# Appendix I.

## A background to the characterisation methods implemented in this study

| Ochres at site/on object | | |
|---|---|---|

| Non-Invasive | | Invasive (samples taken) | |
|---|---|---|---|
| Visual examination, photography on site and in labs in direct light | | Morphology (surface) | ↳ Polarized light microscopy (PLM), ↳ Scanning electron microscopy (SEM) |
| Elemental analysis (major and minor elements): X-ray fluorescence spectroscopy (XRF) | | Elemental analysis (major, minor, trace and rare earth elemental analysis) | ↳ Energy dispersive spectroscopy (EDS) ↳ Inductively coupled plasma – atomic emission spectroscopy (ICP-AES) |
| | | Molecular analysis | ↳ Fourier transform infrared spectroscopy (FTIR), Raman (?) |
| | | Structure | ↳ X-ray diffraction (XRD) |

**Figure App. I - 1.** The analytical methods for chosen characterisation purposes in this project (Lyman 1990; Brundle, Evans et al. 1992; Silbilia 1996; Skoog, Holler et al. 1998; Rouessac and Rouessac 2000; Action 2004; Adriaens 2005; Sackler 2005)

## App. 1.1. Optical methods

App. 1.1.1.   Polarising light microscopy

Light microscopy is chiefly used to provide an extension to the limitations of one's vision, thereby enabling the examiner to observe a small object, sample or particle more distinctly due to the formation of a magnified image. This technique is therefore used to visually observe the physical and optical properties of materials by assessing the way they interact with plane and cross-polarized light.

All microscopes characteristically work on the same principle, whereby waves of, for example, visible light are emitted from a source and proceed to pass through a field diaphragm found in the illuminator. These are focussed onto the sample by means of a

substage condenser. The light waves then interact with the material positioned on the stage, either through reflection or transmission, and the modified wave pattern is subsequently converged and magnified through a chosen objective lens, which produces a magnified and inverted primary (real) image in front of the eyepiece. The eyepiece or ocular magnifies the image further, and the image can then be observed at a total magnification equivalent to the objective lens power multiplied by the ocular magnification (which is always x10). The maximum magnification in a light microscope is x1000, a power that is achieved through oil immersion of the sample.

A polarized light microscope is an enhanced optical microscope system, equipped with a polarising system placed along the optic axis. The system includes a plane-polarising filter or polariser, which is located between the source and the stage, and the analyser is inserted between the objective and the eyepiece. The illumination interacting with the specimen is thereby plane-polarised, and when the analyser or sample is rotated at right angles to the polariser, the sample is said to be viewed under cross polars. This may significantly aid in the visual examination and identification of materials by providing supplementary data inherent to the materials' character and/or composition.
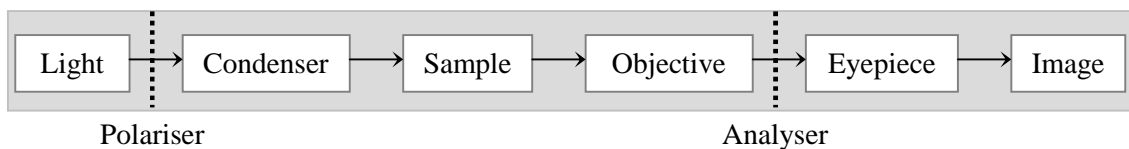
**Figure App. I - 2.** The basic components of a compound microscope

Many professionals consider microscopy as the initial method of choice for the characterisation, studying and identification of the various materials they come across throughout their line of work. The widespread application of this technique is

primarily a result of its low cost and relative ease of use. Although microscopy can positively identify a material without the need of any complex instrumentation, the technique is fairly subjective and the whole process, including sample preparation can be significantly time consuming. Specimens, for instance, must be able to transmit light if they are to be observed under a PLM, and must therefore either be present as single crystals, or as thin sections (Gribble and Hall, 1992; Eastaugh, Walsh et al., 2004; Petraco and Kubic, 2004).

