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Bright Light: Microspectrofluorimetry for the Characterization of Lake Pigments and Dyes in Works of Art

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CONSPECTUS

Color is an important component in the perception of beauty and in an artist's original intent when creating a work. Better conservation of our cultural heritage requires detailed knowledge of artwork materials and the complex evolution they have endured over time. Organic dyes have been used from ancient times, and their characterization is a challenge that has been successfully addressed over the past few years by the development of advanced techniques, such as microspectrofluorimetry.

In this Account, we describe the application of microspectrofluorimetry to the study of medieval illuminations, paint cross sections, millenary textiles, and wall paintings. In our research into color in medieval Portuguese illuminations, we chose to emphasize the importance of the experimental design and the use of microspectrofluorimetry in the context of other analytical techniques, such as microFTIR, microRaman, and micro-X-ray fluorescence (microXRF). Within this framework, we were able to unveil the full complexity of a medieval colorant and to address issues not yet explored, such as the influence of Arab, Jewish, and Christian cultures on the production and underlying technology of Portuguese illuminations. The analysis of individual pigment particles or aggregates (by excitation with an 8 μm diameter spot) in paint cross sections from works by Vincent van Gogh and Lucien Pissarro highlights the technique's advantage of high spatial resolution. Its high spectral resolution proved to be useful not only for better characterizing the dyes used to color Andean textiles but also for detecting mixtures of relevant chromophores; the emission signals for the reds in Paracas and Nasca textiles were shown to be due to the presence of purpurin and pseudopurpurin. Finally, the complexity of the study of yellow dyes and the importance of accurate historical reproductions is addressed in a study of Asian organic colorants on historic Chinese wall paintings.

Microspectrofluorimetry offers high sensitivity, selectivity, fast data acquisition, good spatial resolution, and the possibility of in-depth profiling. It has proved to be an invaluable analytical tool in identifying dyes and lake pigments in works of art. As Saint-Exupéry's protagonist said in *Le Petit Prince*, "L'essentiel est invisible pour les yeux," or "What is essential is invisible to the eye"—but it may be unveiled with kind love, a prepared mind, and a little help from microspectrofluorimetry.

1. Introduction

To create a little flower is the labor of ages.
William Blake

In one of his last public interventions in the 1980s, Brandi challenged scientists to find how to characterize the organic compounds present in works of art. The passionate debate he had opened in the 1960s with "The cleaning of pictures in relation to patina, varnish and glazes"[1] was certainly on his mind. Without this knowledge, how could one argue that in a certain picture a precious, colored, original glaze was still

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there and that color was not a consequence of aging but an artist's choice? How could one avoid the possible irreversible damage in a restoration of a Michelangelo, which includes the removal of an "aged" varnish, without knowing if it is an aged varnish or a colored *velatura*? Brandi's challenge was indeed taken up, and in the past two decades the field of conservation science has grown and matured to the extent that it is regarded as a discipline in its own right, in which new advanced methods are developed to unveil some of the last secrets of the works of art.

Projects such as the *Molart* and *Molab*,[2,3] employing very different strategies, played a crucial role in the development of a critical mass and in the shaping of the field of conservation science. The *Molart* project, with its follower *De Mayerne*, focused on molecular aging studies, whereas the *Molab* proposed a holistic approach to the characterization of the work of art: enough data must be collected *in situ* using noninvasive methodologies in order to be representative of the materials and techniques of a certain work of art. Both approaches are complementary.

In the past few years, we have been particularly interested in the development of methodologies that will enable a complete characterization of the organic colorants used in the past as well as their degradation products. Changes in pigments, whether used pure or mixed with other pigments, can alter the appearance of a painting significantly; as a consequence, the identification and state of degradation of colorants is of fundamental interest, since it provides critical information about the artists' aesthetic perspective, conceptions, and choices, and how the work has changed over time. Therefore, it is desirable to develop methods that can characterize these materials noninvasively or from small samples that may be available from works of art. The potentiality of microspectrofluorimetry in the field of conservation science was tested within this framework: first, in historically accurate reproductions and, finally, in works of art, such as textiles, medieval illuminations, or oil paintings.[4,5] Microspectrofluorimetry offers high sensitivity and selectivity, combined with good spatial resolution and fast data acquisition; it can also be used *in situ* without any contact with the sample or work of art to be analyzed, for movable objects that can be transported in the laboratory. The importance of sensitivity is clear when the following facts are taken into account: some of the dyes used in the past to create bright colors may have faded or may have been applied as very thin coats over, or mixed with, an inorganic pigment or extender, and as a consequence they may be present in very low concentrations. The possibility of *in situ* analysis of ancient colorants is a considerable advantage, particularly when considering that the techniques currently employed for dye analysis (HPLC-DAD-MS, microFTIR, and microRaman) usually require microsampling. Microspectrofluorimetry also presents some drawbacks, namely, the absence of a molecular fingerprint as disclosed in infrared spectra. This limitation may be overcome by using consistent data-basis buildup with historically accurate reproductions.

