光學奈/微米檢測於生醫之應用

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Contents

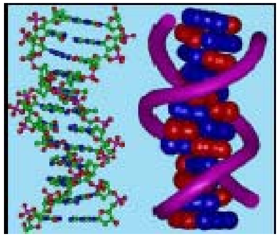
- From Micro to Nano-BioMed
- Why optics
- Micro scale - Optical Coherent Tomograph for optical imaging of Biological tissues
- Nano scale - Surface Plasmon Resonance for Biosensing

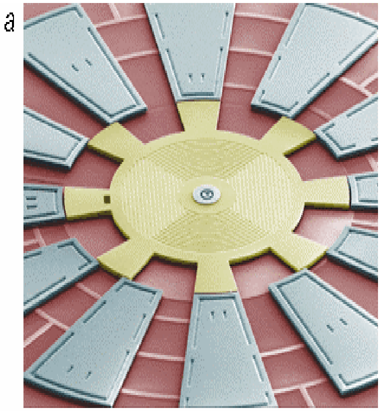
MEMS and Nano History

There's Plenty of Room at the Bottom

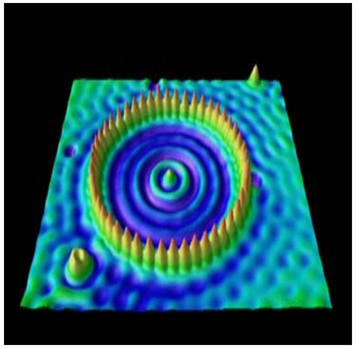
by Dr. Richard P. Feynman at Cal Tech, Pasadena, CA on Dec. 26, 1959

- Miniaturizing devices (information storage, computation, motor)
- Evaporation and lithograph
- Parallel microfabrication by a hundred tiny hands
  - **MEMS technology**
- Rearranging the atoms
- The marvelous biological system (DNA, RNA, Protein, Amino Acid, etc. for information processing, computation)
  - **Nanotechnology, Bio-Nano**





By TI, M Mehregany



by IBM 1993

MEMS and Nano History

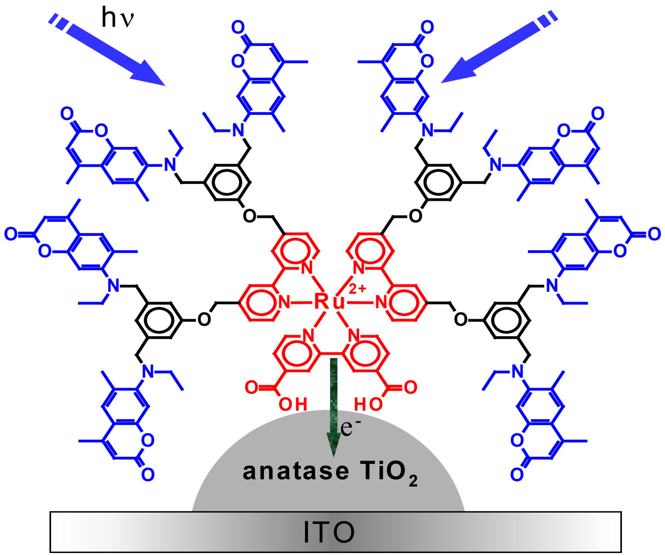
When we get to the very, very small world---say circuits of seven atoms---we have a lot of new things that would happen that represent completely new opportunities for design. Atoms on a small scale behave like nothing on a large scale, for they satisfy the laws of quantum mechanics.

- Dr. Richard P. Feynman

Infinitesimal Machinery

by Dr. Richard P. Feynman at JPL, Pasadena, CA on Feb. 23, 1983

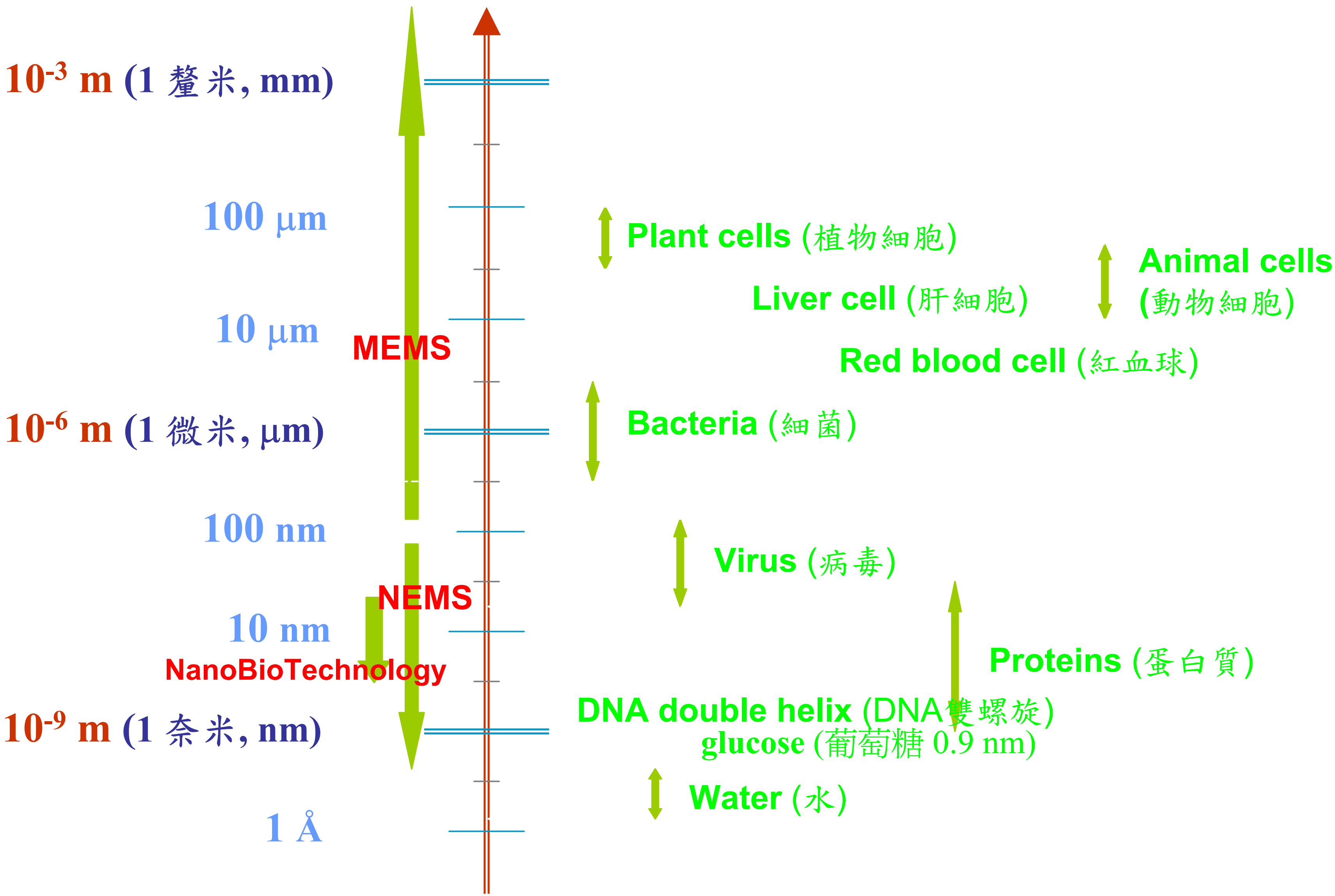
- Make, Use, and Power "Swallowable surgeon"
  - Electrostatic actuation
  - Electromagnetic field
  - Mobile microrobots powered by ATP or Optics (Autonomous machine for cellular operations or
- Friction, Sticking and Shaking of atoms
- Microfabrication by casting or by imprecise tools
- Quantum computation



© J. Fréchet, Berkeley, 2001

- Biology is a guide (but not a perfect guide)
  - Various form changes by applying electrical field, which affect viscosity of fluid.

Size and Technology



Hierarchical structure

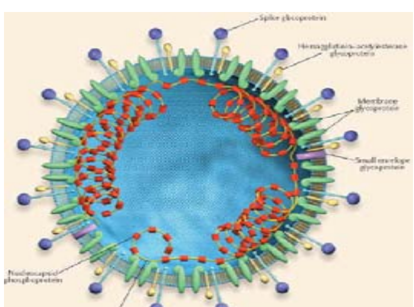
Small molecules
Amino acids
Nucleic acids
Water
Lipids
Ions

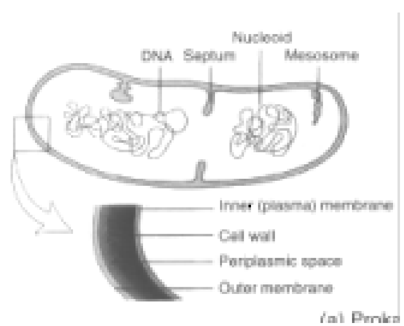
Self assembly to form DNA, RNA and Proteins

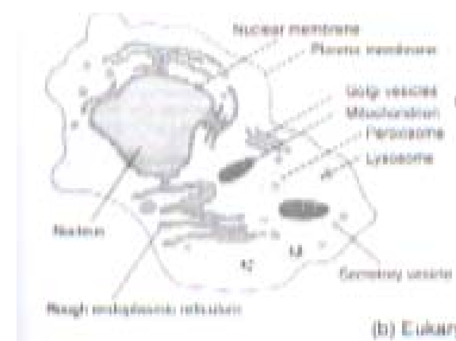
Virus
Size: 20-200 nm
Structure: Single or double strand RNA or DNA
Function: Infectious but not self-replicating

Prokaryotic cells: Bacteria
Size: 1 - 10 um
Structure: Single-cell organism consisting of single closed compartment that lacks a defined nucleus
Function: Free-living and self-replicating

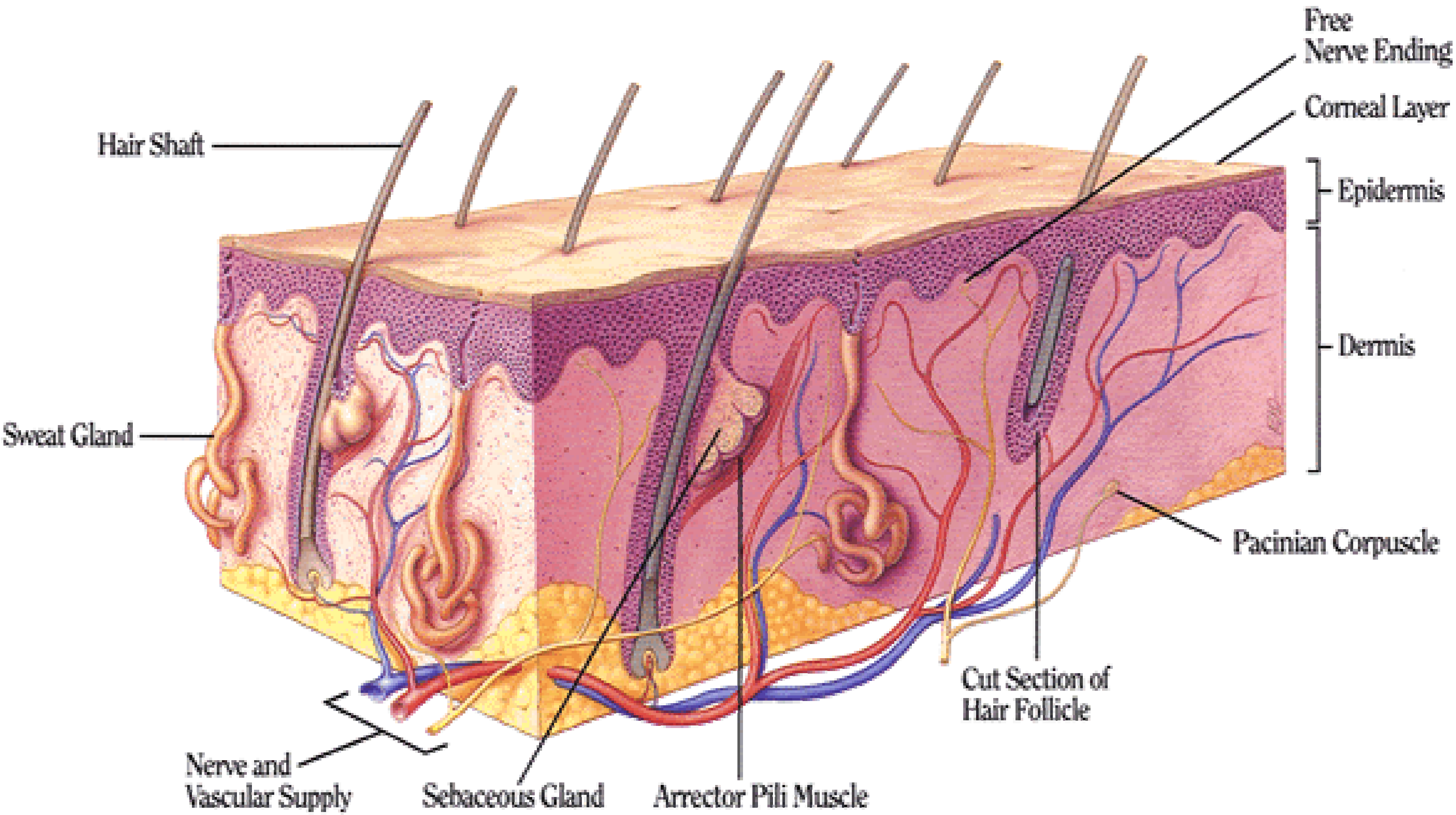
Eukaryotic cells: Animal/plant cells
Size: 10 -100 um
Structure: Complex structure surrounded by a lipid membrane, contains an organized nuclear structure
Function: Self-replicating and able to assemble to for tissues



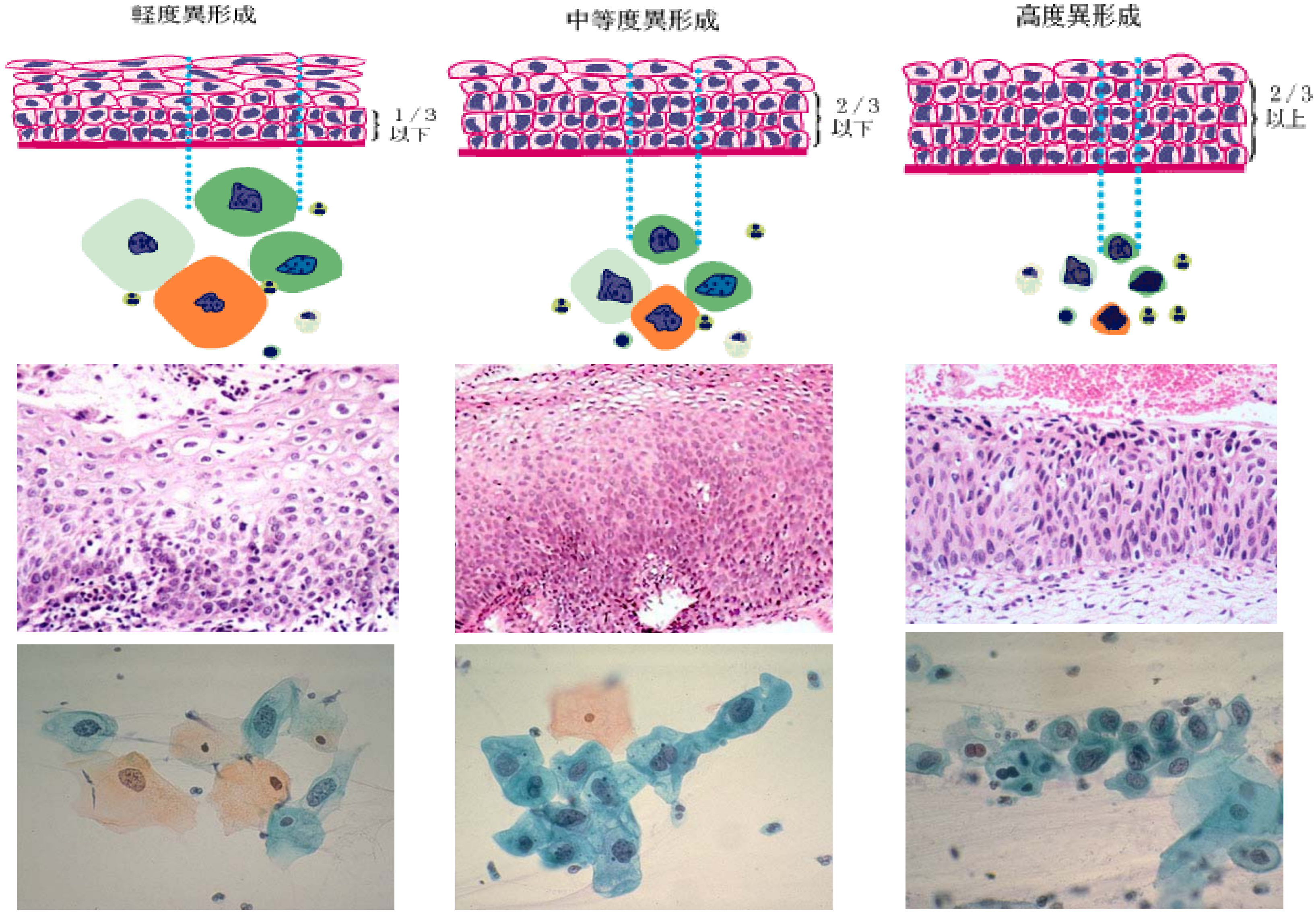




Biological Structure - Skin

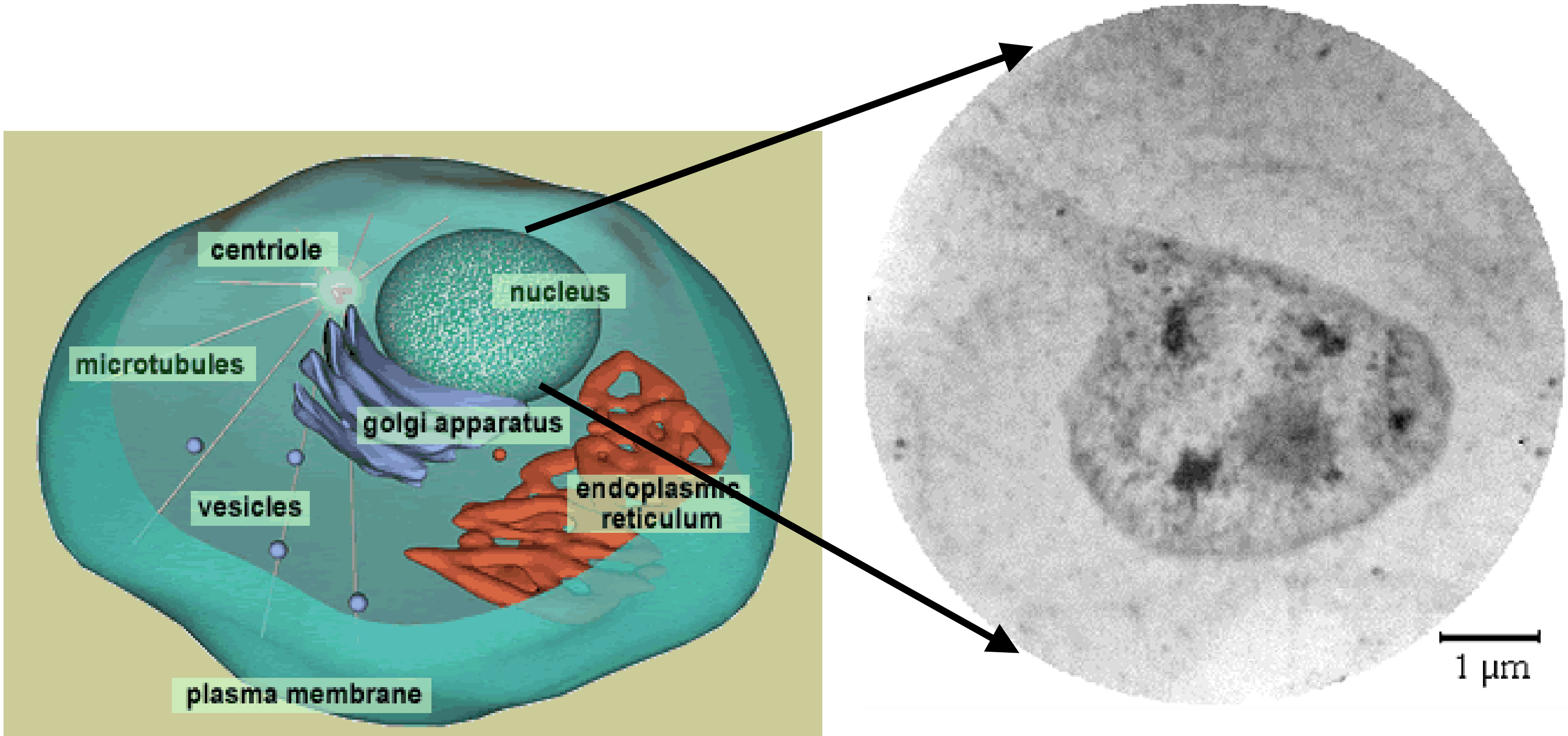


Carcinoma *in situ*



Source - www2.plala.or.jp

Cell Nuclei



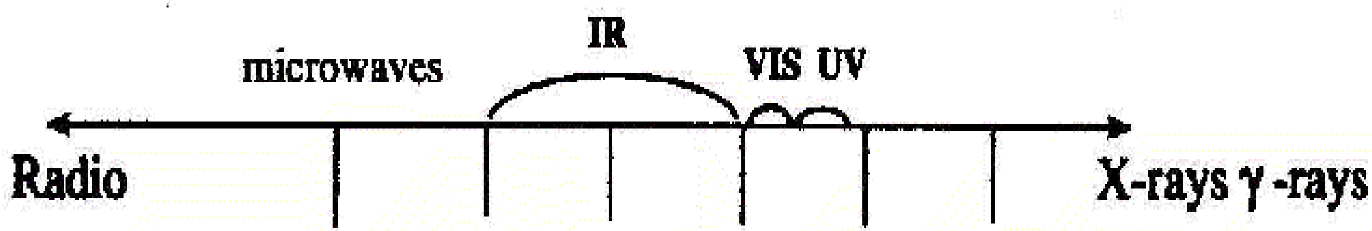
Hydrated mammary epithelial cell nuclei, For the x-ray images

Why Optics?

Optical methods

- Wide spectral range for parallel measurement
- Varieties of spectroscopic methods available for molecules (nano) and cells-tissues (micro) characterization
- Non-ionization radiation
- Non-invasive

Dimensions



	cm	1	0.1	0.01			
λ	µm	1000	100	10	1	0.1	0.01
	nm				1000	100	10
ν	Hz	3×10^{11}			3×10^{14}		3×10^{16}

UV	190 - 400 nm
Visible	400 - 800 nm
IR	2.5 - 16 µm

Energy

Light can be defined as the electromagnetic spectrum in the frequency range of 10^{11} (far infared) to 10^{17} (far ultraviolet). The energy of a single photon is given by, E=hv in J or eV, where h is Planck's constant=6.6261×10^{-34} J · s = 4.1361×10^{-14} eV · S and υ is the frequency of light in Hz, and the wavelength of the light, λ, is given as nm, where c is the speed of light in vacuum = 2.99792458×10^{8} m/s.

Mean lifetime

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

UV-VIS absorption
Fluorescence and phosphorescence emission
Bioluminescence
Chemiluminescence
Internal reflection spectroscopy
Laser light scattering

Ref: G.R. Van Hecke, K.K. Karukstis, "A Guide to Lasers in Chemistry", Jones & Bartlett

Force

The relativistic mass of a photon:

Photon mass at rest is zero. However, when it at speed of light, the mass can be calculated as: $E = m \cdot C^2 \Rightarrow m = E / C^2 = (h/\lambda) \cdot C$

So, for a green light quantum (l = 500 nm, m = 4x10-33 g). This is the first working principle of lasers cooling and of optical tweezers.

The force of the action comes from the translational momentum, T and speed of photon, C.

$$T = m \cdot C = E / C = F \cdot t \qquad F = E /(C \cdot t) = W / C$$

The light pressure P (F/A) $P = F / A = (W / AC) = I / C$

For the case of solvent with refractive index, n , the above equations need to be corrected by multiplying a constant, n.

The Electric fields are also very large, for 1000 W/cm2, E = 28×10^7 V/cm

Force

Example: The intensity of sun at the Earth's surface is about 1350 W/m2 (1 W = 1 J/s, 1 J = 1 Nm). Thus, the pressure exert by sun is P = I/C = 1350 (Nm/s)/m^2/$3x10^8$ m/s = $0.45x10^{-5}$ N/m2. The force, which exert on a $5m^2$ car's area is about PxA, F=$2.25x10^{-6}$ N and about $0.45x10^{-5}$ N/m^2 x $115x10^{12}$ m2 = 500000 N on the surface of Earth. For a 1 W laser, it can exert a force F= W/C = $3.3x10^{-9}$ N. This force can accelerate a bacterium of 1 fmtoliter or a mass of 1 picogram over a short distance. With 1 N = 10^5 g cm/s2, the acceleration a = $3.3x10^9$ N/10^{-12} g = $3.3x10^8$ cm/s2.

This is for a particle that is larger than the wavelength of light (Mie scattering). For a very small particle (Rayleigh scattering), the force will be much smaller.

For dielectric spheres, e.g. cells, gradient force will experience both axial and transversal gradient forces, which pull against the pressure of light into focus. By experiments, one can find out the axial and lateral forces, normally expressed as a percentage of calculated light force (0.4% - 10% for axial force, 13% - 41% for lateral force).

Optics

Cover both micro & nano!

