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Fluorescence Spectroscopy: A Powerful Technique for the Noninvasive Characterization of Artwork

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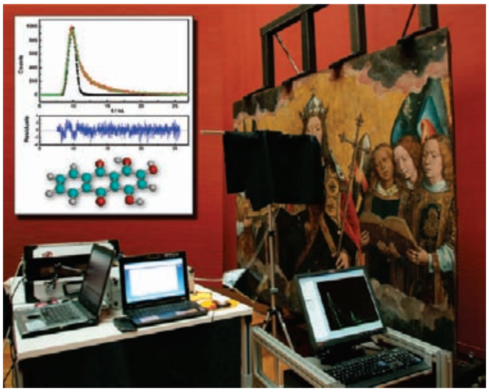

After electronic excitation by ultraviolet or visible radiation, atoms and molecules can undergo thermal or radiative deactivation processes before relaxing to the ground state. They can emit photons with longer wavelengths than the incoming exciting radiation, that is, they can fluoresce in the UV−vis−near-infrared (NIR) range. The study of fluorescence relaxation processes is one of the experimental bases on which modern theories of atomic and molecular structure are founded. Over the past few decades, technological improvements in both optics and electronics have greatly expanded fluorimetric applications, particularly in analytical fields, because of the high sensitivity and specificity afforded by the methods. Using fluorimetry in the study and conservation of cultural heritage is a recent innovation.

In this Account, we briefly summarize the use of fluorescence-based techniques in examining the constituent materials of a work of art in a noninvasive manner. Many chemical components in artwork, especially those of an organic nature, are fluorescent materials, which can be reliably used for both diagnostic and conservative purposes. We begin by examining fluorimetry in the laboratory setting, considering the organic dyes and inorganic pigments that are commonly studied. For a number of reasons, works of art often cannot be moved into laboratories, so we continue with a discussion of portable instruments and a variety of successful “field aplications” of fluorimetry to works of cultural heritage. These examples include studies of mural paintings, canvas paintings, tapestries, and parchments. We conclude by examining recent advances in treating the data that are generated in fluorescence studies. These new perspectives are focused on the spectral shape and lifetime of the emitted radiation.

Recent developments have provided the opportunity to use various spectroscopic techniques on an increasing number of objects, as well as the ability to fully characterize very small amounts of sample, either in a laboratory setting or on site. Thus, a new technological highway is open to scientists; it is still difficult to navigate but offers an enormous potential for investigating objects without touching them. Fluorescence spectroscopy is one of the most important of these techniques.

1. Introduction

In 1982, Renè de la Rie first investigated the potential of fluorescence spectroscopy for the noninvasive characterization of art materials such as binders, pigments, and dyes.[1] After his pioneering studies, the fluorescence spectroscopy in conservation science has been exploited by others. A number of papers concern the understanding of the chemical−physical properties of the molecules by applying luminescence techniques to standard laboratory models and pictorial replicas strictly reproducing works of art. Other works are more

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specifically focused on testing the potential of the different fluorescence-based techniques to obtain diagnostic information in a noninvasive manner, by developing and projecting new instrumentation or experimental setups. These two main pathways are closely connected since the instrumental output recorded from a diagnostic measurement on an original work of art should take into account the chemical–physical properties of the involved systems.

In a recent review, the relevance of fluorescence spectroscopy as a powerful analytical method for the diagnostics of organic dyes in cultural objects has been highlighted.[2]

2. Laboratory Fluorimetry: Knowledge of Materials and Diagnostics

A characterization of the chemical components of artwork should be the first step for the application of luminescence-based techniques in the cultural heritage field.

Spectroscopic studies may provide several pieces of information on the molecular properties of materials used in artwork. Steady-state UV–vis absorption and fluorescence spectroscopy supply information about the electronic excitation energy and the nature of the excited states. Time-resolved techniques provide information concerning their relaxation pathways.

Preliminary investigations have been carried out in solution, homogeneous medium where quantitative determination of parameters, such as fluorescence quantum yields and lifetimes, can be easily achieved and the effects of changes in environment, pH, or different additives can be controlled. Thereafter, laboratory samples, mimicking multiple execution techniques, have been studied, and the results have been interpreted in light of those obtained in solution.

Absorption and luminescence measurements provide complementary information; however, emission is generally more sensitive and selective because detection of each single fluorophore can be achieved using the appropriate excitation wavelength, thus resulting a suitable analytical technique for multicomponent analysis. Moreover, fluorescence excitation spectra may help in recognizing different fluorophores, sometimes present as traces, and unrecognizable by other conventional analytical techniques.

Commercial spectrofluorimeters were used for investigations in solutions, whereas, to obtain emission spectra from surfaces out of the fluorimeter sample holder, measurements were carried out using fiber optic systems. To measure fluorescence lifetimes in the laboratory, commercial instruments based on the phase-shift method or on the time-correlated single-photon counting technique have been used.

