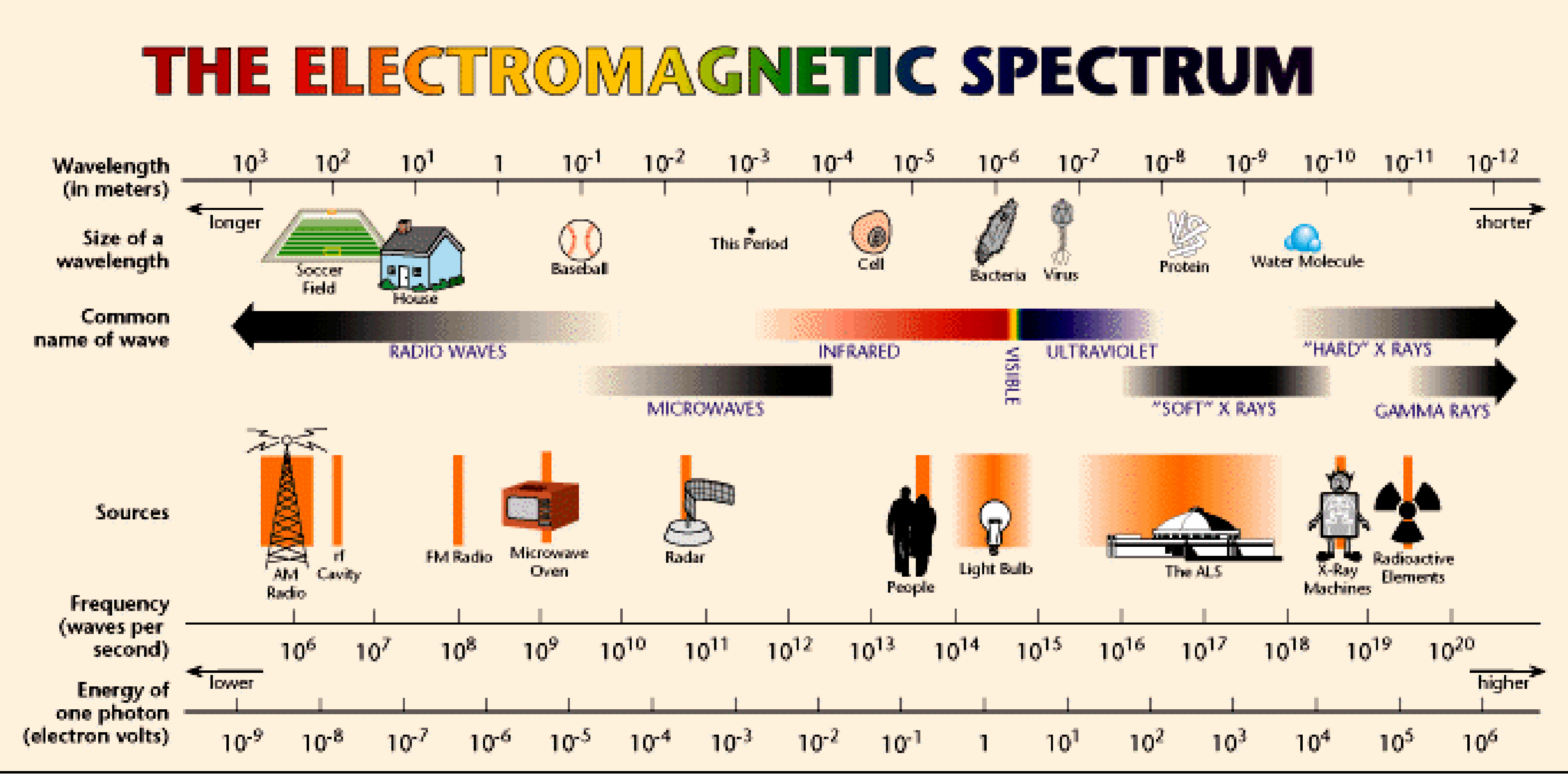
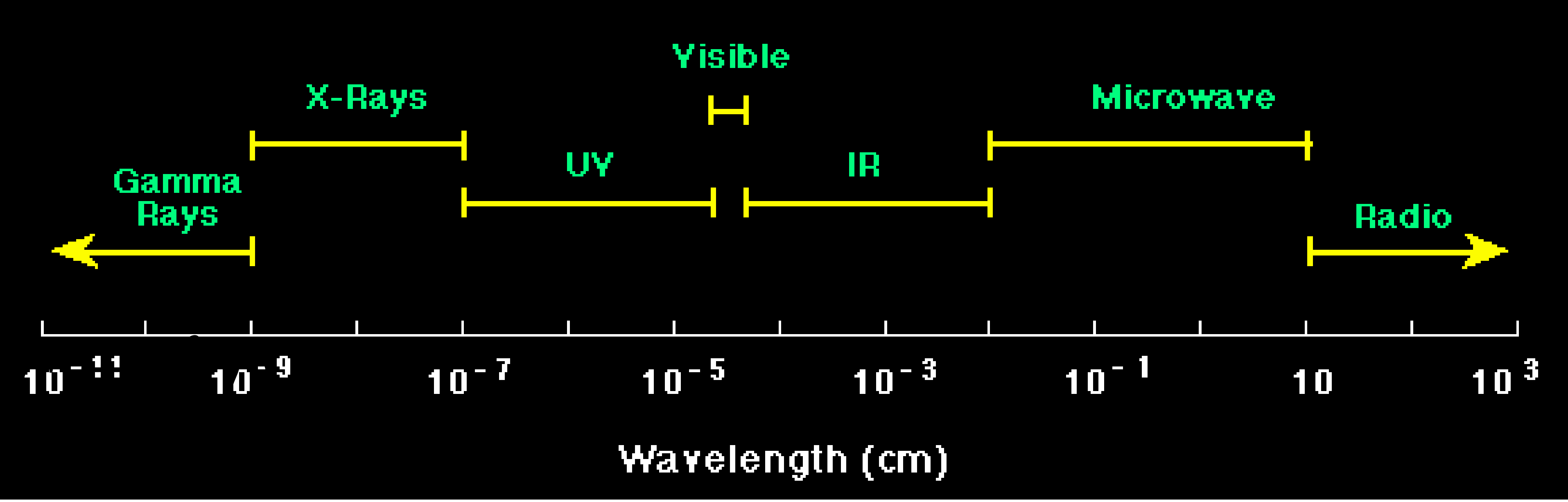
Spectroscopy





