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UV-Vis spectroscopy

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Abstract:
UV-Vis reflectance spectroscopy has been widely used as a non-invasive method for the study of cultural heritage materials for several decades. In particular, FORS, introduced in the 1980s, allows to acquire hundreds of reflectance spectra *in situ* in a short time, contributing to the identification of artist's materials. More recently, microspectrofluorimetry has also been proposed as a powerful non-invasive method for the identification of dyes and lake pigments that provides high sensitivity and selectivity. In this chapter, the concepts behind these spectroscopic methodologies will be discussed, as well as the instrumentation and measurement modes used. Case studies related with different cultural heritage materials (paintings and manuscripts, textiles, carpets and tapestries, glass, metals, and minerals), which show the usefulness of UV-Vis reflectance spectroscopy and microspectrofluorimetry applied to the study of artworks, will also be presented.

Keywords: non-invasive, UV-Vis spectroscopy, reflectance, FORS, microspectrofluorimetry, paintings and manuscripts, textiles, carpets and tapestries, glass, metals, minerals

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1 Introduction

In order to provide the appropriate tools for curators, conservators, and conservation scientists to understand and preserve the cultural heritage, it is important to analyse, identify, and characterise the materials used in the production and restoration of artworks, which are often complex systems [1, 2]. Presently, the study of the materials constituting artworks can be performed using invasive and/or non-invasive approaches including spectroscopic methods which can be classified according to the region of the electromagnetic spectrum used or produced in the measurement [2].

In its broadest sense, ultraviolet-visible spectroscopy is concerned with interactions between electromagnetic radiation in the ultraviolet-visible region and matter. The ultraviolet (UV) region covers approximately the 10–380 nm range of the electromagnetic spectrum. It is commonly divided in three main sub-regions, which are: UVA in the 320–380 nm; UVB in the 280–320 nm; and UVC in the 100–280 nm. In addition, the 10–200 nm range is also named as vacuum ultraviolet (VUV), even though it is only explored if measurements are done in vacuum. The visible (Vis) region comprehends the 380–750 nm spectral range.

UV-Vis spectroscopy is related to excitation of the outermost electrons of the atoms, which are involved in the formation of molecules, and is, therefore, often referred to as "electronic spectroscopy" [3]. Measurements in the UV-Vis region are usually performed in transmittance, reflectance and photoluminescence (fluorescence and phosphorescence) modes. Transmittance and reflectance measurements need to be recorded against a reference material while photoluminescence acquisitions can be considered absolute measurements.

In the art conservation field this spectroscopic technique, unlike the most traditional applications of UV-Vis spectroscopy [3], is mainly applied *in situ* by using portable devices and employing non-invasive methodologies. The studied artworks have, excluding a few cases such as glass items and stained glass windows, an opaque appearance that force them to be measured in reflectance mode. Spectra acquired in reflectance mode on bulk samples or masstone pigments and dyes are usually more difficult to interpret than the corresponding transmittance spectra due to several factors affecting reflectance measurements, such as the surface roughness and texture, particles dimension and distribution, packing density, compositional homogeneity and the thickness of the paint layers.

Spectroscopic measurements in the Vis were introduced to the cultural heritage field by Rawlins at the National Gallery in London in the 1930-1940s [4]. At the same time, Vis reflectance spectroscopy was applied to

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characterise paints and pigments for the first time during the 1930s at the Department of Conservation and Technical Research of the Fogg Art Museum, in Cambridge, Massachusetts [5]. After the World War II, in Rome at the Istituto Centrale del Restauro (now Istituto Superiore per la Conservazione ed il Restauro) Manlio Santini developed a precise and reliable non-invasive methodology for the acquisition of reflectance spectra in the Vis on the painting Maestà by Duccio di Boninsegna to monitor the cleaning procedure during its conservation treatments [6]. In the same years, Vis reflectance spectroscopic methodologies were strongly improved by researchers working mainly in paint formulation laboratories and paint industries [7]. In the years that followed this methodology was further developed at the Conservation Laboratories of the National Gallery in London [8]. Lastly, since the early 1980s, when fibre optical devices became available and starting from the pioneering work of Mauro Bacci at the Istituto di Ricerca sulle Onde elettromagnetiche of the Consiglio Nazionale delle Ricerche (which became Istituto di Fisica Applicata "Nello Carrara" in [9]), the non-invasive technique of fibre optic reflectance spectroscopy (FORS) has been applied and improved in this field including the use of portable instruments and expanding the operating range from the UV up to the near-infrared (NIR, 750–1100 nm) and short-wavelength infrared (SWIR, 1100–2500 nm) regions [10–16].

After the work of E. René de la Rie in [17], fluorescence spectroscopy has also become a powerful analytical technique in the field of cultural heritage [17, 18]. More recently, microspectrofluorimetry, which combines the advantages of fluorescence measurements with those of a confocal microscope, was introduced into the art conservation field particularly for the study of organic colourants and binders [19–24]. Maria João Melo and co-workers have been pioneers of the development of microspectrofluorimetry, combined with data from other analytical techniques, to characterise dyes and lake pigments, and of the creation of a reference database to overcome the absence of a molecular fluorescence fingerprint [25–29]. Microspectrofluorimetry provides high sensitivity and selectivity, combined with good spatial and spectral resolution and fast data acquisition [20]. Moreover, for movable objects that can be transported into the laboratory, it can be used *in situ* without any contact with the artwork or (micro) sample under study [20].

In this chapter, the use of UV-Vis reflectance spectroscopy and microspectrofluorimetry in the study of cultural heritage will be discussed. The principles and theory behind these spectroscopic methodologies will be presented, followed by a section on the instrumentation and measurement modes used. The chapter will end with the presentation of some relevant case studies related with different cultural heritage materials (paintings and manuscripts, textiles, carpets and tapestries, glass, metals, and minerals), which show the usefulness of UV-Vis reflectance spectroscopy and microspectrofluorimetry as *in situ* methods applied to the study of artworks.

2 Principles and theory

Ultraviolet and visible radiation that hit a surface can interact with matter in different ways: it can be transmitted, transmitted in a diffuse way, reflected (in a specular and diffuse way, as discussed below), absorbed, absorbed and emitted as photoluminescence (fluorescence and phosphorescence), or laterally diffused at wavelengths different from those of the incident monochrome radiation (diffusion or Raman effect) [30]. If the frequency of incident radiation corresponds to the difference of energy of a transition between two energy levels that specific frequency is absorbed by the material causing an excitation of resonance which generates a variation in the distribution of the electronic density (electronic spectroscopy). This effect can be represented by the Bohr model:

$$\Delta \mathrm{E} = \mathrm{E}_2 - \mathrm{E}_1 = \mathrm{h}\nu \rightarrow \mathrm{h}\nu = \mathrm{hc}\,\bar{\nu} = \mathrm{hc}/\lambda$$

where E_1 and E_2 are the initial and final energies, respectively; h = Planck's constant (6.62×10^{-34} Js); c = light velocity in vacuum (2.99×10^8 m/s); ν = frequency; λ = wavelength; $\bar{\upsilon}$ = wavenumber. This phenomenon causes, for example in the case of electronic spectroscopy, a variation in the distribution of the electronic density in the outermost orbitals of an individual atom or, in molecular orbitals, an electronic transition from the occupied levels to the unoccupied levels. The Bohr's relation therefore connects the atomic or molecular electronic energy levels with the frequency of the incident radiation.

