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# Surface Topography: Metrology and Properties



## PAPER

### Surface form analysis on complex freeform organic structures – measuring erosive wear on human teeth in vitro

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## Abstract

Natural human enamel (NHE) is a complex freeform surface which has presented significant difficulties in measuring surface form change using non-contacting laser profilometry (NCLP). Measuring surface form change on NHE is a metrology proxy for measuring dental tooth structure loss, and characterising this using non-ISO parameters (volume, surface area, and normalised lesion depth) has been seldom studied due to surface complexity and undetermined measurement errors. This study determines NCLP measurement errors (instrument repeatability and method reproducibility) for non-ISO parameters, characterises change in surface form on NHE following a dietary pH-cycling model. NHE ( $n = 1$ ) was scanned consecutively twenty-times using NCLP with/without sample replacement producing consecutive surface profile data. Residual data was created after subtracting consecutive filtered profile data ( $80 \mu\text{m}$ , Gaussian filter), and mean (SD) volume, surface area, and normalised lesion depth was determined within a  $1.5 \text{ mm}$  circular region of interest (ROI). Volume error (expressed as height variation across ROI surface area) was  $0.022 \mu\text{m}$  (instrument repeatability) and  $0.149 \mu\text{m}$  (method reproducibility), whilst surface area error (expressed as percentage change of the surface area deviation across the entire surface area) was  $0.034\%$  (repeatability error) and  $0.081\%$  (reproducibility error). Sixty-four natural enamel surfaces taped with polyvinyl-chloride tape leaving  $1.5 \text{ mm}$  exposed ROI underwent dietary erosion cycling (three 5-min cycles,  $0.3\%$  citric acid w/v, pH 3.2) generating artificial erosion lesions. Samples were scanned with NCLP before/after each erosion cycle, scans filtered for microtexture, and after-erosion scans were subtracted from before-erosion scans. NCLP results show mean (SD) volume, surface area, normalised depth, and 3D step-height of the eroded area increased significantly after each erosion cycle, with no significant difference in calcium and phosphate release after each cycle. We demonstrate a robust and valid dental model with analysis workflow to measure surface form change in NHE using NCLP, improving understanding of measuring surface form change in complex freeform surfaces.

## 1. Introduction

Surface profilometry remains the gold-standard for measuring surface form changes to characterise enamel loss, provided the loss of tissue is within measurement/operating limits of the profilometer [1–4]. The outer surface of natural human enamel comprises a complex freeform surface consisting of irregular peaks and troughs which have been previously shown to be difficult

to measure accurately. The reason for this difficulty lies in the ability of a profilometer to trace these surface features, either contacting or non-contacting, which can lead to measurement inaccuracies if these features are too steep (beyond the angular tolerance), too deep (beyond the vertical measurement range) or too narrow (outside the lateral resolution) [5–7].

Previous research using natural human enamel has minimised the problems associated with

measuring this complex freeform surface by utilising profile subtraction of before and after scans to produce a residual data set, from which data analysis are conducted [4]. This is a type of form removal, which minimises the impact of the irregular peaks and troughs by subtracting one data set from another, leaves a residual data set containing points of difference [4].

Dental research studying erosive toothwear, have used changes in surface form, measured using non-contacting laser profilometer (NCLP), as a proxy for quantifying enamel loss from natural human enamel in vitro [4]. The quantification of enamel loss using three-dimensional (3D) step height formation has been used on natural human enamel by using the profile subtraction method and analysing the residual data set [4]. This is a feat which was previously limited to research using artificially flattened enamel samples [8, 9].

Accurate and reliable analysis of surface form change on natural human enamel using NCLP has been limited to 3D step height change, because a reference region of uneroded enamel is required for comparison to the erosion lesion [4]. In previous research, the quantification and characterisation of 3D step height as the measure of surface form change on natural human enamel had reliability errors of  $0.28 \mu\text{m}$  (instrument repeatability error) and  $0.43 \mu\text{m}$  (method reproducibility error) respectively [4]. Other characterisation techniques which do not require reference/comparator regions to allow for surface form change characterisation include change in volume, surface area, and normalised depth. The errors associated with measuring these changes have not been studied to the same degree of accuracy and reliability on the natural human tooth surface [1–3].

