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DEPARTMENT OF CONSERVATION

TRACING TEMPERA THROUGH TIME-OF-FLIGHT SECONDARY ION MASS SPECTROMETRY

Analysing samples from four icons from the Nationalmuseum collection

Nina Olivier

Degree project for Master of Science with a major in Conservation
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UNIVERSITY OF GOTHENBURG http://www.conservation.gu.se
Department of Conservation Fax +46 31 7864703
P.O. Box 130 Tel +46 31 7864700
SE-405 30 Gothenburg, Sweden

Master's Program in Conservation, 120 ects
Author: Nina Olivier
Supervisor: Kristina Frenguelli
Cover photo: Heiko Arens/Nationalmuseum

Title: Tracing Tempera through Time-of-Flight Secondary Ion Mass Spectrometry – analysing samples from four icons from the Nationalmuseum collection

ABSTRACT
This thesis will analyze samples in form of cross-sections taken from four icons of the Nationalmuseum collection, two Russian, from the 18th and 19th centuries and two Greek, also from the 18th and 19th centuries, using the Time-of-Flight Secondary Ion Mass Technique.

The objective is to detect, isolate and, if possible, identify the amino acids and the fatty acids in the protein and lipids used in the tempera, the ground, and the olifa using Time of Flight Secondary Ion Mass Spectrometry. By doing so, a view of the distribution of the different substances used in making the icons, can be presented.

Through analysis, the paint layer can be studied to see how it has been affected by later additions such as olifa (varnish) and conservation materials.

This will benefit the knowledge of these icons and their technical composition as well as it will be an aid in the conservation and restoration of icons from a technical point of view.

To provide a background for the analysis, sections describing icons and the materials technological composition thereof, tempera and the chemistry of tempera, and the materials analysis method of Time-of-Flight Secondary Ion Mass Spectrometry, have been included in the thesis. These sections have been based on literature research, articles, seminars, and a conference on tempera, held in Munich in 2018.

The ocular examination of the icons, and the collection of paint samples for the cross-sections took place in the fall of 2022. The analysis of the samples was done in the spring of 2023.

The microscopy of the cross-sections was done in the studio of the author and the analysis was made at RISE research facilities in Borås with help of their Time-of-Flight Secondary Ion Mass Spectrometry researcher.

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This research could never have been done without the help of Nationalmuseum's conservation department. I want to thank them all for allowing me the opportunity to conduct my research on icons from their collection.

Having cross-sections would not have been of much help if I hadn't been able to study and analyze them at RISE in Borås. Researcher Peter Sjövall has been an immense help here, and without him and his knowledge of the Time-of-Flight Secondary Ion Mass Spectrometry, this research would not have happened. Peter Sjövall has a vast knowledge of analyzing data regarding fatty acids and lipids, collected through the secondary ion mass spectrometry. Besides being an important part of the analysis, he has also been cheering me on from the start, sending me more articles to study and valuable feedback in regard to the thesis.

I also want to thank my teachers in iconology, iconography, Christian art, and theology at Enskilda Högskolan for their deep knowledge of icons and their ability to inspire their students to want to learn more. And I want to thank my classmates in these courses for all the knowledge exchange, exchange of valid research material and for supporting one another throughout the courses. You know who you are.

Another acknowledgement goes to my icon painting teacher, Efti Papadopoulou Georlin, for bringing me on many field trips in Greece, studying old icons. These field trips have led me to interviews with monks and nuns in Greek monasteries in Macedonia, such as the Agios Dionysios at Mt Olympus and monasteries in Meteora, actively practicing Greek iconographers and icon conservators at the Byzantine Museum of Thessaloniki. Without these icon courses and trips, my knowledge in the field of icons would be much more limited.

Lastly, but not least, I want to send a thank you to Ikonsällskapet and its founder Ulf Abel, for keeping the knowledge and interest in icons alive in Sweden. Through seminars and excursions arranged by Ikonsällskapet, my own knowledge of icons, has deepened and I learn that the more I learn about icons, the less I actually know.

Preface

As a newly graduated paintings conservator at the Gothenburg University in 2010, I felt I needed to get a deeper practical understanding of how paintings are built up. Icons, being some of the oldest surviving portable paintings, with a history of approximately 2000 years, use a technique in their materials technological composition that make them last and survive for hundreds, even thousands of years. This was very fascinating to me as a conservator. What in the icon painting technique makes them survive so long?

To understand icons better, I started attending icon painting courses. Through these, I learned to build up the icons from scratch and I started to learn more about them. The painting medium used for icons, tempera, especially fascinated me, and I began to dig deeper.

Doing so, I found that tempera was extremely complicated, and there was a general lack of knowledge regarding the material, both among curators, artists, and conservators. So, what made it so complicated?

In 2018, the Doerner Institute in Munich, arranged a tempera conference with over 270 participants from 22 countries, to try to figure out the complexity of tempera, and so I went there. The conference focus was on tempera, used as a painting medium in the art world, where the composition of it often is unknown. In icon painting, however, the techniques are according to tradition the same, and therefore often known. Disregarding unknown factors in the paint medium and the instabilities these can bring, what made the complexity of tempera lead to such a strong material? I wanted to know more.

Research on materials analysis techniques for organic components, led me to Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), and I decided to conduct a research using ToF-SIMS as a materials analysis technique. I asked Nationalmuseum to do the analysis based on their unique icon collection and they approved. To make the research more controllable, I decided to limit my research to four icons from the collection and to analyze the material in these through cross-sections.

1. Introduction

Nationalmuseum in Stockholm holds one of the largest collections of Russian icons outside of Russia and one of the largest icon collections outside the Orthodox world. The collection holds not only 285 Russian icons, but also 2 Byzantine icons, 28 icons from the Greek/Macedonian area, 1 Serbian, 4 Bulgarian, 1 Estonian and 2 Finnish icons[1].

Most of this collection, 241 icons, was given the museum as a donation in 1933 by banker and businessman Olof Aschberg who had been collecting icons throughout his years in Russia[2]. In 1952, Aschberg donated a second round of approximately 30 icons to the museum[3].

Some of the icons from the Nationalmuseum collection, came later, in the 1960's from the collection of Vilhelm Assarsson, which was sold to/taken over by Åke Wiberg before ending up at the museum. Among these are some of the oldest of the museum's Russian icons[4].

With this vast variety of icons, the Nationalmuseum collection of 323 icons, spanning from the 13th to the 20th century, is a very interesting source of material when it comes to research.

Analysis methods when it comes to inorganic materials are many and most of them work well and are reliable. When it comes to organic analysis methods, however, these have not always been as reliable and many of them have an error range that always must be brought into consideration.

Older techniques for analyzing organic materials were often extremely complicated and time-consuming, and required a lot of complicated equipment. Nowadays, technique is developing quicker, offering better and more reliable methods for the organic material analyses as well.

The Time-of-Flight Secondary Ion mass spectrometry (ToF-SIMS) is a surface analysis technique that can identify and spatially resolve the chemical composition, both organic and inorganic, of a sample[5]. Advantages of this technique, in comparison to other techniques, is that it is non-destructive, quick, it presents a visual map of the distribution of different material in a sample, and it has a very small ion beam, which makes it suitable for analyzing very small samples. It is also possible to distinguish between different organic and inorganic materials within a sample at the same time, which is timesaving when it comes to analyzing material.

1.1 Disposition

This thesis will focus on icons and on tempera, and the chemistry there within, as well as of a shorter description of ToF-SIMS, culminating in an analysis of cross-sections from four of the icons from the Nationalmuseum collection, using ToF-SIMS. The thesis is built up by

[1] Ulf Abel with Vera Moore, *Icons*, Nationalmuseum, Stockholm 2002.
[2] Ibid, pg. 9
[3] Ibid, pg. 12
[4] Ibid, pg. 15
[5] Bouvier, Caroline, Vand Nuffel, Sebastiaan, Walter, Philippe and Brunelle, Alain, *Time-of-flight secondary ion mass spectrometry imaging in cultural heritage: A focus on old paintings*, Journal of Mass Spectrometry, 2022;57

different sections providing background information of icons, of tempera and colloidal chemistry, in order to better understand the analysis of the cross-sections.

The first section will provide a background to what icons are, their history, technique, and geographical origin. The purpose of this is to provide an understanding of the uniqueness of icons and the materials technological perspective of these.

The second section will discuss tempera and the chemistry within tempera as a paint medium. This section is important in order to understand how tempera as a painting technique differs from others and how the different substances within the medium affect each other and the characteristics of the paint.

The third section will present the analysis of the samples.

1.2 Aim and objective

The research will aim to analyze material from four icons from the Nationalmuseum collection, two Russian and two Greek, which will be further described in section 3. The objective is to collect samples from the icons in order to isolate and identify amino acids and fatty acids. Determining amino acids and fatty acids will help to identify the proteins and lipids used in the materials of the icons.

The analysis will be done using the ToF-SIMS technique at Research Institutes of Sweden (RISE) in Borås.

There would be an expectation to find amino acids from the protein of the collagen of the animal glue in the ground and there should also be amino acids from the protein used in the tempera paint layer. Fatty acids would be expected to be found in the lipids of the olifa. Possibly, lipids could be found in the paint layer and in the ground. If an isolation of amino acids and fatty acids is possible, then an identification of these should also be possible. By identifying the proteins and lipids, we could get a clue as of which animals the proteins and fats derive from. This could, in turn, present us with more knowledge regarding the recipes and materials technological sources (i.e. animals) for the geographical area where the icon was written[6].

Greece and Russia are not only separated by a geographical distance, but also differ in many other ways regarding materials technological aspects of icon painting. The climate, socio-economic status in the country, trade routes, geography, and social status of icons and iconographia all have an impact on the technical perspective of icons in these regions. Keeping this in mind, it is probable that the paint recipes for Greek iconography will differ from the Russian ones.

Another interesting topic to the research is to see if there are any amino acids from protein left for detection in the samples or whether the olifa[7] or materials from conservation treatments have penetrated the proteinaceous layers, rendering detection impossible. Traditionally, many icons have been treated with wax or sturgeon glue for conservation purposes. How much of these treatments are visible? Is the proteinaceous material detectable?

[6] See section 2.1 Icons.
[7] See section 2.4.6, Olifa.

The goal of the research is to try to reach results from the analysis that can be useful for further research within the heritage science community regarding icons. Hopefully, the results of the analysis can give us some clue of the materials technological aspects of the icons. The results of the analysis could also be beneficial for the knowledge of conservation and treatment of icons and of how material in conservation affects the paint layer.

1.3 Research questions

The research questions of this thesis focus on the chemical-technical composition of the icons and are as follow:

- Using ToF-SIMS, is it possible to detect any amino acids and fatty acids in the samples? By doing so, would an identification of amino acids, and thereby protein, and of fatty acids, and thereby lipids, be possible?
- Using the ToF-SIMS Ion mapping, is it possible to see the distribution of protein and lipids in the samples?
- Will ToF-SIMS show if any later additions, such as olifa or materials from conservation treatments have affected the protein in the samples?

1.4 Method

This thesis has been conducted using mixed methods with methodological, data and investigator triangulation, a technical art history methodology mixed with conservation science, combining historical and contemporary research of icons with materials technological research, comparison, colloidal chemistry, and scientific analysis, to get results that can answer the research questions of the thesis. The strategy of using mixed methods, has been chosen to get a broader, more top-down overview of the research.

The literature research has been a combination of literature from several different fields. It has been based on both primary and secondary sources and in a combination thereof.

Regarding icons, the literature research has spanned between theology of icons, Christian art and iconology, history of icons and techniques of painting and mainly been through books. A small over balance in literature regarding icons, lean towards Russian icons. Possibly this has to do with the broad interest from the West, concerning Russian icons, spurred by an extensive publicity campaign conducted by Russian conservators and researchers in the beginning of the 20th century, as a reaction against the Russian destruction of icons[8].

The literature research on icons has mainly taken place in relation to the icon program at Enskilda Högskolan (EHS), Institute of Eastern Orthodox studies, Stockholm in 2021-2022. The courses were held online.

Seminars concerning icons have also been attended, both online and in person (in Stockholm). These have been via EHS, ikonsällskapet and British Association of Paintings Conservators-Restorers (BAPCR) and spanned over four years, from 2019-2023.

Regarding tempera, the literature research has been focused on tempera as a term, on the contents of tempera, on protein chemistry, the chemistry of lipids, and on colloidal chemistry,

[8] See section 2.2.2, Russia.

and it has been conducted through books and articles. Besides this, research on tempera has also been through online seminars and a conference (in Munich, 2018)[9].

More books and articles have been published on tempera by German researchers than by researchers of other nationalities. Many of them are connected to the Doerner institute in Munich, and they were also the initiative takers to the international conference on tempera in 2018. This over-weight might be due to a tradition of paint research, started by German painters and scientists in the 19th century[10].

The research on tempera has spanned over five years, starting with the tempera conference in Munich in 2018. After this, several seminars on tempera have been attended, both online and in person (in Munich and in Stockholm). Seminar givers have been both artists, curators, conservators, and conservation scientists.

The literature research on materials analysis methods has been through books and articles. Many of the books presenting overviews of the methods, have been a bit dated, while articles have presented newer methods. The research on the ToF-SIMS technique has been through articles published in scientific journals only.

The research on materials analysis methods was helped immensely by a course on materials analysis methods within the field of science, given in 2022-2023 at the Gothenburg University, Institute of cultural heritage.

The analysis of organic material done in this thesis, was conducted using the ToF-SIMS technique. Samples were taken from the four icons chosen for the thesis, from areas that were already damaged. Existing lacunae facilitated getting every layer, including ground (not the support). The sample collecting took place on 25 October 2022 by the author of the thesis, using a sharp scalpel. These samples were cast in methyl methacrylate and then polished in a microtome before the analysis[11]. To ease the understanding of the samples of the analysis, pictures were taken using microscopy. Pictures of each sample were taken in regular light and in UV.

The ToF-SIMS analysis was done at RISE in Borås in the spring of 2023.

A survey was conducted regarding contemporary tempera recipes used by iconographers today. This survey was posted in a closed group on social media for iconographers and artists, with 15.8 thousand members[12].

1.5 Theoretical framework

This thesis will discuss some different concepts. In order to understand these better, a definition of some of the terms of the thesis will follow:

[9] A link to the conference is listed in the List of references, under other sources.
[10] See section 1.5, Theoretical framework.
[11] See section 7, ToF-SIMS analysis of the Icon samples.
[12] See section 4, Tempera.

Icons will in this thesis be defined as the ones used within the Eastern Orthodox religion, as defined at the Seventh Ecumenical Council in 787, AD. and the concept will not include any Catholic icons or icons from any other religion[13].

The definition of an icon will be based on the scriptures of John of Damascus in the 8th century[14];

"The fourth kind of image are the figures and types set forth by Scripture of invisible and immaterial things in bodily form, for a clearer apprehension of God and the angels, through our incapacity of perceiving immaterial things unless clothed in analogical material form.."

"Bodies as having form and shape and color, may properly be represented in image. Now if nothing physical or material may be attributed to an angel, a spirit, and a devil, yet they may be depicted and circumscribed after their own nature. Being intellectual beings, they are believed to be present and to energize in places known to us intellectually."

"If Holy Scripture clothes God in figures which are apparently material, and can even be seen, they are still immaterial. They were seen by the prophets and those to whom they were revealed, not with bodily, but with intellectual eyes."

"We know that it is impossible to look upon God, or a spirit, or a demon, as they are. They are seen in a certain form, divine providence clothing in type and figure what is without substance or material being, for our instruction, and more intimate knowledge, lest we should be in too great ignorance of God, and of the spirit world… God therefore, not wishing that we should be in ignorance of spirits, clothed them in type and figure, and in images akin to our nature, material forms visible to the mind in mental vision. These we put into form and shape…"

In this thesis, the term icons will be limited to portable icons executed in tempera and will not include mosaics, wall paintings, frescoes, seccoes, encaustic or any other technique. Icons will be described further in section 2 of the thesis.

To understand the definition of *Tempera* (Lat. *temperare*, meaning to mix), tempera must first be discussed from a historical perspective.

Traditional recipes on tempera as a painting medium, can be found in Cennini's *Il libro dell'arte* from the 15th century, (and even further back than this). In his book, Cennini described tempera as egg-based paint. He had two recipes for tempera, where the first was intended as a binder for mural paintings (whole egg with fig sap, mixed with water) and the second was intended for painting on walls, on iron and easel paintings (egg yolk mixed with pigments ground in water)[15].

Vasari and Armenini described in their publications from the 16th century, tempera as whole egg mixed with sap from the fig tree. However, the terms were a bit unclear, and tempera could sometimes be used as a word for binder, and oil paints could sometimes be referred to as tempera. To paint "à la tempera" was used as a general term for any painting technique used by the Old Masters prior to the introduction of oil paint to the easel painting[16].

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[13] See section 2.2.1, Greece.
[14] St. John of Damascus, *Three treatises on the Divine Images: Apologia Against Those Who Decry Images",* (2001), pp 30-31
[15] Cennini, Cennino d'Andrea, *The Craftsman's Handbook "Il Libro dell'Arte"*, 1960, pg. 91
[16] Reinkowski-Häfner, Eva, *Tempera: narratives on a technical term in art and conservation*, 2019, pg. 22

18th century sources mention “peinture à détrempe” as paint medium with a base of glue or gum and mixed with size or egg yolk + fig tree sap[17].

The term tempera has often been wrongfully translated when translating paint recipes from one language to another. One example is in England and the US, where tempera many times has been translated as distemper, which is something else[18]. Another mix-up is the term emulsion, which in English and French, is defined as a mixture between egg and water, but in German as a mixture between egg and oil[19]. Both these definitions are right, and at the same time wrong. The definition of emulsion is not as simple[20].

In 19th century Germany, old recipes mentioned tempera as egg yolk with a possible addition of vinegar, but also as egg + fig tree sap. The paint industry in Germany at this time, started to attempt to find ways of inventing a modern, industrially produced tempera, based on oil + tempera[21].

During the beginning of the 20th century, German painting technology researchers in Munich, defined tempera, based on the history and characteristics of the paint, as a paint medium using water-miscible binders where egg or casein had to be ingredients in order to form a paint film that when dry, was resistant to water[22].

Adding oil to the egg yolk, is called a tempera grassa, or as many say: an egg-oil tempera.

Looking at the history of describing tempera, it is easy to see how confusing it has been. It has been referred to both from the material aspects of it, from the technical aspects, from a historical point of view and as a category. Besides this, tempera has also been used as a category of defining anything unclear. Curators and art historians have used tempera as a definition, when looking at a painting which they ocularly conclude not to be an oil, watercolor or acrylics. Or if it is a mixed media painting.

A lot of research has been done on easel paintings said to be tempera, to understand what it is from a material-technical and chemical point of view, and the conclusion has not always been clear. Even artists themselves have not always had the terms right for tempera. An example is research on the works of Paul Klee. In 1911, he started editing a catalogue over his lifetime work in which he noted which works he had painted, using what media, if he had retouched them later, etc. When scientists analyzed the works Klee had noted as tempera, they found that they were often not tempera at all, but instead combinations of media such as watercolor, gouache and oil[23].

Studies on paintings done by the Native Americans on the Canadian West Coast, show a very different sort of tempera. They used eggs from salmon, sometimes fresh, sometimes

[17] Ibid, pg. 23
[18] Ibid
[19] Ibid, pg. 29
[20] See section 4.3, Emulsions.
[21] Reinkowski-Häfner, 2019, pg. 22
[22] Reinkowski-Häfner, 2019, pg. 22
[23] Bäschlin, Nathalie, Zeppetella, Patrizia and Zumbühl, Stefan, *Then egg, then watercolour or tempera paints, then alcohol resin': Paul Klee's tempera painting techniques*, 2019, Archetype Publications Ltd in association with the Doerner Institut, Bayerische, pg. 145

dried, and sometimes chewed together with red cedar bark, as a paint medium[24]. But tempera using fish eggs has so far not been noted in other places.

According to the conservation scientists at the Doerner Institute in Munich, who do a lot of research on tempera, tempera can be classified according to three different categories;

- Visual appearance (it looks as a tempera and not as an oil, watercolor etc.)
- Miscibility/durability (it can be diluted with water)
- Material/chemical compounds (it mainly consists of aqueous binders such as proteins or polysaccharides)

This thesis will define tempera from a material/chemical category as an egg-based paint medium[25].

Names of icon motifs have been marked in bold throughout the thesis. Important terms are mentioned in Italics but are also picked up in a glossary at the end of the thesis.

Iconographia will in this thesis represent the act of creating an icon and not the representation of an image[26]. The act of creating an icon is done by iconographers. For simplifying reasons, the depiction on the icon will in this thesis be referred to as the motif and not as iconography. This is consciously done to prevent confusion of terms. The choice of the term iconographia will be further explained in section 2.1, Icons.

1.6 Ethics

The thesis has been conducted according to research ethics. Laws have been followed, as well as ethical guidelines for both research, sampling, and analysis.

The sampling has been done in accordance with the Ethical Sampling Guidance published by the Icon Heritage Science Group in 2019 and which was based on views from consultation across Icon's professionals and interested parties, such as the European Research Infrastructure for Heritage Science (E-RIHS).

The collection of the samples from the icons, has been done using an invasive method. To fully analyze the materials technological composition of the icons, cross-sections had to be made. Surface analyses were not going to be enough.

The ToF-SIMS analysis technique was chosen in this thesis partly since it is a non-destructive method. The samples can therefore be examined using other materials analysis techniques after the ToF-SIMS analysis has been carried out.

The analyses of organic material such as in the ground and the paint medium, often require invasive techniques. In order to conduct the ToF-SIMS analysis, one sample from each of the four icons was needed.

[24] Leechman, Douglas, *Native Paints of the Canadian West Coast*, Technical Studies, V 1937, pp 206-207, as referred to in Gettens, Rutherford J., and Stout, George L., *Painting materials – a short encyclopedia*, 1966, pg. 19.
[25] See section 4.3, Emulsions.
[26] See section 2.1, Icons.

According to the records at Nationalmuseum, no samples had previously been collected from the icons. Had there been old ones, these could have been used for the analysis, as the ToF-SIMS technique[27] does not require fresh samples. But since no older samples existed, sampling had to be carried out for this research. One sample was collected from each of the four icons, using a scalpel to make sure all the layers (ground, paint layer and olifa), were included. The sampling was done by the author of the thesis, supervised by Nationalmuseum's Director of Preservation and Photography, Kriste Sibul. The sampling was done in situ in the storage of the museum as to move the icons minimally.

As the Nationalmuseum icon collection is one of the largest outside of the Orthodox world, and cross-sections can be saved and re-used for more analyses, collecting samples for cross-sections from these four icons could be defended as a basis for further technical examination of the icons. The results from this analysis, as well as from future ones, could be of importance to a larger field of researchers, from conservators to art historians and conservation scientists. Publishing the results of it will be beneficial to many, including the museum itself.

As the icons are old and rare, an invasive sampling could only be defended if it was done following ethical guidelines of sampling. The samples would be small, preferably collected from already existing lacunae. As the analysis of this thesis focused on isolating and identifying organic substances embedded in the materials of the icons, there were no requirements for the state of the motif of the icons themselves. Sampling was therefore to be done from four icons that were visually not in a perfect state. The olifa on the icons was darkened and had been left without any deeper treatment and the icons had not been exposed to any active conservation treatment in a long time.

The ToF-SIMS technique can detect and analyze extremely small parts of the samples without damaging the surface of the sample. The samples collected for this thesis can therefore be saved as cross-sections and kept in the museum storage after the analysis has been done or used for more research. The samples already collected can be used for future research on these four icons.

The data collected through the analysis is presented as interpreted in dialogue with researcher Peter Sjövall at RISE. It has not been tampered with, nor been interpreted deceivingly. When conducting an analysis using ToF-SIMS, the ion beams can be targeted to specific areas of a sample, and the markers requested for the analysis are looked for (such as amino acids, fatty acids, metal etc.). A data base connected to the ToF-SIMS is used to identify the material the secondary ions come from, and peak graphs and visual ion images are presented to see the markers. The interpretation of the material is done based on this.

The interpretations of the data collected through the analysis is discussed at the end of the thesis. The ion images collected at RISE are attached together with the analysis of the samples. In these images, NMI 158 is mislabeled as NMI 157. Also, methylmethacrylate is mislabeled as epoxy.

A survey was conducted prior to this thesis, where the participants were asked openly, but in a private, closed social media group. They were informed of the purpose of the survey and their answers have been anonymized for this thesis[28].

[27] See section 12, Analysis methods.
[28] See section 4, Tempera.

1.7 Summary of current research

Research on icons is conducted in many countries, especially in those where the Eastern Orthodox church is the predominant one. As the interest in icon painting keeps growing, so has the interest in the national heritage of the Orthodox traditions[29]. There is a pride in the care of icons and research is put highly within the field. However, many of these countries have limited funds due to a poor economy, war, or other conflicts, which have left their marks on all fields of society. Therefore, research on icons from a materials technological aspect, is, just as other disciplines within cultural heritage, in need of funding and support.

Another difficulty is that lot of research coming from the Eastern Orthodox countries is published using Cyrillic, making access to it hard for Western researchers.

Research on tempera, is an ongoing field of interest for conservators, scientists, curators and more. Traditionally, the knowledge of the chemistry of the egg, has been more developed within the food industry, than in paint research, but the latter is starting to catch up and more is learned regarding tempera every year.

The development of new materials analysis methods keeps on striving forward. New technology and inventions, lead to new methods being developed. With the new technology, analyses of organic materials can be more precise, more accurate, cheaper and less time consuming. This will lead to new discoveries and new developments of methods both within the analyses field, but also within the field of conservation-restoration. It will also make research and analyses more accessible to a larger field of professionals.

Newer research shows very interesting results for analyzing old protein in a 15th-century painting by Sandro Botticelli's workshop, using palaeoproteomics[30], (Gr. palaio, meaning old or ancient, and proteomics, the study of proteins produced in organic systems) which is a method of mass spectrometry-based proteomics. This method only requires a small sample, but it can still identify the species that the proteins come from. It can also measure the molecular damage profile in the amino acids[31], which can be very interesting for the field of conservation.

In their study, di Gianvincenzo et al. were able to identify the species of the protein by looking at species-specific peptides[32]. They were able to identify chicken egg yolk in the paint medium through identification of vitellogenin-1, vitellogenin-2 and apolipoprotein B. They also identified peptides in collagen alpha-1 and collagen alpha-2 belonging to sheep and goat in the ground layer[33]. The identified proteins were compared to a reference database.

In their research, di Gianvincenzo et al. also examined deamidation of amino acids in order to get an overview of damage to the proteinaceous substances. They could see that the damages were higher in the samples from the ground layer, where the protein in the animal

[29] Bobreshova, Marina, *Issues and development trends of modern Icon painting,* 2019, pg. 1
[30] F. di Gianvincenzo, D. Peggie, M. Mackie, C. Granzotto, C. Higgitt and E. Cappellini *Palaeoproteomics guidelines to identify proteinaceous binders in artworks following the study of a 15th century painting by Sandro Botticelli's workshop*, in Scientific Reports, 2022
[31] Di Gianvincenzo et al., 2022, pg. 2
[32] Ibid, pg. 3
[33] Ibid, pg. 4

hide glue had been boiled. The amount of protein in the samples, however, was not extensive and might therefore offer an error range[34].

While doing their analysis, di Gianvincenzo et al. also found an error range connected to the pigments used in the samples. Madder lake pigment is prepared by extracting colorant from dyed sheep wool and in this process, peptide bond hydrolysis occurs. Therefore, the pigments also have a presence of protein from sheep[35].

Another thing to keep in mind while analyzing egg protein, is that although only egg yolk has been used in the medium, traces of egg white might still be present due to the process of separating the yolk from the white[36].

1.8 Limitations

The icons of the Nationalmuseum collection are unique, old and they belong to an important collection. As objects from Nationalmuseum, they are not to be moved from the museum facilities and they are to be handled minimally to avoid damaging them and triggering reactions in the materials of the icons. A decision was therefore taken to collect samples from the four icons chosen and to bring the samples to the labs for analysis.

Materials analysis methods are costly and time consuming. No research facility exists today in Sweden, where all the different analyses can be carried out at the same time. Different equipment is scattered in different parts of the country if they even exist within the national borders. There is a huge need for facilities with much different equipment, including the ToF-SIMS for analyses of cultural heritage.

Kulturarvslaboratoriet is operated by the National Heritage Board and is open for projects within the field of cultural heritage. This laboratory has some equipment and tools for materials analysis, but the equipment could be considered somewhat limited. To access these, one must apply to a guest colleague program, and few researchers are accepted. Research conducted here must be of interest to the public and objects belonging to private collections are not granted access. The author of the thesis applied to the program prior to this thesis but was declined. An important note is that Kulturarvslaboratoriet does not have access to ToF-SIMS, which was one of the aspects relevant for this thesis. Only four ToF-SIMS instruments exist in Sweden today; the one at RISE, one belonging to Astra-Zeneca, and two belonging to Chalmers in Gothenburg. Running an analysis using ToF-SIMS is extremely costly. As researchers within the field of cultural heritage, funds are often limited and so many analyses might be limited due to which methods are economically defendable, instead of being chosen after which methods are the best.

Another problem is that researchers working with analysis instruments, often specialize in the ones they have access to. This also limits the range of analyses known to the cultural heritage field, and it might limit analyses to which instrument researchers can operate. Also, the data base connected to the instrument does not always cover every material, and therefore cannot always be used to the fullest.

[34] Ibid
[35] Ibid, pg. 5
[36] Ibid, pg. 6

The best analyses are always the combined, multidisciplinary ones, but due to problems described above, this thesis could only focus on one analysis method. If any combinational analysis would have been conducted, the ToF-SIMS would probably have been carried out first, and then a Fourier Transmitted Infrared (FTIR)[37] and/or an immunofluorescence analysis[38] would have been done to cross-reference the data collected. This would have given the analysis of the thesis a fuller and more complete result. However, since the samples can be used again, additional analyses can still be carried out on them, just not within the limits of this thesis.

When this thesis is written, contact has been made with the Department of Biochemistry and Microbiology at the University of Chemistry and Technology in Prague, Czech Republic regarding a nanoLC-ESI-Q-ToF mass spectrometry with a more detailed and precise analysis of the samples. Through this method, specific binders such as animal glue or egg, can be identified, as well as in which layer they exist. Furthermore, the method can distinguish between egg yolk, egg white, whole egg, casein, animal glue/gelatin, blood, and more.

More methods for analyses of organic material exist than the one described in this thesis.

This thesis does not, other than very briefly, bring up the conservation perspective of icons. The focus is on chemistry and materials technological aspects regarding icons.

1.9 Sustainability perspective

Icons are made to last. They are eternal, just as the theology they represent. Iconographers, according to tradition, transfer their theophany into the icon and with it the energeia that gives the icon life[39]. Icons are made using naturally occurring materials, gathered from the surroundings. The tradition of making icons go back 2,000 years and the technique and recipes haven't changed much[40]. The material used in the icons are still the same as then. In a time where every aspect of life must be considered for climate reasons, icons are still a work of the hand. They are not industrially produced, but contemplatively created by man. The paint medium, tempera, thanks to its complex nature, forms a lasting and durable film, or to quote Daniel V. Thompson: *A well-made painting with egg is about as nearly permanent as any kind of painting that mankind has yet invented… Paintings in egg tempera have generally changed less in five hundred years than oil paintings do in thirty*[41].

Icons are used, and then reused. An icon which has been worn down by kissing and veneration, is taken care of. The popular document on Greek icon painting, the Hermeneia, from c. 1730, written by hieromonk and iconographer Dionysius of Fourna, describes a part

[37] The Fourier transform infrared method can identify classes of both organic and inorganic material. The method uses an infrared source which interacts with bonds in the material, which in turn absorb IR radiation. This causes molecules to shift position, rotate, or atoms in the molecules to change position. It then uses a mathematical operation to convert the data into a spectrum.

[38] Immunofluorescence microscopy uses antibodies labelled with fluorescence and can be used for identification of protein from animal glues or egg, casein, and gum Arabicum. It exists in a direct form where the antibodies react directly with the protein, and an indirect form where a primary antibody binds to the protein and a second antibody reacts with the primary antibody (Ramirez-Barat, Blanca and de la Viña, Sonsoles, (2001), pp 282-288, pg. 282-283).

[39] Tsakiridou, C.A, *Icons in time, persons in eternity, Orthodox Theology and the Aesthetics of the Christian Image*, Chapter 13, *The Theophanic icon*

[40] See section 2.4, Technique.

[41] Thompson, Daniel V., *The Materials and Techniques of Medieval Painting*, 1956, pg. 63

of “washing”, repairing icons, and manipulating the material to protect them from fire. This indicates that post-Byzantine iconographers have had a long tradition of caring for and preserving icons[42].

One version of the much-copied Hermeneia, the version by Father Ioannis Balpis of Cydonia, has a recipe for preparing the support of the icon so that it obtains pesticidal properties. The recipe suggests that the fabric on the panel[43] should be treated with glue containing psaki (which could be interpreted as poisonous aconitum genus plants or black ivy bush, or as a generic term for poison and/or a bitter substance) or ashes (which increases the pH and thereby creates a hostile environment for pests) to avoid growth of worms[44]. This indicates a wish to already in the preparation of the icon, create a durable and lasting material, even though the traditional materials technological preparation of icons is already durable and sustainable in itself.

The same Balpis version, emphasizes the need for using only one glue solution throughout the entire process of preparing the support for the icon, since using various sizes of several concentrations can lead to layers with different mechanical properties, which may in turn lead to cracking and detachment[45].

The various instructions in post-Byzantine Hermeneia-influenced manuals on “how to repair an old and decayed icon” don’t really have equivalents in western painting manuals[46]. This too strengthens the perspective and the uniqueness of icons as a tradition of sustainability and eternal life.

Another example of caring for the icon, is that the application of varnish on an icon would traditionally take place on the first Sunday of Lent, every year[47]. Conservators of today know very well what happens with paintings where the old layer of varnish hasn’t been removed before applying a new one, but there is something sweet and tender in the notion of applying new varnish every year to protect the icon and to keep it lasting longer.

Icons cannot be treated as other works of art. In Russia, treatment has been based on archeological principles, rather than those of art, where the icon is treated as an object of cultural heritage[48]. When it comes to conservation-restoration of icons, treatments are usually based on three approaches[49];

- A material-based one (chemical and aesthetical), where the materials of the icon need stabilizing due to damage.
- A value-based one (psychological), where the intangible values of the icon are considered (e.g. cultural, religious, personal).
- A people-based one (personal), where the focus is on preservation of the icon based on the relationship, or involvement by stakeholders.

[42] Beltsios, Konstantinos G. and Mastrotheodoros, Georgios P., *Sound Practice and Practical Conservation Recipes as Described in Greek Post-Byzantine Painters’ Manuals*, 2019, pg. 43
[43] See section 2.4.1. Support
[44] Beltsios, and Mastrotheodoros, 2019, pg. 45
[45] Ibid, pg. 46
[46] Ibid, pg. 49
[47] Gatrall, Jefferson J. and Greenfield, Douglas, *Alter Icons: The Russian Icon and Modernity*, 2010, pg. 8
[48] Bobrov, Yuri, *Interpretation as an Inevitable Risk in Conservation of Icons,* 1996, pg. 335
[49] Jakobi, Davina Kuh*, The implications of conserving religious icons,* 2014, University College London

Often, these three interact and work together in the decision making of a treatment of the icons.

Conservation treatment of icons has a tradition of being carried out with materials such as wax or animal glue. As wax is a non-reversive treatment and discolors over time, this material has long been discarded for icon restoration in the west. Sturgeon glue, commonly used for the conservation treatment of icons, has no real equivalent within the categories of glues, but in a sustainable perspective, and for the future of life on earth, using isinglass really is not defendable and other, more sustainable sources with equivalent properties of this would be very welcome in the field of conservation-restoration.