Speed of light (v) = **wavelength (λ)** x **frequency ($\bar{\upsilon}$)**

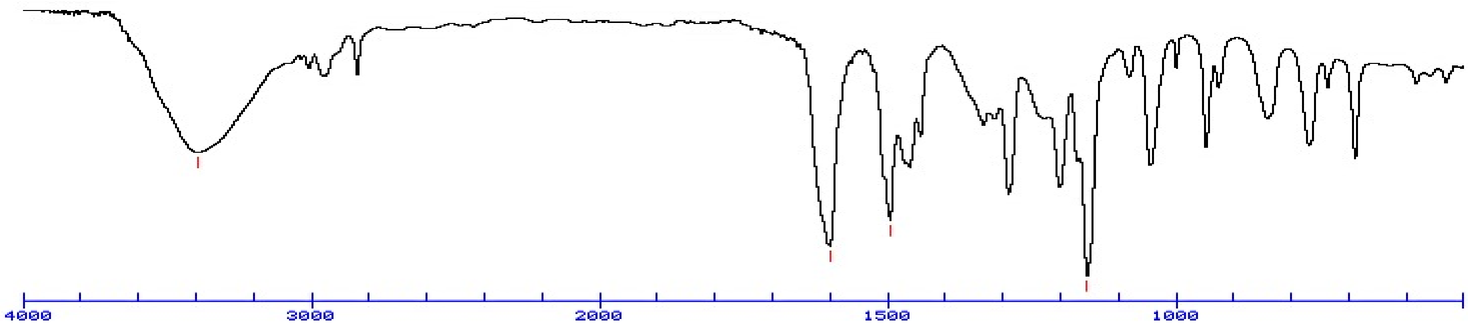
Wavenumber, $\bar{\upsilon} = \frac{1}{\lambda}$, is the number of wave maxima per cm.

Units are cm^{-1}. 700 nm **red light** = $1.43 \times 10^{4}\ cm^{-1}$
420 nm **violet light** = $2.38 \times 10^{4}\ cm^{-1}$

Velocity of light changes in different substances.

Index of refraction of a substance, n = c / v

Light and Matter: Absorption (spectroscopy)
Scattering (image formation)



An Example IR Spectrum

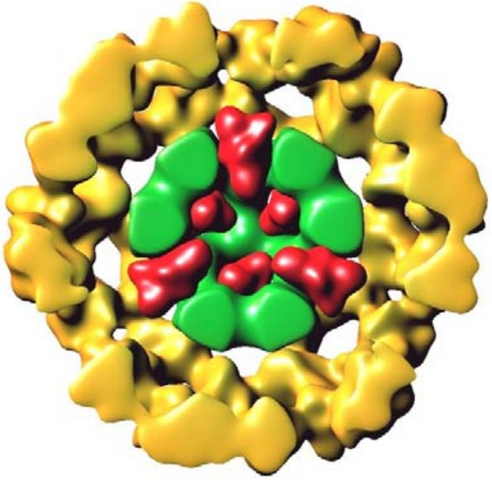
- Light Photography

$\lambda \sim$ **400 - 700 nm**

Abbe (~1878): Limit Res. ~ $\lambda/2$

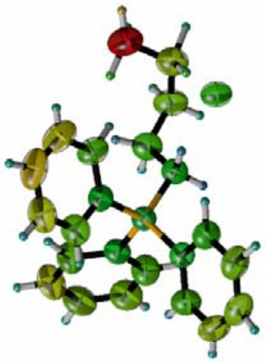
- Electron Microscopy

$\lambda \sim$ **0.001 - 0.1 nm**



- X-Ray or NMR

$\lambda \sim$ **0.1 nm**



Spectroscopy

Particles, particularly **electrons**, also have **wave behavior**.

Question: **How do light waves interact with matter** (electrons)?

Answer: Depends - different frequencies monitor different physical processes.

