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Effects of two separation methods of crown and root on enamel thickness measurements

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Abstract: In computer-aided dental anthropology it is sometimes a regular process to separate the crown from the roots. In order to assess the methodological impact of sectioning crown and roots for the computation of enamel thickness, we compared two digital approaches(separating the crown from the root using the cervical line or a basal plane) for the 3D analysis of enamel thickness on a total number of 82 hominin lower postcanine teeth, including South African fossil hominins(n=26), Neanderthals(n=22), and modern humans(n=34). According to paired t-test, no significant difference is observed in the enamel thickness values between two methods, but subsequent inter-taxa comparisons reveal different results in average enamel thickness(AET) in premolars. Separation based on a basal plane is more operator-dependent, not practical to sinuous cervical margin and might mask between-group distinctions. Besides providing a set of raw data for further investigation, this study reports thinner premolar RET in Neanderthals compared with modern *H. sapiens* and therefore support the notion that Neanderthal has generally thinner relative enamel. Our results show that, for studies aimed at discriminating among different species, using the cervical margin to isolate the crown from the root is a practical option as it considers the anatomical nature of tooth, especially for those specimens(such as anterior dentition, or molars of *Pan* and *Gorilla*) with steep cervical line.

Keywords: Taxonomic discrimination; Enamel thickness; Dental anthropology; 3D virtual measurements; Tooth crown; Tooth root

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1. Introduction

Enamel thickness has provided information when assessing hominid taxonomy, functional morphology and dietary ecology[1-7]. It has been measured from physical sections[3], naturally fractured teeth [8,9] , flat-plane radiograph[10] and medical/micro CT imaging[6,11-14].

In order to measure the enamel thickness(and many other parameters) using virtual models, 3D digital models must be first oriented according to a reference plane section(for 2D analysis) or to separate the crown from the root(s)(for 3D analysis). For 2D analysis, Tafforeau[15] oriented the 3D model based on a reference plane1) that approximates the cervical line, and sections can be made perpendicular to this plane or to the best-fit plane(created using a system of animation) containing the major dentine horn tips. Olejniczak[16] simplified Tafforeau's second method, defining a section perpendicular to the plane intersecting the three main dentine horn tips. Another method, proposed by Feeney et al[17] and later refined by Benazzi et al[18], creates a section passing through two points digitized on the widest labiolingual bi-cervical diameter and the dentine horn tip(or central mamelon in incisors). For 3D measurements, some scholars separated the crown from the root(s) using the best-fit plane of the cervical line, this method was a standardization and automation of Tafforeau's(2004) method of fitting a plane into the cervical line, and was previously used with success in digital analyses of teeth[19-21]; a more complicated method was proposed by Olejniczak[16](referred as 3D-c by Benazzi et al[18]): the most apical plane of section through the cervix that shows a continuous ring of enamel was first located(plane A), next, plane A was gradually moved toward the roots until the most apical plane of section still containing enamel was located(plane B). The plane exactly halfway between plane A and B was taken as the section plane, above which coronal measurements were recorded(Fig.1). This method was also very commonly applied in recent years[22,23], but shortcomings are this method includes operator-dependent error. Benazzi et al[18] suggested separating the crown from the roots based on the cervical line, which was then interpolated by a smooth surface to seal the bottom of the coronal dentine(see Fig.1, referred as 3D-b by Benazzi et al[18]), it has been suggested that this method also suits the morphology of the anterior teeth and premolars, and is anatomically sound.

In a previous study led by Benazzi et al[18], the authors cautioned that Olejniczak's basal(average) plane might return an incongruent representation of the tooth crown if the cervical margin is rather sinuous and therefore suggested 3D-a or 3D-b for isolating molar crowns, but a small sample size(13 teeth) did not allow statistical comparisons, what is not yet clear is the impact of methodological differences on the resulting data, for example, does the enamel thickness data yielded

1) This reference plane defined by Tafforeau(2004) was also named "best-fit plane" by later scholars, although it is not actually created through fitting calculation. Tafforeau(2004) plotted four straight lines(average lines of the cervix) along the cervical margin of four aspects of the molar, as the approximation of the cervical line. The angle between each average line and a horizontal line(with reference to the occlusal surface) was calculated. The average of these angles was used to correct the position of the volume model, so that the cervical margins were made to be as close to the horizontal plane as possible. Sections were made perpendicular to the horizontal plane.

