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Preface

The field of fluorescence continues to grow steadily, both in fundamental aspects and in applications. For instance, the number of scientific articles published every year that contain the word 'fluorescence' in the title has increased approximately linearly in the last 50 years (ISI data), from 150 in 1960 to 3,200 in 2005. These articles are only a small fraction of the total number of publications. A search with the same keyword 'fluorescence' anywhere in the article yielded nearly 16,000 articles for the year 2005, a high number indeed, and that exceeds the corresponding figure for 'NMR,' another powerful spectroscopy.

The present book, which is the fourth in the Springer Series on Fluorescence, collects articles written by speakers of the *9th International Conference on Methods and Applications of Fluorescence: Spectroscopy, Imaging and Probes* (MAF 9), held in Lisbon, Portugal, in September 2005, along with a few invited articles. The meeting, with more than 300 participants from 33 countries, included 18 plenary and invited lectures.

Current issues related to fluorescence are discussed in the present book, including recent advances in fluorescence methods and techniques, and the development and application of fluorescent probes. Historical aspects and an overview of fluorescence applications are also covered. Special emphasis is placed on the fluorescence of artificial and biological nanosystems, single-molecule fluorescence, luminescence of polymers, microparticles, nanotubes and nanoparticles, and on fluorescence microscopy and fluorescence correlation spectroscopy.

Lisboa, October 2007 Mário N. Berberan-Santos

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Part A
History and Fundamental Aspects

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Early History of Solution Fluorescence: The *Lignum nephriticum* of Nicolás Monardes

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"y pediles, me diesen personas habiles,
y esperimentadas con quien pudiese platicar:
... señalaronme, hasta diez, o doze principales ancianos:
y dixeronme, que con aquellos, podia comunicar"

(I requested able and experimented persons to whom I could enquire:
they presented me up to ten or twelve old learned men:
I was told that I could communicate with all of them.)

Fr. Bernardino de Sahagún.
Historia General de las Cosas de Nueva España (ca. 1575–1577).

Abstract The history of molecular fluorescence is closely associated with the emission from plant extracts. N. Monardes, in his *Historia Medicinal* (Seville, 1565), was the first to describe the blue opalescence of the water infusion of the wood of a Mexican tree used to treat kidney ailments. The strange optical properties of the wood, known as *Lignum nephriticum* (kidney wood), were later investigated by Kircher, Grimaldi, Boyle, Newton and many other scientists and naturalists in the ensuing centuries. However, when G.G. Stokes published in 1852 the first correct relationship between light absorption and fluorescence, his observations were based on the emission of quinine sulphate solution, because in Europe the wood of *Lignum nephriticum* was no longer available and its botanic origin was unknown. An inspection of the works of sixteenth century Spanish missionaries and scholars who compiled information on the Aztec culture, such as Fr. Bernardino de Sahagun and Francisco Hernandez, indicates that pre-Hispanic Indian

doctors had already noticed the blue color (fluorescence) of the infusion of *coatli,* a wood used to treat urinary diseases. Coatli wood was obtained from *Eyserhardtia*, a tree of the family of *Leguminosae*, and is the most likely source of the exotic *Lignum nephriticum*. The wood of *Eysenhardtia polystachya* contains large quantities of Coatline B, a rare *C*-glucosyl-α-hydroxydihydrochalcone. This compound gives rise to a fluorescent reaction product, in slightly alkaline water at room temperature, which is responsible for the blue emission of *Lignum nephriticum* infusion.

1 Introduction

For nineteenth century investigators of optical properties, what we now call fluorescent emission of some plant extracts was a well-known, yet unexplained, phenomenon [1, 2]. The enigmatic color of these solutions and of a few mineral samples (as fluorospar) was sometimes envisaged as *internal dispersion*, because it was considered a peculiar instance of light reflection or scattering. It was G. G. Stokes who in 1852 first introduced the term "fluorescence" in his study of the *internal dispersion* from quinine sulfate solution [3]. This work marked a turning point on luminescence research, because Stokes correctly identified fluorescence as an emission process, due to light *absorption*, and taking place at a frequency lower that the exciting one [4, 5]. In the first lines of this long paper (100 pages plus figures), the reader learns that his research was motivated by previous reports on the quinine emission (a *beautiful celestial blue colour*) from Herschel [6, 7][1] and Brewster [8]. Stokes also checked for fluorescence a large variety of plant extracts, inorganic salts and minerals, his own skin, feathers of several birds, Port and Sherry wines, etc. Surprisingly, an exotic wood used to treat kidney and bladder disorders (*Lignum nephriticum*), which had been for centuries the best-known source of fluorescent solutions, is not even mentioned in this long list of emitting materials. Herschel, on the other hand, included only a brief statement about the different colors from this wood infusion, as observed by transmitted and reflected light, noting that "*I write from recollection of an experiment made nearly 20 years ago, and which I cannot repeat for want of a specimen of the wood*" [6, 7]. The intriguing optical properties of *Lignum nephriticum*, which gave rise to the first published observation of fluorescence (v. infra), were afterwards investigated by Boyle, Newton, Priestley and many other naturalists and philosophers. Nevertheless, they could not be analyzed under the new spectroscopic methods and concepts of the nineteenth century; the wood had vanished from the inventories of British apothecaries and druggists.

[1] Sir John Frederick William Herschel (1792–1871) was the son of William Herschel, the noted astronomer and telescope builder who discovered Uranus. John Herschel, a leading scientist of his day, made important advances in mathematics and astronomy. He was also a pioneer researcher on the chemical processes of photography, discovering the hyposulfite fixing reaction.

Here we wanted to summarize the intricate history of this plant extract, which was to play an important part in the development of fluorescence, delving into the early observations of sixteenth century Spanish scholars that compiled ancient Aztec traditions on medicinal herbs. We also present a preliminary notice of the spectral properties of a water-soluble, strongly emitting compound isolated from the Mexican tree *Eysenhardtia polystachya*, the most likely source of *Lignum nephriticum*. The interested reader is referred to previous reports on many historic aspects related with this plant, and the long search to get its botanical source identified [1, 2, 9–12].

2
Medicinal Botany at Nueva España (Mexico) in the Sixteenth Century: Monardes, Sahagún and Hernández

In 1521 Hernán Cortés finally established Spanish control over the Mexico Valley, the home of the Mexica Indians that later became known as Aztecs. The Mexica civilization had a rich history of using plant medicines for treating all sorts of diseases, and this vast new medicinal knowledge very soon aroused the interest of the Spanish colonizers. Dr. Nicolás Bautista Monardes (1508–1588) was at that time a highly respected medical practitioner in Seville [13], who was also engaged in commercial operations with the New World (Fig. 1). Although he never crossed the Atlantic, he profited from his position at the sole Spanish trade port with America to collect samples of the new plant species, that were used with medicinal purposes in the newly discovered territories, from ship officials, travelers and correspondents. Monardes developed over the years a strong appreciation and first-hand knowledge of the therapeutic applications of many exotic plants, some of them cultivated in his own botanic garden. As a result, he published a book with the first description of the medicinal uses of more than 80 American plant species [14]. In this book, which went through several additions and editions [12, 13], Monardes included a section on a tree of Nueva España used to treat kidney and urinary diseases: *Del palo para los males de los riñones, y de vrina*. In the description of the way the wood infusion should be prepared, Monardes wrote: "*They take the wood and make slices of it as thin as possible, and not very large, and place them in clear spring water, that must be very good and transparent, and they leave them all the time the water lasts for drinking. Half an hour after the wood was put in, the water begins to take a very pale blue colour, and it becomes bluer the longer it stays, though the wood is of white colour*". A second description of this blue coloring property is also provided in another part of the book, as a test to distinguish genuine from fake wood samples.

Monardes' *Historia Medicinal* was a great success and was translated very soon to other languages. An early Latin translation (1574) by the influential Flemish botanist Charles de L'Écluse (1526–1609), in which the wood's name

ELOGIO HECHO POR EL
ILLVST. S. GONC, ALO C, ATI-
eco de Molina, al Retrato del Autor que
ſe vee, en ſu Muſeo.

A 3

Fig. 1 Nicolás B. Monardes (1508–1588), from a wood engraving in his Historia Medicinal (1565–1574)

is given as *Lignum nephriticum* [12], helped to extend awareness of its strange optical properties in Europe. Monardes' brief statement is considered the first published record of a fluorescent emission, but there also exists a much lesser known parallel history that took place on the other side of the Atlantic, in the country of origin of the *Lignum nephriticum*.

