Image analysis

AI-enabled multiscale synchrotron imaging and analysis of human bone microstructure

Quantitative investigation of vascular, lacunar, and canalicular networks in human bone using synchrotron µCT/nano-CT and Amira Software

Bone is a hierarchical and dynamic biological tissue whose function depends on structural organization across multiple spatial scales. From millimeter-scale cortical architecture to nanometer-scale canalicular networks, its structure governs mechanical stability, cellular communication, nutrient transport, and remodeling behavior.

At the cellular level, osteocytes regulate bone formation and resorption through the osteocyte lacunar–canalicular network (OLCN). Understanding how this network interacts with vascular porosity and mineralized matrix requires quantitative three-dimensional analysis across scales (Figure 1). However, the complexity of these datasets, particularly at nanometer resolution, poses significant challenges for visualizing the relevant details, and highlights the need to incorporate 3D visualization into the segmentation and analysis workflow.

In this application note, we explore how synchrotron radiation microtomography (SR µCT) and nano-computed tomography (nano-CT) can be combined with quantitative analysis in Thermo Scientific™ Amira™ Software to investigate human bone structure from the micro to nanoscale. In the µCT study, human alveolar and fibula bone from patients undergoing maxillofacial reconstruction surgery were characterized, quantifying vascular networks and osteocyte lacunae [1]. In the nano-CT study, canalicular remodeling patterns were described and quantified across a range of anatomical sites and with varying patient age and sex, as well as healthy and pathological conditions [2].

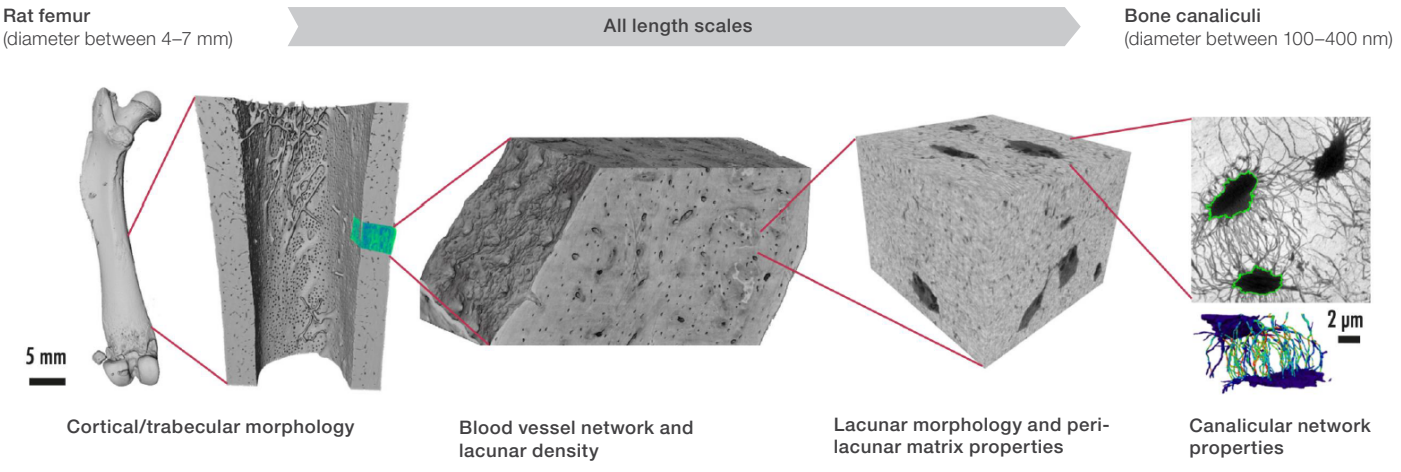

Figure 1. Bone as a hierarchical material spanning cortical structure, vascular networks, lacunae, and canaliculi across length scales.
Adapted from Anuth *et al.*, 2026 [2].

Multiscale synchrotron imaging of human bone

Micron-scale: vascular and lacunar architecture

Synchrotron µCT imaging was performed at voxel sizes of 2.27 µm and 640 nm [1]. These datasets enabled three-dimensional quantification of cortical microarchitecture without destructive sectioning (Figure 2).

