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# LEDμSF: A new portable device for fragile artworks analyses. Application on medieval pigments☆

Aurélie Mounier [a,*], Sylvain Lazare [b], Gwénaëlle Le Bourdon [b], Christian Aupetit [b], Laurent Servant [b], Floréal Daniel [a]

[a] IRAMAT-CRPAA, Institut de Recherche sur les ArchéoMATériaux (IRAMAT), UMR CNRS 5060, Centre de Recherche en Physique Appliquée à l'Archéologie (CRPAA) Université Bordeaux Montaigne, Maison de l'archéologie, Esplanade des Antilles, 33607 Pessac, France

[b] ISM, Institut des Sciences Moléculaires, CNRS / Université Bordeaux, - UMR 5255, - Bâtiment A12, 351 Cours de la Libération, 33405 Talence Cedex, France

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ABSTRACT

Spectrofluorimetry is a method, among others, with the utmost sensitivity suitable for the analysis of organic materials. Commonly available devices allow us to work on small objects, or powders or on samples deposited on quartz plates. However, the study of fragile artworks (canvas paintings, pastels, medieval manuscripts) on which sampling is prohibited and which cannot be moved to laboratories requires light portable equipment for the in situ analysis of the artwork without contact or preparation. The system has to be sensitive, non-invasive, time-effective, and with a small footprint in order to perform the data acquisition within the conservation or restoration room for the sake of minimal disturbance. A new spectrofluorimeter device using LEDs sources (LEDμSF) has been designed and its performance evaluated for the in situ characterization of artworks of our Cultural Heritage components. This equipment, invented by the authors of this paper, has recently been patented.
The system has two excitation sources (LEDs at 285 and 375 nm). The excitation light is focused onto a small spot on the sample and the excited fluorescence is optically collected and coupled into the fiber of the spectrometer. This confocal arrangement of the measurement head eliminates most of the stray light. Filters are associated with the LED to cut their reflection and their second-order effects (570 and 750 nm). The signal emitted by the sample is transmitted by an optical fiber connected to a Thorlabs mini-spectrometer (200–1000 nm). The choice of LEDs is based on various studies of the analysis of binders and pigments. 285 nm is used to study the fluorescence of blue or yellow pigments (lapis-lazuli, azurite, Egyptian blue; orpiment, lead tin yellow, stil-de-grain...) and some organic binders (protein glue, gum Arabic). 375 nm induces fluorescence of lipid binders (egg yolk, linseed oil) and red pigments (red lead, cinnabar, cochineal...). The measurements obtained have been compared with conventional laboratory equipment currently used on pigments in mediaeval paintings.
The analyses have defined the characteristic bands of the fluorescence emission of some red, blue, and yellow pigments (e.g. the red lead gives maximum fluorescence at 580 nm; cinnabar at 610 nm, bucktooth around 530 nm...). The characteristic maxima emission wavelengths allow us to discriminate most of the pigments mixed with different binders, deposited on quartz plates, on parchment or on illuminated facsimile.


## 1. Introduction

In the framework of a project studying a collection of medieval illuminations (Marcadé coll., Treasury of Bordeaux cathedral), a mobile system has been developed in order to overcome the impossibility of sampling the manuscripts and/or displacing them to the laboratory for analysis by hyperspectral imaging or micro-spectrofluorimetry [1–3].

The new device, recently patented, is based on the fluorescence properties of some materials when excited under UV light. Actually, the molecules absorb the incident excitation light, usually in the ultraviolet, to form a short living (ps-ns) excited electronic state which eventually (with quantum yield of a few percent) re-emits a photon of lower energy (larger wavelength) quite rapidly. This re-emission of fluorescent light occurs in any direction at random and with a wavelength spectrum which is quite characteristic of the structure of the molecule. This particular feature of the fluorescence spectra can be used to perform a rapid analysis of the sample of art thanks to fluorescence detectors having adequate sensitivity and a handy configuration adapted to the artwork environment.

Generally, spectrofluorimetry consists of recording emission and excitation spectra in order to identify the fluorophores and is used less frequently for measuring the fluorescence lifetime. High-performance bench top laboratory devices are equipped with high-power xenon

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* Corresponding author.
*E-mail address:* mounieraurelie33@yahoo.fr (A. Mounier).

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sources and both excitation and emission monochromators, providing high sensitivity and selectivity. Sample compartments in a basic configuration are designed to work with cells or slides. They are therefore perfectly adapted for the characterization of model samples, e.g. films made of binder mixed with pigments and deposited on a quartz slide.

However, these systems did not allow us to work on the Marcadé collection which has, among other artworks, 41 illuminations dating from the 14th to the 16th century which cannot be moved from their storage room. Firstly, their fragility and the conditions for conservation of the manuscripts paintings require in situ analysis. Secondly, their sizes vary from 6 to 60 cm and would not fit the requirements of the laboratory systems without a great deal of handling. These considerations led us to the successful development of a new, small, and mobile device adapted to the study of the collection.[1]

This new portable, lightweight device named LEDμSF can be positioned very fast and it quickly collects the emission spectra obtained upon excitation with the ultraviolet radiation of LEDs (light emission diodes), which are small powerful modern light sources. This new system was tested on models of medieval materials (pigments and binders) and the results were compared with those obtained with laboratory instruments. Moreover, a reference database has been constructed for future fast recognition of pigments and binders commonly used in medieval times.

