7. Applications of absorption and fluorescence spectroscopies in photomedicine

7.1 Molecular Energy Levels

A. Different energy levels
B. Electronic (and vibrational) energy levels
C. Population of energy levels

Energy Levels

A. Different energy levels

$$E_{molecule} = E_{translation} + E_{electron\ spin} + E_{nuclear\ spin} + E_{rotation} + E_{vibration} + E_{electronic}$$

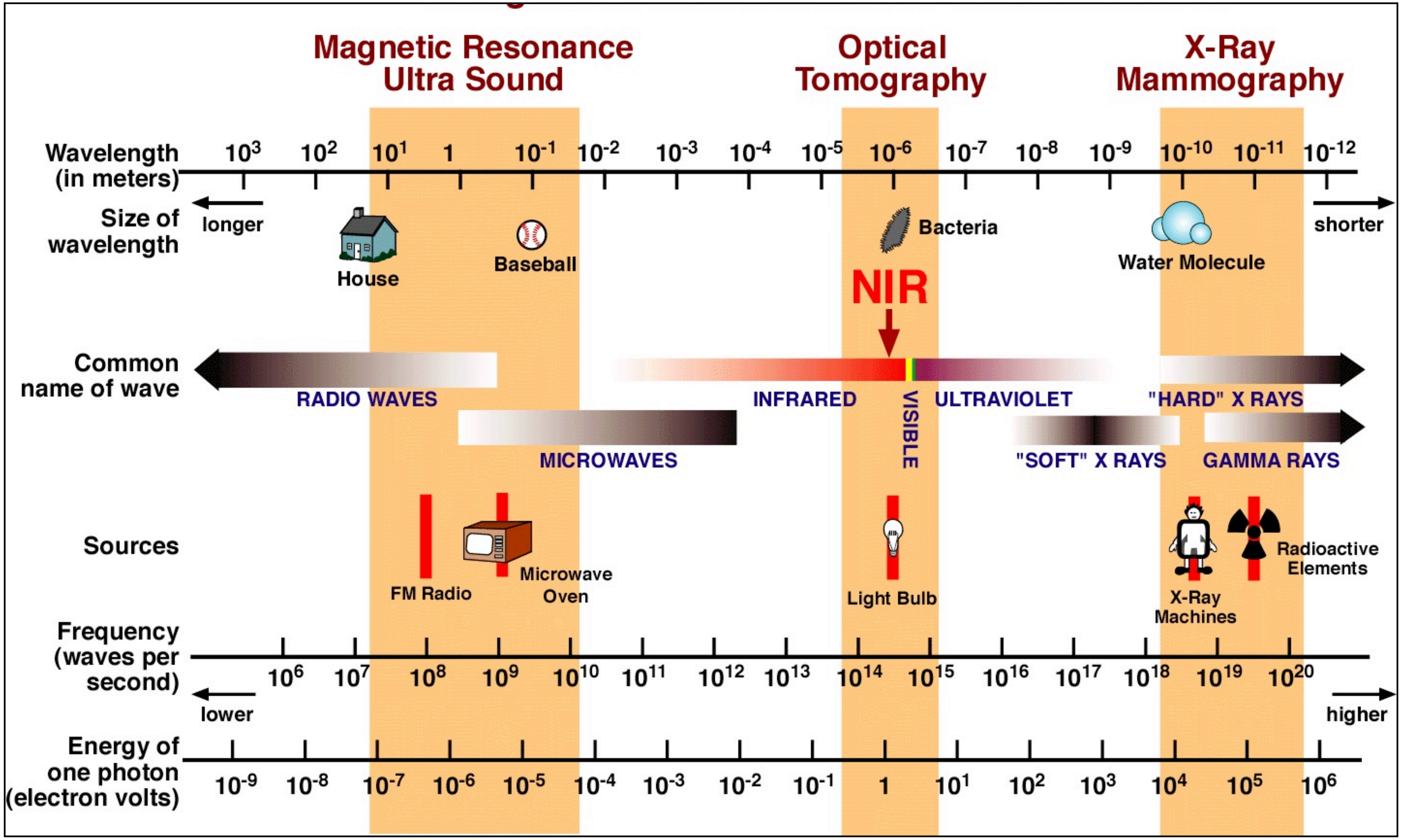
- Translational energy: motion of the molecule's center of mass through space
- Spin energy: nuclear and electron spin
- Rotational energy: rotation of the molecule about its center of mass
- Vibrational energy: vibration of the molecule's constituent atoms
- Electronic energy: mutual interactions of the molecules electrons and nuclei

