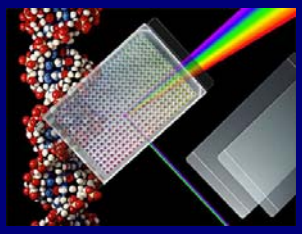
OPTICAL AFFINITY BIOSENSORS

J. Homola



Institute of Photonics and Electronics
Prague, Czech Republic

Institute of Radio Engineering and Electronics (IREE), Prague

- Established in 1954.
- Belongs to Academy of Sciences of the Czech Republic.
- 120 staff members.
- Funding: 2/3 ASCR contribution, 1/3 grants and contracts.

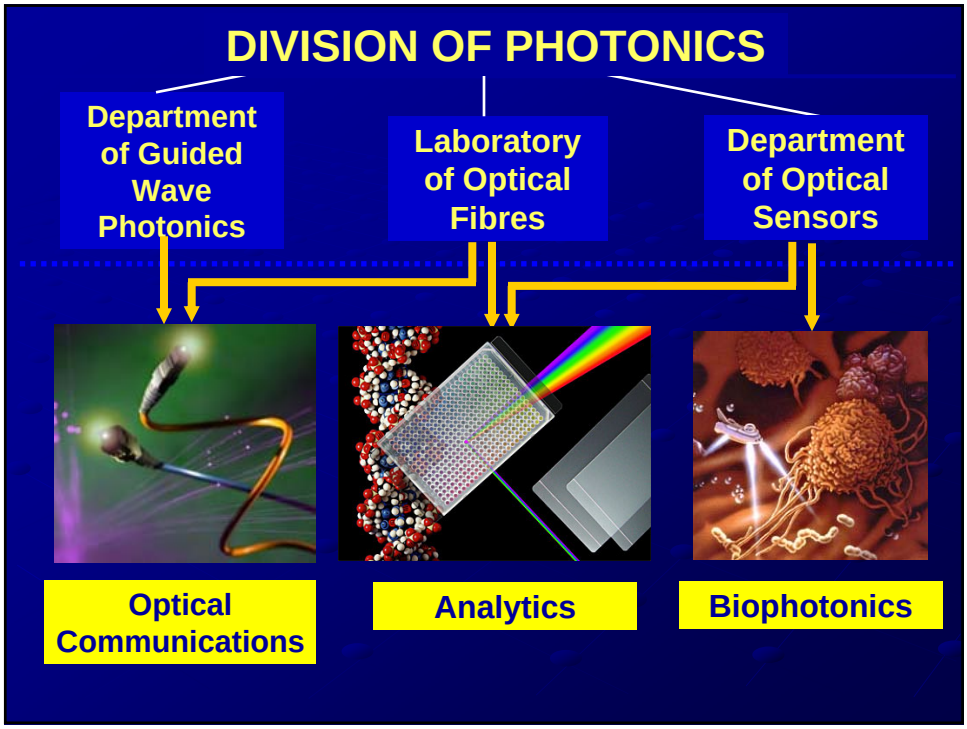
Institute Mission

Basic and applied research in three Thrust Areas:

Area I: Photonics

Area II: Materials for optoelectronics

Area III: Signals and Systems

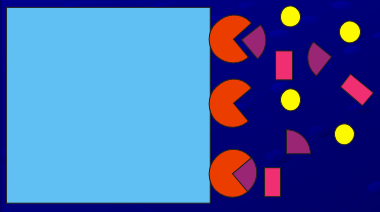


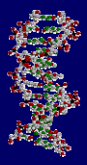
INTRODUCTION TO OPTICAL AFFINITY BIOSENSORS

Affinity Biosensors

Affinity biosensors are devices consisting of a biomolecular recognition element (*e.g.* antibody) and a sensor hardware,

Mechanical
Electrical
Magnetic
Optical





Antibodies
Proteins
DNA, RNA
Peptides
MIPs

which translate the binding event between the target molecules and biomolecular recognition element into an output signal.

Main Types of Optical Affinity Biosensors

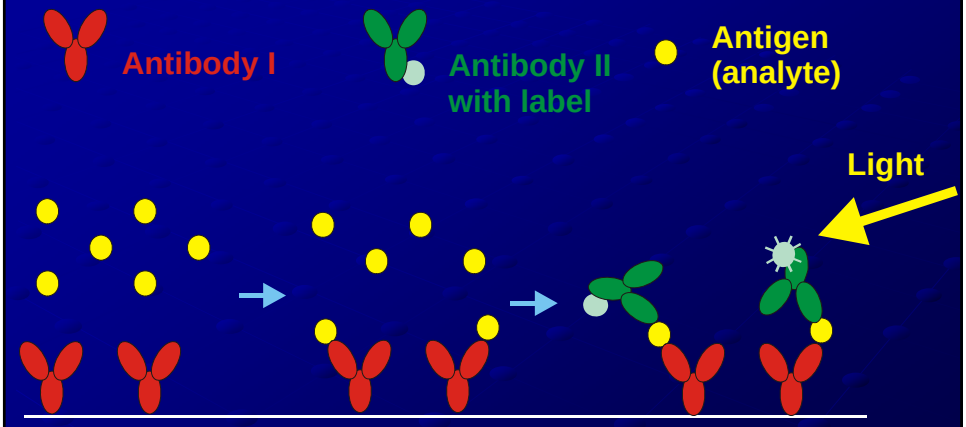
1. Label-based affinity biosensors

- Sensors based on fluorescence spectroscopy.

2. Label-free affinity biosensors

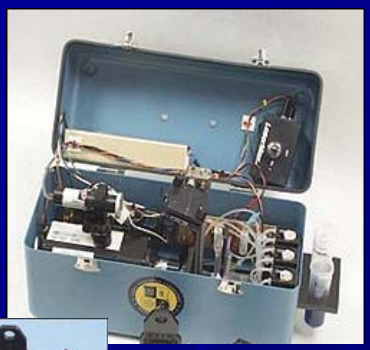
- Interferometric sensors (Mach-Zehnder integrated optical interferometer, white light interferometer).
- Sensors based on spectroscopy of guided waves (grating coupler, resonant mirror, surface plasmon resonance sensor).

Sensors Based on Fluorescence Spectroscopy: Principle of Operation



Principle of fluorescence-based optical affinity biosensors.

Fluorescence-Based Biosensors: Portable Array Biosensor (NRL)



Portable array biosensor developed by the group of F. Ligler, NRL; commercialized by Constellation Technology (USA)

Characteristics:

- Antibodies immobilization by a non-contact array spotter
- Sandwich detection format
- 15 immunoassays/sample
- 6 samples at a time
- Detection time: 10-15 minut

Detection limits (LOD):

- Staph. enterotoxin B (0.5 ng/ml)
- Cholera toxin (1.6 ng/ml)
- B. Anthracis spory (10^4cfu/ml)
- Listeria (10^4cfu/ml)
- E. Coli 0157:h7 (10^3cfu/mL)

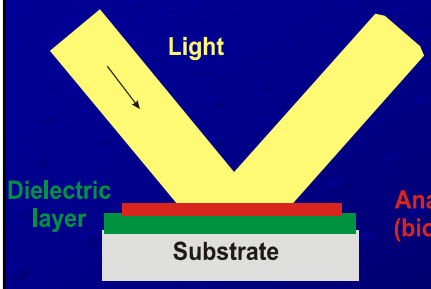
Fluorescence-Based Biosensors: RAPTOR Fiber Optic Biosensor (NRL)

US Marine using the RAPTOR to test for detection of toxins and pathogens in drinking water in Bahrain.

Characteristics:

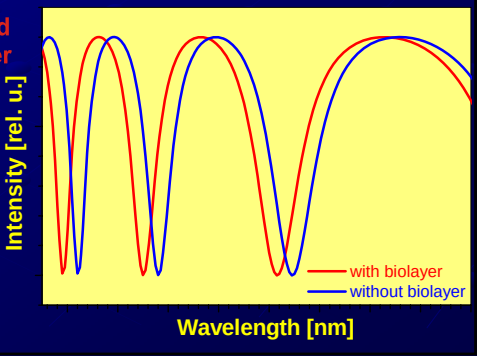
- Simulataneous detection of 4-8 analytes
- Sandwich detection format
- Same disposables used repeatedly until positive response
- Automated assay and readout
- LOD comparable with florescence array sensor

Sensors Based on Multiple-Beam Interference on Optical Multilayers: Principle of Operation



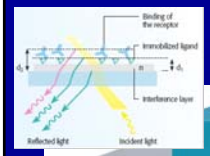
$$n_1 \rightarrow n_2$$
$$\Downarrow$$
$$\lambda_{min}^{(1)} \rightarrow \lambda_{min}^{(2)}$$

Analyzed (bio)layer



Multiple-beam interference on a dielectric optical multilayer (above) and corresponding interference spectrum of reflected light (right)

Sensors Based on Multiple-Beam Interference on an Optical Multilayer: BIAffinity (Analytik Jena)



BIAffinity sensor for biomolecular interaction analysis developed by the group of G. Gauglitz, University of Tuebingen; commercialized by Analytik Jena AG, (FRG).

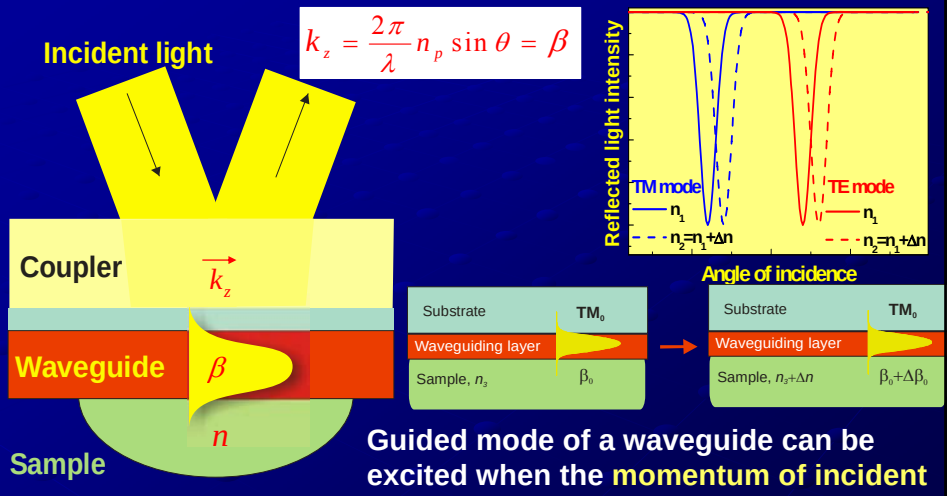
Characteristics:

- Label-free measurement.
- Two-channel system.
- Temperature stabilization.

Aplication:

- Real time study of biomoleculars interactions.
- Measurement of kinetics, concentrations, and association/dissociation constants.

Sensors Based on Spectroscopy of Guided Waves: Principle of Operation

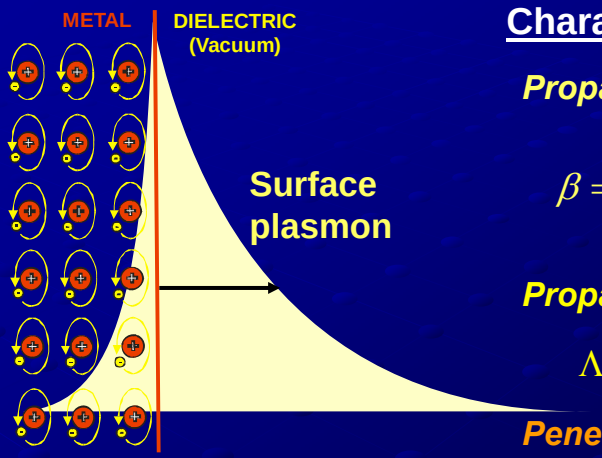


Guided mode of a waveguide can be excited when the momentum of incident light is equal to that of the mode.

This resonant momentum can be determined from the spectrum of reflected light .

SURFACE PLASMON RESONANCE (SPR) BIOSENSORS: PRINCIPLE OF OPERATION

Fundamentals of SPR Sensors: Surface Plasmons



A surface plasmon at a metal – dielectric interface.

