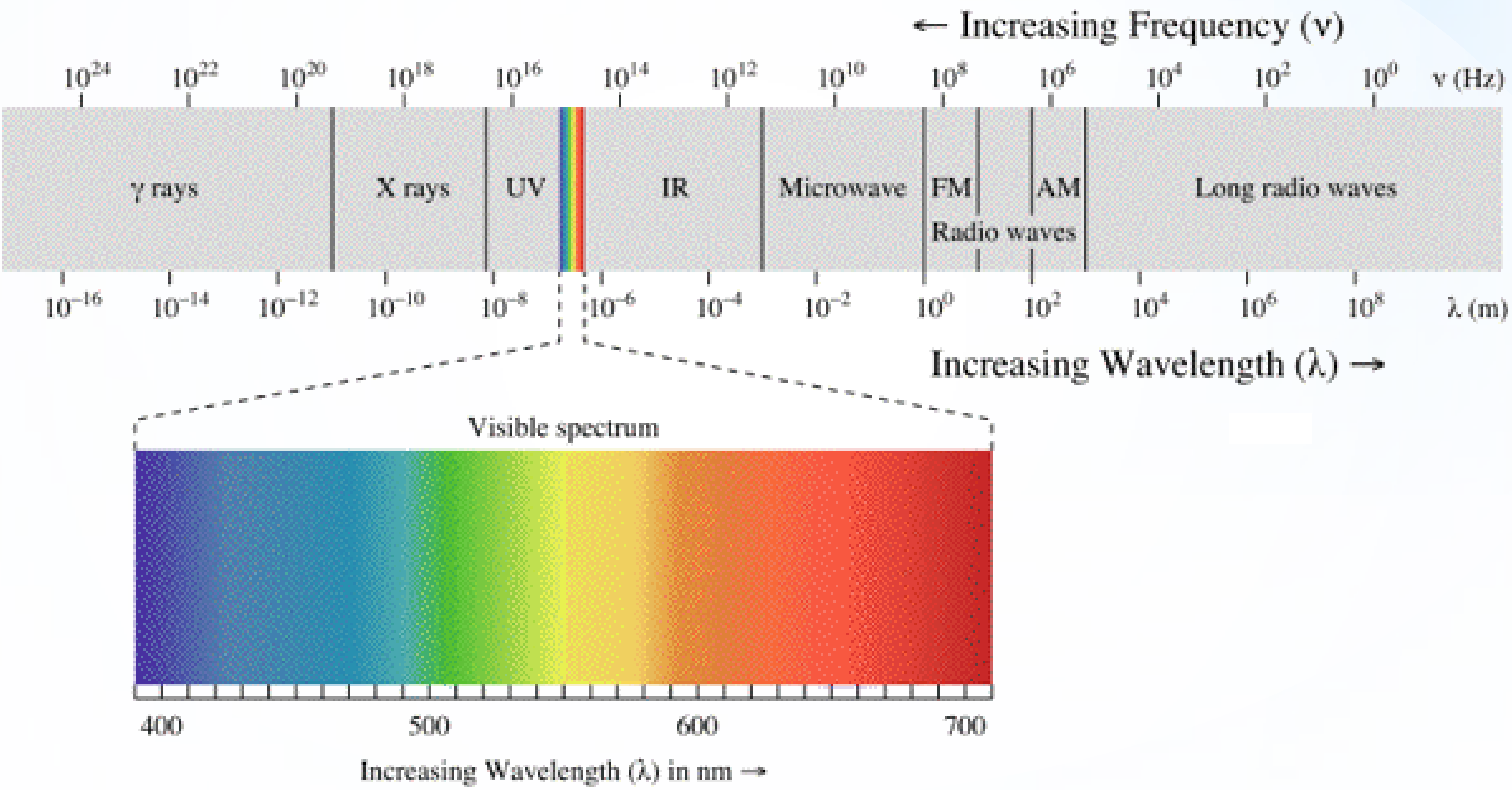
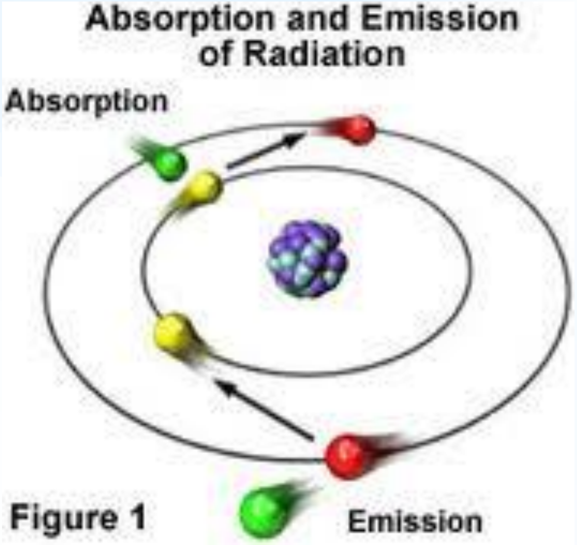
Fluorometric Analysis