Various factors were be observed as part of the experimental protocol when observing pigments under a microscope, and these have been listed in the table below (refer to Appendix II).

| Plane polarized light | | Crossed polars |
|---|---|---|
| Colour | Surface | Anisotropism |
| Size | Diaphaneity | Birefringence |
| Shape | Pleochroism | Interference colours |
| Aggreagation | Refractive index and Relief | Extinction positions |

**Table App. I - 1.** Pigment particle properties to be observed under the PLM

App. 1.1.2. Scanning electron microscopy (SEM)

One of the drawbacks to the light microscope is its resolving power threshold, a factor dictated by the wavelength of light and which limits the amount of observable detail of the sample. The SEM is therefore a clear improvement to this technique: although it works on the same principle as the light microscope, it utilises electrons rather than light waves as the source. These electrons, which work in a vacuum, also have wave properties, including a much smaller wavelength than that of visible light, therefore enabling high-resolution imaging of surfaces and a greater depth of field (100 times) in

comparison to a light microscope. The SEM also has a superior magnification of approximately x100,000. The electrons are typically generated by a thermal emission source (such as a tungsten filament) and are accelerated to a higher energy at the operating voltage. Like the light microscope, the SEM also uses a series of condensers, an objective (or probe-forming lens) and also includes a projector. These components are typically a series of powerful electromagnets and scanning coils used to control and manipulate electron flow for suitable and/or desired electron-specimen interactions. The image is then generated through scanning of the incident electron beam across the sample surface and projecting it onto a screen.

| Characteristic | Inelastic Scattering | Elastic Scattering |
|---|---|---|
| *Responsible for the generation of* | Secondary electrons | Backscattered electrons (BSE) |
| *Electron energy* | Low energy <50 eV | High energy, comparable to incident electron energy |
| *Theory* | Inelastic interaction, where energy is transferred from incident beam electrons to loosely bound electrons | Electrons 'bounce off' specimen: Remaining incident electrons measured after interaction with sample |
| *Responds to* | Surface, relies on roughness | Composition, therefore used for topographical observations |
| *Provides* | A high resolution, 3D morphological image of sample surface | Image contrast as a function of elemental composition and atomic number, as well as surface topography |

**Table App. I - 2.** Electron-specimen interactions in the SEM involved in image production

The improved magnification and resolution of the SEM therefore complements the previous binocular microscope technique by giving accurate particulate sizes, as well as detailed particle morphologies and state of aggregation (Goldstein, 1992; Goodhew, Humphreys et al., 2001; Goldstein, Newbury et al., 2003; Ponting, 2004).

**App. 1.2. X-ray techniques:**

App. 1.2.1. X-ray fluorescence spectroscopy (XRF)

XRF is a non-invasive, fairly sensitive, qualitative and semi-quantitative instrumental technique also used for the elemental analysis of materials. It works on the same principle as the EDS, therefore involving the element-shell transitions described above, but uses primary X-rays originating from an X-ray source rather than electrons for excitation. XRF is actually considered to be an indispensable tool for the study of archaeological materials for several reasons, including its non-invasiveness/non-destructiveness and the rapidity of analysis (Ferrero, Roldan et al., 2002; Burrafato, Calabrese et al., 2004; Ida and Kawai, 2005; Kannigieber, Malzer et al., 2005; Fremout, Saverwyns et al., 2006; Calza, Anjos et al., 2008).

| X-ray tube | X-rays → | Sample | Electron shell transitions + X-ray emission → | Detector |
|---|---|---|---|---|
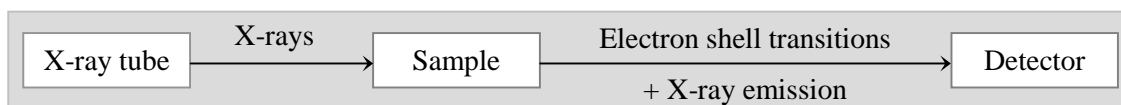
**Figure App. I - 3.** An outline of a basic XRF system

