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To cite this article: J. Alex Huffman, Anne E. Perring, Nicole J. Savage, Bernard Clot, Benoît Crouzy, Fiona Tummon, Ofir Shoshanim, Brian Damit, Johannes Schneider, Vasanthi Sivaprakasam, Maria A. Zawadowicz, Ian Crawford, Martin Gallagher, David Topping, David C. Doughty, Steven C. Hill & Yongle Pan (2019): Real-time sensing of bioaerosols: Review and current perspectives, *Aerosol Science and Technology*, DOI: [10.1080/02786826.2019.1664724](https://doi.org/10.1080/02786826.2019.1664724)

To link to this article: <https://doi.org/10.1080/02786826.2019.1664724>



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Accepted author version posted online: 12 Sep 2019.
Published online: 27 Sep 2019.



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Real-time sensing of bioaerosols: Review and current perspectives

J. Alex Huffman^{a,b}, Anne E. Perring^c, Nicole J. Savage^d, Bernard Clot^e, Benoît Couzry^e, Fiona Tummon^e, Ofir Shoshanim^f, Brian Damit^g, Johannes Schneider^h, Vasanthi Sivaprakasamⁱ, Maria A. Zawadowicz^j, Ian Crawford^k, Martin Gallagher^k, David Topping^k, David C. Doughty^l, Steven C. Hill^l, and Yongle Pan^l

^aDepartment of Chemistry and Biochemistry, University of Denver, Denver, Colorado, USA; ^bMultiphase Chemistry Department, Max Planck Institute for Chemistry, Mainz, Germany; ^cDepartment of Chemistry, Colgate University, Hamilton, New York, USA; ^dAerosol Devices Inc, Fort Collins, Colorado, USA; ^eFederal Office of Meteorology and Climatology MeteoSwiss, Payerne, Switzerland; ^fIsrael Institute for Biological Research (IIBR), Ness Ziona, Israel; ^gApplied Biological Sciences Group, Johns Hopkins University Applied Physics Laboratory, Laurel, Maryland, USA; ^hParticle Chemistry Department, Max Planck Institute for Chemistry, Mainz, Germany; ⁱOptical Sciences Division, Naval Research Laboratory, Washington, DC, USA; ^jAtmospheric Sciences and Global Change Division, Pacific Northwest National Laboratory, Richland, Washington, USA; ^kCentre for Atmospheric Science, SEAES, University of Manchester, Manchester, United Kingdom; ^lCCDC Army Research Laboratory, Adelphi, Maryland, USA

ABSTRACT

Detection of bioaerosols, or primary biological aerosol particles (PBAPs), has become increasingly important for a wide variety of research communities and scientific questions. In particular, real-time (RT) techniques for autonomous, online detection and characterization of PBAP properties in both outdoor and indoor environments are becoming more commonplace and have opened avenues of research. With advances in technology, however, come challenges to standardize practices so that results are both reliable and comparable across technologies and users. Here, we present a critical review of major RT instrument classes that have been applied to PBAP research, especially with respect to environmental science, allergy monitoring, agriculture, public health, and national security. Eight major classes of RT techniques are covered, including the following: (i) fluorescence spectroscopy, (ii) elastic scattering, microscopy, and holography, (iii) Raman spectroscopy, (iv) mass spectrometry, (v) breakdown spectroscopy, (vi) remote sensing, (vii) microfluidic techniques, and (viii) paired aqueous techniques. For each class of technology we present technical limitations, misconceptions, and pitfalls, and also summarize best practices for operation, analysis, and reporting. The final section of the article presents pressing scientific questions and grand challenges for RT sensing of PBAP as well as recommendations for future work to encourage high-quality results and increased cross-community collaboration.

ARTICLE HISTORY

Received 3 July 2019
Accepted 28 August 2019

EDITOR

Tiina Reponen

1. Introduction

The investigation of atmospheric aerosols of biological origin arose in the mid-nineteenth century due to speculations on the origin of diseases afflicting humans and crops (Carnelley, Haldane, and Anderson 1887; Pasteur 1862; Vallery-Radot and Hamilton 1885). Many other applications of aerobiology followed within the first half of the twentieth century, including population biology, aero-allergology, and the detection of biowarfare agents (Gregory 1961; Stackman et al. 1942). Today, research regarding the sources, properties, concentrations, and diversity of bioaerosol is motivated by increasingly diverse

questions and needs (e.g., Burge 1990; Cox and Wathes 1995; Cox et al. 2019; D'Amato et al. 2007; Fröhlich-Nowoisky et al. 2016; Morris et al. 2014a; Núñez et al. 2016; Šantl-Temkiv et al. 2019; Shiraiwa et al. 2017; Sorensen et al. 2019; Womack, Bohannan, and Green 2010). Many applications use the same broad principles of detection, however. The diversity of application means that scientists, engineers, and health practitioners who study and monitor primary biological aerosol particles (PBAPs) are spread widely across relatively unrelated communities with separate networks of collaboration. One motivation for the present journal special issue entitled “*Bioaerosol*

CONTACT J. Alex Huffman ✉ Alex.Huffman@du.edu 📧 Department of Chemistry and Biochemistry, University of Denver, Denver, Colorado, USA; Multiphase Chemistry Department, Max Planck Institute for Chemistry, Mainz, Germany.

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Research: Methods, Challenges, and Perspectives” is to provide broad recommendations to these diverse communities.

Discussion is complicated by the use of variable nomenclature across research communities. Here, we use the terms bioaerosol and PBAP interchangeably, defined as in Després et al. (2012), to describe “solid airborne particles derived from biological organisms, including microorganisms and fragments of biological materials such as plant debris and animal dander.”

Identification of PBAP, e.g., to the genus and species level for many infectious microorganisms, or the genus level for some allergenic fungi and bacteria, is required for many applications (Table 1). Thus, many measurement methods involve collection of particles followed, e.g., by visual identification of cultured microbes or of individual particles under a microscope (Mandrioli et al. 1998), antigen/antibody assay, or polymerase chain reaction. Such manual analysis can be subjective, costly, and time-intensive, which causes delays in data availability, limits the breadth of application, and can result in poor subsampling of measurements. As a result, samplers capable of autonomous and continuous real-time (RT) or near-RT analysis have become increasingly common. The terms online and offline are also used here to refer to RT and manual methods, respectively. In some cases, a distinction is necessary between true direct-reading RT sensors that sample particles and then collect, analyze, and report interpreted data without requiring human input, and sensors that autonomously sample particles and then collect and analyze data in RT, but which require some level of manual human interpretation and analysis at a later stage. The timescale of automatic analysis and reporting leads to further distinction between sensors. In some cases, sampling, analysis, and reporting can be achieved within seconds or minutes, and in other cases, this requires integration times of several hours.

Development of RT analysis of PBAP was driven largely by the need for early warning of airborne threats to public health or national defense, including acts of bioterrorism, for forecasting of aeroallergens, and for climate research (e.g., cloud glaciation). Thus, a wide variety of RT techniques have been developed, as reviewed thoroughly elsewhere (e.g., Ballard, Brown, and Ozcan 2018; Caruana 2011; Després et al. 2012; Griffiths and Decosemo 1994; Ho 2002; Holt and Bennett 2014; Huffman and Santarpia 2017; Lim et al. 2005a; Spurny 1994; Xu et al. 2011). RT detection presents a host of technical

and scientific challenges that may not be obvious to a data or instrument user. Further, no RT analysis can unambiguously provide information about all important aspects of PBAP, including the following: particle size, morphology, species identification, vitality or viability state, and quantitative concentration. Most techniques discussed here utilize physical or chemical properties to infer the biological nature of detected particles. All RT techniques thus apply key analytical assumptions that must be understood, challenged, and adapted to maximize the desired information.

RT instrumentation has been routinely applied with respect to several broad categories of objectives for studying PBAP spread across a variety of basic and applied research fields. Motivations for this research are discussed in more depth within other articles in the special issue (e.g., Cox et al. 2019; Šantl-Temkiv et al. 2019), but are presented briefly here and in Table 1. Four largely separated categories of PBAP are summarized below as targets for RT detection, each with application in multiple scientific disciplines.

- i. Pathogenic aerosols (e.g., viruses, bacteria, fungi) that can infect or spread toxins to humans. Monitoring is required to protect public health and for national security against disease vectors spread naturally or through nefarious intent. Applications can include continuous monitoring in urban areas, within occupied buildings or public transportation, and with respect to defense-related activities (Douwes et al. 2003; Laumbach and Kipen 2005; Lim et al. 2005b). In this case, the need for taxonomic specificity is high in order to filter out false positives from noninfectious PBAPs.
- ii. Pathogenic aerosols that can infect or otherwise damage crops and livestock. Primary motivations include reducing the spread of disease and maximizing crop yield and profit (Aylor et al. 2011; Douglas et al. 2018; Lis, Mainelis, and Górný 2008). A related motivation is to observe spread of disease through natural ecosystems (e.g., forests).
- iii. Pollen and other allergen-containing aerosols, including fungal spores, pet dander, small insects, e.g., dust mites, and their fragments, that can impact human health. Applications include monitoring networks that disseminate information to the public (Buters et al. 2018; Greiner et al. 2011), monitoring pollination of crops, and the natural migration of organisms and biodiversity

in a changing climate (Beggs 2004; D'Amato et al. 2016; Hamaoui-Laguel et al. 2015; Ozanne et al. 2003). In some cases, the need exists for specific taxonomic identification, and in other cases, only generalized information is required.

- iv. PBAP categorized more broadly, e.g., into taxa such as pollen, fungal spores, bacteria, and other PBAP classes. In some cases, the need for taxonomic specificity is much lower, e.g., to understand how certain PBAP types affect cloud physical properties or to investigate PBAP chemistry (Georgakopoulos et al. 2009; Pöschl and Shiraiwa 2015).

A key purpose of this manuscript is to describe techniques that address these objectives and provide critiques, including recommendations for operation and analysis. Each major section discusses current technological limitations and future needs to be addressed by the research community. This review and perspective article will focus specifically on the most commonly applied *real-time techniques for bioaerosol analysis*, with emphasis on civilian, commercially available techniques. Acronym definitions are listed in the nomenclature at end of text.

