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Hierarchical imaging of African bovid tooth cementum using X-ray microtomography

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ABSTRACT

Cementum deposits on mammalian teeth contain layered microstructures associated with the chronological age of an animal and other details of their life history. Hard X-ray tomography data captured this record contained within the cementum deposits from whole teeth without sectioning. We investigated three teeth of African bovids, namely gemsbok (*Oryx gazella*), eland (*Taurotragus oryx*), and African or Cape buffalo (*Syncerus caffer*) using the laboratory-based system nanotom m for measuring each complete tooth to identify relevant regions, which were scanned at the ANATOMIX beamline of Synchrotron SOLEIL, France. Using microtomography in archaeological materials such as teeth, eliminates the need for tooth sectioning, making it a desirable alternative for archaeologists and museum curators. Synchrotron measurements enabled the application of pixel sizes as low as 0.65µm, which generated around 40 TB of data. The three adult bovids investigated here, have a known day of death and season of death, and come from regions with distinct seasonal patterns in temperature and/or rainfall. They also have an estimated age at death based on occlusal wear. The known information serves as a control to determine the applicability of microtomography on whole teeth of large bovids. Preliminary results show that microtomography can successfully replace the need of sectioning in cementum dental analysis. Our future goal is to develop a protocol to standardize procedures of tooth cementum analysis in bovids using microtomography.

Keywords: tooth cementum analysis, incremental annulations, African bovids, zooarchaeology, age-at-death, synchrotron radiation-based micro-computed tomography, hunter-gatherer subsistence strategies, human evolution.

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1. INTRODUCTION

The microscopic analysis of tooth cementum has been applied in archaeology to answer questions about seasonality, mobility, site occupation, and the role of diet in human evolution [1-7]. Teeth, especially molars, are abundant in archaeological sites due to their durability; for this reason, they are key to reconstructing aspects of human food procurement and diet. Cementum found on mammal teeth, microscopically viewed in cross-section to detect tooth cementum annulations (TCA) or incremental layers (ILs), holds information on the chronological age at the time of death, and the season of death of an animal [2-7]. Mammal teeth consist mainly of enamel, dentine, and cementum. Cementum is a tissue surrounding the dentin at the cervical region or neck – located at the dentinoenamel junction (DEJ) and at the root of the tooth. ILs can be found at the roots and around the apices – or the tip of the tooth root, reaching up to the DEJ; its main function is to bond the tooth to the alveolar bone. Cementum growth and deposition takes place during an animal's lifespan [8] creating ILs as it forms, seldom going through remodeling [1, 2]. Cellular cementum is found in the apices, while acellular cementum is found at the DEJ and at the cervical area (Figure 1). ILs found in acellular cementum are thought to better estimate the season of death, and cellular cementum the age at death [3], but age at death is also recorded within the acellular cementum. The banding found on the acellular cementum, generally appears thicker and banding of ILs more regular than those found in cellular cementum. Generally, two layers – a dark and a light one – represent a year of life. The dark layer represents a period of arrested growth, while the lighter layer represents a growth period, where nutrients and food are available and copious.

In zooarchaeology, age at death estimation provides statistical information on the relationship of predator versus prey [9-11] and human preference in relation to food procurement. Age estimates have been traditionally obtained using tooth sectioning, which is destructive and not ideal for valuable archaeological collections. An alternative method for age estimates uses occlusal wear tooth crown attrition [9, 12]; however, the age approximations can be skewed due to untimely tooth wear caused by harsh diets and the environmental settings in the wild. Information found in the last accumulated cementum annulation or ILs have been correlated to seasonal diets [2, 4-7], particularly for temperate mammals [5, 6]. Seasonality relates to the idea that events linked to food procurement, occur seasonally at times when these items become available, and that people procure these items according to their specific needs. Therefore, information obtained from tooth cementum ILs contributes a vital component in the study of human evolution and seasonal strategies surrounding foraging behavior [5, 6]. Recent studies reported observations on life history patterns related to hormonal changes found in cementum ILs [13]. Investigation on the nature of factors involved in the accumulation and appearance of the cementum ILs, and how these differ in each species, has been tested repeatedly, but most involved tooth sectioning. A study using synchrotron radiation microtomography conducted on a group of twenty adult individuals with known ages from an 18^{th} to 19^{th} century collection from England, documented a correlation between the number of estimated ages extracted from the number of ILs and the known age of these subjects [14]. Other studies on human teeth using microtomography also show the same trend [15]. To our knowledge, this is the first time, ungulate (particularly bovid) tooth cementum ILs have been investigated using microtomography.

