Multianalytical non-invasive characterization of phthalocyanine acrylic paints through spectroscopic and non-linear optical techniques.

Alice Dal Fovo1,2,*, Mohamed Oujja3, Mikel Sanz3, Alejandro Martínez-Hernández3, María Vega Cañamares4, Marta Castillejo3, Raffaella Fontana1

1 Consiglio Nazionale delle Ricerche - Istituto Nazionale di Ottica, CNR-INO, Largo Enrico Fermi 6, 50125 Firenze, Italy.
2 Università degli Studi di Firenze, Dip. Chimica, Via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy.
3 Instituto de Química Física Rocasolano, CSIC, Serrano 119, 28006 Madrid, Spain.
4 Instituto de Estructura de la Materia, CSIC, Serrano 121, 28006 Madrid, Spain.

Abstract. The documentation and monitoring of cleaning operations on paintings benefit from the identification and quantification of the materials to be selectively removed. Since the preservation of integrity of materials in artworks diagnosis is a priority, the application of non-invasive techniques is commonly preferred. In this work, we present the results obtained with a set of non-invasive optical techniques for the chemical and physical characterization of six copper-phthalocyanine (Cu-Pc) acrylic paints. Cu-Pc pigments have been extensively used by artists over the past century, thanks to their properties and low cost of manufacture. They can also be found in ancient paintings in form of overpaints/retouchings, providing evidence of recent conservation treatments. The optical behaviour and the chemical composition of Cu-Pc paints were investigated through a multi-analytical approach involving micro-Raman spectroscopy, Fibre Optics Reflectance Spectroscopy (FORS) and Laser Induced Fluorescence (LIF), enabling the differentiation among pigments and highlighting discrepancies with the composition declared by the manufacturer. The applicability of Non Linear Optical Microscopy (NLOM) for the evaluation of paint layer thickness was also assessed using the modality of Multi-photon Excitation Fluorescence (MPEF). Thickness values measured with MPEF were compared with those retrieved through Optical Coherence Tomography (OCT), showing significant consistency and paving the way for further non-linear stratigraphic investigations on painting materials.

Keywords: spectroscopic techniques, non-invasive analysis, phthalocyanine paints, multi-photon excitation fluorescence, optical coherence tomography, stratigraphy.

Corresponding author:

* Alice Dal Fovo, e-mail: alice.dalfovo@ino.it
1. Introduction

Today azo and polycyclic compounds, including phthalocyanine, perylene, diazoxine, DPP (diketopyrrolopyrrole), indanthrene and quinacridone, are among the most important synthetic organic pigments in the worldwide production. Linstead\textsuperscript{1,2} was the first to define the molecular structure of phthalocyanine in 1933, whose name derives from the phthalic acid derivatives used for the synthesis and from the greenish-blue of their hue (cyan from the Greek κυανος). Phthalocyanine (Pc) molecules consist of four isoindole units (C\textsubscript{8}H\textsubscript{7}N) connected by four nitrogen atoms forming a macrocycle. Copper phthalocyanines (Cu-Pc) are metal complexes where a Cu\textsuperscript{2+} cation occupies the central cavity of the macrocycle and forms covalent bonds with the N atoms (Fig. 1). Cu-Pc \(\alpha\)-, \(\beta\)-, and \(\gamma\)- polymorphic forms are widely used as blue and green pigments, referenced as PB15 and PG7 (class 74) in the Colour Index Database (Society of Dyers and Colourists and American Association of Textile Chemists and Colorists).\textsuperscript{3} Various artists, inter alia Magritte, Picasso, Lichtenstein, Mondrian, Kandinsky and Pollock, made use of PB15 pigments between 1935 and 1990.\textsuperscript{4,5} Phthalocyanine paints owe their success to their remarkable properties, such as ease of use, low cost of manufacture, excellent lightfastness, heat stability up to 550 °C and good resistance to most solvents.\textsuperscript{6} The partial solubility of Cu-Pc in aromatic solvents, though, may lead to colour changes (blue towards green) due to crystallization defects occurring during the transition from \(\alpha\)- to \(\beta\)- polymorphic form with concurrent growth in crystals size.\textsuperscript{7} Since aromatic compounds, especially toluene and xylene, are used as solvent agents in several varnishes and adhesives in paintings, the risk of deterioration of Cu-Pc pigments is significantly high. To prevent damages, it is crucial to identify Cu-Pc paints before any restoration treatment involving the removal or application of protectives/consolidants. Modern pigments, like Cu-Pc, can be found in ancient paintings in form of pictorial retouchings, especially when the original colours were toxic or unavailable.\textsuperscript{5} Therefore, the detection of Cu-Pc overpaints, recognizing them in the presence of ancient pigments, may provide evidence of previous conservation treatments. As a rule, a pictorial retouching that infringes the ethical standards of restoration, i.e. overlapping the ancient paint or being excessively recognizable in terms of colour difference, has to be replaced. Like altered varnishes, the removal of modern overpaints requires a selective cleaning, which greatly benefits from a precise quantification of the material present before the process starts, both for documentation and monitoring purposes. An exhaustive chemical-physical characterisation of materials can be achieved through a multi-analytical approach consisting in the application of a variety of techniques.