An alternative luminescence-based instrumentation for the diagnostics of artwork is laser-induced fluorescence spectroscopy (LIF),[3] consisting of a laser excitation source, optics (lenses/fiber optics), a spectrometer, and a sensitive CCD detector. By using an intensified charge-coupled device (ICCD) as detector or a multichannel analysis (MCA) device,[4] time-resolved measurements can be performed.

2.1. Naturally Occurring Organic Dyes. Among the chemical components of artwork, organic dyes are the best candidates to be recognized by fluorimetric methods since they often exhibit fairly intense luminescence.

In this context, colorants used in artistic paintings or miniatures or for dyeing precious textiles of the classes of hydroxyanthraquinones,[5−9] flavonols,[10] phenoxazines,[11] carotenoids,[12] and indigoids[5,13] have been investigated in solution under various experimental conditions by using stationary and time-resolved spectrophotometric and fluorimetric techniques.

Yellow colorants, such as flavonols, absorb at $\lambda \leq 400$ nm and fluoresce in the 420−500 nm wavelength range.

Yellow-orange carotenoids absorb in the 430−450 nm range and exhibit emissions markedly shifted to the red, 650−700 nm.

Typical red colorants, belonging to the family of hydroxyanthraquinones, widely diffused in nature, absorb around 500 nm and emit in a wide spectral region (550−650 nm).

Purple color was often obtained using orcein,[14] a natural dye that is a mixture of several reddish colorants of the family of phenoxazines. Its absorption ranges from 500 to 570 nm. To the best of our knowledge, orcein is the unique purple color dye that exhibits fluorescence emission that occurs at about 590 nm in solution.

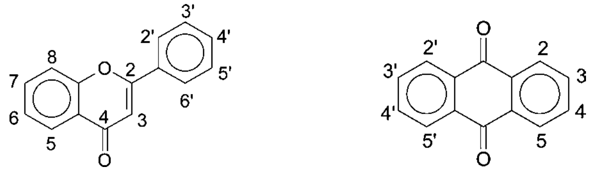
Colorants absorbing in the 600 nm region, typically indigoid dyes, are blue; their emission in solution is rather weak and specular to the absorption band.

In each class of colorants, substituents but also the environment can markedly affect the emissive properties. Some significant examples of these effects will be described in the following.

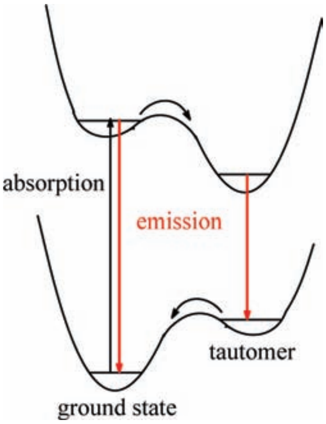
Flavonoid and anthraquinoid colorants are polyhydroxyl derivatives of molecular colorless structures, flavone and anthraquinone (Scheme 1), which absorb visible light and, therefore, appear yellow and red colored.

The number and position of hydroxyls, as well as the surrounding environment, may dramatically affect the fluorescence features. In fact, depending on the above, dual

SCHEME 1



SCHEME 2



fluorescence emissions via intramolecular proton transfer in the excited state (ESPT)[15−17] may arise. Moreover, fluorescence shifts and changes in intensity may occur due to either deprotonation, self-aggregation, or interaction with other molecular or ionic species able to form molecular complexes.

ESPT can occur from a OH group to a carbonyl oxygen in ortho position, thus originating a tautomeric form. From the double minimum energy surfaces of Scheme 2, it can be seen that after absorption, the molecule can emit from the tautomeric form with a marked bathochromic shift. Occurrence of ESPT for alizarin (1,2-hydroxyanthraquinone) but not purpurin (1,2,4-hydroxyanthraquinone) justifies the more intense emission of the latter.[6]

Fluorescence generally is weak and often undetectable for the neutral forms but becomes more intense or shifts to the red as the pH increases, due to stabilization of anionic forms,[6,7,10,18,19] Figure 1.

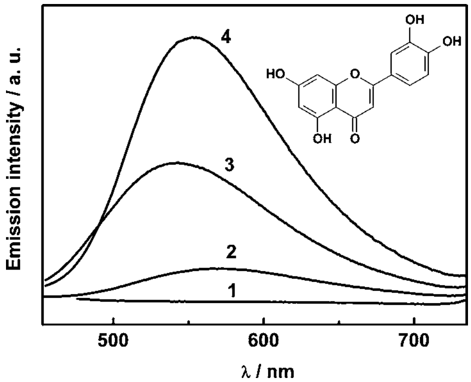


FIGURE 2. Emission spectra of the luteolin−Al^{3+} adducts at increasing concentrations of Al^{3+} ions: (1) $[Al^{3+}] = 0$; (2) $[Al^{3+}] = 5 \times 10^{-6}$; (3) $[Al^{3+}] = 2.5 \times 10^{-5}$; (4) $[Al^{3+}] = 4\times 10^{-4}$ mol dm^{-3}.