Modes of Interactions

- Absorption
- Scattering
- Reflection
- Refraction
- Polarization
- Interference
- Diffraction

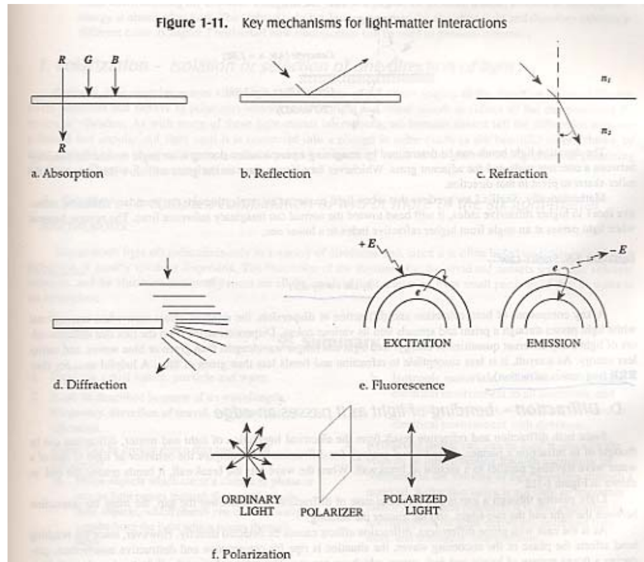
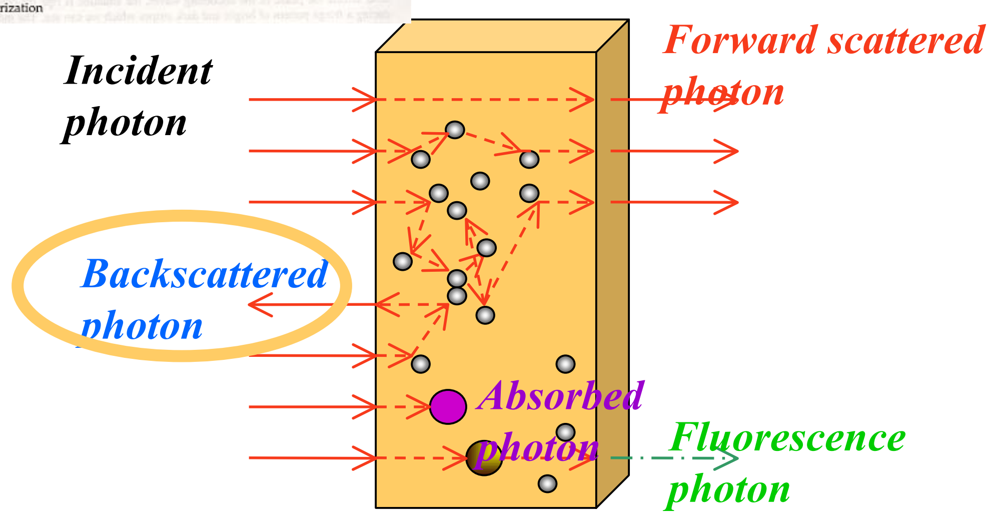


Figure 1-11. Key mechanisms for light-matter interactions



Absorption

通過一物體的光強度低於入射強度時，稱為光被該物體(的物質)吸收了。為了與微觀吸收有所區別，必要時稱它為宏觀吸收。一般光束照射下，該二強度的比值為$e^{-\alpha L}$， α稱為吸收係數，L是光通過的路徑長。經過微觀吸收過程之後，光的能量轉變成介質原子的能量。由於該原子與週遭環境進行各式各樣的交互作用，例如碰撞，所以那些能量除了以自發射方式散射出去之外，也可能轉變成熱或其他形式的能量。有吸收效應時，折射率會變成複數，其實部的意義與一般的折射率相同，虛部則與吸收係數有關。不論實部或虛部，都會因介質中的電磁場等因素而變化。

Scattering

由於吸收光能之後的自發射爲隨機過程，自介質之帶電質點發出之後續光波的行進方向散佈於整個空間，所以帶電質點使光波發生散射的現象。類似的狀況發生在一介質內部，若介質中的物理條件(如密度)有起落(fluctuation) ，散射就會顯著，因而能由光束的側面看到光束的存在。粒徑比光波波長小得多的質點造成散射時，散射光的強度與波長的四次方成反比

Scattering

A.彈性散射與非彈性散射

微小的質點造成的散射波之頻率，與入射波相同，稱爲彈性散射或瑞立(Rayleigh)散射。但是有結構的質點吸收光能之後，可能用掉其中的一部份，產生分子振動等等，於是散射的光波頻率小於入射者；相反地，部份振動能量也可能添加於散射光波，而使散射光波的頻率高於入射頻率，這兩者都屬於非彈性散射，例如Raman 散射。

B.螢光

物質將入射光波的部份能量變成其他形式的能量，而將其餘能量散射出去時，稱爲螢光。與彈性散射對應的螢光爲共振螢光，一般的螢光對應於非彈性散射。

Reflection

介質表面上的反射率，是反射波強度與入射波強度的比值。它隨光波電場方向(偏振)、入射角、兩側介質之折射率而變。如果介面另側的介質是薄膜(一層或多層)，其厚度也會影響自最外面反射的反射率，此時偏振及入射角也有影響。如果涉及導體介質，電導率也是一個變因。

Relfection

介質中會受光波電場影響的電荷之密度，它們與周圍物質交互作用的強弱，以及這些電荷的微觀能量值與光子能量的接近程度，會影響它們隨入射光電場運動的幅度，因而影響續發光波的強度和速度。折射率是真空中的光速與介質中的光速之比值，所以也就受光波頻率的影響。另一方面，折射率是入射角與折射角的正弦值之比，所以不同[頻率的光波折射後會朝不同方向行進，而使多色光波的各成分散開，稱為色散。

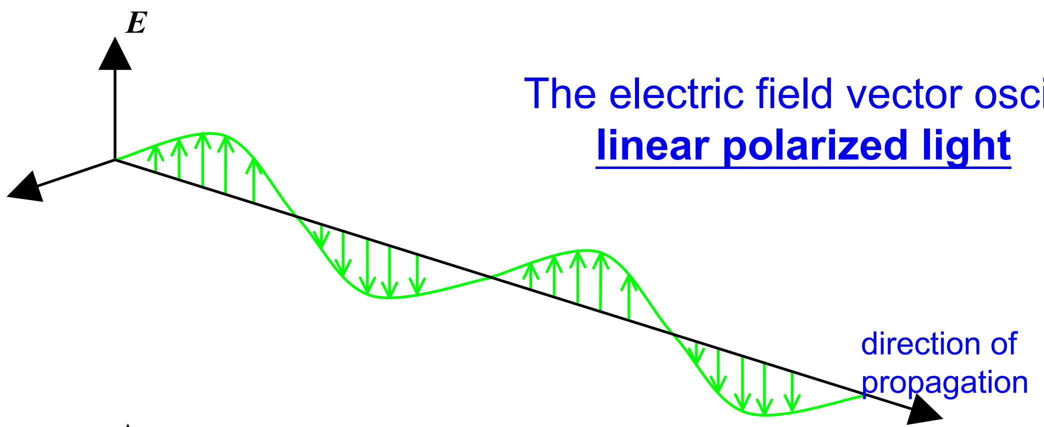
Total Internal Reflection

光波由折射率低的介質(光疏介質)射向折射率高的介質(光密介質)時，折射光線會向法線偏折，折射角小於入射角；反之，則折射角大於入射角。此外，兩介質及頻率固定，而光從光密介質向光疏介質傳播時，入射角愈大則折射角也愈大。於是，入射角達到某個值時，折射角等於90度，此時的入射角稱爲臨界角。入射角再增大，則光波不再折射，而全部反射回光密介質，這就是全內反射，其反射率爲100%。若光疏介質之厚度爲有限值，則可能發生穿隧效應(tunneling effect)。

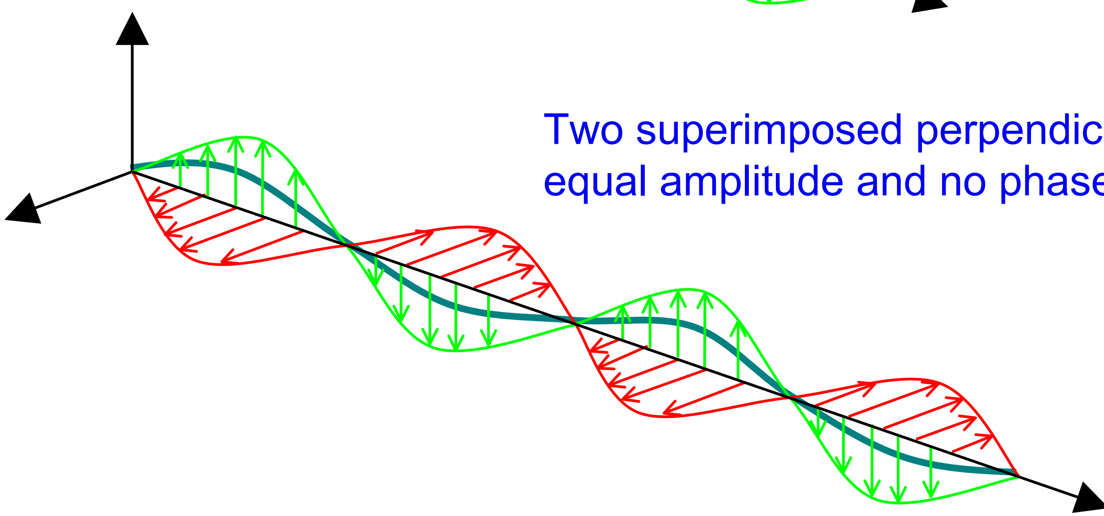
Polarization

偏振是指波的振動量保持以固定方式振動的現象，所謂固定方式又有幾種類型，其中最簡單也最常見最常用的一類，是沿固定方向振動的現象。這種情況的偏振稱爲線偏振，因爲它的振動量平行於空間的一條直線；又稱爲平面偏振，因爲那條線與光線行進方向形成固定的平面。偏振是橫波才會有的性質。絃波與光波都是橫波，所以都能呈現偏振。除了立方晶系的物質之外，其他晶系的物質如塑膠膜偏光鏡，都是非各向同性的(anisotropic)材料。在這種物質中，沿不同方向偏振的光波，會具有不同的折射率及速度，稱爲具有雙折射性(birefringence)。

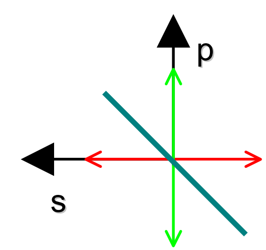
Polarized Light



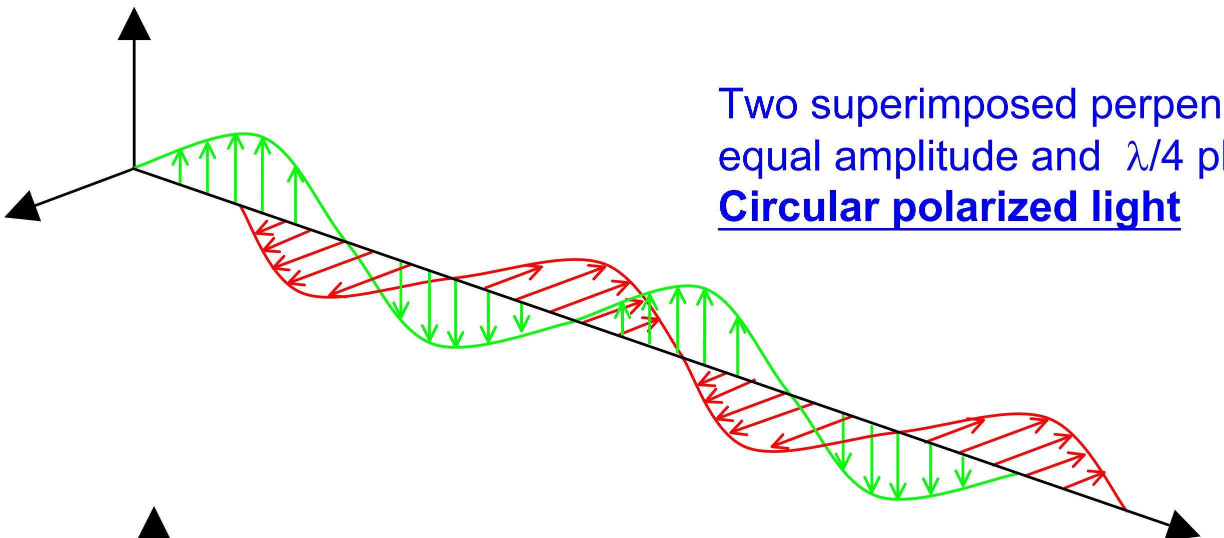
The electric field vector oscillates in one direction only =>
<u>linear polarized light</u>



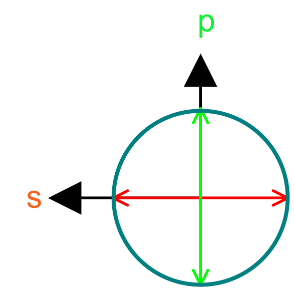
Two superimposed perpendicular light waves with equal amplitude and no phase shift

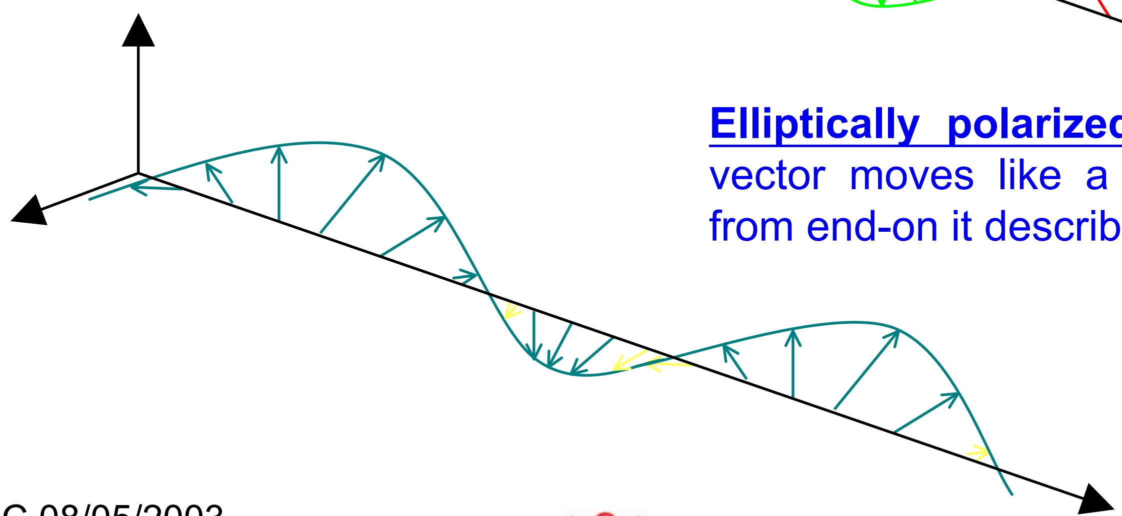


Circular and elliptically polarized light

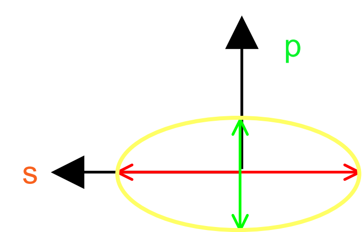


Two superimposed perpendicular light waves with equal amplitude and $\lambda/4$ phase shift => **Circular polarized light**





Elliptically polarized light => the electric field vector moves like a „deformed“ coil, i.e. viewed from end-on it describes an ellipse



Interference

兩個頻率相同的波相遇時，在波峰與波峰(或波谷與波谷)相會處，二者會相長而使振幅增大；相反地，在波峰與波谷相會處，它們會相消而使振幅變小。這個現象稱爲波的干涉，其相長與相消的位置決定於波長及波源的排列和其間的距離。這些位置分佈於空間中，形成干涉圖案。

光譜學上利用干涉效應把不同頻率的光波分開；全像術利用干涉記錄三維資訊；量度學上利用干涉測定物體厚度、平整度、密度、折射率等物理量及其變化；以光纖製成的迴轉儀利用干涉現象測定轉動及標定方向。此外還有許多應用。

Diffraction

改變方向繞過途中障礙物體前進的現象稱爲繞射。使這現象顯著地呈現出來的條件是：障礙物尺寸與波長相近。例如可見光的波長約爲千萬分之五公尺，所以不會繞過一般障礙(如人體)，而可以繞過幾乎併攏的手指間隙、旗幟的紗線。光波的繞射會使原該成影的地方也受光波照射，而使像的邊緣模糊。

光譜上的應用: 減少狹縫的寬度，可以擴大繞射條紋的間隔；增加狹縫的數目，可以使繞射圖案更鮮明，因此光柵(grating)的設計可使光譜的細節顯露出來。

Optical Properties of biological tissues

- When the EM wave of optical ray encounters the biological tissue, there will be multiple effects of reflectance, absorption, and scattering due to inhomogeneity of the sample.
- To characterize the properties of biological tissue, there are four parameters of optical properties can be derived from directed or indrected measurement of biological tissues, e.g. refractive index n, absorption coefficient u_a, scattering coefficient u_s, and anisotropy factor, g.

Optical Properties of biological tissues

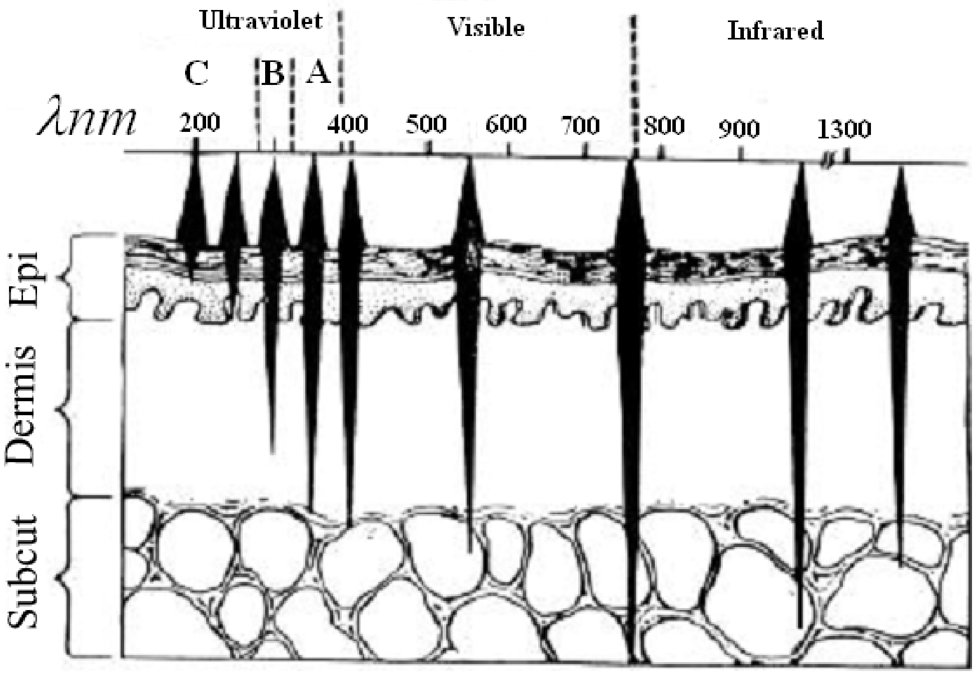
Each tissue has its own characteristic optical absorption spectra, one can approximate the optical properties of tissues with that of water, due to the facts of water is the major composition of human body, > 70%. Both water and saline solution transmit well in the visible range and the absorption is high in the UV (l<300 nm) and the IR (l>2um). Tissue shows similar strong absorption in the UV and the IR.

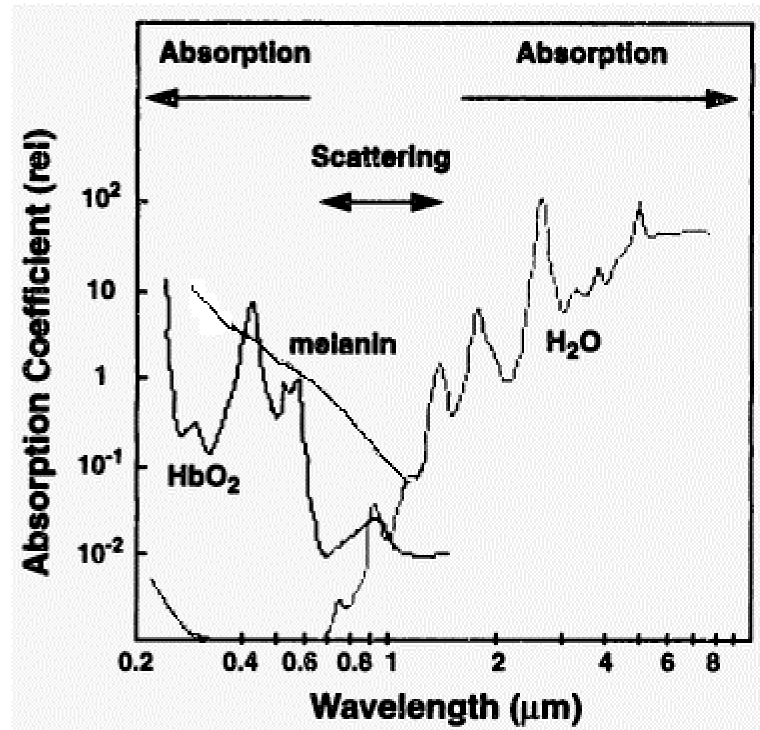
Optical Properties of biological tissues

However in blood, there are strong absorption in the visible range due to chromophores (色素基) such as hemoglobin (血紅素) and bilirubin（膽紅素）. Therefore for a tissue that contains blood, the absorption is dominated by the absorption in the blood. There are also other chromophores that absorb light in the specific spectral range, such as melanin（黑色素）and proteins as shown in the following figure.

•Ref: Cheong et.al. "A review of the optical properties of biological tissues", 1990, IEEE J. Quantum Electronics 26: 2166-2184

Optical Properties of Tissue





Optical method for micron scale

OCT

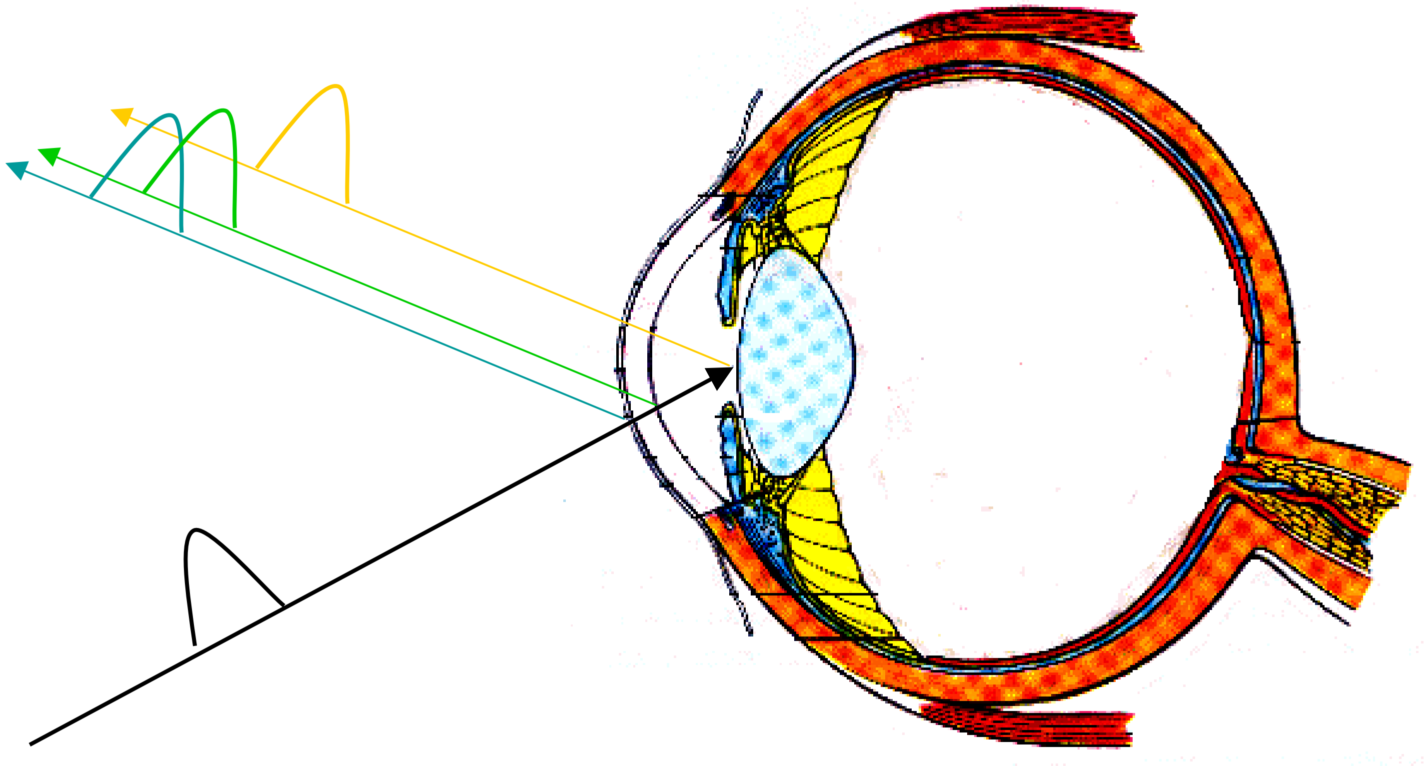
OCT in Bio-medicine

- Internal microstructure information within the subsurface biological tissues
- Diagnostic medical imaging technology
- Optical biopsy
- Functional OCT
  - Color Doppler OCT
  - Polarization Sensitive OCT

Characteristics

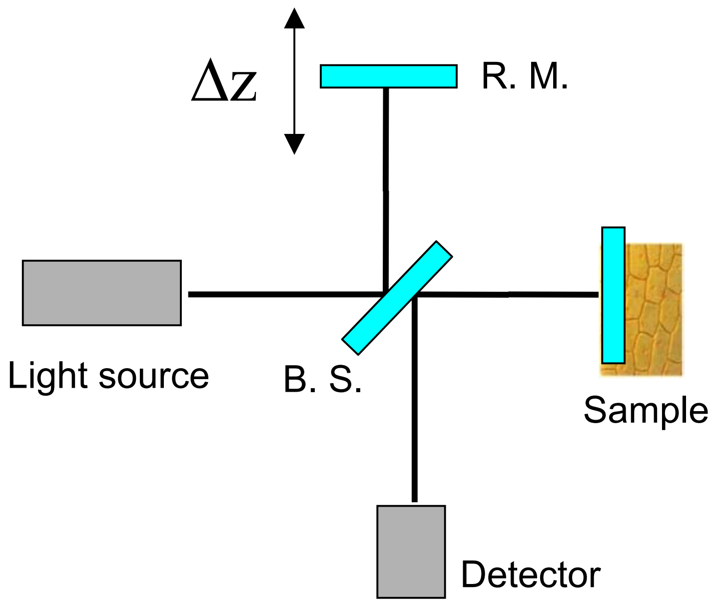
- ***Advantages of OCT***
  - Non-invasive, Minimally-invasive
  - High Spatial Resolution (~μm)
  - High Sensitivity (> 85dB typically)
  - Small Size
  - Safety
  - Reasonable Price
- ***Disadvantages of OCT***
  - Smaller Penetration Depth
    - mm ~ cm

