Multiple-anode PMT makes possible the detection, discrimination, enrichment, and deposition of bioaerosols on-the-fly

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Background for detecting and characterizing bioaerosol particles

Environmental and occupational monitoring of hazardous aerosols, especially bioterrorism threats by way of dispersal of pathogenic bioaerosols, requires advanced systems that can identify harmful bioaerosol particles in quasi real-time and in situ. Instruments presently available to identify airborne biowarfare agents (BWA), mostly based on biochemical technologies, cannot be run continuously and are generally costly in both time and logistical burden. In these instruments, aerosol particles are collected and pretreated first, and then analyzed and identified. There is always a big challenge to get rid of the overwhelmingly dominant background aerosol particles in the pretreating process; a large quantity of background aerosol particles could contaminate the reactions, and disable the biochemical assay within minutes. A different approach, based on the analysis of UV laser-induced fluorescence spectra (UV-LIF), has resulted in instruments that can continually and rapidly discriminate bio-threat-like aerosols from many kinds of ambient aerosols. However, this approach struggles with specific identification and produces high false alarm rates, especially in instruments that rely on only two or three fluorescence bands. To overcome the limitations of each approach, a combined method has been devised, in which the dispersed spectroscopy of the UV-LIF signal is used as a first-stage discriminator to prescreen the background aerosol particles and rapidly get rid of interfering aerosols, while specific biochemical technology is used to identify suspect aerosols with high accuracy for bioaerosol detection and characterization.

A photomultiplier tube (PMT) is one of the most sensitive detectors, and it has a fast response time ~1 ns. The PMT needs no water cooling, is compact, and has been widely used in bioaerosol detection systems. For systems based on fluorescence spectrum, the intensified charge coupled device (ICCD) is generally selected for its high spatial resolution with single-photon sensitivity. However, the slow data-transfer rate of the ICCD detector limits the overall recording speed to tens of spectra per second, whereas the real-time fluorescence detection system needs higher sampling rates (at least a thousand per second) in order to have the ability to capture the hundreds of fluorescence spectra per second required for transient bursts of high concentration aerosols. In addition, a field-portable instrument needs components that are compact and lightweight. A multi-anode PMT assembly that has recently become available (Hamamatsu Corp., model H7260 with 32 anodes) holds promise for building on these recent advances for detection of bioaerosol particles. Replacing the cooled ICCD with this 32-anode PMT detector has dramatically increased the spectrum recording speed and reduced the physical size of the detection system, and has made it possible to detect, discriminate, sort, and collect bioaersol particles, one-by-one, on-the-fly, with a single UV laser shot.

Characteristics of the multi-anode PMT

The multi-anode PMT, shown in Fig. 1, behaves like multiple PMTs within a single housing. Each anode has its own output connection to a pin at the base of the PMT housing. The special design of the electron-multiplication section for the multi-anode makes this a unique detection unit. It consists of nine dynode stages for electron multiplication. The spatial integrity of photoelectrons, which comes from the particular location (x, y) of the photocathode in the region around each anode, is preserved by output from an anode to a corresponding pin. The detail of Fig. 1 illustrates the schematic of this design. Therefore, the signal from photons that strike a particular photocathode will be multiplied significantly (a gain of nearly 10^6 can be achieved when 800V is applied), preserved in the right location, and read out from a pin corresponding to one of the 32 anodes.

