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Optical Techniques for Detecting and Identifying Biological-Warfare Agents

Electro-optical techniques, such as laser-induced fluorescence, can detect and help to identify bio-agents, but laboratory assays are needed before taking medical treatment measures.

By DARRYL P. GREENWOOD, *Fellow IEEE*, THOMAS H. JEYS, BERNADETTE JOHNSON, JONATHAN M. RICHARDSON, AND MICHAEL P. SHATZ

ABSTRACT | Rapid and accurate detection and identification of biological agents is an objective of various national security programs. Detection in general is difficult owing to natural clutter and anticipated low concentrations of subject material. Typical detection architectures comprise a nonspecific trigger, a rapid identifier, and a confirming step, often in a laboratory. High-confidence identification must be made prior to taking action, though this must be traded against regrets stemming from delay. Sensing requirements are best established by posing plausible scenarios, two of which are suggested herein. Modern technologies include the use of elastic scatter and ultraviolet laser-induced fluorescence for triggering and standoff detection. Optical and nonoptical techniques are used routinely in analyzing clinical samples used to confirm infection and illness resulting from a biological attack. Today, environmental sensing serves at best as an alert to medical authorities for possible action, which would include sample collection and detailed analysis. This paper surveys the state of the art of sensing at all levels.

KEYWORDS | Biological agents; fluorescence; medical diagnostics; particles; scatter; standoff sensing

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I. INTRODUCTION

The defense against the use of biological agents is a national security [1] and homeland defense objective [2]. Timely and accurate detection of biological agents is an essential component of the defense because it enables protective measures such as masking, evacuation, avoidance, and early medical treatment. For technical reasons, detection is exceedingly difficult, since very small quantities are infectious and because benign biological matter is common in the environment. Understanding the capabilities and limitations of bioagent detection is the subject of this paper, which surveys the state of the art of optically based bioagent detection and gives a prognosis for this capability into the future.

Use of biological agents as a means for defeating enemies has persisted through the centuries [3]. Following scientific breakthroughs such as the understanding of the germ theory of disease by Koch in the late nineteenth century, bioweapons found increased emphasis, with numerous nation-state programs existing throughout the twentieth century [4], [5], and some into the twenty-first [6]. Over time, the more that was learned of the potential for bioagents to cause disease and death, the more repugnant such potential became to most civilized countries and governments. The United States pursued offensive bio-warfare from 1941 until 1969, when President Nixon abruptly terminated the program [7], [8]. Entered into force in 1975, the Biological and Toxin Weapons Convention (BTWC), which bans the development, stockpiling, production, and use of such weapons, has been ratified by 140 countries. President Gorbachev admitted in 1989 that the former Soviet program had continued well past the

date of signing the BTWC [9]. Currently no country openly admits to having a current offensive biowarfare program. In October 2001, the United States experienced its most severe bioattack when a perpetrator mailed anthrax spores to the news media and the Congress, resulting in 22 casualties, including five deaths [10]. Analysis suggests the quantity of material used, if dispensed more efficiently, could have caused significantly more casualties. The events of 2001 suggest that in addition to concerns over nation-state bioweaponry, the United States should be concerned over biological agents used by terrorists [11]–[13]. Because of the diversity and potential impact of the threat, the United States and many other countries maintain *defensive* military biological programs, aimed at detecting attacks and protecting intended victims from harm [14], [15].

A. The Threat

A biological agent attack could occur anywhere and through a variety of venues, including food, drinking water, insect vectors, and air [16]. (This paper does not address attacks by injection or dermal application, nor vectors, though one such attack was documented during World War II [17].) An aerosol attack with biological agents would work optimally as a fine mist of 1–5 μm -sized particles [18] since particles in this size range find optimum inhalation and retention. According to Pearson [19], as few as one microbe and as many as 10 000 can infect an individual, suggesting that aerosol clouds can be incredibly sparse, thus stressing the limits of detection.

Food and water are at risk due to a long and vulnerable supply chain [20], [21]. Thankfully there is motivation to keep the supply safe, and technologies are employed to provide security and to sense the presence of contamination. An attack on a food supply, as occurred with *Salmonella typhimurium* in The Dalles, Oregon, in 1984, would be considered today an act of terrorism [22]. Though none of the 751 victims died in that attack, others have suggested more potent means of widespread food poisoning [23]. Additional research and development are needed to improve the safety of the food and water supply system, including means of improved detection.

According to Franz *et al.* [24] any of a number of bioagents might be employed, ranging from bacteria such as *Bacillus anthracis* (the causative agent of anthrax), brucella (numerous species), *Yersinia pestis* (“the plague”), and *Francisella tularensis* (tularemia) to viruses such as variola (the causative agent of smallpox) and Ebola. Also of concern are a number of biotoxins, including Staphylococcus Enterotoxin B and botulinum, both of which were researched in the now-defunct U.S. offensive program. According to Zilinskas [25], Iraq researched and developed anthrax, perfringens, botulinum, aflatoxin, numerous viruses, and ricin, though few were carried to a weaponized state. For a more complete list and understanding of the physical and medical characteristics of potential bioagents, we refer the reader to the Centers for Disease Control and

Prevention¹ or to the U.S. Army’s Textbook on Military Medicine [26].

Of importance to this paper are the characteristics of these agents, since it is the purpose of detection to recognize microbes for what they are. Physical characteristics include external features such as shape, morphology, and epitopes (particularly antigens and other proteins), sugars, and fundamental amino acids. Internal features include DNA and RNA. Some techniques look for elemental makeup at the atomic level. Additional matter may appear in the analyte, including natural biologics and inorganics. Optics and electro-optical sensors play a role in all of these approaches.

B. Detection and Identification—Broadly Speaking

A key element of a defensive program is the detection and accurate identification of biowarfare agents at time of use and in postattack. Detection and identification (ID) are essential to the defense because they enable individual and collective protection, correct and timely medical treatment, and identification and apprehension of the perpetrator. Regrettably, bioagent detection and ID are difficult to achieve for a number of reasons.

- 1) The attack could occur anywhere and through any number of physical routes.
- 2) Small quantities of material can infect and cause great harm.
- 3) Significant benign biological matter exists in the environment.
- 4) Any one of a number of agents could be used.
- 5) Natural disease-causing agents could be employed, thus masking whether there was an attack or a natural event.
- 6) Bioagents can be engineered to mask detection and delay correct treatment.

On the positive side, disease caused by these agents can often be treated if diagnosed or detected in time and if there are sufficient therapeutic measures available. For all these considerations, it is important to address attack scenarios that are potentially realizable while emphasizing those incidents that can cause the greatest harm.

The fact that a bioattack may appear as a fine aerosol suggests that optical approaches are of great relevance for direct detection. As depicted in Fig. 1, a typical aerosol-detection architecture comprises early warning (a “trigger”), some form of sample collection, an early stage identifier, and a late stage confirmer (also an identifier, though typically with an alternative technology). Early-warning sensing can comprise point detectors that sample the aerosol, or standoff (i.e., remote) sensors that scan the air at a distance. In both cases, the sensor assesses the probability that the suspect air contains threat agent. A “point” trigger sensor is typically a particle counter of some sort, with techniques including elastic scatter,

¹See www.emergency.cdc.gov/agent/agentlist.asp.

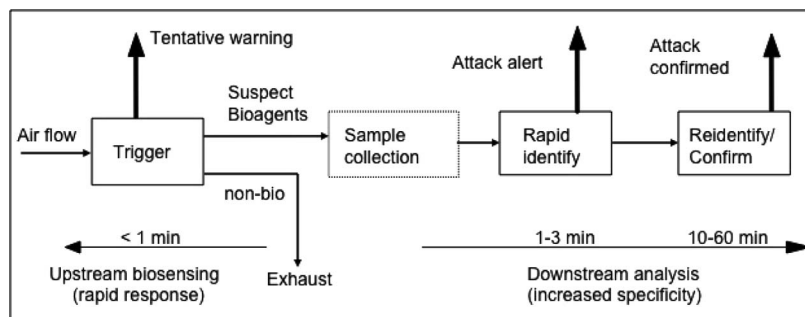


Fig. 1. A typical aerosol-detection architecture, which comprises early warning (a “trigger”), some form of sample collection, an early stage identifier, and a late stage confirmer (also an identifier, though typically with an alternative technology).

laser-induced-breakdown spectroscopy, fluorescence spectroscopy, polarization scattering, and Raman spectroscopy. Standoff techniques, currently limited to lidars that interrogate clouds of particles, have been developed to measure elastic backscatter and fluorescence. While other techniques have been considered (including microwave and millimeter wave), the most promising remain optical in nature, since longer wavelength techniques are less sensitive or less discriminating. Given the presence of background aerosols, all such techniques are subject to false alarms, and given various limitations in atmospheric propagation (attenuation and scattering), these are invariably range-limited. An additional limitation on point and stand-off optical sensors is their inability to discriminate live versus dead microbes, thus opening the potential for false alarms, though this potential objection can also be seen as an advantage in that even a failed attack (e.g., with dead bacteria) would be detected.