Fluorescence spectroscopy:

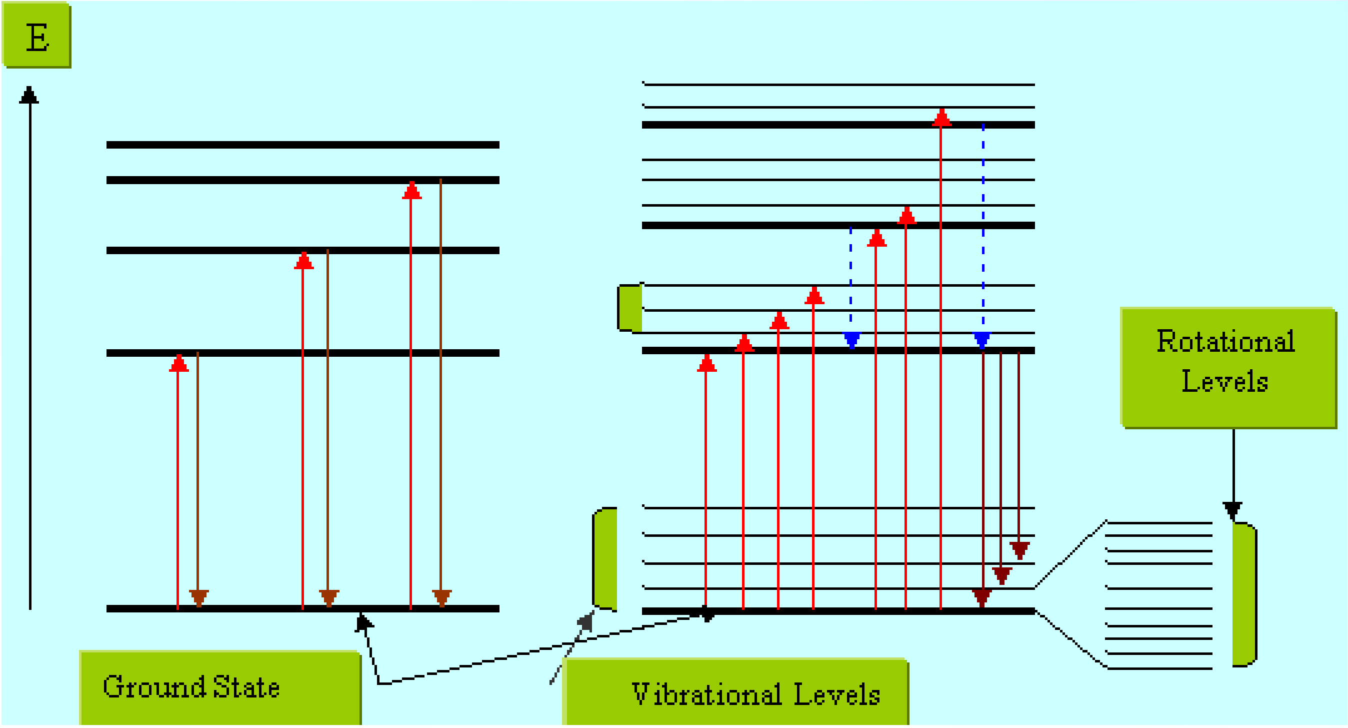
- *Is a type of electromagnetic spectroscopy which analyzes **fluorescence** from a sample.*



- *It involves using a beam of light, usually* ***ultraviolet light*** *that excites the electrons in molecules of certain compounds and causes them to emit light.*



- *Devices that measure fluorescence are called* ***fluorimeters***



- *The solid black lines represent electronic energy level.*
- *Red arrow represent electronic absorption.*
- *Browns arrow represent electronic emission.*
- *Blue level represent relaxation from higher excited levels to first excited electronic level.*

