BIOSENOSRS

BIO 580

Optical Biosensors- theory part 3

WEEK-9

Fall Semester

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Topics that will be covered in the course

- ❑ History of biosensor development, applications and requirements of biosensors and classification
- ❑ Principles of molecular recognition and transduction signal acquisition
  - ✓Sources of Biological Recognition elements – enzymes/proteins, ssDNAs, antibody and Others
  - ✓Design considerations for use of recognition elements in biosensors
  - ✓Modeling of reactions for various biosensor applications- electrochemical, optical, piezoelectric, colorimetric, fluorometric and others.
- ❑ Modification of sensor surfaces and immobilization techniques
  - ✓Covalent modification of surfaces using surface chemistry
  - ✓Self Assembled Monolayers (SAM) and adsorptions
  - ✓Other ways to immobilize biological macromolecules on various solid surfaces
- ❑ Detection methods and Physical Sensors
  - ✓Electrodes/transducers – electrochemical (amperometric, potentiometric, and conductimetric transductions)
  - ✓Other sensors - for e.g., optical sensors (colorimetric/fluorimetric/luminometric sensors), Surface Plasmon Resonance (SPR) sensors, and piezoelectric resonators.
- ❑ Fabrication of biosensors
  - ✓Miniaturization-application of nano-materials, nanoparticles, carbon nanotubes (CNTs) and others
  - ✓Biocompatibility – stability, reproducibility and repeatability of biomolecules on transducer surfaces
- ❑ Data acquisition, statistical and error analysis
  - ✓Inter and Intra-assays and Coefficient of variation (CV)
  - ✓Signal to noise ratio
  - ✓Normalization/optimization and signal retrieval
- ❑ Examples of commercial biosensors

Evanescent wave, Surface Plamon Resonance, and θ_{SPR}

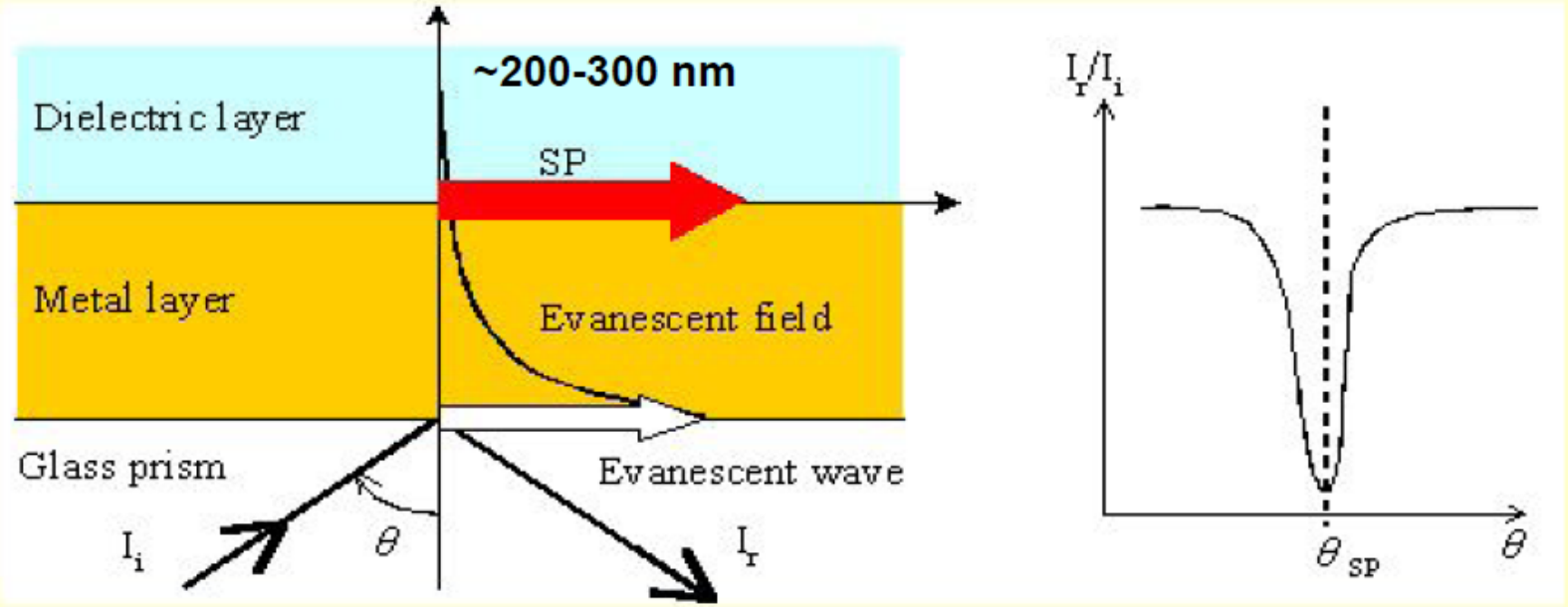
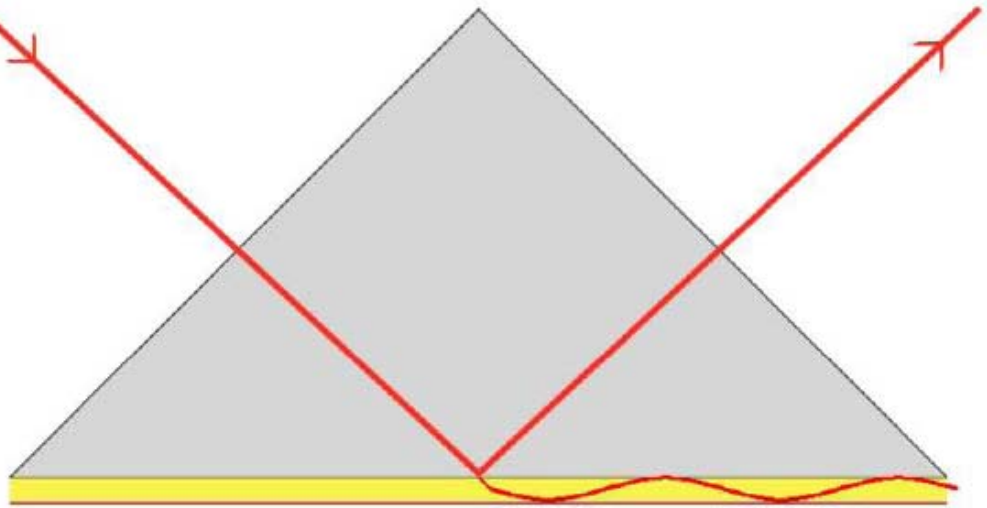


Figure 1. **Mechanism of the SP excitation.**

θ_{SPR} : a specific incident angle where SPR phenomenon occurs

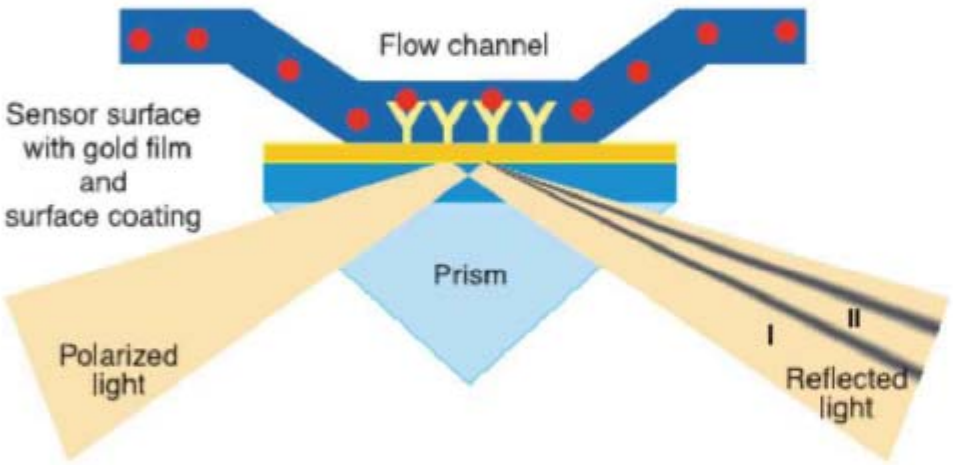
www.sys.eng.shizuoka.ac.jp/~j-kondoh/SP1.GIF

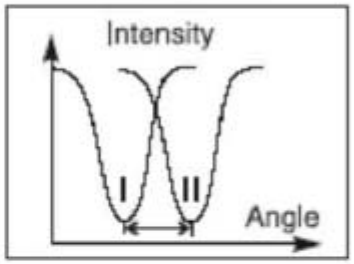
SPR (Surface Plasmon Resonance)



- SPR causes a reduction in intensity of incident light at a specific "resonance angle".
- The resonance angle depends on the local refractive index (n2)
- n2 depends on the local mass density of the sample medium surface

How Does SPR Detect Molecular Interactions





- Resonance angel changes upon ligand-analyte interaction
  (local mass density change→refractive index change→ resonance angel change)

Surface Plasmon Resonance (SPR)

- SPR – optical phenomenon based on evanescent field
- Evanescent wave is sensitive to refractive index (RI) in close proximity to the sensor surface
- Change in RI α solute concentration
- Advantages
  - Label-free technique
  - Highly sensitive to RI change, ~ 3×10^{-7} RU
  - Works in turbid or opaque samples

I. Surface Plasmon Resonance(SPR) Biosensor

A fraction of the light energy incident at a sharply defined angle can interact with the delocalised electrons in the metal film (plasmon) thus reducing the reflected light intensity. The precise angle of incidence at which this occurs is determined by a number of factors.

In biosensor applications the principal determinant becomes the refractive index close to the backside of the metal film. Target molecules are immobilised and addressed by ligands.

If binding occurs to the immobilized target the local refractive index changes, leading to a change in SPR angle, which can be monitored in real-time by detecting changes in the intensity of the reflected light. These changes can be used to determine affinity constants.

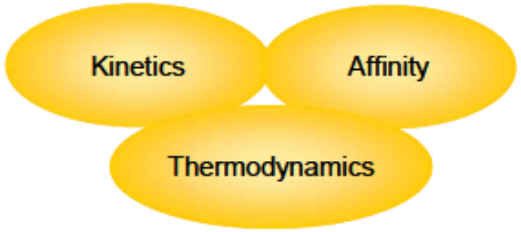
Where are SPR Biosensors used?

- **Surface sensitive optical detection method–interactions between biomolecules:**

  protein-protein, protein-ligand, protein-DNA, protein-membrane

- **Phenomenon that occurs when light is reflected off thin metal films.**
- **Identification and Quantification (association, dissociation and equilibrium constants, and energetics) of these interactions.**

What SPR Biosensors Measures



How fast, strong & why…

Is the binding of a lead compound

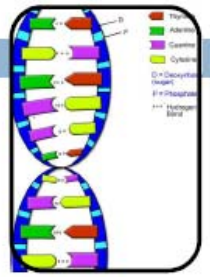
How specific & selective...

Is this drug binding to its receptor?

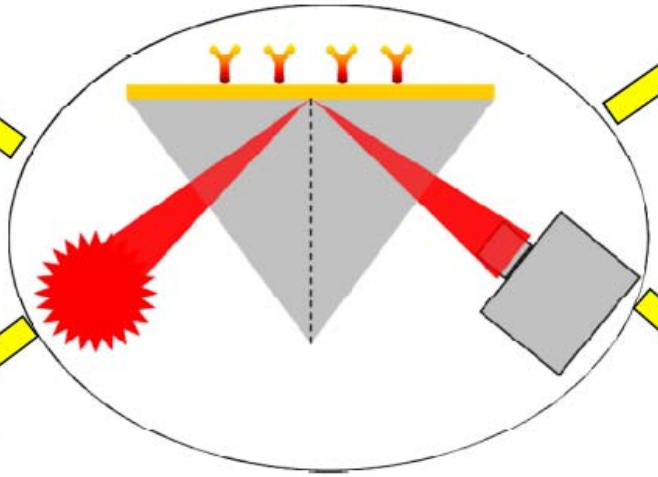
How much...

Biologically active compound is in a production batch?

