

Shedding Light on Real-time Biodetection

The Need for Biodetection

A major factor in reducing our vulnerability to the escalating threat of biological weapons on the battlefield or in civil defence lies in enhancing biological agent detection technology. Indeed, the speed with which released biological agents are detected is paramount to warn of the impending danger in sufficient time to reduce casualties. Clearly, the best defence is for people to avoid contamination altogether (this is known as the "detect to warn" philosophy). However, specific agent identification is also necessary to administer the appropriate medical treatment to those who have been exposed (the "detect to treat" principle).

Biodetection, unfortunately, is not a straight-forward problem and much of the difficulty relates to the tremendous lethality of the agents which are typically a million times more effective on a mass basis than chemical weapons. The lethal dose of inhalation anthrax, for instance, is just 10000 spores – about one billionth of a gram. Consequently, sensors must be exceptionally sensitive to register relatively low concentrations of micron-sized cells. Furthermore, these deadly particles must be distinguished against an enormous concentration of background particles that will inevitably be in the air; typically one is considering a ratio of one target particle against a background of hundreds of others. To make matters even worse, a significant portion of the background, such as pollen or benign bacteria, has similar biological nature to the agent itself and is therefore difficult to discriminate.

Different Biodetection Approaches

The silver bullet sought by everybody is to achieve BW agent detection that is both instantaneous and specific. In reality, though, there is a spectrum of competing technologies, each making compromises between speed, specificity and cost. Perhaps the most specific and sensitive practical technique is PCR (Polymerase Chain Reaction), a biotechnology approach amplifying and matching DNA code from a sample. The method is capable of staggering sensitivity, detecting a single cell in about 30 minutes – too long for the "detect to warn" principle. Similarly, there are immuno-assay approaches that also give specific analysis. The major drawback with specific technologies, however, is the requirement for specialist chemical consumables that add considerably to the logistic burden and the running costs which can reach hundreds of dollars per hour.

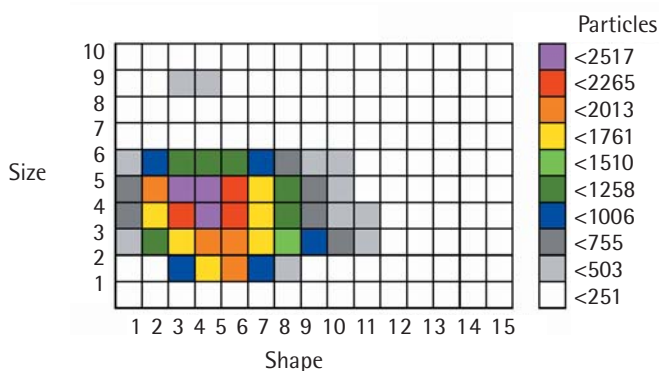
Conversely, at the other extreme, optical sensors have been used for genuine real-time biodetection in military systems for many years. These devices have no requisite for expensive consumables and are therefore cost-effective to run for long periods. The notable disadvantage, however, is the lack of specificity where optical sensors offer, at best, a generic detection. For instance, different species of bacteria, whether highly pathogenic or benign from a natural source, are optically far too similar to distinguish. It follows that the principle of these devices is not to detect hazardous levels of pathogenic material in the environment, but instead to monitor for significant changes in bioaerosols indicating

and civilian situations. In challenging environments, fuel oils can give rise to many tens of fluorescence false alarms per day.

Other concerns relate to these sensors' complexity, power requirement, and lifetime owing to their Q-switched laser sources. Here, the non-linear crystals involved in the UV frequency conversion are susceptible to catastrophic optical damage, limiting the lifetime to just several thousands of hours.

ASAS Technology

Aerosol Size And Shape (ASAS) Technology is a different approach to optical biodetection and has been deployed in the British Armed Forces' Prototype and Integrated Biological Detection Systems (PBDS and IBDS) for over six years. Additionally, Smiths Detection has been using ASAS Technology in biodetection systems such as NBCerberus. The principle of this technique involves passing a stream of single particles through a visible laser beam and analysing the elastically scattered light. The intensity of scattered light indicates the size of the particles and the spatial asymmetry of the scattered light corresponds to the particles' own asymmetry.



Example of Size, Shape and Concentration Signature for an Aerosol Background Using ASAS Technology

Like other optical sensors, ASAS Technology is not capable of identifying agents specifically itself but the technology is especially effective at building up an ambient aerosol "finger print". The background particles are characterised in real-time with respect to size, shape, and concentration. Algorithms and neural networks are then employed to pick out systematic changes in the background indicating the onset of a significant new aerosol source. The fundamental strength of the technology is its excellent sensitivity with the capability of identifying minor changes against a complex background. Additionally, many sources of background particles can be neglected owing to these particle groups' distinct size and shape properties. For instance, fuel oils (such as diesel and aviation fuel), smokes and some bioaerosols such as pollen can be discriminated from the threat, greatly reducing the potential for false alarms.

Next Generation Sensors

There is a now distinct path for how real-time biodetection sensors must mature to enhance their performance and increase their practicality. Indeed, the primary market drivers for the future optical sensors are:

- Reducing false positives (false alarms)
- Decreasing device size, weight and cost
- Improving long-term reliability

For the next generation of fluorescence sensors, manufacturers such as General Dynamics and TSI are now attempting to design out the bulk UV laser sources and integrate new UV semiconductor lasers which offer tremendous advantages in terms of size and cost. Although diode laser technology is progressing quickly towards shorter wavelengths, the current practical options for wavelength are limited to 370nm or greater, constraining the sensors to NADH excitation without capability of toxin and virus detection. There will, of course, be great interest when diode lasers and high power LEDs become available at the preferred wavelength of around 280nm - perhaps in as little as 3 to 5 years.

the onset of an attack. Optical sensors, as a result, are commonly deployed in several major defence scenarios. Firstly, in military point-detection systems integrating different technologies, optical sensors reduce the running costs by triggering the operation of slower and more expensive identification technologies. Another concept of deployment in integrated systems has been to run the identifiers continuously with the real-time sensors which function as "alerters", warning troops to put on protective equipment. The final possibility is an extensive network of real-time sensors incorporating data fusion to offer area protection of a strategic facility such as an airbase or a port.

