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Autor(en): **Dietemann, Patrick / Neugebauer, Wibke / Baumer, Ursula**

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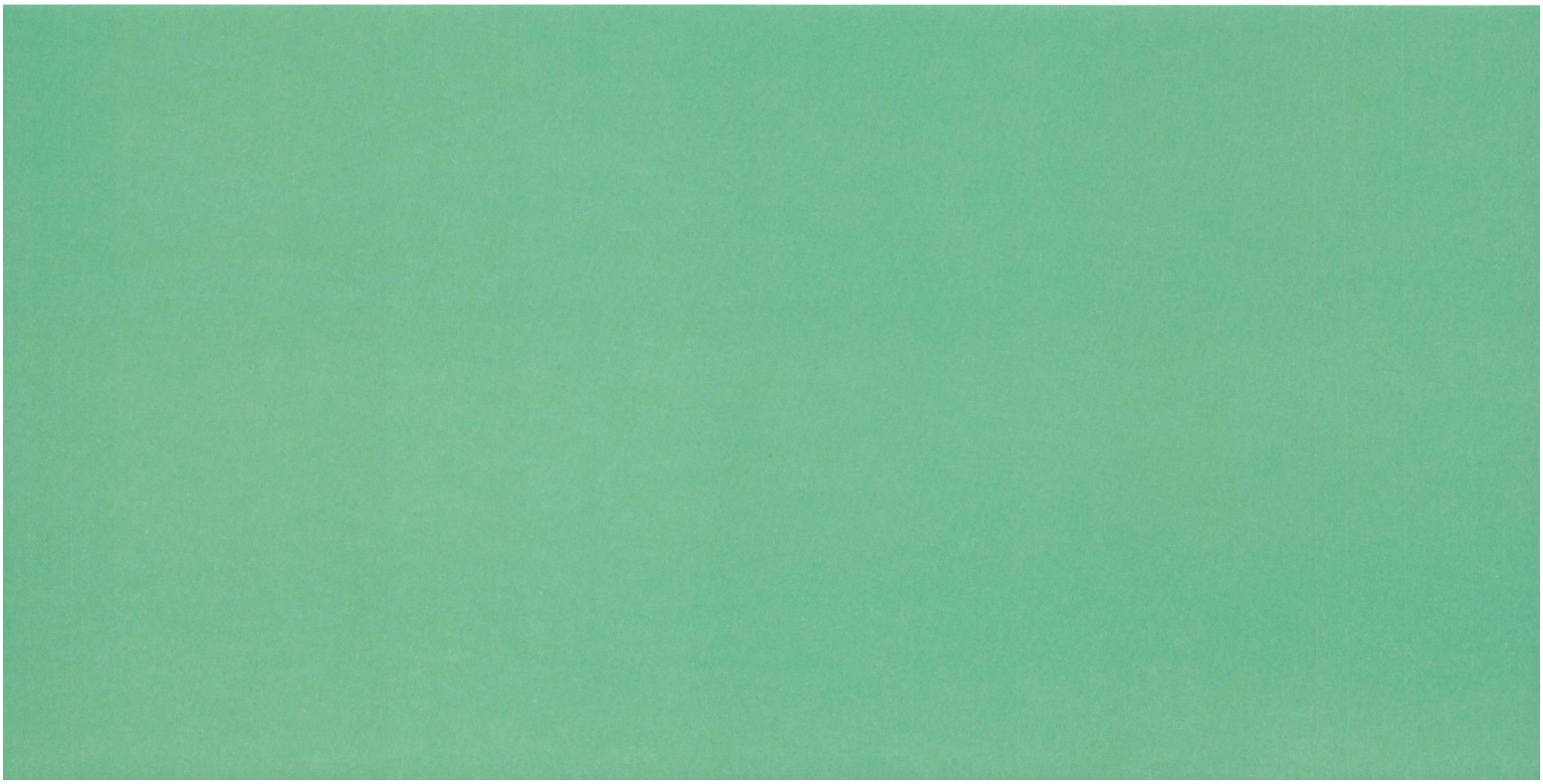
Patrick Dietemann, Wibke Neugebauer, Ursula Baumer, Irene Fiedler, Cedric Beil, Andrea Obermeier, Stephan Schäfer and Stefan Zumbühl



INTRODUCTION

Analysis of tempera paint binding media presents various challenges. Firstly, the binding medium of a particular paint can consist of many components that may be difficult to identify simultaneously within a tiny, single sample. Secondly, many tempera paintings created around 1900 consist of complex layered structures; heterogeneous layers of paint were applied one on top of another, sometimes superimposed with unpigmented intermediate layers whose presence can be difficult to recognise, especially when they are thin. Thirdly, because many tempera paints are somewhat lean, the binders present in the uppermost layers can easily penetrate underlying stratigraphy, making a distinction between actual binding medium and binder contamination from adjacent layers very difficult, if not impossible.

Equally difficult, as reported recently elsewhere, is the problem of choosing an appropriate terminology for various types of media. The common terms 'tempera' and 'oil' can denote different aspects of paint and painting: the solubility or dilutability of the paint (aqueous or non-aqueous) as it is used by the artist, its material composition, and the visual appearance of the painting that is created with it (Neugebauer 2016; Dietemann *et al.* 2015).[1]

A large, interdisciplinary research project on the use of tempera binding media in easel painting around 1900 recently carried out at the Doerner Institut in Munich encountered all of these challenges during the various phases of research.[2] The process of overcoming these challenges was, however, greatly assisted by the fact that very detailed information on the materials and techniques used for specific paintings was available from written sources and could thus be used for comparison with the results of scientific analyses of the same works (Dietemann and Neugebauer 2014).

This paper reports some of the main results of the binding medium analyses carried out on three tempera paintings by Arnold Böcklin (1827–1901), Franz von Stuck (1863–1928) and Franz von Lenbach (1836–1904) respectively. (The paint application techniques employed in the creation of those works is discussed in the contribution by Neugebauer, in this volume, esp. case studies I–III.)

For the analyses of the media of the paintings, an approach was developed that combines several methods in order to obtain a comprehensive identification and localisation of different binding media in a single sample. In the following text, the methods are compared and their possibilities and limitations discussed. At first sight, some of the analytical results appear surprising, but are seen to be reasonable when practical aspects of painting are considered. As noted above, the specific materials used by the artists as binders in the paintings examined were described in more or less detail in written sources, which were studied before sampling and analysis. This provided an extremely useful basis for the interpretation of the analytical results.

METHODS

Gas chromatography-mass spectrometry (GC-MS) is a very powerful method for accurate and detailed identification of binding media. Because binders can contain many different components, at the Doerner Institut samples are usually extracted with several solvents of increasing polarity: isooctane, methanol, methanol/chloroform 3:7, methanol/oxalic acid (anhydrous, 10 wt%) and water. This extraction protocol allows for partial separation of different components; derivatisation procedures and measuring conditions can be adjusted to the main components dissolved in the individual extracts (Koller *et al.* 1998; Dietemann *et al.* 2012, p. 286).[3] Proteins were analysed after hydrolysis with 'constant-boiling' (azeotropic) hydrochloric acid, using ion exchange (liquid) chromatography with post-column derivatisation (amino acid analysis).

Although GC-MS is a powerful technique, it does not allow localisation of different materials within a sample, not even when different layers are separated as far as possible by scraping before analysis. Therefore, two-dimensional Fourier transform infrared focal plane array

imaging (FTIR-FPA imaging) was used, which allows identification of material classes due to characteristic absorptions (Salzer and Siesler 2009). The measurements were performed in attenuated total reflectance (ATR) mode to improve the spatial resolution down to c. 1 µm (Zumbühl *et al.* 2014): a germanium crystal (n_D 4.0) was pressed onto the surface of a cross-section, covering an area of 32 × 32 µm with 64 × 64 pixels. Since the IR penetration depth is < 1 µm (Zumbühl *et al.* 2014), the spectral intensity is dependent on the pressure; therefore if the surface of the cross-section is not completely planar, areas in poor contact with the crystal will result in spectra of low intensity.

Because the manner in which proteins are distributed within (fresh) paints is of decisive importance for their rheological behaviour and optical properties after drying, protein-selective fluorescent staining was used as a second, independent imaging technique. SYPRO Ruby was used as a stain; the staining protocol followed included fixation with formaldehyde (Schäfer 2013; Neugebauer 2016, pp. 30–32). To prepare the samples for this study, generally Micro-Mesh 4000 or 6000 was used to polish the cross-sections prior to staining. The samples were then examined under ultraviolet illumination; fluorescence was observed using the Zeiss filter set no. 9 or Leica filter set I3 (both BP 450–490 nm, LP 515 nm). The stain only exhibits an orange fluorescence under such conditions if it is in direct contact with proteins (Schäfer 2013).