Fig. 2 Fr. Bernardino de Sahagún (ca. 1500–1590)

Bernardino de Sahagún (ca. 1500–1590) was a Franciscan missionary that obtained a scholarly education at Salamanca University before departing for Mexico in 1529 (Fig. 2). Sahagún quickly became fluent in Nahuatl, the Mexica language, and was associated through his long life with the Colegio Trilingüe of Santa Cruz de Santiago de Tlatelolco, the first college of higher education of America, established in 1535 by the Viceroy Antonio de Mendoza. The pupils, sons of Indian nobility, in addition to reading and writing in Spanish and Nahuatl, were taught in Latin, logic, arithmetic, music and native medicine. One of the college's Indian teachers of Aztec traditional medicine was Martinus de la Cruz, who authored the earliest American medical herbal book (*Libellus de medicinalibus Indorum herbis* ..., 1552). The manuscript is

illustrated with beautiful color drawings of vernacular plants and was written in Latin by Juannes Badianus, reader in the same college and also "*by race an Indian*"[2]. This fascinating Mexica pharmacopoeia remained unknown until its discovery at the Vatican Library by Dr. Charles U. Clerk in 1929, and is an indication of the interest of the Spanish colonizers in native medicine. One of the recipes briefly records the name of a plant (*cohuatli*) which might be related with that yielding *Lignum nephriticum*, as shown below. Fr. Bernardino, on the other hand, carried out a life-long ambitious ethnologic research, by compiling the description of Mexica history, religion, agriculture, technology and medical practices with the assistance of selected groups of his former native trilingual students. With that purpose, he interrogated, with the help of a carefully designed questionnaire (in Nahuatl), a large number of "informants", old Indians with expertise on each area of knowledge [15].

Sahagún, after many vicissitudes [15], managed to complete (ca. 1575–1577) his great ethnologic work in the form of a richly illustrated bilingual Spanish-Nahuatl manuscript, which he entitled *Historia General de las Cosas de Nueva España*. Unfortunately, the manuscript, known today as the *Florentine Codex*, was never published; in fact, the first facsimile reproduction had to wait three centuries before it was published [16][3]. Those parts of the *Historia General* concerned with native medicine are to be found in Books X and XI of this impressive compilation. Fr. Bernardino was also careful enough to record the names of his sources, old Mexica doctors, who transmitted and revised the original descriptions[4]. One of the plants described by Fr. Bernardino is the *coatli*, which was used to treat kidney diseases and appears two times in the Nahuatl compilation[5]. The first text, which is the most interesting for our purposes here, is shown in Fig. 3, taken from the corresponding page on the *Florentine Codex*, which also contains in a parallel column a curious (non-literal) Spanish translation. In the Nahuatl text, we

[2] This unique manuscript was first printed in facsimile form and supplemented with outstanding studies of its contents and historical background by Emmart EW (1940) The Badianus manuscript. An Aztec herbal of 1522. J. Hopkins Press.

[3] An additional manuscript, known as Codex Matritense (CM) and containing the materials compiled by Fr. Bernardino up to 1558–1559, was discovered in Madrid split between the libraries of the Royal Palace and the Royal Academy of History (RAH).

[4] "This relationship as placed above of the medicinal herbs, and of the other medicinal things contained above, were given by the old doctors of Tlatelolco Santiago, with a large expertise on medicinal things and all of them public healers. Their names and that of the scribe who wrote this follow. And since they don't know how to write, they asked the scribe to place their names here: Gaspar Mathias, vecino de la Concepción; Pedro de Santiago, vecino de Santa Inés; Francisco Symón, vecino de Santo Toribio; Miguel Damián, vecino de Santo Toribio; Felipe Hernández, vecino de Santa Ana; Pedro de Requena, vecino de la Concepción; Miguel García, vecino de Santo Toribio; Miguel Motolinia, vecino de Santa Inés". Florentine Codex (1575–1577) vol III, f 332v–333.

[5] The first description follows: "Coatli, vacalquavitl, memecatic, pipitzavac, piaztic, pipiaztic, pipinqui oltic atic. patli, yoan aqujxtiloni, matlaltic iniayo axixpatli. Noliui, colivi, tevilacachivi, maquixtia mih". CM–RAH, fol 203v and Florentine Codex (1575–1577) vol III, f 266. The second description follows: "Coatli: is a big tree, is broken in pieces and is put into water to be soaked: its juice must be drunken by who has fever or urine retention, because it liquifies the urine". Florentine Codex (1575–1577) vol III, f 291v-333. Translated by Dr. Bustamante J.

are told that "*coatli ... patli, yoan aqujxtiloni, matlaltic iniayo axixpatli ...*", that is, "*coatli ... is a medicine, and makes the water of blue colour, its juice is medicinal for the urine*", showing that the native healers already noticed the unusual optical properties of the *coatli* infusion[6].

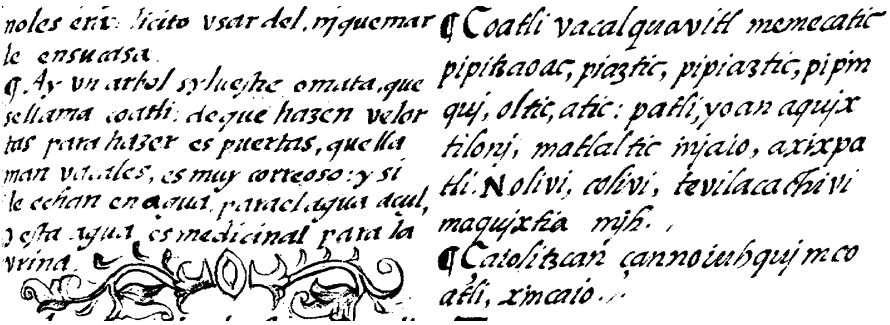


Fig. 3 The Nahuatl text describing *coatli* in Sahagún's *Historia General de las Cosas de Nueva España*, compiled ca. 1558; Florentine Codex, V. III, f. 266

A more elaborated description of *coatli* is provided by Dr. Francisco Hernández (ca. 1515–1587), a learned naturalist and court physician to Philip II [17–19]. The Spanish king commissioned Hernández in 1570 to carry out a 5-year exploration of the natural history and native traditions of New Spain, Peru and Philippines. This was the first scientific expedition in a modern sense, carefully planned and with well-defined tasks as e.g., to undertake "*a survey of herbs, trees and medicinal plants ..., consulting doctors, medicine men, herbalists, Indians and other persons with knowledge in such matters ...*" [19]. An accompanying geographer was expected to map the lands being explored, and local native painters were in charge of drawing plants, animals, minerals, countryside scenes, etc. Hernández spent seven years solely in New Spain and, on his return in 1577, he presented to King Philip six large volumes of Latin text and 10 of paintings, describing more that 3000 plants, 400 animals and 35 minerals, together with collections of living plants and animals, seeds, herbaria, maps, etc. This *Natural History of New Spain* was never published; the manuscripts were reduced to ashes in the great fire of El Escorial in 1671. Fortunately, parts of the work of Hernández were finally printed in Rome and Mexico City in the 17th century (for a detailed account, see [17, 18, 20][7]). In addition, several of Hernández's manuscripts, including his personal fair copy of the Natural History, have been preserved [20]; in this

[6] The number of colors which can be identified in the visible spectrum is, of course, different in every culture. Fr. Bernardino included in his encyclopedic work a description of the pigments and dyes used by the Mexicas. According to his Nauhatl text, the *matlaltic* color would be similar to our turquoise blue and was made from the flowers of a plant (matlalin) whose taxonomy is unclear (translated by Dr. Bustamante J).

[7] Copies of the Hernández manuscripts were kindly made available to us by Dr. J. Bustamante.

last manuscript, the author refers to three *coatli* plants, but only in one of them the blue color property is mentioned (Fig. 4). This specific plant is described as *coatli or water snake*[8] and, after a brief botanical characterization, Hernández goes on to say: "*The water in which chips of this wood have been soaked for some time takes a blue colour and on drinking refreshes and washes the kidneys and bladder*", and later on: "*This wood is being taken to the Spaniards for quite a long time, to whom it produced great admiration to see how the water is instantly tinged of a blue colour*". The author also adds that the infusion has been tried upon himself in various occasions.

