Time-Resolved Fluorescence Spectroscopy and Fluorescence Lifetime Imaging for the Analysis of Organic Materials in Wall Painting Replicas

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Project Report

Organic Materials in Wall Paintings

Edited by Francesca Piqué
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Front cover: Tintori replica resample OL18BIS photographed in raking light. This is one of the wall painting replicas created by Leonetto Tintori and studied as part of the Organic Materials in Wall Painting project. The Tintori replicas are archived at the Laboratorio per Affresco di Vainella, Prato, Italy. Photo: OMWP.

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Daniela Comelli, Austin Nevin, Giovanni Verri, Gianluca Valentini, and Rinaldo Cubeddu

Introduction

Photo-induced luminescence (PL) emission, defined as the spontaneous emission of radiation from an electronically excited species following excitation by electromagnetic radiation, has long been used for the examination of works of art as a simple photographic technique (Hansell 1968; Aldrovandi and Picollo 2001; Mairinger 2004). It is characterized by spectral features and emission intensity, decay time, and polarization (Lakowicz 2006). PL consists of fluorescence and phosphorescence, depending on the nature of the excited state: the former involves the fast radiative emission from a singlet excited state (with typical lifetimes from 10^{-9} to 10^{-7} s), whereas the latter involves the much less probable and slow transition from a triplet excited state (from 10^{-6} to 10^{0} s) (Lakowicz 2006).

With advances in technology and wider use of portable noninvasive luminescence spectroscopy and imaging devices (Comelli et al. 2004; Thoury et al. 2007; Comelli et al. 2008; Romani et al. 2008; Ricciardi et al. 2009; Verri 2009; Verri and Saunders 2014), there has been an increase in applications of laser-based PL spectroscopic techniques for analysis of cultural heritage (Raimondi et al. 2009; Romani et al. 2010; Comelli et al. 2011, 2012).

These applications are related to the analysis of intrinsically luminescent organic and inorganic materials employed in works of art, including proteins, oils, waxes, semiconductor pigments, resins, and varnishes (de la Rie 1982; Miyoshi et al. 1982; Bottiroli, Gallone-Galassi, and Bernacchi 1986; Larson, Shin, and Zink 1991; Anglos et al. 1996; Thoury et al. 2007; Nevin et al. 2007, 2009; Verri 2009; Verri and Ambers 2010).

For the Organic Materials in Wall Paintings (OMWP) project, the analysis of fluorescence emission of organic materials on Leonetto Tintori's replicas was performed by irradiating samples with ultraviolet radiation from a nanosecond-pulsed nitrogen laser emitting at 337 nm (alternative UV-pulsed laser sources include the third and fourth harmonic of a Q-switched Nd:YAG laser emitting light at 355 nm and 266 nm, respectively). The properties of the PL emission (amplitude, spectrum, and lifetime) offer a means of discriminating between some of the organic compounds and assessing the chemical and physical properties of the painted surfaces.

This research is focused on analysis that employed both a time-resolved fluorescence spectrometer and a fluorescence lifetime imaging (FLIM) unit (Comelli et al. 2004, 2005). Here, the main advantage is that the emission lifetime is insensitive to variations in concentration of organic material and independent of the intensity of the fluorescence emission. In addition, changes in fluorescence lifetime may be

related to composition and chemical modifications of organic materials, as well as to the influence of the microenvironment on the selected organic samples (e.g., pigments, varnishes).

It is recognized that in situ identification of organic materials used on painted surfaces using fluorescence spectroscopy is not straightforward (Verri et al. 2008; Romani 2010). This is due to intrinsic similarities in emission spectra of many of these materials. Also, the complex attenuation of emission by optical absorption from other non-emitting materials (e.g., some pigments) is a known problem and may significantly distort fluorescence spectra (Verri et al. 2008; Clementi et al. 2009). Nevertheless, UV-induced fluorescence can be employed as an effective technique for discriminating between different complex mixtures of painting materials. This is a particular advantage of FLIM, which is suited to analysis of large painted surfaces and to assessment of variation in fluorescence lifetime related to heterogeneities rather than to the specific identification of materials, which requires careful interpretation of data and can be better achieved by integration with complementary analysis.

