

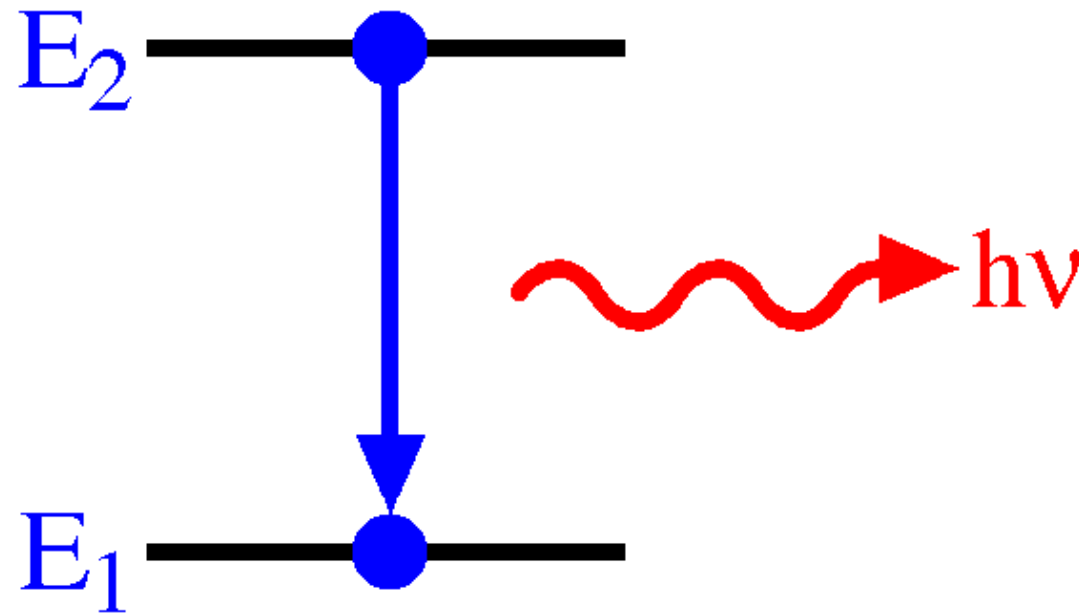
# Fluorescence Spectrometry



**Presented by:**  
**Pooja Dhurjad**

# Emission spectroscopy

Emission spectroscopy is a spectroscopic technique which examines the wavelengths of photons emitted by atoms or molecules during their transition from an excited state to a lower energy state.



# Luminescence

Luminescence is the emission of light from any substance, and occurs from electronically excited states.

Types:

The emission rates of **fluorescence** are typically  $10^8 \text{ s}^{-1}$ , so that a typical fluorescence lifetime is near 10 ns. The emission rates of **phosphorescence** are slow ( $10^3$  to  $100 \text{ s}^{-1}$ ), so that phosphorescence lifetimes are typically milliseconds to seconds.

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Fluorescence is much more widely used for chemical analysis than phosphorescence.

## Fluorescence spectroscopy

Fluorescence spectroscopy (fluorometry or spectrofluorometry), is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample.

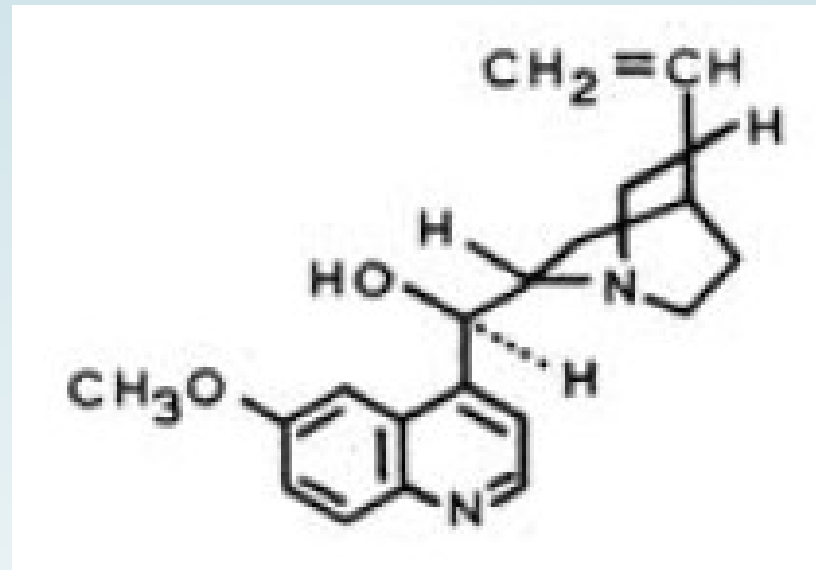
It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy, typically, but not necessarily, visible light. This shift to longer wavelength is called the **Stokes shift**.

