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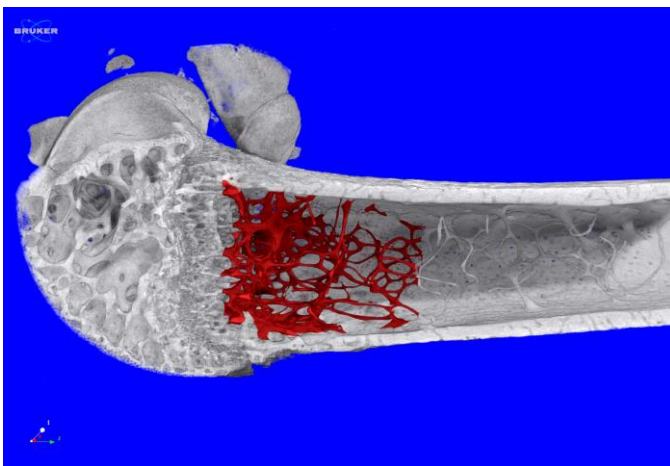
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● **Welcome**

Welcome to the September “back-to-school” Bruker microCT Academy Newsletter of 2016! Best wishes for a successful season of imaging and research! This issue’s topic is one that is important to a large number of bone and orthopedic researchers. The mouse and rat femur and tibia – referred to as the “appendicular” sites, are the standard locations to study the architecture and phenotype of trabecular and cortical bone. This newsletter gives an overview of the key aspects of this methodology and introduces two new method notes that describe how to characterize trabecular and cortical most effectively with the latest Bruker microCT-SkyScan software.

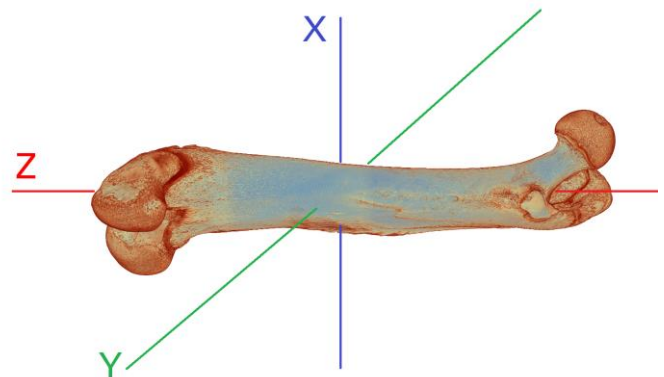
● **MicroCT morphometric analysis of rodent trabecular and cortical bone**

Many readers will already be familiar with morphometric analysis of rodent trabecular and cortical bone. The method notes released with this newsletter bring this established methodology up to date with the latest SkyScan software (DataViewer, CTAn). As with all microCT analysis tasks, bone morphometry of the femur and tibia of preclinical models starts with the region of interest (or volume of interest). Getting this right is perhaps more important than anything else in obtaining good quality unbiased data on developments and changes between experimental groups in biological processes in growing and remodeling bone.



*Figure 1. In the rodent femur, trabecular metaphyseal and cortical diaphyseal volumes of interest, shown in red, are defined by distance from the growth plate in standardized axially aligned microCT datasets.*

Alignment: The first step is checking that the long bone microCT dataset is aligned so that the cross-section plane is perpendicular to the bone axis. This is done in SkyScan DataViewer software. 3-axis viewing both visualizes and allows correction of non-alignment in any axis.



*Figure 2. Femur or tibia datasets need correct alignment along the long axis.*

The growth plate reference: With the dataset axially aligned, the next step is to identify a reference cross-sectional plane representing the growth plate. MicroCT follows the established practice of bone histomorphometry in using the growth plate as the anatomical referent for positioning both the trabecular and the cortical volume of interest (VOI).