**Fig. 1.** Copper phthalocyanine molecule. A Cu\textsuperscript{2+} cation occupies the central cavity of the macrocycle forming covalent bonds with N.
When dealing with works of art, preserving materials integrity is of utmost importance. Therefore, non-invasive procedures, not involving either sampling or pre-treatments, are preferred. Among the various spectroscopic techniques, Raman spectroscopy, Vis-NIR Fibre Optics Reflectance Spectroscopy (FORS), and Laser Induced Fluorescence (LIF) are well-established and widely used for the study of the chemical composition of paint materials. Raman spectroscopy allows for the assignment of molecular composition according to the vibrational frequencies of chemical bonds present in both inorganic and organic compounds. Vis-NIR FORS is used to probe pigments and identify molecular functional groups, i.e. absorption bands due to overtones and combinations of fundamental vibrations. When applied in combination with Raman spectroscopy, FORS facilitates the identification of chemical compounds, especially when the detection of the Raman signal is hampered by the intense fluorescence emitted by binders and varnishes, or when different pigments show similar Raman spectra (like in the case of several phthalocyanine paints analysed in this paper).

On the other hand, LIF is based on the detection of the fluorescence emitted by molecules electronically excited with a coherent monochromatic laser source. Natural organic pigments and dyes, as well as synthetic organic and inorganic pigments, may fluoresce due to the presence of delocalized electrons in molecules containing multiple aromatic rings, long chains of conjugated double bonds or di-azo bonds. Since LIF results are not always unambiguous, the support of other spectroscopic methods may be necessary for a full material analysis.

In the past decades, a significant number of non-invasive optical methods have been applied to paintings for the study of their multi-layer internal structure, with the aim of overcoming detection limits due to the presence of highly scattering and semi-opaque materials. Optical Coherence Tomography (OCT), originally developed for biological applications, provides high-resolution cross-sectional images of semi-transparent objects. It has proven to be effective for the in-depth visualization of low scattering varnish layers, enabling the distinction between aged and new varnish. In the presence of high-reflecting varnishes, the use of an OCT setup combined with confocal microscope optics may facilitate internal visualization, by focusing the beam inside the sample rather than on its outer varnish surface. Apart from OCT, a variety of 3D imaging techniques have been introduced for the study of artistic paintings in the past decade.

Non-linear optical microscopies (NLOM), working in various modalities, including Multi-Photon Excitation Fluorescence (MPEF) and Second and Third Harmonic Generation (SHG and THG), account for cutting-edge, non-invasive analysis based on non-linear optical processes through which molecules simultaneously interact with two or more photons within the same quantum event. These non-linear optical phenomena can be observed when a given material is excited by a tightly-focused femtosecond (fs) pulsed laser, propagating through a high numerical aperture microscope objective, ensuring a good penetration capability and a high axial resolution, in the range of micrometres. NLOM techniques may provide 3D compositional and structural information based on the detection of fluorophores (by MPEF), of crystalline or highly organized structures without inversion symmetry (by SHG) or of local differences in refractive index and dispersion, i.e. interfaces (by THG). NLOM analyses have been applied for 3D imaging of protective layers with the aim of supporting cleaning processes of natural and synthetic varnishes. This allowed the in-depth monitoring of the extent of morphological degradation due to aging or laser ablation. Cross-section pictorial layers were obtained through the NLOM modality of femtosecond pump-probe microscopy and more recently, MPEF
imaging has been applied for the determination of thickness of egg-tempera layers.\textsuperscript{29} A recent work has illustrated the comparison of OCT and NLOM for the study of painting materials.\textsuperscript{22} Based on these antecedents, the main limitation of NLOM imaging seems to be due to the presence of highly scattering and/or absorbing media (pigments), which obstruct the detection of the NLOM signal.

