Accelerated measurement of perikymata by an optical instrument

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Abstract: The proposed device considerably reduces the measuring time of important microscopic features of tooth crown surfaces. The instrumentation is accompanied by a computer program to analyse the results. Tooth enamel is formed by ameloblasts, which demonstrate daily secretory rhythms developing tissue-specific structures known as cross striations, and longer period markings that are referred as striae of Retzius. These striae correspond to linear structures on the enamel surface. This newly developed optical measuring instrument can automatically, precisely and accurately record the number and periodicity of perikymata on the dental crown. Furthermore it can characterize the variability in periodicity of perikymata in hominids. The depth of field can be extended as desired by taking several images with different focus positions and combining them into a single composite image that contains all regions fully focused.

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OCIS codes: (120.4820) Optical systems; (120.2830) Height measurements; (120.6660) Surface measurements, roughness; (240.5770) Roughness; (170.0170) Medical optics and biotechnology; (170.0110) Imaging systems; (170.3890) Medical optics instrumentation; (170.5810) Scanning microscopy, Teeth, enamel, Perikymata, Kromdraai, Hominin, Homo.

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Received 3 Jun 2013; revised 1 Aug 2013; accepted 4 Aug 2013; published 12 Sep 2013
1. Introduction

The study of human physiological development and its variability have been of central interest for anthro-biologists, pathologists, dentists, orthodontists and pediatricians, to name a few. Current heightened societal expectations in the field of biometric identification, which has witnessed a tremendous progress, encouraged a number of contemporary innovations such as the one presented here. Analysis of perikymata is of major theoretical interest, yet inadequately used in biometric identification. Now, the technology, which indeed permits high quality identification, paves the way to predicting more features, enhances more accurate definition and evaluation procedures, and above all encourages constant improvements. Our specific objective in this paper is to design and implement an optical system for measuring and analyzing
perikymata of the human tooth crown. The main contribution is to furnish a non-invasive time efficient methodology to count the number of perikymata. The goal is to determine the spatial periodicity structure of the perikymata for classification of fossil hominins and for the analysis of variable patterns of dental development in extant human populations. Indeed, the number and periodicity of perikymata on the enamel surface are a direct reflection of the timing and duration of crown edification [1, 2].

Quick measuring was designed with the following constraints:

1. automatic and portable setup (Hand-held tool),
2. no operator intervention,
3. measurement time for a tooth not to exceed ten minutes,
4. high spatial resolution,
5. low cost for the components.

A compact, elegant and user-friendly optical system has been constructed by the authors of this paper in the laboratory of the Université de Franche-Comté, France. Our instrument significantly differs from the previous devices based on electron microscopy, confocal microscopy and optical profilometry. Such instruments are not only much more expensive but also of considerably larger in size and heavier weights, which prevented easy portability [3–9]. Also, to add to a significant disadvantage, those techniques are considerably more time consuming in acquiring data.

2. Problem statement

2.1. Characterization of perikymata

The perikymata, which have depth of 2 to 5μm (Fig. 1) with the spatial periodicity ranging from 50 to 100μm, allows estimations of the following aspects:

1. duration and speed of formation of tooth crowns,
2. age determination individuals at the end of dental growth,
3. classification of hominid fossil and forensic identifiers.

Figure 1 show profilometric images on enamel surfaces along with perikymata.

2.2. Previous methods

Due to its highly accurate spatial resolution in measuring perikymata, confocal microscopy drew attention, [10, 11], subsequently, significant measurements have been reported in [9, 12, 13]. In 2000, in his PhD thesis, M. Yuan presented the analysis of 300 teeth via perikymata counting. It consumed a long experimentation time of close to three years [14, 15]. An Optical profilometer can measure the 3D topography of perikymata (see Fig. 1). It requires the tooth to be wrapped in metal. Our method seeks to overcome the drawbacks of the optical profilometer, namely:

1. difficulties in measuring the 3D profile due to the diffusion of light by the tooth;
2. experimentation time is very long (3 to 4 hours for the entire tooth) due to the small field of view of optical profilometer lens (0.64 × 0.48mm²);
3. measurement cannot be automated due to the non flat surface of tooth;
4. high cost of the measuring equipment.
2.3. The proposed method

The significant advancement by the authors is that the proposed optical device demands only a few minutes per tooth while capturing the bidirectional reflectance (BRDF) and bidirectional function of the texture (BTF). Each tooth is favorably guided to improve observations of neat perikymata by selecting the best incidence angle of light. This key idea led to the design of our apparatus and heads, and permitted the measurement of perikymata for detecting a texture to the order of a few micrometers.