Following segmentation, the following morphometric parameters were extracted:

- Lacunar density and volume
- Vessel porosity
- Mineral-to-vessel distance (50% and 95%)
- Vessel orientation (theta/phi distributions)
- Level of alignment (LOA)
- Permeability tensor

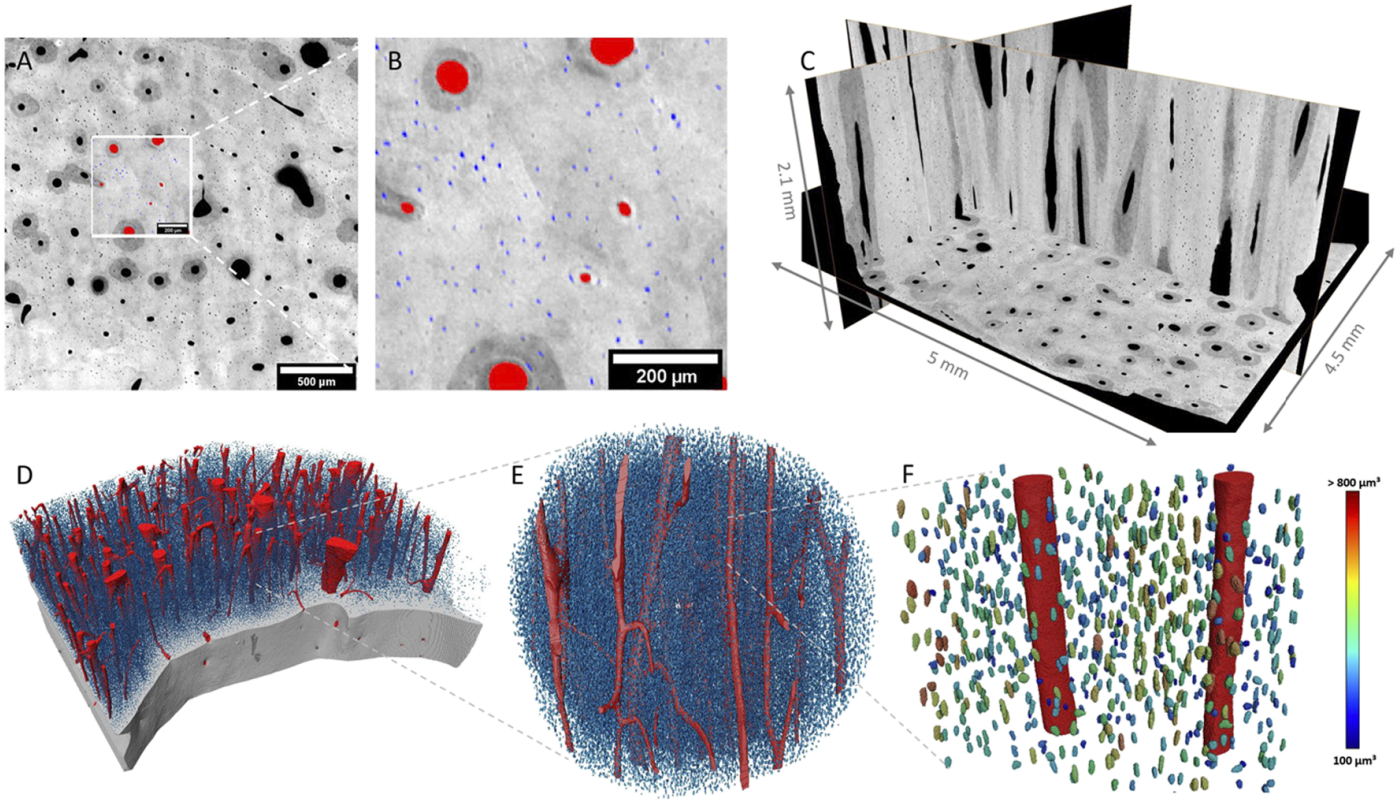

Figure 2. Three-dimensional segmentation of human cortical bone acquired by synchrotron µCT. (A–B) Representative µCT slice showing cortical bone microstructure. (C) 3D rendering of the segmented volume. (D–E) 3D visualization of the vascular network within the mineralized matrix. Mineralized matrix (gray), vascular pores (red), and osteocyte lacunae (blue) are visualized for quantitative morphometric and permeability analysis [1].

Quantitative analysis revealed significantly higher lacunar density in alveolar bone compared to fibula bone, as well as differences in mineral-to-vessel distance [1]. These findings suggest site-specific differences in osteocyte distribution and microstructural organization, which may influence remodeling behavior and graft integration.

