Potential misidentification of in situ archaeological tool-residues: starch and conidia

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Abstract

Microscopic identification of organic residues in situ on the surface of archaeological artefacts is an established procedure. Where soil components morphologically similar to use-residue types exist within the soil, however, there remains the possibility that these components may be misidentified as authentic residues. The present study investigates common soil components known as conidia, fungal spores which may be mistaken for starch grains. Conidia may exhibit the rotating extinction cross under cross-polarised light commonly diagnostic of starch, and may be morphologically indistinguishable from small starch grains, particularly at the limits of microscope resolution. Conidia were observed on stone and ceramic archaeological artefacts from Honduras, Palau and New Caledonia, as well as experimental artefacts from Papua New Guinea. The findings act as a caution that in situ analysis of residues, and especially of those less than 5 μm in size, may be subject to misidentification.
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1. Introduction

The identification of archaeological artefact function via microscopic observation of the morphologies of adhering residues is an established procedure [11]. As a component of such studies, the correlation of use-wear traces with residue types has long been acknowledged as providing important corroborative evidence in strengthening functional determinations. Only when observation of residues in situ on an artefact's surface is not possible, owing to artefact size or other physical constraints, should potential residues be removed without initial microscopic examination of the artefact. This increasing recognition of the importance of in situ observation has meant that in many cases residue identification is made without the aid of chemical tests, which typically require removal of the residue to a microscope slide or vial. For

in situ identification the morphology of the organic or inorganic particles attached to the artefact surface is given paramount importance, along with the reaction of the residue to varying lighting conditions such as bright-field, dark-field and cross-polarised light.

While classification of residues still attached to an artefact provides clear evidence for inferring use-actions through association with use-wear patterns, without further chemical testing the possibility remains that morphological examination may provide erroneous identifications. This possibility increases if there are soil components which are morphologically similar to common residue types, and examinations are undertaken near the limits of resolution of the microscope employed. The present study investigates common soil components known as conidia, fungal spores which may be mistaken for starch grains. The aim of this paper is to raise awareness of the potential for confusion with regard to these objects when employing light microscopy, in a similar manner to that previously done for

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faecal spherulites [2]. Starch grains have formed one of the central foci for residue microscopists, and any optically-similar materials should be recorded to avoid misidentification. Examples here are from residue studies of archaeological stone and pottery samples from Micronesia, New Caledonia and Honduras, as well as a modern sample of oven stones from Papua New Guinea.

2. Starch and conidia

2.1. Starch

Of relevance to the current study are those aspects of storage starch grains observed on an artefact's surface through the incident (or reflected) light microscope. These include size, shape and particularly the rotating-cross effect seen under cross-polarised light. For a more general introduction to starch grains and their formation, role and archaeological importance, see Haslam [8] (also [1,22,26]). The main points to be noted here are that starch grains are carbohydrate polymers from storage organs and other locations within plants, ranging in size from 1 to over 100 μm, and are typically spherical to ellipsoidal in shape. Many archaeologically-important starches, including in the New World *Zea mays* (maize) and *Manihot esculenta* (manioc), in the Pacific *Colocasia esculenta* (taro) (Fig. 1), and in Southeast Asia *Oryza sativa* (rice), have grains which can approach or fall below 10 μm in maximum diameter [5,15,18,20]. Transitory starches not produced in storage organs are also typically <5 μm [8]. It is these small
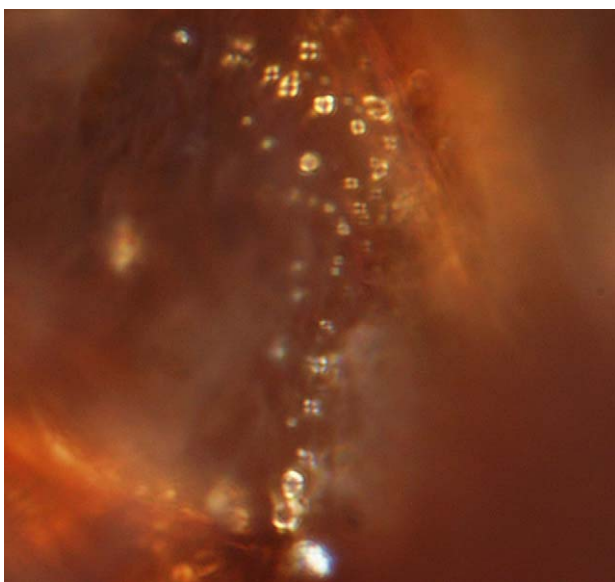

Fig. 1. Starch grains of *Colocasia esculenta* (taro), 1–2 μm in diameter, on an experimental potsherd; note extinction crosses. Cross-polarised bright-field illumination: 1000×.

starches, especially those which have diameters below 5 μm, which are potential candidates for misidentification with conidia.