Context

Analysis of binding media used in paint has been performed with PL spectroscopy on a variety of model samples, films, and mock-ups of oil- and protein-based binding media. The most recently published results of fluorescence spectroscopy for binding media using a variety of laser sources are summarized in table 1 (Nevin, Spoto, and Anglos 2012). In contrast to the fluorescence of resins used for varnishes (Thoury et al. 2007), the fluorescence of proteins is better understood, and the identity of different fluorophores has been suggested (Nevin et al. 2006). Protein-based binding media contain many fluorophores; hence, the emission spectra of proteins are strongly dependent on excitation wavelength.

Spectroscopic discrimination between pure protein-based binding media (egg white and casein; egg yolk and animal glue) requires the use of excitation below 300 nm, which is sufficient to excite emissions from aromatic amino acids (see table 1). When excitation wavelengths greater than 300 nm are employed and emission is detected only above 380 nm, spectra recorded of protein-based media are generally similar in shape but may differ slightly in fluorescence lifetime (Nevin et al. 2007). Egg yolk films emit at 425 nm, whereas the addition of a drying oil causes a shift to 450 nm (Castillejo et al. 2002). The addition of linseed oil causes broad fluorescence emissions, which may shift from 492 to 683 nm (de la Rie 1982; Larson 1991) with prolonged natural aging. Generally, lifetimes of binding media are on the order of 3–6 ns.

The addition of pigments to protein-based and oil-based films may significantly modify the detected fluorescence from the binder (de la Rie 1982; Verri et al. 2008; Nevin et al. 2008). Results suggest that, with low binder-to-pigment ratio, the weak signal of intrinsic fluorescence may limit the application of fluorescence spectroscopy and imaging for the identification of organic binders. In some cases, the optical absorption or quenching effect by the pigment could be so strong as to completely obliterate the fluorescence emission by the binder and therefore produce false-negative results.

TABLE 1
Laser-induced fluorescence spectroscopy of protein-based binding media where Tryptophan (Trp) and Tyrosine (Tyr) attributions are given only when reported (Nevin et al. 2012).

Organic material	Excitation wavelength	Emission wavelength/ nm	Lifetime/ns	Attribution
Animal glues	248	305, 385		Tyr, pentosodine
	337	415	4.7	di-tyrosine
		430–440		
	355	440, 415, ~480		Pyridinoline, di-tyrosine, dihydroxyphenylalanine and related products
Calcium caseinate	337	460	6.0	
Casein	248	340, 420		Trp,di-tyrosine
	266	310, 330		Tyr, Trp
	355	435		Oxidation products
	363.8	456		
Egg white	248	340, 420		Trp, di-tyrosine
	266	310, 330		Tyr, Trp
	337	415	5.3	di-tyrosine
	363.8	588		
	355	435		Oxidation products
Egg yolk	248	515–440		Phospholipids
	266	310, 330		Tyr, Trp
	337	425	4.0	
Egg yolk – linseed oil – tempera	248	450		
Oil – Linseed—aged 1 year	363.8	492		
Oil – Linseed—aged 50 years	363.8	685		
Wax	337	500–550	6.0	

Materials and Methods

Time-resolved laser-induced fluorescence spectroscopy and FLIM data were acquired from the surfaces of the replicas. Results from different replicas are presented in the following sections.

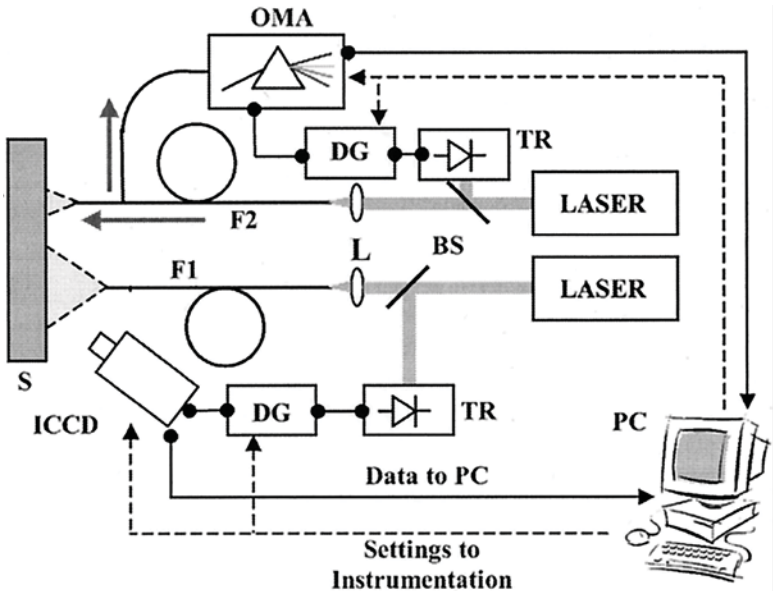
Time-Resolved Laser-Induced Fluorescence Spectroscopy

A time-gated visible spectrometer (Optical Multi-Channel Analyzer, EG&G Princeton Applied Research, Princeton, NJ) with sensitivity in the 370–650 nm

spectral range was employed for estimating the emission decay kinetic of each sample as a function of the emission wavelength (Comelli et al. 2004). A schematic of the device is shown in figure 1.