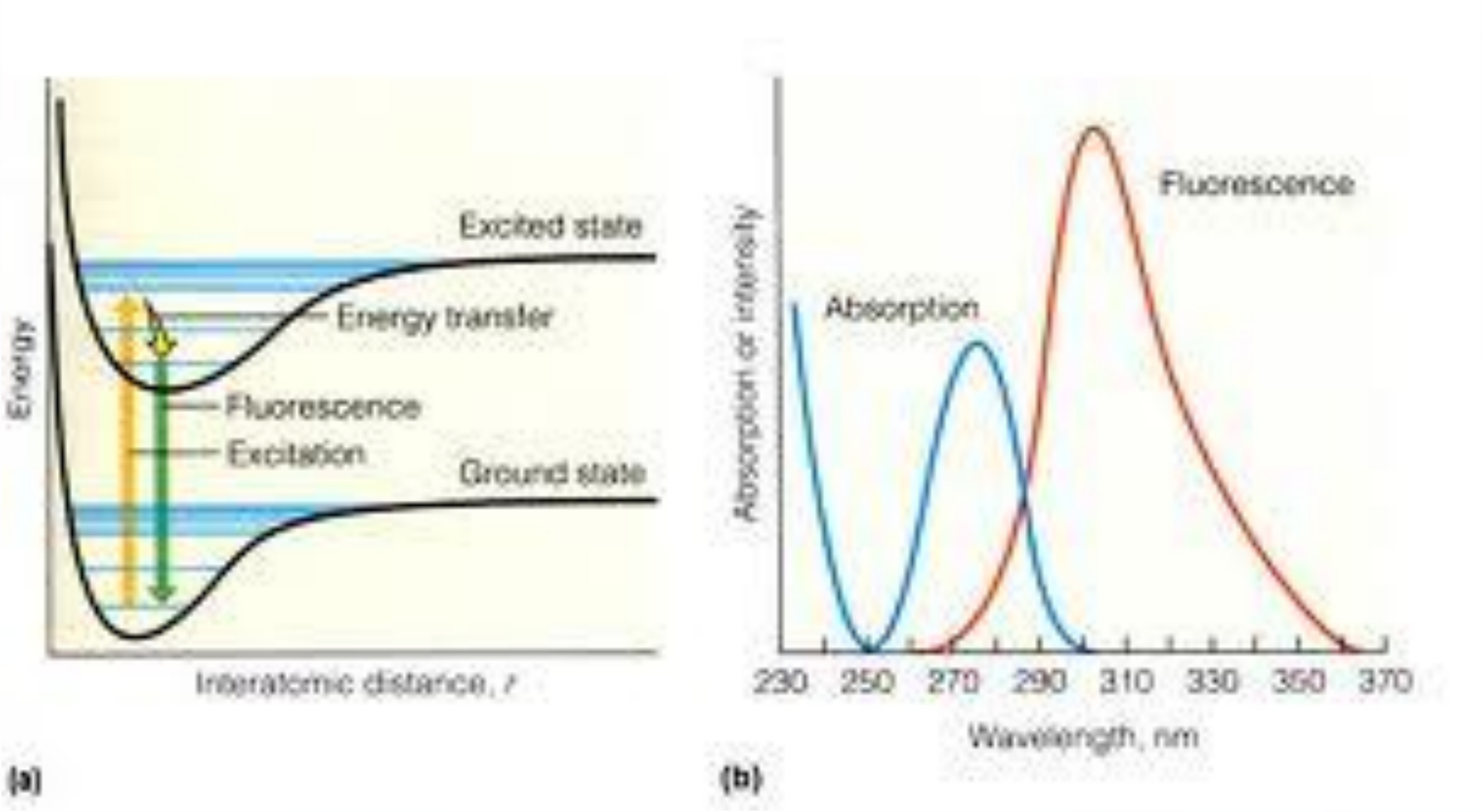
- *Molecules have various states referred to as energy level.*

- *Fluorescence spectroscopy is primarily concerned with electronic and vibrational states.*

- *Generally, the species being examined has a ground electronic state (a low energy state) of interest.*

- *and an excited electronic state of higher energy. Within each of these electronic states are various vibrational states.*

- *In fluorescence spectroscopy, the species is first excited, by absorbing a photon, from its ground electronic state to one of the various vibrational states in the excited electronic state.*