2 Icons

Icons are not to be treated as paintings or as works of art. They are liturgical objects, filling the same function as a chalice might do in a service. Many admire icons because of their special aesthetics and form, but icons are not just images. Instead, icons are created for the enhancement of the relationship with God. They differ from other art in being representations of something holy but are not holy in themselves. Icons are venerated, but never worshipped. Orthodox theologians sometimes refer to icons as "theology in colors"[50] and the icon can be said to be materialized faith[51].

This is why the format and language of icons has been preserved with such a conservatism. The icons are included in the conservatism of the liturgy.

2.1 Icons

Icons (Gr. *eikon,* meaning image or likeness), are religious images used in liturgical contexts. Icons are meant to be "worldly interpretations of heavenly archetypes" and typically depict members of the Holy family, saints, scenes from the Bible or from the lives of holy figures. Motifs on icons are taken from the Bible and from church tradition, such as the Synaxarion. They are according to tradition copied and most iconographers use models for their motifs rather than inventing new ones (with some exceptions, when newly canonized saints or holy events need depicting).

According to church legend, sacred images have existed ever since the time of Christ[52]. The very first icon is said to be the **mandylion** (an *acheiropoieton*, not by human hand painted)[53]. Legend has it that King Abgar of Edessa was suffering from leprosy and asked his servant to ask Christ to come and cure him. If Christ couldn't come, he wanted an image of Christ instead. Abgar's servant met Christ, who washed his face and wiped it dry on a piece of cloth. The image of Christ's face was then imprinted on the cloth. The servant brought the cloth back to his king, who was instantly cured of illness[54]. This original mandylion, was eventually put in a niche and covered, in order to protect it from those who wanted to destroy it. When it eventually was rediscovered in the 540's, A.D, it had made a second imprint on the ceramic tile behind it in the niche (an image now known as the **keramion**)[55]. These images were, according to legend, for a very long time kept in Edessa, until they were sold and moved. Both the mandylion and the keramion eventually ended up in Constantinople[56], the capital of the Byzantine empire. After the crusaders' plundering of the city in 1204, the icons disappeared and have been lost to the public ever since[57]. **The veil of Veronica** (vera eikon – true image) was another acheiropoieton, from when Veronica wiped the sweat off the face of Christ on Golgotha[58]. However, the story of this event is not mentioned in any of the gospels.

[50] Džalto, Davor, *Icons: The Orthodox Understanding of Images and the Influence on Western Art,* 2019, pg. 2
[51] Ibid, pg. 3
[52] Ouspensky, Leonid, *Ikonens teologi,* (2000, translated by Göran Ståhl), pg. 49
[53] Ibid, pg. 62
[54] Ibid, pg. 63
[55] Ibid, pg. 64
[56] Ibid, pg. 65
[57] Ibid, pg. 65
[58] Ibid, pg. 66

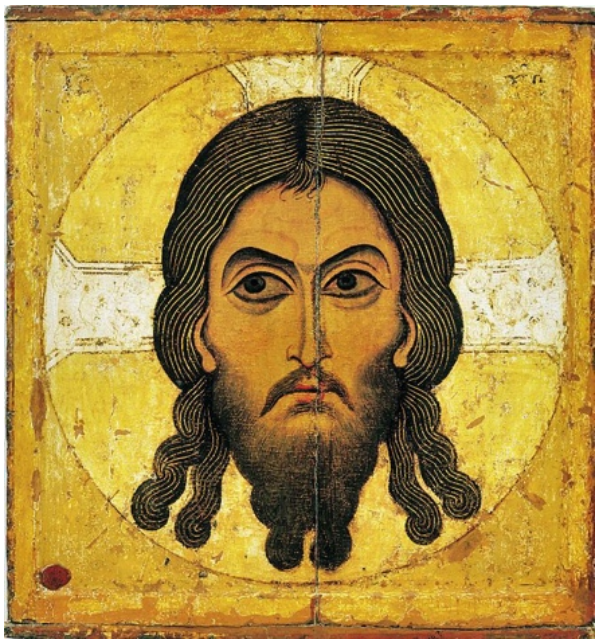
Figure 1. Mandylion, unknown author, Wikimedia commons

The first images of Mary were according to tradition written by the apostle Luke[59]. These are the **Hodegetria**, where Mary is depicted with baby Christ, both facing the viewer, while Mary is pointing her hand towards Christ, showing us the way to salvation, the **Eleousa** (the caressing mother of God) where Mary and baby Christ are cheek to cheek, showing the love and tenderness between mother and child. **The Deiseis** is another icon, where Mary is depicted by the side of Christ, her hands again pointing towards him, aiming her prayers to him[60]. No icons written by Luke, however, exist today[61], but there are Hodegetria icons dated to the 6th century[62].


Figure 2. Hodegetria, unknown author, Byzantine empire 15th century, Wikimedia commons

[59] Ibid, pg. 76
[60] Ibid, pg. 76
[61] Ibid, pg. 76
[62] Ibid, pg. 76


Figure 3. Eleousa, author Nina Olivier, 2017

Profane images were common in earlier times and Egyptian mummy portraits are often said to be the prototypes of the portable icons. As Christianity was legalized by the emperor Constantine in 313 AD., the religion expanded, the sacred art flourished [63] and an urge of portable Christian images rose. People were not satisfied only with seeing the sacred images in church, but also wanted them for their homes, for their loved ones and to keep during journeys and so the tradition of icons grew. Today, icons are kept in the Orthodox churches and homes. They are venerated and prayed to, they are kept for protection, for support and for guidance.

Time and space in icons are sacred, which means that they are existential and liturgical[64]. Because of this, events that did not occur at the same time historically, can be depicted in the same icon. Holy persons who did not live during the same historical time, can stand side by side in an icon. The sacred space allows the Orthodox believer to exist together with Christ at all times[65]. This also enhances the notion that the space around an icon is sacred.

Every detail in an icon is relevant. Iconology and theology of the icons go deep and in many levels. Objects depicted, numbers, colors, geometrical shapes, letters, garments, accessory, and positioning of bodies are all important and intentional in an icon. They are all filled with symbolism in many levels, including theological ones.

In summary, an icon is more than merely an image. It is an archetype, a guidance, and a prayer in one[66]. Because of this, icons are not painted, they are written. *Iconographia* means to write images. Iconographers are sometimes also referred to as authors. Icons are not meant

[63] Ibid, pg. 102
[64] Evdokimov, Paul, *The art of the icon: A theology of Beauty, section II*, chapter II, n. pag.
[65] Ibid, section II, chapter IV, n.pag.
[66] Ouspensky, *Ikonens teologi,* pg. 220

to be looked at, instead, they are read. The icon is meant to be liturgy in portable form, teaching the reader of the icon something new every time they study it. The icon can be read and interpreted with new lessons every time and it is deeply connected with its religious purpose.

2.2 Geographical origin

Eastern Orthodox icons are geographically spread over some regions. They derive from the former Byzantine empire; Greece, Northern Macedonia, Bulgaria, Georgia, and Serbia, as well as from the former Russian empire; Ukraine, Russia, Belarus, Czechia, Romania, Estonia, Latvia, Lithuania, but also from Finland, Egypt, and Ethiopia. This thesis will analyze samples from Greek and Russian icons, and therefore, a deeper description of these geographical regions, as well as their history of iconographia, will be presented briefly in the following sections.

2.2.1 Greece

The earliest icons were written in the Byzantine empire, mainly in the encaustic technique[67], inherited from the Egyptian mummy portraits and the Hellenic culture, but also in tempera.

Due to the early icons' connections to Hellenism, they were questioned by the iconoclasts, who drew parallels between icon veneration and idol worshipping[68]. In 726 A.D., the Byzantine emperor, Leo III, decided to ban icons. This started the iconoclast movement, and with it a dark era for icons, where many of them were purposely destroyed and iconodules (those venerating icons) were persecuted.

The Seventh Ecumenical Council in 787 A.D., settled once and for all that icons were to be allowed in the Eastern Orthodox church. Due to disturbance and disagreement, however, this was not fully imposed until 843 A.D. (still celebrated within the Orthodox church as the Triumph of Orthodoxy).

The oldest surviving icons today are from the 6th century and kept in Rome and St Catherine's monastery on Mount Sinai, Egypt[69].

After the end of iconoclasm in 843, tempera has been the main medium in iconographia[70]. Up until the 6th century, iconographia bore traces of the old, antique, traditions of painting, but later, the Byzantine style of painting evolved[71].

In 1453, the Byzantine empire fell to the Ottoman Turks, and the Byzantine era came to an end. Constantinople was plundered and destroyed, and with it much of the Christian art. This, however, did not mean the end of the Orthodox church, nor of the icons, but the main center of iconographia was instead moved, to Crete[72]. Crete was at this time part of the Venetian

[67] Painting/modelling with hot wax, often beeswax or Punic wax. The wax was heated up and mixed with pigments and then applied very quickly before the wax hardened. The support was a panel.
[68] Ambrosios Giakalis, "Images of the divine, the Theology of Icons at the Seventh Ecumenical Council", (2005), pg.13
[69] Evseyeva et al, *A history of Icon Painting*, 2005, translated by Kate Cook, pg. 31
[70] Ibid, pg. 31
[71] Ibid, pg. 43
[72] Ibid, pg. 97

Republic and iconographers were protected here. The patrons of the vast icon production were Orthodox, the main ones being St Catherine monastery on Mt Sinai, St John the Theologian monastery on Patmos, and the monasteries on Mt Athos.

On Crete, the iconographers were largely influenced by the Italian renaissance painting, and exchange of inspiration, ideas and even artists flourished. The most famous of Cretan iconographers who went into other art, is probably Doménikos Theotokópoulos, El Greco.

With Crete, came many new iconographies, motifs, and types of icons. From documents surviving from this time, it is clear that Crete held a large icon production and that clients were various, from the wealthy citizen to the common folk and not only Orthodox. On Crete, several important motifs of icons, were developed, such as **passion** icons, the Great Humiliation (dead Christ/ **Man of Sorrows**) and **the Pietà**.

Figure 4. Man of Sorrows, author Nikolaos Tzafouris, c. 1487-1500, Wikimedia commons

At the end of the 17th century, Crete was invaded and taken over by the Ottoman empire, and the iconographers were invited to monasteries, especially the ones around Mt. Athos and Meteora. With the moving of icon production to the monasteries, eventually monks became the new iconographers.

During the 17th, 18th, and 19th centuries, Greek iconographia was much inspired by the Western Baroque.

2.2.2 Russia

In 988, Prince Vladimir of Kiev, was baptized and with this action, Russia adopted Christianity, and the Russian Orthodox church was established.

The earliest surviving Russian icons, come from the Cathedral of St Sophia in Novgorod, and are from the 11th century[73].

The Russian churches were often spacious and required large icons in order to fill out the walls. This led to the icons often being more generalized, with more pronounced silhouettes and contours[74], in comparison to Greek icons. Russian icons often bore themes of overcoming suffering, with a promise of hope and salvation. The saints in these icons, are often more expressive than the Byzantine ones, which strove to be more intellectual and Hellenic[75].

As the Russian empire expanded, more churches were built, and more icons were needed. During the 11th and 12th centuries, Greek iconographers frequented Russia, to teach and paint, and the Byzantine icons were copied by the Russian iconographers[76].

In 1223 – c. 1480, Russia was invaded by Tartar-Mongols, and the southern regions, including Kiev, were left devastated. The northern regions, such as Novgorod, Rostod, Tver, Pskov, Yaroslavl, Suzdal and Moscow, kept their icon production going. Despite wars and attacks on Russia, the Russian Orthodox church stood strong and kept Russia united. Motifs depicting the Father as the **Ancient of Days** were developed here.

When the Byzantine empire fell in 1453, the Russian Orthodox church now became the most powerful Orthodox church in any of the Orthodox countries. Russian iconographers continued to develop the iconographia, without the inspiration from the Byzantine, rendering it even more conservative and nationalistic. The local schools of iconographia, in Novgorod, Tver, Moscow, became important[77].

During the 15th century, the most famous of the Russian iconographers, Andrei Rublev, worked in Vladimir and Moscow and his work still inspires iconographers today.

Figure 5. Troitsa (Trinity), author Andrei Rublev, c.1410, Wikimedia commons

[73] Evseyeva et al, 2005, pg. 121
[74] Ibid
[75] Ibid
[76] Ibid, pg. 123
[77] Ibid, pp. 149-150, 155

Russian 16th century was marked by the rule of Ivan IV, "the terrible", who established the Czardom of Russia in 1547. During this period, many wars weakened the nation[78].

During the 17th and 18th century, European elements started to penetrate the Russian conservative life. Polish gifts via former prisoners-of-war, embassies, and other types of exchange, brought German, Dutch, French and Italian works of art[79]. Some smaller icon collections were starting to be built up. The royal armory gathered iconographers from Ukraine, Belarus, Armenia, the Netherlands, Germany and Jewish painters to Russia, and a new artistic concept of icons was brought about[80]. The academic art and life of European universities, and Latin, was held in high regards, and concentrated around the cities, especially Moscow and St Petersburg, and old, medieval iconostases were replaced with new, Baroque, and neoclassic ones[81]. Old icons were removed, rewritten, or replaced. Traditional iconographia was only withheld in villages far from the cities. One example of a new motif from this period is **Divine Sophia**.

18th century new iconographia in Russia, looked very little like traditional iconographia and more like Christian art in general. Instead of depicting heavenly events, they more and more depicted earthly ones. Churches were renovated, which affected early works[82]. During the late 18th century, icons started to reappear a little in traditional forms, but with elements of naturalism[83].

In the 19th century, Russian icon production was fully controlled by the Holy Synod (the supreme canonical authority) to make sure the Orthodox church beliefs were followed to last detail[84]. Early 20th century icons, bore influences of Art Noveau[85].

During the second half of the 19th century, icons were rediscovered in Russia. Old, "black boards" (*chernye doski*) were treated by *ikonniki* – iconographers-conservators and icon masterpieces once again saw the light of day. This was also what happened with Rublev's **Troitsa** (trinity icon), probably the most famous icon of all times, which appeared now. Important books on icons were written, including Rovinsky's "Review of Icon Painting in Russia to the End of the Seventeenth Century" (1856), Buslaey's "General remarks on Russian icon painting" (Obshcie poniatiia o russkoi ikonopsi) (1866) and Kondakov's "The Contemporary State of Russian Folk Icon Painting" (1902).

Many of the old and forgotten 15th and 16th century icons were exhibited in 1913 in "Old Russian Art" in Moscow[86]. The exhibition not only woke the Russian interest in icons again, but also spurred Russian artists into Russian avant-garde art, inspired largely by icons[87].

In 1917, the Russian revolution was a fact, and the new Soviet government began to systematically seize church lands. Icon scholars, such as Alexander Anisimov, then scientific director of the "Commission for the Preservation and Uncovering of Monuments of Ancient

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[78] Ibid, pg. 160
[79] Ibid, pg. 211
[80] Ibid
[81] Ibid
[82] Ibid, pg. 217
[83] Ibid
[84] Ibid, pg. 222
[85] Ibid, pg. 229
[86] Gatrall, Jefferson J. and Greenfield, Douglas, *Alter Icons: The Russian Icon and Modernity*, 2010, pg. 4
[87] Ibid, pg. 5

painting", started to move icons from churches to museums in an attempt of saving them from destruction[88]. Anisimov was important from a modern conservation point of view, as he introduced science to the restoration, or 'uncovering', in his own terms, of icons[89].

In light of the newly found interest in icons, Soviet government wanted to show off the newly re-discovered pieces to the world and brought many important pieces on exhibitions abroad. The uncovering and restoration of the icons was highlighted. In 1929-32, the icons toured in Europe and the U.S. Not all icons returned to the Soviet Union, as quite a few of the icons were sold off in the exhibitions[90].

In the 1980's, Russia experienced a religious revival, and old churches and monasteries were restored, and icons once again started to flourish[91]. Old icons were bought back to Russia from other countries and many new ones were produced.

Today, iconographia is alive in a large-scale in Russia with many workshops and schools, including the St Tikhon Orthodox Theological Institute, which has a Faculty of Church art, where iconographia is taught[92]. The tempo of the modern world and the iconographers' dependency on the market and its requirements, might lead to an eviscerated iconographia, where the original intention and meaning of the icon is lost[93].

2.3 Iconostasis

The iconostasis (Gr. *eikonostási* = icon stand) is a screen that separates the sanctuary from the nave of the Orthodox church. This is where most of the icons in an Orthodox church are placed. Behind it, to the sanctuary, only men are allowed.

The iconostasis can also refer to a smaller, portable, icon stand.

Not all icons in an Orthodox church will be in the iconostasis. Some will be placed alone for veneration; kissing, praying, lighting candles, placing flowers etc. In private homes of the Russian Orthodox church, an icon will often be placed in the corner of the east wall.

Russian iconostases were often large, as the churches were large. They had more icons in them, compared to iconostases of other Orthodox churches[94].

[88] Ibid, pg. 7
[89] Ibid, pp 93-100
[90] Ibid
[91] Evseyeva et al, 2005, pg. 238
[92] Ibid, pg. 242
[93] Bobreshova, 2019, pg. 5
[94] Evseyeva et al, 2005, pg. 121


Figure 6. The Iconostasis of St Sava in Belgrade. Photography: Nina Olivier, 2022

2.4 Technique

Since icons have been around for so long and follow an ancient tradition of materials technology, the system itself hasn't really changed a lot. This gives us a unique insight in the technique of one of the oldest ways of building and constructing portable paintings. Although certain materials of the icon's support, ground, paint medium and pigments have varied and changed over the millennia, the way of making the support and eventually writing the icon, has stayed the same. *Hermenia* from the 18th century, written by the Athonite monk Dionysos of Fourna, describes the steps of painting faces in icons in nine steps[95]. These steps are the same ones that iconographers still follow today.

2.4.1 Support

The support for icons is generally a wooden panel, but it can also be canvas (although this is not so common).

The wood chosen for the panel was traditionally a local one, such as poplar, chestnut, oak, or lime wood[96] (more common in the former Byzantine part of the orthodox world) or linden, juniper, alder, spruce, pine, beech, fir and walnut (more common in the former Russian part of the orthodox world)[97]. Cennini suggested in his The Craftman's Handbook that the panel would be made of whitewood, poplar, linden, or willow.

For the panel not to warp, the wood should be made of dense[98], slowly grown tree and then be left to air dry over a long period of time (preferably a year or two at least)[99][100]. It should

[95] Bobreshova, 2019, pg. 33
[96] Pyatnitsky, Yuri, *The Hermitage Museum of St Petersburg, the Greek Treasures*, pg. 315
[97] The NM collection holds 29 icons on poplar, 181 icons on linden, 12 icons on juniper, 26 icons on spruce, 17 icons on alder, 32 icons on pine, two on beech, four on fir, four on walnut, one on both poplar and linden and one on both linden and spruce. Three icons are on unidentified wood, and seven on other material than wood.
[98] Jörgen Wadum mentions in his article "Historical Overview of Panel-making Techniques in the Northern Countries" from the Getty symposium on the Structural Conservation of Panel Paintings, 1995, pg. 151, studies that show that the year rings in oak wood were narrower before 1630-40, but wider after these years.
[99] Thompson Jr., Daniel V. *The Practice of Tempera painting, materials, and methods,* 1962, pg. 9-10.
[100] According to Wadum, 1995, wood was seasoned for 2-5 years during the 16th and 17th centuries, and for 8-10 years during the 15th century.

have been quarter-sawn (radially cut) to best avoid warping[101]. The best panel is made from a single piece of wood, but sometimes more than one piece of wood needs to be glued or joint together. For glue, Cennini had a glue recipe using cheese and lime, which turned into a cementlike mass[102]. Wooden cleats or splines have been used to join the pieces of wood, lip joins, groove-and tongue joins and wedge-shaped joins have been cut, but also other types of joins, such as butt-joins have been used[103].

2.4.2 Ground

The wooden support is pre-sized with glue and a fabric is glued to the entire surface of the panel or as strips, only covering the joints. The fabric functions both as a foundation for the ground, and to prevent the wood from deforming and the boards to part. Studies show that parchment and vegetable fibers hold the wood poorer than cloth[104]. The ground (*levkas*) consists of animal glue mixed with chalk (northern Europe) or gypsum (southern Europe). The ground is applied in layers. The number of layers vary some according to the different schools of iconographia. A canvas that is grounded on both sides is called a *tabletka*.

Once all the ground layers have been added, they are polished to the smoothness of a mirror to become a perfect ground for the gold and paint layers.

However, studies on icons from the St Catherine's monastery in Egypt and on some Roman icons from the 6th to 9th century, done in München[105], showed that not all the icons from St Catherine had a ground layer. Of the five Roman icons examined, two did not have ground layers. Here, one had a gypsum layer and one a reddish-brown layer, with an unclear function. Only one of the Roman icons was painted in tempera, the rest in wax. On the icons with grounds, the white grounds seemed to be underneath the gilding mainly.

2.4.3 Sketching

Now, the motif is sketched onto the board, freehanded or using *spolvero* technique (or nowadays a form of home-made carbon paper where pigment is brushed onto a paper which is then put pigment down towards the ground. When the sketch is traced on top of it, the motif is transferred to the panel).

The sketch is filled in using paint medium, generally tempera or tempera grassa, with a pigment, generally black, brown or red.

2.4.4 Background

The most common background in icons is the golden one. The gold represents the uncreated light of heaven and is meant to be a welcoming reminder of what awaits the reader in eternity.

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[101] Hoadley, R. Bruce, *Chemical and Physical Properties of Wood,* from the Getty symposium on the Structural Conservation of Panel Paintings, 1995, pg. 19.
[102] Thompson, 1962, pg. 10-11.
[103] Uzielli, Luca, *Historical Overview of Panel-Making Techniques in Central Italy,* from the Getty symposium on the Structural Conservation of Panel Paintings, 1995, pg. 119-120, and Wadum 1995, pg. 155
[104] Ibid, pg. 113.
[105] *Inkarnat und Signifikanz, Das menshliche Abbild in der Tafelmalerei von 200 bis 1250 im Mittelmeerraum,* 2017

If the icon will have a golden background, the gilding is done before the painting, either in oil or in water, or in a mix thereof, depending on which effect is sought after. Icons will often have a polished gold, so that the joints of the gold leaves are invisible. Sometimes, a bole is added to the ground before the gilding.

The background can be painted, usually yellow, but it can also be pale green, pale blue, light brown, umber, red, white or any other color[106]. Sometimes, the background is left without either gold or paint and instead a metal encasing, a *riza*/*oklad* is added.

2.4.5 Paint layers

Paint is applied in multiple layers. When the paint layers are applied, the first to be added is always the bottom hue of the carnation, *sankir*. Next are the bottom hues of the background, the clothes, the objects etc. After this, layers of paint are applied with lighter shades for every layer, so that the clothes and objects with their textures and creases are slowly sculpted. Details are added lastly.

When the entire icon is written, the face is sculpted in the same way as the background, clothes etc. were. Highlights are the last thing to be added.

The sides and the back of the icon are also covered in paint.

2.4.6 Olifa

Optimally, the icon will now rest for a half year until the lacquer/varnish, the *olifa*, is added. The olifa (Gr. *olifa* = boiled oil) is an oil, traditionally a linseed oil, or an olive oil (however, an olive oil does not dry and instead turns rancid, so it does not make the best olifa), sometimes mixed with dammar, and sometimes with additives such as lead white, manganous copper[107] or cobalt acetate, which work as a siccative. The olifa is worked into the painting, often by strokes of the palm, until it is evenly distributed. In archeological findings from the Novgorod and Kiev workshops, researchers have found traces of olifa made of boiling olive oil (imported from the south), with an addition of amber[108]. Later, the Russian olifa has been done with linseed oil, where the one from Northern Russia was considered the best[109]. Olifa applied too thickly tend to wrinkle as the oil in the lower layers dry more slowly than the one on the surface. Since the olifa often is applied by palm strokes, the oil tends to collect at the edges, where it wrinkles[110].

Old recipes recommended allowing the oil to be in the sun before using it as an olifa. By doing so, the oil would polymerize and oxidize, thickening the oil. Other recipes recommend boiling the oil before using it[111].

Sometimes, icons are varnished using egg white (glair), other times with shellac, dammar, or mastic[112]. Some old recipes on icon painting, emphasize the importance of adjusting the

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[106] Ibid
[107] Bentchev, Ivan, *The Varnished icon – A Historical Survey of Icon Varnishes*, 1999, in Jolkkonen et al., *Icon Conservation in Europe*, 1999, pg. 67
[108] Bobreshova, 2019, pg. 39
[109] Bentchev, 1999, pg. 65
[110] Ibid, pg. 67
[111] Ibid, pg. 65
[112] Weissmann, Gilles, *Techniques of Traditional Icon Painting,* 2021, pg. 122

olifa according to the pigments used in the paint layers. Instead of using *trimentina* (resin from coniferous trees – colophony/rosin), they suggest using *spigo* (distilled spike lavender oil). This would be of importance for example with the occurrence of verdigris-type green pigments in the paint layer, which could be solved in coniferous resins (as in trimentina) in combination with solvent mixtures (a property known from the copper resinate production)[113].

The predominance of instructions in old post-Byzantine manuals on how to "wash" icons, suggest that many of the old icons had siccative oil-containing varnishes which had darkened and blackened[114], something also noted with the Russian chernye doski, black boards.

2.4.7 Riza/Oklad

Sometimes, but not always, icons have a metal encasing, called a *Riza* (Ru. = vestment/robe) or *Oklad* (Ru. = covered). The purpose of this is to protect the icon from kisses, candles etc. The metal cover has openings for the faces and hands of the figures depicted, but the rest of the motif is covered.

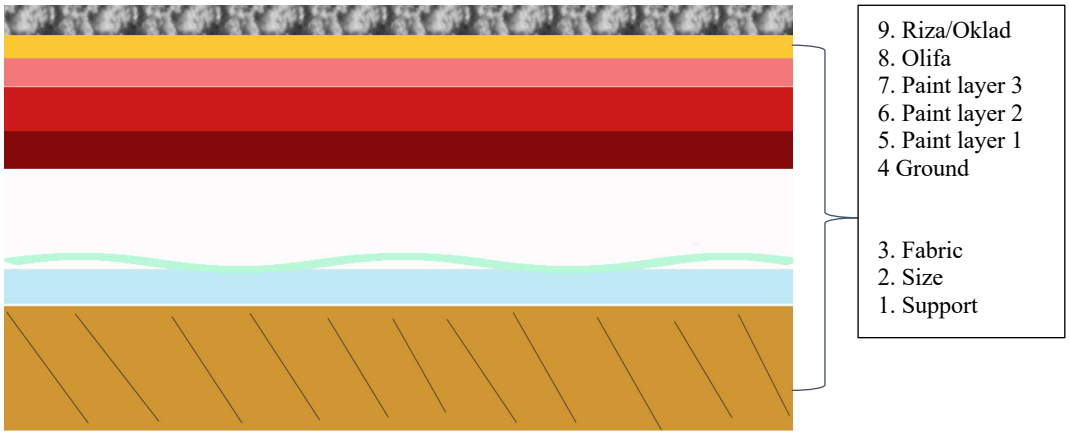

Figure 7. The layers of the icon. Illustration Nina Olivier.

[113] Beltsios, Konstantinos G. and Mastrotheodoros, Georgios P., 2019, pg. 47
[114] Ibid, pg. 49

3 The icons of the thesis

The icons chosen for this analysis were done so based on some different criteria. Firstly, they are from two geographical areas located far from each other. This is interesting, as the choices of material used for the creation of the icons can vary due to access and supply. Secondly, as this analysis aims to research the materials of the icons, the motifs of the icons, as well as the state of them, was not of a great importance. Therefore, icons were chosen that were not in a perfect condition, nor seemed to have been treated excessively, conservation wise. A rough surface with an old and darkened olifa did not matter, but rather enhanced the urge to analyze. Also, the surfaces of the icons chosen were already damaged and had lacunae from which the samples could easily be collected.

None of the icons analyzed in this thesis are on display today.

3.1 NMI 158

The icon with the object number NMI 158, is a Russian icon depicting Saint John the Baptist as the Angel of the Desert. According to National Museum documentation[115], it is from Yaroslavl in the early 18th century. It derives from the Aschberg collection given to the museum in 1933 and measures 32.5 x 27 x 3 cm.

The icon is in egg tempera on canvas, on a wooden linden panel, made of a single board with two splines inlaid from opposite sides. These two are both lost. Both front and back of the icon are grounded and the back is painted brown.

Previous conservation treatment was done before entering the museum collection. It involves some retouches in the center of the lower part of the motif, as well as repainted borders. The lower left corner was damaged and refilled with gesso. Later conservation treatment has included retouching lacunae at the gold border.

The current state of the icon includes a crack on the left edge of the panel. The panel is warped and the olifa is darkened and uneven.

[115] Abel, Ulf, *Icons*, pg. 122

Figure 8. NMI 158. Photo: Nina Olivier

3.2 NMI 232

The icon with the object number NMI 232, is a Russian icon depicting Saint Nicholas of Myra. According to National Museum documentation[116], it is from the 19th century. This icon also derives from the Aschberg collection given to the museum in 1933 and it measures 31 x 24 x 2.5 cm.

The icon is in egg tempera, on a wooden linden panel, made of a single board with two splines inlaid from opposite sides. These two are both replaced. The back is painted brown.

Previous treatment was done before entering the museum collection. It involves retouches and some new wood along a crack going through the middle of the panel.

The current state of the icon includes losses of paint along the crack and on the edges of the icon. The olifa is darkened and uneven.

[116] Ibid, pg. 181

Figure 9. NMI 232. Photo: Heiko Arens/Nationalmuseum

3.3 NMI 286

The icon with the object number NMI 286, is a Greek icon depicting Saint George and the Dragon. According to National Museum documentation[117], it is from the late 18th century, Northern Greece. This icon was given to the museum by Siri Aschberg in 1961 and it measures 35.5 x 27 x 2 cm.

The icon is in egg tempera on canvas, on a wooden spruce panel, made of a single board with two superimposed splines. These two are both lost. The back is painted black.

Previous treatment was done before entering the museum collection. It involves a hole in the top left of the icon having been mended with a wooden dowel.

The current state of the icon includes a crack running through the middle of the icon with losses in paint and ground along the crack. Four holes through the panel, one top left, one top right, one bottom left and one bottom right. There are paint losses and lacunae throughout the surface and the olifa is darkened and uneven.

[117] Ibid, pg. 36

Figure 10. NMI 286. Photo: Heiko Arens/Nationalmuseum

3.4 NMI 287

The icon with the object number NMI 287, is a Greek icon depicting Deesis and selected saints. On the top, we see Christ, with the Theotokos and St John the Baptist on each side. On the bottom, we see St Paraskevi, St Nicholas, and St Catherine of Alexandria. According to National Museum documentation[118], it is from the 19th century. This icon was given to the museum by Siri Aschberg in 1961 and it measures 24.5 x 20.5 x 3 cm.

The icon is in egg tempera, on a wooden fir panel, made of a single board without splines.

The current state of the icon includes paint losses and lacunae throughout the surface. The olifa is darkened and uneven, and the bottom left side of the icon is damaged by fire.

[118] Ibid, pg. 41

Figure 11. NMI 287. Photo: Heiko Arens/Nationalmuseum.

3.5. Reference 1 + 2 (R1 and R2)

A reference panel was also prepared for the analysis. The panel was pre-sized with animal glue from cow hide before it was prepared with a ground made of animal glue from cow hide and chalk. A non-woven was also added as an extra support, to avoid panel warping and cracking along the board. The panel was polished to a smooth surface before painted with egg tempera, prepared with egg yolk (from hen) and white vinegar 1:1. The tempera was prepared and painted in Perea, in the north of Greece, in September 2022, so the egg yolk used for the tempera was made from egg from a Greek hen.

The tempera was left to dry for a half year before half of the panel was brushed with an olifa from olive oil, and the second half of the panel was left without a varnish.

4 Tempera

As already mentioned in section 1.5, Theoretical framework, tempera will in this thesis be defined material/chemically as an egg-based paint medium. This, however, is an extremely simplified definition, which needs further discussion.

As a paint medium, tempera is pleasant to work with. Artists have used tempera for easel painting all the way back to the beginning of painting, up until modern day. In conservation, tempera has sometimes historically been used for retouching. This technique, called a total retouch, was especially popular in the 19th century, among other things for not changing very much in color with time. Also, it's easy to sculpt and thereby imitate paint structures[119]. However, tempera which has been left to dry, forms an impenetrable film, which is hydrophobic and almost impossible to solve. This is why retouching with tempera was eventually discarded.

In iconographia, tempera has not differed very much over the years and the same recipes have been kept according to tradition[120]. The oldest surviving document describing iconographia is from 910 ± 80 years A.D. and describes representations of holy persons in icons. The most cited Greek manual on iconographia is the Hermeneia by Dionysius of Fourna from c. 1730[121]. It contains both materials technological, iconographical and restoration material.

For icons, traditionally, the binding agent has been egg yolk mixed with something else, either alcohol, vinegar, or water. The liquid additives to the tempera serve to cut the greasiness of the yolk, to make it more liquid, and to function as preservatives[122]. Adding oil to the egg tempera, is called a *tempera grassa*.

Prior to this thesis, the author conducted a survey in 2022 on tempera recipe, in a social media group with 15 thousand members, all iconographers and artists. The iconographers in this group, were asked which recipe for tempera they use. The purpose of the survey was to get a contemporary answer to how iconographers today prepare their paint.

No other recipes were given as replies, and therefore also no other paint mediums but tempera was listed in connection to iconographia. These recipes all imply paint medium for portable icons.

[119] Nicolaus, Knut, *Handbok för restaurering av målningar,* 2001, pg. 278
[120] Beltsios, Konstantinos G. and Mastrotheodoros, Georgios P., 2019, pg. 42
[121] Ibid
[122] Thompson, Daniel Varney, *The practice of Tempera painting,* 1962, pg. 96

The different *recipes* given through the survey, for portable icons, were:

1:1 egg yolk/ white wine + some drops of Frankincense[123] for scent

1:1 egg yolk/ water + vinegar or white wine

1:2 egg yolk/ dry white wine + 5 drops of lavender

1:3 egg yolk/ white wine

1:1:1 egg yolk/ holy water/ vodka

1:2 egg yolk/ white wine

1:1 egg yolk/ water

1:1 egg yolk/ vinegar

1:1 egg yolk/ gin

1:1 egg yolk/ water + an addition of vinegar

1:1 egg yolk/ grape vinegar

1:1 egg yolk/ water + acidic acid[124]

Egg yolk + 1 tbsp vodka

Egg yolk + dry white wine measured with the eggshell + some drops of lavender oil

2/3 egg yolk from duck + 1/3 dry white wine + a drop of clove essential oil

Ukrainian icon conservators replied when asked during an online seminar regarding Ukrainian icons, that the common Ukrainian tempera recipe for icons is egg yolk + water and sometimes a little bit of vinegar or wine. During the same seminar, they also mentioned that the recipe of Ukrainian baroque icons (Cossack art), was a tempera grassa[125].

During travels to Greece in 2018 and 2022, the author of the thesis has met and interviewed an iconographer working with large-scale icon paintings in Greek churches. They are following the recipe used today by iconographers for large-scale paintings, which is a distemper made from PVA-c mixed with pigments, painted on large canvases, sized, and primed using acrylics.

[123] Frankincense was traditionally one of the gifts the three wise men gave baby Christ. In theology, it represents man's prayer to God. (Author's note).
[124] NB: the acidic acid could damage ultramarine, Thompson, Daniel V., *The practice of Tempera painting,* pg. 96.
[125] Kravchenko, Valeriia, Khrebtenko, Marharyta, Shashkova, Anneta, *A glimpse into Ukrainian Icon Painting,* BAPCR talk on the 1st of March 2023

Max Doerner (1870-1939, Munich) advised against using vinegar or phenol in tempera, as he noted some discoloration of the pigments due to these. Instead, he recommended using a drop of oil of cloves or small amounts of alcohol.