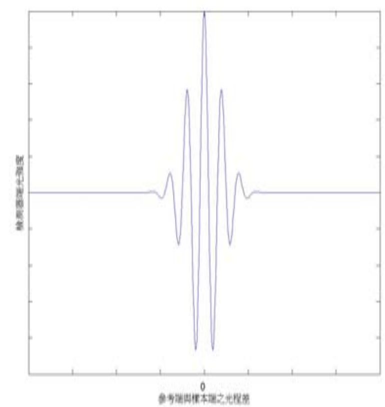
Principle of OCT



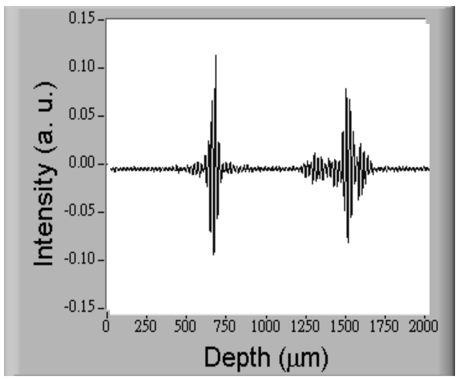
Distance = Delay × Speed of light

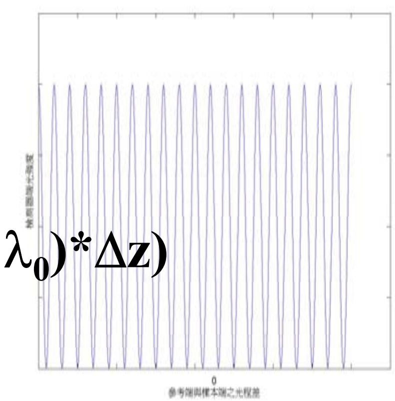
Michelson Interferometer



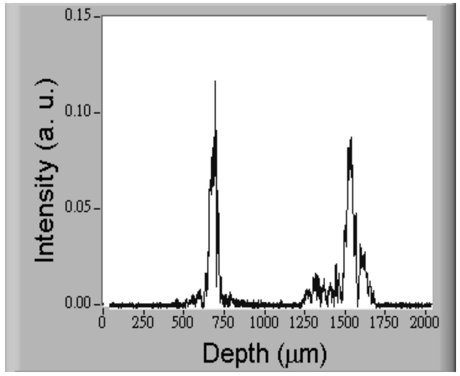


Detected Signal



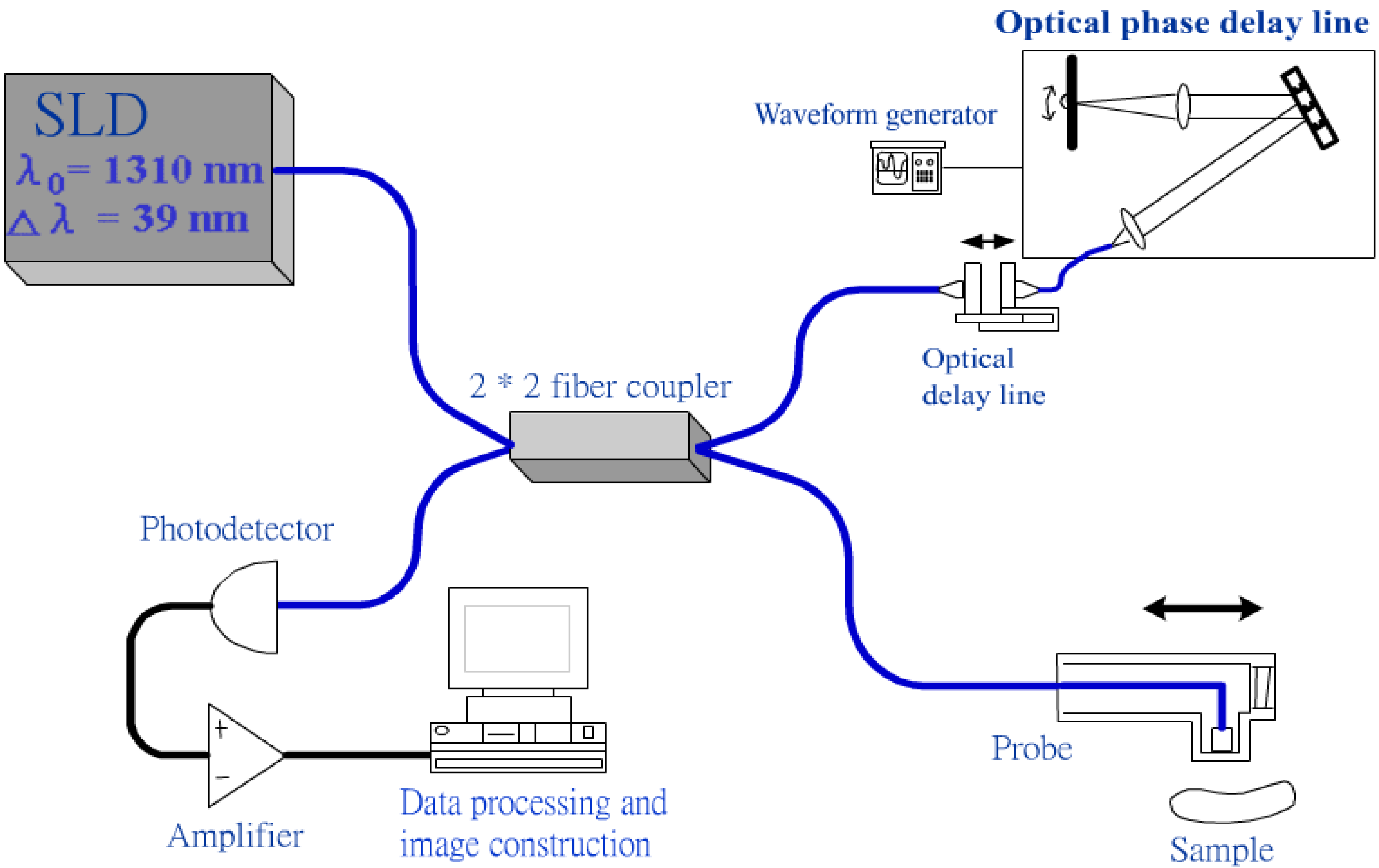


After Demodulation



$$I(t) \sim Er^2 + Es^2 + 2ErEsCOS(2*(2\pi/\lambda_0)*\Delta z)$$

OCT Setup



Optical phase delay line
SLD
λ_0= 1310 nm
$\Delta\lambda$ = 39 nm
Waveform generator
Optical
delay line
2 * 2 fiber coupler
Photodetector
Amplifier
Data processing and
image construction
Probe
Sample

Spatial Resolution

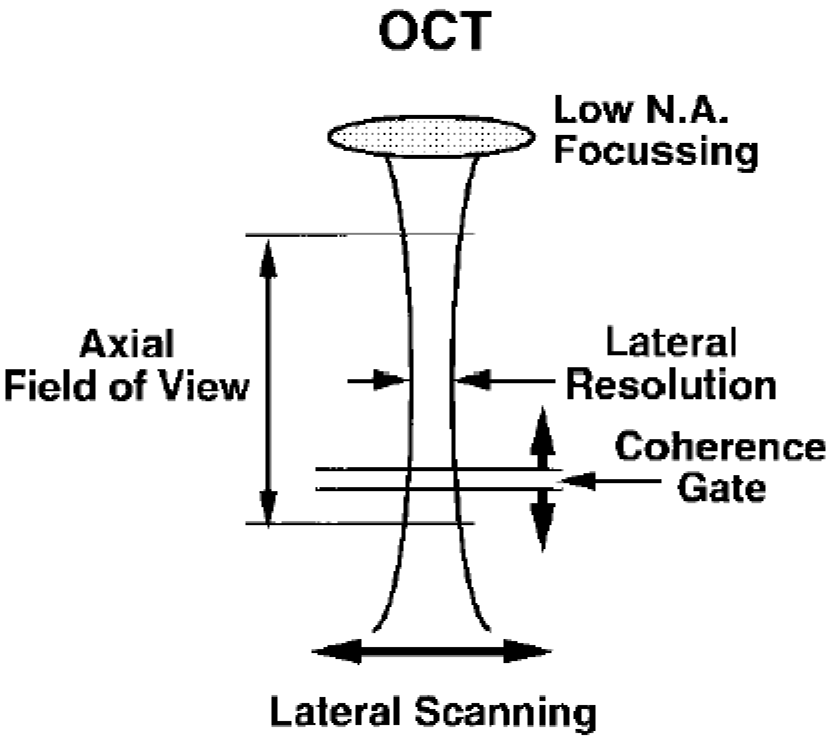
Longitudinal resolution

$$\Delta z = \frac{2\ ln\ 2 \cdot \lambda_0^2}{\pi \cdot \Delta\lambda}$$

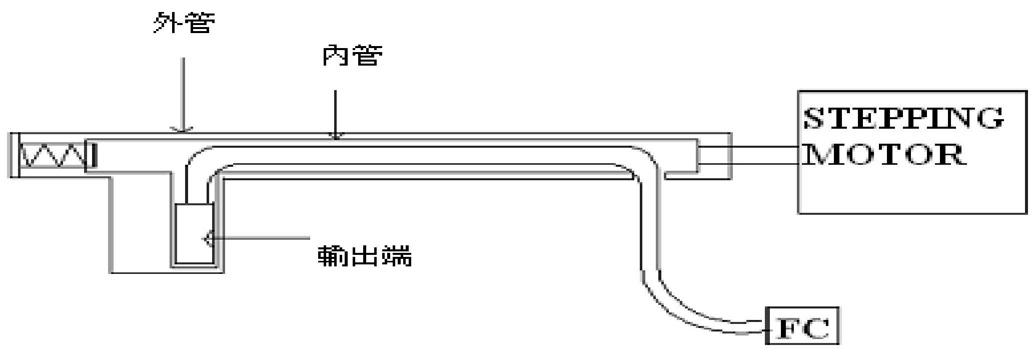
Lateral resolution

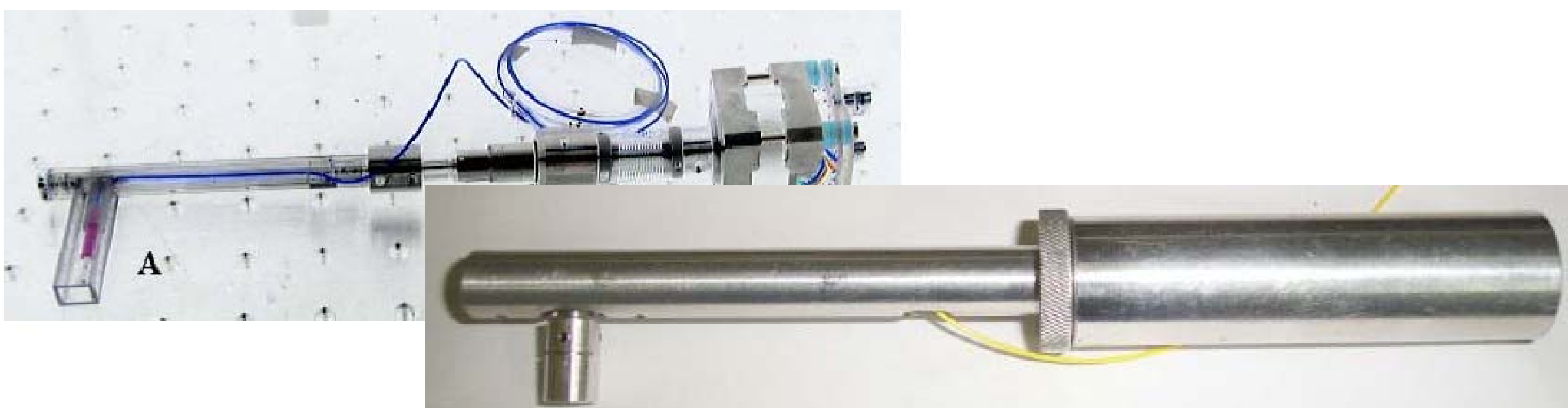
$$\Delta x = \frac{4\lambda \cdot f}{\pi \cdot d}$$

d：Spot size before the lens

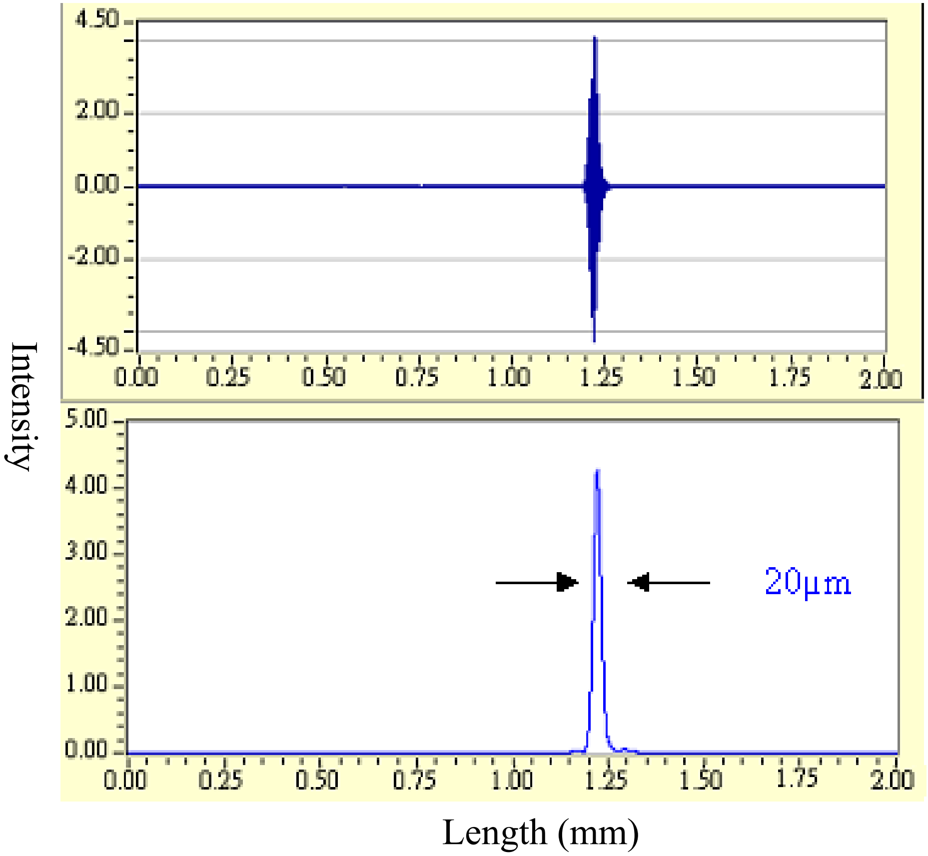


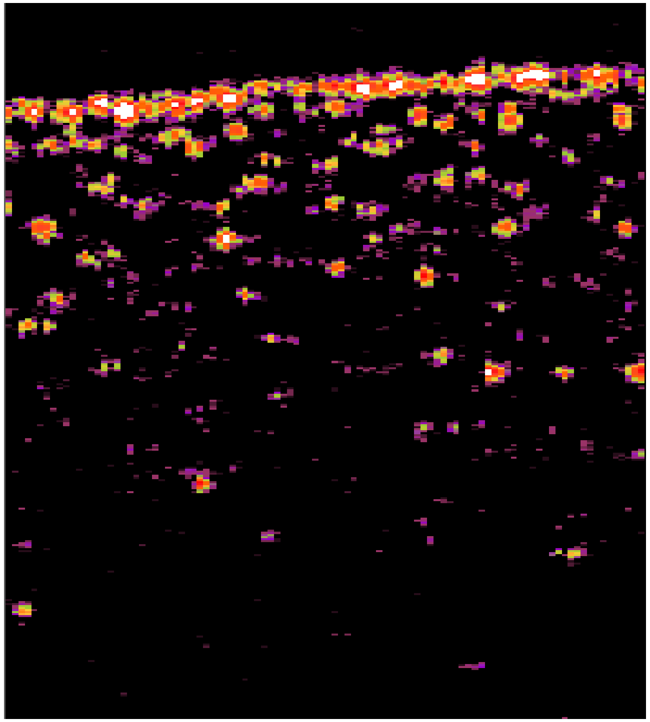
Probe Design





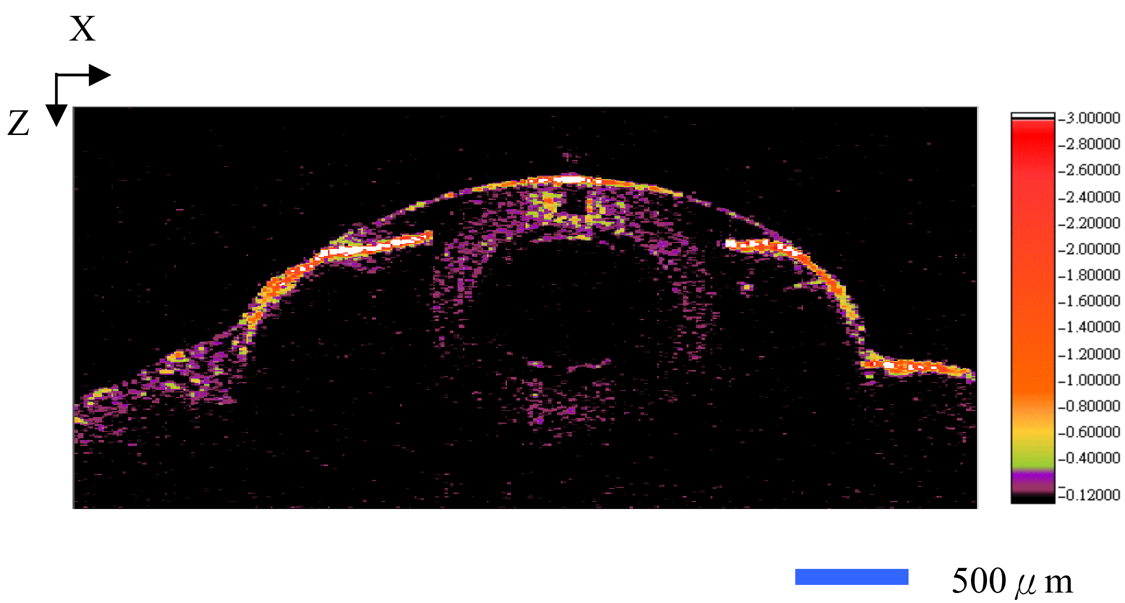
Interference Signal & Onion Image

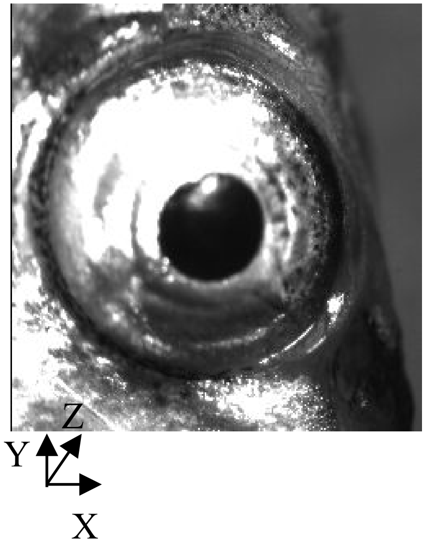




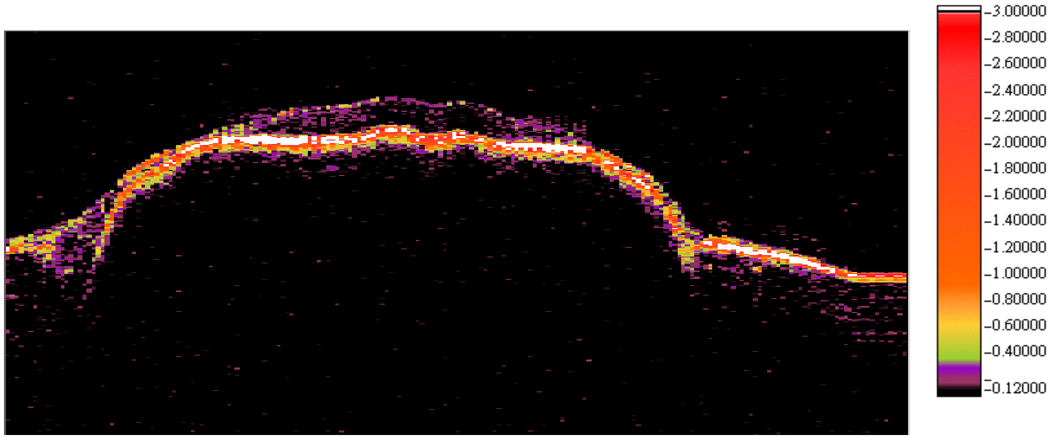
NTU Nano-BioMEMS Group

Fish Eyeball

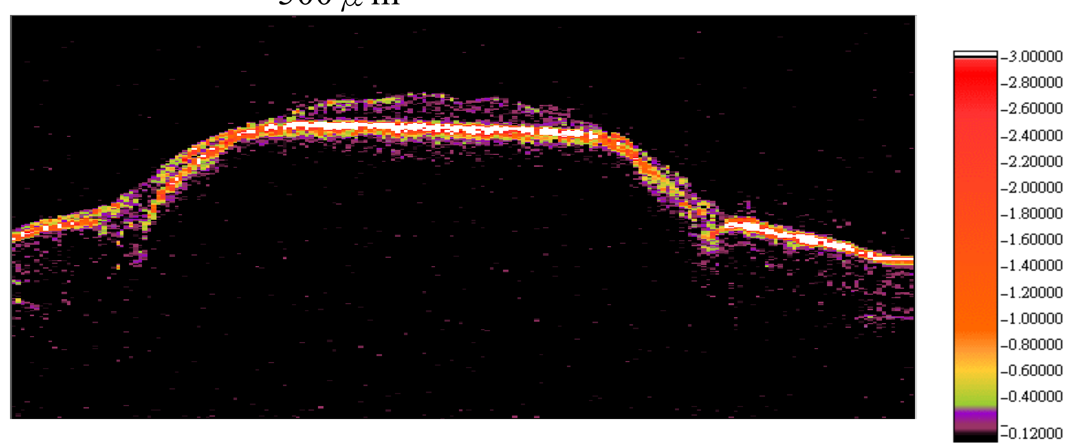




Changed Y-axial

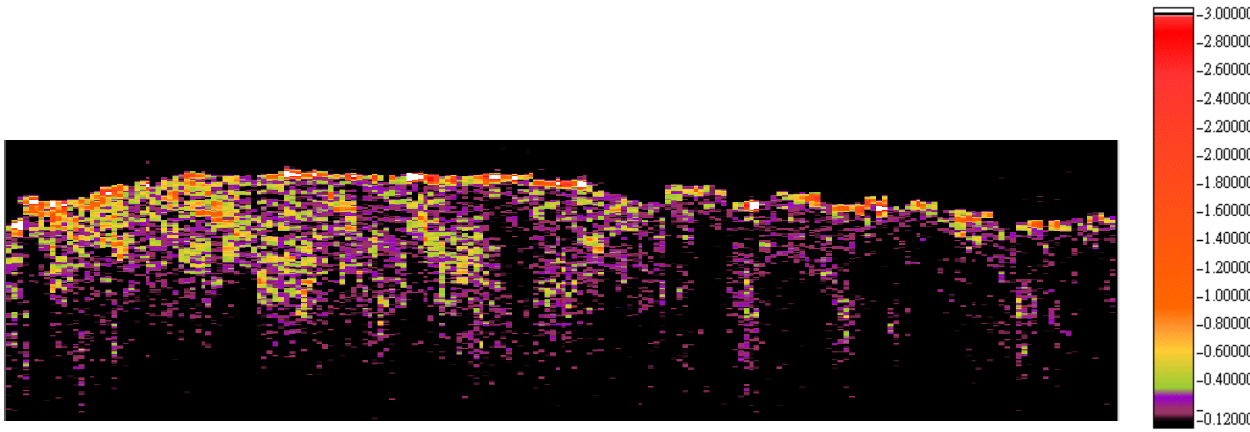


500 μ m

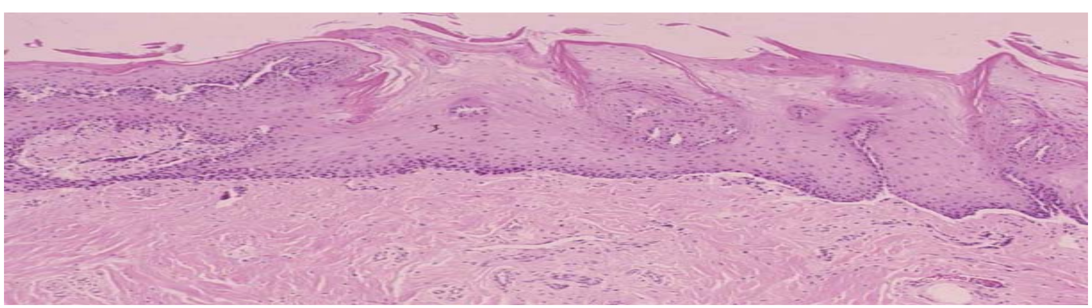


500 μ m

Result (Pig Tongue)

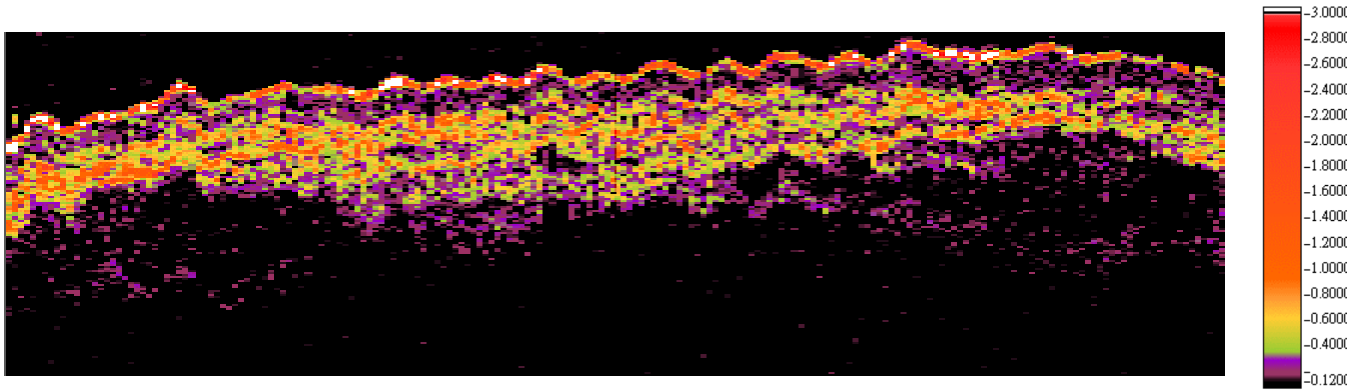


500 μ m

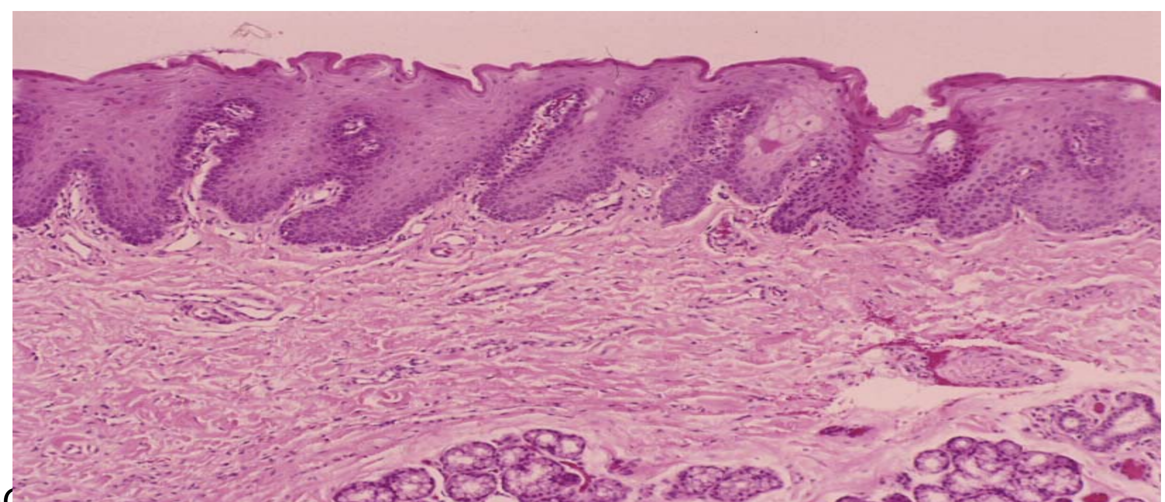


500 μ m

In Vivo Lining Mucosa (Labial 唇內側)

