

VIRTUAL HISTOLOGICAL RECONSTRUCTION OF PRE-NATAL DENTAL ENAMEL IN HUMAN DECIDUOUS TEETH

Alessia Nava^{*1,2}, Luca Bondioli^{†2}, Alfredo Coppa¹, Diego Dreossi³, Luciano Fattore^{2,4}, Lucia Mancini^{‡3} & Clément Zanolli⁵

¹Dipartimento di Biologia Ambientale, Università di Roma 'La Sapienza', Rome, Italy

²Museo delle Civiltà. Museo Nazionale Preistorico Etnografico 'L. Pigorini', Sezione di Bioarcheologia, Rome, Italy

³Elettra - Sincrotrone Trieste S.C.p.A., Basovizza (Trieste), Italy

⁴Dipartimento Ingegneria Chimica Materiali Ambiente, Università di Roma 'La Sapienza', Rome, Italy

⁵Laboratoire AMIS, UMR 5288, Université Toulouse III, Toulouse, France

Keywords: X-ray microtomography, dental virtual histology, human dental enamel, evolution of human life history

Summary: A sample of human deciduous teeth from the Roman Imperial necropolis of Velia (I-II cent. CE) was measured by high-resolution, phase-contrast synchrotron X-ray microtomography at the SYRMEP beamline of the Elettra laboratory. Results show the suitability and potential of the setup available at SYRMEP in paleo-ontogenetic studies on tooth enamel, through visualization of the growth markers in the mineralized tissue.

1. INTRODUCTION

The study of human dental enamel microstructure allows to document its growth parameters, and to identify stressful events during the individual life. Therefore, the quantification of the enamel growth trajectories in fossil teeth is of great importance in understanding human life-history evolution [1]. Moreover, the analysis of enamel that forms *in utero* - which is present in the deciduous human dentition - is critical in understanding the growth of the foetus, and can offer information about the mother's health status during pregnancy.

The quantification of the short (Cross Striations) and long (Retzius lines) period growth markers is usually done destructively by histological analysis of thin slices of the teeth. More recently, human enamel has been imaged non-destructively using synchrotron X-ray microtomography (μ CT) [2-3]. However, in 2016, Immell et al [4] showed the importance to optimize the synchrotron setup reducing the total dose during the fossil and sub-fossil samples' irradiation, thus minimizing the potential deleterious effect of X-rays on ancient DNA.

This contribution presents how the use of the synchrotron X-ray μ CT setup of the SYRMEP beamline, in phase-contrast mode, allows to image growth markers in the cuspal enamel of deciduous central incisors, that forms partly prenatally. Tomographic experiments were carried out by using pixel sizes of 2.0 μ m, 1.3 μ m and 0.9 μ m. It can be shown that fine details in the enamel regions are well visible for images reconstructed with the isotropic voxel size of 1.3 μ m (Fig.1).

2. EXPERIMENTAL METHOD

Thirty-five synchrotron X-ray μ CT measurements were conducted on the deciduous dentition of infants from the necropolis of Velia (I-II centuries CE, Campania, Southern Italy) and of modern teeth. Sixteen different deciduous central incisors were selected among the available teeth, and measured with different parameters.

Experiments were performed in white-beam configuration with a medium energy of 25 keV. The sample-to-detector distance was set to 150 mm. For each scan, we acquired 2000 projections over a 180° rotation with an exposure time/projection of 1.5 s. Projections were recorded with a 16-bit, air-cooled sCMOS detector (2048 x 2048 pixels) coupled to a 15 μ m-thick LSO:Tb scintillator screen. A 1.3 μ m effective pixel size was set, using the variable optical zoom of the detecting system, corresponding to a field of view of 2.6 mm x 2.6 mm.

The slice reconstruction was done by the SYRMEP Tomo Project software [5]. Before reconstruction, a single-distance phase-retrieval algorithm [6] was applied to the sample projections trying to find a good compromise between the visibility of fine details in the tooth enamel and spatial resolution.

* e-mail: alessia.nava@uniroma1.it

† e-mail: luca.bondioli@beniculturali.it

‡ e-mail: lucia.mancini@elettra.eu

3. RESULTS

Twelve measurements were performed on a subsample of 9 individual teeth from Velia. We were able to determine the daily enamel secretion rate - that is the speed at which the ameloblasts move towards the outer surface of the tooth - in 11 cases, and we were able to identify the presence of the Neonatal Line - the first accentuated line marking the birth event - in 6 cases. Two teeth, pertaining to the same individual, showed no Neonatal Line, thus suggesting that the child did not survive enough to form the birth landmark (at least 10-15 days after birth).