In this work, we present the results obtained through the application of a set of non-invasive optical techniques for the chemical and physical characterization of six phthalocyanine acrylic paints (©Maimeri, Brera, IT): Primary Blue Cyan (PBC), Phthalo Blue (PB), Phthalo Green (PG), Cobalt Blue Hue (CBH), Permanent Blue Light (PBL) and Permanent Green Light (PGL). The optical behaviour and the chemical composition of the aforementioned acrylic paints were investigated through a multi-analytical approach involving micro-Raman spectroscopy, FORS and LIF. The results obtained were then compared with the chemical information provided by the manufacturer. This work also aims at further testing the applicability of NLOM techniques and investigating non-linear optical phenomena involved in painting materials. In-depth analyses of thin phthalocyanine acrylic paints layers laid on a glass support were carried out non-invasively by NLOM in the modality of MPEF. Thickness values determined by MPEF were compared by those retrieved through OCT in order to verify the reliability of the NLOM results.

2. Experimental

2.1. Materials

The set consists of six acrylic paints (Extra-fine acrylic colours, ©Maimeri Brera, IT) laid on glass coverslips (2.5 x 5 cm$^2$, 140 μm thickness). Three are pure Cu-Pc: Primary Blue Cyan (PBC), PB15:3 – 74160; Phthalo Blue (PB), PB15:1 – 74160 and Phthalo Green (PG), PG7 – 74260. The remaining three are mixtures of Cu-Pc and different organic compounds, like aniline and acetoacetanilide, and the inorganic compound titanium dioxide: Cobalt Blue Hue (CBH), PB15:3 – 74160, PW6 – 77891, PB29 – 77007; Permanent Blue Light (PBL), PB15:3 – 74160, PG7 – 74260, PW6 – 7789 and Permanent Green Light (PGL), PG7 – 74260, PW6 – 77891, PY97 – 11767. Each pigment is referenced according to the Colour Index Database.\textsuperscript{3} Bright-field images and micro-images, obtained with a Leica DFC 420 charge-coupled detector (CCD) camera, are shown in Figure 2.
Fig. 2. Copper phthalocyanine paint layers applied on glass coverslips (upper row) and micro-images (lower row, 740 x 550 μm²) showing paint micromorphology. CIEL*a*b* values as measured through FORS analysis, are also reported (see text).

2.2. Methods

2.2.1. Micro-Raman Spectroscopy

Raman spectra were collected using a Ranishaw InVia 0310-02 System based on a continuous Nd:YAG laser excitation source at 532 nm that illuminated the surface of the paint layer with a spot diameter of 1 μm. The system is equipped with a Leica microscope and an electrically refrigerated CCD camera. The wavenumber resolution was 4 cm⁻¹ and the acquisition time set for measurements of 10 s. Low laser powers (0.15 – 0.3 mW) were used to prevent damage of the samples.

2.2.2. Fibre Optics Reflectance Spectroscopy (FORS)

FORS spectra were acquired with a Zeiss Multi-Channel Spectrometer, including a MCS 521 VIS NIR-extended module and a MCS 511 NIR 1.7 module with spectral sensitivity in the 304 - 1100 nm and 950 - 1700 nm ranges, with spectral resolution of 3.2 nm and 6.0 nm, in the visible and IR region, respectively. The illumination/observation geometry is 45°/0°. For measurements, each sample was placed on a 100 % reflecting background (Spectralon) and each spectrums was the resulting average of three acquisitions from each of three measurements points that were selected for each sample. The output signal was processed through a dedicated software, providing also CIEL*a*b* coordinates computed using the measured Vis spectrum with standard D65 illuminant and 2° observer (Fig. 2).

2.2.3. Laser Induced Fluorescence (LIF)

Excitation of the fluorescence emission was induced by a Q-switched Nd:YAG laser operating at its 4th harmonic at 266 nm, with pulse duration of 15 ns, and repetition rate of 1 Hz. The luminous emission was collected and dispersed by a 0.30 m spectrograph with a 300 lines/mm grating (TMc300, Bentham) coupled to an intensified CCD (2151, Andor Technologies). The temporal gate (width of 3 μs) was fixed at zero time delay with respect to the arrival of the laser pulse to the surface of the sample.
2.2.4. Optical Coherence Tomography (OCT)

In-depth imaging was performed with a Time-Domain confocal OCT prototype developed at the Istituto Nazionale di Ottica (CNR-INO, Italy), which combines confocal microscope optics with the OCT setup. The system operates at a wavelength of 1550 nm with axial resolution of 10 μm in air and lateral resolution of 2.5 μm. The maximum acquisition length is 25 mm, both in the x and y directions, whereas the in-depth probing length is 1 mm.