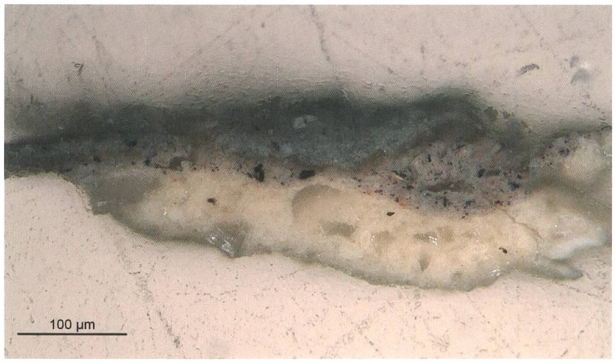
Backscattered electron (BSE) images of cross-sections were produced with a scanning electron microscope (SEM) for comparison with the FTIR-FPA images. However, these images were only used for comparison and are not discussed any further.

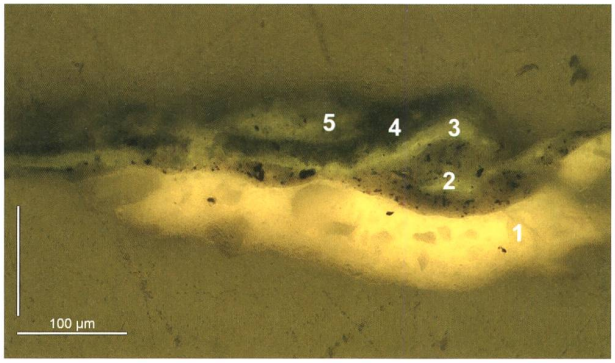
CASE STUDY I: ARNOLD BÖCKLIN, *VILLA AM MEER I* (*VILLA BY THE SEA I*), 1864

The materials and techniques used by Arnold Böcklin for *Villa am Meer I* (*Villa by the Sea I*) (see the contribution by Neugebauer, in this volume, Fig. 1) were described in detail by Rudolf Schick (1840–1887), who worked as an assistant to Böcklin in the late 1860s. Schick recorded Böcklin's use of paints consisting of finely ground frankincense and sandarac resins mixed with water and pigments. After the painting was finished, he impregnated it with molten wax (Schick 1901, pp. 75–77, 104). Although Schick only mentions an application of wax after the completion of the painting ('*nach Vollendung des Bildes*'), our paint cross-sections show that Böcklin also applied intermediate varnishes of wax between paint layers (see the contribution by Neugebauer, in this volume, Figs 4 and 5).

Binding medium analysis was particularly difficult because the painting had been restored: varnished with dammar and lined using a mixture of Venice turpentine (larch turpentine with colophony) and beeswax. For this study, sampling was only possible at the edges of the painting, where the tacking margins had been masked with a tape comprised of an aqueous adhesive based on starch and animal glue. The tape was temporarily removed for sampling. Only two layers of paint (one on top of the other) from the sea were sampled, both from the same location. GC-MS analyses revealed frankincense[4] (with a trace of polysaccharides from this resin) in the upper layer of paint, but not in the lower. Sandarac was not found, either because characteristic peaks were masked in the chromatograms by the presence of diterpenoids of larch turpentine and colophony from the lining adhesive or because it is not present (or only present in negligible quantities). The latter option would be consistent with Schick's account, as he noted that the amount of sandarac in Böcklin's binder varied (Schick 1901, p. 104). Beeswax is present in large quantities but this cannot be attributed unequivocally to either the composition of the original coating or the presence of the lining adhesive. In contradiction to Schick's report both paints also contain egg proteins as well as a lipid material. Because of the fatty acid components of beeswax it could not be ascertained whether the lipid material contained fat from egg yolk, but the high content of dicarboxylic acids clearly allows the identification of a drying oil in both paints. The identification of egg and drying oil is

Figs 1a–1c Arnold Böcklin, *Villa am Meer I (Villa by the Sea I)*, 1864, paint on canvas support, 124.5 × 174.5 cm, Bavarian State Painting Collections, Munich, Schack Collection, inv. no. 11528. Paint cross-section from the sky (close to the left tacking edge), viewed under an optical microscope.





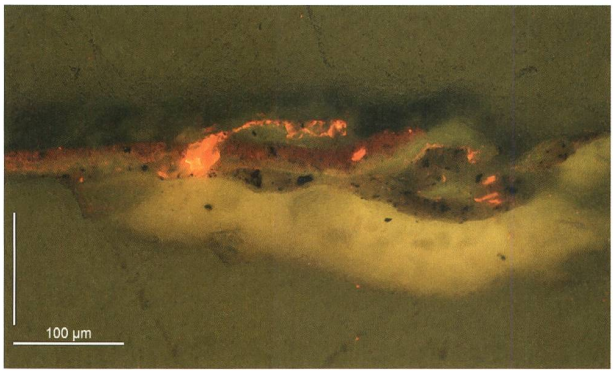


Fig. 1a In visible light.

Fig. 1b Under UV illumination with filter set I3 (Leica), before staining. Layer build-up: 1 ground layer; 2 pale violet paint; 3 intermediate layer; 4 dark blue layer; 5 varnish.

Fig. 1c As Fig. 1b after staining with SYPRO Ruby. The occurrence of orange fluorescence under UV illumination after staining attests to the presence of proteins. Strong fluorescence is seen in a crack that extends into the deeper layers, probably indicating the presence of animal glue used during conservation treatment (consolidation and/or masking tape). The entire upper paint layer 4 and part of layer 2 show a uniform orange fluorescence. This can probably be attributed to an even distribution of egg within these layers.

surprising, as these substances are not mentioned by Schick, whose description of how Böcklin painted *Villa am Meer I* has been found to be reasonably plausible (Neugebauer 2016, pp. 209–218). [5]

An attempt at FTIR-FPA imaging in ATR mode of a sample prepared in cross-section was unsuccessful because the thick, unpigmented, intermediate layers were very soft and slightly below the general surface level after polishing. Accordingly, there was not enough pressure on these regions of the sample to allow for the identification of beeswax. However, staining with SYPRO Ruby was successful. In a cross-section from the sky (Figs 1a–1c) the entire upper paint layer shows a uniform orange fluorescence (Fig. 1c). This can probably be attributed to the presence of egg protein, which is obviously homogeneously distributed in the paint layer. That Böcklin would have added egg to the aqueous paint is not surprising because otherwise it would have almost certainly rolled off when applied on top of a layer of (hydrophobic) beeswax. The addition of egg with its remarkable emulsifying power would not only have improved the wetting ability of the aqueous paint on the wax-based substrate but it would also have stabilised the suspension of the resin particles within it. Accordingly the Munich painter and scholar in the field of painting technology Ernst Berger (1857–1919) described the preparation of such suspensions by 'grinding the resins with water and with addition of egg' (Berger 1912a, p. 247). Max Doerner (1870–1939) recommended the addition of egg especially for emulsions based on polysaccharides and other 'artificial

emulsions',[6] in order to increase their stability and improve their adhesion to the substrate (Doerner edn 1938, p. 183). Therefore the occurrence of egg is not really surprising in such a context, as egg must have been a common additive, as will be shown below.

Although Schick's description of Böcklin's painting process – the use of resins suspended in water, followed by coating with beeswax – seems generally reliable, it should be noted that in one of the two samples of paints studied, egg and possibly drying oil are the only binders identified (the beeswax must derive from intermediate layers or from the lining adhesive, and the animal glue from the masking tape or a later consolidation treatment). As a result, it can be concluded that Schick's description is generally valid but incomplete and that the actual painting process was more complex.

CASE STUDY II: FRANZ VON STUCK, *DER KRIEG* (*WAR*), 1894

According to written sources and to Stuck's own statements, *Der Krieg* (*War*) (see the contribution by Neugebauer, in this volume, Fig. 6) was created with *Syntonosfarben* (Syntonos paints) (Neugebauer 2016, p. 321). Syntonos paints were a commercially available product introduced by the German artist Wilhelm Beckmann (1871–?). Thanks to Beckmann's patent specification, the composition of the Syntonos binding medium is known to be an oil-in-water (O/W) emulsion of linseed oil with gum arabic, which is modified with wax, tallow and green soap as well as glycerol (Patent DE78793, 13 June 1893; see also the contributions by Neugebauer and by Pohlmann *et al.*, in this volume). Fortunately it was possible to obtain samples from Syntonos tube paints (zinc white no. 3) that had survived in a paintbox belonging to James McNeill Whistler (1834–1903) (FitzHugh *et al.* 2011).[7] This enabled us to make a comparison between the pure tube paint, the paint layers of Stuck's *Der Krieg* and the formula given in the patent. The results of the binding medium analyses by chromatographic techniques are shown in Table 1.