Optical systems play an important role in identification of the threat agent, wherein a sample is collected from either the air (or food or water) or infected human subject. Sampling can be triggered by an event such as a positive hit in an early-warning sensor or presentation of an ill person in a doctor’s office or hospital. In either case, the material is collected and provided to one or more in a class of identifiers. The gold standard identifier for many hospital labs and physicians is culture, with optical microscopy the method used to quantify and assess growth of the suspect agent. Culture is, however, slow (days to weeks) and limited in general use since the biomaterial being assayed must be consistent with the culture medium used. More modern techniques include immunoassay and polymerase chain reaction (PCR). Both are much more rapid than culture, with response times in the tens of minutes to hours. Immunoassay involves the binding of target antigens to immobilized antibodies, with binding signaled by an optical or color change (e.g., the physician’s strep test kit). PCR involves a binding of DNA sequences of the sample with a known genetic sequence. Many organizations are working to reduce PCR times to as little as a minute, with labs-on-a-chip and micropore technologies as

approaches. As with all detection techniques, immunoassay and PCR are limited by backgrounds, interferents, and false readings.

The objective of the sensing and identification architecture is to provide sufficiently accurate and timely warning of an exposure so that protective actions can be employed. Current approaches are limited in sensitivity, timeliness, and accuracy; thus there is room for innovation and advances. In particular, trigger sensors are by design generic, trading speed for specificity. A trigger sensor that could not only detect the presence of potential pathogens but also identify the species and pathogenicity would be highly desirable since this would enable rapid and effective protection and treatment. This paper addresses the state of the art and what improvements in the sensors are needed to provide the needed defense at an affordable level.

II. REQUIREMENTS FOR DETECTION TECHNOLOGIES AND APPROACHES

To determine requirements for sensing biological agents, one needs to consider the operational use of such sensing. One must consider not only credible attack scenarios and specific threat use but also how the information produced by the sensors supports a response to obviate, or at least reduce, the effects of the attack and the constraints on the deployment of sensors. There are many possible responses to a biological attack: avoiding the contaminated area, personal and collective physical protection, medical prophylaxis and treatment, and decontamination may all be employed either singly or in conjunction.

The different response options impose different requirements on the sensors. For example, measures to avoid exposure require that the sensor detect the attack before it reaches the people to be protected and respond quickly enough that protective action can be completed before the agent reaches the vulnerable population. On the other hand, a response of treating the population of an urban area does allow time, at least a few hours, for chemical tests (immunoassays or PCR) to detect and identify the agent; however, it has an extremely low tolerance for false

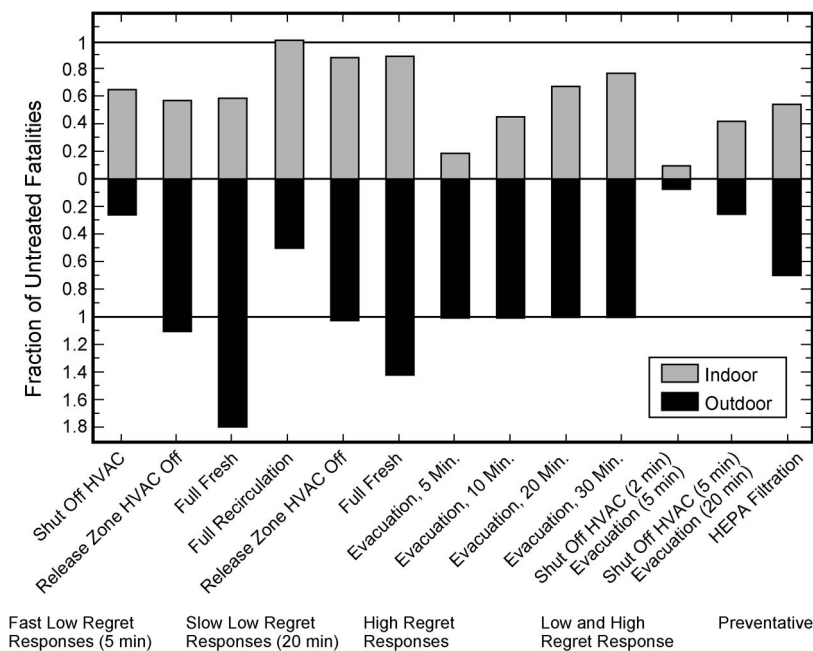


Fig. 2. Effectiveness of different response options for reducing the impact of an attack in an office building. Plotted are the ratios of the number of lethal exposures expected for a variety of responses relative to those that would occur with no response. Above the zero line are the lethal exposures internal to the facility; below the line are those outside. Note that, in some cases, a response can actually increase the number of fatalities (values > 1). The figure was calculated using a CONTAM model for a low-rise office building.

alarm and requires that the agent be identified with enough specificity to determine the correct treatment. (The assumed response time of a few hours in this latter case is predicated on the assumption there are treatments, available and approved by the FDA. This is generally the case for a bacterial agent, provided potential antibiotic resistance can be identified. However, viruses and toxins have few tested approved treatment modalities other than supportive care. For all agents, treatment of a large populace, with a certain fraction of immunocompromised individuals, is problematic and will heavily stress the public health system.)

We will focus on two scenarios in this section: a domestic facility protection scenario and a military scenario defending troops on the battlefield. In each case, we recognize that the conditions of the release, that is, the agent, mass, location, wind, and weather conditions are largely under the control of the attacker. Thus, rather than analyze specific attacks, we generally prefer to analyze classes of attack and attempt to design or develop a system that protects against as large a fraction of the attacks in the class as practical.

For the first type of scenario, we will consider an attack on a domestic facility, which could be a building or transportation facility. The responses that we have found to be most effective in a series of analyses have been some facility-dependent response, such as an HVAC shutdown, to slow the spread of the agent, followed by an evacuation if the attack is confirmed. Typically one would like the first

response to occur within a few minutes after the attack, and the evacuation to occur within 15 min or so; sensor responses must be rapid enough to support this. Fig. 2 shows the ratio of the likely number of lethal exposures that would occur with the indicated response to the number that would occur if the incident were undetected. The figure was calculated using a CONTAM² model for a low-rise office building. The tolerance for false alarms in this scenario is quite low; based on discussions with a number of building operators, typical values are about one per month for HVAC or other changes to slow the spread of agent, and something significantly less than once per year, perhaps once per century, for an evacuation. The example of fire alarms may suggest a higher tolerance for evacuations, but in the case of fire alarms, there are techniques for giving a rapid “all clear” without causing undue panic. This is unfortunately not the case currently for alarms of biological incidents. The sensitivity requirements are more contentious; very small concentrations can pose a hazard for a population breathing them in over a period of an hour or more indoors, and someone wanting to preclude such harm would desire sensor sensitivities far better than can currently be achieved. However, an attack using what would seem to be plausible amounts of material, say, between 1 g and 10 kg of aerosolized agent, released in a brief period, typically results in concentrations ranging from tens of agents containing particle per

²www.bfrl.nist.gov/IAQanalysis/.

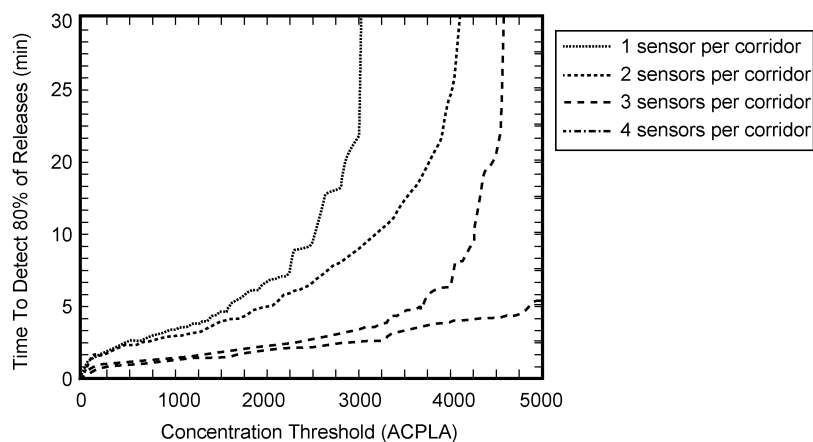


Fig. 3. Latency of potential sensor networks with a probability of detection of 80% as a function of the threshold. Uses same model as Fig. 2 with 1 g releases and shows how the time between the release and point where the concentration at one of the sensors reaches its threshold varies with sensor threshold and density. Sensors are deployed in key portions of the HVAC system, large spaces, and along the corridors of the building.

liter of air (ACPLA)³ to many thousands of ACPLA. An example is shown in Fig. 3; this figure uses the same model as Fig. 2 with 1 g releases and shows how the time between the release and point where the concentration at one of the sensors reaches its threshold varies with sensor threshold and density.