The energy associated with each of these levels is quantized.
They are associated to orbitals

Energy Levels

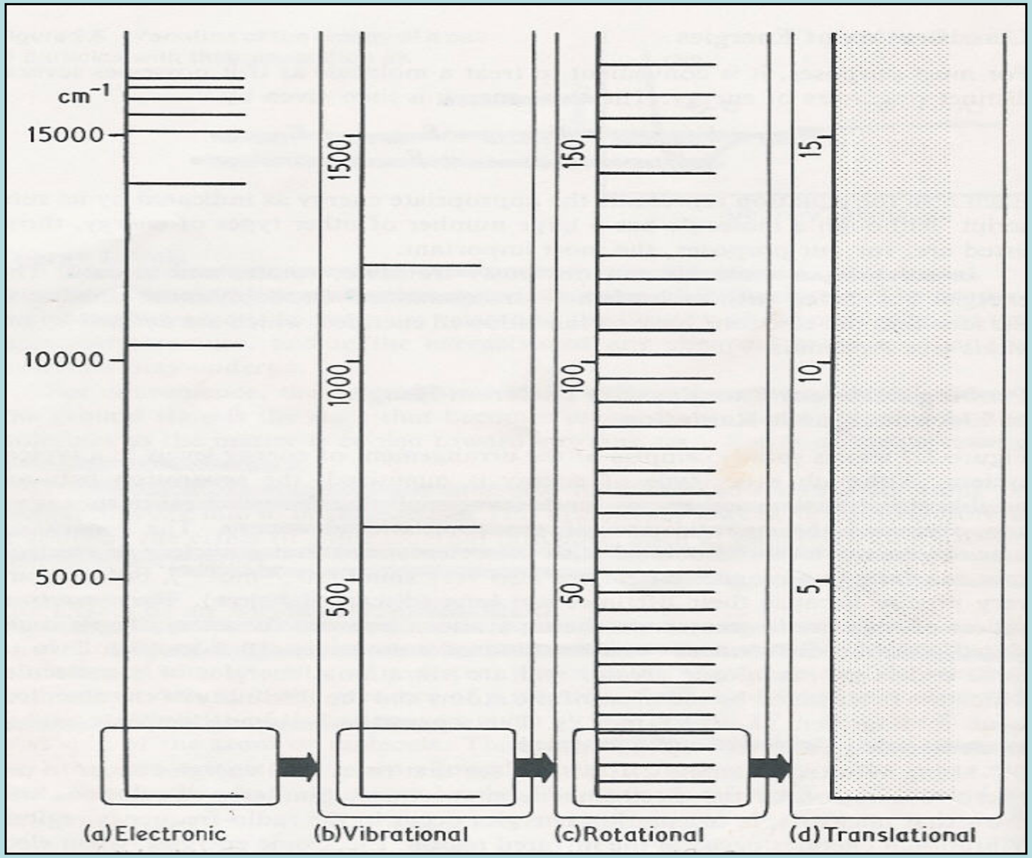
Energy	Energy Level Separation [J]
Translation	Very small
Spin	10^{-25}
Rotation	10^{-22}
Vibration	10^{-20}
Electronic	10^{-19}

Energy Levels



Energy Levels

cm^{-1}



Common spectroscopists unit, wavenumber = 1 / wavelength, so proportional to frequency and energy

Energy Levels

B. Electronic energy levels

(**The most important energy levels in photomedicine**)

- In particular, UV-VIS absorption / fluorescence spectroscopy involves electronic energy transitions