In the interpretation of the results the conservation history of the painting must be taken into account. Documents from the conservation archive at the Doerner Institut of the Bavarian State Painting Collections in Munich reveal an incomplete list of several treatments undertaken on *Der Krieg* (Neugebauer 2016, pp. 616–617). In 1914 a removal or reduction of (several?) varnish(es) is mentioned as is the application of a new coat of 'English coach varnish' ('*englischer Kutschenlack*'). From a photograph taken in 1930 it can be discerned that a coating had at some point been applied to the reverse of the painting; when exactly this was done remains uncertain (possibly 1903). Based on our analyses, the varnish(es) that were removed probably contained mastic: minor amounts of mastic, which is not a component of the Syntonos binder, were found in all samples of the paint, and in notably higher concentrations in the upper layers (Table 1, samples 1 and 6). No mastic was found in the current varnish (Table 1, sample 2A). Among the three or more layers of varnish coatings currently present on the painting,[8] analysis identified at least one dammar layer and an oil-resin layer consisting of boiled linseed oil and colophony (Table 1, sample 8). The latter mixture almost certainly corresponds to the *englischer Kutschenlack* mentioned in the conservation report. Its components have penetrated deeply into the paint layers. The coating that was applied to the reverse was most likely primarily Venice turpentine – it too must have penetrated deeply into the paint, as larch turpentine with colophony was found in all samples, with a high concentration in lower layers and decreasing concentrations in the layers towards the paint surface and varnish interface (Table 1, samples 1C–1A or 2C–2A).

The analytical results obtained for the binder of the paint from the historical Syntonos paint tube (Table 2) correlate very well to the patent specification for that paint brand (as mentioned above, it is an O/W emulsion of linseed oil with gum arabic, which is modified with wax, tallow and green soap as well as glycerol; see Patent DE78793, 13 June 1893). However, it must be noted that the exact composition of 'green soap' is uncertain. As a

Table 1 Franz von Stuck, *Der Krieg (War)*, 1894, paint on canvas support, 245.5 × 271 cm, Bavarian State Painting Collections, Munich, Neue Pinakothek, inv. no. 7941. Overview of the results of binding media analysis by chromatography. Samples with different numbers are from different locations. Samples with the same number but subsequent letters are from the same location and were separated by scraping layer by layer (from A–C, descending – A being the top layer).

Pale green: non-original conservation materials, these may account for a portion of the other samples by contamination. *Dark green*: samples containing original (Syntonos) paints.

+++ = large quantity, ++ = medium quantity, + = small quantity, (+) = trace, • = detected.

Sampled area	Sample number*, **SAMPLE DESCRIPTION (MAIN COMPONENTS ARE IN BOLD PRINT)**	WAX	TRITERPENOIDS	
			Dammar	Mastic
	(9) **coating on the verso**	–		
	(8) **varnish** ('*englischer Kutschenlack*')	–	++	
	(7) **ground layer** without isolation layer, without coating from the verso	–		
	(5) **ground layer** with isolation layer and varnish	–	(+)	
Dark brown from background (upper tacking edge)	(1A) **dark brown paint**, with varnish	–	++	+
	(1B) **blue paint**	–	++	+
	(1C) brown-beige **ground layer**	–	+	
Flesh paint (lower tacking edge)	(2A) yellow **varnish**	(+) paraffin	+	
	(2B) **white flesh paint**	(+) paraffin	+	(+)
	(2C) brown **ground layer**, multilayer build up (contains brown paint layer, c.f. sample 6C)	–	+	(+)
Red from blood (lower tacking edge)	(6A) **varnish** with red paint	–	++	+
	(6B) **red paint**	–	+	(+)
	(6C) **brown-red paint** (from underpainting)	–	+	(+)

* The sample numbers correspond to the chronological order in which the samples were taken. The table is however arranged for optimal clarity of its contents.

LARCH TURPENTINE	FATTY ACIDS**	PROTEINS	GUM ARABIC	GLYCEROL	INTERPRETATION
+++ with colophony	+				**Coating on verso** (larch turpentine with colophony)
+ with much colophony	+++ boiled linseed oil	(+) egg animal glue			**Varnishes** (dammar / boiled linseed oil with colophony), possibly coating on verso
	++ animal fat boiled linseed oil	+++ animal glue	–	•	**Ground layer** (animal glue with boiled linseed oil)
+++ with colophony	+++ animal fat drying oil	+++ animal glue		(•)	Combination of samples 7, 8 and 9
+ with colophony	+++ drying oil animal fat	+++ egg	++		**Syntonos paint** (gum arabic, linseed oil, animal fat) with egg / mastic, varnish / coating on verso
++ with colophony	+++ drying oil animal fat	++ egg animal glue	++	•	Syntonos paint with glue and egg / mastic, varnish / coating on verso
++ with colophony	++ drying oil animal fat	++ animal glue	(+)	•	Ground layer with traces of Syntonos paint / varnish / coating on verso
++ with colophony	++ drying oil	+ egg	+		Varnish, possibly with a trace of Syntonos paint and egg
++ with colophony	+++ ricinoleic acid	++ egg	++	•	Syntonos paint with egg / varnish / coating on verso
+++ with colophony	+++ ricinoleic acid	++ animal glue (egg)	+	•	Ground layer with Syntonos underpainting (6C) / varnish / coating on verso
+	++ linseed oil	+++ egg	–	•	Varnish / red Syntonos paint / egg
++ with colophony	+++ ricinoleic acid	+++ animal glue egg	++	•	Syntonos paint with glue and egg / varnish / coating on verso
++ with colophony	+++ ricinoleic acid	+++ animal glue egg	++		Syntonos paint with glue and egg / varnish / coating on verso

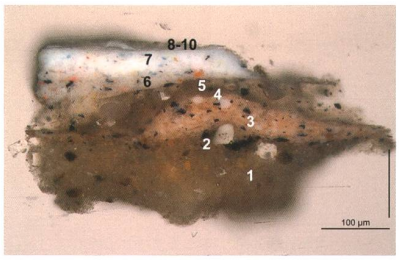
** Fatty acids from drying oils, animal fat and castor oil are only identified together, after hydrolysis and methylation. A retroactive attribution of fatty acids to initial components is not possible (see the text for discussion).

Table 2 Composition of the binder of Syntonos paint – comparison of the patent specification with the analytical results of historical tube paints and of paint samples from *Der Krieg* by Franz von Stuck.

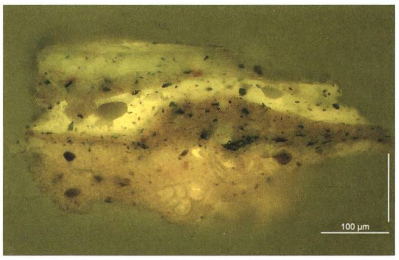
X = detected, (X) = only detected in some samples, ? = attribution ambiguous.

MATERIAL	SYNTONOS PAINT PATENT SPECIFICATION	RESULTS OF ANALYSIS OF SYNTONOS TUBE PAINT	RESULTS OF ANALYSIS OF *DER KRIEG*
gum arabic	X	X	X
linseed oil	X	X (?)	X (?)
glycerol	X	X	X
wax (~1%)	X	X	(X)
tallow (~2%)	X	X	(X)
green soap (~2%)	X	?	?
egg	–	–	X
animal glue	–	–	(X)
castor oil (soap?)	–	X	X

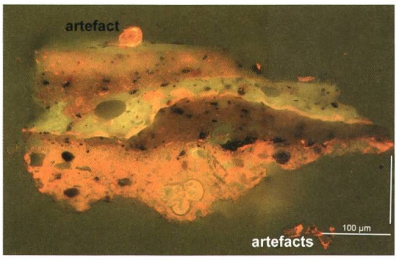
Figs 2a–2d Franz von Stuck, *Der Krieg (War)*, 1894, paint on canvas support, 245.5 × 271 cm, Bavarian State Painting Collections, Munich, Neue Pinakothek, inv. no. 7941. Cross-section of a sample from the flesh (lower leg of the corpse in the foreground, lower tacking edge) viewed under an optical microscope.



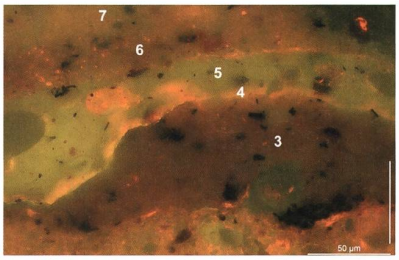
2a



2b



2c



2d

Fig. 2a In visible light. Layer build-up: 1 ground layer; 2 isolation (mentioned in the sources, possibly visible here as slightly higher fluorescence at the top of the ground layer); 3 light brown paint layer; 4 and 5 intermediate layer (4 proteinaceous part, 5 weakly pigmented part); 6, 7 white layers; 8 intermediate layer (varnish); 9 white layer; 10 varnish.

Fig. 2b Under UV illumination with filter set I3 (Leica), before staining.

