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Laser scanning confocal microscopy: a potential technique for the study of lithic microwear

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Abstract

The key problem restricting lithic microwear analysis is the lack of quantitative analysis to support qualitative assessments of different wear traces. This paper presents the reflective laser scanning confocal microscope (LSCM) as a new technique for the study of lithic microwear that has the potential to resolve this problem. Firstly, an example is presented that shows how the LSCM compares with conventional reflected light microscopy and scanning electron microscopy. This shows that images, rivalling that of the SEM, can be produced in similar timescales to conventional photomicrography and with no need for casting or sample preparation. The LSCM is also used to measure surface roughness of use-wear produced from working hide (dry, fresh and greasy), woodworking and antler working. This analysis demonstrates clear differences between the different wear polishes and the potential of the LSCM as a quantitative approach in lithic microwear research.

Keywords: Lithics; Use-wear; Lithic Microwear; LSCM; Confocal microscopy; SEM; Quantification

1. Introduction

The study of prehistoric stone tool use remains a key device in the examination and interpretation of archaeological site function and the small scale behavioural operations of hominine and early human societies. Lithic microwear analysis, along with residue analysis, has been fundamental to addressing these questions (Cahen et al., 1979; Donahue et al., 2002; Hardy, 2004; Keeley and Toth, 1981) and is a technique that relies principally on the use of reflected light microscopy at a range of magnifications; from what is termed low-power approaches (up to 100×), which target principally fracture damage (Tringham et al., 1974), to ‘high-power’ approaches (100–400×), which examine striations and changes in surface morphology in addition to fractures (Keeley, 1980). Scanning electron microscopy (SEM) is also used at magnifications principally ranging between 25 and 800 (Debert and Sherriff, 2007; Mansur-Franchomme, 1983). In all types of lithic microwear analysis data and images derived from experiments using stone tools to work, a range of materials are compared to wear traces observed on archaeological tools.

There are four commonly encountered problems inherent in these approaches: first, the formation processes of wear are still not well understood within the field. Previously, the results of a collection of research projects led Grace to summarise ‘it would now seem conclusive that the (wear) process is abrasive’ (c.f. Grace, 1993). Research using chemical analysis has shown that simple abrasion is unlikely to be the only wear process in operation (Christensen et al., 1998; Evans and Donahue, 2005; Šmit et al., 1999). Alongside this, comments and data presented in Anderson et al. (2006) show persistence of the silica dissolution models. Second, processes in the burial environment produce an array of wear traces that often interfere with the interpretability of wear traces resulting from tool use (Levi Sala, 1986). This remains a significant problem but it has been turned to its advantage and used as a means to improve understanding about site formation processes

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(Burroni et al., 2002). Third, wear accrues and appears in different ways on the various types of raw material used for tools (Lerner et al., 2007). This problem has been addressed by analysts through the use of comparative experimental assemblages produced on the different types of raw materials encountered. Fourth, and the focus of this paper, microwear analysis relies on interpretation of primarily subjective observations.

It has always been acknowledged by proponents of the technique that a move towards more quantitative analyses is needed (Keeley, 1980; MacDonald and Sanger, 1968; Semenov, 1964). Numerous blind test studies over the past three decades have had variable 'success' (Bamforth et al., 1990; Gendel and Pirnay, 1982; Grace et al., 1988; Newcomer et al., 1986, 1987; Newcomer and Keeley, 1979; Rots et al., 2006; Shea, 1987; Shea and Klenck, 1993). Whilst there have been several questions as to the adequacy of the experimental design of such studies and how results may therefore have been skewed (Bamforth, 1988; Hurcombe, 1988; Moss, 1983; Newcomer et al., 1988), these tests indicate the potential for improvement and highlight the need for more objective and reliable methods.