Emission signals from paint layers can be affected by several chemical and physical phenomena. The binder concentration, the formation of different compounds (mixtures of pigments and binder), and chemical-quenching phenomena induced by some pigments can affect the fluorescence spectra. In addition, pigments may scatter and absorb the emission of the surrounding fluorescent binder [4]. Phenomena of auto-absorption between fluorophores of the fluorescent species also occur [5]. All these phenomena can change the spectral distribution and lead to a shift in the emission maxima and/or to the formation of other new bands, causing some difficulties in the interpretation of fluorescence emission [4]. To reduce these impacts on the fluorescence, a mathematical treatment based on the Kubelka–Munk theory should be applied [4–6] to correct luminescence of the binders from the effect of organic materials in paintings.

Correction for fluorescence spectra distorted by the presence of scattering and absorbing particles has not been applied as the aim of this work was essentially to assess the ability of the LED spectrofluorimeter device to measure the emission signal of medieval pigments and binders with a good signal/noise ratio and sensitivity and to show that the spectra, compared with our database, can lead to the identification of the pigments.

## 2. Materials and instrumentation

### 2.1. Materials

The reference pigments are hand-made pigments[2] prepared in our laboratory according to the recipes described in medieval treatises or supplied by the OKHRA Company.

In a first approach, the analyses were focused on

- red pigments: red lead, cinnabar, red ochre, cochineal, and brazilwood;
- blue: azurite, lapis-lazuli, and Egyptian blue ;
- yellow pigments: orpiment, lead tin yellow (type II), buckthorn, bucktooth, stil-de-grain. Organic and inorganic pigments were mixed with either one or several binders including gum Arabic, rabbit skin glue, and egg white, and were deposited on quartz slides.

One hundred fifty pigments were studied and included on a color chart on parchment and one *facsimile* of medieval illumination was prepared by Marlier's workshop. For the colors chart, the pigments were applied with a distemper technique. The *facsimile* is copied from the same Dominican antiphonary (14th). Pigments were applied with proteinic (fish glue) or sugar binders (gum Arabic, honey). The theme represented is the Nativity. The pigments most used in medieval times, blue, yellow, and red, are employed (aforesaid). Gold leaf is used on the background.

### 2.2. Instrumentation

#### 2.2.1. Portable LEDμSF (LED micro-spectrofluorimeter)

The system (Fig. 1) is composed of a Thorlabs spectrometer (CCS200/M) with a range from 200 to 1000 nm and a fiber optic (Ø 400 μm). The UV sources are two low-power LEDs, at 375 nm (5 mW) and 285 nm (300 μW), for the excitation of materials.

LEDs have associated filters: 320 nm for the LED at 285 and 455 nm for the other one. Filters allow the removal of the band due to the reflection of the LED and to the second-order effects, which are respectively 375 and 750 nm and 285 and 570 nm.

Depending on the artworks to be analyzed, it may be necessary to lower the intensity of the UV sources. For fragile objects or when the signal is saturated, it can be adjusted by neutral density filters (0.1, 0.3, or 0.6).

A black occultation system can be added to exclude contributions from external light. The working distance is about 4 cm and adjusted with lasers. The investigation area on the sample is about 1 mm in diameter.

An Arduino module (open hardware circuit including a microcontroller which can be programmed to analyze and produce electrical signals) allows control of the working distance, LED lighting, the choice of parameters (such as integration time), and the start of the analysis. It permits the activation/deactivation of laser pointers, with a red light indicator, to get the adequate working distance and the activation/deactivation of the UV sources with their respective light indicators.

The software allows the selection of the integration time with increments of 1 s to 50 s with a display of the programmed exposure duration, which cannot exceed 50 s. The time depends on the response of the materials analyzed (low or saturated signal).

Each spectrum has been corrected taking into account the background noise during the measurement to improve the signal to noise ratio. The spectra were then subject to smoothing thanks to the Thorlabs software.

The LEDμSF is light (weight < 1.5 kg), portable (15 × 15 × 8 cm) and is powered and controlled by USB connection to a portable computer and by means of a graphic interface. The prototype stand is a tripod on which a motorized translation system is easily adapted to provide a horizontal or vertical motion for accurate positioning without contact on fragile objects such as manuscripts for example. It is continuously adjustable in height up to a maximum of 1.5 m high. It has a central column that can be reversed and a 90° adjustable swivel to adjust the tilt.

To validate the results obtained by LEDμSF, they are compared with those obtained using two benchtop devices.

#### 2.2.2. Benchtop fluorescence systems

Measurements were performed using a Horiba Jobin-Yvon SPEX Fluorolog-2 spectrofluorimeter equipped with double monochromators on both the excitation and emission beams. The excitation lamp is a 450 W high-pressure xenon lamp. A thermoelectrically cooled photomultiplier (Hamamatsu R928) is used for the signal detection, using the photon counting mode. Fluorescence signals are expressed in “counts per second” (cps). Data acquisition and data processing were computer controlled (SpectrAcq Datamax run by the Grams/32

[1] This system is the result of many years collaboration between the Institut des Sciences Moléculaires of the University of Bordeaux and our laboratory, the Centre de Recherche en Physique Appliquée à l'Archéologie of the University of Bordeaux Montaigne (France). The device was patented on 24th December 2014 with the INPI (Institut International de la Propriété Industrielle). ERMA Electronique (France) is the manufacturer of our system.

[2] Workshop of Renaud Marlier, France.