X-rays - scattering

Ultraviolet (uv) and visible (vis) - electronic states

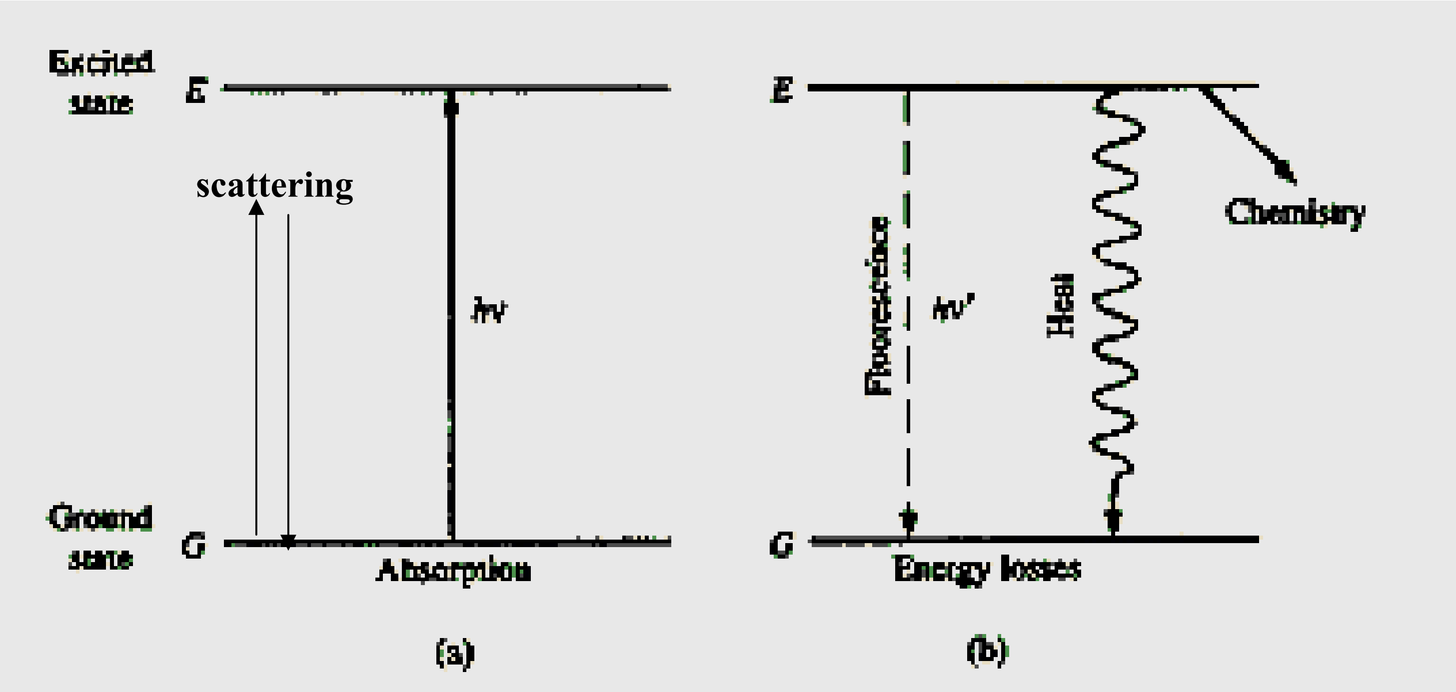
Infrared - vibrations

NMR - radiofrequency (rf) of magnetic nuclei in a magnetic field.

Optical rotation, circular dichroism (birefringence) – different indices of refraction (light speed) of right and left polarized light.

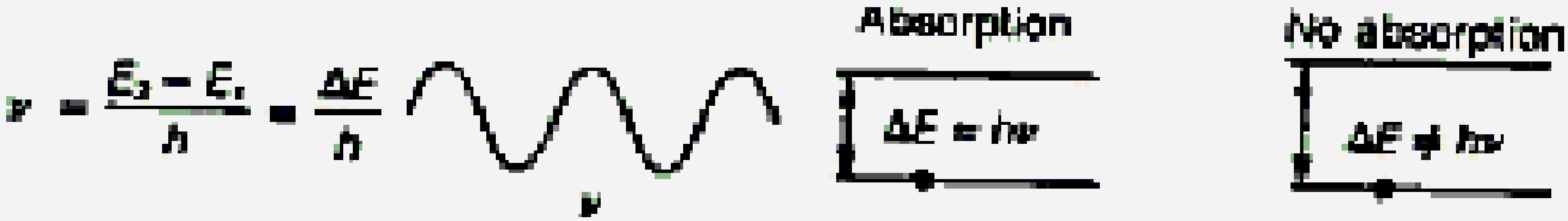
When **light interacts with matter**, there are **two possibilities**:

1. **Scattering** - the light is transmitted but velocity changes.
2. **Absorption** (photons are absorbed)
    - a. they produce heat
    - b. the cause a chemical change
    - c. they are reemitted (**fluorescence**, **phosphorescence**)



The oscillating electric field induces a force on the charges particles (electrons, protons).

If the **frequency of oscillation corresponds to an energy-level difference, the photon will be absorbed** - its energy will change form into electron or nuclear motion.



Time frame for **absorption**:

The **absorption usually occurs in the time it takes one wavelength to pass the molecule**.

the speed of light, $c = 3 \times 10^{17}$ nm sec^{-1}
for uv light, the wavelength, $\lambda \approx 300$ nm.

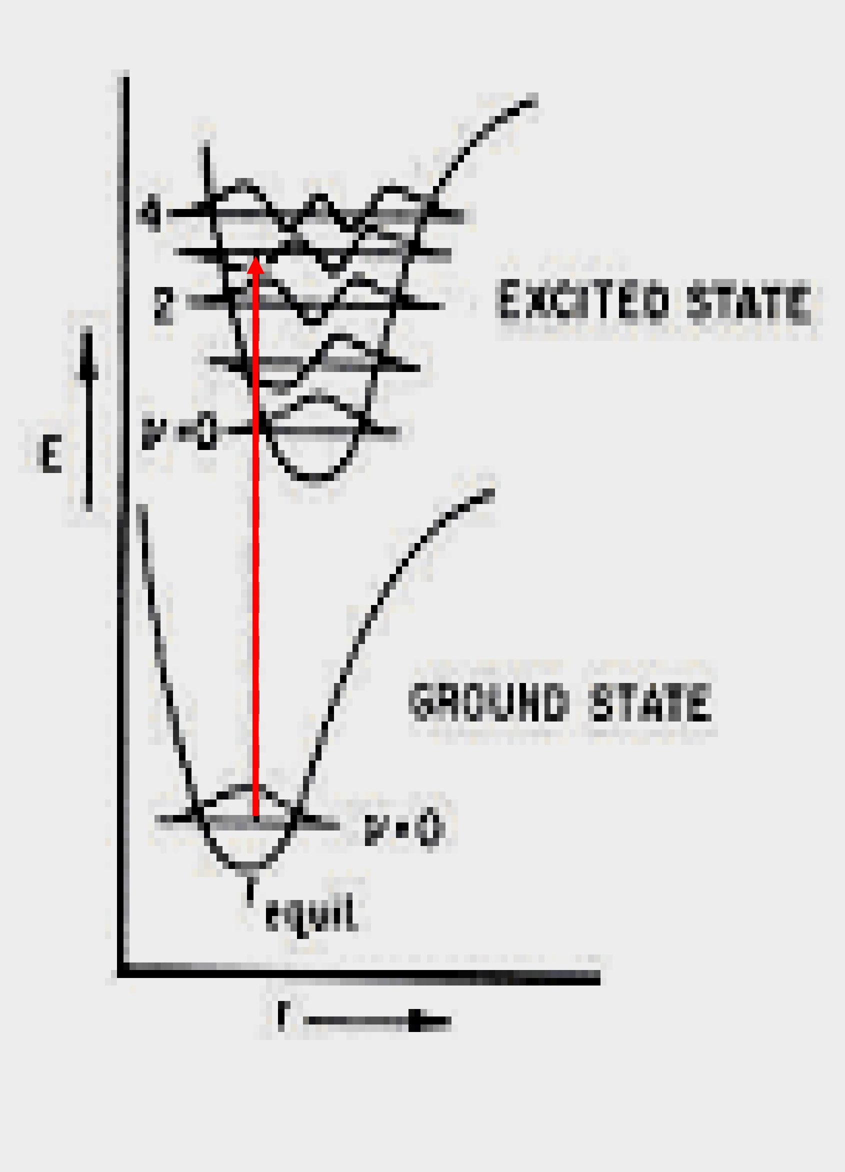
3×10^2 nm sec / 3×10^{17}nm = **10^{-15} sec**

