Physics of Life

PHYS-468

Spectroscopy with NMR and SPR

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LBEM, IPHYS, SB, EPFL

NMR Spectrometry

NMR Spectrometer

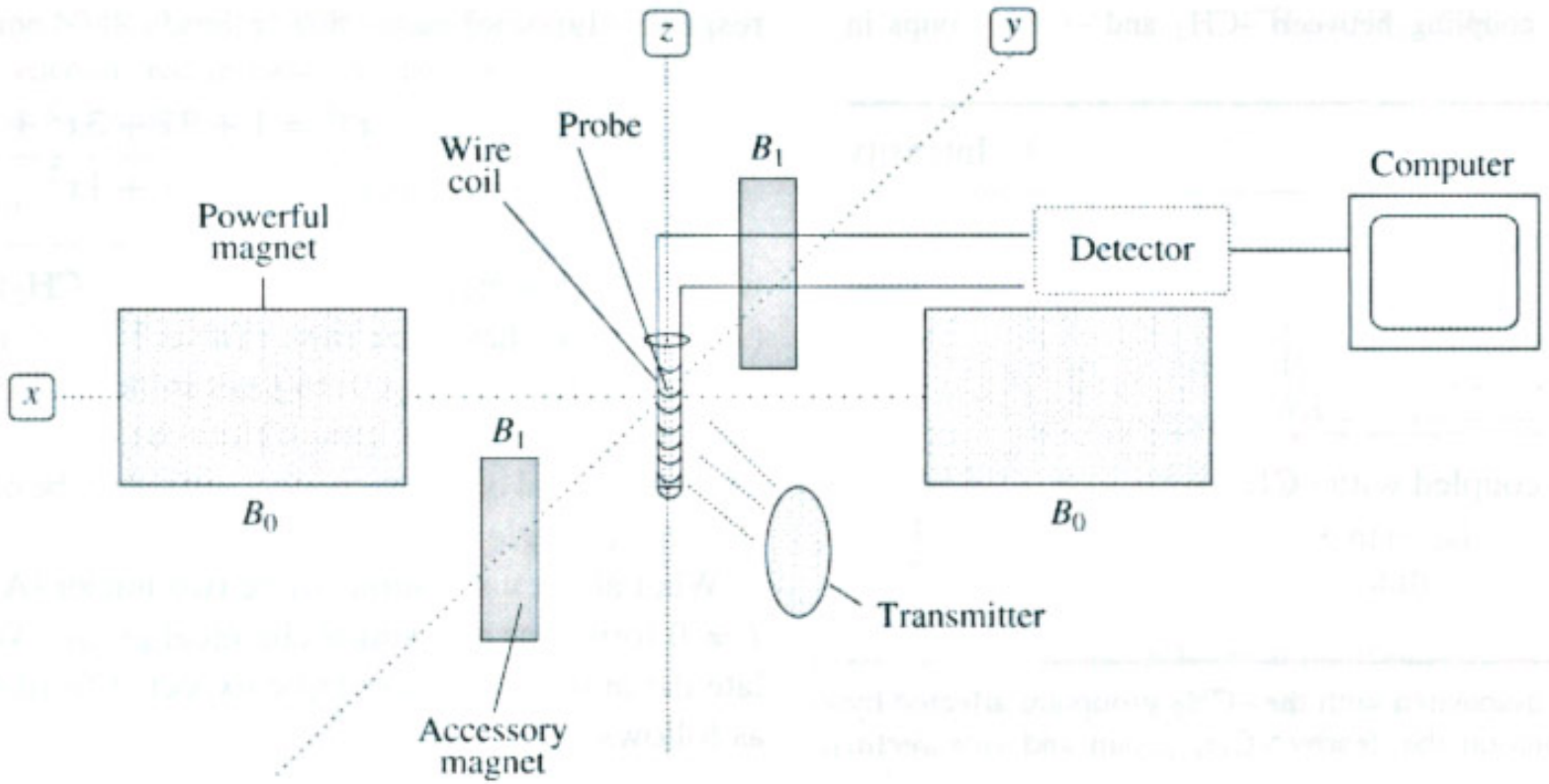

Figure 3.52. Outline design of an NMR spectrometer. The sample is placed in a tube called the probe. This is surrounded by wire coil. A powerful magnetic field (of field strength B_0) is generated in the x-axis. Radiofrequency radiation is generated by the transmitter which generates a magnetic field orientated along the y-axis. When the frequency of this field is the same as the Larmor frequency, the resonance condition is met. Absorption of radiation induces a current in the wire coil along the z-axis which is electronically detected. This current is proportional to the intensity of absorbance.

The accessory magnet typically needs to produce radiowave frequencies (100MHz to 1000MHz), depending on the magnetic B field strength of the machine.

Nuclear Magnetic Resonance (NMR) Spectroscopy

Energy of a wave with frequency ν:

$$E = h \cdot \nu$$

Energy splitting due to magnetic field B:

$$E = \pm \mu \cdot B_0 \cdot \sin\Theta$$

$$\Delta E = 2\mu \cdot B_0 \cdot \sin\Theta$$

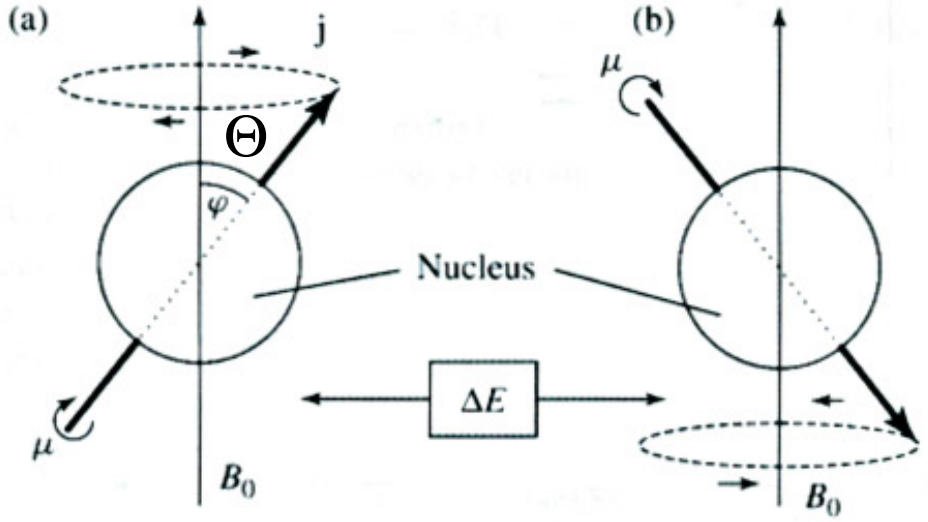

Figure 3.48. Physical basis of NMR. A spinning nucleus generates a magnetic field with a spin moment, μ, which generates an angular momentum, *j*. When placed in an external magnetic field (B_0), the nucleus can align (a) with or (b) against the field. The difference in energy between these orientations is ΔE which corresponds to frequencies in the radiowave part of the spectrum.

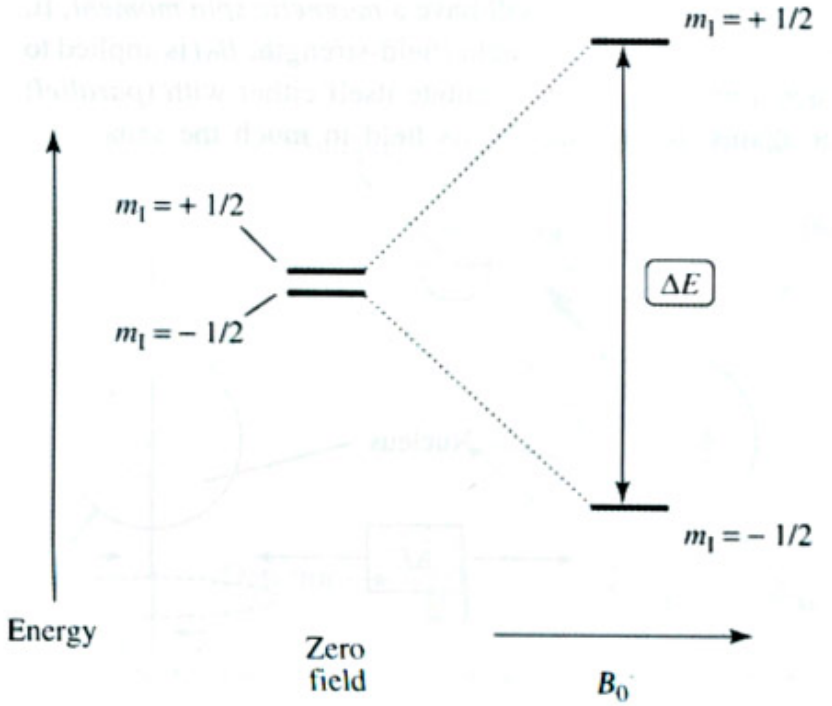

Figure 3.49. Effect of applied magnetic field on spin states. In the absence of an applied magnetic field (i.e. zero field), the energy difference (ΔE) between the two spin states is very small. The stronger the field strength (B_0) of an applied magnetic field becomes the larger ΔE (Equation (3.38)).