The second type of scenario is an off-target attack on troops in the field. Their response in case of an alarm is to employ personal protection (suits and other protective gear) and collective protection (closing the hatches on the vehicles and activating the filters). These responses can take about a minute in favorable cases once a sensor sounds an alarm; this time includes time for a decision to be made, commands to be relayed to the troops, and practiced troops to don the gear and engage the collective protection systems. The sensors need to be placed reasonably close to the protected troops to preclude the attack's being released downwind of the sensors yet upwind of the troops. The sensors should have rapid responses—on the order of one minute or less. Alternatively, the sensor could be a standoff sensor located with the protected troops; in that case, the reaction time requirement will be more stringent if the detection range is short and can be relaxed for long range detections, since the cloud will take longer to reach the troops.

An example of a point sensor deployment is analyzed in Fig. 4, which shows contours for 95% network Pd, the probability of detection, for an ensemble of HPAC [27] plumes, for a variety of sensor sensitivity and spacings. These curves represent sensitivity thresholds; anywhere above the curves represents aerosolized masses that can be detected at the various thresholds. For a variety of attacks

and weather conditions, sensors with limits of detections (LODs) ranging up to 500 particles per liter or so have some utility; sensors with LODs of single particles are probably unnecessary. To achieve a high Pd in high winds, the sensors must be close together. The tolerable false-alarm rate depends on the level of perceived threat. If the perceived threat level is very high, false alarms of once every few days or weeks might be tolerable; if the perceived threat level is low, the tolerance for false alarms will decline proportionately.

Of course, these scenarios do not come close to exhausting the applications of optical sensors. For example, detection of contaminated surfaces, which can be important both for avoidance and decontamination efforts, would benefit strongly from optical-based sensing. In addition, optical sensors are also used to limit the use of consumables in biochemistry-based sensors and to provide a signal transduction or amplification for biochemical binding or recognition. Examples of these applications will be described in subsequent sections.

III. DETECTION AT A POINT

A. Rapid Detection

The rapid detection of an aerosolized biological agent dramatically improves the ability to mitigate the effects of such an attack. Optical detection is presently the fastest means for sensing the presence of aerosolized biological agents and can be divided into individual-particle and multiple-particle detection. Individual-particle detection is characterized by the detection of signals from one particle at a time, while multiple-particle detection is characterized by the detection of integrated signals from multiple particles. These two categories are exemplified by flow

³An ACPLA is a somewhat imprecise unit, though widely used in this field.

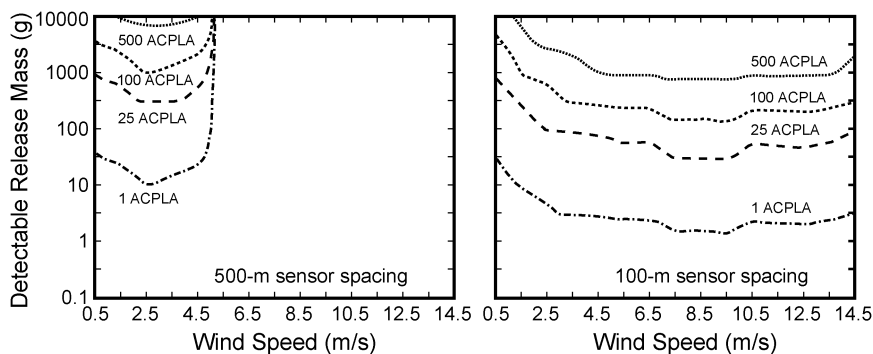


Fig. 4. Plots of the minimum amount of aerosolized agent detectable as a function of the wind speed for a linear array of sensors. The curves are for sensors of different limits of detection. The agent release is 2 km upwind of the sensor array. Contours represent different concentration thresholds at a detection probability of 95%. For the 500 m separation at high wind speeds, there is more than a 5% chance that no agent reaches the sensors on either side of the plume, so no level of increased sensitivity would achieve 95% Pd; more sensors are needed.

cytometry [28] and lidar (see Section IV). For attacks in which the agent concentration is significantly less than the ambient background concentration, individual-particle detection yields better detection and discrimination of agent particles than multiple-particle detection because the agent particle signals are not mixed with background particle signals.

Flow cytometry typically involves the laser illumination of individual particles traveling in a liquid stream and the detection of the resulting elastic scattering [29] and fluorescence [30] in order to detect and discriminate one type of particle from another type of particle. Real-time individual-particle detection of aerosolized biological agents differs from traditional flow cytometry in two ways. First, the particles are in air, not liquid; and secondly, the particles are not modified with fluorescence enhancing chemicals, so only the natural fluorescence from the particle is detected. Because the natural fluorescence from unmodified aerosol particles is much less intense and specific than that from fluorescence-enhanced particles, real-time optically based biological agent detectors are much less discriminating than typical flow cytometers. On the other hand, these biological agent detectors are more rapid and do not require a supply of chemicals that are often toxic. Because elastic scattering and natural fluorescence-based detection are not sufficiently specific, detectors based on these phenomena are used not to identify bioagents but rather to alert the presence of a threat-like aerosol. Depending on circumstances, this alert can be used, for example, to activate a separate identifier system or to divert air flow in a building ventilation system. The key figures of merit [31] for a biological agent detector are then sensitivity (minimum detectable agent concentration), probability of detection, false-alarm rate, and response time.

B. Design Considerations

An important consideration for bioagent sensors is the environment in which they will operate. Typically the

ambient particle concentration greatly exceeds the desired detection concentration of biological agent. This disparity requires that the sensor be very good at discriminating background particles (clay, diesel particulate, pollen, mold, etc.) from bioagent particles in order to achieve a low false-positive rate. For example, in an urban environment the concentration of particles greater than 1 μm diameter may range from 1000 to 100 000 per liter depending on many parameters (e.g., time of year, traffic conditions).

The design of a real-time single-particle optically based biological-agent trigger involves two basic considerations. First, individual aerosol particles must be characterized well enough to discriminate threat-like and non-threat-like particles. Secondly, the threat-like particle concentration must be characterized well enough to support a reliable trigger threshold that maximizes the probability of detection while minimizing false positives. The ability to discriminate different types of particles depends upon both the native difference between particle types for the measured properties and the signal-to-noise ratio of these measurements. The ability to characterize the threat-like particle concentration depends upon the detector air sample rate, the time available to make a concentration measurement, and the level of clutter in the environment.

The ability to optically detect and discriminate particles is partially determined by the amount of light that the particle emits either as elastic scattering or as fluorescence. Equation (1) gives the number of detected photons (N_p) that are emitted by a particle, with an optical cross-section σ , which is illuminated by a light beam with a power of P in a cross-sectional area A for a time τ . The photon collection and detection efficiencies are η_c and η_d respectively, h is Planck's constant, c is the speed of light, and λ is the wavelength of the illuminating light

$$N_p = \eta_c \eta_d \frac{P \tau \lambda}{A h c} \sigma. \quad (1)$$

The number of particles detected (n) is given by (2), where C is the aerosol particle concentration, T is the time available for counting particles, and ϕ is the air sample rate

$$n = \phi CT. \quad (2)$$

For continuous sampling, the air sample rate is given by (3), where A is the cross-sectional area of the sample volume, L is the length of the sample volume, and τ is the time required for a particle to transit the sample volume. The effective air sample rate (also referred to as responsivity) can be calculated by dividing the particle count rate by the particle concentration

$$\phi = AL/\tau = n/(TC). \quad (3)$$

The design of a biological agent detector involves a compromise between maximizing the signal from each particle that enters sample volume and minimizing the detectable agent concentration. For a given light power, the maximum particle signal is obtained by focusing that light into the smallest possible cross-sectional area A so as to increase the particle illumination intensity. However, as the sample volume decreases, the minimum detectable concentration increases.

It is rarely possible to perfectly discriminate ambient background particles from agent particles. Typically, there is some small fraction of the background aerosol that resembles the agent particles. These particles are referred to as clutter. In a low clutter environment, the number of threat-like particles in the measurement threat region dominates the number of clutter particles in the threat region. In a high clutter environment, the number of clutter

particles in the measurement threat region dominates the number of actual threat particles.