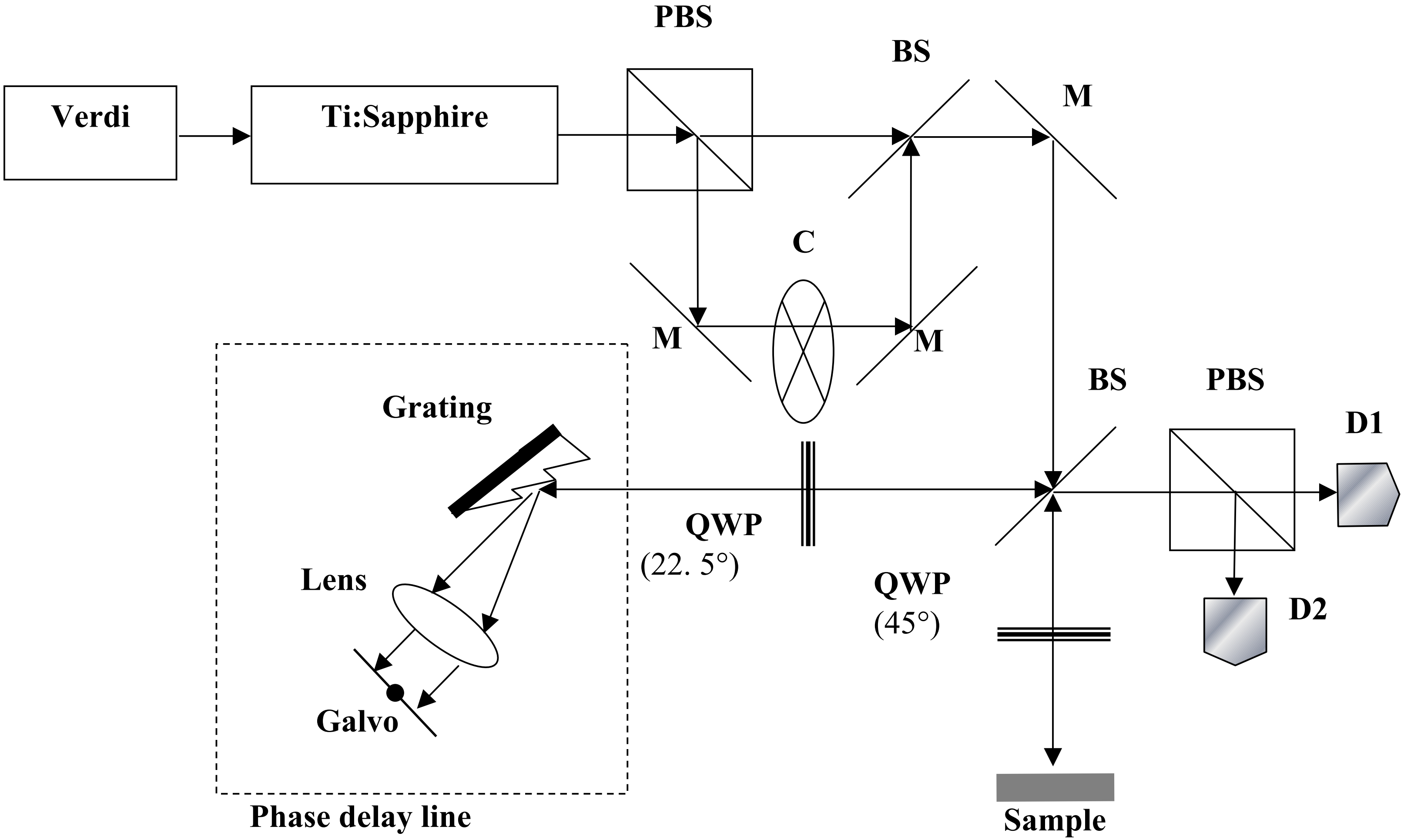


500 μm

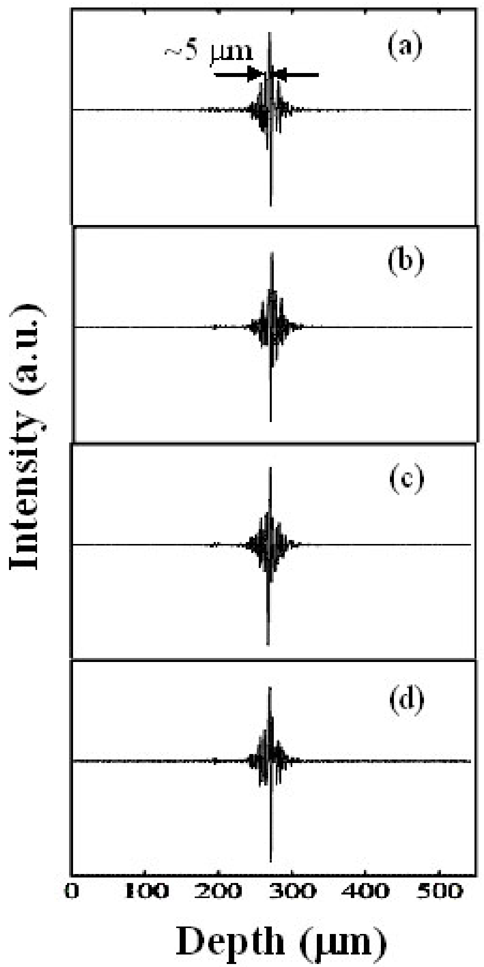


200 μm

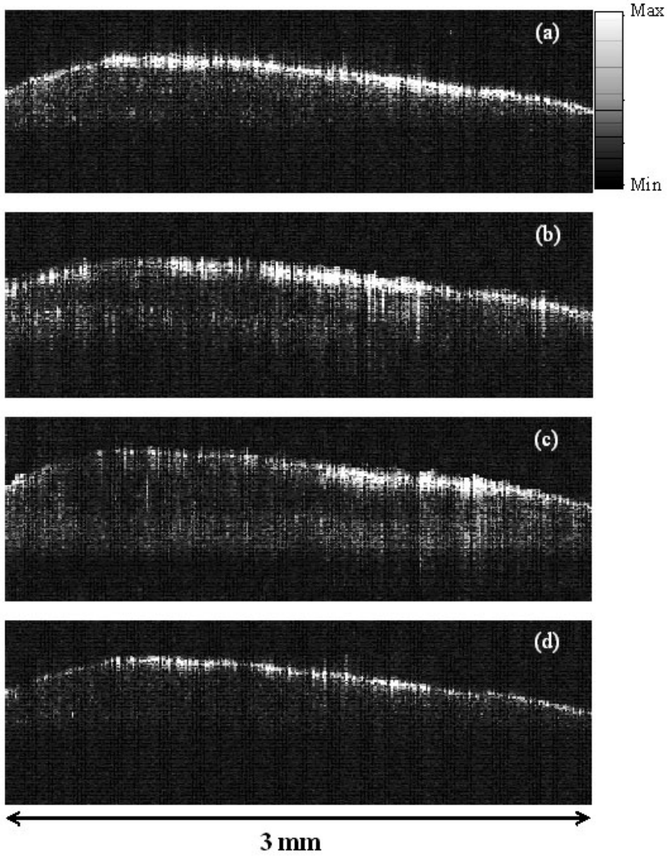
Phase Sensitive OCT

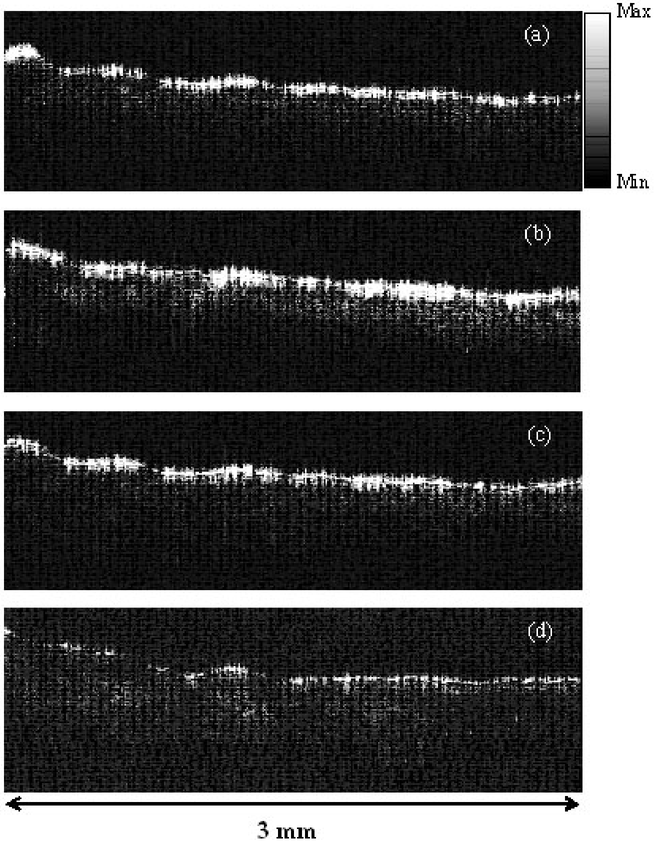


Interference Signal

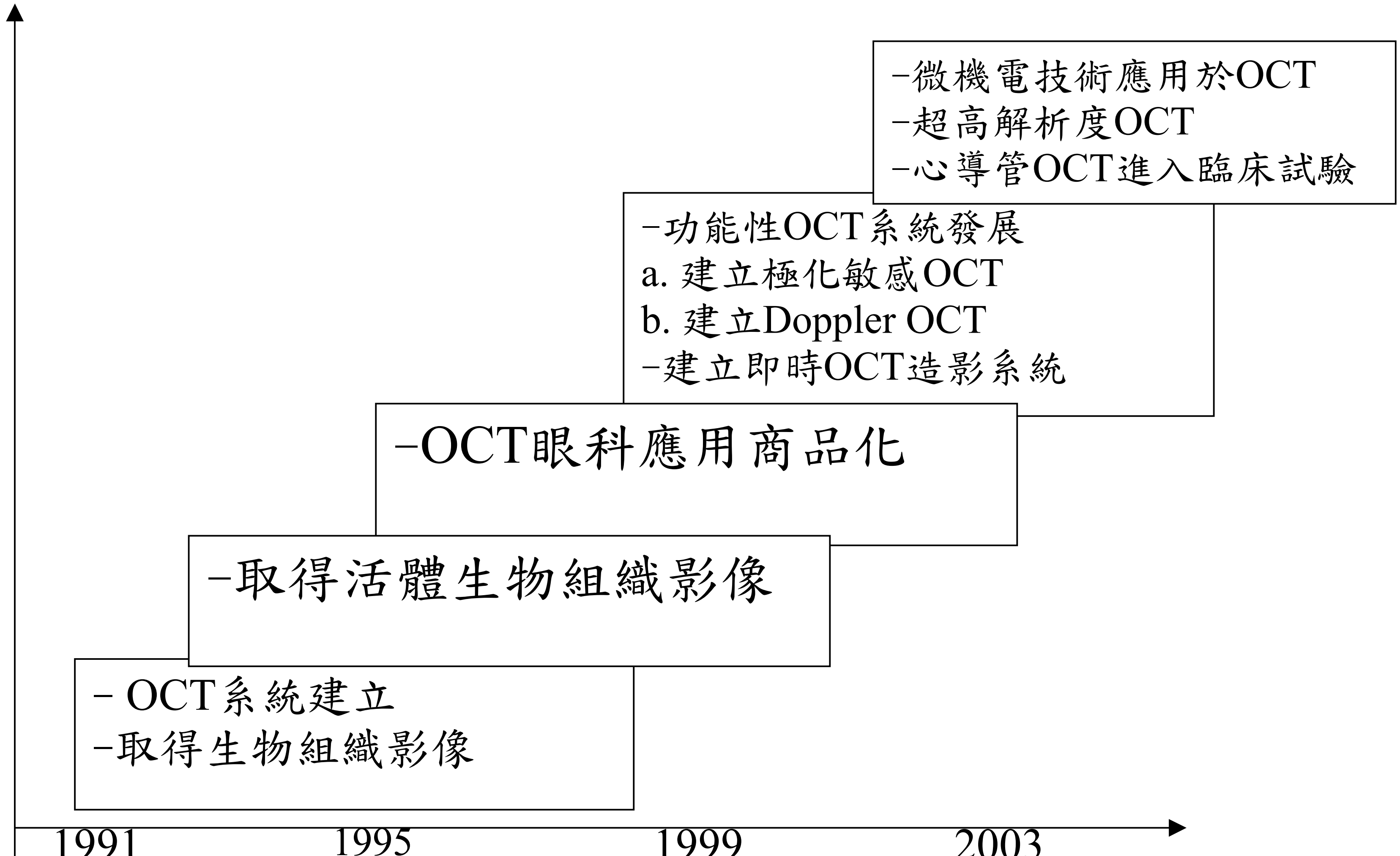


PSOCT images





Road map



-微機電技術應用於OCT
-超高解析度OCT
-心導管OCT進入臨床試驗
-功能性OCT系統發展
a. 建立極化敏感OCT
b. 建立Doppler OCT
-建立即時OCT造影系統
-OCT眼科應用商品化
-取得活體生物組織影像
- OCT系統建立
-取得生物組織影像
1991
1995
1999
2003

Optical method for Nanoscale

Surface Plasmon Resonance

Ellipsometery

表面觀測的生化感測器的種類

1.QCM.

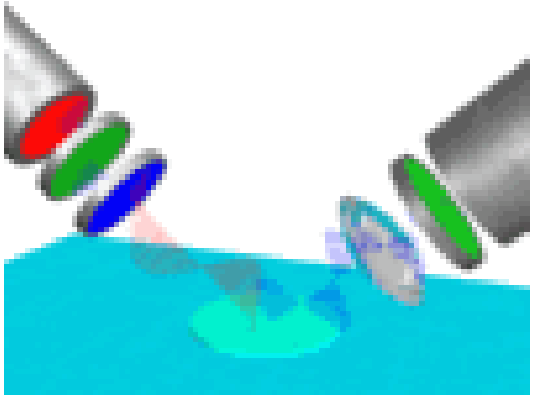
2.Internal reflection spectroscopy

3.SPR.

4.Ellipsometer

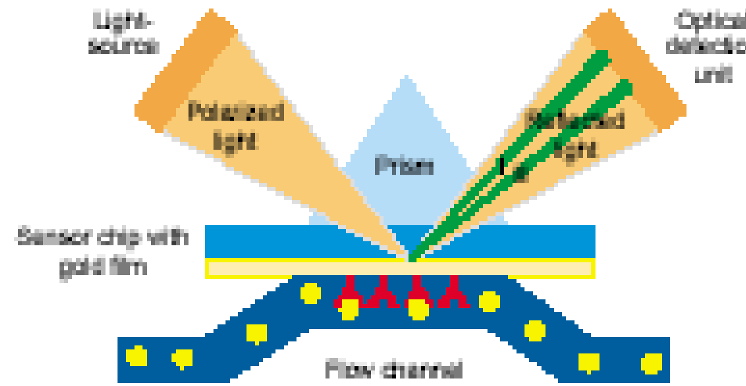
5.STM

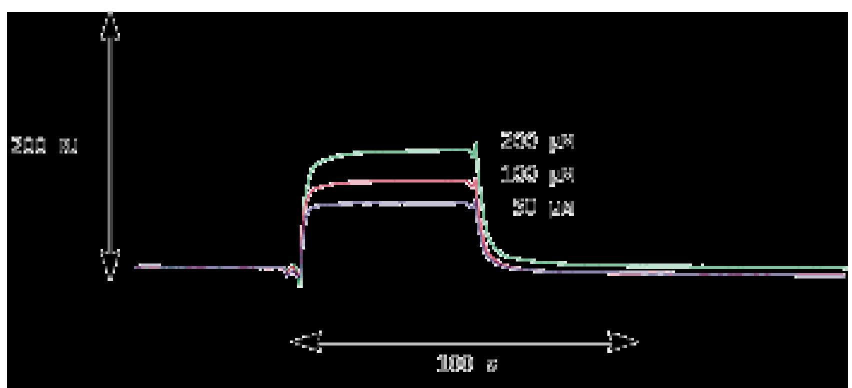
6.AFM……



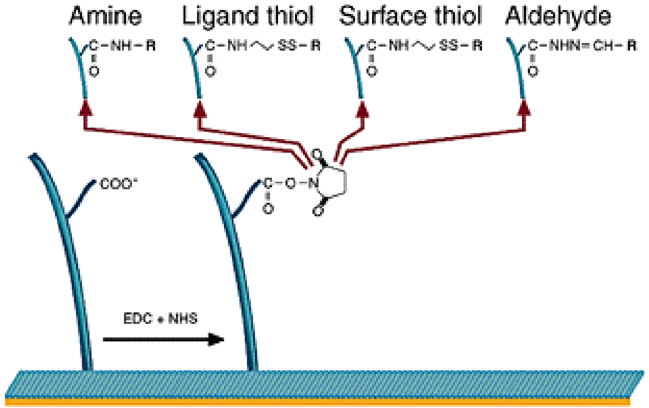
1. H. Raether, "Surface Plasmon on Smooth and Rough Surfaces and on Gratings", Springer-Verlag, Berlin, 1988.
2. J. Homola, et.al. "Surface plasmon resonance sensors: review," Sens. actuators. B, 54: 3-15, 1999.
3. Z. Salamon, et.al., "Surface plasmon resonance spectroscopy as a tool for investigating the biochemcial and biophysical properties of membrane protein systems I: Theoretical principles", BBA 1331: 117-129, 1997

Biacore

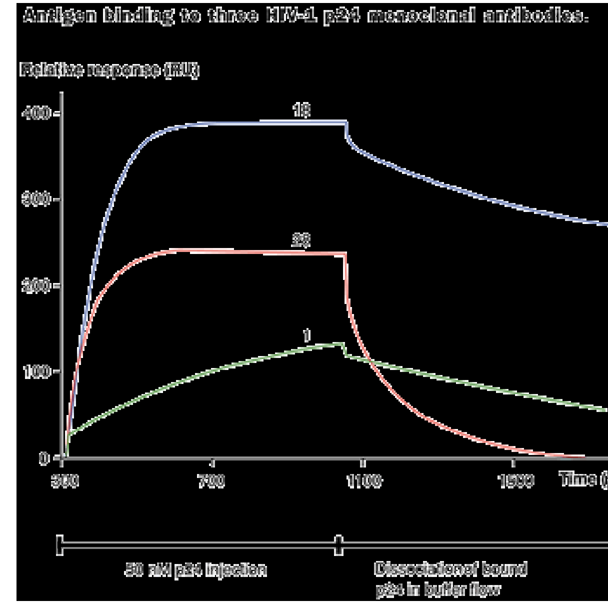




圖片下載自:www.biacore.com



Covalent derivatization



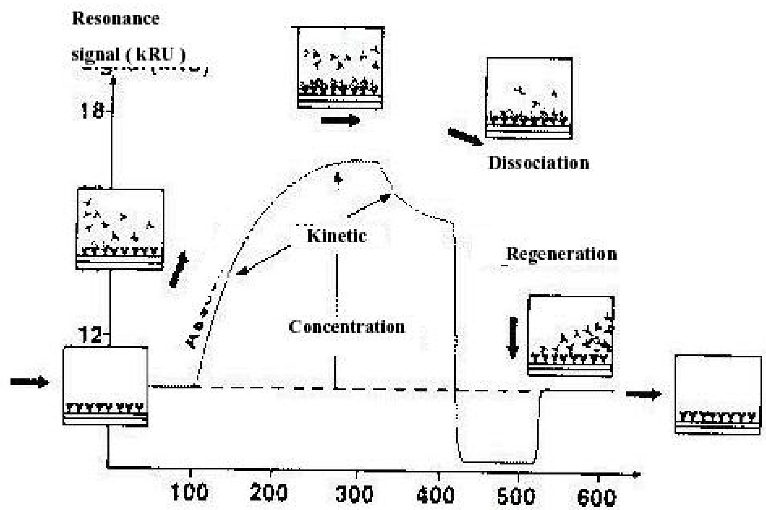
1. Molecular weight detection: >180 Da

2. Required sample volume: injected volume + 20-80 μl

3. Refractive index range: 1.33-1.40

SPR sensor的優點

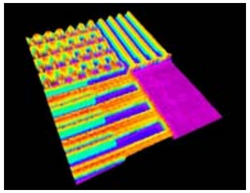
- 免標的物,不用螢光.
- 及時線上監控,
  可量測分子的
  動態鍵結.

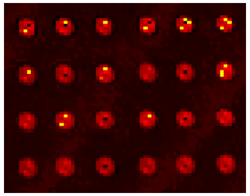


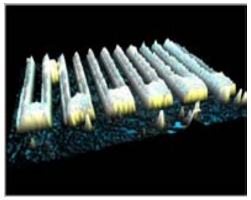
- 靈敏度高.每毫米平方1兆克(1 pg mm^{-2})的濃度變化
- 可量測固液相介面.

EP3

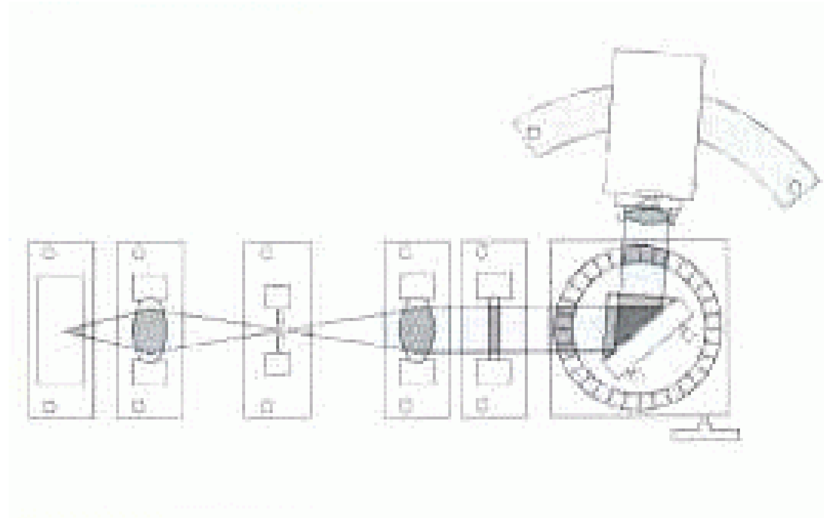
- Materials research
- Biotechnology
- Organic Films (OLED)
- Semiconductors / Displays

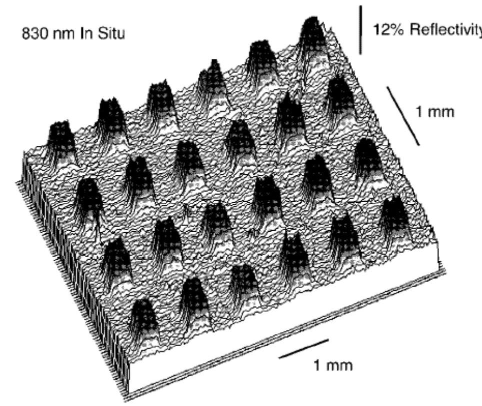




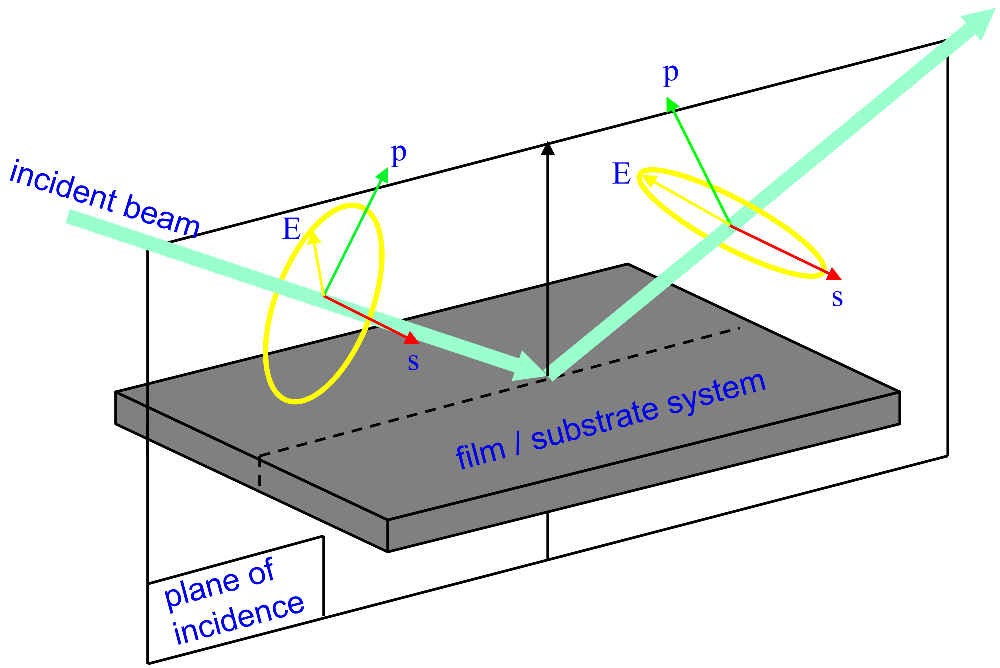


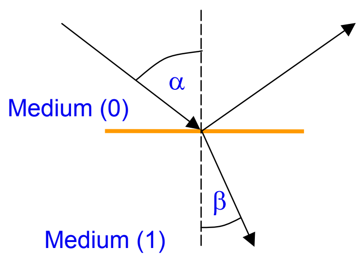
SPRImager





The Diffraction of Light





Index of refraction
(material is not absorbing; k = 0)

$$n = \frac{\sin \alpha}{\sin \beta} = \frac{c_0}{c_1}$$

k = extinction coefficient

n = index of refraction

i = imaginary number (√-1)