He also noted some decomposing in pigments containing sulfur, such as cadmium, vermilion, and artificial ultramarine, as they in combination with the nitrogen and sulfur compounds in the egg, may form hydrogen sulfide[126].

If oil is added to a tempera paint afterwards, as an impregnation, this results in a change in color hue and a change in the opacity of the paints. Because of this, if oil is to be added to the paint medium, it is better to do so from the beginning[127].

To understand the complex nature of tempera, a closer study of the components of it is needed.

4.1 Proteins

Proteins (Gr. *proteios*, meaning first) are built of α-amino acids, consisting of a carboxyl group, an amino group, and a hydrogen atom, with an organic side group attached to a carbon atom[128]. The amino acids can be non-polar, polar-uncharged or polar-charged. Depending on their charge, they fill different functions within the protein. Non-polar amino acids have an aliphatic side chain, which becomes increasingly hydrophobic as the chain length increases. They avoid contact with water and cannot form hydrogen bonds. They are usually found in the interior of proteins, where contact with water can be avoided[129].

Polar-uncharged amino acids have hydroxyl groups in their side chains, which form hydrogen bonds with water. They are also water-soluble[130]. Polar-charged amino acids have side chains that are ionized at neutral PH and can form ion-ion interactions and hydrogen bonds. Because of this, polar-charged amino acids are often found on surfaces of proteins, where they can form interactions or bonds[131].

When protein molecules are formed, the carboxyl groups of the amino acids condensate and form polypeptides. The polypeptides fold due to a hydrophobic effect to create as large a distance as possible between the non-polar amino acids and water[132]. This creates a helix where one side often is polar and the other side non-polar.

The chemical properties of the protein helix thus depend on which of the amino acids are in contact with a solvent. If the polar side is in contact with a solvent, such as water, it is hydrophilic, but if the non-polar side is in contact with water, it is hydrophobic[133].

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[126] Gettens, Rutherford J. and Stout, George L., *Painting materials – a short encyclopedia*, 1966, pg. 18
[127] Dietemann et al., *A colloidal description of tempera and oil paints, based on a case study of Arnold Böcklin's painting Villa Am Meer II (1865),* 2014, pg. 35.
[128] Backman, Lars, *Protein Chemistry,* 2020, 3, n. pag.
[129] Ibid, 3.1.1, n. pag.
[130] Ibid, 3.1.2, n. pag.
[131] Ibid, 3.1.3, n. pag.
[132] Ibid, 4.4, n. pag.
[133] Ibid, n. pag.

The content of an egg[134]:

	Egg white (also called albumen[135])	Egg yolk (also called vitellus)
Water	84.8%	51.5%
Albumin, vitellin, etc.	12%	15%
Fat or oil	0.2%	22%
Lecithin	trace	9%
Mineral matter	0.7%	1%
Other substances	2.3%	1.5%

Figure 12. The content of an egg.

Egg white contains around 149 proteins. The adhesive substance is the protein albumin, which contains carbon, hydrogen, nitrogen, oxygen, and sulfur. Albumin is water-soluble. When dried, it forms a clear and brittle, but over time hygroscopic film[136].

The high water content in egg white does not change if the egg is cooked[137].

Egg yolk contains around 14 different proteins[138]. Vitellin is a phosphoprotein found in egg yolk and it separates from the albumin. Other proteins in the egg yolk are non-polar amino acids (glycine, leucine, isoleucine, alanine, valine, methionine, and proline), polar-uncharged amino acids (serine, threonine) and polar-charged amino acids (lysine, arginine, and histidine). This means that the egg yolk contains proteins that are both hydrophobic as well as hydrophilic and the protein helix will have both polar and non-polar properties.

A fresh egg yolk generally has a pH of 6[139].

4.2 Lipids

Lipids (Gr. *lipos* = fat) are chemical names for substances containing fat and fatlike substances. Fatty oils are one example of lipids. These are generally insoluble in water, but soluble in non-polar solvents. The oils belong to a group called esters. Esters can be formed when an acid and an alcohol react. The acids contain fatty acids such as palmitic acid ($C_{16}H_{32}O_2$, or C16:0), stearic acid ($C_{18}H_{36}0_2$ or C18:0), linoleic acid (C18:2), linolenic acid and oleic acid ($C_{18}H_{34}O_2$ or C18:1) [140]. The alcohol of the oil is called glycerin. Each

[134] Church, A.H, *The Chemistry of paints and painting*, 1901, pg. 20
[135] NB: Albumen (egg white) is not the same as Albumin, which is a protein.
[136] Church, A.H, 1901, pg. 20
[137] Dietemann et al., 2014, pg. 30
[138] Phenix, Alan, *The composition and chemistry of eggs and egg tempera,* 1996, pg. 12
[139] Ibid
[140] Nylén, Paul, Söderberg, Einar, Johansson, Tryggve, *Hantverkets bok,* 1953, pg. 34

glycerin molecule can bind three fatty acids. They then form triacyl glycerides (neutral lipids) These fatty oils with their glycerin and fatty acids form complicated chemical systems[141].

When the oil dries, as in an olifa[142], oxygen is bound to the oil molecules in the fatty acid chains, forming molecule structures in all directions of the film. Eventually, the molecules lose their ability to move, and the film becomes solid, but thanks to the long molecule structures, the film stays elastic and flexible[143].

The lipids found in egg yolk are triacyl glycerides, phospholipids, and cholesterol compounds[144].

The lecithin (Gr. *lékithos* = yolk), which is high in egg yolk, is a fatty substance which contains nitrogen, phosphorus, and phospholipids, but differs from many other fats by being amphiphilic, which means it is both hydrophilic (attracts water and can be dissolved in water) and lipophilic (attracts fat and can be dissolved in other lipophilic substances).

The lipids and proteins in the egg yolk form lipoprotein assemblies. These can be formed as high-density assemblies, HDLs (containing lipovitellins[145]) or low-density assemblies, LDLs (containing water-soluble proteins such as livetins, vitamin-binding proteins and more[146]). In HDLs, the phospholipids are closed off by the protein structure, but in the LDLs, the phospholipids form the outer shell together with the lipoproteins. Since these LDLs can stabilize lipid droplets in an aqueous phase (see next section on emulsions), they function as the emulsifier of the substance[147].

When egg yolk dries, the water content in it, evaporates. The lipids (fats) are suspended in the albuminous matrix and hardens, forming a strong film, which is eventually not affected by water[148]. The lipoprotein structure is also very stable to oxidation[149]. An oil in a tempera grassa will take longer time to dry, as the egg contains antioxidants that will work to prevent the oxidation of the oil. To aid the oxidation, the added olifa often contains siccatives (such as in linseed oil).

While palmitic acid, stearic acid, oleic acid, and linoleic acid are all dominant fatty acids in egg yolk[150], there are also occurrences of unsaturated fatty acids and polyunsaturated fatty acids[151].

Palmitic acid (C16:0) and stearic acid (C18:0) can be detectable even in aged oils[152]. The intensity ratio of these can measure the nature of the oil. Oleic acid (C18:1) can also be measured to see if the oil has been oxidized[153].

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[141] Ibid, pg. 36
[142] See section 2.3.6, Author's note.
[143] Nylén, Paul, Söderberg, Einar, Johansson, Tryggve, 1953, pg. 38 and Bouvier et al, *Time-of-flight secondary ion mass spectrometry imaging in cultural heritage: A focus on old paintings,* 2022, pg. 11
[144] Phenix, Alan, 1996, pg. 13
[145] Ibid, pg. 16
[146] Ibid, pg. 15
[147] Dietemann et al., 2014, pg. 37
[148] Church, A.H., 1901, pg. 20
[149] Phenix, Alan, 1996, pg. 13
[150] Ibid pg. 12
[151] Ibid
[152] Bouvier et al, 2022*,* pg. 11
[153] Ibid

4.3 Minerals and trace elements

Besides protein, lipids, and carbohydrates (which are left out of this thesis), egg yolk is also rich in micronutrients, such as vitamins, choline, minerals, and trace elements.

Egg yolk is rich in sulfur (S), phosphorus (P), calcium (Ca) and potassium (K), has essential trace elements of copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), selenium (Se), iodine (I) and zinc (Zn)[154]. The exact content of mineral and trace elements vary with the diet of the hen.

4.4 Emulsions

An emulsion consists of two fluids, where one is microscopically dispersed in the other. If oil is the dispersed phase in the system, and water is the continuous phase, it is an O/W emulsion (oil in water). If water is the dispersed phase, and oil the continuous phase, it is a W/O emulsion (water in oil). An O/W is generally miscible with water, whereas the W/O emulsion is miscible in non-polar solvents[155].

To this paint medium emulsion, pigments are added, affecting the microstructure of the emulsion and the interactions between the substances in it[156], creating colloidal systems[157]. Colloidal systems are systems with one dispersed substance and where the particles in this dispersed substance vary in dimensions between one nanometer (nm) and one micrometer (μm). The surfaces of the particles (such as their shape and surface structure, their components, their interactions (such as how they respond to changes in the medium, e.g. additives, pH, salt levels etc.) and their properties (such as charge), determine the properties of the entire colloidal system[158].

Tempera, with egg yolk, vinegar (or water, acidic acid, wine etc.) and pigments, forms a multiphase fluid[159]. This type of fluid gets a complex rheology (flow behavior), such as thixotropy (when a fluid becomes less viscous when stress is applied), yield stress (the point when a substance goes from solid to liquid when stress is applied), shear thinning (the behavior of fluids where the viscosity decreases when put under a sideway force) and viscoelasticity (the behavior in a fluid that makes it deform viscosically and elastically at the same time)[160]. The viscosity in a paint is ruled by pigment-pigment interactions, and not by the binder. The viscosity in a paint is not constant[161].

The size of the pigments, the shape of them and the distribution in the medium, the volume of pigments in comparison to the total volume of the fluid, all affect the rheology of the fluid and the colloidal system, as do acids (as in vinegar or acidic acid), which affect the pH of the proteins and the electrical charge of the pigments, and whether or not the pigment has been

[154] Guyot, Nicolas, Nys, Yves and Réhault-Godbert, Sophie, *The Golden Egg: Nutritional Value, Bioactivities, and Emerging Benefits for Human Health*, 2019, section 2.2.2. Minerals and Trace Elements, n. pag.
[155] Nylén, Paul, Söderberg, Einar, Johansson, Tryggve, 1953*,* pg. 51-52
[156] Hodapp, Annika, Dietemann, Patrick and Willenbacher, Norbert, *Flow behavior and microstructure of complex multiphase fluids,* 2019, pg. 58
[157] Dietemann et al., 2014, pg. 29
[158] Ibid, pg. 31
[159] Hodapp, Annika, Dietemann, Patrick and Willenbacher, Norbert, 2019, pg. 57
[160] Ibid, pg. 58
[161] Dietemann, Patrick, *Tempera or oil? Colloidal chemistry and the microstructure of paints,* 2023

pre-coated with protein[162], such as glair[163] (some pigments, such as lead white, can be pre-coated with protein before being ground with oil in order to prevent capillary suspensions due to a high level of humidity within the pigments[164]). The water content in the pigments can also affect the rheology and the colloidal system[165].

Experiments done by Hodapp, Dietemann and Willenbacher in 2019, show that adding just a small amount of egg yolk to a mixture of pigments in linseed oil, drastically increases both viscosity and yield stress[166].

Other experiments have shown that emulsions containing egg tempera with an addition of oil, can consist of almost 90% lipids and 10% egg protein in dry form, but still form O/W emulsions. In other words, even if the egg in the emulsion is oh so little, it still forms an aqueous system in the continuous phase[167]. This has to do with colloid chemistry, where according to Bancroft's Rule, the phase in which the emulsifier is more soluble, is the continuous phase[168]. In their experiments, Hodapp, Dietemann and Willenbacher, have found that the LDLs in the egg yolk, which function as an emulsifier, can exclusively stabilize O/W emulsions[169]. Additionally, adding a small amount of protein to an oil color, such as in pre-coating the pigments with protein, will change the properties of the oil, for example give a higher pigment content, which in turn leads to less ability to degrade, discolor, or darken[170].

When the gel of the aqueous binder, the emulsion, dries, it connects the pigment particles. These interlocking particles strengthen the binder. Pores are formed, but filled with residual water and oil, which eventually dry by autoxidative cross-linking. The evaporation of water from the gel helps the particles to solidify, leading to a rapid gel formation, which render the particles immobile[171]. Oil added to the paint form droplets that are larger than the ones naturally occurring in the egg yolk, leaving big pores after autoxidative cross-linking. These voids can also affect the shrinkage of the paint structure in the drying process.

Paint reconstruction done by Dietemann et al. in 2014, shows that water is of relevance in the formation of an aqueous system. The amount of water added, the pH in the emulsion (because of the water), the temperature of the water, how the water affects the pigment surface and the pigment-binder ratio, are all relevant factors when it comes to the properties of the paint when drying, including the optical properties in the paint[172].

Gel formation of the emulsion can occur both while preparing the paint and after painting, while drying. This is of relevance to the paint properties. If the pigments in the emulsion are immobile in the drying process, the water can pull back from the pigment matrix, first into a inhomogeneous system of water droplets in oil, and later completely, leaving no water in the matrix. If the proteins are not interlocked when the water disappears, they will move with the water and form an inhomogeneous protein-distribution in the paint[173]. The distribution of the

[162]Hodapp, Annika, Dietemann, Patrick and Willenbacher, Norbert, 2019, pg. 61
[163] Beaten or pressed egg white (Author's note)
[164] Dietemann, 2023
[165] Bonaduce et al. *A holistic view on the role of egg yolk in Old Masters' oil paints,* 2023
[166] Hodapp, Annika, Dietemann, Patrick and Willenbacher, Norbert, 2019*,* pg. 64
[167] Dietemann et al. *Analysis and interpretation of binding media in tempera paintings*, 2019, pg. 69
[168] Dietemann et al., 2014, pg. 36
[169] Ibid
[170] Dietemann, 2023
[171]Dietemann et al., 2014, pg. 38
[172] Ibid, pg. 41
[173] Ibid, pg. 43

paint might then look like a W/O emulsion, when it was really an O/W emulsion before the water disappeared.

In short conclusion: tempera paints are colloidal O/W systems where the oil is dispersed in the aqueous continuous phase, even if the oil to water ratio is 90% vs. 10%. Many different factors of the colloidal system affect the properties of the paint, including those of the binder, the pigments, the interfaces, and interactions between these different substances.

When the water withdraws from the O/W emulsion, the paint dries and stabilizes. Due to the polypeptide helixes formed in the protein folding and the lipids in the egg yolk, the dried tempera becomes hydrophobic.

In tests conducted on aged tempera films (by Khandekar in 1994, 1995 and 1996, by Khandekar et al. in 1994, by Mills in 1966, by Mills and White in 1975 and by Schilling et al. in 1996) , the following was noticed: the amount of extractable lipids decreased drastically[174] (oleic acid decreasing the most, stearic acid fairly unaffected), the cholesterol was almost completely gone from tempera paints less than 20 years old[175] (apart from in some samples with verdigris, which could suggest that verdigris protects the oxidation of lipids), amino acid content decreased up to 25%[176].

[174] Phenix, 1996, pg. 16
[175] Ibid, pg. 17
[176] Ibid

5. Analyzing organic material

The analysis methods when it comes to inorganic materials are many and most of them work well and are reliable. When it comes to organic analyzing methods, however, these have not always been as reliable and many of them have an error range that always must be brought into consideration.

Older techniques for analyzing organic materials were often extremely complicated and time-consuming, and required a lot of complicated equipment. Nowadays, technique is developing quicker, offering better and more reliable methods for the organic material analyses as well. For a good reading of the analysis of the organic material, a combination of different methods is preferred. That way, the results can be cross-referenced and deduced with a higher accuracy. As described previously (see section 1.8, Limitations), however, only one materials analysis technique was used in the analysis carried out in this thesis. A description of the technique follows.

5.1. ToF-SIMS

The Time-of-Flight Secondary Ion mass spectrometry (ToF-SIMS) is a surface analysis technique that can identify and spatially resolve the chemical composition, both organic and inorganic, of a sample[177]. The sample material is sputtered (meaning that a metal is deposited on a surface by using fast ions to eject particles of it) and ionized[178]. This leads to an emission of particles such as molecular fragments, molecular complexes, and molecules, where most of the particles are neutral, but some are also electrons and secondary ions[179]. These secondary ions that are generated are then analyzed according to their mass-to-charge ratio. The analyzed information can then be read and mapped in images.

In SIMS, the surface of the sample is bombarded either by pulses of primary ions from a high-energy beam (nowadays usually a liquid metal ion gun, LMIG most commonly using bismuth[180], but also $Ga^+, In^+, Au_n{}^+$)[181] or from a continuous beam. As the beam diameter is 0.4 to 2 μm, it can target very small particles of the sample. Energy is then transferred from the primary ions of the beam to the atoms in the sample. When the kinetic energy is larger than the energy of the surface bond, it causes elements or fragments of molecules to be emitted from the surface. A part of this emission are secondary ions, and they are characteristic of the surface of the material. These secondary ions, either positive or negative, are then separated, accelerated[182] and focused in a TOF mass analyzer[183]. A low-energy

[177] Bouvier, Caroline, Vand Nuffel, Sebastiaan, Walter, Philippe and Brunelle, Alain, *Time-of-flight secondary ion mass spectrometry imaging in cultural heritage: A focus on old paintings*, Journal of Mass Spectrometry, 2022;57
[178] Ibid, pg. 5
[179] Bich, Claudia, Brunelle, Alain and Touboul, David, *Cluster ToF-SIMS imaging as a tool for micrometric histology of lipids in tissue,* Mass Spectrometry Reviews 2013, pg. 442 and Thiel, Volker and Sjövall, Peter, *Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS): Principles and practice in the Biogeosciences*, Royal Society of Chemistry, 2014, pg. 124
[180] Bouvier et al., 2002;57, pg. 6
[181] Thiel, Volker and Sjövall, Peter, 2014, pg. 124, 133
[182] Bouvier et al., 2002;57, pg. 6
[183] Thiel and Sjövall, pg. 122, pg. 134

electron beam is used in between the analyses to neutralize the surface[184]. Since the beam diameter is so small, the technique can acquire data from every pixel of the analyzed area[185].

A pulsed primary beam targets only a small fraction of the surface, which means the surface is not very damaged by the process[186]. With a continuous beam, the secondary ions are rearranged into short pulses before being analyzed[187]. The LMIG can focus the primary beam on a small diameter while maintaining a high-energy current, which is an advantage for the analysis[188]. Often, the primary ion beam is optimized for the surface imaging analysis, and so a second ion source, used for removing material from the sample surface, is used in combination with the primary ion source. This is called a dual beam[189].

The Time-of-Flight (ToF) or delayed extraction (DE), is used applying a delay to the extraction voltage of the secondary ions (which is the same as the duration of the primary ion beam pulse). This delay causes the first of the emitted secondary ions to be less accelerated than the last of the emitted secondary ions[190]. This will give all the secondary ions a time focusing of the same mass-to-charge ratio at the detector[191], which in turn allows for ion imaging of both high mass as well as high spatial resolutions (minimizing the ambiguity of the secondary ion peaks). [192] The delay function was not used in the analysis conducted in the thesis.

Since the primary ion beam can be focused to less than 1 μm, the technique performs both high mass-resolution and high spatial resolution chemical imaging and can detect both inorganic and organic material simultaneously[193]. Using ToF-SIMS imaging, the chemical composition of a surface can be recorded as ion density maps of both organic and inorganic material[194]. This way, not only information regarding the molecular material can be mapped, but also the spatial information[195].

Advantages of this method:

- It works well for analyzing complex organic and/or inorganic samples simultaneously.
- It can analyze and map molecular ions on very small samples.
- It is non-destructive of the cross-section. The analysis is carried out on the outer layer of the surface.
- It can be used to detect both lipids as well as amino acids, both materials often used in painting media.

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[184] Bouvier, Caroline, Glanville, Helen, de Viguerie, Laurence, Merucci, Chiara, Walter, Philippe and Brunelle Alain, *Time-of-Flight Secondary Ion Mass Spectrometry Imaging of Cross Sections from the Baccanals Paintings of Nicolas Poussin,* Analytical Chemistry, 2021, 93, pg. 4465
[185] Thiel and Sjövall, pg. 124
[186] Ibid, pg. 122-123
[187] Ibid, pg. 132, pg. 138
[188] Ibid, pg. 133
[189] Ibid, pg. 136-137
[190] Bouvier et al., 2022;57, pg. 7
[191] Ibid
[192] Bouvier et al., 2021, pg. 4465
[193] Voras, Zachary E., deGhetaldi, Kristin, Baade, Brian, Gordon, Eric, Gates, Glenn & Beebe, Thomas P., *Comparison of oil and egg tempera paint systems using time-of-flight secondary ion mass spectrometry*, Studies in Conservation, Vol. 61, 2016 – Issue 4
[194] Bouvier et al., 2022; 57*,* pg. 6
[195] Bich et al., 2013, pg. 442

- It can characterize different products formed during drying of an oil film, thereby determining whether the ions derive from fatty acids in the paint medium, from filler or from pigments.
- A visual map is presented, so that the distribution of materials within the sample can be traced.
- The preparation process is fairly quick and simple.
- The ion beam can be directed at a specific target within the sample, allowing the analysis to be very controlled.

Disadvantages of this method:

- Certain surrounding compounds can amplify or inhibit the secondary ion emission, causing an error range[196]. One such compound is cholesterol.
- As only a small fraction of emitted particles are ions, the signal intensity can vary in samples due to the chemical environment of the sample[197].
- An error range can also be detected with the concentrations of fatty acids and the distribution between and inside layers (egg/oil mixture)[198], interactions with the environment (such as lead white pigment) and aging within the layers[199].
- The presence of proteins can easily be identified, but to identify the protein, multivariate analyses are often required [200].

[196] Bouvier et al., 2022; 57, pg. 8
[197] Thiel and Sjövall, 2014, pg. 129
[198] See section 4.3 on emulsions (Author's note)
[199] Bouvier et al., 2022;57, pg. 12
[200] Ibid, pg. 13

6. ToF-SIMS Analysis of the icon samples

The analysis with ToF-SIMS was made in April 2023 at RISE, Research Institutes of Sweden, by researcher scientist Peter Sjövall and paintings conservator Nina Olivier.

The samples were prior to the analysis cast in CEM4000 Lightfix, Methylmethacrylatfrei (Cloeren Technology GmbH) and polymerized in a Technotray Blue Light Curing Device with a 70-90°C temperature and a radiation time of 5 x 2 minutes. As sample preparation is an important part of the ToF-SIMS analysis, the samples were better prepared at RISE. For a good analysis with the ToF-SIMS, it is important that the risk of surface contamination is minimalized prior to the analysis[201]. Due to this, the samples were cut and sawed to fit into the very small sample holders for the ToF-SIMS at RISE. The surfaces were polished and cut with a Leica SM2500 microtome with a ratio of 10 µm/ slice.

To ensure the measured data, reference samples were prepared under identical material-technical conditions as the samples from the NM collection. These too were prepared for the ToF-SIMS analysis in the same way as the other samples.

The samples were analyzed in negative and positive ions mode. They were not coated with metal.

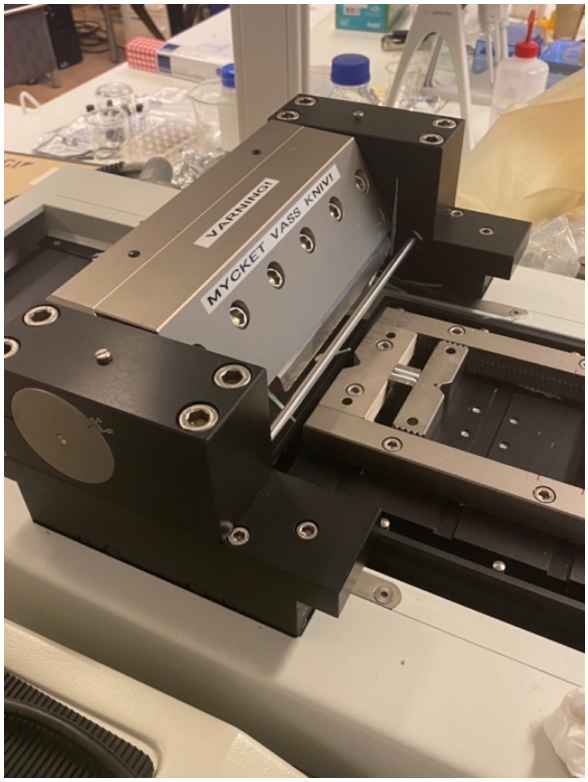


Figure 13. The microtome at RISE. Photography: Nina Olivier on 2023-04-12.

[201] Thiel and Sjövall, 2014, pg. 139

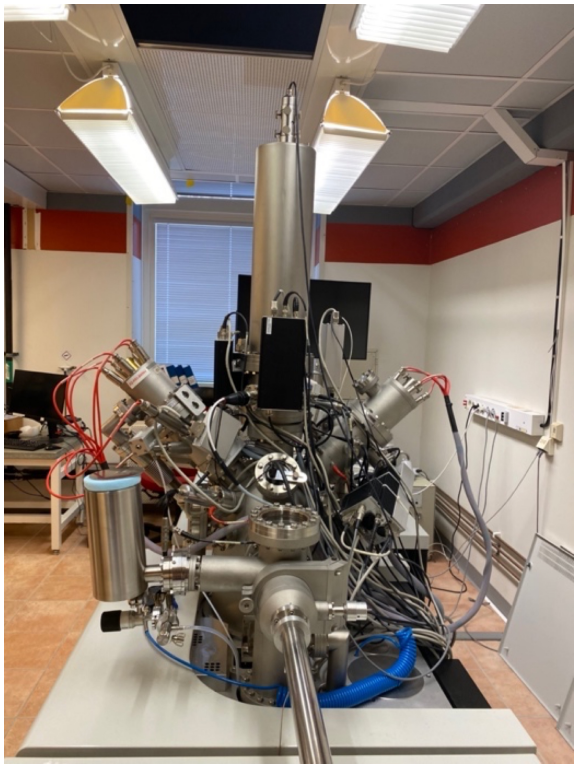


Figure 14. The ToF-SIMS IV instrument, manufactured by ION-TOF Gmbh at RISE.

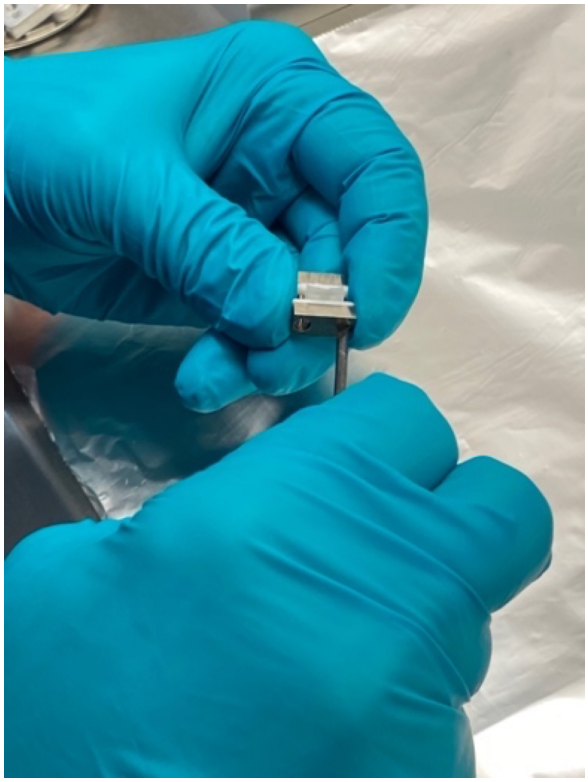


Figure 15. The sample being prepared for ToF-SIMS analysis. Photography: Nina Olivier on 2023-04-12.


Figure 16. The sample in the sample holder. Photography: Nina Olivier on 2023-04-12.

The sample holder containing the samples, was then placed in a load lock of the ToF-SIMS instrument. The load lock was evacuated to $\sim < 10^{-5}$ mbar before a gate valve opened into the main analysis chamber and the sample holder was transferred into it using a transfer arm[202]. Two video cameras using macro and micro view, were used as an aid in positioning the samples before analysis.

Several analysis parameters were selected, such as positive or negative ion detection and more before the analysis was engaged. The observations from the analysis were made by Peter Sjövall and the interpretation of the observations were made by Nina Olivier.

[202] Thiel and Sjövall, pg. 142

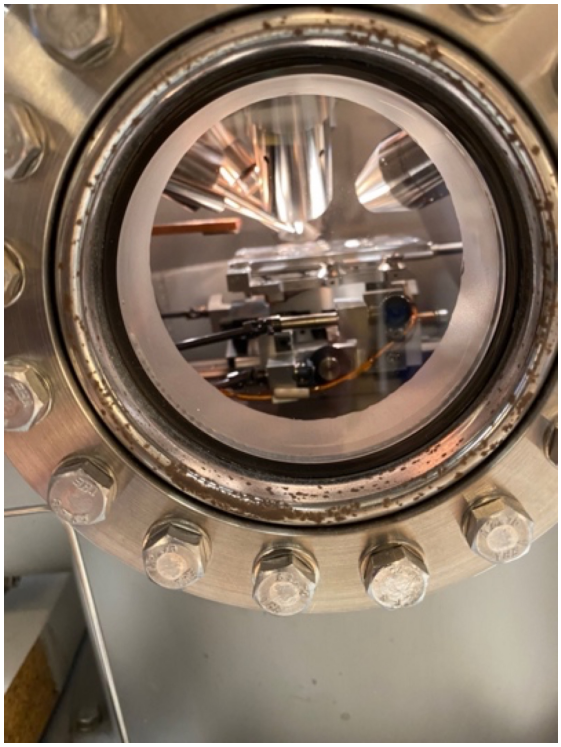


Figure 17. The sample in the ToF-SIMS machine. Photography: Nina Olivier on 2023-04-12.

6.1 NMI 158


Figure 18. Area of sampling indicated with a green circle, NMI 158. Photography: Nina Olivier.

Figure 19. Close-up area of sampling, NMI 158. Photography: Nina Olivier.

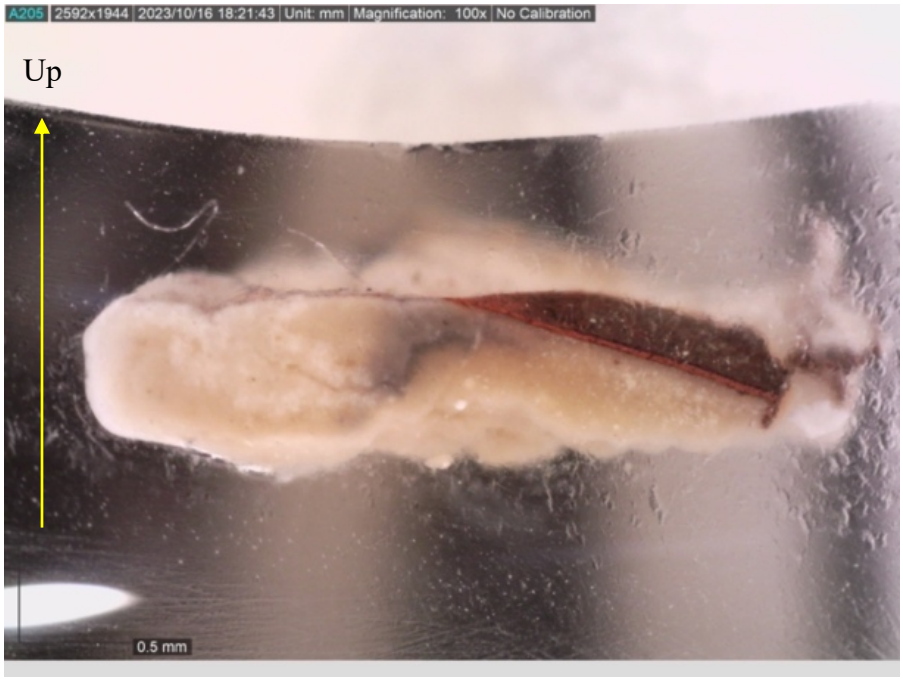


Figure 20. The cross-section in regular light, NMI 158, picture taken 2023-10-16, using a DinoLite at 150x.

Figure 21. The cross-section in UV light, NMI 158, picture taken 2023-10-16, using a DinoLite at 150x.

Microscopy images of the NMI 158 sample, show a single, thin, red paint layer, visible in the top of the sample, underneath a thick, brownish looking layer, and some rather thick, white ground.

UV light shows a yellow fluorescence that indicates that the ground is penetrated with oil.

The NMI 158 sample was analyzed on 2023-04-12. Three areas of the sample were analyzed, and ions were measured in negative and positive mode. Images below show the areas through extremely enhanced microscopy. The targeted areas are both paint layer as well as ground layer.

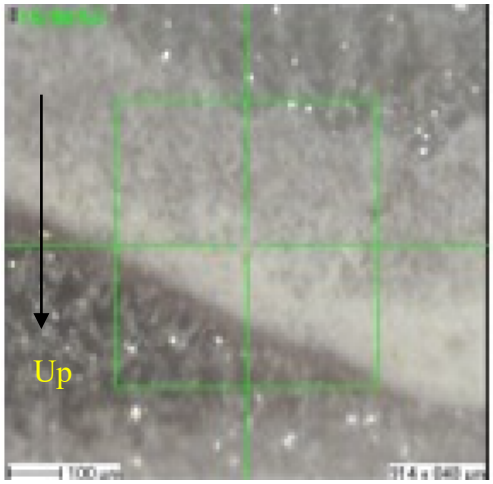


Figure 22. NMI 158, area 1, ground layer targeted with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

Data collected in correlation to the sample NMI 158, area 1:

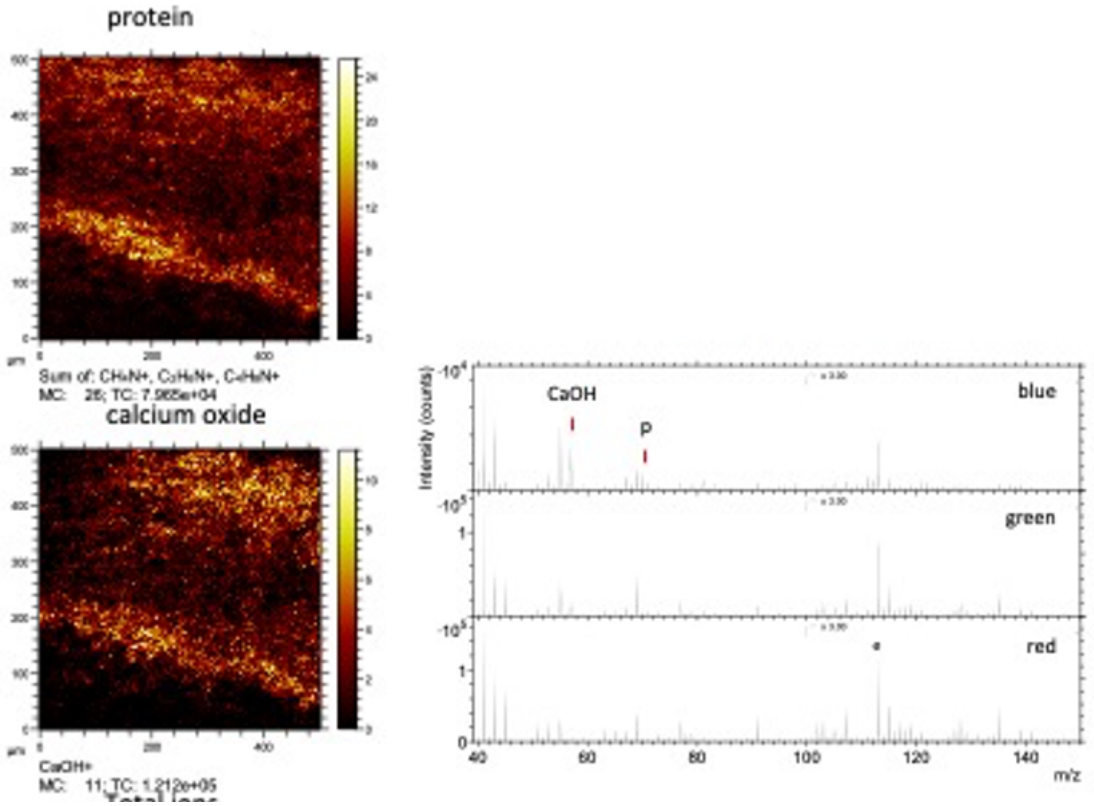


Figure 23. Ion map showing Calcium oxide (CaOH) together with protein in the ground layer (area 1). The bright dots in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and calcium oxide in this area, we can tell that the distribution of the substances is the same.

