

# LITHIC RESIDUE ANALYSIS AT STAR CARR

Volume 1 of 2

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# ABSTRACT

Ancient trace residues left on stone artefacts by people represent a source of potentially fruitful data about diet, technology, and behaviour, but their investigation is not problem-free. Rather, correct identification of degraded residues and determination of their natural or anthropogenic origin remains at the heart of current methodological development in lithic residue analysis. This thesis addresses these issues by examining: 1) 13 modern reference residues on flint flakes, 2) modern residues on 78 experimentally buried flint flakes at Star Carr and off-site, and 3) residue traces on 138 archaeological stone artefacts from Star Carr. The study of modern reference residues showed that only residue types bearing diagnostic structures can be confidently identified by visual analysis alone. The study of experimentally buried flakes showed that tree resin, softwood tissue, and red ochre preserved after both one month and 11 months burial periods and across three burial environments, and were the most likely candidates to be encountered archaeologically. When the archaeological material was examined using reflected visible light microscopy (VLM), hypotheses of residue origin based on visual observations were tested against chemical information collected from the residues. Importantly, the microscopic hypotheses of residue identity based on comparison with reference residues and published literature were, in nearly all cases, falsified by confocal Raman microspectroscopy (micro-Raman) and gas chromatography-mass spectrometry (GC-MS). Key identifications were: iron (III) oxide, gypsum, quartz, pyrite, and organics. Some residue samples also contained compounds consistent with pine tree resin, but this finding is considered preliminary. These results from stone artefacts highlight the need in lithic residue analysis for: 1) more careful consideration of chemical processes in the burial environment, and 2) further incorporation of appropriate scientific techniques to verify microscopic residue identifications.

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Figure 8.18. Red-orange amorphous residue deposit (location 1) on flake tool 94362. Deposit imaged with SEM before and after confocal Micro-Raman. Micro-Raman is destructive, but spot size is very small. Image on right shows the damage to the residue due to penetration of the Raman laser.

Reflected light micrographs of what is reported to be Middle Stone Age resin (a,b,d) with vegetal imprint in the resin (c) on a Levallois end product piece (ME93/373) from Sodmein Cave, Northeast Africa. These deposits are essentially identical in morphology with the bounded amorphous and plant tissue cast deposits identified as iron (III) oxide in this study. From Rots et al. 2011 Fig. 18a-d. Image for examination purposes only, to be removed prior to publication.

Reflected light micrographs of what is reported to be Middle Stone Age animal tissue (a), blood (c), and resin (e) on a quartz tool from Sibudu Cave, South Africa. These deposits are similar in morphology with diffuse amorphous and bounded amorphous deposits identified as iron (III) oxide in this study. From Delagnes et al. 2006 Fig. 6a,c,e. Image for examination purposes only, to be removed prior to publication.

Reflected light micrographs of what is reported to be Middle Stone Age blood (a), and animal tissue (e) on points from Sibudu Cave, South Africa. These deposits are similar in morphology with bounded amorphous deposits identified as iron (III) oxide in this study. From Lombard and Wadley 2009 Fig. 4a,e. Image for examination purposes only, to be removed prior to publication.

Reflected light micrographs of what is reported to be resinous hafting tree gum (a), blood (c), and animal tissue (h) on a quartz segment from Sibudu Cave, South Africa. These deposits are similar in morphology with the bounded and diffuse amorphous deposits identified as iron (III) oxide in this study. From Lombard and Phillipson 2010 Fig. 6a,c,h. Image for examination purposes only, to be removed prior to publication.

## **CHAPTER 9 RESULTS: COLOURLESS CRYSTALS**

Figure 9.1. Lath and rosette crystals found together on blade fragment 108229.

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Figure 9.3. Lath crystals near an edge on flake tool 94362, SEM.

Figure 9.4. Example of fine needle shape, on blade tool 98086. Rainbow needle/lath shaped crystals were also seen as rosettes, along the left distal edge, at cortex transition line.

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Figure 9.9. Originally interpreted as possible tracheids or vessel elements with bordered pits embedded in polish (most convincing instance seen), it is now believed these are actually gypsum in sheet formation. This example was found on core axe tool 99454, proximal end, right and left edges.

Figure 9.10. Fibrous sheet crystals in a parallel arrangement could be mistaken for vascular tissues from plants. This example on tool 99276 was originally interpreted as wood tracheids within a polish, but, no pores or bordered pits could be identified.

Figure 9.11. This example of fibrous sheet crystals is from tool 108237, showing shiny deposits with fibrous sheet crystals arranged in parallel alignment. Right image shows a close up of the fibrous sheet crystals from tool 108237, with lath and twinned swallowtail crystals also visible.

Figure 9.12. A ruffled edge crystalline formation found on bladelet tool 99276, likely a mineral deposit.

Figure 9.13. Rosette 1 and lath crystals forming a large deposit on the blank control buried in the wetland at Star Carr for 11 months.

Figure 9.14. Lath crystal interrogated with Micro-Raman on the blank control buried in the wetland at Star Carr for 11 months.

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Figure 9.17. Location of Raman laser and spectrum collection on a twinned swallowtail crystal on flake tool 99756.

Figure 9.18. Micro-Raman spectrum collected from a twinned swallowtail crystal on flake tool 99756.

Figure 9.19. It is curious that the gypsum crystals were sometimes found distributed along the edge of tools. Here, an example from retouched flake 98306 showing what appear to be lath gypsum crystals on the dorsal left proximal mid edge.

## **CHAPTER 10 RESULTS: SHINY DEPOSITS**

Figure 10.1. Example of wavy line tideline on blade tool 94066, ventral left mid edge. Approximate length of this tideline is 6600  $\mu\text{m}$  (6.6 mm).

Figure 10.2. Example of tideline on blade tool 94066, ventral left mid edge. Note pool-like edges.

Figure 10.3. Core preparation flake 85814 with dull lustre deposit (circled). This deposit is likely silicon dioxide, originating either as a precipitate of dissolved amorphous silica from the soil from or from dissolution of the flint (quartz) itself.

Figure 10.4. Left: Blade 109720 showing macroscopically visible deposit which appears greasy with a dull lustre. Right: Twinned swallowtail gypsum crystals within the dull lustre deposit.

Figure 10.5. This shiny deposit was found on chamfered fragment piece 109735. As with all lithics, the tool was washed with water and left to air dry on a cling film-lined tray. The deposit is located on the dorsal centre surface – this part of the tool was in contact and adhered to the cling film whilst drying and is considered a shiny deposit formed by curation procedures.

Figure 10.6. Shiny deposits related to the washing process on blade 99765. A) Fibrous sheet formation suggestive of gypsum. Steep edges of this shiny deposit suggests its origin is additive, not due to dissolution of the flint. B) A twinned swallowtail crystal embedded in the shiny deposit.

Figure 10.7. Examples of shiny deposits documented in relation to the wash process, containing different types of gypsum crystal shapes. All images show areas on blade 98855 which were in contact with cling film during drying. A) Shiny deposit, exhibiting 'islands' with raised edges, and containing twinned swallowtail gypsum crystals, dorsal left mid edge. B) Lath and rosette crystals embedded within shiny deposit, dorsal left mid edge. C) Rhombus crystal within shiny deposit, ventral right mid edge.

Figure 10.8. Shiny deposits with lines mirroring the folds present in the cling film drying surface. Blade 99765.

Figure 10.9. Blade 99516 and shiny deposits on the cling film. A) Blade 99516 and associated label drying on cling film after first wash with ultrapure water. B) Shiny deposit imprint left on cling film

matching the outline of the blade. C) Shiny deposit imprint left on cling film matching the outline of the plastic tag.

Figure 10.10. Shiny deposit on the cling film left after air drying blade 99516. Circled is the formation of a twinned swallowtail gypsum crystal.

Figure 10.11. Shiny deposit on blade tool 99516 observed after first wash. Deposit contains rhombus and twinned swallowtail gypsum crystals. The deposit was located ventral centre interior part of the blade, slightly distal.

Figure 10.12. Left: Macroscopically visible shiny deposit on blade 99516 after first wash. Right: After the second wash with water, the shiny deposit is still present, but appears to be fainter and less shiny. No gypsum crystals were found in the shiny deposit on the tool after the second wash.

Figure 10.13. The spot on blade 99765 where the spectrum in Figure X was collected. The spot was within a tideline deposit.

Figure 10.14. Raman spectrum of a tideline deposit from blade 99765, showing two polymorphs of silicon dioxide are present:  $\alpha$ -quartz and moganite.

Figure 10.15. Dull lustre deposits (circled) present on core 85009. Flint 85009 is a core piece of knapping waste with cortex that contains a dull lustre shiny deposit in the centre of the flint surface. This piece is unlikely to have been used.

## CHAPTER 11 RESULTS: WHITE ANGULAR CRYSTALS

Figure 11.1. Examples of opaque angular crystalline material. Left: white angular crystalline residue on the ventral right proximal edge of blade 93327. Right: crystalline residue found around a microchip on blade 93312, dorsal right mid edge. From microscopic examination, both traces were originally interpreted as possible tool attrition or bone residue.

Figure 11.2. Residue identified as possible bone or stone attrition from bladelet 98859.

Figure 11.3. Left: Non-bone deposit located within microchip scar on awl 109731, ventral right mid edge. Right: Spot shows the location investigated with the Micro-Raman laser, corresponding to the spectrum below.

Figure 11.4. Raman spectrum of a white crystalline deposit within microchip on awl tool 10973 (image above). The spectrum illustrates that this residue is not bone.

Figure 11.5. Bladelet 110656 with potential bone residue present on edge of tool, just right of dorsal tip. The red dot in the right image shows the location investigated with the Micro-Raman laser, corresponding to the spectrum below.

Figure 11.6. The spectrum illustrates that this white crystalline residue on bladelet 110656 (image above) is not bone, but possibly a mineral related to  $\alpha$ -quartz, based on the peak present at  $464\text{ cm}^{-1}$ .

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Figure 12.1. The shale beads from Star Carr (Museum of Archaeology and Anthropology, Cambridge, accession number: 1953.72). Image from Milner et al. (2016).

Figure 12.2. Left: The engraved side of the pendant. Right: The back of the pendant.

Figure 12.3. Finely engraved sub lines, branching from a long main line. SEM, secondary electron mode, 50 x.

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Figure 12.5. Micro-Raman spectra taken of brown deposit from within engraved line 11. Clear presence of organic material is indicated, likely peat. Image by Konstantinos Chatzipanagis.

Figure 12.6. Gold structures with triangular faces.

Figure 12.7. High density of gold granular spherical crystals located within the nick mark on the non-engraved side of the pendant.

Figure 12.8. Gold angular pyrite from the reference collection.

Figure 12.9. Close up of a pyrite framboid with cubo-octahedral microcrystals. SEM, backscattered electron mode.

Figure 12.10. Micro-Raman spectrum collected from the red spot on the framboidal structure. Image by Konstantinos Chatzipanagis.

Figure 12.11. Fragmentary microfaunal remains, likely part of a copepod. Location 1, line 1.

Figure 12.12. Fragmentary microfaunal remains, likely part of a copepod. Location 1, Line 1. SEM, secondary electron mode.

Figure 12.13. Unidentified biological structure, likely microfaunal remains. Loc 17, Line 1.

Figure 12.14. Perhaps a chitinous insect structure. Loc 32, Line 26.

Figure 12.15. Loc 9. Biological structure, possibly a diatom. Micro-Raman analysis has showed the structure is carbon-rich, and thus likely organic. Loc 9, Line 11.

Figure 12.16. Raman spectrum collected on suspected microfaunal remain in one of three locations demonstrating that this is organic. Image by Konstantinos Chatzipanagis.

Figure 12.17. White crystals within the perforation of the pendant.

Figure 12.18. SEM image of the perforation and crystals within it (right), 200x magnification. Image by Andy Needham.

Figure 12.19. Micro-Raman spectrum collected from a crystal grain located within the perforation of the pendant. Image by Konstantinos Chatzipanagis.

Figure 12.20. Detail of the perforation of bead 110671. Designation of the 'front' face of the bead is based on the gradual widening of the hole and the presence of grooves.

Figure 12.21. Shale bead 110671 found in situ at Star Carr.

Figure 12.22. White line 2 within the shale on the front of the bead. This is part of the natural stone material.

Figure 12.23. White material within the natural shale in a circular formation on the back of the bead.

Figure 12.24. Bead 113830. Left image shows the front surface. Right image shows the back surface. Note groove visible on both sides of the bead.

Figure 12.25. Iron oxide deposit, location 1, front of bead 113830.

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Figure 12.27. Clark's backfill bead. Left: the front surface. Right: the back surface.

Figure 12.28. Comparison of lines on Clark's Backfill bead with lines on the pendant at the same magnification. Left: criss-cross striations on the back of Clark's backfill bead (location 10). Right: wide anthropogenic engraved line with brown sediment infilling (location 1).

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Figure 12.30. Gypsum rosette 1 and lath crystals on the back of Clark's backfill bead (location 12).

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Figure 13.1. Making birch bark tar with airtight vessels experimentally at Star Carr.

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Figure 13.4. Common juniper branch with only minuscule amounts of red resin exuding.

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Figure 13.6. Location of nine lithics which contained pine compounds in their residue extracts. Circles represent housing structures. Image by Becky Knight.

Figure 13.7. Example of a mass spectrum of possible diterpene fragment Unknown 1 from blade 108373.

Figure 13.8. Example of a mass spectrum of possible diterpene fragment Unknown 2 from blade 108373.

Figure 13.9. Black shiny deposit with bubbly smooth appearance, dorsal left mid edge of burin 108205.

Figure 13.10. Black shiny deposit with bubbly smooth appearance, dorsal left mid edge of burin 108205.

Figure 13.11. Some deposits still remain on burin 108205 after solvent extraction, ventral left mid edge.

Figure 13.12. Total gas chromatogram of the trimethylsilylated residue extract from burin 108205. Inset shows mass spectra of Dehydro-7-DHA, DHA, and 7-oxo-DHA, present in trace amounts.

Figure 13.13. Partial gas chromatogram of the trimethylsilylated sample extract from burin 108205, zoomed in to show altered markers of pine resin.

Figure 13.14. Black deposit on blade 108373, dorsal centre.

Figure 13.15. Microcharcoal on blade 108373.

Figure 13.16. Total gas chromatogram of the trimethylsilylated sample extract from blade 108373. Inset shows mass spectra of Dehydro-7-DHA, DHA, and 7-oxo-DHA, present in trace amounts.

Figure 13.17. Partial gas chromatogram of the trimethylsilylated sample extract from blade 108373, showing derivatives of abietic acid.

Figure 13.18. Left: black amorphous residue located on the ventral proximal mid edge of microlith 108397, prior to solvent extraction and GC-MS. Right: Black residue deposit after GC-MS. Most of the deposit was removed but some residue still remains.

Figure 13.19. Total gas chromatogram of the trimethylsilylated sample extract from microlith 108397. Inset shows mass spectra of Dehydro-7-DHA, DHA, and 7-oxo-DHA, present in trace amounts.

Figure 13.20. Partial gas chromatogram of the trimethylsilylated sample extract from microlith 108397.

Figure 13.21. Black granular deposits on the right mid edge on blade 109649.

Figure 13.22. Total gas chromatogram of the trimethylsilylated sample extract from blade 109649. Inset shows mass spectra of Dehydro-7-DHA, DHA, and 7-oxo-DHA, present in trace amounts.

Figure 13.23. Partial gas chromatogram of the trimethylsilylated sample extract from blade 109649.

Figure 13.24. Black deposits and microcharcoal on the right mid edge on bladelet 109691.

Figure 13.25. Total gas chromatogram of the trimethylsilylated sample extract from microlith 109691. Inset shows mass spectra of Dehydro-7-DHA, and DHA, present in trace amounts.

Figure 13.26. Partial gas chromatogram of the trimethylsilylated sample extract from microlith 109691.

Figure 13.27. Black microcharcoal on microlith 109724, right distal edge.

Figure 13.28. Total gas chromatogram of the trimethylsilylated sample extract from microlith 109724. Inset shows mass spectra of Dehydro-7-DHA, and DHA, present in trace amounts.

Figure 13.29. Partial gas chromatogram of the trimethylsilylated sample extract from microlith 109724.

Figure 13.30. Black deposit and white crystalline material on bladelet 110657, ventral right distal edge.

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Figure 13.32. Partial gas chromatogram of the trimethylsilylated sample extract from bladelet 110657.

Figure 13.33. Large black shiny deposit on bladelet 111490, ventral proximal right edge.

Figure 13.34. Total gas chromatogram of the trimethylsilylated sample extract from bladelet 111490. Inset shows mass spectra of Dehydro-7-DHA, and DHA, present in trace amounts.

Figure 13.35. Partial gas chromatogram of the trimethylsilylated sample extract from bladelet 111490.

Figure 13.36. Black deposit showing outlines of plant cells on microlith 113623, ventral left edge.

Figure 13.37. Total gas chromatogram of the trimethylsilylated sample extract from microlith 113623. Inset shows mass spectra of Dehydro-7-DHA, and DHA, present in trace amounts.

Figure 13.38. Partial gas chromatogram of the trimethylsilylated sample extract from microlith 113623.

Figure 13.39. Trimethylsilylated total gas chromatogram of reference *Pinus sylvestris* resin, one year old. The sample was diluted by a factor of 20x.

Figure 13.40. Partial gas chromatogram of reference *Pinus sylvestris* resin, one year old. The sample was diluted by a factor of 20x. Two peaks are labelled in Figure 13. as possible diterpene fragments. The peak occurring at 19.688 min has ions of 257, 73, 75, 91, similar to unknown 2 (but occurring at a different retention time), and the peak at 20.057 min has ions of 257, 91, 93, 79. The closest match to the peak marked as 'related to isopimaric acid?' is isopimaric acid TMS, with ions of 73, 255, and 241.

Figure 13.41. Example of a sediment sample total gas chromatogram collected underneath tool 108373 (context 337). Note Dehydro-7-DHA, DHA, and 7-oxo-DHA are not present.

Figure 13.42. Partial gas chromatogram of the trimethylsilylated sample extract from sediment sample 10873, zoomed in from the previous image to show Dehydro-7-DHA, DHA, and 7-oxo-DHA are not present.

Figure 13.43. Partial gas chromatogram of the trimethylsilylated method blank.

Figure 13.44. Proposed degradation of abietic acid into derivative compounds by oxidation and isomerisation. Compounds identified in this study are circled. Degree of oxidation increases moving down the flow chart. Based on Proefke and Rinehart (1992), Pastorova et al. (1997), van den Berg et al. (1998; 2000), van den Berg (2003), Modugno and Ribechini (2009), and Lattuati-Derieux et al. (2014).

Example of a pollen percentage diagram of 'Star Carr Clark site' monolith (lake-edge), showing *Pinus sylvestris* pollen throughout the sequence. From Dark 2017 Fig. 2. Image for examination purposes only, to be removed prior to publication.

Example of pollen and spore concentrations from 'M1' monolith (lake-edge), showing *Pinus sylvestris* pollen throughout the sequence. From Dark 1998a Fig. 11.3. Image for examination purposes only, to be removed prior to publication.

Figure 13.45. Scots pine tree (*Pinus sylvestris*) in May releasing large quantities of pollen from the male flowers in York, UK.

## **CHAPTER 14 DISCUSSION AND CONCLUSION**

No figures

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# DECLARATION

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as references. All photographs and images are by the author unless noted otherwise.

In cases where group publications have arisen from work this thesis, or work in the thesis contributed to a publication, only the contribution in which I was directly involved is presented and the corresponding publication is cited.

## Publications related to the thesis

Milner, N., Bamforth, M., Beale, G., Carty, J.C., Chatzipanagis, K., Croft, S., Conneller, C., Elliott, B., Fitton, L.C., Knight, B., Kroger, R., Little, A., Needham, A., Robson, H.K., Rowley, C.C.A., Taylor, B., 2016. A Unique Engraved Shale Pendant from the Site of Star Carr: the oldest Mesolithic art in Britain. *Internet Archaeology* 40. doi:10.11141/ia.40.8

Croft, S., Monnier, G., Radini, A., Little, A., Milner, N., 2016. Lithic residue survival and characterisation at Star Carr: a burial experiment. *Internet Archaeology* 42. doi:10.11141/ia.42.5

Needham, A.P., Croft, S., Kröger, R., Robson, H.K., Rowley, C.C.A., Taylor, B., Gray Jones, A., Conneller, C., in press. The application of micro-Raman for the analysis of ochre artefacts from palaeo-lake Flixton.

# PART 1 INTRODUCTION AND METHODS

# CHAPTER 1 INTRODUCTION

## 1.1 Aim, research questions, and objectives

The aim of this study is to accurately identify and interpret residues on stone tools from the site of Star Carr using microscopic and chemical characterisation techniques. This aim led to three research questions:

1. Is there potential for residues to survive on lithics at Star Carr?
2. Which residues can be identified on stone tools from Star Carr and what do they tell us about the lives of Early Mesolithic hunter-gatherers?
3. What techniques are most useful for the discipline of lithic residue analysis?

The key objectives which are addressed in the thesis are:

- 1) To critically review methods of lithic residue analysis in general (Chapter 2), and also review their specific application to Mesolithic material (Chapter 3).
- 2) To test the potential of residue survival experimentally at Star Carr by burial of lithic residues (Chapters 5 and 6).
- 3) To determine which residue types can be identified by visible light microscopy, and which types require chemical characterisation (Chapter 6).
- 4) To identify residues on a sample of flint tools from Star Carr (Chapters 8-13).
- 5) To discuss the methodological implications of the findings (Chapter 14).

My approach to the study of lithic residues has been data-led. Preconceived notions about the residue types that should be found on stone tools from Star Carr, and what

this would tell us about Mesolithic people in Britain, were not imposed. Instead, the approach was to let the evidence speak for itself and follow the data that emerged as the project progressed. Thus, this thesis focuses on direct observations of the lithic material being studied, the techniques used, and their veracity.

This PhD was undertaken as part of the POSTGLACIAL Project, funded by the European Research Council and led by Nicky Milner (PI). The project focused around excavations at Flixton Island and Star Carr and the extraction of palaeoclimate and palaeoenvironmental data in order to understand human behaviour in the context of climate change from 10,000-8000 cal. BC. The PhD directly addressed the third objective of the POSTGLACIAL Project: to set a new benchmark for the high-resolution analysis of archaeological deposits at Star Carr by developing an integrated ‘forensic’ approach to the analysis of the artefactual and molecular debris left by human activity.

More widely, the residue analysis study presented here contributes to strategic theme 3: Scientific Methods, set out in the Historic England Mesolithic Research and Conservation Framework (Blinkhorn and Milner, 2013, p. 33), and in particular S3:12 which states that “Forensic approaches to tool use, such as use-wear analysis and residue analysis, are being used more widely, particularly in other parts of Europe, to help discern the cultural biographies of stone and organic tools. Further applications in Britain are needed in order to understand how tools were used.”

## 1.2 A background to residue analysis

### 1.2.1 What is residue analysis?

Archaeological residue analysis is the study of microscopic and chemical deposits found on artefacts. These traces may or may not have been caused by human interaction with the object (Grace, 1996). Residues can be divided into two categories: 1) residues that can be physically seen and identified with microscopic techniques (sometimes referred to as microfossils), and 2) residues which cannot be seen, such as biomolecules or portions thereof, including lipids, proteins, and DNA. Microscopic trace residues come in many forms and can be recovered from a variety of artefact substrates. Microscopically detectable residues encompass but are not limited to: starch grains, phytoliths, calcium oxalate crystals, pollen, plant cell tissues, resin or tar, fungal and pteridophyte spores, bone flakes, fatty deposits, hair, feathers, and minerals such as red ochre and kaolin.

Residue analysis is a technical subdiscipline of archaeological material culture analysis, chiefly concerned with identifying the function of artefacts. Early in its development, residue analysis separated from usewear analysis as its own entity and both usewear and residue analysis provide an approach to investigate the manufacture and use-life of artefacts. However, usewear and the study of polishes at high magnification, also known as microwear, are concerned with deciphering traces of wear as a result of physical friction, whereas residue analysis seeks to identify additive deposits on artefact surfaces.

Residue analysis can be carried out on a range of artefactual contexts such as pottery, wood tools, ground stone tools, antler tools, calculus from human teeth, and coprolites. This thesis focuses on the study of microscopic and chemical residues found on stone tools.

The ideal goal of lithic residue analysis is to arrive at a detailed account of the use of objects by people in the past, and/or reconstruct the technological choices made during manufacture activities. This goal is complicated by three issues in residue analysis, as outlined by Grace (1996), Haslam (2006), and Langejans (2009):

1) Non-use related residues might contaminate artefacts.

The presence of contaminants on artefacts that originate from the burial environment or from various modern sources after excavation is probably the biggest concern facing residue analysts.

2) Researchers are still unable to describe the mechanisms of residue preservation.

The chemical mechanisms that degrade residues as related to specific burial environments are not well-understood, and this means analysts may be unaware of processes that are affecting the study assemblage. Experimental burial of residues in a variety of well-controlled laboratory burial conditions (pH, water flow, temperature, organic content in soil, etc.) could provide some much-needed insight into the process of residue degradation.

3) Residues on a tool could be a palimpsest of events and it is impossible to distinguish these different events.

Residues of the same or different type are not necessarily related in time, and the tasks to which artefacts are put can vary throughout the course of the objects use.

A major issue that can be added to this list of challenges in residue analysis is:

4) Residues may be identified incorrectly.

The ability to produce secure and reliable residue identifications is of great importance and a foundational methodological issue within the discipline of lithic residue analysis. Recent research by Monnier et al. (2012), Croft et al. (2016), Pedergrana and Blasco (2016), and Pedergrana and Ollé (2017), have highlighted the issue of identification accuracy. This issue is particularly pertinent to visually nondiagnostic residues that are amorphous.

The formation of residues and usewear on stone tools is affected by many factors. Variables influencing the formation of residues (and usewear) on stone tools include: raw material type (hardness, porosity, inclusions), chemical structure (adhesion properties, crystal lattice system), raw material mass, surface area, working load, moisture content in both the material being worked and the substrate, duration of working (time), type of motion (sawing, scraping, boring), direction and angle(s) of working, prehensile pressure or grip. The chemistry and physical movement of the depositional environment also may impart residues and usewear traces on lithics. Environmental factors such as rain, humidity, temperature, UV exposure, pH, oxygen availability, microbial and fungal action, operate both at time of use and post-depositional taphonomy. This list is probably incomplete but begins to indicate the many natural and cultural origins that can lead to, and also alter, the deposition of any given residue. Despite these issues and considerations, lithic residue studies have meaningfully contributed to existing debates about past tool use, technology, and culture.

## 1.2.2 Theory and residue analysis

### 1.2.2.1 Introduction

Residue analysis is theoretically impoverished because it is still grappling with issues of residue identification, reliability, and reproducibility. However, residue analysis has seen some convergent themes in terms of approach, and new concerns have introduced foci for methodological development. Here, theoretical topics foundational to the operation of residue analysis are considered.

### 1.2.2.2 Analogy

In many studies, the process of archaeological residue identification begins with microscopic observation and the search for any patterns in their appearance. This is followed by documentation through notes, drawings, and micrographs of the residues. The observations of the residues are then compared with collections of modern reference residues and/or residue descriptions in the literature and a determination is made. Thus, the interpretation of archaeological lithic residues has relied heavily on analogical reasoning to draw comparisons with modern reference residues to make identifications.

Analogy is an important tool in archaeology, and has been used both for explanation and also as a means of generating hypotheses for testing. The use of analogy to investigate archaeological problems has been debated by Wylie (1985, 1982). Wylie (1985, p. 64) notes that many critics of the use of analogy, have argued that “the use of analogical inference in archaeological research should be strictly limited; analogy should serve only as a means of generating hypotheses whose credibility must be established on independent, non analogical grounds.” Scientific archaeologists have leveled this critique, which is consistent with the emphasis of measuring and testing observable phenomena within the scientific method. However, Wylie (1985, p. 96) rightly points out that archaeology as a discipline is inherently and inevitably analogical, since “...past cultural systems may be different enough from those we know in the present that they cannot be considered part of the same domain...”, and, perhaps most importantly, that archaeological reconstruction necessarily relates the properties we observe from the past to causal forces or human actions.

While I accept that archaeology inherently relies on analogy as an overarching principle, this study shows it is inappropriate as the singular basis of interpretation for lithic residues. The use of analogy on its own to compare reference and archaeological residues, in all cases tested, was inadequate to yield reliable conclusions. This stance agrees with Murray and Walker (1988), who believe that analogies are inescapable in archaeology but should be chosen with care.

### 1.2.2.3 Uniformitarianism

The theory of uniformitarianism was originally proposed to the Royal Society of Edinburgh in *Concerning the System of the Earth, its Duration, and Stability* (1785) by James Hutton as a way to understand geological history. This theory of uniformitarianism was later supported by observational data of the natural world collected by Charles Lyell, published between 1830 to 1833 in three volumes called *Principles of Geology* (1830–1833). It held that geological processes such as the formation and erosion of mountains and sediments, could be explained by invariance of natural laws through time and small gradual changes. This geological concept of uniformitarianism was foundational to Charles Darwin propelling the study of evolutionary biology forward with *On the Origin of Species by Means of Natural Selection* (1859). As relevant to this study, uniformitarianism is the assumption that the natural processes that occurred in the past operate in essentially the same ways today. As Gould (1965) points out, the belief in the invariance of natural laws which characterises uniformitarianism is also the underpinning of all sciences. Bailey (1983, p. 174) defines uniformitarianism as the “...belief in universal principles which apply irrespective of time and place.” It appears that the concept of uniformitarianism has been used in two ways in archaeology: 1) as a concept to understand how geological and biological forces shape the archaeological record (Trigger, 2006, p. 29), and 2) as a concept to draw associative uniformities between ethnographic and archaeological data to explain ancient human behaviours (Cameron, 1993). A prime example of the use of uniformitarianism in the second sense is the ethnoarchaeological work of Binford with the Nunamiut in Alaska (1980), whereby the patterns of faunal bone discard, hearth and living feature arrangements observed in a modern population were used as a reference point to understand the archaeological sites of hunter-gatherers. Here, the use of the concept of uniformitarianism is applied in the former sense, as used in geology and physics. Uniformitarianism is a foundational concept to taphonomic studies of lithic residues, such as the burial experiment presented here (Chapters 4 and 5), and also

taphonomy more generally in archaeology and paleontology. Residue degradation factors such as the movement of water, pH level, and the physical and chemical interactions of the residues with scavenging animals, insects, fungi, and a host of microorganisms, are assumed to act in modern times as they did many thousands of years ago in the Mesolithic.

#### 1.2.2.4 Experimental archaeology

Experiments used in archaeology became popular in the positivist climate of the 1960s to 1980s (Bell, 2009, p. 33). Experiments are used to better understand cultural processes and also the formation/alteration processes that affect the archaeological record. Understanding the decay of artefacts and buildings in the burial environment is particularly important for management of buried heritage and its long-term preservation (Bell, 2009, p. 35). Traditionally, residue analysis has had a close link with experimental archaeology and analogy has been the underlying explanative mechanism, whether made explicit or not. In turn, many experimental archaeology programmes have been inspired by ethnoarchaeology, and ethnoarchaeology has also been used in its own right to collect and study the traces left on stone tools (González-Urquijo et al., 2015, p. 28). Indeed, the use of replica stone tools on plants and animals, intended as proxies, has been common practice to assess archaeological trace evidence (van Gijn, 2010). Some of these lithic residue experiments lack sufficient control and thus the results are of limited use in their ability to be predictive or applied to other situations. More tightly controlled hypothesis-driven experiments have a useful place in archaeological practice, as long as they are empirical and not simply 'xeroxing' previous research and buttressing pre-understandings (Bell, 2015). Experiments specific to lithic residue diagenesis are reviewed in Chapter 4.

### 1.2.2.5 Multiple lines of evidence to move beyond the subjective method

Wylie (2000, 1989), and Killick (2015a, p. 243) have argued that multiple lines of evidence, and specifically independent lines of evidence, used in archaeology are able to yield the strongest interpretations. Wylie (1989) likens independent lines of evidence to strands of cable which when woven together are mutually reinforcing. Choosing methods and data sets that are independent of each other is a profitable methodology for the interrogation of lithic residues, and the validity of the resulting archaeological interpretations is increased. For instance, multi-analytical lithic residue studies that take identification further by using chemical characterisation techniques, in combination with microscopic observations, have offered the most convincing cases of prehistoric tool use.

In their assessment of FTIRM for the identification of animal residues on lithics, Monnier et al. (2017b) believe that it is unlikely that just a single technique will be sufficient for residue analysis and that use of a combination of complementary methods are preferable. They argue that multiple techniques are especially important during the current developmental phase of lithic residue analysis. Likewise, Veall and Matheson (2014, p. 20), also stressed a multi-analytical strategy helps avoid errors in interpretation of residues and provides the most robust approach. The use of multiple lines of evidence has been a long-standing recommendation in archaeology as a way to cross-check individual methods and indeed this seems to be an important emerging strategy relevant to the field of lithic residue analysis.

Multiple lines of evidence are allowing residue analysts to challenge and improve upon subjective methods that produce results that differ from analyst to analyst. The use of a subjective method does not allow us to deal with the problem of equifinality – that several different processes can lead to the same observed phenomenon. This is an apparent lesson in this study. Most often, the hypotheses generated from the

subjective microscopic observations were proved incorrect by the application of chemical characterisation techniques.

#### 1.2.2.6 Scale of analysis

Archaeological studies are carried out at various scales of analysis such as local, landscape, regional, national, and international levels. Haslam (2006a, p. 406) discussed the scale of residue analysis in relation to interpretive power, raising the issue “...of the theoretical appropriateness of asking broad questions of micro-scale techniques”. In doing so, he appears to be cautioning that the questions asked of the lithic residue analysis should be of a scale suited to the data that will be gathered. Indeed this is warranted since there is a natural disconnect between the detailed micro focus of residue analysis conducted on a (usually small) selection of tools and the larger archaeological context. Therefore, residue analysis is inherently ill-equipped to address ‘big questions’ in archaeology, but can more effectively contribute by providing detailed and specific analysis that yield intimate insight into past people’s lives. Residues from lithic tools provide only a snapshot of one or more events in the past. The snapshot taken across the one or more artefacts studied is variable in size: some stone tools are one-time used expedient tools set to one task, and some tools are curated and used for a set of activities over a longer period. Overall, the events that led to anthropogenic residue deposition on the lithic occur on a relatively short-lived timescale.

This study zooms in to consider microscopic and chemical traces on individual lithics collected at the local site level. It is proposed that this micro scale of data capture is appropriate for gaining an in-depth and specific understanding of the objects that people made and used. A close understanding of artefacts is valuable to interpret the past, and can alter previous ideas based on typo-technological analysis.

### 1.2.2.7 Artefact biographies

A biographical perspective to stone tools, and the construction of detailed artefact 'biographies', has been suggested as a potentially productive approach to theorise residue analysis (van Gijn, 2010; van Gijn and Wentink, 2013). The point of a biography is to tell the story of an individual – in this case the narrative of an individual stone tool – over the length of what has been termed its 'life history' (Andrefsky, 2008) or 'use-life' (Seeman et al., 2008). The artefact has also been conceptualised as its own site on which human actions are inscribed (Loy, 1993), similar to the notion of artefact biography. The 'artefact as site' concept can be thought of in terms of discrete 'locales' of residues and usewear existing on each the lithic, which are analogous to the activity areas of an archaeological site. Haslam (2006a) suggested that agency and narrative could be used to make residue analysis more theoretically engaging, increasing the value in an area of archaeology that is technical and based on a wide range of scientific methods. Haslam suggested the specific actions of individual actors are recorded on stone tools and that this record provides a way to incorporate agency into the interpretation of meaning from residue analysis.

There are some issues in attempting to apply the theoretical concepts of object biography, agency, and narrative as interpretive frameworks for residue analysis results. Most importantly, these concepts cannot be utilised until the foundation of residue studies – accurate identification – is secure. Identification is still a contentious area in lithic residue studies and is not entirely methodologically resolved. The other point to make regarding the biographical and narrative approaches is that when one commences research aiming to tell a story, one may ascribe meaning where there is none, simply to fulfil the need of biography construction.

### 1.2.2.8 The fit between usewear analysis and residue analysis

It has been argued that usewear and residue analysis strengthen each other, and it has been generally recommended that they be conducted in tandem (Kealhofer et al., 1999; Lombard, 2005; Rots and Williamson, 2004; van Gijn, 1998). The main benefit suggested of doing both at the same time is that the spatial distributions of residues on tool surfaces can be matched up with the distributions of usewear. Here, the term 'usewear' is used to encompass both macro-fracture (analysis uses unaided eye) and microwear (analysis uses high power microscopes) because both approaches make functional interpretations based on lithic impact and wear traces, albeit at a different scale.

Lombard (Lombard, 2005), Wadley and Lombard (Lombard and Wadley, 2007b, 2007a; Wadley and Lombard, 2007a), and Langejans (2011) have argued that some residue identification issues are overcome by taking a 'multi-analytical', 'multi-stranded' or 'contextual approach', respectively. Lombard (2005) put forward a case for using macro-fracture analysis, usewear analysis, and residue analysis together as corroborating multiple lines of evidence for successful interpretation of stone tool function. Wadley and Lombard (Lombard and Wadley, 2007a, 2007b; Wadley and Lombard, 2007) advocate the use of multi-stranded evidence, including integration of usewear with residue analysis, and the use of multiple related micro-residue types found in the same locations on the tool. For example, if bone residues are found in conjunction with fat and muscle residues, along with usewear, this is a more reliable identification of animal processing than an isolated instance of one residue type. Similar to Wadley and Lombard's multi-stranded evidence, in Langejans' contextual approach (2011), in situ visual residue identifications are made more secure by mapping their patterning on the tool surface and by the co-existence of other types of related residues in the same locations.

The problem with usewear as currently practised is that it is not an independent line of inquiry but rather contingent on the subjective interpretation of each analyst. This subjectivity is a longstanding problem and has not been resolved to the satisfaction

of the wider scientific community. The critique of microwear by Newcomer et al. (1986, p. 216) that “...there has been no convincing demonstration that anyone can consistently identify worked materials by polish type alone...” remains relevant today. More recently, Akoshima and Kanomata (2015, p. 17) also highlighted the subjective nature of the discipline, noting: “Microwear analysts tend to select data that would better serve their goals of reconstruction of specific human activities conducted at the site.” Although attempts at developing objective and quantitative methods in usewear are being tested (Evans et al., 2014; Stemp et al., 2015; Wilkins et al., 2012), overall, usewear has been based on subjective analogical reasoning between modern and ancient phenomena observed on stone tools, which might be considered at odds with the new and multidisciplinary scientific approaches being developed for the analysis of residues. Thus, this study focuses on residue analysis exclusively. Residue analysis is a developing area of research currently at the centre of a debate on methodological, identification, and interpretive grounds. Investigating these issues as applied to usewear analysis were determined to be outside the scope of the present research.

## 1.3 Background to Star Carr

### 1.3.1 The excavations and recent research

Star Carr, along with many other Upper Palaeolithic and Mesolithic sites, were discovered in the Vale of Pickering, North Yorkshire, by John Moore (Moore, 1950). In 1948, Moore found stone tools eroding out of a ditch in a field at the edge of a palaeolake which he recorded and called Lake Flixton (Figure 1.1). Moore excavated Star Carr in 1948-1949, as well as two locations on Flixton Island within the palaeolake (Flixton 1 and Flixton 2) (Milner et al., 2011b; Moore, 1954). Subsequently, a curator at the Scarborough Museum sent a selection of the flint

tools to Grahame Clark at the University of Cambridge. The tools piqued his interest because of their potential to signal a settlement with both lithic and organic preservation, similar to the Mesolithic sites in Denmark (Clark, 1972). Clark directed excavations at Star Carr between 1949-1951 (Clark, 1954), followed by the Vale of Pickering Research Trust led by Tim Schadla-Hall (1985), Paul Mellars (1989) (Conneller and Schadla-Hall, 2003; Mellars and Dark, 1998), and more recently Chantal Conneller, Nicky Milner, and Barry Taylor from 2004-2015 (2004-2011) (Milner et al., 2013, p. 44, in press).

Star Carr rose to prominence due to the excavations and publications of Clark, and the importance of the site is still recognised internationally.

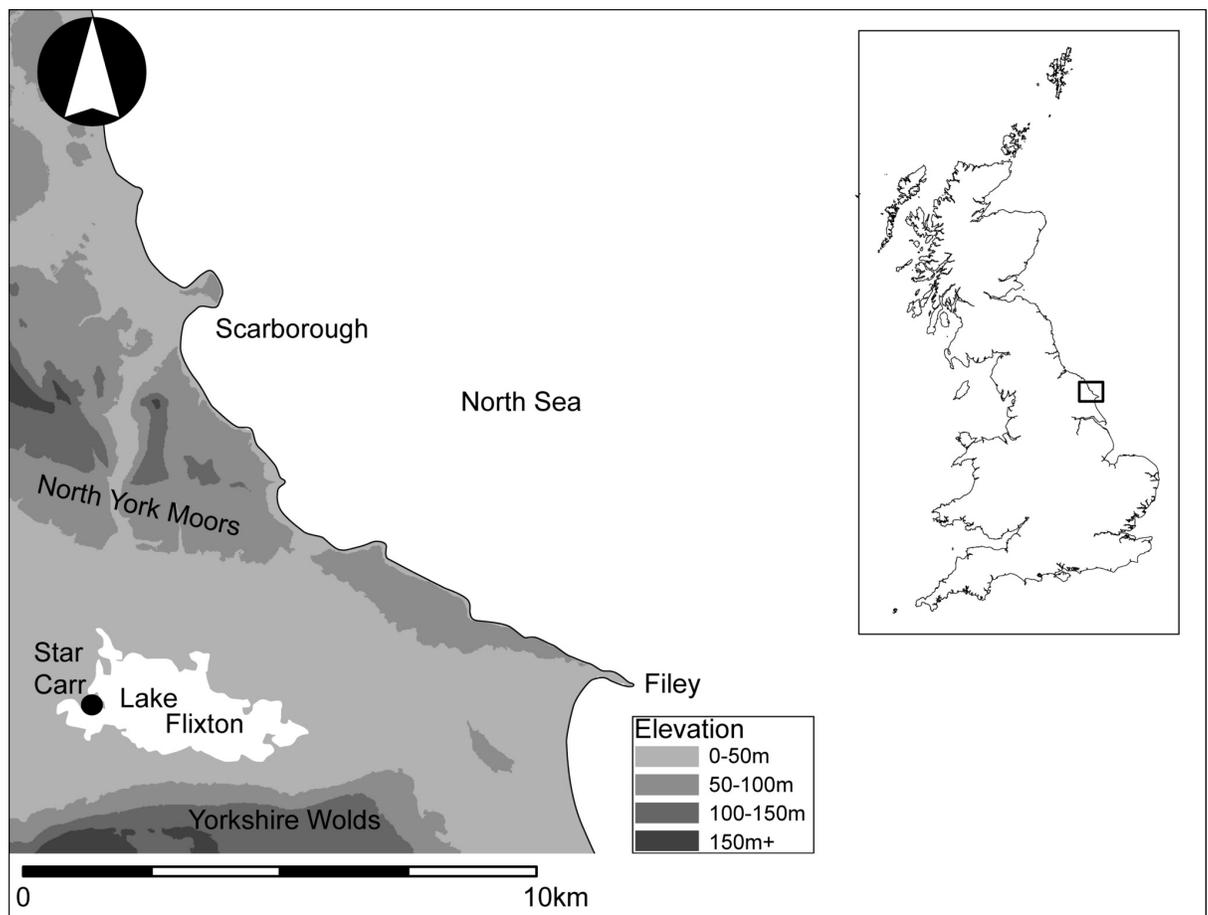


Figure 1.1 Location of the site of Star Carr in North Yorkshire, UK. Image from Milner et al. (in press).

The Mesolithic period in the UK spans from approximately 9,500 to 4,000 cal BC, and Star Carr dates to the early part of the Mesolithic (Conneller and Higham, 2015). People living during this time were hunting and gathering wild animals and plants, and moving from place to place intermittently to exploit different resources. The British Mesolithic is mostly known through Star Carr (Conneller, 2014, p. 7003), and it was considered a 'type-site' for the period. We now think of the site as exceptional because no other known site has the number and variety of artefacts found at Star Carr.

A new high-resolution programme of dating undertaken by Alex Bayliss (Historic England) has resulted in a detailed chronology of site occupation at Star Carr. The first Mesolithic human activity at Star Carr is modelled with radiocarbon dates to start at *9385–9260 cal BC (95% probability)*, and end at *8555–8380 cal BC (95% probability)* (Bayliss et al., in press Fig. 17.2). The new chronology supports human occupation at the site periodically for 735–965 years (95% probability), and indicates Star Carr was a special place in the landscape which was revisited by hunter-gatherers over many generations.

Star Carr has revealed spectacular archaeology. Antler frontlets (Elliott et al., in press; Little et al., 2016), antler barbed points (Elliott, 2012; Elliott and Milner, 2010), thousands of lithics (Conneller, 2000; Conneller et al., in press), shale, amber, and animal teeth beads (Clark, 1954; Milner et al., 2016), birch bark and birch bark tar (Aveling and Heron, 1998; Clark, 1954; Fletcher et al., in press), timber platforms and worked wood (Bamforth et al., in press; Mellars et al., 1998; Taylor, 1998), and four dwelling structures (Conneller et al., 2012; Taylor et al., in press), are some of the important finds.

The animal remains that have been commonly recovered at Star Carr are: red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), elk (*Alces alces*), aurochs (*Bos primigenius*, extinct), and wild boar (*Sus scrofa*) (Clark, 1954; Legge and Rowley-Conwy, 1988), and this consistent with faunal assemblage data from other Early

Mesolithic sites in North-West Europe (Overton and Elliott, in press). Additionally, thirteen fish bones and teeth from northern pike (*Esox lucius*) and European perch (*Perca fluviatilis*), and family Cyprinidae, were also found at the site (Robson et al., 2016). Recently, a more in-depth and detailed faunal analysis at Star Carr taking into account spatial analysis, NISP values, and bone condition was completed by Knight et al. (in press), indicating a complex set of animal processing activities occurred.

Pollen analysis (Albert and Innes, 2014; Dark, 1998a, 1998b; Day, 1996; Innes et al., 2010, 2011; Walker and Godwin, 1954) and detailed auger and borehole survey mapping (Cloutman, 1988; Cloutman and Smith, 1988; Palmer et al., 2015) around the margins of Lake Flixton indicates that the environmental setting of at Star Carr was that of a wetland landscape on the edge of Lake Flixton. Palaeobotanical analysis of macrofossils from the Vale of Pickering by Cummins (2003) and Taylor (2014, 2011), support the picture of a wetland environment, with aquatic (*Chara*, *Cristatella*, *Nymphaea alba*, *Potamogeton*, *Scirpus lacustris*) swamp/fen (*Phragmites*, *Carex*, *Urtica dioica*, *Eupatorium*, *Sparganium*, *Typha* sp., *Thelypteris palustris*), and arboreal (*Betula*, *Corylus*) species present.

### 1.3.2 Overview of soil chemistry at Star Carr

The soils at Star Carr can be described as hydromorphic soils; soils which are poorly-drained profiles, dominated by mottling, dark grey colours or peat accumulation on flat low-lying sites (Young, 1976, p. 130). Hydromorphic soils have been exposed to prolonged water saturation and can have seasonal alternation between waterlogging and drainage, affecting the supply of O<sub>2</sub> to the soil, thus also affecting the oxidation state of elements such as iron, manganese, and sulfur (van Breemen and Buurman, 2002, p. 159). Additionally, Star Carr contains acid sulfate soil; soils in which sulfuric acid is produced. An active acid sulfate soil contains of sulfide minerals (typically iron sulfides and the mineral pyrite) exposed to oxidation,

forming sulfuric acid and consequently the pH drops to ultralow levels (Fanning, 2006, p. 11). This occurs when the water table is lowered and oxygen is introduced into the soil profile.

The major reactive species at play in the soil at Star Carr are sulfur (S) and iron (Fe), as well as aluminium (Al), calcium (Ca), and manganese (Mn) (Boreham et al., 2011a, 2011b; High, 2014; Rowley et al., in press). Sulfur is the prime culprit causing acidification of the soils when it forms oxidation products (sulfates,  $\text{SO}_4^{2-}$ ) (Boreham et al., 2011a). The source of this sulfur is autochthonous, being found in the soil itself, rather than being transported by groundwater or rainwater (Boreham et al., 2011a, p. 2854). More specifically, it seems likely that elevated sulfur concentrations documented in borehole transects throughout Star Carr are present not only from decomposed organics in the peat, but also from sulfur-containing mineral deposits (e.g. pyrite,  $\text{FeS}_2$ , gypsum,  $\text{CaSO}_4$ ) underlying the peat (High, 2014, pp. 54, 72).

The influence of at least three chemically distinct water sources on local site hydrology and exposure of the soils to oxygen are major factors influencing the prevailing chemical conditions and the preservation of organic artefacts at Star Carr (Boreham et al., 2011a; High, 2014).

### 1.3.3 Site deterioration

Site deterioration at Star Carr from initial excavation in 1949 to the latest excavations in 2015 is well documented. When the site was first excavated, the anoxic peat burial environment yielded organic artefacts, for which the site is famous, in excellent condition. More recently, damage has been found to organic material including bone and wood (High, 2014; High et al., 2016, 2015; Milner, 2007; Milner et al., 2011a), soils (Boreham et al., 2011a, 2011b), and even the pollen record (Albert et al., 2016; Dark, 2017). Water levels within archaeological contexts have been shown to be fluctuating with the overall trend of the water table lowering. With oxygen introduction

into what were previously anaerobic conditions in the peat, new chemical reactions which are accelerating decay pose real threats to the preservation of Star Carr. The peat is drying out at Star Carr, and is dramatically evidenced by an 80 cm loss of peat depth since 1950 (Dark, 2017, p. 247).

## 1.4 Thesis organisation

This thesis is divided into three main parts. Part I is the research rationale and background to the project, containing chapters which set the scene by reviewing lithic residue analysis research from the perspective of methodology (techniques used, Chapter 2), residue analysis research specific to the Mesolithic (Chapter 3). With this in mind, the methods selected for use in this thesis are outlined (Chapter 4). Part II presents an experimental investigation of residue diagenesis that took place at Star Carr (Chapters 5 and 6). This study also examined the issue of residue identifiability and produced a guide for the identification of residues with reflected visible light microscopy (Chapter 6). The chapters within Part III illustrate how the residue analysis approach was applied at Star Carr (Chapter 7). The results are presented in individual chapters by residue type (Chapters 8-13). A discussion of the findings, revisiting the original research questions, and the results relevant to the development of the discipline of lithic residue analysis, is followed by concluding remarks with recommendations and implications of this study (Chapter 14).

# CHAPTER 2 LITHIC RESIDUE ANALYSIS REVIEWED

## 2.1 Introduction

Stone tools are one of the main lines of evidence prehistoric archaeologists have at their disposal to interpret past human behaviours. Residue analyses have valuable roles to play in understanding individual stone tool functions and technological choices at a very detailed level. This fine-grained approach can also contribute to a better understanding of broad topics in prehistory such as socio-economic patterns (Bicho et al., 2015, p. 7). Residue analysis is one line of inquiry that complements typological and ethnoarchaeological studies (González-Urquijo et al., 2015), and refitting studies (e.g. Conneller, 2007; Conneller et al., 2007).

As part of this research it was important to critically evaluate previous analyses and the range of methods used in residue studies on lithics (objective 1) before attempting to examine lithics from Star Carr. This chapter covers three areas. The first section provides a history of research for residues on lithics, reviewing some of the key studies. The second part of this chapter will review the specific techniques that have been used in lithic residue analysis, organised by visual and chemical approaches. The third section will offer critiques on methodological issues – the process of how residue analysis is conducted. In doing so, it will address the question: what constitutes best practice in residue analysis? The term ‘residues’ here is considered in the widest sense, including both microscopic and chemical (often termed ‘organic’) residues.

## 2.2 History of research

Lithic residue analysis is a developing subdiscipline in archaeology. Its older counterpart, usewear analysis, began to take shape in the 1960s with the PhD work of Sergei Semenov (and translated into English in 1964, published in Russian in 1957). An interest in this research spawned the subsequent development of archaeological residue research, with Frederick Briuer (1976) publishing the first study on prehistoric lithic residues, in conjunction with usewear.