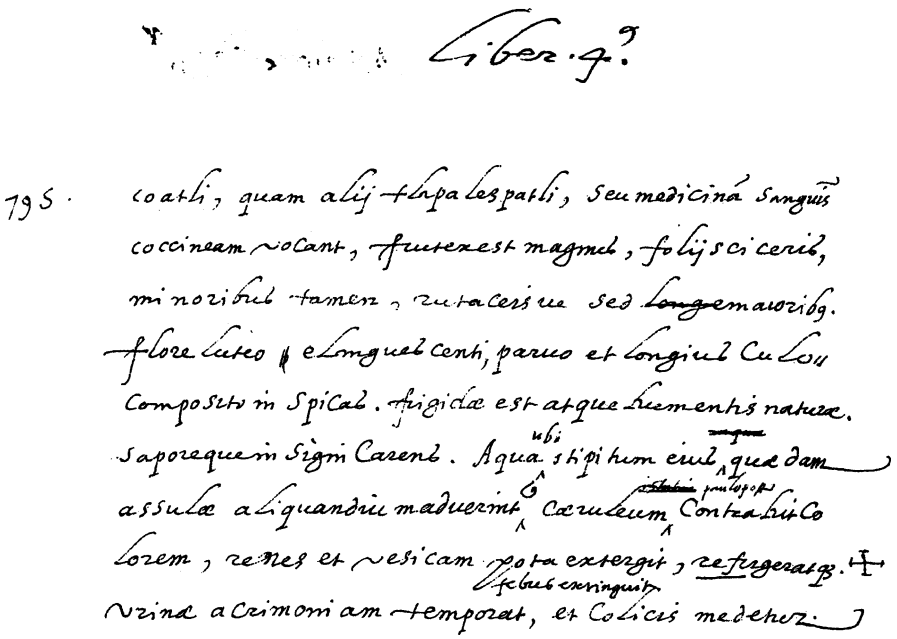


Fig. 4 Handwriting of Dr. Francisco Hernández, ca. 1574, describing *coatli* and remarking the blue colour (*caeruleum*) of its infusion. *De Historia Plantarum Novae Hispania, Liber Quartus,* Ms. 22436, Biblioteca Nacional, Madrid, Spain

Hernández was very likely aware of Monardes small but successful treatise, and it is known that he had access to Fr. Bernardinos manuscripts during his stay in Mexico City. Both, the learned naturalist and the Franciscan anthropology pioneer knew that *coatli* was the source of *Lignum nephriticum*. However, the unfortunate fate of their corresponding great compilations on the New Spain Natural History, which never went to print, contributed to the struggle in tracing the botanic source of the wood by later European botanists.

[8] Hernández was fluent in Nahuatl to such an extent that he left in Mexico a copy (now lost) of part of his manuscripts in this language. For unknown reasons he translated *coatli* as *water snake*; the correct translation is "vara medicinal" (*medicinal stick*). Bustamante J, personal communication.

3
Changing Perspectives: Kircher, Boyle and Newton

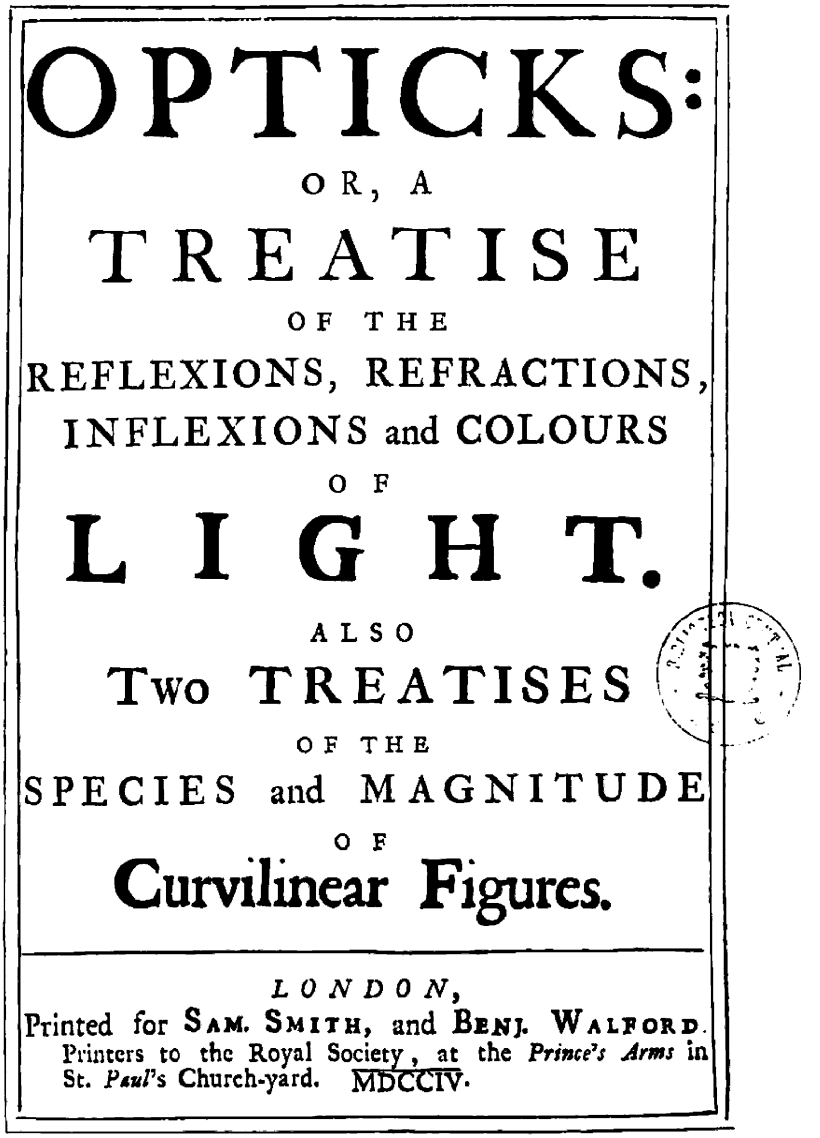
With the advent of the work of Galileo and Newton in the seventeenth century, science in the world was completely transformed. The foundation of scientific societies, such as the Royal Society of London, established in 1660, and the regular publication of research journals, such as the *Philosophical Transactions* of the Royal Society, heralded the dawn of modern science. From the many studies of the fascinating colors of *Lignum nephriticum* in this epoch [1, 9–12], we have selected those of Kircher, Boyle and Newton, which illustrate well the profound changes in the progress of science. At the beginning of the century, the learned Jesuit Athanasius Kircher (1601–1680) published a vivid description of the many colors of the wood's infusion in his *Ars Magna Lucis et Umbrae* [21][9]. Kircher obtained the emitting solution pouring clear water on a cup made of the wood, which was a gift from the procurator of the Jesuits in Mexico. On his account, which was more detailed than those of the early Renaissance observers, Kircher reproduced parts of the *coatli* brief botanic description of Hernández. Later on, in the same optics treatise, he advanced the first explanation of the wood's fantastic colors (*collores illo phantasticos*). Interestingly, Kircher realized ([21], p 176) that the infusion colors became more intense in basic solution (*Cum enim dictum lignum sale ammoniaco* [ammonium chloride] *turgeat*), and from this he concluded that the seeds of all colors were present in the ammonium salt. In fact, Kircher was not very convinced with this involved explanation because he declared his willingness to subscribe a better interpretation, if ever found.

The approach of Boyle (1627–1691) to the same problem was completely different, and we concur with Stapf [9] in considering his account in *Experiments and Considerations touching Colours* [22] as the first scientific description of fluorescence. In this detailed analysis of the infusion colors, Boyle revised previous observations from Monardes and Kircher, reproducing the critical parts of their texts. In addition, he provided a discussion on the origin and morphology of the samples of *Lignum nephriticum*, and stated the descriptive character of his observations[10]. Still, he did not claim to have found a physical explanation of the "*deep and lovely ceruleous colour*" of the infusion. An important contribution from Boyle's experiments is the study of the sensitivity of the infusion fluorescence to the solution pH, noting that the intensity of the blue color can be completely quenched in acidic solutions. After checking that the fluorescence can be restored by adding alkalis, he writes: "*I have hinted to you a New and Easie way of Dis-*

[9] There is an earlier edition, published in Rome in 1646. See ref [9] and [12].

[10] "And I confess that the unusualness of the Phaenomena made me very sollicitous to find out the Cause of this Experiment, and though I am far from pretending to have found it, yet my enquires have, I supose, enabled me to give such hints …". See ref [22], p 203.

covering in many Liquors (for I dare not say in all) wether it be an Acid or a Sulphureous [alkaline] *Salt*" ([22], p 213). This is, probably, the first description of the analytical application of a fluorescent indicator. Boyle



OPTICKS:

OR, A

TREATISE

OF THE

REFLEXIONS, REFRACTIONS,

INFLEXIONS and COLOURS

OF

LIGHT.

