CHARISMA

Cultural Heritage Advanced Research Infrastructures: Synergy for a Multidisciplinary Approach to conservation/restoration

# EVALUATION REPORT ON PROTOCOLS FOR REPRESENTATIVE SAMPLING

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# 1. EXECUTIVE SUMMARY

This document reports the results of the work carried out on Task WP2.1a within Work Package 2 during the first period of the CHARISMA project towards evaluation of protocols for representative sampling and strategies for complementary analyses. It includes specifically:

a) A summary of the detailed information collected through a questionnaire reviewing the current practice among CHARISMA partners including sampling methodology and practice for all types of painting (canvas, panel, wall etc) and painted objects.

b) A literature survey focussing mainly on preparation of paint samples, including general literature, requirements for various FTIR techniques (a subject which has a specific synergy with the practical experiments on this subject being conducted in WP10) and some promising new methods.

# 2. INTRODUCTION

The general theme of Work Package 2 is consideration of best practice and definition of protocols with the aim of working towards common standards in the field of science applied to cultural heritage conservation. The individual tasks within the work package have been chosen to address specific aspects of practice. Task 2.1a, *Methodologies for sampling and sample preparation*, is active during months 0 to 30 of the CHARISMA project and has the goal of formulating guidelines for the methodology of scientific analysis/examination of cultural heritage objects, and specifically the role of sampling within this.

In recent years there have been many developments in advanced equipment for the non-invasive investigation of cultural heritage objects, which are of great advantage for the analytical study of art objects. They provide valuable data that can give a general overview of the materials present, and are often able to answer many of the questions posed during conservation or technical research, avoiding or minimizing the need for sampling. However, for some questions more detailed information on the stratigraphy of the object and chemical composition of the materials present is needed which can only be obtained from the analysis of micro-samples. This is often crucial in answering questions arising during conservation treatment or in understanding the technique used in creating an object. Nevertheless in-situ non-invasive analysis can help to minimise the number of samples that are taken, as well as informing the choice of appropriate places for micro-samples for further in-depth analysis.

Effective technical examination of an object depends on selecting an appropriate strategy for combining the results from multiple analytical techniques, both non-invasive and those that can only be applied to micro-samples. There has been a proliferation of new non-invasive instruments as well as developments in existing techniques. The same is true for those techniques used for analysis of samples. Increasingly multiple complementary techniques are applied to a single sample, and issues such as the best use of samples, as well as integration of the results, become important.

Sample preparation issues have become more critical recently due to new techniques being introduced that are more demanding in this respect. This includes various surface analysis techniques such as SIMS for which the quality of the surface of the sample is very important and strongly influences the success of the analysis. In this project the interest in sample preparation is further stimulated by the work planned in WP10 Task 10.1 where chemical imaging techniques, particularly various types of FTIR imaging and mapping, are being explored. Improving sample preparation should improve the reliability and quality of the results from these analytical techniques, and each type of technique has specific requirements, or is more or less demanding.

More and more techniques are being used on a single sample, especially on cross-sections, as new methods of analysis are being introduced, such as chemical imaging. Although most of the analytical techniques are considered non-destructive to the sample, they can induce damage that might interfere with subsequent analyses. The order in which multi-technique analysis is conducted then becomes important. This is particularly a danger with newer more unfamiliar techniques, as we may not be aware of how they can affect the sample. For example the more intense beam of a Field emission gun scanning electron microscope (FEG-SEM) can more easily create beam-sensitive samples, as can analysis of uncoated non-conducting samples under variable pressure conditions in the SEM, or analysis with synchrotron techniques such as X-ray absorption near edge structure spectroscopy (XANES). Attenuated total reflectance Fourier transform infared microscope (ATR-FTIR) can create a circular depression in the sample. Raman analysis can burn a sample if the power used is not carefully chosen.

These techniques provide complementary information on the sample. In order to accurately interpret the results of analyses on a single sample, effective integration of results is necessary. For example, the many chemical images produced from FTIR imaging/mapping need to be correlated with optical microscope images (normal and ultraviolet light), and SEM-EDX (back scattered images and EDX maps), as do spectra from individual points on the sample. This can be a laborious process, so it would be helpful to consider the best procedure and also what tools are available to achieve and facilitate this.

Task 2.1a aims to consider some of these issues. The work has been formulated under two main headings:

- *Methodology, procedure and ethics of sampling*
- *Best procedures for sample preparation (including new methods), complementary analyses with multiple analytical techniques and integration of the results*

The first of these is more general in character, aiming to consider guidelines for 'best practice' for representative sampling and sample preparation in the context of the general methodology of technical examination of an object, including practical procedures and ethical issues. The second has a more narrow focus and relates to more specific issues such as how the analysis is conducted once the samples have been extracted from the object, how the samples are prepared and issues relating to the use of multiple techniques on a single sample, as well as integration of the results.

*Methodology, procedure and ethics of sampling*

The proposed work under this heading includes:

- Collection of detailed information from each partner in the CHARISMA consortium on the methodology followed for sampling in their institution for a particular type of object (circulation of a questionnaire).

- Compilation of information on the state-of-the-art among CHARISMA institutions for sampling methodology and practice from the information collected by the questionnaire that could be used as a basis for guidelines.
- Survey of literature on sample preparation and methodology, including existing codes of conduct and guidelines.

*Best procedures for sample preparation (including new methods), complementary analyses with multiple analytical techniques and integration of the results*

The work under this heading focuses on current sample preparation practices and procedures through the following actions:

- Review of published literature in the cultural heritage field on this subject
- Collection of information on current practice for sample preparation among CHARISMA partners (survey, exchanges), including any investigation of new preparation methods. This action will harvest specific expertise within the consortium.

This deliverable (D2.1a) reports the work carried out on this task during the first period of the CHARISMA project towards evaluation of protocols for representative sampling and strategies for complementary analyses. Firstly, the results of the questionnaire that was circulated to CHARISMA partners to collect information on current practices relating to sampling methodology and sample preparation for all types of painted object are summarised. Secondly, a literature survey focussing mainly on preparation of paint samples, but also including general literature on methodology has also been conducted and is included as part of this document.

## 3. SAMPLING METHODOLOGY AND PRACTICE: STATE OF THE ART AMONG CHARISMA PARTNERS

A questionnaire surveying the methodologies for sampling, sample preparation and related documentation was circulated in order to compile information on the state-of-the-art on this subject among CHARISMA partners. The partners contributed information on specific practice in their institution relating to paintings on all types of support (canvas, panel, wall), as well as painted objects (sculpture, furniture etc). The template questionnaire is included as an Appendix. The purpose was:

a) To collect information on the procedures/methodology for the analysis of materials in cultural heritage objects followed by CHARISMA partners, particularly with regard to sampling practices (including both ethical and practical considerations) and documentation relating to sampling.
b) To collect information on the specific methods used during sampling and sample preparation.
c) To compare and exchange information on sample preparation methods between CHARISMA partners with the aim of improving these methods.
d) To use this information to formulate a 'best practice' for sampling methodology/protocols including both practical and ethical issues, and to promote discussion among the partners on this subject.
e) To identify areas of development that partners are currently investigating and to find areas of common interest for discussion among the partners. e.g. sample preparation for specific analytical techniques where it is challenging such as SIMS and FTIR imaging.

Some of the issues that the questionnaire was designed to consider include:

- What is the purpose of the technical examination, what questions does it aim to address? (conservation treatment, examination for historical and archaeometric research on materials and technique, degradation problem etc)
- Who is responsible for the decision to sample? Who authorises/initiates it? What factors contribute to this decision?
- What ethical considerations are taken into account?
- How are the sampling points chosen and recorded?
- How is the information from non-destructive analysis integrated into the methodology and how does it contribute to the sampling procedure? What non-invasive techniques are used?
- How is previous knowledge integrated into the procedure?
- What analytical techniques are used? What type/size of sample is required for each technique and how is it prepared?
- How are the results documented? How are they integrated with non-invasive analysis and technical imaging?
- Are the tools/procedures adequate or not? Where is there room for improvement?

- How can new non-destructive methods or new techniques for the analysis of samples such as those being developed in CHARISMA (eg WP9, WP10) contribute to, or be integrated into, this methodology?

The survey comprised three sections. The first section, entitled ‘Background information’ was intended to collect general information on the methodology followed by an institution, the general ethical approach and the purpose of sampling. The second section requested more detailed information on the non-invasive methods used as part of the general methodology of examination of objects and also its role as a preliminary to sampling. Section 3 – sampling, sample preparation and analyses – collected more detailed practical information on sampling methodology, sample preparation and the analytical techniques that were used.

The emphasis on sampling within the methodology in this questionnaire is somewhat artificial, since in practice it may be decided after non-invasive examination of an object that sampling is not necessary. However, it was formulated in this way because the focus and purpose of this task is to examine the place of sampling within the methodology, and also sample preparation, with the aim of ensuring that protocols can be suggested that make the most efficient use of the sample once taken, since it is micro-destructive to the object.

### 3.1 SURVEY SECTION 1: General information

It was decided that this task should initially concentrate on collecting information from the CHARISMA partners on their practice in relation to all types of painted object (paintings on canvas and panel, wall paintings, polychrome sculpture etc). The specific types of object for which information was given from each CHARISMA partner are listed in Table 1. The majority of the information relates to methodology for examination of European old master paintings, since this is a major field of interest for a high proportion of the partners. Wall paintings are also covered by almost as many partners, as well as polychrome sculpture and painted decorative art objects. The methods mentioned in the completed questionnaires are also applied by some partners to modern and contemporary art, works on paper (manuscripts, prints, drawings), icons, Eastern paintings (scrolls, silk) parchment and papyrus, and works from Egyptian and Mediterranean antiquity.

