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Be Here...
Be Vibrant...

OPTICAL TECHNIQUES PART-2



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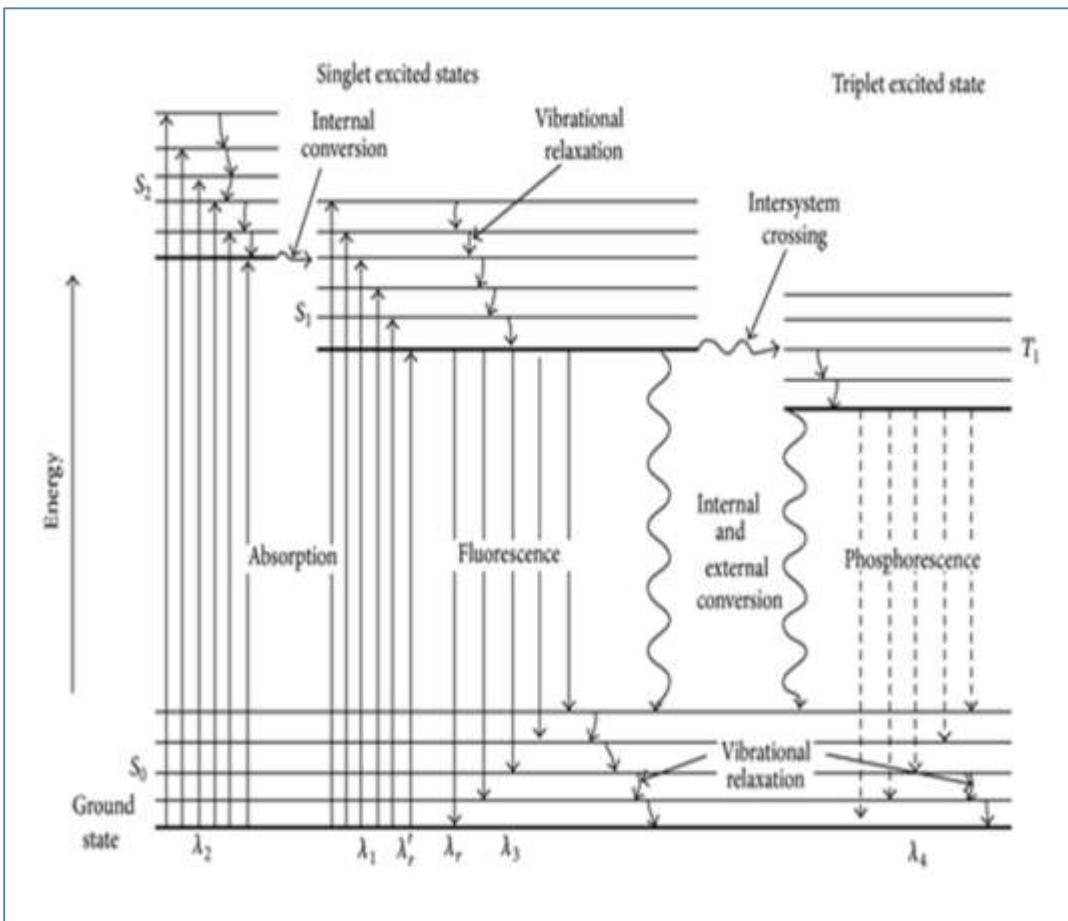
- ❑ **luminescence** family of processes in which susceptible molecules emit light from electronically excited states created by
 - either a physical (Absorption of light)
 - Mechanical (friction),
 - Chemical mechanism.

Photoluminescence Generation of luminescence through excitation of a molecule by **ultraviolet or visible light photons** is a phenomenon termed photoluminescence,

- ❑ depending upon the electronic configuration of the excited state and the emission pathway divided into two categories
- ❑ Fluorescence
- ❑ Phosphorescence

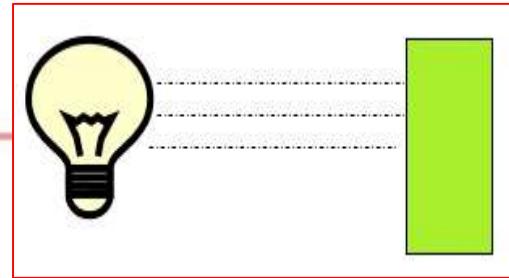
❑ Fluorescence:

Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength after a brief interval, termed the fluorescence lifetime.



- The property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength, but with a much longer excited state lifetime.
- Phosphorimetry: The measurement of phosphorescence
- Emission of phosphorescence light is **longer than** the decay time of fluorescence

Fluorometry



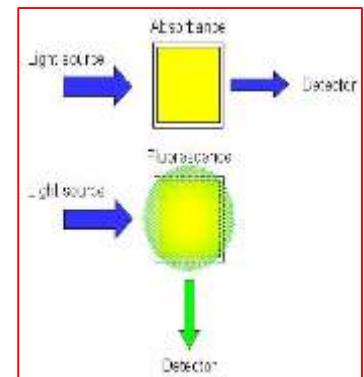
- When a beam of light is incident on certain substances they emit visible light or radiations. This is known as fluorescence.

