Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

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Tutorial 3: Evaluation of Optical Biosensor Response

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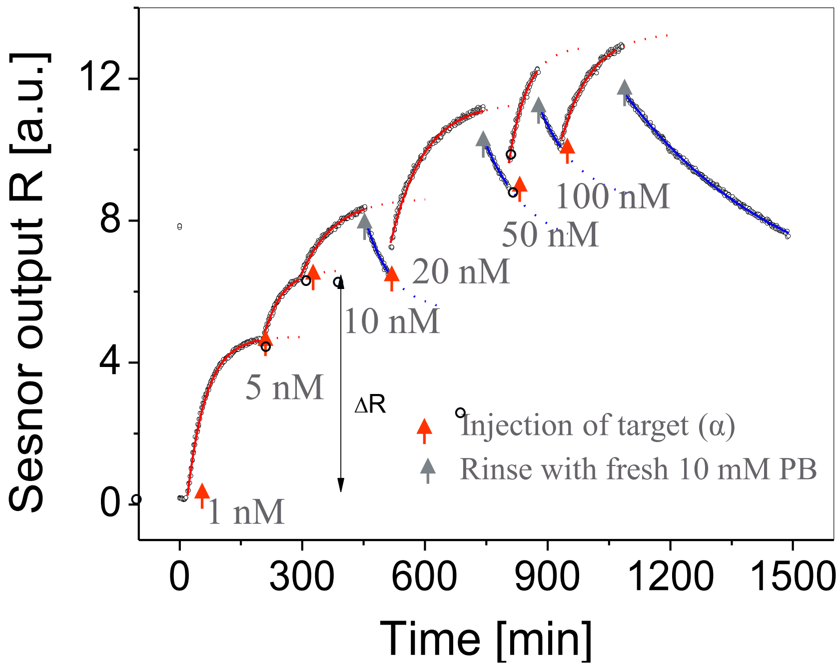
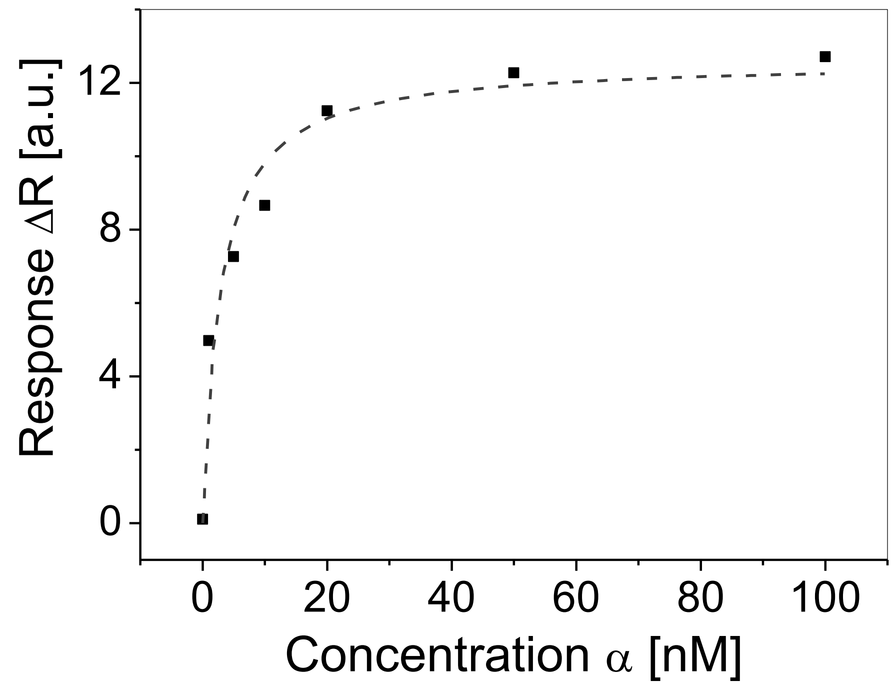
Content

- Definitions of biosensor key characteristics: limit of detection, sensitivity, detection range.
- Tutorial on the evaluation of acquired optical biosensor data for direct and competitive assay, establishing of calibration curve, determining of baseline noise, determining the limit of detection and limit of quantification.