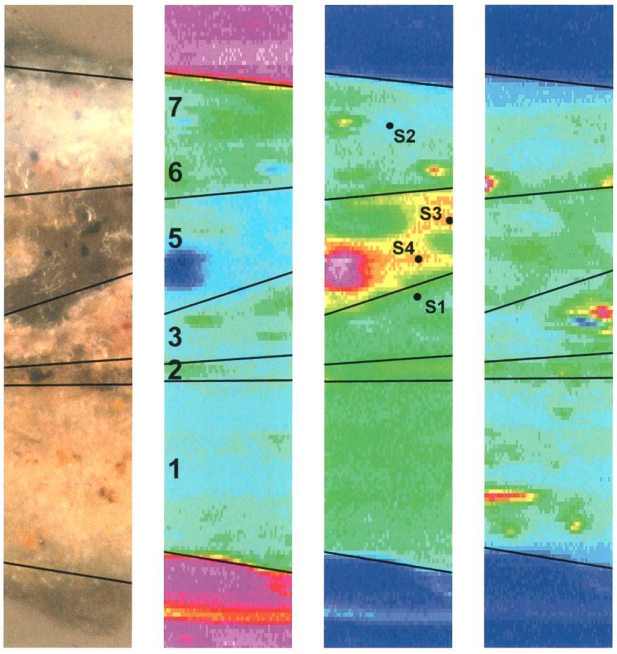
Fig. 2c As Fig. 2b after staining for proteins with SYPRO Ruby (artefacts caused by protein-containing foreign particles on top of the cross-section). The paint in layer 5 must have formed air bubbles during preparation or painting, two of which are visible in this cross-section. One remained empty (dark oval shape), the other (adjacent to the left) was filled with the white paint of the next layer as painting continued.

Fig. 2d Detail of Fig. 2c with layers 3–7.

consequence, identification of linseed oil, tallow and green soap is hampered because their components, the fatty acids, are identified together, in bulk, after hydrolysis, and cannot be unequivocally attributed to one or another of the components. In summary, we may observe that odd-numbered fatty acids as well as ricinoleic acid were found in the binder. Odd-numbered fatty acids are characteristic for animal fats, and therefore of tallow, while ricinoleic acid is a characteristic component of castor oil (Table 1). Based on this finding, we may surmise that the green soap was made of saponified castor oil, while the tallow was added to the binder as indicated in the patent specification (Table 2). Another possible, albeit unlikely, interpretation is that the green soap was made of saponified tallow, while castor oil was added to the paint binder to modify the linseed oil. As the paint samples from Stuck's painting did not always contain the wax and tallow ingredients specified in the patent (Table 2), the inclusion of these additives must have been somewhat variable, dependent on the pigmentation of each specific paint.

The results of our analyses of Stuck's painting are somewhat more complex. In addition to the components present in Syntonos paint binders, egg and sometimes animal glue were identified. As will be demonstrated in the following discussion, these latter materials (especially the egg)[9] are very likely to have been added by the artist (Tables 1 and 2). The cross-sections of the sampled areas show a complex stratigraphy consisting of many layers, thus the question arises of whether the presence of the proteins (both from the egg and the animal glue) is due to their occurrence in individual (isolation) layers (not easily detectable in the cross-sections) or whether they are homogeneously distributed in the paints.

Figs 3a–3d Franz von Stuck, *Der Krieg*: detail of the cross-section shown in Figs 2a–2d after further polishing (as a result of which the structures of the air bubbles and the protein agglomerate no longer correspond to Figs 2a–2d).



3a 3b 3c 3d

Fig. 3a In visible light with auxiliary lines and layer build-up (see also Fig. 2a).

Figs 3b–3d FTIR-FPA (false colour) images of the cross-section detail. The spectrum in each point is integrated between the wave numbers defined in the respective caption of the image. The size of the integrated area is correlated with colours, from large to small: pink, red, yellow, green, light blue, dark blue.

Fig. 3b Absorption of carbonyl (1765–1665 cm^{-1}).

Fig. 3c Absorption of proteins (amide I, 1701–1594 cm^{-1}). The green areas in the otherwise yellow layer are voids (air bubbles).

Fig. 3d Absorption of polysaccharides (1137–980 cm^{-1}).

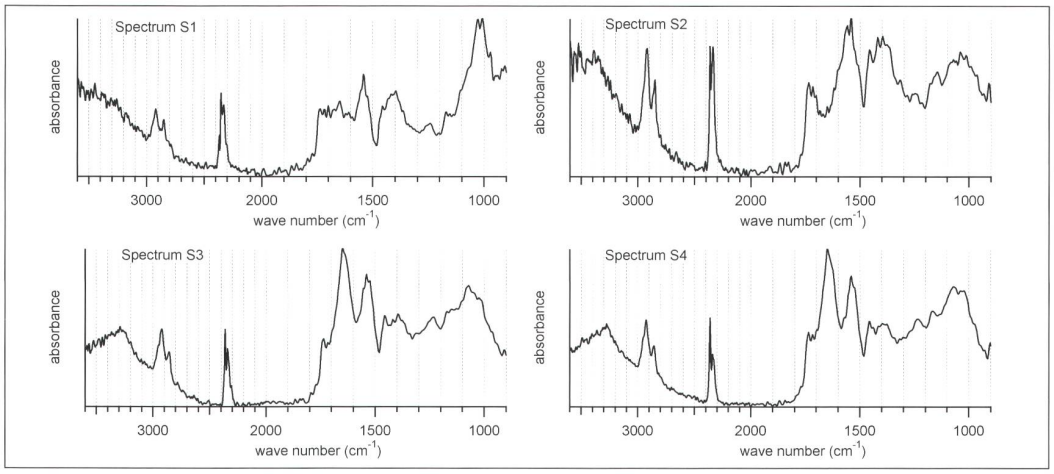


Fig. 3e FTIR spectra of the four spots indicated in Fig. 3c. Spectra S1 and S2 correspond to paints known to be Syntonos paints. The shape of the absorption of polysaccharides at 1100–1000 cm^{-1} is not strictly reproducible, as can be seen in the four spectra (see the text for more details).

Staining of a cross-section with SYPRO Ruby to examine the distribution of protein components was very successful; a variety of detailed information on the distribution of the proteins was revealed (Fig. 2; see also Neugebauer 2016, pp. 326–333, 623–625). The ground layer stained positively, as did the three paint layers: light brown (3) and two layers of white (6, 7). The lower part of the weakly pigmented intermediate layer, labelled (4) in Fig. 2, has clearly reacted with the stain, although the upper part (5) has not. It seems that layer (4) is a proteinaceous layer applied over the light brown paint (3) that was subsequently coated with weakly pigmented material (5).

Figs 3a–3d show a detail of the cross-section and corresponding false-colour images created with FTIR-FPA. The image displaying the carbonyl absorption (for lipids and resins, Fig. 3b) mainly shows the embedding resin and a somewhat uniform occurrence of a carbonyl peak throughout all the layers. The image relating to the area of the amide I absorption (for proteins, Fig. 3c) reveals a protein agglomerate (red area) and an increased signal (yellow area) in the weakly pigmented intermediate layer (5). However, the yellow areas in the white paint layers (6 and 7) show characteristic absorptions of zinc oxalates (1626, 1364, 1317 cm^{-1}; see Monico *et al.* 2013) and are not caused by proteins.

The occurrence of polysaccharides (Fig. 3d) is not easy to establish in the spectra. FTIR reference spectra for gum arabic show a strong absorption reaching from c. 950 to almost 1200 cm^{-1}, usually with a weak double maximum around c. 1050 cm^{-1} and shoulders at c. 970 and 1160 cm^{-1}. Other absorptions in the gum spectrum, e.g. at 1610 or 1430 cm^{-1}, are usually masked by stronger absorptions in the same regions from other materials such as oils and metal soaps.[10] The polysaccharide absorption at 1100–1000 cm^{-1} can be seen in the spectra; their presence was confirmed by gas chromatography (Table 1). However, the shape of the absorption is not strictly reproducible (see Fig. 3e) due to the occurrence of several pigments and fillers that also absorb in the polysaccharide region: the presence of ochre, aluminosilicates, quartz and barium sulphate was determined by scanning electron microscopy (SEM). Nevertheless (in light of the weak pigmentation) it seems reasonable to assume that the intermediate layer (5) contains oil, polysaccharide and protein based on spectra S3 and S4

(Fig. 3e). It would appear, therefore, that this layer contains the same mixture of components as the paint layers which we know to be comprised of Syntonos paints (spectra S1 and S2).

It is interesting to note that the FTIR information derived for proteins is not consistent with the results obtained by staining with SYPRO Ruby (Figs 2c and 2d). The thin proteinaceous layer (4) (which clearly accepted the stain) on top of the light brown paint layer (3) is not resolved in the FTIR image (Fig. 3c) although the protein agglomerates in the intermediate layer (5) are visible both by staining and with FTIR imaging. On the other hand, the clear pattern of proteins visible in the FTIR image in the same bulk layer (5) (Fig. 3c, yellow area; Fig. 3e, spectra S3 and S4) is not resolved with staining. This is a phenomenon that was encountered frequently in the study of tempera painting around 1900 as presented here: positive staining could always be correlated with proteins found by amino acid analysis, but in some cases there was no positive staining in spite of the fact that the presence of proteins could be ascertained by FTIR. We have concluded that a positive stain result can be trusted, but in some cases quenching of the fluorescence may produce a false negative result, in part depending on the nature of the pigments that are present.[11] However, more often, the absence of a positive reaction with the stain seemed likely to be the consequence of the presence of a considerable amount of oil that served to impede staining of the protein, because the stain itself is in the form of an aqueous solution. In such cases the texture of the sample surface – the presence of features such as cracks or roughness – will determine whether or not the proteins in a sample can be successfully accessed by the stain.[12] Accordingly, the surface of a cross-section should not be polished too highly prior to staining.[13] It seems possible that oil components of the paints may be smeared across the surface during fine polishing, creating a thin but continuous hydrophobic layer of oil that will repel the aqueous stain.[14] Whatever explanation(s) might apply in any specific situation, one must bear in mind that a given structure seen after staining might be an artefact of non-uniform access of the stain to the surface, rather than an accurate representation of protein distribution.

