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# Comparative analysis of confocal microscopy objective magnifications on dental microwear texture Analysis. Implications for dietary reconstruction in caprines

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## ABSTRACT

Dental microwear texture analysis (DMTA) is a proxy used for characterising vertebrate palaeodiets in archaeological and palaeontological studies through the acquisition of 3D micro-texture height maps using confocal microscopy. Unlike previous techniques, DMTA is a quantitative method and enables reproducibility and repeatability. However, researchers working on DMTA have used different measurement equipment, softwares and microscopy objectives (magnification and numerical aperture). In the case of the objectives, DMTA widely uses 100x objective magnification, although successful applications at 20x and 50x have been reported. This study investigates the discriminative abilities at different magnifications, and discuss the advantages of each one. For this purpose, a set of 94 modern domestic and wild goats were analysed at 10x/0.30, 20x/0.45, 50x/0.80, and 100x/0.80. Our results demonstrate that all magnifications can discern between the seven groups of modern goats. However, 10x and 20x magnifications showed lower discrimination compared to 50x and 100x. Discriminant analyses revealed progressively refined distinctions at higher magnifications, with 100x yielding the best results. These findings support prior research suggesting the efficacy of 100x magnification in DMTA as the most optimal choice. However, it should be emphasised that high magnification and numerical aperture can present some limitations. Depending on the characteristics of the material studied and the microscope, an objective with lower magnification and numerical aperture may be more suitable.

## 1. Introduction

Dental microwear serves as a proxy for analysing the microfeatures and microtexture resulting from recent feedings, spanning weeks and months, on the occlusal or buccal surfaces of teeth. These features stem from tooth contact with particles during the chewing process (Gordon,

1988; Teaford, 1988; Romero et al., 2012). This approach has been widely utilised in archaeological and palaeontological studies, contributing to the reconstruction of vertebrate palaeodiets, including ungulates (e.g. Rivals et al., 2010; Blondel et al., 2022; Uzunidis and Rivals, 2023), carnivores (e.g. Schubert et al., 2010; Tanis et al., 2018), perissodactyls (e.g. Hullot et al., 2019), primates (e.g. Martínez et al., 2022)

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and elasmobranchs (McLennan and Purnell, 2021). Additionally, this method is also effective for analysing taphonomic features (e.g. trampling) (Uzunidis et al., 2021; Weber et al., 2022; Micó et al., 2024). Early studies in dental microwear analyses utilised Optical Microscopy (OM; e.g. Puech, 1979) and Scanning Electron Microscopy (SEM; e.g. Pérez-Pérez et al., 1994; Romero et al., 2004; Estebarez et al., 2009; 2012; Martínez et al., 2016). These methods entailed qualitatively identifying pits and scratches on the tooth surface, although semi-quantitative methods were also developed (Ungar, 1995). In the 2000s, stereo light microscopy emerged as another technique. Similar to its predecessors, this method relies on the qualitative identification of scratches and pits (LMDA; Solounias and Sempredon, 2002; Sempredon et al., 2004). In the same way that SEM technique, semi-quantitative methods have also been developed (e.g. Merceron et al., 2005; Strani et al., 2018).

In this scenario, dental microwear texture analysis (DMTA) has been developed (Ungar et al., 2003; Calandra and Merceron, 2016; Green and Croft, 2018; Hernando et al., 2020). Unlike preceding techniques, this method employs optical profilometry for collecting 3D topographic data of the dental surface. DMTA offers several advantages over previous methods, including the quantification of dental texture in 3D, and a broader range of parameters that can be assessed. Unlike earlier techniques, DMTA does not identify specific features such as scratches and pits, but quantifies the entire surface. The parameters obtained quantify the topography and geometry of the surface, considering motifs (hills or dales), furrows, roughness, or anisotropy. In this regard, the consideration of entire surface through DMTA proves to be more discriminant to distinguish different diets, such as in domestic ungulates, compared to LMMA (Ibáñez et al., 2020). DMTA can be analysed using Scale-Sensitive Fractal Analysis (SSFA; Ungar et al., 2003; Scott et al., 2005, 2006) or International Organisation for Standardisation (ISO) surface roughness parameters (Schulz et al., 2010, 2013).

DMTA enables repeatability and reproducibility, and alleviates inter- and intra-observer bias (DeSantis et al., 2013) compared to previous techniques (Grine et al., 2002; Galbany et al., 2006). However, researchers working on DMTA have used different measuring equipment (e.g. Leica, Sensofar, Keyence or Zeiss microscopes), software (e.g. Toothfrax and Mountains) and microscopy objectives (magnification and numerical apertures). In the last years, there has been a growing interest in analysing the variability obtained in results according to different microscopes (Kubo et al., 2017), the analysed support (Mihlbachler et al., 2019; Sawaura et al., 2022), the objectives (Calandra et al., 2019), and even the software (Calandra et al., 2022).