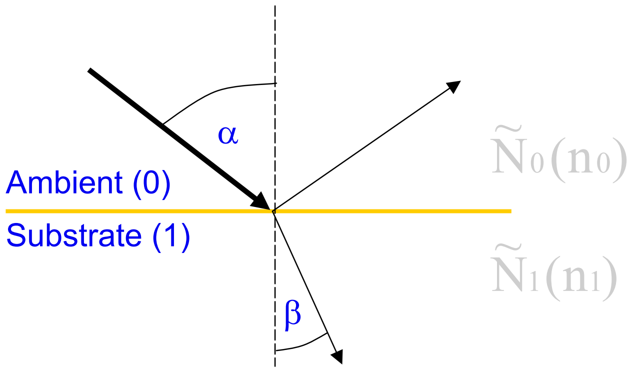
Complex index of refraction
(material is absorbing, k ≠ 0)

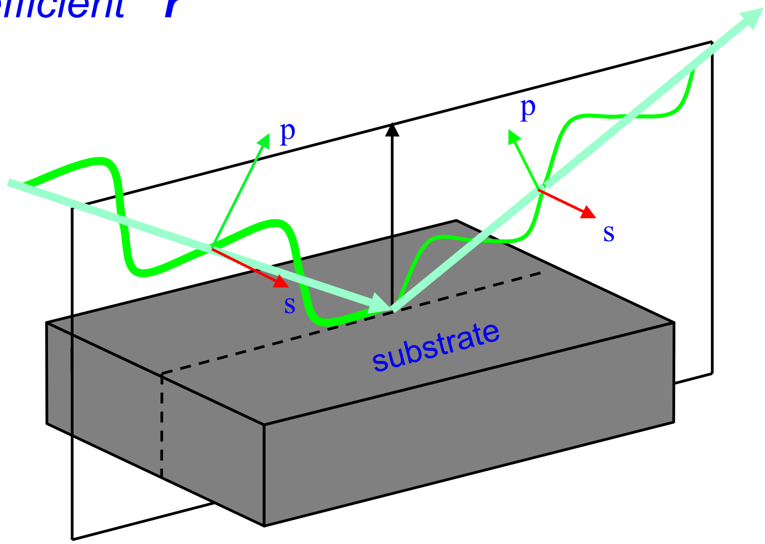
$$\tilde{N} = n - ki$$

Diffraction

➢ A plane wave incident in medium 0 gives rise to a resultant *reflected* wave in the same medium and to a resultant *transmitted* wave in medium 1 (substrate)

➢ The ratio of the amplitude of the outgoing wave to the amplitude of the incident wave leads to the "*Fresnel reflection coefficient*" ***r***

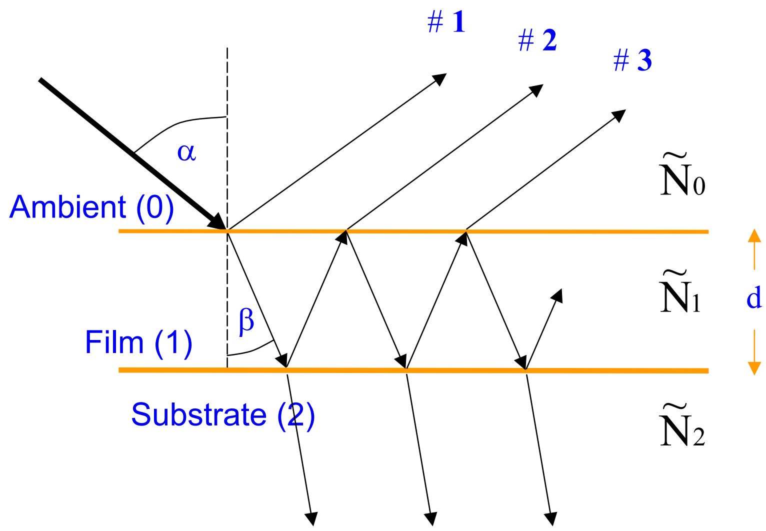


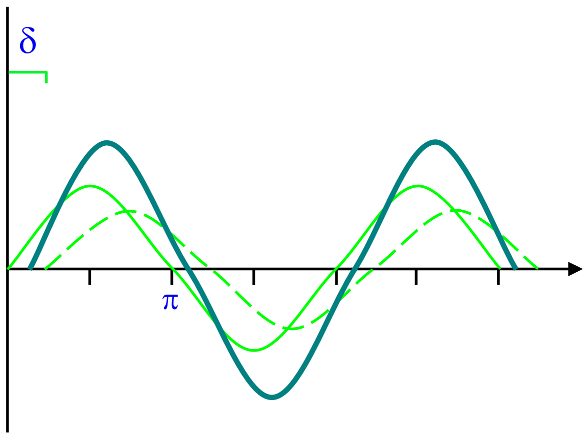


$$r_{01}^{p} = \frac{E_{out}^{p}}{E_{in}^{p}} = \frac{\tilde{N}_1 \cos\alpha - \tilde{N}_0 \cos\beta}{\tilde{N}_1 \cos\alpha + \tilde{N}_0 \cos\beta}$$

$$r_{01}^{s} = \frac{E_{out}^{s}}{E_{in}^{s}} = \frac{\tilde{N}_0 \cos\alpha - \tilde{N}_1 \cos\beta}{\tilde{N}_0 \cos\alpha + \tilde{N}_1 \cos\beta}$$

In a system with more than one interface addition of the reflected waves leads to an infinite geometric series for the "*total reflected amplitude*" ***R*** *(Total reflection coefficient)*





Interference of two waves leads to a resultant wave (green one). The amplitude of it depends on the phase shift (δ).

$$\boldsymbol{R}^p = \frac{E^p_{out\#1} + E^p_{out\#2} + E^p_{out\#3} + \ldots}{E^p_{in}} = \frac{r^p_{01} + r^p_{12}\, e^{(-i\delta)}}{1 + r^p_{01}\, r^p_{12}\, e^{(-i\delta)}}$$

$$\boldsymbol{R}^s = \frac{E^s_{out\#1} + E^s_{out\#2} + E^s_{out\#3} + \ldots}{E^s_{in}} = \frac{r^s_{01} + r^s_{12}\, e^{(-i\delta)}}{1 + r^s_{01}\, r^s_{12}\, e^{(-i\delta)}}$$

$$\delta = \text{phase shift} = 4\pi \left(\frac{d}{\lambda}\right) \tilde{N}_1 \cos\beta$$

Surface plasmon resonance的物理現象

1.plasma

2.plasmon

3.surface plasmon

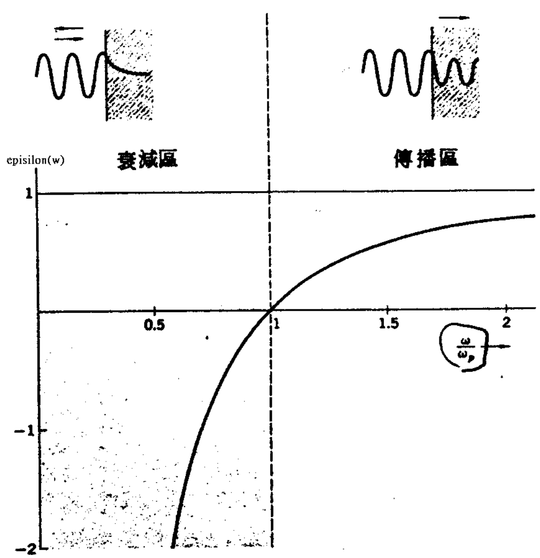
4.TIR & evanescent wave

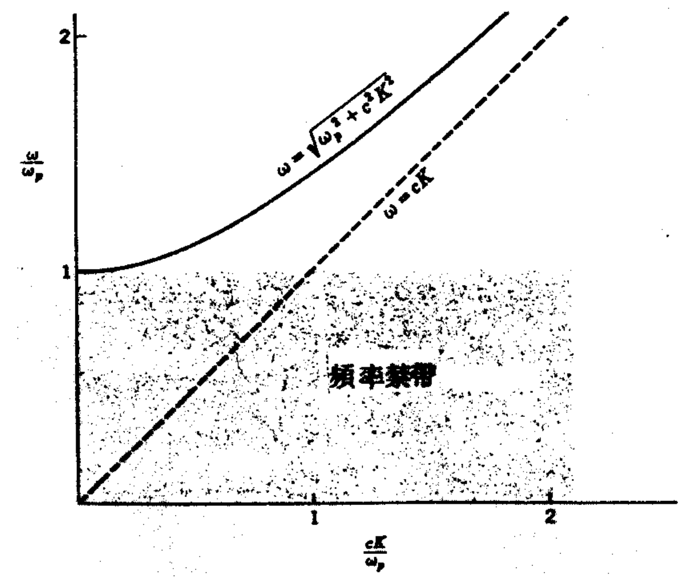
5.fresnel equation

Plasma

定義:具有同樣電子密度和離子密度的游離氣體。

$$\omega_p = \sqrt{\frac{Ne^2}{m\varepsilon_0}} \qquad \varepsilon = \varepsilon_0\left(1 - \frac{\omega_p^{\ 2}}{\omega^2}\right) \qquad r = j\omega\sqrt{\mu\varepsilon_0}\cdot\sqrt{1-\left(\frac{f_p}{f}\right)^2}$$

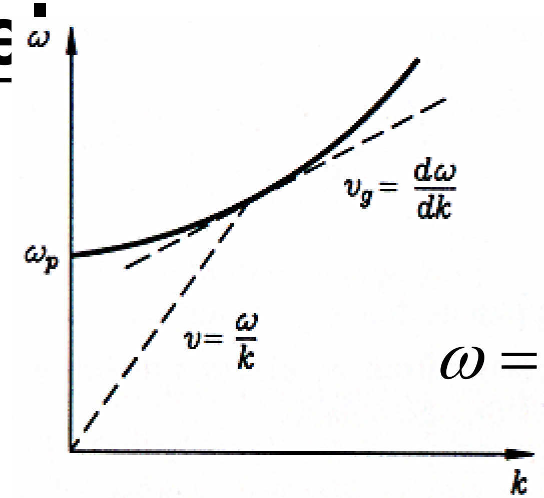




Plasmon

- **Plasma oscillations in metals**
- **The collective plasma oscillation**
- **Consider a metal:<u>positive ions forming a regular lattice</u> & <u>conduction electrons move free</u>**
- **For common metal**

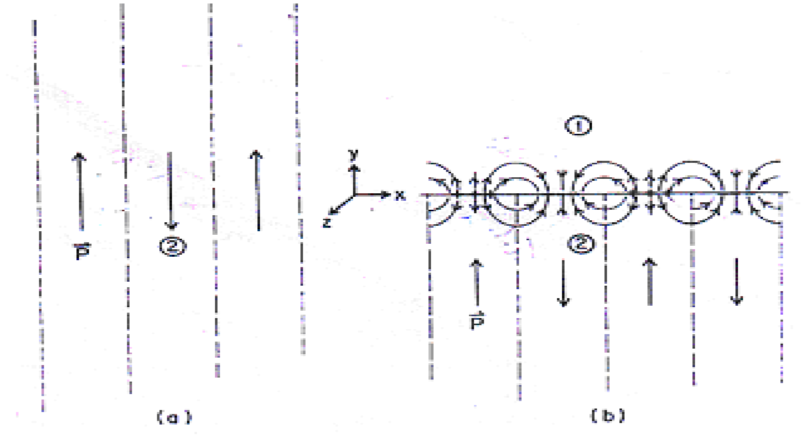
$$\hbar\omega_p \approx 12eV$$



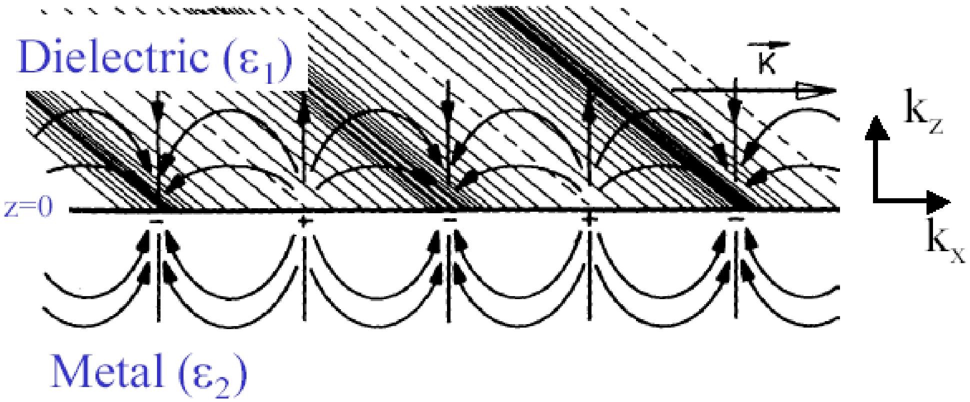
$$\omega = \omega_p^{\ 2} + k^2 c^2$$

Surface plasmons

- Interface between a metal and dielectric may support charge density oscillation.
- Surface plasmon is p-polarized, because the non_continuous Ez will produce surface charge.



資料來源:http://mpi.leeds.ac.uk/index.php



By boundary condition:

$$E_{x1} = E_{x2}$$

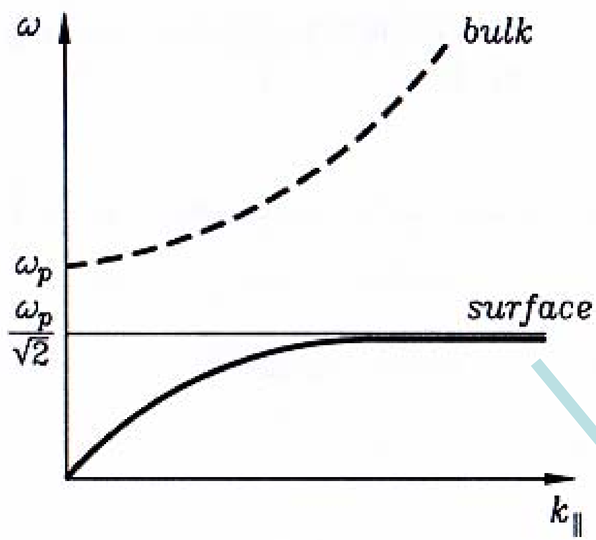
$$H_{y1} = H_{y2}$$

$$\because D_1^n = D_2^n, \varepsilon_1 E_{z1} = \varepsilon_2 E_{z2}$$

Surface plasmon's dispersion relation

$$\because E_{z1} = -E_{z2} \;\therefore \varepsilon_1 = -\varepsilon_2$$

So we choose metal and dielectric



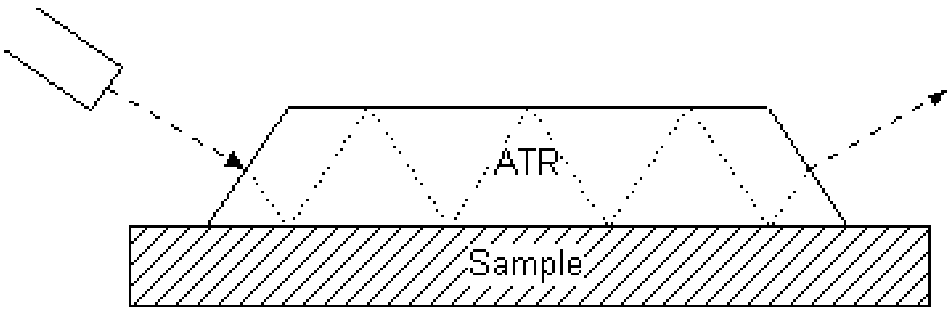
$$K_{x1} = K_{x2} = K_x$$

$$\frac{K_{z1}}{K_{z2}} = -\frac{\varepsilon_1}{\varepsilon_2} \cdots (1)$$

$$\begin{cases} K_{z1} = \sqrt{-K_{x1}^2 + \varepsilon_1 K^2} \\ K_{z2} = \sqrt{-K_{x2}^2 + \varepsilon_2 K^2} \end{cases} \cdots (2)$$

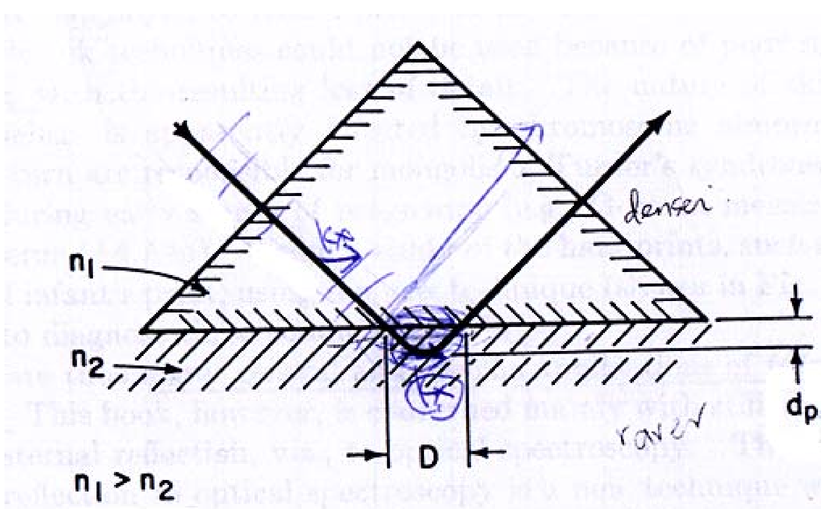
$$K_x = \frac{\omega}{c}\sqrt{\frac{\varepsilon_1 \varepsilon_2(\omega)}{(\varepsilon_1 + \varepsilon_2(\omega))}}$$

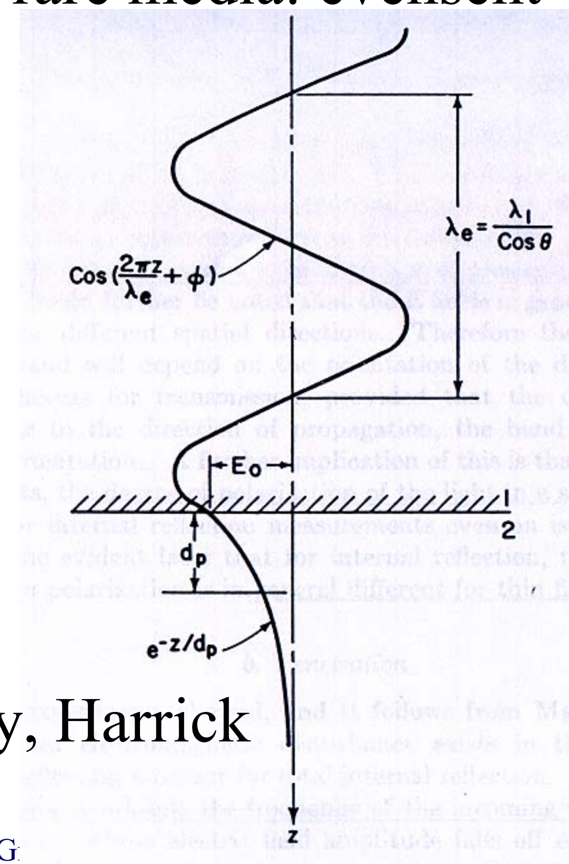
Total internal reflection & evensent wave



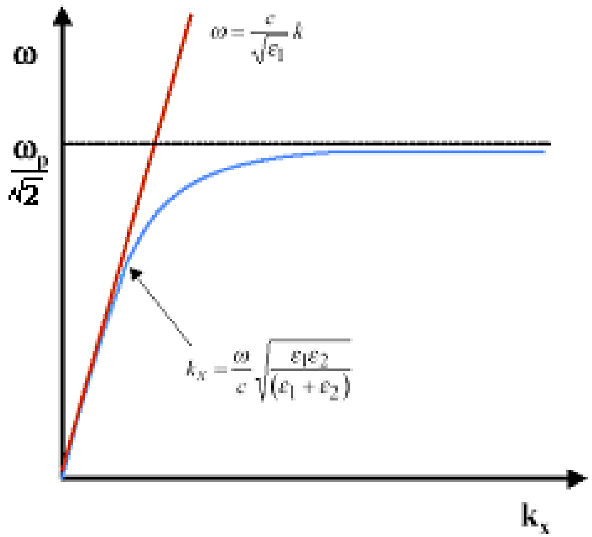
In dense media: standing wave

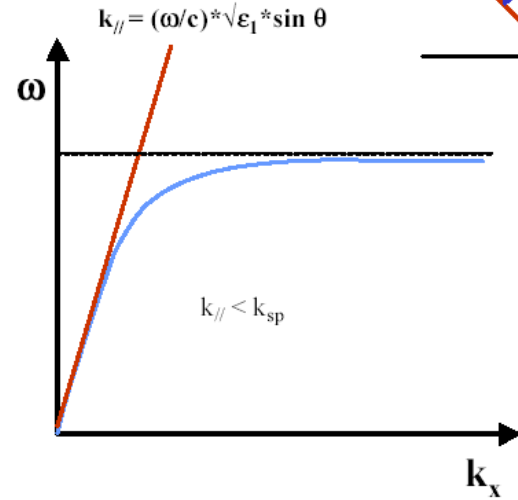
In rare media: evensent wave

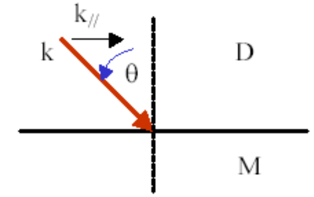


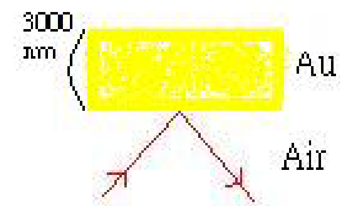


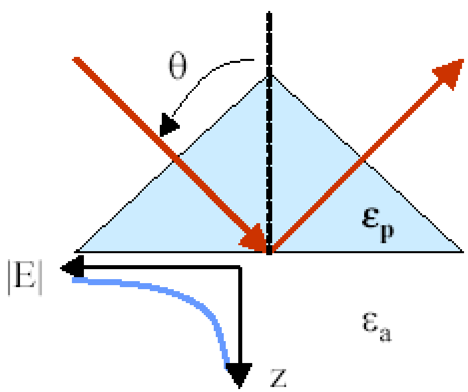
資料來源: Internal reflection spectroscopy, Harrick









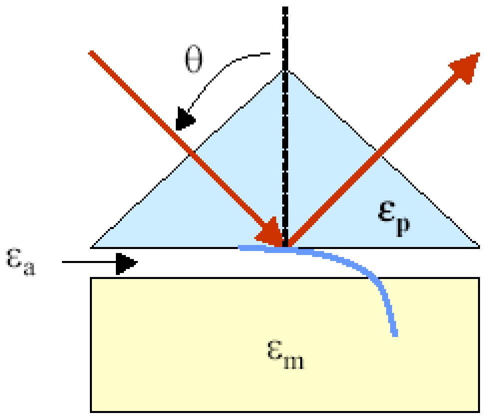


We can't stimulation the SP by light directly, so we use prism coupling.

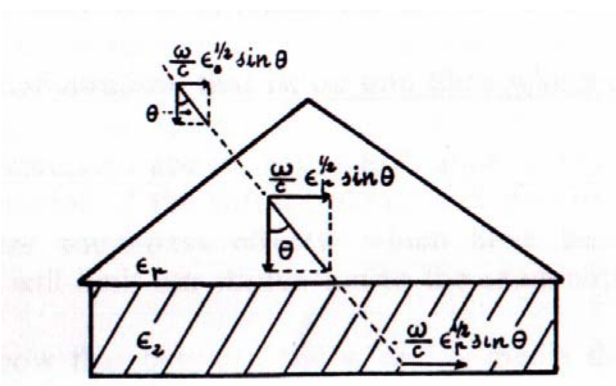
$$k_{//} = (w/c) * \sqrt{\varepsilon_p} * \sin\theta$$

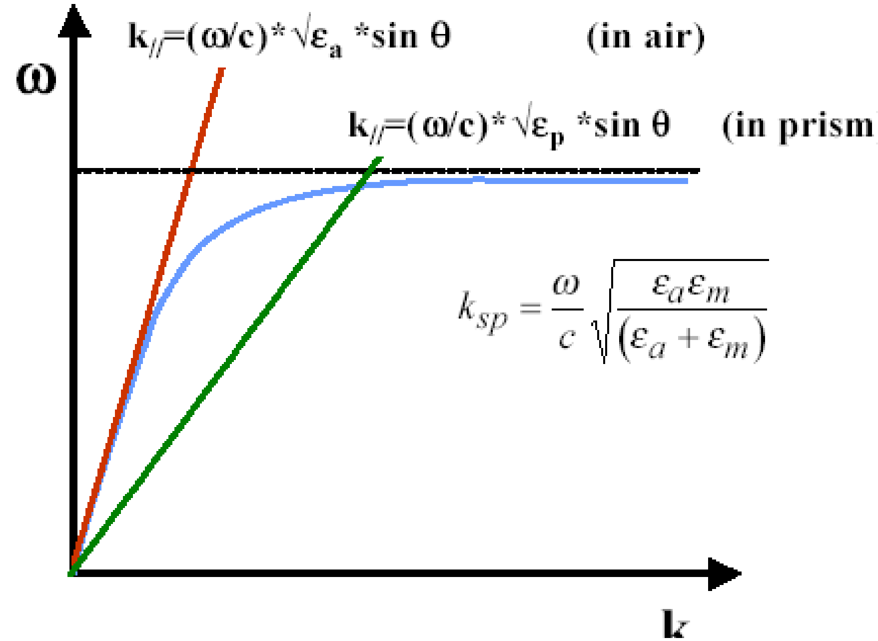
資料來源:http://mpi.leeds.ac.uk/index.php

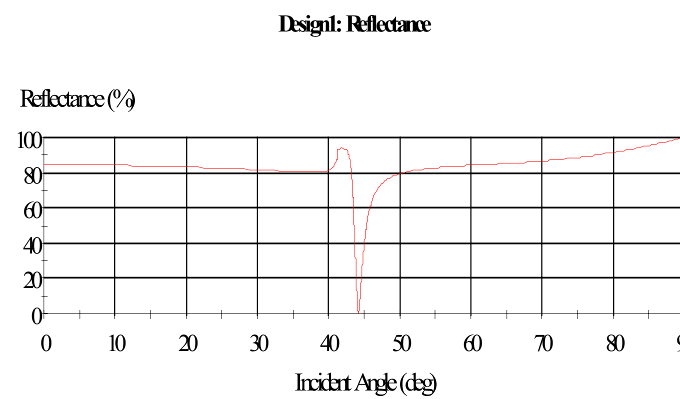
Otto(1968):



Kretschmann(1971):



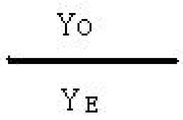




Fresnel equation

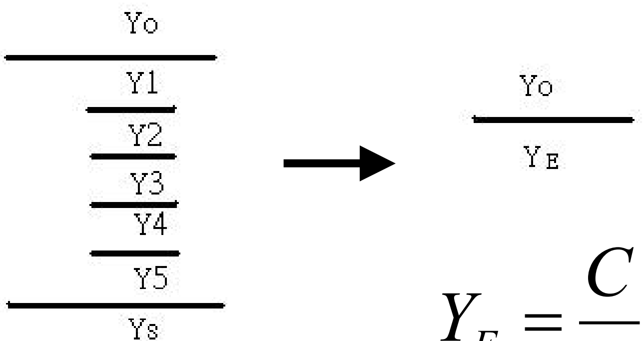
We can use it to determine the reflective percentage.