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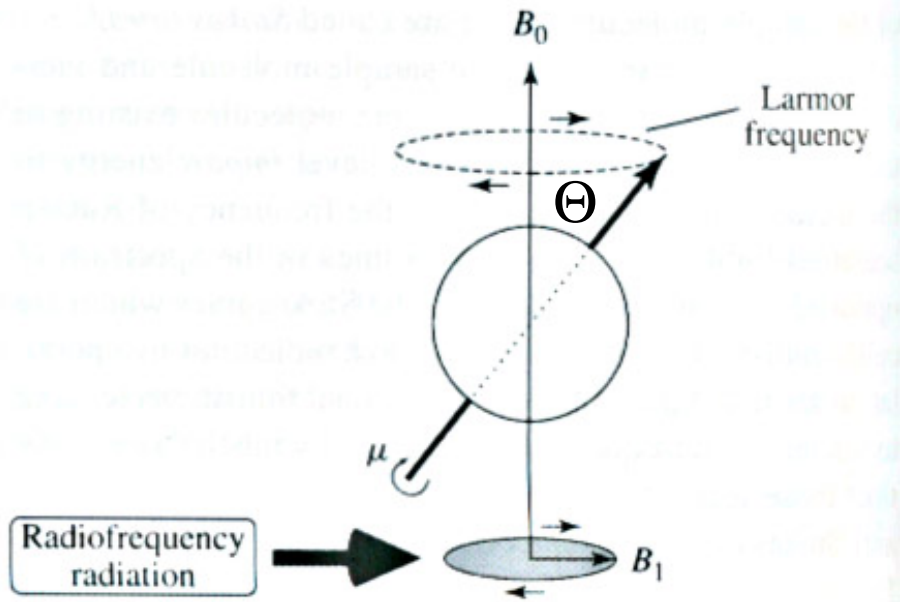

Figure 3.50. The resonance condition. Exposure of the nucleus to radiofrequency radiation sets up a magnetic field (of field strength, B_1, shown in grey) which has a frequency of oscillation. The resonance condition occurs when this frequency equals the Larmor frequency of the spin magnetic moment. Transition between spin states only occurs at the resonance condition.

B_1 = osscillating magnetic field of nucleus

$B_1 << B_0$

Resonance frequency ν of an atom with spin μ in the field B:

$$\nu = \frac{2\mu \cdot B_0 \cdot \sin\Theta}{h}$$

Since μ and sin(Θ) depend on the atom and on the local environment of the atom, the frequency ν is also depending on those factors.

Larmor frequency ω of *resonance condition* is:

$$\omega = 2\pi \cdot \nu$$

Resonance condition for a specific nucleus:
(only for nuclei with spin,
i.e., uneven atomic mass or uneven atomic number,
such as ^{1}H, ^{13}C, or ^{31}P)

$$\Delta E = \frac{\gamma}{2\pi} \cdot B_0$$

B_0 = applied magnetic field

μ = magnetization

γ = gyromagnetic ratio of the nucleus (a constant)

Nuclear Magnetic Resonance (NMR) Spectroscopy

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Table 3.6. Magnetic properties of some nuclei important in biochemistry

Nucleus	I	Natural abundance (%)	γ rad·s^{-1} T^{-1}	**Resonance Frequency in B field of 14.092 Tesla (MHz)**
^{1}H	1/2	99.98	26.752	**600.0**
^{2}H	1	0.015	4.107	**92.1**
^{12}C	0	98.9	—	**–**
^{13}C	1/2	1.10	6.7283	**150.9**
^{14}N	1	99.63	1.9338	**43.3**
^{16}O	0	99.76	—	**–**
^{32}S	0	95.02	—	**–**
^{31}P	1/2	100	10.8394	**242.9**
^{35}Cl	3/2	75.77	2.642	**58.8**
^{15}N	1/2	0.37	−2.7126	**60.8**

I = net spin of nucleus

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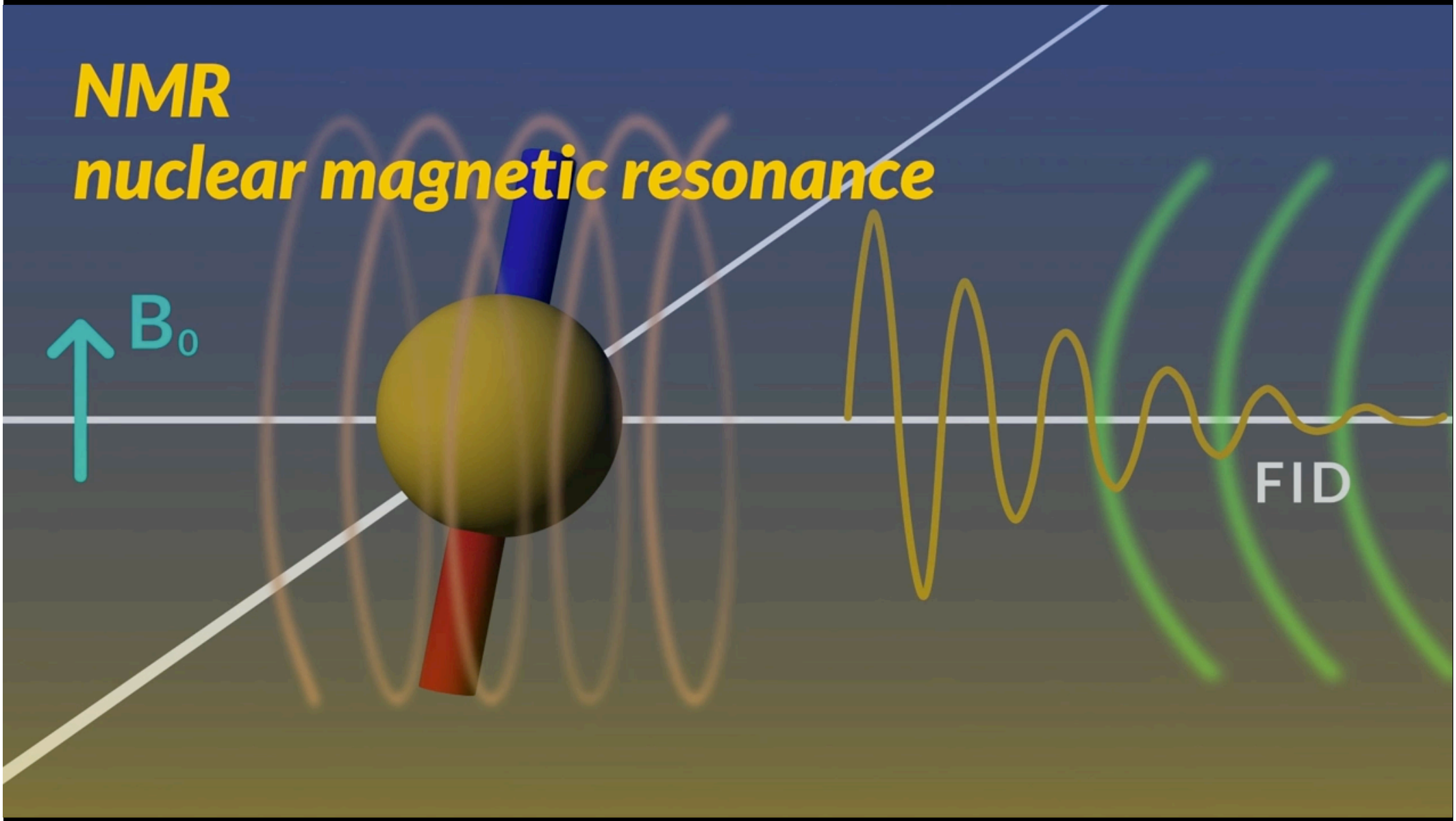
Proton NMR Spectroscopy



https://www.youtube.com/watch?v=RZLew6Ff-JE

NMR: Chemical Shielding

Chemical shielding of magnetic field is effective over distances of 3-8Å only.

$$B_{eff} = B_0 \cdot (1 - \sigma)$$

B_0 = *Initial magnetic field*

σ = *Shielding constant*

B_{eff} = *Effectiv magnetic field for nucleus*

Table 3.7. Proton NMR chemical shift values for some common chemical groups encountered in biomolecules (nucleus under investigation is denoted by arrow) *TMS = tetramethylsilane*

Chemical group	Chemical shift (ppm)
TMS	0
CH_3-Metal	−0.5–0
CH_3-CH_2-	0.8–1
$CH_3-CH_2-CH_2-$	1.2–2
$CH_3-C(=O)-$	1.8–2.2
$H-C\equiv C-$	2.2–3
CH_3-O-	3.5–4.5
$C=CH_2$	4.7–5.5
$-CH=CH-$	4.5–7
Benzene	7

E.g., the resonance frequency of the proton in H-C≡C- is shielded by -C≡C- by 2.2 to 3 ppm.

Measuring the absolute Larmor frequency ω is difficult.
It is easier to measure a frequency in comparison with that of a reference molecules (TMS), yielding a “chemical shift δ”, in parts per million (ppm):

$$\delta = \frac{\nu_S - \nu_{Ref}}{\nu_{Ref}} \cdot 10^6$$

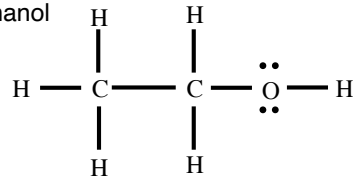
δ = *Chemical shift* [*ppm*]

ν_S = *resonance frequency of sample nucleus*

ν_{Ref} = *resonance frequency of reference nucleus*

Example: What is the NMR spectrum for Ethanol?