ALSO

Two TREATISES

OF THE

SPECIES and MAGNITUDE

OF

Curvilinear Figures.

LONDON,

Printed for SAM. SMITH, and BENJ. WALFORD.

Printers to the Royal Society, at the *Prince's Arms* in St. *Paul's* Church-yard. MDCCIV.

Fig. 5 Title page of Newton's treatise *Opticks*, London 1704

himself used the infusion emission as a test of solution acidity in later experiments [23].

Newton (1643–1727) was also well familiarized with *Lignum nephriticum's* unusual color play, which is referred to in some of his published work. In the first exposition of his color theory [24], Newton was already convinced that he understood the physical basis of the strange optical effects of the wood's infusion, and said: *"those are substances apt to reflect one sort of light and transmit another"* ([24], p 3084). In his influential treatise on optics (Fig. 5), Newton described an experiment in which the infusion was sequentially illuminated with light from which either the red or the blue parts of the spectrum were suppressed [24, 25], to conclude in classifying the blue emission as a kind of reflection. It has been pointed out [12] that this "explanation" was not different from that previously proposed by another Jesuit father, F.M. Grimaldi (1618–1663), professor of mathematics at Bologna, in the same book where he announced his discovery of light diffraction. Newton's misinterpretation of the phenomenon was reproduced without being questioned by later investigators, and the scarcity of the wood samples probably put an end to further experimental quest on the source of its luminescence.

4
The Search for the Botanic Source of *Lignum nephriticum*

Lignum nephriticum is not, obviously, a proper botanical term, but only denotes a plant remedy. When the wood ceased to be used as a drug, that name could no longer be associated with any known botanical species, at least in Europe. The source of the wood became a mystery among European botanists of the 17th–19th centuries, until it was identified after years of research at the beginning of the past century by Stapf [9] and, later, by Safford [11] as the wood of the Mexican tree *Eysenhardtia polystachya* (Ortega) Sarg. Silva:3:29 (1982) (Figs. 6 and 7).

The actual situation in Mexico must have been quite different. A large part of current Mexican folk medicine incorporates the Aztec heritage [26][11], and Mexican popular medicine men (*curanderos*) and peasants have been using *coatli* for centuries for the same therapeutic applications as those indicated by Sahagún, Hernández and Monardes[12]. Thus, Mexican professional natural-

[11] There is an abundant bibliography dealing with Aztec medicine, which dates back to the second half of the sixteenth century. See e.g., the detailed account of Emmart EW in footnote 2.

[12] Several references to the wood as *palo dulce* (*sweet wood*) or *taray* appear e.g., in the recipes of the self-care manual of Esteyneffer J (1712) Florilegio Medicinal, Edition of Anzures MC, 2 Vols. Acad Nac Medicina, Mexico, 1978. The ancient name of the plant (*coatli*) is still used as *cuate* or *cuatle* in several Mexican regions, see Carmona ML, Estudio anatómico, morfológico y etnobotánico de algunas maderas de importancia medicinal en Mexico. Professional Thesis, UNAM, México, 1992. Wood samples can be obtained today from Mexico City plant vendors, who recommend it for bladder diseases and refer to it sometimes as *blue wood* (!)

Fig. 6 *Eysenhardtia polystachya* (Ortega) Sarg. Silva:3:29 (1982), the source of *Lignum nephriticum*

ists and botanists never lost sight of the plant source of *Lignum nephriticum*. For example, Oliva, a Mexican Professor of Pharmacology, noted in 1854 that the "*cuate* (*also Palo Dulce, Taray, Leño Nefrítico*)" corresponds to *Viborquia polystachya*, an old name of *Eysenhardtia* [27].

Fig. 7 Leaves and flowers of *Eysenhardtia polystachya*

The genus *Eysenhardtia* (*Leguminosae*) comprises at least 12–15 species, from shrubs to large trees of 20 m in height[13], although only few of them have been tested for fluorescence. It is very common in several parts of Central America and the SW of the United States, even forming forests in some places[14]. The fortuitous duplication of its botanic description with different names[15] and the noted large variability among species [11] may have rendered it more difficult to identify the true source of *Lignum nephriticum*[16].

[13] Sousa M, Cruz R (2005) Institute of Biology and Botanic Garden, UNAM, Mexico, personal communication.

[14] Cruz R, UNAM, Mexico, personal communication.

[15] *Eysenhardtia polysachya* was first described in 1798 by the Spanish naturalist Gómez Ortega C as *Viborquia polystachya*, from a tree grown in the Madrid Royal Botanic Garden from seeds taken from Mexico. Ortega's name was replaced by the present one, proposed much later (1823) by Humboldt, Bonpland and Kunht in their description of the species (see [11] for details). None of these naturalists noted the wood's fluorescent property nor its relationship with *Lignum nephriticum*.

[16] In fact, Safford suggested that Monardes' fluorescent wood sample was from a large Philippine tree (a *Pterocarpus*) and that a Mexican *Pterocarpus* was also used as a source for *Lignum nephriticum*. The Philippine origin is very unlikely, because regular traffic between New Spain and these islands (the "Manila galleon") started after 1565.

5
The Fluorescent Components of *Lignum nephriticum*

Fluorescent pigments are, of course, ubiquitous components of the vegetal world. In the case of *Lignum nephriticum*, its notoriety was due to the large concentration and easy water solubility of the wood's blue fluorescent dyes. The tree water extract also contains dyes emitting in the yellow spectral range that would not be discussed here. As far as we are aware, the first fluorescent compound isolated from *Eysenhardtia polystachya* was reported in 1978 as a water-soluble glucoside of unknown structure [28]. The tree wood water extract was later investigated by Beltrami and coworkers [29], who isolated two major components (ca. 0.5% dry weight), Coatline A and Coatline B (Fig. 8), with a *C*-β-glucopyranosyl-α-hydroxydihydrochalcone structure. In this work, the fluorescent properties of these compounds were not discussed, although the authors referenced Boyle's work and Safford's paper [11]. Shortly after that, it was claimed [30][17] that Boyle's fluorescent indicator had been finally identified from *Eysenhardtia*, in the form of the compound 7-hydroxy-2′,4′,5′-trimethoxyisoflavone. Apparently, this compound was isolated from a fraction of plant products first solubilized in petrol and, therefore, it is very unlikely that it could be the highly water-soluble dye from *Lignum nephriticum*. Later work on *Eysenhardtia* wood and bark components was motivated by its presumed medicinal applications [31, 32], and no attention was given to fluorescent compounds.

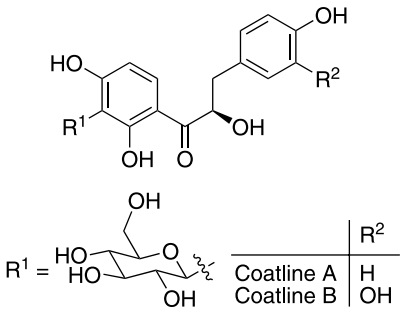


Fig. 8 Molecular structure of the non-emitting *C*-β-glucopyranosyl-α-hydroxydihydrochalcones Coatline A and B, first isolated from *Eysenhardtia polystachya* by Beltrami and coworkers [29]. In a mild alkaline solution, Coatline B yields a strongly blue-fluorescent reaction product, L

We report here the results of a preliminary study of the complex spectral properties in water solution of Coatlines A and B, isolated from *Eysenhardtia polystachya*; a more extended account will soon be published elsewhere[18].

[17] The way the isoflavone was isolated from the wood is far from clear.

[18] Acuña AU, Amat-Guerri F, in preparation.

Since these experiments were prompted by the historical relevance of these compounds, special attention is given only to spectral features that appear in the visible spectrum. Coatline A absorption spectrum in water solution changes in a reversible way from slightly acidic (λ_{max} 282 and 320 nm, pH 6) to basic solution (λ_{max} 258 and 338 nm, pH 10). Both forms are essentially transparent and non-fluorescent in the visible range.

