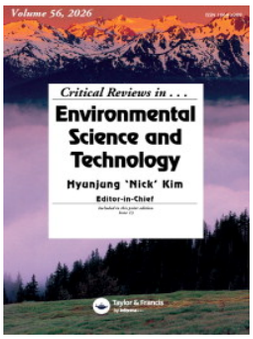

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A critical review of luminescent strategies for bioaerosol analysis: Bridging energy-driven mechanisms to analytical performance for environmental surveillance

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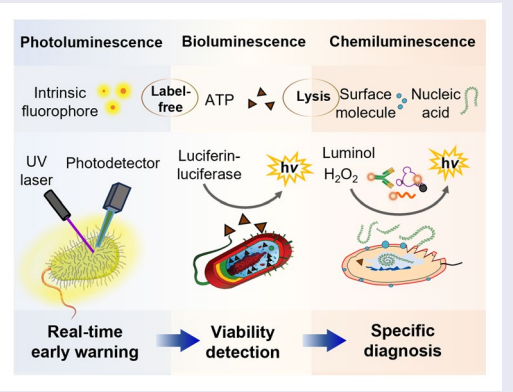
ABSTRACT

Bioaerosols, as dynamic and biologically active components of the atmosphere, impose stringent demands on detection technologies. This review presents an energy-driven framework for understanding luminescent strategies in bioaerosol analysis, highlighting the fundamental tradeoffs inherent in different energy transduction pathways that have been overlooked in recent advances. Here, luminescent detection strategies are systematically categorized into optical excitation-driven photoluminescence, biochemical energy-driven bioluminescence, and chemical reaction-driven chemiluminescence. By dissecting how excitation energy is transferred and converted into measurable signals, this review establishes direct links between underlying luminescence mechanisms and key analytical performance metrics, including sensitivity, specificity, temporal resolution, and operational robustness. It reveals that optimizing individual luminescent techniques in isolation is insufficient to meet the inherently multidimensional requirements of bioaerosol surveillance. Consequently, we propose that future breakthroughs rely on the rational integration of complementary luminescence mechanisms into multimodal sensing architectures, achieving unified rapid screening, viability assessment, and molecular confirmation. Key directions include the development of integrated multimodal platforms, advanced and robust luminescent materials, and the incorporation of microfluidics and automation to enable continuous, field-deployable monitoring. This review provides guidance for the selection, optimization, and future development of bioaerosol monitoring technologies, while also offering insights for applications such as early warning and sensitive screening during infectious disease outbreaks.



KEYWORDS analytical approaches; bioaerosols; bioluminescence; chemiluminescence; luminescent detection; photoluminescence

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1. Introduction

Bioaerosols, comprising airborne particles of biological origin including viable and non-viable microorganisms, bio-fragments, and metabolic products, constitute a ubiquitous component of the airborne atmosphere and play a critical factor in environmental quality and public health (Fröhlich-Nowoisky et al., 2016; Jin et al., 2022). These suspended biological particles have been proved to influence climate processes by acting as cloud condensation and ice nuclei (Wolf et al., 2020), while also serving as carriers of infectious agents, allergens, and toxins that threaten human health, agriculture, and ecosystem stability (Fennelly, 2020; Iqbal et al., 2024; Liu et al., 2020). The rapid global transmission of airborne pathogens, exemplified by the SARS-CoV-2 pandemic, has further highlighted the vulnerability of existing surveillance systems to respiratory disease outbreaks (Peng et al., 2022). Unlike chemical pollutants, bioaerosols exhibit biological activity, infectivity, and adaptability even at extremely low concentrations (Jaumdally et al., 2024), placing stringent demands on monitoring technologies in terms of rapid response, high sensitivity, and reliable discrimination under complex atmospheric backgrounds.

Conventional bioaerosol analytical approaches remain insufficient to meet these demands. Culture-dependent methods, although considered the gold standard for viability assessment, have been experimentally validated to be severely hindered by prolonged incubation times and an inability to capture viable but non-culturable microorganisms (Ghosh et al., 2015; Wang et al., 2026). Microscopy-based methods are useful for morphological characterization, but they lack sensitivity for low-abundance or submicron particles (An et al., 2024; Huang et al., 2024). Molecular assays targeting specific nucleic acids have been developed, demonstrating exceptionally high analytical sensitivity and taxonomic specificity in controlled experiments (Zhang et al., 2022). However, it indicates that traditional nucleic acid amplification methods are often unsuitable for on-site and real-time environmental monitoring due to their heavy reliance on complex sample preprocessing and continuous thermal cycling (Lee et al., 2020). These limitations hamper rapid decision-making in environmental surveillance and risk assessment, which is a particularly serious problem in scenarios such as pathogen outbreaks.

To address the urgent need for rapid and sensitive bioaerosol monitoring, increasing attention has been directed toward optical sensing technologies (Du et al., 2023; Gutiérrez-Gálvez et al., 2022; Yue et al., 2022). Among these, luminescent strategies have emerged as promising solutions for bioaerosol sensing due to their ability to transduce biological recognition events into measurable optical signals with high sensitivity (Dias Neves & Mendes Pinto, 2024; Ko & Hwang, 2026). Representative examples include laser-induced fluorescence for long-term, non-contact monitoring of atmospheric bioaerosol concentrations (Petersson Sjögren et al., 2023), ATP bioluminescence assays for rapid quantification of viable airborne microorganisms (Liu et al., 2024), and immuno-based luminescent assays for pathogen-specific detection (Kim et al., 2025). These advances demonstrate that luminescent techniques can support diverse monitoring objectives, ranging from early warning to targeted identification. However, their analytical performance varies widely, reflecting fundamental differences in how excitation energy is generated, transferred, and converted into light signal. Although an increasing number of studies have summarized techniques for bioaerosol detection, most existing works predominantly focus on individual techniques, specific target organisms, or application demonstrations (Huffman et al., 2020; Wang et al., 2021; Yao, 2022). A systematic framework remains largely absent, which bridges the fundamental energy-driven mechanisms of different luminescent strategies to their intrinsic analytical performance limits. This lack of mechanistic integration hinders rational comparison and technology selection, particularly for environmental applications where heterogeneous aerosol matrices, strong background interference, and temporal variability impose strict constraints on detection reliability and deployability.

In this review, this gap was addressed by critically evaluating luminescent bioaerosol monitoring strategies through an energy-driven perspective. Luminescent strategies are classified into three fundamental categories, including optical excitation-driven photoluminescence, biochemical

energy-driven bioluminescence, and chemical reaction-driven chemiluminescence. The coupling of different biorecognition elements, ranging from intrinsic fluorophores to specific surface molecules or nucleic acid sequences, with these energy-driven pathways was first examined. Excitation mechanisms, energy conversion processes, and signal generation modes were then systematically linked to key analytical performance metrics relevant to environmental surveillance, thereby elucidating why different luminescent strategies exhibit distinct strengths and limitations. Finally, future perspectives are discussed with an emphasis on multimodal luminescent integration from triggering to confirmation, advanced luminescent materials, and microfluidic-based portable platforms. By bridging fundamental energy mechanisms with practical analytical performance, this review mainly provides a mechanistic guidance to the development of integrated and application-oriented bioaerosol monitoring systems tailored to environmental pollution and public health surveillance.

2. Coupling biological recognition elements with luminescent strategies

Biological recognition defines the fundamental interaction pathways between bioaerosol constituents and sensing elements, thereby establishing the molecular and physicochemical basis upon which luminescent signals are generated. The nature of these interactions directly influences how excitation energy is introduced, converted, or amplified in subsequent luminescent detection strategies. Regardless of whether the downstream signal is driven by optical excitation, intracellular biochemical energy, or exothermic chemical reactions, the choice of recognition element acts as the control switch for energy coupling. Consequently, biological recognition critically constrains analytical performance limits, particularly in terms of response kinetics, specificity, and field deployability (Puthussery et al., 2023). Generally, these biorecognitions can be broadly classified into three categories based on their energy-coupling characteristics, including recognition of intrinsic luminescent components, surface-specific molecular recognition, and nucleic acid-based recognition.

