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New taphonomic advances in 3D digital microscopy: A morphological characterisation of trampling marks

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ABSTRACT

The concept of equifinality has become one of the greatest difficulties in the field of taphonomy However, new advances in technology have diminished the margins of error in the interpretation of archaeological sites. The use of multivariate statistics and the most recent advances in microscopic analysis of Bone Surface Modifications (BSMs) have enable a less subjective interpretation of site formation processes. Nevertheless, this broader range of methodological approaches also presents some problems. The capacity of laser scanners in processing inconspicuous and superficial cortical alterations, such as trampling marks, has proven to be problematic. This study presents a new advance towards resolving this problem through the use of the HIROX KH-8700 Digital Microscope, whereby detailed digital three-dimensional (3D) reconstructions are able to pick up such minute BSMs. Through the statistical comparison of the David Laser scanner and the HIROX KH-8700 Digital Microscope, this paper contributes to our understanding of said equipment, followed by a significant advance in the characterisation of superficial BSMs. The combination of advanced microscopy and the application of geometric morphometrics highlights a morphological differentiation between two different types of trampling marks, hereby named scratch and graze trampling marks.

1. Introduction

In recent years taphonomy has become a vital analytical tool for studying paleoanthropological sites. Taphonomy has provided a strong empirical background on which the interpretation of a site can be constructed, contributing to some of the most significant archaeological debates, such as the 'Hunter-Scavenger' debate (Binford, 1981; Blumenschine, 1986, 1995; Domínguez-Rodrigo, 1997; Domínguez-Rodrigo & Barba, 2006, 2007; Blumenschine et al., 2007; Domínguez-Rodrigo et al., 2007).

To many analysts, however, difficulties lie in two major concepts; analogy and uniformitarianism. Taphonomy relies heavily on the concept of uniformitarianism and actualism (Hutton, 1794; Playfair, 1802; Lyell et al., 1830; Whewell, 1847) to construct a hypothesis. Uniformitarianism is, in turn, highly conditioned by multiple factors relying on analogy for theoretical support (Bunge, 1981). In order to combine these concepts in the construction of theoretical frames of reference, experimentation plays a key role in order to provide a middle range theory that aids in our understanding of a site (Merton, 1967; Binford, 1967, 1968; 1981; Gifford-Gonzalez, 1991). Nevertheless, the incorrect use of analogy can condition our reconstruction of the past. Subjectivity plays a major role in this, strongly conditioned by the equifinality present in the taphonomic, paleoanthropological and archaeological record (Domínguez-Rodrigo et al., 2017). Equifinality is a conditioning factor that causes the misinterpretation of different taphonomic traces that can be product of the same agent, or *vice versa*. In recent years, the conflictive equifinality present between trampling and cut marks has become increasingly apparent, causing the misclassification of Bone Surface Modifications (BSMs) (Sahle et al., 2017), as well as the erroneous interpretation of sites (McPherron et al., 2010; Thompson et al., 2015).

Since their initial introduction in scientific literature, trampling

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marks have come to be defined as superficial, irregular and "flat bottomed" linear traces, produced by sedimentary abrasion (Brain, 1967; Behrensmeyer, 1978; Andrews and Cook, 1985). In the 1980's, issues with these definitions, however, led authors in disagreement as to the criteria used in defining naturally produced and anthropic linear striations (Fiorillo, 1984; Oliver, 1984; Andrews and Cook, 1985; Behrensmeyer et al., 1986). From this lack of supporting criteria, many analysts were led to highlight the possible equifinality present in the taphonomic register (Olsen and Shipman, 1988). While more in depth analysis of these traces were able to highlight the importance of different distinguishing variables (Domínguez-Rodrigo et al., 2009), analysts have still found issues using these qualitative variables, providing demand for more empirically objective data (Domínguez-Rodrigo et al., 2017).

Microscopy applied to taphonomy has played a key role in overcoming these issues. In the 1980's a great deal of studies revolved around the use of Scanning Electron Microscopes (SEM) (Potts and Shipman, 1981; Shipman, 1981; Shipman and Rose, 1983; Shipman et al., 1984a, b; Andrews and Cook, 1985; Behrensmeyer et al., 1986; Cook, 1986; Olsen, 1988; Olsen and Shipman, 1988), which took advantage of the high resolution and visual perception of texture. Combined with the introduction of statistical multivariate analyses in archaeology (Domínguez-Rodrigo et al., 2009; de Juana et al., 2010; Moclán et al., 2018), in recent years SEMs have proven to be a considerable tool in empirically processing the taphonomic register (Pineda et al., 2014). Come the 21st century, confocal microscopy (Archer and Braun, 2013; Pante et al., 2017; Otárolla-Castillo et al., 2017; Gümrükçu and Pante, 2018), high resolution optical microscopes (Bello and Soligo, 2008; Bello et al., 2009, 2016, 2013; Bello, 2011), as well as micro-photogrammetric three-dimensional (3D) reconstructions (Maté-González et al., 2015, 2016; Aramendi et al., 2017; Arriaza et al., 2017; Maté-González et al., 2017a, Yravedra et al., 2017a, b, c) have also been key tools in the development of analytical methods.

Further noteworthy developments are based on the analysis of two-dimensional (2D) derived information from 3D models, such as that of a cut mark's cross-section, via different biometric (Bello and Soligo, 2008; Bello et al., 2009, 2013; Bello, 2011) and geometric morphometric (Maté-González et al., 2015, 2016, 2017a) approaches. Multiple studies have also applied these concepts to BSMs produced by animals (Arriaza et al., 2017; Yravedra et al., 2017a). With the introduction of laser scanners, these approaches were then developed via 3D landmark models to further analyse cut marks (Courtenay et al., 2017) as well as other BSMs such as anthropic percussion pits (Yravedra et al., 2018) and carnivore tooth pits (Aramendi et al., 2017).

With such a broad range of techniques, methodological studies, comparisons and debates have been fundamental in contextualising and determining the value of these different methodological approaches. Work by Maté-González et al. (2017b, c), for example, have statistically analysed the reliability of different approaches, arguing the validity of the method. While some doubt has been cast over 2D landmark models (Otárolla-Castillo et al., 2017), further statistical data have strongly supported the reliability of these methods in taphonomic analysis (Courtenay et al., 2018).

The outcome of these methodological debates highlights the potential of laser scanners, such as the DAVID Structured-Light SLS-2 Scanner, as a considerably strong tool for future investigation in this field. The problem with such equipment lies in the lack of resolution when capturing superficial and inconspicuous taphonomic traces (Courtenay et al., 2017; Maté-González et al., 2017b, c), such as trampling marks – a problem that has become increasingly apparent in the taphonomic study of some archaeological and paleoanthropological sites (Domínguez-Rodrigo et al., 2011; Domínguez-Rodrigo and Alcalá, 2016; and further research pending publication). The objectives of this paper are to provide a new complementary approach that is capable of reconstructing and analysing such inconspicuous traces that are susceptible to equifinality.