A brief overview will be presented of the dyes used in the past for dyeing and painting and on the methods currently employed for their analysis. We will address the importance of understanding a dye's photochemistry and photophysics as well as that of historically accurate reproductions. Finally, case studies will be described to highlight the successful stories where emission fluorescence proved invaluable and the incomplete ones where work is still in progress.

1.1. Natural Dyes in Context: Textiles, Medieval Illuminations, and Paintings. Natural dyes and their metal-ion complexes have been used for textiles, manuscript illuminations, paintings, and other works of art. For example, anthraquinones and their hydroxy derivatives have been used as red dyes and pigment lakes from prehistoric times, and we can find written accounts of the use of anthraquinone reds and purples as dyes in ancient Egypt;[6] anthraquinone lakes (e.g., madder red) were also very popular with Impressionist painters, including Vincent van Gogh. Pure dyes such as indigo were also used as painting materials, for example, in medieval illuminations.[7]

To be used as a textile dye, the chromophore must be absorbed as much as possible into the fibers; that is, it must be resistant to washing. Dyes can bind to the surface of the fiber or be trapped within. They are often bound to the textile with the aid of metallic ions known as mordants, which can also play an important role in the final color. Alum, as a source of aluminum ion, is an important historical mordant, and it was widely used in the past.[8] Dyes, such as indigo, are trapped in the fibers due to an oxidation—reduction reaction, without the aid of a mordant.

It is useful to distinguish between dyes and pigments based on their solubility in the media used to apply the color; dyes are generally organic compounds that are soluble in a solvent, whereas pigments, used in painting, are usually inorganic compounds or minerals which are insoluble in the paint medium (oil, water, etc.) and are dispersed in the matrix. Lake pigments can be prepared by precipitating the dye extract with aluminum salts, such as alum.[4,7]

1.2. Chromophores: The Classic Palette. By the time of the founding of the Mediterranean civilizations, what we would consider the *classic palette* for natural dyes had already

