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Raman Microscopy as a Primary Technique for Identifying Micro-residues Related to Tool-use on Prehistoric Stone Artefacts

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Analyses of ancient micro-residues preserved on stone artefacts can potentially provide detailed information about how prehistoric humans used the artefacts to process materials such as food, pigments and/or adhesives. However, prehistoric micro-residues are likely to degrade and there are multiple potential sources of contamination, such as contact with sediments, groundwater, recent handling, storage materials or laboratory conditions, any of which can inhibit reliable identification of micro-residues and other traces of prehistoric use. In this chapter we illustrate the use of Raman spectroscopy as a primary method to identity ancient micro-residues preserved on stone artefact surfaces that are due specifically to prehistoric use as opposed to some form of ancient or modern source of contamination. Stone tools from Liang Bua (Flores, Indonesia) and Denisova Cave (Altai Mountains, Siberia) are used to demonstrate the methodology.

Keywords

related, tool-use, raman, prehistoric, microscopy, stone, artefacts, primary, technique, identifying, micro-residues

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Raman microscopy as a primary technique for identifying micro-residues related to tool-use on prehistoric stone artefacts.

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Abstract

Analyses of ancient micro-residues preserved on stone artefacts can potentially provide detailed information about how prehistoric humans used the artefacts to process materials such as food, pigments and/or adhesives. However, prehistoric micro-residues are likely to degrade and there are multiple potential sources of contamination, such as contact with sediments, groundwater, recent handling, storage materials or laboratory conditions, any of which can inhibit reliable identification of micro-residues and other traces of prehistoric use.

In this chapter we illustrate the use of Raman spectroscopy as a primary method to identity ancient micro-residues preserved on stone artefact surfaces that are due specifically to prehistoric use as opposed to some form of ancient or modern source of contamination. Stone tools from Liang Bua (Flores, Indonesia) and Denisova Cave (Altai Mountains, Siberia) are used to demonstrate the methodology.

X.1 Introduction

Stone tools are the most common artefacts excavated at archaeological sites dating from the Stone Age and are in many instances the only remaining evidence of how people lived in the distant past. Analysis of ancient micro-residues preserved on stone artefacts can provide detailed information on the activities undertaken with such implements and forms a useful tool to reconstruct past human behaviours.

The identification of ancient micro-residues, however, is only the first step in relating it to the original function of the tool, as the presence of a micro-residue may originate from multiple agencies other than transfer of contact material during use. For example, both organic and inorganic micro-residues can be transferred to stone tool surfaces via contact with sediments, groundwater, bacteria, subterranean invertebrates and fungi. Contamination after excavation is also not negligible and can occur through handling by archaeologists during excavation, contact with storage material, or through laboratory conditions and physical contact with analytical instruments and facilities used to study the tools.[1]

Previously, Raman spectroscopy has been used to verify the identification of macro-residues on stone tools detected through optical microscopy by usewear specialists.[2] In the Raman spectroscopic methodology presented here, artefacts were not selected based on the presence of any visible macro-residues attached to the tools, but applied as the primary technique for initially locating and analysing organic and inorganic micro-residues. This strategy was followed to avoid any bias that targeted preferentially larger residues or residues present only on polished edges and surfaces, and focus on particles less than 50 microns in size. These micron-sized residues can only be successfully and systematically analysed by a technique with high spatial resolution, such as Raman microscopy, with the added advantage that both inorganic and organic materials can be identified simultaneously.[1] Furthermore, the technique

is non-destructive and leaves the residues in context on the artefact, allowing for future study of the same artefacts.

In this chapter we use stone artefacts from Liang Bua (Flores, Indonesia) and Denisova Cave (Altai Mountains, Siberia) to illustrate the use of Raman spectroscopy as a primary method to identity ancient micro-residues preserved on stone artefact surfaces that are specifically due to prehistoric use as opposed to some form of ancient or modern source of contamination.

X.2 Archaeological background

Liang Bua is a limestone cave located on the island of Flores, Indonesia, with a cultural sequence spanning the past ~190 thousand years.[3] During this time, the cave was occupied successively by at least two human species, initially by *Homo floresiensis* and later by *Homo sapiens* (modern humans), currently with no evidence of temporal overlap.[3,4] Denisova Cave was used as an occasional occupation site, initially by Neanderthals and Denisovans with some occupation periods overlapping,[5] and later by *Homo sapiens*. The two sites have completely different climatic conditions and it is expected that the preservation of organic residues will be much higher at the lower average temperature in Denisova Cave (which is located in the Altai Mountains of southern Siberia) than in Liang Bua, which forms part of the tropical Indonesian archipelago.