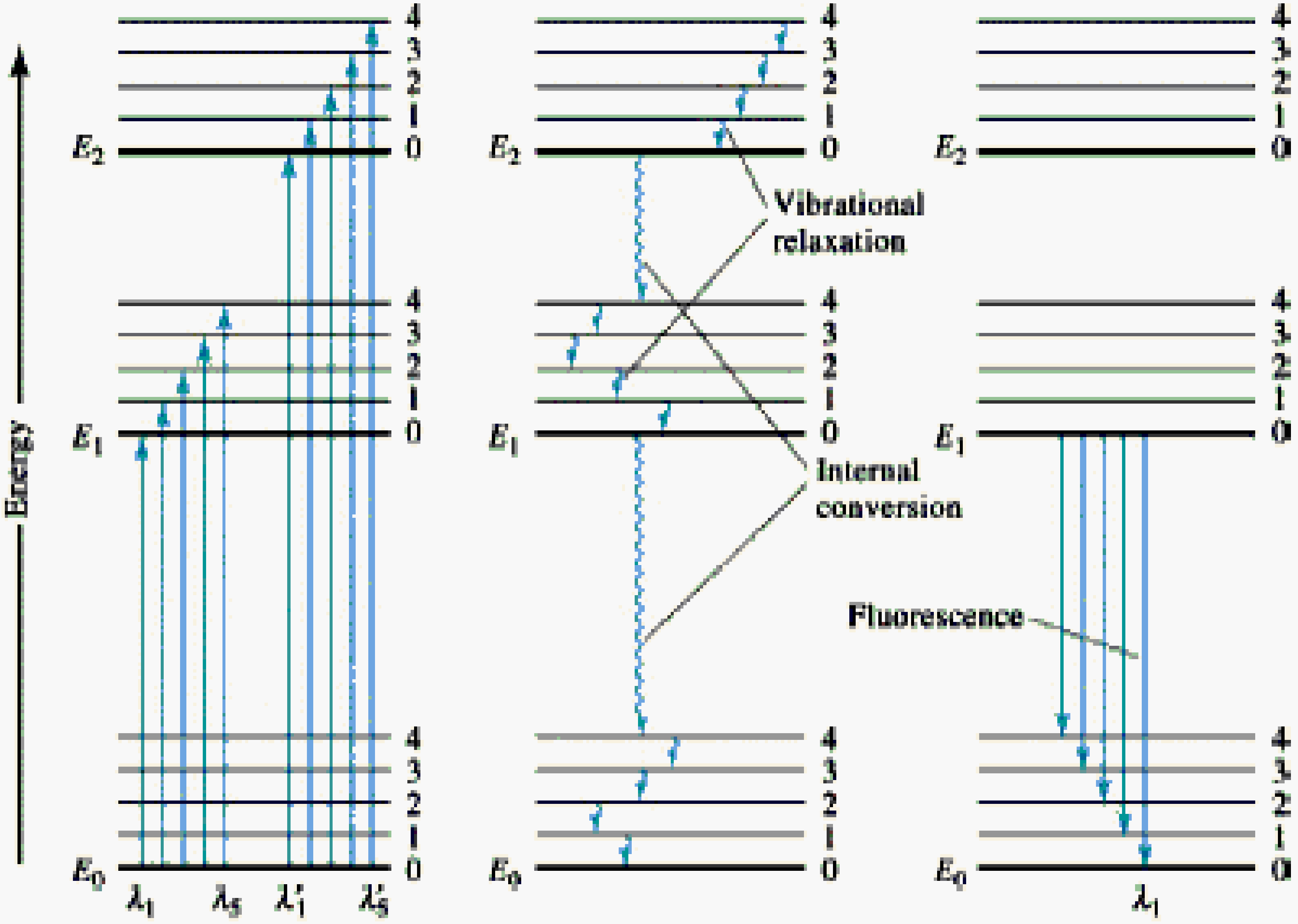
Frank-Condon Principle

- “The nuclear motion (10^{-13} s) is much slower as compared with electronic motion in transition (10^{-15} s), so it is negligible during the time required for an electronic excitation.”
- Since the nucleus does not move during the excitation, the internuclear distance keeps the same, and “the most probable component of a electronic transition involves only the vertical transitions”.

Frank-Condon

Potential energy diagram for a diatomic molecule illustrating Franck-Condon excitation. Note that the equilibrium separation is longer in the excited state than in the ground state, thus the “vertical” transition often results in excitation to a higher vibrational level in the excited state.