TABLE 1: Types of painting or painted object considered by each partner in the questionnaire

| | European old master paintings on canvas, panel, metal etc | Modern and contemporary painting | Wall painting | Polychrome sculpture | Decorative arts and furniture (eg frames etc) | Icons | Egyptian and Mediterranean antiquity | Works on paper: manuscripts, prints and drawings | Eastern paintings (scrolls, silk) | Parchment and papyrus |
|---|---|---|---|---|---|---|---|---|---|---|
| UNIPG | X | X | X | X | | | | | | X |
| C2RMF | X | | X | X | | | X | | | |
| IESL-FORTH | X | | X | | X | X | | | | |
| NGL | X | | | | | | | | | |
| SOLEIL | Type of sample depends on the users of the large scale facility | | | | | | | | | |
| ICVBC-CNR | X | X | X | X | | | | | | |
| NCU | X | X | X | X | X | | | | | |
| ATOMKI-HAS | Type of sample depends on the users of the large scale facility | | | | | | | | | |
| CPP-LRMH | | | X | X | | | X | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| BM | X | | X | X | | X | X | X | X | X |
| DIBS | X | X | X | X | X | | | | | |
| Of-ADC | X | | X | X | | X | | X | | |
| OPD | X | | X | X | | | | | | |
| PRADO | X | | | X | X | | | | | |
| RCE | X | X | | | X | | | X | | |
| KIK-IRPA | | | X | | | | | X | | |
| RISSPO-HAS | Type of sample depends on the users of the large scale facility | | | | | | | | | |
| UNIBO | X | | X | | | | | | | |

*Purpose of the examination and type of information obtained*

The main motivations for technical examination and sampling of a painting or painted object were described in the survey as the following:

- Investigation of the materials and layer structure of both original and non-original materials in support of conservation treatment, to answer questions raised by conservators/restorers, curators, archaeologists and art historians. The aim is to inform and contribute to defining appropriate cleaning and consolidation strategies as well as give information on the state of preservation. This includes examination of the structure of the support, as well as investigation of the paint and coatings.
- Study of the original materials and techniques of execution, research into the history of use of materials etc. Generally the same samples used to inform conservation treatment are used for this purpose. This type of investigation might be carried out in conjunction with an art-historical cataloguing programme or can be related to the preparation of a catalogue for an exhibition. Fundamental studies on occurrences, history of use or characterisation of a particular material, as well as pigment deterioration or paint defects can also sometimes emerge as an extension of this type of study.
- Research into the degradation of materials, including both investigation of changes, with a view to understanding the original appearance of a work and why a particular type of alteration has occurred, as well as more fundamental research into deterioration mechanisms. This type of research is often initiated by conservation scientists.
- Information related to storage environment and conditions, as well as health and safety concerns related to the materials that might be contained in the object.
- Authenticity, attribution and dating.
- Testing of conservation materials and treatments.

A single examination will often encompass all of these aims, providing knowledge of the techniques of original production, ancient reworking and modern conservation intervention.

Other related lines of investigation mentioned in the survey include analysis of reference materials, including historical ones such as tube paints to study both the history of use of particular materials and also test characterisation methods. Analysis of test samples is also sometimes carried out to explore and assess new conservation methods and materials, for example, new adhesives for plastics. Investigation of the application of new analytical techniques is a focus of some of the research in some institutions.

More specifically the type of information that can be gained from analysis of samples includes, according to the survey, the following:

- The technique of the painting or painted object as a whole, as well as specific information on layer structure, paint mixtures and chemical composition of the materials in paint samples (at both elemental and molecular level), including both original material and that relating to past conservation treatments, such as retouchings, overpaint and varnish.
- For identification of pigments, as well as their deterioration products, detailed information on the elemental and molecular composition of pigments can be obtained, as well as their location in the microstructure.
- Detailed information on the composition of paint media and varnishes can be obtained by various analytical techniques. Their location within the microstructure is more difficult to determine, but some information can be obtained in favourable situations or with certain types of analysis.
- Detailed information on the composition of the dyestuff in materials containing organic colorants such as red and yellow lake pigments can be obtained.
- Samples are occasionally taken to identify the wood of a panel painting or for fibre analysis of a canvas painting.
- Dendrochronological analysis of wood can provide information on the date of creation of an object, for specific types of wood.

*Responsibility for the decision to sample and factors contributing to the decision*

The factors contributing to the decision to sample an object and the chain of responsibility for approval of sampling have much in common among the CHARISMA institutions. In many of the CHARISMA partner institutions if conservation treatment has been approved then any scientific investigation and sampling that is needed in support of the treatment is considered integral, and therefore does not require further formal permission. The exact sample sites, number of samples taken, quantity of material per sample and the questions to be explored by the analysis are then discussed between the conservator and the scientist. In other situations, the specific sampling needs will be discussed on a case by case basis with various professionals (conservators, conservation scientists, curators, art historians, archaeologists, architects), to formulate the specific questions that might be answered by sampling and to consider what is ethically acceptable. The ultimate decision to allow sampling usually lies with the curator, art historian or archaeologist that is responsible for the object. In some institutions a more formal process is followed, where an application is made describing the purpose of the technical examination, the proposed methodology and reasons for sampling, which then requires approval by authorities such as the institution responsible for the object or the head of a museum conservation department. It is, of course, generally agreed that sampling should only be undertaken by trained and authorised professionals.

Many different factors are borne in mind when taking the decision to allow sampling and considering how many samples should be taken and from which sites. Firstly, the results from non-invasive analysis should be taken into account and generally it should be established that sampling is necessary to answer the questions that are raised. Sampling is justified where the information obtained is likely to be critical to the conservation treatment being undertaken and will affect conservation decisions, or where the value of the knowledge gained outweighs the risk to the object. The questions need to be well formulated in order to choose the sample sites, sampling method and appropriate analytical technique effectively – for this the different type of expertise contributed by the scientists, conservators, curators, historians and archaeologists is invaluable and good knowledge of the history and context, state of conservation and possible questions relating to cleaning and treatment is crucial for the most effective use and interpretation of the scientific results from analysis of samples. Also key in achieving this aim is knowledge of any comparable studies of, for example, the painting technique of a particular artist or works from a particular geographical region, which can inform decisions as to which areas of the painting or painted object will be explored by sampling, perhaps because a specific material or method is known to be characteristic.

Ethical considerations concerning the exact area or situation in which is it acceptable to take samples must also be taken into account. These can depend on the conservation state and size of the painting – generally samples are not taken from paintings or painted objects where there are no losses, and samples are usually taken from the edge of existing losses or from the edge of the painted surface. Although all the partners surveyed agreed that the sample size should be the minimum possible, it was also noted that it is better not to sample at all than to take a sample that is so small that the analysis is not successful.

In addition to ensuring that the questions that technical examination aims to answer are well formulated, and have a foundation in good knowledge of historic materials and techniques, the sampling needs to be carried out in a way that ensures the highest probability of obtaining good quality data – for example, if the identity of the binding medium of original paint is the goal of the analysis, the sample will be wasted if care is not taken in the initial sampling to avoid contamination with later restoration materials or, where this is not possible, to ensure that the analysis is informed by what is known of the conservation history of the painting.

### 3.2 SURVEY SECTION 2: Non-invasive analysis as part of the examination protocol

The non-invasive imaging and analytical techniques that are applied by the CHARISMA partners listed in the survey questionnaire for the examination of paintings and painted objects are detailed in Table 2. Some imaging techniques, such as infrared reflectography and X-radiography, have a long history and are an important part of the methodology for technical examination of cultural heritage objects, particularly for examination of underdrawing, pentimenti, state of conservation and the structure of the support. The survey also shows that CHARISMA partners often use these images to inform sampling, both to aid in choosing appropriate sampling sites and in formulating the questions that analysis of samples hopes to answer. Infrared reflectography is now carried out mainly with digital technology, but conventional film is still generally used for X-radiography, with the plates being scanned and then mosaiced or otherwise processed in digital form, which allows reduction of interfering features such as cradles on the back of panel paintings or stretchers on the back of canvasses, or to enhance key features of interest.

X-ray fluorescence spectroscopy is also well-established, with most of the institutions having access to at least some form of the technique. The highest number use small portable ‘hand-held’ instruments, which have a fairly large spot size (of a few mm) but are relatively cheap and are useful for acquiring basic information about the elements in a particular area, contributing to pigment identification. Several of the CHARISMA partners also have access to more sophisticated XRF systems with a smaller spot size, although they are usually less portable. XRF analysis is more powerful when combined with examination of the surface of the painting with a stereomicroscope to gain information on pigment mixtures and painting technique (layer structure, features of the method of application). This is usually only possible in the environment of a conservation studio, but is an important tool when sampling in order that good knowledge of the state of the paint at the sample site is gained, which can be crucial in interpreting the results of analysis, especially in determining whether the sample point is representative of the area or the painting as a whole. It is often combined with digital macrophotography.

The techniques mentioned above are all integral to the routine technical examination carried out in CHARISMA institutions, but there are other non-invasive analytical techniques mentioned in the table that are not so universally accessible. In some cases this is because there is no suitable commercially available instrument (although the cost of acquiring and maintaining a large number of scientific instruments is also a factor), and the instruments are prototypes built by the institution themselves. Many of these are, however, available to the cultural heritage community through MOLAB transnational access in the CHARISMA project. By demonstrating which non-invasive techniques are readily available in the field and which are not, the survey shows the value and need for the MOLAB facility. Also listed are the techniques available through FIXLAB, again otherwise not easily accessible. They provide detailed analysis such as identification and quantification of trace elements in a non-invasive way, which would normally necessitate the use of techniques that can only be applied to samples.

A primary reason for taking samples can be the need to obtain information about the layer structure of a painting or painted object. As yet, very few non-invasive techniques offer this ability, although some progress is being made towards this goal. Optical coherence tomography, being developed in a portable form in WP9 and also already used by the partner involved, can give ‘virtual’ cross-section images where reasonably transparent materials such as varnishes and glazes are concerned, and sometimes deeper into the paint structure, depending on the refractive index and other properties of the paint components. Although it does not give as detailed information as is gained from a sample, it can be valuable when used in combination with ‘real’ cross-sections to gain an idea of how representative the sample actually is of the layer structure across a particular area. Some of the other techniques being developed in WP9 also hold out the prospect of progress in this respect. Some depth-resolved information is also possible through certain XRF and spectral imaging systems, as well as LIBS and unilateral NMR.