Starches of different species may exhibit characteristic granule shapes [19] readily distinguished under transmitted light on a microscope slide, but it is not always as easy to discern the exact shape of small grains still attached to an artefact's surface. A faceted or slightly oval-shaped grain may well appear spherical in these circumstances, even leaving aside the fact that some starches in fact present different shapes when rotated in three dimensions [23]. In part the lack of ability to clearly differentiate grain shape may result from grains being lodged in crevices or surrounded by soil and other residues (e.g. [17]), but the resolving power of the microscope also plays a role. The resolution of the microscope will depend on its numerical aperture, with higher numerical apertures providing better resolution, given that artefact microscopy is usually conducted keeping the immersion medium (air) and light wavelength (white, centred at about 550 nm) constant. The highest numerical apertures are only available at high magnification, resulting in a shallow depth of field and short working distance. The use of 'long working distance' lenses, which may be essential in artefact microscopy, can also result in lower numerical aperture values and decreased resolution. A general rule of thumb is that the total magnification (eyepiece × objective) should not exceed 750–1000 times the numerical aperture of the objective lens (as the objective also acts as the condenser in incident microscopy) [21:31–32]), For example, a 100× long working distance objective lens with a numerical aperture of 0.80, when used with a 10× eyepiece gives a magnification of 1000×, well above the 600–800× (calculated as 0.80 × 750–1000) required for ideal resolution with this aperture value. The extra magnification in this case is in effect 'empty' magnification, making the image bigger, but not clearer. The net result is that the shape of small starch grains may not be precisely determined owing to diffraction effects on their outlines.

Regardless of size and shape, the most important diagnostic feature used by archaeological-starch researchers for in situ identification is the presence under cross-polarised light of a rotatable extinction cross, centred on the hilum of the grain [6,11,14,17,27]. The exact positioning and shape of the cross can again be readily determined on larger grains and under transmitted light on a microscope slide, but resolution issues come into play concerning small in situ starch grains. In such cases the best observation that can be made is that a cross is present and does indeed rotate smoothly when rotating the polarising filters, and that interference colours are not visible (in situ starch grains will appear white under reflected light). The latter observation is necessary to rule out potential misidentification of

spherulites [2]. If a group of small, roughly spherical, objects with smoothly rotating extinction crosses are observed together, this may in the past typically have been taken as evidence of contact with starchy material.

2.2. Conidia

Little attention has been given in the archaeological residue literature to fungal structures, either as residues in their own right or in terms of their effects on other residues. It is not the purpose of this paper to redress this issue, but an overview of fungal growth and types is necessary for contextual purposes. The term *fungi* covers a variety of organisms such as the molds, mildews, rusts, yeasts and mushrooms; soil-borne fungi constitute a major part of the soil biomass and are the dominant agents in organic decomposition [16]. With exceptions (for example the unicellular yeasts), the typical fungal morphology consists of filaments known as hyphae (singular hypha), which as a mass may be referred to as the mycelium. Hyphal cell walls are strengthened by the polysaccharide chitin, as opposed to the cellulosic composition of plant structural components. Some fungi possess macroscopically visible fruiting bodies (for example, the mushrooms), however, the majority are individually microscopic, producing spores for either sexual or asexual reproduction. Spore and fruiting structure morphologies remain one of the most useful classificatory mechanisms for fungi [24], along with structural aspects such as the presence of septa in the hyphae. Spore types include zygospores, ascospores and basidiospores (sexual) and conidia (asexual), and it is the latter which form the focus of this paper. Much of the information on conidia presented here is drawn from Cole and Kendrick [4] and Watanabe [24].

