# Real-time biological agent detection using particle size, shape and fluorescence characterisation

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## ABSTRACT

Developments in real time optical biological agent detection and sensing are presented which describe start of the art advances in the detection and warning of these pathogens. The following paper describes the basic operating principles of the current BIRAL ASAS™ (Aerosol Size and Shape) system which measures the optically determined particle properties, on a particle by particle basis, and uses the information to describe the size and shape characteristics of the aerosol. Furthermore, recent development of the existing technology to also encompass fluorescence detection is described, which significantly increases the detection ability of the ASAS™ aerosol suite. This operational improvement is a major advancement in the field of airborne biological agent detection and allows for near generic detection and warning. Applications of this device include all aspects of bio–aerosol monitoring, including the use as a biological agent detector and generic identifier, use as a general bio-agent monitor and also for use as a hazardous environment monitor. Such a device would be particularly useful in the fields of Armed Forces protection and National Defence either as a point detector or as a "plug and play" biosensor detector in a network.

Keywords: Aerosol detection, biological agent detection, particle count detection, size detection, shape detection, fluorescence detection, biological warning, biological trigger, detect to warn, point detection.

## 1. Introduction

Biological agents pose one of the greatest risks to personnel at the current time. Biological agents are extremely lethal to organisms that can be infected by the agent and pose a very high risk due to the very small amount of agent which constitutes a lethal dose. For example, a lethal dose of inhaled anthrax is a very small fraction of a gram. Such agents are typically many orders of magnitudes more effective on a personnel level than chemical agents. As a consequence of this high degree of lethality, biological agent detectors need to be exceptionally sensitive. They must be capable of detecting low levels or even single particle agents against a constantly changing background aerosol distribution, which may represent background to agent ratios of hundreds to one or lower. A further problem arises from the fact that biological agents are also intrinsically similar to naturally occurring biological backgrounds, including pollens, spores and benign bacteria. Given these challenges, biological agent detectors need to be sensitive enough to detect and differentiate on a level where the agent poses a threat to human health and also to be able to warn about an impending attack in sufficient time for appropriate action to be taken.

The field of biological agent detection has received considerable interest recently due to the relative ease with which an agent can be manufactured and disseminated. The threat is high on the list of possible attack scenarios due to the extremely small amounts of agent required to cause widespread disruption and devastation. While there are considerable problems in attempting a bio-agent attack, such as ensuring that the agent is still viable when human

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contact occurs and the fact that the production and transportation of agents is rigorously controlled, the high impact of such an assault makes bio-agents a potential method for terrorist activity. An additional problem with bio-agents is the similarity between very lethal pathogens and seemingly benign organisms. The effect of an agent combining properties of both could result in extreme lethality. For example a highly worrying scenario would be the combination of elements from a lethal agent with a benign but highly infectious organism, such as a flu or common cold virus or similarly infectious bacteria. In such a situation the effect of the combined bio-agent would result in considerably more damage than the original due to higher infection rates and possibly increased longevity in the environment. The genetic engineering of such a pathogen is not currently possible although new forms of naturally occurring diseases and methods available for the artificial manipulation of bacteria are increasing.

## 2. Different detection approaches

The ideal solution for a real-time detector is a biological organism specific response that results in almost instantaneous, specific and repeatable identification. However, there are considerable technological and practical difficulties in the development of sensors that provide a real-time response for all three of these criteria. As a consequence, the need for real-time information on which to make an informed decision concerning a potential threat results in a relaxation of the need for specific identification to one of a more general nature. In the most likely scenario where specific identification is not possible, it is likely that very similar organisms would result in the 'detection' of a bio-agent, even though some of these 'agents' do not actually pose a threat. While such a situation is not ideal, the extreme lethality of bio-agents means that such a semi-specific detection would be acceptable as a warning device. In situations where there is a high level of disruption it is likely that a generic detection coupled with a small probability of false alarms is required. However, in a dangerous environment or if there will be little disruption, a much-reduced specificity would allow for a high degree of protection to personnel who could be potentially exposed. There are two main detection strategies involved in the protection of personnel – "detect to warn" and "detect to treat". The former is used in situations where there is sufficient time for personnel to be protected or even evacuated, and the latter in situations where exposure has already occurred, or is imminent and treatment is the only remaining cause of action.