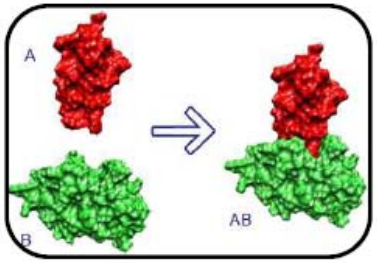
Biological Applications of SPR



DNA-DNA
or
DNA-RNA

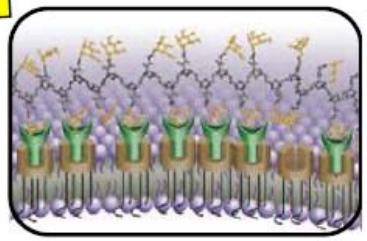


Antigen-Antibody



Protein-
protein

- Ligand - Receptor interaction
- RNA - Protein interaction
- Cell - Protein interaction
- Drug Screening



Carbohydrates

SPR detection

Principle

SPR detects refractive index changes close to the surface

E.g. accumulation of 1 pg/mm^2 gives a change of 1 µRIU or 1 RU

All biomolecules have refractive properties, so no labeling required

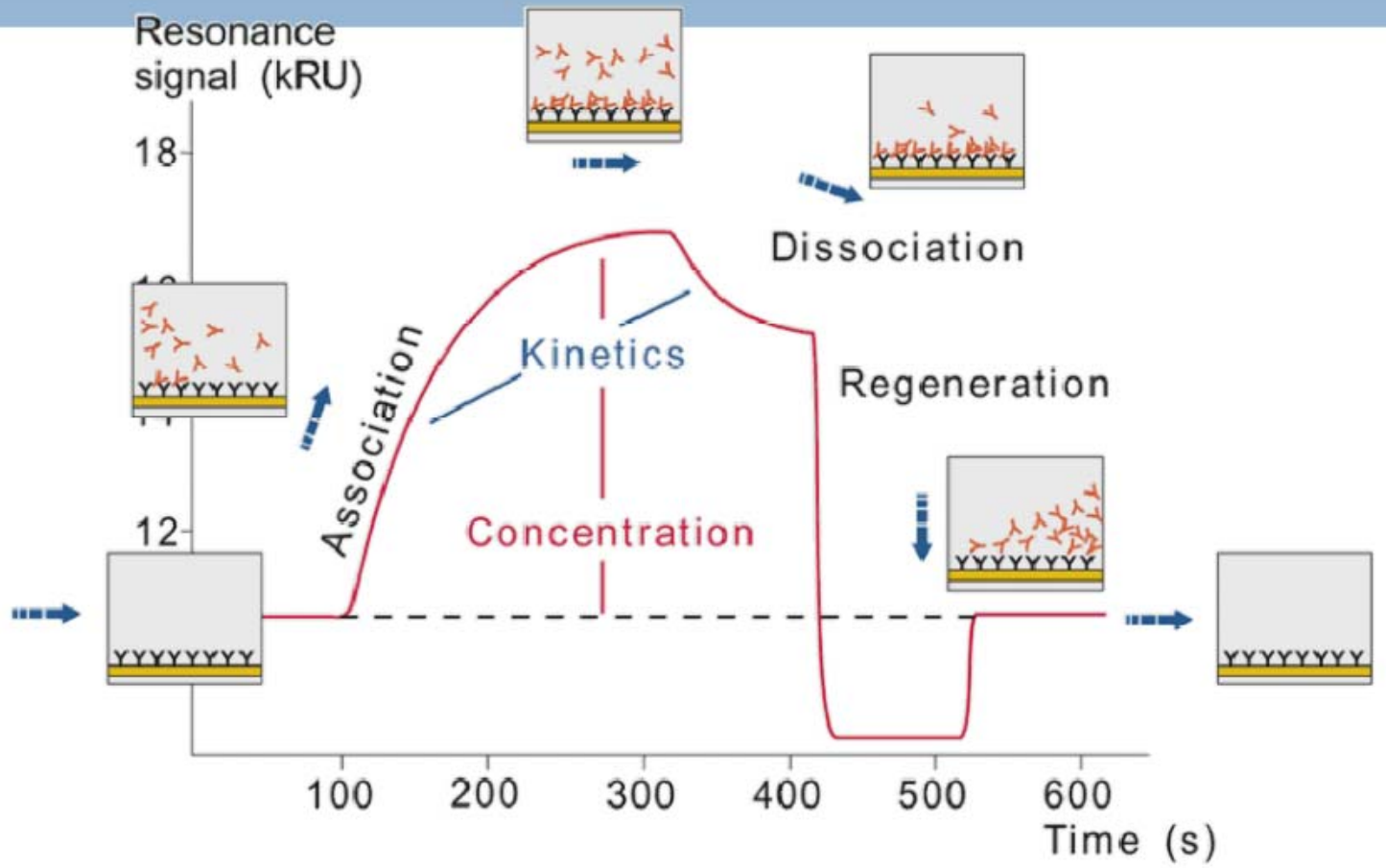
Result

No need to separate bound from free

This facilitates real-time measurements as a basis for taking kinetic data

Work with un-altered analytes possible

SPR Sensogram



www.astbury.leeds.ac.uk/facil/SPR Biacore SPR

Analysis of SPR with examples

- Specificity
- Concentration Assays
- Affinity Analysis

Specificity Analysis

- **Do two molecules interact with each other? Yes/No Answer**

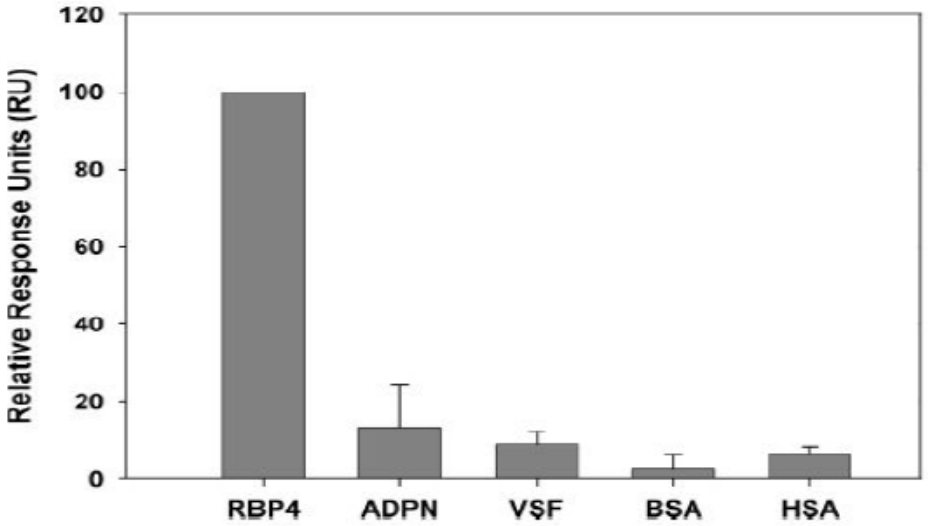


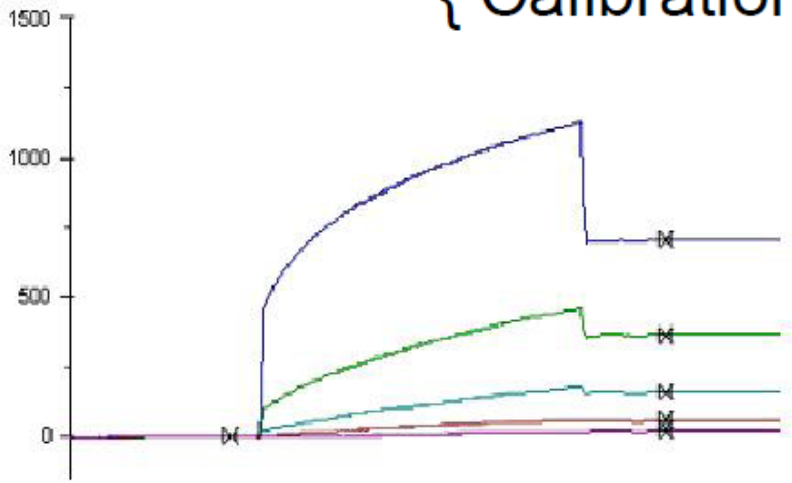
Figure 4. Specificity of aptamer no. 38 to RBP4. Specificity of the aptamer was determined with SPR analysis using a constant amount of ssDNA immobilized on the gold chip and by varying the concentrations of retinol binding protein 4 (RBP4), adiponectin (ADPN), visfatin (VSF), bovine serum albumin (BSA), or human serum albumin (HSA). The response units (RU) were obtained with 1 μM protein solutions at 25 °C and are shown relative with the RBP4 results.

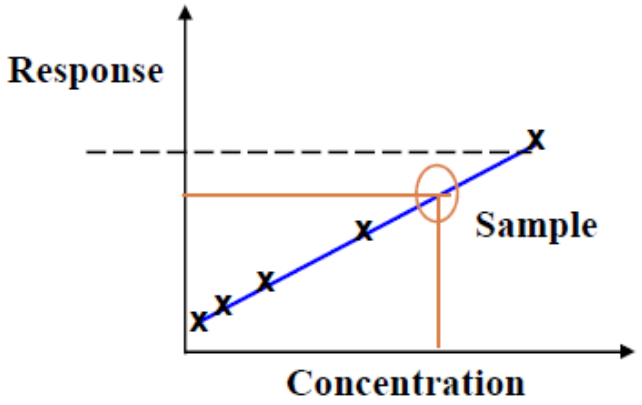
Lee et al., *Anal Chem*, 80, 2008

Concentration Assays

- Concentration based on biological activity
- All concentration assays require a calibration curve
- Concentrations of unknowns samples are calculated from this 4 - 7 concentrations in duplicate
- Direct binding formats

{ Calibration Curves }





Sample matrix for calibration curve

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Sample matrix for unknown samples

Affinity Analysis

Equilibrium and Kinetic Constants are related

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

» Association rate: $\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B]$

» Dissociation rate: $-\frac{d[AB]}{dt} = k_d \cdot [AB]$

» At equilibrium: Association = Dissociation

$$k_a \cdot [A] \cdot [B] = k_d \cdot [AB]$$

» The equilibrium constant:

$$K_A = \frac{[AB]}{[A] \cdot [B]} = \frac{k_a}{k_d} \qquad K_D = \frac{[A] \cdot [B]}{[AB]} = \frac{k_d}{k_a}$$

Rate Constants

	Association rate constant k_a	Dissociation rate constant k_d
Definition	$A + B \xrightarrow{k_a} AB$	$AB \xrightarrow{k_d} A + B$
Unit	$[M^{-1}s^{-1}]$	$[s^{-1}]$
Describes	Rate of complex formation, i.e. the number of AB formed per second in a 1 molar solution of A and B	Stability of the complex i.e. the fraction of complexes that decays per second.
Typical range	$1x10^{-3} – 1x10^{7}$	$1x10^{-1} – 5x10^{-6}$

Equilibrium Constants

	Equilibrium dissociation constant K_D	Equilibrium association constant K_A
Definition	$\frac{(A).(B)}{(AB)} = \frac{k_d}{k_a}$	$\frac{(AB)}{(A).(B)} = \frac{k_a}{k_d}$
Unit	[M]	[M^{-1}]
Describes	Dissociation tendency High K_D = low affinity	Association tendency High K_A = high affinity
Typical range	$1x10^{-5} – 1x10^{-12}$	$1x10^{5} – 1x10^{12}$

Kinetics- Analysis of Experimental SPR Curves

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} A\text{-}B \text{ complex}, \qquad K = \frac{k_a}{k_d} = \frac{[A.B]}{[A][B]}$$

Fit the experimental curve into various reaction models* (non-linear regression model) and get the kinetic parameters from the best fit.

(1) Pseudo first-order reaction model

(2) Mass transport limitation model

(3) Inhomogeneous ligand model

(4) Inhomogeneous analyte model

*J. Luo *et al. J. Biochem.* 130, 553-559 (2001).

Reaction rate equations

ZERO ORDER REACTION: A **0-order reaction** has a rate which is independent of the concentration of the reactant(s).

$$r = k$$

where r is the reaction rate, and k is the reaction rate coefficient with units of concentration/time.

$$2NH_3(g) \rightarrow 3H_2(g) + N_2(g)$$

FIRST ORDER REACTION: A **first-order reaction** depends on the concentration of only one reactant (a **unimolecular reaction**). Other reactants can be present, but each will be zero-order. The rate law for an elementary reaction that is first order with respect to a reactant A is $r = -\frac{d[A]}{dt} = k[A]$

$$\mathrm{H_2O_2}(l) \rightarrow \mathrm{H_2O}(l) + \frac{1}{2}\mathrm{O_2}(g)$$

$$\mathrm{2N_2O_5}(g) \rightarrow \mathrm{4NO_2}(g) + \mathrm{O_2}(g)$$

$$\mathrm{SO_2Cl_2}(l) \rightarrow \mathrm{SO_2}(g) + \mathrm{Cl_2}(g)$$

SECOND ORDER REACTION: A **second-order reaction** depends on the concentrations of one second-order reactant, or two first-order reactants.

For a second order reaction, its reaction rate is given by: either of the following 3 eqns.