TABLE 2 Non-invasive analytical techniques mentioned by the partners in the questionnaire (P = portable)[1]

| | Optical stereomicroscopy | Infrared imaging | X-radiography | Beta radiography | UV fluorescence imaging (UV-induced visible luminescence imaging) | Multispectral imaging | Visible induced luminescence imaging | Digital holographic speckle pattern interferometry + Optical holographic interferometry | Infrared thermography | Optical coherence tomography | Optical holographic interferometry | X-ray fluorescence spectroscopy | Laser induced fluorescence spectroscopy | Laser induced breakdown spectroscopy | mid and near FTIR spectroscopy or microscopy | Raman spectroscopy or microscopy | UV-VIS-NIR-mid-IR spectroscopy (various configurations including with fibreoptics) | NMR profiling | PIXE | Nuclear reaction analysis | Rutherford backscattering spectrometry | Prompt Gamma activation analysis | XRD-XRF | Large chamber variable pressure Scanning electron microscope | Time-of-flight Neutron Diffraction (TOF-ND) | Small angle Neutron scattering (SANS) | Triple axis spectrometer (TAS) | Dendrochronology |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UNIPG | X | X | | | X | | | | | | | X (P) | | | X (P) | X (P) | X (P) | X (P) | | | | | | | | | | |
| C2RMF | X | X | X | | X | | | | | | | X (P) | | | | X (P) | X | | X | X | X | | X (P) | | | | | |
| IESL-FORTH | X | | | | | X | | X (P) | | | | | X | X (P) | | X (P) | | | | | | | | | | | | |
| NGL | X | X | X | | X | | | | | | | X (P) | | | | | | | | | | | | | | | | |
| ICVBC-CNR | X | | | | X | | | | | | | X (P) | | | | | X(P) | | | | | | | | | | | |
| NCU | X | X | X | | X | | | | X (P) | X | | | | | | | | | | | | | | | | | | X |
| RWTH | | | | | | | | | | | | | | | | | | X | | | | | | | | | | |
| ATOMKI-HAS | | | | | | | | | | | | X | | | | | | | X | X | X | | | | | | | |
| CPP-LRMH | X | X | | | X | | | | X | | | X | | X (P) | | | X | | | | | | | | | | | |
| BM | X | X | X | X | X | X | X | | | | | X (P) | | | X | X (P) | | | | | | | | X | | | | |
| DIBS | X | X | X | | | | | | | | | X (P) | | | | | | | | | | | | | | | | |
| Of-ADC | X | X | X | | X | | | | | | | X (P) | | | | | X | | | | | | | | | | | |
| OPD | X | X | X | | X | | | | | | | X (P) | | | | | X | | | | | | | | | | | |
| PRADO | X | X | X | | X | | | | | | | | | | | | | | | | | | | | | | | X |
| RCE | X | | X | | | | | | | | | X (P) | | | | | | | | | | | | | | | | |
| KIK-IRPA | X | X | X | | | | | | | | | X | | | | X | | | | | | | | | | | | X |
| RISSPO-HAS | | | | | | | | | | | | X (P) | | | | | | | X | | | X | | | X | X | X | |
| UNIBO | | | | | | X | | | | | | X | | | | | | | | | | | | | | | | |

[1] The techniques listed here are not necessarily a reflection of availability within the institution as a whole but are only those listed for application to the particular type of painting or painted object for which the respondent institution provided information. In particular the KIK-IRPA entry only relates to techniques applied to the examination of wall paintings. The NCU data was provided by the Institute of Conservation and Restoration of Cultural Property of NCU.

## 3.3 SURVEY SECTION 3: Sampling, sample preparation and analyses

Table 3 gives the techniques listed by CHARISMA partners as those that they use for the analysis of samples. Optical microscopy, scanning electron microscopy with energy dispersive X-ray analysis, various FTIR spectroscopic techniques, micro-Raman spectroscopy and Gas Chromatography - Mass Spectrometry are the techniques that almost all the CHARISMA partners apply routinely. The particular instrument specifications detailed by the partners in the survey reflect developments in these techniques. Variable pressure SEM, for example, has become common in this field and avoids the need for carbon coating since charge compensation is given by the chamber gas; this makes it feasible to now to analyse cross-sections with other techniques after SEM without having to repolish the sample. Large area EDX detectors, a recent introduction owned by a few partners, allow far lower beam currents to be used reducing beam damage, and so also conserve the sample in a state where it is still suitable for further analysis.

It is also noticeable that FTIR mapping and imaging techniques are now reasonably widespread, allowing spatial information about the location of both inorganic and organic components in the paint samples to be obtained from all types of samples, including directly from cross-sections. The actual instrumentation with which this is achieved varies among the partners – mapping with a single element detector, scanning with a linear array or imaging with a focal plane array. These are combined with various different modes of analysis – transmission, reflectance and ATR objectives of various types. These techniques are proving their potential to improve analytical capabilities in the field, particularly for analysis of organic components within cross-sections, but there is development to be done in optimising sample preparation (the subject of WP10 task 1), with the requirements being slightly different for each type of instrument. FTIR is also used to give molecular information on inorganic components of the samples, as is micro-Raman spectroscopy. XRD is useful for giving structural information on crystalline materials but may need a separate sample, the quantity depending on the type of equipment available; however, the technique is not destructive to the sample so that it can still be analysed with other techniques such as Raman or FTIR or used for further analysis of organic materials within the sample.

Although FTIR gives a great deal of useful information on the samples analysed, for detailed characterisation of organic components other techniques are needed, the most common being GC-MS and HPLC. For these it is necessary to take a powdered sample, which is consumed during the analysis. This is also an area where the number of different techniques that are being applied has increased. To make best possible use of the sample, it is therefore necessary to carefully consider which technique is most suitable for the specific type of materials contained in the sample. Certain techniques are only used by a few partners, for example ESI-MS and DTMS for the analysis of varnishes and binding media. The size of the sample for these techniques needs to be balanced against the quality of the information that can be obtained, since if it is too small contamination can become more of an issue. Some GC-MS protocols separate the sample into fractions that are analysed in a series of steps, which can allow identification of many different classes of materials but may also have implications for the amount of sample that is required. A separate sample is usually used for HPLC analysis of dyes in lake pigments, but sample size can be an issue, especially where the lake is not the main component in the paint. New Raman methods that reduce fluorescence hold out the prospect of smaller samples and even identification of the dyes directly on cross-section samples.

The techniques that are mentioned by only a few partners often have very specific analytical aims; neutron activation analysis for trace elements in, for example, lead white, which can help to establish the origin of the pigment, or ion chromatography for determination of soluble salt content in the efflorescence from wall paintings. The new immunological methods are still in development and only used occasionally. Immuno-fluorescence microscopy and especially chemiluminescence techniques have the potential to gain detailed information on organic components directly from cross-sections. ELISA is another technique relatively new to this type of sample that can allow further characterisation of protein materials.

The techniques only available at the synchrotron or neutron large-scale facilities are also not used routinely, of course, but are available by application for beamtime. They can give information that is not obtainable by other means and often the same samples that have been prepared for other types of analysis can be used.

*Who takes the samples?*

Most commonly amongst the CHARISMA partners it is the scientists that will be undertaking the analysis that actually take samples from objects. This has significant advantages, since a deep knowledge of the nature of the sample, its exact location on the object and the appearance of the sample site is extremely valuable in interpretation of the results of the analysis. In this way, also, the most appropriate sample in terms of type and size can be taken and the scientist is perhaps best placed to ensure that the sample is extracted and stored in such a way as to avoid contamination. The survey reports that conservators also sometimes take samples, usually with the scientist present, and occasionally also with the curator or archaeologist, so that the decisions about sampling sites and numbers, as well as the questions that it is hoped will be addressed, can be made together and in full understanding and agreement. In a few cases, especially where analysis is being undertaken for conservators or curators external to the consortium partner, scientists are not allowed to take samples and instead they must be taken by a conservator. Sampling is a delicate and skilled process and it is implicit that the ability to take the correct quantity from an appropriate location, and to choose the site in a responsible way, relies on it being undertaken only by skilled and trained professionals. This gives the best possible prospect of most effective use being made of the sample.

TABLE 3. Techniques for the analysis of samples mentioned by the partners in the questionnaire (brackets imply more occasional use)

| | Optical microscopy | Scanning Electron Microscopy –Energy Dispersive X-ray analysis | Transmission electron microscopy | Fourier Transform infrared spectroscopy or microscopy | Raman spectroscopy or microscopy | Ion chromatography | Gas Chromatography | Gas Chromatography – Mass Spectrometry | Electrospray ionisation mass spectrometry | Direct temperature resolved mass spectrometry | High Performance Liquid Chromatography | X-ray Diffraction | Liquid Chromatography – Mass Spectrometry | Immunological methods (IFM, ELISA etc) | Thin layer chromatography | Ion exchange chromatography | Staining tests | Microchemical tests | Neutron activation analysis analysis | Nuclear Magnetic Resonance spectroscopy | Synchrotron techniques |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UNIPG | X | X | | X | X | | | X | | | | (X) | (X) | (X) | | | | | | | |
| C2RMF | X | X | | X | X | | | X | | | | X | | | | | X | | | | |
| IESL-FORTH | X | X | X | X | X | | | X | | | | X | | | | | | | | X | |
| NGL | X | X | | X | (X) | | | X | | | X | (X) | | | | | (X) | (X) | | | |
| SOLEIL | X | X | | X | X | | | | | | | X | | | | | | | | | X |
| ICVBC-CNR | X | X | | X | X | | | X | | | | | | | | | | | | (X) | |
| NCU[2] | X | (X) | | | (X) | | (X) | | | | (X) | | | | (X) | | X | X | (X) | | |
| CPP-LRMH | X | X | | X | | X | | X | | | X | (X) | | | | | | X | | | |
| BM | X | X | | X | X | | X | X | | | X | (X) | | | | | | | | | |
| DIBS | X | X | | X | X | | | X | | | (X) | X | | | (X) | X | | | | | |
| Of-ADC | X | (X) | | X | X | | | X | | | X | (X) | | | | | | | | | |
| OPD | X | X | | X | (X) | X | | (X) | | | | | | | | | X | X | | | |
| PRADO | X | X | | X | | | | X | | | X | | | | | | X | | | | |
| RCE | X | X | | X | X | | | X | (X) | (X) | | X | | | | | X | X | | | |
| KIK-IRPA | X | X | | X | X | | | X | | | (X) | X | X | | | | | | | | |
| UNIBO | X | X | | X | X | | | X | | | | X | | X | | | | | | | |

[2] This data was provided by the Institute of Conservation and Restoration of Cultural Property of NCU.