- Fluorescence starts immediately after the absorption of light and stops as soon as the incident light is cut off.

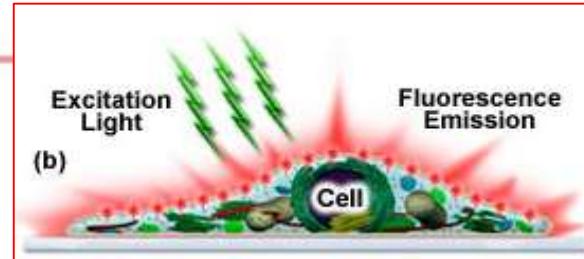
- The substances showing this phenomenon are known as fluorescent substances.

- The measurement of fluorescence is called.

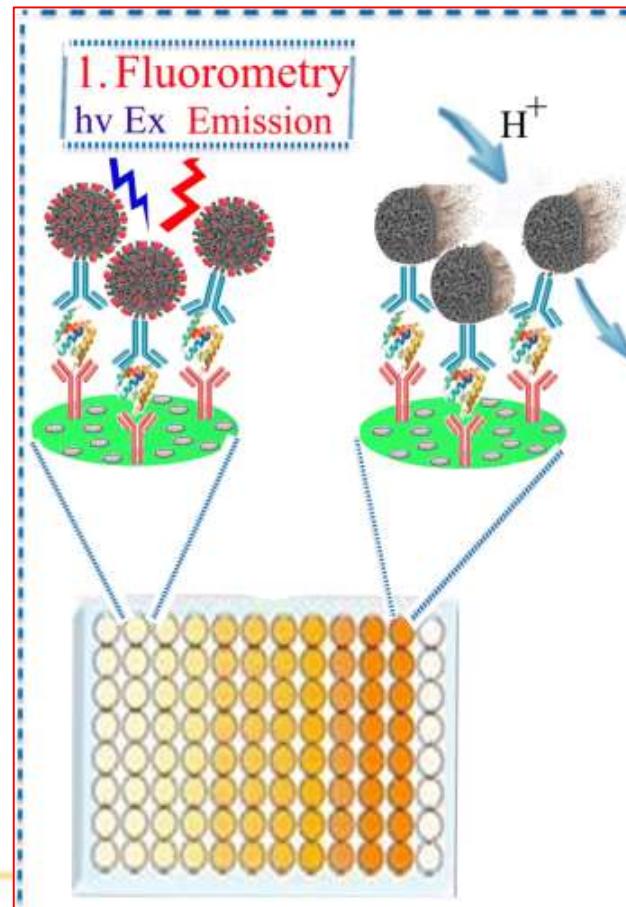
Fluorometry



Fluorometry



- Quantify biological analytes as a function of fluorescence.
- Requires the sample to be bound to a specific fluorescent agent
- An extensive range of nucleic acid, cell function dyes, and fluorescent proteins are commercially available worldwide.



Fluorometry

- Fluorophores absorb light of a distinct excitation wavelength and emit, or fluoresce, light of reduced energy thus a longer wavelength.

- This behavior can be modified so that the fluorescent reagents are restricted from emitting light unless bound to a specific molecule, such as dsDNA.

