

A New Real-time Biological Agent Characterisation System

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Biological Agent Detection, Biological Agent Trigger, Detect to Warn, Point Detection, Biological Detection Algorithm

ABSTRACT

The following paper describes the core technology of a real time optical biological agent detection system which has been developed from Biral's existing and proven ASAS™ family of biological agent triggers and sensors. The sensor for the system has additional capabilities based on the introduction of a fluorescence sensor providing greater discrimination than previously available. The new sensor will be incorporated in the Integrated Sensor Management System (ISMS) which utilises complex real-time data tracking algorithms that simultaneously monitor the different aerosol characteristics. The system is capable of tracking and adjusting the sensor alert levels to take into account the constantly changing aerosol environment and thus significantly reduces the risk of false alarms. The use of two measurement systems, ASAS™ and fluorescence in one sensor is a unique combination and a major advancement in the field of airborne biological agent detection and warning.

INTRODUCTION

Biological warfare agents are difficult to detect. They are specific pathogenic organisms that cause disease in humans but chemically and physically they are very similar to the wide range of, largely innocuous, particles of biological origin that are found in the atmosphere. Traditionally the only way of detecting them with any certainty was by utilising their binding properties to specific antibodies or, more recently, by their genetic fingerprint. Such techniques are still required to specifically identify a disease causing organism but they are relatively time-consuming, require collection into liquid and use highly specialised consumables.

It has been recognised for many years that there is a need for rapid analysis techniques that can be carried out in airborne suspension that generically identify particles that are likely to contain agent material. The first developments to achieve this, at least in part, are incorporated in Biral's ASAS™ technology and this has been deployed by the UK armed forces in both the Prototype and Integrated Biological Detection Systems (PBDS and IBDS).

Both PBDS and IBDS are, in effect, mobile laboratories housed in military vehicles as shown for IBDS in Figure 1. They contain a suite of analytical techniques that have a range of responses from the real-time non-specific recognition of a new source of particles in the atmosphere to specific identification using antibody biochemistry that takes around 30 minutes. They also require a skilled crew and all the military

systems needed to enable them to survive independently in the battlefield. As the systems are costly to operate and require skilled manpower their numbers are necessarily limited.



Figure 1: Integrated Biological Detection System

To supplement IBDS there is a requirement for a much simpler system that can be deployed over a wide area, can operate without the need for constant attendance, and can deliver a warning of the presence of an agent cloud with a high level of confidence. To meet this requirement Biral have developed VeroTect™ and, in partnership with Serco Assurance Ltd, are producing a networked biological sensor for the Integrated Sensor Management System (ISMS).