The HIROX is a powerful tool for visualisation purposes, providing high-resolution photographic and observational data which may aid in studies regarding use wear patterns (Stemp et al., 2015; Fernández-Marchena et al., 2016; Martín-Viveros, 2016; Marciani et al., 2018; Martin-Viveros et al., 2018; Martin-Viveros and Ollé, 2018; Wierer et al., 2018), residues (Revedin et al., 2010; Ronchitelli et al., 2015), dental micro-wear analysis (Oxilia et al., 2015), taphonomy (Crezzini et al., 2015; Malassé et al., 2016; Pérez et al., 2017; Rufa et al., 2017; Duches et al., 2018; Moclán et al., 2018; Pineda et al., Under Revision) as well as other sub-disciplines within the archaeological field. Developments in taphonomic morphological analysis using high-tech digital reconstruction methods have made a significant change from the initial approximations to cut mark morphology proposed by Walker and Long (1977). While the HIROX KH-8700 3D Digital Microscope has been used before in similar studies (Blasco et al., 2016; Malassé et al., 2016; Maté-González et al., 2017b, c; Duches et al., 2018), these papers have been limited to either biometric, 2D or even mostly qualitative results. Further issues with this microscope can be seen in its' dependency on a light source to provide accurate 3D reconstructions. In this study we confront these topics, demonstrating the capacity of the 3D digital microscopes, such as the HIROX KH-8700, in processing superficial marks and further highlighting the first morphological characterisation of trampling marks.

2. Materials and methods

In order to compare the quality and efficiency of the HIROX KH-8700 3D Digital Microscope in comparison to the DAVID Structured-Light SLS-2 Scanner, an experiment was devised to see how the positioning of light can affect the morphology of the digitally reconstructed cut mark under observation. Both the 2D 7-landmark cross section model described by Maté-González et al. (2015) and the 3D 13-landmark study as described by Courtenay et al. (2017) were tested in the preliminary phase of this study. Once the most accurate lighting position had been established, the second phase of our experimentation confronted the morphological classification of trampling marks using the HIROX KH-8700 3D Digital Microscope.

2.1. HIROX KH-8700 3D digital microscope

The microscope used in this study is the HIROX KH-8700 3D Digital Microscope (Fig. 1), located at the *Institute of Human Paleoecology and Social Evolution* (IPHES) of Tarragona. This microscope is equipped with a MXG-5000REZ triple objective revolving lens (Fig. 1b), with a magnification range from 35x to 5000x plus a field of view from 8 mm to 0.06 mm at an operable distance of 3.5 mm–10.0 mm (Table 1). The microscope is accompanied by a high intensity LED light source that can be positioned around the object (Fig. 1c and d). This light source provides a temperature of 5700 k, closely portraying daylight colour and producing the highest quality real-time images with no warm up time needed. The HIROX microscope provides the possibility of combining both ring and coaxial light (Fig. 1c), while presenting the possibility of using polarised filters. The built in compact CCD camera projects these images onto a high definition LCD 21.5" monitor with high intensity pixel reproduction as well as the capacity to display up to 16.77 million colours with a contrast ratio of 1000:1 and brightness of 300 cd/m^2. The combination of state of the art hardware and the Genex Engine graphics processor ensures maximum quality when carrying out any type of microscopic analysis. The HIROX is capable of quickly producing 3D digital reconstructions using a combination of quick auto focus and depth synthesis functions. The additional use of the HIROX's tiling function is used to create a mosaic and complete digital reconstruction of the subject under analysis.

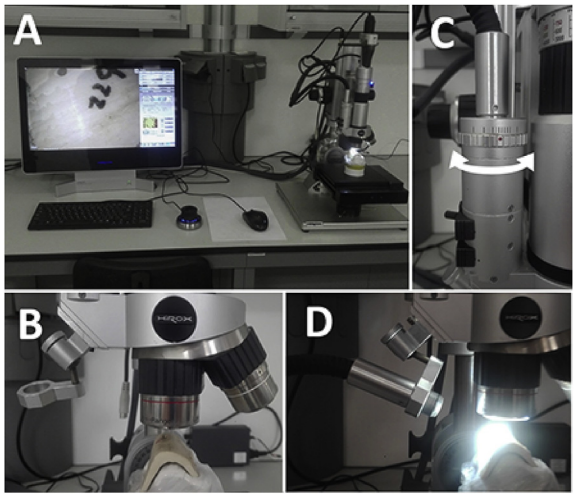

Fig. 1. (A) The HIROX KH-8700 3D Digital Microscope located at the IPHES lab (Tarragona, Spain). (B) The MXG-5000REZ triple objective revolving lens. (C) Lighting position from above, providing the option of combining ring (turning the wheel to the right) and coaxial (turning the wheel to the left) lighting conditions. (D) Lighting position from the side, movable using the HIROX's adjustable light support.

Table 1
Details regarding each of the lenses of the MXG-5000REZ triple objective revolving lens.

Lens Magnification	HFV (Horizontal Field Of View (FOV))	Depth of field	Working distance
35x - 250x	8.76–1.22 mm	0.72–0.072 mm	10 mm
140x - 1000x	2.18–0.31 mm	0.09–0.007 mm	10 mm
700x - 5000x	0.44–0.06 mm	0.01–0.0007 mm	3.5 mm

2.2. DAVID Structured-Light SLS-2 scanner

The DAVID Structured-Light SLS-2 Scanner, located at the TIDOP research group in the University of Salamanca, is a powerful tool in providing real reproductions of external bone topography in less than 1 min. The equipment consists of a DAVID USB CMOS Monochrome camera, an ACER K132 projector and a calibration marker board. This equipment is able to produce a density of up to 1.2 million points while providing a high-resolution 3D model that can be later imported into different graphics software such as Avizo (Visualisation Sciences Group, USA). Use of this equipment in 3D reconstructions of taphonomic traces have already proven to be successful and can be consulted in Courtenay et al. (2017, 2018), Maté-González et al. (2017c) and Yravedra et al. (2018b). Tests regarding superficial taphonomic traces, however, have proven to be unsuccessful (Courtenay et al., 2017).

2.3. Testing the efficiency of the HIROX digital microscope

A total of 5 lighting positions were initially considered (Fig. 1c and d); from the left of the mark, from the right of the mark, a fixed position above the mark using a ring light, a fixed position above the mark using a combination of ring and coaxial light (mix) and finally the use of two light sources from either side of the mark. The use of two lights intended to reduce the amount of shadow cast over the cut mark in an attempt to provide sufficient illumination of the entire incision. The sheer strength of the HIROX Digital Microscope's own light source, however, might present some problems. Multiple types of secondary lights were considered and tested, however, none of these solutions were able to match the intensity of the LED light provided by this microscope. Because of this, the 5th lighting position was discarded.

In order to reduce variability produced by other factors, once the bone was positioned prior to analysis, neither the bone nor the platform could be moved or altered, ensuring that the only changing variable within our analysis was the positioning of the light. Cut mark cross-section profiles were captured using the mid-range lens at a 600× magnification. Between 110 and 130 photos were taken for each profile, combined and constructed into a 3D model using the HIROX's quick auto focus and depth synthesis functions.

For the digital reconstruction of the entire mark, the necessary magnification, as seen by the analyst, was selected in order to capture every feature of the mark, depending on the incisions' depth and size. Each cut mark was digitally reconstructed using the Mosaic 3D Tiling function within the HIROX. The number of photos taken per tile for each mark was used as suggested by the microscope's own internal software, however for higher quality reconstructions a minimum of at least 30 photos is recommended. The total number of tiles was entirely dependent on the microscope and cannot be altered manually by the analyst.