**Fig. 1.** 375 nm LED in action with our lab prototype on a facsimile reproduced in accordance with the medieval techniques. The analytical head of the LEDμSF device build by the manufacturer Erma Electronique. The head is finely positioned by means of a micrometric X-Y stage in particular to adjust the working distance and the analyzed spot. The system supported by a tripod for in situ analysis.

software). Both excitation and emission spectra (recorded with 4 nm bandwidth) were corrected for instrumental factors. The measurements are made in the "front face" mode. The emitted light is collected within an angle of 22.5° with respect to the excitation beam.

Micro-spectrofluorimetry was carried out using a system Horiba Jobin-Yvon Fluorolog-3, coupled to an Olympus BX51 microscope via optical fibers. It was also equipped with a pressure xenon excitation lamp at 450 W lamp, a double monochromator for excitation, and a single monochromator for the emission beam. Spatial resolution was controlled via pinholes to select the analysis zone of microscopic size (from 5 to 100 μm using an X20 objective). Similar systems equipped with fiber optic are not yet popular and reports of applications on Cultural Heritage are scarce [7,8].

Emission spectra were collected exciting at wavelength ($\lambda_{exc}$ = 366 nm) using a dichroic filter of 375 nm and from a 100 μm spot.

The validation of the system was done on reference samples (NIST) and then on models of medieval samples. The recorded spectra were corrected to take the instrumental function into account.

The question of the impact of UV light on the materials studied is essential. Considering the portable system, LEDs deliver low power and very short time analysis (50 s maximum against 5 min for benchtop devices). Furthermore, density filters were used to reduce the intensity of the incident beam on the sample. In a previous study with benchtop devices, cinnabar proved to be the most fragile pigment, so density filters were added to reduce the power sent on to the sample. 375 nm is a commonly used excitation wavelength (wood lamp, for example) to view repairs and damages on paintings that may be invisible in ordinary light. As LED at 285 nm is more harmful, the integration time is increased progressively, depending on the nature of the fluorescence. Iterations tests (between 5 and 10 iterations) on a same point of analysis showed that the maximum wavelength does not change. As powerful xenon lamps used in some laboratory instruments give off heat, a cooling system is necessary. LEDs emit no or little heat.

Regarding microspectrofluorimetry measurements, the effect of the excitation wavelength, when exciting at 366 nm, was studied and allowed us to determine the maximum power acceptable below the damage threshold for the fragile objects. The irradiance is 400 $\mu W.mm^{-2}$ and attenuation grids were used to minimize the power.

The test was dictated by the cinnabar which appears to be the most photosensitive pigment and could present blackening when power is too high. This phenomenon was observed and became known after using analytical techniques with lasers such as Raman spectroscopy [9]. An irradiance of 55 $\mu W.mm^{-2}$ was found to be the maximum supportable for the cinnabar. So, the analyses and spectra presented here were obtained with these conditions set up within the system.

## 3. Results and discussion

Results are organized by color. First, we give the results obtained with the portable LEDμSF and laboratory instruments. The validation of the emission spectra is provided with a benchtop spectrofluorimeter allowing the acquisition of both excitation and emission spectra on materials prepared on quartz slides. Microspectrofluorimetry was also applied to validate emission spectra from small areas and to work on model samples and details of the *facsimile*.

### 3.1. Red pigments

Emission spectra of 5 red pigments mixed with gum Arabic were analyzed using the portable LEDμSF (Fig. 2) and compared to the spectra acquired using the laboratory instrument (Figs. 3 & 4).

With the LEDμSF, the emission spectra were obtained using the excitation wavelength $\lambda_{ex}$ at 375 nm. Cinnabar gives a maximum emission wavelength at 612 nm, red lead at 587 nm, cochineal at 640 nm, and brazilwood at 630 nm. Red ochre gives a very low fluorescence, and no characteristic band appears.

These results show that these five red pigments could be distinguished on the basis of their maximum of fluorescence emission band and by the shape of the spectrum (narrowband, wide band, etc.).

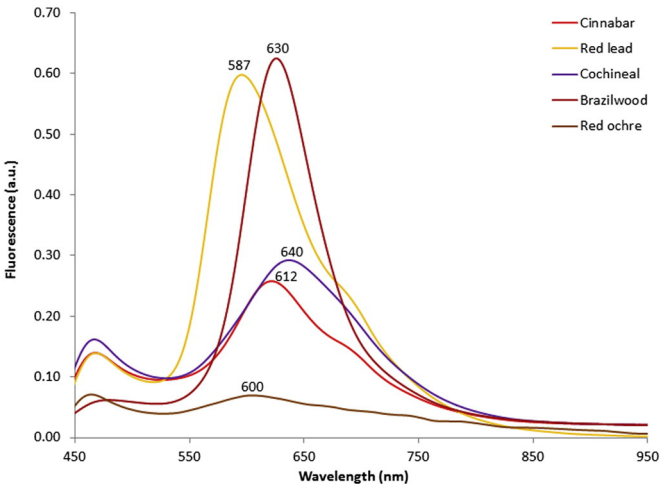


**Fig. 2.** Emission spectra of the five red pigments (red lead (integration time: 30 s), cinnabar (50 s), red ochre (30 s), brazilwood (10 s), and cochineal lake (30 s)) obtained with the LEDμSF at exc375 nm. We note that the five spectra are different and therefore pigments could be distinguished. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