![Image of micro-roughness off the crown: (a) Image of perikymata, (b) 3D profile of the selected area in (a), (c) 2D profile segment on figure(b).]

2.4. Reflectance and texture surfaces (BRDF/BTF)

The visual appearance of a surface is related to viewing and illumination directions, which can be characterized by the BTF (Bidirectional Texture Function) [16, 17]. At a fine scale, surface undulations cause the local intensity and image texture to vary. At a sufficiently coarse scale the local texture is the not resolvable and local image intensity stays uniform. The dependence of this image intensity on illumination and viewing direction is described by the BRDF (Bidirectional Reflectance Distribution Function) [18–25]. For each camera position, the sample is so oriented that its normal is directed towards the vertices of the faces, as illustrated in Fig. 2. With this arrangement, a considerable number of measurements is made in the plane of incidence when the directions of the light source and viewing lie in the same plane as the sample normal. Furthermore, for each camera position, a specular point is included where the sample normal bisects the angle between the viewing and source directions. Sample orientations with corresponding viewing angles or illumination angles greater than 85° (degrees) are excluded from the measurements to avoid self-shadowing [16].
2.5. **Illumination Model**

The physical principles utilized in our novel device is summarized here especially emphasising the effects of shadows and measurements based on the detection of the specular component of light. We select the best eligible angle that generates the shadows on the perikymata. Illuminating the tooth surface by a normal incidence causes reflection and diffusion that will decrease the image contrast. Selecting proper illumination angle we optimize the contrast, when the source of light is placed on the opposite angle of the camera relative to the normal of the surface measured (according to the Snell-Descartes law). The Shape-From-Shading (SFS) [26] model, which reconstructs the shape of objects from multiple images, yields a collection of normals (to boundary surfaces) and surface-depth vectors, termed to be ‘normal-maps’ and ‘height-maps,’ respectively. Our method illuminates the physical object in the right angle limit. Ambient light, shadows and specular are the three most important aspects that can contaminate the results determined from the Lambertian model. Two types of shadows, attached and cast (see Fig. 2), are respectively associated with the surface normal pointing away from the light source, and certain relief preventing the light from reaching some sample areas.

![Fig. 2. Illustration of Shadow Types.](image)

3. **Profile**

To achieve the measurement time not exceeding ten minutes a feedback control of the CCD lens position relatively to the surface of the tooth is devised. We deliberately added a metrology tool to determine the profile, which governs the feedback control of the camera angle and that of illumination, of the tooth surface and ensure the reproducibility of the measurement.

3.1. **Feedback control of the CCD lens position relatively to the tooth surface**

This method is best suited to help a profilometer trace back the profile of the tooth surface that will be used to automatically control the camera angle. The principle of confocal laser triangulation is our choice because we need precisely only the shape and not the height of the
profile. This option drastically reduces the number of images \( N_{\text{images}} \) given by:

\[
N_{\text{images}} = \frac{\Delta Z}{\delta}; \quad \delta: \text{the depth of field}
\]  

(1)

and \( \Delta Z \) is defined in Fig. 3(a), it appears as the period sampling of the 2D profile along \( Z \) axis. Steps \( \Delta x \) are chosen according to the slope of the profile. The camera will always be under an angle to the normal of the profile average slope, which depends on the curvature of the tooth surface, for the region to be explored, calculated according to the laser triangulation method.

3.1.1. Laser spot size incident in the laser triangulation

The resolution depends on the spot image obtained using the CCD camera that plays the role of a point sensor that aids to calculate the centroid of the spot. The thinnest diameter of the laser spot without distortion of the image spot is most desirable. For a good focusing of the diode laser beam a doublet of focal length 25 mm yielding a spot size approximately \( \sim 30 \mu m \) is used, vide Fig. 4. A standard procedure of image processing circumscribes the stain and calculates the centroid of the spot image obtained by using the microscope 10× objective lens on the CCD camera. The initial adjustment of the microscope objective captures the spot perfectly at the center of the sensor. While scanning for \( x_w \) or \( y_w \), to determine the tooth profile, the stain will move along the direction the axis motion. Our algorithm then acts on an engine displacement

Fig. 3. (a) Determination of the number of images \( N_{\text{images}} \) with zero viewing angle. (b) Determining the number of normals for a viewing angle \( \theta \).

