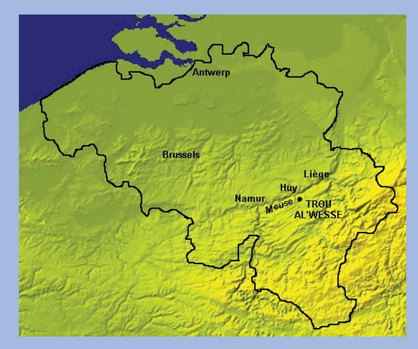
The ArcheoNIR Project
Archaeological Application of Near Infrared (NIR) Spectroscopy: Analysis of Fauna from Trou Al'Wesse (Belgium)



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Introduction

The ArchaeoNIR project combines archaeometric and archaeological analyses, testing the application of near-infrared spectroscopy (NIRS) combined with hyperspectral chemical imaging (NIR-HCI) to faunal samples from the Pleistocene and Holocene sequence at Trou Al'Wesse. NIRS can rapidly screen, in a non-destructive way, sediment for the presence of bone and (potentially) may also be a tool to evaluate the degree of collagen preservation. The quality attributes of samples can be assessed using both spatial and spectral (and therefore chemical) information obtained from the HCI systems.
The current project developed out of an earlier project run by the CRA-W team to respond to the problem of bovine spongiform encephalopathy (BSE or mad cow disease). They developed a technique using near infrared spectroscopy (microscopy and HCI) to test for contamination in animal feed by identifying the presence of bone particles. Discussion of this project with Matthew Collins, John Stewart and Rebecca Miller regarding potential archaeological applications led to the development of the pilot project at Trou Al'Wesse in Belgium.
The stratigraphic sequence (Fig. 1) on the terrace covers the period from ca. 50,000 to 5,000 uncal BP and includes Mousterian, Aurignacian, Mesolithic and Neolithic occupations. Non-archaeological layers, notably one intermediate between the Mousterian and Aurignacian, and others covering the Last Glacial Maximum and early Lateglacial, also contain fauna to provide a continuous sequence. This sequence thus enables us to address, among others, one of the main questions in current archaeological research: the role of climate change on human occupations during the Palaeolithic and Early Holocene. Data obtained from the fauna, combined with systematic dating, will help to refine the sequence of climatic oscillations throughout this period and situate the presence and absence of humans within paleoenvironmental context. Climate and environment play a key role in explanations of human adaptation, survival and extinction when hominids are confronted with changing climate, particularly rapid oscillations. NIRS-HCI analyses of the fauna will contribute to these questions by clarifying differences in faunal taphonomy within and between strata, leading to further analyses that will refine the palaeoenvironmental and climatic sequence observed in the TAW sequence.
Comparative analyses of bone samples within and between strata will provide taphonomic information with respect to post-depositional processes affecting bone and collagen preservation.
Finally, NIRS-HCI analysis of archaeological bone samples will enable selection of samples for subsequent analyses that require good collagen preservation, including radiometric dating and ancient DNA analysis.

Objectives

The overall objective of the project is to develop the NIR-HCI technique to evaluate the relative degree of collagen preservation in bone recovered from archaeological contexts. Statistical analyses of the NIR spectra signatures will have direct implications for archaeological applications: 1) taphonomic analyses of archaeological sites using differences in the NIR signatures of the different strata and 2) sample selection for subsequent planned analyses requiring collagen preservation, including aDNA, radiometric dating and ZooMS. The ArcheoNIR project is thus the first stage in a series of related analyses of the faunal assemblages at Trou Al'Wesse.