Briuer (1976) employed microscopic as well as chemical methods to identify residues, finding microscopic plant and animal remains from a sample of 37 prehistoric Arizonan stone tools from rockshelters and open air sites. In doing so, the study showed lithic residues can be used to understand the function of artefacts, and thus the activities, of past peoples. Several types of analyses were conducted:

- 1) usewear with low magnification stereomicroscopy (30x)
- 2) microscopic examination to identify in situ residues based on morphology
- 3) removal of residue sub-samples and testing with a series of botanical chemical reagents to identify plant lignin, cellulose, suberized or cutinized tissue, and starch
- 4) the collation of a reference collection based on the combustion and measuring of several C13/C12 ratios of known plant and animal taxa (not used in the study)
- 5) mass spectrometry on two extracted residue samples (returning no clear results due to the complexity of the compounds)

Plant parts identified morphologically and with stains included: starch granules, stellate hairs, pollen, calcium oxalate crystals, raphides, cell walls (lignified, suberized and cutinized), cell lumen, tracheids, fiber tips, spiral vessels, and hair vessels (trichomes). In spite of this nascent stage of residue analysis, Briuer tried to exclude natural phenomenon as causal factors for the presence of the residues on the tools. For instance, he showed that the locations of in situ residues were

associated with worked tool edges. Briuer (1976) also tested randomly selected items, from on and off-site, as controls. The off-site items, including rocks and macrobotanical remains, had no similar residues to those found on the archaeological tools. Twenty on-site non-cultural items were tested with chemical indicator reagents, with three (one woodrat bone, two plant twigs) giving positive reactions showing plant material was present.

Shafer and Holloway (1979) followed Briuer and conducted a functional analysis of 25 Archaic chert flakes from Hinds Cave, Texas, to investigate possible animal and plant residues, applying residue analysis together with usewear. Usewear was conducted with low power stereoscope while wearing surgical gloves, and residue samples were extracted with razor blades and mounted on slides for viewing with transmitted light microscopy. A total of 11 of 25 specimens examined were found to contain micro-residues. They found starch granules, phytoliths, pollen, tracheids, calcium oxalate crystals, plant fibers, charcoal, rodent and other mammal hairs, and epidermal cellular fragments from lechuguilla (*Agave lechuguilla*), agave (*Agave* sp.), sotol (*Dasyllirion* sp.), and yucca (*Yucca* sp.) (1979, p. 395). Shafer and Holloway drew comparisons between modern replicas used for various tasks, using experimental archaeology to better understand the archaeological record. Most of the tools they examined were multi purpose, and usewear patterns were often not distinct or attributable to specific uses. The multipurpose nature of tools used by hunter-gatherers was an important insight; it made clear that archaeologists should not impose functional categories on tools based on gross morphology and also not assume that one tool was used for only one type of task. The conclusion drawn from their study is that people used tools in a generalised and sometimes expedient fashion.

The study by Anderson (1980) was the first to examine non-organic residues morphologically and chemically. Anderson examined prehistoric flint tools and replica tools used on plant and animal remains and modern biological samples, which were studied with a metallographic microscope and SEM. Experimental

samples viewed with SEM were also subject to energy dispersive X-ray spectroscopy (EDS), used to characterise the mineral components of the reference samples. Anderson dealt specifically with durable polish residues from the minerals within plant and animal tissues that are laid down on flint during tool use, including calcium oxalate crystals, silica phytoliths, silica casts of softwood tracheids, hardwood fibres, and vessel elements.

Although Anderson (1980) claims that animal cellular residues were found, they do not appear to be morphologically distinctive in the archaeological pieces or the modern specimens shown in her SEM images. Anderson's SEM work illustrates that the structure of plant micro-residues are much easier to identify than animal residues such as antler, bone, and periosteum tissue.

This study also contributed to a question pertinent to both usewear and residue analysis: is polish formation on the lithic surface due to additive and/or subtractive processes? The answer from Anderson's (1980) experimental SEM work clearly showed pieces of worked materials transferred and adhered, with amorphous and distinctive crystalline residues building up as they accumulate on the stone tool. However, she also found that the flint surface is partly dissolved due to the combination of friction, heat, abrasion, and water during tool use. Although Anderson's study was groundbreaking for its time, EDS is arguably not the most appropriate means to investigate residues that have a similar chemical signature to the stone substrate. For instance, it is difficult to prove that the residue signals collected from EDS originate from the plant being worked and not the flint tool, both of which also contain silicon and calcium. Additionally, none of the X-ray spectra collected from residues were presented for the reader to evaluate.

Archaeological residue studies gained momentum in the 1980s. Fullagar explored tool function by using residue and usewear techniques in Oceania (Fullagar, 1989, 1988, 1986), while Cosgrove (1985) used SEM-EDS on Tasmanian lithics, and Hill and Evans (1987) used SEM, infrared spectroscopy, high performance liquid

chromatography (HPLC) on Oceanian pottery. Loy and Nelson (1986) and Hall et al. (1989) advocated the use of lithic residue analysis and outlined potential research directions for the sub-discipline. Loy investigated starch from stone tools in the 1990s (Loy, 1994; Loy et al., 1992), although his greatest impact was in generating interest in the study of blood residues (Broderick, 1982; Coughlin and Claassen, 1982; Loy, 1983; Newman and Julig, 1989; Richards, 1989).

In the past few years, residue analysis has entered a new stage of development as it continues to borrow from the natural sciences. This is evidenced by two recent publications on usewear and residue analysis: 'An Integration of the Use-Wear and Residue Analysis for the Identification of the Function of Archaeological Stone Tools' (2014), edited by Lemorini and Cesaro, and the book 'Use-wear and Residue Analysis in Archaeology', edited by Marreiros, Gibaja Bao, and Bicho (2015). Studies presented in the 2014 collection of reports are part of a trend indicating that usewear, and particularly residue analysis, are moving in a new direction, toward the incorporation of ever more sophisticated chemical and elemental analyses, such as FTIRM, Micro-Raman, XRF, SEM-EDS, GC-MS. The 2015 book also makes it clear that functional analysis is on a new path that draws on more scientific techniques.

## 2.3 Techniques

### 2.3.1 Introduction

There are two ways in which residue analysts have approached analysis:

- 1) examination of residues as they are found in situ on the lithic surface.
- 2) extraction of the residues physically and/or chemically.

Both visual and chemical analyses have been carried out in situ on tool surfaces and by extraction. For residues to be examined in situ they must be detectable at least microscopically. However, it has been shown experimentally that residues can be harboured deep within microcracks of the stone, which are invisible with traditional light microscopy and require extraction (Shanks et al., 2001). This section will review the techniques used by analysts to investigate lithic residues.

## 2.3.2 Microscopy

### 2.3.2.1 Introduction

Many residue researchers have approached residue identification by visual methods alone, using stereo microscopes and reflected visible light microscopes (here, abbreviated as reflected VLM), and sometimes scanning electron microscopes (SEM), for in situ viewing. In this approach, residues are identified based on qualitative morphological traits which are compared with a reference collection and/or existing literature (Langejans and Lombard, 2015). Low power stereomicroscopes (about 5x to 100x) and/or high power reflected VLM (about 50x to 1600x) are the oldest and most affordable techniques to interrogate putative traces of human activity left on stone tools. Scanning electron microscopy (SEM) (about 10x to 500,000x) has also been used for imaging residues and is considered a high power microscopic approach. During the initial phase of functional analysis for the study of usewear and residues, there was some debate as to whether a low power approach (Tringham et al., 1974), or high power approach (Keeley, 1980) should be taken. Today low and high power approaches are no longer dichotomised and it is accepted that both are complementary. Laser scanning confocal microscopy (LSCM) is an additional type of microscopy that has found recent applications in archaeology. LSCM has been used by some researchers to create 3D images of stone tools and document the presence of usewear and residues. This section will

detail the types of microscopy used in lithic residue analysis using relevant case studies and critique these microscopic methods.

### 2.3.2.2 Microscopy: in situ

#### Reflected VLM

Reflected VLM microscopes, make use of the the reflection of light off the stone tool, so residues can be examined in situ without the need for extraction and mounting on glass slides. Reflected VL microscopes are also referred to in the literature as incident light, optical, metallographic, or metallurgical microscopes. Many studies have identified residues in situ on the artefact surface with a reflected VLM (Eales et al., 1999; Fullagar et al., 2006; Hardy, 2004; Hardy et al., 2001; Hardy and Garufi, 1998; Hardy and Moncel, 2011; Haslam, 2006b; Kononenko, 2011; Langejans, 2012a, 2010, 2009; Lombard, 2007, 2005; Robertson et al., 2009; Weisler and Haslam, 2005).

In order to illustrate some of the problems with in situ identification of lithic residues based on reflected VLM, the case of the visual identification of erythrocytes, or red blood cells (RBCs) will be examined. Ancient RBCs have reportedly been found in situ on stone tools with reflected VLM (Lombard, 2014, 2011, 2008; Loy and Dixon, 1998; Loy and Hardy, 1992) and also on pottery (Matheson et al., 2009, p. 194). These RBC identifications have large interpretative implications, so this is an issue of interest not just to lithic residue analysts, but to the wider archaeological community.

Wadley et al. (2004) used experimental flakes on blood containing items then examined them with reflected VLM. One flake was used to cut raw beef then dried in an oven and stored in a plastic bag, the other smeared with beef blood and allowed to dry overnight and stored in a plastic bag. Wadley et al. (2004) present an image

the residue on the flake used to cut raw beef, in which a preserved blood vessel with intact RBCs is purported. When the images are examined, the supposed blood residues illustrated are perhaps actually fungal spores in a chain formation (sometimes referred to as a 'string of pearls'). The circular items identified as blood are similar to fungal spores found on reference collection grass sample of *Phragmites* sp. leaves (Figure 2.1). Barton (2009, p. 135) also illustrated in situ fungal spores in a chain formation, in that case they were found surrounding starch granules on an experimentally buried tool.

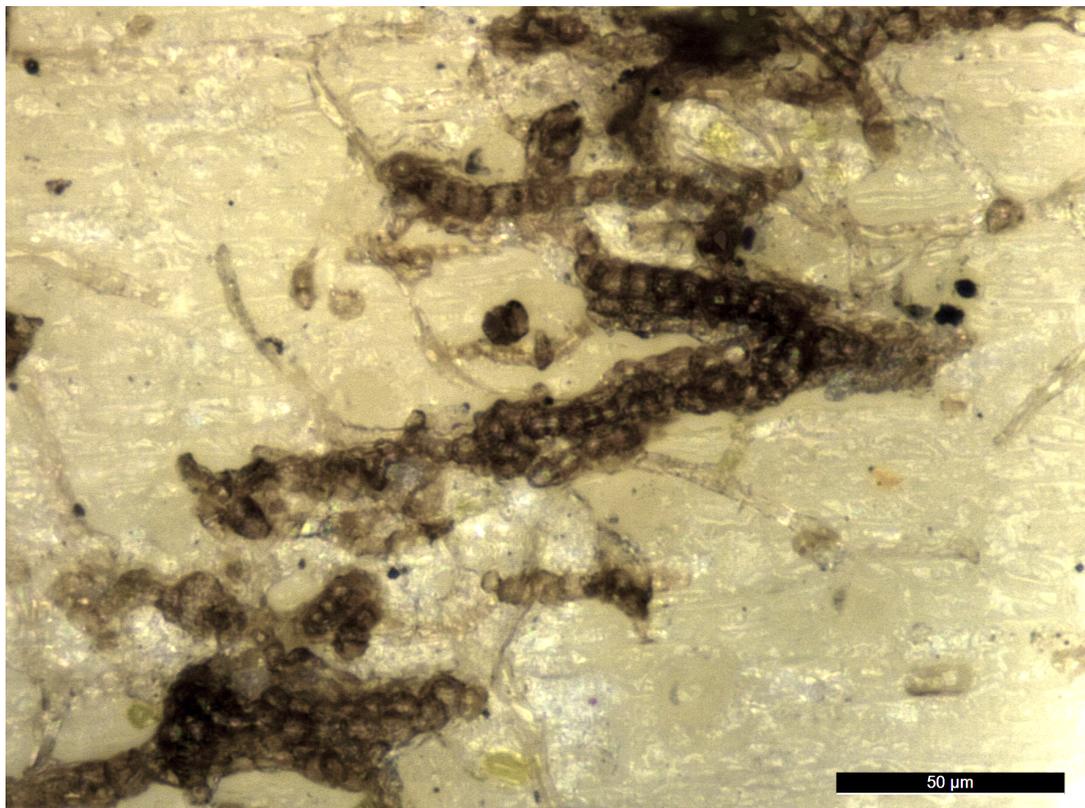


Figure 2.1. Fungal material on *Phragmites* sp. leaves.

In general, the RBCs of fish, amphibians, reptiles and birds are nucleated (Claver and Quaglia, 2009), but mammal RBCs do not have nuclei or any other cytoplasmic organelles at maturity (Telen, 2009, p. 126), an evolutionary advantage that allows more oxygen to be carried by each RBC. Nonmammalian RBCs are oval, whereas the morphology of RBCs seen in fresh mammalian blood are flattened biconcave

discs (discocytes). Adult human RBCs have an average diameter of about 7.2-7.4  $\mu\text{m}$  (Price-Jones, 1933) and a max thickness of 2.2  $\mu\text{m}$  (Smith and Wilson, 2001). Most animal RBCs fall within 5-10  $\mu\text{m}$  diameter (Seaman, 1975, p. 1183).

A recent residue analysis (Rots et al., 2017) was conducted on 17(?) tools (or perhaps 15 tools, both sample numbers were noted on p. 11 in the paper) from Middle Stone Age Sibudu Cave, South Africa. Several white blood cells extracted from a serrated point occurring together were reported (Rots et al., 2017 Fig. 22a) (see image). On inspection, these items are more likely to be fungal spores or pollen from the burial environment or post-excavation contamination, and many species of fungal spores overlap in size with mammalian blood cells (Agashe and Caulton, 2009; Watanabe, 2002). Additionally, aggregations are a natural form of spore genesis in several groups of fungi. Also, even if blood cells did preserve on the point, it is unlikely that several white blood cells could ever be recovered in a multi-cellular clump since white blood cells are not abundant even in fresh whole blood, accounting for only 1% of the total blood volume.



Transmitted light micrograph of what are proposed to be Middle Stone Age white blood cells from a rock hyrax, extracted from serrated point (875) from Sibudu Cave. From Rots et al. 2017 Fig. 22a. Image for examination purposes only, to be removed prior to publication.

Rots et al. (2017) also claimed in situ 'RBC-like inclusions' were found visually on three points (1136, 551, 875). It was suggested that a circular item on tool 875 (Rots et al., 2017 Fig. 22c) (see image) was a RBC, and that it originated from zebra or impala blood, based on its diameter.



Reflected light micrograph of what is proposed to be Middle Stone Age red blood cell from a zebra or impala, on serrated point (875) from Sibudu Cave. From Rots et al. 2017 Fig. 22c. Image for examination purposes only, to be removed prior to publication.

Visual methods for the identification of archaeological lithic residues have been criticised for the often subjective basis for identifications. Visual methods have also been questioned on the grounds that archaeological residues are fragmentary and degraded, unlike the reference specimens used to make comparative identifications. The reliance on subjective interpretation in the field may explain why microscopic residue analysis has yet to become a mainstream approach in scientific archaeology. In addition, some studies have failed to demonstrate, with micrographs or adequate descriptions, the characteristics that were used to identify each residue type found by the analyst, which make the results impossible for the reader to evaluate.

## Scanning electron microscopy (SEM)

A scanning electron microscope (SEM) bombards the sample with electrons in order to create an image. SEM is an excellent tool to capture three dimensional microtopography of lithic surfaces and residues in high-resolution. Because it is a non-light based technique, the images are presented in grayscale and no colour information is collected. Light microscopy can yield unwanted imaging artefacts, which can sometimes lead to misinterpretation of the morphological features of residues. SEM imaging is accomplished with electrons, so no imaging artefacts are produced by the interaction of light with the sample. Typical SEMs operate in high vacuum conditions within the sample chamber. During analysis in SEM, there are several types of electrons that are discharged from the sample that are detectable. One of the useful types for screening lithic residues are backscattered electrons (BSE). Using a SEM in BSE mode provides good imaging quality for uncoated specimens because the signal is less sensitive to the effects of charging (Goldstein et al., 2003, p. 202).

SEM imaging of stone tools is limited by the size of the chamber, which will only accept small artefacts, so large lithics are not possible to analyse. Other drawbacks of SEM include the investments of training, time for analysis, and the expense required, which effectively limits the number of samples. Conventional SEM requires the object's surface to be prepared with a conductive sputter coating – a layer of gold, gold-palladium, platinum, graphite, carbon, or tungsten, in order to avoid charging and improve image resolution (Bozzola, 2007, p. 450). Obviously these treatments are not ideal for rare archaeological artefacts. To avoid this damaging processing, most lithic residue studies to date have used what are called environmental SEMs, variable-pressure SEMs, nature SEMs or some other similar term (Goldstein et al., 2003, p. 221). These SEM instruments can accommodate the analysis of large non-coated samples in chambers with higher ambient pressure (low-vacuum) conditions (Pollard and Heron, 2008, p. 49).

VP-SEMs are non-destructive to artefacts, since no sputter coatings are required for imaging; a major advantage to conventional high vacuum SEM analysis. Application of conductive coatings were applied to stone tools to image both prehistoric and experimental residues with conventional high vacuum SEM in earlier work by Anderson (1980), van Gijn (1984), and Hortolà (2002). However, conventional SEMs have also been adapted for use like a VP-SEM when used in secondary electron mode for examining experimental residues (Hortolà, 2008, 2005).

Borel et al. (2014, p. 49) compared the use of secondary electrons and backscattered electrons for SEM imaging. They found backscattered electrons penetrate deeper into the sample, and provide information about chemical homogeneity and texture of the sample which is helpful to identify points of interest for EDS microanalysis. Residue experiments with replica quartzite tools by Pedergrana and Ollé (2014, p. 56) also noted the usefulness of the backscattered electron detector to scan tool surfaces rapidly in the SEM, as organic residues stand out in contrast to the stone surface due to differences in atomic number. Monnier et al. (2013) favours the use of backscattered electrons to produce compositional images of residues because any organic residues, being primarily composed of carbon, appear dark, and the stone, being primarily composed of silicon, appears light. The stone appears bright because it is composed of heavier atoms, and emits more backscattered electrons than organic residues which are composed of lighter atoms. This is useful information for screening artefacts for organic versus inorganic residues, although it cannot identify the nature of the residue. However, it is a practical first step in creating an accurate 'map' of natural and cultural residues on the tool.

SEM research by Hortolà (2005, 2002, 2001, 1992) on recent and aged blood films on stone substrates identify mammalian RBC morphologies, and what are described as negative replica impressions of RBCs. Hortolà (2002) used SEM to explore the morphological changes human RBCs undergo over time in experimental blood smears on obsidian, limestone, and chert. The stones were stored in a room at

ambient temperature for periods ranging from 7.5 to 10.17 years. The in situ blood residues were then coated with carbon and gold and imaged with SEM. Hortolà observed that most RBCs retained morphological characteristics similar to fresh cells, but also alternate morphologies, including moon-like shapes, and negative impressions left by the blood were visible with SEM. Obviously, the results of this study may not be comparable to blood residues on archaeological blood buried for thousands of years and exposed to long-term taphonomic effects. Nevertheless, it does establish that RBCs can preserve for at least a decade in unburied indoor conditions. Conceivably, there may be special preservation contexts that allow for blood cells to survive archaeologically, for example erythrocytes protected in environments within the microtopography of the stone, or in extremely dry, cold, or anaerobic sediments with low microbial activity.

In contrast to observations by Hortolà, a study on the microtaphonomy of blood in parietal bone by Cappella et al. (2015) found RBCs were morphologically distinct for only a week after death of the individual. In the archaeological bone Cappella et al. (2015) tested, RBCs were not detected by either: 1) histological staining and microscopic viewing, or 2) immunohistochemical testing with Glycophorin A.

It is difficult to reconcile claims of microscopically-identified intact RBCs from both ancient and modern aged blood residues with what is known about blood from biological and medical literature. Problematic is the fact that RBC membranes are highly elastic (Mohandas and Gallagher, 2008) and denature when dried out or when exposed to different osmolarity conditions, causing turgidity and eventual lysis or shrinkage due to movement of liquid into or out of the blood cell. Indeed, a number of morphological changes to animal RBCs can be expected once they are outside the body as residues on stone. The diameter of fresh RBCs begins to decrease in the presence of air after only a day (Liao et al., 1998, p. 193). It is also known that upon dehydration or placement in a hypertonic solution, normal biconcave RBCs are altered – cell structure changes to shriveled and crenated (an RBC morphology called an echinocyte that has many evenly spaced projections) (Bain, 2006, p. 91;

Nikinmaa, 1990, p. 72; Reinhart and Chien, 1986, p. 1111; Telen, 2009, p. 127). Thus, RBCs on experimental stone tools should change in size and morphology if they dry out, from biconcave discs to echinocytes. On the other hand, when RBCs are placed in a hypotonic solution such as water, RBCs undergo a different type of morphological transformation, first swelling and becoming cup shaped (stomatocytes), then spherical (spherocytes), eventually leading to hemolysis (ruptured cells) (Telen, 2009, p. 127).

#### Laser scanning confocal microscopy (LSCM)

Laser scanning confocal microscopy (LSCM) is a type of optical light microscopy that takes images point-by-point at selected depths of field and reconstructs it as one image which can be seen in three dimensions. LSCM has high z-axis resolution and is used in ophthalmology to take images of living cells within patients eyes (Guthoff et al., 2006). LSCM was used by Shanks et al. (2001) to image experimental residues in microcracks of the lithic surface, and Evans and Donahue (2008) used this imaging technique to document the microtopography of lithic usewear. Stemp et al. (2009) used LSCM with length-scale fractal analysis to quantify micro-topographical patterns on the surfaces of lithics experimentally used on shell, wood, dry hide, and soaked antler. They found that they produced surface wear profiles that were different enough from each other to be able to be discriminated. Recently, this research was furthered by Stemp et al. (2015), who used LSCM with area-scale fractal complexity calculations to quantitatively document surface texture in 3D on 30 basalt flakes used to cut an oak branch. The working loads applied during use of the flakes was documented in a range between 150g to 4.5kg. Stemp et al. (2015) showed that working load impacts the development of microwear and that differences between experimental working load could be identified when the working load was above ~100g.

The usefulness of LSCM for objective quantifiable documentation usewear extent was also tested by Evans et al. (2014). Evans et al. (2014) used flint flakes

experimentally on dry beech wood (n=2) and soaked deer antler (n=2), and each tool was examined with LSCM prior to first use, and after 1000, 2000, and 3000 strokes to the worked material. Evans et al. (2014, p. 74) found that they could not determine whether surface texture of the worked areas of the flint was unique to contact material type, but that the wood-working wear was not statistically different over the course of flint use as more strokes applied.

Whether LSCM can successfully be applied to archaeological lithics to distinguish anthropogenic worn surface features from post-depositional or post-excavation wear, remains to be seen. Evans et al. (2014, p. 75) state that for the automation of lithic usewear quantification, it will be important to develop analytical techniques to differentiate between worn and unworn parts of the tool edge. A UK Arts and Humanities Research Council (AHRC) project titled 'Fragmented Heritage' From the Kilometre to the Nanometre: Automated 3D Technology to Revolutionize Landscape, Site, and Artefact Analyses (lead research organisation: University of Bradford, PI: Dr Randolph Donahue) is currently underway and appears poised to contribute to quantitative method development in the study of lithic usewear.

Quantification using International Organization for Standardization (ISO) measures including surface roughness (Sa), scalar quantisation (Sq), maximum height of the scale-limited surface (Sz), and area scale fractal analysis, and collaborations with surface metrologists may be productive avenues for tool usewear/microwear studies (Ungar and Evans, 2016, p. 3). Improved quantification of lithic surface alterations might be helpful direction for usewear, however, the main issue for usewear from a methodological standpoint remains obtaining an accurate and reliable identification of ancient anthropogenic versus natural versus modern modifications to the stone surface.

### Combined microscopic approaches

Scanning electron microscopy (SEM) used in conjunction with reflected VLM microscopes for imaging residues was adopted early on in the development of residue studies (Anderson, 1980; Jähren et al., 1997). Over the years, improvements in imaging quality and the wider accessibility of SEMs have enhanced our ability to identify residues morphologically. Borel et al. (2014) conducted an experiment where micrograph images from used replica tools were compared by optical (reflected) VLM and SEM. They concluded that both imaging techniques should be used together and provide complementary information to assist the identification of residues and usewear. Similarly, Monnier et al. (2012) also compared the use of reflected VLM versus SEM to identify different types of experimental residues. In their blind tests, they found that SEM did not improve the identification of hide or bone scraping residues, but did diminish the ambiguity of ash (a hardwood) whittling residues, starch, and antler more than the reflected VL microscope. A recent experimental study by Pedergnana and Ollé (2017) compared a metallographic reflected VLM to VP-SEM for their ability to identify several plant and animal residues, including bone, meat, and wood, finding that VP-SEM highly improves the accuracy of residue identification. This contrasts with the experiment of Croft et al. (2016), who found that VP-SEM did not significantly reveal additional diagnostic structures beyond those observed with reflected VLM in the twelve plant and animal residues tested in their experiment.

### 2.3.2.3 Microscopy: extraction and viewing with transmitted or reflected VLM

#### Introduction

Residues on a stone tool to be analysed visually can be sampled by extraction from specific spots ('spot sampling') or the entire stone surface, and this has usually been done with distilled water or 5% ammonium hydroxide (NH<sub>4</sub>OH). Extraction can be

accomplished by mechanical extraction with scalpel, tweezers, or needle (Fullagar, 2006), air displacement micropipette with polypropylene disposable tips (Barton and White, 1993; Perry, 2005; Zarrillo and Kooyman, 2006), partial immersion of the tool in the vibrating waves of an ultrasonic bath (Fullagar et al., 2006; Summerhayes et al., 2010 supporting material), or total immersion of the tool in an ultrasonic bath (Croft, 2012; Kononenko, 2011). These extracted residues are then usually mounted on glass slides and viewed with a transmitted light microscope. The testing of different residue extraction techniques for optical microscopic analysis of lithic residues was recently experimentally reviewed by Cnuts and Rots (2017). They recommended that the types and distribution of the residues be considered first by conducting on-tool residue observations with reflected VLM (both stereo and metallurgical microscopes), then pipette extraction for most residues, or ultrasonic bath for dispersed, poorly adhering residues. Cnuts and Rots (2017) also tested the use of an ultrasonic dental scaler that uses a stream of pressurised water, but this instrument was not recommended for residue extractions since it left polish and striations on experimental flints. Additionally, use of a dental scaler for extraction probably sprays the residue in many directions because the water spray operates at high pressures, conceivably causing sample loss. As a way to target a potentially residue-containing area of a stone tool, Cnuts and Rots (2017 Fig. 1) proposed that just the edge of a lithic can be held in an ultrasonic bath, and then the extract prepared for microscopic viewing.

Extraction, preparation of glass slides, and viewing with transmitted light microscopy is a common method employed when starch and phytoliths are the residues of interest (Denham et al., 2003; Dominguez- Rodrigo et al., 2001; Hayes et al., 2017/2; Kealhofer et al., 1999; Pearsall et al., 2004). The morphological features of starch granules and phytoliths cannot be discerned when viewed in situ on the artefact surface with stereomicroscope or reflected VLM microscope, thus transmitted light microscopy is necessary to obtain any taxonomic identifications.

Starch residues in particular have been found associated with archaeological artefacts by many researchers (Weiner, 2010, p. 222), and have yielded new information about the plant resources people used for food and technology. There have also been reports of very old starch found on stone tools, suggesting it can persist for thousands of years (Fullagar and David, 1997; Loy et al., 1992; Mercader, 2009; Piperno et al., 2004, 2000; Piperno and Holst, 1998; Revedin et al., 2010; van Peer et al., 2003).

For starch, the majority of studies have used morphological characteristics and/or chemical stains for identification, although an unequivocal test is the application of  $\alpha$ -amylase, known to digest starch granules (Hardy et al., 2009, p. 249). Other destructive tests for starch involving application of chemical reagents to glass slides include: chloral hydrate-iodine, pyrogallol acid, ferric chloride, sulphuric acid, and hydrogen peroxide (Hall et al., 1989, p. 144). Both starch and phytoliths require the establishment or use of an extensive reference collection of different plant parts and species for comparative identification (Fullagar et al., 1996).

Damaged starch has been hailed as a way to identify human processing such as grinding/milling, cooking, or fermentation, with researchers claiming that the damage is activity-specific (Henry et al., 2009; Messner and Schindler, 2010), even when recovered from archaeological contexts (Babot and Apella, 2003; Henry et al., 2011; Wang et al., 2017). However, modifications to starch granules can occur by natural degradation factors (Haslam, 2004). Collins and Copeland (2011) pointed out that we should expect a portion of archaeological starch to be damaged, since gelatinisation can occur in situ in the sediment. Rather, naturally aged and taphonomically damaged starch granules may display damage similar to human-modified starch. This point is well-illustrated in experimental work by Babot (2003). Babot (2003, p. 74) showed potato starch granules that have been frozen in soils, dehydrated by air, or dehydrated by heat display a flattened relief, loss of lamellae, loss of extinction cross, and regions of the granules that have lost birefringence – features that might be misinterpreted as evidence of human modification. The action

of soil bacteria on several plant starches has also recently been examined by Hutschenreuther et al. (2017). Hutschenreuther et al. (2017) found the damage pattern left by multiple species of soil bacteria on starch granules appears the same as the damage pattern left by just one species of bacteria. However, the damage pattern varied between plant species. Thus, it appears the bacterial strain does not impact damage pattern on granules, but the species of plant starch does influence damage pattern. This is likely because starch granule morphology varies genetically according to plant species, with some species producing granules more crystalline than others (containing more difficult-to-digest amylose than amylopectin), whereas the bacterial community is using similar enzymes (amylases) to attack starch. The mechanisms of ancient starch preservation and diagenesis are not well-understood, but this is an active area of research (Barton, 2009; Haslam, 2008, 2004; Therin, 1998).

### Staining

Another method for examining residues using light microscopy is staining. Dyes mixed with water have been used as a means to stain, and thus identify, archaeological lithic residues or refute/confirm previous microscopic observations. The application of stains requires the residue to be removed from the tool stone surface, mounting the material on glass slides, then application of a stain for viewing with transmitted light microscopy. Several types of anthropogenic residues have been reported as possible to stain: starch, pollen, cellulosic tissues, lignin, resins, suberized and cutinized cell walls, fat, muscle tissue, and collagen (Briuer, 1976, p. 482; Loy, 1994, p. 100; Rots et al., 2016, p. 10).

Kasten (2002, p. 5) defines a dye as “an organic aromatic molecule containing the requisite groups that provide visible color and permit molecular binding to a material,” and use of a dye in solution is known as staining. There are two main groups of dyes used in cell biology and histology: basic dyes and acid dyes (Cormack, 2001, p. 7). A basic dye carries a net positive charge on its coloured

portion [ $\text{dye}^+\text{Cl}^-$ ] and binds to cell and tissue structures that are negatively charged, such as the phosphate backbone of DNA, some cytoplasmic components (ribosomal RNA and rough endoplasmic reticulum), glycosaminoglycans and acid glycoproteins. There are three chemical reactive groups found on biomolecules which can react with basic dyes: phosphate groups of nucleic acids, sulfate groups of glycosaminoglycans, and carboxyl groups of proteins (Ross and Pawlina, 2011, p. 5). Some examples of basic dyes are: Toluidine blue, methylene blue, methyl green, pyronin G (Ross and Pawlina, 2011, p. 5). An acidic dye carries a net negative charge on its coloured portion [ $\text{Na}^+\text{dye}^-$ ]. Acidic dyes bind to cellular and extracellular structures which are positively charged, such as mitochondria, cytoplasm, secretory granules, collagen, and proteins in the extracellular matrix. Acidic dyes are less specific and less precise than basic dyes and stain a broader range of molecules within and around cells (Ross and Pawlina, 2011, p. 5). Examples of acid dyes are: Orange G, eosin, acid fuschin, and aniline blue (Ross and Pawlina, 2011, p. 5).

### *Staining of starch*

Starch residues have been identified or more easily viewed by extraction and application of a number of dyes. In an exhaustive study of modern starch species, Reichert (1913, p. 55) found that different types of starch were stained by dyes differentially. The intensity and uniformity of colouration, as well as certain features of the granule are affected differently. Potassium iodide (KI) in solution (iodine potassium iodide, IKI) produces a blue/purple colour and is the most widely employed stain for the indication of intact native starch granules from archaeological contexts. IKI was used in early lithic residue studies by Briuer (1976), and later by Hall et al. (1989), Loy (1994), Balme et al. (2001), Babot and Apella (2003), and Barton (2007) to detect the presence of native starch granules. Iodine will stain as little as  $1\mu\text{g}$  per ml of starch, the stain strongly adhering to the crystalline amylose component of starch producing a blue colour, and weakly-staining the less-ordered and highly branched amylopectin component a red-purple (Bailey and Whelan, 1961, p. 969; Haslam, 2004, p. 1716). However, other substances besides starch can be

dyed by IKI and starch granules can turn a variety of colours: blue, violet, brown, red, or nearly black (Hall et al., 1989, p. 146). Starch damaged by factors such as crushing and gelatinisation also may not take up the IKI stain. The application of iodine on starch granules interferes with the ability to examine birefringent characteristics such as the presentation of the extinction cross (Hall et al., 1989, p. 147), and dyeing of starch grains with periodate (oxidised iodine), results in a total loss of birefringence after 60 minutes of application of the stain (French, 1984, p. 228). Safranin (safranin O) stains xylem and cork (Locquin and Langeron, 1983, p. 235), and can also be used to stain starch granules (Hall et al., 1989). Stains used to identify modified or damaged starches have also been used to identify archaeological starch residues. Congo red ( $C_{32}H_{22}N_6Na_2O_6S_2$ ) was tested by Lamb and Loy (2005) to identify starch granules which are fragmentary or damaged by gelatinisation. Trypan blue was used by Barton (2007) to identify starch modified by swelling or crushing.

#### *Staining other plant residues*

Other plant residues can also be stained. Sudan III ( $C_{22}H_{16}N_4O$ ) stains cork, cutin, fats, resin, waxes, and oils (Locquin and Langeron, 1983, p. 235). Briuer (1976) tested lithic residues by application of Sudan III to extracted residues, which stains waxy plant cell walls that contain suberin or cutin bright red-yellow.

#### *Staining animal collagen*

Stephenson (2015), Fullagar et al. (2015), and Rots et al. (2016) all report the use of Orange G and Picro-Sirius-Red (PSR) to visually identify residues considered to be collagenous material originating from animal tissues. Stephenson (2015) used PSR stain to identify the presence of collagen (animal protein). A solution of .25% PSR ( $C_{45}H_{26}N_{10}Na_6O_{21}S_6$ , see Figure 2.2) was applied to residues extracted from 10 ten grindstones from Australia. It was claimed (Stephenson, 2015, p. 237) not only that collagen was found by staining, but also specifically that fibres, amorphous collagen, sheet collagen and collagen fibrils were identified microscopically within samples,

although no features diagnostic to the structure of these tissue types were described. Kemp and Nicoll (1996) stained the attachment cones of conodont fossils (extinct agnathan chordates) with a variety of stains that would indicate the presence of collagen and chondroitin sulfate proteins. However, they found the histological structure of the attachment cones indicates that they do not contain cartilage proteins, showing that non-proteins can be incorrectly stained, yielding false-positive reactions.

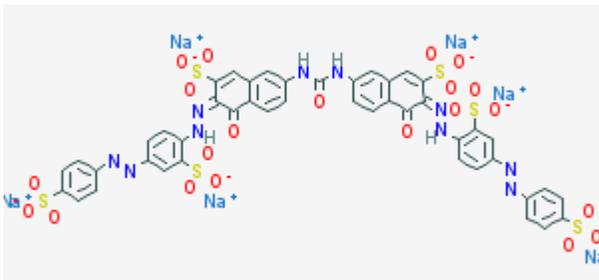


Figure 2.2. Chemical structure of Picro-Sirius-Red (PSR).

### *Staining red blood cells*

There are reports of ancient red blood cells (RBCs, erythrocytes) being identified in situ on stone tools with reflected VLM without any stains or proteomic analysis (Kononenko et al., 2016/8; 2014, 2011, 2008; Loy, 1983; Loy and Dixon, 1998; Loy and Hardy, 1992; Robertson et al., 2009) and also on pottery (Matheson et al., 2009, p. 194), and within the tissues of an Egyptian mummy (Zimmerman, 1973) and the Iceman (Janko et al., 2012). Lombard (2014) recently identified putative archaeological blood residues on on 62,000 year old South African lithics visually. For comparison, Lombard (2014, 81) experimentally applied blood to a replicated quartz tool and allowed it to dry out for several days. Reflected VLM micrographs were then taken that illustrate round biconcave-looking discocyte RBCs in situ – a morphology that would be expected of blood in the fresh state. The putative ancient blood residues identified visually were subject to a presumptive test with luminol, a chemiluminescent forensic product (brand name BLUESTAR®) that reacts with iron-containing catalysts (such as RBCs) in the presence of an oxidising agent such as

hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and exhibits a blue glow in low-light conditions (Caudullo et al., 2017; King and Miskelly, 2005). Luminol will react with iron-based catalysts, such as potassium ferricyanide (Feng et al., 2005). Luminol will also produce false positives with: sodium hypochlorite (found in bleaching agents) (Creamer et al., 2005; Quickenden and Cooper, 2001); turnip, parsnip, horseradish, carrot, and onion (Quickenden and Creamer, 2001). Luminol is also used to identify *Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Salmonella typhimurium*, and *Listeria monocytogenes*, bacteria found in faecal matter and contaminated meat (Magliulo et al., 2007).

The use of presumptive tests such as luminol for the identification of blood by visual means, even at the scene of modern violent crimes, are not always reliable. Hence other more specific molecular approaches are being developed in forensics, such as protein analysis by matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS) (Bradshaw et al., 2014). Determination of blood source can be achieved by examining small differences in the protein amino acid sequence of haemoglobin chains with MALDI-MS. This method has been further refined by use of a shotgun bottom up proteomic approach to increase the reliability of protein identification, yielding information that allows aged blood stains and blood from several sources in a mixture to be identified (Patel et al., 2015).

### *Methylene Blue*

Methylene blue (C<sub>16</sub>H<sub>18</sub>N<sub>3</sub>SCl) stain is typically used for animal tissues including blood, bone marrow, lymph nodes, mammary tissues, nucleic acids, and eye lens (Sabnis, 2010, p. 293). However, methylene blue can also be used on plant tissues, and it stains cellulose, tannin, xylem, and cork (Locquin and Langeron, 1983, pp. 235, 237). Additionally, methylene blue is used as a general bacterial stain because it is cationic (Horobin, 2002a, p. 299).

### *Orange G*

Orange G ( $C_{16}H_{10}N_2Na_2O_7S_2$ ) (also known as orange gelb) has a net negative charge and binds to acidic tissues. Orange G is typically used as a background or cytoplasmic stain and is a component of many biological stains (Horobin, 2002a, p. 114). It has been applied to the staining of fungi, food, drinks, candies, keratin, hair, and skin (Sabnis, 2010, p. 352) and is used industrially to colour textiles, plastics, leather, paper, wood, inks, and pencils (Horobin, 2002a, p. 114). It also has been used to stain plant protein bodies and fecal protein particles for identification with light microscopy (Barber et al., 1991; Perlnemolnar et al., 1985). Orange G is used in clinical contexts and stains erythrocytes, hemosiderin crystals and asbestos bodies, pyknotic nuclei, and cells with highly keratinised cytoplasm (Boon and Suurmeijer, 1996, p. 259). Orange G has also been mixed with other stains to stain pollen (Alexander, 1969; Peterson et al., 2010). Orange G is used as a colour marker to monitor the process of agarose gel electrophoresis and polyacrylamide gel electrophoresis. Orange G is also a pH indicator, showing orange in neutral and acidic pH or red in pH greater than 9.

### *Critique of the use of stains*

Rots et al. (2016, p. 10) claim that the use of stains to identify lithic residues is 'established', citing Stephenson (2015) and her use of Pico-Sirius-Red for the identification of collagen residues on lithics, and Matheson and Veall (2014) for their use of Hemastix (a colourimetric test) with EDTA (a chelating agent) for the identification of red blood cells. Despite the use of a variety of stains to identify archaeological lithic residues, there are some issues. Firstly, any removed residues might actually be sediment adhering to artefact surfaces from the burial environment. This was illustrated with blind tests by Rots et al. (2016, p. 23), which showed that in addition to staining experimentally applied use-residues, it is also possible for incidental contaminants on the tool to be stained, and then incorrectly interpreted as related to tool use. Thus, it follows that a comparison of the stained microfossil items in the residue to stained items in a sediment sample associated with the artefact

should be a requirement to help establish that the items seen in the purported anthropogenic residue are not sediment contaminants. Secondly, stains are not very specific. Any items which fit the prescribed action of the dye will be stained, including not only any potential archaeological residues, but also contaminant residues from modern sources and the burial environment. For instance, Orange G will not only bind to animal proteins, but also plant proteins, and fungi (Sabnis, 2010, p. 352), so it is impossible to confidently identify animal collagen from lithic residues with this stain alone. Stains are not specific enough to identify the presence of particular molecules or compounds, but rather stain a host of possible biological structures. Only general categories of residue types can be identified, such as collagen, plant cellulose, or starch. These general categories do not let the analyst know what species of animal protein, plant cellulose, or plant starch is present, and thus may mask the presence of modern contaminants. So, rather than being a well-established method for the identification of artefact residues in archaeology, stains cannot be considered problem-free. There are more reliable methods available for the rapid screening of potential anthropogenic lithic residues, such as viewing with low power microscopy, or use of a VP-SEM.

## 2.3.3 Chemical characterisation

### 2.3.3.1 Introduction

There are currently three main ways of investigating the chemical nature of lithic residues:

- 1) bombardment with electrons and analysis of emitted X-rays to identify elements present in the sample, called energy dispersion X-ray spectroscopy (EDS or EDX),

- 2) striking the sample with infrared radiation and subsequent detection of the types of vibrations between chemical bonds which are collected and correlated to functional groups (infrared spectroscopy),
- 3) and by separation of atoms and molecules and identification by their mass to charge ratio (mass spectrometry).

These methods have been carried out by extracting the residue or analysing the residue in situ on the stone surface.

### 2.3.3.2 Chemical characterisation: in situ

#### Introduction

There are two groups of in situ techniques of chemical characterisation for archaeological residues: those which identify the presence of specific elements, and those which identify functional groups. Elemental analysis techniques provide basic information as to the chemical nature of archaeological residues. These techniques indicate which elements are present in the sample but not how they are combined. Although elemental analysis cannot provide a specific chemical identification for the residue at hand, they make an excellent first step in order to assess whether the residue is organic or inorganic and can direct further, and more in-depth investigations. The second group of in situ chemical characterisation techniques produce a chemical signature or 'fingerprint' which correlates to functional groups. These can provide powerful detailed evidence which are very specific to the sample tested.

Fourier transform infrared microspectroscopy (FTIRM) and Raman microspectroscopy use infrared radiation (IR) to measure the interaction between light and matter. The unit of measurement is wavenumbers, the number of waves in one cm within the electromagnetic spectrum. FTIR and Raman operate by

bombarding the sample with IR radiation (the excitation energy source) and detecting the characteristic movements or vibrations between atom bonds. This information is correlated to functional groups, and hence these two techniques provide a chemical 'fingerprint' of the compounds present in the residue investigated.

For analytical purposes, the most useful area of the IR spectrum is the 'fingerprint region' at 2.5-15  $\mu\text{m}$  (wavenumbers 4000-650  $\text{cm}^{-1}$ ), within which many organic compounds produce characteristic absorption spectra (Pollard and Heron, 2008, p. 66). "When a molecule is irradiated with electromagnetic radiation, *energy is absorbed if the frequency of the radiation matches the frequency of the vibration,*" (McMurry, 2011, p. 438), meaning that bonds between atoms stretch, compress, or bend in specific molecular motions which allows functional groups to be identified in a sample.

In situ techniques can use analytical instruments which chemically characterise a sample in conjunction with microscopy, such as SEM-EDS, FTIRM, and confocal Raman microscopy, but there have also been reports of the use of in situ ATR-FTIR without microscopy for large macroscopically visible residues (Matheson and McCollum, 2014).

An argument can be made for the development of in situ chemical techniques to analyse residues, as there are drawbacks to destructive analyses. It seems desirable for lithic residue analysts to use methods that will not consume the residue in a single destructive test since it foregoes replication. For rare artefacts of high artistic, historical, or spiritual value, preserving the integrity of the residue and artefact may be essential. For example, descendent groups may be willing to accept scientific investigations that do not require artefacts under their jurisdiction to be drilled, cut, or exposed to chemical solvents. Conservators have explored a number of approaches to analyse museum objects where destructive analyses are not an option (Derrick et al., 1999). Non-destructive approaches to collect chemical information in the field are also being used, for instance to study pigments on

Palaeolithic on stone plaquettes (Roldán et al., 2013), and pigments on Neanderthal perforated shells (Zilhão et al., 2010). Residues that remain in situ on the artefact after analytical analyses leave the possibility of applying improved chemical characterisation methods and technologies.

The ethics of destructive sampling and chemical characterisation of rare residues from stone tools was questioned by Galanidou (2006), with particular reference to GC-MS and py-GC-MS. She highlights the issue that typically only interpretable and definitive results are published in the hard sciences, leading to a false optimistic view of science and that researchers own motives may not be aligned with artefact preservation. Galanidou (2006, p. 358) describes the problem: “As the pressure to build up the sort of scientific and academic capital required to fulfill the demands of academic and funding bodies increase, it becomes more and more obvious that the rules of the game need to be made clear”. Galanidou points out here that although there are general ethical conduct guidelines of archaeological practice, these guidelines need to be made more explicit for particular situations, such as when it is (and isn't) appropriate to carry out destructive sampling.

#### Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS)

When a solid sample is bombarded with the electron beam within the SEM chamber, it emits an X-rays spectrum. Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS, also SEM-EDX or SEM with EDAX) is the use of SEM fitted with a X-ray detector within the SEM chamber to collect elemental information, also referred to as X-ray microanalysis or analytical electron microscopy. Particular interest points of defined dimension on the lithic tool can be selected for analysis by aiming the electron gun beam.

The bombardment of the sample with the incident electron beam causes the electrons associated with each atom in the sample to excite and eject electrons. When electrons from outer shells replace electrons that were removed from the inner

shells, an X-ray is produced. Each X-rays energy is equal to the energy difference between the shells in which the electron transition takes place, which are characteristic to specific elements (Roomans and Dragomir, 2007, p. 508). The ED detector within the SEM chamber then picks up the reading and produces a qualitative spectra showing which elements are present in the selected area simultaneously (Pollard et al., 2007, p. 111). The instrument can produce a distribution map of different elements over the stone surface. It is important to be aware that uneven or rough surfaces will cause scattering of the primary electron beam which will affect the quality of the spectra able to be obtained. The simplest way to ameliorate this problem is to rotate the specimen so the point or feature of interest faces the EDS detector (Goldstein et al., 2003, pp. 476–480). Samples prepared for conventional SEM-EDS are usually sputter coated with carbon (e.g. Jahren et al. 1997). More recently, however, there are studies that are taking EDX spectra from lithic residues without coating the artefacts (e.g. Pawlik and Thissen, 2011; Pedergrana and Ollé, 2014).

There are many examples of the use of SEM-EDS for elemental analysis of in situ archaeological lithic residues (Cristiani et al., 2009; Dinnis et al., 2009; Hardy and Shiel, 2007; Helwig et al., 2014; Monnier et al., 2013; Ollé et al., 2014; Pawlik and Thissen, 2011; Pedergrana and Ollé, 2014; Yaroshevich et al., 2013), the earliest being Cosgrove (1985). He found a resinous pitch like material distributed along the edge of a quartzite flake in Tasmania. Although the specific chemical source was not identified, the residue was interpreted as evidence of a hafting material.

The use of SEM-EDS was applied to experimentally used tools by Jahren et al. (1997), and more recently by Pedergrana and Ollé (2014). Jahren et al. (1997) used six chert flakes on bone and bamboo that were exposed to a treatment to simulate diagenesis during artefact burial: 35% hydrogen peroxide for 24 hours at room temperature. This was an aggressive treatment that removed organic matter and left mineral components. After the exposure to H<sub>2</sub>O<sub>2</sub>, the residues on the chert pieces were analysed visually by SEM and elemental analysis carried out by EDS. Jahren

et al. (1997) interpreted the EDS analysis as able to differentiate between bone and bamboo residues by the presence of qualitative 'elemental signatures'. However, the presence of specific elements in an unknown archaeological residue (such as Al, Si, P, K, and Ca) cannot act to specifically identify the residue, but it may provide a line of evidence that complements other analyses. For example, Jahren et al. (1997, p. 249) show their experimental bone residue as having Ca and P present, but this is not a 'signature' unique to bone, as many substances may also show these peaks on an EDS spectrum, such as limestone from the stone tool cortex, or inclusions of the natural soil in the residue. Particularly problematic is the attribution of silicon to silica rich plant residues, since any usewear polish originating from the underlying stone (e.g. chert, flint, obsidian, or quartzite) is also primarily composed of SiO<sub>2</sub>, and will thus give a silicon signal.

Rots et al. (2017) claim to have confirmed the presence of protein residues, on the simple basis of finding the elements carbon, nitrogen, and phosphate together using SEM-EDS. EDS analysis identifies elements, not how they are combined at the molecular level, meaning that it cannot specifically identify the amino acids or peptides required to confirm the residue in question is a protein. SEM-EDS is better regarded as a screening method for lithic residues prior to further chemical analysis. In this case, current high-resolution methods available in palaeoproteomics might be used to investigate suspected ancient proteins.

Elemental analysis by microprobe in a scanning electron microscope with energy dispersive X-ray spectroscopy (SEM-EDS) is not appropriate for the specific identification of carbon based organic residues because all organic materials are composed of the same main elements (C, O, N) and elemental ratios do not provide a precise method to identify each residue in question. However, SEM-EDS can provide a line of evidence that supports visual analysis or other chemical analyses of organic residues. SEM-EDS might usefully be applied to help differentiate between inorganic residues such as hematite and metals.

EDS is not able to identify a lithic residue specifically, because it does not identify chemical compounds. However, EDS does provide basic elemental information that is collected in seconds about the chemical nature of residues and may help inform further chemical investigations. Overall, variable pressure SEM-EDS is an ideal first step to provide useful preliminary elemental information about each unknown residue, as well as excellent imaging without any optical artefacts from lighting conditions that can occur with reflected visible light microscopy.

$\mu$ -XRF spectroscopy and synchrotron-based 2D  $\mu$ -XRD coupled with  $\mu$ -XRF ( $\mu$ -XRD -  $\mu$ -XRF)

Another technique of elemental analysis by microprobe is laboratory-based  $\mu$ -XRF spectroscopy. Lombardo et al. (2016) used this technique as a preliminary analysis to find out if suspected evidence for pyrite residues in the form of iron and sulphur were present on flint pieces, supporting the identification of these tools as 'strike-a-lights' or fire-starters. They took the positive result from this first test further by using two other further in situ techniques. Synchrotron-based 2D  $\mu$ -XRD coupled with  $\mu$ -XRF ( $\mu$ -XRD -  $\mu$ -XRF) was used to create images that mapped the elements present on a microscopic scale across the surface of suspected strike-a-lights. Additionally, micro-Raman spectroscopy was used as a spot analysis, with all collected spectra showing peaks consistent with pyrite and iron sulfide minerals.

Fourier transform infrared microspectroscopy (FTIRM)

Fourier transform infrared spectroscopy benches have been used in connection with microscopes (FTIRM) to conduct chemical analysis of lithic residues. A FTIRM instrument can be used locate residues with the microscope objective and then to collect IR measurements on particular spots of the residue. It is possible to collect infrared spectra with a FTIRM from a sample as small as  $\sim 10\mu\text{m}$  (Griffiths and de Haseth, 2007, p. 303). The technique can identify organic and inorganic (if infrared active) compounds (Prati et al., 2017, p. 130). With FTIR microspectroscopy, the

residues can be examined in situ on the tool surface non-destructively or removed from the tool.

A FTIRM can collect spectra in reflectance or transmission mode (Monnier et al., 2017b), but when the residue is examined in situ, only reflectance spectra can be collected, since transmitted light cannot usually pass through both the sample and the underlying stone to the detector. FTIR obtains IR spectra of absorption or emission of photons (molecules transition from high energy to lower energy states, and the energy difference is measured).

There are three main modes for FTIR analysis of a solid sample: transmission, absorbance, and reflectance (Prati et al., 2017, p. 130). The information from transmission and absorbance spectra are equivalent, and one can be changed into the other format by mathematical transformation. FTIRM is an attractive approach to develop for lithic residue analysis for several reasons: it is non-destructive, reproducible, no sample preparation is required, very small samples can be analysed in situ, and organic and inorganic residues can be analysed simultaneously (Bunaciu et al., 2014, p. 271). In spite of all these benefits, however, FTIRM is a new technique and there are only five studies so far that have used it to characterise archaeological lithic residues (Cesaro and Lemorini 2012; Monnier et al. 2013; Solodenko et al. 2015; Zupancich et al. 2016; Aleksandrova et al. 2014) and only three studies have investigated the technique from the standpoint of methodological development for application to stone tool analysis, using experimentally-produced residues and substrates (Prinsloo et al. 2014; Monnier et al. 2017; Monnier et al. 2017).

