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Screening in situ bone and teeth preservation by ATR-FTIR mapping

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ABSTRACT

Bones and teeth are highly hierarchically structured and hererogeneous materials, and post mortem processes can reinforce this heterogeneity. It is therefore important to consider this heterogeneity to better understand diagenetic processes. In this study, ATR-FTIR mapping was applied to several heated and un-heated archaeological samples, and to similar modern references in order to test the potential of this method. ATR-FTIR mapping can provide spatially resolved information on alteration state of mineral and organic matter. This technique allowed to describe the spatial distribution of organic and mineral matter preservation in unheated Palaeolithic bones (Bize-Tournal, France) characterized by a better preservation in the centre of the cortical bone. Spatial variations in the chemical composition of an archaeological heated bone (Abri Pataud, France) compared to a modern reference suggested taphonomical uptake of carbonate in the most external part. This pattern could correspond to a process of re-carbonatation of the calcined mineral matter in the outermost part of the sample due to combustion in a CO_2 rich atmosphere. FTIR-ATR is a powerful tool that allows for identifying and characterizing local heterogeneities in bone preservation. This technique open new prospects to reconstruct the taphonomical history of ancient samples.

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1. Introduction

Bones and teeth are characterized by a wide heterogeneity of composition and a complex hierarchical structure from the macro to the micro-scale (Weiner and Traub, 1992; Fratzl et al., 2004). Composition and structure are more heterogeneous for teeth than for bone due to the association of enamel, dentin and cement in teeth (Hillson, 2009). These biomaterials can be extensively affected by various taphonomical and diagenetic processes that can modify the structure and the composition of these biomaterials (Lee-Thorp, 2002; Trueman et al., 2004). These different processes reinforce the initial heterogeneity in structure and composition of these materials, and can limit the use of bio and geochemical proxies (Lee-Thorp, 2002; Lee-Thorp and Sponheimer, 2003; Bocherens et al., 2008).

Various techniques have been applied to fossil remains in order to characterize taphonomic alterations, evaluate preservation states and understand diagenetic processes. The molecular and structural properties of fossil bone mineral fraction have been widely investigated by means of scanning and transmission electron microscopy (SEM/TEM), X-ray diffraction (XRD) and small angle X-ray scattering (SAXS) as well as infrared (FTIR) and Raman spectroscopy (Weiner and Bar-Yosef, 1990; Person et al., 1995; Reiche et al., 2002b; Turner-Walker and Syversen, 2002; Hiller et al., 2003; Pucéat et al., 2004). These studies have highlighted the impact of dissolution/recrystallization processes inducing an increase in apatite crystallinity and a decrease in carbonate content. Organic matter composition can be monitored by various techniques in order to investigate the preservation state of the collagen matrix, non-collagenous proteins, lipids or DNA (Evershed et al., 1995; Nielsen-Marsh et al., 2002; Tuross, 2002; Geigl et al., 2004).

Most of these analytical techniques have been applied to bulk bone powder, thus limiting the consideration of tissue heterogeneity. The main approach used to investigate bone and teeth micro-structures is the application of optical and electronic microscopies (SEM or TEM). Qualitative and quantitative analyses were performed to evaluate the preservation state of histological structures that was considered as a proxy of preservation of the bone chemical composition (Hedges et al., 1995; Turner-Walker and Syversen, 2002). Besides providing an evaluation of the preservation state of the samples, the examination of histological microstructures reveals important information to reconstruct the taphonomical history and better understand the complex accumulation processes of sedimentary and archaeological records (Nielsen-Marsh et al., 2007; Turner-Walker and Jans, 2008; Hollund et al., 2012). More recently, localized and spatially resolved elemental and isotopic analyses have been performed on bones and teeth from various archaeological and palaeontological contexts. These studies provide elemental or isotopic profiles or maps using two different analytical strategies: micro-sampling or direct analyses using micro-ablation system coupled to mass spectrometry (Cerling and Sharp, 1996; Reiche et al., 1999, 2002a;

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Brady et al., 2008; Olivares et al., 2008; Duval et al., 2011; Aubert et al., 2012). Cathodoluminescence was also employed to map trace element distribution in fossil remains (Ségalen et al., 2008). These studies highlighted the presence of a large spatial heterogeneity in the elemental and isotopic composition related to alteration processes and more particularly uptake and diffusion of exogeneous elements in the samples. They also demonstrated that the composition of the different mineralized tissues (enamel *vs.* bone, dentine and cement) and their structure influence the uptake of trace elements inside bone during diagenesis (Gaschen et al., 2008; Hinz and Kohn, 2010; Suarez et al., 2010).

Spatially resolved analyses of the molecular and structural composition of bones are commonly used in the biomedical field and have brought valuable information regarding the development, maturation and pathologies recorded in mineralized tissues (Paschalis et al., 1996; Fratzl et al., 2004; Boskey et al., 2005). Until today, there has been very little work on ancient material. Microfocus small angle X-ray scattering was applied to archaeological bones to detect changes in crystal properties during diagenesis and the impact of heating in archaeological context (Wess et al., 2002; Gourrier et al., 2011). Collagen preservation in bone was explored by ATR-FTIR micro-spectroscopy (Chadefaux et al., 2009), ion beam analysis (Beck et al., 2012) or near infrared spectroscopy imaging (Vincke et al., 2014). In order to investigate simultaneously the composition and properties of the mineral and organic phases, Fourier transform infrared micro-spectrometry (FTIRM) was recently applied to archaeological bones. FTIR is one of the most used techniques applied to fossil bulk samples and FTIRM was widely applied to bone samples in biomedical studies (Paschalis et al., 1996; Fratzl et al., 2004; Boskey et al., 2005). The first results obtained by FTIRM demonstrated the ability of this analytical approach to screen diagenetic alterations such as collagen loss, modification of carbonate content and mineral recrystallization of fossil bone at the histological scale (Reiche et al., 2010; Lebon et al., 2011). Moreover, mapping offers the advantage of providing several hundreds of measurements for each sample, thus allowing to obtain statistical data to investigate the relationship between the different parameters used to evaluate diagenetic processes. This technique has the potential to better understand diagenetic processes at the micro-scale. However, these studies have highlighted difficulties related to sample preparation. The application of FTIRM needs the preparation of very thin sections by ultra-microtomy (2–5 μm thick), requiring the impregnation of the sample in resin in order to fill all porosities before cutting. Such a preparation is time consuming and the presence of resin inside bone porosities can limit the data treatment. Moreover, even if the size of thin sections obtained in previous work was quite acceptable considering the use of ultra-microtomy, the size of the larger histological sections did not exceed 1×1 mm^2, preventing from appreciating variations of composition at different scales. Finally, the preparation of hard and brittle tissues such as enamel or heated bones is not possible using ultra-microtomy.

In order to overcome these analytical difficulties FTIR mapping in attenuated total reflection mode (ATR-FTIR mapping) was applied to investigate bones and teeth composition. ATR-FTIR spectroscopic imaging has been developed in the last decade and applied to investigate biomedical samples and art materials (Spring et al., 2008; Porto et al., 2010; Kazarian and Chan, 2013). This mode of measurement requires minimal preparation as analyses are realized at the surface of the samples. This method has the potential to be applied on samples cross sections, i.e. on the same support than other methods like SEM, ion beam analysis or LA-ICPMS. A first application of micro-ATR-FTIR spectroscopy to archaeological bones was realized by Chadefaux et al. (2009) to evaluate collagen denaturation from the inner to the outer part of Neolithic bone sections. The aim of the present paper is to examine whether ATR-FTIR mapping has the potential to provide spatially resolved information on the alteration state of mineral and organic matter of archaeological bone and tooth samples. This method will be first applied to a modern tooth and a modern heated bone selected for their heterogeneous composition. The modern references will then be compared to archaeological samples to examine the alteration state of the mineral and organic matter and to reconstruct their taphonomical history.