A random selection of cut marks were reconstructed using all 4 different lighting positions. These cut marks, 4 in total, came from previously studied material by Courtenay et al. (2017) and were reproduced using simple quartzite flakes on a *suid* femur diaphysis. These were later processed following the methodological approaches described by both Maté-González et al. (2015) and Courtenay et al. (2017) in order to see how the lighting position affected both the morphology of cut mark section profiles as well as the entire morphology of each mark. Through this, both methods could be assessed, observing the variation in quality of the 3D digital reconstructions of each incision. Additionally, the reliability of the HIROX and its use for geometric morphometrics could be tested.

Notes on the amount of photos taken per tile, the number of tiles used to reconstruct the full incision, the time taken to reconstruct the mark and the time taken for the analyst to process the mark were taken. The number of pixels and the distance between the lowermost and uppermost positioning of the lens were also noted.

2.4. Collecting of landmark data

Cut mark profiles were exported into the free tpsDig2 (v.2.1.7) software where the allocation of 7 homologous landmarks were carried out following the geometric morphometric model described by Maté-González et al. (2015, Fig. 2a).

In order to collect the location of the 13 landmarks according to Courtenay et al. (2017)'s 3D model (Fig. 2b), the position of each point was recorded through a series of measurements. Each measuring tool is provided by the HIROX's own internal software, thus providing accurate results with little to no human error. The self-calibration select sensor within the HIROX's system automatically configures and applies the appropriate lens settings according to the lens and magnification being used, eliminating the need for further calibration. Measurements can be taken using various different tools and simple mouse operations via the monitor's display. The accuracy of these measurements are as small as 1μ.

Landmark coordinate data was first collected using the HIROX's 'XY-Width' function, measuring and plotting across a 2D graph the location of each point (Fig. 3a), followed by a measurement in depth using the 'point height' function (Fig. 3b). In doing so, the landmark's position was established along the *z*-axis of a 3D plot. These landmark coordinates were recorded in a database and later imported into R for statistical analysis.

2.5. Statistics

All statistics were carried out in the free software R (www-rproject.org, Core-Team, 2018).

In order to assess the efficiency of the KH-8700 3D Digital

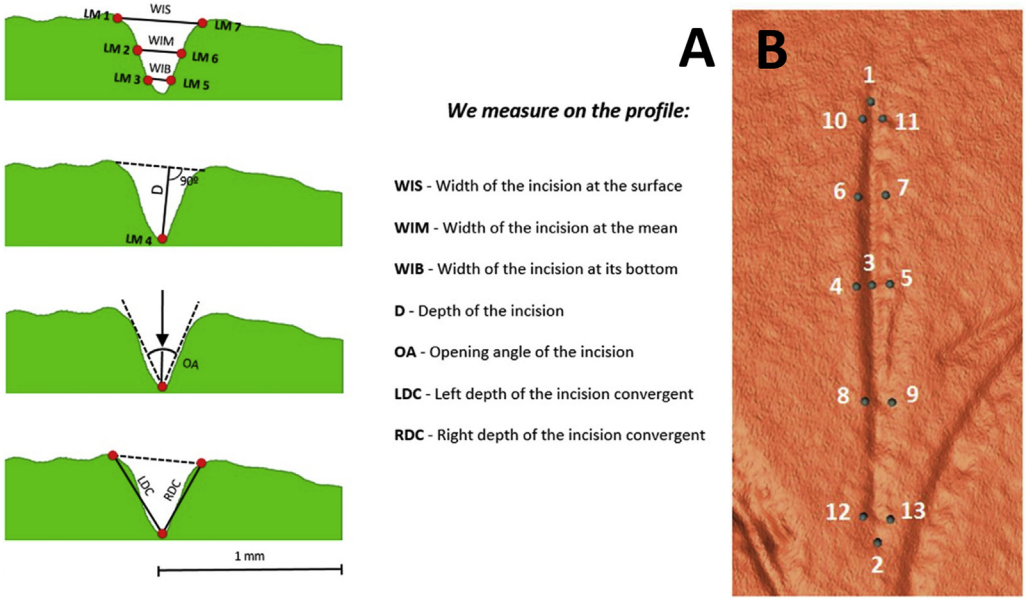

Fig. 2. (A) Location of the seven landmarks used in the 2D morphometric analysis of cut mark cross sections (Maté-González et al., 2015) and the measurements taken for each cut mark profile (Bello and Soligo, 2008). (B) Location of the 13 landmarks used to capture the shape of entire incisions, as described by Courtenay et al. (2017).

Microscope, various polynomial multiple regressions were performed to assess the weight and importance of each variable in the time taken to create a full 3D digital reconstruction of each mark. A step regression was also used to assess which variables were conditioning these results and which were not.

Comparisons of the different lighting conditions on cut mark profiles were firstly performed measuring the thickness, depth and angles of each incision (Fig. 2) following the quantitative methodological approach proposed by Bello and Soligo (2008). This biometric data was obtained using the free software tpsDig2 (v.2.1.7) and later imported into R. This was done in order to test the differences presented in the 3D reconstructions of the cut mark morphology using the different techniques. These measurements were firstly tested for normality according to the Shapiro-Wilk normality test before being subjected to further multivariate analysis (MANOVA). Following this, a jack-knifed linear discriminant analysis was used to determine whether significant differences were present through the calculation of a confusion matrix. For these tests, both the MASS (Venables and Ripley, 2002) and the RVAideMemoire (Hervé, 2018) R packages were used.

Considering previous observations (Maté-González et al., 2017b; Courtenay et al., 2018) on the variables suggested by Bello and Soligo (2008) had a major impact on the variance among groups, these tests were also performed including or excluding the opening angle of the incision (OA).

Geometric morphometric analyses of the cut mark profiles were based on 7 homologous landmarks, as specified above. Files containing