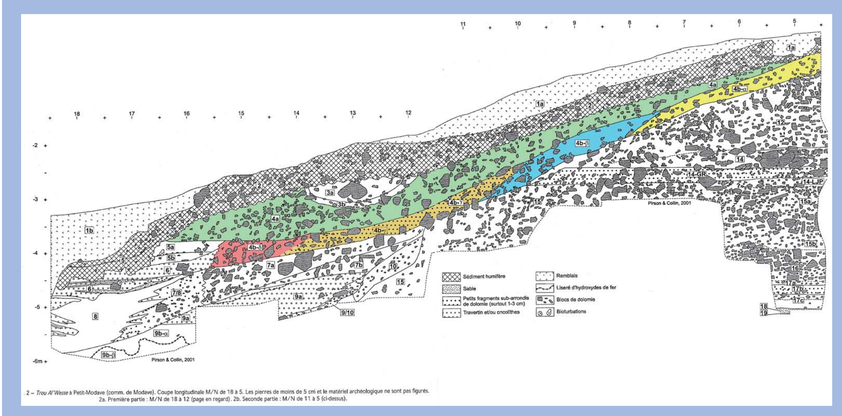


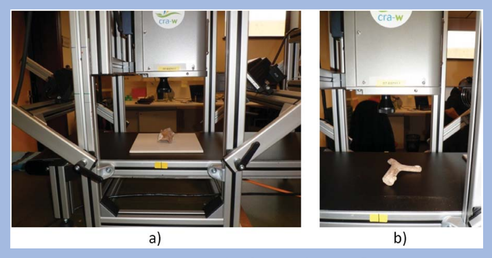
Figure 1. Stratigraphic sequence at Trou Al'Wesse, longitudinal profile M/N 5-18 (Pirson & Collin 2005).

Material and methods

Near infrared spectroscopy (NIRS) is based on absorption of light (absorbance) at selective wavelengths of the electromagnetic spectrum by the organic molecules constituting the analyzed samples. In this work, NIR is combined with hyperspectral images. These images are collected using a NIR hyperspectral line scan or push-broom imaging system, combined with a conveyor belt (BurgerMetrics SIA, Riga, Latvia). The instrument used for this study is an SWIR XEVA CL 2.5 320 TE4 camera from XENICS (Fig. 2-3) using an ImSpector N25E spectrograph that includes a cooled, temperature-stabilized Mercury-Cadmium-Telluride (MCT) detector from SPECIM Ltd, Oulu, Finland. The system projects a beam of light onto a two-dimensional Focal Plane Array (FPA) and each image consists of 320-pixel lines acquired at 209 wavelength channels: 1,100-2,400 nm at 6.3 nm intervals with 32 scans per image. The resulting images provide a reflectance spectrum for each pixel. The acquisition is done using HyperProVB software (BurgerMetrics SIA, Riga, Latvia).
The push-broom (also called line scan) imaging system has several advantages. This technique can be applied in on line analysis and allows acquisition of large datasets in a short time whatever the sample size. It permits quantitative or semi-quantitative detection of chemical compounds in samples. And finally, it provides information on the distribution of chemical compounds in the sample. Thus, all these advantages make line scan particularly well suited for applications in this study.
Prior to analysis, the spectral imaging system is calibrated with a dark image (by blocking the lens entrance) and a white image (background) collected from a standard white reference board (empty teflon plate). The spectra are then automatically corrected accordingly. This procedure is performed to compensate for offset due to the dark current, the light source temperature drift, and the lack of spatial lighting uniformity.
After image acquisition a spectral library is created for each bone scanned using HyperSee software (BurgerMetrics SIA, Riga, Latvia). This library contains spectra selected at random places on the bones (Figs. 3 and 4). The selection of spectra is made in such a way to cover all the spectral diversity of the bone (i.e. the different sides of the bone).
Once the spectral library is constructed, chemometric analyses can be run (Principal Components Analysis or PCA). PCA allows extracting the maximum information from the data and then enables comparison of different areas on a single bone, bones from a single stratum, bones from different strata, etc.

Tables 1 and 2 describe the samples used to build chemometric models (Table 1) and the samples used for prediction of potential collagen content (Table 2). For model creation a total of 216 spectra per sample are selected. Samples are scanned twice in order to ensure camera stability. For each scan 108 spectra are extracted from each sample (54 spectra per side). Then, for predicted samples (layer 15) 198 spectra are extracted from each sample (99 spectra per side).

