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Error Rates in Buccal-Dental Microwear Quantification Using Scanning Electron Microscopy

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Summary: Dental microwear, usually analyzed using scanning electron microscopy (SEM) techniques, is a good indicator of the abrasive potential of past human population diets. Scanning electron microscopy secondary electrons provide excellent images of dental enamel relief for characterizing striation density, average length, and orientation. However, methodological standardization is required for interobserver comparisons since semiautomatic counting procedures are still used for micrograph characterization. The analysis of normally distributed variables allows the characterization of small interpopulation differences. However, the interobserver error rates associated with SEM experience and the degree of expertise in measuring striations are critical to population dietary interpretation. The interobserver comparisons made here clearly indicate that the precision of SEM buccal microwear measurements depends heavily on variable definition and the researcher's expertise. Moreover, error rates are not the only concern for dental microwear research. Low error rates do not guarantee that all researchers are measuring the same magnitudes of the variables considered. The results obtained show that researchers tend to maintain high intrapopulation homogeneity and low measurement error rates, whereas significant interobserver differences appear. Such differences are due to a differential interpretation of SEM microwear features and variable definitions that require detailed and precise agreement among researchers. The substitution of semiautomatic with fully automated procedures will completely avoid interobserver error rate differences.

Key words: dental microwear, error rates, scanning electron microscopy, quantification

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Introduction

Phytolyths are abundant not only in plant foods, such as leaves, shoots, fruits, or medullas, but also in dust and ashes that can be incorporated into food items during food handling and processing. The siliceous nature of phytolyths means that they are able to produce microscopic damage, in the form of scratches and pits, on the enamel surfaces of teeth during food chewing. Such damage can be observed using scanning electron microscopy (SEM), and the analysis of dental microwear patterns can be correlated to food consumption and dietary habits (Teaford 1994). Dental microwear research has proved to be a good indicator of the ecological adaptations of extant and extinct primates, including fossil Hominin species, both on occlusal tooth surfaces (Daegling and Grine 1999, Dennis *et al.* 2004, Grine 1981, 1986; M'Kirera and Ungar 2003; Teaford 1985, 1994; Teaford and Oyen 1989; Teaford *et al.* 1996; Ungar 1990, 1992, 1996, 1998; Ungar and Kay 1995; Ungar and Spencer 1999; Ungar and Williamson 2000) and on buccal ones (Galbany and Pérez-Pérez 2004, Lalueza and Pérez-Pérez 1993, Pérez-Pérez *et al.* 1994, 1999; Puech 1981, 1984; Puech and Albertini 1984; Puech *et al.* 1983, 1989). However, the semi-automatic procedures (Pérez-Pérez 1999, Ungar 1995) most frequently employed in counting and measuring microwear features (i.e., pit and scratch widths and lengths) are accompanied by unavoidable interobserver error rates (Grine *et al.* 2002) which depend heavily on a number of factors: SEM brightness and focus, precision in variable definition, the overlap of microwear features, the researcher's expertise and fatigue during the analysis, and observation conditions (room lighting, temperature, quietness, etc.). Until highly automated microwear measuring procedures are developed (Grine *et al.* 2002), detailed analyses of intra- and interobserved error are required for methodological standardization and reliability. Recently, error rate estimations were provided for occlusal microwear analyses (Grine *et al.* 2002), showing that both intra- and interobserver errors should not be neglected and that a certain bias can be as-

sociated with the semiautomatic characterization of microwear patterns on teeth. The present paper seeks to determine the magnitude of error rates associated with buccal microwear analyses in order to compare them with those reported for occlusal tooth surfaces. The methodological procedures for characterizing buccal and occlusal microwear are significantly different. Occlusal microwear research requires characterization of both pits—of various shapes and sizes, and frequently overlapping—and scratches, usually at 500× magnification; in contrast, buccal microwear analysis involves the characterization at 100× magnification of striations only, since no pits are observed on the buccal surfaces of teeth. The typical field width of a 100× image is 1196×972 μm, whereas the 500× image field is much narrower, 240×196 μm.

The analysis of both occlusal and buccal microwear error rates is relevant for the methodological standardization of microwear measuring techniques and for making interobserver comparisons of dental microwear research.