2. Material

In order to test the potential of micro-ATR-FTIR spectroscopy, modern and archaeological samples showing various states of crystallinity, collagen and carbonate contents were selected. Modern samples were collected in a butchery. They consist in a modern horse tooth (MHT) and an ox bone. The ox bone was then heated at 700 °C for 40 min (MHOB) and displayed a partial calcination state: the external part appear calcined whereas the internal part is still carbonized. The modern tooth sample will was chosen to screen variation of composition between enamel, dentin and cement. MHT and MHOB will allow to obtain references of low (cement/dentin), medium (enamel and carbonized bone part) and high crystallinity (calcined part of bone sample) associated or not with organic matter (cement and dentin vs. enamel for example). Archaeological samples are a bone from the Magdalenian level of Bize-Tournal Cave (Aude, France; ~15 ka) thereafter BZ-2011-24; and a partially calcined bone coming from a Gravettian layer of Abri Pataud (Dordogne, France, ~22 ka) thereafter AP55a. Previous analyses were realized on bulk samples coming from the same sites and archaeological layers (Lebon et al., 2010; Zazzo et al., 2013).

3. Method

Samples were sectioned into 2–4 mm thick cross sections using a low speed diamond saw (Struer Minitom) and cross sections were then polished prior to analysis (Micro-Mesh polishing products). Cross sections were cleaned in an ultrasonic bath with ethanol for a few minutes and air dried overnight prior to analysis. Cross sections were analysed using a Hyperion 2000 microscope coupled with a Vector 22 FTIR spectrometer. The microscope is equipped with a nitrogen cooled MCT-A single element detector collecting radiation in the infrared range from 4000 to 650 cm^{-1}. The micro-ATR-FTIR mapping was realized using an ATR Germanium crystal mounted on a 20× cassegrain objective. The ATR Ge crystal is 100 μm in diameter and IR beam is focused on a 32×32 μm area. Cross sections were raster-scanned in a rectangular grid pixel by pixel with various steps size increments (from 80 μm to 150 μm step size per pixel). Spectral analyses were performed using OPUS software (Bruker). The distribution of the main components and structure of the mineral and organic matter were monitored from the absorbance ratios in order to avoid variations of raw intensities due to the quality of the contact between ATR crystal and the sample. The organic content was monitored from the Amide I/Phosphate band ratio (Trueman et al., 2004). Carbonate/Phosphate band ratio was calculated to evaluate carbonate content (Wright and Schwarcz, 1996). Hydroxyl groups were monitored in calcined samples from the weak absorption band near 3570 cm^{-1}. Apatite crystallinity is usually monitored based on the ν_4PO_4 vibration bands at 605 and 565 cm^{-1}, using the IR splitting factor (IRSF) defined by Weiner and Bar-Yosef (1990). As the ν_4PO_4 vibration bands are below the cutoff of the MCT-A detector (~650 cm^{-1}), apatite crystallinity was evaluated using the ν_3 and ν_1PO_4 vibration bands. In this work, crystallinity was monitored from 1060/1075 ratio (ν_3PO_4 domain) which provides similar information than the IRSF. In addition to the 1060/1075 intensity ratio position ν_1PO_4 band (~960 cm^{-1}) was also measured. The position of the ν_1PO_4 band can provide further information since a wavenumber shift can be observed following ionic substitutions that modify mineral crystallinity and lattice strain (Freeman et al., 2001; Penel et al., 2005; Antonakos et al., 2007; Lebon et al., 2010). The enhancement of apatite crystal perfection due to a decrease of carbonate content and/or fluor uptake in apatite results in a positive wavenumber shift of the ν_1PO_4 band. On the contrary, an incorporation of carbonate or other bivalent ions in apatite lattice resulting in a reduction of crystallinity induces a decrease of ν_1PO_4 band frequency. All bands and corresponding baselines used are

Table 1
Infrared intensity ratios used to evaluate bone sample properties, and related baselines and errors.

Parameter	Intensity ratio (cm^{-1})	Baseline (cm^{-1})	Error
Collagen/phosphate	$I_{1660}/I_{max\sim1030}$	1300–1800/800–1200	<0.01
Carbonate/phosphate	$I_{1417}/I_{max\sim1030}$	1300–1800/800–1200	<0.01
Crystallinity	I_{1060}/I_{1075}	800–1200/800–1200	0.01
Hydroxyl/phosphate	$I_{3570}/I_{max\sim1030}$	3550–3590/800–1200	<0.001
Ionic substitutions	ν_1PO_4 position	–	0.1

summarized in Table 1 and typical spectra collected for each samples are presented in Fig. 1. In order to estimate the error on the calculated indices, we selected three areas for each specimen, and analysed them three times each. The standard deviations calculated for each triplicate were averaged to estimate the precision of the measurements. Errors are reported on Table 1.

4. Results

4.1. Modern horse tooth (MHT)

Differences in the composition of cement, enamel and dentin are observed in the MHT section by ATR-FTIR imaging (Fig. 2). As expected, no trace of organic matter is detected in enamel and this area shows high crystallinity values (mean = 1.53) and low carbonate contents (mean = 0.16; SD ± 0.02). The ν_1PO_4 band is centred around 958.6 cm^{-1} (SD ± 0.3). In the dentine part of the section, carbonate content varies between 0.16 and 0.23 with a mean value at 0.20 (SD ± 0.02), and crystallinity varies between 1.32 and 1.35 (mean = 1.34; SD ± 0.01). Collagen content values vary between 0.13 and 0.19 with a mean value at 0.17 (SD ± 0.01). These values are close to that observed on modern bone for crystallinity, collagen and carbonate content, respectively. The ν_1PO_4 band is centred around 960.3 cm^{-1} (SD ± 0.6 cm^{-1}) with a position varying between 959.7 and 960.8 cm^{-1}. In the cement, carbonate content varies between 0.22 and 0.51 with a mean value at 0.28 (SD ± 0.04), and collagen values vary between 0.15 and 0.19 with a mean value at 0.18 (SD ± 0.01). Crystallinity varies between 1.30 and 1.35 with a mean value at 1.33 (SD ± 0.01). The ν_1PO_4 band is centred around 960.0 cm^{-1} (SD ± 0.6 cm^{-1}) with a position varying between 958.7 and 961.2 cm^{-1}.

4.2. Modern heated ox bone (MHOB)

The carbonized and calcined parts of the sample display large differences in crystallinity and carbonate contents (Fig. 3). No collagen was detected in this sample. The carbonized part presents crystallinity values varying between 1.40 and 1.50 (mean = 1.49; SD ±