There are various strategies currently employed to detect bio-agents – all of which result in a compromise between the specificity, speed and the cost of ownership to the user. Biotechnology offers the most specific detection approach and the Polymerase Chain Reaction (PCR) technique is capable of the amplification and detection of a DNA sample from a single bio-agent cell within 30 minutes. This technique is too slow to be used as a 'detect to warn' sensor, but could be used as a specific sensor once an agent attack is in progress and used to identify appropriate medical treatment. Immuno-assay techniques also give a similar specific analysis. However, an additional drawback, on top of the long response time, is the requirement for specialist chemical consumables that add considerably to the logistic burden and running costs that can add hundreds of dollars per hour to the operational cost.

At the other extreme, optical technologies intrinsically result in real-time bio-detection and devices based on these technologies have been available to military and civil defence organisations for a number of years. The response time for these systems arises from the computational processing of the data and as a consequence can be considered as genuinely real-time – Figure 1. These devices have little or no consumables and hence a low logistics burden on the deployment, can be run continuously to provide 24 hour detection capability over periods of months or longer and are therefore extremely cost effective. However the common drawback of this type of sensor is the lack of specificity, with sensors mostly offering a generic detection capability at best, since the optical similarity of the target particles with benign, naturally occurring backgrounds makes them difficult to distinguish. As a consequence of this lack of specificity this type of sensor has been used to monitor changes in the ambient aerosol concentration and distribution and thus provide an indication of the onset of an attack. One major advantage of these systems is that they can be used as an early warning device and used to trigger more specific detector technologies, such as PCR, thus significantly reducing running costs. Also, networked sensors could be implemented where the response to an 'attack' could be looked at over the network and responses due to localised background changes or localised and benign man-made aerosols ignored. This type of technology represents the principle of 'detect to warn' and is

generally used as a trigger for other technologies or as networked point detection sensors. Common applications include the protection of an area of importance such as a military battlefield, airbase or port, or a civilian area such as a country's major population centre or major airport.

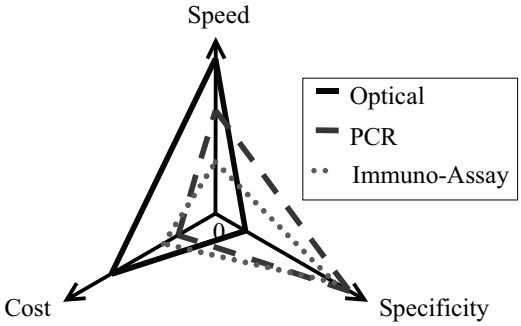


**Figure 1**. Advantages of different detection strategies.
The figure graphically describes the relative abilities of the optical sensors and the biotechnological approaches of PCR and Immuno-assay. For example the optical technique produces a faster response than either the biotechnologies and is more cost effective but is not as specific.

### 2.1. Historical optical detection philosophies

The practical design of optical systems to characterise aerosol distributions uses lasers to determine independent parameters of the particle distribution as a whole. In this manner a map of the background can be constructed using the independent parameters and any changes to this map can then be considered as a change in the aerosol background. However, the situation is complicated by the fact that the aerosol background is constantly changing and any sampling of the aerosol distribution needs to be sufficiently large so as to reduce statistical variations in the sample with respect to the background. As a consequence of this sampling strategy, sensors that characterise the aerosol in as many different independent parameters as possible have a higher degree of differentiation between the background and potential agents.

There have been two separate historical strategies that have dominated in the US and UK. The US approach has been to analyse particle distributions in terms of the count and size. In contrast the UK's MoD has used a strategy which is based on the philosophy of count, size and shape in the form of the ASAS™ technology manufactured by BIRAL, UK. More recently the US has adopted the use of fluorescence as well as the size and particle count, with established commercial sensors including the BAWS and BARTS devices from General Dynamics and FLAPS manufactured by TSI.