$r = k[A]^2$ or $r = k[A][B]$ or $r = k[B]^2$

$$\mathrm{2NO_2}(g) \rightarrow \mathrm{2NO}(g) + \mathrm{O_2}(g)$$

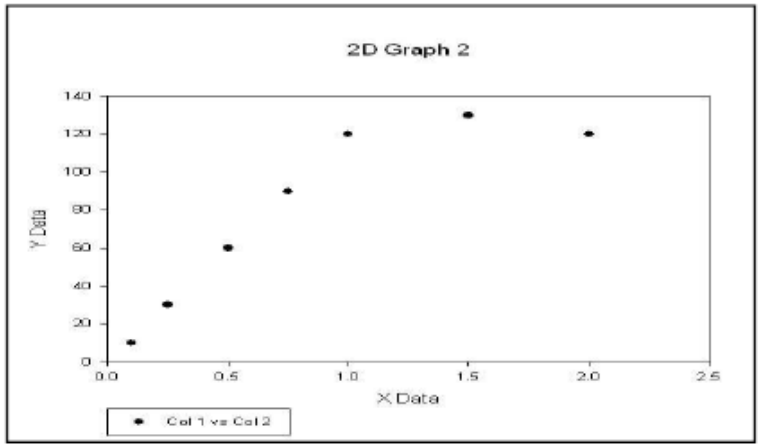
PSEUDO FIRST ORDER REACTION: Measuring a second order reaction rate can be problematic: the concentrations of the two reactants must be followed simultaneously, which is more difficult; or measure one of them and calculate the other as a difference, which is less precise. A common solution for that problem is the **pseudo first order approximation**

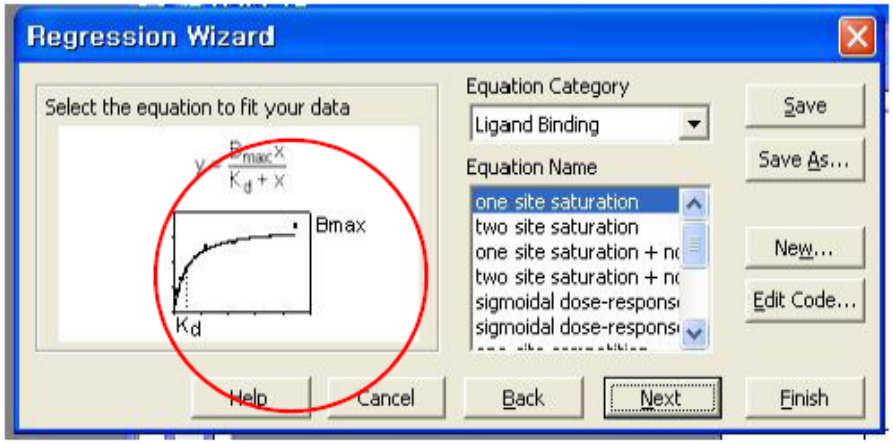
If *either* [A] or [B] remain constant as the reaction proceeds, then the reaction can be considered **pseudo first order** because in fact it only depends on the concentration of one reactant. If for example [B] remains constant then:

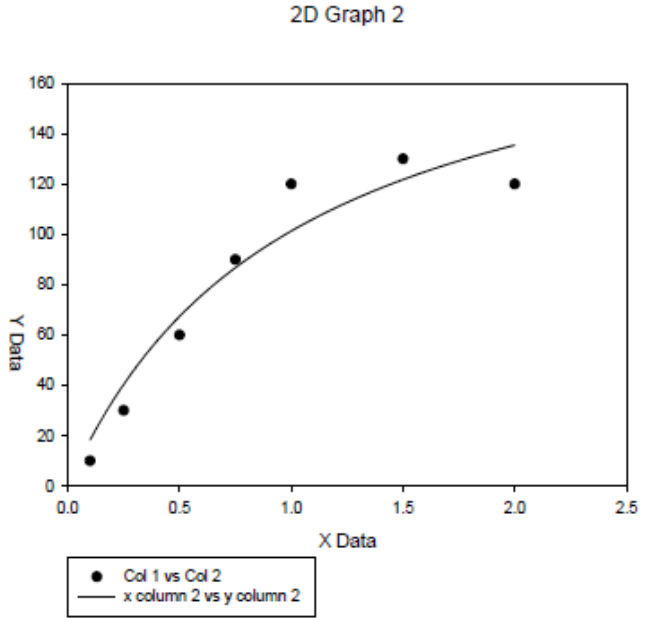
$$r = k[A][B] = k'[A]$$

Data 1*

	uM	RU
	1	2
1	0.1000	10.0000
2	0.2500	30.0000
3	0.5000	60.0000
4	0.7500	90.0000
5	1.0000	120.0000
6	1.5000	130.0000
7	2.0000	120.0000

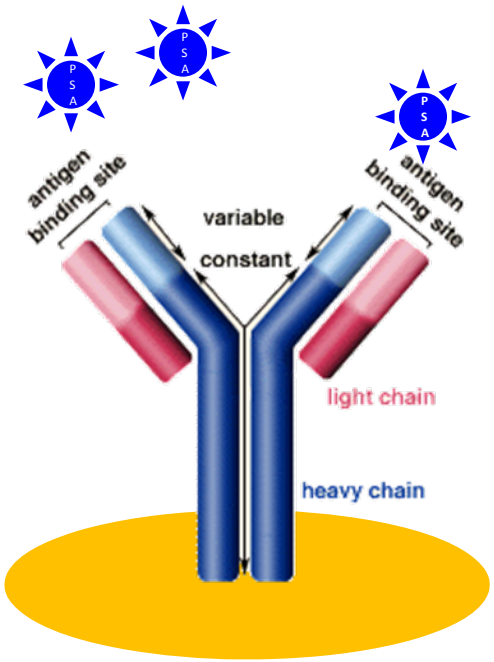






Use of Sigma Plot

Eg., PROSTATE SPECIFIC ANTIGEN (PSA) BINDING TO MONOCLONAL ANTIBODY (MAB)



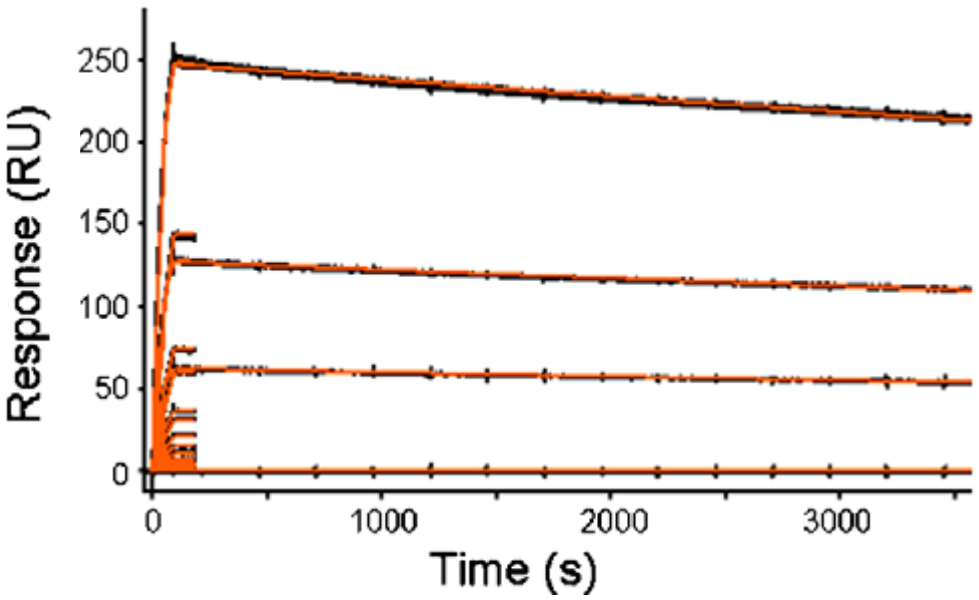
- PSA- 30 kDa protein - routinely used marker in the diagnosis of prostate cancer.
- In this study, 22 participants measured the binding of PSA to a mAb by SPR.
- mAb-immobilized on carboxymethyl dextran surface-amine-coupling chemistry using EDC and NHS.
- Three different densities of mAb immobilized-varying contact times and dilution.
- [PSA] used in 2.5-600 nM range for k_a calculation.

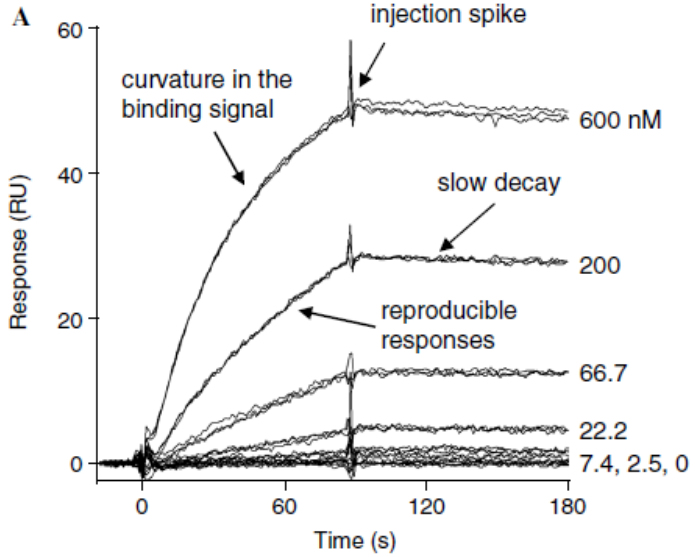
y

- [PSA] of 600 nM for k_d experiment.
- Global fitting of data using 1:1 interaction model.

http://www.biology.arizona.edu/IMMUNOLOGY/tutorials/antibody/structure.html

Analysis - 1:1 interaction model (A+B=AB), Scrubber software





Black lines = experimental (increasing concentrations of PSA)

Orange lines = model fit

Katsamba et al. Analytical Biochemistry 352 (2006) 208–221

Advantages

- Real time analysis & Label free technique – No need for radioactive, fluorescent or any other labelling.

- The Change in SPR signal - specific to the binding event - no need for purified sample – antigen in extracts can be used.
- Highly sensitive (RI changes $<10^{-5}$ with time resolution of few seconds) and simple construction.

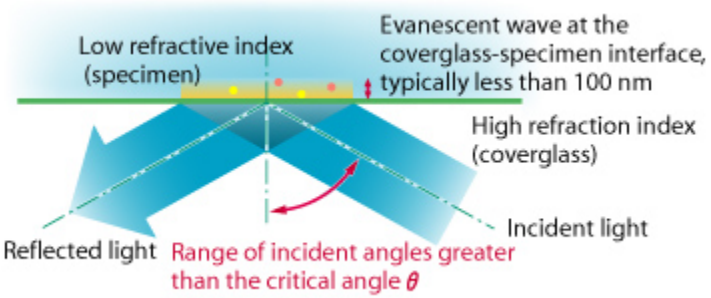
Disadvantages

- Mass transport can affect kinetic analysis.
- Any artifactual RI change other than from the interaction can also give signal.
- One of the interacting molecules should be immobilized on the surface.
- Thickness of the metal film (thin film is preferred).

SUMMARY

- Surface plasmon resonance detects binding events as changes in mass at the chip surface
- Real-time kinetic measurements
- Qualitative rankings
- Measurement of concentrations
- Information about structure-activity relationships
- No labeling and low volumes samples needed

Evanescent wave



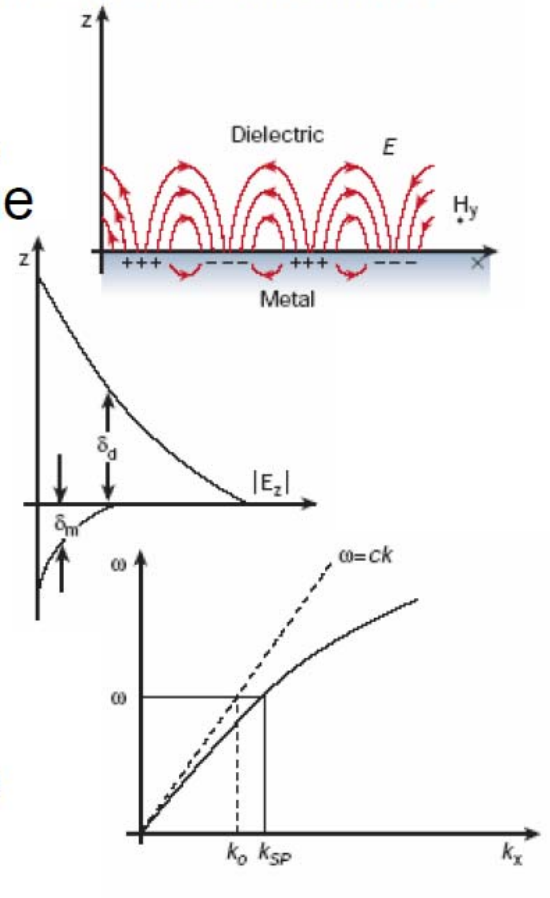
Evanescent Wave Illumination method
Certain objects make it possible to introduce laser illumination at incident angles greater than the critical angle (θc) resulting in an evanescent wave immediately adjacent to the coverglass/metal-specimen interface.