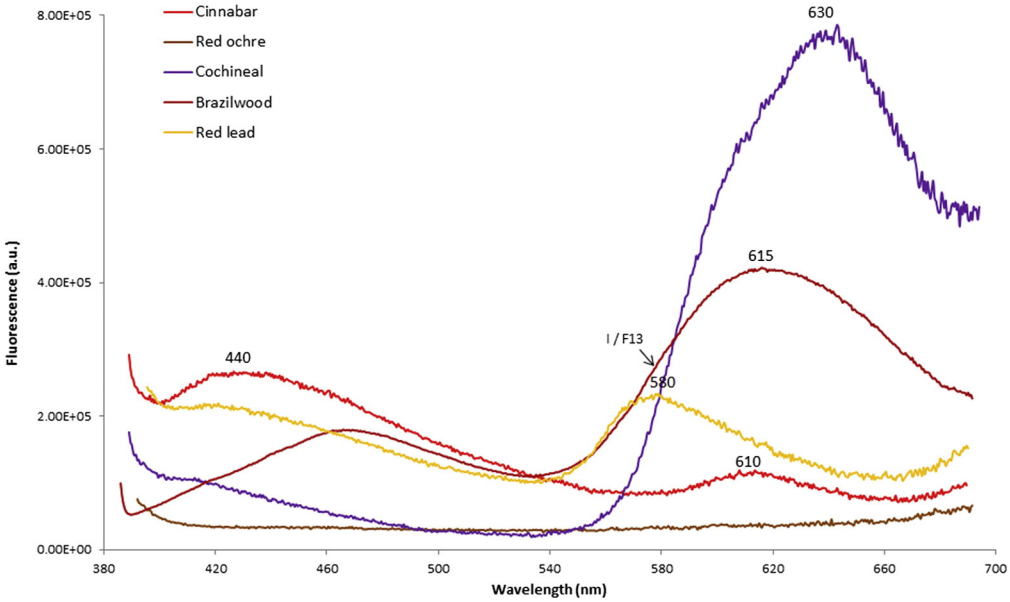


**Fig. 3.** Emission spectra of the five red pigments (red lead, cinnabar, red ochre, brazilwood, and cochineal lake) obtained with the benchtop system ($\lambda_{exc}$ 366 nm). The five spectra present different maxima wavelengths and pigments could be distinguished. To facilitate the comparison of the 5 spectra, the brazilwood spectrum intensity was divided by a factor 13. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Given the lower resolution of the LEDμSF than the benchtop devices, these results can be in good agreement with those previously obtained and published [1,10].

With the benchtop devices (Figs. 3 & 4), cinnabar (HgS) shows a fluorescence emission with a maximum wavelength at 610–612 nm which corresponds to the semiconductor HgS [11,12]. Red lead ($Pb_3O_4$) presents a band at 578–580 nm that could be associated with irregularities in the crystal structure or the presence of impurities [13].

Regarding the organic pigments, a broad and more intense band is observed at 615–618 nm for the films containing brazilwood and at a longer wavelength (630–633 nm) for cochineal. For brazilwood, the main chromophore is a weak organic acid called brazilein. The maximum observed depends on the substrate used for the fixation of the dye. In our case, we used alum and we showed in a previous article [1] that excitation and absorption spectra of brazilwood gave a maximum at 510 nm. Brazilwood studied with other substrates such as chalk or lead showed that the maximum could be different and moved toward larger wavelengths (560 nm) when the substrate is different [14].

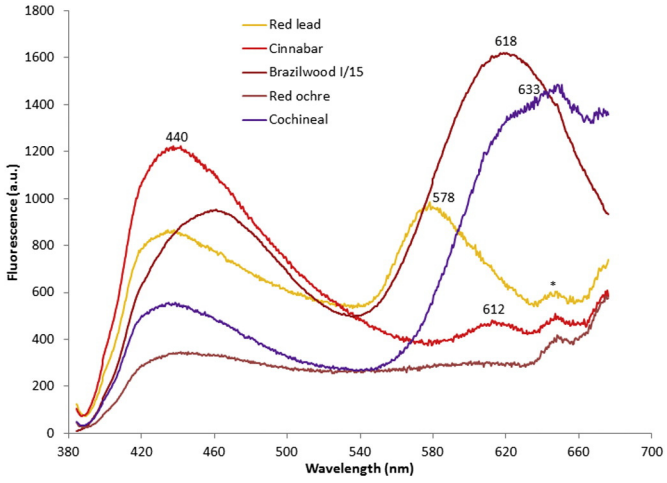


**Fig. 4.** Emission spectra of the five red pigments (red lead, cinnabar, red ochre, brazilwood, and cochineal lake) obtained with the microspectrofluorimeter ($\lambda_{exc}$ 366 nm). The maxima of the five spectra are the identical to those obtained with the two others system. Pigments are distinguished. The band noted * is an artifact. To facilitate the comparison of the 5 spectra, the brazilwood spectrum intensity was divided by a factor 15. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In the case of cochineal, this emission can be attributed to its fluorescent anthraquinone-based constituents which are predominantly carminic, kermesic, and flavokermesic acids, and also other recently identified minor anthraquinones [15,16]. Several fluorimetric studies of anthraquinoid colorants have been performed. They show the same maximum (>630 nm) for cochineal [5,17,18], such as on purpurin and alizarin currently used as madder lake [7,19].

Red ochre ($Fe_2O_3$) does not show any significant characteristic fluorescence emission. Where there is a mixture with gum Arabic, a decrease is observed in the fluorescence of the binding medium that could be explained by a quenching effect [20].

In order to confirm the attribution of the emission bands observed in the 540–700 nm region to the pigment, acquisition of excitation spectra was performed in addition to the emission spectra. For example, the excitation spectra carried out on cochineal and brazilwood films are similar to the absorption spectra measured from the pigment powders, even if some slight differences can be observed and are explained by the nature of the pigments (mixtures containing absorbing but not fluorescent species). However, this confirms that the observed fluorescence peaks in the 540–700 nm mainly originate from the red pigments [1].

