Spectroscopies

Overview

- In this lecture you will learn,
- Some common spectroscopic techniques
- Linear and non-linear light-matter interactions
- Keywords: spectroscopies, spectroscopic instrumentation, non-linear optical phenomena

Singlet and Triplet States

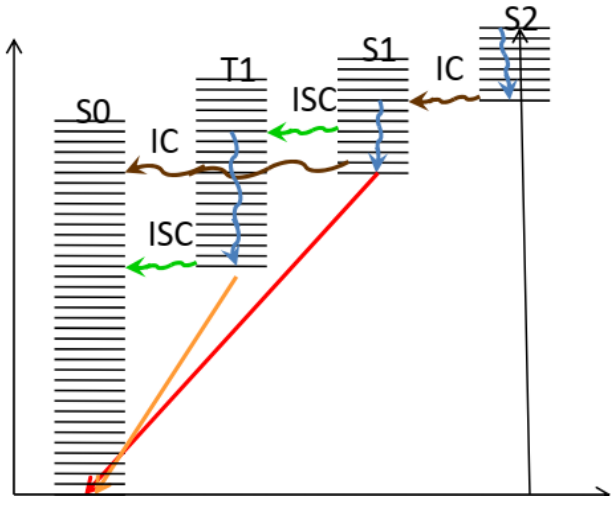
- In the ground state, electrons are paired giving rise to a net 0 spin. Paired states are called singlet states. When an electron is excited from the ground state, it can go into an excited state where it remains paired with net 0 spin or unpaired with net spin of 1. The unpaired state is called a triplet state. As we have seen in the case of atomic orbitals electrons first fill the unpaired states and then fill in pairs. In the same manner the triplet state has a lower energy than the next higher singlet state.

Singlet and Triplet State

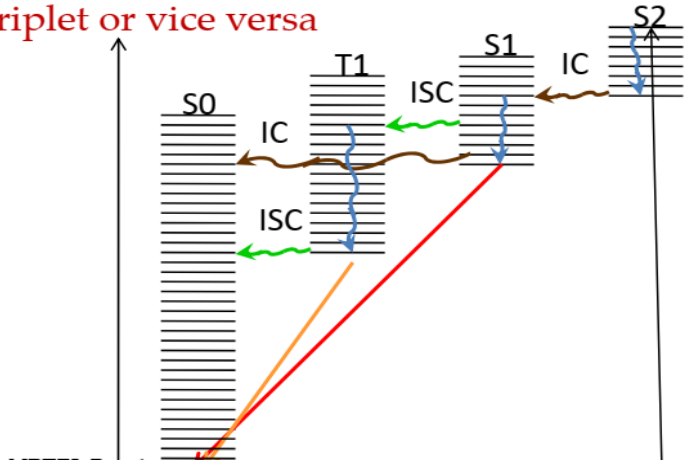
- The singlet and the triplet state differ in their relaxation to the ground state. The singlet state can come back to the ground state quickly because it is already spin paired. For the triplet to come to the ground state a spin flip is required which is a longer process. Singlet to singlet transition accompanied by photon emission is called fluorescence and triplet to singlet transition with spin flip and photon emission is called phosphorescence

Jablonski Diagrams

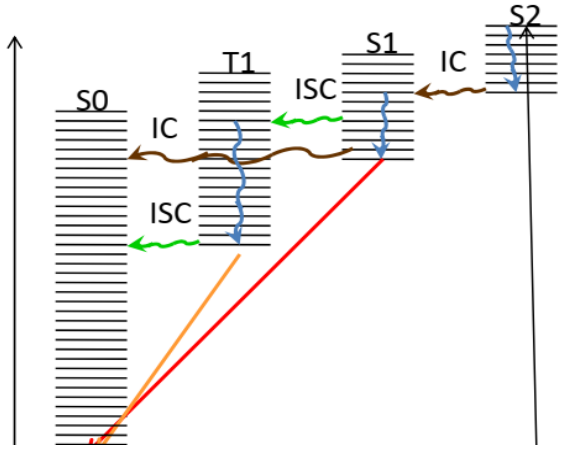
- Jablonski diagrams depict various processes that can change the energy levels of the excited state. The excited state can undergo various transitions, some which are radiative, i.e. emit photons, and some that are non-radiative, i.e. energy of the excited state is lost into heat or vibrational levels as shown in the diagram below. S and T refer to singlet and triplet states respectively