2. Real-time techniques

2.1. Fluorescence spectroscopy

2.1.1. Overview

Among the most common RT technique for PBAP detection is the use of laser- (or light)-induced fluorescence (LIF). This technique typically uses monochromatic light (continuous or pulsed) to investigate the fluorescent properties of individual particles flowing in air through the instrument. The resulting fluorescent signals of sufficient intensity are then broadly interpreted according to general assumptions made about the molecular source of fluorescence in particular bands. Autofluorescence (intrinsic fluorescence without tags or dyes) from certain biomolecules, especially those containing substituted aromatic rings (i.e., riboflavin and several amino acids), can indicate the presence of biological material, whereas the intensity from most non-biological aerosol is quite low (e.g., Pöhlker, Huffman, and Pöschl 2012 and references therein). Wavebands of excitation and emission are frequently chosen to coincide, e.g., with the peaks of tryptophan and NADH emission, though many other fluorophores contribute to the signature of atmospheric PBAPs. As discussed in more detail below, LIF detection generally allows discernment of biological

from non-biological aerosol and possibly more detailed differentiation, depending on instrumental capabilities.

Fluorescence spectra from individual molecules are broad, by nature of the distribution of photon energies following electron relaxation within excited molecules. Bioparticles can vary by 10^4 in diameter (e.g., 10 nm virus – 100 μ m pollen) and thus 10^{12} in volume. Emission spectra from PBAP typically have contributions from huge numbers of molecules, including complex mixtures of fluorophores. As a result, related individual classes of PBAP (e.g., all bacteria) can have similar LIF spectra, making species-level identification challenging from fluorescence alone. Furthermore, fluorescence is subject to interferences from non-biological aerosols that contain aromatic hydrocarbons (e.g., industrial chemicals and engine exhausts). Laboratory studies have shown that LIF spectra of PBAP can be strongly influenced by growth conditions or agglomeration with leftover growth media or other materials (Pan et al. 2014b; Sivaprakasam et al. 2011), or by exposure to ultraviolet light and ozone (Pan et al. 2014a). Using multiple excitation wavelengths can improve discrimination and reduce false positives.

In earlier systems, excitation was generally performed by relatively high-powered lasers. More recently, the use of smaller diode-pumped lasers, flash lamps, and light-emitting-diodes (LEDs) as light sources has greatly reduced the system size, weight, and power consumption (Cabalo et al. 2008; Davitt et al. 2005; Pan et al. 2003a). Fluorescence spectra are also measured by several additional instrument types discussed below, but these are not presented in this section because they also rely heavily on other optical information for particle characterization.

A number of useful summaries and comprehensive reviews discuss the background spectroscopy of LIF as applied to bioaerosols (Ammor 2007; Campbell et al. 2005; Hill et al. 2014; Hill et al. 2009; Hill et al. 2013; Hill et al. 1999; Jeys et al. 2007; Kopczynski et al. 2005; Pöhlker et al. 2013; 2012; Wlodarski et al. 2006) and the instrumentation used in this subfield (Caruana 2011; Fennelly et al. 2017; Huffman and Santarpia 2017).

2.1.2. LIF for early warning of human pathogens

National security concerns over biowarfare agents (e.g., anthrax, plague, or tularemia) have driven the development of RT bioaerosol detection by fluorescence. Since fluorescence alone cannot differentiate between harmful and benign bioaerosols, these RT

Table 1. List of PBAP classes applied to RT detection.

Particle category	Application information				Particle information			Detection information		
	Application classes	Application examples	Scientific communities represented	End-point for written results	Bioparticle classes	Physiological state	Example(s)	Taxonomic specificity required	Temporal window necessary	Selected detection challenges
1 Human pathogens	Indoor pathogen monitoring	Hospitals, public transport, schools, homes, workplace	Public health, medical professionals, national security	Trade-specific publ.; peer-reviewed journals	Viral, bacterial, fungal	Viable	<i>Mycobacterium tuberculosis</i> (bacteria), influenza virus, Measles, <i>morbillivirus</i>	High (species)	Fast (minutes)	RT detection of viruses; Rapid identification of bacterial pathogens at low cost
2 Human pathogens	Outdoor pathogen monitoring	Water and waste treatment, municipal composting and trash, urban areas	Public health, national security	Trade-specific publ.; peer-reviewed journals	Viral, bacterial, fungal	Viable	<i>Aspergillus fumigatus</i> , <i>coccidioides</i>	High (genus or species)	Slow (hours to days)	RT detection of viruses; Rapid identification of bacterial pathogens at low cost
3 Human pathogens	National, military, homeland security	Airports, military bases, dense public areas	Public health, national security	Governmental reports; peer-reviewed journals	Viral, bacterial, fungal	Viable	<i>Bacillus anthracis</i> , <i>Pasteurella pestis</i> , <i>Francisella tularensis</i>	High (species)	Fast (minutes)	RT detection of viruses; Rapid identification of bacterial pathogens at low cost
4 Allergens	Indoor allergen monitoring	Residential, workplace monitoring	Indoor air research, built environment engineering	Peer-reviewed journals; Trade-specific publ.	Fungal, pollen, insect fragments/ feces, pet dander	Any	<i>Alternaria alternata</i> , <i>Blomai tropicalis</i> , <i>Dermatophagoides</i> sp. (house dust mites)	Medium-high (species or allergenic groups)	Slow (hours to days)	Sufficient taxonomic differentiation autonomously at low cost
5 Allergens	Outdoor allergen monitoring	Municipal monitoring and forecasting, pollination spread (invasives or with climate)	Allergen dispersal monitoring, biodiversity, climate research	Transmitted directly to public; peer-reviewed journals	Pollen, fungal	Any	<i>Ambrosia artemisiifolia</i> pollen (ragweed pollen), <i>Alternaria alternata</i> spores (mold)	Medium-high (species or allergenic groups)	Slow (hours)	RT identification of fungal spores
6 PBAP reproductive units	Outdoor PBAP ecology	Biodiversity, biogeography, organism dispersal range, climate changes	Biodiversity, ecology, climate research	Peer-reviewed journals	Pollen, fungal, bacterial, viral (viable)	Viable or dormant	Any from bioparticle classes	Medium to high (species or groups; viable)	Very slow (days or longer)	RT identification of fungal allergens
7 Plant pathogens	Crop and agriculture monitoring	Dispersal, movement of crop disease	Agricultural engineering, farmers	Proprietary info.; trade-specific publ.; peer-reviewed journals	Fungal, bacterial	Viable	<i>Puccinia graminis</i> (rust); <i>Phytophthora infestans</i> (potatoe blight)	High (species)	Slow (hours to days)	Sufficient taxonomic differentiation autonomously at low cost

8	Animal pathogens	Livestock, domestic animal care	Monitoring of airborne disease vectors, indoor and outdoor livestock facilities	Agricultural engineering, farmers, ranchers	Proprietary info.; trade-specific publi.	Viral, bacterial, fungal	Viable	Foot-and-mouth disease virus	High (species)	Medium (minutes to hours)	RT detection of viruses
9	All PBAP	Industrial monitoring	Pharmaceutical production	Manufacturing, industrial hygiene	Proprietary info.; trade-specific publi.	Bacterial, fungal	Any	Any contaminant	Low (general)	Fast (minutes)	RT detection and rapid identification of fungal and bacterial particles
10	All PBAP	Industrial monitoring	Cleanroom manufacturing, e.g., computer chip assembly	Manufacturing, industrial hygiene	Proprietary info.; trade-specific publi.	Bacterial, fungal	Any	Any contaminant	Low (general)	Fast (minutes)	RT detection and rapid identification of fungal and bacterial particles
11	All PBAP	Aerosol-cloud-precipitation-climate interactions	Hygroscopic or ice-nucleation active PBAP	Atmospheric and climate scientists	Peer-reviewed journals	Bacteria, fungal, pollen, fragments	Any	<i>Pseudomonas syringae</i> (bacterial); <i>Mortierella alpina</i> (fungal)	Low in some cases, high in others. Often unknown.	Fast (minutes)	Rapid measurement of INA-relevant properties, diversity of PBAP, low concentrations at high altitude
12	All PBAP	Atmospheric chemistry	Atm. aging, surface or organic aerosol surface coating	Atmospheric and climate scientists	Peer-reviewed journals	Bacterial, fungal, pollen, other PBAP surfaces	Any	Any PBAP	Low (general classes) to High	Very fast (seconds/minutes) to slow (hours to days)	PBAP identification after atmospheric processing
13	Metabolic PBAP	Airborne cell metabolism	Active cell metabolism or reproduction in cloud droplets, dry aerosol	Atmospheric and climate scientists, cell biologists, diverse discipline	Peer-reviewed journals	Bacteria	Metabolically active	<i>Pseudomonas syringae</i> , <i>Pseudomonas graminis</i>	Medium (groups or species)	Fast (minutes)	Measuring airborne reproduction, cell metabolism conditions, metabolic state, biochemical cycling

methods are referred to as frontends or triggers and are used to determine when to turn on more specific optical detection techniques or to employ further analyses for the identification of specific agents (e.g., using antibodies or nucleic acid sequences).

Initial measurements by the U.S. Army Research Laboratory (ARL) of fluorescence of individual particles transiting a 488-nm laser beam indicated that single-particle fluorescence from *Bacillus anthracis* was far larger than that from a common mineral dust (kaolin) and other particles (Pinnick et al. 1995). Subsequently, the system was used to measure fluorescence spectra of laboratory-generated particles (Hill et al. 1999; Pan et al. 2001; Pan et al. 2003b; Pan et al. 2010; Pan et al. 1999) and atmospheric aerosol (Pan et al. 2007; Pinnick et al. 2004). The Canadian Defense Research Establishment teamed with TSI, Inc. (Shoreview, MN, USA) to develop the first commercially available fluorescence-based RT bioaerosol detector, the UV-APS or FLAPS (Agranovski et al. 2003b; Hairston, Ho, and Quant 1997; Ho 2002; Ho, Spence, and Hairston 1999). MIT Lincoln Laboratory (LL, Lexington, MA, USA) developed a 266-nm-based fluorescence and elastic scattering detector, the Biological-Agent Warning Sensor, BAWS (Primmerman 2000). The BAWS sensor was employed beginning in the year 2000 as the trigger on the U.S. Department of Defense deployed Joint Biological Point Detection System, JBPDS (Grometstein 2011; Lynch et al. 2005). In parallel, the Naval Research Laboratory (NRL) demonstrated an elastic scattering-cued fluorescence sensor at 266 nm (Eversole et al. 1999; Seaver et al. 1999). A collaboration between LL, NRL, and Edgewood Chemical and Biological Center led to an improved breadboard capability under the Rapid Agent Aerosol Detector (RAAD) program initiated in 2002 (DeFreez 2009; Jeys et al. 2007) that employed an 808-nm structured beam as the cueing laser (Herzog et al. 2007), 355-nm polarized elastic scattering, dual fluorescence excitation at 266 and 355 nm (Sivaprakasam et al., 2004) that triggered laser-induced breakdown spectroscopy (Hybl et al. 2006) at suspect events. The RAAD sensor was slated in 2017 to be the detector under the Enhanced Maritime Biological Detection (EMBD) program. A team from the University of Hertfordshire and U.K. Defense Ministry developed a fluorescence and light-scattering instrument for bioparticles, later commercialized as the WIBS (Foot et al. 2008; Kaye et al. 2000; Kaye et al. 2005).