(a) Molecular absorption (b) Nonradiative relaxation (c) Fluorescence

Fluorescence

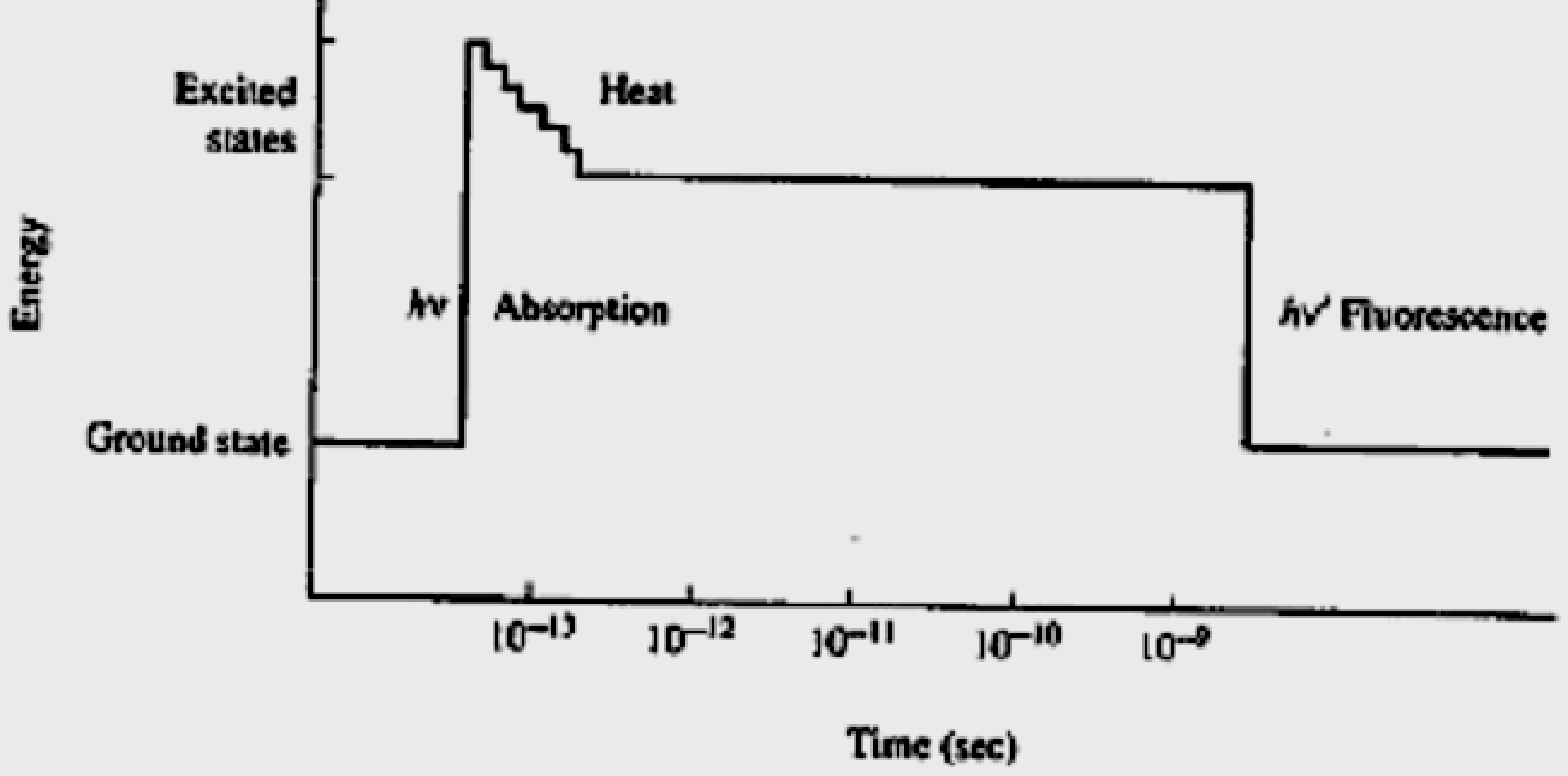
When atoms and molecules absorb UV/vis radiation, electrons are promoted to higher energy states. Various processes lead to relaxation of the excited atoms or molecules. In the case of molecules, this involves vibrational relaxation, internal conversion, and emission (**fluorescence** and **phosphorescence**).

Typical time frames:

absorption: 10^{-15} s
vibrational relaxation: 10^{-11}-10^{-10} s
internal conversion: 10^{-12} s

luminescence processes

fluorescence: 10^{-5}-10^{-10} s
phosphorescence: 10^{-4}-10^{4} s



The molecule can relax from the ground vibrational state of the excited electronic state by fluorescence.

Because of the loss of energy, the emitted photon will have a lower energy than the absorbed. This means a lower frequency and longer wavelength.