This paper details the application and appraisal of a new device for the study of microwear traces on stone tools; the material science laser scanning confocal microscope (LSCM). This type of microscope was developed in 1950s but did not come into use until mid-1980s and, by combination with fluorescent techniques, has become well established in biomedical research (Pawley, 1999). Fluorescent LSCM has been used previously in archaeological research, where dyes have been used to highlight microcracks in stone tool surfaces (Derndarsky and Ocklind, 2001; Shanks et al., 2001). White-light confocal microscopy has been applied recently to study the texture of tooth surfaces (Scott et al., 2006, 2005). In contrast, reflected LSCM has had limited application in metrological (surface science) and tribological (wear) research (e.g. Ebersbach et al., 2006; Pohl and Stella, 2002). Here, we present results from LSCM analysis of the surfaces of experimental stone tools used on a variety of different materials: hide (fresh, greasy, and dry), antler, and wood to illustrate the imaging and surface characterisation abilities of the technique. Measurements of surface texture are presented along with micrographs demonstrating the quality of images produced by the LSCM.

2. Background

2.1. Surface characterisation in lithic microwear analysis

Early attempts at quantification involved simple measurement of striation direction, the crude recording of surface reflectivity and use of interference patterns (Dumont, 1982; Keeley, 1980; MacDonald and Sanger, 1968). Some attention has been placed on image processing as different polished surfaces have different topographies and reflect different levels of light (brightness). Grace et al. (1987) documented the comparison of greyscale histograms and others (Gonzalez-Urquijo and Ibanez-Estevez, 2003; Vila and Gallart, 1993) have attempted to develop this further. It has also been applied to validate observations of microwear traces resulting from butchery (Mitchell, 1997). However, it is clear that several ongoing issues need to be resolved with this approach before it becomes useable. These problems relate to the inability to control for orientation, lighting and material reflectivity and the ubiquitous problem of the post-depositional modification of wear features.

Chemical analysis of tool edges has also been an avenue of exploration that has also displayed some potential (Christensen et al., 1998; Evans and Donahue, 2005; Šmit et al., 1999). These studies have contributed to our understanding of lithic microwear formation processes and have identified the possibility of characterising certain wear features by studying surface chemistry.

Another promising avenue of research is highlighted by Kimball et al.'s (1998, 1995) much overlooked studies using atomic force microscopy to directly measure surface topography. This was applied to study tools used for 1 h each on meat, dry hide, wood, and antler. A series of 1×1 μm areas were selected for the measurement of surface roughness (Ra), five from 'peaks' and five from valleys from within 15×15 μm area scans of tool edges. The worn surfaces on the used tools were quantitatively distinct from each other and unused surfaces. Whilst the types of use-material studied were limited, it can be seen that harder materials smooth the more exposed parts of the tool more than softer materials. The valleys within the polished surfaces on the used tools have the same roughness of unused flint except for the tools used on wood and antler; here, these regions appear to be partially smoothed. Kimball et al. (1995) argue that this supports a model of polish formation, which incorporates silica dissolution and re-deposition. The atomic force microscope produces very good three-dimensional surface micrographs but tool size is restricted to those less than 10 mm in size and scan depth is limited, so application to true assemblages or larger areas of wear is not feasible when archaeological tool size and shapes are to be studied.

Anderson with others has experimented with optical rugosimetry (Anderson et al., 1998) and optical interferometry (Anderson et al., 2006). While these methods were only applied to plant working tools, there is a clear demonstration of potential for applying them to the problem of wear quantification. Optical interferometry has also been applied to study stone working tools (Astruc et al., 2003) and again shows a limited but positive application.

Stemp and Stemp (2001) introduced laser profilometry and experimented by measuring along 4 mm long transects at tool edges, recording surface roughness at 1 μm resolution. They showed that different stone types have different roughness and, by studying tools used to saw shell, pottery and antler, demonstrate the method's potential. Later work (Stemp and Stemp, 2003) compared tools used over different numbers of strokes to saw wood and pottery by measuring surface roughness (Rq), over different length-scales, and fractal dimension. Wood sawing showed no quantifiable difference in surface roughness between unused, little used, and heavily used tools.

Pottery sawing showed quantifiable changes in surface roughness; becoming smoother as the tools were used for longer. Further development of this method is clearly needed although the two-dimensional nature of the measurement and the scale over which it operates may be a significant limitation. Areas of surface polishing are often limited to within 50 μm of the edge in patches less than 1 mm in width.

The method introduced here, laser scanning confocal microscopy, combines the surface metrology features of the atomic force microscope and the laser profilometer along with high magnification and high depth of field imaging capabilities usually associated with scanning electron microscopy.

