Research Article

Title:

Material characterisation of a painted beehive panel by hyperspectral imaging in combination with advanced spectroscopic and chromatographic techniques

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Abstract:

In this study, a painted beehive panel from the collection of Slovene Ethnographic Museum was examined with respect to its material composition with the aim to reveal the painting technique. Due to the state of degradation due to outdoor weathering (UV irradiation, rainfall, extreme temperature and humidity fluctuations), as well as past conservation interventions, the object represented a complex analytical challenge. We aimed for non-invasive techniques (FTIR in reflection mode, Raman spectroscopy and hyperspectral imaging in the range of 400 to 2500 nm), however in order to explore paint layers, cross-sections were also analysed using Raman spectroscopy. FTIR spectroscopy in transmission mode and gas chromatography coupled to mass spectrometry were also used on sample fragments. Various original materials were identified such as pigments and binders. The surface coating applied during restoration interventions was also characterised. Additionally, organic compounds (oxalate, carboxylate), representing transformation products, were found. The potential use of Prussian blue as a background paint layer is discussed.

Keywords: painted beehive panels, cultural heritage, reflection FTIR spectroscopy, transmission FTIR spectroscopy, non-invasive Raman spectroscopy, GC-MS analysis, hyperspectral imaging

1. Introduction

Painted beehive panels are a folk art form unique to certain Slovenian-speaking regions from the mid-18th century on. The colourfully painted flat wooden panels (ca. 13 x 30 cm) decorated the
fronts of wooden beehives stacked under a roof, forming an open-air apiary or “bee-house”. As such, the objects had to withstand outdoor weathering conditions with intensive UV irradiation, rainfall, extreme environmental fluctuations, as well as mechanical degradation, which resulted in extensive degradation.

Painted beehive panels are testaments of popular creativity and significant collections exists in the Slovene Ethnographic Museum (Ljubljana, Slovenia, ca. 1000 objects) and The Museum of Apiculture (Radovljica, Slovenia, ca. 1500 objects), most of which can be digitally accessed [1, 2].

Given the sizes of these collections, a general analytical strategy to explore the available materials evidence would be advantageous to have in order to better manage the collections including their conservation.

Most existing research is art-historical or ethnographical and our current understanding of the painting process is based on 20th century texts and observational studies [3, 4, 5], which present following possible characteristics. A base layer of paint, most often calcium carbonate [4], might have been applied directly across the entire front of a wooden panel forming the background of the composition. Once this foundation layer dried, the composition was stencilled or drawn with a pencil; this was then painted out such that forms were filled with mostly basic colour tones (modelled or not), and then painted further with details and contours. Each layer of paint was left to dry before the next one was applied. Oil paints, probably hand-made of locally available linseed oil or oil of turpentine and durable mineral pigments [5], might have been used in most cases, although tempera paints and binders such as poppy seed oil, mastic resin, egg white and egg-
varnish tempera were mentioned as well [3, 4]. Painted panels might have even been originally coated/varnished with bleached linseed oil mixed with mastic resin [4].

Spectroscopic and separation techniques such as Raman, FTIR and GC-MS are well established in the investigation of heritage materials [6, 7] including in the analysis of panel paintings [8, 9], while Delaney et al. explored the used hyperspectral imaging and imaging spectroscopy [10]. In a study of an Italian early renaissance panel painting, a combination of different molecular and elemental spectroscopic imaging methods was shown to provide insight into artistic materials, pigment distribution and underdrawings [11], while hyperspectral imaging and other spectroscopic techniques were used for another similar object [12], where mapping of wax and other organic materials is described and discussed.

For characterisation of drying oils gas chromatography coupled to mass spectrometry (GC-MS) is often used to determine the palmitic-stearic and azelaic-palmitic acid ratios, on the basis of which the most frequently used drying oils (linseed oil, poppyseed oil and walnut oil) can be reliably identified [13, 14] and other organic materials such as waxes and triterpenoid resins can also be revealed [14].

