# Applications of Fluorescence Spectroscopy

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**ABSTRACT**

Fluorescence spectroscopy is a rapid, sensitive method for characterizing molecular environments and events. Fluorescence spectroscopy is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. In this spectroscopy generally light passes issued by the excitation source through a unified candidate or waves and collides with the sample of fluorophore, the sample absorbs part of the incident light and fluorescence is happening in the sample. There are a relatively small number of compounds that have a characteristic fluorescence such as aromatic hydrocarbons. This has contributed to the use of the spectroscope successfully in the detection of many of organic compounds, aromatic numerous active substances in drugs in the field of research chemicals, biochemical, and medical analysis of organic compounds.

A great deal of research has been done on the development of new fluorescent dyes for visualizing finger prints with a laser in the area of forensic science. Fluorescence has also been applied extensively to questioned-document analysis, principally in characterization of inks. Other types of evidence that use fluorometric analysis include drugs, glass, petroleum products, and biological samples. Fluorescence spectroscopy may be an excellent diagnostic as well as excellent research tool in medical microbiology field with high sensitivity and specificity. This article presents a brief overview of the theory of fluorescence spectroscopy, together with some examples of applications of this technique in food analysis, forensic science, medical diagnosis, medical science etc.

**Key words:** Fluorescence spectroscopy, Fluorometry, analysis, spectrofluorometer

## 1. INTRODUCTION

Spectroscopy has made an outstanding contribution to the present state of atomic and molecular chemistry and lasers have always played an important role in this area. During the past 20 years there has been a remarkable growth in the use of fluorescence in the biological sciences. Fluorescence spectroscopy and time-resolved fluorescence are considered to be primarily research tools in biochemistry and biophysics.

Fluorescence is the molecular absorption of light energy at one wavelength and its nearly instantaneous re-emission at another, usually longer, wavelength. Fluorescent compounds have two characteristic spectra: an excitation spectrum (the wavelength and amount of light absorbed) and an emission spectrum (the wavelength and amount of light emitted). These spectra are often referred to as a compound's fluorescence signature or fingerprint. No two compounds have the same fluorescence signature. It is this principle that makes fluorometry a highly specific analytical technique. Fluorometry is the measurement of fluorescence. The instrument used to measure fluorescence is called a fluorometer or fluorimeter. A fluorometer generates the wavelength of light required to excite the analyte of interest; it selectively transmits the wavelength of light emitted, then it measures the intensity of the emitted light. The emitted light is proportional to the concentration of the analyte being measured. Fluorometers employ monochromators (a spectrofluorometer), optical filters (a filter fluorometer), or narrow band light sources like LED’s or lasers to select excitation and emission wavelengths.

Fluorometry is chosen for its extraordinary sensitivity, high specificity, simplicity, and low cost as compared to other analytical techniques. Fluorometry is ordinarily more sensitive than absorbance measurements. It is a widely accepted and powerful technique that is used for a variety of environmental, industrial, medical diagnostics, DNA sequencing, forensics, genetic analysis, and biotechnology applications. It is a valuable analytical tool for both quantitative and qualitative analysis.

## 2. PRINCIPLE OF FLUORESCENCE SPECTROSCOPY

Fluorescence and phosphorescence are photon emission processes that occur during molecular relaxation from electronic excited states. These photonic processes involve transitions between electronic and vibrational states of polyatomic fluorescent molecules (fluorophores). Fluorophores play the central role in fluorescence spectroscopy. Fluorophores are the components in molecules that cause them to fluorescence. Majorly fluorophores are the molecule which contain aromatic rings such as Tyrosine, Tryptophan, Fluorescein etc.

The Jablonski diagram (Figure 1) offers a convenient representation of the excited state structure and the relevant transitions. Molecules that have become electronically excited subsequent to the absorption of visible (400~700nm), UV (200~400nm), or NIR(700~1100 nm) radiation. Excitation process to the excited state from the ground state is very fast, on the order of $10^{-15}$ s. After excitation, the molecule is quickly relaxed to the lowest vibrational level of the excited electronic state. This rapid vibrational relaxation process occurs on the time scale of femto seconds to picoseconds. Fluorescence emission occurs as the fluorophore decay from the singlet electronic excited states to an allowable vibrational level in the electronic ground state. The fluorescence excitation and emission spectra reflect the vibrational level structures in the ground and the excited electronic states, respectively.

**Fluorescence**: **Prompt fluorescence**: $S_1 \rightarrow S_0 + h\nu$
The release of electromagnetic energy is immediate or from the singlet state.
**Delayed fluorescence**: $S_1 \rightarrow T_1 \rightarrow S_1 \rightarrow S_0 + h\nu$
This results from two intersystem crossings, first from the singlet to the triplet, then from the triplet to the singlet.
**Phosphorescence**: $T_1 \rightarrow S_0 + h\nu$
Delayed release of electromagnetic energy from the triplet state.