Chemical analysis measuring calcium ( $\text{Ca}^{40}$ ) and phosphate ( $\text{P}^{31}$ ) released into acidic solution have been previously studied on polished enamel studies using mass spectroscopic [10–12] and photometric [13] techniques and have varied by method of reporting. In early erosion models, typically calcium release values have been reported between  $2.4\text{--}28.6 \text{ nmol}$  [13] and  $0.18\text{--}0.87 \text{ mg ml}^{-1}$  [10], and  $1.4\text{--}17.2 \text{ nmol}$  for phosphate release [13], whilst others have reported their release normalised by the surface area of enamel exposed to acid with calcium release reported at  $3\text{--}33 \text{ nmol mm}^{-2}$  [12]. However, inductively coupled plasma mass spectroscopy (ICP-MS) has not been used to analyse acid erosion of natural enamel surfaces.

The aims of this work were to determine the measurement errors of volume, surface area, and normalised depth on natural human enamel using NCLP and compare the output to chemical analysis.

## 2. Materials and methods

Extracted, caries free, human permanent molar teeth were collected under ethical approval (REC: 12/LO/

1836) with patient informed written consent. Sixty-four enamel sections from the buccal or palatal/lingual portions were produced, with approximate equal dimensions ( $5 \text{ mm} \times 5 \text{ mm} \times 3 \text{ mm}$ ), using a  $300 \mu\text{m}$  diamond wafering blade, and fixed on  $76 \text{ mm} \times 26 \text{ mm} \times 1 \text{ mm}$  glass microscope slides (Marienfeld GmbH & Co. KG, Germany) using epoxy resin adhesive (EverStick, Everbuild Products Ltd., UK). All samples were cleaned of any organic and inorganic surface contaminants using a 10-minute immersion in 4.7% sodium hypochlorite, followed by 30-minute ultrasonication in distilled water, air drying, followed by a 2-minute alcohol wipe using cotton buds [4].

Clear polyvinyl chloride (PVC) protective barrier (tape) with a 1.5 mm diameter central circular hole was applied to each specimen over the maximum bulbosity of each tooth section using the tandem scanning confocal microscope (TSM) to detect the peak in focus under  $\times 20$  magnification [4].

A NCLP, with 655-nm confocal displacement laser, mounted on an automatic motion system (XYRIS 2000CL, TaiCaan, Southampton, UK) was used to scan the tooth surfaces. The NCLP had a  $2 \mu\text{m}$  laser spot size with spatial (X,Y) resolution  $< 1 \mu\text{m}$ , 10 nm axial (Z) resolution and 0.6 mm Z-range [4, 14]. Scanning was conducted in a temperature ( $21.0 \pm 0.5 \text{ }^\circ\text{C}$ ) and humidity ( $47 \pm 5\% \text{ RH}$ ) controlled room, and followed good metrology scanning guidelines [15]. To ensure minimisation of thermal variation on scanning, the samples were allowed to thermally equilibrate for a period of 30 min prior to initiating each scan [4, 14]. Instrument repeatability and method reproducibility error in measuring 3D step height using NCLP was previously determined as  $0.28 \mu\text{m}$  and  $0.43 \mu\text{m}$  respectively. This was defined as the mean variation in height across the entire surface of the scanned tooth surface between repeated scans of the same tooth sample [4].

Surface form measurements using the NCLP consisted of a  $3.5 \times 3.5 \text{ mm}^2$  rectilinear grid with points measured at  $10 \mu\text{m}$  intervals ( $351 \times 351$  points); the data consisted of 123,201 individually scanned measurement points.

NCLP repeatability error (error due to uncertainty in the instrumentation) and reproducibility error (error due to instrumentation and operator accurately locating the sample) for measuring volume/surface area/normalised depth were first determined. A single human natural, unpolished, enamel sample was scanned with NCLP twenty times—10 times without moving (instrument repeatability) and then the sample was picked up and replaced 10 times (method reproducibility). Surface profile data filtered to remove microtexture using an  $80 \mu\text{m}$  Gaussian filter. This produced 20 surface form data sets; an example is seen in figure A1. Each surface form data was subtracted from the previous one to produce 18 residual data sets which represented the uncertainty between measurements.

These residual data sets were restricted to the central 1.5 mm circular region and measurements for volume ( $\mu\text{m}^3$ ) and surface area ( $\mu\text{m}^2$ ) of the region of interest were determined; normalised lesion depth ( $\mu\text{m}$ ) was then calculated by dividing the volume by surface area. The mean and standard deviation for each measurement was calculated from the 18 residual data sets (nine instrument repeatability and nine method reproducibility residual data). The instrument repeatability and method reproducibility error for volume was determined dividing the deviation in volume across the surface area of the residual data sets and expressed as a height variation across the surface area. Whilst the repeatability and reproducibility error for surface area was calculated by dividing the standard deviation by the mean values for surface area for the repeatability and reproducibility and expressed as a percentage of the sample surface area.

