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Research Article

Analysis of Correlation between Atmospheric Fluorescent Particles and Biomaterials

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ABSTRACT Bio-aerosols are important components of atmospheric aerosols, which can affect human health depending on the origin of biological particles. A real-time monitoring technology for bio-aerosols can rapidly provide information on the current state of biological particles in ambient environment, which substantially minimize the impact of hazardous biological particles. The aim of this study is to evaluate LIF (laser-induced fluorescence) technology on real-time monitoring of bio-aerosols by investigating the correlation between biomaterial concentration (cultured microbes or extracted DNA) and fluorescent particle concentration in ambient environment. For this, we used LIF instrument, BDS (Bio-aerosol Detection System), and analyzed the correlation coefficients from the collected data (the non-fluorescent particle concentration, the fluorescent particle concentration, the cultured microbial concentration, the extracted DNA concentration). Pearson's correlation coefficients (r) from this study are as follows: 0.85 ($p < 0.001$) between the fluorescent particle concentration and the cultured microbial concentration, 0.93 ($p < 0.001$) between the fluorescent particle concentration and the extracted DNA concentration, while 0.42 ($p < 0.01$) between the non-fluorescent particle concentration and the cultured microbial concentration, 0.49 ($p < 0.01$) between the non-fluorescent particle concentration and the extracted DNA concentration. It was also found that the size range of the fluorescent particles, which exhibited the highest coefficient for the extracted DNA concentration and the cultured microbial concentration, was 2 to less than 10 micrometer (μm), and their coefficients were 0.89 ($p < 0.01$) and 0.8 ($p < 0.001$), respectively. These results indicate that the fluorescent particles, especially in the range of 2 to less than 10 μm, are highly correlated with bio-aerosols rather than the non-fluorescent particles. Taken together, LIF technology is great for real-time monitoring of bio-aerosols by counting fluorescent particles and resolving particle sizes at ambient atmospheric environment.

KEY WORDS Bio-aerosol monitoring, Fluorescent particles, Non-fluorescent particles, LIF (Laser-induced fluorescence), Fluorescent signals, Scattering signals, Pearson's correlation coefficient

1. INTRODUCTION

Bio-aerosols often contain biological particles such as virus, bacteria, and fungi. It is frequently transported attached to other particles, such as skin flakes, soil, dust, or dried residues from water droplets. Aggregation of cells into dumps can

enhance the survival while dwelling in ambient atmospheric environment. Major sources of bio-aerosols in outdoor are wind action on soil, agitation of open water, and raindrop impaction. Other farming activities, cattle, swine animal house will also generate bio-aerosols. Thus, bio-aerosols contain numerous different kinds of biological particles of different size. If some biological particles such as influenza, pneumoniae, or tuberculosis are transmitted via bio-aerosols, it can cause to health risk for human and animals. Especially, bio-aerosols, ranging from 2 to 10 μm, enter the lung through respiratory tract so that can cause a serious illness (Labiris and Dolovich, 2003). Therefore, it is important to monitor the concentration and sizes of bio-aerosols in real-time to ensure the health of human and animals. However, the real-time monitoring of bio-aerosols has been a difficult task. Traditional approaches involving culture and microscopic technologies require days or complex procedures to get results (Denoya, 2016). Moreover, culture underestimates the concentration of bio-aerosols because of the viable but nonculturable bio-particles which may affect human health (Li *et al.*, 2014; Fakruddin *et al.*, 2013; Du *et al.*, 2007). There are many efforts to overcome the disadvantages of traditional approaches by adopting optical technologies (Wei *et al.*, 2016; Huffman *et al.*, 2009). One of these technologies is LIF (laser-induced fluorescence) which uses a high intensity light source to induce light scattering and auto-fluorescence of bio-particles (Agranovski *et al.*, 2003; Hairston *et al.*, 1997). Auto-fluorescence is derived from internal cellular fluorophores naturally present in the molecules such as pyridine nucleotides (NADH, NADPH) and riboflavin (Harrison and Chance, 1970). Accordingly, the detection of auto-fluorescence under atmospheric conditions may indicate the presence of viable bio-particles (Laflamme *et al.*, 2005; Setlow and Setlow, 1977). Recently, LIF technology has been applied in fields requiring the real-time bio-aerosol monitoring (military, pharmaceuticals) (Denoya, 2016; Choi *et al.*, 2014). And LIF technology was evaluated by performing the detection of harmless air-released biological particles, mainly for military purpose, however, little has been done to evaluate the technology by monitoring bio-aerosols occurring naturally. In the present study, to demonstrate the possibility of LIF as a real-time bio-aerosol monitoring technology, we investigated the correlation between atmospheric fluorescent particles and biomaterials. For this, we used LIF instrument, BDS (Bio-aerosol Detection System). It was installed outside (on the top of our research building), and monitoring and sampling were performed simultaneously. We investigated the relationship between fluorescent particles and biomaterials from monitoring and sampling data. To our knowledge, this is the first study on evaluation of LIF technology for real-time monitoring of bio-aerosols, by analyzing correlations between monitoring data (the fluorescent particle concentration, the non-fluorescent particle concentration) and sampling data (the cultured microbial concentration, the extracted DNA concentration).