Ethanol



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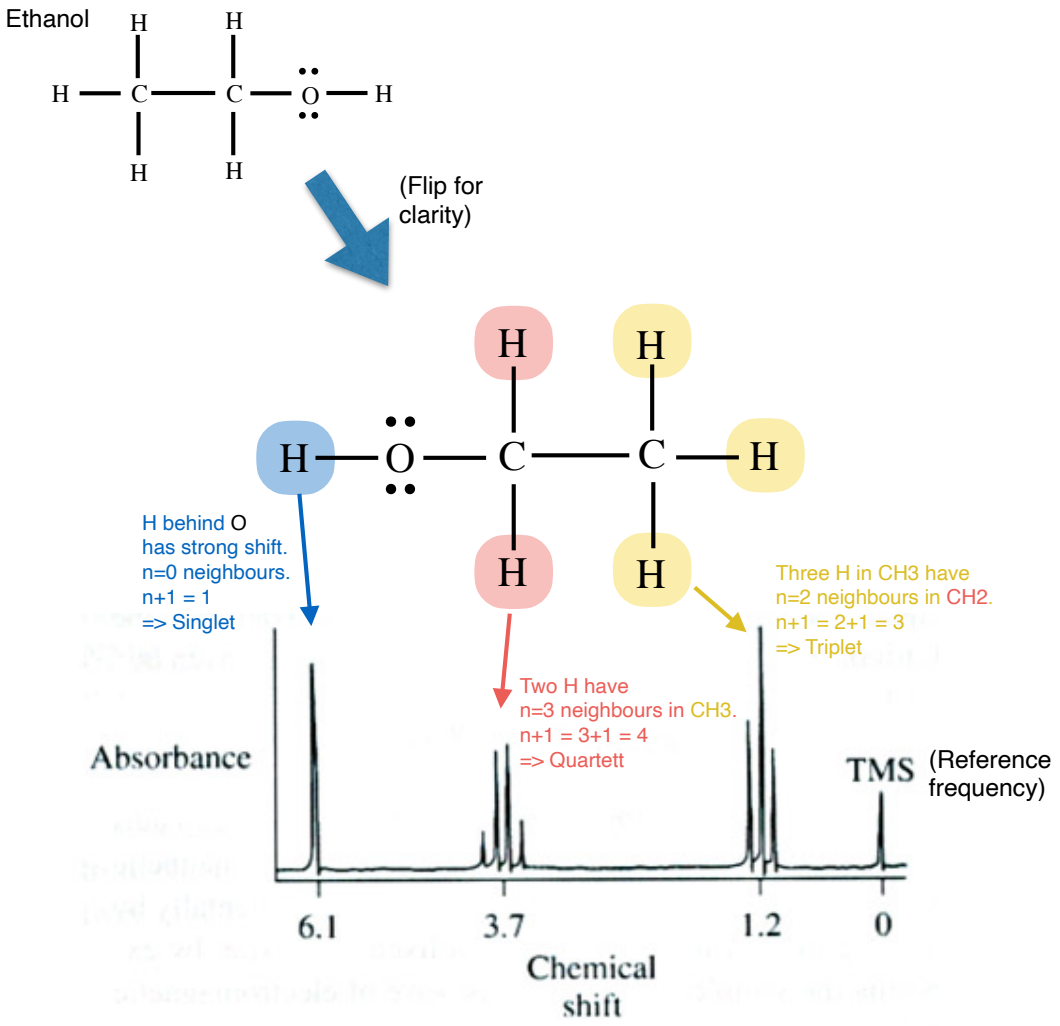


Figure 3.51. Proton NMR spectrum of ethanol. An NMR spectrum consists of a plot of absorbance intensity versus chemical shift (d). Peaks arising from each of the three types of proton in the structure are labelled. In the case of $-CH_3$ and CH_2 groups, multiple peaks arise as a result of spin coupling. Butler and Harrod (1989), *Inorganic Chemistry: Principles and Applications*, Addison Wesley Longman, Reproduced with permission.

NMR: Spin Coupling

$$Intensities\ of\ peaks = coefficients\ of\ binomial = (1+x)^n$$

$$(1+x)^2 = 1+2x+1x^2$$

$$(1+x)^3 = 1+3x+3x^2+1x^3$$

$$(1+x)^4 = 1+4x+6x^2+4x^3+1x^4$$

$$Number\ of\ peaks = (2 \cdot n \cdot I)+1$$

n = *number of nuclei in chemical group*

I = *spin*, (1/2, 1 ...)

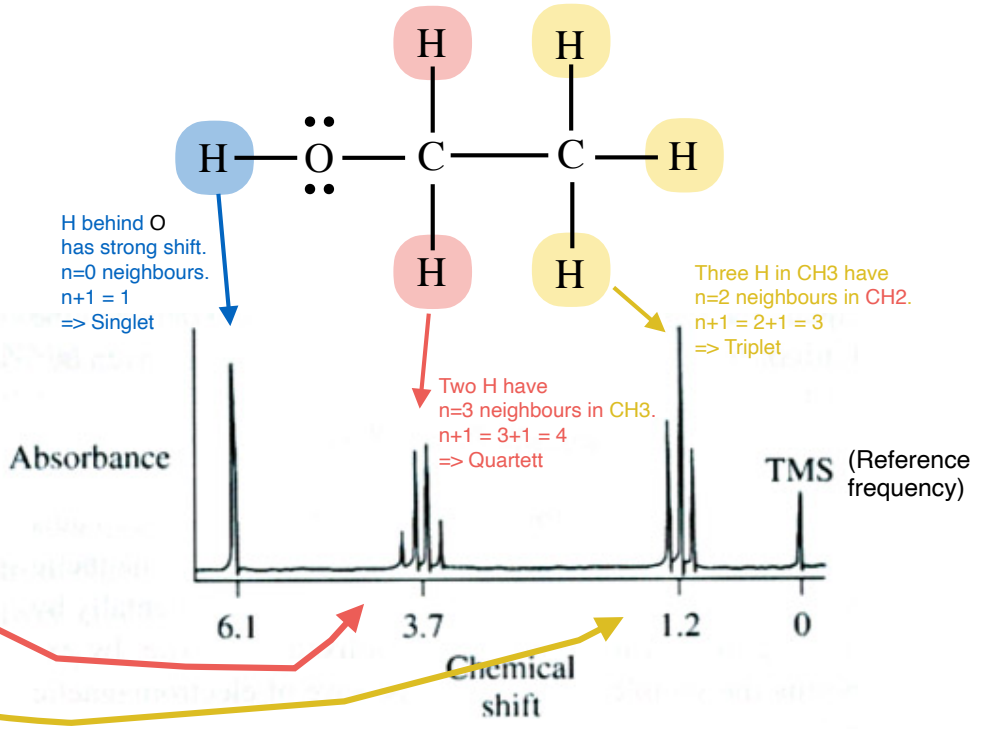
Table 3.8. Spin coupling between $-CH_2$ and $-CH3$ groups in ethanol

Arrangement	Intensity
CH_2 resonances coupled with $-CH_3$	
All up ↑↑↑	1
Two up ↑↑↓ ↑↓↑ ↓↑↑	3
One up ↓↑↓ ↑↓↓ ↓↓↑	3
All down ↓↓↓	1
CH_3 resonances coupled with $-CH_2$	
All up ↑↑	1
One up ↓↑ ↑↓	2
All down ↓↓	1

Note: Resonances associated with the $-CH_2$ group are affected by the spins of protons on the nearby $-CH_3$ group and *vice versa*. This is called spin-spin coupling. The number and type of possible spin arrangements on the coupled group is shown with arrows. The NMR peak due to the $-CH_2$ group splits in the ratio 1 : 3 :3: 1 while that due to the $-CH_3$ group splits 1 : 2 : 1. This ratio depends on the number of possible spin arrangements in the coupled nuclei.

Table 3.9. Pascal's triangle

n	Relative intensities
0	1
1	1 : 1
2	1 : 2 : 1
3	1 : 3 : 3 : 1
4	1 : 4 : 6 : 4 : 1
5	1 : 5 : 10 : 10 : 5 : 1
6	1 : 6 : 15 : 20 : 15 : 6 : 1



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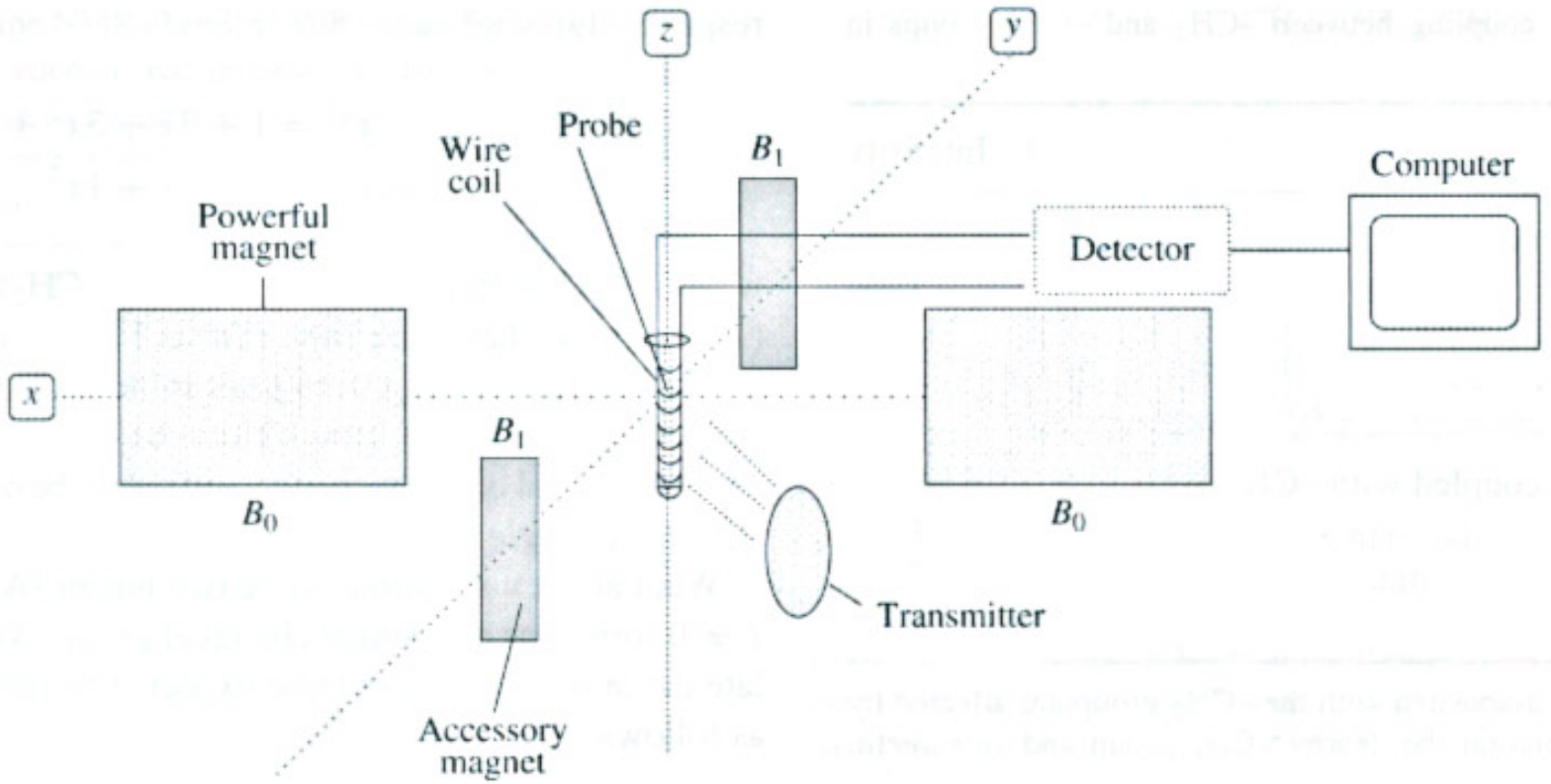
NMR Spectrometer