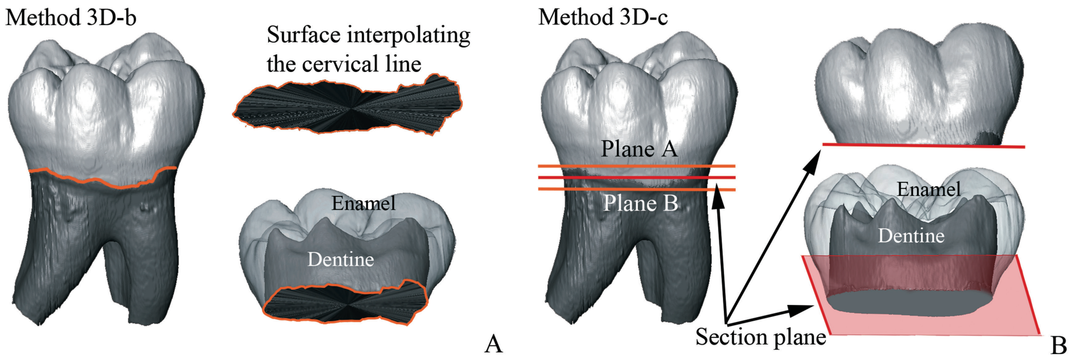

Fig.1 A sketch of two protocols(modified from Benazzi et al[18])

图 1 两种测量方法示意图 （修改自 Benazzi 等 [18]）

A. Method 3D-b digitally isolates the crown using the cervical line(orange), the bottom of the crown was sealed by a smooth surface interpolating the cervical line. B. Method 3D-c sections the teeth using a basal plane(red), which is halfway between Plane A and Plane B(orange). See Introduction for the explanation of Planes A and B // A. 3D-b 方法根据齿颈线（橙色）分离齿冠，随后软件自动根据齿颈线的形状差值生成成一光滑曲面覆盖齿冠底部，计算其上的釉质体积、齿质体积等。B. 3D-c 方法建立一个与平面 A 和平面 B（橙色）距离相等的平面（红色），以它作为基底平面切分齿冠和齿根。平面 A 和 B 的建立方法见 " 引言 "

using 3D-c differ significantly from 3D-a and/or -b, could such differences bias an interspecific comparison? Based on a larger sample size, this paper uses lower postcanine dentition from a variety of hominin taxa, provides an example of testing the performance of digital protocols to handle 3D models, compares the enamel thickness data yielded following 3D-b and 3D-c methods. Due to practical constraints, this paper only offers a preliminary comparison between 3D methods.

2. Materials and methods

2.1 Study Sample

The composition of sample used in this study is presented in Tab.1. We investigated a total number of 73 lower postcanine teeth derived from 5 taxa: *Paranthropus robustus*(19), *Australopithecus africanus*(3), South African early *Homo*(4), Modern *H. sapiens*(34), and Neanderthals(22). The South African fossil hominins derived from Sterkfontein, Kromdraai B and Swartkrans have an age range from 2.8 Ma to 1.36 Ma[24,25], the Neanderthals come from Montmaurin, Krapina and La Chaise, with an age range from OIS 11 to OIS 5e [26-28], the modern *H. sapiens* are European and East Asian populations. It is important to note that part of the enamel thickness data has been published, along with detailed descriptions of those samples in question [29]. Only specimens showing occlusal wear stages 0-2 according to Molnar[30] were selected.