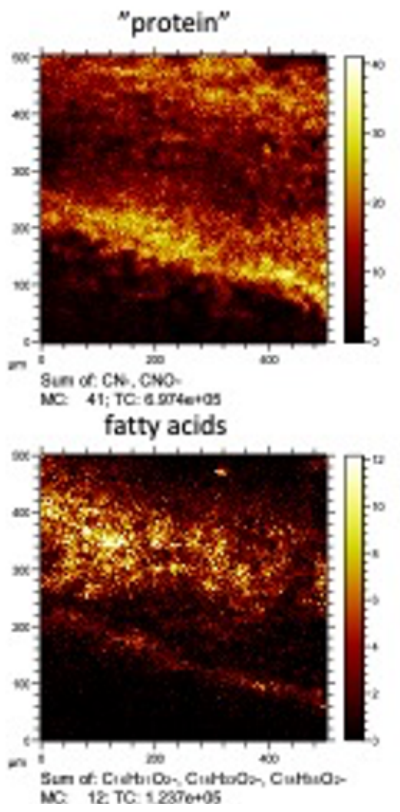


Figure 24. Ion map showing fatty acids inside the protein cluster in the ground layer (area 1). The bright dots in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and fatty acids in this area, we can tell that the fatty acids are distributed inside the protein cluster.

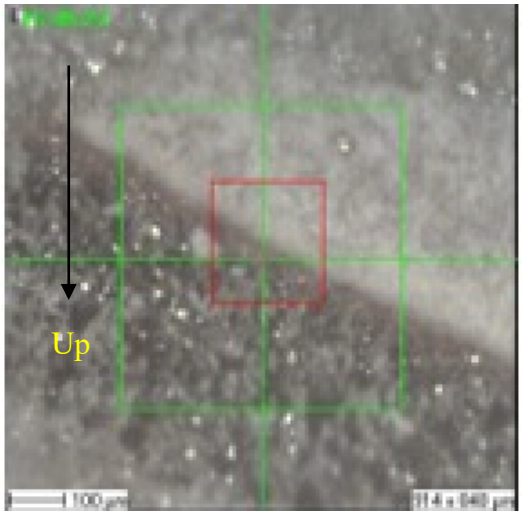


Figure 25. NMI 158, area 2, paint layer, targeted with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

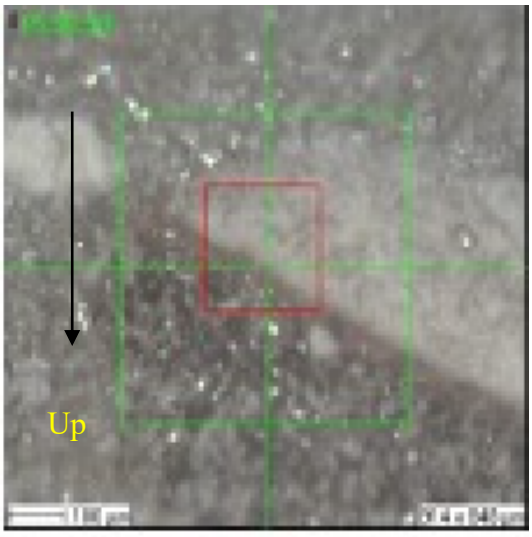


Figure 26. NMI 158, area 3, paint layer, targeted with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

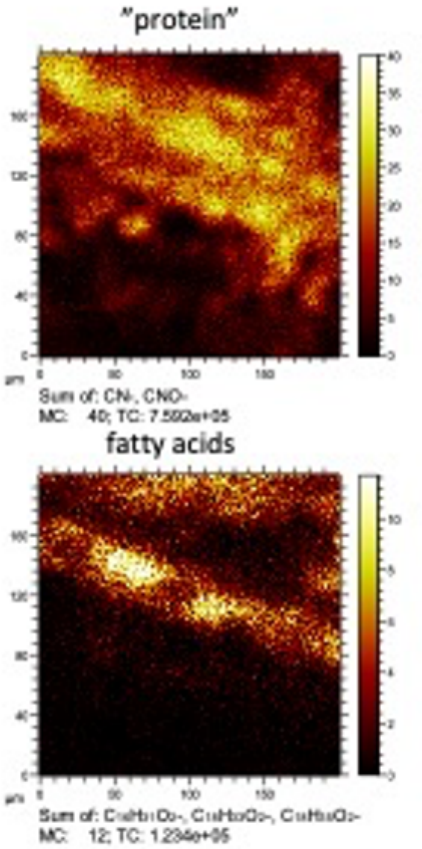


Figure 27. Ion map showing protein together with fatty acids in the paint layer (area 2). The bright dots in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and fatty acids in this area, we can tell that protein is found inside and with clusters of fatty acids.

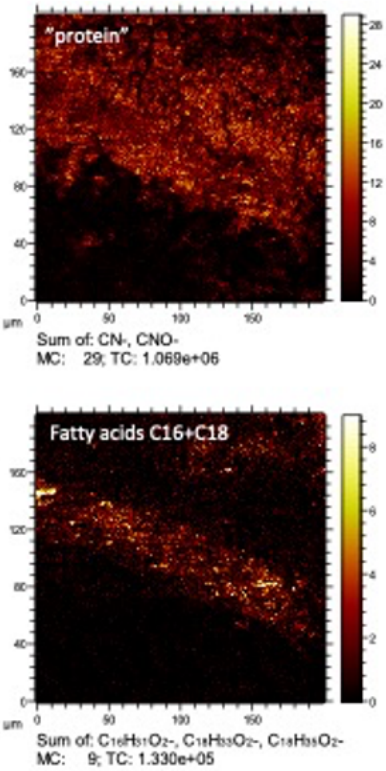


Figure 28. Ion map showing protein together with fatty acids in the paint layer (area 3). The bright dots in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and fatty acids in this area, we can tell that protein is found inside and with clusters of fatty acids.

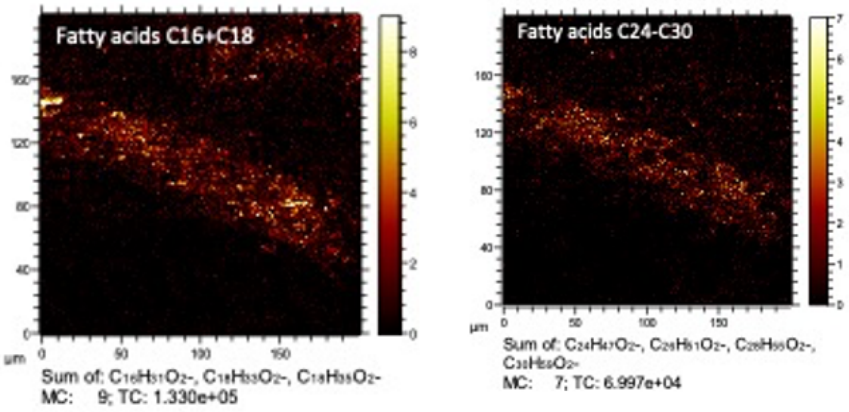


Figure 29. Ion map showing fatty acids palmitate (C16), stearate (C18), tetracosanoic acid (C24) and triacontanoic acid (C30) in the paint layer (area 3). The bright dots in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and fatty acids in this area

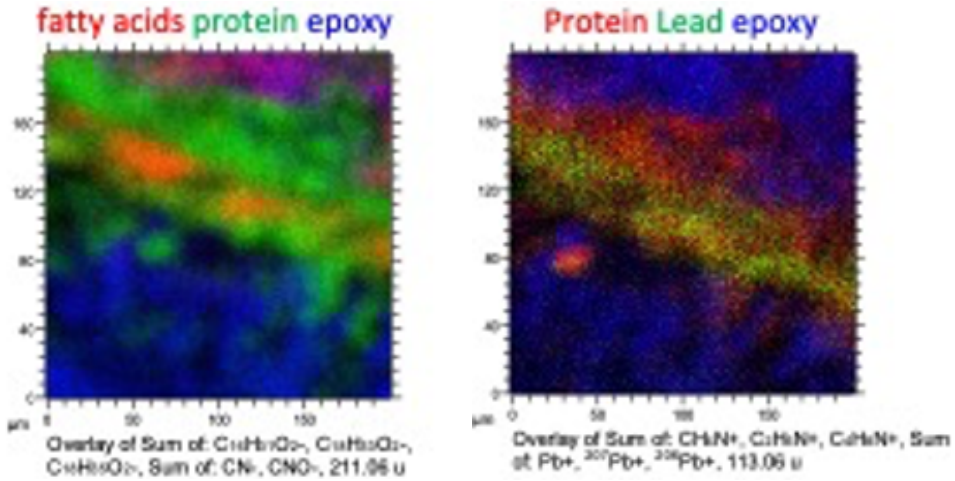


Figure 30. Ion map showing the distribution of fatty acids, protein, lead oxide (PbO) and methylmethacrylate in the paint layer, area 2. The distribution of fatty acids and lead oxide overlap.

In the NMI 158 sample from an early 18th century Yaroslavl Russian icon the following was observed;

In the analysis, calcium oxide (CaO) was found together with protein in the ground layer. (See area 1, figure 31 and 32). This would indicate a ground layer made of chalk ($CaCO_3$) and animal glue. Fatty acids were found inside clusters of protein. (See area 1, figure 31).

Protein was found in the paint layer with fatty acids inside clusters of protein. (See area 1 and 2, figures 33 and 35). The protein in the paint layer, suggest that tempera was used as a paint medium of the icon. The fatty acids found in the sample were palmitate (C16:0) and stearate (C18:0) with almost no oleate. Since both these fatty acids occur naturally both in the egg yolk and in oil, it is hard to say if the fatty acids found come from the egg yolk or from an addition of oil, either to the paint medium or as an olifa. There were also traces of longer chain fatty acids, C24:0 and C30:0. C24:0, tetracosanoic acid, occurs in waxes and some seed oils. C30:0, triacontanoic acid occurs in beeswax. This suggests that the icon has been treated with wax in a previous conservation.

That the fatty acids were found inside clusters of protein, both in the ground- and the paint layer, (See area 1 and 2, figures 39 and 41), could indicate that they came from the egg yolk and had been folded inwards in the protein helix the polypeptide folding process. However, it could also be interpreted as pores in the aqueous paint system which have been filled with oil from the olifa after the evaporation of the water from the paint.

The finding of lead oxide in combination with fatty acids on the surface of the paint layer (See area 2, pos. mode, figure 34), indicates that most of the fatty acids came from an olifa, which had been mixed with a lead white (2PbCO) as a siccative.

Fatty acids and amino acids were both found in negative mode in the sample. Only amino acids, however, were found in the positive mode. According to Bouvier et al, 2021, fatty acids found in negative ion-mode can indicate that the binder is oil- or egg yolk-based. Amino acids found in positive-ion mode can indicate that the binder is glue- or egg-based[203]. If both fatty acids in negative ion-mode and amino acids in positive ion-mode are found, then the binder

[203] Bouvier et al., 2021, pp 4463-4471, pg. 4467

can be egg yolk or mixtures[204]. This same occurrence of fatty acids and amino acids was also found in the reference samples, which were both prepared using an egg yolk tempera.

The small amounts of metals in the paint layer, could be from the pigments used for the coloring. Iron (Fe) and copper (Cu) were both found (See area 2, pos mode, figure 34). As the paint layer of the sample is red, this could indicate iron pigments (e.g. English red, Red Sienna, or Red Ochre, Fe_2O_3), but as lead oxide was also present in the paint layer, lead pigments e.g. red lead oxide/minium, Pb_3O_4) could have been used. No traces of manganese (Mn), cadmium (Cd) or mercury (Hg) were found.

Small traces of chlorine (Cl2), phosphate (PO_4) and sulfate (SO_4), found in the paint layer could come from traces elements in the egg yolk. This could also be the case of the copper (Cu).

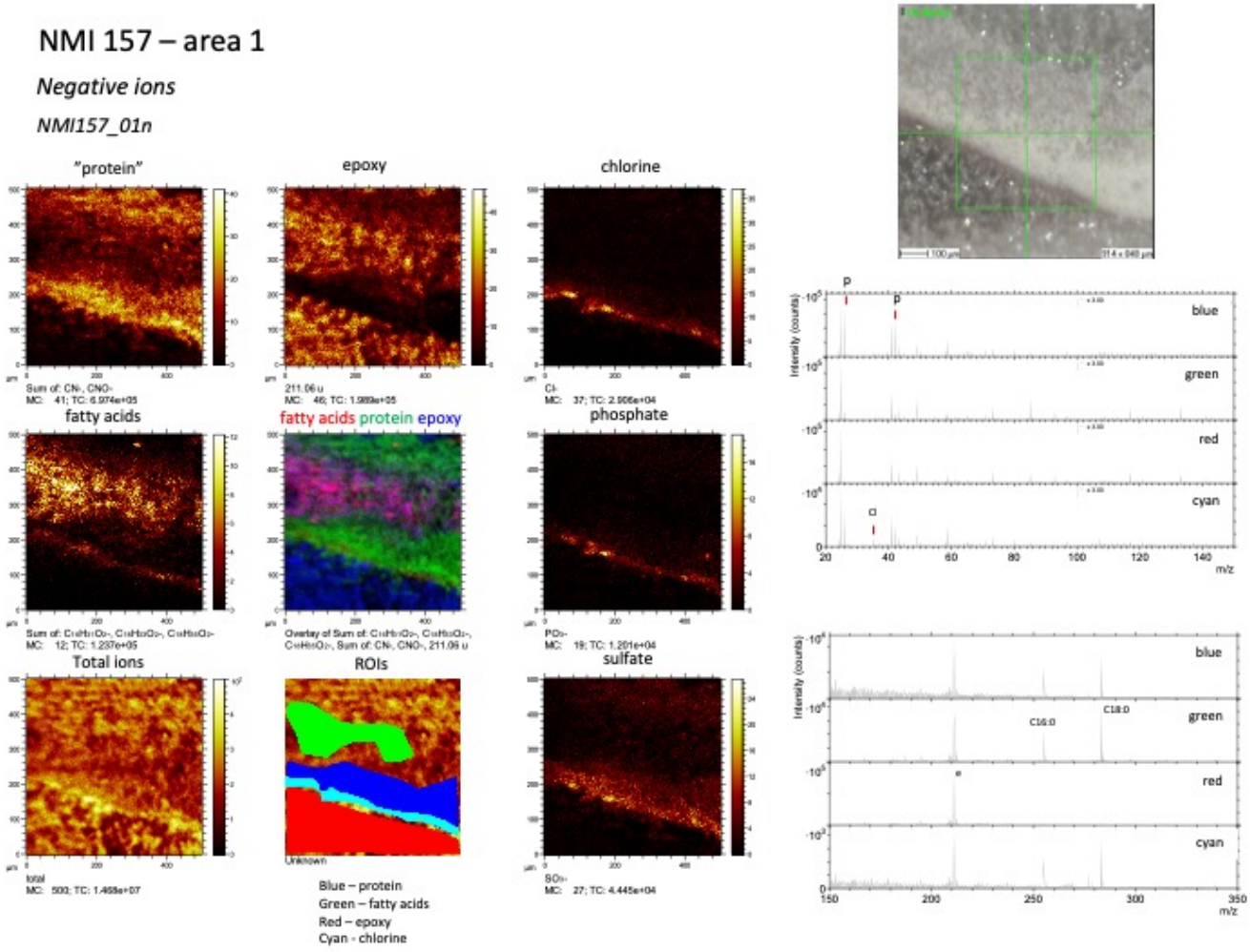


Figure 31. Data from NMI 158 (mislabeled as 157), area 1, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

[204] Ibid

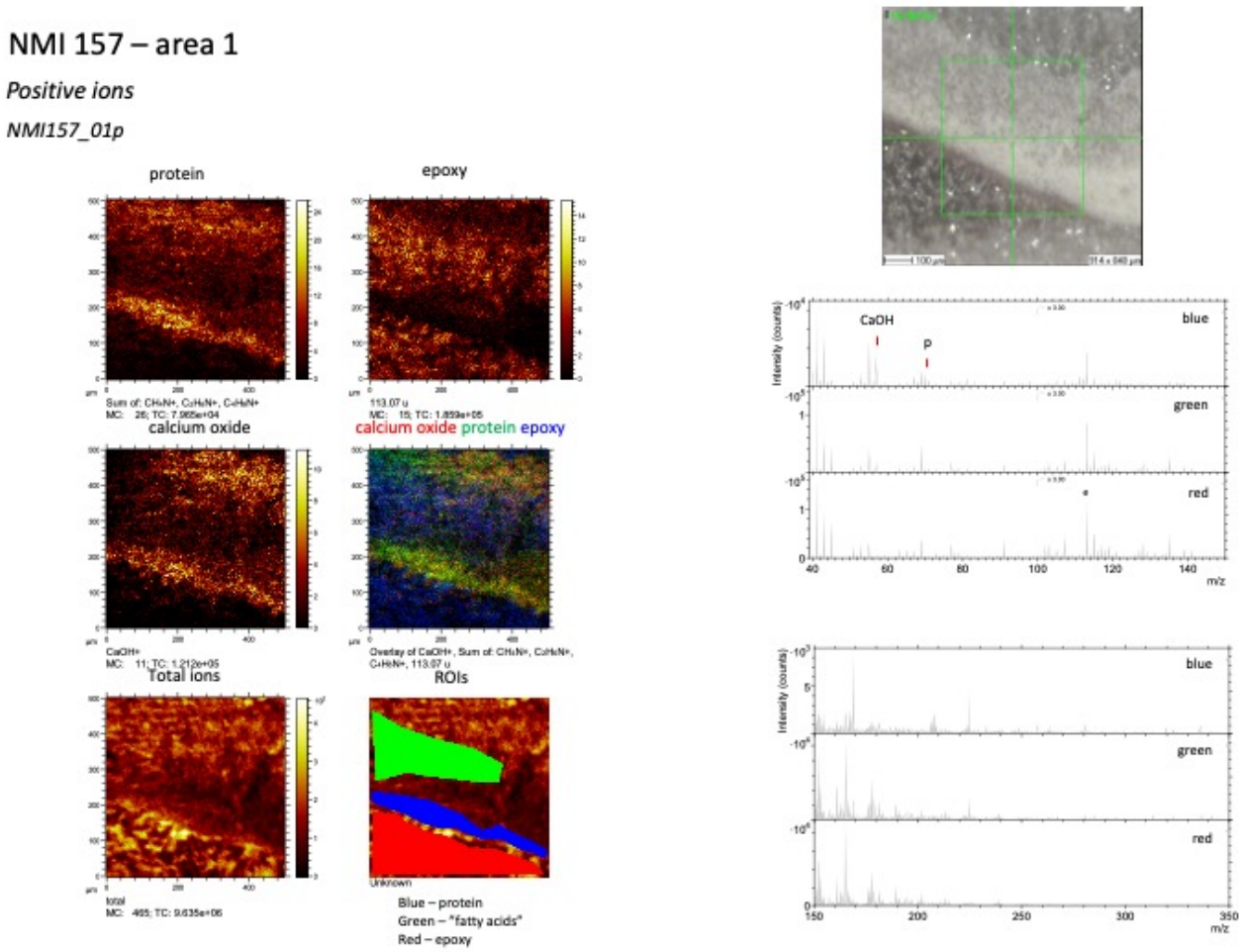


Figure 32. Data from NMI 158 (mislabeled as 157), area 1, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle row, right side of the chart, shows the distribution of calcium oxide and protein within the analyzed area.

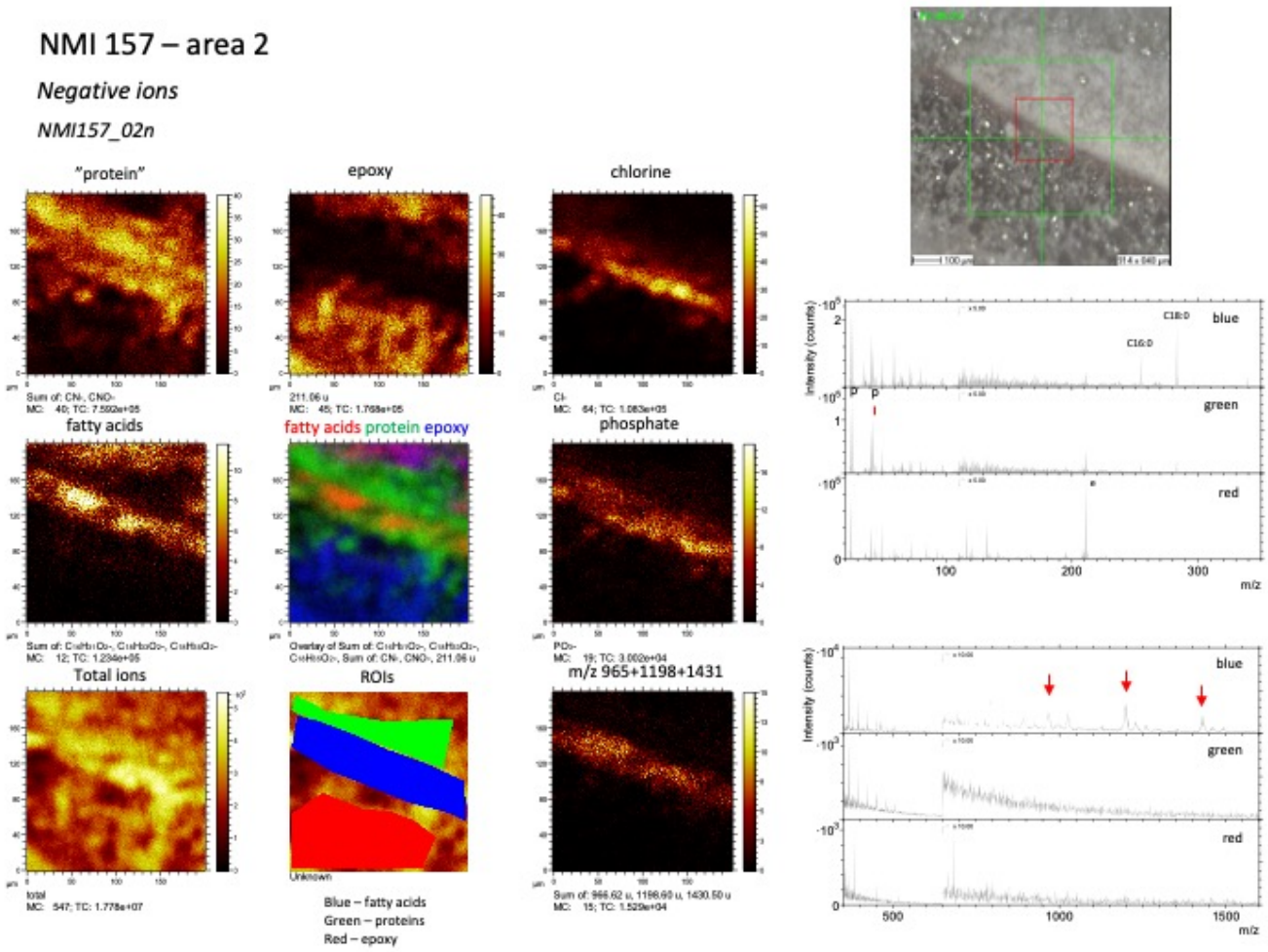


Figure 33. Data from NMI 158, area 2 neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

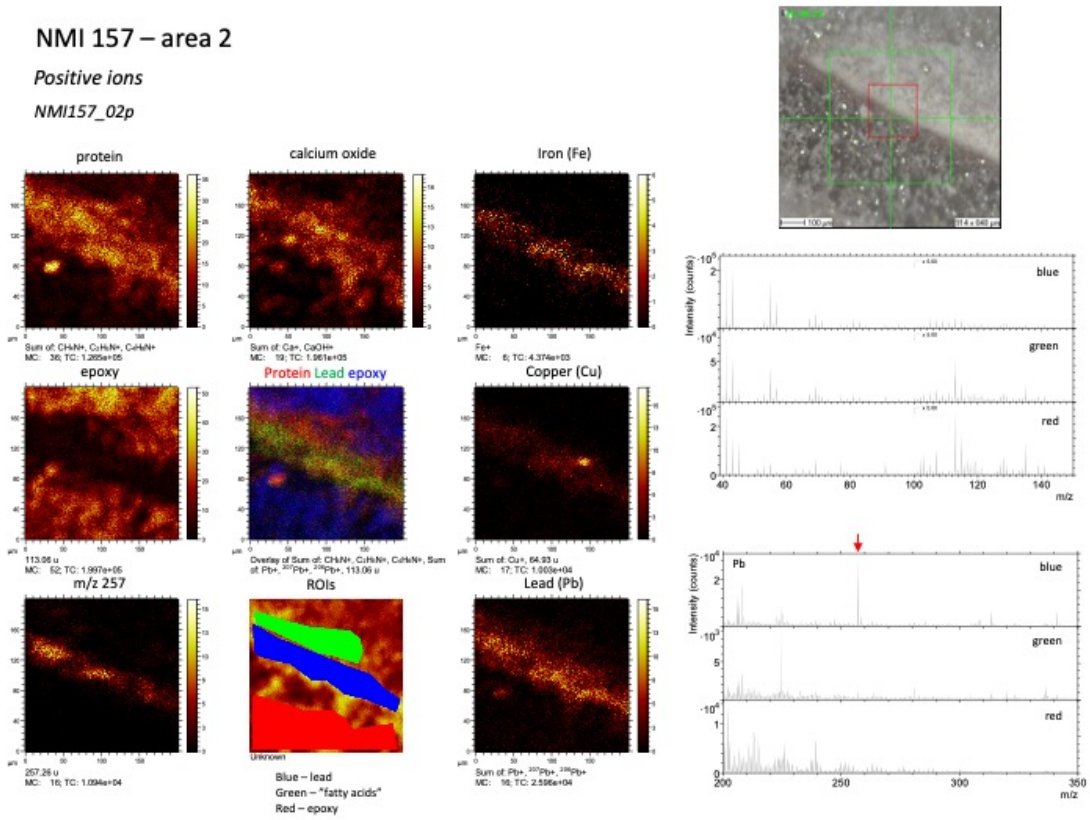


Figure 34. Data from NMI 158, area 2, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of lead and protein within the analyzed area.

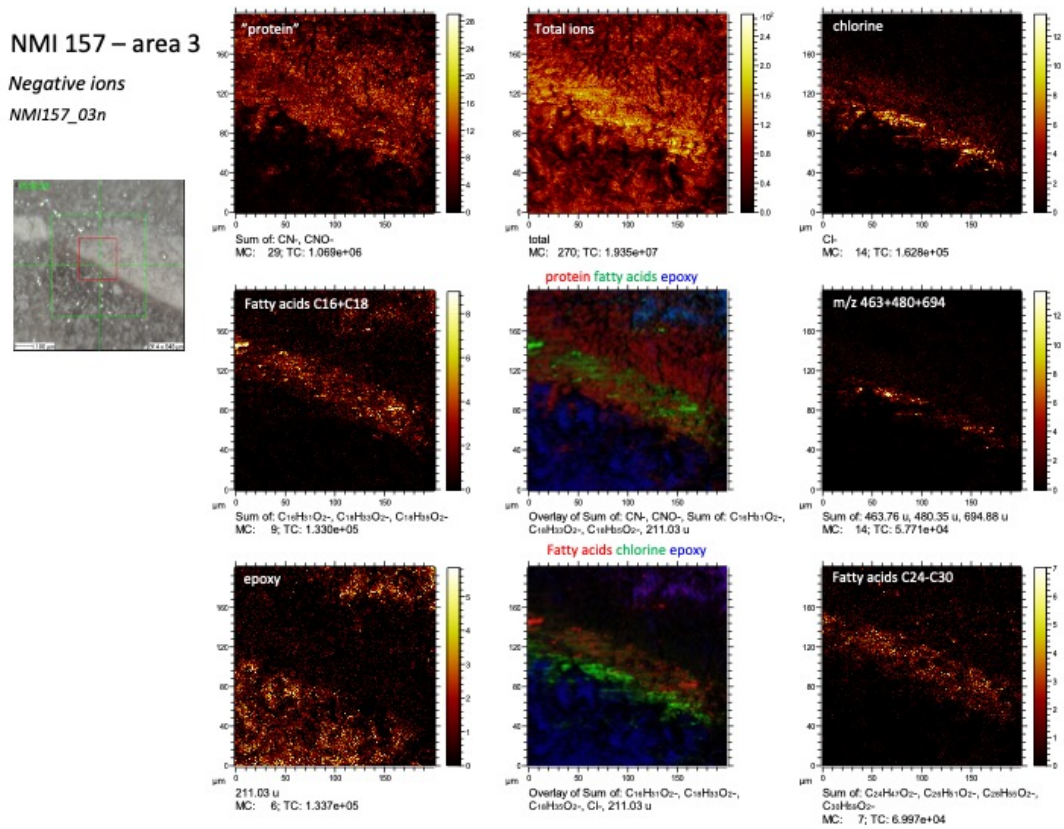


Figure 35. Data from NMI 158, area 3, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area, and the colored picture in the middle bottom row, shows the distribution of fatty acids and chlorine within the analyzed area.

6.2 NMI 232

Figure 36. Area of sampling indicated with a green circle, NMI 232. Photography: Nina Olivier.

Figure 37. Close-up area of sampling, NMI 232. Photography: Nina Olivier.

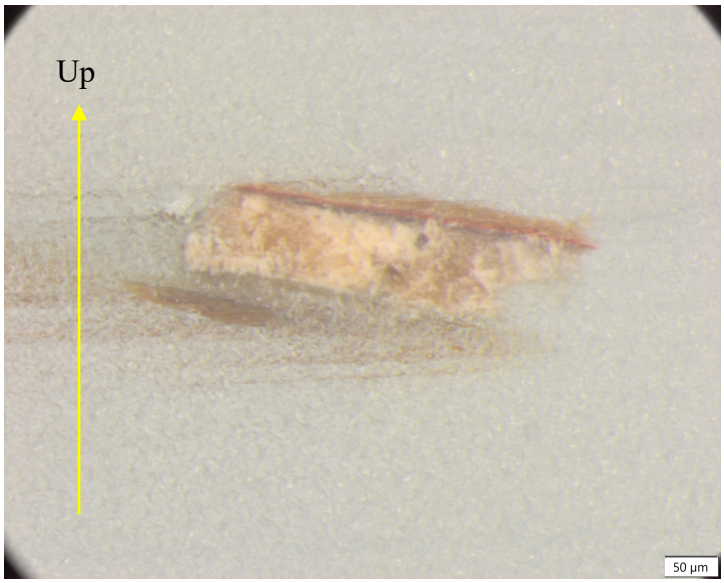


Figure 38. Cross-section from NMI 232, picture taken in microscopy at RISE by Peter Sjövall on 2023-04-25, in regular light and 50 µm.

Figure 39. Cross-section from NMI 232, picture taken in regular light at 150x using a DinoLite.

Figure 40. Cross-section from NMI 232. Picture taken in UV light 150x, using a DinoLite.

Images show a single, thin, red paint layer in the top part of the sample. On top of the paint layer, a yellow/beige substance with an uneven structure is shown. The ground layer underneath it is rather thick and white. In the bottom, a thin, dark layer is shown. Between the ground layer and the paint layer is an extremely thin, dark/grey layer. This could indicate a thin layer of surface dirt and be an indication that the prepared panel rested some time before the paint layer was applied. If the sample had been collected in the motif area, such a layer could have indicated a charcoal sketch, but as it is outside of the motif area, this option is unlikely.

Through UV light, the image shows that the ground is saturated with oil.

The NMI 232 sample was analyzed on 2023-04-25. Six areas of the sample were analyzed, and ions were measured in negative and positive mode. Images below show the all the areas, except for area 2, where no good image was saved, through extremely enhanced microscopy.

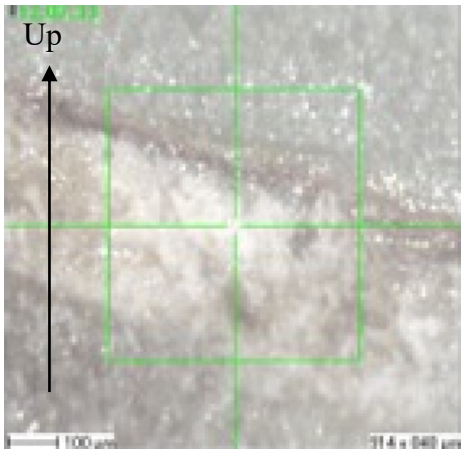


Figure 41 NMI 232, area 1, ground layer targeted with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

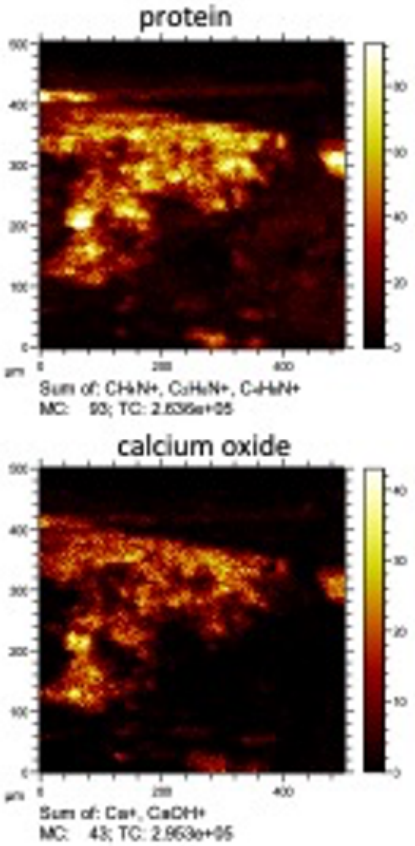


Figure 42. Ion map showing calcium oxide (CaOH) together with protein in the ground layer (area 1). The bright dots in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and calcium oxide in this area, we can see that they overlap each other, indicating distribution in the same area.