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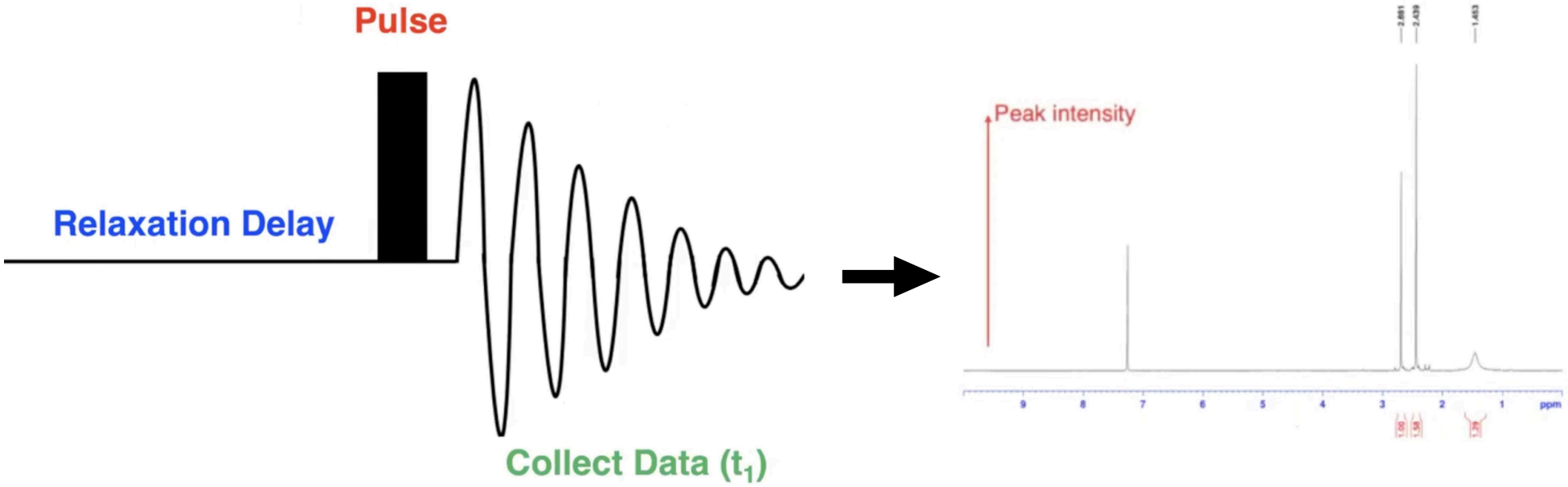
2D NMR Spectroscopy



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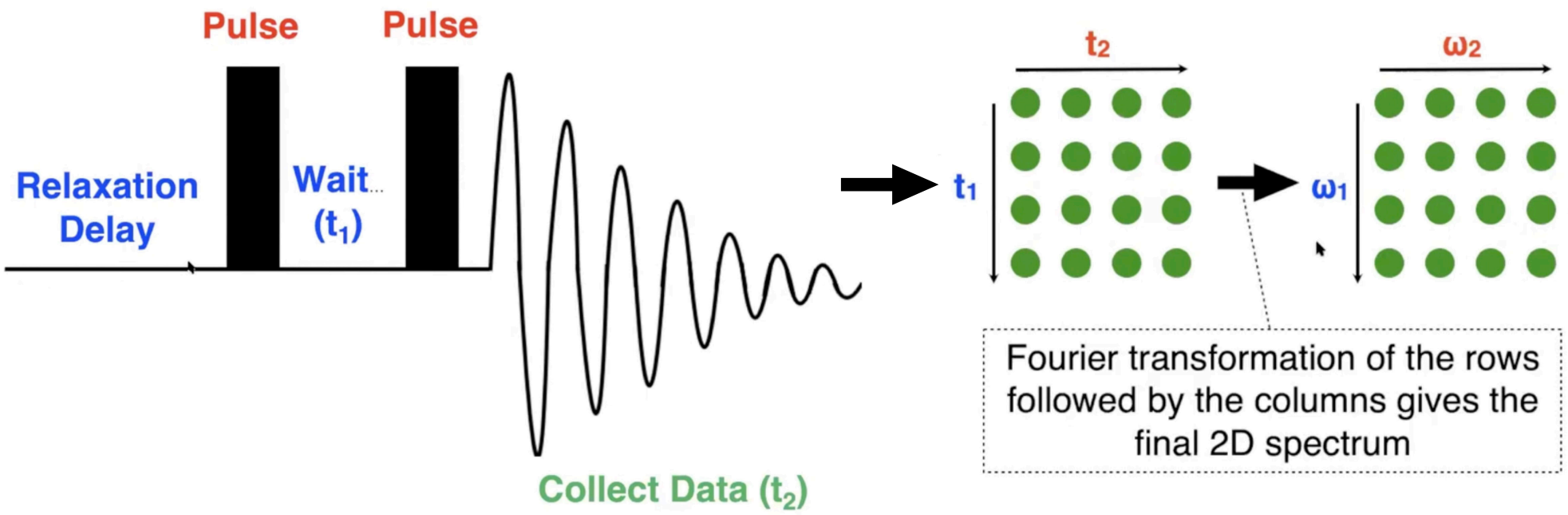
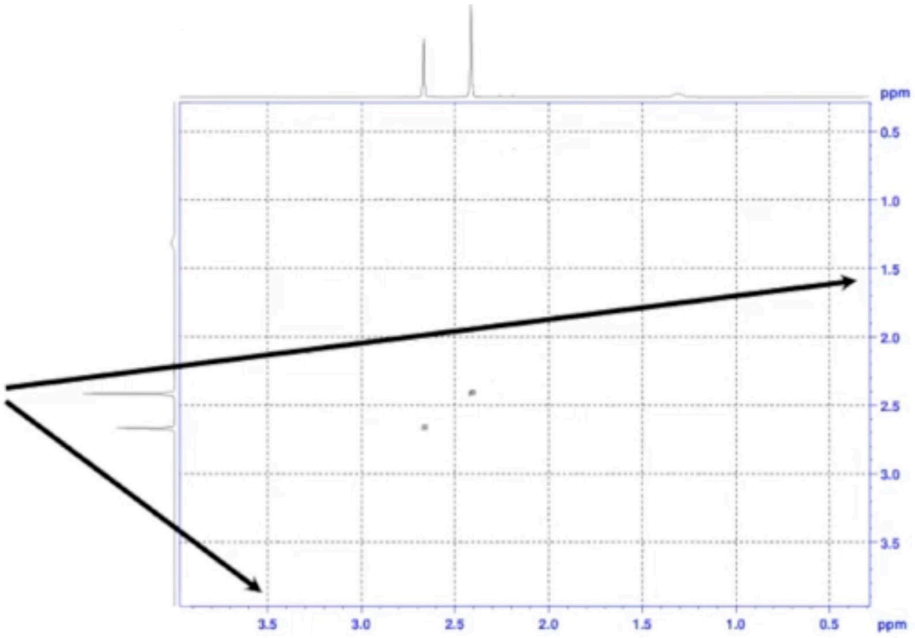
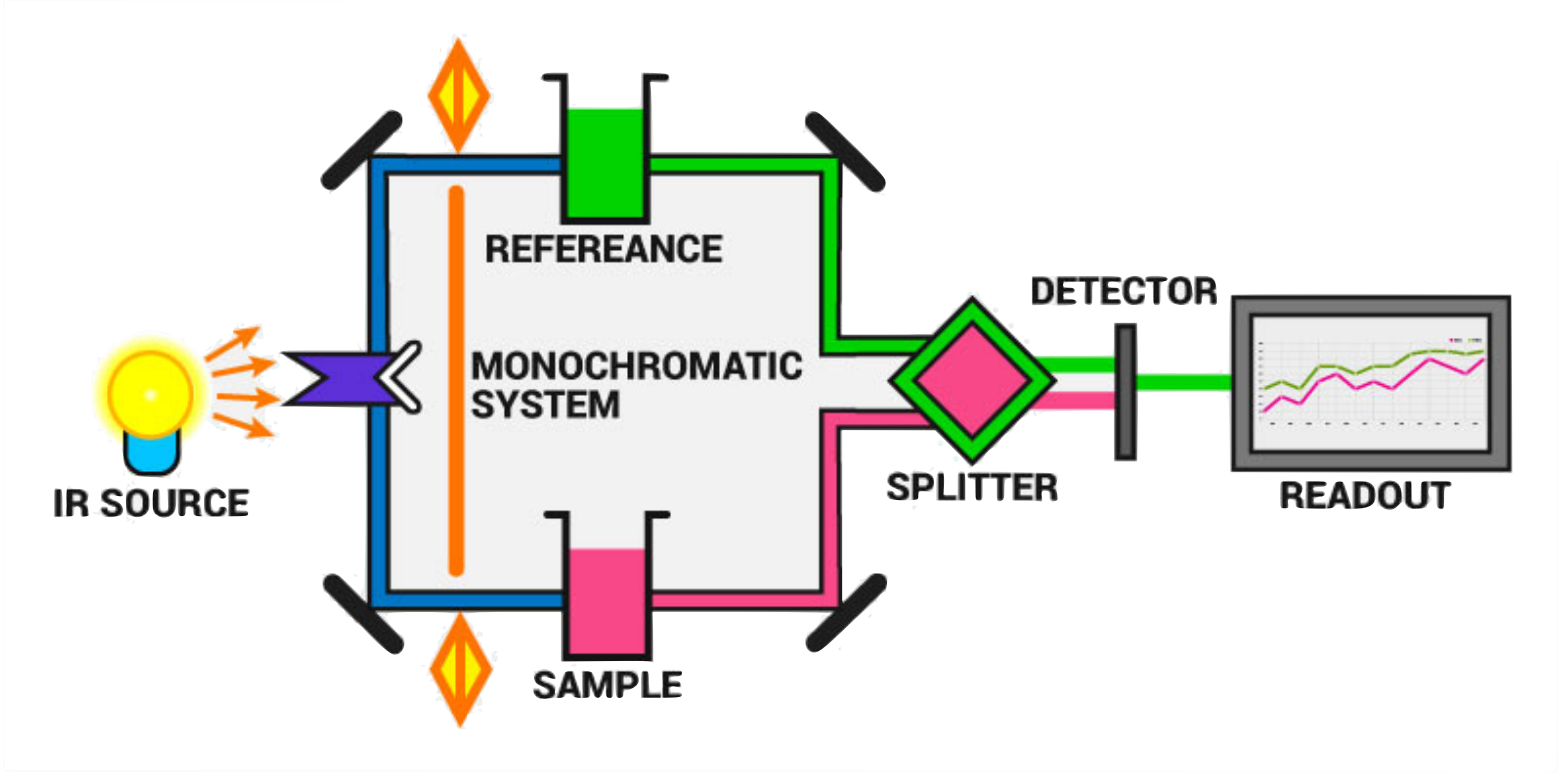


