Laser induced fluorescence and its applications

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What is fluorescence

Simply the property of certain materials to absorb radiation at one wavelength then re-emit that radiation at another wavelength usually longer wavelength.
ABSORPTION OF LIGHT

$hv$

ABSORPTION OF LIGHT
Types of electronic Transitions cont…

- $\pi^* - \pi$ transitions: A bonding $\pi$ electron is excited into an antibonding $\pi^*$ orbital.
  - A C=C double bond acts as a chromophore by providing the empty $p^*$ orbital for electron promotion.
  - Conjugating the chain causes the molecular orbitals to lie closer together, increasing the absorption wavelength.
Types of electronic Transitions …

- **n - π* transitions**: a non-bonding electron is excited to an antibonding p* orbital.
  - The transition for absorption of a carbonyl (CO) comes from one of the O lone pair electrons (non-bonding) being excited into an empty π* orbital within the carbonyl group.
Table (2) lists many of possible chemical pathways that can be taken by an excited molecule.
Excitation scan em 324 nm

28 nm Stokes Shift

296 324

Emission scan excitation 291 nm

INT

nm

260 280 300 320 340 360 380 400
RELATIVE POSITIONS OF ABSORPTION, FLUORESCENCE, AND PHOSPHORESCENCE EMISSION BANDS
LASERs (Light Amplification by Stimulated Emission of Radation)

- several of the higher electronic and vibrational energy levels of the active species are populated.

- **Must have the existence of a metastable state that has a long enough lifetime for stimulated emission to occur.**

- **To get a net emission from the metastable state, there must be a greater population in this state than the lower state.**

  - pumping

![Diagram of laser principles]

**Equations:**

1. **Excitation**
   - $E_x$

2. **Partial relaxation**
   - $E_x ightarrow E_y$

3. **Metastable excited state**
   - $E_y$

**Pumping** (excitation by electrical, radiant, or chemical energy)
LASER

- spontaneous emission

  • species in an excited electronic state may lose all or part of its excess energy by spontaneous emission of radiation

  • path of the emitted photon varies from different excited molecules

  - produces incoherent monochromatic radiation

  \[ \begin{align*}
  &\text{(1)} \\
  &\text{(2)} \\
  &\text{(3)}
  \end{align*} \]

  \begin{figure}
  \centering
  \includegraphics[width=\textwidth]{spontaneous_emission.png}
  \caption{Spontaneous emission}
  \end{figure}
LASER

- stimulated emission

• excited laser species are struck by photons that have the same energies as the photons produced by spontaneous emission.

• cause the excited species to relax immediately to the lower energy state
LASER

- stimulated emission

  - simultaneously emit a photon of exactly the same energy as the photon that stimulated the process

  - emitted photon travels in exactly the same direction and is precisely in phase with the photon that caused the emission

![Diagram of stimulated emission](image)
LASER INDUCED FLUORESCENCE:

Is The Optical Emission From Atoms, Or Molecules That Have Been Excited To Higher Energy Levels By Absorption Of Laser Radiation.

In This Case Laser Provides A Very Selective Means For Populating Excited States, Which Gives More Accurate And Sensitive Measurements.

Two types of Laser Induced Fluorescence:

1-LASER INDUCED ATOMIC FLUORESCENCE
2-LASER INDUCED MOLECULAR FLUORESCENCE:
**LASER INDUCED ATOMIC FLUORESCENCE**

This method is now increasingly being used for trace elemental analysis. It involves depend on the change of the sample to free atoms and ions and subsequently exciting the free atoms and ions to higher energy states using a laser sources.

**LASER INDUCED MOLECULAR FLUORESCENCE**
Dispersed Fluorescence from CH₂O
Laser Induced Breakdown Spectroscopy (LIBS)

• It is developed for use in the environmental analysis,

• Laser-Induced Breakdown Spectroscopy utilizes a pulsed laser that rapidly heats (~5000 K) and ablates the sample forming a plasma. The spectral lines emitted from the plasma indicate the constituent elements can be analyzed to indicate which atomic species are present.
Laser-induced plasma

Nd:YAG Pulsed Laser

Pulsed laser

Fiber optic

Emission collection

Sample

Detector

Atomic emission lines provide species identification

Emission Intensity

Wavelength (nm)
Representative LIBS Spectra of heavy metals
arsenic is a potent human carcinogen and Toxicant. Epidemiological studies have shown associations between arsenic in drinking water and different forms of cancer, skin lesions, vascular diseases and diabetes mellitus.

1- Adsorption of heavy metals.

Arsenic (200ug) removal by dried water hyacinth roots 0.1gm doped in polymer as a function of time (min.)
The Advantages Of Laser Induced Fluorescence

1- Laser induced fluorescence is very sensitive where the many atoms and radical can be detected.

2- Laser induced fluorescence distinguished any optical emission and it’s more sensitive than the absorption spectroscopy.

3- Laser induced fluorescence can population in any state.

4- Laser induced fluorescence can identify the species and it’s concentrations.

5- Absolute measurements of concentration can be obtained by laser induced fluorescence.
Applications:

*Fundamental research:*

1- Studies in energy transfer, particularly in the field of proteins and membranes.
2- Rate study of the decay processes of excited state species.
3- Effect on fluorescence of molecules due to the environment, e.g. solvent, pH, ... 
4- Change in fluorescence due to structural changes in the molecules.

*Biochemistry:*

1- Biochemists are involved in studies relating to the metabolism, e.g. catecholamines, tryptophan metabolites.
2- Determination of proteins orientation.
3- Determination of binding site of proteins.
4- Membrane studies.
5- Enzymes and protein
6- Glucose and metabolites can determine in blood.
7- Porphyrins are monitored to detect porphyria,

8- Steriods, e.g. plasma cortisol and oestrogen levels

9- Urea and uric acid in plasma

10- Vitamins

**Industrial chemistry:**

1- Analysis of dyes and pigments for fluorescence and phosphorescence

2- Optical brighteners on papers,

3- A very easy method to distinguish between different crude oils is by fluorescence analysis. It is possible to determine the source of oil when investigating oil spills. An example of excitation emission matrices for two different oils.

**Forensic analysis:**

1- Detection of drugs and poisons in tissue.

2- Determination of the age of a body from the fluorescence of organs and tissues.

3- Detection of blood and sperm.

4- Detection of gun grease.
Fluorescence microscope

Figure 1. The fluorescence microscope.
(a) Epi-illumination fluorescence microscopes use the objective both to illuminate and image the specimen. Shown is an upright microscope with the slide at the bottom. The light source, in this case an arc lamp, sends full-spectrum light to the specimen by way of a fluorescence 'cube' that selectively illuminates the specimen with a wavelengths that excite a particular fluorophore (shown: green light to excite rhodamine). The red fluorescence that is excited sends photons in all directions and a fraction are collected by the objective and sent through the cube to the eye or camera port above. The cube has two filters: the dichroic mirror and barrier filter, to prevent the exciting wavelengths from reaching the detector. (b) The details of a cube designed by Chroma Technologies to excite and detect GFP. The three main components (labeled 2, 3 and 4) have specific spectral features that are ideal for GFP. Note that the dichroic mirror splits between reflection and transmission right between the absorption and emission peaks of the GFP, which are superimposed in blue and green, respectively.