2.2. Laser scanning confocal microscopy

2.2.1. Principles of the system

Fig. 1 shows a schematic of an LSCM system. The microscope used here was an Olympus LEXT 3100 laser scanning confocal microscope (LSCM) designed for metrology in material science. The basic principle of a confocal microscope is that it forms images by means of reflected light from a discrete focal plane. All light reflected from surfaces away from the focal plane are discarded by way of a pinhole aperture that is optically conjugate with the focal plane. The depth of a focal slice is a function of the diameter of this pinhole and the wavelength of the incident light. The incident light used by the LEXT system is a laser at 408 nm which is scanned across the surface using a microelectromechanical resonant galvano mirror in the laser beam's path. By way of a motorized focusing head the objective lens is displaced through the vertical axis and slices of optically focussed sections are produced. As the position of each point of light recorded is known, the slices can be processed together to create three-dimensional representations of the object or surface data (cloud data). The planar resolution that can be achieved with this system is 0.12 μm and up to 10 nm vertical resolution. Magnification depends on the objective lens used and as standard the highest magnification objective is 100× (0.8 NA), which allows magnifications of 2000× to be achieved.

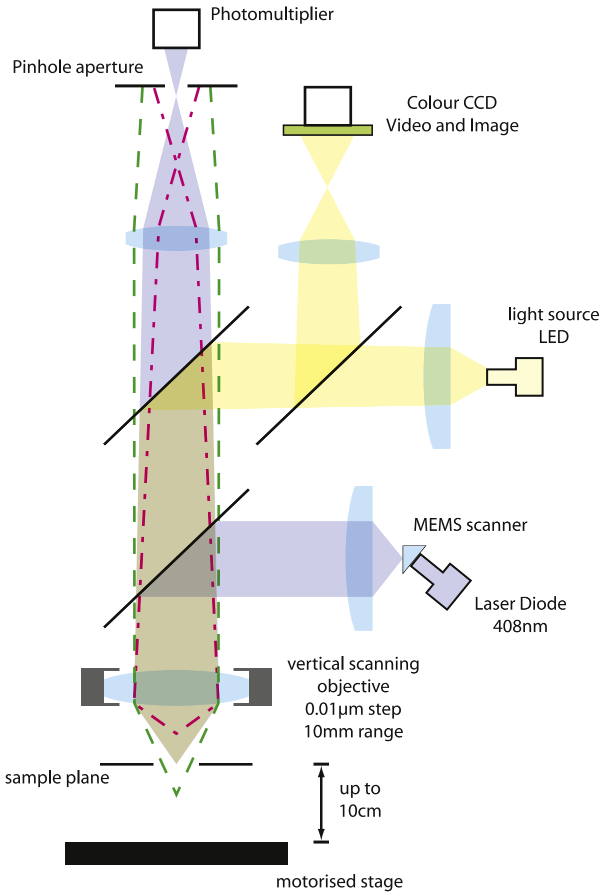

Fig. 1. A schematic illustration showing the basic LSCM system and a simplified light path for laser and conventional light emission and detection. Dashed lines show the effect of the pinhole aperture on laser light reflecting from surfaces above and below the focal plane. Only light that is in focus is detected by the photomultiplier.

The Olympus LEXT system has a dual head, combining the LSCM with a standard light microscope system. This feature allows it to be used with the familiarity of a conventional reflected light microscope either alone or at the same time as LSCM.

2.2.2. Utility to qualitative analysis

As the LEXT can be used as a reflected light microscope it is initially used to scan across the surfaces and around the edges of tools at 200×, as is typical for traditional lithic microwear analysis. Switching on the confocal laser requires no movement of the sample and after scanning through the *z*-axis and setting the objective at a focal depth below the surface, the instrument produces a three-dimensional surface model by continually scanning upward until the entire surface has been scanned. The amount of time this takes is dependent on the amount of depth to the surface of interest and the adjustable vertical resolution. The maximum resolution is dependent on the numerical aperture of the objective lens used; lower resolution scans can be produced, but at a cost to the quality of the topographical data and resulting image.