2D NMR spectra have two frequency dimensions

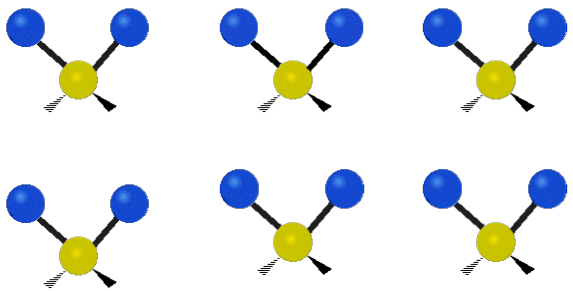


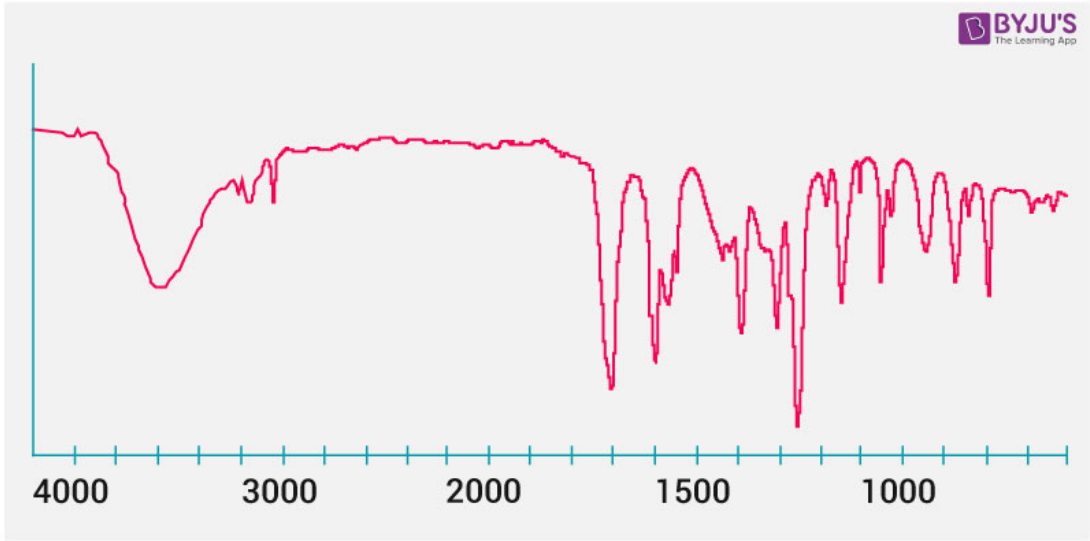
Infrared Spectroscopy



Infrared spectroscopy (IR spectroscopy or vibrational spectroscopy) is the measurement of the interaction of infrared radiation with matter by absorption, emission, or reflection. It is used to study and identify chemical substances or functional groups in solid, liquid, or gaseous forms. It can be used to characterize new materials or identify and verify known and unknown samples.

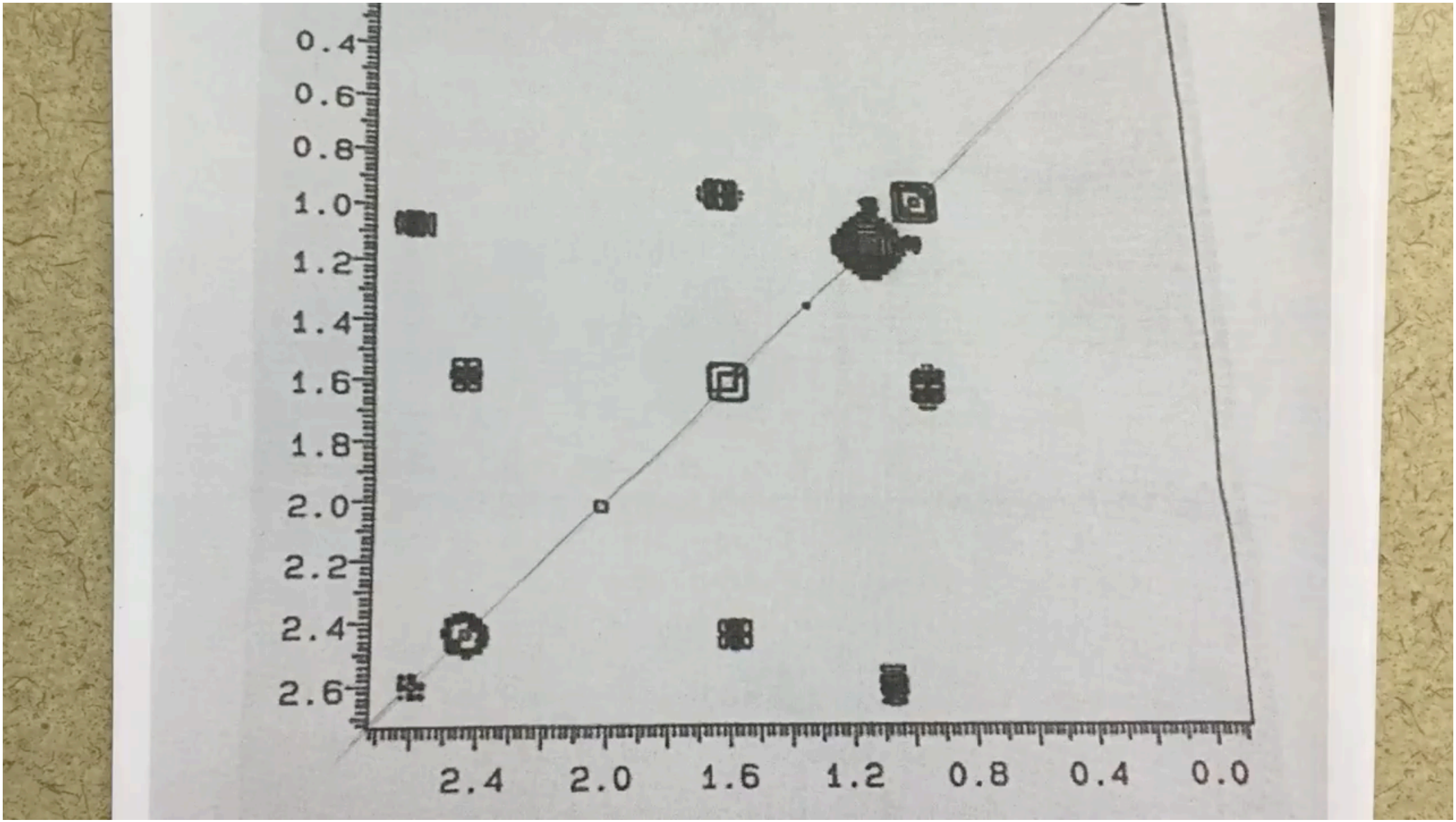
Different vibrational modes absorb IR light in a way that is characteristic for the molecule.





https://en.wikipedia.org/wiki/Infrared_spectroscopy

2D NMR Spectroscopy



https://www.youtube.com/watch?v=UlLEEjIB_Zo

NMR Spectroscopy

NMR Spectroscopy is used for:

- Determination of an unknown chemical structure
- Non-invasive measurement of the concentration of a chemical
- Analysis of chemical pathways and the production of chemical compounds
- Determination of binding constants
- Determination of the kinetics of a chemical reaction
- Analysis of conformational changes of a protein as a function of external parameters (such as pH, temperature, pressure, ionic strength, ligand binding)
- Structure determination of the active site of an enzyme
- Interaction with other molecules (e.g., enzyme-subtrate, protein-protein, protein-DNA)
- Structure determination of proteins in solution
- Determination of the kinetics of conformational changes or of folding dynamics of proteins
- Tomography, imaging.