Coatline B is also non-fluorescent in acidic water solution, and its absorption spectrum is similar to that of the A analog. However, on mild alkalinization, a series of slow spectral changes take place, which finally result in the irreversible formation of a strongly blue-emitting species, L. The new compound L presents an intense absorption at 429 nm (pH 8–10), with and absorption coefficient much larger than that of the original Coatline B. As a result of that, the water solution takes a golden yellow color. This new species L shows (Fig. 9) the characteristic intense pale-blue fluorescence (λ_{max} 466 nm) of the wood's infusion, with an emission quantum yield

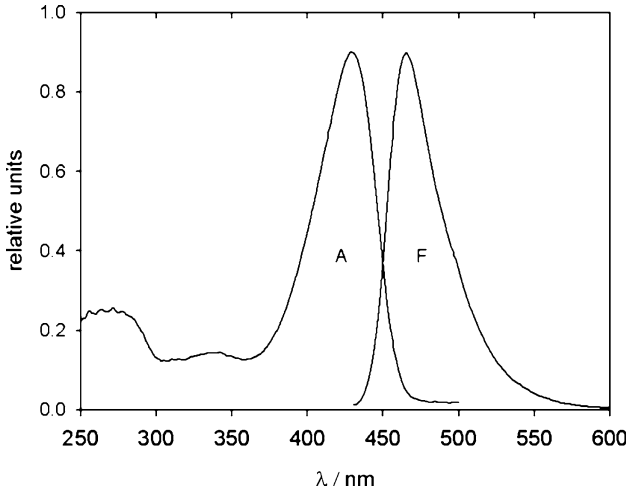


Fig. 9 Absorption and corrected emission spectra (water, pH 10) of Coatline B reaction product L, that yields the blue fluorescence of *Lignum nephriticum*

in the 0.8–1.0 range. The fluorescence is completely quenched on acidification. Obviously, the full identification of the molecular structure of L would require much more detailed studies. Nevertheless, it is clear that the blue fluorescence of *coatli-Lignum nephriticum* infusion is due to its large contents of the water-soluble *C*-glucosyl hydroxydihydrochalcone Coatline B, and the later conversion of this glucoside to the strongly emitting compound L[19]. The

[19] The wood of *Eysenhardtia polystachya* contains additional glycosides with the same hydroxydihydrochalcone group as Coatline B, see [32]. It seems very likely that these compounds also contribute to the formation of blue-fluorescing products.

fact that the blue emission is largely enhanced in alkaline water was also an additional complicating factor in the search for the true source of *Lignum nephriticum*. The *C*-glucose group of Coatline B explains its large water solubility, as well as one of the wood's contemporary popular names (*sweet wood)*.

6 Conclusions

The ancient pre-Hispanic Aztec doctors first noticed the bluish color (fluorescence) of the infusion of the *coatli* wood, meaning *medicinal stick*, which was used to treat kidney and bladder disorders. This early description has been preserved by Fr. Bernardino de Sahagún in his monumental *Historia General de las Cosas de Nueva España*. In 1565 Nicolás B. Monardes first published in Europe the medicinal usage and the unusual optical properties of this wood, that was known since then in the Old World as *Lignum nephriticum*.

The original source of *coatli-Lignum nephriticum* is, most likely, a tree of the genus *Eysenhardtia*, which is well distributed in Mexico and other regions of Mesoamerica. The infusion of the wood of *Eysenhardtia polystachya* contains a large amount of water-soluble fluorescent compounds. The intense blue fluorescence observed in water extracts of *E. polystachya* is due to the presence of the *C*-glucosyl hydroxydihydrochalcone Coatline B, first isolated from this tree by Beltrami and coworkers [29]. The glucoside is converted to a strongly blue-emitting compound in slightly alkaline water solution[19]. The multiple color effects that attracted the attention of many investigators over centuries result from the combination of an intense absorption in the visible range (429 nm) and a very large emission yield in the blue spectral range (466 nm) of the Coatline B reaction product.

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Autónoma, Mexico) in our botanic pursuit. Ramiro Cruz guided one of us (AUA) to collect *Eysenhardtia* branches and provided the tree images shown in this work (Figs. 6 and 7), and Silvia Salas (SERBO, Oaxaca) even set up an expedition to procure for us the wood of *Pterocarpus acapulcensis*. We are also indebted to Benjamín Rodríguez (Institute of Organic Chemistry, CSIC) for isolating pure samples of Coatline A and B from *Eysenhardtias* wood, and to Marisela Vélez for procuring "sweet wood" from Mexico City herb markets. We also thank Puri Morcillo (Institute of Organic Chemistry, CSIC) for laboratory assistance, Guillermo Bernabeu (Institute of Physical Chemistry, CSIC) for skilful help in the spectroscopic experiments, and, last but not least, to Don Carlos López-Bustos for his valuable suggestions at the early stages of this research. Figure 3 was taken from the facsimile edition of the Florentine Codex [16], ms. Laur. Med. Palat. 220, Biblioteca Medicea Laurenziana, Firenze, and is reproduced with permission from the Ministero per il Beni e le Attività Culturali (Italy). Permission to reproduce Fig. 4, from ms. 22436, was granted by the Biblioteca Nacional, Madrid (Spain). Work financed, in part, by Project BQU 2003/4413, from the Spanish Ministry of Education and Science.

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From Well-Known to Underrated Applications of Fluorescence

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Abstract Molecular fluorescence is extensively used in physical, chemical, material, biological, and medical sciences as a tool for detection/analysis, visualization, investigation of local properties, diagnosis, etc. In fact, fluorescent compounds can be used not only for mere visualization, but also as probes, indicators, sensors, and tracers for providing

information on local concentrations of ionic or neutral species, and on the structure and dynamics of matter or living systems (see B Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, 2002). New compounds with improved characteristics in terms of sensitivity, selectivity, and photochemical stability appear almost daily.

The present review does not intend to be exhaustive: some applications of fluorescence relevant to fundamental and applied research will be illustrated with pertinent examples. In addition, technological or industrial applications will be exemplified.

1
Early Applications of Fluorescence

In 1565, Nicolas Monardes reported the first observation of fluorescence[1]: he described the bluish color of an infusion of wood *Lignum nephriticum* under certain conditions of observation (see the article by U. Acuna in this book). He wrote: "Make sure that the wood renders water bluish, otherwise it is a falsification. Indeed, they now bring another kind of wood that renders the water yellow, but it is not good, only the kind that renders the water bluish is genuine." Therefore, such a method for the detection of a counterfeited object can be considered as the very first application of fluorescence.

Another old application is the fluorescent tube. In 1857, Edmond Becquerel[2] was the first to conceive the idea of coating the inner surface of an electric discharge tube with luminescent materials. Such tubes are similar to the fluorescent tubes that are made today. In fact, the inner coating is nowadays made of Eu^{II}, Eu^{III}, and Tb^{III}, so that addition of blue, red, and green light yields white light.

Fluorescence has long been used as an analytical tool for the determination of the concentrations of various species, either neutral or ionic. What are the early applications of fluorescence with this aim? The answer can be found in the famous book *History of Luminescence* by E. N. Harvey [1]: the first paper was published by Victor Pierre [2] who was a professor in Prague, and later in Vienna. In a series of papers, he studied solutions of single fluorescent compounds and mixtures. He noticed that bands of fluorescent spectra were characteristic of a particular substance. He noted also the effect of solvent and acidity or alkalinity.

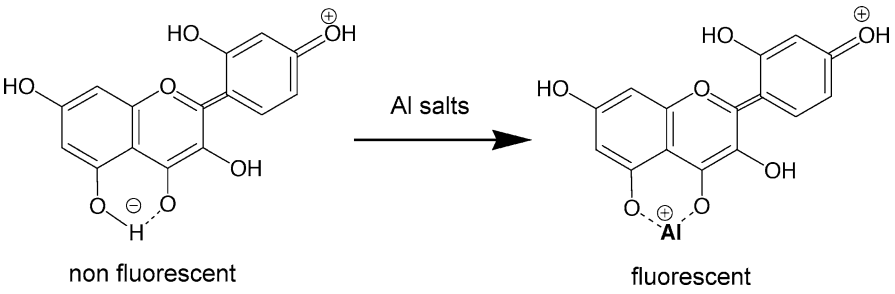
G. G. Stokes certainly had the same idea in mind. In fact, he lectured "On the application of the optical properties to detection and discrimination of organic substances" before the Chemical Society and the Royal Institution in 1864. The content of this lecture is likely to have been quite general and not

[1] The term fluorescence was not known at that time. It was introduced by G. G. Stokes in 1851 in the seminal paper "On the refrangibility of light".

[2] Edmond Becquerel is the father of Henri Becquerel who discovered radioactivity. Edmond Becquerel invented the famous phosphoroscope that bears his name. He was professor at the Museum National d'Histoire Naturelle and at the Conservatoire Impérial des Arts et Métiers in Paris.

restricted to fluorescence. In 1883, 1884, and 1885, Stokes gave his famous Burnett lectures "On light" [3], and one of the topics of Lecture I in the second course entitled "On light as a means of investigation" is "Fluorescence—its use as a means of discrimination". One can read on page 154: "... the observation of fluorescent substances in a pure spectrum exhibits features by which they may be followed and detected in spite of other substances even in large quantity". However, no specific example was given in the text.