In the case of the objectives used, DMTA researchers have employed a wide range of objectives with different magnifications and numerical apertures (NA). Firstly, the NA determines the lateral and axial resolution. A higher NA allows for the measurement of deeper features and, consequently, a better resolution of the texture. In DMTA, objectives with different NA have been used, including 0.90 (Merceron et al., 2016) and 0.80 (Winkler et al., 2019; Weber et al., 2022; Jiménez-Manchón et al., 2023). It should be noted that the NA used has not been specified in all papers on DMTA. Calandra et al. (2019) analysed a same sample using a 50x/0.95 and 50x/0.75 objectives and found different results. The authors confirmed that this parameter can produce varying results, as expected, and that a higher NA should produce more accurate results.

The magnification used in confocal microscopy determines the spatial sampling size. The images obtained, regardless of magnification, will have the same total number of pixels. However, objectives with higher magnification analyse smaller areas, so the quantity of pixels per surface unit is higher. This characteristic is shown by a smaller spatial sampling size in the objectives with higher magnification. In DMTA studies, the use of 20x has been found to be useful for discriminating modern populations of primates (e.g. Ramírez-Pedraza et al., 2022; Martínez-Martínez et al., 2022) and domestic caprines (Ibáñez et al., 2020). A 50x objective lens has also been successfully tested for discriminating extant populations of suids (Souron et al., 2015), deer (Kubo et al., 2017), and caprines (Jiménez-Manchón et al., 2023).

However, the 100x objective has been most commonly used in DMTA (e.g. Bestwick et al., 2021; Kubo et al., 2021; Winkler et al., 2022; Berlioz et al., 2023; Louail et al., 2023). From a theoretical standpoint, higher magnification, such as 100x, should produce more accurate results, as the number of pixels for the same area analysed is greater. Kubo et al. (2017) analysed the same dataset of extant sika deer at different magnifications (50x and 100x). Despite mentioning that “higher magnification guarantees the better 3D data”, they found that significant differences between the two sets at different magnifications were small, and both were able to discriminate different diets (Kubo et al., 2017, page 162).

In this context, it should be stressed that in quantitative artifact microwear analysis (QAMA), a method focused on analysing micro-features and microtextures on lithic and bone artifacts to determine the materials they were used for (Ibáñez et al., 2016), researchers typically use 20x magnification in lithics (e.g., Rosso et al., 2017; Ibáñez et al., 2019; Ibáñez and Mazzucco, 2021; Pichon et al., 2021) and bones (e.g. Martisius et al., 2018); although 50x magnification has also been employed (Macdonald et al., 2019).

The effect of analysing DMTA using different magnifications, considering NA, has not been extensively studied. The primary questions are, firstly, whether the use of high magnification, such as 100x, provides superior characterisation and results of dietary pattern compared to lower magnification (e.g., 20x and 50x). Secondly, it remains to be determined, in an archaeological or palaeontological context, what the advantages and disadvantages are of using 100x magnification compared to lower magnification objectives.

To address these questions, in this paper, we aim to explore the variability of DMTA in a set of wild and domestic goats, previously analysed (Jiménez-Manchón et al., 2024), using different magnifications: 10x, 20x, 50x and 100x and the same microscope (Sensofar Plu Neox). The specific aims of this study are: (1) to determine whether different dietary regimes can be discriminated at various magnifications, (2) to identify which of the four magnifications achieves better characterisation and discrimination between dietary groups, and (3) to discuss the advantages and disadvantages of each objective lens.

## 2. Material and methods

### 2.1. Material

A set of 94 modern domestic and wild goats has been analysed (Supplementary material 1). Three species of wild goats from three different regions were examined: Alpine ibex (*Capra ibex*, CI henceforth) from Val d'Aosta (Italy), Iberian ibex (*Capra pyrenaica*, CP) from southern Iberian Peninsula, and Nubian ibex (*Capra nubiana*, CN) from Sinai Peninsula and southern Jordan (Table 1). Domestic goats (*Capra hircus*), managed differently, come from four regions: goats fed in the steppe (CH1) from the Laghouat steppe (Algeria), in an overgrazed and wooded landscape (CH2) from north-eastern Iberian Peninsula, in grasslands (CH3) of the southern side of the Pyrenees, and in a wooded environment dominated by grasses and woody plants (CH4) of the Causse de Larzac in southern France. In previous studies (Jiménez-Manchón et al., 2023; 2024), DMTA conducted at 50x/0.80 successfully discriminated among the seven groups of modern goats. For further details, we refer the reader to the original publications (Jiménez-Manchón et al., 2023, 2024).

