Time out—states and transitions

Spectroscopy—transitions between energy states of a molecule excited by absorption or emission of a photon

$$h\nu = \Delta E = E_i - E_f$$

Energy levels due to interactions between parts of molecule (atoms, electrons and nucleii) as described by **quantum mechanics**, and are

characteristic of components involved, i.e. electron distributions (orbitals), bond strengths and types plus molecular geometries and atomic masses involved

Spectroscopic Regions

Typical wavelength (cm)	Approximate energy (kcal mole-1)	Spectroscopic region	Techniques and Applications
10^{-11}	3×10^{8}	γ-ray	MÖssbauer
10^{-8}	3×10^{5}	X-ray	x-ray diffraction, scattering
10^{-5}	3×10^{2}	Vacuum UV	Electronic Spectra
3×10^{-5}	10^{2}	Near UV	Electronic Spectra
6×10^{-5}	5×10^{3}	Visible	Electronic Spectra
10^{-3}	3×10^{0}	IR	Vibrational Spectra
10^{-2}	3×10^{-1}	Far IR	Vibrational Spectra
10^{-1}	3×10^{-2}	Microwave	Rotational Spectra
10^{0}	3×10^{-3}	Microwave	Electron paramagnetic resonance
10	3×10^{-4}	Radio frequency	Nuclear magnetic resonance

Adapted from Table 7-1; Biophysical Chemistry, Part II by Cantor and Schimmel

Spectroscopic Process

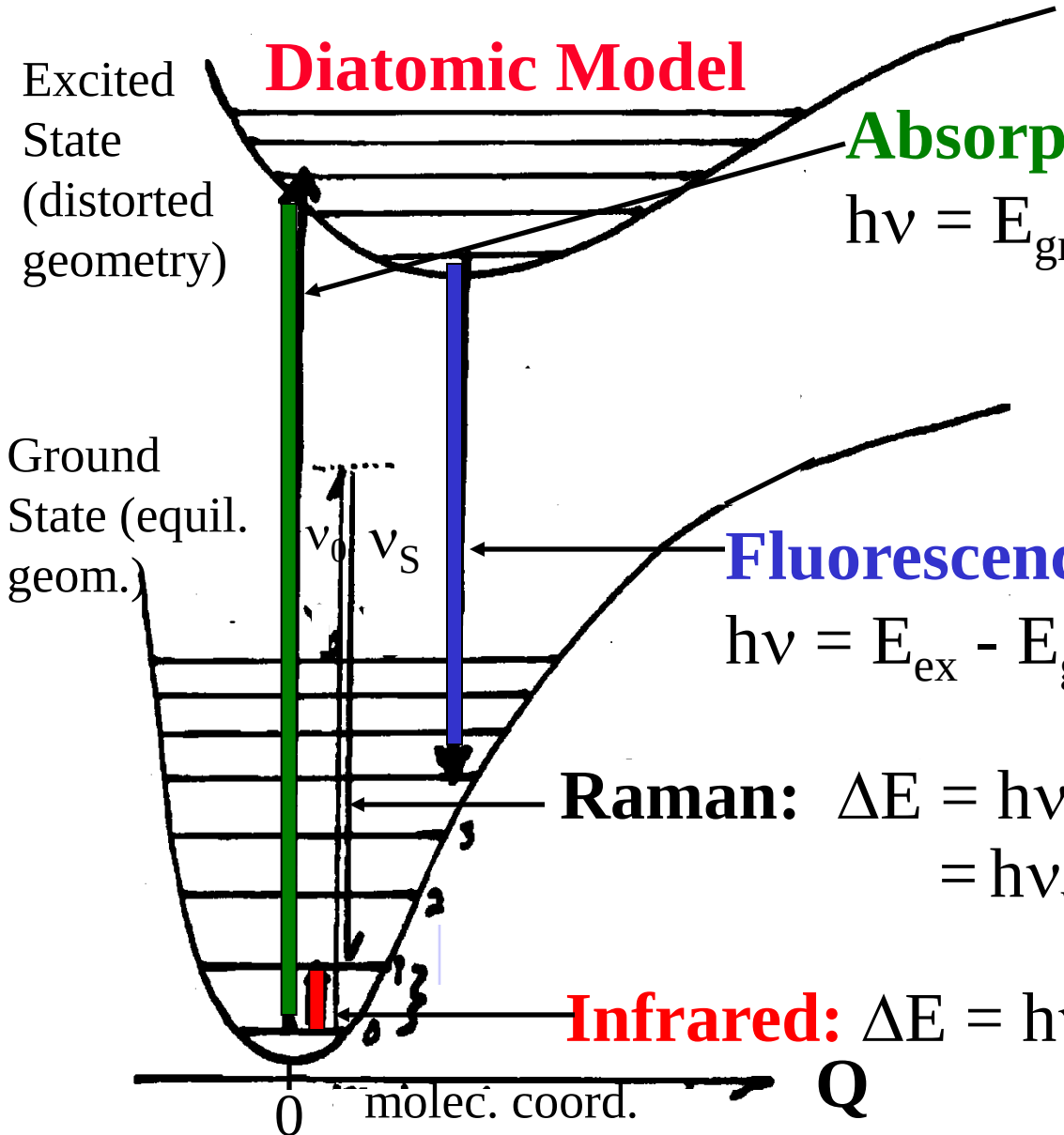
- Molecules contain distribution of charges (electrons and nuclei, charges from protons) and spins which is dynamically changed when molecule is exposed to light
- In a spectroscopic experiment, light is used to probe a sample. What we seek to understand is:
  - the RATE at which the molecule responds to this perturbation (this is the response or spectral intensity)
  - why only certain wavelengths cause changes (this is the spectrum, the wavelength dependence of the response)
  - the process by which the molecule alters the radiation that emerges from the sample (absorption, scattering, fluorescence, photochemistry, etc.) so we can detect it

