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Identification of fragmented bones and their state of preservation using near infrared hyperspectral image analysis

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This preliminary work comprises examples where near infrared (NIR) hyperspectral imaging has been applied to identify animal bone material in complex sieved soil–sediment matrices from an archaeological excavation at a Stone Age site in northern Scandinavia. NIR hyperspectral image analysis has been performed, as a fast and non-destructive technique, on whole bone and tooth samples, as well as on soil from the excavation containing fragmented skeletal material in order to identify fragmented bones, to provide information about the skeletal material's chemistry–mineralogy within the site and the different layers as well as studying the possibility of describing their different state of preservation.

Keywords: hyperspectral, near infrared imaging, bone, teeth, archaeology, principal component analysis

Introduction

In the field of archaeology, scientists are often faced with a great amount of data derived from analyses of a variety of findings or artefacts. Archaeological materials such as ceramics, metallic objects, slags and bones have been submitted to various forms of analysis using archaeometry and osteology.[1–4] Materials, often smaller in size and incorporated in the soil matrix, such as ashes, highly fermented or oxidised organic matters, fragmented bones and ceramics, carry a lot of information which could prove to be valuable. However, due to the fact that identification and quantification is a laborious task when numerous bulk samples are to be handled, assessment of these materials may become a low priority. These materials can play an important role in the study of the condition and state of degradation of archaeological materials, which may be necessary in order to assess the need for *in situ* preservation as a reasonable alternative to excavation. For this, more research is needed on the factors affecting conservation of different archaeological materials, including bone.[5] Various analytical techniques can be applied in order to assess the condition and state of degradation of archaeological materials. Chemical methods are usually very accurate and allow a detailed interpretation to be made of the degradation process, but their destructive nature, cost and time consumption limit their applicability in archaeology.[6] There is a need to develop screening methods based on non-destructive techniques. Recently, van Doorn *et al.* suggested that non-destructive techniques in archaeology are needed for certain situations where damage to samples is especially undesirable.[7] In this direction, several studies have been performed to identify bones and their state of preservation due to chemical or thermal factors, using, for instance, a variation of the Zooarcheology by Mass Spectrometry (ZooMS) technique[8–9] that foregoes an acid demineralisation step, thus avoiding the destructive character of the method.[7] ZooMS and its varia-

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tions are based on the measurement of collagen peptide in the sample instead of DNA extraction, which is usually used for faunal identification in archaeology.[10] Alternatively, recent developments in the fields of optics and electronics have opened new possibilities for the non-destructive measurement of the physical and chemical properties of archaeological materials. Non-destructive spectroscopic techniques such as near infrared (NIR) spectroscopy have been applied for different purposes in the study of archaeological materials, mainly focusing on the conservation and ageing degradation of the materials, as paintings or wood.[11–12] In their book, Derrick *et al.* present infrared spectroscopy as the most widely used method in most museum laboratories and in conservation science.[13] Classical near infrared spectroscopy techniques give a very accurate overall characterisation of samples analysed, but not of the distribution of their constituents and do not provide information about the heterogeneous nature of all samples.[14] This has to be considered as a limitation of classical NIR technology, which is used in the development of indirect methods requiring a large number of reference samples and the construction of empirical models. In order to overcome some of the limitations of classic NIR technology and to benefit from the spatial information as well as the heterogeneity information of samples, development of multispectral or hyperspectral techniques, such as NIR microscopy and NIR imaging have been proposed as elegant solutions.[15] Multispectral imaging is produced by sensors that measure reflected energy within several (usually between 3 and 20) specific bands of the electromagnetic spectrum. Hyperspectral sensors measure energy in narrower and more numerous bands (20 or more), which provides a continuous spectral measurement across sections of the electromagnetic spectrum.[16] The huge increase in the number of spectral bands when working with hyperspectral imaging offers a substantial challenge when it comes to the work of mapping features on the basis of chemical composition, especially if little or no knowledge is available beforehand concerning the area being mapped.[17]