Material and Methods

Four different SEM micrographs of buccal-dental enamel surfaces were selected from the collection of primate and hominid photographs previously obtained by our research group (Galbany *et al.* 2004a). None of the seven researchers involved knew in advance which specimen or species was being analyzed. Two of the selected SEM images belonged to a baboon (*Papio anubis*), a Cercopithecoidea primate, and both were lower left second molars (LM_2) of adult females from the National Museums of Kenya (NMK om6992 and om7288). The other two SEM micrographs were obtained from *Hominidae* teeth: an upper left first molar (LM^1) of OH-13, assigned to an immature female hominin of *Homo habilis* from Olduvai (Tobias 1991), and a lower right third premolar (RPm_3) of LH-4, assigned to a hominin of *Australopithecus afarensis* from Laetoli (White 1978) (Fig. 1). The dental casts of the analyzed teeth were obtained from the original museum collection specimens using the regular-body polyvinylsiloxane President MicroSystem™ (Coltène® AG, Altstätten, Switzerland). Positive casts were made using the epoxy resin Epo-Tek #301 (QdA). The tooth replicas were mounted on aluminium stubs and a colloidal argent belt (Electrodag 1415M, Acheson Colloiden Co., Ontario, Calif., USA) solution was applied to allow electron dispersal and prevent the accumulation of electrostatic charges during SEM observation (Rose 1983). Finally, the samples were sputtercoated with a thin, nonobliterating 400 Å gold layer to allow observation by SEM.

All SEM images were obtained at 100× magnification on the middle third of well-preserved buccal surfaces of tooth crowns, avoiding the occlusal and cervical thirds, and using secondary electrons in a Cambridge Stereoscan S-120 scanning electron microscope. The electron acceleration used was relatively low, around 10–15 kV, and each image was obtained at 72 ppi digitalization resolution with the Image Slave software, 1024×832 pixel images being obtained (Galbany *et al*. 2004b). Each SEM micrograph was cut off to include a 0.56 mm^2 square surface area (748.33 μm of field width), in which scratches were counted manually following standard methodological procedures for buccal microwear research (Galbany *et al.* 2004b, Pérez-Pérez *et al.* 1999). Microwear features were quantified with the Sigma Scan Pro V Statistical Package for Social Sciences (SPSS Inc., Chicago, Ill., USA). All objects longer than 15 μm and with a minimum length-to-breadth ratio of 3:1 on the enamel surface of teeth were measured without considering curvature. Objects with smaller length-to-breadth ratios were considered as pits and were not counted. Each of the four selected images was characterized four times by each of the seven researchers, who showed various degrees of expertise in measuring buccal microwear: five of them had more than 3 years of experience (R1 to R5), one was a fairly inexperienced researcher (R6), and one (R7) had some experience using Ungar's Microwear software (Ungar 2001). Six researchers (R1 to R6) used SigmaScan Pro 5.0 by SPSS for microwear feature characterization, while researcher R7 used Ungar's Microwear software. Each image was measured only once by a single researcher in the same measuring session, and a minimum

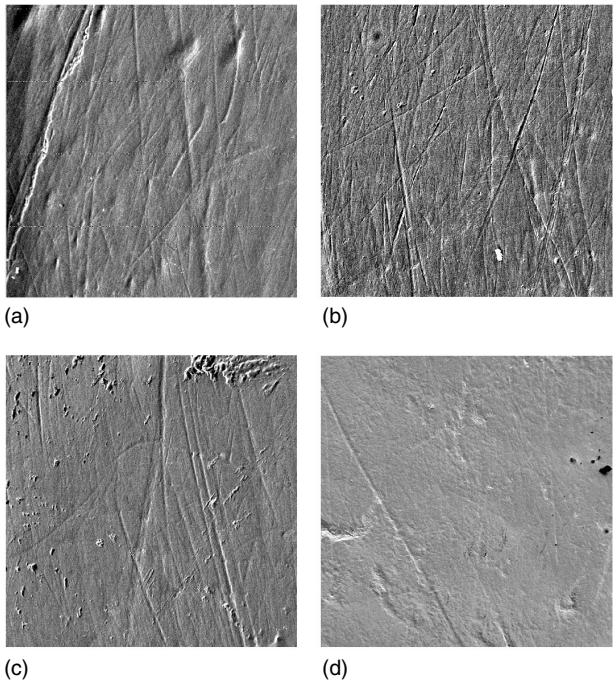

FIG. 1 Micrographs repeatedly measured in the intra- and interobserver error analysis, belonging to (a) *Australopithecus afarensis* hominin from Laetoli–LH-4 (513×513 pixels); (b) *Papio anubis* (baboon) from National Museums of Kenya—om6962 (600×600 pixels); (c) *Papio anubis* (baboon) from National Museums of Kenya—om7288 (600×600 pixels); and (d) *Homo habilis* hominin from Olduvai—OH-13 (513×513 pixels). All four analyzed images included a 0.56 mm^2 square area with a field width of 748.33 μm.