In general, when matter absorbs part of the incident radiation and is excited to higher energy levels, this state is not stable and the species returns to its initial equilibrium. This ground state can be reached through "deactivation" processes that can take place in several ways: without emission of radiation (for example, internal conversion of energy in order to obtain a thermal equilibrium, i. e. dissipation as heat), with spontaneous emission of radiation with a lower energy than that of the absorbed radiation (fluorescence, phosphorescence), with photochemical processes, etc. Depending on the molecular environment and the "deactivation" process, the excitation states can exist for 10^{-13}-10^{-3} sec. Lambert and Beer developed a relation that correlates the intensity of an absorption with the concentration and thickness of the absorbing species (Beer-Lambert Law or

Beer’s Law, which is strictly observed only for absorbing materials in diluted homogeneous medium):

$$A = \varepsilon bc = -\log T = \log(1/T)$$

where A = absorbance; **ε** = molar absorptivity or molar absorption coefficient; b = thickness of the sample; c = concentration; T = transmittance.

When dealing with measurements on opaque materials, different parameters play an important role in the definition of the spectral features [3, 31]. The reflected radiation from a generic surface, or a paint layer, that can be considered as a multicomponent polycrystalline assemblage, is generally called “total reflectance” (R_T), which is the sum of the specular (or surface, R_S), diffuse superficial (R_{DS}), retro-diffused (R_{RD}), and diffuse (or volume, R_V) reflectance (Figure 1). The specular component is the portion of the reflected radiation that has not penetrated into the paint layer, whereas the diffuse component is that portion of radiation which has penetrated into the paint layer and interacts with one or more pigment’s particles [32]. According to Vincent and Hunt, R_V depends on several parameters, such as wavelength, particle dimensions, packing density, real refractive index *n* and absorption coefficient *k*, scattering coefficient *s*, and optics geometry [33].

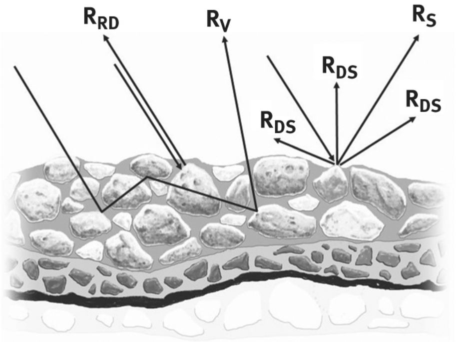

Figure 1: Total reflection modes (courtesy of Miguel Ángel Herrero Cortell).
No credits it’s our own figure.

The volume reflectance carries with it the major part of spectral information that is used to identify the materials. If one is interested in quantitative information, such as in the measurement mode in transmittance, the reflectance spectra can be expressed as apparent absorbance:

$$A' = \log(1/R)$$

or as Kubelka-Munk (KM) function [34], $f(R_\infty)$, from a homogeneous material with infinite thickness (R_∞). The KM infinitive reflectance function is given by:

$$f(R_\infty) = (1 - R_\infty)^2/2R_\infty = k/s$$

where the parameters *k* and *s* are the absorption and scattering coefficients, respectively. This equation assumes that the incident radiation is monochromatic, the scattering processes show no wavelength dependence, and the two parameters *k* and *s* are constant in a homogenous medium. Both formulae try to represent the relationship between the intensity of absorbance and the concentration of the compound in a linear way. A’ is usually more favourable in the NIR-SWIR region and for highly absorbing samples (low dilution), while KM is applied when condition of infinite dilution in a non-absorbing matrix (high dilution) is present. Even though the KM phenomenological theory [34] is widely used for multiple scattering calculations in the paint industry, it implies certain assumptions and hence has several limitations. The most significant assumption is the isotropic scattering within the sample. Each particle that composes the paint layers, such as pigments, dyes, fillers, etc., has to be far enough apart from one another in order to scatter or absorb the incident radiation independently from all the others, which means that they have to obey to the “no dependent scattering” rule. Moreover, the paint layer is assumed to have plane, parallel surfaces, sufficient in extent to ensure that edge effects can be ignored in the measured reflectance spectra. The incident radiation and its reflected component have to be diffuse, and no generation of radiation, such as fluorescence and related processes, within the paint layer is expected. All these assumptions make the application of KM function to reflectance measurements acquired on paintings, and artworks in general, very problematic as the paint layers constituting these objects barely fit any KM theory requirements.

2.1 Electronic transitions

In the UV-Vis spectral range transitions between electronic energy levels can be observed, which determine the absorption bands in the UV-Vis region. An electron is excited when the frequency of the incident electromagnetic radiation is the same as the difference of energy between two electronic states. This difference of energy depends on the electronic structure of the molecule and of its “environment”. For a transition to happen after absorption of radiation it is necessary to have a dislocation of charge and some rules, such as Laporte Selection Rule, Spin Multiplicity Selection Rule, and Coupling Interaction with Neighbour Cations, have to be respected [32].

The electronic transitions can be related to transitions between molecular orbitals and they are often classified according to the nature of the molecular orbitals involved. Based on the symmetry of the orbitals involved and the electromagnetic field, it is also possible to assess the intensity of a transition. One of the most important rules in this case is related with the spin multiplicity of the orbitals involved in the transition. Transitions between states that have the same multiplicity of spin are allowed, whereas transitions between levels with different spins are forbidden. The consequence of this rule is that the allowed transitions produce spectral characteristics which are more intense, while the forbidden transitions are absent or create weaker absorption bands.

The absorption bands of pigments are generally related to Ligand-Field (LF), Charge Transfer (CT), and Valence-Conduction band transitions (VC), also known as Band theory, while for organic materials delocalised Molecular Orbital (MO) band transitions are considered [30, 32, 35]. In many cases, the spectral features enable the identification of the nature of the compounds analysed and, in particular, to identify pigments, dyes, alteration products, and some other organic compounds.

LF involves inorganic compounds containing metal ions with unpaired electrons in *d* or *f* orbitals, such as the *3d* transition metals: titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), and copper (Cu) [30, 32]. It explains absorption of the UV and Vis radiation by the transition-metal compounds or impurities of many minerals, gems, and pigments, such as azurite, ruby, and chromium sesquioxide (chrome green or chromium oxide green). The outermost electrons of the transition metals located in orbitals *3d* are responsible for the transitions between the energy levels. Several features cause the degeneracy of the *d* or *f* orbitals of the transition-metal ions, such as the anions or dipolar groups (defined as ligands) which surround the transition metals, the symmetry and geometrical arrangement of the ligands as well as their number and type. These features determine the number of permitted transitions and the line shape of the registered spectra, which can lead to different absorption spectra (and therefore different transmittance or reflectance) for the same transition metal (for example as can be observed for Cu^{2+} in azurite and in malachite). However, not all possible transitions between levels have the same intensity. As previously stated, the selection rules determine which transitions, and their intensities, are permitted and measured. The LF transitions are usually not so intense since they are only partially permitted by the selection rules [32].

CT generally produces intense absorptions since these transitions are fully allowed by the selection rules [30]. Here, processes in which the absorbed energy determines the migration of one electron between close ions or between an ion and ligand are found. Although there is a real transfer of an electron, it remains located in its new position, which means that this electron is linked to one specific ion. In the crystalline structure, an electron mainly located in an orbital of a ligand can be excited and transferred to an orbital mainly located in the metal ion or vice-versa (CT between ligand-metal, LMCT, or metal-ligand, MLCT, respectively), as in chrome yellow pigment (lead chromate, $PbCrO_4$), where the absorption of radiation causes the transfer of one electron from one oxygen of the chromate ion to the chrome ion. These transfers can also happen between ions adjacent to the same metal in different valence states, and in this case the process is essentially a photochemical oxidation-reduction reaction. This is often the case of ion pairs such as Fe^{2+} and Fe^{3+}, Mn^{2+} and Mn^{3+}, or Ti^{3+} an Ti^{4+}, when they are placed next to each other, as for example in Prussian blue pigment, $Fe_4[Fe(CN)_6]_3 \cdot nH_2O$, where the charge transfer occurs between two non-equivalent Fe ions ($Fe_A{}^{2+} + Fe_B{}^{3+} \rightarrow Fe_A{}^{3+} + Fe_B{}^{2+}$).