The spectrally and lifetime-resolved device is based on a time-gated detector in the nanosecond time range coupled to the visible spectrometer. UV-pulsed excitation (at 337 nm) is provided by a pulsed nitrogen laser (VSL-337ND-S, Laser Science Inc., Franklin, MA). The laser beam is sent in a fiber bundle that is placed in gentle contact with the sample through a metallic spacer covered with a Teflon ring. The fiber bundle is made of a central fused silica fiber (Ø=600 µm) that delivers excitation radiation to the sample surface, and of 20 fibers (Ø=200 µm) arranged in two circular rings, which collect the emitted fluorescence and deliver it to the detector. The time-gated detector is a linear intensified camera characterized by a fixed gate width of 10 ns and a temporal jitter close to 0.2 ns. To reduce the noise component, the detector is cooled down to 5°C with an internal Peltier. A cutoff filter (B+W UV 010) placed before the entrance slit of the detector is used to remove the excitation light from the recorded spectra. A simple trigger unit based on a fast photodiode permits synchronization of the detector with the laser pulse. Completing the apparatus is a delay generator capable of delays from 0 to 100 ns in 0.5 ns step. The automatic setting of all instrumental parameters during measurements is controlled using a PC with dedicated software, implemented within the LabWindows environment (National Instruments, Austin, TX). To analyze the kinetics of the emission, a set of fluorescence spectra is acquired after different delays with respect to the excitation pulses and processed according to a monoexponential decay model. A program for offline analysis has been developed using Matlab (MathWorks, Natick, MA).

FIGURE 1
Schematic of fluorescence devices used at the Politecnico di Milano. LASER = pulsed nitrogen laser; BS = beam splitter; L = focusing lens; F1 = silica fiber; F2 = silica fiber bundle, ICCD = time-gated intensified camera; OMA = time-gated spectrometer; TR = optical trigger circuits; DG = delay generators; S = Tintori replica surface; PC = personal computer.



Fluorescence Lifetime Imaging

The FLIM system (Comelli et al. 2004) is based on a time-gated intensified camera (ICCD225, Photek, St. Leonards-on-Sea, England, UK) exhibiting a minimum gate width of 10 ns. A sequence of images is acquired by activating the gate of the image detector at different delays with respect to excitation pulses. In this way, the temporal behavior of the fluorescence emitted by each pixel is recorded. Then, by applying a suitable fitting procedure based on a monoexponential decay model, the fluorescence lifetime map of the field of view is reconstructed.

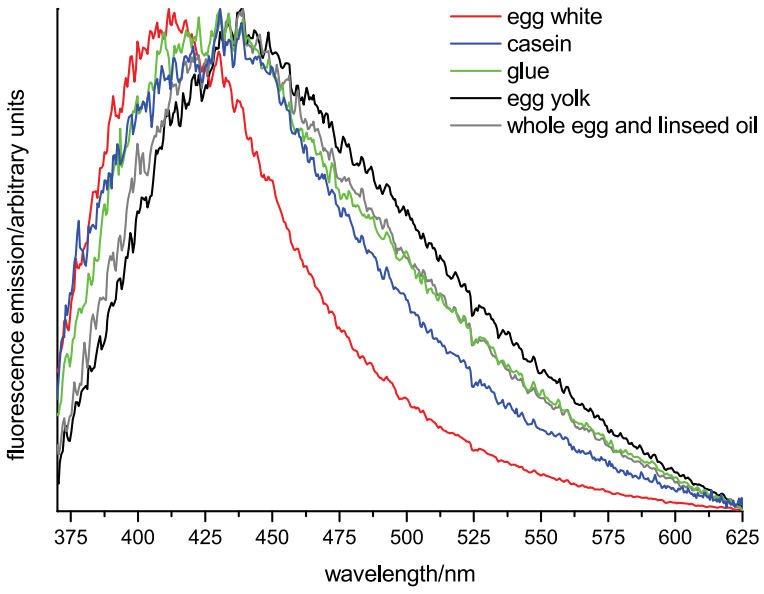
The UV excitation light (=337 nm) is provided by a second nitrogen laser (LN203C, Laser Photonic, Orlando, FL) that generates 1-ns-long pulses, synchronous with the gated camera, at a repetition rate of 50 Hz. The excitation beam is coupled to an optical silica fiber having a core diameter of 600 µm and delivered to the replica in a circular area of about 20 cm in diameter. Typical irradiance is kept below 3 µW/cm^2. A custom-made trigger circuit and a precision delay generator (DG535, Stanford Research Systems, Sunnyvale, CA) allow temporal sampling of the emitted fluorescence. The entire system has been assembled in a portable rack of about 60 × 60 × 70 cm.