After determining NCLP instrument repeatability and method reproducibility error in measuring volume, surface area and normalised depth on one natural enamel sample, the remaining 64 enamel samples were all scanned with NCLP before erosion cycling to produce baseline scans.

The samples underwent pH-cycling which consisted of three 5-minute cycles of immersion in citric acid (0.3%, pH 3.2) at room temperature ( $22 \pm 0.5$  °C) using an orbital shaker (62.5 RPM, Stuart mini-Orbital Shaker SSM1, Bibby Scientific, England), followed by a 2-minute wash with deionised water. The PVC tape was then carefully removed and the samples allowed to air dry for 30 min, before being scanned using the NCLP to produce after-erosion scans [4].

The NCLP data (before and after erosion) was filtered to remove micro-texture (80  $\mu\text{m}$  Gaussian filter) to leave only surface form data. The after-erosion data was subtracted from the before-erosion data to produce a residual data set corresponding with each 5-minute erosion cycle.

This data was loaded into surface metrology software (MountainsMap, DigitalSurf, France), and the boundary of the erosion wear scar was manually mapped. Volume loss ( $\mu\text{m}^3$ ), surface area ( $\mu\text{m}^2$ ), normalised depth ( $\mu\text{m}$ ), and 3D step height ( $\mu\text{m}$ ) were calculated; with means and standard deviations calculated. These were generated by comparing a reference surface to the 1.5mm region of interest created automatically by the metrology software (MountainsMap, DigitalSurf, France).

Percentage change compared to 5 min erosion was calculated as  $\left(\frac{B - A}{A}\right) \times 100$  where A is the value for volume or surface area or normalised depth at 5 min erosion, and B is the value for volume or surface area or normalised depth at 5, 10, or 15 min.

The citric acid used in each pH-cycle was collected in 15 ml polypropylene tubes and stored immediately in cold storage ( $-80$  °C). The citric acid was analysed for total calcium 40 and phosphate 31 release using

inductively coupled plasma mass spectroscopy (ICP-MS, PerkinElmer NexION 350D), and expressed as  $\mu\text{g l}^{-1}$ .

Statistical analysis was conducted using GraphPad Prism 7 (GraphPad Software Inc, California, USA). Data was initially assessed for normality using Komogorov-Smirnov and Shapiro-Wilks tests and were deemed normally distributed; mean and standard deviations were therefore reported. Parametric testing one-way repeated measures ANOVA with post-hoc Tukey test for multiple comparisons was conducted to determine differences for each measurement output and erosion cycle.

### 3. Results

The repeatability and reproducibility error for volume was as  $0.022 \mu\text{m}$  ( $\pm 1 \sigma$  of instrumentation repeatability uncertainty) and  $0.149 \mu\text{m}$  ( $\pm 1 \sigma$  of instrumentation and methodology reproducibility uncertainty) over the measurement area region. The repeatability and reproducibility error for surface area were respectively 0.034% and 0.081% across the enamel surface. A representative example of the erosion lesion visualisation and analysis is shown by figures A2 and A3.

Mean (SD) erosion lesion volume ( $\mu\text{m}^3$ ) and surface area ( $\mu\text{m}^2$ ) both increased after 5, 10, and 15 cumulative minutes and are shown in table A1 and figure A3. Mean (SD) volume loss ( $\mu\text{m}^3$ ,  $n \times 10^6$ ) after 5, 10, and 15 cumulative minutes were 1.91 (0.78), 3.03 (0.80), and 3.76 (0.67)  $\mu\text{m}^3$  respectively, whilst the mean (SD) surface area ( $\mu\text{m}^2$ ,  $n \times 10^6$ ) were 1.72 (0.25), 1.80 (0.27), and 1.87 (0.34), respectively. Multiple comparisons revealed statistically significant ( $p < 0.01$ ) differences in volume loss between each erosion time, and surface area between 10 and 15 min when compared to 5 min erosion ( $p < 0.05$ ).

Percentage change in erosion lesion volume and surface area for 10- and 15-min erosion increased sequentially when compared to values obtained at 5 min; mean erosion lesion volume increased by 58.7% (10 min) and 97.0% (15 min), whilst mean erosion lesion surface area increased by 4.6% (10 min) and 8.3% (15 min).

Mean (SD) normalised depth ( $\mu\text{m}$ ) after 5, 10, 15 min were 1.11 (0.43)  $\mu\text{m}$ , 1.70 (0.50)  $\mu\text{m}$ , and 2.05 (0.42)  $\mu\text{m}$  respectively which represented a 53.2% (10 min) and 84.7% (15 min) increase when compared to normalised depth at 5 min. This was statistically significant between each erosion time ( $p < 0.05$ ).

