Spectroscopic studies of some Organic Fluorescent Molecules

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Abstract

This paper attempts to study how **the Spectroscopy of organic Fluorescent Molecules**method iseffective for obtaining information on the microenvironment at the molecular level near a probe molecule. Fluorescence spectroscopy analyzes fluorescence from a molecule based on its fluorescent properties.Fluorescence is a type of luminescence caused by photons exciting a molecule, raising it to an electronic excited state.

Fluorescence spectroscopy uses a beam of light that excites the electrons in molecules of certain compounds, and causes them to emit light. That light is directed towards a filter and onto a detector for measurement and identification of the molecule or changes in the molecule. Fluorescence spectroscopy is used in, among others, biochemical, medical, and chemical research fields for analyzing **organic fluorescent moleculescompounds.** There has also been a report of its use in differentiating malignant skin tumors from benign. In the field of water research, fluorescence spectroscopy can be used to monitor water quality by detecting organic pollutants. Recent advances in computer science and machine learning have even enabled detection of bacterial contamination of water. Kasha's rule dictates that the quantum yield of luminescence is independent of the wavelength of exciting radiation. This occurs because excited molecules usually decay to the lowest vibrational level of the excited state before fluorescence emission takes place.

Fluorescence is the temporary absorption of electromagnetic wavelengths from the visible light spectrum by fluorescent molecules, and the subsequent emission of light at a lower energy level. Stimulating light excites an electron, raising energy to an unstable level. This instability is unfavorable, so the energized electron is returned to a stable state almost as immediately as it becomes unstable. This return to stability corresponds with the release of

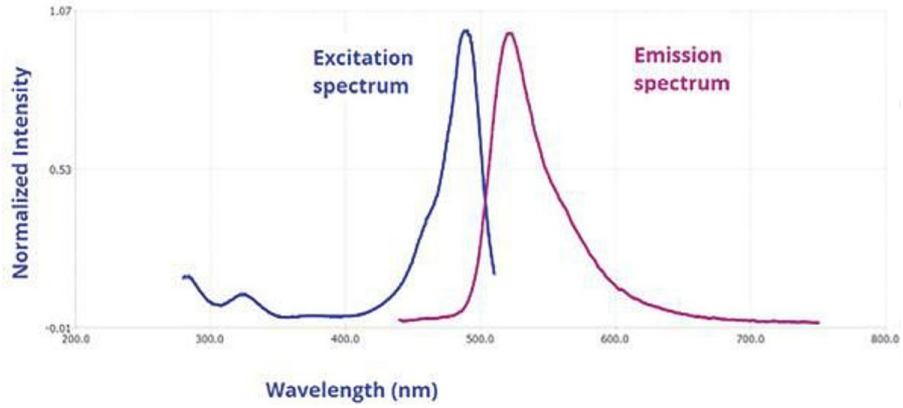
excess energy in the form of fluorescence light. This emission of light is only observable when the stimulant light is still providing light to the organism/object and is typically yellow, pink, orange, red, green, or purple. Fluorescence is often confused with the following forms of biotic light, bioluminescence and biophosphorescence. The Kasha–Vavilov rule does not always apply and is violated severely in many simple molecules. A somewhat more reliable statement, although still with exceptions, would be that the fluorescence spectrum shows very little dependence on the wavelength of exciting radiation. For many fluorophores the absorption spectrum is a mirror image of the emission spectrum.

Key words: Fluorescence, emission spectrum, exciting radiation, Spectroscoy, organic

Introduction

Most organic molecules have at least some bonds that can be ruptured by energies of this strength. Consequently, fluorescence due to sigma∗→σsigma∗→σ transitions is rarely observed. Instead such emission is confined to the less energetic π∗→ππ∗→π and π∗→nπ∗→n processes. Fluorescence commonly occurs from a transition from the lowest vibrational level of the first excited electronic state to the one of the vibrational levels of the electronic ground state. This is known as the mirror image rule and is related to the Franck–Condon principle which states that electronic transitions are vertical, that is energy changes without distance changing as can be represented with a vertical line in Jablonski diagram. This means the nucleus does not move and the vibration levels of the excited state resemble the vibration levels of the ground state.

The term fluorescence refers to one type of luminescence. Luminescence, broadly defined, is light emission from a molecule. There are several types of luminescence.



Photoluminescence is when light energy, or photons, stimulate the emission of a photon.