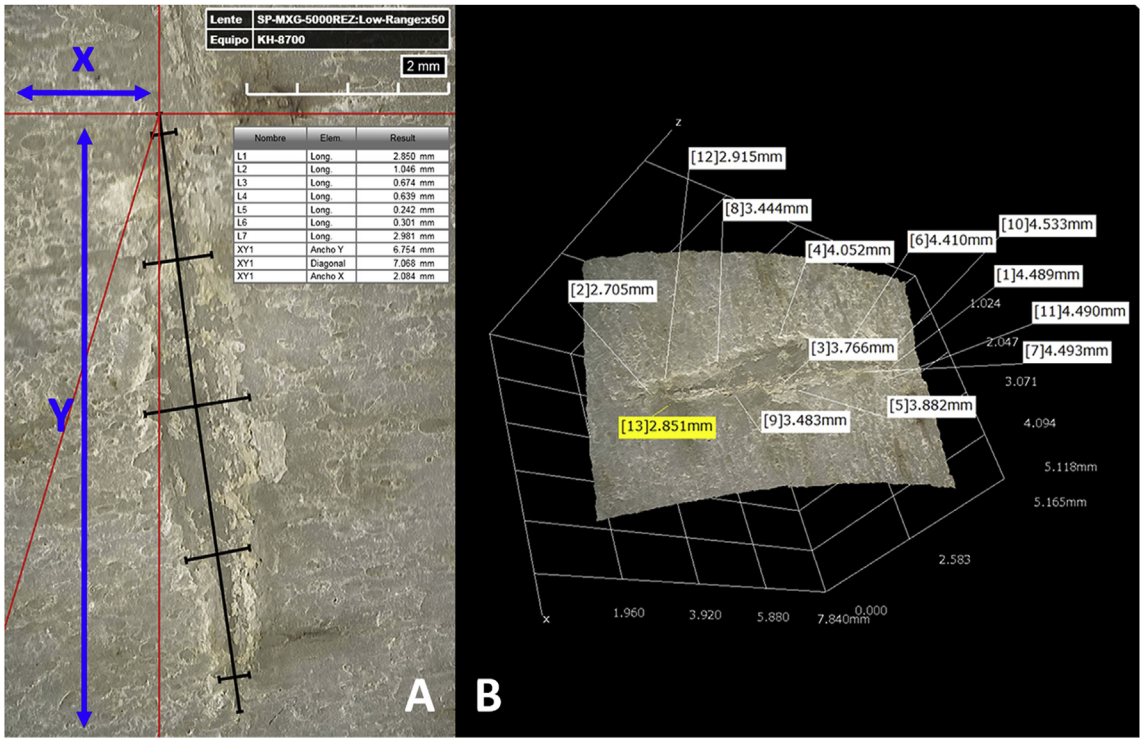

Fig. 3. Description of the measurements taken in order to plot the 3D coordinates of each of the 13 landmarks across the (A) x, y and (B) z axes.

Table 2
–Table presenting the different variables taken when carrying out a 3 Dimensional Reconstruction using the HIROX KH-8700 Digital Microscope and the time taken to process each cut mark under the different lighting conditions.

Cut Mark Number	Lighting Position	Length of Incision (mm)	Width of Incision (mm)	Magnification	Focus Range (Lens Height) (mm)		Number of Pixels		Number of Photos Taken per Tile	Number of Tiles	Tiling Time (Min)	Time Taken to Place Landmarks (Min)	Total Processing Time (Min)
					Sup.	Inf.	X	Y					
1	Ring/ Coaxial	8.494	0.935	200	815.95	−1583.05	2411	9844	25	42	20:47	06:22	27:09
	Coaxial	8.494	0.935	200	815.95	−1583.05	2411	9844	25	42	20:50	04:59	25:49
	Left	8.494	0.935	200	815.94	−1583.05	2411	9844	25	39	19:22	04:59	24:21
	Right	8.494	0.935	200	815.95	−1583.05	2411	9844	25	39	19:10	03:17	22:27
2	Ring/ Coaxial	11.552	1.455	100	3763.6	−470.7	2496	7175	30	27	13:31	07:52	21:23
	Coaxial	11.552	1.455	100	3763.6	−470.7	2496	7175	30	30	14:55	06:15	21:10
	Left	11.552	1.455	100	3763.6	−470.7	2496	7175	30	30	14:40	06:05	20:45
	Right	11.552	1.455	100	3763.6	−470.7	2496	7175	34	23	12:33	07:17	19:50
3	Ring/ Coaxial	6.521	0.682	200	1020.9	−253.3	1731	7479	25	22	08:07	05:20	13:27
	Coaxial	6.521	0.682	200	1020.9	−253.3	1731	7479	25	23	05:32	07:18	12:50
	Left	6.521	0.682	200	1020.9	−253.3	1731	7479	25	23	08:39	05:09	13:12
	Right	6.521	0.682	200	1020.9	−253.3	1731	7479	25	30	08:04	05:30	13:34
4	Ring/ Coaxial	9.000	1.197	150	1531.2	−841.8	2807	7960	25	34	13:24	07:12	20:36
	Coaxial	9.000	1.197	150	1531.2	−841.8	2807	7960	25	34	13:50	06:54	20:44
	Left	9.000	1.197	150	1531.2	−841.8	2807	7960	25	34	14:33	07:48	21:21
	Right	9.000	1.197	150	1531.2	−841.8	2807	7960	25	31	13:23	05:24	18:47

the landmark data were edited and imported into R where a full Procrustes fit and an orthogonal tangent projection (Dryden and Mardia, 1998) were used to normalise the data for further multivariate statistical analyses. This technique, commonly referred to as Generalized Procrustes Analysis (GPA) is used to standardize the form information through the application of superimposition procedures including translation, rotation and scaling. Any remaining differences are exposed through patterns of variation and covariation that can be assessed through several statistical tests (Slice, 2001; Rohlf, 1999).

A principal component analysis (PCA) in shape space and form space on the Procrustes superimposed landmarks was performed using the geomorph package in R (Adams et al., 2017). Changes in both shape and form were visualized with the aid of transformation grids (Bookstein, 1989). PC scores were then extracted and used to examine the variance between samples by means of a MANOVA test and a jack-knifed LDA. A canonical variance analysis (CVA) using 1000 permutations tests was used to determine further morphometric differentiations between the techniques.

3D landmarks were statistically analysed in a similar manner to the 2D landmarks model. This 3D landmark configuration consists of 13 identical points on the exterior and interior surface of each mark, following Courtenay et al. (2017)'s methodological approach. Geometric morphometric analysis began, as before, with a GPA based on the natural logarithm of centroid size. PCAs were later produced in order to assess patterns of variation, with the help of transformation grids and warpings, thus revealing any differences that may be produced through the change in lighting position. PC scores were later extracted and used to assess the similarities and differences between methods based on MANOVA tests, LDA jack-knifed confusion matrices and finally CVA tests, as described before.

Once the best lighting position was established, all subsequent digital reconstructions followed this exact method.

2.6. Trampling experiments

Trampling experiments were carried out using sieved sediments from levels 2 and 3 from the archaeological site of Peña de Estebanvela (Segovia, Spain) (Yravedra, 2005, pp. 249–253). This site has been dated using calibrated AMS ^{14}C to around 10–14 Ka (Jordá Pardo and Cacho, 2013), consequently being attributed to various phases of the Magdalenian (Cacho et al., 2016; Yravedra et al., 2018). Sediment samples obtained from this site consist of a mixture of compact and loose quartz sandy sediments that were used to bury the remains of an *Ovis* skeleton. These sandy sediments are composed of quartz granules, presenting an angular morphology with an average granular size of 250 μm. In total 14 bones of both axial and appendicular skeletal elements were buried. These consisted of mandibulae (Number of Elements (NE) = 2), femorae (NE = 2), tibiae (NE = 2), and radiae (NE = 2). All the bones were dry and meatless when buried while the sediments used to bury the bones were also dry. Once the bones were buried, the area was trampled on by a single individual for a total of 5 min before being uncovered, cleaned and studied.

A total of 56 trampling marks were produced, however, not all presented clear morphologies where all 13 landmarks could be easily located and processed. For the purpose of this study, and under the premise of obtaining a statistically significant sample for morphological characterisation, 30 trampling marks were carefully selected and processed.