Conidia (singular conidium) are usually formed at hyphal tips from specialised structures called conidiophores, although these may be lacking in some species. The conidiophore is itself composed of various other cell types, and may include branches, metula, and the phialides, which produce the actual conidia. The arrangement of these various cells provides taxonomic information, as does the appearance and size of each component. Conidia may be formed singly as the terminal components of the conidiophore in a number of configurations, or may be successively produced to form a linked chain [3]. Two of the more ubiquitous fungal genera, *Aspergillus* and *Penicillium* (used as examples throughout this paper), contain species which exhibit small, spherical spores joined in such chains. Conidia are typically hyaline or a shade of brown, and surfaces may be smooth to very rough, with minute projections from the conidial surface. They are present in soils, fresh water, salt water and can also be airborne. Additionally, as discussed further below, under cross-polarised light conidia may exhibit extinction crosses.

With reference to the criteria for in situ starch identification outlined earlier, therefore, conidial sizes, shapes, and reaction to cross-polarised light all allow for potential confusion.

There are many thousands of conidia-producing fungal species (see for example [9]), and the descriptions in this paper are necessarily generalised. Conidia form in a wide variety of shapes and sizes, including coiled and elongated forms, and can reach $> 50\ \mu m$ [25], but it is those conidia formed as roughly spherical spores $< 10\ \mu m$ in size which are of concern here. Similarly, while conidia may be coloured, it is the hyaline spores which are more likely to present the opportunity for misidentification as starch. Even with these restrictions there remain at least dozens of conidia which are $< 5\ \mu m$, and even more $< 10\ \mu m$, which form individually or in chains, are ovate or spherical [24], and on which any surface ornamentation is unlikely to be recognisable during in situ artefact microscopy. For example, *Aspergillus parasiticus* conidia are globose, $3.7–5.5\ \mu m$ in diameter and develop from phialides arranged radially on a rounded extension from the hypha, while *Penicillium nigricans* conidia are globose or subglobose, $2.7–4\ \mu m$ in size, and develop typically in chains running sub-parallel to the hyphal axis [24]. Conidia do possess internal structure, including a nucleus and mitochondria (see, for example [3,7]), but these are unlikely to be visible when viewing small spores via in situ light microscopy. Unfortunately the fungal literature does not discuss fungal appearance on irregular surfaces such as artefacts, which makes direct comparison of in situ fungal residues with established keys or published photographs difficult.

3. Archaeological and modern examples

Conidia described here were observed on archaeological artefacts from three countries: Palau, New Caledonia and Honduras. The Palauan and Honduran artefacts were flaked chert and obsidian tools, typically $< 4\ cm$ in maximum dimension, whereas the New Caledonian artefacts were pottery sherds. The various geographical locations and artefact material types are included to emphasise the prevalence of fungal interaction with archaeological objects, and the similarity between these disparate occurrences in appearance and context of the conidia when viewed in situ. All artefacts were analysed with an Olympus BX60 polarising microscope with light- and dark-field capacity, at magnifications of 100, 200, 500, and $1000\times$.

In all three archaeological cases, conidia were initially identified owing to their occurrence in groupings consisting of half a dozen to several hundred individual spores. In many instances they were associated with transparent hyphae approximately $1–3\ \mu m$ in diameter

(Figs. 2 and 7). Conidia were often noted adhering to these hyphae, and clusters appeared to originate on hyphal side-branches. The most distinctive form of cluster was the group of conidia 'chains' growing in either parallel or radial formations. The conidia were typically 1—4 μm, appearing smoothly spherical or slightly ellipsoid (taking into account resolution issues noted earlier), and the majority displayed an extinction cross to some extent (Figs. 3—10). The cross appears to rotate smoothly about the centre of the grain when the polariser is rotated. In plane-polarised light the spores appear translucent and globose, without discernible structural components. The conidia appear typically white in cross-polarised light owing to birefringence. For every artefact on which conidial concentrations were recorded, subsequent observation also revealed the presence of individual spores and isolated conidia chains, separated from any other fungal elements on the artefact surface. These isolated conidia are of particular concern, as there is no contextual information to warn the analyst that these may not be starch grains. One species of fungus observed on the New Caledonian artefacts possessed conidia which did not exhibit an extinction cross, and these would not be confused with starch (Fig. 6).