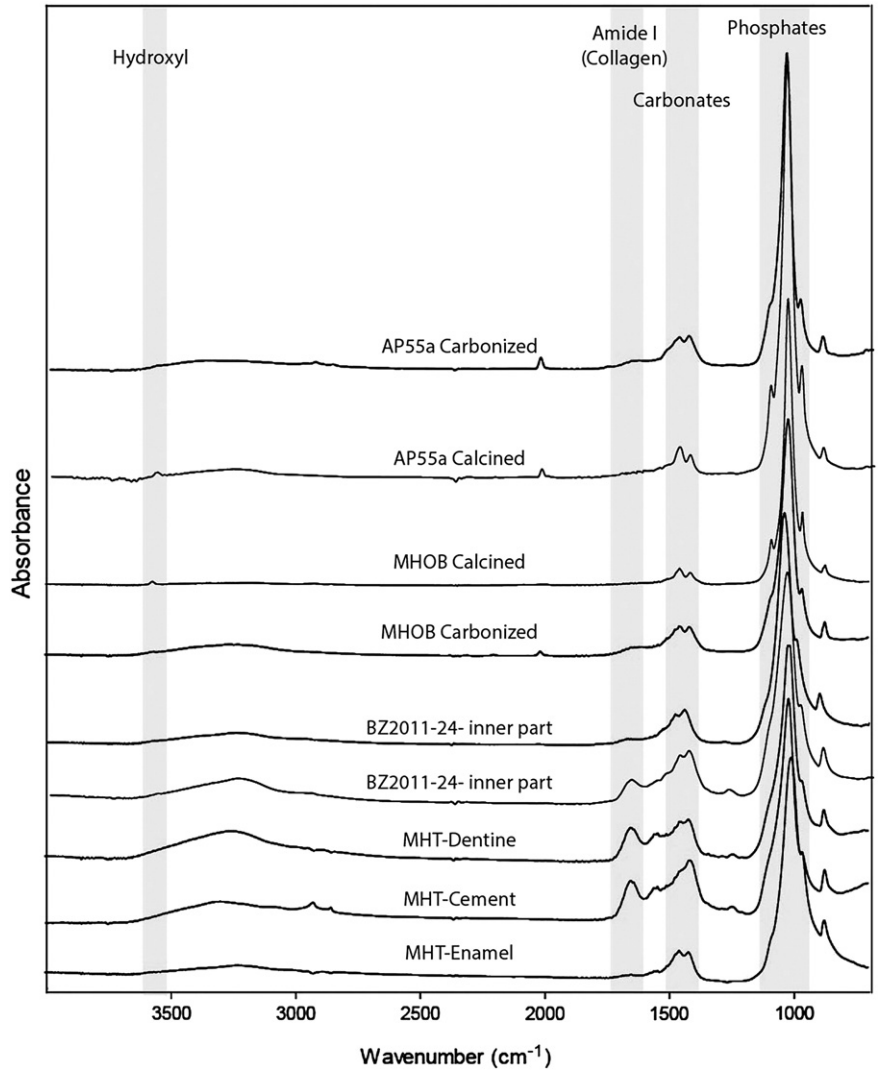

Fig. 1. Typical spectra obtained for Modern Horse Tooth (MHT) samples in the enamel, cement and dentin parts, for sample BZ2011-24 in the inner and outer parts, and in the calcined and carbonized parts of Modern Heated Ox Bone (MHOB) and archaeological heated bone (AP55a).

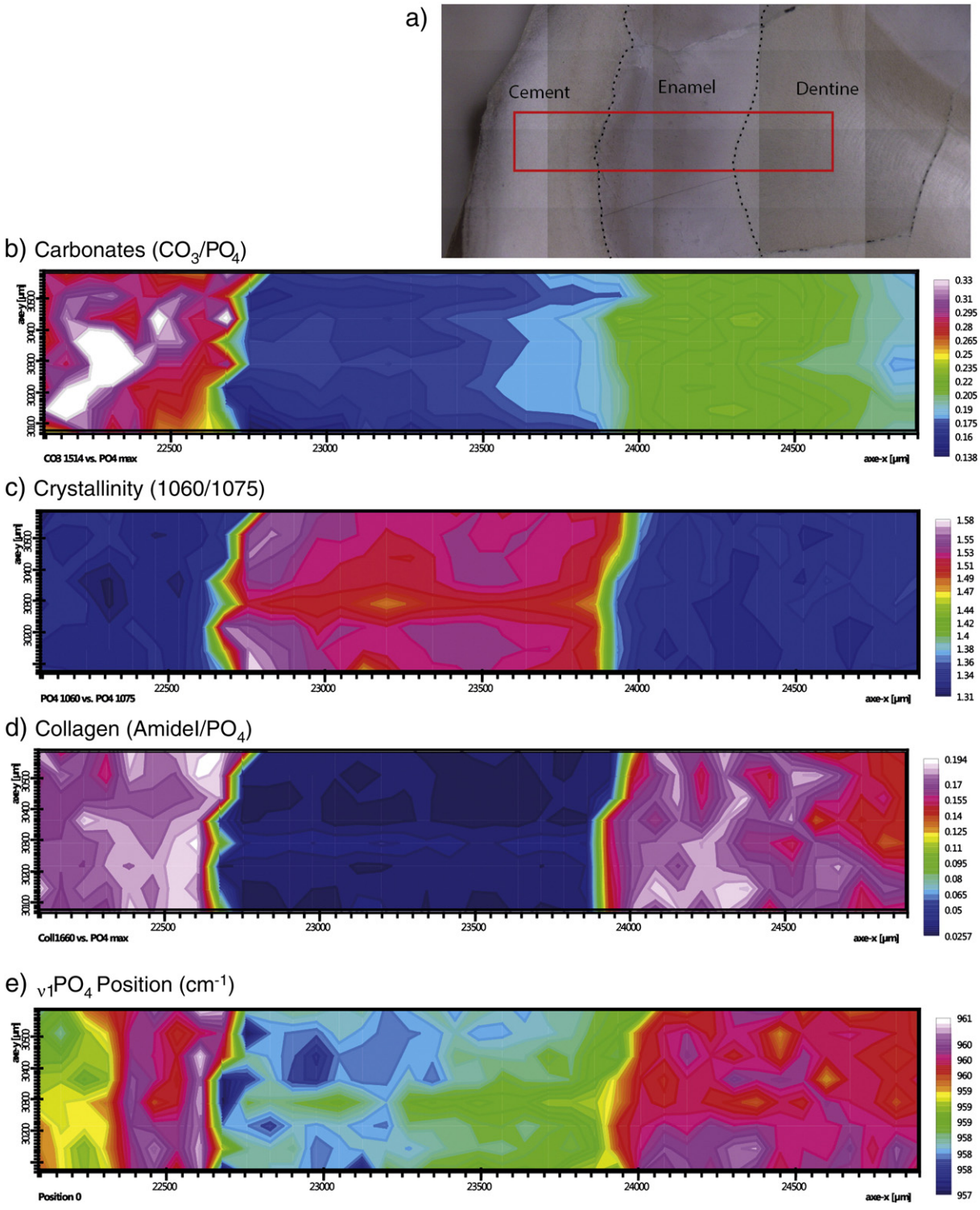

Fig. 2. a) Optical view of the analysed area of the modern horse teeth cross section (MHT) and spatial distribution of b) carbonate content, c) crystallinity, d) collagen content, and e) position of ν_1PO_4 band determined by ATR-FTIR mapping.

0.01), whereas values exceed 1.6 in the calcined part (mean = 1.65; SD ±0.03). Carbonate content varies between 0.07 and 0.13 in the carbonized part (mean = 0.10; SD ± 0.01) whereas values are lower than 0.05 in the calcined part of the sample (mean = 0.04; SD ± 0.01). The hydroxyl band is detected in the calcined part. The position of the ν_1PO_4 band is also clearly distinct in the calcined and carbonized areas with mean positions at 962.8 cm^{-1} (SD ± 0.3 cm^{-1}) and 961.8 cm^{-1} (SD ± 0.4 cm^{-1}) respectively.

4.3. Archaeological bone from Bize-Tournal (BZ-2011-24)

A low collagen content is detected in the sample BZ-2011-24 with a mean value of AmideI/PO_4 ratio at 0.04 (Fig. 4). The highest values are observed in the centre of the cross section with AmideI/PO_4 ratio values up to 0.12. Crystallinity values vary between 1.27 and 1.58 (mean = 1.44; SD ± 0.06). High crystallinity values are observed in the external part of the sample and decrease in the inner part of the shaft. The