It is our opinion that the weakly pigmented layer (5) contains protein throughout, in homogeneous mixture ('overlooked' by staining, but evidenced by FTIR-FPA imaging), and that there is a proteinaceous layer (4) on top of the light brown paint (3) (evidenced by staining, but 'overlooked' by FTIR-FPA imaging). There could be several explanations as to why the staining reveals the thin proteinaceous layer (4) while FTIR-FPA imaging does not: it may be too thin to be resolved with FTIR, or the protein component may not be sufficiently present at the surface of the cross-section for detection by FTIR. Due to its nature, the aqueous staining solution can have a deeper penetration depth in the sample mass than that achieved by IR radiation.[15] Indeed a careful comparison of the UV pictures before and after staining clearly reveals that layer (4) was swollen by the action of the staining solution and subsequently pushed layers (3) and (5) apart as it expanded. Accordingly, it can be concluded that layer (4) has a different response to the staining solution than layer (5), and furthermore that it is possible that this is less a reflection of the distribution of protein within the layers, but rather of the distribution of oil.

In terms of the paint layers we know to be comprised of Syntonos paints (3, 6, 7) we take the view that the staining confirms a uniform distribution of egg protein. Because those paint layers also contain a quantity of oil, which tends to prevent staining, it seems illogical to assume that trace amounts of protein from the oil or gum arabic would stain positive in such a context, but not in layer (5). In addition, as previously mentioned (in case study I), the addition of egg to tempera paints by the artist would have been of practical value in this particular situation. The presence of protein in the (Syntonos) paint layers may be inferred in spectrum S1 in Fig. 3e, because the area of the amide I absorption (1650 cm^{-1}) between the oil carbonyl signal (c. 1720 cm^{-1}) and the zinc soaps (c. 1540 cm^{-1}) is filled

in. However, it is surprising that spectrum S2 in Fig. 3e does not likewise suggest the presence of protein in this area, although staining does. Obviously, interpretation of FTIR spectra alone can be very difficult due to the spectral overlap with the partially hydrolysed oil and an unknown quantity of metal soaps. In other words, based on the results of FTIR-FPA imaging, we would infer that the egg protein identified by chromatography would only be located in the weakly pigmented intermediate layer (5) (Figs 3a–3d), while based on staining with SYPRO Ruby (Figs 2c, 2d), we would suggest its presence only in layers (3), (4), (6) and (7). For the various reasons discussed above, we conclude that each analytical method gives an incomplete picture so should be consulted simultaneously to obtain a more precise overview of protein distribution, and that in this example, all the layers contain egg protein.

CASE STUDY III: FRANZ VON LENBACH (COPY AFTER TITIAN), *DAS KONZERT (THE CONCERT)*, 1865

Das Konzert (*The Concert*) by Franz von Lenbach (see the contribution by Neugebauer, in this volume, Fig. 10) is one of several copies by that artist of Old Master paintings. As noted by Neugebauer (in this volume), contemporary sources indicate that it was painted in '*Wasserfarben*' (water paints) which, in this context, indicates 'tempera'. The results of the binding medium analyses by gas and liquid chromatography are shown in Table 3.

Today, the painting is coated with at least three, presumably original,[16] varnishes. Based on careful sampling and GC-MS analyses, the first seems to consist of linseed oil with a little copaiba balsam (Table 3, sample 3A), the

Table 3 Franz von Lenbach (copy after Titian), *Das Konzert* (*The Concert*), 1865, paint on canvas support, 109.8 × 123.2 cm, Bavarian State Painting Collections, Munich, Sammlung Schack, inv. no. 11486. Overview of binding media identified by chromatography. Samples with different numbers are from different locations. Samples with the same number but subsequent letters are from the same location and were separated by scraping layer by layer (from A–C, descending – A being the top layer).

Pale green: contamination by later varnish.

+++ = large quantity, ++ = medium quantity, + = small quantity, (+) = trace, • = detected, n.a. = not analysed.

SAMPLED AREA	SAMPLE NUMBER*, SAMPLE DESCRIPTION	COLOPHONY	COPAIBA BALSAM
	(4) varnishes applied on the framed painting	+++	–
White from sleeve, person on the right (see Fig. 4)	(3A) varnish under frame rabbet, with glaze	(+)	+
	(3B) grey paint, with residues of varnish and glaze (sample 3A)	+	+
	(3C) lower grey paint (sample presumably includes some ground layer)	(+)	(+)
Brown from harpsichord	(5A) varnish and brown glaze		(+)
	(5B) brown paints		+
	(5C) red–brown paint (from underpainting)		++
	(6) red–brown paint (from underpainting) with ground layer, without any varnish		–

others of linseed oil and colophony (Table 3, sample 4). The ground layer is very thin; it does not form a continuous layer as it occurs primarily in discreet pockets, filling the gaps between the threads of the canvas. Traces of the ground are present in paint cross-sections (Fig. 4, see also Neugebauer 2016, p. 302), and it also seems to be present in samples 6, 3C and 5C (Table 3). Analysis by gas and liquid chromatography revealed that the ground is bound with flour paste, as glucose (from starch) was found during sugar analysis and an amino acid profile showing characteristics of gluten, the protein glue in flour, was also noted. A positive result for starch was obtained by staining the ground layer in cross-sections with iodine and potassium iodide. The samples containing the ground layer also contain walnut oil, however it is unclear whether this material should be assigned to the paint layers or to the ground in those samples (Table 3).

In general it can be stated that the paints are bound with drying oils with some copaiba balsam and egg. In some samples, the drying oil was identified as walnut oil, while in others both linseed and walnut oil seem to be present, possibly in different layers. The copaiba balsam is of the type *Copaifera langsdorfii L.*, because kauranic acid and pinifolic acid were detected (van der Werf *et al.* 2000). The same type of copaiba balsam was also found in two paintings by Arnold Böcklin: *Villa am Meer II* (*Villa by the Sea II*) from 1865 (Dietemann *et al.* 2014, p. 34) and *Triton und Nereide* (*Triton and Nereid*) from 1873/1874.[17]

Examination of the samples prepared as cross-sections showed that most samples taken for chromatographic analysis contain more than one paint layer (compare, for example, samples 3A–3C with Fig. 4). This raises the question of how oil, resin and egg are distributed in the stratigraphy of the painting. As can be seen in paint

OIL AND FAT**	PROTEINS	POLYSACCHARIDES	GLYCEROL	OTHERS
++	n.a.	n.a.		modern plasticisers
+++	–	–		modern plasticisers
+++	(+) egg	–	•	modern plasticisers
++	++ gluten and egg	• starch	–	modern plasticisers
++ walnut oil	+ egg	n.a.	–	
+++	++ egg	n.a.	–	modern plasticisers
+++	++ egg	• starch	–	
+++ walnut oil	++ gluten	• starch		

* The sample numbers correspond to the chronological order in which the samples were taken. The table is however arranged for optimal clarity of its contents.

** The oil binders studied here contain some animal fat and some drying oil containing dicarboxylic acids with chain lengths of 11 to 13 carbon atoms (D_{C11}–D_{C13}), which are not components of natural drying oils (possibly they derive from dehydrated castor oil or rapeseed oil).

Figs 4a–c Franz von Lenbach (copy after Titian), *Das Konzert (The Concert)*, 1865, paint on canvas support, 109.8 × 123.2 cm, Bavarian State Painting Collections, Munich, Schack Collection, inv. no. 11486. Cross-section of paint sample from the white sleeve of the person on the right (cf. Table 3, samples 3A–3C).

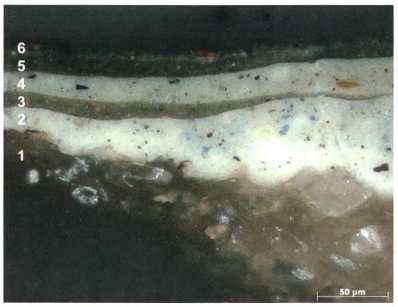


Fig. 4a In visible light. Layer build-up: 1 ground layer; 2, 4, 6 opaque layers; 3, 5 glazes. (Varnishes not indicated.)