Figs. 2–4 show different images of the same tool used to whittle antler tine for 40 min. Fig. 2 was produced using a modified metallurgical microscope (Olympus BH2) through a 20× objective lens (0.40NA). The theoretical focal depth achieved by this system is 6.09 μm. The image shows the worn edge and illustrates typical 'antler polish'. High points on the surface are smoothed and the general texture undulates perpendicular to the edge, parallel to the working direction. Fig. 3 is a typical example of an image produced by the LSCM. A 100× objective was used with a numerical aperture of 0.8. Vertical stepping was set at 20 nm, and 205 vertical slices were taken. The LEXT scans at two slices per second; this image took 3 min 25 s to produce. Scanning to the area of interest was quick as one could manually manipulate the tool and/or stage, so the area of wear was under the objective. Fig. 4 shows a similar area of wear on the tool but was acquired using a scanning electron microscope. This is clearly a high quality image but the production of this image required gold coating and fixing to a stub, a process not suitable for most archaeological material. A further limitation of SEM is the restrictive size of this chamber. Casting can negate this

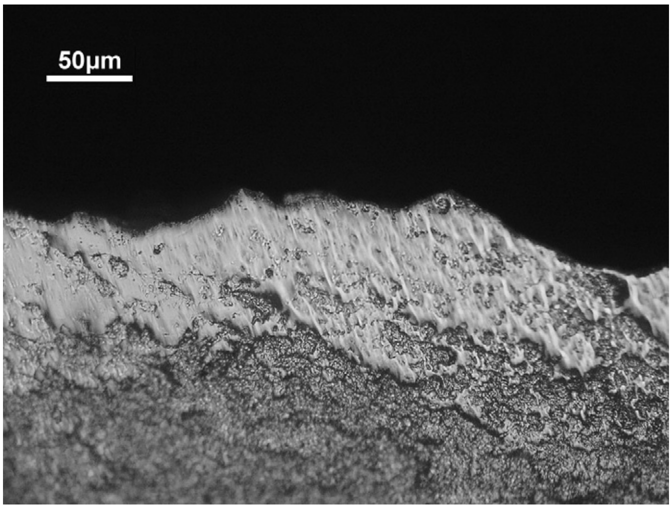

Fig. 2. A photomicrograph of the dorsal edge of a tool used to whittle smooth soaked antler for 40 min (taken using a 20× objective lens).

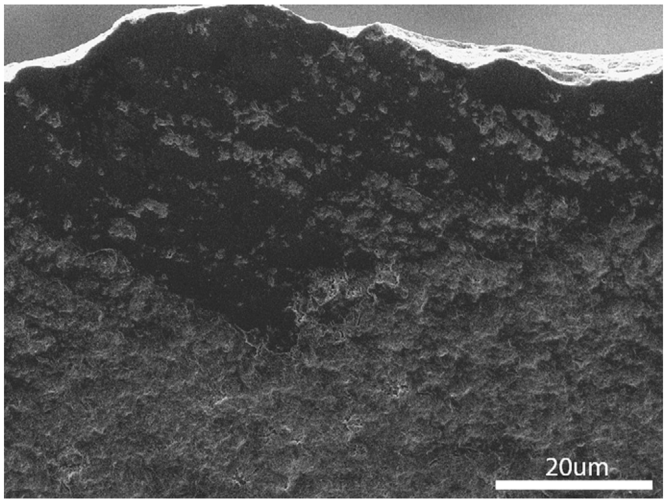

Fig. 4. SEM image of a similar area to that shown in Fig. 3. The working edge can be seen at the top of the image and the worn surface appears dark.

problem by replicating areas of tool edges (which would have to be identified in advance using reflected microscopy). Samples are placed under vacuum in the SEM chamber (which takes 1 min to open and 3 min to pump down). The stage is moved so the area of interest is in view and this process can take some time as the lowest magnification is 100×. One is unable to see the tool directly to position it under the electron beam; this has to be done by viewing a coarse live image. Therefore, it can take over 20 min before the sample is positioned. From here image acquisition takes around 2 min depending on the settings used. Fig. 4 took a total of 40 min excluding coating to produce and this was after having identified the area of interest using standard microscopy in advance.