*Recording of sampling site(s)*

The survey indicates that common practice among CHARISMA partners is to record sample sites on a hard copy image of the painting or object, generally a colour print of a digital image. In the recent past a black and white photograph was often used, and in those partners who have been conducting this type of analysis over decades, this has proved to be a durable method of documentation. The same may not be true of conventional colour prints, and there is not much evidence that their permanence for this purpose has been tested. In quite a high number of the institutions surveyed these sample sites are then transferred on to a digital image and marked using standard imaging software such as Photoshop. Detail images are sometimes used – either hard copy or digital – in order to mark the sample sites more accurately. Some institutions routinely photograph the sample point before and after sampling to record it, while others take photomicrographs more occasionally. Other types of image such as infrared images or fluorescence images might also be used to mark the sample points. X,Y coordinates of the location are recorded by some of the institutions.

Detailed notes are made to describe each sample point and to record the identifying number or code that it has been given, as well as to record any additional information about the appearance of the surface of the painted object or painting and the question that the analysis hopes to answer, both of which are useful when interpreting the analytical data. It is also good practice to make a note of any information on previous conservation treatments, or other aspects of conservation history, known surface coatings or consolidants etc. This information is usually recorded by hand initially, or in laboratory notebooks that are afterwards archived. Usually this information is then transferred into reports in electronic form.

Only a few institutions have an integrated structured database of their records of technical examination in which the sample sites and related information is inserted. This is, however, a developing area of interest for many CHARISMA institutions and would therefore be a suitable area for future networking. It has links to other tasks concerned with integration of data, including both Task 2 in WP2 and WP8.

*Sampling process; tools, containers and method*

Slightly different sampling methods are used depending on the type of sample that is taken and what analysis is intended. Samples that are taken to prepare a cross-section and examine the stratigraphy of the paint and surface coatings are usually taken with a no.11 scalpel blade. The size depends very much on the size and state of conservation of the object. A sample size of around 300 microns was around the average reported from easel paintings, but from wall paintings a larger sample can be necessary to be able to obtain the complete stratigraphy in such a way that it retains its coherence. Various sample containers are suggested; cupped microscope slides protected with a further ordinary microscopy slide placed on top, gelatine capsules, glass vials, small polypropylene tubes with conical ends. Quartz tubes are needed where neutron activation analysis is intended. The sample is generally transferred either with the tip of the scalpel blade or a brush.

Samples are taken as powdered scrapings for FTIR microscopy in transmission mode, GC-MS, HPLC, Raman and XRF using a small rounded blade scalpel (no. 15). For organic analysis, contamination is more of an issue and so chemically-cleaned glass vials are universally used. The sample size is very variable and depends very much on the types of analysis that will be used. Where analysis of varnish is required during conservation treatment it is sometimes collecting by dissolving it with a cotton swab or Japanese paper soaked in a suitable solvent. This does not of course allow differentiation between different layers, and sometimes this can be achieved by carefully scraping of the surface coatings layer by layer from a small area. Cleaning of all tools such as blades and brushes between samples to avoid cross-contamination is important.

For samples for wood identification, rounded scalpel blades (no.10 or 20) are used. Ideally a transverse, axial and tangential shaving would be obtained but usually this is not possible and the identification relies on a single transverse thin section. For samples for identification of canvas fibres both warp and weft threads are taken.

*Preparation of cross-sections*

At this stage of Task 1 in this work package attention will be given to the preparation of samples as cross-sections (rather than the various preparation and derivatisation methods used for chromatographic and mass spectrometric analysis). This is due to the interest in Task 1 of Work Package 10 (based around improvement of techniques for the analysis of organic materials in cross-sections) in compiling this particular information. It will also be valuable for the planning of the next part of the task, which will aim to compare and define the advantages and disadvantages of various methods. In the future work possible new methods or variations to established practice will also be explored, but in advance of this it is useful to compile information on the current preferred methods of embedding, grinding and polishing cross-sections.

There is some variation in the exact techniques or materials used, but also a lot of common ground among the CHARISMA partners, summarised in Table 4. Three types of resin are mainly used for embedding samples; polyester is the most common, followed by epoxy, and polymethyl methacrylates (in one case in the form of Plexiglas® blocks). The advantages and disadvantages of each type, and their specific properties, will be considered in more detail in future work in this task, particularly in relation to application of various chemical imaging techniques to the mounted samples, but some concerns seem to be formation of air bubbles (particularly for epoxy), as well as setting time.

Main variations in the method of grinding and polishing concern whether the cross-section is created by microtoming the block or by grinding with silicon carbide paper. Usually this is carried out with water as a lubricant. Where water-sensitive materials are present the samples are often ground and polished dry, but this does not always give a satisfactory surface. Micromesh® polishing cloth, either with water or dry, seems to be the most common polishing method, although other types of abrasive cloth are also used. The other sample preparation processes mentioned in the survey – impregnation with cyclododecane before mounting to avoid infiltration of resin into porous samples, or use of KBr as an embedding medium – relate very much to samples where it is intended to carry out analysis of organic components directly on the cross-sections. They do avoid difficulties in interpretation of the analysis that can result, for example, from smearing of the embedding resin across the sample surface during polishing, but can be difficult for small samples or for long-term storage and these methods are not necessarily appropriate for routine use.

Cross-section preparation methods are often a combination of tradition and habit, passed on through example rather than any formal teaching process. The aim in constructing guidelines or a protocol for this aspect of sampling methodology is not therefore to be prescriptive, but to provide information on the advantages and disadvantages of each material or process and their suitability for particular types of analysis. The aim in this first deliverable of this task is to simply collect together the information on established practice to use as a basis for testing and comparison, so that guidelines can be formulated in the second deliverable at month 30. In this way new developing methods can also be discussed, tested and incorporated into the suggested methodology.

TABLE 4: Sample embedding resins, grinding and polishing methods used in the CHARISMA consortium for the preparation of thick cross-sections

| | **Embedding resin** | **Grinding method** | **Polishing method** | **Comments** |
|---|---|---|---|---|
| UNIPG | Epoxy resin (Epofix™) | Resin block is cut with diamond saw, SiC paper also used | Micromesh® polishing cloth and felt cloth for final polish (water or dry) | Samples sometimes pre-treated by impregnation with cyclododecane to avoid penetration with resin |
| C2RMF | Polyester resin (H59, Sodemi) | SiC paper | Fine grades of SiC paper | |
| IESL-FORTH | Epoxy resin (Epofix™) | SiC paper (with water or dry) | Velvet cloth with diamond paste | |
| NGL | Slow setting polyester resin (Tiranti) | SiC paper (with water) | Micromesh® polishing cloth (water or dry) | |
| SOLEIL | Polyester resin (Serifix)<br>Epoxy resin (Epofix ™) | SiC paper (with water or dry) | Micromesh® polishing cloth for hand polishing or diamond paste/colloidal silica with cloth on an automatic polisher | |
| ICVBC-CNR | Epoxy resin (Epofix™)<br>Polyester resin (Mecaprex) | SiC paper (with water or dry) | LAM-PLAN 431 abrasive cloths with water | |
| NCU | Polyacrylic resin (Spofacryl O, Duracryl Plus O) | SiC paper (with water) | Felt cloth | |
| CPP-LRMH | Polyester resin (H59, Sodemi) | SiC paper (with water) | Velvet cloth with diamond paste | |
| BM | Slow-setting polyester resin (Tiranti) | SiC paper (with water) | Micromesh® polishing cloth (dry) | |
| DIBS | Methacrylate resin (Technovit 2000 LC) | SiC paper (with water) | Micromesh® polishing cloth (dry) | |
| Of-ADC | Polyester resin (Serifix)<br>Epoxy resin | SiC paper (with water) | Felt cloths (OP-nat and OP-felt), wet, with or without diamond paste | |
| OPD | Polyester resin (Mecaprex SS) | SiC paper (with water) | Fine abrasive paper with with water or Ligroin (hydrocarbon solvent) | |
| PRADO | Cold setting methacrylate resin (Technovit 4004) | SiC paper (with water) | Micromesh® polishing cloth (dry) | |
| RCE | Polyester resin (Polypol PS230) | SiC paper (with water) | Micromesh® polishing cloth (dry) | Sample holder used for hand polishing to achieve an even surface with both sides parallel, an advantage for ATR-FTIR imaging |
| KIK-IRPA | Sample is first glued to the surface of a small Plexiglas cube with methacrylate resin (Spofacryl), then covered with a layer of the same resin. A second small Plexiglas cube is then placed on top before | SiC paper (with water) | Micromesh® polishing cloth | Resin can sometimes be smeared over the surface during polishing which interferes with ATR-FTIR imaging |

| | | | | |
|---|---|---|---|---|
| | the resin has hardened. | | | |
| UNIBO | Polyester resin<br>Epoxy resin | SiC paper (with water or dry) | Micromesh® polishing cloth (dry) | Sample holder used for polishing.<br>Samples for FTIR embedded in KBr pellet and then polished with micromesh (see WP10) |

*Analysis of cross-sections by several complementary methods*

Cross-section samples are generally examined and analysed by a series of complementary methods. They are always examined first by optical microscopy, and SEM-EDX and Raman are also routinely used. Increasingly, quite a number of the institutions also have access to FTIR instrumentation that can be used directly on cross-sections and this will become more common in the future. Considerations that need to be taken into account are that SEM-EDX analysis can result in beam damage (particularly after EDX mapping), which might affect subsequent analysis of organic materials, for example by ATR-FTIR microscopy. Conversely ATR-FTIR microscopy can create a crater of varying depth caused by contact of the sample with the ATR objective and this change in the sample topography might affect other analyses. An increasing number of different techniques, including those available at the synchrotron or other large-scale facility, are used on the same sample. Although they are non-destructive to the sample in that it is not destroyed or consumed, they can even so damage the sample in a way that affects subsequent analyses and it is important to be aware of the particular features of each technique. A series of different types of images are often created – visible light, ultraviolet fluorescence, EDX maps and FTIR maps and images – and need to be integrated. Guidelines or protocols for this aspect of technical examination will not be dealt with in detail here and are instead planned for future work in the task.

