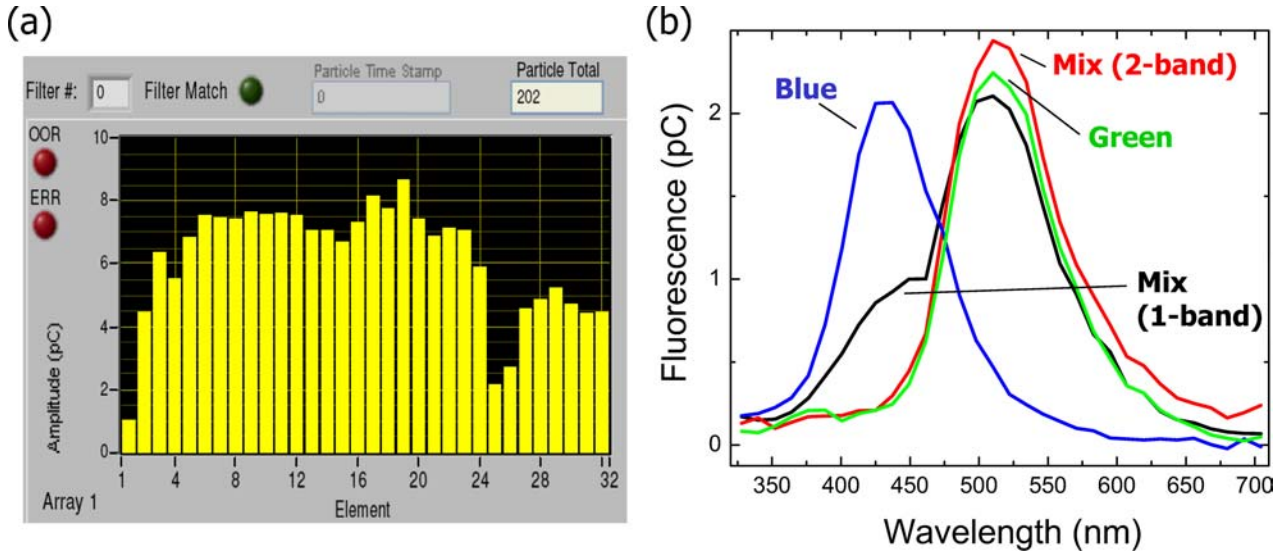

FIG. 2. (a) Portion of PC display during data acquisition showing scatter intensity detected per LED element, and (b) fluorescence spectra averaged over flagged PSL particles.

spectra but, also, scatter intensities and LED illumination intensities, all in real time. Both the optical hardware and present electronics acquire these optical signals, and the design of more sophisticated algorithms targeted to bioaerosol fluorescence using this data is under development. Application and testing of new algorithms is enabled by the reprogrammable nature of the DSP.

Results of the algorithm were used to trigger the mini-aerodynamic deflector located downstream from the LED array illumination, a distance corresponding to approximately 6 msec after the particle entered the optical system. Figure 3 is an epi-fluorescence image of the deflected and undeflected aggregates of spheres in the case where green PSL were selected using the two-band algorithm. In this configuration, the area containing deflected particles is smaller than 250 um in diameter. Among 5,000 doped water droplets, 185 were flagged as having green fluorescence. Counting deflected particles yields nominally 180 green and 8 mistakenly removed blue ones. The particle generation rate was such that successive water droplets were well separated in space and hence unaffected by gas bursts intended for adjacent particles. Thus, deflected blue particles indicate that the algorithm targeted to green particles yielded a flag for a blue one, or that clusters of multiple PSL were present in a single water drop. However, undeflected particles are exclusively blue, hence no "suspicious" (green) particles were falsely categorized as "safe" (blue), indicating that upon receipt of a trigger, the deflector is extremely reliable in removing suspect particles from the jetstream. Thus, an improvement in the particle concentration ratio may be achieved with refined algorithms.

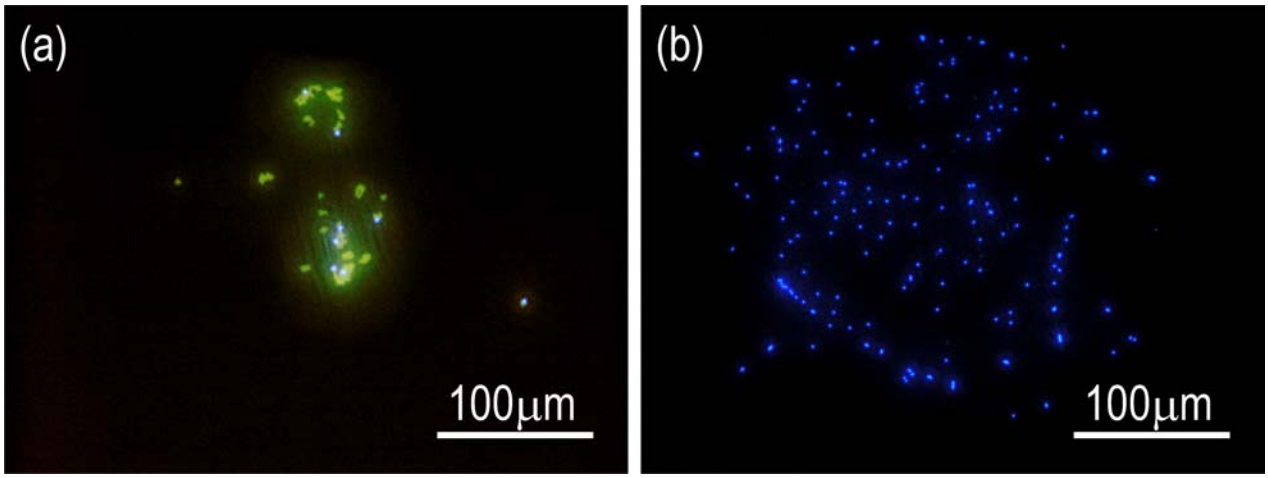

FIG. 3. Epi-fluorescence images of (a) deflected green and (b) undeflected blue PSL spheres.

COMMENTS

By replacing all of the fundamental components with smaller and potentially more cost-effective alternatives, we have demonstrated the capability of a genuinely portable aerosol particle sensor to perform tasks that are traditionally the realm of tabletop-size systems. Application of emerging UV semiconductor light emitters and use of novel detector arrangements enables compaction of the core optical components. In conjunction with custom-designed real-time electronics and a miniaturized aerodynamic deflector, the entire sensor system is now significantly reduced in size while maintaining both the capability of real-time fluorescence spectral detection and analysis and physical sorting of aerosol particles. This opens the possibility of creating truly portable aerosol warning sensors or front-end sensor networks.

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