Results and Discussion

Analysis of Pure Binders: replica M4bis

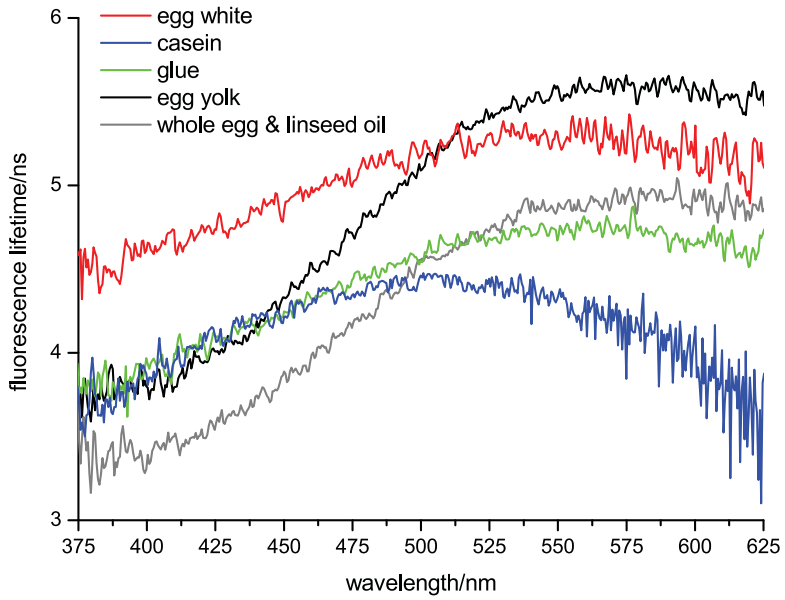
Different sectors on replica M4bis (see appendix 1), prepared with egg, egg white, glue, and proteins mixed with oil, have been analyzed using time-resolved fluorescence spectroscopy. For binders, fluorescence excited at 337 nm is ascribed to the presence of different fluorophores in the binding media (see table 1 for published attributions). Figure 2 shows the normalized emission of the sectors painted with pure binder without the addition of a pigment. The fluorescence emission of pure egg white is hypsochromically shifted (at 410 nm) with respect to that of the other binding media; mixtures containing both protein and lipids (egg yolk, and egg mixed with linseed oil) yield the broadest fluorescence with maxima at approximately 435 nm. Collagen-based glue and casein are not easily distinguished from protein-lipid mixtures on the basis of the spectral emission at 337 nm excitation (Nevin et al. 2007).

FIGURE 2
Normalized fluorescence spectra of organic binder from replica M4bis (excitation wavelength 337 nm).



Fluorescence lifetime of the same binders shows a complex behavior varying with emission wavelength—that is, shorter lifetimes for shorter wavelengths and longer lifetimes for longer wavelengths—hence suggesting the presence of fluorophores with different decay kinetics in each binder. Focusing on the 375–500 nm spectral range, where all binders show a maximum fluorescence emission, egg white exhibits the longest lifetime (4.5 ns at 400 nm) and glue and casein have shorter lifetimes close to 4.2 ns at 430 nm. Egg yolk and whole egg mixed with oil show a similar trend in the spectral variation, which increases with emission wavelength for both binders. More specifically,

FIGURE 3
Fluorescence lifetime as a function of emission wavelength of different binding media from replica M4bis.



egg mixed with oil has the shortest lifetime (3.7 ns at 430 nm), while egg yolk exhibits a lifetime of 4.1 ns at 430. These observations are in agreement with the trends reported for the analysis of binding media on quartz substrates (Nevin et al. 2007). Results are shown in figure 3.