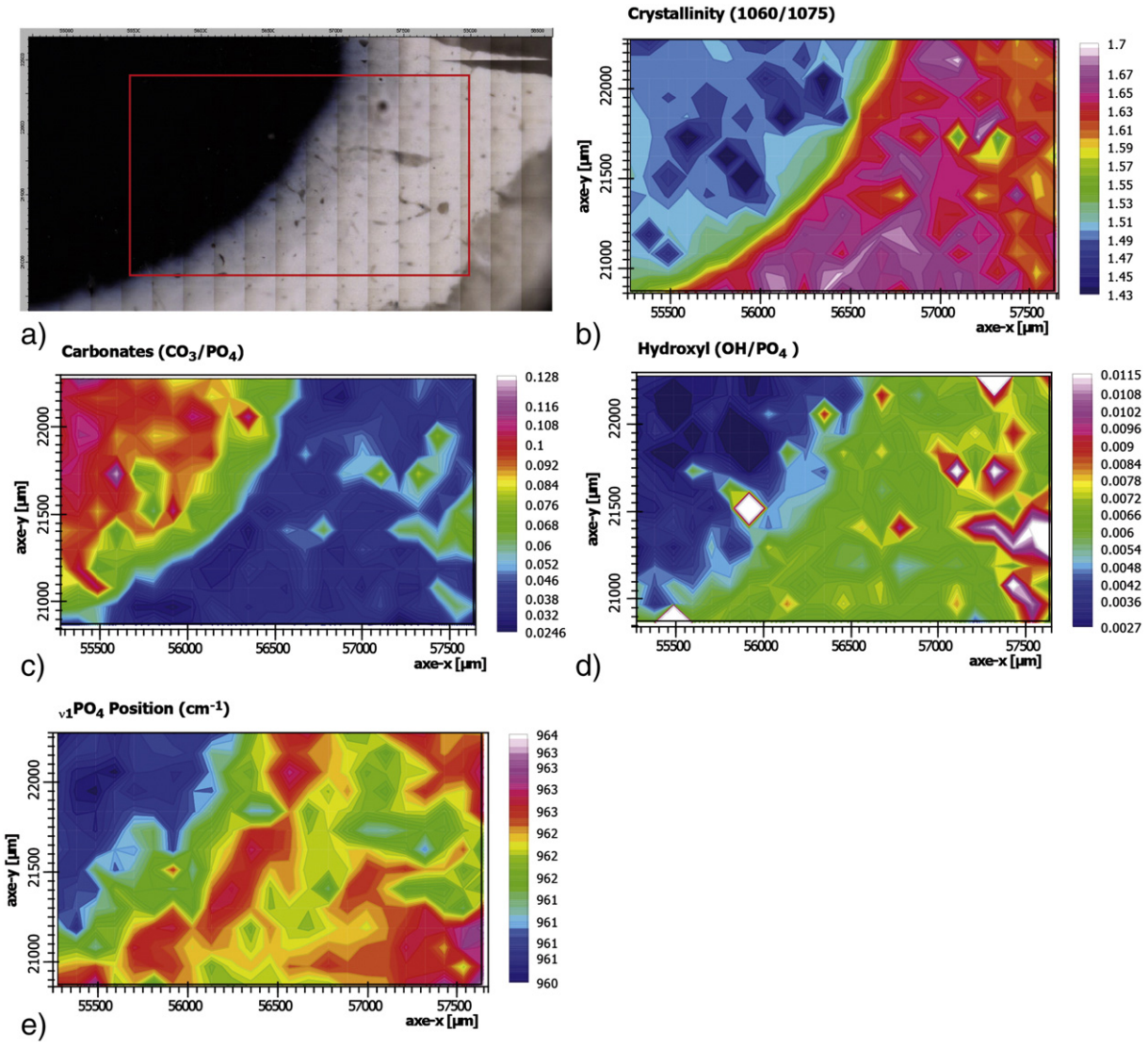

Fig. 3. a) Optical view of the analysed area of the heated modern ox bone cross section (700 °C) and spatial distribution of b) crystallinity, c) carbonate content, d) hydroxyl content and e) position of ν_1PO_4 band determined by ATR-FTIR mapping.

carbonate/phosphate ratio varies between 0.08 and 0.25 (mean value = 0.16; SD ± 0.02). The carbonate content distribution is heterogeneous especially in the centre of the sample. The position of the ν_1PO_4 band varies between 957.9 and 961.8 cm^{-1} (mean value = 959.7 cm^{-1}; SD ± 0.7 cm^{-1}). The distribution of this parameter is similar to that of the crystallinity value.

4.4. Archaeological heated bone from Abri Pataud (AP55a)

The carbonate and crystallinity distributions clearly differ between the calcined and the carbonized part of the sample (Fig. 5). The hydroxyl band was not detected in the calcined part due to a low signal/noise ratio in the 4000–3000 cm^{-1} spectral range. No collagen was detected in this sample. In the carbonized area, the carbonate content varies between 0.05 and 0.13 (mean = 0.09; SD ± 0.01). Crystallinity values are homogeneous and vary between 1.54 and 1.60 (mean = 1.58; SD ± 0.01). The position of the ν_1PO_4 band varies between 961.7 and 963.2 cm^{-1} (mean = 962.3 cm^{-1}; SD ± 0.3 cm^{-1}) with higher values observed in the outer part of the carbonized area. The calcined part displays higher crystallinity values varying between 1.62 and 1.86 (mean = 1.73; SD ± 0.05). The carbonate content varies between 0.02 and 0.11 (mean = 0.05; SD ± 0.02). Two sub layers (white and grey in colour) can be distinguished by their crystallinity and carbonate contents within the calcined part. Surprisingly the internal (grey) part displays a lower carbonate content and a higher crystallinity than the external part. The position of the ν_1PO_4 band varies between 961.2 and 962.9 cm^{-1} within the calcined part. The mean value (mean = 962.3 SD = 0.3) is similar to that observed in the carbonised area. Lowest values were observed in the outer part of the calcined area whereas highest values were observed in the inner part.

5. Discussion

ATR-FTIR mapping provides a coherent set of data on different types of samples, relevant to previous studies by our group carried out on artificially heated bones and fossil samples (Lebon, 2010; Lebon et al., 2010; Reiche et al., 2010; Lebon et al., 2011). However, results slightly differ from previous studies and may provide new insights on the conservation states of samples and alteration processes. The pixel values of maps obtained for each parameter screened are presented in histograms (Fig. 6).

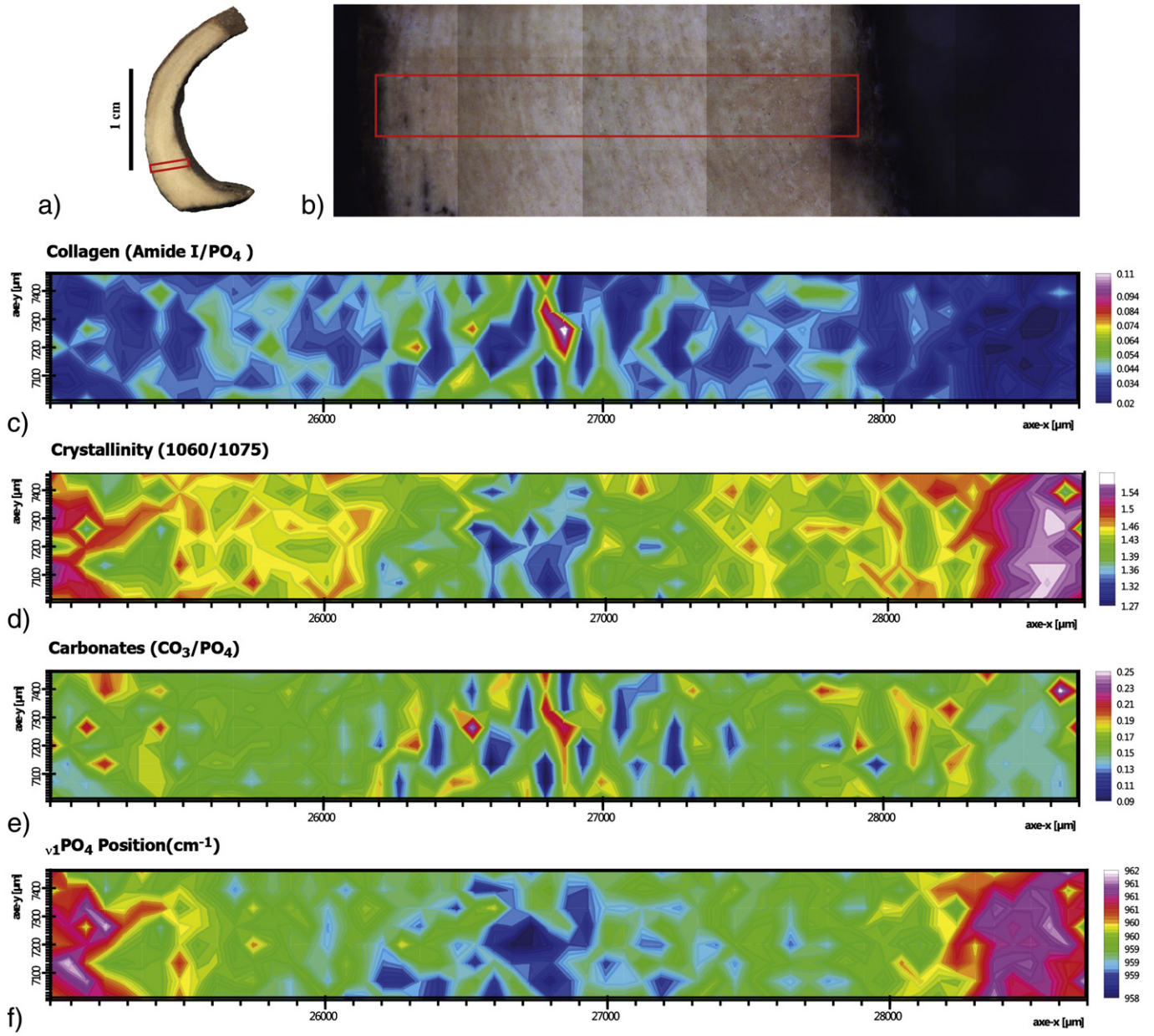

Fig. 4. a) Optical view of the cross section and b) the analysed area of the un-heated archaeological bone (BZ-2011-24) and spatial distribution of c) collagen content, d) crystallinity, e) carbonate content, and f) position of ν_1PO_4 band determined by ATR-FTIR mapping.