Due to their superficial nature, these trampling marks present a practically non-existent cross section to study. As a product of this, the 3D 13-landmark model proposed by Courtenay et al. (2017) was solely used to process each trampling mark. The landmark data was then statistically processed in the free software R, as previously described.

3. Results

3.1. Comparing the David SLS-2 versus the HIROX KH-8700

3.1.1. Time

The HIROX was able to recreate each incision in a time range of 5:32 to 20:50 min with an average time of 13:30 min (Table 2). Compared with the processing time required by the DAVID SLS-2 (less than a minute) (Courtenay et al., 2017; Maté-González et al., 2017c), the HIROX is substantially slower (Table 3).

The use and comparison of multiple linear, logistical and polynomial regressions have shown that the most important conditioning variables in the time taken to reconstruct each mark are, in order of importance; cut mark length, magnification, the number of tiles and finally the cut mark's width (AIC = −1.62). The use of stepwise regressions and consequent comparison of Akaike Information Criterion

Table 3
Comparisons between different reconstruction techniques. Updated from Maté-González et al. (2017c).

Technique	System	Measuring Procedure	Classification	Portability	Full 3D Reconstruction Time (Min)	Operable Distance	Resolution	Cost (Eur)
Microscope	KH-8700	3D Digital Microscope	Active Sensor	Low	Aprox. 13.5	1–10 mm	0.15–0.01 μm	< 100,000
Laser Scanner	David SLS-2	Structured Light	Active Sensor	Medium	> 1	0.15–5.00 m	0.02 mm	3000
Photogrammetry	Reflex + Macro Objective	Micro-photogrammetry	Passive Sensor	High	Aprox. 25	10–50 cm	0.02 mm	1000

(AIC) values confirm that these 4 variables have a significant ($p < 0.05$) effect on the processing time to reconstruct the cut marks. Since the number of tiles is entirely dependent on the magnification, the length and width of each incision, a clear correlation between these variables and the reconstruction conditions of the taphonomic traces can be observed.

In contrast, the time required for the landmarking process does not vary substantially between the two methods (HIROX vs DAVID SLS-2). Therefore, the time taken to landmark the traces has not been accounted for in this study.

3.1.2. Lighting positions

Measurements taken from cut mark profile reconstructions present limited variability. Results obtained through MANOVA ($p \approx 1$) and LDA tests support this statement (Table S1). This proves that the reconstructions generated using different lighting positions with the HIROX are practically indistinguishable from those obtained using the laser scanner. The exclusion the OA measurement provides a slightly higher level of classification (15%) in the LDA (Table S1), however MANOVA results still highlight the similarities of both reconstruction methods ($p \approx 0.9$).

2D geometric morphometric analysis produced a total of 10 PC scores (Fig. 4a), and a 2D plot where most groups overlap with a wide dispersion of individuals across tangent space regardless of the reconstruction technique used. In general, these tests do not show a clear differentiation between each cut mark, making it hard to distinguish between the different profiles. MANOVA results present insignificant differences between the lighting positions (Table 4) yet are also inconclusive in distinguishing between the different cut marks regardless of the technique used for the reconstruction (Table 5). Considering distance in shape space across the PCA graph, lighting position from above (both mix and ring) present the highest level of similarities with the laser scanner. The same can be said considering MANOVA results.

CVA results represent a large dispersion of points (Fig. 4b), where the differentiation between groups appears to be slightly clearer; probably because CVA tests tend to overestimate differences. LDA classification/misclassification tables present a relatively high percentage of correctly classified cut marks (Table S4), while the reconstruction techniques in general are considered highly similar to those of the scanner (Table S5).

The PCA on the 3D reconstructions is defined by a total of 19 PC scores. The scatter-plot containing the first two PCs shows that cut marks can be clearly differentiated regardless of the lighting position (Fig. 5a). These results are strongly supported by MANOVA, which present high similarities amongst all 5 reconstruction techniques ($p > 0.4$) and are still able to distinguish between the cut marks in most cases ($p < 0.05$). The only exceptions are found in two of the cut marks that can be considered practically identical in morphology. CVA results present 3CV scores (CV1 and CV2 = 94% cumulative variance) that clearly differentiate between all cut marks (Fig. 5b), tightly grouping all 4 reconstruction techniques. LDA jack-knifed classification/misclassification results highlight that all 4 cut marks are completely distinguishable, indicating that despite the use of the HIROX or the David SLS-2, the 13-landmark 3D model is capable of distinguishing perfectly between cut marks (Table S4). LDA results comparing reconstruction techniques present high percentages of misclassification in all cases, highlighting a lack of differentiation between techniques and thus implying the similarities in reconstruction quality for all cases (Table S5).

2D variations in shape (Fig. 4) are mostly defined by differences in cut mark depth (PC1), and in the angle of the mark and irregularities on the incision wall (PC2). 3D transformation grids along PC1 indicate changes in the curve and depth of the cut mark, whereas PC2 underlines a slight variation in the positioning of landmark 3 (indicating a slight change in angle) and in the width of the cut mark. Considering results presented by transformation grids, changes in directionality tend to cast a shadow over the profile, thus affecting the angle of the mark. While this can be problematic for 2D reconstructions, the effects on 3D landmark models is minute and reflected through one single landmark. These results are strongly supported by both significant numerical and graphic results, concluding that the most accurate reconstruction technique employed the use of a lighting position from above. The mixture of coaxial and ring lighting were considered optimum for higher magnifications, such as those required for the study of superficial taphonomic traces. Thus, this technique was preferred for the rest of this study.

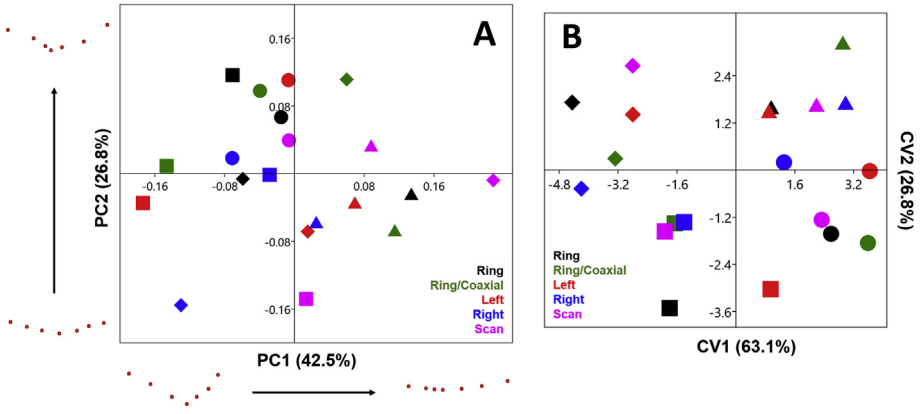

Fig. 4. Two scatter plots presenting (A) PCA and (B) CVA graphs comparing the 5 different reconstruction techniques of different cut mark cross sections according to the 2D 7-landmark model as described by Maté-González et al. (2015). Each cut mark is represented by a different symbol while variance in shape is presented for the extremities of both PC scores along their respected axis.

Table 4
MANOVA p values comparing the different reconstruction techniques, both using the 2D landmark model of cut mark profiles (in non-bold typeface) and using the 13 landmark 3D model of the entire incision (in bold typeface).