X.3 Experimental methods

X.3.1 Sample preparation

Working with micron-sized residues increases the possibility of contamination from various sources that can interfere with the correct interpretation of the archaeological relevance of a residue. For instance, during the course of this study, indigo was identified[6] in fibres on artefacts originating from Liang Bua, attracting considerable interest, as the island of Flores

has a long history of batik work; a prehistoric presence of native *Indigofera* plant material (which has medicinal properties) would thus not have been out of place. Unfortunately, indigo fibres were also identified on glass slides placed in the same Raman laboratory for a week to test for airborne pollution sources. The results were positive and the conclusion was that micron-sized indigo-coloured cotton fibres are quite common in the air, which led to the re-classification of the indigo fibres on the stone tool as a contaminant.[7]

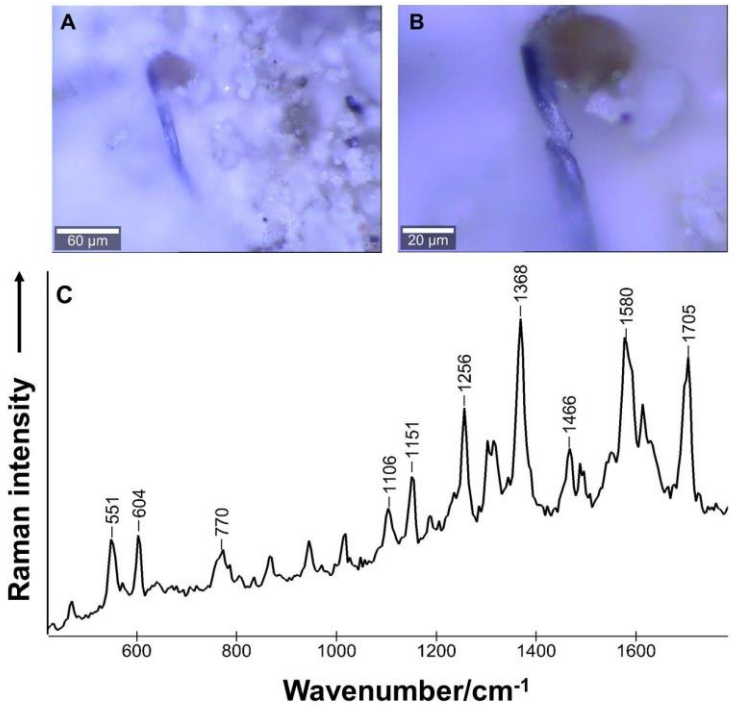

Figure 1. Images of indigo-coloured fibre on Liang Bua artefact and Raman spectrum of indigo using 532 nm excitation.

Aware of the many sources of contamination that can influence the interpretation of archaeological data, we requested that artefacts collected at Denisova Cave and Liang Bua should be excavated encased in sediment, eliminating exposure to ambient conditions at the excavation site. The stone tools were only removed from the sediment under clean laboratory conditions and for each artefact, sediment in contact and 3 cm away from the artefact surface was collected. The artefacts were handled with nitrile gloves (latex, powder and protein free)

and placed on a support fashioned with Blu-Tack® (a synthetic rubber compound) to accommodate its shape. This enabled the positioning of each sample under the Raman microscope with the incident laser perpendicular to the point of analysis. The support was covered with a piece of nitrile glove to prevent contamination from the Blu-Tack® (contamination from the nitrile glove is easy to recognise). Basic precautions, such as storing the samples in clean bags and boxes, were taken before and after analysis. Raman reference spectra were recorded for any material that was in contact with the artefacts and, together with spectra collected from glass slides placed in strategic positions in the laboratory to screen for air pollution, forms a database of possible contaminants.[1]

X.3.2 Raman analysis

Raman spectra were recorded with a WITec® alpha 300R confocal Raman microscope (WITec® Instrument Corp., Germany) equipped with two UHTS300 spectrometers and two CCD detectors: (1) a visible DV401 detector for use with 532 nm excitation, and (2) a DV401 detector for 785 nm excitation. The excitation sources were two diode lasers operated at 532 nm and 785 nm wavelengths with 38 mW and 120 mW maximum power output respectively. Zeiss® microscope objectives (20X and 50X magnifications) were used, achieving a sub-micron spatial resolution. The samples were placed on a piezo-driven, feedback-controlled scanning stage.