Almost the same maxima are obtained for these five red pigments even if differences are observed for organic pigments with a shift of the band of about 10 or 15 nm toward short wavelengths (Table 1). Despite the loss in signal intensity, measurements using microspectrofluorimetry demonstrate the good correlation between the results obtained on the different devices and offer in addition a spatial resolution of major interest for the study of small areas. Other studies noted a very significant loss of intensity with the fiber system [7]. What is important is that the maxima band of emission fluorescence of pigments and the shape and intensity ratio for the spectra are equivalent.

### 3.2. Blue pigments

Emission spectra obtained on three blue pigments mixed with gum Arabic (azurite, lapis-lazuli, and Egyptian blue) are given in Fig. 5.

**Table 1**
Maxima wavelengths of emission of fluorescence observed for each red pigments depending the device employed. We note a good correspondence of the max wavelengths between the three devices. The two benchtop devices ($\lambda_{exc}$ 366 nm) present the same max (Δmax 3 nm) and the same shape of the curves. For inorganic pigments, the portable device ($\lambda_{exc}$ 375 nm) presents a good matching with the benchtop devices. The bigger variations are for the organic pigments (Δmax 15 nm). *nb: Narrowband; w.i: wide & intense; m: medium.

| Pigments | LEDμSF ($\lambda_{max}$) | Benchtop spectrofluorimeter ($\lambda_{max}$) | Microspectrofluorimeter ($\lambda_{max}$) |
|---|---|---|---|
| Cinnabar | 612 nm *nb** | 610 nm *nb* | 612 nm *nb* |
| Red lead | 587 nm *m.nb* | 580 nm *m.nb* | 578 nm *m.nb* |
| Red ochre | — | — | — |
| Cochineal | 640 nm *w* | 630 nm *w.i* | 633 nm *w.i* |
| Brazil wood | 630 nm *w.i* | 615 nm *w.i* | 618 nm *w.i* |

With the LEDμSF, the excitation at 285 nm was chosen to stimulate the common band of the three blues near 440 nm. Azurite presents a characteristic band at 440 nm. Lapis-lazuli presents a second band at 710 nm. And the Egyptian blue exhibits two extra bands at 560 and 890 nm. These data show that the three pigments can be discriminated (Fig. 5).

With the *benchtop system*, spectra of these three blue pigments were acquired in an emission range which did not allow us to observe the features reported on the spectra measured using the LEDμSF above 560 nm. Beyond, there is no information while the three blue pigments differ between 560 and 900 nm with the bands at 560, 710, and 890 nm (Table 2).

### 3.3. Yellow pigments

The five yellow pigments investigated were widely used in medieval manuscripts: orpiment, lead tin yellow, bucktooth, buckthorn, and stil-de-grain.

"Buckthorn" or "nerprun" and bucktooth are extracted from *Rhamnus* shrubs, whose botanical *Rhamnaceae* family includes nearly 100 species in Asia and Central Europe. Their use started at the end of the Middle Ages until the 19th century, employed as dyestuff for dyeing textiles and lake for paintings [21]. From a chemical point of view, the colored compounds are flavonoids (green fruits), anthraquinones (red berries), and anthocyanins (black fruits). The origin of the different maxima observed on fluorescence spectra is usually associated with the appearance of the ripeness of the fruits of these vegetal species. "Stil-de-grain" is the result of a mixture of "buckthorn" with tin, aluminum, and chlorine [22]. The fluorescence could be modified by the maturity of the berries with the formation of sugar and the glycosylation which perturbs the hydrogen bond and perturbs the absorption spectrum [23].

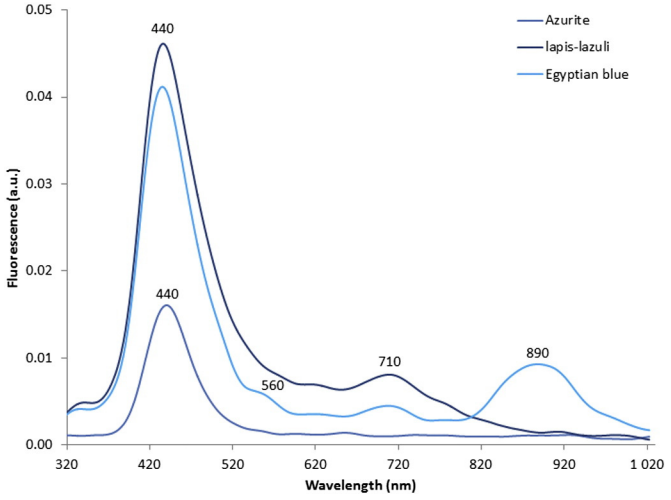


**Fig. 5.** Emission spectra of three blue pigments with an integration time of 30 s (azurite, lapis-lazuli, and Egyptian blue) obtained with the LEDμSF (exc 275 nm). Azurite gives one fluorescence band, lapis-lazuli, two and the Egyptian blue three fluorescence bands which allow us to distinguish them. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

With the LEDμSF, spectra have been acquired using the excitation wavelength 285 nm in order to detect the emission bands of pigments from 400 nm. Lead tin yellow presents a maximum of fluorescence emission around 543 nm, orpiment at 523 nm, bucktooth at 520 nm, buckthorn at 571 nm, and stil-de-grain at 550 nm (Fig. 6). The maximum emission bands of orpiment and bucktooth are similar but the orpiment spectrum is wider spread toward the short wavelengths. The system enables us to properly distinguish them.

