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Journal of Archaeological Science 31 (2004) 1491–1501

Journal of Archaeological SCIENCE

http://www.elsevier.com/locate/jas

The first residue analysis blind tests: results and lessons learnt

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Received 29 July 2003; received in revised form 2 March 2004

Abstract

Twenty-eight stone flakes were produced, sterilised and then used for a variety of tasks involving the processing of plants and animal products. Precautions were taken to avoid contaminating the residues. One set of used flakes was stored in sealed plastic bags; the other set was buried in compost for a month and then exposed to open-air conditions for three days. The bagged tools were used for a blind test (Test One) to assess the identification skills of the residue analyst who was not provided with any information prior to conducting the analysis. She obtained a high score for recognition of residues and tasks performed. Test Two used the tools that had been buried in compost and here the aim was to study the effects of acidic, organic-rich deposits on plant and animal residues. The Test Two results intimate that animal residues are more sensitive to certain burial and exposed conditions than plant residues, but more closely controlled experiments are needed before definite conclusions can be drawn.

Keywords: Residue analysis; Residue preservation; Blind test; Replication studies; Stone tools

1. Introduction

This is the first (to our knowledge) published report of blind tests for residues that were deliberately put on replicated stone tools. Blind tests in archaeological context are, however, standard practice for many microwear analysts [2,3,9,16,17,20,23,26]. The most obvious reason for conducting such tests is to verify the skill of an analyst regarding the identification and interpretation of residues adhering to stone tools. Valuable additional benefits can be gained from such inquiries with careful project design. Investigating conditions under which residues preserve is one example of data that can be examined and documented during replication studies conducted for blind testing. Thus, Test One was designed to test the skills of the residue analyst (ML) and Test Two was designed to investigate the effects on residues of burying residue-coated flakes in organic-rich, slightly acidic soil.

One of the most provoking questions for any science, including archaeology, is: How do you know? Archaeological case studies often lack the means necessary to provide accurate evaluations and confidence tests for interpretations and interpreters ([26]: p. 66). A blind test is an objective method to evaluate the accuracy of information retrieved by a specific method ([23]: p. 76). Not surprisingly residue analysis, as in the case of the well established discipline of use–wear analysis ([19]: pp. 50–56), has met with some scepticism in archaeological circles. However, problems of the method are continuously being addressed as more results become available and as better testing methods are developed for residue studies. The potential of residue research is therefore increasingly being recognised [1,4,7,10,11–14,18,21]. Time, effort and costs of residue analysis usually constrain the analyst's ability to investigate large archaeological samples, but the method has nonetheless become a valuable analytical approach in lithic studies and in the study of other archaeological material such as ceramics [15].

This first set of blind tests comprises only one of an ongoing series designed to address issues regarding the accurate visual identification of modern and ancient

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doi:10.1016/j.jas.2004.03.010

residues as well as the potential of organic material to survive a variety of post-depositional agencies.

2. Method

2.1. Preparation of the residue samples

Twenty-eight stone flakes made on raw materials often encountered in the southern African context such as hornfels, dolerite, chert and chalcedony were selected for the tests. The flakes were first rinsed under running water and then soaked for 48 h in a 1:10 bleach solution in a synthetic container to remove adhering bacteria. The flakes were again rinsed and then were sun-dried in a clean synthetic container. They were subsequently numbered on the ventral side, using correction fluid and indelible black ink. This operation was performed wearing latex gloves to prevent finger-lipids (Fig. 1) from contaminating the flakes.

Latex gloves were also used for all subsequent tasks, except where otherwise stated in the inventory (Table 1). This turned out to be a mistake because the gloves were powdered and they left unwanted starch grains on some of the tools (see the reference to starch from gloves in Tables 1–3). The practice will be discontinued in future. The error was only realized during the tests. After the tests were completed a comparative sample was created from the glove powder so that it could be easily recognised in future. This sample is illustrated in Fig. 2 where numerous starch grains are evident. The glove powder was manufactured from cornstarch (Smith and Nephew Ltd, pers. comm.).

The replicated flakes were used for cutting raw and cooked beef and bones, for cutting raw and cooked potato and for scraping twigs. Blood and plant exudates were smeared on flakes without performing any activities. Some of the tools were placed for 2 h in a closed oven that was switched off after it was preheated to 30°. This was done to dry the tools. When the residues were dry the flakes were stored individually in new zip-lock plastic bags. A number of flakes were not used but the tools without residues were curated in the same way to the utilised specimens. All activities were duplicated and both sets of samples were treated in the same way for one month. Thereafter the set of flakes

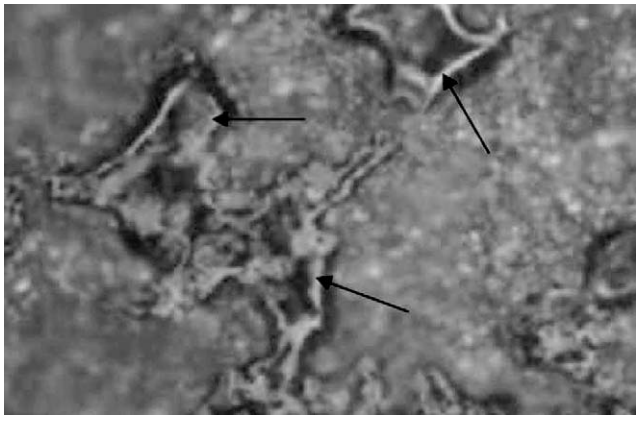

Fig. 1. A photomicrograph of "finger-grease" (lipids) on the surface of a replicated artifact, taken at 50× magnification.