In the US strategy an ultra-violet (UV) laser is commonly used to illuminate a stream of particles to excite fluorophores contained within bio-agent cells. In this manner only those particles that contain the fluorophores of interest can be differentiated from the background by the fluorescence signature of the particles. When this information is linked with the size and count differences between the background, the bio-agents can be distinguished and alarms triggered. While this type of sensor has been used in major point detection systems, such as the US military's JBPDS (Joint Biological Point Detection System), it has limitations such as susceptibility to false alarms and long term reliability and cost issues.

There have been two historic approaches to the wavelength of laser system used in this form of detector, which are based on common solid state bulk laser systems to produce 266 and 355 nm radiation. These laser wavelengths stimulate different molecules associated with biological cell activity, with the 266 radiation stimulating the amino acid tryptophan, a component of proteins and therefore present in agents of interest (e.g. bacteria, viruses and toxins). A sample fluorescence profile is presented in Figure 2. The 355 nm wavelength excites NADH, (Nicotinamide Adenine Dinucleotide), an aerobic respiration-involving molecule and therefore present in biologically active bio-agents, e.g. bacteria. The limitations of the different wavelengths are that the 266 nm source is harder to control and suffers lifetime damage problems, while the 355 nm sources are not as efficient exciters of the bacteria's NADH with respect to 266 and tryptophan. One specific advantage of the 355 nm excitation strategy is the large fluorescence

from bacterial growth media – however this can be removed from the bacteria by cleaning. Furthermore it is questionable whether viruses and toxins (no NADH) can be detected using the 355 method. Additionally, these laser sources are expensive and require large amounts of electrical power due to their inefficient 'wall-plug' operation. Also these systems require cooling and in some cases this is achieved using water and heat exchangers and the systems also require regular maintenance.

Another of the limitations of the fluorescence technique is that commonly occurring aerosol particles also emit similar fluorescence to the bio-agents of interest, and thus these particles are likely to confuse the sensor and result in too many false alarms when a challenging environment is encountered. Perhaps one of the most worrying of these interferants is fuel oil, which would be prevalent in any situation where such a sensor is likely to be placed, whether military or civilian. One way to reduce the risk of false alarms from fluorescing interferants is to combine the data with other properties of the particle. Even so false alarms can occur many tens of time a day with this type of sensor.

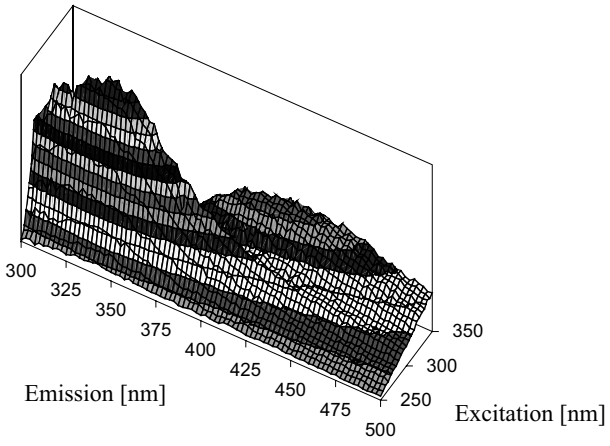


**Figure 2**. Emission spectra for a sample bacteria for different excitation wavelengths.
From the figure it can be seen that the fluorescence response is much stronger for excitation wavelengths around 280 nm. At this wavelength there is a strong response over the 300 – 400+ nm range. For excitation around 350 the fluorescence emission is significantly reduced and is shifted to higher wavelengths. Furthermore lots of biological and non-biologically active materials fluoresce in the emission band for 350 nm excitation and thus this wavelength produces a large number of false alarms. 280 nm excitation is the preferred source for optical fluorescence detection of biological materials.
(Results kindly supplied by DSTL, UK).