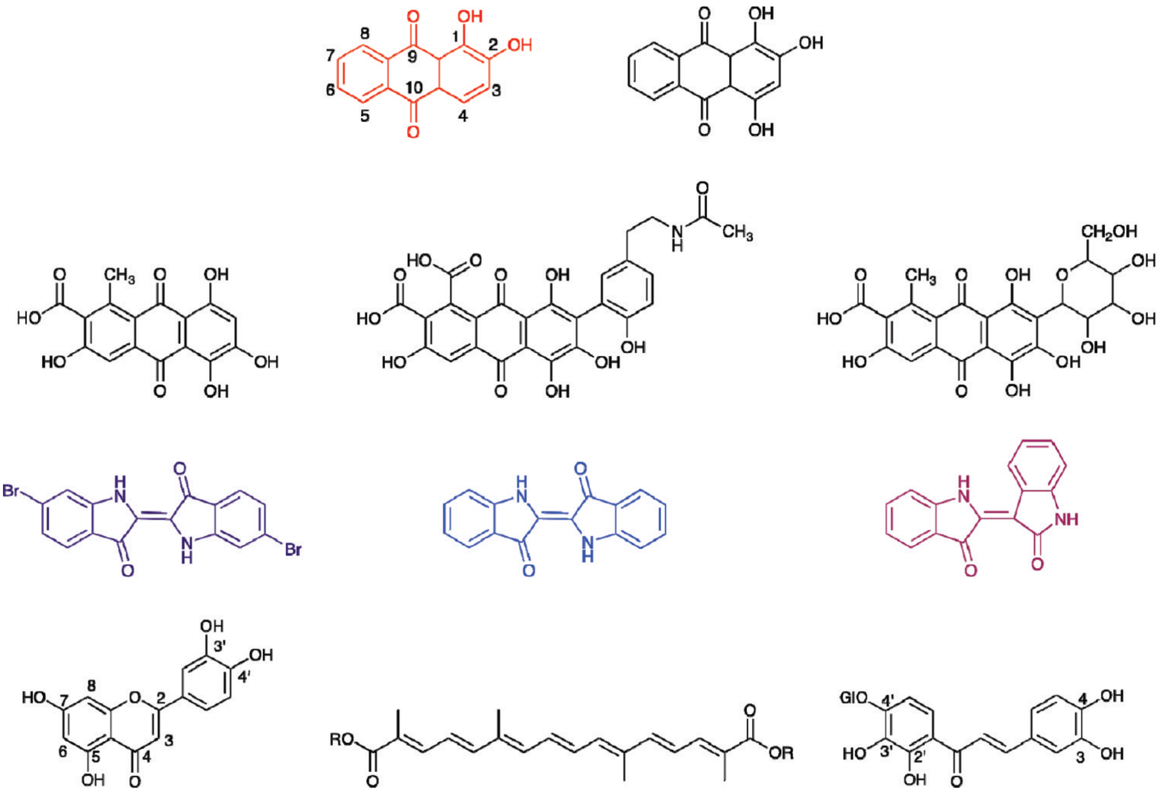

FIGURE 1. Anthraquinone reds: (i) from plants, alizarin (1,2 dihydroxy anthraquinone) and purpurin (1,2, 4 trihydroxy anthraquinone), first row; (ii) from insects, kermesic acid, carminic acid, and laccaic acid A, second row. The blues and purple chromophores are depicted in the third row: indigo (indigotin), purple (6,6′-dibromoindigotin), and indirubin. For the yellows, the flavone luteolin, the carotenoid crocetin, and the chalcone marein (okanin 4′-*O*-glucoside), fourth row.

been established, and most valued colors were indigo for the blues, anthraquinone-based chromophores for the reds, and 6,6′-dibromoindigo for purple[7] (Figure 1). The natural sources for yellows were much more diverse, so yellows could generally be obtained locally (Figure 1). With the exception of some browns, all other colors, including green and orange, could be obtained with these blue, red, purple, and yellow dyes. This classic palette was preserved over centuries, if not millennia. The first adjustment resulted from the loss of Tyrian purple following the fall of Constantinople and the subsequent collapse of the Roman social and commercial web. This was followed by a new entry, cochineal red, brought by the Spanish from the New World.[7] However, even with the introduction of cochineal, the chemical nature of the classic palette was maintained, as carminic acid is still a substituted 1,2-dihydroxy anthraquinone. This classic palette was only challenged by the audacity of chemists, who created new molecules, and colors never seen before, from the mid-19th century on.[9]

1.3. Fading, Lifetimes, Photochemistry, and Photophysics. The processes taking place in the excited state, after absorption of light by a molecule, are usually complex and in heterogeneous systems such as the ones offered by works of art they are still a challenge. This may explain why so few systematic studies have been carried out and published.[10] Both photochemistry and photophysical studies will be necessary to fully exploit the strength and test the limitations of UV−VIS-NIR emission spectroscopy as an analytical technique for the identification of dyes in works of artistic and historic importance. Also, this knowledge will enable more information on the dye's environment and its conservation state to be extracted.

1.4. Current Methods and the Development of New Methods of Analysis. Identifying the ancient dyes and dye sources has only been possible with the development, in the past two decades or so, of sensitive new microanalytical techniques.[11] It is possible to group the methods as *in situ* (UV−VIS reflectance and florescence) or requiring microsampling (Raman, IR, HPLC-DAD-MS). Currently, the most efficient method for unequivocal dye characterization is HPLC-DAD-MS, but it requires sampling (Table 1). Chromophores are first extracted and then separated chromatographically and characterized by UV−VIS spectrophotometry and mass spectrometry; whenever possible, a comparison is made with authentic references. Currently, the use of HPLC-DAD enables dyestuff characterization from as little as 0.1 mg of thread. Recently,

TABLE 1[a]

	microfluorimetry	microFTIR	microRaman	HPLC-DAD-MS**
alizarin lake	*	d	SERS**, ***	√
purpurin lake	√	d	SERS**	√
carminic acid lake	√	√	SERS**	√
laccaic acid A	*	√	SERS**	√
luteolin lake	√	d	SERS**	√
rutin lake	√	d	SERS**	√
crocetin lake	√	d	SERS**	√
6,6'-dibromoindigo	*	√	√	√
indigo	*	√	√	√

[a] * Emission and excitation spectra may not allow for an unequivocal identification due to the low S/N. Together with lifetimes, identification is possible. ** It is necessary to extract the dye. *** In textiles, madder lake was identified by shifted Raman spectroscopy. [15d] (d) Depends on the concentration of the dye in the alumina matrix; usually unequivocal identification is not possible.

developed mild extraction methods allow more detailed chemical information to be obtained on the historical natural dyes, and as a consequence it is sometimes possible to identify the natural sources, even for the more complex analyses of yellow dyes. Raman microscopy has also been actively explored for dye analysis and new methods, that allow for higher S/N, developed, such as surface-enhanced Raman scattering (SERS).[12]

Most promising are the analytical techniques based on fluorescence detection that take advantage of the opportunity for high sensitivity and selectivity, combined with *in situ* analysis.[4,5,13] Microspectrofluorimetry as well as transportable fiber-optic spectrofluorimeters have been systematically tested in the past few years.