Fig. 5 shows a simplified schematic for a real-time optically based bioagent detector. Light from a source of cross-sectional area d^2 is imaged into a sample volume (d^3) through which ambient air passes. Particles that pass through the sample volume scatter light elastically and emit fluorescence. Some of this radiation is directed to a photodetector with an area d^2 .

Combining (1)–(3), we can solve for the detectable bioagent concentration as a function of the incident optical power, as shown in (4). The light beam cross-sectional area (A or d^2), the particle transit time (τ) through the sample volume, and the air sample rate have dropped out of this equation

$$C = \frac{n}{\sigma LT} \frac{N_p}{\eta_c \eta_d} \frac{hc}{P\lambda}. \quad (4)$$

In addition to elastic scatter, a variety of inelastic scatter techniques have been pursued, the most prominent of which is laser-induced fluorescence. Biological matter fluoresces due to the presence of aromatic amino acids (tryptophan, tyrosine, and phenylalanine), which fluoresce in response to excitation in the near ultraviolet (UV) (around 260–280 nm), and nicotinamide adenine dinucleotide (NADH), which can be excited using a relatively broad range of UV wavelengths in the 300–360-nm range. For a fluorescence-based single-particle detector, Fig. 6 shows the detectable concentration as a function of optical power for a signal-to-noise ratio of ten and for illumination wavelengths of 280 and 340 nm assuming the values for the other variables as shown in Table 1. The fluorescence cross-section (σ), for a given particle size, is one of the key parameters that determine the minimum detectable particle concentration. The fluorescence cross-section of

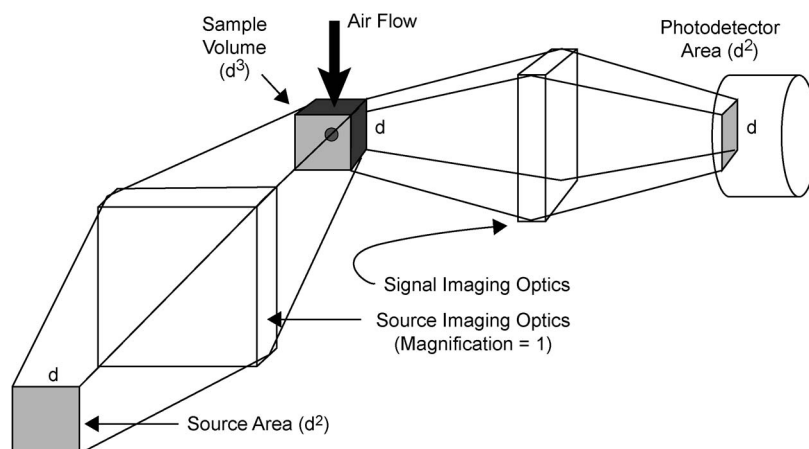


Fig. 5. Schematic of generic optically based biological agent particle detector.

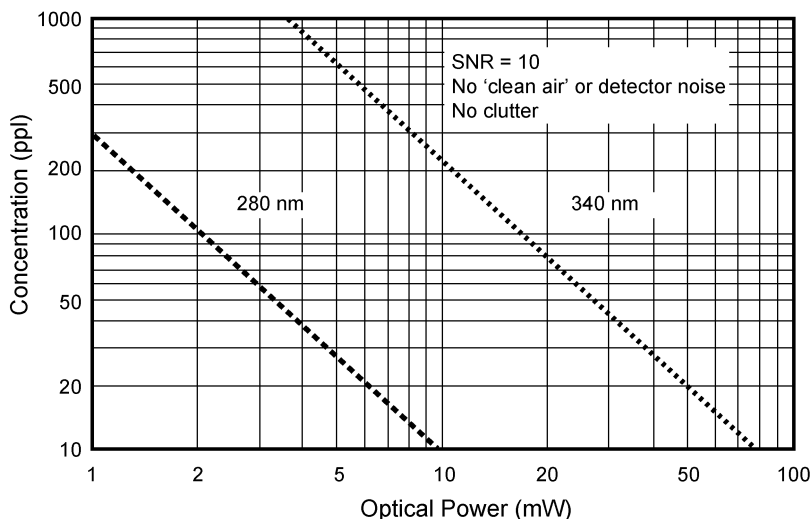


Fig. 6. Calculated detectable particle concentration utilizing light induced fluorescence as a function of optical power for 280- and 340-nm illumination wavelengths. See Table 1 for other variables in calculation.

Table 1 Variables Used in Calculation of Fig. 6

Number of detected particles (N_p)	100 particles
Particle fluorescence cross-section (σ)	$(50, 5) \times 10^{-12} \text{ cm}^2 @ (280, 340) \text{ nm}$
Number of detected photo-electrons (N_{pe})	100 photoelectrons
Photon collection, detection efficiency (η_e, η_d)	30%, 15%
Threat detection time (T)	60 s
Sample transit time (τ)	1 ms

biological particles has been measured by several groups [32]–[35] and the values in Table 1 are typical for 1 μm *Bacillus subtilis* spores. However, it should be noted that the cross-section strongly depends on the method of preparation.

C. Historical Developments

The development of real-time optically based bioagent sensors goes back to the early 1990s. Yee and Ho [36] demonstrated that it was possible to detect anomalous aerosols by temporally monitoring the concentration and particle-size distribution of the ambient aerosol via an elastic-scattering-based particle detector (see also Stroud *et al.* [37]). In 1991, Kaye *et al.* [38] demonstrated the detection and discrimination of anomalous aerosol particles based on the elastic-scattering measurement of particle concentration, size, and shape measurements. The particle shape measurement was based on the asymmetry of elastic scattering. Spherical particles generate a cylindrically symmetric elastic-scattering pattern, while elliptical particles generate an asymmetric pattern. The degree of asymmetry indicates the degree of ellipticity. While elastic-scattering-based detection can discriminate anomalous aerosols when the concentration of these aerosols is a significant fraction of the background aerosol concentra-

tion, this technique alone has not proven to be very useful for detecting anomalous aerosols that are a minor constituent of the background aerosol.

In 1993, Ho *et al.* [39] showed that the native fluorescence from individual biological particles could be detected. In this work, they demonstrated the fluorescence detection of *Bacillus subtilis* spores in a liquid flow cytometer. They also suggested that this native fluorescence could be used to directly detect bioagents in the atmosphere. In 1996, Ho [40] reported the detection of aerosolized individual bioagent simulant particles utilizing an elastic-scattering-based detector [41] that was modified to include a 325-nm induced fluorescence measurement. This instrument was referred to as the fluorescence aerodynamic particle sizer (FLAPS). In many environments, most background aerosols are composed of inorganic material that fluoresces very weakly as compared to organic particles. Thus, fluorescence greatly increases the optical discrimination of biological agent particles from background aerosols and reduces the false positive rate of agent detection. The design and performance of the FLAPS was detailed in 1997 [42]. In 1998, the FLAPS was modified to be smaller, power efficient, and field portable, and the 325-nm source was replaced with a 349-nm source [43]. Development of the FLAPS

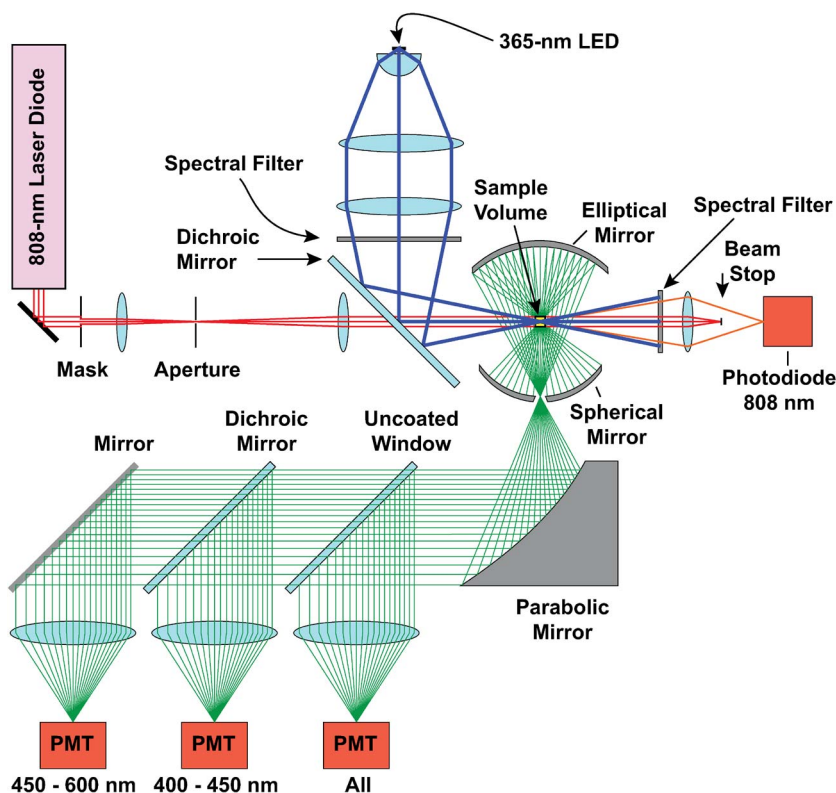


Fig. 7. Optical diagram of BAST showing key features of excitation wavelengths and sensing modalities.