By contrast the UK has traditionally adopted the use of size, shape and count information for the aerosol distribution to distinguish the particle distribution. This technique uses the elastically scattered light from a stream of particles to calculate the size and shape of the particles and uses the information to produce a "finger print" for the aerosol distribution. In this way changes to the aerosol profile can be easily detected in the size and shape information and the particle count used to set alarm thresholds when a change in the size-shape distribution has been confirmed. This device, or variants on it, have been used by the UK Armed Forces for over six years in PBDS (Prototype Biological Detection System) and will be deployed in the upgraded IBDS (Integrated Biological Detection System). It has been also used by companies such as Smiths Detection in bio-detection systems such as NBCerberus. The device itself is not capable of specific identification but is very effective at detecting changes in the particle distribution. Algorithms and neural networks can then be utilised to pick out tell-tale changes in the distribution indicating the onset of a significant new aerosol source. Fundamentally this technology is extremely sensitive due to the capability to determine minor changes in aerosol backgrounds. Additionally, a crucial advantage of this sensor is the ability to differentiate between the many sources of bio-agent interference and potential agent releases due to the distinctive size-shape distributions. Common interferants such as fuel oils (diesel, aviation fuel and petrol), smokes, combustion products and natural bio-aerosols such as some pollens can be discriminated and hence this ability greatly reduces the risk of false alarms over situations where this level of discrimination is not available – see Figure 3.

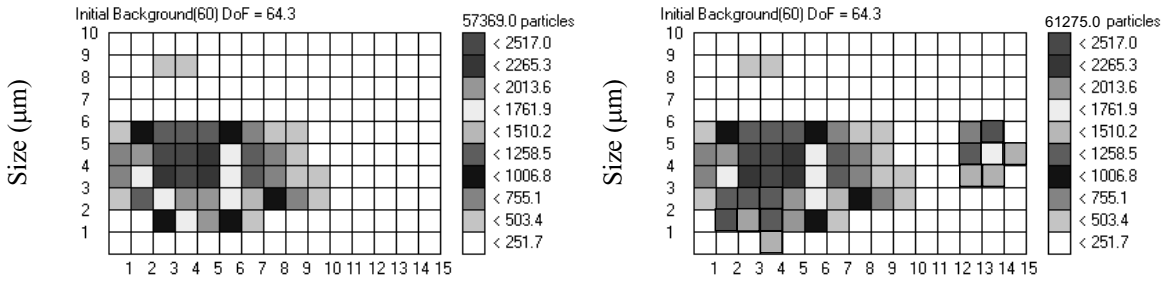


Figure 3a. Figure 3b.

**Figure 3**. Example of ASAS™ size & shape discrimination.
The size and shape information can be used to determine the background particle profile (Figure 3a). While this profile is constantly changing, it is possible to track the changes and to detect relatively small aerosol events that do not match the background profile (Figure 3b). Once a sufficient number of particles representing a different aerosol profile have been recorded a warning will be produced. In this figure the change in the lower left-hand side represents a potential threat and the change in profile in the middle right represents an obscurant or other non bio-agent aerosol.
(Images for illustration purposes only.)

### 2.2. Next generation detector capabilities

The next generation of biosensor to reach the market has to achieve considerable advancements over the current standard. Sensors must be capable of providing a mature technological base that is capable of large improvements in performance and practicality, with the major market forces being:

- Reduction of false alarms (both *positive* and *negative*)
- Increased discrimination
- Improved reliability and reduced sensor down-time
- Reduced device cost, weight and size

One method currently being considered / employed is to design out the need for large, complicated and expensive bulk solid state lasers with the use of new semiconductor lasers. Given the advancements currently underway in the field of semiconductor lasers and with the benefits including reduced costs, size and complexity it appears that these devices would be a useful alternative to the conventional solid state laser. However, despite the current high level of anticipation these sources are limited in their wavelength capabilities and are only capable of producing wavelengths around 400 nm in a reliable and cost effective package suitable to this type of application. Given the current state of development it is likely that a source in the desired 266-300 nm emission wavelength band would be capable of integration into bio-sensors sometime within the next 5 years.