The practical optical techniques that have been used in systems to date have employed lasers to physically characterise aerosol particles in terms of independent parameters and so neglect the major part of a variable background. Two separate optical philosophies have dominated which have developed on either side of the Atlantic. The approach in the US has been to analyse particles in terms of size, fluorescence, and concentration where the best known devices are BAWs and BARTS (General Dynamics) and FLAPS (TSI). In contrast, the UK's Ministry of Defence has used a different philosophy characterising particle size, shape, and concentration in the form of ASAS Technology (manufactured by Biral).

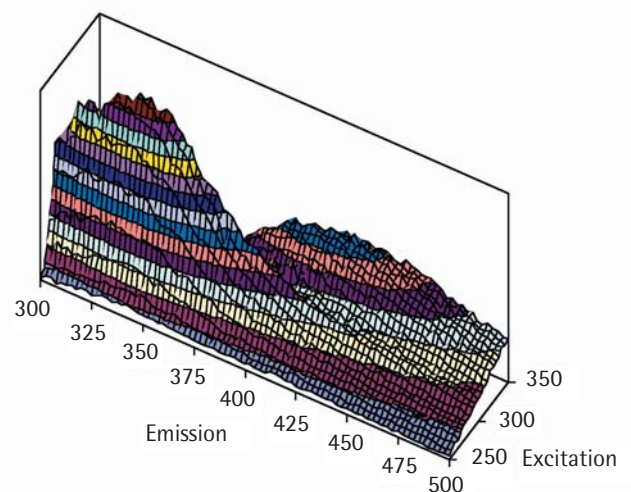
Fluorescence Technology

Fluorescence sensors illuminate a stream of single particles with ultra-violet lasers to excite biofluorophors – molecules that are only present in biological particles and emit a signature fluorescence. The measurement of significant fluorescence indicates that a particle is biological. By monitoring for systematic changes in the concentration of bioaerosols, larger fluctuations than those explained by natural processes, this generic sensor triggers an alarm. Fluorescence sensors have already proven themselves effective and have been deployed in major point detection systems such as the US military's

JBPDS. The current designs, however, have important limitations – most notably susceptibility to false positives, long-term reliability issues and cost.

Historically, two commonly available varieties of pulsed UV lasers have been employed in sensors which are differentiated by wavelength (266nm and 355nm). 266nm laser light primarily stimulates the amino acid tryptophan, a component of proteins and therefore incorporated in bacteria, toxins and viruses. In contrast, 355nm sensors seek to excite NADH, a molecule involved in aerobic respiration within cells. It is notable that at this longer wavelength, the bacteria's intrinsic fluorescence is much weaker and additionally it has not been demonstrated that toxins and viruses (which contain no NADH) are detectable.

One major hurdle with UV fluorescence techniques is that other commonly-occurring particles can emit a similar fluorescence, confusing the sensor. Perhaps the most important of these interferents are fuel oils such as diesel and aviation fuel – both very common materials in military



Excitation and Emission Spectra of Bacteria Showing the Absorption Peaks at around 280nm and 350nm. (Results kindly provided by Dstl, UK)

At Biral, our strategy is to introduce a family of real-time biodetection sensors with a number of technical innovations enhancing performance and practicality. Self-evidently, one variety of device will not fit all biodetection applications and we believe that a range of optical sensors with a differing performance / cost mix is the best way to address the needs of networks of low-cost sensors along with those of point-detection triggers.

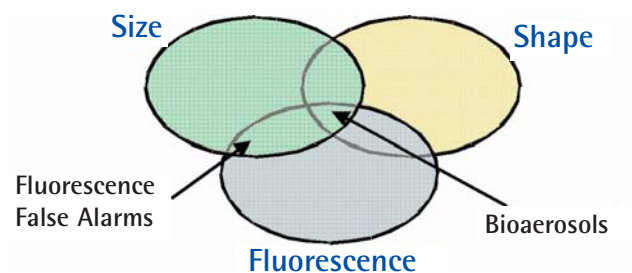
Our approach to reducing the probability of false alarms has been to increase the number of measured independent particle parameters, giving more information to reject insignificant background particles from the data. The new Biral VeroTect sensor unites the US and the UK optical detection philosophies by characterising particle size, shape, concentration and a set of fluorescence parameters. No other real-time sensor analyses as many particle parameters. Furthermore, we were motivated to retain the advantages of 280nm tryptophan excitation (for detection of bacteria, toxins and viruses) and we have incorporated an innovative low-cost light source with a lifetime of many years



Summary

Optical techniques for biological agent detection have been deployed by the UK and US military for many years. Although these sensors are only capable of non-specific or generic detection, they have notable advantages over biotechnology approaches for identification including speed of response and low operating costs. Consequently, there are two major applications for these devices, either as a real-time trigger / alerter in integrated point-detection systems or in a network of low-cost sensors.

Optical biodetection technology is developing fast and there is now a move away from high-cost bulk lasers. Furthermore, new designs will physically characterise a greater number of particle parameters to achieve a higher level of generic discrimination.



Characterising More Independent Physical Parameters of Particles Leads to Enhanced Discrimination