These tell us about molecular identity, structure, mechanisms and analytical concentrations

Magnetic Resonance—different course

- Long wavelength radiowaves are of low energy that is sufficient to 'flip' the spin of nuclei in a magnetic field (NMR). Nuclei interact weakly so spectral transitions between single, well defined energy levels are very sharp and well resolved. NMR is a vital technique for biological structure studies.
- Higher energy microwaves can promote changes in the rotational motions of gas phase molecules, which is the basis of microwave rotational spectroscopy (not a method of biological importance).
- Microwaves are also used for spin-flips of electrons in magnetic fields (ESR or EPR), important for free radicals and transition metal systems (open shell). Magnetic dipole coupling can be used to measure distances between spins—growing importance in peptides and proteins.

Optical Spectroscopy - Processes Monitored
UV/ Fluorescence/ IR/ Raman/ Circular Dichroism



Analytical Methods

UV-vis absorp. & Fluorescence. move e^- (change electronic state) high freq., intense

CD – circ. polarized absorption, UV or IR

Raman –nuclei, inelastic scatter very low intensity

IR – move nuclei low freq. & inten.

Optical Spectra--topic of the course

- Infrared radiation excites molecular vibrations, i.e. stretching of bonds and deformation of bond angles. Molecule has 3N-6 internal degrees of freedom, N atoms. States characterize the bound ground state.
- Radiation in the visible (Vis) and ultraviolet (UV) regions , will excite electrons from the bound (ground) state to more weakly bound and dissociative (excited) states.
- Changes in both the vibrational and rotational states of the molecule can be associated with this, causing the spectra to become broadened or have fine structure.

These motions are sampled in absorption, emission or scattering

Optical Spectroscopy – Electronic, Example Absorption and Fluorescence

Essentially a probe technique sensing changes in the local environment of fluorophores

