ESRF	**Experiment title: Exploring the limits of dental evolution: Phase contrast tomography of the Eurasian least shrew**	**Experiment number**: EC 769
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Shifts: 3	**Local contact(s)**: Paul Tafforeau	*Received at ESRF:*

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Report:

In this ID19 experiment we imaged the internal microstructure of the smallest teeth of one of the worlds smallest mammals – the Eurasian least shrew *Sorex minutissimus* - using phase contrast. The measurements were very successful, and the results are currently being analysed and written up for submission to Nature or an equivalently ranked journal. We used 0.34 micron voxel resolution scans to image just the principal, tallest cusp (the protoconid) of the third and smallest molar tooth of the lower jaw, and 0.677 micron voxel resolution scans of the whole teeth of second and third lower molar teeth. Additional scans of the same cusp and tooth positions from other, closely related species of shrews (*S. minutus* & *S. caecutiens*) were obtained to enable inter-species comparisons. From the resulting data we resolved enamel and dentine tubules, hollow tube shaped objects, within the mineralized teeth hard tissues. These were segmented out to allow us to follow their direction away from the boundary between the enamel and dentine (the enamel-dentine junction, EDJ) (Fig. 1). These tubules represent the paths of odontoblast (in dentine) and ameloblast (in enamel) cells during tooth shape formation, and as such are a historical record of the distributions and movements of cells recorded in hard, mineralized, unchanging mature teeth.

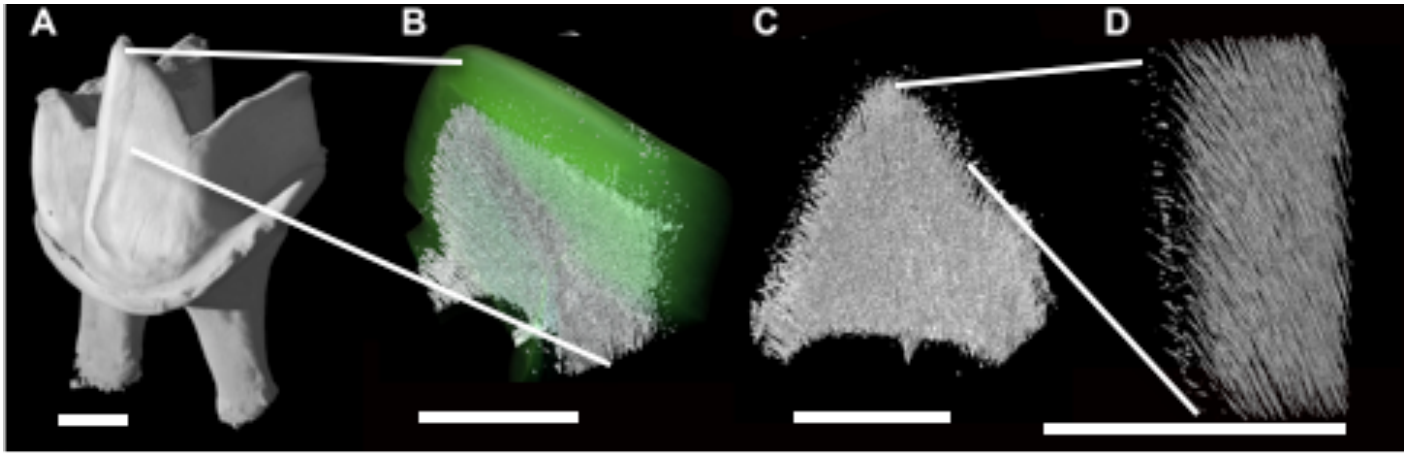

Fig.1 A: Pseudo-3D rendering of whole *S. minutissimus* lower 3rd molar tooth; **B:** Protoconid cusp tip from A showing enamel (green outer layer) and dentine tubules (white). **C:** Dentine tubules from cusp tip in (**B**). **D:** Individual dentine tubules reaching enamel-dentine junction (left edge), showing uncertain relationship to enamel tubules (short, far left). Scale bars = 100 µm. A = 0.677, and B-D = 0.34, micron voxel resolution.

To address the hypotheses posed in the proposal (relating to the patterns of tubules, similarity in these patterns between left and right teeth, the nature of any coordination of dentine and enamel tubules, and the ability of a computational model of tooth development to predict some of these features), we have initially quantified numbers and densities of dentine tubules within the protoconid cusp, and the whole teeth, of a

number of samples. Results (Fig. 2) suggest a relatively high degree of variation in both number and density of dentine tubules between samples, and that left–right tooth pairs from the same individual show smaller differences than comparisons to the same tooth position from a different individual. However, differences in cuspal dentine area and tubule density between left–right tooth pairs from the same individual do exist i.e. specimen 232 568. It appears that the asymmetry between dentine areas is however compensated for by a reciprocal amount of asymmetry between enamel areas, such that the external surface areas and volumes of the left and right lower third molar teeth of an individual can be very similar even when large differences exit between dentine areas, volumes, tubule numbers and tubule densities. This suggests that there is an unknown mechanism for control of the enamel thickness (and hence cusp and tooth area and volume) which enables equal sized teeth and cusps to be produced from more variable dentine volumes. One hypothesis is that tooth size is limited by physical constraints of the location of the developing tooth germ. The crypt, bone surrounding the tooth as it develops, may be of constant size and limit maximum size of the tooth even where there is variation in the size of the dentine and pre-dentine matrix. This unexpected finding, which we believe is a novel discovery made possible by ESRF expertise and facilities, suggests that investigations into the contribution of physical constraints to final tooth shape and size would be a fruitful area of further research.