X.3.3 Cleaning and analysis procedures

Once removed from the sediment crust, the samples were first photographed and macro-residues characterised using Raman spectroscopy. Samples were then cleaned by ultrasonication for 10 s in Milli-Q® water. A systematic search and analysis of micro-residues on washed artefacts were undertaken, concentrating on the edges of the artefact perimeter using a 50X objective, within a strip approximately 200 μm from the edge (on both ventral and dorsal

sides). This is a time-consuming task and in general ~100 Raman spectra were recorded for each artefact from ~500 spots probed by the oscilloscope. On encountering potentially significant micro-residues, investigations of the adjacent surfaces were conducted to document residue distributions and, if possible, Raman mapping was undertaken on small areas. Finally, a random check of both surfaces, away from the edges, was conducted with a 20X magnification objective, to document any other residue area(s) and to confirm the extent and depth of micro-residue concentrations. Sediment samples taken from the sediment surrounding the samples and removed during ultrasonication were placed on microscope slides and also analysed.

X.3.4 Reference material

Micro-residues originating from both animal and plant material are often complex mixtures of nucleotides, proteins, lipids and carbohydrates and it is not expected that Raman spectroscopy will be able to identify all constituents, as is possible with techniques based on mass spectrometry.[8] A first step in using Raman spectroscopy as a tool is to evaluate the range of residues that can be detected through their Raman spectra on artefacts with known micro-residues.[9] Addressing this need, replicate stone tools used to imitate tasks regularly performed in the Stone Age during food processing were used to detect the type and distribution of micro-residues related to specific tasks (e.g. sawing, cutting, scraping) on different materials (e.g. meat, wood, hide, bone). Some of these tools were used nearly 30 years ago as the basis for documenting residue and wear patterns and were used on a variety of materials, including animal flesh, bone and hide.[10] New tool-use experiments were undertaken for comparison with the 30 year-old tools. Studying samples with known residues and functions forms a reference base to aid interpretation of the results from archaeological tools.

X.4 Results and Discussion

X.4.1 Sediment

It is expected that residues associated with a stone tool will also occur in the surrounding sediment as degradation processes and dissolution through the working of groundwater might detach some original residues from the artefacts. Comparing the frequency of occurrence of a specific residue on a tool with the three sediment samples collected for each artefact is helpful in deciding if a residue present in the sediment was deposited on the artefact or, conversely, if the occurrence of a residue in the sediment is due to dissolution from the artefact. Sediment from Liang Bua consisted mainly of feldspar and α-quartz grains, but other common minerals such as calcium carbonate, goethite and anatase were frequently observed. Sediment attached to the artefacts from Denisova Cave consists mainly of amorphous phosphate particles interspersed with α-quartz and feldspar grains, with varying amounts of calcite present for some of them. Although kaolinite clay was identified in sediments from both Denisova Cave and Liang Bua using FTIR spectroscopy, it was not observed in the sediment using Raman spectroscopy.

X.4.2 Experimental tools

Table 1 summarises the results obtained from the experimental tools. The most common residues that were detected on tools used to process animal products 30 years ago were identified as collagen (amide I and amide III bands), bone (PO_4 stretch at 962 cm^{-1}) and saturated fatty acids (SFA) with CH_2 and CH_3 stretching vibrations between 2800 and 2950 cm^{-1}, bending CH_2/CH_3 vibrational bands at 1463 and 1443 cm^{-1}, a CH_2 twisting mode at 1300 cm^{-1}, and C-C stretching at 1133, 1105 and 1067 cm^{-1}.[11,12] Collagen fibres, small pieces of bone and individual fatty acid residues could be recognised visually (Fig. 2). Saturated fatty acids and bone appeared smeared in areas where pressure was applied during the processing activity.

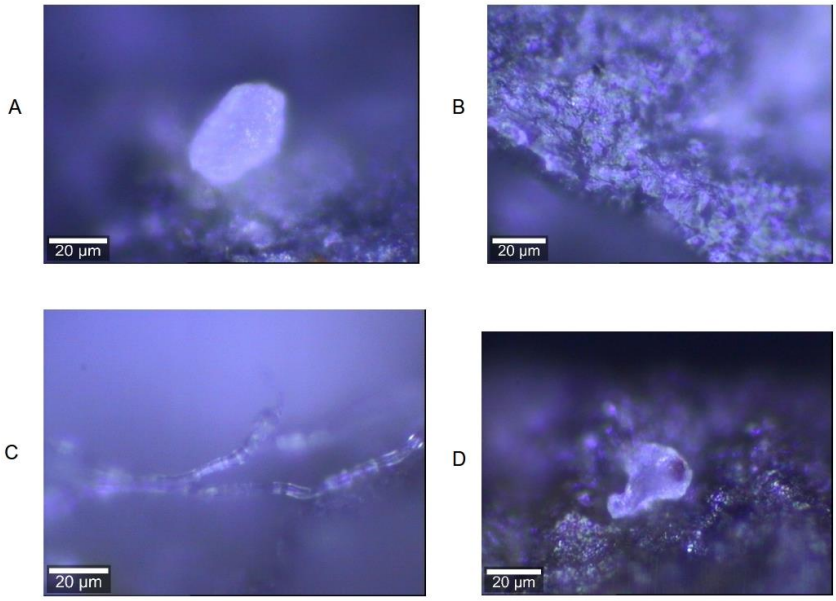

Figure 2: Micro-residues associated with processing animal material: (A) Discrete saturated fatty acid, (B) Smeared saturated fatty acid, (C) Collagen micro-fibres and (D) Bone fragment.