Figure 2: Instrument used for image acquisition.



a) b)

Figure 3: Image acquisition for samples on teflon plate (a) or on conveyor belt (b).

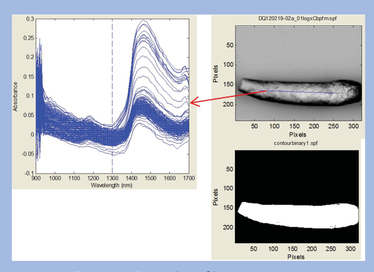


Figure 4: Examples of bone images.

Layers	Samples
4b-δ	I12.237
	I12.257
AC	K13.149
	K13.152

Table 1: Samples used for model construction.

Layers	Samples
15(a)	L8.143
	L8.151
15(g)	L9.102
15(f)	L9.65
15	N5.72
15	N5.74
15(c)	N6.102 dec 26
15(c)	N6.96 dec 35
15(f)	N7.184 dec 40
15(c)	N7.174 dec 38
15(a)	N8.132
15(b)	N8.172

Table2: Samples from layer 15 used for model prediction.

Analyses

Initial comparisons have been done on small samples to evaluate the potential for observing the range of variability in NIR spectra in bone samples from the Trou Al'Wesse stratigraphic sequence. The first stage is thus to determine whether the NIR spectra from bone samples vary. Comparison of the NIR spectra obtained on sample sets within and between strata using PCA will identify potential groups. At present, groups can be isolated without as yet identifying the underlying factors. However, these groups can be used to identify differences and similarities in faunal assemblages in the sequence, and to address certain taphonomic questions.
The second stage will be to explain the observed variability by identifying these underlying factors. Very preliminary results suggest that difference in the degree of collagen preservation is likely to be a major factor, but others can also be hypothesized.
Such explanation and archaeological interpretation of groups will be proposed later on in the project, based on the results of technical developments for the interpretation of the NIR spectra. Alongside these comparative analyses to address taphonomic questions, the ArcheoNIR project also focuses on means to evaluate the qualitative and/or quantitative degree of collagen preservation in archaeological bone. To do so, several aspects must be clarified, including:
- is there any characteristic wavelength directly related to the presence of collagen?
- can the degree of collagen preservation be quantified?
- can relative distances between groups be related to differential collagen preservation?

Collagen

Figure 5 (Baykal et al., 2010) illustrates some characteristic wavelengths (with asterisks) related to the presence of collagen. Figure 6 represents the mean spectrum of samples from layer AC (a) and layer 4b-δ (b). Layer AC is considered to have poor collagen preservation, based on several failed attempts to data fauna; layer 4b-δ, however, has provided successful dates for the Late Mesolithic and is considered to have sufficient collagen preservation. Both layers are Mesolithic. Comparison between the literature and spectra from samples from layer 4b-δ seems to show similar characteristic wavelengths (dashed red lines). Moreover, spectra from AC samples differ from the spectra from 4b-δ samples. Figure 7 illustrates the spectral differences between the AC and 4b-δ samples.

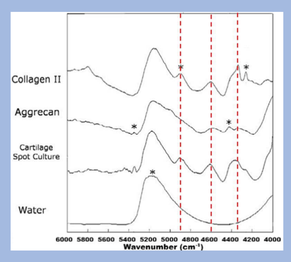


Figure 5: Characteristic wavelengths related to the presence of collagen.

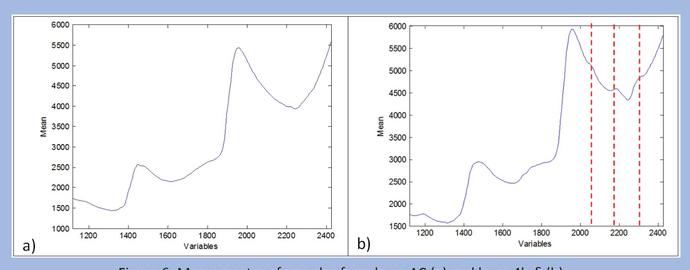


Figure 6: Mean spectra of samples from layer AC (a) and layer 4b-δ (b).