In the present study, the material composition of the painted beehive panels was studied in order to explore the painting technique. Paint stratigraphy was researched to investigate the hypothesis that contours are generally better preserved as they are constituted of several paint layers, and to explore the background paint layer which is mostly deteriorated.
2. Materials and Methods

2.1 Description of the painted beehive panel, preparation of the removed samples and analytical strategy

The panel depicting the theme of “A fight over a pair of man’s trousers” (ref. no. PK2, 1882) (Figure 1) from the collection of the Slovene Ethnographic Museum has no catalogued information regarding its provenance and history, as is the case with many painted beehive panels [5]. The panel lacks many areas of the painted composition, as evident from Figure 1a. The most preserved are the contours, along with some other parts of the composition; however, the panel almost entirely lacks any hint of the paint film depicting the background. It is executed on wood with dimensions ca. 13 x 35 x 1.5 cm.

The photographs of the panel in visible light and under ultraviolet (UV) radiation are presented in Figure 1. For UV fluorescence photography, UV-A fluorescent bulbs Osram L 36W/73 and a Canon EOS 350D camera with EF-S18-55 mm f/3.5-5.6 II lens and UV-cut-off filter were used.

The locations of the point-based non-invasive analyses are marked in Figure 1a (“A” indicates FTIR spectroscopy in reflection mode, “R” indicates Raman spectroscopy). Destructive sampling locations (PK2-1, PK2-2, PK2-2b, PK2-3, PK2-4) are denoted in Figure 1a as well. A part of these samples containing strata from the support to the uppermost layer was used for FTIR spectroscopy (PK2-1, PK2-2, PK2-3), GC-MS analyses (PK2-1 and PK2-2), and for preparation of cross-sections used in optical and Raman microscopy. The exception was sample PK2-4 used in its raw form for Raman spectroscopy only. Sample PK2-2b (as a cross-section) was investigated using optical and Raman microscopy only.
To prepare the cross-sections, samples were embedded in the casting resin Kristal PS (transparent two-component copolymer resin, Samson Kamnik, d.o.o., Slovenia), and then polished using silicon carbide abrasive papers and paraffin oil. Finally, the samples were cleaned in petroleum ether in ultrasonic bath.

Figure 1. Photographs of the painted beehive panel “The fight for a pair of trousers” (Slovene Ethnographic Museum, ref. no. PK2, 1882): (a) in visible light and (b) ultraviolet-induced visible fluorescence. The sampling locations for FTIR spectroscopy in reflection mode (A), Raman spectroscopy (R) and destructive sampling (PK2-1, PK2-2, PK2-2b, PK2-3, PK2-4) are indicated.
2.2 Fourier Transform Infrared (FTIR) spectroscopy

FTIR transmission spectra were recorded using a Perkin Elmer Spectrum100 FTIR spectrophotometer coupled to a Spotlight FTIR microscope equipped with nitrogen cooled mercury-cadmium telluride (MCT) detector. The samples investigated by FTIR transmission mode were taken from the painted beehive panels, placed between the windows of a diamond anvil cell and examined under microscope.

Non-invasive FTIR analysis of the panel’s surface was carried out with a portable Alpha-R spectrometer from Bruker Optics. The pseudo-absorption spectra ($A' = \log (1/R)$, $R$=reflectance) spectra were collected in reflection mode between 7500 and 400 cm\(^{-1}\), at 4 cm\(^{-1}\) spectral resolution. 160 scans per sample were averaged and for the background measurement, a gold mirror was used. An integrated video camera controlled and monitored the sampling area.

Processing of the FTIR data was implemented using Bruker OPUS software.