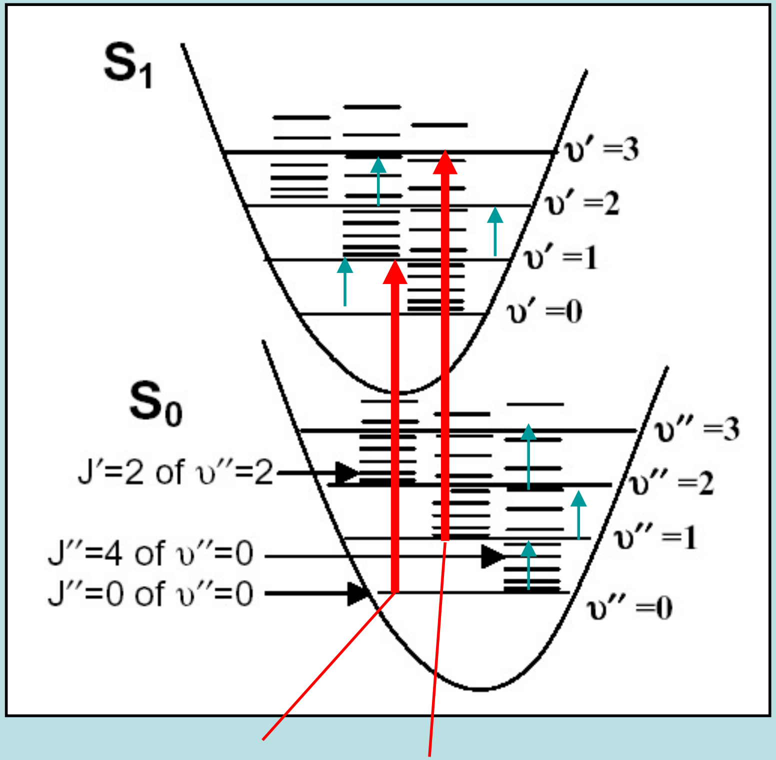
----

- Electronic energy levels of molecules are described by molecular orbitals
- When an electron undergoes an electronic transition, it is transferred from one molecular orbital to another

Infrared Absorption Spectroscopy

Infrared absorption

IR radiation does not have enough energy to induce electronic transitions as seen with UV. Absorption of IR is restricted to compounds with small energy differences in the possible vibrational and rotational states.