Results are coherent with the known growth parameters of the same dental population [7] and highlight the suitability of the microtomographic setup of the SYRMEP beamline at Elettra for the characterization and quantification of human dental enamel microstructures in fossil and extant human teeth.

References

- [1] Dean MC. Tooth microstructure tracks the pace of human life-history evolution. *Proceedings of the Royal Society B: Biological Sciences* 273(1603), 2799-2808, 2006.
- [2] Le Cabec A, Tang N, Tafforeau P. Accessing developmental information of fossil hominin teeth using new synchrotron microtomography- based visualization techniques of dental surfaces and interfaces. *PLoS One* 10:e0123019. doi:10.1371/journal.pone.0123091, 2015.
- [3] Tafforeau P, Zermeno JP, Smith TM. Tracking cellular-level enamel growth and structure in 4D with synchrotron imaging. *Journal of Human Evolution* 62, 424-428, 2012.
- [4] Immel A, Le Cabec A, Bonazzi M, Herbigl A et al. Effect of X-ray irradiation on ancient DNA in sub-fossil bones – Guidelines for safe X-ray imaging. *Scientific Reports* 6, 32969, doi: 10.1038/srep32969, 2016.
- [5] Brun F., P., Accardo A., Kourousias G., Dreossi D., Mancini L., Tromba G., Pugliese R., Enhanced and flexible software tools for X-ray tomography experiments at the Italian synchrotron radiation facility Elettra. *Fundamenta Informaticae* 141(2-3), 233-243, 2015.
- [6] Paganin, D., Mayo, S.C., Gureyev, T.E., Miller, P.R., Wilkins, S.W. Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object, *Journal of Microscopy*; 206(1), 33-40, 2002.
- [7] Nava A, Bondioli L, Coppa a, Dean C, Rossi PF, Zanolli C. New Regression Formula to Estimate the Prenatal Crown Formation Time of Human Deciduous Central Incisors in Pre-industrial Populations. Submitted to *Plos One*.

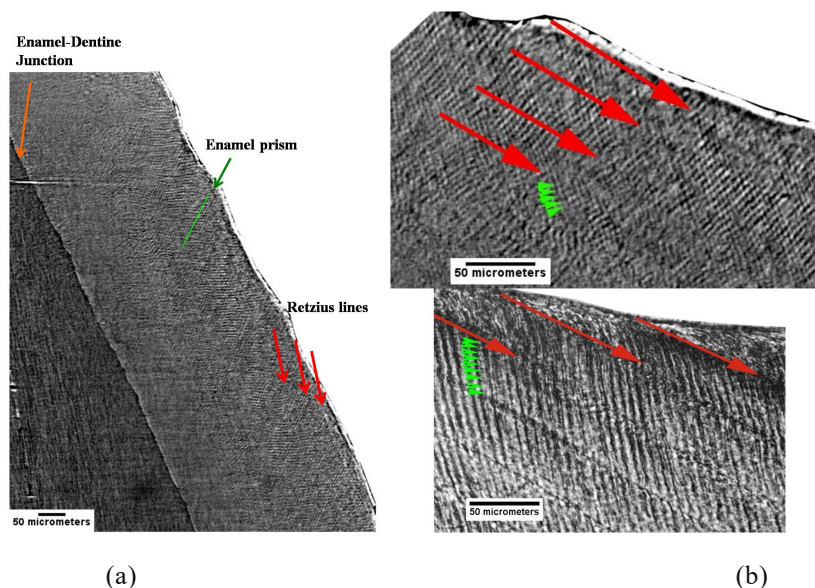


Figure 1: Reconstructed X-ray images of the sample Velia 387, cuspal portion of the upper deciduous central incisor showing the enamel microstructures (isotropic voxel size 1.3 μm): (a) the Enamel-Dentine Junction, Retzius lines and enamel prism are highlighted; (b) upper panel, same as in (a) magnified, Retzius lines highlighted by red arrows, enamel prisms with daily cross striations pointed by green arrows; lower panel in (b) shows, for comparison, a histological thin section imaged in transmitted light microscopy.