7. Applications of absorption and fluorescence spectroscopies in biomedical optics

7.1 Molecular Energy Levels

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

Energy Levels

A. Definitions

- Ground state is state of lowest energy
- States of higher energy are called excited states
- If two or more states of a molecule or atom have the same energy value, they are degenerated

- **Energy levels are characteristic states of a molecule or atom**

Energy Levels

A. Different energy levels

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

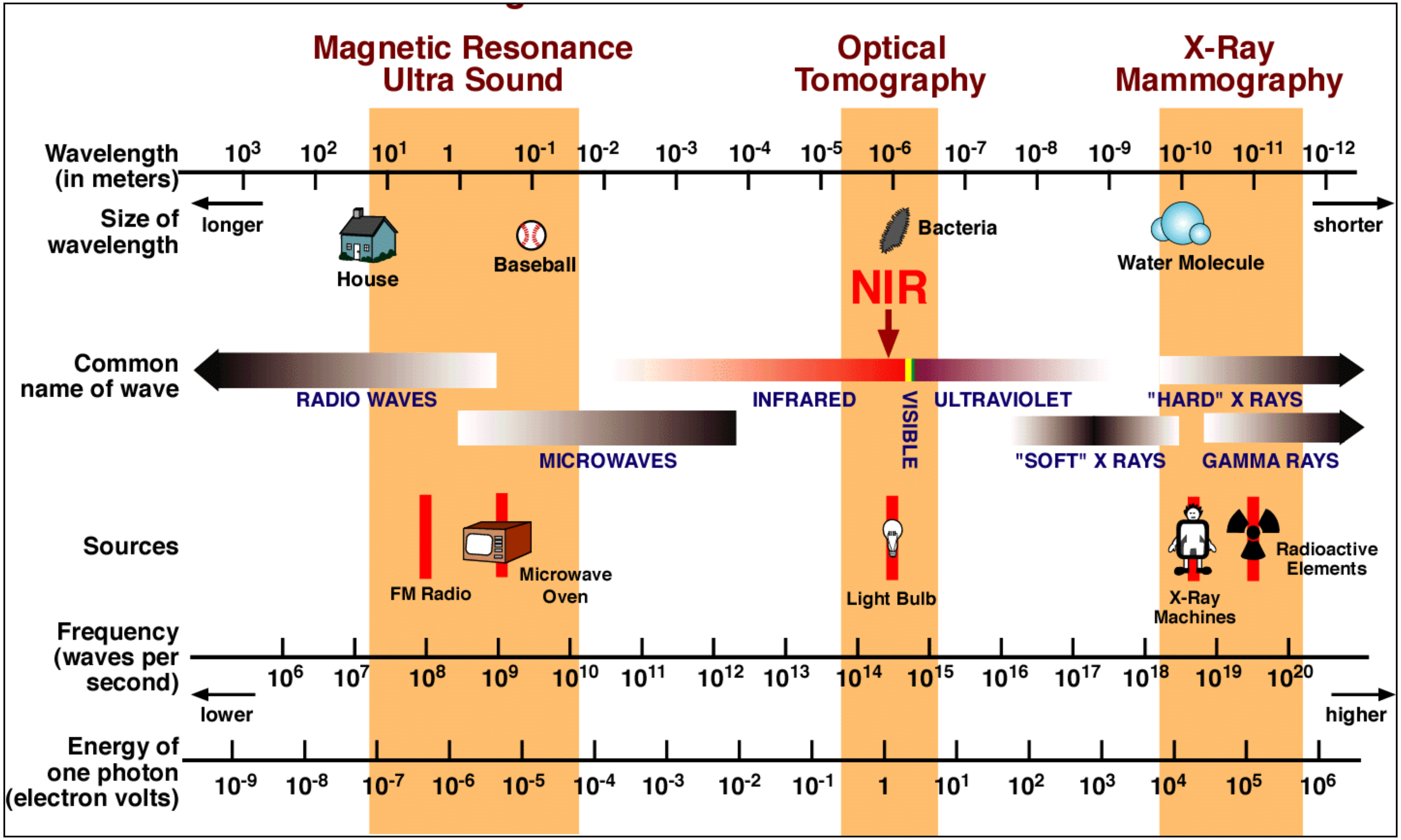
- Translational energy: motion of the molecule’s center of mass through space
- Spin energy: nuclear and electron spin
- Rotational energy: rotation of the molecule about its center of mass
- Vibrational energy: vibration of the molecule’s constituent atoms
- Electronic energy: mutual interactions of 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 (HOMO-LUMO)	10^{-19}