Characteristics of SP:

Propagation constant, β

$$\beta = \frac{\omega}{c}\sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}}$$

Propagation length, Λ

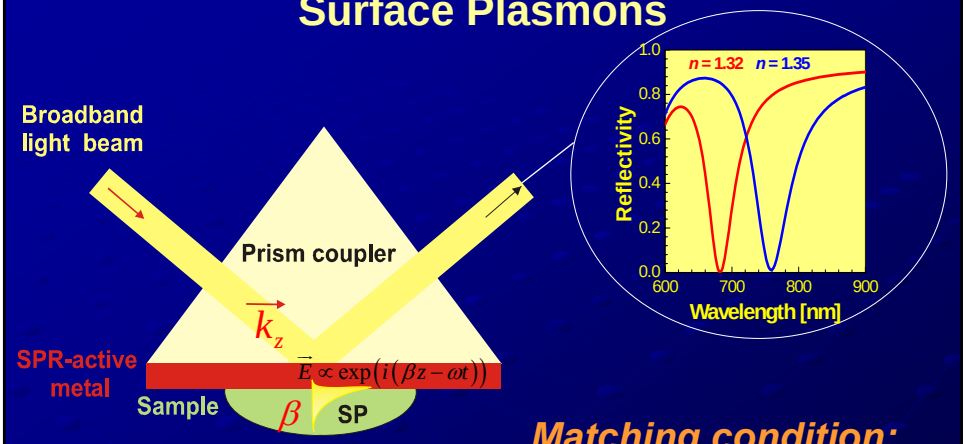
$$\Lambda = 3 - 30\ \mu\text{m}$$

Penetration depth, L

$$L_{metal} \simeq 20\ \text{nm}$$

$$L_{diel} \simeq 150 - 400\ \text{nm}$$

Fundamentals of SPR Sensors: Excitation of Surface Plasmons

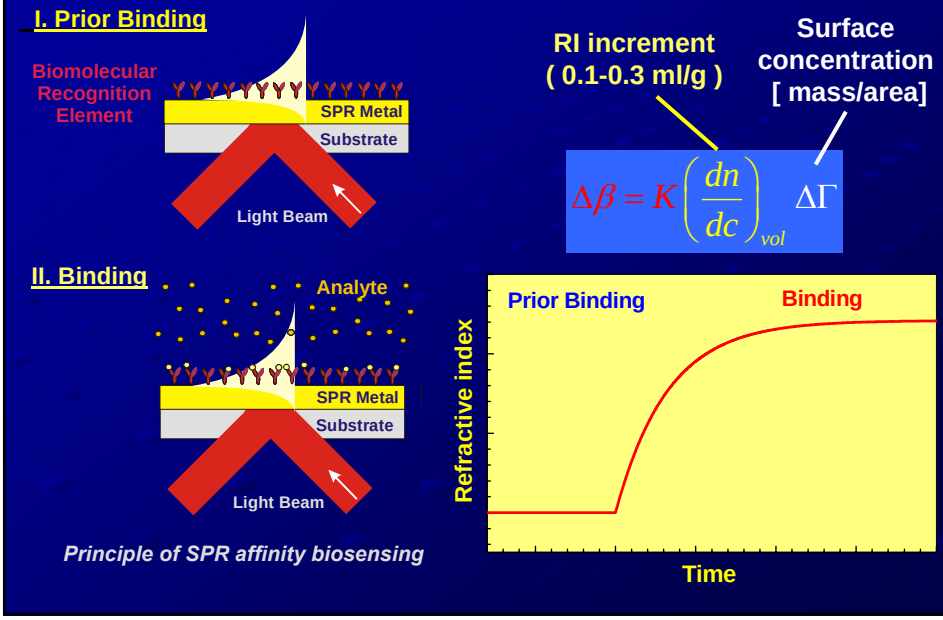


Excitation of a surface plasmon occurs when the matching condition is fulfilled.

Matching condition:

$$k_z = \frac{2\pi}{\lambda} n_p \sin\theta = \mathrm{Re}\{\beta\}$$

Surface Plasmon Resonance Affinity Biosensing



Principle of SPR affinity biosensing

Surface Plasmon Resonance Biosensors: Main Features and Benefits

- **Direct** (can detect analyte in one-step).
- **Real-time** (binding of analyte to sensor surface can be continuously monitored).
- **Label-free** (no fluorescent or radioactive labels are required for detection of analyte).
- **Minimum interaction length/volume required** (small sample volumes can be analyzed).
- **Generic technology** (combines generic optical technology with receptors ***specific*** against particular target analytes).

SURFACE PLASMON RESONANCE SENSOR PLATFORMS

Main Configurations of SPR Sensors

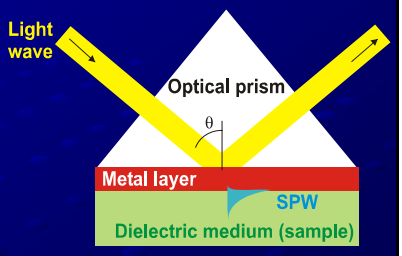
Coupling conditions:

$$\sqrt{\varepsilon_P}\sin(\theta) = \mathrm{Re}\left\{\sqrt{\frac{\varepsilon_M \varepsilon_D}{\varepsilon_M + \varepsilon_D}}\right\}$$

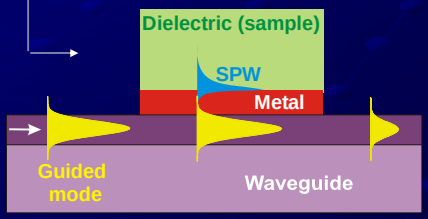
$$N_{ef} = \mathrm{Re}\left\{\sqrt{\frac{\varepsilon_M \varepsilon_D}{\varepsilon_M + \varepsilon_D}}\right\}$$

$$\sqrt{\varepsilon_D}\sin(\theta) + m\frac{\lambda}{\Lambda} = \pm\mathrm{Re}\left\{\sqrt{\frac{\varepsilon_M \varepsilon_D}{\varepsilon_M + \varepsilon_D}}\right\}$$

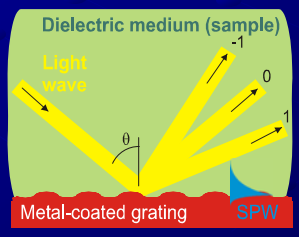
I. Prism coupler-based



II. Waveguide coupler-based

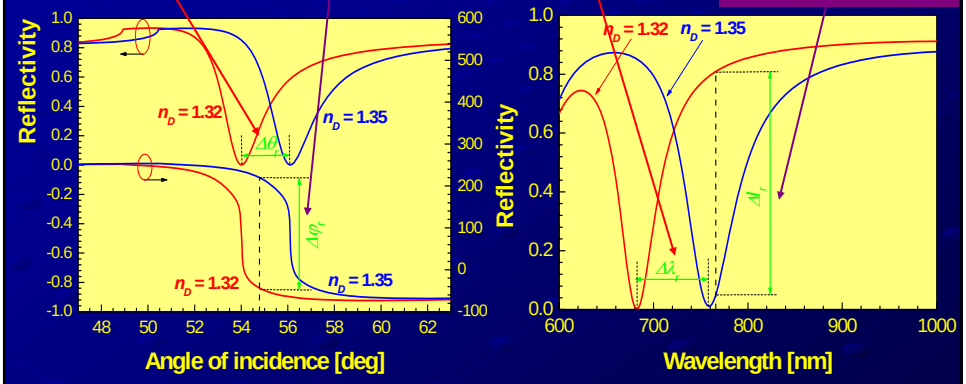


III. Grating coupler-based



Detection Approaches Used in SPR Sensors

Angular modulation | **Phase modulation** | **Wavelength modulation** | **Intensity modulation**



Reflectivity and phase for light wave exciting surface plasma waves via a prism coupler (SF14 glass prism – 50 nm thick gold layer – dielectric) as a function of the angle of incidence (a) and wavelength (b) for two different refractive indices of the dielectric; wavelength – 682 nm.

What do we require from an SPR sensor platform?

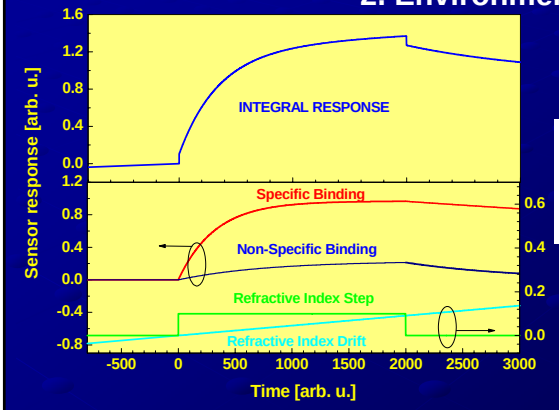
- **Performance** (sensitivity, resolution, reproducibility).
- **Robustness** (reliability in real-world conditions).
- **Multiplexing capability / throughput** (capable of performing multiple measurements at a time).
- **Size** (appropriate for application).
- **Cost** (low equipment and supply costs).

Motivation for Development of Multichannel SPR Sensing Devices

- **Multichannel devices** increase **throughput** of SPR sensor systems and allow for simultaneous detection of **multiple analytes** for complex sample analysis.
- **Multichannel devices** improve **quality of SPR measurements** by referencing out effects interfering with SPR measurements (temperature fluctuations, sample composition variations, non-specific adsorption from complex samples, *etc.)*.

Robustness: SPR Biosensing in Realistic Conditions

Interferences: 1. Sample matrix
2. Environment



Multichannel SPR sensors with referencing channels

Fig. SPR biosensing with sample and environmental effects interfering the measurement.

Sensors Based on Spectroscopy of Surface Plasmons: State of the Art

Sensors based on angular spectroscopy of surface plasmons : BIAcore S51 (left), BIAcore 3000 (middle), Spreeta sensor, TI, (right) .

Features:

- High resolution (10^{-7} RIU)
- Temperature controlled
- Multiple sensing channels

Applications: biomolecular interaction analysis

Multichannel SPR Sensors with Spatial Multiplexing: Parallel Channel Approach

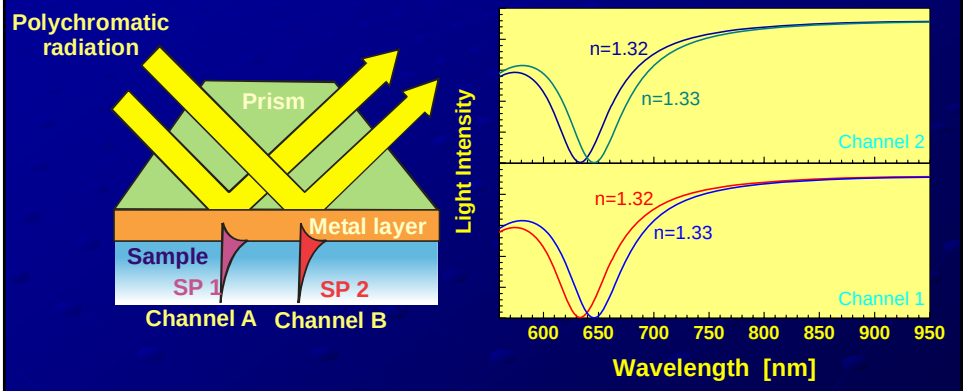


Fig. Concept of multichannel SPR sensor with parallel sensing channels and typical SPR spectra observed with such a sensor.

Multichannel SPR Sensors with Wavelength Division Multiplexing: Overlayer Approach

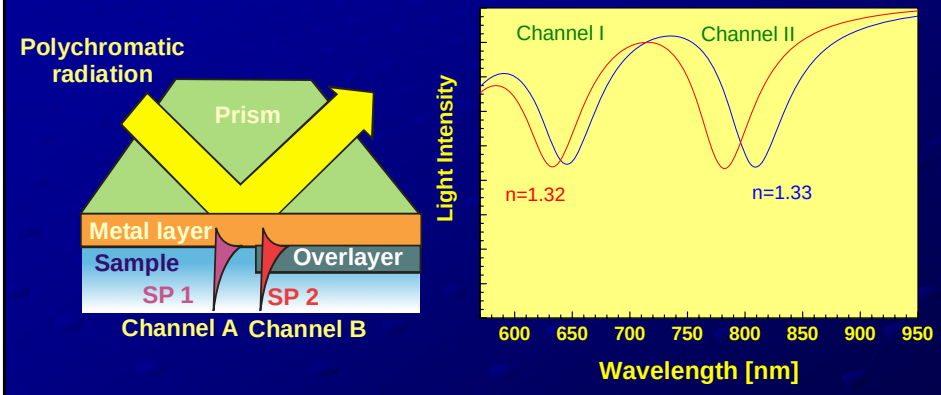


Fig. Concept of multichannel SPR sensor with an overlayer shifting response from one of the sensing channels to a longer wavelength and typical SPR spectra observed with such a sensor.

Multichannel SPR Sensors with Wavelength Division Multiplexing: Serial Channel Approach

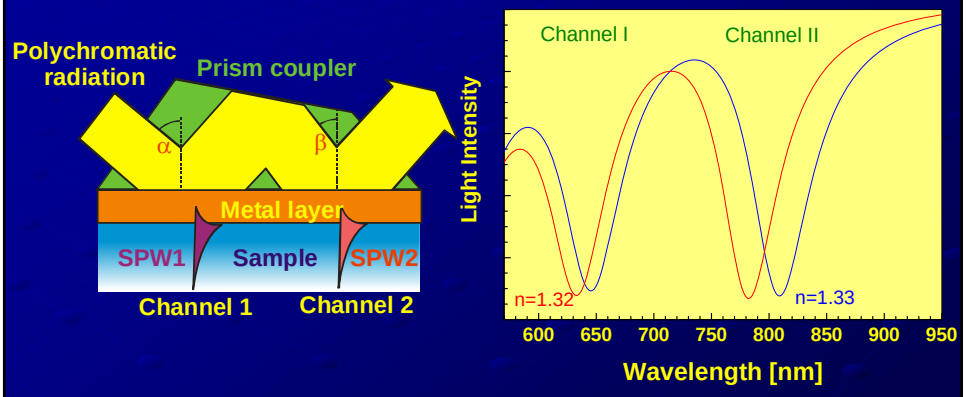


Fig. Concept of multichannel SPR sensor with serial sensing channels and typical SPR spectra observed with such a sensor.

