

# I Am the Light Beneath Your Eyes...\*

## A Short History of the Discovery and Properties of Fluorescence

*Amrit Krishna Mitra*

**This article forays into the history of fluorescence and discusses its occurrences along with the applications that have evolved over the years.**

### 1. Who Am I

You might have seen me in the sparkles of rubies, emeralds, and the Hope diamond when under short wave UV light. You might have marvelled at me when you noticed a greeneye swim in the water. You might have caught a glimpse of me in the iridescent light-emitting ‘black box’ entertainments and environments of theatres and shrines. Have you ever wondered who or what I am?

### 2. Sites of My Occurrence

Hello, ‘I am fluorescence’. I was discovered in a Mexican tree in 1565 when Nicolás Monardes, a Spanish physician and botanist, discovered a peculiar blue tinge that I added to water contained in cups made from the wood of the Mexican tree. Contemporarily, Bernardino de Sahagún, a Franciscan missionary, also reported the presence of a blue colour proclaiming my powers to be beneficial for the urine. I was also called ‘kidneywood’ (*Lignum nephriticum*) by Charles de L’Écluse because I was considered effective in treating kidney ailments. The chemical compound responsible for my existence is matlanine, which is the oxidation product of one of the flavonoids found in this wood.



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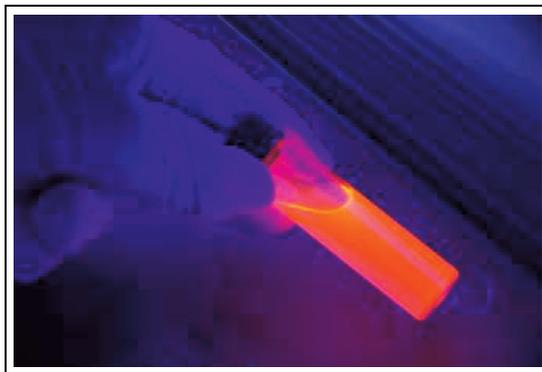
#### Keywords

Fluorescence, UV light, fluorophores, fluorescent dyes, Stokes shift.

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**Figure 1.** Representative solution of an organic fluorophore.



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In my presence, the visible light component – sometimes known as the ‘white light’ – tends to be reflected and perceived normally as colour, while the UV component of light is modified, and ‘stepped down’ energetically into longer wavelengths, producing additional visible light frequencies, which are then emitted alongside the reflected white light. Human eyes perceive these changes as the unusual ‘glow’ of fluorescence (*Figure 1*).

<sup>1</sup>Not the same as bioluminescence.

My type of luminance<sup>1</sup> is significantly different from the natural bioluminescence of organisms such as bacteria, insects, fish and fireflies. This involves no reflection at all, but true living generation of light (via the chemistry of luciferin).

The other wondrous properties that I have include making white light appear yellow when made to pass through an aqueous infusion of Mexican wood. In contrast, I can reflect blue light from the solution.

You might wonder as to why I was restricted to botanical pursuits alone, but you may be pleased to know that more recently, several highly fluorescent glucosyl-hydroxychalcones were isolated from Mexican wood.

Have you heard of lapis solaris? It is a widely known stone oth-



erwise called barium sulphate. Galileo Galilei, after having received inspiration from the work of a Bolognian shoemaker, Vincenzo Casciarolo, confirmed my presence in the famous stone. Galilei said, “It must be explained how it happens that the light is conceived into the stone, and is given back after some time, as in childbirth.”

Recall what I talked about kidneywood a little while ago. My existence was further investigated by Robert Boyle in 1664, when he was inspired by Monardes’s report and investigated this system more fully. He discovered that after multiple infusions, the wood lost its power to give colour to the water. He thus concluded that there was some ‘essential salt’ in the wood responsible for the effect. He also discovered that the addition of acid abolished the colour, and the addition of alkali brought it back.

Now, if you thought that I could limit myself to yellow and blue light alone, you are mistaken, my friend, for how can the colours not multiply when something as ‘moving’ as alcohol is around. And who could brew alcohol better than Mr Brewster himself? David Brewster, in 1833, described that when a beam of white light passed through an alcohol solution of leaves, a red beam could be observed from the side [which was, of course, chlorophyll fluorescence (*Figure 2*)]. He considered the effect due to ‘dispersion’.

John Herschel went a step further by calling me ‘epipolic dispersion’ when he observed me for the first time in quinine sulphate. In 1852, George Gabriel Stokes published his massive treatise *On the Change of Refrangibility of Light*. He initially used the term ‘dispersive reflection’ to describe the phenomenon presented by quinine sulphate. Stokes used a prism to disperse the solar spectrum and illuminate a solution of quinine. He noted that there was no effect until the solution was placed in the UV region of the spectrum.