Energy Levels



Energy Levels

B. Electronic energy levels

(The most important energy levels in photomedicine)

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

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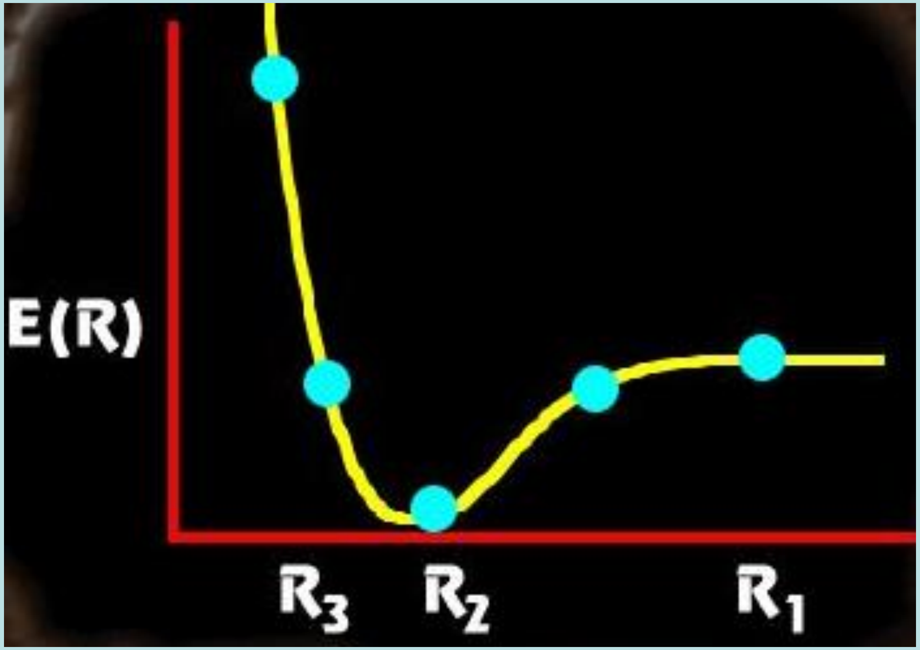
- Electronic energy levels of molecules are described by molecular orbitals
- When an electron undergoes an electronic transition, it is transferred from one molecular orbital to another

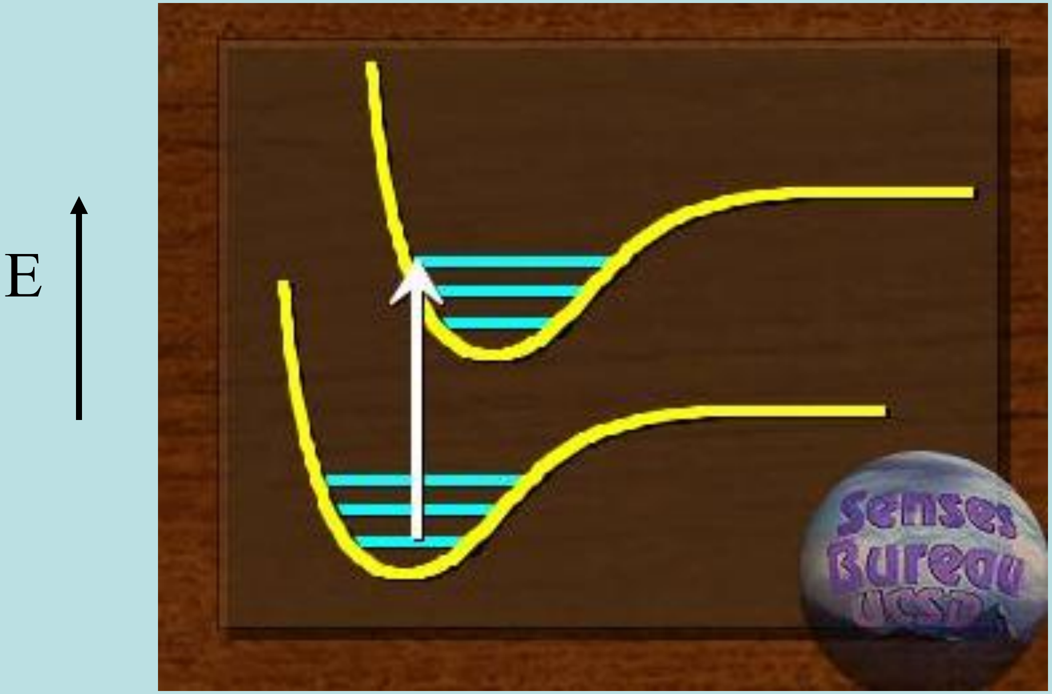
Energy Levels

Energy level associated to one orbital varies with inter-nuclear distance (R) in a molecule



