

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

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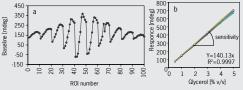
### **Objectives**

- · 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 Four flow channels (4FC) Flow chanel area=1.2 mm Array area ≈ 6 mm<sup>2</sup> Biacore 3000 60 spots array Spot area ≈ 0.064 mm² Array area ≈ 9 mm² SMP6 pin Carboxy-methylated hydrogel SPR chip Microgrid II 100 spots array Spot area ≈ 0.008 mm<sup>2</sup> Array area ≈ 6.5 mm<sup>2</sup> SMP3 pin Surface activation for amine coupling

#### Evaluation of IBIS iSPR performance



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).

Table 1: Measured instrumental parameters

· Sensitivity over the surface is

. LOD is sufficient for biomolecular

Variation in baseline over the surface

Figure 3: Glycerol calibratio

Variation in sensitivity over the surface (100 ROI) Variation in baseline measurements over the surface  $\Delta 0.0041 (43pg/mm^2)^*$ Limit of detection (LOD)

 $\Delta =$  change in refractive index calculated from change of 1% glycerol in solutio variation=avg StDev/avg responcex100 LOD= baseline noise + 3 StDev \*based on: 1mdeg=10.8 pg/mm²

31.675Ln(x) + 202.47 R<sup>2</sup> = 0.9994

y = 18.328Ln(x) + 129.06

#### 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).

is very high

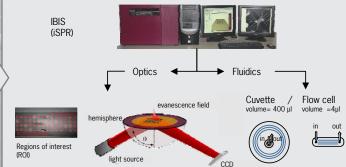
uniform

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

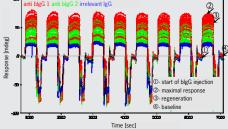
#### **Conclusions**

- · 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



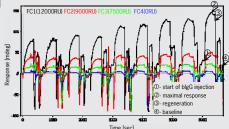
#### Sensorgrams



#### 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 then 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

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180.0

160.0

140.0

100.0

60.0

20.0

0.0

120.0

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