The occlusal surface of all teeth was cleaned using acetone, followed by 96 % ethanol using cotton wool to eliminate any remaining dust and acetone residues. Subsequently, the occlusal surface was moulded using high-precision polyvinylsiloxane silicone (President Light Body, Coltene Waledent). A band on the enamel of the protonocid or hypoconid of the second lower molar (LM2) was carefully cut. When it was not possible, it was extended to include the first (LM1) and third lower molar (LM3).

**Table 1**  
Overview of the dataset of domestic and wild populations goats analysed.

Group	Species	Number of specimens	Status	Dietary regime/management strategy	Geographical location
CH1	<i>Capra hircus</i>	18	Domestic	Algerian steppe	Laghouat (Algeria)
CH2	<i>Capra hircus</i>	15	Domestic	Overgrazed wooded area	Tarragona (Spain)
CH3	<i>Capra hircus</i>	3	Domestic	Grassland	Pyrenees (Spain)
CH4	<i>Capra hircus</i>	15	Domestic	Grasses and woody plants	Larzac (France)
CI	<i>Capra ibex</i>	12	Wild	Grasses and woody plants	Val d'Aosta (Italy)
CN	<i>Capra nubiana</i>	12	Wild	Grasses and woody plants	Sinai Peninsula (Egypt) and Petra region (Jordan)
CP	<i>Capra pyrenaica</i>	19	Wild	Woody plants	Sierra Nevada and Sierra de Cazorla (Spain)

## 2.2. Methods

### 2.2.1. Acquisition

The enamel band previously cut was analysed using a Sensofar Plu Neox white-light scanning confocal interferometer microscope at the Institut Milà i Fontanals-CSIC (Barcelona, Spain) and utilising the SensoSCAN® acquisition software package. For each enamel band, four 3D micro-texture height maps (n total = 376) were captured using four objective lenses (Fig. 1; Table 2). The 10x/0.30 and 20x/0.45 objectives have a lower NA and optical resolution than the 50x and 100x objectives, both of which have the same NA (0.80). The consistent NA in the latter two objectives (50x and 100x) allows to directly address the issues of magnification and pixel size on the surface (spatial sampling, see Table 2). In all cases, the field of view (FOV) is not considered since the same surfaces have been subsequently selected (see 2.2.2).

Images were acquired as follows: initially, a unique image was taken using a 50x/0.80 objective in topography mode from the cut mould. The obtained images were analysed and published in a previously work (Jiménez-Manchón et al., 2024). Subsequently, the area of the cut enamel band was analysed using other objectives (Table 2). For the 100x objective, a 2x2 stitching was performed to obtain a larger image size. The light source was automatically selected in each case using the SensoSCAN software.

### 2.2.2. Processing 3D texture surface

All images were processed using Mountains 7© (Digital Surf). An area of 150 x 150 µm was extracted in the centre of each image. The samples were inverted along the z-axis, given that negative silicone

moulds were analysed. The extreme measured points were eliminated (upper and lower 0.5 %) to remove outliers. A least-square (LS) levelling method was employed in order to correct the lack of horizontality of the sample. Subsequently, a polynomial function (power 8; Francisco et al., 2018) was performed to remove the curved morphology of teeth. Finally, the non-measured points were filled.