2.2.5. Multi-Photon Excitation Fluorescence (MPEF)

In-depth analysis was also performed with a non-linear optical microscope developed at the Instituto de Química Física Rocasolano (IQFR-CSIC, Spain) that allows the collection of the MPEF signals from the focal volume at the sample plane in the reflection mode. The setup is depicted in Figure 3. The excitation light source is a mode-locked Ti:Sapphire femtosecond laser emitting at 800 nm, with average power of 680 mW, delivering 70 fs pulses at a repetition rate of 80 MHz. A variable neutral density filter (NDC-50C-2M, Thorlabs) was used to control the laser power reaching the sample. For the present measurements, the average power was in the range of 1-15 mW, values that ensure absence of damage on the paint layers. The laser beam was conducted to the sample through the aperture of a microscope objective lens (M Plan Apo HL 50X, Mitutoyo, NA 0.42) by using a dichroic beam splitter (FF750-SDi02-25x36, Semrock) with a high reflection at 800 nm.

The laser focal plane was selected with motorized translation XYZ stages (Standa 8MVT100-25-1 for XY and Standa 8MTF for Z). The lateral and in-depth resolutions achieved are of 1 and 2 μm, respectively. A LabView interface was used to control both scanning and data acquisition procedures. The MPEF signals were collected in the backward direction through the microscope objective lens and a beam splitter (70/30) and measured using a photomultiplier tube (9783B, ET Enterprises) connected to a lock-in amplifier (SR810 DSP, Stanford Research Systems) to ensure high amplification and signal-to-noise ratio. A short pass filter (335-610 nm, Thorlabs FGB37S) was placed at the entrance of the photomultiplier to cut off the reflected laser light. The remaining 30% of the MPEF signal was sent to a CCD camera (Thorlabs DCC1645C) for online visualization of the sample surface and the signal collection process.

![Fig. 3. Layout of the non-linear optical microscope used for MPEF measurements.](image-url)
3. Results and discussion

3.1. Micro-Raman Spectroscopy

The chemical composition of the six analysed paints was determined through comparison with spectral information reported in literature and databases available online.\textsuperscript{33-35} The main Raman bands assigned for each Cu-Pc pigment, together with their respective intensities, are reported in Table 1, while the chemical compositions are summarized in Table 2. As an example, Figure 4 includes the Raman spectra acquired in the PB and PBL samples.

Spectra of PBC and PB display numerous common bands (the most intense ones are centred at about 590, 680, 1451 and 1527 cm\(^{-1}\)), hampering the distinction between the two pigments. Concerning PG, the most significant bands appear at 685, 1503 and 1536 cm\(^{-1}\). Raman analysis on these three pure phthalocyanine pigments (PB, PBC, PG) allowed assignment of the bands in accordance with literature.

Table 1. Main Raman bands of six analysed copper phthalocyanine paints.

| Paint (acronym)          | Main Raman bands [cm\(^{-1}\)] and relative intensity*, \(\lambda_{ex} = 532\) nm |
|-------------------------|------------------------------------------------------------------|
| Primary Blue Cyan (PBC) | 231w, 255w, 590m, 680m, 747w, 951w, 1037w, 1137w, 1341w, 1451m, 1527s, 1595w, 2672w, 2870w, 2976w, 3056w |
| Phthalo Blue (PB)       | 230m, 260m, 548s, 583m, 680m, 747w, 808w, 951w, 1096m, 1145w, 1450m, 1530s, 1584w, 1644w, 2192w |
| Phthalo Green (PG)      | 142w, 162w, 505m, 685s, 818w, 978w, 1080m, 1284m, 1340w, 1388m, 1503s, 1536s |
| Cobalt Blue Hue (CBH)   | 143w, 231m, 255m, 450s, 590s, 609s, 680s, 747w, 831w, 951w, 1008w, 1037w, 1137m, 1341s, 1451s, 1527s, 1595w, 2870w, 3056w |
| Permanent Blue Light (PBL)| 153w, 309m, 505w, 615w, 685s, 818w, 978w, 998w, 1008w, 1080m, 1240m, 1332w, 1340m, 1388s, 1455w, 1503s, 1536s, 1591w, 1668w |

* s: strong; m: medium; w: weak

Table 2. Chemical composition of the six analysed copper phthalocyanine paints.

| Paint (acronym)          | Inorganic Copper Phthalocyanine
|-------------------------|----------------------------------|
| PB15:1                  | α [C\(_{32}\)H\(_{24}\)CuN\(_{8}\)] |
| PB15:3                  | β [C\(_{32}\)Cl\(_{2}\)CuN\(_{8}\)] |
| PG7                     | Organic Chlorinated Phthalocyanine
|                         | Sodium Polysulphide-Aluminosilicate |
| PB29                    | Inorganic Ultramarine [azo coupling of aniline and acetoacetanilide] |
| PY97                    | Organic Arydile yellow |
| PW6                     | Inorganic Titanium Dioxide [TiO\(_{2}\)] |