2.2 Methods

East Asian specimens used in this study were scanned using a 225 kV-μXCT scanner housed at the Institute of Vertebrate Paleontology and Paleoanthropology(IVPP, Chinese Academy of Sciences) and were segmented using Avizo 8.1(Visualization Sciences Group, www.vsg3d.com). Isometric voxel size ranged from 10 to 70 μm.

A variety of protocols have been proposed to measure enamel thickness in its full three-dimensional form, as mentioned in Introduction, they differ mainly in the way to identifying sections between crown and roots. For comparative reasons, we employed two protocols, referred as 3D-b and 3D-c by Benazzi et al[18](Fig.1). The 3D-b method separates the crown from the root(s) based on the cervical line, and the coronal dentine tissue was sealed by a smooth surface following the curve of the cervical line(we used “Surface Editor-Fill hole” option on Avizo 8.1 to interpolate the bottom of the crown). The 3D-c method uses the section plane defined by Olejniczak et al[6], and Olejniczak[16] to separate the crown from the roots.

Three variables were measured for each specimen using “surface area volume” option in Avizo 8.1: the volume of the enamel cap(Ve, mm^3), the volume of the coronal dentine that includes the volume of the coronal pulp(Vcdp, mm^3), and the surface area of the enamel dentine junction(SEDJ, mm^2). We then calculated two indices of enamel thickness: 3D AET(Ve/SEDJ), the 3D average enamel thickness(mm), and 3D RET($100\times$3D AET/[$Vcdp^{1/3}$]), the scale-free 3D relative enamel thickness [22,31].

Tab.1 Composition of the study sample

表 1 本文使用的牙齿标本

Taxa	$P_3(n)$	$P_4(n)$	$M_1(n)$	$M_2(n)$	$M_3(n)$	Provenance	Enamel thickness data
P. robustus	2	2	2	6	7	Swartkrans Members 1, 2; Kromdraai B	Pan et al[29]
Au. africanus		1		1	1	Sterkfontein Member 4	Pan et al[29]
Early *Homo*	1	1	1		1	Swartkrans Members 1, 2	Pan et al[29]
Neanderthals	5	4	5	3	5	Montmaurin; La Chaise Abri Suard; Krapina Level 8	Pan et al[29] ; original data
Modern humans	7	7	9	6	5	Central Europe; East Asia	Pan et al[29]; original data

Tab.2 Average and range of enamel thickness values using 3D-b and 3D-c methods

表 2 两种方法所得牙齿釉质厚度的平均值和区间

		AET-b(mm)		AET-c(mm)		RET-b		RET-c	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Au. africanus	Premolars	1.35	-	1.53	-	22.38	-	24.67	-
	Molars	1.82	1.81-1.83	1.94	1.88-1.99	26.91	25.74-28.08	29.50	28.00-31.00
P. robustus	Premolars	1.83	1.70-2.06	1.95	1.83-2.17	29.87	23.38-35.46	32.84	27.09-41.17
	Molars	2.06	1.66-2.70	2.18	1.64-2.78	24.83	19.32-38.00	27.51	19.44-48.56
Early *Homo*	Premolars	1.64	1.48-1.79	1.65	1.46-1.85	31.00	29.03-32.97	32.46	28.45-36.48
	Molars	1.57	1.38-1.77	1.58	1.37-1.79	23.78	20.05-27.52	26.31	20.23-32.39
Neanderthals	Premolars	0.73	0.59-1.10	0.80	0.60-1.11	12.80	9.96-19.03	13.92	10.27-19.07
	Molars	1.31	1.10-1.54	1.30	1.04-1.61	19.08	16.05-23.73	18.84	15.06-24.77
H. sapiens	Premolars	1.13	0.81-1.61	1.14	0.85-1.65	25.31	18.19-31.72	24.73	19.84-32.75
	Molars	1.36	1.06-1.69	1.37	1.12-1.60	22.53	17.56-28.39	23.06	19.04-27.26

We used paired t-test to check if there is any differences in the results of enamel thickness in 3D-b and 3D-c methods done on the sample. The results of t-test are presented in Tab.2. Interspecies comparisons were done using the rank-based Kruskal–Wallis test with post hoc comparisons(Tab.3). Summary and boxplots of 3D AET and RET in each taxon are shown in Tab.4 and Fig.2, results are reported with regard to dental classes.