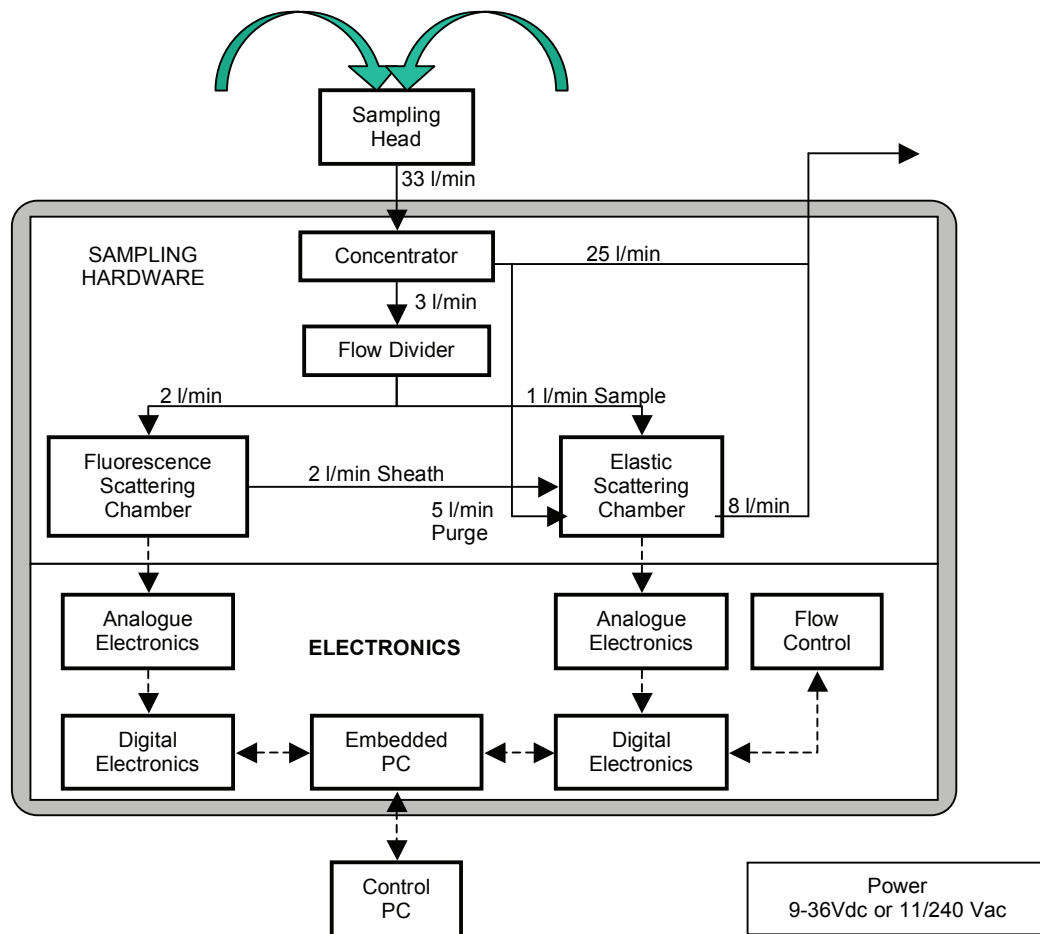
PRINCIPLES OF VEROTECT

VeroTect integrates two measurement systems: the Biral ASAS system that was developed first for PBDS and subsequently extensively refined for IBDS together with a novel technique for the measurement the fluorescence characteristics of the aerosol. The Verotect sensor is shown in figure 2a and 2b. A simple functional block diagram is shown in figure 2c.



Figures 2a, 2b: VeroTect bio-sensor

Figure 2c: Functional block diagram of the Verotect bio-sensor



PARTICLE SHAPE AND SIZE MEASUREMENT

The ASAS systems use the spatial analysis of the light scattered by a particle to classify its shape in terms of an asymmetry factor. The integrated intensity of the scattered light is used to determine a size parameter for the particle and the number concentration of the aerosol is determined from the number of pulses of light that are counted. A schematic diagram of the ASAS chamber as used VeroTect is shown in figure 3.

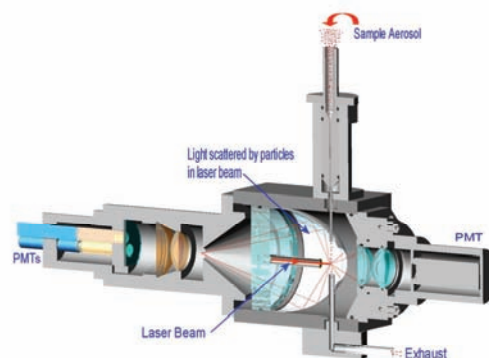


Figure 3: ASAS Scattering Chamber

The value of ASAS is that it provides a non-specific, multi-parameter classification of a mixed aerosol that enables new sources of particles to be recognised and quantified against a variable background of existing sources. Figure 4 shows examples of the types of data that is generated by ASAS for particles of different sizes and shapes.

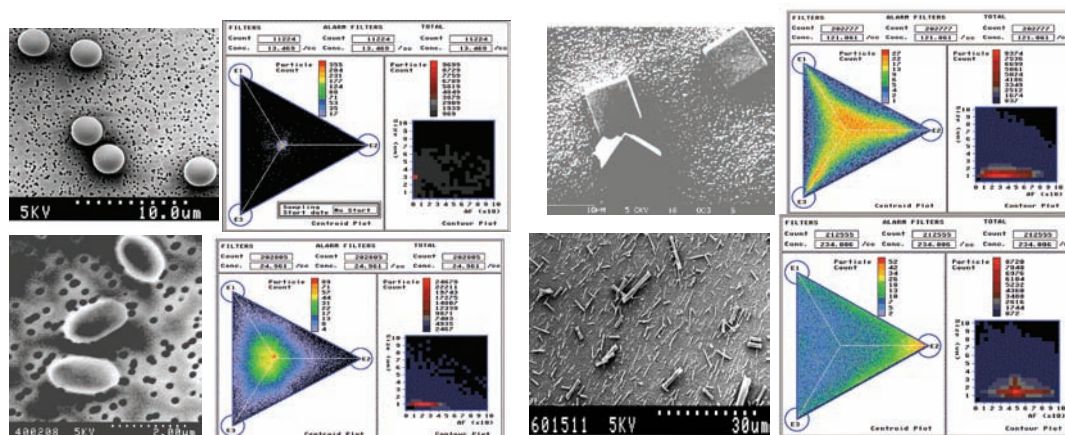


Figure 4: Examples of ASAS data from particles of different shapes and sizes

The data produced by ASAS has been shown to be highly suitable for training artificial neural networks, particularly those of the Kohonen map type. Once trained the network can continuously analyse the data from an instrument and monitor the concentration of particles that fall within the parameter space of each of the maps.