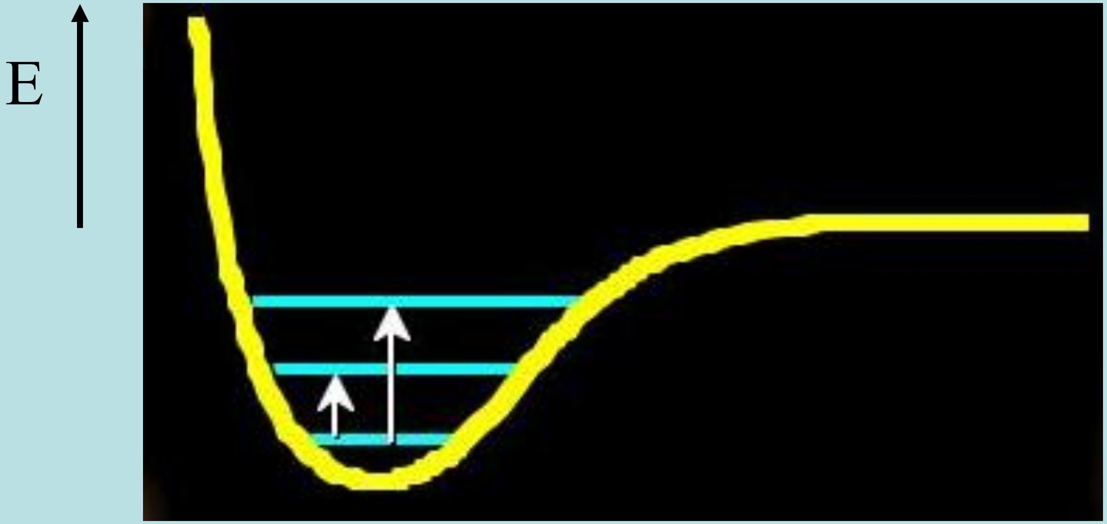
Energy Levels

Electronic spectroscopy involves transitions between electronic energy levels



Energy Levels

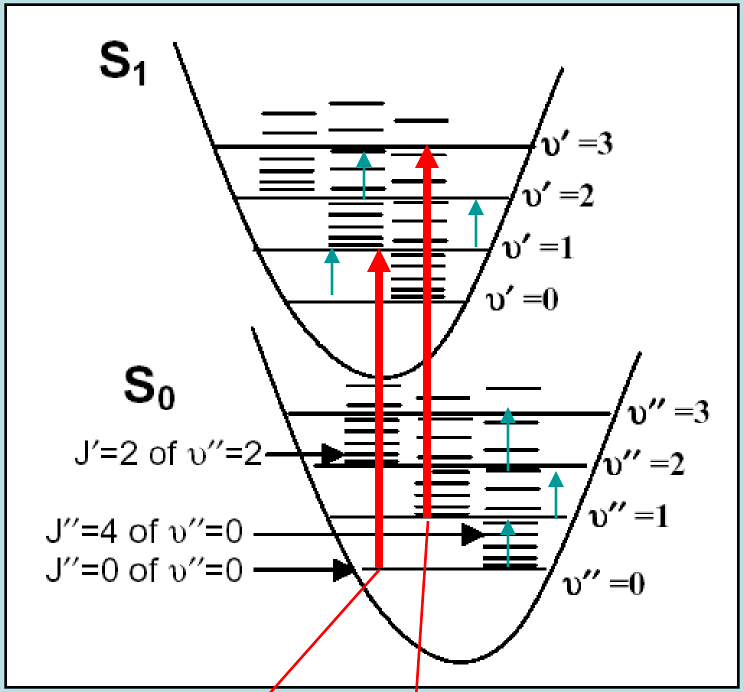
Vibrational spectroscopy involves transitions within an electronic energy state



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.