Table 1
Inventory of residues, tasks performed and artifact curation

1	*Ozoroa paniculatum* exudates on flake platform, dried then stored in plastic bag
2	Flake edges scraped on *Ozoroa paniculatum* bark from a thin twig, dried then stored in plastic bag
3	One edge scraped with bark from twig of *Lannea discolor*, dried then stored in plastic bag
4	Cut raw beef fat and small quantity of uncooked meat, dried on glass in oven (30°), then buried in compost
7	Cut groove into outer cortex of fresh beef bone; some blood on bone. Dried then buried in compost.
10	Cut raw beef dried on glass in oven (30°), then stored in plastic bag
12	One lateral used to cut deep groove into centre of fresh cut beef bone; some blood and fat on bone. Dried on glass overnight in cold oven, then stored in plastic bag. Gloves not used.
13	Cut raw beef, dried on glass in oven (30°), dried then buried in compost
14	Cut raw potato, dried on glass in oven (30°), then stored in plastic bag
16	Cut cooked meat, dried on glass in oven (30°), then stored in plastic bag
17	Cut cooked potato not using gloves, dried in oven (30°), then stored in plastic bag
18	Cut cooked potato not using gloves, dried on glass in oven (30°) then buried in compost
19	Cut cooked fat, dried on glass in oven (30°), then stored in plastic bag
21	Beef blood smeared on dorsal and ventral surface. Dried on glass overnight in cold oven, then buried in compost. Gloves not used.
22	Cut *Acacia karoo* wood from tree where bushbabies (*Galago*) live, dried and stored in plastic bag
24	Cut raw potato, dried on glass in oven (30°), then buried in compost
25	Cut groove into outer cortex of fresh beef bone; some blood on bone. Dried on glass overnight in cold oven, then stored in plastic bag.
27	Cut cooked fat dried on glass in oven (30°), then buried in compost
28	Cut *Acacia karoo* wood from tree where bushbabies live, dried and stored in plastic bag
29	No residues, stored in plastic bag
30	Beef blood smeared on dorsal and ventral surface. Dried on glass overnight in cold oven, then stored in plastic bag. Gloves not used.
31	No residues, stored in plastic bag
33	Cut raw beef fat and small quantity of uncooked meat, dried on glass in oven (30°), then stored in plastic bag
40	No residues, dried then buried in compost
41	Scraped marine shell, stored in plastic bag
44	One flake edge scraped on *Ozoroa paniculatum* bark of thin twig, dried then buried in compost
45	*Ozoroa paniculatum* exudate smeared on flake platform, dried then buried in compost

Table 2
Results of blind test (Test One) by ML: items stored in plastic bags

Flake #	Plant residues identified	Animal residues identified	Mineral residues identified	Comments on identifications	Actual task or residue
1	Starch grains, starchy residue, plant tissue, plant fibers	None	Possible starch from gloves	Contact with fibrous plant material. Starch 2 possibly from latex gloves on proximal part.	Plant exudates
2	Resin, starchy residue, raphides, starch grains, plant fibers	None	Possible starch from gloves	Processing of resinous plant material	Scraped bark and wood
3	Woody residue, epidermal cell tissue, resin	None	None	Processing of wood and bark	Scraped bark and wood
10	Woody/plant tissue and cells (fibrous overlays), plant fibers, starch grains	None	None	Processing of woody/fibrous plant material	Cut raw meat
12	None	Animal fat, possibly cooked, hair	None	Processing of animal fat, possibly cooked	Cut fatty bone
14	Starch grains, starchy residue	None	Possible starch from gloves	Processing of starchy plant material	Cut raw potato
16	None	Animal tissue	None	Processing of faunal tissue	Cut cooked meat
17	Plant tissue, plant fiber	None	None	Processing of plant material	Cut cooked potato
19	None	Animal fat, animal tissue	Possible starch from gloves	Processing of fatty faunal tissue	Cut cooked fat
22	Woody residue, plant tissue	Animal tissue	None	Contact with plant and animal material	Scraped *Acacia* wood from tree with *Galago*
25	None	Animal tissue	None	Processing of faunal tissue	Cut cortex of bone
28	Plant fiber, starchy residue	Animal tissue, animal fat	Possible starch from gloves	Processing of animal material, contact with starchy and/or plant material	Scraped *Acacia* wood from tree with *Galago*
29	Plant fibers	None	Possible starch from gloves	Processing of fibrous plant material	No tasks performed
30	None	Blood	None	Contact with blood	Blood smeared on flake
31	Plant fibers, plant tissue, starchy residue	None	Possible starch from gloves	Processing of plant material	No tasks performed
33	None	Animal tissue, cooked animal fat	None	Processing of fatty animal tissue, possibly cooked	Cut raw fat
41	Plant tissue, plant fibers, starchy residue	None	Possible starch from gloves	Processing of fibrous plant material	Scraped marine shell

with duplicated residues (and one pristine tool that had not been used) was removed from the individual plastic bags and buried together in a large synthetic bag containing dry organic compost. The dry compost was considered to represent the sort of conditions that might prevail in a cave site where the deposits contained a high proportion of organic material. The bag was sealed in a lightproof container and placed indoors.

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. Notwithstanding the circumstances LW decided to present these tools to ML as a blind test without advising her of the conditions that they had been subjected to. Because the controls had been removed from this experiment, Test Two could clearly not be used to measure reliably ML's ability to recognise residues on tools. Instead Test Two was to be viewed only as an investigation into the effects of acidic soil on residues on stone tools. Residues observed by ML were then to be checked and confirmed by BW.

Both the protected and the exposed flakes were analysed in the University of the Witwatersrand archaeology laboratory three months after their production. No information whatsoever was provided to the residue analyst (ML) prior to the blind test. During the analyses a catalogue of comparative residues, compiled by BW,

Table 3
Results of blind test (Test Two) by ML: items buried in compost

Flake #	Plant residues identified	Animal residues identified	Mineral residues identified	Comments on identifications	Actual task or residue
4	Starchy residue	Animal tissue	Possible starch from gloves	Processing of animal tissue	Cut raw fat
7	Plant fibers, woody residue, plant tissue	None	None	Processing of plant material	Cut into cortex of raw bone
13	None	Animal tissue, animal fat, hair	None	Processing of animal tissue	Cut raw meat
18	Woody residue, resin, plant fibers, plant tissue	None	None	Processing of plant material	Cut cooked potato
21	Plant fibers, resin, plant tissue	None	None	Few residues, possibly only contact with plant material	Blood smear
24	Plant tissue, resin, woody residue, plant fibers	None	None	Contact with plant material	Cut raw potato
27	Plant tissue, exudates, plant fibers, starchy residue	None	Possible starch from gloves	Processing of plant material, possibly starchy	Cut cooked fat
40	Starch grains, starchy residue, plant tissue	None	Possible starch from gloves	Processing of starchy plant material	No tasks
44	Plant tissue, resin, starch grains	None	None	Processing of resinous plant material	Scraped bark
45	Plant tissue, plant fibers	None	None	Few residues, possibly only contact with plant material	Plant exudates smeared on flake

was used as a source of optical reference. This represents standard procedure during residue analyses conducted by ML. A clean microscope slide was left on a shelf in the microscope laboratory to monitor possible airborne contaminants. Only dust particles were observed, but no airborne starch grains. The flakes were not left exposed when they were not being analysed.

