Sensors and Materials, Vol. 34, No. 9 (2022) 3371–3381
MYU Tokyo

S & M 3046

Multi-wavelength-excitation Laser-induced Fluorescence for Real-time Bioaerosol Detection: A Mini Review of Applications

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(Received February 22, 2022; accepted May 23, 2022; online published July 11, 2022)

Keywords: laser-induced fluorescence, bioaerosols, multi-wavelength excitation, detection

The development of real-time online bioaerosol detection technologies and instruments for the prevention and control of infectious diseases, environmental quality supervision, biological safety, and health protection has attracted increasing attention. Compared with single-wavelength-excitation laser-induced fluorescence technology, laser-induced fluorescence technology based on multi-wavelength excitation possesses major advantages in detection sensitivity and aerosol species discrimination. This article summarizes the progress of research on instruments based on multi-wavelength-excitation laser-induced fluorescence technology, and forecasts future research and development prospects, as well as provides a reference for the technical research and application of real-time online bioaerosol detection, especially for harmful pathogenic microbial aerosols, in the field of public safety.

1. Introduction

An aerosol is a stable dispersion system composed of solid or liquid particles suspended in a gas medium and has the potential to spread over long distances.(1) Bioaerosols are an important part of atmospheric aerosols, containing solid or liquid particles of life-active substances such as microorganisms and biological macromolecules. Bioaerosol particles include bacteria, fungi, viruses, mycoplasma, dust mites, pollen, spores, animal- and plant-derived proteins, various fungal toxins, and their fragments and secretions,(2–4) with a particle size in the range of 0.01–100 μm. There are hundreds of harmful pathogenic microorganisms spread by aerosols, which can cause large-scale pollution in the short term. Meanwhile, biological warfare agents released in the atmosphere in the form of bioaerosols can cause great harm to personnel as well as military and civilian facilities. Therefore, strengthening the monitoring of bioaerosols is of great significance for the early warning of bioterrorism attacks and reducing the spread of respiratory diseases and related infections.(5)

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https://doi.org/10.18494/SAM3871

ISSN 0914-4935 © MYU K.K.
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At present, the main technical method used for the real-time online detection of bioaerosols is laser-induced fluorescence (LIF).[6–8] The proteins, viruses, bacteria, and other bioorganic substances contained in bioaerosol particles have fluorescent groups, and these substances can produce fluorescence under the excitation of a laser of the corresponding wavelength. The fluorescence emitted from these biological substances shows significant differences in the spectral characteristics. For example, typical bioluminescent molecules such as tryptophan[9] (excitation wavelength λ_{ex} = 280 nm), riboflavin[10] (λ_{ex} = 450 nm), and coenzyme-nicotinamide adenine dinucleotide (NADH)[11] (λ_{ex} = 350 nm) have different excitation-emission fluorescence bands, allowing the bioaerosols to be monitored by detecting their fluorescence spectra. LIF possesses the advantages of high detection sensitivity, easy operation, and a large detection range.

At present, many LIF-based commercial particle spectrometers use a single-wavelength laser to determine the size and fluorescence of aerosol particles, and can only obtain limited fluorescence information and few excitation-emission fluorescence bands. Thus, it is difficult to eliminate the interference of non-biological fluorescent particles as well as identify the species of fluorescent particles. Therefore, analysis with multi-wavelength-excitation LIF technology combined with multi-excitation-emission fluorescence band signals has become a research priority with prospects of improving the distinguishing ability and detection efficiency of aerosols. This article reviews the current development of multi-wavelength-excitation LIF technologies and related instruments.

2. Development of Multi-wavelength-excitation LIF Instruments

2.1 Fluorescence particle spectrometers

Pan and coworkers[12,13] reported a fluorescence particle spectrometer (FPS) for real-time aerosol fluorescence spectral measurement (Fig. 1). The FPS can determine fluorescence spectra and aerosol particle size at the same time: 635 and 670 nm diode laser beams are focused and

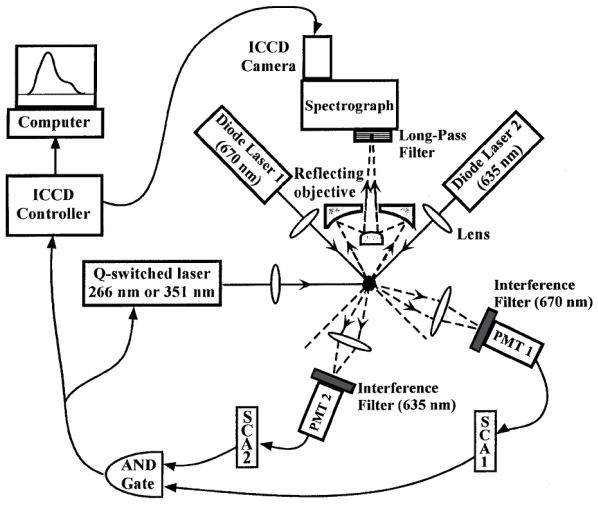

Fig. 1. Structure of FPS. Reprinted/Adapted with permission from Ref. 12 (© Optica Publishing Group).