	Ring	Mix	Left	Right	Scan
Ring		0.98689 **0.94135**	0.86246 **0.77183**	0.70995 **0.90411**	0.85949 **0.40608**
Mix	0.98689 **0.94135**		0.72821 **0.98715**	0.62274 **0.99989**	0.83721 **0.61758**
Left	0.86246 **0.77183**	0.72821 **0.98715**		0.9868 **0.99242**	0.57261 **0.74994**
Right	0.70995 **0.90411**	0.62274 **0.99989**	0.9868 **0.99242**		0.47208 **0.64374**
Scan	0.85949 **0.40608**	0.83721 **0.61758**	0.57261 **0.74994**	0.47208 **0.64374**	

3.2. Digital reconstruction protocol

Through the statistical results presented here and experience using the HIROX, the ideal conditions for 3D digital reconstruction are as follows:

After having calibrated and centralised the main table, the piece should be placed on a sturdy support, specimen mount or directly on the table with the area of interest positioned as flat as possible. This ensures that in the process of reconstruction the piece is unable to move, thus preventing blurry photos. The cut mark should be positioned either vertically or longitudinally as straight as possible, therefore reducing the number of tiles needed in the digital reconstruction. Additionally, the reconstruction should require the least amount of focal depth possible. In this experiment, the focal range oscillated between 1 and 3 mm (Table 2), however, superficial marks that require high levels of magnification ideally require the lowest focal depth possible. This particular variable, however, is highly dependent on the natural topography of the piece, its' positioning on the table and can also vary due to the magnification used. The analyst is therefore advised to ensure that the piece is as flat as possible, while consulting the depth of focus of the lens used, thus adjusting accordingly (Table 2).

In order to capture the entire morphology of the object under study, sufficient magnification is essential in order to clearly observe, photograph and thus capture the base, walls and surrounding cortical of each incision. For 2D reconstruction of cross sections, 600× magnification (FOV = 505 µm, Table 1) using the mid-range lens is ideal. The section profile produced should be captured between 30% and 50% of the entire mark's length, as initially described by Maté-González et al. (2015). In this case the mosaic function is not necessary. For 3D reconstructions of entire marks, under most circumstances the ideal magnification can be considered between 100x (FOV = 1516 µm, Table 1) and 200x (FOV = 3032 µm, Table 1) magnification. This can be performed using either the low-range or the mid-range lens. The entire mark can be captured using the mosaic function, however the analyst has no control over the number of tiles necessary to fully reconstruct the mark. Depending on the magnification, number of tiles and the dimensions of the object under study, the time required to create the 3D reconstruction will vary.

The number of photographs taken per tile is strongly recommended to be as few as 30, thus ensuring the best perception of depth for each mark. The optimum lighting condition is with the light source placed directly above the sample, using a mixture of coaxial and ring light according to the magnification used. No polarising filters are advised at magnifications lower than 1000x.

3.3. Analysis of trampling

The HIROX KH-8700 performed efficiently enough to reconstruct each trampling mark (n = 30) and to collect the required 3D landmark data. During the analysis of trampling marks, a series of patterns were observed, possibly highlighting the presence of two different groups of trampling marks in the sample. Both groups can be qualitatively characterised by their width and the quantity of internal striae either on the floor or along the wall of the mark. The first group consists of thinner traces with very few or no internal striations (Fig. 6a), whereas the marks included in the second group are wider and present a high number of internal striations (Fig. 6b). The qualitative identification of these two groups was further tested through the use of a K-Means clustering model (Table 6). This test was carried out on the PC scores obtained through PCA which, in turn, confirmed the presence of two

Table 5
MANOVA p values comparing the differentiation of different cut marks using varied reconstruction techniques, both using the 2D landmark model of cut mark profiles (in non-bold typeface) and using the 13 landmark 3D model of the entire incision (in bold typeface).

	Cut Mark 1	Cut Mark 2	Cut Mark 3	Cut Mark 4
Cut Mark 1		0.64485 **0.007125**	0.15693 **0.009103**	0.14058 **0.001474**
Cut Mark 2	0.64485 **0.0071251**		0.15665 **0.078724**	0.15431 **0.002631**
Cut Mark 3	0.15693 **0.0091033**	0.15665 **0.078724**		0.40692 **0.00753**
Cut Mark 4	0.14058 **0.0014735**	0.15431 **0.002631**	0.40692 **0.00753**	

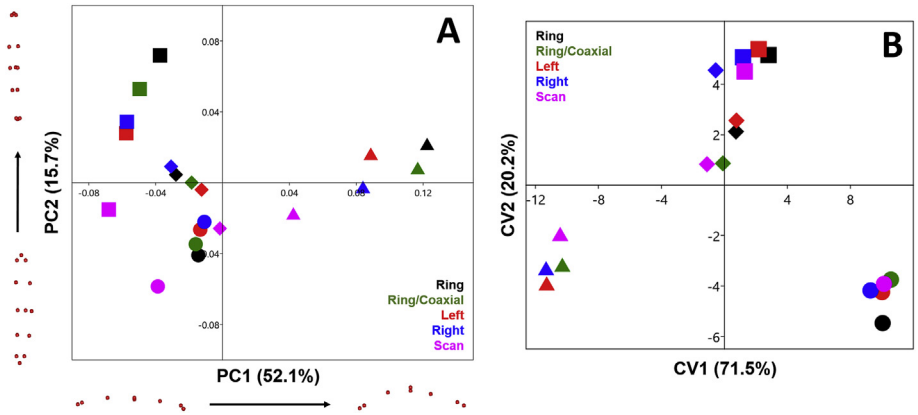

Fig. 5. Two scatter plots presenting (A) PCA and (B) CVA graphs comparing the 5 different reconstruction techniques of different cut marks according to the 3D 13-landmark model as described by Courtenay et al. (2017). Each cut mark is represented by a different symbol while variance in shape is presented for the extremities of both PC scores along their respected axis.

Fig. 6. Examples of the two different types trampling marks: (A) scratches and (B) grazes.

different groups of trampling marks based on their morphology.

The results for the K-Means clustering model were then applied to future morphological analysis to further classify these two different trampling groups. In total the clustering model separated 21 of the 30 trampling marks into one group and the remaining 9 were separated into the second group. The first group, consisting of the much finer trampling marks, were named '*scratches*' (Fig. 6a), whereas the wider marks with abundant internal striae were named '*grazes*' (Fig. 6b). The term *scratch* was assigned based on the etymological definition of the word as a "score or mark [of] the surface of (something) with a sharp pointed object" (The Oxford English Dictionary, 2018). The term *graze* was selected as a result of its definition as a "scrape or break [of] the surface of [something]" or a "touch or scrape lightly in passing" (The Oxford English Dictionary, 2018), thus deemed appropriate, considering the connotations associated with the word *graze*.

PCA results and transformation grids (Fig. 7) confirm the original hypothesis that these marks can be morphologically characterised and separated through their width. Additionally, the variance in shape of the mark's trajectory is reflected across PC2 as an important variable to define trampling marks. This confirms Domínguez-Rodrigo et al. (2009)'s original characterisation of trampling marks through their either sinuous or curvy groove trajectory.