2.1. Intrinsic luminescent component recognition: Direct energy transduction

Recognition strategies based on intrinsic luminescent components represent the most direct coupling between biological identity and luminescent output. Endogenous biomolecules inherently present in bioaerosols serve simultaneously as recognition elements and signal transducers, eliminating the need for exogenous labels and minimizing kinetic barriers to signal generation. This direct energy transduction maximizes temporal resolution, making intrinsic recognition particularly suited for real-time early warning applications.

Bioaerosols contain abundant endogenous fluorophores, including aromatic amino acids and metabolic coenzyme factors, which passively absorb high-energy photons and instantaneously re-emit lower-energy fluorescence through electronic relaxation processes (Gratzl et al., 2025) (Figure 1a). The nanosecond-scale lifetime of this absorption-emission cycle enables continuous monitoring of single airborne particles without reagent consumption. However, reliance on external excitation inevitably introduces elastic scattering and non-biological fluorescence background, imposing a fundamental physical limitation on signal discrimination. In contrast, adenosine triphosphate (ATP) recognition represents an active biochemical energy-coupling mechanism. As the universal intracellular energy carrier of living organisms, ATP serves as the chemical fuel that drives the luciferin-luciferase reaction to release yellow-green photons without external excitation (Liu et al., 2024) (Figure 1b). This dark-field emission intrinsically links luminescent output to viable biomass. Nevertheless, the enzymatic nature of ATP-driven luminescence introduces vulnerabilities, including reagent instability and sensitivity to environmental

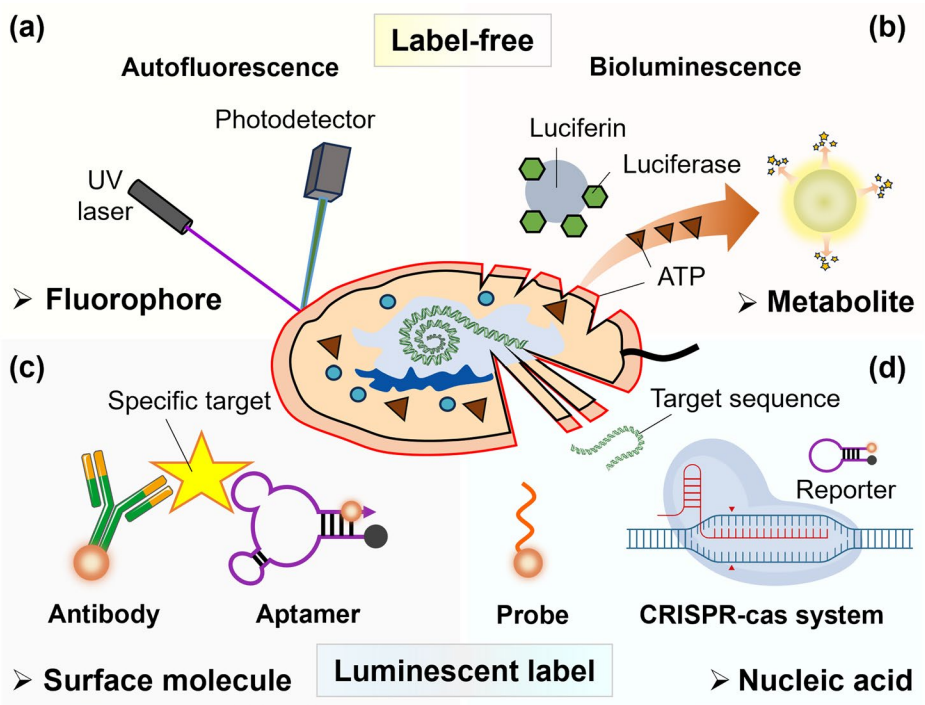

Figure 1. Biological recognition elements in luminescent analysis. (a) Autofluorescence detection based on endogenous fluorophore recognition. (b) Bioluminescence detection based on intracellular metabolite recognition. (c) Surface-specific molecular recognition. (d) Nucleic acid-based recognition.

stress, which complicate long-term field deployment when compared with the robustness of intrinsic photophysical processes.

2.2. Surface-specific molecular recognition: Affinity-mediated energy conversion

Surface-specific molecular recognition exploits high-affinity interactions between exogenous probes and specific microbial surface structures, such as membrane proteins, lipopolysaccharides, and polysaccharide antigens (Li et al., 2024). This recognition mode preserves the structural integrity of whole cells or viral particles and serves as a versatile interface for converting molecular binding events into luminescent signals through either photoluminescent or chemiluminescent pathways. Typical examples are immuno-recognition and aptamer-based recognition (Figure 1c).

In practice, antibodies or aptamers are conjugated with luminescent reporters, including organic fluorophores, quantum dots, or enzyme labels such as horseradish peroxidase (HRP) and alkaline phosphatase (AP), forming the basis of fluorescence and chemiluminescence immunoassays (Carpenter & Bartkowiak, 1989; Morimoto et al., 2017). Although antibodies have long been considered the gold standard for immuno-recognition, their structural stability is sensitive to temperature, pH, and oxidative or alkaline microenvironments (Gao et al., 2026). These conditions are frequently encountered in chemiluminescence reactions, where reactive intermediates can compromise antibody integrity and disrupt signal transduction. Aptamers, synthetic single-stranded oligonucleotides, provide a chemically robust alternative. They can be engineered to undergo target-induced conformational changes (Yoshikawa et al., 2023) and can recognize a broad spectrum of analytes, including proteins, toxins, whole cells, and viral particles that are challenging targets for antibodies (Li et al., 2015; Zhou & Rossi, 2017). Importantly, aptamers exhibit high thermal and chemical stability, retaining functionality after drying or thermal stress (Ellington & Szostak, 1990). Their resilience under harsh reaction conditions ensures faithful

coupling between recognition and light generation, making aptamers particularly attractive for field-deployable chemiluminescent biosensors (Weng et al., 2024).

2.3. Nucleic acid-based recognition: Enzyme-powered signal amplification

Nucleic acid-based recognition targets unique genetic sequences to achieve species- or strain-level discrimination. Unlike intrinsic luminescent component or surface recognition, this strategy typically requires substantial external energy input to overcome kinetic barriers associated with target accessibility and signal amplification. Consequently, nucleic acid recognition is almost invariably coupled with enzymatic amplification mechanisms in luminescent detection schemes to achieve trace-level detection (Figure 1d).

Conventional approaches rely on polymerase chain reaction (PCR) to amplify target sequences, converting molecular recognition into detectable fluorescence signals (Tyagi & Kramer, 1996). Although this strategy offers unmatched specificity, the need for cell lysis, thermal cycling, and multistep processing extends response times to minutes or hours. More recently, clustered regularly interspaced short palindromic repeats and associated proteins (CRISPR-Cas) systems (e.g., Cas12/Cas13) have introduced an alternative amplification pattern, which utilize the chemical energy of enzymatic collateral cleavage to trigger signal amplification (Fozouni et al., 2021). The Cas enzyme is activated to perform indiscriminate *trans*-cleavage of surrounding reporter probes upon binding to a target sequence, effectively converting a single recognition event into thousands of cleavage reactions that release fluorophores or trigger chemiluminescent substrates (Yan et al., 2025). Experimental applications of the CRISPR system have achieved ultra-high sensitivity detection of airborne pathogens at the attomolar level, which overcomes the inherent sensitivity limitations associated with traditional single-fluorescence readouts (Su et al., 2025). However, the dependence on multi-step biochemical energy transduction, including sequence recognition, enzymatic amplification, and luminescent signal emission, introduces complexity in fluidic handling and automation. Therefore, nucleic acid recognition remains a tradeoff between exquisite specificity and system simplicity in bioaerosol monitoring.

