Optical microchip sensors for multiplexed detection of biological pathogens

D. Bhatta*[a], A. Michel[a], M. Marti Villalba[a], G. D. Emmerson[a], I. J. G Sparrow[a], M. B. McDonnell[b], E. A. Perkins[b] , R. W. Ely[c], G. A. Cartwright[c]

a Stratophase Ltd., Unit 10a, The Quadrangle, Premier Way, Romsey SO51 9DL, UK

b Defence Science and Technology Laboratories, Porton Down, Salisbury, Wiltshire SP4 0JQ, UK

c Bristol Industrial and Research Associates Ltd., Unit 8, Harbour Road Trading Estate, Portishead, Bristol BS20 7BL, UK

*Corresponding author. Tel: (+44) 1794 511 226; Fax: (+44) 8704 580 754, E-mail: devaki.bhatta@stratophase.com

Summary

SpectroSens, a multi-channel optical microchip sensor system suitable for rapid, label-free multiplexed detection of a wide range of bio-hazardous agents is presented. Optical chips containing multiple high-precision planar Bragg gratings are exploited as low-cost, robust refractive index sensors. Sensitivity to biological agents is conferred by functionalising individual sensing regions with different antibodies selected against various targets of interest. Antigen binding to the surface-immobilised antibodies results in localised changes in refractive index, which manifest as increases in wavelength of light reflected from the sensor chip. Real-time detection of multiple bio-hazardous agents including bacterial cells/spores, viruses and toxins has been demonstrated. This multi-analyte capability, coupled with its inherent robustness, portability and ease of use highlights the potential use of SpectroSens technology in applications ranging from bio-hazard detection for security and defence purposes to point-of-care medical diagnostics .

SpectroSens Technology

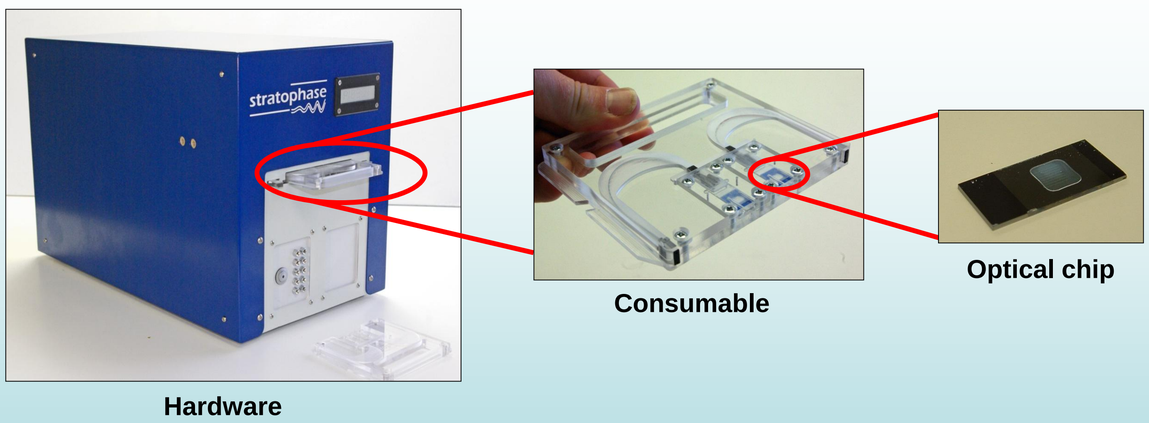

Figure 1: SpectroSens Technology A). SpectroSens detector with plastic plug-in consumable comprising two multi-channel optical sensor chips [1]. **B)** Schematic illustration of the principles of SpectroSens biological detection: target pathogens in the liquid sample are captured by specific antibodies immobilised on individual sensing channels within the optical sensor chip. Changes in optical spectra associated with selective pathogen binding are captured by the SpectroSens detector[2].