Other than on archaeological artefacts, conidia were observed on two basalt cobbles collected from a river bed in West New Britain Province, Papua New Guinea. One of the cobbles was used following collection as a heating stone in an experimental earth oven, the other was unused. These cobbles were analysed using an Olympus BHMJ polarising microscope at 100, 200, 500
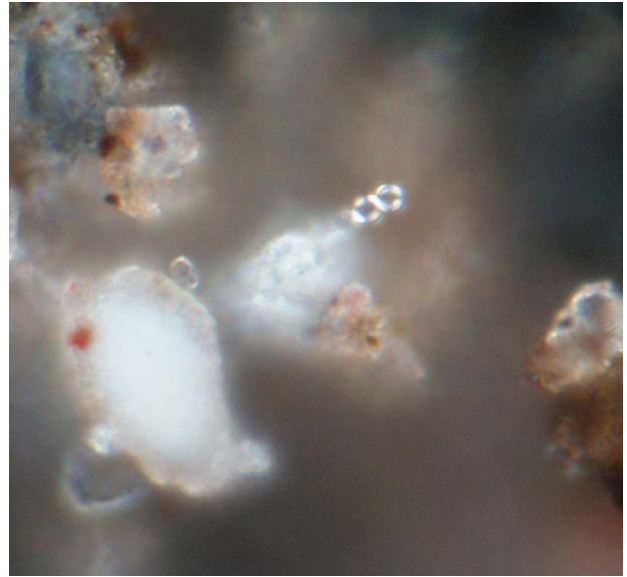

Fig. 3. Conidia (3 μm) in chain formation. Cross-polarised bright-field illumination: 1000×. Artefact FS7/13 from Copan, Honduras.

and 800×. The observed spores again closely resemble the *Aspergillus* radial chain form (Figs. 11—13), and conidia from the heating stone were used in the transmitted light observations described below. The conidia averaged 2—3 μm in diameter, were spherical to ovate, and possessed extinction crosses which appeared to rotate around a central point when observed in situ. The presence of these spores on the unused cobble was somewhat unexpected as it was collected from the bed of a running river, although conidia are produced by some

Fig. 2. Conidia (2.5—3 μm) on transparent hypha 3 μm wide; note extinction cross. Cross-polarised bright-field illumination: 1000×. Artefact FS7/13 from Copan, Honduras.

Fig. 4. Conidia (1—2 μm) in chain; note extinction crosses. Cross-polarised bright-field illumination: 1000×. Sherd #16 from Nouville, New Caledonia.

Fig. 5. Conidia (1–1.5 μm) concentration. Cross-polarised bright-field illumination: 1000×. Sherd #16 from Nouville, New Caledonia.
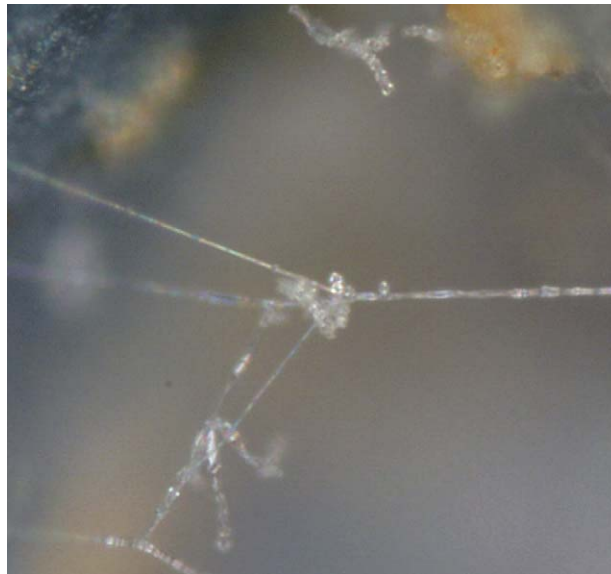

Fig. 7. Conidia (2–3 μm) attached to transparent hyphae 1–2 μm wide. Plane-polarised bright-field illumination: 500×. Artefact #1571 from Palau.

fungal species underwater [25]. It is possible that the fungus has grown on the artefact surface in the two years since collection.