2.3 Raman spectroscopy

The spectra were recorded using a 785 nm and 514 nm laser excitation lines with a Horiba Jobin Yvon LabRAMHR800 Raman spectrometer coupled to an Olympus BXFM optical microscope. The spectra were recorded using ×50 LWD objective lens and/or ×100 objective lens and a 600 grooves/mm grating. A multi-channel, air-cooled CCD detector was used. Experimental parameters (time of exposure, accumulation, power at the sample etc.) were adjusted according to the specifics of the samples. The specific parameters used for the collection of the presented spectra are included in the Figures captions.
The dimensions of the panel allowed investigation directly under the microscope in a non-invasive manner. The panel was placed directly under the objective and the spectra were then collected using the x50 LWD objective from the locations of the interest. Further analysis was done also on the cross-sections of the samples. In such case, the cross-section was placed under the microscope and investigated using x100 objective lens. Spectral interpretation and identification of the materials were done in comparison with own spectral database and the literature [15, 16].

2.4. Hyperspectral imaging

The system comprises of a high-resolution ClydeHSI Hyperion Art Scanner that can accomplish scan areas up to 2.2 m x 2.2 m with an optical spatial resolution better than 25 μm. This scanner was used with either a push-broom VNIR (400 to 1,000 nm, Δλ = 3 nm (FWHM)) or SWIR (900 to 2,500 nm, Δλ = 10 nm (FWHM)) hyperspectral cameras each capable to provide a spatial resolution on the panel of better than 0.3 mm. The scanner was fitted with a dual distance sensor to ensure that even curved surfaces will remain in focus. Illumination was made using a tungsten light source that has a smooth spectral emission from approx. 350 nm to 3,500 nm. The illumination level in the visible spectrum was ca. 2,000 lux. At the start and end of each scan a reflective white tile was measured to record the instrument spectral-spatial response function, and this was used to convert the raw data signal into reflectance and absorption data. Subsequently, data analysis was made using Principle Components Analysis (PCA) and Spectral Angle Mapping (SAM) methods to extract the location of materials and their distributions across the panel.
2.5 Optical microscopy

The cross-sections of the samples were examined using an Olympus BX 60 microscope connected to an Olympus SC-50 video camera using visible and ultraviolet (UV) illumination, the latter emitted from a Hg bulb Ushio USH-1030L.

2.7 GC-MS Analysis

A sample (0.5–2 mg) was treated with 3 mL of 0.5 M methanolic solution of NaOH and 300 µL of dichloromethane. After flushing with nitrogen, the closed vials were heated for 10 min at 90 °C. The vials were cooled briefly before addition of 3 mL of a 12% methanolic solution of H₂SO₄. After nitrogen flushing, the closed vials were heated for 10 min at 90 °C. The vials were then cooled to room temperature. 3 mL deionized water and 1.5 mL hexane were added to the vials and then fatty acids methyl esters (FAMEs) were extracted by vigorous shaking for about 1 min. Following centrifugation, the top layer was transferred into a vial for GC-MS analysis.

GC–MS analyses were carried out using Thermo Scientific Focus GC with a mass spectrometric detector Thermo Scientific ISQ. Chromatographic separation was achieved using a Supelco, Omegawax 320 capillary column (bonded polyethylene glycol stationary phase; 30 m x 320 µm x 0.25 µm). The injector temperature was set at 200 °C and the interface temperature at 250 °C. The oven was programmed from 185 °C, then increased at 1 °C/min to 215 °C, stayed constant for 9 min and then decreased at 10 °C/min to 185 °C. The injection volume was 2 µL and the inlet was operated in split mode, with a 1:5 split ratio. The carrier gas was helium at a constant flow of 2 mL/min.
3. Results and discussion

The painted beehive panel was examined to obtain a comprehensive picture of the applied materials and their potential transformations (Table 1).