delay of 2 days was required for repeating the characterization of an image. Thus, each observer counted scratches on a total of 16 images and invested a minimum of 8 days for completing the measurements.

All researchers were required to distinguish between natural, antemortem microwear and postmortem enamel damage based on previous knowledge of buccal microwear research (Martínez *et al.* 2001). All observed scratches on the enamel surface >15 μm were measured by defining their initial and final points and without considering curvature. Scratch lengths were then automatically recorded and striations shorter than 15 μm were removed from the database before measures of scratch density and average length (in μm) were derived. All statistical analyses of intra- and interobserver error rates were made with the SPSS v.11 statistical package.

Results

In the first instance, the interobserver error rates were computed as the mean absolute percentage difference (MAPD), as described in Grine *et al.* (2002). The error rate values obtained for the seven researchers, measuring four images four times, ranged between 4.26 (R2) and 15.33% (R6) for the density of striations on the buccal surfaces, and between 3.63 (R2) and 19.41% (R7) for the average length of the striations. Grine *et al.* (2002), analyzing four replicas of two micrographs by one researcher, report MAPD values of 4.1 and 12.9% for the density of scratches on occlusal surfaces, and 4.6 and 7.0% for the length of scratches, similar to those found in the present study.

However, the MAPD is not a precise measure of the dispersion of repeated measurements. Rather, the standard error of the repetitions (Jamison and Zegura 1974, Page 1976, Sokal 1995, Utermohle and Zegura 1982) may better reflect a researcher's reliability in characterizing metric features (Pérez-Pérez *et al.* 1990). Thus, the standard errors of the repeated measurements were computed. Table I shows the average density and length of scratches, along with their standard error (e_x) and variance (V_x), for each analyzed micrograph (M1, M2, M3, M4) and by researcher (R1 through R7). The mean standard error and variance values range, respectively, from 3.52 striations and 2.77% (R2) to 16.01 striations (R7) and 10.21% (R6) for striation density; and from 3.21 μm (R5) and 2.39% (R2) to 12.51 μm and 13.07% (R7) for striation length. Researchers with standard error values below five striations and 5 μm were R2 and R5, while those with variance values < 5% are R1, R2, and R5. Researchers with standard error values for both variables between 5–10 striations and 5–10 μm were R1 and R3, while R3 and R4 showed variance values in the range 5–10%. Researchers R6 and R7 showed the highest standard error and variance values for all variables measured (Table I, Fig. 2). If the least experienced researchers are excluded from the analysis, the maximum standard error and variance values obtained are 10.30 striations and 6.09% (R4) for striation density, and 5.97 μm and 5.66% (also R4) for striation length.

If the repeated measurements reported by Grine *et al.* (2002) are used to compute the standard errors (e_x) and variances (V_x) of the repetitions, the calculations yield a standard error of 5.33 striations with a variance of 5.34% for striation density, a standard error of 0.60 μm, and a variance of 3.99% for striation length (these values are included in Fig. 2 for comparison). Note that the magnitudes of the striation lengths differ greatly between our 100× magnification research and the report of Grine *et al.* (2002) with 500× magnification.

To test whether all researchers were measuring the same magnitudes in each micrograph considered, a multiple analysis of variance (MANOVA) designed for repeated measurements was performed, using SPSS v. 11 and considering two repetition factors (seven researchers and four replicas for each image considered). The MANOVA tests for homogeneity of means showed highly significant differences (F test) among researchers, whereas the replica factor had no effect (Table II). The univariate intersubject effect comparisons (for each of the 15 variables involved in the buccal striation pattern analysis) showed the same patterns: no significant differences among the replicas and highly significant differences among researchers. In fact, a tendency of researcher R7 to measure longer striations was evident (Fig. 3) for all images, since striation fragmentation was seldom considered. However, researcher R1 paid great attention to striation fragmentation and showed the smallest average striation lengths, also for all images; this researcher was followed by R6, R2, R3, R4, and R5. It is significant that those researchers who were conservative in measuring long striations within each image also showed smaller measurement errors. There was also a tendency of researcher R7 to measure a high density of striations in all images, whereas R1, R2 and R3 generally measured low striation densities (Fig. 3).