This study uses hierarchical imaging, which enables the visualization of structures at microscale resolution from large volumes [16]. These methods deliver high-resolution images that are ideal for capturing dental composition, providing valuable information for seasonality, life history, age estimates and mortality studies without the need of sectioning. The goal of this pilot study is to test the applicability of microtomography to modern African bovid teeth, before applying it to archaeological samples to investigate seasonality and seasonal resource procurement as a subsistence strategy in Stone Age archaeology.

2. MATERIALS AND METHODS

2.1 Fauna samples

The modern sample consists of three adult bovid teeth of two different group sizes [17] belonging to gemsbok (*Oryx gazella*), eland (*Taurotragus oryx*), and Cape buffalo (*Syncerus caffer*), belonging to group sizes 3a/b, and 4 respectively (Table 1). These samples were obtained during field research in the mid-1980s through early 1990s in the Kalahari Desert, Botswana (1985-86), near Lake Eyasi, Tanzania (1984-present), and from the Athi River Game Ranch, Kenya (1991-92). These specimens belong to the collections curated in the Laboratory of Zooarchaeology directed by H.T. Bunn at the University of Wisconsin – Madison.

2.2 Sample Preparation

The samples were placed inside clear plastic test tubes with a tight fit to prevent any movement during the rotation generated during scanning. The bottom of the plastic test tubes was glued to a mounting disk, which was then attached to the sample stage.

Table 1. Properties of samples starting from left to right. Catalogue number (Cat #); species; skeletal element with M= molar; subscript = lower/mandibular; superscript = upper/maxillary molar; known day of death (DOD); season of death; age estimate in years using occlusal wear and crown height method.

Cat #	**Species**	**Skeletal Element**	**Known DOD**	**Season of Death**	**Age Estimate (yrs) occlusal wear/crown heigh**
X2 #205	Gemsbok (*Oryx gazella*)	Left M_1	26-Mar-86	wet season	~11
GK #68	Eland (*Taurotragus oryx*)	Left P_4	27-Jul-86	dry season	~10
EY #077	Cape Buffalo (*Syncerus caffer*)	Left M^1	26-Oct-86	end of dry season	~9-10

2.3 Synchrotron radiation-based X-ray micro-computed tomography (SRμCT)

Tomographic imaging of the bovid teeth was performed at the X-ray phase-contrast microtomography setup of the ANATOMIX beamline at Synchrotron SOLEIL, 91190 Saint-Aubin, France [18]. For this study, we set the undulator gap to 5.5 mm and used 20 μm gold and 200 μm copper filters to obtain a white beam with an average photon energy around 40 keV. The propagation distance between object and detector was 50 mm. The detector consisted of a 20 μm-thick LuAG scintillator coupled via a 5× or 10× optics to a Hamamatsu Orca Flash 4.0 V2 scientific CMOS camera with 2048 × 2048 pixels [19]. The 5× and 10× objectives provided effective pixel sizes of 0.65 μm and 1.3 μm, respectively. The exposure time was set to 200 ms per projection. Tomographic acquisition consisted of 5,900 radiographs during continuous rotation over an angular range of 360 degrees. Thus, scan time for each local region was about 20 minutes. In some cases, the rotation axis was offset so that the volume imaged had a diameter of nearly twice the width of the detector's field-of-view, i.e., about 5.2 mm for the 5× and 2.6 mm for the 10× configurations. The data were reconstructed using the standard processing pipeline at the ANATOMIX beamline. Projections were processed with a Paganin filter [20] using a Paganin length of 9, 19, or 32 μm [21]. A double flat-field ring-artifact correction was applied [21].