CVA results clearly separate the two trampling groups based on their shape through significant Mahalanobis ($D = 19.7491$, $p < 0.0001$) and Procrustes ($D = 0.0570$, $p < 0.0001$) distances. This separation is clearly supported by MANOVA results ($p = 9.12e\text{-}06$) as well as jack-knifed LDA classification/misclassification tables with a correct classification range between 93.33% of the samples (Table 6).

Differences in form space do not vary much from results obtained in shape space (Fig. 8), except for a slight overlapping between groups. In this case, transformation grids accentuate a change in the width of the trampling marks across both PC1 and PC2. A slight difference can also be seen in the distance between landmarks 10/11 and 12/13 with the corresponding end of each groove, indicating that the length of *scratches* is longer than those of *grazes*. MANOVA results still identify significant differences between groups ($p = 1.0543e\text{-}05$) while jack-knifed LDA classification tables correctly classify 76.67% of the sample (Table 6).

Table 6
LDA Classification/Misclassification table presenting the possibility of correctly associating each mark to the respected K-Means cluster group.

	Shape			Form		
K-Means	LDA	Graze	Scratch	LDA	Graze	Scratch
Scratch	Scratch	0%	100%	Scratch	1%	99%
Scratch	Scratch	31%	69%	Graze	66%	34%
Scratch	Scratch	2%	98%	Scratch	27%	73%
Graze	Graze	83%	17%	Scratch	83%	17%
Scratch	Scratch	0%	100%	Graze	9%	91%
Scratch	Scratch	0%	100%	Scratch	2%	98%
Scratch	Scratch	0%	100%	Scratch	2%	98%
Graze	Graze	100%	0%	Scratch	20%	80%
Graze	Scratch	47%	53%	Graze	86%	14%
Scratch	Scratch	5%	95%	Scratch	1%	99%
Graze	Graze	97%	3%	Scratch	14%	86%
Scratch	Scratch	22%	78%	Graze	100%	0%
Scratch	Scratch	0%	100%	Scratch	1%	99%
Scratch	Scratch	0%	100%	Scratch	1%	99%
Graze	Graze	100%	0%	Graze	100%	0%
Scratch	Scratch	0%	100%	Scratch	2%	98%
Graze	Graze	69%	31%	Graze	78%	22%
Scratch	Scratch	10%	90%	Scratch	7%	93%
Scratch	Scratch	2%	98%	Scratch	5%	95%
Scratch	Scratch	1%	99%	Scratch	5%	95%
Scratch	Scratch	1%	99%	Scratch	1%	99%
Scratch	Scratch	0%	100%	Scratch	6%	94%
Graze	Graze	99%	1%	Graze	94%	6%
Graze	Graze	98%	2%	Graze	87%	13%
Graze	Scratch	1%	99%	Scratch	29%	71%
Scratch	Scratch	0%	100%	Scratch	1%	99%
Scratch	Scratch	1%	99%	Scratch	10%	90%
Scratch	Scratch	0%	100%	Scratch	1%	99%
Scratch	Scratch	5%	95%	Scratch	11%	89%
Scratch	Scratch	0%	100%	Scratch	0%	100%

4. Discussion

Microscopy in archaeology is an important tool when considering the amount of information available through minute BSMs that remain invisible to the naked eye. This study presents the potential that the 3D digital microscopes have to offer, providing a solution to the problems in resolution presented by laser scanners such as the DAVID SLS-2 when studying superficial taphonomic traces. Through this we have been able

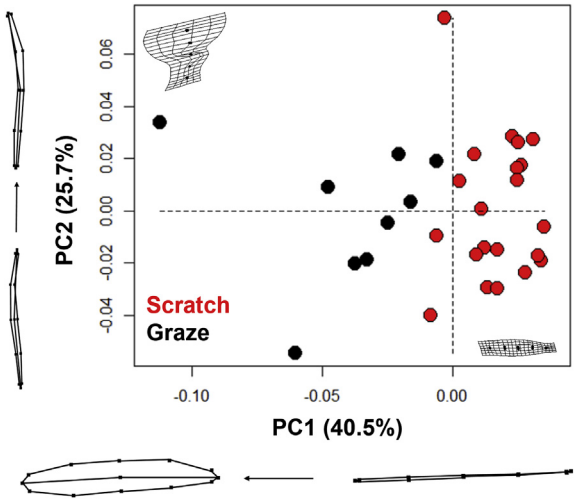

Fig. 7. Scatter plot of the PCA results comparing the morphology of both scratches and grazes in shape space. Variance in shape is presented for the extremities of both PC scores along their respected axis.

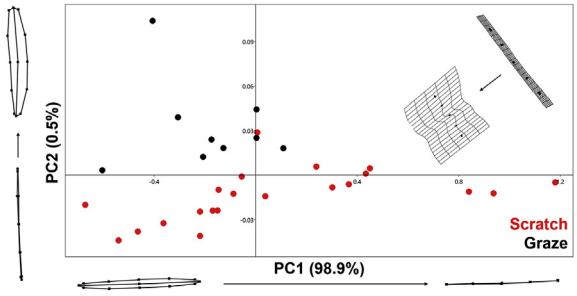

Fig. 8. Scatter plot of the PCA results comparing the morphology of both scratches and grazes in form space. Variance in form is presented for the extremities of both PC scores along their respected axis.

to characterise trampling marks through their morphology, revealing two new categories of naturally produced marks, hereby named *scratch* and *graze* trampling marks.

The impact the two new trampling marks may have on taphonomy is still to be tested. Traditionally, it would be assumed that the nature of trampling marks are conditioned by sedimentological and geological features (Schiffer and Shipman, 1987; Olsen and Shipman, 1988; Fisher, 1995; Marín-Monfort et al., 2013; Reynard, 2014). This may be the explanation behind the occurrence of two different types of morphologically comparable trampling marks. Depending on the abrasive nature of the sediment, different frequencies of *scratch* and *graze* marks could be produced. In our experiment, a mixture of different fine sands have been used, however, gravel like sediments and layers of large quartz granules are more likely to cause more damage to bone cortical surfaces (Marín-Monfort et al., 2013; Reynard, 2014). Additionally, other factors such as bone density, cortical hardness and time exposed to trampling processes may also need to be considered (Öhman et al., 2012; Walden et al., 2017). These variables, however, require a wide range of experimental studies in order to empirically answer these questions.

Methodologically, while the HIROX KH-8700 3D Digital Microscope provides some limitations (Maté-González et al., 2017a, b), considering the price and analytical speed of this equipment, our study highlights a series of advantages. Information revealed through statistics as well as the comparison of transformation grids and warpings highlight that regardless of the lighting position, the HIROX does not provide too much variation in digital reconstructions. While 3D reconstructions appear to be the least affected by the difference in light source, 2D analysis of cut mark cross sections are more susceptible to change when positioning the HIROX's LED light at either side of the mark. This might be explained by the shadows cast across the section of each mark. Statistically, the changes in reconstruction techniques are mostly insignificant. However, in order to present a more accurate representation of mark morphology, the lighting position must remain homogenous throughout any study. With regards to an ideal lighting condition we recommend using a lighting position from above, using a combination of ring and coaxial light in order to properly illuminate the entire mark.