2. MATERIALS AND METHODS

2.1 Instrument (BDS)

BDS (Bio-aerosol Detection System, Samyangchemical Co., Ltd, Korea) was employed in this study, which was developed based on the BAMS (Biological Aerosol Monitoring System) developed with the authority of the South Korean government, Agency for Defense Development, using LIF technology for military requirement. It consists of a detector and a collector. A detailed description of BDS has been given elsewhere (Jeong *et al.*, 2017; Choi *et al.*, 2014), and so only brief explanation is given here. The detector consists of a concentration module and a monitoring module. The concentration module is designed to enrich aerosol particles sized such that can most efficiently be deposited in human respiratory system, the particle sizes range from 2 to 10 μm. The concentrated particles pass through the monitoring module. The principles of monitoring is based on two kinds of fluorescent signals (430–550 nm, 500–600 nm) and scattering signals emitted from particles by 405 nm laser source. In this study, the fluorescent signals of 430–550 nm and 500–600 nm emitted from aerosol particles are measured to count the fluorescent particles and the scattered signals are measured not only to count the non-fluorescent particles but also to distinguish particle size (Fig. 1a). The optical properties of the detector of the BDS are similar to those of an OPC (Optical Particle Counter). Particle sizing of the detector of the BDS like the OPC, is based on the principle of single particle elastic light scattering following the Mie theory (Rosenberg *et al.*, 2012). And then the detector is calibrated by a reference PSL (Polystyrene latex spheres, Duke Scientific Corp., Palo Alto CA 94303, USA) to get the relationship between the response and reference particle size. From