5.1. Modern vs. archaeological samples: identification of alterations

BZ-2011-24 sample mapping reveals the presence of collagen in the inner part of the sample (Fig. 4). Maximal values of Amide I/PO_4 ratio (~0.12) indicate the presence of substantial collagen content even if the mean value is very low (0.04). Compared to Amide I/PO_4 ratio obtained for modern bone like tissues (0.2), we can assume that more than 50% of collagen is still preserved in the centre of the cortical bone whereas a bulk analysis on bone powder would probably not have allowed detecting any trace of remaining collagen. Crystallinity values and the position of the ν_1PO_4 band are modified by diagenesis and present values approaching that observed for modern enamel (Fig. 6). The distribution patterns of collagen, crystallinity values and the position of the ν_1PO_4 band indicate a classical alteration profile with a better preservation in the centre of the cortical bone. The higher position of the ν_1PO_4 band in the outer part of the cross section is not correlated with the lower carbonate content. Such higher wavenumbers may indicate a fluorine uptake in the outer part of the sample (Lebon et al., 2010; Thomas et al., 2011).

The archaeological sample AP55a presents a composition pattern typical of high temperature heated samples (Fig. 5). As shown on the artificially heated sample, heating induced important compositional changes in bone tissues. During heating, the organic matter mainly composed by collagen matrix is progressively carbonized between 200 °C and 450 °C. Organic carbon is removed beyond 650 °C. The composition of the mineral phase is also deeply modified. From 250 to 300 °C, carbonate content decreases rapidly. This loss of carbonates induces an enhancement of the mineral lattice order that allows the sharp increase of crystallinity observed between 650 and 700 °C. Beyond 700 °C, the presence of hydroxyl groups is characterized by the recrystallization of the initial carbonate apatite in hydroxyapatite (Shipman et al., 1984; Rogers and Daniels, 2002; Pasteris et al., 2004; Lebon et al., 2010).

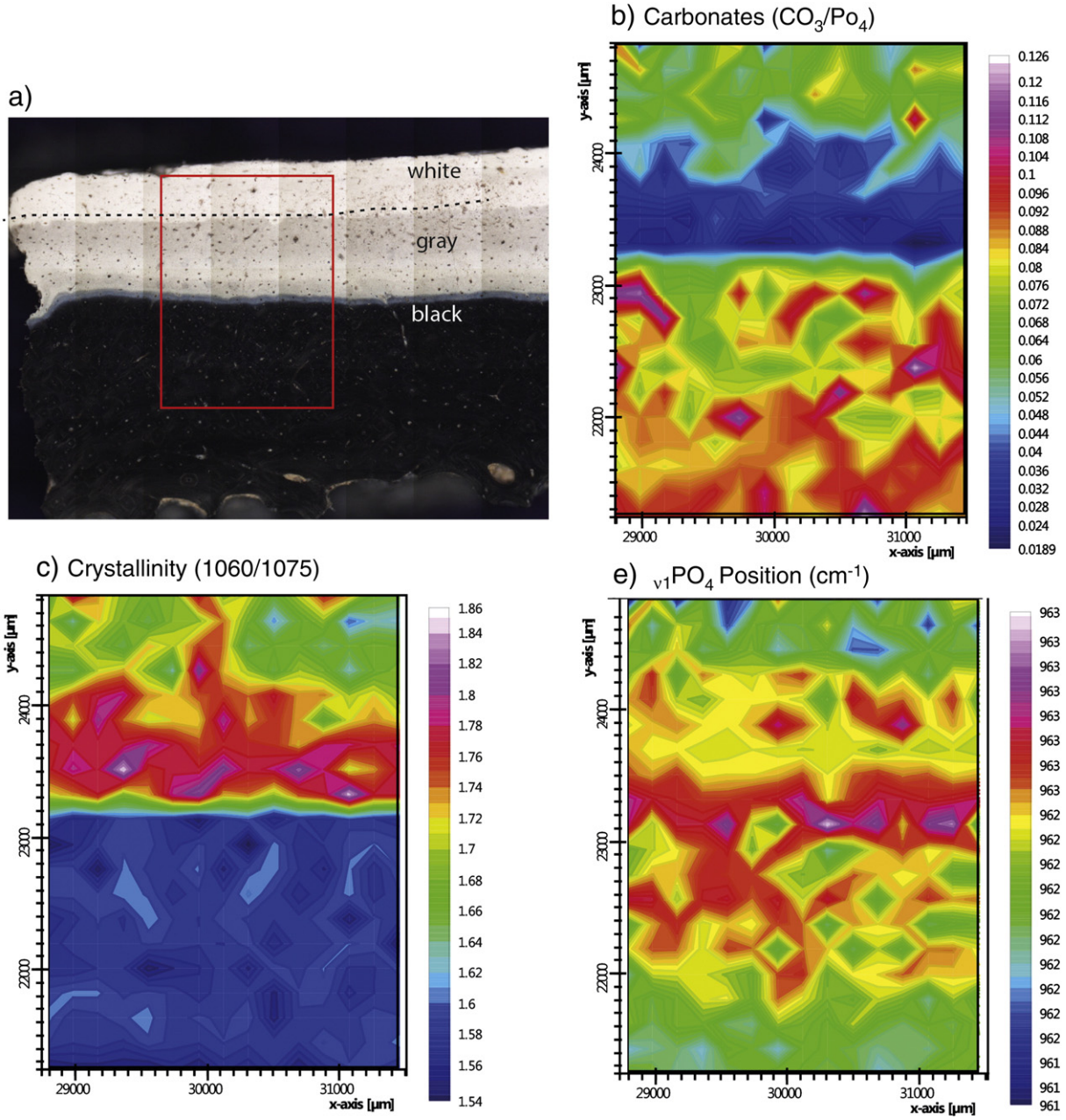

Fig. 5. a) Optical view of the analysed area of the heated archaeological bone (AP55a) and spatial distribution of b) carbonate content, c) crystallinity, and d) position of ν_1PO_4 band determined by ATR-FTIR mapping.

Temperature is not the only parameter that influences the composition of heated bones. It can also be influenced by the environment of combustion, the initial state of the heated bones (fresh vs defleshed), and the duration of combustion. Thus a short combustion (<1 h) can result in an incomplete heating and the presence of a gradient of composition between the inner and the outer part of sample (Hanson and Cain, 2007; Walker and Miller, 2005). Such a gradient is observed in the modern sample heated at 700 °C for 40 min (Fig. 3).