Chelation with metal ions has an effect similar to deprotonation since it induces fairly intense fluorescence emission. For example, luteolin (5,7,3′,4′-tetrahydroxyflavone), the emission of which is undetectable in the molecular neutral form, fluoresces up to unitary quantum yield attainment upon additions of Al^{3+} ions,[18] Figure 2. The intense fluorescence of chelates explains the light-fastness of the colorant on textiles dyed using a mordant,[20] since the absorbed light is mostly given up as emission, reducing photochemical degradation.

Metal complexation of hydroxyl dyes is used to obtain lakes, insoluble organic pigments used in painting. Emission spectra of lakes, prepared from weld (*Reseda luteola* L.) following different recipes,[21] allow one to distinguish lakes obtained by different procedures based on the shift of the emission maximum, Figure 3. Contributions to fluorescence of residual chlorophylls from dye extraction are also evident in lakes 1 and 2 at 663 and 675 nm, respectively.[21] Although

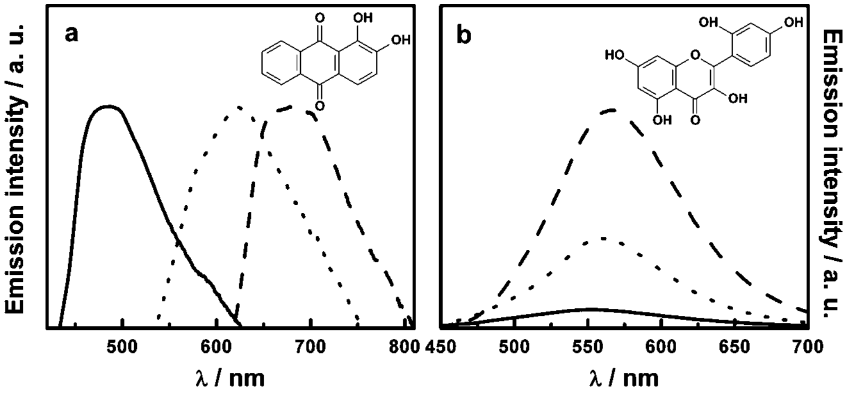


FIGURE 1. (a) Example of alizarin fluorescence red shift upon deprotonation (normalized spectra): neutral (pH = 2, —), monoanion (pH = 9, ···), and dianion (pH = 14, −−−) forms. (b) Example of enhancement of the morin fluorescence upon deprotonation: neutral (pH = 7.5, —), monoanion (pH = 10, ···), and dianion (pH = 13, −−−) forms.

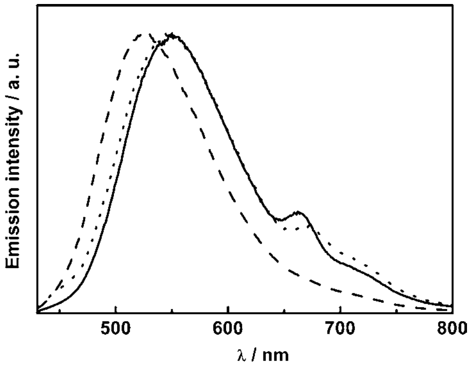


FIGURE 3. Normalized emission spectra (λ_{exc} = 366 nm) of powdery weld lakes (lake 1, —; lake 2, ···; lake 3, – – –) prepared according to three different historical recipes.

it is not probable to find chlorophylls in ancient works of art, due to their instability, this confirms the selectivity of the technique.

Studies of purpurin and alizarin lakes, spread on different supports and with various binders, have been also carried out using confocal microfluorescence spectroscopy by Melo et al.[22] The results obtained by the authors showed microfluorescence to be a promising analytical tool for the identification of red lake pigments combined with various binding media.

Of particular interest was the study of dyed textiles where, in addition to the colorant, a mordant (generally alum) may be present. For example, alizarin dyed wool samples[8] do not fluoresce in the absence of mordant. When alum mordant is present (Figure 4, top), the emission matches that detected in solution at pH ≈ 9 (monoanion).

Silk and wool samples dyed with orcein showed the fluorescence of both the fiber (~440 nm) and the colorant (~630 nm). These samples were also used to reproduce the changes that textiles undergo in time when irradiated with a 175 W xenon lamp for 100 h in order to produce an accelerated aging, which induces decoloration accompanied by decrease of dye emission and increase of fiber emission, Figure 4, middle.[8]

The aged sample emission was exploited to identify orcein on an original fragment of Renaissance tapestry from Bruxelles (16th century), Figure 4, bottom.[11]

Another interaction affecting fluorescent properties is self-aggregation, which generally results in a bathochromic shift of the absorption and emission spectra. Detected for indigo in concentrated solution (λ_{max} = 720 nm) and in powders (λ_{max} = 750 nm),[5] its knowledge was useful to recognize indigo on tapestry (see below).