VC transitions are characteristic of metals and some inorganic pigments that show properties of semiconductors [30]. In some crystalline lattices, the discrete energy levels of the external electrons of the ions constituting the lattice, are extended to energy bands that exist close to them. There are therefore two energy bands in which the electrons can exist: one at lower energy called “valence band” and one at a higher energy called “conduction band”. The electrons in the conduction band have higher energy than those in the valence band and, in practice, are not linked to any ion, but are free to move in the crystalline lattice, from which comes the definition “free electrons” or “conduction electrons”. Between the valence band and the conduction band there is an energy gap, called “band gap”, in which the electrons cannot be found. In the semiconductor materials, such as vermilion, HgS, red lead, Pb_3O_4, and cadmium yellows, CdS, (Cd,Zn)S, the width of the forbidden band is in between that of the metals and the dielectric compounds, and the passage of electrons from the valence band to the conduction band is seen as an intense absorption in the UV-Vis range. This type of transition produces characteristic absorption bands with an “S” shape and the transition energies can be approximately defined by their inflection points, i.e. the point of the curve at which the sign of the curvature changes, which is determined by looking at the first derivative of their reflectance spectra [16].

Finally, delocalised molecular orbital (MO) theory explains the electronic transition in organic molecules [30]. The electronic orbitals in covalent or weakly ionic bonding are capable of overlapping between themselves and form molecular orbitals called bonding molecular orbitals σ or π and anti-bonding molecular orbitals σ^* or π^*. In this representation, the bonding orbitals represent the maximum positive overlapping between symmetric wave functions and are more stable than the individual atomic orbitals of the single atom. The corresponding anti-bonding molecular orbitals, on the other hand, are less stable than the individual atomic orbitals of the single atom. Absorption of radiation of appropriate energy can promote one of the σ or π electrons to an anti-bonding orbital σ^* or π^* (transition $\sigma \rightarrow \sigma^*$ or $\pi \rightarrow \pi^*$), respectively.

The vast majority of dyes, natural and synthetic, can be viewed as containing an extended conjugated chromophore system to which are attached electron donor and electron acceptor groups (auxochromes). A chromophore is the atom or group of atoms of a molecule in which the electronic transition responsible for a given spectral band is approximately localized; the term referred originally to the groups in the molecule that are responsible for its colour (IUPAC definition,http://goldbook.iupac.org). Chromophores include $C = C$ bonds as well as the azo group (-N = N-), thio group (C = S), and nitro group (-N = O). In turn, an auxochrome is an atom or group of atoms which, when introduced into a chromophore, causes a bathochromic shift (red shift) and/or a hyperchromic effect in a given band of the chromophore, usually in that of lowest frequency (IUPAC definition). Auxochromes have the capability to donate to, or accept from, one or more electrons, through σ- or π-bonds. Those belonging to the first group are electron donor groups while those in the second group are electron acceptor groups. The choice of σ- or π-bonds depends on the electronegativity, the presence of multiple bonds or the existence of lone pair of electrons. The substituents $-NH_2$, X (F, Cl, Br, I), $-CH_3$, Ar, $-NR_2$ and -OR, where R represents an organic group, are electron donors through π-bonds, while $-NO_2$, -R(C = O), $-C \equiv N$, $-CO_2H$ are electron acceptors. To have colour generation, the energies involved in the MO transitions have to be moved from the ultraviolet into the visible, which can be done by increasing the size of the conjugated system and/or with the addition of auxochromes. For example, a red shift induced by -OH groups is observed from the colourless anthraquinone to the coloured alizarin (1,2-dihydroxy-9,10-anthraquinone) and purpurin (1,2,4-trihydroxy-9,10-anthraquinone). Generally speaking, the absorptions occurring in organic coloured molecules are based on π bonding in the extended conjugated systems and the colours observed are modulated by the energy gap between the ground and excited electronic states involved in the transition.

2.2 Microspectrofluorimetry

When a species is excited through the absorption of a photon, it causes electronic transitions and brings the absorbing species into an electronic excited state. In the case of organic molecules, the emission of photons accompanying de-excitation is called photoluminescence, which can be divided into two phenomena depending on the nature of the excited state and the lifetime of the phenomena: fluorescence (excited singlet states) and phosphorescence (triplet excited states) [36]. The phenomenon of fluorescence as an emission of radiation following absorption of radiation was demonstrated by George Gabriel Stokes in the middle of the nineteenth century [37]. Stokes stated that the emitted radiation is always of longer wavelength (lower energy) than the exciting one (Stoke's law).

Fluorescence is measured by exciting a species at an absorption wavelength, also called the excitation wavelength, and measuring the emission at a longer wavelength called the emission wavelength. The characteristics of fluorescence of a given species are affected by any excited-state process involving interactions of the excited species with its close environment, which can provide information not only on the emitting species but also on the respective microenvironment. For this reason, and for being highly sensitive, fluorescence spectroscopy has been widely used in many scientific and technological fields, such as physics, chemistry, materials science, biology, and medicine.

Spectrofluorimeters hyphened to confocal microscopes give the possibility to acquire both fluorescence excitation and emission spectra. In the excitation spectrum, that resembles an absorption spectrum, fluorescence emission is collected at a fixed emission wavelength, which usually corresponds to the maximum fluorescence, while the excitation wavelength is scanned. The fluorescence emission spectrum is obtained by keeping a fixed excitation wavelength (commonly corresponding to the maximum of the excitation/absorption spectrum) while the emission wavelengths are scanned. Emission is generally highly sensitive and selective since detection of different species can be achieved using the appropriate excitation and/or emission wavelengths. The high sensitivity is important for detecting materials which may be present in low concentrations due to, for example, degradation processes. Moreover, the confocal microscope helps to avoid emission from the support or other layers possibly present in the sample. This is achieved with the use of a pinhole aperture at a focus point that coincides with the focal plane of the objective lens. The light from the focal plane of the objective lens will pass through the pinhole aperture, while light which does not come directly from the focal plane (out-of-focus) gets blocked by the pinhole [38].