2.4 Improving the appearance of incremental layers

Cementum incremental layers (ILs) occur in cellular and acellular cementum. Cellular cementum, typically found in the apical part of the tooth root is not well mineralized and its deposition not constant [22]. On the other hand, acellular cementum, located primarily at the DEJ is mineralized and is deposited at a slower and more constant rate [2]. At the DEJ, the cementum band is thicker and ILs are best observed. Due to the large size of bovid teeth and their roots, we first obtained sections at 4 height steps from the DEJ to the apex of the root at 1× magnification (6.5 μm), covering a height of 26 mm. Then, we obtained local scans at 5× (1.3 μm), and 10× magnification (0.65 μm), which were chosen based on observations on total cementum width and overall quality after each scan and volume reconstruction were performed onsite. Previous work shows it is possible to improve the visibility of incremental layers by integrating along the layer direction [15, 23]. Since incremental layers are curved and hardly aligned with the imaging direction, we automatically straightened the cementum before optimizing the integration direction.

3. RESULTS

3.1 Imaging of tooth cementum incremental layers

The approach of imaging selected local regions and enhancing ILs is illustrated in Figs. 1 and 2. The overview scans in the lab on the nanotom (Fig. 1a) help to identify the height steps to be acquired at 1× magnification at the synchrotron radiation facility (Fig. 1b). Local regions are selected on the 1× images (green frame in Fig. 1b) to be scanned at 5× magnification (Fig. 2a). The cementum region was manually selected (Fig. 2a) and then automatically straightened and enhanced to enable annotations of ILs (Fig. 2b).

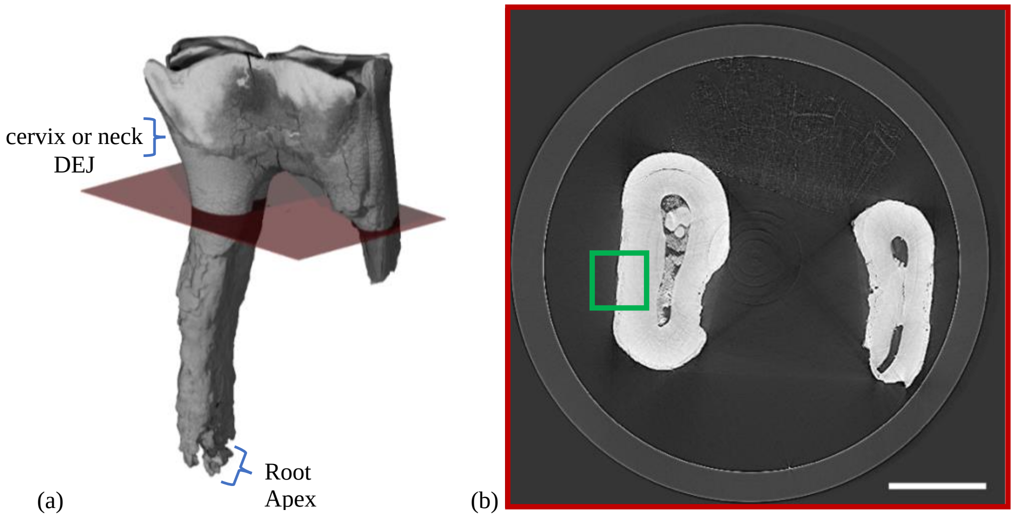

Figure 1. Gemsbok (a) Rendering of tooth from nanotom image with location of further investigated virtual stack shown in red. (b) Corresponding slice from 1× magnification synchrotron image with region of interest scanned with 5x magnification marked in green. The scale bar corresponds to 5mm.