FOR one layer:



$$R=\left(\frac{Y_0-Y_E}{Y_0+Y_E}\right)^2$$

FOR multilayer:



$$\begin{bmatrix} B \\ C \end{bmatrix} = \prod_k^q \begin{bmatrix} \cos\delta & \frac{i}{\eta}\sin\delta \\ i\eta\sin\delta & \cos\delta \end{bmatrix} \begin{bmatrix} 1 \\ Y_s \end{bmatrix}$$

$$Y_E=\frac{C}{B}$$

$$\delta=\frac{2\pi N_1}{\lambda}\cos\theta_1\cdot d_1$$

- 資料來源:thin film optical filters ,Macleod

Introduction -Concept

- 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*.
- SPs can be produced by electrons or by light in the attenuated total reflection (ATR) device.

Introduction – SPR itself and Variations

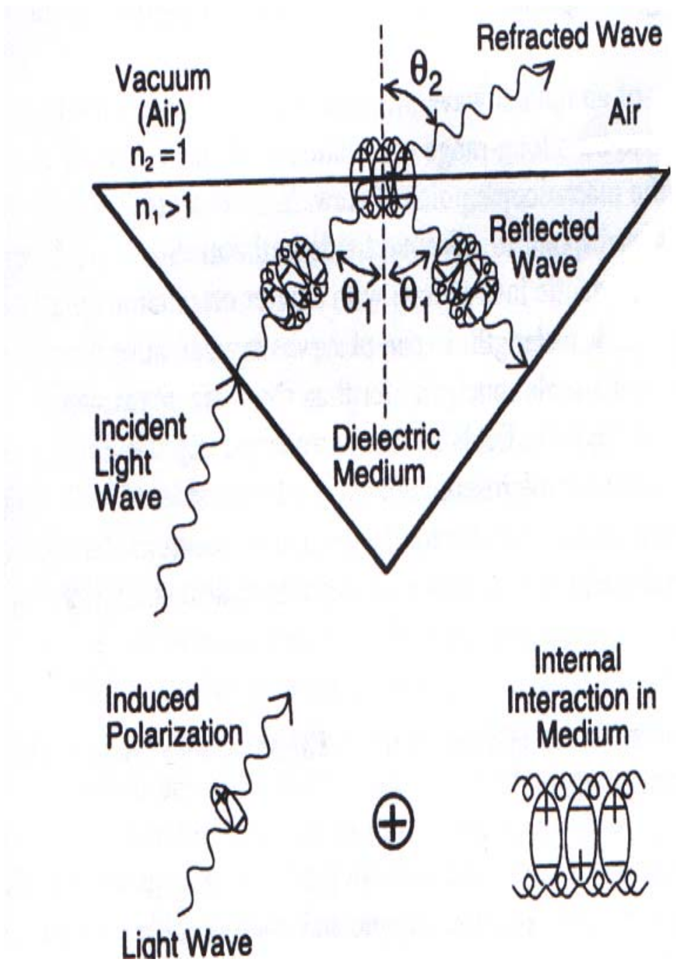
- With the excitation by light, a strong enhancement of the electromagnetic field in the surface (resonance amplification) can emit up to 100 times stronger in the resonance than out of resonance. This enhancement is correlated with a strong reduction of the reflected intensity up to a complete transformation 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

Introduction - Reality

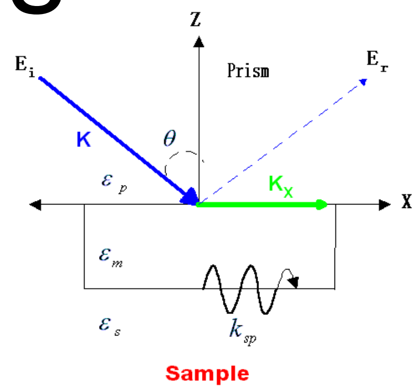
- It is observed by Woods Lamp on metal grating early in the 20th century.
- "Surface Plasmon" appears in 1960s to explain the existence of such a phenomena.
- Definitions of Surface Plasmon
  - A quantized oscillation of an electron on a planar surface of a metallic film and dielectric interface
- It can be excited by various forms of energy, e.g. optical, electrical, chemical.
  - Surface plasmon is excited by a resonant interaction, momentum match condition ($K_{sp}=K_x$) 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.

Introduction – Probing the matter

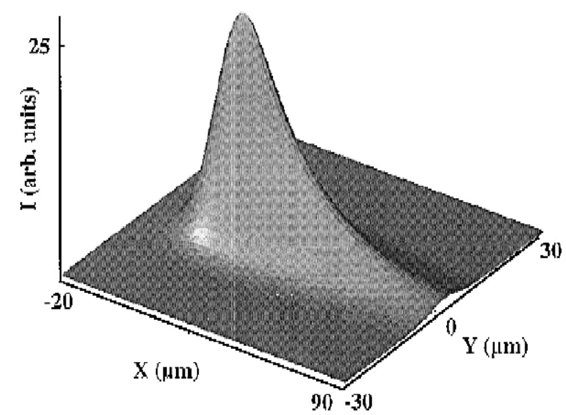
- Electromagnetic Interaction in a Dielectric System
- Light propagating in a dielectric medium induces polarization in dielectric medium.
- The total energy & momentum transport in the medium in the form of a coupled mode of electromagnetic field with matter.
- No labelling of the biomolecules is necessary for their detection.
- SPR allows the measurement of the kinetics of biomolecular interactions in real time with a high degree of sensitivity



Introduction - Behaviors



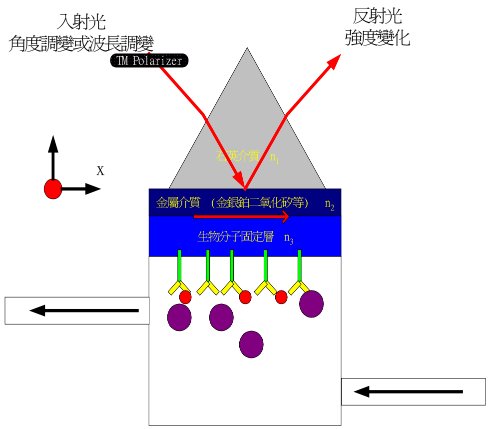
- Propagation length of the SP
  - In z direction intensity decrease to 1/e, $L=1/k_z$, (@600 nm, air:280 nm, Au:31 nm)
  - In x direction intensity decrease to 1/e, (@ 515 nm, ~22um)
- Material dielectric constant
  - Gold →-72+i2
  - Silver →-81+i5
  - Copper →-72+i7
  - Aluminum →-173+i32

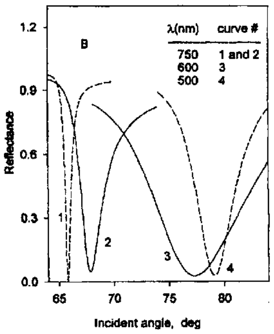


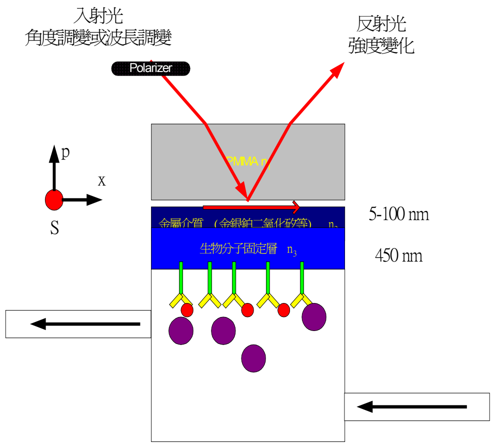
Metal	Ag		Au	
Wavele ngth nm	630	850	630	850
x (um)	19	57	3	24
- Z (nm)	24	23	29	25
+ Z (nm)	219	443	162	400

Introduction - Systems

- 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 dielectric layer (SiO_2) (~ 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





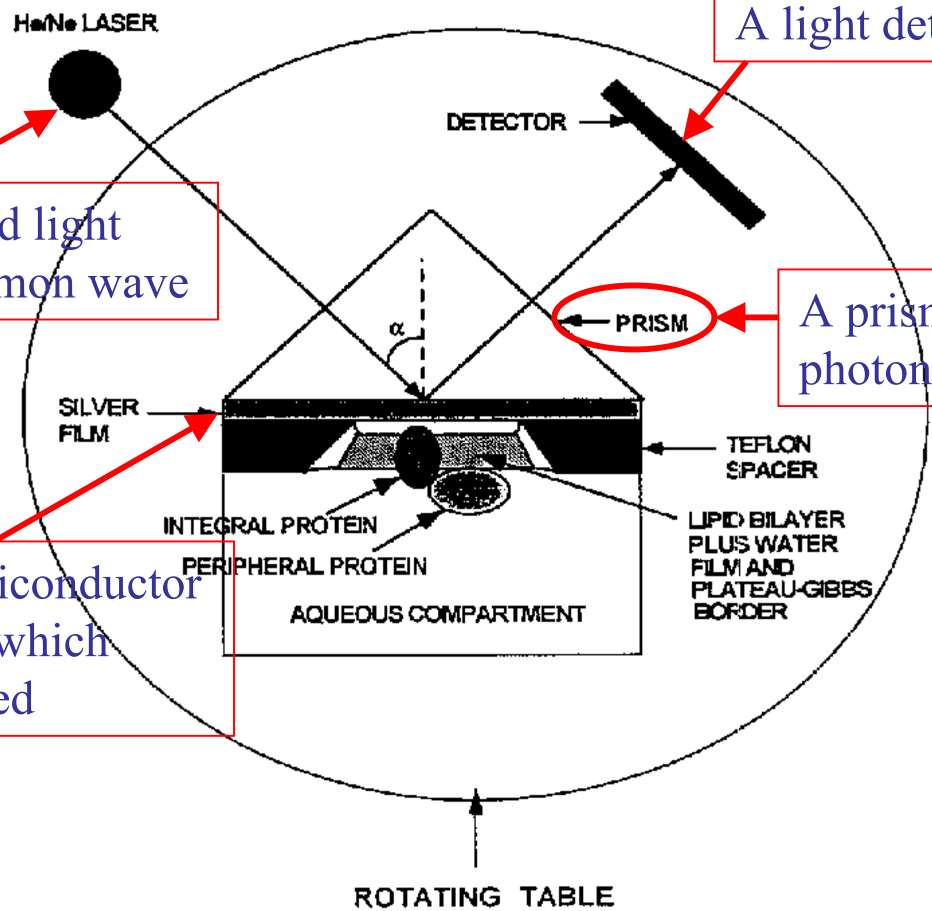


A light detector

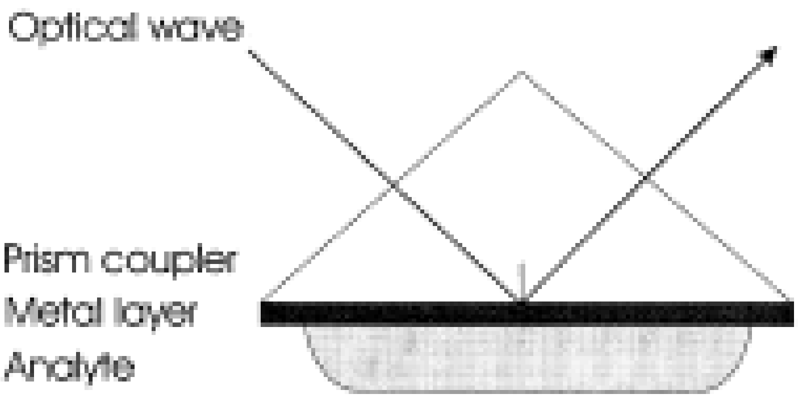
A source of p-polarized light used to excite the plasmon wave

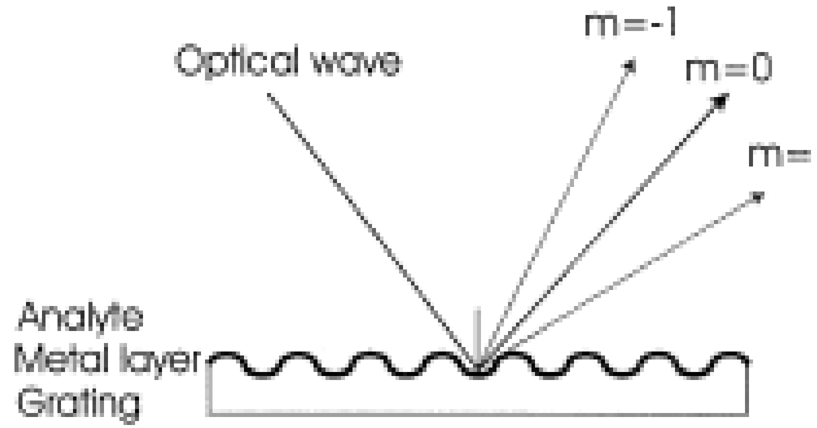
A prism which couples light photons to plasmons

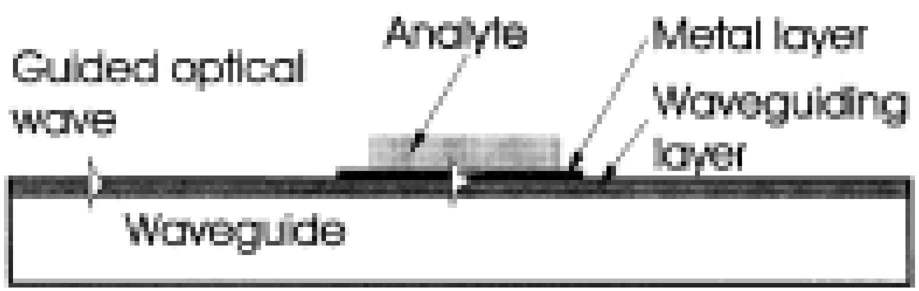
A thin metallic or semiconductor film on the surface of which plasmons can be excited



Modes of excition



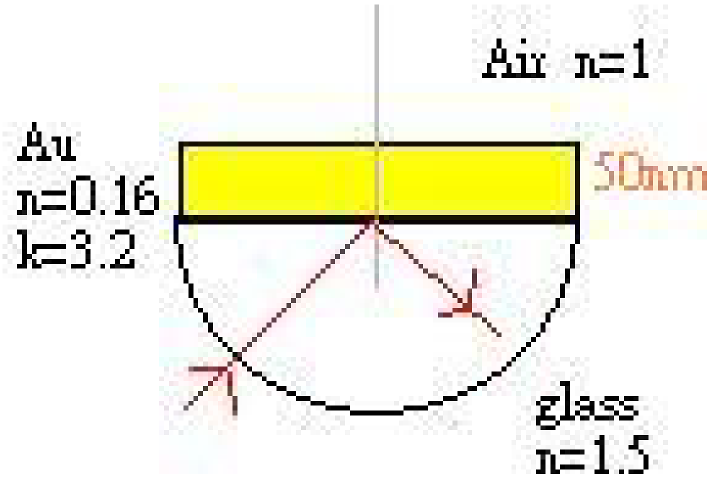


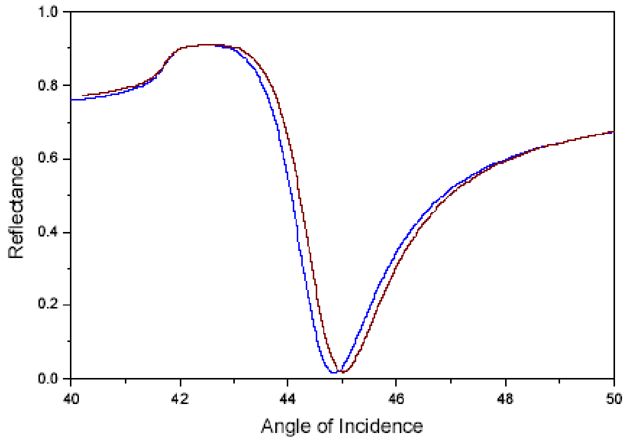


Modes of SPR Detection

- 角度變化(最常用)
- 波長變化
- 強度變化
- 相位變化(最準確)

Angular interrogation	
Sensitivity (deg RIU^{-1})/ Resolution (RIU)[b]	
$\lambda = 630$ nm	$\lambda = 850$ nm
191	97
5×10^{-7}	1×10^{-6}





Introduction – Measurement Modes

- Intensity[1]
- Momentum[2]
- Phase[3]
- Polarization[4]
- Wavelength[5]
- Image[6]
- Prism coupler
- Grating coupler
- Fiber
- Wave guide[7]
- Dielectric coupler[8]

1) B. Liedberg et.al., Sen. Act. B, 4: 299-304, 1983
2) K. Matsubara et.al., Appl. Opt. 27: 1160-1163, 1988
3) S.G. Nelson et.al, Sen. Act. B, 35: 187-191, 1996
4) A.A. Kruchinin et.al., Sen. Act. B 30: 77-80, 1996
5) L.M. Zhang et.al., Electron. Lett. 23: 1469-1470, 1988
6) C. E. Jordan et.al., Anal. Chem., 69: 1449-1456, 1997.
7) A. Miliou et.al., IEEE J Quantum Electron, 25: 1889-1897, 1989
8) Z. Solomon et.al., Biophy., 73:2791-7, 1997

Introduction - Applications

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.

Volume Mass Density	$d = M / A[(n_{av}^2 - 1)/(n_{av}^2 + 2)]$ $\quad n_{av}^2 = (n_p^2 + 2n_s^2)/3$
Surface Mass Density	$m = dt = 0.1M / At[(n_{av}^2 - 1)/(n_{av}^2 + 2)]$
Thickness, t (nm)	$k = \beta(c\lambda / 4\pi)$
Heterogeneous mixtures (Lorentz-Lorenz relation)	$m_p = 0.3tf(n)(n - n_b)/[A_p / M_p - V_p(n_b^2 - 1)/(n_b^2 + 2)]$ $f(n) = (n + n_b)/(n^2 + 2)(n_b^2 + 2)$

- Maxwell's Equations

$$\nabla \times \overline{H} = \frac{4\pi \overline{j}}{c} + \frac{1}{c}\frac{\partial \overline{D}}{\partial t}$$

$$\nabla \times \overline{E} = -\frac{1}{c}\frac{\partial \overline{B}}{\partial t}$$

$$\nabla \cdot \overline{D} = 4\pi\rho$$

$$\nabla \cdot \overline{B} = 0$$

- Source Free Medium

$$\nabla^2 \overline{E} = \frac{\varepsilon\mu}{c^2}\frac{\partial^2 \overline{E}}{\partial^2 t} + \frac{4\pi\sigma\mu}{c^2}\frac{\partial \overline{E}}{\partial t}$$

$$\frac{c^2}{v^2} = \varepsilon\mu - i\frac{4\pi\sigma\mu}{\omega}$$

- Solution

$$\overline{E} = \overline{E}_0 e^{i\omega(t - x/v)}$$

$$N^2 = \varepsilon\mu - i\frac{4\pi\sigma\mu}{\omega}$$

- Solution

$$\overline{E} = \overline{E}_0 \exp[i(\omega t - 2\pi N x/\lambda)]$$

A plane wave, with wavelength λ , traveling along x-axis

- A plane wave from the direction given by direction cosines (α,β,γ)

$$\overline{E} = E_0 \exp\{i[\omega t - \frac{2\pi N}{\lambda}(\alpha x + \beta y + \gamma z)]\}$$

$$\overline{E} = E_0 \exp(-\frac{2\pi k}{\lambda}x)\exp[i(\omega t - \frac{2\pi n x}{\lambda})]$$

- Characteristic admittance of medium

$$N = \frac{c}{v} = n - ik \qquad n: refractive\ \ index \qquad k: extinction\ \ coefficient$$

- Boundary condition

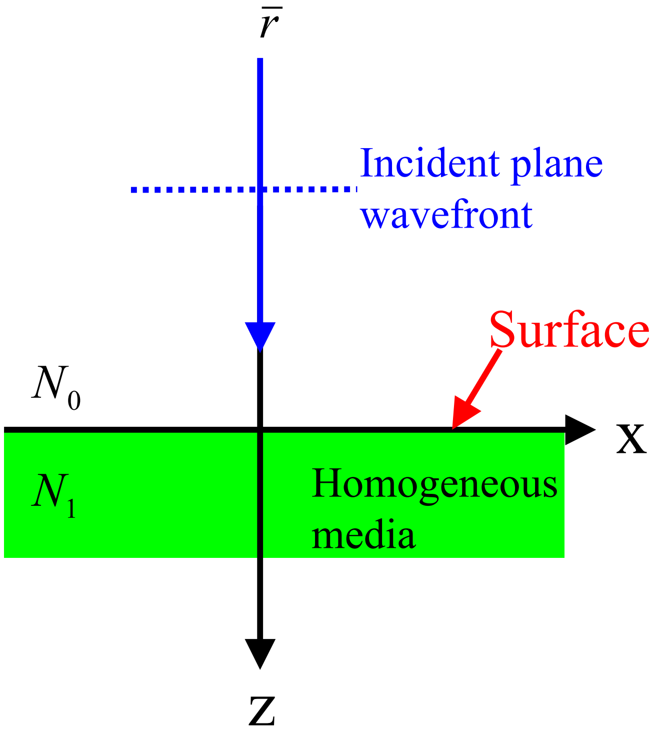
$$\overline{H}_1 = \overline{H}_t \qquad \overline{E}_1 = \overline{E}_t$$

- Normal incident

$$\begin{cases} \overline{E}_1 = \overline{E}_1^+ = \overline{E}_0^+ + \overline{E}_0^- & at \quad z = 0 \\ \overline{H}_1 = \overline{H}_1^+ = \overline{H}_0^+ + \overline{H}_0^- & at \quad z = 0 \end{cases}$$

$$\overline{E}_0^- = \frac{N_0 - N_1}{N_0 + N_1} \overline{E}_0^+$$

r: reflectivity



- **Reflectance**

$$R = \left(\frac{N_0 - N_1}{N_0 + N_1}\right)\left(\frac{N_0 - N_1}{N_0 + N_1}\right)^* \Rightarrow R = \left|\frac{N_0 - N_1}{N_0 + N_1}\right|^2$$

- TM (p) and TE (s) incident wave

√-$\overline{E}$ in the plane of incidence

(TM or p-polarized wave)

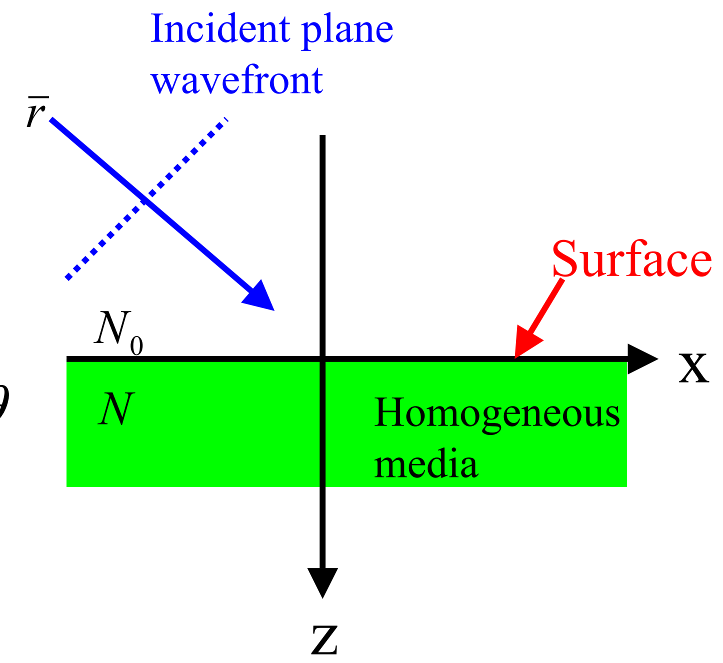
\- $\overline{E}$ normal to the plane of incidence

(TE or s-polarized wave)