Emission spectra measured with the *benchtop device* were obtained using the excitation at 366 nm. The reason is that excitation source at 285 nm cannot be used because the spectra are cut at 570 nm (spectrometer grating 2nd-order diffraction of 285 nm) masking the band of some yellow pigments. On the opposite of the results obtained with LEDμSF ($\lambda_{exc}$ 285 nm), at the excitation wavelength of 366 nm, the spectra from orpiment and lead tin yellow do not show any characteristic emission band. Spectrum from bucktooth gives a maximum emission wavelength at about 524 nm, buckthorn at 580, and stil-de-grain at 550 nm (Fig. 7). These emission maxima wavelengths seem to be in accordance with the appearance of the maturity of the berries. The bucktooth prepared with green fruits presents a band of shorter wavelength (524 nm). The maximum observed for stil-de-grain is intermediate and can be explained by the maturity of the fruit or of the alum and/or the tin added to fix the pigment. Then, the buckthorn is prepared with fruit at full maturity (red or black fruit) and presents its maximum emission toward the larger wavelength (580 nm).

Only a few papers report the fluorescence of stil-de-grain but results from Romani et al. [24] indicate that many factors can influence the fluorescence emission such as pH, the substrate and the maturity of the berries. In the paper, the authors indicate that the emission spectrum can be attributed to quercitrin and emits at 425 nm in their case. Fluorophores could be flavonoids with maxima absorption spectrum at 256 and 350 nm.

Concerning the results obtained for yellow pigments (Table 3), the comparison of fluorescence maxima indicates there is a good agreement between the two sets of data.

**Table 2**
Comparison of the maxima wavelengths of the fluorescence emission of the three blue pigments between the LEDμSF ($\lambda_{exc}$ 285 nm) and the benchtop system ($\lambda_{exc}$ 285 nm). For azurite, we obtain a spectrum with the portable device which allows us to identify it. With the benchtop system, bands are attributed to the binder (gum Arabic) and no to the pigment. The lapis-lazuli spectra are equivalent with the two devices. Egyptian blue was just identified with the portable system, no spectrum was obtain with the benchtop device. *nb: Narrowband; w.i: wide & intense; m: medium.

| Pigments | LEDμSF ($\lambda_{max}$) | Benchtop spectrofluorimeter ($\lambda_{max}$) |
|---|---|---|
| Azurite | 440 nm *i.nb* | 418, 439 nm *w* (gum Arabic) |
| Lapis-Lazuli | 440 i.nb, 710 nm *m.nb* | 476 nm *w.i* |
| Egyptian blue | 440 *i.nb*, 560, 714 *m.nb* & 890 nm *m.nb* | — |

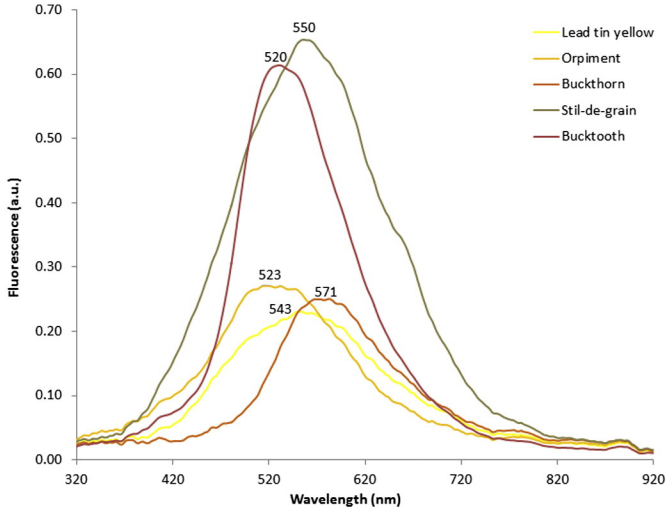


**Fig. 6.** Emission spectra of five yellow pigments with an integration time of 50 s (orpiment, lead tin yellow type II, buckthorn, bucktooth, and stil-de-grain) obtained with the LEDμSF (exc 275 nm). We note that the five spectra are different and pigments could be distinguished. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### 3.4. Application to facsimile

Red and blue pigments were investigated using the portable system on the *facsimile*.

The mobile system (LEDμSF) enabled to easily distinguish the three red pigments (Fig. 8) by comparison between the reference spectra and those obtained on red pigments of the facsimile ($\lambda_{ex}$ 375 nm). The red of Mary's dress is identified as cinnabar because the spectrum presents a maximum emission at 613 nm. For the bed cover, red lead presents a band with a maximum at 590 nm. And for the purple bed, the spectrum indicates cochineal with a maximum at 630 nm which could be attributed with brazilwood regarding its reference emission maximum. The shift of the maximum toward short wavelength could be explained by the mixture with lead white to give a pink color.

Blue colors on the facsimile analyzed using the LEDμSF also present characteristic features. The blue pigment on the top left corner of the image presents one band at 440 nm, attributed to azurite after comparison with a reference spectrum of this pigment. A second blue pigment was identified on the bottom right corner. The spectrum presents an intense band at 445 nm and a little one at 730 nm. These two bands correspond to the lapis-lazuli of our database (440 and 710 nm). Finally, a third blue pigment was identified on Joseph's blue coat. Thanks to the three bands of the spectrum (440, 700, and 890 nm), the pigment can be identified as Egyptian blue. These results are consistent with pigments used to make this facsimile. This demonstrates the potential of the LEDμSF device to record fluorescence emission spectra and by comparison with our database, to enable the identification of pigments.