Mean (SD) 3D step height ( $\mu\text{m}$ ) after 5-, 10-, and 15-minutes erosion were 1.26(0.33)  $\mu\text{m}$ , 1.89 (0.43)  $\mu\text{m}$ , and 2.51 (0.65)  $\mu\text{m}$  respectively. This represented a 62.5% (10 min) and 116.3% (15 min) increase in 3D step height when compared to the value obtained at 5 min erosion. Multiple comparisons revealed

statistically significant ( $p < 0.0001$ ) differences in mean 3D step height between each erosion time ( $p < 0.0001$ ).

Total calcium and phosphate release for each erosion cycle can be seen in figure A4. Mean (SD) total calcium release ( $\mu\text{g l}^{-1}$ ) after 5, 10, and 15 min was 2749.1 (820.5)  $\mu\text{g l}^{-1}$ , 2646.3 (841.0)  $\mu\text{g l}^{-1}$ , and 2709.8 (1058.1)  $\mu\text{g l}^{-1}$ . Similarly, mean (SD) total phosphate release ( $\mu\text{g l}^{-1}$ ) after 5, 10, and 15 min was 83.6 (34.8)  $\mu\text{g l}^{-1}$ , 52.2 (40.1)  $\mu\text{g l}^{-1}$ , and 61.3 (46.4)  $\mu\text{g l}^{-1}$  respectively. There was no statistically significant ( $p > 0.05$ ) difference between erosion times for either calcium or phosphate release.

#### 4. Discussion

The dental model utilised in this study allowed for the determination of the measurement capabilities of NCLP in measuring surface form change on a complex organic freeform surface using natural tooth surfaces. By determining the repeatability and reproducibility errors for measuring volume, surface area, normalised depth, and 3D step height, we have been able to demonstrate the validity and robustness of the dental model in measuring changes to surface form over time. This study demonstrated that erosion lesion volume, surface area, normalised depth, and 3D-step height could be calculated on natural human enamel samples and increased with increasing erosion time.

Additionally, we demonstrated and validated a unique method to characterise the progression of dental erosion on natural enamel, over time, using changes in volume, surface area, normalised depth and 3D step height. Additionally, chemical analysis confirmed that total calcium and phosphate release remained consistent for each 5-minute erosion cycle. This corroborates the formation of the initial erosion lesion together with erosion lesion volume, surface area, normalised depth, and 3D-step height.

In dental research, the study of erosive toothwear has proved a technical and difficult challenge owing to the complex topography of the tooth surface. The study of early signs of erosion remains a lasting goal to allow for the development of new strategies for diagnosis and management. Typically, the characterisation of early erosion lesions has been previously studied on polished enamel specimens from different biological sources primarily of human and bovine origin [1, 2, 16–20]. Polished enamel has been, and still remains, the substrate of choice in erosion research due to the ease and standardisation of sample preparation, ease of sample protection to allow differential wear analysis, and ease of post-erosion analysis [6]. However, this approach uses a polishing step which removes the outer mineral rich layer. To determine the presence and subsequent progression of these natural surface erosion lesions, changes in surface form, surface roughness, and surface optical properties have been studied previously [18, 21–25].

A significant issue overcome in this study was in the localisation and characterisation of surface form change in the dental tooth model. Previous studies that utilised polished enamel samples and non-contacting measurement systems such as NCLP [1, 9, 19, 21, 26], confocal laser scanning microscopy (CLSM) [27, 28], and vertical scanning interferometry (VSI) [29, 30] have utilised single post-erosion scans to determine bulk enamel loss using a number of different surface form outcomes including mean single point step height [19, 26, 31] and mean 3D step height [1, 2, 32]. These outcomes are based on ISO 5436–1 which calculates step height according to the difference in height between the base of a given wear scar and one or two flat reference plane(s) either side of the wear scar [33]. For studies utilising polished enamel this is relatively simple as the protected regions of enamel will already be relatively flat making comparison with the eroded scar easy to calculate; and if there are any deviations in form then these can be mathematically corrected for to allow step height determination using form removal [1, 2, 34]. However, complex freeform surfaces do not have a mathematically definable form that can be used for form removal purposes during analysis [35, 36].

In our study we utilised the gold standard measurement technique for determining profile change, NCLP, using whole unpolished natural enamel samples. We could obtain similar results by utilising profile subtraction, between baseline and after erosion scan data, to characterise changes in surface form using changes in volume, surface area, normalised depth and 3D step height.