The strategy at BIRAL is to introduce a family of real-time bio-detection sensors with a number of technical innovations enhancing performance and practicality. No single instrument will fit all biodetection applications and it is our belief that a range of optical sensors giving a variety of performance versus cost options is the best way to address the needs of networks of low cost sensors along with those of point detection triggers.

Biral's response to the aforementioned market forces has been to increase the number of measured independent particle parameters giving more information to reject irrelevant background particles from the data and reducing the probability of false alarms. The result is a bio-sensor that is capable of detecting four independent particle characteristics; size, shape, fluorescence and particle count – see Figure 4. To the best of our knowledge no other optical biosensor is capable of real-time determination on as many particle discriminators. For this new development we have retained the advantages of 280 nm excitation of tryptophan by the introduction of a novel light source. This new source is an innovative introduction to the bio-sensor detection capability of the instrument and will provide a

low cost alternative to the conventional solid state laser source. Furthermore, the new device has a lifetime of years, requires no maintenance, is reliable and also of importance is that the optical performance degradation is negligible over the lifetime of the device.

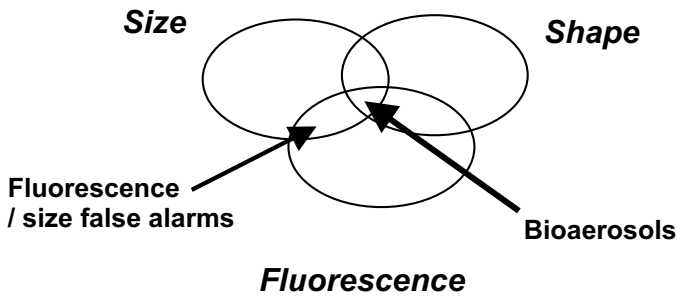


**Figure 4**. Representation of the reduction in false alarms through the use of multiple particle characteristics.
The false alarm rate of a sensor can be reduced by increasing the number of independent aerosol characteristics. The specific advantage of the introduction of the fluorescence characterisation is that the false alarms that would occur between the size-shape, or the size-fluorescence information are significantly reduced. The intention behind this strategy is remove as many of the false alarms as possible and only leave those alarms that actually look like potential bio-agents. It can not be assumed that a previously unseen (or uncharacterised) bio-agent is not present and so the systems should not be over-designed in such a way that these agents are missed. This can be achieved by the cross-correlation of as much information as possible: i.e. size, shape, fluorescence and count characterisation.

It is the symbiosis of these two detector strategies (UK and US) and the experience BIRAL has gained with the UK's ASAS™ technology that makes us believe that the addition of fluorescence measurements will lead to a large step forward in the field of biological aerosol detection. The new detector called VeroTect™, has been and is currently involved in a series of in-house and external trials, including tests with bacterial and viral agent simulants. In addition to these tests, the device has also been characterised for common interferants. The device has shown very good trials performance and the combination of the ASAS™ size and shape characterisation with the fluorescence detection has proved to be capable of the detection of bio-agent simulants. It is not our intention to provide a detailed performance for the VeroTect™ bio-sensor in this paper, however a brief summary of the expected performance based on the current design is described.