ASAS now has a long pedigree as the technique of choice for the real-time, non-specific characterisation element of BW agent detection systems. It was originally chosen for the UK's first BW detection system, the Prototype Biological Detection System (PBDS), and has operated successfully in that system for close to ten years. Shortly after this it was also employed in the Interim Naval Biological Detection System (INBDS) Such was its success in these roles that it was selected for further development as the non-specific detector for the Integrated Biological Detection System (IBDS), that is about to enter service.

BIOLOGICAL CHARACTERISATION BY INTRINSIC FLUORESCENCE

Although the ASAS technology has clearly demonstrated its value as a non-specific detector of biological aerosols it cannot differentiate the arrival of a cloud of particle containing biological agent from a new source of innocuous material with a similar parameter set. In PBDS and IBDS the generic differentiation of cellular organisms from other components of the atmospheric aerosol is achieved using luminometry. However, this is a biochemical technique that requires collection into liquid and the use of delicate and expensive consumables. It is therefore unsuitable for use in a robust, compact and unattended sensor.

There is only one candidate technique that is capable of differentiating micro-organisms from the majority of the components of the atmospheric aerosol while they remain in airborne suspension and that is intrinsic fluorescence. Fluorescence occurs when electron bonds within a molecule are moved from their ground state to an excited state by high-energy photons, such as those supplied by UV light. There is then a decay process back to the ground state that emits photons with a lower

energy (generally in the long wavelength UV or visible parts of the spectrum) and this is the fluorescence output.

Bacteria, viruses and proteins all fluoresce when excited by UV light. The major fluorophore in cellular organisms is the amino-acid tryptophan with lesser contributions from proteins such as NADH and the flavins. These materials are common to all cellular organisms and so, although fluorescence may be used to differentiate organisms from other particles, it is very unlikely that it could be used reliably to identify specific organisms. Figure 5 illustrates the fluorescence emission spectra of three very different bacteria, including one that has been grown in two different nutrient broths

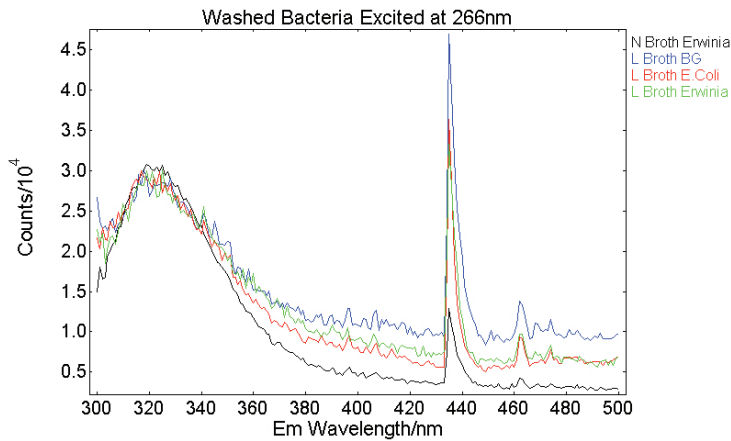


Figure 5: Emission spectra for washed bacteria excited at 266nm

Cultivated bacteria, that have not been cleaned as part of the production process, are likely to have growth medium in the aerosol particles. This growth medium will also fluoresce though with different characteristics. Figure 6 shows the excitation and emission spectra of the same bacterium (a) washed and (b) unwashed.