Due to small sample size, our Early Pleistocene specimens do not permit paired t-tests and interspecific comparisons(nonparametric tests) for each molar and premolar position, all the statistical analyses were conducted with reference to dental classes(premolar/molar). As one

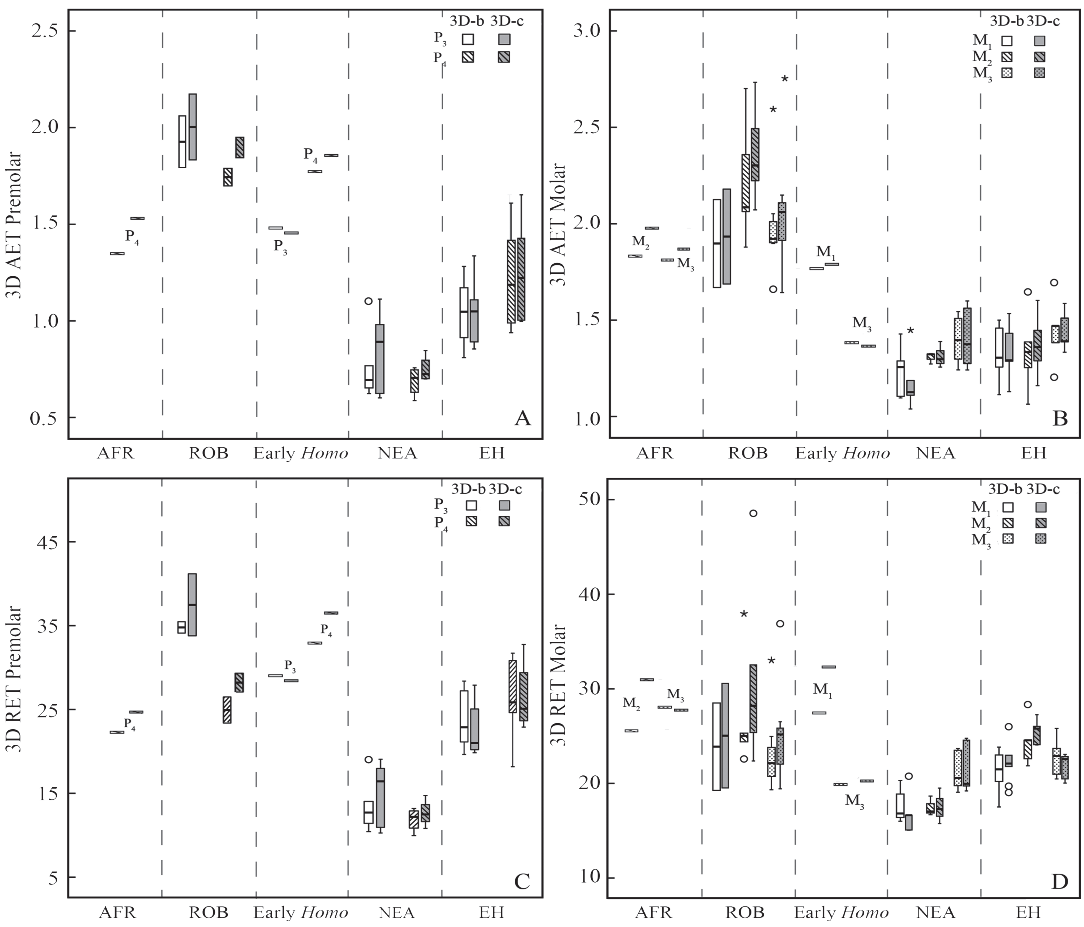

Fig.2 Average and relative enamel thickness(AET and RET) values in each taxon, provided by 3D-b and 3D-c methods