Fig. 4. (×10).

Fig. 4. Spot image obtained (×10).
micrometer to vary the camera position along the axis $Z_{CCD}$ in the right direction to align the spot centroid with the center of CCD.

### 3.2. Saving the tooth profile

![Principle of reading 2D profile of the tooth](image1)

To save the 2D profile, say $z_{CCD}(x_w)$ of a tooth, we rely on the feedback control of the position of the target at a distance that equals the tooth working distance while moving the tooth axially along the $x_w$ direction (Fig. 5(a)). The incremental step of moving the tooth is $50 \mu m$ and at a speed that matches the acquisition time of an image and calculating the displacement of the spot, which is proportional to $Z$ and has a value of the approximately (100 ms). Figure 5(b) shows the 2D profile of the permanent right central incisor mandibular $KB5223$, a fossil remnant from the site of Kromdraai B (South Africa).

### 4. Measurement of tooth texture

![Medium shot profile of a step profile total tooth](image2)

Fig. 6. (a) Medium shot profile of a step profile total tooth, (b) Representation of the medium plane of the 3D topography of a region, the crown of the tooth.

We start with a prior tooth profile, see Fig. 5(b), subsequently, we calculate the medium plane of that profile as shown in Fig. 6(a). We measured the 3D profile, see Fig. 6(b) corresponding to the 1D profile of Fig. 5(b) by performing a shift, in working coordinates, $x_w$ and $y_w$ directions see Fig. 3(b).

The spatial resolution of an image is $50 \mu m$, it allows us to evaluate the error we make on the approximation of the mean plane of the tooth. We determine the normal to the plane through the point as $x_w = \frac{\Delta x}{2}$ (see Fig. 3(b)), $\Delta x$ is the sampling period in $x_w$ axis. And then, we direct
our measuring head (CCD camera and light source) such that it bisects the angle between the camera and the source and is aligned with the normal.

We then measure the texture of the tooth by recording \( n \) images of the surface of the tooth (see Fig. 7), according the equation 1, the whole perpendicular planes to the camera between the plane of the highest profile (see Fig. 8) and the plane of the lowest profile. This series of topographic textures pertaining to the searched area. For each image only very clear and neat areas, according to an algorithm based on shapelet, are identified and saved. An example is illustrated in Fig. 9 which gives the texture obtained for sample step of 50 \( \mu m \).

![Fig. 7. Principle of image reconstruction of the texture of an area.](image)

![Fig. 8. Alignment of the normal mean plane, with the bisector of the angle between the camera and the source.](image)

![Fig. 9. Surface texture of the lower right permanent central incisor of KB5223 (see Fig. 13), a fossil hominin from Kromdraai B (South Africa)(see Fig. 5(b)).](image)
4.1. System for measuring tooth texture

The optical system was designed (see Fig. 10(a)) to examine the surface quality of a set of teeth a 2 Myr (million years old) fossil from Kromdraai B (South Africa) which was attributed to Homo habilis by Braga and Thackeray 2003 [27].

![Optical system for measuring perikymata](image)

To realize the extent of the profile and texture of teeth we have to move a tooth by $x_w$, $y_w$ and $z_w$ and CCD camera by $z_{CCD}$ and rotation of the measuring head. The optical system comprised of a microscope $10 \times$ objective lens of long working distance enhanced by appropriate corrections of the fluorite aberration and flat field optics. The field of view of the CCD camera, is approximately, $2.5 \times 2 \text{ mm}^2$ and $90 \text{ μm}$ of depth of field. The CCD camera was placed on a translation stage of $25 \text{ mm}$ travel distance and $0.05 \text{ μm}$ of minimum incremental motion.

This versatile instrumentation was limited to perikymata measurements.

5. Results

5.1. Reconstruction of tooth along the measured profile

Figure 11 shows the 2D texture of the tooth after juxtaposing images of all its areas.

