Lecture 5

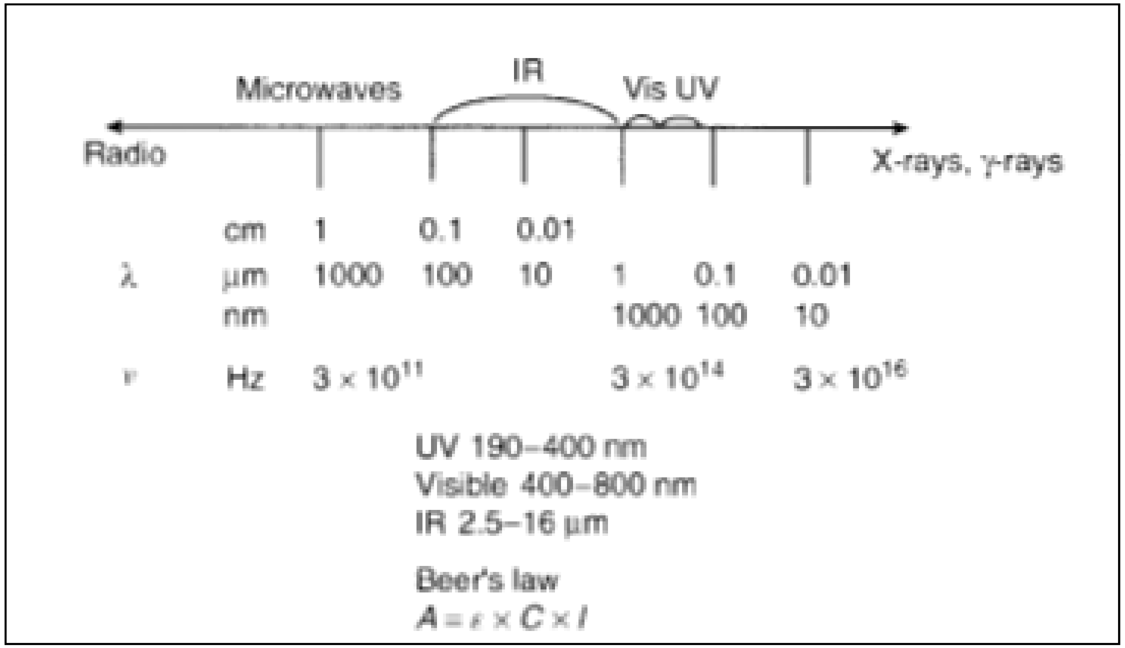
Optical sensors.

SPR Sensors: Principle and Instrumentation.

Optical sensors

What they can be based on:

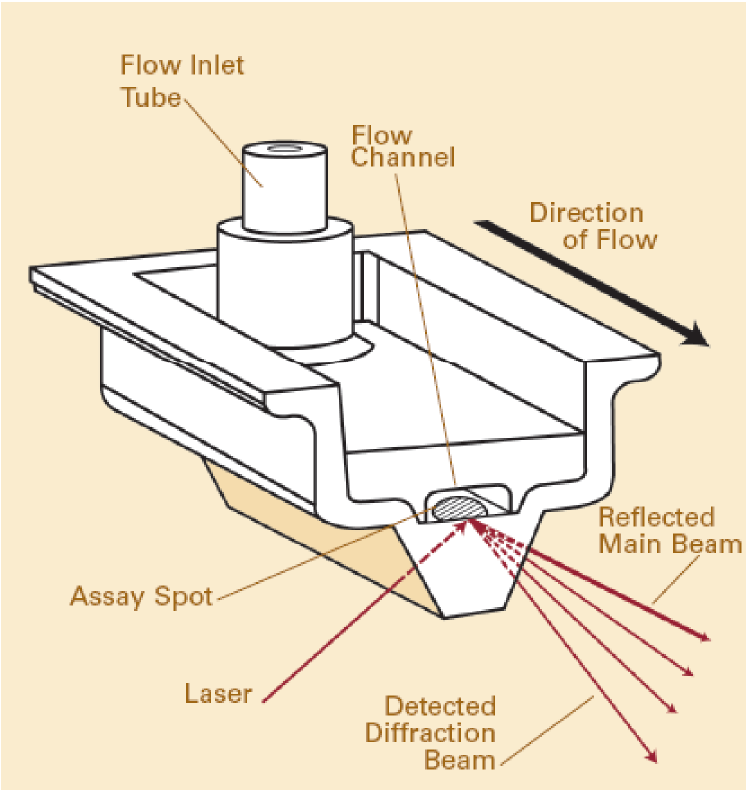
- Absorption spectroscopy (UV-VIS, IR)
- Fluorescence/phosphorescence spectroscopy
- Bio- and chemiluminescence
- Refractive index sensing
- Laser light scattering

