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Accurate measurement of airborne biological particle concentration based on laser-induced fluorescence technique

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ABSTRACT

We develop a biological particle counter based on laser-induced fluorescence for accurate measurement of biological particle concentrations in the air. Pure water, NaCl particles, polystyrene latex spheres, and standard fluorescent particles are used as media to evaluate the performance of the counter. *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are used as representative biological particles to evaluate the measurement accuracy of the counter. In experiments, the results measured by the counter are consistent with he results obtained with the culture method; For each bacterium, good linear agreement is observed between the two results, and the values of the coefficient R^2 are all more than 0.97. Because of particle superposition errors, system error, etc., at low concentrations, the number measured by the counter divided by the number measured by the culture method, $\eta = N_{counter}/N_{culture}$, is larger than that at high concentrations. For same type of test sample, although the distribution is different, the η values at same concentrations are similar. Finally, the repeatability of the two methods is tested. The results obtained using the counter are found to be more stable, with a relative standard deviation (RSD) of 8.14%; this is less than the RSD of 15% obtained using the culture method.

1. Introduction

Bioaerosols are suspensions of airborne particles that contain living organisms or particles released from living organisms (Watches & Cox, 1995). Examples include bacteria, fungi, pollens, viruses, and protein allergens from animals or plants. Intrinsic fluorescence is an inherent characteristic of bioaerosols, and it is usually generated from organic substances [such as amino acids, riboflavin, and reduced nicotinamide adenine dinucleotide (NADH)] under excitation by ultraviolet (UV) light (Lakowicz, 2006).

Since the discovery of the intrinsic laser-induced fluorescence (LIF) of bioaerosols, mixing techniques in combination with the single-particle scattering technique and LIF technique have been rapidly developed over the last two decades (Yong-Le et al., 2007). Many companies, colleges, and research institutions have studied the intrinsic fluorescence of various bioaerosols and developed systems and equipment for identification and classification of bioaerosols. In 1997, Hairston and his colleagues from TSI Inc. in the US designed a fluorescence detection system based on the APS-3310 particle counter using two lasers (Hairston, Ho, & Quant, 1997). In 1998, Pinnick et al. created an experimental device for detecting the fluorescence spectrum of a single biological aerosol particle,

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which could characterize biological particulates; this system has an additional two lasers (488 and 266 nm), unlike that of Hairston et al. The 488 nm laser is used not only for scattered light detection but also the detection of the intrinsic fluorescence. The 266 nm laser determines whether there are biological particles (Pinnick et al., 1998). In 2000, Brosseau designed and developed a fluorescence detector, the UV aerodynamic particle sizer. The instrument employs a pulsed, 355 nm laser rather than a continuous-wave UV laser such as that described by Hairston et al. Brosseau et al. (2000). Azbil BioVigilant Inc. introduced the IMD-A series, in which scattered light detection and fluorescence excitation are performed by the same laser, a semiconductor laser with a near-UV wavelength of 405 nm (Product Specifications of Azbil BioVigilant. Inc, 2013a, 2013b). The semiconductor laser can greatly reduce the cost and allow for miniaturization of the equipment. Evenstad et al. developed FLAPS-3317, a new fluorescence sensor based on a previous system. In this system, scattered light detection and fluorescence excitation are also performed by the same 405 nm semiconductor laser. However, the fluorescence is separated into two wave bands to identify bioparticles (Jim, Dahu, Hairston Peter, & Darrick, 2013). Many current systems and instruments resemble FLAPS-3317. These include the biological agent warning sensor (Primmerman, 2000) and the single-particle fluorescence analyzer (Eversole, Hardgrove, Cary, Choulas, & Seaver, 1999). Pan et al. designed an experimental device called the dual-excitation-wavelength particle fluorescence spectrometer. It has double-wavelength excitation and a multichannel receiver, and 16 varieties of atmospheric particles have been tested (Pan et al., 2011). In 2004, Kaye et al. introduced the wide issue bioaerosol sensor (WIBS) for networked deployment (Kayea et al., 2004). One year later, Kaye et al. developed WIBS-2 (Kaye et al., 2005). Several years later, Droplet Measurement Technologies developed a new instrument called WIBS-4. In 2012, Toprak and Schnaiter evaluated its performance in the laboratory and in the field (Toprak & Schnaiter, 2012). In recent years, Droplet Measurement Technologies developed a new instrument called WIBS-4A. WIBS uses pulsed UV xenon lamps for fluorescence excitation, and it generates two wave bands during operation. It also has a laser diode at 635 nm and is used for scattered light detection. The two wave bands for fluorescence excitation are at 280 and 370 nm, which correspond to the tryptophan and NADH spectral absorption peaks, respectively, and are used for intrinsic fluorescence detection (Product Specifications of Droplet Measurement Technologies). In addition, countries such as Germany, Switzerland, and Norway have also made great advances in the development of light-induced fluorescence detection technology (Bundke, Reimann, Nillius, Jaenicke, & Bingemer, 2010; Farsund, Rustad, Kasen, & Haavardsholm, 2010; Kiselev, Bonacina, & Wolf, 2011).

Almost all of these studies focused on the identification and classification of bioaerosols. However, sometimes the concentration of microorganisms is more important than the identification and classification of bioaerosols. When the concentrations of infectious bacteria or viruses exceed a certain limit in the environment, these microorganisms cause diseases and adversely affect human health. Therefore, it is important to measure the concentration of bioaerosols accurately and promptly in various environments.

In this paper, we present a counter with optical and detection systems, and conduct a series of experiments to evaluate its performance, focusing on its accuracy in measuring bioparticle concentrations. Finally, we compare the counter's results with those of the culture method.

2. Description of counter

Many counters using LIF and light scattering are described in detail elsewhere. Our counter, with a new optical system and detection system, is described here. The exterior of the instrument is shown in Fig. 1. The dimensions of the device are 245 mm (width) × 220 mm (height) × 145 mm (depth)

2.1. Optical system

The optical system is shown schematically in Fig. 2. The system consists of the input optics for the 405 nm near-UV laser diode beam used for both the scattered light and LIF signals. Receiving optics are also needed to collect light from the illuminated particles

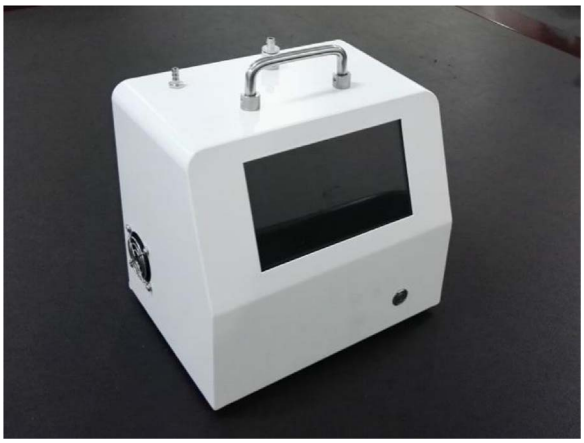

Fig. 1. Exterior of instrument.