Detection of fluorescence of individual aerosols with two sequential excitations and broad band

emission channels (Kaye et al. 2005; Sivaprakasam et al. 2004) has been shown to better discriminate against diesel soot, a common interferent (Sivaprakasam et al. 2011). A dual excitation system with 32-channel spectral resolution provides more specific bioaerosol classification (Huang et al. 2008; Pan et al. 2010). Suspect particles, identified based on fluorescence, can be sorted and collected by an air puffer deflection technique (Pan et al. 2001; Pan et al. 2004) or an electrostatic charging technique (Sivaprakasam et al. 2009) for further analysis.

2.1.3. Further commercial development of 1-3 channel LIF

Here, we discuss a few selected commercial LIF instruments, the evaluation of which can apply to other LIF technologies.

2.1.3.1. UV-APS. The Ultraviolet Aerodynamic Particle Sizer (UV-APS; TSI, Shoreview, MN, USA) was marketed widely and, though discontinued, is still used for research purposes. The UV-APS has been applied to both indoor (Bhangar et al. 2016; Kanaani et al. 2008; Pereira et al. 2017) and outdoor PBAP analyses (Hallar et al. 2011; Huffman et al. 2013; Pöschl et al. 2010; Schumacher et al. 2013; Valsan et al. 2016; Wei et al. 2016) as well as to investigate airborne microorganism viability and dynamics (Agranovski et al. 2003a; Agranovski et al. 2004; Pan et al. 2014a; Saari et al. 2015). Pulsed 355-nm light from a Nd:YAG laser excites fluorescence and the integrated intensity from 420 to 575 nm is measured (Brosseau et al. 2000; Hairston, Ho, and Quant 1997). In contrast to most other LIF instruments, the commercial UV-APS (Model 3314) does not report single-particle information, but rather summed totals over an user-defined time period (seconds to minutes). The detector response of the fluorescence background is also not routinely monitored or corrected for, which could significantly influence the fluorescence intensity and overall interpretation of the data.

Particle size is measured as the aerodynamic diameter (0.7–20 μm), and the size resolution is generally higher than for optical sizing instruments. Because the UV-APS reports fluorescence in a single emission band, overall particle discrimination is generally poorer than for multichannel instruments and particles may be more likely to escape detection, depending on the lowest channel of fluorescence intensity used for analysis (Healy et al. 2014; Huffman et al. 2012). Data from ambient aerosol measurements have

shown that the UV-APS number concentration scales well with the FL3 channel of the WIBS, as discussed below (Gosselin et al. 2016; Healy et al. 2014).

2.1.3.2. Wideband Integrated Bioaerosol Spectrometer (WIBS).

The Wideband Integrated Bioaerosol Spectrometer (WIBS) is a widely used, commercial, three-channel LIF spectrometer developed by the University of Hertfordshire (UH), now licensed to and manufactured by Droplet Measurement Technologies (DMT; Longmont, Colorado). Several noncommercial WIBS versions were produced (i.e., revisions 3 and 4; UH) with slightly different optical and electronic configurations, and DMT has manufactured two commercial models (4A and NEO). Detailed technical descriptions can be found elsewhere (Foot et al. 2008; Gabey et al. 2010; Kaye et al. 2007, Kaye et al. 2005; Könemann et al. 2019; Perring et al. 2015). All WIBS versions are generally similar: Elastic scatter from a 635-nm laser is used to determine particle size and asymmetry, two xenon flashlamps are filtered to produce narrow excitation wavebands centered at 280 and 370 nm, and two wideband photomultiplier detection channels ($\sim 310\text{--}400\text{ nm}$ and $\sim 420\text{--}650\text{ nm}$) provide three channels of fluorescence detection.

Early publications analyzing data from WIBS or similar sensors made a binary distinction between non-fluorescent and fluorescent aerosol with thresholds determined by fluorescence signal in the absence of particles (“forced trigger” mode) (Gabey et al. 2010). More recently, a typing scheme has been applied, which classifies particles into eight categories based on which channels display fluorescent signal (Perring et al. 2015). Hernandez et al. (2016) and Savage et al. (2017) used this method to characterize certain PBAP of interest, showing pollen, bacteria, and fungal spores to have distinct patterns; however, repeatability between instruments was relatively poor.

The Instascope (DetectionTek; Boulder, Colorado, USA), used commercially to monitor mold and fungal spores inside homes, is very similar to the WIBS-4A. Although the two descend from the same design, the Instascope has some technical differences (e.g., lacks asymmetry measurement and has a different physical configuration) and is primarily marketed to non-scientists.

2.1.4. Commercial multichannel LIF

2.1.4.1. Multiparameter Bioaerosol Sensor (MBS).

The Multiparameter Bioaerosol Sensor (MBS, UH) is similar in design to the WIBS, but features enhanced spectral resolution and morphological information

(Ruske et al. 2017). A xenon flashlamp provides excitation at a single wavelength (280 nm), and the fluorescence signal is split with a grating and detected over eight channels (310–638 nm) by a multichannel photomultiplier. A dual CMOS linear array records the spatial patterns of elastic scattering to provide morphological information. Work is ongoing to improve real-time statistical analysis of the scattering patterns (e.g., mirror symmetry and peak-to-mean intensity) and to aid discrimination between particles with similar fluorescent spectra.

2.1.4.2. Spectral Intensity Bioaerosol Spectrometer (SIBS).

The Spectral Intensity Bioaerosol Spectrometer (SIBS, DMT) is a commercial LIF instrument built on the optical block of the WIBS, modified to disperse emission spectra into 16 channels (300–720 nm) following excitation pulses from filtered xenon flashlamps centered at 285 and 370 nm (Könemann et al. 2019). The instrument has been described in detail, characterized in the laboratory (Könemann et al. 2019), and recently applied to ambient aerosol (Könemann et al. 2018a; Nasir et al. 2018). The SIBS provides significantly increased spectral resolution relative to WIBS units and may be able to discriminate between aerosol types with higher certainty once appropriate data analysis techniques (e.g., clustering or machine learning) are applied. Technical challenges and benefits are discussed by Könemann et al. (2019).

2.1.4.3. Rapid-E.

The PA-300 (Plair SA, Geneva, Switzerland), described by Kiselev, Bonacina, and Wolf (2011; 2013), can provide real-time observations of total pollen and grass pollen concentrations for particles 0.5–100 μm (Crouzy et al. 2016). An updated model called the Rapid-E can be used to identify further pollen taxa (Šaulienė et al. 2019) and consists of a blue laser (400 nm) used to produce time-resolved scattering patterns across 24 detectors at different angles ($\pm 45^\circ$ forward to backward). A second UV laser (337 nm) excites a fluorescence signal, which is measured across 32 channels with a spectral range of 350–800 nm and eight sequential acquisitions (0.5- μs interval). The fluorescence lifetime is recorded for four bands at nanosecond resolution.

Results from the Rapid-E were presented and compared to manual Hirst-type analyses (Hirst 1952) by Šaulienė et al. (2019). They found that the instrument has the potential to identify pollen morphotypes in RT; however, they also highlighted a number of remaining issues, including the need to generalize algorithms across instruments and to include more

pollen taxa. Plair also manufactures a Rapid-C focused on detection of viruses, bacteria, and fungi which is applied to bio-contamination in industrial processes and clean rooms.

2.1.5. Particle differentiation and fluorescence analysis techniques

The objective of particle analysis by fluorescence techniques is frequently to separate PBAPs from non-PBAPs, with the addition of broad taxonomic detail when possible. Detection of aerosols is typically performed automatically in RT, whereas analysis is usually performed offline, though simplified data processing is possible in RT. Instruments that use fluorescence alone can be limited in their ability to differentiate between aerosol classes. Species-level discrimination of fungal spores or bacteria is not possible, and sensitivity is limited for individual viral particles that are tens of nm in size. LIF instruments are frequently applied with the goal, e.g., to broadly understand atmospheric PBAP trends without fine-level classification or to monitor concentrations of a specific list of pollen species. Fluorescence-based techniques are also frequently paired with other RT or offline sensors to increase the level of specificity that can be achieved.

LIF bioaerosol data are generally analyzed in one of a few ways: (i) counting particles above a given threshold as fluorescent, and, often, interpreting this as a lower limit proxy for PBAP (e.g., Gabey et al. 2010; Huffman, Treutlein, and Pöschl 2010), (ii) assigning types based on response in different fluorescent channels (Perring et al. 2015; Wright et al. 2014), or (iii) using classification algorithms utilizing resolved emission spectra from multichannel instruments (Könemann et al. 2019; Ruske et al. 2017). The uncertainties associated with each strategy can dramatically affect interpretation of data, as discussed below.

For methods (i) and (ii) above, or to reduce input particle number for (iii), a first step is to determine if a particle is fluorescent in a given channel. For example, for WIBS data the forced trigger background + n standard deviations (σ) is commonly used to determine a threshold. Historically 3σ has been used (Gabey et al. 2010), but this relatively low threshold may allow detection of a fraction of some classes of non-biological particles (e.g., certain mineral dusts and soot) with significant frequency, overestimating the true PBAP concentration (Crawford et al. 2017, 2016; Toprak and Schnaiter 2013). Laboratory characterizations by Savage et al. (2017) demonstrate

that a 9σ threshold for WIBS data more effectively excludes many interferents without significantly impacting PBAP concentration. Some interferents (e.g., diesel soot and textile fibers) remain, however, even using a higher threshold. Another approach to determining the fluorescent threshold is to fit a Gaussian-constrained probability distribution to the ambient data (Perring et al. 2015). A variety of other threshold strategies have been explored with respect to WIBS data (Gabey et al. 2011, 2010; Savage et al. 2017; Toprak and Schnaiter 2013; Wright et al. 2014). The choice of threshold depends on the dataset; sampling locations with low expected interferent concentrations will be more tolerant of lower thresholds. However, computational demands of processing large datasets at lower thresholds using methods such as cluster analysis are high due to the inclusion of a vastly dominant interferent population.