These observations led Stokes to proclaim that I am of longer wavelength than the exciting light, which led to this displacement being called the ‘Stokes shift’. He also seems to have been the

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– Galileo Galilei on lapis solaris, the stone widely known as barium sulphate.



**Figure 2.** Fluorescence of chlorophyll under UV light.



first to propose, in 1864, of my use as an analytical tool, in a lecture titled, ‘On the application of the optical properties to detection and discrimination of organic substances’.

Note that I did not create this illumination from quinine sulphate alone. I went on to emit light from calcium sulphate also upon being excited in the UV. This was noted by Becquerel where he says that the emission occurs at a wavelength longer than that of the incident light.

In the year 1852, fluorite gave its name to the phenomenon of fluorescence, which is prominent in fluorites from certain locations, due to certain impurities in the crystal.

I owe my origin to fluorite (the mineral form of calcium fluoride,  $\text{CaF}_2$ ). The mineral fluorite was originally termed flourspar, and it belonged to the halide minerals. Fluorite usually crystallizes in isometric cubic manner, often in octahedral and in complex isometric forms. In the year 1852, fluorite gave its name to the phenomenon of fluorescence, which is prominent in fluorites from certain locations, due to certain impurities in the crystal.



Adolph Von Baeyer, a German chemist, synthesized Spiro[isobenzofuran-1(3H), 9'-[9H]xanthen]-3-one, 3', 6'-dihydroxy derivatives in 1871. He apparently coined the name 'fluorescein', from 'fluo' and 'resorcin' (resorcinol), which he reacted with phthalic anhydride.

All these famous scientists have also received several accolades because of the efforts that they have put in for me. Adolph Von Baeyer was awarded the Nobel Prize in Chemistry "in recognition of his services in the advancement of organic chemistry and the chemical industry, through his work on organic dyes and hydroaromatic compounds".

Following this were several incidents which resulted in the discovery and production of various compounds where I happened to be the principal characteristic factor.

In 1856, at the age of 18, William Henry Perkin set out with the idea of making quinine by oxidizing allytoluidine, instead he accidentally produced the synthetic dye – mauve – a derivative of coal tar with an aniline base.

I can be present in the form of paints or dyes emitting the 'glow' that you so marvel at. When I am present in such paints, they are called fluorescent paints.

Fluorescent paints offer a wide range of pigments and chroma which also 'glow' when exposed to the long-wave UV frequencies. These UV frequencies are found in sunlight and some artificial lights. But they and their glowing-paint applications are popularly known as black light and 'blacklight effect', respectively.

There are both visible and invisible fluorescent paints. The visible appear under white light to be any bright colour, turning peculiarly brilliant under black lights. Invisible fluorescent paints appear transparent or pale under daytime lighting but will glow only under UV light and in a limited range of colours. Since these can seem to 'disappear', they can be used to create a variety of clever effects.

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Both types of fluorescent painting benefits when used within a contrasting ambience of clean, matte-black backgrounds and borders. For eg., a ‘blackout’ effect minimizes other awareness and cultivates the peculiar luminance of UV fluorescence. Both types of paints have extensive applications where artistic lighting effects are desired, particularly in black box entertainments and environments such as theatres, bars, shrines, etc. Out-of-doors, however, UV wavelengths are rapidly scattered in space or absorbed by complex natural surfaces, dulling the effect. Furthermore, the complex pigments degrade quickly in sunlight.

### 3. List of Compounds

- H Caro (1874) – eosin.
- C Liebermann (1880) – polycyclic aromatic hydrocarbons.
- Paul Erlich (1882) used uranin (the sodium salt of fluorescein) to track the secretion of aqueous humour in the eye. First *in vivo* use of fluorescence.
- K Noack (1887) published a book listing 660 compounds arranged according to the colour of their fluorescence.

Could one have imagined that one of the first uses of fluorescein could be found from groundwater? In 1877, in a major groundwater tracing experiment in southern Germany using 10 Kilograms of fluorescein, it was found that River Danube and the Rhine are connected by underground streams!

Now, there had to be the classification of compounds and chemical groups having me as a strong property in them. So, R Meyer (1897) used the term ‘fluorophore’ to describe the chemical groups which tended to be associated with fluorescence; this word was analogous to ‘chromophore’ which was first used in 1876 by O N Witt to describe the groups associated with colour.

In 1867, F Goppelsröder introduced the term ‘fluoreszenzanalyse’ and performed the first fluorimetric analysis in history: the determination of Al(III) by the fluorescence of its morin chelate.