Bulk Optic Laboratory SPR Sensor

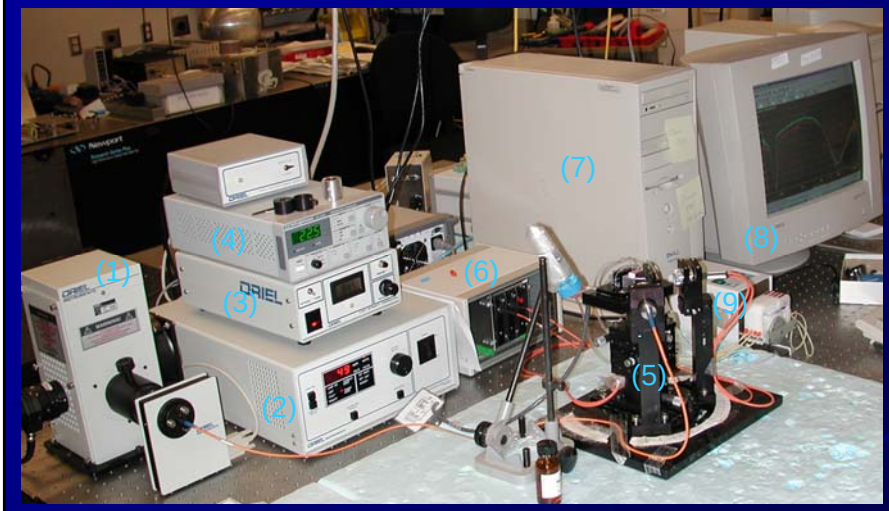


Fig. Laboratory SPR sensor. (1) lamp, (2) power supply, (3) intensity controller, (4) temperature controller, (5) SPR assembly, (6) spectrograph, (7) PC, (8) display, (9) pump.

RI resolution:
2×10^{-7} RIU

Reference-Compensated SPR Biosensing I: Compensating Slow RI Variations

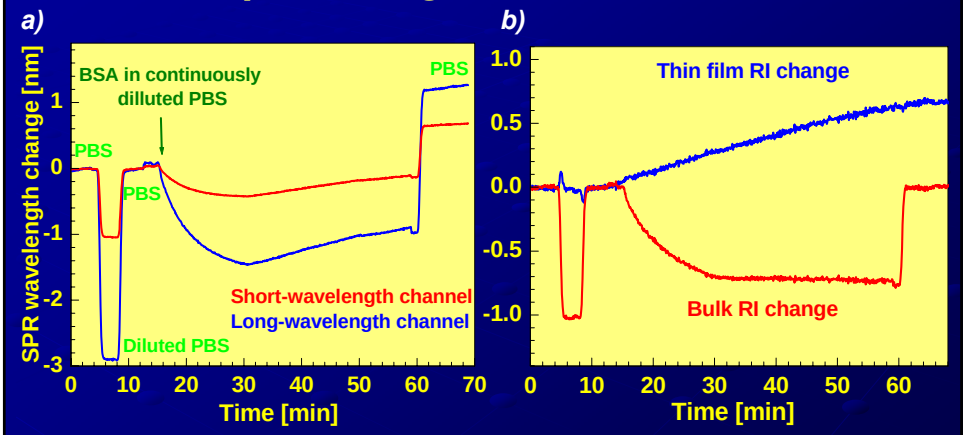
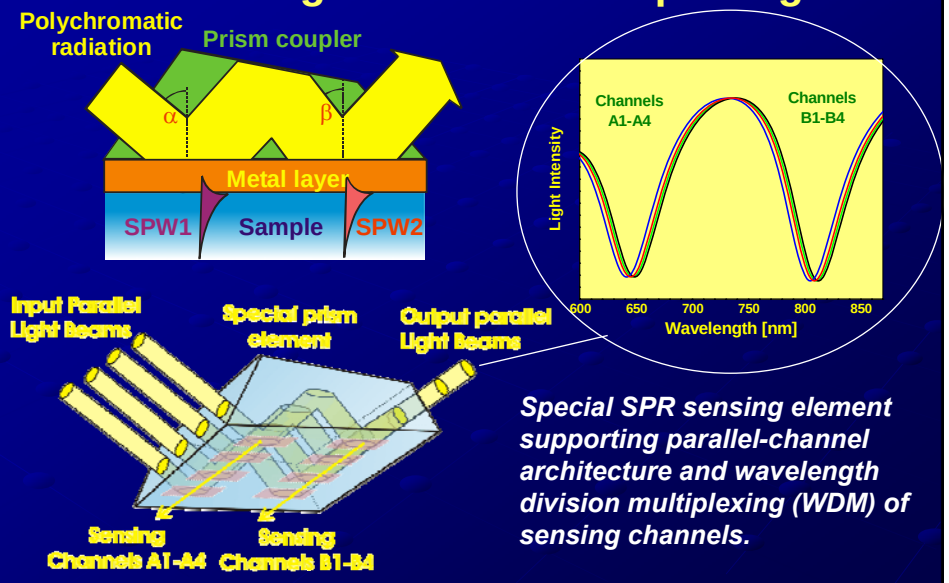


Fig. a) Response of the SPR sensor with two serial channels to formation of a thin BSA layer in the presence of slow background refractive index drift.
b) Deconvoluted surface and bulk refractive index responses.

DEVELOPING MOBILE SPR SENSING SYSTEM

Combining Parallel Architecture with Wavelength Division Multiplexing



Special SPR sensing element supporting parallel-channel architecture and wavelength division multiplexing (WDM) of sensing channels.

SPR WDM Sensor Platform: Implementation

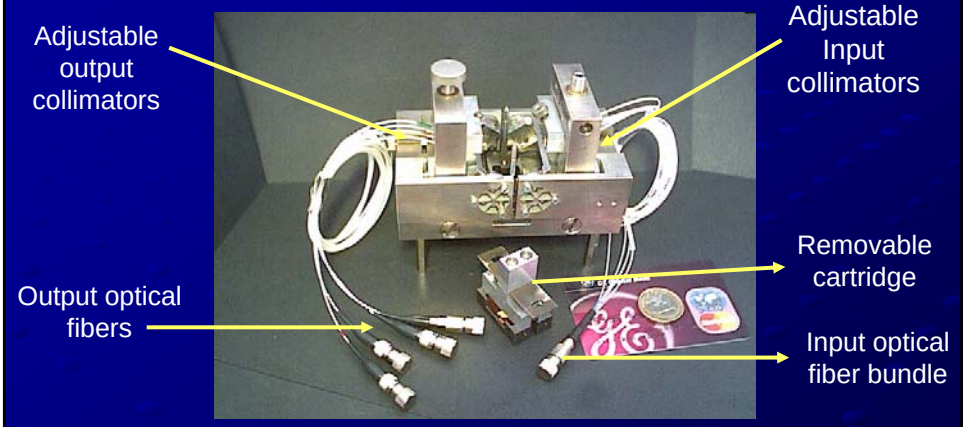
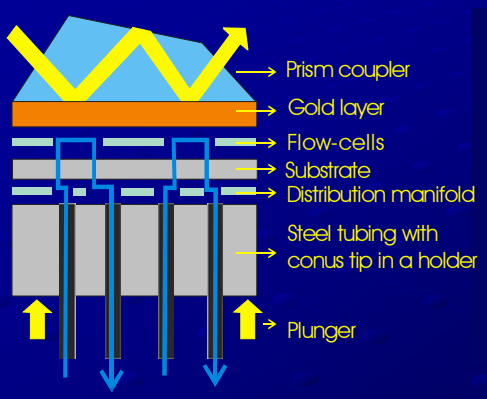


Fig. Miniature optical bench of an 8-channel SPRWDM sensor and a removable sensor chip cartridge.

Sensor Chip Cartridge and Fluidic System: Concept



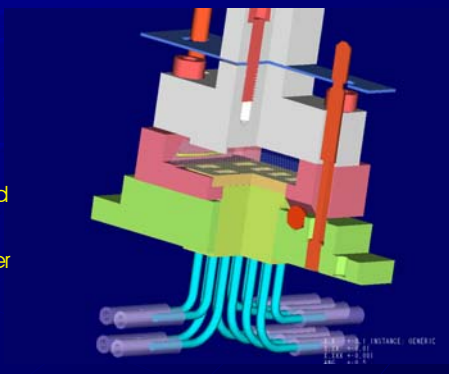


Fig. Concept of multilayer fluidic system for distribution of liquid samples to eight independent sensing channels.

Sensor Chip Cartridge: Implementation

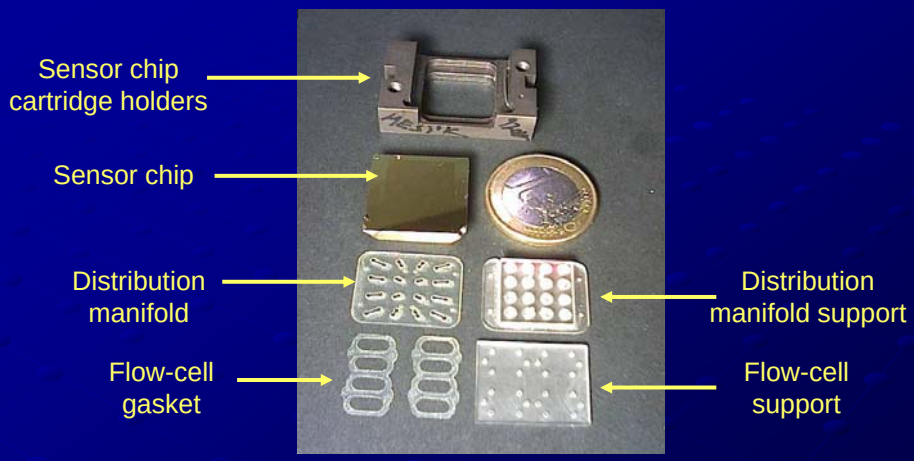


Fig. Elements of SPR sensor chip cartridge for 8-channel SPRWDM sensor.

System Integration

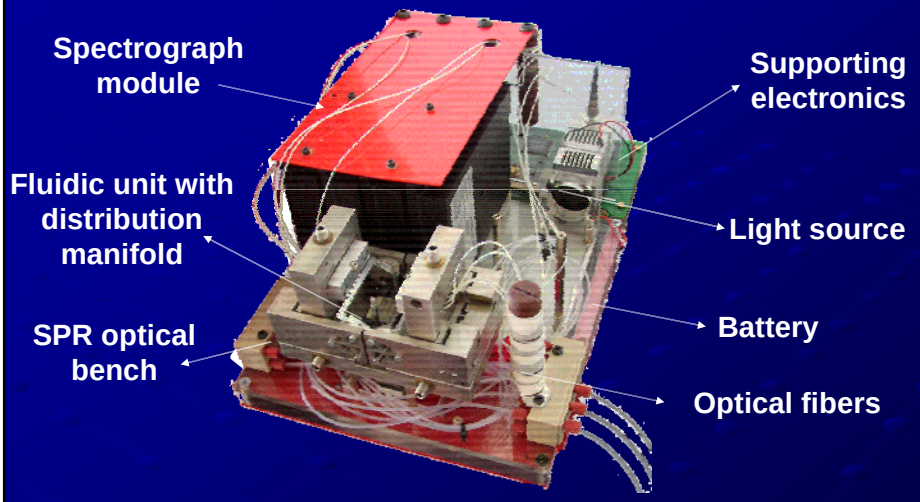


Fig. An eight-channel mobile SPR sensor system integrating miniature SPR optical bench, light source, spectrometer module and microfluidics.

Data Acquisition and Processing Software

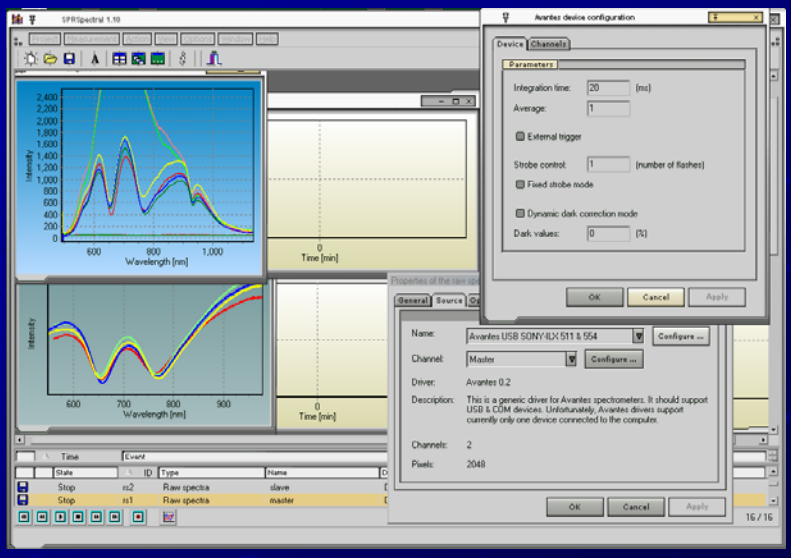


Fig. SPR Spectral – software for data acquisition and processing.