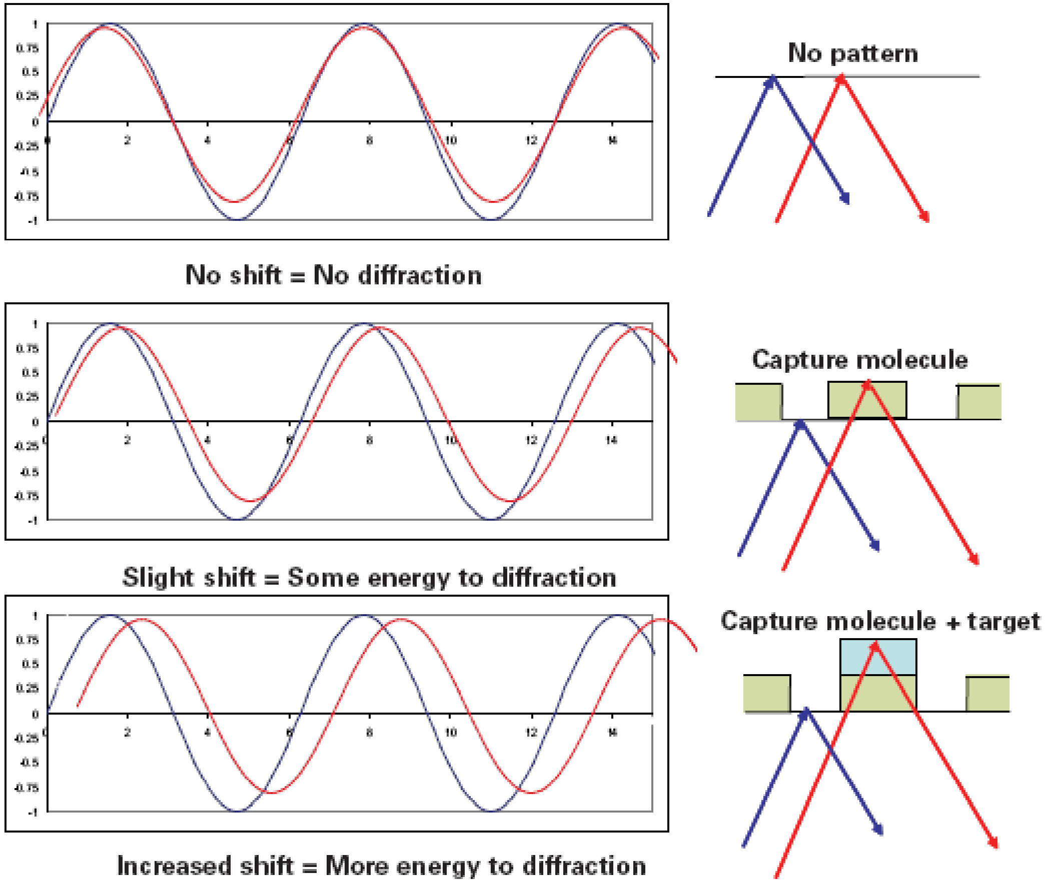


Detecting Refractive Index Changes

- Grating based biosensors

Axela's Diffractive Optics "Dot"- technology





Detecting Refractive Index Changes

- Grating based biosensors

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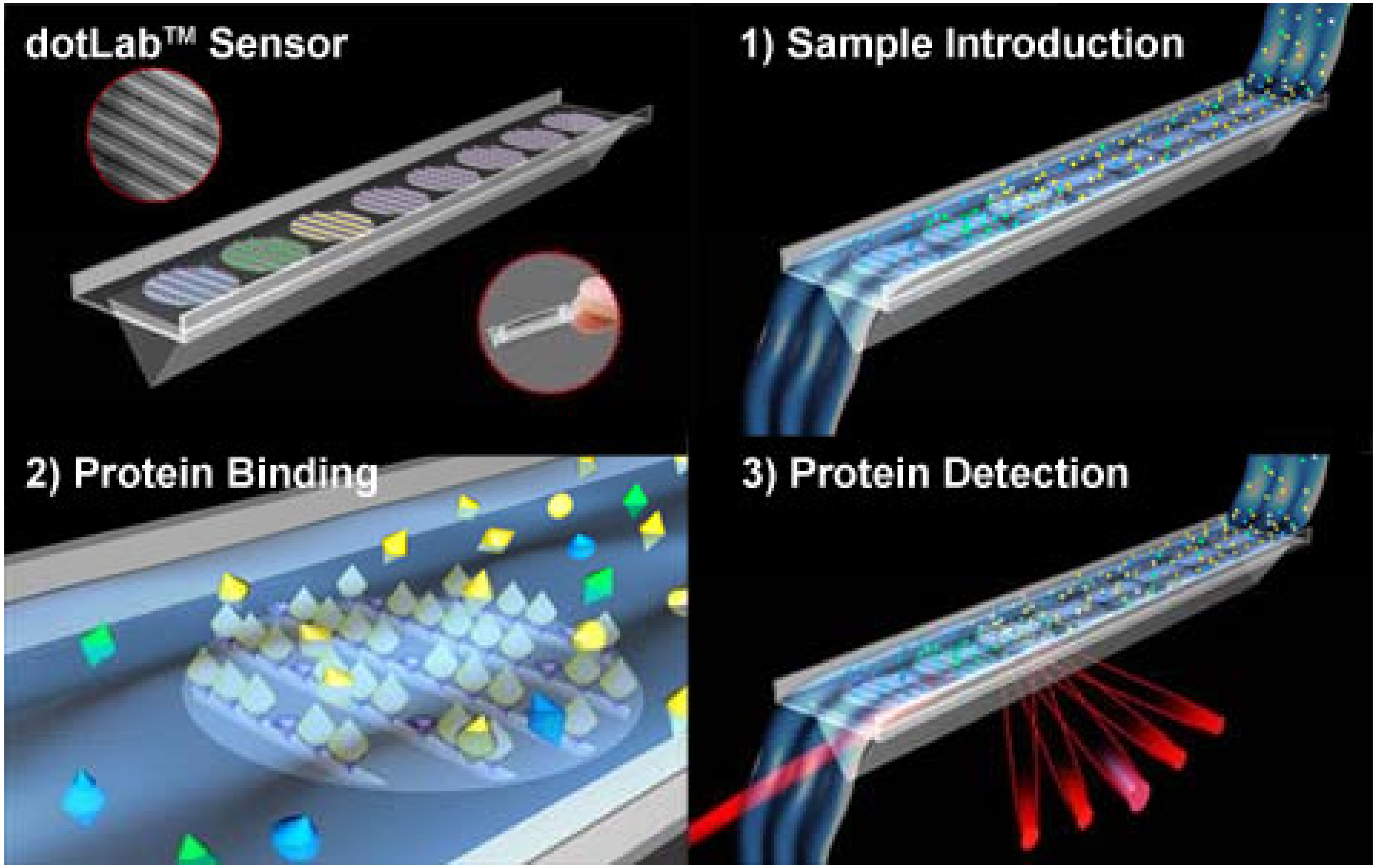
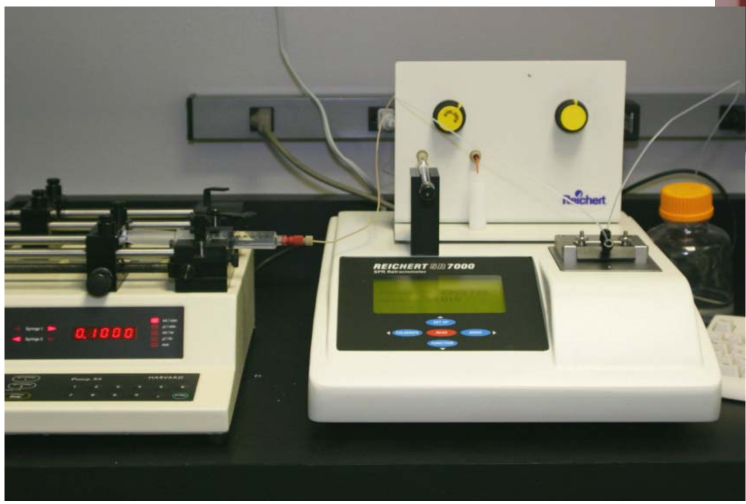


Detecting Refractive Index Changes

- SPR
  - the most sensitive technique $\Delta n<10^{-7}$.
  - detect changes in a thin layer adjacent to the sensor surface

BIAcore 3000

IBIS-iSPR



Reichert SR7000

SPR Phenomenon

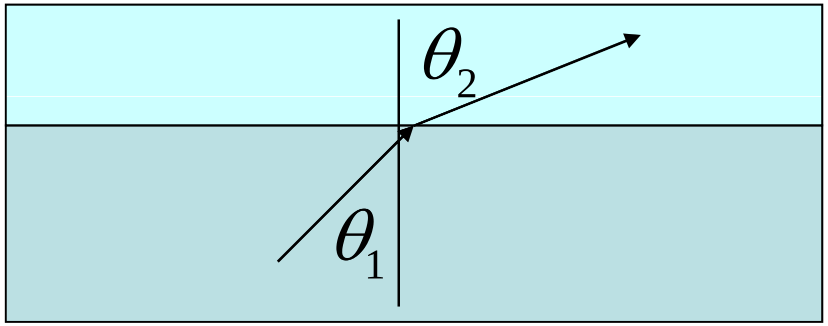
Brief History of Surface Plasmons

- first observed in 1902 by R. Wood as narrow dark bands in the spectrum of metal gratings
- observed in thin metal films as a drop in reflectivity by Thurbadar in 1958 and explained by Otto, Kretchmann and Raether in 1968.
- 1970s plasmons used to characterize metal films and study processes on the metal surfaces.
- 1990 first commercial SPR (Surface Plasmon Resonance sensor is launched by BIAcore AB.
- Currently, SPR becoming a major tool for characterizing and quantifying biomolecular interactions

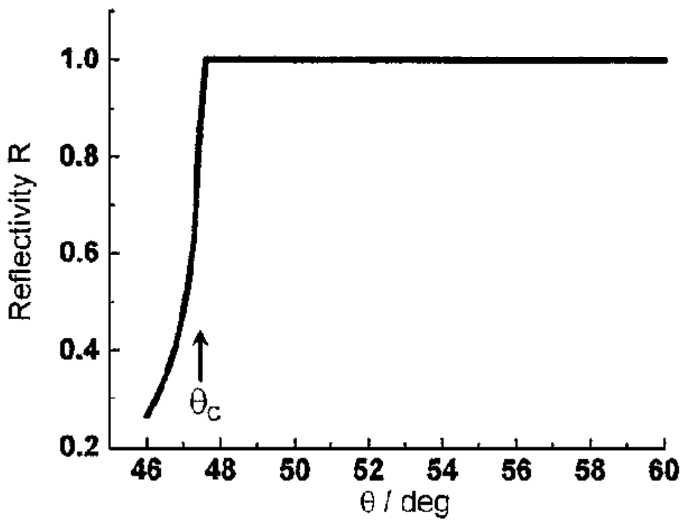
Evanescent field

- Snell's law

$$\frac{\sin\theta_1}{\sin\theta_2} = \frac{n_2}{n_1}$$

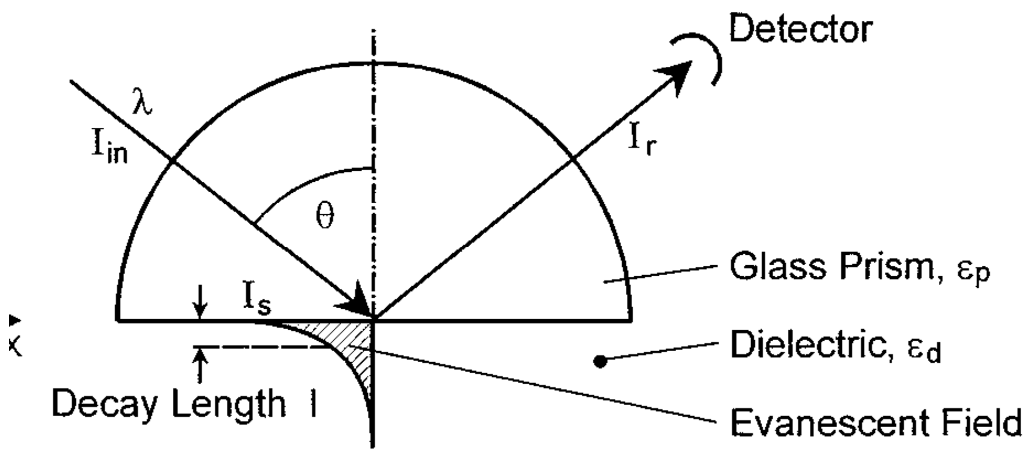


$$\sin\theta_c = \frac{n_2}{n_1}$$

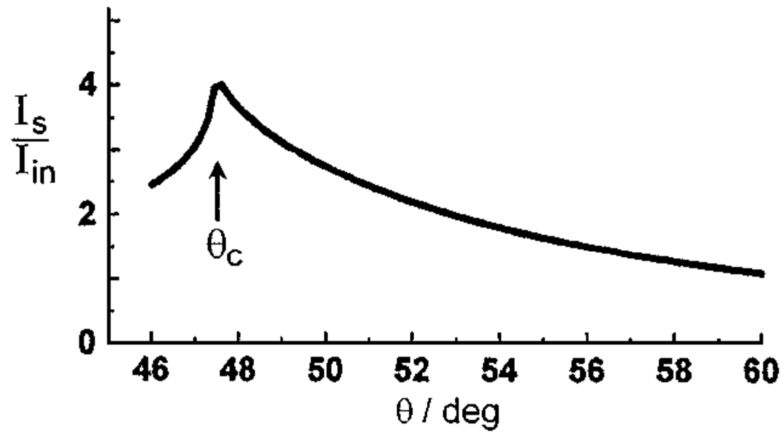


Evanescent field

- Consider light propagating from higher refractive index to lower refractive index media