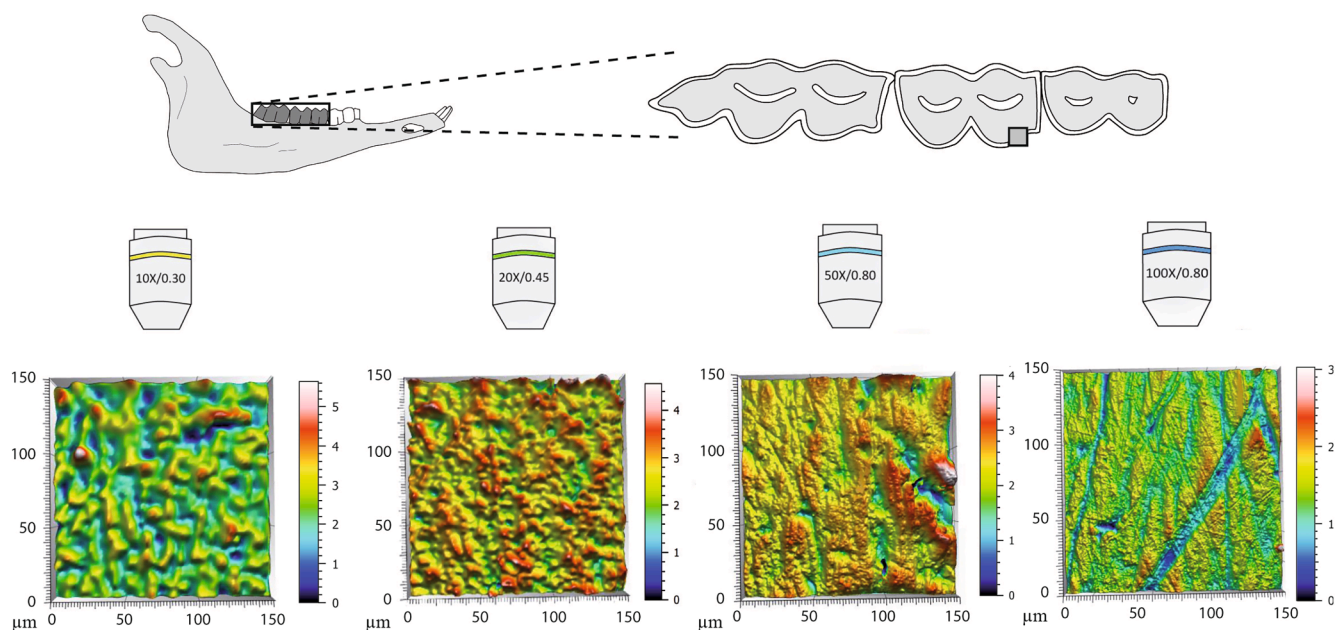
SSFA and ISO parameters were performed using MountainsMap 7 (Table 3). Three SSFA parameters were considered: *epLsar* (anisotropy), *Asfc* (complexity) and *HASfc9* (heterogeneity of complexity, 3x3 sub-regions). Regarding ISO 25178 parameters, a total of 30 parameters were analysed, covering height, function, plateau size, space, volume, feature and area.

### 2.2.3. Statistical analysis

Parameters were log-transformed in order to increase normality and homoscedasticity. After evaluating the normality of data (Shapiro-Wilk's test), significant differences ( $p \leq 0.05$ ) among seven goat populations for each magnification group were evaluated using one-way ANOVA ( $p \leq 0.05$ ), followed by Tukey's pairwise comparison. When homogeneity of variance (Levene's test) showed significant differences, Welch's ANOVA was used. Subsequently, a discriminant analysis was conducted for each dataset to explore how the seven groups are discriminated across different magnifications. The analyses were carried out using IBM SPSS Statistics 29.0.

## 3. Results

All data obtained are available in the [Supplementary material 1](#). The



**Fig. 1.** Exemplary 3D micro-texture height maps of the tooth surface texture of an extant domestic goat's lower molar obtained at 10x/0.30, 20x/0.45, 50x/0.80 and 100x/0.80 objectives.



**Table 2**

Summary of objective lenses specifications (NA = numerical aperture, WD = word distance, FOV = field of view).

Measuring equipment	Measurement array	Objective	NA	WD (mm)	FOV (μm)	Spatial sampling (μm)	Optical resolution (μm)	Maximum slope (°)	System noise (nm)	Measurement type	Analysis time (min)	Step size (μm)
Sensofar Plu Neox	768 x 576 pixels	Nikon EPI 10x	0.30	17.5	1689 x 1413	0.69	0.47	17	30	Topography	0.5	0.20
		Nikon EPI 20x	0.45	4.5	845 x 707	0.34	0.31	27	8	Topography	1	0.20
		Nikon EPI 50x	0.80	2	338 x 283	0.13	0.18	53	4	Topography	1	0.20
		Nikon ELWD 100x	0.80	4.5	169 x 141	0.07	0.18	53	3	Extended topography – stitching	≈ 4–5	0.20

**Table 3**

Description and functional groups of the ISO and SSFA parameters.

Abbreviation	Description	Functional group
epLsar	Anisotropy	Direction
Asfc	Area-scale complexity	Complexity
Hasfc9	Heterogeneity	Heterogeneity of complexity
Sq	Root mean square height	Height
Sku	Kurtosis of the height distribution	Height
Sp	Maximum peak height	Height
Sv	Depth between the mean plane and the deepest valley	Height
Sz	Height between the highest peak and the deepest valley	Height
Sa	Arithmetic mean height	Height
Smr	Peak material proportion	Functional
Smc	Inverse areal material ratio	Plateau size
Sal	Auto-correlation height	Density
Str	Texture aspect ratio	Spatial
Vm	Material volume at a given material ratio	Volume
Vv	Void volume of the core	Volume
Vmp	Material volume of the core at given material ratio	Volume
Vmc	Material volume of the core at given material ratio	Volume
Vvc	Void volume of the core	Volume
Vvv	Dale void volume	Volume
Spd	Density of peaks	Feature
Spc	Arithmetic mean peak curvature	Feature
s10z	Ten-point height	Height
s5p	Five-point height	Height
s5v	Five-point pit height	Height
Sda	Closed dale area	Area
Sha	Closed hill area	Area
Sdv	Mean dale volume	Feature
Shv	Mean hill volume	Feature
Sk	Core height	Functional
Spk	Peak reduced height	Functional
Svk	Reduced dale height, Reduced valley depth	Functional
Smr1	Low peak material portion	Functional
Smr2	High peak material portion	Functional

**Table 4**

Number of correctly identified specimens with the corresponding percentage in brackets using the classificatory rule obtained from a Discriminant Analysis at 10x magnification.