Effects of Pigments on Fluorescence of Organic Media

The influence of pigments on fluorescence depends on a number of factors:

1. Optical absorption of the luminescence emission by the colored pigment (Verri et al. 2008; Clementi et al. 2009)
2. Possible luminescence of the pigment (Clementi 2009)
3. Modification of the binder microenvironment, which could induce quenching phenomena and chemical interactions or reactions between binder and pigment and between binder and substrate (Lakowicz 2006)

Binders mixed with ocher pigments: replicas Z- and N-

In the Z- series (see appendix 1), the addition of increasing concentrations of ocher pigment leads to a decrease in the intensity of the fluorescence of the various organic binders and to a modification of fluorescence spectra (see Verri 2008 for a detailed description of the fluorescence emission of replica Z8). This is caused by selective optical absorption of the fluorescence of egg (the binder used in Z8) by the yellow-colored pigment. However, no significant differences are observed in the fluorescence lifetime between the unpigmented and pigmented areas. This is ascribed to the lack of influence on the fluorescence decay from the inert yellow pigments (i.e., no influence of the microenvironment on the binder fluorophores). As observed for pure egg samples, replicas painted in egg yolk mixed with yellow ocher exhibit a dependence of fluorescence lifetime with wavelength, which increases from 4.0 ns at 380 nm to 5.0 ns at 580 nm. Casein mixed with the same pigment exhibits a smaller variation in lifetime with wavelength, while an average

fluorescence lifetime of approximately 4.9 ns is observed in the different sectors of replica Z9.

Interesting results have been observed on samples painted *a fresco*, *a stanco*, and *a secco*: for *stanco* replica N16, painted in yellow ocher mixed with egg yolk (see appendix 1), there is a decrease in fluorescence lifetime in comparison with the lifetime of samples painted *a fresco* and *a secco*. It is not known for certain the reason behind this phenomenon; however, it has been shown that calcium ions increase fluorescence lifetime of fluorophores (Ross et al. 1991). Therefore it may be suggested that the longer lifetime in sectors painted *a fresco* could be caused by the interaction between Ca^{2+} and fluorophores present in binding media. Chemical modifications of binding media (hydrolysis) due to the alkalinity of the plaster are also possible, but the fluorescence emission spectra of the sectors in N16 are the same. In sectors in replica N34 (see appendix 1) painted with animal glue, the average fluorescence lifetime increases to 4.6 ns (*fresco*) compared to 4.0 ns (*secco*); the same trend is found in sectors painted with casein in replica N25 (see appendix 1) with fluorescence lifetimes of 4.7 (*fresco*) and 4.5 ns (*secco*).

Binders mixed with lead white and copper carbonate pigments: replicas AZ- and OL-

The fluorescence spectrum and lifetime of lead white, calcium carbonate, malachite, and azurite (AZ) mixed with whole egg in replica AZ2bis (see appendix 1) are strongly influenced by the phase of application and show noticeable changes from *fresco* to *stanco* (48 hours). The *fresco* substrate contributes to a slight hypsochromic shift of the fluorescence spectrum in comparison with that of pure whole egg. This outcome could be the result of a chemical reaction between the alkaline substrate, pigments, and binding medium or optical absorption (fig. 4). Fluorescence lifetime varies, with binders applied *a fresco* exhibiting a longer emission with respect to those applied *a secco* (fig. 5). A similar effect was observed in samples containing ocher pigments (see appendix 1).

FIGURE 4
Normalized fluorescence spectra of sectors painted at different times from replica AZ2bis containing whole egg, b1 (*a fresco*), and b4 (*a secco*) (excitation wavelength 337 nm).