3 Instrumentation, accessories and measurement modalities

The basic optical configuration for a spectrometer working in the UV-Vis region includes one or more polychromatic light sources, one or two wavelength selectors for splitting the radiation into its different components (wavelengths), and one or more detectors for measuring the radiation that interacts with the sample [3, 39, 40]. Depending on the type of instrumentation, an internal or external sample holder can also be present. Reflectance spectra are usually measured using an integrating sphere linked to the spectrometer for working in total and/or diffuse reflectance modes. Most of the compact-portable devices are also equipped with fibre optic bundles to send radiation to the measured spot and to collect its reflected/transmitted component. Spectrometers are split in dispersive and non-dispersive. The latter are based on interferometers, such as the Michelson interferometer, while the former need one or two monochomators for spectrally dispersing the incident radiation. However, in the UV-Vis regions the interferometric-based systems have not been very widespread for routine analysis. Dispersive spectrometers are divided in spectrophotometers and spectroanalysers, the sequence of the optical path of the former being defined as a) light source, b) monochromator, c) sample, and d) detector, while that of spectroanalysers is characterised for having the sample placed right after the light source, before the monochromator. Traditional spectrometers are also grouped in “single beam” and “double beam” devices. The latter group of instruments have two light paths, through the sample and the reference, which are automatically and continuously interchanged during the acquisition of the spectra. The single beam spectrometers make the acquisition of the sample and reference at different times, which means that the devices require well stabilised light sources and electronics to keep the measurement conditions steady during the acquisition of the spectra. Starting from the 1990s a new generation of spectroanalysers also became available. They include multiplex detectors, made of linear photodiode or charge-coupled devices (CCDs) arrays, which allow to measure a complete spectrum in fractions of second up to few minutes, depending on the device, experimental set-up, and the analysed sample. In the last years, Texas Instruments has produced a new set of low cost micro electromechanical system (MEMS) digital micromirror devices (DMD) working in the UV-Vis and NIR-SWIR ranges. The core of these devices, the DMD, consists of an array of thousands/millions of tiny micromirrors, which pick the individual wavelengths by selectively turning columns of mirrors on or off, in order to reflect only the desired wavelengths to the single detector [41].

The most common detectors and light sources are reported in Table 1.

Table 1: Most common detectors and light sources used in UV-Vis spectroscopy.

Detectors	Sensitivity	Radiation source	Emission range
Photomultiplier tube	< 190–850 nm	Tungsten-Halogen filament lamps With the addition of iodine, a higher filament temperature is obtained	320–3200 nm
Silicon photodiodes	200–1100 nm	Deuterium lamp	185–400 nm
Silicon charge-coupled devices (CCD)	200–1100 nm	Xenon arc lamp	270–1000 nm

[1] there is no table footnote

As previously stated, reflectance measurements are “relative measurements” which means that these spectra need to be referenced against a reflectance standard, such as barium sulphate (barite) plates or fluoropolymer (PTFE) targets, which have high diffuse reflectance and are virtually free of spectral features over the UV-Vis and NIR-SWIR ranges [32, 42]. These standards have to be stable with time, temperature, etc., show a homogenous and smooth surface, present no fluorescence and be opaque (no transparency).

Fluorescence measurements are made with the aid of fluorimeters or spectrofluorimeters, the latter comprising an excitation and emission monochromator and recording both excitation and emission spectra [3]. Spectrofluorimeters hyphened to confocal microscopes offer the possibility to acquire fluorescence spectra *in situ*, from samples placed directly on a programmable x-y-z microscope stage [22]. A continuous xenon lamp is used, which is directed into a double-grating excitation monochromator that selects the desired wavelength of the excitation beam. The beam passes through a dichroic mirror and all-reflective optic mirrors which direct the incident light to the microscope. Within the microscope, a pinhole-turret containing holes of various diameters controls the spatial resolution collimating the illumination, which then illuminates the sample on the programmable microscope stage. Fluorescence from the sample is directed back up into the microscope, passing through the optic mirrors and dichroic mirror to a spectrometer or spectrograph with one or two detectors (a photomultiplier tube and a CCD array). The dichroic filters which transmit certain wavelengths and reflect others enable the separation of emission and excitation light. Fluorescence signals are expressed in "counts per second" (cps). Optimization of the signal is made through mirror alignment in the optic pathway of the microscope using strong emitters references such as rhodamine.

4 Case studies

Most of the applications of UV-Vis diffuse reflectance spectroscopy in the field of cultural heritage are carried out using fibre optic systems (FORS). Other applications are carried out using handheld systems working in contact with the object or, to a lesser extent, inside laboratories using systems equipped with integrating spheres.

4.1 Paintings, manuscripts

A major part of FORS applications are related to paintings and illuminated manuscripts, for a couple of reasons. Firstly, these artworks are generally flat, which is a favourable situation for analysis; secondly, the identification of coloured materials is usually straightforward if the artist's technique, the geographic area and the period in which the object was produced are known. Moreover, FORS can contribute to the identification of organic colourants such as dyes and lake pigments in a non-invasive way, which is difficult to do with other analytical techniques. Therefore, there are several works in which FORS has been applied in the identification of colourants on historical paintings, such as easel paintings [11, 16] and mural paintings [15, 43–45], and on illuminated manuscripts [46–49]. While most of these FORS studies on colourants aim at a qualitative identification, some evaluate the possibility of obtaining quantitative data on the colourants present in a paint [50, 51], using specific mathematic algorithms.

In the analysis of paintings and illuminated manuscripts, the spectral range used is the most important feature. Vis range instruments are able to identify some of the "coloured" colourants, but will not provide information on certain groups of materials, such as the white and black pigments. The extension into the UV region (200–380 nm) adds spectral features useful for discrimination among white pigments [16, 52]. On the other side, extension into the NIR region adds spectral features useful for identification of specific materials, such as irongall ink, which can be distinguished from carbon pigments according to its typical rising above 700 nm [53]. Finally, the possibility of extending the spectral range up to the SWIR range strongly widens the diagnostic power of the technique, adding information provided by vibrational band overtones and combinations which allows identifying pigments containing hydroxyls, water molecules, and carbonates [14].

A different situation is the analysis of contemporary art. The number of colour materials used by artists is perhaps hundreds of times larger than those used from the past up to the beginning of the twentieth century. Moreover, many synthetic compounds show chromatic features similar or identical to historic artist's materials. One example is shown in Figure 2 in which the inflection points of vermillion and other four modern colourants (alizarin crimson, irgazin scarlet, permanent red, cadmium red) are all located at ca. 600 nm. Despite these premises, some studies recently proposed the use of FORS in the characterisation of contemporary painted artworks [54, 55], at least as a complementary technique. In these cases, the existence of spectral databases is particularly useful [56].

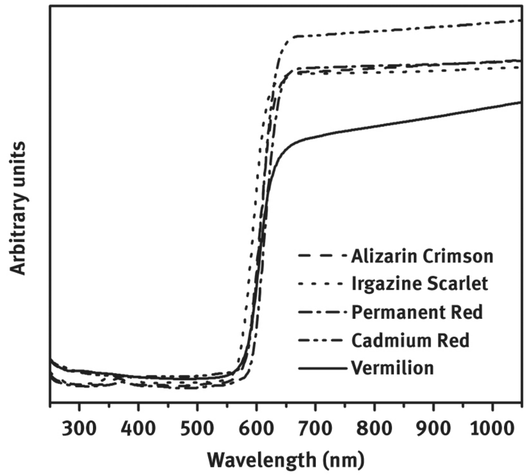

Figure 2: FORS spectra of vermillion and modern red colourants.
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Of great interest in the field of conservation of paintings is the possibility of monitoring colour changes by means of periodic reflectance measurements [8, 52, 57]. Data can be collected at regular intervals and possible degradation trends can be easily highlighted. Some recent works have also evaluated the potential of FORS in the characterisation of polymers used in paintings and manuscripts, with particular concern to binders [58, 59] and plastics [55, 60].

Complementary to reflectance spectroscopy, microspectrofluorimetry has been particularly applied to the identification of dyes and lake pigments in illuminated manuscripts and paintings, including analysis in paint cross-sections and *in situ* [19, 24]. It has been used to study brazilwood [27], see Figure 3, lac dye [28], cochineal [29], alizarin, purpurin and eosin [22], and orcein [25]. A recent work has applied microspectrofluorimetry together with a chemometric approach to discriminate between the different chromophores in a reference database [26].