图 2 分别使用 3D-b 和 3D-c 测得各类群的平均厚度 (mm) 和相对釉质厚度

A, B. AET; C, D. RET. Standard box and whisker plot revealing the interquartile range(25th-75th percentiles: boxes), 1.5 interquartile ranges(whiskers) and the median values(black line). Outliers more than 1.5 interquartile ranges from the box are signified with circles, extremes more than 3 interquartile ranges from the box are signified with asterisks. AFR: *Au. africanus*; ROB: *P. robustus*; NEA: Neanderthals; EH: Extant human//A, B. 平均釉质厚度；C, D. 相对釉质厚度。标准箱线图显示了 50% 的样本数据、样本数据的中位数和上、下四分位数。对于极个别超出了理论上、下限的数据（极端异常数据），则以圆圈或星标表示。缩写：ROB：粗壮傍人；AFR：南方古猿非洲种；EH：现代人；NEA：尼安德特人

Tab.3 Conover’s post hoc pairwise comparisons after the Kruskal-Wallis test are reported below(significant results only)

表 3 **Kruskal-Wallis** 非参数检验后的成对比较结果，仅显示釉质厚度存在显著性差异的类群

Tooth position	Group 1	Group 2	AET-b	AET-c	RET-b	RET-c
Premolars	Neanderthals	*P. robustus*	<	<		
	Neanderthals	Early *Homo*	<			
	Neanderthals	*H. sapiens*			<	<
	Neanderthals	*P. robustus*			<	<
	Neanderthals	*Au. africanus*			<	<
Molars	*Au. africanus*	Neanderthals			>	>
	P. robustus	*H. sapiens*	>	>		
	P. robustus	Neanderthals	>	>	>	>
	Early *Homo*	Neanderthals				
	H. sapiens	Neanderthals			>	>

Note: a. Significant differences(group 1-group 2) are indicated by the directions, when $p≤0.05$ / 组 1 与组 2 的显著性差异（$p≤0.05$）及差异的方向性由符号表示

Tab.4 Paired *t*-test for differences in the enamel thickness values between 3D-b and 3D-c methods

表 4 两种测量方法所得牙齿釉质厚度的配对 *t* 检验结果 (Alpha=0.05)

Dental classes	Premolar AET	Premolar RET	Molar AET	Molar RET
Sig.(p)	0.06	0.27	0.15	0.1

might be interested in inter-taxa differences of enamel thickness between two methods at each tooth position, such information is presented only as boxplots in Fig.2.

3. Results and discussion

The two methods provide very similar results, although small magnitude of differences exist(Tab.2 & 3; Fig.2), paired *t*-test shows that switching one method to another, AET and RET do not change importantly(Tab.4). Between two methods, the differences in the premolar mean AET and RET are 2.8% and 4.2%, respectively; the differences in the molar mean AET and RET are 1.7% and 3.6%. As for interspecific comparison, among five taxa, inconsistent results lie in the comparison of premolar AET values: significantly thicker enamel in early *Homo* than Neanderthals is observed in AET data provided by 3D-b method, but such significance is rejected using data acquired by 3D-c. Both methods are consistent in showing significantly larger premolar AET for *P. robustus* than Neanderthals Both methods show thinner premolar RET for Neanderthals than extant *H. sapiens*, *P. robustus* and early *Homo*. Significantly thicker molar AET is seen for *P. robustus* than *H. sapiens* and Neanderthals, also Neanderthals have thinner molar RET than modern humans and australopiths(Tab.3). Overall, between-taxa overlap in enamel thickness is abundant(Fig.2). As previous studies identified thinner enamel for Neanderthal canines and molars when compared with modern humans, and related it to a larger volume of coronal dentine[11,22,32,33], here we report thinner premolar RET in Neanderthals and thus support the general findings that Neanderthals have relatively thinner enamel than modern humans.

