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# Raman spectroscopy of lipid micro-residues on Middle Palaeolithic stone tools from Denisova Cave, Siberia

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ABSTRACT

Raman spectroscopy is a powerful method for detecting micro-residues on stone tools. To further develop techniques for determining stone tool function, we devised a methodology using Raman microscopy to analyse in situ micro-residues before conventional usewear study. We analysed 18 stone artefacts collected in situ from Denisova Cave in Siberia, where excellent organic residue preservation is expected. We report here details of saturated and unsaturated fatty acids identified on eight stone tools from the Middle Palaeolithic levels. The spatial distribution of smeared fatty acids shows strong correlation with spatial distributions of usewear (particularly use-polish, but also striations, edge rounding and scarring) on each tool, demonstrating that these micro-residues are likely associated with prehistoric tool contact with animal tissue. We compared Raman spectra and the types, abundance and distribution of micro-residues on the Denisova Cave artefacts with those on modern experimental stone tools (with known function). The results provide further support for Middle Palaeolithic processing of animal tissue and probable skin scraping at Denisova Cave.

## 1. Introduction

As underscored by previous analysts, the visual characterisation of micro-residues using optical microscopes is challenging when the residues lack distinct shapes or structures (Langejans, 2012; Monnier et al., 2012, 2017a,b; Wadley and Lombard, 2007). In these conditions, micro-residues resulting from stone tool use also pose challenges because they are more difficult to distinguish from modern contaminants, mineral background or from the effects of post-depositional processes (Langejans, 2010).

Spectroscopic analyses have been applied previously to characterise visible traces of glue and adhesive compounds, after macroscopic or low-magnification observations (e.g., Cârciumaru et al., 2012; Vahur et al., 2011). Similarly, microscopic usewear studies have been complemented by subsequent application of spectroscopic techniques (e.g., infrared and Raman spectroscopy) to residues potentially linked with utilised tool edges (e.g., Cesaro and Lemorini, 2012; Hogberg et al., 2009). Other studies have applied Raman or infrared spectroscopy at a later stage of functional analysis, to confirm origins of organic residues that were previously identified as distinct structures by optical microscopy (Monnier et al., 2013, 2017a; b). To confirm that micro-residues are related to prehistoric tool use and not the outcome of another agency (such as contamination from handling or sediments), it is important to assess multiple lines of evidence (e.g., Lombard and Wadley, 2009), including micro-residue abundance and meaningful distributions (Rots et al., 2016). For example, micro-residues that are distributed widely on artefact surfaces may potentially be a consequence of contact with sediment (or various taphonomic processes) rather than tool use, which typically constrains impacted residues close to used tool edges.

Previous studies have shown that it is usually appropriate to record and document residues before undertaking detailed usewear analysis, which often requires cleaning of tools to observe wear on tool surfaces (Keeley, 1980). In situ, non-destructive study of residues should be undertaken before residues are removed for chemical and other testing. A common first step in study of tool residues is optical microscopy to identify tools and residues that may then be subjected to further testing. In this study, prehistoric stone tools were not first selected on the basis of optical microscopy or any macro-residues. Our study aimed to evaluate Raman microscopy (Raman spectroscopy with optical

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microscope capabilities) as a non-destructive method of in situ residue analysis independent of microwear analysis—'independent' in the sense that Raman microscopy is undertaken first and, thus, tools are not subject to residue alteration, removal or contamination by prior analysis. This independence, however, poses particular challenges when initial functional interpretations are not available to limit the areas of observation, or to suggest potential utilised materials, modes of use and utilised edges. First, the analyst is not assisted by prior optical microscopy cues or related techniques (e.g., chemical staining) to infer the nature or origin of residues. Second, to be viable and effective, the analytical technique must be rapid enough to efficiently scan and probe hundreds of micro-residues on stone surfaces and edges. The analytical technique should have the capacity for rapid chemical analysis (within a few seconds) to provide adequate sample coverage of tools, commonly with surface areas on the order of ~10–50 $cm^2$. Relocating the same tool residue under different instruments would be very time consuming, so the use of a single instrument for optical microscopy and chemical analysis is highly advantageous.

We argue that Raman microscopy is a viable 'independent' methodology for initially locating and analysing in situ micro-residues on prehistoric stone artefacts. Recently, we confirmed that Raman microscopy has particular advantages for the early examination of stone artefacts after initial screening with conventional usewear/residue observations (Bordes et al., 2017). Bordes et al. (2017) showed that Raman microscopy of unwashed artefacts can identify archaeologically significant organic traces with minimal artefact handling (to reduce the chances of contamination) and with minimal cleaning (< 10 s ultrasonication in water); the latter removed loosely adhering sediment without dislodging residues related to tool use.