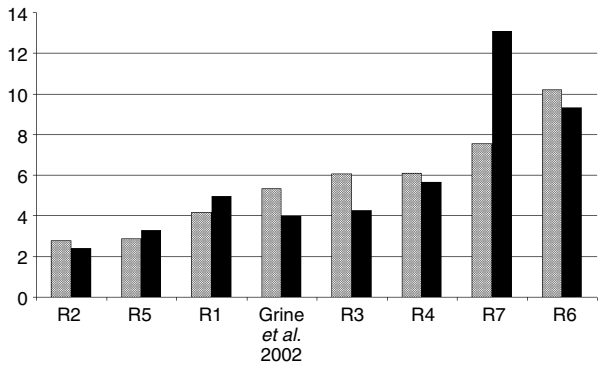

FIG. 2 Bar-plot of the repeated measurements variances in percent as estimation of error rates of striation density and average length for all researchers and including values from Grine *et al.* (2002) for comparison. ▒=density, ■=length.

TABLE I Summary statistics by researcher of error rates for the density and length of measured striations in each measured micrograph

	R1			R2			R3			R4		
	x	e_x	V_x	x	e_x	V_x	x	e_x	V_x	x	e_x	V_x
P1												
Density	112.25	2.39	2.13	111.00	2.48	2.23	112.75	2.95	2.62	155.50	5.61	3.61
Length	112.83	4.29	3.80	146.81	3.90	2.66	119.54	3.15	2.64	109.15	5.19	4.75
P 2												
Density	196.75	2.78	1.41	209.00	6.10	2.92	172.50	8.91	5.17	253.75	25.28	9.96
Length	115.45	7.13	6.18	149.24	2.07	1.39	131.10	6.16	4.70	118.72	8.26	6.96
P3												
Density	146.50	9.54	6.51	124.25	2.95	2.37	86.25	10.81	12.53	173.00	7.54	4.36
Length	93.86	3.53	3.76	134.22	4.40	3.28	124.40	4.09	3.29	97.62	5.41	5.54
P4												
Density	90.50	8.09	8.94	64.50	2.53	3.92	50.75	2.93	5.77	93.25	2.75	2.95
Length	94.68	5.69	6.01	146.15	3.37	2.31	130.18	8.09	6.21	96.72	5.03	5.20
Mean												
Density		5.70	4.18		3.52	2.77		6.40	6.06		10.30	6.09
Length		5.16	4.95		3.44	2.39		5.37	4.25		5.97	5.66

	R5			R6			R7			Mean of group		
	x	e_x	V_x	x	e_x	V_x	x	e_x	V_x	x	e_x	V_x
P1												
Density	132.25	8.48	6.41	133.00	6.38	4.80	193.00	8.69	4.50	135.68	5.28	3.89
Length	93.75	3.69	3.94	112.72	13.99	12.41	105.59	11.32	10.72	114.34	6.36	5.56
P2												
Density	326.75	7.05	2.16	219.50	25.44	11.59	312.75	30.08	9.62	241.57	15.09	6.25
Length	109.98	2.84	2.58	125.75	12.67	10.08	100.68	13.17	13.08	121.56	7.47	6.15
P3												
Density	140.75	2.32	1.65	111.75	13.21	11.82	204.00	6.47	3.17	140.93	7.55	5.36
Length	97.62	5.41	5.54	119.19	6.65	5.58	85.18	8.94	10.50	108.49	5.38	4.96
P4												
Density	75.00	1.58	2.11	78.25	10.38	13.27	136.50	18.81	13.78	84.11	6.72	7.99
Length	83.85	1.67	1.99	96.91	10.07	10.39	91.43	16.60	18.16	105.70	7.22	6.83
Mean												
Density		4.86	2.88		13.85	10.21		16.01	7.57		8.66	5.75
Length		3.21	3.27		10.60	9.33		12.51	13.07		6.60	5.87

Symbols are indicative of: x = mean values of the repeated measurements; e_x = standard error of the repeated measurements, and V_x = variance of of the repeated measurements.