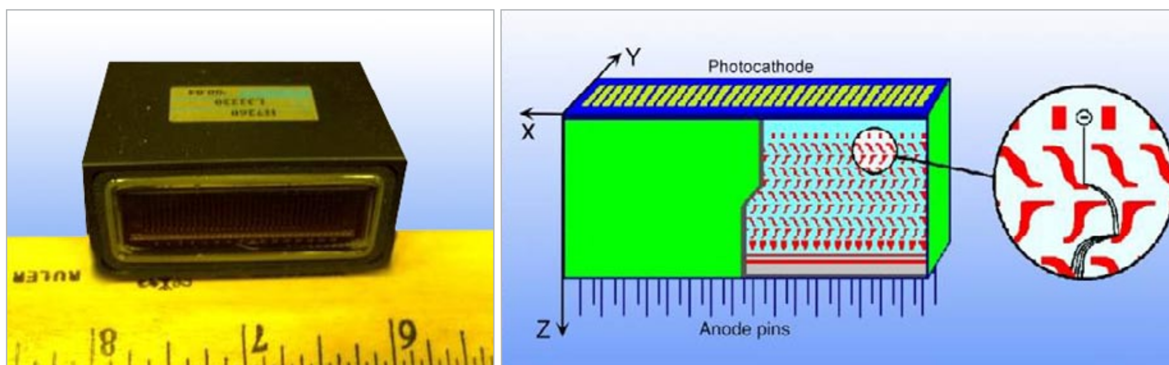

Figure 1. Illustration of the 32-anode photomultiplier tube, a rectangular photocathode (1.0 cm x 3.5 cm), and 32 output pins. Electron multiplication is achieved via a 9-stage dynode unit that preserves the spatial integrity of the photoelectroncs emitted along the x-axis of the photocathode.

The conventional PMT is a zero-dimensional detector without any spatial information of the intensity distribution of the incident photons along the x- and y-dimensions. All the photons incident on a big photocathode are summed, and the corresponding photoelectrons are multiplied and then read out from a single anode. By contrast, the 32-anode PMT is able to differentiate the varying intensities of incident photons along its x-axis, but without spatial resolution along y-axis (see detail in Fig. 1). However, since the fluorescence spectra from biological materials generally have pretty broad spectral profiles, dividing the spectrum from 300 nm to 600 nm into 32 bands supplies enough resolution for discriminating these spectra. Thus, the 32-anode PMT is an ideal choice for fluorescence spectra detection.

Overview of the bioaerosol identifier system

The schematic of the bioaerosol identifier system is shown in Fig. 2. It consists of two stages: the first stage is the developed bioaersol detecting, discriminating, enrichment, and deposition subsystem; and the second stage is the biochemical assay subsystem for identifying the strain of the biowarfare agent (BWA).