A further advantage of µCT for bone analysis is that the volumetric data, once segmented, enables true 3D modeling and simulation of fluid flow through the system. Here, the Xlab module of Amira Software was used to perform fluid flow simulations in the blood vessel system and to compare the absolute permeability tensors between samples, revealing more unidirectional permeability in fibular bone compared to alveolar bone samples. Skeletonization of the vessel network enabled quantification of vessel network alignment, which is critical for graft integration into the recipient bone tissue (Figure 3).

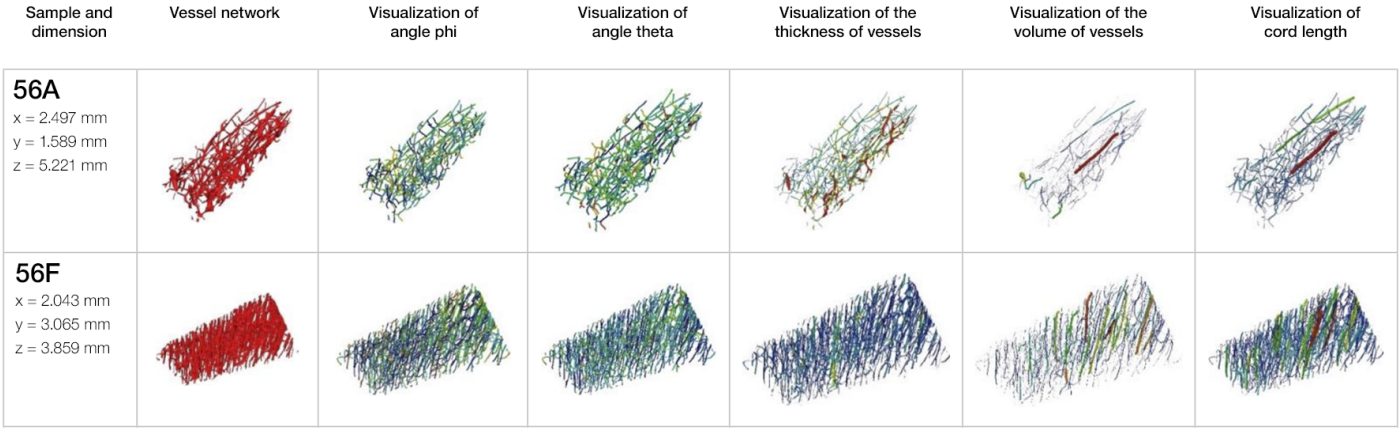

Figure 3. Skeletonized vessel networks from synchrotron µCT data color-coded by orientation. Representative alveolar (56A) and fibula (56F) samples illustrate anisotropic vessel alignment used for level-of-alignment and permeability tensor calculations. Adapted from Wüster *et al.*, 2023 [1].

Nanometer-scale: canalicular network remodeling

To investigate remodeling behavior at the cellular level, synchrotron nano-CT imaging was performed at voxel sizes down to 50 nm [2]. At this resolution, individual canaliculi (100–400 nm in diameter) become resolvable, enabling direct visualization of the osteocyte communication network (Figure 4).