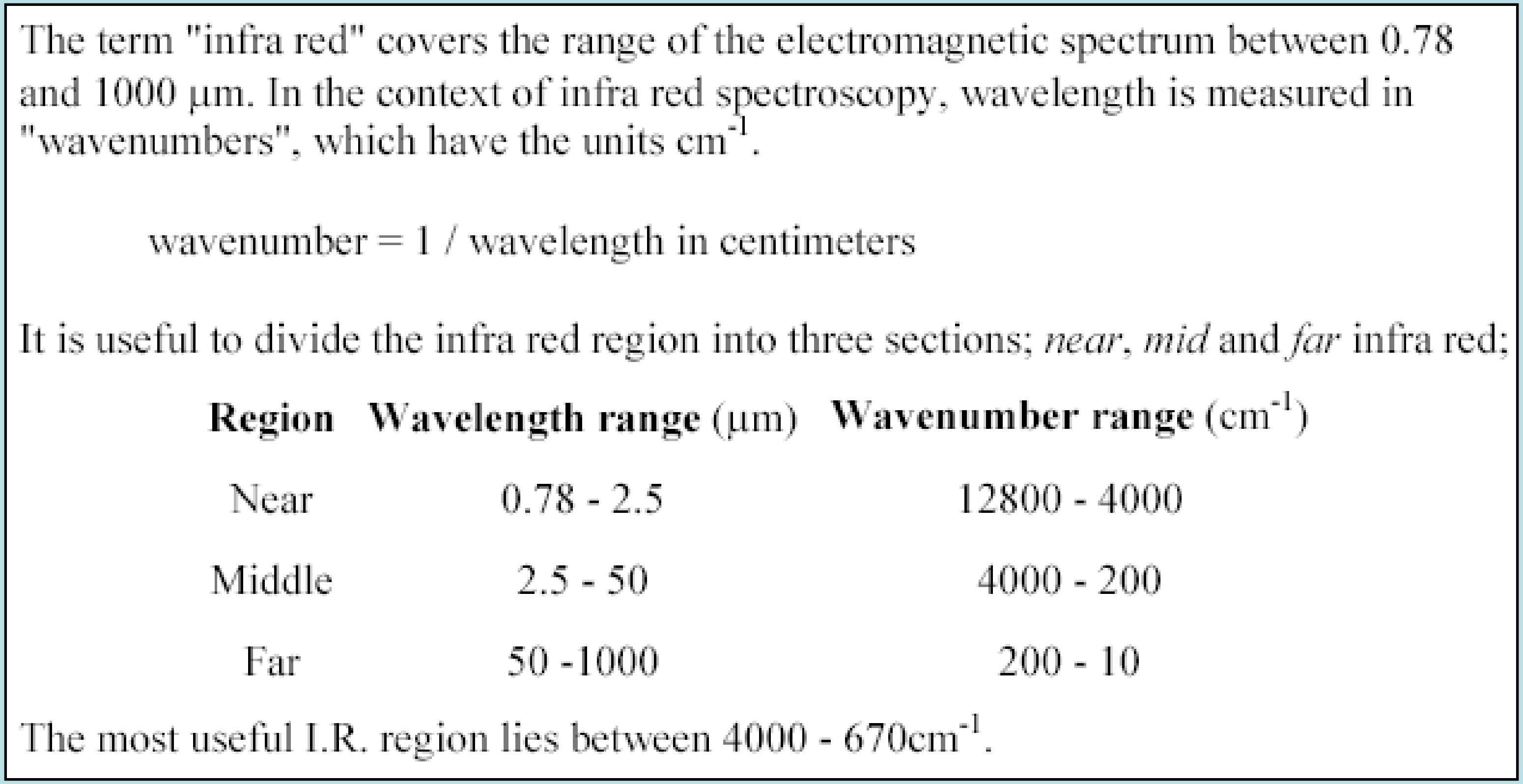


Electronic transition

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

Infrared Absorption Spectroscopy
(due to changes of rotational and vibrational states)

Introduction



The term "infra red" covers the range of the electromagnetic spectrum between 0.78 and 1000 µm. In the context of infra red spectroscopy, wavelength is measured in "wavenumbers", which have the units cm^{-1}.

wavenumber = 1 / wavelength in centimeters

It is useful to divide the infra red region into three sections; *near*, *mid* and *far* infra red;