Several methods have been proposed for the digital computation of enamel thickness. Apart from above-mentioned whole-crown enamel thickness measurements, scholars are also determined to investigate occlusal, cuspal and lateral enamel thickness in hominids, and create enamel thickness mapping to make developmental/functional inferences [5,31,34], including some Chinese fossil specimens [35,36].

Current study compares two methods of sectioning crown from the root(s), to assess the impact of methodological differences on the enamel thickness measurements. It is reasonable to assume, which has also been suggested in the technical note by Benazzi et al[18] based on a small sample size, that the results differ more in teeth with sinuous cervical margin(anterior teeth and premolars) than in teeth with almost planar cervix. However, according to our paired *t*-tests, the differences between enamel thickness value yielded by 3D-b and 3D-c fail to reach the significant level, in either premolars or molars(Tab.4). Nonetheless, our interspecies comparisons output different results with regard to premolar RET, when using enamel thickness values yielded by different protocols.

Even though 3D-b and 3D-c reach very similar results, subsequent between-taxa comparisons show different results in premolar AET. Therefore the author is inclined to suggest that systematic error and subtle differences in sectioning virtual models might undermine analyses aimed at interspecies comparisons. This study supports previous assertion that sectioning the crown using a virtual plane(3D-c) may mask the potential differences existing between closely related species [18]. Biases can be introduced when dental sample have steep cervical margin.

In conclusion, we believe that section using the cervical line(3D-b) is preferred for the measurements of enamel thickness for all dental classes with well-preserved cervical margin/basal portion of the crown. We recommend this protocol because it suits the morphology of anterior teeth and premolars, and is far less operator-dependent. As suggested by Benazzi et al[18]: "Olejniczak's basal(average) plane(3D-c) might return an incongruent representation of the tooth crown if the cervical margin is rather sinuous". Although two methods output similar enamel thickness value, we cautioned that inter-taxa discrimination, especially of the RET, might be underestimated when using the method 3D-c compared with 3D-b. We hope the comparisons of methods conducted above will bring valuable insights into the computation of 3D enamel thickness.

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人类牙齿齿冠和齿根分离两种技术方法对牙釉质厚度测量的影响

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摘要：在基于计算机断层扫描技术（CT）和虚拟图像处理技术的灵长类牙齿测量学研究中，经常需要分离三维虚拟模型的齿冠和齿根，再进行后续测量工作，如计算机辅助的生物力学分析、釉质厚度测量等。而分离齿冠和齿根这一步骤，目前有多种方法，如，1) 根据齿颈线切分齿冠，或 2) 人工建立基底平面切分齿冠。为了评估这两种不同的处理方式对后续的牙齿测量学上的影响，本文使用三维方法测量了 82 例化石和现代人类下颌后部牙齿的釉质厚度，包括南方古猿、早期人属、尼安德特人和现代人。使用配对 *t* 检验对比发现，两种方法得到的釉质厚度数值上没有显著差别，但随后进行的种间比较发现，使用基底平面切分齿冠的方法比较费时，更依赖于测量者的人工操作，并且可能弱化了物种间前臼齿绝对釉质厚度的差异，造成系统误差。其原因是对于前臼齿和前部牙齿等齿颈线形状不规则的标本，基底平面难以建立或误差较大。在未来对釉质厚度的种间差异的研究中，特别对齿颈线形状不规则的标本（如人类前部牙齿及猩猩、黑猩猩的牙齿等），本文推荐使用齿颈线分离齿冠和齿根，测量和计算齿颈线之上的釉质厚度。釉质厚度有一定的分类学、功能形态学和系统发育学意义。本文积累了一批可供未来对比研究的原始数据，并且发现尼安德特人前臼齿的相对釉质厚度显著小于现代人，这与前人利用臼齿、犬齿所做的对比研究结果相同，支持了尼安德特人拥有较薄的相对釉质厚度这一观点。

关键词：分类学差异；釉质厚度；牙齿人类学；三维虚拟测量；齿冠；齿根