2.2. Inorganic Pigments. Since the 1990s, fluorescence spectroscopy has been applied to organic materials in the field of cultural heritage; only recently has attention also been devoted to luminescent inorganic pigments. They include ancient synthetic compounds, like some copper based silicates, and more recent semiconductor materials, zinc oxide and cadmium sulfoselenides. While emissions of organic molecules are generally due to $\pi^* \rightarrow \pi$ electronic transitions, the color and the luminescence of inorganic compounds may originate from d–d transitions, charge transfer transitions, or transitions between the conduction and the valence band in semiconductors.

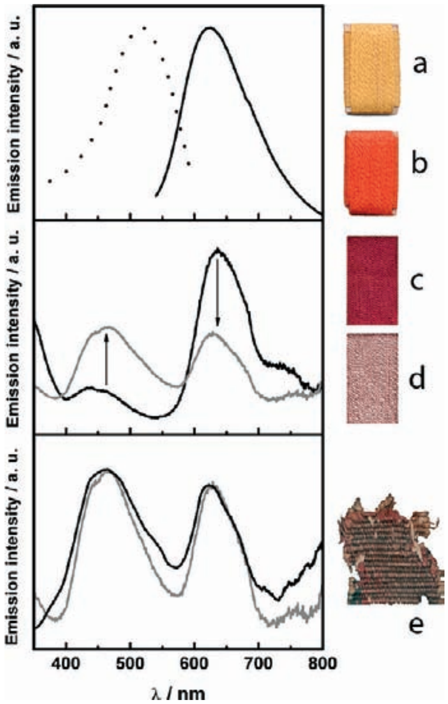


FIGURE 4. Left column, top, reflectance emission spectrum (—, λ_{exc} = 440 nm) and excitation spectrum of the emission (···, λ_{em} = 620 nm) of a wool sample dyed with alizarin in the presence of mordant; middle, emission of a natural orcein dyed silk sample before (black) and after (gray) 100 h accelerated aging; bottom, comparison between normalized emission spectra from the original tapestry fragment (black) and the orcein-dyed wool standard aged for 100 h (gray). Right column, alizarin dyed wool without (a) and with (b) mordant, orcein dyed silk before (c) and after (d) aging, and (e) original 16th century tapestry fragment.

Egyptian blue is considered to be the first synthetic pigment; it was widely used in the Mediterranean basin from the Fourth Dynasty in Egypt until the end of the Roman period and beyond. Based on X-ray diffraction data, the currently accepted chemical structure for Egyptian Blue corresponds to the naturally occurring mineral cuprorivaite. The absorption and luminescence properties of this pigment have already been assigned by Pozza and co-workers.[23] Recently reinvestigated, it revealed the highest quantum efficiency of luminescence (10.5%) ever found for a fluorophore emitting in the

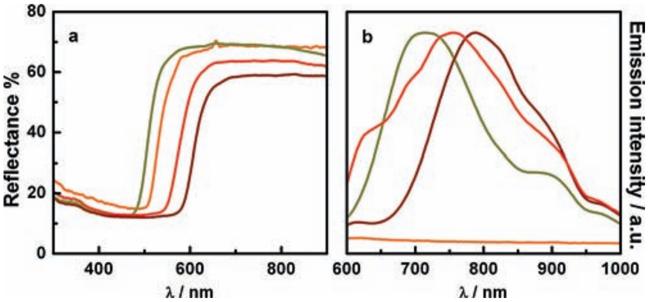


FIGURE 5. Diffuse reflectance (a) and normalized luminescence (b) spectra ($\lambda_{exc} = 500$ nm) of cadmium-based pigments of different chemical composition.

800−1100 nm range.[24] This characteristic has important diagnostic implications in the field of cultural heritage. In fact, Egyptian blue can be easily identified by means of both luminescence imaging[25] and spectrofluorimetry (see below, *Domus Aurea*), because its intense NIR emission does not overlap signals of other luminescent pigments.

Among more recent materials, a peculiar emission behavior is shown by zinc oxide. This compound, known and produced since the Roman age, was first used as an anti-inflammatory agent. After the mid-19th century, it became a white pigment widely used in modern and contemporary art. Zinc oxide, in the hexagonal wurtzite structure, is a semiconductor with a band gap energy of 3.3 eV at room temperature. Two emissions are reported in the literature, one in the UV and the other in the visible. The UV emission, assigned to the band gap transition, is associated with exciton annihilation, whereas the broad band in the visible, called the green emission, seems to be related to oxygen vacancies.[26] When zinc oxide is applied on a painted surface with a binder, the green emission is generally undistinguishable from the binder emission; nevertheless, the band gap can be assumed as a fingerprint of zinc oxide presence.