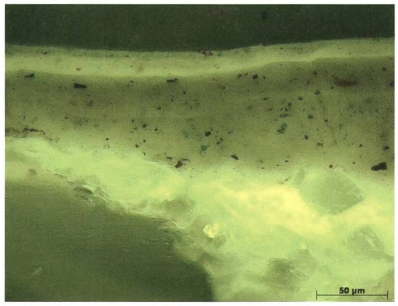


Fig. 4b Under UV illumination with filter set 09 (Zeiss), before staining for proteins with SYPRO Ruby.

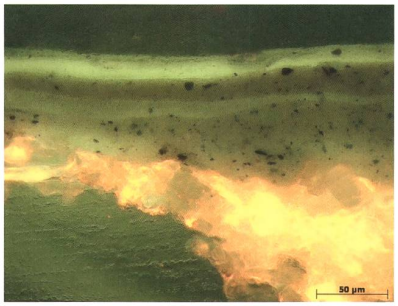


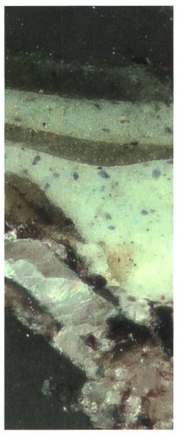
Fig. 4c As Fig. 4b after staining.

cross-sections (Fig. 4) (see also the contribution by Neugebauer, in this volume, Figs 11 and 12), Lenbach used alternating opaque paints and transparent glazes to create his copy after Titian (Neugebauer 2016, p. 298). Staining with SYPRO Ruby was positive in all opaque paints but not in the glazes. A positive result for staining can be very clear and unambiguous, as is the case in the opaque paint layers (2, 4, 6) in a cross-section from the left hand of the figure on the left (see the contribution by Neugebauer, in this volume, Fig. 12); however, it can also be difficult to discern, as in layers (2, 4, 6) in the cross-section from the sleeve of the figure on the right (Fig. 4c). In the latter cross-section, staining with SYPRO Ruby has resulted in a marked increase in contrast between two of the opaque paint layers (2, 4) and the glaze layer (3) between them (compare Figs 4b and 4c), which confirms a uniform orange fluorescence in the opaque strata (2, 4). Therefore it can be deduced that the egg identified by means of chromatography (Table 3) is homogeneously distributed in the two opaque layers (2, 4). However, as seen above (case study II), this does not necessarily mean that the glazes do not contain some egg.

The results of the FTIR-FPA measurements are given in Figs 5d–5g. Due to the complex layer build-up it was not possible to obtain consistent FTIR images as a good and uniform pressure could not be maintained over the entire surface of the cross-section. The resulting areas of low signal intensity are rendered dark in Figs 5c–5g; these regions of lower values in the false-colour images should be ignored.

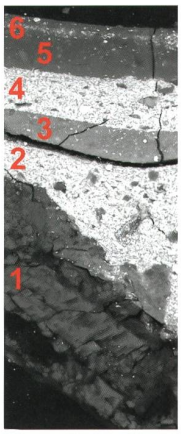
In addition to the pigments present, the FTIR spectra of the ground layer also show evidence of the presence of starch (1145, 1076, 1024 cm^{-1}) and variable amounts of protein (1650 cm^{-1}). Due to penetration of the embedding resin (acrylate) into the sample, no clear conclusion about the presence of drying oils in the ground could be drawn (Fig. 5h). An inhomogeneous distribution of protein can be seen in the FTIR image of the amide I absorption (Fig. 5d). The amide II absorption overlaps with the absorption of the lead soaps (1530 cm^{-1}) and the FTIR image is clearly dominated by the latter. The formation of lead soaps correlates clearly with the amount of lead white (1400 cm^{-1}) in the opaque paint layers (2, 4, 6) (Figs 5e and 5f). The spectra of the glazes (3, 5) are mainly characterised by the absence of IR-active pigments. Accordingly, the spectra (S1 and S2) of one of the glazes (5) looks quite similar to (for example) a spectrum (S3) of one of the paint layers (4), considering that they contain less lead carbonate and lead soaps.

Figs 5a–5g Franz von Lenbach, *Das Konzert*: detail of the cross-section shown in Figs 4a–4c polished after staining with SYPRO Ruby (the polishing has altered the appearance of the cross-section).



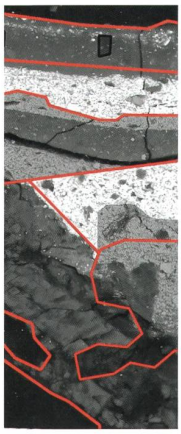
5a

Fig 5a Stained with iodine for starch, in visible light



5b

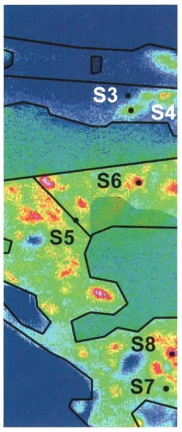
Fig. 5b Backscattered electron (BSE) image, SEM. Layer build-up (in red) as in Fig. 4.



5c

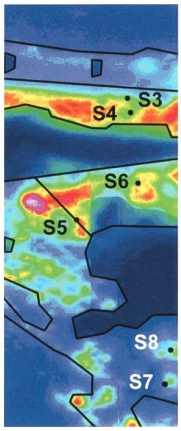
Fig. 5c As Fig. 5b with auxiliary lines.

Figs 5d–5g FTIR-FPA (false colour) images of the same detail with auxiliary lines. Areas of low signal intensity are rendered dark and should be ignored.



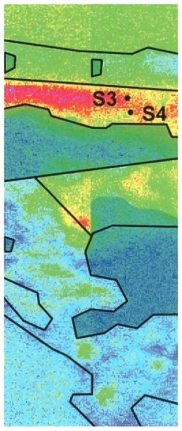
5d

Fig. 5d Absorption of proteins (amide I, 1696–1605 cm^{-1}).



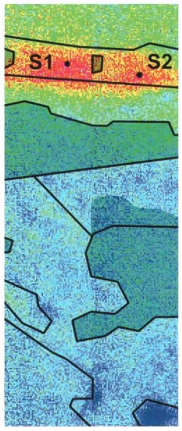
5e

Fig. 5e Absorption of carbonate (1480–1321 cm^{-1}).



5f

Fig. 5f Absorption of lead soaps and proteins (carboxylates and amide II, 1558–1493 cm^{-1}).



5g

Fig. 5g Absorption of carbonyl (1720–1696 cm^{-1}).

Possibly the glazes contain more resin (1710–1700 cm^{-1}) than the paints, but this cannot be determined with certainty due to the highly variable amounts of metal soap ageing products. The position and shape of the C–H stretching vibrations (2928 / 2854–2856 cm^{-1}) (Fig. 5h) indicates a very low resin content in the glaze (5), which accords with the low amounts of copaiba balsam that were detected by means of GC-MS (Table 3).

It might be thought that the glazes (3, 5), which according to Neugebauer's conclusion were applied as oil paints (Neugebauer 2016, pp. 298–299, 306–307), do not contain proteins in light of the negative outcome of staining with SYPRO Ruby. However spectra S1 and S2 recorded within glaze (5) (Fig. 5h) do show absorbance in the region of 1650 cm^{-1} that is not characteristic of oil-resin binders. In a similar manner, the opaque

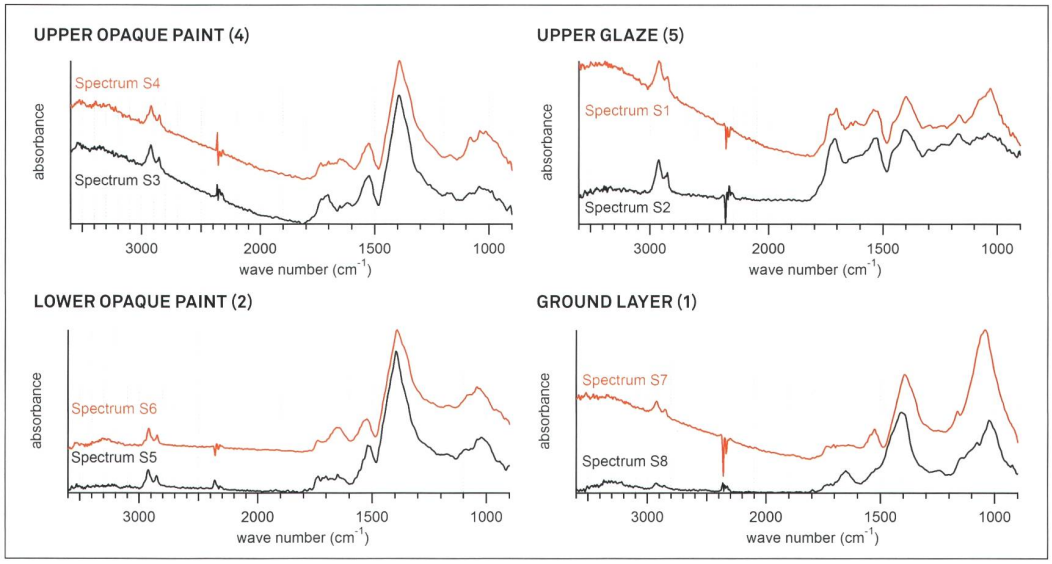


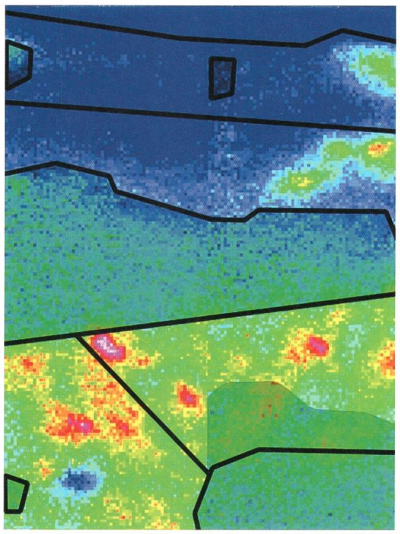
Fig. 5h FTIR spectra of the eight spots indicated in Figs 5d–5g.

paints that obviously contain protein, the area around 1650 cm^{-1} also reveals some absorption in protein-depleted regions (spectra S3 and S5, Fig. 5d), not unlike the spectra obtained from the glazes. Thus, the presence of proteins in the glazes cannot be excluded unequivocally.