The three mixtures (CBH, PBL and PGL) and the PBC paints show bands ascribed to additional components. The bands at 1332, 1455, 1591 and 1668, cm\(^{-1}\) in the PGL spectrum correspond to the pigment Arydile Yellow, PY97.\textsuperscript{36} Bands at 260, 548, 583, 808, 1096, 1644 and 2192 cm\(^{-1}\) in the CBH spectrum allow identifying the presence of Ultramarine, PB29. Bands at 143, 231, 450, 609 cm\(^{-1}\) in PBL, CBH, and PBC (traces) are due to Titanium White, PW6, in rutile form. We found some discrepancies with the composition declared by the manufacturer. The most significant one concerns PGL paint, in which bands of barium sulphate (at 998 cm\(^{-1}\)) and calcium sulphate (at 1008 cm\(^{-1}\)) where observed, their presence possibly related to a component added as filler. Also in this case, bands assigned to the Cadmium Yellow pigment (at 153, 309, 615 cm\(^{-1}\)) were detected.\textsuperscript{37} Furthermore, no traces of titanium dioxide were found in PGL, in contrast with what was reported by the manufacturer.
Table 1: Ceramic Pigments and their Characteristics

| Pigment                     | Characteristics |
|-----------------------------|-----------------|
| Primary Blue Cyan (PBC)     |                 |
| Phthalo Blue (PB)           | ✓               |
| Phthalo Green (PG)          | ✓               |
| Cobalt Blue Hue (CBH)       | ✓               |
| Permanent Blue Light (PBL)  | ✓               |
| Permanent Green Light (PGL) |                 |

3.2. **Fibre Optics Reflectance Spectroscopy (FORS)**

The reflectance spectra of the six acrylic paints studied in this work are reported in Figure 5. Previous studies have shown that Cu-Pc pigments present specific spectral features, which upon combined analysis by Raman spectroscopy allow discriminating between different compounds. It is observed that all analysed paints display varying degrees of transparency at 800 nm, the wavelength of the femtosecond laser used for NLOM measurements in the MPEF mode.

![Figure 4: Micro-Raman spectra of a) Phthalo Blue (PB) and b) Permanent Blue Light (PBL) acrylic paints upon excitation at 532 nm.](image)

![Figure 5: Reflectance spectra of Cu-Pc pigments in the 300-1700 nm spectral range.](image)

Figures 6 and 7 show specific spectral zones for better differentiation of the paint materials. Figure 6 displays the spectral region between 400 and 920 nm for the Primary Blue Cyan, Phthalo Blue and Phthalo Green paints. PG (red line in Fig. 6) shows a reflectance peak centred at around 500 nm, whereas the absorption band is in the 550-790 nm range (absorption edge). In turn, PB and PBC (blue and orange lines in Fig. 6, 8.
respectively) both exhibit a reflectance peak around 460–470 nm and absorption in the 580–720 nm region. Differentiation between these three paint materials is based on identifying the inflection points of the second derivative of the reflectance function in the NIR region between 700 and 900 nm (Fig. 6b) which are located at 800, 860 and 900 nm for PBC, PB and PG respectively.

**Fig. 6.** a) Reflectance spectra in the 400-920 nm of Primary Blue Cyan, Phthalo Blue, Phthalo Green; b) second derivative curves in the 700-920 nm range (as selected by the grey dotted square in a), where the zero-cross points correspond to the position of the inflection points.

Figure 7 shows the spectra of all Cu-Pc paints in the 350-700 nm region. Further confirmation of the chemical composition of these paints is based on the detection of a shoulder at 410 nm attributed to titanium dioxide in rutile form.\(^\text{38}\)

**Fig. 7.** Reflectance spectra of Cu-Pc paints. The shoulder at 410 nm highlights the presence of TiO\(_2\) only in Primary Blue Cyan, Cobalt Blue Hue and Permanent Blue Light.

### 3.3 Laser Induced Fluorescence (LIF)

LIF spectra of the six Cu-Pc paints are displayed in Figure 8. The common broad emission band between 280 and 380 nm is attributed to the acrylic binder and it is consistent with previous published studies.\(^\text{10,39,40}\) The
emission bands at higher wavelengths between 380 and 580 nm are characteristic of each pigment. Specifically, CBH and PBC show very similar fluorescence emissions, with maxima at 400 and 440 nm, PBL displays an emission band centred at around 400 nm, PGL shows an emission band slightly shifted towards longer wavelengths (460-570 nm, centred at 500 nm) with respect to the other pigments and PG emits a relatively less intense fluorescence band located between 380 and 550 nm. Finally, the PB spectrum is characterised by a nearly featureless broad emission band.