Portable SPR Sensor for Field Use

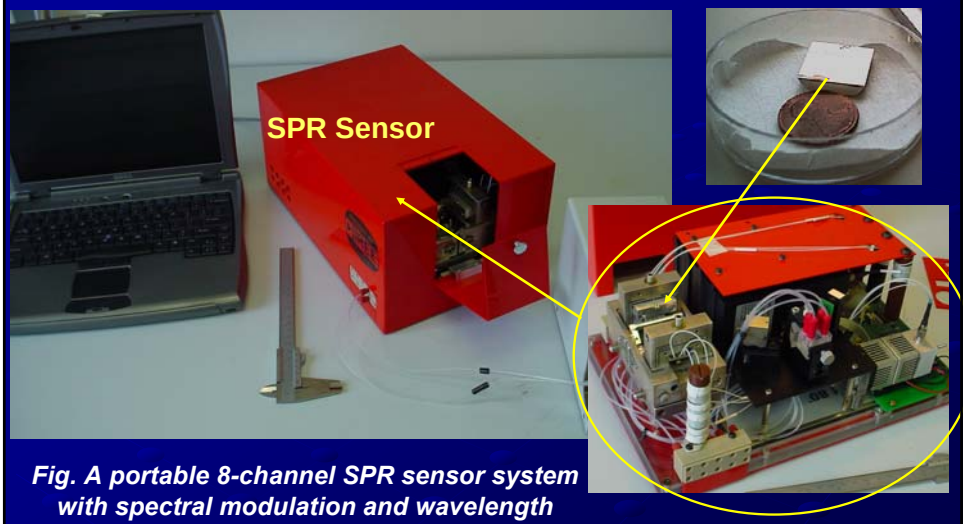
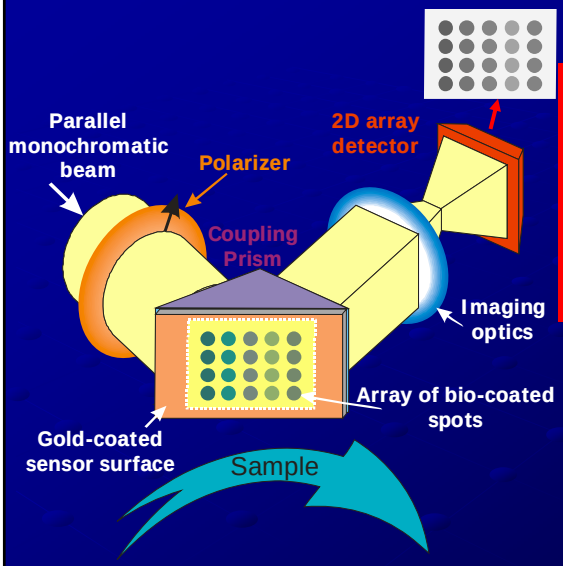


Fig. A portable 8-channel SPR sensor system with spectral modulation and wavelength division multiplexing (WDM) of sensing channels.

RI resolution:
4×10^{-7} RIU

DEVELOPING HIGH-THROUGHPUT SPR SENSING SYSTEMS

SPR Imaging: Features and Performance



Typical performance:

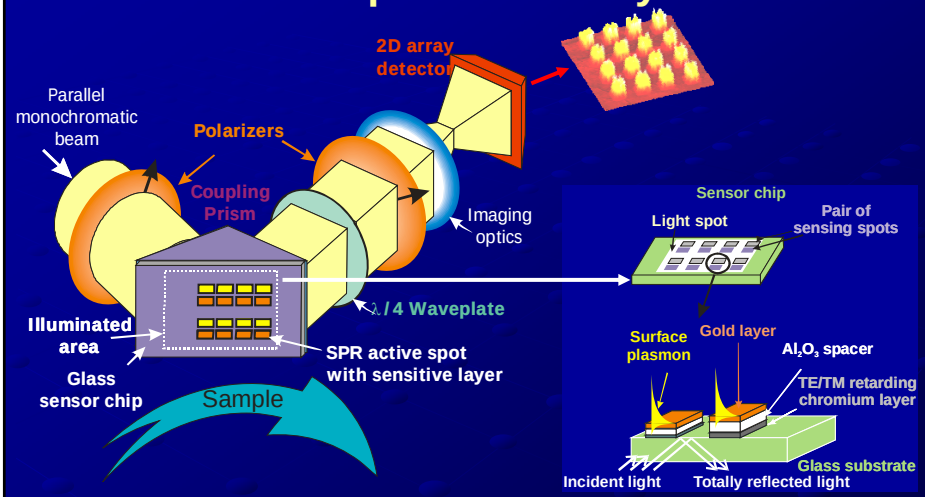
RI resolution:
$3x10^{-5}$ RIU *
Detection of nucl. acids
10nM (18-mer)**
Detection of proteins
1nM (anti-FLAG)***

Challenges:

- **Operating range. Noise due to light level fluctuations.**
- **Image contrast.**

* Fu, E., et al., *Review of Scientific Instruments*, 75, 2300 - 2304 (2004).
** Lee, H.J., T.T. Goodrich, and R.M. Corn, *Analytical Chemistry*, 73, 5525 - 5531 (2001).
*** Wegner, G.J., H.J. Lee, and R.M. Corn, *Analytical Chemistry*, 74, 5161 - 5168 (2002).

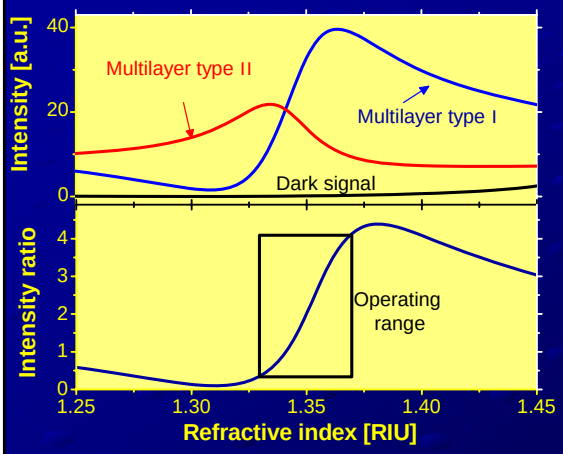
SPR Imaging Utilizing Polarization Contrast and Special Multilayers



SPR imaging with polarization contrast and the detail of the SPR sensor chip with series of pairs of sensing spots.

M. Piliarik, H. Vaisocherova, J. Homola, *Biosens. & Bioelectron.*, 20, 2104 - 2110 (2005).

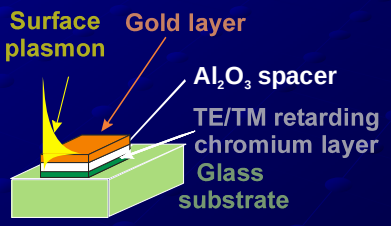
SPR Imaging Utilizing Polarization Contrast and Special Multilayers: Theory



Multilayer type:

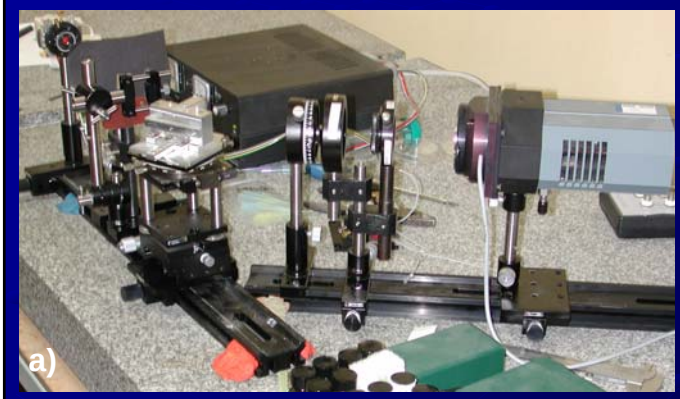
I. Cr thickness – 4 nm

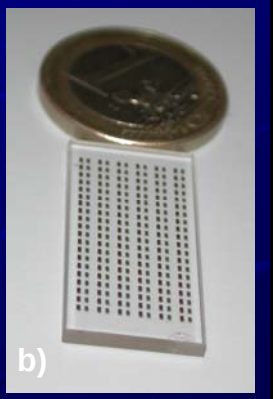
II. Cr thickness – 14 nm



Dependence of light intensity on the refractive index for the two different SPR multilayers and their ratio. Multilayer structure: chromium layer, aluminum oxide layer (100 nm), and SPR-active gold layer (40 nm).

SPR Imaging Utilizing Polarization Contrast and Special Multilayers: Experimental





a) View of the SPR imaging system with polarization contrast, b) detail of SPR imaging chip with pairs of different SPR multilayers.

High-Throughput SPR Sensor Based on SPR Imaging in Polarization Contrast

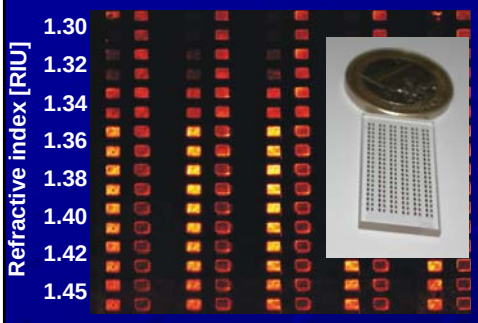


Fig. Image of the SPR sensing chip for rows of sensing spots exposed to liquids of different refractive indices.

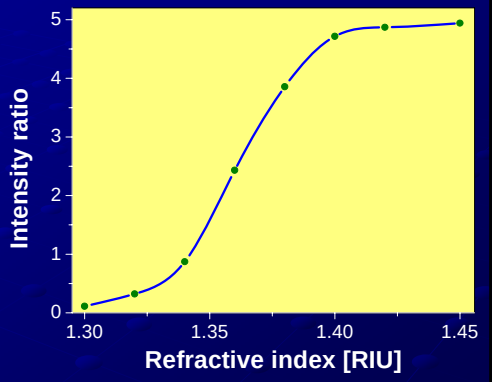
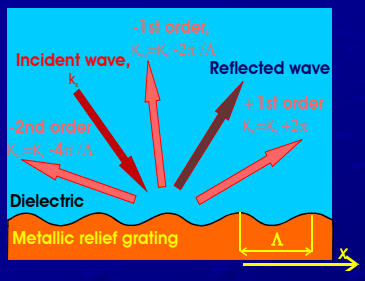


Fig. Ratio of intensities at the two multilayers vs refractive index.

RI resolution:
1×10^{-6} RIU

SPR Sensor Based on SP Spectroscopy on Array of Diffraction Gratings



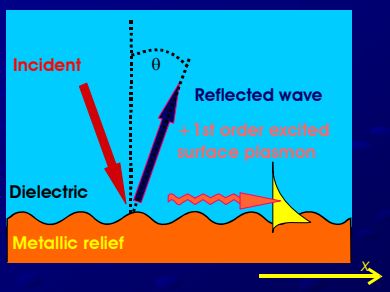
Diffraction of light on a diffraction grating.

Coupling condition:

$$k_0 \sin(\theta) + m\frac{2\pi}{\Lambda} = k_{xm}$$

$$k_{xm} = \mathrm{Re}\{\beta_{SP}\}$$

a)



b)

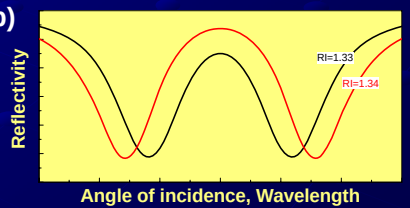
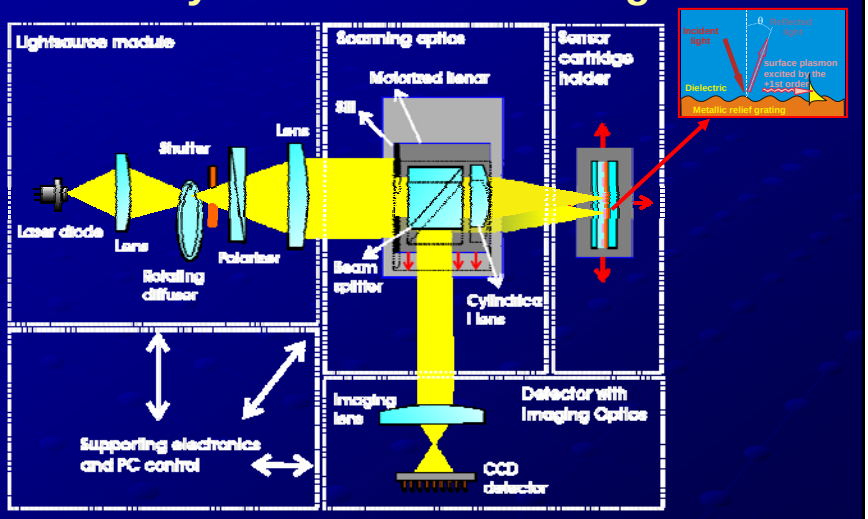


Fig. Excitation of SP on a diffraction grating: a) grating structure, b) reflectivity

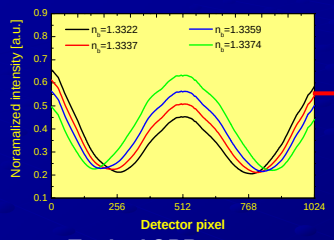
SPR Sensor Based on SP Spectroscopy on Array of Diffraction Gratings



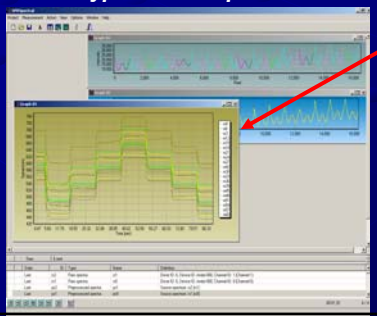
SPR sensor based on surface plasmon spectroscopy on an array of diffraction gratings and detail of illumination of the chip.

J. Dostálek, M. Miler, J. Homola, *Sensors and Actuators B*, 107, 154 - 161 (2005).

SPR Sensor Based on SP Spectroscopy on Array of Diffraction Gratings: Data Processing



Typical SPR spectra.



Special software for acquisition and processing of angular SPR spectra from multiple diffraction gratings.