3. Optical excitation-driven photoluminescence strategies for bioaerosol analysis

3.1. Intrinsic fluorescence sensing: Real-time screening with limited specificity

Intrinsic fluorescence sensing utilizes external optical energy to excite endogenous biological fluorophores, including aromatic amino acids such as tryptophan and tyrosine, coenzymes like nicotinamide adenine dinucleotide (NADH/NADPH), and flavin-containing molecules such as riboflavin (Fontal et al., 2025). This photon-driven mechanism confers an intrinsic kinetic advantage, where fluorescence generation occurs almost instantaneously after excitation (Song et al., 2025), rendering intrinsic photoluminescence the only luminescent strategy capable of real-time detection of airborne particles. These endogenous fluorescent substances exhibit unique excitation-emission characteristics, in which tryptophan typically emits light at approximately 340 nm when excited at 280 nm, NADH emits light at about 460 nm when excited at 340 nm, and riboflavin-related compounds emit light near 520 nm when excited by blue light (Forde et al., 2019) (Figure 2a). The relative abundance and emission characteristics of these fluorescent substances vary across different types of bacteria, fungi, and pollen, constituting the intrinsic fluorescence fingerprint of microorganisms and forming the basis for fluorescence-based bioaerosol classification. For instance, bacterial cells typically display dual emission peaks around 340 and 450 nm corresponding to tryptophan and flavins, respectively (Yue et al., 2022), while fungal spores often exhibit broader emissions in the 420–550 nm region due to the abundance of NADH and riboflavin (Forde et al., 2019). The relative intensity ratios of these spectral bands can therefore be used to differentiate bacterial, fungal, and pollen particles under UV excitation.

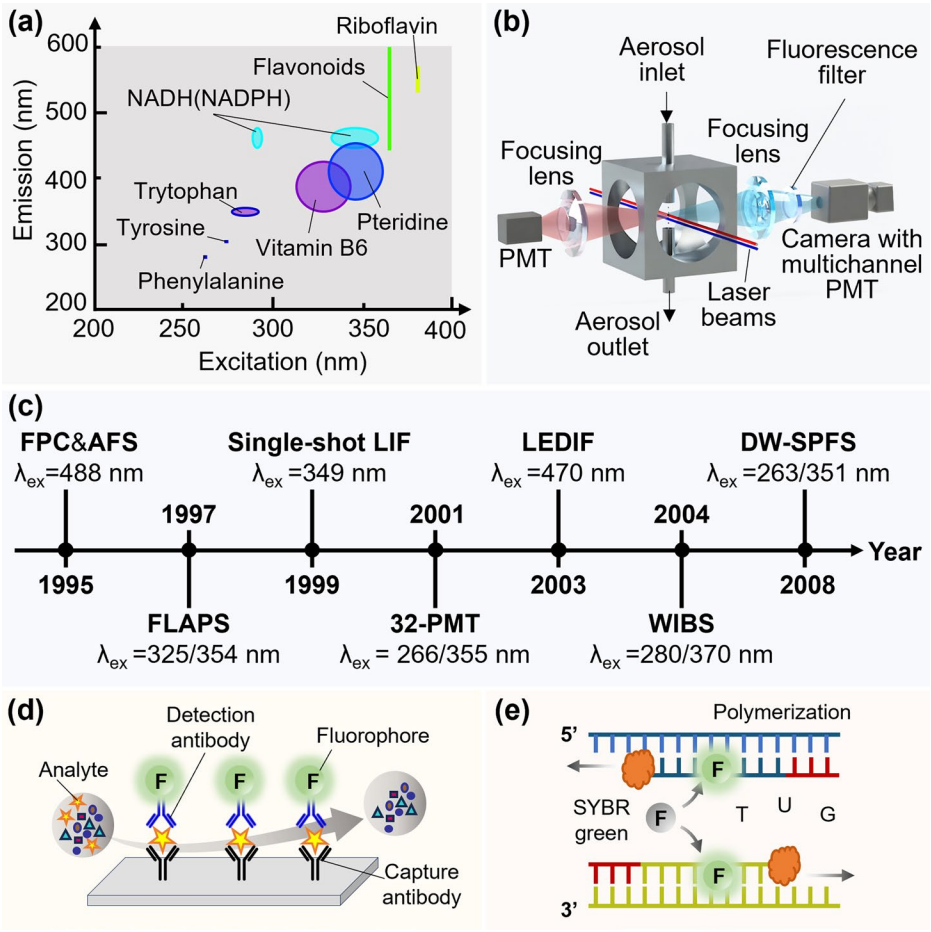

Figure 2. Optical excitation-driven photoluminescence strategies for bioaerosol analysis. (a) Fluorescence emission-excitation matrices of intrinsic biofluorophores in bioaerosol. (b) LIF detection principle for bioaerosol analysis. Reprinted with permission from (Kwaśny et al., 2023). Copyright (2023) MDPI. (c) Historical perspective of the development of LIF-based bioaerosol detection technology. (d) Schematic diagram of fluorescence immunoassay. (e) Schematic illustration of fluorescence-based amplification.

LIF is capable of analyzing single particles in ambient air without sample collection (Saari et al., 2013). The detection principle involves accelerating bioaerosols through a designed micro-nozzle, guiding them along a specific path and radiation focus point. The suspended particles are interrogated by a pulsed or continuous-wave laser beam, and their particle size can be estimated by the measurement of their flight time along this path, while particle fluorescence is measured through a photomultiplier tube or spectrometer equipped with optical filters (Figure 2b) (Kwaśny et al., 2023). LIF instruments have undergone over two decades of iterative evolution (Figure 2c). The foundational development of bioaerosol triggers and their underlying science is largely catalyzed in the early to mid-1990s through the pivotal collaboration between the U.S. Army Research Laboratory (ARL) and Yale University (Richard, 2009). This partnership establishes the earliest milestones in real-time detection, notably introducing the first fluorescence particle counter (FPC) capable of detecting airborne bacteria (Pinnick et al., 1995) and the aerosol-fluorescence spectrum analyzer (AFS) to capture the emission spectra of individual airborne biological particles using an excitation wavelength (λ_{ex}) of 488 nm (Hill et al., 1995). Concurrently, the applied feasibility of discriminating bioaerosols from ambient interferents, such as dust or soot, is demonstrated by the first-generation Fluorescent Aerosol Particle Sensor (FLAPS I) in 1997, which integrates aerodynamic sizing with ultraviolet (UV) laser-induced

endogenous fluorescence (λ_{ex} = 325 or 354 nm) (Hairston et al., 1997), setting a crucial precedent for subsequent commercial UV-APS system (λ_{ex} = 349 nm) (Ho et al., 1999).

As the surging demand for field-deployable and highly sensitive bio-warning systems, subsequent technological breakthroughs focus primarily on source miniaturization, spectral fidelity, and multi-modal integration. A critical scientific inflection point occurs in 1999, when the Yale-ARL team utilizes crossed-diode laser triggering and UV lasers (λ_{ex} = 266 or 351 nm) to acquire the first high-signal-to-noise, reproducible single-shot fluorescence spectra of bioaerosols (Pan et al., 1999). To overcome inherent data acquisition bottlenecks, a 32-anode photomultiplier tube (PMT) is integrated in 2001, endowing these systems with single-photon sensitivity and enabling an unprecedented full-spectrum recording frame rate exceeding 1,400 Hz (Pan et al., 2001). This enhances detection capability paved the way for highly complex, multi-modal platforms. A prime example of which is the Rapid Agent Aerosol Detector (RAAD), which uniquely combines elastic light scattering, multi-wavelength fluorescence (λ_{ex} = 266 and 355 nm), and laser-induced breakdown spectroscopy (LIBS) to perform up to 14 independent measurements per particle (Jeys et al., 2007).