**Fig 8.** LIF spectra acquired by excitation at 266 nm of the Cu-Pc pigments.

The identification of the LIF emission bands collected by excitation at 266 nm, as described above, is necessary to ensure the proper detection of the MPEF signals. Given the wavelength of the femtosecond laser source, it is expected that three-photon absorption at 800 nm would yield similar fluorescence spectra than those collected by one-photon absorption at 266 nm. The detection range of the non-linear optical microscope is limited by the filter in front of the photomultiplier to the region of 335-610 nm. According with LIF measurements, this implies that MPEF signals include emission from the binder and pigment components of the paints.

### 3.4. Optical Coherence Tomography (OCT)

For each sample, two OCT profiles were acquired along two perpendicular lines in the middle of the painted surface and indicated by a) and b) in Figure 9. The real thicknesses of the paint layers were evaluated as an average of six values measured along a 1 mm OCT profile, taking into account the refractive index of the medium (n = 1.5). The signal coming from the glass support was taken as a reference, assuming that 1 pixel corresponds to 1 μm along the in-depth direction and to 5 μm along the scanning direction of the tomographic image.

It is worth mentioning that the acquired OCT profiles enable the visualization of the lower glass/air interface due to the transparency of the Cu-Pc paints at the wavelength of the OCT source of 1550 nm. Differently, no distinction between paint and glass is possible, due to the negligible difference in refractive index between the two materials. For that reason, the thickness of the paint layers is assessed taking as reference the glass surface visible at the border of each sample. Table 4 displays the thickness values determined by OCT.
3.5. Multi Photon Excitation Fluorescence (MPEF)

MPEF signals were collected for all the six Cu-Pc paints in an area of 5 x 5 mm$^2$ approximately located in the middle of each sample in order to ensure a correct comparison with the OCT tomograms. On the basis of signal intensity, the detection was optimized in each case by setting the laser power to the most suitable range, although it never exceeded 15 mW to ensure non-damaging measuring conditions. The dependence of the MPEF signal with the depth below the surface of the paint (Z-scan) was measured at five different sample positions of the analysed area. MPEF profiles were normalized and fitted with a Lorentzian function, and the full width at half maximum (FWHM) was taken as an estimation of the paint layer apparent thickness. To obtain the real thickness, the FWHM values where subsequently corrected by applying the apparent depth correction factor (F), according to the formula

$$F = \frac{1 - \sqrt{1 - NA^2}}{n - \sqrt{n^2 - NA^2}} = 1.54$$

where $n$ is the refractive index of the sample (1.5), computed following ref. $^{35}$, and NA the effective numerical aperture of the focusing objective lens (0.42). The collected MPEF signals as a function of depth are represented in Figure 10, together with Lorentzian function fits. The thickness of the paint layers after correction (FWHM$^{corr}$) are reported in Table 4 with standard errors and OCT measured thickness for comparison.
Table 4 brings evidence of the good agreement between the paint thickness values determined in this work through MPFE and OCT. Liang et al.\textsuperscript{22} have discussed the complementary capabilities of NLOM and OCT and have presented some evidence of damage on non-transparent painting materials under the high laser intensities often used in NLOM. Dal Fovo et al.\textsuperscript{29} examined tempera paint layers by MPEF upon femtosecond excitation at 740 nm and showed that their thickness was underestimated due to the strong absorption and attenuation of the emitted fluorescence. The NLOM measurements presented in this work are a more favourable case. First, the average laser power used to measure the MPEF signals are far from the damage threshold of the paint materials (as monitored through CCD online visualization of the sample surface during femtosecond laser excitation). Additionally, excitation at 800 nm enables an adequate in-depth penetration to generate the MPEF signal in all phthalocyanine acrylic paints that also display sufficient degree of transparency in the spectral range where the MPEF signal is expected. Notwithstanding the usefulness of the THG modality of NLOM to determine the layer boundaries, the results shown herein demonstrate that the proper choice of laser excitation wavelength and average power and of the spectral range for collection of the fluorescence signals is crucial to ensure a correct estimation of thickness paint layers by MPEF.