SPR Sensor Based on an Array of Diffraction Gratings: Experimental Setup

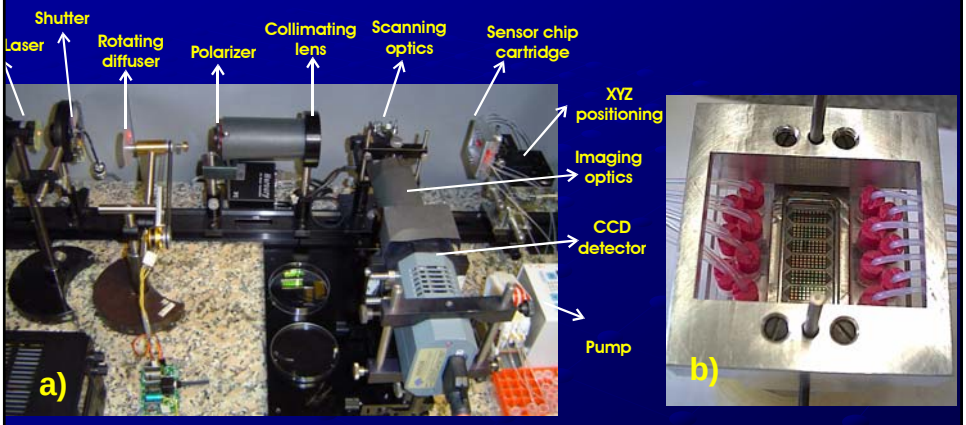


Fig. a) View of the optical system for SPR spectroscopy on an array of miniature diffraction gratins, b) detail of a sensor chip with microfluidic cartridge.

RI resolution:
7×10^{-7} RIU

MULTI-SURFACE-PLASMON SPECTROSCOPY

Multichannel SPR Sensors with Wavelength Division Multiplexing: Serial Channel Approach

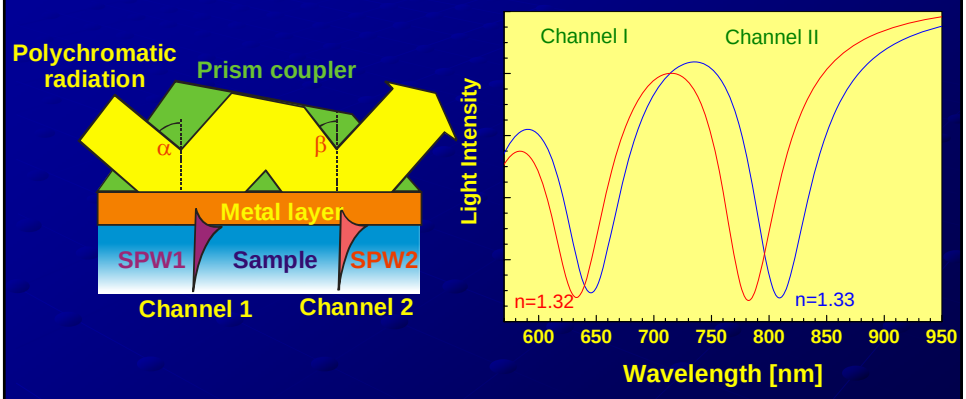


Fig. Concept of multichannel SPR sensor with serial sensing channels and typical SPR spectra observed with such a sensor.

Reference-Compensated SPR Biosensing I: Compensating Slow RI Variations

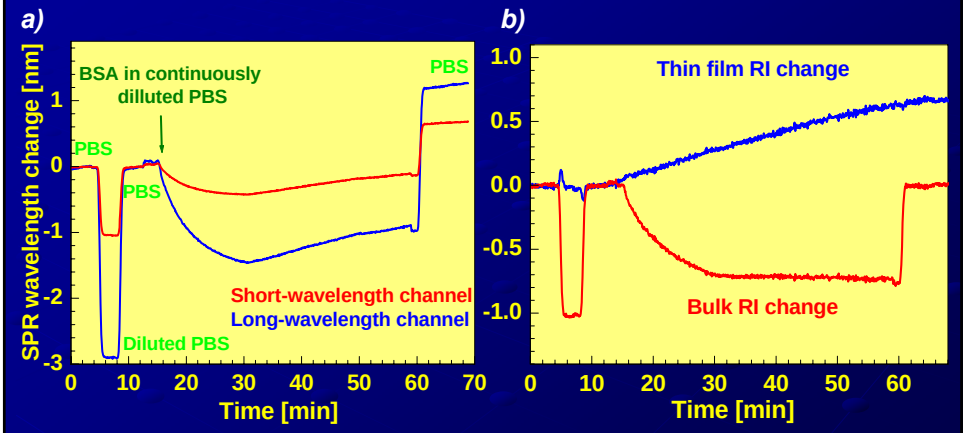
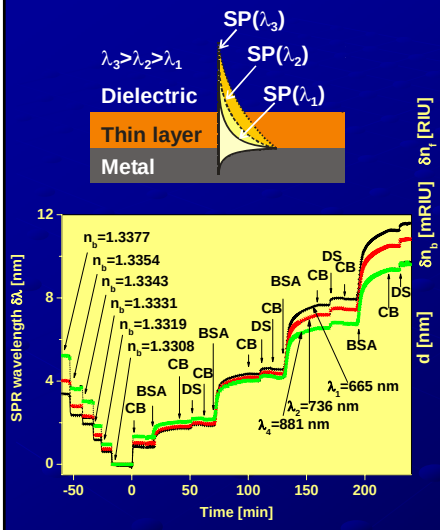
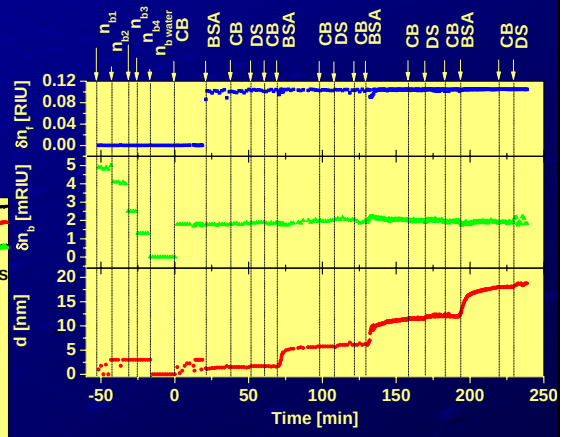


Fig. a) Response of the SPR sensor with two serial channels to formation of a thin BSA layer in the presence of slow background refractive index drift.
b) Deconvoluted surface and bulk refractive index responses.

Multi-Surface Plasmon Spectroscopy for Determination of Refractive Index Distribution



SPR response to formation of BSA multilayer.

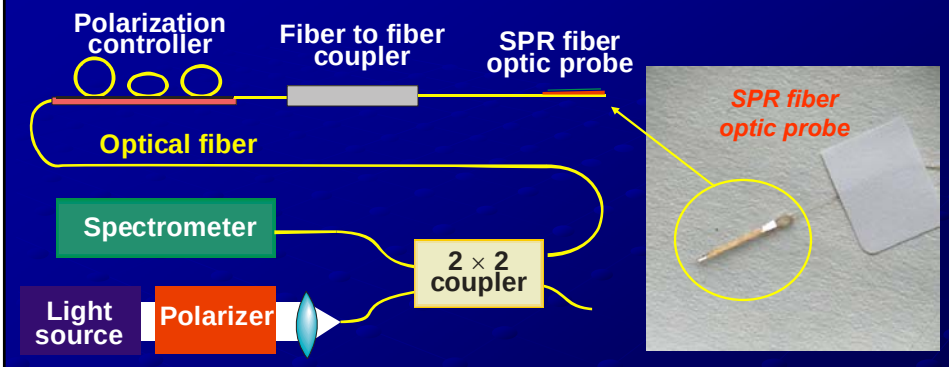


Deconvoluted refractive index of background, thickness and refractive index of a thin BSA multilayer.

Sensors and Actuators B 113, 774-781 (2006).

MINIATURIZED FIBER OPTIC SPR PROBE

Miniature Fiber Optic SPR Sensor



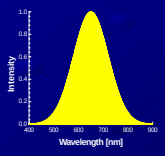
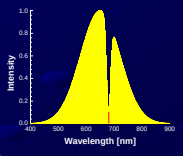


Fig. SPR fiber optic sensor with wavelength modulation (transmission mode).



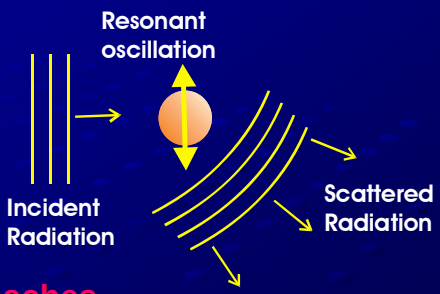
Homola, J., et. al, Proc. SPIE 4416, 82 (2001).
R. Slavík, J. Homola, Sensors and Actuators, B54, 74 (1999).

LOCALIZED SURFACE PLASMONS ON NANOPARTICLES

Localized Surface Plasmons on Metallic Nanoparticles

LSP – EM oscillation on small metallic objects:

$$\mathrm{Re}\{\varepsilon_m\} + 2 = 0$$



Theoretical analysis: approaches

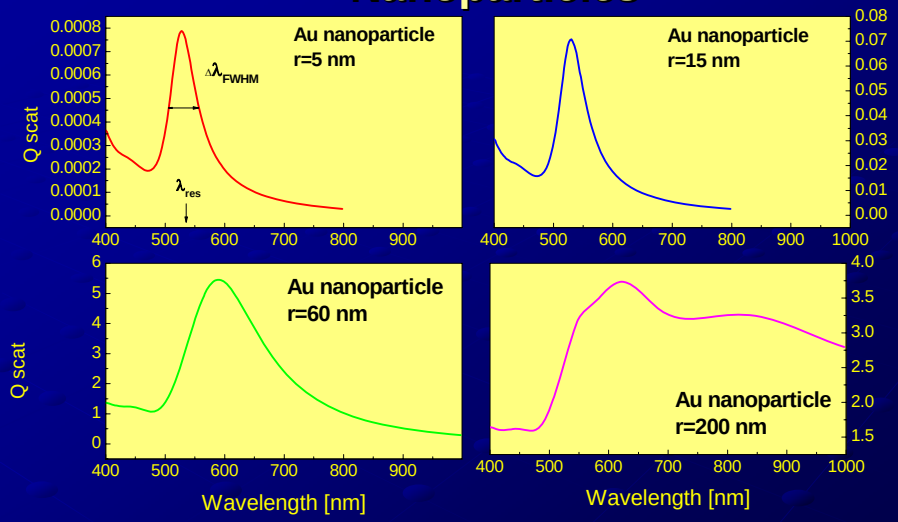
a) Mie theory

Exact analytical solution, valid for spherical particles

b) Discrete Dipole Approximation

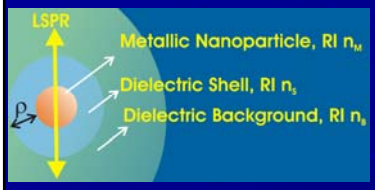
Numerical calculation of scattering on particles with arbitrary shape, limited for particles with $\left|\sqrt{\varepsilon_m}\right| k_0 d < 1$

Resonant Scattering of Light on Metallic Nanoparticles



Dependence of the total scattering efficiency Qscat on the wavelegth for four radiuses of nanoparticle (gold) in water medium calculated using Mie theory.

Sensor Based on Spectroscopy of Localized Plasmons on Spherical Nanoparticles



	S_B [nmRIU^{-1}]	S_S [RIU^{-1}]	χ [1/nmRIU]
ATR λ=800 nm	8000	40	0.6
DG λ=800 nm	600	3.5	0.5
LP λ=550 nm	50	3.8	0.07

Definitions:

$$d\lambda_{res} = S_B dn_B$$

$$d\lambda_{res} = S_S dn_S \rho$$

$$\chi = \frac{S_S}{\Delta\lambda_{FWHM}}$$

Comparison of sensitivity and χ parameter for SPR sensors using: attenuated total reflection (ATR), diffraction coupling (DG) and localized surface plasmons (LP).

BIOMOLECULAR RECOGNITION ELEMENTS AND THEIR ATTACHMENT

Biomolecular Recognition Elements and Their Immobilization

Biomolecular recognition elements used in SPR biosensors include: antibodies, peptides, proteins, DNA, RNA, etc.

Choice of immobilization technique depends on the type of ligand, size of analyte and specifics of application.