- The various processes that can occur to an electron that has been pumped to an excited state (in this case the single S2 level are
  - Vibrational relaxation (shown by blue wiggles)
  - Internal conversion (IC) shown by brown wiggles. These are processes where the electron moves to another singlet state with similar energy. Inter system crossing (ISC) where an elctron changes the spin state, singlet to triplet or vice versa



- All the processes described above are non-radiative processes, i.e. there is no emission of photons
- There can also be radiative processes accompanied by the emission of a photon. Fluorescence which is radiative relaxation from a singlet to another singlet state. Phosphorescence which is radiative relaxation from a triplet state to singlet state



Spectroscopy

- Probing the energy levels of molecules is possible due to the interaction of the photons with electrons occupying those energy levels. Typically electron energy levels are probed in the UV and visible part of the spectrum while vibrational energy level structure is probed in the IR region. The probing of energy levels is called spectroscopy and it is done using spectrometers.

- Spectrometers can probe, reflectance, transmission or absorbance of materials. One could also look at angular spectra to characterize various scattering processes.

- Spectrometers can be made using a scanning diffraction grating as described earlier or by using a technique called FTIR (Fourier Transformed Infrared) spectroscopy where taking the fourier transform of an interference pattern produces the complete spectrum without the need for scanning. This technique allows for fast recording of spectra.

FTIR Spectrometer

- In the scanning spectrometer narrow band wavelength region is sampled to construct the spectrum point by point. In the FTIR instrument shown in the diagram, a Michelson interferometer is used. Where a moving mirror imparts a varying phase difference between two beams which are recombined to produce the probe beam. From basic interference phenomena, we know that the intensity of the probe beam is proportional to $\varepsilon(k)\cos(k\delta d)$ where δd is the optical path difference between the two mirrors.

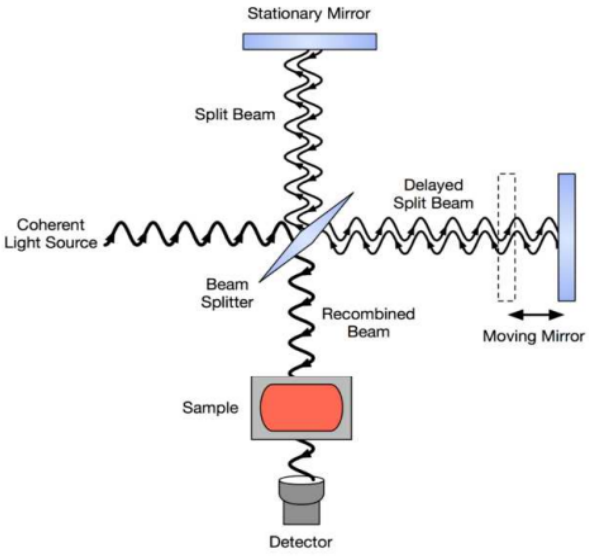


Image courtesy: Wikipedia Commons

FTIR Spectrometer

- Here, $\varepsilon(k)$ is the absorption at wavenumber k, Using orthogonality of the cosine function, in other words, using a fourier transform, one can then obtain $\varepsilon(k)$ from the interferogram, i.e. the pattern observed at the detector as a function of moving mirror position.

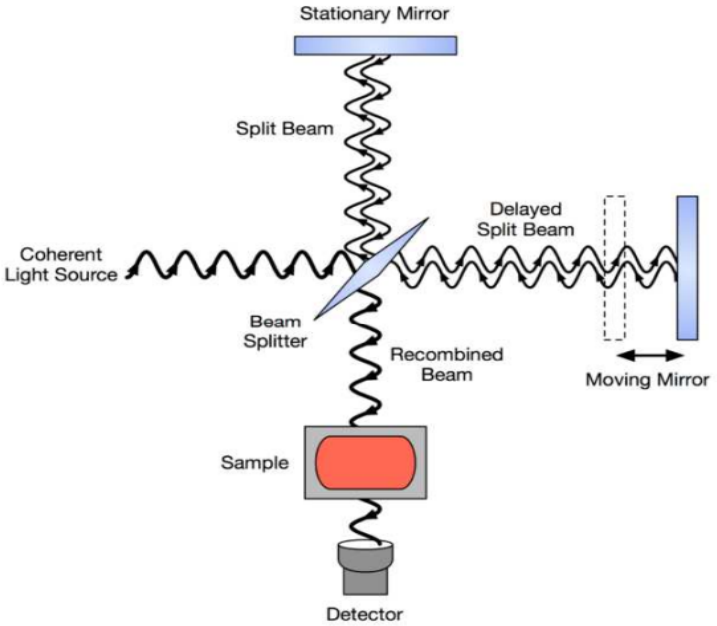
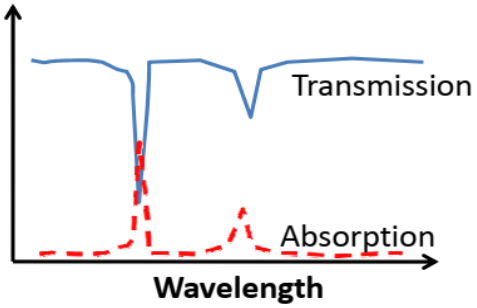