![Surface texture of the lower right permanent central incisor](image)

Following this reconstruction, we can quantify the variation of the perikymata periodicity.
5.2. Counting perikymata

For counting perikymata, we have developed a workable algorithm based on conventional methods of image processing, namely, binarization, filtering, segmentation and finally squeletisation. Satisfactory results were obtained after user interventions to correct the deviations of the results from the original ones. We adopted a more convenient procedure that consists of calculating the profile of each line, then the minimum for each profile, and corrections based on averaging periods of twenty profiles. As shown in Fig. 12(a) and Fig. 12(b), the minimum of the profiles corresponds to perikymata. After manual correction of this result, we obtain the score of perikymata shown in Fig. 12(b).

![Fig. 12. (a) Images of perikymata and profile of a line. (b) Perikymata tally of the tooth. (c) Periodicity of perikymata area explored.](image)

Finally the number of perikymata are counted and the spatial variation frequencies of these perikymata, as shown in Fig. 12(c), were determined.

5.3. Periodicity of fossil perikymata

The Kromdraai B site (South Africa) is located 1.5 km east of the Sterkfontein cave and has yielded 27 hominid specimens representing a minimum of nine individuals [28], that have been the subject of a number of significant studies. Since the work of Bromage and Dean (1985), several researchers have emphasized that the periodicity of perikymata (often referred as ‘perikymata packing pattern’) represent a good morphological indicator for distinguishing hominin taxa (genera or species) with different patterns of dental development which, in turn, help to establish their phylogenetic relationships. The method currently used to measure this periodicity is to divide arbitrarily the height of the crown in ten segments (or deciles) of equal length. For each segment, the number of perikymata is then counted and displayed on a graph [1]. The drawback of this method is the reproducibility of intra- and inter-observer, the counting accuracy the perikymata, particularly on the other edges of the deciles. One of the most contentious points about the identification of fossil hominins is related to their allocation to the earliest members of the genus *Homo*. As regards the periodicity of the perikymata at the cervix, the first representatives of the genus *Homo* could be characterized, as opposed to either *Paranthropus* or *Australopithecus*, by a tightened periodicity near the cervix. It is generally accepted that Plio-Pleistocene hominins, compared to extant humans, are characterized by a smaller number of perikymata, for the same type of tooth crown. The smaller number of perikymata means a shorter duration of crown formation. The periodicity of perikymata provides information on the rate of formation of the crown along its length. The results published so far indicate that in extant humans, whereas perikymata are widely spaced near the cusp tip, the periodicity decreases towards the cervix. This decrease in the periodicity along the crown rom the cusp tip...
to the cervix indicates that the lateral component of enamel growth (the extension rate) slows down towards the end of crown edification (Dean and Reid, 2001 [1]). In *Paranthropus*, the extension rate remains constant (and relatively high, as compared to extant humans) as indicated by equally spaced perikymata along crown length (Dean and Reid, 2001 [1]; Lacruz, 2007 [9]). However, the variability in the perikymata periodicity remains largely unknown in extant human populations, as demonstrated by Guatelli-Steinberg et al 2005 [29] when their considered broad comparative sample. Therefore, the range of extant human periodicity of perikymata is still largely not known enough to identify reliably and accurately when our uniquely pattern of crown growth arose in the course of evolution. A specific example of our study is now presented.

![Fig. 13. The lower right permanent central incisor of KB5223, a fossil hominin from Kromdraai B (South Africa)(views 1 to 4), (1) vestibular, (2) distal, (3) lingual, (4) mesial.](image)

pertaining to the periodicity of perikymata on anterior permanent teeth of the specimen KB5223 that has been examined by confocal microscopy, Lacruz [9]. This comparison, verification, and the discussion and interpretation of results is limited to the four permanent incisors described for KB5223 (Grine 1982; Braga and Thackeray 2003 [27]) These 4 teeth are isolated. The tooth (the right central incisor) for which Lacruz [9] counted the number of perikymata (and obtained the figure of 86) has an incomplete crown. The left central incisor also shows an incomplete crown. For the right central incisor studied in [9], the periodicity was not specifically mentioned. The author simply observed a: ‘... perikymata distribution on the I1 of KB5223 just a few microns from the cervix, displaying a pattern of nearly evenly spaced perikymata near the cervix, which appears to be a feature associated with Paranthropus and not with Homo.’ (p. 180). In the present study, we recorded a strong variation of the periodicity between 59 and 94 microns at mid-crown height (Fig. 14, striae 25 to 32). Near the cervix (Fig. 14, striae 5 to 9), the values decreased significantly and ranged between 32 and 42 microns. A simple visual comparison of two histograms clearly shows that from the middle of the crown up towards the cervix, the periodicity increased from about 60 microns to 40 microns where the specific area of the crown was not preserved. In the light of Lacruz’s conclusion our precision of measuring the periodicity should be further discussed. We can apply our system not only to other teeth of KB5223 but also to any other type of measurable fossil tooth. The KB5223 lateral incisor deserves more attention because the crown area near the cervix is very well preserved, hence more precisely measurable. Figure 15 and 16 show results obtained on the right lateral incisor of KB5223. In this case, we record 97 perikymata, a much higher number as compared to the 86 perikymata encountered on the right central incisor of the same specimen. In Fig. 16 the perikymata closest to mid crown height (striae 35 to 42) are observed in the area of the crown and the periodicity lies between 42 and 84 microns. Again, this strong variation is worth noting. In our observation of the periodicity for the preserved area near the cervix area (striae 1 to 9), values decreased significantly and ranged between 17 and 37 microns. As for the central
incisor, the periodicity decreased clearly between the middle part of the crown and the cervix area.