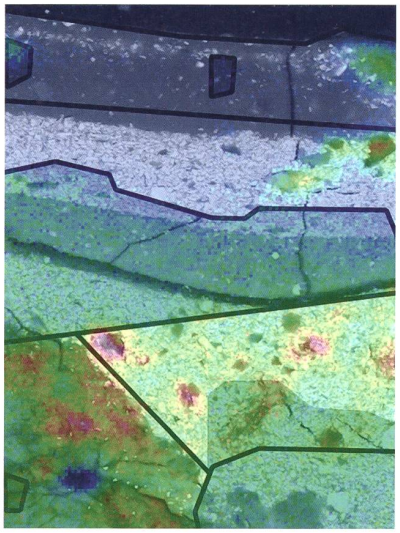
The inhomogeneous distribution of proteins in the opaque paints requires further explanation. If the FTIR image (Fig. 5d) is compared to the SEM image (Fig. 5b), it appears that areas of high amide I absorption correlate with areas of lower pigment density (Figs 6a–6c). The behaviour of oil-protein mixtures considered in light of colloid chemistry has been discussed in some detail in an earlier paper (Dietemann *et al.* 2014). Five different ways to create a paint containing egg and oil as a binder are described, and assumptions about the resulting distribution of protein in oil have been made (Dietemann *et al.* 2014, p. 42). Unfortunately it is still unclear how to understand the relationship between these observations with the structures seen in Fig. 6c. It may be assumed that regions of elevated protein content and reduced pigment density are formed when the protein components in a liquid egg tempera paint start to form gel aggregates due to low water content following continuous evaporation of water during, or subsequent to, the grinding of the paint. It might be expected that the pigment particles are pushed into the regions with increased concentration of protein aggregates during gel formation by the action of the muller or the brush. However, the colloidal processes of protein gel formation, its influence on the rheological properties of paints and the resulting distribution of pigments, proteins and oils in a dried paint (Dietemann *et al.* 2014) cannot be discussed here in detail but will provide a topic for future study. Nevertheless, a single example of a paint reconstruction is described in order to indicate the correlations suggested.

A fresh tempera paint was prepared from lead white and egg yolk (Neugebauer 2016, pp. 509–510). As part of the experiment, SYPRO Ruby staining solution was added to the pure egg yolk before grinding to ensure that the stain would be evenly distributed in the binder. When viewed under UV radiation, increased fluorescence intensities could therefore be attributed to increased protein concentrations, not to uneven access of the stain, as may have been the case in the

Figs 6a–6c Franz von Lenbach, *Das Konzert*: details of the cross-section shown in Figs 5a–5g.



6a

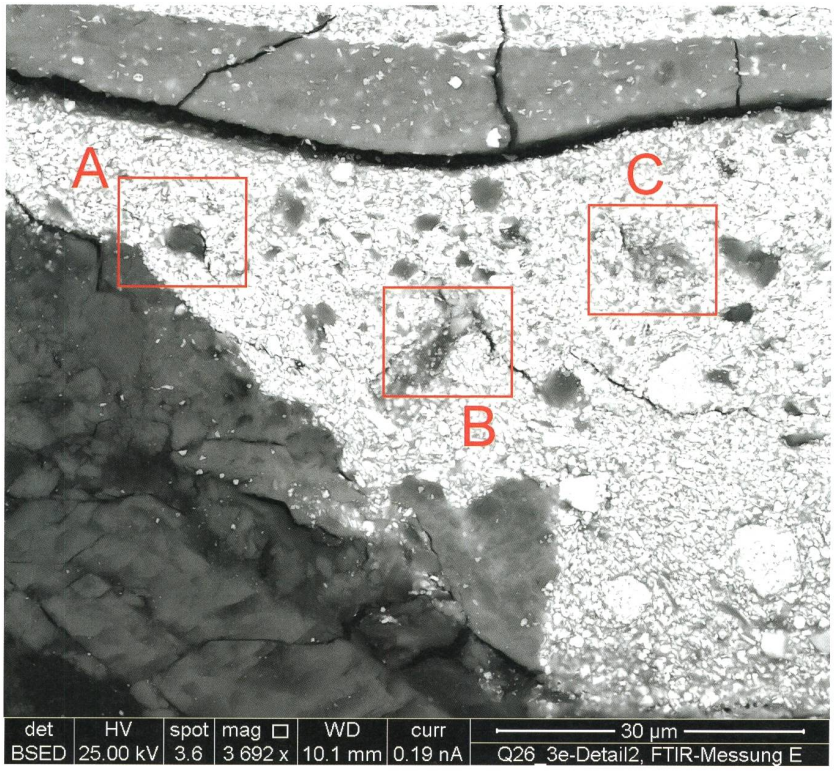


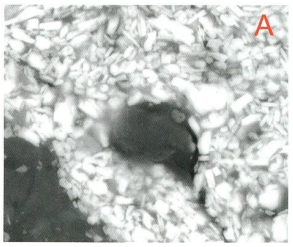
6b

Fig. 6a Detail of Fig. 5d. FTIR-FPA (false colour) image, absorption of proteins (amide I).

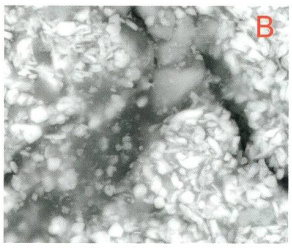
Fig. 6b As Fig. 6a superposed with the SEM image.

Fig. 6c SEM image and three details in higher magnifications (A, B, C). The areas of lower pigment density visible in these images correlate with the areas of amide I absorption (Fig. 6a).

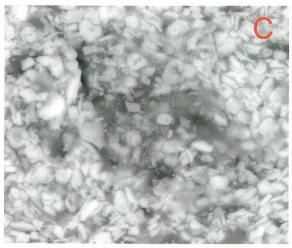




6c A



6c B



6c C

Figs 7a–7d Liquid tempera paint made of egg yolk, mixed with SYPRO Ruby, and lead white, viewed under an optical microscope. Areas of higher binder concentration display a stronger orange fluorescence under UV illumination. Observed in visible light, the same areas reveal a lower pigment density.

Fig. 7a In visible light, magnification 100×.

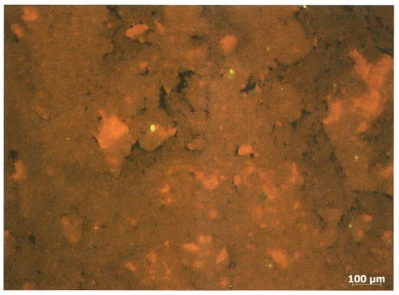


Fig. 7b As Fig 7a under UV radiation with filter set 09.

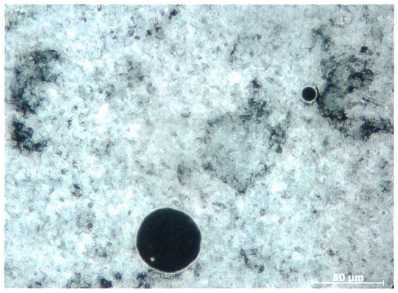


Fig. 7c In visible light, magnification 500×.

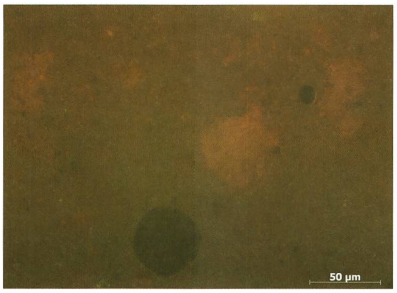


Fig. 7d As Fig 7c under UV illumination with filter set 09.

cross-sections described above (Figs 2c and 4c). Indeed examination of the liquid test mixture under the stereomicroscope revealed that the protein distribution was inhomogeneous in the liquid egg tempera paint (Figs 7b and 7d), which is perhaps unsurprising in light of the strong colloidal properties of proteins. It is apparent that areas of high protein content have lower pigment concentrations in the liquid paint (Figs 7a–7d), a pattern that can also be seen in the dried paint of *Das Konzert* by Lenbach (Figs 6a and 6c). Nevertheless it might be unwise to use this as proof that the opaque paints in the cross-section of *Das Konzert* (Figs 4a–4c, 5a–5c and 6a–6c) represent 'normal' egg tempera paints similar to the paint we prepared and examined in its liquid state (Figs 7a–7d); paint systems containing egg, oil, resins and pigments are very complex and there might be many more ways to produce such structures. Clearly more work is needed for a better and more comprehensive understanding of these types of systems.

