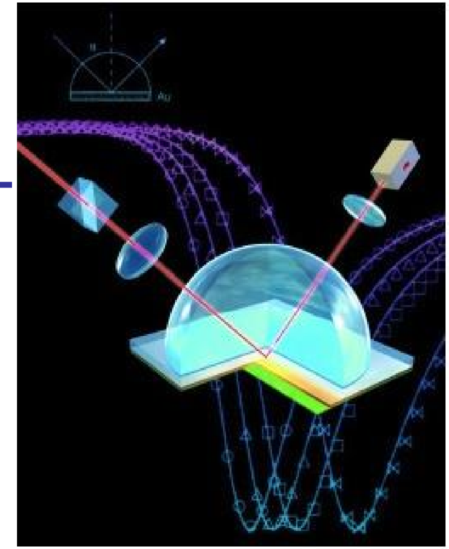
光學生物晶片



Outline

1. **Optical methods**
2. **Surface Plasmon Resonance (SPR)**

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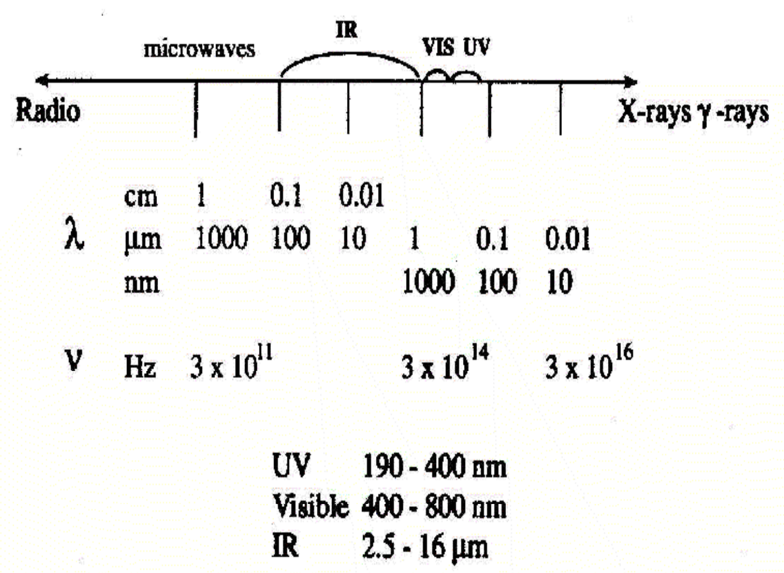
II. Optical Method

Optical methods

Light can be defined as the electromagnetic spectrum in the frequency range of 1011 (far infared) to 1017 (far ultraviolet). The energy of a single photon is given by, E=hv in J or eV, where h is Planck's constant=6.6261x10-^{34} J · s = 4.1361x10^{-14} eV · S and υ is the frequency of light in Hz, and the wavelength of the light, λ, is given as , where c is the speed of light in vacuum=2.99792458x10^{8} m/s.



1. UV-VIS absorption
2. Fluorescence and phosphorescence emission
3. Bioluminescence
4. Chemiluminescence
5. Internal reflection spectroscopy
6. Laser light scattering

Terms

- *Quantum efficiency* (Q): defined as the number of carriers generated per incident photon.
- *Gain*: defined as the ratio of the total current that flows in response to photo excitation to the current that flows in direct response to impinging photons (the primary photocurrent). This is a measure of the carrier multiplication or other mechanisms that go on in the sensor.
- *Bandwidth or frequency response*: the frequency range over which the photo detector responds, with a cut-off frequency at which the output signal amplitude is reduced by 3dB.
- *Responsivity*: relating to the output signal amplitude to input power, defined as $R_I = \frac{\text{output current}}{\text{optical input power}} = \frac{I_p}{P_{opt}} = \frac{Qq}{h\upsilon} = \frac{Q\lambda(\mu m)}{1.2398} \quad (A/W)$
- *Noise equivalent power (NEP)*: defined as the amount of light required to get a signal equivalent in power to that of the noise (S/N=1). For a current-output transducer: $NEP = \frac{\text{RMS noise current}(\frac{A}{\sqrt{Hz}})}{R_I(\frac{A}{W})} \text{ in}(\frac{W}{\sqrt{Hz}})$
- Detectivity (D*): defined as the reciprocal NEP, correcting for the proportionality of . in $D^* = \frac{\sqrt{A}}{NEP}$

Absorption

- Beer Lambert's Law $A = \varepsilon CL$

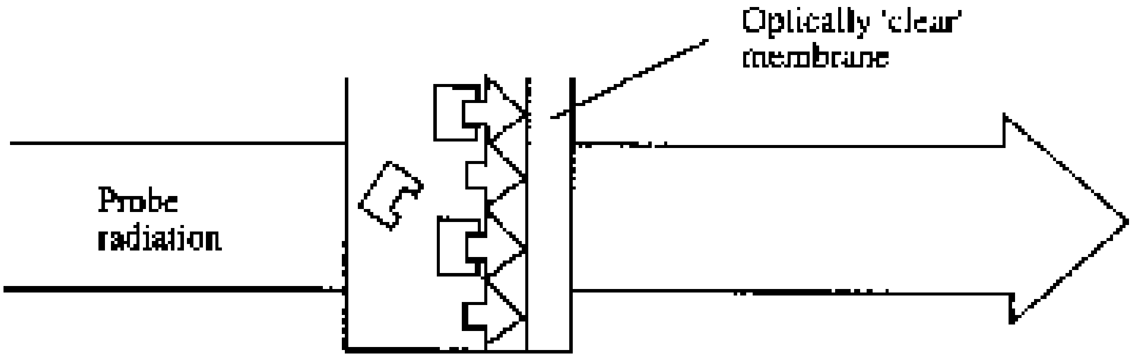
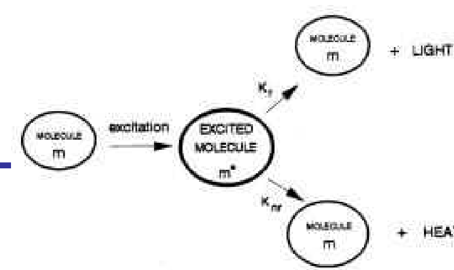


Photo-Luminance

The First observation of luminescence 1565

The first paper on luminescence 1852
(Sir G.G. Stokes)

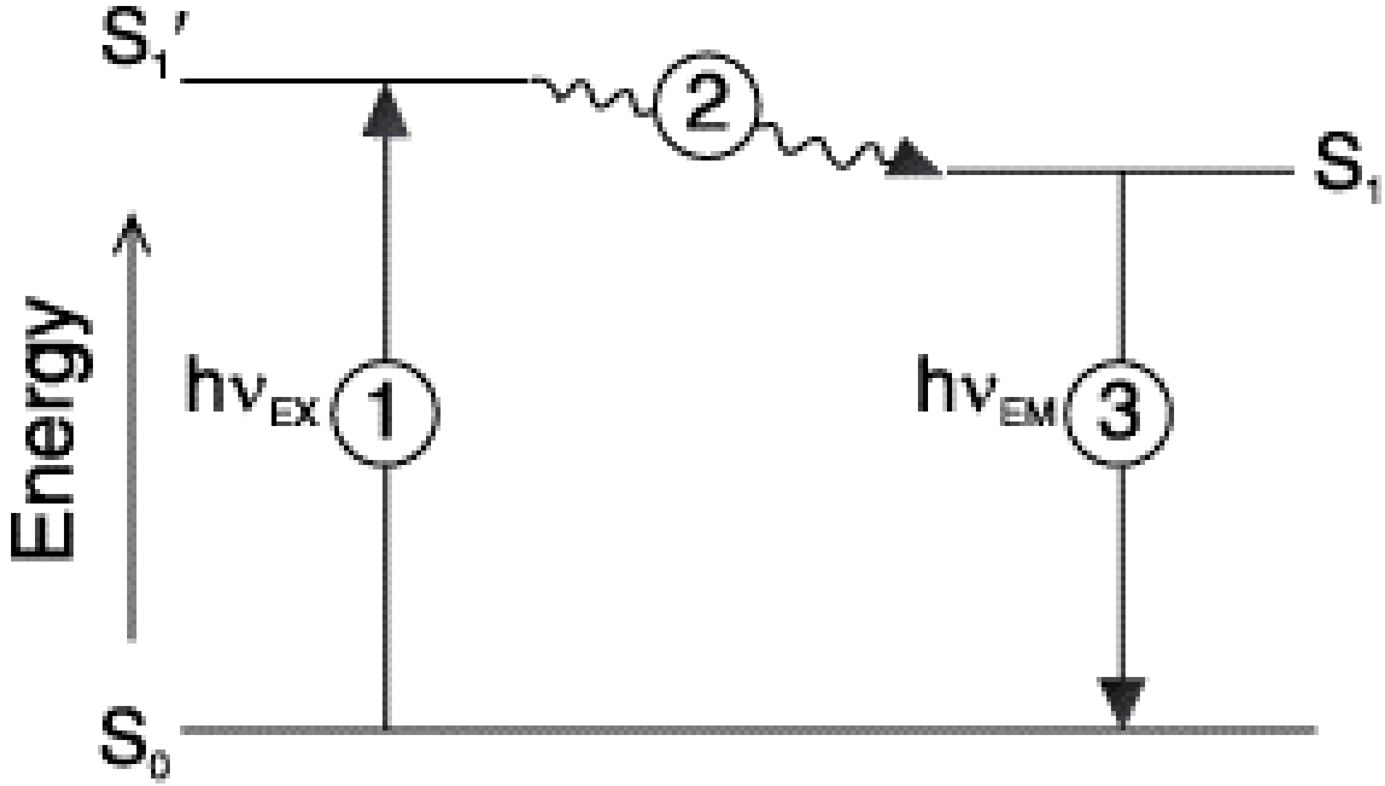
Luminance is a general term used to describe the emission phenomenon, which caused by energy absorption then followed by a radiation emission with lower energy. Depending on the type of feeding energy, there are many kinds of luminescence can be distinguished: bio-, cathodo-, chemo-, electro-, photo-, radio-, thermo-, luminescence



Mean lifetime of molecules in the excited state

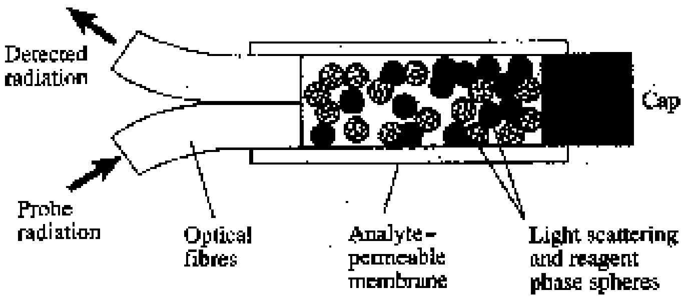
Photon absorption	10^{-15} sec
Electronic transition (S – S)	10^{-9} sec
Electronic transition (T – S)	10^{-6} sec
Vibrational transition	10^{-3} sec
Rotational transition	10^{-2} sec

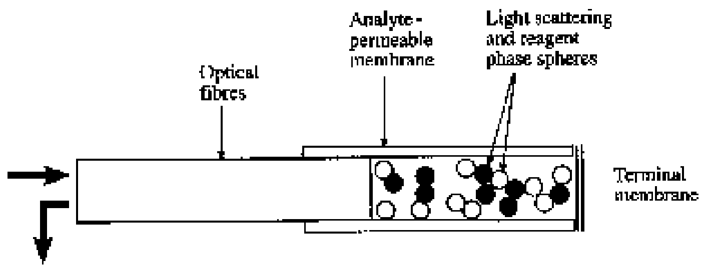
Fluorescent process

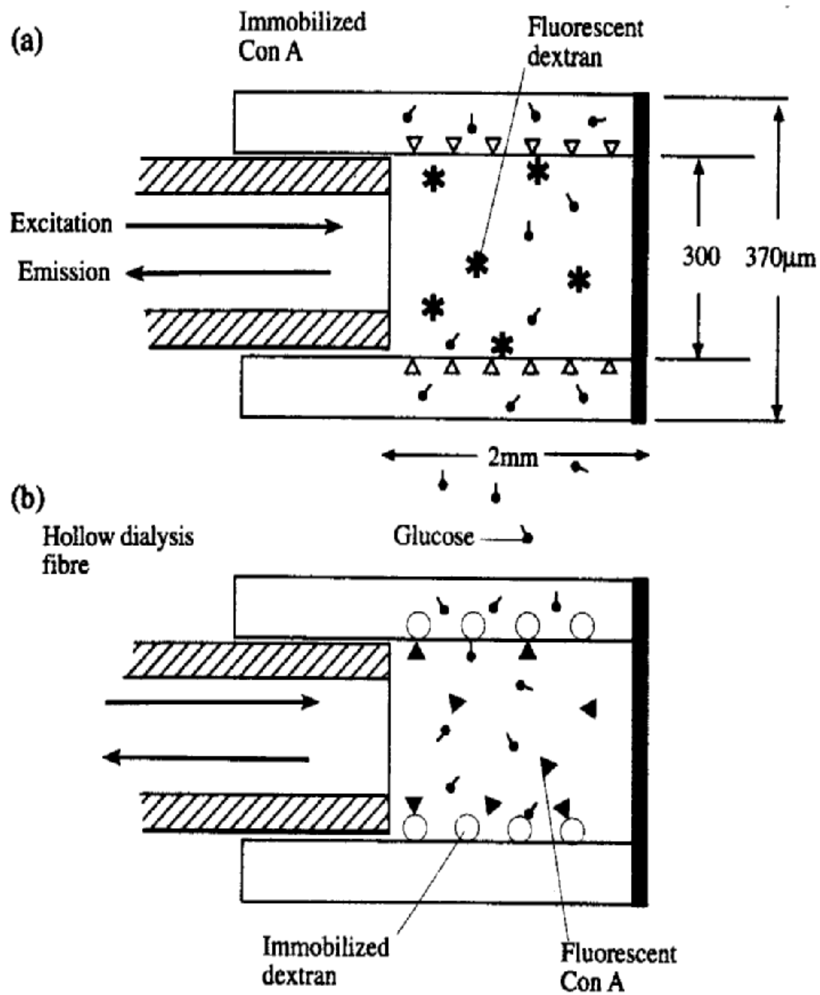


Jablonski diagram illustrating the processes involved in the creation of an excited electronic singlet state by optical absorption and subsequent emission of fluorescence.