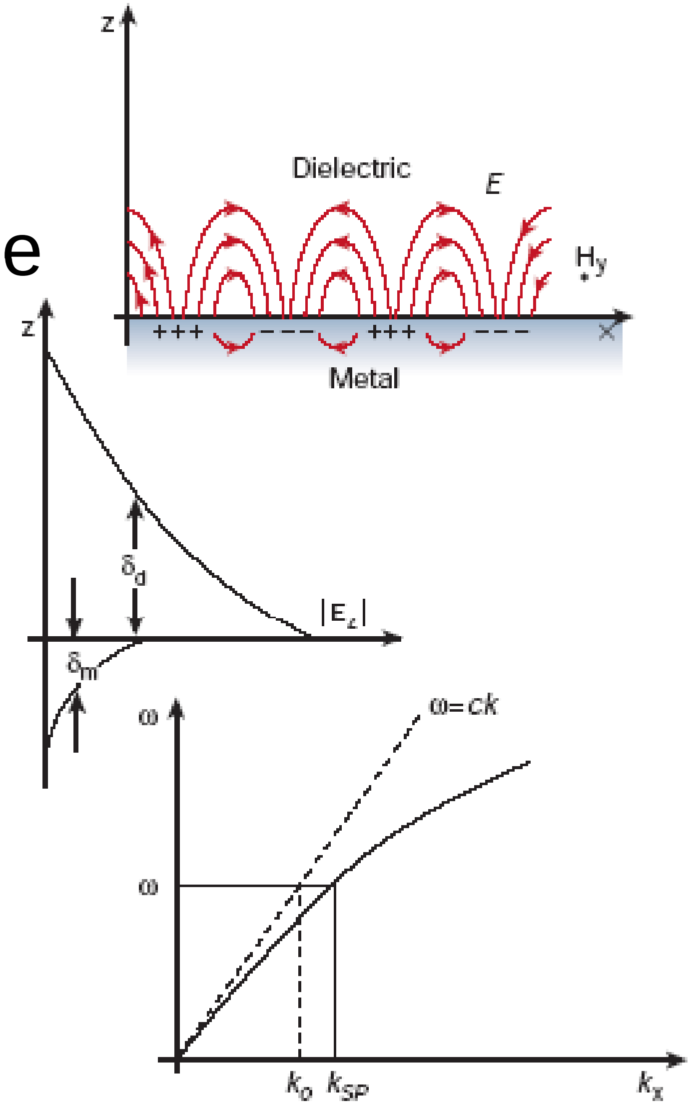


$$l = \frac{\lambda}{2\pi\sqrt{(n\sin\theta)^2 - 1}}, \quad \theta > \theta_c$$



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



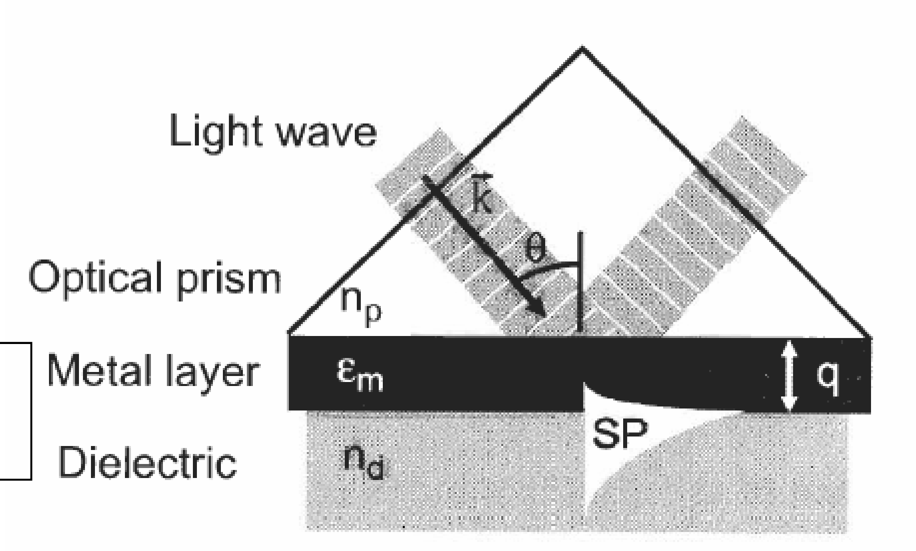
Excitation of Surface Plasmons

- Kretschmann geometry (ATR)

for the surface plasmon wave:

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

correction for prism and finite metal thickness



for the evanescent field:

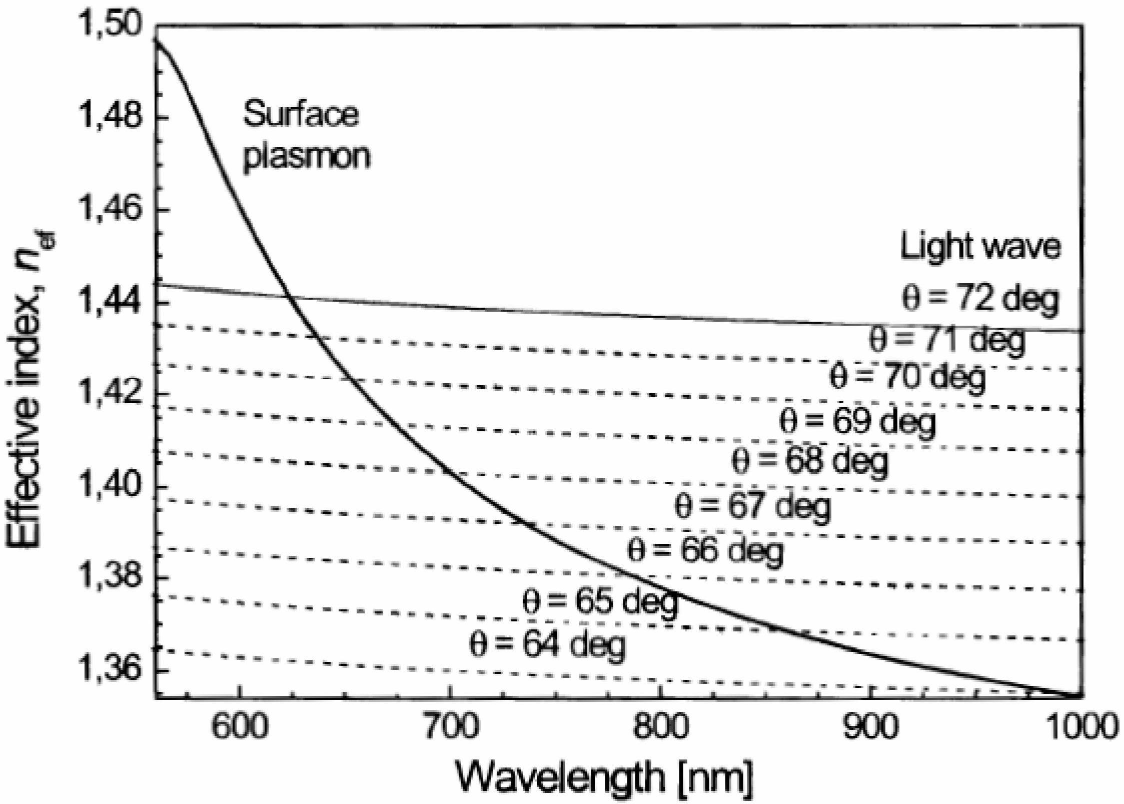
$$\beta^{EW} = \frac{\omega}{c} n_p \sin\theta$$

matching the momentum:

$$n_p \sin\theta = \mathrm{Re}\left\{\sqrt{\frac{\varepsilon_d \varepsilon_m}{\varepsilon_d + \varepsilon_m}}\right\} + \Delta n^{SP}$$

Excitation of Surface Plasmons

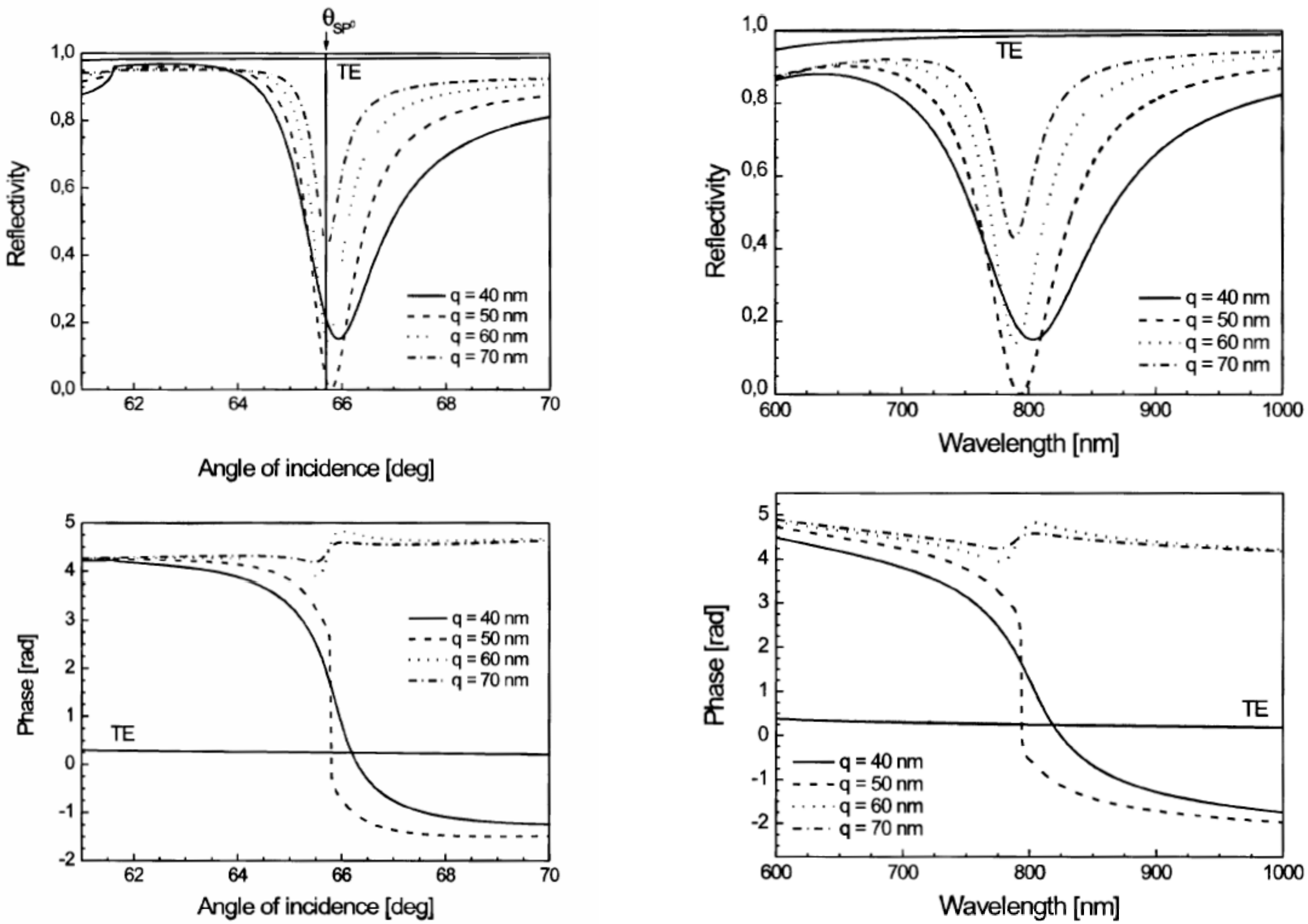
- effective index is a monotonous function of the wavelength, so there is a matching condition for the angle at the fixed wavelength or for the wavelength at fixed angle



effective index of surface plasmons and evanescent field for gold on BK7

Excitation of surface plasmons

- **Example**: gold on BK7 glass



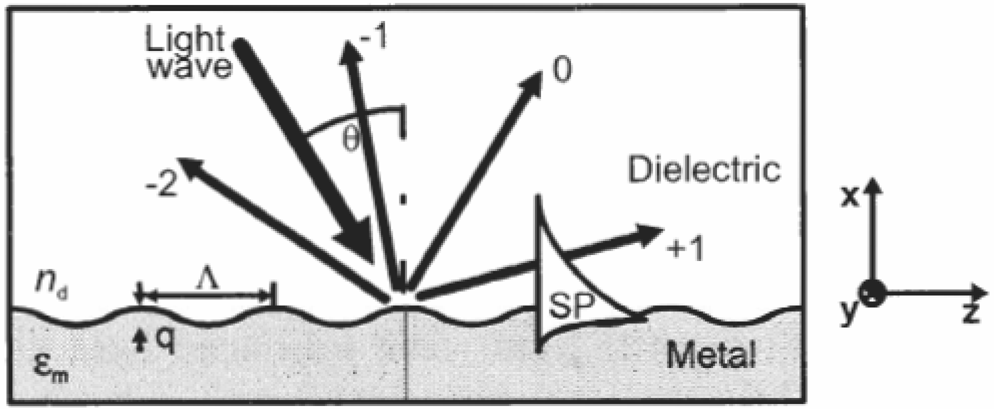
- fixed wavelength (800nm), angle varied
- fixed angle (66 deg), wavelength varied