Chemiluminescence, is defined as when chemical energy stimulates the emission of a photon, and this includes bioluminescence, as seen in fire flies and many forms of sea life.

Electroluminescence, is when electrical energy or a strong electric field, stimulates the emission of a photon, such as in some lighting applications.

Fluorescence, specifically, is a type of photoluminescence where light raises an electron to an excited state. The excited state undergoes rapid thermal energy loss to the environment through vibrations, and then a photon is emitted from the lowest-lying singlet excited state. This process of photon emission competes for other non-radiative processes including energy transfer and heat loss.

When the term "fluorescence" is used, the same methods of measurement can typically be applied to any of the above categories of luminescence.

Objective:

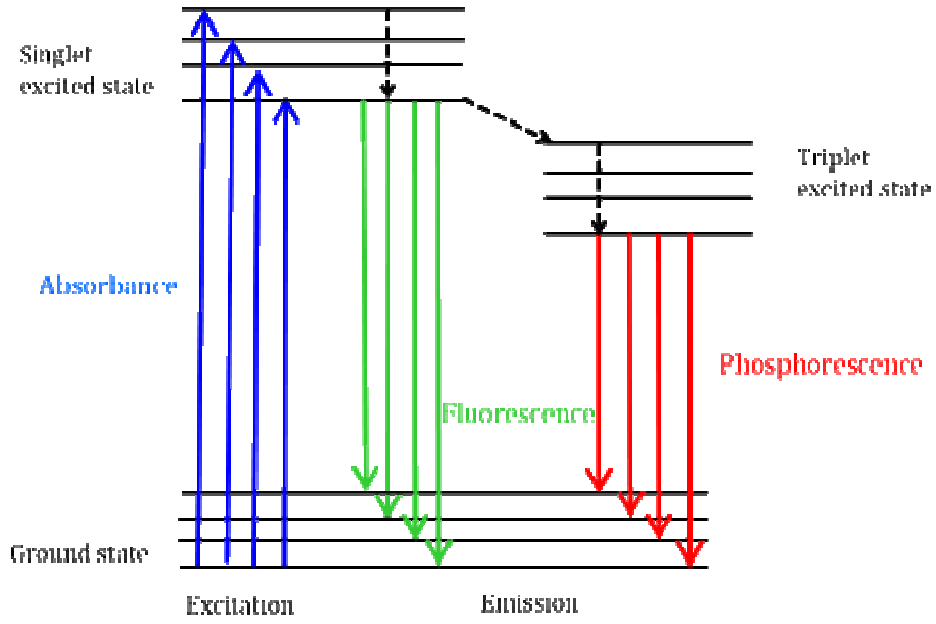
This paper intends to exploreand analyze **Fluorescence spectroscopy of few Organic Molecules ;**a technique that relies on the presence of a fluorophore, which is a chemical group in an analyte that absorbs radiation (normally in the ultraviolet region) and emits light with a specific, longer wavelength.

Fluorescence Spectroscopyof Organic Molecules

Fluorescence spectroscopy is routinely used for studying structural changes in conjugated systems, aromatic molecules, and rigid, planar compounds due to alterations in temperature, pH, ionic strength, solvent, and ligands. A single fluorophore can generate thousands of detectable photons that can be repeatedly excited and detected, making fluorescence spectroscopy is a highly sensitive technique.

Fluorescence is a type of radiative emission that occurs when a molecule absorbs energy at a wavelength where it has a transition dipole moment. The excitation energy provided to the

molecule at the ground state promotes photons to an excited singlet state, where they then decay to the lowest vibrational energy level of this excited singlet state. This energy further relaxes back to the ground state of the molecule, emitting photons in the process, as shown in



Jablonski diagram illustrating different transitions between a molecule's energy states.

Fluorescent molecules can also undergo there are three methods of nonradiative relaxation where the excitation energy is not converted into photons: (1) internal conversion, (2) external conversion, and (3) intersystem crossing. Internal conversion occurs when there is a relatively small energy gap between two electronic states and the electrons transition from a higher electronic state to one of lower energy. Here the energy is transferred to the vibrational modes of the electronic state. Since vibrational processes are thermally driven, increasing temperature leads to decreases in fluorescence intensity. In external conversion, energy is lost through collisional quenching with solute molecules in the fluorophore's environment. Intersystem crossing arises when vibrational levels of the singlet and triplet excited states overlap in energy and electrons transition from the lowest singlet excited state to the first excited triplet state. The photons emitted as they return back to the ground state is known as phosphorescence (Figure 1). The triplet state is lower in energy than the singlet state so phosphorescence peaks are found at longer wavelengths than fluorescence. Since these transitions are also forbidden,

phosphorescence exhibits a longer lifetime (~10-4 – 102 seconds) compared with fluorescence (~10-9 – 10-6 seconds). The longer lifetimes also lead to thermal deactivation via oxygen quenching, solvent movement, and intermolecular collision so phosphorescence typically cannot be observed at room temperature and samples must therefore be cooled at liquid nitrogen temperature.