Readings for the pH of the tap water (15 ml) and the compost (5 ml in 15 ml ultrapure deionised water) were taken in triplicate using a standard electronic laboratory pH meter.

2.2. The residue catalogue

The residue catalogue is currently used as both a teaching and an analytical device. The importance of reference collections of plant materials, faunal materials and other potential sources of residues that may adhere to prehistoric stone tools cannot be over-emphasized. The use of such a catalogue during analysis ensures consistent identification of residues that may have preserved on the replicated flakes as well as on archaeological specimens.

First prehistoric activities were replicated using a variety of replicated flakes. The flakes were used to process various materials of plant and animal origin [27]. An additional set of reference material was mounted on microscope slides and photographed at various magnifications. An Olympus BX40 stereo binocular metallographic microscope with analysing and polarising filters and bright and dark field incident light sources was used. Detailed sketches recording the exact positions of the different residues were made. Magnifications that were used ranged from 50× to 800×. The right eyepiece of the microscope was fitted with a measuring graticule so that the dimensions of residues like starch grains, hairs and blood cells could be measured.

General categories within the catalogue are as follows: plant residues, animal residues, miscellaneous residues and possible post-depositional contaminants. Cases where cross-polarised light was used to capture the characteristic features of certain residues were also documented. Cross-polarised light can sometimes be used to distinguish between residues of similar appearance, for example collagen and cellulose fibers.

The catalogue's current images were generated from daylight color slides exposed directly through the incident light microscope using an Olympus SC35 camera. Recently a digital camera (Olympus DP10) has been fitted to the microscope, which will be used for future data capture and storage. Images are in full color because color variation in residues is sometimes conducive to correct identification. Each section of the catalogue begins with a general description of the residue type, followed by more detailed information gained from experimentally replicated tools, the reference collection on glass microscope slides and previously analysed archaeological examples.

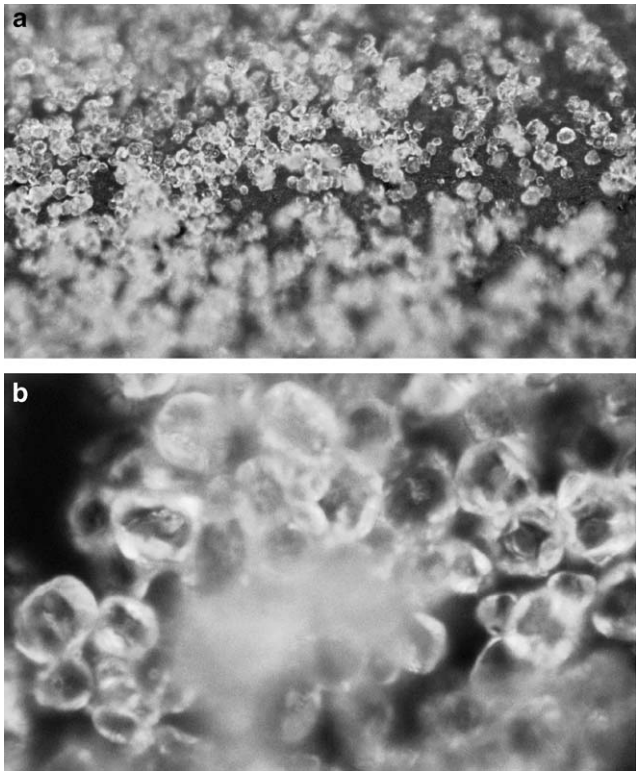

Fig. 2. Grains of cornstarch from powdered latex gloves used during preparation of the samples for the blind test. (a) 100× magnification; (b) 500× magnification, under cross-polarised light. The characteristic extinction cross observed on small unmodified starch grains (up to 5 μm diameter) is not always visible on larger grains (8–10 μm), especially if they have been altered by a refining or heating process or are lying at the wrong angle.

The first part of the catalogue contains descriptions and optical reference material of plant residues. It is subdivided into sections containing images and descriptions of plant tissue, plant fibers, starch grains, white starchy deposits, raphides, resins, exudates, and residues obtained by processing nuts and seeds. More detailed descriptions of some of the residue types applicable to this paper are provided in Appendix A. Various tasks were performed to ensure that the residues would adhere to the replicated stone tools and that they would have a distribution that could be authentic. Such tasks included scraping epidermal cell tissue (bark) from fresh wood, scraping and cutting bulbs, roots and starch-rich tubers, cutting fruit, cutting and scraping leaves and stems, cutting various grass and grain species, and contact with resins and exudates. Images and data were gathered from some of these materials in fresh, cooked, dried and/or weathered conditions. Indigenous species such as *Hypoxis*, *Acacia*, and a variety of endemic grasses were used, but other specimens were also included as a control measure. Ongoing work is being conducted in order to create a more extensive indigenous and ethnographically representative collection.

In the second part of the catalogue animal residues are discussed and depicted. The subsections include images of animal tissue, blood films, red blood cells, collagen (sheet and fibrous collagen), bone fragments, lipids, nucleated cells, hair samples, feathers, shell fragments, fish scales and hoof fragments. Optically, even at high magnifications, blood and tissue residues from modern species are similar to those from prehistoric specimens, but more accurate identification (at species level) is only possible with DNA extraction and sequencing. Materials used for the documentation of animal residues include commercially available sources and fresh, cooked and dried samples were processed.