Cesaro and Lemorini (2012) was the first study to use FTIRM to investigate archaeological lithic residues, looking at Neolithic flint and obsidian lithics from two sites in Southern Italy: Masseria Candelaro and Sant'Anna di Oria. For comparison with spectra obtained from the archaeological material, Cesaro and Lemorini (2012) made a reference collection of spectra based on flint and obsidian tools used for

various animal processing tasks such as butchering, hide scraping, and bone working. Contact materials included antler, bone, teeth, shell, fleshy tissues, tendons, marrow, brain, hide, and some combinations of these items. They also took reference samples not only from the residues, but also on areas of the tool showing no residues or usewear. This was done in order to collect a baseline or 'blank' chemical signature of the stone material of each artefact and account for this in their interpretations of the residues. They identified adipocere, lipids, proteins, bone, and calcite residues on archaeological tools.

As Cesaro and Lemorini (2012) focused on the use of FTIRM on animal residues, Lemorini et al. (2014) focused on plant residues, using lithics from three environmental zones and several sites. Lemorini et al. (2014) collected FTIRM reflectance spectra to complement previous low and high power usewear analysis which indicated that archaeological tools from all environmental zones were used for plant working, specifically on wood, reeds, cereals, and herbaceous plants. Lemorini et al. (2014) made experimental flint and obsidian tools and used them on a reference collection of 25 plants to produce spectral standards, created with domestic and wild plant materials from Israel, Italy, and Ukraine. The part processed from each plant in the reference collection (i.e. fruit, seeds, stems, bark) was not noted, although this is a key piece of information since it impacts the resulting spectra. Three cluster groups of reeds, cereals, and grasses were produced based on FTIRM signatures from archaeological material in their study, and then compared to the reference collection. However, many of the reference collection plants did not match the archaeological material. Lemorini et al. (2014) decided to treat lithic residue spectroscopic data statistically by Principal Component Analysis (PCA) because spectral features were too complex to assign, and they found the reproducibility of the experimental data was low. They suggest these problems arise from the small amount of residues present on the tools, overlapping spectral contributions, and the reflectivity and variable micromorphology of the stone.

The study by Prinsloo et al. (2014) was the first to specifically focus on the development of FTIRM for the purpose of investigation of lithic residues. Prinsloo et al. (2014) assessed the use of a FTIR attached to a microscope with attenuated total reflectance built into the optics (ATR-FTIRM) for in situ lithic residue analysis. They used this instrument to collect spectra on specific spots of six experimentally produced tools (quartzite, mudstone, chert, and two types of hornfels) used on blue wildebeest muscle, bone and fat residues. Prinsloo et al. (2014) found that in addition to specific spectral information on residues, FTIRM can also help characterise the mineralogy of the stone substrate. An important conclusion of Prinsloo et al.'s (2014) experimental study is that visual microscopic analysis and FTIRM are complementary, and that study of residues with FTIRM does not constitute a replacement for microscopic morphological identifications. They argue for the use of non-destructive in situ techniques to investigate lithic residues, stating it is not only the most responsible approach, but also the most informative since the micro-context is maintained.

A novel application of FTIRM was used by Horrocks et al. (2014) for the identification of morphologically-ambiguous starch extracted from dental calculus. The spectra collected on the suspected starch granules from teeth were compared with spectra from archaeologically-relevant reference species starches. The FTIRM spectra collected from the calculus were consistent with the identification of starch, but it appears it was not possible for Horrocks et al. (2014) to suggest the specific species of plant(s) involved. The FTIR spectra of the reference starch species presented in the paper do appear relatively similar. More work is required on this topic to nail down if FTIRM has the capability to differentiate species of starch by their chemical structure.

A recent (2017) project led by Gilliane Monnier at the University of Minnesota has also investigated the potential of FTIRM for lithic residue analysis. This systematic experimental project was much-needed, since there have been recent FTIRM reports with potentially inaccurate interpretations of spectra, calling into question

whether FTIRM is a valid method for lithic residue analysis. In particular, Cesaro and Lemorini (2012), Solodenko et al. (2015), and Zupancich et al. (2016), all present spectra of proposed archaeological residues masked by the signal from the underlying stone.

Two publications from Monnier et al.'s project report on experimental plant residues and stone substrates (Monnier et al., 2017a), and animal residues (Monnier et al., 2017b). Monnier et al. (2017a) carefully compared each peak assignment of reflected and transmitted spectra collected on modern plant residues (spruce bark, spruce pith, rosin or colophony (conifer resin), raw taro root, and lemongrass leaves) and stone types (light English flint, dark English flint, Texas chert, dacite, and obsidian). They found reflected and transmitted spectra to be comparable. Monnier et al. (2017a) also compared these reflected spectra to conventional transmission spectra available from a reference library (Kimmel Center for Archaeological Science, Weizmann Institute of Science), which demonstrated the comparability of reflectance FTIRM spectra of the plant residues with transmission spectral standards (KBr pellet). The stone substrates themselves were closely comparable, but contained *reststrahlen* bands (residual rays). Reststrahlen bands can occur when FTIR measurements are taken in reflection mode. They are produced when the frequency of light hitting the sample is nearly equal to the frequency of vibration of the atoms within the sample "when the wavelength of light being reflected by a material matches the wavelength of its absorption". Importantly, Monnier et al. (2017a) also examined the effect of the underlying stone substrate by collecting spectra of residues in situ on the stone. This showed that different stone substrates impart no interference signals to the IR measurements of the residue, but that minor differences located above  $900\text{ cm}^{-1}$  wavelength portion of the spectrum were attributable to differences in the chemical composition of the residues themselves. This finding is of great importance since it implies that FTIRM spectra of the same residue type found on different substrates are comparable.

Monnier et al. (2017a) created an online reference library of the FTIR spectra collected on various substrates, plant and animal residues, together with microscopic images of the residues (<http://z.umn.edu/ftir>). This will be a useful resource for other researchers to compare their FTIR residue data to these standards. Monnier et al. (2017a, 2017b) also systematically compared FTIRM reflectance spectra collected on mirrored slides to transmission spectra collected on sodium chloride (NaCl) plate across all plant and animal residues tested experimentally, showing that they are generally comparable. Practical operational notes for FTIRM analysis of lithic residues brought to light by Monnier et al. (2017a, p. 175), are: 1) narrowing the aperture of the FTIRM to only include the residue essentially eliminates the effect of the underlying stone (reduces interfering signals), and 2) increasing the number of scans of the residue reduces noise. These steps improve the quality of the spectra collected and hence the ease of interpretability of peaks. They also recommend that an exhaustive peak-by-peak analysis of each lithic residue FTIRM spectrum is carried out to account for any differences present between the observed spectrum and the standard(s) used for identification.

The study by Monnier et al. (2013) applied multiple techniques to the characterisation of black residues from three flint Palaeolithic Mousterian tools from the Hummal site in Syria, including FTIRM. These residues were suspected to be hafting residues and they were visible with the naked eye.

The usewear and residues study by Solodenko et al. (2015) used FTIRM to analyse three Palaeolithic tools with visible residues. The lithics were found in a context associated with elephant rib bones with cut marks at Revadim, a Lower Palaeolithic Late Acheulian site in Israel. Solodenko et al. (2015) claim to have identified adipocere on two of three tools examined with FTIRM. Unfortunately, this work has several methodological issues and inconsistencies. They do not explain the method they used to identify residues with FTIRM, such as comparison with spectra obtained from reference collection residues or use of an IR spectral database. No micrographs of the visually-identified residues tested further with FTIRM were

presented, so the nature of the putative residues is unclear. For instance, 'red dots' on a flint flake with no usewear were visually identified, but they do not state if the red dots were investigated with FTIRM, and the gross images of the tool do not show any red residues. Instead, bone residues are suggested to be present on the flake from FTIRM investigation (2015, p. 8), residues that are later described as contamination from the sediment. Their suggestions about ancient worked materials from macroscopic usewear and microwear are also not adequately explained. For instance, the basis for their interpretation of domed polish found on a flint scraper as formed by working animal tissues and wood is unstated. Additionally, Solodenko et al. (2015) do not provide any discussion of the age of the adipocere residues identified on two tools and the mechanisms of preservation that allowed them to survive for 300,000-500,000 years. Finally, it has been shown experimentally that adipocere have different IR spectra depending on the burial context (Stuart et al., 2005).

In sum, the technique is still being developed. Some limitations with the use of FTIRM for lithic residue analysis are beginning to be fleshed out. FTIR applied to residues produces reflectance spectra. However, most reference libraries are of absorption spectra. This is why it is important to establish reference collections of IR reflectance spectra for archaeology, based on used stone tools, both buried and in a 'fresh state'. Use of FTIR equipment can also be costly, which may be inhibitive. The spectra of lithic residues obtained from FTIRM are affected by several variables: the thickness of the residue, the reflectiveness or shininess of the residue, the microtopography of the residue and the underlying stone (flat surfaces giving better results), and the aperture size during spectrum collection. This technique is still being developed (Monnier et al., 2017a). What is known is that the quality and reliability of the spectra obtained can be greatly increased by decreasing the aperture or area exposed to the IR beam so that none of the stone substrate is incorporated, and using a very high number of scans (on the order of 5,000 scans and a spectrum collection time of 50 min).

### Confocal Raman microspectroscopy (micro-Raman)

Another in situ technique for chemical characterisation of lithic residues being used is confocal Raman microspectroscopy (micro-Raman), also called  $\mu$ -Raman spectroscopy ( $\mu$ -RS). Raman spectroscopy is used to identify inorganic and organic molecular species of solid, liquid, or gas samples. A unique 'fingerprint' of a specific molecule is provided by IR and Raman spectra, based on the mass of the atoms, their geometric arrangement, and the types of chemical bonds present in the molecule (Larkin, 2011, p. 2). Like FTIR, Raman spectroscopy is also a type of vibrational spectroscopy and deals with the interaction between light and matter, although each arises from different processes. The techniques are considered complementary since some materials which are Raman active are not IR active, and vice versa.

The sample is irradiated with a visible or near-IR monochromatic laser, and the resulting scattered radiation is measured (ideally at  $90^\circ$ ) with a spectrometer (Skoog et al., 2007). Samples are irradiated with particles of visible light (photons) from an intense laser beam in the UV-visible region that excites the molecules within the sample, causing interatomic bonds to move or vibrate. The energy of the photons applied to the sample causes photons to scatter as both Rayleigh scattering (elastic scattering) and Raman scattering (inelastic scattering), which is a very weak phenomenon (Rull, 2012, p. 2). The incident laser excites the bonds between atoms by disturbing the electronic charge distribution in the molecule (Dietze et al., 2010, p. 24). This energy from the laser causes the bonds between atoms in the sample to move in predictable and measurable ways (e.g. symmetric/asymmetric stretching, bending, scissoring, twisting, and deformation) and causes photons of light to scatter. The charge-coupled device (CCD) detector in the Raman microscope detects photons from the scattered Raman signal (Hollricher, 2010, p. 51). The resulting Raman spectrum plots the difference between the incident photons from the laser and scattered photons, represented as shifts in wavenumbers ( $\text{cm}^{-1}$ ) from the incident frequency (Movasaghi et al., 2007, p. 495). An unknown molecule can be identified because it exhibits a unique spectroscopic pattern of frequencies, or

'fingerprint', (Koenig, 2000, p. 16) that can be recognised by comparison with spectral libraries and published literature.

There are four major benefits of using micro-Raman to investigate microscopic lithic residues: 1) micro-Raman can identify the specific molecular nature of microscopic residues with a high degree of spatial and spectral resolution (Smith and Clark, 2004, p. 1140); 2) it is minimally invasive to the residue and is considered a non-destructive technique, with a tiny laser spot size only  $\sim 1 \mu\text{m}$  burned by the incident laser beam during analysis; 3) the residue can be analysed in situ on the non-uniform surfaces of the stone tool; and 4) no sample preparation is required. While it is not necessary to polish a sample flat (Edwards and Chalmers, 2005, p. 41), it is my experience that the signal-to-noise ratio and the quality of the Raman spectrum is better and the when undulating surfaces of artefacts can be positioned in such a way so that the laser hits the residue of interest at close to  $90^\circ$ . To facilitate this, a bed of moldable Blu-Tack® with a new surface of Parafilm M® covering it may be used on the microscope stage to support and position the artefact. Since the residue can be analysed without removal from the artefact surface, it is still available for other types of analyses, and its spatial position is maintained.

However, there are several limitations of the use of micro-Raman for lithic residue analysis. Firstly, there is the requirement for the gross morphology of the artefact or sample to be of sufficient small size to fit within the working distance of the microscope objectives used. Flat samples are preferred so that a  $90^\circ$  angle can be made with the excitation laser, which improves the clarity and quality of the spectra able to be collected. Obviously, stone tools and other artefacts often have considerable microtopography, so this might pose a challenge if residues are examined in situ. Not all compounds are Raman active, and micro-Raman cannot detect some materials such as sodium chloride. Fluorescence also presents the most common issue in Raman spectroscopy (Weiner, 2010), and can be caused by organic materials with chromophore groups (very often conjugated double bonds) (Casadio et al., 2016, p. 62). Fluorescence interference is caused by photons

emitted from the sample, sample impurities, or sample decomposition during analysis. Fluorescence is more intense than the weak Raman signal, and hides Raman features (Bellot-Gurlet et al., 2006, p. 962). Sometimes fluorescence is generated while trying to collect a spectrum of a particular lithic residue, but can be removed by quenching, whereby the laser burns off the top layer of the residue and reaches further into the residue sample (e.g. Bordes et al., in press). At other times, fluorescence is impossible to overcome, even after adjusting the laser strength and collection time. For instance, when the experimental pieces containing birch bark tar, pine resin, and fish residues were tested, their spectra were very fluorescent with no clear Raman peaks discernible in the attempts made. Micro-Raman is limited by the working distance between the objective and the stage of the microscope available on the instrument being used. Thus, residues on large artefacts may be difficult or impossible to analyse in situ. One of areas requiring further work in the application of micro-Raman to lithic residues is amassing spectral reference libraries which are relevant to archaeological questions. Creating spectra of modern reference collection residues may be very helpful for drawing comparisons and identification of unknown archaeological residues.

Applications of micro-Raman spectroscopy have been used to investigate pigments in paintings (Badillo-Sanchez and Baumann, 2016; Li et al., 2009; Osticioli et al., 2006; Saverwyns, 2010; Tomasini et al., 2012), wall paintings (Holakooei et al., 2016; Nevin et al., 2008; Sawczak et al., 2009), rock art (Gomes et al., 2013; Prinsloo et al., 2013; Stuart and Thomas, 2017), wallpaper (Colomban, 2011), and statues (Cosano et al., 2017). micro-Raman analysis of glasses, ceramics and porcelain (Akyuz et al., 2008; Colomban, 2005; Sendova et al., 2005), silicates in jewellery (Colomban et al., 2006), pigment in dental calculus (Radini et al., forthcoming), anthropogenic pyrite traces on flint fire-strikers (Lombardo et al., 2016), and naturally-formed authigenic pyrite crystals on the surfaces of a stone pendant (Milner et al., 2016). Although Raman is typically used in archaeology to characterise inorganic crystalline materials, it has also been applied to identify residues that are organic, such as bitumen on stone tools (Monnier et al., 2013).

Raman has also been used in a cursory way in archaeological studies without sufficient explanation of how Raman data was interpreted. For instance, Chriazomenou et al. (2014) present a Raman spectrum to support a claim of resin being found on a flint tool from Crete. However, no analysis of the Raman peak pattern, intensity, or the assignments of vibrational modes was provided to support their claim.

Bradtmöller et al. (2016) used micro-Raman to identify both organic and inorganic components in Upper Palaeolithic residues suspected to originate from hafting, identifying hematite, charcoal, and resin. Schmidt et al. (2015) used a combination of microscopic ATR-FTIR and micro-Raman spectroscopy to identify black organic 'tempering-residue' on Middle Stone Age lithics. They found this residue most likely results from a heat treatment of silcrete stone in embers of green wood to improve its flaking qualities, which leaves resin from the fuel wood behind on the tools. Based on experiments, Schmidt et al. (2016) recommend the use of micro-Raman as the best method to investigate tempering-residues. Conservation scientists and art historians have also used Raman for conservation and authenticity studies (Smith and Clark, 2001). A recent study by Bordes et al. (in press) analysed five stone tools using micro-Raman from Liang Bua cave, Indonesia, two tools with dates associated with *Homo floresiensis* (60-100 kyr ago, Sutikna et al., 2016), and three tools associated with modern humans (ranging from 3-12 kyr ago). Bordes et al. (in press) identified iron oxide and manganese oxide deposits on both the tools and cave sediments, showing the presence of contaminants on the tools. Additionally, micro-Raman was used to identify apatite, calcium nitrate, proteins, and lipids on tools, and was also used to confirm the visual identification of plant fibres and starch granules, and investigate an unidentified black micro-residue.

In addition, a type of Raman analysis – surface enhanced resonance Raman scattering (SERS) – has been used to detect specific DNA sequences without enzymatic polymerase chain reaction (PCR) amplification (Graham and Faulds, 2008; Xu et al., 2015), and is proposed for application to highly damaged ancient

DNA (aDNA) in archaeological samples (Feuillie et al., 2014). It might be feasible to use this technique for the analysis of residues on lithics, but this has not yet been explored.

#### In situ methods for usewear

A brief mention of in situ methods used to chemically investigate usewear are included here. These are worth considering because some usewear polishes originate from the worked material and are thus technically residues (Christensen et al., 1998), or are combinations of residue trapped in a hardened silica gel (Ollé and Vergès, 2008), although it should be noted that the precise mechanisms of usewear formation remain to be resolved (Evans, 2014; Evans and Donahue, 2005; Odell, 2001; Ungar and Evans, 2016; Unger-Hamilton, 1984). After Anderson's (1980) SEM-EDS study, the chemical nature of usewear was investigated with ion beam analysis (IBA) techniques by Andersen and Whitlow (1983), followed by Christensen et al. (1998, 1993, 1992). IBA is a blanket term covering several techniques, among them Rutherford backscattering spectrometry (RBS), elastic recoil detection analysis (ERDA), nuclear reaction analysis (NRA), particle-induced X-ray emission (PIXE), and ion channeling spectrometry, all of which provide the elements present in the sample (Nastasi et al., 2015). RBS hydrogen-depth profiles were collected by Andersen and Whitlow (1983) on experimental stone tools with usewear polishes from working various plants and animals and 'neutral' unused surfaces. The hydrogen-depth profile of elk skin usewear did not differ much from the signature of the neutral stone surface, whereas the fresh willow usewear showed a greater difference with the stone. This seemed to suggest that some types of usewear polish cannot be differentiated easily from the stone substrate, whilst others can. Christensen et al. (1998) used micro-PIXE to investigate if usewear is produced by additive, subtractive, or both processes by implanting a ~0.1 µm thick layer of copper (Cu) marker on flint blades then using them to work bone. They found that the Cu marker persisted on the used edge after bone working, so it was not removed by the friction of abrasive force. Additionally, the amount of calcium (Ca) present in the

spectrum after use on bone spiked. Christensen et al. (1998) thus came to the conclusion that usewear formation is primarily an additive process from the worked material to the stone surface. Šmit et al. (1999) used the elemental analysis offered by micro-PIXE to investigate experimental tools (used on bone, oak wood, birch wood, pine wood, trout, rabbit, deer, hide, and unripe wheat) and five Slovenian Mesolithic end-scrapers. Reference points with no signs of polish on the tools were collected. These points differed from the polish areas in that they contained very small amounts of minerals which were interpreted as originating from the worked material, including two main groups of phosphorus (P), Ca and sulfur (S), potassium (K). In terms of understanding polish formation, Šmit et al. (1999, p. 567) suggested that shiny polish deposits are not simple layers on top of the stone, but that they are mixed and diffused or melted into the flint. Evans and Donahue (2005) carried out in situ investigations of usewear on experimental and archaeological lithics using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). In this minimally destructive technique, a microprobe is used to detect elemental concentrations on points of the stone tool surface in parts per million. Disappointingly, SEM-EDS used by Ollé and Vergès (2008, p. 44) to investigate usewear formation found no experimental polish samples showed signals different from the underlying rock composition.

The above research indicates chemical characterisation of usewear polishes has been exploratory and has not developed to a point where standardised techniques are used. Chemical characterisation of usewear polishes is certainly a worthwhile pursuit, and is here considered far more reliable than visual identification of the texture of usewear polish types based on microscopy alone. Whether the elemental analysis techniques used above are appropriate for this task today is questionable in two respects. Firstly, the proportions of elements present may not be specifically characteristic to a single usewear polish type. Also, the chances of encountering a single usewear polish type on archaeological lithics is perhaps doubtful. Rather, elemental analyses are probably complicated by stone tools being used on multiple items (e.g. reeds, wood, bone, meat), which leaves an undistinctive mixed cocktail of

elements behind in the polish. Secondly, more specific (and perhaps more diagnostic) information about the biomolecules and minerals present in usewear polishes can be collected using other techniques such as Micro-Raman and FTIRM.

### 2.3.3.3 Chemical characterisation: extraction

#### Introduction

Archaeological residues can be extracted from stone tools and subsequently tested based on their chemical properties, and there are a variety of techniques that have been used. Extraction with chemical characterisation techniques covered in this section are: FITR, PIXE, GC-MS, DNA techniques, presumptive tests, and immunological methods. FTIR is an IR vibrational spectroscopy technique that has been used to investigate extracted lithic residues, although it has traditionally been applied to identify crystalline and amorphous minerals and organics in sediment samples (Weiner, 2010, p. 265). PIXE, as described previously, is an elemental analysis technique and is one of several ion beam analysis (IBA) techniques, but here it is dealt with in terms of its application to extracted residues.

One of the best methods currently used for assessing organic residues is gas chromatography mass-spectrometry (GC-MS). This requires removal of the residue and is appropriate for the identification of small molecules such as lipids. The technique provides results of high chemotaxonomic resolution because it is possible to characterise an archaeological sample down to individual molecular species. This is done using chemotaxonomy, whereby the presence of a compound (a 'molecular marker') or the distribution of compounds is compared and matched to the closest modern reference. However, interpretation can be complicated by the molecular marker being present in multiple substances, and also the fact that preparation of the substance by people in the past or degradation during burial changes the chemical composition.

Presumptive tests (haemoglobin crystallization, urinalysis test strips) that were used in the early days of blood protein residue detection on lithics are no longer employed, but are reviewed for historical completeness. In the hunt for traces of ancient blood proteins, these tests were replaced by immunological methods (cross-over immunoelectrophoresis, enzyme linked immunosorbent assay, radioimmunoassay), that operate based on the detection of the formation of the antibody-antigen complex. Some studies are still using immunological methods to make claims of ancient proteins on lithics, but overall the area of research has been quiet, perhaps readying itself for what may be the next major breakthrough in the search for blood on stone tools – palaeoproteomics. It has already been shown that proteins can survive for millions of years in association with mineral material (within a biological tissue, ostrich eggshell) (Demarchi et al., 2016), but whether such stunning preservation of protein residues harboured in stone tools is yet to be seen.

#### Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy is a type of infrared spectrometry that measures the absorption of infrared radiation by the sample and identifies the stretching and bending between molecule bonds (McMurry, 2011, p. 434). Information provided by an FTIR spectrum includes the functional groups of molecules and the structure of molecules, arising from deformations between the bonds of the molecule during exposure to the IR beam. Different molecules have different characteristic vibrational spectra because they absorb the infrared radiation at specific wavelengths. Sample preparation of solids for traditional FTIR is destructive, as part of the item being analysed is ground and pressed into a pellet or disc, usually with potassium bromide (KBr). The sample is placed on a small window or holder in the FTIR spectrometer, then exposed to a beam of infrared radiation, which is selectively absorbed by the sample and sensed by the detector. However, it is also possible to prepare a sample by simply squeezing it between two diamond plates in a device called a diamond anvil cell for

analysis (Smith, 2011, p. 177). Once an IR absorption spectrum is obtained, it is compared to standards in a computer database, or a reference spectrum from a known material. Today, FTIR instruments with an attenuated total reflectance accessory attached (ATR-FTIR) have become popular, since they operate quickly with essentially no sample preparation required. Thus, a small amount of the residue of interest from an artefact can be extracted and placed directly on the ATR-FTIR plate for analysis.

FTIR is a qualitative analysis that can be applied to inorganic and organic archaeological material, for instance food residues such as lipids, proteins, and carbohydrates (Cummings, 2007). FTIR has been used by archaeologists to exclude residue contamination from the burial environment by testing sediments associated with lithics to see if the residues identified were present in the soil matrix (Monnier et al., 2013). FTIR is relatively quick and inexpensive to conduct, and has been recommended as a good initial screening technique for archaeological samples to determine if further analytical tests should be carried out (Shillito et al., 2009). For instance, FTIR has been used to analyse extracted black crusts of unknown origin on Neolithic pottery, (Maniatis and Tsirtsoni, 2002).

FTIR has also been applied to lithic residue analysis. For example, Cârciumaru et al. (2012) extracted black residue from one tool and divided the residue for number of analytical tests. Part of the residue was ground with KBr into pellets for standard FTIR. The FTIR spectrum suggested that the black residue was bitumen, based on interpretation of various bands. There were three areas in the spectra suggesting bitumen: 1) bands from 2929 to 1456  $\text{cm}^{-1}$  from  $\text{CH}^3$  and  $\text{CH}^2$  vibrations, suggesting a hydrocarbon structure, 2) bands typical of aromatic structures, from 1600 to 1500  $\text{cm}^{-1}$ , at 1731  $\text{cm}^{-1}$  specific to  $\text{C}=\text{O}$  carboxylic aromatic acids which are oxidation products of aliphatic chains, and 3) bands at 1242 to 1178  $\text{cm}^{-1}$  are probably acid resins, since etheric and alcoholic groups show these peaks. Based on the FTIR results, Cârciumaru et al. (2012, p. 1947) were able to exclude a plant or animal origin for the putative hafting material, since they did not find a strong sharp carbonyl

bands at 1750-1740  $\text{cm}^{-1}$  and 1715  $\text{cm}^{-1}$ , as would be expected of vegetable oils or animal fats.

Yaroshevich et al. (2013) also used FTIR to examine two Upper Palaeolithic scalene triangles from Israel, one with white calcareous deposits on the surface, the other with black carbonaceous deposits. It was not stated if the FTIR spectra were obtained by extraction from the tools and analysed with traditional FTIR analysis, or in situ on the stone surfaces with FITRM. The FTIR result was not conclusive in determining the major organic component of the black residue, but FTIR did show that mineral calcite dominated the white residue. Despite the lack of data, Yaroshevich et al. (2013) suggested that both residues were adhesives deliberately applied by humans.

Helwig et al. (2014) removed samples of residue with scalpels from 16 stone tools from northwestern Canada for FTIR and other analyses. The samples were analysed in transmission mode with either a normal FTIR spectrometer or a FTIR microscope. They compared the FTIR spectra of two types of adhesives from two tools, one golden-yellow residue, and one dark red resin residue. FTIR spectra showed that the yellow residue was consistent with conifer resin of the Pinaceae family, and the red resin residue contained lignans (a large group found in plants), and the major absorptions for pinoresinol (in *Pinus* spp.) matched a published reference spectrum. Additionally, FTIR also identified amide I and II bands suggestive of protein on four artefacts. The combined results of all analyses employed (FTIR, GC-MS, micro-Raman, SEM-EDS, polarised light microscopy) showed spruce (*Pinaceae*) conifer resin was present on 11 of 16 tools sampled and that fats and cholesterol and possible red iron oxide (red ochre) were also present within some of the adhesive residues.

#### Particle-induced X-ray emission (PIXE)

Particle-induced X-ray emission (PIXE) is similar to X-ray fluorescence (XRF) in that

they are both rapid non-destructive elemental analysis techniques and have been used in archaeology to examine rocks, metals, and ceramics (Verma, 2007, p. 1). In both techniques, the sample is bombarded with an excitation source of energy (X-rays,  $\gamma$ -rays, or charged particles) which causes electrons to drop from higher shells around atoms to lower shells and X-rays photons to be emitted. The X-rays are measured by a spectrometer (Johansson, 1995, p. 1). PIXE has been used by archaeologists as part of functional studies of stone tools (1998, 1993, 1992).

The application of in situ microscopic PIXE as a way to determine the elemental composition of lithic residues, previously identified microscopically, was attempted by Langejans (2007). However, the background character of the stone interfered with the readings so she instead extracted samples from six prehistoric tools and four experimentally used tools as reference. The results were not very successful, as 1) the residues slid around between the mylar sheets they were mounted on and fragmented during analysis, so many samples (about half) did not retrieve usable readings, 2) the mylar sheets interfered with the readings, 3) many kinds of unpredicted elements were found in both the modern and archaeological residues, and 4) the results did not provide a good match between modern reference and archaeological materials. In any case, there are major issues with solely relying on elemental characterisation techniques to identify the residue in question. Lithic residues of organic origin are composed of the same main elements in different ratios, but a ratio does not provide a specific 'signature' to provide a specific and reliable identification. Also, the elemental ratios obtained from fresh reference samples will not be similar to the ratios from archaeological residues that have undergone diagenesis, so they are not easily compared.

#### Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography mass-spectrometry provides information about the types of compounds present in heterogeneous archaeological samples. By using unique compounds as identifiers, or biomarkers, the biological origin of the sample can be

identified, sometimes very specifically. According to Philp and Oung (1988, p. 887), molecular biomarkers are organic compounds present in the original source material whose carbon skeleton is preserved throughout the geological record. Peters et al. (2007, p. 3) state biomarkers have three main traits that distinguish them from other organic compounds: 1) biomarkers have structures composed of repeating subunits, indicating that their precursors were components in living organisms; 2) each parent biomarker is common in certain organisms. These organisms can be abundant and widespread; 3) the principal identifying structural characteristics of the biomarkers are chemically stable during sedimentation and early burial. As used by Regert and colleagues (Regert, 2004; Regert et al., 2005, p. 130, Regert et al., 2003a), biomarker refers to the original naturally biosynthesised component, and alteration marker denotes a molecular constituent formed by degradation of the precursor biomarker. Here, this classification is adopted.

In simple terms, mass spectrometers have five major components: the sample inlet, ion source which transforms molecules in the sample into charged gas phase ions, mass analyser that separates ions based on their mass-to-charge ( $m/z$ ) ratio, detector which counts ions, and computer which receives and outputs the data as a mass spectrum (Pavia et al., 2015, p. 108). In the GC-MS, the sample once ionised is carried by a stream of gas through a heated column to the detector. The  $m/z$  of the ions present in a mass spectrum are examined using digital databases within software programs, which are able to quickly compare the peaks in the sample to a library of potential compounds which match. The largest and most widely used mass spectral library is the National Institute of Standards and Technology (NIST) reference database, which is often integrated into mass spectral analysis software. Mass spectra can also be identified by comparison with published literature. Due to their structure, molecules break in predictable fragments when ionised and thus the fragmentation pattern of a compound is reproducible.

GC-MS has been used by many researchers to characterise the chemical nature of lithic residues, in nearly all cases to investigate putative resinous or bituminous

residues that contain lipids. Besides lipids, the use of GC-MS to characterise food carbohydrates within lithic residues has also been reported (Dhakal and Armitage, 2013). This class of residues have been removed physically from the stone tool and then usually exposed to chemical solvents.

Adhesives have been chemically identified from several hunter-gatherer sites. From the Palaeolithic, birch bark tar (Koller et al., 2001; Mazza et al., 2006), conifer resin (Breede River yellowwood) (Charrié-Duhaut et al., 2013), and bitumen (Boëda et al., 2008, 2002, 1996; Cârciumaru et al., 2012; Hauck et al., 2013) were discovered. From the Mesolithic, birch bark tar (Aveling and Heron, 1999, 1998; Roberts et al., 1998), and pine resin and beeswax (David, 1998) have been found. Neolithic sites have contained birch bark tar and pine resin (Regert, 2004; Regert et al., 1998), and animal hide glue (Bleicher et al., 2015).

Mazza et al. (2006), Charrié-Duhaut et al. (2013), Hauck et al. (2013), and Helwig et al. (2014) all used GC-MS as one method among a set of techniques to characterise putative hafting residues on lithics, taking a multi-analytical approach. GC-MS can be applied to investigate potential organic residues and support the interpretation of hafting adhesives. Additionally, a good level of specificity in residue identification is achievable with GC-MS: botanical taxon or geological source of the residue can often be identified by comparison with known modern samples. For instance, tree taxa, such as birch, (*Betula* sp.), from Britain and Italy (Aveling and Heron 1998; Mazza et al. 2006), spruce, (*Picea* sp.) and pine, (Pinaceae sp.), from the Canadian Yukon and Northwest Territories (Helwig et al., 2014, 2008), and bitumen sources from the Bichri Mountains, Syria (Boëda et al., 2008), and Shaaf tar sands, Syria (Hauck et al., 2013) have been possible to identify in lithic residues using GC-MS.

Archaeological resinous residues are a valuable residue type to study with GC-MS since their chemical identification can provide evidence for the collection and production of adhesives, sealants, or the working of fresh resinous wood. A natural tree resin is a type of plant exudate that is viscous or solid, flammable, and non-

water soluble, but lipid or spirit soluble (Langenheim, 2003, p. 45). Secreted tree resin seals injuries from wind, fire, lightning, and herbivory, and prevents invasion of fungi and insects (Figure 2.3). Here, the wide-encompassing term 'resinous residue' is used to mean any sticky substance (processed or unprocessed) that originated from plant exudate or natural coating on the stems, leaves, flower heads or cones.

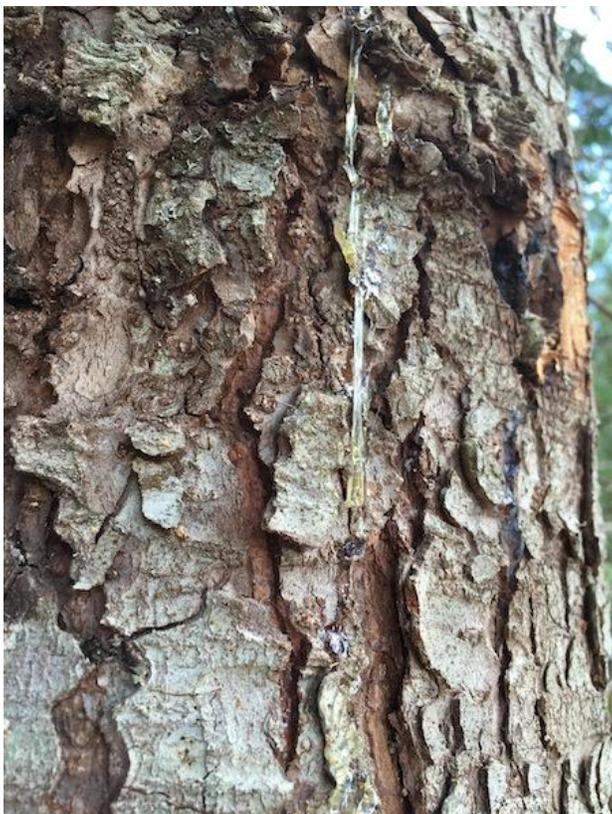


Figure 2.3. A fresh 'bleed' resin readily exudes from a wound in a pine tree. Naturally exuding resins like this may have been collected and used by Mesolithic hunter-gatherers.

Tar is described in early dictionaries as a viscid brown-black oleaginous product of the distillation of pinewood that dries to a flexible film by the evaporation of solvents (Child, 1995, p. 112). 'Tar' is also used to describe the product of the distillation process of birch bark (Aveling and Heron, 1998) and also mixtures of birch bark tar with animal fat (Dudd and Evershed, 1999). 'Pitch' is a term still used sometimes to refer to crude resin, but has a variety of definitions (Child, 1995, p. 112), and due to confusion will not be used here. Processed resin from pine trees is called turpentine

if it is the distilled, volatile mono- and sesquiterpene fraction, and rosin or colophony if it is the nonvolatile diterpene fraction (Langenheim, 2003, p. 307).

Archaeological biomarkers we might expect in Star Carr resinous residues include triterpenoids from birch bark tar, and di- and triterpenoids found in plant resins, which can be diagnostic to genus or species (Evershed, 2008, p. 898). Birch bark specifically is expected to contain the following triterpenoid biomarkers: betulin, betulinic aldehyde, betulone, lupenone, and lupeol (Urem-Kotsou et al., 2002, p. 964). The GC-MS biomarkers of 13 modern angiosperm trees for purposes of chemotaxonomy of ancient wood tars were described by Hayek et al. (1990). Beech, oak, and alder contain  $\alpha$ - and  $\beta$ -amyrin, and  $\beta$ -sitosterol biomarkers (Hayek et al., 1990).

Theoretically, it should be possible to extract and characterise lipids from only microscopic amounts of organic residue on stone tools. Since GC-MS works on a molecular level and is a very sensitive technique, it presumably should be able to detect minute (nanogram) quantities of lipids. However, to the author's knowledge, conclusive results have only been obtained on stone tools which contain large, visible deposits of residue. There may well be a limit or threshold on the residue sample size.

Luong et al. (2017) have recently proposed methodological improvements to organic residue analysis for lithics, using 14 lithics between ~14 000 and 1000 years old from Liang Bua cave, Indonesia. Luong et al. (2017) first conducted low magnification microscopic analysis on all stone tools and identified those which appeared to show traces of use or not. They recommend isolating stone edges containing residues for extraction, thereby allowing testing residues from a general location on the tool. Luong et al. (2017) used chloroform/methanol (3 : 1 v/v) as the solvent for extraction on seven tools that appeared to contain microscopic residues and managed to hold just the edges of interest in a sonic bath with the solvent with tweezers for 20 min, then performed a second total immersion sonication for 20 min.

It should be noted that holding tool edges in solvent requires the use of a fume hood to avoid inhalation of dangerous chemicals, and might not be practical if a large number of lithics were to be tested this way. In addition to testing seven lithics which were thought to potentially contain anthropogenic residues, Luong et al. (2017) tested seven unused lithics as a negative control in which no anthropogenic residues were expected to be found. This was a good control step, but could be furthered by testing sediment samples directly associated with lithics positive for anthropogenic residues, which is likely the best test for potentially contaminating compounds. Luong et al. (2017) used a technique that is more sensitive than traditional GC-MS – gas chromatography-tandem mass spectrometry (GC-MS-MS) and compounds found were quantified. The use of GC-MS-MS allowed monitoring for selected target compounds and a better detection of non-volatile compounds present in the sample.

The GC-MS technique is limited by the fact that it only works to separate organic and inorganic compounds of low molecular weight that can be made volatile (i.e. put into a gaseous state) (Kyle, 2017, p. 132). Non-volatile compounds cannot be analysed with GC-MS, however, liquid chromatography-mass spectrometry (LC-MS) and pyrolysis GC-MS (py-GC-MS) are being used to help overcome analytical gaps (Hübschmann, 2015, p. 2). Compounds for GC-MS analysis must also be thermally stable and not decompose under the conditions employed. These two prerequisites of the sample mean only about 20% of known compounds are able to be analysed by GC (Pollard and Heron, 2008, p. 61).

A further drawback of GC-MS in lithic residue analysis is the requirement that the sample be extracted from the surface of the tool. This is done either by physical removal if the sample is macroscopically visible, for instance by sterile scalpel, or by chemical removal by immersing part of or the whole lithic in solvent to lift the residue. Although GC-MS requires extraction of the residue, it might be argued that destructive sampling is justified in cases where there is high potential to obtain valuable detailed chemical information. For instance, as Monnier et al. (2013, p. 3723) pointed out, Hauck and colleagues (2013) approach to chemical

characterisation of residues by GC-MS was not ideal, since most of the original residue was removed from the Palaeolithic tools tested from the Hummal site, Syria. Monnier and her colleagues argue that lithic residues can be identified chemically using non-destructive methods such as SEM-EDS, FTIRM, Micro-Raman.

There are some other mass spectrometry techniques that may prove valuable in the future for application to characterisation of lithic residues. Liquid Chromatography (LC-MS), also referred to as high-performance liquid chromatography (HPLC-MS), is not limited by the need for the compound to vaporise and be thermally stable, and can be used to characterise a wider range of compounds such as dyestuffs, paint samples, lipids (Degano and La Nasa, 2016), and perhaps proteins (Barker et al., 2015). Pyrolysis coupled with gas chromatography-mass spectrometry (Py-GC-MS) analyses a solid sample directly, by heating it to the point of decomposition to gaseous decomposition products (sample is pyrolysed) prior to GC-MS analysis (Hübschmann, 2015, p. 824). This is useful for analysing non-volatile sample compounds of high molecular weights. With Py-GC-MS only 1 to 100 µg sample size is needed and minimal sample preparation is required (Meier and Faix, 1992, p. 177), usually an extraction of the sample with organic solvent to remove any unbound low molecular weight components that could obscure the high molecular weight components of interest. Py-GC-MS was used by Bonaduce and Andreotti (2009) to examine organic paint binders in artworks, and Scalarone and Chiantore (2009) used it to examine natural and synthetic resins. Evershed, Van Bergen, and Stankiewicz (in Galanidou, 2006) used Py-GC-MS to investigate two microsamples of potential resin from two Upper Palaeolithic flint stone tools (a red-brown microlith and a grey bladelet) from Kastritsa, Greece. No components from either stone tool could be assigned to resin. Interestingly, the fact that no resin compounds were found contradicts an earlier IR spectroscopic analysis of residue from the grey bladelet reported by Adam (1989) that the earliest organic adhesive in Greece was found. The analysis in Galanidou (2006) however, indicated that the second tool analysed contained lignin, and compounds consistent with plant or algae cuticle wax or beeswax. Orsini et al. (2017) used evolved gas analysis mass spectrometry

(EGA-MS), Py-GC-MS, and double shot pyrolysis/gas chromatography/mass spectrometry (DSP-GC-MS) to study proteins in reference materials (egg white, casein, animal glue), easel paintings, mural paintings, and a sample from a Egyptian mummy. Orsini et al. (2017) found that the use of analytical pyrolysis allowed a better understanding of ageing and degradation of proteins. Soft ionisation techniques that cause low fragmentation of large molecules in the gas phase, such as electrospray ionisation (ESI-MS) and matrix assisted laser desorption ionization (MALDI-TOF-MS), are also suitable for nonvolatile polar and high molecular weight constituents (Regert et al., 2003b) such as compounds in ancient beeswax (Garnier et al., 2002). Gas chromatography combustion-isotope ratio mass spectrometry (GC-C-IRMS) measures the carbon stable isotope ratios ( $\delta^{13}\text{C}$ ) in individual compounds and may allow an extra level of specificity to be obtained in characterising palmitic and stearic acids (common to both animals and plants). For instance, GC-C-IRMS is able to differentiate between porcine and ruminant adipose fats, and between lipids from C3 and C4 plants (Bonaduce et al., 2017). The application of these newer techniques may be productive in the future for lithic residue analysis.

### DNA techniques

DNA (both mitochondrial and nuclear) has been shown to survive in ancient archaeological tissues in conditions where microbial and chemical degradation are limited. In terms of DNA residues on stone tools, a few studies have reported the extraction, PCR amplification, and identification of modern ethnoarchaeological mitochondrial DNA (mtDNA) (Kimura et al., 2001), and ancient mtDNA (Hardy et al., 1997; Shanks et al., 2005). Experiments with modern DNA from cow blood applied to stone tools by Shanks and colleagues (2001) looked at the issue of feasibility of DNA recovery from microcracks in the stone, suggesting it is possible to recover DNA even after stone surface washing procedures. DNA in the cow blood residues on obsidian was detected by using fluorescent 4,6-diamidion-2-phenylindole (DAPI) to stain DNA and view it with confocal microscopy. In another experiment, Shanks et al. (2004) used PCR to amplify two target fragments of cytochrome b mtDNA from

stone microcracks in three chert flakes used thirteen years previously for experimental butchery, finding matches with the canid family. These studies have been questioned since the ability of stone tools to hold proteins (and hence likely also DNA) was shown to be doubtful (Craig and Collins, 2002). The PCR technique has not been widely applied to lithic residue studies, and PCR amplification of aDNA is sensitive to contamination from modern sources (Malainey, 2011c, p. 247). PCR also provides little sequence information, only yielding small fragments of up to 100-150 base pairs (Der Sarkissian et al., 2015).

Recent years have seen a major shift in aDNA studies from targeting a few nuclear markers to whole genome sequencing (Der Sarkissian et al., 2015; Shendure and Ji, 2008). Although difficult, it has been shown it is possible to sequence contiguous aDNA sequences and reconstruct entire genomes, and several examples have been published such as woolly mammoth (Miller et al., 2008) and neanderthal (Prüfer et al., 2014). The traditional PCR method used to amplify individually targeted segments of aDNA is now rarely used with the arrival of new DNA sequencing platforms (Orlando et al., 2015). High-throughput sequencing methods (HTS), also known as next-generation sequencing (NGS) or second-generation sequencing (2ndGS), generates millions of sequence reads per run. However, HTS may produce limited lengths of sequences.

Commonly used today in archaeology is shotgun sequencing, where all DNA in the sample is sequenced. Reads are identified by comparing them to a modern reference genome using computer software. Shotgun sequencing is well-suited for metagenomic studies where DNA is present from several sources, such as bacterial communities and ancient microbiomes such as those preserved in dental calculus (Warinner et al., 2014). Shotgun sequencing is also useful for studies of past diet and disease in the past using dental calculus (Weyrich et al., 2017), and understanding the diet and ecology of extinct species such as of the extinct moa (Aves, Dinornithiformes) via coprolite material (Wood et al., 2008).

Greens and Speller (2017, p. 4) have recently noted that NGS approaches may provide new avenues for exploring DNA within stone, and metagenomic analysis has recently been applied to study the microbiomes of brick and stone of historic buildings to better understand biodeterioration (Adamiak et al., 2017; Gutarowska et al., 2015). Shotgun sequencing to analyse lithic residues that potentially contain multiple sources of DNA has not yet been explored. Such a test was beyond the scope of this thesis, but may be worth testing experimentally. This test might include flints used on known materials that are buried for a period of time (or chemically processed to mimic archaeological ageing and degradation), and then compared to the species composition found in the sediment sample collected directly underneath each flint. The ability of aDNA to preserve in non-porous lithic surfaces, perhaps in microcracks on tools found in cold and dry burial environments, requires further study.

#### Presumptive tests

The seemingly excellent prospect of blood protein residue identification from ancient lithics, first by biochemical presumptive tests (haemoglobin (Hb) crystallisation and urinalysis test strips), then other techniques, caused much excitement initially (Bahn, 1987). Several approaches have been used to attempt to identify animal proteins, especially blood proteins.

#### *Haemoglobin (Hb) crystallization*

This method is based on 'salting-out' - any Hb proteins present form crystals when in solution with high salt concentrations. This is meant to be a species specific test where identifications are made based on the crystal shapes formed. Loy (1983) reported animal species identifications such as moose, caribou, grizzly bear, and sea-lion based on the shape of the crystals produced from prehistoric lithics from west coast and boreal forest in British Columbia.

This and other studies using haemoglobin crystallization (Garling, 1998; Loy, 1993; Loy and Dixon, 1998; Loy and Hardy, 1992; Loy and Wood, 1989) were seriously critiqued (Gerlach et al., 1996; Gurfinkel and Franklin, 1988; Remington, 1994; Smith and Wilson, 2001, 1992), due to multiple problems with the technique. Even pure, non-archaeological haemoglobin from humans and analytical grade reagents can produce a variety of shapes due to impurities (Smith and Wilson, 1992), and thus it is not possible to obtain reliable species identifications via haemoglobin protein crystallization on degraded archaeological blood. A high quantity of the original protein must be present for the technique to work, which experiments have shown does not preserve on buried artefacts (Gurfinkel and Franklin, 1988). Also, the crystal shapes are required to undergo X-ray diffraction study to be confidently identified, which requires enough of the protein to be present to make crystals of a relatively large size ( $100 \mu\text{m}^3$ ), containing intact folded protein.

#### *Urinalysis test strips*

Urinalysis test strips (under the brands Chemstrip®, Hemastix®, Accutest®, Ecur-Test®, and Labstix®), are used medically to detect the presence of blood, leukocytes, nitrite, urobilinogen, protein, ketone, bilirubin, glucose, pH, and specific gravity, in urine. Hemastix® in particular has been used to the greatest extent to study putative archaeological blood residues because it is a haemoglobin-specific chemical reagent test strip (Hb-CRTS). When the haem group comes into contact with chemicals on the test strip, a change in colour is seen. Urinalysis test strips were used by Loy (1985, 1983), Fredericksen (1985), and Loy and Wood (1989), to identify the presence of blood on stone tools. The urinalysis test strip method to investigate putative blood residues was called into question due its propensity to produce false positives (Custer et al., 1988; Downs and Lowenstein, 1995). These can come from soil samples from archaeological sites, microbial peroxidases, chlorophyll, and contaminants such as hypochlorite (Eisele et al., 1995; Smith and Wilson, 2001, p. 314). False positives may be easily obtained because the reaction that causes the colour change is based on weak bonding of the chemical reagent

and the protein by electrostatic attraction and/or hydrogen bonds (Gurfinkel and Franklin, 1988); these types of bonds can easily occur with clay, soil, and humic acids (Malainey, 2011b, p. 220).

The use of Hemastix® was tried again by Loy and Dixon (1998), but this time with the addition of a chelating agent, ethylenediaminetetraacetic acid (EDTA). EDTA sequesters metal ions, such as iron-containing materials or compounds (ferric, Fe<sup>3+</sup>), thus removing sources of metal ion contamination so that the test strip will only react with haemoglobin. Loy and Dixon (1998) used Hemastix® with EDTA as one line of evidence to identify blood on Palaeoindian fluted points from eastern North America, although it is not clear how many tested positive with this technique. In her PhD, Williamson (2000) used the Hemastix® with EDTA procedure to detect blood residues within two of 15 pigments on South African rock art. Gibson et al. (2004) reported positive results for blood on two previously washed and handled Middle Stone Age stone tools from Rose Cottage Cave, South Africa, but these results are not discussed. Most recently, Matheson and Veall (2014) report on testing the use of Hemastix® with sodium EDTA on experimental materials including six experimentally degraded samples, 177 experimental replicas, 238 reference samples (on microscope slides) and 39 mixtures of substances. They claim to have validated the Hemastix® plus EDTA technique for screening for blood in archaeological material, but the method will need to be tested by other researchers before its efficacy can be assessed.

### Immunological methods

The use of presumptive tests was followed by immunological methods to identify predefined specific targets in the archaeological material. The researcher chooses which specific proteins they think are possible or likely to be present in an unknown archaeological sample.

Whereas urinalysis tests and Hb crystallization focus on the identification of red blood cells, immunological methods focus on the identification of blood plasma proteins. Ouchterlony, cross-over immunoelectrophoresis (CIEP), enzyme linked immunosorbent assay (ELISA), and radioimmunoassay (RIA) are methods that have been used to detect blood proteins on stone tools. These immunological techniques all rely on the natural binding of antibodies to protein antigens from known animals targeted in the archaeological residue, causing the formation of antibody-antigen complexes, which are detected by various means. The receptor interaction between antibody and antigen is generally thought of as specific; the antibody will in theory only bind to an antigen with the correct type of epitope binding site present on the antigen (Malainey, 2011b). However, cross reactions which yield false positives have been highlighted as a potential source of misidentification, as different proteins can share common sequences of up to eight amino acids (Child and Pollard, 1992, p. 41). In an experiment that modeled the diagenesis of skeletal glycoproteins, present naturally in fossil shells and bones, Collins et al. (1992) demonstrated that several types of sugars spontaneously form covalent bonds to proteins through condensation reactions, yielding brown melanoidins. The melanoidins were cross-reactive with antibodies raised against 75 Kyr fossil shells (*Mercenaria mercenaria*). Thus, the specificity of immunological methods have been called into question, since similar epitopes on related proteins such as serum albumin from humans and bovines can both cause positive reactions.

#### *Cross-over immunoelectrophoresis (CIEP)*

Ouchterlony, immunodiffusion, or gel diffusion is the simplest and least sensitive immune response detection technique. The antigen and antibody are placed at opposite ends of an agar gel plate and allowed to diffuse at room temperature. Cross-over immunoelectrophoresis (CIEP) greatly improved on the ouchterlony technique. A series of paired wells are punched into the agarose gel. The unknown solution (the antigen) is placed into one well and the antisera containing known antibodies is placed in the other well. This gel is put into an electrophoresis tank and

an electric current is applied to pull the antigen and antisera together. The formation of the antibody-antigen complex and the strength of the reaction is qualitatively detected with staining and magnification, giving a 'yes or no' answer (Smith and Wilson, 2001).