Requirements:

1. High, controlled ligand density
2. Non-fouling background

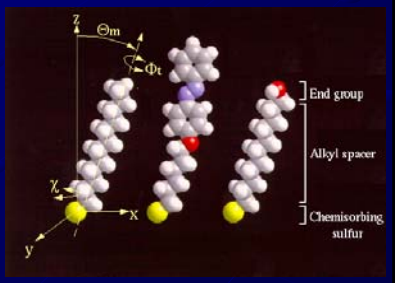
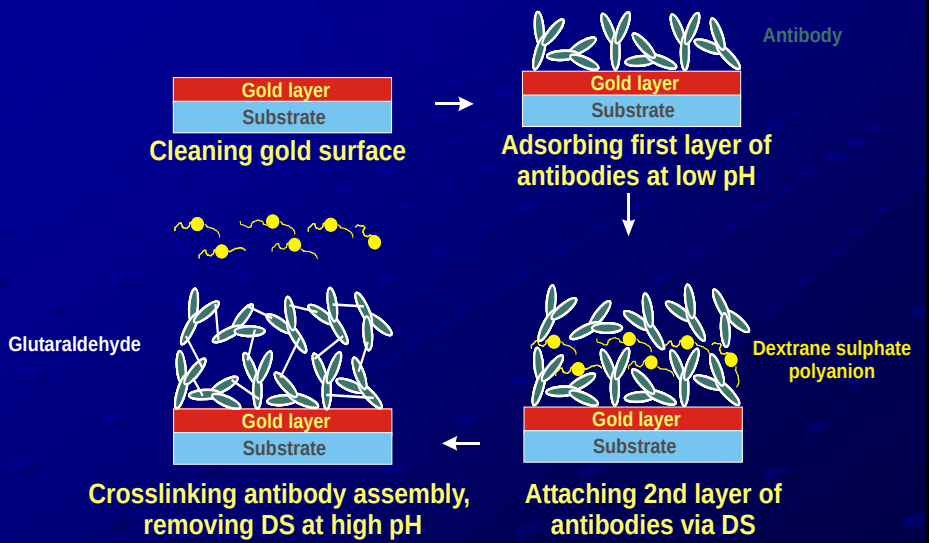
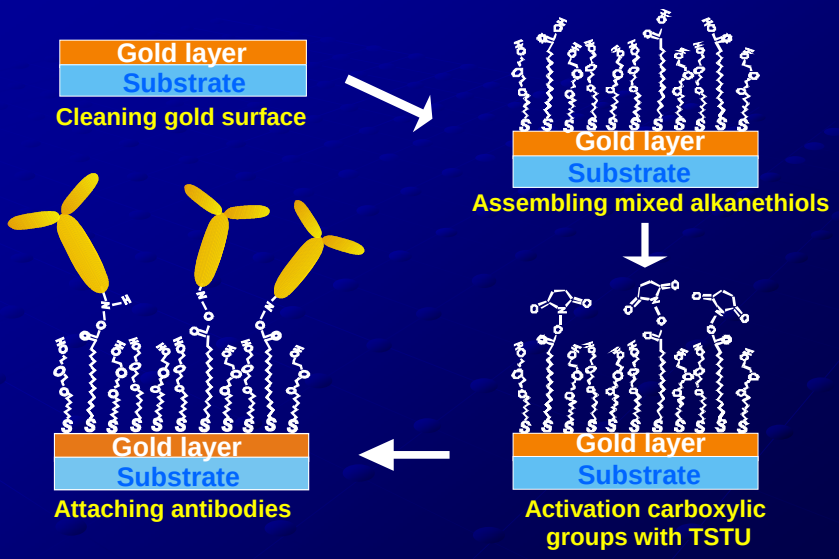


Fig. Structure of alkylthiol self-assembled monolayers.

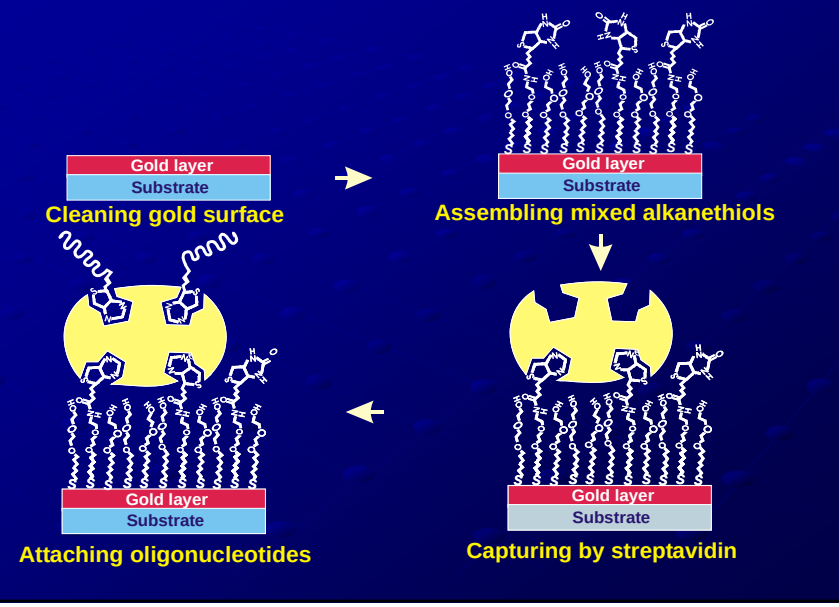
Functionalization of SPR Biosensors: Crosslinked Antibody Assemblies



Functionalization of SPR Biosensors: Alkanethiol Attachment Chemistry



Functionalization of SPR Sensors Using Streptavidin – Biotin Chemistry



APPLICATIONS OF SURFACE PLASMON RESONANCE BIOSENSORS

Applications of SPR Biosensors

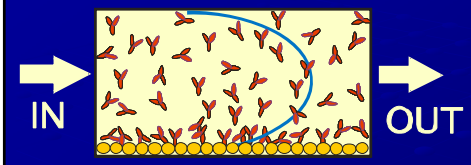
1. Investigation of molecules and their interactions

SPR offers a platform for real-time study of macromolecules and their interactions allowing determination of specificity, interaction models, kinetic rates, equilibrium constants, thermodynamic constants, and epitope mapping.

Existing and potential applications:

- Fundamental research.
- Development of drugs and therapies.
- Development/optimization of existing detection technologies (*e.g.* immunoassays).

Observing Biomolecular Interactions under Realistic Hydrodynamic Conditions



Sample flowing though a flow-cell in the laminar flow approximation.

Diffusion equation and first-order kinetic equation:

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial^2 x} + \frac{\partial^2 C}{\partial^2 y}\right) - 4v\frac{y}{h}\left(1-\frac{y}{h}\right)\frac{\partial C}{\partial x}$$

$$D\frac{\partial C}{\partial y} = \frac{\partial B}{\partial t} = k_a C(B_{Max} - B) - k_d B$$

B – concentration of bound analyte
C – concentration of free analyte
v - flow rate
h – flow-cell depth

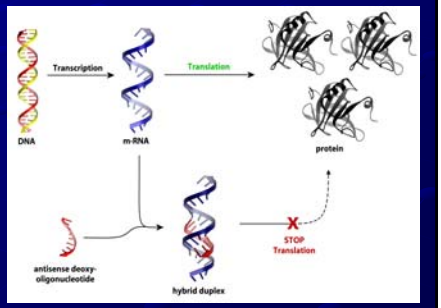
Investigation of Oligonucleotides for Antisense Therapy

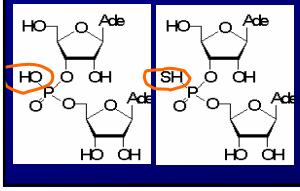
The principle of antisense drug is a sequence-specific binding of antisense oligonucleotide to the target "sense" mRNA, resulting in blocking of the expression of pathological gene information.

WHAT IS CRITICAL?

Natural oligonucleotides do not have sufficient resistance to cell nucleases

IT IS NECESSARY TO MODIFY CHEMICAL STRUCTURE





Current chemical modifications of internucleotide linkage have not optimal properties (hybridization with RNA, non-toxicity, etc.)

New modifications are investigated to develop an optimal antisense drug

Screening of Oligonucleotides as Potential Candidates for Antisense Therapy

29 oligonucleotides (dT_{15}) with different structural modifications at concentration of 100nM were bound to rA_{23} immobilized on the sensor surface.

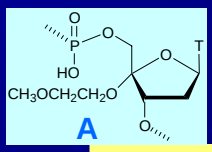
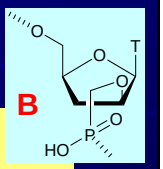
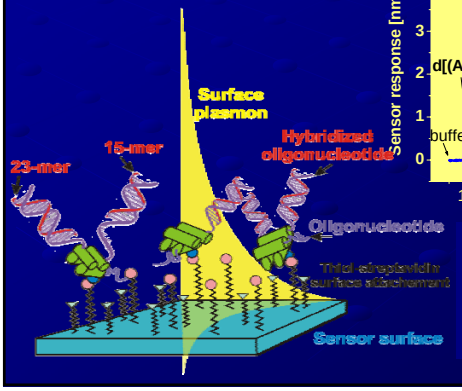
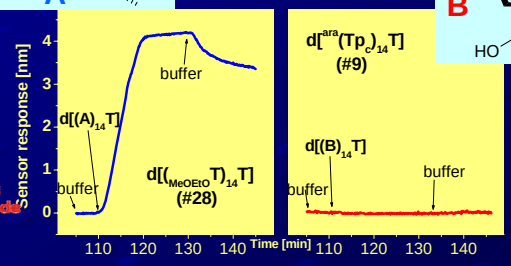


Fig. Sensor response to binding of oligonucleotides with selected modified linkages to natural rA_{23}

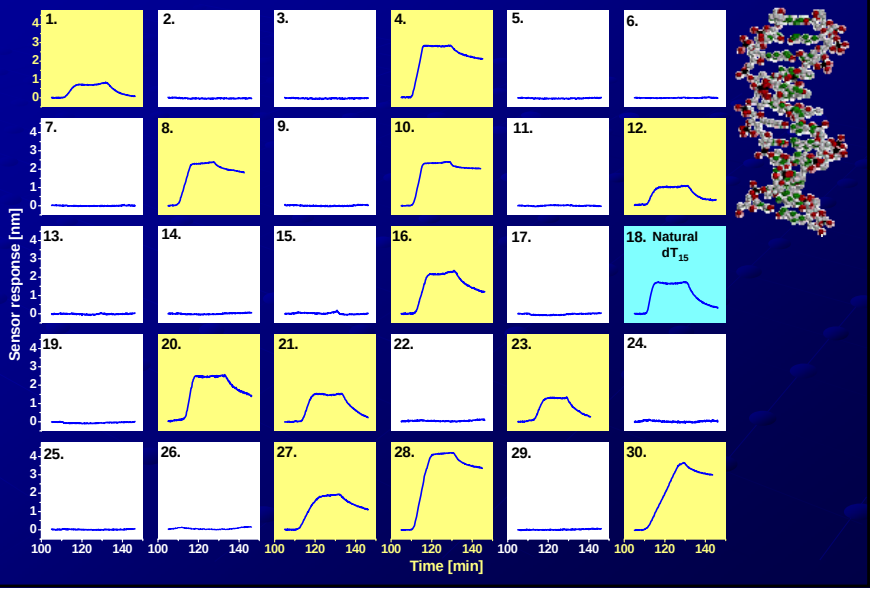




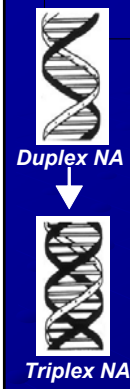


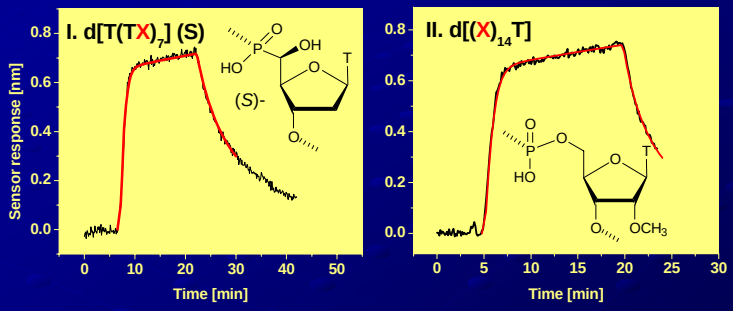
- Rapid screening (YES or NO in 15 min)
- Small sample consumption (0.1-100nM)
- Monitoring kinetics of the oligonucleotide interactions

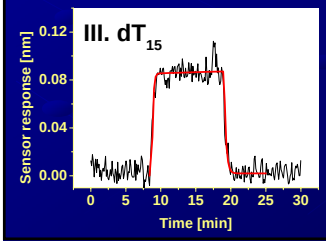
Primary Screening of Oligonucleotides with Modified Linkage for Antisense Therapy



Kinetic Analysis of Synthetic Modified Oligonucleotide Hybridization







Oligo #	k_{aD} [$M^{-1}s^{-1}$]	k_{dD} [s^{-1}]	k_{aT} [$M^{-1}s^{-1}$]	k_{dT} [s^{-1}]	K_{dD}[nM]	K_{dT}[μM]
I	4.96e5	3.16e-3	2e3	5.7e-5	6.4	0.03
II	4.76e5	7.21e-3	2.87e3	1.54e-4	15.2	0.05
III	7.05e5	7.94e-2	4.09e2	1e-4	112.7	0.24

Applications of SPR Biosensors (cont.)

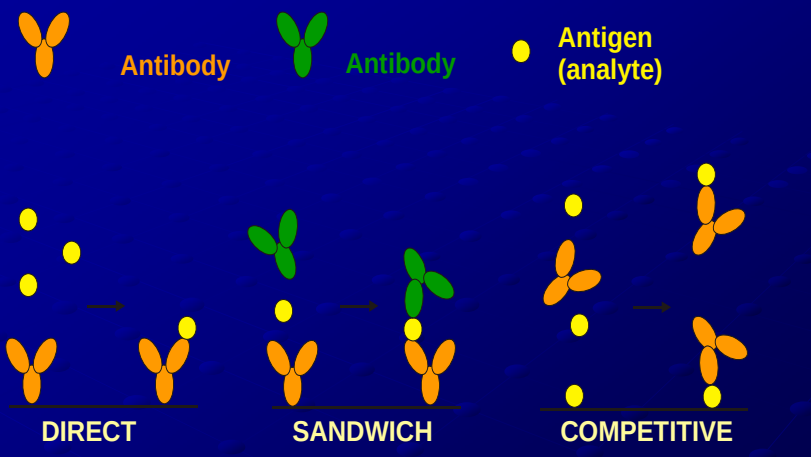
2. Detection, identification and quantification of chemical and biological substances.