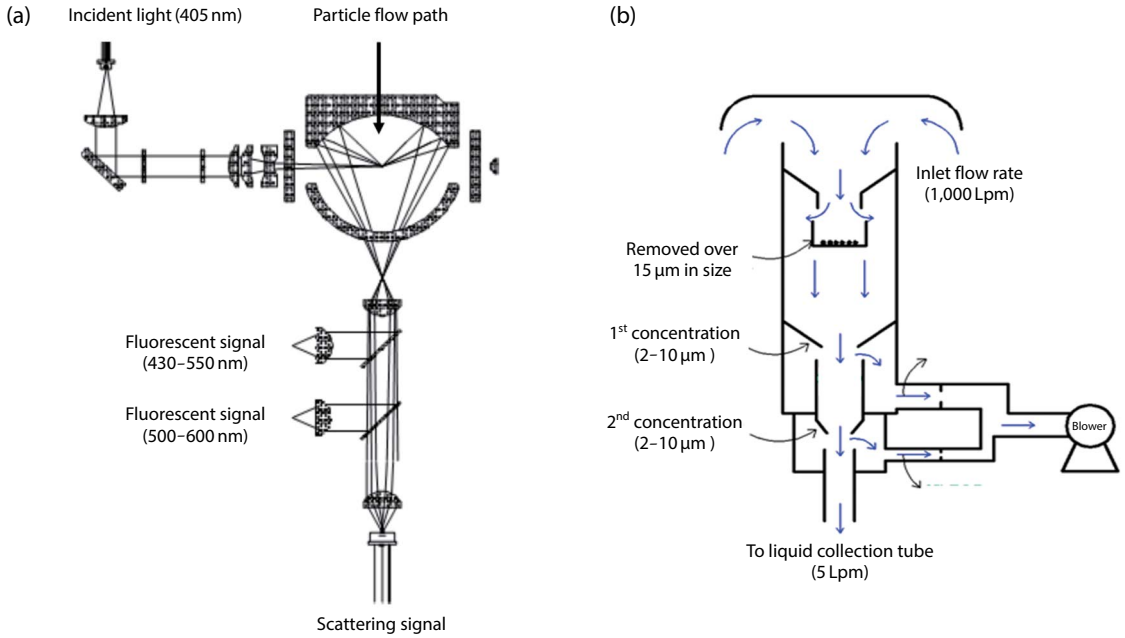

Fig. 1. Schematics of the detector showing emitted light path (a) and the collector showing aerosol flow path (b).

the calibration results, the particle sizing of the detector of the BDS is divided by the size range of 0.23–40 μm in up to 32 bin sizes. Finally, it is calibrated with the reference equipment, APS3321 (TSI, MN55126, USA), using the reference PSL. The collector consists of a liquid sampler and a concentration module with the same function as the concentration module of the detector. The concentration module of the collector is connected directly to the liquid sampler (Fig. 1b).

2.2 Bio-aerosol Monitoring and Collecting

The instrument, BDS, was operated outdoor on the rooftop of Samyangchemical research institute (~10 m above ground) (Fig. 2). Before operating, it was calibrated using the test dust SAE J726 for blank test, and using PSL (0.7–20 μm) (for particle size determination. Aerosol sampling was performed at an inlet flow rate of 90 L min^{-1} (LPM) for monitoring, at an inlet flow rate of 1,000 LPM for collecting, at ambient pressure and temperature. The particles of 2–10 μm size are concentrated by passing through the three-stage nozzles, the concentration efficiency is about 60–65% at 3 μm size. The detector and the collector sampled air for 1 hour, 3 times a day (AM 10–11, PM 1–2, PM 4–5) on April 24, 27, May 8, 18, 26, June 2, 2017. Scattering and fluorescent signals are obtained from the detector to identify non-fluorescent particles and fluorescent particles, and liquid samples are obtained from the collector for microbial culture and DNA extraction. Data from the detector and the collector were converted to the value of the same

Fig. 2. BDS was installed on the top of the building to monitor and collect bio-aerosols in real-time: the detector (right) and the collector (left).

volume to interpret the results.

2.3 Culture of Liquid Samples

Some of the liquid samples were taken for microbial culture (bacteria, fungi). General bacteria were cultivated on AC (aerobic count) petri-film (3M Science. AL, MN, USA) for 24 hrs at 37°C. Fungi were cultured on YM (yeast mold) petri-film for 48 hrs at 25°C. The collected liquid samples are cultivated for 3 repetitions of each sample, the average value of triplicate of each sample is used for further analysis. The developed colonies were counted as CFU (colony forming unit) which is a measure of viable bacterium or fungus.

2.4 DNA Extraction from Liquid Samples

The remaining liquid samples used for culture were centrifuged at 16,000 × g for 25 min, and the pellets were suspended in 400 μL of 100 mM sodium phosphate buffer (pH 8). DNA extraction was performed according to the Fast Prep Cell Homogenization method (DeSantis *et al.*, 2005), but only a single bead-beating velocity and duration was used (6.5 m s^{-1} for 45 s) (Brodie *et al.*, 2007). Extracted DNA was quantified by using NanoDrop Lite (Thermo-fisher scientific, Wilmington, DE, USA) according to the manufacturer's protocol. The DNA concentration is measured three times for each sample and the average is used for further analysis.

2.5 Statistical Analysis

The data on the number of cultured microbes, the amount of extracted DNA, and the numbers of fluorescent particles and non-fluorescent particles were consolidated in a spreadsheet (Microsoft Excel; Microsoft Corporation, Redmond, Washington, USA) and organized for analysis, Pearson's correlation coefficient is used to analyze the relationship between parameters (cultured microbial concentration and fluorescent particle concentration, extracted DNA concentration and fluorescent particle concentration, cultured microbial concentration and non-fluorescent particle concentration, extracted DNA concentration and non-fluorescent particle concentration), and to determine the most correlated size range as bio-aerosols. The significant test of the correlation coefficient is performed to decide whether the linear relationship between two parameters is meaningful or not. The correlation is significant at the level of 0.01 or less ($p < 0.01$).