The first use of CIEP for the detection of archaeological protein residues was by Newman and Julig (1989), and many other studies also have used CIEP (Allen et al., 1995; Downs and Lowenstein, 1995; Gerlach et al., 1996; Högberg et al., 2009; Kooyman et al., 2001, 1992; Leach and Mauldin, 1995; Newman et al., 1996, 1993; Newman and Julig, 1989; Petraglia et al., 1996; Tuross et al., 1996; Yohe et al., 1991). Yohe and Bamforth (2013) used CIEP on 83 stone tools from Colorado, finding four tools positive for sheep, bear, horse, and camel. The stone tools came from a cache of unknown age, but based on tool typology they believe the tools are Clovis. Yohe and Bamforth (2013, p. 2340) used a solution of 5% ammonium hydroxide to remove proteins from the tools, an extraction reagent which Craig and Collins (2002) found to be ineffective in removing bovine serum albumin (BSA) protein from ground ceramic. Craig and Collins (2002) state that 5% ammonium solution has a high pH, which causes major functional groups to deprotonate, and also displaces positively charged residues from surfaces. Additionally, Craig and Collins (2002) found a 5% ammonium solution applied to BSA for 24 hours actually reduced the amount of BSA present, suggesting that ammonium actually damages protein (Craig and Collins, 2002, p. 1080). Yohe and Bamforth also did not mention if purified antigens and antibodies were used or whether these were tested for specificity.

Shanks et al. (2004) also reported the use of a 5% solution of ammonium hydroxide for successful protein and DNA removal from three experimental chert flakes 13 years after they were used to butcher an animal. Similarly, 5% ammonium hydroxide was recently used to extract aDNA of cougar (*Puma concolor*), from a 1,300 year-old scraper from the site of Bridge River, British Columbia, Canada (Super, 2017, p. 14). Moore et al. (2016) applied CIEP and usewear analysis to 25 Palaeoindian and Early

Archaic chert stone tools from Flamingo Bay, South Carolina, USA. Turkey, chicken (gallinaceous fowl), bovid and deer protein residues were identified.

CIEP was also recently used on residues extracted from 17 tools approximately 250,000 years old from Shishan Marsh 1, Jordan, by Nowell et al. (2016), finding matches for rhinoceros, duck, horse, camel, and bovine proteins. Again, 5% ammonia was used as the extraction reagent to remove protein residues from the stone tools, which is probably ineffective for removal of protein.

### *Enzyme linked immunosorbent assay (ELISA)*

A further immunological method for protein recognition, enzyme linked immunosorbent assay (ELISA), detects the formation of the antigen-antibody complex either qualitatively or the reaction is measured quantitatively. The test antigen is exposed to the antibody labeled with an enzyme that reacts in the presence of a chromogenic or fluorogenic substrate that is optically detected (Cartechini et al., 2017, p. 245). A positive reaction in the immunoassay is the formation of the antigen-antibody complex, denoted by the presence of the colour or fluorescent signal. The strength of the colour signal is a measure indicating how much of the target antigen is present. There are three main types of ELISA immunoassay: direct, indirect, and sandwich (aka capture) (Crowther, 2001, p. 11). The specific procedure varies according to the procedure utilised, but the main general stages are: solid phase (plates, plastics), coating (desorption, binding capacity, non-specific binding, covalent attachment), washing (dipping, wash bottle, multichannel pipettes, reservoir, handheld devices, plate washers), addition of blocking buffer solutions, incubation with reagents (antigens, antibodies, conjugates), and reading (by eye, spectrophotometer) (Crowther, 2001). ELISA has been used by Hyland et al. (1990) to detect cervid blood proteins on a Palaeoindian uniface from Shoop, Pennsylvania; Cattaneo et al. (1992) to detect human albumin in British bone samples from the Bronze Age (2200-1700 B.C. cal.) to the English

Civil War (A.D. 1644); Cattaneo et al. (1994) to detect human albumin in cremated bone from Iron Age, Roman, and Saxon contexts in Britain and Italy; Tuross and Dillehay (1995) to detect proboscidean (elephant family) haemaglobin traces on one of seven Palaeoindian lithic artefacts from Monte Verde, Chile; and by Marlar et al. (2000) to detect human muscle tissue residues in ceramic vessels.

### *Radioimmunoassay (RIA)*

Finally, Radioimmunoassay (RIA), or protein radioimmunoassay (pRIA), is a form of ELISA in which the detecting antibody is radioactively labelled (Brown and Brown, 2011). With RIA, the inhibition of binding of radiolabeled antigen to a known antibody is compared to inhibition by standard solutions of unlabeled antigen (Yalow, 2012, p. 2). The technique is quantitative because amount of radioactivity from the formation of the antigen-antibody complex is measured by a scintillation counter. RIA was first developed and used medically to measure the concentration of insulin in the liquid blood plasma from humans (Yalow and Berson, 1959). Lowenstein (1980) applied the technique to ancient materials, identifying albumin and collagen proteins from fossils, and the technique was also used in the forensic sciences for human blood detection (Butt, 1983). Reuther et al. (2006) used an improved pRIA to detect blood residues on experimental lithics. Lowenstein et al. (2006) also conducted an experimental blind test of pRIA to identify species. The study involved six unidentifiable bone fragments and 43 tools stained with blood from ungulates, carnivores, a fish and a bird, with a list of possible species given to testers for each bone and bloodstained tool. The results of Lowenstein et al. (2006) seem to show a high success rate for the use of pRIA, since all of the bone and 40 of 43 tools species were identified correctly, and only one false positive no false negatives were found. Lowenstein used RIA on historical archaeological bones of unknown origin, at the site of Donner Lake to investigate cannibalism, two of them found to be human (Hardesty et al., 2005, p. 48). RIAs have high specificity, detectability and sensitivity, but were criticised by Hyland et al. (1990) for use in archaeology on the grounds that

expensive equipment is required, reagents have a short shelf-life, there are potential health hazards from radiation, and radioactive materials must be disposed of safely.

### *Problems with immunological methods*

There are several serious critiques that have been raised regarding immunological methods to detect protein residues.

1) It has not yet been established that archaeological protein antigens can in fact survive on stone tools

Ancient proteins are degraded and may not react in the same way modern intact materials do in clinical tests. And yet, some lithic residue researchers believe that blood residues not only survive on ancient stone tools, but are also visible using light microscopy (Fullagar, 2014; Langejans and Lombard, 2015; Lombard, 2014). This stance is challenged by the immunological literature. Antibodies will often *not* react with denatured proteins (Benjamin et al., 1984, p. 68; Landsteiner, 1936), and degradation would be expected in most archaeological situations.

Furthermore, the ability of proteins to be sorbed or bind to the mineral surfaces of stone tools in the first place has been called into question. Craig and Collins (2002) were highly sceptical of the ability of proteins to bind to lithic surfaces such as flint, chert, and obsidian. They noted that surface charge and available surface area of the stone are important factors impacting the ability of the protein residue to bind to the stone substrate, and concluded that lithics have a low ability to possess protein residues.

In addition, Craig and Collins' (2002) experimental study tested different protein extraction techniques that researchers have used to obtain proteins from archaeological material. They showed that previously reported techniques to extract proteins from stone tools, including water, urea, guanidine-HCl, 5% ammonia, PBS,

EDTA, all failed to extract the proteins that were baked onto ground ceramic. However, they did show that the detergent sodium dodecyl sulfate (SDS) works to extract bovine serum albumin (BSA) proteins applied to ground ceramic in Milli-Q water solution, heated at 85°C for 7 days. SDS detergent removes protein from mineral surfaces, but also denatures the protein and is difficult to remove from the protein after binding. The SDS detergent method to extract protein had previously only been used by Gurfinkel and Franklin (1988).

2) cross-reactions are possible due to lack of specificity of commercially available polyclonal antisera

Nonspecific cross-reactions are a major issue in applications of immunochemical techniques to archaeological proteins. Child and Pollard (1992, p. 45) advise that the archaeological antigen and the antibodies used must be purified to be made as pure as possible to avoid incorrect reactions, and antibodies must be tested for specificity and any cross-reactions must be known. In their view, these two steps provide the necessary checks to increase the reliability of immunochemical detection of archaeological proteins. Different species within a genus are actually very closely related genetically and in terms of proteins, with few differences in the protein code between species. For instance, Prager et al. (1980) and Lowenstein et al. (1981) found that there was less than 1% difference between the albumins of an extinct mammoth compared to living African and Asian elephants (Lowenstein and Scheuenstuhl, 1991).

3) false-positives

False-positives originating from the sediment are possible when using immunological techniques. In particular, CIEP has been noted for false positives, caused by chlorophyll, bacteria, modern animal feces, and metal cation such as manganese, copper, and iron oxide originating from the soil (Högberg et al., 2009, p. 1731).

#### 4) blind tests show identification inaccuracies

Downs and Lowenstein (1995) conducted blind tests of archaeological tools and control material using three blood identification techniques, finding essentially no agreement between the results obtained by ouchterlony, CIEP, and RIA. They suggested that broken up, degraded proteins may react in unpredictable ways during testing. Another blind test of CIEP by Leach (1998) of plant residues applied to 19 experimental lithics, groundstone, and ceramic pieces resulted in only one correct identification from the laboratory tested. Again, this result casts considerable doubt on CIEP as an effective technique for residue identification.

5) some results obtained by immunological methods do not agree with other lines of evidence collected at the site (Odell, 2001, pp. 56–59).

Fiedel (1996) outlined several cases where the immunological results at the archaeological site did not agree with other lines of evidence. At the prehistoric site of Vidiitshuu (Trout Lake), Northwest Territories, no trout was found by CIEP (Nolin et al., 1994), which is incongruous with the fact that trout are abundant in the lake and also the finding of fatty acids from fish in the GC-MS and thin-layer chromatography analyses of two humus samples collected from two pit features. CIEP detected chickens from several Archaic period sites in Oregon (Fiedel, 1996, p. 141; Williams, 1992). These Native American sites pre-date European contact, so the presence of chickens does not fit with what is known archaeologically (and ethnographically). Another improbable CIEP result was obtained by Newman and Julig (1989), who found guinea pig antisera produced a positive precipitate from one stone point from the Palaeoindian site of Cummins, at Thunder Bay, Ontario.

6) Immunoassay requires destructive sampling from the residue.

Depending on the artefact, extraction for immunoassay may not be feasible or desirable. In cases where the residue items under investigation are microscopic, the small quantities available may not be sufficient for accurate collection.

### *Future work on ancient proteins*

In sum, all protein residue extraction and identification techniques used on lithics to date in archaeology are controversial, but some researchers remain optimistic about their application. Proteins, and potentially also DNA (Shanks et al., 2001), remain attractive objects of study within lithic residues since they are able to offer identifications of the highest specificity. Archaeological use of Hb crystallization to identify blood on stone tools has been abandoned. It also appeared that the use of urinalysis test strips was completely discredited, but Matheson and Veall (2014) recently suggest Hemastix has potential when used with the addition of EDTA. Successful results using CIEP were also reported by Seeman et al. (2008), and more recently by Yohe and Bamforth (2013), and Moore et al. (2016). Lombard (2014) reports success with using luminol for in situ identification of ancient red blood cells. However, luminol is a presumptive test, not a confirmatory test, so further chemical characterisation techniques are required to prove blood is present.

Immunochemical methods have been argued as providing a simple and cost-effective way to identify proteins in heritage materials, particularly for paintings and museum objects (Cartechini et al., 2017, p. 242). However, immunological methods to investigate ancient proteins provide only indirect identifications of defined targets, and antibody-based immunoassays are set to be eclipsed by new mass spectrometry techniques developing in the field of palaeoproteomics. Child and Pollard (1992, p. 45), and Malainey (2011b, p. 232) consider immunological methods suitable only for screening for archaeological proteins, as a starting point to which biochemical verification should follow. Thus, immunological methods can no longer be considered to provide decisive detection of proteins.

Protein sequencing with mass spectrometry may offer some exciting options for protein identification on lithics in the future. Proteins may be good items to study because they preserve over a wider spectrum of archaeological settings than aDNA and they are specific to particular tissues (Cappellini et al., 2014b). Protein analysis through mass spectrometry can be used to identify taxonomic origin. For instance, peptide mass fingerprinting (PMF) can reveal family or subfamily taxonomic levels (Buckley et al., 2009; Buckley and Kansa, 2011; Solazzo et al., 2013; Stewart et al., 2013). In this method, proteins in an unknown sample are digested into short peptides, that are separated and quantified by mass spectrometry. Examples of mass spectrometry-based techniques include gel electrophoresis combined with liquid chromatography tandem-mass spectrometry (LC-MS-MS) (Rao et al., 2015), matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Scalarone et al., 2005; Solazzo et al., 2013), and desorption electrospray ionisation mass spectrometry (DESI-MS) (Heaton et al., 2009). Good results have already been obtained experimentally from a range of in situ protein residues on flint with DESI-MS, with optimised conditions found to be an angle of 70° and a distance of 5 mm between the stone tool surface and the nebuliser (Heaton et al., 2009, p. 2150). Protein sequencing also appears promising (Cappellini et al., 2014b), and use of direct sequence comparisons of established reference databases can provide even higher taxonomic resolution (Cappellini et al., 2012, 2014a). The sequence of peptides in mass spectra are inferred by using software databases (Lu et al., 2009). These new techniques allow a bottom-up approach to be taken with unknown proteins in complex mixtures.

A promising way forward in the area of protein residue analysis from stone tools is to focus on the development of successful extraction techniques and then sequence the peptides. In this way, the analysis of proteins from lithics can move beyond the focus on on blood proteins, and work with other protein types might prove fruitful. Collagen protein in bone is highly repetitive and survives in many archaeological deposits, as evidenced by the success of its use in stable isotope analysis and <sup>14</sup>C dating (Collins et al., 2002, p. 384). Aiming to recover collagen, which is the most

abundant protein in the animal kingdom (Lodish et al., 2012), found in bone, dentin, connective tissue, cartilage, ligaments, skin, muscle, and other locations, may be a good macromolecule to focus research upon, although the survivability of collagen from non-bone sources is not well studied.

#### 2.3.3.4 Chemical characterisation: summary

Each of the techniques reviewed above has limitations. For this reason, at this stage of development of lithic residue analysis, it is justified to use multiple independent techniques to allow cross-checking the information obtained by one technique against others. Of the emerging residue techniques, SEM-EDS, micro-Raman, and FTIRM appear to hold the most promise as in situ techniques, and GC-MS is the extractive technique of choice due to the high specificity with which biomarker compounds can be identified. All four methods provide lines of evidence which are complementary to visual analysis of lithic residues by traditional reflected VLM. The impact a technique has on the archaeological material can be an important consideration for rare or unique artefacts, or in cases where indigenous groups call for the use of non-destructive methods. The search for archaeological protein residues on stone tools represents a special case, with extraction and identification methods needing to be tested further to demonstrate reliable results.

Some studies have used new techniques to characterise residues chemically. These chemical techniques are at their best when merged with results from traditional light microscopy residue identifications (e.g. Monnier et al. 2013). The application of chemical methods is desirable because they provide a means to test residues identified visually. Whilst the development of new chemical techniques applied to lithic residues holds promise, they are only as good as their interpretive value for the research questions, and should not be used uncritically. Table 2.1 shows some of the possible benefits and drawbacks of the use of new chemical characterisation techniques for the study of lithic residues.

Table 2.1 Advantages and disadvantages of application of chemical characterisation techniques in lithic residue analysis.

<b>Advantages</b>	<b>Disadvantages</b>
Can help identify residues more securely, particularly residues that have no diagnostic structure	Training needed to operate equipment or tethered to the expense of hiring technician
Can provide independent lines of evidence to test visual observations of residues	Risk of inappropriate use of results. Overinterpretation of results, selective presentation of the data
Offers an avenue for collaboration between archaeologists and researchers in chemistry, physics, biology, soils science, and materials science	May need to seek expertise for interpretation of results. Results may be difficult for other researchers to assess if they do not have experience with the techniques used

## 2.4 Methodological critiques

### 2.4.1 Introduction

What constitutes best practice in residue analysis? Residue analysis is a developing area in archaeology, and there is no single set of rules or ‘recipe’ of procedures to pick off the shelf. Researchers differ in the methods and techniques they employ, and this is partly due to the differing specificity of questions being asked of the residues, and limited by the equipment they have at their disposal both in terms of microscopes and chemical analytical techniques. Yet, there is some level of agreement developing as to appropriate methods. There are nine areas of methodological issues identified that are reviewed here in analytical order: 1) quality control, 2) the use of artefacts from insecure contexts (anti-contamination), 3) collection and use of soil samples, 4) in situ versus extractive approach, 5) storage,

6) cleaning, 7) documentation and mapping, 8) problems with identification by analogy, and 9) the presentation of visual results. The choice of methods used in this study (detailed in Chapter 4) cannot be described without due consideration of these methodological issues.

## 2.4.2 Quality control

The wide diversity of quality present in lithic residue studies is perhaps a result of the relatively unstandardised discipline of residue analysis. Some functional studies are unfortunately still suffering from basic reporting issues such as failure to report the number of artefacts analysed by each particular method. It is also important to report all findings, not just a selection of the artefacts that had 'successful' residue results within a study. We cannot assess the success of each residue analysis method if the artefacts which returned no or uninterpretable results are omitted. Furthermore, the same residue analysis methods need to be applied to all artefacts wherever possible. In other words, the whole tool assemblage sample needs to receive the same analysis treatment. However, it may be difficult to apply the same residue method to the whole sample where expensive or very time-consuming techniques are involved, but it should always be possible where residue identification is made on a morphological basis using traditional visible methods.

## 2.4.3 Use of artefacts from insecure contexts

Should we only use artefacts specifically collected with residue analysis in mind? One of the main methodological trends emerging is tighter control over residue samples from the point of artefact recovery to analysis, taking steps to prevent post-excavation contamination (Barton and White 1993, 171; Hall et al. 1989, 148; Lints and Surette 2010; Livarda and Kotzamani 2006; Newman and Julig 1989, 120-121; Cummings 2007). In this way, the handling history is well-documented and

controlled. The main areas of convergent recommendations are: 1) bagging artefacts individually and immediately as they are exposed on site to avoid handling, 2) leaving artefacts unwashed and without labels that adhere to their surfaces. Some researchers argue that good residue results can be obtained from artefacts which have undergone curation processes such as washing and labelling (Barton 2007; Cârciumaru 2012; Helwig et al. 2014) and some researchers have performed residue analysis on surface-collected artefacts which have been exposed to the elements (Barton, n.d.; Kononenko et al., 2015). However, the use of artefacts that were not collected from primary contexts and/or artefacts that were previously handled and curated can complicate microscopic analysis. Identifying contaminants such as corn starch from artefacts adds a level of background 'noise' to be dealt with, which is costly in terms of time required to locate and examine each one during microscopic analysis. It is recommended to avoid these issues by using unwashed artefacts that have never been handled prior to residue analysis. To obtain artefacts with minimal exposure to modern contaminants, residue analysis must be planned from the outset as part of the archaeological research program and excavation design, not as an afterthought.

Being able to exclude modern contamination as much as possible is worthwhile with the design and use of standard anti-contamination protocols. However, maintaining high levels of anti-contamination control while doing SEM and in situ chemical characterisations, such as with FTIR microscope, is difficult. For instance, the carbon sticky stub used to mount the lithic for viewing in the SEM chamber leaves a modern residue behind on the sample.

#### 2.4.4 Collection and use of soil samples

There seems to be a general consensus that soil samples should be taken at the same time as the artefacts for analysis are recovered. This is done so that artefact residues can be compared with any residues found in the soil, and environmental contaminants can be identified (Wadley et al., 2004, p. 1492). However, the best

locations to sample and an appropriate number of comparative soil samples that should be taken is less clear. Researchers have taken soil samples off site, from the topsoil, and within the context or matrix where artefacts were recovered (Cummings, 2007; Mercader, 2009; Wadley et al., 2004). Mercader (2009, supporting material, p. 3) systematically tested the sediment in direct contact with the stone tool surface, by removing the sediment during jet bath cleaning and subsequent mounting on slides.

Examining past research, it is clearly essential that the collection and testing of soil samples associated with each lithic is a key component of conducting any residue analysis. Regardless of the type of residue (microscopic or chemical) being investigated, it is important to compare the natural burial environment to the residues found.

If a residue found on a tool surface is also found in the associated soil sample, should it be interpreted as present on the tool due to processes unrelated to tool use? Hardy et al. (2001, p. 10973) eliminated all tools from analysis where the same residue was found in both the soil and on the tool. This may be the strictest stance to take, but the most scientifically rigorous. Earlier studies by Hardy and Garufi (1998, p. 179), Lombard and Wadley (2007a), and Wadley and Lombard (2007) emphasised that the frequency of the residues on the tool surface is a key way to inform the interpretation of contaminant versus authentic use-residue. The argument was that the presence of 'few' of the residue type should be interpreted as contaminants, and 'many' of residue should be interpreted as a clue to their being archaeologically authentic. However, arguments for genuine anthropogenic residues based on frequency are no longer convincing and quantification of residues may be impossible in cases where there are no individual structural units or cells that can be discerned microscopically, for instance resinous and fat residues. Rather, sediment samples associated with lithics need to be tested and compared to the residues found on the analysed tools.

## 2.4.5 Storage

Studies examining different soil storage conditions have been conducted in palynology and soil biochemistry. For example, DeForest (2009) conducted an experiment to test enzyme activity in acidic soils that were cold stored at 4°C and -20°C. Three types of forest soils of pH 5, 4.1, and 4.0 were sampled at 2, 7, 14 and 21 days. Interestingly, he found no major difference in enzyme activity between soils stored at 4°C and -20°C, which are nearly identical to the fridge and freezer temperatures debated for the storage of Star Carr artefacts. Although the soils tested in DeForest's storage experiment are of different pH and composition than the soils in the wetland (about pH 2.4) and dryland (about pH 6.1) areas of Star Carr, studies like this may provide a guide in the absence of site-specific storage experiments.

Often, no special care of artefacts for residue analysis is taken beyond the standard practice of storing unwashed artefacts in airtight zip polyethylene bags and not handling them until microscopic analysis, e.g. Lombard (2008, p. 29); Wadley and Langejans (2014, p. 31); Wadley and Lombard (2007, p. 1002).

There is hardly any information available concerning why some residue analysts refrigerate their samples, but it is probably an attempt to maintain original burial conditions as closely as possible prior to residue analysis. In her thesis, Langejans (2009, pp. 72–73) recommended storing artefacts in sealed containers in a refrigerator with dry cold conditions, with the logic that doing so would slow microbial decay. She also refrigerated experimentally used tools and slides containing residues, excluding transport (Langejans, 2010, p. 974). Thirty Swedish Early Neolithic artefacts were collected and analysed by Högberg et al. (2009, pp. 1728–1729) from the site of Almhov-delområde 1. The stone tools were collected using powder-free antiseptic gloves, were not cleaned, placed in a covered box immediately, and then into a fridge. Högberg et al. (2009, 1729) did not make their reasoning for refrigeration explicit, but state “This treatment made sure that the artefacts and soil control samples were not damaged and were exposed to as little

contamination as possible”. The reason for cold storage of the lithics and associated soil samples is likely the belief that refrigeration discourages fungal growth and bacterial decay, although there are no tests available yet to confirm this.

## 2.4.6 In situ vs extractive approach

Veall and Matheson (2014, p. 14) show removal of the residue from the artefact as a necessary step in the process of residue identification. Fullagar and Matheson (2014, p. 7064) also present residue analysis as including steps of low and high power microscopy which culminates with the residue removal for microscopic or further analysis. Removal of the residue in question may take place by pipette, sonication, dry removal (such as a sterile razor blade), or with solvents. These processes to extract the residue can harm the artefact by introducing modern usewear or modern chemical residues, both of which may also compromise or influence the interpretation of tool function. However, there are other non-destructive approaches currently being used and developed which preserve residue context and have a minimal impact on limited archaeological resources. For example, work by Wadley et al. (2009), Lombard (2011), Langejans (2012a, 2011) have identified residue types in situ on tool surfaces while importantly conserving contextual information, such as related ‘suites’ of residue types and usewear. This information is brought together as multiple lines, which gives a better picture of tool function than a single residue type alone. In situ chemical approaches that do not require extraction are also considered non-destructive. For instance, Monnier et al. (2013), Prinsloo et al., (2014), and Lemorini et al. (2014) all advocate the use and development of non-destructive FTIRM spectroscopy for the investigation of lithic residues.

## 2.4.7 Cleaning

For residue analysis, cleaning of artefacts may not be obligatory, depending on the nature of soil matrix from which the artefacts are recovered. In fact, some analysts suggest it is essential that no washing take place before the examination of residues because it may unintentionally remove them (Hardy et al. 2001, p. 10973). However, other researchers have found that residues may be visually masked by fine sediment when they are examined in situ (Mercader, 2009 supporting material). Fullagar (2006) recommends that artefacts be analysed microscopically for residues such as phytoliths and starch prior to any cleaning. However, this is not always possible or practical, particularly when excavated lithics contain heavy deposits from wetland contexts.

Several methods of artefact preparation prior to microscopic examination have been used by residue analysts, ranging from no washing whatsoever to use of harsh chemicals and sonication. An assessment of the diversity of artefact cleaning protocols indicates that there are no standardised methods for cleaning.

Early cleaning methods – designed for usewear studies – were more aggressive with the use of chemicals to remove sediments and sometimes organics as well. Chemical cleaning is not advised as Van Gijn (1990, p. 5) showed it can remove residues from flint surfaces. She used an experimental bone working tool cut in half to compare two cleaning conditions 1) a wash with water and detergent, and 2) chemical cleaning (10% HCl solution, rinse with KOH). Both samples were analysed with a scanning electron microscope (SEM) equipped with an energy dispersion analysis system (EDAX, today usually called EDS), to examine elemental composition of spots on the stone surface. Van Gijn found that the sample that had only been washed with detergent and water maintained high peaks of phosphorus, carbon, and calcium, which were bone residues, whereas the elemental traces had been obliterated from the sample that underwent chemical cleaning, which showed silica peak only, as did the surrounding flint surface. Van Gijn also experimentally scaled pike (a soft fish) and then cleaned the tools with 10% HCl, noting the rough

polish and striations that were present prior to the treatment disappeared after chemical cleaning.

Borel et al. (2014, p. 47) examined experimental and archaeological artefacts for usewear and residues before and after cleaning. Samples were put in an ultrasonic bath with neutral phosphate-free detergent (Derquim® LM 02) for 10 minutes, then in an ultrasonic bath in acetone for 2 min, following a protocol used by Ollé and Vergès (2008). This is also an aggressive approach to cleaning that is better suited for the study of usewear only, as the ultrasonic bath removed residues.

The use of brushes to remove excess sediment from artefacts has been used by several workers, but there are risks of introducing abrasive marks to the tool surface, adding contaminants, and cross contamination between artefacts. Indeed, Lombard (2007, p. 409) found plastic fragments or toothpaste remains on South African Middle Stone Age tools that had been brushed. Langejans (2011, p. 986) removed sediment attached to experimental flakes by lightly brushing them. It was not stated if the same brush was used on all experimental flakes or what type of brush used for cleaning (i.e. synthetic or animal hair), which could have introduced contaminants to the flakes.

Artefacts can also be exposed to a small stream of water from a squeeze bottle to remove soil (called a jet bath). Jet bath cleaning of stone tools is gentle and an effective means of cleaning since it does not impart any marks or introduce any contaminants when ultrapure water is used. A jet bath with deionised water was used by Mercader (2009, supporting material) to remove loose sediment adhering to Mozambican Middle Stone Age stone tool surfaces, that was microscopically analysed separately for starch. However, the results of the analysis of sediment in contact with each tool in Mercader (2009), was not reported in the paper or supporting material. In consequence, we do not know if any starch (which was the target archaeological residue in the study) was found in the sediment and thus

cannot assess the if starch from the sediment contributed to the starch found on the lithics.

#### 2.4.8 Residue distribution mapping

Traditionally, it has been argued that documenting the location of residues on the artefact surface and establishing their spatial relationship to usewear is the best way interpret tool function (Hardy et al., 2013; Hardy and Moncel, 2011; Langejans, 2011; Langejans and Lombard, 2015; Lombard, 2004; Lombard and Wadley, 2007a; Rots et al., 2011; Rots and Williamson, 2004; Wadley and Lombard, 2007).

Several methods have been used to map residues, including the use of various grid patterns overlaid on drawings and photographs of the dorsal and ventral sides of each tool analysed (Cnuts and Rots, 2017; Langejans, 2012b, p. 211; Lombard, 2007, p. 411, 2004, p. 39; Rots et al., 2006, p. 937; A. L. van Gijn, 1990, p. 16; Wadley and Langejans, 2014, p. 26). Here, the underlying assumption has been that residues left by human action are always distributed on lithic surfaces in meaningful patterns which analysts today can reconstruct.

However, the idea that humans deposit residues in predictable ways on the lithic cannot be assumed. Rots and Williamson (2004, p. 1298), found that experimental residues occurred in locations on the tool away from working edges. A recent large-scale experimental study involving working 15 plant taxa with 99 pieces of red jasper by Xhaufclair et al. (2017) found that residue distribution was typically random across lithic surfaces and not spatially associated with usewear. Xhaufclair et al. (2017) indicate that real residues can occur in unexpected places on the tool and should not automatically be considered contaminants. This is an important finding that breaks with previous thought, which regarded patterned residue distribution and association with usewear as important preconditions to designate residues as anthropogenic (e.g. Hardy and Garufi, 1998). Further experimentation is required to build on the

results of Xhaufclair et al.'s (2017) study, but the fact that the ideas about residue/usewear patterning are being explored and questioned is beneficial for the methodological development of residue analysis.

There are good reasons to maintain a record of residue locations on stone tools which are being analysed for residues. Noting locations of residues on a map allows them to be reexamined and relocated for further microscopic analyses, for instance SEM. Mapping during preliminary microscopic analysis also simplifies sampling for chemical analyses at a later time. Location documentation of residues should preferably occur on photographs rather than drawings which are subject to human error or misrepresentation when reconstructed.

#### 2.4.9 Problems with identification by analogy

The basic method for microscopic identification of residues involves the use of modern reference collection images of residues to draw analogies with the archaeological materials. This has inherent problems since the 'best match' available between the reference collection and archaeological material does not exclude other possible items which are not in the reference collection. Reference collection images are usually in a fresh state and thus do not reflect the taphonomic processes which took place over hundreds of years or more on the archaeological material. Thus, the comparability between modern and archaeological residues is easily called into question due to degradation differences, especially where residue morphologies are ambiguous. Some researchers have created diagenetically altered residues experimentally to provide more realistic proxies to compare with archaeological residues (Anderson, 1980; Jähren et al., 1997; Langejans, 2009).

As multiple examples in this chapter have shown, assertions of microscopic traces of blood, animal tissue, and bone residues identified on stone tools many thousands of

years old are still being published, even when there are no diagnostic biological structures present to allow visual identification.

A fuller recognition of the difficulties surrounding microscopic residue identification and reliability are beginning to be flagged up (Monnier et al., 2012), as well as the issue of identification of contaminants (Bordes et al., in press; Crowther et al., 2014; Haslam, 2006b; Pedergrana et al., 2016). Misinterpretation of residues can quite easily occur. Arguably, the trend in recent years shows that the standards for residue identification are overall becoming higher, with multiple techniques being used to confirm identifications. The correct identification of archaeological residues cannot be accepted without scepticism when the method of identification relies on microscopy alone.

There is a problem of lack of available standards for archaeological microscopy generally, making it difficult for researchers to check reported results (Killick, 2015). Some progress in this area is being made with the publication of online databases which are growing as more researchers contribute to them (e.g. UCL phytolith online gallery, PhytCore Phytolith Database, Starch Grain Database, starch-id.edu).

The problem with analogy also extends to chemical residue analysis. Often an analogy is drawn between reference library spectra available in databases and the archaeological signal. This is an issue because most references are 'pure' samples, not archaeological ones. Archaeological samples are likely to be heterogeneous complex mixtures which can be more difficult to decipher and identify with confidence.

## 2.4.10 Presentation of visual results

Good presentation of microscopic and macroscopic images is important for residue studies. After all, if the evidence of residues is presented visually, the reader must be

able to see it. Images are often presented as small figures in publications, and sometimes not in colour or in suitable quality to portray key morphological features of degraded archaeological residues. This is problematic because readers cannot judge the visual evidence presented at the same standard that the researcher observes. The issue of the reader not being able to 'see' what the researcher does is a methodological one, as it diminishes the ability to compare and replicate results across the discipline. Limitations of the printing process such as small size, grayscale, or low pixel count of images can be overcome by publishing image results online so they are accessible digitally.

A second, albeit fading, presentation issue is the illustration of residue images taken at the metallographic microscope without z-stacking, which are 'one shot' images. One shot images do not represent the whole area observed by the researcher during analysis, since multiple depths of field cannot be presented in focus simultaneously. Again, this undermines the reader's ability to visually comprehend the results being presented. As imaging technology improves and becomes more widely available and affordable, the issues around presentation of residue visual results are lessened. Advancements in imaging technology and increasing digital availability of information is certainly a trend impacting all disciplines that collect and analyse images.

Almost all usewear and residue studies make use of visual morphological characteristics as the first, or only, step for identification. Since the correct visual identification of the type of usewear and/or residue is the basis for archaeological interpretations, a clear demonstration of this evidence should figure centrally in publications. Many studies could benefit readers much more by illustrating the match that was made between reference collection or replica images, and annotating diagnostic features, and the archaeological lithics.

## 2.5 Conclusion

This chapter has presented a wide variety of techniques that have been used to analyse lithic residues. If we want residue analysis to become more widely accepted in archaeology and the scientific community, we need to better develop standards of methodological practice. Rather than portraying the methods of artefact residue analysis as well-established and accepting them uncritically, residue analysts should more carefully consider every aspect of the methods employed and be honest about their limitations. Archaeological researchers should not be expected to become biochemists (Nigra et al., 2015). Rather, close collaborations with specialists in the sciences on archaeological problems can bridge two areas of expertise and provide exciting, but also reliable, results.

# CHAPTER 3 LITHIC RESIDUE ANALYSIS IN THE MESOLITHIC

## 3.1 Introduction

There are few residue analyses that have been carried out on Mesolithic artefacts, and even fewer that have investigated residues on stone tools specifically. In fact, just eleven lithic residue studies from Mesolithic contexts could be found (Table 3.1), and only those published in English will be considered here. It is possible that the grey literature contains other lithic residue studies, but no site reports relevant to the topic were found after searching several terms within the Archaeological Data Service (ADS) website. These studies have investigated lithic residues by both microscopic and/or chemical means.

In addition to reviews of the studies on lithic residues from Mesolithic sites, the methods being used in terms of their efficacy will be assessed. The last part of this chapter critiques Mesolithic residue studies to date and offers suggestions for improvement. The use of (?) in this chapter denotes that the sample size is unstated or unclear in the literature reviewed. Where the same data is published in multiple locations, it is considered one study.

Table 3.1. Published studies on Mesolithic stone tool residues, investigated by microscopic and/or chemical means.

Source	Location	Mesolithic sites	Publication	Number of Mesolithic lithics studied for residues	Residues found	Technique(s) appropriate for interpretations made?
Richards 1989	UK	Thorpe Common rockshelter	Book, Breaking the Stony Silence, Sheffield Lithics Conference 1988, BAR British Series	Stereomicroscope, reflected VLM: 50. Urinalysis test strips: 25	41 tools blood	No
Roberts, Barton, and Evans 1998	UK	Thatcham III, Oakhangar VII	Book, Stone Age Archaeology: Essays in honour of John Wymer	Microscopy: 0. IR spectroscopy, GC: 2	1 tool probable resin, 1 tool inorganic concretion	Not enough information presented to assess
David 1998 (microscopy by Moss, chemical analysis by Evans)	UK	Seamer Carr	Book, Stone Age Archaeology: Essays in honour of John Wymer	Microscopy: 33. Differential IR spectroscopy, GC-MS: 33. HPLC: 1	1 tool possible hafting adhesive ( <i>Pinus</i> resin and beeswax), protein	Not enough information presented to assess
Aveling and Heron 1998	UK	Star Carr	Journal, Ancient Biomolecules	Microscopy: 0. GC, GC-MS: 1	1 tool birch bark tar	Yes
Pawlik 2004	Austria and Germany	Ullafelsen, Henauhof-Nord II	Book, Lithics in Action: Papers from the Conference Lithic Studies in the Year 2000	Reflected VLM: 110 from Ullafelsen, unspecified number of tools from Henauhof-Nord II. SEM: ? EDX: ?	Unspecified number of tools birch bark tar, ash	No
van Gijn 2007	UK	Goldcliff sites A and J	Book, Prehistoric coastal	Stereo microscope, reflected VLM:	2 tools black wood tar	No

			communities: the Mesolithic in western Britain	19(?). Chemical testing: 0		
K. Hardy and Shiel 2007	UK	Howick	Book, Mesolithic Settlement in the North Sea Basin. A case study from Howick, Northumberland	Reflected VLM: ? SEM-EDX:12 (?)	5 tools meat or seeds; 5 tools oil, wax, fat, or carbonised organic material; 2 tools mineral residues; 3(?) tools wood ash or charred timber	No
B. Hardy and Svoboda 2009	Czech Republic	Pod zubem, Pod křídlem	Book, Archaeological science under a microscope: studies in residue and ancient DNA analysis in honour of Thomas H. Loy	Reflected VLM: 70. SEM: ? chemical testing: 0	12 tools plant; 4 tools wood; 3 tools starch; 2 tools feathers; 2 tools hair; unspecified number of tools resin	No
Vahur et al. 2011	Estonia	Pulli	Estonian Journal of Archaeology	Microscopy: 0. ATR-FTIR: 1	Birch bark tar; probably fat and conifer resin	No
Cristiani et al. 2009; 2014	Italy	Riparo Gaban rockshelter (Mesolithic/Neolithic)	Journal, Documenta Praehistorica; Book, An Integration of the Use-Wear and Residue Analysis for the Identification of the Function of Archaeological Stone Tools, BAR International Series	Stereo microscope, reflected VLM: 92(?). VP-SEM- EDX: ? ATR- FTIR: ?	iron oxide (Fe <sub>2</sub> O <sub>3</sub> ), calcite (CaCO <sub>3</sub> ), organics	No
Aleksan	Russia	Dvoinaya	Journal,	Stereo	6 tools	No

drova, Kireeva, and Leonova 2014		Cave	Archaeology, Ethnology and Anthropology of Eurasia	microscope, fluorescent LM, polarised LM: 153 (?), ATR-FTIR, FTIRM, XRF: 9(?)	conifer resin; 3 tools carbohydrate (likely from tree gum or plant sap); 4 tools animal protein; 1 tool red ochre; 9 tools calcite; 7 tools clay minerals	
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### 3.2 Residue analysis at Mesolithic sites

The earliest exploration of Mesolithic residues was carried out by Richards (1989), who claimed blood residues were preserved on lithic tools from Thorpe Common Rockshelter, South Yorkshire. First, 50 tools were examined with a stereomicroscope and reflected VLM. According to Richards (p. 82), remaining soil matrix on the tool is easily differentiated and "... can be visually distinguished from blood residues". 41 of the 50 tools were found to contain blood residues based on microscopy alone. Identifications of blood based on microscopic visual appearance are insufficient by today's standards for lithic residue evidence. Putative blood residues were extracted with a stainless steel blade under the stereomicroscope from 25 lithics and placed on urinalysis test strips (BM-Test-7). This is a test that is used medically to detect the presence of hemoglobin as a proxy for blood in urine. Richards obtained positive results for the presence of blood from 22 of 25 lithics. However, the urinalysis test strip method to investigate putative blood residues was called into question due to its tendency to produce false positives, as discussed in Chapter 2.

In the 1990s, three residue studies were undertaken in the UK. In 1998, Roberts et al. (1998) investigated the chemical composition of four samples of potentially resinous material from Early Mesolithic sites in Britain: 1) a suspected adhesive from an unretouched flake from Thatcham III (UK), 2) a suspected adhesive from an oblique point microlith from Oakhanger VII (UK) (Rankine et al., 1960), 3) one of the 'resin cakes' from Star Carr, as well as 4) a 'resin block' from Lackford Heath (UK). Samples of 2 mg were extracted from each artefact. Slightly different types of methods were applied to the analysis of the samples, with gas chromatography (GC) carried out on all samples, but infrared spectroscopy (IR) carried out on the Thatcham and Oakhanger samples and differential infrared spectroscopy (DIR) carried out on the Star Carr and Lackford Heath samples. Additionally, the Thatcham sample was examined with a SEM for the presence of organic material or pollen, however, none was visible and SEM was not carried out on the other three samples. The traditional IR spectroscopy involved grinding the mastic samples and use of a single KBr pellet/disc. The DIR used two beams simultaneously on both the sample KBr pellet and a reference pellet, whose spectrum is removed from the sample spectrum. The DIR method is not used for lithic residue analysis today, as there are software programs that easily subtract selected components out of the sample spectrum mathematically.

The chemical analyses of the Thatcham sample suggested the potential adhesive contained traces of probable resin, but no further specificity in terms of taxon was able to be attained. A sample for chemical analysis was taken from the interior of the Star Carr resin cake, avoiding the surface which was treated for conservation with plastic polyvinyl chloride (PVC), after which IR spectroscopy and GC and were carried out. The chemical analyses found traces of a resinous material, which was suggested to be birch resin with clay and possible beeswax. The potential mastic sample from the Oakhanger VII microlith showed it to be an inorganic concretion.

David (1998) reported residue analysis of two groups of spatially-related flint microliths (33 total) from the peat at Seamer Carr. Chemical characterisation tests were carried out despite the preceding results of the reflected VLM examinations by Emily Moss that showed an absence of microscopic residues on the microliths. Nonetheless, the chemical characterisation by differential IR spectroscopy and GC carried out by John Evans found one scalene triangle microlith out of 33 tools contained possible trace of hafting adhesive on it, composed of *Pinus* resin and beeswax (1998, p. 200). HPLC was also conducted on residues from the same microlith, showed it contained the amino acids glutamic acid and glycine, which are both very common in proteins and hence a more specific identification was not possible. It is not clear if residues were removed from tool surfaces to conduct the differential IR spectroscopy, and it is not stated how residues were extracted for GC or HPLC, i.e. types of solvents used. This report is very scant on information – none of the IR, GC, or HPLC data is presented so the reader cannot assess if the claims of *Pinus* resin, beeswax, glutamic acid, and glycine in the microlith residue are supported by the data.

Aveling and Heron (1998) chemically characterised suspected tarry materials from Star Carr. Clark had previously noted residues were preserved on one microlith (see photograph) and two barbed points from Star Carr, and five thin flat 'resin cakes' were identified as oxidised wood pitch or natural resin at the British Museum by M.H. Hey (Clark 1954, 167, plate XX). The microlith hafting tar, five resin cakes, and three birch bark rolls were analysed by extracting samples using solvent and conducting lipid residue analysis by GC and GC-MS. Aveling and Heron (1998) compared the resulting spectra with modern experimentally-prepared birch bark extracts, birch bark tar, and bark samples from other Mesolithic contexts. They found that all of the tarry substances contained triterpenoids derived from heating birch bark. Perhaps surprisingly, the three unburned birch bark rolls from Star Carr produced chromatograms more similar to heated laboratory-produced birch bark tar than modern untreated birch bark. This led Aveling and Heron (1998, p. 77) to suggest that alteration of triterpenoid markers in bark can occur due to processes other than

burning. The issue of the origin of altered bark compounds might be further explored since some resin biomarkers are assumed to be indicative of thermal treatment when in reality, they may originate from natural chemical ageing.



A microlith from Star Carr with a visible birch bark tar deposit (from Clark 1954, 167, plate XX F). Image for examination purposes only, to be removed prior to publication.

It has been suggested that birch bark tar production occurred at Star Carr (Clark, 1954; Fletcher et al., in press), given the numerous charred birch bark rolls found on site. On a wider scale, it seems that birch bark tar was a relatively common product in Mesolithic Europe. Amorphous black lumps, sometimes with teeth marks, have been found from early, middle, and late Mesolithic sites in Sweden (Huseby Klev, Segebro, Bökeberg, and Ringsjöholm), the UK (Star Carr, Lackford Heath), Denmark (Barmose), and Norway (Øvre Storvatnet). Microsamples from these lumps tested with GC-MS are consistent with birch bark tar (Aveling, 1998; Aveling and Heron, 1999). Mesolithic microliths from sites in Germany have also been found with black 'pitch' on them, which could indicate the use of birch bark products (Bokelmann et al., 1981; Bokelmann, 1994).

In a functional study incorporating usewear and residue analysis, Pawlik (2004) investigated suspected birch bark tar hafting residues. These were identified as 'residue spots' from lithics from Mesolithic Ullafelsen, Austria and Mesolithic Henauhof-Nord II, Germany. From Ullafelsen, it appears that a total of 110 lithics (mostly microliths, made from a variety of siliceous raw materials), were examined with reflected VLM for residues at the same time as usewear analysis was conducted, and 25 lithics were found to contain residues. An unspecified number of suspected tar microsamples were extracted from the Ullafelsen tools for SEM-EDX. Hafting material was also extracted from an unspecified number of Neolithic hafted blades (intact composite tools) from several unspecified sites in Switzerland (but definitely including the Burgäschisee-Süd – it was the only site named), was used for SEM-EDX comparison to the Mesolithic tools. At least two experimental birch bark tars were also made and examined with SEM-EDX. One of the experimental birch bark tars was made in an oxygen-excluding an airtight container, and the other tar was made in an environment that did not completely exclude oxygen.

From Henauhof-Nord II, an unspecified number of blades were previously examined, published in two German reports (Pawlik, 1997, 1995), presumably using VLM. Some putative hafting residues were examined in situ from Henauhof blades, with SEM, but it is unclear how many tools were examined. SEM images of the purported birch bark tar from Henauhof-Nord II blades showed the residue contained plant remains: "... primary plant material, deformed cellular parts and fibres could be recognized in the amorphous matrix" (Pawlik, 2004, p. 175). The analysis was done in situ since the potential hafting material was in quantities too small to extract, unlike the sampled residues from Ullafelsen. One control sediment sample from Henauhof-Nord II was examined visually with SEM to see if it appeared similar to the residues on the suspected hafting residues on the tools.

The elemental analyses (EDX) were performed on an unstated number of samples from Ullafelsen, Henauhof-Nord II, Burgäschisee-Süd, and at least two experimental birch bark tars. Pawlik found the main constituents in both experimental birch bark

tar and archaeological residues were potassium (K) and calcium (Ca), which it is suggested to be contamination from ash during tar production. Pawlik concludes that the residues on tools from both Ullafelsen and are hafting mastics, and that they are weapon inserts. Pawlik (p. 178) also concludes that Mesolithic people did not use air-tight containers in a dry distillation because the archaeological residues look like experimental tars produced by incomplete distillation, seeming to suggest that the presence of plant materials within the archaeological residues proves this point (p. 179). However, “fibrous plant material” was also found within the soil sample from Henauhof-Nord II examined with SEM (p. 174).

There are several major problems in this study. The most apparent issue is the fact that the number of residue samples, lithics, and archaeological sites is unclear, so it is unknown which analyses were conducted on which tools from which archaeological sites, and leaving the reader unable to gauge the extent of the study. The reader is also given no indication if some of the samples examined with VLM and/or SEM-EDX were determined as not originating from birch bark tar. The presence of K and Ca do not indicate the residues examined are birch bark tar, and thus cannot confirm Pawlik’s assertion (p. 176) that they are associated with birch bark tar. Also, no control microanalysis points from areas on the tools with no visible residues were taken for comparison with the residue EDX spectra, to see if they also contained K and Ca. In any case, elemental analyses techniques, such as EDX and XRF, are inappropriate for the confirmation of the presence of birch bark tar, since they provide only the elements present in the sample, not chemical compounds which can be traced to a specific source. Additionally, only items referred to as ‘residue spots’ that appeared morphologically similar were reported by Pawlik, so the reader is left wondering whether other residue types were found.

A functional study of usewear and residue analysis was carried out by van Gijn on 19 Mesolithic stone tools from Goldcliff sites A and J, UK. Tools were examined with a stereo microscope and reflected VLM, although it is not clear if all 19 tools received the same analysis treatment. Black deposits were found on two tools, which

were interpreted as wood tar used in the hafting arrangement. Resinous residues are amorphous, so visual identification is insufficient. However, no chemical characterisation was carried out to test what should be a hypothesis, rather than a definitive assertion, as to residue origin.

Research by K. Hardy and Shiel (2007) examined residues with VLM on an unspecified number of lithics from the site of Howick, UK. Also presented in the study, separate from the residue analysis, was a usewear analysis of 100 lithics found inside the Howick hut and experiments used to inform the interpretation of usewear. It was not stated if any of the same tools from the residue study were also analysed in the usewear study. Also, it was not stated if different people conducted the residue analysis and the usewear analysis, which might explain why these datasets appear disjointed. The overall interpretation from the usewear study was: “a wide range of activities was taking place across the site, exploiting many different raw materials” (p. 135).

For the residue study, an unstated number of tools were first screened for in situ residues using VLM (Meiji ML 2305), and this was followed by microanalysis with SEM-EDX. The SEM was used in back-scattered electron mode (BSE) to collect images. Interpretations of residue origin were made using the EDX spectra collected on residues, and obtaining C:N ratios. C:N ratios were then compared to ratios of five known generalised categories of natural materials:

- 1) wood, straw, oils and fats= C/N >30
- 2) green plant tissue= C/N around 25
- 3) wheat and other seed grains= C/N around 15
- 4) sediment and fungi= C/N around 10
- 5) most animal tissues (excluding fatty acids), bacteria and actinomycetes= C/N 5 and below

Where the above reference standards came from, i.e. their own work or from another source, is unstated. K. Hardy and Shiel (2007, 121) found that residues on tools fell into two groups based on their low C:N ratios were suggested to be meat or pulse seeds and high to infinite C:N ratios were interpreted as evidence of wood. The results from 12 Howick lithics are listed by K. Hardy and Shiel (2007), however, it should be noted the sample size is stated as 10 lithics later in the paper (p. 135). Their interpretations from each tool are presented below:

3162 animal protein or seeds

4697 animal protein or seeds

4410 Copper mineral with zinc, suggested to be from either secondary copper mineralisation or pigment use by Mesolithic people

4382 cordierite (mineral), as well as an oil, wax, fat, or carbonised organic material

4986 animal protein or seeds and an oil, wax, fat, or carbonised organic material

4395 an oil, wax, fat, or carbonised organic material

1621 potassium from ash or charred timber, sodium might be from seaweed or driftwood

2758 an oil, wax, fat, or carbonised organic material

4548 potassium, chloride, and sodium suggest wood ash or charred timber, also animal protein or seeds

4294 animal protein or seeds

4652 potassium, chloride, and sodium suggest wood ash or charred timber

4063 an oil, wax, fat, or carbonised organic material

In total, the residues on five tools were interpreted as used on meat or seeds; five tools had residues interpreted as oil, wax, fat, or carbonised organic material; two tools had evidence of mineral residues; and three tools were interpreted as used on wood ash or charred timber (although it is later stated on page 135 that “residues on two tools had possible evidence for wood ash or charred wood”). K. Hardy and Shiel interpreted residues with C:N ratios of 5 or below as animal protein or seeds. However, by their own guide, anything with a C:N ratio below 5 was stated as

originating from several sources: animal tissues, bacteria, and actinomycetes, and 'seeds' were not listed in this category. Another inconsistency is the interpretation of tool 4410. Originally the residue deposit containing copper with zinc inclusions on tool 4410 was interpreted as either due to secondary copper mineralisation (natural origin) or pigment use by Mesolithic people (cultural origin), and then it is stated in the conclusion that the use of copper was "probably for pigment" (p. 136).

Although many EDX readings were taken, K. Hardy and Shiel (2007, 122) stated "... only the positive, residue related results will be discussed", also stating that many residues examined were determined to be soil or had low X-ray counts (poor quality spectra). However, it would have been useful to have both positive and failed readings reported, so an assessment of the success of the technique could be made. Additionally, the C:N ratio values, which are their basis for residue interpretation, are presented for only five of 12 tools. No results from sediment controls were presented by K. Hardy and Shiel, and it is not stated if they examined any. However, reference is made to general 'bulk soil' C:N ratios; perhaps they are referencing C:N values used in soil chemistry.

The study by K. Hardy and Shiel (2007) is honest about the preliminary nature of their findings. However, why the values of C and N were used to interpret residue origin is not clear. C:N ratios are used in soil chemistry and their ability to chemically characterise archaeological residues is questionable. C:N ratio measures the relative nitrogen content of organic materials and is an indicator of soil decomposition by microorganisms, particularly relevant to agricultural studies (Conklin, 2013; Hazelton and Murphy, 2016; Stevenson and Cole, 1999).