Among semiconductors, cadmium sulfide-based pigments have received particular attention by artists since the early 19th century thanks to the wide variety of shades they offer, from yellow, through orange and red, to maroon.

Cadmium sulfide (CdS) is a yellow pigment with a band gap energy of 2.42 eV (513 nm) at room temperature.[27] The corresponding transition is due to direct electron−hole recombination, while a second transition, in the 670−800 nm range, known as red emission, is related to a deep-trap resulting from structural defects or impurities like sulfur vacancies.[28] The replacement of different ions in the lattice produces a variation of band gap energy that reflects in color (Figure 5a) and emission maximum (Figure 5b) changes. From a diagnostic point of view, it is important that several cadmium-based pigments emit in the NIR and can be thus distinguished from yellow and red organic pigments whose emissions typically fall in the visible.

For the diagnostics of inorganic pigments in works of art, LIF has also been employed to examine a set of CdS and CdSe sulfide-based pigments in some oil painting replicas. The technique was suitable for differentiating among various cadmium pigments and identifying individual components in mixtures.[3]

3. Portable Fluorimetry

The investigation of works of art that cannot be moved from their location due to their dimension or for insurance purposes necessitates on-site measurements through the use of portable instrumentation.

Under the stimulation of results obtained in diagnostics using laboratory luminescence-based techniques and taking advantage of the technological development in the miniaturization of almost all instrumental parts, a great effort has been addressed to create portable instruments suitable for *in situ* measurements.

3.1. The Instruments. A portable fluorimeter has been assembled consisting of a xenon lamp as an excitation source, a monochromator for selecting the excitation wavelength, and a fiber-optic cable that directs the exciting light on the surface under study. The emission signals are revealed and transferred to the detector passing through suitable filters, if necessary.[11,29]

A further improvement in the luminescence-based techniques applied to cultural heritage diagnostics was the realization of a patented prototype of a portable instrument, based on the time-correlated single-photon counting (TCSPC) method, purposely assembled for on-site measurements of luminescence lifetimes on artwork surfaces.[30]

The usefulness of coupling steady-state and time-resolved luminescence techniques in the diagnostics of artwork emerges when different colorants having similar emission spectra have to be recognized. For example, two structurally similar red dyes belonging to the anthraquinoid family, laccaic acid and carminic acid, have fluorescence maxima at the same wavelength (~625 nm). They come from far geographical areas and are extracted from different insects (laccaic acids from *Kerria lacca* Kerr, an Indian insect; carminic acid from *Dactylopius coccus* Costa, a Mexican insect). Even if not distinguishable from spectra, they can be distinguished on the basis of their different lifetimes ($\tau_1 = 0.9$, $\tau_2 = 2.6$, and $\tau_1 = 1.5$, $\tau_2 = 5.0$, respectively).[31,32]

Other fluorescence techniques, such as laser imaging detection and ranging (LIDAR), used with application of fluores-

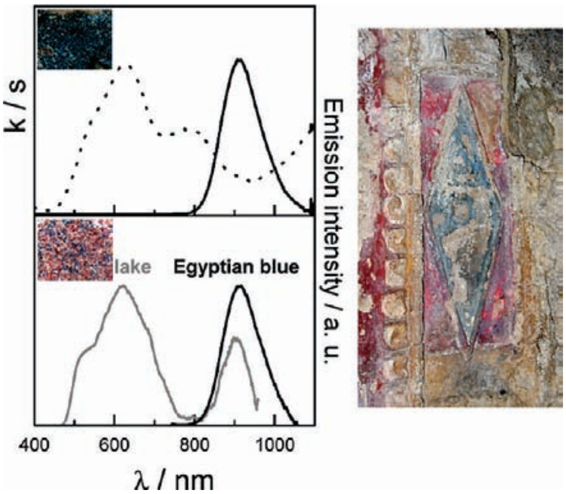


FIGURE 6. Right, decoration details of the *Gilded vault* room of *Domus Aurea*. Left top, absorption (···) and emission (—, $\lambda_{exc} = 540$ nm) spectra collected in the blue area. Inset: video-microscopy image (magnification 50×) of the blue area. Left bottom, emission spectra (gray, $\lambda_{exc} = 440$ nm; black, $\lambda_{exc} = 540$ nm) collected on the purple area. Inset, video-microscopy image (magnification 50×) of the purple area.

cence-multispectral imaging,[33,34] and fluorescence lifetime imaging and spectroscopy,[4] are amenable to be set up in a portable format.

3.2. The Applications. During the past few years several interventions on original works of art have been carried out using portable instrumentation within the transnational access activity of MOLAB.[35] In the following, meaningful applications of portable fluorimetry to objects of artistic and historical importance (painting, tapestry, parchment) are reported.

