

Towards multi-analyte biosensor for food screening, based on IBIS imaging SPR

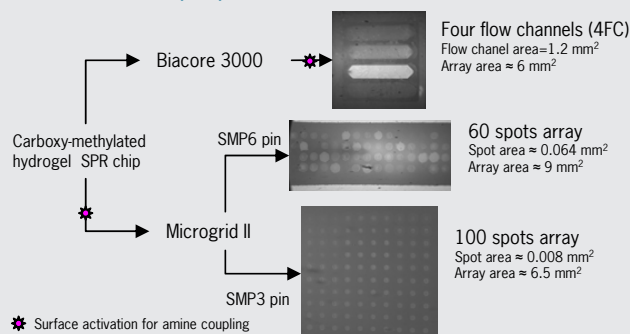
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Objectives

- To develop and test the multi-analyte biosensor based on imaging surface plasmon resonance (iSPR) system in microarray format.
- Multiple immunoassays will be implemented, using the described system, for real time, label free and simultaneous analysis of several food contaminants.

Methods and Results

Sensor surface preparation



Evaluation of IBIS iSPR performance

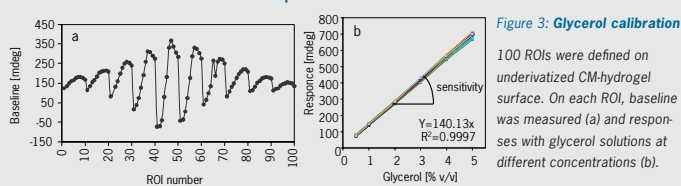


Figure 3: Glycerol calibration

100 ROIs were defined on underivatized CM-hydrogel surface. On each ROI, baseline was measured (a) and responses with glycerol solutions at different concentrations (b).

Parameter	Value
Sensitivity	$\Delta 0.1429 = 140$ mdeg
Variation in sensitivity over the surface (100 ROI)	1.5%
Variation in baseline measurements over the surface	50%
Limit of detection (LOD)	$\Delta 0.0041$ (43pg/mm ²)*

Table 1: Measured instrumental parameters

- Variation in baseline over the surface is very high
- Sensitivity over the surface is uniform
- LOD is sufficient for biomolecular interaction analysis

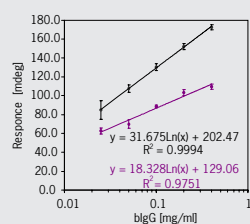


Figure 4: Comparison between blgG detection assay in IBIS iSPR and in Biacore 3000

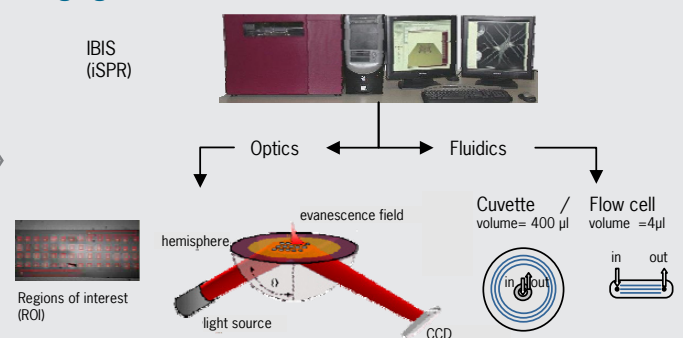
blgG calibration curves were measured using the same Biacore 4FC chip (immobilized with anti blgG) in Biacore 3000 (black) and in IBIS iSPR in flow cell configuration (purple).

- Assay sensitivity and variation between replicates is comparable between Biacore 3000 and IBIS iSPR

Conclusions

- IBIS iSPR sensitivity and reproducibility is comparable to Biacore. Flow cell configuration offers better shaped sensorgrams and lower standard deviation between replicates.
- Sensitivity of different ROI doesn't vary much, in spite of the high differences in baseline.
- Immobilization of big molecules (e.g. proteins) in high amount per spot is challenging, due to restrictions in combination of amine coupling chemistry and spotting technique on CM hydrogels.

Imaging SPR measurements



Sensorgrams

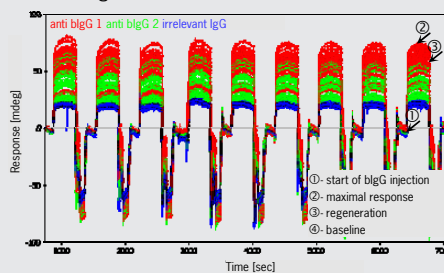


Figure 1: bovine IgG replicates in cuvette (100 spot array)

Bovine IgG (blgG) injection and regeneration cycles over 100 spot array of anti blgG antibodies in cuvette configuration of the iSPR.

- Reproducible baseline and maximal response
- High variation between spots
- Anti blgG 1 gives higher responses than anti blgG 2

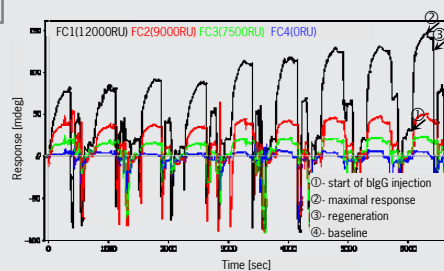


Figure 2: blgG calibration curve in flow cell (4FC Biacore chip)

Injections of different concentrations of blgG over Biacore chip immobilized with different levels of anti blgG, in flow cell configuration of the iSPR.

- Response level corresponds to immobilization level of a ligand on the surface and analyte in the solution
- Sensorgrams contain kinetics information