Unsupervised and/or supervised classification techniques can identify distinct bioaerosol populations from measured spectra, size, and morphology more accurately than manual analyses. Methods applied to LIF data include k -means, random forest, hierarchical agglomerative clustering (HAC), convolutional neural networks, support vector machines, and several machine learning and artificial-intelligence-based strategies (Crawford et al. 2015; Pan, Huang, and Chang 2012; Pinnick et al. 2004; Robinson et al. 2013; Ruske et al. 2017; Swanson and Huffman 2018, 2019). Validation studies of classification techniques have shown high sensitivity to data preparation and algorithm choice. As such users are encouraged to follow published guidelines (Ruske et al. 2017; Ruske et al. 2018; Savage and Huffman 2018).

Supervised methods can be used to classify each detected particle into a distinct type, but performance depends on the applicability of the data used to train model parameters. Once trained, supervised methods can achieve significantly faster processing times and higher accuracy. The quality and variety of data used for training are key, however, as are the organism growth conditions and aerosolization methods. Many relevant PBAP cannot be appropriately aerosolized for laboratory study (e.g., wet-discharge fungal spores), and standard aerosolization methods can damage or kill bacterial cells (Heidelberg et al. 1997). Forde et al. (2019) recently highlighted remaining challenges in the use of laboratory data for training and validation of analysis methods. The co-benefits of unsupervised and supervised methods suggest that the choice of one or a combination depends on the required speed of classification and quality of training data used. The

development of efficient and accessible open-source libraries allows users to more easily move from, e.g., unsupervised methods to neural networks or advanced supervised methods. However, the high discriminatory power of supervised methods often comes with the risk of overtraining algorithms due to the recognition of unwanted features. Access to raw data is essential to allow meaningful quality control with respect to supervised methods of analysis and for comparisons between devices. If possible, deterministic approaches should be used concurrently with black box machine learning techniques (e.g., deep neural networks).

2.1.6. Fluorescence calibration methods

The interpretation of bioaerosol LIF usually assumes that instrumental response is repeatable in time and across instrumental platforms. Fluorescence intensity, however, varies with, e.g., instrument sensitivity, excitation wavelength, and particle size. Therefore, there is a need to establish standards for aerosol fluorescence intensity and comparison of instrument response. Performance can degrade for various reasons including detector drift, reduction of excitation irradiance, temperature dependence of the laser, or accumulation of particulates on optics. An established calibration protocol would greatly reduce the logistical support burden for fielded instruments, while simultaneously increasing reliability and confidence of measurements. Another common need is for calibration aerosols at specified concentrations to ensure repeatability of instrument counting response.

In some cases, instruments are used with relative fluorescence intensity calibration or are calibrated using unreliable standards. One example is using freshly grown fungal spores or bacteria, but this method suffers from the high variability of microorganism spectral properties based on age, size, physiological health, and growth conditions, as well as the fact that growth media can perturb the fluorescence response. In other cases, standard polystyrene microspheres doped with fluorescent dyes are used for both sizing and fluorescence intensity calibration. While these particles produce consistent sizing results, the fluorescent dyes degrade over time and can be inconsistent from batch-to-batch. Further, surfactants, included in varying concentrations, can have intense fluorescent properties that influence LIF results (Könemann et al. 2018b). One example of a standard particle that has been used for fluorescence calibration is a polymer bead coated only on the surface with blue dye (B0200; Thermo Fisher Scientific, Inc.,

Sunnyvale, CA, USA), which can exhibit relative stability (<20% fluorescence variability over a year) once surfactants are washed off before aerosolization (unpublished data, author Sivaprakasam).

One method developed for fluorescence intensity verification can also be used to validate particle concentration and establish traceability (Li et al. 2014). Particles size selected by a differential mobility analyzer (DMA) is sampled by an optical particle counter, collected on a wafer, and scanned for number count verification (Linnell et al. 2016). A prototype instrument developed by MIT LL and NRL is used to deliver controlled concentrations of monodispersed aerosol to calibrate LIF sensors and is commercially available (C-CAG, L2 defense, Edgewood, MD, USA). An alternative method for fluorescence intensity calibration also uses a DMA to produce monodispersed aerosol in order to determine a relationship between fluorophore mass and detector intensity (Robinson et al. 2017). Such instrumentation is unlikely to be available to many LIF users, however. While Robinson et al. (2017) suggested that instrument performance could be checked using polydisperse distributions, this requires using the instrument sizing, which may introduce errors; thus, primary calibrations are still recommended. It is also not clear whether the calibrations detailed in Robinson et al. (2017) are suitable for higher-resolution spectrometers or whether the sensitivity changes with environmental conditions (e.g., relative humidity after drying).

The current lack of a gold standard fluorescent calibration and standardized operational parameters within the LIF community is among the most significant barriers to directly comparing results and providing reliable sub-classifications. The ideal calibration standard would (i) be easily aerosolized, (ii) have consistent fluorescent properties (i.e., intensity and wavelength of emission band), (iii) minimize the need for expensive equipment, (iv) not involve complex chemical, biological, or physical laboratory requirements, (v) be stable over weeks to years at room temperature, and (vi) be non-hazardous for use or transport. It is unlikely that individual calibration standards would be suitable for all excitation wavelengths and all emission ranges, but having suitable standards for several ranges would be acceptable. It is also important for fluorescence to be calibrated and reported as a function of particle size, given the strong relationship between fluorescence intensity and particle size (Hill et al. 2001; Hill et al. 2015; Savage et al. 2017; Sivaprakasam et al. 2011).

2.1.7. Perspectives and general challenges for fluorescence analysis

2.1.7.1. Instrument design and characterization.

Könemann et al. (2019) demonstrated significant performance differences between LIF instruments due to variations in flashlamp irradiance (e.g., ~220% difference between two lamp units observed). Electronic gain applied to sources such as xenon lamps or power associated with laser or LED units determines the photon flux that promotes fluorescence; thus, decreases in photon flux reduce fluorescence signals. Because fluorescence signals are frequently not normalized to source intensity for commercial instruments, perturbations or drift in irradiation power density can significantly influence data interpretation. Further, detector sensitivity, whether altered through degradation or electronic gain, determines the ability to detect emitted light (e.g., Robinson et al. 2017). These factors often go unmonitored and can change over time. The lack of protocols for setting or reporting instrument gains complicates comparisons between units (e.g., Hernandez et al. 2016) and poses a significant barrier to the adoption of more sophisticated classification tools. These effects highlight the need to characterize and calibrate LIF instrumentation.

LIF spectrometers are now commercially available that offer greater spectral resolution than previous generations of instruments. There is still utility in 1-3 channel instruments to determine bulk PBAP concentrations, with some level of broad sub-classification possible when data are treated and caveated appropriately. Modification of the excitation and detection bands to increase sensitivity to pollen, or other specific PBAP of interest, may be beneficial for monitoring purposes (e.g., changing WIBS FL3 to match the chlorophyll peak emission) (O'Connor et al. 2014). Moving from fluorescent intensity to photon counting may also be beneficial and should be explored if feasible (Sivaprakasam et al. 2004). For instruments with higher-emission spectral resolution, normalizing to maximum spectral intensity is one way to cope with differences between instruments, although this is not a suitable long-term solution.

Many RT LIF spectrometers have very low flow rates (e.g., 0.2 L min^{-1}) causing poor counting statistics when airborne PBAP concentrations are low ($<10^5 \text{ s L}^{-1}$); thus, averaging intervals should be chosen, in part, with respect to particle concentration. In some newer instruments, the conversion from light-scattering signal to calculated particle size is hidden within proprietary software, which can lead to sizing errors or drifting offsets. In all cases, particle

sizing should periodically be calibrated and monitored, e.g., using NIST-traceable PSL particles. Various instruments provide proxies for particle shape, but the quality of these measurements varies between instrument types and their application should involve careful calibration with known particle shapes (Gabey et al. 2010; Healy et al. 2012; Könemann et al. 2019; Savage et al. 2017).

2.1.7.2. Data interpretation. Physical properties of particles influence their classification and data interpretation. Emitted fluorescence intensity generally varies by the second to third power of diameter, depending on the absorptivity, excitation and emission properties, and composition of the particle (Hill et al. 2001; Hill et al. 2015; Sivaprakasam et al. 2011). The dependence of the fluorescence versus size relationship on LIF penetration depth makes normalizing fluorescence measurements by particle size impossible without additional information about each particle. When weakly fluorescent particles are excluded from further analysis, the strategy for threshold determination also influences particle categorization. For example, by increasing the threshold of discrimination a particle may no longer be considered fluorescent in one channel, while retaining fluorescent status in another, thus changing categorization. These factors combine to make the comparison of individual particle types (i.e., Perring et al. 2015) complex, unless using instruments calibrated to a common fluorescence standard, or with matching gain, limiting to a narrow and matching particle size, and using the same threshold strategy (Savage et al. 2017).

Particles that saturate the detector pose an unresolved issue. In early efforts, saturating particles were removed from cluster analysis-classification schemes to avoid conflating particle types. Large and brightly fluorescent PBAP (i.e., pollen), however, frequently saturates one or more channels; thus, their removal may result in misrepresentation of aerosol composition. Weakly fluorescing PBAPs can also challenge quantitative estimation of particle number (Healy et al. 2014; Huffman et al. 2012), and the problem is exacerbated when higher-fluorescence thresholds are used. The goal of a particular analysis will largely define the strategy.

Non-biological interferences pose a significant ongoing challenge. Many particles can be detected as fluorescent, including some mineral dust, aged organic aerosol, soot, or textile fibers (Gabey et al. 2013; Hill, Mayo, and Chang 2009; Huffman, Treutlein, and Pöschl 2010; Savage and Huffman 2018; Savage et al.

2017; Sivaprakasam et al. 2011; Toprak and Schnaiter 2013). Care should be taken when sampling in locations with potentially high concentrations of interferents, especially in urban or built (i.e., indoor) environments. Laboratory characterization of LIF instrument response to PBAP has been foundational, but differences between lab-grown or generated particles and ambient PBAP are likely significant. These differences may affect interpretation, especially when supervised algorithms trained on lab data are utilized. Lastly, a strong association has been made between fluorescence in certain detection bands and viability of bacterial or fungal aerosol. This association can be useful in controlled laboratory environments, but is almost totally invalid when analyzing complex mixtures of ambient aerosols.

An additional challenge with some emerging LIF instruments is that they limit the complexity of raw data output to streamline data analysis or for proprietary reasons. These practices may improve consistency in data analysis, but may also introduce errors by hindering the double-checking of analytical assumptions.

2.2. Elastic scattering, microscopy, and holography

Each of the sensors discussed specifically below includes the capability to sample, analyze, and report aerosol data automatically and in RT, which means that data are made available with a period of seconds to several hours after samples are taken. For all sensors discussed, the focus is primarily on allergenic pollen detection.