1.5. The Importance of Accurate Historical Reproductions and a Database. When studying the materials used to create a work of art, complexity may be addressed not only by using complementary techniques but also by knowing the materials and techniques employed in the original process of art creation. Recreating old recipes with as much historical accuracy as possible provides representative samples that may be used as standards.[14] Having the *Historical Recipe Reconstructions* assembled in a database is essential when operating with *in situ* techniques. Model samples prepared with pure materials will in turn enable both a better understanding of the results obtained with the historical reproductions and the testing of the analytical methodologies. The information necessary to produce the historical reproductions is to be found both on documentary sources covering technical information contemporary with the period being investigated as well as on analytical data obtained by the works themselves.[14,15]

2. Microspectrofluorimetry

2.1. The Technique. The measurements were obtained with a microSPEX instrument using a setup described elsewhere,[16] where the Spex Fluorog apparatus 3-2.2 is connected to an Olympus BX51 M confocal microscope, with spatial resolution controlled with a multiple-pinhole turret, corresponding to a minimum 2 μm and maximum 60 μm spot with a 50× lens. For steady-state fluorescence spectra, a continuous 450 W xenon lamp, providing an intense broad spectrum from the UV to near-IR, is directed into a double-grating monochromator. The incident excitation beam is directed onto the sample, and its fluorescence is directed back up into the microscope. To view the sample's fluorescence directly, a binocular eyepiece and a digital camera are used. Beam-splitting is obtained with standard dichroic filters used at 45°; they are located in a two place filter holder. For a dichroic filter of 570 nm, excitation may be carried out until about 560 nm and emission collected after about 580 nm. For the study of red and yellow dyes, two filter holders with two sets of dichroic filters are employed, 500 and 570 nm in one set and 430 and 470 nm in the other set. This enables both the emission and excitation spectra to be collected with the same filter holder for each group of dyes. Spectra are collected after focusing on the sample (eye view) followed by signal intensity optimization (detector reading).

The pinhole aperture that controls the area of analysis is selected based on the signal-to-noise ratio. For weak to medium emitters, it is set to 8 μm, which is appropriate for the analysis of individual pigment particles or aggregates in a paint film, and more generally it offers an optimized signal-to-noise ratio and good spatial resolution for artists' paints.

2.2. Comparison with Fiber Optic. With regard to the comparison of the method with the currently available transportable fiber-optic spectrofluorimeters, the more significant advantages of microspectrofluorimetry are as follows: (i) the possibility to acquire both emission and excitation spectra, (ii) high spatial resolution, (iii) high signal-to-noise ratio, enabling high spectral resolution and to obtain very well resolved spectra, and finally the possibility of (iv) in-depth profiling. It is important to stress the fact that an excitation spectrum repro-

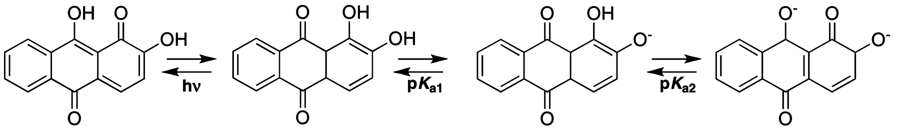

FIGURE 2. Acid base equilibria for alizarin. The 1.10 keto tautomer, formed in the excited state (alizarin is a 9.10 quinone) is also depicted. For more details please see ref 4.

duces the chromophore absorption spectrum; a relevant parameter for the characterization of a dye or a pigment. The simultaneous acquisition of emission and excitation (absorption) spectra together with high spectral resolution facilitates a more accurate identification of dye molecules and lakes. In common with what is obtained using a fiber-optic setup, microemission fluorescence displays high sensitivity and very good reproducibility together with the possibility of performing semiquantitative analysis. When working with the fiber-optic and higher areas of analysis, a higher light throughput is achieved and this may be an advantage when studying very weak emitters. On the other hand, in the absence of confocal excitation, emission from the support or underlayers may turn signal interpretation difficult.