A plane scan hyperspectral imaging system gathers spectral and spatial data simultaneously by recording sequential images of a sample. One of the advantages of this technique is that assessment is completely independent of the analyst's expertise. This technology is gradually finding its place in analytical laboratories and in industry, and in recent years hyperspectral imaging has become an important analytical approach in several areas, including the quality control of pharmaceutical, agricultural and mineralogical products.[18] It has been successfully used for the identification, mapping and quantification of materials of different origins.[19–22] In archaeological research, the use of hyperspectral imaging is less well known.[23] A large number of studies included the use of satellite remote sensing,[24,25] however, most of the well-known satellite sensors, such as Landsat, Quickbird or Spot use multispectral sensors, which can vary from 4 spectral bands as in Landsat I to 14 spectral bands as in the Aster sensor.[26–28] In spite of the low resolution and the necessary and sometimes cumbersome post-processing of data, the use of spectral sensors on board satellites and planes has shown to be useful in archaeological research.[29] Only recently has hyperspectral imaging been applied for archaeological purposes. Alexakis *et al.* used data from four sensors, including Hyperion, which contains 220 spectral bands, to detect Neolithic settlements in a low relief region in Greece.[30] Also, Kwong *et al.* used these hyperspectral images in support of an archaeological study in the Oaxaca Valley of Mexico to better understand soil and vegetation types related to the ancient settlement.[31] More recently, Thomas *et al.* applied NIR spectroscopy directly on fossil bones to map constituents within bones and to assess its burial history.[32] Linderholm *et al.* have demonstrated the utility of NIR technology and NIR hyperspectral imaging to distinguish between human-affected and non-affected soils in samples from a Bronze Age settlement.[33]

This study was undertaken to demonstrate the potential of hyperspectral imaging spectroscopy combined with simple chemometric tools to provide information about the chemical and/or mineralogical differentiation within archaeological materials, which could otherwise not be established using conventional methods. The specific aim of this study was to describe possible applications of NIR hyperspectral imaging in the analysis of archaeological materials in terms of identifying fragmented bones and also to define a first step for describing their different states of preservation. To do so, several examples are presented where NIR hyperspectral image analysis was performed on bone and teeth material retrieved during an archaeological excavation at a Stone Age site in northern Scandinavia.

Materials and methods

Archaeological materials

In this study, numerous bones and teeth from elks (*Alces alces*) from the Stone Age site Bastuloken (raä 183 parish of Ramsele) in the county of Ångermanland in northern Sweden were subjected to analysis. The site is situated on the shore of Lake Bastuloken and was first excavated in 2005;[34] the material analysed in this study was retrieved during this excavation. At the site (Figure 1), three elliptic aggregations of fire-cracked stones were visible (15 × 7 m), each surrounding a depression. These aggregations were thought to be constructions for elk hide processing (possibly tanning), which required the use of other products such as brain- and bone-derived fat.

During the 2005 excavation, an area of 1 m^2 was excavated to a depth of 0.9 m (Figure 2). The excavated stratigraphy revealed unusually large amounts of unburned bone matter, both intact and fragmented, as well as significant amounts of fire-cracked stones. Soil and bone samples were subsequently collected from soil columns taken from levels at intervals of 0.05 m. The ^{14}C-dating from the lower levels (DN5) gave a date about 4600 cal before present (BP) and from the upper levels (DN2) a date of about 2200 cal BP.[34] Most of the

Figure 1. The Bastuloken site, with the oval stone embankment (photo Johan Olofsson).

site, however, was assessed as belonging to the late Stone Age (6000–4000 BP), based on the stone tools and ^{14}C dates retrieved during later excavations.[35]

Material chosen for further NIR image analysis included two main categories:

1) Sixteen bulk soil samples from the stratigraphy that were collected from levels at intervals of 0.05 m and sieved to 1.25 mm. This residue consisted of a mixture of coarse material such as minerals, fragmented rock, bones, teeth etc. From one of the layers (DN7) a small piece of elk bone was crushed and homogenised in order to acquire an "internal" reference.
2) Larger objects, bones and artefacts that were retrieved from all seven documentation levels (DN 1–7, 10 cm each) of the stratigraphy. A selection of bones and teeth from the various levels was used in this study.

