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Using Light to Detect . . .

biological organisms in the atmosphere

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Until quite recently the only way to detect micro-organisms in the atmosphere was to collect them, along with all the other types of particle present, into liquid or onto a surface. They could then be grown on nutrients, examined microscopically or subjected to a range of bio-chemical assays to determine their characteristics. These procedures took hours and often days or even weeks to complete. Largely driven by renewed concerns over the use of biological weapons, work began in the late 1980s to look at how micro-organisms might be detected without the need to remove them from the atmosphere for analysis.

Analysing organisms in airborne suspension gives very clear time advantages. However, when the work began there were very few principles that might be developed to differentiate them from other types of particle. One of the major problems is that the micro-organisms of interest are likely to be a small proportion of the total particulate content of the atmosphere and so must be detected against a highly variable background of unknown materials both organic and inorganic. A second problem is that harmful organisms are chemically, and sometimes physically, very similar to benign materials that are very likely to be part of the background aerosol.

The techniques that had been developed for characterising airborne particles were extremely limited. The most common requirement was to count and size particles. Techniques had been developed, starting in the 1930s, to use the light scattered by particles when they passed, individually, through the focus of a light beam to both enumerate them and assign a size parameter. The size of a particle may be inferred from the intensity of the light it scatters but it is a relative measure. The light scattered varies in intensity depending on the size and shape of the particle, its optical properties and the angle from which it is viewed.

As the scattered light is a function of a range of properties of airborne particles it was an obvious starting point for improving the specificity of characterisation. For particles that are the same order of size as the wavelength of the light they are scattering, Mie theory predicts the angular pattern of intensity of the scattered light around the particle. The cylindrical symmetry of the light pattern can be shown to be a function of the geometrical symmetry of the particle and this was something that it was possible to measure by using an array of sensors.

An instrument that measured the size, shape and number concentration of aerosol particles demonstrated much superior performance in detecting new classes of particle in a mixed aerosol and has been incorporated in a number of systems for detecting biological agents. However, it cannot distinguish micro-organisms from other particles or even differentiate organic from inorganic material.

Researchers began to look at other properties that could provide a more specific discrimination and attention was quickly focussed on the intrinsic fluorescence, which is characteristic of all organisms. This was of particular interest, being an optical property that could feasibly be measured in parallel to the light scattering of particles. Fluorescence is a form of inelastic scattering where high energy photons excite electron bonds in a molecule, which then release a lower energy photon as they return to their stable, ground state. To excite fluorescence in the fluorophores that are present in micro-organisms requires ultra-violet light in the wavelength band 260-360 nm band.

It was soon demonstrated that the fluorescence from single particles containing micro-organisms could be detected and measured using techniques adapted from the light scattering instruments. To excite the fluorescence it was necessary to add a high intensity source of UV light and this could only be provided by relatively complex optical systems that used the 3rd or 4th harmonics of solid state lasers such as Nd:YAG to give wavelengths around 355 nm or 266 nm.

Although the systems developed are optically complex they have demonstrated very clearly that they are effective in differentiating particles that fluoresce from those that do not. When combined with the particle characterisation parameters obtained from the elastically scattered light, they can reliably detect a new source of micro-organism against the normal atmospheric background aerosol. Such instruments are now beginning to be incorporated into systems for the detection of biological weapons.

However, they are not perfect as a number of common environmental pollutants as well as some natural atmospheric components can be mistaken for micro-organisms, as they also fluoresce and may have similar light scattering properties. The drive now is to improve characterisation once again by spectral analysis of the fluorescence. The fluorescence spectrum varies from material to material and the evidence obtained from laboratory measurements indicates that effective differentiation from most of the likely interferents should be possible with broadband spectral differentiation.

There is also a drive, in parallel, to develop simpler, more robust and lower cost instruments. The focus here is the development of UV light sources that do not require highly complex non-linear optics. There are reasons to hope that, in the reasonably near future, UV laser diodes with the required wavelengths, power and lifetimes will become available. Until this day arrives it has been demonstrated that light generated from flashlamp sources can excite fluorescence from aerosols of micro-organisms in a system that can be both compact and robust.

Significant progress has been made in developing instrumentation for the detection of biological organisms in airborne suspensions and such instruments are commercially available. Ongoing research promises even better capabilities in the future.

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Figure 1

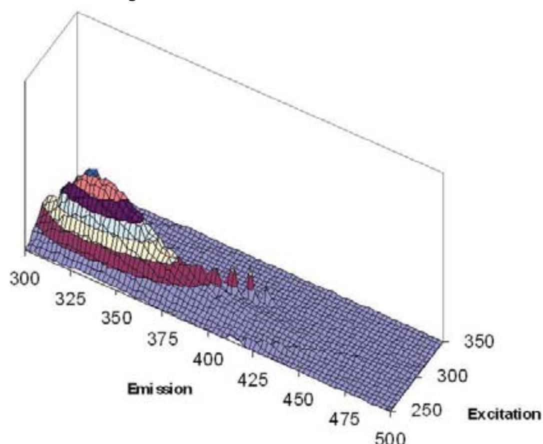
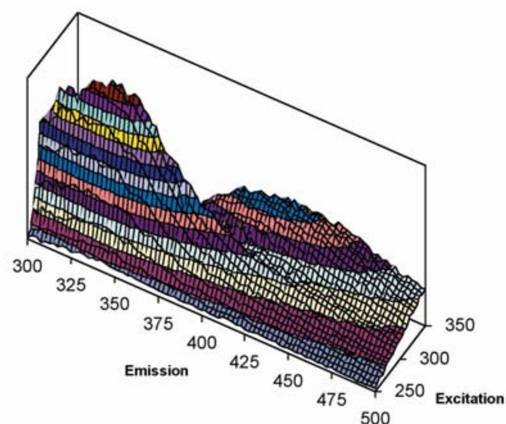


Figure 2



These figures highlight the noticeable difference in fluorescence response between washed bacteria (figure 1) and unwashed bacteria (figure 2). It is likely that washed bacteria will be undetected by commonly used 355 nm excitation sources.

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