Discussion

It is evident that any observational science involving the measurement of continuous variables, such as characterizing tooth microwear patterns, implies a certain degree of measurement error that needs to be controlled for, or at least minimized. Device error is frequently small since modern measuring equipment shows great precision, while clerical error is seldom a problem since the measurements can be directly inputted into a computer database without the need for data handling. However, intra- and interobserver errors still need to be carefully considered. As measuring procedures become more and more sophisticated, researchers' expertise and objectivity are of major concern. The variables to be measured need to be clearly and comprehensively defined so that different observers may replicate the measurements. Fully automatic measuring procedures would eliminate interobserver error, but the characterization of microwear patterns is still far from becoming automatic, at least where the aim is to measure microwear patterns as a combination of individual feature density, size, and orientation. Efforts should therefore focus more on automatic measures of surface roughness and relief.

The standard error of a series of repeated measurements is the best measure of intraobserver error because it determines a confidence interval around the actual variable value. Also, the standard deviation of a sample may be significantly reduced if the average of at least four repetitions is used as the actual variable measurement (Pérez-Pérez *et al.* 1990). Thus, the smaller the standard error the smaller the between-population differences that one can discriminate significantly between populations. The standard error of repeated measurements can be directly compared among researchers, given a significant number of repetitions, and their variance can be used as indicative of a researcher's measure of dispersion, with lower values expected for more experienced and precise researchers. However, the mean absolute percentage difference (MAPD), computed

TABLE II Multivariate and univariate general lineal model contrasts comparing the repeated measurements for the two factors considered (four replicas and seven researchers) of each image analyzed

	Multivariate effects					
	Replica (4 repetitions)			Researcher (7 repetitions)		
Image	Wilks λ	F	p value	Wilks λ	F	p value
1	0.133	3.273	0.060	0.000	7.729	***0.000***
2	0.335	0.994	0.533	0.000	5.353	***0.000***
3	0.213	1.850	0.210	0.000	5.975	***0.000***
4	0.243	1.560	0.284	0.000	5.222	***0.000***

	Intersubjects effects							
	Image 1				Image 2			
	Replica		Researcher		Replica		Researcher	
Variable	F	p value	F	p value	F	p value	F	p value
NH	0.208	0.653	11.146	***0.000***	0.786	0.386	12.379	***0.000***
NV	0.053	0.821	21.523	***0.000***	0.459	0.506	12.182	***0.000***
NMD	1.789	0.196	8.446	***0.000***	2.527	0.128	5.421	***0.002***
NDM	0.634	0.435	9.789	***0.000***	1.357	0.258	2.512	0.056
NT	0.365	0.553	25.356	***0.000***	1.460	0.241	10.484	***0.000***
XH	0.010	0.921	4.560	***0.005***	1.690	0.208	5.231	***0.002***
XV	0.790	0.385	4.274	***0.006***	0.582	0.454	2.437	0.062
XMD	0.210	0.652	6.165	***0.001***	0.404	0.532	3.250	***0.021***
XDM	0.002	0.964	3.781	***0.011***	1.089	0.309	7.155	***0.000***
XT	0.369	0.550	4.791	***0.004***	0.506	0.485	3.373	***0.018***
SH	0.051	0.824	1.290	0.306	2.336	0.142	1.598	0.200
SV	0.343	0.565	8.958	***0.000***	0.059	0.811	13.037	***0.000***
SMD	0.025	0.875	5.030	***0.003***	0.061	0.807	8.560	***0.000***
SDM	0.101	0.754	1.798	0.151	0.015	0.905	8.010	***0.000***
ST	0.052	0.822	8.013	***0.000***	0.028	0.869	13.573	***0.000***