Excitation of surface plasmons

- grating coupling

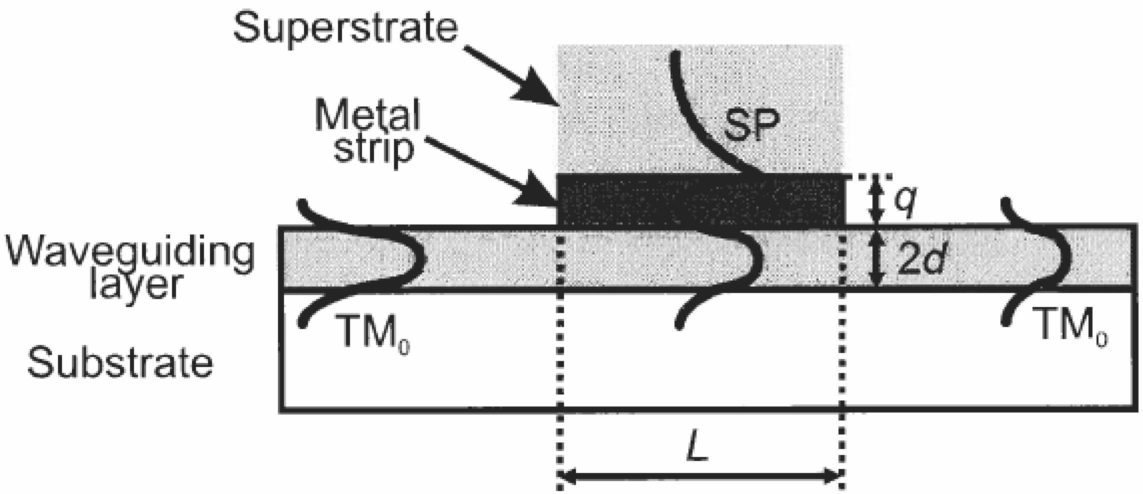
$$\vec{k}_m = \vec{k} + m\vec{G} \qquad \vec{G} = \frac{2\pi}{\Lambda}\vec{z}$$

$$n_d \sin\theta + m\frac{\lambda}{\Lambda} = \pm\left(\operatorname{Re}\left\{\sqrt{\frac{\varepsilon_d\varepsilon_m}{\varepsilon_d+\varepsilon_m}}\right\} + \Delta n^{SP}\right)$$