The treatment of the lithic sample is unclear. K. Hardy and Shiel (2007, 120) first state that tools went through VLM scanning prior to SEM analysis. It is later stated (2007, 122) that artefacts were immediately bagged on site and "Bags were not reopened until the artefacts were ready to be placed in the SEM at which point their surfaces were lightly brushed to remove any surface soil". So, the reader is left

wondering how many tools went directly to SEM analysis, and how many were preliminarily scanned with VLM? Also, the light brush treatment leaves sediment on tools – sediment that could have been mistaken for archaeological residues during analysis. One good thing methodologically in K. Hardy and Shiel (2007, p. 121), is the collection of a background EDX spectra for every tool on an area with no visible residues. This allows for comparison to the residue spectra.

B. Hardy (Hardy, 1999; Hardy and Svoboda, 2009) conducted usewear analysis and analysis of in situ residues with reflected VLM analysis of 70 lithics (40 blades and bladelets, 28 flakes, one unifacial point, and one crescent) from two Mesolithic sites in Czech Republic: Pod zubem (long-term occupation) and Pod křídlem (short-term occupation). Residues and usewear were photographed and documented on line drawings of each artefact. Sediment samples from each level of both sites were examined for the presence of residues, although their spatial relationship with the residue-containing artefacts was not stated. Residue identifications were made based on comparison with published and reference residues. An unspecified number of residues were selected for SEM analysis, removed with double sided adhesive tape, coated with gold, and examined with traditional SEM.

The following residue identifications were presented: plant, wood (general categories), starch granules, resin, feathers, and hair (specific identifications). At Pod zubem, 46 stone tools were examined and plant fragments, wood fragments, starch grains, feathers, hair and resin (interpreted as mastic) were found. From Pod křídlem, 24 stone tools were examined and plant fragments and starch grains, were found.

Starch granules were found on three tools from both sites. The starch granules were not counted but recorded on a presence/absence basis for each tool. In one case (a bladelet from Pod křídlem), light micrographs (see image) are presented of round items with extinction crosses, identified as starch granules, in association with what are identified as parenchymal cells.

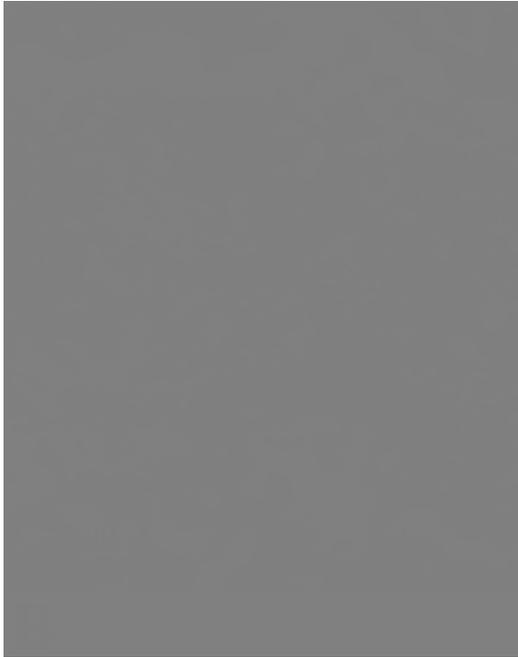


Light micrograph of purported Mesolithic in situ starch granules located on the centre of the ventral surface of a flake from Pod zubem, Czech Republic. From Hardy and Svoboda 2009 Figure 8B, C. Image for examination purposes only, to be removed prior to publication.

Presumably, these images were taken in cross polarised light, since the circular items seem to exhibit extinction crosses, but this is not noted. The bigger problem is

that these items may not be starch at all. There are a number of items that are starch look-alikes, including fungal spores (Haslam, 2006b), coprolitic spherulites, bordered pits (tracheids and vessel elements), spiral wall thickenings (tracheids and vessel elements), air bubbles, spores, pollen, coccoliths, some diatom frustules, zooplankton with calcareous shells including bivalve veligers (larvae), and ostracods (Croft, 2012, p. 73). B. Hardy and Svoboda did not examine the purported starch granules with transmitted light microscopy to view 3D shape, hilum placement, colour, rotation of the arms of the cross, or other pertinent features. The presence of an extinction cross under cross polarised light is not sufficient for starch identification, since many of the above items also exhibit such a cross in the same conditions. None of the starch granules were assigned to a plant taxon or even described in terms of morphotypes, although genus or family level identification is usually possible. And, the only confirmatory starch test known to date is to extract suspected starch from the tool and digest it with  $\alpha$ -amylase (Hardy et al., 2009).

An unspecified number of tools contained what was identified by Hardy and Svoboda (2009) as resin used as a hafting mastic (see image). These reported resin residues may be entirely possible, but microscopic observations of experimental resin residues (Croft et al., 2016) show that resins are amorphous with no diagnostic structures present to allow them to be identified, necessitating chemical characterisation.



Light micrograph of purported Mesolithic in situ hafting resin on a bladelet from Pod zubem, Czech Republic. From Hardy and Svoboda 2009 Figure 5B, C. Image for examination purposes only, to be removed prior to publication.

From the Pod zubem lithics, Hardy and Svoboda (2009) found bird feathers on one blade and one flake from Pod zubem. The single feather barbule found on the flake (PZ 824) was used to interpret the tool as being used for cutting avian tissue. This claim is a stretch because there is nothing to indicate that the barbule is not an incidental contaminant from handling or airborne dust particulates.

A major issue with this B. Hardy and Svoboda's (2009) study is that it appears that no modern contaminant sources were considered in the interpretation of results. It is possible that some or all of these residue types, and especially the hair and feather remains, are unrelated to ancient human activities. Also, the light micrographs in this study were taken at one plane of focus, so it is hard for the reader to see the evidence presented. However, it should be noted that z-stacking technology was not widely available and the quality of lithic residue imaging has vastly improved since this study.

Cristiani et al. (2014, 2009) investigated usewear and residues in situ on one tool type: trapezes from Late Mesolithic/Early Neolithic Riparo Gaban rockshelter, Italy, a site that represents the transition between the two periods. A total of 182 microliths were examined, 92 trapezes from the Late Mesolithic layer, and 90 trapezes from the Early Neolithic layer. Optical microscopy, SEM-EDX, and ATR-FTIR were the techniques used to investigate residues. First, a stereo microscope and reflected VLM were used to locate brown, red, and white residues on 27 of 182 trapezes. Cristiani et al. (2009, p. 198) state that all residues were found in the 'hafting zone' of the trapeze tools.

An unspecified number of tools were further investigated in situ with SEM-EDX and in situ ATR-FTIR. From one EDX spectrum collected on a red residue (Fig. 9B), Cristiani et al. (2009, p. 201) infer the presence of iron oxide ( $\text{Fe}_2\text{O}_3$ ) and calcite ( $\text{CaCO}_3$ ), which they interpret as evidence of red ochre, also suggesting that the white material is a pigment selected by people that was added to the red ochre to change the intensity of the colour. As mentioned previously, EDX is a technique for elemental analysis and thus does not indicate the presence of either iron oxide ( $\text{Fe}_2\text{O}_3$ ) or calcite ( $\text{CaCO}_3$ ); EDX tells you which individual elements are present in a sample, not how they are combined as compounds. In addition, even if iron oxide and calcite were to be identified with an appropriate technique, such as Micro-Raman, it must be considered that minerals also occur naturally in sediments, thus this possible natural contamination source would need to be excluded for an anthropogenic origin to be plausible. The brown residue was suggested to be an organic residue since the EDX spectrum shows it contained an intense carbon peak. This could actually signal the presence of organics from sediments on the tools from the burial environment.

In situ analysis of lithic residues took place with ATR-FTIR, but number of tools analysed in this way is unspecified. A brown residue (unclear if it was the same residue that was examined with SEM-EDX) was subject to ATR-FTIR analysis. It was not stated what type of spectral information was collected (absorption,

transmission, reflection) and the two spectra presented (of a brown residue on a trapeze and a reference residue composed of beeswax mixed with natural bitumen) are illegible. C-H stretching vibration features at  $2919\text{ cm}^{-1}$  and  $2850\text{ cm}^{-1}$  were found in the spectrum, meaning the sample contains organics. The brown residue spectrum obtained was compared to a spectrum reference library of unknown size, to which they found bitumen and beeswax sample was the 'best match' for the brown residue (2014, p. 102), although the two spectra do not appear similar. Also, it is not clear if the C-H stretching peaks from the brown residue and the bitumen and beeswax reference are even validly comparable. The brown residue FTIR spectrum was taken in reflectance mode (as labeled on Fig. 9e), and the bitumen and beeswax reference material spectrum is presented in %Transmittance (Fig. 9f) (Cristiani et al., 2009). Light reflection is an interface, or surface measurement, whereas light transmittance is a bulk measurement. It is unclear whether these different methods of FTIR data collection on residues are completely comparable, but Monnier et al. (2017b) have begun to explore this issue with experimental animal tissue residues on stone tools.

Like many studies reviewed here, Cristiani et al. (2014, 2009) did not include any controls from sediment samples in association with the tools examined for residues, or collect EDX and ATR-FTIR spectra from areas of the stone tools without any residues. No anti-contamination measures are mentioned in terms of excavation, storage, cleaning, or post-excavation handling, so the history of modern contaminants that could have come in contact with the archaeological material are unknown.

Macroscopically visible black deposits from an Early Mesolithic flint 'insert' from the Pulli site, southwestern Estonia, were tested by Vahur et al. (2011) with ATR-FTIR. Vahur et al. assumed the flint artefact was part of a composite tool, calling the microlith an 'insert', and also assumed the residues were an adhesive layer prior to chemical testing. Two microsamples of the black residue were extracted with a scalpel and tweezers and placed on the ATR crystal. For comparison to the

archaeological residue, ATR-FTIR spectra were taken of modern reference birch bark tar (*Betula alba*), pine resin (*Pinus sylvestris*), and fir resin (*Picea abies*). No microscopy was conducted.

Vahur et al. (2011) concluded that the residue on the flint artefact was birch bark tar, and probably also containing fat and conifer resin. The two black residue FTIR spectra from the microlith show similarities with the reference birch bark tar, particularly the strong bands present at  $\sim 2920\text{ cm}^{-1}$  and  $\sim 2850\text{ cm}^{-1}$ , which were assigned as aliphatic C-H stretch (alkyl groups), and the strong band at  $\sim 1730\text{ cm}^{-1}$  assigned as C=O stretch (esters, ketones, aldehydes), a medium band at  $\sim 1456\text{ cm}^{-1}$ , assigned as C-H deformation (alkyl groups), medium at  $\sim 1170\text{ cm}^{-1}$ , assigned to C-O-C stretch (esters). It is not clear if the bands at  $\sim 2920\text{ cm}^{-1}$  and  $\sim 2850\text{ cm}^{-1}$ ,  $\sim 1730\text{ cm}^{-1}$ ,  $\sim 1456\text{ cm}^{-1}$ , and  $\sim 1170\text{ cm}^{-1}$  uniquely identify birch bark tar. Although the match between the IR spectra from the black residue and the reference birch bark tar appears pretty good, only three reference materials were compared to the archaeological residue, so other possible options were not excluded.

No sediment sample controls were analysed by Vahur et al. (2011), but a background spectrum was taken of an area of the flint microlith without any residues. Interestingly, the background spectrum of the flint surface showed it contained two aliphatic C-H stretch peaks at  $\sim 2920\text{ cm}^{-1}$  and  $\sim 2850\text{ cm}^{-1}$ , just like the extracted black residue and the reference birch bark tar, although they are weaker. This shows that there are some organic compounds present on the area of the tool with no visible residues. The fact that these bands found at the same locations in both the background flint and the residue spectra are not discussed in the paper somewhat undermines the interpretation that the black residues are archaeological. No anti-contamination measures were mentioned in the paper, so it might also be possible that these bands at  $\sim 2920\text{ cm}^{-1}$  and  $\sim 2850\text{ cm}^{-1}$  in the background flint spectrum originate from modern contamination with organic aliphatic compounds.

Aleksandrova et al. (2014) conducted usewear and residue analysis on 765 Early Mesolithic stone tools from Dvoynaya Cave, Northwestern Caucasus Mountains, Russia. Tools were washed with a soft brush and alcohol prior to analysis with a metallographic microscope at magnifications ranging from 50-500x. Residues were visually identified on 153 (20%) of the assemblage. Four major groups of residue types were identified during microscopic analysis. The first residue group consisted of long gray porous and fibrous material, suggested to be carbonised plant fibres, possibly from pine. It was noted that pine wood fibres were found in the Early Mesolithic cultural layer. The second group were semi-transparent light yellow to opaque dark brown and reddish residues, and it was suggested that they were resinous. These residues were a few millimeters to 1.5 cm in diameter, and up to 3 mm thick. The third group of residues consisted of light reddish-brown 'stripes' that occurred in the same direction as usewear. Residues in the fourth group were spots and stripes in red, brown, black, and whitish colours. These residues did not occur in any pattern over the surface of the tools, and were reasoned to be natural formations.

Nine lithics (four blades, three lunates, a laminar flake, and an end-scraper on a flake) were selected for further IR analyses. The following instruments were used for analysis: stereo microscopes, fluorescent light microscope, polarised light microscopes, FTIRM, handheld XRF, and ATR-FTIR. It was not clear if all nine tools containing residues were subject to all of these techniques because the data is not presented or summarised. Nevertheless, a table (p. 4) shows data was collected in 1-3 'IR probes' per tool, so these are the results of either the FTIRM or ATR-FTIR analysis. The interpretations of the residue groups were as follows:

Group 1. These residues were interpreted as conifer exudate (resin) and such minerals as macrocrystalline calcite and reddish-brown clay minerals.

Group 2. Like group 1, this residue also was characterised as containing organic and mineral components and conifer resin. Additionally, animal protein and carbohydrate

containing material were inferred by the presence of absorption bands at  $\sim 1650$  and  $\sim 1550 \text{ cm}^{-1}$  and  $\sim 1607$  and  $\sim 1620 \text{ cm}^{-1}$ , respectively. The carbohydrate material was suggested as likely from fruit tree gum or other carbohydrate-containing plants. The interpretation of this residue overall was it represented the remains of glue with fibrous impressions from wrapping the tools on shafts.

Group 3. There were variable findings in this group, but overall the same components were identified as present in Group 2: conifer resin, animal protein, carbohydrates, and minerals.

Group 4. This reddish-brown fine-grained powder residue was interpreted as minerals ochre and calcite based on the results from microscopy, XRF, spectroscopy (unstated whether it was FTIRM or ATR-FTIR) and crystal field analyses. No organics were found.

It is notable that although residues in groups 1-3 were grouped according to their morphological distinctiveness, they were chemically similar. This might suggest the chemical components are actually contamination sources from similar sources, perhaps from the burial environment or modern post-excavation origins.

Aleksandrova et al. (2014) concluded that their results were helpful for understanding ways of hafting and that they had partially illuminated the composition of glues. They also found that the chemical characterisation analyses supported the usewear interpretations. Aleksandrova et al. (2014) do not state if any residues were extracted, so it is assumed that they were examined in situ, even when data was collected with the ATR-FTIR. Absorption spectra are presented, so these were probably collected with the ATR-FTIR and not FTIRM, since FTIRM data is collected in reflection mode if the residue is analysed in situ. Similar to other Mesolithic residue analysis studies, this paper lacks clarity in terms of exactly what methods were used and how the data was used to arrive at interpretations. There is also no mention of attempting to involve any controls along with the analysis of the residues,

such as from sediment sampling or taking spectra from parts of the tools with no residues as a background. Since no anti-contamination measures were taken, the interpretations of the presence of animal protein and carbohydrates from fruit tree gum or other plants are in question, since these could have originated from skin flakes from handling (Pedergnana et al., 2016), and starch present in the lab (Crowther et al., 2014). It is also unclear if the water, brushes (unclear whether natural or synthetic bristles were used), or alcohol used had any impact on the outcome of the analysis. Also, the identification made in the study for 'carbohydrate' is not specific enough to support Aleksandrova et al.'s (2014) suggest that it originates from Mesolithic fruit tree gum or plant sap. For instance, cellulose is also carbohydrate present in the cells of all green plant, many algae, urochordate animals (tunicates), and some bacteria (Nobles and Brown, 2007, p. 1). This means cellulose is expected to be common in the sediments containing any plant organics, and it was mentioned in the paper (p. 3) that wood fibre from pine was present within the Early Mesolithic layer in which the sample lithics were collected.

### 3.3 Discussion

Only eleven studies could be found that examine residues found on Mesolithic stone tools. The reasons for this dearth are twofold. Firstly, research in the Mesolithic has been seen by many academics and the public as a backwater – conceptualised as a static period where not much changed, garnering little scholarly attention historically, but this is improving currently (Henson, 2016; Milner et al., 2015). Secondly, residue studies on Palaeolithic stone tools have drawn more interest. Palaeolithic material is attractive for study since the period covers evolution and extinction of several species related to anatomically modern humans. Examples of the 'oldest' residues or residues associated with other *Homo* species tools can be published in high-ranking journals. Thus, Palaeolithic artefacts are more appealing for their ability to improve

researchers career impact and funding opportunities. Clearly, Mesolithic stone tools could be investigated more for microscopic and chemical residue traces that might yield novel information about Mesolithic lifeways.

The main focus in research on Mesolithic stone tool residues has been resinous residues from birch and pine trees, traces which are always interpreted as evidence of hafting. However, other organic residue types, such as blood, animal protein, fats, beeswax, feathers, hair, starch granules, carbohydrates (suggested to be from plant gum or sap), wood, and charcoal. Additionally, some mineral residues have been reported by Aleksandrova et al. (2014), including calcite, clay minerals, and red ochre.

A key issue for development in lithic residue analysis in the Mesolithic (and all residue analysis) is specificity. We are coming to a stage in residue analysis where it is no longer tenable to make archaeological interpretations based on nonspecific data. For instance, as was discussed, the IR identifications of 'animal protein' and 'carbohydrate' by Aleksandrova et al. (2014) are not specific enough to allow their attribution to anthropogenic residue sources. Rather, the general functional groups identified could easily originate from sediment, handling, or laboratory contamination sources.

Pigment use by Mesolithic people, for both symbolic and functional purposes, is an area that warrants further investigation, particularly since mineral residues can be more chemically stable and thus preserve better than organic residues. There have been interesting examples of what appears to be a practice of harvesting red ochre and perhaps other pigments such as kaolin at several Mesolithic sites, including Stainton West, Cumbria (Clarke, 2014), and Flixton School House Farm, North Yorkshire (Needham et al., 2014). Nodules of pyrite with usewear or signs of abrasion from Star Carr (Clark, 1954, p. 167) and Mesolithic sites in the Netherlands could have been used as strike-a-lights (van Gijn et al., 2001; van Gijn and Houkes, 2001), and sulphuric iron microwear traces appear to be detectable on flints used as

part of strike-a-lights (Sorensen et al., 2014, p. 481). Christiani (2014) and Aleksandrova et al. (2014) are the only studies on Mesolithic lithic residues to report the presence of red ochre on a stone tool, but mineral lithic residues requires further work with appropriate methodology.

The 'hafting residues' theme is present in Mesolithic studies of stone tool residues, (Aveling and Heron, 1998; David, 1998; Hardy, 1999; Hardy and Svoboda, 2009; Pawlik, 2004; Roberts et al., 1998; Vahur et al., 2011; van Gijn, 2007). The study of hafting residues has been a dominant focus in lithic residue studies internationally, due to their durability persistence in varied archaeological contexts, with reports from France (Dinnis et al., 2009), Germany (Koller et al., 2001; Pawlik, 2004; Pawlik and Thissen, 2011; Sandermann, 1965), Austria (Pawlik, 2004; Schäfer et al., 2006), Switzerland (Rottländer, 1991), Italy (Mazza et al., 2006), Romania (Cârciumaru et al., 2012), Syria (Hauck et al., 2013; Monnier et al., 2013), Israel (Yaroshevich et al., 2013), Egypt and Sudan (Rots et al., 2011), South Africa (Charrié-Duhaut et al., 2013; Delagnes et al., 2006; Gibson et al., 2004; Lombard, 2011, 2007, 2006, 2005; Lombard and Phillipson, 2010), and Yukon and Northwest Territories, Canada (Helwig et al., 2014). Chemically, the terpenoids found in resinous adhesives are known to survive for long periods in a variety of archaeological contexts (Pollard and Heron, 2008, p. 235).

The analysis of organic residues by GC-MS can identify the constituents involved in hafting recipes, and can also identify which type of tree resin was exploited. This and other applications of GC and GC-MS studies on stone tool hafting residues (e.g. Cârciumaru et al., 2012; Charrié-Duhaut et al., 2013; Helwig et al., 2014; Mazza et al., 2006) have potential for future identifications of the nature of hafting residues at Star Carr.

GC, GC-MS, and ATR-FTIR have only been conducted on stone artefacts that exhibit relatively large residue deposits – large enough to be visible to the naked eye. Whether it is necessary for the residues to be visible macroscopically to obtain

successful GC-MS results is unknown. Microscopic amounts of a residue on a stone tool should be sufficient for normal GC-MS, since only 1 nanogram of pure organic material is required for injection into the column. However, in practice sampling from stone tools may require substantially more material in order to detect biomarkers, perhaps about 1 milligram, to take into account degradation and the incidental inclusion of non-organic components in the sample.

### 3.4 Critique

The quality of Mesolithic residue studies speaks to the limited adoption of residue analysis by Mesolithic researchers. Frustratingly, it is often unclear how many samples were analysed for residues, which methods of analysis artefacts were subject to, what equipment was used, and if usewear and residue analysis was conducted on the same set of artefacts. Furthermore, it is often unstated where samples were collected on site, negating the ability for spatial interpretation or taking into account the type of sediments present in the archaeological context(s). Without knowing the locations of artefacts, the results gained from residue analysis cannot be considered representative of all activities across the site, but site function has sometimes been suggested despite this. There is also a general lack of use of controls to understand contamination or if the residues identified as archaeological are actually modern. This review of Mesolithic residue studies makes it clear that uncontrolled curation and handling has been typical and contamination is a concern. Studies usually drew analogies between the observed residues and modern reference material or replicas produced by experimental archaeology.

### 3.4.1 Research questions posed

The research questions posed by UK Mesolithic residue studies encompass the following: confirmation of microscopic blood residues (Richards 1989), chemical identification of the source of putative hafting adhesive (Roberts et al. 1998; David 1998; Aveling and Heron 1998; Pawlik 2004), identification of animal vs. plant residues and activities carried out at a site (Hardy and Shiel 2007), functional analysis by microscopy alone (Hardy and Svoboda 2009), functional analysis by combination of microscopy and in situ chemical characterisation (Cristiani et al. 2009; 2014; Aleksandrova et al. 2014).

When posing research questions, it is important to consider the small scale of residue analysis as related to interpretive power. This point was articulated by Haslam (2006a, p. 406), who flags the issue “...of the theoretical appropriateness of asking broad questions of micro-scale techniques,”. It will be critical for the research questions asked of residues and usewear to be explicit, appropriate and fitting with the number of tools analysed and the microscopic nature of the evidence. This means that residue studies, which are usually based on very small artefact numbers, need to be conservative and not overstretch interpretation for publishing impact. For instance, it would be unreasonable to suggest site function based on residue analysis of a sample 10 lithics from the whole assemblage.

A strategic sampling of several stone tools from different areas across site has not been attempted at any Mesolithic site, which could potentially capture and better describe activity areas and hunter-gatherer life in the Mesolithic. Although it appears Hardy and Shiel (2007) took samples from different features and phases, they did not interpret their residue results with reference to possible spatial patterning of activities across the Howick site.

## 3.4.2 Sampling strategies for residues

### 3.4.2.1 Choosing which types of artefacts to analyse

Because the analysis of residues – particularly microscopic residues – from archaeological artefacts is time-intensive, artefacts are usually chosen on a subjective non-random basis. Sometimes a particular area of the site such as a feature (e.g. roasting pit, hearth, occupation layer sampled at Howick, Hardy and Sheil 2007) or specific artefact type (e.g. trapeze microliths, Cristiani et al., 2009) is targeted for residue analysis. This sampling strategy means that sampled artefacts are not representative of the tool types present or activities across the site, but this is not necessarily a ‘bad thing’. In fact, strategic selective sampling with specific research questions in mind is likely the most profitable approach to residue analysis, given it is usually impossible to complete an exhaustive analysis of the total assemblage recovered from a site. Research questions that focus on defining activity patterns spatially or provide clarification of morpho-technological questions are positive contributions to the study of Mesolithic residues. Comparison of activities between sites has been investigated via functional analysis incorporating residues and usewear, but also with reference to other lines of evidence, such as the faunal assemblage (e.g. Hardy 1999; Hardy and Svoboda 2009).

### 3.4.2.2 Choosing the number of artefacts

Sample sizes in stone tool residue studies have tended to be very small in general. In fact, Haslam (2009, p. 49) found that over a thirty year period from 1976-2006 stone tool microscopic residue studies had small sample sizes, with a mode of only three artifacts. The largest residue study done to date on Mesolithic stone tools appears to have been conducted by Cristiani et al. (2014, 2009), who examined 92

trapezes from the Late Mesolithic layer of Riparo Gaban rockshelter, Italy. However, it is not clear if all 92 trapezes were analysed by both stereo microscope and reflected VLM and it is not stated how many trapezes underwent VP-SEM-EDX and in situ ATR-FTIR.

### 3.4.2.3 Controls

Controls are imperative to understand contamination sources, both from the burial environment and/or modern origins. Mesolithic residue studies have yet to take a rigorous approach to the issue of contamination. Nearly all studies carried out to date took no measures to prevent or identify contamination of the artefacts during excavation or post-ex processing (except Richards use of plastic gloves during artefact analysis (1989, 80). No studies included anti-contamination protocols from the point of excavation, through handling, microscopic examination, or any other tests to prevent modern contaminants from contact with the artefacts being examined for residues.

#### Sediment controls

Also, sediment samples in direct association with each artefact recovered for residue analysis were not collected in any of the studies reviewed. Sediment samples in contact or just adjacent to each artefact are important to collect so that the presence of the residues identified on each artefact is not falsely attributed to human activity. These sediment samples can exclude natural contamination from the burial environment and are essential for determination of legitimately use-related residues.

No study reviewed here systematically tested sediment samples associated with each artefact for the presence of residues identified on the artefacts. Some non-

Mesolithic microscopic lithic residue studies have sampled sediments near and within the same depositional context as the lithics to check for background environmental contaminants (e.g. Barton et al., 1998; Hardy, 1999; Mercader, 2009), but this has not yet become a widespread practice. The examination and testing of sediment directly associated with artefacts is an imperative step necessary to support claims of anthropogenic residues being found on the artefact.

## Controls for chemical testing

When in situ chemical characterisation analyses are carried out on a stone tool, for instance EDX, Micro-Raman, or FTIRM, measurements should be taken at points on the stone tool where no visible residues are present. Doing this not only informs the researcher the background signal from the stone substrate, which might be incorporated into the spectra collected from the residue, it also acts as a negative control since no chemical components from the residue are expected to be present on the bare stone. Negative controls are also applicable to extraction-based chemical characterisation techniques. Extractions can be taken on tools identified as containing no microscopic residues, a relevant test when GC-MS is used.

## Lab controls

Lab controls to test for contamination should be used and reported. For instance when GC-MS is the technique used, Aveling and Heron (1998) reported that their solvent blanks did not contain the peaks found in the archaeological materials tested. Another example of a lab control is the use of blank glass slides in the rooms where residues are extracted and examined with transmitted light microscope, which gives the researcher an idea of the composition of dust particulates in the room, which may contain items that can be mistaken for anthropogenic residues when they land on stone tools. Dust can contain starch, hair, feathers, all of which were

reported by Hardy and Svoboda (2009) as Mesolithic residues. No papers reviewed here used blank glass slide controls to test for the presence of ambient contamination sources.

### 3.5 Conclusion

It is clear that residue analysis on Mesolithic lithics is only just beginning to be explored, and its contribution to functional interpretation has yet to be fully realised and integrated into wider debates. Mesolithic residues are being explored and identified with different techniques and different levels of reliability. Variable techniques used to investigate Mesolithic residues, both in situ and by extraction, have included low and high power microscopy (identification by residue morphology by stereo microscope and reflected VLM), MS, GC-MS, SEM-EDX, FTIRM, ATR-FTIRM, and SEM-T-FTIR. Although Mesolithic organic and inorganic residues have been analysed using a wide range of methods, it seems the most reasonable starting point to a functional analysis is to first visually examine residues with microscopy, and document their locations on the tool. Hypotheses can be made as the nature of any amorphous residues with no diagnostic structure. After this foundational work, residues identified can be tested via chemical characterisation techniques to test initial microscopic observations. GC-MS is probably the most robust approach to chemically characterise lithic organic (carbon-based) residues that has been used so far, however it is destructive, extraction may introduce scratches and scars to the artefact, and it can require a relatively large sample to be removed from the artefact that must contain enough organic material to be successful. If the microscopic pilot study of lithics from Star Carr and Flixton (n=48) is taken at face value, then it can be predicted that other artefacts in the assemblage are likely to contain only small, microscopic residue deposits, which may be insufficient quantities for MS and GC-MS sampling. Organic compounds can be difficult to interpret, and this situation is likely made more confusing when the use-

history of the artefact is multifold and the residues present are overlapping, mixed, and bound with sediments. An early attempt to differentiate plant and animal lithic residues by MS found the patterns of the organic elements in the spectra were too complex to identify (Briuer 1976, 478). However, this does not preclude trials of MS and GC-MS if a sufficient amount of residue can be removed from the stone surface.

Only one of eleven studies examined here (Aveling and Heron 1998) presented data that is appropriate to the specificity of the archaeological interpretation provided. This flags a major issue in Mesolithic lithic residue analysis, but is also applicable to lithic residue analysis as a whole. It seems that many archaeologists either have a poor understanding of the limitations of the techniques they are using, or alternatively, are knowingly stretching their results to fit a storyline. Either way, the research presented thus far on Mesolithic lithic residues is unreliable and in some cases simply incorrect.

There are a number of improvements to be made in terms of methodological consistency and interpretation and this assessment of the literature has drawn out some key points in order to gain the most from residue analysis:

1. Be clear about how many artefacts are receiving which type of analysis (stereo microscope, reflected VLM, SEM, chemical analyses), and report the *total* number of artefacts analysed, not just positive results. This improves research transparency and allows the efficacy of the techniques applied to be assessed.
2. When morphologically amorphous residues are identified (such as 'hafting residues' or 'birch bark tar'), a testable hypothesis should be made, using chemical characterisation techniques.
3. Collect and test sediment samples directly associated with each artefact to rule out contamination.
4. When chemical characterisation is carried out, collect 'background' readings on the areas of the tool with no residues and compare it to the residue.

5. Reduce the amount of modern sources of contamination during excavation and post-excavation by taking anti-contamination steps.
6. Make the scale of interpretation based on microscopic evidence explicit.

Due to the developing nature of Mesolithic residue studies, any generalisations about work done to date are necessarily limited. Overall, Mesolithic residue studies can be characterised as afterthoughts to the excavations. These studies can at times seem disorganised and haphazard due to the use of inappropriate or unclear methods, as well as reporting discrepancies. On the other hand, there seems to be a good effort to use comparative material (both in the forms of experimental replicas and modern reference spectra), which is deemed necessary to assist in identifications made by comparison. Lithics are the predominant artefact class preserved at Mesolithic sites, so their careful study is important to improving our knowledge of activities during this period. Residue analysis on stone tools have been applied extensive around the world to investigate many questions. Examples of residue research on Mesolithic stone tools are few and far between and its potential is far from being fully realised. Thus, lithic residue analysis studies in the Mesolithic are much-needed.

# CHAPTER 4 METHODS

## 4.1 Introduction

This chapter will outline the methods used in this thesis. The overall approach to the identification of lithic residues was based firstly on examination of residues in situ with reflected visible light microscopy (VLM) and the identification and grouping of similar residue types, followed by the application of chemical characterisation techniques (as reviewed in Chapter 2).

## 4.2 Anti-contamination protocols

This thesis included buried experimental residues on stone tools (Chapters 5 and 6), reference residues on stone tools (Chapters 5 and 6), and archaeological stone tools (Chapter 7). Each experimental flake, reference residue, and archaeological artefact was handled with care to prevent contamination from hands and cross-contamination between samples. The handling method was consistent for all analyses.

### 4.2.1 Excavation

A total of 614 stone tools and 614 sediment samples were excavated at Star Carr specifically for residue analysis. Excavators collected lithics for residue analysis by inserting their trowel into the soil just below the find and lever it directly into an

appropriate polyethylene zip bag, and did not touch samples. A small sediment sample (~ 5 g) was taken below each flint with a trowel and placed in a zip bag. Residue samples were then stored on site in a chilled picnic box. After each day of excavation, the lithics for residues analysis and their soil samples were transferred to a fridge. Experimentally buried lithics and their associated sediment samples were also recovered in the same way. The work order for lithic analysis in the Star Carr project was arranged to minimise contamination. No other types of analyses, such as lithic typo-technological analysis, morphometrics, or refitting took place prior to residue analysis, preventing the introduction of modern contaminants from handling or curation practices such as the addition of tape, ink, glue, or nail polish.

## 4.2.2 Storage

The reasons for the methods of artefact storage are rarely explained in residue analysis studies. Due to the lack of information on suitable protocols for archaeological residues, several experts were consulted to determine the most appropriate method of storage for lithics intended for residue analysis in this study. Bioarchaeologist Oliver Craig suggested freezing the stone tools and soils to stop fungal and microbial activity altogether. However, there was concern that this might alter the stone material by causing expansion of ice in microcracks, impairing planned microwear analysis. Freezing might also rupture any water-containing plant cell walls and thus destroy morphological characteristics used for identification. Conservator Ian Panter (York Archaeological Trust) advised against freezing due to the risk of post-excavation damage to the artefacts. However, Panter indicated that cold storage at 5°C is not always effective in halting the action of bacteria, worms, and insects. Similarly, palaeoecologist Rolf Mathewes advised that the cold storage level for core sediment samples (palynological analysis) at 4°C provides a temperature at which water is densest and microbial activity is low, but not absent (Mathewes 2014, pers. comm.). Thus, cold storage in a fridge at 5°C was the method chosen to slow the digestion of any potential archaeological residues by

fungi, bacteria, microorganisms, and/or worms and insects. In this way, the original cold and wet burial conditions were maintained as closely as possible. Unfortunately, the fridge containing the samples in BioArCh was inadvertently turned off by lab users at some point between January 30, 2015 and February 18, 2015. The amount of time the samples went without refrigeration is unknown, but samples were slightly cold when discovered February 18.

### 4.2.3 Cleaning

After excavation, all archaeological tools and tools buried in the experimental phase of research had peat or clay-rich deposits adhering to their surfaces. A cleaning treatment was required to allow residues to be seen during microscopic examination, but it was unknown which cleaning method might be best suited to the Star Carr assemblage. Thus, a literature review of cleaning treatments used in lithic residue analysis studies was conducted (Accompanying Material 1). Additionally, several cleaning treatments were also trialled on four lithics from wetland context (310), excavated in 2013 at Star Carr, listed below. A 'jet bath' refers to a gentle stream of ultrapure water expelled from a plastic squeeze bottle.

#### 1) Tool left damp, jet bath

- SC13 93229 TR34 (310) A18
- Appears to be an effective treatment that is non-abrasive and does not leave any chemical residues on the tool surface

#### 2) Tool air dried on tray lined with cling film, jet bath

- SC13 94099 TR34 (310) A26
- Some original sediment adhering to tool cracks and falls off when dried on cling film
- Does not appear to remove as much sediment from tool surface as jet bath from damp

- 3) Tool air dried on tray lined with cling film, compressed air (5 Star brand, HFC free)
- SC13 93254 TR34 (310) A17
  - Some sediment still visible macroscopically along the edges of tool
  - Compressed air appears to be relatively effective at removing sediment, but leaves invisible chemical residues from the propellant behind
- 4) Tool air dried on tray lined with cling film, rubbed in clean plastic bag
- SC13 93238 TR34 (310) A18
  - Any abrasive items in the adhering sediment adds unwanted microwear to the tool
  - Leaves sediment adhering. Unsatisfactory

From the literature review in conjunction with the results of the cleaning trial, it was determined that a wash with ultrapure water and gentle rub with a gloved hand (starch-free) would be the most suitable preparation for lithics prior to microscopic analysis.

Cleaning was conducted by first laying down a long strip of cling film as a working surface on the counter. Lithics were set out in their bags for cleaning, evenly spaced on the cling film. One gloved hand removed each lithic from its bag. The lithic was held with the gloved hand whilst exposed to a gentle jet bath stream of ultrapure water from a squeeze bottle, keeping a distance of 10 cm between the artefact and the nozzle. Then, each lithic was set to dry next to its associated bag on a tray lined with a new sheet of cling film, making sure no samples or bags touched each other. Gloves were changed with every handling of each lithic, both during and after cleaning, to prevent cross-contamination.

#### 4.2.4 Handling during analysis

Each artefact and experimental flint was handled with care to prevent contamination from hands and cross-contamination between samples. No handling of samples with bare hands took place prior to residue analysis. Powder free gloves that tested negative for starch were used for handling during microscopic and chemical residue analyses. Careful handling also limited the introduction of common modern contaminants on stone tools, such as skin flakes from bare hands and modelling clay (Pedergrana et al., 2016), and starch (Crowther et al., 2014).

Blu-Tack® was used as the base support material during microscopic examination of the experimental flakes. Blu-Tack®; can transfer to the flint, and can also stick to and remove residues from the stone surface. To prevent contamination or loss of residues, the microscope stage was prepared by placing a bed of Blu-Tack® on the stage and then overlaying a new layer of plastic paraffin film (Parafilm M®) to create a fresh unused surface that separated the mouldable Blu-Tack®; from each flake. Parafilm M® was chosen instead of cling film to place on top of the Blu-Tack® for the mounting surface because it is flexible and able to withstand stress without tearing or puncturing. Thus, the experimental flakes never came into direct contact with the Blu-Tack®. The paper-covered side of the parafilm was removed and placed facing upward to be in contact with the specimen and the parafilm was orientated by touching the edges only. Each flake was placed on the microscope stage and manoeuvred using a new powder free glove each time. These procedures helped to minimise, but did not eliminate, the presence of occasional modern contaminants. For example, blue and pink fibres from clothing were sometimes observed, and starch granules within the glue of the double-sided tape used to mount soil samples on slides were found (Figures 4.1 and 4.2).



Figure 4.1. Contaminant pink and blue threads found in a soil sample from the 11 month alkaline unit.

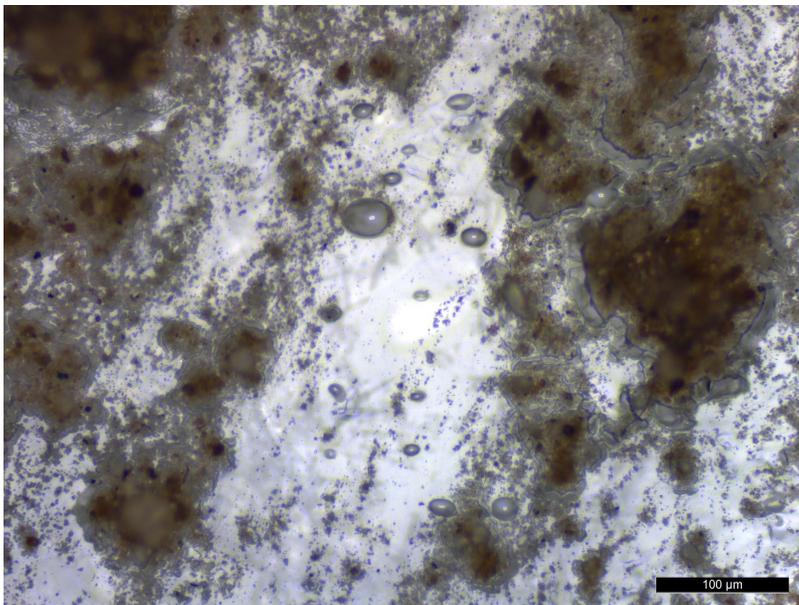


Figure 4.2. Example of starch granules underneath a mounted soil sample, originating from double sided tape.

## 4.3 Reflected visible light microscopy (VLM)

Reflected VLM was the first step prior to any chemical characterisation techniques being applied to identify residues. A low power stereo microscope with magnification from 5x to 40x, with an eyepiece magnification of 10x was used as an initial means to examine potential residues on tool surfaces. High power reflected VLM analysis was then carried out using a Leica DM1750 M, with objectives ranging from 5x to 100x, and an eyepiece magnification of 16x (Figure 4.3).

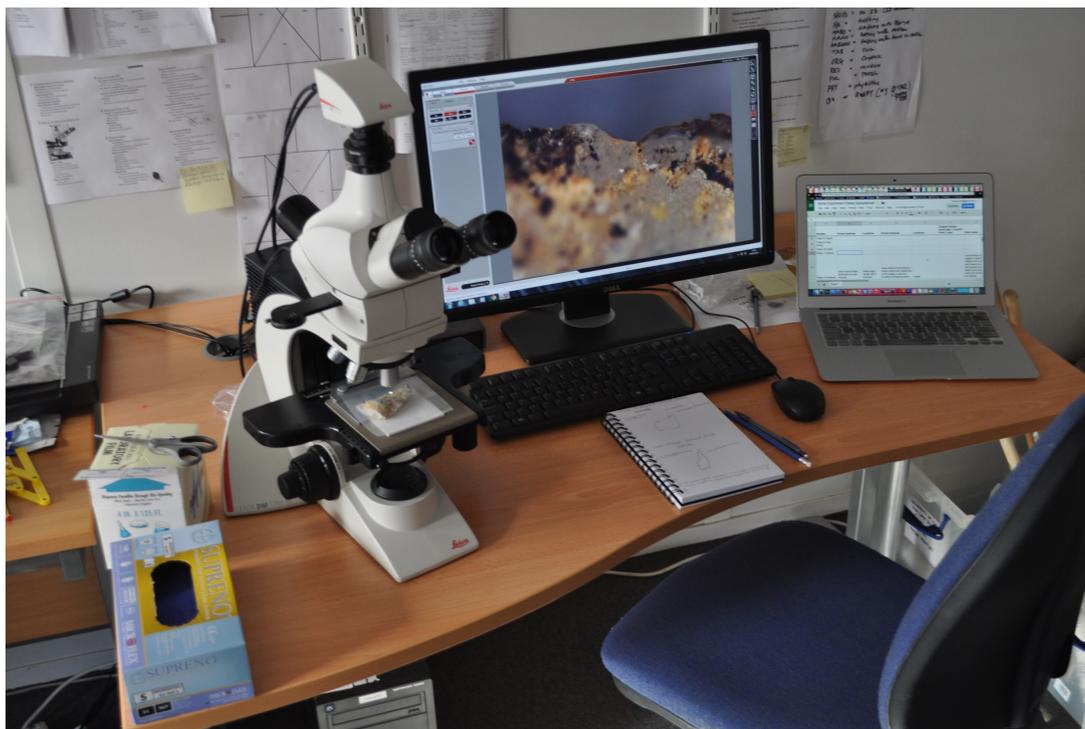


Figure 4.3. Reflected visible light microscope (VLM). A Leica DM1750 M with digital camera. Lithic being examined for residue traces in situ (Archaeology Department, University of York).

All edges on dorsal and ventral sides were examined, as well as several transects through the centre. Each stone tool was systematically examined and the locations of microscopic residues were documented. At least one composite extended focus microscopic image was taken for each tool, but more were taken if

residue deposits of interest were encountered. A series of z-stacked micrographs were taken for each microscopic residue to make a composite extended focus image, using Leica LAS Montage software. The program stitches together micrographs taken at different planes of focus of the residue in the z axis, resulting in an in-focus composite image which captures microtopography. Sediment sample controls were prepared by direct mounting on glass slides with double-sided tape and examined with reflected VLM.

Printed photographs of artefact surfaces were used to map numbered locations of residues (Figure 4.4), which were subject to further chemical analyses or SEM. The location number of each residue on each artefact was also noted in the file name of micrographs. Using annotated printed photographs during reflected VLM analysis made later relocation of specific residues possible. The numbered locations of residues also enabled comparison of reflected VLM images with microscopic images taken with other methods (SEM, FTIRM, and Micro-Raman).

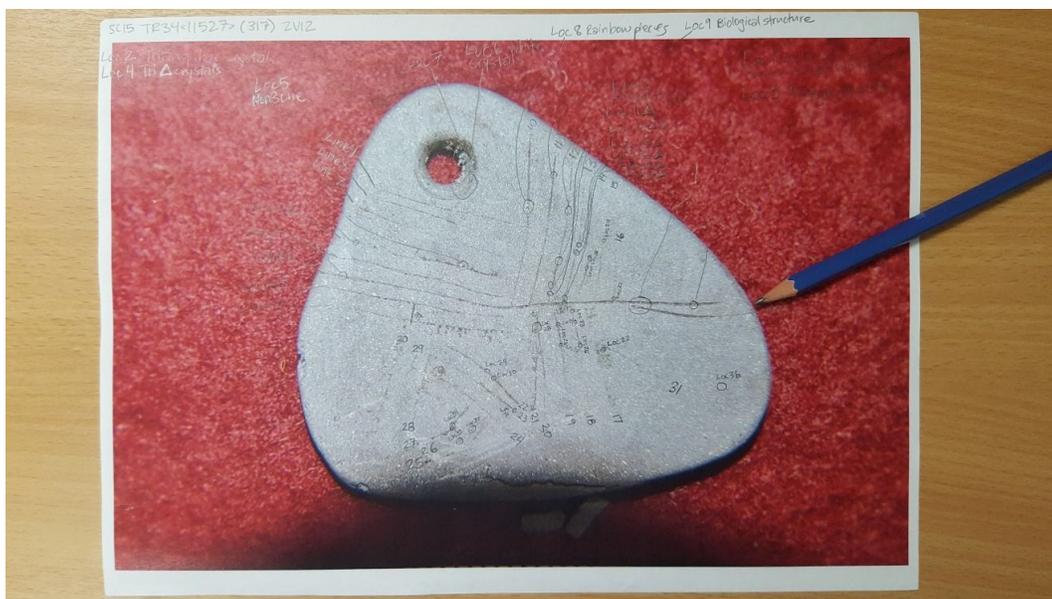


Figure 4.4. Example of the use of printed photograph to document the location of residues for further examination.

### 4.3.1 Microscopic data collection

The description of residues found on each stone artefact are presented in Accompanying Material 3. The following categories of observation were noted for each artefact:

- Artefact number, year of excavation, context, grid square
- Artefact type
- Contains possible resinous residues?
- Contains other possible anthropogenic residues?
- Dorsal residues, locations
- Ventral residues, locations
- Microwear (microchipping, polish, striations, edge rounding)
- Was the tool used?
- Other notes
- Foraminifera in stone
- Lath or rosette crystals Q gypsum or selenite
- Pyrite framboids or triangles
- Red-orange deposits Q iron oxide
- Clear shiny tideline
- Cling film or wash deposit
- Soil sample prepared?
- Does the tool need to be photographed/mapped?
- Recommend for GC-MS testing?
- Potential resinous residue removed by GC-MS solvent extraction?
- Other processing/analyses
- Initial interpretations

## 4.4 Scanning electron microscopy (SEM)

Two variable pressure or 'environmental' tabletop SEMs equipped with X-ray detectors to carry out EDX spectrometry were used (Hitachi TM-1000 and Hitachi TM3030Plus) to analyse modern residues on flint and residues on stone tools from Star Carr (Figures 4.5 and 4.6). VP-SEMs are non-destructive to artefacts. No sputter-coatings (such as gold, carbon, palladium) are required for imaging using VP-SEMs; a major advantage to traditional high vacuum SEM analysis. All SEM images were collected in backscattered electron mode or secondary electron mode and from 25x to 3000x magnification. Both SEMs used were coupled with energy dispersive X-ray spectrometers (EDS or EDX), and were capable of elemental microanalysis. Points of nominally .2  $\mu\text{m}$  on lithic residues were targeted for elemental microanalysis.



Figure 4.5. Variable-pressure Hitachi TM-1000 SEM equipped with an EDX detector (LACORE, University of Minnesota).

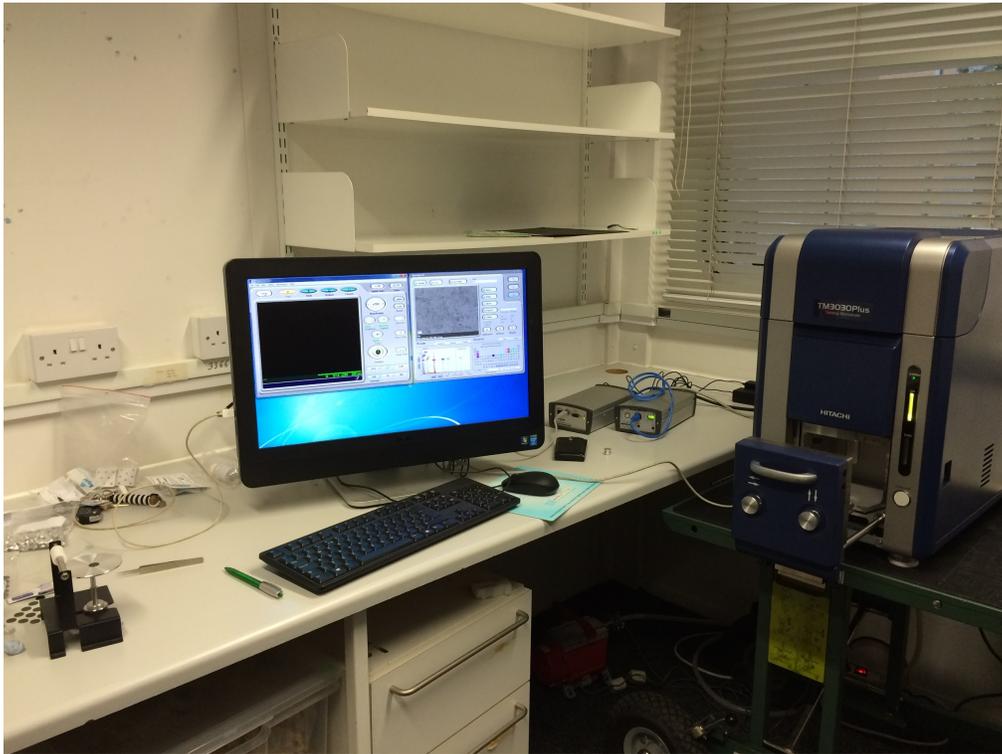


Figure 4.6. Variable-pressure Hitachi TM-3030Plus SEM equipped with an EDX detector (demonstration at BioArCh, University of York).

Three other traditional SEMs available at the University of York in the JEOL Nanocentre and Biology Department were trialed for examination of residues on lithics. These instruments were not designed to accommodate uncoated samples at low vacuum and were time-consuming and difficult to operate. The images produced on the traditional SEMs were fuzzy and of poor quality due to high levels of charging, and thus were not used further for investigating residues.

## 4.5 Fourier transform infrared microspectroscopy (FTIRM)

The Fourier transform infrared microspectroscopy (FTIRM) method was very attractive to apply to residues because of its potential to leave residues in situ on the lithic surface and to chemically characterise them non-destructively. Training was received from Gilliane Monnier in April-May 2015 using the FTIRM on in situ experimental and archaeological residues on flint at the Characterization Facility, University of Minnesota. A Thermo Scientific Nicolet Continuum FTIR microscope paired with a Nicolet iS50 FTIR bench with a liquid nitrogen-cooled MCT detector and a KBr beamsplitter, was used (Figure 4.7).



Figure 4.7. FTIRM. Nicolet Continuum microscope connected to a Nicolet Series II Magna-IR System 750 FTIR bench (Characterization Facility, University of Minnesota). The dewar flask contains liquid nitrogen for cooling the detector.

From the tests conducted, it was determined that overall, the spectra obtained from both experimental and archaeological residues did not show an adequate signal-to-noise ratio, and thus did not provide clear chemical information. There were several origins of poor signal quality, meaning there are many variables that can negatively influence spectra or make spectra so poor as to be simply not collectable. These factors which impact spectrum quality are: microtopography of the stone (FTIRM works best on polished flat surfaces, such as resin-impregnated sediment thin sections used in micromorphology), reflectance of the residue, the interaction of the stone material with the residue, residue thickness. However, Monnier et al. (2017a, 2017b) recently improved upon the FTIRM method for residue analysis by increasing the number of scans taken on difficult residues to very high amounts, and also narrowing the aperture to capture signals from just the residue, avoiding interference from the stone substrate.

## 4.6 Confocal Raman microspectroscopy (Micro-Raman)

Confocal raman microspectroscopy (Micro-Raman) is a spectroscopic technique utilised for the identification of crystal and molecular structures employing lasers to excite vibrational and stretching modes within the samples; this technique can suggest the chemical nature of microscopic residues with a high degree of specificity. Micro-Raman is minimally destructive to the residue in that an area of the residue of interest, about 20  $\mu\text{m}$ , is burned by the incident laser beam during analysis. No sample preparation is necessary to conduct Micro-Raman, and residues can be analysed in situ. Inclusion of water in residue samples does not impact the ability of Micro-Raman to obtain high-quality spectra, unlike FTIR

techniques. However, the artefact must be small enough to fit within the working distance between the Raman microscopic objective and stage.