2.3. Photophysics and Photochemistry as a Tool to Explore with Higher Efficacy the Information Obtained. Anthraquinone derivatives and yellow dyes, flavonoid or carotenoid based, are complex systems displaying photophysical properties that may be strongly influenced by pH and metal ion complexation.[4,5,17,18] As regards indigo, a full understanding of its photophysics as well as photochemistry has emerged from the comprehensive studies on the photophysics of the keto and leuco forms of indigo and its derivatives carried out during the past 5 years.[10a,b]

Photophysical and photochemistry studies provided important information for the understanding of the photostability of indigo blues as well as of 1,2-dihydroxy anthraquinone reds. For both systems, efficient radiationless processes, possibly involving excited state proton transfer (ESPT) and excited state intra- and intermolecular proton transfer (ESIPT), play a determinant role in the overall stability of the molecules. ESPT and ESIPT with excited state tautomer formation (Figure 2) can be considered to induce a photoprotective mechanism for the molecule, enabling a very fast and effective dissipation of the excess energy of the excited state.

Less is known about the excited state properties of pigment lakes in the solid state,[4,5] and only recently were the first systematic studies published on the solid state photophysics and photochemistry of these ancient colorants, reds,[4] blues,[10] and yellows.[18] Nevertheless, this positive trend will enable the disclosure of the full potential of fluorescence spectroscopy applied to the study of ancient dyes.

From these first photophysical studies on alizarin and purpurin lakes, it emerged that more is needed to be known from the structure of the aluminum and other metal ion lakes in order to have a full rationalization of the observed excited state properties. From the studies we are presently conducting, by solid state NMR, a complex pattern emerged, where the final structure depends on the methods used for the lake formation.[19]

3. Case Study: Medieval Portuguese Illuminations

Portuguese codices date from the formation of Portugal as a kingdom and are testimonies to medieval ideas, religion, and politics. Color use and production was a consequence of the technology available as well as of cultural and artistic options; defining the specificities of color will contribute to fingerprint the influences of the three different cultures that coexisted in Portugal at that time, Arab, Jewish, and Christian. This subject is approached within an interdisciplinary framework, from an art history and molecular sciences point of view, aiming to explore issues related with the symbolic and social meaning of color in medieval Portuguese illuminations, produced during the twelfth and first quarter of the thirteenth century in Portuguese monasteries.

In the past 5 years, we have undertaken a systematic study of the materials and methods of Portuguese medieval illuminations,[20] where microspectrofluorimetry has been used in context with other techniques.

3.1. Microspectrofluorimetry in Context: Experimental Design and Modus Operandi. MicroRaman, microFTIR, microXRF, and microXRD are powerful complementary techniques for the characterization of colorants in medieval manuscripts. More recently, analytical techniques based on emission fluorescence have also been experimented with for the identification of dyes and lake pigments with rewarding results, and were used to study the color of Portuguese medieval illuminations from three important Portuguese monasteries.[21]

The global approach and experimental design for a comprehensive study on color production for medieval Portuguese illuminations will be briefly described (Figure 3). Missions, where the equipment is transported to the institutions where

Macro photos are taken (x10-x80), that will contribute to characterizing the paintings' surface and mixtures

Microsampling is performed under the microscope, after careful selection of the colors that will be studied in more depth in the laboratory.

Statistical relevance is achieved with microEDXRF. Its spatial resolution, 70 μm, allows a first comprehensive screening. Elemental analysis

Raman is performed in selected folios due to its high spatial resolution, 1 μm. Molecular characterization

FIGURE 3. Main steps in a mission for the study of the medieval manuscripts from Alcobaça monastery (BNP, May 2009). MicroXRF and microRaman are techniques complementary to microspectrofluorimetry, that are used *in situ*. Microsamples will be afterward analyzed in laboratory, by microFTIR, microspectrofluorimetry, and microXRF. Photos by Duarte Belo.

the manuscripts are preserved, are prepared after a careful selection by the art history experts. From these manuscripts, a relevant number of folia will be analyzed in order to ensure statistical relevance.

During the mission, the first screening is carried out by microXRF, which indicates the possible colorants and extenders present and allows a first quantification of these elements; moreover, its 70 μm enables us to obtain data that are representative for the distribution, in the manuscript, of a certain paint color. MicroRaman, which allows for high spatial resolution (1−5 μm spot) and where the diverse paint components may be excited separately, as well as emission fluorescence techniques will be used to address specific points, such as the molecular characterization of an inorganic pigment or a dye, respectively. Together with the spectroscopic investigation, paints are also analyzed by optical microscopy which allows us to understand how the final color is built up (by layers or by mixture), to detect possible degradation phenomena, and to sample the color paints that will be subjected to a more detailed characterization in the laboratory, as regards the colorants, binders, and additives. Designed microsampling presents several advantages, namely it keeps to a minimum the handling of the manuscript and allows for more detailed studies in the laboratory, without the time constraint that *in situ* mission implies. Typically, in the laboratory, a sample will be first analyzed by microFTIR, allowing for binder characterization and to gain an insight into the full paint formulation. If a dye is present, microspectrofluorimetry may be carried out first, as it requires no contact with the sample and employs a low-intensity radiation. MicroRaman and microXRD may be used to address specific points; with both methods, sample destruction may occur.