Table 1. The materials as identified on the painted beehive panel (PK2) at the different sampling locations as marked in Figure 1a, along with the methods used.

| Material                        | Raman reflection | FTIR-microscopy | Raman microscopy | FTIR-transmission | GC-MS   |
|--------------------------------|------------------|------------------|------------------|-------------------|---------|
| lead white                     |                  |                  | PK2-1, PK2-2b, PK2-3, PK2-4 |
| anatase                        |                  |                  | PK2-2b           |                   |         |
| barium sulfate                 | R4, R5, R7, R18, R19 | A1, A3, A4, A5, A7 | PK2-1, PK2-2, PK2-2b, PK2-3, PK2-4 | PK2-1, PK2-2, PK2-3 |
| calcium carbonate              | R6               |                  | PK2-1           |                   |         |
| chrome yellow                  |                  |                  | PK2-2*           |                   |         |
| iron oxide (likely haematite type) | R2, R3, R16, R17 |                  | PK2-1, PK2-2b    |                   |         |
| iron hydroxide (likely goethite type) | R19         |                  | PK2-2b           |                   |         |
| cinnabar/vermilion             | R1, R3, R8       |                  | PK2-1, PK2-4    |                   |         |
| lead oxide (minium)            |                  |                  | PK2-4            |                   |         |
| ultramarine                    | R9, R10, R11, R12, R14 |                  | PK2-1           |                   |         |
| Prussian blue                  | R5, R18          | A1, A2, A3, A4, A5, A7 | PK2-1, PK2-2, PK2-2b, PK2-3, PK2-4 | PK2-1, PK2-2, PK2-3 |
| Emerald green                  |                  |                  | PK2-1*           |                   |         |
3.1. Inorganic materials (pigments, fillers, extenders, adulterants)

3.1.1. General investigation of paint layers

Several different pigments and other components of paint layers such as fillers, extenders or adulterants were detected (Table 1). Among these are cinnabar/vermillion, iron oxide (likely of the haematite type), lead oxide (minium), iron hydroxide (likely of the goethite type), carbon-based black, Prussian blue, ultramarine, lead white, calcium carbonate, barium sulfate and anatase. The presence of Emerald green and chrome yellow was suggested but could not be completely confirmed.

The number of detected pigments is significant for an object of folk art, where the colour palette was usually limited. As these objects faded due to outdoor weathering, they tended to get frequently “refreshed”, i.e. painted over, while in use; this could have been done in situ by folk

| Pigment Type                  | Detected Components | Identification |
|-------------------------------|---------------------|----------------|
| carbon-based black            | R3, R13, R15, R16, R17 |                |
| lipids                        | A1, A2, A3, A4, A5, A6 (at the back), A7 | PK2-1, PK2-2, PK2-3 |
| triterpenoid resin (mastic/dammar) | A1, A2, A3, A4, A5, A7 | PK2-1, PK2-3 |
| beeswax                      | A1, A2, A5, A6, A7, A3, A4** | PK2-2, PK2-3, PK2-1, PK2-2 |
| oxalate                       | A1, A2, A3, A4       | PK2-1, PK2-2, PK2-3 |
| carboxylate                   |                     |                |
| carnauba wax                  |                     | PK2-1, PK2-3 |
| Identifications:              |                     | Identification uncertain

*Identification uncertain

**Present in traces
artists themselves or by owners. Once acquisitioned, curators or conservators may have done the same [4].

The best preserved areas of PK2 are the contours of the painted figures and objects, mostly executed in a darker red-brown colour, along with the draperies painted in red, and some of the partially remaining whitish, green, brown, blue and black painted sections. In the green coloured regions such as tree foliage (R4, R5, R18, A5, Figure 1a) and the lighter green apron of the female figure (R7, R19, A3), barium sulfate was detected using Raman spectroscopy, along with Prussian blue (R5, R18). Both were present in the FTIR spectra as well. Raman analyses corroborated the detection of cinnabar in the red draperies (R1, Figure 1a), although in one red area (A1, Figure 1a) FTIR showed the presence of Prussian blue as well. Whether the latter is a component of the same or of an underlying stratum could not be determined.