Devices that measure fluorescence are called fluorometers or fluorimeters.

# Theory of molecular fluorescence

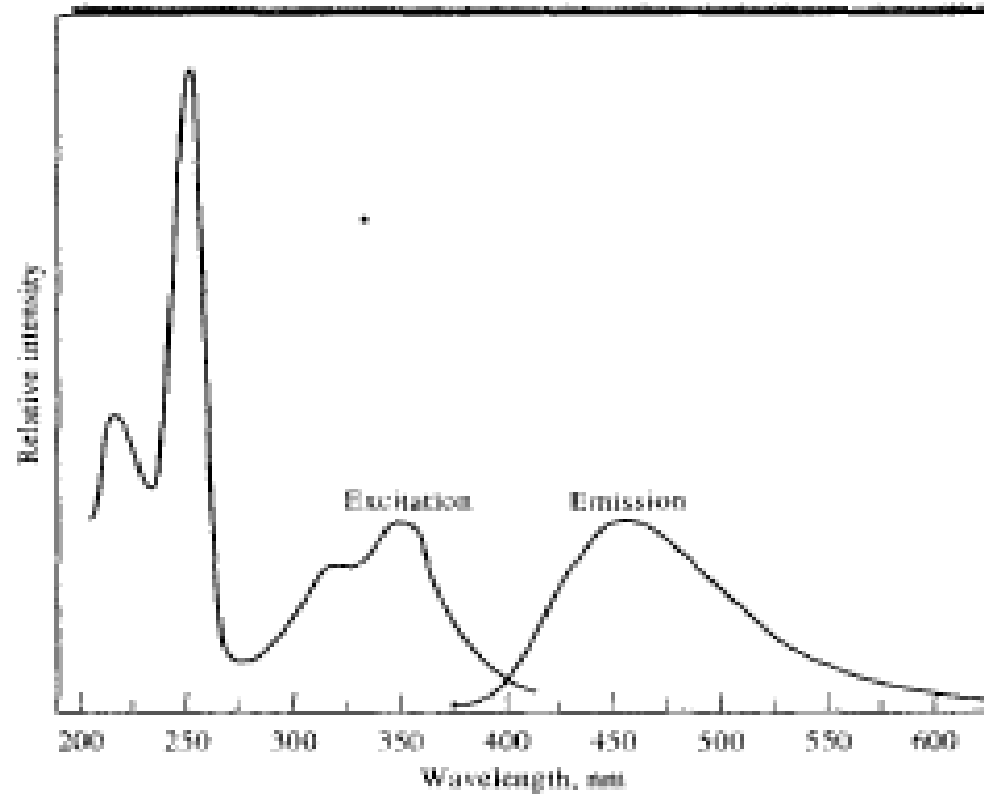
Molecular fluorescence is measured by exciting the sample at the absorption wavelength, also called the excitation wavelength, and measuring the emission at a longer wavelength called the emission or fluorescence wavelength.

The first observation of fluorescence from a **quinine** solution in sunlight was reported by Sir John Frederick William Herschel in 1845.



Quinine

The quinine in tonic water is excited by the **ultraviolet light** from the sun. Upon return to the ground state the quinine emits **blue light** with a wavelength near **450 nm**.



Fluorescence excitation and emission spectra for a solution of quinine.