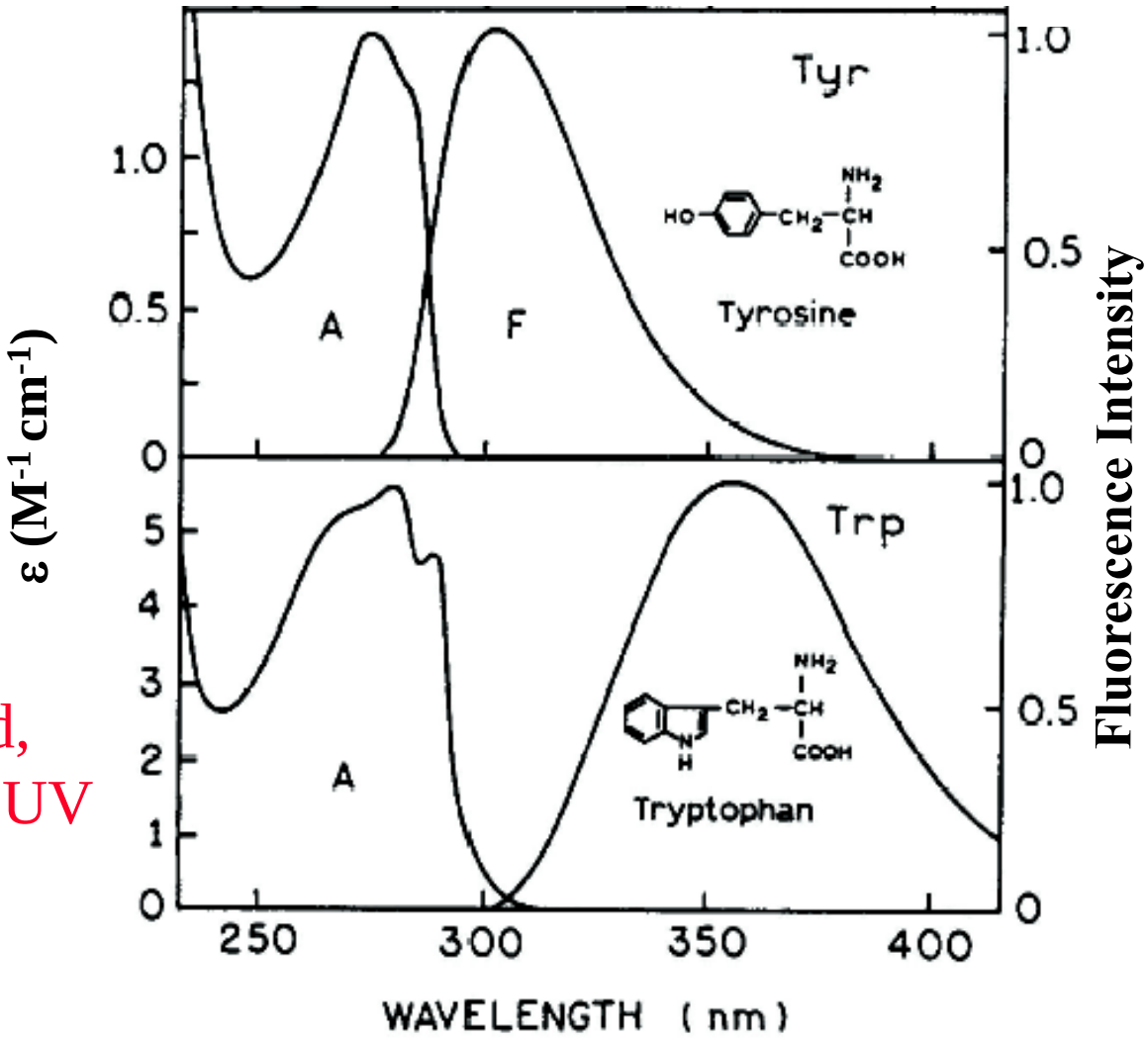
What do you see?

Intrinsic fluorophores

eg. Trp, Tyr

Change with tertiary structure, compactness

Amide absorption broad, Intense, featureless, far UV ~200 nm and below



Optical Spectroscopy - *IR Spectroscopy*

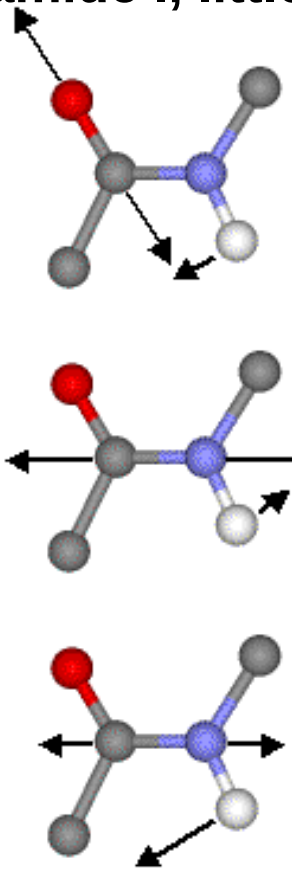
Protein and polypeptide secondary structural obtained from vibrational modes of amide (peptide bond) groups

Aside: Raman is similar, but different

amide I, little amide II, intense amide III

What do you see?