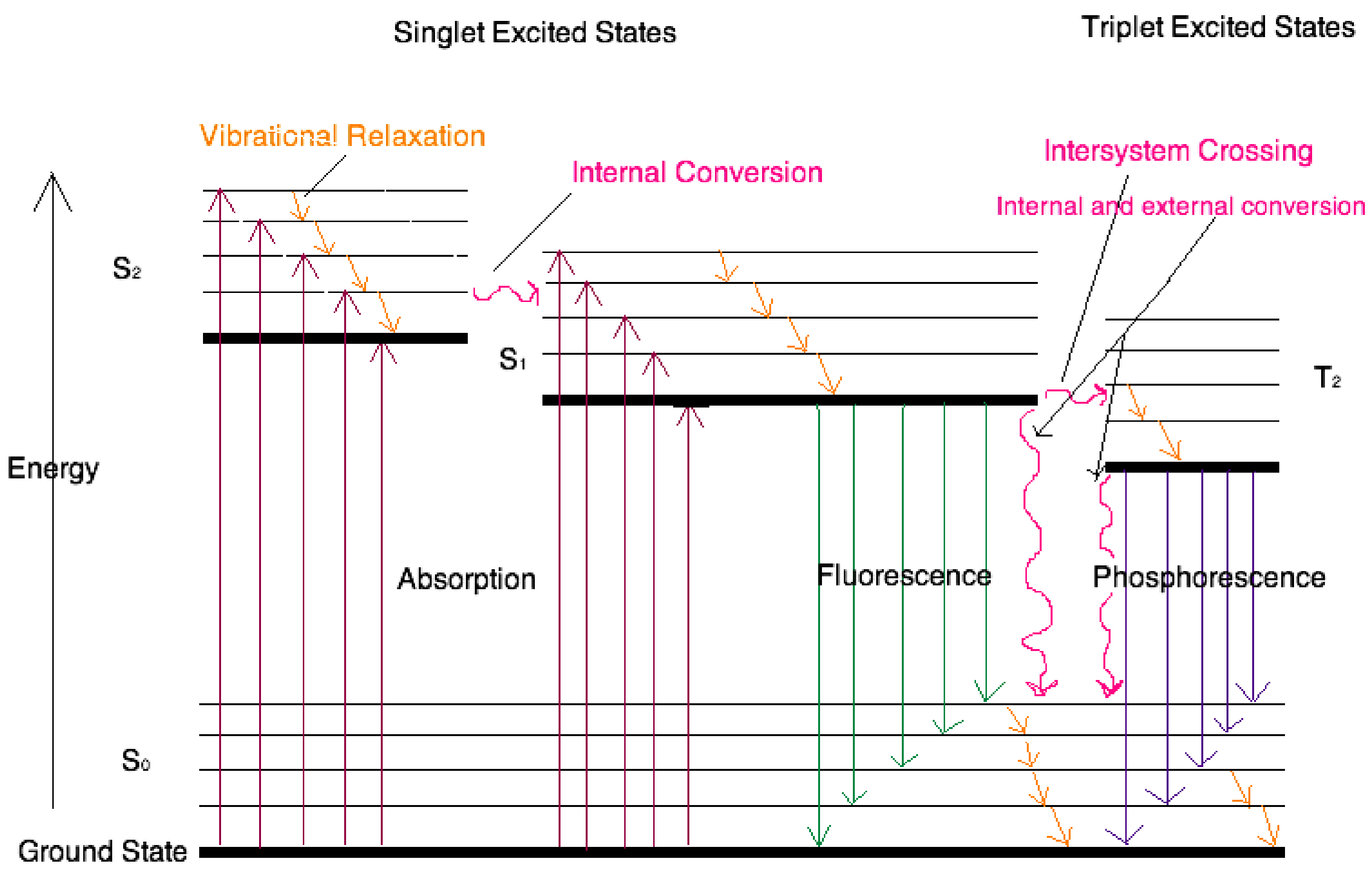
- *Collisions with other molecules cause the excited molecule to lose vibrational energy until it reaches the lowest vibrational state of the excited electronic state.*

- *The molecule then drops down to one of the various vibrational levels of the ground electronic state again, emitting a photon in the process.*

- *As molecules may drop down into any of several vibrational levels in the ground state, the emitted photons will have **different energies**, and thus **frequencies.***

- *Therefore, by analysing the different frequencies of light emitted in fluorescent spectroscopy, along with their relative intensities, the structure of the different vibrational levels can be determined.*

Theory:



Jablonski energy diagram

1. Absorption:

- *The absorption of UV-Vis radiation is necessary to excite molecules from the ground state to one of the excited states.*