For the heated archaeological sample (AP55a), crystallinity values observed for both the calcined and carbonized area are higher than values observed for the modern sample artificially heated at 700 °C (Fig. 5). This pattern could be caused by diagenetic alteration although recrystallized apatite is usually considered very resistant to alteration processes as the improvement of the perfection and size of crystals limits the reactivity of crystallites with the burial environment (Reiche et al., 2002a; Zazzo and Saliège, 2011) . These high crystallinity values could also correspond to higher temperature of heating of the archaeological sample. Indeed, the AP55a sample presents a particular pattern of composition in the calcined part that may result from a temperature of heating above 750 °C. The outermost calcined part (white) presents lower crystallinity and lower ν_1PO_4 band position than the underlying grey part (Fig. 5). The outermost part of the heated sample is directly exposed to the source of heat and should have shown the most important state of recrystallization whereas for this sample, it is the underlying grey part. Previous work showed that maximal crystallinity values are measured around 700 °C. Crystallinity values then slightly decrease from 750 °C to 900 °C (Lebon et al., 2010). The pattern of composition for this sample could thus correspond to an exposure of the surface to a temperature higher than 750 °C while the light grey part could correspond to heating around 700 °C. However, this hypothesis cannot explain the spatial distribution of carbonates which are more important in the outermost part of the sample. Calcination experiment indicates that the carbonate content continues to gradually decrease beyond 700 °C (Lebon et al., 2010). It is noteworthy that this experimental

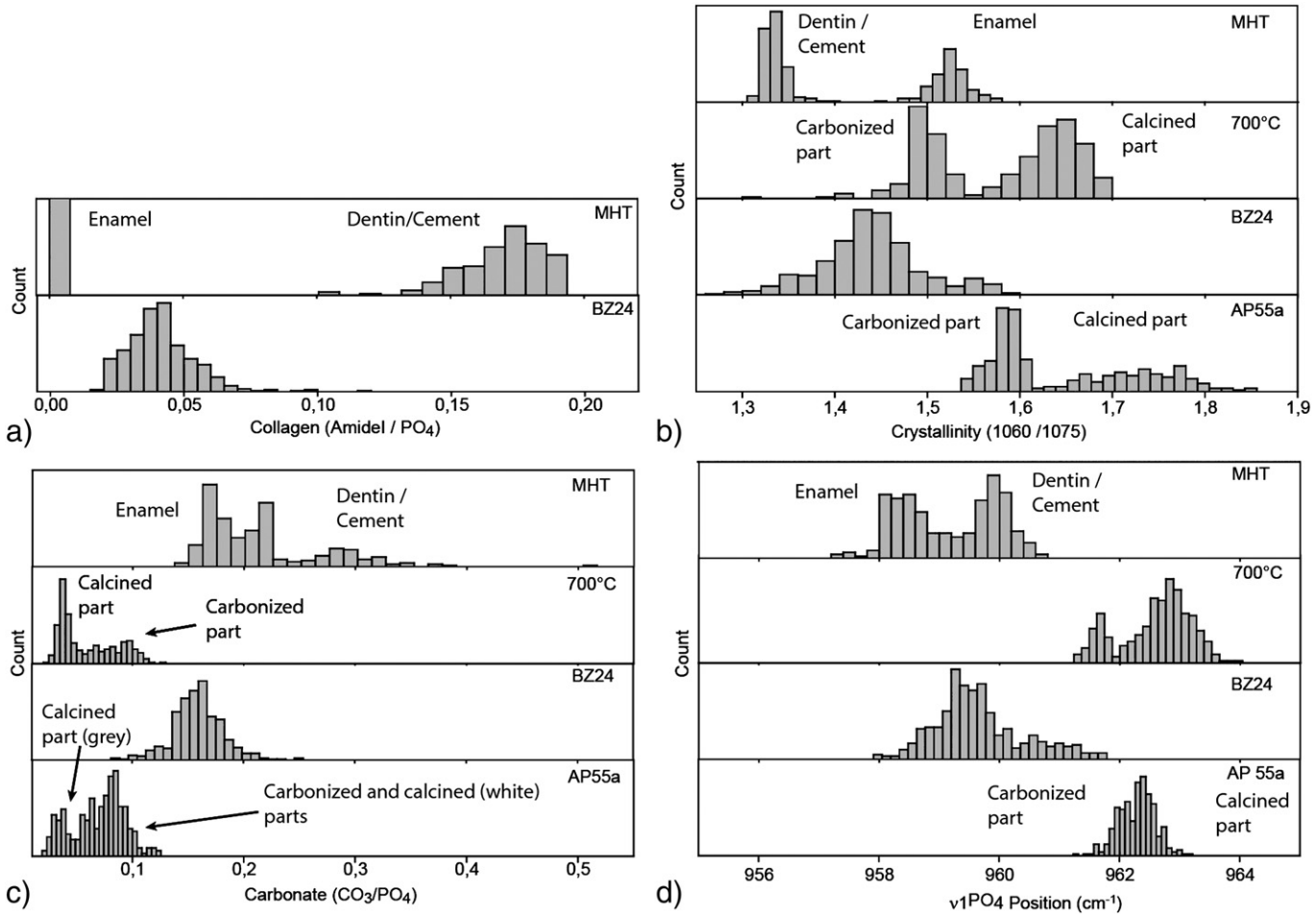

Fig. 6. Histograms of map pixel values for a) collagen content, b) crystallinity, c) carbonate content and d) position of the ν_1PO_4 band (cm^{-1}).

work was realized in a muffle furnace in oxidizing condition which does not reflect exactly hearth conditions where charcoals and carbonized organic matter produce more reducing conditions. Radiocarbon dating of modern and archaeological bones indicates that up to 90% of the initial bone carbonate can be replaced by carbon from the atmosphere of combustion (Hüls et al., 2010; Zazzo et al., 2012). Zazzo et al. (2012) also showed that bones heated in a hearth under natural conditions for 2 h can present higher carbonate contents than bones heated in the same condition for 1 h only. These results might suggest that, in addition to an exchange, carbon uptake can also occur in calcined bone mineral during heating and/or cooling depending on the heating conditions. The pattern of carbonates distribution could thus correspond to a process of re-carbonatation of the outermost part of the sample due to combustion in a CO_2-rich atmosphere. In a previous study on heated bone from Abri Pataud, carbon isotopic composition ($\delta^{13}C$) of the calcined bones from the same level than the sample analysed here already suggested a fuel composed by a mixture of wood and bones (Zazzo et al., 2013) . The crystallinity and carbonate content pattern observed for the sample AP55a could thus attest for heating in a reducing environment. There is currently not enough data on the modifications of the mineral composition induced by heating in natural condition and the number of samples analysed here is too limited to confirm this hypothesis.

5.2. Potential of ATR-FTIR spectroscopy in archaeological studies

The results obtained here by FTIR-ATR mapping demonstrate for the first time that it is possible to characterize both organic and mineral matter on large areas for such samples. The application of this method to archaeological and fossil samples will contribute to select the better preserved part of a sample for further analysis. Even if the ability of crystallinity to provide a reliable marker of alteration of elemental or isotopic composition is debated (Pucéat et al., 2004; Trueman et al., 2008), the presence of remaining collagen can be considered as a good proxy for the preservation of the biogenic composition of bones. As shown for BZ-2011-24, although the bulk preservation of the organic matrix is very poor, some parts can be well preserved (Fig. 4). ATR-FTIR mapping could thus contribute to select the best preserved parts likely to provide reliable isotopic signals involved in paleaoenvironmental and paleaoclimatic reconstruction. Moreover, in addition to collagen content and crystallinity values, the spatial distribution of the carbonate content can also be investigated. Trueman et al. (2008) suggested that the proportion of structural carbonates offer more potential than crystallinity to screen alteration of the stable isotope signal. As shown for the archaeological heated sample (AP55a), the carbonate content distribution can provide additional information that can be used to reconstruct taphonomical history. Further analyses are needed but carbonate content screening might be used to better choose the parts of calcined apatite used as support of ^{14}C dating.

Compared to previous FTIR techniques employed to screen diagenetic changes affecting bones and teeth tissues at the histological level, ATR-FTIR spectroscopy requires very limited preparation of the sample. Indeed, only a slight polishing of the surface is required to obtain a good contact between ATR crystal and sample. This measurement mode can be applied to thick samples without resin embedding, and microtomy is not required contrarily to measurement by transmission. Large areas are available for analysis thus allowing a multi-scalar approach. Moreover, sample preparation is similar to that used for light and electronic microscopy, elemental analysis such as X-ray fluorescence,

ion beam analysis or ICPMS. The preparation protocol is also compatible with other structural techniques such as X-ray diffraction and small angle X-ray scattering. We would like to emphasize, however, that a good contact between the surface of the sample and the ATR crystal is needed for obtaining reliable ATR-FTIR maps. This contact is achieved by applying a pressure on the sample that can produce surface deformation at the surface. This potential alteration of the sample must be taken into account if further analyses are planned. Another disadvantage of ATR-FTIR spectroscopy is that the depth of the sample screened is proportional to the wavelength. This results in a very low intensity of IR band in the range of 3000–4000 cm^{-1} which makes it difficult to detect weak band such as the hydroxyl group (~3570 cm^{-1}) in sample AP55a (Fig. 1). Despite these limitations, ATR-FTIR spectroscopy presents the major advantage of combining more easily these different analytical techniques and levels of information, and better characterize alteration features and processes. The first results obtained here open new prospects in the study of bone and tooth diagenesis.