All the NIR-analysed bones were subjected to osteological analysis,[36] and the species and body parts were determined. The bone specimens were air dried and bone surfaces lightly cleaned, manually, during the osteological analysis. Adsorbed mineral matter as well as secondary minerals were not removed and have therefore contributed to the NIR image spectra.

Instrumentation

Hyperspectral images, or hypercubes, are three-dimensional datasets containing light intensity measurements where two dimensions (x and y) represent spatial distances and the third dimension (λ) represents spectral variation such as wavelength. They can be interpreted as stacks of, typically, hundreds of two-dimensional spatial images at different wavelengths, or as tens of thousands of spectra, aligned in rows and columns.

The instrument used in this study was a sisuCHEMA pushbroom shortwave infrared hyperspectral imaging system

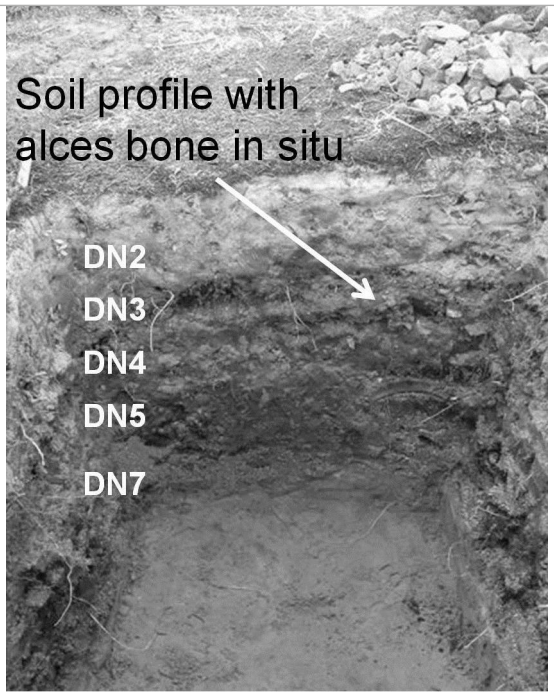

Figure 2. Section profile from the excavation in 2005. DN indicates documentation levels (photo Krister Efverström).

(Spectral Imaging Ltd, Oulu, Finland) for acquiring images (320 × 430) from 1000 nm to 2498 nm at intervals of 6–7 nm. The images obtained with this instrument were transformed into pseudo-absorbance using Evince image analysis software (UMBIO AB, Sweden, http://www.umbio.com). When analysing sieve residues, the samples were placed on a tray (5 × 5 cm), while when introducing bone and teeth samples to the NIR camera, the samples were placed on a conveyor belt, allowing larger samples to be analysed.

All data were pre-processed by the standard normal variate (SNV) in order to correct for light scattering, and centred prior to chemometric principal component analysis (PCA).

Results and discussion

In archaeological fieldwork, the sieving of soils and sediments is the most common way of retrieving artefacts and other materials. Figure 3 shows a standard and a false RGB image of such a residue fraction and presents the complexity and variety that is often encountered in archaeological contexts. Standard RGB images are made of three colours, or channels (Red, Green and Blue). The colour of each pixel within the image is coded by these three values (one for each channel) ranging from 0 to 255. The combination of the values of each channel gives the colour of the pixel. In contrary, in a false RGB image, the values of the three channels are values of absorbance at a given wavelength instead of values for the three previous colours. However, in order to visualise the image easily, the three values of absorbance are coded in the RGB scale and in this case we used the