- TM (p) incident wave - $\overline{E}$ is titled at θ

$$\left|\overline{E}^{+}_{\tan}\right| = \left|\overline{E}^{+}\cos\theta\right|$$

$$\eta_M = \frac{N}{\cos\theta}$$



- reflectivity

$$r = \frac{\eta_0 - \eta_1}{\eta_0 + \eta_1}$$

$$\eta_1 = \frac{N_1}{\cos\theta_1}$$

- Snell's law

$$N_0 \sin\theta_0 = N_1 \sin\theta_1$$

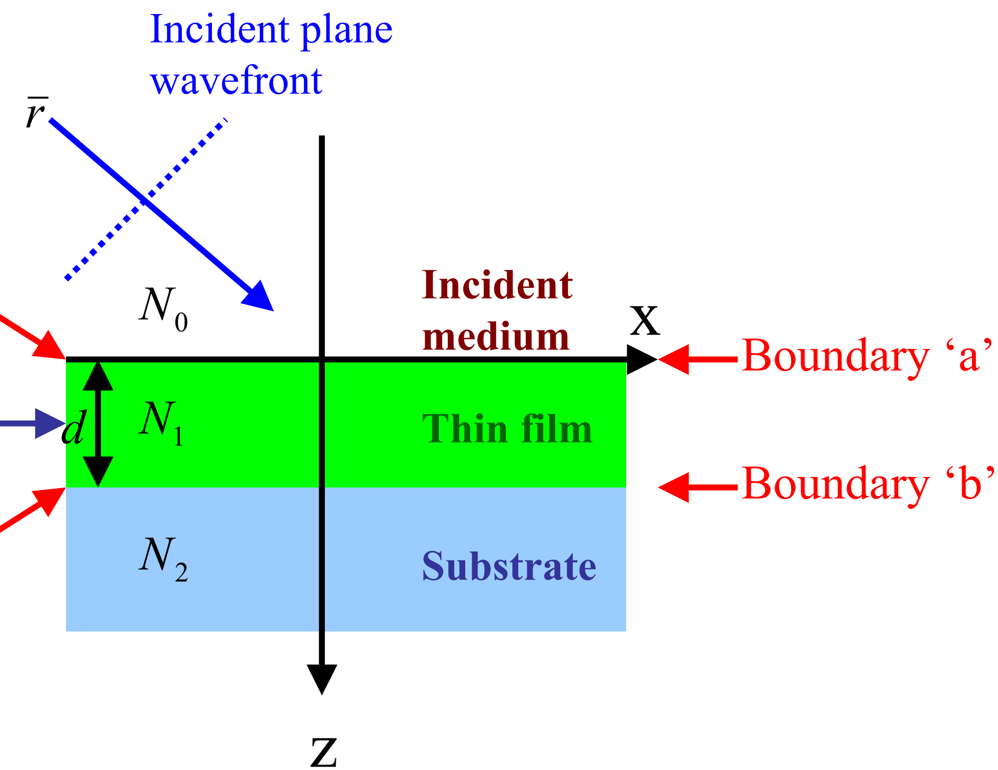
$$\overline{E}_{1a}^{+} = \overline{E}_{1b}^{+} e^{j\delta_1} \qquad \overline{E}_{1a}^{-} = \overline{E}_{1b}^{-} e^{-j\delta_1}$$

$$\overline{H}_{1a}^{+} = \overline{H}_{1b}^{+} e^{j\delta_1} \qquad \overline{H}_{1a}^{-} = \overline{H}_{1b}^{-} e^{-j\delta_1}$$

$$\delta = \frac{2\pi N_1}{\lambda} \cos\theta_1 d$$

$$\overline{H}_b = \overline{H}_{1b}^{+} + \overline{H}_{1b}^{-}$$

$$\overline{E}_b = \overline{E}_{1b}^{+} + \overline{E}_{1b}^{-}$$



$$\begin{bmatrix} \overline{k} \times \overline{E}_a \\ \overline{H}_a \end{bmatrix} = \begin{bmatrix} \cos\delta_1 & (i\sin\delta_1)/\eta_1 \\ i\eta_1 \sin\delta_1 & \cos\delta_1 \end{bmatrix} \begin{bmatrix} \overline{k} \times \overline{E}_b \\ \overline{H}_b \end{bmatrix} \Rightarrow \begin{bmatrix} B \\ C \end{bmatrix} = \begin{bmatrix} \cos\delta_1 & (i\sin\delta_1)/\eta_1 \\ i\eta_1 \sin\delta_1 & \cos\delta_1 \end{bmatrix} \begin{bmatrix} 1 \\ \eta_2 \end{bmatrix}$$

- medium admittance

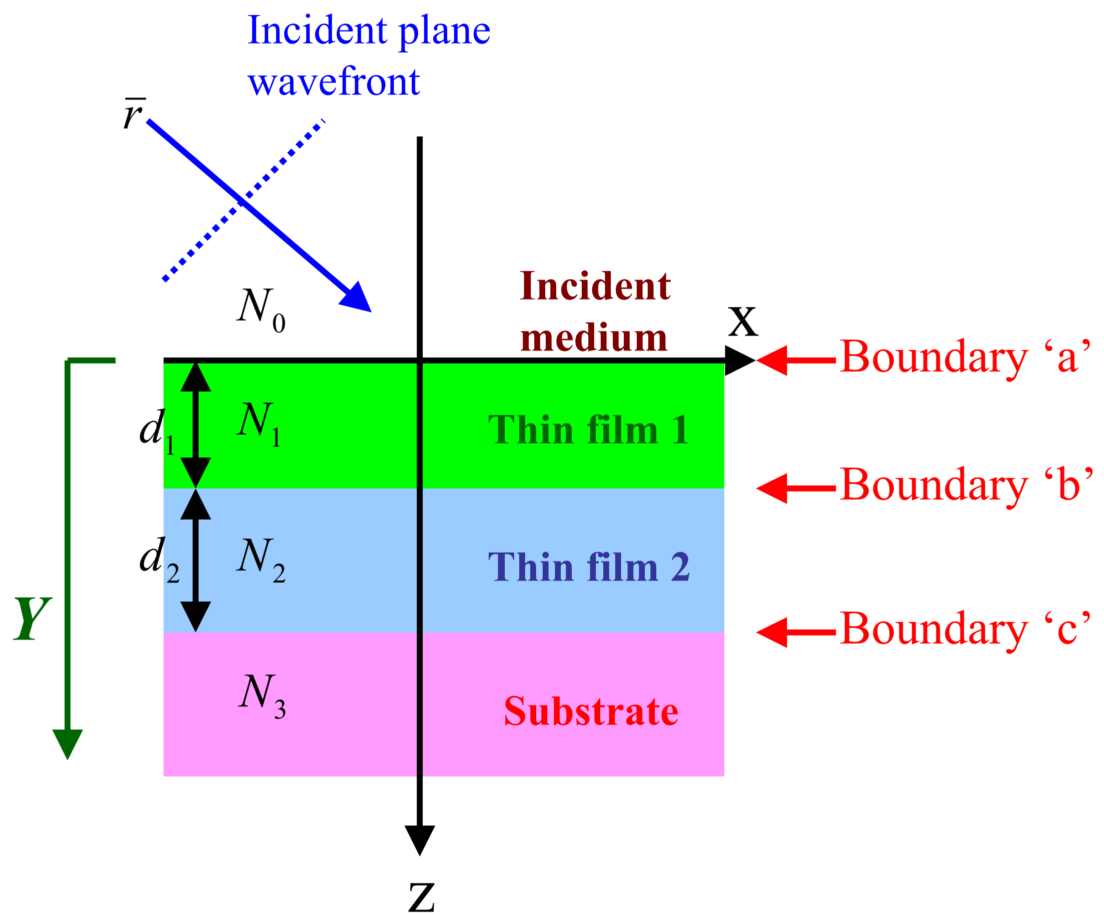
$$Y = \frac{C}{B}$$

- reflectivity

$$r = \frac{\eta_0 - Y}{\eta_0 + Y}$$

- Reflectance

$$R = \left| \frac{\eta_0 - Y}{\eta_0 + Y} \right|^2$$



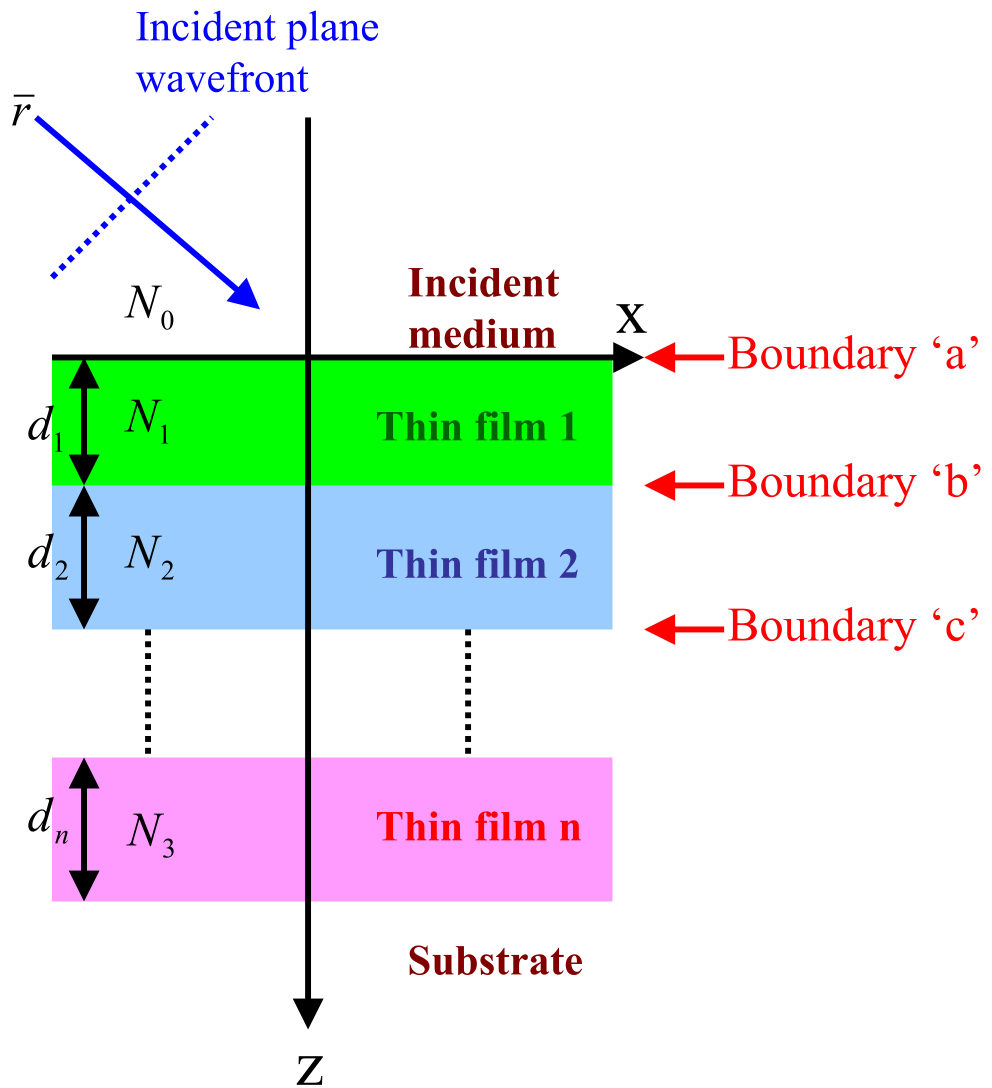
$$\begin{bmatrix} B \\ C \end{bmatrix} \rightarrow \begin{bmatrix} \bar{k} \times \bar{E}_a \\ \bar{H}_a \end{bmatrix} = \begin{bmatrix} \cos\delta_1 & (i\sin\delta_1)/\eta_1 \\ i\eta_1 \sin\delta_1 & \cos\delta_1 \end{bmatrix} \begin{bmatrix} \cos\delta_2 & (i\sin\delta_2)/\eta_2 \\ i\eta_2 \sin\delta_2 & \cos\delta_2 \end{bmatrix} \begin{bmatrix} \bar{k} \times \bar{E}_c \\ \bar{H}_c \end{bmatrix} \leftarrow \begin{bmatrix} 1 \\ \eta_3 \end{bmatrix}$$

$$\begin{bmatrix} B \\ C \end{bmatrix} = \left\{ \prod_{r=1}^{n} \begin{bmatrix} \cos\delta_r & (i\sin\delta_r)/\eta_r \\ i\eta_r \sin\delta_r & \cos\delta_r \end{bmatrix} \right\} \begin{bmatrix} 1 \\ \eta_{n+1} \end{bmatrix}$$

where $$\delta_r = \frac{2\pi d_r \cos\theta_r}{\lambda}$$

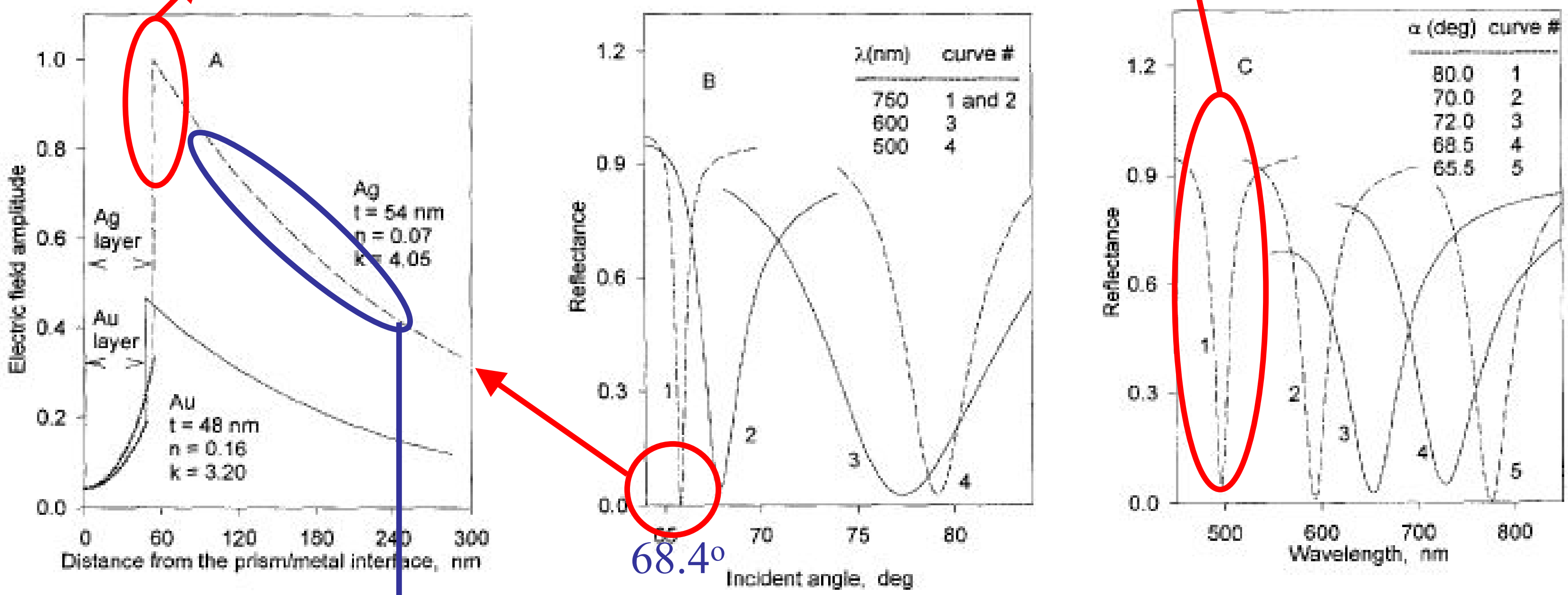
$$\eta_r = \frac{N_r}{\cos\theta_r}$$

$$N_0 \sin\theta_0 = N_r \sin\theta_r$$



Enhanced at the metal/dieletric interface

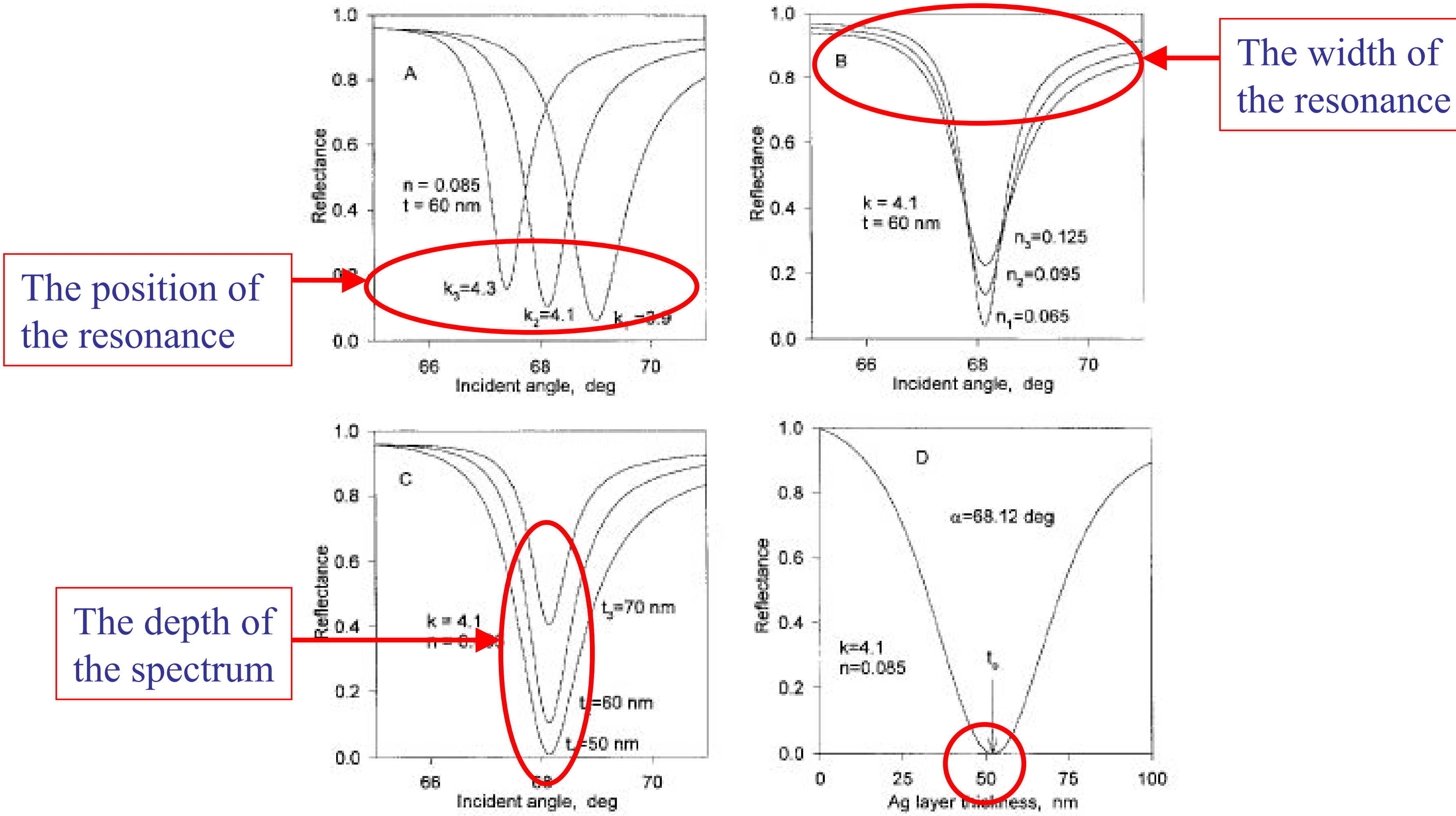
Silver has much narrower and deeper resonance spectra



Rapidly decays with increasing distance from the metal surface

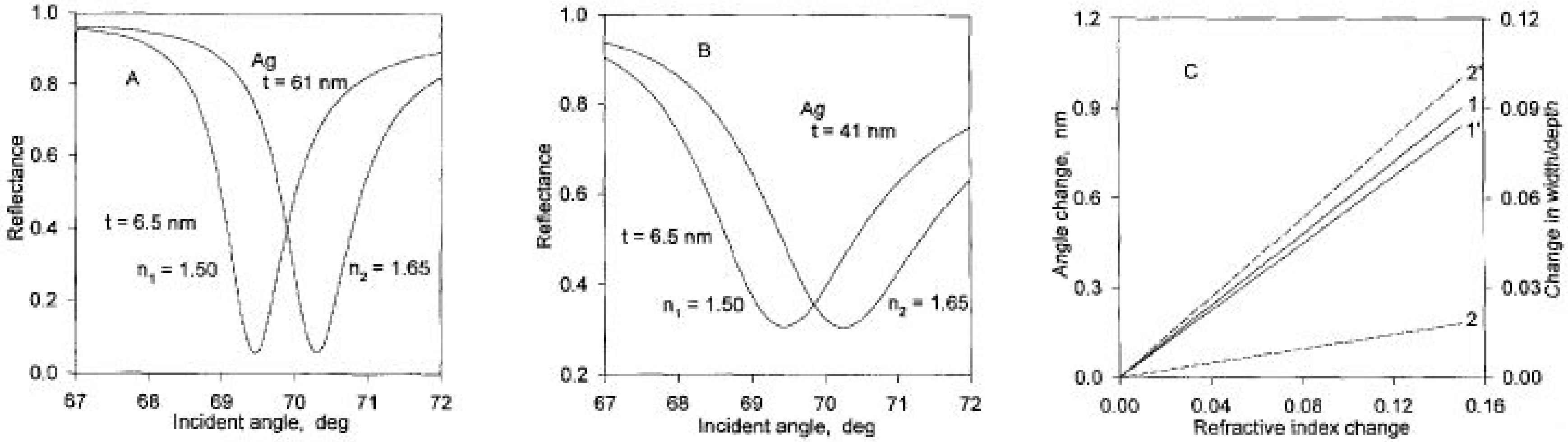
Increasing wavelength being equivalent to increasing angle of incidence

- A single metal layer with no additional deposited dielectric layer

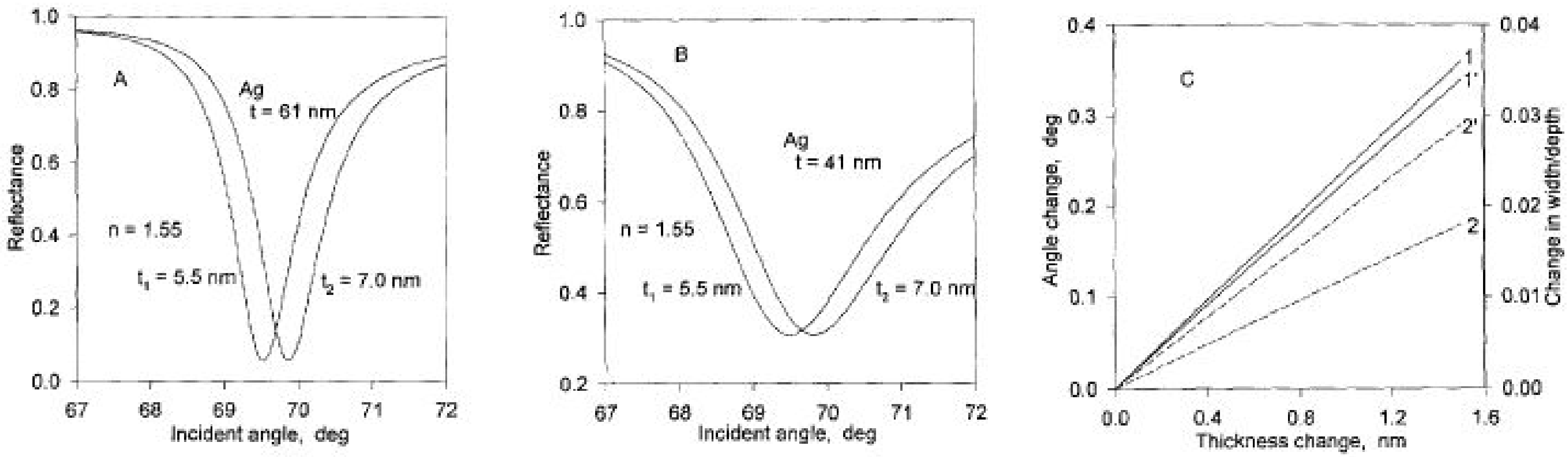


• SPR spectra obtained with non-light absorbing dielectric layer (k=0)

SPR resonance vs. *n* of deposited dielectric layer

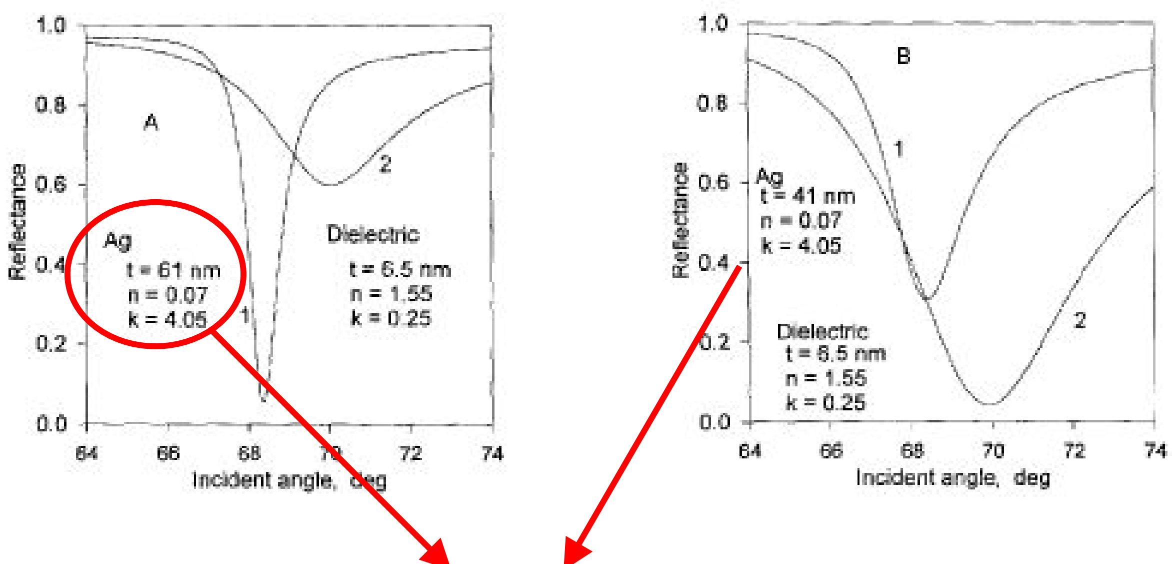


SPR resonance vs. *t* of deposited dielectric layer



NTU Nano-BioMEMS Group

• Influence of optical parameters on SPR spectra obtained with light-absorbing dielectric layer

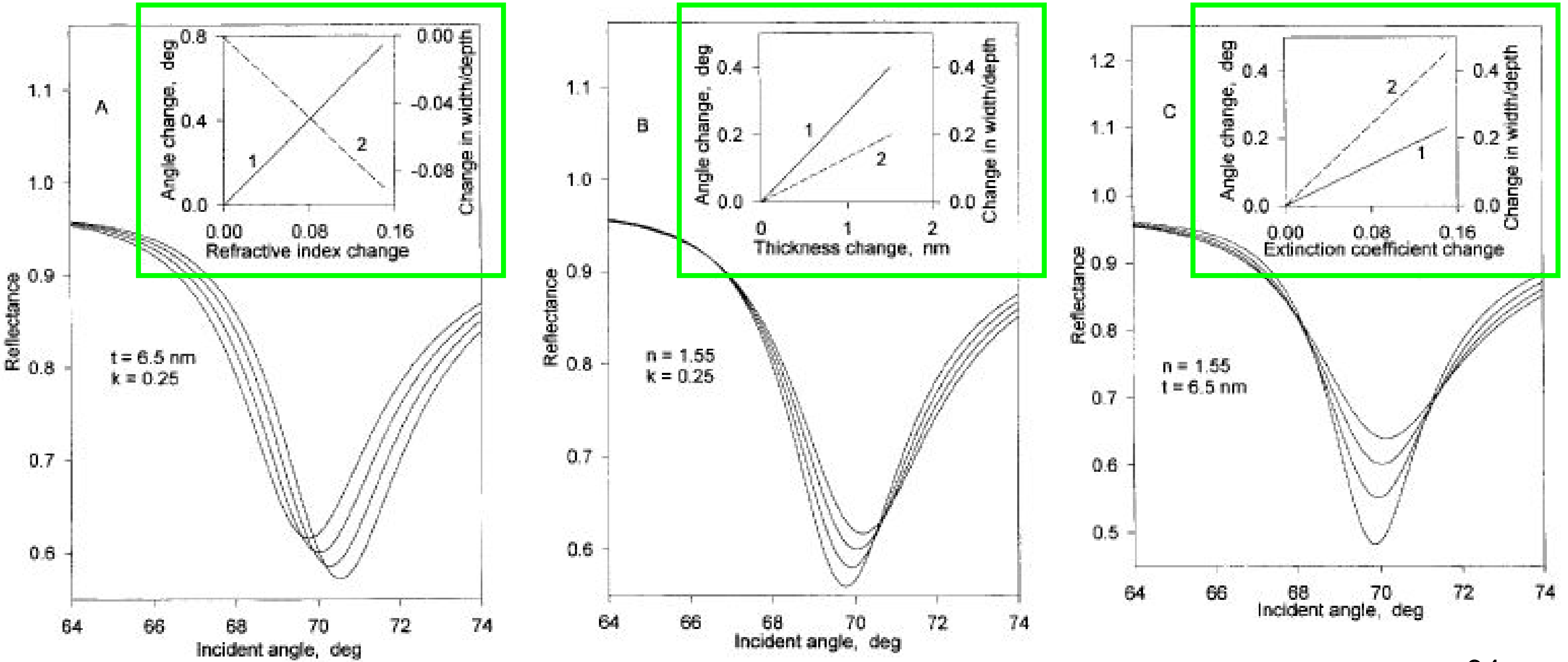


A non-zero k parameter has opposite effects on the resonance depending upon whether the metal film is thicker or thinner than t_o