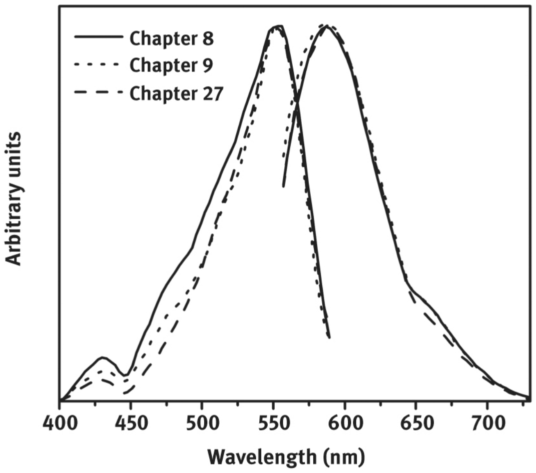

Figure 3: Excitation (λem 610 nm) and emission (λex 530 nm) spectra of Brazilwood lake pigments produced from different medieval recipes in *Livro de como se fazem as cores* [27].
No Credits

4.2 Textiles, carpets, tapestries

The main application of FORS to textile materials is related with the identification of the dyes used to give colour to fibres. From the analytical point of view, there is similarity between the identification of dyes on a painting, illuminated manuscript or textile since the chromophores involved are mostly the same. The analysis of textiles is however more difficult for at least two reasons: firstly, the surface of a textile artwork is usually more irregular than that of a painting or manuscript, which is an unfavourable situation when using a surface technique such as reflectance spectroscopy; secondly, in the art of textiles the most used dyes, the so-called mordant dyes (e.g. madder, kermes, cochineal, weld, logwood) were applied on fibres with different mordants (copper, tin, iron and aluminium salts), a feature that can introduce variability in the spectral characteristics useful for identification. An example of the great variability in the identification of cochineal is reported in a study by Morales and Berrie [61], which includes both absorbance and fluorescence spectroscopies.

Due to the great performances of FORS as a survey method, in the last years several works have been published concerning the analysis of carpets [62, 63], clothes [64] or various garments [65–67]. The fast response of FORS can address further investigation such as sampling and analysis with more powerful techniques (HPLC-MS, SERS, etc.). Ancient textiles have also been successfully studied with microspectrofluorimetry [19].

4.3 Glass

The use of FORS in the characterisation of coloured vitreous materials cannot yield information on their global composition. However, it can provide information on the nature of the chromophore system in a simple way, including the oxidation state of the species responsible for light absorption, an information that would need more powerful and expensive analytical techniques such as Mössbauer, Auger or X-ray photoelectron spectroscopies. The characterisation of the oxidation state of the chromophores can also yield information on the firing conditions used in glassmaking, in particular with concern to Fe^{2+}/Fe^{3+} ratios. Most of the chromophore systems in glasses can be identified due to their absorption positions and spectral shapes. Table 2 presents a selection of these data.

Table 2: Absorption maxima of chromophore systems in glass.

Ions	Absorption maxima	References
Ag(0)	420–500 nm depending on particle size	[68]
Au(0)	525–550 nm depending on particle size	[68]
Co^{2+}	three bands in the 525–650 nm range	[12]
Cr^{3+}	430–460 nm, bands in the 630–680 nm range	[69]
Cr^{6+}	365 nm	[69]
Cu(0)	band centred at 450 nm, peak at 570 nm	[70]
Cu^{+}	no bands	[71]
Cu^{2+}	700–900 nm	[72]
Fe^{2+}	450 nm (sh), band in the 900–1200 nm range	[72]
Fe^{3+}	a series of bands in the 375–450 nm range	[72]
Fe^{3+}-S_2^{-}	405 nm	[73]
Mn^{2+}	430 nm, very weak	[71]
Mn^{3+}	470–520 nm	[69]
Mn^{4+}	450 nm	[69]
Ni^{2+}	three bands in the 430–640 nm range	[69]
UO_2^{2+}	416 nm, bands in the 410–475 nm range	[74]
V^{3+}	425, 645 nm	[71]
V^{4+}	1100 nm	[71]
V^{5+}	350 nm	[71]

[1] There is no footnote

Several studies on glass artworks exploited the identification of chromophores by means of absorption spectrophotometry, using either the transmission or reflectance mode. The pioneering works by Bamford [71] and by Schreus and Brill [73] set the basis for the measurement of the absorption properties of a glass system in transmission mode. Subsequent works exploited the same method [12, 16, 75], using either sunlight or specific light sources, while other studies exploited the reflectance mode [76–78]. Both transmittance and reflectance, in fact, display the same electronic transitions [79]. The lack of scattering centres renders the reflectance mode less advantageous for transparent glasses, while it is more advantageous (or even mandatory) when studying translucent or opaque glass systems such as enamels [80].

4.4 Metals

Due to the intrinsic features of FORS, which is a technique mostly devoted to the characterisation of coloured artworks, its contribution in the study of metals is poor. Most metals used in artworks can be identified only when they are pure and unaltered [14]. In this context, a preliminary study proposed the possibility of monitoring the conservation state of metal swords [81].

4.5 Minerals

With concern to the identification of mineral phases, FORS has already demonstrated its potential as a preliminary technique. Several papers have shown its usefulness in the characterisation of iron oxides and hydroxides [82] and some databases are available online (https://speclab.cr.usgs.gov/spectral-lib.html). Yet, from the mineralogical point of view, the most relevant information provided by FORS in the field of cultural heritage is the identification of coloured gemstones. As in the case of glass, FORS provides the identification of the chromophore system which, in many cases, leads to the identification of the gemstone itself. It cannot be used, though, for the identification of uncoloured gemstones such as diamond and quartz. Another point in common with the analysis of glass artworks is the fact that transparent gemstones usually yield poor spectral responses, due to the lack of scattering centres, while much better spectra can be obtained when analysing opaque gemstones. Usually only the reflectance mode can be exploited in the analysis of gemstones, particularly when complete jewellery artworks are studied in which gemstones cannot be taken away from their settings. Table 3 lists the absorption features that can be useful for identification of the most precious gemstones by means of FORS.

Table 3: List of spectral features for the identification of gemstones.

Gemstone	Spectral features	Chromophore system	References
amethyst	540 nm (absorption)	Fe^{3+} (colour centre)	[83]
emerald	430/600–615 nm (absorption)	Cr^{3+} (ligand field)	[84]
garnet	500/525/575/695 (absorption)	Fe^{2+}, Fe^{3+}, Mn^{2+}, Mn^{3+}, Cr^{3+}	[85]
ruby	413/564 (absorption), 693/694 nm (fluorescence)	Cr^{3+} (ligand field)	[86]
sapphire	385/450/578 nm (absorption)	Fe^{2+}, Fe^{3+}, Ti^{4+} (ligand field, intervalence charge transfer)	[87]
turquoise	425/625–915 nm (absorption)	Fe^{2+}, Fe^{3+}, Cu^{2+} (ligand field)	[88]

[1] There is no footnote

4.6 General comments

Independently from the type of material analysed, it is important to point out that the characterisation given by reflectance spectroscopy is based on the position of the minimum/minima of reflectance, which become maximum/maxima values when the spectrum is turned into apparent absorbance or Kubelka-Munk coordinates. The reflectance maximum/maxima, in fact, only provide information concerning the colour of the area analysed, not the chromophore system. The reflectance spectra of cold colours (blue, green, turquoise, etc.) usually have one or more minima and the identification of the spectral features is relatively simple. When the spectrum has no minima, generally the characterisation is based on the inflection point, which is a typical situation for warm colours (red, orange, yellow, brown, etc.). In these cases, an accurate identification of the spectral features is based on the first derivative of the spectrum, where the inflection point becomes a maximum.