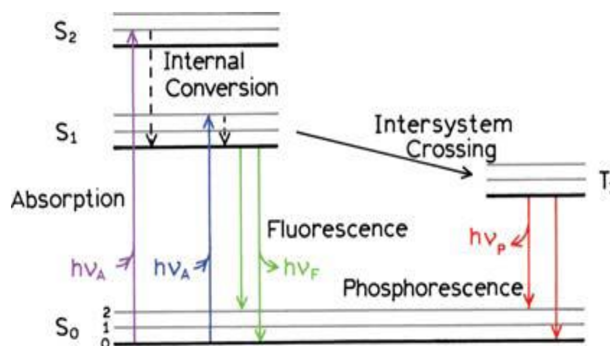


**Figure.1.Jablonski Diagram**

**2.1. Types of fluorescence and emission processes:** Stokes fluorescence is the reemission of less energetic photons, which have a longer wavelength than the absorbed photons. Stokes shift is the rapid decay to the lowest vibrational level of $S_1$. Dilute gases at high temperature emission at shorter wavelengths than those of absorption occur. This is called Anti-Stokes fluorescence. It occurs when thermal energy is added to an excited state or a compound has many highly populated vibrational energy levels. Resonance fluorescence is the reemission of photons possessing the same energy as the absorbed photons. This type of fluorescence is never observed in solution because of solvent interactions, but it does occur in gases and crystals.

## 3. INSTRUMENTATION OF SPECTROFLUOROMETER:

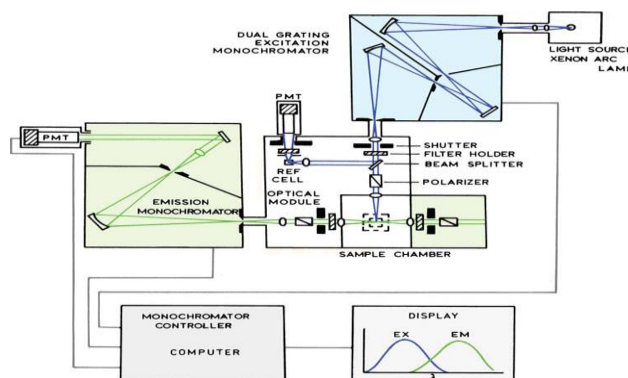


**Figure.2.Schematic diagram of a spectrofluorometer**

A diagram of a typical fluorimeter is shown in Figure 2. It consists of a continuous ozone-free xenon lamp generating a continuum of visible and ultra-violet light, a monochromator to select the required wavelength for excitation, a sample compartment, and a second monochromator coupled with a photomultiplier tube (PMT) to analyze the fluorescence signal. The grating in the excitation and emission monochromator can disperse light from 200 to 900 nm. The entrance and exit slits of each monochromator control the intensity and wavelength spread (band pass) of the light. Illumination from the Xenon-lamp is collected by an elliptical mirror and directed toward the entrance slit of the excitation monochromator. The excitation monochromator selectively delivers a narrow band of wavelengths of excitation light that strikes the sample. A portion of the incident light is absorbed by the sample, and some of the molecules in the sample fluoresce. The emitted light enters the emission monochromator, which is positioned at 90° angle with respect to excitation light path in order to minimize the risk of transmitted or reflected incident light reaching the detector. No monochromator is perfect and it will transmit some stray light, that is, light with other wavelength than targeted. The emitted light is transmitted in a narrow range of centered about the specified emission wavelength and exits through the adjustable slits, and finally striking the detector. The signal is amplified and generates a voltage that is proportional to the measured emitted intensity. The right angle geometry is used when the sample is transparent. There is geometry for collecting fluorescence, known as front faced geometry. In this geometry the fluorescence is collected from the sample from the same surface at which the sample is illuminated by excitation light. The front face geometry is used primarily when the sample is opaque or solid.

A fluorescence spectrum of a sample is recorded by scanning the emission monochromator for a constant wavelength of the excitation light ($\lambda ex$). Similarly, an excitation spectrum is acquired by scanning the excitation monochromator at a fixed emission wavelength ($\lambda em$). The source light has different intensities at all wavelengths, and it may vary over time during each experiment or between each experiment. This can distort the spectrum and corrections are necessary to get an instrument-independent spectrum. In order to correct this, fluorimeter is equipped with an additional reference photodiode (PD) which measures a fraction of source light separated using a beam splitter (BS) after the exit slit of excitation monochromator before it enters the sample compartment. By rationing the fluorescence signal to the reference signal, correction is made for variation in excitation light intensity as a function of wavelength.