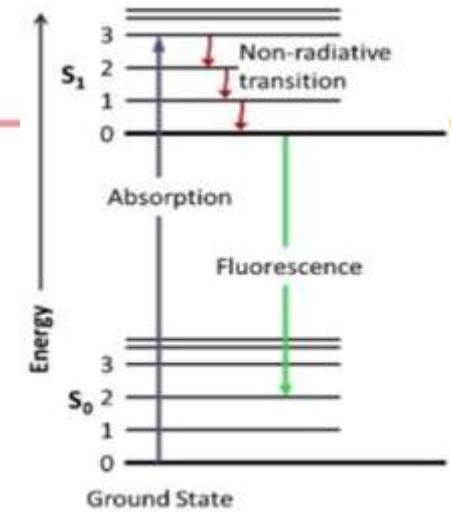
Fluorometry

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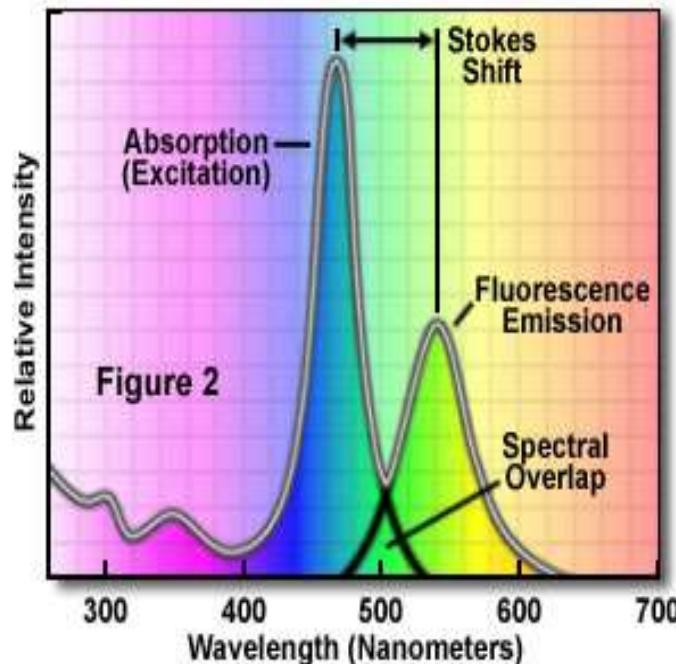
- Before fluorescence, some loss of the excitation energy occurs
- The emitted fluorescence light is of less energy but longer wavelength than excitation light

Stokes shift:

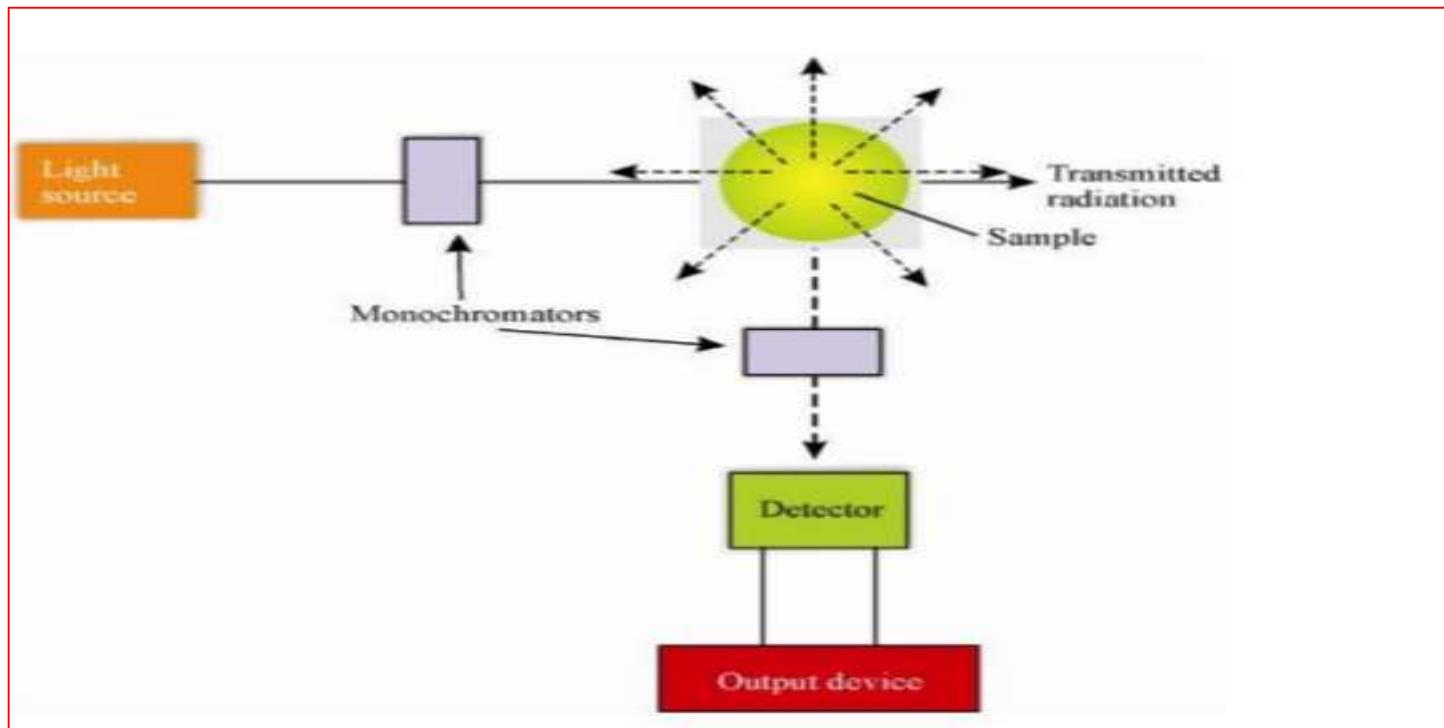
The difference between the maximum wavelength of the excitation light and the maximum wavelength of the emitted fluorescence light