Groups	CH1	CH2	CH3	CH4	CN	CI	CP	Total
CH1	12 (66.7)	3 (16.7)	0	3 (16.7)	0	0	1 (5.6)	18
CH2	2 (12.3)	8 (53.3)	2 (13.3)	1 (6.7)	1 (6.7)	0	0	15
CH3	0	0	3 (100)	0	0	0	0	3
CH4	0	1 (6.7)	0	14 (93.3)	0	0	0	15
CN	3 (25)	0	0	1 (8.3)	6 (50)	1 (8.3)	1 (8.3)	12
CI	0	0	0	0	1 (8.3)	8 (66.7)	3 (25)	12
CP	1 (5.3)	0	1 (5.3)	3 (15.8)	3 (15.8)	1 (5.3)	10 (52.6)	19

results by objective are presented below.

### 3.1. 10x magnification

All parameters except for five (Str, Std, Smr2, epLsar and HASfc9) showed significant differences among the seven goat populations (Supplementary material 2). Pairwise comparisons (Tukey *post-hoc* test) revealed significant differences between ibexes and domestic goats, as well as among the wild goat populations. However, no significant differences were observed among the four management strategies (Supplementary material 2). A discriminant analysis was performed on parameters that showed significant differences. The predictive model achieved a correct classification of 64.9 % of specimens (Table 4). In the LDA texture-dietary plot (Discriminant function 1 = 41.8 %, Discriminant function 2 = 17.8 %), three out of the four groups of domestic goats (CH1, CH2 and CH4) predominantly occupy negative values along the first axis, while three wild goats (CP, CN and CI) and one domestic group (CH3) occupy positive values (Fig. 2). According to discriminant coefficients (Supplementary material 2), domestic goats exhibit lower values of height (e.g. Sq), plateau size (Smc) and complexity (Asfc) compared to wild goats.

### 3.2. 20x magnification

ANOVA's test detected significant differences among seven goat groups in all parameters except for five (Sku, Str, Smr1, Smr2 and epLsar) using 20x magnification (Supplementary material 2). The Tukey's pairwise comparisons revealed significant differences between domestic and wild goats, as well as among wild goats. In the case of domestic goats, differences were only found between CH1 and CH4 (Supplementary material 2). The Discriminant Analysis showed a correct classification of 67 % of specimens (Table 5). All centroids of the domestic goat groups present negative values in the Discriminant Function 1 (58.7 %), while those of wild goats have positive values. The negative ones correspond to lower values in height (e.g. Sq) and volume (e.g. Vm) than those of wild goats.