In animal processing experiments, where tools were analysed 1 month after use (Table 1), similar residues were detected, but in some cases Raman spectra of discrete and smeared fatty acid residues had additional peaks at 1656 and 3010 cm^{-1} (C=C stretch and =C-H stretching, respectively) and a shoulder centred at 1260-1270 cm^{-1} (=C-H deformation) that can be attributed to unsaturated fatty acids.[11,12] The presence of another C=C stretch band centred at 1679 cm^{-1} suggests the presence of a trans-isomer[11] and a band at 1610 cm^{-1} (Fig. 3) might be attributed to a conjugated cis-isomer mode with multiple C=C-C=C modes or an aromatic ring.[13] It can be concluded that for the older samples unsaturated fatty acids were degraded to their saturated counterparts during the 30 years of storage. The frequency and residue type on the tools used for processing animal products varied according to tool function. On artefacts used to saw or cut bone, bone and collagen residues were the most common; on tools used to cut meat, collagen fibres and smeared protein were the most common. Scraping animal skin

resulted in mostly fatty acid residues, some as discrete residues with a specific shape and others spread over a larger area (Table 1).

Table 1:

Material worked	Age	Function	Residue type	Frequency
Fresh possum skin	30 years	Scraping	Smeared SFA	Common
			Discrete SFA	Common
			Protein fibre	Common
			Protein	Common
			Starch grain	Rare
Dry animal bone	30 years	Sawing	Bone + collagen	Very common
			Smeared bone	Very common
			Smeared SFA + bone	Very common
			Smeared SFA	Rare
Meat	30 years	Cutting	Collagen fibre	Very common
			Smeared protein	Very common
			SFA fibre	Uncommon
			Discrete SFA	Uncommon
Fresh bone	30 years	Scraping	Bone and collagen	Very common
			Collagen fibre	Very common
			Lipids	Uncommon
			Plant fibre	Rare
Fallow deer	3 months	Cleaning	Smeared bone and collagen	Very common
		Extracting bone marrow	Bone and collagen	Very common
		Scraping	Smeared SFA/UFA mixture	Uncommon
Fallow deer	3 months	Sawing	Bone and collagen	Very common
			Smeared bone and collagen	Very common
			Smeared SFA/UFA mixture	Uncommon
			Protein	Rare
Fallow deer skin	3 months	Scraping	Smeared SFA/UFA mixture	Common
			Discrete SFA/UFA mix	Common
			Collagen fibre	Uncommon
			Protein	Rare
Fern	2 weeks	Cutting	Plant fibre	Common
			Carotenoid pigment	Common
			Smeared carotenoid pigment	Common
Rowan	2 weeks	Cutting and scraping	Wood fibre	Common
			Smeared wood fibre	Common
			Oxalate	Rare
			Discrete SFA	Rare
Siberian pine	1 month	Cutting and scraping	Smeared wood	Common
			Wood fibre	Common
			Plant fibre	Uncommon
			Smeared resin	Uncommon
Birch bark	1 month	Cutting	Smeared wood	Common
			Wood fibre	Common
			Plant fibre	Uncommon
Nettle	9 month	Cutting	Plant fibre	Common
			Smeared SFA/UFA	Common
			Discrete SFA/UFA	Common

Very common: Micro-residue widespread
Common: Micro-residues occur in limited areas or with specific distributions
Uncommon: Infrequently detected
Rare: Only once or twice detected

On most artefacts that were used to process fresh plant material (fern, rowan, Siberian pine and birch bark), the most common residues detected were plant fibres (high cellulose content, main bands at 1093 and 1121 cm^{-1})[14], wood fibres (high lignin content, main band at 1600 cm^{-1})[14] and carotenoid pigments (only for fern). Although discrete fatty acids were detected on these tools (saturated and saturated/unsaturated mixtures) resulting in Raman spectra exactly the

same as on tools used to process animal products, their numbers were not significant and they were not smeared or spatially distributed in recognisable patterns that can be linked to usewear. However, on the artefact used to cut nettle, a plant rich in natural oils, saturated/unsaturated fatty acid mixtures were commonly recorded as discrete and smeared residues (Table 1).