*Long-term storage (archiving) of samples*

Long-term storage of samples from cultural heritage objects is an issue that needs to be considered because, as the survey shows, samples from earlier examinations (including both cross-sections and any unmounted fragments that remain) are routinely re-examined over the course of many years, and are always considered before any further sampling is undertaken. Several of the institutions in the consortium have stored samples from as long ago as the 1950s that are still used for further investigations, either because new analytical techniques now exist that allow new information to be extracted, or perhaps as part of research projects that re-use the information for other purposes than the original examination, such as cataloguing programmes or investigations into the history of use of a particular material or occurrences of a particular form of degradation.

For cross-sections to be re-examined and new information to be gained from them as instrumental techniques improve, they have to remain in sufficiently good condition, which could depend to some extent on storage conditions, but also on the ageing characteristics of the resins used for embedding the samples. In several of the institutions a number of different resins have been used over various periods of time. Those mounted in epoxy resins seem to have become rather yellow and brittle, although these were also the oldest archived samples, from the 1950s, so this might not necessarily indicate that epoxies are a particularly poor choice of embedding medium from this point of view. Even so, it is usually still possible to examine the cross-sections, and younger samples have often survived well and only require a light polish in preparation for further examination or analysis. A past practice that can be more problematic is the fixing of cover slips on the cross-sections with a mounting medium such as DPX. This can sometimes react with the materials in the sample, although again it is often possible to recover the sample by polishing. The samples are also often not in such good condition if staining tests have been applied to them, or if they have been carbon coated for SEM analysis. Some of the more specialist mounting methods, for example KBr which has been used where FTIR analysis of the cross-section is intended, require particular storage conditions and this may have implications for their long term survival. Storage conditions might also be a factor in how well samples in other types of embedding media have survived; the survey reports methods of storage being wooden or metal cabinets on glass microscope slides or in plastic boxes or bags. One partner uses plastic acid–free cabinets with small silica gel bags for humidity control and it may be that this more careful approach has advantages. Another important aspect that needs to be considered is adequate documentation of the samples, including descriptions and records of sampling points and labelling of the actual samples in a manner that will survive long term.

# 4. SAMPLING METHODOLOGY AND PRACTICE: A LITERATURE REVIEW

## 4.1 Motivations and scope of the literature review

The survey questionnaire has collected information on sampling practice and methodology within the CHARISMA consortium, which can form a good basis for compilation of guidelines for best practice in this area. The aim of this literature review is to supplement this by acquiring a broader view of practice outside the CHARISMA consortium. For this first deliverable under this Task in Work Package 2 the focus of this review has been on certain subjects. The first part concerns more general literature on sampling methodology and sample preparation techniques. A review has also been made of existing documents on guidelines and standards relating to sampling.

The second half of this literature review concentrates on sample preparation techniques. There is a heavy emphasis on FTIR analysis because this has become of particular interest in the field of science applied to cultural heritage due to the development of FTIR imaging and mapping instruments that can be used directly on cross-sections. This is also a field of research being explored in Task 1 of Work Package 10, and hence this literature review can form a valuable foundation for the design of some of the practical experimental work. In the same way as the survey questionnaire, this first deliverable under this task concerns mainly the literature relating to paint samples. It considers the sample preparation requirements that various different FTIR techniques impose (FTIR mapping and imaging in transmission and reflectance, ATR-FTIR mapping and imaging, synchrotron FTIR mapping in transmission or reflectance), and the advantages/disadvantages of each. Analysis of cross-sections with Secondary Ion Mass Spectrometry (SIMS) is even more demanding with respect to sample preparation. A review of this technique provides a useful supplement to the information taken from the FTIR literature. The final section deals with the relatively new sample preparation technique of argon ion beam polishing, which has not yet been widely used in the cultural field but which appears to hold some potential.

## 4.2 General literature on sampling methodology, protocols and practice

There has, for a long time, been much discussion in the cultural heritage sector of the ethical and methodological issues surrounding the taking of samples from precious museum objects. However, until fairly recently, very little of this debate has been published or formalised in a written context. In general, individual institutions have been responsible for generating their own guidelines for the sampling of objects and the subsequent analysis and documentation. In 1998 the American Institute for conservation formulated one of the first codes of practice in this area which deals briefly with this subject. This document states that the appropriate consent must be given by the owner or custodian prior to any investigation being carried out. Only the minimum of material, necessary for the intended study, should be removed and that proper documentation to record both the process of sampling and the results obtained is required. Subsequently, other professional bodies in many countries have adopted similar codes (Khandekar 2003). More recently, however, The European Committee for Standardization (CEN) have drafted a more complete document providing a set of guidelines for the methodology of sampling materials from cultural property. This document deals more thoroughly with every aspect of sampling from the criteria that should be used to evaluate the need for sampling, through to the process of taking samples and their subsequent documentation and storage. In addition a project funded by the Mellon foundation, ConservationSpace, http://www.conservationspace.org, recently investigated this area, conducting research into the methodology of conservation-related work projects, including analysis and sampling. They have examined the use of business process modelling to look at ways of documenting all of the stages involved, and the decisions that need to be taken when a conservation project is being considered. A flow chart illustrating the methodology is available on the project website.

Mounting samples from painted objects as cross-sections for microscopy has become a routine method of examination. The technique was initially pioneered in the early twentieth century and rapidly developed into a standard method used by museums and institutions all around the world. Initial work was carried out by Laurie (1914) and was then extended by Gettens at the Fogg Art Museum, Cambridge, Massachusetts (see Gettens 1936). In these early experiments samples were often embedded in a wax medium and cut with a microtome to make thin sections.

Plesters's seminal article for *Studies in Conservation*, published in 1956 and still much referred to, details the history of the technique up until that point and takes the discussion forward with a description of the preparation of embedded thick cross-sections, much the same as that still routinely in use today. Plesters clearly lays out the advantages of being able to study micro-samples of paint in cross-section. A minute amount of material yields a great deal of information; the sequence of paint layers in the picture, their colour, texture, thickness and the size and appearance of pigment particles can all give valuable insight into the artist's technique. In addition, specific questions relating to the restoration or conservation of a particular passage may be answered. However, in this article Plesters also touches on some of the ethical discussions surrounding the sampling of paintings. She states that only a minimum of material should be removed (i.e. as small a sample as possible), and that a work is usually only sampled when some information of use to the restorer might be gained.

Derrick et al. (1994) published a study of different types of embedding resin used for the preparation of cross-sections. The authors found that while polyester resin offered advantages over other synthetic resins available such as epoxies, which yellow and become brittle, or acrylics which tend to shrink on curing, there are still some problems with this method. Polyester was found to soak into, or infiltrate, porous samples and the authors also noted that certain types of component were found to be soluble in the polyester medium – some waxes and some organic dyes used in modern synthetic pigments. The study evaluated different types of embedding media used in other fields such as forensic science and tested several different barrier methods with the aim of reducing the penetration of the polyester resin. Contamination from the embedding resin can potentially cause difficulties if the samples are used for particular types of analysis, especially if the organic materials in the sample are of interest. This issue is one that is mentioned frequently in the FTIR literature, so will be discussed in more detail in the section in which it is reviewed below. Ultimately, the method of sample preparation chosen usually depends mainly on the aim of the study. Preventing the infiltration of the polyester resin may be important if the organic materials are being studied, or for certain types of samples such as those from wall paintings, but in turn using a barrier method to achieve this may make samples structurally weaker and less easy to microtome to produce thin sections for study in transmitted light or by transmission FTIR.

In the same paper Derrick et al. also give a useful description of the practicalities of preparing thin sections using a RMC Model 7000 microtome with a glass knife. Thin sections of 5 µm were prepared from embedded samples in this way. The authors state that smaller samples are easier to microtome and that for larger samples it helps to cut the sample into a pie shape before positioning it in the mould so that the point of the sample can be microtomed first. An embedded sample is easier to successfully microtome if the excess embedding medium is trimmed from the resin block before cutting. Too large a cutting surface tends to cause the thin section to curl up and the sample can then de-bond from the embedding medium. The authors state that the orientation of the layers in the sample to the knife edge makes a considerable difference to the quality of the cut. They found that for a horizontal knife edge the initial cut was best made with the layers of the sample orientated at 10° from the vertical, with the most important layer positioned closest to the knife. If the layers were orientated directly vertically curling of the thin section and particle loss increased, while horizontal orientation resulted in the compression of the sample. However, different samples are likely to behave differently on cutting and sample orientation may need to be adjusted. A further discussion of microtoming to produce thin sections is included on the section related to the preparation of samples for analysis by FTIR techniques below.