Beer's Law and Concentration Effects

While absorption occurs on the timescale of less than 10-15 seconds, the relaxation process from the excited to the ground state is much slower. Therefore, fluorescence can provide information on a fluorophores' interactions with surrounding molecules and solvents, unlike absorption.

Fluorescence intensity is directly proportional to the excitation light intensity

$F=2.303 * K * I0 * \varepsilon bc$

where K is a constant based on instrument geometry, I0 is the intensity of the excitation light, e is the fluorophore's molar absorptivity, b is the pathlength, and c is the concentration. Since the fluorescence intensity is not ratioed to the incident light intensity like with absorption measurements, the fluorescence sensitivity is much greater because it is not limited by the instruments ability to differentiate between the incident and detected intensities. Consequently, smaller concentrations are required for measurements.

The above equation is only linear when the sample absorbance is less than 0.05 AU. If a sample is too concentrated, the emission light can be reabsorbed by the fluorophore, attenuating the fluorescence signal at shorter wavelengths. Excitation light may also not fully penetrate the full width of a highly concentrated sample, which will also lead to decreased fluorescence intensities.

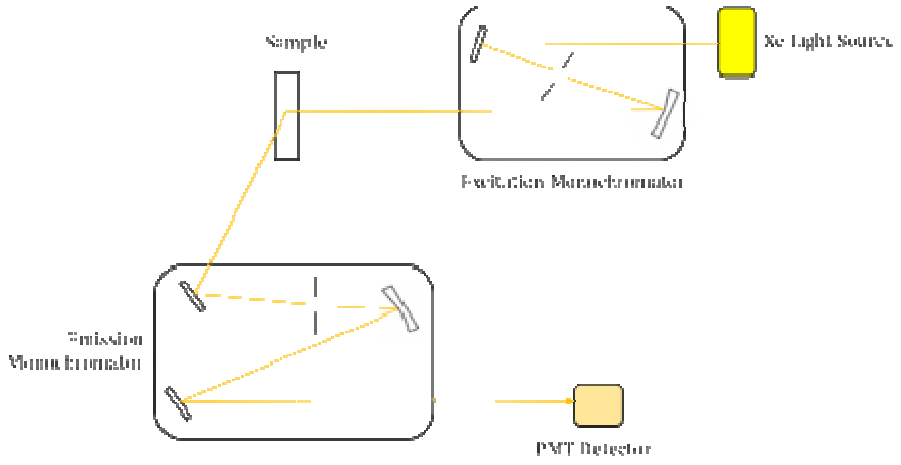
Instrumentation of Fluorescence Spectroscopy

Characteristics of a Fluorescence Spectrum

Fluorometers are composed of an excitation and emission monochromator, allowing users to obtain both excitation and emission spectra. A measurement made by a fluorometer is unique to

the individual instrument's excitation and emission monochromators. Fluorescence is directly related to luminous flux and the efficiency of measurement and therefore dependent on the instrument design and components such as the light source, monochromator optics, and photomultiplier tube. Each light source will have a different spectral output (both shape and power) which will vary and decrease over the lifetime of the source.

Excitation spectra plot the intensity at a fixed emission wavelength while varying the excitation wavelengths. Since most emission spectra are independent of the excitation wavelength, the excitation spectra are frequently duplicates of the fluorophore's absorption spectrum.



Cartoon schematic of a fluorometer.

Conversely, an emission spectrum plot the intensity at a fixed excitation wavelength while scanning through varying emission wavelengths. These emission scans provide information on the molecular structure of the fluorophore and the local environment surrounding it. Since the fluorescence emission always occurs from the lowest excited state to the ground state, the shape of the emission spectrum is independent of the excitation wavelength. More energy is also required to excite a molecule from the ground to the excited state, resulting in emission peaks at longer wavelengths (ie smaller energies) than their corresponding excitation wavelengths. This difference in energy between the excitation and emission wavelengths is known as the Stokes shift.