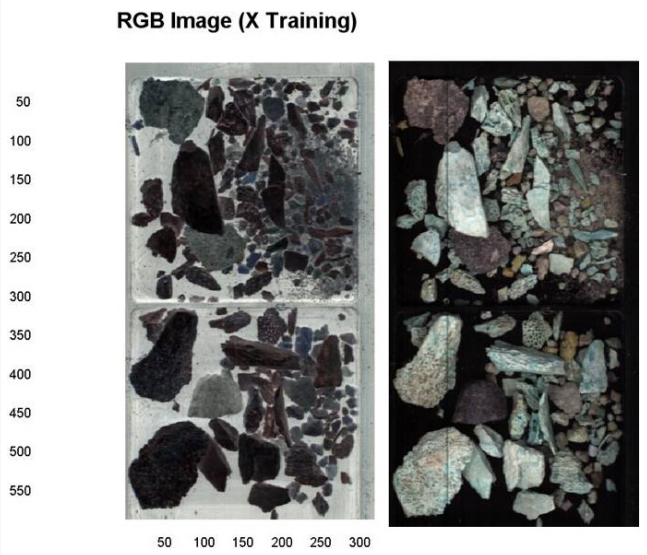

Figure 3. RGB and false RGB images on a sieved sediment fraction from DN7 (a mixture of minerals and fragmented bone material).

default settings of the Evince software (R 1350 nm, G 1750 nm and B 2150 nm). In Figure 3 it is possible to visually identify minerals and skeletal remains such as bones (both the inner spongy part as well as the outer compact bone). This example is taken from the DN7 bottom layer, after sieving a sediment sample to a 1.25 mm fraction. In the field, fractions of less than 2 mm are not usually catered for, and are analysed only as subsamples under laboratory conditions, mainly because of limited resources but also because identification is laborious.

When applying PCA on this image combined with the image of the small ground elk bone, the first two components explain more than 99% of the variation in the data. Bones and bone residues could be easily identified in the sieved fraction simply by selecting a hot-spot area in the score plot of the first and second principal component. Points selected in the hot spot area of the PCA model are specifically coloured and can be visualised in the processed image (Contour 2D) and in the original image. This selection highlights clusters from the ground elk bone that appear as stains and some bone fragments in the residue fraction (Figure 4). In addition, various mineral phases (possibly iron oxides, quartzite and flint) exhibit bands in the NIR range. The spectra of these phases seem isolated from the main cloud within the PCA plot (Figure 5) and are clearly distinguished from the matrix as a whole.

With regard to the analysis of the larger prehistoric elk bones from the excavation, a set of femur and long bones (ossalonga) was submitted to the NIR hyperspectral imaging system. A false RGB image is presented in Figure 6, where bones from different levels of depth may be observed. The bone specimens are all 3–6 cm in length. Horizontal and vertical scales on the images give the position of every pixel on both axes.

When building a three-component PCA model (PC 1–3, 74%, 7% and 4% of explained variability, respectively) on the NIR spectra obtained from this image, interesting patterns are obtained. Figure 7 shows the reconstructed image using the third principal component where changes in colour indicate differences in scores values, suggesting changes in the physical and possibly the chemical composition of the bones. In the studied stratigraphy, the bones showed an interesting trend, as illustrated by the PC models, as there was a distinct change in the PCA model for bone samples coming from deep (earlier date) to superficial (later date) layers. In Figure 7, samples from layer DN7 appear darker in the third PC compared to the other samples, indicating a potential group differentiation within the sample set. One of the post-depositional taphonomic (degradation) processes affecting the bone matter was increasing soil acidification over time through podsolisation.[37] This was evident in the bone matter coming from the upper

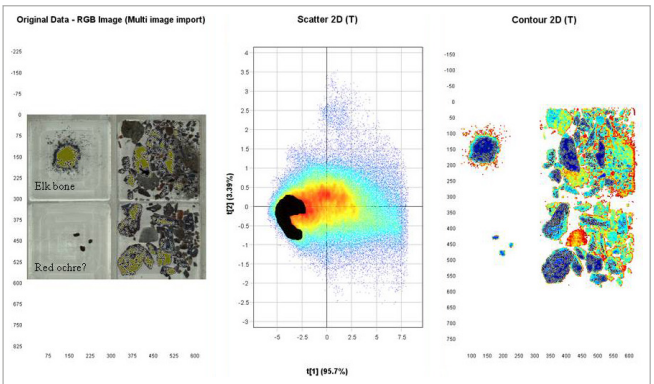

Figure 4. Composite images of ground elk bone and a sieved sediment fraction from DN7 > 1.25 mm (a) and the corresponding PC plot (PC 1 and 2) (b) and false RGB image (c). The black cluster in the score plot (b) marks the elk bone powder and identifies all other substances with similar response in the NIR spectrum (a and c).

