

Moving particles with ultrasonic standing waves

Optical evanescent biosensors such as Light Scattering Surface Plasmon Resonance [1] (LS-SPR) and Metal Clad Leaky Waveguide [2] (MCLW) are being developed by Dstl for potential application in the detection of BW agents in the field (Figure 1).

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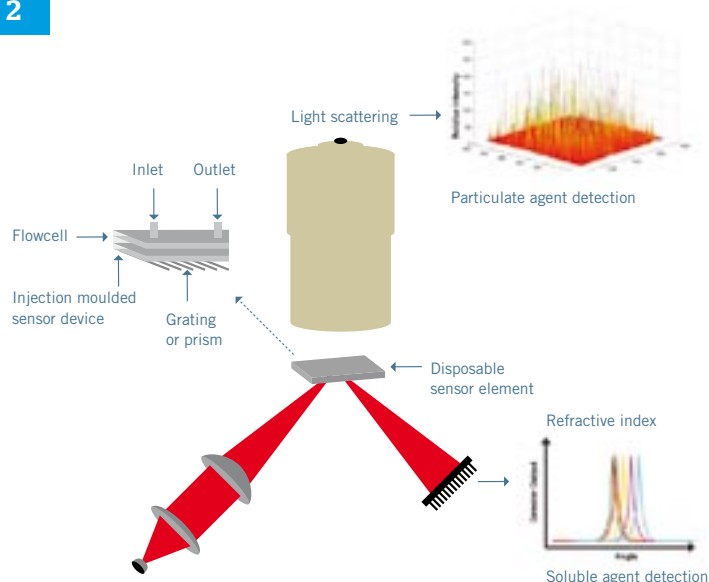


MCLW (left) and LS-SPR (right)

By monitoring arrays of antibodies on a disposable sensor surface it is possible to directly detect the capture of soluble agents (protein toxins such as ricin or botulinum), through the resulting refractive index change, or particulate agents (bacteria and viruses, for example anthrax or smallpox) by light scattering (Figure 2). As no additional antibody or other reagents are required the technology has a very low logistics burden compared with currently deployed systems and offers good potential for unattended operation in the field.

In operation these detectors would be linked up to an aerosol collection system, such as the Horizontal Wetted Wall Cyclone, which would feed a continuous liquid sample stream into which particles in the atmosphere in the 1–10 μm size had been concentrated. This offers the potential for near-real time

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monitoring of the environment for the presence of BW agents. However, there are a number of challenges to be overcome for optimum performance. One is the complex nature of the sample (Figure 3). As well as possible BW agent, the air contains large numbers of other particles including biologicals, such as fungal spores, pollens or non-pathogenic bacteria and viruses, and non-biological material. All of these could interfere with the detection process, for example by binding non-specifically to the sensor surface. Clean-up of the sample prior to reaching the detector should considerably increase confidence in its performance but should not significantly delay the rapid detection process or introduce the risk of blockages within the fluidics system. Ideally such an approach could also be applied to the selective concentration of bacteria for improved limits of detection of optical evanescent biosensors or any other biological detection technology.

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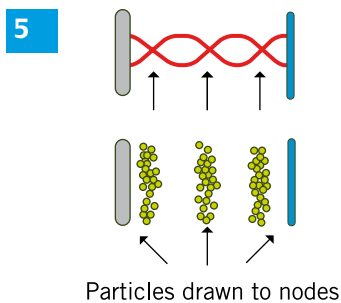
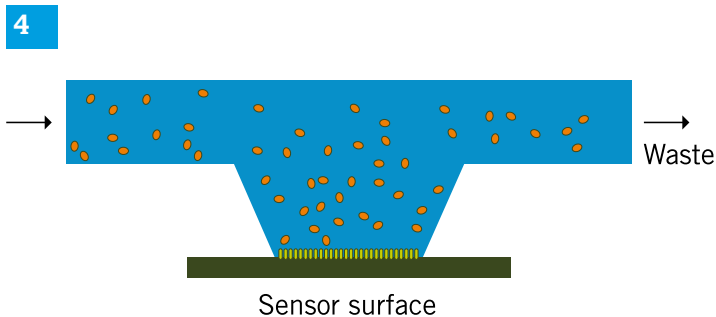


Another challenge results from the use of surface immobilised antibodies for selective agent capture. Although this limits reagent requirement compared with antibodies added to the bulk sample stream, relatively slow mass transport of the particles to the surface located antibodies limits the sensitivity of the technique (Figure 4a). To meet both challenges a method of applying a force to particles suspended in a liquid was required (Figure 4b). One method investigated by Dstl was the application of ultrasonic standing waves (USW).

Standing Waves

An ultrasonic standing wave may be set up using a transducer and reflector. Within the standing wave there are antinodes, where the pressure amplitude is at a maximum, and nodes, where the pressure amplitude is zero. Particles suspended in fluid within the standing wave experience a primary force that drives them towards positions of minimum acoustic energy, which in the case of cells and similar material suspended in water is close to the pressure nodes (Figure 5). The radiation force that drives the

particle depends on the wavelength of the ultrasound, the volume of the particle, its density and its compressibility. Frequencies greater than 1 MHz ($\lambda < 1.5\text{mm}$ in water) are preferred so that high pressure amplitudes may be employed without inducing damaging cavitation.



In collaboration with the late Professor Coakley and his group at Cardiff University [3,4], Dstl investigated the application of ultrasound to the manipulation of bacterial-sized particles. In this research, use of an ultrasonic chamber of pathlength $\lambda/2$, allowed the concentration of bacteria at the centre of a sample channel at the resulting single pressure node (Figure 6a). Also, a system was set up with a $\lambda/4$ pathlength combined with a reflector of suitable thickness. This for the first time demonstrated the establishment of a pressure node close to the reflector wall. By coating antibodies on this wall, capture of bacterial spores was enhanced 200-fold compared with no acoustic field applied (Figure 6b). By integrating

the ultrasonic transducer into the MCLW biosensor [5], orders of magnitude improvements in the limits of detection in a five minute assay were demonstrated (Figure 7).