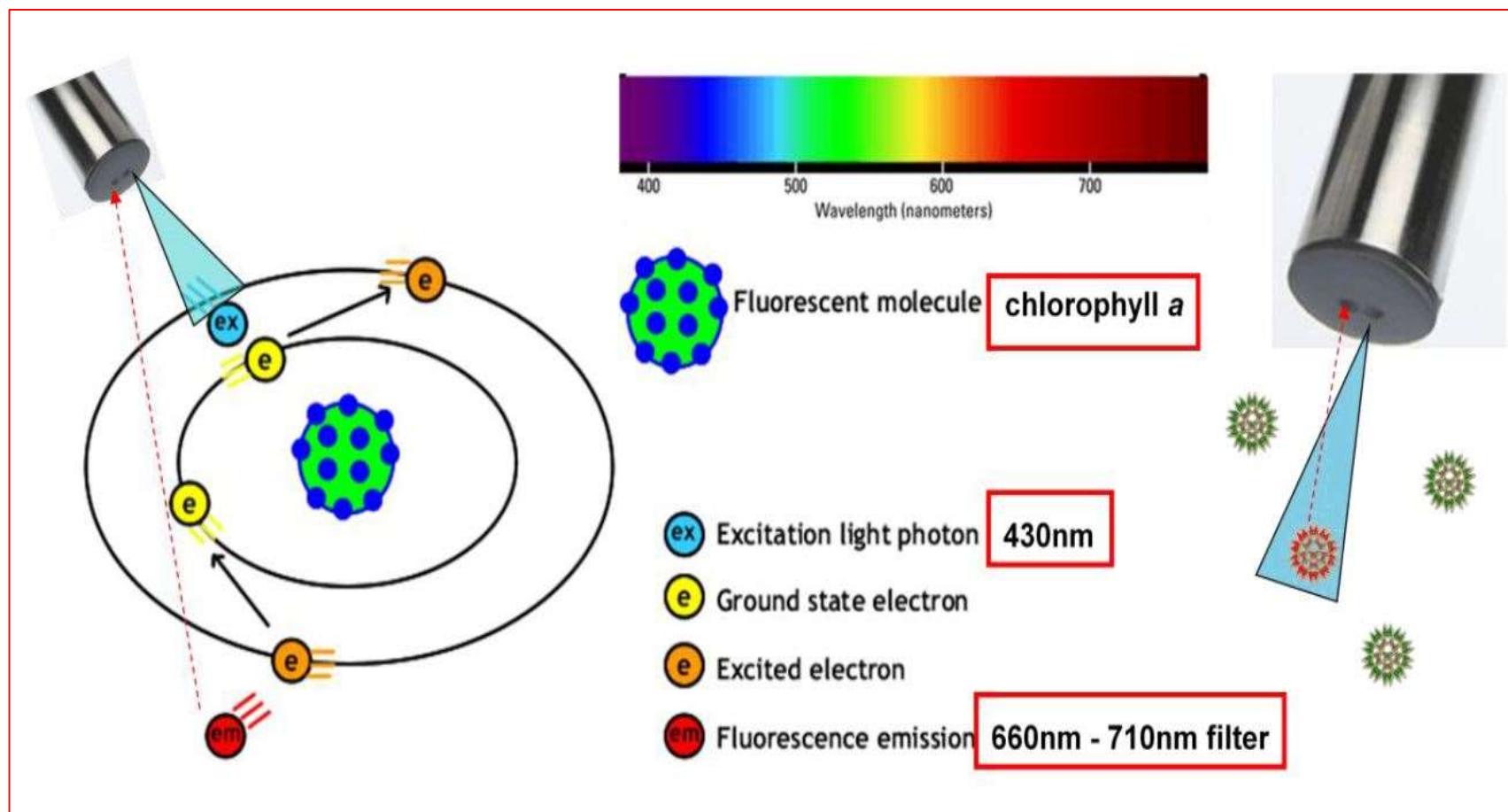


Excitation and Emission Spectral Profiles

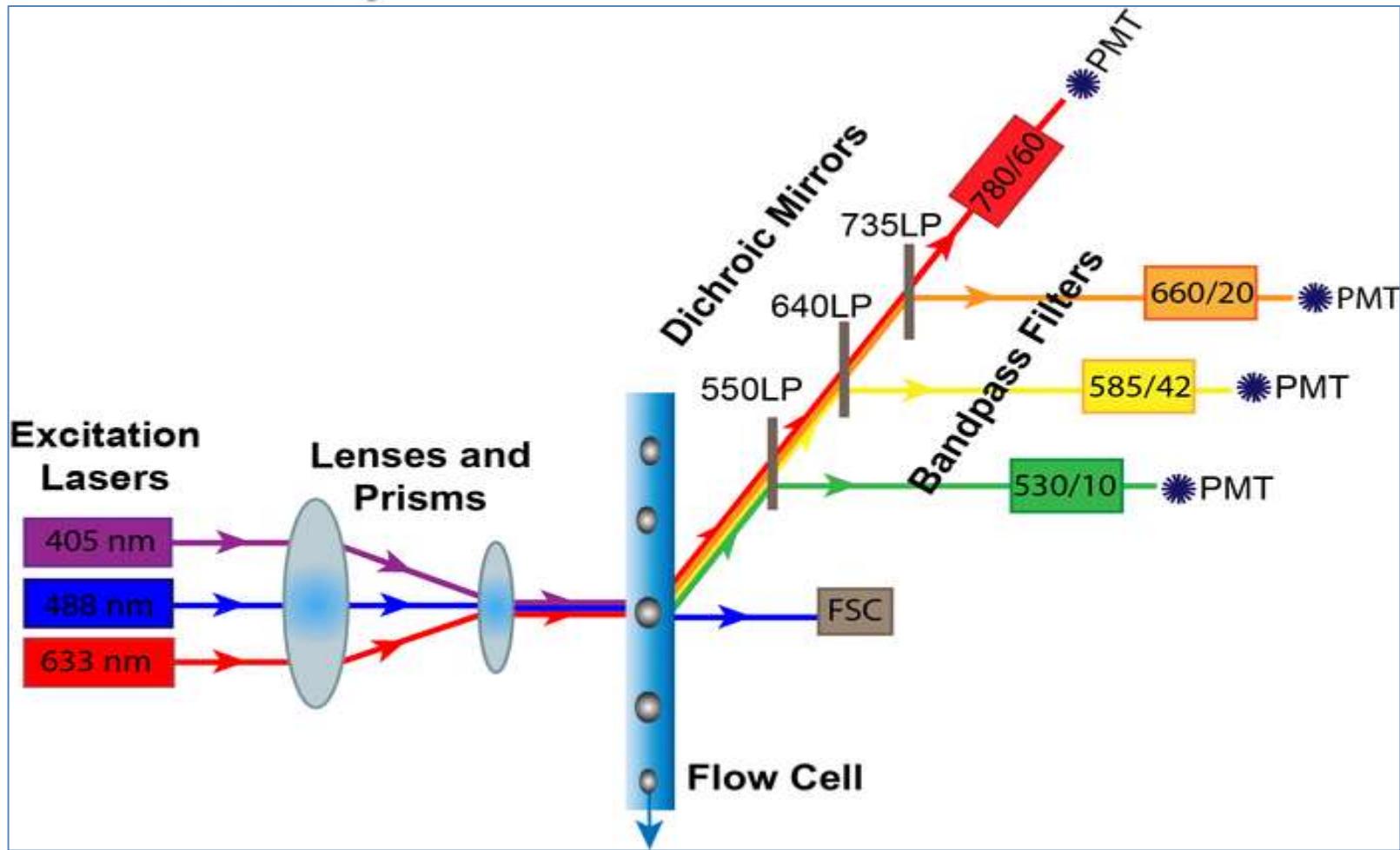


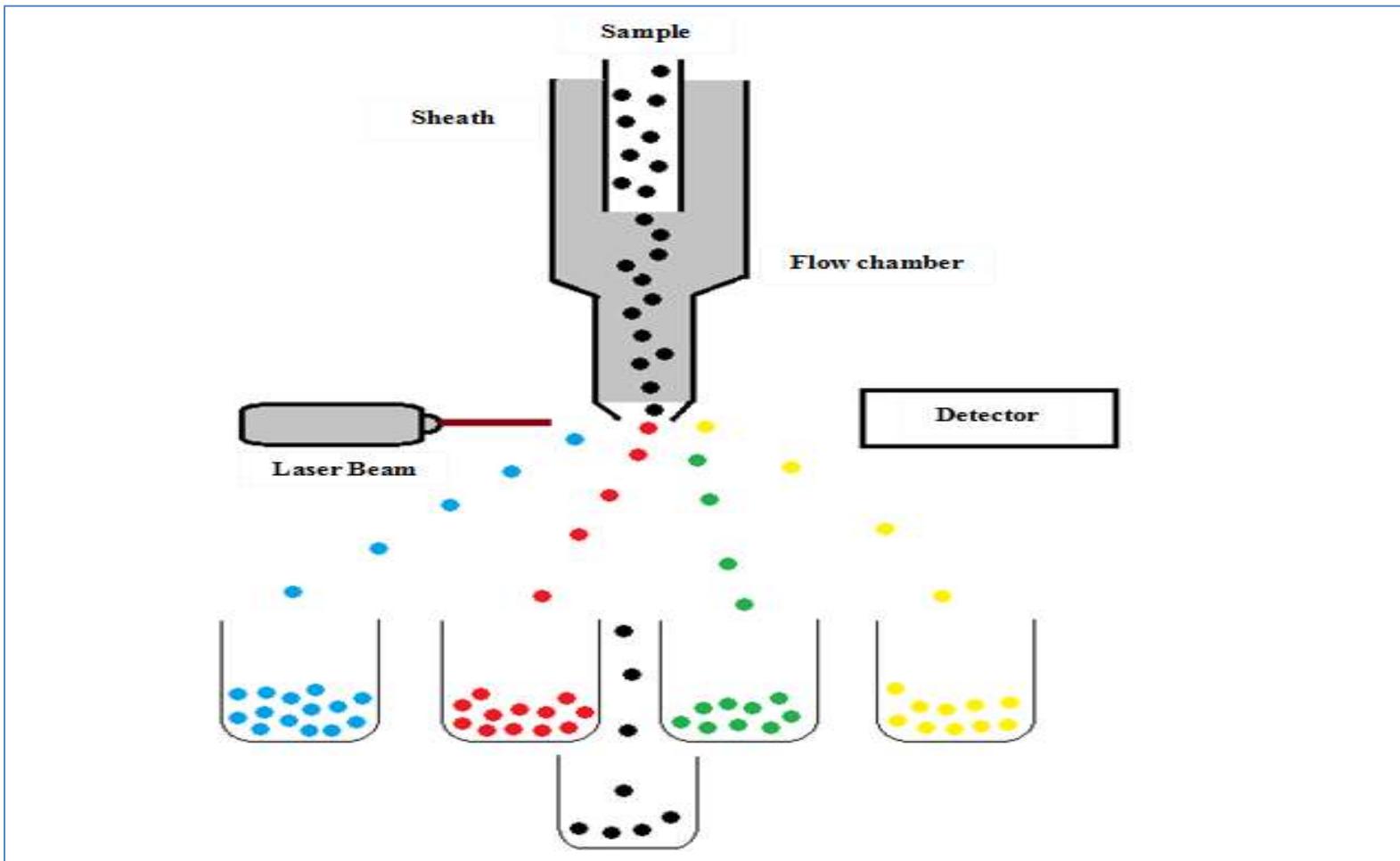
Fluorometry





- ❑ Cytometry refers to the measurement of physical or chemical characteristics of cells.
- ❑ Flow cytometry is a process in which measurements are made