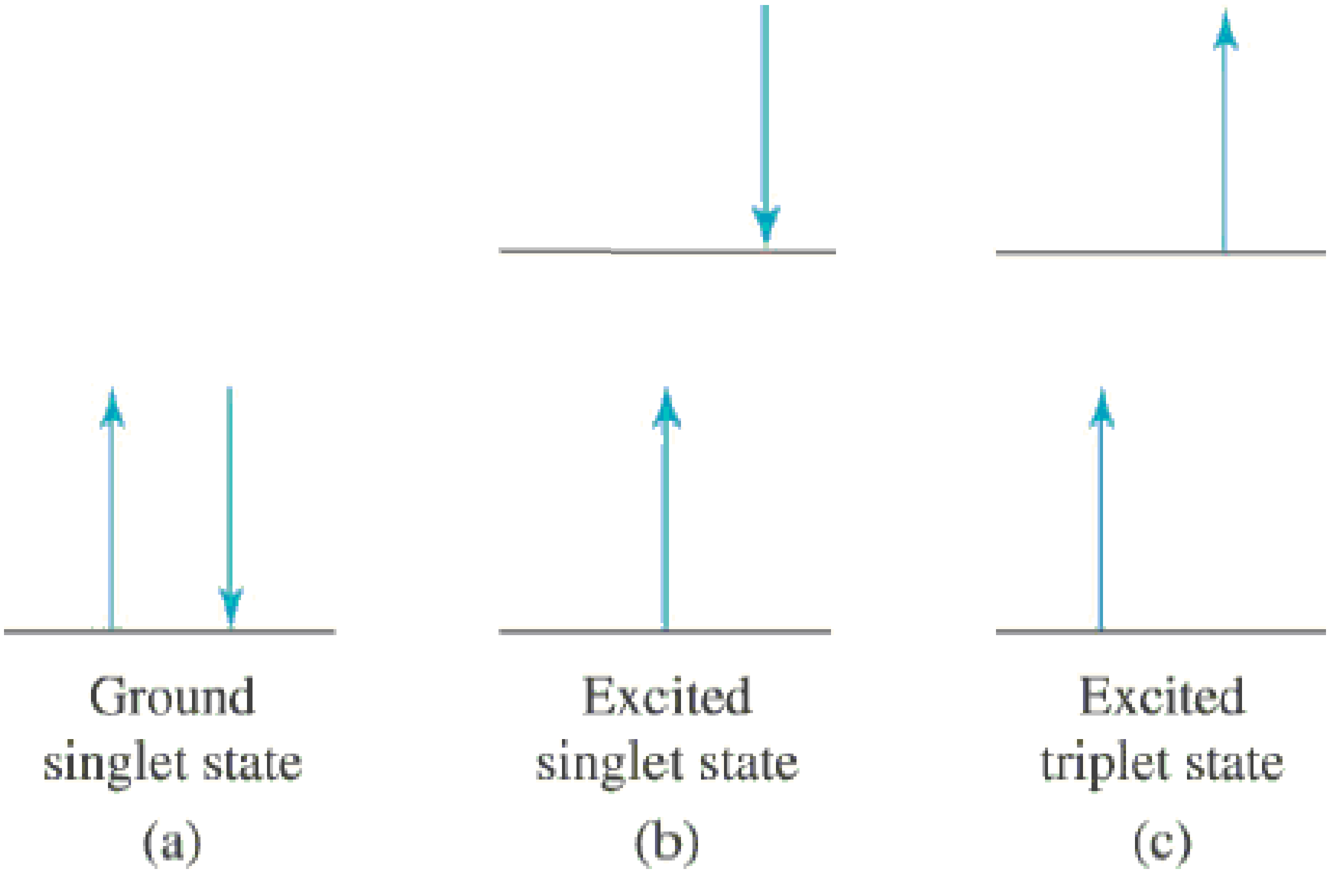
Electronic transitions

- **Selection rules**: allow **S→S**, and **T→T** processes but **not S→T and T→S**. Ground states are usually singlets; thus most excitations are to singlet excited states, like $S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$, …
- **Triplet states are usually formed by intersystem crossing** from an excited singlet state, such as S_1, rather than by direct excitation from the S_0 ground state.

Phosphorescence

Fluorescence takes place from an **excited singlet state**

Phosphorescence takes place from an **excited triplet state:**



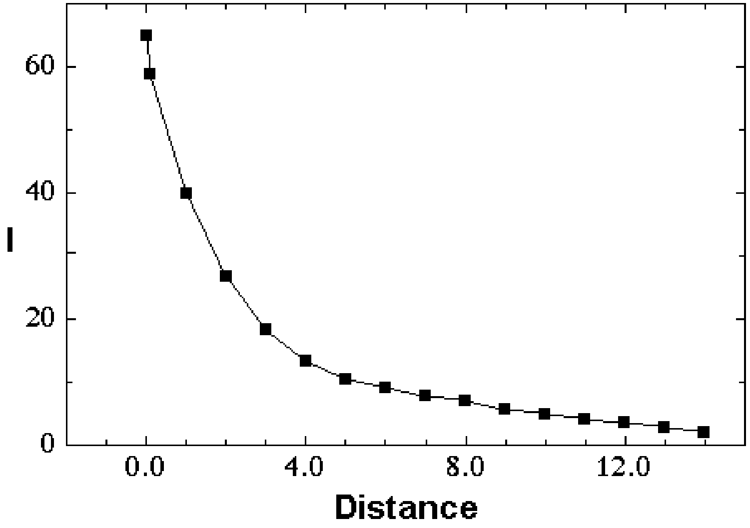
Beer-Lambert Law

When light passes through a homogeneous sample, the *fractional* decrease *(not absolute)* in light intensity is the same across any interval, dx. This resembles **1st order kinetics or radioactive decay**. The change in light intensity, I, with distance is

$$\frac{dI}{dx} = -I\alpha c$$

$$-\frac{dI}{I} = \alpha c dx$$

or $$I_t = I_0 e^{-\alpha c l}$$



dI/I is the fractional decrease in light intensity, α is a constant and c is the concentration. We can integrate this to get:

$$\ln(I_0/I_t) = \alpha\ c\ l,$$

where I_0 is the initial intensity and I_t is the intensity of the light transmitted at a distance l.

$$I_t = I_0 e^{-\alpha c l}$$

Note that c and l are both in the exponential factor.
Instead of looking at variation of intensity with distance at a constant concentration, we can consider a constant path length and varying concentration.

It follows that the **transmitted intensity decreases exponentially with concentration**.

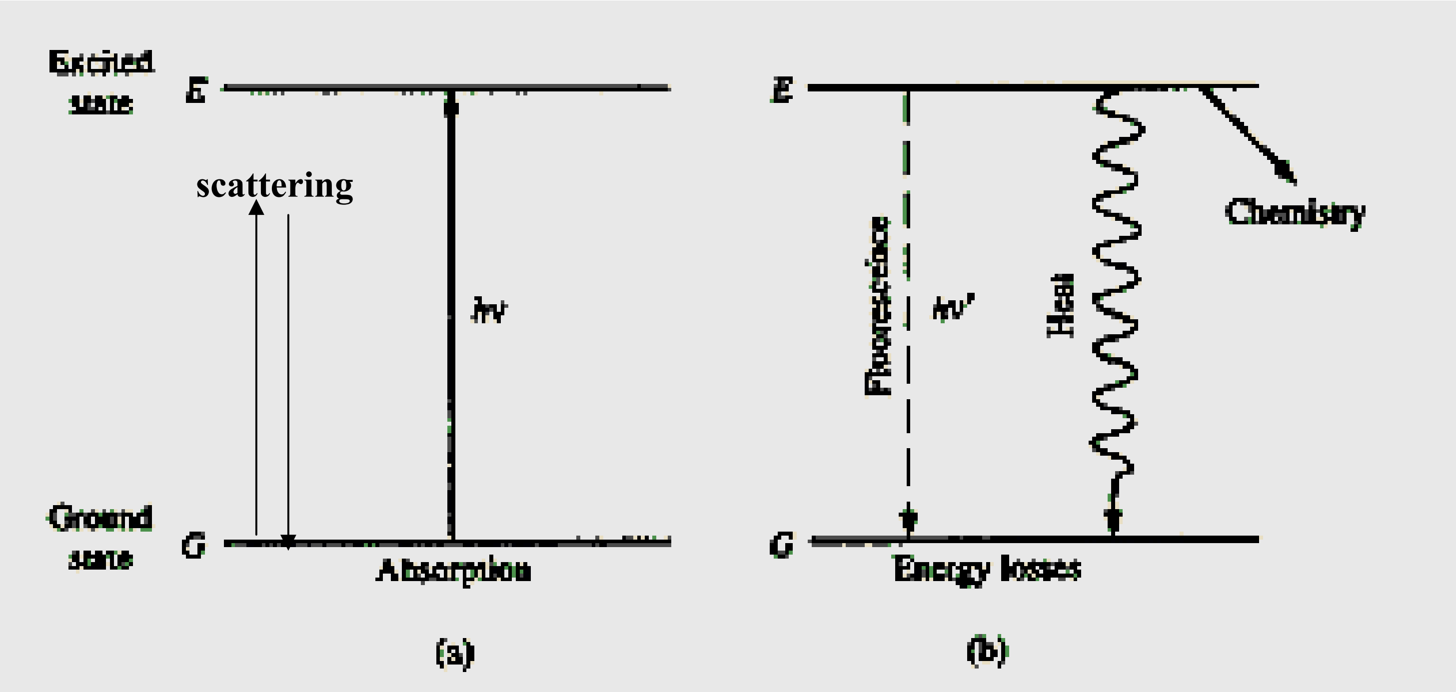
$$A = \log \frac{I_0}{I_t} = \varepsilon c l$$