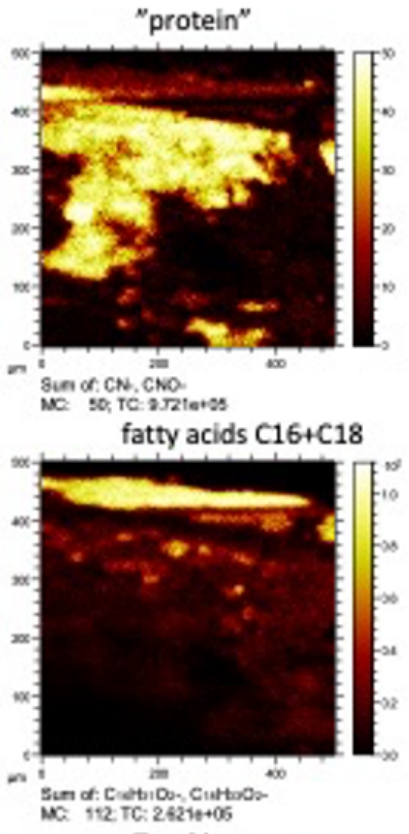
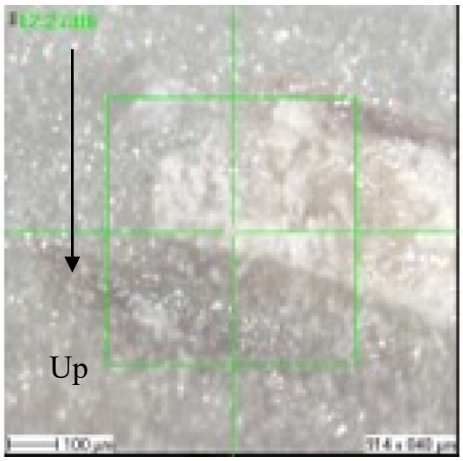


Figure 43. Ion map showing fatty acids on top of the protein cluster in the ground layer (area 1). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and fatty acids in this area, we can see that the fatty acids are mainly distributed on top of the protein.



NMI 232, area 3, ground layer targeted with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

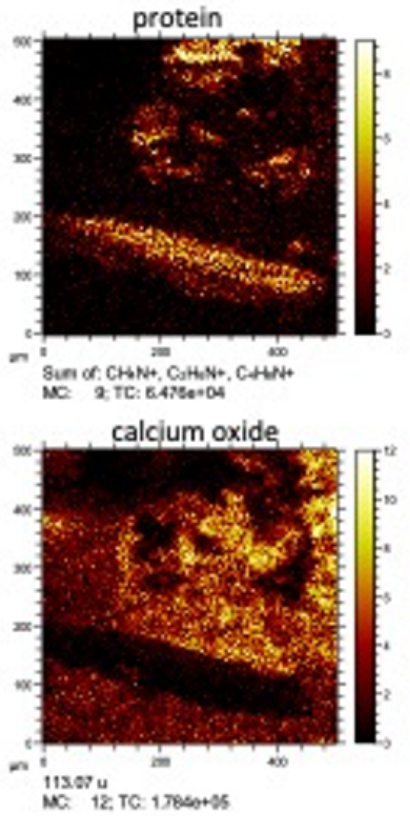


Figure 44. Ion map showing the distribution of calcium oxide in the ground layer and protein in the paint layer (area 3). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and calcium oxide in this area, we can see that although there is protein also in the ground layer, where the calcium oxide is, the main part of protein is in the paint layer.

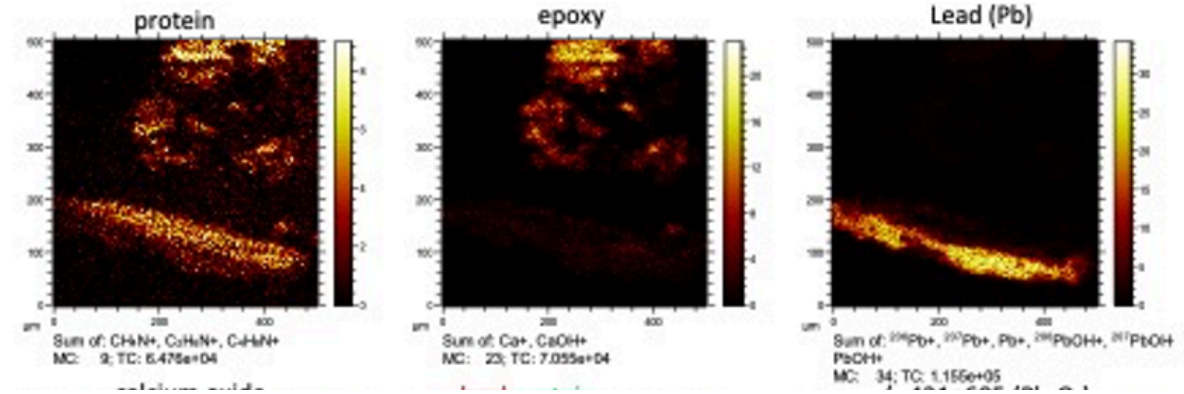


Figure 45. Ion map showing the distribution of protein and lead (Pb) in the paint layer (area 3). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and lead, we find the distribution of lead to be in the paint layer, just as the main part of the protein in this targeted area.

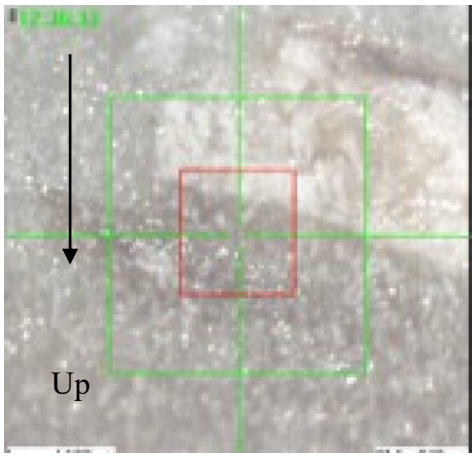


Figure 46. NMI 232, area 4, paint layer targeted with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

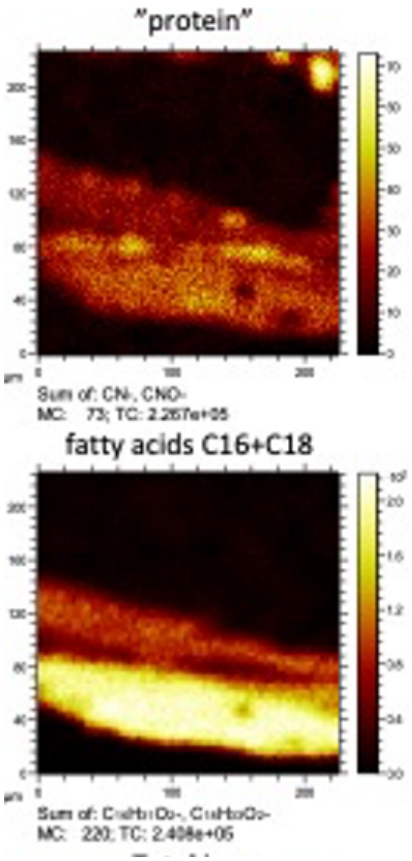


Figure 47. Ion map showing the distribution of protein and fatty acids in the paint layer (area 4). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and fatty acids, we find that the distribution of protein is in the same area as the fatty acids.

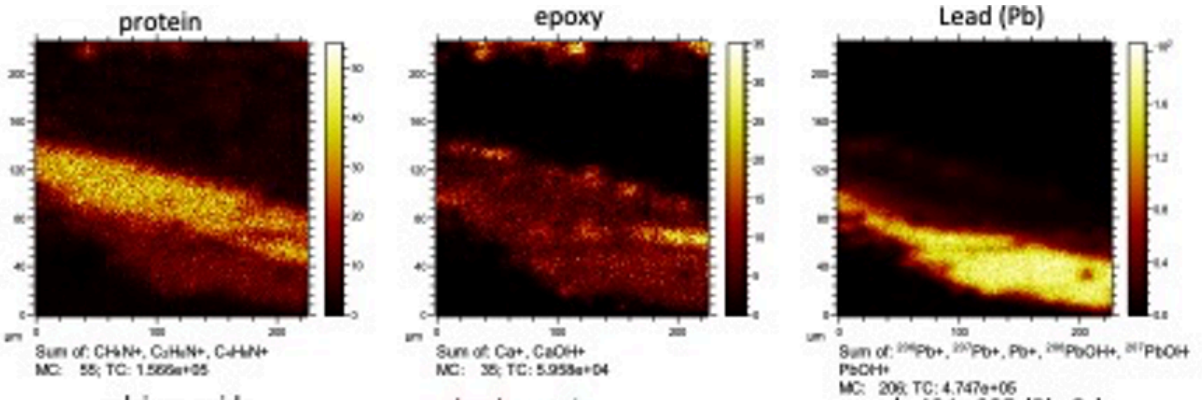


Figure 48. Ion map showing the distribution of protein and lead (Pb) in the paint layer (area 4). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and lead, we find the distribution of the materials to be within the same area.

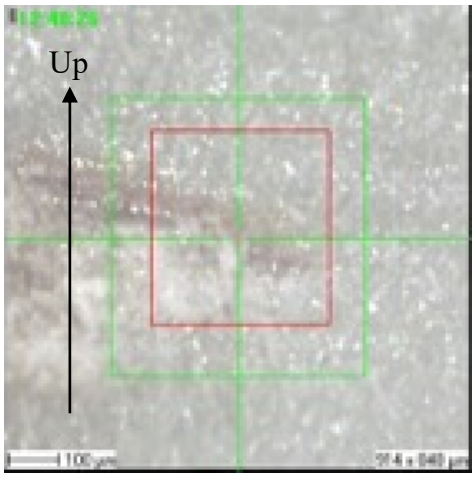


Figure 49. NMI 232, area 5, paint layer and olifa targeted with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

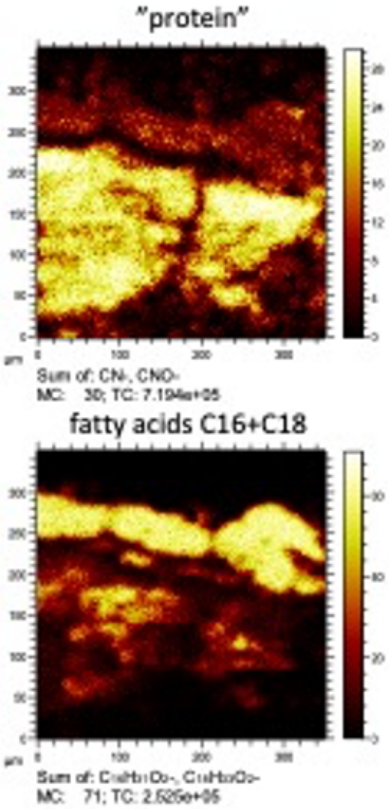


Figure 50. Ion map showing the distribution of protein and fatty acids in the paint layer and olifa (area 5). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and fatty acids, we find that that the fatty acids are distributed on top of the paint layer, and that the major part of the protein is in the paint layer in this area.

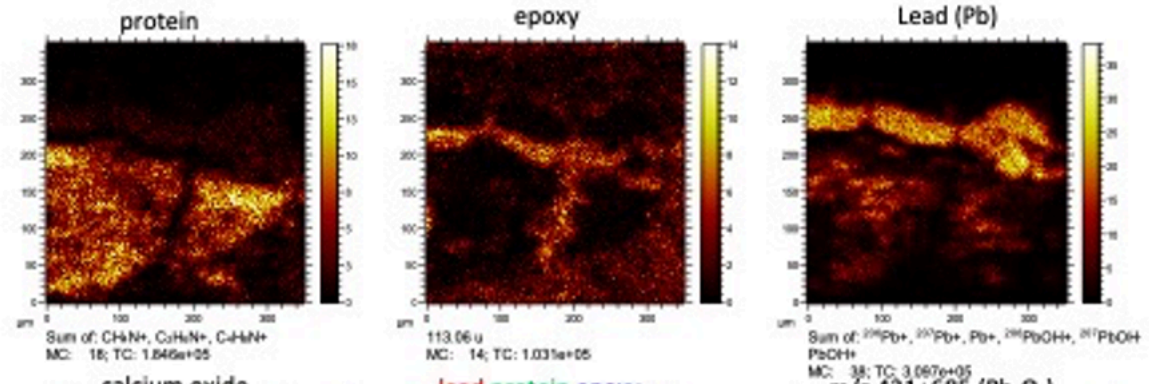
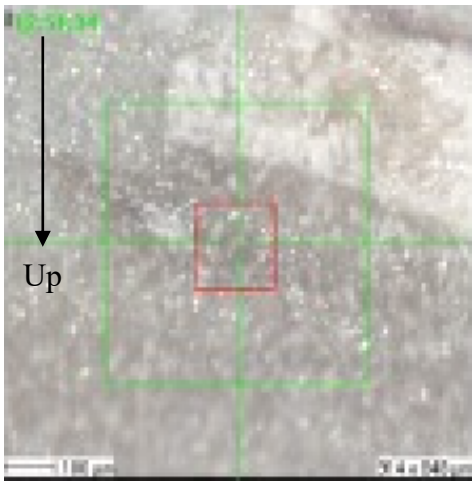


Figure 51. Ion map showing the distribution of protein and fatty acids in the paint layer (area 5). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and lead, we find that the distribution of lead follows that of the olifa, and the protein is in the paint layer in this area.



NMI 232, area 6, paint layer targeted with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

The NMI 232 sample from a 19th century Russian icon shows that;

Just as in the sample from NMI 158, calcium oxide (CaO) was found together with protein in the ground layer. This indicates a ground made of chalk ($CaCO_3$) and animal glue.

Fatty acids and lead oxide were found on top of the paint layer. (See area 5, figures 57 and 58). This could indicate that an olifa mixed with lead white (2PbCO) as a siccative had been used as a varnish for the icon.

The paint layer showed protein inside clusters of fatty acid and lead oxide, but no traces of protein were found outside of the clusters. (See area 4, figure 47). This could indicate that an olifa with lead white siccative has worked its way through the paint layer and filled the empty pores in it.

Small traces of chlorine (Cl2), phosphate (PO_4) and hydrocarbons (C_xH_y), were visible in the protein area. These could come from trace elements of the egg yolk tempera. The hydrocarbons could be an effect of the diet of the hen[205], but they could also indicate alkyl chains, produced by aliphatic hydrocarbons (occurring in aliphatic compounds, such as white spirit, turpentine etc.) and fatty acyl lipids[206]. This in turn, could possibly be traces of a previous conservation treatment.

There were areas of separated protein and methylmethacrylate in the center of the paint layer. This could indicate that the methylmethacrylate used for the samples, also managed to work its way through pores in the aqueous paint layer.

The high contents of other material in the paint layer, fatty acids, lead oxide and methylmethacrylate, could suggest that the tempera used for the paint layer, had a high content of water, which when evaporated, left many pores in the paint structure.

Also corresponding to the NMI 158 sample, fatty acids and amino acids were both found in negative mode in the sample, while only amino acids were found in the positive mode. This indicates, as in the former sample, that the binder in the paint layer was egg yolk or an egg

[205] Ushakova, T.M. et al., *Carbohydrates of the chicken egg yolk*, 1979, abstract (article in Russian) and Hwang, Keum Taek et al., *Hydrocarbons detected in irradiated and heat-treated eggs*, 2001, abstract.
[206] Thiel and Sjövall, 2014, pg. 145

yolk mixture. The same result was given in the analyses of the reference samples, where the binder was egg yolk.

As in NMI 158, the main fatty acids found in the sample, were palmitate (C16:0) and stearate (C18:0).

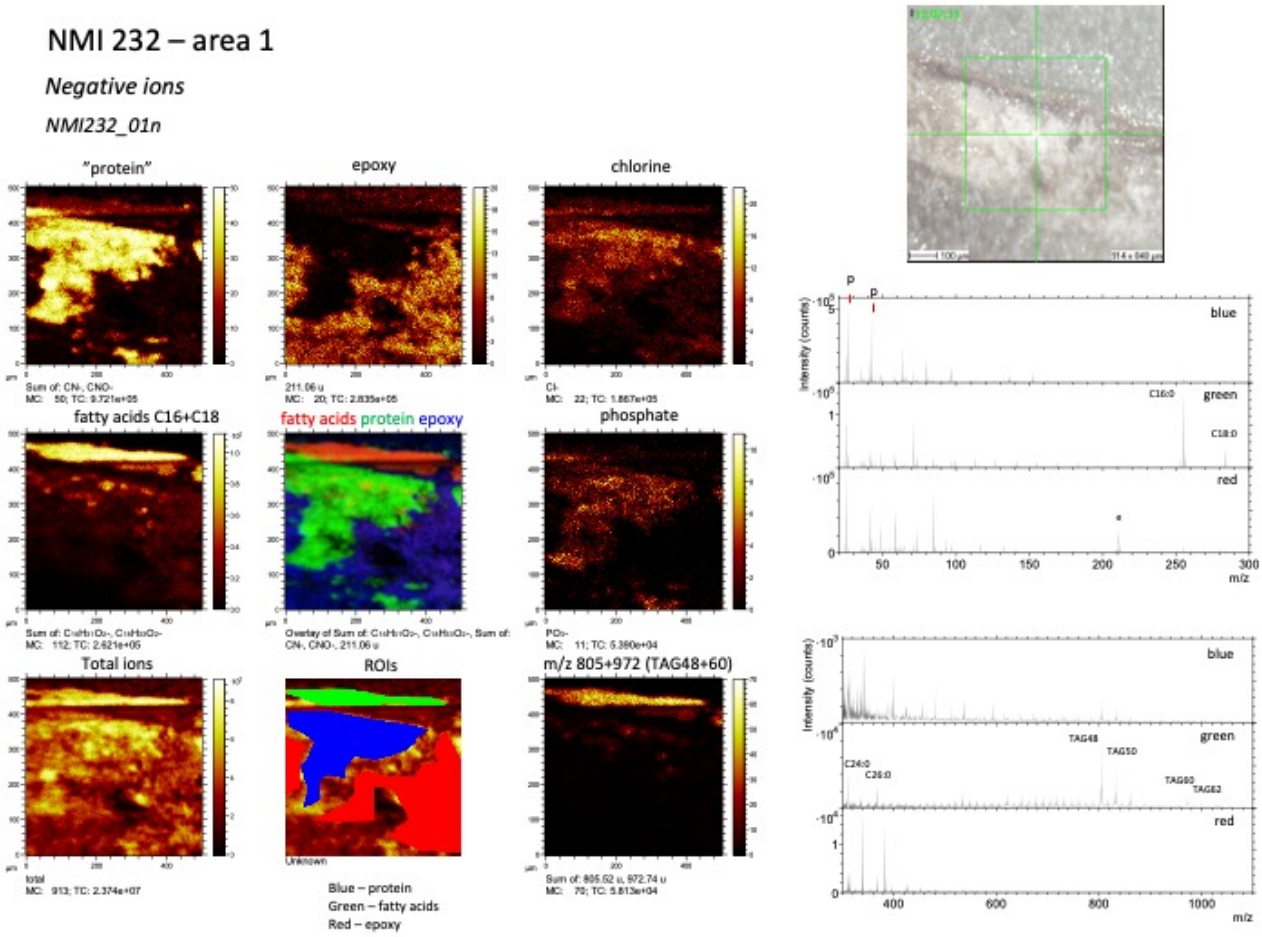


Figure 52. Data from NMI 232, area 1, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

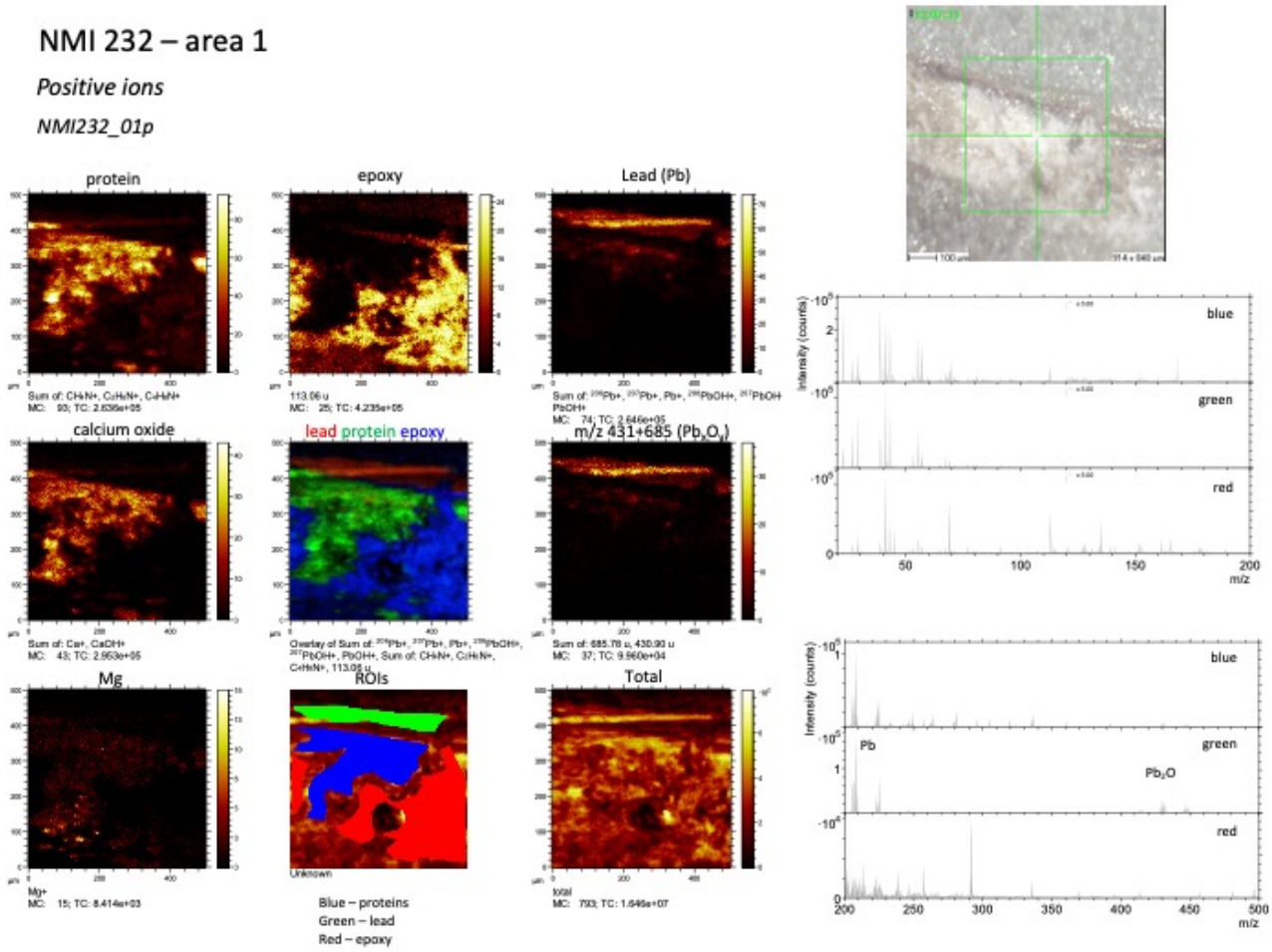


Figure 53. Data from NMI 232, area 1, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of lead and protein within the analyzed area.

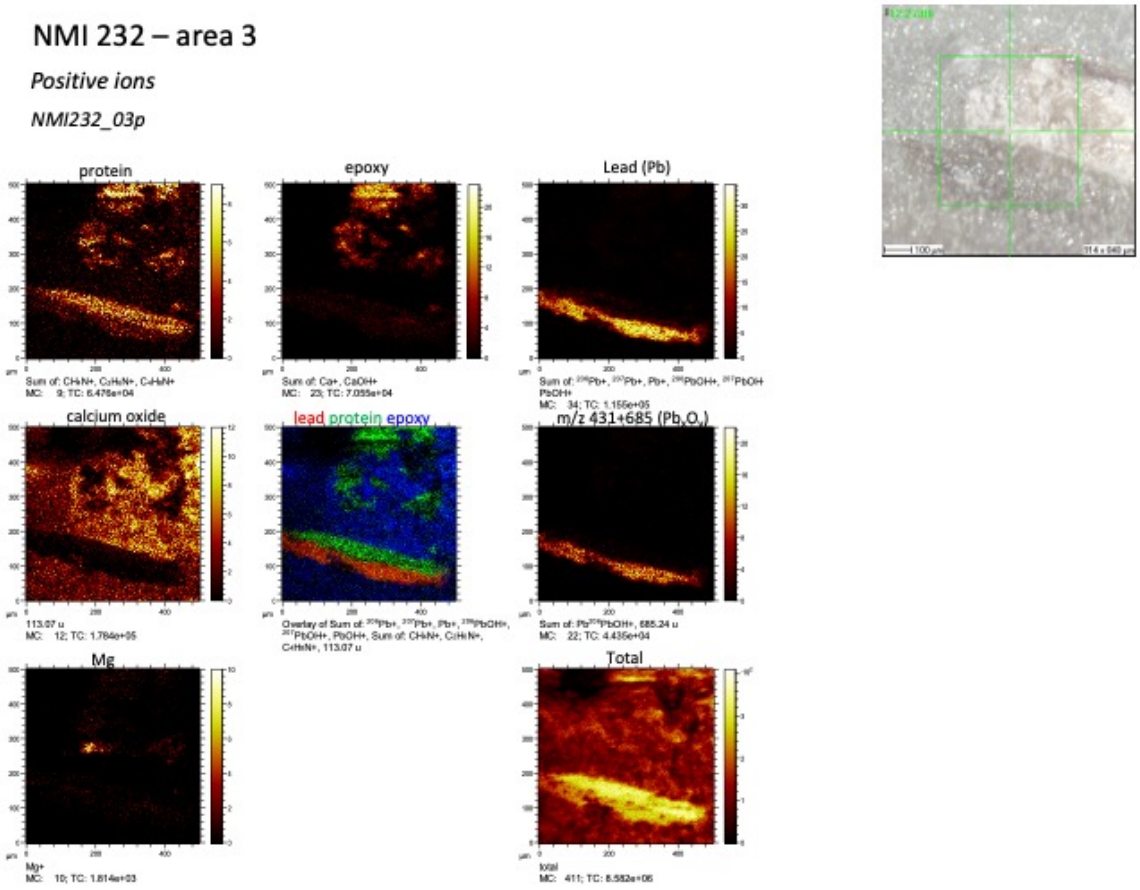


Figure 54. Data from NMI 232, area 3, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of lead and protein within the analyzed area.

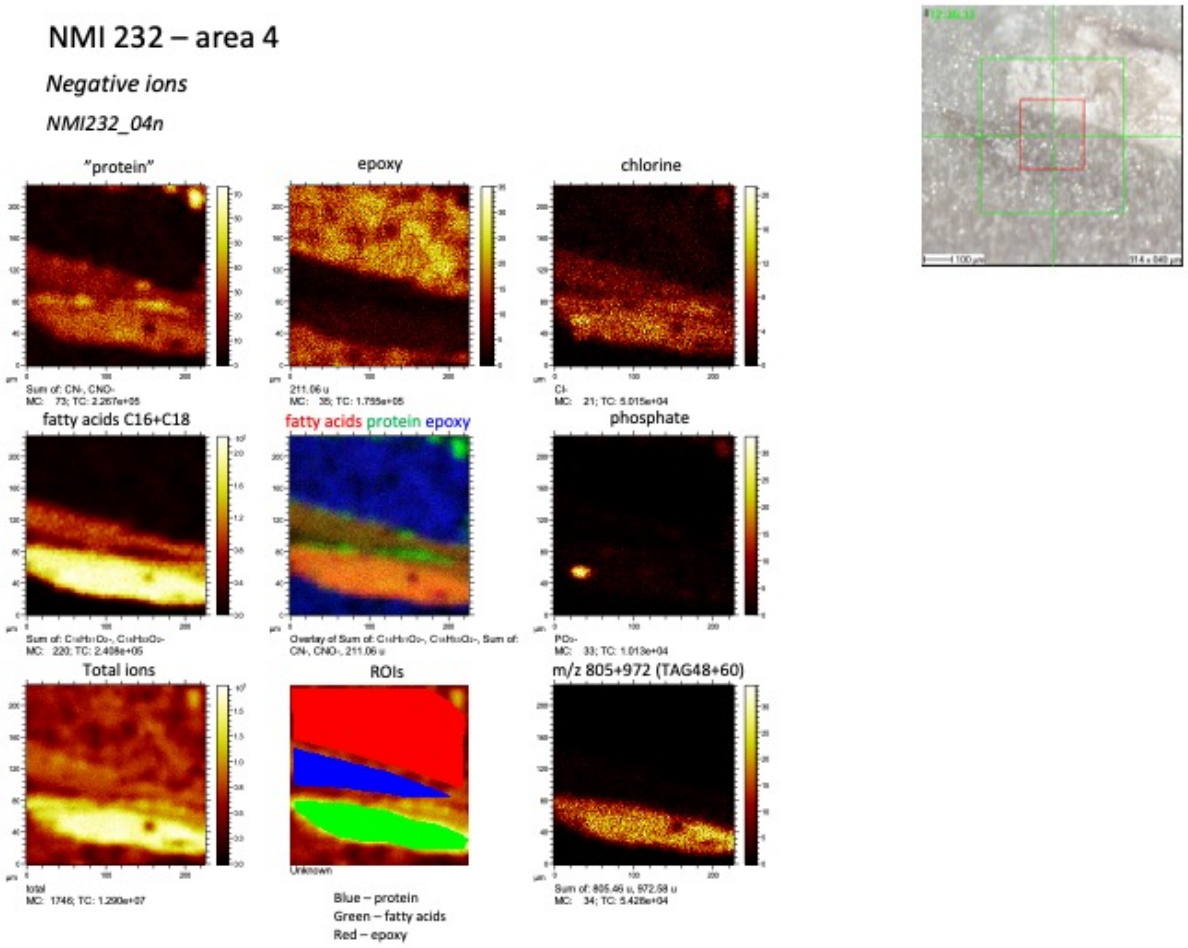


Figure 55. Data from NMI 232, area 4, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

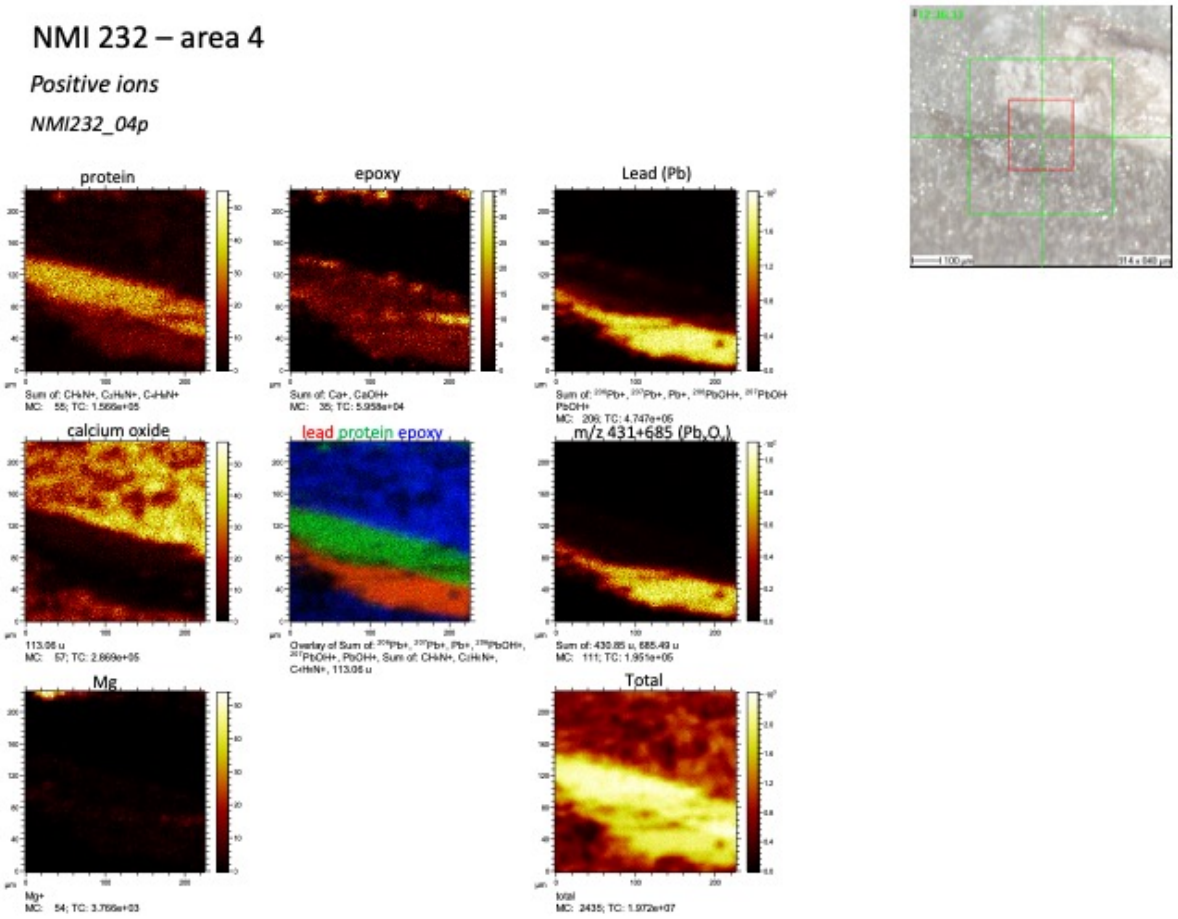


Figure 56. Data from NMI 232, area 4, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of lead and protein within the analyzed area.