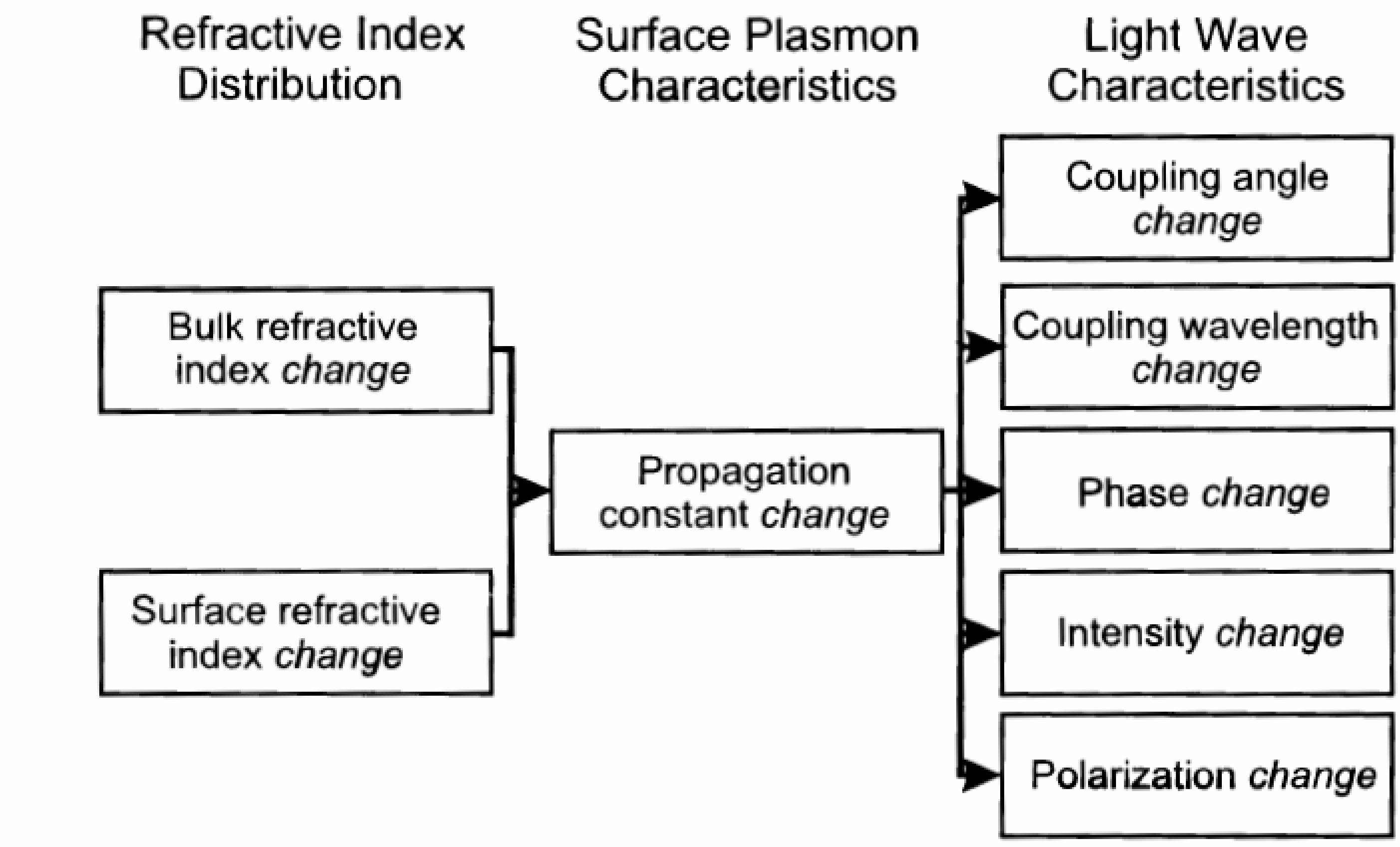
- waveguide coupling

$$\beta_M = \operatorname{Re}\{\beta_{SP}\}$$



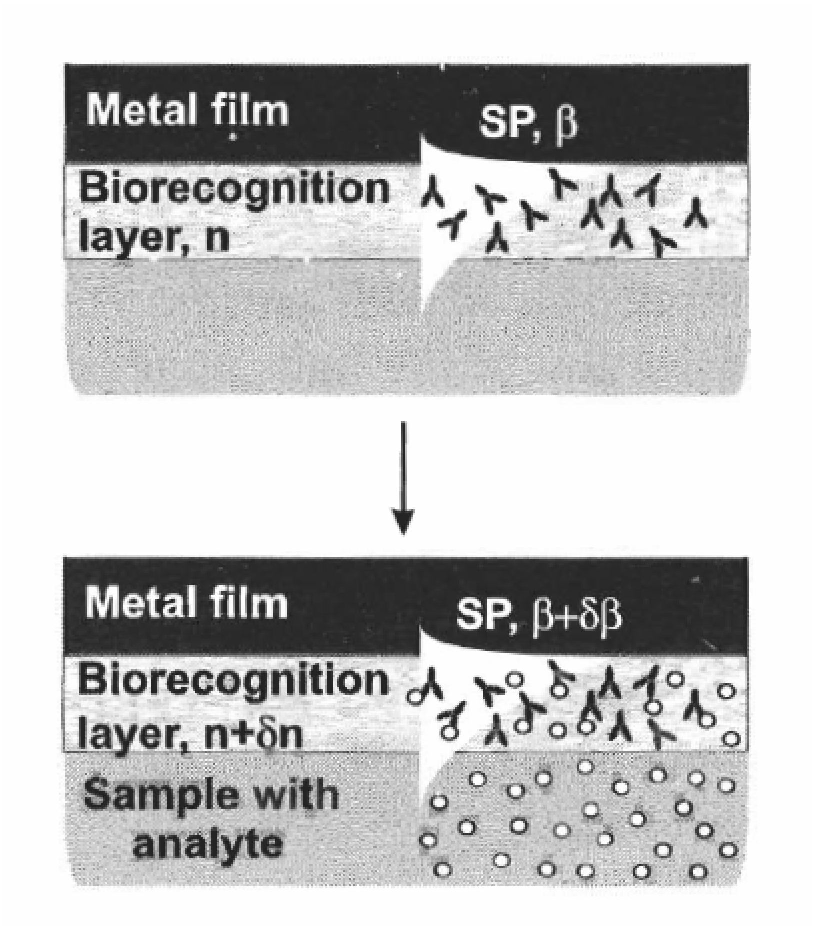
Surface plasmon sensor

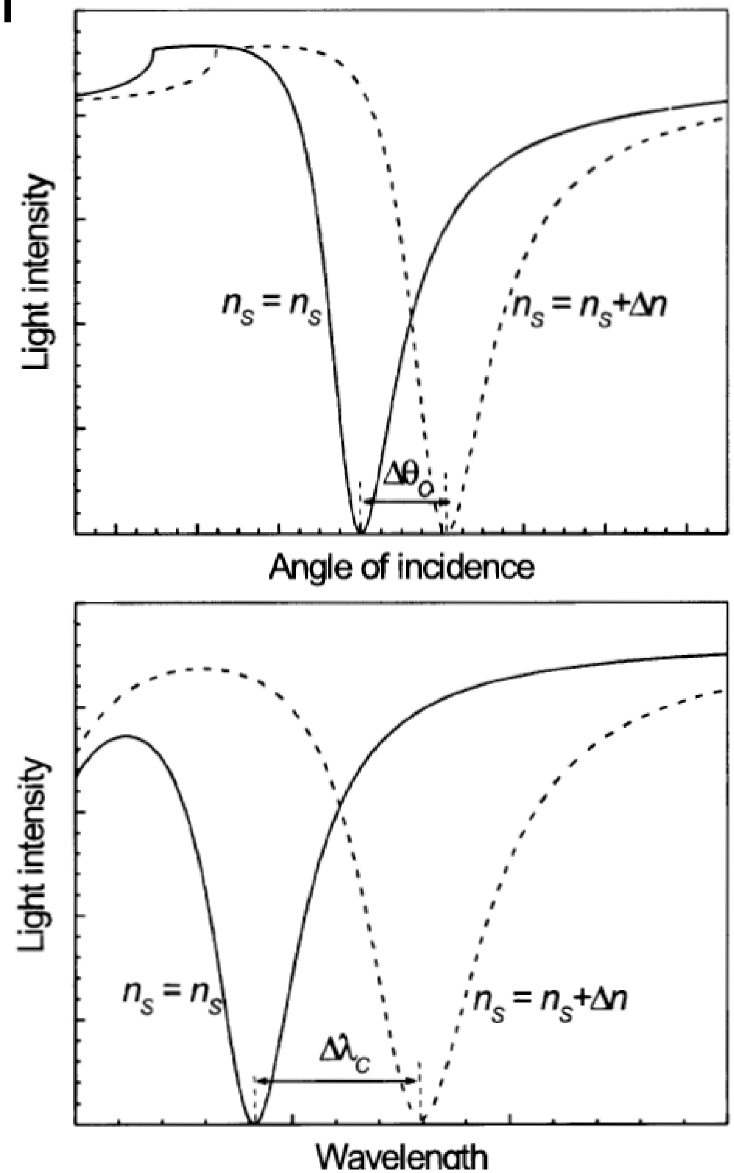
- The concept



Surface plasmon sensor

- Principle of affinity SP biosensor





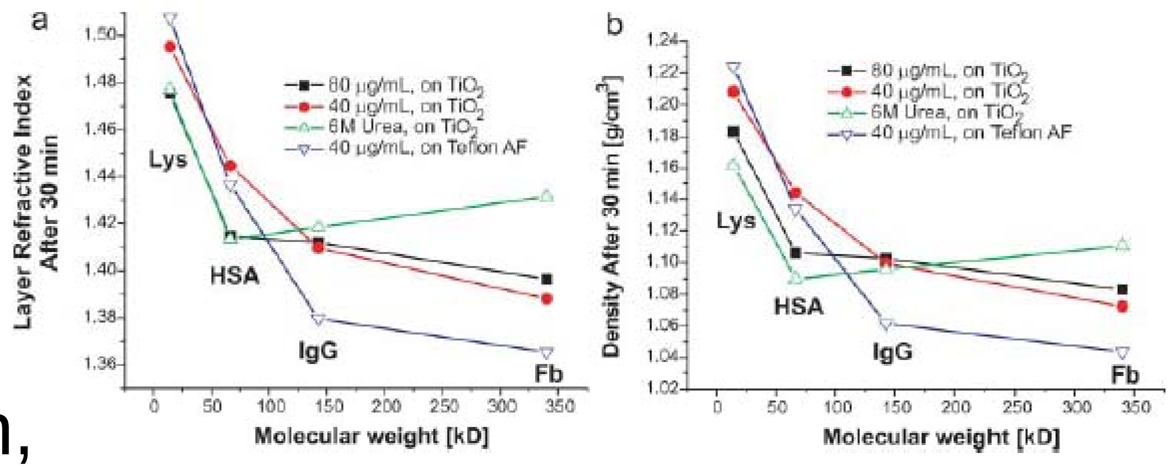
Performance characteristics of SPR

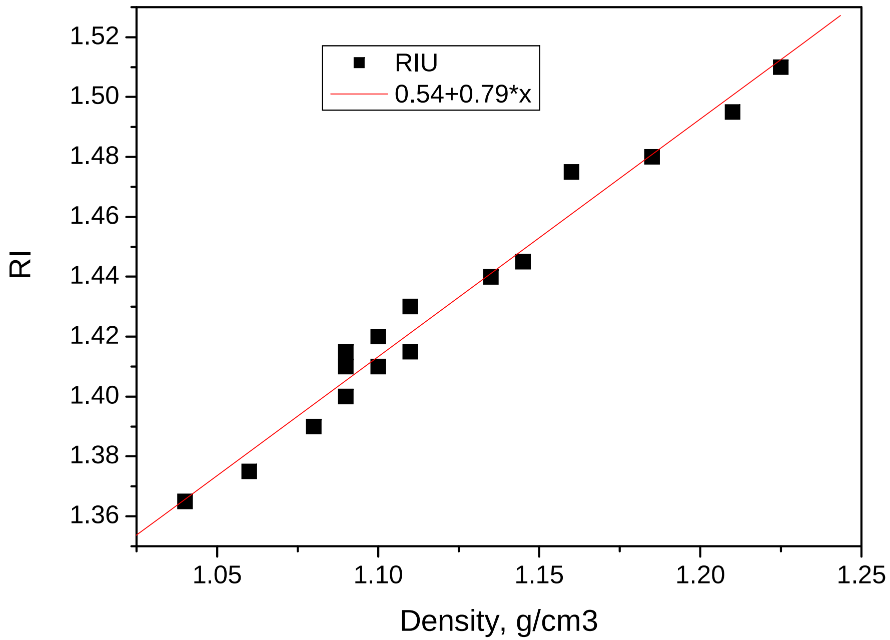
- sensitivity – slope of the calibration curve
- linearity – maximum deviation from linear transfer function within the dynamic range
- resolution – smallest change in refractive index that produces detectable output change
- accuracy – agreement between the measured value and the actual value
- reproducibility – ability to produce the same output over a period of time
- dynamic range – range of analyte concentrations that can be measured with a given accuracy
- limit of detection – concentration at which one can decide if the analyte is present

Sensitivity of SPR biosensor

$$S = S_{RI} \frac{dn_b(c)}{dc}$$

RI vs adsorbed density of proteins





measured: fibrinogen, g-immunoglobulin, albumin, and lysozyme on hydrophilic and hydrophobic surfaces

Voros, Biophys.J, 87, 553-561.

Sensitivity of SPR biosensor

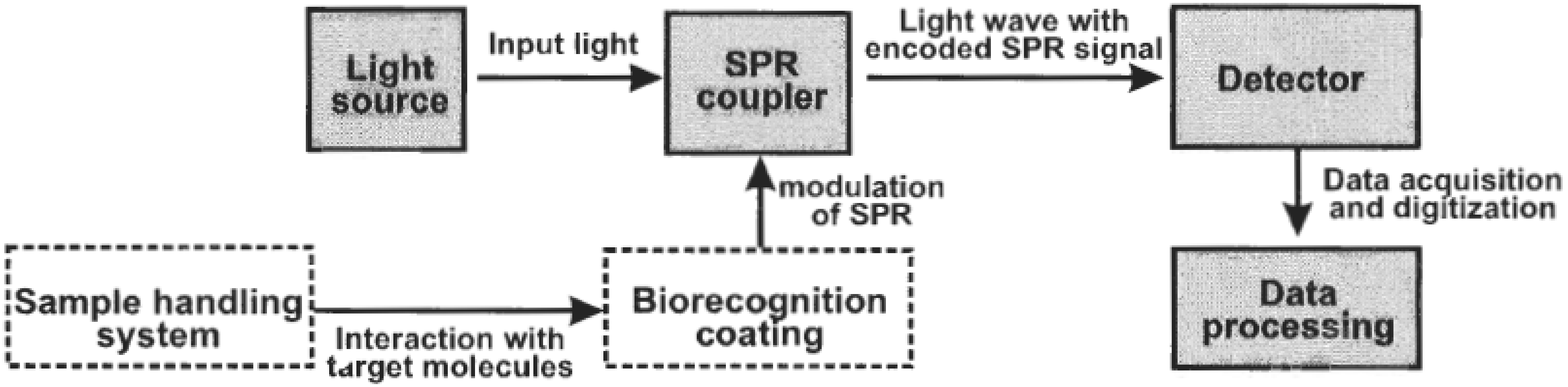
$$S = S_{RI} \frac{dn_b(c)}{dc} = S_{RI} \cdot \gamma \cdot [C]$$

- 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)