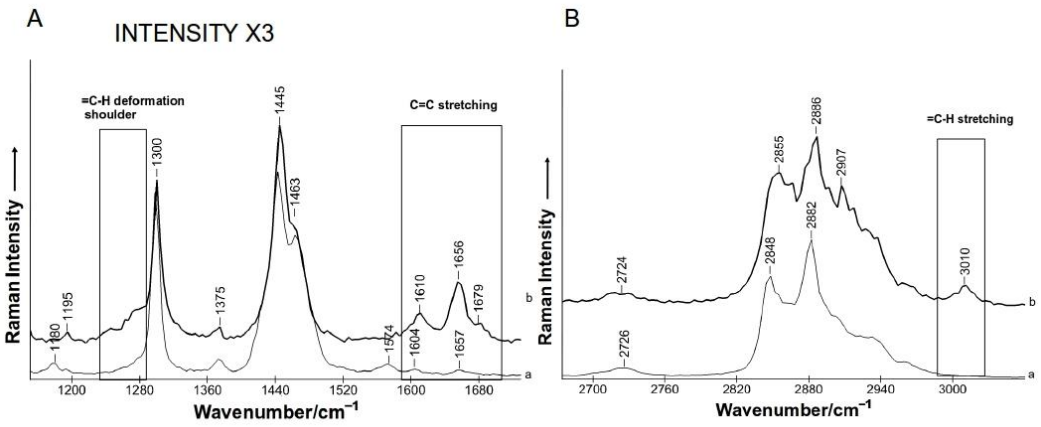

Figure 3: Comparison between Raman spectra of saturated fatty acid and saturated/unsaturated fatty acid mixtures recorded on stone tools used to process fresh animal skin (Figure 6 from reference 15).

Therefore, there is no single Raman spectrum that makes a chemical distinction between artefacts used on animal and plant material, so the distribution of the residues and association with other materials become very important. Although discrete and smeared saturated/unsaturated fatty acids commonly occur on the artefact used to cut nettle, they occur in patches interspersed with plant fibre bundles and the smeared fatty acid residues are not as thick as for the artefacts used to process animal products.[15]

X.4.3 Archaeological artefacts

Stone tools used repeatedly to scrape, cut or saw particular materials sustain diagnostic use wear, including polish associated with changes in surface micro-topography, that can be visually identified under a light microscope.[16] It has been shown experimentally that micro-

residues are commonly found in areas with usewear[17] and therefore an in-depth study of polish distribution for each artefact was undertaken after the Raman analyses and compared to the distribution patterns of the residues.[15]

Three different micro-residue groups were distinguished among the artefacts so far studied. The first group, for artefacts from Denisova Cave, consists of smeared fatty acid residues (Fig. 4A), similar to those obtained on experimental tools used to process animal products and some plants, in addition to discrete fatty acid and protein residues.[7,15] In general, these fatty acid residues are closely associated with areas of high polish (Fig. 4B) and in some instances include unsaturated fatty acid residues similar to the 1 month-old experimental tools (see section X.4.2 and Fig. 3). This indicates a high degree of preservation, as expected for the cold conditions in Denisova Cave. Although the very nature of a smeared residue classifies it as originating from the past use of a tool, a good correlation between polish and smeared fatty acid areas offers supporting information (Fig. 4B). A closer look at the distribution patterns on the tool shown in Fig. 4B shows that the correlation between polish and fatty acid distribution is very high and on the left long edge, smeared fatty acids and polish appear on both sides, implying that pressure was distributed to both sides of the tool during use, which is consistent with a cutting/sawing action. On the other edge of the tool, the smeared fatty acid zones and polish occur only on one side, corresponding to a scraping action.

On the experimental tools used to process some animal products, small pieces of bone as well as areas of smeared bone were identified (Table 1). Although particles of amorphous phosphate (PO_4 stretch at 950 cm^{-1}, FWHM 25-35 cm^{-1})[18] were found widespread on all the artefacts from Denisova Cave, they do not show any specific spatial distribution; as amorphous phosphate is the most common mineral identified in the sediment, it was probably transferred from the sediment to the artefact surfaces and on some artefacts formed a patina (phosphate skin). Collagen fibres associated with bone are commonly found on experimental stone tools used to

work bone and meat, but were not found on Denisova stone artefacts in association with lipid micro-residues. Taking into account the prominent presence of both discrete and smeared fatty acid micro-residues, together with the absence of bone and collagen (which is well preserved in some bones excavated at Denisova Cave), a comparison with Table 1 suggests that these tools were most likely used to scrape animal skin.