The reddish-brown contours show the presence of iron oxide and/or carbon-based black (R2, R3, R16, R17, Figure 1a) with possible presence of cinnabar/vermilion as well (R3, R8, Figure 1a). All the remaining blue segments on the panel were identified as ultramarine with Raman spectroscopy (R9-R12, R14, Figure 1a); however, using FTIR in reflective mode, Prussian blue was also detected (A2, Figure 1a) which could either be a part of the same or of an underlying layer.

There are several whitish areas still preserved on the panel; however, it wasn’t possible to identify the pigment using Raman analysis, with the exception of a possible presence of calcium carbonate in one location (R6, Figure 1a). Furthermore, FTIR again showed the presence of Prussian blue in one of the white areas (A4, Figure 1a). Calcium carbonate was detected also on PK2-1 in the layer containing ultramarine, although this could be considered as an impurity. Using Raman
spectroscopy, lead white was detected as a white pigment by means of invasive analysis only in the cross-sections of PK2-1, PK2-2b and PK2-3, in the paint layer closest to the wooden support. Barium sulfate was found in the majority of areas of both darker and lighter green colour, and in particular in all point analyses where Prussian blue was identified as well (R5, R18, A1, A3–A5, A7, Figure 1a) and might be present as an adulterant or extender of the main pigment(s) used in these paints.

Anatase was identified only in the cross-section of the sample PK2-2b with a single green paint layer on the wooden support, but could be considered as an impurity. In the same cross-section an ochre-coloured particle, identified as iron oxide hydroxide, likely of goethite type, was detected in only one point. Lead oxide (minium) was detected on the surface of the sample PK2-4 and could have been used in the pigment mixture (along with the identified lead white and cinnabar/vermillion) for skin tones of the figures. The detection of Emerald green and chrome yellow could not be confirmed; the former was indicated in the bottom paint layer of PK2-1 and the latter in the lower part of the seemingly single paint layer in PK2-2. Additional information is needed to verify the presence of these two pigments.

3.1.2. Investigation of green paints

It was not possible to obtain the exact pigment composition of the green paints using either FTIR spectroscopy in reflection mode or non-invasive Raman spectroscopy. In the locations R4, R5, A5 (Figure 1a) barium sulfate and/or Prussian blue were detected. These locations were additionally
examined with hyperspectral imaging (Figure 2), with areas with similar spectral features highlighted in purple. R4, R5 and A5 are therefore likely to have similar mixtures of pigments.

Figure 2. Detail of the panel with R4, R5 and A5 sampling locations: (a) RGB image, (b) false-colour VNIR PC1, (c) false colour SWIR PC1.

Based on the identification of Prussian blue using spectroscopic analyses in R5 and A5 (Figure 1a), the green areas could be composed of a mixture of blue (Prussian blue) and yellow pigments. To gain better insight, samples PK2-2 and PK2-2b were removed to examine the cross-sections (Figure 3a,b). The presence of one of the possible forms of Prussian green was established in the course of further research [17], while optical microscopy of PK2-2 (Figure 3a) showed what appear like large pigment particles (some measuring ~10 µm and more) that appeared to be of emerald rather than blue colour, with almost no visual indication of a possible presence of yellow pigments (particles of blue and yellowish to reddish colour are noticeable within the sample, however, they are barely visible).

The presence of yellow pigments could not be detected using the analytical techniques employed within this study. One exception is the possible presence of chrome yellow (lead chromate) detected by Raman spectroscopy using excitation at 514 nm in the cross section of PK2-2 (Figure 3a). Although the Raman signal (Figure 3c) was obscured by fluorescence, a weak band at ~840
cm\(^{-1}\) could be attributed to chromate symmetric stretching mode [18, 19]. Although the 785-nm line offers more spectral information on the structure of lead chromate-based compounds [19] and it could lead to reduction of fluorescence, no satisfactory results enabling unambiguous confirmation of chromate were obtained. Since the majority of particles appear greenish, and the analysis confirmed the presence of Prussian blue overall, as well as barites with the possible presence of chrome yellow, a plausible interpretation could be the use of chrome green. The other possible green pigments, such as pigments/mixtures under the term “Prussian green”, need further investigation. The same can be concluded for PK2-3 (Section 3.3) of a lighter green colour, composed of a lower greenish and an upper yellowish layer, where the results of Raman and transmission FTIR spectroscopy indicate the presence of Prussian blue, barium sulfate and possibly lead white.