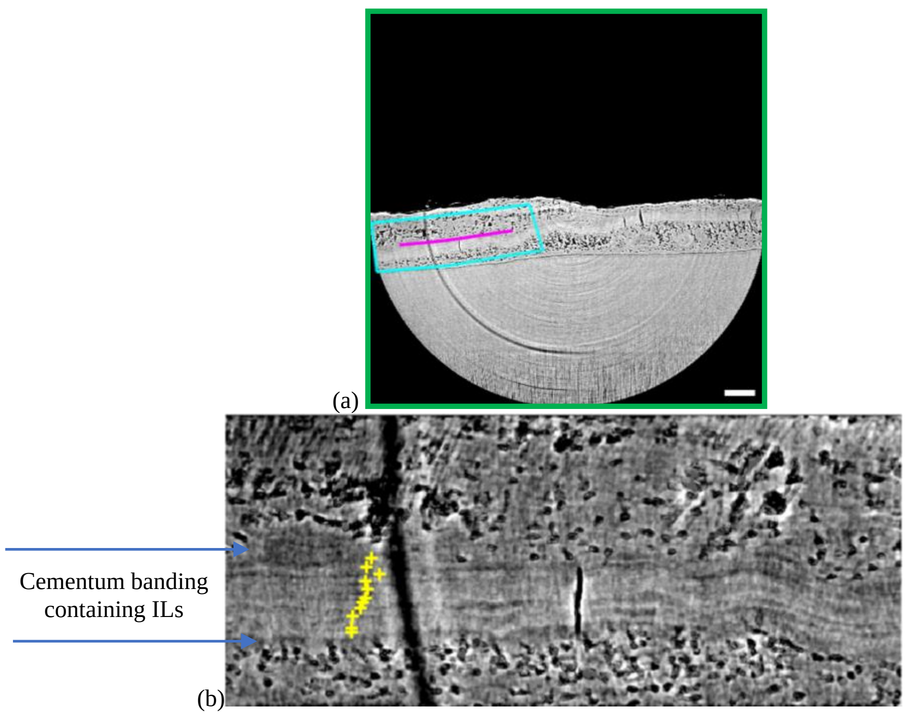

Figure 2. Gemsbok (a) Synchrotron image at 5× (1.3 µm) magnification showing selected acellular cementum region in cyan and centerline in magenta. (b) Straightened and enhanced cementum region based on centerline with annotations (yellow crosses) provided by observer AV marking 11 incremental layers. The scale bar corresponds to 200 µm.