6. Discussion

First of all, our method provides reliable results, since they are very close to those obtained by other authors on the same fossil with confocal microscopy [1] [9]. Moreover, our count of perikymata is fully automatic and very fast (a few minutes). Finally, in addition to general statements on periodicity of fossil teeth (e.g., Lacruz, 2007 [9]), we can provide a precise measures of periodicity. Hillson and Bond in 1997 [30] [11] provided data on changes in the periodicity in extant humans. Our values obtained for \textit{KB}5223 in the cervical area of the crown are very similar to those known for extant humans in homologous areas of crown height. Regarding the genus \textit{Paranthropus}, the data are most often expressed in deciles, and it is therefore difficult to compare our results to these data. Nevertheless, if we consider data graphically provided by Dean and Reid in 2001 [1], it seems clear that the periodicity of perikymata at the cervix in \textit{Paranthropus}, is hardly less than 100 microns. This \textit{Paranthropus} value is much higher to the one measured on the two lower permanent incisors of \textit{KB}5223. In the cervical areas of both crowns (the right central or the right lateral incisor), the periodicity was found to be 50 microns.
The variation of the periodicity of perikymata is only very partially known in extant humans. Extant human values can overlap with the currently observed values in fossil hominin. Our tool then provides an objective tool to increase of knowledge using larger extant comparative samples, to compare these results with fossil hominin values, and to interpret them in terms of developmental patterns and phylogenetic relationships. However, to reach this goal, the redefinition of the reference plane (which should be the medium plane of the tooth crown) needs to be defined by more than one 2D parallel profile measurement (see Fig. 5(a) for better determination). This is essential for more complex instrumentation.

7. Conclusions

Studies on enamel microstructures are important to assess dental development mechanisms in fossils. We aimed to develop a noninvasive, simple and efficient optical profilometer in order to offer a new approach to measure and to highlight the perikymata spatial periodicity. We developed algorithms of image processing associated with an optical instrumentation. Our method allows the determination of 3D crown surface profiles with perikymata periodicities of 40 to 100 \( \mu m \) and heights of a few microns. Our measurements can be achieved in only a few minutes per tooth. Our setup considerably accelerates and improves the accuracy of the count and periodicity of the perikymata. Thus the instrumentation is anticipated to be widely used to characterize the variability in extant humans and to classify fossil hominins. In this study, we demonstrate with observer-independent, accurate and reliable measurements, that the periodicity of perikymata decreases towards the cervix in two fossil hominin teeth from Kromdraai B: one central and one lateral permanent incisor of KB5223. It is difficult to compare our results with data on \textit{Paranthropus} because they are most often expressed in deciles. Nevertheless, if we observe data graphically provided by Dean and Reid in 2001 [1], it seems that the periodicity of perikymata at the cervix in \textit{Paranthropus} is hardly less than about 100 microns; i.e. much higher than the one measured on KB5223. The measuring time of a tooth was close to ten minutes and permitted a practical and objective assessment. Given the results presented here, we can emphasize the relevance of our approach to immensely facilitating the measurements of perikymata of fossil hominin teeth and in extant samples.
Acknowledgments

This study was partly supported by the European Commission Marie Curie Research Training Network (European Virtual Anthropology Network; http://www.evan.at), according to the Contract MRTNCT – 2005 – 019564 (FP6).