A clear advantage is presented here for the LSCM over reflected microscopy and SEM. With the use of software (such as CombineZM) high depth of field images can be produced from reflective light photomicrographs taken at different focal planes through a sample. Standard stages can also be fitted with piezoelectric motors to automate the vertical sampling process and this is the principle behind devices such as the Alicona Infinite focus system used at the Natural History Museum, London to study cut-marks on bones (Bello et al., 2007). These systems, however, do not afford the same accuracy of the LSCM as they use normal light and the slices are computed using next-neighbour pixel computations to determine parts of images which are on the focal plane. The SEM also produces images of extensive focal depth but the images produced appear flat and take significant amounts of time and effort to produce. The LSCM on the other hand can produce high focal depth colour images by combining colour reflected light photomicrographs with LSCM slice data. These images are not simple photomicrographs but contain topographic surface data, which can be studied in numerous ways.

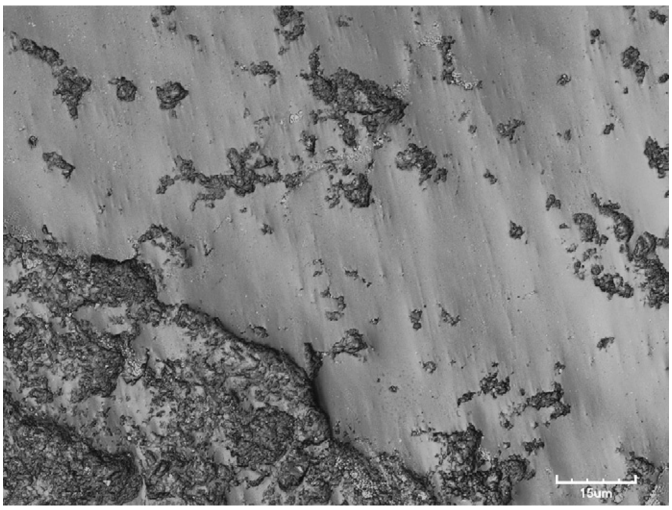

Fig. 3. An LSCM image of the dorsal edge of the same tool shown in Fig. 2. The working edge is just out of the field of view to the top of the image (scanned using a 100× objective lens).

3. Quantitative application

3.1. Method

To allow for a basic appraisal of the surface mapping capabilities of this equipment, a series of simple stone tools was produced and analysed. Flakes were knapped from a nodule of English chalk flint and each was randomly assigned to one of the five use groups or an unused control group. Two flakes were assigned to each of these six groups. Tools were used to work on antler, wood, and three different conditions of hide (fresh, dry and greasy). Tools selected were all used for 40 min, and were all used in similar motions. Antler and wood were whittled and the hides were scraped unidirectionally. These motions differ in many subtle ways, but, for the purposes of this experiment they were considered similar enough to have little effect on the results. The duration and consistency of use-direction ensured that wear traces would likely be well developed while the number of variables likely to produce differences between wear characteristics was reduced. The inclusion of an unused control group along with randomisation in tool assignment allows the assessment of natural surface roughness variation within the experimental set.

Tools (including the control group) were cleaned following use with detergent (Decon) and a soft bristled brush before 10 min baths in ammonium hydroxide (10%) and hydrochloric acid (10%). These baths were followed by a 10-min bath in deionised water.

Ventral surfaces of tools were initially observed at 200×, as it is standard for microwear analysis, and areas where wear had developed were scanned by switching to confocal laser scanning at high magnification. This was done with the working edge of the tools just outside the top edge of the field of view as this avoided complications with scanning blank areas (past the edge).

Analysis of the surface cloud data for comparison was carried out using the operating software provided with the microscope (LEXT software, Olympus) and the surface texture measurement statistic used was root mean square roughness (Rq) of sampled areas on the tool surface. Rq is calculated as the square root of average squared height values over the surface (Eq. (1)). N_s corresponds to the number of data points studied and $Z_{s,i}$ corresponds to the height data for any given point. The Rq statistic is one of several standard summary measurements for characterising surface roughness in engineering (ASME, 2002).