3.2.1. Mural Paintings: *Domus Aurea*. The *Domus Aurea* (Golden House) is the largest imperial palace built in the heart of Ancient Rome by the Roman emperor Nero after the Great fire of Rome in 64 A.D. The huge complex is unique for its architectural composition and richness in fresco paintings and stuccoes that burial hid and preserved for centuries. The composition and execution techniques of the precious decorations were, for the first time, studied in 2007, during a MOLAB access.[35]

In some blue decorations of the *Gilded vault* room, the absorption and emission features of Egyptian blue were observed (Figure 6). The colorant could be identified even when it was mixed with other luminescent pigments like hydroxy-anthraquinone red lakes that were found in some purple-violet areas.

3.2.2. Canvas Paintings: *Victory Boogie-Woogie* (Piet Mondrian, 1942−1944). The *Victory Boogie-Woogie* is the last unfinished work by Piet Mondrian, which is recognized as one of the most important works of modern art. The painting consists of 574 colored areas, most of which are painted and others made up of composite materials such as paper, commercial tapes, homemade tapes, and cellophane. The study of the pigments employed in this masterpiece is a relevant example of a multitechnique on-site noninvasive approach to modern materials.[36]

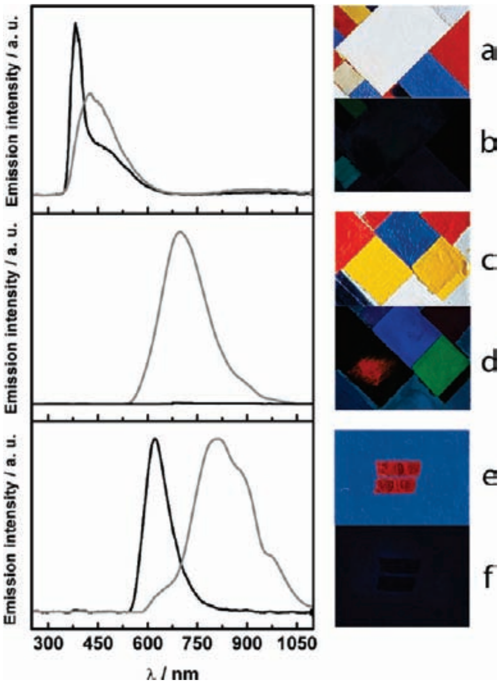


FIGURE 7. Mondrian's *Victory Boogie-Woogie*. Left column, fluorescence emission spectra of a (top) white rectangle, (middle) a yellow rectangle, and (bottom) a red painted tape on a blue rectangle. Right column, visible light (a, c, e) and UV-fluorescence (b, d, f) images corresponding to the areas where the spectra were obtained.

The UV-induced fluorescence image of the surface highlighted different luminescence emissions in localized areas.

Fluorescence spectral analysis of some white paints (apparently homogeneous) revealed the presence of two different emissions, Figure 7, top. XRF measurements carried out on white paints recognized the presence of zinc, titanium, and barium. Titanium and barium can be related to the use of titanium dioxide and barium sulfate, the latter also identified by mid-FTIR spectroscopy. The presence of zinc may be due to ZnS and ZnO whose discrimination is by far more complicated. However, UV−vis fluorescence spectroscopy allowed for the identification of zinc oxide through the characteristic band gap emission.

In some yellow squares, where cadmium and sulfur were revealed by XRF measurements, red fluorescent spots were detected under UV light (Figure 7, middle). The red fluorescing areas showed an intense emission at about 700 nm,

FIGURE 8. The portable fluorimeter at work on the tapestry *Earthquake in Filippi*.

which is related to a deep-trap transition resulting from structural defects or impurities in the cadmium sulfide pigment.

According to elemental analyses, red squares are rich in cadmium, selenium, and sulfur, suggesting the use of sulfoselenide cadmium red. Fluorescence spectra showed in fact a broad emission centered at about 810 nm, due to structural defects or impurities. A different emission was observed on several transparent cellophane tapes, probably painted in red and glued by Mondrian himself. An emission maximum at 620 nm and the absence of key elements suggest the presence of an organic pigment, Figure 7, bottom.

3.2.3. Tapestries. In-situ fluorimetry was applied to the Renaissance tapestries, designed by Raffaello Sanzio, which are exposed in the Gallery of Tapestries at the Vatican Museum. Most colorants used for dyeing fibers were recognized through their luminescence.[37]

With a 350 nm excitation, a band around 450 nm was always present, and it was assigned to the wool fiber.

Different color regions were analyzed on the observe and reverse sides; the latter was often preferred due to partial bleaching of color on the externally exposed side. Three tapestries (*Earthquake in Filippi*, *Emmaus′ supper*, and *Listra′s Sacrifice*) were analyzed; the portable fluorimeter at work on *Earthquake in Filippi* is shown in Figure 8.