3. RESULTS

3.1 Size Distribution and Number Concentration of Non-fluorescent Particles and Fluorescent Particles

Fig. 3a presents the size distribution and number concentration of non-fluorescent particles measured with the BDS on the top of our building. It measured 3 times a day for 1 hour during the experiment. The size distribution of non-fluorescent particles, which ranges from < 1 μm to 30 μm, showed little change during the experiment period. The number concentration pattern of size-resolved non-fluorescent particles was no big change either during the experiment except the yellow dust day, May 8 (www.airkorea.or.kr). Even though particles entering the detector are highly concentrated into specific particle size ranges (2–10 μm), the number concentration of < 1 μm is slightly higher than the other size ranges except the yellow dust day, it decreases as the size increases (Fig. 4a). Whereas, the yellow dust day, the 1–< 2 μm sizes are much higher than the other size ranges (Fig. 4a). Fig. 3b shows the size distribution and number concentration of fluorescent particles measured with the BDS on the top of our building. The size distribution of fluorescent particles, which ranges from 1 μm to 30 μm, showed little change during the experiment period. However, as shown in Fig. 4b, unlike the number concentration of the non-fluorescent particles, the number concentration of the fluorescent particles is the highest in the range of 2 to less than 10 μm, it decreased gradually from 10 μm or more and decreased sharply from below 2 μm. Especially, the highest concentration of 2–< 10 μm fluorescent particles was observed on April 27, regardless of the yellow dust, which means the yellow dust is not directly associated with the fluorescent particles.

3.2 Concentrations of Fluorescent Particles, Non-fluorescent Particles, Cultured Microbes and Extracted DNA

Table 1 presents daily, hourly concentrations of size-integrated (< 1 μm to 30 μm) non-fluorescent particles and fluorescent particles, and those of cultured microbes (the sum of bacteria and fungi) and extracted DNA. To analyze the relationship between the fluorescent particles and the cultured microbes (or the extracted DNA), the concentration of fluorescent particles was compared with that of cultured microbes or extracted DNA. As shown in Table 1, two days with the highest fluorescent