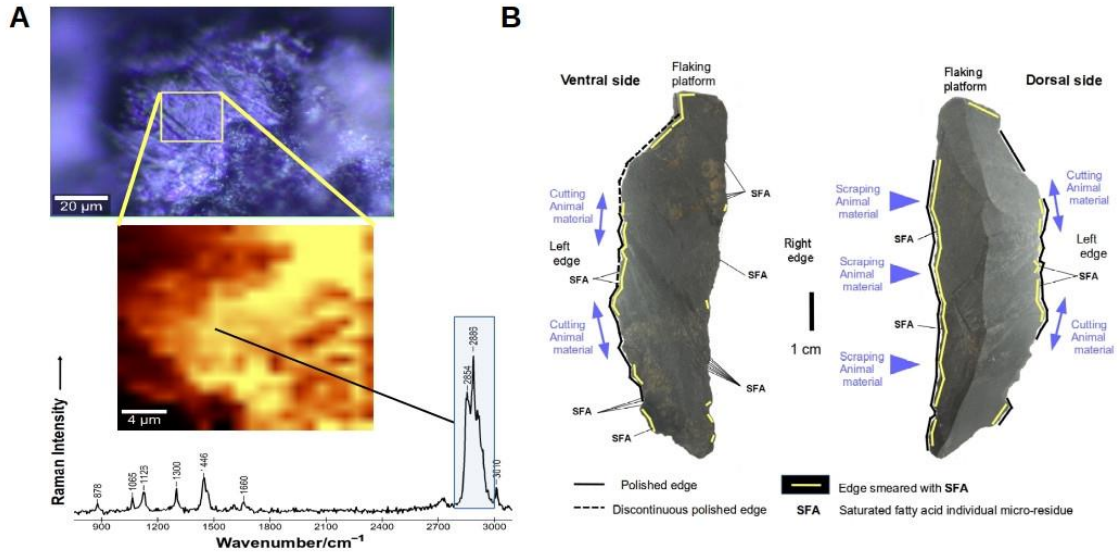

Figure 4. Map (using the strong C-H stretch band) of smeared fatty acid residue on a stone tool from Denisova Cave (A) and comparison of the distribution patterns of polish and saturated fatty acid residues on the same tool from Denisova Cave (B).

Residues most common for the second group of micro-residues are discrete fatty acids and proteins, protein/fatty acid mixtures and plant fibres. This group is typical for some artefacts from Liang Bua and the fatty acid residues commonly occur on polished areas, while the proteins are generally more randomly distributed. Fig. 5 shows a typical distribution pattern of saturated fatty acids and plant fibres on an artefact and representative spectra of plant/wood fibres. The main chemical components of plant fibres are cellulose (including hemicelluloses), moisture, lignin and pectins, which vary in abundance between species and growth conditions. Main cellulose bands occur at 1093 and 1121 cm^{-1} (C-O and O-C-O stretching modes) and the C-H deformation mode at 903 cm^{-1}.[14] Although Raman spectroscopy is not able to identify

specific plant species, the aryl ring stretching bands characteristic of lignin are useful to broadly distinguish between wood and plants richer in cellulose. For example, a higher intensity of the lignin aryl stretching vibration at 1597 cm^{-1} accompanied by a shoulder at 1654 cm^{-1} indicate that fibres probably originate from wood.[14] The presence of plant fibres and the absence of a strong presence of fatty acids and bone suggest that these artefacts were used to process plant material.