\textbf{Table 4.} Comparison between thicknesses measured by MPEF and OCT.
4. Conclusions

This paper describes the characterization of a set of purposely developed samples of six phthalocyanine acrylic paints using non-invasive spectroscopic, interferometric and non-linear optical microscopy techniques. Chemical-physical characterization of the paint materials was obtained by laser micro-Raman spectroscopy and Fibre Optics Reflectance Spectroscopy (FORS), which provided complementary information for the identification of molecular compounds in each paint and for highlighting in some cases discrepancies with the chemical composition declared by the manufacturer. The application of these two techniques made also possible to differentiate between the two pure phthalocyanine Phtalo Blue and Primary Blue Cyan (PB15:1 and PB15:3 pigments, α- and β- polymorphic forms, respectively), having very similar composition but different reflectance behaviour. The measurement of the laser induced fluorescence (LIF) spectra of the paints upon excitation at 266 nm allowed for discriminating the bands emitted by the acrylic binder from those of the pigments, while non-invasive stratigraphic analysis by optical coherence tomography (OCT) yielded an estimation of the paint thicknesses. The results obtained using these techniques served to select the adequate excitation and signal collection conditions for studying the phthalocyanine acrylic paints by Multi-Photon Excitation Fluorescence, a modality of non-linear optical microscopy (NLOM), a cutting-edge non-linear technique seldom applied on painting materials. Upon femtosecond laser excitation at 800 nm, and applying average powers far below the paint damage thresholds, it was possible to determine the paint layers’ thickness through this technique. The good agreement between the thickness values estimated with OCT and MPEF serve to validate the latter technique for paint materials that display sufficient degree of transparency to the excitation laser wavelength and in the spectral range of the emitted fluorescence. Further research is in progress on samples simulating the real structure of a painting, and by combining MPFE with other modalities of NLOM such as Second and Third Harmonic Generation (SHG, THG).

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The research leading to these results was funded by the EU Community's H2020 Research Infrastructure program under the IPERION CH Project (GA 654028) and by Ministerio de Economía, Industria y Competitividad (MINECO) of Spain under Project CTQ2016-75880-P.
References