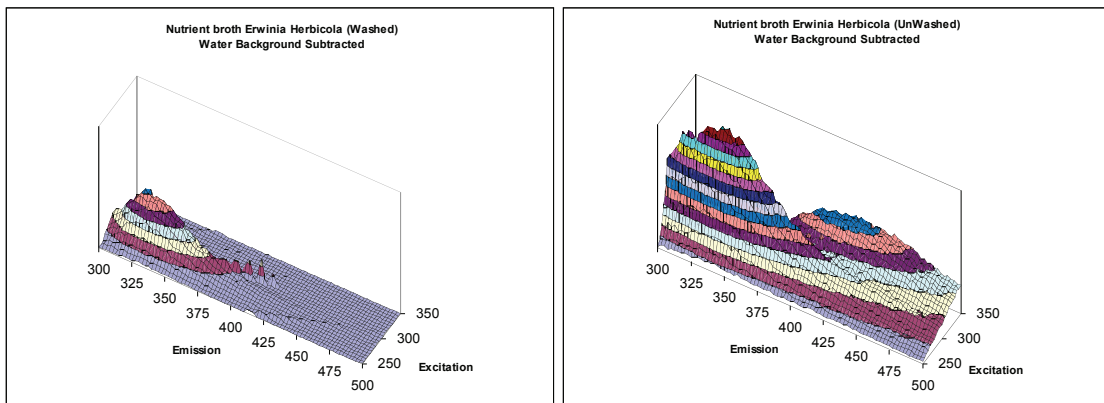


Figure 6a: *Erwinia herbicola* – washed

Figure 6b: *Erwinia herbicola* - unwashed

ULTRA-VIOLET LIGHT SOURCES

The fluorescence yield from the cellular fluorophores is low and their concentration in micro-organisms is also small and so in order to excite detectable levels of fluorescence a high intensity UV light source is required. The earliest systems (1,2)

for detecting fluorescence in airborne particles used CW ion lasers but these were large, fragile and operated at wavelengths that were too long for efficient fluorescence generation.

The optimum excitation wavelength for cellular organisms has been shown, in a number of laboratory studies, to be around 280nm. However, there is a fairly broad wavelength band that is capable of producing fluorescence, albeit with a lower yield. It is also well established that the excitation and emission bands are changed if there is growth medium attached to the organism; the growth medium both broadening and intensifying the response.

Using the best available laser technology there are only two UV wavelengths that can be produced that give a high intensity beam from a reasonably compact and robust package. They are the third and fourth harmonics of the solid state family of lasers and these yield wavelengths around 355nm and 266nm. The two wavelengths have competing advantages and limitations: 266nm, being closest to the optimum excitation wavelength excites fluorescence most efficiently and is the only one of the two that can produce fluorescence from organisms that have been washed clean of growth medium. However, 355nm may be generated more efficiently and is effective in detecting the growth medium associated with unwashed organisms.

The current generation of solid-state harmonic lasers are complex, delicate and expensive components that are not suitable for incorporation into systems where multi-point detection or widespread field monitoring may be required. Because of this, considerable effort is presently going into the development of compact and robust semiconductor sources capable of delivering continuous wave sub-300 nm radiation. In particular, the US SUVOS (Semiconductor Ultraviolet Optical Sources [3]) programme, which commenced in 2002, is making significant gains towards this objective and has already demonstrated prototype LED (light emitting diode) devices capable of room temperature continuous 280nm emission at milliwatt power levels.

Published results from existing particle fluorescence systems allow the determination of an approximate lower limit for the satisfactory excitation and detection of fluorescence from typical individual particles containing micro-organisms. This shows that, in general, a particle must experience a UV fluence of the order of 200-300 $\mu\text{J}/\text{cm}^2$ for each steradian of fluorescence collection angle for adequate signal-to-noise in the acquired signal. Where the fluorescence is separated into spectral bands an increase in the fluence in proportion to the number of spectral bands will be required.

The required UV energy may be delivered to the particle in a few tens of nanoseconds from a Q-switched, solid-state harmonic laser. However, for continuous-wave lower power sources such as the early generation UV LED's, millisecond timeframes would be required. In the latter case the need to retain a single particle within the focused beam for such relatively long periods would be technically difficult and would compromise the overall effectiveness of the instrument. Therefore, while UV LED's, and ultimately UV diode lasers, offer the best prospects for compact, low power and lower cost bio-aerosol fluorescence sensors in the future, device output powers and lifetimes must be increased very significantly before they can be regarded as a viable option.

The only alternative sources of UV light of the required intensity are Xenon flash tubes. These are broadband sources but the output can be tailored to provide the required excitation wavelength band by the use of appropriate filters. The fluorescence characterisation element of VeroTect employs a miniature Xenon tube

the output of which is tailored to provide an excitation pulse centred on 280nm in order to achieve the optimum fluorescence yield from particles containing micro-organisms.

THE FLUORESCENCE MEASUREMENT CHAMBER

To accommodate the different characteristics of xenon lamp illumination, as compared to laser excitation, a large measuring volume is used in which there may be many particles present. The particles will be a representative sample of the atmospheric aerosol and may include fluorescing interferences such as diesel oils or other fuels and lubricants. The fluorescent signal is collected with maximum efficiency from all the particles in the measurement volume using optical techniques analogous to those used in light scattering nephelometers. The integrated fluorescence signal is separated from elastically scattered light from the excitation source by highly efficient filtration and is split into two detection bands. A schematic of the optical arrangement of the chamber is shown in figure 7. The ratio of the intensity of the bands together with geometric characterisation from the ASAS system provides the data required by detection algorithms to reliably distinguish agent material from potential interferences.