Competitive Fluorescent







Chemilumescence

Table 5.2 Examples of chemiluminescent immunoassays

Analyte	Label	Chemiluminescent reactants
Human IgG	Luminol	H_2O_2–haemin
Testosterone	Luminol derivative	H_2O_2–Cu(II)
Thyroxine	Luminol derivative	Microperoxidase
Biotin	Isoluminol	H_2O_2–lactoperoxides
Hepatitis B	Isoluminol derivative	Microperoxidase–peroxide
Rabbit IgG	Isoluminol	Microperoxidase–peroxide
Cortisol	Isoluminol	Microperoxidase–peroxide

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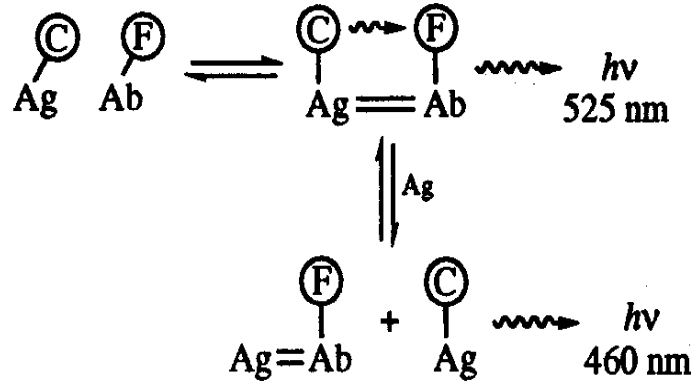
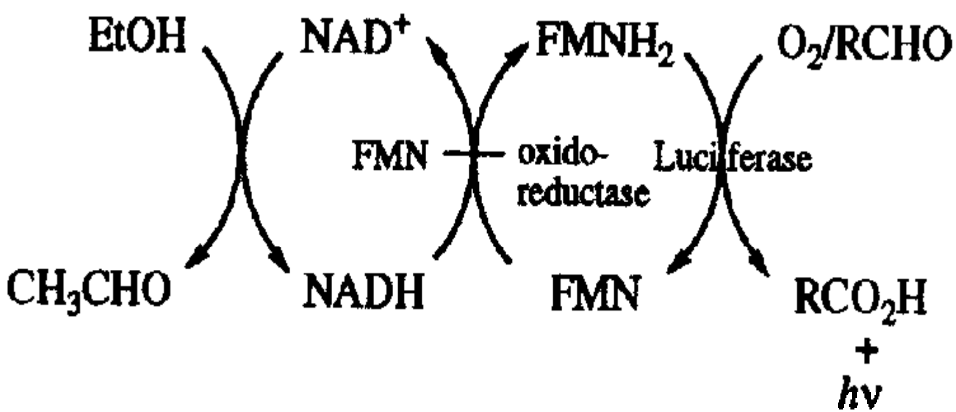
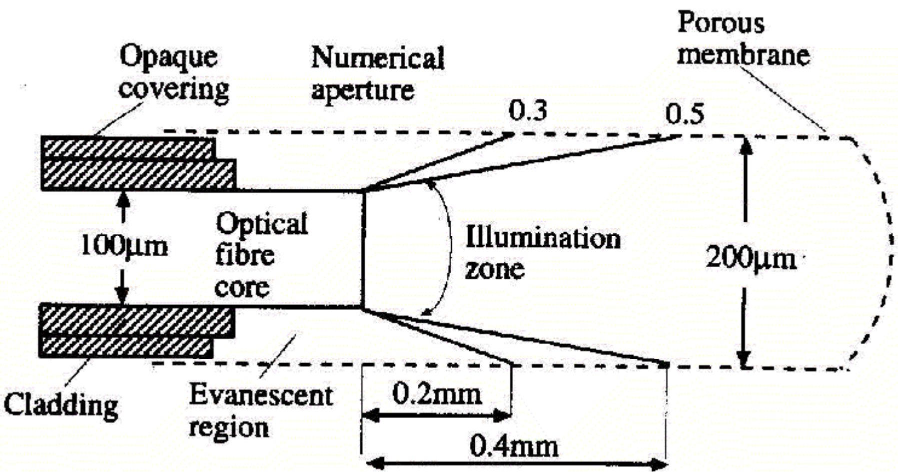
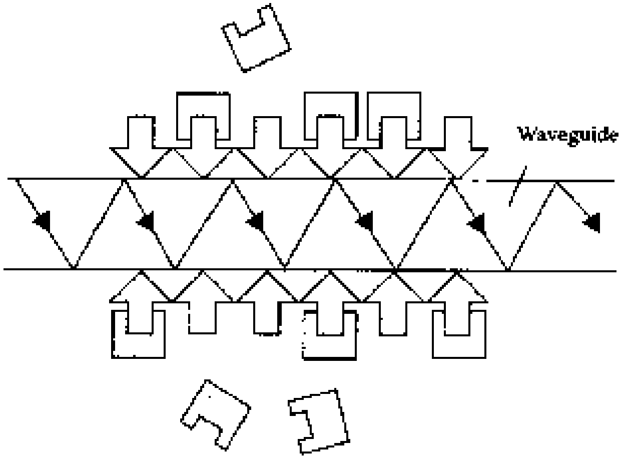
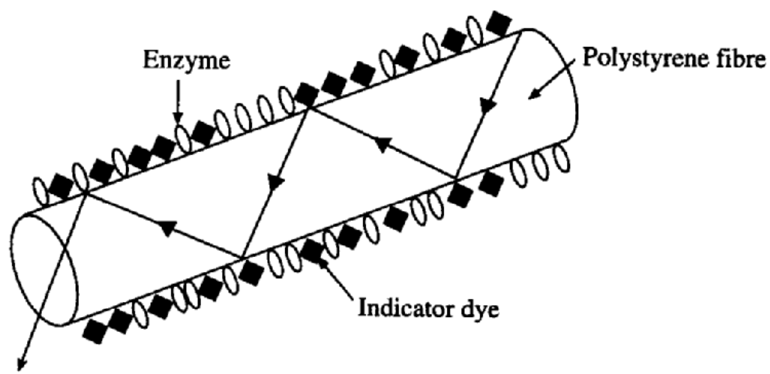