while the cells pass through the measuring apparatus in a fluid stream
- ❑ Cells are labelled with different specific fluorescent labels such as **B-phycoerythrin , fluorescein isothiocyanate, rhodamine-6G** and dye-labeled antibodies
- ❑ Most flow cytometers incorporate **two or more fluorescence emission detection system** for multiple fluorescent labels can be used



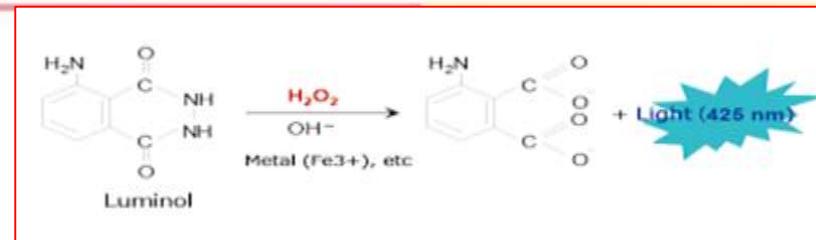


- ❑ Luminescence in which the excitation event is caused by chemical, biochemical or electrochemical reaction and not by photo illumination.

Instrument: Luminometers

- ❑ Chemiluminescence
- ❑ Bioluminescence
- ❑ Electrochemiluminescence

Chemiluminescence (CL) :