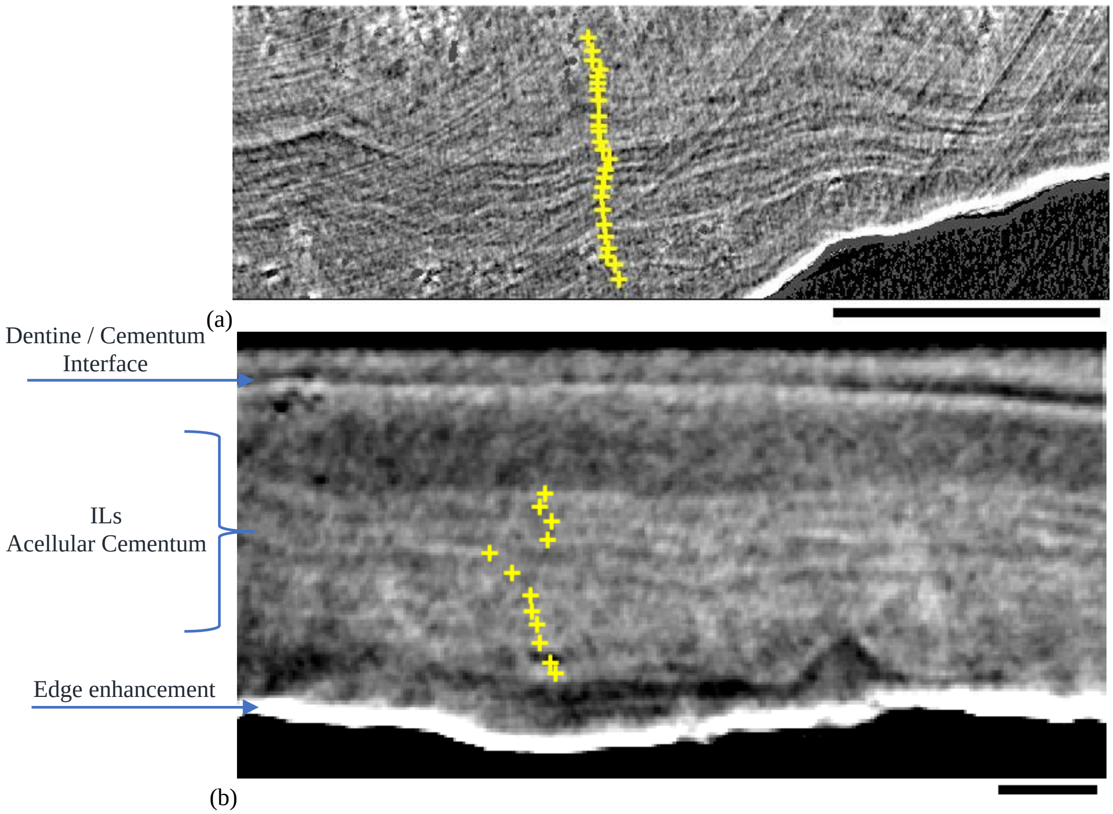

Figure 3. (a) Eland image at 5× (1.3 µm effective pixel size). Straightened and enhanced cementum region with annotations (yellow crosses) provided by observer AV marking 25 incremental layers. (b) Cape buffalo with annotation marks marking the brighter incremental layers and a thicker dark layer below the last marked bright layer [23]. Both scale bars correspond to 200 µm.

3.2 Interobserver Agreement of Incremental Layers

Four people investigated four regions containing cementum ILs, which were annotated using MATLAB (R2020b) with the purpose of observing the margin of error between the individuals annotating the ILs (Table 2). The difference in mean number of ILs was analyzed using one-way ANOVA – a tool used to analyze variance to determine statistically significant differences between means of three or more groups, in this case independent observers [24, 25]. The mean number of incremental layers was significantly different at the 0.05 level between observers JJ and MH, and between JJ and CT (Table 3).

Table 2. Mean number of incremental layers per region and observer.

ID	Region	AV	JJ	MH	CT	Mean	expected
1	018 1gemsbok205	9.0	15.0	8.0	13.0	11.3	11.0
2	019 1gemsbok205	7.0	14.3	6.7	7.3	8.8	11.0
3	115 2eland68	23.0	19.3	12.0	12.7	16.8	10.0
4	012 2eland68	15.7	24.0	14.7	15.3	17.4	10.0
	mean	13.7	18.2	10.3	12.1	13.6	10.5

Table 3. ANOVA multi-comparison of means tests for 4 observers doing 3 annotations for 4 regions. Statistically significant differences at the 0.05 level are marked with † and shown in bold font.

Observer 1	AV	AV	AV	JJ	JJ	MH
Observer 2	JJ	MH	CT	MH	CT	CT
Probability	0.121	0.346	0.855	**0.002†**	**0.019†**	0.814

4. DISCUSSION AND CONCLUSIONS

Tooth cementum has been investigated using optical microscopy as early as the 1950's [26], and it is still used today. Preparing the samples includes submerging them in epoxy, cutting, slicing, polishing, dyeing, and decalcifying [27]. In forensic anthropology and archaeology, this has been the standard procedure; however, it is destructive and not ideal for invaluable archaeological material. The use of microcomputed tomography enables investigating of questions about human evolution, specifically questions surrounding seasonal exploitation of resources. Cementum found on teeth, microscopically viewed in 3D cross-sections, as tooth cementum annulations, like those found in annular growth rings of trees, holds seasonal information in regions with marked seasonal weather patterns, e.g., tropical dry and rainy seasons, or temperate winter and summer seasons. Narrower, denser annulations correspond to the less-productive, stressful dry or winter seasons during which less growth occurs, while wider, less-dense annulations characterize rainy or summer seasons. The season at the time of death is recorded in the last deposited layer of the tooth cementum. This deposition takes place prior to the animal's death. The age at death is equal to the number of incremental layer pairs deposited during periods of growth throughout the animal's life (with any needed adjustment for the eruption schedule of the tooth) [1-6]. The expectation is that if humans are exploiting specific targets in their peak season, and using knowledge gained from past experiences, then, bone assemblages comprising an abundance of animal deaths from that season will result. Conversely, a more opportunistic and less seasonally focused, annual hunting strategy will yield evidence of animal mortality more evenly distributed throughout the year. Using microtomography in archaeological teeth could provide an opportunity to document patterns of seasonal site use and resource exploitation in archaeological settings, that would allow us to create models of human behavior in relation to human choice and available resources. The method first needed to be tested in modern bovids with a known season of death and estimated age at death, before attempting its use on archaeological teeth.