Given the features of reflectance systems, with concern mainly to portable FORS systems which can provide several spectra in short times, it can be useful to manage the spectral responses using pattern recognition chemometric methods. Mathematical techniques such as Principal Components Analysis (PCA), Cluster Analysis (CA) or Discriminant Analysis (DA) can be used for the purpose, allowing to group objects (reflectance spectra in this case) which are more similar and therefore providing a semi-automatic way to process large amounts of spectral information. This is particularly important in cases when reflectance spectroscopy is used as a survey method to address further, more powerful diagnostic techniques or the subsequent withdrawal of samples. The most critical point of the pattern recognition process is the normalisation of spectral data, in order to extract the best of the information contained in it. The most suitable methods are *range scaling* (scaling all spectra between 0 and 1), *mean centering* and *autoscaling* (mean centering and normalisation to the standard deviation) [89]. Some examples of the application of pattern recognition methods to spectral information obtained with FORS are reported in the literature with concern to paintings [90, 91] and textiles [9, 64].

5 Conclusions (advantages, drawbacks)

For the technical features that it presents, UV-Vis reflectance spectroscopy has been particularly suitable for diagnostic studies on a wide range of artwork typologies. Portable systems, in particular when coupled with fibres, can be used for easily acquiring hundreds of spectra in short time; this amount of information can then be used to address further measurements by means of more powerful techniques. Particularly relevant is the possibility of identifying colourants on paintings and textiles in a non-invasive way, a feature which is unique among spectroscopic techniques. The major drawbacks of reflectance spectroscopy can be resumed as follows: it is hard to manage spectral responses from mixtures of substances (e.g. mixtures of colourants); the spectral responses can be poor in artworks or samples with irregular surfaces or that are partially transparent; it is generally not possible to discriminate colourants having similar absorption features. In such cases, the complementary use of other analytical techniques such as Raman and infrared spectroscopy or mass spectrometry is mandatory. On the other hand, microspectrofluorimetry is also presented as a powerful technique for the non-invasive identification of colourants, particularly if supported by a reference database and by the complementarity of other analytical methods.

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References

[1] Taft WS, Mayer JW. The science of paintings. New York: Springer-Verlag, 2000.
[2] Pinna D, Galeotti M, Mazzeo R, editors. Practical handbook on diagnosis of paintings on movable support, European project ARTECH. Firenze: Centro Di, 2009.
[3] Gauglitz G. Ultraviolet and visible spectroscopy. In: Gunzler H, Williams A, editors. Handbook of analytical techniques. Vol. 1. Weinheim: Wiley-VCH, 2001.
[4] Rawlins FI. Studies in the colorimetry of paintings: a note in conclusion. Tech Stud Field Fine Arts. 1942;10:230–31.
[5] Barnes NF. A spectrophotometric study of artists' pigments. Tech Stud Field Fine Arts. 1939;7:120–30.
[6] Santini M. Applicazione del metodo spettrofotometrico nella determinazione colorimetrica sui dipinti. Boll dell'Istituto Centrale del Restauro. 1959;23/24:95–129.
[7] Duncan DR. The identification and estimation of pigments in pigmented compositions by reflectance spectrophotometry. J Oil Colours Chem Assoc. 1962;45:300–24.
[8] Bullock L. Reflectance spectrophotometry for measurement of colour change. Natl Gallery Tech Bull. 1978;2:49–55.
[9] Cazenobe I, Bacci M, Picollo M, Radicati B, Bacci G, Conti S, et al. Non-destructive spectroscopic investigations of dyed textiles: an application to yellow dyed wool samples. In: Preprints of the 13th triennial ICOM meeting. Rio de Janeiro, ICOM Committee for Conservation, and Roy Vontobel Eds. London : James & James. 2002: 238–44.
[10] Dupuis G, Elias M, Simonot L. Pigment identification by fiber-optics diffuse reflectance spectroscopy. Appl Spectrosc. 2002;56:1329–36.
[11] Leona M, Winter J. Fibre optics reflectance spectroscopy: a unique tool for the investigation of Japanese paintings. Stud Conserv. 2001;46:153–62.
[12] Bacci M, Picollo M. Non-destructive detection of Co(II) in paintings and glasses. Stud Conserv. 1996;41:136–44.
[13] Delaney JK, Ricciardi P, Deming Glinsman L, Facini M, Thoury M, Palmer MR, et al. Use of imaging spectroscopy, fiber optic reflectance spectroscopy, and X-ray fluorescence to map and identify pigments in illuminated manuscripts. Stud Conserv. 2014;59:91–101.
[14] Aceto M, Agostino A, Fenoglio G, Idone A, Gulmini M, Picollo M, et al. Characterisation of colourants on illuminated manuscripts by portable fibre optic UV-visible-NIR reflectance spectrophotometry. Anal Meth. 2014;6:1488–500.
[15] Bacci M, Baldini F, Carlà R, Linari R. A color analysis of the Brancacci Chapel Frescoes. Appl Spectrosc. 1991;45:26–31.
[16] Bacci M, Picollo M, Trumpy G, Tsukada M, Kunzelman D. Non-invasive identification of white pigments on twentieth century oil paintings by using fiber optic reflectance spectroscopy. J Am Inst Conser. 2007;46:27–37.
[17] de la Rie ER. Fluorescence of Paint and Varnish layers (Part I). Stud Conserv. 1982;27:1–7.
[18] Romani A, Clementi C, Miliani C, Favaro G. Fluorescence spectroscopy: A powerful technique for the noninvasive characterization of artwork. Acc Chem Res. 2010;43:837–46.
[19] Claro A, Melo MJ, Seixas de Melo JS, van Den Berg KJ, Burnstock A, Montague M, et al. Identification of red colorants in van Gogh Paintings and Ancient andean textiles by microspectrofluorimetry. J Cult Heritage. 2010;11:27–34.
[20] Melo MJ, Claro A. Bright light: microspectrofluorimetry for the characterization of Lake Pigments and Dyes in works of art. Acc Chem Res. 2010;43:857–66.
[21] Mounier A, Lazare S, Le Bourdon G, Aupetit C, Servant L, Daniel F. LEDμSF: a new portable device for fragile artworks analyses. application on medieval pigments. Microchem J. 2016;126:480–87.

[22] Claro A, Melo MJ, Schäfer S, Seixas de Melo JS, Pina F, van Den Berg KJ, et al. The use of microspectrofluorimetry for the characterization of Lake Pigments. Talanta. 2008;74:922–29.

[23] Matteini P, Camaiti M, Agati G, Baldo MA, Muto S, Matteini M. Discrimination of Painting binders subjected to photo-ageing by using microspectrofluorometry coupled with deconvolution analysis. J Cult Heritage. 2009;10:198–205.

[24] Melo MJ, Otero V, Vitorino T, Araújo R, Muralha VSF, Lemos A, et al. A Spectroscopic study of Brazilwood Paints in medieval books of hours. Appl Spectrosc. 2014;68:434–44.

[25] Melo MJ, Nabais P, Guimarães M, Araújo R, Castro R, Oliveira MC, et al. Organic Dyes in illuminated manuscripts: a unique cultural and historic record. Philos Trans R Soc A Math Phys Eng Sci. 2016;374:1–20.