The evanescent wave reaches maximally a few hundred nanometers into the specimen and its energy drops off exponentially.

www.nikon.com/.../application/tirf2/index.htm

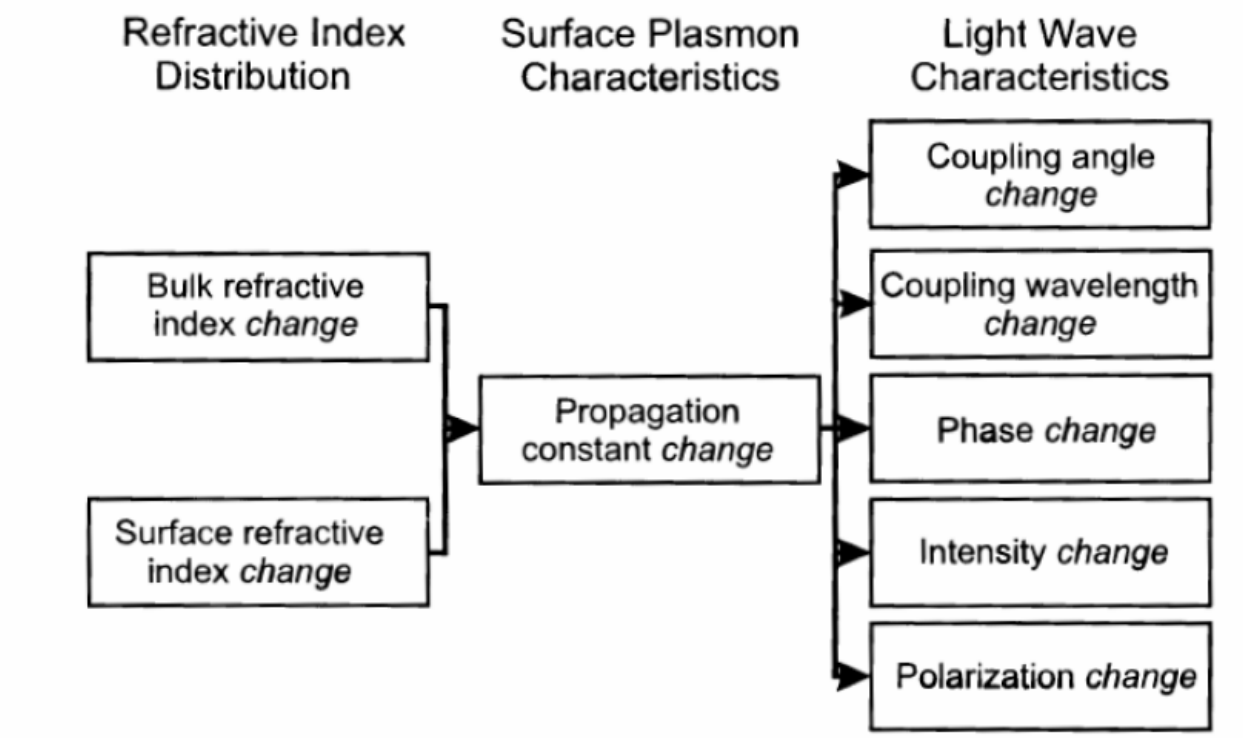
What is surface plasmon?

- collective excitation of the electrons at the interface between metal and dielectric
- transverse magnetic in character, electric field is perpendicular to the interface
- localized at the interface, evanescent in perpendicular direction
- experience higher (and non-linear) refractive index, cannot be directly coupled to free radiation



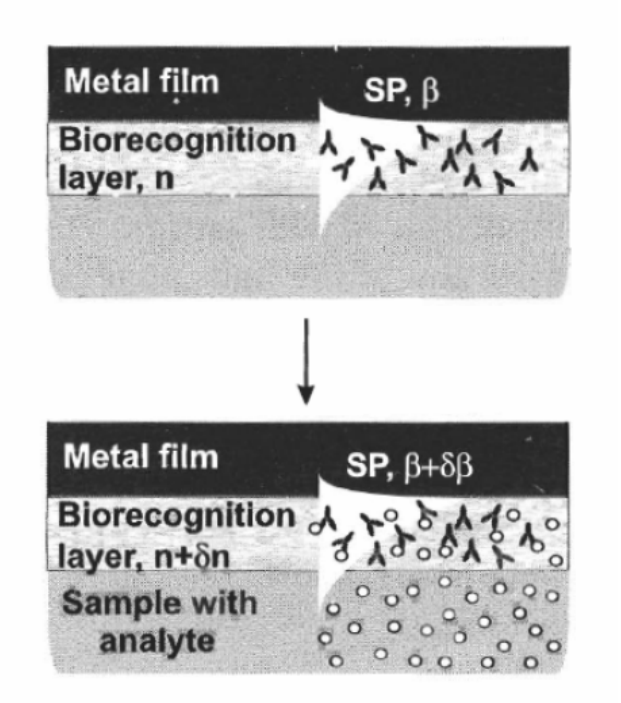
Surface plasmon sensor

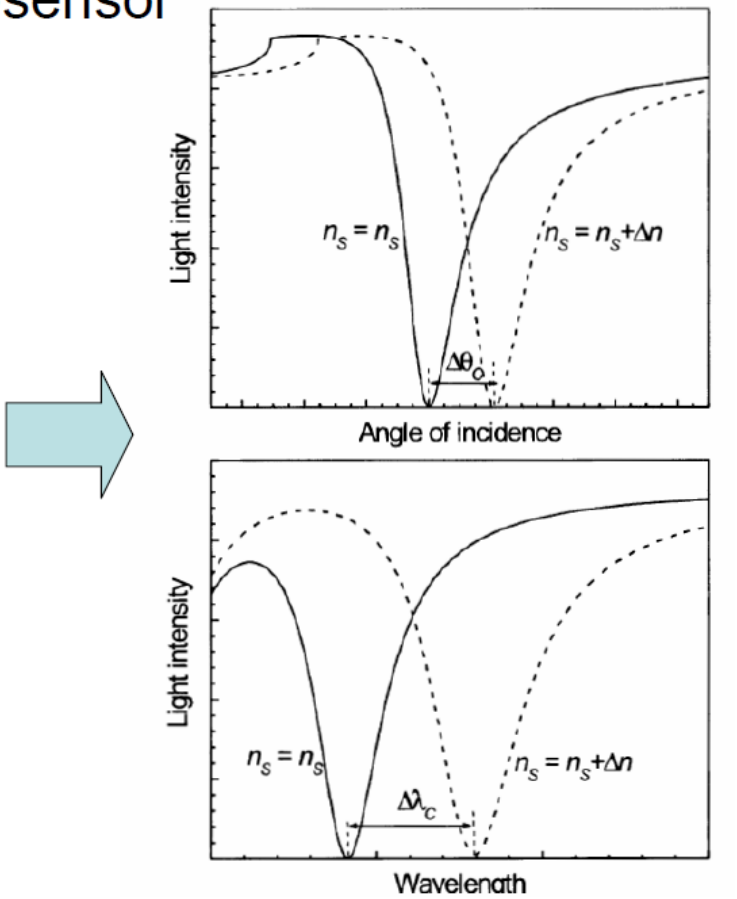
- The concept



SPR biosensor

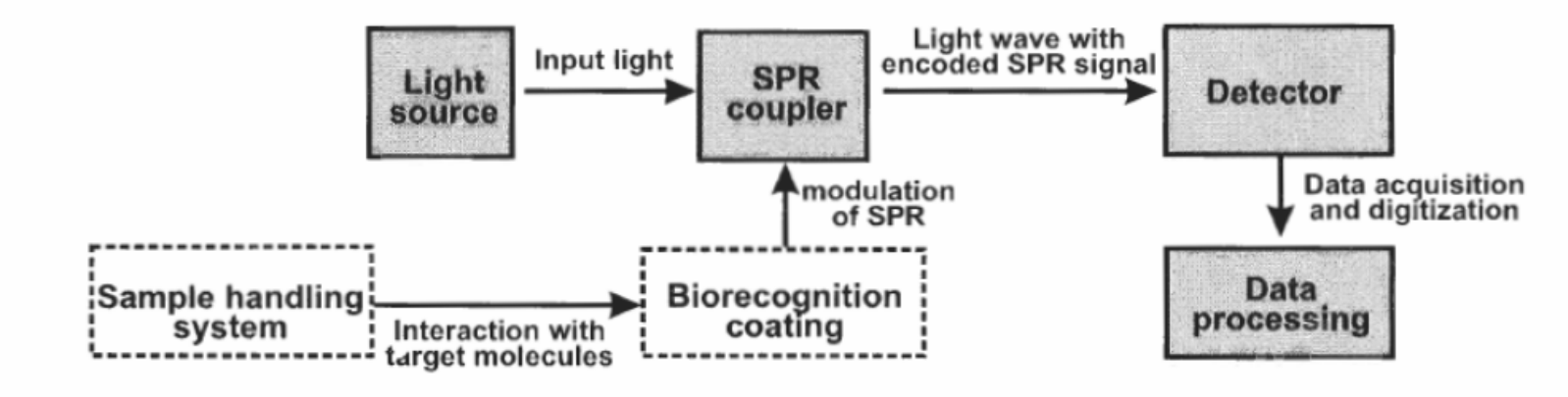
- Principle of affinity SP biosensor



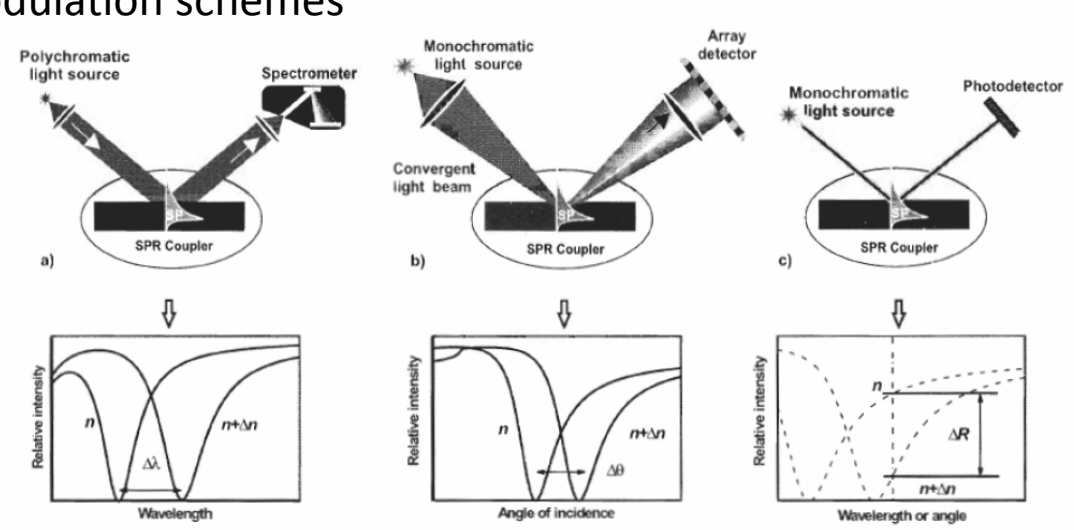


SPR instrumentation

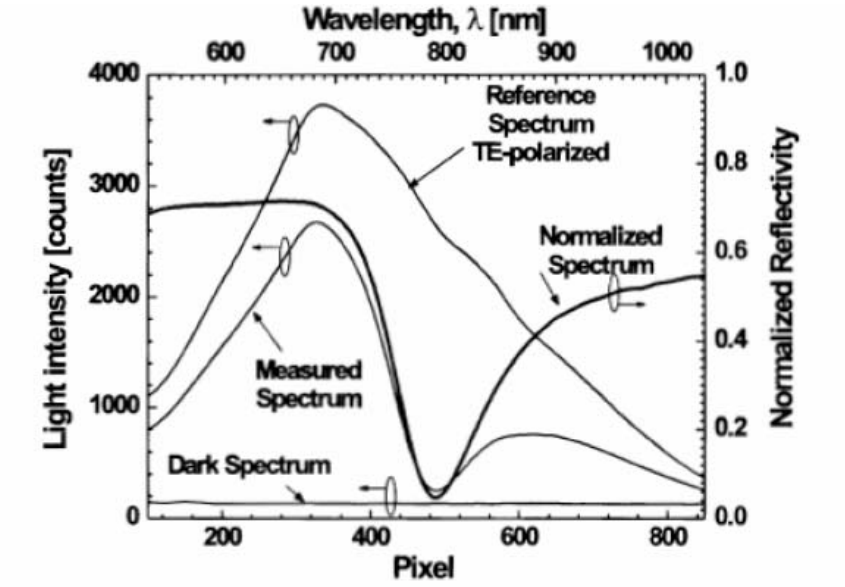
Scheme of an SPR biosensor



Optical modulation schemes



Data processing for SPR



1. Signal normalization
   - subtracting dark signal
   - normalizing intensity to TE or air scan
2. Finding minimum position
   - direct measurement
   - polynomial extrapolation
   - centroid position

} sub-pixel precision!

Sensitivity of SPR biosensor

$$S = S_{RI} \frac{dn_b(c)}{dc} = S_{RI} \cdot \gamma \cdot [C]$$

- for given folding state of the protein (fixed density) the refractive index is proportional to the amount of proteins absorbed (g/cm^2)
- Rule of thumb: change of 10^{-6} RI = approx. 1 pg/mm^2 of adsorption.