Red-purple regions of the three tapestries exhibited emission maxima in the 624−630 nm range, which were assigned to orcein by comparison with laboratory standards (Figure 9, top). A modest blue shift of the emission from the tapestries with respect to the standard was attributed to aging, based on comparison with aged standards.

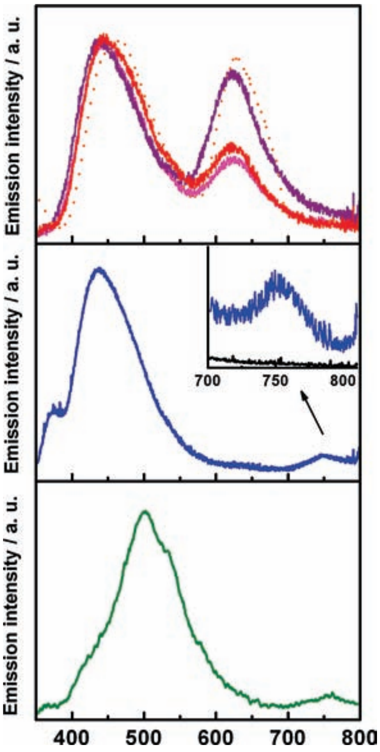


FIGURE 9. Top, emission spectra ($\lambda_{exc} = 350$ nm) monitored in purple regions on the reverse side of different tapestries, compared with the emission of an aged wool laboratory sample dyed with orcein (orange dotted line). Middle, emission spectra monitored on the reverse of the blue frame decoration of *Listra′s Sacrifice* tapestry ($\lambda_{exc} = 350$ nm). Inset, zoom on the long wavelength region ($\lambda_{exc} = 650$ nm); the signal from the warp is completely flat (black line). Bottom: emission spectrum monitored on green regions ($\lambda_{exc} = 350$ nm) of the frame decoration of the *Earthquake in Filippi* tapestry, front side.

Emission spectra monitored on blue regions (350 nm excitation), in addition to the band of the fiber, exhibited a batochromic, very weak band ($\lambda_{max} \approx 750$ nm), Figure 9, middle, which was assigned to the colorant. By comparison with laboratory samples, this emission was assigned to indigo.

Two emissions were found in green areas; one centered at 500 nm and the other, much weaker, at 750 nm (Figure 9, bottom). The latter is due to indigo, while the hypsochromic one corresponds to emission from some yellow dye, probably a flavonol, partially overlapped to the emission of the fiber. This indicates that green color was obtained by a mixture of some yellow dyes and the blue indigo.

3.2.4. Parchment: The Book of Kells. The precious Book of Kells is an early medieval manuscript from around the 800, held at the Trinity College Library of Dublin. Investigations were carried out on selected parchment folios of the Book, coupling the portable fluorimeter with the time-correlated single-photon counting equipment.[31] Two colorants, blue and purple, were recognized from their emission spectra and lifetimes. The blue colorant showed a very weak emission at about 730 nm, matching that of a laboratory standard of natural indigo in Arabic gum, while the emission spectrum of the purple colorant (~630 nm) was consistent with the fluorescence of orcein.[11] These assignments were confirmed by lifetime determinations ($\tau_{\text{indigo}} = 2.4$ ns; $\tau_{\text{orcein}} = 2.2$ ns).

4. New Perspectives in Data Treatment

Generally, experimental data have to be treated and elaborated in order to obtain the widest information concerning the object of interest, especially when analyzing a complex matrix such as a painted layer. Therefore, several works have been focused on searching for the right approach and the best interpretation of the instrumental raw data.

Recent advances in this field have been orientated in two directions: the spectral shape and the lifetime of luminescence.

4.1. Corrections of Emission Spectra. In reading emission spectra, the most important improvements have concerned a correct definition of fluorescence maxima to identify either the colorants or the surrounding substances. For identifying on-site some different natural varnishes, the use of both maximum position and full width at half-maximum of the emission spectra as a function of the excitation wavelength have been considered.[38] Recognition of binders and varnishes has been also carried out using laser-induced fluorescence coupled with total emission analysis.[39]

Concerning colorants, self-absorption effect, that is, absorption by the colorant itself of the emitted light, thus erasing a portion of the emission spectrum on the short wavelengths side, is the main source of uncorrected maximum wavelength reading. This effect can be obviated in solution by decreasing the probe concentration, but it is unavoidable for fluorophores in the solid state.

The problem of the correction for self-absorption of fluorescence spectra collected on pictorial works has been recently addressed, and a method for the treatment of the fluorescence signals has been developed.[40,41] In Figure 10, an application of the self-absorption correction to several carmine lake emission spectra collected on *Pala Albergotti* by Vasari is shown.