SPR affinity biosensor technology shows potential for rapid, label-free, sensitive detection and identification of a large variety of chemical and biological agents and pathogens.

Existing and potential applications:

- **Environmental monitoring – field detection of chemical pollutants and contaminants such as pesticides.**
- **Medicine - detection of analytes such as antibodies and hormones (*in vitro*, *in vivo*).**
- **Food safety - detection of chemical food contaminants (antibiotics) and foodborne pathogens (bacteria).**
- **Security, counter-terrorism – rapid detection of chemical and biological warfare agents (toxins, bacteria) in the field.**

Affinity SPR Biosensing: Detection Strategies



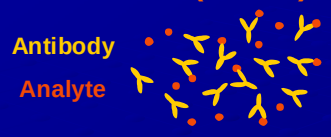
Medium size and large analytes.

Small and medium size analytes at low levels. Analyte verification.

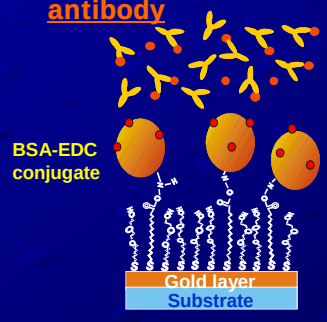
Small analytes at low concentrations.

SPR Sensor-Based Detection of Endocrine Disruptors

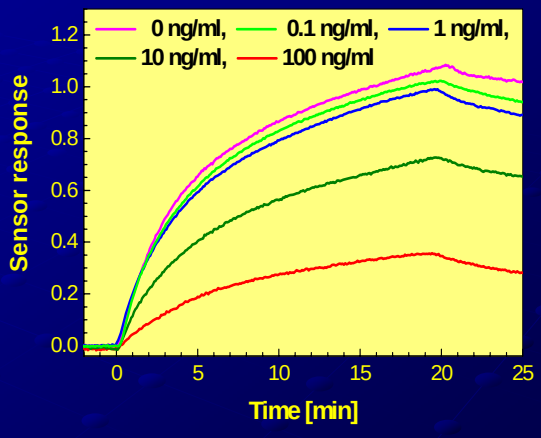
I. Incubation (20 min)



II. Detection of free antibody

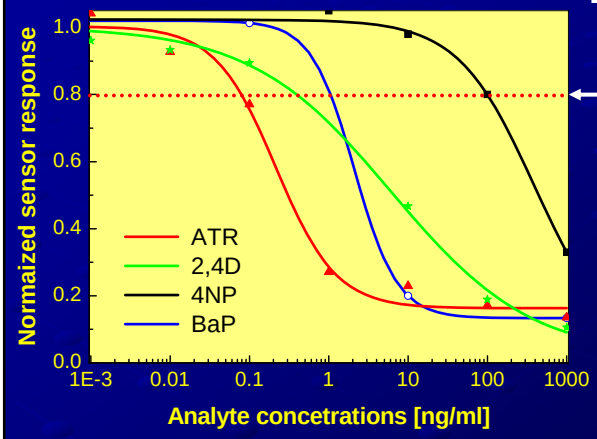


Inhibition assay detection format.



Detection of atrazine using inhibition assay. Kinetic response to unreacted antibody.

SPR Sensor-Based Detection of Endocrine Disruptors: Calibration & Limit of Detection



Calibration curves for detection of atrazine (ATR), 2,4-dichlorophenoxyacetic acid (24D), 4-nonylplhenol (4NP) and benzo[a]pyrene (BaP)

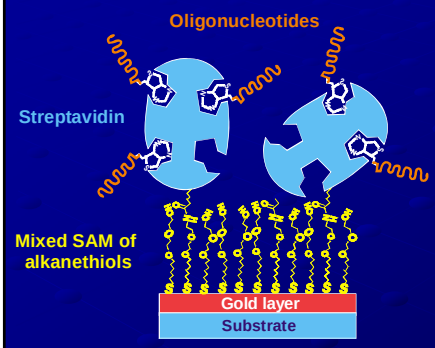
Limit of detection (LOD)

$$c_{LOD} = \frac{m\sigma_{blank}}{S_{c\to 0}}$$

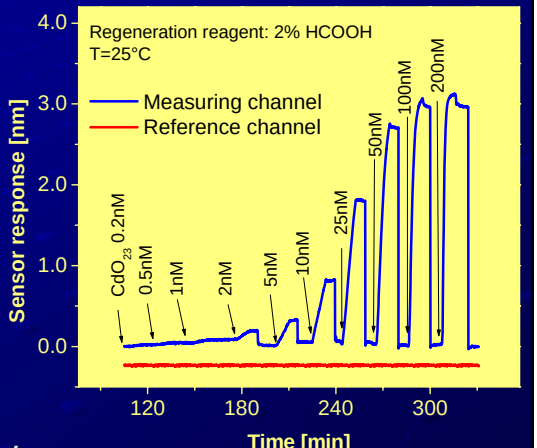
Achieved LODs:

- **ATR – 0.08 ng/ml**
- **2,4-D – 0.4 ng/ml**
- **BaP – 1 ng/ml**
- **4NP – 100 ng/ml**

SPR Sensor-Based Detection of Oligonucleotides



Immobilization of oligonuclotides via streptavidin attached to alkanethiols.



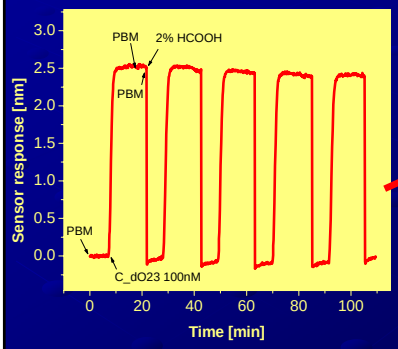
Binding of 23-mer oligonucleotide to complementary probe (blue) and to non-complementary base sequence (red).

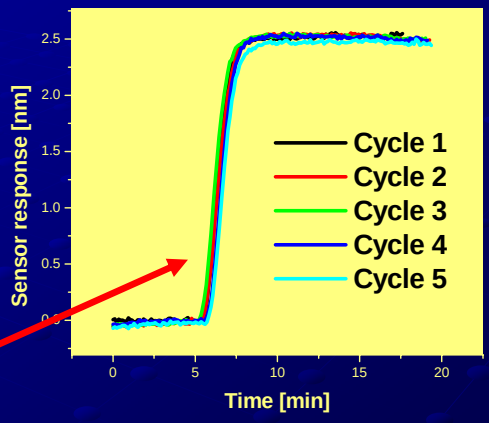
H. Vaisocherová et al., *Biopolymers,* in print.

SPR Sensor-Based Detection of Oligonucleotides: Regenerability

Regeneration reagent:

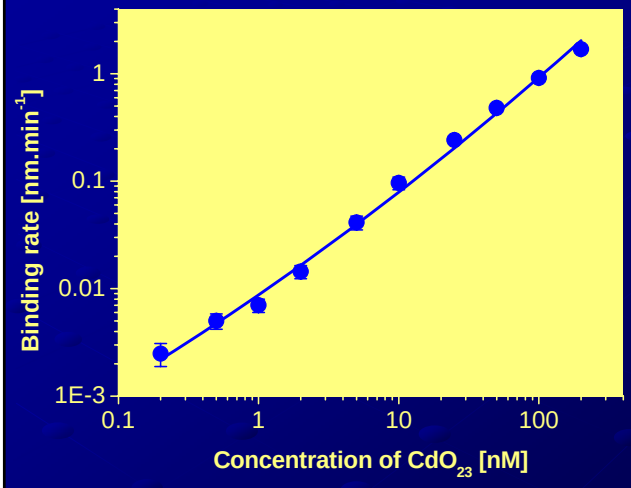
Formic Acid (2%)





Five detection/regeneration cycles obtained for detection of 23-mer oligonucleotide using a complementary probe.

SPR Sensor-Based Detection of Oligonucleotides: Limit of Detection

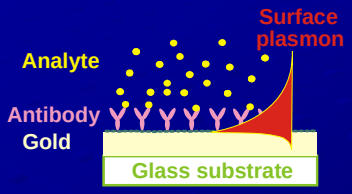


Achieved LOD:
100 pM
(0.70 ng/ml)

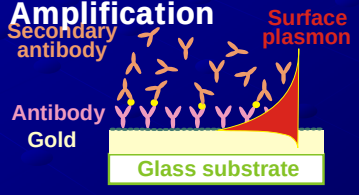
Calibration curve for detection of model oligonucleotides.

SPR Sensor-Based Detection of Staphylococcal Enterotoxin B

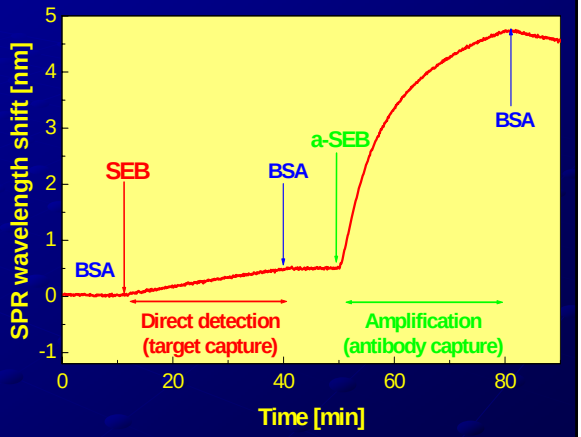
I. Direct detection



II. Amplification



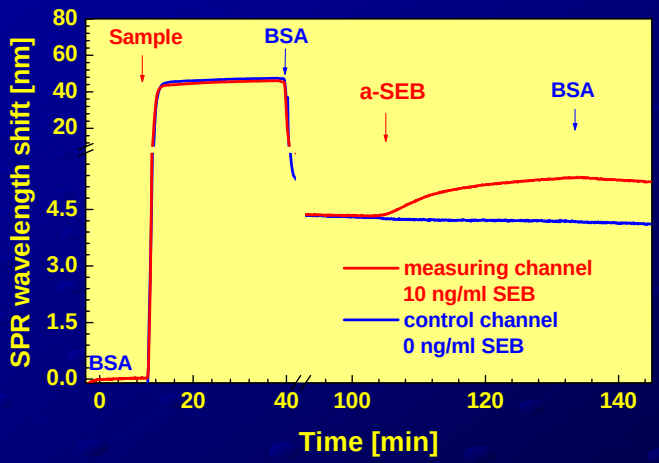
Sandwich assay detection format.



SPR sensor response to SEB. (SEB concentration - 25 ng/ml, a-SEB concentration – 3 μg/ml).

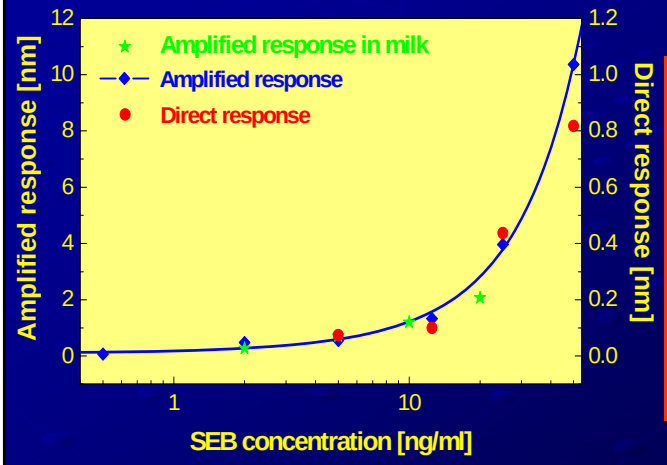
J. Homola et al., *Int. Journal of Food Microbiology*, 75, 61-69 (2002).

SPR Sensor-Based Detection of Staphylococcal Enterotoxin B in Milk



Sensor response to milk spiked with SEB. Sensor response to SEB (10 ng/ml in milk) and control solution (milk without SEB).

SPR Sensor-Based Detection of Staphylococ. Enterotoxin B: Limit of Detection



SPR sensor response to different concentrations of SEB in buffer and milk. Direct detection and sandwich assay.