2. Vibrational Relaxation:

- *Absorption of radiation will excite molecules to different vibrational levels of the excited state.*
- *This process is usually followed by successive vibrational relaxations (VR) as well as internal conversion to lower excited states.*

3. Fluorescence:

- *After vibrational relaxation to first excited electronic level takes place, a molecule can return to the ground state by emission of a photon, called fluorescence (FL).*

- *The fluorescence lifetime is much greater than the absorption time and occurs in the range from 10^{-7} to 10^{-9}s.*

- *However, not all excited molecules can show fluorescence by returning to ground state.*

4. Internal and External Conversion:

- ***Internal conversion :***

can result in a phenomenon called predissociation (PD) where an electron relaxes from a higher electronic state to an upper vibrational energy of a lower electronic state.

- ***External conversion:***

is a process whereby excited molecules lose their energy due to collisions with other molecules or by transfer of their energy to solvent or other unexcited molecules.

5. Intersystem Crossing:

- *The process of intersystem crossing involves transfer of the electron from an excited singlet to a triplet state.*

6. Phosphorescence:

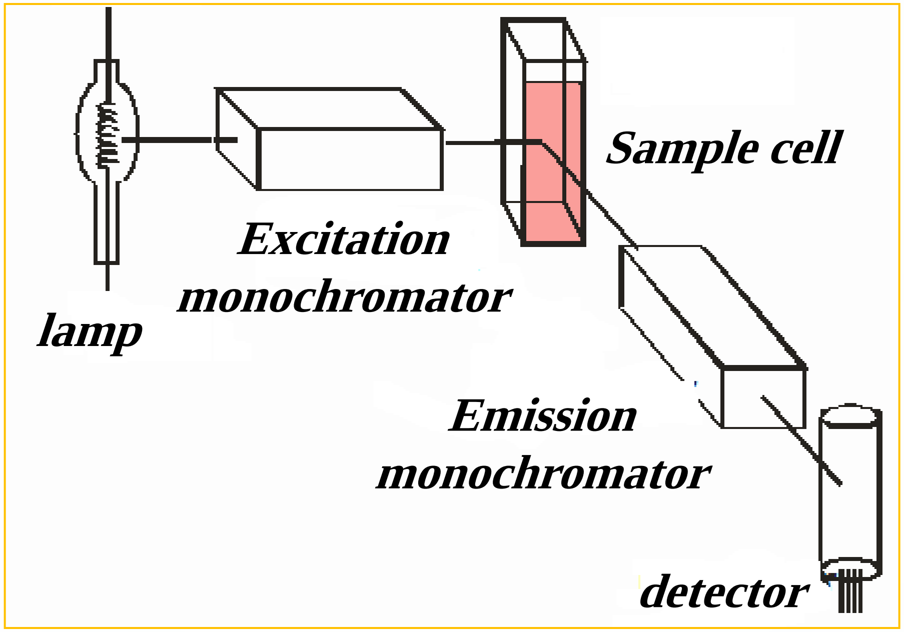
Electrons crossing the singlet state to the triplet state can follow one of three choices including:

- *returning to the singlet state.*
- *relax to ground state by internal or/and external conversion.*
- *Lose their energy as a photon **(phosphorescence)** and relax to ground state. It requires a much longer time than fluorescence (10^{-4}s to up to few s).*

Instrumentation :

Two general types of instruments exist:

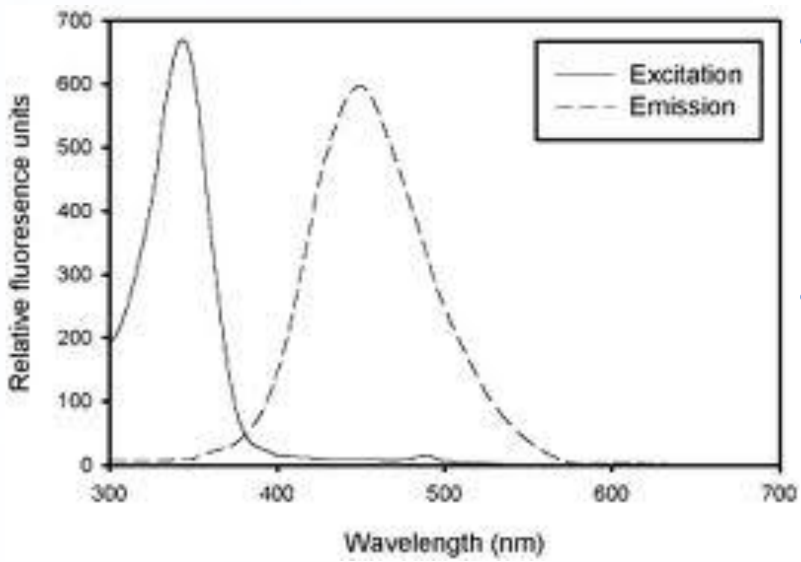
- ***filter fluorometer*** *: use filters to isolate the incident light and fluorescent light.*
- ***Spectrofluorometer*** *: use diffraction grating monochromator to isolate the incident light and fluorescent light.*