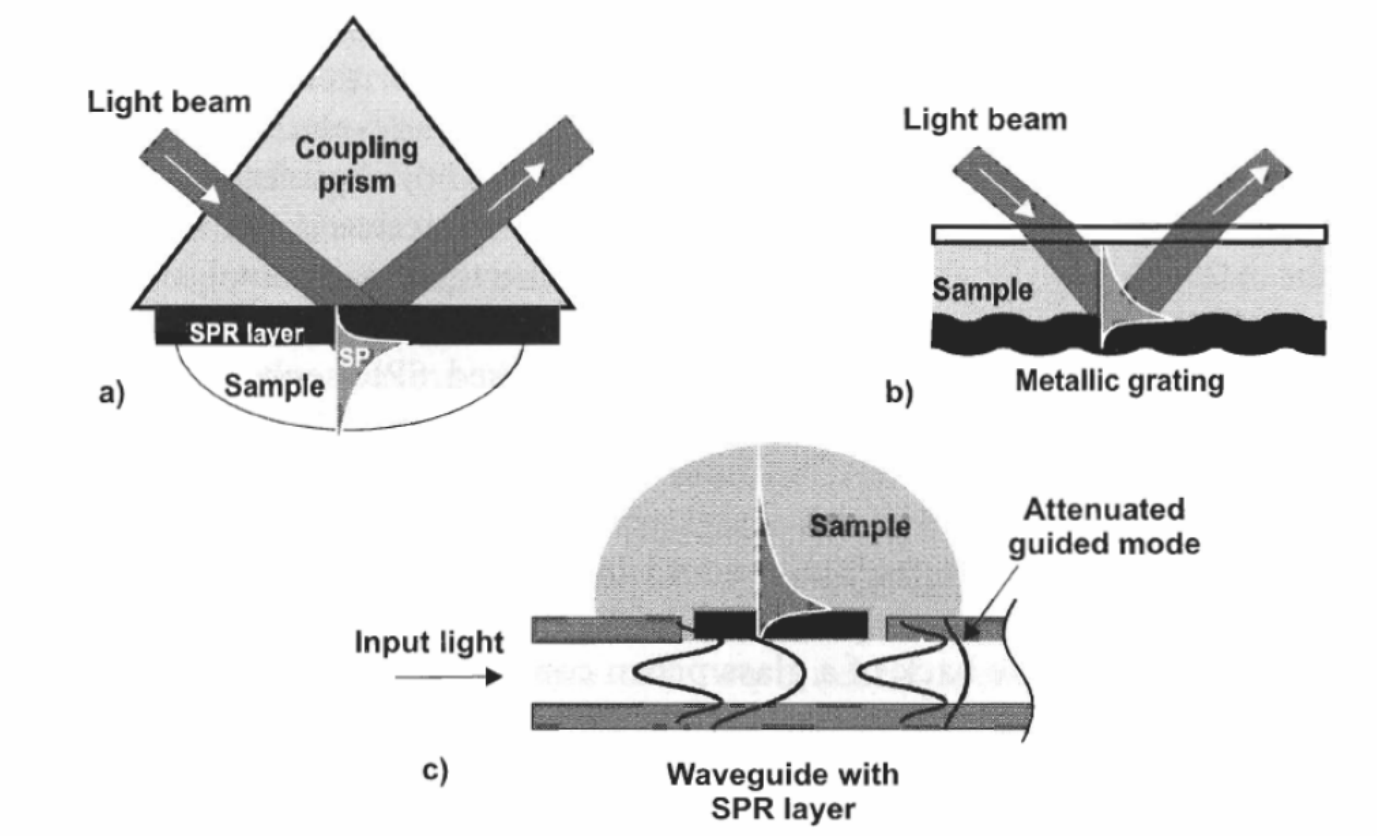
- S_{RI} – sensitivity to refractive index change, includes:
  - modulation method (angle scan, wavelength scan,etc.)
  - hardware
  - software (e.g. method of locating the minimum)

Problem

- Calculate position of the SPR minimum for a prism-based setup involving
  - a light source at 780nm,
  - BK7 optical prism (refractive index 1.511 @780nm),
  - gold film (refractive index 0.1420+i*4.7571 @780nm)
  - a water-based buffer on the sensor side (n=1.33).

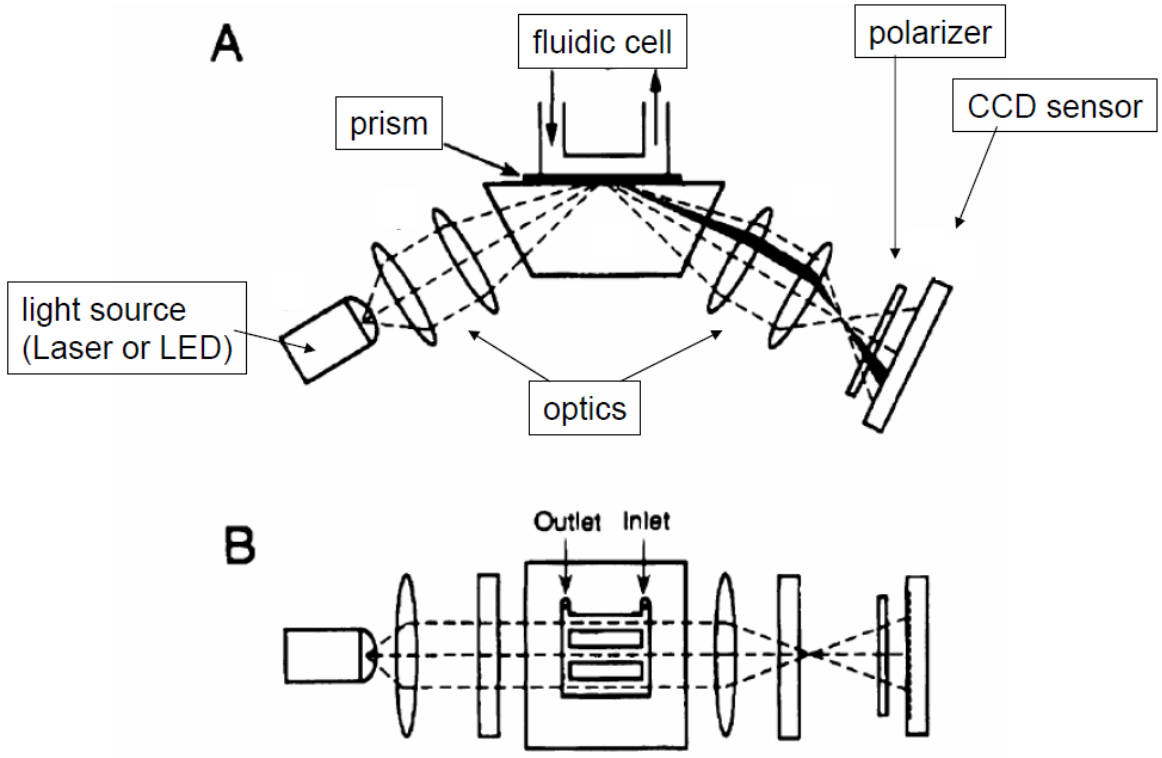
  What change in the absorption minimum we expect when the refractive index of buffer changes by 10^{-4}?