2.2.1. BAA500

The BAA500 (Hund-Wetzlar, Wetzlar, Germany) is currently used in the ePIN pollen monitoring network established in Bavaria, Germany (Oteros et al. 2015). The device uses a method inspired by the workflow of human operators carrying out manual pollen monitoring. Samples are collected and fed through a microscope system, which measures images at eight focal positions (z-scan) for identification of a wide range of pollen taxa. Imaging and analysis are performed while the next sample is collected, which means that the data are made available several hours after the sample is taken. The scope of the device is mostly restricted to pollen ($>10\ \mu\text{m}$) and some spores. Smaller particles are excluded by the sampling mechanism to increase the performance of image analysis by keeping slides clean. Training of the recognition algorithms can be

performed using live monitoring data by labeling events manually. The strong focus on pollen and the use of microscopy builds on the knowledge base and quality standards traditionally applied by aerobiologists (Galán et al. 2014).

2.2.2. Poleno

Similar to other LIF devices, the recently commercialized Poleno (Swisens AG, Horw, Switzerland) utilizes fluorescence (LED excitation at 280 and 365 nm), but is unique due to the use of digital holography to reconstruct in-focus images of airborne particles. The quality of the holographic images makes it possible for the trained human eye to manually distinguish, e.g., between pollen and non-pollen particles, as well as to recognize certain pollen taxa. This provides the possibility for external verification of analyses applied to the raw data (either LIF measurements, images, or both). The availability of raw images also means that more classically trained aerobiologists are not required to completely change their analysis paradigm. Algorithms can be trained on-line by manually labeling events; however, the advantages of on-the-fly training are somewhat in contradiction with the need for reproducibility and traceability, which are difficult to attain with evolving algorithms. Convolutional neural networks have successfully been applied to images from a Poleno, with up to ten different pollen taxa identified (Sauvageat et al. 2019). The focus of the first tests was pollen, and the ability of the Poleno to measure other (bio)-aerosols remains to be tested as does the extension of classification algorithms to identify further pollen species. Data from the Poleno are analyzed automatically and reported to the user within seconds.

2.2.3. KH-3000

The commercially available KH-3000 (Yamatronics; Japan) measures the forward- and side-scattering signals from a 780-nm laser beam, and data are reported immediately (Kawashima et al. 2007). The device has been used since 2002 across the Japanese national automatic pollen monitoring and forecasting network “Hanakosan,” the first of its kind. The requirements for pollen monitoring in Japan, however, are somewhat unique: The dominant allergenic species (*Cryptomeria japonica*, Japanese Cedar) can easily be discriminated from other pollen taxa due to the specificity of winter season emission and the exceptionally large size and smooth surface of the pollen grains (Beug 2004). Although attempts were made to extend the scope of the KH-3000 (Kawashima et al. 2007;

Kawashima et al. 2017), the potential is limited. The robustness and the low cost of the device make it, however, a good candidate to complement the spatial resolution of a network backbone consisting of more precise (but more expensive) devices. This is important, since a relatively dense network of real-time pollen monitoring would open up an avenue for data assimilation in numerical forecast models (Sofiev et al. 2006; Vogel, Pauling, and Vogel 2008), justifying the existence and development of cheaper simple instruments.

2.2.4. PollenSense

A newly available sensor from the company PollenSenseTM (<https://pollensense.com>) detects and identifies pollen automatically through a proprietary imaging and deconvolution process after collecting particles onto a substrate, and the sensor provides information within one half to several hours of measurement (Lucas et al. 2018). The manufacturer Web site claims that a fraction of ambient pollen species can be identified and that the sensors can be purchased or leased for home or community use. Nothing has been published in peer-reviewed literature at this time.

2.2.5. Portable sensors

New embodiments of relatively inexpensive, portable sensors for RT bioaerosol detection based on optical scattering or emission are in constant development (Ballard, Brown, and Ozcan 2018; Huffman and Huffman 2019; Huffman, Swanson, and Huffman 2016; Kühnemund et al. 2017; Navruz et al. 2013; Tsuruzoe and Hara 2015; Wei et al. 2014; Wu, Shiledar, et al. 2017; Wu et al. 2018), enabled, e.g., by improvements in additive manufacturing (i.e., 3D printing), battery technology, and optical source cost and quality (i.e., more powerful LEDs at reduced cost). In some cases, these emerging sensors are not yet paired with automatic collection systems, but together are sure to provide significant improvements to PBAP detection capabilities in at least the next decade as products become commercially available.

2.3. Raman spectroscopy

Techniques for measuring Raman spectra (RS) of atmospheric aerosol particles and the use of RS for particle characterization continue to be developed (Deboudt et al. 2010; Hiranuma et al. 2011; Ivleva et al. 2007; Ivleva, Niessner, and Panne 2005; Rosasco, Etz, and Cassatt 1975; Rosen and Novakov 1977), and

include high-throughput techniques and automated analysis methods (Craig, Bondy, and Ault 2017; Doughty and Hill 2017). Peaks in RS indicate the vibrational frequency modes of molecules. The number of particles that can be measured in a given time period using existing RT instruments applying Raman spectroscopy is far smaller than can be measured using fluorescence or mass spectrometry, because the intensity of RS of typical atmospheric particles is weak and so long integration times are necessary (e.g., seconds to minutes for a 1- μm particle). Thus, particles must either be trapped in air electrostatically (Vehring and Schweiger 1998) or optically (Thurn and Kiefer 1984; Wang et al. 2015), or collected onto a substrate and then analyzed (Rösch et al. 2006). Techniques such as surface-enhanced Raman spectroscopy (SERS) that increase Raman signal by orders of magnitude (for molecules within nanometers of SERS-active substrates or nanoparticles) have potential to enable real-time measurement (Craig, Bondy, and Ault 2015; Sivaprakasam, Hart, and Eversole 2017). The information content of RS is far larger than that of fluorescence spectra. For example, an ensemble of RS can indicate hundreds of different vibrational frequencies. The assignment of a RS to a material such as a mineral or mineral salt can be unambiguous (Nyquist, Putzig, and Leugers 1997). RS has also been shown effective in identifying biological particles. For example, in tests with clean spores and vegetative cells of *Bacillus cereus*, *B. anthracis* Sterne and *B. thuringiensis*, RS were assigned correctly to species with greater than 96% probability (Ronningen et al. 2014).

The only commercially available instrument designed for identification of specific types of infectious aerosols is the automated aerosol Raman spectrometer termed the Rapid Enumerative Bioidentification System (REBS), developed by Battelle (Columbus, Ohio). Its Raman spectrometer and data analysis techniques for characterizing bacteria and bacterial spores have been described by Ronningen et al. (2014). The REBS was used to measure atmospheric aerosol in 15-min intervals over a 7-h period, as reported by Doughty and Hill (2017). Aerosol particles are collected from air automatically onto a tape, and RS are measured using excitation at 643 nm. The instrument collects particles while simultaneously measuring RS of particles collected in the preceding period. The laser beam is focused to a line on the tape. The resolution of the line-scanning imaging spectrometer (dispersion-mated with a CCD detector) allows ~ 40 RS to be acquired simultaneously (each corresponding to a position along the beam line in

approximately 1 μm steps). The laser line is then stepped, e.g., every 2 μm across the sample to get a subsampling of deposited particles. If three replicate RS are measured for each pixel interrogated, typically the three-replicate RS of 100,000 pixels can be measured in a day of continuous instrument operation. To reduce the chance of particles overlapping on the tape, the REBS is run so that typically 5,000 to 25,000 RS measured per day exhibit intensities above a given threshold. In urban/suburban areas, near roads, or downwind from combustion sources, the fraction of RS dominated by the D and G peaks of black carbon (BC) or combustion soot is well over 50%. The large number of BC particles in many outdoor air samples may partially account for the high fraction of RS that were indicated, e.g., by Doughty and Hill (2017) to be BC, but spectral properties of the ambient particles are also likely to play a role. The differential Raman cross sections (DRSL) of diesel soot, for example, are hundreds of times larger than those of benzene or toluene (Le, Lefumeux, and Pino 2017) and even larger than the DRSL of most non-aromatic materials. The REBS has been shown to be able to measure RS from particles as small as 300-nm polystyrene spheres (unpublished data, authors Doughty and Hill), with aromatic rings similar to benzene or toluene. These facts suggest that BC particles with mass equivalent diameters smaller than, e.g., 100 nm could be detectable and thus could contribute to the large numbers of RS observed in atmospheric samples. In contrast to this, the large majority of other particle types have smaller Raman cross sections and minimum detectable sizes that are a few to many times larger.

Most intact pollen grains and many fungal spores are too large to be collected by the REBS in its standard operating mode, thus challenging application of the instrument for ambient bioaerosol detection. The particle collection system could be modified to collect and measure larger particles, e.g., by changing the microscope objective, but that would reduce its capability in measuring smaller particles.

Fluorescence of bioaerosols, minerals, and other particles can overwhelm relatively weak Raman peaks. In measurements made using an REBS, a significant fraction of RS were so dominated by fluorescence that no Raman peaks were apparent (Doughty and Hill 2017). Many of these fluorescent particles are likely to be bioaerosol. Photo-bleaching can reduce the fluorescence so that the Raman peaks appear more clearly, but the time required for it reduces the sample rate. Alternately, longer wavelength illumination could be used, but that also requires longer illumination times.

The combination of fluorescence and Raman signals present in large fraction of measured RS may yield more information than can be obtained from RS alone. At λ_{ex} 643 nm, fluorescence is dominated by different fluorophores (e.g., chlorophylls) than those observed at wavelengths commonly used in LIF detection.

The number of spectra measurable in a short time period by RT Raman instruments is also limited because some particles can be charred or physically modified by higher laser intensities (e.g., $> 1 \text{ mW } \mu\text{m}^2$) (e.g., Blaha, Rosasco, and Etz 1978; Lai et al. 2016). Thus, there is a complex tradeoff between laser intensity (scales positively with potential for burning), laser wavelength (Raman intensity scales as $1/\lambda^4$, but also fluorescence is generally stronger at shorter excitation wavelengths), and imaging/photo-bleaching time versus sample rate. In RT systems, the choice is more difficult because it must be made *a priori* or automatically at the time of measurement. Good databases exist for RS of minerals (Lafuente et al. 2015), many biological materials (De Gelder et al. 2007), and of a few microorganisms (Guedes et al. 2014), but there is a great need for more complete databases of RS of atmospheric bioaerosols. More study is needed of RS of mixed and aged particles, as well as of differences in ambient and laboratory-generated bioparticles.