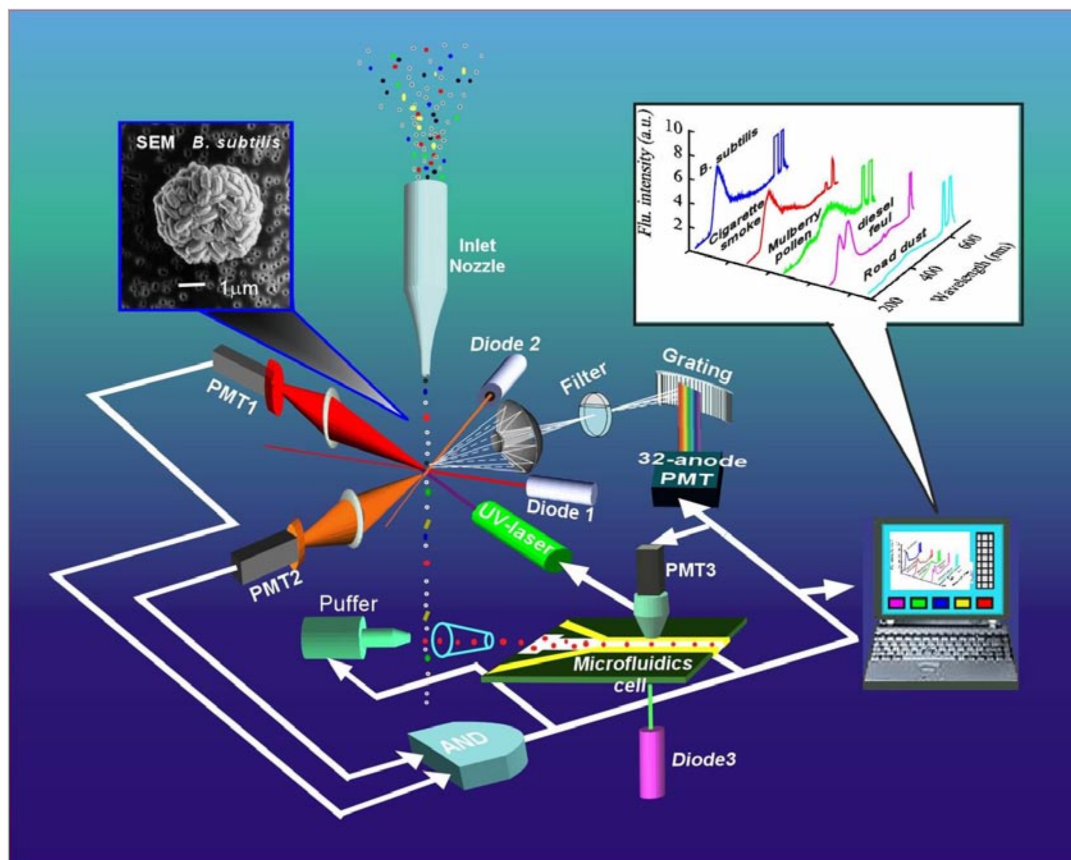

Figure 2. Schematic of the bioaerosol identifier system. The microfluidics cell and its associated optics are used for high-accuracy identification of the strain of the biowarfare agents. The UV-laser-induced fluorescence spectrum and the puffer are used for high-speed discrimination, on-the-fly, of bioaerosol particles. The scattering signal from the trigger volume (defined by the intersection of the color laser diodes) and the fluorescence from the biochemical assay are detected by a conventional single-anode PMT. The dispersed spectra for discriminating bio- or nonbio-aerosol particles are detected by a 32-anode PMT.

The first stage has the following key elements. (1) A concentrator that draws in air at about 300 liters/min and utilizes the virtual impact principle. The concentrator keeps most of the particles with a 1 µm to 10 µm diameter in the minor exit flow at a rate of 1 liter/min. (2) By using a specifically designed nozzle, the particles are forced to flow in a straight trajectory. They are localized within a cylindrical area of 600 µm diameter for a distance of at least 1 cm. (3) The exiting particles are aligned to flow through the intersecting volume (referred to as the trigger volume) of two diode laser beams with different wavelengths. Only if the particles travel through this intersection will the elastic scattered signals at two PMTs coincide, resulting in an AND gate output. (4) This output serves as a trigger to the UV laser (263 nm, the 4th harmonic of YLF), which is synchronized to illuminate the detected particle from the trigger volume flying through the sample volume (defined as the intersection of the UV-laser beam and the focal point of the fluorescence collection lens). When this particle is irradiated with UV radiation (λ = 263 nm), the UV-LIF spectrum is

dispersed by a compact spectrograph covering a wavelength span of 250 nm to 700 nm. This wavelength span of 450 nm is aligned with the detector elements from the 32-anode PMT (Hamamatsu H7260-20). (5) Every aerosol particle that transits through the trigger volume and subsequently transits though the sample volume is irradiated. The resulting fluorescence spectrum for each of these particles is captured and analyzed by the readout and processing electronics made by Vtech Engineering Corporation. (6) The on-board processor determines whether or not a particle has the characteristic spectrum of known bio-threat aerosols. If a particle matches pre-determined signature criteria, the electronics trigger the air puffer to blast out a puff of air, which then deflects that particular particle as it flows further down stream. (7) The "puffed" potential threat particles is delivered into the entry well of a microfluidic cell with the particle aerodynamic localizer (PAL). The second stage consists of these steps: (1) tag the threat particles by fluorescence-labeled biorecognition elements, such as antibodies in solution which are continually supplied to the entry well of the microfluidic cell; (2) hydraulically focus and pull the liquid, which contains threat particles tagged by biorecognition elements, into a stream with a 20 µm x 20 µm cross section; and (3) continuously identify threat particles present by fluorescence emission from attached biorecognition elements.