Date: May 17th

Titration Experiment





By fitting the dependence of equilibrium sensor response R on the analyte concentration α the association affinity constant K_a can be fitted from a function:

$$\Delta R = const \frac{K\alpha}{1 + K\alpha}$$

$$K_A = \frac{k_a}{k_d}$$

Surface Reaction with Mass Transfer

Reaction kinetics become a function of mass transfer rate k_m.

$$\frac{d\gamma}{dt} = k_{on}\alpha\left(\beta - \gamma\right) - k_{off}\gamma$$

$$k_{on} = \frac{k_a}{1 + k_a\left[\beta - \gamma\left(t\right)\right] / k_m}$$

$$k_{off} = \frac{k_d}{1 + k_a\left[\beta - \gamma\left(t\right)\right] / k_m}$$

Fast diffusion

$k_m >> k_a\beta$

Reaction is affinity-controlled and $k_{on} \approx k_a$, $k_{off} \approx k_d$

Slow diffusion

$k_m << k_a\beta$

Reaction is diffusion-controlled, $k_{on} \approx k_m\beta^{-1}$ and $k_{off} \approx k_m k_d (k_a\beta)^{-1}$

(low probe / ligand density, high flow rate)

Equilibrium Sensor Response

$$\frac{d\gamma}{dt} = k_{on}\alpha\left(\beta - \gamma\right) - k_{off}\gamma = 0$$

$$\gamma = \frac{k_{on}\alpha\beta}{k_{off} + k_{on}\alpha}$$

$$\boxed{\begin{gathered}\gamma = \beta\frac{K\alpha}{1 + K\alpha} \\ K = k_{on} / k_{off}\end{gathered}}$$

Analytical function that can be used for the fitting of calibration curve in order to determine the equilibrium constant.

Performance Characteristics

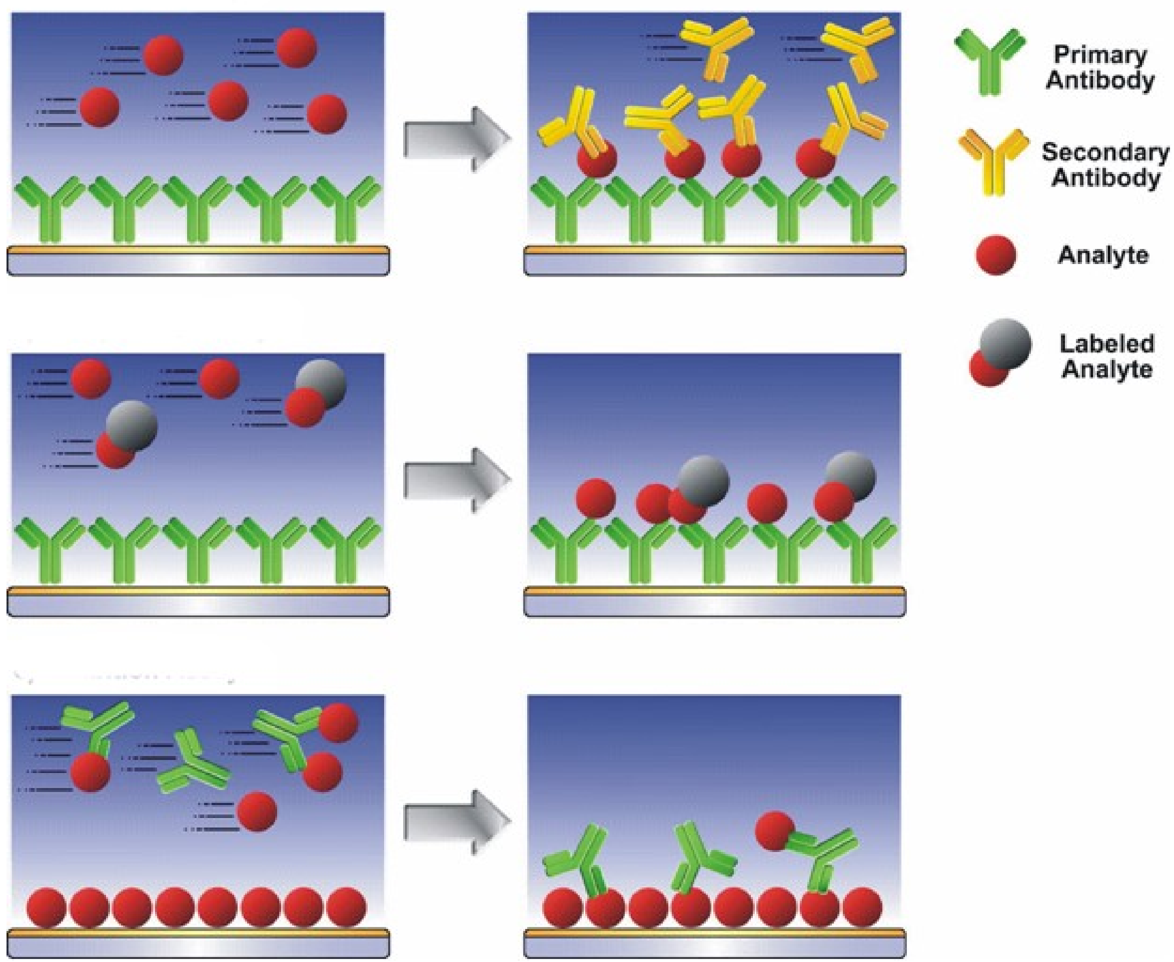
Detection range:	Concentrations of analyte that can be determined.
Sensitivity:	The value of the sensor response per analyte concentration.
Limit of detection	Minimum concentration of analyte that can be detected
Specificity / selectivity:	Interference of the presence of other compounds must be minimized for obtaining the correct result.
Matrix effect	Detection in real samples (e.g. blood serum) is rather more difficult than in model ones (e.g. buffer)
Analysis time:	The necessary time to carry out the analysis
Reusability:	Sensor chips are used only once or can be regenerated for multiple detections.