The development of LIF instruments subsequently shifts toward cost-effectiveness and expanded spectral dimensionality. In 2003, the Yale-ARL team demonstrates the feasibility of using compact light-emitting diode (LED) linear arrays to excite flowing micro-particles, marking a paradigm shift in excitation architecture and laying the foundation for modern miniaturized UV-LED triggers, thereby circumventing the limitations of bulky, high-power pulsed lasers (Pan et al., 2003). This evolution facilitates the widespread adoption of multi-wavelength excitation techniques, such as the Wideband Integrated Bioaerosol Sensor (WIBS) introduced in 2004, which employs dual-wavelength excitation (λ_{ex} = 280 and 370 nm) (Kaye et al., 2004). In 2008, the method is significantly improved with the development of the dual-wavelength single-particle fluorescence spectrometer (DW-SPFS) (λ_{ex} = 263 and 351 nm). By efficiently utilizing a single spectrometer and a PMT array, this instrument enables continuous, real-time spectral measurements of individual suspended particles (Huang et al., 2008). Building on this rich historical framework of optical and instrumental advancements, state-of-the-art analyzers, such as Rapid-E and the Spectral Intensity Bioaerosol Sensor (SIBS), have continued to optimize multichannel detection and real-time data processing (Könemann et al., 2019; Lieberherr et al., 2021). Supported by recent breakthroughs in high-efficiency excitation sources, ultra-sensitive detectors, and machine learning-driven spectral analysis algorithms (Leśkiewicz et al., 2018; Saito & Kawai, 2022), these foundational innovations have firmly established LIF as the preeminent technology for the real-time, *in situ* characterization and early warning of bioaerosols.

The bottleneck of LIF is not instrument sensitivity but severe signal interference. Significant spectral overlap exists not only among different microorganisms but also between biological particles and non-biological atmospheric components. Recent field studies demonstrate that non-biological atmospheric constituents, particularly polycyclic aromatic hydrocarbons, soot, and humic-like secondary organic aerosols, exhibit excitation-emission matrices that almost perfectly overlap with biological particles in the 350–500 nm range (Yue et al., 2022; Zhang et al., 2021). Although statistical and machine-learning approaches can improve probabilistic classification, they cannot overcome the physical reality that autofluorescence signatures lack molecular uniqueness. Consequently, intrinsic fluorescence excels at rapid screening and total biological particle counting but remains inadequate for reliable pathogen-level identification.

3.2. Extrinsic fluorescence labeling-based sensing: Molecular specificity enabled by the cost of speed

Extrinsic fluorescence labeling strategies enhance analytical specificity by coupling optical excitation with molecular recognition, in which exogenous fluorophores are linked to antibodies, aptamers, or nucleic acid probes. In these systems, highly specific recognition events are transduced into fluorescence signals, enabling species- or even strain-level discrimination. However, this

enhancement in specificity is achieved at the expense of temporal performance. Sample collection, reagent incubation, washing, and signal integration introduce inherent delays ranging from minutes to hours, fundamentally departing from the instantaneous nature of intrinsic photoluminescence.

Fluorescence immunoassays represent a widely adopted labeling strategy, leveraging fluorescently tagged antibodies or aptamers that selectively bind to specific molecules on microbial surfaces. In a typical sandwich configuration, capture probes immobilized on a substrate bind the target analyte, followed by the introduction of fluorescently labeled detection probes targeting a different epitope (Figure 2d). The surface is irradiated with light after removal of unbound reagents through washing steps, fluorescence intensity is quantified and correlated with target concentration (Augustine et al., 2024). This principle of fluorescence immunoassay detection has been integrated into numerous platforms, including fluorescence-based microarrays capable of multiplex detection and quantitative fluorescence-based lateral flow immunoassays (Lin et al., 2020; Parolo et al., 2013). Representative examples in the analytical application of bioaerosols include fluorescence-based lateral flow assays enabling sensitive detection of airborne viral pathogens within approximately one hour (Wang et al., 2020). Although such approaches significantly improve signal-to-noise ratios compared to autofluorescence, sensitivity can still be influenced by substrate scattering and matrix effects.

Fluorescence-based nucleic acid amplification strategies provide the highest level of analytical specificity by targeting unique genomic sequences and are widely regarded as the gold standard for pathogen identification. The analysis process begins with sample collection, followed by cell lysis to release the nucleic acids. Quantitative polymerase chain reaction (qPCR) combines exponential target amplification with real-time fluorescence monitoring (Li et al., 2020) (Figure 2e). Currently, two fluorescence strategies are widely used in qPCR, including intercalating dyes (such as SYBR Green), which can emit strong fluorescence after binding to any double-stranded DNA (Oh et al., 2023), and sequence-specific hydrolysis probes (e.g., TaqMan), which only release fluorescence signals when the target sequence is amplified (Holland et al., 1991).

To address the limitations of thermal cycling, isothermal amplification methods such as loop-mediated isothermal amplification (LAMP) have been developed, enabling faster detection under constant-temperature conditions and facilitating integration into portable devices (Notomi et al., 2000; Tomita et al., 2008). More recently, fluorescence-labeled CRISPR-based reporters have further enhanced specificity and sensitivity by coupling target recognition with collateral cleavage-mediated signal amplification, enabling rapid detection of airborne pathogens at ultra-low concentrations (Yan et al., 2025). Although these approaches offer unparalleled molecular precision, their reliance on multistep processing hampers continuous, real-time monitoring.

4. Biochemical energy-driven bioluminescence strategies for bioaerosol analysis

4.1. ATP bioluminescence assays: the direct metrics for viable biomass

ATP bioluminescence extracts biochemical energy directly from the sample, yielding an effectively zero-background optical signal that correlates directly with viable biomass. The foundational reaction for emitting light at approximately 560 – 580 nm, involving the oxidation of D-luciferin catalyzed by luciferase in the presence of ATP, O_2, and Mg^{2+}, is well established (Figure 3a) (Kim et al., 2019). This unique energy conversion pathway offers exceptional background suppression capabilities and biological relevance, making ATP bioluminescence the definitive indicator of viable biomass in airborne microbial monitoring. However, the primary challenge of ATP bioluminescence for bioaerosol analysis lies not in the enzymatic reaction itself, but in the preservation of the intracellular ATP during the sampling and detection process.

4.2. Sample processing of airborne microbes: Preserving biochemical energy

The analytical reliability of ATP bioluminescence critically depends on the preservation and efficient release of intracellular biochemical energy throughout bioaerosol sampling and sample