Could one have imagined that one of the first uses of fluorescein could be found from groundwater? In 1877, in a major groundwater tracing experiment in southern Germany, it was found that River Danube and the Rhine are connected by underground streams. I was placed in the form of fluorescein in the Danube and about 60



hours later, I appeared as an affluent in the Rhine. 10 Kilograms of fluorescein was used!

Now, naturally, with all these discoveries, one needed to experiment directly with me and thus needed to observe me. Otto Heimstaedt and Heinrich Lehmann (1911–1913) developed the first microscopes for studying my properties as an outgrowth of the UV microscope (1901–1904). The instrument was used to investigate the autofluorescence of bacteria, protozoa, plant and animal tissues, and bioorganic substances such as albumin, elastin, and keratin. Stanislav Von Prowazek (1914) employed the fluorescence microscope to study dye binding to living cells.

In the inorganic field, Nichols and Slattery (1929) reported the first observation of intense fluorescence of uranium in a NaF matrix. Hieger (1930) found that the fluorescence spectrum (measured photographically) of 1, 2-benzanthracene resembled the spectra of some carcinogenic coal tars. Cook and his associates (1933) isolated a few grams of 3, 4-benzpyrene from two tons of coal tar and demonstrated that its fluorescence spectrum was identical to that of the active tars. Much later, Gregorio Weber (1952) synthesized dansyl chloride for attachment to proteins and used polarization to study protein hydrodynamics – these studies initiated the field of quantitative biological fluorescence.

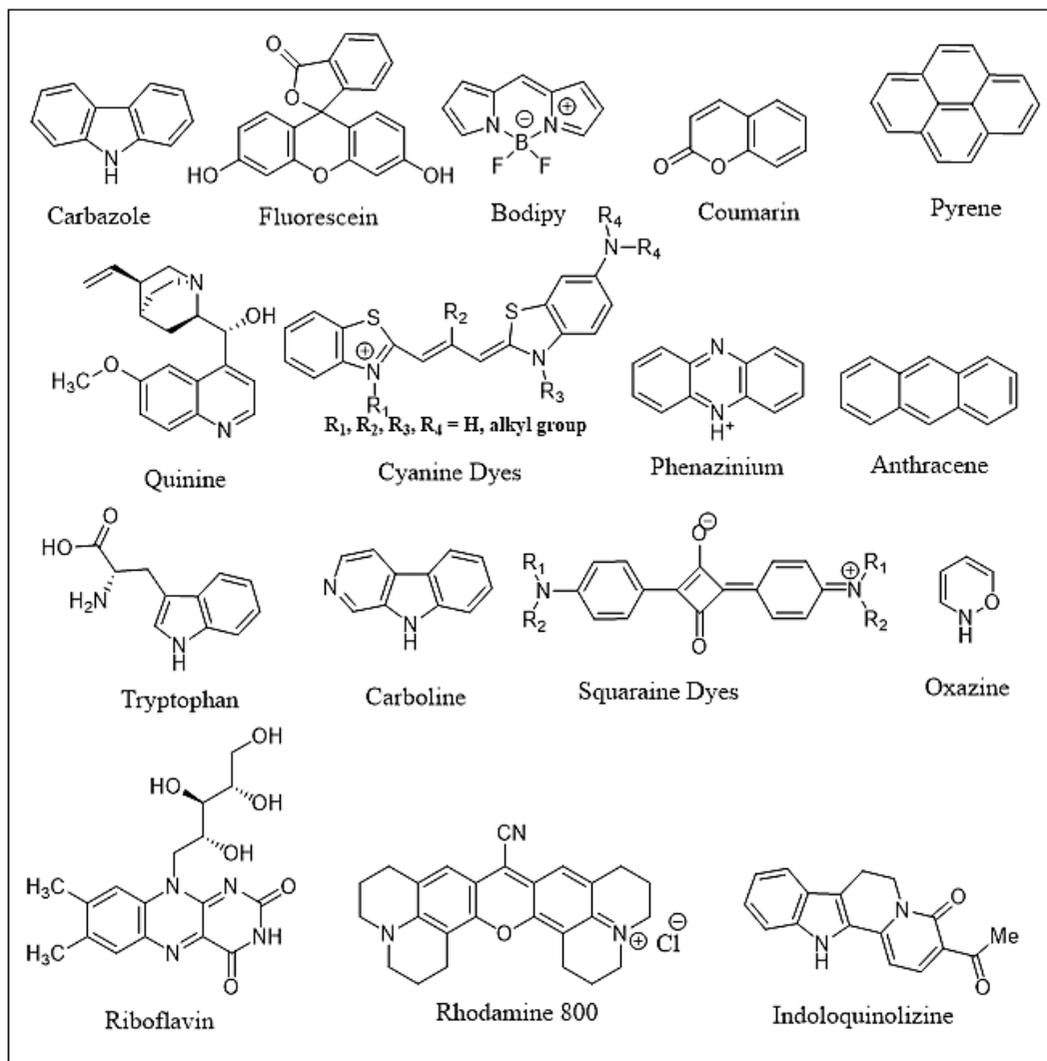
If we now look at the field of drugs, it will be interesting to note that Albert Coons (1941) labelled antibodies with FITC<sup>2</sup>, thus giving birth to the field of immunofluorescence. Brodie and Udenfriend (1943) introduced a simple method for the determination of quinine, and its dextro-rotatory stereoisomer quinidine, in plasma. Saltzman (1948) introduced fluorimetric methods for salicylates in blood. Shimomura, Johnson, and Saiga (1962) discovered the green fluorescent protein in the *Aequorea victoria* jellyfish.