Khandekar (2003) published a review paper which provides a useful history of the literature relating to the preparation of cross-sections and a detailed discussion of the techniques commonly used to embed and polish samples as thick cross-sections. The paper provides descriptions of the methods used for removing minute samples of paint, as well as the systems employed for the embedding process and the types of resin that have been used, including waxes, polyesters, epoxies, acrylics and polypropylene/polyethylene co-polymers. Khandekar also briefly discusses the issue of long-term storage of cross-section samples. There is little information published about this subject despite the serious implications for research in this field. Cross-sections are precious and unique samples that contain valuable information about the objects from which they have been removed. In many cases there may not be another opportunity to obtain a further sample and the ethics of doing so when samples already exist should be considered. However, for cross-sections to be re-examined and new information to be gained from them as instrumental techniques improve, they have to remain in sufficiently good condition. Khandekar notes that early samples mounted in wax at the Harvard University Art Museum survive remarkably well. In contrast some of the samples prepared in epoxy resin do not survive. Adhesives, such as fir balsam or DPX, a mixture of distyrene, tricresylphosphate and xylene, which were sometimes used to fix cover slips in place, have also caused problems, especially where copper green pigments are present. How well samples will last is a factor to take into consideration when evaluating any new developments in the methods of preparing cross-sections.

### 4.3 Sample preparation issues relating to Fourier Transform Infrared spectroscopic techniques

The analysis of materials, both organic and inorganic, by infrared spectroscopy, has been exploited by conservation scientists since soon after the advent of the technique. Initial measurements were carried out on dispersive instruments and allowed the study of bulk materials either as nujol dispersions or in KBr pellets. However, the large sample size required and the poor spectral resolution achieved meant results were limited. The development of Fourier transform infrared spectroscopy (FTIR) which gave better signal to noise ratios and allowed data to be obtained from extremely small samples, and also the introduction of FTIR microscopes, led to far greater possibilities, especially for the examination of complex samples such as those encountered in the cultural heritage sector.

Casadio et al. (2001) provide a useful review of the history of IR spectroscopic techniques applied to analytical problems in the field of science applied to cultural heritage, up until the year 2000. The introduction of the diamond anvil micro-compression cell, in which small samples are pressed between two diamond windows, made great improvements. This allowed for much smaller sample sizes and avoided the sample manipulation involved in preparing a dispersion or a KBr pellet. The sample is flattened between the diamond windows until it is thin enough for analysis in transmission mode. In conjunction with an IR microscope, some degree of spatial resolution of the FTIR data within the sample can be achieved and different areas of the sample can be analysed separately, with the smallest possible area being around 30 microns with a conventional globar source. An approach that is commonly used is to separate the layers within the microstructure of a paint sample with a scalpel under the microscope for analysis in the diamond cell. This is often very effective, but the complex combinations of materials that can be encountered can be challenging and it is not always possible to be certain that material from only a single layer is contributing to the spectrum.

For this reason there has been much interest in development of methods that allow FTIR analysis to be performed directly on samples prepared as cross-sections, which preserve the layer structure intact. This involves either the preparation of some form of thin section for analysis in transmission mode or instead, reflection techniques can be used on thick cross-sections. Both approaches require particular attention to the preparation of samples. Thin sections need to be both thin enough for transmission and structurally strong enough to remain intact, whereas the surface quality of thick-sections has a big impact on the data obtained by reflection FTIR. Attenuated Total Reflection (ATR) FTIR has also been employed for analysis of both un-mounted fragments and samples prepared as cross-sections. With this technique it is critical that good contact is achieved between the sample and the ATR crystal, which involves a slightly different set of sample preparation considerations.

The advances in the capabilities of the instrumentation available and new FTIR techniques for imaging or mapping, as well as synchrotron techniques, have allowed improvements in the data obtained from samples from the point of view of both spatial resolution and quality of the spectra, but in turn this has led to the need for more careful sample preparation. To a certain extent the preparation method employed will be dictated by the instrumentation available and the type of FTIR analysis to be carried out. The materials being studied and the particular question of interest will, of course, also play a role.

There are a number of techniques that are capable of identifying the inorganic components in cross-sections that are routinely used, such as SEM-EDX and Raman microscopy. Interest in FTIR imaging and mapping techniques that can be used on cross-sections has been driven to a great extent by their ability to analyse not only the inorganic components (pigments, fillers etc) but also the organic components (binders, coatings etc), as well as compounds that result from interaction between the pigments and binding media. Nevertheless, organic components within samples are inherently less strongly absorbing of infrared radiation than many of the inorganic materials commonly present, and there is much discussion in the literature about the difficulties that this can cause. Contamination of the sample by the resin in which it is mounted for preparation as a cross-section is another issue often mentioned in the literature. In practice it is often possible to obtain reasonable results from a thick cross-section prepared and polished in the traditional way, but problems can be encountered with porous samples if they have been infiltrated with resin, and absorbance bands from the resin can often appear in spectra from the uppermost or lowermost layers nearest the embedding medium. These issues have motivated much of the work described in the literature on investigation of alternative mounting methods or improvement of the cross-section surface.

Early experiments in this field with alternative embedding media that would not interfere with the FTIR analysis were carried out by Pilc et al. (1995). A system was developed for embedding paint samples in a silver chloride block that could then be microtomed using a glass knife to make thin cross-sections, analysed in transmission mode. These could be flattened further in a diamond cell if they were still too thick to allow good transmission spectra to be collected. The use of silver chloride overcame some of the problems encountered when attempting to microtome samples embedded in epoxy or polyester resin blocks, where the process of cutting tended to smear a thin layer of the resin across the surface of the sample, which contributed to the overall FTIR spectrum so that interpretation, particularly of organic components, was compromised. However, the disadvantage associated with this technique is that silver chloride is photosensitive and darkens on exposure to light, making location of the sample within the block, and accurate microtoming to produce thin sections, difficult. Pilc et al. (1995) also experimented with KBr as an embedding medium, but found that samples mounted in this way were difficult to microtome to form thin sections as they tended to fragment and break up.

Pilc et al. analysed only single points on the cross-section, but subsequently instruments became available that were capable of mapping the sample by collecting spectra from a sequence of points in the shape of a grid. The data could then be processed to give a ‘map’ of the distribution of an absorbance band that was characteristic of a particular component of the sample. A further development was the advent of focal plane arrays. Heeren et al. (1999) reported the application to paint cross-sections of an FTIR microscope with a 64 x 64 pixel mercury-cadmium-telluride infrared imaging array, which allowed spectra to be collected from the whole field of view simultaneously, so that ‘images’ of the distribution of an absorbance band could be produced far more quickly than with the mapping approach. Heeren et al (1999) carried out the analysis in reflection mode on thick cross-sections already prepared for light microscopy in the conventional way, embedded in resin and polished. The value of correlation of the FTIR images with the results of other analysis on the same sample, such as SEM-EDX mapping has been demonstrated in many subsequent papers in this research group.

Van der Weerd et al. (2004) described a new method of preparing thin sections embedded in KBr by polishing the KBr pellet from both sides. The authors evaluated the differences in the transmission spectra obtained by FTIR imaging on a multi-layered sample prepared in this way compared to the data obtained from a similar sample compressed in a diamond anvil cell. Microtoming of thin sections from a sample embedded in a synthetic resin was not successful in this study due to the brittle nature of the aged paint in the sample used. The transmission results were compared to those obtained by reflection techniques on conventionally embedded cross-sections. The quality of the specular reflectance spectra was found to be poor and complicated by peak shifts and distortions, which can hinder interpretation. The transmission spectra obtained from both the KBr thin section and the diamond cell were of good quality. Nonetheless Cotte et al. (2009) mention some drawbacks of this technique. For example when polishing a KBr pellet from both sides to obtain a thin section it is difficult to obtain a homogeneous thickness across the sample and, without great care and experience, it is also possible to lose the sample altogether. In addition samples prepared in this way may have little cohesion and poor structural strength making them extremely fragile and difficult to handle. Cotte et al. (2009) also experimented with an alternative to microtoming, placing a sample on its side in a diamond micro-compression cell in such a way that when it is crushed the layers remain in sequence. This approach was used for a wall painting sample which was examined with synchrotron based µFTIR mapping and allowed some specific information about the different layers to be gained.

Paint samples from historic cultural objects are not easy to section by microtoming. The samples tend to be inhomogeneous and multi-layered, with the various layers of paint behaving differently during cutting. In addition, hard pigment particles, such as haematite or azurite, are difficult to cut and may be roughly fractured or knocked out completely. Older paint films tend to be brittle and hard, or can be crumbly and lacking in cohesion, although modern paint is likely to be easier to section. Even if it is not possible to achieve a perfectly coherent and complete cross-section it can still be a useful method of sample preparation depending on the goal of the analysis, especially if it is a particular layer that is of interest; for example, microtomed thin sections of red lake glazes have been used in the past for microspectrophotometry to identify the class of dyestuff used to prepare the lake pigment, and in this case it is not so critical to obtain a section that includes all layers. Preparation of thin sections can be particularly problematic, however, for certain sample types, such as those from wall paintings (Cotte et al. 2008).

Despite the inherent problems of preparing extremely thin sections of fragile materials, there are good reasons for this avenue of research. All reflection FTIR techniques are extremely dependent on the surface quality of the sample, which can lead to noisy or distorted spectra. Furthermore, reflectance spectra do not always correspond well with library spectra, which are usually collected in transmission mode. Transmission FTIR analysis tends to produce more reproducible results. Various authors give different estimates of the thickness required for microtomed thin sections in order to achieve good transmission FTIR measurements. In reality this probably depends on the materials involved. Cotte et al. (2009) suggest that for effective transmission FTIR analysis, microtomed thin sections should be no more than 5 µm thick to avoid saturation of the most intense vibration modes. Other authors, for example Tsang et al. (1991), Lluveras et al. (2010) and Gruchow et al. (2009) use slightly thicker slices of 10-15 µm thickness.