This technique couples a Raman spectrometer to a standard optical microscope, allowing visualisation and micro-analysis. Initial testing of experimental and archaeological residues on flint surfaces took place at the Characterization Facility, University of Minnesota, using a Witec alpha300 R confocal Raman microscope with UHTS300 spectrometer and DV401 CCD detector (Figure 4.8).

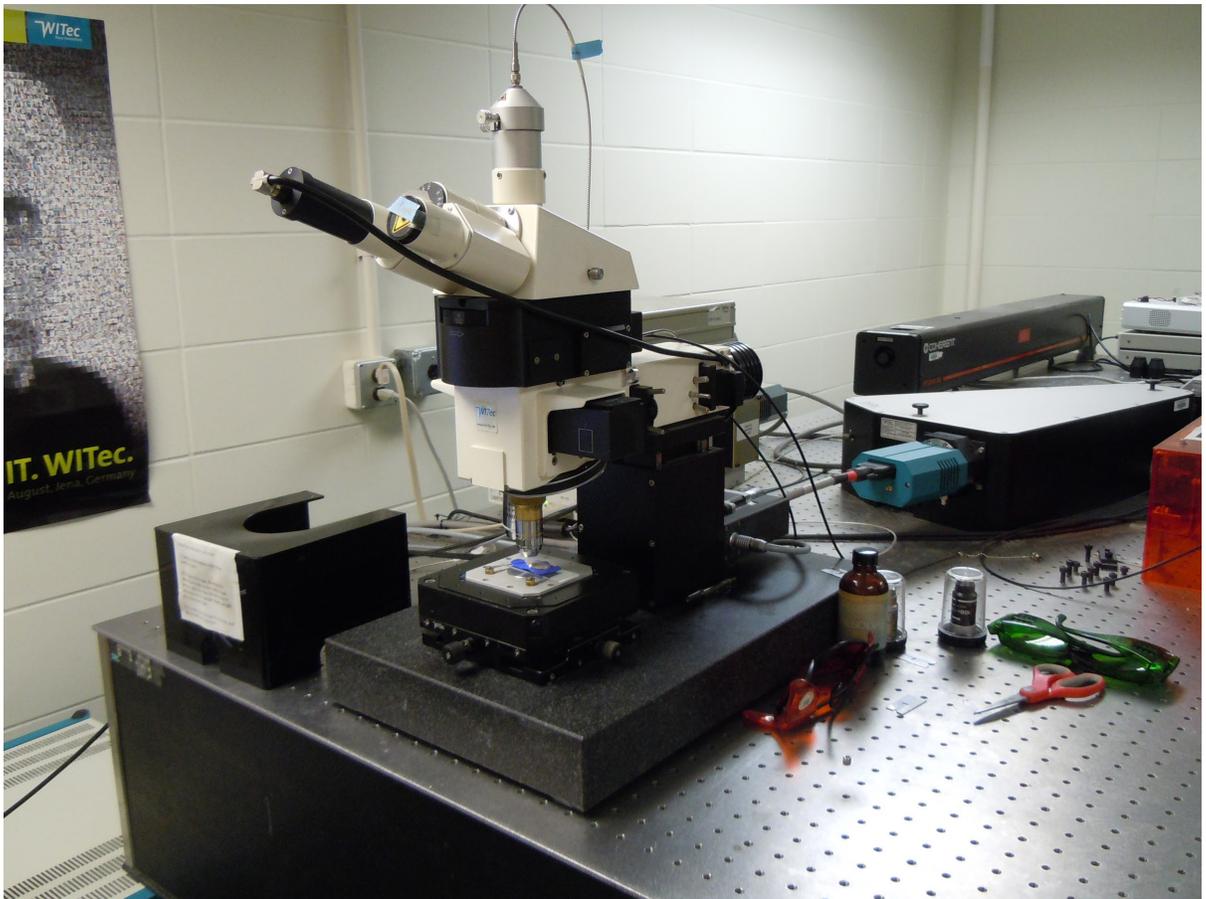


Figure 4.8. Micro-Raman. WITec alpha 300R confocal Raman microscope equipped with a UHTS300 spectrometer, a DV401 CCD detector and piezo-driven, feedback-controlled stage. Excitation source here is an Ar laser operated at 514.5 nm wavelength and 10 mW (Characterization Facility, University of Minnesota).

It was discovered that fluorescence was a common problem for organic residues, such as birch bark tar, making it impossible to collect useful spectra on these types of samples. The majority of the Micro-Raman analysis was conducted at the University of York, with support from Konstantinos Chatzipanagis and Roland Kröger (Physics Department, University of York). A HORIBA Jobin Yvon XploRA confocal Raman microscope (Figure 4.9) was used with a Nd-YAG laser (532 nm) energy excitation source. LabSpec (version 6) and OriginPro 2016 software were used to collect and evaluate spectra.

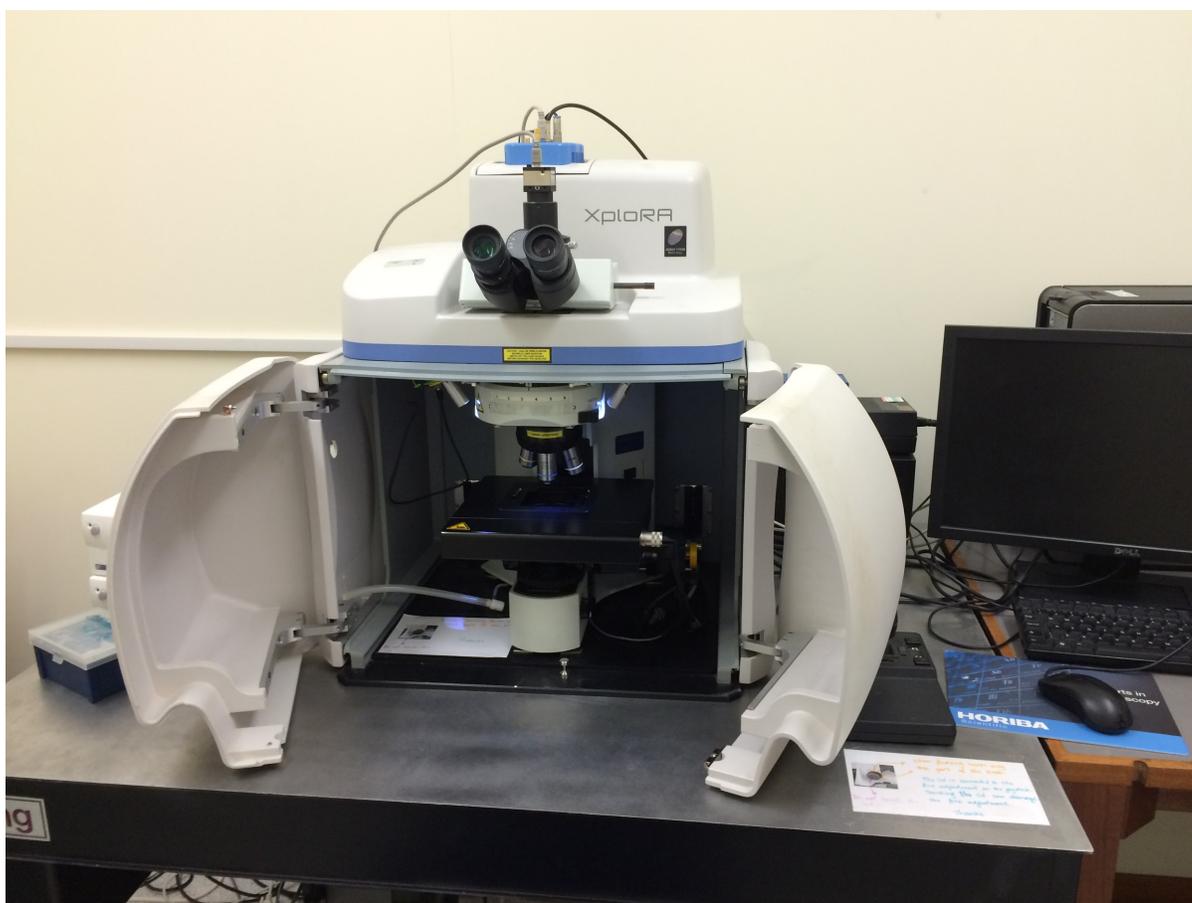


Figure 4.9. Micro-Raman. HORIBA Jobin Yvon XploRA confocal Raman microscope. Excitation source here is a green Nd:YAG diode laser operated at 532 nm wavelength at a power of 20 mW (Physics Department, University of York).

A laser spot size of nominally 1  $\mu\text{m}$  was used. Once a Raman spectrum was collected, the bands present were compared to spectral database reference libraries,

as well as published literature, to identify the residue. In this study, confocal Raman microscopes were always used for spot microanalysis, although sampling in a line and mapping an entire area are possible. Polynomial baseline corrections were applied to all Raman spectra using the software LabSpec (version 6), thus all spectra presented in the thesis are altered to show a better signal-to-noise ratio because the background noise was subtracted. OriginPro 2016 software was used to plot data.

## 4.7 Gas chromatography-mass spectrometry (GC-MS)

As a destructive test, residue samples cannot be submitted to other forms of analysis after GC-MS, and thus if several techniques are being used, GC-MS should always be last in the order of analytical techniques being performed. Samples used in this study were prepared for this study by a total extraction of residues from the lithic by immersion of the tool in organic solvents and sonication. Accompanying Material 4 details the step-by-step standard operating procedure for sample preparation. Sampling by drilling, knife, or swabbing with solvent were not practical options since the residues concerned are microscopic. Unfortunately, this means the extract obtained cannot be locationally pinpointed to specific spots on the tool. Prepared lithic residue samples were injected into an Agilent 7890A Series chromatograph with 5975 C Inert XL mass-selective detector with a quadrupole mass analyser (Biology Department, University of York) (Figure 4.10). Gas chromatograms were analysed and comparisons made with the use of the NIST library within Agilent MSD Chemstation software, (version G1701EA E.02.02.1431). Unknown peaks within sample gas chromatograms and mass spectra were also compared to available literature.

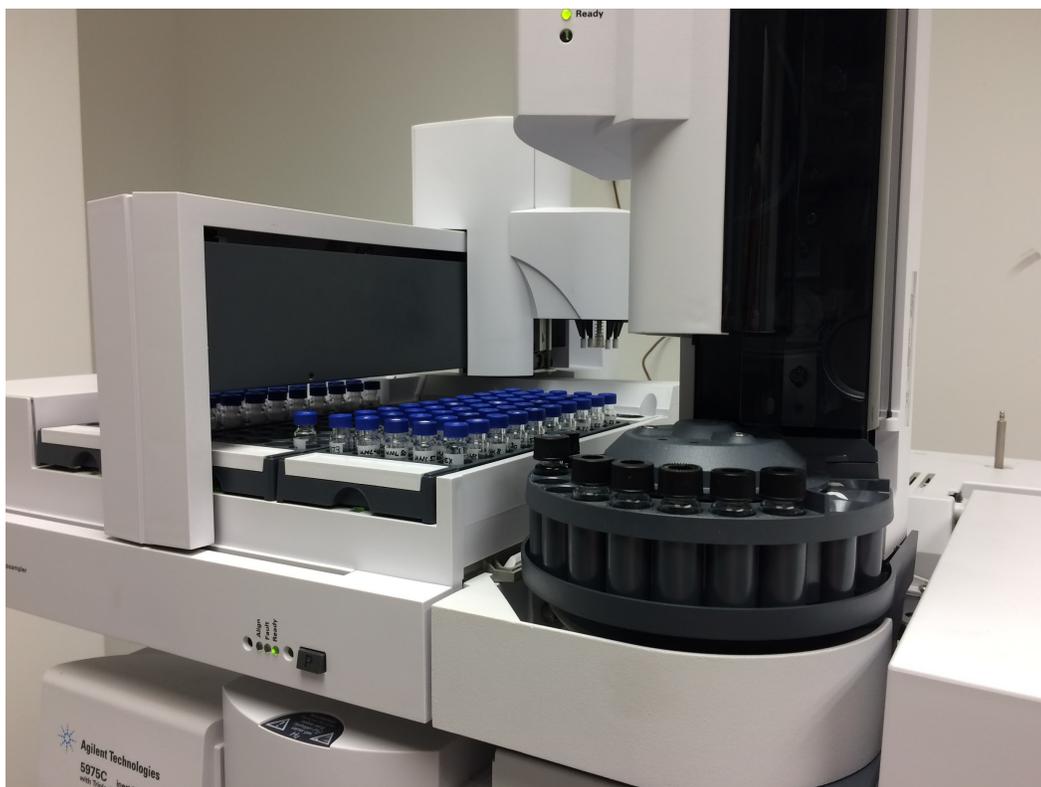


Figure 4.10. GC-MS. Agilent 7890A series chromatograph attached to a 5975 C Inert XL mass-selective detector with a quadrupole mass analyser (Biology Department, University of York).

## 4.8 Conclusion

In sum, stereo microscopy, reflected VLM, VP-SEM-EDX, FTIRM, confocal Micro-Raman, and GC-MS have been used to investigate and identify the reference residues on flint, experimental lithics containing residues, and archaeological artefacts. These techniques are applied in the following two parts of the thesis.

# PART 2 EXPERIMENTAL INVESTIGATIONS OF LITHIC RESIDUES

# CHAPTER 5 SET UP OF RESIDUE DIAGENESIS BURIAL EXPERIMENT

## 5.1 Introduction

Microscopic residue analysts rely on visual observations to interpret ancient residues, sometimes assuming anthropogenic residues have been identified when possible contaminants have not been excluded. Often, the morphological characteristics specific to residue identifications are not described and illustrated. A further issue arises when biological descriptions of organic tissues based on specially prepared histological stained and thin-sections are used as a guide for residue analysts (e.g. Langejans and Lombard, 2015), which misleadingly gives the impression that these biological features can be found in archaeological material. Biological descriptions are often ill-suited to residue analysis, since they describe features that are not present or simply invisible on degraded archaeological residues and absent even on reference collection residues (e.g. residue on a stone substrate). Therefore, an important question which should be addressed is: 'Are there any specific visual characteristics that identify the residue in question unambiguously?'. This issue was highlighted in blind tests by Monnier et al. (2012), which showed that even modern residues that have undergone no diagenetic alterations can be ambiguous and difficult to identify.

Currently, data concerning taphonomically-altered lithic residues is scarce. Basic experiments to assess the reliability of archaeological residue analysis from varied burial environments are still needed. Grace (1996), Haslam (2006a, p. 208), and Langejans (2009, p. 15) have all noted that the building of 'significant body of results' is required to form a reference base for residue analysis as a discipline and allow larger questions to be posed, such as resource exploitation and social changes.

Langejans (2009) argues that contributions to the 'significant body of results' can only be accomplished with large-scale studies. Calls for work on residue diagenesis specific to archaeological sites were put forth by Lombard and Wadley (2007a, p. 156): "Well-designed and controlled research programs need to be constructed to address particular questions about diagenesis in archaeological sites".

This chapter is the first of two chapters which sets out the design and implementation of an experiment to investigate lithic residue preservation and identifiability in advance of analysis of flint from Star Carr. The experiment is reported in full in Croft et al. (2016). The information collected assisted in the targeting of particular residue types on archaeological material in the following stage of research. In addition, the experiment added much-needed information to the body of knowledge regarding residue preservation within the discipline of residue studies. This chapter first evaluates other lithic residue burial experiments. The objectives, experimental design and methods for the burial experiment are then presented. The results of the experiment are set out and evaluated in Chapter 5.

## 5.2 Review of lithic residue diagenesis experiments

### 5.2.1 Introduction

There are very few examples of experiments that have interpreted microscopic lithic residues after diagenetic processes have occurred (Table 5.1). Here, studies which used the following three criteria will be reviewed:

1. Residues were placed on stone and/or glass substrates.

2. Residues were exposed to factors which would cause diagenesis to occur: by burial in sediment or by application of chemical treatments to simulate natural diagenesis.
3. The method of residue detection includes microscopy.

## 5.2.2 Evaluation

Table 5.1. Past lithic residue diagenesis experiments.

Reference	Total number of pieces (including blanks, replication, and pieces lost during experiment)	Substrate type(s)	Residues applied	Diagenesis treatment(s)	Duration of treatment
Anderson 1980	23	flint tools	wood, grass, cartilage, fresh and cooked bone, soaked antler, dry hide	chemical: hydrogen peroxide and hydrochloric acid, sulfuric acid, sodium hydroxide, alcohol, and ether	unknown
Jahren et al. 1997	6	chert flakes	bamboo, bone	chemical: 35% hydrogen peroxide	one day
Hortolà 2001	2	stone tools: knife, projectile point	blood	exposure outdoors (1 week), exposure indoors, burial	1 year
Wadley et al. 2004	10	flakes of hornfels, chert, dolerite, chalcedony	raw muscle, raw fat, raw blood, raw bone, cooked fat, cooked and uncooked starch, tree bark, and tree exudates	burial and outdoor exposure. Buried in a bag with compost for 30 or 60(?) days indoors. Thereafter tools mistakenly scattered outdoors in a garden where they were watered daily for a period of three days. Tools subsequently sun-dried and examined	63 days(?)
Barton 2009	8	silcrete and silicified tuff flakes	starch	burial and outdoor exposure at soil surface	4 months, 2 years
Langejans 2009, 2010	~ 370	flakes of hornfels, chert, quartzite, Meuse flint; microscope	bone, fat, blood, muscle tissue, starch, woody tissue	burial and outdoor exposure at soil surface, covered in or outside caves, microscope slides buried facing up or down	4 weeks, 1 year

		slides with 3 grades of sandpaper			
Rots et al. 2016	3	flint tools: blades and an endscraper	wood, bone, meat	burial	2 months

As part of her study of lithic polishes and residues, Anderson (1980) used 23 experimental flint tools on five species of temperate wood and one species of grass (in both fresh and dried states), fresh cartilage, long bones and ribs, cooked bone, soaked antler, and dry hide. The effect of archaeological diagenesis was simulated by cleaning the experimental tools containing residues with a number of diluted chemicals. The tools underwent cleaning treatments with one or more of the following: hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydrochloric acid (HCl), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), sodium hydroxide (NaOH), alcohol, and ether. Microwear polishes and residues were examined with a reflected light microscope and then coated with gold or carbon and examined with two SEM with EDX spectroscopy. Although it is also stated by Anderson (1980, p. 183): “X-ray dispersive analysis gave qualitative indications of the mineral components of the samples” these results were not systematically reported in the study. However, it is noted that “Residue materials on the archaeological and experimental tools were found to be composed principally of silicium or calcium” (1980, p. 194). The individual results of each of the 23 experimentally used tools were unfortunately not detailed in the article, although SEM images of seven experimental residue conditions were illustrated.

Jahren et al. (1997) conducted an experiment where lithic residues were not buried but a simulation of the effects of diagenesis took place. Then, residues were analysed morphologically with traditional SEM and analysed elementally with EDS. The part of the experiment of concern here involved bamboo and deer bone residues on chert flakes (the other part examined the residues without stone substrate). One flake contained bamboo residue, one flake contained deer bone residue, and one flake was blank, and all were examined with light microscopy prior

to chemical treatment. The three flakes were soaked in 35% hydrogen peroxide for 24 hours at room temperature and then rinsed with deionised water. Hydrogen peroxide was chosen to mimic the oxidation and destruction of organic matter in an aerated burial environment, leaving the mineral components of the residues intact. After the simulated diagenesis treatment, the three flakes were fractured into smaller pieces for mounting on SEM stubs and gold coatings were applied for analysis. Jähren et al. (1997) found that the structure of prismatic fibrous crystals in the bamboo residues were not significantly altered after chemical treatments. The bone residue was amorphous and greasy, showing no characteristic structure. Bone residues were reduced on the surface of the chert after the hydrogen peroxide treatment. The whole experiment was repeated twice, which showed reproducible results.

Hortolà (2001) used two replica stone tools to examine mammal blood residues with SEM. He buried a projectile point replica with a gazelle blood smear, whilst a stone knife with peccary blood was exposed outdoors for one week (about an hour a day in the sunlight), thereafter exposed indoors. The duration of the experiment was one year. The burial environment was a vegetal soil ( $6 < \text{pH} < 7$ ) in an open-air vivarium container at the Botanical Institute of Barcelona. The point was buried 7.5 cm down in the soil but due to clayey soil accumulation, it was recovered at a depth of 10.5 cm. During burial, the mean air temperature ranged from about 2°C to 34.5°C, with a mean of 16.5°C, amount of absolute rain was 545 mm, and mean air relative humidity was 61.5%. It is worth noting that the burial was not truly a natural sediment environment with normal taphonomic factors (such as bioturbation by earthworms and insects, or natural percolation of moisture in the sediment), as the experiment was contained. When the piece was examined macroscopically, it was found that clear blood staining remained which was lighter in colour than the original blood smear. With traditional SEM, microscopically visible red blood cells of at least three morphologies were identified, with varying levels of confidence (suspected near-discocyte, suspected echinocyte, discocyte or spherocyte, and near spherocyte).

Thus, Hortolà presents a case for intact red blood cells surviving a near-neutral and vegetation-rich burial environment for at least 1 year.

Wadley et al. (2004) conducted blind tests to assess the reliability of high-power microscopic residue identification. The relevant part of this study is Test 2, in which stone flakes containing plant and animal residues were buried in a bag of compost. Flakes were used for cutting raw beef, cooked beef and bones, cutting raw and cooked potato and scraping twigs. The blood and plant exudates were smeared on flake surfaces. The tools were used (and residues were applied) while the analyst was wearing with cornstarch powdered latex gloves, except for two experimental flakes which were used without gloves (the reason for this is unstated). The experimental conditions for each flake prior to burial in compost are listed below:

- Cut raw beef fat and meat, dried in oven (30°C)
- Cut fresh beef bone with blood present, dried
- Cut raw beef, dried in oven (30°C)
- Cut cooked potato, dried in oven (30°C). Gloves not used.
- Smeared beef blood on dorsal and ventral surfaces, dried. Gloves not used.
- Cut raw potato, dried in oven (30°C)
- Cut cooked fat dried on glass in oven (30°C)
- No residues, dried
- Scraped tree bark (*Ozoroa paniculatum*), dried
- Smeared tree exudate (*Ozoroa paniculatum*), dried

The stone flakes used in the experiment were made from several raw material types: hornfels, dolerite, chert, and chalcedony. It was not stated which stone raw material type was used with which residue type. This means the substrate conditions used across the experiment were variable and hence incomparable in terms of residue diagenesis results. Substrate is an important variable to control since grain size of the stone type could have impacted residue preservation. Some of the used flakes were dried in an oven at 30°C for 2 hours, and others were simply 'dried'. Again, a

lack of experimental control is apparent – the flakes were dried in different ways and are thus not comparable because they did not receive the same treatment.

The stone flakes with residues were then buried in a large synthetic bag containing acidic, organic-rich dry compost for 60 days indoors. The bag was stored in a lightproof container. However, the experiment was derailed when “After a period of 60 days the compost and the tools were inadvertently scattered outdoors in a garden where, for three days, they were exposed to the elements. In addition, they were watered daily. This exposure of the flakes was not intentional; the bag was discovered and removed by someone who was not aware of the intended experiment. When the error was discovered the tools were sun-dried and placed individually in new zip-lock plastic bags” (Wadley et al., 2004, p. 1493). Despite this major mistake that resulted in a total loss of experimental control, these tools were presented anyway as a residue diagenesis blind test to Lombard.

One of the key problems is that because of the mistake, the experimental tools experienced two different burial environments, so it is impossible to assess which environment caused diagenetic alterations in the residues. There are also basic discrepancies in reporting in this article. The duration of burial is unclear within the article, with both one month (page 1491) and 60 days (page 1493) being cited as the period that the flakes were present in the compost. The text notes a total of 28 flakes were experimentally prepared (Wadley et al., 2004, p. 1492), but Table 1 which provides an inventory of each item and experimental treatment lists a total of 45 flakes. To add to the confusion, Table 3 shows 10 flakes were used for Test 1 and Table 2 shows 17 flakes took part in Test 2, which does not add up to 28 either. Wadley et al. (2004) were critiqued for flaws in design and loss of experimental control by Crowther and Haslam (2007).

Barton (2009) conducted a burial experiment with sweet potato starch (*Ipomoea batatas*) placed on eight flakes of two raw materials: four silcrete flakes and four silicified tuff flakes. It is not reported how long the sweet potato residues were left to

dry on the experimental flakes after use or how they were stored prior to burial. Each flake was labelled with a metal tag tied to the flake with metal wire. Half the sample was buried in two 10-20 cm deep squares and half were left on the soil surface in 1993 at a site about 10 km west of the town of Singleton, New South Wales, Australia. The burial environment was slightly acidic, and was composed of wind and water borne decayed sandstone bedrock, making a sandy soil. Plastic mesh was pegged down on top of the buried samples which did not prevent disturbance from an animal or person, since one of the buried experimental flakes (silcrete flake, buried two years) was not recovered. Samples and adhering sediment from the tool surfaces were collected after a) four months and b) two years, and placed in separate clip lock plastic bags. The level of rainfall recorded at Singleton and during the experiment time intervals was 279 mm from May to September, and 1,214 mm from May 1993 to August 1995. The sample left on the soil surface, was exposed to rewetting by rain, direct sun, and ultraviolet light. After recovery, the experimental flakes were stored (presumably indoors at room temperature) until 2007, when microscopic analysis took place.

For analysis, starch grains were first located with a reflected light microscope, then spot samples were removed. Spot samples were removed with a 20 microlitre droplet of ultrapure water placed on the tool surface left undisturbed for about 30 seconds, then agitated with the nylon pipette tip. Then, each sample was retrieved and mounted on a glass microscope slide and viewed with a transmitted light microscope.

Barton reports that the results of the experiment were counterintuitive since more sweet potato starch grains were recovered from flakes that were laid on the surface (total= 196) compared to the buried sample (total= 132). However, the silcrete flake buried for two years which was not recovered could have contributed to the total number of starch grains from buried contexts, and this was not taken into account. Interestingly, Barton found that the level of preservation was completely different between the two burial squares which were only two metres apart: one for the four

month specimens, the other for the two year specimens. No anti-contamination measures were reported in the experiment beyond the use of individual plastic bags being used to store flakes after recovery. Barton (2009, p. 134) noted that there were considerable differences in starch preservation between individual flakes. It was suggested that the variability between individual flakes might play a role, presumably in terms of microtopography of the stone, the original amount of starch residue applied to each flake, and the surface area covered by the residue.

The first burial study conducted on a large-scale that specifically deals with lithic residues, their preservation, and microscopic identification, was carried out by Langejans (2010, 2009), although other researchers currently have large lithic residue burial experiments in progress. Because Langejans burial experiments were pivotal to the methodological development of microscopic residue analysis, they are reviewed here in detail.

Langejans (2009) burial experiments were designed to understand the diagenesis of modern lithic residues, and thus provide a more informed and appropriate analogical method to interpret archaeological microscopic residues. In this way, Langejans' study moved out of the laboratory and beyond the use of modern reference collection residues, which are not exposed to the taphonomical decay and post-depositional changes that archaeological residues undergo. For analysis, tools were rated on scale called a 'preservation index' (Langejans, 2010, p. 978, 2009, p. 51), a score from 0-5 given for each experimental lithic indicating how much of the original residue was left. A score of 5 is described as 'the situation just after use' involving thick residue deposits, and a score of 0 is 'no observed residue left'.

A total of six groups of burial experiments took place in four locations: Sibudu and Sterkfontein (South Africa) and Zelhem and Wilhelmina Polder (The Netherlands). An overview of the conditions of each experiment are provided in Table 5.2. Sibudu and Sterkfontein are archaeological sites, and the Zelhem and Wilhelmina Polder locations are not. Considering all experiments in the study, the residue types

examined were: bone, fatty bone, blood, muscle, starch, and woody tissue. The substrates to which residues were applied were: flakes made of hornfels, chert, quartzite, Meuse flint, as well as glass slides with and without three grades of sandpaper attached. After residues were added to the substrates, some experimental samples were buried in the soil and some left exposed and unburied.

In the South African experiments, bone, fatty bone, muscle tissue, starch, woody tissue residues were placed on freshly knapped chert, quartzite, and hornfels flakes. At Sterkfontein (total experimental pieces= 80), two sets of the experimental chert and quartzite flakes (one set buried at a depth of 5 cm, one set unburied), were placed inside the cave on top of and within a “modern talus cone” (Langejans, 2009, p. 23) and another two sets were placed outside the cave (one set buried at a depth of 5 cm, one set unburied). In the Sterkfontein experiments, it is clear that the burial of experimental material did not take place in any archaeological contexts. At Sibudu (total experimental pieces= 80), two sets of experimental hornfels flakes (one set buried at a depth of 5 cm, one set unburied), were placed inside the rock shelter on/in a rock fall (Langejans, 2009, p. 30) and another two sets were placed outside the rock shelter at the drip line (one set buried at a depth of 5 cm, one set unburied). In the Sibudu experiments, burial of experimental material did not take place within the excavated area containing archaeology, but rather in what appears to be a modern rock fall. After burial periods of 1 month and 1 year, the samples at Sterkfontein and Sibudu were recovered and examined with reflected light microscopy, and the distribution of all residues and contaminants were mapped on drawings of each flake.

The first group of Dutch experiments took place with potato starch and marrow/bone residues on previously knapped and washed Meuse flint. At Zelhem, 13 experimental flints were buried at a depth of 15-20 cm and 13 flints (table reports 12, Langejans 2009, 36) were deposited on sand under the organic surface layer. At Wilhelmina Polder, 13 of the experimental flints were buried at a depth of 15-20 cm,

and 13 flints (table reports 12, Langejans 2009, 35) were exposed on top of the surface. All samples were recovered after 6 months.

The next group of Dutch experiments took place on glass slides and used the following residue types: starch, blood, and fat. The glass slides were fitted with coarse, medium, and fine sandpaper, and some glass slides had no sandpaper. At Zelhem and Wilhelmina Polder, 56 slides were buried with the residues facing down, and 24 slides were buried with the residues facing up (tables in the thesis report 32 slides faced up at both Zelhem and Wilhelmina Polder, Langejans 2009, 37-40). The Zelhem slides were deposited 5 cm below the surface in sand (Langejans, 2010). The Wilhelmina Polder depth of burial in marine clay was not reported but was presumably also 5 cm below the surface. The burial period was 6 months.

When compared, the six groups of experiments explored many variables that could impact residue decay: anti-contamination treatments prior to burial, open air locations vs covered rock shelter vs semi-protected cave, burial or exposed at soil surface, at rock shelter drip line or covered, burial time intervals (1 month, 6 months, 1 year), substrate type, orientation of residues on slides (facing up or down), pH, precipitation, sediment types, sediment moisture, temperature, burial depths (5 cm, 15-20 cm, surface, deposited under the organic surface). Sample recovery also differed across experiments, with some pieces unfortunately lost or destroyed in five out of six experiments. The South African experimental residues were compared to a relevant archaeological sample. The experiments in the Netherlands were not compared to archaeological samples, but rather were used to understand residue preservation in burial environments which were significantly different from the South African sites.

Table 5.2. Summary of Langejans (2009) residue burial experiments.

	Sibudu	Sterkfontein	Zelhem	Wilhelmina Polder	Zelhem	Wilhelmina Polder
<b>Country</b>	South Africa	South Africa	Netherlands	Netherlands	Netherlands	Netherlands
<b>Materials worked</b>	fresh bone, muscle, potato, wood	fresh bone, muscle, potato, wood	marrow/bone, blood, potato	marrow/bone, blood, potato	marrow fat/bone and potato	marrow fat/bone and potato
<b>Residue types</b>	bone, fatty bone, muscle tissue, starch, woody tissue	bone, fatty bone, muscle tissue, starch, woody tissue	fat, blood, starch	fat, blood, starch	fat, bone, fatty bone, starch	fat, bone, fatty bone, starch
<b>Substrate</b>	hornfels flakes	chert and quartzite flakes	Facing down: glass slides, glass slides fine-grained sandpaper, glass slides with medium-grained sandpaper, glass slides with coarse-grained sandpaper. Facing up: glass slides	glass slides, glass slides fine-grained sandpaper, glass slides with medium-grained sandpaper, glass slides with coarse-grained sandpaper	Meuse flint	Meuse flint
<b>Anti-contamination steps pre-burial</b>	hornfels flakes knapped specifically for this project	chert flakes knapped specifically for this project	none noted	none noted	tools washed and dried prior to use	tools washed and dried prior to use
<b>Anti-contamination steps post-excavation</b>	samples not washed, stored in plastic ziplock bags	samples not washed, stored in plastic ziplock bags	samples not washed, stored in plastic ziplock bags	samples not washed, stored in plastic ziplock bags	samples not washed, stored in plastic ziplock bags	samples not washed, samples stored in plastic ziplock bags
<b>Number of samples</b>	80 (including blanks)	80 (including blanks)	80? (including 16 blanks)	80? (88?) (including 16 blanks)	25	25 (including 5 blanks)
<b>Burial time</b>	1 month, 1	1 month, 1	6 months	6 months	6 months	6 months

<b>intervals</b>	year	year				
<b>Recovery</b>	2 tools used on bone inside the shelter not recovered	2 tools used on bone placed inside and outside cave not recovered. Sample buried outside cave lost (in 2009 PhD it suggests the whole sample was lost, but 2010 says four tools used on starch outside cave were lost), which was replaced with quartzite instead of chert	All slides recovered	All slides lost/destroyed due to ploughing	2 tools used on starch and exposed on the surface not recovered	12 (?) tools not recovered (results from only 13 tools reported). 9 tools recovered lost their labels, some of which could have belonged to the blank sample. These tools of unknown experimental treatment were analysed anyway
<b>Location</b>	40 inside rock shelter, 40 outside shelter at the drip line	40 chert inside cave, 36 (?) chert and 4 quartzite outside cave at the surface	outside	outside	outside	outside
<b>Soil cover</b>	20 buried inside cave, 20 exposed inside cave, 40 outside cave	20 buried inside cave, 20 exposed inside cave, 40 outside cave	all samples buried	all samples buried	13 buried, 12 (13?) deposited on sand under the organic surface layer	13 buried, 12 (13?) exposed
<b>Sample orientation</b>	unknown	unknown	56 slides with residues facing down, 24 (32?) slides with residues facing up	56 slides with residues facing down, 24 (32?) slides with residues facing up	unknown	unknown
<b>Burial depth for half of sample</b>	5 cm	5 cm	5 cm	burial depth not noted in 2009	15-20 cm	15-20 cm
<b>Burial depth for other half of sample</b>	N/A	N/A	N/A	N/A	deposited on sand under the organic surface layer	deposited on surface
<b>Mean annual precipitation (mm)</b>	1000 inside (?) and outside shelter	660 inside (?) and outside cave	758	721	758	unknown

<b>pH soil</b>	7.2 inside, 6.4 outside	7.8 inside, 7.2 outside	3.9	7.4	humus top layer 3.94, sediment under top layer 3.87	unknown
<b>Soil type</b>	ash	sandy loam	sand	marine clay	sand	marine clay
<b>Soil moisture (Pa)</b>	5 inside, 13 outside	22 inside, 82 outside	97	not measured, estimated to be high	97	not measured, estimated to be high
<b>Mean winter monthly min temp (°C)</b>	18.5 inside, 18.5 outside	-2 inside, -2 outside	2.1	monthly min temperature 3.7	monthly min temperature 2.1	monthly min temperature 3.7
<b>Mean summer monthly max temp (°C)</b>	23.5 inside, 23.5 outside	27 inside, 27 outside	17	monthly max temperature 17.8	monthly max temperature 17.0	monthly max temperature 17.8
<b>Compared to archaeological sample?</b>	yes	yes	no	no	no	no
<b>Results</b>	good preservation of residues on both the experimental and archaeological tools inside the cave	good preservation of residues on the experimental tools inside the cave. All residues disappeared after one year burial outside cave.	sandpaper-covered slides have a positive effect on residues preservation, preservation on the fine- and medium-grained paper was better than the coarse-grained paper. Residues on glass preserve poorly. Residues underneath slides have a better chance of survival	sandpaper-covered slides have a positive effect on residues preservation, preservation on the fine- and medium-grained paper was better than the coarse-grained paper. Residues on glass preserve poorly.	few or no residues preserved	few or no residues preserved
<b>Reference</b>	Langejans 2009; 2010	Langejans 2009; 2010	Langejans 2009; Langejans 2010	Langejans 2009	Langejans 2009	Langejans 2009

From the burial experiments results, Langejans predicted which lithic residue types might survive in the archaeological contexts at Sterkfontein and Sibudu. The residue types which Langejans (2009, pp. 172–173) states preserve poorly (and thus are

less likely to preserve archaeologically) were starch and muscle tissue. Those with better preservation were animal bone and plant tissue, which she expected higher preservation of in the archaeological assemblage. In at least some experimental conditions, Langejans found that bone, muscle tissue, woody tissue, and starch residue types survived and were microscopically identifiable, suggesting an overall optimistic outlook for residue identification archaeologically.

The above predictions made by taking experimental degradation information and using analogical reasoning to form expectations of the archaeological material. In the experiments, Langejans assumes the variables influencing diagenesis in non-archaeological sediments are comparable to the conditions in the archaeological sediments. However, the sediment conditions which were used in experiments appear to be recent, not archaeological. At Sterkfontein, the experimental pieces were on top of and buried in a modern talus cone, and at Sibudu, the experimental pieces were on top of and buried in a rock fall, outside the limits of the archaeological excavation.

One of Langejans predictions stemming from the experimental results was that stable environments, such as those inside the cave and rockshelter would preserve residues better than outside, unstable environments (Langejans, 2009, p. 175). The samples inside the caves at Sterkfontein and Sibudu were better preserved than the samples deposited outside the caves. Inside the Sterkfontein cave it is dry and microbial activity is low, and experimental residues were found to preserve well. Thus, if tools in the past were deposited inside the cave, their residues were expected to preserve well archaeologically. It is known however, that a number of archaeological tools spent a good deal of time outside Sterkfontein cave before an event at the end of the interglacial washed them inside the cave. On these tools which washed into the cave, Langejans predicted no residues would be expected to preserve because exposed residues deteriorate rapidly.

Langejans (2010) also presents the experiment that took place on glass slides buried in Zelhem, Netherlands, alongside the experiments that took place on stone flakes buried in South Africa. The Zelhem experiment on glass slides did not use all the same residue types as the stone flake experiments at Sibudu and Sterkfontein. Additionally, the Zelhem burial experiment took place on a different time scale (recovery of slides after 6 months) compared with the Sibudu and Sterkfontein experiments (recovery of flakes at 1 month, 1 year).

Although the Zelhem experiment “stands on its own” (Langejans, 2010, p. 974), it could have been reported separately because it addresses a different question (the effect of substrate grain size on residue preservation). A separate report would also clarify that results from stone flakes, slides, and sandpaper are not comparable, which seems to be implied in the thesis (Langejans, 2009, p. 34).

Values presented within the 2009 thesis, and also between the thesis and the 2010 publication are unclear. For instance, a table within the 2010 article (Langejans, 2010, p. 975) presents the temperature and precipitation conditions as being the same both in and outside the caves, which seems unlikely to be realistic since caves are at least partially sheltered and often colder than the surrounding environment. The same table records temperature for Sibudu, Sterkfontein, and Zelhem, expressed as ‘mean winter monthly temperature’ and ‘mean summer monthly temperature’, but in the 2009 thesis Zelhem temperatures were expressed as ‘monthly minimum temperature’ and ‘monthly maximum temperature’.

The experiments attempted to test a variety of variables that could impact residue preservation. It must be noted that it will never be possible to control all variables in a burial experiment that uses the natural environment. However, it seems the experiments could have been better controlled and more systematic. For instance, it seems illogical to use different stone material types as substrates within experiments whose results are later compared. Additionally, there are inconsistencies in reporting, such as number of samples, and lack of agreement between figures

reported in 2009 and 2010. The value of the results are difficult to assess since so many variables were incorporated and it is not always clear which experiments were compared to draw conclusions.

Langejans stressed the use of the 'contextual approach' (Lombard and Wadley, 2007a; Wadley and Lombard, 2007) to identify both residues and contaminants. The key tenets of this approach are association and distribution. Association refers to documentation of all residues present on each tool in situ, as residues may occur as suites of related residues which reinforce identifications. Distribution refers to documenting the positioning of all residues on a tool, and the patterns are used to determine the authenticity of the archaeological residue. For residues to be considered 'use-related' they must show a logical distribution on the stone tool surface, with contaminants typically being found all over the tool. The implication of the contextual approach is that mapping the locations and types of residues across the stone surface is essential. However, other researchers have found that residues can occur anywhere on the stone tool surface, as discussed in Chapter 2.

Langejans' (2009) residue diagenesis experiments, carried out in South Africa and the Netherlands, were used as a comparative basis to understand preservation and identification of archaeological residues from two sites in South Africa. However, no experimental residues were buried within the same contexts as the archaeological residues to which they are later compared. This means the experimental residues were not exposed to the same diagenetic alteration conditions as the archaeological residues, making comparison difficult. Importantly, the lithic residue burial experiments by Langejans cannot be generalised as applicable to archaeological settings. It is important to understand residue diagenesis within the specific burial conditions present at the archaeological site(s) being studied.

A residue blind test experiment was carried out by Rots et al. (2016), to 1) examine the issue of residue contamination and misinterpretation, and 2) evaluate the accuracy in identifying tool use between in situ residues (examined with reflected

VLM), and extracted residues (examined with transmitted LM). Part of the experiment included a residue diagenesis aspect by burial of used tools. Three experimental flint tools containing wood (BT11), bone (BT24), and meat (BT29) residues were buried for two months. The type of sediment and burial conditions were not described, such as indoor vs outdoor, precipitation, temperature, pH, and bioturbation – variables that very much influence the survival and identifiability of the residues. Also, it is not stated what species of wood, bone, or meat residues were used, which may have been relevant to their preservation and identification. The extent to which the wood, bone, and meat residues preserved can be assessed based on the information presented Table 2 in the paper (2016, p. 12). The following blind test results were obtained by microscopic analysis of in situ residues on the three buried flints: 1/2 analysts identified the presence of wood residues (BT11), 0/2 analysts identified the presence of bone residues (BT24), and 0/2 analysts identified the presence of meat residues (BT29). Thus, only the wood residue was correctly identified by Analyst 1. Regarding the experimentally used and buried tools, Rots et al. (2016, p. 24) note “... it is immediately clear that flakes that were buried after use caused most problems”, and “... sediments acquired during the deposition phase of the tool’s use-life obscured any of the use-related residues, thus making them difficult to document”. Their results suggest that sediments have a large impact on the ability of an analyst to correctly identify the residue on the lithic.

### 5.2.3 Experiment design improvements

Experimental design improvements can be made for lithic residue diagenesis studies. In particular, greater concern for experimental control by the presence of fewer variables is essential. There are several key ways the study presented here differs from previous residue burial experiments:

- The same stone raw material type was used throughout the experiment thus allowing comparability. Additionally, the flint material chosen for experimental

tools (Yorkshire Wolds flint) is comparable to a portion of the flint assemblage found at Star Carr.

- No marks were applied to the experimental pieces: sample numbers were written on separate labels.
- Experimental pieces were buried in archaeological sediments. Thus, experimental residues were exposed to similar soil chemistry, pH, biological degradation factors, temperature, and precipitation variables as the residues on archaeological artefacts.
- Burial of an off-site control group. The experiment repetition group and burial at an off-site location makes sure observed changes in the experimental residues at the archaeological site are not due to random occurrences.
- Sample recovery 100%. All pieces buried were recovered.
- No disturbance to experiment from digging animals, ploughing, or mistaken human interference.
- Sediment samples taken and analysed directly underneath each buried flake. This allowed an assessment of the extent of mobility of each tested residue in the sediment.
- Anti-contamination steps taken. No lithics for residue analysis touched with bare hands in the field. Lithics were handled with powder free and starch free gloves for cleaning and all analyses.

## 5.3 Objectives

The residue diagenesis experiment accomplished four objectives:

1. It assessed the extent of lithic residue preservation at Star Carr. The experiment was used to analyse which types of residues preserved within the experimental timeframe and in which burial environment conditions. Site specific information about residue preservation was obtained, which informed expectations of the archaeological material.

2. It established which residue types contain diagnostic traits that can be used for microscopic identification of residues. Experimentally produced reference residues were examined in the fresh state, which made it possible to distinguish between residues that have either 1) microscopically diagnostic characteristics, 2) distinctive but not diagnostic characteristics, or 3) no distinctive characteristics.
3. It assessed the rate at which residue diagenesis occurs at Star Carr by considering change over one month, and 11 month intervals.
4. It assessed if SEM improved identifiability of lithic residues over reflected visible light microscopy (VLM).

## 5.4 Methods

### 5.4.1 Introduction

The methods used and the selection criteria of experimental variables is described in this section. There are two main areas of interest at Star Carr. The first is the dryland where there is evidence for everyday occupation in the form of structures and debris; this is slightly acidic clay-rich sediment (~pH 6.4). The second is the waterlogged lake edge zone where wooden platforms were built and artefacts and ecofacts were deposited; here the sediment is highly acidic organic peat (~pH 3.3). A nearby non-archaeological control location on the Yorkshire Wolds was chosen for the third burial unit because it provided an alkaline sediment (~pH 8.4) to be tested and compared to the Star Carr burial units.

Flint was chosen as the substrate onto which experimental residues were applied because flint artefacts dominate the assemblage at Star Carr. Twelve residue types of bone, antler, muscle, fish, bird, mammal, reeds, geophyte, softwood, hardwood, tree resin, and red ochre were selected because they are broadly representative of the evidence from Star Carr in terms of the diet and craft tasks hunter-gatherers carried out there. These materials were worked with flint flakes to produce visible residues, plus unused blank control flakes were used. A total of 78 flint flakes were made and divided into 6 experimental groups, each group containing 13 flakes (12 used flakes, 1 unused control flake). In addition, two reference collection groups (n= 26) were made, totalling 104 replica flint flakes. Anti-contamination procedures were taken to avoid contamination at every stage of the experiment and during analysis. These steps are detailed in the relevant sections below.

#### 5.4.2 Creation of experimental flakes

The stone tools at Star Carr were made from two major types of flint: glacial till flint and Yorkshire Wolds flint. The Wolds flint was more easily accessible and thus was chosen for the experiment. Unmodified flakes (n= 104) were produced from flint nodules by Andy Needham in York in the winter of 2013/2014. The flint was light grey in colour with a chalky white cortex. Gloves were not worn during knapping due to the limits it places on maneuverability; however, Mr Needham washed his hands before each knapping session. The impact of the use of bare hands leaving finger grease and perhaps skin flakes on the flint flakes was thought to not impact the microscopic results significantly since the residues applied in quantities that would overwhelm the hand contaminants. Flakes were caught on the surface of a plastic tarp and were immediately collected and placed in individual polyethylene zip plastic bags. The flint flakes were stored at room temperature prior to their use. Flakes were thereafter handled only with gloved hands to 1) reduce the addition of contaminants, and 2) reduce the exposure of residues to physical and chemical alteration due to variables not associated with the experiment.

### 5.4.3 Adding residues to experimental flakes

Each flake was removed from its bag and handled with a new set of powder free nitrile gloves during the experiment and laid on a clean surface of cling film. The contact materials used in the experiment were chosen to provide proxies that reflect the animal, botanical, and mineral, remains found on site at Star Carr, and thus likely to have been used by ancient hunter-gatherers. General residue classes reported in the literature, such as feathers, hair, and starch, were also included to assess the likelihood of encountering these residue types microscopically on stone tools from Star Carr. We know people butchered mammals with hair, such as deer and aurochs, and birds, including waterfowl with feathers. Potato starch was included since nutritious starch-rich geophytes available to Mesolithic people were probably exploited and it is plausible their remains could be found on tools at the site. It should be noted that the skin of the potato was sliced as well as the fleshy tuber during the use of experimental flakes, and therefore other micro-remains could be transferred onto the flint. For instance, it is known that sweet potato (*Ipomoea batatas*) incorporates materials from the sediment such as phytoliths directly into the skin (Tromp and Dudgeon, 2015). Here it is taken as a possibility that the phenomenon of incorporation of sediment particulates into the skin may occur in all tubers, roots, corms, and rhizomes.

Twelve contact materials were used with the flint flakes to create visible residues prior to burial (Table 5.3, Figure 5.1.). Blank control flakes were added to each group of used flakes to: 1) examine the variation in the micromorphology of the raw stone substrate, 2) observe any possible transfer of sediment material that might be mistaken for a residue, and 3) document changes to the flake due to burial condition, independent of residue applied. These controls were not used but otherwise treated identically to the experimentally used flakes.

Table 5.3. Twelve contact materials added to flint flakes.

<b>Material</b>	<b>Taxon</b>	<b>Motion</b>
Bone	<i>Bos taurus</i>	Fresh cow bone including periosteum tissue was cut in a sawing motion
Antler	<i>Cervus elaphus</i>	A small amount of ultrapure water added to dry antler, then cut in a sawing motion
Muscle	<i>Bos taurus</i>	A cutting motion was used on a piece of fresh beef steak
Fish	Family Cyprinidae	The flake was cut through fish skin and scraped against the few large scales that were present on the body. It should be noted that scales did not easily stick to flint surfaces during use of the tools
Bird	<i>Anser anser</i>	The skin, feathers and muscle of the goose were cut through with the flake
Squirrel	<i>Sciurus carolinensis</i>	The flake was used in a cutting motion through animal skin, hair, and tissues
Potato	<i>Solanum tuberosum</i>	The flake was used in a slicing motion through the skin and center of the potato
Reeds	<i>Iris pseudacorus</i>	The leaves were sliced and scraped
Softwood	<i>Picea</i> sp.; cf. <i>Picea abies</i>	Conifer branches with bark were cut and scraped
Hardwood	<i>Salix alba</i>	Withes were cut and scraped
Resin	<i>Pinus thunbergii</i>	Natural unmodified pine resin was applied with gloved hands to the non-cutting edge of flakes at the proximal end
Red ochre	N/A	Natural red ochre pigment powder was obtained from an art supplier. According to the supplier, it had no additives, and contained 20-70% iron oxide. The ochre was heavily applied with gloved hands all over flint pieces



Figure 5.1. Flakes after use on plant tissues, animal tissues, and ochre, drying trays lined with cling film.

An acute edge angle of each flake was used to cut into each contact material until residue was visible on the surface. The used flakes were placed on trays lined with cling film. Residues were left for three days to dry on the surfaces of the flake in order to ensure the residues were adhering to the stone substrate. The trays were placed in a lab space with no opening windows and away from air vents to avoid air particulate accumulation. After the three days, each flake was placed in its own zip-lock bag prior to burial, with new gloves used for every flake.

To avoid directly marking or labelling the flint flakes and potentially contaminating or obscuring residues, a laminated label was created for each specimen, which stayed with each specimen throughout the experiment and analysis. Each label contained a unique number, the residue type on the flake, and length of burial.

## 5.4.4 Burial

### 5.4.4.1 Burial locations

The experiment was repeated in three burial units and at two time intervals of one month and 11 months. Each burial unit contained 24 used flakes (two sets containing 12 residue types) and 2 controls, for a total of 72 used flakes and 6 controls. Table 5.4 outlines the experimental conditions. The burial took place on May 19, 2014. The flakes were transported to site in a picnic box to keep samples cool and block out sunlight.

Table 5.4. Summary of experimental conditions.

<b>Group</b>	<b>No. of flakes</b>	<b>Burial unit</b>	<b>Time</b>
Group 1	13	Dryland	1 month
Group 2	13	Dryland	11 months
Group 3	13	Wetland	1 month
Group 4	13	Wetland	11 months
Group 5	13	Alkaline	1 month
Group 6	13	Alkaline	11 months

The experiment was repeated in three locations (Figure 5.2). At Star Carr, two areas were chosen for burial: one on the dryland and one in the waterlogged area which would have been the lake edge during the Mesolithic. These units had been excavated prior to burial of the experimental material. At the third location, a third set of utilised flakes were buried as a control in slightly alkaline conditions on the Yorkshire Wolds at Manor Farm in Thixendale, North Yorkshire. In each of the three locations, a 1 x 1 m square unit was excavated to a depth of about 10 cm into which the experimental flakes were deposited (Figure 5.3).



Figure 5.2. Map of East Yorkshire with locations of units 1 and 2 at Star Carr and unit 3 at Manor Farm.



Figure 5.3. The excavated dryland 1 x 1 m unit 1 prior to burial of experimental flakes.

Exactly the same experiment was carried out on six groups of flakes, conducted twice for each burial unit. Two additional groups contained reference collections, one set stored in the fridge and one set stored in the freezer.