A well-known application of fluorescence to analysis was reported by Göppelsröder in 1868 [4, 5]: the complexation of morin (a hydroxyflavone derivative) with aluminum is accompanied by a dramatic enhancement of fluorescence intensity (Scheme 1), thus offering a straightforward way to detect this metal. It was the first time that the term *fluorescence analysis* was employed.



Scheme 1

Among the old applications of fluorescence, it is worth mentioning that uranin (the disodium salt of fluorescein) was used for the first time in 1877 as a tracer for monitoring the flow of the Danube. On all maps, it is shown that the Danube springs in the Black Forest and, after many hundreds of kilometers, flows into the Black Sea. But there are several sinks (swallow holes) in the bed of the Danube. The biggest one is near Immedingen. Ten liters of a concentrated solution of uranin were poured by Knop into the bed of the upper current of the Danube, and 50 hours later, the fluorescence could be observed in the water of the river Aache 12 km to the south. This river flows into Lake Constanz that feeds the Rhine. Therefore, only a small part of the water from the Danube spring arrives at the Black Sea. Most of it flows into the North Sea! Nowadays, fluorescence tracing is currently used in hydrology, especially to simulate pollution.

At the beginning of the twentieth century, numerous applications of fluorescence were developed and reported in several books. For instance, in the second part of the book by Radley and Grant [6], the list of applications using fluorescence analysis is impressive (Fig. 1). Dake and De Ment also described interesting applications [7]. Dake was so fond of fluorescence that he faced

FLUORESCENT LIGHT
and Its Applications

INCLUDING LOCATION AND PROPERTIES OF FLUORESCENT MATERIALS . . . A THEORETICAL AND PRACTICAL EXPOSITION OF FLUORESCENCE AND SIMILAR PHENOMENA.

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Fig. 1 Contents of the book by Radley and Grant published in 1933

his fireplace with fluorescent minerals that were illuminated by a UV lamp attached to the ceiling. In their famous book, Pringsheim and Vogel [8] gave various examples of application, including fluorescent carpets and ceilings in theaters, or fluorescent ballets (Fig. 2).

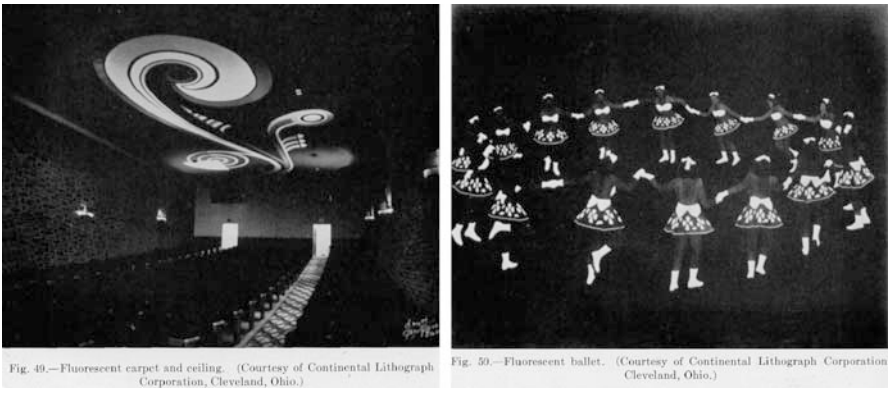


Fig. 49.—Fluorescent carpet and ceiling. (Courtesy of Continental Lithograph Corporation, Cleveland, Ohio.)

Fig. 50.—Fluorescent ballet. (Courtesy of Continental Lithograph Corporation Cleveland, Ohio.)

Fig. 2 Photographs published in the book by Pringsheim and Vogel published in 1943

After these historical aspects, let us give some examples of well-known and underrated applications of fluorescence that are currently used today.

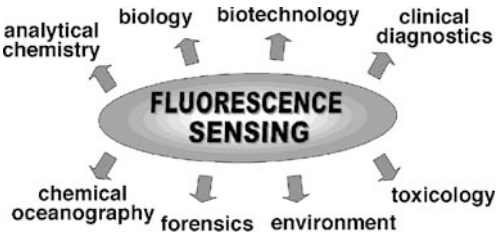
2
Monitoring of Excited-State Processes

Steady-state and time-resolved fluorometries are widely used to determine the rates of photoinduced electron transfer, proton transfer, and energy transfer in artificial or living systems. In fact, the time constants of these processes fall into the experimental time window around the excited-state lifetime, so that analysis of the fluorescence decays allows one to calculate the rate constants [9]. This well-known use of fluorescence will not be further discussed.

3
Fluorescence Sensing

Sensing is one of the most important applications of fluorescence, as confirmed by the recent special issue of the *Journal of Materials Chemistry* devoted to fluorescent sensors [10], and the book *Optical sensors* [11] in which fluorescence appears to play a great role.

The extensive use of fluorescence sensing in many fields (Scheme 2) can be explained by the distinct advantages of this technique in terms of sensitivity, selectivity, response time, local observation under the microscope, and remote detection by means of optical fibers.



Scheme 2

3.1
Fluorescent Molecular Sensors

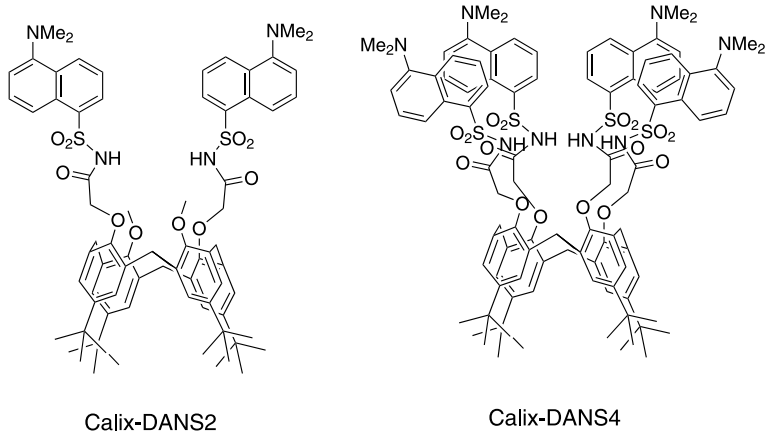
The design of a fluorescent molecular sensor that is selective of a given analyte is actually a work of molecular engineering that involves many disciplinary fields: photophysics, photochemistry, analytical chemistry, physical chemistry, coordination chemistry, and supramolecular chemistry [9, 12–14].

Numerous fluorophores have been used for fluorescence sensing: naphthalene, anthracene, pyrene, aminonaphthalimide, diaminonaphthylsulfonyl, coumarins, fluorescein, eosin, rhodamines, benzidine, alizarin, seminaph-

thofluorescein, oligo- and polyphenylenes, porphyrins, ruthenium complexes, etc. The number of analyte targets is still increasing with much progress in sensitivity and selectivity:

- Cations: H_3O^+ (pH), alkali, alkaline earth, transition, and post-transition
- Anions: fluoride, chloride, carboxylates, phosphates, ATP, nitrates, etc.
- Molecules: hydrocarbons, amino acids, sugars, urea, ammonia, amines, alcohols, O_2, CO_2, H_2O_2, etc.

As an illustration of selectivity in fluorescence sensing, the detection of toxic metal ions in the environment will now be briefly presented. Calix[4]arenes bearing two or four dansyl fluorophores, called Calix-DANS2 and Calix-DANS4, respectively (Scheme 3), exhibit outstanding complexing abilities [15]. Calix-DANS2 shows a high selectivity toward Hg^{2+} over interfering cations (Na^+, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}) and a sensitivity in the 10^{-7} mol L^{-1} concentration range. The complexation of Hg^{2+} induces a strong fluorescence quenching due to a photoinduced electron transfer process from the fluorophore to the metal center. Calix-DANS4 exhibits an extremely high affinity for Pb^{2+} with a high selectivity over various competing ions (Na^+, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+}). The unprecedented detection limit (4 mg L^{-1}) is fully compatible with the level defined by the World Health Organization. The affinity of Calix-DANS4 for Pb^{2+} can be rationalized by the activation of the inert pair of electrons on Pb^{2+}.