intersected to determine the size of particles, and when a particle passes through both these beams, a 266 nm pulsed UV laser is triggered. In this equipment, the fluorescence spectra are recorded by an image-intensified CCD (ICCD). The FPS is sufficiently sensitive to detect aerosol particles with sizes from 3.5 to 11 μm.

Pinnick *et al.*[14] improved the FPS system by using a virtual impactor particle concentrator as an inlet. In their study, the fluorescence spectra measured by a gated ICCD were grouped by hierarchical cluster analysis with the threshold parameters chosen. Pan *et al.* improved their previous apparatus;[15] their improved particle-fluorescence spectrometer (PFS) was equipped with a pulsed 263 nm laser to excite LIF and used a 32-anode photomultiplier tube (PMT) detector to measure dispersed LIF spectra in the wavelength range from 280 to 600 nm. Meanwhile, an aerodynamic-focusing sheath inlet nozzle module was installed to improve the sampling rate. The elastic scattering of each particle can be detected by the significantly improved PFS, and the fluorescence spectra of bacterial particles with sizes as small as 1 μm can also be measured.

Huang *et al.*[16] reported an *in situ* aerosol detection system that can rapidly measure dual-wavelength-excitation LIF spectra of single flowing aerosol particles. In this system, particles flow through the intersection of 650 and 685 nm laser beams, and the scattered light is detected by two PMTs. These lasers and PMTs are utilized as a trigger module. When PMTs detect the scattered light, 263 and 351 nm UV lasers are triggered immediately in rapid succession to illuminate the aerosol. Afterwards, the scattered UV laser light is blocked by filters with cutoff wavelengths of 295 and 380 nm that are positioned at the entrance slit, and the fluorescence emitted from the aerosol dispersed by the spectrometer is recorded by a 32-anode PMT array. Their study demonstrated potential applications of this dual-wavelength LIF-based aerosol detection system in environment monitoring, especially for aerosols containing harmful bacteria, fungal spores, or pollen.

A particle fluorescence spectrometer (DPFS) system that evolved from the work of Huang *et al.* was designed by the US Army Research Laboratory (Fig. 2). Instead of the visible lasers in their aerosol detection system, the DPFS uses crossed 785 and 830 nm lasers as the LIF trigger. Its LIF assembly also consists of 32-anode PMTs and 263 and 351 nm pulsed lasers.[17] Pan *et al.*[18] measured the fluorescence spectra of *Bacillus subtilis* and other aerosol particles by using

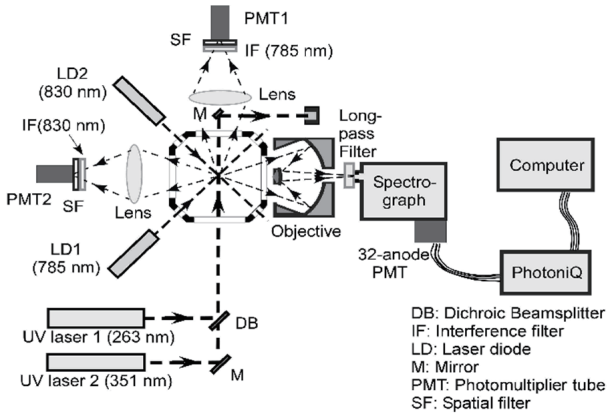

Fig. 2. Top-view schematic of DPFS. Reprinted/Adapted with permission from Ref. 18 (© Optica Publishing Group).

the DPFS, the data were analyzed with six different algorithms, and the increase in its discrimination capability was investigated.