For comparative purposes, several conidia were removed from the basalt cobble used in the earth oven by applying 30 μl of ultrapure water, agitating briefly, then transferring to a microscope slide. The slide was examined under transmitted light at 400 and 1000× using an Olympus BX50 microscope fitted with a rotating stage and polarising filters. The conidia were

observed for the most part singly (the transfer having disrupted the conidial chains), appearing in plane-polarised light as practically transparent objects approximately 2.5 μm in diameter. Under cross-polarised light many, but not all, of the grains displayed an extinction cross to some degree. It was apparent, however, that the cross does not rotate smoothly in transmitted light, but instead the central portion of the cross elongates in one of two perpendicular axes depending on the clockwise or anticlockwise rotation

Fig. 6. Chain of conidia (2 × 3 μm); this fungal species is clearly different from that in Figs. 4 and 5, and does not exhibit an extinction cross. Cross-polarised bright-field illumination: 1000×. Sherd #16 from Nouville, New Caledonia.

Fig. 8. Conidia (2–2.5 μm) in parallel chains. Cross-polarised bright-field illumination: 1000×. Artefact #1194 from Palau.

Fig. 9. Conidia (2–2.5 μm) in chain formation. Cross-polarised bright-field illumination: 1000×. Artefact #1194 from Palau.

Fig. 11. Dense cluster of 2.5 μm conidia. Cross-polarised bright-field illumination: 800×. Artefact #3 from West New Britain, Papua New Guinea.

of the polariser. This subtle elongation is not evident during in situ incident light microscopy, and may be an artefact of the passage of light through the internally differentiated spore body. As a further test, iodine potassium iodide (IKI) stain was applied to the slide, with no reaction observed from the conidia. Since IKI stains starch a red-brown to blue-black colour, depending on the amylose/amylopectin ratio in the grains [8], this test should be useful in distinguishing starch from conidia during transmitted light microscopy.
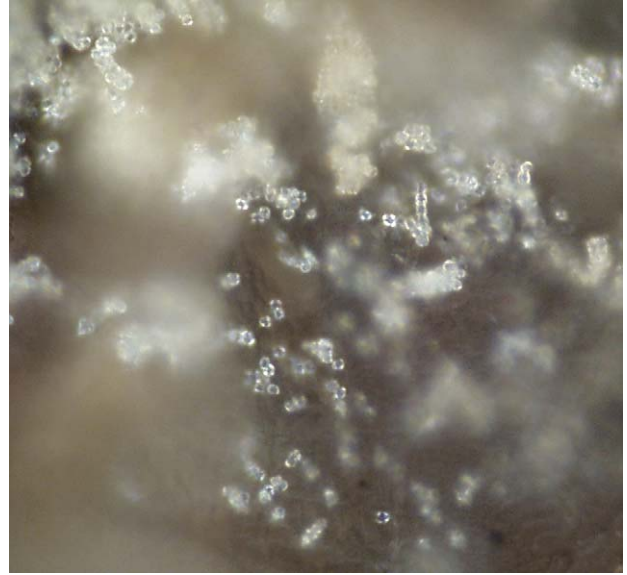
4. Discussion

On many of the analysed artefacts conidia were observed adhering to the artefact surface away from soil deposits or hyphae. There is no reason to expect therefore that the spores will only be found within a soil matrix or obviously associated with other fungal elements. For the purposes of this study it was not possible to remove, culture and observe the fungi to record the level of detail required by mycologists for
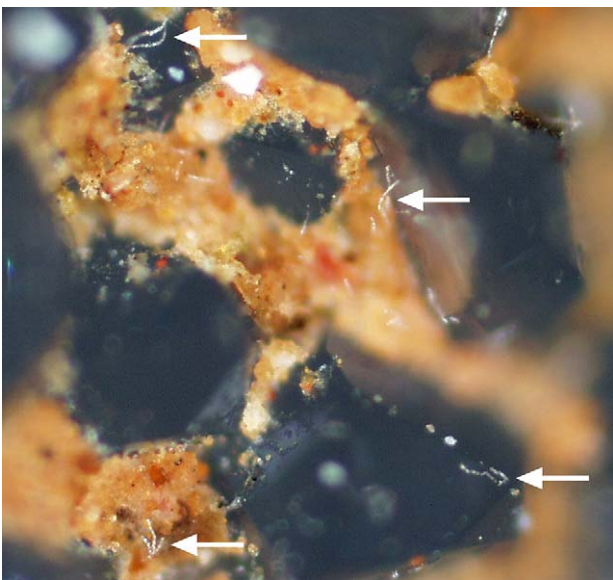

Fig. 10. Conidia chains (arrowed) on artefact surface and adhering soil. Cross-polarised bright-field illumination: 100×. Artefact #1194 from Palau.

