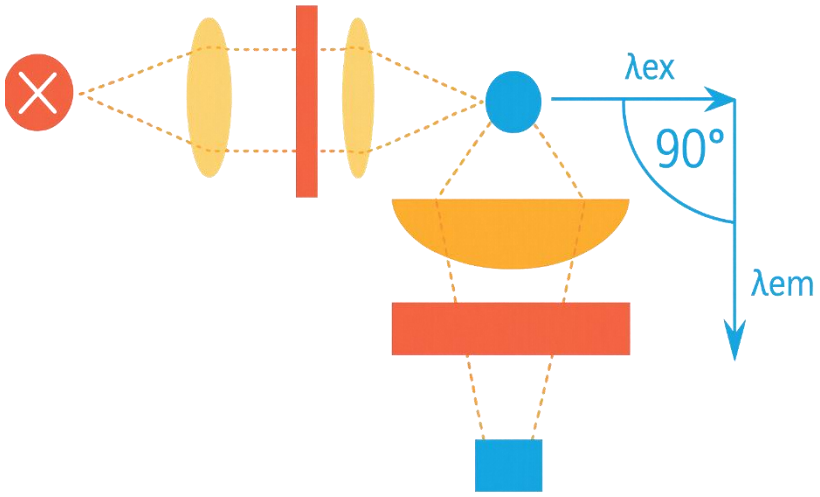
Fluorimetric Analysis

Theory of Fluorimetric Analysis

What is Fluorimetric Analysis?

- Fluorimetry measures fluorescence emitted by a substance after absorbing light or radiation.
- It involves exciting molecules to higher energy states and detecting the light emitted as they return to lower energy states.



Basic Principle

- A fluorophore absorbs photons, exciting its electrons to higher states.
- As electrons return to the ground state, they emit lower-energy (longer wavelength) photons.
- The emitted light's intensity and characteristics reveal information about the fluorophore's environment, concentration, and interactions.

Step-by-Step Process:

1. **Excitation**: A photon is absorbed by the molecule, promoting an electron to a higher energy level.
2. **Internal Conversion**: The excited electron may lose a bit of energy through non-radiative processes before emission.
3. **Emission**: The electron drops back to the ground state, releasing a photon — this is the observed fluorescence.

Key Characteristics:

- **High Sensitivity**: Detects very low concentrations.
- **Selectivity**: Distinguishes fluorophores by their unique excitation and emission wavelengths.
- **Dynamic Range**: Measures a wide concentration range.
- **Non-Destructive**: Preserves sample integrity during analysis.

Components:

1. **Excitation Source**: UV or visible light source to excite molecules.
2. **Monochromator**: Selects specific excitation wavelengths.
3. **Sample Cell**: Holds the sample for analysis.
4. **Emission Monochromator**: Filters emitted light to isolate desired wavelengths.

5. **Detector**: Measures the intensity of emitted fluorescence.
6. **Impurity Detection**: Identifies trace fluorescent impurities undetectable by other methods.

Mathematical Relationship:

$$F = K \cdot \Phi \cdot I_0 \cdot \varepsilon \cdot c$$

- **Where:**
  - F= Fluorescence intensity
  - K= Instrumental constant
  - Φ= Quantum yield (fraction of absorbed photons that are emitted as fluorescence)
  - I_0= Intensity of the excitation light
  - ε= Molar absorptivity of the compound
  - c= Concentration of the fluorescent species

Advantages of Fluorimetry:

- Very high sensitivity (detects nanogram or even picogram levels)
- Selectivity (only naturally or derivatized fluorescent compounds respond)
- Quick and non-destructive

Limitations:

1. Limited to fluorescent compounds or those that can be tagged.
2. Fluorescence influenced by pH, temperature, and quenching agents.

Luminescence: Definition & Types

Definition

- Luminescence is the emission of light by a substance not resulting from heat.
- It is a **radiative process** where a substance emits light due to electronic transitions.

Types of Luminescence

- There are several forms, classified by the source of excitation:

Type	Cause of Excitation	Description
Photoluminescence	Light	Includes **fluorescence** and **phosphorescence**
Chemiluminescence	Chemical reaction	No external light; energy from a chemical reaction causes emission

Bioluminescence	Biological reaction	Light emission in biological systems (e.g., fireflies)
Electroluminescence	Electric field	Used in LEDs
Triboluminescence	Mechanical action	Light emitted when crystals are crushed or rubbed

Fluorescence vs. Phosphorescence

Feature	Fluorescence	Phosphorescence
Duration	Short (10^{-9} to 10^{-7} sec)	Long (10^{-6} to minutes)
State Involved	Singlet → Singlet	Triplet → Singlet
Energy	Higher energy	Lower energy due to loss over time
Applications	Analytical detection	Imaging, glow-in-the-dark materials

Mechanism in Fluorimetry

- In fluorescence, the excited molecule returns directly from the singlet excited state (S_1) to the singlet ground state (S_0).
- In phosphorescence, the excited electron undergoes intersystem crossing to a triplet state (T_1) before returning to the ground state, resulting in delayed emission.