Surface Plasmon Resonance (SPR)

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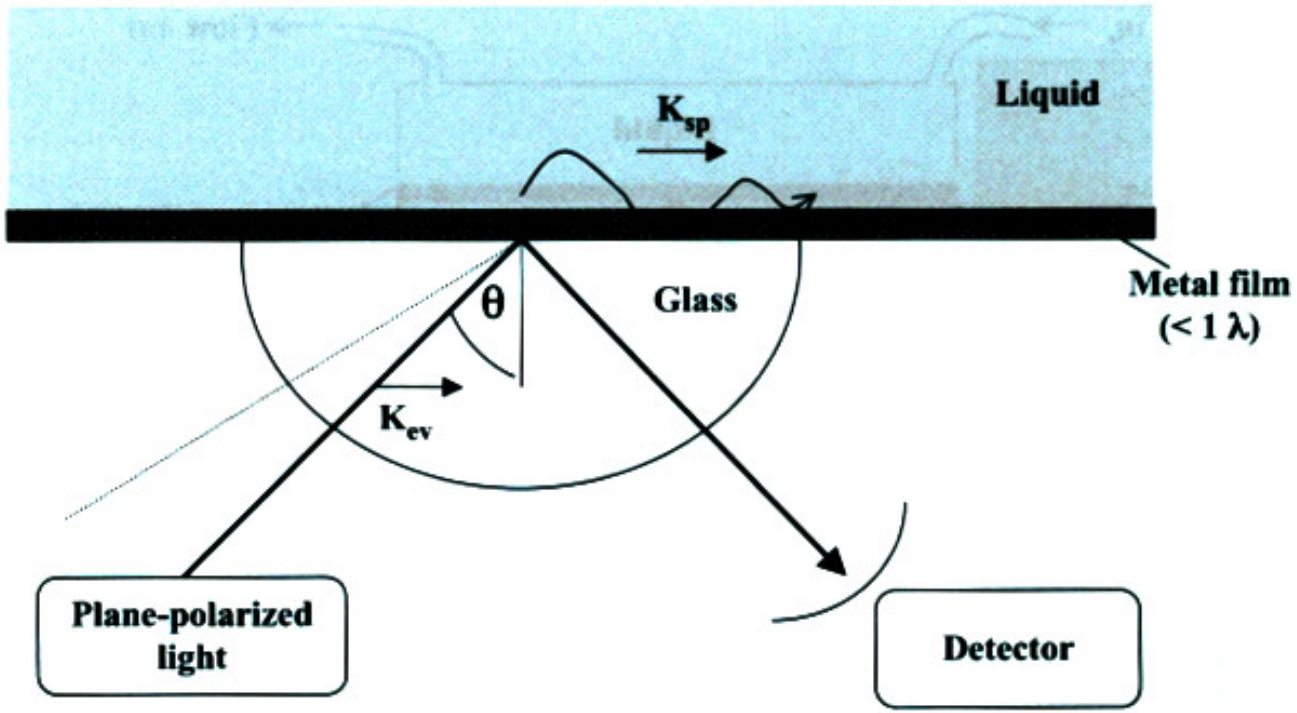


Figure 3.61. Surface Plasmon resonance. Plane-polarized light (wavelength λ) arriving at a thin layer (thickness <λ) of metal between a more (glass) and less (liquid or air) optically dense material is reflected by total internal reflection. An evanescent wave enters the metal interface to a depth <λ. At a particular angle of incidence, θ, the evanescent wave (vector K_{ev}) couples with free oscillating electrons (plasmons; vector K_{sp}) within the metal. Energy absorbed in this process is detected as a sharp reduction in intensity of the reflected light at a specific value for θ. K_{sp} depends strongly on the refractive index of the liquid or air immediately above the metal layer to a depth <300 nm.

Surface plasmon resonance occurs, when the evanescent wave equals the surface plasmon wave, i.e. $K_{ev}=K_{sp}$

This happens at the angle Theta under the following condition:

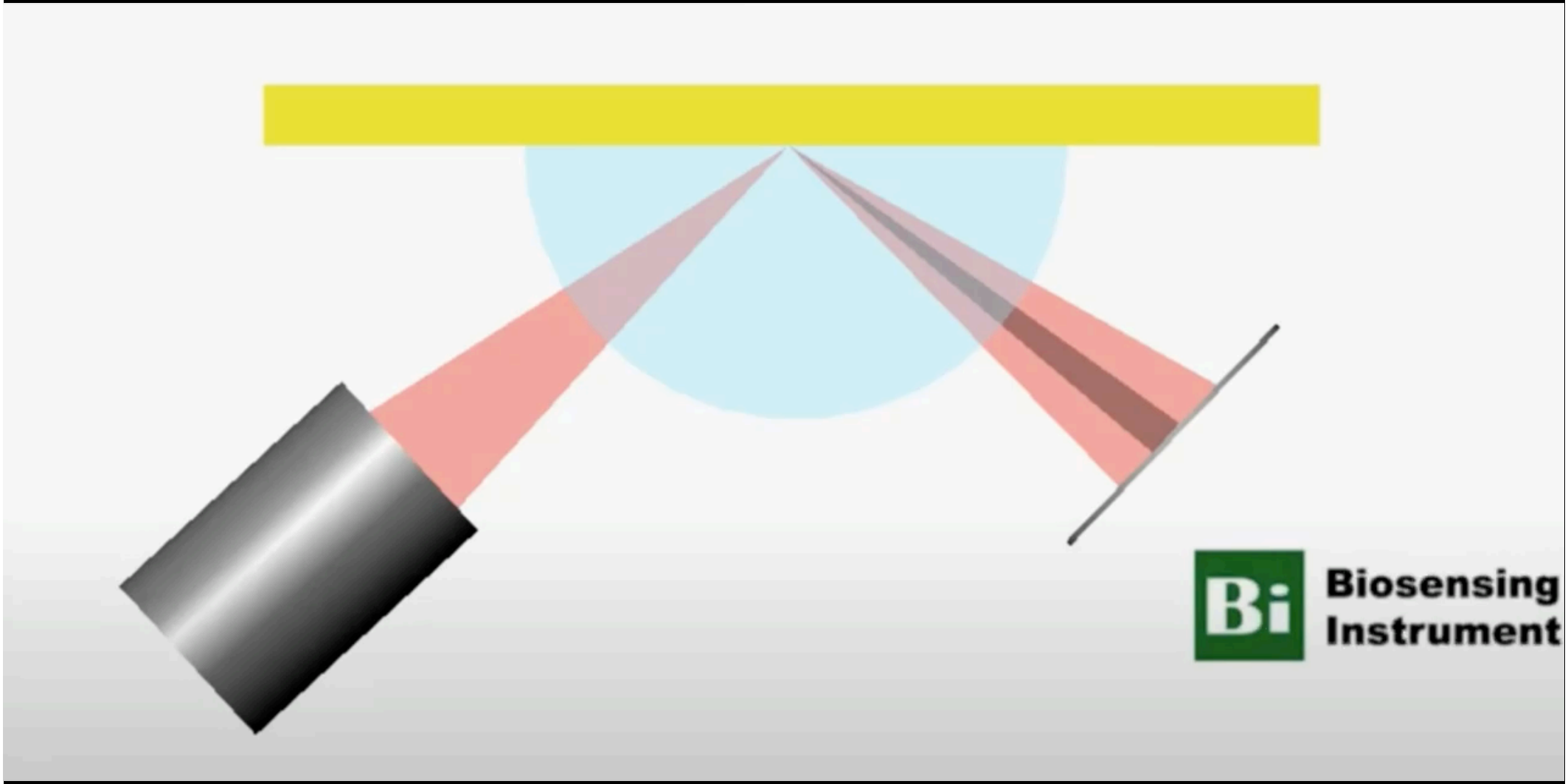
$$\sin\Theta \bullet \sqrt{\varepsilon_1(\lambda)} = \sqrt{\frac{\varepsilon_2(\lambda) \bullet \varepsilon_3(\lambda)}{\varepsilon_2(\lambda) + \varepsilon_3(\lambda)}}$$

ε_1 = dielectric constant of the glas

ε_2 = dielectric constant of the metal film

ε_3 = dielectric constant of the sample solution

Surface Plasmon Resonance (SPR)