Molecular fluorescence is measured by exciting the sample at the absorption wavelength, also called excitation wavelength, and measuring the emission at a longer wavelength called the emission or fluorescence wavelength. Usually, fluorescence emission is measured at right angles to the incident beam so as to avoid measuring the incident radiation. The short-lived emission that occurs is called fluorescence, whereas luminescence that is much longer lasting is called phosphorescence.

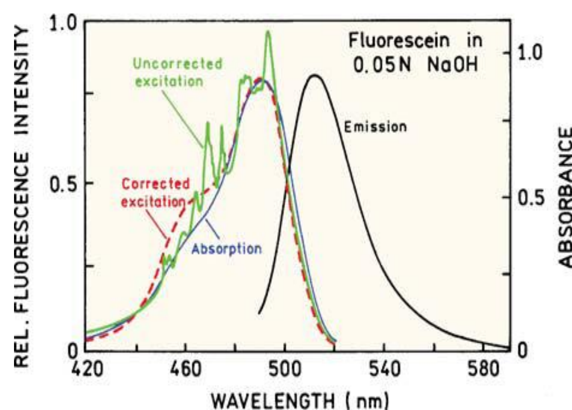


**Figure.3.Excitation and Emission Spectra of fluorescein**

## 4. APPLICATIONS OF FLUORESCENCE SPECTROSCOPY

**4.1. Laser induced fluorescence spectroscopy of human tissues for cancer diagnosis:** Cancer is one of the most dreaded diseases of our time and has a very high incidence. Early tumours often arise from tissue which have a rapid turnover of cells and are active in repair like transformed mucosa on the surface of hollow organs (oral cavity, gastrointestinal tract, female reproductive organs etc.). Laser spectroscopic techniques have the potential for in-situ, near real time diagnosis and the use of non-ionizing radiation ensures that the diagnosis can be made repeatedly without any adverse side effects.

Laser Induced Fluorescence (LIF) has been used for diagnosing cancer in two ways. One approach involves systemic administration of a drug like hematoporphyrin derivative (HpD) which is selectively retained by the tumour. When photo excited with light of appropriate wavelength the drug localized in the tumour fluoresces. This fluorescence is used for detection and imaging of the tumour. Photo excitation also leads to populating the triplet state via intersystem crossing. The molecule in excited triplet state can directly react with bio-molecules or lead to generation of singlet oxygen which is toxic to the host tissue. The resulting destruction of the host tissue is exploited for photodynamic therapy of tumour.

**4.2 Study of Marine Petroleum Pollutants:** Fluorescence spectroscopy is one of the good technique to detection of oil slicks on the water surface, determination of petroleum contaminants in seawater and determination of particular petroleum derivative compounds as well as identification of pollution sources. Main components of any oil are hydrocarbons. The other components are primarily derivatives of hydrocarbons containing single atoms of sulfur, oxygen or nitrogen. Only a few of hydrocarbons fluoresce, while the major of them show no ability to luminescence. The content of compounds able to fluorescence rarely exceeds 10% of the oil mass. At the same time the petroleum strongly absorbs radiation, especially the ultraviolet and blue light. In spite of this petroleum is a luminescent medium and fluorescence is a phenomenon which allows testing oils. Fluorescence of oils has wavelength over then 260 nm and covers a spectral area of ultraviolet and visible light. The phenomenon is most significant in the 270–400 nm range.

**4.3. Accurate determination of glucose:** Glucose is considered as a major component of animal and plant carbohydrates in biological systems. Furthermore, blood glucose levels are also an indicator of human health conditions: the abnormal amount of glucose provides significant information of many diseases such as diabetes or hypo glycemia. Fluorophotometry was used widely owing to its operational simplicity and high sensitivity. Recently bio-molecule-stabilized Au nanoclusters were demonstrated as a novel fluorescence probe for sensitive and selective detection of glucose.

## 5. CONCLUSION

Fluorescence spectroscopy is a rapid, sensitive method for characterizing molecular environments and events. Fluorescence output is linear to sample concentration over a very broad range. Fluorometry is a relatively simple analytical technique. Fluorometry is chosen for its extraordinary sensitivity, high specificity, simplicity, and low cost as compared to other analytical techniques. It is ordinarily more sensitive than absorbance measurements. It is a widely accepted and powerful technique that is used for a variety of environmental, industrial, medical diagnostics, DNA sequencing, forensics, genetic analysis, and biotechnology applications. It is a valuable analytical tool for both quantitative and qualitative analysis.

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