6. Conclusion

This study is the first application of ATR-FTIR mapping to fossil bones and teeth. This technique can be directly applied on a cross section with limited preparation of the samples and will allow combining ATR-FTIR mapping with other techniques such as elemental or isotopic microanalysis. The results obtained demonstrate the ability of this method to map the distribution of the structure and chemical composition of the organic (collagen content) and mineral (carbonate content and crystallinity) fractions of bones and teeth. Using this method, it was possible to detect the local preservation of collagen for an upper Palaeolithic bone. For a partially calcined bone sample, the distribution of crystallinity and carbonate content suggest a process of uptake of carbonates due to combustion in a reducing atmosphere. This information can be used to identify the areas that are best preserved, to better understand alteration processes and to reconstruct the taphonomical history of samples in order to verify the reliability of the biogenic information provided by the geochemical composition of ancient bones and teeth.

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References

Antonakos, A., Liarokapis, E., Leventouri, T., 2007. Micro-Raman and FTIR studies of synthetic and natural apatites. Biomaterials 28, 3043–3054.
Aubert, M., Williams, I.S., Boljkovac, K., Moffat, I., Moncel, M.-H., Dufour, E., Grün, R., 2012. In situ oxygen isotope micro-analysis of faunal material and human teeth using a SHRIMP II: a new tool for palaeo-ecology and archaeology. J. Archaeol. Sci. 39, 3184–3194.
Beck, L., Cuif, J.P., Pichon, L., Vaubaillon, S., Dambricourt Malassé, A., Abel, R.L., 2012. Checking collagen preservation in archaeological bone by non-destructive studies (Micro-CT and IBA). Nucl. Inst. Methods Phys. Res. B 273, 203–207.
Bocherens, H., Drucker, D.G., Billiou, D., Geneste, J.-M., Kervazo, B., 2008. Grotte Chauvet (Ardèche, France): a "natural experiment" for bone diagenesis in karstic context. Palaeogeogr. Palaeoclimatol. Palaeoecol. 266, 220–226.
Boskey, A., DiCarlo, E., Paschalis, E., West, P., Mendelsohn, R., 2005. Comparison of mineral quality and quantity in iliac crest biopsies from high- and low-turnover osteoporosis: an FT-IR microspectroscopic investigation. Osteoporos. Int. 16, 2031–2038.
Brady, A.L., White, C.D., Longstaffe, F.J., Southam, G., 2008. Investigating intra-bone isotopic variations in bioapatite using IR-laser ablation and micromilling: implications for identifying diagenesis? Palaeogeogr. Palaeoclimatol. Palaeoecol. 266, 190–199.
Cerling, T.E., Sharp, Z.D., 1996. Stable carbon and oxygen isotope analysis of fossil tooth enamel using laser ablation. Palaeogeogr. Palaeoclimatol. Palaeoecol. 126, 173–186.
Chadefaux, C., Le Hô, A.-S., Bellot-Gurlet, L., Reiche, I., 2009. Curve-fitting micro-ATR-FTIR studies of the amide I and II bands of type I collagen in archaeological bone materials. e-PRESERVATIONScience 6, 129–137.
Duval, M., Aubert, M., Hellstrom, J., Grün, R., 2011. High resolution LA-ICP-MS mapping of U and Th isotopes in an early Pleistocene equid tooth from Fuente Nueva-3 (Orce, Andalusia, Spain). Quat. Geochronol. 6, 458–467.
Evershed, R.P., Turner-Walker, G., Hedges, R.E.M., Turos, N., Leyden, A., 1995. Preliminary results for the analysis of lipids in ancient bone. J. Archaeol. Sci. 22, 277–290.
Fratzl, P., Gupta, H.S., Paschalis, E.P., Roschger, P., 2004. Structure and mechanical quality of the collagen–mineral nano-composite in bone. J. Mater. Chem. 14, 2115–2123.
Freeman, J.J., Wopenka, B., Silva, M.J., Pasteris, J.D., 2001. Raman spectroscopic detection of changes in bioapatite in mouse femora as a function of age and in vitro fluoride treatment. Calcif. Tissue Int. 68, 156–162.
Gaschen, A.A.M., Döbeli, M., Markwitz, A., Barry, B., Ulrich-Bochsler, S., Krähenbühl, U., 2008. Restrictions on fluorine depth profiling for exposure age dating in archaeological bones. J. Archaeol. Sci. 35, 535–552.
Geigl, E.M., Baumer, U., Koller, J., 2004. New approaches to study the preservation of biopolymers in fossil bones. Environ. Chem. Lett. 2, 45–48.
Gourrier, A., Bunk, O., Müller, K., Reiche, I., 2011. Artificially heated bone at low temperatures: a quantitative scanning-small-angle X-ray scattering imaging study of the mineral particle size. ArchéoScience 35.
Hanson, M., Cain, C.R., 2007. Examining histology to identifiy burned bone. Journal of Archaeological Science 34, 1902–1913.
Hedges, R.E.M., Millard, A.R., Pike, A.W.G., 1995. Measurements and relationships of diagenetic alteration of bone from three archaeological sites. J. Archaeol. Sci. 22, 201–209.
Hiller, J.C., Thompson, T.H.U., Evison, M.P., Chamberlain, A.T., Wess, T.J., 2003. Bone mineral change during experimental heating: an X-ray scattering investigation. Biomaterials 24, 5091–5097.
Hillson, S., 2009. Teeth, 2nd ed ed. Cambridge University Press, New York.
Hinz, E.A., Kohn, M.J., 2010. The effect of tissue structure and soil chemistry on trace element uptake in fossils. Geochim. Cosmochim. Acta 74, 3213–3231.
Hollund, H.I., Jans, M.M.E., Collins, M.J., Kars, H., Joosten, I., Kars, S.M., 2012. What happened here? Bone histology as a tool in decoding the postmortem histories of archaeological bone from Castricum, The Netherlands. Int. J. Osteoarchaeol. 22, 537–548.
Hüls, C.M., Erlenkeuser, H., Nadeau, M.J., 2010. Experimental study on the origin of cremated bone apatite carbon. Radiocarbon 52, 587–599.
Kazarian, S.G., Chan, K.L.A., 2013. ATR-FTIR spectroscopic imaging: recent advances and applications to biological systems. Analyst 138, 1940–1951.
Lebon, M., 2010. Caractérisation des ossements chauffés en contexte archéologique—etude comparative de matériel moderne et fossile par spectroscopie infrarouge. In: Théry-Parisot, I., Chabal, L., Costamagno, S. (Eds.), Actes de la Table Ronde Taphonomie des résidus organiques brûlés et des structures de combustion en milieu archéologique, CEPAM - UMR 6130, 27 au 29 mai 2008. P@lethnologie 2, pp. 149–162.
Lebon, M., Müller, K., Bahain, J.J., Fröhlich, F., Falguères, C., Bertrand, L., Sandt, C., Reiche, I., 2011. Imaging fossil bone alterations at the microscale by SR-FTIR microspectroscopy. J. Anal. At. Spectrom. 26, 922–929.
Lebon, M., Reiche, I., Bahain, J.J., Chadefaux, C., Moigne, A.M., Fröhlich, F., Sémah, F., Schwarcz, H.P., Falguères, C., 2010. New parameters for the characterization of diagenetic alterations and heat-induced changes of fossil bone mineral using Fourier transform infrared spectrometry. J. Archaeol. Sci. 37, 2265–2276.
Lee-Thorp, J., 2002. Two decades of progress towards understanding fossilization processes and isotopic signals in calcified tissue minerals. Archaeometry 44, 435–446.
Lee-Thorp, J., Sponheimer, M., 2003. Three case studies used to reassess the reliability of fossil bone and enamel isotope signals for paleodietary studies. J. Anthropol. Archaeol. 22, 208–216.
Nielsen-Marsh, C.M., Ostrom, P.H., Gandhi, H., Shapino, B., Cooper, A., Hauschka, P.V., Collins, M.J., 2002. Sequence preservation of osteocalcin protein and mitochondrial DNA in bison bones older than 55 ka. Geol. Soc. Am. 30, 1099–1102.
Nielsen-Marsh, C.M., Smith, C.I., Jans, M.M.E., Nord, A., Kars, H., Collins, M.J., 2007. Bone diagenesis in the European Holocene II: taphonomic and environmental considerations. J. Archaeol. Sci. 34, 1523–1531.
Olivares, M., Etxebarria, N., Arana, G., Castro, K., Murelaga, X., Berreteaga, A., 2008. Multielement μ-ED-XRF analysis of vertebrate fossil bones. X-Ray Spectrom. 37, 293–297.
Paschalis, E.P., Jacenko, O., Olsen, B., Mendelsohn, R., Boskey, A.L., 1996. Fourier transform infrared microspectroscopic analysis indentifies alterations in mineral properties in bones from mouse transgenic for type X collagen. Bone 19, 151–156.
Pasteris, J.D., Wopenka, B., Freeman, J.J., Rogers, K., Valsami-Jones, E., van der Houwen, J. A.M., Silva, M.J., 2004. Lack of OH in nanocrystalline apatite as a function of degree of atomic order: implications for bone and biomaterials. Biomaterials 25, 229–238.
Penel, G., Delfosse, C., Descamps, M., Leroy, G., 2005. Composition of bone and apatitic biomaterials as revealed by intravital Raman microspectroscopy. Bone 36, 893–901.
Person, A., Bocherens, H., Salihge, J.F., Paris, F., Zeiton, V., Girard, M., 1995. Early diagenetic evolution of bone phosphate: an X-ray diffractometry analysis. J. Archaeol. Sci. 22, 211–221.
Porto, I.M., Saiani, R.A., Chan, K.L.A., Kazarian, S.G., Gerlach, R.F., Bachmann, L., 2010. Organic and inorganic content of fluorotic rat incisors measured by FTIR spectroscopy. Spectrochim. Acta A Mol. Biomol. Spectrosc. 77, 59–63.
Pucéat, E., Reynard, B., Lécuyer, C., 2004. Can crystallinity be used to determine the degree of chemical alteration of biogenic apatites? Chem. Geol. 205, 83–97.
Reiche, I., Favre-Quattropani, L., Calligaro, T., J, S., Bocherens, H., Charlet, L., Menu, M., 1999. Trace element composition of archaeological bones and postmortem alteration in the burial environment. Nucl. Inst. Methods Phys. Res. B 150, 656–662.
Reiche, I., Lebon, M., Chadefaux, C., Müller, K., Le Hô, A.-S., Gensch, M., Schade, U., 2010. Microscale imaging of the state of preservation of 5000-years-old archaeological bones by synchrotron infrared microspectroscopy. Anal. Bioanal. Chem. 394, 2491–2499.