The methods in this study expand on work previously conducted by Mylonas et al [8]. Both our studies utilised similar Gaussian filtering to remove microtextural features and noise on surface form data before subtracting before/after scan data to obtain residual data. Our previous study demonstrated the instrument repeatability error and method reproducibility error of using 3D step height to measure surface form change, in the present study we additionally determined the instrument repeatability and method reproducibility errors for measuring non-ISO parameters volume, surface area, and normalised depth. Our study therefore helps better understand the limit of NCLP when applied to measuring surface change using non-ISO parameters. By subtracting after erosion data from its pre-erosion scan, this served as a form of form fitting which together with the Gaussian filter, produced a more stable form datum from which to measure any changes in form. The process of data filtering is an accepted method to ensure removal of any unwanted data (background, noise, or other sources) and isolation of specific data sets according to the type of analysis to be performed [37]. However, it is unknown whether different filter lengths or parameters have an impact on the accuracy of final data analysis and thus would require future study to

determine its impact on the analysis of data obtained from NCLP.

Our data after 5 min erosion indicated changes in volume, normalised depth, and 3D step height above the stated reproducibility and repeatability error of the NCLP for each measurement. Interestingly, only surface area measurement was lower than the associated reproducibility and repeatability error. As a result, for the methods used in this study, the NCLP method gave robust data after just 5 min erosion time for changes in volume, normalised depth, and 3D step height; whereas 10 min erosion time was required before robust data could be obtained for surface area change.

Our data indicated the process of surface form change increased with each pH erosion cycle. This was demonstrated by the increasing percentage change versus 5 min erosion, in volume, surface area, normalised depth, and 3D step height. This suggests the erosion process in natural human enamel increases even though the erosive solution, erosion time, and temperature were consistent. The erosion characteristics of natural human enamel differs according to depth away from the amorphous surface layer (outermost layer of enamel) towards the amelodentinal junction (ADJ, the boundary marking the end of enamel layer and start of dentine layer of a tooth); rate of erosion increases as the ADJ is reached due to the decrease in Fluoride ( $F^-$ ) and Calcium ( $Ca^{2+}$ ) content and increase in impurities (Magnesium ( $Mg^{2+}$ ) and Carbonate ( $CO_3^{2-}$ )) [5, 38, 39]. Our results also agree with Zheng et al [38, 39] who also demonstrated the wear characteristics of human enamel differed according to the region being eroded with the amorphous outer enamel layer exhibiting most resistance to citric acid erosion versus enamel close to the DEJ [38, 39].

The calcium and phosphate release indicated no statistically significant difference between erosion times, indicating for the same erosive conditions, the same quantity of calcium and phosphate was released from the surface of natural human enamel into the citric acid solution after each 5 min cycle. This is contrary to findings from previous studies, utilising polished enamel, which demonstrated that calcium and phosphate release increases with increasing erosion time [10, 11]. This may be explained chemically and in terms of differences in erosion cycling method. Chemically, if conditions for each erosion cycle are kept consistent—same acid solution, same temperature of acid solution, and same erosion time—the total calcium and phosphate released into the erosive solution should remain consistent. As a result, an erosion cycling method where all samples are eroded equally under the same conditions will result in similar/same amount of calcium and phosphate release—as seen in our study. However, in a previous study by Rakhmatullina et al [40], used a erosion cycling method where samples were grouped and eroded independently with 8 different erosion times (with the same acid solution and acid temperature). This study demonstrated a linear

increase in calcium release with increasing erosion time, which is to be expected if different groups under erosion cycling with different erosion times [40]. If the data in our study was considered according to cumulative erosion times i.e. 5, 10, 15 min, then indeed our study also demonstrated a linear increase in calcium/phosphate release. Additionally, Hookham et al 2020 demonstrated that calcium/phosphate loss following acid attack (1% citric acid) on bovine enamel resulted in deeper penetration of rhodamine B dye and thus an increase in fluorescence volume measuring using confocal laser scanning microscopy. Their study showed that as erosion lesion depth increased, so did the fluorescent volume detected due to the deeper penetration of rhodamine B dye into the eroded enamel [41]. However, whilst their study used polished bovine enamel, the principle outcome they demonstrated could potentially translate to the human enamel model; though this would require future study.