Optical coupling schemes



SPR sensor based on prism coupler and anglular modulation

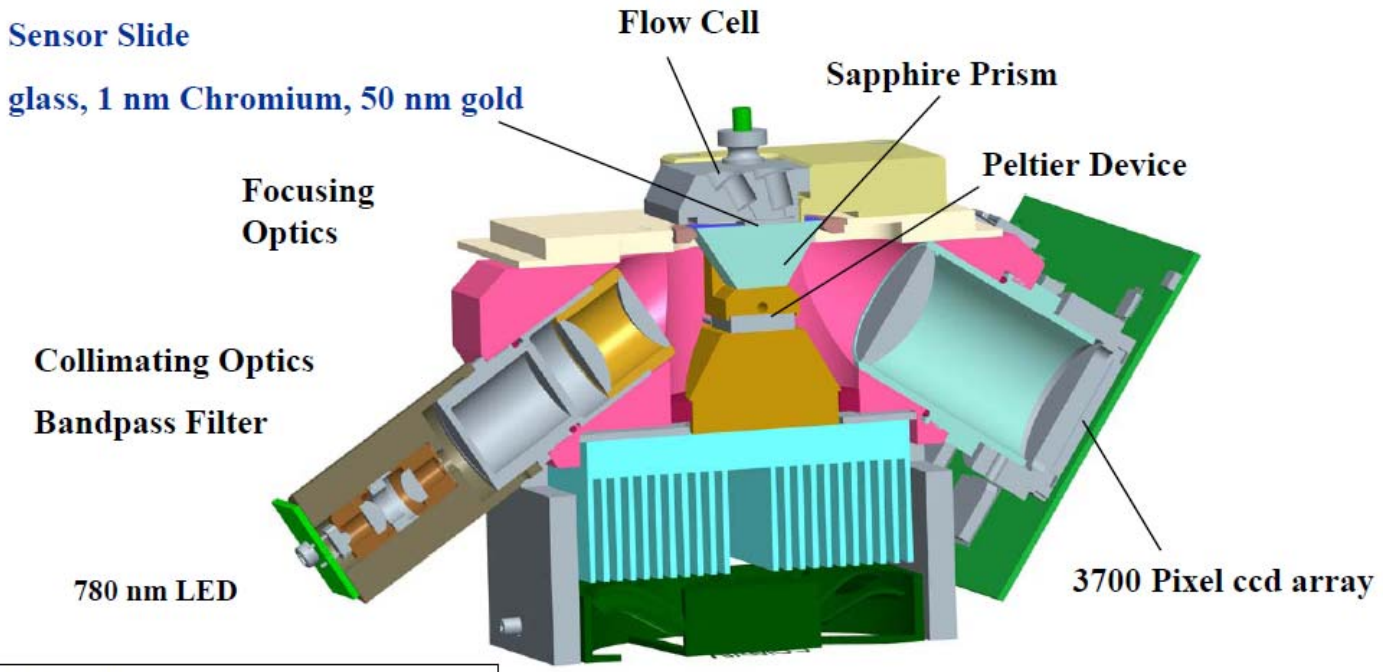
- Sensor schematics



SPR sensor based on prism coupler and anglular modulation

Eg., 1

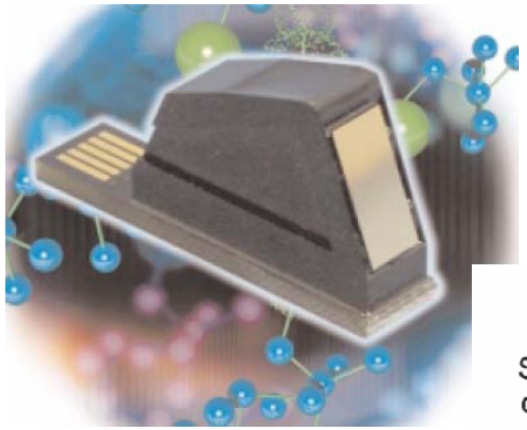
- Reichert SR7000

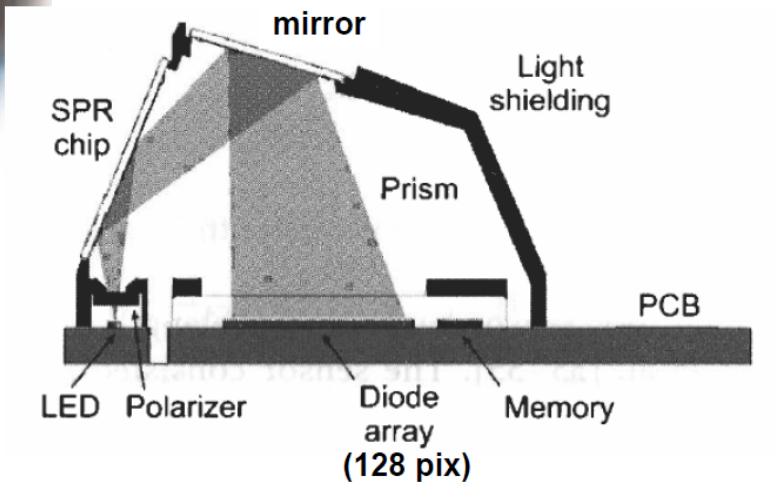


Specifications:
- sensitivity $2*10^{-7}$ RU
- dynamic range 1.3 – 1.6

Eg., 2

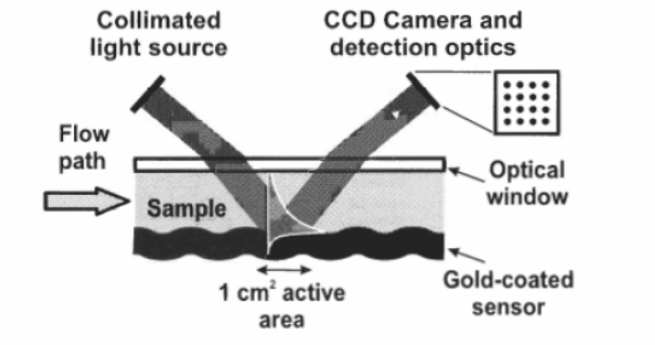
- Texas Instr. SPREETA

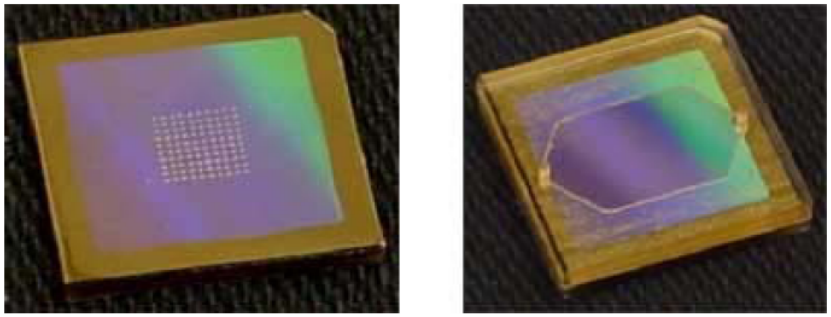




SPR sensor based on grating coupler and intensity modulation

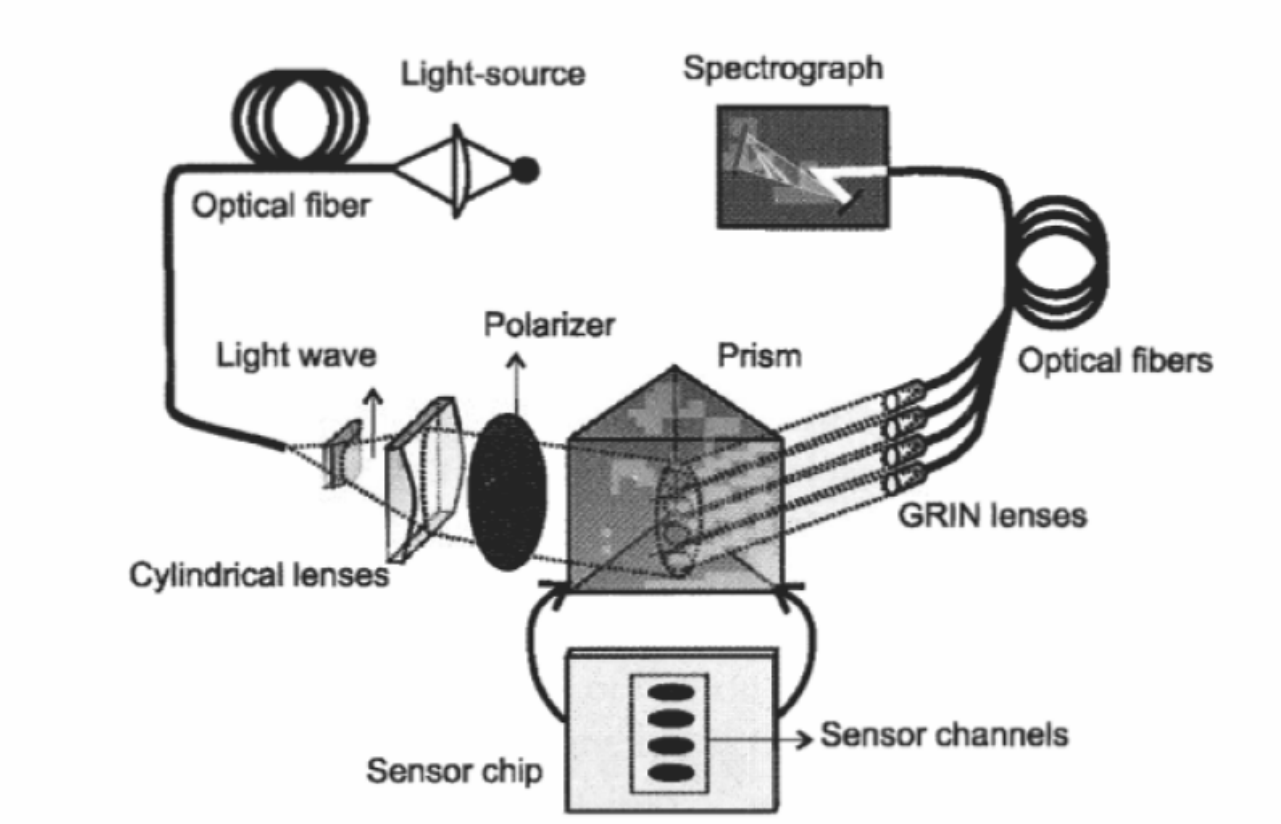
FLEX chip, HTC Biosystems (acquired by BIAcore)





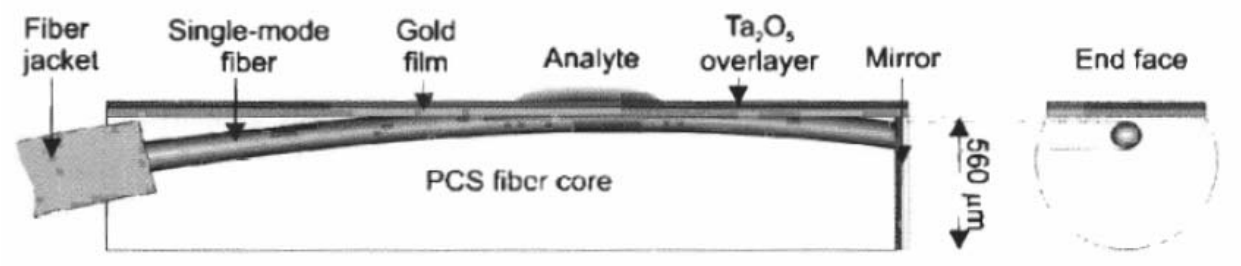
Eg., 3 fiber optic

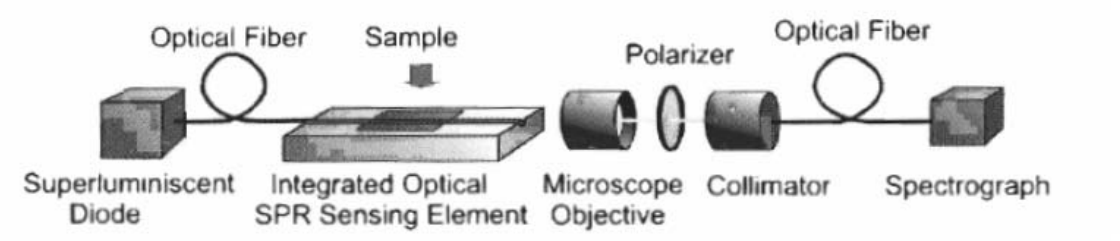
- Schematics of a 4 channel sensor with wavelength modulation



Integrated optical SPR sensor

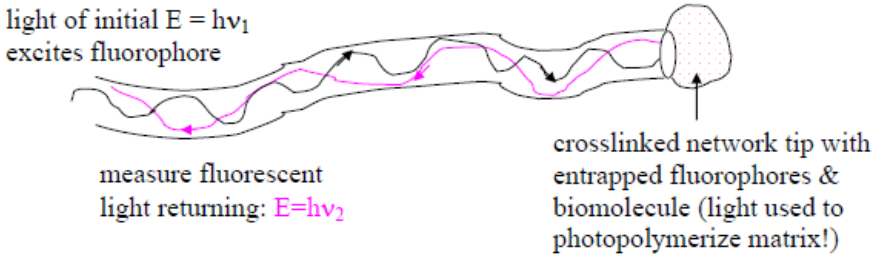
- SPR probe using a side polished optical fiber





- sensitivity (w. wavelength modulation) $<10^{-6}$;
- sensitivity (w. intensity modulation) $5*10^{-5}$;

Example 2: fiber optic sensors: fluorophores incorporated into tip change fluorescence level depending on level of target present



Typically:

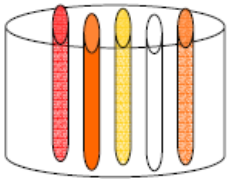
- Oxygen present at tip quenches fluorescence from trapped fluorophore (ex., tris(4,7-diphenyl-1,10 phenantroline) Ru(II) dichloride = $Ru(dpp)_3^{2+}Cl_2$)

- Action of trapped oxidase (biological element, ex., GOD) depletes O_2, causing ↑ fluorophore emission

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{GOD}]{} \text{Gluconic acid} + H_2O_2$$

How can we account for natural O_2 fluctuations?

Multichannel fiber optic: 1. enhancing selectivity and/or
2. multianalyte detection

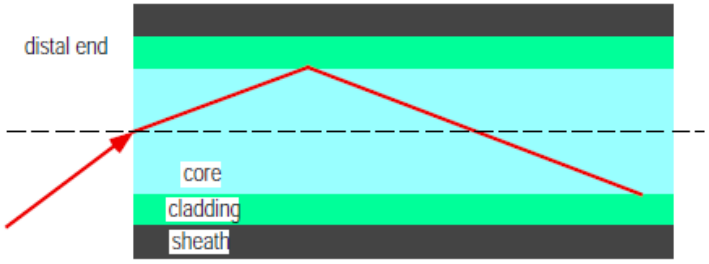


How can we measure multiple analytes?

MD. Marazuela et al., "Fiber-optic biosensors- an overview". *Anal. Bioanal. Chem.* **372**, 664 (2002).

Optical waveguides

A waveguide is defined as any component which guides a light wave through it by a series of total internal reflections (TIR). Provided that the angle of incidence into the waveguide is not too great, the light "couples" into the waveguide, finally emerging from the other end.



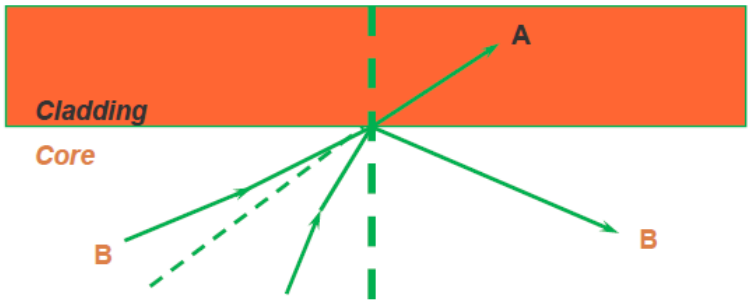
Light propagation through a fibre optic waveguide

The "angle of acceptance" of a wave guide is therefore a function of the critical angle since only light that is totally internally reflected (or is aligned along the axis of the waveguide) will propagate.

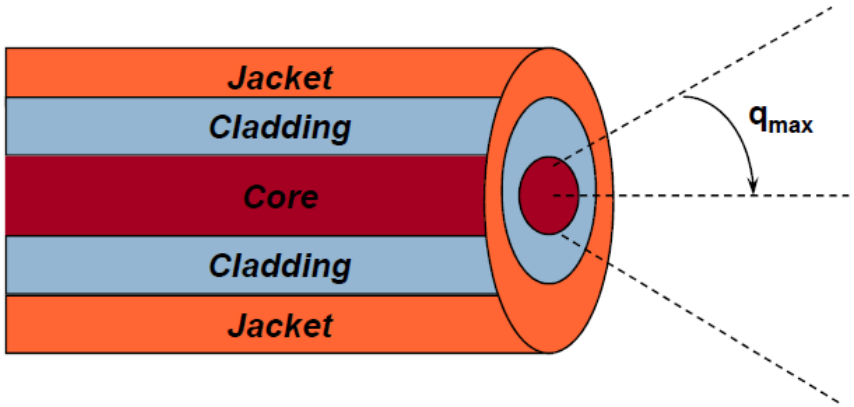
The greater the RI of refraction, the greater the range of values that θ_i can have for a given wave guide.

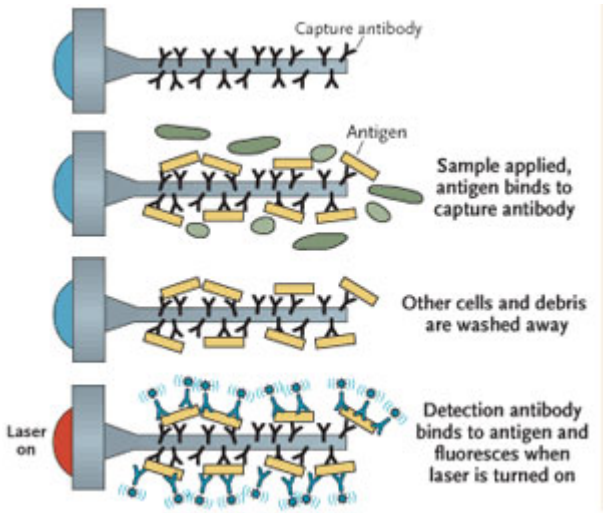
For a cylindrical geometry waveguide (fibre optic), the acceptance angle can be modified by (1) the density of the fibre optic itself, and (2) by placing a high RI material, or "cladding", along the length of the waveguide.