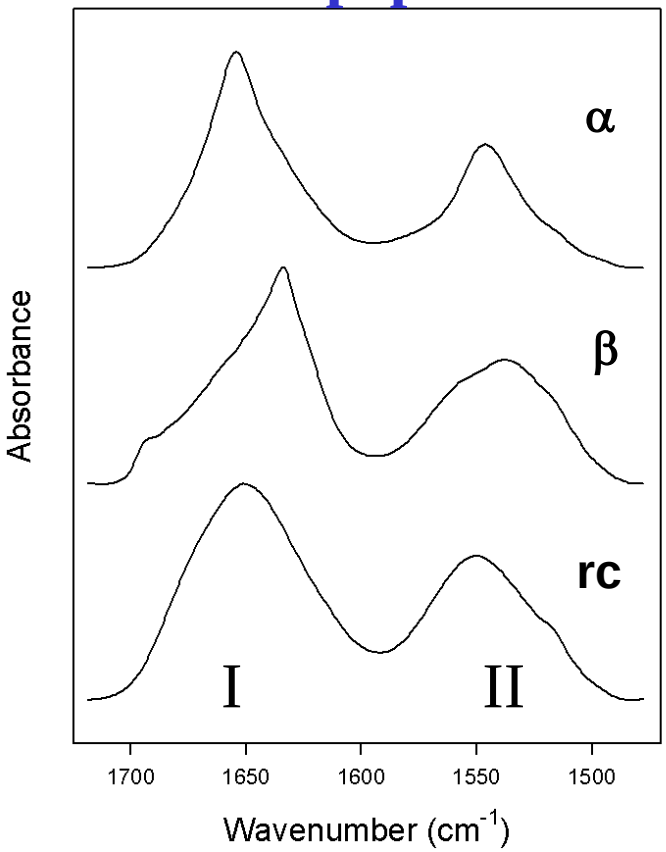
Model peptide IR



Amide I
(1700-1600 cm^{-1})

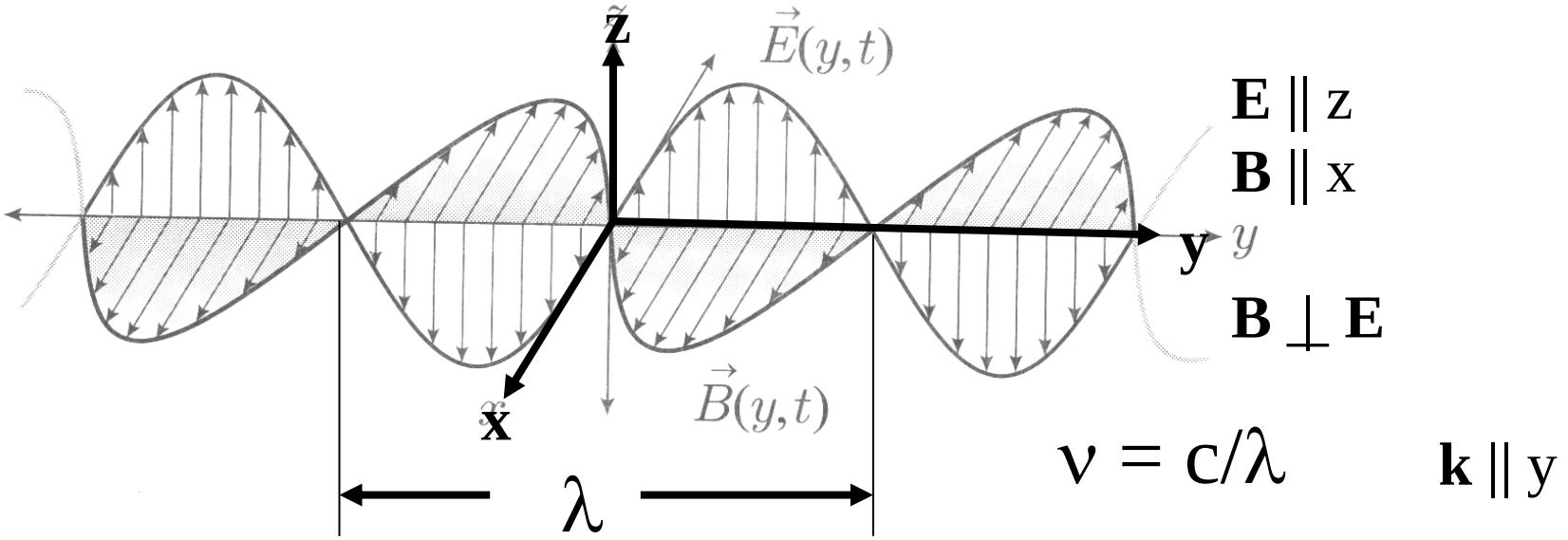
Amide II
(1580-1480 cm^{-1})

Amide III
(1300-1230 cm^{-1})