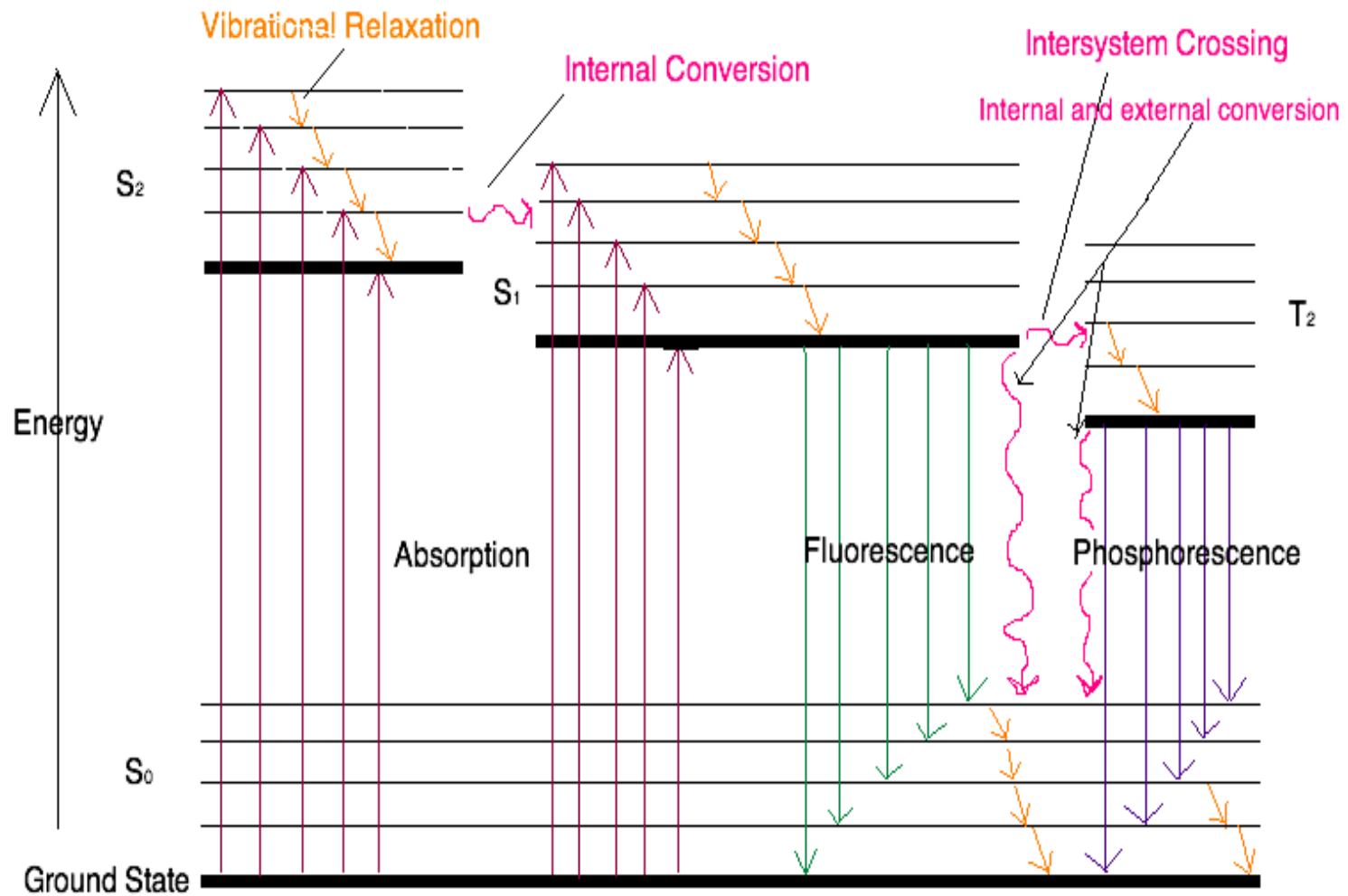
# Relaxation processes

Once the molecule is excited to  $S_1$  or  $S_2$  several processes can occur that cause the molecule to lose its excess energy.