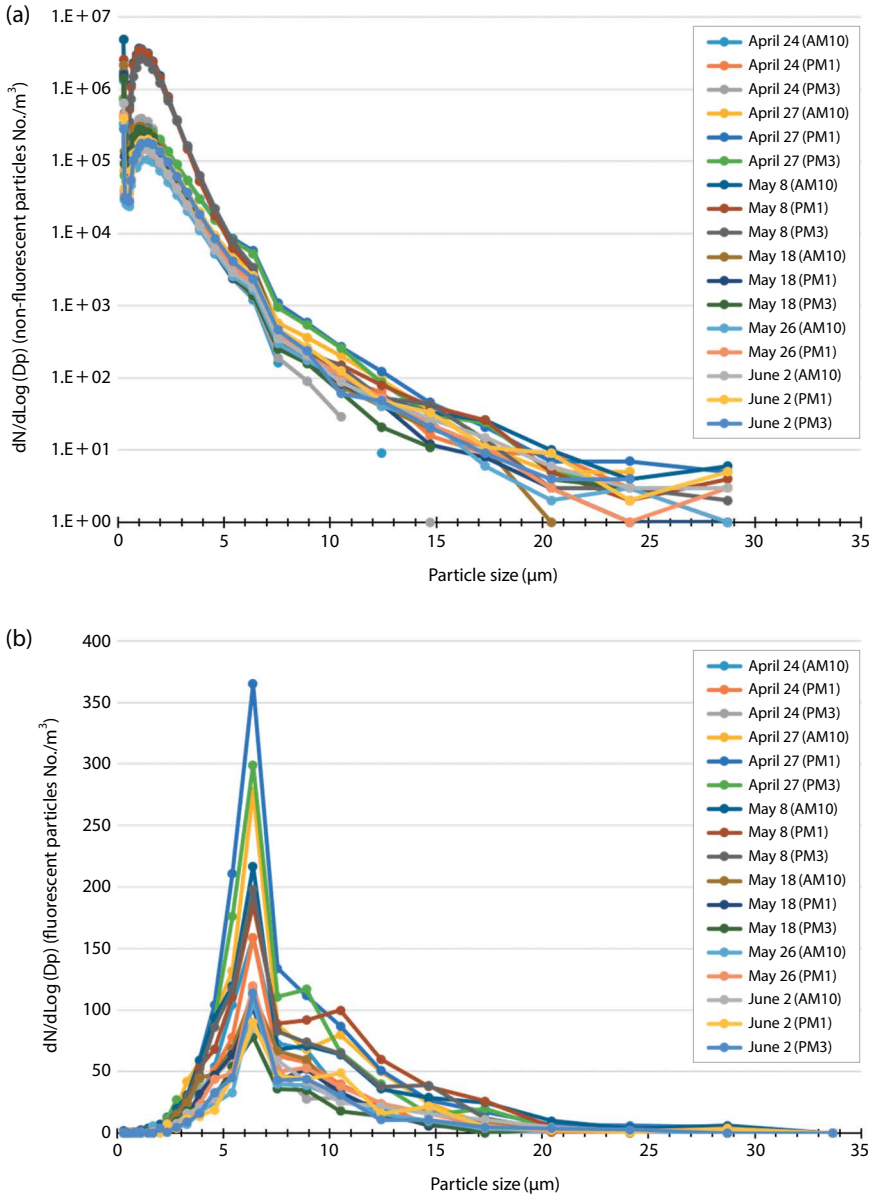

Fig. 3. Size distribution and number concentration of non-fluorescent particles (a) and fluorescent particles (b) in each sampling duration.

particle concentration were April 27 and May 8, and the concentrations of extracted DNA and cultured microbes also tend to be higher in the two days than in the other days. But on May 8, the yellow dust day, which is the highest concentration of non-fluorescent particles (about 10 times compared to April 27), the fluorescent particle concentration tends not to be increase proportionally to the non-fluorescent particle concentration, although the fluorescent particle concentration is relatively higher. The results indicate that the fluorescent particles are closely related to the cultured microbes and the extracted DNA, but is less related to the non-fluorescent particles.

3.3 Correlation Analysis between the Parameters

In order to accurately analyze the relationship between the parameters, the Pearson's correlation coefficient (r) between the fluorescent particle concentration and the biomaterials (the extracted DNA concentration and the cultured microbial concentration) was first calculated. There were significant linear correlations, where the r of the extracted DNA concentration to the fluorescent par-