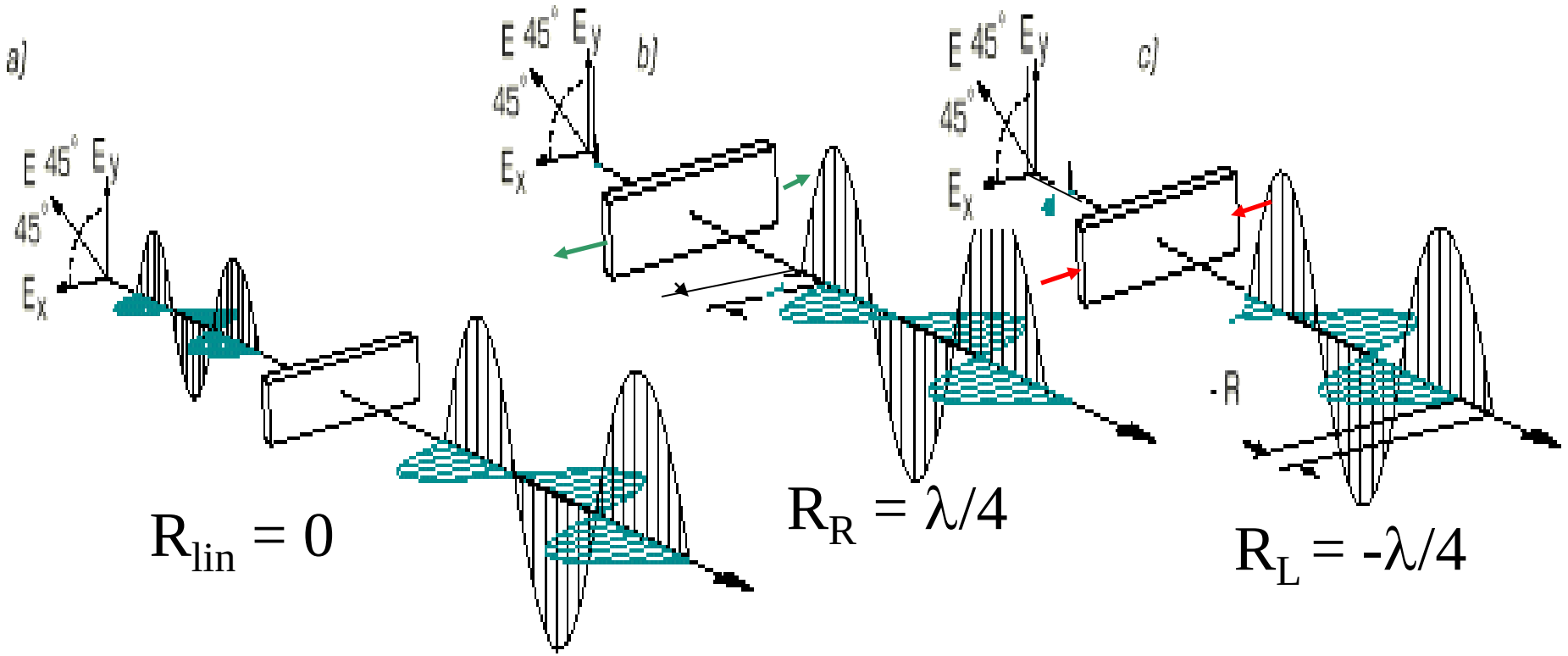
Spectroscopy

- Study of the consequences of the interaction of electromagnetic radiation (light) with molecules
- Light beam characteristics - wavelength (frequency), intensity, polarization - determine types of transitions and information accessed



Light Polarization

[courtesy Hinds Inc. brochure]