## 5. Conclusion

Surface form change was evaluated successfully and robustly in a dental tooth model after determining NCLP repeatability and reproducibility errors for measuring volumetric, surface area, normalised depth, and 3D step height. Erosion lesions increased in volume, normalised depth, and 3D step height after each erosion cycle. The applications of the methods utilised in this dental model can be applied in many different areas where form analysis of organic freeform structures is required; this has potential for application in medical, dental, and engineering research.

## Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

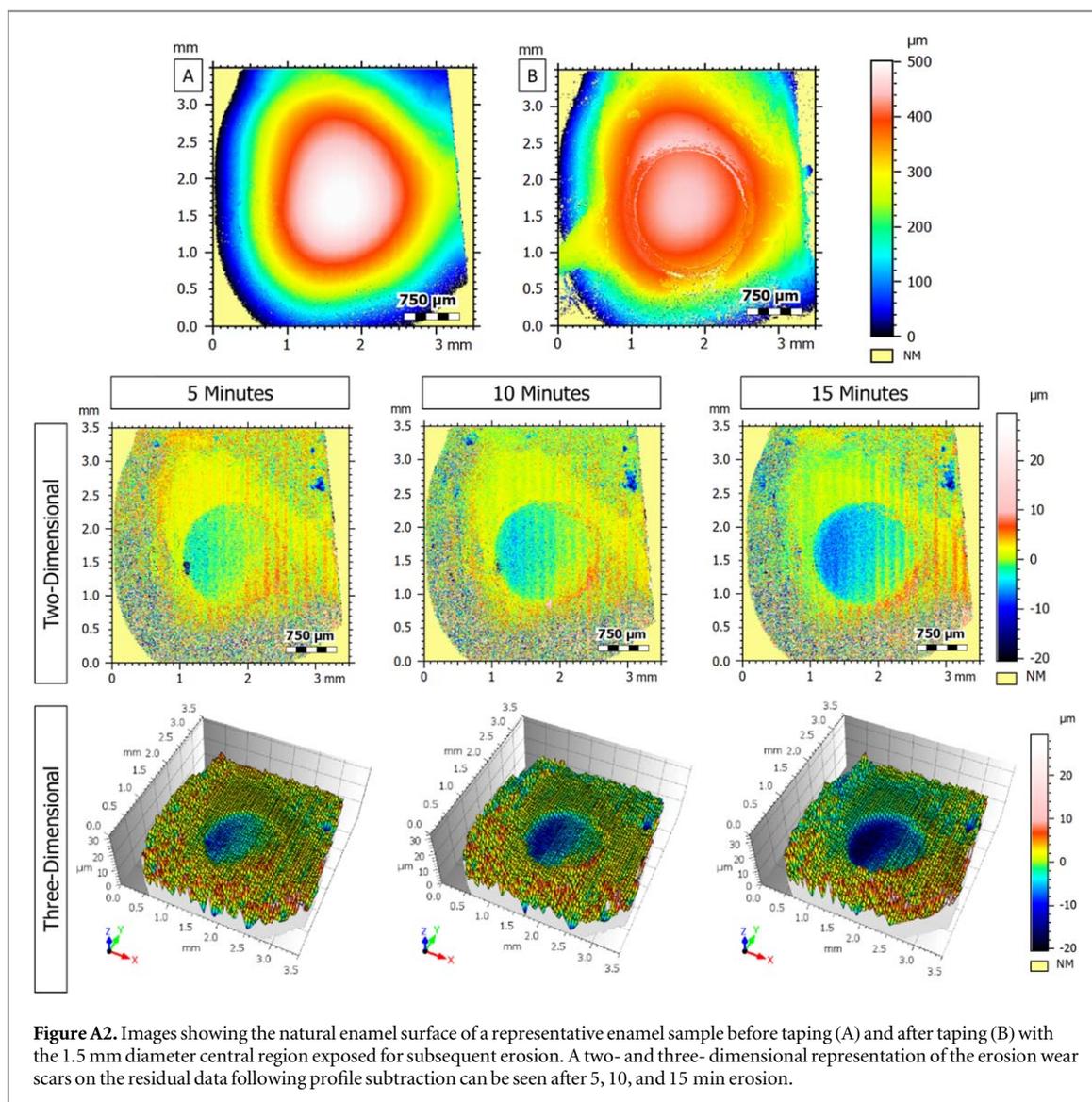
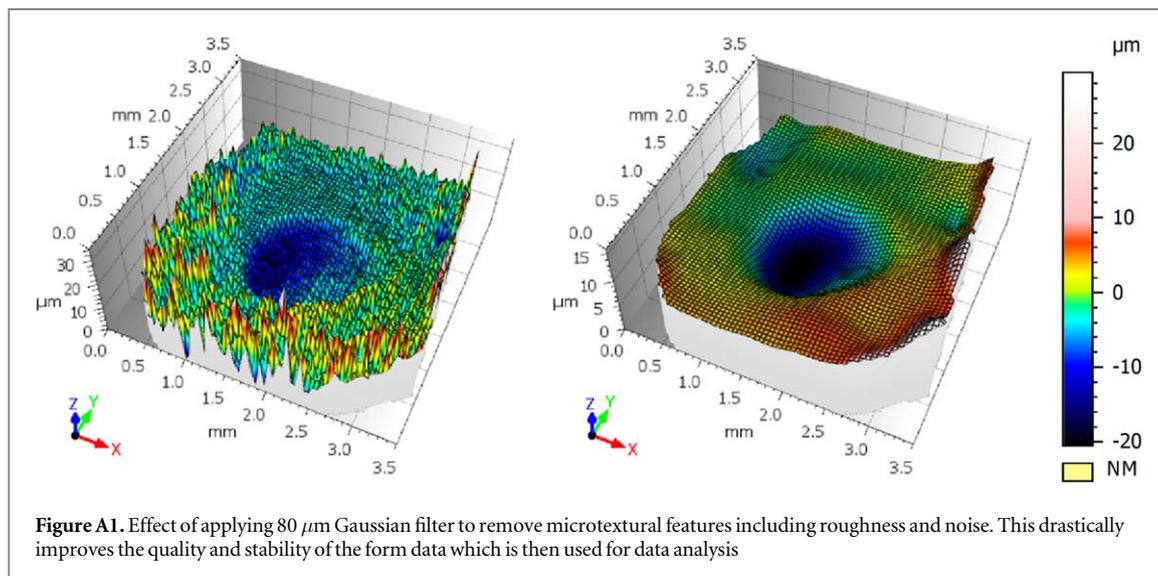
## Author contributions