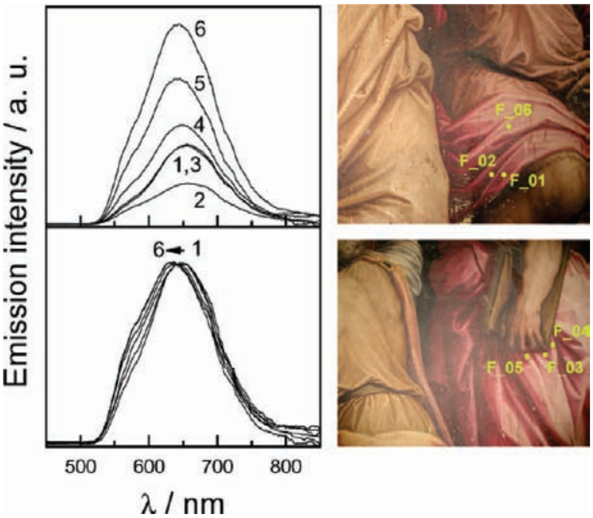


FIGURE 10. Top, fluorescence spectra ($\lambda_{\text{exc}} = 480$ nm). The emission maximum moves from 642 nm on the first spectrum to 657 nm on the sixth. Bottom, normalized fluorescence spectra corrected for self-absorption and multiple scattering phenomena. The correction moves the spectra toward shorter wavelengths making them quite similar. Photos show the examined points.

4.2. Reading of Fluorescence Decays. A kinetic approach to the study of luminescence of artistic objects may have a determining role in the diagnostics of colors on surfaces. Compared with previous works,[4] the home-assembled TCSPT portable instrument has introduced important improvements by increasing the time resolution by about 1 order of magnitude and applying more advanced methods for the interpretation of the experimental decay profiles. In the approach, proposed in 2004 by Comelli et al. based on MCA, fluorescence decays had been handled by first-order fitting algorithms. This application, although useful to distinguish chemically different fluorophores, is not completely exhaustive when describing the complexity of a painted layer, where several different emitting species could coexist. In the TCSPC-based instrument, multiexponential fit of fluorescence decays allows for a better description of such kinetics. To gain more insights into kinetically complex systems, the maximum entropy method (MEM),[42] which considers a distributed set of logarithmic functions, is now being applied to luminescence decay analysis. The heterogeneity of the environment surrounding a fluorophore embedded in a painting layer gives rise to complex decays that can originate from different fluorophores but also from the same chemical species in a different microenvironment. In this context, MEM can be used to recover the right distributions of luminescent lifetimes from the experimental data.[43]

5. Conclusion

Over the last 15 years, the scientific community has provided restorers and conservators with a wide range of completely noninvasive techniques able to disclose several secrets concerning the chemical nature of the materials used in creating works of art in the course of the centuries. Luminescence-based spectroscopic techniques were developed, following different approaches, in order to extract the greatest amount of information on several artwork components such as binders, varnishes, and pigments in paintings and organic dyes in tapestries and manuscripts. At first, each luminescence-based technique was generated and tested as a stand-alone investigation method, able to provide information for diagnostic purposes. Then, scientists coupled this with other complementary spectroscopic methods (for instance vibrational spectroscopy) or complementary applications in the same field (for example, time-resolved luminescence measurements) to collect the widest data sets to design at best the investigated materials. Consequently, today, to the pressing requests by conservators and restorers for noninvasive diagnostic approaches, each piece of information obtainable from any on-site technique must be taken into account: luminescence-based techniques are among the best candidates for this role. The strong engagement into the double pathway of instrumental and technical development, combined with ever increasing knowledge of material properties, have become general rules of the scientific effort in the world of conservation science, and this is what is going to occur for luminescence techniques applied to works of art.

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BIOGRAPHICAL INFORMATION

Aldo Romani graduated in Chemistry at the University of Perugia in 1987 and received his Ph.D. in Chemistry in 1992. Currently, he is a Researcher at the Chemistry Department of the University of Perugia. He has authored about 70 scientific papers concerning both basic and applied subjects principally involving characterization of the excited states of organic molecules by means of the parameters that govern their radiative and nonradiative processes using spectroscopic techniques in absorption and emission. These techniques have been also applied, for nondestructive diagnostic purposes, in the field of the cultural heritage.

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Gianna Favaro graduated in Chemistry from the University of Padua in 1958; she has been lecturer in Physical Chemistry and related subjects at the Universities of Padua, Bologna, and Perugia. Full professor of Physical Chemistry at the University of Perugia, she has authored over 130 articles concerning electronic spectroscopy, photoinduced energy transfer, photosensitization, photokinetic studies on photochromic compounds, and photochemical and photophysical characterization of materials of interest for cultural heritage. Retired since 2008, she is still involved in several scientific engagements.

FOOTNOTES

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