Biosensor Functionalisation

Surface-immobilisation of the detection antibodies ensuring optimal antigen recognition capability is critical to the performance of the biosensor. Target-specific antibodies were immobilised via secondary anti-species antibodies, which were oriented by recombinant Protein A/G covalently immobilised to the aminosilanised titanium oxide sensor surface using an amine-reactive homo-bifunctional cross-linker (BS^3).

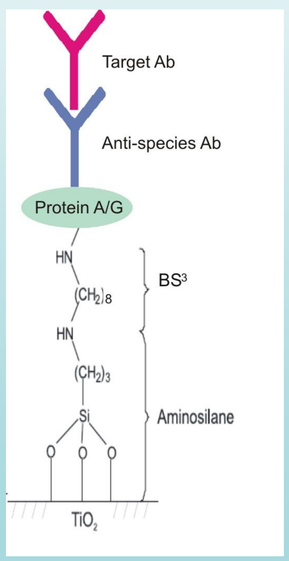

Figure 2: Schematic Illustration of the bio-recognition layer

Multiplexed Bio-hazardous Agent Detection

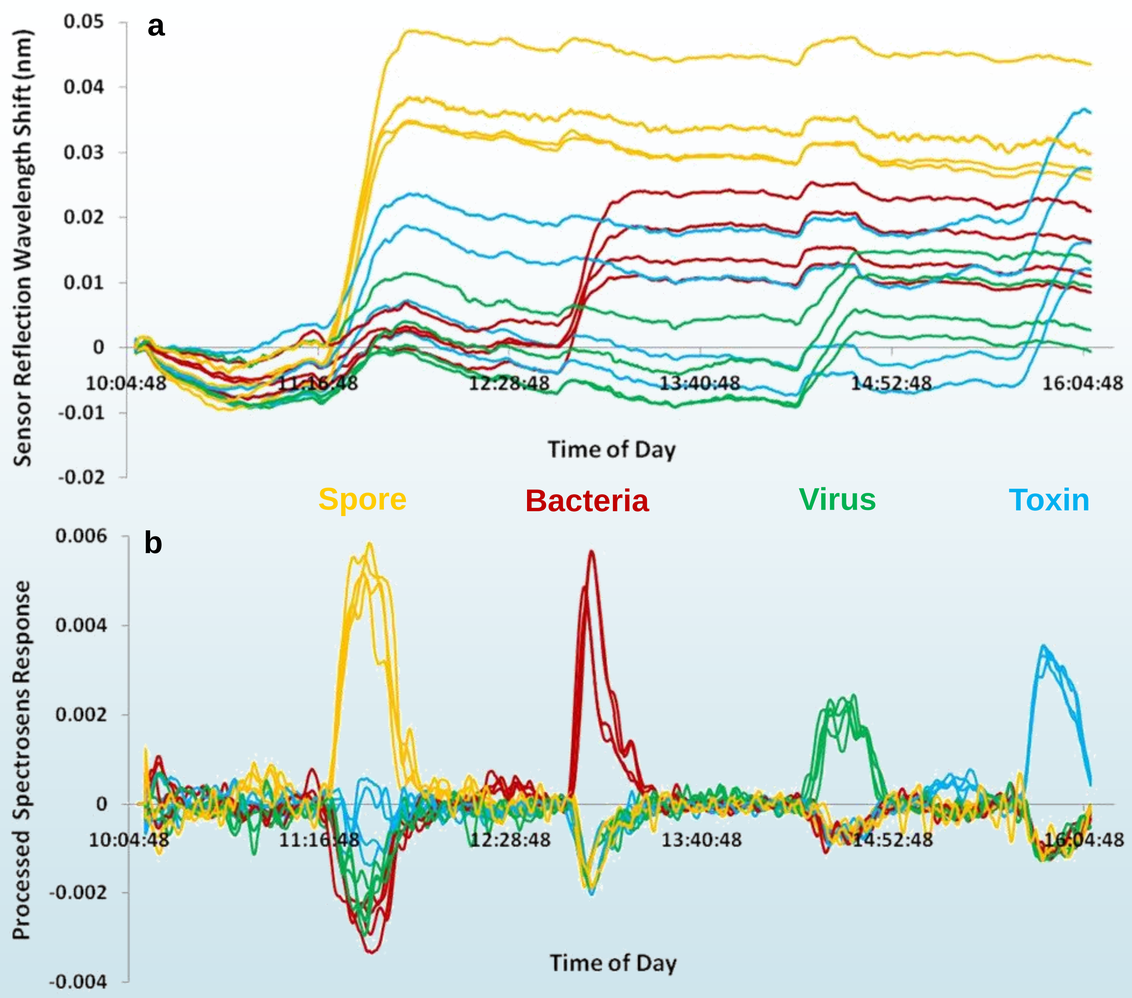

Figure 3: Real-time (a) and processed (b) SpectroSens sensorgrams demonstrating consecutive detection of a) *B. Anthracis* (BA) spores – UM23CL2 strain ($5x10^7$ cfu/ml), b) *F. Tularensis* (FT) cells – live vaccine strain ($2x10^8$ cfu/ml), c) Vaccinia viruses – heat-killed (10^7 pfu/ml) and d) ricin toxin (250 ng/ml) using a single consumable. (-) Anti-BA channels, (-) anti-FT channels, (-) anti-vaccinia channels, (-) anti-ricin channels.

Performance Enhancements

Ultrasonic disruption can be used to release smaller, more readily detectable fragments from large particulate antigens. Experimentation on the resonant mirror (RM) device (similar technology to SpectroSens) using ultrasonic disruption demonstrated a 100-fold improvement in the detection of bacterial spores[3].

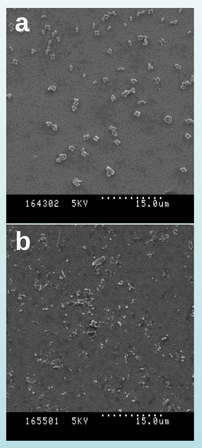

Figure 4: SEM images of surface-bound *B. atrophaeus* spores a) before and b) after ultrasonic disruption

This technique is easily transferred onto the SpectroSens system and is one of many approaches being investigated with a view to increasing sensor response time and sensitivity.

Conclusions