![Figure 3](image)

**Figure 3:** Optical micrographs of the cross-sections of (a) PK2-2 with the location of Raman analysis indicated, (b) PK2-2b, and (c) a Raman spectrum obtained on the location marked in (a), implying the presence of lead chromate (\(\lambda_0 = 514\) nm).

3.1.3. Blue paints and a possible background paint

It appears, upon visual inspection, that the best preserved areas might be those that were originally composed of more than one paint layer. It is impossible to identify the colour of the
painted background since it is almost completely absent (likely applied as a single-layer application across the entire panel surface as is often the case with painted beehive panels [4]). However, on closer visual inspection, a bluish or perhaps a greenish layer appears to have been applied, as visible also on the faces of the female figures, where the uppermost flesh colour tones are present only as minute residues.

Non-invasive investigations using reflection FTIR and Raman spectroscopy (locations R11, A2, Figure 1a) of a dark blue contour of a dress led to different results. Namely, Raman spectroscopy (Figure 4a) showed the presence of ultramarine on R11 (characteristic band at ~547 cm⁻¹), and FTIR spectroscopy indicated Prussian blue on A2. The presence of Prussian blue was confirmed by the reflection infrared spectrum (Figure 4b), based on the characteristic C=N stretching vibration at 2085 cm⁻¹ and derivative–like spectral features of the Fe–O group located at 630–430 cm⁻¹ [20]. In addition, the presence of Prussian blue was confirmed by reflection FTIR spectroscopy in all the investigated locations (A1, A2, A3, A4, A5, A7). These areas are of different colours (red, blue, white, brown, light and dark green), it seems less likely for Prussian blue to be consistently present as part of the pigment mixtures. A possible interpretation would be the presence of Prussian Blue in the lower layers, e.g. as a potential background paint layer.
Figure 4. (a) Raman spectrum at location R11, Figure 1a, identifying ultramarine based on its characteristic band placed at 547 cm$^{-1}$ ($\lambda_0=785$ nm). (b) A reflection FTIR spectrum obtained at A2 (Figure 1a), identifying vibrations characteristic for Prussian blue, lipids and triterpenoid resin.

To investigate the matter further, the sample PK2-1 was taken from the dark blue contour of a garment (Figure 1a). This consisted of all stratigraphic layers – from the wooden support to paint layers as evident in the optical micrograph of the cross-section (Figure 5a). Two paint layers are present: a lower one of lighter blue/greenish-blue colour and an upper one of darker blue colour.

The Raman spectrum (Figure 5b) at the spot R1 in Figure 5a revealed the characteristic ultramarine band at 547 cm$^{-1}$, while the spectrum at R2 confirmed the presence of Prussian blue based on the characteristic bands at 276, 537, 2092, 2155 cm$^{-1}$. At R2, barium sulfate (band at 990 cm$^{-1}$) and lead white (band at 1049 cm$^{-1}$) were detected as well. The sample PK2-1 was investigated also by infrared spectroscopy in transmission mode (Figure 5c), where the band at 2087 cm$^{-1}$ indicated the presence of Prussian blue. In addition, lipids (2927, 2854, 1739 cm$^{-1}$), oxalate (1321 cm$^{-1}$), carboxylate (1550, 1536 cm$^{-1}$) and barium sulfate (1079, 637, 611 cm$^{-1}$) were also detected.
3.2. Organic materials (binders, coating)