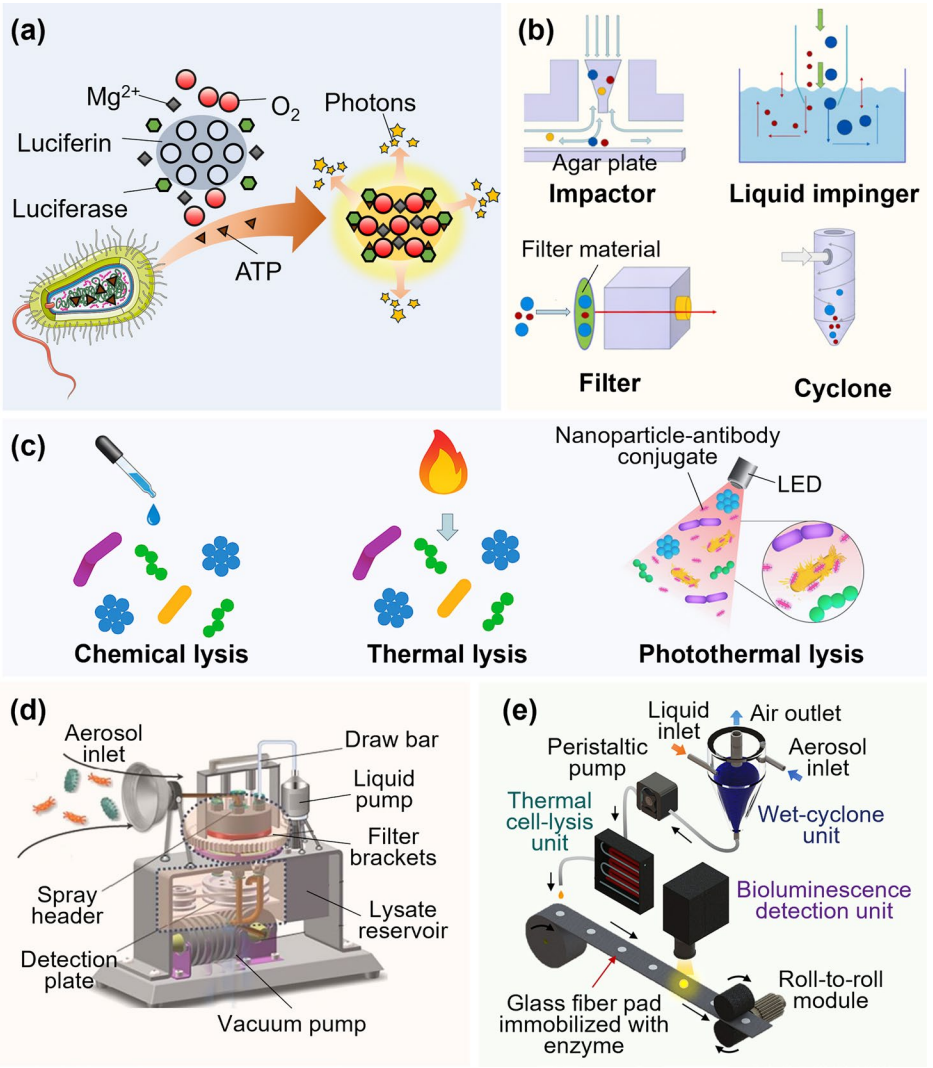

Figure 3. ATP bioluminescence detection for bioaerosol analysis. (a) The reaction mechanism of ATP-based bioluminescence assay. (b) Schematic illustration of the impactor, liquid impinger, filter, and cyclone sampler. Reprinted with permission from (Liu et al., 2024). Copyright (2024) Elsevier. (c) Schematic illustration of cell lysis by chemical, thermal, and photothermal approaches. Reprinted with permission from (Kim et al., 2018). Copyright (2018) American chemical society. (d) The schematic diagram of online bioaerosol monitoring system based on ATP bioluminescence. Reprinted with permission from (Chen et al., 2024). Copyright (2024) Elsevier. (e) Schematic diagram of the automated bioaerosol-monitoring system based on ATP bioluminescence. Reprinted with permission from (Cho et al., 2020). Copyright (2020) American chemical society.

preparation. Common bioaerosol collectors, including impactors, filters, liquid impingers, and cyclones, offer different tradeoffs between collection efficiency, mechanical stress, and compatibility with downstream enzymatic assays (Liu et al., 2024) (Figure 3b). Impactor deposits particles onto agar plate, which is suitable for subsequent surface swabbing or on-substrate ATP extraction. Filter sampler traps particles on membrane or fibrous substrates, which is simple and robust but require efficient elution or swabbing to recover ATP prior to assay. Liquid-based samplers, such as liquid impingers and wet cyclones, provide direct compatibility with luciferase reagents, enabling rapid transition from collection to analysis. However, the high-velocity impingement required to capture micron-sized particles can impose substantial shear stress, potentially damaging cells or perturbing intracellular ATP levels (Han et al., 2015). Such stress-induced ATP fluctuations may decouple luminescence intensity from true airborne biomass.

Cell lysis is a prerequisite for liberating ATP and thus represents a critical control point in biochemical energy-driven detection. Three dominant cell lysis modalities have been implemented in ATP-based bioaerosol analysis systems, including chemical lysis using detergents, degreasers, or enzyme mixtures, thermal lysis achieved by rapidly heating to disrupt cell membranes, and photothermal lysis utilizing localized heating mediated by plasma or nanoparticles (Figure 3c). Chemical lysis using detergents or enzyme cocktails offers operational simplicity but requires strict compatibility with luciferase activity (Liu et al., 2024). Thermal lysis, typically implemented *via* resistive heating, avoids chemical inhibitors and is widely adopted in automated monitoring systems, enabling ATP extraction within minutes (Cho et al., 2020). Emerging photothermal lysis strategies employ near-infrared irradiation of plasmonic or carbon materials to induce localized heating and membrane rupture (Kim et al., 2018). This non-contact approach enables rapid and efficient ATP release, particularly suited for miniaturized and portable platforms. Notably, any failure to fully rupture the cell membrane results in a false negative, regardless of the luciferase sensitivity.

4.3. The application of ATP bioluminescence in bioaerosol real-time analysis

Several automated systems have been developed to integrate modules of bioaerosol sampling, cell lysis to release ATP, and enzymatic luminescence measurement into near real-time monitoring platforms, where the detection limits can reach 100 – 1000 CFU/m^3. For instance, Chen et al. reported an online bioaerosol monitoring system comprising a rotatable filter sampler, a chemical pyrolysis unit, and a fluorescence unit based on a single-photon detector, achieving detection limits of 2.9×10^3 CFU/m^3 for *Staphylococcus aureus* aerosols and 292 CFU/m^3 for *Candida albicans* aerosols within an 8-min detection time (Figure 3d) (Chen et al., 2024). Similarly, Cho et al. developed an on-site automated bioaerosol-monitoring system integrating a wet-cyclone sampler, a thermal lysis unit, an ATP detection unit featuring a glass fiber pad coated with luciferin/luciferase for bioluminescence reactions, and a detector equipped with a photomultiplier tube, enabling continuous detection of *E. coli* bioaerosol with a minimum detection limit of approximately 130 CFU/m^3 at 5 min intervals (Figure 3e) (Cho et al., 2020). These efforts underscore that ATP bioluminescence offers a rapid, sensitive metric of viable biological content in aerosols, and has been deployed in portable and continuous monitoring systems.

Nevertheless, the thermodynamic instability of luciferase remains a fundamental constraint of biochemical energy-driven luminescent strategies. As a protein enzyme, luciferase is highly sensitive to temperature, humidity, and chemical environment, leading to rapid activity loss under typical outdoor monitoring conditions. This intrinsic fragility limits maintenance cycles and long-term deployment compared to purely physical photoluminescence systems. Recent developments in microfluidic automation (Park et al., 2016) and enzyme-stabilized reagent formulations (Martínez-Pérez-Cejuela et al., 2023) partially mitigate these challenges, expanding the feasibility of ATP bioluminescence for field-based screening of viable bioaerosol loads.

5. Chemical reaction-driven chemiluminescence strategies for bioaerosol analysis

Chemiluminescence (CL) represents the most sensitive luminescent strategy for bioaerosol analysis by directly converting chemical potential energy into optical signals. Unlike photoluminescence or bioluminescence, chemiluminescence does not require external photon excitation or intracellular metabolic activity. Instead, light emission originates exclusively from exothermic chemical reactions, which theoretically eliminates optical background and enables an effectively infinite signal-to-noise ratio (Ke et al., 2022; Klüpfel et al., 2022). This unique thermodynamic advantage makes chemiluminescence particularly powerful for quantifying extremely dilute bioaerosol-derived biomarkers in complex environmental matrices.

5.1. Generation of chemiluminescence for trace-level detection

The generation of chemiluminescent signals relies on highly efficient chemiexcitation pathways that populate electronically excited intermediates. Several classical chemiluminescent systems form the foundation of CL-based biosensing (Figure 4a). The luminol-HRP system is among the most widely employed platforms, in which luminol is oxidized by hydrogen peroxide to generate excited-state 3-aminophthalate that emits blue light upon relaxation (Joung et al., 2014). HRP acts as a catalyst, markedly enhancing reaction efficiency and photon yield, making this system particularly suitable for enzyme-linked immunoassays. Acridinium ester systems constitute a second major class of chemiluminescent labels. These compounds undergo rapid oxidative cleavage in alkaline hydrogen peroxide, generating intense flash-type emission without requiring enzymatic catalysis (Zhao et al., 2021). Their high chemical stability and strong luminescent output enable low-background and high-throughput detection. In contrast, dioxetane-based substrates such as 3-[2-spiroadamatane]-4-methoxy-4-[3-phosphoryloxy]-phenyl-1,2-dioxetane (AMPPD) generate long-lasting emission following enzymatic deprotection by AP and subsequent thermal decomposition of unstable intermediates (Haris et al., 2021), offering extended signal integration windows.