Reiche, I., Vignaud, C., Favre-Quattropani, L., Menu, M., 2002a. Fluorine analysis in biogenic and geological apatite by analytical transmission electron microscopy and nuclear reaction analysis. J. Trace Microprobe Techn. 20, 211–231.
Reiche, I., Vignaud, C., Menu, M., 2002b. The crystallinity of ancient bone and dentine: new insights by transmission electron microscopy. Archaeometry 44, 447–459.
Rogers, K.D., Daniels, P., 2002. An X-ray diffraction study of the effects of heat treatment on bone mineral microstructure. Biomaterials 23, 2577–2585.
Ségalen, L., de Rafélis, M., Lee-Thorp, J.A., Maurer, A.-F., Renard, M., 2008. Cathodoluminescence tools provide clues to depositional history in Miocene and Pliocene mammalian teeth. Palaeogeogr. Palaeoclimatol. Palaeoecol. 266, 246–253.
Shipman, P., Foster, G., Shoeninger, M., 1984. Burnt bones and teeth: an experimental study of color, morphology, crystal structure and shrinkage. J. Archaeol. Sci. 11, 307–325.
Spring, M., Ricci, C., Peggie, D., Kazarian, S., 2008. ATR-FTIR imaging for the analysis of organic materials in paint cross sections: case studies on paint samples from the National Gallery, London. Anal. Bioanal. Chem. 392, 37–45.
Suarez, C.A., Macpherson, G.L., González, L.A., Grandstaff, D.E., 2010. Heterogeneous rare earth element (REE) patterns and concentrations in a fossil bone: implications for the use of REE in vertebrate taphonomy and fossilization history. Geochim. Cosmochim. Acta 74, 2970–2988.
Thomas, D.B., McGoverin, C.M., Fordyce, R.E., Frew, R.D., Gordon, K.C., 2011. Raman spectroscopy of fossil bioapatite—a proxy for diagenetic alteration of the oxygen isotope composition. Palaeogeogr. Palaeoclimatol. Palaeoecol. 310, 62–70.
Trueman, C.N., Privat, K., Field, J., 2008. Why do crystallinity values fail to predict the extent of diagenetic alteration of bone mineral? Palaeogeogr. Palaeoclimatol. Palaeoecol. 266, 160–167.
Trueman, C.N.G., Behrensmeyer, A.K., Tuross, N., Weiner, S., 2004. Mineralogical and compositional changes in bones exposed on soil surfaces in Amboseli National Park, Kenya: diagenetic mechanisms and the role of sediment pore fluids. J. Archaeol. Sci. 31, 721–739.
Turner-Walker, G., Jans, M., 2008. Reconstructing taphonomic histories using histological analysis. Palaeogeogr. Palaeoclimatol. Palaeoecol. 266, 227–235.
Turner-Walker, G., Syversen, U., 2002. Quantifying histological changes in archaeological bones using BSE-SEM image analysis. Archaeometry 3, 461–468.
Tuross, N., 2002. Alterations in fossil collagen. Archaeometry 44, 427–434.
Vincke, D., Miller, R., Stassart, É., Otte, M., Dardenne, P., Collins, M., Wilkinson, K., Stewart, J., Baeten, V., Fernández Pierna, J.A., 2014. Analysis of collagen preservation in bones recovered in archaeological contexts using NIR Hyperspectral Imaging. Talanta 125, 181–188.
Walker, P.L., Miller, K.P., 2005. Time, temperature, and oxygene availability : an experimental study of the effect of environmental conditions on the color and organic content of cremated bone. American Journal of Physical Anthopologie Supplement 40, 222.
Weiner, S., Bar-Yosef, O., 1990. States of preservation of bones from prehistoric sites in the Near East: a survey. J. Archaeol. Sci. 17, 187–196.
Weiner, S., Traub, W., 1992. Bone structure: from angstroms to microns. FASEB J. 6, 879–885.
Wess, T., Alberts, I., Hiller, J., Drakopoulos, M., Chamberlain, A.T., Collins, M., 2002. Microfocus small angle X-ray scattering reveals structural features in archaeological bone samples; detection of changes in bone mineral habit and size. Calcif. Tissue Int. 70, 103–110.
Wright, L.E., Schwarcz, H.P., 1996. Infrared and isotopic evidence for diagenesis of bone apatite at Dos Pilas, Guatemala: palaeodietary implications. J. Archaeol. Sci. 23, 933–944.
Zazzo, A., Lebon, M., Chiotti, L., Comby, C., Delque-Kolic, E., N. R., Reiche, I., 2013. Can we use calcined bones for radiocarbon dating the paleolithic? Radiocarbon 55, 1409–1421.
Zazzo, A., Saliège, J.-F., Lebon, M., Lepetz, S., Moreau, C., 2012. Radiocarbon dating of calcined bones: insights from combustion experiments under natural conditions. Radiocarbon 54, 855–866.
Zazzo, A., Saliège, J.F., 2011. Radiocarbon dating of biological apatites: a review. Palaeogeogr. Palaeoclimatol. Palaeoecol. 310, 52–61.