2.2 Wideband integrated bioaerosol spectrometers

As a three-channel LIF spectrometer, the wideband integrated bioaerosol spectrometer (WIBS) developed by the University of Hertfordshire is a widely used aerosol fluorescence sensor. WIBS features three fluorescence channels, includes several versions, and is now manufactured by Droplet Measurement Technologies. Kaye *et al.*[19] developed a multi-channel single-particle aerosol fluorescence sensor, WIBS2, which was employed to establish large-area bioaerosol detection networks (Fig. 3). Particles as small as 1 μm can be detected by a 660 nm continuous-wave (CW) diode laser beam, then the scattered light generated triggers the sequential firing of 280 and 370 nm lasers to illuminate the particles. Different from WIBS2, WIBS3 uses a 632 nm diode laser beam to generate elastically scattered light. A PMT with bands of 310–400 nm and 400–600 nm is used to measure the fluorescence excited by the 280 nm laser beam, and the emission excited by the 370 nm beam is recorded in the 400–600 nm band. WIBS3 was used to measure ambient aerosols in Manchester, UK and Borneo, Malaysia, and the research results indicate that the 310–400 nm band in Manchester gives greater discrimination and can better distinguish between different datasets.[20]

The latest version of WIBS is WIBS-NEO, which can measure the light scattering and fluorescence of a single particle, as well as the particle size and particle asymmetry factor. Particle sizes in the range of 0.5–30 μm can be detected by WIBS-NEO. Laser beams with wavelengths of 280 and 370 nm are used to excite the fluorescence of bioaerosol particles, and wavebands of 310–400 nm and 420–650 nm are measured.

The Multiparameter Bioaerosol Spectrometer (MBS) developed by the University of Hertfordshire has been used in many real-time bioaerosol monitoring experiments.[21] MBS is similar to WIBS in its design and operation. When an aerosol flows through the sensing region, aerosol particles of 0.5–20 μm size are first detected and sized by a 635 nm low-power laser beam, then particles larger than a threshold size trigger a 637 nm high-power laser beam to irradiate these particles. Different from WIBS, high-resolution details of particles' spatial light-

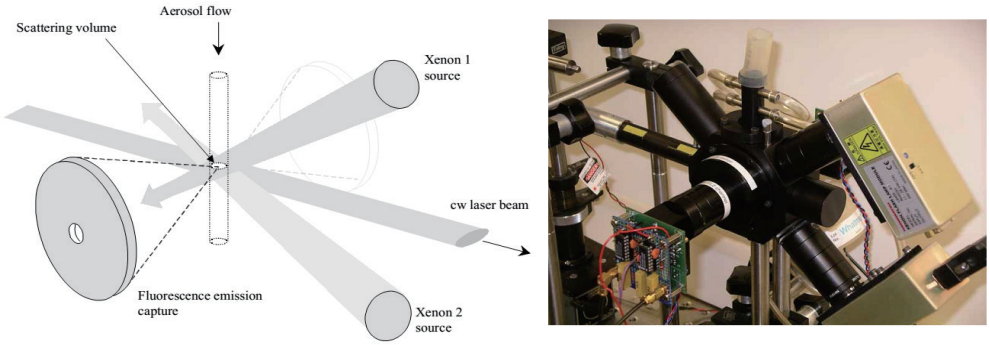

Fig. 3. (Color online) Schematic and photograph of WIBS2. Reprinted/Adapted with permission from Ref. 19 (© Optica Publishing Group).

scattering patterns are recorded by a dual CMOS linear array installed in MBS, and the spectral resolution is increased by an eight-channel PMT, which records eight equal-wavelength bands of fluorescence covering the range of 310–640 nm. Moreover, the dual CMOS linear array can both provide the morphology of particles and identify particle surfaces with potentially useful features. Furthermore, this modification can enhance particle classification and reduce false-positive bioparticle detection.

The Spectral Intensity Bioaerosol Sensor (SIBS) manufactured by Droplet Measurement Technologies is an improvement of WIBS, and it shares fundamental units with the latest versions of WIBS. SIBS uses 280 and 370 nm as excitation wavelengths and measures fluorescence emission in the range from 298 to 735 nm, covering 16 bands, providing additional spectral information and sufficient spectral resolution.[22] Könemann *et al.*[23] used SIBS to record the fluorescence spectra of 16 reference compounds, providing a means of distinguishing the spectra of bacteriochlorophyll, chlorophyll a, and chlorophyll b. This indicates that SIBS can resolve integrated spectral signals originating from relevant bioluminescent molecules.

2.3 Other instruments

A two-wavelength-excitation single-particle fluorescence analyzer (2-SPFA) has been developed (Fig. 4). For this instrument, elastically scattered light of a single particle produced by a 785 nm CW laser beam is used to measure the particle size as well as trigger 266 and 355 nm UV pulsed lasers. Different from other LIF instruments, the 785 nm CW laser beam and both UV pulsed laser beams are directed collinearly into the aerosol chamber, and fluorescence signals are detected by PMTs in three bands (350, 450, and 550 nm) as well as the scattered light. 2-SPFA has been applied to characterize individual biological aerosol particles, including biological warfare agent surrogates,[24] with the particle aerodynamic diameter ranging from slightly below 1 μm to about 8 μm.