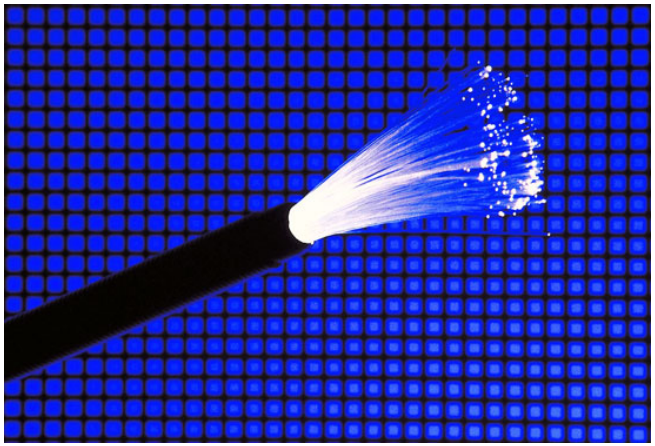
OPTICAL Fiber-Optic BIOSENSORS



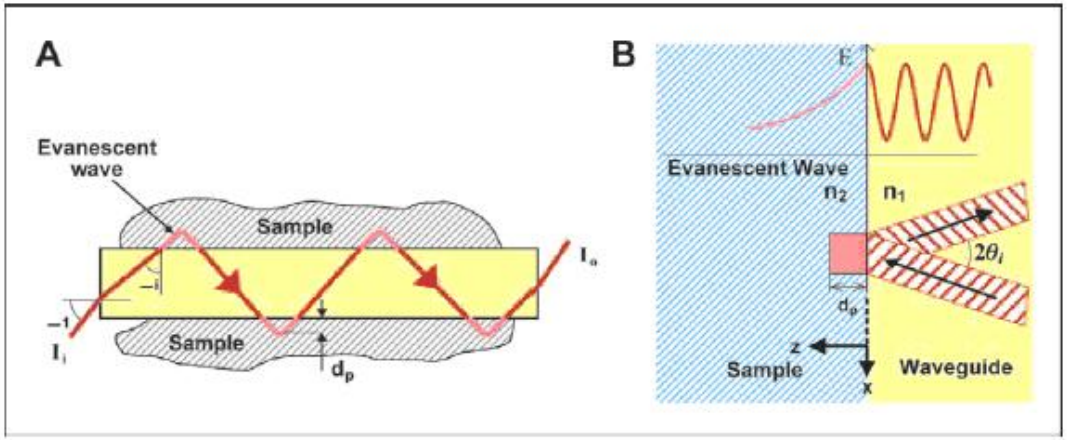
Total Internal Reflection



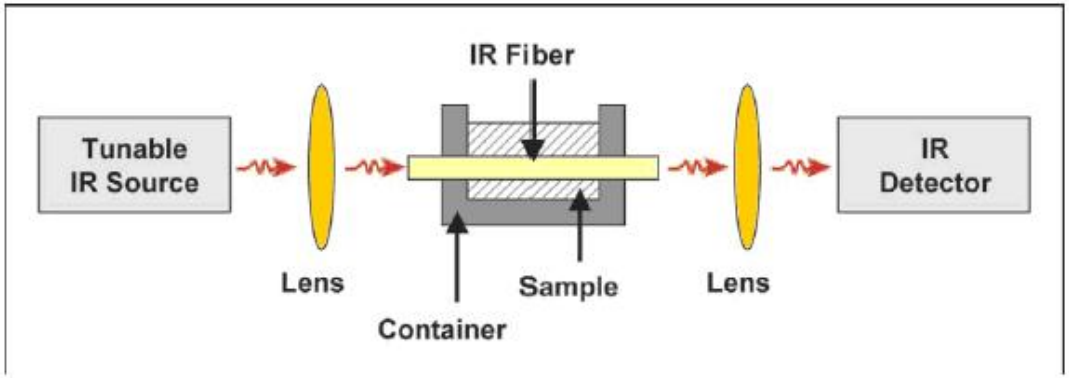

Capture antibody
Antigen
Sample applied, antigen binds to capture antibody
Other cells and debris are washed away
Laser on
Detection antibody binds to antigen and fluoresces when laser is turned on



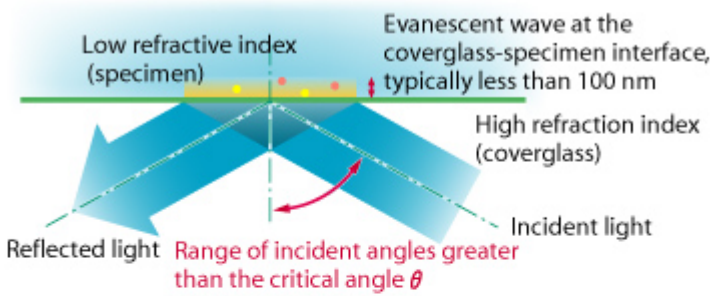
evanescent wave on FO

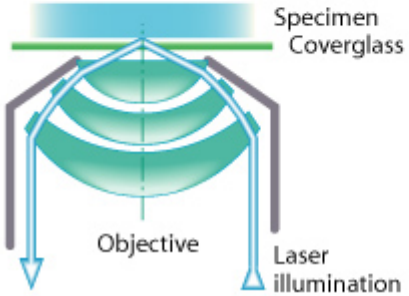


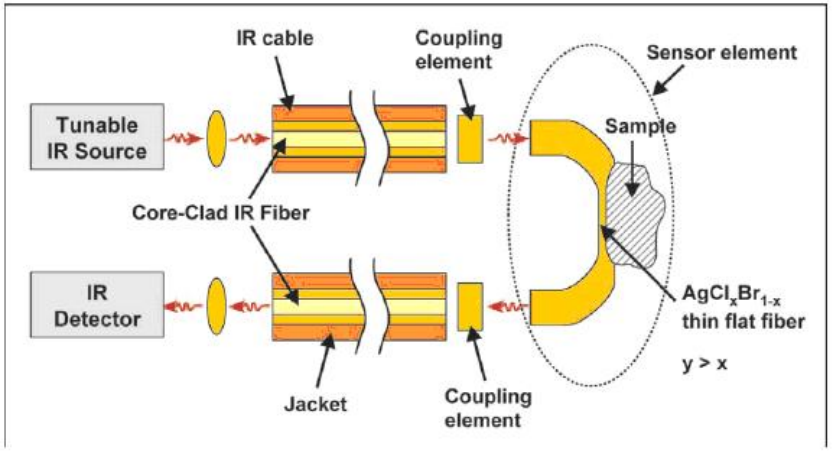
(A) *Evanescent wave in a waveguide, in contact with a sample.* (B) *Evanescent wave at the interface between two media, under total internal reflection.*



The experimental setup of an "inner sensor".

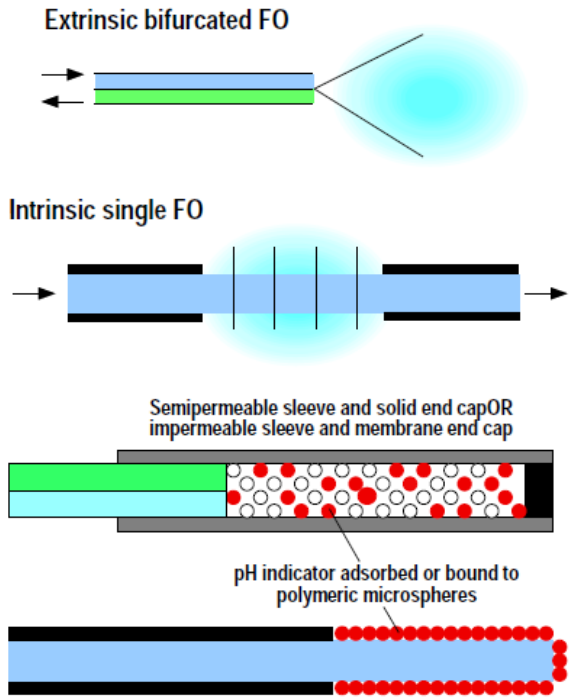




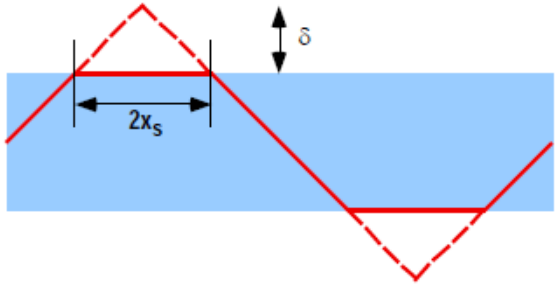


The experimental setup of a "remote sensor".

Eg., of fibre optic sensors



Examples of different fibre optic chemical sensor configurations



Lateral shift of a totally internally reflected beam and resulting effective width of the waveguide

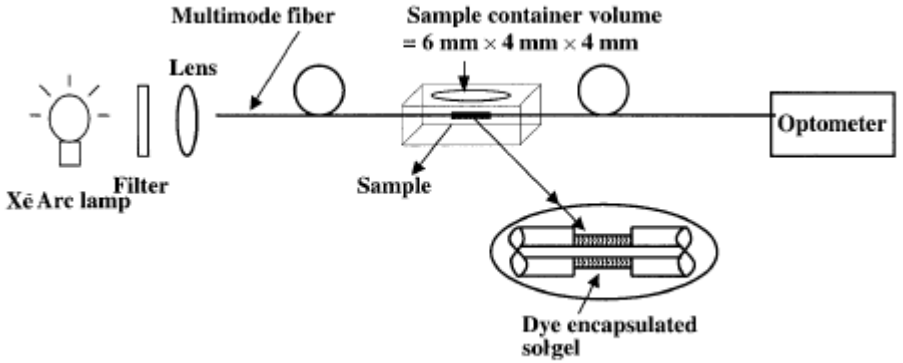
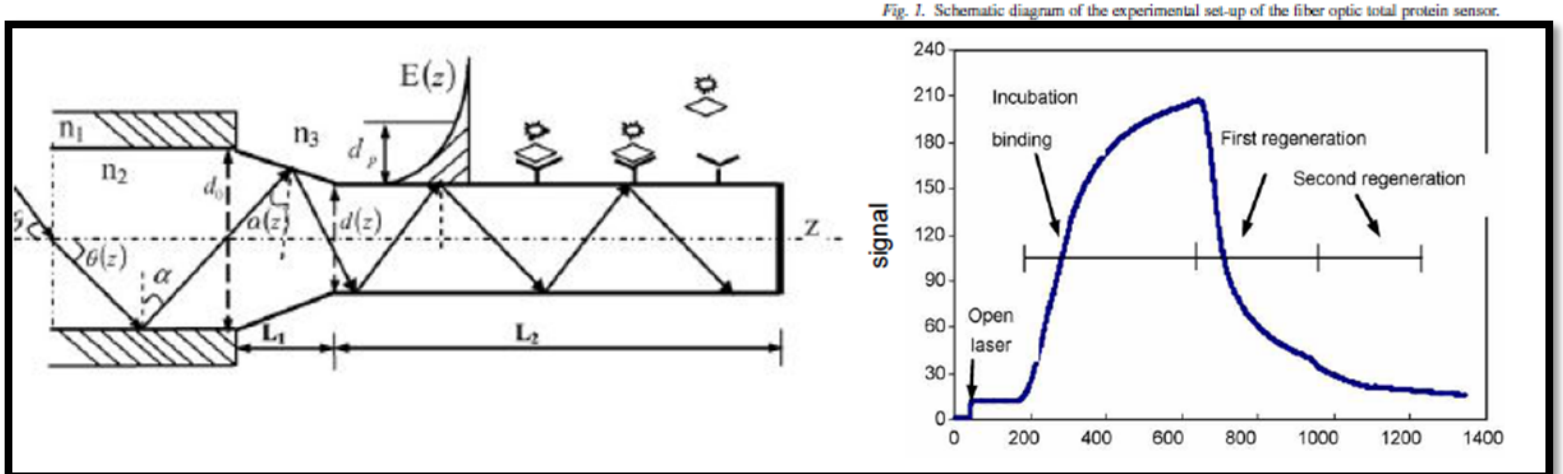


Fig. 1. Schematic diagram of the experimental set-up of the fiber optic total protein sensor.



Biochemical-optical

The interaction with light

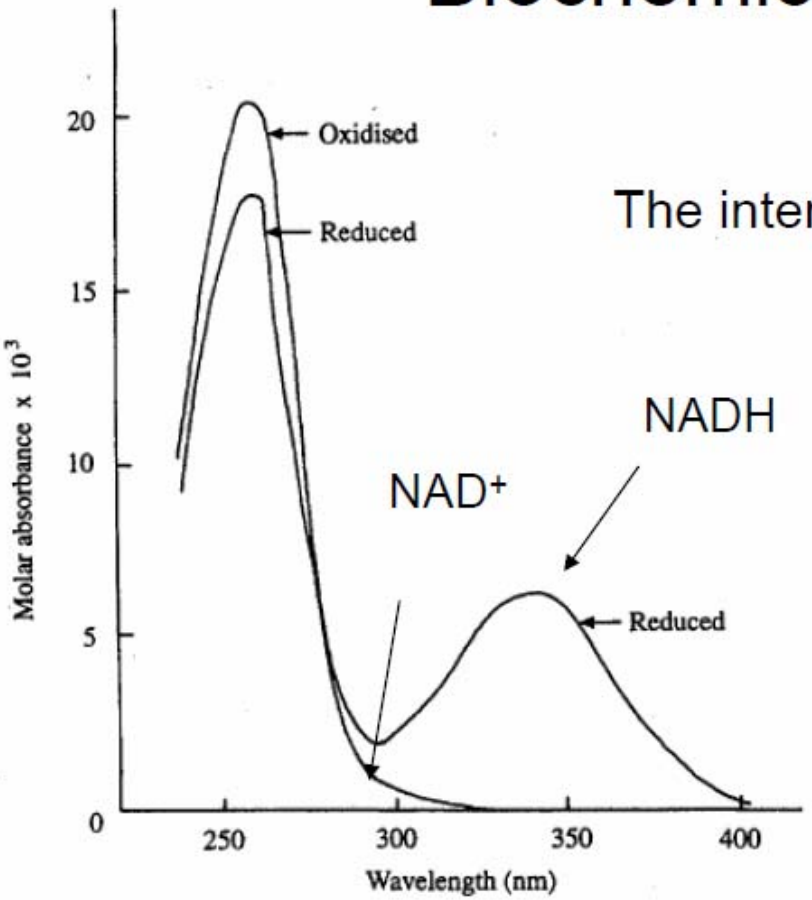
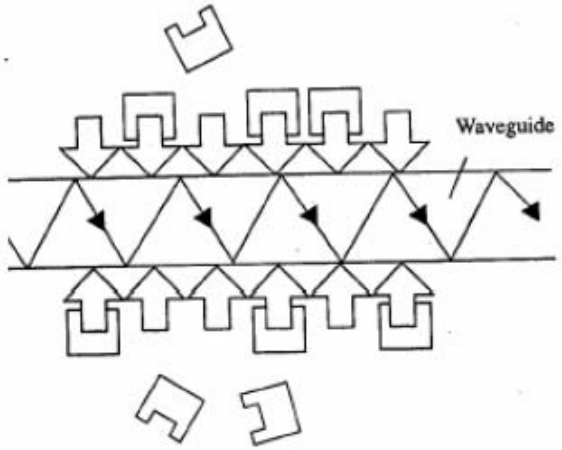