## 3. VeroTect bio-sensor

As described above the new bio-sensor manufactured by BIRAL envelops the detection strategies of size, shape, fluorescence and particle count. The device is a complete USB interfaced detector that is designed with remote operation and surveillance in mind. The device can be operated via battery power, has a low power consumption and a near zero logistics burden. As with the ASAS™ devices on the market today, the detectors are factory calibrated and the size and shape detector channels are not adjustable. However, in order to increase the usability of the device the fluorescence detection system is user programmable. The fluorescence detection works on the bulk media principle and is supplied by the same sample airflow used to measure the size and shape profile of the aerosol sample – see Figure 5. In this way the fluorescence and size-shape information can be directly related. Furthermore, the rate at which the bulk fluorescence information is calculated can be adjusted to increase the time resolution of the instrument – a function that will produce more temporally accurate information in times of heightened risk of bio-activity. In addition to this functionality it is proposed that the instrument can be used in a manner that will allow automatic gain control of the fluorescence measurements so that when there is very high biological activity the device will provide a reduced sensitivity. This would ensure that the information gained has a large dynamic range, but is still capable of the required resolution. By using such an automatic gain control it is envisaged that the device will be capable of producing fluorescence information in even the most challenging situations and as a consequence would exhibit no loss of detection capability due to the presence of large amounts of fluorescent interferants.

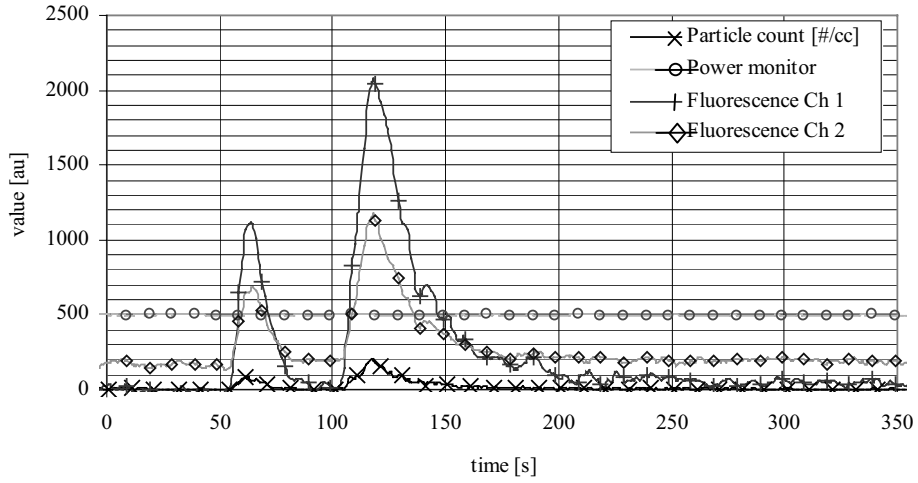


**Figure 5**. Measured bulk fluorescence on an aerosol sample by the VeroTect™ instrument.
The data displayed shows the particle count recorded (×10), the Ultra-violet (UV) power monitor and the measured response of two fluorescence channels. Fluorescence Channel 1 shows the response in the near UV and Fluorescence Channel 2 the response in the near UV to Visible. The relative responses of the fluorescence channels can be used to describe the fluorescence properties of the bulk aerosol and it is clear for this sample that the UV response is significantly larger than the response of the visible channel shown. In this example the sampling rate was 2 Hz, although this is user variable. Note the data points shown are for illustrative purposes only and different fixed off-sets have been applied to the data sets to aid in the presentation of the graph.

The sensor has a detection capabilty that exceeds that of the existing ASAS™ systems. While the underlying operating principles are the same for the size-shape information for both instruments, the VeroTect™ instrument has additional software functionality that allows for greater processing control of the data. Both systems are capable of detecting size particles in the detection range of 0.5 – 15 microns in diameter and shaped particles are represented in asymmetry factor or aspect ratio format, with values ranging from 0-100 (0 being perfectly spherical). The VeroTect™ software allows for the adjustment of the size-shape information displayed to the user and as a consequence the user can concentrate on the area that is of most interest[*1]. This ability allows for an individual instrument to be calibrated for a preferred detection range (likely detection ranges are 0.5-10, 0.5-15 or 0.5-20 microns diameter, although other ranges could be offered as custom designs). Furthermore additional graphical displays have been incorporated that allow for greater ease of use and are simpler to understand by the user. The operational ability to detect fluorescent bio-particles has been assessed by the use of simulants (both biological and non-biological) and the device is expected to be capable of a resolution down to counts of viable bio-agent particles of approximately $10\ell^{-1}$ as measured in the local aerosol environment. In principle the bio-detection capability is limited to the fact that the actual aerosol distribution measured is dependant on statistical variations and only measurements on the level of single particles or greater can be detected. This resolution uses the 'laboratory' based determination of aerosol concentration for discretely generated aerosol samples (both biological and non-biological in nature) and an instrument with a concentrator to increase the sampled particular count. The concentrator used in this calculation was ×10 over the size range of particles sampled and the concentrator was taken to be 100% efficient[*2].