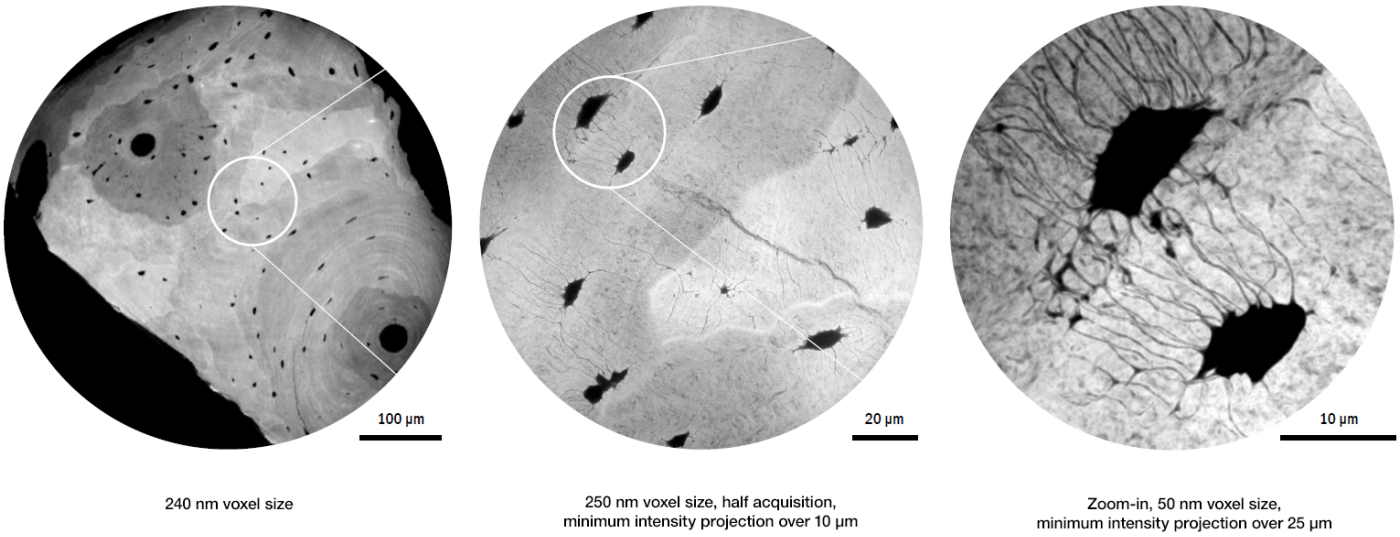

Figure 4. Synchrotron nano-CT imaging of human bone from 240 nm to 50 nm voxel size, resolving osteocyte lacunae and canalicular structures. Adapted from Anuth et al., 2026 [2].

Nanoscale analysis revealed distinct remodeling behaviors at cement lines and newly formed bone interfaces. When newly formed canaliculi encounter pre-existing bone, they either integrate into the existing canalicular network or form loop-like structures when no viable connection point is available [2]. These canalicular loops represent structural adaptations that may influence mechanotransduction and local signaling (Figure 5).