App. 1.2.2. Particle- (or proton-) induced X-ray emission spectroscopy (PIXE)
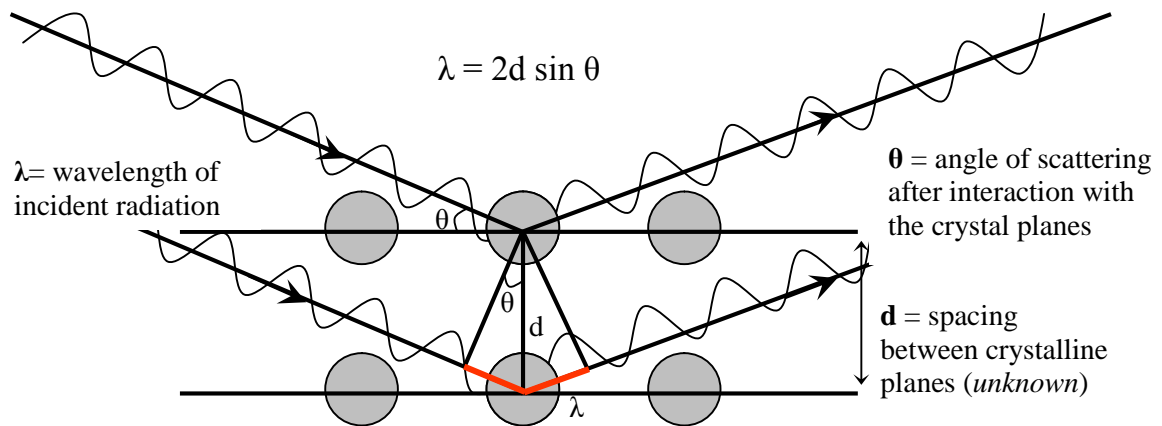
The aforementioned mechanism of electron displacement, excitation and transfer is also the principle involved in element identification in the PIXE. This technique in fact works on the same principle as the EDS and XRF, but irradiates the sample with an incident beam of collimated high-velocity protons rather than by electrons. The proton beam is typically generated with an energy of between 1-5 MeV within a particle accelerator (such as Van de Graff instruments), and is directed towards the sample which may be mounted externally in an airpath line, or in the sample chamber in a vacuum: though the former is the non-invasive method of analysis, the vacuum

gives more accurate results. The advantage of using a particle source is the mass/size ratio of the protons, which are 1836 times bigger than an electron. This property gives the instrument a higher spatial resolution since the beam is scattered less within the sample as well as a lower X-ray background (*bremsstrahlung*) caused by deceleration of the incident beam. This consequently gives the technique a good signal-to-noise ratio, high trace element sensitivities (when compared to XRF) and an excellent detection limit of about 1 ppm when utilized in vacuum mode and calibrated with well-chosen standards (Kim, Simon et al., 2003; Brenner, Lill et al., 2004; Grassi, Migliori et al., 2005; Reiche, Britzke et al., 2005; Weber, Martinot et al., 2005).

## App. 1.2.3.  X-ray diffraction (XRD)

This X-ray technique utilizes different material characteristics for identification purposes. Rather than establishing which elements are present, the XRD exploits the diffraction of an incident X-ray with a substance whose structure is crystalline and is therefore composed of repeating units. When the monochromatic X-rays interact with the crystalline material whose planes are comparable to the incident X-ray wavelength, constructive or destructive interference is experienced, depending whether the waves are in phase or in opposition. Constructive interference occurs if Bragg's law of crystal diffraction is satisfied. The material identity is established as follows: (i) The wavelength, $\lambda$, of the incident radiation is known as a set monochromatic, collimated and generally fixed X-ray source is used, while the (ii) angle of scattering, $\theta$, is measured by the diffractometer whose detector rotates along a pre-programmed angular range with a particular angular velocity around the same axis. (iii) Diffraction depends upon the atomic arrangement in the crystal, which is determined by the different spacings, d, between the crystal planes. This results in varied diffraction and

generates (iv) a diffractogram with peak profiles that can be used to determine the sample constituents by (v) comparison with a peak database (Cullity, 1978; Delhez, de Keijser et al., 1988; Hammond, 1990; Broadhurst, 2002; Rendle, 2003; Nel, Lau et al., 2006).