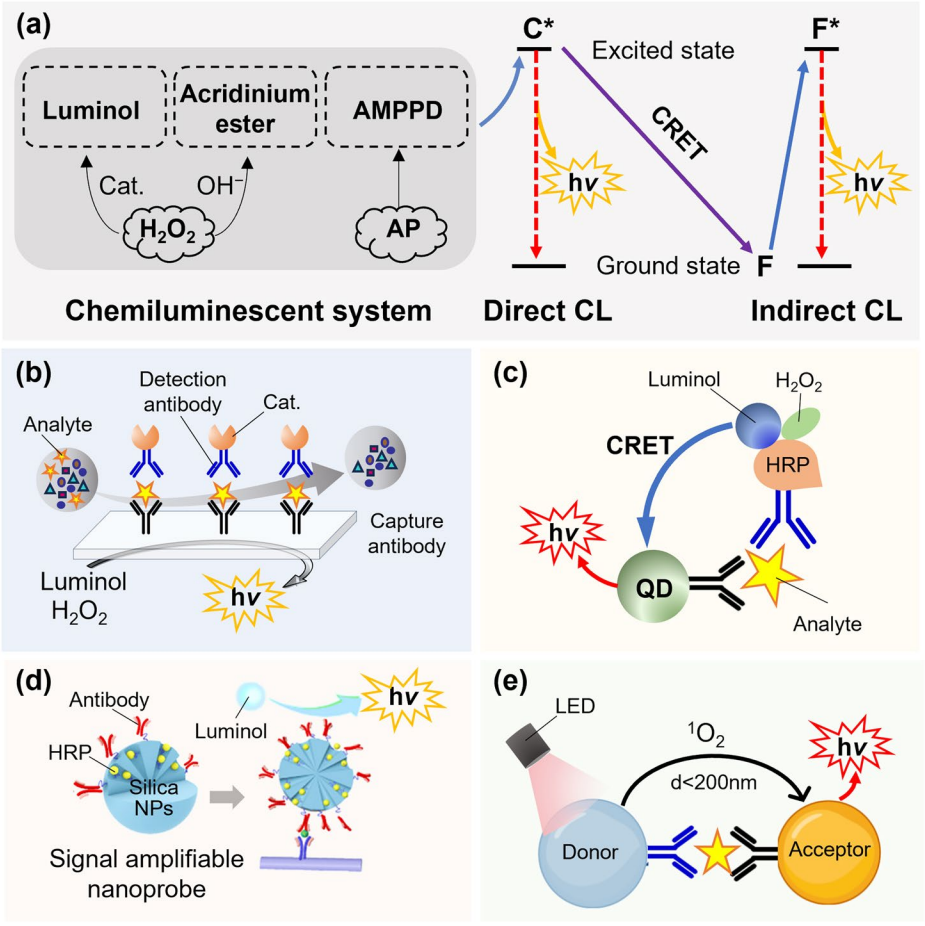

Figure 4. Chemical-driven luminescence strategies for bioaerosol analysis. (a) The common chemiluminescence systems and modes. (b) Schematic diagram of chemiluminescent immunoassays based on direct production mode. (c) Schematic diagram of chemiluminescent immunoassays based on chemiluminescence resonance energy transfer process. (d) Signal-amplifiable biosensing platform based on size-selective biomolecule immobilization strategy. Reprinted with permission from (Jung et al., 2021). Copyright (2021) American chemical society. (e) Schematic illustration of LICA biosensing strategy.

Functionally, chemiluminescence can operate in either direct or indirect modes (Figure 4a). In direct CL, the reaction products themselves emit photons. In indirect CL, chemical energy is non-radiatively transferred to secondary fluorophores through chemiluminescent resonance energy transfer (CRET), producing wavelength-shifted and photostable emission (Huang et al., 2006). However, because chemiluminescent signals are intrinsically transient and governed by reaction kinetics, precise synchronization between reagent mixing and photon detection is required, increasing instrumental and operational complexity.

5.2. Coupling biomolecular recognition with chemiluminescence

The analytical specificity of chemiluminescence-based bioaerosol detection arises from the coupling of molecular recognition events with controlled chemical energy release. Chemiluminescent immunoassays (CLIA) represent the most established implementation of direct CL. In classical enzyme-linked immunosorbent assays (ELISA), experimental designs frequently employ a dual-antibody sandwich structure, where target analytes are firmly captured by immobilized antibodies and subsequently recognized by enzyme-labeled detection antibodies to trigger precise light emission (He et al., 2024) (Figure 4b). The exceptionally wide dynamic range and sensitivity of CLIA have made it indispensable in clinical diagnostics and environmental monitoring, albeit at the cost of multiple washing steps and prolonged assay times.

Indirect CL strategies based on CRET provide greater flexibility in signal transduction. In CRET systems, chemiluminescent donors transfer energy to proximal fluorescent acceptors, with quantum dots emerging as superior acceptors due to their high quantum yield, tunable emission wavelengths, and strong quenching efficiency (Xu et al., 2016). Biological recognition events, such as nucleic acid hybridization or immuno-binding, are engineered to modulate the distance between chemiluminescent donors and quantum dot acceptors (Huang et al., 2006), thereby controlling energy transfer efficiency (Figure 4c). This approach enables substantial signal amplification and multiplexed detection by employing quantum dots with distinct emission colors.

5.3. Strategies for signal amplification and sensitivity enhancement

Chemiluminescence sensitivity can be further enhanced by amplifying chemical energy turnover. The emergence of nanozymes, nanomaterials with enzyme-like catalytic activity, has significantly advanced CL signal amplification (Xie et al., 2021). It has demonstrated that CeO_2 nanocomposites can efficiently drive cascade luminescent reactions, achieving exceptionally high sensitivity for the detection of *Escherichia coli* (Hu et al., 2025). Studies have explored how Au nanoparticles actively participate in and drive luminol-based chemiluminescence, resulting in a massive enhancement of the optical signal (Wang et al., 2023). To maximize these catalytic effects, researchers have engineered nanoprobes with specific porous structures (Figure 4d), in which the engineered microenvironments and extremely high specific surface areas maximize enzyme immobilization and catalytic efficiency, ultimately enabling the ultrasensitive detection of trace pathogens (Jung et al., 2021; Li et al., 2025). Magnetic nanozymes additionally facilitate rapid enrichment and separation of bioaerosol-derived targets, improving on-site operability (Qu et al., 2022).

Light-initiated chemiluminescent assays (LICA) represent a transformative advance that addresses kinetic and operational limitations of traditional CL (Huang et al., 2025). LICA employs light to excite photosensitizers on donor beads to generate singlet oxygen or other reactive oxygen species, which subsequently trigger chemiluminescent reaction in spatially confined acceptor beads (Figure 4e). Although light is used as a trigger, the emitted photons originate from chemical potential energy release, resulting in a unique anti-Stokes shift and preserving the infinite signal-to-noise advantage of chemiluminescence (Guo et al., 2020; Ullman et al., 1994). This mechanism allows for a strict temporal separation between the excitation light pulse and the signal measurement. The short diffusion distance of reactive species suppresses

background from unbound reagents, enabling wash-free operation and greatly simplifying automated workflows (Boso et al., 2016).

Although chemiluminescence offers superior sensitivity, conventional chemiluminescence reaction mechanisms rely heavily on continuous mass transfer and the dynamic interactions among multiple components (Cheng et al., 2025). This reliance intrinsically restricts the maximum achievable emission intensity and physically hinders the potential for continuous, long-lasting light emission. Recent innovations, including wash-free LICA formats (Zhang et al., 2026), chip-integrated sampling and CL modules (Wang et al., 2021; Xiong et al., 2021), and reagent stabilization strategies (Hao et al., 2024), are progressively transforming chemiluminescence from a laboratory-based technique into a viable component of autonomous, high-sensitivity bioaerosol monitoring systems for pathogen surveillance and emergency diagnostics.