Miscellaneous residues include ochre of different hues from various sources, charcoal and mineral deposits similar to those found on archaeologically recovered tools from Sibudu Cave, KwaZulu-Natal. The reference materials in this section, furthermore, contain table salt, blackboard chalk, and manganese dioxide. Post-depositional contaminants include mycohyphae, rootlets, spores and crystalline mineral deposits. Post-excavation contaminants, which need to be well characterized so that they do not confuse the identification and interpretation of archaeological residues, can include metal scratches from the use of trowels during excavation, finger-lipids from handling of the artifacts, traces of labels applied during curation, aluminum foil marks, shed insect skins and cobwebs, as well as modern, processed fibers from clothing and storage media such as paper, cotton–wool, silk and polyester. These are all included in the reference collection.

An important aspect of the residue catalogue is that it is work in progress. As more archaeological material and reference materials are analysed, the catalogue is continuously improved and expanded upon. In the future, different soil types and depositional conditions will be investigated and images of the residues generated in experiments will be added to the catalogue. This database provides a valuable foundation for the identification of residues, both in a teaching environment and for practicing analysts.

2.3. The blind tests

ML undertook the blind tests in October 2002. At the time her background comprised two years of experience in microscopic methodology and residue analysis with BW as her mentor. Clean laboratory conditions were adhered to, but latex gloves were not worn during analysis.

A high-power metallographic microscope (Olympus BHS-2UMA) with a binocular viewing head, bright and dark field incident internal lighting, rotating polarising filters and a movable X–Y stage was used for the analysis. Various lenses facilitated magnifications of 50, 100, 200, 500 and 800×. Initially each flake was scanned

at 50×. Where residues were observed, magnifications of up to 800× were used to enable the identification of residue types. Further aids included the manipulation of the light source, such as bright and dark field illumination, as well as the polarisation of light. A photographic record was kept of most of the observations in order to add reference material to the catalogue and to assist in future identification and interpretation of residues. The observations were then tabulated using a format similar to the tables expressing the results in this paper. No changes have been made to the original observations prior to publication. Definitions of the various residue types identified are provided in Appendix A.

Good results were obtained for Test One (Table 2), where the flakes were dried and stored in plastic bags, and it was possible to identify contact residues accurately on 15 out of 17 flakes (88%). As indicated in Table 2, accuracy beyond simple animal/plant distinction was attained for some of the residues. Although Flakes 29 and 31 were not used for any tasks, they have plant remains on them because of the use of powdered latex gloves during the marking of the flakes. The residues were thus correctly identified. Flakes 22 and 28 provide an interesting case study. They were used to scrape the outer bark and wood from live *Acacia karoo* trees, which are occupied by arboreal *Galago moholi* (lesser bushbaby). The presence of animal tissue and fat on the tools, in addition to the plant residues left by replication work, cannot yet be satisfactorily explained, but future research will explore incidental residues left by animals.

Flakes 10 and 41 were incorrectly identified. Flake 10 showed a highly birefringent residue that was interpreted as fibrous overlays (Fig. 3d) of plant origin. According to the catalogue, information from Loy (pers. comm.), and previous experience these characteristics are ascribed to plant material, and they were documented as such. Subsequent research into the phenomenon showed, however, that longitudinal sections in faunal tissue may also account for the regular, structured appearance especially where collagen bundles and/or skeletal muscle are encountered. Observed with a light microscope, under polarised light, such muscle cells or fibers show cross-striations of alternating light and dark bands. The darker bands are anisotropic, and therefore highly birefringent under polarised light due to the ordered myosin molecules of the thick filaments ([8]: p. 118, pp. 182–184).

Residues on Flake 41, used to scrape marine shell, were incorrectly identified as fibrous plant material.

Residues on the Test Two flakes were mostly duplicates of Test One residues yet only 60% of the original residues were accurately identified in Test Two (Table 3). These results most likely indicate the extent to which depositional and post-depositional conditions influence the preservation of residues. Residues from animal products were especially badly affected by the treatment of the Test Two tools, but because the experiment was not well controlled we do not know to what extent the residues were affected by burial in the compost and to what extent they were affected by subsequent watering. The results of the pH readings for the tap water were: pH = 7.87; 7.90 and 7.79 with an average of 7.85. The pH readings for the compost were: pH = 6.03; 5.97 and 6.06 with an average of 6.02. The acidity of the compost, which is rich in humic acids, may well have destroyed some of the animal residues. Plant residues may also have transferred from the compost to the tools, though the starch grains evident on Tool 44 (Table 3), which was not used for any tasks, may alternatively have come from the latex gloves.

3. Discussion

This first residue blind test has demonstrated that it is possible to identify residues on stone tools with an acceptable degree of accuracy using microscopy alone. The analyst obtained a score of 88% for identification of the Test One residues, which had been curated under controlled conditions. This score would have been higher if the analyst had, in addition to microscopy, used the Hemastix® test [28] for the recognition of blood. Hemastix® testing is always performed on archaeologically recovered lithics that are suspected of having blood residues and the method is invaluable for distinguishing blood from other residues of similar appearance. Hemastix® testing will be allowed in future blind tests.

It is essential that archaeologists providing the material for residue identifications supply the analyst with a full history of the excavation and duration of the artifacts. With proper background knowledge it is possible to take some precautionary measures which may limit errors of identification. The powdered gloves are a case in point. Likewise, when residue analysis is intended, clear instructions must be given by the analysts to archaeologists, not only for the excavation and curation of the tools that will be analysed but also for their subsequent handling. Our Test Two has shown that residues are sensitive to environmental conditions and are easily contaminated, causing their removal or alteration. Thus, in archaeological contexts utmost care must be taken to avoid post-excavation corruption of residues.