Calix-DANS2 Calix-DANS4

Scheme 3

Calix-DANS2 can be chosen to illustrate the importance of immobilization of the molecular sensor when designing a sensing device. Calix-DANS2 was grafted on a large-pore mesoporous silica material (via two long alkyl chains containing triethoxysilane groups). Addition of mercury ions to water results in fluorescence quenching, and the good selectivity is maintained, as shown by the relative variation in fluorescence intensity that starts at higher

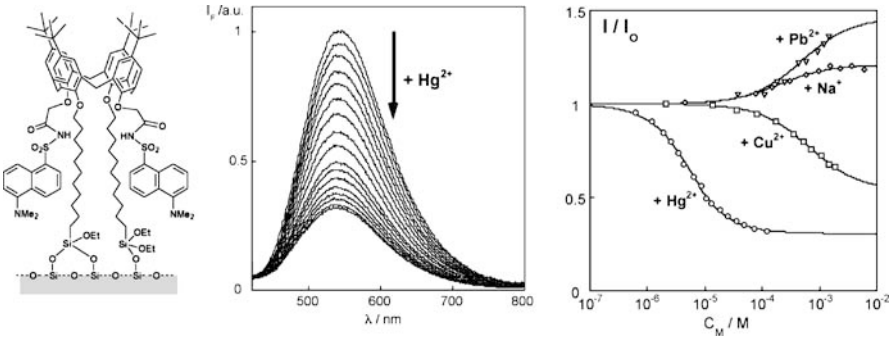


Fig. 3 Immobilization of Calix-DANS2 on a mesoporous silica surface via two arms (*left*). Effect of addition of mercury ions on the fluorescence spectra (*middle*). The effects of other cations appear at much higher concentrations (*right*) (adapted from [16])

mercury concentrations for other possible interfering ions (Fig. 3) [16]. The response time is a few seconds and the detection limit is 3.3×10^{-7} mol L^{-1} in water.

3.2 Microfabricated Analysis Systems

Considerable effort is made toward miniaturization of sensing devices with the following advantages: high speed, online monitoring, and low consumption of samples and reagents, which is a distinct advantage especially in the case of biological samples. The development of lab-on-chips, in particular the DNA gene chips, is impressive. Microfabricated analysis systems, called μ-TAS (micro total analysis systems), are based on microfluidic technology using microchip channels, microreactors, valves, and pumps [17, 18]. Fluorescence is one of the optical detection methods employed in these systems.[3] Many applications, especially in the life sciences, are being developed: clinical diagnostics, immunoassays, DNA separation and analysis, sequencing, etc.

A very sophisticated microfluidic device developed by Richard Mathies and coworkers at the University of California in Berkeley aims at detecting amino acids in future missions on Mars [19] (Fig. 4). It is based on capillary electrophoresis, and fluorescamine is used as a fluorescence marker for amino acids. The first step of the analysis is sublimation of amines and amino acids onto an aluminum disk spin-coated with fluorescamine. This device was successfully tested in Atacama Desert in Chile, one of the most arid regions in the world: it is 50 times more arid than Death Valley in

[3] The other detection methods are electrochemical or based on chemiluminescence, electrochemiluminescence, or mass spectrometry.

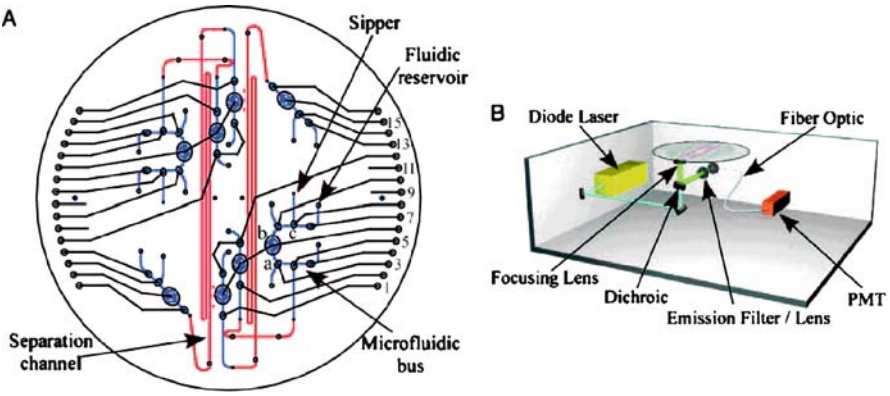


Fig. 4 Microdevice for amino acid biomarker detection and analysis on Mars. **a** Top view of the microdevice showing registration of the capillary electrophoresis channel (*red*), pneumatic manifold (*black*), and fluidic bus wafers (*blue*). **b** Schematic of the instrument showing confocal excitation and detection optics [19]

California, and it can thus be considered as the best Mars analog site. Sensitivity ranges from micromolar to 0.1 nM, corresponding to parts-per-trillion sensitivity.

3.3 Fluorescence Imager for Sensing

Still with the aim of detecting life, but not based on a microfabricated system, a robot called Zoe (in Greek, Zoe ($\zeta\omega\eta$) means life) has been developed by Alan Waggoner and coworkers at NASA Ames Research Center in collaboration with the Carnegie Mellon Institute. This is one of the most beautiful applications in the field of fluorescence sensing. The robot has under its carriage an outstanding fluorescence imager for the detection of chlorophyll-based life (e.g., cyanobacteria in lichens), as well as proteins, nucleic acids, lipids, and carbohydrates, using four fluorescent dyes with different ranges of excitation and emission. The optical system consists of a flash lamp, a selection of filters for excitation and emission, and a camera. Successful tests have been carried out in Chile's Atacama Desert.

3.4 Fluorescence LIDAR

LIDAR is the acronym of LIght Detection And Ranging (which is a transposition of RADAR for RAdiowave Detection And Ranging). LIDAR has long been used for atmospheric monitoring, and in particular for the detection of SO_2 and NO_2 in the atmosphere. The technique is based on

back-scattering and absorption of these species. Extension to fluorescence detection is possible by using appropriate emission filters. The applications concern:

- Status of vegetation
- Probing of plankton in seawater
- Imaging of the façades of historical monuments

In all these applications, a Nd:YAG laser is used for providing excitation pulses of about 10 ns duration at 532 and 355 nm thanks to frequency doubling and tripling, respectively.

Vegetation

The aim is the remote detection of chlorophyll fluorescence upon excitation at 532 nm, which is of interest for monitoring and measuring the chemical activities and the status of trees and forests. Early detection and mapping of damaged vegetation as a consequence of pollution can also be made. Remote estimation of chlorophyll concentration as a function of time is possible by taking the ratio of the intensity of the images at 740 and 685 nm [20]. The test is validated by the comparison of the average intensity ratio and the amount of chlorophyll determined by chromatography. The average intensity ratio follows the chlorophyll concentration that increases in summer and decreases in autumn.

Seawater

From aircrafts or ships, the detection of fluorescence from chlorophyll in phytoplankton and from phycoerythrin-containing plankton (e.g., cyanobacteria) (both excited at 532 nm) permits evaluation of the biomass. In addition, the fluorescence of dissolved organic substances can be detected upon excitation at 355 nm. NASA has developed an airborne oceanographic LIDAR and a shipboard laser fluorometer to achieve such evaluations.

Façades of Historical Monuments

The mobile system is placed in a truck at a distance of about 60 m from the façade. A 355-nm pulsed laser beam is swept over the façade row by row. The spectrally resolved fluorescence signals are recorded on each point and allow [21]:

- Visualization of areas of biodeterogen (lichens, green algae)
- Identification of different stone types with natural surface aging or crust and pollution deposited layers

Façade status assessment is thus obtained in view of restoration planning.

4
Clinical Diagnostics

Clinical diagnostics is also relevant to sensing, but this topic deserves a separate section with a distinction between in vitro and in vivo methods.