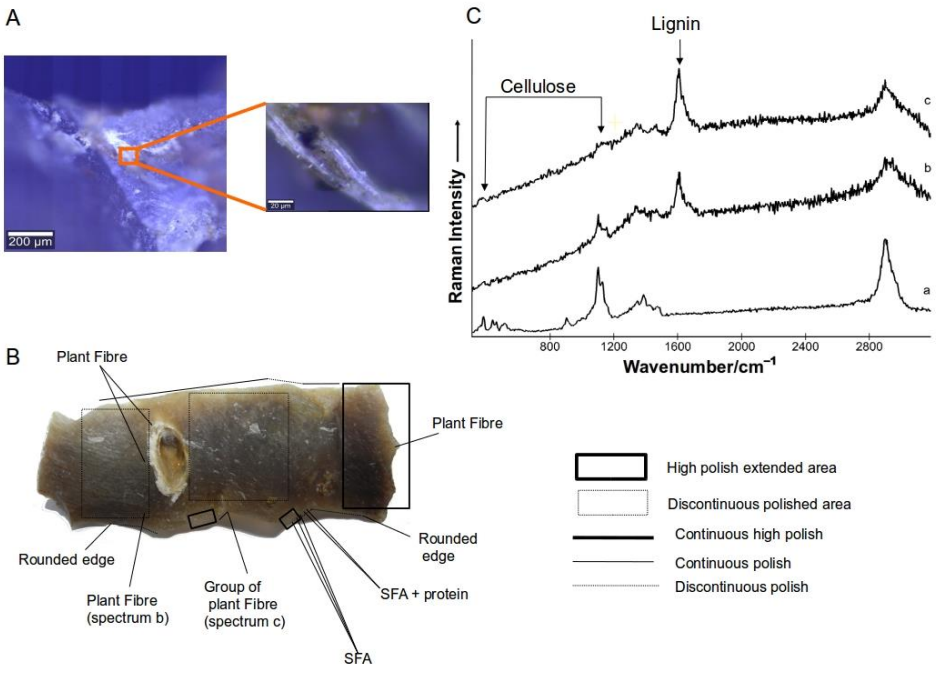

Figure 5: (A) Plant fibres detected on an artefact from Liang Bua, and (B) shown in relation to polished edges. (C) Raman spectrum of cellulose fibre suspected to be contamination due to high signal-to-noise ratio (a), and Raman spectra of other plant fibres analysed on stone tools from Liang Bua (b, c).

The third set of micro-residue types and distribution patterns is extremely rich in a variety of residues and an example can be seen in Figure 6, where polish distribution is also indicated. Small pieces of bone commonly occur and are sometimes smeared; discrete as well as smeared fatty acid residues are common and in some instances mixed with bone. Plant fibres occur in

clusters and concentrations of protein residues were also detected. The dorsal distal part of this stone tool has the most intense polished area and also the highest number of bone apatite and lipid micro-residues. Plant fibres that occur in clusters were identified, but appear outside the main working zone, so their presence might be incidental to the main use of the tool. Artefacts with this group of residues are also from Liang Bua and, comparing the residue suite to Table 1, were probably used for butchering.

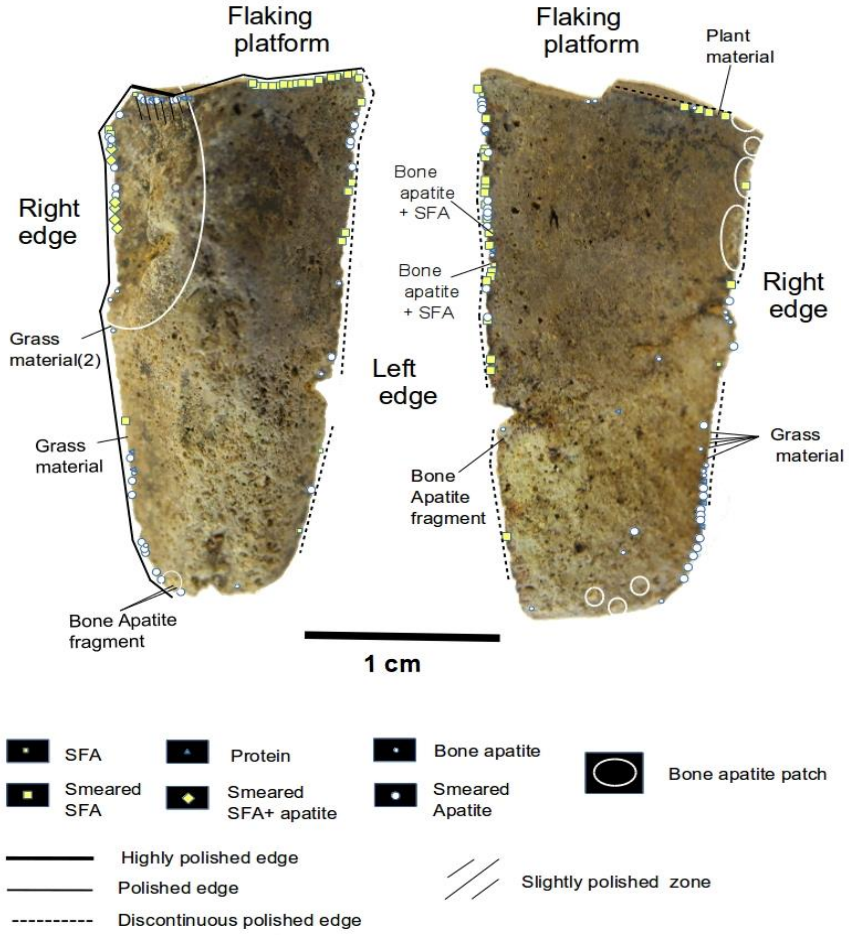

Figure 6. Example of the third set of residue types and distribution pattern in comparison with polish distribution.