It is considered standard practice to collect a sample of the deposit from the archaeological context where an artifact intended for residue analysis is recovered. This enables the analyst to establish whether starch grains or geologically derived residues migrated from the deposit to the artifact. In the case of Flake 21 (Test Two) plant remains were the only residues remaining on the flake although blood had been liberally smeared on it. In this case it is possible that blood was destroyed by the acidic conditions of the compost in which the flake was buried,

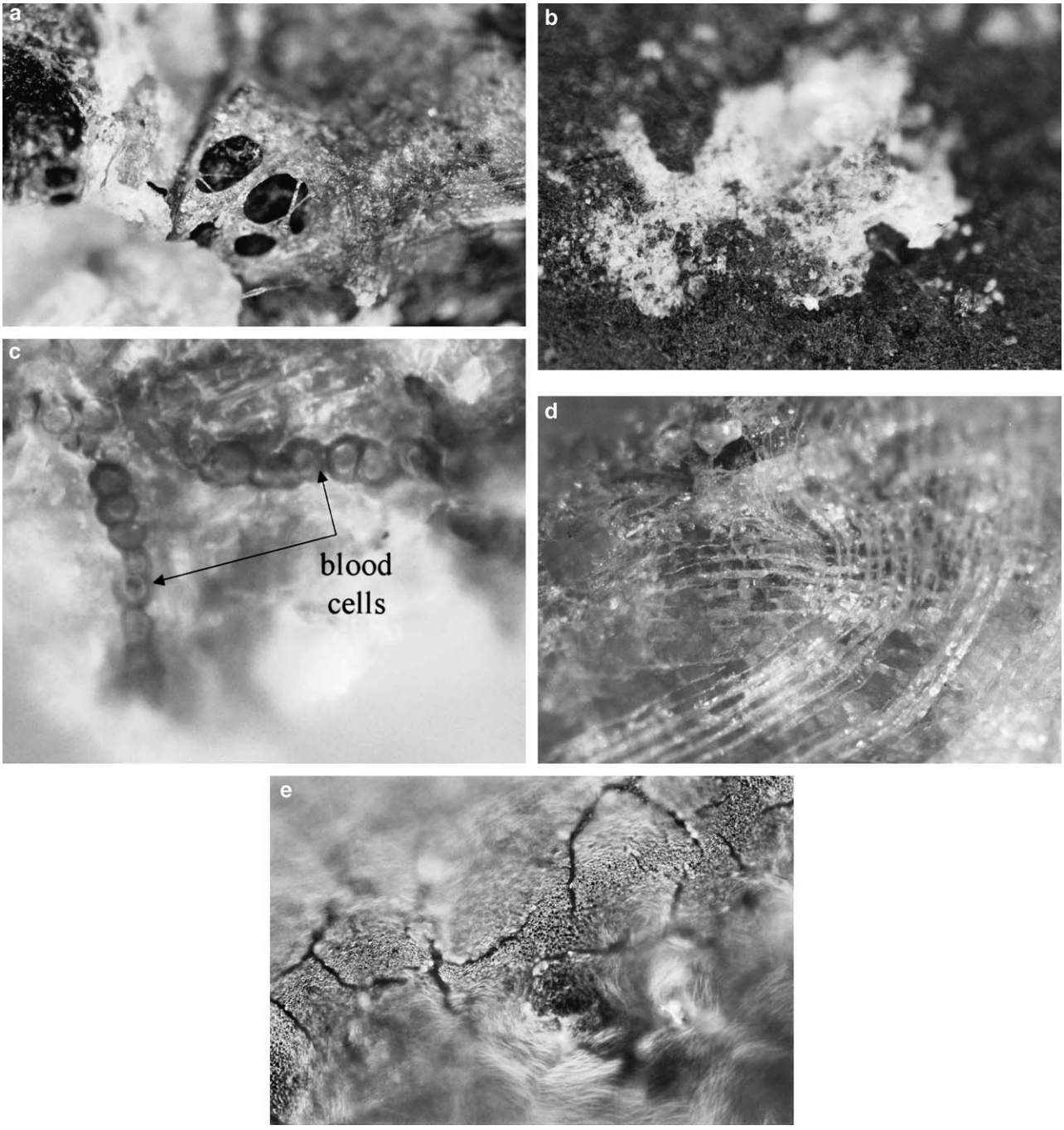

Fig. 3. Residues of animal origin found on the tools used in the blind test: (a) animal tissue and fat on Flake 19 that was used to cut cooked fat (50× magnification); (b) a fat residue on Flake 13 that was used to cut raw meat (100× magnification); (c) a blood vessel with intact red blood cells found on Flake 10 (500×, cross-polarised light) from cutting raw meat; (d) skeletal muscle tissue also found on Flake 10 (200× magnification, cross-polarised light) and (e) fresh blood smeared onto Flake 30 resulted in this blood film (200× magnification, cross-polarised light).

but more experimental work must be done to confirm this suspicion. The compost may also have accounted for the plant residues that were recorded by ML and later confirmed by BW. In the experience of BW such incidental residues are usually superficial when they occur on archaeological specimens and can readily be distinguished from ancient residues that are lodged firmly in cracks and smeared on the surface of the tool because of extended use.

The results of Test Two (Table 3) suggest that contamination with plant material in organic-rich archaeological horizons is a real threat to the accuracy of residue analysis. The commercially purchased compost used for this blind test is probably more organic-rich than the