Heterogeneous Assays

Sandwich

Competitive

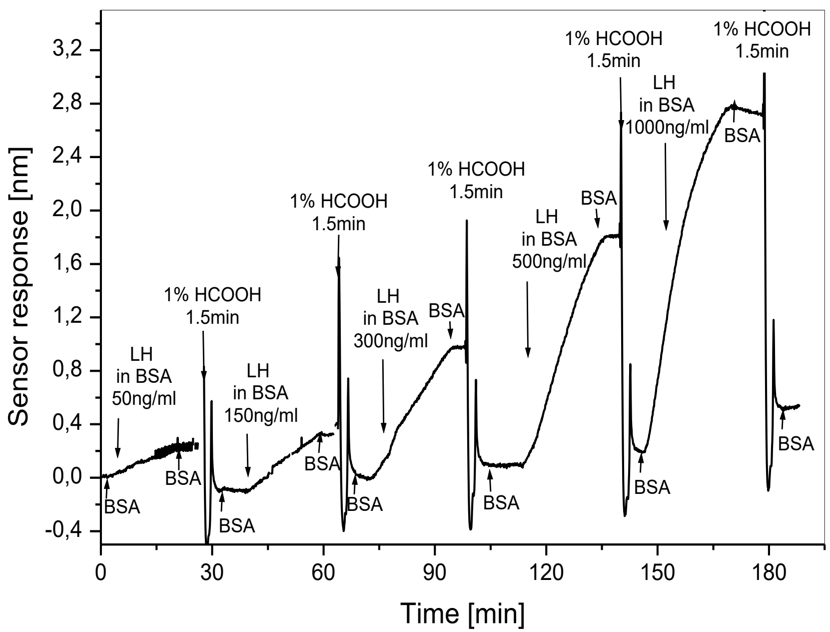
Inhibition

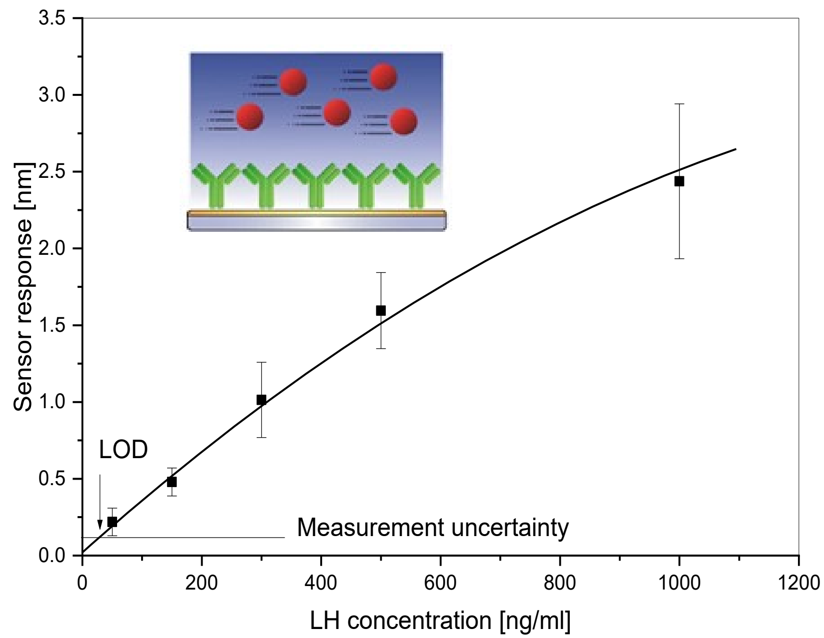


J. Homola (editor): Surface Plasmon Resonance Based Sensors, Springer, 2006.

Example of Direct Assay

Direct detection of luteinizing hormone (LH, triggers ovulation). Protein with molecular weight of 29 kDa.