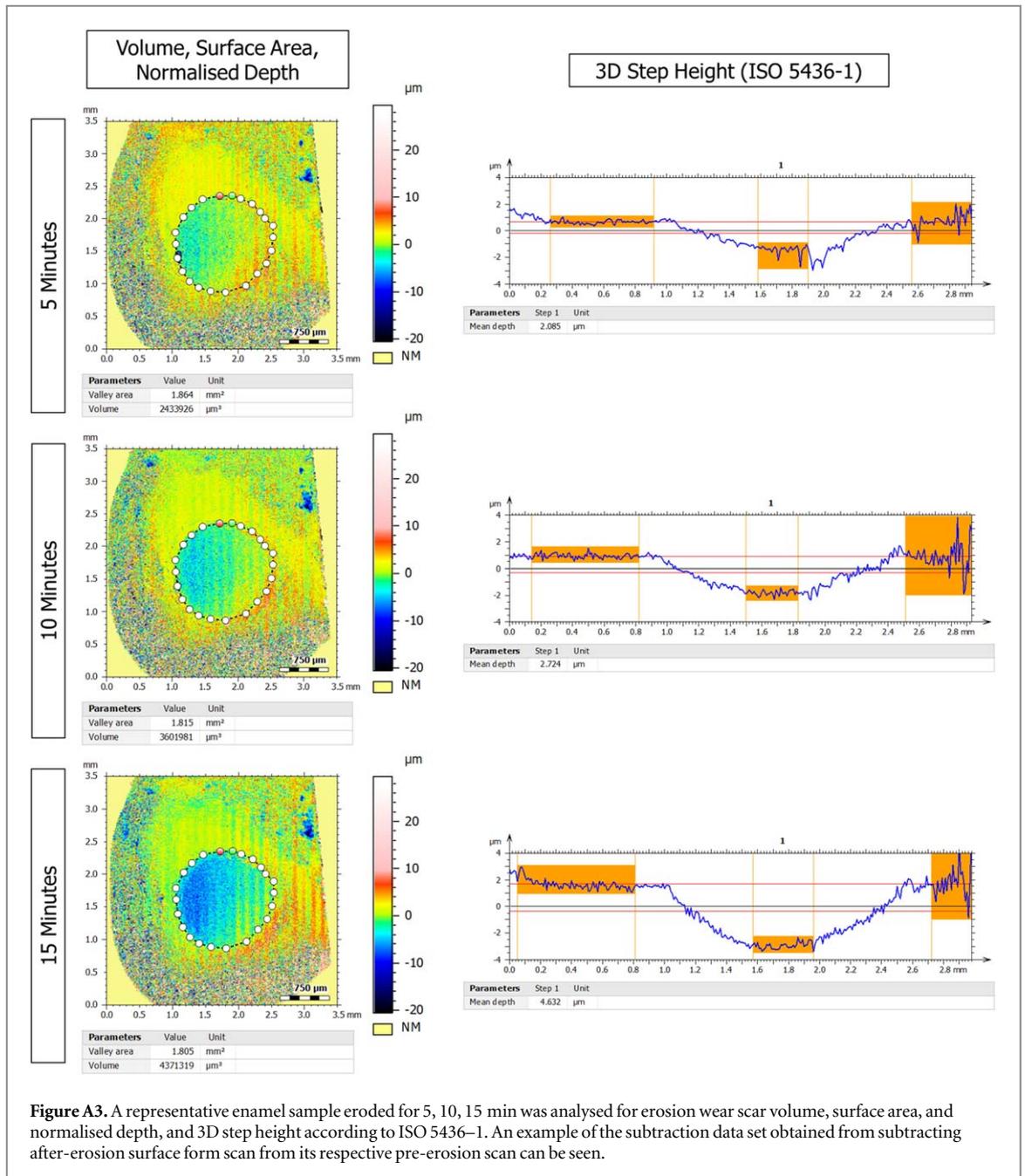
P Mylonas, R Moazzez, A Joiner, D Bartlett, T Bull, and J McBride contributed to conception, design, data collection, analysis and interpretation, as well critical revision of the manuscript. All authors gave final approval and agreed on the contents of the work.