[26] Nabais P, Melo MJ, Lopes JA, Vitorino T, Neves A, Castro R. Microspectrofluorimetry and chemometrics for the identification of medieval lake pigments. Heritage Sci. 2018;6:1–11.

[27] Vitorino T, Melo MJ, Carlyle L, Otero V. New insights into Brazilwood lake pigments manufacture through the use of historically accurate reconstructions. Stud Conserv. 2016;61:255–73.

[28] Castro R, Pozzi F, Leona M, Melo MJ. Combining SERS and microspectrofluorimetry with historically accurate reconstructions for the characterization of Lac Dye Paints in medieval manuscript illuminations. J Raman Spectro. 2014;45:1172–79.

[29] Vitorino T, Otero V, Leslie C, Melo MJ, Parola AJ, Picollo M, 2017, Nineteenth-century Cochineal Lake Pigments from Winsor & Newton: insight into their methodology through reconstructions. In: Bridgland J, editors. ICOM-CC 18th Triennial Conference Preprints. Copenhagen. Paris: International Council of Museums, 4-8 Sep 2017. 1–9.

[30] Nassau K. The physics and chemistry of color, the fifteen causes of color. New York: Wiley & Sons; 1983.

[31] Mitton PB. Opacity, hiding power, and tinting strength. In: Patton TC, editor. Pigment handbook volume III characterization and physical relationships. New York: Wiley & Sons, 1973: 289–329.

[32] Burns RG. Mineralogical applications of crystal field theory. 2nd ed. Cambridge Topics in Mineral Physics and Chemistry Vol. 5. Cambridge: Cambridge University Press, 1993.

[33] Vincent RK, Hunt GR. Infrared reflectance from mat surfaces. Appl Opt. 1968;7:53–59.

[34] Kubelka P, Munk F. Ein Beitrag Zur Optik Der Farbanstriche. Z Tech Phys. 1931;12:593–601.

[35] Hollas JM. Modern spectroscopy. 2nd ed. New York: John Wiley & Sons, 1992.

[36] Lakowicz JR. Principles of fluorescence spectroscopy. Berlin/Heidelberg: Springer Science+Business Media, LLC; 2006.

[37] Valeur B. Molecular fluorescence: principles and applications. Weinheim (Germany): Wiley-VCH Verlag GmbH, 2001.

[38] Semwogerere D, Weeks ER. Confocal microscopy. In: Wnek GE, Bowlin GL, editors. Encyclopedia of biomaterials and biomedical engineering. 2nd ed. Vol. I. Boca Raton, Florida, USA: Taylor & Francis, 2008: 705–14.

[39] Workman J. Ultraviolet, visible, and near-infrared spectrometry. In: Workman J, Springsteen A, editors. Applied spectroscopy. A compact reference for practitioners. San Diego: Academic Press, 1998: 29–48.

[40] Springsteen A. Reflectance Spectroscopy: an overview of classification and techniques. In: Workman J, Springsteen A, editor(s). *Applied spectroscopy. A compact reference for practitioners*. San Diego: Academic Press, 1998:193–224.

[41] Feather GA, Monk DW, 1995, The digital micromirror device for projection display. In: Proceedings IEEE International Conference on Wafer Scale Integration (ICWSI). San Francisco, California, USA: Tewksbury and Chapman (Eds.), 18-20 Jan 1995. 43–51.

[42] Springsteen A. Standards for reflectance measurements. In: Workman J, Springsteen A, editor(s). *Applied spectroscopy. A compact reference for practitioners*. San Diego: Academic Press, 1998:247–67.

[43] Appolonia L, Vaudan D, Chatel V, Aceto M, Mirti P. Combined use of FORS, XRF and Raman spectroscopy in the study of mural paintings in the Aosta Valley (Italy). Anal Bioanal Chem. 2009;395:2005–13.

[44] Bacci M, Baldini F, Carlà R, Linari R, Picollo M, Radicati B. A color analysis of the Brancacci Chapel Frescoes. part II. Appl Spectrosc. 1993;47:399–402.

[45] Bacci M, Picollo M, Radicati B, Casini A, Lotti F, Stefani L. Non-destructive investigation of wall painting pigments by means of fibre-optic reflectance spectroscopy. Sci Technol Cult Heritage. 1998;7:73–81.

[46] Aceto M, Agostino A, Fenoglio G, Baraldi P, Zannini P, Hofmann C, et al. First analytical evidences of precious colourants on mediterranean illuminated manuscripts. *Spectrochim Acta A*. 2012;95:235–45.

[47] Delaney JK, Ricciardi P, Deming Glinsman L, Facini M, Thoury M, Palmer MR, et al. Use of imaging spectroscopy, fiber optic reflectance spectroscopy, and X-ray fluorescence to map and identify pigments in illuminated manuscripts. Stud Conserv. 2013;59:91–101.

[48] Aceto M, Agostino A, Fenoglio G, Idone A, Crivello F, Griesser M, et al. Analytical investigations on the coronation gospels manuscript. Spectrochim Acta A. 2017;171:213–21.

[49] Cucci C, Bracci S, Casini A, Innocenti S, Picollo M, Stefani L, et al. The illuminated manuscript corale 43 and its attribution to Beato Angelico: non-invasive analysis by FORS, XRF and hyperspectral imaging techniques. Microchem J. 2018;138:45–57.

[50] Dupuis G, Menu M. Quantitative characterisation of pigment mixtures used in art by fibre-optics diffuse-reflectance spectroscopy. Appl Phys A. 2006;83:469–74.

[51] Fernandez Rodrıguez JM, Fernandez Fernandez JA. Application of the second derivative of the Kubelka–munk function to the semiquantitative analysis of Roman Paintings. Color Res Appl. 2005;30:448–56.

[52] Bacci M, Casini A, Cucci C, Picollo M, Radicati B, Vervat M. Non-invasive spectroscopic measurements on the Il ritratto della figliastra by Giovanni Fattori: identification of pigments and colourimetric analysis. J Cult Heritage. 2003;4:329–36.

[53] Aceto M, Calà E. Analytical evidences of the use of iron-gall ink as a pigment on miniature paintings. Spectrochim Acta A. 2017;187:1–8.

[54] Cucci C, Bartolozzi G, De Vita M, Marchiafava V, Picollo M, Casadio F. The colors of Keith Haring: a spectroscopic study on the materials of the mural painting Tuttomondo and on reference contemporary outdoor paints. Appl Spectrosc. 2016;70:186–96.

[55] Cucci C, Bigazzi L, Picollo M. Fibre optic reflectance spectroscopy as a non-invasive tool for investigating plastics degradation in contemporary art collections: A methodological study on an expanded polystyrene artwork. J Cult Heritage. 2013;14:290–96.

[56] Montagner C, Bacci M, Bracci S, Freeman R, Picollo M. Library of UV–Vis–NIR reflectance spectra of modern organic dyes from historic pattern-card coloured papers. Spectrochim Acta A. 2011;79:1669–80.

[57] Saunders D. The measurement of colour change in paintings. Eur Spectrosc News. 1986;67:10–18.