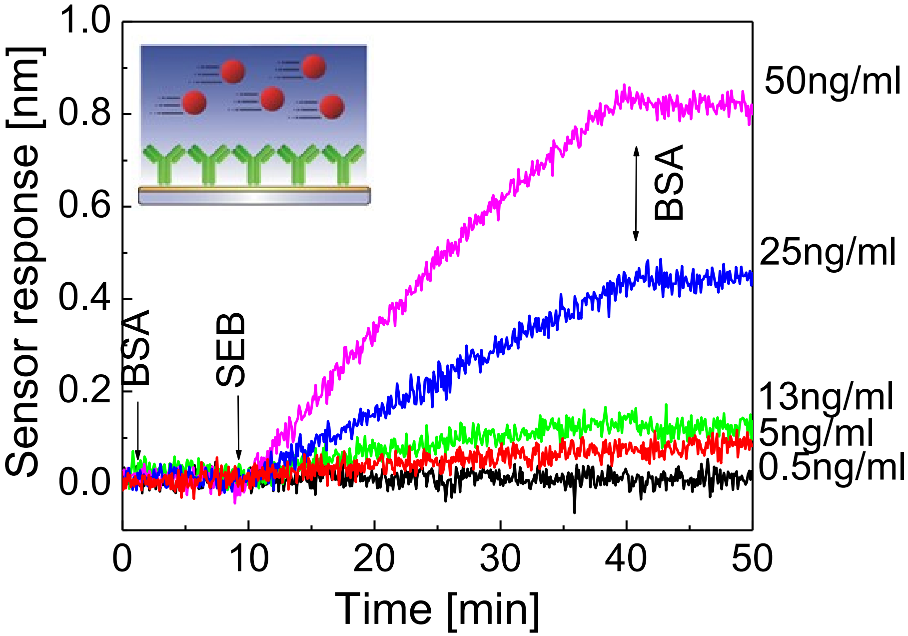


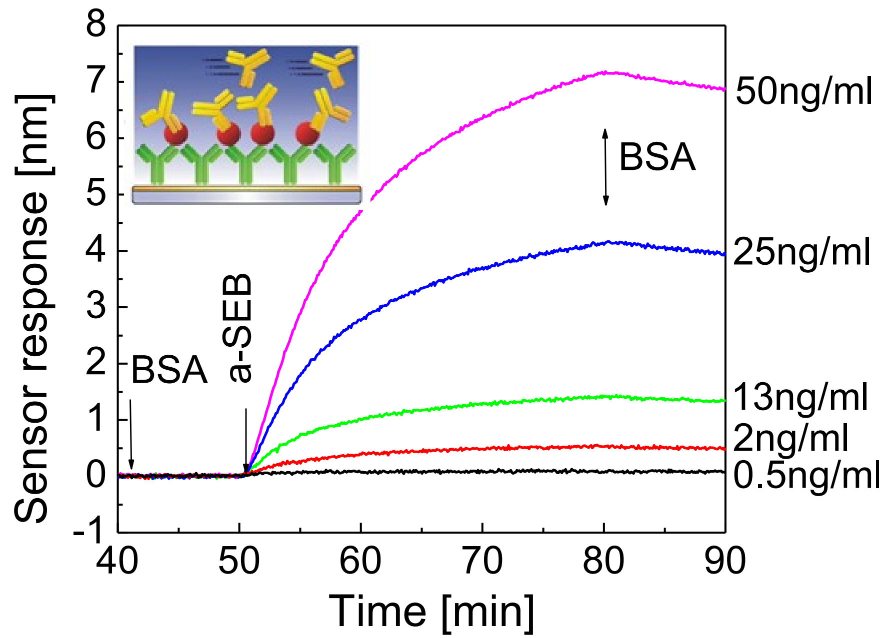


Binding kinetics for increasing concentrations of LH and regeneration between detection cycles (left) and the calibration curve (right).

Example of Sandwich Assay

Staphylococcal enterotoxin B (SEB) – toxin that commonly causes food poisoning, with severe diarrhea, nausea and intestinal cramping. Molecular weight 28 kDa. Response amplified by secondary polyclonal IgG with molecular weight of 160 kDa.