1. R.P. Linstead, J. Chem. Soc., 1934, 1016–1017, DOI:10.1039/JR9340001016.
2. R.P. Linstead and A.R. Lowe, J. Chem. Soc., 1934, 1022–1027 DOI:10.1039/JR9340001022.
3. https://colour-index.com/. Accessed February 2018.
4. N. Khandekar, C. Mancusi-Ungaro, H. Cooper, C. Rosenberger, K. Eremin, K. Smith, J. Stenger and D. Kirby, Stud. Conserv., 2010, 55, 204–215.
5. C. Defeyt and D. Struvu, e-Preservation Science (e-PS), 2014, 11, 6–14, ISSN: 1581-9280 web edition.
6. F. H. Moser and A. L. Thomas, 1964, 41 (5), 245, DOI: 10.1021/ed041p245.
7. F. M. Smith and J. D. Easton, J. Oil Col. Chem. Assoc., 1966, 49, 614–630.
8. H. Edwards, J. M. Chalmers, RSC Analytical Spectroscopy Series, The Royal Society of Chemistry, London, 2005.
9. E. Cloutis, L. Norman, M. Cuddy and P. Mann, J. Near Infrared Spectrosc., 2016, 24, 119–140.
10. M. Marinelli, A. Pasqualucci, M. Romani, G. Verona-Rinati, J. Cult. Herit., 2017, 23, 98–105, doi.org/10.1016/j.culher.2016.09.005.
11. R. de la Rie, E. Fluorescence of paint and varnish layers (Part I, II, III). Stud. Conserv. 1982, 27, 1-7; 65-69; 102-108.
12. D. Anglos, M. Solomidiou, I. Zergiotti, V. Zafiropulos, T. Papazoglou, C. Fotakis, Appl. Spectrosc., 1996, 50, 1331–1334.
13. A. Nevlin, G. Spoto, D. Anglos, Appl. Phys. A, 2012, 106, 339–361, DOI 10.1007/s00339-011-6699-z.
14. P. Targowski, M. Góra, M. Wojkowski, Hindawi Publishing Corporation, Laser Chemistry, 2006, Article ID 35373, 11 pages, doi:10.1155/2006/35373.
15. H. Liang, M. Cid, R. Cucu, G. Dobre, A. Podoleanu, J. Pedro, D. Saunders, Optics Express, 2005, 13, 6133–6144.
16. R. Fontana, A. Dal Fovo, J. Striøva, L. Pezzati, E. Pampaloni, M. Raffaelli, M. Barucci, Appl. Phys. A, 2015, 121, issue: 3, 957-966, DOI: 10.1007/s00339-015-9505-5.
17. B. Blümich, J. Perlo, F. Casanova, Progress in Nuclear Magnetic Resonance Spectroscopy, 2008, 52, 197.
18. N. Proietti, D. Capitani, V. Di Tullio, Sensors, 2014, 14(4), 6977.
19. F. Presciutti, J. Perlo, F. Casanova, S. Gliöggler, C. Miliani, Appl. Phys. Lett., 2018, 93, 033505.
20. G. Filippidis, M. Massaouti, A. Selimis, E.J. Gualda, J.M. Manceau , S. Tzortzakis, Appl. Phys. A, 2012, 106(2), 257–263, DOI:10.1007/s00339-011-6691-7.
21. A. J. L. Adam, P. C. M. Planken, S. Meloni, J. Dik, Opt. Express, 2009, 17(5), 3407.
22. H. Liang, M. Mari, C. S. Cheung, S. Kogou, P. Johnson, and G. Filippidis, Opt. Express, 2017, 25, 19640–19653.
23. G. Filippidis, G. J. Tservevelakis, A. Selimis, C. Fotakis, Appl. Phys. A, 2015, 118, 417–423, DOI: 10.1007/s00339-014-8357-8.
24. G. Latour, J.P. Echard, M. Didier, M.C. Schanne- Klein, Opt. Express, 2012, 20, 24623–24635, DOI:10.1364/OE.20.024623.
25. G. Filippidis, M. Mari, L. Kelegkouri, A. Philippidis, A. Selimis, K. Melessanaki, M. Syletou, C. Fotakis, Microsc. Microanal., 2015, 21, 510–517, doi:10.1017/S1431927614013580.
26. M. Oujia, S. Psilodimitrakopoulos, E. Carrasco, M. Sanz, A. Philippidis, A. Selimis, P. Pouli, G. Filippidis and M. Castillejo, Phys.Chem.Chem.Phys., 2017, 19, 22836, DOI: 10.1039/c7cp02509b.
27. T. E. Villafana, W. P. Brown, J. K. Delaney, M. Palmer, W. S. Warren, M. C. Fischer, Proc. Natl. Acad. Sci. USA, 2014, 111, 1708–1713, DOI:10.1073/pnas.1317230111.
28. T. E. Villafana, W. P. Brown, W. S. Warren, M. Fischer, Proc. of SPIE, 2015, 9527, 9, DOI: 10.1117/12.2184758.
29. A. Dal Fovo, R. Fontana, J. Striøva, E. Pampaloni, M. Barucci, M. Raffaelli, R. Mercatelli, L. Pezzati, R. Cicci, Lasers in the Conservation of Artworks XI, Proceedings of LACONA XI, P. Targowski et al. (Eds), NCU Press Torun, 2017, DOI: 10.12775/3875-4.10.
30. R. G. Kuehni, Color: An Introduction to Practice and Principles, Wiley, New York, USA, 1997.
31. R. G. Kuehni, Color Space, and Its Division, Wiley, New York, USA, 2003.
32. CIE (1974a). CIE44; 1979. E. Blinowska, K. Collaer, and M. T. Stork, B. Schmied, J. Photochem. Photobiol. B, 2006, 83(2), 197–212, DOI:10.1016/j.jphotochem.2005.09.009.
33. L. Burgio, R. Clark, Proc. SPIE, Acta Part A, 2001, 57, 1491–1521.
34. M.C. Caggiani, A. Cosentino, A. Mangone, Microchem. J., 2016, 129, 123-132, http://dx.doi.org/10.1016.
35. N. Marchetti, A. Atre, F. Benetti, N. Proietti, V. Di Tullio, M. Mascalchi, I. Ostioli, S. Siano, I. Turbanti Memmi, 2013, 29, 2, 153-159, DOI:10.1179/1743294412Y.0000000065.
36. C.S. Nadim, S. Zumbuehl, F. Delavy, A. Fritsch, R. Kuchnenn, Spectrochim. Acta, Part A, 2009, 73, 505–524, doi.org/10.1016/j.saa.2009.11.029.
37. B. W. Singer, D. J. Gardener and J. P. Derow, The Paper Conservator, 1995, 19, DOI:10.1080/03094227.1993.9638401 published online 2010.
38. G. Poldi and S. Caglio, Opt. Spectrosc., 2013, 114, 929–935.
39. V. Spizzichino, F. Angelini, L. Caneve, F. Colao, R. Corrias, L. Ruggiero, STUD CONSERV. 2015, 60, S178-S184, DOI: 10.1179/0039363015S178-S184.
40. R. Fantoni, L. Caneve, F. Colao, L. Fiorani, A. Palucci, R. Dell’Erba, V. Fassina, J. Cult. Herit., 2013, 14S, S59–S65, https://doi.org/10.1016/j.culher.2012.10.025.
41. S. Lawman and H. Liang, Appl. Opt., 2011, 50, 32, 0003-6935/11/326039.
42. G.W. White, Microscope, 1970, 18, 51–9.