ESRF	**Experiment title: The evolution of tooth replacement rate in the largest ever land animals - phase contrast tomography of sauropod dinosaur dentine increments**	**Experiment number**: ES-753
Beamline: ID19	**Date of experiment**: from: 31/05/2018 to: 04/06/2018	**Date of report**: 05/03/2020
Shifts: 12	**Local contact(s)**: Paul Tafforeau; Elodie Boller; Lukas Helfen	*Received at ESRF:*

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Report:

In this ID 19 experiment we used single distance phase contrast x-ray microtomography to study the teeth of a range of species of sauropod dinosaurs from the Jurassic and Cretaceous time periods. The purpose was non-destructive identification, mapping and quantification of tooth dentine growth increments (von Ebner lines and/or Andresen lines), in order to estimate tooth development and replacement rates and reconstruct the evolution of the sauropod dentition across the group as a whole. The data collected are being analysed for inclusion in the PhD thesis of Seela Salakka, Department of Geosciences, University of Helsinki; has been presented at an international conference; and will provide data for a series of stand-alone papers on tooth replacement in individual sauropod species, a methods paper on estimating and validating rates of tooth development and replacement in sauropod dinosaurs from tomographic data, plus a high impact paper summarising these results to show the evolution of sauropod tooth replacement and its links to diet, feeding adaptations etc. across the entirety of Sauropoda.

During the experiment, we used two different voxel resolutions, principally 2.26µm but also 13.06µm where larger specimens required an increased field of view. Energies used were 91keV and ~151keV. 6000 projections were taken per measurement, and the recently developed accumulator acquisition mode was used throughout the experiment.

Approximately 145 volumes/measurements were made at 2.26µm and 240 at 13.06µm, though many especially at 13.06µm were multiple volumes in the vertical dimension in order to image the whole length of an entire sauropod tooth. 35 sauropod individuals were imaged in total. The majority of specimens were scanned at both 2.26µm 13.06µm voxel resolutions, to allow all of the dentine growth increments in a region of the tooth to be imaged at the smaller voxel size, and the overall geometry of the dentine growth increments (but not every single increment) to be imaged across entire teeth and multiple teeth within jaws at the larger voxel size.

We detected dentine growth increments in the teeth of every specimen imaged, which is an unusually high percentage (e.g. **Fig 1A**). This is a considerably higher percentage than the current gold standard of histological thin sectioning of specimens and microscope imaging, in which a variable percentage of specimens do not show increments due to damage during the sectioning process or the nature of only being able to image individual slices of a much larger 3D volume with the high potential of cutting through a region with poor preservation of increments due to biological or taphonomic (the process of fossilisation) processes. It is also testament to the usefulness and power of phase contrast synchrotron tomographic imaging in such non-destructive analysis.