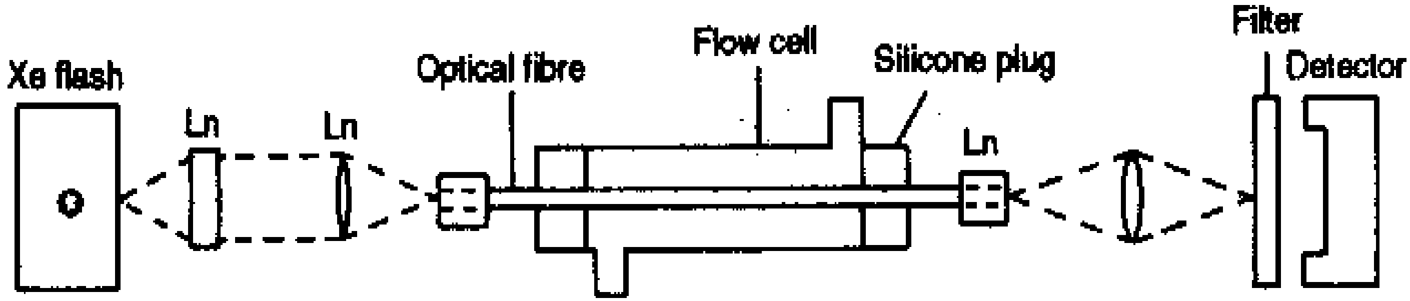
Optical Fiber



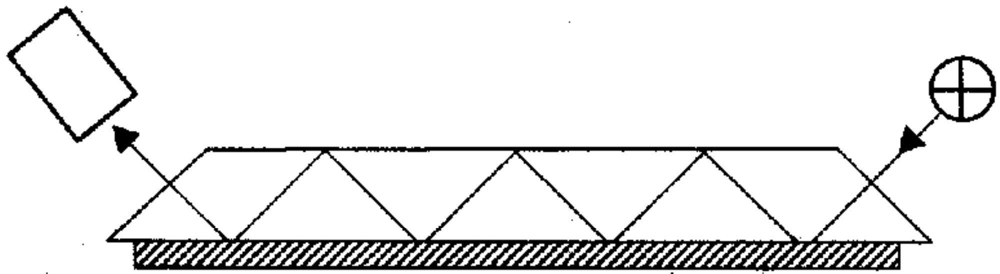


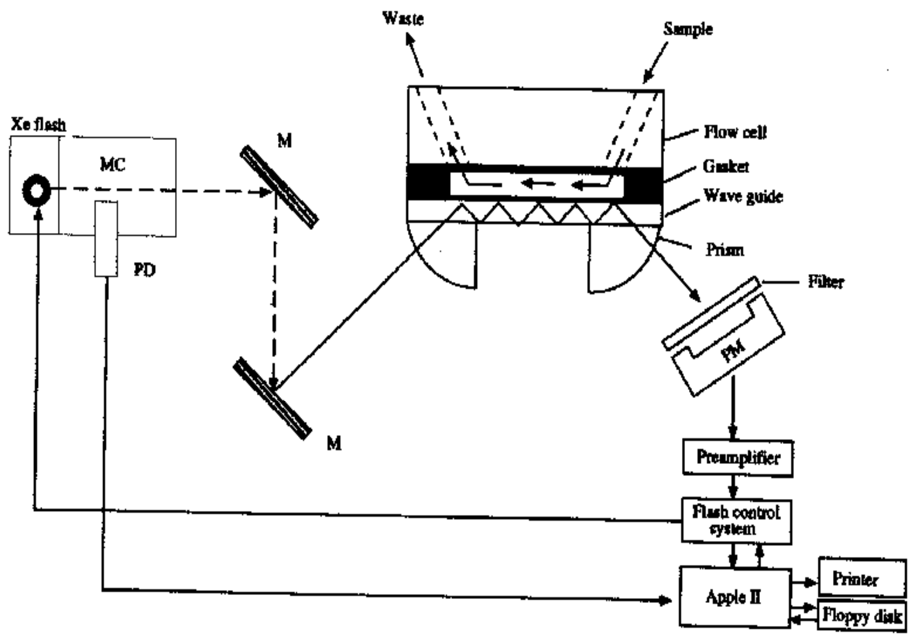


Flow through

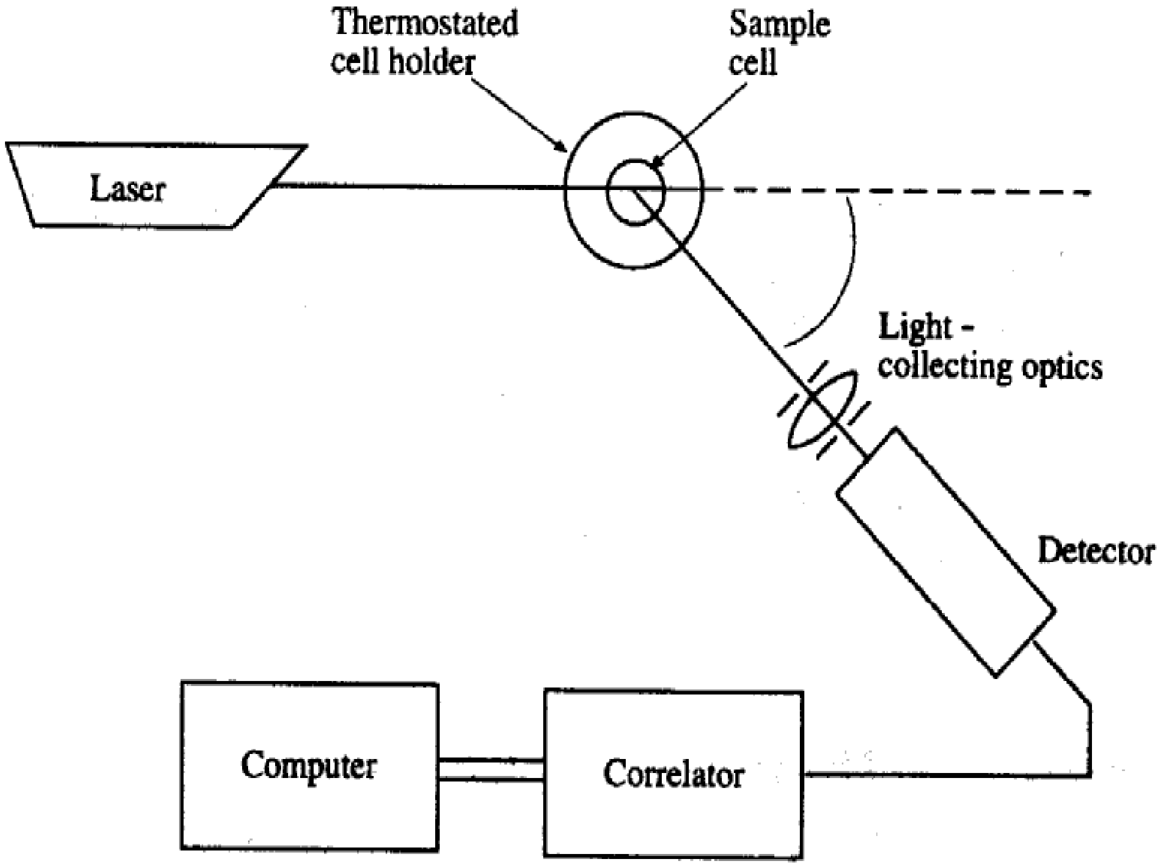


Attenuated Total Reflection

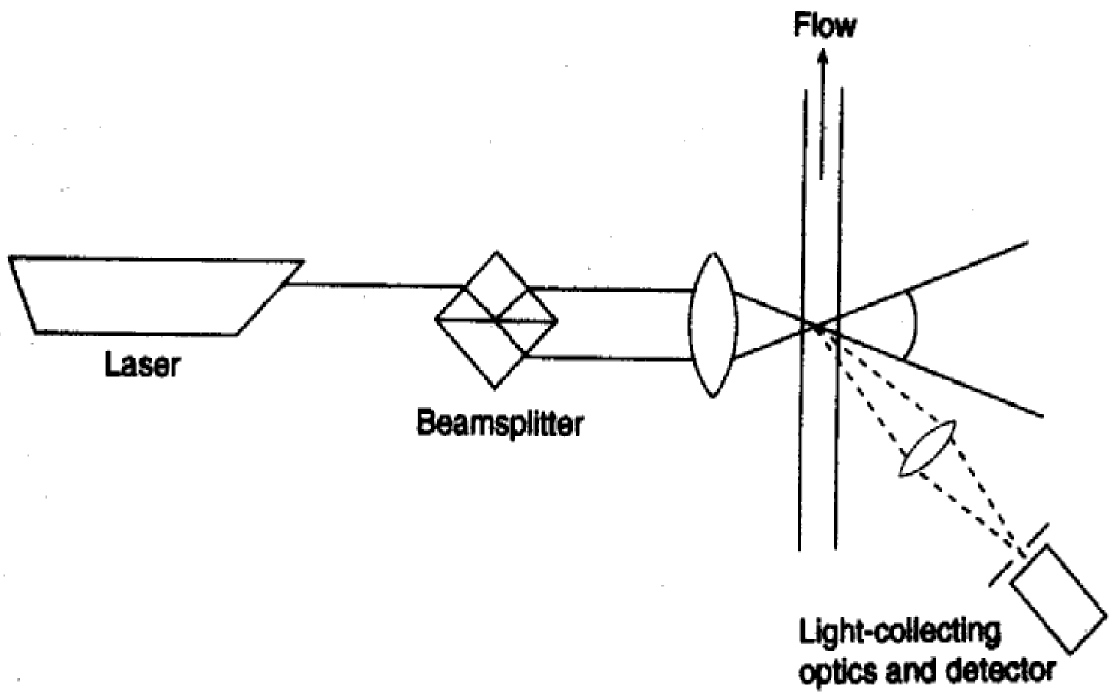




Laser scattering



Laser Doppler flow meter



III. SPR

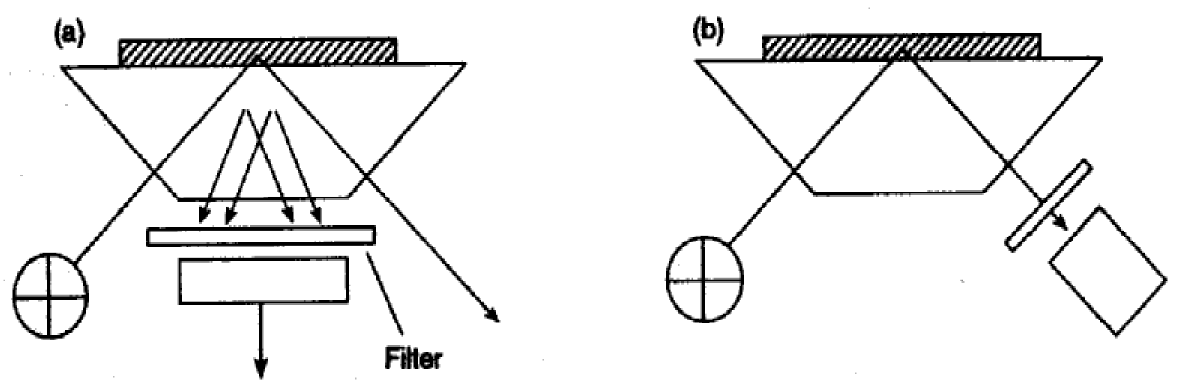
1. Introduction

- Solid state electronic properties can be studied by using two different approximation:
  - Electrons moving in the periodic array of atoms, or
  - High density of free electron liquid in a metal (~10^{23} cm^{-3}), ignoring the lattice. (plasma concept)
- It thus allow the longitudinal density fluctuation, plasma oscillations, propagate through the volume of metal.
- The quanta energy of these "volume plasmons" is in the order of 10 eV. ($\hbar\omega_p = \hbar\sqrt{4\pi ne^2/m_0}$), which has been studied in detail theoretically and experimentally with electron-loss spectroscopy.
- Maxwell's theory shows that SP can propagate along a metallic surface with a broad spectrum of eigen frequencies for $\omega = 0 \cdots \omega_p/\sqrt{2}$, which depends on the wave vector k.

I. Introduction

- SPs represent electromagnetic surface waves that have their intensity maximum in the surface and exponentially decaying fields perpendicular to the surface.
- They can be produced by electrons or by light in the attenuated total reflection (ATR) device.
- With the excitation by light, a strong enhancement of the electromagnetic field in the surface (resonance amplification) can emit up to 100 times stronger in the resonance than out of resonance. This enhancement is correlated with a strong reduction of the reflected intensity up to a complete transformation of the incoming light into SP. It thus provides an important tool for the studies of metal optics on smooth and corrugated surfaces. The measurement of its intensity and its angular distribution allows determination of the surface roughness, r.m.s. height and correlation length. On structured surface, the angular distribution of diffusely scatter light can be changed engineeringly.
- The applications include
  - Enhanced photoeffect
  - Localized plasomons effect results in large field enhancement (10^4-10^6) in Nonlinear second harmonic generation (SHG) and Surface enhanced Raman Scattering (SERS)
  - Scatter light amplification at Rayleigh waves
  - Light emission from tunnel junction
  - High frequency mode with ultra thin film

Surface Plasmon Resonance

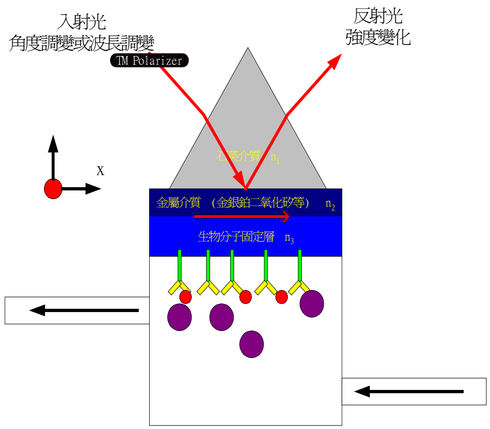


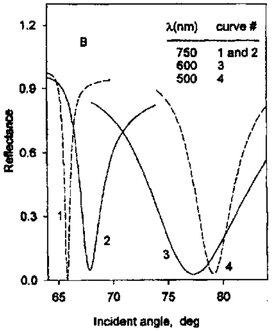
SPR Principle - General

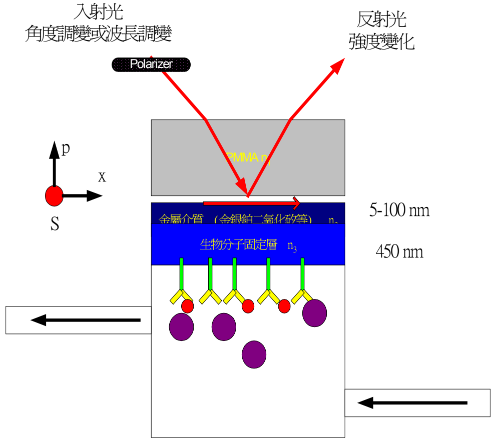
- "Surface plasmon resonance spectroscopy as a tool for investigating the biochemical and biophysical properties of membrane protein systems. I: Theoretical principle", BBA, 1331 (1997) 117-129
  - Excellent review by Z. Salamon, H.A. Macleod, G. Tollin,
- Observed by Woods Lamp on metal grating early in the 20th century.
- "Surface Plasmon" appears in 1960s to explain the existence of such a phenomena.
- Definitions of Surface Plasmon
  - A quantized oscillation of an electron on a planar surface of a metallic film
  - charge density waves propagating along a metal-dielectric interface
- It can be excited by various forms of energy, e.g. optical, electrical, chemical.
  - Surface plasmon is excited by a resonant interaction, momentum match condition (Ksp=Kx) with an evanescent field. K_{sp}: wave vector of surface plasmon, K_x : parallel component of photon wavevector
- Extensively used to study the changes in refractive index of thin film (metal, dielectric) and its vicinity surface properties (nm – sub-um) in physics and recently in biochemistry and biomedicine.