has continued [44], and it is now available as a commercial product from TSI Inc.⁴

The original FLAPS influenced the development of many subsequent fluorescence-based bioagent sensors. These include sensors developed at the Naval Research Laboratory (NRL), the Army Edgewood Chemical and Biological Center (ECBC), MIT Lincoln Laboratory, and the U.K. Defense Science & Technology Laboratory (DSTL). NRL has focused on the development of sensors for measuring the elastic scattering and fluorescence characteristics of biological aerosols including multiwavelength excitation of these aerosols [45]–[47]. ECBC has focused on the development of low-cost sensors, including providing the initial funding support for the Biological Agent Warning Sensor (BAWS) and, more recently, developing light-emitting diode (LED)-based sensors [48]. DSTL has extended the original work of Kaye *et al.* and developed sensors based on particle shape and fluorescence detection [49]–[51]. In addition to the FLAPS produced by TSI Inc., other companies have also developed fluorescence-based sensors [52]–[55].

The state of the art in a fielded real-time optically based individual-particle biological agent detector is the BAWS [56]–[58]. This detector was originally developed at MIT

Lincoln Laboratory and has proven itself in many sensor competitions. It is now part of the U.S. Joint Biological Point Detection System (JBPDS), which is manufactured by General Dynamics Inc.⁵ The JBPDs is the first fully automated military bioagent detection system. In the JBPDs, the BAWS performs the function of cueing an air-to-liquid particle collector to begin particle collection for the biological-agent-identifier subsystem.

D. Looking Forward

Efforts to improve biological-agent detectors have focused on reducing cost and improving the performance of these sensors. These are objectives that can find themselves in conflict. MIT Lincoln Laboratory has been developing the Biological Agent Sensor and Trigger (BAST) [59], [60] to meet the needs of low-cost biological agent detection, and the Rapid Agent Aerosol Detector (RAAD) [61] to meet the needs of high-performance biological agent detection.

The BAST utilizes low-cost UV LEDs in place of high-cost UV lasers. Fig. 7 shows an optical schematic of the BAST. Particles entering the BAST sample volume are illuminated by both an inexpensive 820-nm diode laser and a 365-nm LED. There are four different measurements of the particle light emission (820-nm forward elastic

⁴<http://www.tsi.com>.

⁵www.gdatp.com/products/detection_systems/BAWS/BAWS.htm.

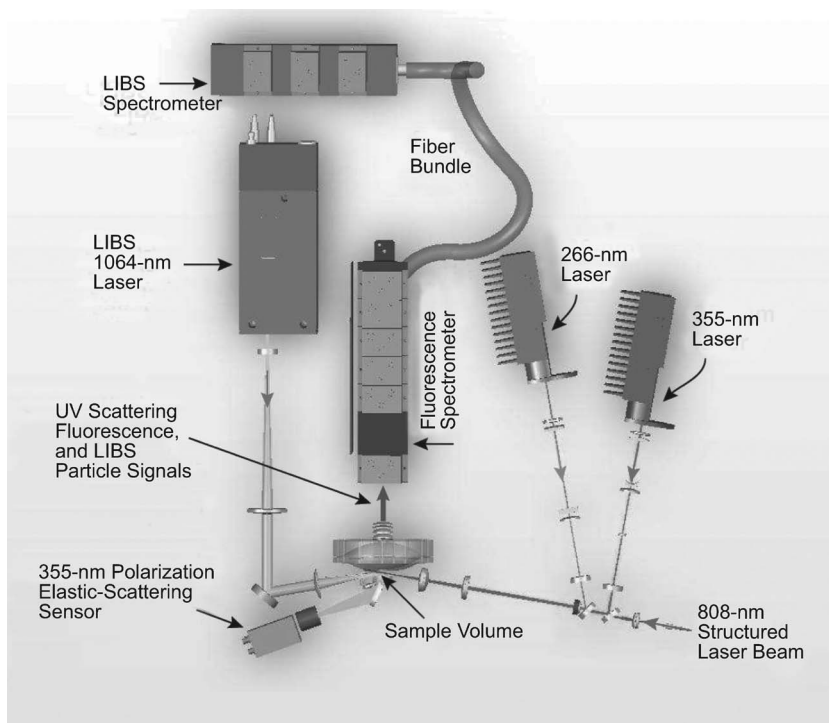


Fig. 8. Optical schematic of the RAAD.

scattering, 365-nm side elastic scattering, 400- to 450-nm fluorescence, and 450- to 600-nm fluorescence). The 820-nm forward scattering measurement detects the presence of a particle and gives a measure of the particle size. The 365-nm induced elastic scatter and fluorescence gives additional information with which to discriminate threat and nonthreat particles.

The RAAD effort is aimed at dramatically improving the performance and reliability of fluorescence-based biological agent triggers (see schematic in Fig. 8). The performance is increased by incorporating up to 14 measurements that are made on each particle that flows through the system. Table 2 lists the series of measurements taken on aerosol particles by RAAD. These additional measurements dramatically increase the ability to discriminate threat and nonthreat particles, therefore reducing the false positive rate. Reliability is improved by keeping the optics clean with sheath air flow and by increasing laser lifetime by operating lasers only when particles are present in the sample volume. RAAD is not specifically intended to be a low-manufacturing-cost instrument. However, because of a lower false-positive rate and improved reliability, it should be a low-operating-cost instrument. This is important since much of the cost associated with bioaerosol triggers comes from the cost of reacting to false triggers and from the costs associated with maintaining the sensor. This approach aims to dramatically reduce both of these costs.

IV. STANDOFF BIO-DETECTION

A. Early Warning

Standoff detection (i.e., detection at a distance) of biological agents offers many potential capabilities not easily provided by localized (point) detection systems. Although point sensors are a more mature technology offering higher localized sensitivity [62], [63], standoff systems can provide sensitivity over a wide area and may be simpler to deploy where this capability is needed. Standoff systems also provide the capability of mapping the course of an aerosol hazard, allowing for advance warning to downwind assets. Mapping and tracking of the hazard, particularly when combined with plume modeling, can help to determine what assets have been contaminated, thus guiding treatment and decontamination efforts. Lastly, mapping can provide the capability of determining the point(s) of origin of an attack, which could be vital for identifying those responsible. Ultimately, the best solution for protecting at-risk locations might be an integrated system that combines high-sensitivity point sensors (trigger and confirmatory) with a wide-area coverage standoff detection system, creating a system-of-systems with the best possible performance [64].

The primary technology that has been exploited is light detection and ranging (lidar), which has the capability of mapping a threat in space, usually defined in terms of range, azimuth, and angular elevation relative to

Table 2 Staged Detection Architecture of the RAAD

STAGE 1 Detection	STAGE 2 Discrimination: multi-band elastic scattering and fluorescence	STAGE 3 Discrimination: laser induced breakdown spectroscopy
Illumination Source: 808-nm laser continuous operation	Illumination Sources: 266-nm pulsed laser 355-nm pulsed laser pulsed operation when particle with appropriate size is present.	Illumination Source: 1064-nm pulsed laser pulsed operation when particle is present that is part of a threat-like aerosol as indicated by fluorescence measurements
Detection Channel: 808-nm elastic scattering	Detection Channels: 266-nm elastic scattering 355-nm elastic scattering 290-330 nm fluorescence 340-370 nm fluorescence 380-410-nm fluorescence 420-600-nm fluorescence 355-nm polarized elastic scattering	Detection Channels: 285-nm magnesium emission 422-nm calcium emission 589-nm sodium emission 766-nm potassium emission
Measurements: particle size particle position	Measurements: particle relative reflectivity particle relative fluorescence spectra particle absorption	Measurements: particle atomic composition

the position of the sensor. Range information relies on timing the return optical signal relative to a transmit pulse. It is useful to note that a round-trip of 1 km at the speed of light requires $\sim 6.7 \mu\text{s}$. Using fast digitizers, it is easily possible to sample the return signal at a rate equivalent to 1 m of range, which most likely exceeds the requirements for biodetection. Angular resolution is determined by the instantaneous field of view (FOV) of the system, which can be as small as $100 \mu\text{rad}$, giving a linear resolution of 0.1 m at 1 km, which exceeds the requirements. (There are still good reasons to limit the FOV, as discussed below.)