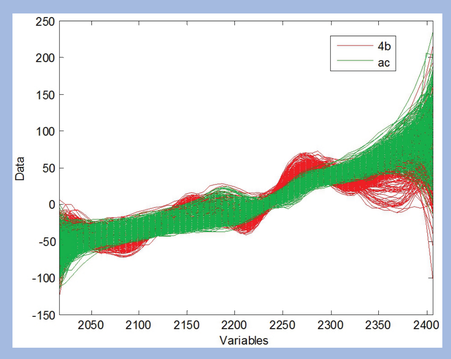


Figure 7: Spectral differences between the AC and 4b-δ samples.

For comparison and potential quantification, an independent bone sample standard was recently obtained from Matthew Collins (University of York). This is a sample of a medieval cow tibia (Fig. 8) that has been extensively analyzed at York, considered to have nearly 100% collagen preservation. Unlike modern bones that would contain lipids that also affect the spectra, these are no longer present in the bone standard. PCA analysis comparing different sample sets against this standard should indicate at minimum the relative degree of collagen preservation.

Figure 8: Sample from a medieval cow tibia (scale in cm).

Preliminary results

A series of comparative PCA analyses is in progress to address different taphonomic questions of archaeological relevance.

1. **Model development**. An initial test compares samples that are considered to collagen-rich or collagen-poor, based on successful and unsuccessful dating of previous samples from two strata at Trou Al'Wesse: stratum AC/ACOF, an alluvial deposit, and stratum 4b-δ, a colluvial deposit. Both are Mesolithic in age. Results shown here are thus preliminary and not independent of the archaeological context. Prediction plots for sample sets from the TAW sequence can be compared to the standard to evaluate the degree of collagen preservation.

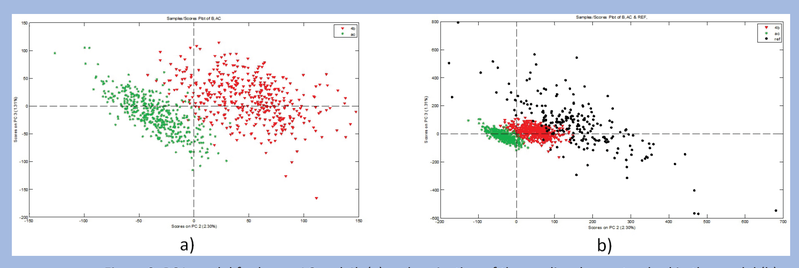


Figure 9: PCA model for layers AC and 4b (a) and projection of the medieval cow standard in the model (b).

The left graph above (Fig. 9a) shows the PCA results for the model with AC and 4b-δ layers. Two clear groups appear - green for samples with insufficient collagen (layer AC) and red for samples with sufficient collagen (layer 4b-δ). The variance is explained by PC 2 and PC 3. The presence of two groups suggests that collagen preservation is a factor distinguishing between these groups, but further analysis is required for rigorous demonstration. The right graph (Fig. 9b) shows the PCA results for the medieval cow sample projected in the model. The reference sample would appear to be a third distinct group, but is close to the 4b-δ group in the model, which may provide support to confirm that the differences in groups are linked to collagen content.

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A second model was built by PLS-DA (Partial Least Squares Discriminant Analysis) on the same dataset. Figure 10 shows the projection of the medieval cow sample in the model. This model also tends to classify the medieval cow sample in the same class as samples from layer 4b-δ.

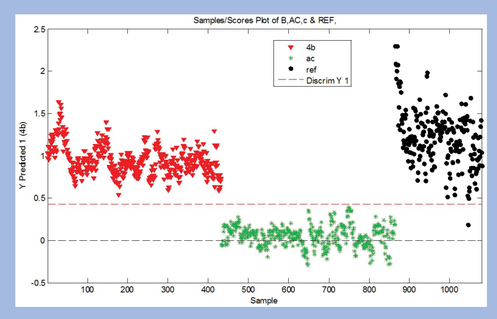


Figure 10: Projection of the medieval cow sample in the PLS-DA model.