## Ethical Statement

All teeth were obtained with written consent from patients attending for Oral Surgery at Guy's Hospital, UK (REC:12/LO/1836). This research was funded by Unilever Oral Care, UK to whom we are grateful. A Joiner is an employee of Unilever Oral Care, UK.

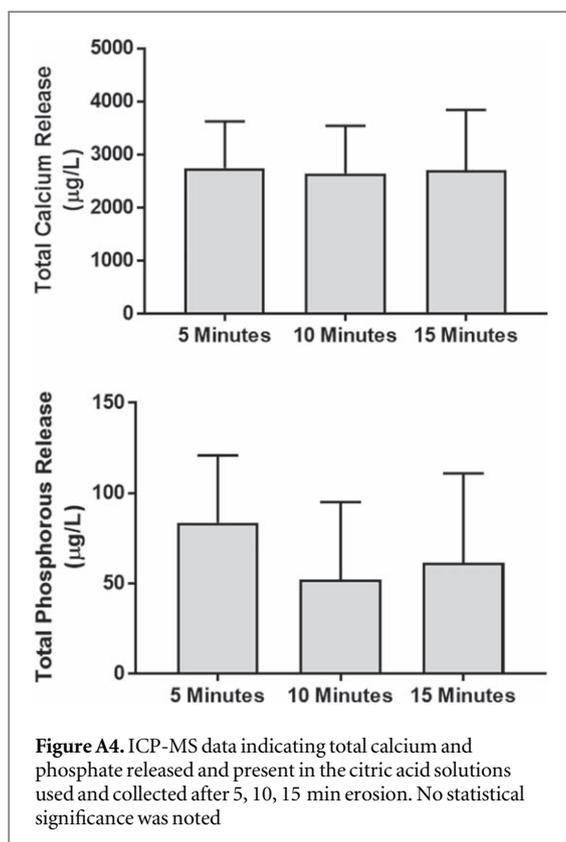
## Appendix





**Table A1.** Measurement of erosion lesion mean volume, surface area, normalised depth and 3D step height are shown and calculated as a percentage change versus 5 min erosion to demonstrate progression of erosion. Standard deviations (SD) are also shown in brackets for each measurement.

Cumulative Erosion Time (min)	Mean (SD) Volume ( $\mu\text{m}^3$ ) ( $n \times 10^6$ )	% Change versus 5 min	Mean (SD) Surface Area ( $\mu\text{m}^2$ ) ( $n \times 10^6$ )	% Change versus 5 min	Mean (SD) Normalised Depth ( $\mu\text{m}$ )	% Change versus 5 min	Mean (SD) 3D step height ( $\mu\text{m}$ )	% Change versus 5 min
5	1.91 (0.78)	0.0	1.72 (0.25)	0	1.11 (0.43)	0	1.16 (0.33)	0.0
10	3.03 (0.80)	58.7	1.80 (0.27)	4.61	1.70 (0.50)	53.2	1.89 (0.43)	62.5
15	3.76 (0.67)	97.0	1.87 (0.34)	8.31	2.05 (0.42)	84.7	2.51 (0.65)	116.3



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