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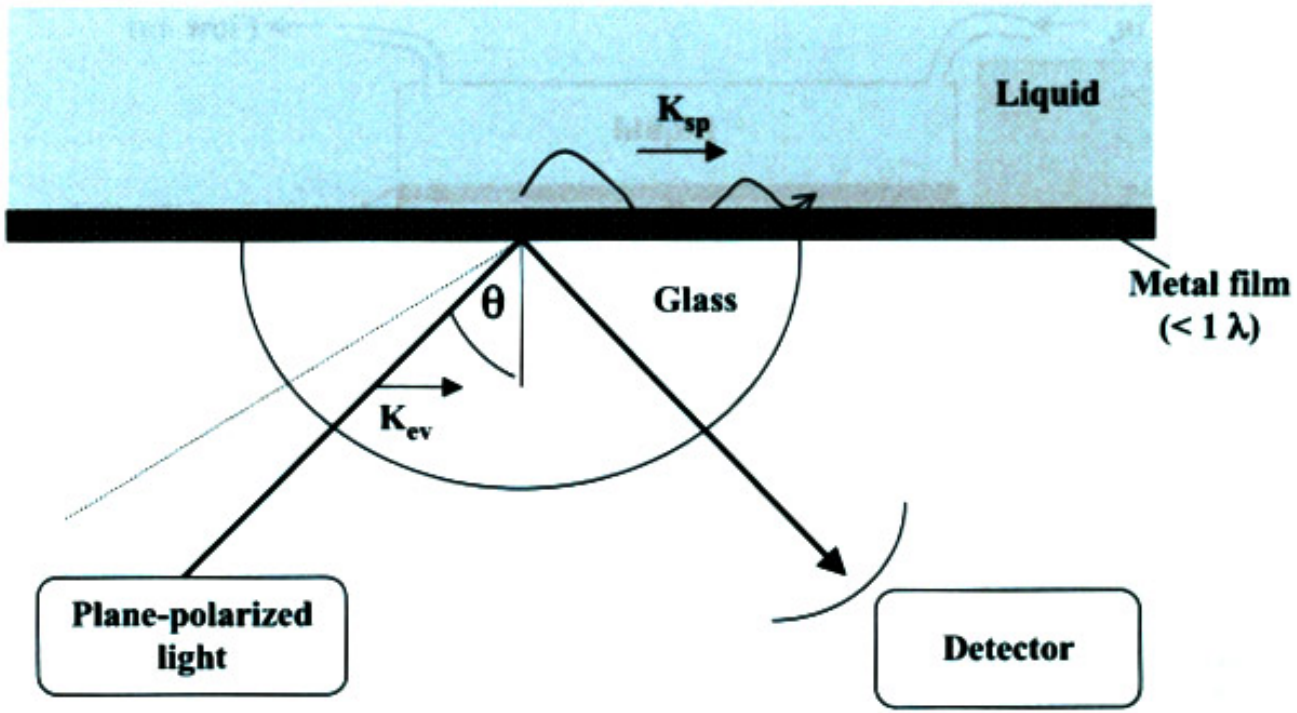

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Surface Plasmon Resonance (SPR)

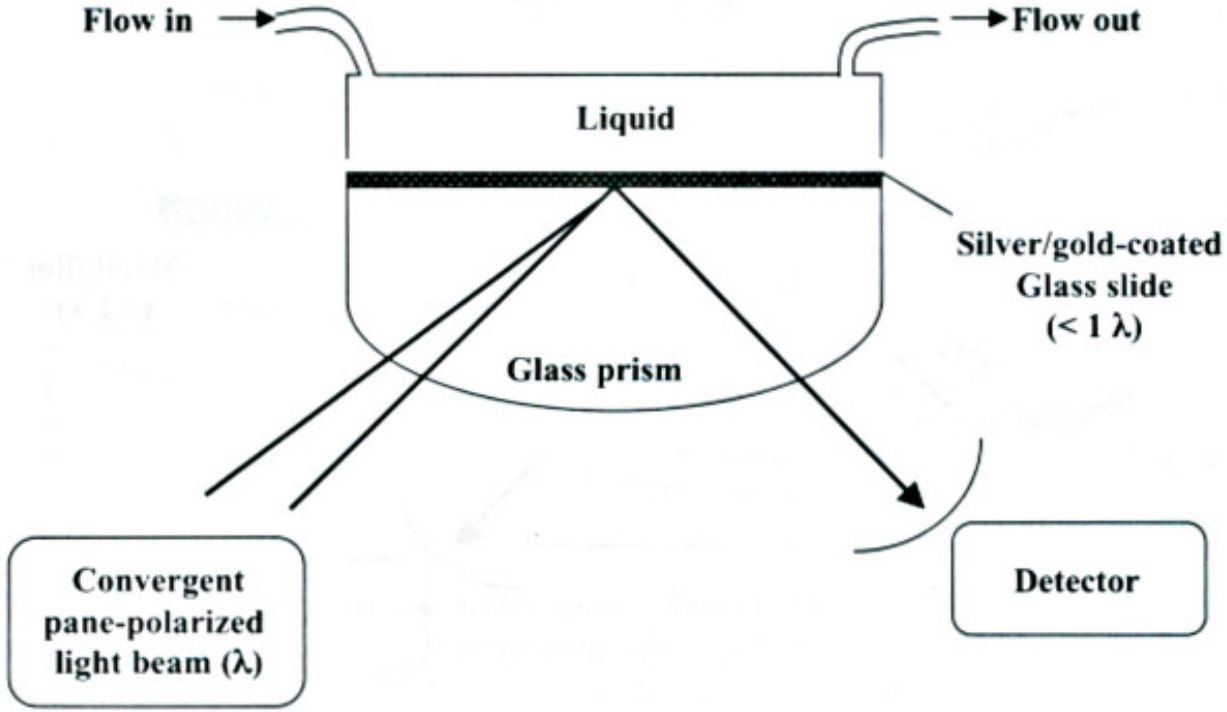

Figure 3.62. A typical SPR instrument. A convergent beam of plane-polarized light (wavelength, λ) is focused through a glass prism onto a silver/gold-coated glass slide and reflected to a detector. Solutions can pass through a flow-cell at defined values of flow-rate and temperature. Protein flowing through the flow-cell can alter the surface immediately above the metal layer.

μ_0 = magnetic susceptibility constant
n_g = refractive index of glas
c = light speed
Θ = light incidence angle

$$K_{ev} = \frac{\mu_0}{c} \cdot n_g \cdot \sin\Theta$$

$$K_{sp} = \frac{\omega_0}{c} \cdot \frac{\delta_m \cdot n_s^2}{\delta_m + n_s^2}$$

n_S = refractive index of solution
δ_m = dielectric const. of metal film
ω_0 = angular frequency of waves

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Surface Plasmon Resonance (SPR) Time Curve

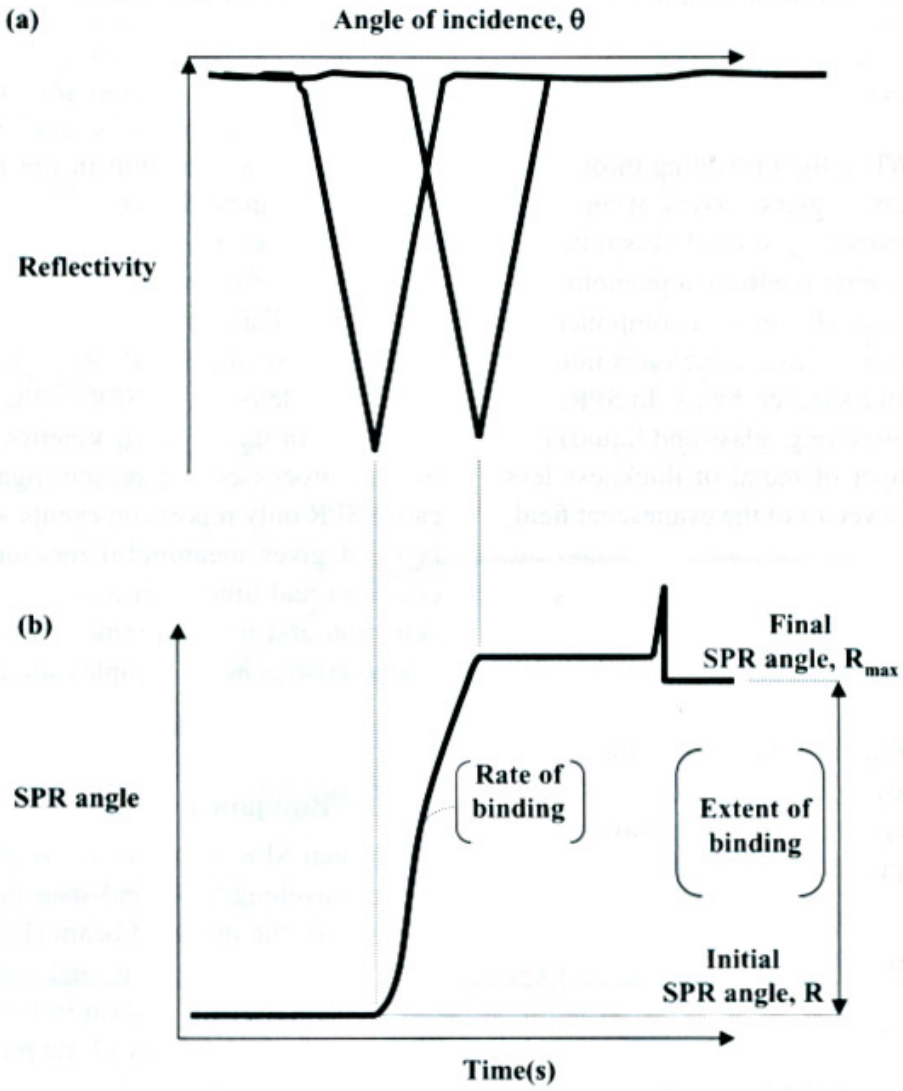


Figure 3.63. Schematic of SPR protein binding experiment. The sensor is equilibrated with buffer before addition of a protein solution. This changes n_s immediately above the sensor surface giving a sharp decrease in reflectivity. Protein binding is followed as a change in SPR angle (θ) over time. When the surface becomes saturated, the SPR angle reaches a maximum. Loosely bound protein is removed by washing with buffer. Extent of adsorption is given by the difference between initial and final SPR angles while the rate of binding may be measured from the steepest part of the positive slope.