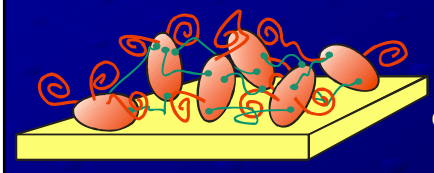
Achieved LOD:

Direct:
5 ng/ml

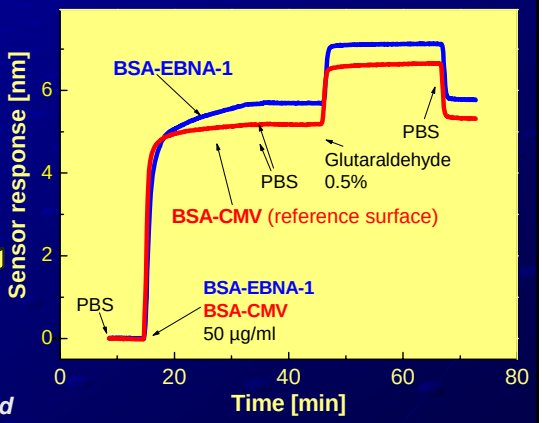
Sandwich:
0.5 ng/ml

SPR Sensor-Based Detection of Antibody Against Epstein-Barr Virus

Short (20 amino acids) synthetic peptide (EBNA-1) used as a receptor



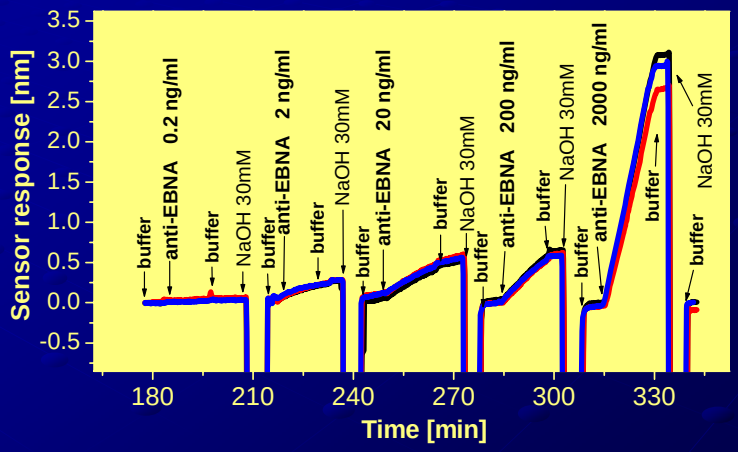
BSA-EBNA conjugate immobilized via hydrophobic interactions on gold



Sensor response to immobilization of BSA-peptide conjugates

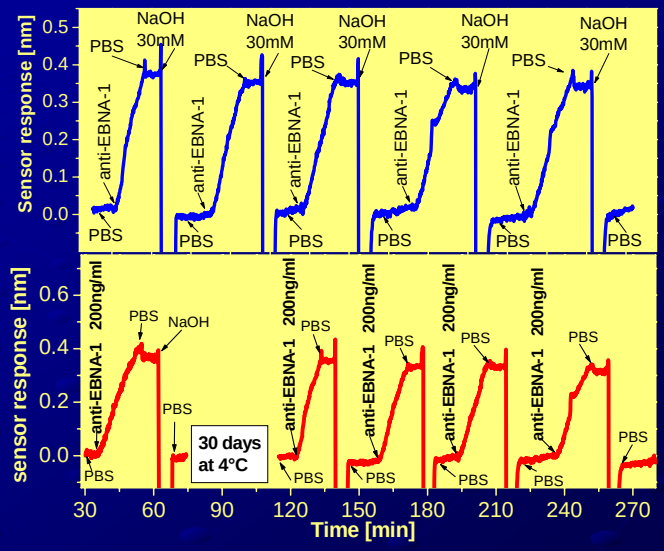
H. Vaisocherová et al., *Biosensors and Bioelectronics*, in print.

SPR Sensor-Based Detection of Antibody Against Epstein-Barr Virus: Concentration Dependence



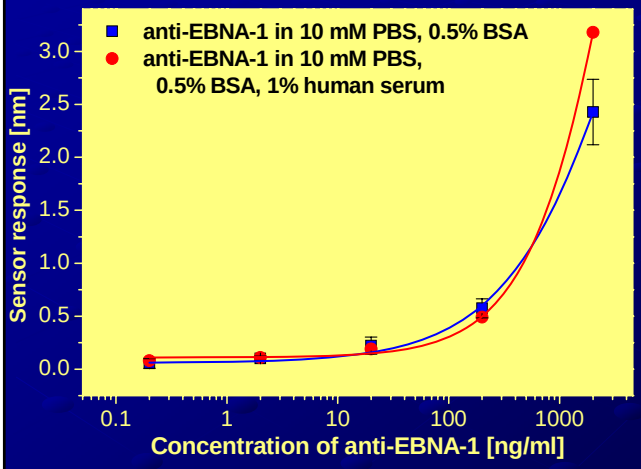
SPR sensor response to increasing concentration of anti-EBNA using regenerated peptide surface.

Detection of Antibody Against Epstein-Barr Virus: Sensor Regenerability and Storability



SPR sensor response to series of detection/regeneration cycles on freshly functionalized sensor (upper) and sensor after 30-day storage (lower).

SPR Sensor-Based Detection of Antibody Against Epstein-Barr Virus: Limit of Detection and Reproducibility



Achieved LOD:
0.2 ng/ml

Reproducibility
85 per cent

Sensor response to anti-EBNA in buffer (14 binding experiments for each concentration) and in serum.

SPR Sensor-Based Detection of Human Chorionic Gonadotropin: Concentration Dependence

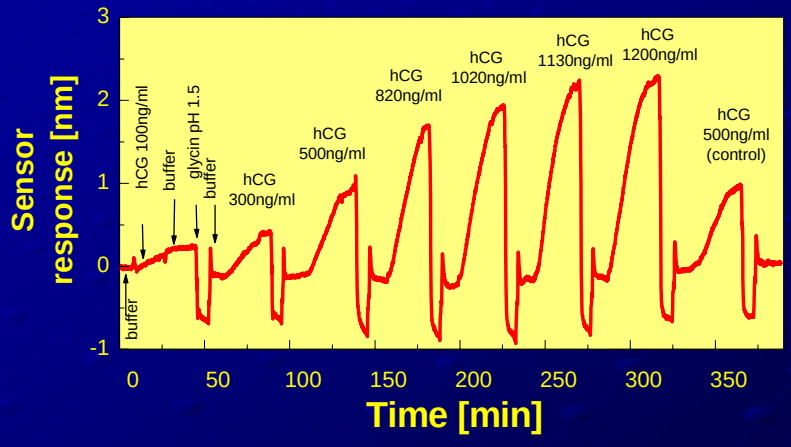


Fig. Typical sensorgram with SPR response to samples with increasing concentration of hCG (upper). Calibration curve for hCG (lower).

SPR Sensor-Based Detection of Human Chorionic Gonadotropin: Regenerability

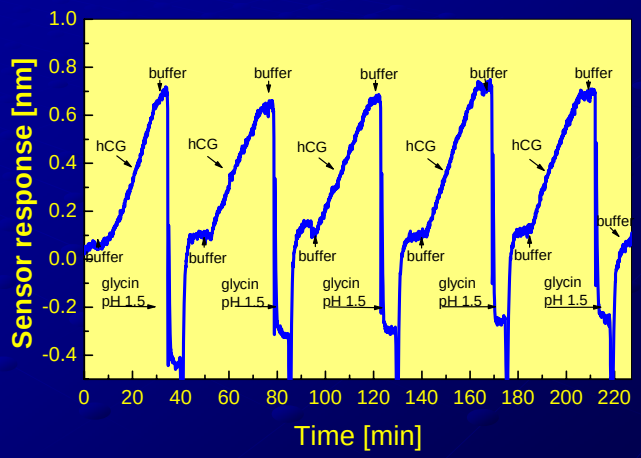
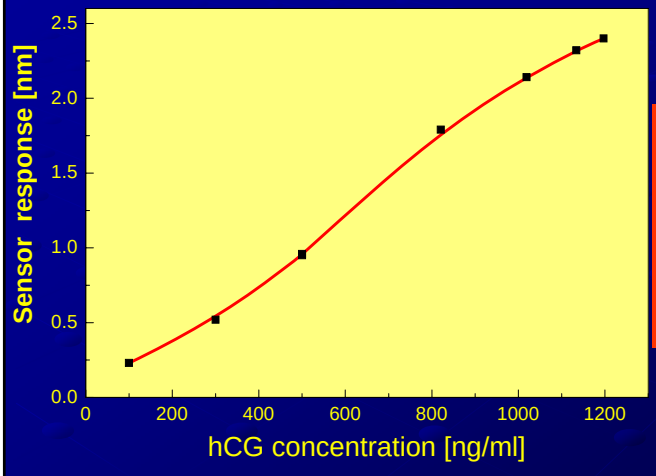


Fig. Sensor response to series of measurement/regeneration cycles. Regeneration agent: glycin pH 1.5.

SPR Sensor-Based Detection of Human Chorionic Gonadotropin: Limit of Detection

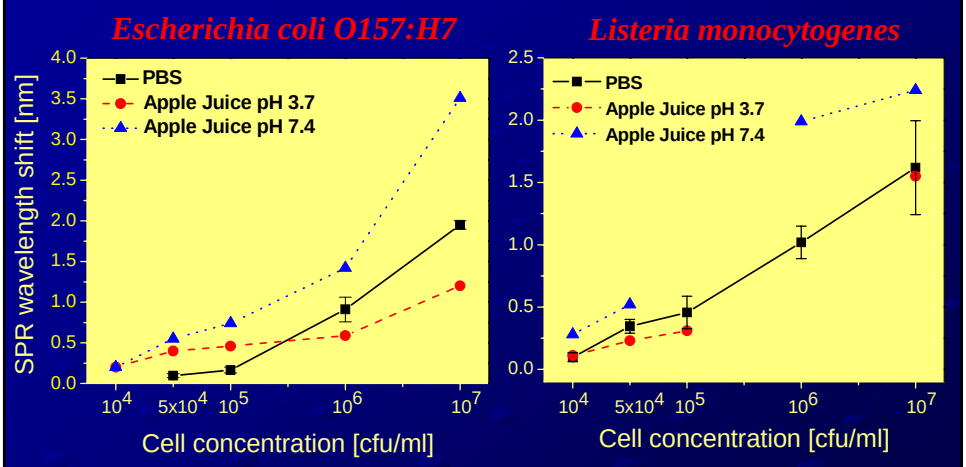


Achieved LOD:
50 ng/ml

Reproducibility
90 per cent

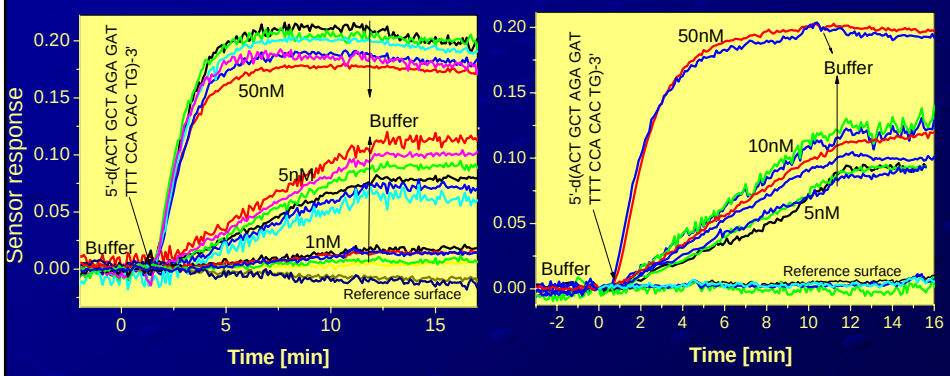
Sensor response to different concentrations of hCG on buffer.

SPR Sensor-Based Detection of Bacterial Pathogens



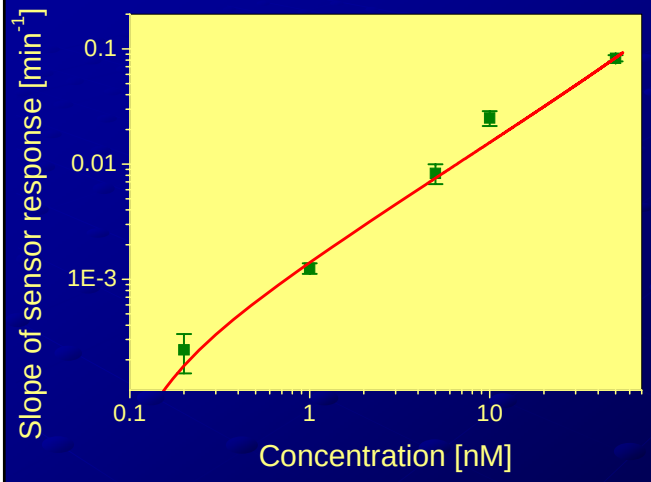
SPR sensor response to different concentration of selected bacteria present in buffer and in apple juice.

Applications of High-Throughput SPR Sensors: Observing DNA Hybridization



Observing DNA hybridization for SPR chips coated with oligonucleotides using in situ functionalization and microspotting.

High-Throughput SPR Sensors: Observing DNA Hybridization



Sensor response to different oligonucleotide concentrations.

Achieved LOD:

200 pM

1.5 ng/ml

Number of channels:

64

Measurement time:

3 - 10s

SPR Biosensor Technology: Future

- **Increase number of sensing channels to increase the throughput of the SPR sensor technology for massively parallelized screening.**
- **Develop robust referencing methods for (potentially direct) robust detection of bioanalytes in complex samples (food, blood, etc.).**
- **Advance miniaturization of SPR sensor technology towards in vivo real-time diagnostic tools.**
- **Improve SPR sensor sensitivity to increase speed of analysis and improve detection limits for large bacterial pathogens.**
- **Introduce microfluidics to SPR sensors and optimize interaction conditions to increase the speed of analysis.**
- **Advance development of biomolecular recognition elements and attachment chemistries to improve SPR sensor specificity and shelf life.**

Summary

- Optical biosensors based on spectroscopy of surface plasmons provide label-free, fast, specific and sensitive alternative to traditional laboratory analytical techniques.
- Various SPR sensor platforms and functionalization chemistries are available to meet needs of specific applications.
- SPR sensors can detect small and medium size analytes at practically relevant concentrations (0.1-1 ng/ml).
- SPR biosensor technology can benefit many important areas such as medicine, environmental protection, food and drug screening and security.