You must be knowing that during World War II, there was a huge breakout of malaria. The US government issued a desperate call to scientists and doctors to find a treatment for malaria. Since Japan had taken over most of the world's supply of quinine – the

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<sup>2</sup>Fluorescein isothiocyanate.





**Figure 3.** List of few reputable organic fluorophores.

best-known treatment – Allied forces in the Pacific theatre needed a new drug, and fast. With an instrument called the fluorometer, Brodie and Udenfriend could measure how much of the drug was in a patient's plasma sample. Much later, the spectrophotofluorometer was devised by Aminco–Bowman (Silver Spring, MD) and Farrand Optical Co. (Walhalla, NY). Several scientists have contributed their lives towards discovering my properties and occurrences.

## Where I Stand in the Modern Days!

In recent times, design and characterisation of small molecule, environment-sensitive fluorophores are extremely significant from the viewpoint of biochemical and biophysical studies. These are extremely precious owing to their capability to probe micro-environments which can decipher bulk information in the study of molecular biology, drug discovery, material science, tissue diagnostics, environmental indicators, enzyme substrates, and cellular staining agents. Although numerous fluorophores are known in the form of coumarins, fluoresceins, cyanines, oxazines, pyrenes, quinines, BODIPY dyes (*Figure 3*), etc., the quest for newer ones is still on as these are extremely essential to visualise a biochemical process. These organic frameworks are particularly beneficial for fluorescent labelling as they encompass a broad array of photophysical properties including molar absorptivity, quantum yield, Stokes shift, lifetime, and ability to respond towards the immediate environment. Generally, fluorescent probes can broadly be divided into two main categories: intrinsic and extrinsic probes. Aromatic amino acids, flavins, coumarins, etc., are examples of intrinsic fluorophores. Many instances can be cited, where the molecules under consideration are non-fluorescent (e.g. DNA, RNA) or their intrinsic fluorescence is inadequate. These are the cases when the molecules are labelled with fluorescent extrinsic probes. Extrinsic probes can be labelled via covalent bonding or through non-covalent interactions. Dansyl chloride or TRITC (tetramethylrhodamine-isothiocyanate) are the reactive dyes, that bind covalently to any reactive amine groups present in a protein. Fluorescent maleimide dyes like rhodamine red<sup>TM</sup> C2-maleimide offer efficient site-specific labelling of proteins via coupling to a cysteine. In some cases, when a molecule is weakly fluorescent or non-fluorescent in water at a certain wavelength region but fluoresces to a considerable extent when bound to the macromolecule, the importance of labelling with a non-covalently associated dye is observed. For example, we can consider ANS (1-anilino-8-naphthalenesulfonate) for protein labelling, DPH (diphenylhexatrienyl) for membrane labelling, and EB (ethidium

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bromide) for DNA labelling. Often, conventional organic fluorophores suffer from poor photostability, narrow absorption spectra, and broad emission features. Here comes the utility of semiconductor nanocrystals (also called quantum dots). They are highly photostable with broad absorption spectra and narrow-size, tunable emission spectra. Recently, advances in the synthesis of these materials have resulted in bright, sensitive, extremely photostable, and biocompatible semiconductor fluorophores. Again, it is worthwhile to mention that salts of certain lanthanides are fluorescent. Many examples can be cited where lanthanum-containing reagents were employed for the fixation of nucleic acid structures. Many complexes of europium, terbium, and samarium were discovered to exhibit unusual luminescence properties when excited by UV light.

Would you like to know more? Following is a list of suggest reading.

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### Suggested Reading

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