Both types use the following scheme:

- *The **light from an excitation source** passes through a **filter** or **monochromator,** and strikes the sample.*
- *The fluorescent light is emitted in all directions.*
- *Some of this fluorescent light passes through a **second filter or monochromator**, which is usually placed at 90° to the incident light beam to minimize the risk of transmitted or reflected incident light reaching the detector.*
- *Various light sources may be used as excitation sources, including lasers, photodiodes, and **lamps**; xenon arc and mercury vapor lamps in particular.*

- *The **detector** can either be single-channeled or multi-channeled.*
- *The single-channeled detector can only detect the intensity of one wavelength at a time.*
- *while the multi-channeled detects the intensity at all wavelengths.*



- *When measuring fluorescence spectra, the wavelength of the excitation light is kept constant, preferably at a wavelength of high absorption, and the emission monochromator scans the spectrum.*
- *For measuring excitation spectra, the wavelength passing though the emission filter or monochromator is kept constant and the excitation monochromator is scanning.*

Differece between fluorometer and spectrophotometer

DIFFERENCE	*FLUOROMETER*	*SPECTROPHOTOMETER*
Source of light	*Xenon arc lamp or mercury vapor lamp* ***(Perpendicular to sample)***	*Tangeston or Deturium* ***(Straight line)***
Sample cell	*Cuvette completely transparent* *Cell compartment is painted black*	*Only two side transparent*
Detector	*Two monochromator*	*One monochromator*

What is Quenching?

- ***Quenching*** *refers to any process which decreases the fluorescence intensity of a given substance.*

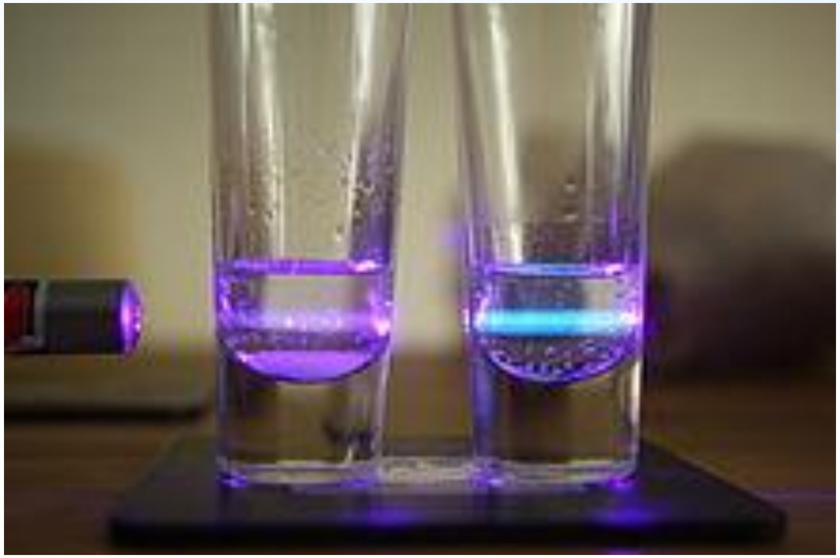
- *A variety of processes can result in quenching:*

such as excited state reactions, energy transfer, complex-formation and collisional quenching.

- *As a consequence, quenching is often heavily dependent on pressure and temperature.*

- *Molecular* ***oxygen , iodide ions and acrylamide*** *are common chemical quenchers..*

Example :



- *Two samples of quinine dissolved in water with a violet laser (left) illuminating both.*
- *Typically quinine fluoresces blue, visible in the right sample.*
- *The left sample contains **chloride ions** which quenches quinine's fluorescence, so the left sample does not fluoresce visibly.*

Application:

- ***Fluorescence spectrocopy*** *is used in biochemical, medical, and chemical research fields for analyzing organic compound.*

- *There has also been a report of its use in differentiating malignant, bashful skin tumors from benign.*