Currently work is under way on an ultrasonic two-stage filter and concentrator for the Output 3 Portable Integrated Battlefield Biological Detector (PIBBD). This project aims to produce an automated BW system that can demonstrate an unattended specific detection capability with the performance comparable to the currently deployed truck mounted Integrated Biological Detection System (IBDS) in a man-portable piece of equipment. As the system is intended to carry out continuous monitoring the filter and concentrators must work on a flow through basis. In the filter step particles of $10\ \mu\text{m}$ are pushed to the chamber wall with a lower ultrasonic pressure and removed in 10 per cent of the flow. These particles are larger than the bacterial particles of interest and so are directed to waste. The smaller bacterial particles which are not moved by the low ultrasonic force pass through the filter unaffected. This sample then moves on to the concentration stage which applies a higher acoustic pressure, sufficient to direct the particles to the chamber wall. It is intended that all the bacteria are thus concentrated and removed in 10 per cent of the flow and directed on to the biosensor and over the antibody array coated sensor surface. Although these units are currently quite large, the team at Southampton have also produced microfabricated concentrators under a Joint Grant Scheme funded project.

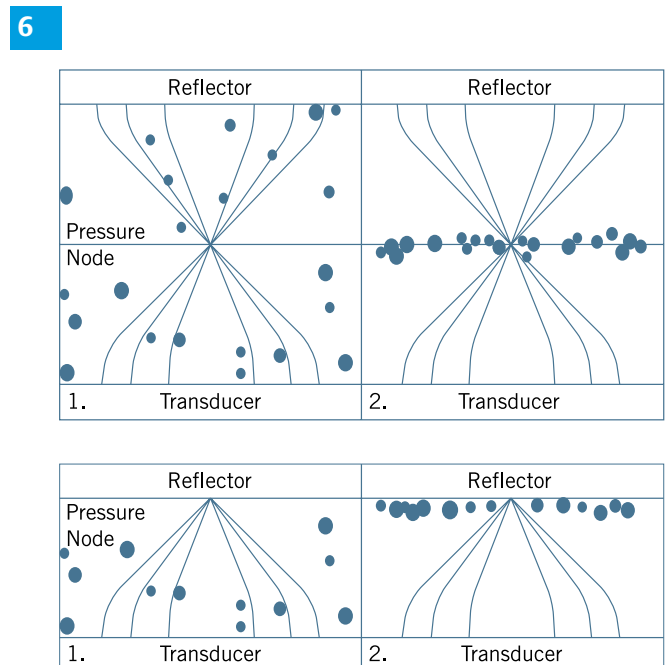
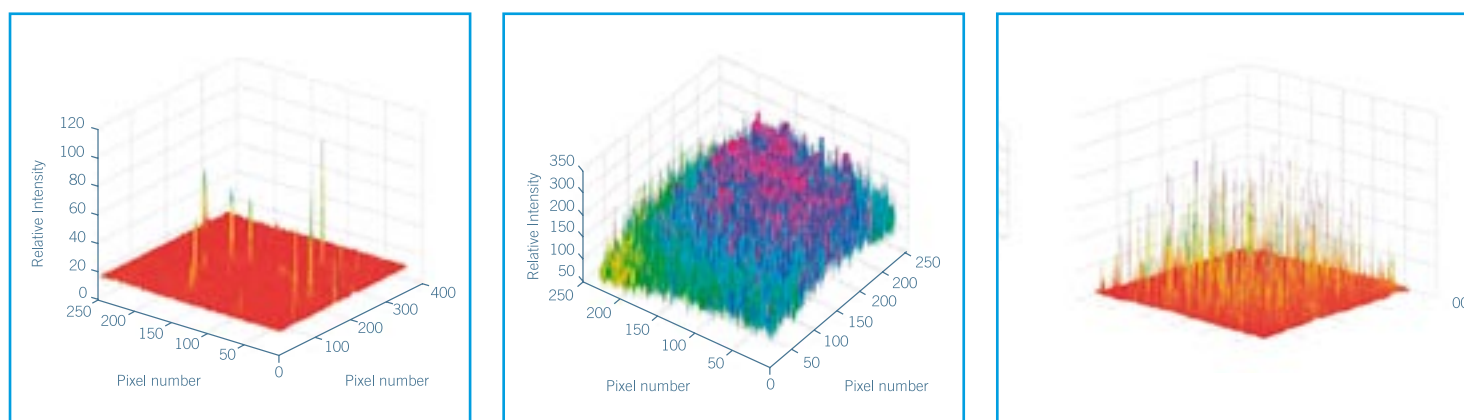


Figure 6a (top), Figure 6b (bottom)

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Before, during and after application of ultrasound

Although this technology has been developed by Dstl for application in the monitoring of atmospheric aerosols for BW agents, it has clear applications in the detection of bacteria in other specimens such as blood, faeces, soil and water. The technology has the potential to reach very disparate sectors all with common challenges. Many of these different sectors could be addressed by a single platform. Such a platform could take a microbe containing sample and enrich and purify the microbes in a non-selective rapid manner to allow the subsequent identification of the microbes present. Ploughshare has created a dedicated company, ProKyma™ Technologies Limited, to develop the ultrasound technology towards this latter area. ProKyma™ has received seed corn investment from the Rainbow Seed Fund and is finalising the next round finance.

References

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*First published in Distillation in 2007 –
Distillation is Dstl's in-house publication.*