	Image 3				Image 4			
	Replica		Researcher		Replica		Researcher	
Variable	F	p value	F	p value	F	p value	F	p value
NH	0.338	0.567	28.089	***0.000***	3.786	0.066	16.495	***0.000***
NV	0.141	0.711	13.548	***0.000***	0.787	0.386	13.392	***0.000***
NMD	0.170	0.685	4.763	***0.004***	2.518	0.128	4.423	***0.005***
NDM	0.245	0.626	13.069	***0.000***	5.098	0.035	5.313	***0.002***
NT	0.028	0.869	20.569	***0.000***	2.906	0.104	10.338	***0.000***
XH	1.701	0.207	13.527	***0.000***	0.004	0.947	2.451	0.061
XV	0.989	0.332	8.063	***0.000***	0.418	0.525	5.266	***0.002***
XMD	0.443	0.513	5.333	***0.002***	0.509	0.484	2.853	***0.036***
XDM	0.209	0.652	3.783	***0.011***	0.172	0.683	9.519	***0.000***
XT	0.932	0.346	9.973	***0.000***	0.001	0.980	6.905	***0.000***
SH	0.045	0.833	2.295	0.075	0.830	0.373	0.823	0.566
SV	0.015	0.904	26.376	***0.000***	0.594	0.450	7.200	***0.000***
SMD	0.200	0.660	11.683	***0.000***	0.690	0.416	2.414	0.064
SDM	2.082	0.165	0.479	0.816	0.864	0.364	17.311	***0.000***
ST	0.575	0.457	24.894	***0.000***	0.117	0.736	8.938	***0.000***

N= density of striations, X= average length of striations, S= standard deviation of the striation lengths, V= vertical striations, H= horizontal striations, MD= mesio-distal oblique striations, DM= disto-mesial oblique striations.

as the observed value minus the sample mean divided by the sample mean (Grine *et al.* 2002), can only be considered to be a measure of the maximum amount by which a given set of researchers under- or overestimate the mean value (Grine *et al.* 2002); for a reduced number of observers this may not even approximate the actual measurement. The MAPD statistic is highly sensitive to reduced numbers of repetitions and researchers, and varies greatly with the magnitude of the measured variable (for identical dispersion ranges, different MAPD values are obtained if the sam-

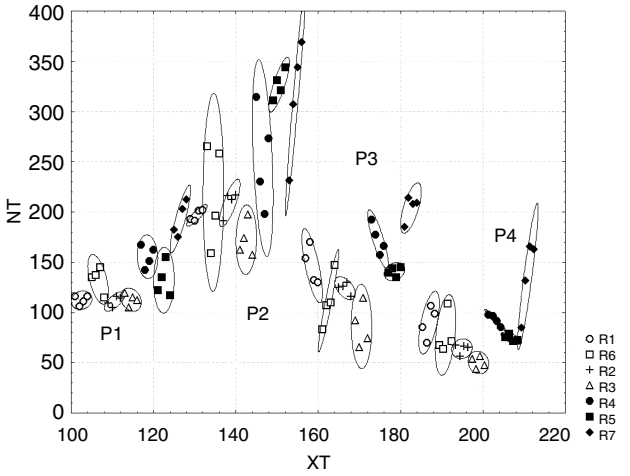

FIG. 3 Plot of striation density (NT) versus striation average length (XT) of all the repeated measurements for each picture (P1, P2, P3, and P4) and each researcher (R1, R2, R3, R4, R5, R6, R7). The ellipses include 50% confidence intervals [x±aσ] of all samples compared.

ple means differ). This makes comparisons between researchers difficult, and a set of only two repetitions is unlikely to be representative of overall measurement error.

The standard errors and coefficients of variance obtained for the buccal microwear analyses performed here (Table I, Fig. 2) show that error rates do indeed vary among researchers, with the least experienced ones (R6 and R7) showing the highest values. The variances among the most experienced researchers (with at least 3 years in buccal microwear research) do not exceed 6%, and this may represent a deviation of about nine striations in density and 10 μm in average length for the images studied here. Therefore, between-group differences in buccal microwear patterns can only be discriminated if such interpopulation differences exceed the error estimations by a large amount. In addition to these error rate estimations, the analysis of the interobserver variability also needs to consider whether all researchers are in fact measuring the same magnitudes of the variables, especially if comparisons between two independent researchers are to be made. From our analysis, it seems clear that although high intraobserver homogeneity is observed for some researchers, they are in fact measuring different things. Despite standardization of measurement procedures, the semiautomatic characterization of dental microwear involves considerable degrees of interobserver error, as well as differences in variable magnitudes (Grine *et al*. 2002), not least if different techniques are also used. Certainly, these results seem discouraging as they suggest that only one experienced observer should make all microwear measurements, thus avoiding interobserver comparisons. However, the results also provide clear guidelines for further methodological standardization among researchers, at least until more precise, automatic surface characterization procedures become widely used in dental microwear characterization.

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