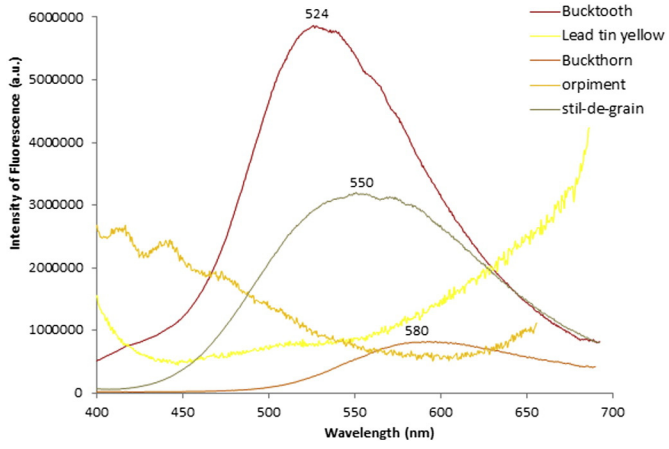


**Fig. 7.** Emission spectra of five yellow pigments (orpiment, lead tin yellow type II, buckthorn, bucktooth, and stil-de-grain) obtained with the benchtop system ($\lambda_{exc}$ 366 nm). No emission spectrum was obtained for orpiment and lead tin yellow. For the three organic pigments, we point the same maxima that of those obtained with the portable device. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 3**
Comparison of the maxima wavelengths obtained on yellow pigments with the LEDμSF ($\lambda_{exc}$ 285 nm) and the benchtop system ($\lambda_{exc}$ 366 nm). With the portable device, we obtain five different spectra which allow us to distinguish them. With the benchtop device, we just obtain the same bands for the organic pigments, no band appears for lead tin yellow or orpiment. *nb: Narrowband; w.i: wide & intense; m: medium.

| Pigment | LEDμSF ($\lambda_{max}$) | Benchtop spectrofluorimeter ($\lambda_{max}$) |
|---|---|---|
| Lead tin yellow | 543 nm *m.w* | – |
| Orpiment | 523 nm *m.w* | – |
| Bucktooth | 520 nm *i.nb* | 524 nm *w.i* |
| Buckthorn | 571 nm *i.nb* | 580 nm *w* |
| Stil-de-grain | 550 nm *w.i* | 550 nm *m.w* |

### 3.5. Binders

Three types of organic binders were used on this *facsimile*: fish glue, distemper (composed of gum Arabic, egg white, and honey) and a mixture of distemper and fish glue (Fig. 9). They were analyzed with the LEDμSF.

Using an excitation at 275 nm, fish glue shows a maximum at 440 nm, the distemper a large band (440–490 nm), and the distemper with glue at 470 nm. As regards the normalized spectra, the three types of binders could not be easily distinguished by the LEDμSF. The emission wavelength of the protein seems to always be below 470 nm [25–27]. A previous study of binders used in medieval paintings showed that gum Arabic, glues, and egg white give maximum emission wavelengths between 430 and 460 nm [28]. The fluorescence of protein can be attributed to the products of photo-oxidation, combinations, and modifications of amino acids from proteins found in collagen (tryptophan-free) [26,29–31].

More generally, the principal binders used in medieval times have been characterized using the LEDμSF system (Fig. 10). Gum Arabic and fish glue spectra present the same shape with a large band and a maximum of fluorescence emission around 440 nm and it is impossible to distinguish them with the aid of emission spectra only.

Fresh linseed oil emits at 430 nm and aging effect induces a shift of the maximum wavelength toward longer wavelength, here to 560 nm. This phenomenon can be explained by the aging process which transforms the molecules in contact with air and light by oxidation and photochemistry. Two phenomena take place: the formation of small compounds which absorb in the UV and the formation of compounds which absorb in the blue (≈400 nm) and emit in the yellow range [32]. The yellowing is correlated to an increase of the absorbance of the oil in the range of 300–400 nm and the increase of the fluorescence intensity towards 500 nm [33]. In the same way, egg yolk shows a maximum at 520 nm. The yellowing of the linseed oil is the result of a succession of oxidation and auto-oxidation phenomena which provoke the appearance of photo-unstable compounds, in particular combined polycetones during the oxidation [32,34].

The identification of the binders is more complicated when they are mixed together and combined with pigments, and is more difficult due to aging. We have now to proceed to the study of mixed materials and considering the aging effect.