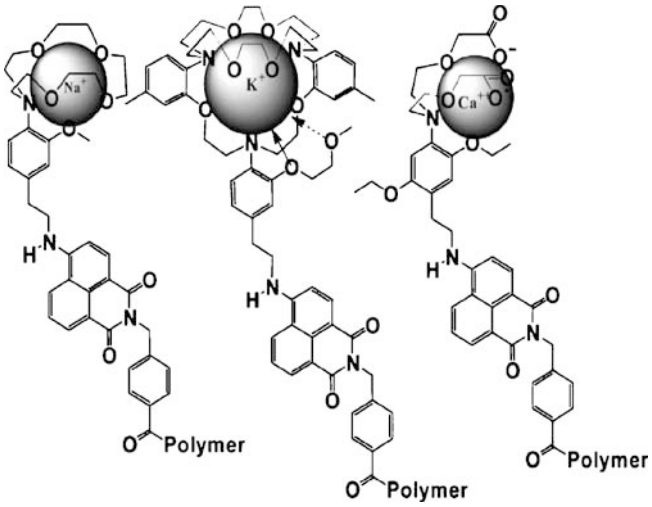
4.1
Critical Care Analysis

A nice example of in vitro analysis is the Osmetech/Roche OSPI critical care analyzer that can measure up to eight critical care analytes in whole blood [22]: pH, CO_2/Hb, O_2, Na^+, K^+, Ca^{2+}, or Cl^-. A disposable cartridge possesses six disks containing the appropriate fluorescent molecular sensors. Using 120 μL

Table 1 Fluorescent sensors for critical care analytes (from [22])

	Normal range	Pathological range	Fluorescent sensor
pH	7.35–7.45	6.7–7.7	HPTS (pyranine)
$P(CO_2)$/Torr	30–45	10–200	HPTS (pyranine)
$P(O_2)$/Torr	70–100	20–600	Oxygen-sensitive dye
Na^+/mM	135–145	100–180	Naphthalimide dye–aza crown[a]
K^+/mM	3–5	1–10	Naphthalimide dye–cryptand[a]
Ca^{2+}/mM	1.0–1.4	0.3–2.5	Naphthalimide dye–chelator[a]

[a] See Scheme 4



Scheme 4

of whole blood, it takes no more than 2 min to get the result. Table 1 shows a list of the analytes, the normal range, the pathological range, and the relevant molecular sensors. Pyranine is used as a fluorescent pH indicator.

For CO_2, a membrane blocks the passage of H_3O^+ and other ions into the sensor but allows CO_2 through, where it forms carbonic acid, increasing the local concentration of H_3O^+. For cation recognition, the naphthalimide fluorophore is linked to an aza crown for Na^+, a cryptand for K^+, and a chelating moiety for Ca^{2+} (Scheme 4). The dissociation constants are consistent with the pathological ranges.

4.2 Angiography

Angiography is a good example of in vivo diagnostics that permits visualization of the blood vessels of the retina in order to detect anomalies: lesions, aneurisms, or occlusions resulting from specific eye diseases. After taking a normal photograph of the retina, a fluorescent dye (fluorescein or indocyanine green) is injected into a vein in the arm of the patient. The dye is conveyed by the circulatory system and reaches the retina vessels. The photograph shown in Fig. 5 is an angiogram observed after injection of fluorescein: the existence of bright points leads to the diagnosis of an early diabetic retinopathy showing microaneurisms.

More recently, indocyanine green has been used as a tracer. It absorbs near 800 nm and emits in the near infrared, so that it can reveal deeper vessels called *choroidal vessels*. In the case of age-related macular degeneration,

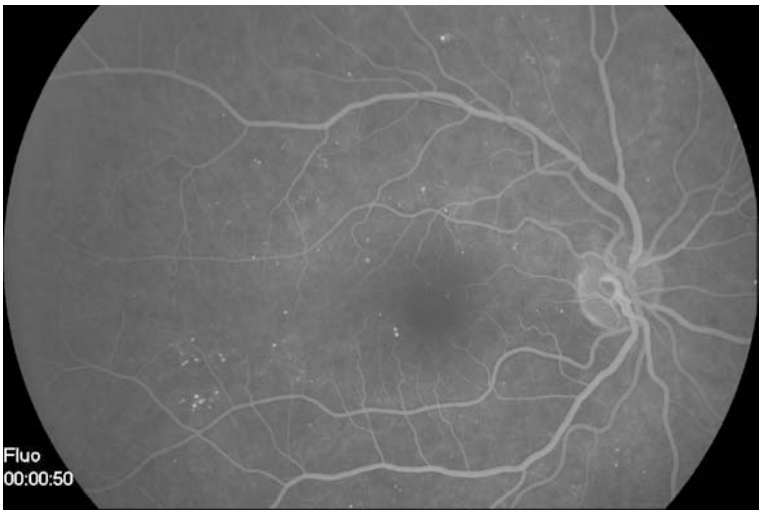


Fig. 5 Angiogram showing microaneurisms (*bright points*) (Courtesy of Dr. J. C. Hache, Lille Hospital, France)

leaky vessels, i.e., choroidal neovascularization, can be visualized, while the angiogram with fluorescein also shows fluid leakage for the same patient.

4.3 Bladder Tumor Detection

Early detection of bladder tumors in situ is possible by looking at the relative fluorescence intensities of tryptophan and NADH by endoscopy. A catheter contains optical fibers to convey light pulses from an excimer laser (308 nm), and other fibers collect the fluorescence which is analyzed by a monochromator and a photomultiplier (Fig. 6) [23].

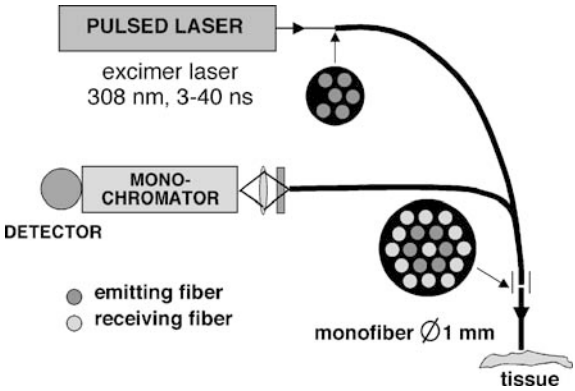


Fig. 6 Experimental setup for the detection of bladder tumors

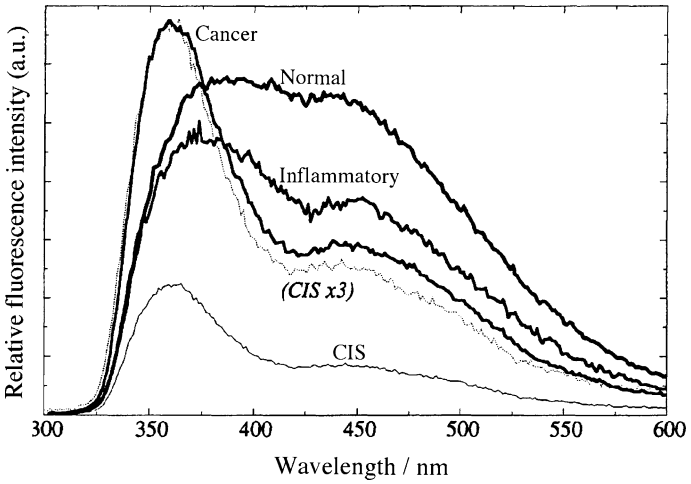


Fig. 7 Fluorescence spectra of normal, inflammatory, and cancerous tissues upon excitation at 308 nm (CIS: carcinoma in situ)

The fluorescence spectra are different according to the state of the tissue (Fig. 7). For tumors and carcinomas in situ, the ratio of the fluorescence intensities at 360 and 440 nm is statistically greater than 2. This test is reliable and can be run during a routine visit.

4.4 Human Skin

Measurements on human skin can be performed with a fiber-optic spectrofluorometer.[4] Double monochromators are necessary to isolate the fluorescence signal from strong scattering samples such as human skin.

Measurements of the fluorescence from tryptophan in epidermis and collagen in dermis provide a tool for:

- Evaluation of skin aging and photoaging
- Detection of skin disease
- Determination of epidermal proliferation (tryptophan)
- Control of drug delivery in photodynamic therapy

5 Imaging and Tracing

Imaging and tracing is a most important application of fluorescence not only in biology, but also in many other fields. In biology, organic fluorophores or fluorescent antibodies have long been used as tracers; they will not be described here, whereas new methodologies using semiconductor nanocrystals and fluorescent proteins deserve particular attention.

5.1 Semiconductor Nanocrystals

Semiconductor nanocrystals, also called quantum dots, were first studied in the 1970s, but applications emerged in the 1990s. Their photoluminescence properties are due to quantum confinement; the emission wavelength ranges from the ultraviolet to the infrared depending on the nature of the semiconductor and the size of the nanocrystal. For quantum dots made of cadmium selenide with a protective coating of zinc sulfide, the emission wavelength ranges from the blue (diameter of 2 nm) to the red (diameter of 7 nm).

Quantum dots offer distinct advantages for imaging, as a result of the following characteristics:

- Broad absorption spectrum allowing a common excitation wavelength for nanocrystals having different emission wavelengths

[4] SPEX SkinScan manufactured by HORIBA Jobin-Yvon.