3.2. Microspectrofluorimetry in Context: The Medieval Portuguese Palette. From the study of all three monasteries' manuscripts, carmine and bottle-green colors emerged as possibly characteristic of the Portuguese production, and the first medieval Portuguese palette was proposed (Figure 4).

In the Lorvão manuscripts, namely, in *The book of birds* (1183−1184), the carmine color is based on an organic dye, and infrared analyses indicate that it was possibly obtained from lac dye. The paint is of a beautiful deep red color, displaying a glassy appearance when observed under the microscope. Paint historical reconstructions were produced based both on the infrared results and the recipes found in the medieval treatise "The book on how to make colors". The emission and excitation spectra obtained from three original samples matched well the signals obtained with a paint reconstruction where lac was extracted from the raw material at pH = 11 and the paint solution was applied with a pH = 6. Even if the infrared fingerprint was also similar, it was not possible to reproduce the glassy appearance of the original paint. Moreover, taking into account the quantum yields of fluorescence, measured in solution, for laccaic acid A[22] ($\phi_f \approx 10^{-3}$) and its aluminum complex ($\phi_f \approx 10^{-2}$), it is possible to suggest that the lac dye was not applied as a pigment lake. In this case, microspectrofluorimetry enabled the dye identification as well as to gather information on the characterization of the paint recipe used. Historical reconstructions based on medieval Arabic sources are currently in progress, and eventually will enable to obtain both a match for the molecular fingerprint and the original color appearance.

The study of color in the Santa Cruz monastery collection was carried out in the framework of a *Molab* mission, and,[23] with the aid of a portable fluorimeter, fluorescence emission spectra and lifetimes were collected *in situ* when there was evidence for the presence of a dye or pigment lake. Indigo is easily identified both by fluorescence emission ($\phi_{f,DMF} \approx 10^{-3}$) as well as by Raman spectroscopy, and it was possible to conclude that the dark blues were obtained mixing lapis lazuli with indigo. With regard to the carmine or dark red color, the presence of emission was detected

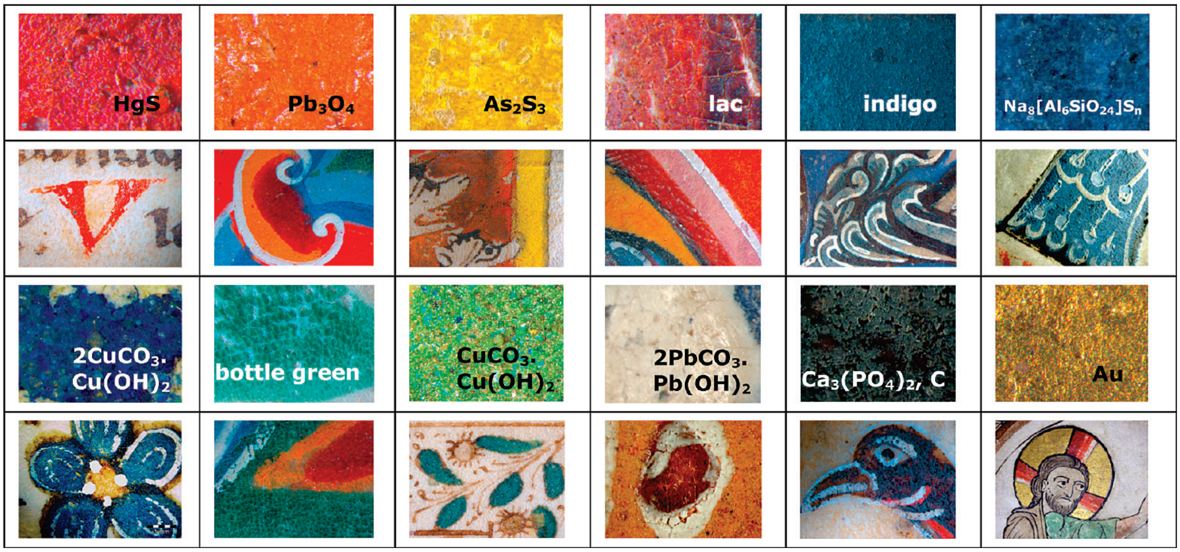

FIGURE 4. The Lorvão palette. Macrophotos for the most relevant colors and respective details, highlighting the way color is constructed in the Lorvão illuminations.

but it was low and no match was found in the Molab database. However, it was possible to conclude that the dark red was obtained again by mixing an inorganic pigment, vermilion, with a dye, not yet identified. The detection of these mixtures for the dark blue and red colors is in itself important evidence for the characterization of the *Santa Cruz* palette, as it was not detected in the manuscript collections of the two other monasteries, Lorvão and Alcobaça.