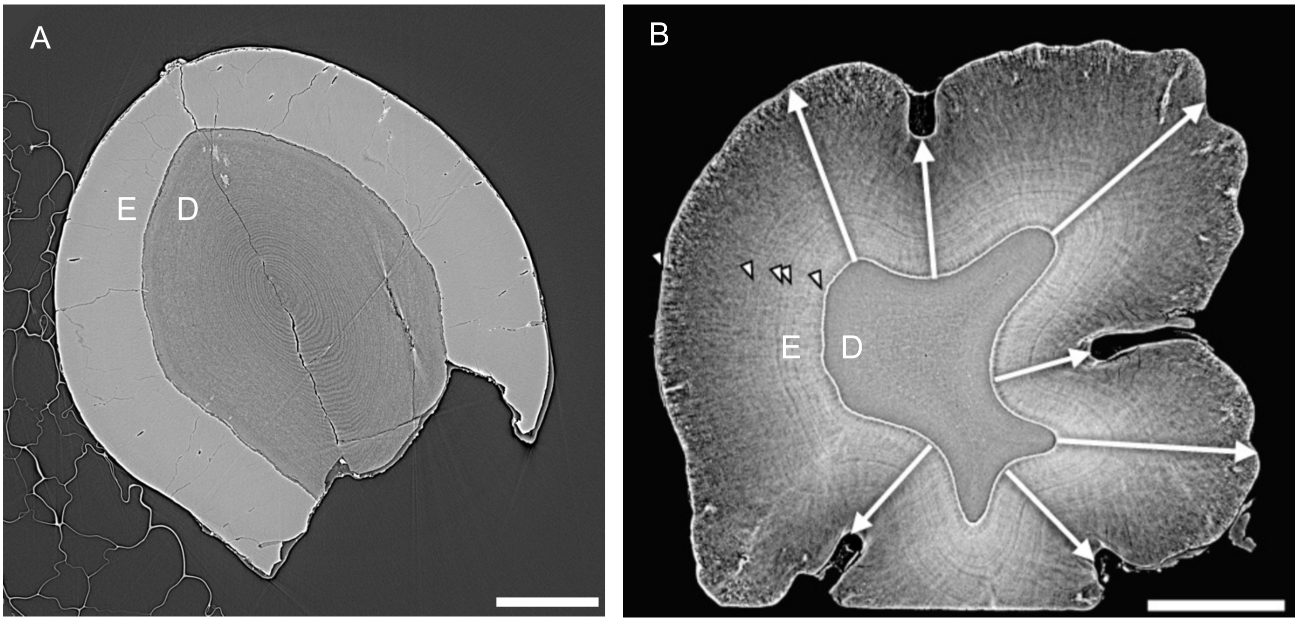

Fig 1 – Growth increments in teeth. A – Horizontal virtual slice through conical tooth of the sauropod dinosaur *Giraffatitan*, showing approximately concentric growth increments in the dentine. **B** – Horizontal virtual slice through irregularly shaped cusp of a molar tooth of a domestic pig, showing growth increments in the enamel. Arrows show direction of enamel growth during tooth development and mineralisation, arrow heads denote prominent increments. D = dentine, E = enamel. Both images – voxel size 2.26μm, scale bar = 1mm**.**

Additionally, pilot scans were made of two partial domestic pig molar teeth (one adult fully mineralised tooth, one developing tooth from a juvenile specimen, specimens procured according to ethical requirements of University of Helsinki animal welfare board) to examine the growth increments in the dentine and enamel tissues. These were made at the same 2.26μm/91keV settings as the sauropod teeth. The scans were succesful (**Fig 1B**), and the resulting data was published in Plos Computational Biology as part of a paper describing a computational model and hypothesis for enamel formation:

Hakkinen TJ, Sova SS, Corfe IJ, Tjaderhane L, Hannukainen A, Jernvall J (2019) Modeling enamel matrix secretion in mammalian teeth. PLoS Comput Biol 15(5): e1007058. https://doi.org/10.1371/journal.pcbi.1007058

Abstract
The most mineralized tissue of the mammalian body is tooth enamel. Especially in species with thick enamel, three-dimensional (3D) tomography data has shown that the distribution of enamel varies across the occlusal surface of the tooth crown. Differences in enamel thick- ness among species and within the tooth crown have been used to examine taxonomic affili- ations, life history, and functional properties of teeth. Before becoming fully mineralized, enamel matrix is secreted on the top of a dentine template, and it remains to be explored how matrix thickness is spatially regulated. To provide a predictive framework to examine enamel distribution, we introduce a computational model of enamel matrix secretion that maps the dentine topography to the enamel surface topography. Starting from empirical enamel-dentine junctions, enamel matrix deposition is modeled as a diffusion-limited free boundary problem. Using laboratory microCT and synchrotron tomographic data of pig molars that have markedly different dentine and enamel surface topographies, we show how diffusion-limited matrix deposition accounts for both the process of matrix secretion and the final enamel distribution. Simulations reveal how concave and convex dentine features have distinct effects on enamel surface, thereby explaining why the enamel surface is not a straightforward extrapolation of the dentine template. Human and orangutan molar simula- tions show that even subtle variation in dentine topography can be mapped to the enamel surface features. Mechanistic models of extracellular matrix deposition can be used to pre- dict occlusal morphologies of teeth.