In addition, absorption and emission spectra are frequently mirror images of one another due to the equal distribution between the vibrational energy levels of the excited and ground states (Figure 3). The Franck-Condon principle explains that because the nuclei are relatively large and the electronic transition involved in emission and absorption occur on such fast timescales, there is no time for nuclei to move and the vibrational energy levels and therefore remain roughly the same throughout the electronic transition.

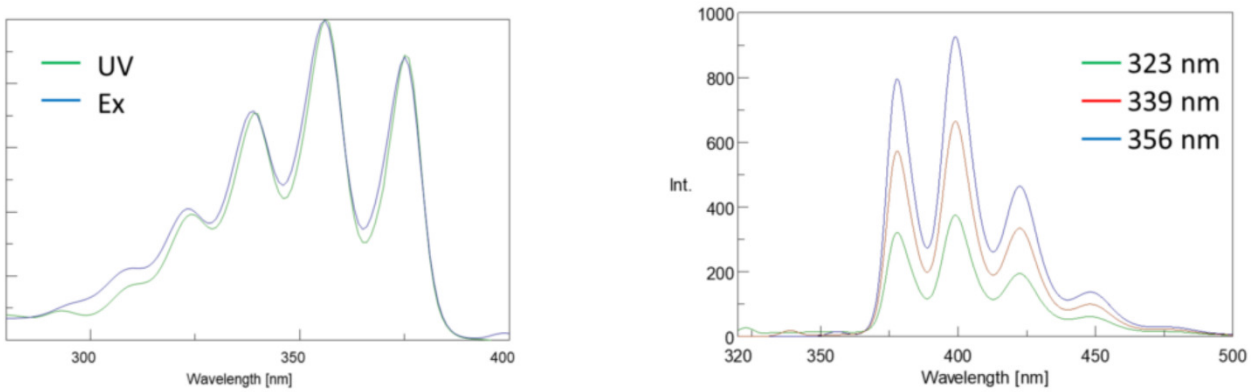


Figure 3. Absorption and excitation spectra (left) and the corresponding fluorescence spectra (right) at different excitation wavelengths.

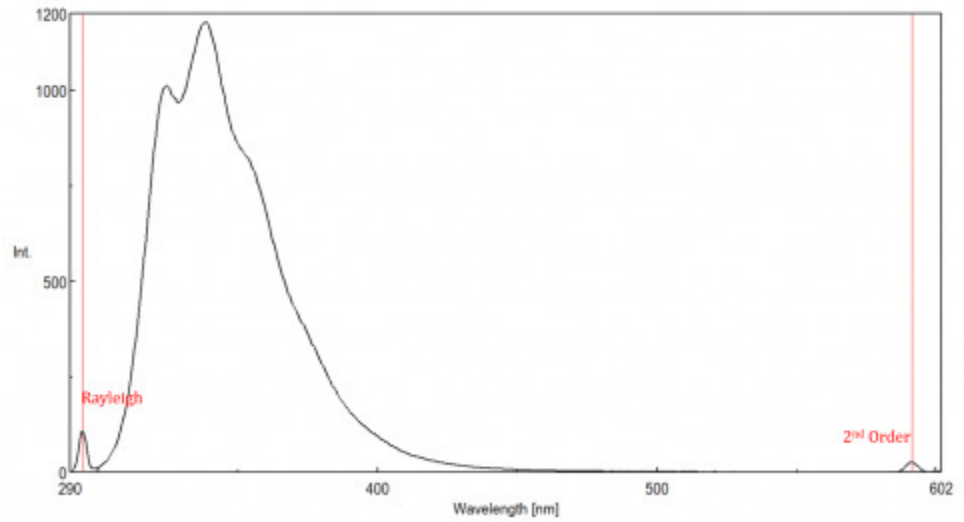
Spectral Bandwidth

Since the fluorescence intensity is proportional to the input light intensity, the amount of light passed through the monochromator will greatly affect the intensity. The sum of the excitation and emission bandwidths should be about the spectral bandwidth (SBW) of the peak being monitored so that all peaks are well resolved. As long as this rule of thumb is followed, the bandwidths can be opened to increase the amount of light throughput for samples with low fluorescence. The SBW can also be impacted by the Stokes shift of the fluorophore. Narrower Stokes shifts may limit the range of acceptable SBWs that can be used.