In order to provide microtomed thin sections with some additional structural strength and protect against the unregulated cutting shock involved in their production, Tsang et al. (1991) describe a method for adhering a Mylar film to the surface of the sample with either water or cold paraffin prior to microtoming. Before analysis the film is removed from the thin section by briefly dipping the sample into liquid nitrogen. This process embrittles the paraffin and enables the thin section to be removed from the film without tearing the fragile sample.

Nonetheless the production of thin sections whilst it may help to improve the quality of the spectral data in comparison to specular reflection measurements by allowing analysis in transmission, does not overcome the issue of contamination from organic embedding resins. Lluveras et al. (2008) describe the use of a glass knife microtome for the preparation of thin sections embedded in an epoxy medium, for analysis by synchrotron FTIR mapping. The thin sections produced were pressed between two diamond windows in order to flatten them and sample thicknesses of 4 and 12 microns were achieved for the two samples reported. However, the authors state that it was impossible to avoid the ingress of the embedding medium into the sample. In addition the microtoming produced some holes in the surface of the sample so data could only be collected from a portion of the sample surface.

A further attempt to overcome contamination from the embedding resin was investigated by Cotte et al. (2009). They demonstrated that an effective barrier could be created between the sample and the embedding resin by first wrapping the sample in aluminium foil, before embedding it and subsequent microtoming. However, this approach was applied to relatively fresh paint samples taken from a reconstruction where it was possible to take large samples. It may not be feasible to apply this method to real samples since the handling difficulties may be too great. In addition the authors state that, once wrapped, it was difficult to accurately position the sample to ensure that microtoming would produce thin slices perpendicular to the layer structure.

The use of thin sections can require an additional infrared transparent support. Various papers mention the use of different materials for this purpose. Gruchow et al. (2009) used a $CaF_2$ support which they found more appropriate than KBr since it is less soft and water soluble. However, they report that since $CaF_2$ is only penetrable by infrared radiation in the range 1050-4000 $cm^{-1}$ the spectral range is reduced. This is less of an issue when using an FPA detector where the spectral range only extends down to 950 $cm^{-1}$, but could be regarded as more of a disadvantage when using an MCT detector. Other authors suggest other infrared transparent materials for use as sample supports for microtomed thin sections; Echard et al. (2008), for example, report the use of a ZnS window when performing synchrotron FTIR analysis.

A further technique is reported in the literature for the analysis of thin sections which avoids the need for an IR transparent support by studying the thin section in transflection mode (sometimes referred to as reflection-absorption mode). Echard et al. (2009) and Bertrand et al. (2010) report the study of varnishes from musical instruments by synchrotron FTIR using this technique. 2 µm thin sections, from samples which had not been embedded in any mounting medium, were prepared by ultramicrotomy (Leica EM UC6, Diatome Histo diamond knife). The ultra-thin sections were deposited directly onto silver coated low-emissivity microscope slides (MirrIR, Kevley Technologies) and the analysis was carried out in transflection mode. This provides a practical and cheap alternative to the use of expensive IR transparent supports such as ZnS or ZnSe and avoids some of the problems mentioned above when using KBr or $CaF_2$. The system described in these papers, of microtoming thin sections without embedding the sample, may only be possible for large samples but in certain circumstances the ability to place the ultra-thin sample slices directly onto a microscope slide may be a practical advantage. However, the spectra generated may not be directly comparable to spectra collected in transmission since the contribution of some reflection signal, overlayed with the absorption signal, can cause spectral distortions.

There are inherent problems with preparing thin sections, particularly for historic paint samples and despite the specific issues associated with specular reflection FTIR, it can be a useful technique. The scientists working in the conservation field at the FOM Institute, AMOLF, in Amsterdam have applied this technique to the study of thick cross-sections on a routine basis. They also worked on improving the polishing techniques employed, which has allowed better quality specular reflectance FTIR data to be collected. The use of a specially designed and engineered sample holder which enabled more controlled and uniform pressure to be applied to the sample during polishing, resulting in a more uniform surface, was reported by Wyplosz et al. (2000). A good discussion summarising these developments is provided in the thesis by Van Loon (2008). She discusses the enhancement in the quality of specular reflectance FTIR data that was achieved by improving the sample surface using this specialised polishing technique. The final stages of the polishing were executed dry, without water or any alternative lubricant.

In addition Cotte et al. (2009) also demonstrated that, in reflection mode, more reproducible spectra could be obtained from the cut surface of an embedded thick cross-section produced with an ultra-microtome, as compared to a similar cross-section produced by polishing with silicon carbide (grade 1200). The more regular microtomed surface led to fewer distortions and less variation between spectra. On the other hand the repeated spectra obtained from the polished cross-section were far more irregular with much greater variation. The authors make the point that this is likely to be a more critical problem for experiments using small beam sizes, for example synchrotron FTIR analysis.

Following on from the work carried out by Van der Weerd et al., Mazzeo et al. (2007) demonstrated an improved ability to map the distribution of organic materials within the layer structure of paint samples when they were embedded and polished as cross-sections in KBr compared to the traditional method of preparation in a synthetic resin. In this case the authors made a thick cross-section from the KBr pellet using dry polishing with successively finer silicon carbide. The results obtained were compared to those from a cross-section embedded in a polyester resin, which was wet polished. The intention of this study was to evaluate the contaminating effect of the polyester resin on the sample and the authors describe the improved stratigraphic detection of the organic materials in the sample embedded in KBr. Despite the potential advantages of embedding cross-sections in KBr, particularly where the organic components are of interest, it is worth mentioning that the longer-term storage of samples prepared in this way is likely to be more difficult.

In this case the analysis was carried out with ATR-FTIR, which has the advantage that the spectra are more similar to the transmission spectra contained in most libraries than reflectance spectra.

Although Mazzeo et al. do not discuss issues of sample preparation relating specifically to ATR-FTIR, the quality of the surface does influence the success of the analysis since good contact needs to be achieved between it and the ATR crystal. For mapping and imaging in particular it is important to be aware when interpreting the FTIR images produced that contact may not be uniform across the surface, and that this might be influencing the results. This is not an issue that has so far been much addressed in the literature.

Prati et al. (2009) evaluated whether other infrared transparent inorganic salts could be used for embedding cross-sections in order to overcome the hygroscopic nature of KBr, which can cause the spectral quality to be reduced during the data acquisition as moisture is gradually absorbed by the sample. This could be particularly problematic if analysis is carried out using a mapping technique where the data acquisition requires a longer period of time and in this situation is likely to cause problems with the subtraction of background spectra. $CaF_2$ and $BaF_2$ were tested, alongside KBr, as alternative embedding media for the preparation of cross-sections. The sample prepared in $BaF_2$ appeared to be rather more fragile than the KBr sample and furthermore showed no particular improvement in signal to noise ratio after a period of ~60 minutes. Tests with $CaF_2$ were unsuccessful and the authors conclude that, of the materials tested, KBr offers the best solution.

The preparation of wall painting samples for analysis by FTIR can be particularly problematic since these samples tend to be characterized by high porosity and long-term exposure to severe environmental conditions. Furthermore these samples often contain only small amounts of organic material and the infiltration of embedding resin can compromise the data obtained. To overcome these problems Martin de Fonjaudran et al. (2008) describe a novel sample preparation technique which involves the consolidation of porous samples with a saturated solution of cyclododecane in toluene (80% w/v) applied drop-wise under vacuum, followed by coating the sample with melted cyclododecane to ensure complete encapsulation, before embedding the sample in an acrylic resin (Technovit LC2000). After microtoming to reveal a cross-section of the sample, the cyclododecane sublimes leaving a gap around the sample, which can reduce the stability of the sample within the resin block. In turn the sample preparation does not lead to photogenic stratigraphies in comparison to traditionally embedded cross-section samples. However, interference from embedding materials which might compromise the FTIR data, particularly for organic materials within the sample, was successfully limited. Nonetheless the sample preparation procedure requires significant manipulation of the sample and a high degree of dexterity and may not be suitable in all cases, particularly for small samples.

### 4.4 Sample preparation issues relating to secondary ion mass spectrometry (SIMS)

Secondary ion mass spectrometry (SIMS) is a highly sensitive chemical analysis technique suitable for investigating both organic and inorganic materials. SIMS can be used to produce chemical images of the sample to give spatially resolved information about the distribution of the different materials involved. The technique has been applied to the study of a wide range of objects within the cultural heritage field. SIMS studies of materials including glass, textiles, dyestuffs, metals, wooden artefacts, stone and paint are all reported in the literature.

Static SIMS is a surface analysis technique exploring only the first one or two atomic layers of the sample and, as such, is virtually non-destructive to the sample, although as with all mass spectrometry some material from the sample is inevitably consumed. Dynamic SIMS is capable of bulk analysis and of depth profiling. Both static SIMS and dynamic SIMS analysis have been used although static SIMS has been applied more often and is particularly useful for surface analysis of embedded samples. On occasion nano-SIMS, a technique which uses higher ion dosages and energies, has been utilised.

SIMS techniques offer advantages for the study of complex samples. They provide very high depth and lateral resolutions in the range of nanometres and they offer molecular specificity and detection of trace components present in only ppm-ppb quantities, undetectable by SEM-EDX analysis. However the literature available on this area also highlights some of the disadvantages and problems encountered.

Since SIMS is an extremely sensitive analytical technique surface contamination can be problematic and many papers in the literature discuss the need for a clean sample preparation, with minimal handling of the sample to avoid contamination with fatty acids. For paint cross-sections Keune (2005) suggests that better results are achieved when no liquid has been used to wet out the sample for light microscopy and that silicon putty to fix samples onto glass microscope slides should be avoided. Van Loon (2008) mentions that contamination with polydimethyl siloxanes can be reduced by rinsing cross-section samples with hexane. Delamare et al. (2005) also state that contamination with polydimethyl siloxanes seems to be due to the microtome knife they used for the preparation of cross-section samples and is hard to avoid. In addition contamination from embedding resin being smeared across the sample surface is also mentioned on several occasions. Reducing the size of the resin block around the sample is suggested by Keune (2005) as a helpful way to minimise this risk. This may also help to increase signal strength by limiting the amount of non-conducting material.