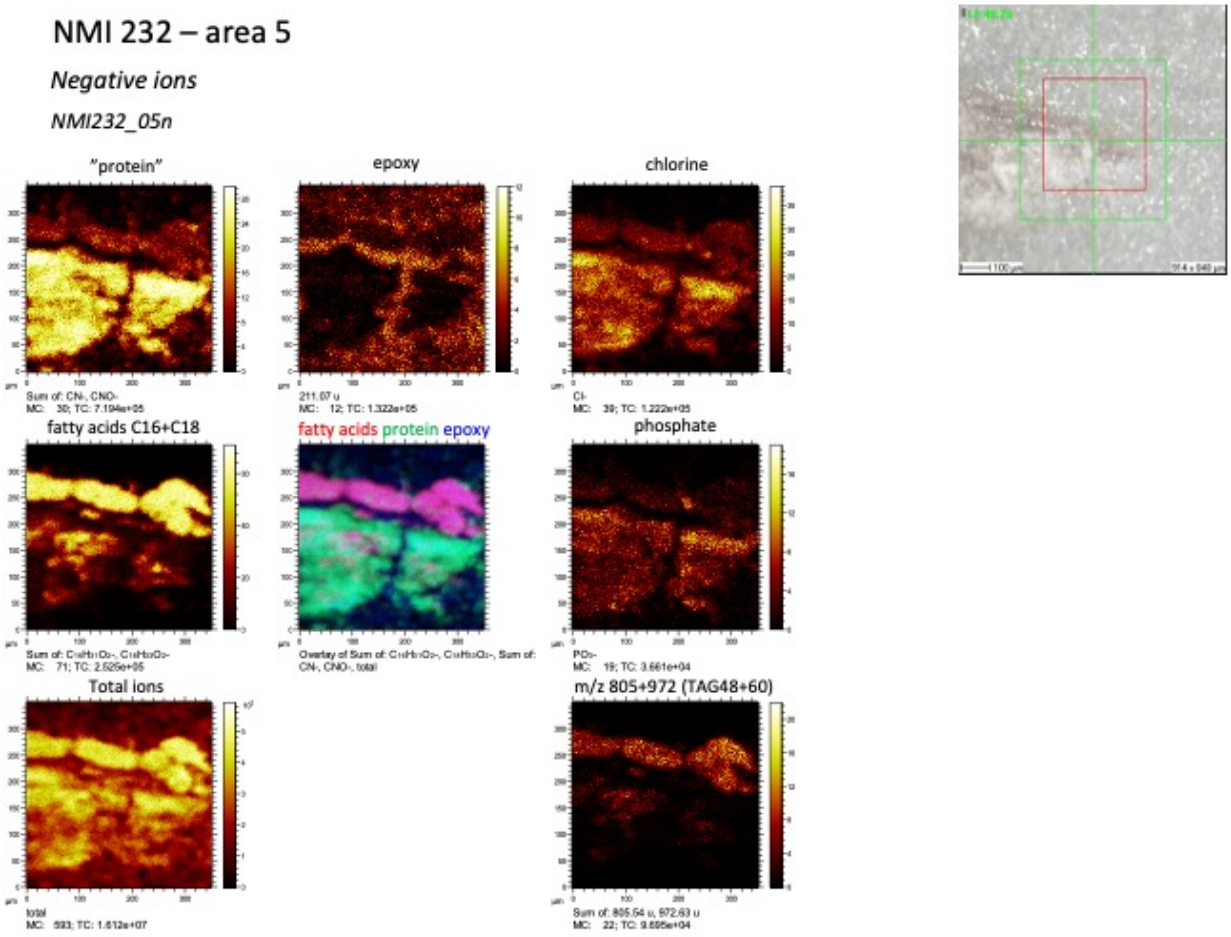


Figure 57. Data from NMI 232, area 5, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

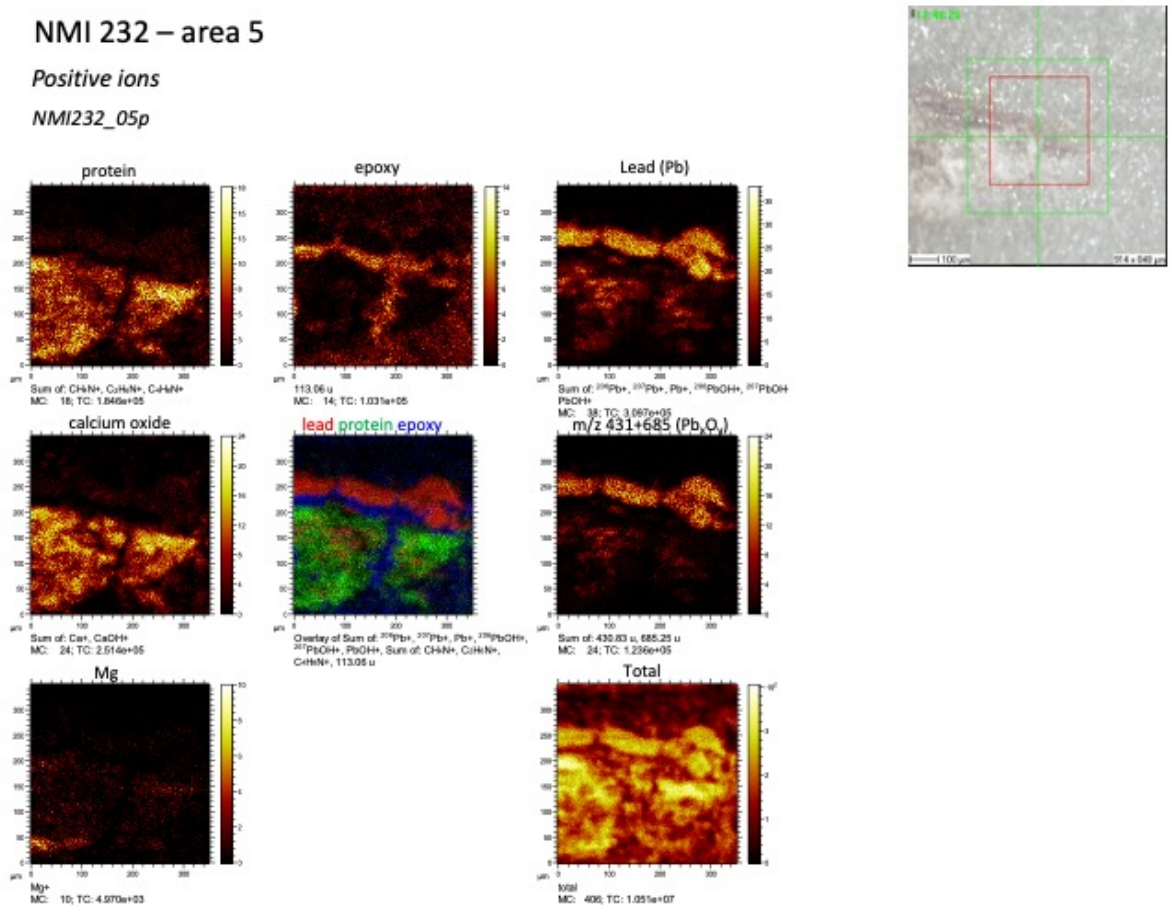


Figure 58. Data from NMI 232, area 5, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

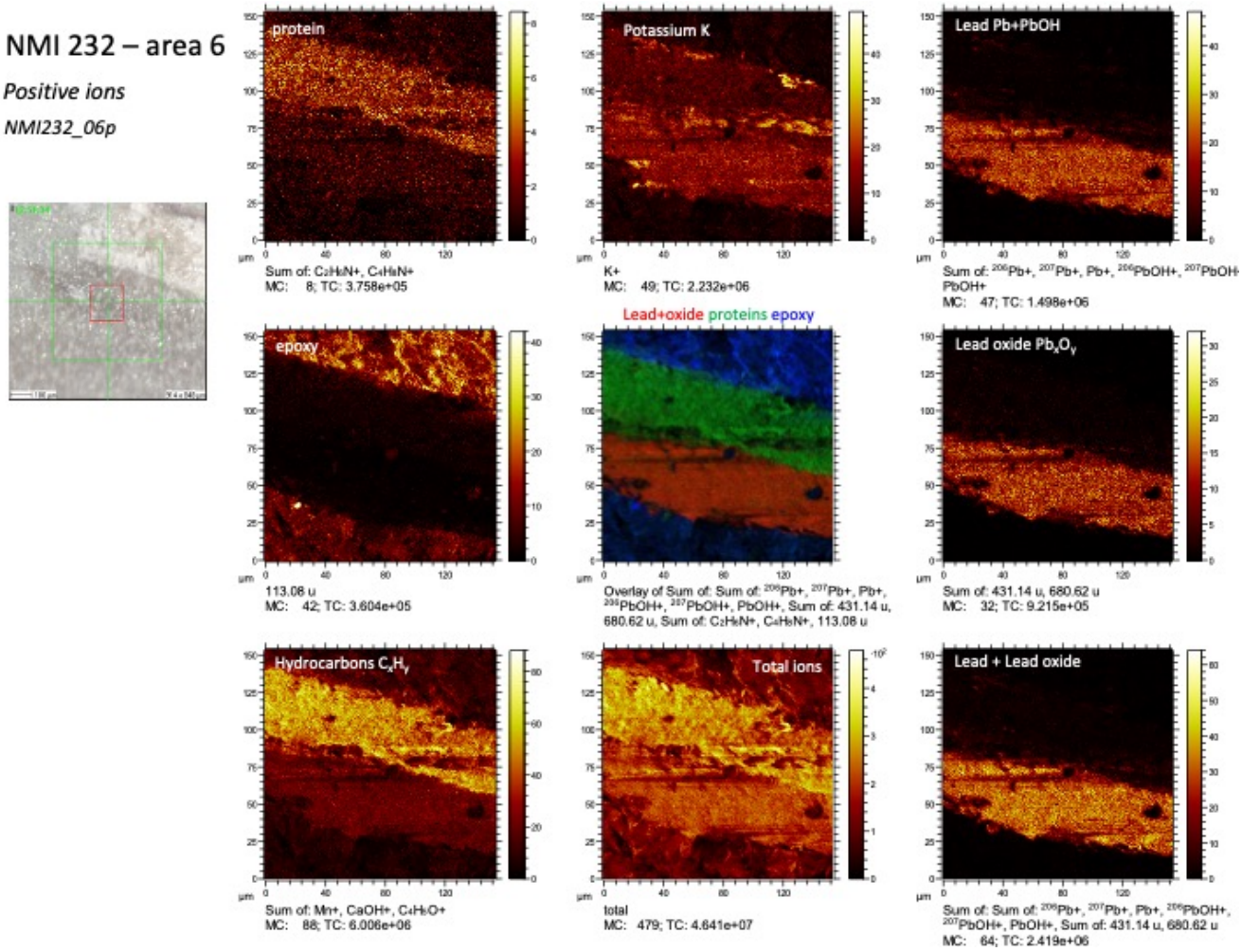


Figure 59. Data from NMI 232, area 6, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

6.3 NMI 286

Figure 60. NMI 286, Area of sampling indicated with a green circle. Photography: Nina Olivier.

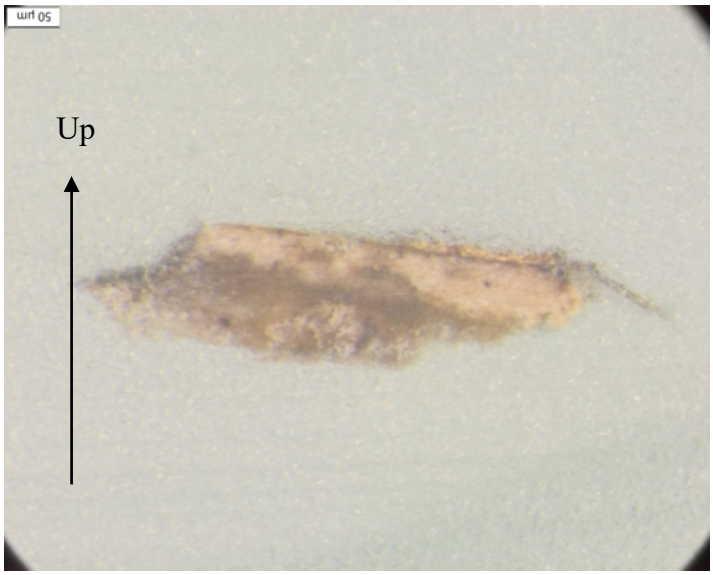


Figure 61. Cross-section from NMI 286. Picture taken at RISE on 2023-04-25 by Peter Sjövall, in regular light and 50 µml.

Figure 62. Cross-section from NMI 286. Picture taken with a DinoLite in regular light and 150x on 2023-10-16.

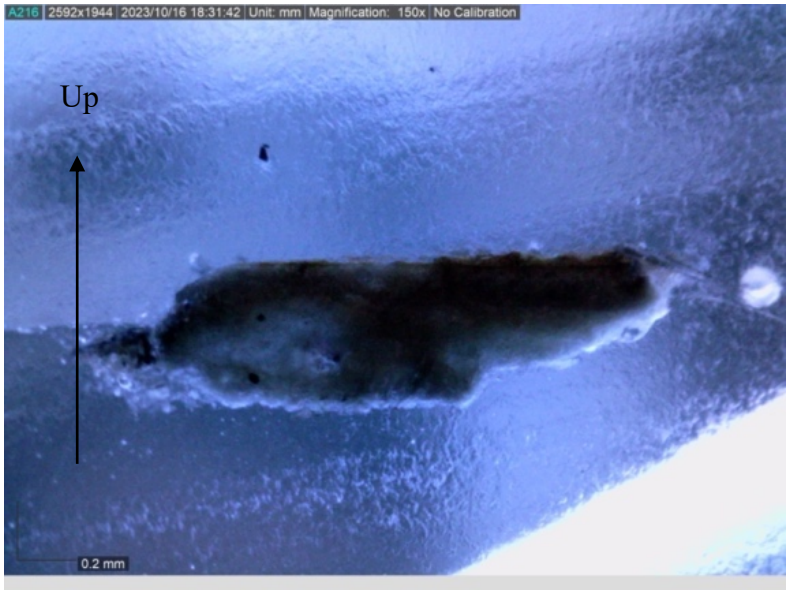


Figure 63. Cross-section from NMI 286. Picture taken in UV light with a DinoLite on 2023-10-16.

Images show a thin, red paint layer on the top of the sample. Underneath the paint layer is one thicker layer of ground, and another layer of something else. This could be a different type of ground than the one closer to the paint layer. In the ground layer, there are some pockets, where paint layer has simmered in.

The picture in UV light shows only a very dark sample.

The NMI 286 sample was analyzed on 2023-04-25. Four areas of the sample were analyzed, and ions were measured in negative and positive mode. Images below show area 2, 3 and 4 through extremely enhanced microscopy.

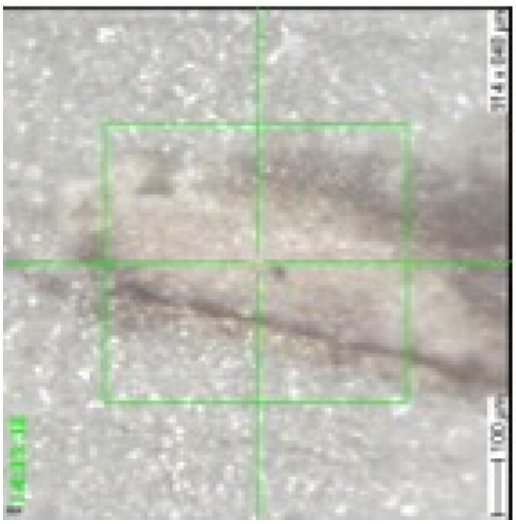


Figure 64. NMI 286, area 2, ground layer with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

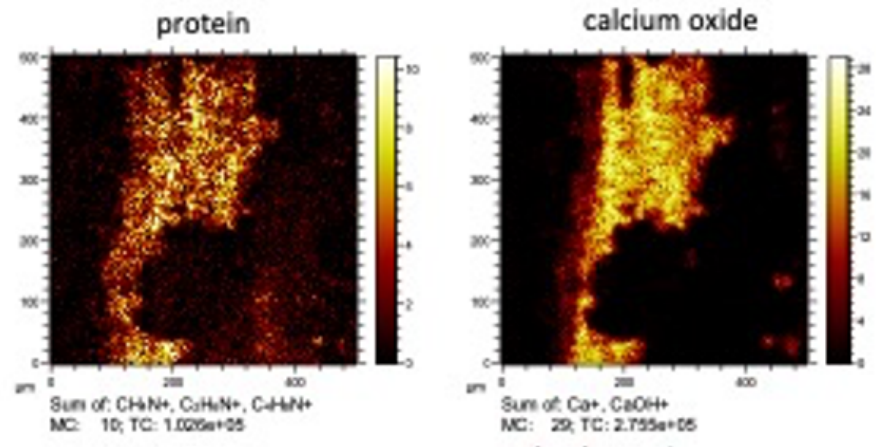


Figure 65. Ion map showing the distribution of protein and calcium oxide in the ground layer (area 2). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein and calcium oxide, we can see that they are distributed in the same way within this area.

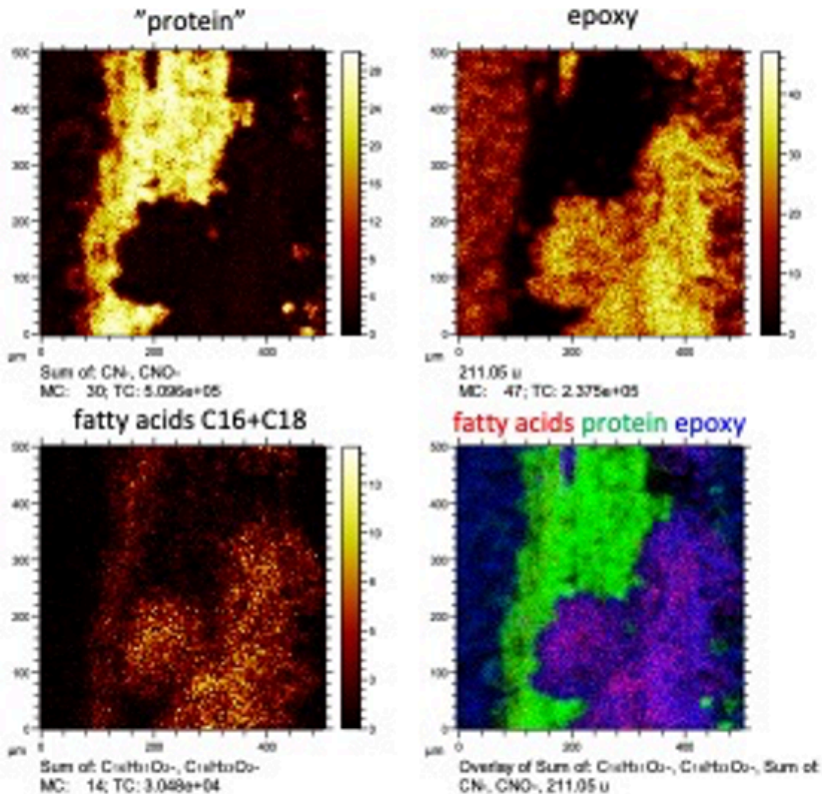


Figure 66. Ion map showing the distribution of protein, fatty acid and methylmethacrylate in the ground layer (area 2). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of fatty acids and methylmethacrylate (labeled epoxy), we can see that they are distributed the same way within this area.

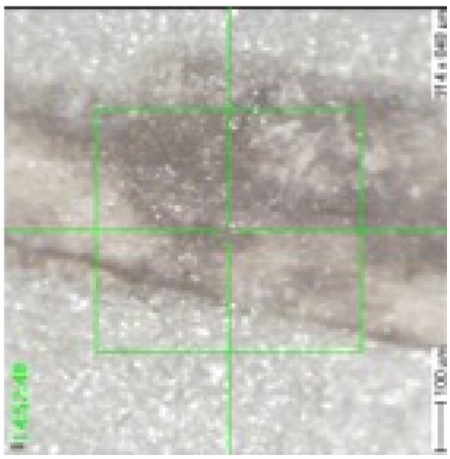


Figure 67. NMI 286, area 3, ground and paint layer with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

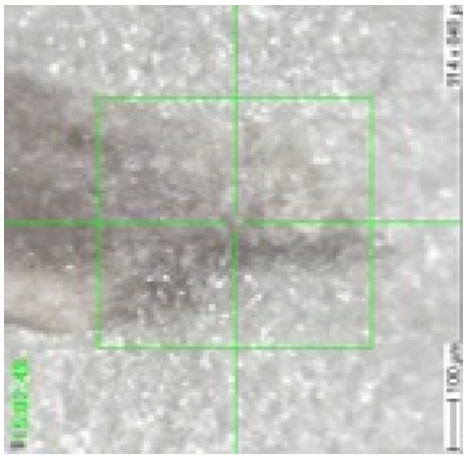


Figure 68. NMI 286, area 4, paint layer with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

The analysis of the NMI 286 sample, deriving from a late 18th century Greek icon, shows that;

Just as the other samples, calcium oxide (CaO), was found together with protein in the ground layer. This indicates a ground made of chalk ($CaCO_3$) and animal glue.

No major metal compounds (Pb, Fe, Cu, Mn, Hg etc.), were found in the paint layer, but there were trace elements of chlorine (Cl2) and phosphate (PO_4) in negative mode, and sodium (Na) and potassium (K) in positive mode, which could be trace elements from egg yolk.

Corresponding to all the other samples, fatty acids and amino acids were both found in negative mode in the sample. Only amino acids, however, were found in the positive mode. As in the other samples, this would indicate an egg yolk binder, or a mixture containing egg yolk.

The fatty acids found in this sample were mainly oleate (C18:1) and stearate (C18:0), where oleate was the predominant one. As the studies by Bouvier et al., 2022;57, indicate, the oleate ratio will be higher in egg yolk than in drying oils. This could indicate an egg yolk tempera in the NMI 286 sample, but it could also be an indication of an olive oil having been used as an olifa.

Fatty acids were also found in the methylmethacrylate. (See area 2, figure 52). This could possibly indicate that some loose substances from the surface, broke loose with the impact of the casting process.

Very interestingly, the NMI 286 sample was highly similar to the reference sample R2, which was prepared with a pre-size of animal glue (cow hide) and a ground of chalk and animal glue. A tempera made from egg yolk + vinegar (1:1) mixed with pigments was used for the paint layer.

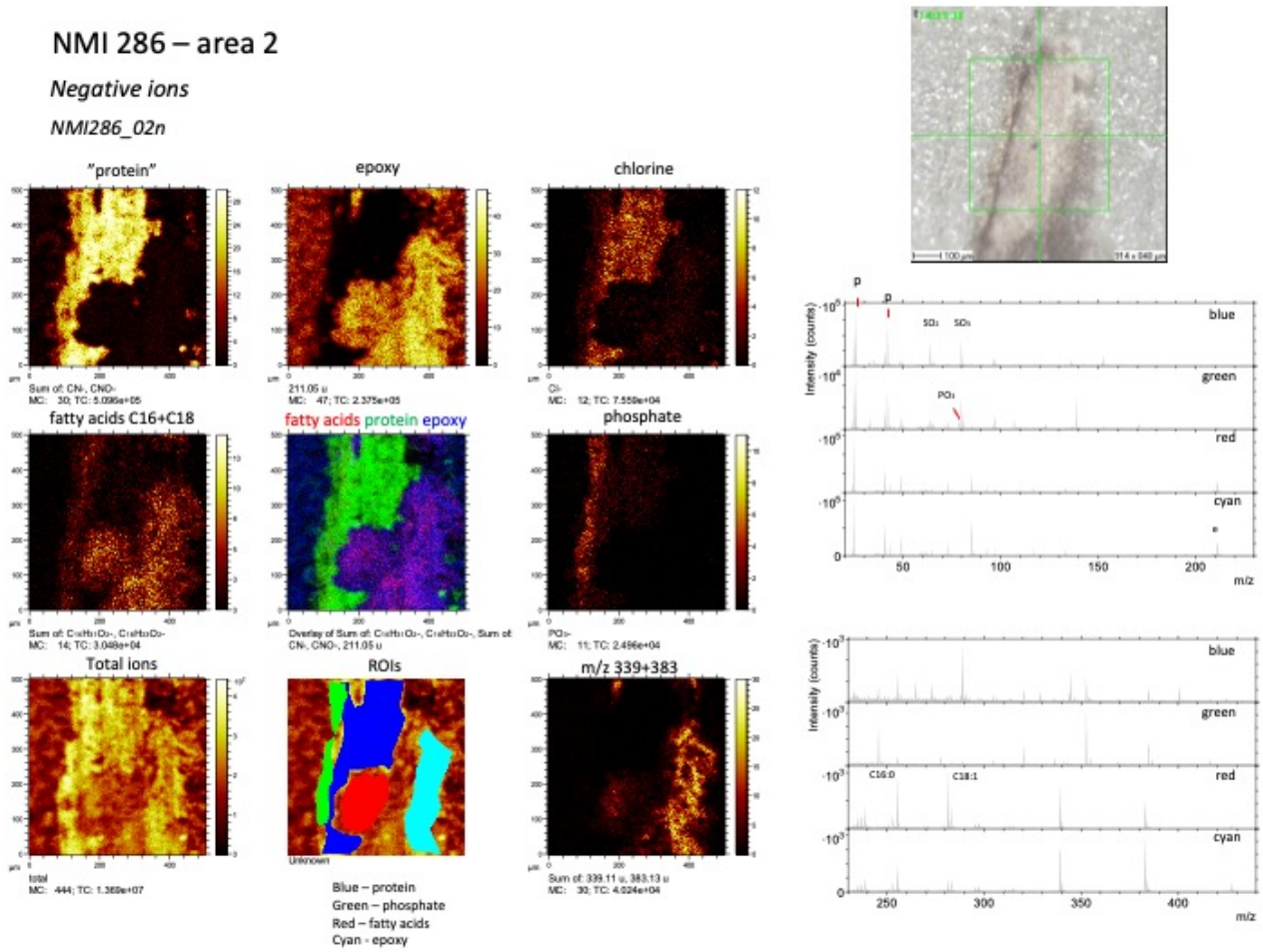


Figure 69. Data from NMI 286, area 2, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

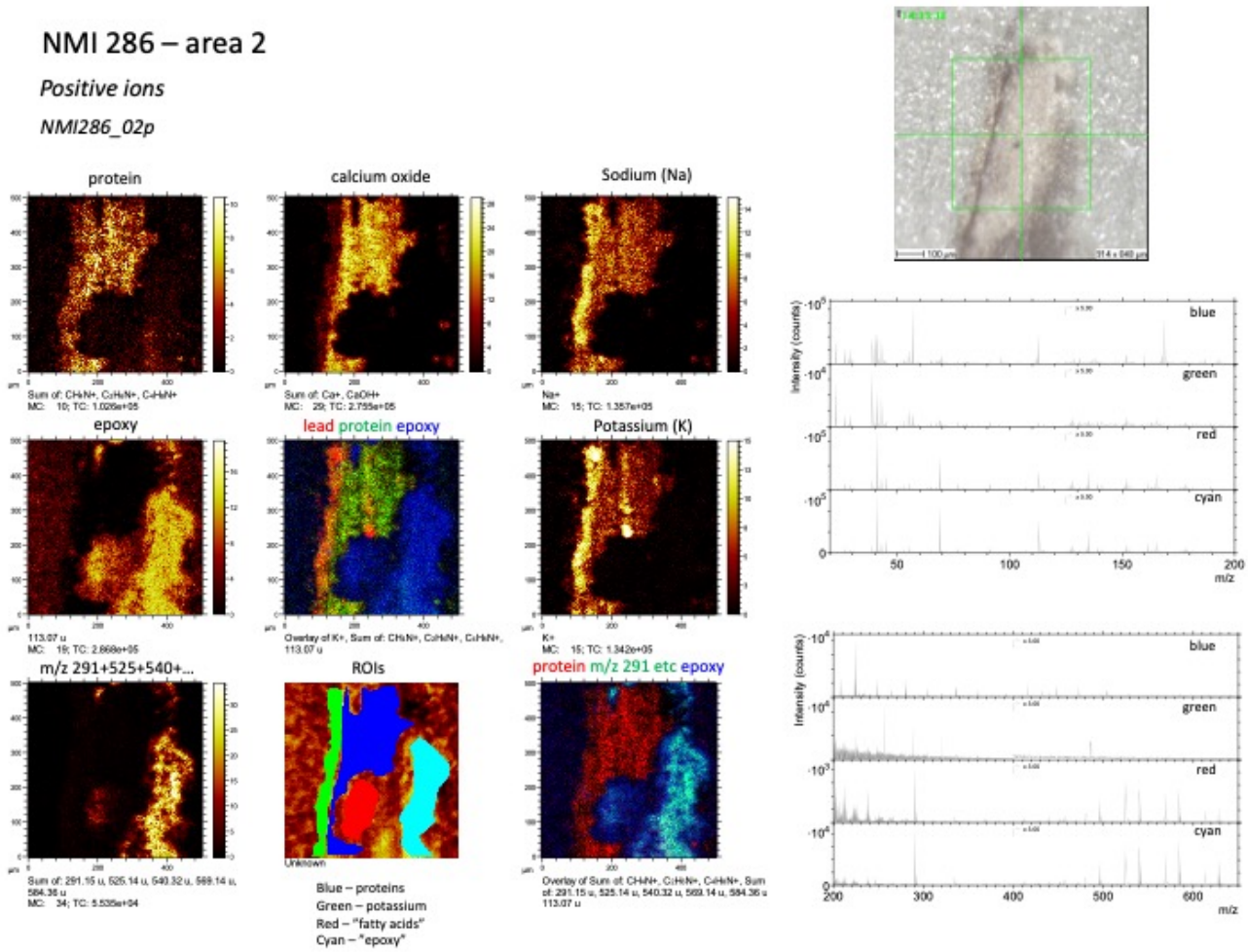


Figure 70. Data from NMI 286, area 2, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of lead and protein within the analyzed area.

6.4 NMI 287

Figure 71. NMI 287. Area of sampling indicated with a green circle. Photography: Nina Olivier.

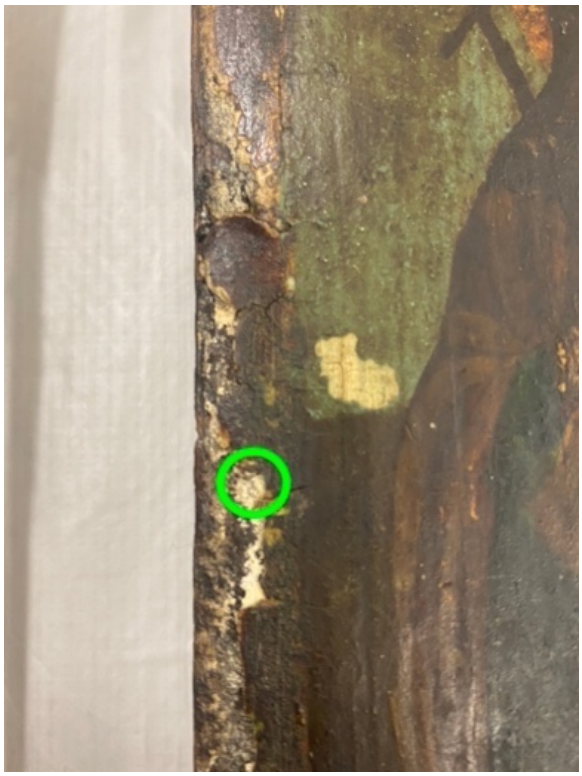


Figure 72. NMI 287. Close-up of area of sampling. Photography: Nina Olivier.

Figure 73. Cross-section from NMI 287. Picture taken with a DinoLite in regular light ca 40x on 2023-05-17 by Nina Olivier.

Figure 74. Cross-section from NMI 287. Picture taken in UV light in ca 75x on 2023-05-17 by Nina Olivier.

The NMI 287 sample was analyzed on 2023-04-12. In the preparation process of making the sample even smaller to fit in the sample cradle for the ToF-SIMS analysis, the sample broke and only a small fragment was left of the already from the beginning small sample. The reading of this sample was therefore not as informative as the analyses of the other samples. As the sample NMI 287 was very small, microscopic images were hard to take. Nevertheless, both a dark paint layer and some fragments of ground, are visible. One area of the sample was analyzed and ions were measured in negative and positive mode. The image below shows the area through extremely enhanced microscopy.

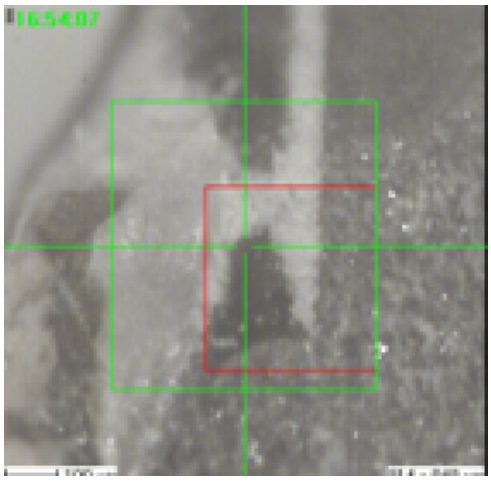


Figure 75. NMI 287, area 1, ground and paint layer with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

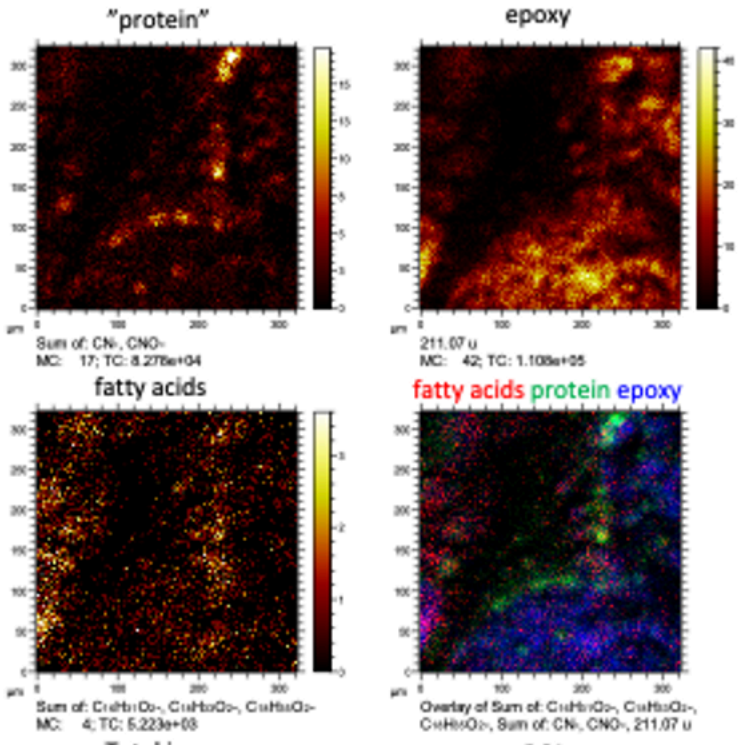


Figure 76. Ion map showing the distribution of protein, fatty acid and methylmethacrylate (labeled epoxy) in area 1. The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images of protein, fatty acids and methylmethacrylate, we can see that the methylmethacrylate dominates the area, but that protein is nevertheless following a thin layer.

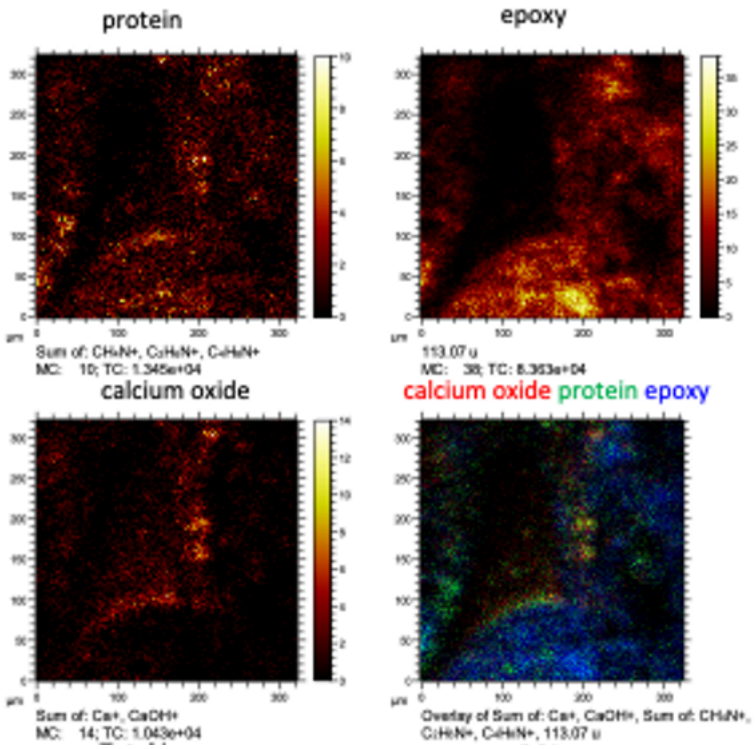


Figure 77. Ion map showing the distribution of protein, calcium and methylmethacrylate (labeled epoxy) in area 1. The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images, we can see that the distribution of calcium oxide in this area follows that of the protein.

The sample from NMI 287 coming from a Greek 19th icon was not as conclusive in the analysis as the other samples. This could probably be explained by the fact that the sample broke in the preparation process and was hard to polish. It also was too small to go in the microtome for extra preparation beforehand.

The paint layer was dominated by methylmethacrylate. This probably has to do with the fact that the sample could not be polished the same way as the other samples, due to the break in the preparation process.

Distorted protein layers made it hard to distinguish different components. But indication of protein was nevertheless there. It followed a very thin layer. In the same layer, calcium oxide was found in positive mode. This could suggest that the protein layer found in the sample, was close to the ground layer, or that the layer is a ground layer made of animal glue and chalk. Small traces of chlorine (Cl2) and sulfate (SO_4), (and extremely small, scattered traces of phosphate (PO_4)) were also found in the same layer. These could be trace elements found in egg yolk.

Corresponding to all the other samples, fatty acids and amino acids were both found in negative mode in the sample. Only amino acids, however, were found in the positive mode. As in the other samples, this would indicate an egg yolk binder, or an egg yolk mixture.

The fatty acids observed in the sample were mainly palmitate (C16:0) and stearate (C18:0). The fatty acids were scattered in the sample, but predominant on one side of the sample, which could be indicated to be the surface of the sample and a suggestion of fatty acids from an olifa on the surface of the icon.

There were unidentified organic ions at m/z 368 (pos) and m/z 666 (neg) in the paint region.