Xu *et al.*[25] constructed a dual-channel bioaerosol detection system that uses 280 and 365 nm lasers to excite the intrinsic fluorescence of different biological substances. The fluorescence

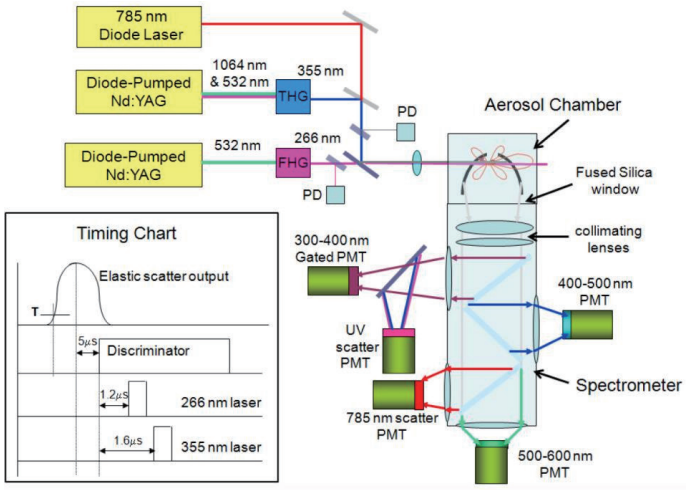

Fig. 4. (Color online) Schematic of 2-SPFA and electronic timing diagram. Reprinted/Adapted with permission from Ref. 24 (© Optica Publishing Group).

spectra and intensity of tryptophan and NADH were measured to detect bioaerosols, and the ratio of the fluorescence intensities of the two channels was used to discriminate these substances. Their research results demonstrate that the use of multi-channel LIF technology can improve the accuracy of detection and expand the detection range of aerosol particles.

Under the sponsorship of the Defense Threat Reduction Agency and the Joint Program Executive Office for CBRN Defense, Massachusetts Institute of Technology (MIT) Lincoln Laboratory developed the Rapid Agent Aerosol Detector (RAAD).[26,27] RAAD uses four lasers with emissions of 808, 266, 355, and 1064 nm. The presence, size, and trajectory of a single aerosol particle are detected by the 808 nm near-IR laser beam and then a structured trigger is generated. If the aerosol particle is sufficiently large (roughly 1–10 μm), then the particle's elemental content is characterized by laser-induced breakdown spectroscopy (LIBS), which is induced by the 1064 nm IR laser beam. For RAAD, each particle is measured up to 14 times on the fly to ensure accurate results. RAAD is an upgrade of the Joint Biological Point Detection System (JBPDS), and it possesses high reliability and discrimination for biological threats.

Taketani *et al.*[28] described a LIF-based instrument for the online detection of organic aerosol particles. First, scattered light of aerosol particles produced by a 635 nm CW laser beam is measured, and then LIF is produced by illuminating the particles with a 266 nm pulsed laser beam. The fluorescence emission in the 300–600 nm band is spectrally dispersed by a grating spectrometer and successively detected by a 32-anode PMT. Their research revealed that pure tryptophan particles as small as 0.3 μm can be detected using the fluorescence of this LIF-based instrument.

The Fluorescent Data Acquisition Instrument (FDAI) is a LIF-based bioaerosol monitor. For FDAI, particle sizes in the range of 0.15–15 μm are measured using a 650 nm laser beam, and then the aerodynamic particle size is determined from the flight time. A 266 nm laser beam irradiates bioaerosol particles to generate a fluorescence signal. The fluorescence pulse signal of 405 nm is subsequently detected and then the intensity and quantity of the fluorescence pulse signal are classified. Li *et al.*[29] obtained the background concentration of bioaerosols in Changsha, China by using FDAI, and a back-propagation neural network with principal component analysis was utilized to investigate the correlation between the bioaerosol concentration and meteorological factors. The derived model is considered promising, with an average relative error of 10.55% in forecasting the bioaerosol concentration. They also developed a simple method based on a wavelet-denoising back-propagation neural network model for forecasting the bioaerosol concentration with higher accuracy; the average relative error was 8.75%.[30]

Rapid-E is a commercial instrument designed and produced by Plair SA, Switzerland, for monitoring atmospheric aerosol particles in real time. It uses a 400 nm near-UV laser beam to irradiate particles, and the scattering image is recorded using 24 time-resolving detectors distributed at different angles to determine the morphology of particles, such as their size and shape. A 320 nm UV laser is utilized to generate LIF.[31] Rapid-E has two detection models for the detection of particles with sizes in the ranges of 5–100 μm and 0.5–100 μm, allowing the distinction of pollen by the former model and spores, particulate matter, and bacteria by the latter model. The latest version, Rapid-E+, can analyze the full spectrum of bioaerosols in the particle size range of 0.3–100 μm.