Furthermore, as can be seen through these results, the HIROX digital microscope is much more powerful at analysing more inconspicuous marks than the DAVID SLS-2. A clear disadvantage of this approach, however, can be seen in the amount of processing time required. An approximately 2 s procedure with the laser scanner can be considered much more efficient when analysing large samples, however, when regarding problems related to equifinality, time should not be favoured over resolution. A further advantage of the DAVID SLS-2 over the HIROX is the cost (Table 3). Nevertheless, the visual advantages presented by the HIROX microscope are much greater, especially when considering the perception of texture and the versatility for further qualitative analysis (Fig. 9).

In addition to this, the HIROX's own mosaic tiling functions provide a much more efficient means of capturing larger surface areas, as opposed to microscopes such as the SEM (Vergès and Morales, 2014), or even photogrammetry (Maté-González et al., 2015; González-Aguilera et al., 2016), that rely on longer additional steps in order to fully reconstruct the area of study. The recent development of SEM 3D Images (Eulitz and Reiss, 2015; Tafti et al., 2015), however, present a powerful advance for microscopy. The use of microscopes such as the HIROX to extract linear mark cross sections from archaeological remains has increased over the past years, generating qualitative conclusions drawn from arguably objective means of obtaining this information. Work by Blasco et al. (2016) combine qualitative criteria (Domínguez-Rodrigo et al., 2009) with observations of a homogeneous \/shaped groove to argue their classification of taphonomic traces as cut marks. Rodríguez-Hidalgo et al. (2018) further classify the \/shaped cross section by calculating mean shapes (via landmark data) along the length of the

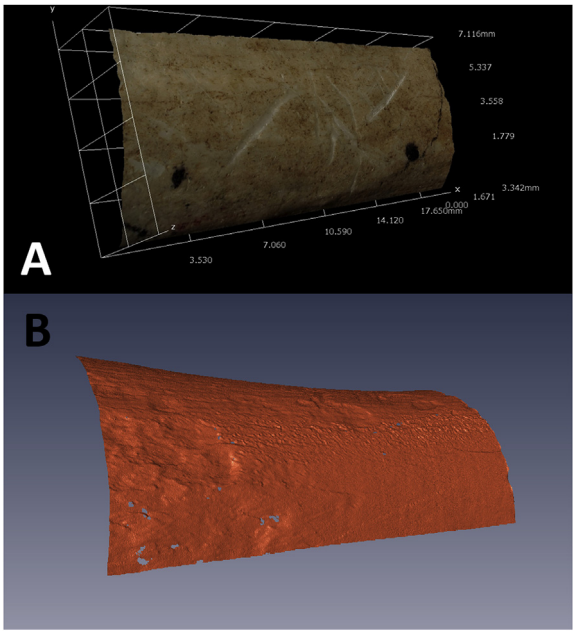

Fig. 9. A comparison of different 3D digital reconstructions using (A) the HIROX KH-8700 3D Digital Microscope and (B) the David Structured-Light SLS-2 Scanner.

groove. This particular approach is strongly supported by quantitative data, yet with the support of an experimental comparative sample has the potential of revealing very important information about the taphonomic register of this site. Similar studies use simpler measurements and calculations to withdraw interesting results (Moretti et al., 2015; Fuentes-Sánchez et al., 2017; Rufa et al., 2017; Duches et al., 2018; Stinnesbeck et al., 2018) that bring taphonomic analysis into the 21st century.

Other notable publications in the field of taphonomy have presented different means of classifying and distinguishing taphonomic traces using quantitative methods, relying on 3D digital reconstructions using confocal profilometers (Pante et al., 2017; Gümrükçu and Pante, 2018; Orlikoff et al., 2018). While data regarding trampling and fluvial altered surfaces provide promising results, comparisons between carnivore tooth marks and cut marks do not respond to any real archaeological questions. Furthermore, distinguishing between these traces can be considered unnecessary when little equifinality is present between the two (Domínguez-Rodrigo and Baquedano, 2018), and are easily macroscopically distinguishable. Observations made by said authors, with the aid of an increased sample size, could begin to respond to important taphonomic issues that have been highlighted in recent years (Pineda et al., 2014; Under Revision).

While microscopy provides a solid methodological groundwork on which to build upon, caution is still advised when relying solely on this data, especially in cases where high-powered technology is used to withdraw rather questionable results (Malassé et al., 2016) based on almost purely qualitative data and inadequate experimental comparative samples. In recent years, some works have diminished the subjectivity present in archaeological and taphonomical studies (Arriaza and Domínguez-Rodrigo, 2016; Egeland et al., 2018; Domínguez and Baquedano, 2018), based on autocritical research presented by Domínguez-Rodrigo et al. (2017, 2018). The level of subjectivity in our work is arguably dependent on an analyst's experience and knowledge, however the use of advanced technology have begun to present ways of overcoming analyst subjectivity. Advances in Artificial Intelligence (AI) and Machine Learning (ML) methods applied to archaeology have begun to break new grounds (Domínguez-Rodrigo, 2018), presenting powerful statistical means of reanalysing data and confronting taphonomic issues (Arriaza and Domínguez-Rodrigo, 2016; Domínguez and Baquedano, 2018), additionally drawing to light interpretation problems that require further attention (Egeland et al., 2018).

5. Conclusions

The 21st century has seen the arrival of technological advances that have revolutionised science. In archaeology, the impact of this paradigm shift can be observed in the application of new technologies to the study of archaeological sites. This paper presents the HIROX KH-8700 3D Digital Microscope as an important tool that is capable of analysing superficial taphonomic traces alongside a new characterisation of naturally produced trampling marks through their morphologic features. The methodological approach described here can be combined to decipher the taphonomic register present within a site from a much more objective perspective. Additionally, applied statistics and the use of landmark data in geometric morphometric analyses are decreasing the degree of equifinality present in taphonomic assemblages.

While the majority of efforts have been focused on the analysis of cut marks, and more recently on carnivore BSMs, few efforts have gone into the geometric morphometric characterisation of naturally produced marks such as trampling. While this study begins to confront these concepts, an ideal progression from this would be to combine these models with our current understanding of cut marks, thus beginning to eliminate a great cause for confusion that many taphonomists face. Differentiation between these two taphonomic traces via geometric morphometrics would be a considerable advance in taphonomic research. Additionally, further investigation into the nature behind *scratch* and *graze* marks may also provide valuable information into effects of sedimentology on trampling marks.

Nevertheless, the impact microscopy has on archaeology is not exclusively limited to taphonomy. Our newfound understanding of this equipment can be applied to other fields of research as well, including traceology, anthropology, and many more disciplines in prehistoric research.

These results present an interesting starting point, especially when employing a combined usage of the 3D digital microscopy with other equipment such as structured light scanners. Considering the results produced in previous research comparing these pieces of equipment, it would be interesting to further include other techniques such as confocal profilometers in this field, to fully understand the advantages of each method. Confocal profilometers are yet to be fully compared with microscopes and, taphonomically, have only been used to test macroscopically distinguishable marks where little equifinality is present. It would be of great interest to investigate the degree of resolution that such equipment can provide, especially if applied to superficial taphonomic traces such as trampling marks.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quaint.2018.12.019.

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