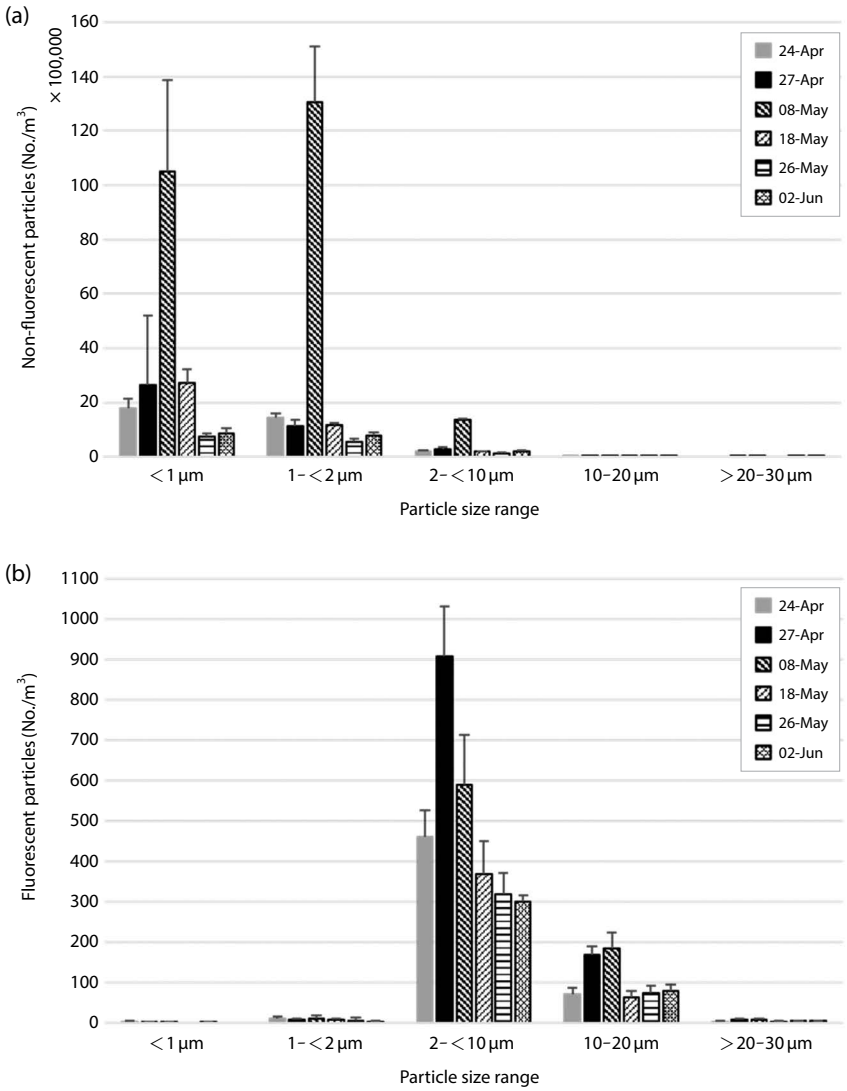

Fig. 4. Concentration distribution of non-fluorescent particles (a) and fluorescent particles (b) depending on particle size range.

ticle concentration was 0.93 ($p < 0.001$) (Fig. 5a) and the r of the cultured microbial concentration to the fluorescent particle concentration was 0.85 ($p < 0.001$) (Fig. 5b). The r between the fluorescent particle concentration and the extracted DNA concentration is slightly higher than the r between the fluorescent particle concentration and the cultured microbial concentration, which indicates many microbes in ambient air may not be cultured, even viable. The correlation between the non-fluorescent particle concentration and the extracted DNA concentration (or the cultured microbial concentration) was also analyzed, where the r was 0.49 ($p < 0.01$) {or 0.42 ($p < 0.01$)} (Fig. 5c, d). The results mean that the fluorescent particle concentration is more closely related to biological materials (the extracted DNA concentration, the cultured microbial concentration), rather than the non-fluorescent particle concentration.

3.4 Effects of Particle Size Range on Pearson's Correlation Coefficient

To analyze the effects of particle size range on the r between the fluorescent particle concentration and the cultured microbial concentration (or the extracted DNA concentration), whole size range was divided into 4 sections, namely 1-<2 μm, 2-<10 μm, 10-<20 μm and 20-30 μm. The size ranges of <1 μm was not included in the analysis as there were little fluorescent particles. As shown in Table 2, the size range with the highest r

Table 1. The concentration of non-fluorescent particles, fluorescent particles, cultured microbes and extracted DNA in each collected sample.

Date		Non-fluorescent particles (No./m^3)	Fluorescent particles (No./m^3)	Cultured microbes (CFU/m^3)	Extracted DNA (ng/m^3)
4. 24	AM 10	11,734,657	1,580	17 (±0.03)	936 (±46)
	PM 01	9,915,761	1,485	19 (±0.06)	692 (±33)
	PM 03	10,343,264	1,171	9 (±0.23)	850 (±38)
4. 27	AM 10	24,155,916	2,520	18 (±0.1)	1,167 (±24)
	PM 01	7,267,838	3,235	25 (±0.07)	1,947 (±51)
	PM 03	9,060,689	2,741	22 (±0.11)	1,520 (±19)
5. 08	AM 10	87,268,382	2,282	16 (±0.12)	1,150 (±140)
	PM 01	74,067,131	2,266	19 (±0.13)	1,400 (±22)
	PM 03	54,165,719	2,152	17 (±0.11)	1,967 (±42)
5. 18	AM 10	15,613,584	1,387	10 (±0.03)	620 (±38)
	PM 01	12,342,232	1,138	15 (±0.07)	420 (±35)
	PM 03	11,812,941	882	7 (±0.05)	108 (±8)
5. 26	AM 10	3,857,346	915	4 (±0.03)	47 (±13)
	PM 01	4,985,910	1,157	3 (±0.03)	58 (±8)
	PM 03	–	–	–	–
6. 02	AM 10	5,859,009	1,012	10 (±0.03)	333 (±17)
	PM 01	6,039,492	994	1 (±0.05)	183 (±6)
	PM 03	5,066,116	981	1 (±0.05)	167 (±12)

between the fluorescent particle concentration and the cultured microbial concentration was 2 to less than 10 μm. There was also same tendency in the r between the fluorescent particle concentration and the extracted DNA concentration, suggesting the fluorescent particles, especially in the range of 2 to less than 10 μm, are highly correlated to bio-aerosols. Furthermore, the correlation between the fluorescent particle concentration and the cultured microbial concentration is much lower than the correlation between the fluorescent particle concentration and the extracted DNA concentration, which means that there are many microbes not to be cultured, even viable, as mentioned above (Fig. 5a, b).