SPR Principle - General

- Four basic elements
  1. Light source (polarization, beam geometry wavelength, angle, intensity, and phase modulation)
  2. A prism (couple photons to plasmons)
  3. A thin film of metal (Au, Ag, Cu, Al, Pd, Pt, Ni, Co, Cr, W) or semiconductor (S (~ 50 nm)
  4. A light detector
- Two basic configurations
  - ✓ Kretschmann type (often used)
  - – Otto type (air or dielectric gap)
- Three features in the responsive curve
  - The (angular or wavelength) position
  - The (angular or wavelength) width
  - The depth of the resonance

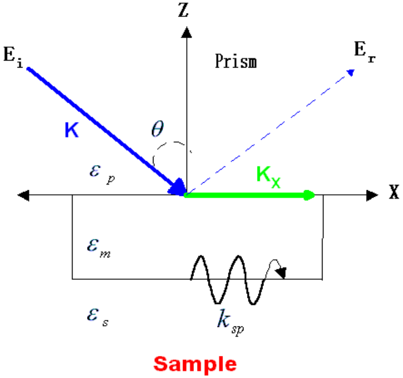


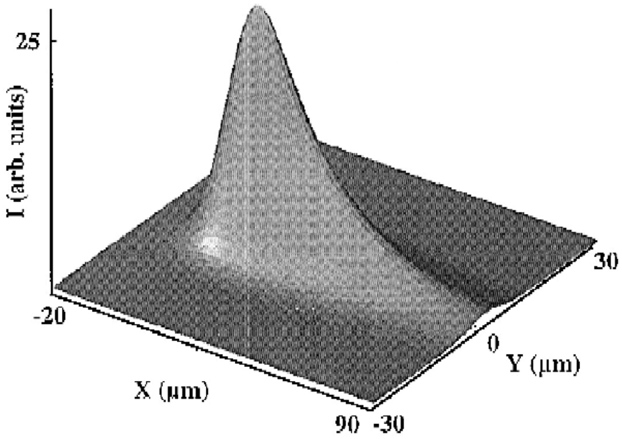




SPR Principle - General

- Propagation length of the SP
  - –In z direction intensity decrease to 1/e, L=1/k_z, (@600 nm, air:280 nm, Au:31 nm)
  - –In x direction intensity decrease to 1/e, (@ 515 nm, ~22um)
- Material dielectric constant
  - –Gold →-72+i2
  - –Silver →-81+i5
  - –Copper →-72+i7
  - –Aluminum →-173+i32
- For SPR signal, Ag is larger than Au (~4 times)





Types of SPR

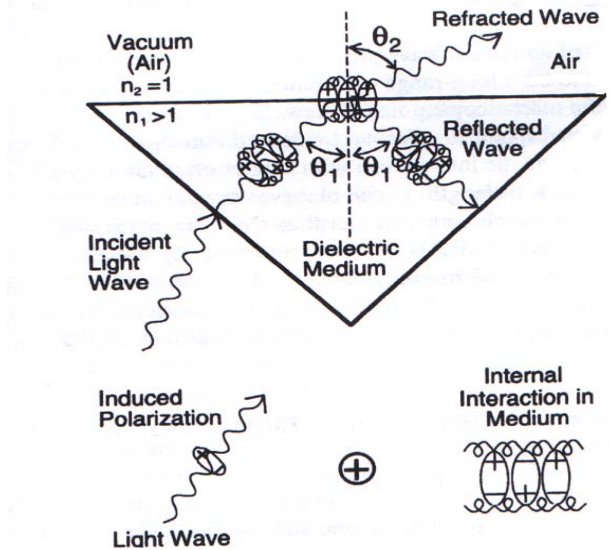
- Time-resolved SPR
- Steady-state SPR
- Fiber SPR
- SPR Imaging

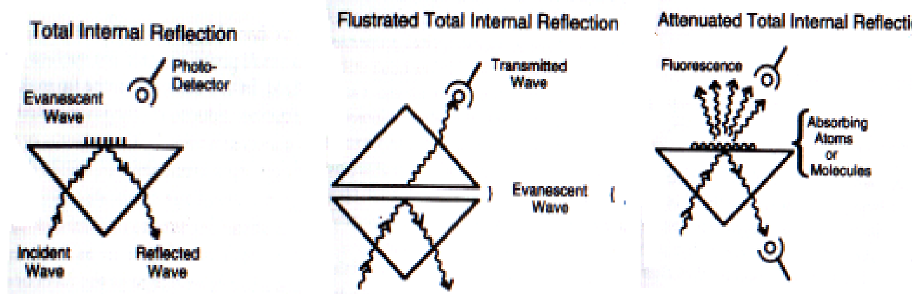
➢ Biomolecules Immobilization
  - Monolayer transfer onto metal film (LB film, lipid vesicles)
  - Assembly techniques
    - surface chemical modification: alkylsilane on hydroxylated surface (SiO_2, Al_2O_3); alkanethiolates on Au, Ag, and Cu; alcohols and amines on Pt; Carboxylic acid on Al and Silver oxide; dextran hydrogel (Pharmacia, BIACore)
    - self-assembly lipid bilayer

SPR Principle - General

Electromagnetic Interaction in a Dielectric System

- Light propagating in a dielectric medium induces polarization in dielectric medium.
- The total energy & momentum transport in the medium in the form of a coupled mode of electromagnetic field with matter.





SPR Principle - General

Light (EM Wave) in a linear homogeneous medium
Governed by Maxwell Equation

$$1.\ \nabla \cdot E = 0 \qquad 3.\ \nabla \times E = -\frac{\partial B}{\partial t}$$

$$2.\ \nabla \cdot B = 0 \qquad 4.\ \nabla \times B = \mu\varepsilon \frac{\partial E}{\partial t}$$

Assume no free charge or free current

Light Propagation velocity (wave function) in medium

$$\nabla^2 E + k_c E = 0 \qquad k_x = \frac{\omega}{c}\left(\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}\right) \qquad k_x = k_x' + ik_x''$$

$$v = \frac{1}{\sqrt{\mu\varepsilon}} \qquad n = \sqrt{\frac{\mu\varepsilon}{\mu_0 \varepsilon_0}}$$

$$c = \frac{1}{\sqrt{\mu_0 \varepsilon_0}} \qquad v = \frac{c}{n}$$

ε :permittivity

μ :permeability

n: refractive index

Theory of SPR – Maxwell eq.

A. Generation of evanescent wave

For a p-polarized wave propagates in the x direction

2: dielectric (z>0)

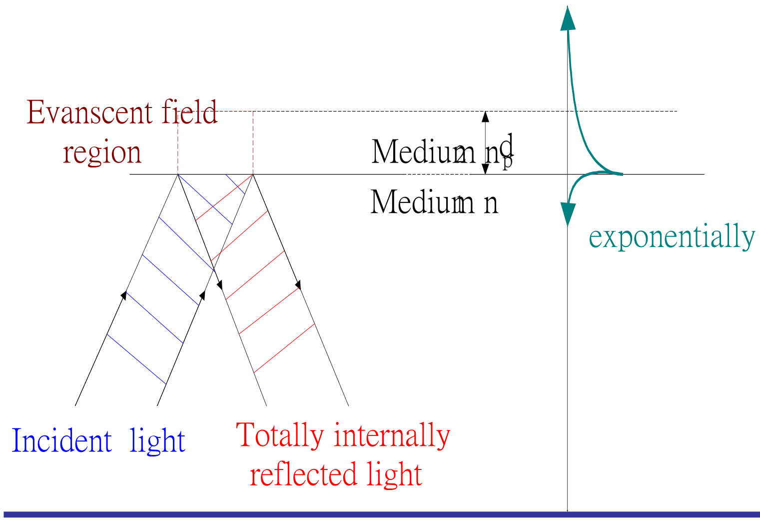
1: metal (z<0)

$$z>0,\quad \overrightarrow{H_2} = [0,\ H_{y2},\ 0]\ e^{i(k_{x2}x + k_{z2}z - \omega t)}$$

$$\overrightarrow{E_2} = [E_{x2},\ 0,\ E_{z2}]\ e^{i(k_{x2}x + k_{z2}z - \omega t)}$$

$$z<0,\quad \overrightarrow{H_1} = [0,\ H_{y1},\ 0]\ e^{i(k_{x1}x - k_{z1}z - \omega t)}$$

$$\overrightarrow{E_1} = [E_{x1},\ 0,\ E_{z1}]\ e^{i(k_{x1}x - k_{z1}z - \omega t)}$$



Maxwell's eqs.

$$\nabla \times \overrightarrow{E_i} = \frac{-1}{c}\frac{\partial \overrightarrow{H_i}}{\partial t} \quad (1)$$

$$\nabla \times \overrightarrow{H_i} = \varepsilon_i \frac{-1}{c}\frac{\partial \overrightarrow{E_i}}{\partial t} \quad (2)$$

$$\nabla \bullet \varepsilon_i \overrightarrow{E_i} = 0 \quad (3)$$

$$\nabla \bullet \overrightarrow{H_i} = 0 \quad (4)$$

Continuity relations (boundary conditions)

$$E_{x1} = E_{x2} \quad (5)$$

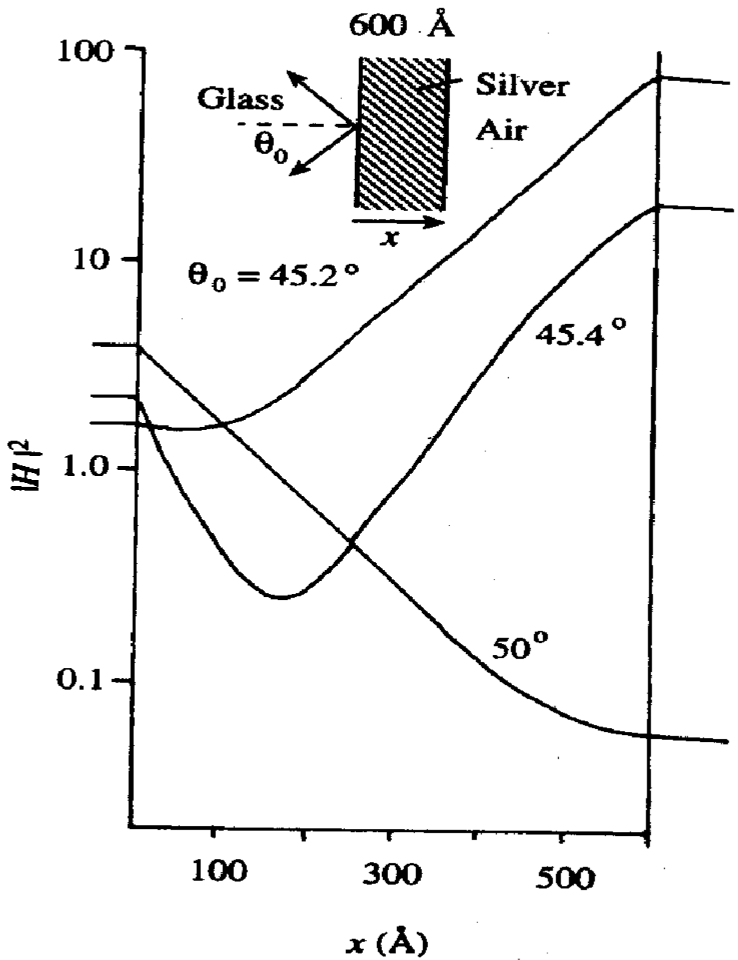
$$H_{y1} = H_{y2} \quad (6)$$

$$\varepsilon_1 E_{z1} = \varepsilon_2 E_{z2} \quad (7)$$

$$(2) \rightarrow \frac{\partial H_{yi}}{\partial z} = -i\,\varepsilon_i \frac{\omega}{c} E_{xi}$$

$$\left.\begin{aligned} \rightarrow\ k_{z1} H_{y1} &= \frac{\omega}{c}\varepsilon_1 E_{x1} \\ k_{z2} H_{y2} &= -\frac{\omega}{c}\varepsilon_2 E_{x2} \end{aligned}\right\} \quad (8)$$

Field intensity within metal



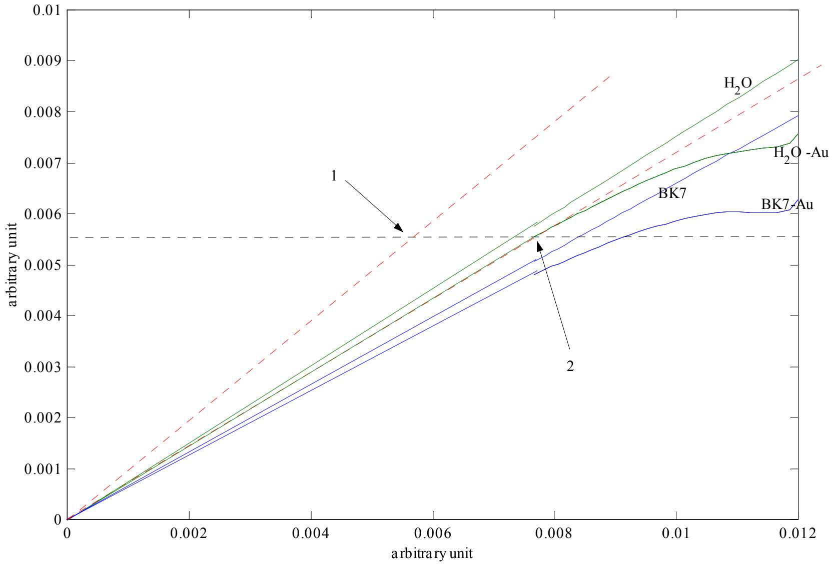
Theory of SPR - Dispersion Relation

substitute (5) for (8) & (6) $\rightarrow$

$$\begin{cases} H_{y1} - H_{y2} = 0 \\ \dfrac{k_{z1}}{\varepsilon_1} H_{y1} + \dfrac{k_{z2}}{\varepsilon_2} H_{y2} = 0 \end{cases}$$

$\therefore$

$$\begin{vmatrix} 1 & -1 \\ \dfrac{k_{z1}}{\varepsilon_1} & \dfrac{k_{z2}}{\varepsilon_2} \end{vmatrix} = 0$$