Fluorescence Artifacts

Scattered light can give rise to artifacts, distorting the fluorescence spectrum. The three most common types of scatter seen in fluorescence are Rayleigh, 2nd order, and Raman scatter (Figure 3). Rayleigh scattering is the scattered excitation light and therefore peaks at the excitation wavelength. 2nd order scatter is higher-order scatter observed at twice the excitation wavelength.

Raman scattering is inelastic scatter due to solvents and peaks at a fixed energy from the excitation wavelength. To differentiate Raman scattering from a fluorescence peak, the excitation wavelength can be varied in 5 to 10 nm increments and if the peak in question shifts with the excitation wavelength and decreases in intensity, then that peak, is due to Raman scatter. You can also check to see if the peak is in the blank solvent spectrum. If it is, there is a chance it is a Raman peak. If the fluorescence peak is too close or overlapping with either the Raman or Rayleigh scatter, the bandwidths and/or excitation wavelength can be adjusted to shift the scatter off the fluorescence peak. These effects are most prominent for very low fluorophore concentrations and especially highly scattering solutions, like proteins, microsphere, nanoparticles, as well as solids.



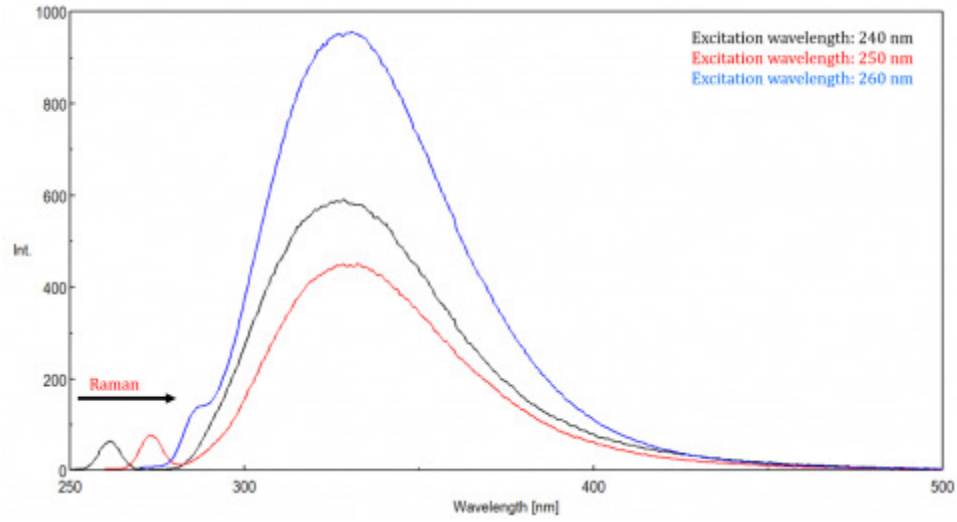
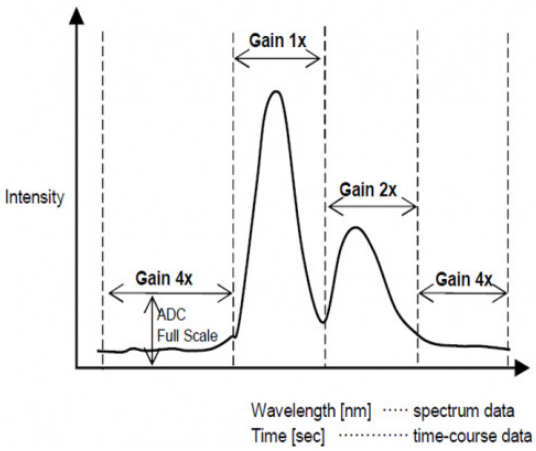


Figure 3. Rayleigh and 2nd order scatter (top) and Raman scatter (bottom) in fluorescence emission spectra.

Dynamic Range

The Automatic Gain Control function automatically adjusts the gain of a signal from the detector based on the fluorescence intensity. This optimizes the signal to noise throughout the entire scanned range for spectral or time course measurements so that peaks with different intensities are automatically adjusted to improve the S/N and assure result accuracy.



Adjustment of peak intensity using the automatic gain control function.

Automatic Sensitivity Control System (SCS)

The Automatic Sensitivity Control System(SCS) expands the dynamic range of the detected fluorescence signal by automatically adjusting the detector voltage according to the fluorescence intensity. This allows for fixed wavelength or quantitative analyses measurements of sub-picomolar to micromolar concentrations without manually changing the instrument.