$$\mathrm{Rq} = \sqrt{\frac{1}{N_s}\sum_{i=1}^{N_s} Z_{s,i}^2} \tag{1}$$

Two samples of roughness data were taken from the cloud data produced by the LSCM imaging process. In the first instance, measurements were taken from $3.8 \times 3.8\ \mu\text{m}^2$ (1024 data points) areas (referred to later as small scale data). In the second instance, measurements were taken using $10.1 \times 10.1\ \mu\text{m}^2$ (7225 data points) areas (referred to later as large scale data). Taking data from areas rather than linear transects has the advantage that they are less biased by orientation of wear features that result from the working direction. Ten areas were measured from smoothed ‘peak’ surfaces at worn edges of each tool. Using two independent scales of measurement allows for better differentiation of the wear types because of the characteristics of polished surfaces produced by the various use-materials. Specifically, surface microtopography which can be appreciated as dominant features and texture, which includes stria and pitting, occurs at a much smaller scale. The two scales used here reflect the authors’ choice on what was apparently appropriate; further experimentation may find that other scales are far better suited to this application.

3.2. Results

Data from the analysis of Rq from sampled areas within polished surface areas are illustrated in Fig. 5. LSCM micrographs of areas of wear from the experimental tools are shown in Fig. 6. There are some overlaps between some of the Rq data derived from the different polished surfaces, however, when both sets of data are used in combination all wear types can be distinguished.

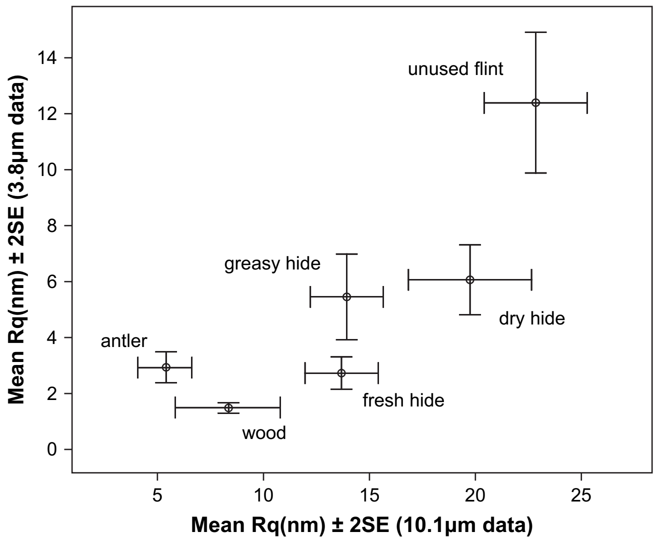

Fig. 5. Roughness data (Rq) derived from small (3.8 μm) and large (10.1 μm) sample areas of wear polish. Five different wear polishes were assessed along with unused flint.

Visually the enhanced depth of field and high magnification allow identifying and easily differentiating the wear types presented in the experimental set. This alone is a substantial result as one of the main issues surrounding microwear analysis is the inability to convey the features of the wear in a photomicrograph. The unmodified flint surface appears rough, dull and unpolished. The surface is clearly not flat, reflecting the micro crystalline nature of the material. Unsurprisingly, both sets of roughness data suggest the natural unused surface of flint shows the greatest degree of roughness and also the greatest variability. The dry hide polished surface is visually distinct from an unused surface; the worn surface is still rough but shows signs of modification including the smoothing of high spots. The roughness measurements on the large scale indicate that the dry hide polish has a similar roughness to the unmodified surface which is slightly smoother but more variable. This fits with expectations as the roughness in troughs is unmodified and variation across the surface is vast. Also fitting with expectations is that the use of the smaller scale roughness measurement allows the two surfaces to be differentiated ($t = 3.879$, $p<0.05$). These measurements are focused on peak regions where the surface has been modified. Similarly in the small scale, wear from greasy hide and dry hide measure very comparably in terms of roughness and this reflects their visual appearance. The greasy hide wear has a greater mean Rq (60.7 nm) than the dry hide (54.5 nm). These overlap with each other and cannot be differentiated statistically, however, the larger scale analysis can be used to differentiate these types of wear ($t = 3.438$, $p<0.05$) allowing for quantitative discrimination. It is, therefore, variation over larger areas that differentiates these two kinds of hide wear. The fresh hide wear is both qualitatively and quantitatively distinct to wear produced by dry hide and greasy hide. The fresh hide wear is more pronounced

