Multispectral Reflectance and UV Fluorescence Microscopy to study painting’s cross sections

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Abstract. UV Fluorescence microscopy is a powerful mean for visualizing and identifying structures in a specimen, and it has long been used in many applications, including pigment identification in artworks. Typical painting cross section samples are the result of multiple layers and especially for paintings produced from the thirteen to sixteen centuries, it is possible that for different pigments a different binder has been used, resulting in a complex stratification of materials. In this paper we propose a novel multispectral microscopy imaging system, which is specially designed for cultural heritage application, capable of acquiring both reflectance and fluorescence images in different wavebands, so to be able to document UV fluorescence emission and reflectance spectra.

1. Introduction
In the cultural heritage field, since the discovery of the Wood filter [1], UV fluorescence has been applied both for imaging the artwork’s surface and for microscopy of paint cross-sections. On the painting surface, UV fluorescence imaging can be used to detect varnishes and retouches [2]. The first ones, in fact, are typically strongly fluorescent. Moreover, the emission of most painting materials commonly used, increases with aging. Thus retouches, more recent that the rest of the paints, would appear darker under the UV light. Multispectral imaging techniques also for UV fluorescence imaging, have been developed to introduce new assessment possibilities [3] [4]. At the same time, also fluorescence microscopy has been widely used to analyse cross-sections of micro-samples, often complemented by SEM microscopy with EDS.

In fact, UV fluorescence microscopy is a powerful mean for visualizing and identifying structures in a specimen. However, like for UV fluorescence imaging, in order to be able to properly analyze the fluorescence emission of a sample, and not to stop at a qualitative examination, the microscopy system needs to be calibrated. Moreover, in order to extract the fluorescent signature, the emission at various wavelengths needs to be recorded, together with the corresponding reflectance signal. Currently, to the best of our knowledge, there is no proposed method that implements such requirements.

In this paper we propose a novel multispectral microscopy imaging system, which is specially designed for cultural heritage application. Although reflectance multispectral imaging microscopy...
have already been investigated [5], the novel microscope presented is capable of acquiring both reflectance and fluorescence images in several wavebands, chosen ad hoc to highlight the most relevant features, which are generally in the blue region, of the fluorescence spectra of paint samples. In addition, the method developed is designed to provide calibrated images. This is particularly useful when, like most of the times, samples exhibit multiple layers in cross sections. The underlying layers are in fact not visible on the painting surface. However, their understanding is extremely useful both for studying the artist’s technique and for conservation purposes. Especially for paintings of the thirteen till sixteen centuries, it is possible that for different pigments a different binder has been used, leading to a complex stratigraphy that cannot be easily studied otherwise. This is especially true for what concerns the organic matter of each layer. In fact, if inorganic components can be quite successfully investigated by SEM-EDS and microRaman spectroscopy, only micro-reflectance FTIR might provide some information about the organic compounds like binders and varnishes. Limitations owing to low signal-to-noise ratio from layers as thin as few tenths of micrometers, interference of strong overlapping peaks of inorganics and shape and intensity distortions in the reflectance spectra make micro-reflectance FTIR not suitable for a widespread use when applied to a painting stratigraphy. The immuno-fluorescence assay and staining tests [6] does not permit the identification of binders different from proteins and it leads to irreversible changes of cross-sections.

Documenting the reflectance and UV fluorescence emission for each layer in different wavebands on the other side, enables a detailed study of the painting’s inner structure. Binders, like egg tempera or linseed oil exhibit typical UV fluorescence emissions in the visible range, however other pigments are generally not fluorescent [7].

In this paper we analyze the multispectral UV fluorescence microscopy images acquired with this novel technique.

2. Test samples

2.1. Paint samples from the OPD collection

A set of samples has been prepared at the Opificio delle Pietre Dure to be used as reference for testing diagnostic techniques. The samples have been made according to the techniques described by Cennino Cennini and other ancient treatises on arts and reflect also the evidences collected from actual paintings studied at the OPD [8].

Two preparation coatings made with rabbit glues and calcium sulphate dihydrated were applied on wood. An “imprimitura” layer, made of rabbit glue and water, overlaps this ground layer. Pictorial layers with different pigments, pure or in mixture, as thin as few tenths of micrometers were applied over the abovementioned ground layer (preparation + "imprimitura"). Pigments were supplied by Zecchi (Italy) and checked by FTIR spectroscopy and/or SEM-EDS as for their actual composition. For some samples, “stand” linseed oil (Zecchi, Italy) was used as binding medium, while for other ones tempera was prepared by mixing egg yolk, egg white and vinegar with a 2:1:1 volume ratio. On top of such samples, a glazing layer with a semitransparent organic colorant, like madder lake or saffron, with linseed oil, was also applied.