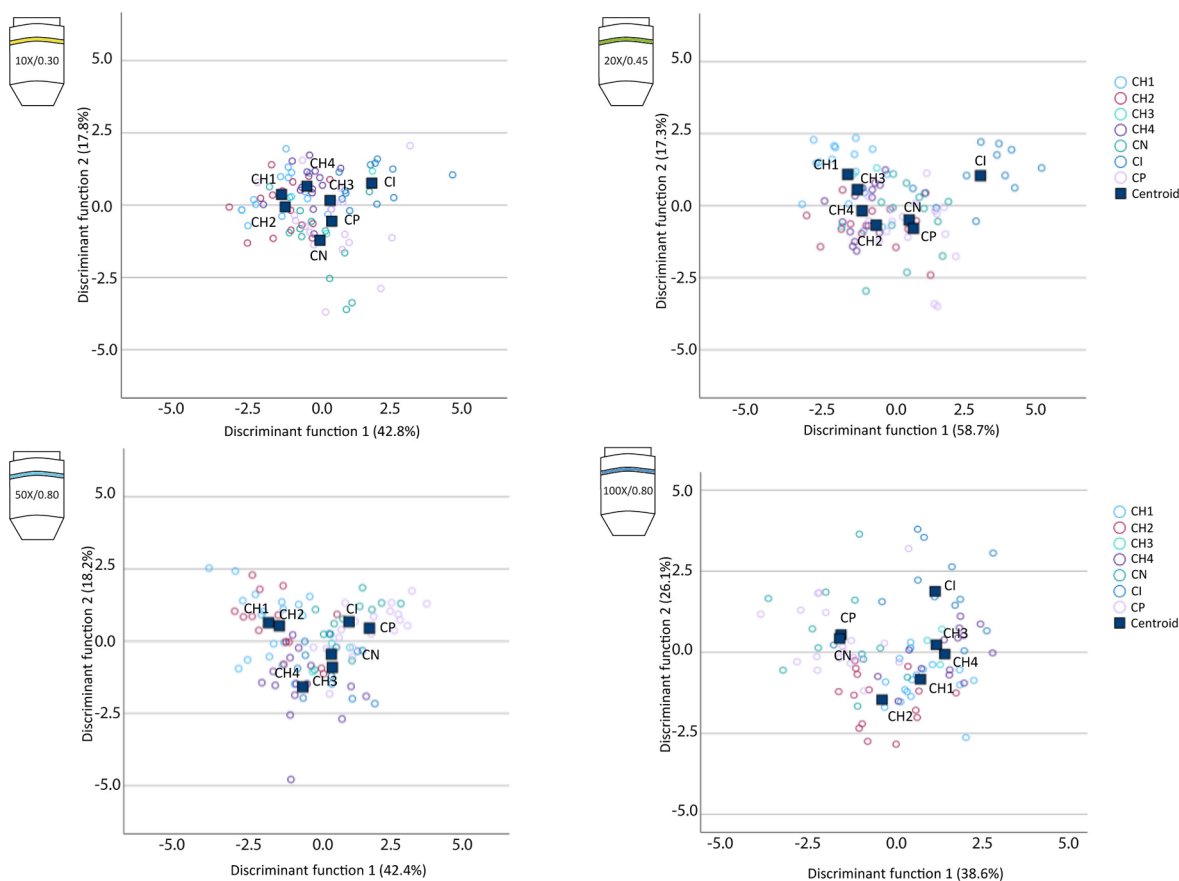


Fig. 2. Discriminant analyses conducted on domestic and wild extant goats at 10x/0.30, 20x/0.45, 50x/0.80 and 100x/0.80.

Table 5

Number of correctly identified specimens with the corresponding percentage in brackets using the classificatory rule obtained from a Discriminant Analysis at 20x magnification.

Groups	CH1	CH2	CH3	CH4	CN	CI	CP	Total
CH1	12 (66.7)	1 (5.6)	1 (5.6)	4 (22.2)	0	0	0	18
CH2	1 (6.7)	8 (53.3)	1 (6.7)	2 (13.3)	3 (20)	0	0	15
CH3	0	0	3 (100)	0	0	0	0	3
CH4	2 (13.3)	2 (13.3)	3 (20)	8 (53.3)	0	0	0	15
CN	0	3 (25)	0	0	7 (58.3)	0	2 (16.7)	12
CI	0	0	0	0	2 (16.7)	10 (83.3)	0	12
CP	1 (5.3)	2 (10.5)	0	0	1 (5.3)	0	15 (78.9)	19

### 3.3. 50x magnification

All parameters exhibit significant differences except for six (Sal, Str, Sda, Sha, Smr1 and epLsar; Supplementary material 2). Tukey’s pairwise comparison reveals differences between wild and domestic goats, as well as among the wild goat groups. Among the four domestic groups, differences were found only between CH1 and CH4, and CH2 and CH4

(Supplementary material 2). The discriminant analysis has shown a correct classification of 71.3 % of the specimens (Table 6). In the texture-dietary plot, it can be observed that the domestic goat groups CH1, CH2 and CH4 display negative values, while the wild goat groups CP and CH exhibit positive values in Discriminant Function 1 (42.4 %). Group CH3 and CI have values close to ‘0’ (Fig. 2).

Table 6

Number of correctly identified specimens with the corresponding percentage in brackets using the classificatory rule obtained from a Discriminant Analysis at 50x magnification.

Groups	CH1	CH2	CH3	CH4	CN	CI	CP	Total
CH1	14 (77.8)	2 (11.1)	0	1 (5.6)	0	1 (5.6)	0	18
CH2	3 (20)	7 (46.7)	0	1 (6.7)	1 (6.7)	3 (20)	0	15
CH3	0	0	3 (100)	0	0	0	0	3
CH4	1 (6.7)	1 (6.7)	1 (6.7)	11 (73.3)	0	1 (6.7)	0	15
CN	0	0	1 (8.3)	0	10 (83.3)	0	1 (8.3)	12
CI	1 (8.3)	0	0	0	1 (8.3)	9 (75)	0	12
CP	1 (5.3)	0	1 (5.3)	1 (5.3)	2 (10.5)	2 (10.5)	13 (68.4)	19