[58] Dooley KA, Lomax S, Zeibel JG, Miliani C, Ricciardi P, Hoenigswald A, et al. Mapping of egg yolk and animal skin glue paint binders in early renaissance paintings using near infrared reflectance imaging spectroscopy. Analyst. 2013;138:4838–48.
[59] Pallipurath A, Skelton J, Ricciardi P, Bucklow S, Elliott S. Multivariate analysis of combined Raman and fibre-optic reflectance spectra for the identification of binder materials in simulated medieval paints. J Raman Spectro. 2013;44:866–74.
[60] Cucci C, Bacci M, Picollo M, Olmi R. Non invasive techniques for identification and characterisation of polymers in contemporary artworks. In: Berchtold T, editor. Future talks 009. The conservation of modern materials in applied arts and design. Munich: Die Neue Sammlung, 2011: 178–84.
[61] Morales KM, Berrie BH. A note on characterization of the Cochineal Dyestuff on wool using microspectrophotometry. E Preserv Sci. 2015;12:8–14.
[62] Pozzi F, Poldi G, Bruni S, De Luca E, Guglielmi V. Multi-technique characterization of dyes in ancient Kaitag textiles from Caucasus. Archaeol Anthropological Sci. 2012;4:185–97.
[63] Angelini LG, Tozzi S, Bracci S, Quercioli F, Radicati B, Picollo M, 2010, Characterization of traditional dyes of the Mediterranean area by non-invasive UV-Vis-NIR reflectance spectroscopy. In: Conservation and the Eastern Mediterranean Proceedings of the IIC Istanbul Congress. Istanbul (Turkey),Sep 20-24 2010. 184–89.
[64] Gulmini M, Idone A, Davit P, Moi M, Carrillo M, Ricci C, et al. The Coptic textiles of the Museo Egizio di Torino: a focus on dyes through a multi-technique approach. Archaeol Anthropological Sci. 2016;9:485–97.
[65] Maynez-Rojas MA, Casanova-González E, Ruvalcaba-Sil JL. Identification of natural red and purple dyes on textiles by fiber-optics reflectance spectroscopy. Spectrochim Acta A. 2017;178:239–50.
[66] de Ferri L, Tripodi R, Martignon A, Ferrari ES, Lagrutta-Diaz AC, Vallotto D, et al. Non-invasive study of natural dyes on historical textiles from the collection of Michelangelo Guggenheim. Spectrochim Acta A. 2018;204:548–67.
[67] Gulmini M, Idone A, Diana E, Gastaldi D, Vaudan D, Aceto M. Identification of dyestuffs in historical textiles: strong and weak points of a non-invasive approach. Dyes Pigm. 2013;98:136–45.
[68] Quinten M. The color of finely dispersed nanoparticles. Appl Phys B. 2001;73:317–26.
[69] Terczynska-Madej A, Cholewa-Kowalska K, Laczka M. The effect of silicate network modifiers on colour and electron spectra of transition metal ions. Opt Mater. 2010;32:1456–62.
[70] Schreiber HD, Stone MA, Swink AM. Novel red–blue dichroic glass containing copper nanocrystals. J Non-Cryst Solids. 2006;352:534–38.
[71] Bamford CR. Colour generation and control in glass. Amsterdam: Elsevier, 1977.
[72] Meulebroeck W, Baert K, Ceglia A, Cosyns P, Wouters H, Nys K, et al. The potential of UV-VIS-NIR absorption spectroscopy in glass studies. In: Meulebroeck W, Nys K, Vanclooster D, Thienpont H, editors. Proceedings of SPIE Vol. 8422, integrated approaches to the study of historical glass - IAS12. Vol. 8422. Belgium: SPIE Brussels 2012: 842208–1-11.
[73] Schreus JW, Brill RH. Iron and sulfur related colors in ancient glasses. Archaeometry. 1984;26:199–209.
[74] Reiche I, Röhrs S, Salomon J, Kanngießer B, Höhn Y, Malzer W, et al. Development of a nondestructive method for underglaze painted tiles - demonstrated by the analysis of Persian objects from the nineteenth century. Anal Bioanal Chem. 2009;393:1025–41.
[75] Meulebroeck W, Cosyns P, Baert K, Wouters H, Cagno S, Janssens K, et al. Optical spectroscopy as a rapid and low-cost tool for the first-line analysis of glass artefacts: a step-by-step plan for Roman green glass. J Archaeol Sci. 2011;38:2387–98.
[76] Arletti R, Conte S, Vandini M, Fiori C, Bracci S, Bacci M, et al. Florence baptistery: chemical and mineralogical investigation of glass mosaic tesserae. J Archaeol Sci. 2011;38:79–88.
[77] Galli A, Poldi G, Martini M, Sibilia E, Montanari C, Panzeri L. Study of blue colour in ancient mosaic tesserae by means of thermoluminescence and reflectance measurements. Appl Phys A. 2006;83:675–79.
[78] Mirti P, Davit P, Gulmini M. Colourants and opacifiers in seventh and eighth century glass investigated by spectroscopic techniques. Anal Bioanal Chem. 2002;372:221–29.
[79] Möncke D, Papageorgiou M, Winterstein-Beckmann A, Zacharias N. Roman glasses coloured by dissolved transition metal ions: redox reactions, optical spectroscopy and ligand field theory. J Archaeol Sci. 2014;46:23–36.
[80] Agostino A, Aceto M, Fenoglio G, Operti L. Analisi non invasive sugli smalti limosini medievali di Palazzo Madama. In: Castronovo S, editor. Smalti di Limoges del XIII secolo. Savigliano, Italy: L'Artistica Editrice, 2014: 211–39.
[81] Grazzi F, Iannaccone R, Barzagli E, Civita F, Salvemini F, Picollo M, et al. 2011. UV-VIS-NIR specular reflectance spectroscopy: a non invasive and portable technique for the characterization of the conservation status of ancient swords. Application to the Japanese blade collection of the Stibbert museum. In: Proc. ART11, 10th International Conference on non-destructive investigations and microanalysis for the diagnostics and conservation of cultural and environmental heritage. Florence: CD-ROM. 13-15 Apr 2011.
[82] Torrent J, Barròn V. Diffuse reflectance spectroscopy of iron oxides. In: Hubbard AT, editor. Encyclopedia of surface and colloid science. New York: Marcel Dekker, 2002: 1438–46.
[83] Rossman GR. Colored varieties of the silica minerals. Rev Mineral. 1994;29:433–68.
[84] Wood DL, Nassau K. The characterization of beryl and emerald by visible and infrared absorption spectroscopy. Am Mineral. 1968;53:777–800.
[85] Izawa MRM, Cloutis EA, Rhind T, Mertzman SA, Poitras J, Applin DM, et al. Spectral reflectance (0.35–2.5 µm) properties of garnets: implications for remote sensing detection and characterization. Icarus. 2018;300:392–410.
[86] Liu Y, Lu T, Mu T, Chen H, Ke J. Color measurement of a ruby. Color Res Appl. 2013;38:328–33.
[87] Bristow JK, Parker SC, Catlow CRA, Woodley SM, Walsh A. Microscopic origin of the optical processes in blue sapphire. Chem Commun. 2013;49:5259–61.
[88] Qiu JT, Qi H, Duan JL. Reflectance spectroscopy characteristics of turquoise. Minerals. 2017;7:1–10.
[89] Aceto M, Agostino A, Fenoglio G, Gulmini M, Bianco V, Pellizzi E. Non invasive analysis of miniature paintings: proposal for an analytical protocol. *Spectrochim Acta A*. 2012;91:352–59.
[90] Aceto M, Agostino A, Fenoglio G, Picollo M. Non-invasive differentiation between natural and synthetic ultramarine blue pigments by means of 250–900 nm FORS analysis. Anal Meth. 2013;5:4184–89.

[91] Orlando A, Picollo M, Radicati B, Baronti S, Casini A. Principal component analysis of near-infrared and visible spectra: an application to a XIIth century Italian work of art. Appl Spectrosc. 1995;49:459–65.