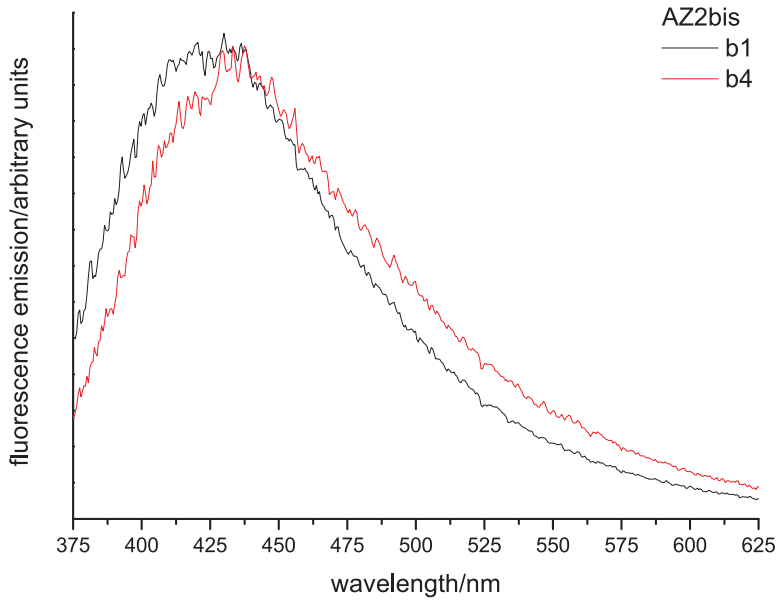
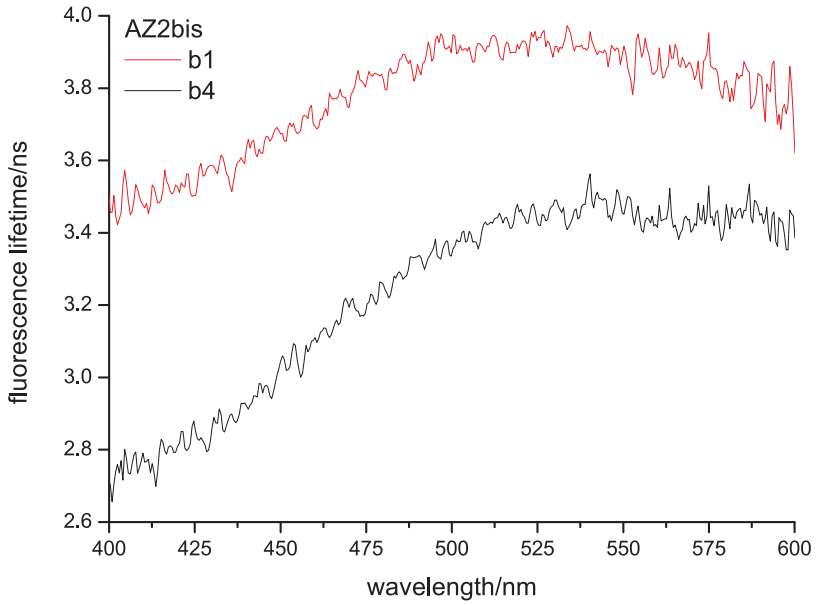


FIGURE 5
Fluorescence lifetime as a function of emission wavelength of sectors painted at different times from replica AZ2bis containing whole egg, b1 (*a fresco*), and b4 (*a secco*).



In the replica painted in glue with the same mixture of pigments used in replica AZ1bis (see appendix 1), smaller differences are observed in the average lifetime values for paint bound in glue and painted *a fresco* and *a secco*. However, the same trend is noted: fresco paint has a longer lifetime than the same paint applied a secco. In addition, the paint bound in glue mixed with the complex mixture exhibits a shorter lifetime than that observed in pure binder (M4bis), and it is possible that, in the AZ1bis replica, copper ions (Cu^{2+}) from malachite and azurite act as possible quenchers for the fluorescence emission, giving rise to a decrease in fluorescence lifetime (Lakowicz 2006).

The addition of oil to the paint in AZ1bis also influences the detected fluorescence. For example, in sector b3 (painted with only glue) an average lifetime of 3.3 ns is observed, whereas glue mixed with oil in sector c3 exhibits a lifetime of 3.6 ns, suggesting that the addition of drying oil (which also fluoresces) leads to an increase in average lifetime. Fluorophores from fatty acid oxidation may contribute to this fluorescence (de la Rie 1982). It is also possible that fatty acid soaps formed upon reactions between basic lead carbonate and lipids could contribute to the fluorescence of the organic materials.

The most complex replica analyzed (see appendix 1) contains whole egg mixed with linseed oil as binder with several pigments (lead white, significant concentrations of copper-containing carbonate pigments—malachite and azurite—and madder lake), painted *a fresco* and *a secco*. The interpretation is complicated by several factors: copper (II) ions are well-known quenchers for fluorescence (Lakowicz 2006), and it has been shown that they influence the fluorescence spectrum of protein-based binding media (Nevin et al. 2008). Basic lead carbonate (lead white) can react with fatty acids to form lead soaps (Keune and Boon 2007). Madder is a fluorescent pigment that emits at around 610 nm (Clementi et al. 2008) and has a short lifetime (on the order of a few nanoseconds) (Romani et al. 2008, 2010).