Units on ε:
M^{-1} cm^{-1}

where **A** is "**absorbance**" or "**optical density**" and **ε** is the "**molar absorptivity**" or "**molar extinction coefficient**" and $\varepsilon = \alpha / 2.303$

When **light interacts with matter**, there are **two possibilities**:

1. **Scattering** - the light is transmitted but velocity changes.
2. **Absorption** (photons are absorbed)
   - a. they produce heat
   - b. the cause a chemical change
   - c. they are reemitted (**fluorescence**, **phosphorescence**)

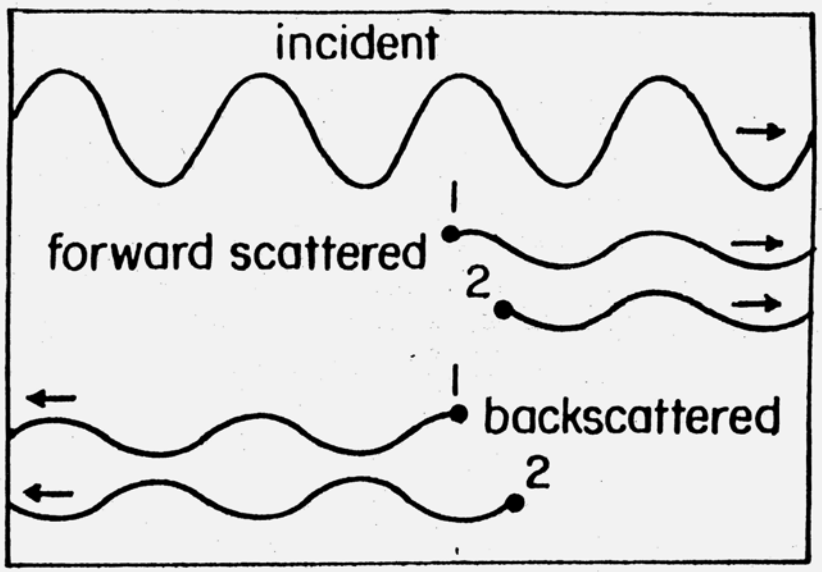


Raleigh (elastic) Scattering

All molecules scatter light. **Elastic scattering occurs when a photon interacts with the molecule but no absorption occurs.** There are no selection rules. **The polarizability, α, is a measure of the ease with which the electrons are distorted** (i.e. a dipole is induced by an electric field.)

$$\mu_{ind} = \alpha E$$

Schematic for forward scattering



Refractive Index

The ratio of the speed of light in a vacuum to the speed of light in a medium.

$$n = c / v$$

Light speed is affected by the **polarizability** of the medium. Incident light induces an oscillating dipole with the same frequency as the incident light. The induced dipole radiates light at the same frequency but there is a **phase delay**, apparently slowing the light.

The more polarizable, the more the phase lag.

At optical frequencies, the **polarizability increases with frequency**. (**Blue light has a greater refractive index, thus is bent more by a prism.)**

Excitation Transfer

Fluorescence **R**esonance **E**nergy **T**ransfer (**FRET**) is an important tool for studying macromolecular structure and dynamics in solution. Some amino acids or reporter groups fluoresce and the **energy transfer strongly depends on distance between donor and acceptor**, making it a valuable tool to study protein folding and other dynamics.

Consider an excited donor, **D^***, and an acceptor (A) that can be excited to a fluorescent state, **A^*.**

$D \rightarrow D^*$ (absorption of light, hv, by donor)

$D^* \rightarrow D$ (other de-excitation)

$D^* \rightarrow D + hv'$ (donor fluorescence)

$D^* + A \rightarrow D + A^*$ (excitation transfer)

$A^* \rightarrow A + h\,v''$ (acceptor fluorescence)

Primary Conditions for FRET

- Donor and acceptor molecules must be in close proximity (typically 10–100 Å).
- The absorption spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor (see **Figure**).
- Donor and acceptor transition dipole orientations must be approximately parallel.

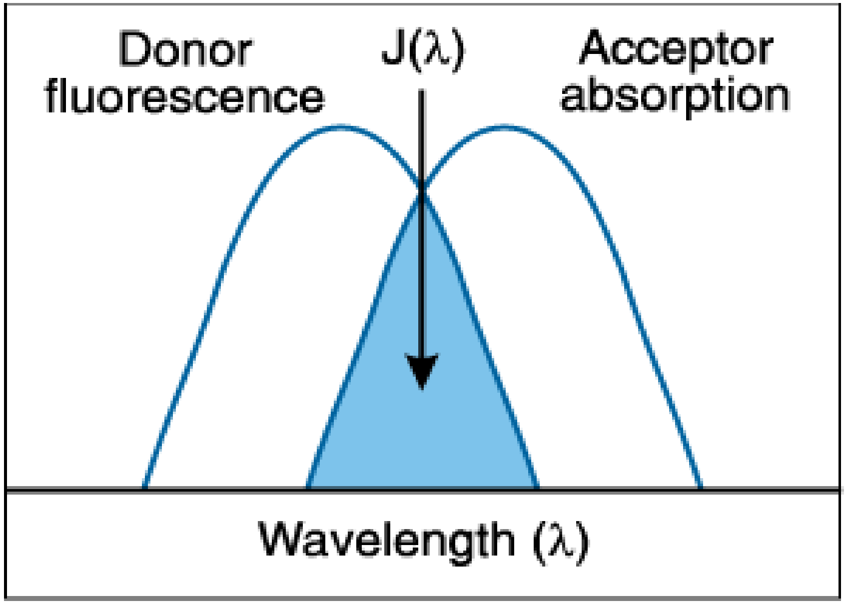


Figure. Schematic representation of the FRET spectral overlap integral.