4. Case Study: Cross Sections

Paint cross sections are used to gain more insight into the technique used to create a painting, its stratigraphic structure, from the preparation layer covering the support, into the color construction and varnish protection. Microsamples are removed from the object and mounted as cross sections, which involves embedding in a synthetic resin followed by careful polishing. Paint cross sections are commonly examined by microscopy, and therefore, the strengths of microspectrofluorimetry were tested for their analysis, first in model samples, in which a chalk ($CaCO_3$) preparation and a white lead ($PbCO_3 \cdot Pb(OH)_2$) *imprimatura* were used for a fluorescent vinyl commercial paint, GEO.[4] Remarkably good excitation and emission spectra were acquired with a high spatial resolution of 8 μm, which is appropriate for the analysis of individual pigment particles or aggregates in a paint film. This was followed by analysis in cross sections from paintings by Vincent van Gogh (Van Gogh Museum) and Lucien Pissarro (Courtauld Institute of Art) (Figure 5). Red lake pigments and dyes were characterized by microspectrofluorimetry; emission and excitation spectra were obtained with high spatial resolution (8−30 μm). The fluorophores, purpurin, and

FIGURE 5. Painting cross section from *Old Mark's field* by Lucien Pissarro (Courtauld Institute), detail from a Paracas mantle (Museum of Fine Arts-Boston), and coupon reproducing the materials and techniques used in a wall painting of the Mogao Grottoes (Getty Conservation Institute).

eosin lakes were identified by comparing their spectra with those from historical reconstructions assembled in a database.

Some of these samples were also analyzed by other techniques requiring microsampling such as HPLC-DAD and SEM/EDS, and the results were consistent with those found by microspectrofluorimetry.

5. Case Study: Textiles

Pre-Columbian textiles are unique as a cultural and historical record, representing the longest continuous textile record in world history[24] (Figure 5). Fortunately, in extremely arid archeological sites, the cultural heritage of different Andean cultures such as Paracas, Nasca, Chancay, and Lambayeque has been preserved. The reds used to dye in Peru before the Inca Empire (14th−15th century) were based on purpurin chromophore obtained from *Relbunium* sp.[8,25]

Microspectrofluorimetry was used to analyze the 76 microsamples taken from different Andean textiles, dated from 200 B.C. to A.D. 1476, in the collection of the Museum of Fine Arts-Boston. The majority of the samples present a red color, but fibers with pink and purple color were also analyzed. SEM-EDX screening enabled them to confirm the use of aluminum ion, Al^{3+}, as a mordant and also to conclude that all the red samples studied were made of camelid fibers.[5]

Emission and excitation spectra were obtained in a 30 μm spot. The fluorophores were identified by comparing their spectra with those from historical reconstructions assembled in a database. In the Paracas and Nasca textiles, dated from 200 B.C. to A.D.1476, purpurin and pseudopurpurin were the red dyes used.[25] Carminic acid was detected in textiles dated close to the Inca Empire, A.D. 1000−1476. The results obtained with this new technique were confirmed and are consistent with those obtained with conventional methods, requiring microsampling, such as HPLC-DAD-MS and SEM-EDX. It is worth stressing that it was the excellent spectral resolution obtained for the reds dyed with *Relbunium* that enabled to assess both purpurin and pseudopurpurin in the emission as well as in the excitation spectra (Figure 6). This was confirmed by HPLC-DAD-MS and by comparison with the spectra obtained from a reconstructed pseudopurpurin lake (obtained after extraction followed by HPLC separation and finally complexation with Al^{3+}). In this study, emission and excitation spectra were obtained directly from fiber set in the microscope stage, but analysis could have been carried out on the entire textiles.