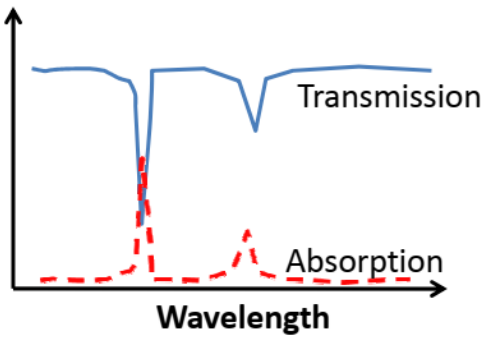
Image courtesy: Wikipedia Commons

Absorption

- The electrons from lower energy levels absorb photons to move up to higher energy levels. This phenomena depends on the wavelength of the incident photon and the availability of higher energy states in the molecule. Absorption is characterized by a parameter called the absorption coefficient α. The absorption coefficient of any material can be found using the Beer-Lambert law. When light passes through a sample of thickness L, the intensity of incident light reduces exponentially. After length L, the intensity can be written as $I = I_o \exp(-\alpha L)$.



- By measuring the transmission coefficient $T = I/I_o$, for different wavelengths, one can estimate the absorption coefficient at different wavelengths. As pointed out earlier, probing with UV or Visible wavelengths, called UV-Vis absorption spectroscopy, probes the electronic energy levels. The peaks observable in the absorption spectra indicate electronic transitions from lower to higher energy levels.



Photon Emission

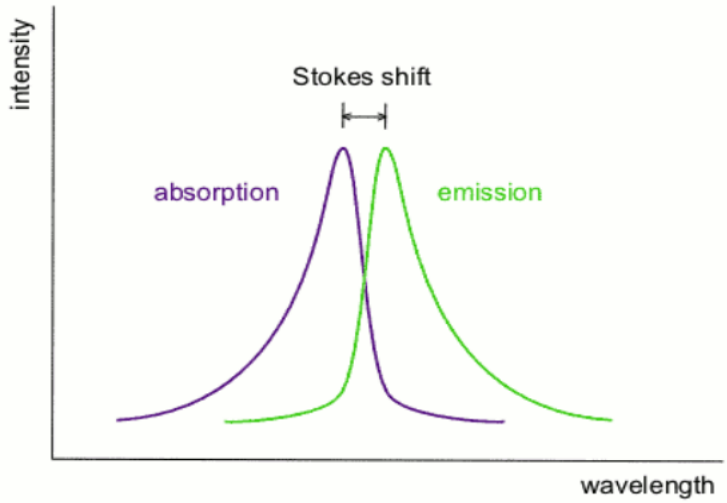
- From the Jablonksi diagram described earlier, we see that there are two radiative processes which can cause emission of photons from molecules. These are fluorescence, which is a transition from an excited singlet state to a ground singlet state, and phosphorescence which is a transition from an excited triplet state to a ground singlet state.

- As the triplet to singlet transition requires a spin flip, the process is slow with half-lives on the order of ms to seconds. On the other hand, fluorescence is a fast process with excited state lifetimes of the order of a few nanoseconds (ns). Fluorescence is a very useful tool in biophotonics. It is extensively used in imaging and sensing of molecules, cellular structures and tissues.

Fluorescence

- The diagram below shows the absorption and emission spectrum of a typical fluorescent dye (fluorophore).

Image courtesy: Wikipedia Commons



-

Stokes Shift

- Fluorescent emission peak is always red-shifted (higher wavelength) compared to the excitation or the absorption peak. This is due to various energy loss mechanisms depicted in the Jablonski diagram. The difference between the excitation and emission peaks is called Stokes shift. There are fluorophores available all the way from violet to red region of the spectrum. There are several efforts to make efficient near IR fluorophores for in-vivo imaging.

Fluorescent Measurement Methods

- Fluorescence from molecules can be used in various measurement techniques to probe different aspects of a given system

- Fluorescence imaging can be used to visualize cellular organization or to track molecules within a volume.