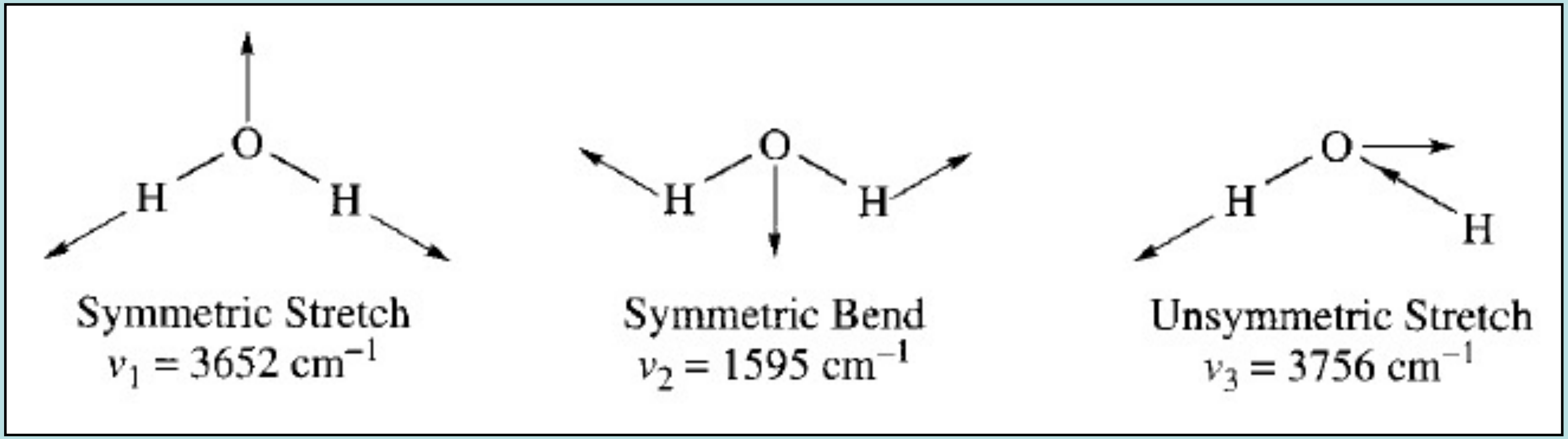
Electronic transition

http://www.shu.ac.uk/schools/sci/chem/tutorials/molspec/irspec1.htm

Vibrational States of a Molecule

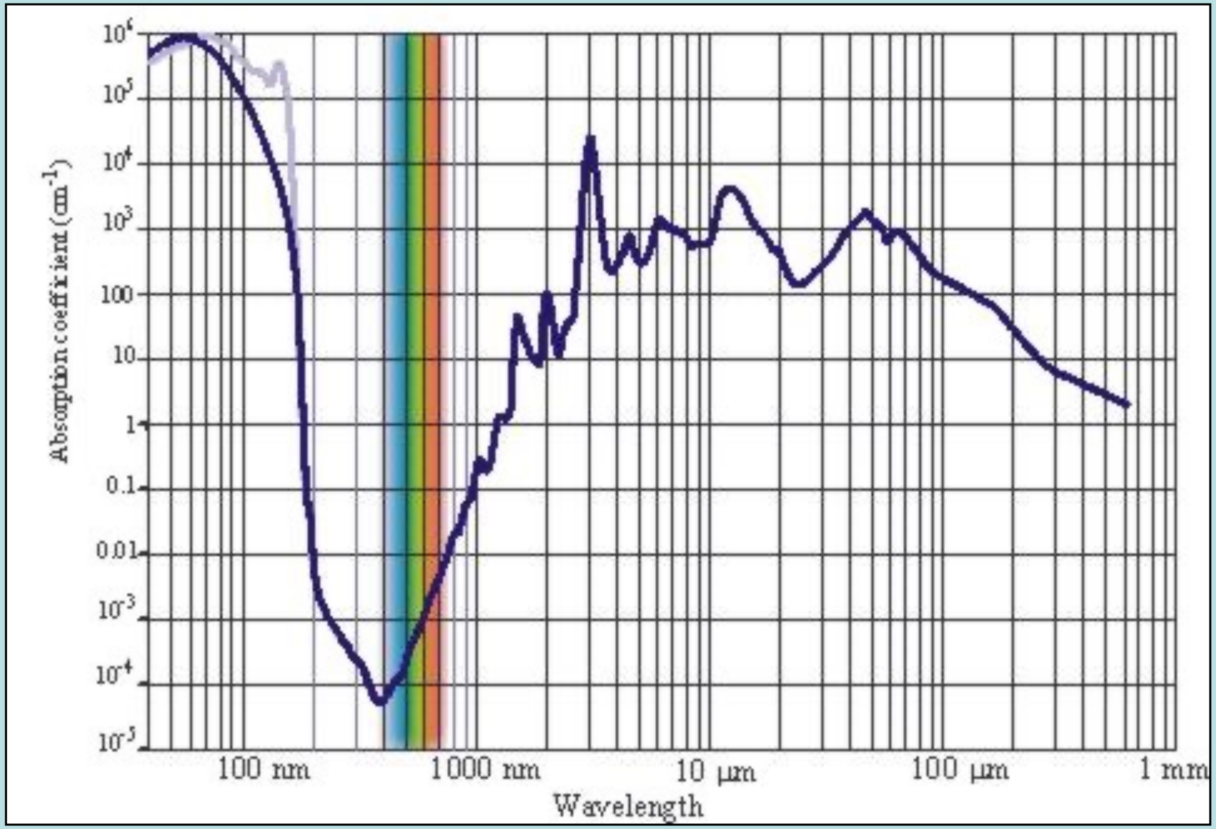
More general cases

Normal modes of vibration of a water molecule:



Infrared Absorption Spectroscopy

Water (major chromophore in biological samples) absorption



Water absorption spectrum - note absorption peaks at 1.45, 1.94, 2.94, 4.5 and 6 microns !