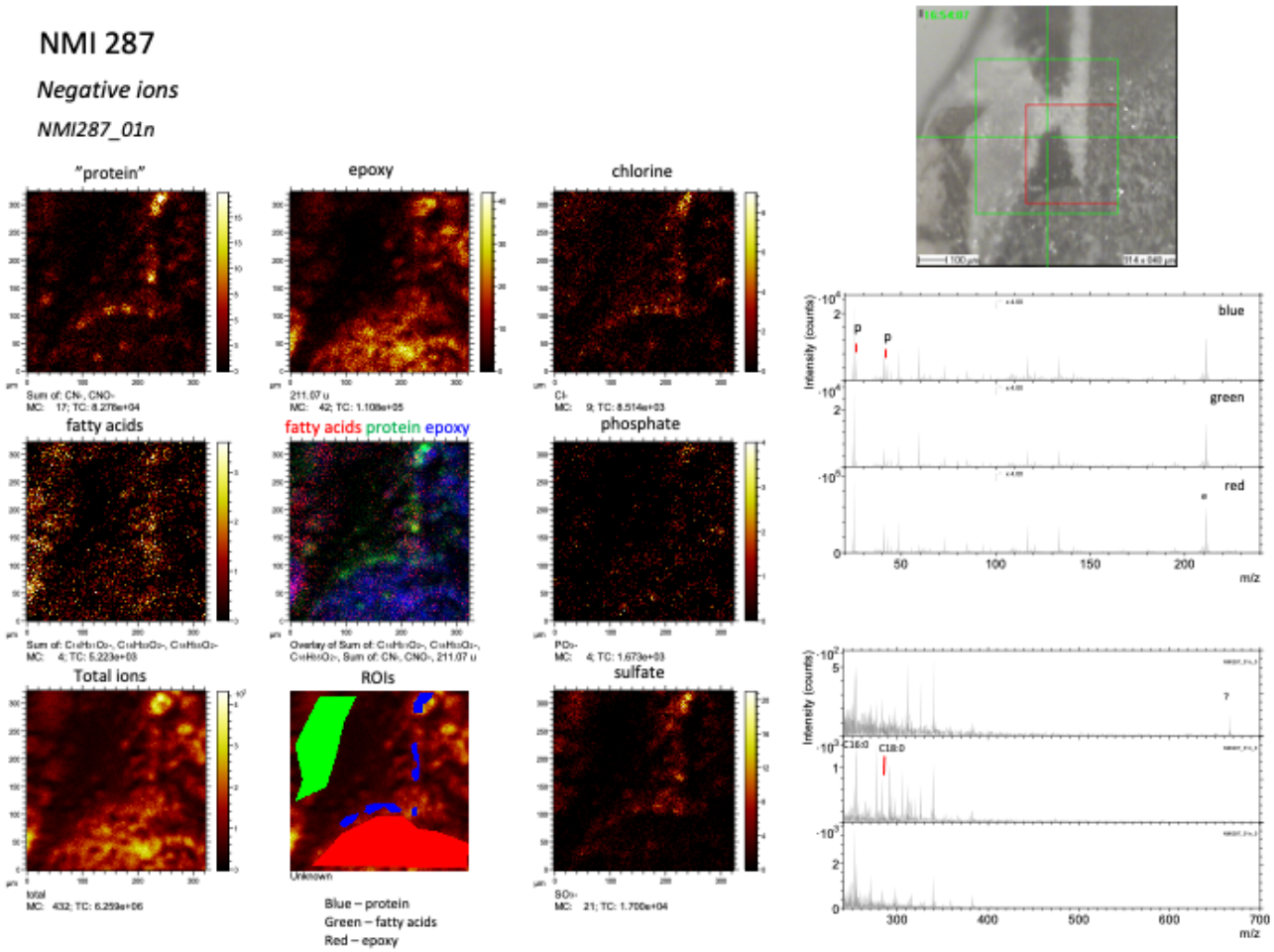


Figure 78. Data from NMI 287, area 1, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

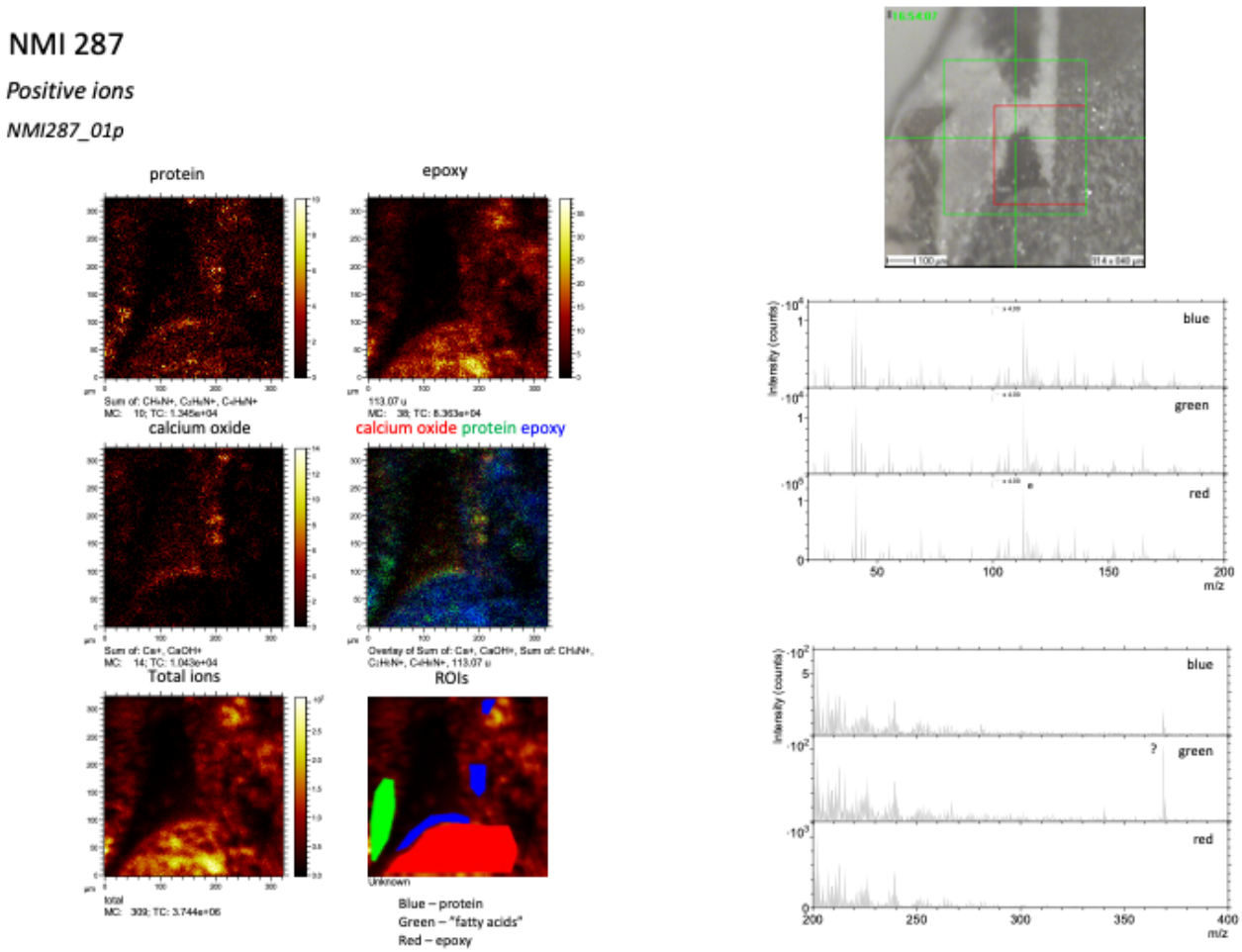


Figure 79. Data from NMI 287, area 1, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of calcium oxide and protein within the analyzed area.

6.5 R1

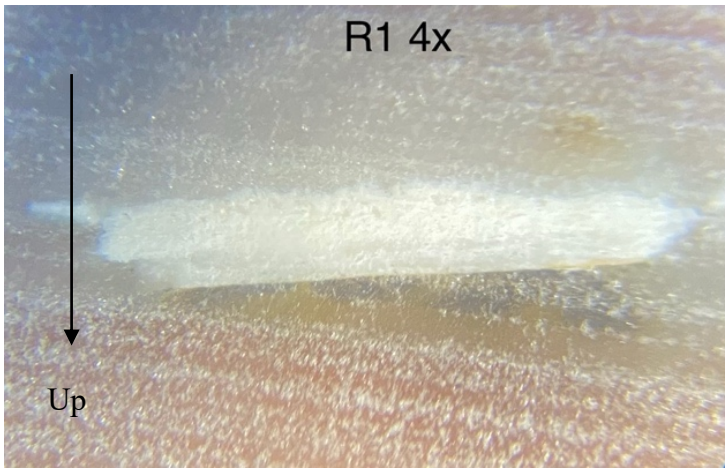


Figure 80. Cross-section from R1, picture taken in regular light, 4x by Nina Olivier on 2023-05-15.

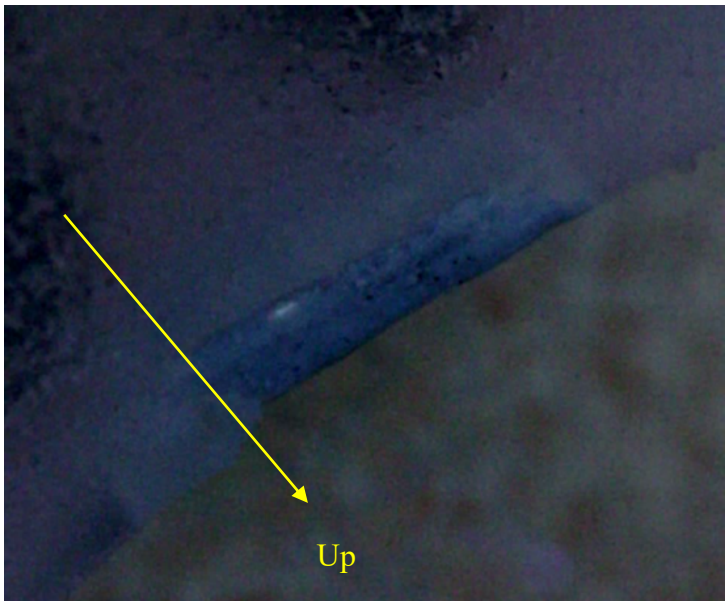


Figure 81. Cross-section from the R1 sample, picture taken in UV light ca 90x using a DinoLite by Nina Olivier on 2023-05-17.

The image of the reference sample R1, in regular light shows an extremely thin paint layer, in the bottom of the picture (more visible in the picture taken in UV light). The ground is on top, showing two layers of application, where a thin layer of surface dust is between the two layers, indicating that some time passed between the application of the layers.

The R1 sample was analyzed on 2023-04-12. Two areas of the sample were analyzed and ions were measured in negative and positive mode. The image below, shows area 2 through extremely enhanced microscopy.

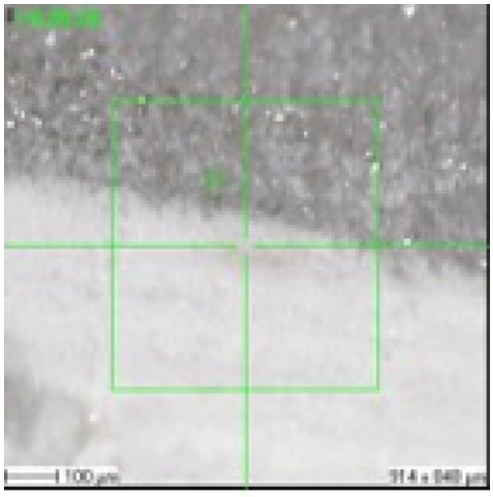


Figure 82. R1, area 2, ground and paint layer with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

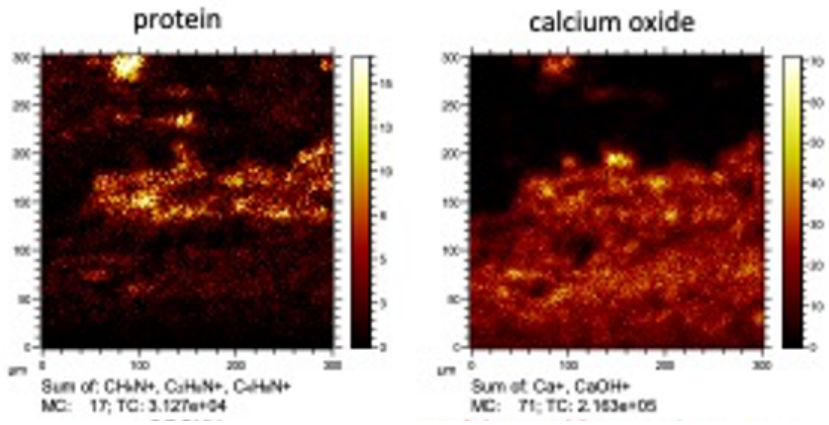


Figure 83. Ion map showing the distribution of protein and calcium oxide in the ground layer (area 1). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images, we can see that protein is found together with calcium oxide in the targeted area.

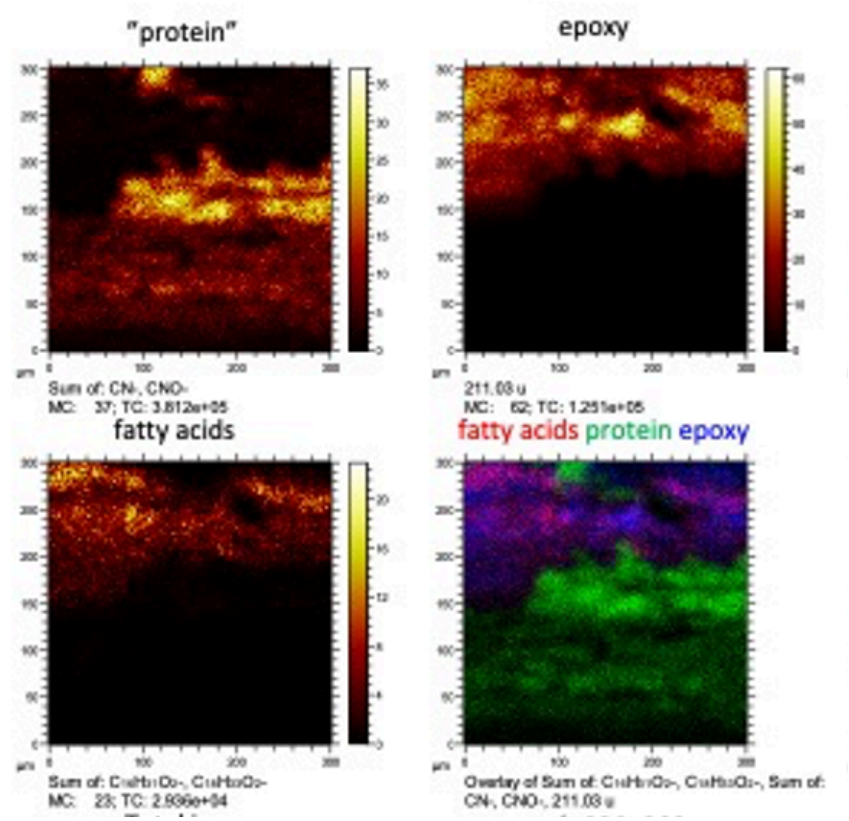


Figure 84. Ion map showing the distribution of protein, fatty acids and methylmethacrylate in area 2. The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images, we can see that fatty acids and methylmethacrylate are distributed in the same area.

The R1 sample was prepared using a pre-size consisting of animal glue, a ground of chalk mixed with animal glue, a tempera consisting of egg yolk + vinegar (1:1) and pigments.

Traces of calcium oxide was found both inside and outside of protein clusters. (See area 1, figure 56). This is an indication of the chalk ($CaCO_3$) and animal glue from the ground. In this reference sample, the ground was indeed a chalk and animal glue mixture.

Amino acids were found in both negative and positive ion mode, but fatty acids were only found in negative mode. In this reference sample, the binder is egg yolk, as indicated by the results of the analysis.

Fatty acids found in the sample were mainly palmitate (C16:0), and about an equal content from oleate (C18:1) and stearate (C18:0). These fatty acids are all from the egg yolk in the paint layer, as no olifa was added to this reference sample.

No major metal content was found, but some traces of chlorine (Cl2), phosphate (PO_4) and sulfate (SO_4), were found in the paint layer, indicating an egg yolk tempera. Also, very small traces of magnesium and aluminum could be found in the paint layer. These too would come from the egg.

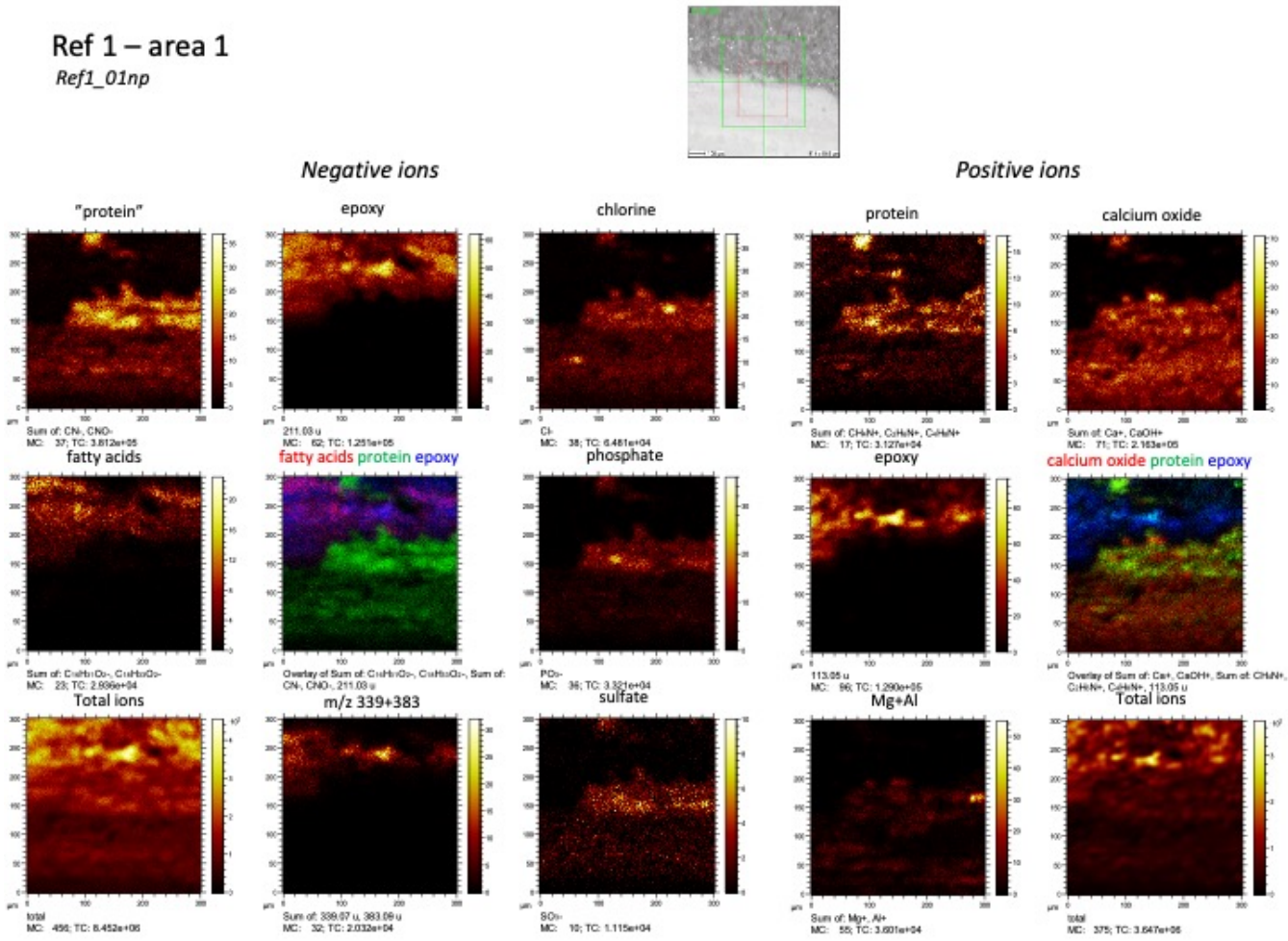


Figure 85. Data from R1, area 1, neg. and pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored pictures in the middle row of the chart, show the distribution of fatty acids and protein, as well as the distribution of calcium oxide and protein within the analyzed area.

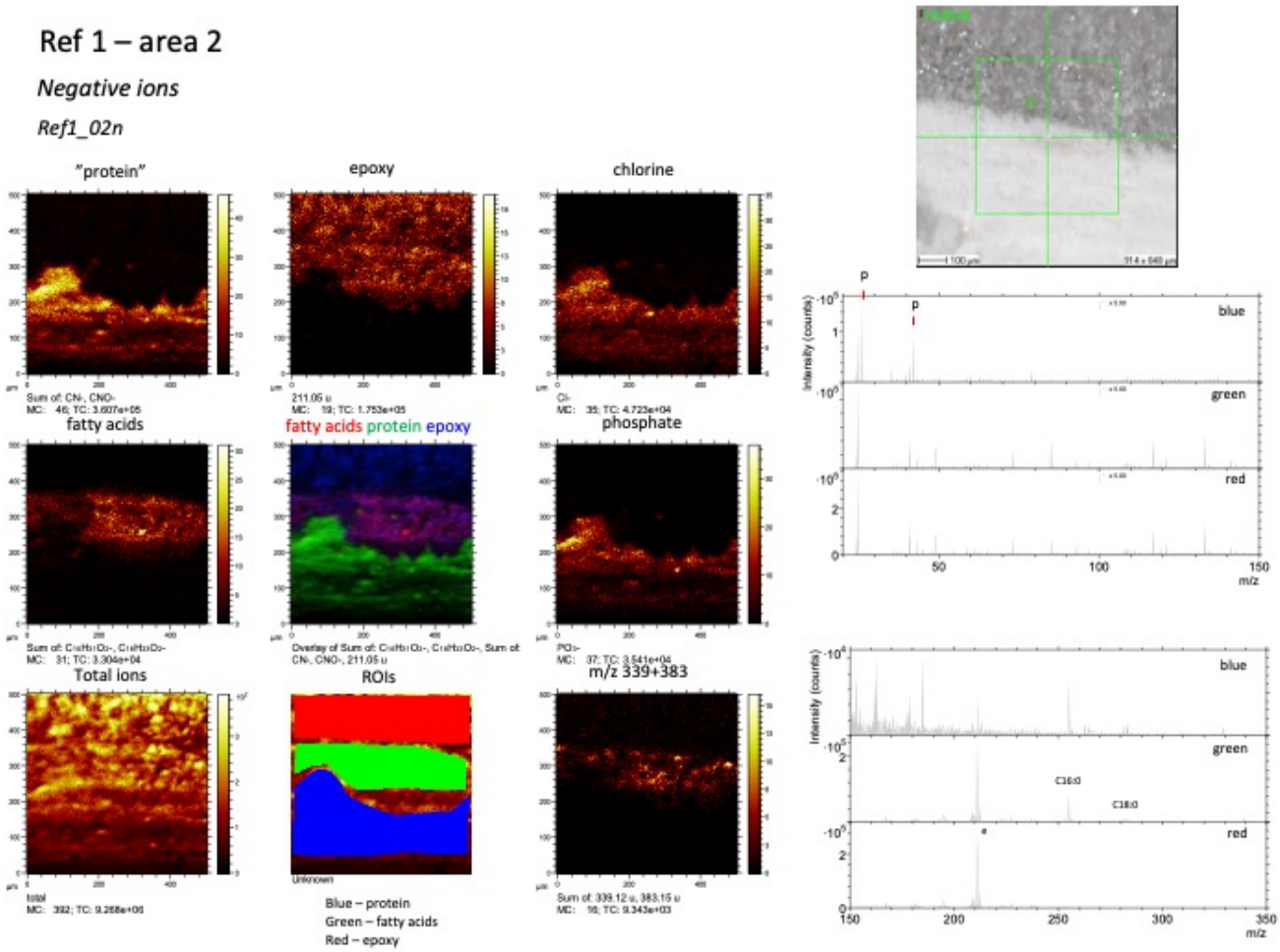


Figure 86. Data from R1, area 2, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

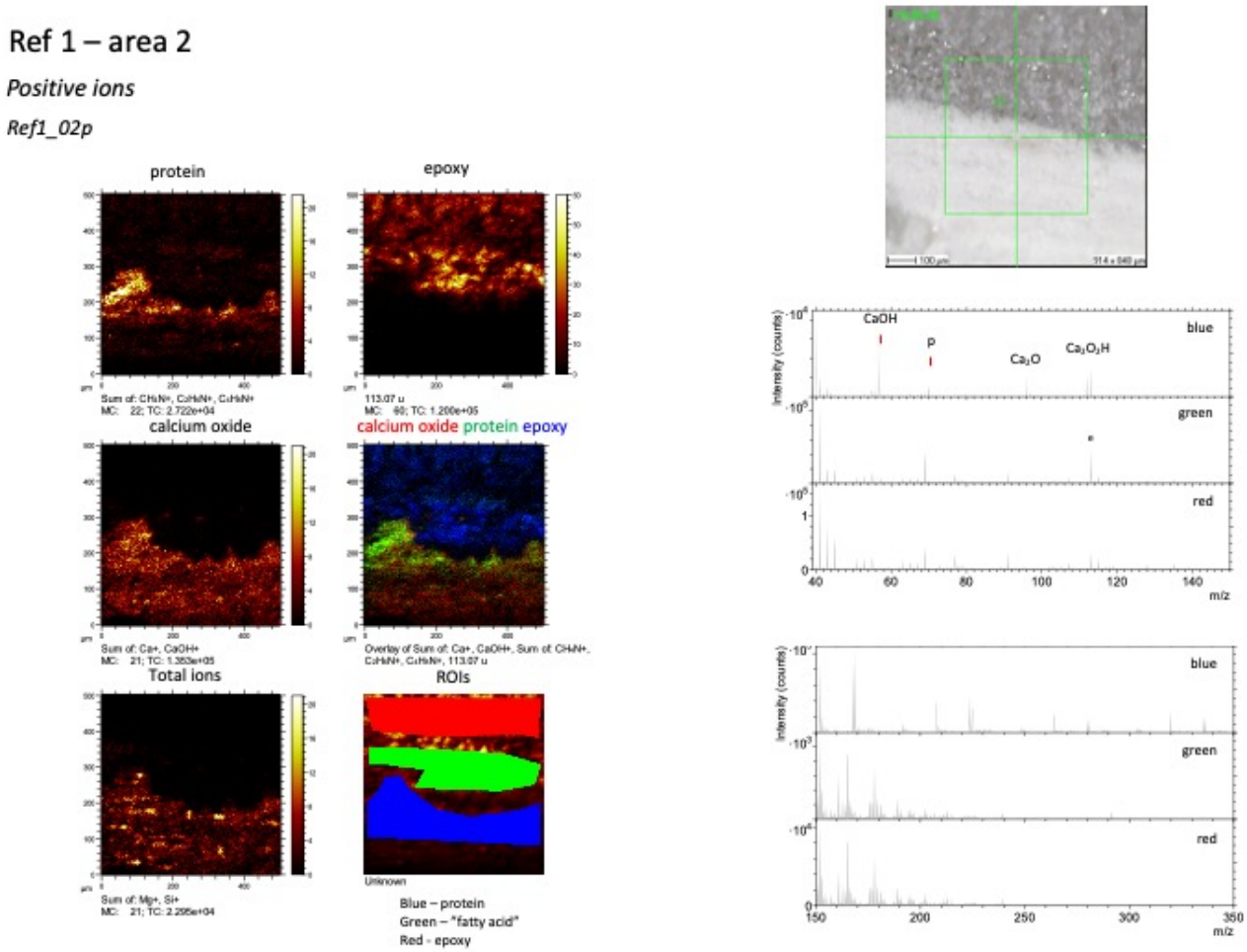


Figure 87. Data from R1, area 2, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of calcium oxide and protein within the analyzed area.

6.6 R2

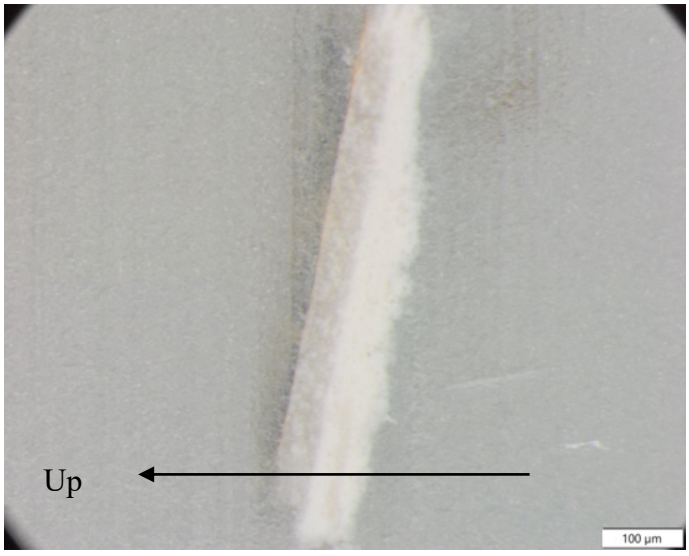


Figure 88. Cross-section from the R2 sample in regular light. Picture taken at RISE by Peter Sjövall in 100 µm.

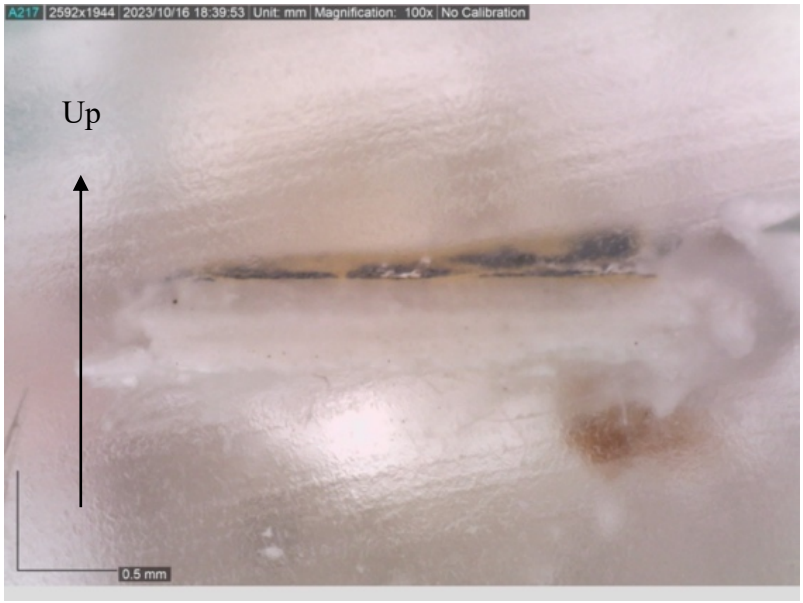


Figure 89. Picture of cross-section from the R2 sample, taken in regular light, 100x with a DinoLite on 2023-10-16.

Figure 90. Cross-section from the R2 sample in UV light at 100x taken with a DinoLite on 2023-10-16.

Just as in the reference sample R1, the image of reference sample R2 in regular light shows a thin paint layer, visible in the top of the two bottom pictures. The ground is below it, showing two layers of application, where a thin layer of surface dust is between the two layers, indicating that some time passed between the application of the layers.

The R2 sample was analyzed on 2023-04-25. Three areas of the sample were analyzed, and ions were measured in negative and positive mode. Images below show the areas through extremely enhanced microscopy.

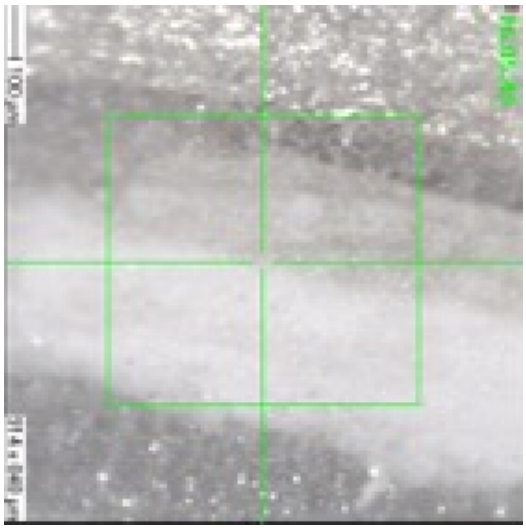


Figure 91. R2, area 1, ground and paint layer with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

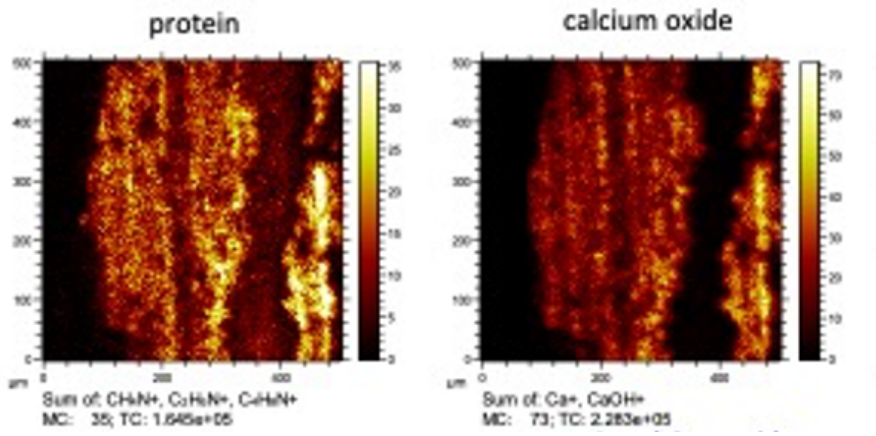


Figure 92. Ion map showing the distribution of protein and calcium oxide in the ground layer (area 1). The bright areas in the image indicate the distribution of the material within the targeted area. Comparing the images, we can see that protein is found together with calcium oxide in this area.

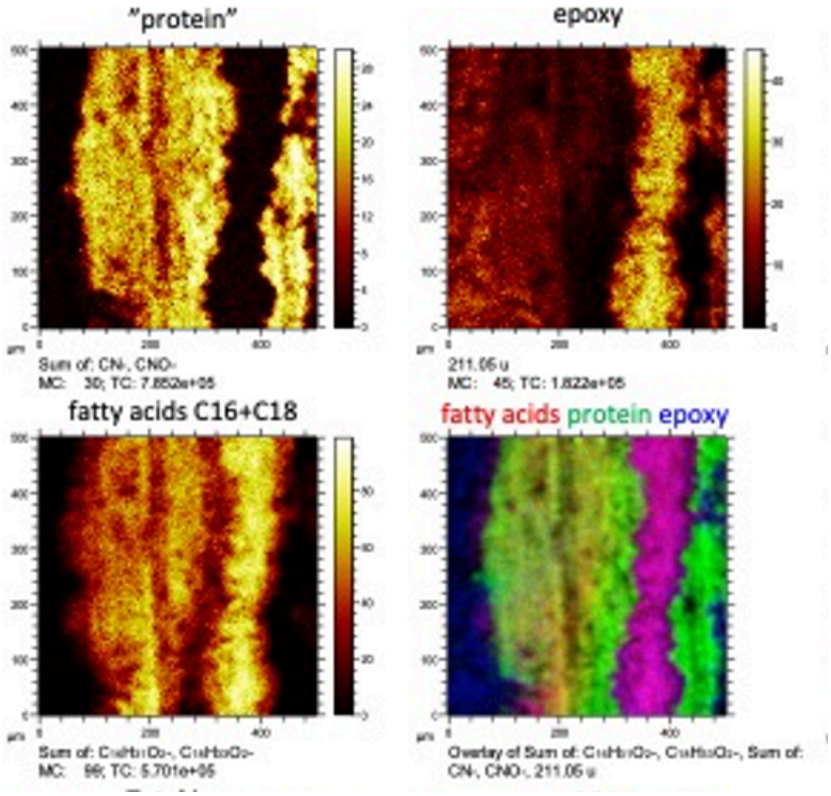


Figure 93. Ion map showing the distribution of protein and fatty acids in the ground layer (area 1). The bright areas in the image indicate the distribution of the material within the targeted area. From the images we can see that protein is distributed together with fatty acids within the area. Next to the protein, fatty acids are found together with methylmethacrylate (labeled epoxy).