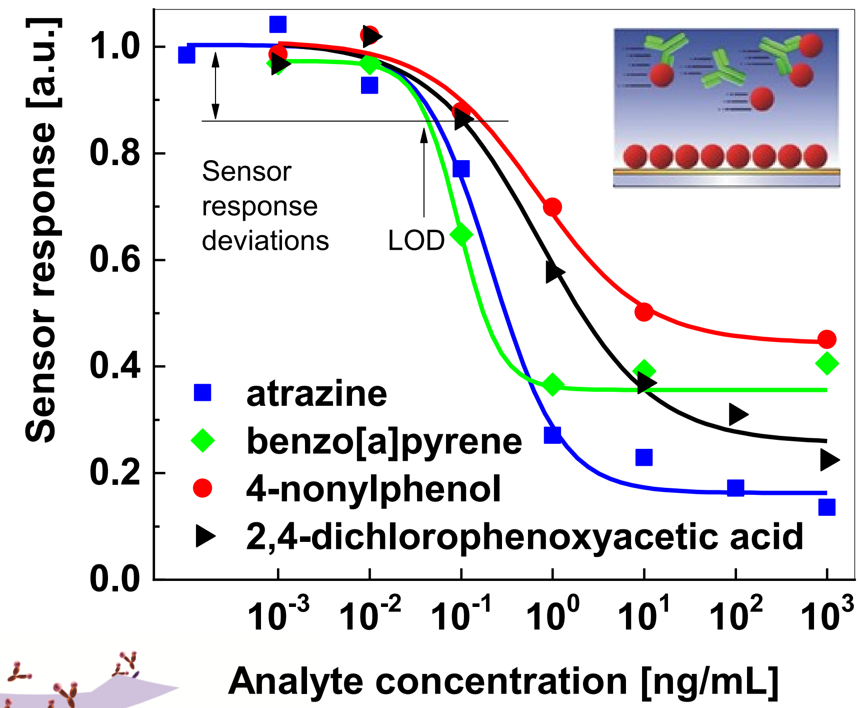


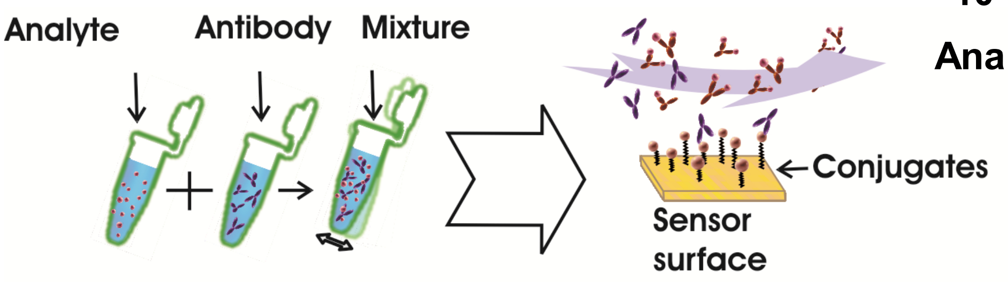


J. Homola, J. Dostalek, S. Chen, A. Rasooly, S. Jiang, S. S. Yee: Spectral Surface Plasmon Resonance Biosensor for Detection of Staphylococcal Enterotoxin B (SEB) in Milk , Journal of Microbiology, 75, (2002) 61-69.

Example of Inhibition Assay

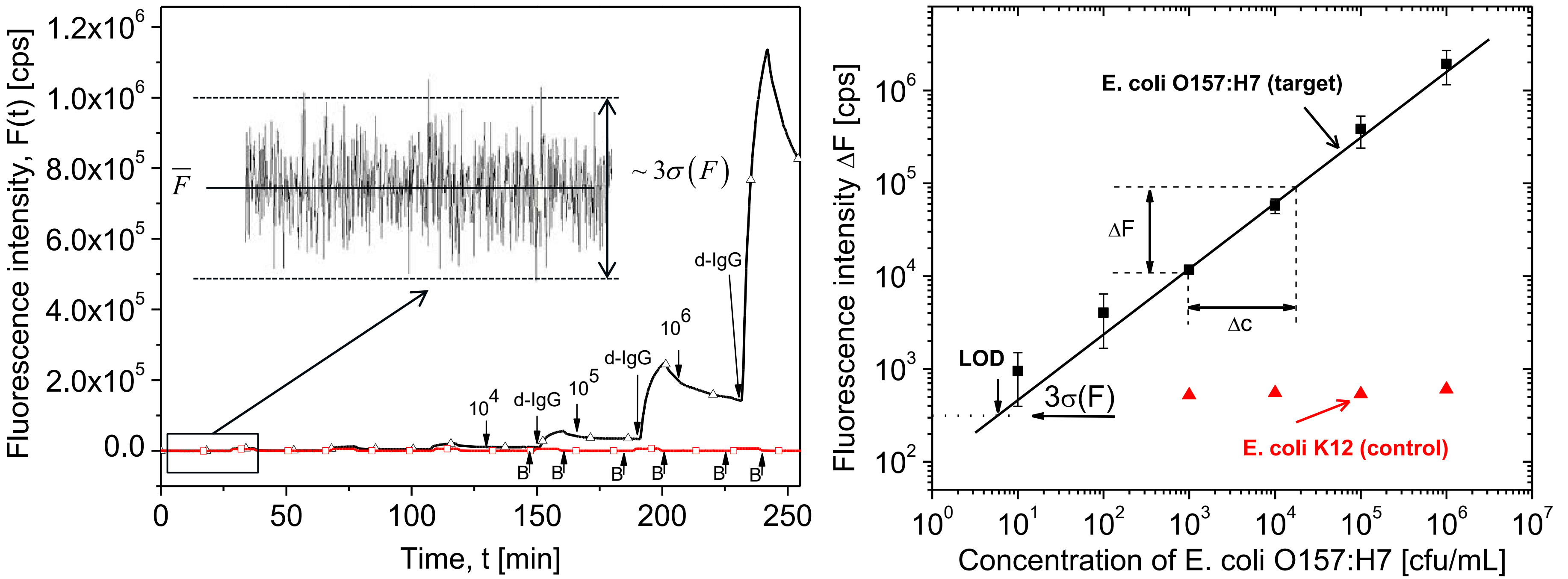
Atrazine – pesticide with molecular weight of 0.2 kDa. Too small to be detected directly and thus inhibition or competitive assays are used.