The efficiency of the energy transfer is defined as the fraction of **D*** that is deexcited by energy transfer to A.

Efficiency = kT / (kT + kd)

kd is the rate constant for deexcitation.

$$\text{Efficiency} = k_T / (k_T + k_d)$$

In the range of 1 to 10 nm (10 to 100 Angstroms), FRET occurs. The efficiency depends on the inverse sixth power of intermolecular distance (like dispersion interactions).

$$\text{Eff} = r_0^6 / (r_0^6 + r^6)$$

where r_0 is the characteristic distance for which Eff = 0.5

Förster Radius, r_0

The distance at which energy transfer is 50% efficient (i.e., 50% of excited donors are deactivated by FRET) is defined by the Förster radius (R_o). The magnitude of R_o is dependent on the spectral properties of the donor and acceptor dyes:

$$R_O = [8.8 \times 10^{23} \cdot \kappa^2 \cdot n^{-4} \cdot \phi_d \cdot J(\lambda)]^{1/6}\ \text{Å}$$

where κ^2 = dipole orientation factor (range 0 to 4; $\kappa^2 = 2/3$ for randomly oriented donors and acceptors)

ϕ_d = fluorescence quantum yield of the donor in the absence of the acceptor

n = refractive index of the medium

$J(\lambda)$ = spectral overlap integral

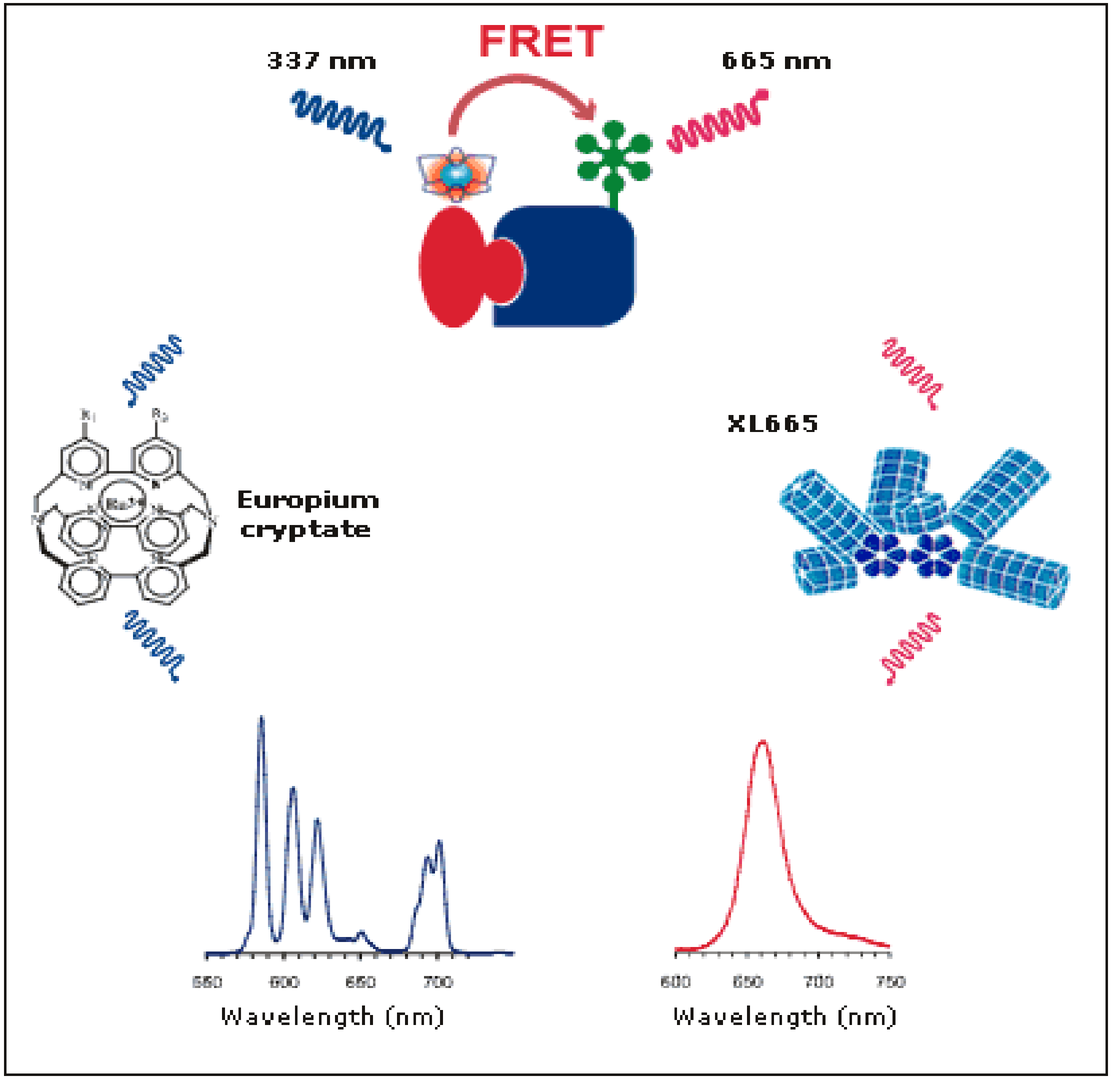
$$= \int \epsilon_A(\lambda) \cdot F_D(\lambda) \cdot \lambda^4 d\lambda\ \text{cm}^3\text{M}^{-1}$$

where ϵ_A = extinction coefficient of acceptor

F_D = fluorescence emission intensity of donor as a fraction of the total integrated intensity

Table. Typical Values of R_o.

Donor	Acceptor	R_o (Å)
Fluorescein	Tetramethylrhodamine	55
IAEDANS	Fluorescein	46
EDANS	Dabcyl	33
Fluorescein	Fluorescein	44
BODIPY FL	BODIPY FL	57
Fluorescein	QSY 7 and QSY 9 dyes	61



When the two entities come into close proximity and upon excitation, **FRET** occurs and XL665 re-emits a specific long-lived fluorescence at 665 nm.