Fig. 12. Radial conidia formations 50 μm in diameter. Dark-field illumination: 200×. Artefact #3 from West New Britain, Papua New Guinea.
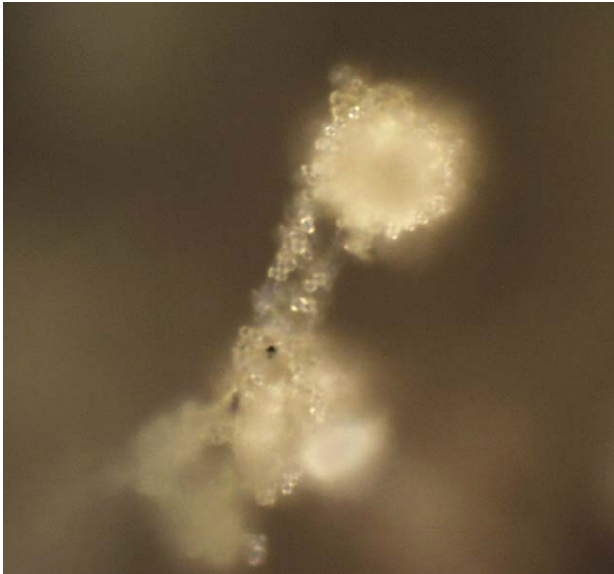
Fig. 13. Radial conidia (50 μm in diameter) showing density of cluster; note extinction crosses visible on some spores. Cross-polarised bright-field illumination: 500×. Artefact #3 from West New Britain, Papua New Guinea.

firm taxonomic classification. It is likely, given the complexities and subtleties involved, that fungal taxonomy is beyond the skills and time of even those archaeologists engaged in microscopic residue analysis. It may be possible to narrow the possibilities using factors such as climate (for example *Aspergillus* is relatively more abundant in tropical climes and *Penicillium* in temperate [10,13:72]), but these are broad guides at best. The possibility that conidia of different species will display reactions to lighting conditions and stains which differ from those observed in this study must also be considered.

The observation that naturally-occurring materials present in soils may be potentially confused with important archaeological indicators is not obviously limited to any residue type. Below the resolution threshold of the microscope employed the prospect of misidentification becomes increasingly real, and for in situ residue analysis the likelihood is that any objects below 5 μm in size may be difficult to characterise precisely. For example, Kennedy [12] observed experimentally that grinding rhyodacite on sandstone left silica particles of ≤3 μm on the surface of the rhyodacite. These particles occasionally exhibited an extinction cross, mimicking small starch granules during in situ observation but not under transmitted light. Future work in this area should therefore aim to develop a reference collection of objects which may not have been targeted by past people, but which may be present on artefacts for taphonomic reasons. Further analyses are already planned to continue study of the staining characteristics of conidia, and to investigate the role of

fungal decomposition of artefact residues in biasing the suite of materials observed in archaeological analyses.

5. Conclusion

Two main conclusions may be drawn from this focused study. First, fungal activity on artefact surfaces is more than a distraction to archaeological residue analysis, and may in fact contribute additional residues leading to potential misidentifications. This point leaves aside the possibility that fungi may be contributing to the decomposition and removal of authentic organic residues present on artefact surfaces and resultant from past use of the object, although the prospect that this is occurring should not be ruled out. Fungal concentration may therefore provide an indicator of past residue location, even if the residue (structurally at least) has now all but vanished from that location. In any case, analysts should be familiar with the form, habit and optical characteristics of common fungal elements, including hyphae and spore bodies. In particular, starch residue researchers who have not previously considered conidia in their analyses should be aware of possible misidentifications when interpreting both their past and present studies.

The second conclusion is that in situ analysis clearly does not provide an adequate means to identify or interpret residues below a certain size range. Just what that range is depends on the resolving power of the analyst's microscope, as well as other factors such as residue preservation and any obscuring components, including other residues. The present study suggests that particular care must be taken in residue interpretation below approximately 5 μm. In such cases, the best alternative is to remove the residue to a microscope slide, where improved optical conditions and the ability to stain observed objects can help determine composition. Despite this limitation, in situ examination remains a necessary first step in any artefact residue study to ascertain a concrete relationship between the residue and its location on a tool.

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