### 3.4. 100x magnification

Significant differences were found in all parameters except for seven (Sku, Sal, Str, Smr1, Smr2, epLsar and HAsfc9; [Supplementary material 2](#)). Tukey's pairwise comparison revealed differences between wild and domestic goat groups, as well as among the wild goat groups. Among domestic goats, differences were observed between CH1 and CH4, and between CH2 and CH4 ([Supplementary material 2](#)). The Discriminant Analysis showed a correct classification of 77.7 % of specimens ([Table 7](#)). All domestic goat groups exhibited positive values in Discriminant Function 1 (38.6 %), except for CH2, which showed values close to '0' ([Fig. 2](#)). Wild goats have negative values except for CI. Regarding Discriminant Function 2 (26.1 %), domestic goat groups showed low values. Only CH3 exhibit positive values, albeit close to '0'. Wild goats present positive values. According to the discriminant function coefficient, high values of features (Smc and Smr) and height (Sa and s10z) have negative values in Discriminant Function 1. In Discriminant Function 2, high values of complexity (Asfc) and height (Sq) show positive values.

## 4. Discussion

DMTA is widely used as a proxy for characterising the palaeodiet of vertebrates in archaeology and palaeontology. In recent years, the comparability of DMTA acquired by different teams and laboratories has been one of the most discussed topics. Thus, variability has been explored between analyses conducted on original specimens and casts (Mihlbachler et al., 2019), the type of silicone used for analysis (Goodall et al., 2015; Sawaura et al., 2022), the inter-microscope variability (Arman et al., 2016; Kubo et al., 2017) and the software for the surface texture analysis ([Calandra et al. 2022](#)).

DMTA has been widely used at 100x magnification, although 20x and 50x have also been successfully utilised. Additionally, QAMA extensively employs 20x and 50x magnifications (e.g. [Ibáñez et al., 2016](#); [Macdonald et al., 2019](#)). However, the performance between different magnifications in DMTA, as well as their differing discriminative capabilities, has not been explored. Only [Kubo et al. \(2017\)](#) addressed the presence of significant differences between 50x and 100x on a single set, finding few differences.

In this paper, we analysed the variability of DMTA in a set of wild and domestic goats at different magnifications: 10x/0.30, 20x/0.45, 50x/0.80 and 100x/0.80. All analysed areas were standardised to 150 x 150  $\mu\text{m}$ . The results obtained from this comparative study show that all objectives, even the one providing lower magnification (10x/0.30), are capable of discriminating the seven groups of domestic and wild goats. As previously observed in a previous study conducted at 50x/0.80 ([Jiménez-Manchón et al., 2024](#)), all objective lenses shows the same trends: domestic goats present a more abrasive dental texture, less complexity and lower height than ibexes. Among domestic goats, those grazing in grass-rich areas with a probable higher dust intake (CH1) present a more abraded dental texture compared to those feeding in woody plant-rich areas (CH4). Among wild goats, *C. nubiana*, inhabiting an arid environment, shows a less complex and more abraded dental texture surface than *C. ibex* and *C. pyrenaica*.

**Table 7**

Number of correctly identified specimens with the corresponding percentage in brackets using the classificatory rule obtained from a Discriminant Analysis at 100x magnification.

Groups	CH1	CH2	CH3	CH4	CN	CI	CP	Total
CH1	14 (77.8)	2 (11.1)	1 (5.6)	1 (5.6)	0	0	0	18
CH2	0	11 (73.3)	1 (6.7)	1 (6.7)	1 (6.7)	0	1 (6.7)	15
CH3	0	0	3 (100)	0	0	0	0	3
CH4	0	0	1 (6.7)	12 (80)	0	1 (6.7)	1 (6.7)	15
CN	0	2 (16.7)	0	0	10 (83.3)	0	0	12
CI	0	0	1 (8.3)	1 (8.3)	0	8 (66.7)	2 (16.7)	12
CP	1 (5.3)	0	0	0	2 (10.5)	1 (5.3)	15 (78.9)	19

Although all objectives are capable of discrimination, Tukey pairwise comparison showed that 10x/0.30 is insufficient to distinguish among domestic goats, and 20x/0.45 only found significant differences between two groups of domestic goats (CH1 and CH4). On the other hand, 50x/0.80 and 100x/0.80 objectives revealed more pronounced differences among the domestic ones, highlighting differences between CH1 and CH4, as well as between CH2 and CH4 ([Supplementary material 2](#)). These results indicate that while 10x/0.30 and 20x/0.45 can discriminate different dietary patterns between wild and domestic goats, higher magnification and NA (50x/0.80 and 100x/0.80) provide greater differentiation. The results obtained from the discriminant analysis have shown increasing discrimination rates: 10x/0.35 (correct classification = 64.7 %), 20x/0.45 (67 %), 50x/0.80 (71.3 %), and 100x/0.80 (77.7 %). Therefore, the capacity for discrimination increases with higher magnification and NA.