Investigation of the front side (A2, Figure 1a) using reflection FTIR revealed the presence of characteristic spectral features of lipids at 4324, 4253, 3010-2900, 2890-2800 and 1743 cm\(^{-1}\) (Figure 4b). More specific analysis of lipid components was possible using GC-MS, using the characteristic palmitic/stearic (P/S) and azelaic/palmitic (A/P) ratios for linseed oil (typically 1.2 for P/S and 1.3 for A/P) [14]. In the samples, higher P/S and lower A/P ratios were observed, which could be explained with the presence of wax mixed with linseed oil as palmitic acid is the most
abundant compound in hydrolysed wax [14]. Further peaks are present in the chromatograms at longer retention times (Figure), representing long-chain compounds characteristic for wax. This was confirmed with GC-MS analyses of pure beeswax. The presence of beeswax in the samples was also indicated with FTIR analysis (spectra not shown).

![Figure 6](image.png)

(a) GC-MS chromatograms of the extracts of (a) sample PK2-2 and (b) beeswax (A=azelaic acid, P=palmitic acid, S=stearic acid, *=long chain compounds).

A triterpenoid resin was confirmed on all investigated locations (A1-A5, Figure 1a) on the front side of PK2 beehive panel, as indicated by the distinctive bands at 1477 ($\delta$CH$_3$) and 1390 ($\delta$CH$_2$) cm$^{-1}$ (Figure 4b) [21]. Within the samples PK2-1 and PK2-3, the resin was identified solely in the uppermost stratum, indicating that the resin component is probably part of a coating.

Hyperspectral imaging offered further information on the distribution of the triterpenoid resin (Figure 7a) and the beeswax (Figure 7b). It can be observed that intensity of the spectral features for triterpenoid resin is higher compared to the spectral features of the beeswax. This is in agreement with the item used for surface protection by the Slovene Ethnographic Museum, i.e.
triterpenoid resin dammar in turpentine with addition of beeswax for matte appearance. The usual ratio of resin to wax is 10:1.

In Figure c, the ratio of characteristic wavelengths for beeswax (2309 nm) [12] and resin (1702 nm) is presented. Based on its homogeneity, it can be concluded that the mixture of resin and beeswax was applied all over the panel. This is not surprising as coatings based on a triterpenoid resin such as dammar, and likely including beeswax to decrease shine, are a commonly applied conservation treatment. The coating is also seen as a fluorescent film under UV-radiation and covers paint losses as well (Figure 1b). The resin and the beeswax were detected in the uppermost transparent layer of the raw samples as analysed by transmission FTIR. In only one location (PK2-2), carnauba wax was detected, which often accompanies beeswax in wax pastes, further indicating that waxes were part of a surface layer.

The hyperspectral images showed a uniform distribution of wax on the panel surface; therefore, waxes are most likely part of the resin varnish. However, beeswax could have been secreted by bees as well, depending on how thorough conservation cleaning was. Important information could potentially be obtained from such “original” wax. Chemical characterisation of beeswax using GC-MS can enable nest-mate recognition based on fatty acid composition. Beeswax plays a role in the chemical communication within a honey bee colony and provides a chemical signature so that subtle differences in its composition may be important and have never been studied historically. Isotope ratio mass spectrometry (IRMS) was found to be very useful in establishing the geographical origin of beeswax [22]. All of this indicates that much can still be learned from
historical beehive panels provided that contemporary coatings have not obscured such crucial information.

(a)

(b)
Figure 7. False colour image for (a) triterpenoid resin (dammar) at 1702 nm, (b) beeswax at 2309 nm and (c) hyperspectral data showing the ratio of intensities at 1702 nm (characteristic of resin) over 2309 nm (characteristic of beeswax). Higher numbers (red colours) represent higher reflectance intensity.