Both static and dynamic SIMS techniques are very dependent on surface topography and hence the preparation of the sample surface becomes extremely important. For static SIMS in particular, since this is a surface analysis technique, the ability to produce a flat sample surface is crucial. Even when the sample surface appears to be uniform and flat by optical microscopy, scratches and pits in the surface can dramatically reduce the quality of the data. The ionisation probability of a particular species, and therefore its detection yield, is dependent on the local primary ion impact angle so will be affected by surface topography. In addition both the mass and spatial resolution of the data are reduced for roughened surfaces.

Van Loon (2008) describes the protocol developed in the FOM-AMOLF laboratory for dry polishing of paint cross-sections and demonstrates the improvement in mass resolution of static SIMS data after re-polishing a sample to improve the surface. The polishing method employed uses a custom-made sample holder to ensure that even pressure is applied to the sample during polishing. Van Loon suggests that short straight movements be used to avoid as much friction, smearing and heating as possible and that the sample should be regularly turned through 90°. She also found that better results were achieved with round, or square, shaped sections. EasySections (VWfecit, England) which have a pronounced rectangular shape give an uneven resistance and are harder to polish to a flat surface.

Mazel et al. (2006) describe a system to produce a clean, flat surface for samples embedded in a polyester resin (H59 Sodemi, France) by ultramicrotomy using a Diatome diamond knife (Leica Microsystems, France). However the authors state that it was impossible to conserve intact the thin (0.5 µm) sections produced in this way so the SIMS analyses were carried out on the cut surface remaining on the sample block.

Dowsett et al. (2004) describe how the quality of the data obtained in dynamic SIMS experiments is also very dependent on the nature of the sample surface. Dynamic SIMS has been used for depth profiling in the study of glass. Obsidian glass was found to present a very favourable case since obsidian artefacts were often produced by conchoidal fracture which left locally smooth surfaces. Depth profiling of other glasses can be more problematic than for obsidian because of the rough surfaces produced by weathering and corrosion. This tends to make the interpretation of dynamic SIMS data more complicated since the roughness of the surface can produce non-uniform sputtering and hence changes in the secondary ion intensities which may not be related to real changes in composition.

SIMS analysis has also been applied to textile samples. Delamare et al. (2005) state that although ToF-SIMS provided interesting results relating to the surface composition of wool fibres before and during dyeing the simultaneous non-planar and non-conducting surface of the samples leads to very weak SIMS signals, making SIMS analysis difficult.

In addition to the requirements for good sample preparation there are some difficulties associated with the SIMS analysis of organic materials. The technique is non-quantitative. Certain components, particularly fatty acid moieties such as those in oil, have low emission yields and escape detections. SIMS analysis of oil paints gives no information on the polymeric oil network and only low yields of diacids are obtained although these are known to be a prominent component from GC-MS analysis, Keune (2005). Furthermore emission yields can be strongly influenced by matrix effects i.e. the sample chemistry at the specific sputtering site, Dowsett et al. (2004). Keune (2005) demonstrated that the detection of fatty acids in oil paint samples was greatly increased by the presence of elements like lead, silver and gold which both increase the ionisation of organic compounds and suppress the fragmentation. This implies that fatty acids are often detected in relative high yields in paint layers containing lead white and simultaneously the presence of fatty acids in lead deficient layers may be missed. The organic ion yields can be improved by coating the sample surface with an ultrathin layer of gold, Keune et al. (2004), but this in turn can suppress the detection of inorganic components.

SIMS analysis does not enable detection of molecular ions for large molecules such as proteins and polysaccharides but characteristic fragments can be identified. However the mass spectra generated are often complicated and require careful interpretation, Mazel et al. (2006) and Delamare et al. (2005).

It is worth stating the SIMS has been used to greatest effect when combined with other techniques such as scanning electron microscopy – energy dispersive X-ray analysis (SEM-EDX) and Fourier transform infrared spectroscopy (FTIR) imaging techniques. In most of the published studies of complicated multi-component samples from cultural heritage objects this has been the case.

## 4.5 Ion beam milling and polishing techniques for sample preparation

Focused Ion Beam (FIB) systems use a finely focused beam of gallium ions operated at low beam currents for imaging and at high-beam currents for site-specific milling. The most common application has been the preparation of thin sections for Transmission Electron Microscopy (TEM). I the cultural heritage field, it has been used to prepare paint samples containing barium sulphate for TEM analysis (Haswell et al. 2008), for metal samples for the investigation of the corrosion of historic organ pipes (Oertel et al. 2008), for historic geological samples (Johnson et al. 2011 forthcoming), for samples of altered medieval glass (Krawczyk-Bärsch 1997) and for historic ceramics (Sciau et al. 2009). A disadvantage of focussed ion beam systems, however, is that it is only feasible to prepare a very small area of a sample, of the order of tens of microns square. Also, the sample can also become contaminated by embedding of the Ga ions from the beam.

Another type of system utilises a broad unfocussed argon ion beam either to mill the sample to remove a significant amount of material, or to ‘polish’ the cross-section, removing only a few microns over a larger area than is possible with FIB, up to 1 mm in size. The low mass of Ar, and the glancing angle at which the beam hits the sample, mean that embedding of ions from the beam is not a significant problem. It is particularly effective for difficult samples that are either very soft or very hard, or comprise a number of different materials that vary in hardness. Cross-sections of coated paper have been made, for example, a challenging type of sample that is often damaged by mechanical preparation methods such as microtoming. The boundaries between the soft organic layers could be seen more clearly than in conventionally prepared samples, as smearing of the layers is avoided (Ström et al. 2010).

In the cultural heritage field, it was first used by Boon et al. to improve the surface preparation of paint cross sections for SEM imaging with impressive results, particularly at high magnifications. Smaller particles within the paint matrix became much more visible and the particle shapes better defined (Boon et al. 2006). In other samples Boon and van der Horst (2008) showed how it could reveal differences in the porosity of a chalk ground. It has also been used to polish cross-sections of historic samples of glass (Bellendorf et al. 2010). As well as giving a surface that allows better imaging, analysis is improved, particularly for Electron back-scattered diffraction (EBSD), where the quality of the surface has a significant effect on the results and the technique is sensitive to mechanical damage on the surface from conventional polishing techniques as well as surface contamination, since it only interrogates the uppermost 10–50 nm of the sample. EBSD has not yet been successfully applied to historic paint samples, however, and it is likely that this would still be a challenge even with a sample polished in this way. This preparation technique has been used almost exclusively for samples that will be examined in the SEM, but, as noted by Boon (Boon and van der Horst 2008). It also improves imaging under the optical microscope. It may also potentially be useful for achieving a better surface for FTIR imaging and mapping techniques in the future.

Boon et al 2006 describe the practicalities of applying this type of polishing to small paint samples. The sample needs to be adhered to a small silicon wafer to allow it to be mounted in vacuum chamber of the argon ion polisher. An unmounted fragment can be used; Boon et al. suggest that the sample can be sputter-coated first to avoid the glue infiltrating the sample. Alternatively it is possible to polish an embedded cross-section in this way, although it needs to be first ground down to make a very small block. A shield shapes the ion beam allowing a few microns to be polished from the surface. The sample is rocked to try to avoid artefacts such as beam curtaining (furrows produced in a pattern radiating out from the centre of the beam in a pattern that has the appearance of a draped curtain). Cryo stages are available to cool the sample if necessary to prevent heating damage, although this is more of a problem for harder materials such as metals. Polishing of a single cross section does however, take a long time (up to a few hours) and the cross-section polisher is an expensive piece of equipment, so it is likely to remain, for the time being, something that is used occasionally rather than routinely.

However, as noted above, polishing artefacts can occur, particularly in samples that vary in hardness, which is true of many cultural heritage samples. The most common are ‘curtaining’ and redeposition (Römer et al. 2009). The Ar beam deviates inwards at pores and this can cause furrows in the surface – curtaining, as already described above. In addition, material sputtered away from the sample surface by the argon beam can be redeposited inside the pores. It is possible to minimise some of these effects by careful choice of the parameters employed for the polishing. For example, most systems rock the sample during polishing to try to reduce curtaining, and for composite materials with components of different hardness that are therefore thinned at different rates, slower polishing can be advantageous. The success of the polishing can therefore depend to some extent on the skill and experience of the operator. Differences in the specifications of the system used might also be important – for example the positioning accuracy of the sample is generally around 10-20 microns, but in some systems a higher magnification viewing set-up allows an accuracy of ± 2 microns.

So-called ‘ion slicers’ are now becoming available where an unfocussed argon ion beam is used to make thin sections by polishing the sample from both sides. This allows larger thin sections to be made, up to a few hundreds of microns, than is possible with focused ion beam milling (Stojic and Brenker 2010).

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## 5. CONCLUSIONS

The compilation and summary of the results of the questionnaire circulated to CHARISMA partners, collecting information on the sampling methodology and practice followed in their institutions, provides a solid foundation that can be used as a basis for the guidelines on practice that will be formulated and finalised at the end of this task in the deliverable at month 30.

The survey also serves to identify particular expertise held within the CHARISMA consortium and to better define areas of practice that could be improved and that would benefit from exchange of knowledge through networking. One particular area that seems open to improvement is the recording and documentation of sample points; at the moment this is often done in several steps, or on a number of different images (either hard copy or digital) and development of tools to streamline the process could be considered.

The more specific information collected on sample preparation practices will be valuable for the design of work and experiments being developed in future work in the project, particularly in WP10 task 1 where these methods are being tested to improve analysis directly on cross-sections with chemical imaging techniques. The literature search in this deliverable extends and expands the results from the survey.