Microtomography has been previously used to investigate human tooth cementum [13-15] with successful results. The challenge here, was to test if the method could be applied successfully to bovid teeth, which are considerably larger than human teeth. To put this in perspective, an adult human, weighs on average 50 – 100 kg. An adult eland weighs between 340 – 600 kg [28], gemsbok weighs on average between 116 – 209 kg [28, 29], and Cape buffalo weighs between 425 – 870 kg [28]. Due to this difference in size, the preliminary inference was that the photon energy of X-ray beams needed to be higher than for human teeth for X-rays to penetrate the thick tissue to reach the focused region; however, this was not critical for these samples. Tooth cementum ILs are discernible and countable in a resolution as low as 1× (6.5 µm) for the buffalo [23], and at 5× (1.3 µm) for the gemsbok and eland. Due to size of these teeth and the limited field of view at higher resolution, measurements at the exact regions of interest are required, and knowledge of the exact locations on cementum deposits containing ILs, is critical for successfully capturing well-resolved ILs.

It is known that ILs are not necessarily recorded or clearly seen in all samples, and that there are variations found between individuals. Investigating larger controlled samples of several taxa can solve this issue [27], or at least provide information on what factors contribute to these variations, other than ascribing them to sample damage.

These three samples offer descriptive information to evaluate the purpose of our investigation, which is to test if observing the ILs on large bovids without the need of sectioning or destroying the sample was feasible. Four members of the team made annotations based on their observations. It is worth noting that no guidelines were provided on how to count the lines, what to exclude and what to include. The results were significantly different from one observer to another, and from the expected results, which was the expected number of ILs based on the estimated age of the animal at death. Observer variation can be due to observer's error, in addition to lack of guidelines and protocol to count the lines correctly, and the degree of exposure or experience each volunteer has in counting ILs. Nevertheless, the gemsbok's expected age is approximately 11 years of age ± 2, and the mean number of observed ILs from four observers for gemsbok region ID 1 is 11.3 (Table 2). The eland (Fig. 3a) presents thin, uneven lines across the cementum banding, which was measured on the distolingual side of the tooth. For this specimen, the mean number of ILs does not match the estimated age at death, and the irregular appearance of these layers suggests that further investigation is needed for this sample as there may be better locations of accumulation and countable ILs.

Seasonal information is inconclusive based on these three samples since a defined last accumulated layer must be discernable. For example, in the gemsbok (Fig. 2), it appears as if in some regions the layers continue beyond the annotations. The last layer (top of the image) marked with a yellow cross is lighter and thin. The gemsbok died during the wet season, which would correlate with the beginning of a lighter and thinner band. Thus, the last annotated band correlates with the known seasonal information for this specimen. Despite this correlation, it is not clear which is the last accumulated layer. On the eland, edge enhancement glared the last accumulated layer. For this reason, it was not possible to evaluate the appearance of the last accumulated layer, which might have provided seasonal information at the time of death. At 1×, the buffalo last counted layer marked by a yellow cross (Fig. 3b) is bright. This buffalo died at the end of the dry season, so the expected accumulated layer is a bright one, which correlates with the known seasonal information (Fig. 3b) [2]. These are mainly comparisons between observations based on the images and the known seasonal information and age estimates. Conclusions cannot be reached at this point, but the observations are encouraging for future investigations. A larger sample could provide information on the correlation between ILs and known date and season of death. Further image enhancement could improve the visualization of the layers in cases where is needed.

Developing ways to traverse the data that can be effectively applied in archaeological materials require a systematic protocol that facilitates time optimization, minimizing scanning and processing time. A standard procedure for tooth cementum analysis specifically designed for bovid teeth, is the direction we are heading. One challenge to overcome is image processing. For example, ring artifacts can interfere with the visualization of the layers, hindering the accurate assessment of the layers and other features. Additionally, edge enhancement often prevents the visualization of the last accumulated layer, although not in all cases. Image enhancement techniques in combination with other methods that are continuously developing, can contribute significantly to addressing these issues, but this requires expertise beyond the field of anthropology, making interdisciplinary, collaborative work essential for the application of this method. In conclusion, microtomography of tooth cementum ILs is a promising technique with the potential of reducing the need for using destructive methods such as tooth sectioning, while answering questions about patterns in ungulate mortality and the seasonality of hunting activities in prehistory.

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