Figure 11 shows the PCA loadings or the weight of the variables (wavelengths) that most influence the PLS-DA model. The pink circles in the left plot (Fig. 11a) are the variables that explain the separation of the samples when looking at component 2 (PC2). These wavelengths are presented in the right graph (Fig. 11b).

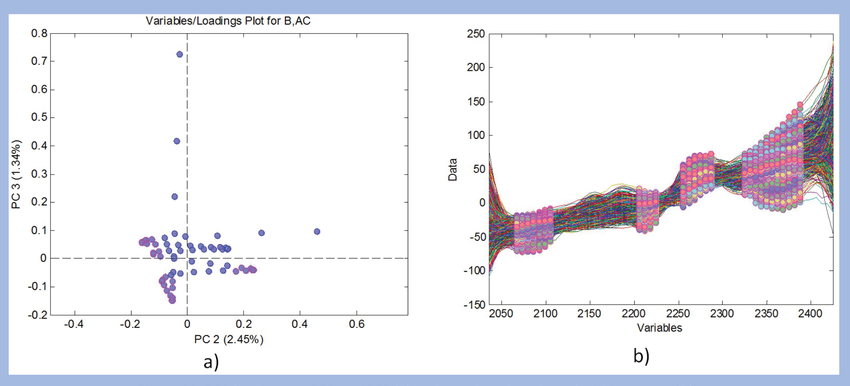
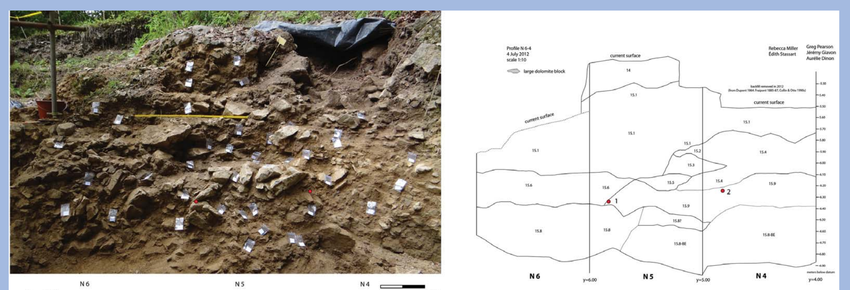


Figure 11: PCA loadings on PC2 and PC3 (a) and wavelengths selected within spectra (b).

2. **Identification/confirmation of geological strata**. In 2012, one of the aims of the field season was to examine stratigraphic unit 15, which contains Aurignacian lithic artifacts and fauna, in order to identify potential sub-divisions. Vertical excavation of unit 15 from top to bottom in a 3-meter wide section (N/O 6-N 4) and squares K-L 8 led to the identification of 10 distinct sub-layers in the field, based on geological variables. Geological analyses of sediment samples are in progress to confirm or refute the distinctions between different sub-layers. To test whether the fauna in each sub-layer is different, reflecting different (as yet unknown) post-depositional conditions and thus supporting the division of the sub-layers, bone samples from each were analyzed by NIR and prediction plots created using the PCA and PLS-DA models previously developed.



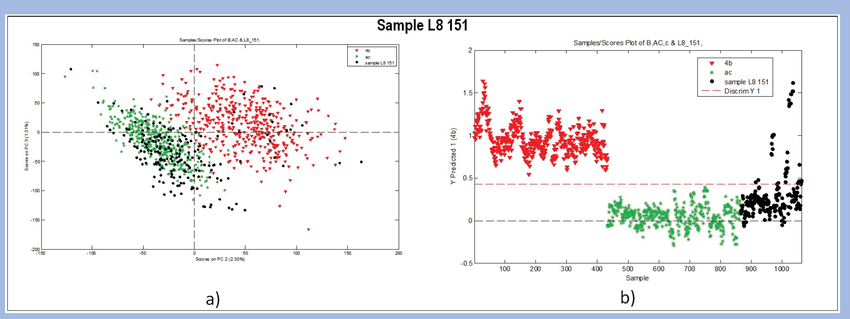


Figure 12: Projections of sample L8 151 in PCA model (a) and in PLS-DA model (b).