CONCLUSION

This study is based on detailed and reliable written sources together with technological examination of the actual tempera paintings dating from around 1900 that are described in these sources (see also Neugebauer 2016; Dietemann *et al.* 2014) and as such is able to establish a comparison with and verification of binding medium analyses and vice versa. Thanks to the written sources, the outcome of the analyses was pre-assigned to a considerable extent.

Three different analytical methods were applied to the same samples. It could be shown that although these methods are quite successful, they work best when employed in a multi-analytical protocol; isolated, each delivers only incomplete and sometimes misleading information. For example, analysis of a painting by Franz von Stuck painted with Syntonos paints indicated the use of egg as an additive to the commercial

binder ingredient that had not been mentioned in the source text. Judging from FTIR-FPA imaging, egg might have been expected to be part of a weakly pigmented intermediate layer seen in a cross-section, rather than in the Syntonos paints. In contrast, staining with SYPRO Ruby would have led to the conclusion that egg is contained in the Syntonos paints but not in the weakly pigmented intermediate layer. The combined, complementary use of all of the methods was necessary to establish a comprehensive knowledge of the binders used in the different layers because each analytical method provided only partial insight when used on its own.

While gas and liquid chromatography allowed a precise and accurate identification and differentiation of the complex mixtures of binders, problems were sometimes encountered when the proteins were strongly degraded. In such cases, it was unclear whether the residual amino acids that were detected should be attributed to contamination or to the presence of a proteinaceous component in the binder. Equally, localisation of the materials within a sample is not possible with chromatographic methods. FTIR-FPA imaging provided helpful information on the distribution of the materials in many cases, but cannot convey all relevant information. The identification of proteins is difficult or impossible in many cases where other materials are present: absorptions of resins, aged and hydrolysed oils and metal soaps, as well as oxalates can all mask protein absorptions in the spectra. This is a major problem because knowledge of whether or not proteins are present is often critically important for the understanding of a paint system. Identification of polysaccharides by FTIR-FPA can also be problematic due to spectral overlap with many pigments and fillers. With regard to the characterisation of proteins, staining with SYPRO Ruby can often provide very helpful and sometimes crucial information. Nevertheless it must be stressed that the outcome of staining must be interpreted with great care and ideally as part of a larger multi-analytical protocol, as there is evidence for repeated false negative results,[18] presumably due to quenching or limited access to the proteins by the stain when applied.

From the cross-comparison of text with analytical results, we were able to determine that the written sources used in the three case studies are on the one hand quite reliable, but on the other hand somewhat incomplete and quite general in nature (Neugebauer 2016, pp. 397–400). We concluded that while they provide valuable guidelines, they should not be taken too literally. This caveat is especially relevant in the context of the information provided on the composition of paints, because samples from actual paintings can be more complex than tube paints or their written formulations. A painter may add components to tube paints for a variety of reasons: egg for example, could be added to stabilise a paint, modify its rheological properties or rate of drying, improve adhesion to underlying layers or to enhance resistance to re-solubilisation during overpainting. Nonetheless, written sources can provide highly useful, almost indispensable information for the interpretation of binding medium analyses.

It has been shown that the properties of paints correlate not only with the materials used but are sometimes influenced even more directly by the way they were combined in the preparation of paints: for example a mixture of egg and oil with water forms an aqueous paint (O/W emulsion), but without water can easily turn into an oil paint that can no longer be diluted with water (Dietemann *et al.* 2014). Accordingly the identification of aqueous and oil-based binders in the same sample is usually insufficient for an unambiguous identification of the paint system, while quantification alone of the components present does not solve the problem. Knowledge of the distribution of the components in the context of the layers of the painting and within individual layers is of vital importance. Consideration of the principles of colloid chemistry led to the theory that the distribution of the various materials in paints might give indications of the initial nature and properties of the paint system under study. This approach was able to offer insight into a number of aspects of the paints that were analysed (see also the contribution by Neugebauer, in this volume). However, at this point in time, the systems studied are far too complex to be described or understood in full. Clearly, more work is needed to achieve this goal.

To conclude, each different analytical method contributed an important and unique piece of information that was not accessible by other means. For very complex objects, such as tempera paintings created around 1900, the use of more than one analytical method is necessary in order to obtain sufficient information to form an interpretation of a binder system. Chromatography, FTIR-FPA and staining can be used as the basis of a multi-analytical approach as they offer complementary data sets that can provide a useful combination of facts on which to interpret the nature of complex paint media.

1 The apparent contradiction that a paint may be water-soluble and therefore a 'tempera', although its material composition is mainly oil, can be explained using colloid chemistry models (Dietemann *et al.* 2014). However, in practice, it is a longstanding convention that a painting is commonly assessed to be a 'tempera' or an 'oil' painting based on its visual appearance: usually aspects such as opacity of the layers, surface mattness or gloss as well as the presence or absence of wet-in-wet colour blending are used as indicators. As described in a previous study (Dietemann *et al.* 2015, pp. 288–289), the above-mentioned properties of paints correlate not only with their material composition and dilutability but also relate decisively to the handling of the individual components (binders, diluents, paint media, pigments) during paint production and painting, and the resulting observed rheological properties that are so important for painting are produced by colloidal interactions.

2 Project 'From Böcklin to Kandinsky: Technological and Analytical Research into Complex Binding Media Mixtures in Munich Tempera Painting around 1900' ('*Von Böcklin bis Kandinsky. Maltechnische und analytische Forschungen zu komplexen Bindemittelmischungen in der Münchner Temperamalerei um 1900*'), funded by the German Research Foundation (Deutsche Forschungsgemeinschaft DFG, project no. DI 1575/1-1, funding period: 1 March 2009–29 February 2012) and by the Studienstiftung des Deutschen Volkes.

3 Polysaccharides were determined following a special sample work-up described in Bonaduce *et al.* 2007.

4 Frankincense was identified by characteristic triterpenoids such as 3-acetyl boswellic acid and 11-oxo-3-acetyl boswellic acid.

5 Schick's description seems plausible not only because the described materials were found, but also because of the visual appearance of the paints: in some areas they contain little lumps of a yellowish, transparent material, presumably particles of finely ground resin. Where upper paint layers are missing on the tacking edge of the painting, it is possible to see that the presence of these lumps leads to porous paint textures and very coarse paint surfaces (illustrated in Neugebauer 2016, p. 210); this appearance is explicitly described by Schick for frankincense and sandarac containing paint (Schick 1901, p. 76).

6 In Max Doerner's terminology 'artificial emulsions' ('*künstliche Emulsionen*') are emulsions based on plant gums, animal glue and starch, and emulsions stabilised by soaps derived from oils or beeswax. They remain water-soluble even after ageing (Doerner edn 1938, pp. 182–188).

7 The paintbox belongs to the Library of Congress, Washington, DC. We are indebted to Elisabeth West FitzHugh and Lynn Brostoff (Library of Congress) for providing the samples.

8 In the SEM images of cross-sections, it was possible to identify up to three layers in the varnish. The composition of individual layers is unclear because the materials were only sampled together.

9 As far as we know, in the 20th century in Germany, egg was not used in painting conservation.

10 The metal soaps mentioned in this article are ageing products of the zinc oxide or lead white pigments reacting with the fatty acids of the oil binder.

11 The presence of copper pigments is notably problematic in the context of the quenching of fluorescence.

12 In some cases, a scratch extending through a paint layer stained positively for the presence of proteins, although the layer itself was not otherwise stained.

13 Micro-Mesh 4000 or 6000 was used in this study (see 'Methods').

14 If the oil binder was indeed smeared across the sample during polishing, the layer is clearly very thin because a continuous layer was not obvious in the FTIR-FPA images, which are based on a penetration depth of < 1 µm (see above).

15 This effect can sometimes be observed when the depth of sharpness in the microscope is moved from the surface of the stained cross-section into deeper areas and fluorescent spots come into focus.

16 The varnishes show the same craquelure as the paints. Lenbach often applied more than one varnish (see Neugebauer 2016, pp. 283, 290, 300).

17 Arnold Böcklin, *Triton und Nereide* (*Triton and Nereid*), 1873/1874, paint on canvas support, 105.3 × 194.0 cm, Bavarian State Painting Collections, Munich, Schack Collection, inv. no. 11534.

18 For example Neugebauer 2016, pp. 227, 528, 529 (cross-section QS P36/1), and pp. 245, 246, 538, 540 (cross-section QS P38/10).