Applications of the bioaersol detecting, discriminating, enrichment, and deposition system

By using the 32-anode PMT as the key detector for dispersed fluorescence spectra, the system can run continuously and autonomously for long periods (e.g., one week) and has the ability to monitor up to 90,000 particles/sec. using an on-board processor (developed by Vtech Engineering Corporation) and an optimized algorithm to discriminate threat-like particles based on UV-LIF. This high-speed discriminator allows only the threat-like particles, which comprise only a very small fraction of all the particles queried via UV-LIF, to continue into the second-stage identifier, minimizing background clutter and the possibility of clogging the microfluidic cell. Therefore, the BWA aerosol particles will then accurately identify threat particles in the second stage.

Now, the high-speed, highly sensitive, real-time, in situ bio-aerosol detecting, discriminating, sorting, and collecting system for micron-sized respirable particles (1 µm to 10 µm), one-by-one, on-the-fly, excited by a single UV laser shot has been successfully tested in various indoor and outdoor fields. The technological advancements have been transitioned to the Defense Advanced Research Project Agency (DARPA), and one of the cloned systems is currently operating in the San Francisco International Airport facility by Sandia-Livermore National Laboratories for calibrating other fluorescence-based biosensors.

Figure 3 shows 500 successive fluorescence spectra from ambient aerosol particles (NRL on Aug.18, 2004) illuminated by the 263 nm Q-switched Nd:YLF laser (50 µJ/pulse). Among them, 19 particles had been assigned to be bio-threat-like aerosols and got deflected and collected; all of them had a strong fluorescence peak around 330 nm. The other 481 particles with their corresponding fluorescence spectra did not meet the criteria conditions, were considered as non-suspect aerosols, and hence were not deflected. The corresponding collections of the deflected aerosols with different criteria definitions of fluorescence spectra underwent analysis by Fourier Transform Infrared (FTIR) spectroscopy. In a blind test at Applied Physics

Laboratories, Johns Hopkins University for the DARPA Spectral Sensor for Biological Agent program, this system was used as the front-end to prescreen the aerosol particles, and collected the suspect particles on a substrate for further analysis by visible Raman, UV Raman, and fluorescence imaging. Results revealed that the biological simulants were recognized and identified with much higher detection probability and a lower false alarm rate with the help of the preselection of bio-threat-like particles than by the direct deposit of all aerosol particles without any sorting process.

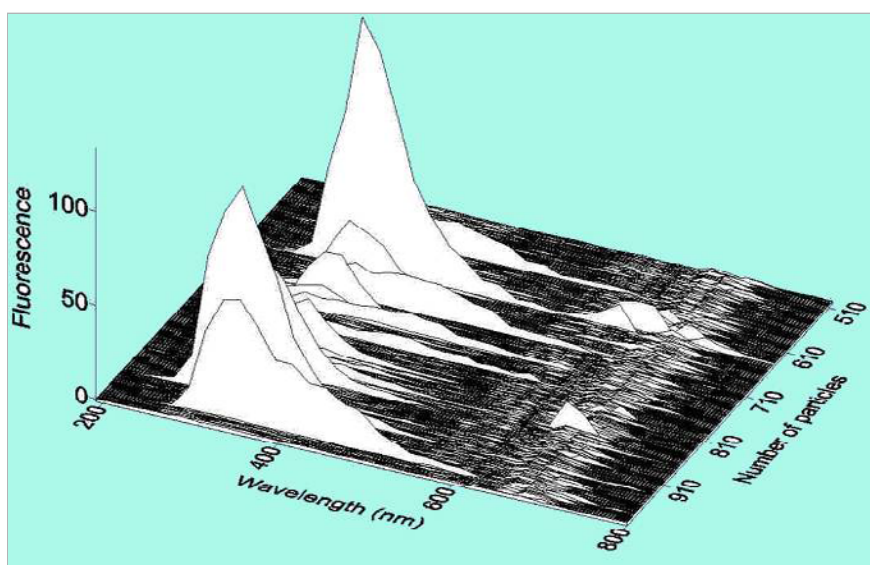

Figure 3. 500 successive single-shot fluorescence spectra from ambient aerosol particles (NRL on Aug. 18, 2004) illuminated by the 263 nm Q-switched Nd:YLF laser (50 μJ/pulse). Any detected ambient aerosol particles that have bio-threat-like fluorescence spectra are deflected and collected for further analysis.

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