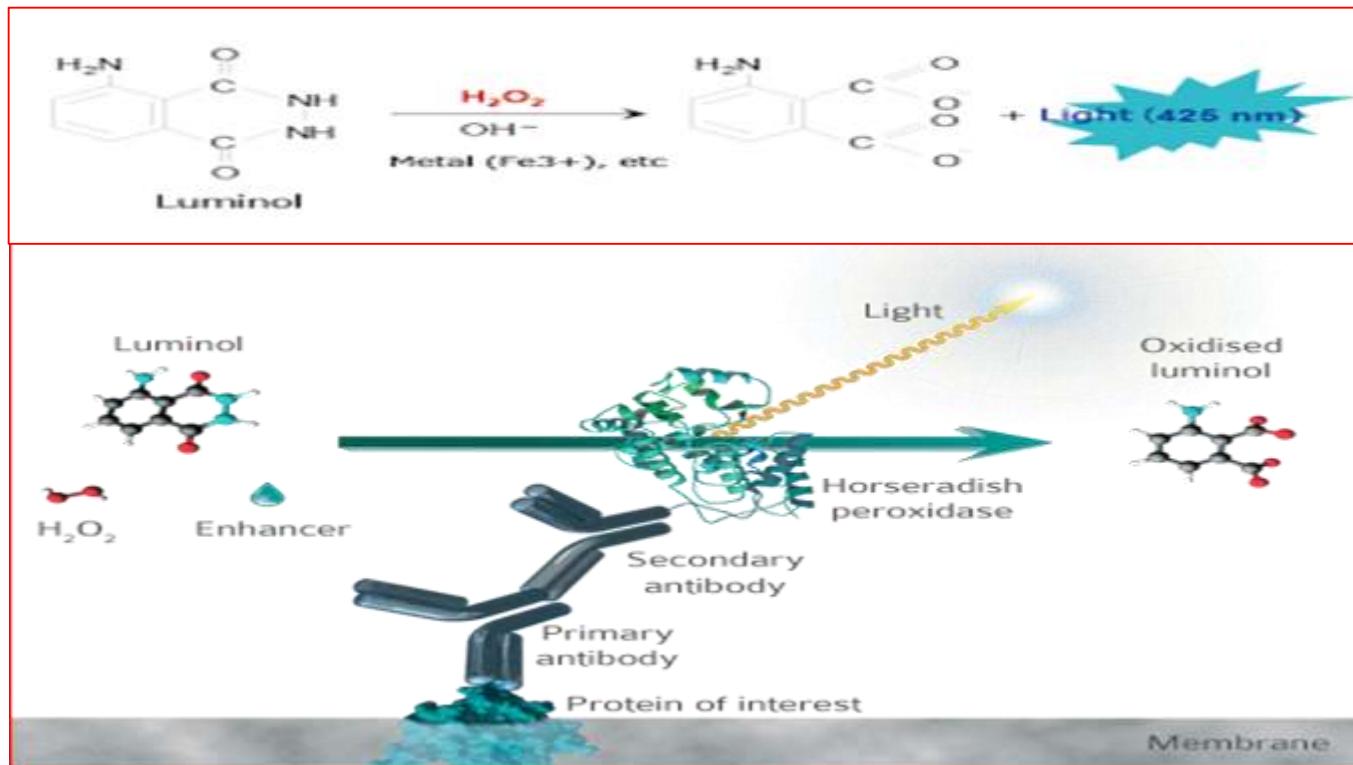


- Emission of light when an electron returns from an excited or higher energy levels to a lower energy levels
- The excitation event is caused by a chemical reaction
- Such as oxidation of an organic compounds (**luminol**, **isoluminol**) by an oxidant (hydrogen peroxide, hypochlorite or oxygen)
- Light is emitted from excited product form in the oxidation

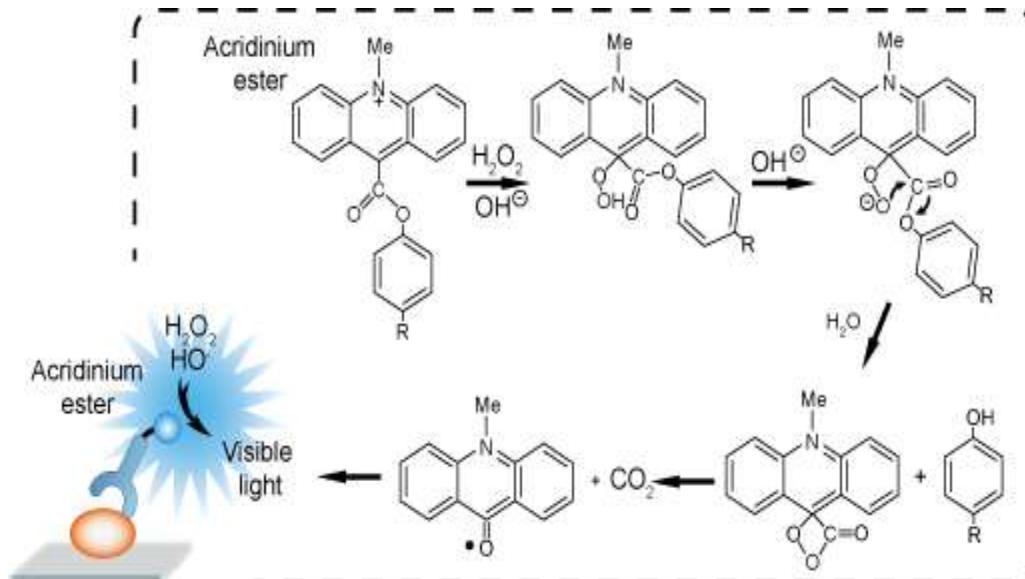
Chemiluminescence (CL) :

Reaction occurs in the presence of

- Enzymes (alkaline phosphatase, horseradish peroxidase)
- Metal ions (Cu and Fe)



- Acridinium labeled compounds have 100 times stronger chemiluminescence intensity compared to luminol labeled ones,
- acridinium esters have the dominant feature that they do not lose the luminescence efficiency even after binding to antigen or antibody



Bioluminescence

- ❑ Chemiluminescence found in biological systems.
- ❑ An enzyme photoprotein increases the efficiency of the luminescence reaction.
- ❑ Luciferase
- ❑ Aequorin
- ❑ Sparkling Squid,
- ❑ luminous mushrooms,
- ❑ Radiant bacteria,
- ❑ Glowing fish.