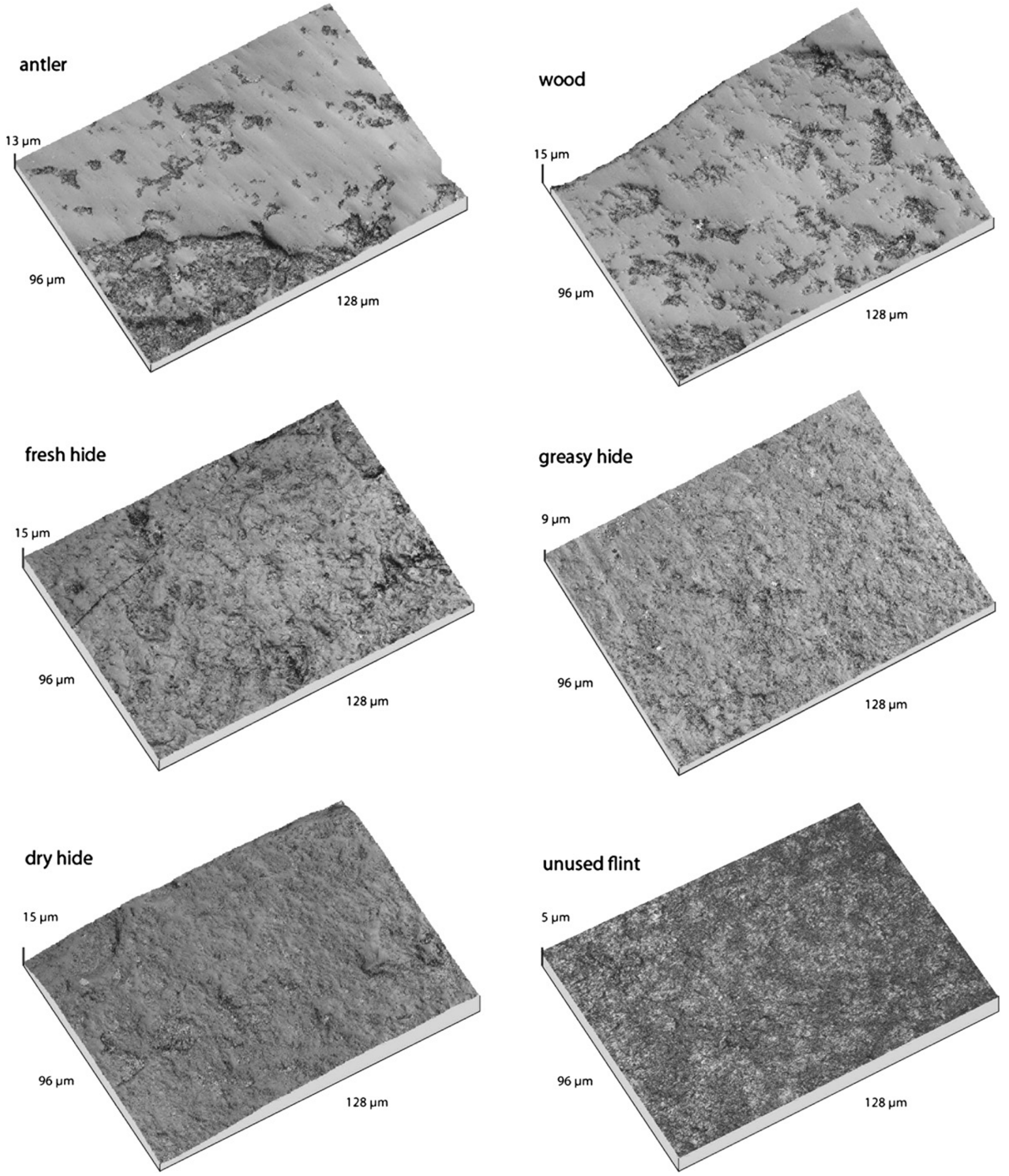

Fig. 6. LSCM micrographs showing examples of the five different wear types and the unused flint. These were produced using the laser scanning confocal microscope with a 100× objective lens.