4. DISCUSSION

Real-time monitoring for the number of microbes in aerosols is important for civilian health (Douwes *et al.*, 2003). But traditional technologies such as culture and microscope are difficult for real-time monitoring because of its culturing time and complex procedures. Many efforts have been made to develop capabilities which can provide real-time information on the concentration of airborne microbes. One of technologies that enables real-time monitoring of airborne microbes is LIF. LIF instrument uses high intensity light source to induce light scatter and fluorescence in the particles passing through an appropriate detecting cell, resulting in the real-time detection of both inert particles and bio-particles such as bacteria, yeasts and fungi. Several papers have demonstrated that LIF instrument is a novel monitor capable of measuring the size and number of bio-aerosols by performing in an aerosol chamber using cultured bacteria or fluorophores derived from cells (Huffman *et al.*, 2009; Agranovski *et al.*, 2004; Hairston *et al.*, 1997). But, it really hasn't been studied very much in atmospheric environment. Therefore, this study aimed at the evaluation of LIF technology for real-time bio-aerosol monitoring by performing in ambient atmospheric environment, not in an aerosol chamber, using the BDS as LIF instrument. The analysis was carried out by correlating the parameters. First, we monitored non-fluorescent and fluorescent particle concentrations for 1 hr, 3 times a day, and measured the concentrations of cultured microbes and extracted DNA from samples collected simultaneously with the monitoring. As shown in Fig. 4, except May 8, when the yellow dust happened, the non-fluorescent particles of <1 μm were the most abundant, even though the BDS strongly enriched the size range of 2–10 μm. Meanwhile, the fluorescent particles is a peak range in 2–<10 μm. There were also similar evidences