The comparison of the results obtained with the 50x/0.80 and 100x/0.80 objectives allows to evaluate the variability of results with objectives that have the same NA (0.80) but different spatial sampling. The spatial sampling of the 50x/0.80 objective is 0.13  $\mu\text{m}$ , whereas that of the 100x/0.80 is 0.07  $\mu\text{m}$  ([Table 2](#)). Consequently, within the same analysis area of 150x150  $\mu\text{m}$ , the number of pixels is higher using the 100x objective, even though the resolution is the same (NA=0.80). The results from this study indicate better discrimination using 100x (77.7 %) compared to 50x (71.3 %). This can be explained by the fact that a greater number of pixels in the same analysed area provides a higher capacity to discriminate texture microfeatures. Therefore, 100x is the objective that allows for better discrimination between wild and domestic goats. This is consistent with [Kubo et al. \(2017\)](#), wherein 100x provides better results, although similar to those of 50x.

All objectives showed discrimination between wild and domestic goats. Therefore, in order to choose an objective in DMTA studies, it has to be considered the aims of the analysis, the compromises and the limitations between the sample analysed, the characteristics of the microscope and the objectives. One of these limitations is the working distance (WD). The use of silicone moulds allows to approximate the lens to the surface analysed, reducing the WD. This is the case of the 50x/0.80 objective that has a low WD of 2 mm and a great NA (0.80). For materials that cannot be moulded using silicone or a replica of the tooth is used, lower magnification with lower NA, and therefore greater WD, could be suitable. The time required can be a constraint with the 100x objective, as the analysed surface area is smaller and stitching can be necessary. In our case, the images obtained with the 100x objective were four times longer than those obtained with the other objectives.

Another factor to be considered is the issues related to sample preparation and taphonomic bias. In this study, we analysed extant collections without taphonomic bias. However, it could be expected that higher magnification and spatial sampling could be more sensitive to these issues, although these aspects have not been explored in this paper.

It is important to note that the interpretations derived in this study are based on goat diets, which cannot be entirely extrapolatable to hominis or other species. However, the enhanced discriminating capacity using higher magnifications, which is linked to greater NA and smaller spatial sampling size, can give clues for choosing the more convenient objectives for other studies.

The results of this work suggest that using higher magnification provides better discrimination. Thus, the use of 100x magnification, which is widely employed in DMTA, could be recommended as a standard. However, it should be noted that this study utilised a NA of 0.80 at 50x and 100x, and using a higher NA (e.g. 0.90 or 0.95) could yield better results, potentially even a magnification of 50x. To enhance the reproducibility and repeatability, it is crucial to provide detailed of the microscope and objectives used, as indicated by Calandra and colleagues (2019).

## 5. Conclusions

In this paper, we explored the use of different objective lenses using a confocal interferometer microscope to distinguish dietary behaviours between wild and domestic goats. DMTA applied to a modern dataset of caprines through different objectives (i.e. 10x/0.35, 20x/0.45, 50x/0.80 and 100x/0.80) in a single confocal microscope (Sensofar Plu Neox) has shown the same trends regardless of the magnification. This indicates that dietary texture can be observed even at low resolution, including at 10x, although the results obtained using 50x and 100x have shown a better discrimination. The results obtained with 100x allowed for slightly better discrimination than those using 50x. Both have the same numerical aperture, so the better discrimination can be due to the higher number of pixels on the same surface, which can help to discriminate diets better. This demonstrates that, at least in DMTA applied on small-sized ungulates, 100x may be the most appropriate and effective.

However, high magnification can present some limitations, such as a shorter working distance, which can require the use of an objective with low magnification and numerical aperture depending on the material analysed. Additionally, high magnification can be more sensitive to taphonomic bias. Thus, this paper shows for the first time the higher level of discrimination of dietary patterns at 100x in ungulate DMTA compared to lower magnifications (i.e. 10x, 20x and 50x) and numerical aperture, and contributes to improving the standardisation, repeatability and reproducibility of this approach.

## CRedit authorship contribution statement

**Sergio Jiménez-Manchón:** Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lionel Gourichon:** Writing – original draft, Investigation, Data curation, Conceptualization. **Laura M. Martínez:** Writing – original draft, Conceptualization. **Ferran Estebarez-Sánchez:** Writing – original draft, Conceptualization. **Rose-Marie Arbogast:** Writing – original draft, Data curation, Conceptualization. **Allouen Evin:** Writing – original draft, Data curation. **Marie Meister:** Writing – original draft, Data curation. **Faiza Tekkouk:** Writing – original draft, Data curation. **Silvia Valenzuela-Lamas:** Writing – original draft, Data curation. **Juan José Ibáñez:** Writing – original draft, Methodology, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

All data are available in the [supplementary material](#).

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jasrep.2024.104716>.

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