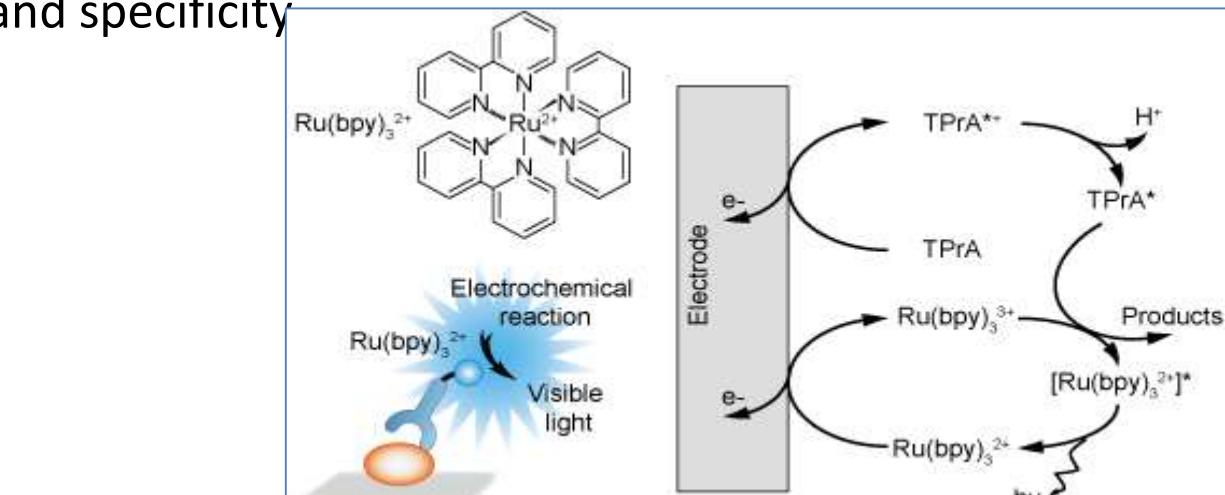
foxfire mushroom



Electrochemical luminescence

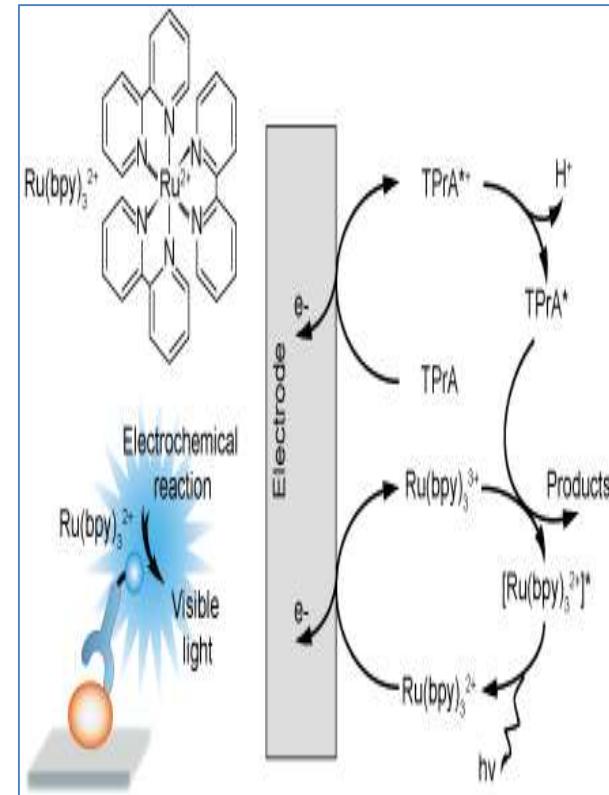
Redox Reaction Mediated Light Emission Reaction

- This system utilizes **Ruthenium tris-bipyridine (bpy)** as label, involves reaction of $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{Ru}(\text{bpy})_3^+$ to produce an excited state of $\text{Ru}(\text{bpy})_3^{2+}$, a stable species which decays to the ground state by emitting an **620 nm orange emission**.
- $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{Ru}(\text{bpy})_3^+$ can be electrogenerated from $\text{Ru}(\text{bpy})_3^{2+}$ by reduction at approximately -1.3 V, and oxidation at approximately + 1.3 V.
- Ultrahigh sensitivity and specificity



Electrochemical luminescence

- Reactive species produced in the Chemiluminescent reaction are electrochemically generated from stable precursor at the surface of an electrode.
- Ruthenium tris (bipyridyl) chelate is Chemiluminescent label.
- Generated at an electrode via oxidation-reduction



Tietz *Fundamentals of Clinical Chemistry,* Optical Techniques

Parul University

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Thank You!