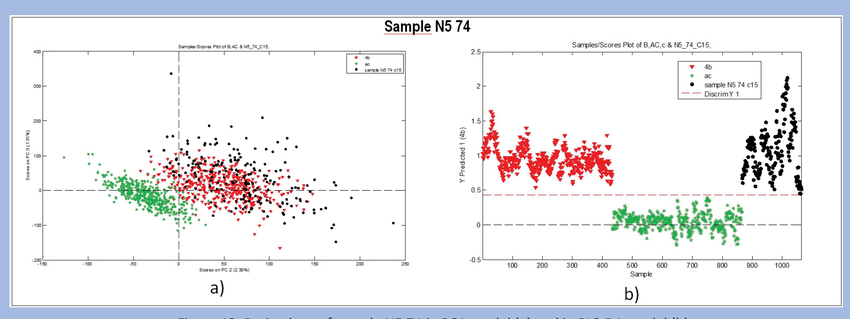


Figure 13: Projections of sample N5 74 in PCA model (a) and in PLS-DA model (b).

The four graphs above (Figs. 12 and 13) show samples from different sub-layers in layer 15 (black circles) predicted using the previous PCA and PLS-DA models respectively. The first sample (L8.151), from sub-layer 15.6 rich in carbonate concretions, suggests that it is more similar to collagen-poor layer AC than collagen-rich layer 4b-δ. This would further suggest that samples from sub-layer 15.6 are also likely to be collagen-poor. The second sample (N5.74), from sub-layer 15.1, is more similar to collagen-rich layer 4b-δ, suggesting that faunal samples from this sub-layer are likely to contain collagen. These initial results show that differences exist in NIR spectra between the sub-layers identified in the field. The combination of geological data in the field to identify potential sub-layers and NIR analysis of bone samples thus provides arguments supporting the division of unit 15 into a series of geologically distinct sub-layers.

These results are, however, still merely indicative and will be refined using larger, representative samples for each sub-layer. Differences between strata could potentially be interpreted as reflecting different post-depositional processes affecting the bones, and thus perhaps changes in climate and environment.

3. **Stratum integrity**. Comparisons of bone samples within a single stratum are also planned to evaluate the integrity or degree of homogeneity in NIR spectra within a single assemblage. If the bones have a similar signature (one group), this is hypothesized to indicate that all were subject to the same post-depositional conditions. This would further indicate that little or no reworking or mixing occurred. If, however, several groups are isolated, this would indicate that different post-depositional conditions affected each group. The different groups contributing to the assemblage would thus have come from different contexts, their admixture showing that more significant reworking or mixing took place. Comparison with strata above and below such a mixed stratum could indicate the source of some of these groups.

4. **Sample selection**. To select the most suitable samples for other analyses, the prediction plots obtained via PCA analysis of NIR spectra show the position of the range of samples (black circles) in relation to groups with sufficient or insufficient collagen. Samples falling within the sufficient collagen group (red triangles) would be considered suitable. NIR analysis would thus provide a rapid, non-destructive method for evaluating bone samples prior to analyses requiring collagen.

References

Baykal D. et al., 2010, Nondestructive Assessment of Engineered Cartilage Constructs Using Near-Infrared Spectroscopy. *Society for Applied Spectroscopy* 64(10): 1160-1166
Pirson S. & Collin F., 2005, Contribution à la stratigraphie du Trou Al'Wesse à Petit-Modave (comm. de Modave, prov. de Liege). *Notae Praehistoricae* 25:39-47.

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