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[*1] The information used in the detection algorithms uses the raw particle data and the accuracy of the algorithms are not effected by the resolution of the graphical displays in any way.

[*2] Please note that the concentrator efficiency, although not wholly accurate, need not necessarily over-estimate the performance as continual on-going development to the device would more than compensate for any errors in this approximation.

As well as the ASAS™ functionality described above the new sensor has a suite of discrimination algorithms which use the comparative analysis of data to information stored in a library, such as common interferants (e.g. fuel oils). The library can be updated and new aerosol information introduced, either by BIRAL or the user, to enhance the identification capabilities of the device. For the purpose of security the library is stored on the control computer and not onboard the instrument. This security feature serves two main purposes. Firstly the instrument can be positioned in sensitive areas where the risk of tampering is large without the risk of sensitive information, for example discrimination algorithms or bio-agent properties, falling into the possession of unauthorised personnel. Secondly the library can be updated remotely and access to the library is available to new sensors added to a network without the need of modification to existing or new sensors. Such functionality leads to a much increased sensor capability and as a consequence a far wider range of applications for the user. Additional functionality is also likely to be available with the device, with possible modular additions including the use of Global Positioning Systems (GPS), meteorological sensors and other commercial devices. BIRAL already has experience of, and is a manufacturer of meteorological sensors and produces ruggedised, fully weather proof and reliable sensors in the well-known HSS sensor range. It is anticipated that the device will find markets in applications where bio-agent detection is required due to health concerns. Furthermore, the device need not be used only in military and civilian bio-detection projects, as the technology also lends itself to general national defence applications, particular monitoring and monitoring the level of bacterial activity in the environment. Further applications are in the field of general particle monitoring, including the sensing and description of combustion products, leading to the ability to detect personnel and vehicle movements remotely and automatically.

## 4. Conclusion

In conclusion optical sensors have been developed and used for military applications in bio-sensing and hazardous environment monitoring for many years. More recently, advancements in the knowledge of optical biodetection have lead to a new generation of sensors that allow wider deployment and greater detection capabilities. Optical bio-detection offers advantages over other forms of bio-agent detection, most notably in the speed of response and the low logistics burden and operating costs. As a consequence of the advancements in optical detection these devices are now at the forefront of detector capabilities and allow for applications as real-time triggers for more specific technologies in integrated point-detection systems and also in networked systems of low cost sensors.

Optical capabilities are advancing in the form of new technologies and there is now a general move away from the bulk solid state laser employed in the early generations of optical fluorescence biological agent detection. The development and availability of low cost optical sources will further enhance the detection capabilities of the next generation sensor. These new sources also offer the advantages of a significantly reduced cost of ownership, both initially and with respect to the continuing logistics burden, are more compact, are likely to offer increased lifetimes and will offer reduced power budgets. The advent of these new optical sources and accompanying technologies will pave the way for bio sensors with increased detection capabilities and a greater degree of discrimination.

BIRAL believe that the introduction of the new range of biosensor heralds the move towards finely targeted generic or near-specific detection. Furthermore, the use of multiple parameters to characterise the aerosol distribution leads to the better discrimination of aerosol events and that the use of this increased detection capability will lead to an improved ability to detect and warn when such aerosol events occur. A new product soon to be launched by BIRAL, VeroTect™, will be the first of a new generation of such sensors and that with the on-going development of the underlying sensor technologies BIRAL will continue to remain at the forefront of development in this field.

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