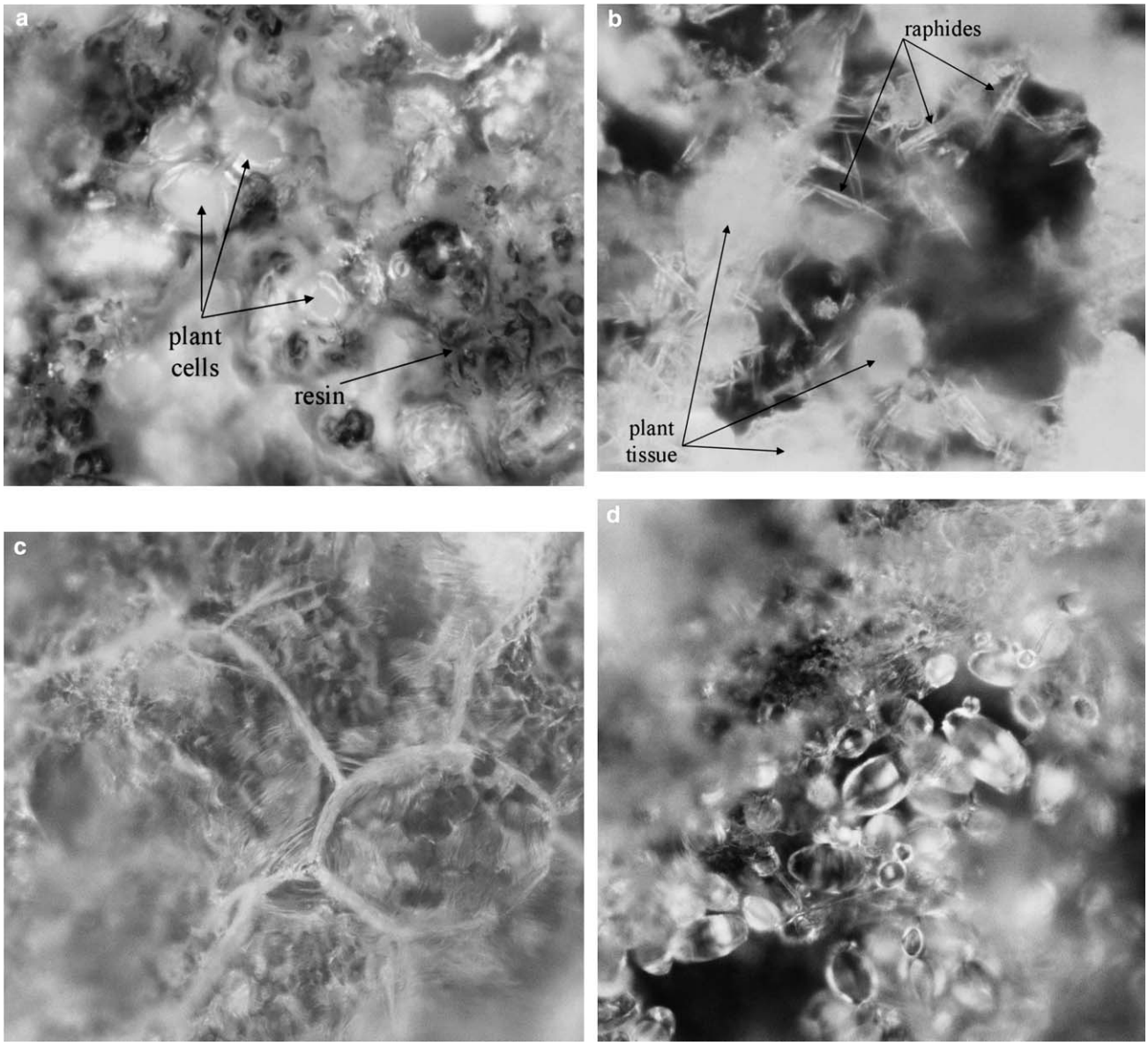

Fig. 4. Residues of plant origin found on the tools used in the blind test: (a) resin and epidermal cell tissue on Flake 2 that was used to scrape bark and wood from a twig (500× under cross-polarised light); (b) raphides and plant tissue cells, also on Flake 2 (500× under cross-polarised light); (c) plant cells from cooked potato on Flake 17 (200× under cross-polarised light) and (d) starch grains on Flake 14 that was used to cut raw potato (taken at 200× magnification under cross-polarised light).

deposits in most archaeological sites. Nonetheless, it is evident that plant residues are greatly over-represented in the test sample that was buried in compost and subsequently exposed and watered for three days. An assessment of the depositional conditions and forms of diagenesis at an archaeological site is therefore recommended before residue analysis is conducted.

Animal products might be more sensitive to post-depositional conditions than plant remains and future work will explore this possibility further. It is noteworthy that only two out of five animal residues survived the compost and watering, whereas all of the seven animal residues survived the protected environment provided by the zip-lock plastic bags. Conditions in the stored plastic bags might not be greatly different from circumstances in some caves or rock shelters that are relatively dry and protected. Thus there is, hypothetically, a good prognosis for the preservation of animal residues on lithics from such sites. Fat, blood and bone residues survived on Flakes 10, 12, 16, 19, 25, 30 and 33 (Fig. 3), which were stored in dry, clean plastic bags. In contrast, meat products did not survive on Flakes 7, 21 and 27 and this may be due to their burial in the acid compost and their subsequent exposure and watering. Archaeological sites with acid soils that are exposed to rain may present problems for residue

preservation and future replication work will address this issue. We are reluctant to draw firm conclusions based on our preliminary tests.

Plant residues may survive conditions that destroy animal residues: all of the buried flakes with plant remains on them retained their original residues, but may have collected more plant residues while buried in the compost (Fig. 4). The caveat here is that a predominance of plant residues at a site need not imply that there was more plant than animal processing at a site: diagenesis rather than human behavior may be indicated. Future replication and experimental residue work will examine the effects of various soil and water types on residues. Deposits will be collected from cave sites as well as open-air contexts. This project is designed to improve our understanding of post-depositional agencies on residues. The tools from Test Two are thus only a small part of the projected study.

A further outcome of the blind tests was the realization that, contrary to previous assumptions, animal tissues on archaeological or replicated tools can be birefringent. Through replication studies we shall further investigate the occurrence of birefringency and its occurrence in materials other than those of plant origin. This is a contentious issue and extensive investigations into the nature of pleiochroism (the property of a crystal of having a different color depending upon the direction of transmitted light through the crystal [25]) and birefringency are needed to establish how useful they are in the identification of residues. Furthermore, examples of different longitudinal and transverse sections of a variety of animal tissues need to be included in the catalogue.

Another lesson that we have learnt is that residue analysts should be cautious about differentiating cooked and raw animal or plant products. The results here show that it is safer to record only the presence of, for example, animal fat or starchy residue, without making further claims. It is possible that the difference between raw, cooked and/or dried residues that preserve on stone tools cannot be confidently established. More examples of cooked versus raw products are needed in the catalogue before attributes of each type can be rigorously investigated.

Some of the problems of residue analysis were highlighted during this blind test, but more importantly, the exercise demonstrates that a relatively high success rate is obtainable for residue identification where suitable conditions have preserved the original residues. It also shows that an extensive modern reference catalogue is indispensable for the identification of residues. The tests reported here are the first of a planned series. Additional research projects will be designed to improve the techniques used for residue analyses of prehistoric lithics. Future research to investigate conditions that preserve or destroy residues is planned. Such work will develop interpretative skills that will ultimately result in clearer insights into past human behavior.

Acknowledgements

We thank two anonymous reviewers for their helpful suggestions. We thank the NRF and the University of the Witwatersrand for ongoing financial support for residue studies. Conclusions reached here are the responsibility of the authors and should not necessarily be attributed to the funding agencies.