• The effect of changes in each parameter on the SPR spectrum

a silver film of 61nm thickness

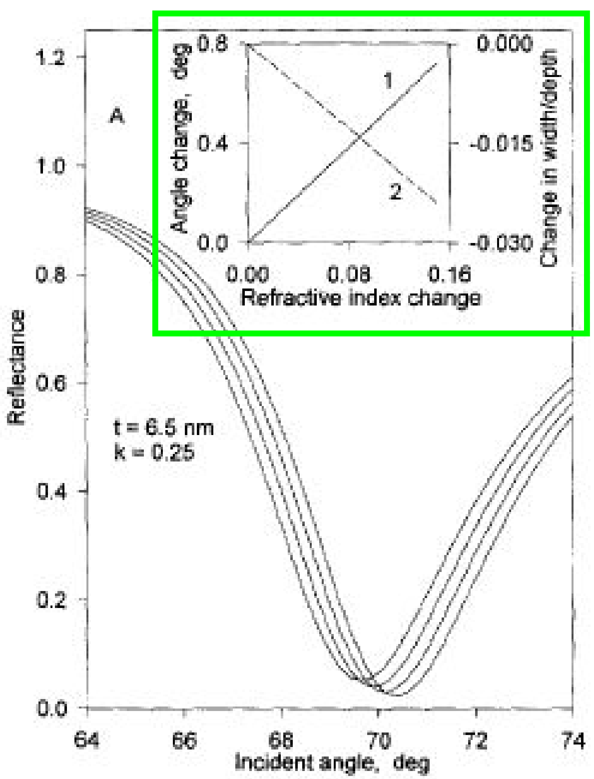
Changing *n* | Changing *t* | Changing *k*



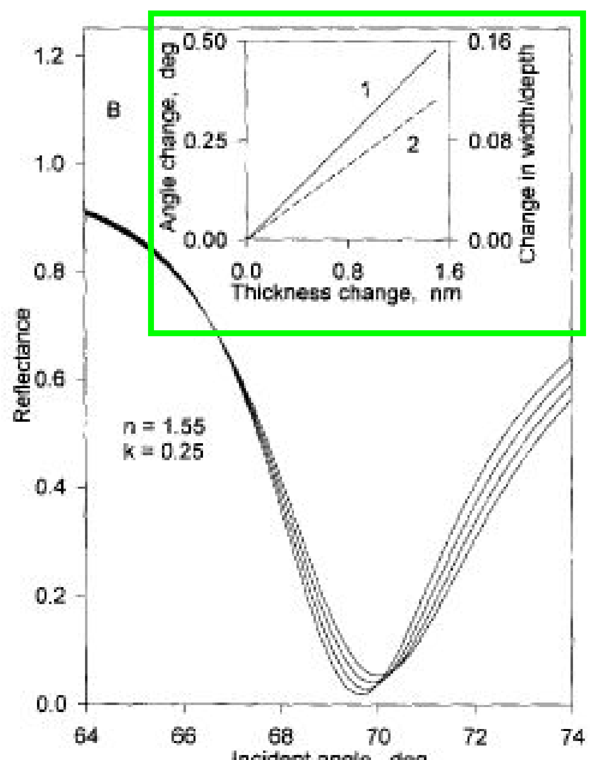
- The effect of changes in each parameter on the SPR spectrum

a silver film of 41nm thickness

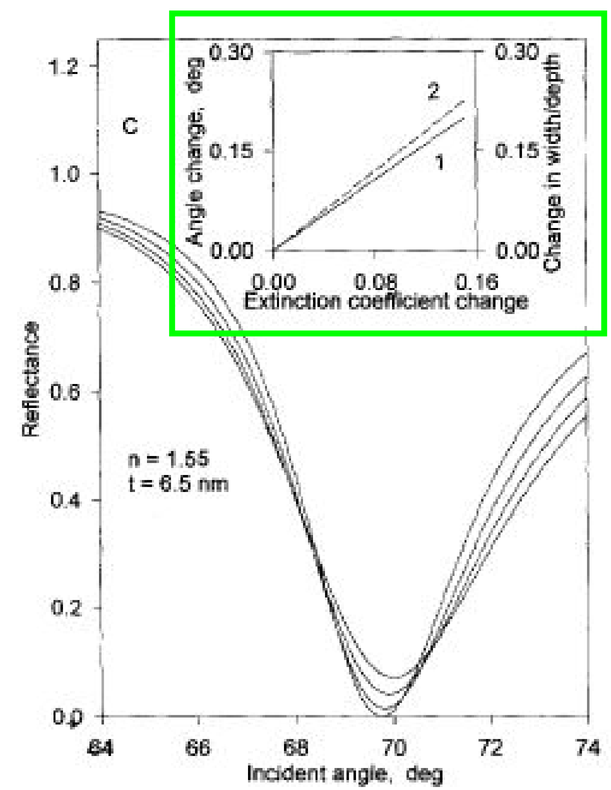
Changing *n*



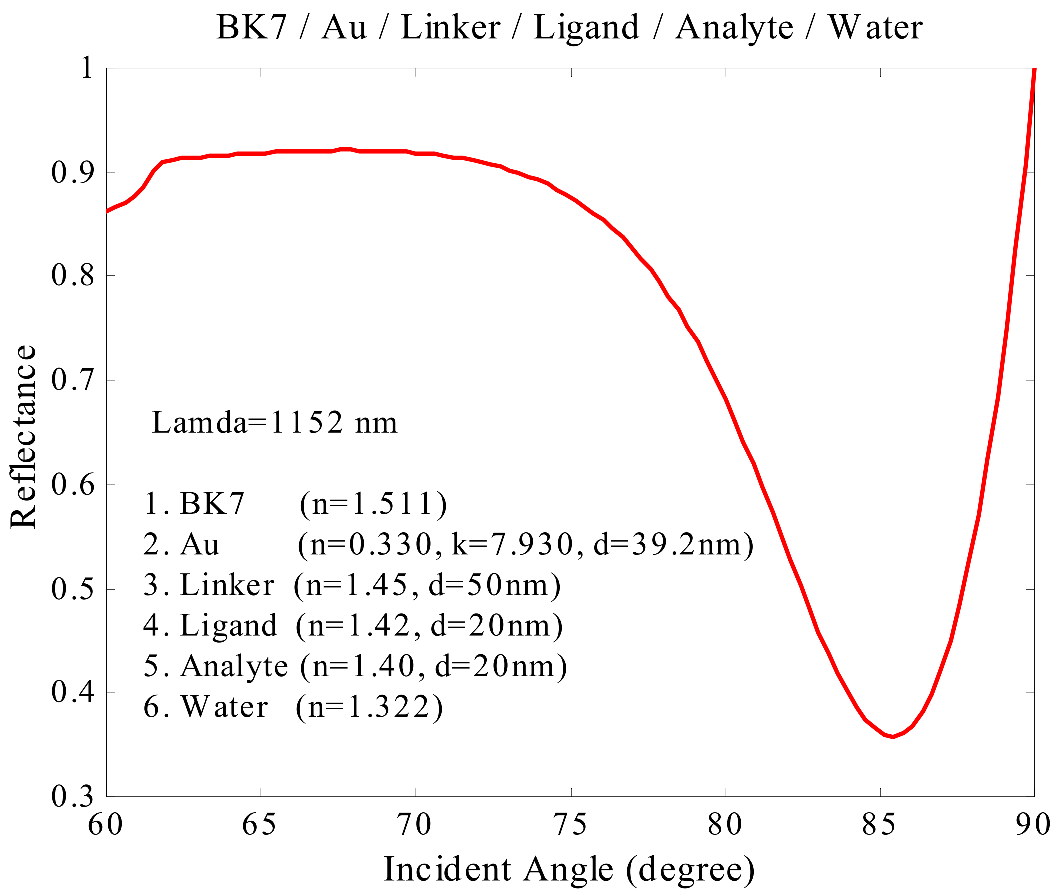
Changing *t*

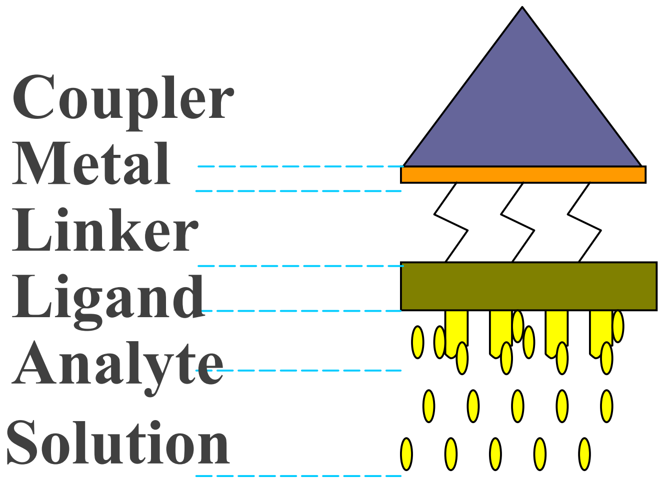


Changing *k*

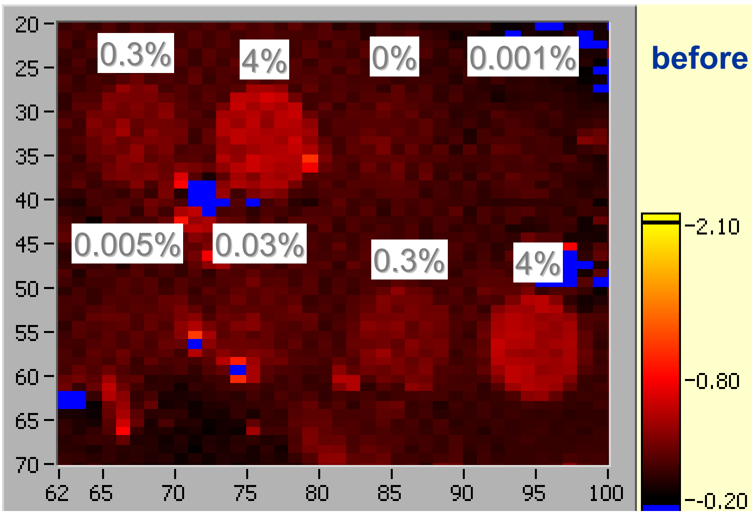


Simulation results

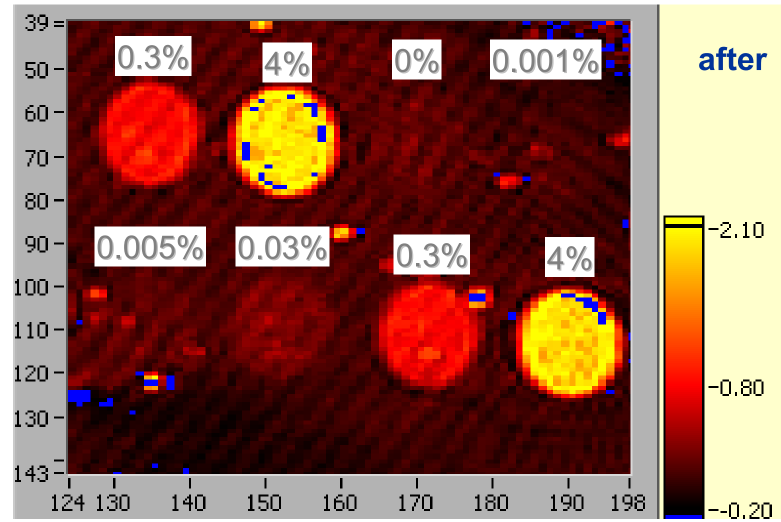




IMAGING SURFACE PLASMON RESONANCE (SPR) FOR BIOCHIPS



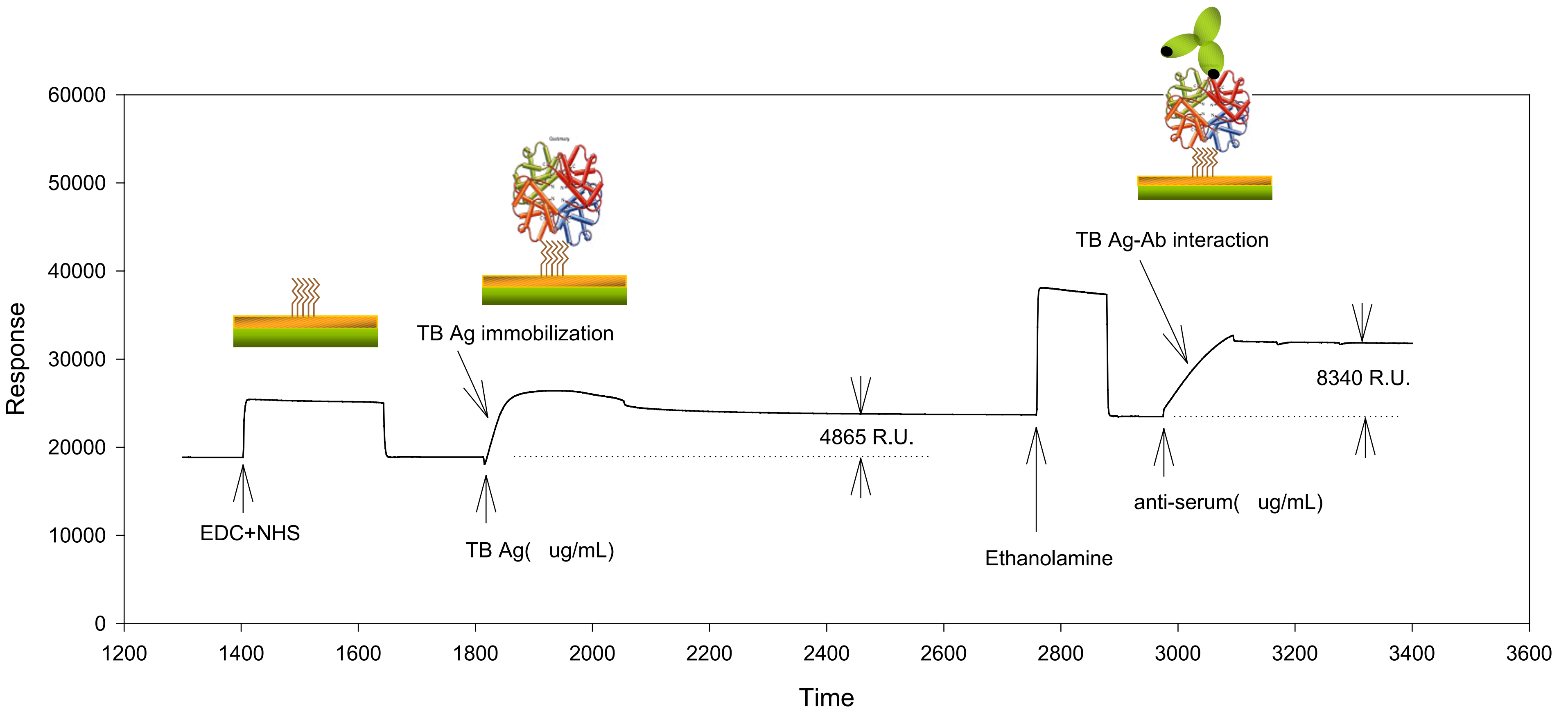
Before the Binding of avidin to immobilized biotin
(The biotin concentration varies between 0 and 4%)



After the Binding of avidin to immobilized biotin
(The biotin concentration varies between 0 and 4%)

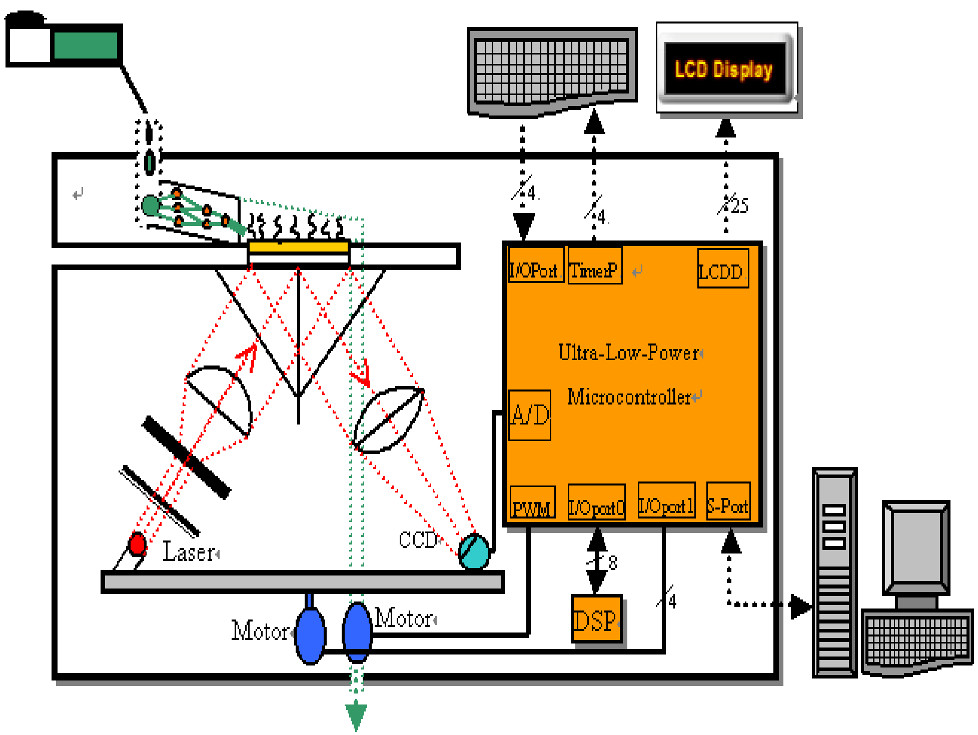
Data from EP3

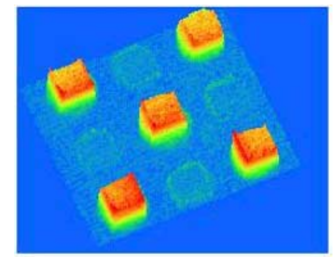
Ab –Ag (ESAT-6) interaction kinetics by SPR Sensorgram

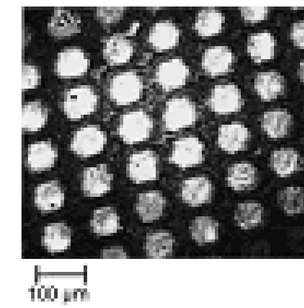


SPR現今的應用和未來的發展

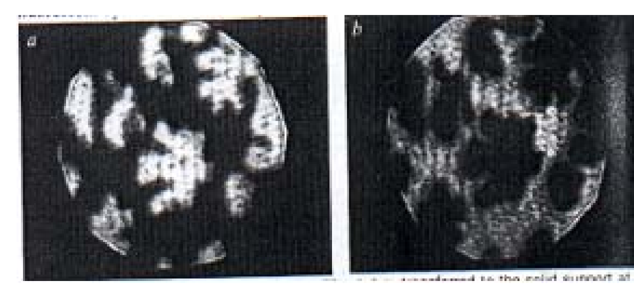
- 1.影像式SPR.



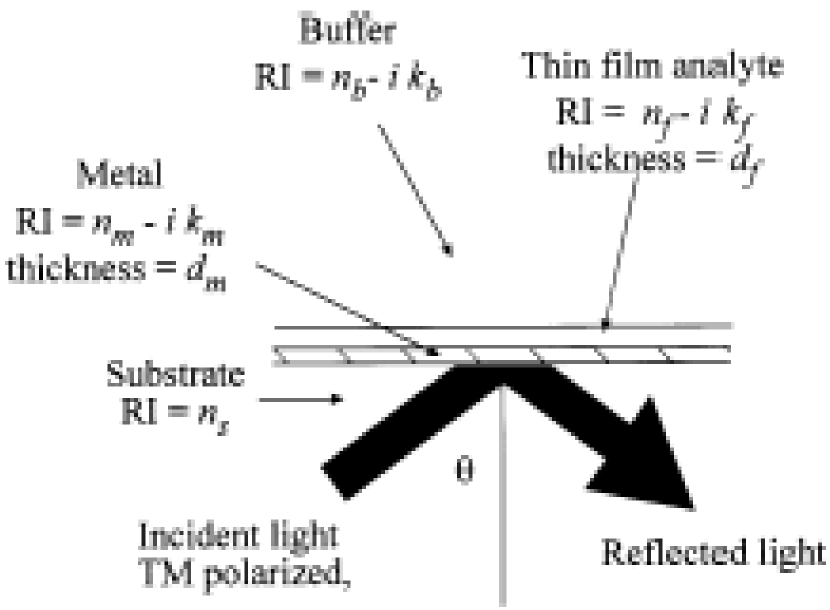




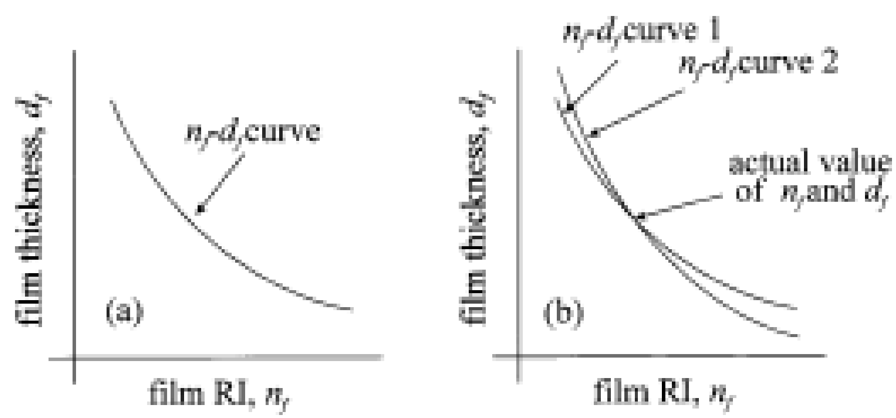
配合螢光顯微術



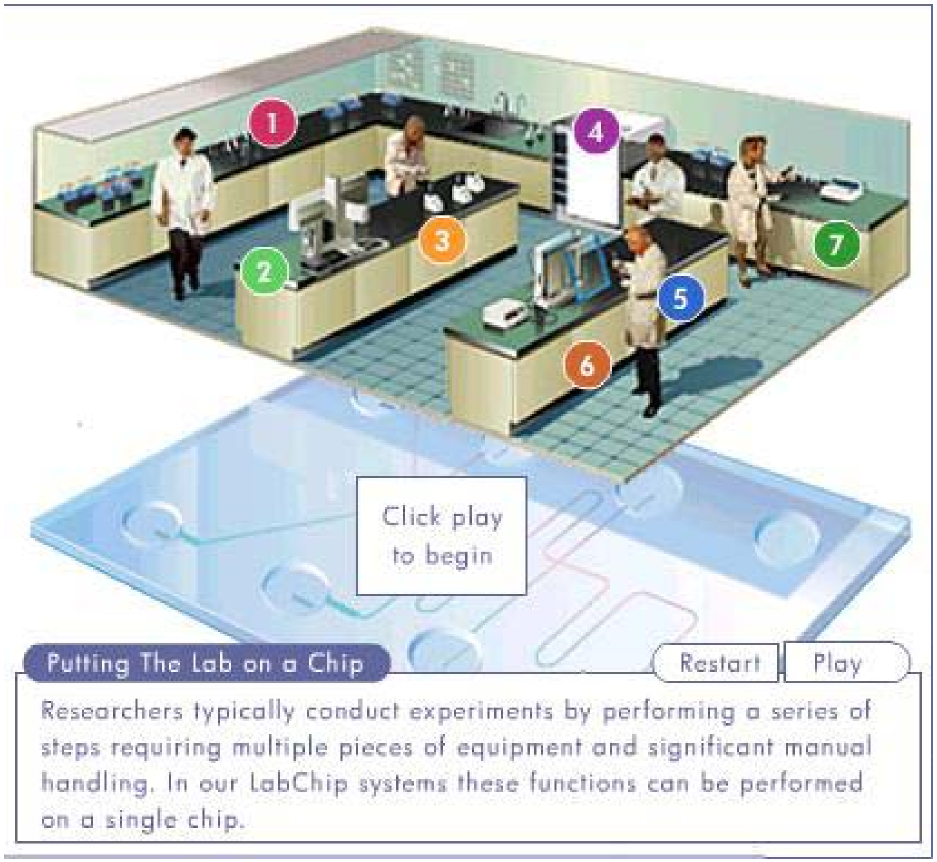
2.計算表面沾黏物質的厚度.

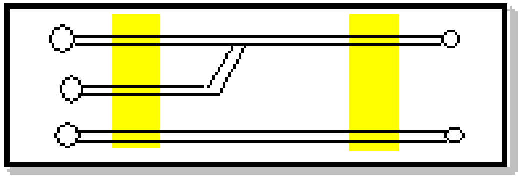


$$\Delta k_{sp} = \frac{\omega^2}{c^2}\frac{(\varepsilon_2\varepsilon_3)^{3/2}}{\varepsilon_2^2-\varepsilon_4^2}\frac{\varepsilon_3-\varepsilon_4}{\varepsilon_3}d_3$$



3.結合微流道.


1
2
3
4
5
6
7
Click play
to begin
Putting The Lab on a Chip
Restart
Play
Researchers typically conduct experiments by performing a series of steps requiring multiple pieces of equipment and significant manual handling. In our LabChip systems these functions can be performed on a single chip.



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