We have chosen this set of samples because it has been widely studied and analysed, and we could access a variety of data on it (see eg. fig. 2).

![Fig. 1 A set of samples of pictorial layers. The triangle on the top of each swatch has been detached to be analysed under the standard optical microscope, SEM, and with the novel multispectral microscope.](image-url)
Fig. 2 Cross section of sample 40D observed under standard optical microscope with (a) visible and (b) UV light and (c) under scanning electron microscope (SEM-EDS). Not all the materials used can be detected using the current analytical techniques. The EDS spectrum of rubia lake shows the presence of aluminium, related to alum in the lake. The larger grains of layer 2 (in c) are composed by cinnabar, whereas the smaller ones consist of lead white. No information can be retrieved on the binder used.

Fig-3 Cross-section of two of the samples prepared for the current study. The yellow circles highlight the position of the Barium Sulphate references.

2.1.1. Sample preparation
We took micro-fragments from near the edge of the sample (see fig. 1 and fig. 2). The sample were then embedded in an alkyd resin and polished with abrasive paper. A micro hole was made in the resin where Barium Sulphate powder was pressed (see fig. 3). Barium Sulphate exhibits a very uniform reflectance from UV to Near IR. Moreover it does not exhibit any UV fluorescence emission.

3. Novel multispectral microscopy

3.1. Multispectral microscope
The acquisition set up is composed by two UV lamps, a fiber optic illuminator with a ring-light guide (PL3000, A-1170, Photonic), an optical system, a CCD camera and a set of band-pass filters, as shown in Fig. 4a. Each UV lamps has inside 3 UV LEDs (High Power LED M365D1, Thorlabs, characterized
by a spectral emission peaked at 365 nm with a FWHM of 10 nm, and an optical output power of 190 mW each LED. The sources were placed at 45° with respect to the normal samples.

The camera is a monochromatic QSI 583w camera with a CCD full-frame sensor (KAF8300) with 3326x2504 pixels (pixel size of 17.96mm x 13.52mm). The CCD camera is equipped with an internal filter wheel. The filter selection reflects the known characteristics of paint reflectance and fluorescence [7] They are narrow bandpass filters with a FWHM of 10 nm with central wavelengths of 450, 470, 500, 532, 550, 600, 650, 750 nm (Thorlabs) (Fig. 4b). The optical system is composed by a zoom body tube (with 6.5x magnification, 1-60135, Navitar), a lens attachments (with 2x magnification, 1-600113, Navitar) and an adaptor for the CCD camera (1-6015, Navitar).

4. Calibration procedures
We aimed at acquiring reflectance images, thus images that at every pixel present the reflectance value of the paint sample, and calibrated UV fluorescence emission images, thus images that, at every pixel, record an emission value independent from the system used to acquire them, and from environmental conditions. In order to reach this goal, several steps are needed.

4.1. Image registration and calibration
The registration procedure removes geometrical misalignments among the images. These are mainly due to small changes in the optical path, between the object and the CCD sensor for the various filters. The algorithm is based on mutual information maximisation [9].

All acquired images have to be corrected for uneven illumination and for stray light. For fluorescence images it is also mandatory to un-mix the contribution of UV fluorescence emission in the visible range from the stray light due to visible environment radiation (although the measurements were taken in a dark room, some stray light is unavoidable).

For each filter, we acquired with the novel microscopy system a “raw” fluorescence image ($I_{R_{UV}}$) of the cross section. We recorded in this portion of the image, values directly proportional to a known percentage of the incident stray-light.

Two “flat” images i.e. image of a uniform surface placed in the same position as the cross section were also acquired for each wavelength, one using UV lamps ($I_{F_{UV}}$) and another using white light ($I_{R_{R}}$). The camera automatically subtracted a “dark” image for each band.

We computed the Corrected Fluorescence+StrayLight Image ($I_{C_{UV&S}}$), and the Corrected Radiance Image ($I_{C_R}$) according to the following:
\[
I_{C_{UV+S}} = (a_{F_{UV}})^*(I_{R_{UV}})/(I_{F_{UV}})
\]
\[
I_{C_R} = (a_{F_{R}})^*(I_{R_{R}})/(I_{F_{R}})
\]

where \(a_{F_{UV}}\) is the average value of \(I_{F_{UV}}\) over the whole image, and \(a_{F_{R}}\) is the average value of \(I_{F_{R}}\) over the whole image.

4.1.1. Reflectance images

The Reflectance Image \(I_{Ref}\) can then be computed as

\[
I_{Ref} = I_{C_{R}} / s_{lr} \times (100/R)
\]

where \(s_{lr}\) is the average of the pixels’ values in \(I_{C_{R}}\) over the part where the reflectance standard was imaged and \((R)\) is the reflectance percentage of the reflectance standard used.