Do the same procedure from (1) & combine the above result, one ca

$$k_{zi} = \sqrt{\varepsilon_i \left(\frac{\omega}{c}\right)^2 - k_x^{\ 2}} \qquad (9)$$

$$\boxed{k_x = \frac{\omega}{c} \sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}}}$$

dispersion relation

Theory of SPR - Penetration Depth

From (9) k_z is imaginary $\therefore$ $k_z = |k_z|\,i$

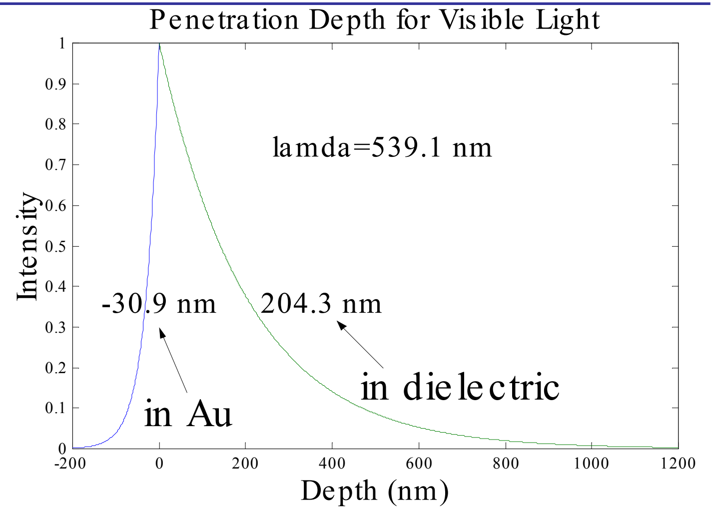
$\because$ $E = E_0\, e^{i(k_{x1}x - k_{z1}z - \omega t)}$

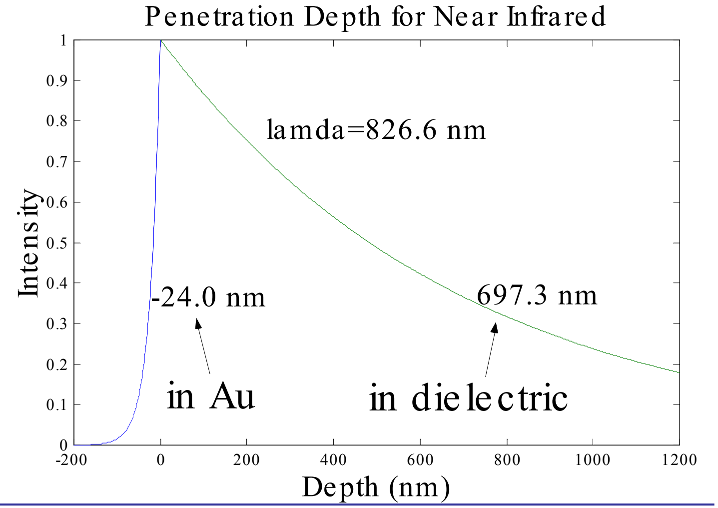
$\therefore$ $E = E_0\, e^{-|k_z||z|}$, exponential decay

penetration depth in metal : $\dfrac{1}{|k_{z1}|} = \left| \dfrac{\lambda}{2\pi} \sqrt{\dfrac{\varepsilon_1' + \varepsilon_2}{\varepsilon_1'^2}} \right|$

penetration depth in dielectric : $\dfrac{1}{|k_{z2}|} = \left| \dfrac{\lambda}{2\pi} \sqrt{\dfrac{\varepsilon_1' + \varepsilon_2}{\varepsilon_2^2}} \right|$

where the dielectric function of the metal $\varepsilon_1 = \varepsilon_1' + i\ \varepsilon_1''$





SPR spectrum: R vs theta & R vs lamda

B. Excitation of Surface Plasmons by Light

ATR Coupler

momentum : $\frac{\hbar\omega}{c} \longrightarrow \frac{\hbar\omega}{c}\sqrt{\varepsilon_0}$

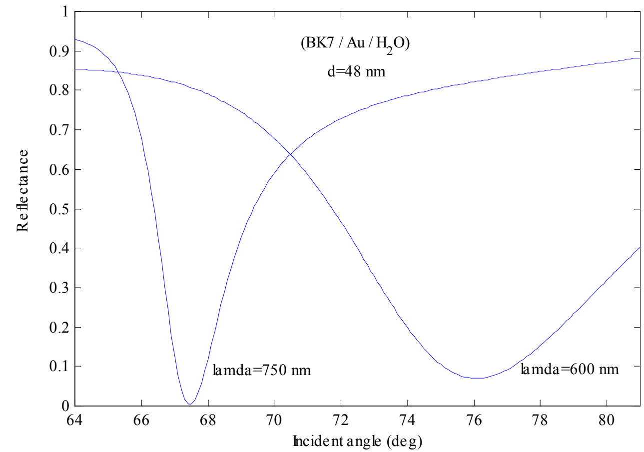
$k_x = \sqrt{\varepsilon_0}\,\frac{\omega}{c}\sin\theta$ (θ :incident angle)

From Maxwell's eqs & Boundary Conditions

for a p-polarized light:

$r_{ik} = (k_{zi} / \varepsilon_i - k_{zk} / \varepsilon_k) / (k_{zi} / \varepsilon_i + k_{zk} / \varepsilon_k)$

[Fresnel's equations]

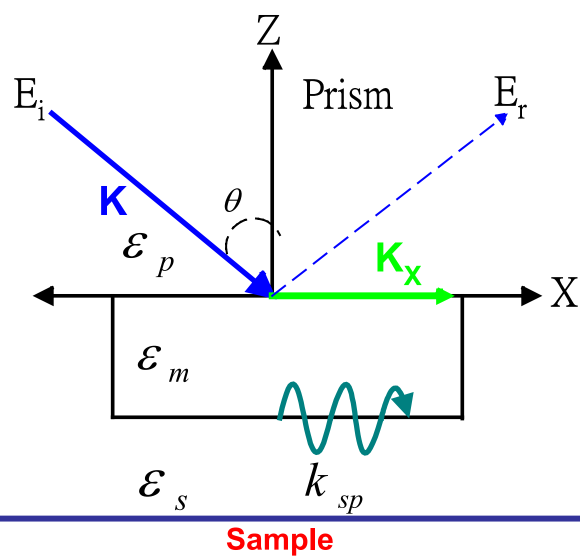


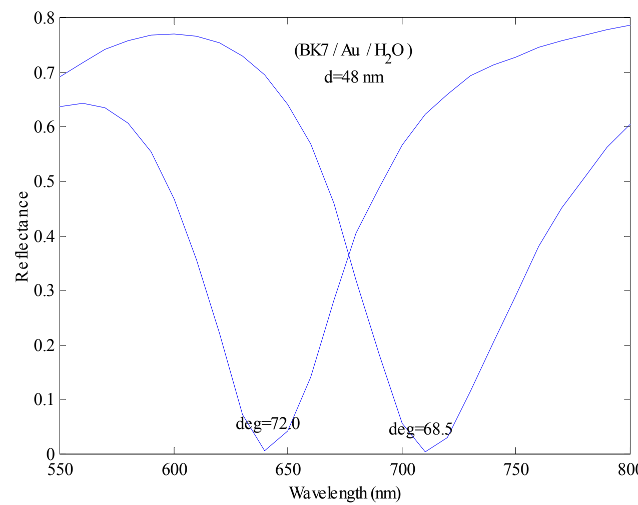
$r_{012} = \frac{E_r}{E_0} = (r_{01} + r_{12}\, e^{2i\alpha})/(1 + r_{01}\, r_{12}\, e^{2i\alpha})$