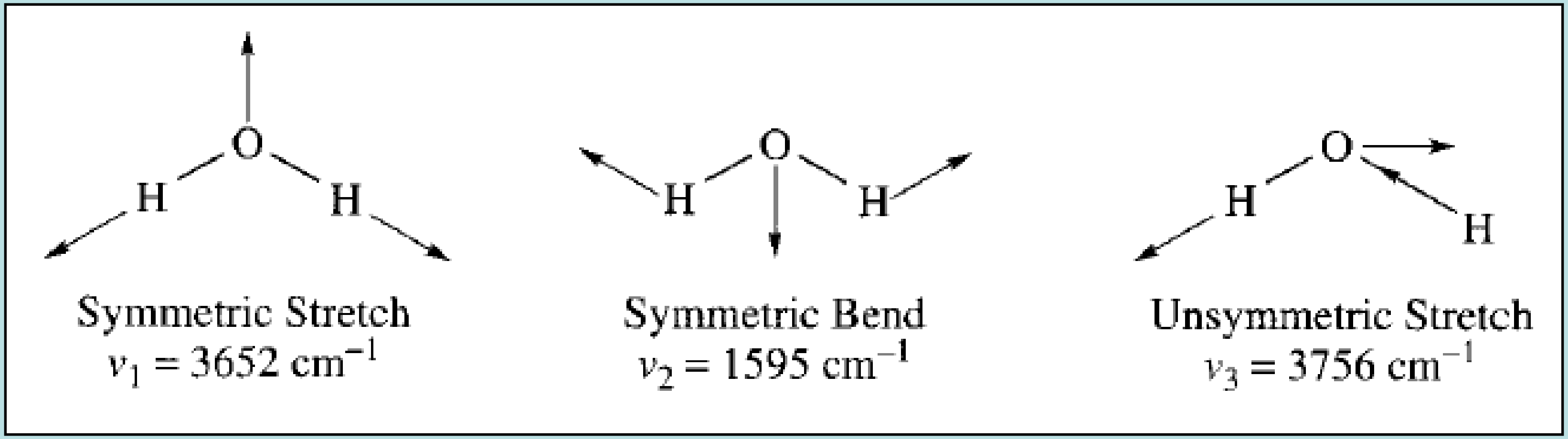
Region	**Wavelength range** (µm)	**Wavenumber range** (cm^{-1})
Near	0.78 - 2.5	12800 - 4000
Middle	2.5 - 50	4000 - 200
Far	50 -1000	200 - 10

The most useful I.R. region lies between 4000 - 670cm^{-1}.

Vibrational States of a Molecule

More general cases

Normal modes of vibration of a water molecule:



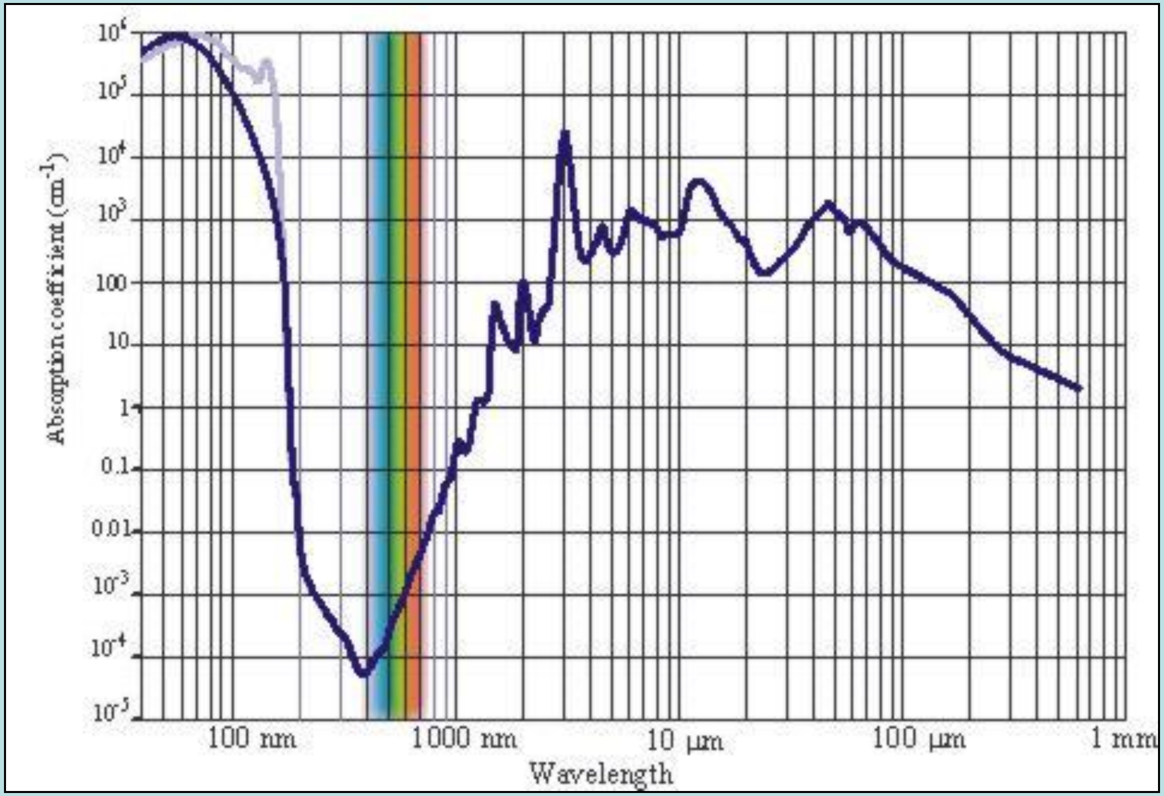
Symmetric Stretch
$\nu_1 = 3652\ \mathrm{cm}^{-1}$