2.4. Mass spectrometry

2.4.1. Online mass spectrometry techniques

Single particle mass spectrometry (SPMS) refers to a collection of techniques that ablate and ionize single aerosol particles with a pulsed laser and analyze their chemistry with time-of-flight mass spectrometry. Instruments tend to be custom-built, with examples including ATOFMS (Gard et al. 1997; Pratt et al. 2009b), SPLAT (Zelenyuk and Imre 2005; Zelenyuk et al. 2015), ALABAMA (Brands et al. 2011), and PALMS (Cziczo et al. 2006; Thomson, Schein, and Murphy 2000). Some have been commercialized, e.g., ATOFMS (TSI), Livermore-SPAMS (Livermore Instruments, Oakland, California) (Frank et al. 2011; Morrical, Balaxi, and Ferguson 2015), Hexin-SPAMS (Hexin Instrument Co, Ltd., Guangzhou, China) (Li et al. 2011; Zhang et al. 2012), and LAAPTOF (AeroMegt GmbH, Solingen, Germany) (Gemayel et al. 2016; Shen et al. 2018). The details of instrument construction, including the wavelength of the ionization laser, vary across different instruments, making direct spectral comparisons challenging.

Among the advantages of SPMS is its ability to detect nearly all atmospherically relevant aerosol types, including soot, dust, and sea salt. One disadvantage, however, is the challenging interpretation of SPMS mass spectra, which are semi-quantitative and strongly influenced by matrix effects (Murphy 2007). Bioaerosol detection presents an attractive target for SPMS, but it can be complicated by these difficulties.

The thermal desorption aerosol mass spectrometer technique, most famous through the series of Aerodyne (Billerica, MA, USA) Aerosol Mass Spectrometer (AMS) instruments (Canagaratna et al. 2007; DeCarlo et al. 2006; Drewnick et al. 2005; Jayne et al. 2000), has been used for RT detection of bioaerosol, but to a lesser extent than SPMS. The main reasons were the restriction to submicron aerosol particles in the first generations of AMS instruments due to the particle size cutoff of the aerodynamic lens (Liu et al. 2007) as well as the uncertain degree of evaporation of PBAP components at the operational AMS vaporizer temperature of about 600–700 °C.

By most online mass spectrometry techniques, aerosol can be collected from the atmosphere and analyzed in the instrument automatically and continuously. Interpretation of the complex mass spectral data generally requires human interaction, though first approximation determinations can be applied using interpretation algorithms operated in RT.

2.4.2 Bioaerosol detection by online mass spectrometry

The interest in bioaerosol detection in the SPMS community was precipitated by the importance of bioaerosols for ice formation in clouds and with respect to national security concerns. While the earliest reports of single particle bioaerosol mass spectra come from laboratory standards (Ferguson et al. 2004; Gieray et al. 1997), SPMS has been increasingly applied to the detection of bioaerosols via aircraft or at high alpine research stations, i.e., to measure the chemical composition of ice residuals and aerosols both in and outside of clouds (Creamean et al. 2013; Pratt et al. 2009a; Schmidt et al. 2017). SPMS has also been applied for the detection of agents of biological warfare and for public health monitoring (Czerwieniec et al. 2005; Frank et al. 2011; Russell et al. 2004; Steele et al. 2006; Tobias et al. 2005). The system deployed by Steele et al. (2006), in particular, paired SPMS with LIF detection of PBAP.

Traditionally, the prominence of negative phosphate ions (PO_2^- , PO_3^- , sometimes PO_4^-) in a single-particle mass spectrum is used to identify bioaerosols.

Often, the phosphate markers are combined with organic nitrogen fragments (CN^- and CNO^-). This was found to match laboratory signatures of bioaerosols well (Ferguson et al. 2004; Pratt et al. 2009a; Schmidt et al. 2017; Sultana, Al-Mashat, and Prather 2017; Suski et al. 2018; Zawadowicz et al. 2017). However, there is also recent evidence that misclassifications with phosphate-rich dust and ash are possible, and a marker ratio-based approach combined with machine learning can improve bioaerosol identification and allow uncertainty analysis (Zawadowicz et al. 2017; Zawadowicz et al., 2019). Recent work using this method was found to compare well with the WIBS sensor in one deployment at a mountaintop research station (Zawadowicz et al. 2019).

The attempts to detect and quantify bioaerosol with the thermal desorption technique are mainly based on nitrogen-containing marker ions, inferred from laboratory studies using reference compounds such as amino acids. The technique was used to estimate the PBAP fraction of submicron organic aerosol mass to be ~20% in both the Amazonian rainforest (Schneider et al. 2011) and a sub-Antarctic marine environment (Schmale et al. 2013). Wolf et al. (2015) used a new aerodynamic lens transmitting up to 3 μm (Peck et al. 2016), thereby enabling the AMS to make $\text{PM}_{2.5}$ measurements. To obtain marker ions for biological material, they used an aerosolized suspension of *Pseudomonas* bacteria. The identified marker ions were also characterized by nitrogen, of the general form $\text{C}_x\text{H}_y\text{N}_1^+$. Wolf et al. (2017) used positive matrix factorization (PMF) to estimate bacteria-like components in measured ambient air to comprise ~2% of the $\text{PM}_{2.5}$ mass.

2.4.3. Perspectives on bioaerosol mass spectrometry

Outstanding issues in detection of bioaerosols using online mass spectrometry include the need for direct comparisons of bioaerosol ion markers and differences in detection between existing mass spectrometric techniques, but also between mass spectrometers and other bioaerosol-specific techniques. Such comparison efforts would ideally include both known bioaerosol populations in a controlled laboratory setting and comparisons between co-located instruments in the field. For the laser ablation SPMS method, differences in ionization laser wavelengths can produce large changes in the resulting single-particle mass spectra, and therefore, there is no guarantee that one combination of spectral markers applies equally well to all existing SPMS instruments.

The selection of laboratory standards to compare against ambient mass spectra is of critical importance, as laboratory-generated standards do not reflect the complexity of real-world aerosol. This especially applies to complex particle matrices, such as sea salt aerosol with fragments of marine bioaerosol (Sultana, Al-Mashat, and Prather 2017) and soil-derived aerosol. Additionally, Wolf et al. (2015) and Suski et al. (2018) recently showed difficulties in separating laboratory bacteria standards from their culturing medium, which changed the properties of sampled aerosols. Laboratory reference data are partly missing for the AMS technique, e.g., pollen, viruses, fungal spores. Reference mass spectra of laboratory-generated biological aerosol particles need to be recorded and published. Ideally, this should be conducted in parallel with AMS and SPMS instruments, such that a direct comparison of marker ions is possible.

It is important to consider the operational definition of bioaerosol and how it compares with other detection techniques. The exact molecular origin of the commonly used phosphate spectral markers (for SPMS) is unclear, but they are likely to be connected to phospholipids and nucleic acids in cells. Nitrogen-containing marker ions may originate from nucleic acids, but are very likely not specific to nucleic acids. PBAPs that lack these components and are instead composed of mostly carbohydrates (e.g., cellulose) will not be classified as bioaerosol with current SPMS detection schemes. Additionally, some carbohydrates (especially levoglucosan, which is readily detected by the AMS) also originate from biomass burning, such that separation between biomass burning aerosol and PBAPs can be difficult.

For both mass spectrometric techniques, but especially for the AMS, the particle size range needs to be extended to larger particles. An upper size limit of 3 μm is in the middle of the size range frequently reported for fluorescent biological aerosol particles (e.g., Huffman, Treutlein, and Pöschl 2010); thus, an extension to even larger particle sizes (up to 10 μm and beyond) should be pursued. The AMS technique additionally suffers from the uncertain evaporation of bioaerosol particles. A new “capture” vaporizer was recently introduced (Hu et al. 2018) which in combination with an extended size range may lead to better PBAP results; however, this needs to be verified. Furthermore, for both techniques, the detection efficiency needs to be determined so that the number concentration of atmospheric biological particles can reliably be calculated.

2.5. Breakdown spectroscopy

Laser-induced breakdown spectroscopy (LIBS) is a laser ablation technique, by which a high-energy laser beam is focused to create a microplasma to induce breakdown of aerosol particle material. The electronic emission from the electronic excited states provides elemental composition of the particle, which can be characteristic of individual material types. LIBS capability provides an orthogonal measurement technique that is proven to improve the false alarm rate of bioaerosol sensors based on fluorescence; however, it does not offer enough discrimination as a standalone technique due to the abundance of detected elements in the environment and the high variability in signal strength due to non-uniformity of the plasma formed (Hybl et al. 2006; Martin, Cheng, and Martin 1999; Saari et al. 2016). A similar technique of spark-induced breakdown spectroscopy (SIBS_b; not to be confused with the fluorescence technique with matching acronym) determines elemental composition, but using energy input from an electrically sparked plasma, which offers a cost-effective alternative to LIBS (Tysk, D'angelo, & Herzog 2015). SIBS_b has been applied for non-RT bioaerosol analysis (Schmidt and Bauer 2010). The breakdown spectroscopy process can be implemented for RT analysis. Particle throughput for this technique is less than ten particles per second, however, due to the low repetition rate of the plasma generation source and the plasma persistence time. This results in inefficient sampling of the aerosol population, adding to the short-comings of this technique. Neither LIBS nor SIBS_b have been commercialized for bioaerosol analysis; however, LIBS is employed in the US DoD RAAD program (see Section 2.1.2). Efforts to produce a predictable plasma source could propel the technique toward more practical implementations and better discrimination. Due to the commonality of the elements detected in the environment, however, it is hard to envision LIBS or SIBS gaining footage as a standalone technique.

2.6. Remote sensing

Stand-off detection systems are used to interrogate atmospheric composition, e.g., for the purposes of both environmental research, national security, and agricultural monitoring and have been engineered for both ground-based and airborne application (Buteau et al. 2010; Christesen et al. 1994; Gelbwach and Birnbaum 1973; Joshi et al. 2013; Richardson, Aldridge, and Milstein 2008). Their advantages over point detectors are principally due to the ability to

scan wide areas, e.g., tens of kilometers, with high spatial resolution (e.g., meters) in RT and with no need to physically reach the target. Quantitative, spatially resolved measurements of bioaerosol concentration and properties are predominantly provided by active methods of Light Detection and Ranging (LIDAR) (Buteau et al. 2008). LIDAR systems have been used for monitoring PBAP of various types (Brydegaard et al. 2009; Saito et al. 2018; Sassen 2008).