Surface Plasmon Resonance (SPR) Imaging Device

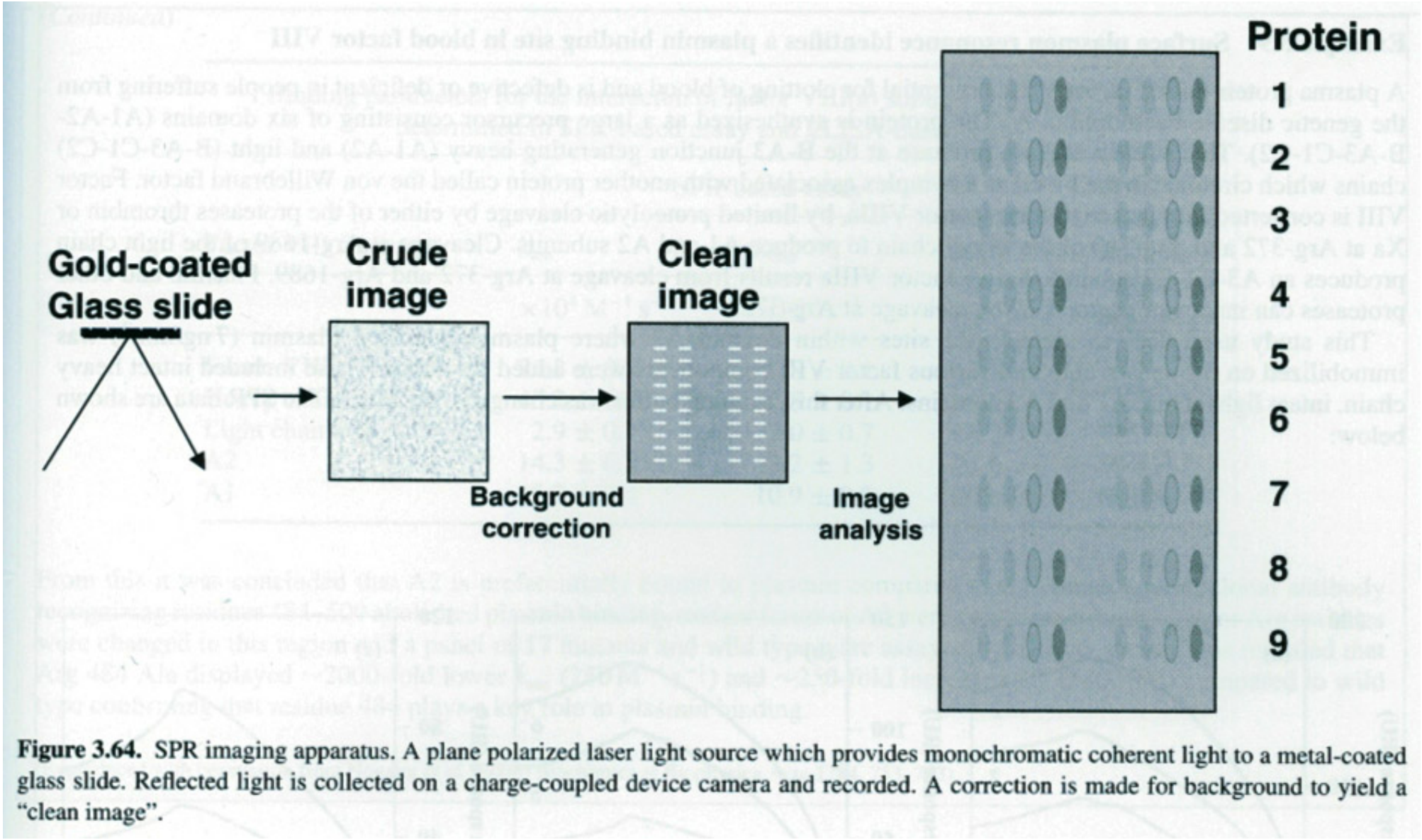


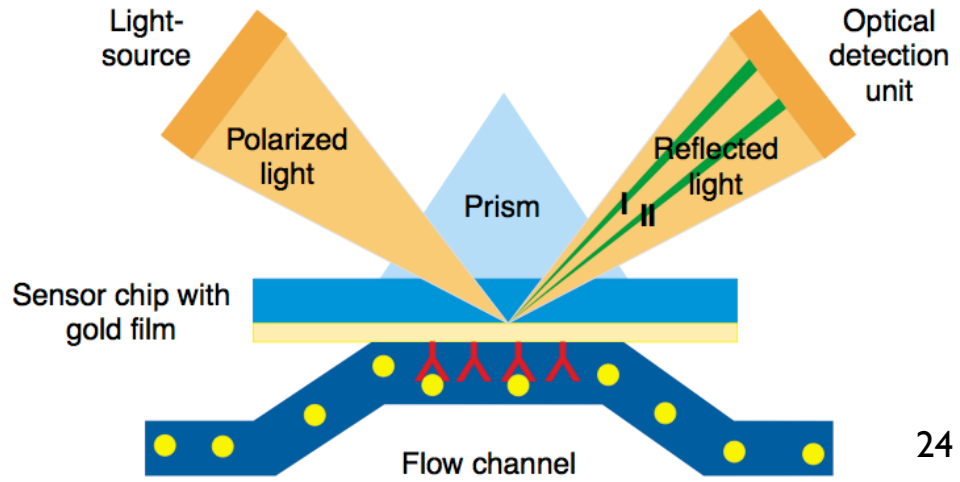
Figure 3.64. SPR imaging apparatus. A plane polarized laser light source which provides monochromatic coherent light to a metal-coated glass slide. Reflected light is collected on a charge-coupled device camera and recorded. A correction is made for background to yield a "clean image".

Surface Plasmon Resonance (SPR)

Biacore®3000

The high performance research system

- Work with high sensitivity
  - direct detection of small molecules at < 1 nM concentration
  - increased resolution for kinetic analysis
  - measurement of weak affinities
- Recover bound analyte in high concentration
- Deliver recovered analyte to vial or direct to MALDI target
- Shorten analysis times
- Minimize sample consumption
- Study binding in non-aqueous and aqueous samples
- Use Biacore Wizards to simplify and accelerate analysis
- Develop specialized applications efficiently
- Perform the most advanced kinetic evaluation



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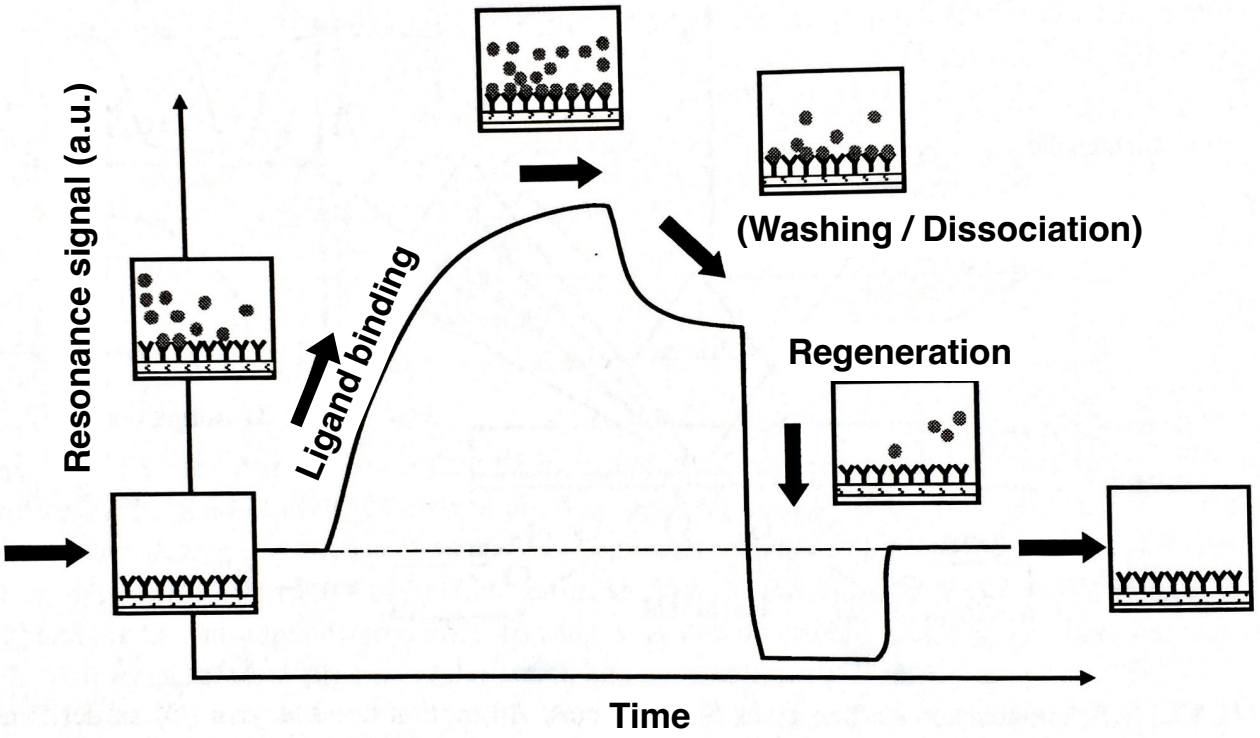

SPR Example:

- A sensor chip surface is covered with antibodies.
- Ligands are added, resulting in an increase of the SPR signal, until ligand binding is saturated.
- Washing with buffer solution leads to partial dissociation of ligands that are not firmly bound.
- Regeneration of the surface by harsh surface treatment and addition of new antibodies restores the chip.