To evaluate Raman microscopy as the first step of in situ residue observation (i.e., prior to conventional usewear/residue observations), we selected in situ artefacts that had been carefully collected from the Denisova Cave deposits for residue analysis. This site was chosen for study because it has excellent conditions for organic preservation over many tens of millennia, as shown by the survival of ancient DNA and collagen peptides in the skeletal remains of archaic hominins (Brown et al., 2016; Meyer et al., 2012; Prüfer et al., 2014; Reich et al., 2010) and in the cave sediments (Slon et al., 2017). To assist in interpreting our results, modern experimental tools were also analysed with Raman microscopy.

## 2. Denisova Cave and artefact selection

Denisova Cave is located on the northwest slopes of the Altai Mountains in southern Siberia. It provides an excellent geographic environment for evaluating our methodology, given the cold, relatively stable conditions in the buried cave deposits and an archaeological record that extends as far back as the late Middle Pleistocene (Derevianko et al., 2003, 2005; Slon et al., 2017). Archaic hominins (Denisovans and Neanderthals) and modern humans have occupied the cave at various times.

The 18 stone tools reported here were collected in 2014 from Layers 15 to 11.4 in the East Chamber of the cave. These layers have yielded Middle Palaeolithic lithic artefacts, as well as the remains of Denisovans and Neanderthals (Brown et al., 2016; Meyer et al., 2012; Prüfer et al., 2014; Reich et al., 2010; Slon et al., 2017). The stones were removed, with minimal handling, from the section walls and stored in plastic bags, which were left open to air dry before being sealed for transport to the University of Wollongong for analysis. Sediment samples were collected for each stone, and included: (1) sediment in contact with the stone surface ('inner sediment sample') and (2) sediment up to 3 cm distant from the stone surface ('outer sediment sample').

Our objective was to assess how these stone tools were used and what plants and animals they were used to process. Preliminary studies of Pleistocene mammal remains in the East Chamber (Vasiliev et al., 2008, 2010, 2013, 2017) indicate the dominance of forest taxa (especially roe deer and Siberian red deer) in Layers 15 and 14, followed by a progressive increase in the proportion of steppe taxa in the overlying Late Pleistocene deposits. These environmental changes may have influenced hominin subsistence strategies and, hence, tool function.

## 3. Methods

### 3.1. Raman and optical microscopy

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 (20× and 50× magnifications) were used, achieving a sub-micron resolution. The samples were placed on a piezo-driven, feedback-controlled scanning stage.

To avoid contamination, artefacts were handled with nitrile gloves (latex, powder- and protein-free), and placed on a support fashioned by Blu-Tack® (a synthetic rubber compound) to accommodate its shape. This enabled the positioning of each sample under the Raman microscope with the incident light (laser) normal (i.e., perpendicular) to the point of analysis. The support was covered with a piece of nitrile glove to prevent contamination from the Blu-Tack®. Samples were stored in clean bags and boxes before and after analysis.

Optical microscopes included an Olympus stereozoom SZ61 with external fibre optic light source, and an Olympus BX51 metallographic microscope with vertical incident illumination and 5×, 10×, 20× and 50× objectives.

### 3.2. Analytical steps

Artefacts were analysed in six steps (analyst(s) indicated below in parentheses), with extra Step 1a for the first five artefacts examined (details in Table 1):

(1) Catalogue unwashed artefacts (E.H. and R.F.).
  (1a) Optical microscopy of microwear and residues on DC2, DC12, DC22, DC42 and DC52 (E.H. and R.F.). Following study of these initial five artefacts, Step 1a was eliminated to reduce the total analysis time, render the Raman microscopy independent of prior optical study, and reduce the chance of modern contamination.
(2) Raman microscopy of residues on artefacts prior to cleaning (L.B.).
(3) Ultrasonication of artefacts (L.B.).
(4) Raman microscopy of residues and sediments (L.B.).
(5) Optical microscopy and mapping of use-polish (L.B.).
(6) Optical microscopy of usewear and residues (R.F. and E.H.).

### 3.3. Experimental tools

Experimental stone-tool residues of more recent age were analysed to aid in the interpretation of residues on the Denisova Cave artefacts. The experimental tool residues included two sets, each of a different age: tools used ~30 years earlier (Set 1; Fullagar, 1986) and tools used up to ~9 months earlier (Set 2). See Table 2 for details.

Set 1 included residues on four tools used to process animal tissue: (1) hornfels X290, used for scraping fresh skin (possum, *Trichosurus vulpecula*) for 5 min; (2) flint X284, used for sawing dry bone (cow, *Bos taurus*) for 20 min; (3) flint X288, used for cutting fresh meat (cow) for 20 min; and (4) flint X309, used for sawing fresh bone (cow) for 45 min. Residues on these four experimental tools were investigated specifically to study lipid preservation and characterise their Raman spectra after ~30 years of storage in stable conditions (details in Table 2).

Set 2 included residues on nine tools made of stones of similar rock