Figure 5.1 Absorption spectra of NAD in oxidised and reduced forms. Reproduced by permission of the Open University Press from Hall (1990)



cal sensor: evanescent field monitoring. Reproduced by permission
ersity Press from Hall (1990)

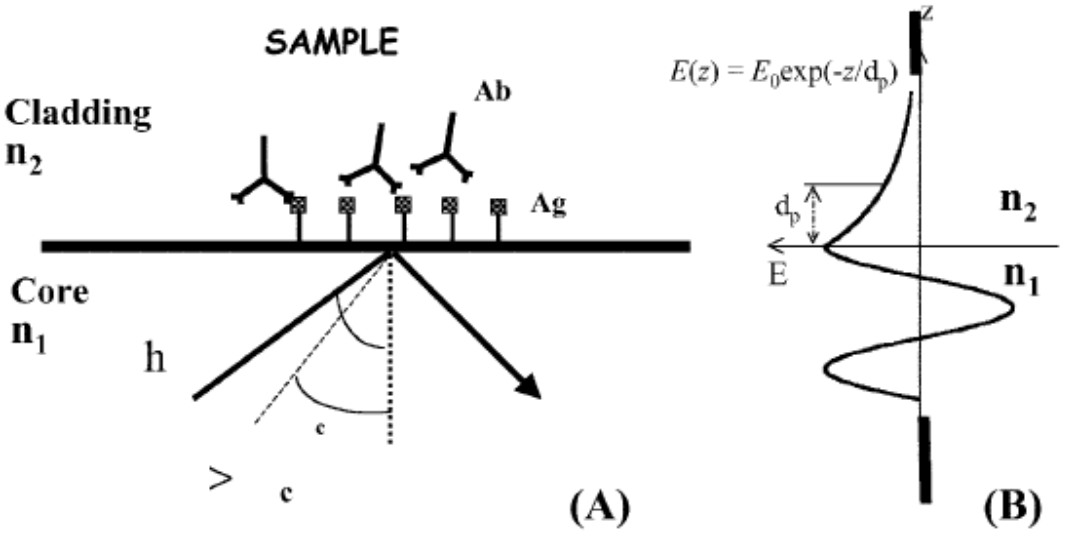
Wave guide

Pyruvate+NADH+H^+ → L-lactate+NAD^+

Biosensors:an Introduction

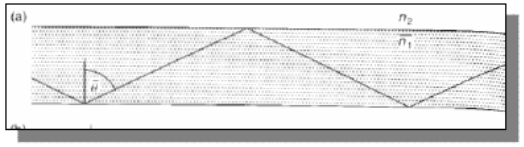
Fiber-optic biosensor

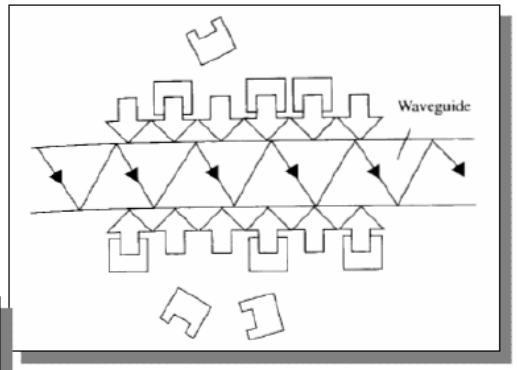
Fig. 1 (A) Total internal reflection of light at the interface between phases of refractive indices n_1 and n_2, where $n_1 > n_2$. Incident rays at an angle greater than the critical angle, θ_c, will be totally internally reflected at the interface. (B) Electric field amplitude E, on both sides of the core/cladding interface of a waveguide. In the lower index medium (n_2, cladding) the electric field amplitude of the evanescent wave decays exponentially, with a penetration distance, d_p, that depends on λ, θ, n_1, and n_2 (see text)

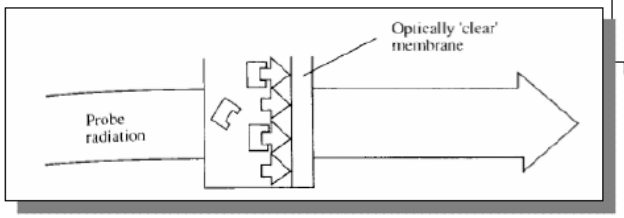


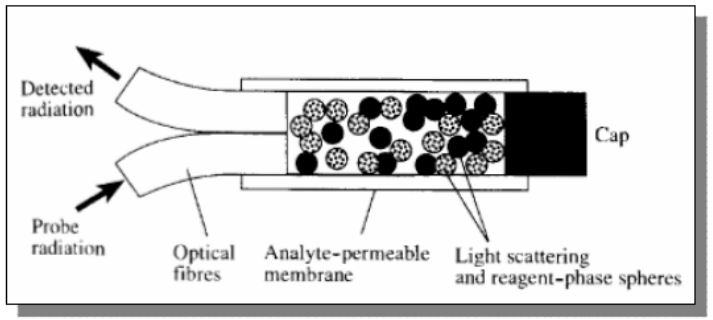
FO biosensor designs

Design examples:





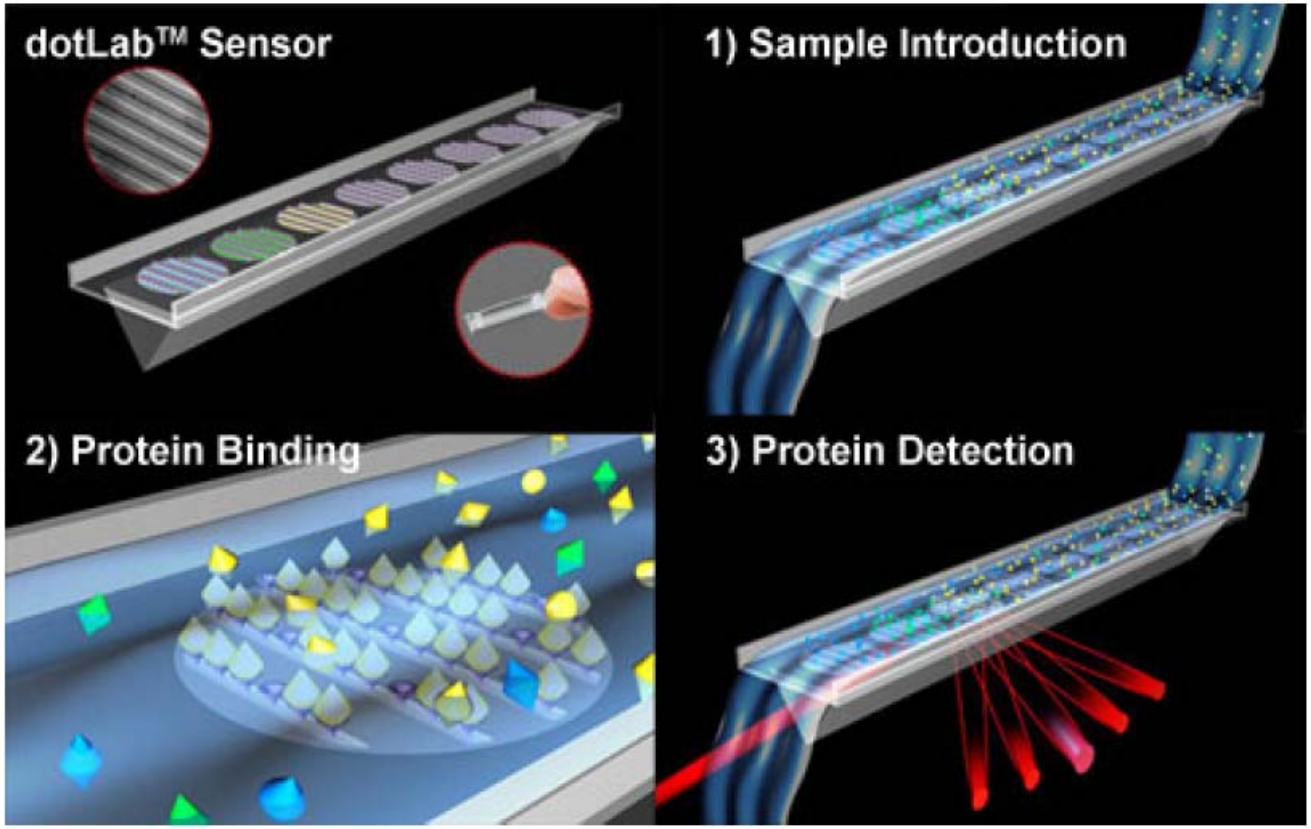




Detecting RI changes

- Grating based biosensors

Axela's Diffractive Optics "Dot"- technology



Application of FO enzyme sensors

Table 2 Fiber-optic enzyme sensors based on different types of transduction mechanisms

Analyte	Enzyme (s)	Solid support	Transducer	Indicator/Reagent	Ref.
Glucose	Glucose oxidase	Acrylamide gel	Oxygen	$Ru(phen)_3Cl_2$	[38]
Glucose	Glucose oxidase	Controlled-pore glass	Oxygen	Pt-octaethylporphine	[42]
Bilirubin	Bilirubin oxidase	Acrylamide gel	Oxygen	$Ru(dpp)_3Cl_2$	[39]
Phosphatidylcholine	Phospholipase/Choline oxidase	Nylon mesh	Oxygen	$Ru(dpp)_3Cl_2$	[40]
Cholesterol	Cholesterol oxidase	Graphite powder	Oxygen	$Ru(dpp)_3Cl_2$	[41]
Penicillin	Penicillinase/penicillin-G amidase	Polyvinyl alcohol matrix	pH	Aminofluorescein	[58]
Creatinine	Creatinine iminohydrolase	Polyvinyl alcohol matrix	pH	Aminofluorescein	[58]
Glucose	Glucose dehydrogenase	Polyvinyl alcohol matrix	pH	Aminofluorescein	[58]
Urea	Urease	Polyvinyl alcohol matrix	pH	Aminofluorescein	[58]
Urea	Urease	Polyacrylamide/PPY film	pH	Polypyrrole (PPY)	[59]
Pesticides	Acetylcholinesterase	Isothiocyanate glass	pH	Thymol blue	[62]
Carbamates	Acetylcholinesterase	Controlled-pore glass	pH	Chlorophenol red	[63]
Paraoxon	Acetylcholinesterase	Langmuir–Blodgett film	pH	Litmus dye	[64, 65]
Organophosphates	Cholinesterase	Sol–gel glass	pH	Indoxyl	[66]
Glutamate	Glutamate dehydrogenase	Optical fiber surface	NADH	-	[48]
Ethanol	Alcohol dehydrogenase	Langmuir–Blodgett film	NADH	-	[49]
Pyruvate	Lactate oxidase/dehydrogenase	Polyvinyl alcohol matrix	NADH	-	[50]
AMP, ADP, ATP	Adenylate/creatine kinase/ firefly luciferase	Collagen membrane	ATP	Luciferin/Mg^{2+}	[53]
Chlorophenols	Horseradish peroxidase	Collagen membrane	H_2O_2	Luminol	[52]
Lactate	Lactate oxidase	UltraBind/Immunodyne memb.	H_2O_2	Luminol	[56]
Choline	Choline oxidase	DEAE Sepharose-PVA gel	H_2O_2	Luminol	[57]

Anal Bioanal Chem (2002) 372 : 664–682
DOI 10.1007/s00216-002-1235-9

REVIEW

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Fiber-optic biosensors – an overview

Application of FO immunosensors

Table 3 Applications of fiber-optic evanescent wave immunosensors

Analyte	Application(s)	Assay format	Label(s)	Ref.
Explosives (TNT and RDX)	Environmental analysis	Competitive	Cy5-labeled antigens	[91, 92, 93, 94]
Cyclodiene insecticides	Environmental analysis	Competitive	Fluorescein-labeled antigens	[98]
Herbicides	Environmental analysis	Competitive	HRP-labeled antibodies	[99]
Isoproturon	Environmental analysis	Competitive	Cy5.5-labeled antibodies	[100]
2,4-D	Environmental analysis	Competitive	Cy5/FITC-labeled antigens	[101]
Cocaine	Clinical analysis	Competitive	Cy5-labeled antibodies	[103]
Coca alkaloids	Clinical analysis	Competitive	Fluorescein-labeled antigens	[104]
Benzo[*a*]pyrene	Clinical analysis	Direct	-	[105, 106]
Protein C	Clinical analysis	Sandwich	Cy5-labeled antibodies	[107, 108]
D-dimer	Clinical analysis	Competitive	Fluorescein-labeled antibodies	[110]
Salmonella spp.	Clinical and food analysis	Sandwich	Cy5-labeled antibodies	[115]
Staphylococcus aureus	Clinical and food analysis	Sandwich	FITC-labeled antibodies	[116]
Enterotoxin B	Clinical and food analysis	Sandwich	Cy5-labeled antibodies	[117]
E. coli O157:H7	Food analysis	Sandwich	Cy5-labeled antibodies	[119]
Fumonisin B_1	Food analysis	Competitive	FITC-labeled antigens	[120]