4.1.2. Calibrated UV fluorescence images

The Corrected Fluorescence Image \(I_{C_{UV}}\) can be obtained subtracting the Stray-Light image \(I_{S}\) from the \(I_{C_{UV+S}}\) image. \(I_{S}\) can be computed as

\[
I_{S} = I_{Ref} \times s_{l_{uv}} \times (100/R)
\]

where \(s_{l_{uv}}\) is the average of the pixels’ values where reflectance standard is in \(I_{C_{UV+S}}\).

Signal acquisition was performed by a CCD array. Its response depends not only on the signal energy but also on the system behaviour for each filter, in particular: the spectral transmittance of the optical systems in front of the detector array \(\Phi(\lambda)\), the spectral transmittance of the \(k\)-th optical colour filter \(\Phi_k(\lambda)\) and the spectral sensitivity of the CCD array \(\alpha(\lambda)\).

To determine the calibration factors \(W_k\) \((k=1,2,...7)\) that need to be introduced in order to derive the calibrated images \(I_{Cal_{UV}}\) from \(I_{C_{UV}}\) images, which are independent of the specific camera and filters used, we proceeded with the indirect method as described in [4].

5. Image analysis

Two samples, 20D and 40D, of the set described in [7] were chosen to illustrate how the method works. The visible image of sample 20D, together with the UV fluorescence image, reconstructed starting from the multispectral images acquired, are shown in fig. 5 and fig. 6 respectively. The single reflectance images in the 8 bands chosen and the images of the UV fluorescence emission are presented in fig. 7. The visible image of sample 40D together with the UV fluorescence are shown in figures 9 and fig. 10, respectively. The reflectance images in the 8 bands chosen and the images of the UV fluorescence emission are presented in fig. 11.

Both samples exhibit, over the white preparation, a red layer made of a mixture of cinnabar and lead white, but the binder is tempera for sample 20D and linseed oil in case of sample 40D. Above the red layer with cinnabar, a glaze layer with madder lake and linseed oil is applied. Since the fragments were taken near the edge of the samples, in some cases the paint dropped down along the end side of the stratigraphy and to a certain extent entered below the layer underneath.

![Fig. 5 Visible image of the cross-section of sample 20D](image1)

![Fig. 6 UV fluorescence image of the cross-section of sample 20D](image2)
**Fig. 7** Reflectance and UV fluorescence multispectral images of Sample 20D. The numerical reference is to the peak of the interferential filter used (see fig 4b).

**Fig. 8** UV fluorescence image at 650 nm of sample 20D. The cross section exhibit a complex fluorescence emission. The Cinnabar layer in particular shows rather difference emission within the layer (highlighted by the blue line), probably due to a penetration of the binder of the top layer (linseed oil) in the Cinnabar layer, which was originally a tempera. Strong fluorescence emission is also displayed by some areas in the preparatory layer. The reason for this needs further investigations.
5.1. Spectral features

The multispectral images acquired document the inner structure of the samples, showing the reflectance and the UV fluorescence emission in different wavebands. We were expecting to find a
clear distinction in the emission peaks of each layer, depending on the paint and on the binder used, corresponding to the known behavior of the reference materials.

However, in reality, the cross sections displayed a much more complex scenario. Different emissions are also present within each layer, possibly because of occurred interactions. For example one binder could have migrated from one layer to the next, as it seems to have happened e.g. for the case illustrated in fig. 8. Such cross-sections had also been coated to be analysed with SEM technique, and then uncoated. This could also have introduced unwanted.

6. Conclusions and future work

To the best of our knowledge, the method presented is novel and addresses an unmet goal in the analysis of paint materials. Sample microscopy has been used in CH field for many years, however not in a multispectral fashion, and generally providing only qualitative and not quantitative data. Multispectral reflectance and UV fluorescence emission microscopy presents several clear advantages for the understanding of a complex stratigraphy. For example organic matter, like that of binders and varnishes, cannot be easily studied with conventional techniques. In fact, only micro-reflectance FTIR might provide some information about the organic compounds, however, the signals of layers few tenths of micrometers thin, generally display a low signal-to-noise ratio, interference of strong overlapping peaks and shape and intensity distortions. On the other side, documenting the reflectance and calibrated UV fluorescence emission for each layer in different wavebands, enables a detailed study of the painting’s inner structure. Understanding the different emissions and correlating them with the presence of some materials will be extremely useful both for studying the artist’s technique and for conservation purposes. We plan to further develop the technique proposed, increasing the magnification and possibly the number of interferential filters, to reach its full potential.

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