Dostalek, J. Pribyl, P. Skladal, J. Homola, Multichannel SPR biosensor for detection of endocrine disrupting compounds, Analytical and Bioanalytical Chemistry, (2007) 389:1841-1847

Calibration Curve



C.J. Huang et al , Biosensors and Bioelectronics (2010), 26, 4, 1425-1431.

Sensitivity S=ΔF/ Δc

Sensor signal noise described by stand. deviation $\sigma(F)=\sqrt{\frac{1}{N-1}\sum_i\left(F_i-\overline{F}\right)^2}$

Limit of detection (LOD) determined from sensor noise as LOD=3σ(F)/S

Limit of quantification (LOQ) determined from sensor noise as LOD=10σ(F)/S

Origin and Reducing of Noise

Shot noise – fundamental property of (coherent) light beam. The flux of photons exhibits statics that leads to $\sigma \sim I^{1/2}$

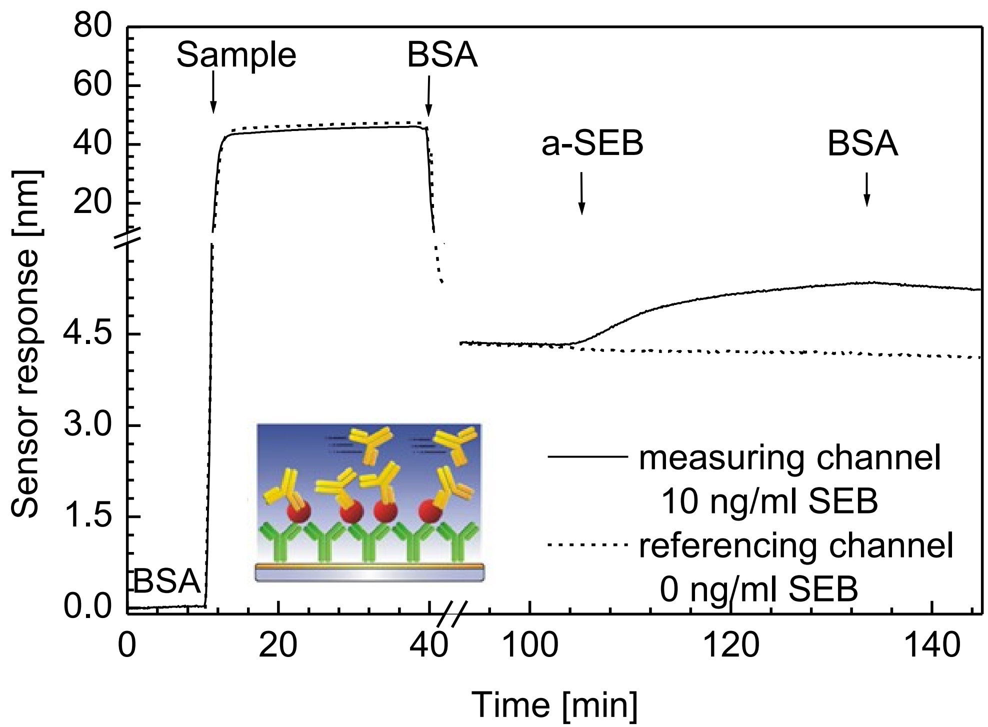
Additional (additive) noise – typically occur at the electronic devices (photo detector, analog to digital convertor).

Reducing noise by averaging – sampling of N measurement and their averaging decreases the baseline noise by fact or $N^{1/2}$. Nowadays rather fast detectors are available – e.g. cameras with MHz repetition rate.

Sensor drift - Useful to distinguish between sensor response noise σ (e.g. nm/Hz) and stability (leading to a long term drift). Sensor stability can be improved by reference-compensated measurements.