Water absorption in the visible

Absorption imaging in the visible and IR

Energy Levels

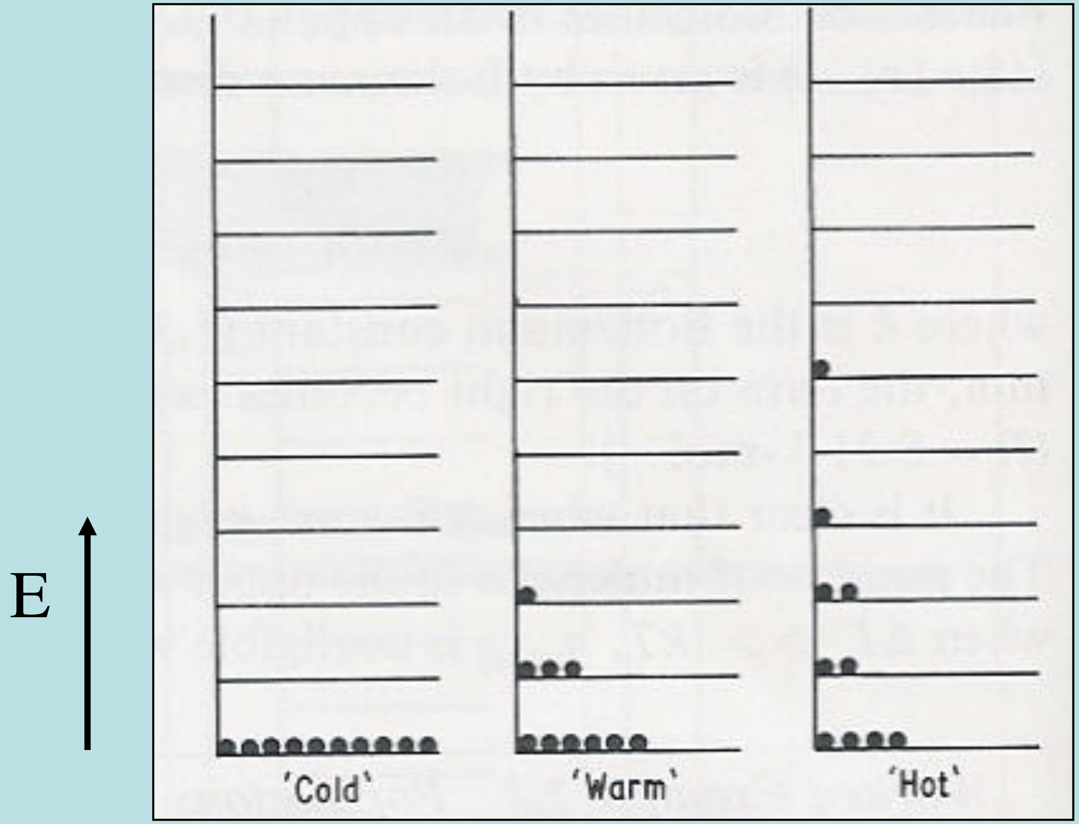
C. Population of energy levels

- At any finite temperature (T), molecules will be distributed among available E levels due to thermal agitation

- The exact distribution among energy levels will depend upon the temperature and separation between energy levels

Energy Levels

Population of energy levels



Energy Levels

Population of energy levels

- At any finite temperature (T), the molecules in an upper state (n_{upper}) relative to the number in a lower state (n_{lower}) is given by the Boltzmann's distribution:

$$\frac{n_{upper}}{n_{lower}} = \exp\left(-\Delta E / kT\right)$$

$k=1.38*10^{-23}$ JK^{-1} (Boltzmann's constant)

ΔE = separation in energy level (HOMO–LUMO gap)

Energy Levels

$$\frac{n_{upper}}{n_{lower}} = \exp\left(-\Delta E / kT\right)$$

k=1.38*10^{-23} JK^{-1} (Boltzmann's constant)

ΔE = separation in energy level (HOMO–LUMO gap)

If ΔE = 1 eV (energy difference between electronic states)
at physiological temperature: n_{upper}/n_{lower} = exp(-40) = 1.6.10^{-17}

If ΔE = 0.1 eV (energy difference between vibrationnal states)
at physiological temperature: n_{upper}/n_{lower} = exp(-4) = 0.02

If ΔE = 0.001 eV (energy difference between rotational states)
at physiological temperature: n_{upper}/n_{lower} = exp(-0.04) = 0.96