A single16-channel SpectroSens consumable has been used successfully for the detection of the following bio-hazardous agents: *B. anthracis* spores, *F. Tularensis* cells, Vaccinia viruses and ricin toxin; these spanned a size range from a few nanometres (toxin) to a few microns (bacterial spores/cells). SpectroSens responses manifested as increases in sensor reflection wavelength from specific sensing channels as a consequence of localised changes in refractive index elicited by selective binding of target pathogens to corresponding antibody-functionalised channels; in contrast, minimal changes were observed from control channels, indicating no appreciable issues with non-specific binding. Toxins were more readily detected, with significant sensor responses to low antigen concentrations occurring within minutes of sample introduction, whilst confident detection of the larger particulate antigens within minutes was demonstrated at (relatively) high antigen concentrations. Ongoing work is focussed on investigating various methods to improve sensor response time and sensitivity. The versatility of the SpectroSens platform enables its exploitation in numerous applications including point-of-care medical diagnostics, environmental/water monitoring, food safety and detect-to-warn/treat military operations.

Acknowledgments

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References

[1] Emmerson, G. D., Watts, S. P., Gawith, C. B. E., Albanis, V., Ibsen, M., Williams, R. B. and Smith, P. G. R., *Electron. Lett.* 38, 1531-1532 (2002).

[2] Bhatta, D., Stadden, E., Hashem, E., Sparrow, I. J. G. and Emmerson, G. D., *Sens. Actuators B Chem*. 149, 233-238 (2010).

[3] Borthwick, K.A.J., Coakley, W.T., McDonnell, M.B., Nowotny, H., Benes, E. and Groschl, M., *J. Microbiol. Methods* 60, 207-216 (2005).