Focus on Photoluminescence:

There are two main types:

A. Fluorescence

- Very fast emission (10^{-9} to 10^{-8} seconds)
- Stops immediately when excitation source is removed
- Involves **singlet–singlet transitions** (no spin change)

B. Phosphorescence

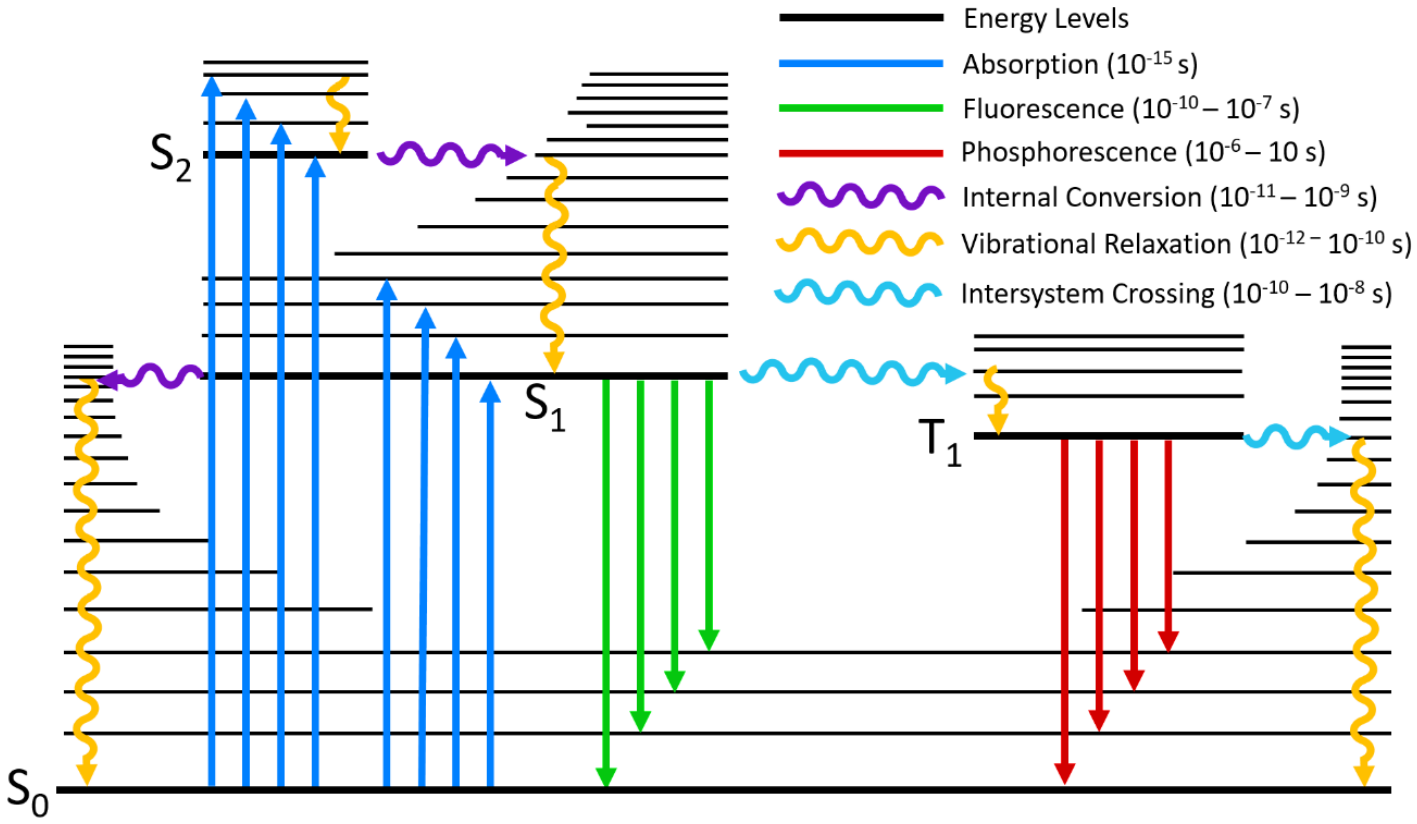
- Slower process (can last milliseconds to minutes)
- Continues even after the excitation source is removed
- Involves **singlet–triplet transitions** (spin-forbidden transitions), hence slower

Energy Diagram: (Jablonski Diagram)

A Jablonski diagram illustrates the electronic states of a molecule and the transitions between them:

- **Vertical Transitions**: Represent absorption of photons leading to excitation.
- **Horizontal Transitions**: Indicate non-radiative processes like internal conversion and intersystem crossing.