$R_{lin} = 0$

$R_R = \lambda/4$

$R_L = -\lambda/4$

Linear Polarization
Preserved in isotropic medium

Right Circular
Polarization

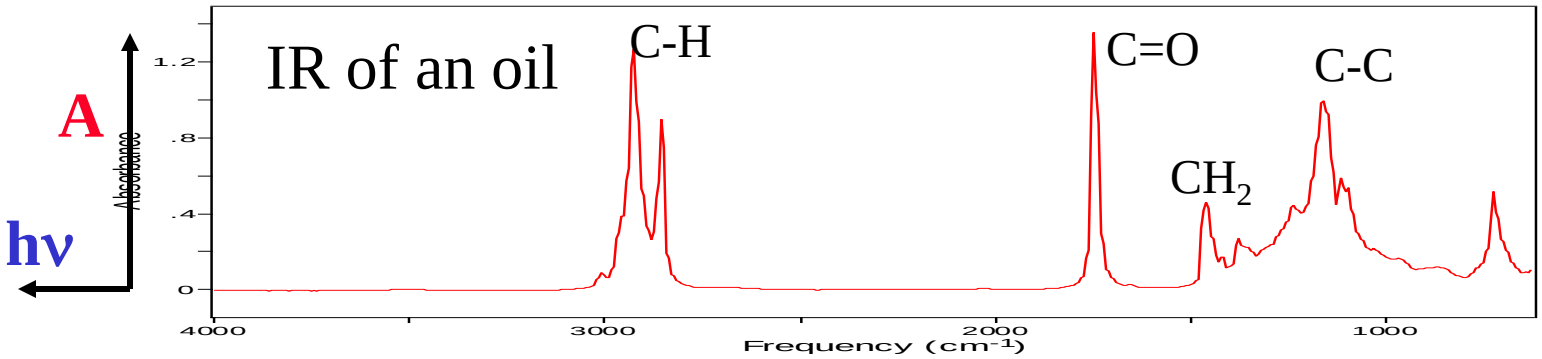
Left Circular
Polarization

Phase retard orthogonal polarizations
forward or back with birefringent medium

Light (E-M Radiation) Characteristics

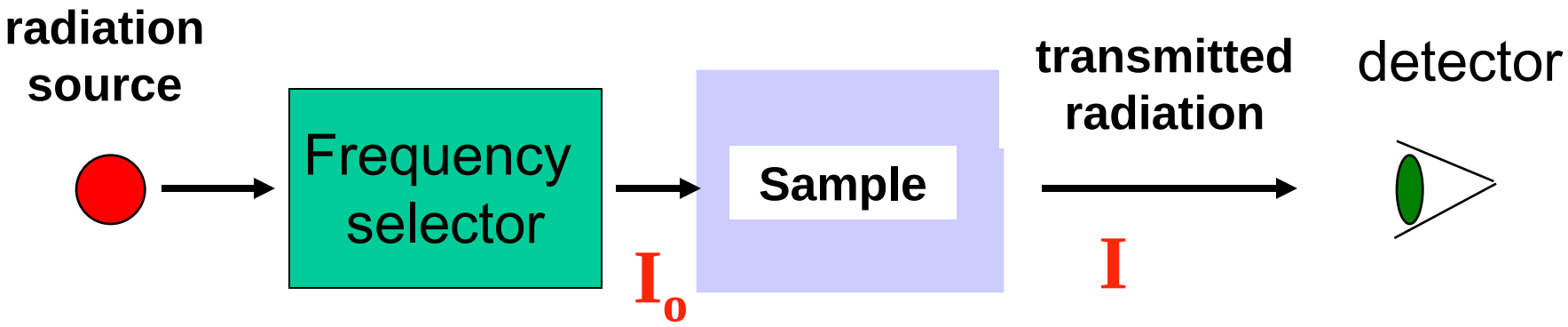
- **Frequency** matches change in energy, type of motion
  → **$E = h\nu$**, where $\nu = c/\lambda$ (in sec^{-1} or Hz)
- **Intensity** increases the transition probability— Absorbance
  → $I \sim \mathcal{E}^2$ –where $\mathcal{E}$ is the Electric Field strength in the radiation
- Absorbance is ratio $A = -\log(I/Io)$
- **Linear Polarization** aligns to direction of dipole change
  → $A \sim [\delta\mu/\delta Q]^2$ where **Q** is the coordinate of the motion

  Circular Polarization results from an interference:
  → $R \sim Im(\mu \bullet m)$ **μ** and **m** are electric and magnetic dipole



Techniques of Absorption Spectroscopy

UV-vis and Infrared spectroscopy deals with absorption of radiation--detect attenuation of beam by sample at detector