Lipids are the likely paint binding medium as they were detected in all non-invasive reflection FTIR measurements and in paint layers of PK2-1, PK2-2 and PK2-3 using transmission FTIR. Other organic compounds such as oxalate were also found in the reflection and transmission and reflection FTIR spectra (distinctive absorption band at ~$1322\text{ cm}^{-1}$ in the spectrum of PK2-1 (Figure 4b and Figure 5c). According to the literature, metal oxalates are often found on cultural heritage objects [23, 24] as products of deterioration. They form during environmental degradation of organic compounds, possibly mediated by micro-organisms [25]. In the IR spectrum of PK2-1, metal carboxylates were also confirmed (sharp bands at $1550$ and $1536\text{ cm}^{-1}$, Figure 5c) and these compounds were already described in the literature [26].
3.3. Investigation of the support

Optical micrograph of the cross-section of PK2-3 (Figure) shows that all the paint layers were removed as the wood structure is clearly visible. This sampling location is marked with a white circle A in Figure 9, and was used to derive reference spectral features for wood in order to perform hyperspectral analysis of distribution of exposed wood. The red areas in the false colour image in Figure 9 represent such features. Sampling was also undertaken in the locations B and C, confirming the validity of this analysis of hyperspectral images. Another areas with high intensities (red) match to the most degraded surface of the object, where only wood is present.

Figure 8. Optical micrograph of the cross-section of the sample PK2-3.

Despite the intensive signal for wood, signals can be observed for other materials, superimposed on wood. These are in areas that are best preserved, such as contours and certain painted areas. These areas are probably less UV sensitive; typically, the areas with carbon-based black.
Figure 9. False colour image highlighting wood spectral features. Higher numbers (red colours) represent higher reflectance intensity.

Correlations between resin (Figure 7a), wax (Figure 7b) and wood (Figure 9) distribution can explain a more intensive deposit of the resin and wax mixture in the porous areas of exposed wood compared to areas coated with paint.

4. Conclusions

Relatively large collections of painted beehive panels, which are relatively uniform objects, represent an interesting source for systematic studies under various aspects. Therefore, a comprehensive material characterisation of a painted beehive panel was carried out using FTIR and Raman spectroscopy, gas chromatography coupled to mass spectrometry and hyperspectral imaging. The investigated object was degraded, likely as a consequence of outdoor weathering. As such it represented a complex analytical problem requiring complementary analytical techniques. Many different compounds were identified representing pigments and their components (cinnabar/vermillion, iron oxide, lead oxide, iron hydroxide, carbon-based black, Prussian blue,
ultramarine, lead white, calcium carbonate, barium sulfate, anatase, chrome yellow and possibly
Emerald green) as well as binders (linseed oil). A surface conservation varnish was identified as a
mixture of a triterpenoid resin and beeswax, with a possible addition of carnauba wax.
Compounds indicative of degradation were identified such as oxalates and carboxylates.
Prussian blue was identified in many locations using FTIR and Raman spectroscopy and the
conclusion was made that it was used as a background colour.
Beeswax, however, could have two origins and some of it could have been deposited by bees
themselves. Studying these depositions might allow us to understand bee activities at the entry
into the beehive or their affinity for different colours. Beeswax may also hold clues as to the origin
of panels, however, this may no longer be possible due to the conservation varnish, which
represents the second source of beeswax.

This study represents a comprehensive material study of a painted beehive panel and will serve as
the blueprint for further analysis of objects in the extensive collections of such panels in Slovenian
museums, with the aim to develop a procedure for condition evaluation. Based on this preventive
conservation strategies and storage recommendations could be developed in the future.

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Authors' Contributions

I.K.C. and K.R. are developed the concept of the research and drafted the manuscript. Y.G. and J.G. carried out hyperspectral imaging and data processing. K.R., M.K., L.L, P.R. were involved in FTIR and Raman analyses. I.K.C. carried out GC-MS analyses. All authors contributed to data interpretation and writing. All authors have read and agreed to the published version of the manuscript.

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Competing interest

The authors declare that they have no competing interest.

Availability of data and material

All data generated or analyzed during this study are included in this published article.
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