A second technology that has been considered is passive infrared (IR) spectroscopy (single or multipixel), which offers no range information but has been exploited primarily as a detector for chemical vapor threats.⁶ To date, passive IR has demonstrated a sensitivity to bioagents that is > 10 times higher (worse) than can be achieved with lidar [65]; however, if it is to be deployed as a chemical vapor sensor, it would be very useful to determine its use as a biosensor as well. There have been recent efforts to combine passive IR with lidar to provide a combined standoff detection system for both bio- and chem-agents [66]. Since lidar shows the most promise for standoff (early warning) detection, we limit our technical descriptions in what follows to this technology alone.

B. Design Considerations

The signal for a standard backscatter/fluorescence lidar system (in the units of energy/meter) can be expressed

most simply as

$$E(\vec{R}, \lambda_0, \lambda_1) = \xi \frac{E_L A_0}{|\vec{R}|^2} O(\vec{R}) T(\vec{R}, \lambda_0) T(\vec{R}, \lambda_1) \times \left(\beta_{amb}(\vec{R}, \lambda_0, \lambda_1) + \beta_{thr}(\vec{R}, \lambda_0, \lambda_1) \right) \quad (5)$$

where parameters are defined in Table 3.

The goal of the lidar sensor and algorithm is to detect and map β_{thr} regardless of the values of β_{amb} and $T(\vec{R}, \lambda_{0/1})$. Where applicable (e.g., for an aerosol comprising similar particles, such as bioparticles), the backscatter coefficient can be related to a cross-section $\beta = n\sigma$, where n is the density (in $\#/\text{m}^3$) and σ is the backscatter or fluorescence cross-section (in m^2/Sr). (Note this is a differential cross section as compared with the previously

Table 3 Parameters in (5)

Parameter	Meaning
\vec{R}	Range vector from sensor (m)
$\lambda_{0/1}$	Wavelength transmitted/received
ξ	Detection efficiency
A_0	Receiver aperture (m^2)
E_L	Transmitted optical energy (Joule)
$0 \leq O(\vec{R}) \leq 1$	Overlap of transmitted light and receiver FOV
$T(\vec{R}, \lambda_{0/1})$	Transmission of transmitted/received radiation along the vector \vec{R}
$\beta_{amb/thr}(\vec{R}, \lambda_0, \lambda_1)$	Ambient/threat backscatter (or fluorescence) coefficients (m^2/Sr)

⁶http://www.jpeocbd.osd.mil/page_manager.asp?pg=7&sub=15.

defined total cross section in (1).) It should be noted that the concept of a cross-section is less useful for aerosols that have a broad range of sizes such as dusts. It is difficult to determine n from lidar data without additional information or assumptions concerning the cross-section.

Transmission of radiation to and from a target is a key factor in the performance of a system under specific local conditions. The transmission functions can be expressed as

$$T(\vec{R}, \lambda) = \exp\left(-\int_0^R (\alpha_{amb}(z, \lambda) + \alpha_{thr}(z, \lambda)) dz\right) \quad (6)$$

where $\alpha_{amb/thr}(z, \lambda)$ are the ambient/threat extinction coefficients (with units of m^{-1}) at each point. In the case of elastic scattering, $\lambda_0 = \lambda_1$ and the transmission becomes $T^2(\vec{R}, \lambda_0)$. When the threat is small and the ambient transmission coefficient is sufficiently constant, the total transmission reduces to

$$T^2(\vec{R}, \lambda) \approx \exp(-2|\vec{R}|\alpha_{amb}(\lambda)) \rightarrow (1 - 2|\vec{R}|\alpha_{amb}(\lambda)) \quad (7)$$

where the arrow indicates the limiting case for low extinction. Under such conditions, and with the additional assumptions about the relationship of α and β , (5) can be solved for the total α and β . These so-called lidar inversion

methods become increasingly difficult as the transmission becomes low. For a good review, see [67].

Fig. 9 illustrates that the backscatter coefficient of a potentially dangerous aerosol threat could be small when compared to that of ambient aerosols. This also demonstrates that it is important to have a method to discriminate biological matter from ambient aerosols since simply looking for an excess of aerosols will not likely offer sufficient information in many cases. It is also clearly advantageous to be able to detect a threat near its release point, where the density is highest.

As a starting point, one can envision a standoff system that offers aerosol ranging only, leaving discrimination to other (e.g., point) detectors. The choice of wavelength will be determined by several factors: 1) the backscatter cross-section of the threat, 2) atmospheric transmission, 3) in-band ambient light, 4) eye-safety limitations, 5) efficiency of detectors, 6) availability of sources, and 7) unobserved (e.g., stealthy) operation. For a typical 1–2 μm -sized aerosol-particle threat, the cross-section decreases dramatically with increased wavelength [68]. At wavelengths much larger than the particle, the rate of decrease approaches the Rayleigh limit ($1/\lambda^4$). In the UV-visible near-infrared (NIR) range, there is a tradeoff between atmospheric transmission, which increases at longer wavelength, and cross-section, which is decreasing at longer wavelengths. At longer wavelengths, the solar background is also decreasing. Finally, there is the concern of eye safety. It is not practical to have all personnel wear laser safety goggles at all times when the sensor is operating.

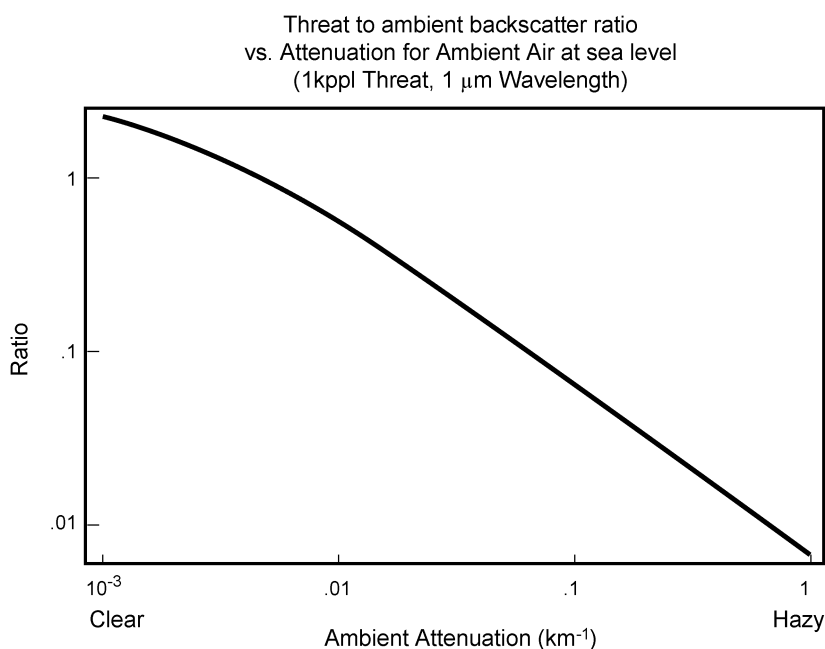


Fig. 9. The ratio of the backscatter coefficient of a 1000 particle per liter (kppm) bioaerosol threat to the backscatter from ambient aerosols ($\beta_{thr}(\vec{R})/\beta_{amb}(\vec{R})$) over a range of ambient conditions (clear to hazy). Even under clear conditions, backscatter from this threat is only ~2 times that of the ambient aerosols and molecules. In hazy conditions, the backscatter from this threat drops well below that of ambient aerosols.

This leads to the preferred use of a source of wavelength just above 1.5 μm over the more commonly available 1.06- μm Nd:YAG, which is transmitted and focused by the human eye. It is also possible to use an ultraviolet source (< 400 nm) where the eye does not transmit and thus the allowed power level is higher than in the visible. The range at this wavelength is greatly reduced over the NIR, however. Using an NIR source, aerosol releases in the 1 kpl (thousand particle per liter) range have been detected at ranges up to 15 km under clear conditions. Regarding the issue of background light, it is important to reject as much of the out-of-band signal as possible using an optical filter. It is also advantageous to narrow the instantaneous FOV as much as is practical (matching also to the transmitted light), thus raising the signal over the background.

Detector efficiency is also a consideration. The goal is to detect as many of the received photons as possible with minimal additional noise. In UV-visible wavelengths, one can use photomultiplier technology, which offers high quantum efficiency (QE) and very low dark current. In the NIR, photomultipliers are no longer responsive, leaving the choice between a photodiode (PD) and an avalanche photodiode (APD). These can be silicon through about 1.1 μm but must be InGaAs at longer wavelengths (such as 1.5 μm). Since the received signal is typically small, the APD is typically preferred over the PD, allowing for good QE, but suffering from relatively high dark current. A fairly recent development has been the photon-counting (Geiger-mode) APD, which detects a single photon at a time, providing a sensitivity that approaches the ideal “white noise” limit [69].