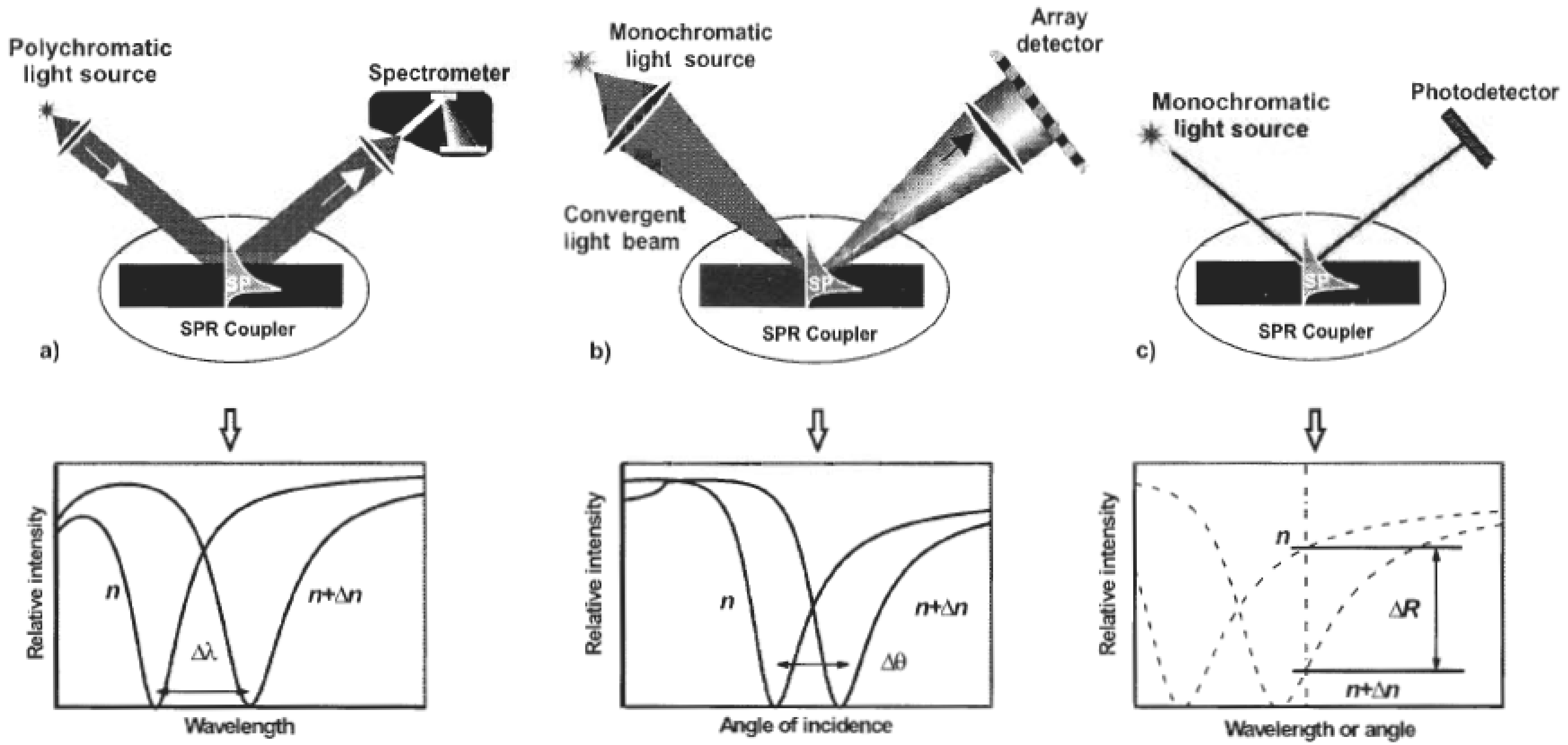
SPR Instrumentation

- Scheme of an SPR biosensor

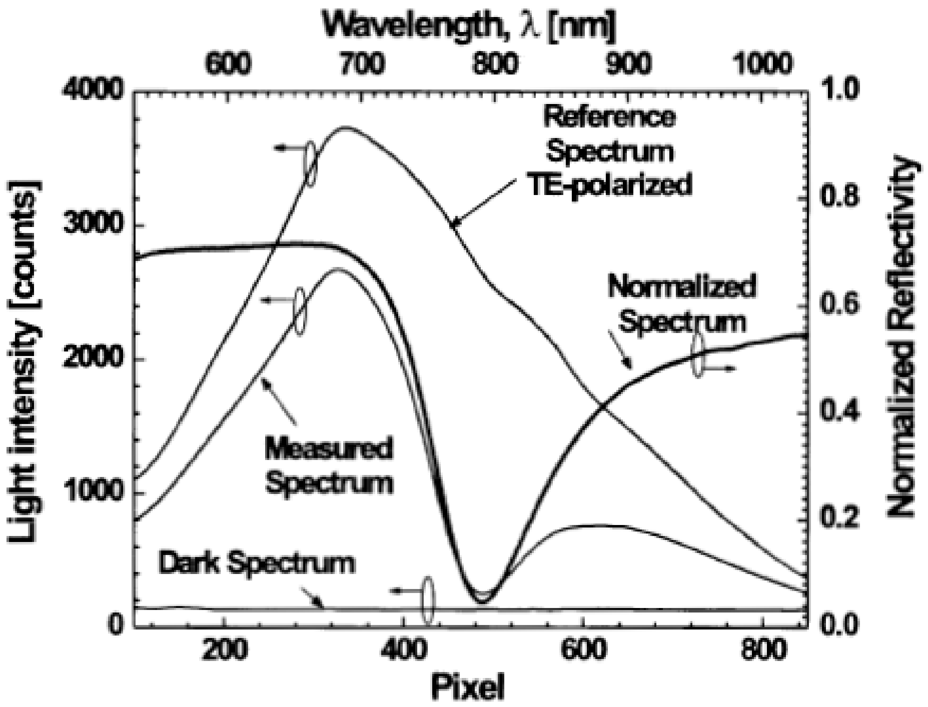


SPR Instrumentation

- Optical modulation schemes



Data processing for SPR

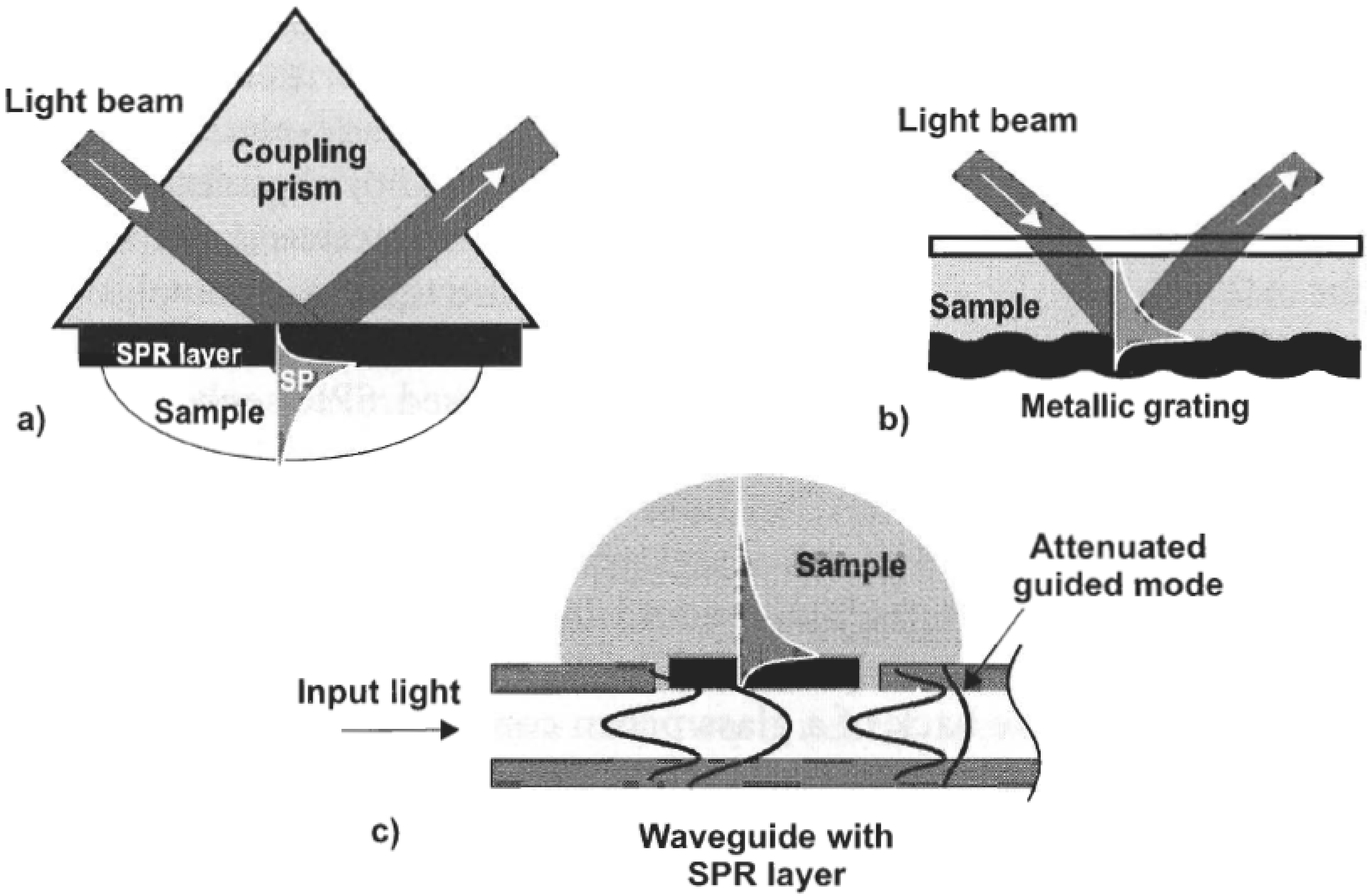


1. Signal normalization
   - subtracting dark signal
   - normalizing intensity to TE or air scan
2. Finding minimum position
   - direct measurement
   - polynomial extrapolation
   - centroid position

} sub-pixel precision!