6. Comparative assessment of energy-driven luminescent strategies for bioaerosol monitoring

6.1. Application-oriented performance benchmarking

To visualize these intrinsic tradeoffs, the three luminescent strategies were comparatively benchmarked across four critical performance dimensions, including sensitivity and limit of detection, specificity and discrimination capability, response time and temporal resolution, and instrumentation complexity and field deployability (Figure 5a). To facilitate a clear and standardized comparison, the performance within each dimension is evaluated using a three-tier qualitative scale, where "high" indicates superior capability, "moderate" indicates acceptable performance with certain practical limitations, and "low" indicates significant operational or analytical constraints.

Sensitivity and limit of detection are critical for analyzing bioaerosols, as the targets are usually present in extremely low concentrations. chemiluminescence-based systems, particularly those employing enzymatic or nanomaterial-assisted signal amplification, achieve the lowest detection limits, reaching attomolar or even single-molecule sensitivity, thereby demonstrating high sensitivity. Bioluminescence offers moderate sensitivity under practical sampling conditions, constrained by ATP degradation during bioaerosol collection. Intrinsic photoluminescence exhibits excellent particle-level detectability but remains vulnerable to optical background interference, resulting in relatively low analytical sensitivity for trace target detection.

Specificity and discrimination capability are primarily dictated by the biorecognition strategy rather than the signal transduction mode. Chemiluminescence and labeled photoluminescence approaches that employ antibodies, aptamers, or nucleic acid recognition achieve the highest taxonomic discrimination, including strain-level identification, reflecting high specificity. Intrinsic photoluminescence enables broad biological classification through spectral pattern recognition but cannot reliably resolve species, reflecting moderate specificity. Bioluminescence provides binary discrimination between viable and non-viable biomass, serving as a confirmation tool rather than an identification method, which translates to low taxonomic specificity.

Response time and temporal resolution are crucial for real-time monitoring. Intrinsic photoluminescence supports continuous, millisecond-scale monitoring of single particles, thus demonstrating high temporal resolution. Bioluminescence and chemiluminescence inherently require aerosol-to-liquid transfer and chemical processing, resulting in response times ranging from minutes to hours. Although microfluidic integration has reduced assay times, these methods cannot replicate the continuous real-time capability of optical excitation-based systems. Considering the absence of complex recognition element incubation, bioluminescence exhibits moderate temporal resolution, while chemiluminescence suffers from even poorer reaction kinetics and complex fluidic handling, resulting in low temporal resolution.

Instrumentation and field deployability reflect system robustness in real-world environments. Photoluminescence systems require lasers and optical components but benefit from reagent-free

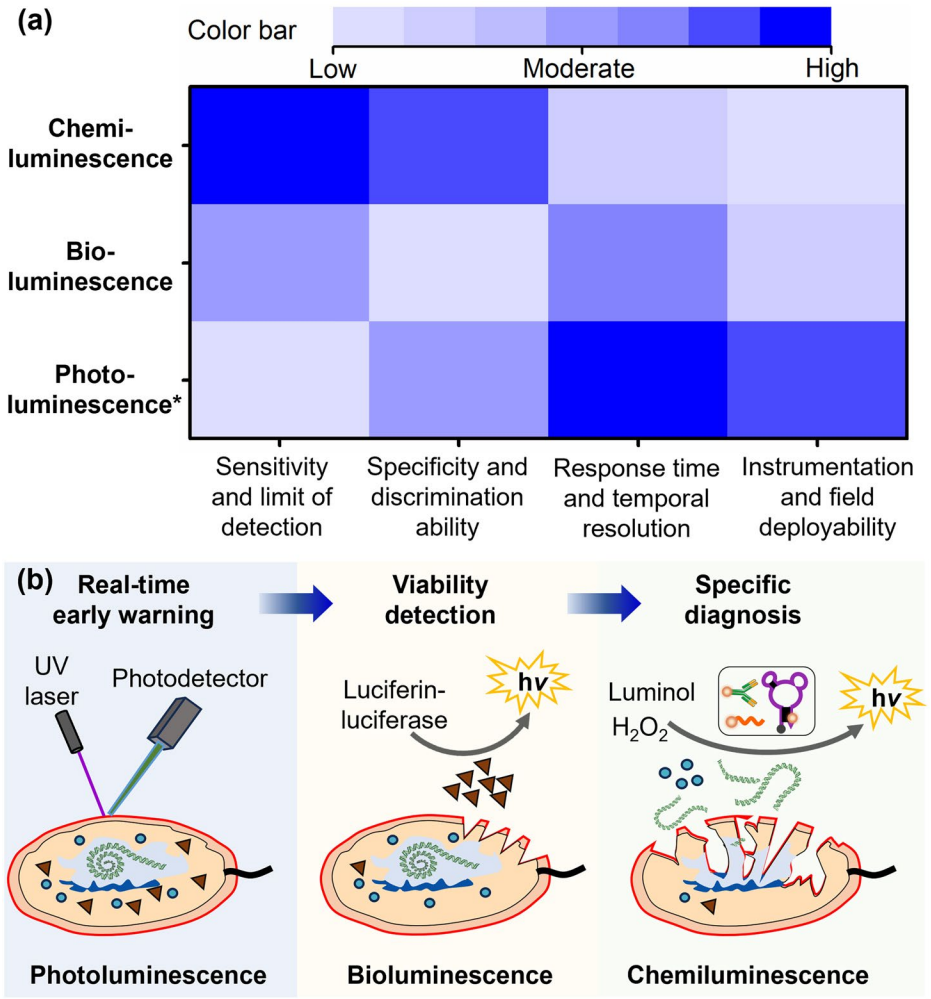

Figure 5. (a) Performance assessment of photoluminescence, bioluminescence and chemiluminescence strategies for bioaerosol analysis. *Refers to photoluminescence based on intrinsic fluorescence. (b) Schematic illustration of the multimodal bioaerosol analysis platform combining photoluminescence, bioluminescence and chemiluminescence.

operation and long-term mechanical stability, exhibiting high field deployability. In contrast, chemiluminescence and bioluminescence systems simplify optical detection but depend on fluidic handling, reagent storage, and enzyme stability, which collectively limit field robustness and autonomous operation, resulting in low field deployability.

6.2. Technology status, performance, and composite cost of luminescent strategies imposed by the energy sources

A comparative analysis reveals that the fundamental performance limits and economic viabilities of luminescent bioaerosol monitoring strategies are dictated by the nature of the energy source driving signal generation. As summarized in Table 1, no single luminescent strategy can simultaneously fulfill three core requirements of bioaerosol monitoring, which are real-time response, high taxonomic specificity, and ultra-high sensitivity.

Photoluminescence relies on externally supplied optical energy, and intrinsic fluorescence-based approaches excels in rapid, real-time detection of individual airborne biological particles. Consequently, it is currently the primary technology that has achieved widespread application

for continuous real-time bioaerosol monitoring (e.g., WIBS, SIBS, Rapid-E). While these platforms bear a very high instrument cost due to the necessity of utilizing sophisticated UV lasers and high-sensitivity photomultiplier tubes, their reagent-free nature dictates an exceptionally low operational cost. This makes them highly economical for continuous, long-term atmospheric surveillance.

In contrast, strategies such as bioluminescence and chemiluminescence, which do not require external light sources, offer superior biological relevance and specificity but face different operational and economic constraints. Bioluminescence exploits intracellular biochemical energy stored in ATP to detect living bioaerosols, achieving detection limits on the order of 130 CFU/m^3 with minute-level temporal resolution (Cho et al., 2020). Chemiluminescence harnesses exothermic chemical reactions to enable ultimate sensitivity in the picomolar range for viral and bacterial biomarkers *via* molecular recognition and signal amplification strategies (Jung et al., 2021). However, because both bioluminescence and chemiluminescence strictly require complex sample extraction procedures and the continuous consumption of specific reagents (e.g., luciferin-luciferase, luminol, antibodies), continuous monitors based on these principles are predominantly in the laboratory research and development stages. Although their optical instrumentation is generally simpler and potentially offers a lower instrument cost than photoluminescence systems, their composite cost over time is dominated by a significantly high operational cost. This continuous reagent dependency makes their independent, long-term operation economically challenging.

