	Experiment title: A tangle of tubules and prisms: spatial relationships of enamel prisms, enamel tubules, and dentine tubules, in mineralised tissues of the Eurasian least shrew	**Experiment number**: EC 1064
Beamline: ID22	**Date of experiment**: from: 07/10/2012 to: 09/10/2012	**Date of report**: 14/07/2014
Shifts: 6	**Local contact(s)**: **Heikki Suhonen**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Ian Corfe[1]*, Aki Kallonen[1]*, Jukka Jernvall[1]*, Keijo Hämäläinen[1]

[1]University of Helsinki

Report:

In this ID22 experiment we used single and multiple propagation distance phase contrast nanotomography to image the internal microstructure of the teeth of *Sorex minutissimus*, the Eurasian least shrew, and *Mus musculus*, the house mouse. The results are currently being written up for submission to a high-ranking journal, have been presented at a number of international conferences, and form part of a PhD successfully completed in mid-2016 (Kallonen 2016).

S. minutissimus is one of the smallest known mammals, and we further examined its smallest molar tooth in order to provide sub-cellular resolution of internal structures of the mineralised dental tissues of this complex structure. *M. musculus* has a quite different, derived and modified tooth macrostructure or overall shape/morphology, so comparison with *S. minutissimus* will allow us to examine the degree to which macrostructure and microstructure/ultrastructure are linked. Very high-resolution internal structure (local tomography) imaging was successfully achieved by using principally a 25-nanometer voxel resolution and a 29.6 keV, pink beam. Generally 1999 images were taken over 360 degrees, with 0.5s exposure time and a 1.5mm Al filter. In some cases multiple adjacent regions of interest were imaged at this voxel resolution in order to build larger merged datasets post scanning. This allowed us to reconstruct images of structures beginning within the internal dentine, across the enamel-dentine junction/boundary, and out to the external enamel surface, in merged single volume reconstructions. A total of 19 high projection number measurements were performed, with 13 in holotomography and six in single-propagation distance modes. Voxel resolutions used were 25nm (x12 measurements), 100nm (x1), 321 nm (x5) and 378nm (x1).

The project was initiated in order to identify, quantify, and map the spatial relationships of enamel prisms, enamel tubules and dentine tubules, especially with regards to their orientation and relationships with each other across the enamel-dentine junction (EDJ). The dentine and enamel tubules are hollow, tube shaped objects within the mineralised tooth hard tissues, which represent the paths of odontoblast and ameloblast cells during mineralisation of the dentine and enamel respectively. In contrast, the enamel prisms are elongate rods formed from bundles of hydroxy-apatite, the basic crystalline constituent of mineralised enamel. Their orientation is thought to represent both the pattern of mineralising ameloblasts as they travel in a wave from the EDJ outwards through a protein matrix scaffold, and also an evolutionary adaptation to resist forces applied to the tooth during eating. However, the nature of the spatial relationship between enamel and dentine tubules across the EDJ, and especially of the enamel tubules to enamel prisms, and enamel prisms in complex three-dimensional patterns, has previously been uncertain.

From the experiment data we have successfully resolved all three of these micron-scale features, as

well as additional sub-micron-scale divisions, branches and links between dentine and enamel tubules at the EDJ, and between individual dentine tubules within the dentine and individual enamel tubules within the enamel (**Fig.1a**). The reconstructions show that in *S. minutissimus* most dentine tubules split into two or more branches as they approach the EDJ (mean = 2.24, standard deviation = 0.55). They also change angles from an acute approach to the EDJ within the dentine to an almost perpendicular angle as these branches cross the EDJ (**Fig.1b**). There are clear connections and continuations across the EDJ between these branches of the dentine tubules and the enamel tubules. Almost every enamel tubule is linked to one of these dentine tubule branches, suggesting a ~twofold higher density of enamel tubules than the principal dentine tubules.