- Fluorescence lifetime imaging can be used to probe the environment near the fluorescent molecule as the lifetime can be perturbed by environmental factors such as pH.

Fluorescence Methods

- Fluorescent resonant energy transfer (FRET) between two fluorophores occurs as a function of their separation. Therefore, FRET can be used to study conformational changes in molecules where certain conformational changes can bring the fluorophores close enough for FRET to occur.

- Fluorescence correlation spectroscopy (FCS) refers to measurements made with very dilute solution of fluorophores where the auto-correlation of the detected signal can be used to study single molecule dynamics.

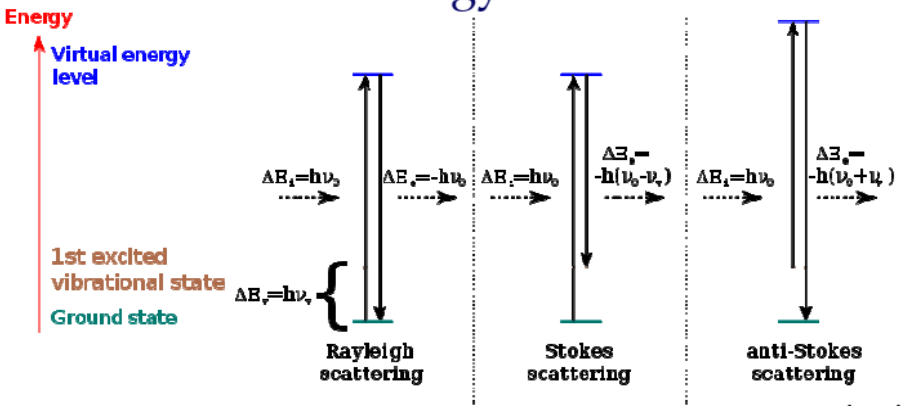
- We will discuss these techniques in detail later.

Vibrational Spectroscopy

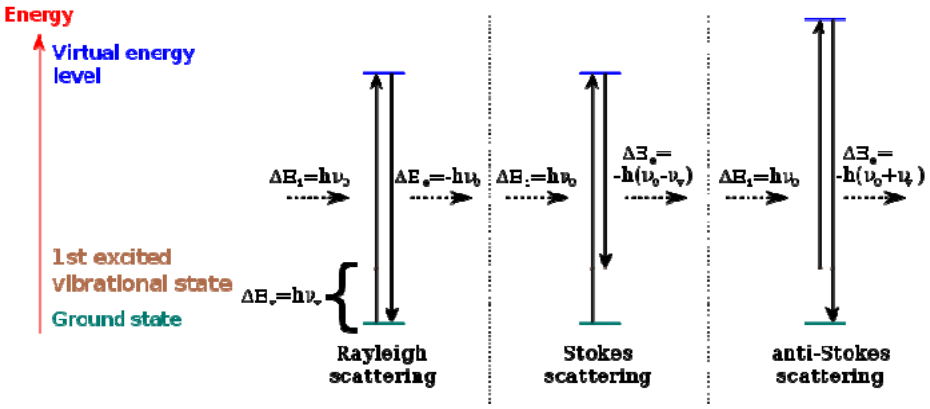
- Just as one probes electronic energy levels using UV or visible light, one can probe the lower energy vibrational levels by using light of higher wavelength. There are two major techniques to probe the vibrational energy levels. Infrared (IR) spectroscopy using a scanning spectrometer or FTIR and Raman spectroscopy where the Raman response of molecules can be probed by using light of any wavelength. Vibrational spectroscopy provides a powerful tool for molecular identification as the coupled vibrational modes create a nearly unique pattern specific to different molecules. In contrast, the electronic energy level spectra of most bio-molecules are relatively featureless.

Raman Scattering

- The diagram below illustrates the process of Raman scattering. Most of the incident photons are elastically scattered (Rayleigh scattering) with no loss in energy. However a small fraction of photons are scattered inelastically where the energy of the emitted photon may be lesser (Stokes Raman) or larger (Anti-stokes Raman). The difference in energy is transferred to a phonon, which is a quantized vibrational energy state.



- This process although similar to fluorescence is very different because the excited state is a 'virtual state' and not a real quantum state. Therefore this process can happen for any wavelength unlike fluorescence which is favorable only when the photon energy is equal to the difference in energies of the real excited state and the ground state.