- **Downward Transitions**: Depict emission processes such as fluorescence and phosphorescence.
- Fluorescence: $S_0 \rightarrow S_1 \rightarrow S_0$ (with light emission)
- Phosphorescence: $S_0 \rightarrow S_1 \rightarrow T_1 \rightarrow S_0$ (with intersystem crossing and delayed emission)



Factors Affecting Fluorescence

Fluorescence is influenced by several chemical, physical, and environmental factors:

1. Quantum Yield (Φ_x)

- **Definition**: The ratio of the number of photons emitted to the number of photons absorbed.
- **Formula**:

$$\Phi_x = \frac{\text{Number of photons emitted}}{\text{Number of photons absorbed}}$$

- **Influence**: Higher quantum yields indicate more efficient fluorescence.

2. Molecular Structure

- **Conjugation**: Extended π-electron systems enhance fluorescence.
- **Rigidity**: Rigid structures reduce non-radiative decay pathways.
- **Functional Groups**: Electron-donating groups can increase fluorescence intensity.

3. Solvent Effects

- **Polarity**: Polar solvents can stabilize excited states differently than ground states, affecting energy gaps.
- **Viscosity**: Higher viscosity reduces molecular motions, potentially increasing fluorescence.
- **Hydrogen Bonding**: Can alter electronic energy levels and fluorescence properties.

4. Temperature

- **Effect**: Increased temperature enhances molecular collisions, promoting non-radiative decay and decreasing fluorescence.

5. pH

- **Ionization States**: Protonation or deprotonation can change electronic structures, influencing fluorescence.
- **Applications**: Useful in studying pH-dependent fluorescence of compounds.

6. Concentration

- **Self-Quenching**: At high concentrations, interactions between fluorophore molecules can lead to quenching.
- **Inner Filter Effect**: Reabsorption of emitted light by other molecules in the sample reduces observed fluorescence.

7. Presence of Quenchers

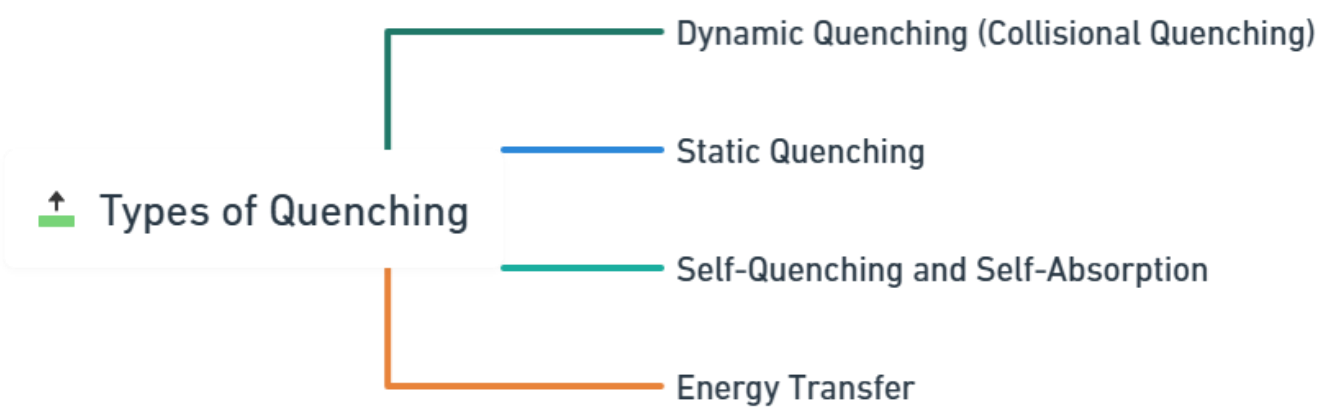
- **Oxygen**: A common quencher due to its paramagnetic nature.
- **Halide Ions**: Heavy atoms can enhance intersystem crossing, reducing fluorescence.

Quenching of Fluorescence

Definition

- Quenching refers to any process that decreases the fluorescence intensity of a sample.
- It can be due to various mechanisms, both dynamic and static.

Types of Quenching



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