$\alpha = k_{z1} \cdot d_1$

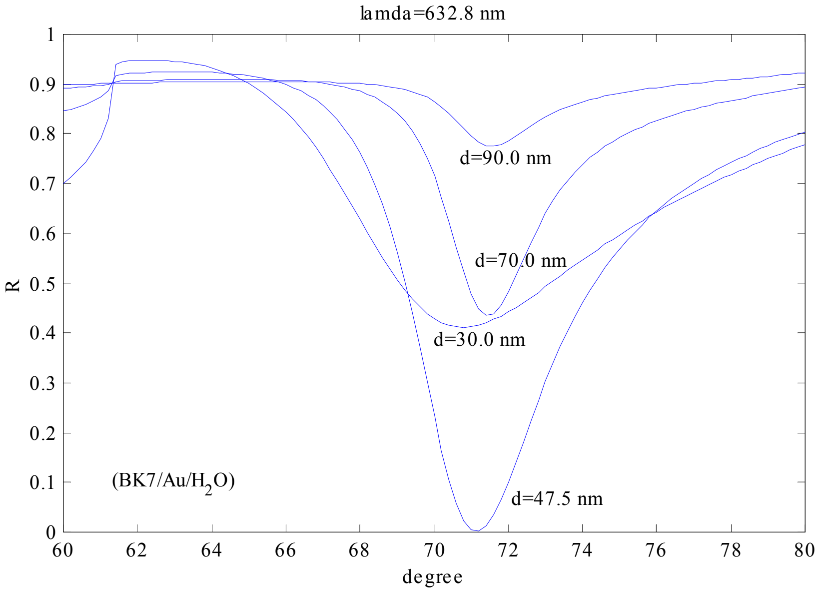
d1 : thickness of the metal film

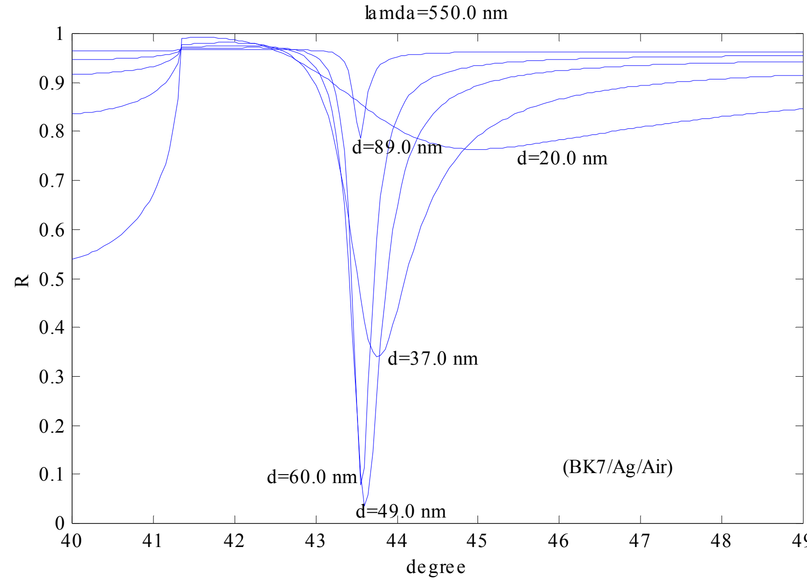
$R = |r_{012}|^2$



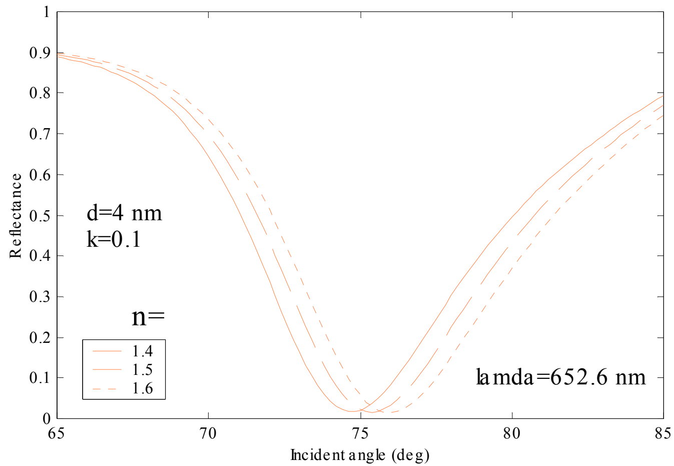


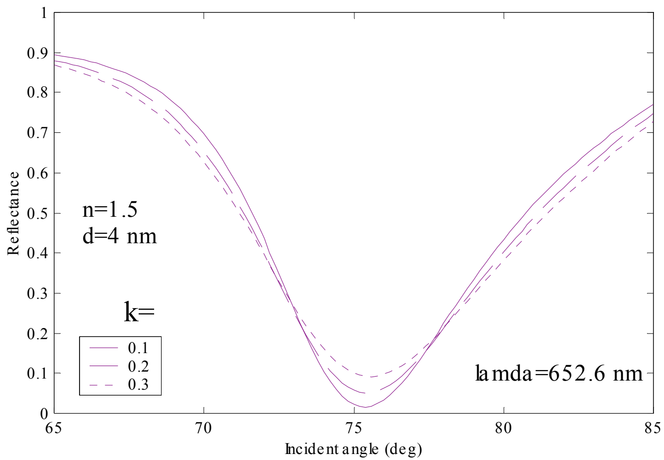
SPR spectrum : different metal

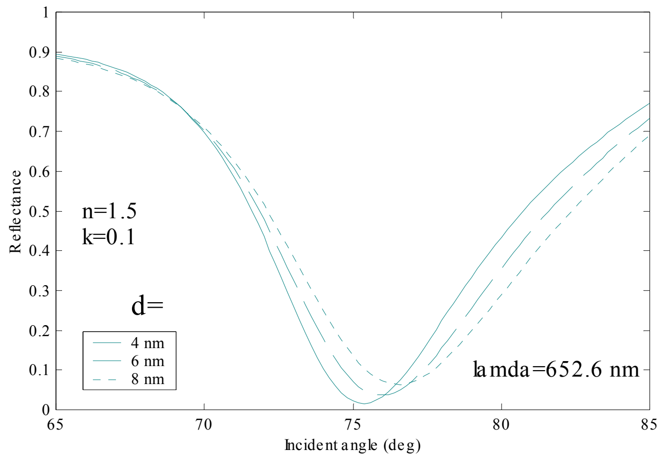




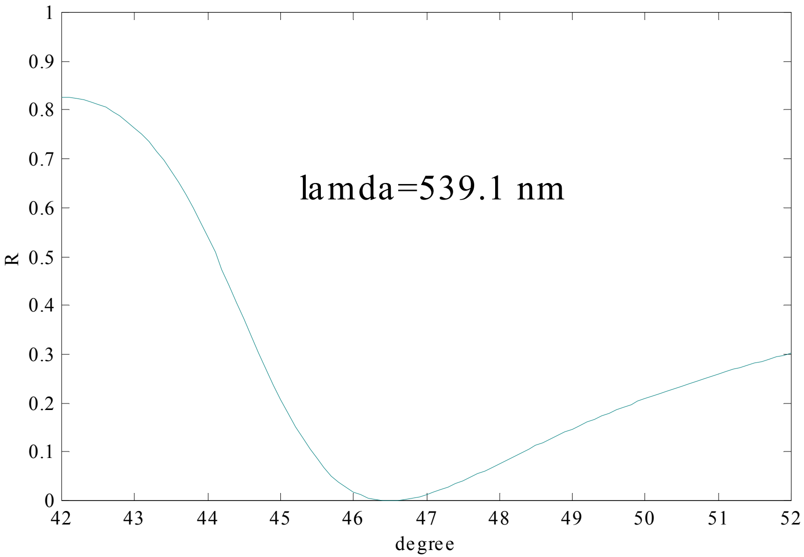
SPR Spectrum : different n, k, d

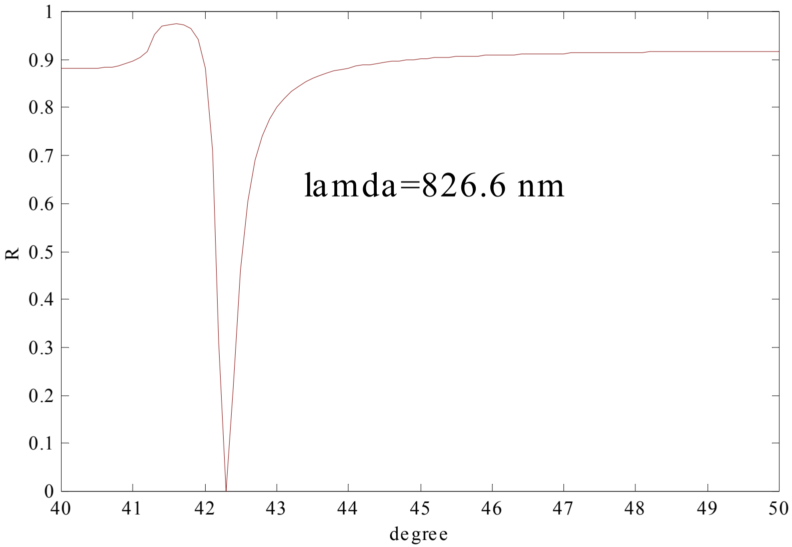




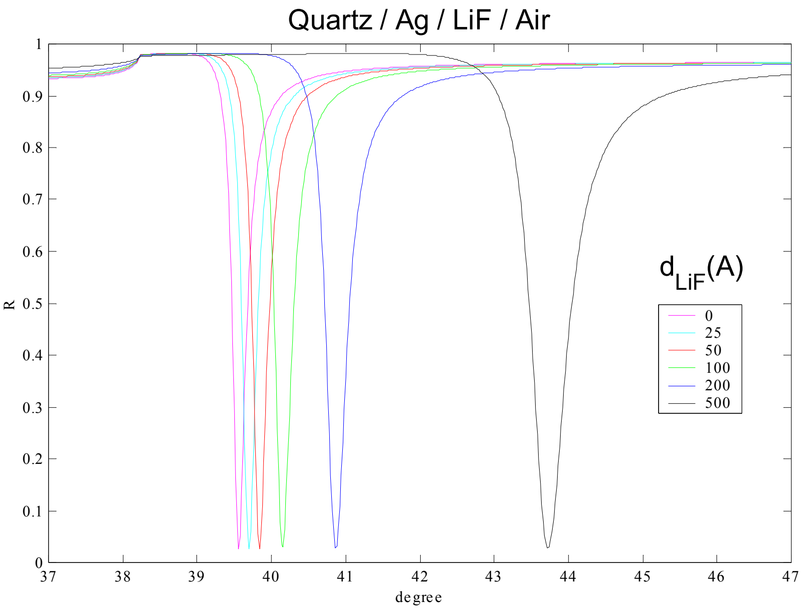


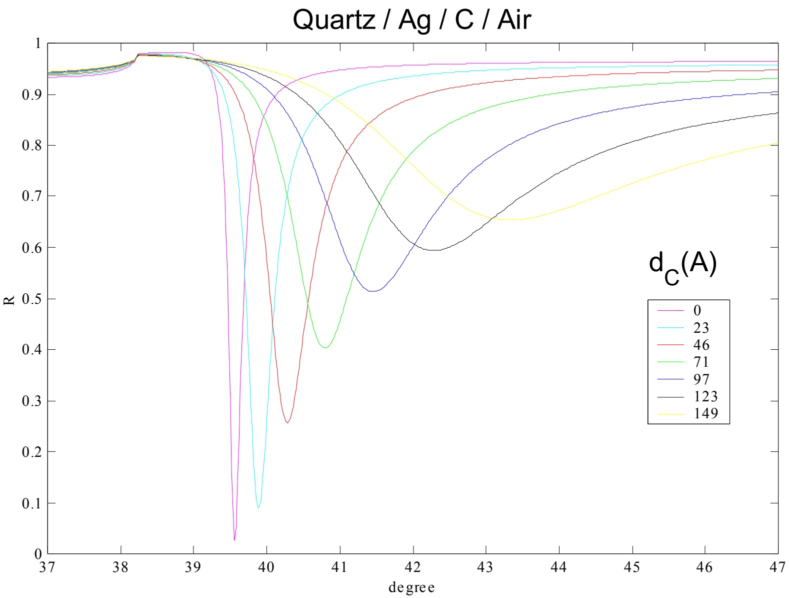
SPR Spectrum : VISIBLE vs NIR



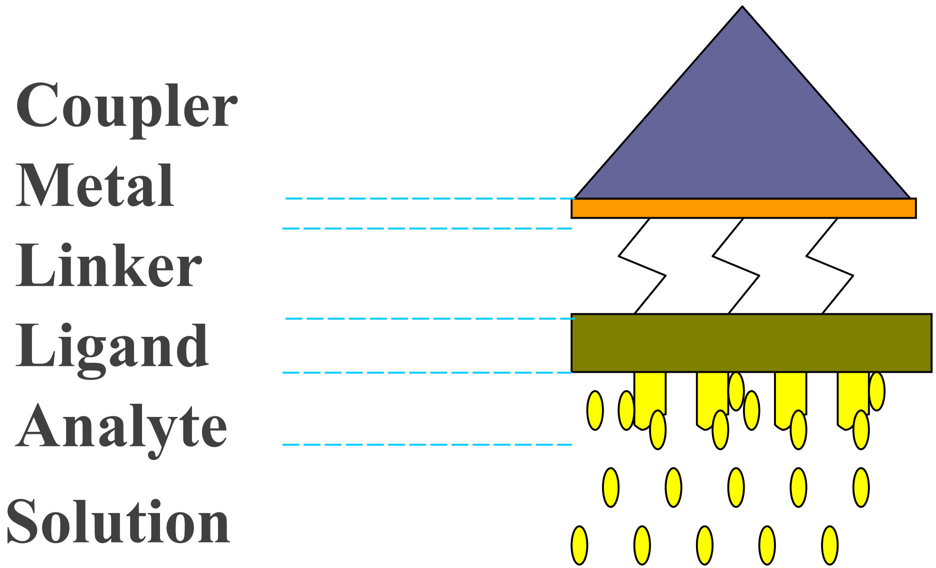


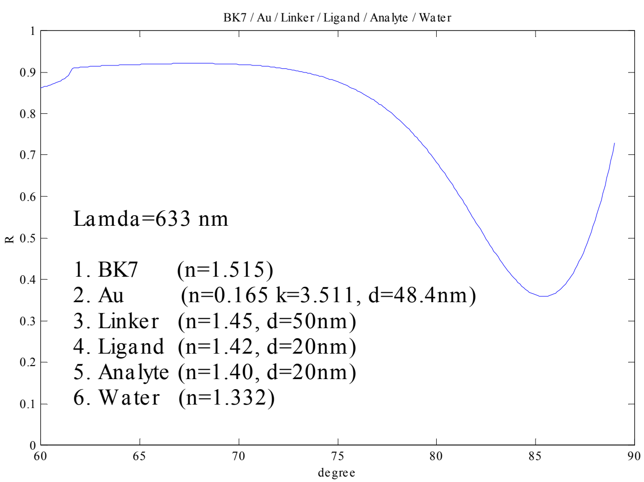
4 Layers : Transparent vs Absorbing

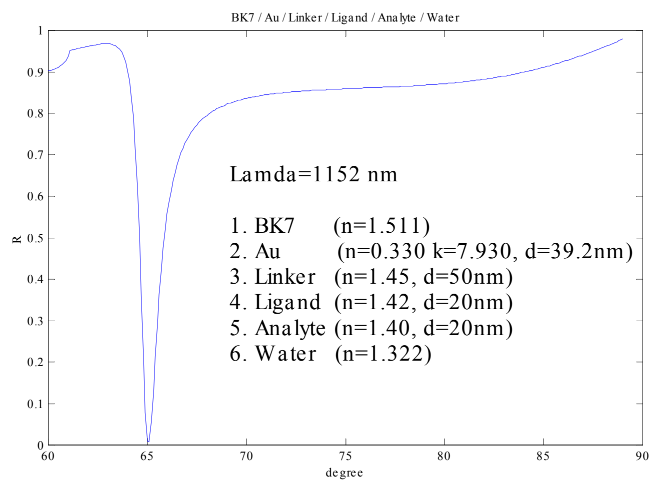




6 Layers : Biochip configuration







Surface Plasmon Resonance (SPR)

The *n*, *t*, and *k* values of a dielectrical layer (e.g. proteins) contain information about the amount (mass) of material in the deposited layer. This provides the means for a measurement of the binding parameters of interacting biological molecules, and together with the thickness of the layer, allows an evaluation of the structural arrangement of the molecules which form the film.