$$\lambda = 2d \sin \theta$$

$\boldsymbol{\lambda}$= wavelength of incident radiation

$\boldsymbol{\theta}$ = angle of scattering after interaction with the crystal planes

$\theta$

$\theta$

d

$\boldsymbol{d}$ = spacing between crystalline planes (*unknown*)

$\lambda$

**Figure App. I - 4.** Bragg's Law of diffraction

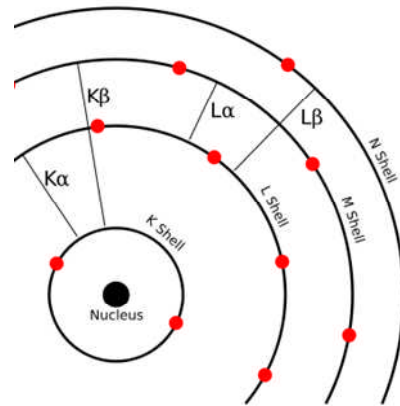## App. 1.3. Elemental analysis

### App. 1.3.1. Energy dispersive X-ray spectroscopy

The displacement of sample electrons by primary electrons triggers the release of a series of other signals besides secondary and backscattered electrons. The most important are the X-ray photons, which correspond to the elastic scattering and electron-shell excitation interactions, and which are characteristic to the atom from which they are released and can therefore be used to identify which elements they originated from. The X-ray generation process is summarised as follows (Goldstein, 1992; Goodhew, Humphreys et al., 2001; Goldstein, Newbury et al., 2003; Ponting, 2004):

- An electron beam is projected onto and hits the sample (composed of atoms)

- The electron beam excites inner shell electrons, resulting in the 'knocking-off', or displacement of constituent electrons from the inner orbit (e.g. the K shell in Figure App. I - 5)

- The atom is excited due to shell vacancy

- An electron from the outer shell replaces 'knocked off' electron

- Energy is given off in the form of X-ray photons, whose energy is characteristic of the parent element.



**Figure App. I - 5.** Electron shell transitions and resultant energy lines

Detection and measurement of the energy therefore permits qualitative elemental analysis; and semi-quantitative analysis with adequate standards. Results are typically presented as oxide %, though they may also be used to form maps or line profiles, showing the elemental distribution along a sample surface.

App. 1.3.2. Inductively coupled plasma – atomic emission spectroscopy (ICP-AES)

ICP-AES, sometimes also referred to as inductively coupled plasma – optical emission spectrometry (ICP-OES), is an emission spectrophotometric technique which can provide extremely flexible and rapid analysis of a number of chemical elements if well calibrated and standardized. It exploits the same principle explained previously whereby excited electrons emit energy at a given wavelength as they return to their ground state, with elements emitting energy inherent to their chemical character. Thus, by determining which wavelengths are emitted by a sample and by determining

their intensities, the analyst can quantify the elemental composition of the given sample relative to a reference standard.

The ICP-AES is a hyphenated technique, encompassing two major components; namely the ICP nebulizer, which generates the plasma, and the AES, which induces photon diffraction and dispersion. The fluid sample is pumped through the nebulizer, which generates an aerosol mist containing ions, electrons and neutral particles. The nebulizer injects humidified Ar gas into the chamber along with the sample. As this mist accumulates, particles are subsequently focused, collimated (by means of lenses and slits) and eventually swept into the AES torch assembly. The sample ions/atoms are promoted to higher energy levels, emitting wavelengths of radiation specific to their composition. The diffraction grating in the AES dispersion system sequentially moves through specified pre-requested wavelengths, and the energy intensity at each wavelength is measured by the detector, a photomultiplier tube, to provide a quantitative result that can be compared to a reference standard (Lyman, 1990; Brundle, Evans et al., 1992; Silbilia, 1996; Skoog, Holler et al., 1998; Rouessac and Rouessac, 2000; Action, 2004; Adriaens, 2005; Sackler, 2005).
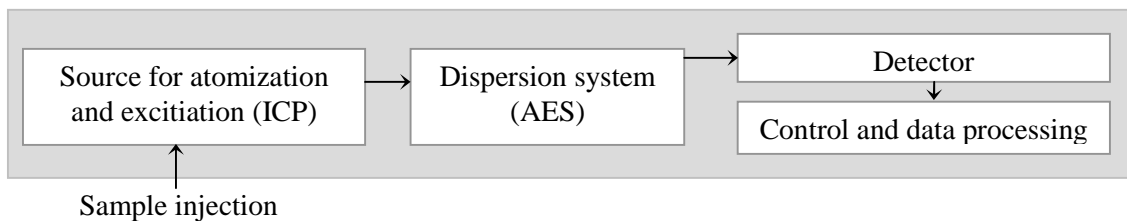