Various mechanism of relaxation:

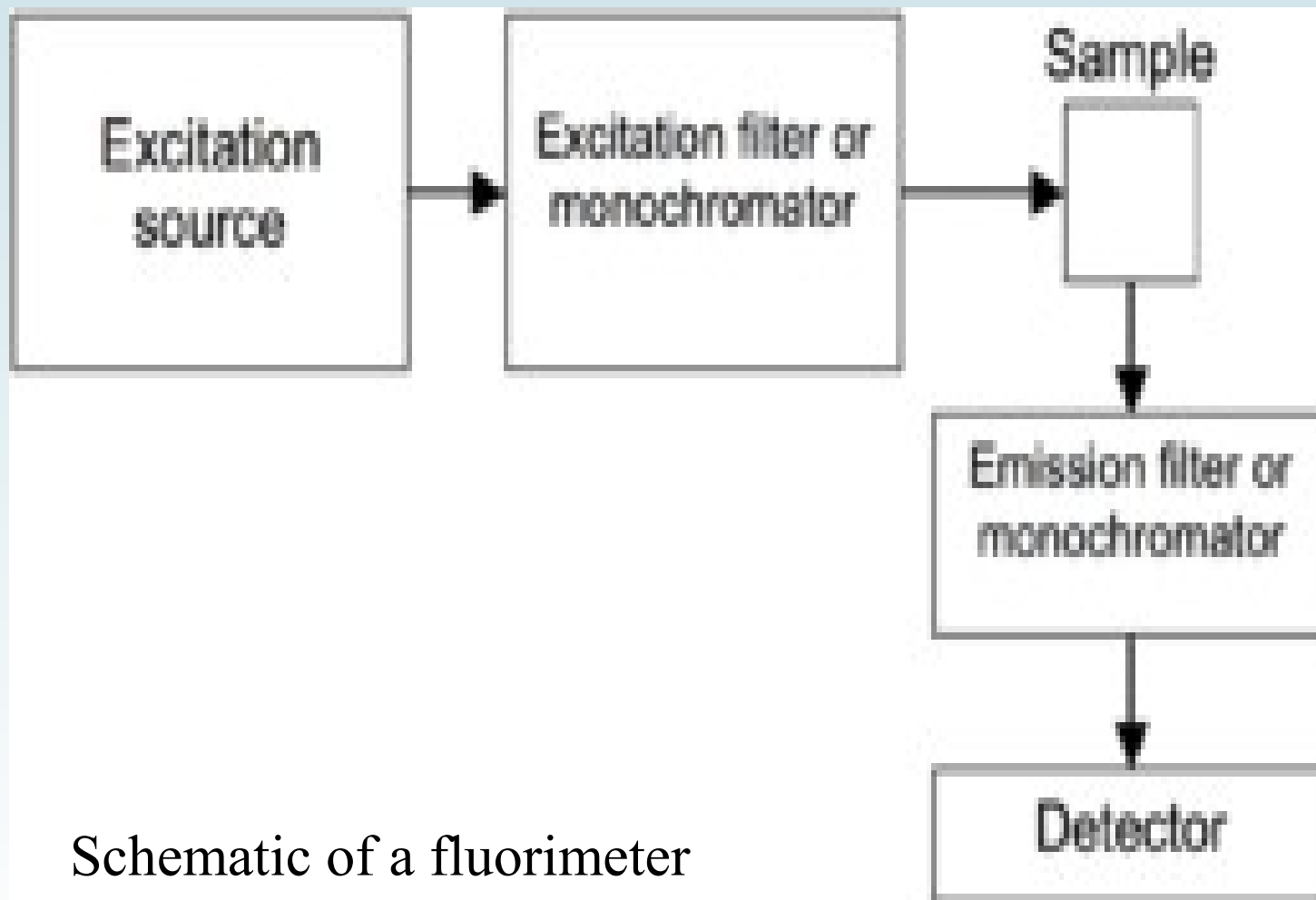
1. Fluorescence
2. Phosphorescence
3. Vibrational relaxation
4. Internal conversion
5. External conversion
6. Inter system crossing





Jablonski diagram

# Instrumentation



Schematic of a fluorimeter

## **Light source**

Xenon lamps

Mercury-vapour lamp

## **Filters and/or monochromators**

Grating monochromator

## **Detectors**

Photomultiplier tube

## Factors interfering with fluorescence intensity

1. Concentration
2. Transition type in fluorescence
3. Structure
4. Temperature and solvent
5. Impurities present in the solution

# Applications

1. Determination of fluorescent drugs in low-dose formulations in the presence of non-fluorescent excipients.
2. In carrying out the limit tests where the **impurity** is fluorescent.
3. Useful for studying the **binding of drugs** to component in complex **formulations**.
4. Widely used in bioanalysis for measuring small amounts of drug and for studying **drug-protein binding**.