Chiral Spectroscopy

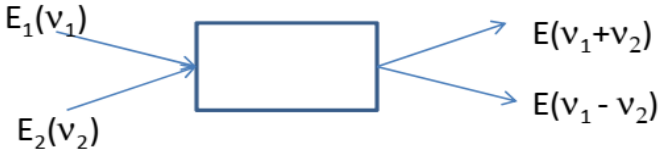
- Chiral molecules have different refractive indices for right circular and left circular polarized light. This means that when a linearly polarized light (which can be considered as a superposition of right and left circular polarizations) is incident on a chiral media it would rotate the plane of polarization. In addition to this, chiral molecules also have different absorption coefficients for the different circular polarization. This behavior is called circular dichroism, by measuring the difference in absorption between left and right circular polarization one can characterize a chiral medium. This technique is called CD spectroscopy.

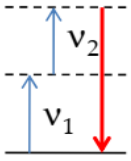
Non-linear Polarizability

- So far we have dealt with linear polarization where the induced dipole moments are linearly depended on the electric field. In reality there are higher order terms whose effects appear as intensity increases. We describe non-linear effects with respect to a parameter called dielectric susceptibilty χ. We expand the polarization density P as $P = \chi^{(1)}E + \chi^{(2)}E^2 + \chi^{(3)}E^3 + \ldots$
- $\chi^{(1)}$ is the linear term with the higher order terms representing second order and third order processes respectively.
- We discuss some of these processes briefly in the following slides.

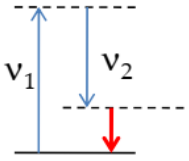
Second Order Processes

- Second order processes can be considered as a two-wave mixing, which produces frequencies which are the sum and difference of the frequencies of mixing waves. In the energy level picture, we explain these processes using the concept of 'virtual energy levels' (dashed lines instead of solid lines used for real energy levels). These are not real energy levels. These processes are only observable if the incident intensity is really large, as possible with ultrafast lasers.





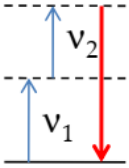
Sum Frequency Generation



Difference Frequency Generation

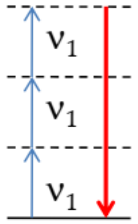
Second Harmonic Generation

- . Second harmonic generation (SHG) is a special case when the two frequencies v1 and v2 are equal. We can then use this process to double the frequency of the incident frequency.

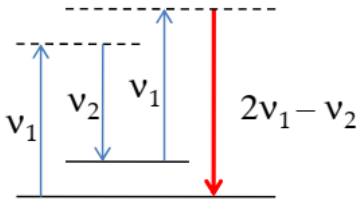


Third-order Processes

- Similar to the second order process, it is also possible to have higher order processes such as third order processes. Third order processes can generate new frequencies such as $2\nu_1 - \nu_2$ and so on which can not arise out of second order processes.



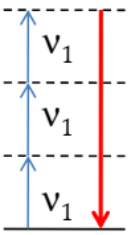
Third Harmonic Generation



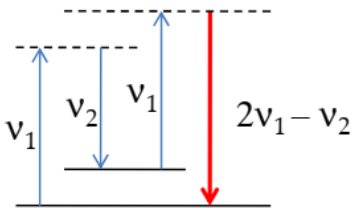
CARS Process

CARS

- Third harmonic generation and Coherent Anti-Stokes Raman Spectroscopy (CARS) processes are third order processes. In CARS, there is a real energy level difference $\nu_1 - \nu_2 = \nu_R$ corresponding to a Raman excitation. By tuning one of the frequencies with respect to the other, say ν_2, one can obtain the Raman response.



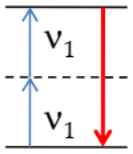
Third Harmonic Generation



CARS Process

Two and Multi-Photon Microscopy

- Another important class of non-linear processes are called multiphoton processes. Lets take the example of a two photon process whose energy level picture looks very similar to second harmonic generation (SHG). However the difference is that the excited state is a real energy level and the two-photon absorption efficiency is related to third-order non-linear term $\chi^{(3)}$. Multiphoton processe yield an 'upconverted' signal, i.e. the signal photons have higher frequency than the incident photons.



Two Photon Absorption

Multiphoton Microscopy

- Two photon or similar multiphoton microscopy is useful for high resolution imaging because two photon excitation is proportional to I^2, where I is intensity of excitation. This means that only regions very close to the focal point will be excited. Also, multi-photon processes make it possible to use longer wavelengths for excitation. Longer wavelengths penetrate deeper into tissue as they are scattered less. Therefore multi-photon microscopy is useful for in-vivo imaging.