LIF-LIDAR has been most successful for PBAP detection, though several other techniques have been successfully applied in parallel for improved discrimination, e.g., longwave infrared differential scattering, femtosecond filamentation, and depolarization ratio LIDAR. The LIF-LIDAR technique utilizes powerful UV lasers (up to tens of watts), usually at excitation wavelengths either 266 or 355 nm (e.g., via pulsed Nd:YAG laser) to photo-excite an atmospheric line of sight and analyze the transient back-fluorescence spectra at >10-ns resolution. The intrinsic challenge in such stand-off detection is that the signal-to-noise ratio (SNR) decreases with square of the distance and exponentially with atmospheric attenuation. Similar to single-particle LIF systems discussed above, increasing spectral resolution of the LIDAR emission detector improves spectral differentiation between biological species in the air, but increasing the number of wavelength channels (N) reduces SNR by \sqrt{N} . Choice of fluorescence excitation and emission bands interrogated follows the same physical reasoning as discussed above with respect to single-particle LIF instruments. Contrary to single-particle instruments, however, the sensing distance, e.g., of a LIDAR system requires the user *de facto* to give up specificity of bioaerosol chemical classification. Further, the process integrates signal over many particles and thus cannot separate contributions from individual aerosol types within the interrogation volume of the LIDAR field of view. For this reason, the primary use of such remote sensing systems is as means for warning about plumes of biological warfare agents.

Portable LIF-LIDARs have existed for more than a decade; however, no LIF-LIDAR systems are commercially available. Existing systems are generally used as research instruments for atmospheric measurements, with no standardized outputs. Stand-off detection of PBAPs based on fluorescence properties is valuable, but faces similar technical challenges to single-particle LIF techniques, e.g., poor ability to differentiate between aerosols due to broad fluorescence spectra. Using LIDAR systems with multiple excitation

wavelengths and combining with other techniques may improve the contributions that LIDAR systems can provide.

2.7. Microfluidic techniques

Emerging microfluidic techniques offer promise for near-RT, species-level identification of infectious or toxic bioaerosols. Microfluidics techniques are based on controlled manipulations of femtoliter to microliter fluid volumes, often in a lab-on-a-chip environment. Microfluidic devices aimed at bioaerosol detection have recently been developed (Choi et al. 2017; Novosselov et al. 2014), usually consisting of a collector, delivering bioaerosols into microfluidic liquid volumes, integrated with a biological assay. Aerosol is collected via impaction into microdroplets, directing aerosols into winding microchannels of a chip, or depositing aerosols onto a small substrate for later recovery (Foat et al. 2016; Han, An, and Mainelis 2010). After collection, a variety of assays may be applied including ATP measurement, PCR, immunoassay, or genomic sequencing (Mairhofer, Roppert, and Ertl 2009). Advantages of microfluidics platforms for bioaerosol detection include sensitivity, adoption of established microfluidic assays, low reagent consumption, rapid assay kinetics, and enhanced aerosol concentration ratio. Most studies have investigated single-phase microfluidics, wherein aerosols are collected into a single-phase aqueous collection liquid, but recent research has introduced bioaerosol detection in a droplet microfluidic environment (Damit 2017). Droplet microfluidics involves the creation of discrete aqueous microdroplets containing reagents that are suspended in a carrier oil phase in the chip (Garstecki et al. 2006; Shang, Cheng, and Zhao 2017). This configuration can further accelerate assay reaction kinetics, enable precise fluidic manipulations (e.g., electrowetting), and may support single-particle assays.

Microfluidics-based bioaerosol detection remains in its infancy, with several technical hurdles needing to be overcome to facilitate robust detection. Paramount among these are lack of maturity of such systems, contamination, and miniaturization of supplementary equipment (e.g., air sampling pump). Microfluidic bioaerosol literature has focused primarily on aerosol collector design, with minimal attention given to the back-end assay. For the purposes of RT detection, it is beneficial for future efforts to emphasize selection and integration of a rapid assay, and to demonstrate the entire detection workflow – from aerosol sampling to

detection of a target organism. Subsequently, these systems must undergo laboratory evaluation as well as field testing to prove their real-world application. Interfacing with advances in the broader field of microfluidics, especially progress in droplet microfluidics, biochemical assays, and microelectromechanical fluidic manipulations, could resolve limitations and improve assay-based bioaerosol detector approaches. No microfluidic systems for bioaerosol analysis are commercially available.

2.8. Paired aqueous techniques

A number of historically offline analysis techniques have been applied for RT bioaerosol detection and analysis, with relatively limited scope of application, and currently no commercial availability of paired sampling analysis, although the Autonomous Pathogen Detection System (APDS) has been deployed by the U.S. Department of Homeland Security as part of its BioWatch program (Dzenitis and Makarewicz 2010; Regan et al. 2008). In particular, collection of airborne PM directly into liquid, e.g., via an impinger, particle-into-liquid-sampler (e.g., wet cyclone), or Spot SamplerTM aerosol particle collector (Aerosol Devices, Fort Collins, Colorado), allows for coupling to RT chemical and biological analyses (Cho et al. 2019; Morris et al. 2014b; Pan et al. 2016, 2018; Park et al. 2014; Seshadri et al. 2009). These strategies require post-processing of data and so do not provide complex data about bioaerosol in RT.

One example of this is aqueous flow cytometry, routinely used in many fields of biology and environmental science, yet applied only infrequently to RT bioaerosol analysis (Chen and Li 2007; Ho and Fisher 1993). Flow cytometry detects microorganisms or other cellular material after attaching fluorescent probes for more selective detection. Identification is possible, e.g., after PCR amplification (Dzenitis and Makarewicz 2010; Regan et al. 2008). Performing these steps autonomously in RT has rarely been achieved (i.e., APDS as an exception; Regan et al. 2008).

Another hybrid example is pairing the type of samplers mentioned above for aerosol collection with ion chromatography separation and detection (Eiguren Fernandez, Lewis, and Hering 2014; Sarda-Estève et al. 2015). This can provide detailed information about chemical tracers in the sampled aerosol, e.g., mannitol and arabitol as tracers of fungal material. The technique requires constant monitoring of the flowing system and is challenging to deploy for field investigations.

3. Broad summary and grand challenges

Recently increased commercial availability of instruments for RT analysis of PBAPs has allowed a surge of studies in a host of environments. Many of these techniques utilize properties based on either chemical (e.g., identification of molecule, functional groups, or constituent elements; absorption and emission properties) or physical (e.g., elastic light scattering) principles as a proxy for detecting PBAP classes. A challenge in the application of these technologies is to carefully understand the link between the response of a given instrument and the PBAP of interest, including interfering species or technical limitations that pose challenges to discrimination between types. The spectrum of PBAP types can span orders of magnitude in physical size and includes large differences in chemical composition, taxonomy, physiological state, natural biological variability, and many other factors. As a result, techniques should be well matched to questions of interest, and users should take care to understand the challenges associated with a set of measurements. Listed below are grand challenges, pressing scientific questions, and community needs with respect to RT detection of bioaerosols.

3.1. Technical needs common across techniques

- i. *Standardization of PBAP operational definitions* – Classes may be as broad as PBAP versus non-PBAP or as specific as genus and species. Individual instruments are sensitive to only a fraction of PBAP classes and differ in the specificity that can be achieved. For some instrument classes, detected aerosol may be defined in terms of instrument response (i.e., fluorescent aerosol as a proxy for some fraction of total PBAP), which underscores standardization needs listed below. Reports should explicitly clarify the classes of PBAP implied for detection.
- ii. *Standardization of calibration and operation* – Adopting particle calibration standards (e.g., for fluorescence and mass spectrometry) and developing standardized protocols for calibration, instrument operation, and analysis are each imperative to improve comparisons between both similar and dissimilar instruments. The use of central facilities that have access to microorganism growth, aerosolization, and safety equipment could be helpful to compare instruments during dedicated workshops by subjecting them to a wide variety of ambient and lab-generated aerosols to validate instrument performance.

- iii. *Standardization of reporting* – Communities of researchers are encouraged to develop and adopt standardized analysis and reporting procedures to better enable inter-comparison of results across all instrument types, with special attention to explicit reporting of key operational parameters (e.g., gain settings) and data processing procedures, including justification for use of any nonstandard methods. Similar to other emerging techniques, data interpretation is evolving using, e.g., machine learning analysis techniques (e.g., Vapnik 1998) and through comparison to traditional bioaerosol measurement methods. In particular, the raw data, exact processing steps, and processing code should be made available whenever possible.
- iv. *Standardization of analysis algorithm application* – Continued improvement in the types and recommended application of advanced analysis algorithms (e.g., supervised classification, machine learning, neural networks) is required. The recommendations with respect to fluorescence-based instruments given above have broad application to many technique classes.

3.2. Need for improved links between technique outputs

There is a need for improved and affordable RT instruments which measure particle types at sufficiently high time resolution with a useful specificity (in some cases to species level as needed for pathogen detection) along with the numbers of particles in each type. In many cases, RT technologies provide rich quantitative information, e.g., estimated number concentrations of classes of particles at high-time resolution, often with high-particle size resolution, and sometimes on a single particle basis. The qualitative information these RT techniques provide, e.g., detailed identification of the particle type or source, however, is often quite low. This is in contrast to many well-developed offline techniques for PBAP detection that provide great qualitative detail, even to the species or strain level, but generally with far lower time resolution or with significantly lower quantitative detail. Recently developed metagenomics analyses, for example, offer relatively rich detail on *what types* of organisms (i.e., species or functional traits) are involved without providing much information on *how many* (i.e., concentration) of those particles or organisms may be present (e.g., Frohlich-Nowoisky et al. 2014). The need to bridge the gap between primarily quantitative techniques and primarily qualitative

techniques thus represents a grand challenge associated with the development and application of RT PBAP sensing. In many cases, combination of techniques (e.g., LIF with holography, breakdown spectroscopy, or antibody-based sensing) helps to provide both adequate counting and discrimination.

3.3. Need for PBAP database development

Open databases should be developed for the techniques listed here, including microscopy, elastic scattering, Raman spectroscopy, fluorescence spectroscopy, mass spectrometry, and LIBS. These should include as many bioparticle and non-biological types as possible, with organism growth, aerosolization, and analysis conditions clearly reported. Input to these databases should also include particles analyzed from ambient air, with breadth of geography, latitude, ecosystem type, season, and weather conditions. Database development should also include the effects of atmospheric aging and processing.