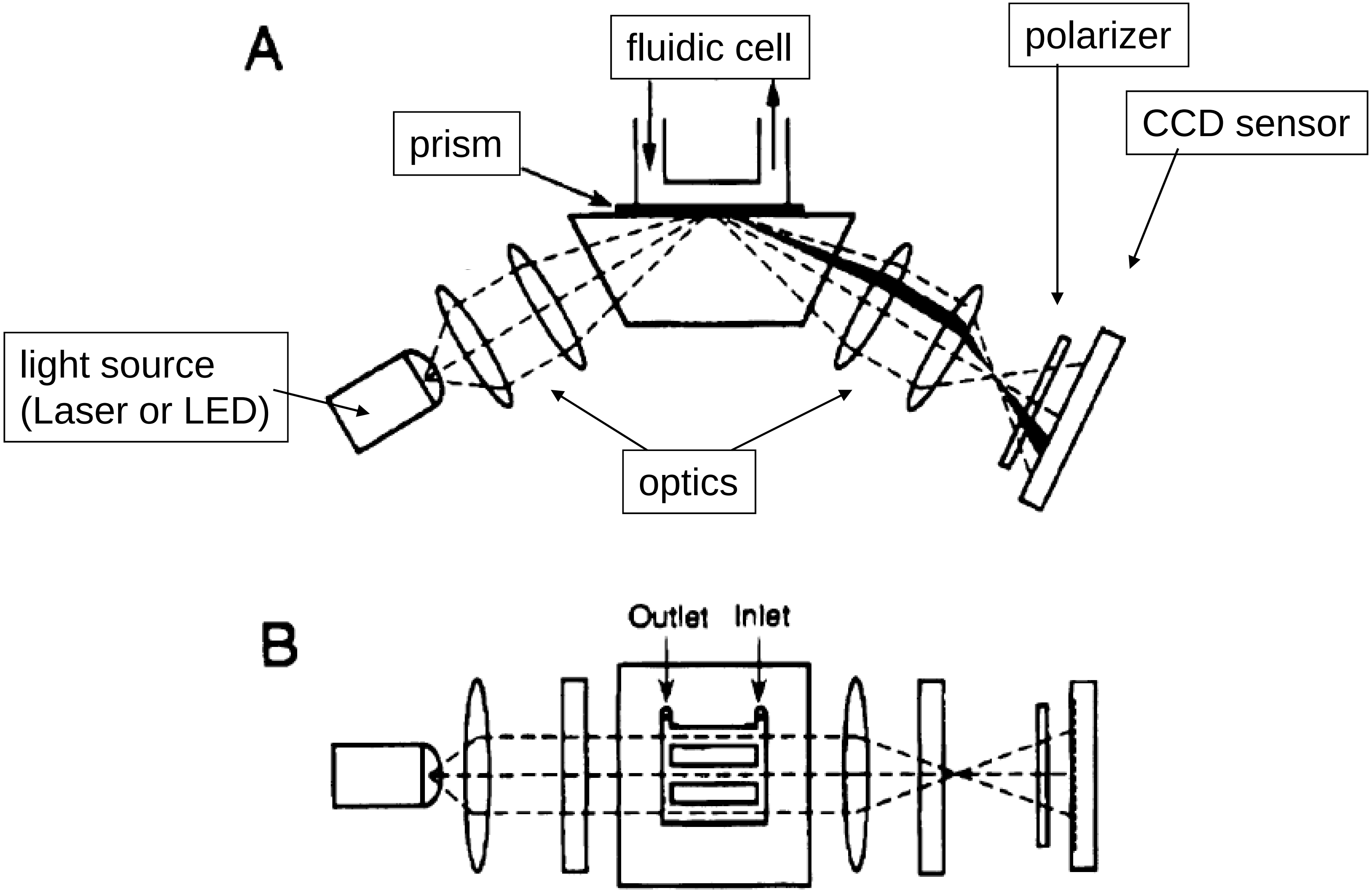
SPR Instrumentation

- Optical coupling schemes



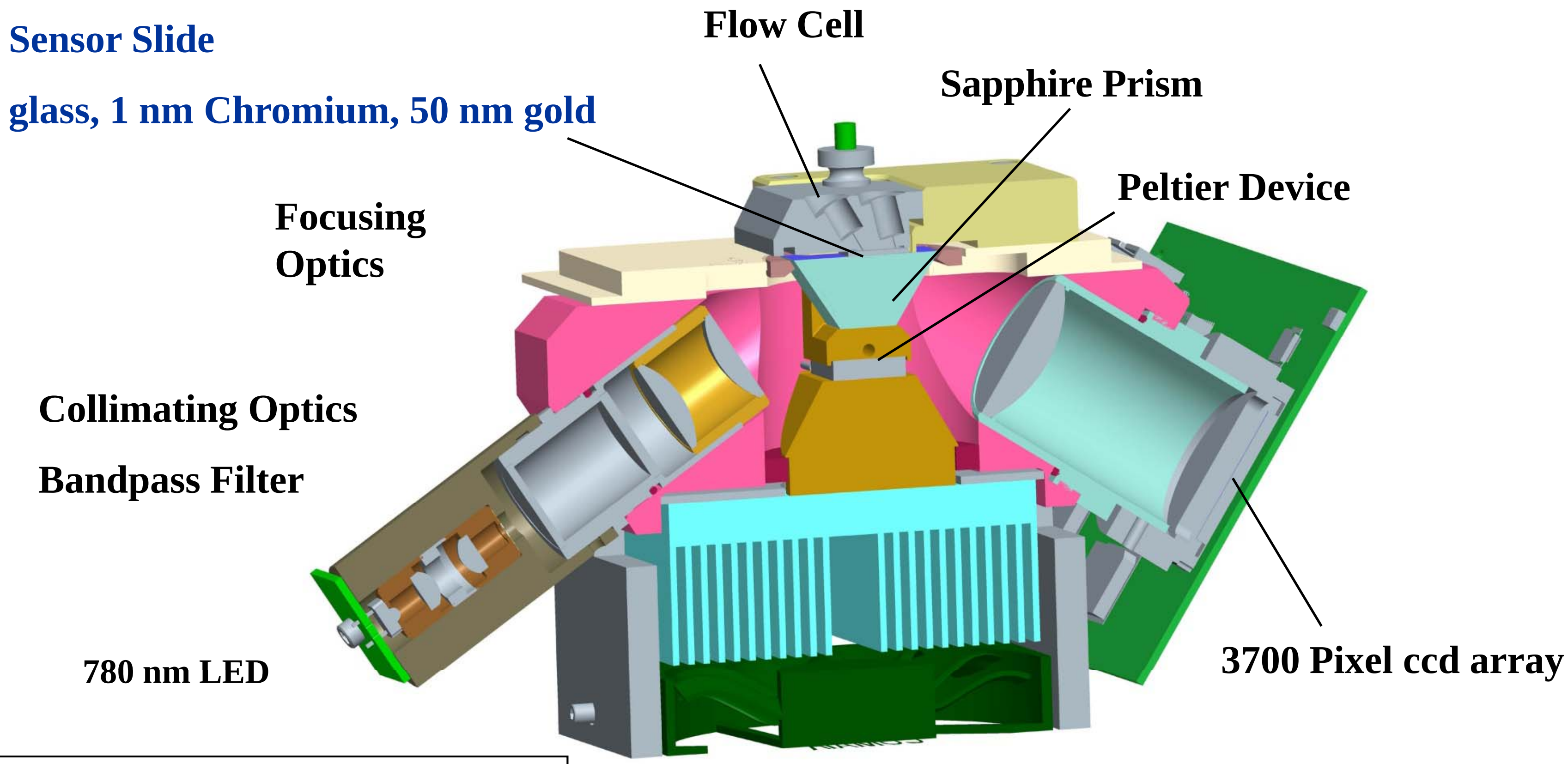
SPR sensor based on Prism Coupler and Angular modulation