As a classic aerosol detector, the Ultraviolet Aerodynamic Particle Sizer (UV-APS)[32,33] spectrometer (model 3314) produced by TSI Inc. uses a 680 nm laser diode to measure the aerodynamic size of aerosol particles and a 355 nm pulsed UV beam to generate LIF. An avalanche photodetector and PMT are respectively used to detect scattered light and fluorescence, and particles with sizes in the range of 0.5–20 μm can be measured. Table 1 summarizes the LIF-based aerosol detectors discussed in this section.

Table 1
Parameters and technical components of the multi-wavelength-excitation LIF-based aerosol detectors.

Model/author	Measured parameters	Laser for scattered light (nm)	Fluorescence excitation laser (nm)	Fluorescence detector/ wavelength range (nm)
FPS	Particle size Fluorescence	635 670	266 351	ICCD 200–650
Pinnick	Particle size Fluorescence	635 670	266	ICCD 295–605
PFS/Pan	Particle size Fluorescence	650 685	263	anode PMT 280–600
Huang	Particle size Fluorescence	650 685	263 351	Spectrometer 32-anode PMT
DPFS	Particle size Fluorescence	785 830	263 351	32-anode PMT 300–600 400–700
WIBS2	Particle size Fluorescence	660	280 370	PMT 320–600 420–600
WIBS3	Particle size Fluorescence	632	280 350	PMT 310–400 400–600
WIBS-NEO	Particle size Fluorescence	635	280 370	16-channel PMT 310–400 420–650
MBS	Particle size Fluorescence	635 637	280	PMT 315–640
SIBS	Particle size Asymmetry factor Fluorescence	785	285 370	16-channel PMT 302–721
2-SPFA	Particle size Fluorescence	785	266 355	PMT 400–500 300–400
Xu	Fluorescence	—	280 365	PMT 450–650 350–650
RAAD	Particle size Fluorescence LIBS	808	266 355	—
Taketani	Particle size Fluorescence	635	266	32-anode PMT 300–600
FDAI	Aerodynamic particle size Fluorescence	650	266	—
Rapid-E	Particle size Fluorescence	400	320	32-channel PMT 350–800
UV-APS	Aerodynamic particle size Fluorescence	680	355	PMT

3. Conclusions

This article reviews the development of multi-wavelength-excitation LIF technology and instruments in recent years. Compared with single-wavelength-excitation LIF, most of the above-mentioned instruments use IR or near-IR laser beams to irradiate aerosol particles. The scattered light signals are subsequently used to trigger UV or near-UV lasers to induce the fluorescence of bioaerosol particles, and highly sensitive PMTs and CCDs are selected to detect the fluorescence and scattered light. The strategy of detecting scattered light and fluorescence produced by different laser beams has distinct advantages over single-wavelength-excitation LIF. This strategy can reduce the interference of scattered light with the fluorescence, and can also be used to determine the particle size by measuring the scattered light signal. It can also decrease the interference of non-target particles by judging the particle size to determine whether to trigger LIF. It is noteworthy that some instruments use dual lasers to irradiate particles to excite fluorescence in different wavelength bands, and provide more spectral information on the particles by combining multiple excitation wavelengths and multiple detection channels, thus improving the ability of distinguish aerosol particles and expanding the detection range of aerosol particles.

However, compared with single-wavelength-excitation LIF-based aerosol detectors such as the Fluorescence Aerosol Particle Sensor (FLAPS) (model 3317), which only uses a 30 mW, 405 nm laser diode as the excitation light source, the optical paths of current multi-wavelength-excitation LIF-based aerosol detectors that use multi-wavelength irradiation/excitation lasers are very complicated. Additionally, different lasers are prone to interference when aerosol particles are excited; in particular, the excitation wavelength may be within the detection range of the fluorescence detector and cause the detector to saturate. Moreover, multi-wavelength-excitation LIF-based aerosol detectors are difficult to miniaturize owing to their complex optical paths and numerous lasers and detectors. Therefore, future research should focus on optical path optimization, laser miniaturization, and aerosol particle classification and identification algorithms. For the real-time monitoring of biological aerosols, multi-wavelength-excitation LIF is expected to play a more important role in the future in improving the accuracy of distinguishing biological and non-biological particles and species of biological particles in environmental monitoring, public safety, and other fields.

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