Care was taken to ensure experimentally used flakes were never touched with fingers as they were being laid in each 1 m x 1 m unit for burial (Figure 5.3). Each specimen bag was opened and the flake was gently eased out close to the ground surface and placed with its laminated label prior to being covered with sediment.



Figure 5.4. Placing used flakes in wetland unit 2.

To better understand sediment conditions at the time of burial and recovery, five pH and oxidation reduction potential (ORP) measurements were collected before the flakes were inserted (Table 5.5 and Table 5.6). Four sediment test locations were taken in each corner of the square, and one taken in the centre. A small well was made in each test location into which ultrapure water was added and mixed with the sediment prior to taking each sediment reading. The pH tester was calibrated on the same day as use, and both probes were cleaned with paper towel and ultrapure water between each reading. A visual estimate of sediment bioactivity including the

presence of plant organics, worms, insects, arthropods, gastropods, burrowing, and root action, was also recorded.

#### 5.4.4.2 Description of the three burial conditions

##### Temperature and precipitation

The National Meteorological Archive of the UK Met Office was consulted to obtain precipitation and temperature data relevant to the burial units for the duration of the experiment. Precipitation and temperature information was not collected within the burial environment itself, and it is assumed that ambient weather conditions provide a reasonable indication of the conditions the flakes were exposed to during the course of the the experiment.

The temperature and precipitation data for burial units 1 and 2 is presented in Figure 5.5. The nearest weather observation station to Star Carr is at Scarborough (NGR = 5028E 4874N, Latitude = 54:27N, Longitude = 00:42W, Altitude = 110 m). The two burial units at Star Carr experienced 75.8 mm of rainfall from the time of burial to the first recovery after a month (Met Office, 2014). The average temperature during the one month period was 13.5°C. The average air temperature for the total burial time of the experiment was 9.8°C and the total amount of rainfall that fell on the units was 549.0 mm.

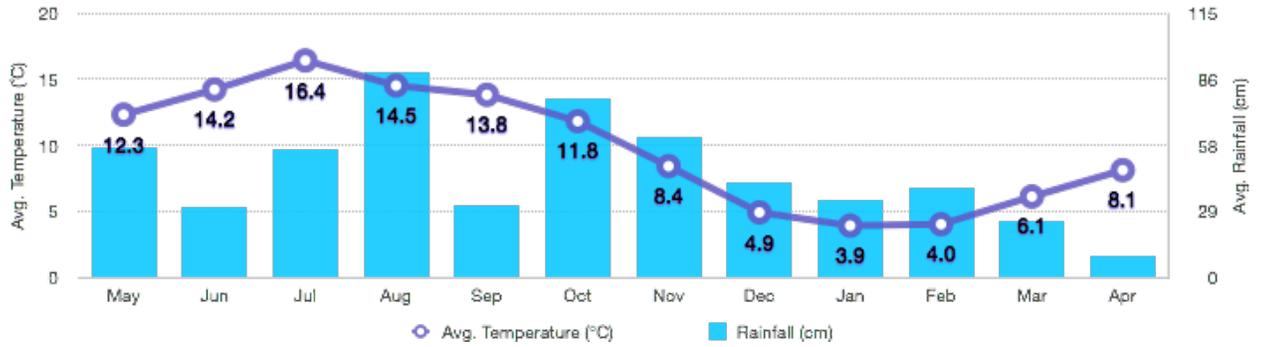


Figure 5.5. Star Carr units 1 and 2 average monthly temperature and precipitation.

The temperature and precipitation conditions that occurred at unit 3 are presented in Figure 5.6. The nearest weather observation station to the burial unit at Manor Farm in Thixendale is High Mowthorpe (NGR = 4888E 4685N, Latitude = 54:10N, Longitude = 00:64W, Altitude = 175 m). At this location, the total rainfall that the alkaline unit saw from the time of burial to the first recovery after a month was 102.0 mm (Met Office, 2014). The average air temperature during the one month burial period was 13.3°C. The average air temperature at unit 3 for the total burial time was 9.4°C and total rainfall was 650.8 mm.

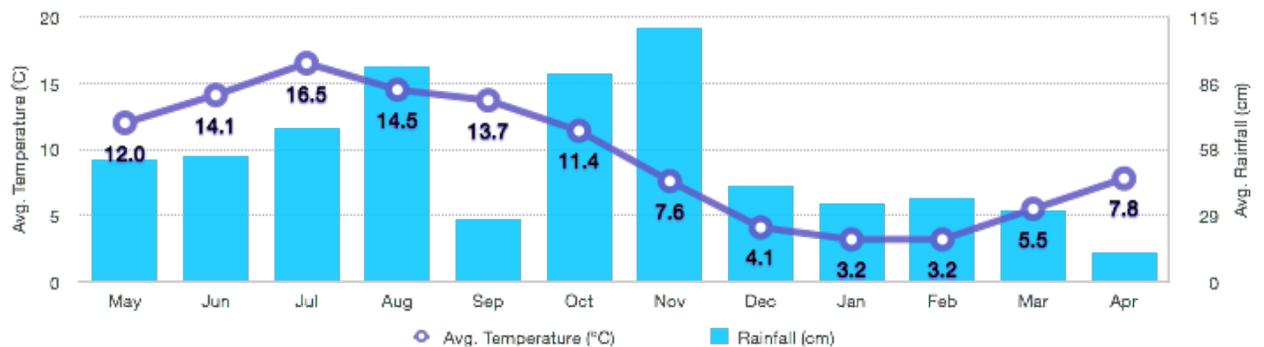


Figure 5.6. Manor Farm (Thixendale) unit 3 average monthly temperature and precipitation.

#### Star Carr unit 1: slightly acidic dryland

Burial deposit context (308) sediment is a heavy grey clay with orange mottling. Recent micromorphological investigations have shown (308) is a mineral deposit, the nature of which varies across the site (Milner et al., in press). The top of deposit (308) is likely to have been a calcitic, very fine sand/silt of a brown earth (Milner et al., in press). Cultural material has been found in this deposit, both flint and highly degraded bone. The base of the 1 m x 1 m unit was very high in clay, with small flecks of peat observed mostly in the northern half of the unit. Plant organics were minimal. Beetles, ants, spiders, centipedes, worms, land snails, and slugs were found in the unit. sediments analysis has shown this context is heavily bioturbated by worms, moles, and roots. Mesolithic bone and antler survives in the dryland in a poorly preserved, fragmentary state.

#### Star Carr unit 2: acidic wetland

This deposit context (312) is a coarse dark green reed peat, with fragments of reed leaves visible within the matrix. It also contains seeds of bogbean *Menyanthes trifoliata*, fruits of the pondweed *Potamogeton* sp. and arboreal bud scales. The proportion of fine grained organic sediment increases towards the base of the deposit. Unit 2 contained no macroscopically visible biological activity at any point. This context contains cultural material: flint, bones, wood and antler; the organic artefacts survived due to anaerobic conditions. Examples of bone found in the wetland at Star Carr are often demineralised with little hydroxyapatite or intact collagen fibrils surviving, known as 'jelly-bones' (Milner et al., 2011, p. 2821).

#### Manor Farm unit 3: slightly alkaline

The off-site control unit in Thixendale, North Yorkshire contained sediment rich in calcareous chalk, which was very stony, loose, and uncompacted. The quantity of plant organics in the sediment was not high, although many living hawthorn roots and weeds were present. The sediment was very biologically active, with worms,

snails, ants, beetles, centipedes, millipedes, woodlice, and insect tunnelling observed. At the one month recovery, an ant nest with eggs was encountered.

### 5.4.5 Collection

The first collection took place on June 16, 2014 after one month, and then nearly a year later on April 21, 2015. The one year recovery was set for May 19, 2015, however, recovery at this date was not possible due to the site excavation schedule, thus the flakes were recovered early at 11 months. Before burial of the experimentally used flakes and at each collection point, the pH and redox potential of the sediment was taken, in three locations over each burial unit (two corners and centre of square) and averaged (Tables 5.5 and 5.6).

The acidity values in unit 1 on the dryland at Star Carr were consistently slightly acidic, and unit 3 at Manor Farm was consistently alkaline at all burial and collection points. The values obtained in the wetland unit 2 at Star Carr require some explanation. A major decrease in acidity was noted between the pH values taken at the original burial and 11 months recovery point. As with all units, the wetland unit was covered with the original sediment excavated to make the pit, but several meters of backfill were added on top of the unit at the close of the excavation season. This backfill contained alkaline calcium-rich marl. Percolation of water through the backfilled sediment could have mobilised higher pH components down to the surface of unit 2 and thus explain the higher pH values obtained after 11 months. Despite these elevated pH values, unit 2 remained acidic throughout the duration of the experiment. It should be noted that within this experiment it was not possible to capture long-term changes in soil chemistry. Chemical analysis of sediments from Star Carr have indicated that annually fluctuating water tables impact their acidity, but acidity is also variable across the site (Boreham et al., 2011b).

Table 5.5. pH measurements in burial units (averaged).

	<b>AT TIME OF BURIAL</b>	<b>AT 1 MONTH RECOVERY</b>	<b>AT 11 MONTHS RECOVERY</b>
Star Carr Unit 1	6.12	6.53	6.65
Star Carr Unit 2	2.43	2.86	4.52
Manor Farm Unit 3	8.59	8.25	8.38

Table 5.6. Oxidation reduction potential, also known as  $E_h$  (measured in mV), in burial units (averaged).

	<b>AT TIME OF BURIAL</b>	<b>AT 1 MONTH RECOVERY</b>	<b>AT 11 MONTHS RECOVERY</b>
Star Carr Unit 1	253	184	362
Star Carr Unit 2	399	457	298
Manor Farm Unit 3	164	183	207

All used experimental flint pieces were recovered from all units (Figure 5.7). The flakes were excavated with trowels following standard residue collection protocols used during excavation at Star Carr. As soon as a flake could be seen, it was removed by inserting the trowel below the flake and levering it out into a new zip bag. Retrieval methods were rigorous, with care to ensure that hands did not touch the flakes at any time. Associated sediment samples were taken directly below each flake and placed in new zip-lock bags. The samples were delivered to a fridge on the same day where they remained in cold storage prior to analysis.



Figure 5.7. Recovery of all experimental flakes in unit 1 after 11 months.

### 5.4.6 Cleaning

The flint flakes recovered after burial were too dirty to observe residues microscopically and thus some cleaning was required to remove excess sediment. Each experimental flake was held with a new non-powdered nitrile gloved hand while a gentle stream of ultrapure water removed sediment, referred to as a jet bath wash. Each flake with its associated bag was set out to dry on a tray lined with new cling film. No samples or bags touched each other.

## 5.5 Conclusion

There have been few rigorous experimental studies on residue survival on stone tools and those that have been conducted have been variable. Analysing the methods used previously enabled a research design to be set out for an experiment related to lithics from Star Carr. The methods were successful in that all samples were retrieved at the end of each experiment and there were no known contamination issues. Chapter 6 presents the results of the experiment.

# CHAPTER 6 RESULTS OF RESIDUE DIAGENESIS BURIAL EXPERIMENT

## 6.1 Introduction

This chapter presents the results of the residue diagenesis burial experiment that took place at Star Carr and Manor Farm (see Croft et al., 2016). Firstly, the procedure used to analyse the experimental residues is described. The second section provides a synthesis of the results and the individual results from each flake recovered from each experimental condition (n= 78) are detailed in Appendix 1. Thirdly, a group of lithic residue types are proposed as possessing morphological characteristics which make them visually diagnostic (Appendix 2). This is followed by a discussion that hypothesizes the residue types which are likely to be encountered in the archaeological assemblage from Star Carr. To conclude, the objectives of the experiment posed in Chapter 5 are revisited and assessed.

## 6.2 Microscopic analysis

Tools were microscopically analysed as detailed in Chapter 4. Additionally, during microscopic analysis, a qualitative scoring system for experimental residues that underwent burial, similar to Langejans preservation index (Langejans, 2009, p. 51) was adopted. This scoring system was used to estimate survival of different residue types. Langejans 'preservation index' (2010, p. 978) was used to rate the state of residue preservation on a scale from 5 (situation just after use) to 0 (no residues

observed).

In addition to the z-stacked images collected with reflected VLM, all 12 reference collection residues and the blank flint were also imaged with SEM. This allowed an assessment of residue identifiability with reflected VLM compared with SEM. A Hitachi TM-1000 VP-SEM was used at (LacCore Facility, University of Minnesota). Each residue was imaged at multiple magnifications ranging from 25x to 5,000x and a total of 140 SEM micrograph images were collected and examined.

### 6.2.1 Reference collection

A reference collection (not buried), consisting of the same twelve residue types and control flakes used in the experimental groups, was created and studied prior to analysis of the buried material. The reference collection was examined and any diagnostic visual characteristics of the residues were identified. The reference collection observations provide baseline data of each residue in a 'fresh' unaltered state. The reference collection was repeated twice creating a total of 26 flakes, with the one set of 13 stored in a freezer at -18°C, and one set of 13 stored in the fridge at 5°C. It was thought that different storage practices might influence residue preservation on stone tools differently, particularly fungal growth and microbial degradation. The reference samples in both storage conditions were periodically monitored and compared macro and microscopically for fungal growth or changes in the appearance of the residue. No major changes were noticed macroscopically between the reference collection group stored in the freezer versus the fridge over a period of one year (May 2014 to May 2015). Microscopically however, fungal hyphae with fruiting bodies were found growing on the freezer-stored flake that was used on a squirrel (Figure 6.1), so some fungal growth occurred between use of the flake and its placement in the freezer.

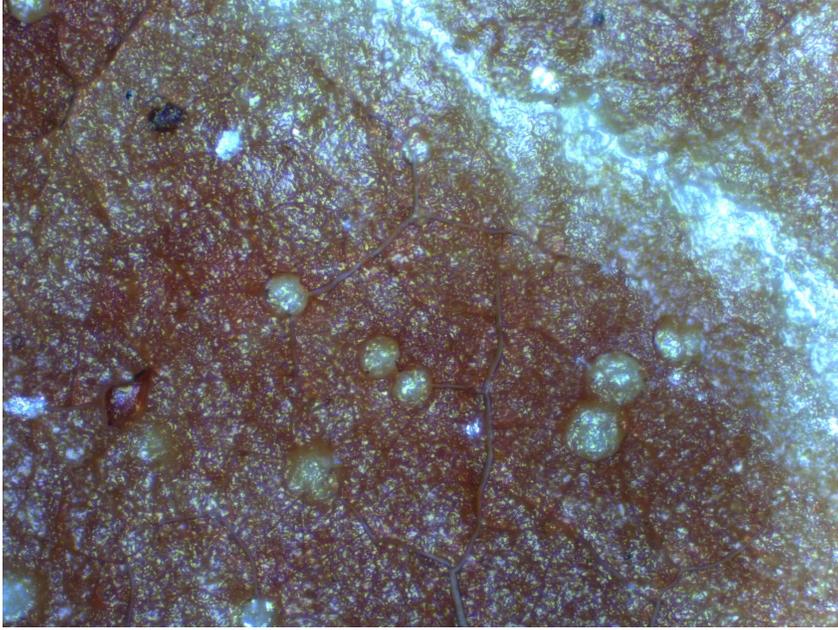


Figure 6.1. Fungal hyphae with round fruiting bodies spreading out over a squirrel blood encrustation from a sample stored in the freezer.

## 6.3 Results

### 6.3.1 Summary of results

This summary is based on the detailed results from the microscopic observation of each experimental tool from the three experimental burial conditions, published in Croft et al. (2016), and Appendix 1. Table 6.1 below summarises residue survival by residue type, burial type, and burial time. If the original residue was identified after excavation, it is colour-coded green. If the original residue was not found, it is marked in red. Yellow boxes indicate cases where identification of the original residue is likely, but no diagnostic features were present to make a confident identification.

The residue types that survived across all burial conditions and time intervals were softwood tracheids, tree resin, bird feathers, squirrel hair, and red ochre. Tree resin and red ochre preserved best, both scoring 4 out of 5 on the preservation index in every burial condition. Although it is not possible to carry out a burial experiment of comparable time depth to archaeological material, these results suggest the above types of residues have some potential to preserve archaeologically at Star Carr. The residue types that preserved most poorly overall were bone, antler, muscle, and potato starch and the results of this experiment suggest these are unlikely to survive on the archaeological material. The assessment of preservation is clearly linked to the visual identifiability of the residue: if the residue is not morphologically distinct from the soil and the stone substrate, preservation cannot be accurately determined. For instance, bone, antler, and muscle residues lack diagnostic traits and thus determining their level of preservation was challenging.

The pattern of preservation documented for the Cyprinid fish scales requires some comment. As a part of the suite of fish residues, including skin and blood, the scales were not distributed equally when applied to flakes, with some pieces unfortunately receiving no scales during butchery of the fish. This accounts for their presence in the wetland after 11 months burial, and absence after only 1 month burial.

Table 6.1. Residue survival.

	1 month dry land	11 months dry land	1 month wetland	11 months wetland		1 month alkaline	11 months alkaline
<b>Bone</b>	Yellow	Red	Red	Red		Yellow	Yellow
<b>Antler</b>	Yellow	Red	Red	Red		Yellow	Yellow
<b>Muscle</b>	Yellow	Red	Yellow	Yellow		Red	Red
<b>Fish</b>	Yellow	Red	Red	Green		Green	Red
<b>Bird</b>	Green	Green	Green	Green		Green	Green

Squirrel	Green	Green	Green	Green	Green	Green	Green
Potato	Yellow	Red	Green	Red	Red	Red	Red
Reeds	Green	Green	Yellow	Yellow	Green	Green	Green
Softwood	Green	Green	Green	Green	Green	Green	Green
Hardwood	Green	Red	Yellow	Green	Green	Yellow	Yellow
Resin	Green	Green	Green	Green	Green	Green	Green
Red ochre	Green	Green	Green	Green	Green	Green	Green

Table key: Original residue found? red=no, green=yes, yellow=likely.

No residues were found on the six blank flint controls from all units and burial intervals. This demonstrates that: 1) no cross-contamination transfer occurred due to horizontal movement of residues on experimental pieces to controls, and 2) no soil contaminants (e.g. reeds, wood, fungi) were identified as residues.

### 6.3.2 Visual identifiability of residues in situ

The modern reference residues were assessed in terms of their degree of identifiability and the individual results are presented in Croft et al. (2016), and Appendix 2. This assessment led to one of the main findings of this experiment: that the visual appearance of many residue types is insufficient to make a confident identification when in situ analysis with reflected light microscopy is used. Unless diagnostic feature(s) are present and can be discerned with the type of microscopy employed, only a suggestion, not a reliable identification, can be made as to the origin of the residue. However, visual characteristics can often be suggestive of residue type, but not securely identify it. Examination of the reference collection in conjunction with experimentally degraded residues has revealed that there are few residues with morphologically diagnostic structures (Table 6.2). These residue types have potential for archaeological identification by reflected light microscopy if found

in an adequate state of preservation. Those residue types falling in the 'distinct' and 'no distinct structure observed' categories require further techniques for accurate identification. Further techniques to identify the residue in question might include: viewing with transmitted light microscopy (extraction of residue required), SEM (with residues preferably examined in situ), or chemical characterisation with EDS (preferably in situ), Micro-Raman (in situ or extraction), GC-MS (extraction required), or ATR-FTIR (possible in some circumstances to use on small artefacts with in situ residues, but extraction recommended where the residue is large enough to permit due to risk of physical damage to artefact).

Table 6.2. Potential for visual identification of residues post-burial with reflected VLM.

	<b>Diagnostic</b>	<b>Distinct</b>	<b>No distinct structure observed</b>
Bone			X
Antler			X
Muscle			X
Red blood cells			X
Animal fat			X
Fish scales	X		
Bird feathers	X		
Mammal hair	X		
Potato starch		X	
Reed cells		X	
Softwood tracheids		X	
Hardwood vessel elements		X	
Resin			X
Red ochre			X

## 6.4 Discussion

### 6.4.1 What residues are likely to survive at Star Carr?

This experiment has shown that some residues were preserved at Star Carr in both slightly acidic clay and very acidic peat conditions, as well as in calcareous alkaline soils located off-site. The experiment has helped inform expectations about which residue types might survive archaeologically. Bird feathers, squirrel hair, softwood tissue, tree resin, and red ochre were preserved after both 1 and 11 month burial periods and across all three burial environments. If these results are taken at face-value, one might hypothesise that all of the aforementioned residue types are identifiable on Star Carr lithics. Yet it would be unreasonable to make this assumption based on an experiment whose maximum duration was only 11 months. The experimental results are, however, sufficient for assessing the microscopic survivability of at least some archaeological residue types, particularly those which did not survive the duration of the experiment. For example, the bone and antler residues on flints in the wetland were not detected with reflected VLM after 11 months burial, suggesting that these residues will not preserve archaeologically in this acidic peat burial environment.

A more considered interpretation of the results questions the preservation of feathers and hair in the archaeological record at Star Carr, since no examples of keratinised organic matter have been found to date, for instance keratin overlying the bone portions of hooves or horns. Taking this into account, it can be hypothesised that the residue types that might be encountered on archaeological tools from Star Carr are tree resins, wood residues, plant cell walls, and red ochre, based on their good preservation on experimental tools (as indicated in Table 6.1). All of these residues were found to require secondary methods in addition to in situ viewing with reflected VLM to securely identify them. As such, it is argued that other lines of evidence that

move beyond suggestive visual characteristics are necessary for residue identification in many cases. This means that any amorphous residues suspected to be anthropogenic should also be investigated through chemical analysis to understand their nature.

Additional techniques can be used to provide more secure identifications of residues observed in situ with reflected VLM. These might include viewing with transmitted light microscopy (extraction of residue required); SEM (with residues preferably examined in situ in a low-vacuum SEM chamber so no coatings are applied to the tool); or chemical characterisation with an SEM capable of energy dispersive x-ray spectroscopy microanalysis or SEM-EDS (preferably in situ); microscopic Raman spectroscopy, Micro-Raman (in situ or extraction); gas chromatography mass spectrometry or GC-MS (extraction required); microscopic Fourier transform infrared spectroscopy or FTIRM (in situ or extraction); attenuated total reflectance Fourier transform infrared spectroscopy or ATR-FTIR (it is possible in some circumstances to use on small artefacts with in situ residues, but extraction is recommended where the residue is large enough to permit sample recovery due to avoid the risk of physically damaging the artefact).

The reed tissues that were buried were only securely identified if both the cell wall structure and green chlorophyll pigments were present. The chlorophyll was an obvious sign that the original reed residue had been relocated, but chlorophyll is likely nonexistent in archaeological plant residues. At Star Carr, the finding of plant cell residues on ancient stone tools might be problematic if recovered from the organic peat contexts since the peat contains both reeds and wood. Worked wood planks (Taylor, 1998) – the earliest example of carpentry in Northern Europe (Milner et al., 2013, p. 58) – are preserved at Star Carr, and it is very plausible that stone tools on site will contain traces of associated wood residues.

The preservation of tree resin residues is particularly noteworthy. Terpenoids, the main compounds in resins (Mills and White, 1977, p. 13; Versteegh and Riboulleau,

2010, p. 17), are chemically stable over long periods of time and degradation-resistant (Brettell, 2017, p. 35). Terpenoids are so durable they have been identified by GC-MS in Eocene and Miocene conifer seed cone fossils that are millions of years old (Otto et al., 2002). Resins are also protected from oxidation in anaerobic environments (Mills and White, 1977, p. 24). Identifications of pine resin, used as a sealant for ships, as coatings for pottery, as fumigants or incense, and as a hafting material for stone tools, are also well-known in the archaeological record (Pollard and Heron, 2008).

Forming an assessment concerning the rate at which residue diagenesis occurs in the acidic archaeological environments at Star Carr is complicated by the fact that not all residues examined have diagnostic characteristics that allow for easy comparison between burial times of 1 month versus 11 months. However, some basic observations are possible. In the dry land unit, within the time between 1 and 11 months burial, bone, antler, muscle, fish and potato changed in designation from likely present to absent. Also in the dry land unit, hardwood changed from positively identified at 1 month to absent after 11 months. In the wetland unit, starch residues from potato were present after 1 month, but absent after 11 months. In the alkaline unit, hardwood was positively identified after 1 month, but became less identifiable after 11 months and was determined to be likely, but not convincingly, still present.

## 6.4.2 Other observations

### Raw material

After both phases of the experiment, it is clear that some items within the flint are possible to mistake for residues at first glance by visual analysis. This highlights the need to examine the raw stone material from which artefacts are crafted carefully before looking for residues. It is important to document the range of 'non-residues'

and examine the possibilities for misidentification. These non-residues may take the form of natural inclusions in the stone, such as fossil foraminifera or chalk vesicles, as well as discolourations and patinas. For example, the flint stone itself sometimes displayed mineral inclusions on the surface of the flint that appear similar to antler or bone (Figure 6.2).

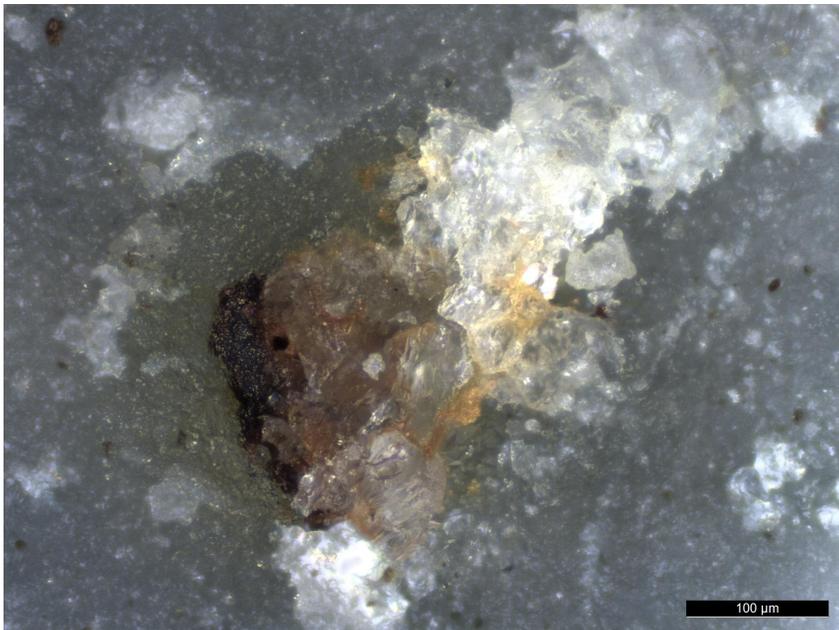


Figure 6.2. Crystals seen in the raw flint material on an unused flake from the reference collection.

## Crystal growth

Authigenic crystal growth was present on all experimental flint pieces buried in the wetland, regardless of burial time length. These crystals grow in rosette and lath shapes that are consistent with gypsum. The crystals were often found growing on top of the residues in the wetland unit. These crystals were not observed on flint pieces from the dry land and alkaline units. White powder was observed on bones from Star Carr by High (2014), who chemically and visually characterised the powder as crystal gypsum (calcium sulfate) by x-ray diffraction and SEM, respectively. These crystals found on Star Carr bones also provide a match morphologically to rosette shapes of crystal gypsum (High, 2014, p. 143; Shih et al., 2005, p. 259).

## Starch preservation

Interestingly, potato starch granule residues were identified from the flake buried in the wetland for a month, but were not convincingly identified from any other unit or time period. These residues were accompanied by clear and extensive crystal formations, which may have increased preservation. After 11 months, no starch was found on the flake used on potato in this unit. There is very little research about the taphonomic processes that starch undergoes when it enters the soil, and there is no clear explanation for its survival archaeologically (Barton and Matthews, 2006). In addition, not much is known about the preservation of starch in wetland environments. However, starch granules were found within the macroremains of a potato (*Solanum maglia*) from Monte Verde, an open, wet, and oxygen-poor site dated to c. 12,500 BP in Chile (Ugent et al., 1987). On the other hand, starch-hydrolysing enzymes have been found in peat bog environments (Haslam, 2004, p. 1721; Lähdesmäki and Piispanen, 1988).

## Similar trace, different source

Sometimes the visual appearance of residue traces produced by different sources appeared similar. For instance, apart from slightly different colouration, probable antler buried for 11 months in the alkaline presented a similar appearance to probable fish residue buried for 11 months in the wetland (compare Figures 6.3 and 6.4). This note of caution is also applicable to residue identification of archaeological residues that lack diagnostic features.

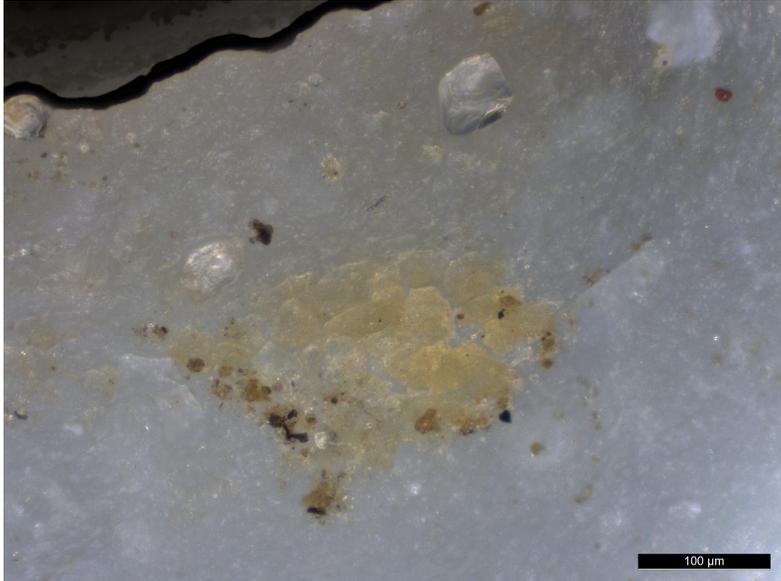


Figure 6.3. Probable antler on flake buried 11 months in the alkaline unit.

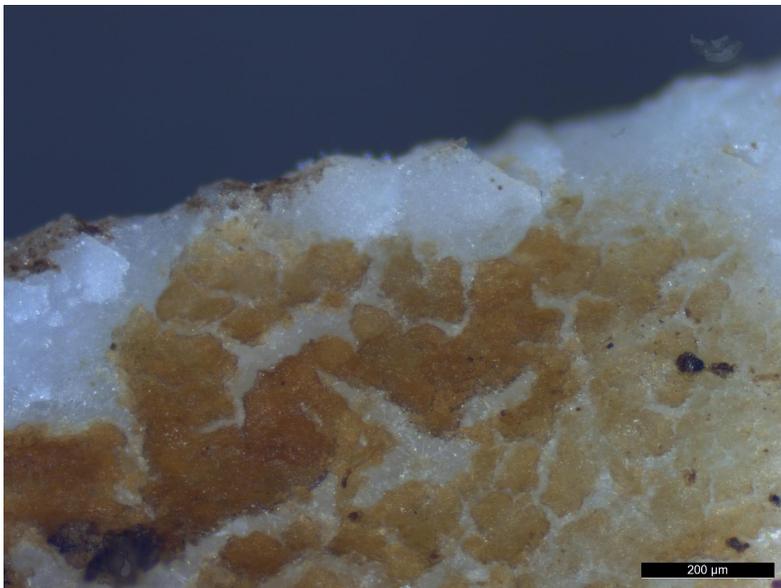


Figure 6.4. Probable fish residue on flake buried 11 months in the wetland unit.

### Lack of blood cellular preservation

Red blood cells (RBCs) were not visually identified on any flakes used in the experiment to process animal tissues, even at magnifications of 1,600x with reflected

VLM (Figure 6.5). Additionally, no RBCs were identified with the VP-SEM on any of the 'fresh' never-buried animal residues in the reference collection, using magnifications up to 5,000x (see Figures 6.6 and 6.7). As discussed in Chapter 2 (section 2.3.2.2), there are unresolved issues with claims of RBC preservation on modern experimental material, let alone ancient stone tools, so it is perhaps not surprising that cellular elements of blood did not identified.

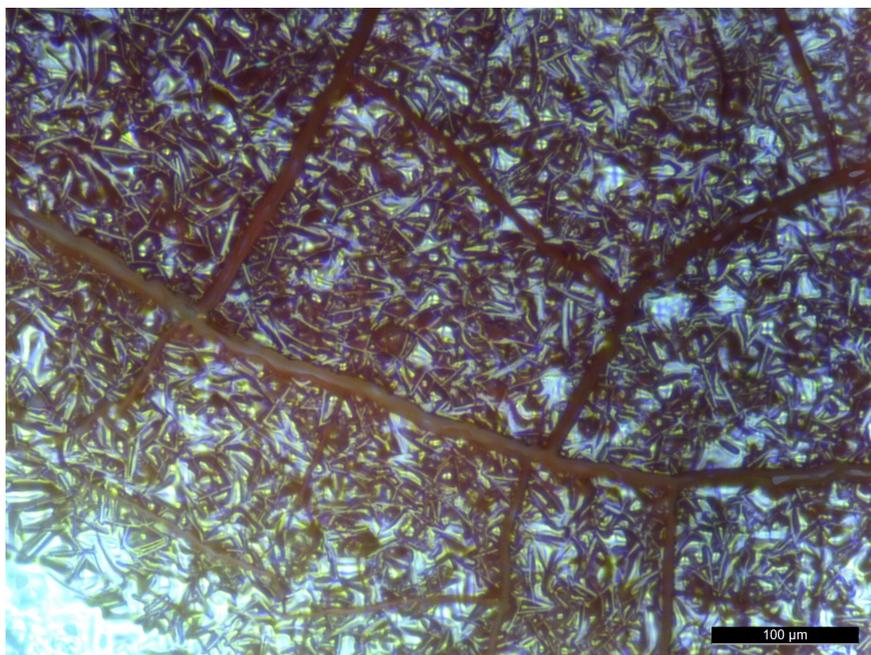
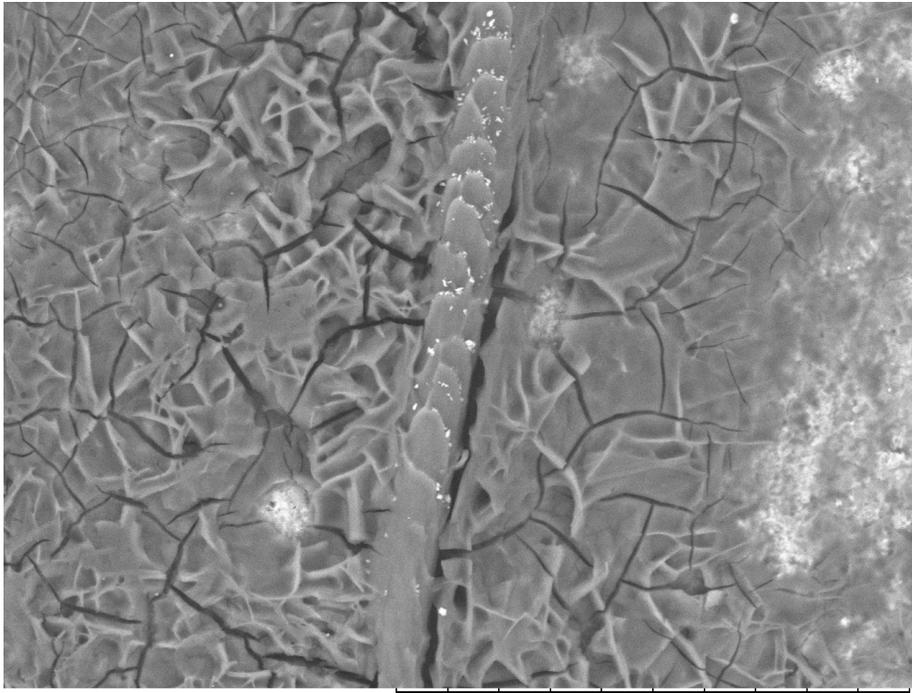
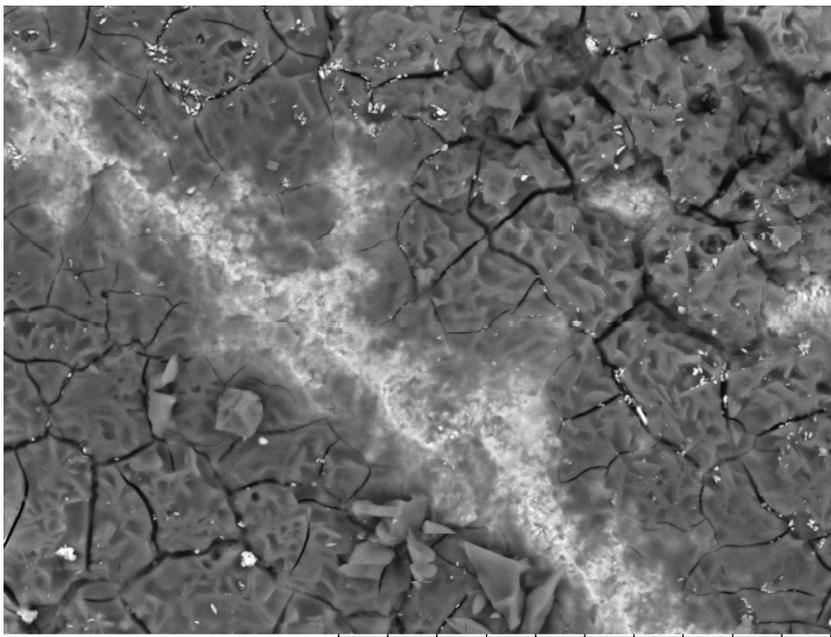


Figure 6.5. Dry squirrel blood film encrustation viewed in situ on flint. Note lack of recognisable RBCs.



Squirrel0012 2015/04/30 L x1.0k 100 um  
LacCore, UMN, funded by NSF

Figure 6.6. Dry squirrel blood encrustation and a hair on experimental flint, stored in fridge for 11.5 months prior to imaging. Note lack of recognisable RBCs.



-- NM D7.0 x1.0k 100 µm

Figure 6.7. Dry squirrel blood encrustation on experimental flint stored for 17 months in fridge. Note lack of recognisable RBCs.

The fish, bird, and squirrel were unfortunately frozen and then thawed prior to being used in the experiment. As a result, no intact cells were located, due to lysis of the cell membranes (Hortolà, pers. comm. 2016). However, it does not explain why no RBCs were found in the beef muscle residues, which was purchased as a fresh steak and stored in the fridge prior to use in the experiment. The flint containing reference beef muscle residues was stored in a fridge then imaged with SEM after nearly a year (11.5 months) after initial tool use. No RBCs were found. This seems incongruous with observations of Hortolà (2002), who used SEM and found RBCs in a human blood film on an experimental chert fragment, dried and stored indoors, that was 10 years 2 months old.

There are three potential reasons for the lack of visible RBCs in the reference beef muscle residues:

- 1) Individual RBC units are no longer present on the flake because they have lysed upon drying. Thus, the borders or edges of RBCs cannot be observed.
- 2) Perhaps the beef muscle tissues have somehow hidden or disguised the RBCs. However, it is important to note that in the case of the beef muscle residues used here, a suite of animal cell types and tissues were present on the flint (muscle, fat, blood), whereas Hortolà applied a single tissue type – blood – to the stone. The presence of blood which was not overlaid by other tissue types probably made the visibility of the RBCs on the chert fragment by Hortolà (2002) easier to view than the mixed suite of muscle, fat, and blood residues present in the beef muscle residues on flint observed in this experiment.
- 3) It might be the case that individual RBCs are present in blood stains, but the morphology of RBCs is so altered they are unrecognisable. This seems unlikely as no individual cell units of any shape (such as echinocytes or variants thereof) could be observed.

### 6.4.3 Review of the experiment

One of the issues with the experiment is that some residues were actually a combination of types. For instance, the original residues on flakes used on the bird included: feathers, muscle tissue, fat, and blood, and there are several kinds of plant tissue represented within 'reeds', 'softwood' and 'hardwood'. This probably created complications for understanding each residue type individually because residue types were overlapping. Future residue burial experiments could refine the design used here to better isolate individual residues for diagenesis testing and avoid using residue types which are actually suites of residues.

Additional techniques can be used to provide more secure identifications of residues observed in situ with reflected VLM. These might include viewing with transmitted light microscopy (extraction of residue required), SEM (with residues preferably examined in situ in a low-vacuum SEM chamber), or chemical characterisation with EDS (preferably in situ), ATR-FTIR (possible in some circumstances to use on small artefacts with in situ residues, but extraction recommended where the residue is large enough to permit due to risk of physical damage to artefact), Micro-Raman (in situ or extraction), GC-MS (extraction required).

### 6.4.4 Assessment of the objectives

This experiment has shown that residues preserved at Star Carr in both slightly acidic clay and very acidic peat conditions, as well as in alkaline soils at Manor Farm. In doing so, it has helped inform expectations about which residue types might

survive archaeologically. Bird feathers, squirrel hair, softwood tissue, tree resin, and red ochre were preserved after both one month and 11 months burial periods and across all three burial environments. However, direct analogy with archaeological material should be avoided. Rather, the experiment should be considered in light of the fact that its duration was less than a year, and the preservation of keratin-based tissues (feathers and hair) is unlikely within archaeological timescales at Star Carr. The tree resin, softwood tissues, and red ochre remain as potential lithic traces that could be encountered archaeologically, but require methods in addition to in situ viewing with reflected VLM to securely identify them.

There were not many residues that had sufficient morphological characteristics to make a secure identification. The residue types that were found to have diagnostic traits were fish scales, bird feathers, and mammal hair. Overall, it was found that the identification of lithic residues by visual means alone is frequently not adequate, thus requiring other lines of evidence.

It is often presumed that the identification of microscopic residues visually is possible only by experts who have extensive experience. Experience is important, but residue analysts need to demystify how microscopic residue identifications are reached and provide convincing evidence that is understandable and accessible (Grace, 1996, p. 216). This may mean taking a more conservative approach to residue identification, and thus interpretation of archaeological residues.

Forming an assessment concerning the rate at which residue diagenesis occurs in the acidic archaeological environments at Star Carr is complicated by the fact that not all residues examined have diagnostic characteristics to allow easy comparison between burial time of one month versus 11 months time intervals. However, some basic observations are possible. In the dry land unit, within the time between one month and 11 months of burial, bone, antler, muscle, fish and potato went from likely present to absent. In the dry land unit, hardwood changed from positively identified at the one month mark to absent after 11 months. In the wetland unit, starch

residues from potato were present after one month and found surrounded by gypsum crystals, but starch was absent after 11 months, likely digested by microbes. In the alkaline unit, hardwood was positively identified after one month, but became less identifiable after 11 months, determined to be 'likely' found.

The identifiability of residues was often not improved with the use of VP-SEM compared to reflected VLM. It was thought that SEM would reveal additional microscopic structural features of residues beyond the features visible with reflected VLM. The exception was wood residues, since the SEM improved the ability to identify the presence of pit features, their arrangement in xylary elements, and the presence of crystals (likely CaOx).

## 6.5 Conclusion

The assessment of lithic residue preservation experimentally in varied burial conditions at Star Carr and Manor Farm has demonstrated and emphasised the complexities of residue identification. As stated by Monnier et al. (2012), it is important that residue researchers acknowledge the limitations of their microscopic observations and report cases where identification of particular residues is ambiguous or problematic. Residue analysts need to demonstrate on what basis microscopic residues are identified, with publication of both positive and negative results. This may mean taking a more conservative approach to residue identification, and thus interpretation of archaeological residues.

Analysis of microscopic residue deposits on stone tools and their chemical characterisation is an emergent sub-discipline in archaeology, and the identifiability of different residues is still an ongoing methodological issue. This issue needs to be examined since reliable discrimination of ancient anthropogenic lithic residues from natural and modern contaminants begins with accurate identification of residues.

Residue analysts are continuing to improve the accuracy and specificity of their identifications, particularly with the wider availability of new technologies and collaborations with chemists, physicists, biologists, and material scientists.

PART 3 ARCHAEOLOGICAL APPLICATION AT  
STAR CARR

# CHAPTER 7 ARCHAEOLOGICAL APPLICATION

## 7.1 Introduction

Based on the results from the experimental phase of research (Chapter 6), several expectations can be tested using the archaeological assemblage in the applied phase of research. Of the residues experimentally buried, the tree resin, red ochre, and softwood tissue residues preserved the best and might potentially preserve on the archaeological lithics. However, it was also found that they require methods in addition to in situ viewing with reflected VLM to securely identify them. It was with these particular residue types in mind that the archaeological lithics were examined during microscopic scanning. Once potential anthropogenic residues were located on the lithics, they were tested further by chemical characterisation techniques: SEM-EDS, FTIRM, confocal Micro-Raman spectroscopy, and GC-MS. This chapter will discuss sampling procedures, the artefacts examined, a microscopic pilot study, and the trialing of methods to investigate residues.

## 7.2 Sampling procedures

### 7.2.1 Considerations

What is the appropriate number of stone tools to examine within a residue analysis study? As seen in Chapters 2 and 3, very often only a handful of lithics are examined

in residue studies, and this may not be sufficient data upon which to base interpretations and conclusions. On the other hand, for this study, it would be untenable to conduct residue analysis on the complete assemblage from recent excavations at Star Carr due to the large time investments required for microscopic and chemical analysis of residues.

Haslam (2009) raised the issue of sample size in lithic residue analysis, reviewing published stone tool residue studies over a 30 year period from 1976-2006. Haslam found that residue analysis as a discipline has been characterised by small sample sizes. In fact, Haslam (2009, p. 49) reports “The most common sample size in the entire dataset (the mode), and therefore the most commonly reported microscopic residue artefact sample size, is three artefacts”. The number of artefacts examined for residues per study ranged from one (Babot and Apella, 2003; Fullagar, 1993; Jones, 1990; Loy, 1985; Pawlik, 2004; Piperno et al., 2004; Slack et al., 2004) to 2,722 (Boot, 1999) artefacts. It should be noted that the number of artefacts examined in studies is often much larger than the number of artefacts actually containing residues. Very small sample sizes reported by Haslam (2009) of usually only three lithics have been the norm in published residue analysis studies. Overall, Haslam’s assessment of residue literature indicates that studies between 1976-2006 were usually not extensive. Rather, these publications generally appear to be novel 'one-offs' that are peripheral to a larger project.

Haslam (2009, 2006a) cautioned against the temptation for residue analysts to over-extend their interpretations based on residue data collected from small numbers of lithics. Haslam’s call on residue analysts to reflect and be critical about the fit between sample sizes and research questions was a valuable contribution to the methodological development of the discipline. Langejans (2011, p. 996) specifically recommended that the sample size for a lithic residue analysis study should be larger than 20 tools. She states sample sizes over 20 allow more in-depth questions to be asked that move beyond: 1) the impact factor of studies that analyse only a few rare artefacts, and 2) the broad questions of studies that are pilot in nature.

Langejans (2011, p. 996, 2009) also takes the view that focusing the residue sampling strategy to just one stone tool type (such as trapezoids microliths (2014, 2009)) in an assemblage facilitates comparison and allows behavioural conclusions to be drawn.

Croft (2012, p. 62) considered the sample size issue in lithic residue analysis as a trade-off between quantity and quality. The analysis of high numbers of lithics will yield results that are more representative of the total assemblage and are thus arguably more valid, while the analysis of fewer lithics produces more detailed data and allows the application of sophisticated techniques. Clearly, the number of lithics that can be examined within a study is dependent on the research questions at hand, time, money, and the techniques and expertise available.

It is recommended that the time required for photography of the whole artefact, microscopy, documenting residue locations, the collection of micrographs, note-taking, and chemical investigations of lithic residues be taken into consideration. Van Gijn (1990, p. 9) estimated that the number of pieces that can be examined for usewear per day with microscopy alone is about 6-10 artefacts, which includes unused pieces. Van Gijn (1990) also noted that large artefacts can take an entire day of microscopy to analyse. Weisler and Haslam (2005) quoted a time of 1-2 hours per lithic for in situ residue analysis, depending on the magnifications used and artefact size.

I have found that each stone tool from the Star Carr assemblage, the majority of which are small microliths, takes on average 2-3 hours to analyse with stereo microscope and reflected VLM, and large tools such as axes take up to about 12 hours to completely scan and document. This estimate includes documentation of residue locations, notes, and the collection of z-stacked images. Each z-stacked image can take up to 10 minutes to capture, since a series of micrographs covering the entire depth of field of the residue and surrounding microtopography are required to make the composite image.

## 7.2.2 Lithic sample selection for Star Carr

In the study here, a broad sampling strategy was considered appropriate since there was very little previous knowledge about which tool types from Star Carr might contain residues. Aveling and Heron (1998) found birch bark tar residues on a microlith from Star Carr, but no other information was available to inform expectations of residue presence on tool types at the site. Considering the broad and exploratory nature of research question 2 posed in Chapter 1, 'Which residues can be identified on stone tools from Mesolithic Star Carr?', it was important for the selection of the lithic sample to reflect the variation present in the assemblage. It was reasoned that taking a typologically varied sample would capture as many residue types as possible and hence we would learn more about tool uses and site activities.

During excavations at Star Carr, a total of 610 artefacts (including all lithic and stone items) and soil samples were collected on site for residue analysis. These artefacts were reviewed and a subsample of 138 was taken and analysed with reflected VLM (listed in Appendix 3). A pilot study of tools from the sites of Star Carr and Flixton Island II took place early on in the project, which was followed by the main study, focusing exclusively on material from Star Carr. For both the pilot study and the main study, sample selection was not random, but rather was based on an assessment of tool form, the presence of macroscopically visible wear, and a subjective judgement of their likelihood of harbouring anthropogenic residues. Lithic expert and co-director of the Star Carr excavations, Chantal Conneller, was consulted to assist with the selection of artefacts suspected to have a high potential for anthropogenic residues. Artefacts which showed macroscopic usewear were preferentially selected, as were formal tools. However, artefact types from every major lithic category represented on site were included in the sample. Tools were sampled from both the dryland and wet land areas of Star Carr. A total of 139 pieces from Star Carr were selected overall and examined using reflected VLM.

## 7.3 The artefacts

In-depth study of the lithics from Star Carr bringing together typological, metric, spatial, and refitting analyses of the lithics at Star Carr has been conducted by Chantal Conneller (Conneller, 2000; 2017). Star Carr-type assemblages are characterised by simple obliquely blunted points, large isosceles and scalene triangles and trapezoids (Conneller et al., 2016; Radley et al., 1974; Reynier, 2005). According to the most recent radiocarbon modelling, stone tool assemblages identified as Star Carr-type were found starting from 9805–9265 *cal BC* (95% probability), and disappearing between 8230–7520 *cal BC* (95% probability), (Bayliss et al., in press; Conneller et al., 2016). The lithic assemblage from Star Carr is comprised of glacial till flint, Wolds flint, and a small amount of chert, with the till flint dominating the assemblage. The lithic sample examined for microscopic residues consisted of glacial till flint and Wolds flint. Foraminifera fossil tests were often found as inclusions within the flint (Figure 7.1).



Figure 7.1. A) Foraminiferan within flint of tool fragment 98950. Globular shape of the test suggests it is of the Family Polymorphinidae (Barnard, 1962; Lloyd, 1962), B) Foraminiferan within blade fragment 93871. The shape of the test suggests it is from the Family Nodosariidae, based on the linear uniserial arrangement of the chambers (Hylton, 2000).

A total of 138 lithics from Star Carr were examined (listed in Appendix 3). All analysed stone tools were collected specifically for residue analysis, and were never touched with bare hands at the point of excavation or during analysis. Lithics were systematically scanned with reflected VLM for residues and usewear. Any lithics that had potential residues were flagged for chemical investigations or further microscopy.

The typological categories ‘spall’ and ‘nodule’ were excluded from analysis, but otherwise all other major tool types are represented within the sample selection (Table 7.1). Some tool categories contain fragmentary artefacts in addition to complete tools.

Table 7.1. Artefact types represented in the residue sample from Star Carr.

<b>Tool type</b>	<b>Number</b>	<b>Percent</b>
Blade	37	28
Flake	18	13
Bladelet	15	11
Microlith	11	8
Blade fragment	10	7
Burin	8	6
Scraper	8	6
Meche de foret	5	4
Fragment	4	3
Axe	4	3
Bladelet fragment	3	2

Shale bead	3	2
Core tablet	2	1
Crested blade	2	1
Wedge	2	1
Engraved pendant	1	0.7
Awl	1	0.7
Hammer stone	1	0.7
Chamfered fragment	1	0.7
Flake fragment	1	0.7
Shatter fragment	1	0.7
<b>Total</b>	<b>138</b>	

The small sample of twelve tools from Flixton Island II excavated in 2013 were examined as part of the initial microscopic pilot study (Accompanying Material 2).

The pilot study was carried out in 2014 to assess if any potential residues could be located on Mesolithic archaeological lithics from two sites in the Vale of Pickering. The sample consisted of a total of 48 lithic tools (36 pieces of flint from Star Carr and 12 pieces of flint from Flixton Island II) all from the 2013 excavation season and from a variety of grid square locations and contexts. Macroscopic observations and photography, as well as low and high power microscopy, and mapping of residues was conducted. The detailed results from stereo microscope and reflected VLM observations of residues on Star Carr artefacts can be found in Supplementary Material 3.