C. Historical Approaches

As in point trigger systems, the most widely studied standoff biosignature is UV laser-induced fluorescence (LIF) [70]. The principal standoff fluorescence signature is due to the presence of NADH, which can be excited using UV wavelengths in the 300–360-nm range. The fluorescence lifetime is on the order of 1–2 ns; thus it can be range-resolved with the backscatter signal [71]. Atmospheric transmission is a major concern in choosing the excitation wavelength for a standoff UV-LIF system. A commonly transmitted wavelength is the third harmonic of Nd:YAG (355 nm), since Nd:YAG is a highly reliable solid-state laser source. Other sources have been suggested as providing a higher signal [72]. Some systems have opted to use shorter wavelengths (such as the fourth harmonic of Nd:YAG, 266 nm), which excite additional fluorophores (such as tryptophan), but this further limits the range/sensitivity of the system. Of course, one could consider using multiple excitation wavelengths, but it is not clear that the payoff would justify the additional complexity.

For the past several years, the DoD has been developing the lidar-based Joint Biological Standoff Detection System.⁷ The goal of this effort is to produce a system that

is capable of near-real-time “generic” (nonspecific) bi-discrimination over a wide area (up to 3 km from sensor). An additional capability is detection and tracking of aerosols—without any specificity—out to 15 km.

As a standoff signature, fluorescence has the complication that it is relatively small (when compared to backscatter) and lies within the solar band. The return signal can be either collected into a single detector using a broadband optical filter or dispersed spectrally into multiple detectors using (for example) a grating [73]. Spectral dispersion offers the possibility of additional discrimination between aerosol species in cases where there is adequate signal to interpret the resulting spectrum. The spectra of many biological species are all quite similar. They are broad and contain no sharp features and, unfortunately, are also similar to that of diesel exhaust. The problem of ambient light is particularly difficult in the case of fluorescence. This is due to the relatively small cross-section and because of the broad fluorescence band. In contrast, elastic backscatter occurs at the transmitted wavelength, so that the receiver may include a narrow-band optical filter that rejects a large fraction of the ambient light. In both cases, it is advantageous to reduce the divergence of the transmitted beam and corresponding FOV of the receiver as much as possible.

Given the limited range of UV, it is advantageous to combine IR ranging with UV-LIF, creating a standoff sensor with at least three channels: UV scatter, UV-LIF, and IR scatter. This is analogous to point trigger sensor technologies described in Section III. Such a sensor can map aerosols at many kilometers and discriminate at shorter ranges (e.g., 1 km) with greatly improved sensitivity in dark conditions. The backscatter return of the two transmitted wavelengths can be compared to get a rough determination of particle size, and the UV-LIF signal can be normalized by either or both of the scatter channels.

D. Looking Forward

In the future, it would be advantageous to develop methods that do not rely on fluorescence, still emphasizing methods that suggest improved sensitivity and selectivity. Recent work has demonstrated that polarization-sensitive infrared lidar may be useful for standoff biodiscrimination [74]–[77]. Polarization lidar is sensitive to the size, shape, and index of refraction of the aerosols and has been exploited for differentiating ice crystals from water droplets in clouds [78]. One feature of biospecies is that their shape, at least in their natural form, is highly regular. Even in aggregate form, the underlying shape of individual species is retained. The question of whether this signature is sufficiently selective must be answered through field and laboratory measurements. Another emerging technology is multiwavelength differential scatter (DISC) in the 9–11- μm range [79]. This technology has been demonstrated for detecting chemical vapors and has recently shown promise as a biodetector as well. The method utilizes a frequency-agile CO₂ laser, which transmits a burst

⁷http://www.jpeocbd.osd.mil/page_manager.asp?pg=7&sub=19.

of 18 different wavelengths in rapid succession, with separate range returns from each. The process is fast enough that the returns from each wavelength can be directly compared, giving a backscatter spectrum as a signature.

Given the wide variety of ambient conditions and the high infectivity or toxicity of some biological threats, it is probably impossible to develop a standoff system that will have acceptable performance for detecting small, yet still dangerous, releases. Standoff systems are better suited for detecting releases that would inflict massive casualties if countermeasures were not taken. Added benefits include rapid deployment, mobile deployment, wide area coverage, and hazard mapping.

V. ENVIRONMENTAL AND MEDICAL DIAGNOSTICS

A. Confirmation

It is tempting to speculate that, if we can detect clouds of bioaerosol in open air, then we should be able to use that information to determine how to treat an exposed populace. While some medically useful information can be derived from environmental sensing (such as the extent of exposure and contamination), one drawback to applying the optical techniques that have been developed for aerosol detection to the area of clinical diagnostics is the relatively nonspecific nature of the optical signatures of agents themselves. Fluorescence, Raman spectroscopy, and Fourier transform IR, for example, are all good candidates for biological/nonbiological discrimination of aerosols and even for classification of agents into likely subgroups, such as bacteria, viruses, etc. However, they cannot at this time provide the specificity required to effect appropriate treatment. Until such time as new signatures and *in vivo* optical techniques are developed, treatment-driving diagnostics will rely on acquisition and assay of a sample (the *confirmation* step). That sample may be an aerosol collection into liquid or onto a filter, a surface wipe from a contaminated area, a nasal swab from a suspected exposed person, or a clinical sample, such as blood or sputum, from a person presenting with symptoms at a hospital or other point of care. In general, the assay techniques to perform identification fall in the same few categories regardless of the sample medium, thus, we will focus the subsequent discussion on assays in use or development for medical diagnostics. The techniques described in this section range from direct optical detection, such as microscopy, to optical signal transduction of chemical or electrochemical processes. We attempt to summarize the methods in common use and note where advances are being made or are needed to better address the identification problem.

B. Biological Culture

Many of the technologies developed for the identification and confirmation stages of a biodetection system (as

posited in Section I) stem from those developed for clinical diagnoses. In particular, acquisition of a sample and subsequent culture and examination provide the basis for the most definitive agent identification—it is the “gold standard.” Culture is used not only to provide some initial confirmation of the suspected agent type (by selection of a compatible culture medium) but also to establish the viability of the suspect agent sample and to provide abundant copy numbers for subsequent assays. While bacterial agents will propagate in a variety of media, viral agents can only propagate in a cell culture; thus some *a priori* suspicion of agent type is usually required to select the appropriate culture medium. Various aspects of the cultured colonies, such as their color, shape, surface features, and growth patterns, are all used to aid in identifying the agent and derive primarily from optical microscopic evaluation. Despite the wealth of information obtainable from culture, and despite the reality that it is still the prevailing diagnostic tool in the medical arsenal, it suffers from a serious drawback. Results can often take days to acquire, and those days are either spent not treating a sick person or presumptively (and possibly incorrectly) treating a patient without sufficient information to do so.

One way to address this shortcoming has been the development of automated culture machines that can offer faster turnaround (< 1 day) and culture of hundreds of different bacterial species as well as determine antibiotic resistance to tens of common antibiotics [80], [81]. Many major hospitals have these machines; while they occupy less space than a microbiology laboratory, they are still out of the price range and availability for most medical facilities. Other systems have been developed to provide more rapid bacterial culture identification on a more limited basis. These rely on introduction of a clinical sample to a liquid culture medium in a small vial, for example [82]. Changes in the medium color are optically sensed as the microorganisms grow, and results are often available within several hours. Again, these systems are not commonly used, but their existence suggests that industry recognizes the need.

C. Immunoassay

Immunoassay tests rely on immunological recognition of an infectious agent or protein marker, typically through the use of agent-specific antibodies and some form of optical signal transduction. The assays are usually rapid (15 min to an hour) and can vary in sensitivity depending on the sample medium, the agent under suspicion, and the assay format. Common formats include wicking tickets (such as is used in a home pregnancy test) and reagent color-change (rapid Strep A test). A patient presenting with flu-like symptoms in a typical U.S. hospital will likely be given a rapid influenza test in the Emergency Department; a sore and reddened throat would prompt a rapid Strep test. If a rapid test is negative, a specimen would be sent to a microbiology laboratory (sometimes in the hospital), where the specimen would undergo a screening

panel for standard respiratory viruses. These panels are typically some form of enzyme immunoassay, such as Enzyme-linked Immunosorbent Assay (ELISA), or some kind of fluorescent-antibody-based stain [83]–[85]. The basic mechanisms for signal reporting derive from binding of the suspect antigen (which is typically immobilized on a substrate) with enzyme-bound antibodies with fluorescent or colorimetric properties. Trained personnel are required to run these assays; and, due to staffing limitations and cost, they tend to be batched and conducted only once or a few times per day. The specimen is often cultured for bacteria as well, depending on the treating physician's observations and recommendations. While antibodies, and corresponding immunoassays, have been developed for most of the biological threat agents, these are not currently part of the screening panels in clinical use. Thus, some other indication that an event had taken place, or the progression of illness of the exposed populace, would be required to alert points of care to conduct bioagent testing.