Appendix A. Description of residue types

Only the residue types relevant to this study will be discussed here. It must be pointed out that not all residues can be identified and the present authors do not claim to be able to recognise all residues.

A.1. Plant residues

A.1.1. Starch grains

Starch grains are probably the most diagnostic of all residue types. A magnification of at least 500× is needed to see small (<5 μm) starch grains, while a magnification of 200× is adequate for viewing larger (5–10 μm) starch grains. Starch grains are made up of alternating layers of amylase and amylopectin in a kind of onion formation giving a spherical grain. Under cross-polarised light the birefringence of the grain results in a characteristic extinction cross which centres on the hilum, or growth point, of the grain. The hilum is that part of the starch grain around which the starch is laid down in more or less concentric layers. The extinction cross will not always be visible if the starch grain has been modified by heating or degradation, or if the orientation of the starch grain in the residue deposit is such that the hilum is not at the top of the grain. Birefringence is defined as the double refraction of incident light by some substances and this phenomenon can be used to distinguish between the structures of certain biological molecules ([5]: pp. 570–572). Hufford ([6]: p. 333) explains that starch and the cell wall tissues in plants are made up of essentially the same cellulose molecules but that they are joined together in different ways. Hence, they are both birefringent under cross-polarised light, but they differ macroscopically.

A.1.2. Starchy residues

This is a white, granular deposit that is amorphous in the sense that it has no plant cell structure visible. Sometimes it has a few identifiable starch grains, but these can be slightly decomposed especially if the deposit is moist, and the concentric layers within the starch grain have been disrupted. It is possible that the starchy looking residues are the result of cooking or heating plant material rich in starch. The grains within these starchy residues are smaller (1–10 μm) than sand grains

and they cannot be confused with fat deposits because they are not reflective and oily like fatty residues.

A.1.3. Plant fibers

Plant or cellulose fibers are not only birefringent, but the broken ends of the fibers tend to look shattered or abruptly broken. These properties allow them to be distinguished from animal fibers and fibers from modern potential contaminants such as wool, cotton or silk. Plant fibers can occur singly or can be grouped together in bundles.

A.1.4. Raphides

These appear as elongated crystals or styloids and can be distinguished on the basis of their narrow width and their appearance in bundles, they are a type of phytolith. Phytoliths are the crystalline substances that are contained in specialised cells within the plant tissue or in the cell lumen [24].

A.1.5. Plant tissues and cells

Plant tissues are generally distinguished from collagen and animal tissues by the principle of birefringence (but see other discussion of this issue), together with other indicators of vegetal origin for the particular residue (like resin and starch grains). Epidermal or woody plant tissue and bark have characteristic rectangular or squarish cells [22] that are often found in an arrangement resembling bricks in a wall. More specific descriptions of plant tissues (e.g.: the presence of tracheids) are often not feasible given the fragmented and sometimes degraded nature of the residues.

A.1.6. Resins and exudates

Two forms of plant residue, which can often be confused with blood films, are resins and exudates (or "plant juices"). These films can have a similar glassy, fluid appearance and can be dark in color (the Hemastix® test is definitive in distinguishing between resins and blood films [28]). Starch grains (usually small, for example 1–2 μm in diameter) can sometimes be seen in the resin film. Plant exudates are less well defined and can be starchy or fluid in appearance.

A.2. Animal residues

A.2.1. Blood films and red blood cells

When a blood film dries on the surface of a stone tool it forms a relatively hydrophobic layer. This layer is caused by the tertiary denaturation of serum albumin molecules and the subsequent formation of an unordered polymer, as reactive sites on different molecules form weak bonds. Provided that the tool remains in a relatively stable environment, such as a dry archaeological deposit, this blood residue will remain intact on the tool and still be biochemically active after hundreds and even thousands of years. Some of the molecular structures of the biomolecules will have been altered from their "native" states, however. Blood films may be found anywhere on the surface of the tool and not necessarily along the working edge. Even if tools had been cleaned after being used (once the blood had dried) or after they were excavated, blood deposits may still be found in cracks and recesses on the tool surface. Blood films appear relatively reflective and may range in color from straw yellow, for thin films, to very dark red or black, for thicker deposits. If the blood film is thick then characteristic "mud-cracking" appears on the surface. Blood films are also multi-component and may contain serum albumin and immunoglobulins as well as other biomolecules that make up blood. It is the protein component of the blood that usually forms the reflective layer, which can be seen to fill in microscopic hollows in the tool surface and often cover intact red blood cells.

Blood cells are recognised by their biconcave shape and generally range in size from 3 to 5 μm in diameter. Red blood cells are sometimes found singly but they can also occur in clumps or groups and are, on occasion, stacked one behind the other. On drying, the edges of the red cells may become crenated or ruffled.

A.2.2. Animal tissue

Animal tissue often accompanies blood films or collagen. No clear cell structure (as with plant tissue) is visible and animal tissues' cells do not have rigid cell walls. Animal tissue deposits are dull or opaque under cross-polarised incident light, although striated muscle tissue can display elements of birefringency under certain conditions [8].

A.2.3. Animal fat

Fat (adipose tissue) and marrow cells have distinctive globular shapes. When crushed or smeared the tissue becomes amorphous, but the smear itself can be detected. Unilocular (common or yellow) adipose tissue is composed of cells that contain one large central droplet of yellow fat in the cytoplasm. Multilocular (or brown) adipose tissue comprises cells with numerous lipid droplets and abundant brown mitochondria [8]. In marrow deposits, bright red erythrocytes occur, which are responsible for platelet formation [29]. In archaeological samples these brown and red spots can sometimes be detected within a whitish mass and indicate the faunal origin of the residue. Fat and marrow appear opaque and are sometimes bluish under cross-polarised light.

A.2.4. Collagen

Next to red blood cells, collagen is the most diagnostic of animal residues and it is found in most supporting tissues. It is mostly fibrous. Parallel collagen fibrils are arranged into strong bundles 2–10 μm in diameter [29]. Thus, collagen fibers often have the

appearance of rope and the ends can look unravelled. The orientation of the elongated tropocollagen molecules in collagen fibers can make them birefringent. Some collagen bundles may display structured layers [8]. Certain collagen types do not form fibrils, but rather have a mesh-like structure [29].