- Sensor schematics



SPR sensor based on Prism Coupler and Angular modulation

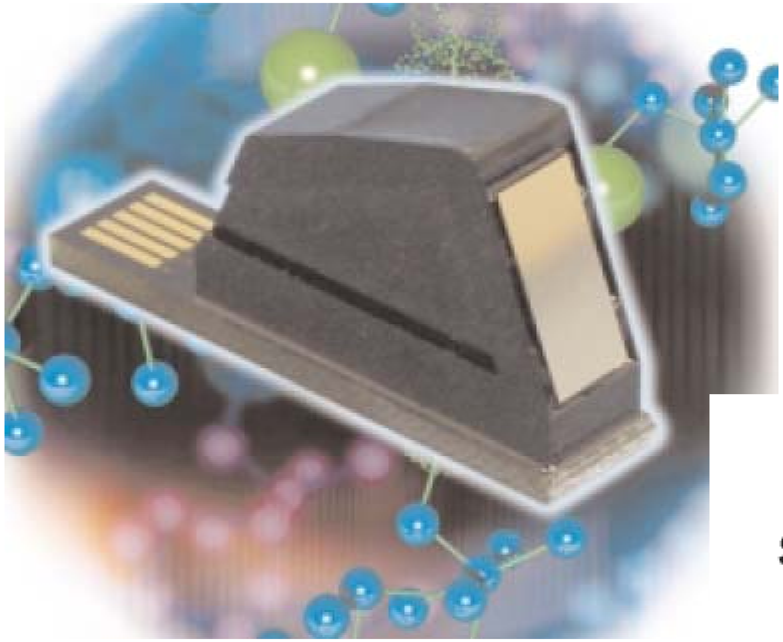
- Reichert SR7000

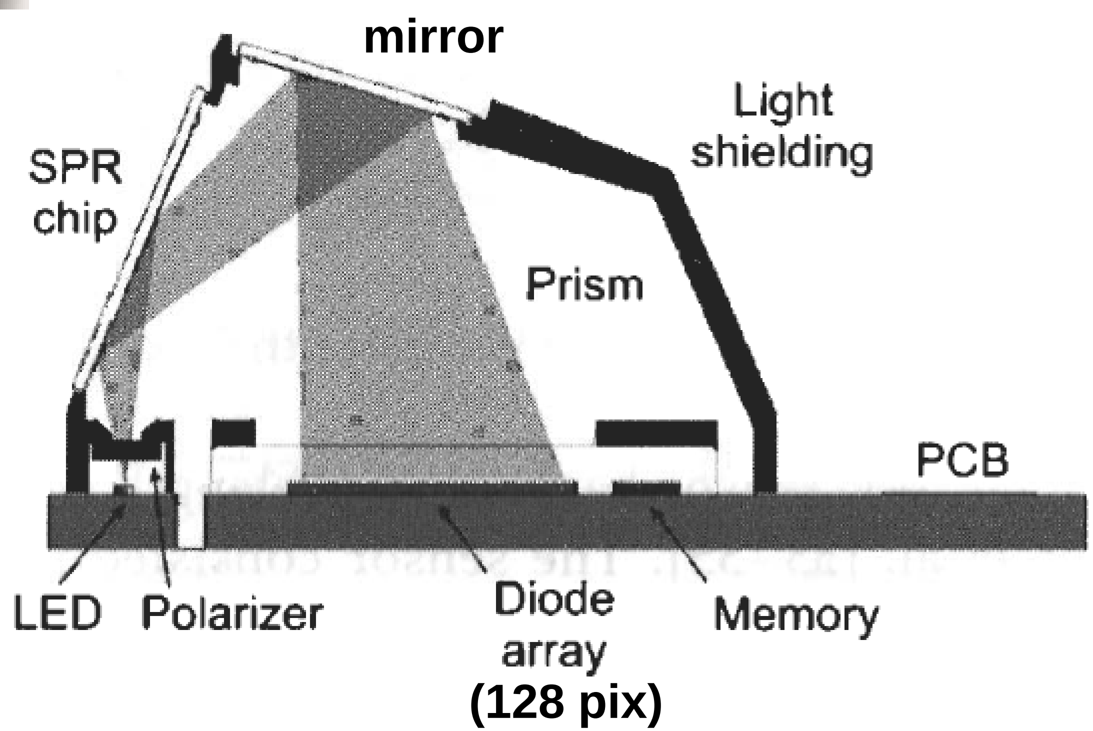


Specifications:
- sensitivity $2 \cdot 10^{-7}$ RU
- dynamic range 1.3 – 1.6

SPR sensor based on Prism Coupler and Angular modulation

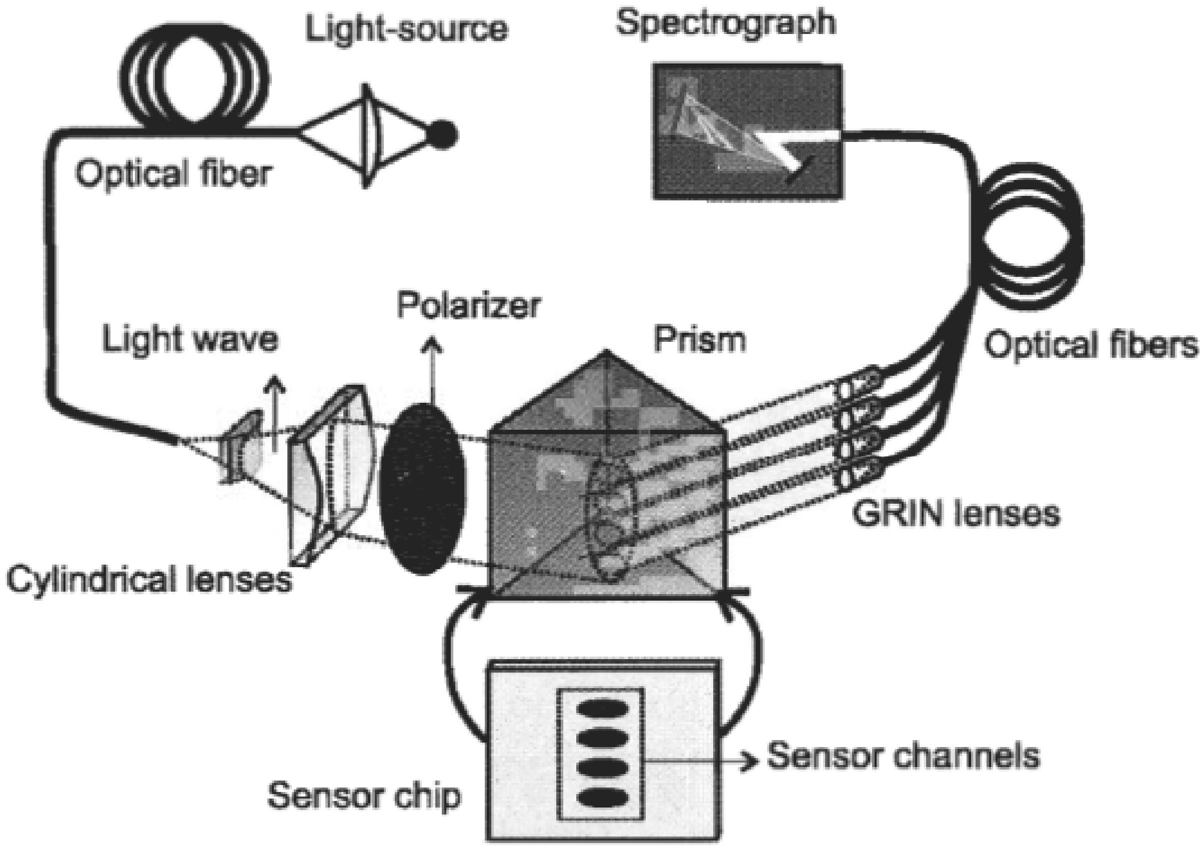
- Texas Instr. SPREETA





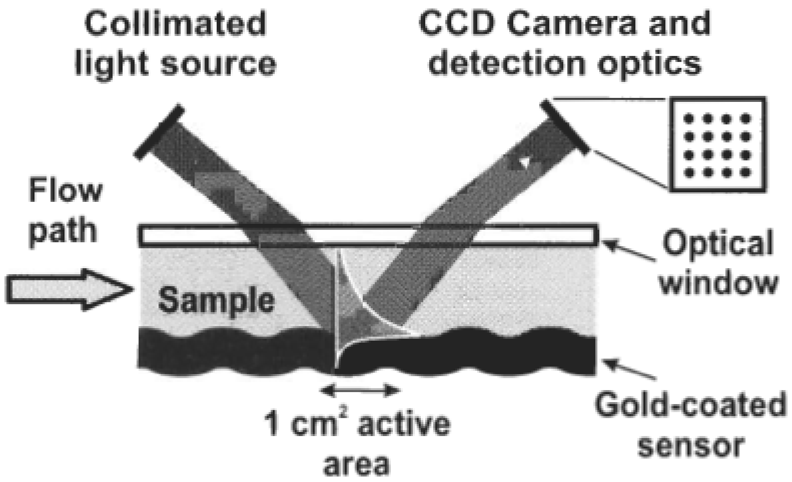
SPR sensor based on Prism Coupler and Wavelength modulation

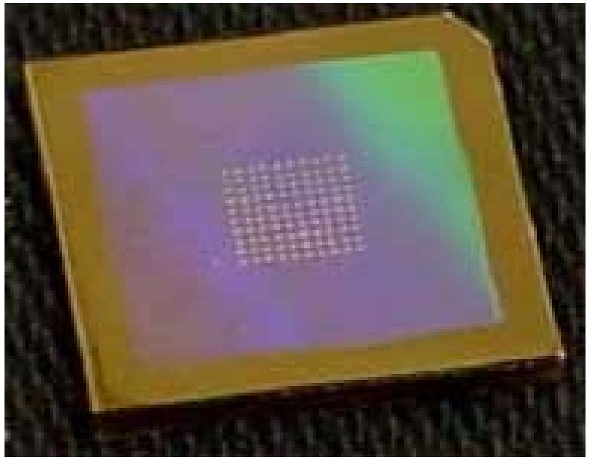
- Schematics of a 4 channel sensor with wavelength modulation

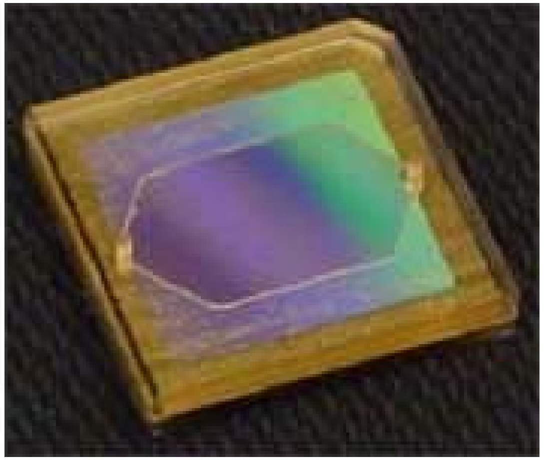


SPR sensor based on Grating Coupler and Intensity modulation

FLEX chip, HTC Biosystems (acquired by BIAcore)

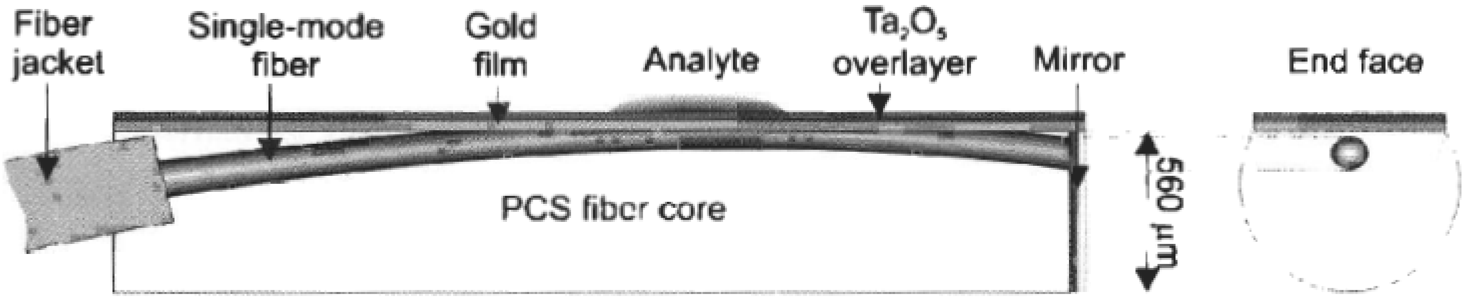


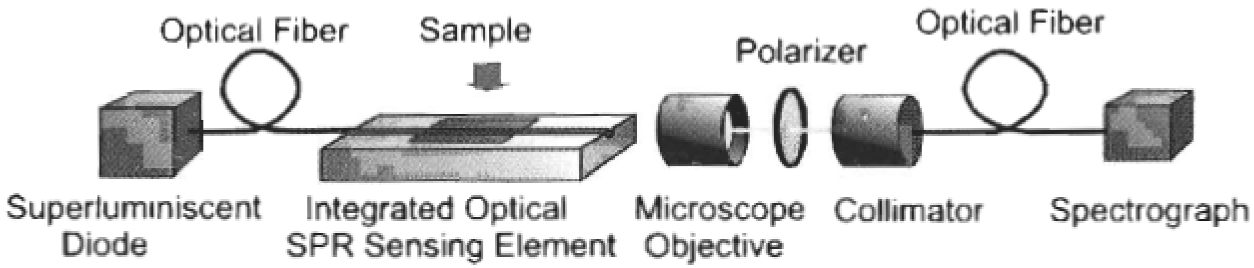




Integrated Optical SPR sensor

- SPR probe using a side polished optical fiber





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

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}?