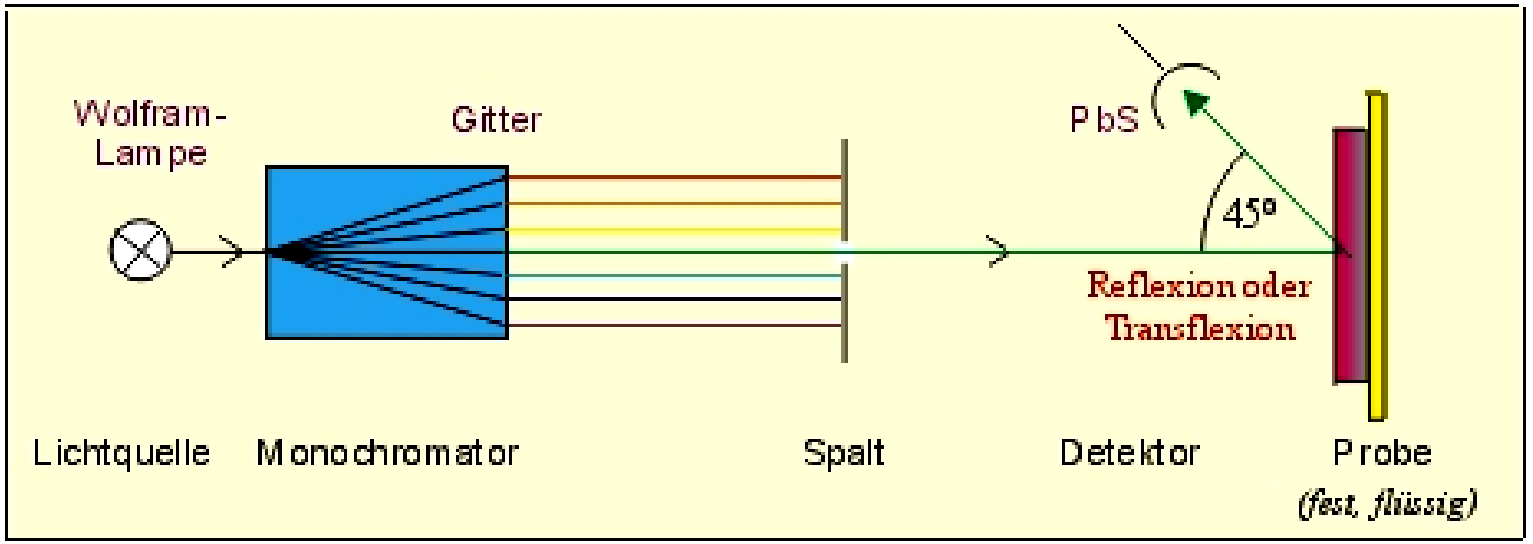


$T = I/I_o$ $A = -\log_{10}(T)$

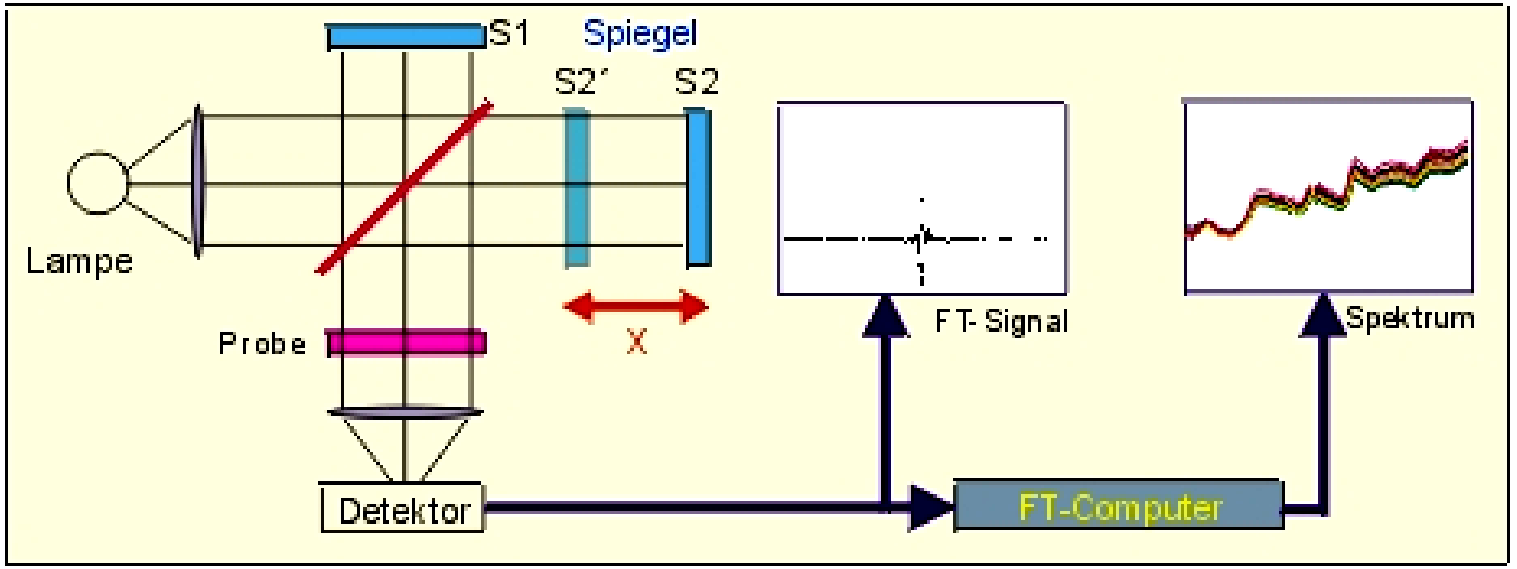
Dispersive spectrometers measure transmission as a function of frequency (wavelength) - sequentially--same as typical CD

Interferometric spectrometers measure intensity as a function of mirror position, all frequencies simultaneously--Multiplex advantage

Dispersive and FT-NIR Spectrometer



Wolfram-Lampe(Tungsten lamp); Gitter(Grating); Spalt(Slit); Lichtquelle(Light source); Spiegel(Mirror), Detektor(Detector); Probe(Sample), Spektrum(Spectrum)



