

Multi-Channel Patterning of Antibodies on a Sensor Surface for Simultaneous Detection of Pathogens and Toxins

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Optical evanescent biosensors have shown considerable promise for the rapid detection and identification of pathogens and toxins. However while the ability to simultaneously monitor for a wide range of these biological agents, without the need for additional reagents, is highly desirable it does impose stringent requirements on the quality and accurate location of the immobilised antibodies on the sensor surface.

Initial studies on the deposition of antibodies on a sensor chip, using microarray contact (BioDot™) and non-contact (NanoPlotter™) methods, showed poorer response levels on the commercially available BIAcore™ 3000 surface plasmon resonance (SPR) biosensor when challenged with the appropriate antigen compared with flow immobilisation of the same antibodies within the instrument.

The drawback of immobilisation on the BIAcore™ is the limitation to 4 separate antibody stripes and the use of BIAcore™ sensor chips. To address this we have developed a number of patterning devices that allow the deposition of up to 16 different antibodies with a flexible approach that should allow an increase beyond 16 and future application on a wide range of sensor surfaces.

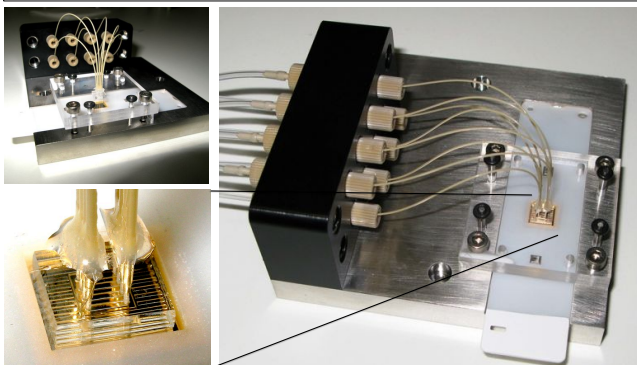


Figure 1

A 4 channel composite patterning device, compatible with the BIAcore™ 3000 flow cell, was developed. Microstructured and CNC milled PDMS, manufactured by combining standard rapid prototyping methods^a with CNC machined fluid connections, was bonded to CNC milled PMMA using an independently developed, but similar, technique to that published by Vlachapoulou et al^b.

Antibody patterning was performed on BIAcore™ CM5 slides using either a BIAcore™ 3000 or the composite PDMS/PMMA patterning device (Figure 1). Ovalbumin and *Bacillus atrophaeus* (formally known as *Bacillus globigii* (BG)) spores were used as simulants for a protein toxin and for *Bacillus anthracis* spores, respectively. Antibodies to ova and BG were immobilised on alternate channels of each chip using a concentration of 30 µg/ml in sodium acetate buffer (pH 4.5) and conventional EDC/NHS coupling^c. To ensure that the comparison between the patterning head and BIAcore™ immobilisations could be made, the same times and flow rates were used throughout.

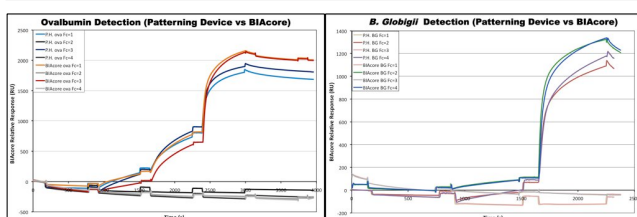


Figure 2. a).

b).

Initial tests were conducted to compare the response between BIAcore™ slides patterned with anti-ova & anti-BG, using either the BIAcore™ system or the composite patterning device. Figure 2 a) & b) shows example SPR traces of the antibody capture of ovalbumin and BG respectively. The responses from all channels are shown.

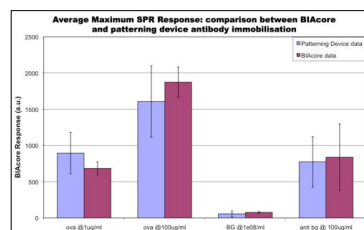


Figure 3.

Measuring the maximum SPR response of antigens binding to immobilised antibodies acts as a method of determining the antibody saturation point. The average antibody saturation points of the, necessarily, limited number of slides patterned using the BIAcore™ system and the composite patterning device were found not to be significantly different.

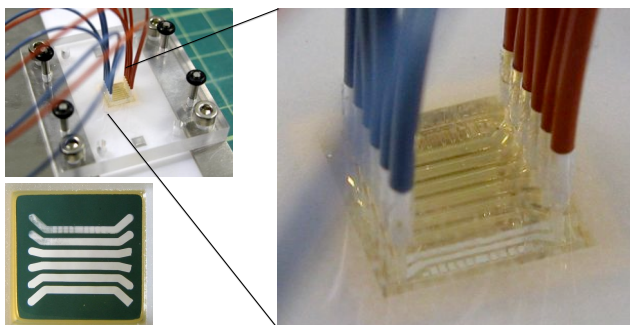


Figure 4

A 6 channel patterning device (Figure 4) has also been designed, to enable flow immobilisation within the BIAcore™ slide format, for use with alternate detectors. Preliminary experiments have been performed, however comprehensive binding experiments with suitable antigens remain to be conducted.

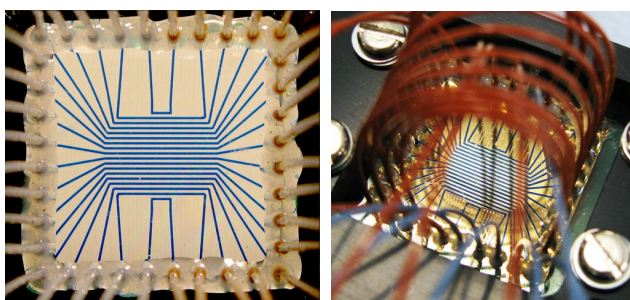


Figure 5

A patterning device enabling the immobilisation of 16 different antibodies onto a microscope slide for use in alternate detector systems has also been developed (figure 5). This patterning device also incorporates 2 shorter channels for deposition of materials to act as markers for automatic alignment of the other 16 channels.

References

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- Vlachapoulou, M., Tserepi, A., Pavli, P., et al, *J. Micromech. Microeng.*, 2009, **19**, 015007.
- Fägerstam, L.G., Frostell, Å., Karlsson, R., et al, *J. Mol. Recog.*, 1990, **3**, 208-214.