6.3. Limitations and challenges of luminescent strategies under real-world field conditions

Although luminescence-based strategies demonstrate excellent performance in specific analytical aspects, their application to bioaerosol detection still faces significant challenges in real-world

Table 1. The comparative characteristics of energy-driven luminescent strategies.

Category	Photoluminescence (Intrinsic LIF)	Bioluminescence	Chemiluminescence
Energy source	External optical excitation	Biochemical reactions	Chemical reactions
Signal origin	Intrinsic fluorescence of biological chromophores or the fluorescence of molecular probes	ATP-dependent luciferase reaction	Light emission from exothermic chemical reactions
Typical recognition elements	Intrinsic fluorophores and labeled molecular probes	ATP	Antigen-antibody/aptamer interactions, nucleic acid hybridization
Advantages	Rapid, multiplexed, and real-time	Ultralow background, high sensitivity, and microbial viability	High specificity, strong amplification, and high sensitivity
Limitations	Sensitive to scattering and autofluorescence, spectral overlap	Enzyme instability, limited continuous detection	Short-lived signals, reagent instability
Best application scenarios	Early warning systems, rapid response events	Viability detection, high-sensitivity total load monitoring	Ultra-trace detection, pathogen-specific monitoring, emergency diagnostics
Example of target	*S. cerevisiae*	*E. coli*	*H3N2* virus
Limit of detection	Size limit of 0.3 μm	130 CFU/m^3	5 pM
Response time	Real-time (< 1 ms)	5 min	20 min
Reference	(Könemann et al., 2019)	(Cho et al., 2020)	(Jung et al., 2021)
Application status	Widely commercialized	Laboratorial	Laboratorial
Representative platforms	WIBS, SIBS, Rapid-E	Automated impingers coupled with PMTs/microplate readers	Microfluidic chips / Luminescence microplate readers
Instrument cost	High (requires expensive UV lasers and detectors)	Low to moderate (simpler optics, no excitation laser)	Moderate (complex fluidics/ extraction needed)
Operational cost	Very low (reagent-free, continuous operation)	High (continuous luciferin/ luciferase consumption)	Very high (costly specific antibodies/substrates)

field environments. These limitations stem from the complexity and variability of environmental matrices. The major limitation of intrinsic photoluminescence lies in the lack of specificity under ambient conditions. In field measurements, numerous non-biological particles, such as mineral dust, combustion-derived aerosols, and secondary organic aerosols, also exhibit autofluorescence that significantly overlaps with biological signals, inevitably leading to elevated false-positive rates. In addition, environmental variables, such as particle aging, humidity fluctuations, and photobleaching effects, can significantly alter fluorescence intensity, increasing the uncertainty in signal interpretation.

ATP bioluminescence approaches typically require the prior collection and extraction of bioaerosols, making it difficult to achieve real-time online detection. The sampling process may introduce losses and induce physiological stress, leading to fluctuations in intracellular ATP levels. Furthermore, ATP measurements represent only total biological activity and cannot distinguish between different types of bioaerosols, such as bacteria, fungi, or pollen. The presence of extracellular ATP released from damaged or dead cells may also result in overestimation of viable biomass. Similarly, chemiluminescence-based strategies also rely on sample collection and multi-step reaction processes, which constrain their temporal resolution and real-time applicability. Undefined background chemical reactions and reactive species may interfere with signal generation in complex environmental samples, increasing the uncertainty of quantitative analysis. Moreover, the field stability of reagents under fluctuating outdoor temperatures poses severe challenges for long-term field deployment.

Therefore, luminescent strategies are currently more suitable for rapid screening and estimates of total biomass, whereas accurate identification of airborne microorganisms typically requires integration with complementary techniques, such as microscopy, culture-based methods, or molecular assays. Future developments should focus on improving robustness against environmental interference and enabling multimodal integration for more accurate and reliable field applications.

7. Conclusions and future perspectives

Luminescent strategies provide powerful analytical tools for bioaerosol monitoring, yet their performance in environmental applications is significantly constrained by how excitation energy is generated, transmitted, and converted into detectable signals. Based-on energy-driven comparative analysis, this review mainly demonstrates that optically excited fluorescence, bioluminescence, and chemiluminescence exhibit distinct strengths and inherent limitations with respect to sensitivity, specificity, temporal resolution, and operational robustness under complex atmospheric conditions. Bioaerosols are intrinsically heterogeneous, temporally dynamic, and embedded in chemically complex atmospheric backgrounds. No single luminescent strategy can simultaneously satisfy all analytical requirements imposed by real-world bioaerosol monitoring. Instead, each strategy occupies a distinct position defined by physical speed, biological relevance, and chemical selectivity. Therefore, future research should focus on integrating complementary energy-driven mechanisms and developing key technologies that can enhance stability, efficiency and deployability.

1. Synergistic integration of multimodal luminescent platforms. A multimodal sensing platform that links real-time early warning, viability screening, and specific confirmation, represents a promising pathway to overcome the limitations of individual luminescent strategies. In such a framework, intrinsic photoluminescence can serve as a reagent-free trigger to provide real-time surveillance for sudden spikes in biological particle concentrations. Once particle concentration becomes abnormal, bioluminescence modules can be selectively activated to assess microbial viability, thereby filtering out non-viable biological events and reducing false-positive alarms. For samples identified as both viable and suspicious, chemiluminescence can then be deployed to achieve definitive taxonomic

identification with high-precision quantification (Figure 5b). This integrated approach maximizes analytical accuracy against environmental interference, and strictly minimizing the unnecessary waste of expensive reagents, thus paving the way for cost-effective and highly reliable bioaerosol surveillance systems.

2. Engineering advanced luminescent materials with enhanced stability and energy efficiency. The long-term deployment of luminescence-based bioaerosol sensors is currently limited by the environmental instability of biological reagents and conventional fluorophores. Future development should therefore emphasize advanced functional materials that decouple luminescent performance from fragile biological components. Natural enzymes in bioluminescence and chemiluminescence systems can be progressively replaced or supplemented by nanozymes and metal-based catalysts, which offer superior thermal and chemical stability, lower production costs, and tunable catalytic activity. Similarly, traditional organic fluorophores used in labeling strategies can be substituted with quantum dots or carbon dots, which provide high quantum yields, broad excitation windows, and narrow emission bands. By tailoring the physicochemical properties of these materials, luminescent detection performance can be maintained with greater reliability under harsh atmospheric monitoring conditions.
3. Miniaturization via microfluidic-based portable system. Portable and autonomous bioaerosol monitoring requires analytical systems that tightly integrate sampling, processing, and detection. Microfluidic system offers a practical route toward this goal by enabling chip-level aerosol collection, rapid cell lysis, controlled reagent handling, and efficient luminescence readout within a unified architecture. This integration minimizes sample loss, shortens the delay between collection and analysis, and significantly reduces reagent consumption. Moreover, microfluidic systems support automation and parallelization, allowing simultaneous screening of multiple targets to enhance detection efficiency. When combined with compact optical detectors, these platforms provide a foundation for portable field monitoring, occupational exposure assessment, and rapid response during public health emergencies.

Author contributions

CRediT: **Xiaorong Yang**: Data curation, Writing – original draft, Writing – review & editing; **Huahao Ye**: Validation, Visualization; **Linghui Peng**: Data curation, Visualization, Writing – review & editing; **Zhishu Liang**: Validation, Visualization; **Guiying Li**: Validation, Visualization, Writing – review & editing; **Taicheng An**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Disclosure statement

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