## 7.4 Trial of methods to investigate residues

In order to address research question 3: 'What techniques are most useful for the discipline of lithic residue analysis?', it was important to test various chemical characterisation techniques and assess their utility. Lithic residue analysis is in a stage of development where a diversity of methods are currently being reported (Chapters 2 and 3). It was an aim of this study is to devise and use protocols based on the best methods currently available and apply these to the analysis of stone tools from Star Carr. Thus, several options were explored for the characterisation of lithic residues.

## 7.5 Conclusion

Having sampled artefacts from Star Carr for the first phase of residue investigation – VLM microscopic analysis and identification of different residue types – various chemical characterisation methods were applied (SEM-EDS, FTIRM, confocal Micro-Raman, GC-MS), as described in Chapter 4. The results of these analyses are presented in the following chapters.

# CHAPTER 8 RESULTS: RED-ORANGE DEPOSITS

## 8.1 Introduction

Red to orange shiny deposits were commonly encountered on the surfaces of stone tools during microscopic analysis. Just over half (54%) of all artefacts examined contained red-orange deposits, from contexts in the waterlogged and dryland areas of Star Carr. Red-orange deposits identical to those found at Star Carr were also documented during the residue pilot study of lithics from Flixton Island and therefore may be present on lithics from other Mesolithic sites in the Vale of Pickering.

This raised the question: are the shiny amorphous red-orange deposits found on lithics from Star Carr resinous residues? It is plausible that hafting residues remain on Mesolithic tools, and particularly in the burial conditions at Star Carr, since birch bark tar was previously identified by GC-MS by Aveling and Heron (1998), and conifer resin was shown to preserve well under experimental burial conditions at the site (Croft et al., 2016 Chapter 13). If the red-orange deposits are resinous residues, biomarkers for tree resin terpenes would be detectable. In order to test this hypothesis, red-orange deposits located on flint tools with reflected VLM were subject to several chemical characterisation techniques.

## 8.2 Methods

Several methods were used to investigate the red-orange deposits, and these methods are detailed in Chapter 4. Reflected VLM was used to scan tools, z-stacked composite micrographs were produced, and locations of residues for testing were mapped. This was followed by examinations with a variable pressure SEM-EDS

(Hitachi TableTop TM-1000). A basic chemical characterisation of the elements present in the red-orange deposits on a flake tool was carried out with EDS microanalysis. FTIRM was then used to determine if functional groups could be detected and used to elucidate the identity of the red-orange deposits. Confocal Micro-Raman spectroscopy was performed with two instruments (at University of York and University of Minnesota) on different examples of the red-orange deposits. Finally, destructive GC-MS was performed as the last chemical characterisation technique. The extraction method used for GC-MS was designed to completely remove all polar and nonpolar compounds from all surfaces of each lithic tool. By using multiple techniques to investigate the same residue type, each technique could be compared in terms of its ability to describe the residue and also whether the results across techniques were in agreement.

## 8.3 Results

### 8.3.1 Presence of red-orange deposits in relation to burial context

A total of 75 of 138 (54%) of the sample analysed from Star Carr with reflected VLM were found to contain red-orange deposits, detected on artefacts from contexts (301), (302), (308), (310), (312), (317), (320), (325), (326), (337), (415), (466), and two unknown contexts (Table 8.1). Red-orange deposits were found on artefacts from all burial contexts except (320). From these a subsample was analysed using different chemical characterisation techniques.

Table 8.1. Contexts containing artefacts with red-orange deposits.

<b>Context</b>	<b>Number of tools with red-orange deposits/total number of artefacts examined from context</b>	<b>Percentage %</b>
<b>301</b>	3/3	100
<b>302</b>	1/1	100
<b>308</b>	21/27	78
<b>310</b>	25/46	54
<b>312</b>	12/38	32
<b>317</b>	3/11	27
<b>320</b>	0/2	0
<b>325</b>	3/3	100
<b>326</b>	2/2	100
<b>337</b>	1/1	100
<b>415</b>	1/1	100
<b>466</b>	1/1	100
<b>unknown context</b>	2/2	100
<b>Total</b>	<b>75/138</b>	<b>54</b>

### 8.3.2 Microscopic description

During analysis with reflected VLM, at least four main morphologies or types of red-orange deposits were observed: 1) bounded amorphous, 2) diffuse amorphous, 3) tideline, and 4) 'casts' of plant tissues.

### 8.3.2.1 Bounded amorphous

Amorphous red-orange deposits with boundaries usually exhibited a liquid pool or droplet-like appearance (Figures 8.1, 8.2). However, some bounded amorphous deposits showed cracking and a plate-like appearance, sometimes with microlamination visible (Figure 8.3). Sometimes, layered red-orange deposits appeared to be flaking or chipping away like old paint.

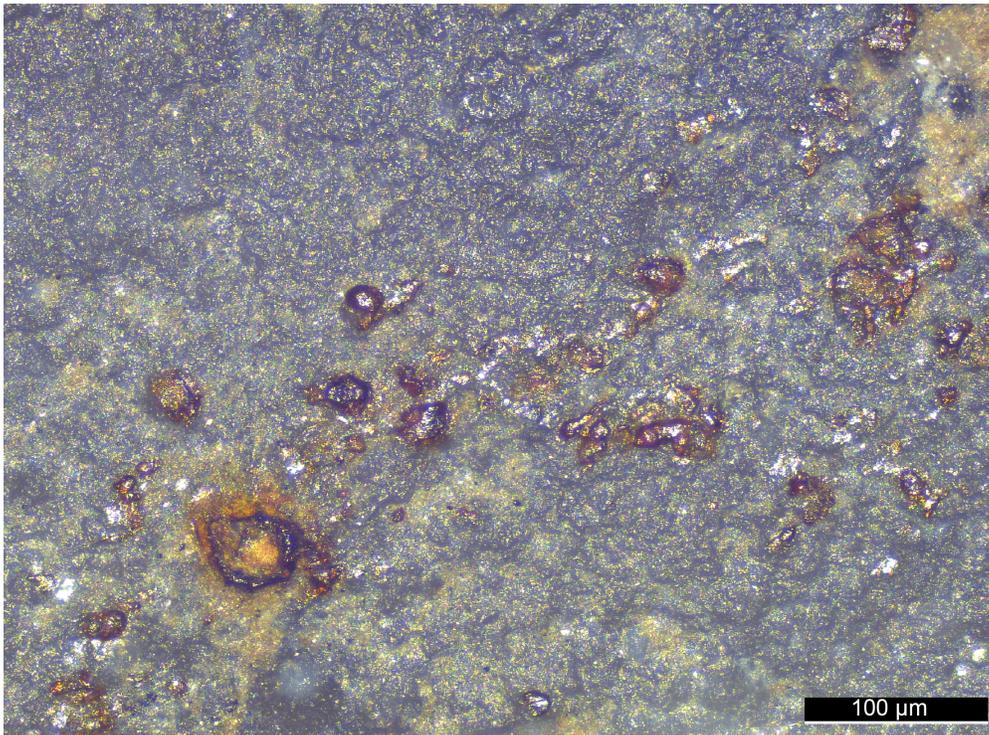


Figure 8.1. Red-orange bounded amorphous deposits with droplet-like appearance on blade tool 109699.

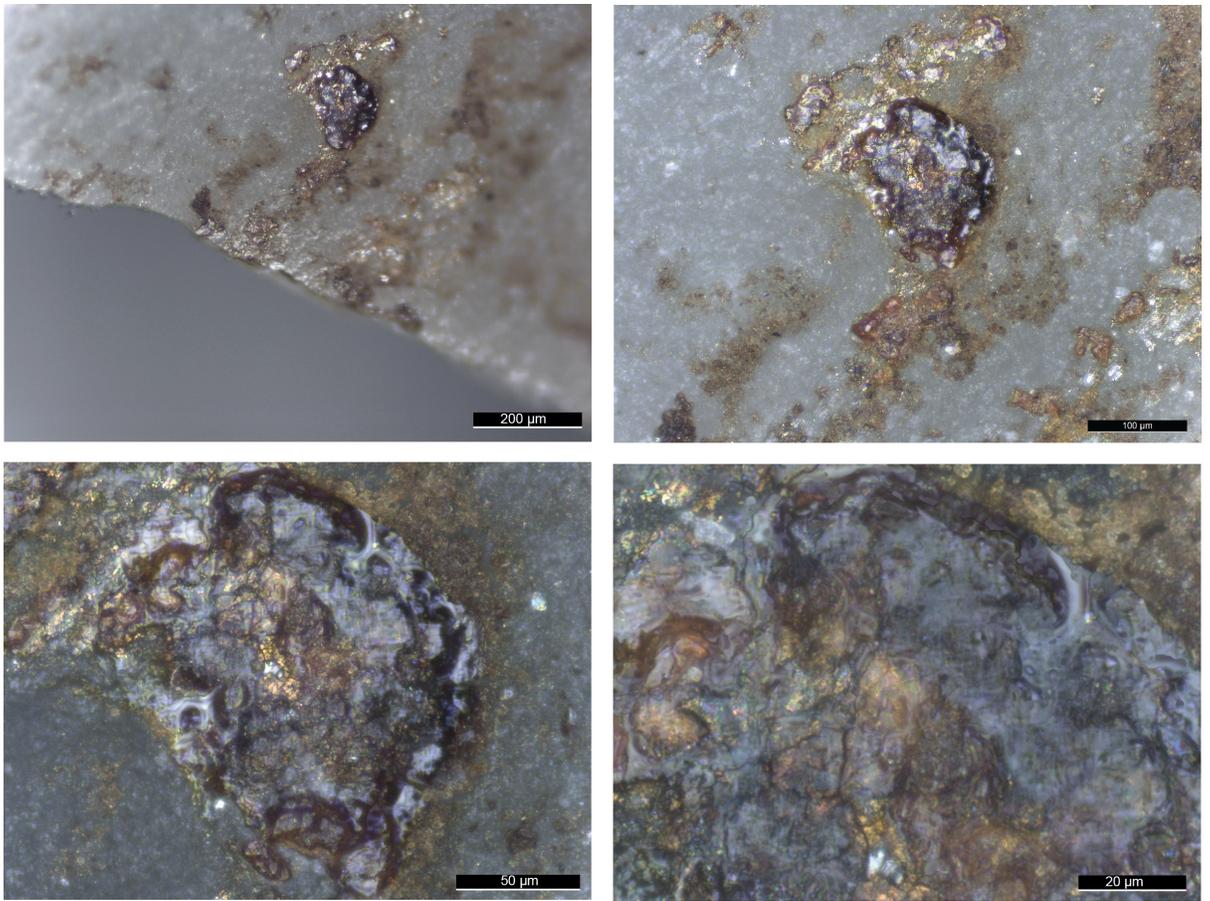


Figure 8.2. Red-orange bounded amorphous deposit with droplet-like appearance on flake tool 94362.

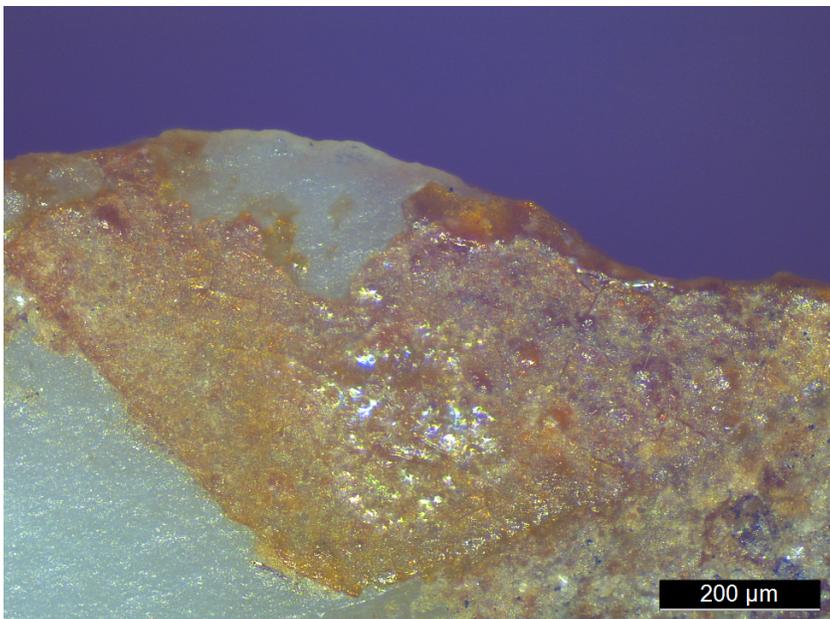


Figure 8.3. Example of red-orange bounded amorphous deposit on blade tool 94445. A laminar appearance and some cracking is evident in the top left area of this deposit.

During recent excavations at Star Carr, red-orange deposits were also seen macroscopically on a large proportion of the bone in the wetland part of the site, and also occasionally on bones in the dryland. Although organic artefacts were not systematically examined as part of this study, bounded amorphous red-orange deposits on a barbed antler point were also observed (Figure 8.4).

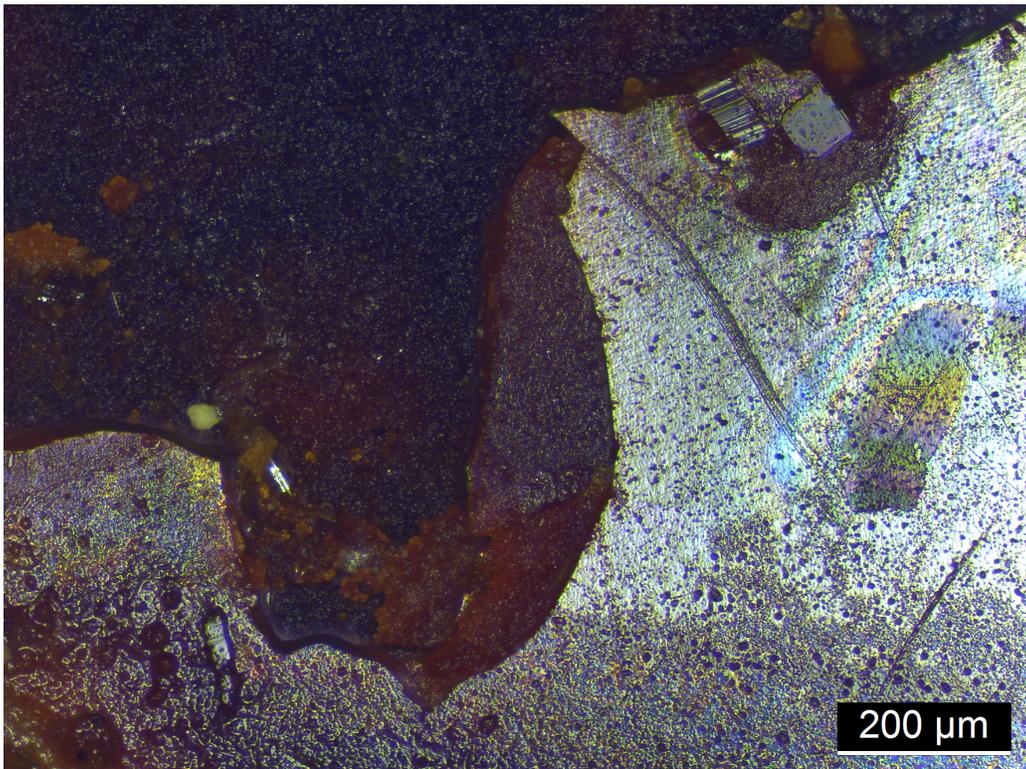


Figure 8.4. Large red-orange bounded amorphous deposit on antler barbed point 108789, note laminar appearance.

### 8.3.2.2 Diffuse amorphous

The diffuse amorphous deposits appeared like localised areas of coating applied with a spray can. This diffuse morphology of red-orange deposit was always found in combination with the more discrete bounded amorphous deposits. Sometimes the

diffuse deposits were seen emanating outwards from the bounded deposits (see examples in Figure 8.5).

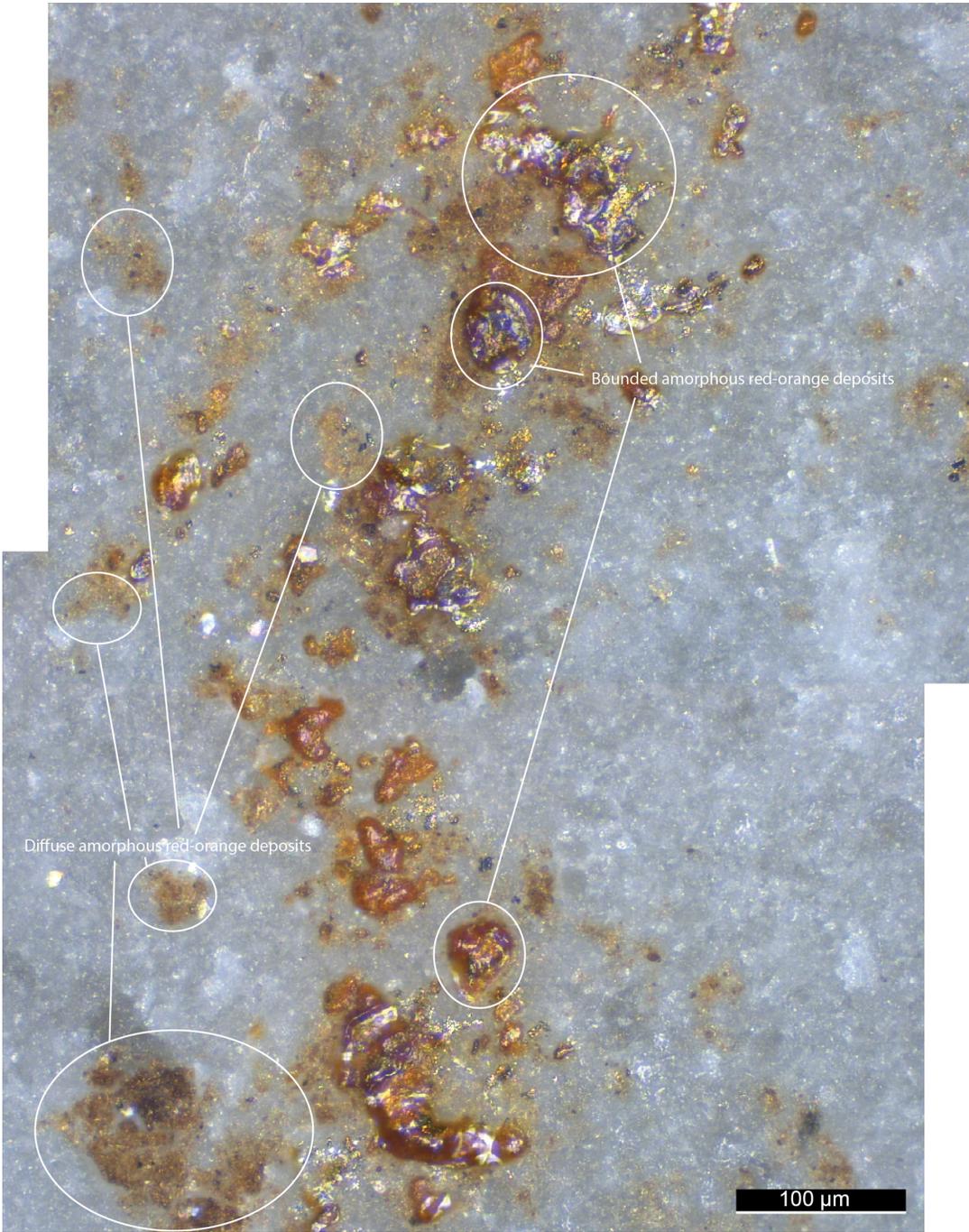


Figure 8.5. Diffuse amorphous red-orange deposits, in combination with bounded amorphous deposits on blade tool 93327.

### 8.3.2.3 Tideline

The red-orange tideline deposit appeared as a slightly wavy line (Figures 8.6, 8.7). It is possible that these represent the edge or extent where dissolved iron minerals precipitated out of the liquid groundwater. This might be similar to the process that occurs when a salt oasis or lake undergoes drying, leaving salt evaporites in place where the margins were. The tideline deposit was also often found associated with diffuse amorphous deposits (Figures 8.6, 8.7).

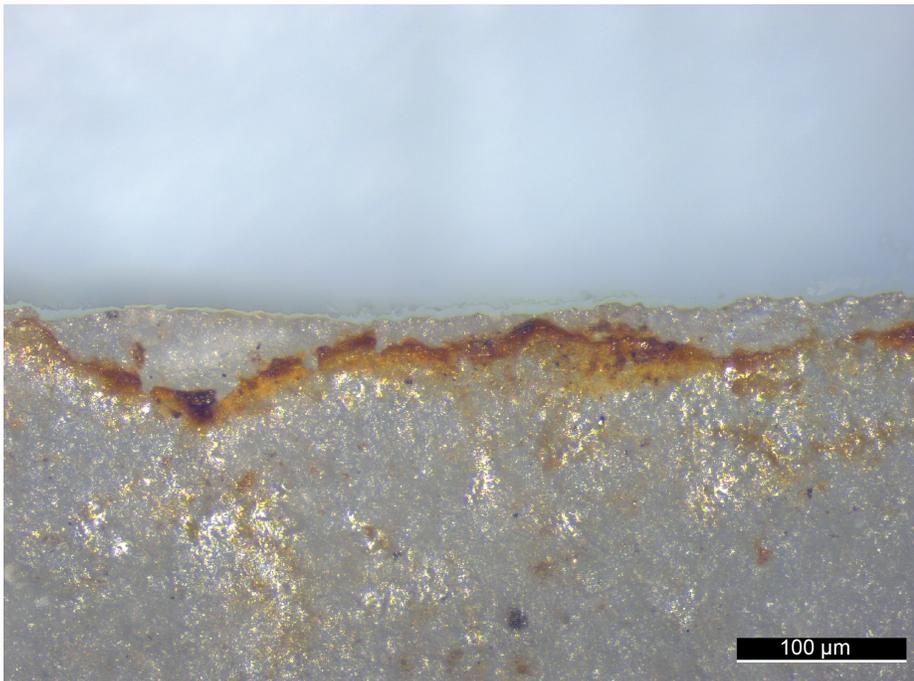


Figure 8.6. Red-orange tideline deposit in association with diffuse deposits on edge of microlith 113623.

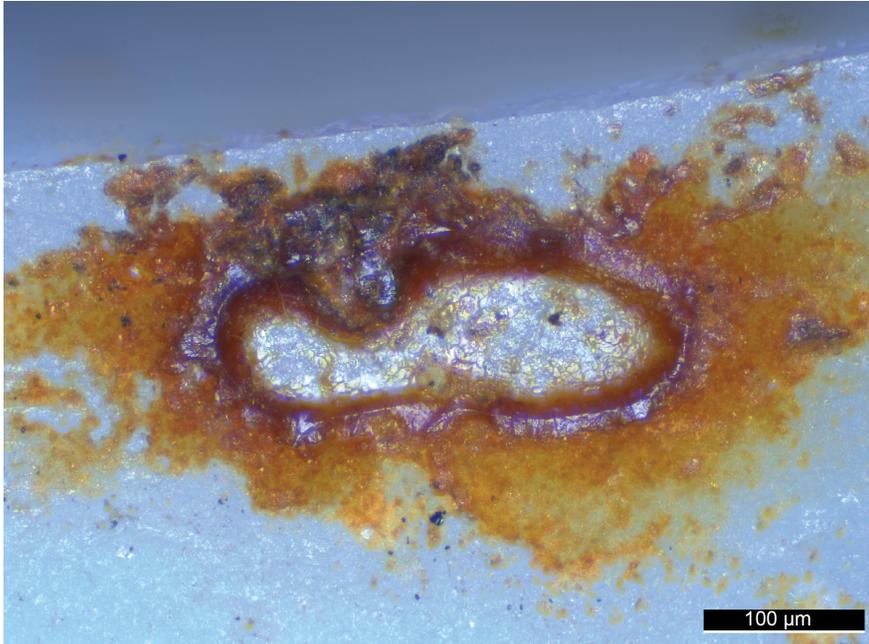


Figure 8.7. Three related morphologies of red-orange deposits found together on microlith 110679. Here, a circular tideline is surrounded by diffuse deposits, with cracked plate-like deposit inside the tideline.

#### 8.3.2.4 Plant tissue casts

The red-orange plant tissue casts had visible cell walls (Figures 8.8, 8.9). Elongate brick-like cell structure is typical of epidermal plant tissue. The cell walls were raised and sometimes partially infilled, not an impression in resinous material, as has been reported in the literature.

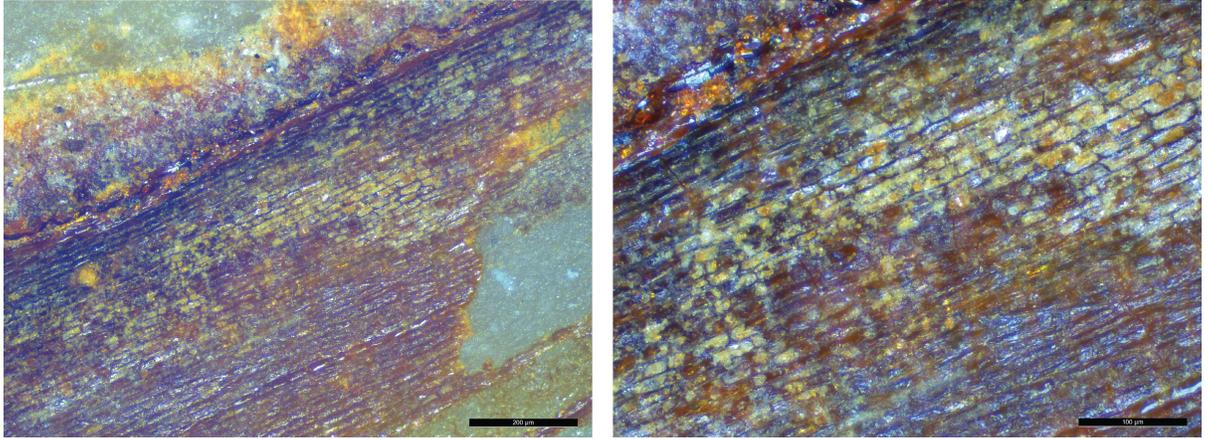


Figure 8.8. Micrograph of red-orange deposit which appears to have infilled the cell walls of plant tissues, creating a cast on blade 108228. Deposit located on dorsal mid centre surface of blade. Right image is a close up of the left image.

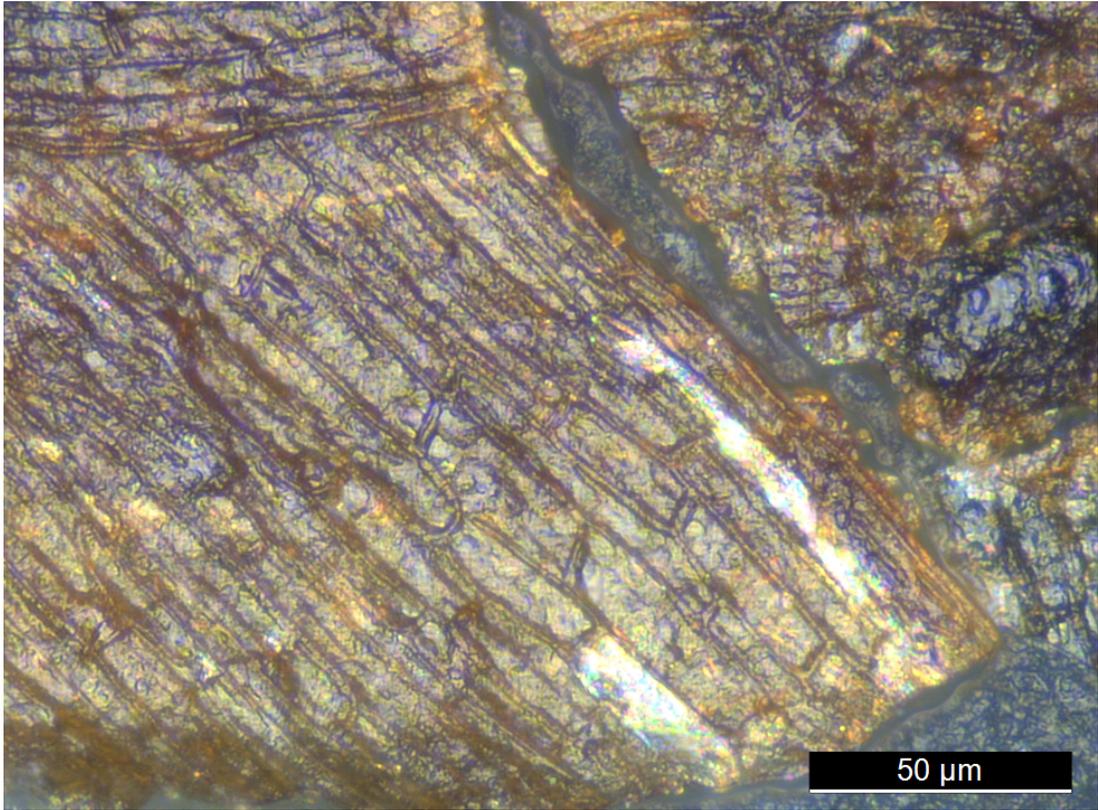


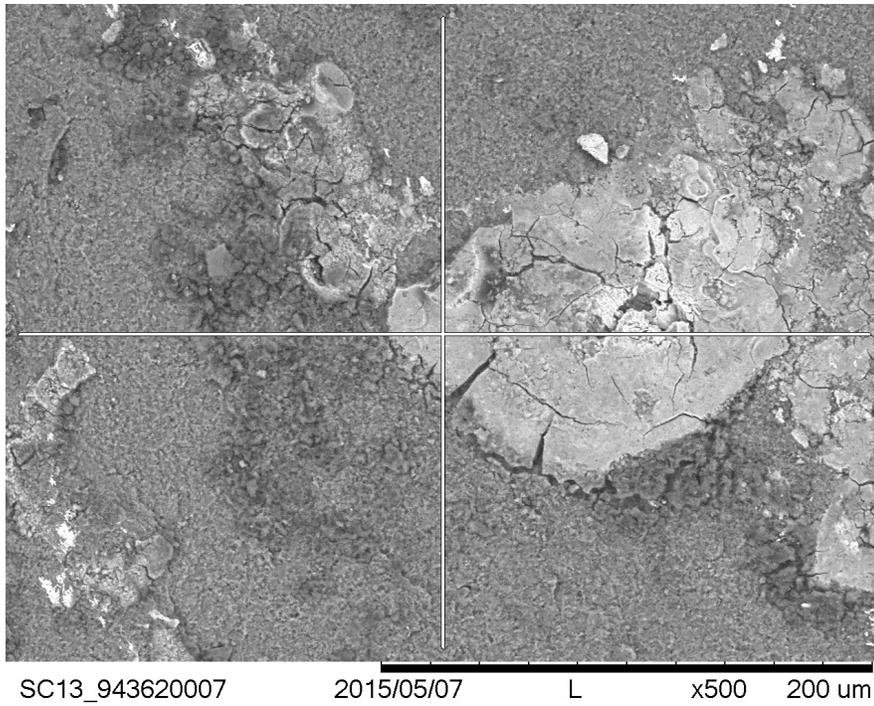
Figure 8.9. Micrograph showing red-orange plant tissue cast (possibly epidermal cells of reed leaves such as *Typha* sp.) on 98333, located ventral central distal surface of blade.

### 8.3.3 SEM-EDS

Microanalysis within the SEM chamber using energy dispersive X-ray spectrometry (SEM-EDS) was used to identify the elements present in the amorphous red-orange deposits. SEM-EDS provided a preliminary investigation into the chemical makeup of this commonly-encountered residue type.

#### 8.3.3.1 Flake tool 94362

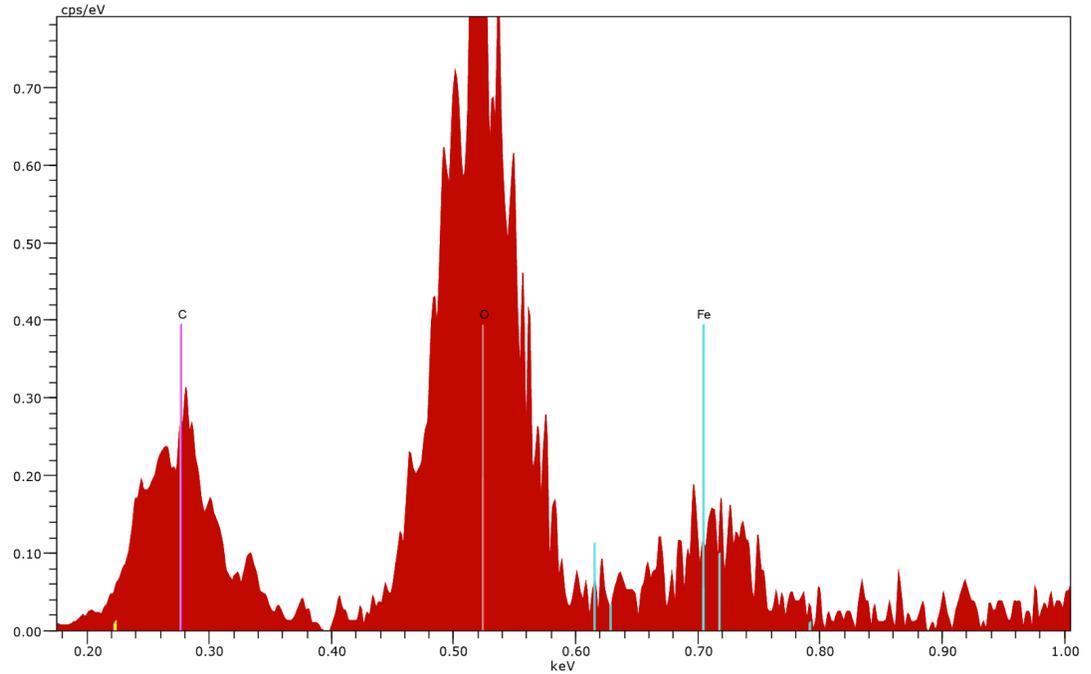
On the ventral side of tool 94362 at location 1, a red-orange amorphous deposit was investigated with SEM-EDS. Backscattered electron images from the SEM display compositional contrast, showing the red-orange deposit as appearing lighter than the flint substrate. This means the residue contained atoms with a higher atomic weight than the flint, and thus the residue was likely a non-organic material. A spot of nominally .2  $\mu\text{m}$  within the red-orange deposit at location 1 was chosen for elemental microanalysis with the electron beam (Figure 8.10). Spectra of the flint surface without any visible residues were collected to serve as a background; these were compared to the red-orange deposit to take account of any effect from the underlying stone. All spectra were replicated three times.



LacCore, UMN, funded by NSF

Figure 8.10. Red-orange deposit (location 1) on flake tool 94362, SEM. EDS microanalysis taken at the point where lines intersect.

The preliminary results of EDS microanalysis of location 1 on a red-orange deposit showed it contained iron, oxygen, carbon, silicon, and sulfur (Figure 8.11). The spectra collected from the flint surface showed that the flint contained oxygen and silicon, but not iron like the residue (data not shown). It should be noted that a small amount of carbon and aluminium are expected in the EDS analysis. This is due to X-ray scattering causing collisions with air molecules in the low vacuum SEM chamber (it contains 'environmental' air), resulting in detection of aluminum from the sample mounting stub and carbon from the sticky tabs used to hold the sample in place. It is therefore difficult to assess the contribution of the carbon content in the residue since it also is added to the spectrum from the carbon sticky tabs used.



Spectrum: SC13\_94362\_Loc1Amber

Element	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
Iron	26	K-series	31.81	31.98	13.58	1.2
Aluminium	13	K-series	18.69	18.80	16.52	1.0
Oxygen	8	K-series	17.54	17.63	26.13	3.4
Carbon	6	K-series	15.16	15.24	30.10	4.1
Silicon	14	K-series	14.98	15.06	12.72	0.8
Sulfur	16	K-series	1.29	1.29	0.96	0.1
Total:			99.46	100.00	100.00	

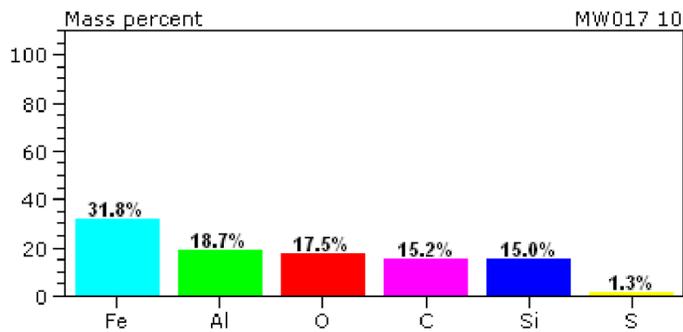


Figure 8.11. Results of microanalysis of .2  $\mu\text{m}$  spot within a red-orange deposit (location 1) on flake tool 94362. Elements present are: iron, aluminium, oxygen, carbon, silicon, and sulfur.

### 8.3.4 FTIRM

FTIRM was used to non-destructively investigate the red-orange deposits and test if chemical characterisation of the residue, and perhaps identification, was possible. The FTIRM instrument was used in reflectance mode and the resolution was  $4\text{ cm}^{-1}$ .

#### 8.3.4.1 Flake tool 94362

A red-orange deposit on the tool at location 3 on flake tool 94362 was studied with FTIRM (Figure 8.12). An aperture size of  $90 \times 75\ \mu\text{m}$  was used and the number of scans was 250.

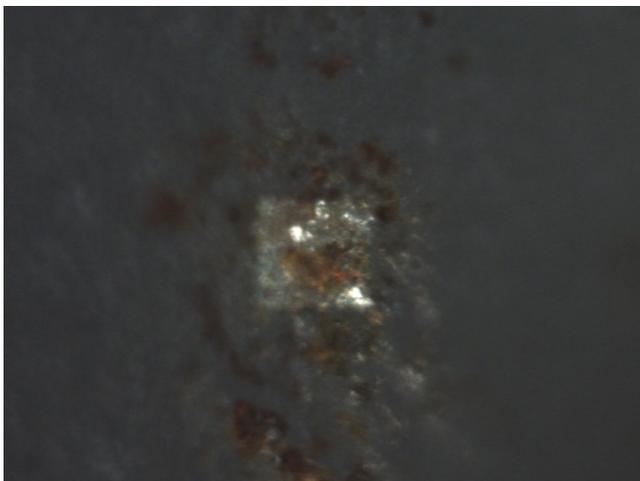


Figure 8.12. Red-orange deposit (location 3) on flake tool 94362. Lit square of  $90 \times 75\ \mu\text{m}$  shows the area analysed to produce the spectrum presented below in Figure 8.13.

The spectrum collected on the red-orange deposit was relatively 'noisy' compared with the spectrum of the flint surface (Figure 8.13). A spectrum of the flint surface substrate with no visible residues was also collected for comparison. The spectrum of the residue shows some weak correspondence to a standard for haematite (Kimmel Center Infrared Spectra Library, Weizmann Institute), but it is not entirely clear. The peaks at  $1106$  and  $795\text{ cm}^{-1}$  in the haematite reference are also present in the red-orange deposit, however, these seem to closely overlap with peaks seen in

the flint as well. Peaks from the underlying flint are present in the residue spectrum of the residue at 798 and 780  $\text{cm}^{-1}$ , which is not surprising given that the capture area (shown in Figure 8.12) includes the stone as well as the residue.

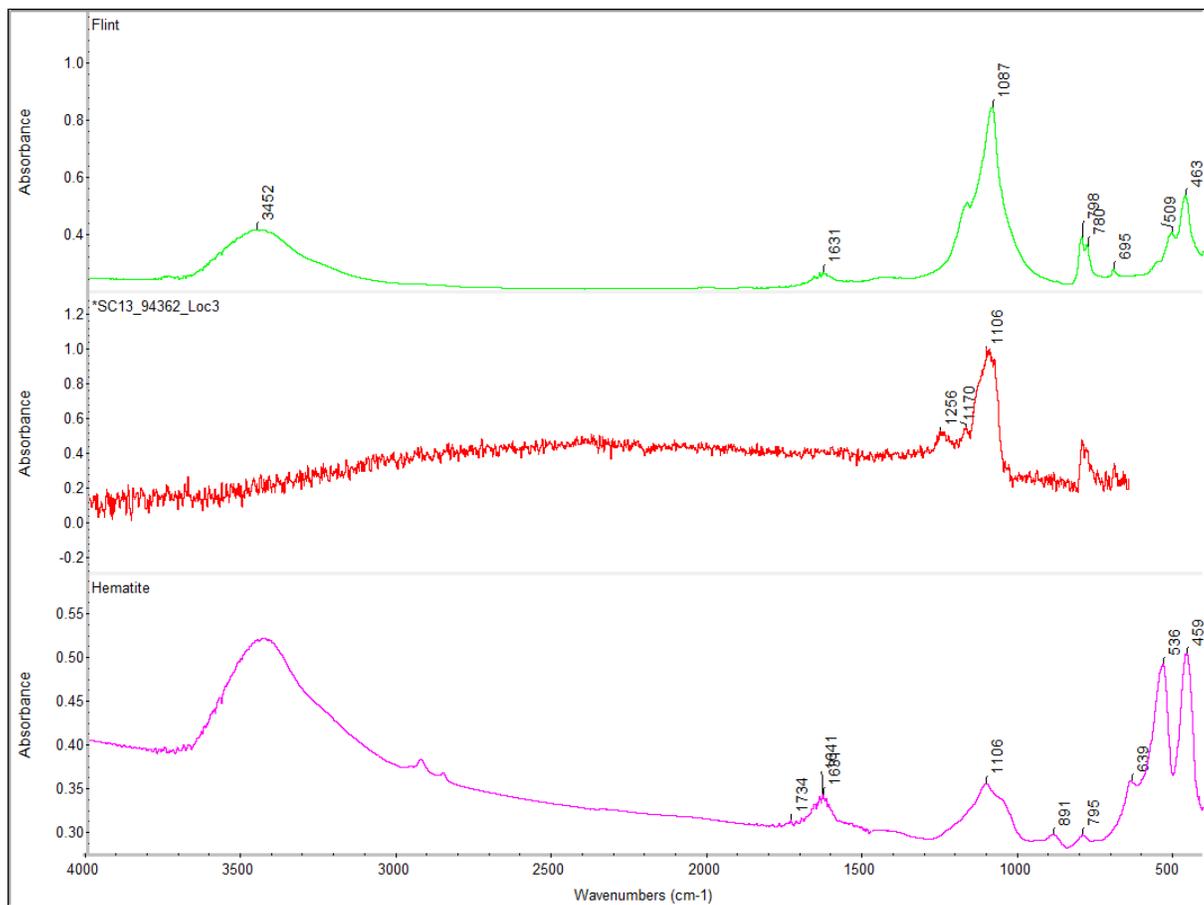


Figure 8.13. FTIR spectrum collected on red-orange deposit (location 3) on flake tool 94362, compared with an area of the flint with no visible residues as a background and a haematite reference.

### 8.3.5 Micro-Raman

Red-orange deposits on three flint tools (94362, 94445, and 95828) were investigated using confocal Micro-Raman spectroscopy. The technique is minimally

destructive and spectra collection can be directed on specific points of the residues whilst in situ on the stone.

#### 8.3.5.1 Blade 94445

Shiny red-orange deposits were visible macroscopically on blade 94445 (Figure 8.14). On the dorsal side these deposits appeared as lines several centimeters long and about 3 mm wide.



Figure 8.14. Blade 94445 showing red-orange plant tissue cast deposits on the dorsal surface.

During microscopic analysis of the blade with reflected VLM, all four morphologies of red-orange deposits (bounded amorphous, diffuse amorphous, tideline, and plant tissue casts) were noted. Some of the deposits appeared layered and cell wall structure was evident in some locations (Figure 8.15).

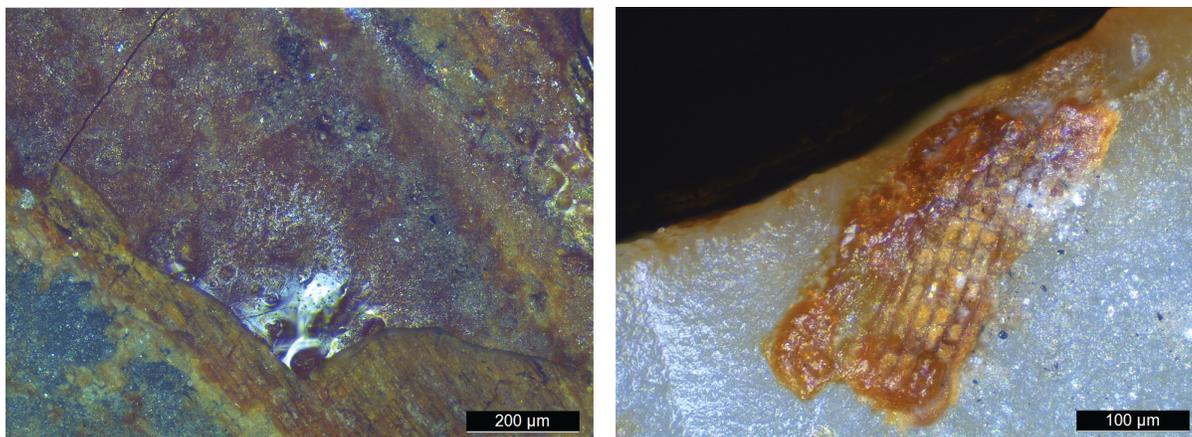


Figure 8.15. Two examples of red-orange deposits found on blade 94445. Left: Bounded amorphous exhibiting microlamination and flaking, plant tissue cast, and diffuse amorphous morphologies were found together. Right: Bounded amorphous and plant tissue cast deposits in close association.

Whilst manipulating the blade 94445 on the microscope mount and stage, three small pieces of the red-orange deposits were unintentionally dislodged from the flint. These fragments were collected from the clean parafilm surface on which they landed and then placed in a sterile glass vial with non-powdered gloves. These residue fragments were prepared for Micro-Raman spectroscopy by placement on a microscope slide with a potassium bromide KBr plate (Figure 8.16).



Figure 8.16. Left: Fragment pieces of the red-orange deposit collected from blade 94445. Right: Pieces of the red-orange deposit on a glass slide with KBr plate prepared for Micro-Raman spectroscopy.

The KBr plate was used since it gives no Raman signal and hence does not interfere with signal collection from the sample. The flattest fragment (mid size piece) was investigated, with the red side facing the laser, and a selected spectrum is presented in Figure 8.17. Table 8.2 shows the correspondence of Raman bands in the sample with reference wavenumber values.

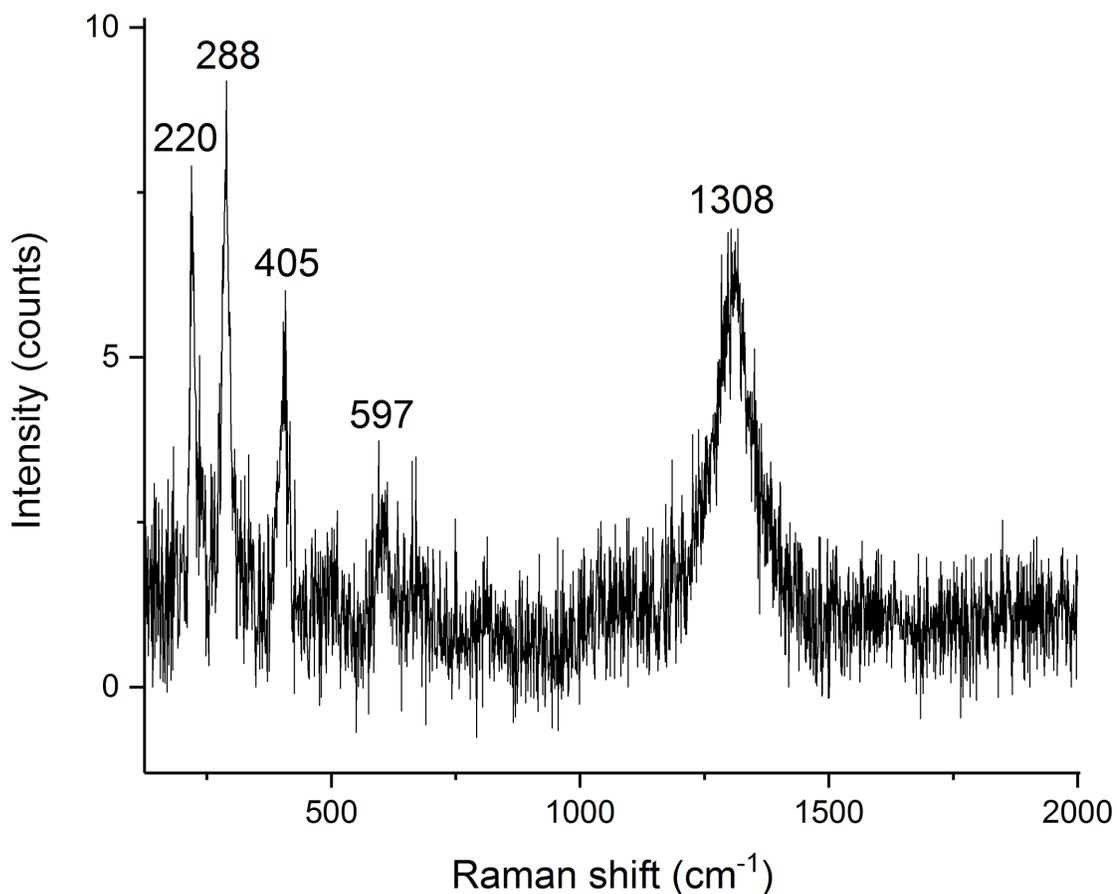


Figure 8.17. A Raman spectrum collected from a red-orange deposit on blade 94445, illustrating the deposit is iron (III) oxide.

### 8.3.5.2 Comparison of red-orange deposits on archaeological flint with haematite reference values

The Raman spectra collected on the red-orange deposits were compared to iron (III) oxide reference values. Iron (III) oxide ( $\text{Fe}_2\text{O}_3$ ) has several known polymorphs, or forms. Polymorphs share the same chemical formula, but have different crystal structures due to different arrangements of the molecules, resulting in different vibrational properties. The four polymorphs of iron (III) oxide are: alpha phase  $\alpha\text{-Fe}_2\text{O}_3$  (hexagonal corundum structure), gamma phase  $\gamma\text{-Fe}_2\text{O}_3$  (cubic spinel structure), beta phase  $\beta\text{-Fe}_2\text{O}_3$  (cubic bixbyite structure), and epsilon phase  $\epsilon\text{-Fe}_2\text{O}_3$  (orthorhombic structure) (Zboril et al., 2003, p. 21, 2002, p. 442).  $\alpha\text{-Fe}_2\text{O}_3$  is the most frequent polymorph of iron (III) oxide existing in nature as the mineral haematite (Zboril et al., 2003, p. 21). Haematite is the mineral form of iron oxide ( $\text{Fe}_2\text{O}_3$ ). There are two main types of haematite: 1) black to metallic steel gray specular crystalline haematite, and 2) red to red-brown soft earthy varieties of haematite. Haematite also naturally occurs in clays (Torres, 2014, p. 177). Haematite, specifically the alpha phase  $\alpha\text{-Fe}_2\text{O}_3$ , is well-studied due to its economic importance in mining, steel manufacturing, and pigment production industries, so many reference sources were able to be compared to the sample. Haematite is also an important material in prehistoric archaeology, since it has been found as ochre in graves, cave paintings, and on personal adornments. Thus, archaeological examples of haematite identified with Micro-Raman were also compared to the sample.

The Raman spectra obtained are consistent with the alpha phase  $\alpha\text{-Fe}_2\text{O}_3$  of iron (III) oxide (known as haematite) (Beattie and Gilson, 1970, p. 983; Bonneau et al., 2017, p. 6; Burgio and Clark, 2001, p. 1504; Courtin-Nomade et al., 2009, p. 716; Das and Hendry, 2011, p. 104; de Faria et al., 1997, p. 875; de Faria and Lopes, 2007, p. 119; de Tercero et al., 2014, p. 2350; Edwards et al., 2001, p. 19; Froment et al., 2008, p. 563; Legodi and de Waal, 2007, p. 164; Mortimore et al., 2004, p. 1183; Oh et al., 1998, p. 63; Ohtsuka et al., 1986, p. 478). Specifically, the Raman wavenumbers ( $\text{cm}^{-1}$ ) shown in Figure 8.17 at 220, 288, 403, 494,  $\sim$ 605, and 1305 match well with the reference values for the iron (III) oxide  $\alpha\text{-Fe}_2\text{O}_3$ . In Table 8.2,

slight differences between wavenumbers in the sample and reference values are due to differences in the laser power used, sample orientation, temperature, and level of crystallinity of the sample (de Faria et al., 1997, p. 875)