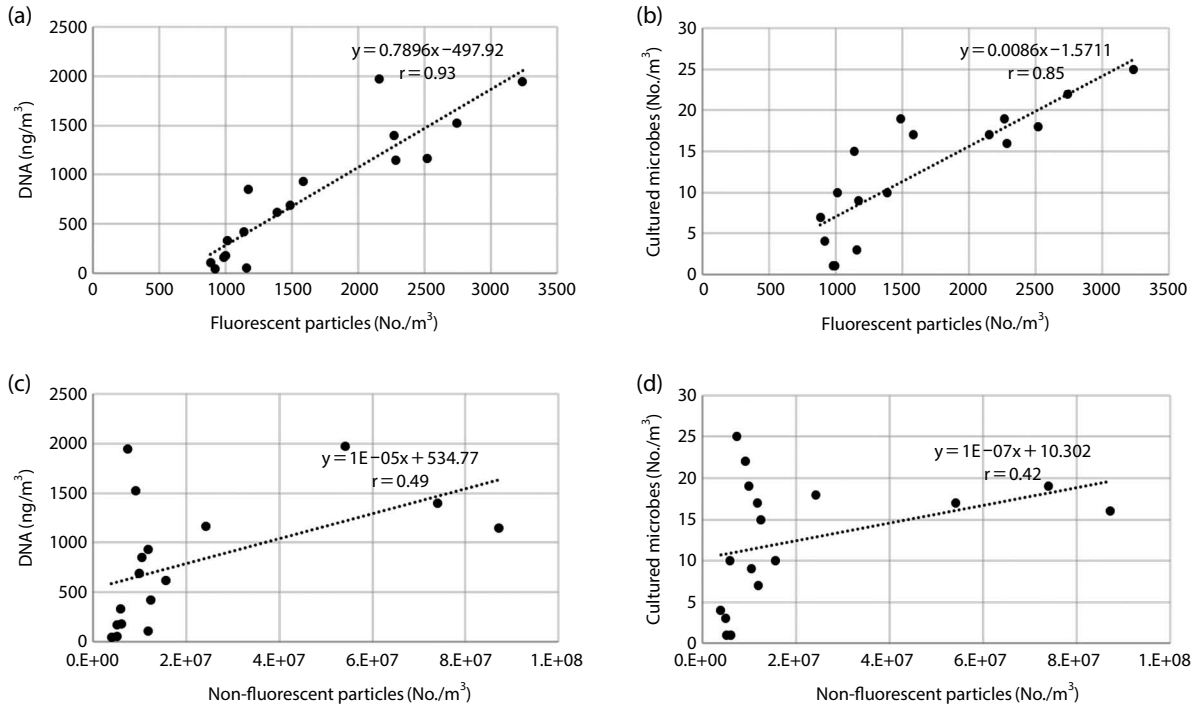

Fig. 5. Correlation between the fluorescent particle concentration and the extracted DNA concentration (a), the fluorescent particle concentration and the cultured microbial concentration (b), the non-fluorescent particle concentration and the extracted DNA concentration (c), and the non-fluorescent particle concentration and the cultured microbial concentration (d). The dashed line is the linear regression line. Pearson's correlation coefficient (r) is 0.93 ($p < 0.001$) (a), 0.85 ($p < 0.001$) (b), 0.49 ($p < 0.01$) (c), and 0.42 ($p < 0.01$) (d).

Table 2. The effect of aerosol particle size on Pearson's correlation coefficient.

Particle size range (μm)	Pearson's correlation coefficient (r)	
	Between fluorescent particle concentration & cultured microbial concentration	Between fluorescent particle concentration & extracted DNA concentration
1–<2	0.46**	0.52*
2–<10	0.8**	0.89*
10–<20	0.58*	0.83*
20–30	0.4**	0.63*

*: $p < 0.01$, **: $p < 0.001$

that the peak range of outdoor fluorescent bio-particles is observed in the range of 3 to 4 μm (Bhangar *et al.*, 2014; Huffman *et al.*, 2009). In yellow dust event, May 8, the number of non-fluorescent particles of <2 μm was significantly increased, comparing to other days, however, the number of fluorescent particles did not increase proportionally with the number of non-fluorescent particles, which indicates the concentration of fluorescent particles is not heavily influenced by that of non-fluorescent particles. This result has same tendency with the study that the fluorescent signal from the LIF instrument, UVAPS (TSI, MN55126, USA), is not affected by the episodic nature of non-fluorescent particles arrival (Huffman *et al.*, 2012). Next, in order to investigate the relationship between fluorescent particles and cultured microbes, or between fluorescent particles and extracted DNA, Pierson's correlation coefficient was calculated. From these results, we observed that the correlation coefficient of the fluorescent particle concentration to the cultured microbes concentration was much higher than the non-fluorescent particle concentration, the correlation of the fluorescent particle concentration to the extracted DNA concentration also tended to be the same as that of the fluorescent particle concentration to the cultured microbes concentration, suggesting that extracted DNA and cultured microbes are highly related to fluorescent particles rather than non-fluorescent particles (Fig. 5). Along with this, the correlation coefficient of the extracted DNA concentration to the fluorescent particle concentration was slightly higher than the correlation coefficient of the cultured microbes (Fig. 5). From

this result, it can be assumed there are many viable, but nonculturable microbes which is highly related to bio-aerosols, in ambient environment. A study shows that the nonculturable microbes accounted for 99.13% of the total microbes in an outdoor bio-aerosol (Li *et al.*, 2011), which means that it is more accurate to extract DNA than to cultivate microbes for bio-aerosol monitoring, however, DNA extraction is also time-consuming and is not suitable for real-time monitoring. If fluorescent signals are used, it will have more advantages in real-time monitoring as well as accurate counting. We also investigated the effect of aerosol size range on the correlation. The concentration of biomaterials (cultured microbes, extracted DNA) were highly correlated with fluorescent particle concentration in the range 2 to less than 10 μm, which suggests the prominent size range as bio-aerosol is 2 to less than 10 μm, although the correlation coefficient of the cultured microbial concentration to the fluorescent particle concentration is considerably lower than that of the extracted DNA concentration to the fluorescent particle concentration (Table 2). Consequently, atmospheric fluorescent particles are highly related to bio-aerosols, especially those that are 2 to less than 10 μm. Taken together, LIF is a great technology for real-time monitoring of bio-aerosols by counting fluorescent particles and resolving particle sizes.

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