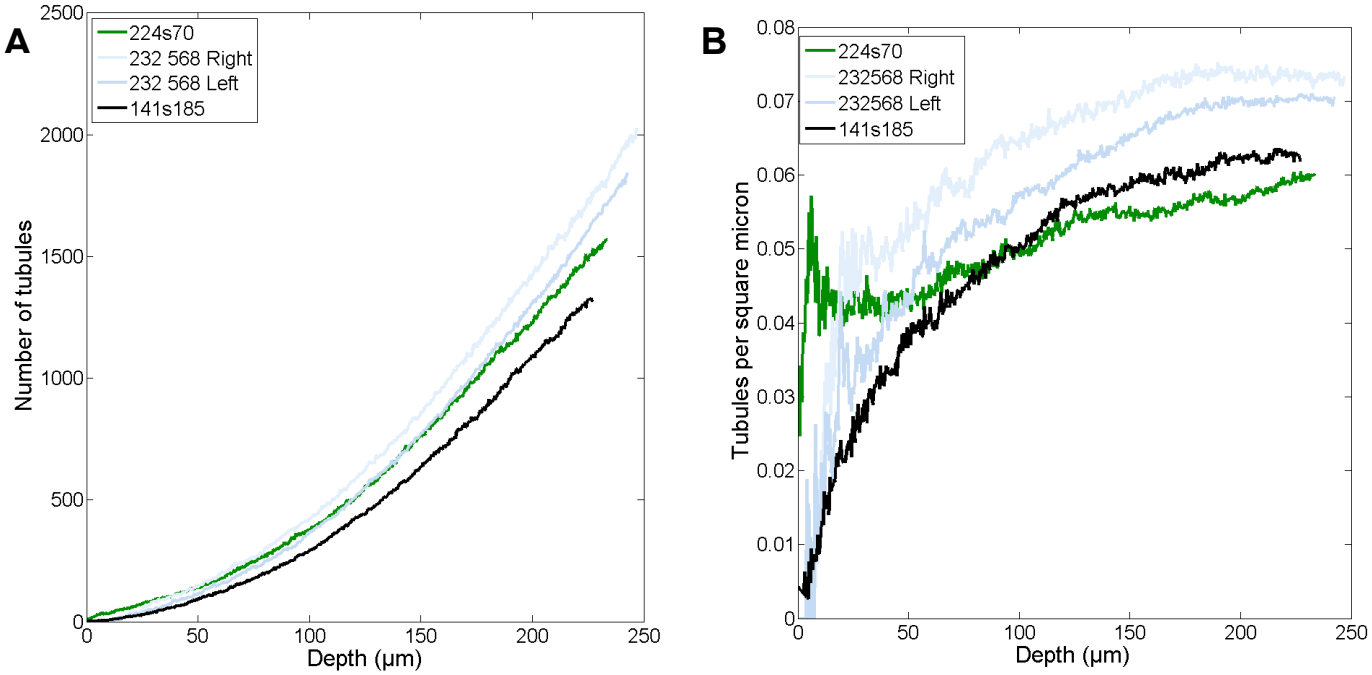

Fig.2: Graphs of **A:** dentine tubule number and **B:** dentine tubule density plotted against depth from the tip of the dentine horn, for third molar protoconid cusps of *S. minutissimus*.

In addition to the dentine and enamel tubules we were expecting to see, we were able to image enamel prisms, the basic structural unit of enamel, at the EDJ and towards the external face of the enamel (Fig. 3). However, noise at the EDJ and insufficient resolution (Fig. 1D; Fig. 3) has made identification of the relationship of the enamel tubules to the dentine tubules, difficult. In order to address this aspect of our proposal, and to investigate the relationships of the unexpectedly imaged prisms to enamel tubules, we intend to apply for beamtime on beamline ID22 at 50-100nm resolution. A test scan of the required region has been successfully carried out on ID22 (Tafforeau pers. comm.) with results indicating that the required region can be imaged at sufficient resolution and with minimal noise to allow questions that could not be answered using data from ID19 to be investigated.

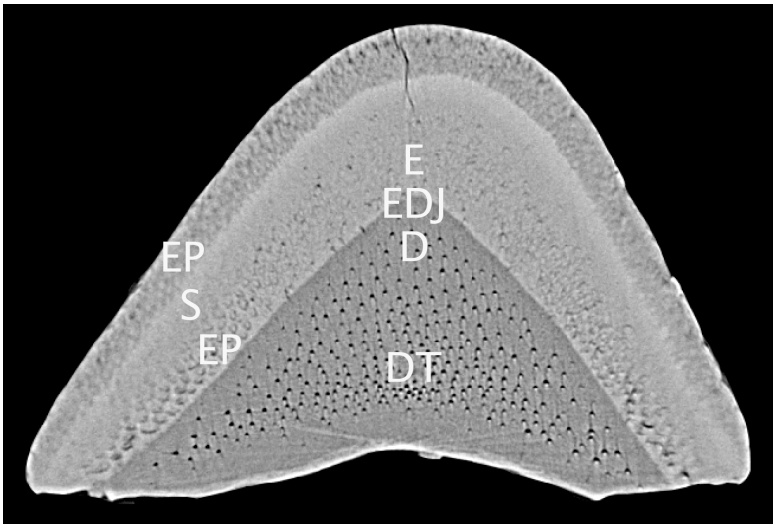

Fig.3: Horizontal slice through protoconid cusp showing enamel (E), enamel prisms (EP), dentine (D), dentine tubules (DT), enamel-dentine junction (EDJ), siderose (S).