Control Experiments

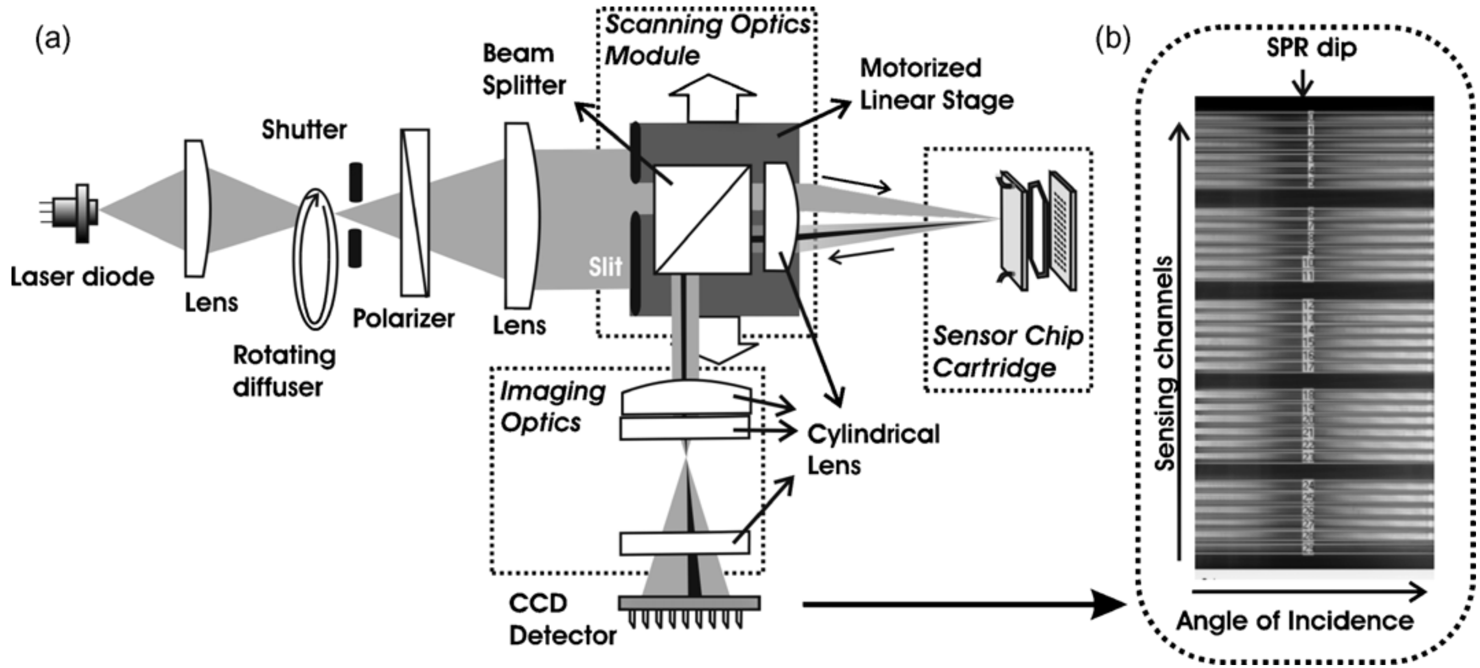
J. Homola, J. Dostalek, S. Chen, A. Rasooly, S. Jiang, S. S. Yee: Spectral Surface Plasmon Resonance Biosensor for Detection of Staphylococcal Enterotoxin B (SEB) in Milk , Journal of Microbiology, 75, (2002) 61-69.



Example of reference – compensated measurement for suppressing of the effect of unspecific sorption and drift.

Multichannel SPR sensor

J. Dostalek, J. Homola, Surface plasmon resonance sensor based on an array of diffraction gratings for highly-parallelized observation of biomolecular interactions, Sensors and Actuators B, (2008), 129/1, 303-310



Example optical system for rapid scanning of SPR response over a 2D arrays of sensing spots with angular interrogation.

Reference Compensated Measurements

J. Dostalek, J. Homola, Surface plasmon resonance sensor based on an array of diffraction gratings for highly-parallelized observation of biomolecular interactions, Sensors and Actuators B, (2008), 129/1, 303-310