(Estimated error $\Delta n = \pm\ 0.02$; $\Delta t = \pm\ 1$; $\Delta k = \pm\ 0.02$)

Volume Mass Density $\quad d = M / A[(n_{av}^2 - 1)/(n_{av}^2 + 2)] \quad n_{av}^2 = (n_p^2 + 2n_s^2)/3$

Surface Mass Density $\quad m = dt = 0.1M / At[(n_{av}^2 - 1)/(n_{av}^2 + 2)]$

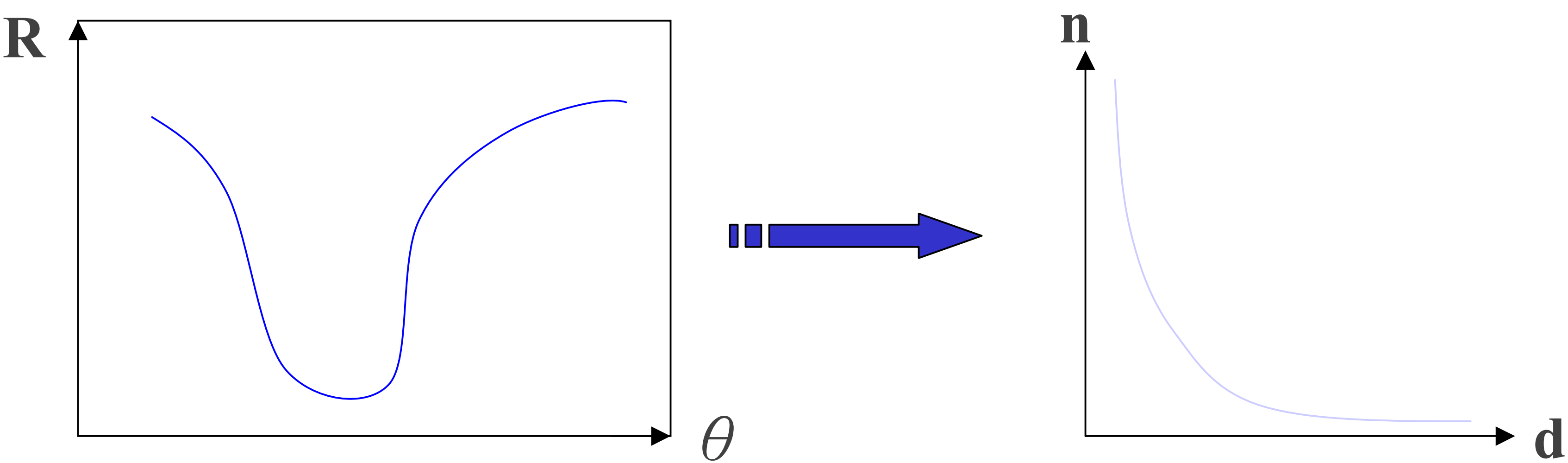
Thickness, t (nm) $\quad k = \beta(c\lambda / 4\pi)$

Heterogeneous mixtures (Lorentz-Lorenz relation)

$$m_p = 0.3tf(n)(n - n_b)/[A_p / M_p - V_p(n_b^2 - 1)/(n_b^2 + 2$$

$$f(n) = (n + n_b)/(n^2 + 2)(n_b^2 + 2)$$

Spectrum Ambiguity



Not unique (n, d) for Analyte Layer

To Solve the problem:

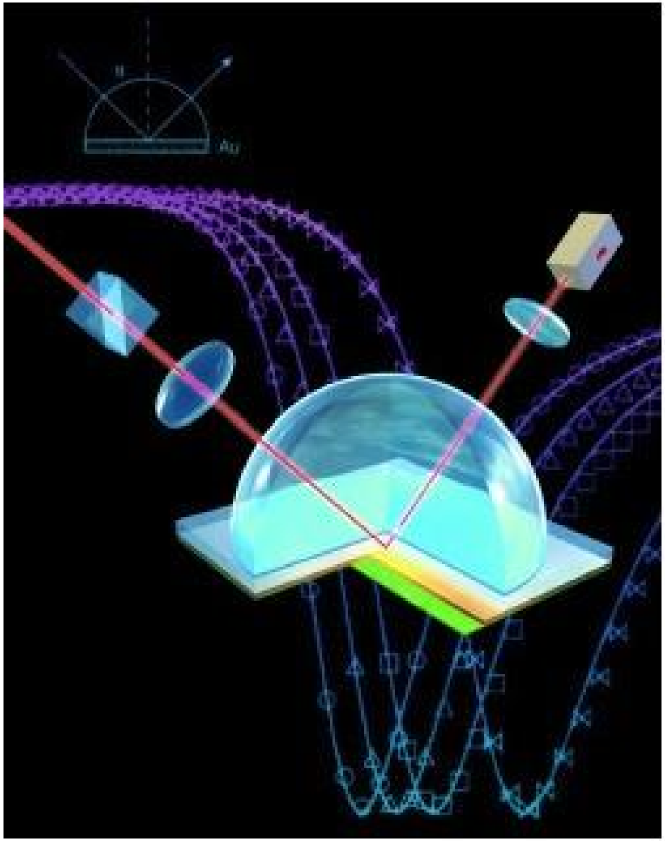
1. Multi-solvent approach
2. Two-color spectroscopy

Near-Infrared Surface Plasmon Resonance Measurements of Ultrathin Films

Angle Shift and SPR Imaging Experiments

Analytical Chemistry.1999,71,3928-3934

Bryce P. Nelson, Anthony G. Frutos,
Jennifer M. Brockman,and Robert M. Corn
Department of Chemistry,
University of Wisconsin-Madison



Application of Surface Plasmon in Microscopic Technique-Surface Plasmon Microscopy(SPM)

Advantages: improve resolution in

1.low-contrast thin-film samples(such as monolayer molecule)

2.low index variations

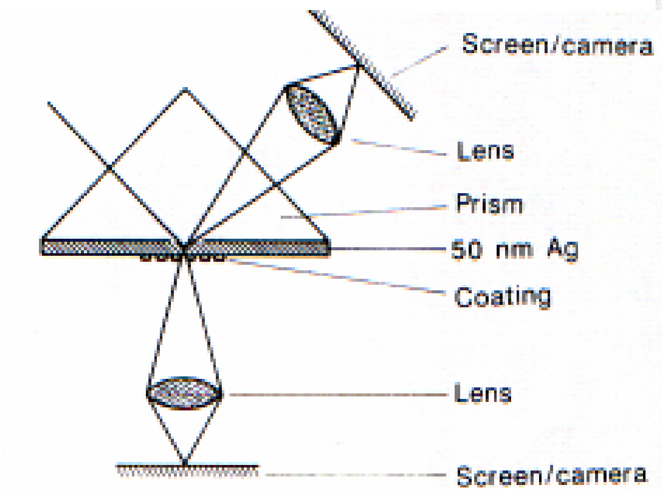


Fig. 1 Schematic of the optical set-up of SPM. Not to scale.

Knoll(1989)

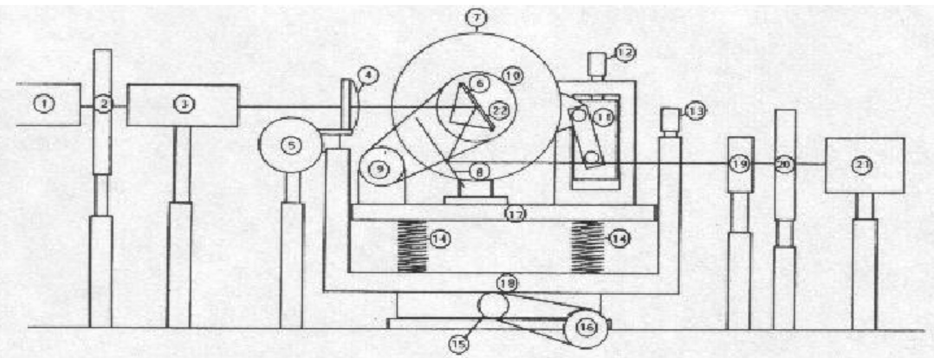
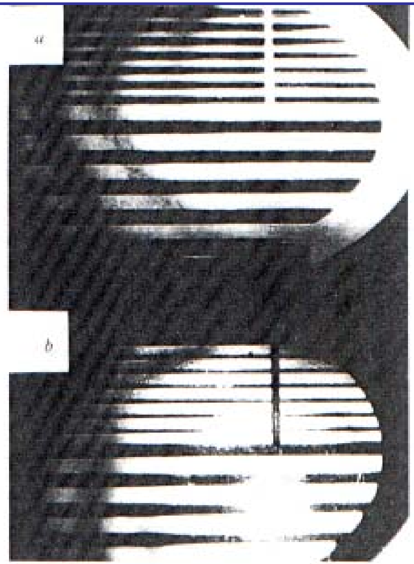
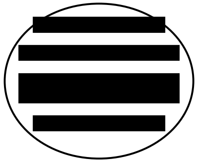


Figure 11. Apparatus used for scanned surface plasmon measurements, with components as labelled below:

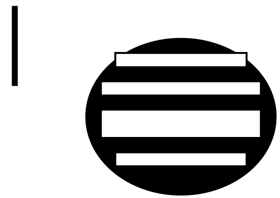
1. HeNe laser (1 mw).
2. Polarizer.
3. Beam expander.
4. Focussing lens.
5. Focus control.
6. Prism.
7. Rotating platform.
8. Mirror.
9. Servo potentiometer.
10. Drum for potentiometer belt.
11. Rotation control link mechanism.
12. Rotation control micrometer.
13. Vertical translation micrometer.
14. Support springs.
15. Horizontal translation micrometer.
16. Servo potentiometer.
17. Vertically translating platform.
18. Horizontally translating cradle.
19. Lens.
20. Polarizer.
21. Photodetector.
22. Ag film on glass slide.

Surface Plasmon Microscopy Image





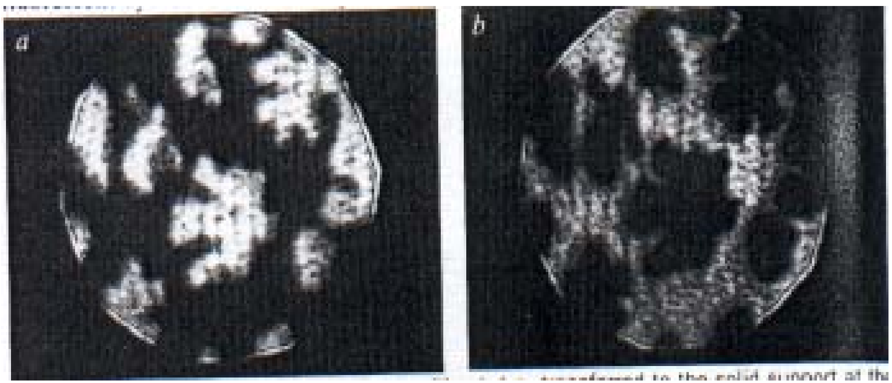
bare silver surface



SiOx coating

Monolayer of DMPA(dimyristoylphosphatidic acid)

47.20° 47.70°



Fluorescence microscop

Surface Plasmon Resonance Imaging System

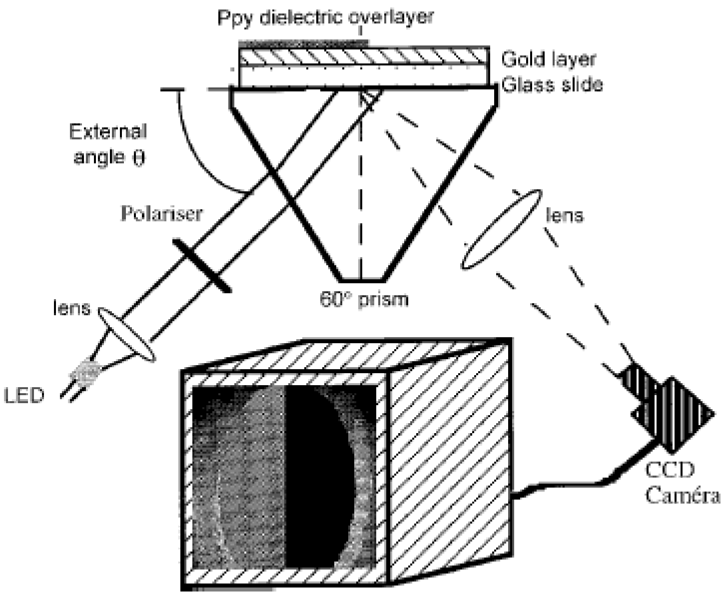


Figure 2. Schematic representation of the SPR imaging device. A collimated, p-polarized LED beam illuminates the sensor surface via a coupling prism ($n = 1.7170 \pm 5 \times 10^{-4}$ at $\lambda = 633$ nm). Reflected light, which contains all of the SPR response information is imaged on a CCD camera. The example shows on the monitor, at a fixed angle, a great contrast due to plasmonic enhancement. The angle of the resonance is adjusted for the bare gold layer giving a dark field, whereas at the same angle, the reflectivity of the dielectric coating has reached a higher gray-level depending on the optical thickness of the film.

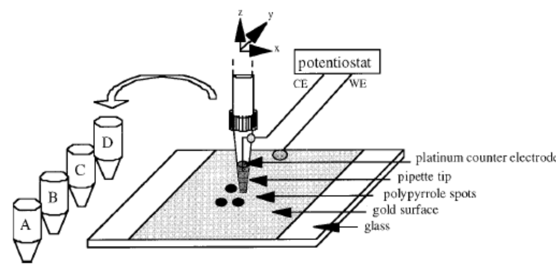


Figure 1. General scheme of the polypyrrole electrospotting methodology: The different tubes A–D containing different pyrrole–ODN and pyrrole monomer solutions are on the left. The spotting is carried out on the gold surface via the plastic tip containing the solution to be copolymerized.