A.2.5. Hairs

Hairs that are not too degraded can have very diagnostic features but they are always recognised, even when degraded, by their cylindrical structure and by the presence of a medulla. The outer sheath of the hair has a distinctive scale pattern that is specific at least to the genus level, although an extensive reference collection is needed for such identification.

Notes:

1. It is often not a single feature of a residue that is used to decide whether it is of plant or animal origin, but rather a combination of features and attributes.
2. Although the Hemastix® test was not expressly used in this study, it may be important to point out its usefulness and state that it was not used because we wanted to preserve the residues for use by other students wishing to do the blind test later on. The Hemastix® test is a fundamental tool for distinguishing between resin and blood.

References

[1] J. Atchison, R. Fullagar, Starch residues on pounding implements from Jinmium rock shelter, in: R. Fullagar (Ed.), A Closer Look: Archaeological Methods Series 6, Sydney University, Sydney, 1998, pp. 109–125.

[2] D.B. Bamforth, Investigating micro-wear polishes with blind tests: the institute results in context, Journal of Archaeological Science 15 (1988) 11–23.

[3] D.B. Bamforth, G.R. Burns, C. Woodman, Ambiguous use traces and blind test results: new data, Journal of Archaeological Science 17 (1990) 413–430.

[4] M. Dominguez-Rodrigo, J. Serrallonga, J. Juan-Tresserras, L. Alcala, L. Luque, Woodworking activities by early humans: a plant residue analysis on Acheulian stone tools from Peninj (Tanzania), Journal of Human Evolution 40 (2001) 289–299.

[5] D.C. Giancoli, Physics: Principles with Applications, second ed., Prentice-Hall International, USA, 1985, pp. 570–572.

[6] T.L. Hufford, Botany: Basic Concepts in Plant Biology, Harper Row, New York, 1979, 333 pp.

[7] A.H. Jahren, N. Toth, K. Schick, J.D. Clark, R.G. Amundson, Determining stone tool use: chemical and morphological analyses of residues on experimentally manufactured stone tools, Journal of Archaeological Science 24 (1997) 245–250.

[8] L.C. Junqueira, J. Carniero, R.O. Kelly, Basic Histology, ninth ed., Lange Medical Books, New York, 1998.

[9] L. Keely, M. Newcomer, Micro-wear analysis of experimental flint tools: a test case, Journal of Archaeological Science 4 (1977) 29–62.

[10] B. Kooyman, M.E. Newman, H. Ceri, Verifying the reliability of blood residue analysis on archaeological tools, Journal of Archaeological Science 19 (1992) 265–269.

[11] T.H. Loy, The artifact as site: the biomolecular analysis of organic residues on prehistoric tools, World Archaeology 25 (1993) 44–63.

[12] T.H. Loy, E.J. Dixon, Blood residues on fluted points from eastern Beringia, American Antiquity 63 (1998) 21–46.

[13] T.H. Loy, M. Spriggs, S. Wickler, Direct evidence for human use of plants 28 000 years ago: starch residues on stone artifacts from northern Solomon Islands, Antiquity 66 (1992) 898–912.

[14] T.H. Loy, A.R. Wood, Blood residue analysis at Cayonu Tepesi, Turkey, Journal of Field Archaeology 16 (1989) 451–460.

[15] Y. Maniatis, Z. Tsirtsoni, Characterisation of a black residue in a decorated Neolithic pot from Dikili Tash, Greece: an unexpected result, Archaeometry 44 (2002) 229–239.

[16] E.H. Moss, A review of investigating micro-wear polishes with blind tests, Journal of Archaeological Science 14 (1987) 473–481.

[17] M. Newcomer, R. Grace, R. Unger-Hamilton, Investigating micro-wear polishes with blind tests, Journal of Archaeological Science 13 (1986) 203–217.

[18] M.E. Newman, H. Ceri, B. Kooyman, The use of immunological techniques in the analysis of archaeological materials—a response to Eisele: with report on studies at Head-Smashed-In Buffalo Jump, Antiquity 70 (1996) 677–682.

[19] G.H. Odell, Stone tool research at the end of the millennium: classification, function, and behavior, Journal of Archaeological Research 9 (2001) 45–100.

[20] G.H. Odell, F. Odell-Vereecken, Verifying the reliability of lithic use-wear assessment by blind tests: the low-power approach, Journal of Field Archaeology 7 (1980) 87–120.

[21] D.R. Piperno, I. Holst, The presence of starch grains on prehistoric stone tools from the humid Neotropics: indications of early tuber use and agriculture in Panama, Journal of Archaeological Science 25 (1998) 765–776.

[22] P.H. Raven, R.F. Evert, S.E. Eichhorn, Biology of Plants, fifth ed., Worth Publishers, New York, 1992.

[23] V. Rots, Hafting traces on flint tools: possibilities and limitations of macro- and microscopic approaches, PhD thesis, Katholieke Universiteit, Leuven, 2002.

[24] H.J. Shafer, G. Holloway, Organic residue analysis in determining tool function, in: B. Hayden (Ed.), Lithic Use-wear Analysis, Academic Press, New York, 1977, pp. 385–399.

[25] D.W.A. Sharp, The Penguin Dictionary of Chemistry, Penguin Books, Ltd, UK, 1983.

[26] J.J. Shea, Methodological considerations affecting the choice of analytical techniques in lithic use-wear analysis: tests, results and applications, in: S. Beyries (Ed.), Industries lithiques traceologie, vol II, aspect methodologiques archeologoques, BAR International Series 411 (II), 15, 1988, pp. 73–82.

[27] B.S. Williamson, Prehistoric stone tool residue analysis from Rose Cottage Cave and other South African sites, PhD thesis, University of the Witwatersrand, Johannesburg, 2000.

[28] B.S. Williamson, Direct testing of rock painting pigments for traces of haemoglobin at Rose Cottage Cave, South Africa, Journal of Archaeological Science 27 (2000) 755–762.

[29] B. Young, J.W. Heath, Wheather's Functional Histology: a Text and Color Atlas, fourth ed., Churchill Livingstone, London, 2002.