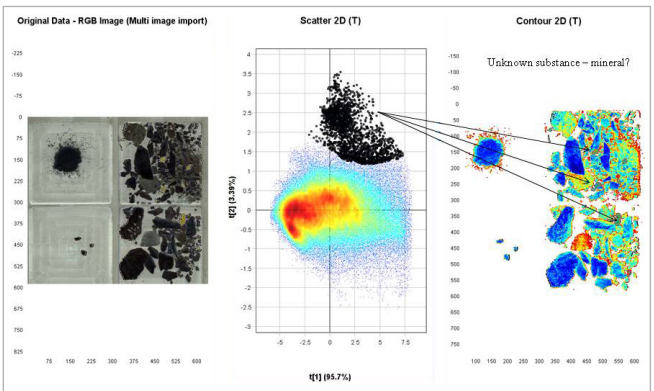

Figure 5. Composite images of ground elk bone and a sieved sediment fraction from DN7 > 1.25 mm (a) and the corresponding PC plot (PC 1 and 2) (b) and false RGB image (c). Here, the cluster of strong PC2 values is highlighted in black, picking out rock fragments/minerals (a and c).

Figure 6. False RGB image of elk bones retrieved from various depths during the excavation.

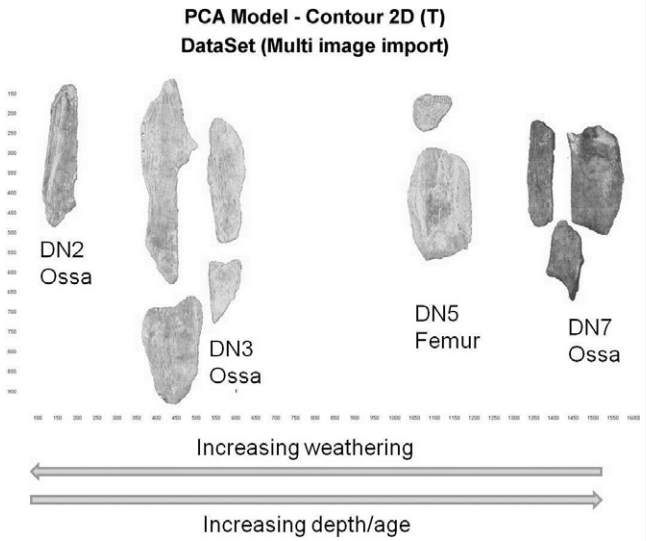

Figure 7. Corresponding (to Figure 6) 2D contour (PC3).

part of the excavation column because of the more apparent weathering of these bones, shown in the NIR image analysis. In addition, at depth the sheer buffering capacity of the large amount of deposited bones prevents them from being weathered. As elk hide processing (tanning) was suggested as one main process on site, this could indicate that the bone matter, although not burned, might have been subjected to cooking (boiling); given the large amount of fire-cracked stones at the site, this is quite likely. In order to investigate if this has taken place, possible cooking of the bones could be further elucidated by alternative approaches as have been put forward by Koon *et al.* on how to analyse bone matter in this respect.[38] Conducting experiments where different types of bone matter are treated by heat in various forms and degree is one way of simulating the possible prehistoric processes, and that could be subsequently analysed by NIR spectroscopy. Investigating burned bones, from mild charring to full combustion would also be of interest and would be relevant in forensics, for instance, to identify human casualties in fires.