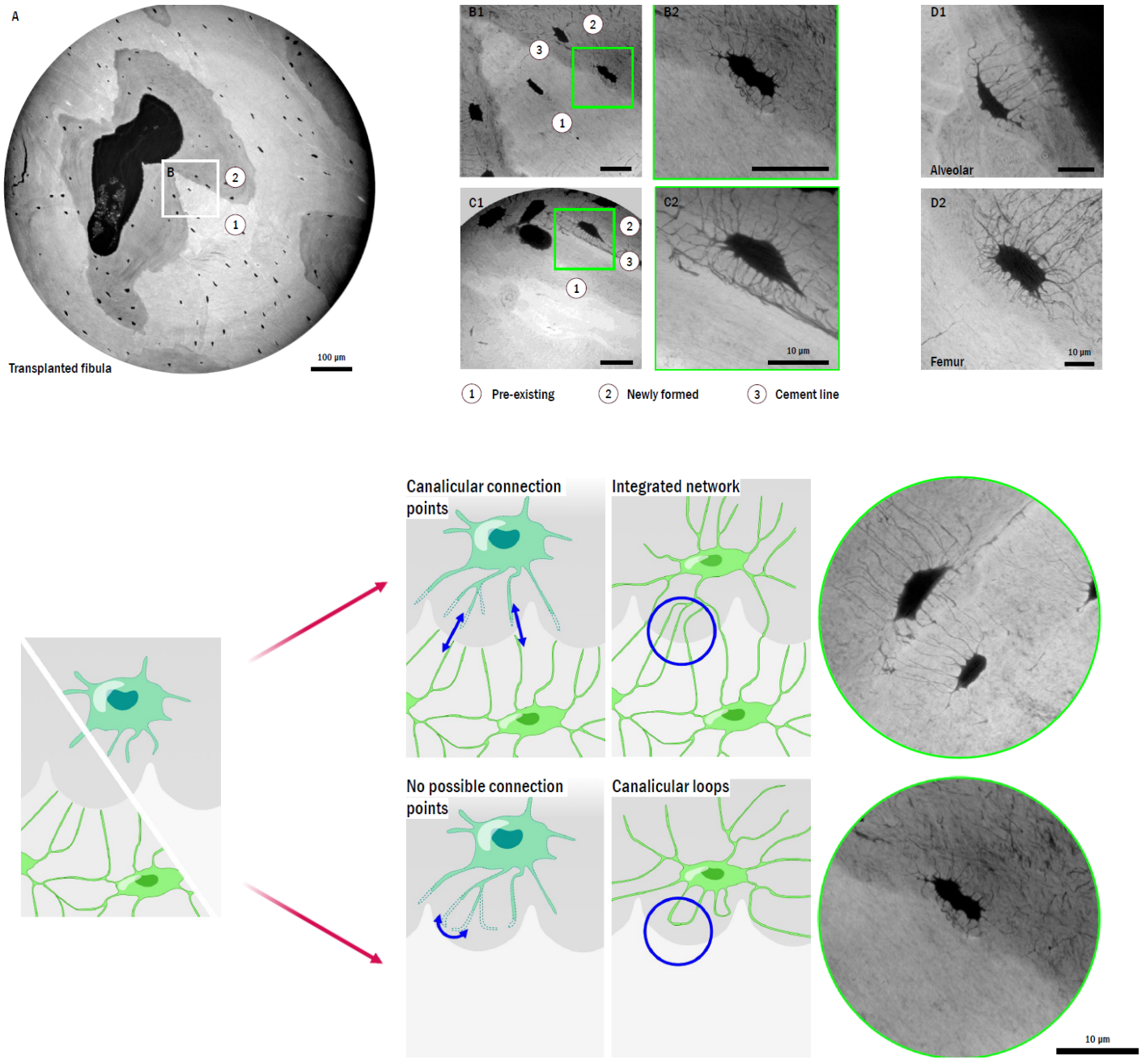

Figure 5. Canalicular remodeling patterns showing network integration or loop formation at cement line interfaces.
Adapted from Anuth *et al.*, 2026 [2].

Integrated multiscale quantitative analysis in Amira Software

A key strength of Amira Software lies in its ability to unify segmentation and quantitative analysis with powerful visualization tools within a single environment. By simultaneously rendering the raw grayscale volumes for anatomical context, segmented blood vessels, and quantification of the osteocyte lacuna size, Amira Software reveals features of the 3D data not easily grasped by simple viewing of 2D slices in different directions.

In this study, the Amira platform was used to:

- Perform skeletonization and orientation analysis
- Estimate permeability tensors
- Segment and analyze canalicular connectivity at nanometer resolution
- Visualize the quantitative results in their anatomical context

Handling large synchrotron datasets requires high-performance data management and multiresolution workflows. Amira Software enables efficient processing of large volumetric datasets, and allows tackling even more challenging segmentation tasks through focused AI training on subvolumes and scalable application to full datasets. This integration eliminates fragmented toolchains and enhances workflow reproducibility.

Why Amira Software for life sciences research?

Life sciences research increasingly depends on quantitative 3D imaging of complex cellular networks. Amira Software offers a powerful set of capabilities tailored to these workflows.

A key strength of Amira Software is its broad segmentation toolkit. In many cases, researchers can use algorithmic segmentation methods and the segmentation editor to generate results faster than building and refining an AI model.

When AI-based segmentation is useful, it can be incorporated directly into the same workflow, supporting a flexible transition between manual annotation, classical segmentation, and automated classification.

Amira Software also provides advanced tools for morphometric analysis and network characterization, including skeletonization, orientation distribution analysis, and quantitative extraction of structural features. In addition, it supports large, high-resolution datasets typical of synchrotron imaging facilities, enabling multiscale workflows within a single software environment.

For researchers investigating bone biology, tissue remodeling, or regenerative medicine, this integration of segmentation, analysis, and 3D visualization helps turn complex imaging data into biologically meaningful insight.

Conclusion

Multiscale synchrotron µCT and nano-CT imaging, combined with the segmentation, analysis, and visualization tools in Amira Software, enable quantitative investigation of human bone remodeling across spatial scales.

Micron-scale analysis reveals differences in lacunar density, mineral distribution, and vascular orientation [1]. Nanometer-scale imaging exposes canalicular remodeling behaviors, including network integration and loop formation [2]. Together, algorithmic segmentation, AI-assisted workflows, and interactive 3D visualization help make these analyses scalable, reproducible, and publication-ready.

To learn more about how Amira Software can support your research, please contact our Amira Software specialists to discuss your specific needs and imaging workflows.

References

1. Wüster, J., Hesse, B., Rothweiler, R., et al. Comparison of the 3D-microstructure of human alveolar and fibula bone in microvascular autologous bone transplantation: a synchrotron radiation µCT study. *Front Bioeng and Biotechnol.* 2023;11:1169385. doi:10.3389/fbioe.2023.1169385.
2. Anuth, S., Bortel, E., Villanova, J., et al. Nano-scale evidence for osteocyte network integration across bone remodeling interfaces in human bone revealed by synchrotron nanoCT. *Mater Today Bio.* 2026;37:102813. doi: 10.1016/j.mtbio.2026.102813.

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