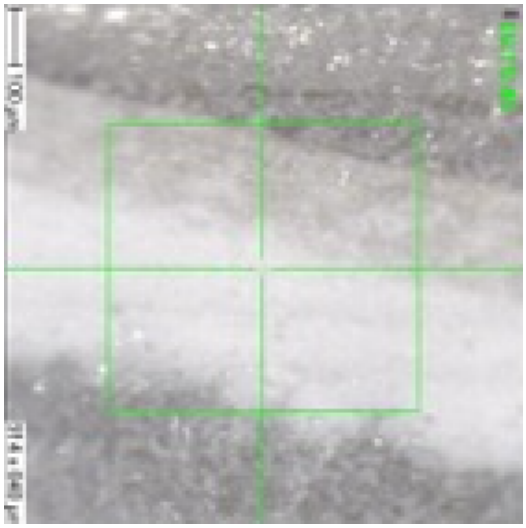


Figure 94. R2, area 2, ground layer with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

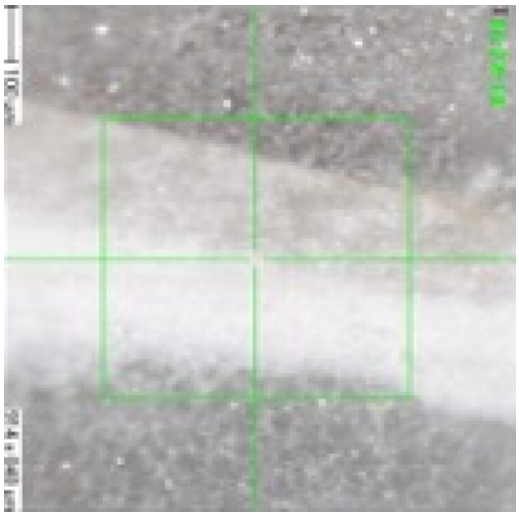


Figure 95. R2, area 3, ground layer with ToF-SIMS. Microscopic image via ToF-SIMS. The crosshair in green shows the target for the ion beam.

The R2 sample was prepared in the same way as the R1 sample, with the addition of an olifa made of olive oil.

Calcium oxide (CaO), was found together with protein in the ground layer. (See area 1, figures 59 and 60). This indicates a ground made of chalk ($CaCO_3$) and animal glue.

Fatty acids were found in the methylmethacrylate, indicating that some of the fatty acids in the material were not bound and therefore could break loose in the impact of the casting process.

Amino acids were found in both negative and positive ion mode, but fatty acids were only found in negative mode. In this reference sample, the binder is egg yolk, as indicated by the results of the analysis.

The fatty acids found in the sample were mainly oleate (C18:1) and stearate (C18:0), where oleate was the predominant one. This would probably be due to the olive oil olifa, which was only a month old by the time of the analysis.

No major metal compounds, such as Pb, Fe etc. were found in the paint layer, but some traces of chlorine (Cl2), phosphate (PO_4) were found in negative mode, as were sodium (Na) and potassium (K) in positive mode.

The R2 sample was very similar to the NMI 286 sample, which could indicate that the material-technical process and the chemical content of the two icons were very similar.

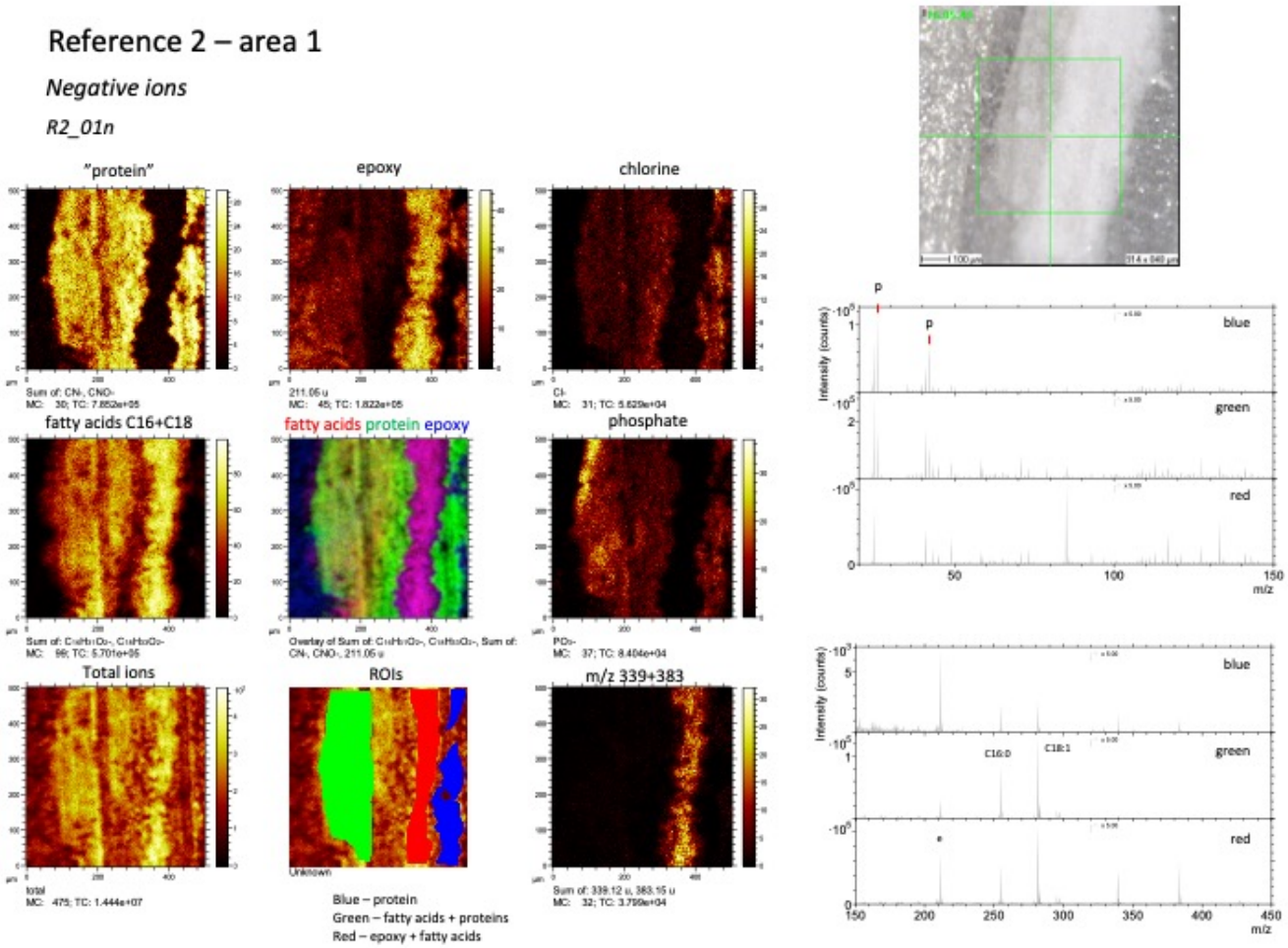


Figure 96. Data from R2, area 1, neg. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of fatty acids and protein within the analyzed area.

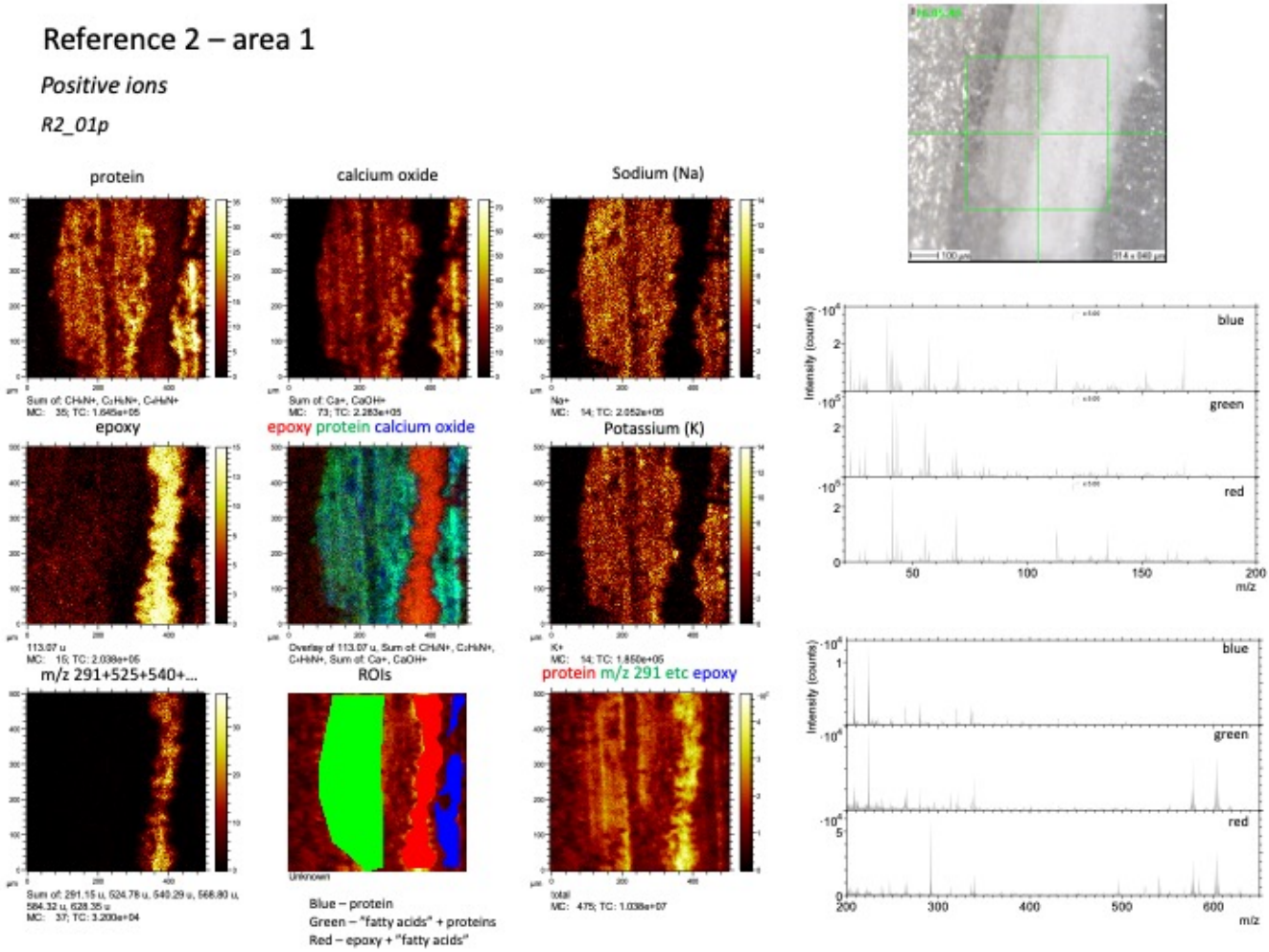


Figure 97. Data from R2, area 1, pos. mode. The brighter parts in each image show the occurrence of the analyzed material they are also labeled with. The colored picture in the middle of the chart, shows the distribution of calcium oxide and protein within the analyzed area.

8. Discussion

Studies by Dietemann et al (2019), show that detection of protein in old paint can be difficult, since protein degrades during ageing and often the analytical methods are more sensible in measuring aged lipids than protein[207]. This results in more findings of lipids than proteins in analyses.

Their studies also show that aqueous binders form gel networks when drying, with empty pores where the water has been, and these pores can be filled with later applied substances, such as varnishes (or olifa), preservation material etc.[208]. The proteins can therefore be harder to detect than other substances, such as lipids.

Di Gianvincenzo et al. (2022) also point at challenges in analyzing protein, which are that proteins in old paintings often are damaged due to degradation, that the proteinaceous substances in a paint medium represents no more than 10% of the total material, that samples often are very small and that paint mediums, such as emulsions, often are complex matrixes consisting of various material. Inorganic material in the paint, such as pigments, can have cations, which can affect the detection of protein in the samples[209].

Bouvier et al, 2021 show that the ion-mode is of importance in interpreting the samples of old paintings. Fatty acids found in negative ion-mode can indicate that the binder is oil- or egg yolk-based. Amino acids found in positive-ion mode can indicate that the binder is glue- or egg-based. If both fatty acids in negative ion-mode and amino acids in positive ion-mode are found, this indicates that the binder can be egg yolk or mixtures[210].

Bouvier et al., 2022;57 show a detection of lipids and protein in the samples, using ToF-SIMS. Their studies show that the O/S level of the oleic acid in old paintings often is between 0.2-0.5 on an oxidation index, but that the proportion of oleic acid will be higher in egg yolk than in drying oils[211]. They also observe that fragment patterns of lipids are more saturated in egg than in oil[212] and that although phospholipids and cholesterol fragments can be detected in ToF-SIMS, the molecules of these in old paintings will have degraded and therefore do not produce the secondary ions of interest to the analysis[213].

Just as in the experiments done by Dietemann et al. in 2019, the ToF-SIMS analysis done for this thesis, proved sensible to lipids. The fatty acids from the samples were easily detected.

[207] Dietemann et al., *Analysis and interpretation of binding media in tempera paintings*, 2019, pg. 69
[208] Ibid, pg. 73
[209] Di Gianvincenzo et al., *Palaeoproteomics guidelines to identify proteinaceous binders in artworks following the study of a 15th century painting by Sandro Botticelli's workshop,* 2022, pg. 2
[210] Bouvier et al., 2021, pp 4463-4471, pg. 4467
[211] Bouvier et al, 2022;57, pg. 11 and 13
[212] Ibid, pg. 13
[213] Ibid

9. Conclusion

Using the ToF-SIMS technique, it was possible to see that the ground layer in all the samples (except for in the fragmented sample of NMI 287, where very little information was left), had a content of calcium oxide together with protein, indicating a ground made of chalk and animal glue.

In all the samples, fatty acids were found alongside protein in negative mode, whereas amino acids were found in positive mode, indicating a paint medium containing egg yolk or an egg yolk mixture.

In two of the samples, NMI 158 and NMI 232, lead oxide was found together with fatty acids, which could indicate the occurrence of an olifa mixed with lead white as a siccative.

In these two samples, NMI 158 and NMI 232, some indication was towards an aqueous paint system with pores that had been filled with other substances.

All of the samples contained the fatty acid stearate (C18:0). Four of the samples (NMI 158, NMI 232, NMI 287 and R1) contained the fatty acids palmitate (C16:0), whereas NMI 286, R1 and R2 also contained oleate (C18:1).

The sample from NMI 158 also contained traces of longer chain fatty acids, such as C24:0 and C30:0. These fatty acid chains also had triglycerides, containing C16:0, C18:0, C24:0 and C26:0, indicating a previous conservation treatment with wax.

The sample from NMI 232 contained traces of hydrocarbon, which could possibly be from a previous conservation treatment.

All the samples contained trace elements that could derive from egg. All the samples contained chlorine (Cl2) and phosphate (PO_4). Three of the samples (NMI 158, NMI 287 and R1) contained sulfate (SO_4), two of the samples (NMI 286 and R2) contained sodium (Na) and potassium (K), whereas NMI 158 also contained copper (Cu), NMI 232 contained hydrocarbons (C_xH_y), and R1 contained magnesium (Mg) and aluminum (Al).

The samples from NMI 286 and R2, were very similar in the analysis, indicating that the material and technique used for these two samples were also similar.

The conclusion of the analysis is that both amino acids and fatty acids could be found in the samples, using the ToF-SIMS technique. While identification of fatty acids from lipids was not a problem, that of specific amino acids from proteins proved to be harder. This might not necessarily be due to the technique, but to lack of information regarding specific amino acids in the data base used for this analysis. The ion imaging of the ToF-SIMS gave a visible mapping of the distribution of proteins and lipids in the samples, which is a very good aid in studying the qualities of the materials of the icons. Some traces of wax treatment on the icon NMI 158 were found, but no other later additions were found in the samples of the thesis. (Although the sample NMI 287 contained traces of unidentified organic material). This shows that the ToF-SIMS technique also can aid in tracing previous conservation treatments.

As no identification of amino acids in the samples was possible, it is impossible to say whether all the protein in the paint layer came from the paint medium. Icons are traditionally treated with isinglass, which would also contain amino acids.

Tempera is a complicated paint system, which changes every time it is formed, due to the ratio of egg, an aqueous material, and the pigments. Many factors affect the system, such as amount, pH charge, electrical charge, pigment size, interfaces, interactions between these, and more. Thus, every tempera batch will differ from another. All the liquids and substances in the paint form a colloidal O/W system, where the oil is dispersed in the aqueous continuous phase. When the water withdraws from the O/W emulsion, the paint dries and stabilizes. Due to the polypeptide helixes formed in the protein folding and the lipids in the egg yolk, the dried tempera becomes hydrophobic. This, in turn, makes the paint system extremely stable, almost impossible to solve.

Any addition of oil to the tempera will change the nature of the paint, regardless of the oil being added to the paint recipe or as an olifa after painting. Added oil droplets are bigger than those found in egg yolk. If oil is added to the paint, the oil droplets could leave voids within the paint structure, possibly rearranging the paint structure. Although the oil of the O/W emulsion will be dried when an olifa is added and the protein helix will have folded, the oil will coat the protein and penetrate the voids of the dried paint. This will change the visible characteristics and the aesthetic perception of the paint as the pigments will be coated with oil, leaving a glossy reflection, rather than a matte one.

The same thing will happen to any conservation or consolidation material added. The material will coat the paint and penetrate the voids of the paint structure. Therefore, the choice of materials for treatment of tempera painting, should be chosen with much care. Thought should be put into not adding material that will change the characteristics of the paint structure too much.

The combination of the support, the ground and the paint layer makes the icon painting technique a long lasting one. Olifa darkens over time due to oxidation, and will need to be removed and changed, just as varnish on any painting.

In the analysis, both proteins and lipids could be determined. It was, however, difficult to see whether or not oil had been added to the tempera or if it had been added only as an olifa afterwards. The two Russian icons analyzed, showed traces of a lead white siccative having been added to the olifa. The two Greek icons did not show the same.

ToF-SIMS is a good technique for mapping and detecting protein and lipids, even if the sample is very old. No conclusions could therefore be drawn regarding the variations in recipe due to geographical, economical or other conditions. It was, however, possible to determine that all of the icons analyzed from the National Museum collection, were painted in tempera, with an olifa added afterwards.

10. Summary

The research questions of this thesis focused on the chemical-technical composition of the icons and were as follow:

- Using ToF-SIMS, is it possible to detect any amino acids and fatty acids in the samples? By doing so, would an identification of amino acids, and thereby protein, and of fatty acids, and thereby lipids, be possible?
- Using the ToF-SIMS Ion mapping, is it possible to see the distribution of protein and lipids in the samples?
- Will ToF-SIMS show if any later additions, such as olifa or materials from conservation treatments have affected the protein in the samples?

All of the research questions were answered in this thesis. It was possible to detect both amino acids and fatty acids in the samples from the icons of the thesis. Identification of the fatty acids was possible, but not that of amino acids. Using the ToF-SIMS, a visible mapping shows the distribution of protein and lipids in the samples. In the sample from the icon NMI 158, traces of wax treatment were found. No signs of the protein itself having been affected by later additions, such as olifa or materials from conservation treatments, were found.

No focus was put in this thesis in the analysis of the pigments used in the paint of the icons from the National Museum. Additional analyses of these could be interesting. Additional analyses to identify the species of the protein, could also be of great interest for researching recipes and local variations in these.

Icons are liturgical objects, used in a liturgical context. Just as the theology icons represent, the objects themselves are also made to be eternal.

They are built up using traditional techniques and materials, in the same way as icons have always been made, over the past 2,000 years. These techniques and materials have proven immensely durable and lasting. The durability of the egg yolk tempera used in the paint layers of icons, can be attributed to the extremely complicated colloidal O/W emulsion which consists of egg yolk, an aqueous additive and pigments. The properties of the tempera will change every time it is made, due to many different factors. These properties will also change with any later addition of an olifa or conservation treatment materials.

Although there are many tools available for analysis of organic material, many of these fail when it comes to determining proteinaceous material. Error ranges are common, and few of the techniques can be used for extremely small substances.

Using ToF-SIMS as a tool for analyzing samples from cultural heritage objects, such as icons, can therefore be an important aid in the analysis of organic material, as well as in mapping previous conservation treatments. Moreover, the visual ion mapping of the ToF-SIMS can be beneficial in understanding how the distribution of substances in a sample is.

Although the proteinaceous substances left in a dried tempera might be as little as 10% of the material, finding and locating the protein is not a problem using a ToF-SIMS technique. The primary ion beam can be focused to less than 1 μm, which is little enough to be precise in the detection of protein.

Using the ToF-SIMS technique for the analyses of this thesis, conclusions could be made from the samples analyzed. It proved possible to detect both amino acids (from protein) and

fatty acids (from lipids) in the samples. However, identification was only possible with the lipids, not with the amino acids. The distribution of protein and lipids in the sample was visually aided by the ion maps provided by the ToF-SIMS instrument. The analysis also showed indications of olifa in the samples, and in one of the samples, detection of materials from a previous conservation treatment was made.

Thanks to the properties of the ToF-SIMS technique, with the small and extremely focused ion beam, that can target very small substances in the material, the possibility to detect and map both organic and inorganic materials in the same sample, simultaneously, the ease in the preparation process for samples for the technique, the visual aid of the ion map and the fact that the technique is a non-destructive analysis tool, ToF-SIMS is a great asset in the world of cultural heritage.

List of figures

A short timeline of icon history

313 AD Christianity is legalized.

730-843 AD Iconoclasm.

787 AD The Seventh Ecumenical Council.

988 AD The Russian Orthodox church is formed.

867-1056 AD The Macedonian dynasty is predominant in the Byzantine icons. (No icons have survived from the first period of this time).

1054 The Great Schism, leading to the division between the Eastern Orthodox and the Roman Catholic faiths.

1204 AD Constantinople is raided by invaders.

1223-1480 AD Russia was invaded by the Tartar-Mongols.

1453 AD The fall of the Byzantine empire.

17th century Crete was invaded by the Ottoman empire.

17th and 18th century Icons were inspired by Baroque and neo-Classical influences.

19th century The Russian icon production went under control of the Holy Synod.

1913 The “Old Russian art” exhibition in Moscow

1917 Russian revolution

Glossary

A guazzo:	Pigments bound with glue or egg rather than with oil.
Chernye doski:	"Black boards" – a Russian term used for old icons that had darkened over time in a combination of oxidized olifa and soot from candles and charcoal.
Encaustic:	An ancient technique of painting with melted wax.
Glair:	Egg white prepared by beating or squeezing through a cloth, used e.g. for pre-coating of pigments.
Iconoclast:	A person who criticizes the veneration of icons.
Iconodule:	A person who favors the veneration of icons.
Iconographia :	Writing icons.
Iconostasis:	A screen in the Orthodox church, separating the nave from the sanctuary. Can also mean a smaller, portable icon stand.
Ikonniki:	Russian icon painter-restorers in the beginning of the 20th century.
Levkas:	The ground of the icon. consisting of animal (hide/skin) glue and either chalk or gypsum.
Olifa:	A sort of varnish on the icon, usually oil based.
Riza/ Oklad:	A metal encasing covering the icon.
Sankir:	The basic shade of carnation on the icon.
Spolvero:	An ancient tracing technique used for transferring sketches to the grounded surface. Lines are traced by stenciled marks on a transfer sheet. A pigment or chalk is then dabbed through the holes, creating an exact image of the sketch.
Tabletka:	A canvas grounded on both sides.
Tempera grassa:	Adding oil to the egg yolk.

List of references

Written publications - Books

- *A history of icon painting*, Lilia Evseyeva, Natalia Komashko, Mikhail Krasilin, Hegumen Luka (Golovkov), Elena Ostashenko, Olga Popova, Engelina Smirnova, Eanna Vakovleva and Eirina Yazykova, Translated by Kate Cook, 2005, Orthodox Books Ltd, UK
- *Alter Icons: The Russian Icon and Modernity,* Jefferson J. Gatrall and Douglas Greenfield, 2010, University Park: The Pennsylvania State University Press
- *Handbok för restaurering av målningar,* Knut Nicolaus, Könemann Verlagsgesellschaft, 1999, (Translated into Swedish by Ulla-Märta Westerståhl in 2001). Printed in Slovenia.
- *Hantverkets bok Måleri,* Redaktion Paul Nylén, Professor vid Tekniska Högskolan, Stockholm, Einar Söderberg, Målerikonsulent vid Statens Hantverksinstitut, Stockholm, Tryggve Johansson, Laborator vid Försvarets Forskningsanstalt, Stockholm, 1953, femte upplagan, Natur och Kultur, Stockholm
- *Icons*, Ulf Abel with Vera Moore, Nationalmuseum, Stockholm 2002
- *Ikonens teologi,* Leonid Ouspensky, (2000, translated by Göran Ståhl)
- *Inkarnat und Signifikanz, Das menschliche Abbild in der Taffelmalerei von 200 bis 1250 im Mittelmeerraum,* Herausgeber, Lehrstuhl für Restaurierung Kunsttechnologie und Konserveirungswissenschaft Technische Universität München, Forschnungsstelle Realienkunde Zentralinstitut für Kunstgeschichte München, Doerner Institut Bayerische Staatsgemäldesammlungen, Opificio delle Pietre Dure Florenz, München 2017
- *Painting materials – a short encyclopedia,* Rutherford J. Gettens and George L. Stout, Dover Publications, Inc., New York, 1966
- *Pratical Protein Chemistry, a handbook*, edited by A. Darbre, 1986, John Wiley & Sons Ltd, Great Britain
- *Techniques of Traditional Icon Painting,* Gilles Weissman, 2021, Search Press Ltd, Kent, Gret Britain
- *Tempera painting 1800-1950, Experiment and Innovation from the Nazarene Movement to Abstract Art,* Edited by Patrick Dietemann, Wibke Neugebauer, Eva Ortner, Renate Poggendorf, Eva Reinkowski-Häfner and Heike Stege, 2019, Archetype Publications Ltd in association with the Doerner Institut, Bayerische Staatsgemäldesammlungen, Munich
- *The Art of the Icon: A Theology of Beauty*, Paul Evdokimov, translated by Fr. Steven Bigham, Oakwood Publications, Pasadena, California
- *The chemistry of paints and painting*, A.H. Church, 1901, 3d ed, London: Seeley and co, Ltd
- *The Good Research Guide for small-scale social research projects,* Martyn Denscombe, 2010, fourth edition, Open University Press, Berkshire, England
- *The Materials and Techniques of Medieval Painting*, Daniel V. Thompson, 1956, Dover Publications Inc., New York
- *The practice of Tempera painting,* Daniel V. Thompson Jr, 1936, Dover Publications, Garden City, New York, unabridged edition published in 1962

- *The Structural Conservation of Panel Paintings,* Edited by Kathleen Dardes and Andrea Rothe, 1995, The Getty Conservation Institute, Los Angeles

Written publications - Articles

- *Analysis and interpretation of binding media in tempera paintings,* Patrick Dietemann, Wibke Neugebauer, Cedric Beil, Irene Fiedler and Ursula Baumer 2019 in *Tempera painting 1800-1950, Experiment and Innovation from the Nazarene Movement to Abstract Art (see books)*
- *Chemical and Physical Properties of Wood,* R. Bruce Hoadley, 1995, in *The Structural Conservation of Panel Paintings (see books)*
- *Flow behaviour and microstructure of complex multiphase fluids*, Annika Hodapp, Patrick Dietemann and Norbert Willenbacher, 2019 in *Tempera painting 1800-1950, Experiment and Innovation from the Nazarene Movement to Abstract Art (see books)*
- *Historical Overview of Panel-making Techniques in Central Italy,* Luca Uzielli, 1995, in *The Structural Conservation of Panel Paintings (see books)*
- *Historical Overview of Panel-making Techniques in the Northern countries,* Jörgen Wadum, 1995, in *The Structural Conservation of Panel Paintings (see books)*
- *Past, recent and future developments in the analysis and interpretation of tempera-based paint systems* by Kristin deGhetaldi 2019 in *Tempera painting 1800-1950, Experiment and Innovation from the Nazarene Movement to Abstract Art (see books)*
- *Tempera: narratives on a technical term in art and conservation,* Eva Reinkowski-Häfner 2019 in *Tempera painting 1800-1950, Experiment and Innovation from the Nazarene Movement to Abstract Art (see books)*
- *The Varnished Icon – a Historical Survey of Icon Varnishes,* Ivan Bentchev, 1999 in *Icon Conservation in Europe*, Edited by Nina Jolkkonen, Auli Martiskainen, Petter Mariskainen and Helena Nikkanen, 1999, The Valamo art conservation institute. pp. 64-88
- *Then egg, then watercolour or tempera paints, then alcohol resin': Paul Klee's tempera painting techniques* by Patrizia Zeppetella, Stefan Zumbühl and Nathalie Bäschlin 2019 in *Tempera painting 1800-1950, Experiment and Innovation from the Nazarene Movement to Abstract Art (see books)*

E-publications – Books

- *Apologia of St John of Damascus Against Those Who Decry Holy Images,* John of Damascus, St. (c. 675-749), 2001, Grand Rapids, MI: Christian Classics Ethereal Library, London
- *Icons in time, persons in eternity, Orthodox Theology and the Aesthetiscs of the Christian Image, C.A. Tsakiridou,* 2013, Ashgate Publishing Limited, Surrey, England
- *Images of the divine, the Theology of Icons at the Seventh Ecumenical Council,* Ambrosios Giakalis, 2005, e-book
- *Protein Chemistry,* Lars Backman, 2020, Walter de Gruyer GmbH, Berlin/ Boston, e-book
- *Scientific Methods and Cultural Heritage,* G. Artioli, 2010, Oxford: Oxford University Press
- *The Hermitage Museum of St Petersburg, the Greek Treasures*, 2004, Ephesus publishing

E-publications - Articles

- *A colloidal description of tempera and oil paints, based on a case study of Arnold Böcklin's painting Villa Am Meer II (1865)*, Patrick Dietemann, Wibke Neugebauer, Luise Lutz, Cedric Beil, Irene Fiedler, Ursula Baumer, in e-Preservation Science, 2014, no 11, pp 29-46
- *A Holistic view on the role of egg yolk in Old Masters' oil paints,* Ilaria Bonaduce, Emilia Bramanti, Patrick Dietemann, Celia Duce, Ophélie Ranquet and Norbert Willenbacher, in Nature Communications, (2023) 14:1534
- *Applications of SIMS to cultural heritage studies,* A. Adriaens, M.G. Dowsett, 30 June 2006, Applied Surface Science 252 (2006) 7096-7101
- *Carbohydrates of the chicken egg yolk,* T.M. Ushakova et al., 1979, Vopr Pitan, (article in Russian), National Library of Medicine, National Center for Biotechnology Information, PubMed Advanced
- *Characterization of protein in paint media by immunofluorescence. A note on methodological aspects* by Blanca Ramirez-Barat and Sonsoles de la Viña in Studies in Conservation 46 (2001) pp 282-288
- *Cluster ToF-SIMS imaging as a tool for micrometric histology of lipids in tissue* by Claudia Bich, David Touboul and Alain Brunelle, "Mass spectrometry Reviews", 2013, pp 442-451, Published online 21 November 2013 in Wiley Online Library
- *Comparison of oil and egg tempera paint systems using time-of-flight secondary ion mass spectrometry,* Zachary E. Voras, Kristin deGhetaldi, Brian Baade, Eric Gordon, Glenn Gates & Thomas P. Beebe, "Studies in Conservation", Vol. 61, 2016 – Issue 4
- *Icons: the Orthodox Understanding of Images and the Influence on Western Art*, Davor Džalto, 2019, European History Online
- *Infrared Spectroscopy in Conservation Science,* Michele R. Derrick, James M. Landry and Dusan Stulik, 1999, The Getty Conservation Institute
- *Interpretation as an Inevitable Risk in Conservation of Icons,* Yuri Bobrov, 1996, ICOM-CC, 11th Triennial Meeting, Edinburgh September 1-6
- *Issues and development trends of modern Icon painting,* Marina Bobreshova, 2019, SHS Web of Conferences 72, 02003, Don State Technical University 344022, Rostov-on-Don, Russia
- *Hydrocarbons detected in irradiated and heat-treated eggs,* Keum Taek Hwang, Sook Bae Kim, Choong Ki Kim, Tai Boong Uhm and Ji Hyun Yoo, 2001, Food Research International, Vol. 34, Issue 4.
- *On the way to tempera grassa: Unraveling the properties of emulsion-based paint binders,* Romain Bordes, Côme Thillaye du Boullay, Maïwenn Le Denic, Floriane Gerony, Maguy Jaber, Guillaume Mériguet, Anne-Laure Rollet, Laurence de Viguerie and Philippe Walter in Colloids and Surfaces A: Physiochemical and Engineering Aspects 673, (2023), 131816
- *Palaeoproteomics guidelines to identify proteinaceous binders in artworks following the study of a 15th century painting by Sandro Botticelli's workshop*, F. di Gianvincenzo, D. Peggie, M. Mackie, C. Granzotto, C. Higgitt and E. Cappellini in Scientific Reports (2022), 12:100638 Nature portfolio
- *Pilot study into the effects of solvents on artificially aged egg tempera films,* Naryan Khandekar, Alan Phenix and Julia Sharp, 1994, The Conservator, no 18, 62-72

- *Sound Practice and Practical Conservation Recipes as Described in Greek Post-Byzantine Painters' Manuals,* Georgios P. Mastrotheodoros and Konstantinos G. Beltsios, June 7th 2018, Studies in Conservation 64:1, 42-53
- *The composition and chemistry of eggs and egg tempera*, Alan Phenix, Early Italian Paintings: Techniques and Analysis, Symposium, Maastricht, 1996, ed. T Bakkenist, R. Hoppenbrouwers and H. Dubois, Limburg Conservation Institute, 1997, pp. 11-20
- *The Golden Egg: Nutritional Value, Bioactivities, and Emerging Benefits for Human health,* Nicolas Guyot, Yves Nys and Sophie Réhault-Godbert, on March 22nd 2019, National Library of Medicine, National Center for Biotechnology Information, PubMed Central
- *The implications of conserving religious icons,* Davina Kuh Jakobi, 2014, University College London
- *Time-of-flight secondary ion mass spectrometry imaging in cultural heritage: A focus on old paintings* by Caroline Bouvier, Sebastiaan Vand Nuffel, Philippe Walter, Alain Brunelle, Journal of Mass Spectrometry, 2022;57
- *Time-of-Flight Secondary Ion Mass Spectrometry Imaging of Cross Sections from the Bacchanals Paintings of Nicolas Poussin* by Caroline Bouvier, Helen Glanville, Laurence de Viguerie, Chiara Merucci, Philippe Walter and Alain Brunelle in Analytical Chemistry, 2021, 93, 4463-4471
- *Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS): Principles and practice in the Biogeosciences* by Volker Thiel and Peter Sjövall, published on 27 August 2014 on http://pubs.rsc.org I doi: 10-1039/9781782625025-00122

Other sources

- *A glimpse into Ukrainian Icon Painting,* Valeriia Kravchenko, Marharyta Khrebtenko, and Anneta Shashkova, BAPCR talk on March 1st, 2023
- *Lipid Glossary*, online lipid glossary provided by Oklahoma State University at https://extension.okstate.edu/fact-sheets/lipid-glossary.html, used on May 3rd, 2023
- Tempera conference in Munich, 2018, http://www.iperionch.eu/tempera-2018-conference-postprints-now-published/
- *Tempera or oil? - Colloid chemistry and the microstructure of paints,* online IAP virtual seminar with Patrick Dietemann, March 16th, 2023