Time-resolved fluorescence spectroscopy was employed to assess the influence of pigments on the fluorescence spectrum and fluorescence lifetime. FLIM was carried out to assess the technique for differentiating larger areas from the different sectors on the replica. The detected fluorescence emission depends on the presence of concentrated malachite and azurite, as well as fluorescent pigment madder. The emission spectra are reported in figure 6. For sectors containing malachite and azurite, the fluorescence spectrum of the binder is attenuated by both quenching and optical absorption by the green and blue pigment, causing a bathochromic shift in the detected emission (especially noticeable in the malachite sector, a2) and a general broadening of the spectrum (Verri et al. 2008). The emission from the sector painted with madder (a4) is clearly visible in the red range; madder absorbs UV radiation and part of the fluorescence from the binder between 350 and 450 nm. Both contribute to the overall fluorescent emission between 500 and 600 nm (Clementi et al. 2008), appearing as a shoulder in the fluorescence spectrum in shown in figure 6.

The fluorescence lifetime measurements of the OL- series (see appendix 1) are more difficult to interpret. Although the general shape of the spectral dependence of fluorescence lifetime of the different sectors containing the oil-egg mixture is similar, the lifetime is different for each sector. The presence of madder in sector a4 reduces the fluorescence lifetime above approximately 480 nm; this is ascribed to the contribution of emissions from the pigment, which, on its own, has a lifetime of approximately 1.5 ns (Romani et al. 2008). Copper-based pigments (sectors a2 and a3) significantly reduce the fluorescence lifetime, possibly due to the quenching of fluorescence by Cu^{2+} ions, which may occur by a Förster resonance energy transfer (FRET) mechanism (Hötzer et al. 2011). The fluorescence lifetime of the sector containing malachite is shorter than that observed in paint containing azurite. This may be related to the greater solubility of the pigment in the oil-egg mixture (Preis and Gamsjäger 2002). Finally, as observed in AZ- replicas, sectors

FIGURE 6
Fluorescence spectra of sectors painted *a secco* from replica OL17b (excitation wavelength 337 nm).

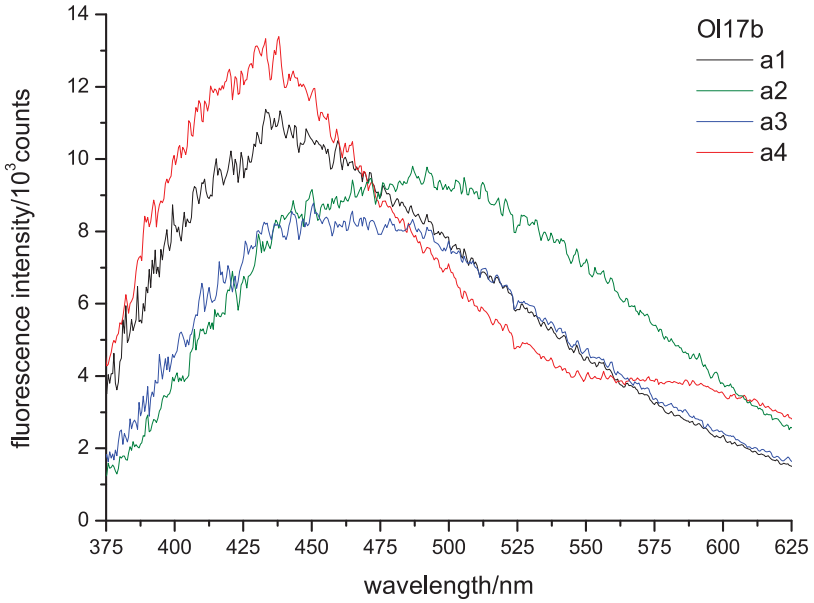


FIGURE 7
False-color FLIM of replica OL17b. The colors of the circles represent different lifetimes.

painted out *a fresco* have decreased fluorescence lifetime with respect to those painted out *a secco*.

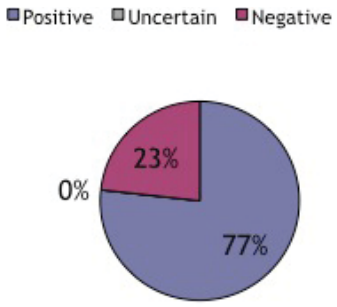
Although FLIM analysis produces images calculated based on the decay in the amplitude of fluorescence integrated in the whole visible range, results acquired with this setup yield trends similar to those reported above for time-resolved fluorescence spectroscopy. False-color images of different sectors, shown as small circles in figure 7 and indicating average decay time, yield average lifetimes that decrease with the addition of copper-based pigments, following the trend observed in spectroscopy (see fig. 6). Furthermore, FLIM measurements suggest that sectors painted *a fresco* have longer fluorescence lifetimes.