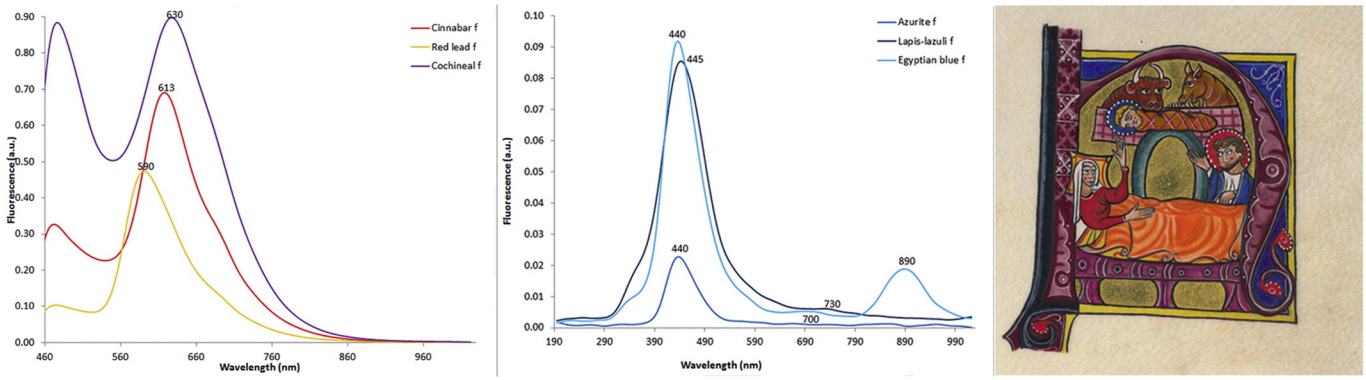


**Fig. 8.** Emission spectra of the three red pigments, (integration time: 30 s), from the facsimile measured with the LEDμSF ($\lambda_{exc}$ 375 nm). Spectra match with the reference pigments: cinnabar for the red dress of Mary, red lead for the cover the bed, and cochineal for the frame of the bed. For blue spectra ($\lambda_{exc}$ 275 nm) (integration time: 30s), azurite spectrum match with the spectra obtained on the blue in the left top corner, lapis-lazuli with the blue in the right down corner and Egyptian blue for the Joseph blue coat. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Using the two excitation wavelengths, the system enables to distinguish the two families of binders. If the maximum wavelength is ≤500 nm: proteinic or sugar binder could have been used while if the max wavelength is ≥500 nm, it can refer to a lipidic binder.

## 4. Conclusions

This methodology allows us to validate parameters and the choice of LED and to collect emission spectra of most pigments and binders used in medieval times. Analyses were carried out on model samples with both benchtop and portable systems to demonstrate the consistency of the techniques and the good matching between them in regard to the maximum wavelength of fluorescence on emission spectra. However, the study of raw and mixed materials is needed mostly to solve the binders' identification which by its colorless nature is more difficult. The study of the emission and excitation spectra initially proved to be essential in choosing the most suitable excitation LED and has permitted the constitution of a database of reference spectra in order to assign the maximum wavelength of fluorescence emission measured with the LEDμSF. The LEDμSF presents the advantage of being mobile, light, and easy to use and to implement. It allows in situ analyses and could be used on fragile artworks. Time for analyses is short. LED and filters are easily interchangeable and density filters could be added to minimize the power of the signal. However, the system only records emission spectra. Excitation spectra cannot be recorded to identify fluorophores at the origin of the fluorescence. Analyses on benchtop devices are required in order to explain and to determine the origin of the fluorescence (fluorophores…).

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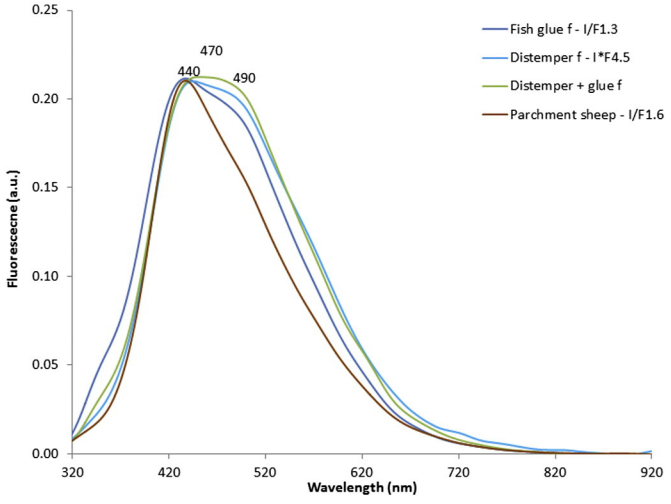


**Fig. 9.** Emission spectra of the three binders with an integration time of 30 s (fish glue, distemper, and distemper + glue) used on the facsimile obtained with the LEDμSF ($\lambda_{exc}$ 275 nm). To facilitate the comparison, the 4 spectra were normalized. The fish glue spectrum intensity was divided by a factor 1.3, the distemper spectrum intensity was multiplied by a factor 4.5 and the parchment spectrum intensity was divided by a factor 1.6.

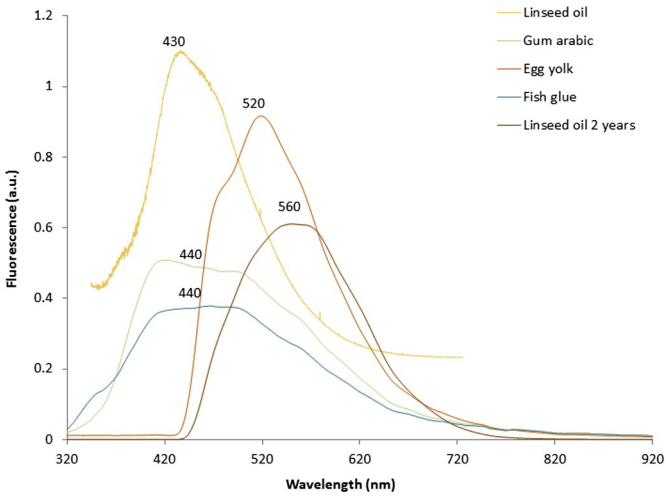


**Fig. 10.** Emission spectra on linseed oil (fresh (5 s) and 2 years old (50 ms), gum Arabic (40 s), egg yolk (5 s), and fish glue (10 s) currently used in medieval times obtained with the LEDμSF ($\lambda_{exc}$ 275 nm for proteinic or sugar binder and $\lambda_{exc}$ 375 nm for oil). Each spectrum has well characteristic shapes which make them easily recognizable.

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