For environmental sensing, military systems have been developed that automatically collect an aerosol sample upon action of an optical trigger (as described in Section III). The collected sample is then injected onto an array of immunoassay tickets, which are then read by an optical reader and by visual inspection. These tickets, called handheld assays, are useful for first identification of a suspicious biological release event but are typically neither sensitive nor specific enough for clinical-diagnostic applications. There are also immunoassay formats where, for example, the antibodies are immobilized on fiber-optic probes [86]. These can offer higher sensitivity than the ticket format and have been developed into field-portable instruments for biological agent detection [87].

In general, immunoassays can be limited in sensitivity and in the specificity required to provide adequate information for appropriate treatment (such as antibiotic resistance), as well as in their utility, given the current assays' one-agent-per-test designs. There are commercial systems, such as those based on electrochemiluminescence (ECL) and surface plasmon resonance (SPR) [88], [89] and developmental systems, such as CANARY [90], that have demonstrated higher sensitivity than typical immunoassay. ECL systems exploit antibody binding for specificity, but the binding reaction is measured using chemiluminescence on an electrode surface, thus the optical signal is generated in the absence of any native background. SPR refers to the interaction of light (at specific angles) with delocalized electrons in thin metal films (plasmons); analytes bound to the back surface of the film can alter the local refractive index and thereby change the deflected angle of the incident light. CANARY (developed at MIT Lincoln Laboratory) represents a novel assay that consists of genetically modified murine B-cells engineered to express antibodies for biological warfare agents and to emit photons in the event of pathogen binding. The B-cell assay, which is suitable for aerosol as well as clinical

samples, responds within seconds to a pathogen binding and offers sensitivities rivaling that of DNA analysis (see Section V-D). The problem of single-agent responsivity can be addressed by developing multisite microarrays of bound antibodies or other antigenic recognition compounds. Multisite antibody microarrays looking for protein markers for parasite infection, for example, have been commercialized [91]. While the research community has developed some multianalyte arrays for bioagent detection [92], the medical diagnostic community has yet to develop broad-spectrum multiagent antibody (or protein) microarrays for bioagent screening, and it may be that the only real impetus to do so would be if they served a more general respiratory-disease diagnosis function. In other words, there is no real commercial market for bioagent detection assay panels, but a more general panel that included sites for bioagent markers might inspire investment.

D. Nucleic-Acid-Based Assays

The primary mechanism for DNA analysis is via polymerase chain reaction (PCR) [93], which is an amplification scheme for producing abundant copy numbers of agent-specific genetic sequences. When dealing with environmental or clinical samples, such as blood or sputum, some sample preparation is required to separate the DNA under test from the enzymes and other chemicals that degrade the DNA and/or inhibit the PCR amplification process. When presented with purified DNA, PCR can provide rapid (on the order of an hour), highly sensitive (fewer than ten gene copies), highly specific (subspecies level, in some cases) identification of a biological organism. While it is not necessary to know the exact sequence of the gene being amplified, it is necessary to know the sequence that flanks the amplified regions (primers). These sequences have been determined for all the high-priority bioagents on the CDC threat list⁸ and have been developed to work with PCR devices ranging from laboratory-grade to handheld. Originally, PCR devices served only to amplify gene copy number; sequence determination usually required an additional step, such as gel electrophoresis or fluorescent assay. Modern real-time PCR machines serve both functions, and do so by incorporation of a fluorescent label (probe) during the amplification process [94]. Thus, as the copy number increases (twofold with each PCR thermal cycle), the fluorescent signal can be tracked in real-time. Specificity derives from the primer sequence being used (only the correct sequence will amplify); sensitivity from the amount of light output reported in a given number of thermal cycles. Although PCR is a DNA amplification process, RNA can also be analyzed through the process of reverse transcriptase, enabling identification of both bacterial and viral agents. At this time, PCR is primarily a laboratory analysis, although the number and use of field-portable devices is

⁸<http://emergency.cdc.gov/agent/agentlist-category.asp>.

growing, especially by the military in environmental and clinical sample analyses.⁹

As with immunoassay, a limitation to PCR is that one must know which agent one is looking for (*a priori* knowledge of the primer sequences) and that each reaction is usually specific to a single agent (although there are multiplex PCR processes that query for several possible agents at once). There are DNA microarrays comprising thousands of probes labelled with complementary DNA (cDNA) that have been developed for (originally) gene expression studies and (eventually) also for host expression studies [95]–[97]. The microarray format could permit hundreds to thousands of concurrent pathogen screening assays to be conducted, provided that one has enough organism-specific knowledge with which to select the cDNA probes. A small pilot study, funded by the U.S. Air Force and the Defense Threat Reduction Agency, called Epidemiological Outbreak Surveillance, developed a pathogen DNA microarray that was used to test for respiratory diseases among recruits in training at a U.S. Air Force base [98]. Results were encouraging, in that early diagnoses of highly contagious diseases such as adenovirus were enabled, thereby providing information to recommend isolation of infected individuals and limit the subsequent spread of the disease. To our knowledge, however, the Holy Grail of the bioagent or even pathogen genome chip has yet to be realized. The chips are expensive, the optical readers are expensive, and the assay times for the large-site-number chips are often several hours. Thus, while DNA microarrays are promising means of conducting large numbers of concurrent tests from a single clinical sample, they have not yet matured to the practical level of common use.

E. Looking Forward

The aforementioned schemes for identifying biological agents from environmental and clinical samples have many variations, designed to make them more sensitive, faster, cheaper, etc. In general, there is a tradeoff between the speed of the assay and the specificity/sensitivity. The faster a correct diagnosis is made, the sooner the patient can receive appropriate treatment and the less likely that a patient will die. Furthermore, the first diagnosis of a biological warfare agent will set in motion a public health response to determine if the patient was the victim of an attack or of a natural exposure. Thus, timely diagnosis is critical not only for the presenting patients but also to minimize fatalities in the (possibly much larger) exposed populace. Techniques to increase assay speed need to be developed, but it is essential that the assays also provide

⁹For example, the R.A.P.I.D and RAZOR systems from Idaho Technologies Inc.

treatment information so that appropriate therapeutics can be administered. A rapid diagnosis of a bacterial infection is useless without a concurrent understanding of what antibiotics that bacteria is responsive to. In addition, the limits of detection of the assay must correspond to clinically relevant levels, ideally even those found in exposed but pre-symptomatic individuals. An ideal medical system would comprise a diagnostic device cheap enough to be used at every point of care (POC), that screened for many common and uncommon (including bioagent-based) infectious diseases, that offered direct patient benefit while a presenting patient was still at the POC (say, 15 min or less), and that relayed relevant information to local and regional networks in real time, so that infectious disease patterns would be detected as they occurred. An ideal environmental monitoring system would perform an identification assay in real-time, following a trigger event, or frequently enough to influence a treatment distribution decision in a timely manner. Although there are many novel approaches under way to address these requirements (including single-molecule chemical detection, nanoparticle detection techniques, chip-based mass spectrometry, etc.), optical technologies that are suitable for use under a variety of environmental conditions, that are minimally invasive to the patient, that use simple chemistries and optical sources (such as LEDs), and that offer robust high-throughput operation will be at the vanguard of the next generation of rapid diagnostics.

VI. SUMMARY

Biological agents have features that an adversary may find attractive as an agent of war or terrorism. To protect potential victims from infection and harm requires rapid and accurate detection and identification of bioagents. Since such agents could be used at any time or place, it is well to consider plausible scenarios that help to guide sensor system design. Two such scenarios were outlined here. For detection and identification of biological-warfare agents in the environment and in the laboratory, electrooptical techniques have found widespread application. The principal approach to early warning sensing today is laser-induced fluorescence, taking advantage of ubiquitous biological constituents that emit light in response to excitation in the UV (particularly tryptophan and NADH). However, since UV-LIV sensing is not specific, additional steps of identification must take place. Even the most robust present-day field sensors require a final confirmation step prior to taking any medical measures. Today, that final confirmation is accomplished through laboratory assays that may involve culture, PCR, immunoassay, or a combination of these. ■

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