

## Nanoscale Spatial and Chemical Exploration of Porcine Trabeculae Bone using Atom Probe Tomography

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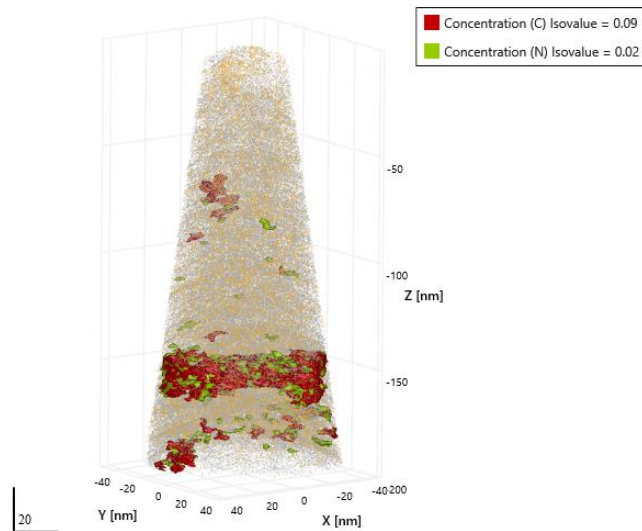
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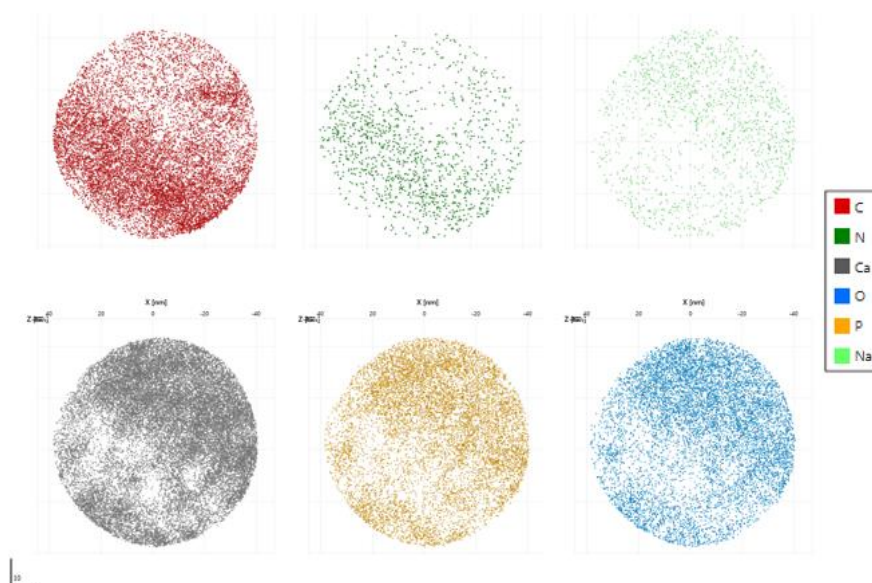
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Bone provides structure and support for vertebrates, and it is the largest ion exchanger in the body to maintain homeostasis. Bone is a complex and heterogeneous composite mainly composed of an inorganic phase (mineral), organic phase (collagen, non-collagenous proteins) and water. [1]. Understanding the spatial structure and chemical composition of bone at different scales is of great significance for elucidating its biomineralization mechanism, mechanical support, bone pathological treatment and bone scaffold development. However, simultaneous characterization of structure and chemical information of bone at the nanoscale presents many limitation, especially the 3D spatial structure exploration and the quantification of low atomic mass elements. Atom probe tomography (APT) is a 3D characterisation technique with a unique combination of high spatial and chemical resolution. While originally developed for the study of metals, pioneering studies have recently adapted APT to explore human bone and leporine bone, at the atomic scale. In those studies, samples were taken from cortical bone, which has a relatively dense structure [2, 3].

Here, we use APT to reveal the spatial structure and chemical composition of porcine trabecular bone at the atomic level. Trabecular bone, also called spongy bone, is highly vascularized and characterised by a largely porous structure. Compared to cortical bone, trabecular bone is lighter, less dense, and more elastic, but cannot support large compressive stresses [4]. This presentation will highlight APT sample preparation strategies for biological samples such as bone, i.e. organic-inorganic hybrid materials, and will discuss challenges with the related data reconstruction of porcine trabecular bone and with quantitative chemical composition measurements. Our 3D reconstruction of porcine trabecular bone by APT reveals co-localisation of C and N, and an interesting complementary spatial relationship between organic and inorganic phases, with collagen fibres spanning across mineral phases observed at atomic resolution (Figure 1). APT measurements from within different porcine trabecular bone specimens found that the concentration obtained from similar collagen and mineral phases, respectively, were relatively consistent from sample to sample. The average Ca/P ratio in the mineral phase of porcine trabecular bone calculated by APT is 1.815, as compared to the theoretical Ca/P value of 1.67. This work may provide new insights and further develops and demonstrates the potential of APT for exploring the spatial structure and chemical composition of bone.



**Figures 1.** APT 3D reconstruction of porcine trabecular bone with an iso-concentration surface of C (red, >9 at%), N (green, >2 at%). The volume in which C and N are highly co-located resemble a fibre, presumably collagen.



**Figures 2.** Ion maps of different elements in selected z-axis cross section through the C–N-enriched fibre of Fig. 1. A complementary spatial relationship between organic and inorganic can be observed. On atomic length scale, the aggregation of C and N ions in the collagen fibre can also be obviously observed.

#### References:

- [1] AL Boskey, *Elements* **3**(6) (2007), p. 385.
- [2] B Langelier, X Wang and K Grandfield, *Scientific reports* **7**(1) (2017): p. 9.

- [3] BEJ Lee, B Langelier and K Grandfield, *Advanced Biology* **5**(9) (2021), p. 2100657.
- [4] JK Gong, JS Arnold and SH Cohn, *The Anatomical Record* **149**(3) (1964), p. 325.