Symmetric Bend
$\nu_2 = 1595\ \mathrm{cm}^{-1}$

Unsymmetric Stretch
$\nu_3 = 3756\ \mathrm{cm}^{-1}$

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 !

Absorption imaging in the visible and IR

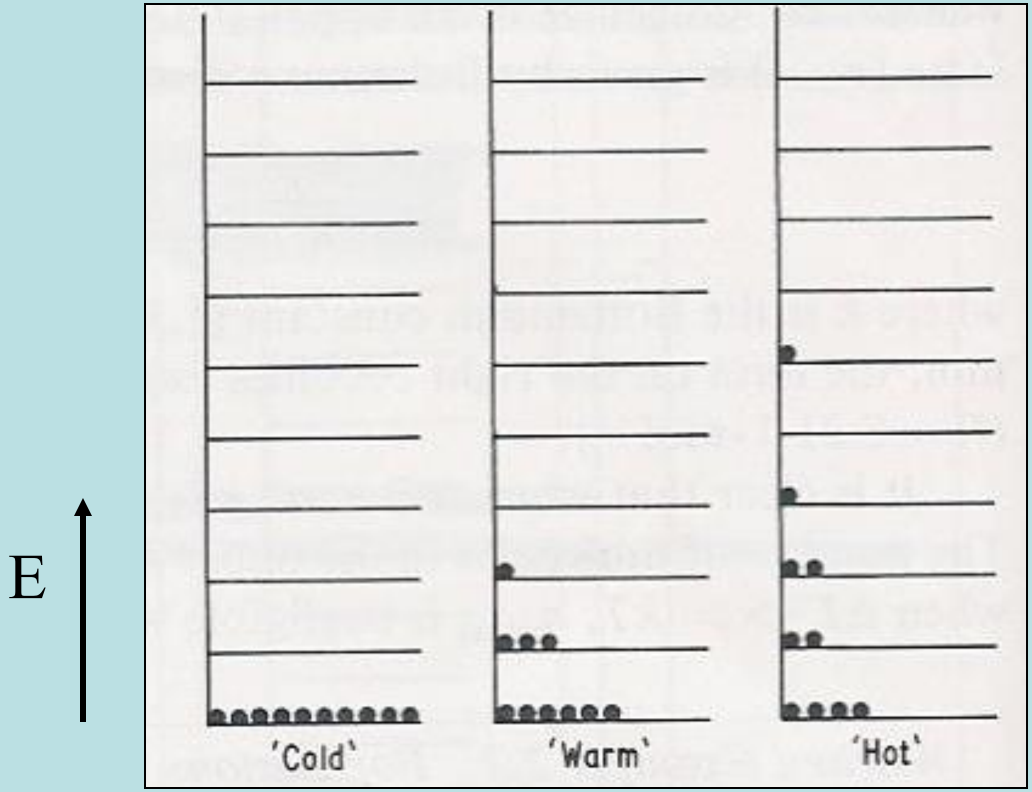
Energy Levels

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: $n_{upper}/n_{lower} = \exp(-0.04) = 0.96$