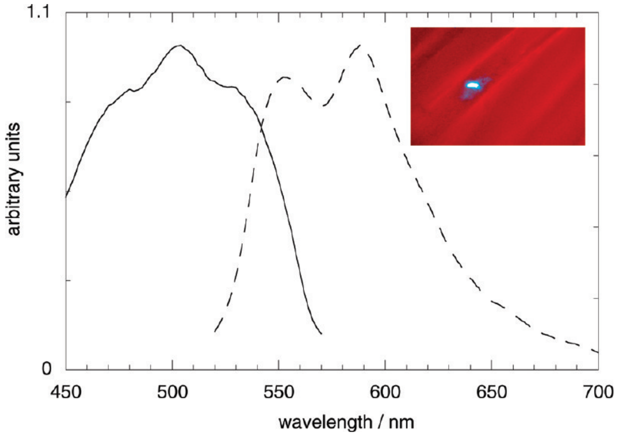

FIGURE 6. Emission and excitation spectra, acquired in a 30 μm area, by exciting directly on a single fiber for the Paracas skirt (MFA 21.2581), 200B.C. to 200 A.D.; $\lambda_{exc} = 500$ nm and $\lambda_{em} = 590$ nm. In the inset, a detail showing the 30 μm excitation spot during data acquisition.

6. Case Study: Wall Paintings

Located near the old Silk Road, in the oasis city of Dunhuang (northwestern China), a group of caves covered with wall paintings, the Mogao Grottoes, were studied by the Getty Conservation Institute (GCI). In order to develop strategies to identify the possible Asian organic colorants applied, a collection of reference samples was built, in the GCI laboratory, with several Asian biological sources (Figure 6). Those could be used in different sorts of samples, among them: pigments, dried extracts, and wall painting mock-ups of different paint combinations.[26]

HPLC-DAD-MS and FTIR were used, and good results were obtained for most of the reference samples and paint samples collected in Cave 85,[26] although some of them are still to be discovered. Without the requirement of microsampling, microspectrofluorimetry proved to be a good analytical technique to characterize even small quantities of colorant material not detectable with the traditional techniques, such as *Gardenia augusta*.

In order to be able to identify the botanical species applied in the mock-ups, two kinds of lakes were prepared with the main chromophores of each species: the first ones in homogeneous media (solution) and the other ones in solid state (powder and painted with aqueous media).

Emission and excitation spectra were obtained in an 8 or 30 μm spot for both reconstructions and the mock-up. It was possible to obtain the two spectra for all the paint combinations containing a *Gardenia augusta* layer and realize that the underlayers (clay and ground) do not interfere with the emission signal from *Gardenia augusta*. However, some small shifts in the emission spectra were observed when *Gardenia augusta* glaze was applied over the inorganic pigment paints.

The painted mock-ups provided the creation of a database which in the future can be useful to determine the presence of *Gardenia augusta* glaze over complex stratigraphies, including alum layer, seven different pigments (vermillion, atacamite, red lead, carbon black, red earth orpiment, and azurite), ground, and clay.

7. Future Perspectives

Work is currently in progress to maximize the extraction of the information present in fluorescence emission and excitation spectra acquired by microfluorimetry. The possibility of obtaining the chromophores' lifetimes together, in the same microspot, will also be fully explored. Research on the photophysics and photochemistry of historic dyes is necessary to exploit better this powerful tool and is being actively pursued. Although the molecular structures of lakes are difficult to determine by fluorescence emission alone, combined with data from other analytical techniques information about the environment of the colorant and its state of degradation can be obtained. This is one of the most promising issues to be explored, because it will enable us to characterize the structure of the metal—dye complex, which in turn may be used to fingerprint particular color recipes and to gain an insight into the paint technology of past times, a technology that did not leave any records other than the objects themselves. It will also enable us to understand better the degradation mechanisms responsible for color fading. Together this information and knowledge will promote the development of better and sustainable conservation strategies: *it is only possible to keep safe what we know well*.

The importance of historically accurate reconstructions assembled in a database was evidenced during this account. This pool of standards will be enriched by the entering of "original" data that will have been fully processed and rationalized. In a symbiotic process, the original data will enable advances in art technological source research that, in turn, will enable a deeper understanding of the work of art and its conservation state. When sufficient data have been collected, and the consistency of the database fully tested, search algorithms will be developed making this new advanced analytical tool accessible to the conservation community, and not just to the photophysics experts.

The works of art we have patiently studied are preserved in museums, archives, and other institutions; the research we have carried out has been possible by the generous support of the directors and staff from DGARG-TT (Torre do Tombo), BPMP (Biblioteca Pública Municipal do Porto), and BNP (Biblioteca Nacional de Portugal); the friendly environment they offer us, their trust in our work and in what science may contribute for a better conservation has been a continuous stimulus. It is a pleasure to thank Meredith Montague, Richard Newman, Aviva Burnstock, Klaas Jan van den Berg, Cecily Grzywacz, Jan Wouters, and the Molab team for all the colorful discussions and Professor Heather Lechtman for her kind interest. To them, Blake's little flower.

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FOOTNOTES

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