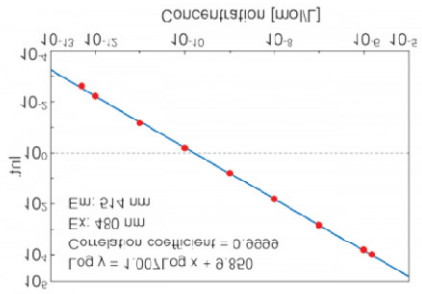
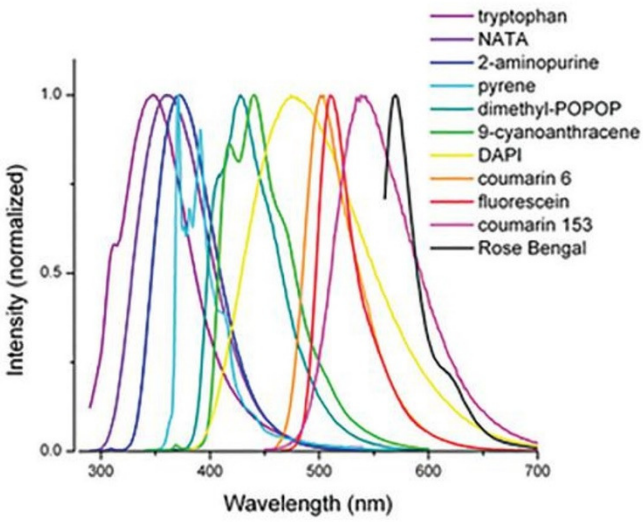


Figure 5. Calibration curve of fluorescein solutions from 5·10-13 to 1.5·10-6 M using the auto-SCS function.

What types of molecules and materials exhibit fluorescence?



Fluorescence emission spectra of some common fluorophores across the UV and visible spectrum

Fluorescent molecules and materials come in all shapes and sizes. Some are intrinsically fluorescent, such as chlorophyll and the amino acid residue tryptophan (Trp), phenylalanine (Phe) and tyrosine (Tyr). Others are molecules synthesized specifically as stable organic dyes or

tags to be added to otherwise non-fluorescent systems. There are entire catalogs of these available. Typically, organic fluorescent molecules have aromatic rings and pi-conjugated electrons in them. Depending on their size and structure, organic dyes can emit from the UV out into the near-IR.

Here are a random sampling of a few common fluorophores that span the UV and Visible range. Some rare earth elements, or lanthanides, have higher electronic orbitals filled, where electrons transition due to metal ligand charge transfers happen between 4f-5d and even 4f-4f orbitals. (Bunzli, 1989) There are many molecules that are luminescent in nature such as a few of the amino acids, chlorophylls, and natural pigments. Others are highly engineered for very specific uses of fluorescence spectroscopy.

A few of the categories of fluorescent molecules and materials are:

- Amino acids (Trp, Phe, Tyr)
- Base pair derivatives (2-AP, 3-MI, 6-MI, 6-MAP, pyrrolo-C, tC)
- Chlorophylls
- Fluorescent Proteins (FPs)
- Organic dyes (fluorescein, rhodamine, N-aminocoumarins and derivatives of these)
- Rare earth elements (lanthanides)
- Semiconductors
- Quantum dots
- Single Walled Carbon Nanotubes (SWCNTs)
- Solar cells
- Pigments, brighteners
- Phosphors

Other molecules and materials such as fluorescent proteins, semiconductors, phosphors, and rare earth elements are among the commonly used fluorescent samples. Polymers with

conjugated aromatics or dienes also commonly have fluorescent properties. Of course, new materials are being created all the time.

Conclusion

Organic Fluorescence is a member of the ubiquitous luminescence family of processes in which susceptible molecules emit light from electronically excited states created by either a physical (for example, absorption of light), mechanical (friction), or chemical mechanism.

Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is a phenomenon termed photoluminescence, which is formally divided into two categories,fluorescence and phosphorescence, depending upon the electronic configuration of the excited state and the emission pathway. 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 process of phosphorescence occurs in a manner similar to fluorescence, but with a much longer excited state lifetime. Although the entire molecular fluorescence lifetime, from excitation to emission, is measured in only billionths of a second, the phenomenon is a stunning manifestation of the interaction between light and matter that forms the basis for the expansive fields of steady state and time-resolved **fluorescence spectroscopy**.

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