FLIM has the advantage of providing spatial information for each measurement, as each pixel contains information related to the temporal decay of fluorescence; consequently, FLIM is less sensitive to the positioning of the fiber in comparison with time-resolved fluorescence spectroscopy. In addition, the technique can be used to rapidly detect differences between larger areas; this discriminating power is its strength. Results from complementary techniques, such as time-resolved fluorescence spectroscopy and multispectral fluorescence imaging (Comelli et al. 2008), can be used to further explain specific changes in fluorescence emission between different samples.

Levels of Identification

Through fluorescence lifetime spectroscopy, it is possible to assess the presence of an organic binder (level 1). While the lifetime might provide some indications of the nature of the binder (level 2), more research is necessary to establish the potential of the technique. Therefore, the criteria used to identify the presence or absence of an organic binder in level 1 include the following:

FIGURE 8
Chart representing the correlation factors for the level of information obtained.



- Organic material present: presence of an emission band with a clearly defined maximum
- No organic material present: absence of an emission band

Figure 8 charts the results obtained after the evaluation of 142 sectors as blind tests and using the above criteria. Level 1 is reached with good confidence (77% of positive results). The negative results are attributed mainly to the presence of false negatives due to the absorption or quenching of fluorescence by pigments.

Conclusions

Analysis of organic materials in wall paintings using fluorescence spectroscopy presents challenges based on the difficulty in interpreting variations in emission spectra, modifications in fluorescence lifetime, and interference from pigments and other materials present in and on the painted surface. Analysis of the naturally aged and well-characterized Tintori replicas suggests possible explanations for the observed fluorescence of different samples. In the absence of pigments, it may be possible to discriminate between different binding media on the basis of spectral lifetime and fluorescence emission. Nevertheless, the use of only one excitation wavelength limits the discrimination capabilities of the technique; excitation spectra recorded with various excitation wavelengths have been shown to be extremely useful in achieving this goal (Nevin et al. 2006). Moreover, complementary analysis of fluorescence lifetime using shorter UV excitation wavelengths (e.g., the fourth harmonic of an Nd:YAG laser at 266 nm), coupled with sensitivity in the UV range, would be advantageous and would allow detection of signal from amino acids.

When binding media are mixed with pigments, modifications of fluorescence spectra often occur due to optical absorption of the emitted radiation by the colored pigment. In some cases, this effect may be mathematically corrected on the basis of visible reflectance analysis (Verri et al. 2008; Clementi et al. 2009). Pigments may also interact chemically with binders. For example, from the analysis of fluorescence lifetime, it is evident that copper-containing pigments tend to reduce the lifetime of emissions. Finally, paint applied *a fresco* exhibits longer lifetime than paint *a secco*; this effect may be caused by the presence of calcium ions in the binding matrix/substrate.

This work has demonstrated the extreme sensitivity of fluorescence. However, its use as an analytical technique for the identification of binding media is not straightforward. Many unanswered questions remain regarding the fluorescence of organic materials, the influence of their preparation, their interaction with other painting materials, and aging and degradation. Another issue not investigated in this study is the interference of commonly used conservation materials and consolidants on the fluorescence of surfaces.

In conclusion, the attribution of fluorescence spectra to specific compounds is not possible at this stage of the research. However, complementary optical spectroscopic technique, including Raman spectroscopy and fiber optic reflectance spectroscopy (FORS), may provide key data for the interpretation of fluorescence.

FLIM analysis has the advantage of providing spatial information on variations in fluorescence lifetime, which is independent from its intensity. This has allowed

the detection of the same organic material irrespective of concentration (e.g., on N- and Z- replicas). One difficulty in applying the technique is the interpretation of results, which often requires integration with other complementary techniques, imaging, and statistical analysis (Comelli et al. 2011). The instrumentation could be improved, such as the use of more compact and transportable laser sources (Comelli et al. 2012) to make the application of FLIM more straightforward, even if the instrumentation itself is research grade and not commercially available.

When carefully interpreted, fluorescence and lifetime spectroscopy and imaging can thus provide useful information related to organic materials and their application in wall paintings.

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