3.4. Need for improved pathogen detection

RT detection of plant, animal, and human pathogens (both infectious and toxic) presents a significant technical challenge that has yet to be widely solved. Infectious virus aerosols are generally too small to be detected individually in the air by the techniques discussed. Development of instrumentation to detect viral particles in RT thus represents a significant community need. Infectious or toxic bacterial or fungal aerosols can often be detected, but differentiating in RT with sufficient taxonomic quality to identify as a potentially harmful species or strain is considerably more complicated. Detection techniques that pair multiple types of analysis have shown increased success, but are expensive and often not commercially available. One objective in this area includes operation of sensors continuously and affordably in locations such as hospitals, public schools, and airports, with little operator input. The identification probability must be very high in most cases to be effective, i.e., before shutting down a public area in case of an infectious aerosol outbreak. Related techniques could be applied to human, agricultural, and livestock applications.

3.5. Need for improved allergen-containing particle detection

Development of techniques for autonomous detection of pollen is more mature than for RT pathogen

Table 2. List of RT bioaerosol technique classes discussed in the text. Values listed are approximate and instrument properties are listed as general attributes. Text sections and individual research manuscripts should be consulted for more detail.

RT Measurement Type	Example Instrument(s)	Detection Principle	Measured Parameters	Particle size range (μm)	Discrimination level (example of best case published)
LIF (1–3 channel)	WBS, UV-APS, BioScout	Fluorescence and elastic scattering	Particle size, fluorescence intensity (1–3 channel spectral resolution)	~0.5 to 10–50	Biological vs non-biological particles
LIF (multichannel)	Rapid-E, MBS, SIBS, DPFS	Fluorescence (and elastic scattering for some instruments)	Particle size, fluorescence intensity (8–32 channel spectral resolution)	~0.5 to 10–100	Rapid-E: different pollen morphotypes (mostly at the genus level) DPFS: <i>Bacillus subtilis</i> spores
LIF (multichannel) + holography	Poleno	Fluorescence + elastic scattering + holography	Particle size, fluorescence in multiple channels, holographic images	~0.5 to 100	Different pollen morphotypes (mostly at the genus level)
LIF (multi-channel) + LIBS + elastic scattering	RAAD Biodetector	Fluorescence + LIBS + polarized elastic scattering	Particle size, fluorescence intensity, elemental composition, and absorption	~0.5 to 10	Broad classification among biological materials, e.g., spores vs. vegetative cells
Optical microscopy	BAA500, PollenSense	Optical microscopy or high-definition photography	Microscope images	>5	BAA500: different pollen morphotypes (mostly at the genus level)
Elastic scattering	KH-3000	Elastic scattering	Forward and side scattering	> 10	Select pollen morphotypes
Raman spectroscopy	REBS	Raman scattering (+ background fluorescence)	Images from which Raman spectra are extracted	< 10	<i>Bacillus</i> spores.
Mass spectroscopy	SPMS, AMS, SPAMS, PALMS, ATOFMS, ALABAMA	Mass spectrometry	Mass spectra of fragment ions from single or ensembles of particles	~0.05 to 5	PBAP by certain marker ions (PO^{3-} , CN^- , CNO^- , K^+)
Breakdown spectroscopy	LIBS	Atomic spectroscopy	Atomic spectra	1 to 100	Limited as a stand-alone technique

detection, but technical advances and improvement in cost are still required. In contrast, the autonomous detection of other allergenic or toxic PBAP classes (e.g., fungal spores, endotoxin-containing particles, pet dander, or fragments of pollen and spores) is not well established. Development of instrumentation toward detection of these particle classes in both indoor and outdoor air is required. Consideration should also be given to detection of both whole particles and ruptured particle fragments (i.e., sub-pollen particles) that can penetrate deeper into respiratory airways to exacerbate negative health responses (e.g., Miguel et al. 2006; Taylor and Jonsson 2004).

3.6. Need for improved cross-disciplinary cooperation

A fundamental challenge associated with PBAP detection is the wide diversity of scientific objectives and motivations across communities, as briefly summarized in Section 1. Each community commonly requires measurements of different PBAP types, with varying quantitative and qualitative detail, and each present unique technical challenges. An associated challenge is that communities are frequently separated by differences in society meetings and networks of collaboration. Cross-disciplinary interaction is further challenged by broad differences in funding sources. Some communities are supported to investigate basic scientific questions and thus publish in relatively openly available academic journals, whereas other communities are funded by governmental agencies tasked with protecting national security or by organizations focused on profit maximization (see Table 1), and so open sharing of results and experience can be limited by proprietary limitations or national security regulations. Transformative advancement in the development and application of RT bioaerosol sensing will require additional multi-disciplinary interaction; thus, the differences in approach, motivation, and funding barriers across communities will need to be overcome. Šantl-Temkiv et al. (2019) discusses these issues in more detail.

3.7. Need for improved links between bioaerosol processes and modeling efforts

Emission of all types of bioaerosols from terrestrial surfaces is influenced, in a complex way, by biological, meteorological, and physical processes. Relatively little is known about emissions and deposition fluxes of most bioaerosol types, because

techniques for measuring surface flux (e.g., by eddy covariance) have only infrequently been successfully applied to bioaerosol measurement. Instrument development work is therefore needed to improve techniques to enable direct flux measurements over various land and water surfaces. Related to this is the need to measure bioaerosol concentrations and properties in the vertical dimension, rather than only at single altitudes. Detailed measurement of these properties will provide improved inputs to dispersion models of various kinds (e.g., from allergen forecasting to global transport and radiative climate effects). Continued and increased communication between modeling and measurement communities is encouraged so that instrument development and monitoring efforts are focused on the collection of datasets that can most efficiently reduce model uncertainties.

3.8. Need for understanding the effects of atmospheric aging on PBAP

In the ambient atmosphere, PBAPs experience photochemical aging/oxidation as well as coating by low-volatile organic and inorganic compounds (e.g., Franze et al. 2005; Huffman et al. 2012; Santarpia et al. 2013). Residence time in the ambient atmosphere can also expose aerosols to UV flux, desiccation, and multiple cycles of freeze-thaw or water activation evaporation. These effects impact microorganism viability and may influence detection properties across a variety of techniques. The community would thus benefit from improvements in the ability to directly measure aerosol viability in order to improve understanding with respect to effects on public health, agriculture, and ecology. Laboratory experiments also need to consider aging processes to verify that biological particles can be recognized after a realistic residence time in the atmosphere.

In summary, real-time sensing of bioaerosol has progressed considerably in recent years, with a broad range of techniques becoming established or under development. The challenges outlined above, however, highlight that that RT techniques are still far from being applied as standardized, universal detection schemes. In particular, techniques vary significantly in the bioaerosol types and properties that can be detected and to what level of taxonomic specificity (Table 2). RT techniques can offer significant advantages in terms of fast response, sensitivity, and lack of sampler perturbation. These factors are advantageous for many different communities with diverse motivations for measuring various types of bioaerosols.

Further development and refinement of RT techniques will continue to open new opportunities in both routine monitoring and research.

Nomenclature

ALABAMA	aircraft-based laser ablation aerosol mass spectrometer
AMS	aerosol mass spectrometer
ARL	Army Research Laboratory
APDS	Autonomous Pathogen Detection System
ATOFMS	aerosol time-of-flight mass spectrometer
ATP	adenosine triphosphate measurement
BAWS	Biological-Agent Warning Sensor
CMOS	complementary metal-oxide semiconductor
DMA	differential mobility analyzer
DMT	Droplet Measurement Technologies
EMBD	Enhanced Maritime Biological Detection
FLAPS	fluorescence aerodynamic particle sizer
HAC	hierarchical agglomerative clustering
INA	ice nucleation active
JBPDS	Joint Biological Point Detection System
LAAPTOF	laser ablation aerosol particle time-of-flight mass spectrometer
LED	light-emitting diode
LIBS	laser-induced breakdown spectroscopy
LIDAR	light detection and ranging
LIF	laser- (or light)-induced fluorescence
LL	MIT Lincoln Labs
MIT	Massachusetts Institute of Technology
MS	mass spectrometry
NRL	Naval Research Laboratory
MBS	multi-parameter bioaerosol sensor
NADH	nicotinamide adenine dinucleotide
NIST	National Institute of Standards and Technology
PALMS	particle analysis by laser mass spectrometry
PCR	polymerase chain reaction
PBAP	primary biological aerosol particle
PMF	positive matrix factorization
PMT	photomultiplier tube
PSL	polystyrene latex sphere
RAAD	Rapid Agent Aerosol Detector
REBS	resource effective bioidentification system or rapid enumerative bioidentification system
RS	Raman spectra
RT	real-time
RUV	three-letter UV-APS terminal code to set the UV pulse detector voltage
SERS	surface-enhanced Raman spectroscopy
SIBS	spectral intensity bioaerosol spectrometer
SIBS _b	spark-induced breakdown spectroscopy
SNR	signal-to-noise ratio
SPLAT	single-particle laser ablation time-of-flight mass spectrometer
SPAMS	single-particle aerosol mass spectrometry
SPMS	single-particle mass spectrometry
SVP	three-letter UV-APS terminal code to set the PMT detector gain
UH	University of Hertfordshire
UV-APS	ultraviolet aerodynamic particle sizer

WIBS wideband integrated bioaerosol spectrometer
YAG yttrium aluminum garnet

Acknowledgments

The authors acknowledge organizers of this special issue, including Shanna Ratnesar-Shumate and Alex Huffman, as well as the AAAR Bioaerosol Working Group and the Bioaerosol Standardization Workshop at the International Aerosol Conference in St Louis, Missouri, in September 2018.

Disclosure statement

The authors declare no conflicts of interest.

Funding

JAH acknowledges support by University of Denver College of Natural Sciences and Mathematics and the Max Planck Institute for Chemistry. Open access article processing charges were paid by the Max Planck Digital Library (MPDL).

ORCID

J. Alex Huffman  <http://orcid.org/0000-0002-5363-9516>

Anne E. Perring  <http://orcid.org/0000-0003-2231-7503>

Nicole J. Savage  <http://orcid.org/0000-0001-7869-6472>

Bernard Clot  <http://orcid.org/0000-0003-3935-6509>

Benoît Crouzy  <http://orcid.org/0000-0002-9911-415X>

Fiona Tummon  <http://orcid.org/0000-0002-6459-339X>

Johannes Schneider  <http://orcid.org/0000-0001-7169-3973>

Vasanthi Sivaprakasam  <http://orcid.org/0000-0002-1633-8108>

Ian Crawford  <http://orcid.org/0000-0003-4433-7310>

Martin Gallagher  <http://orcid.org/0000-0002-4968-6088>

David Topping  <http://orcid.org/0000-0001-8247-9649>

David C. Doughty  <http://orcid.org/0000-0001-7336-636X>

Steven C. Hill  <http://orcid.org/0000-0002-7217-1795>

Yongle Pan  <http://orcid.org/0000-0003-0220-2725>

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