In another example, an elk jaw and teeth were analysed in the same way (Figure 8). Apatite is the mineral giving specific NIR spectra in bone matter,[39] and was evident when looking at the PC cluster (first component) characterising the enamel part of each tooth (Figure 9). Also, there were distinct differences in the three average spectral composition of the elk jaw bone, the enamel and dentine parts of the teeth specimens, which, as known, appears due to differences in crystallinity, water and organic content etc. and which also lead to differential degradation of skeletal remains during diagenesis (Figure 10). This may also be useful for identification of more fragmented materials. The strong band that appears in Figure 10, around 1950–1970 nm, may be related to bone chemistry, as possible amine compounds.[40]

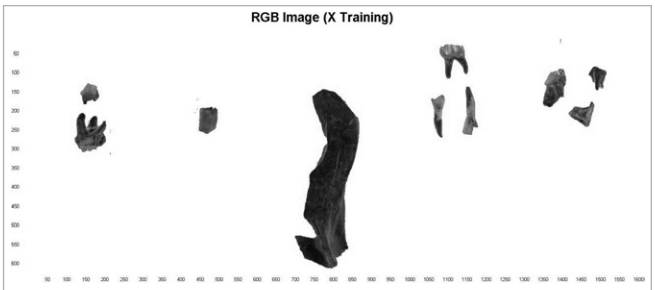

Figure 8. False RGB image of elk teeth and jaw bone retrieved from various depths during excavation.

Conclusion

NIR hyperspectral imaging has great potential in many fields of research, including feed and food analysis, archaeology and forensics. To identify bones in different contexts is a challenging task. This study corroborates previous studies which showed that NIR hyperspectral imaging is a useful technique for detecting the presence of bones in feed and

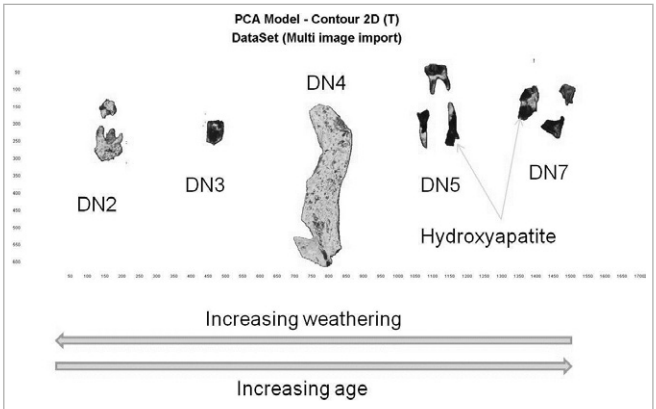

Figure 9. Corresponding (to Figure 8) 2D contour (PC1) highlighting the enamel as darker areas.

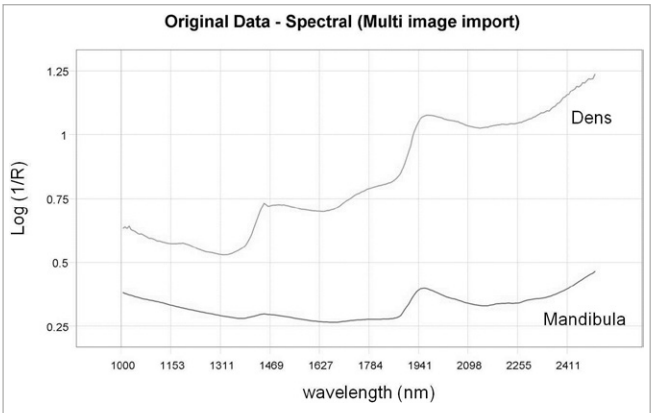

Figure 10. Average NIR-spectra from whole jaw, enamel and dentin part of teeth specimen (all from DN5).

has great potential in archaeological research. In particular, the study produced promising results in terms of showing different traits of elk bones and their state of preservation. Hyperspectral imaging can be useful for the identification of the presence of bones even including variation of the state of preservation, i.e. using NIR hyperspectral image analysis of sieved soil and sediment samples will make it possible to quantify large amounts of bone matter in various states of decay in the soil–sediment matrix.

Further studies need to be performed on bone matter of different ages, subjected to different treatments in prehistory, taphonomic and diagenetic processes and from different species. In this way, it might be possible to build a taphonomic reference model so that bone depositions and their context are better understood.

It is evident that NIR imaging has great potential in widely differing fields of research, as shown in this study, and will become an even more significant analytical approach.

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