and more extensive; almost the entire surface has been modified and the general appearance is a series of domed features with a much smoother transition between smoothed peaks and lower surfaces. The larger scale data wear from fresh hide working overlaps only with that of greasy hide working, however, by using the complimentary data set produced through the small scale analysis these two wear types can be easily differentiated ($t = 5.112$, $p < 0.0001$). The antler and wood polished surfaces again appear entirely different from any of the other surfaces. The polished surfaces produced on tools that worked these two materials are generally much smoother than those produced by any of the hide kinds. They also appear qualitatively distinct from each other: wear from whittling antler has an undulating character that is parallel to the working direction and also presents distinct striations. The wood polish on the other hand lacks striations, appears more flat and more reticulated than the antler polish. The large scale roughness data serve to segregate the wood and antler wear types as being clearly smoother than the other wear types studied. Through the use of the small scale data these two types can be distinguished ($t = 4.999$, $p < 0.05$). The analysis of large and small scale roughness analysis mirror the visual characteristics one associates with the different wear types detailed in this study. The polished surface from working wood and antler appears on the large scale smoother than others. These two wear types are often hard to distinguish and it is often the presence of small scale features, stria or the microscale undulating nature of the antler wear polish that helps one to make this distinction. This pattern is reflected directly in the data presented here thus providing a quantitative measurement of a qualitative distinction. The same can be said for the different hide wear types.

4. Discussion

The comparison between the different microscope techniques along with the presentation of several micrographs of different lithic microwear polishes shows the quality of the laser scanning confocal microscope for the production of high quality micrographs which convey the features that are being presented by the microwear analyst. This resolves a long standing problem for the presentation of lithic microwear data, as traditional photomicrographs with their low depth of field often cannot convey the full suite of features the microwear analyst is trying to show. This technique also presents a significant advantage in the amount of time it takes to produce these high quality images; in contrast to the long and destructive or damaging methods involved in the use of the scanning electron microscope.

As presented, the laser scanning confocal microscope does not just produce high quality images, but digitised high resolution three-dimensional surfaces that lend themselves well to metrological analysis. At this point it would be naive to suggest that the results of small scale area roughness analysis from the experimental tools presented here is anything more than preliminary. The presentation of experimental tools, used for equal lengths of time, is in contrast with the reality of the archaeological record. This experiment also only included one raw material type for tools and incorporated only a limited range of worked materials. It is expected roughness measurements on used material would differ depending on the raw material involved and it has been shown that roughness varies across natural surfaces between some North American cherts (Lerner et al., 2007). This is a problem already acknowledged with traditional approaches and is easily avoided with the use of comparable experimental data sets regardless of these shortcomings, which are part and parcel of preliminary studies; these experiments have highlighted the potential that surface features, in particular the smoothed areas within polished regions, can be characterised using roughness measurements; each different kind of wear having textures that, when measured using roughness analysis at two scales, can be quantitatively differentiated. The trend within these data broadly agrees with data acquired using an atomic force microscope (Kimball et al., 1998, 1995) with the harder materials greatly reducing the roughness of the flint surface.

There are several different measurements that one can make to summarise the topography of a surface and a very common method (Rq) has been presented here. One line of research which is currently under examination is the use of area-scale analyses. These are the three-dimensional counterparts to those presented in preliminary research by Stemp and Stemp (2003) and are the same as those currently being employed in research attempting to quantify dental microwear traces. This more complex approach has proven useful for differentiating primate dental wear produced from different dietary habits (Scott et al., 2006, 2005) and this hints that application to lithic microwear could be successful.

5. Conclusion

The LSCM is a new tool for the imaging and modelling of artefact surfaces. This microscope combines the ease of use and speed of a metallurgical microscope, traditionally used by lithic microwear analysts, with the high focal depth, magnification and resolution of the SEM. The quality of images produced by the LSCM rival that of the SEM, it requires no casting or coating, handles artefacts of all sizes, and is a far quicker method.

The LSCM produces topographical data and preliminary results presented here show that wear, or surface polishes, produced by working different materials can be characterised using simple summary roughness measurements. These results show that the technique has great potential for the advancement of lithic microwear analysis where quantification of wear features might be carried out within a timeframe not much greater than that normally used by a lithic analyst to produce a photomicrograph of a worn surface.

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