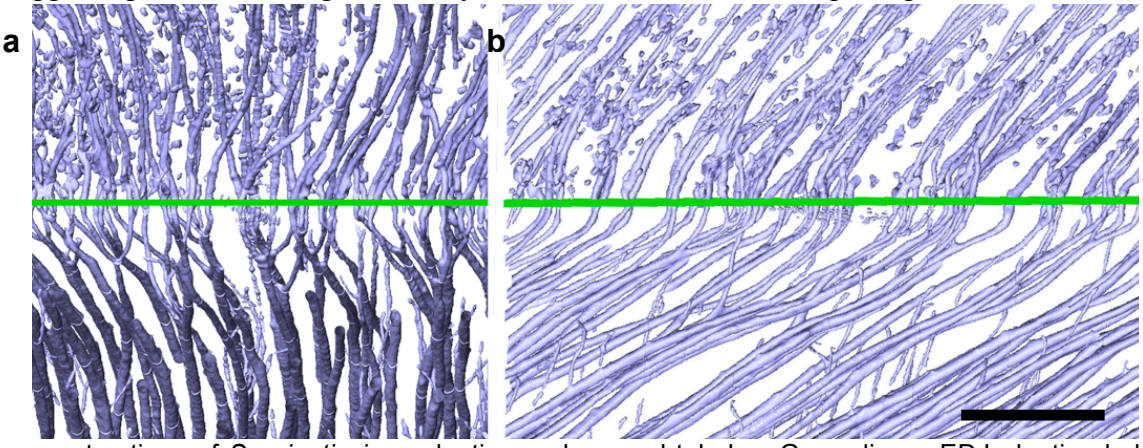

Fig. 1. 3D reconstructions of *S. minutissimus* dentine and enamel tubules. Green line = EDJ, dentine below, enamel above. Scale = 10µm. **1a**: Branching pattern in dentine tubules showing 2-3 branches occurring ~5µm from EDJ, continuous across EDJ with enamel tubules. **1b**: angle variation in dentine and enamel tubules either side of EDJ.

Enamel prisms are clearly visible within the enamel, with the hypomineralised prism sheath marking the boundary of adjacent prisms (**Fig. 2a**). The spatial relationships between enamel prisms and tubules are revealed, with almost all enamel tubules associated with a single prism for their entire length, and few un-associated tubules. In general, enamel tubules are located within enamel prisms, usually towards their exterior edge and just inside the prism sheath (**Fig. 2b**).

In contrast, in *M. musculus*, most dentine tubules split into ten or more branches about twice the distance away from the EDJ than in *S. minutissimus* (10um vs 5um). These dentine tubule branches terminate within globular structures a short distance away from the EDJ within the enamel (**Figs 2c,d**). No enamel tubules extend past these globular structures, so the precise relationship between enamel tubules and prisms cannot be determined in *M. musculus*.

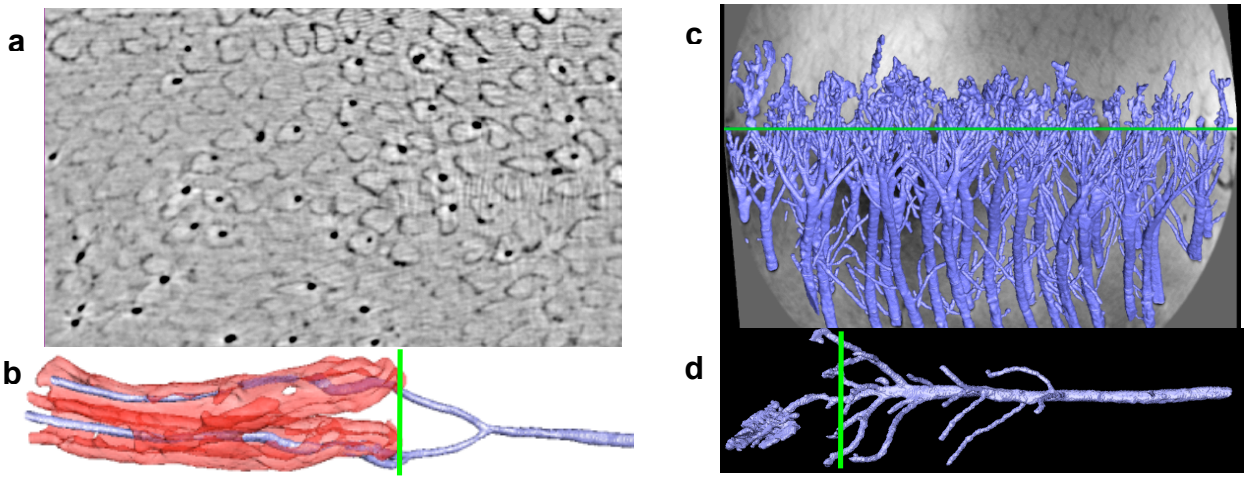

Fig 2a: 2D slice data showing horseshoe shaped enamel prism sheaths and circular enamel tubule air spaces. **2b**: 3D reconstruction of enamel tubules (blue) inside enamel prism sheaths (red), green line = EDJ. **2c,d**: 3D reconstructions of *M. musculus* dentine and enamel prisms, showing multiple dentine tubule branches, and short enamel tubules and globular structures.

Pilot data was also collected on the microstructure of increments of the tooth root cementum of a fossil mammal, and was used for further successful beamtime applications to beamlines ID19 and ID16A (projects **ES152** (completed) & **ES502** (upcoming)), and has been used in a completed MSc thesis (Newham 2014) and to secure funding for a PhD.