**Figure App. I - 6.** Block diagram showing the basic components of an ICP-AES (Ferretti, 1993 P.26)

## App. 1.4. Molecular bond analysis:

App. 1.4.1. Fourier transform infrared spectroscopy (FTIR)

The theory of infrared (IR) spectroscopy is based on characteristics of the IR spectrum and extends from a frequency range of 14,000 cm$^{-1}$ to 10 cm$^{-1}$. It is divided into three regions; the near-, mid- and far- infrared, named for their relation to the visible region in the EM spectrum. The mid-infrared region (4,000 cm$^{-1}$ to 400 cm$^{-1}$) is of most interest for chemical analysis as it corresponds to changes in vibrational energies within molecules ($E_{total}$) where:

$$E_{total} = E_{electronic} + E_{vibrational} + E_{rotational} + E_{translational}$$

FTIR is essentially a measurement technique used to detect the preferential absorption/transmission of given infrared radiation frequencies by the chemical bonds present in powdered samples of the ochre. IR radiation generated from an electrically heated source is essentially guided through an interferometer and allowed to pass through a sample. The sample needs to transmit the IR radiation, and is therefore generally mounted in a potassium bromide disc or between two sodium chloride crystals. Each molecular bond absorbs the IR radiation at particular radiation frequencies that correspond to the vibrational frequencies of the molecules and molecular bonds present. The detector measures the transmitted signal, or interferogram, and outputs an absorbance or transmitted spectrum after performing a mathematical fourier transform on this signal.

Although it is rarely possible to identify an unknown compound by using IR/FTIR spectroscopy alone, this technique has several advantages. Besides being a quick and relatively cheap spectroscopic technique, IR spectroscopy is useful in the identification

of certain functional groups in molecules following reference to correlation charts and tables of infrared data. It can also provide fingerprints for compounds since the IR spectrum of a particular material is unique (Nyquist and O'Kagel, 1971; Derrick, 1995; Edwards, Gwyer et al., 1997; Edwards, Drummond et al., 1999; Rull Perez, Edwards et al., 1999; Beveridge, Fung et al., 2001; van der Weerd, Heeren et al., 2004; van der Weerd, van Loon et al., 2005; Drake and Moore, 2006).

## App. 1.4.2.   Raman spectroscopy

Raman spectroscopy is the measurement of the wavelength and intensity of inelastically scattered light from molecules.  Like FTIR, this technique is used to determine molecular structures, but it utilizes a different wavelength of radiation (a laser source), and it is concerned with the scattering of radiation by the sample, rather than the absorption process.  Light therefore impinges onto the sample and interacts with the electron cloud of the bonds of molecules in the sample.  The incident photon of light excites electrons in the electric dipole of the molecule, which affects the molecule's electric field and generates various forms of scattering, including what is known as Stokes Raman scattering.  The scatter is then reflected onto a detector and a spectrum of Raman shift (x-axis) against counts (y-axis) characteristic of the molecular bonds is obtained.It therefore works in a similar way to FTIR except that the information obtained is different and generally complementary (Barbe and Smith, 2001; Castro, Rodriguez-Laso et al., 2001; Smith and Clark, 2001; Bartick, 2002; Hsu, 2002; Burrafato, Calabrese et al., 2004; Kendix, Nielsen et al., 2004; Vandenabeele, 2004; Buzzini, Massonnet et al., 2006; Eremin, Stenger et al., 2006; Goodall, Hall et al., 2006; Kaminska, Sawczak et al., 2006; Osticioli, Zoppi et al., 2006).