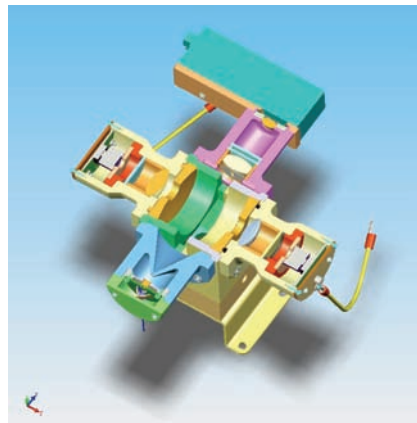


Figure 7: Schematic of the fluorescence chamber

SENSOR NETWORKING TO REDUCE FALSE ALARMS

All instruments designed to alarm in response to the detection of specific events will be subject to some level of false alarms, both false positives and false negatives. In most cases, and particularly the detection of biological warfare agent, the objective is to detect all events that could have an effect on the target. So the frequency of false negatives above the detection threshold must approach zero. To eliminate false negatives requires the sensitivity of the sensors to be maximised. However there is invariably an inverse relationship between sensitivity and false positives as moving the sensitivity towards the threshold increases the number of false events that fall within the detection sensitivity band.

Where a detection system consists of an array of similar sensors the problem of false alarms is greatly increased as the number of such alarms is the product of the number of detectors and the frequency of individual sensor false alarms:

$$\phi_A = n\phi_S$$

where: ϕ_A is the false alarm rate of the array, ϕ_S is the false alarm rate of the single sensor and n is the number of sensors in the array. So, for example, if a sensor is expected to produce one false alarm per day and this is used in a 12- element array it will produce a false alarm every 2 hours. One false alarm per day may just be acceptable to a user but one every 2 hours is unlikely to be. This problem is further enhanced for larger numbers of sensors.

Most groups that are developing detectors based on an array of sensors have elected to overcome this problem by networking the sensors so that they act as a single detector.

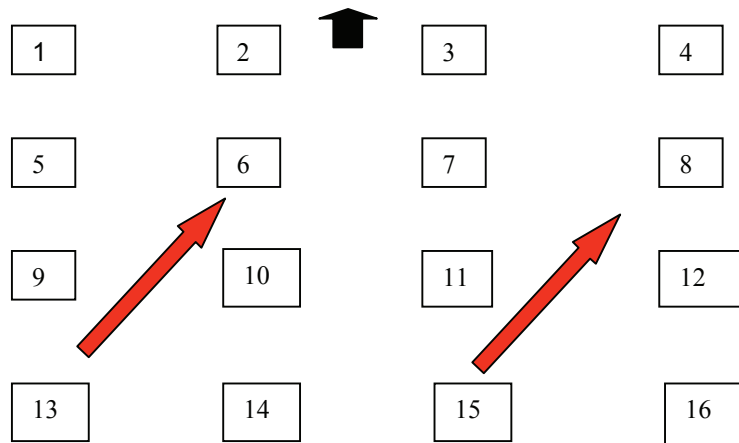


Figure 8. Networked sensor array

Figure 8 shows a 4 x 4 array of sensors with the wind crossing them from the southwest. In the case of wind-borne agent attack it may be expected that one of the sensors 9,13 or 14 would be the first to alarm. If the wind speed and direction are known then it will be possible to predict the time at which the other elements within the array will alarm. If the other sensors alarm within their expected time windows then it is highly probable that it is due to a real cloud passing across the array. If, on the other hand, there is no correlated response then the first alarm is most likely to be false.

This technique enables the false alarm rate from the array, treated as a single detector, to be reduced significantly below that for the single isolated sensors. Hence:

$$\phi_A = \frac{\phi_S}{f(n)}$$

So where the sensor may give one false alarm per day the array may only produce one per week.

The output from the sensors may be linked by algorithms of variable complexity, which may take into account the uncertainties in the wind patterns, the geography of the terrain, structures within or in the region of the array and the micro-meteorology.

CONCLUSIONS

A new sensor for the real time generic detection of biological agents has been developed that is compact, robust, suitable for unattended operation, and requires no reagents.

The use of two measurement systems, ASAS™ and fluorescence, in one sensor is a unique and innovative combination and a major advancement in the field of airborne biological agent detection and warning, providing significantly reduced false alarm rates.

The sensor has been selected for incorporation into Integrated Sensor Management System (ISMS) for the UK MoD on the strength of these technological advances.

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