Bone consists of an inorganic part that is chemically similar to carbonate apatite ($Ca_5(PO_4, CO_3)_3OH$) and an organic part consisting mostly of collagen. Over time, bone undergoes

taphonomic and diagenetic processes, influenced by environmental and burial conditions, which cause alteration of both the organic and inorganic components.[19] Collagen deterioration, microbiological alteration, bioapatite dissolution and recrystallisation, ion depletion or uptake and the precipitation of secondary mineral phases can occur and has an influence on the Raman spectrum.[20]

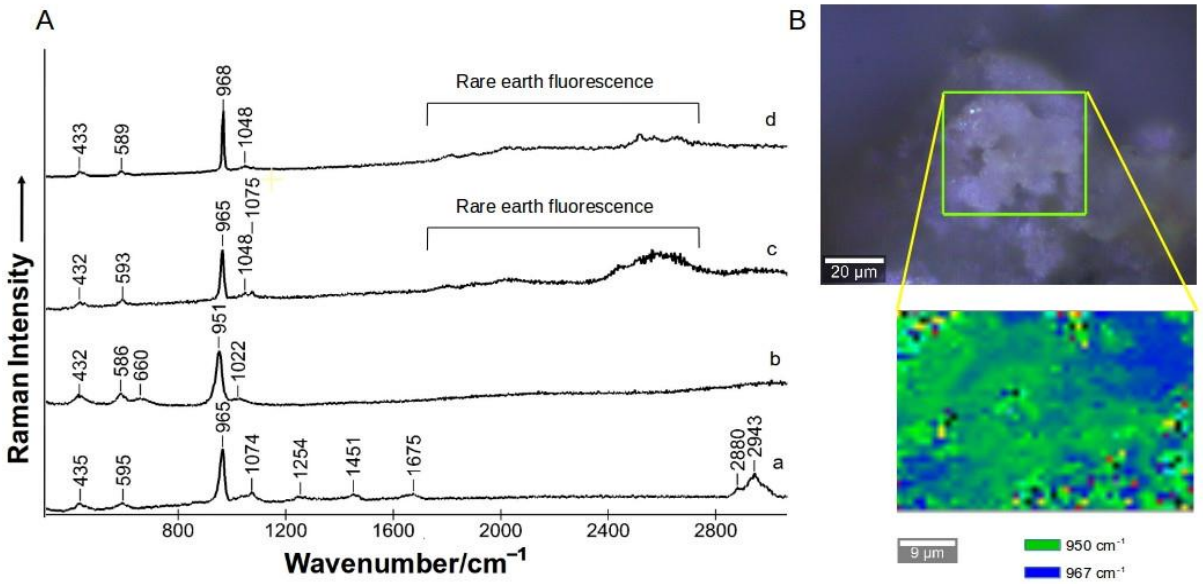

Figure 7: Raman spectra of bone recorded on a stone tool from Liang Bua (A, b-d) and Raman map of the position of the P-O stretch vibration on a small fragment of bone on the tool (B). Raman spectrum (A, a) is from a modern bone sample.

Depletion of collagen can be seen by the disappearance of the peaks at 1254, 1451, 1675, 2880 and 2943 cm^{-1} present in the spectrum of a modern bone sample (Fig. 7A, a) and absent in spectra recorded on a bone fragment on a tool from Liang Bua (Fig. 7A, b-d). The presence of rare earth elements can be identified in two of the spectra (c, d) by fluorescence bands. A closer look at Raman spectra recorded on the bone residue shown in Fig. 7B shows the presence two different phases of phosphate. In one part of the fragment, the symmetric stretch vibration of phosphate occurs at 950 cm^{-1} (FWHM 25-35 cm^{-1}) and in other parts at 967 cm^{-1} (FWHM 15

cm^{-1}). The appearance of both types of phosphate signals on a small bone fragment firmly attached to the stone tool clearly illustrates the coexistence of ongoing degradation or recrystallisation processes of bone.

In classifying the residues as originating from tool use, we have followed very stringent guidelines, as set by researchers using optical microscopy to identify residues; namely micro-residue abundance and meaningful distributions.[21-22] As all the artefacts were washed by 10 s sonication and the residues left on the artefacts are strongly attached, there is a high probability that many of the micro-residues that we rejected as possible contamination might in fact be archaeologically meaningful. On many artefacts we did not detect any significant numbers of micro-residues, but not all stone artefacts excavated at archaeological sites have actually been used as tools and might, instead, be stones produced during the process of lithic reduction and include shatter and production debris, and production rejects.

X.5 Conclusions

The most common residues identified on stone tools from the three different groups are quite distinct. This can be attributed to different degrees of preservation due to climatic differences, but might possibly also be linked to distinct behaviours between human species. Precise archaeological interpretation is not possible at this stage, due to a small sample size, but the results of the present study have laid an important foundation for further work on the identification and interpretation of micro-residues. Comparison with GC-MS results on the same set of artefacts and a study on bone degradation at both sites (currently being conducted) will also contribute to a better understanding of the nature of the micro-residues on the artefacts.

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