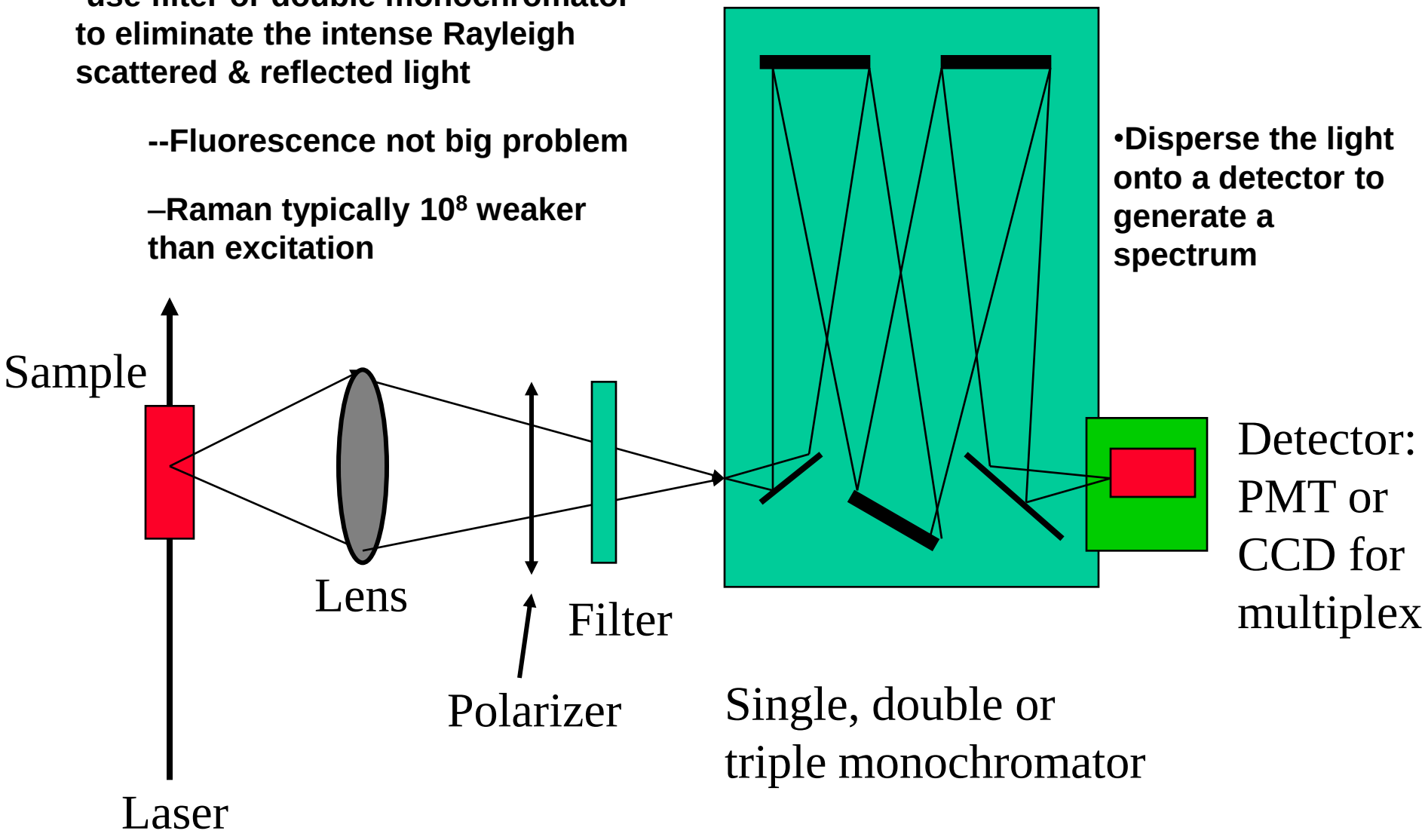
Dispersive Fluorescence or Raman

-use filter or double monochromator to eliminate the intense Rayleigh scattered & reflected light

--Fluorescence not big problem

–Raman typically 10^8 weaker than excitation

•Disperse the light onto a detector to generate a spectrum



Detect intensity, I, against zero background--ideal

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- **Intensity** increases the transition probability
- **Linear Polarization** aligns to direction of dipole change

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Comparison of UV-CD, VCD and IR

	UV-CD	VCD	IR
Measurement	$\Delta A = A_L - A_R$		A
Theoretical	$R = Im(\boldsymbol{\mu \cdot m})$		$D = \boldsymbol{\mu \cdot \mu}$
Experimental	$R = 0.23 \times 10^{-38} \int \Delta\varepsilon/\nu \, d\nu$		$D = 0.92 \times 10^{-38} \int \varepsilon/\nu d\nu$
Sensitivity to 3-D structure	high		low
Molecular transitions	$\pi - \pi^*$, $n - \pi^*$	**C=O, C=C, C=N** **PO^{2-}**, C-O, N-H, etc	
Chromophore	delocalized	localized, each bond	
Nucleotide	weak	negligible	strong
Helical polymer	strong	strong	strong
Observed signal size (A=1)	$10^{-2} - 10^{-3}$	$10^{-4} - 10^{-5}$	1