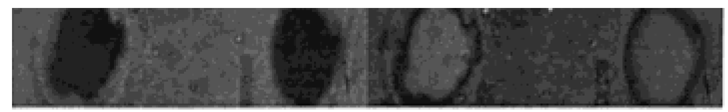
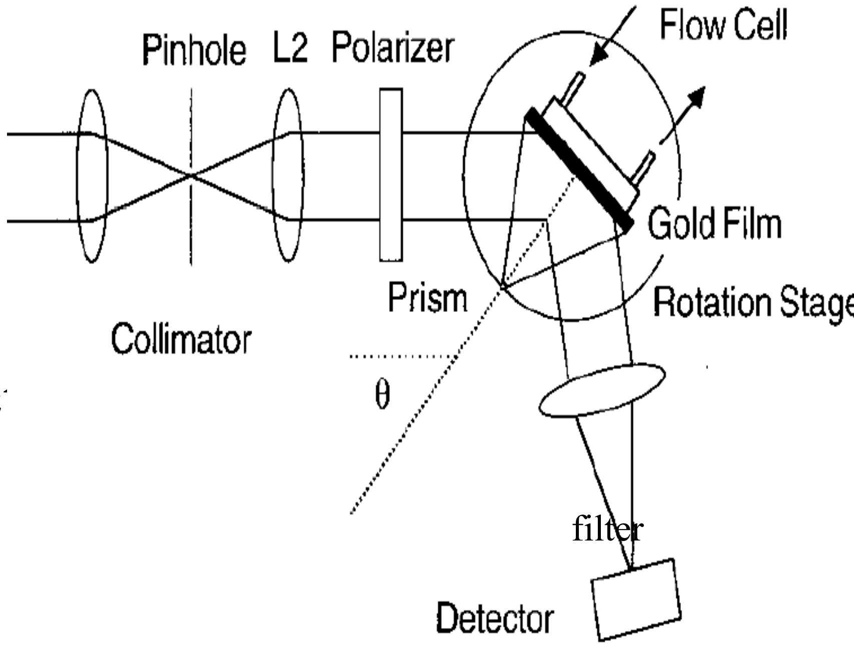


Figure 3. Pictures of the four-element sensor: (a) at the angle of resonance for the four spots, giving the minimum of reflectivity and (b) at the angle at which the kinetics are monitored. Gold gray level appears in the background of the pictures. Dark rings are revealed on the periphery of each spot.

Anal. Chem. 2000,72,6003-6009

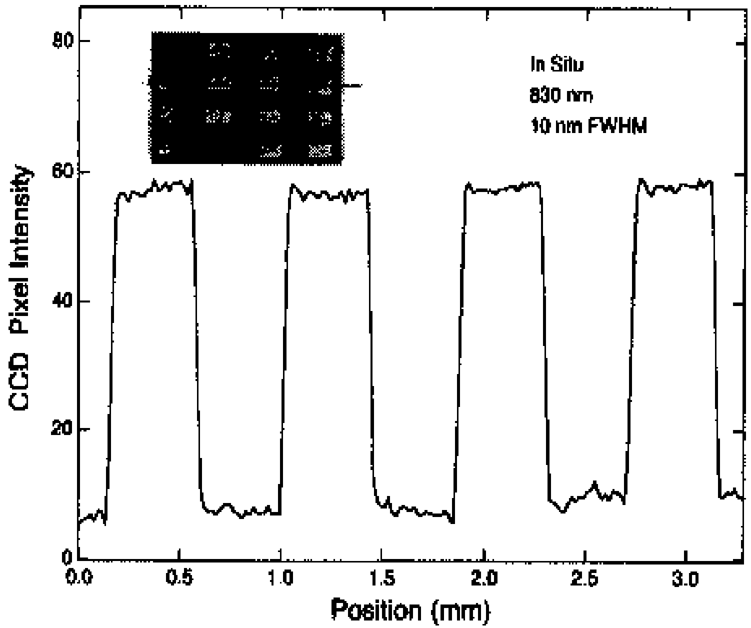
SPR Imaging Measurements Why NIR ?

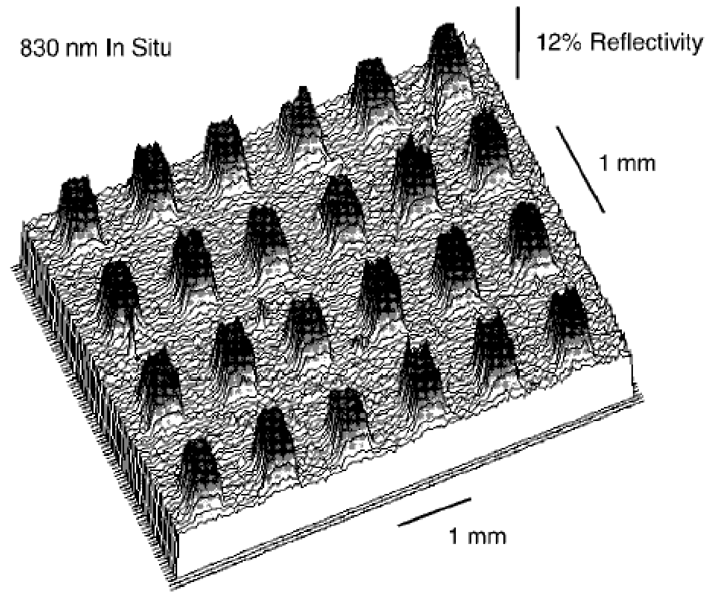
- Incoherent Light Source avoid laser fringe
- NIR white light/interference filter increase reflective light intensity SN ratio
- Better Image Contrast due to increase light intensity
- Fixed incident angle and detec reflective intensity by CCD camera
- Differential adsorption measurement on DNA/biopolymer arrays attached on gold surface



SPR Imaging Measurements

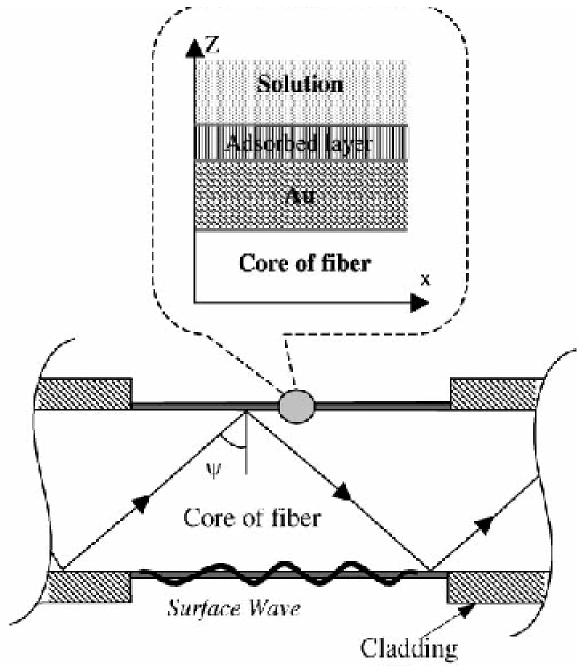
single-stranded DNA binding protein adsorption

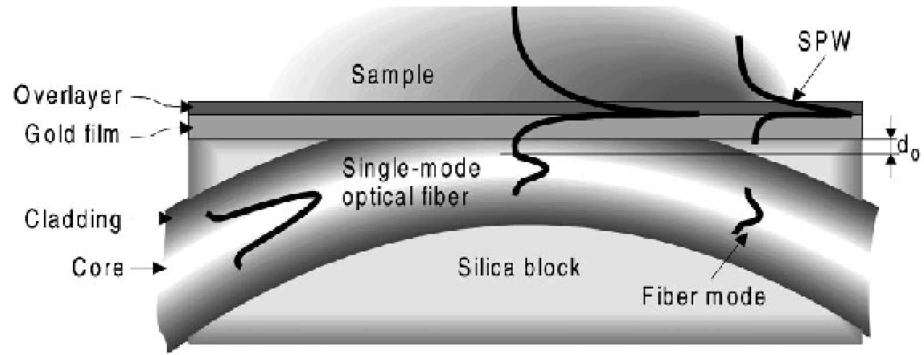




Fiber Optic SPR Sensor-
A Novel Stride in Sensor Design

From Chip-Based to Optical Fiber-Based

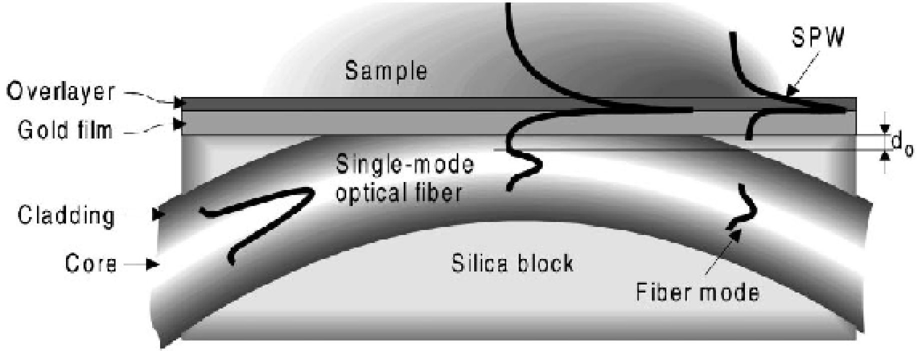


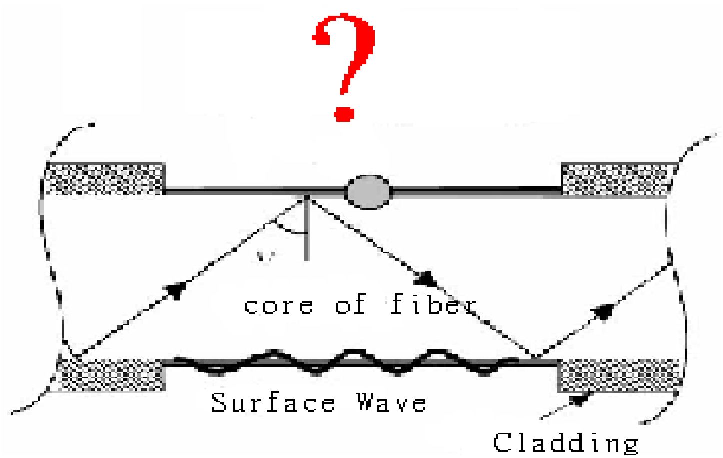


- ❖Portability
- ❖Flexibility
- ❖Rapid Analyses
- ❖Interchangeable

Fiber Optic SPR Sensor

- Analogy to Planar Waveguide Device





Fiber Optic SPR Sensor
Theoretical Analysis

J. Homola , R. Slavik(1996)

- Planar Waveguide Approach & Transfer Matrix Method
- Numerical Complex-Zero-Finding Procedure
- Calculate Complex Propagation Constant

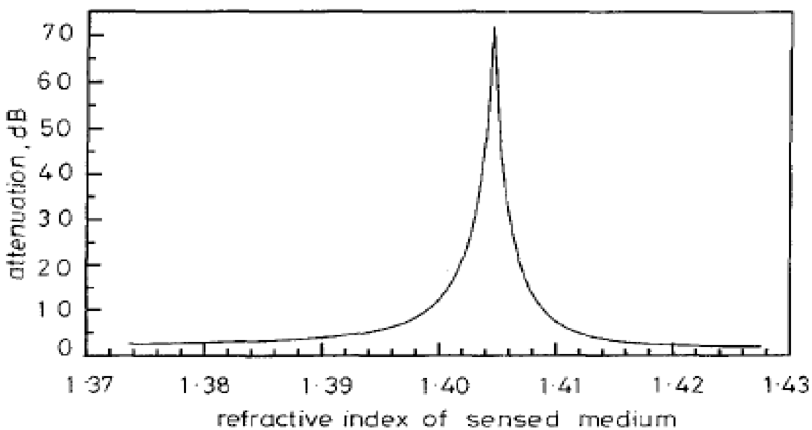
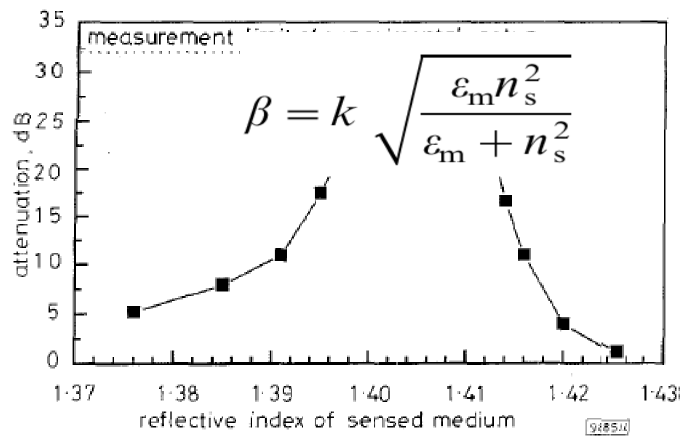


Fig. 2 *Dependence of fibre mode attenuation on refractive index of sensed medium*



$$\beta = k\sqrt{\frac{\varepsilon_m n_s^2}{\varepsilon_m + n_s^2}}$$

Fig. 1 *Measured mode attenuation against refractive index of sensed medium*

Simulation Result

Experiment Result

Fiber Optic SPR Sensor
Design Consideration

- Optic Fiber Consideration

  Fiber material-glass fiber , HiBi fiber

  Single mode or Multimode-

- Sensor Geometry Design
  - Residual cladding depth(d_0)-
  - Polish Side-
  - Tip angle-resonant angle?
  - Metal film thickness

  45-75nm