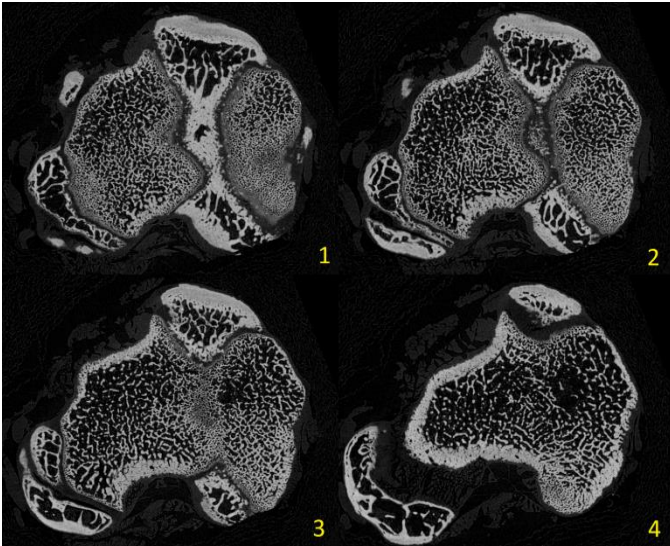


Figure 3. Finding a single planar cross-section reference within the convoluted growth plate structure requires finding a landmark. One often used is the first appearance of a bridging connection of low density growth plate chondrocyte seam, when moving slice by slice from the metaphysis towards the knee growth plate. This approach works in both the femur and tibia.

With the reference level identified, two parameters define the location and extent of the volume of interest relative to this reference. The offset is the interval in number of cross-sections between the growth plate reference level and the start of the VOI range. The height is the extent of the VOI, again in number of cross-sections. With the reference level, the offset and the height, the range of crosssections of the trabecular or the cortical VOI is set.

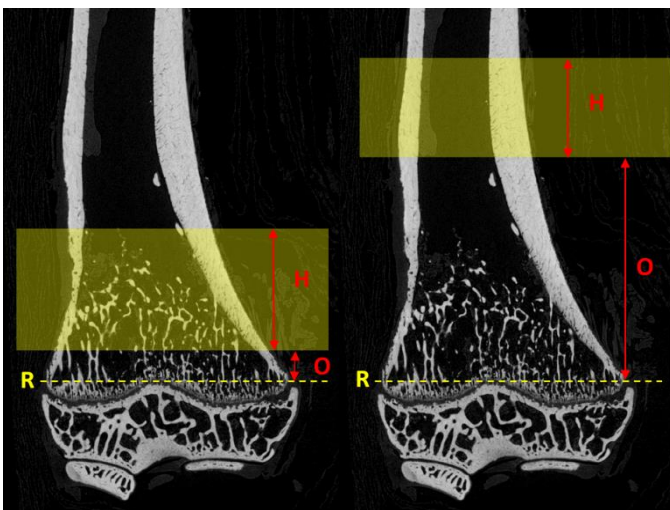


Figure 4. The same system of reference level, offset and height determines the correct positioning both trabecular VOI in the metaphysis and the cortical VOI in the diaphysis.

**ROI delineation for trabecular bone:** In all the cross-sections within the trabecular VOI, an ROI shape must be drawn to separate trabecular from cortical bone. This can be done either manually or automatically with morphological and Boolean operations within SkyScan CTAn. For manual ROI drawing, only a small number of separated crosssections require a drawn shape. The CTAn software interpolates intermediate ROI shapes

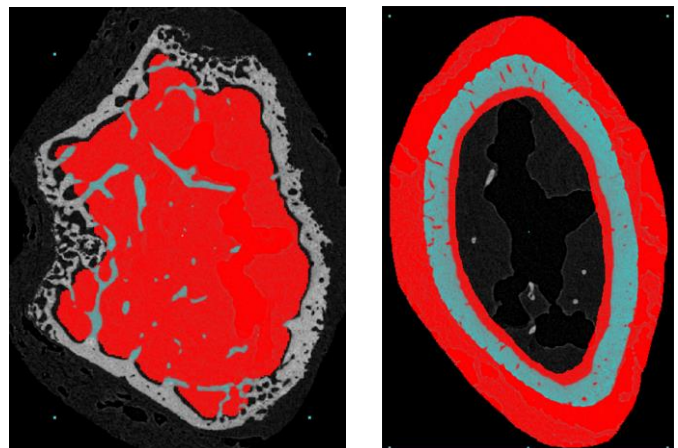


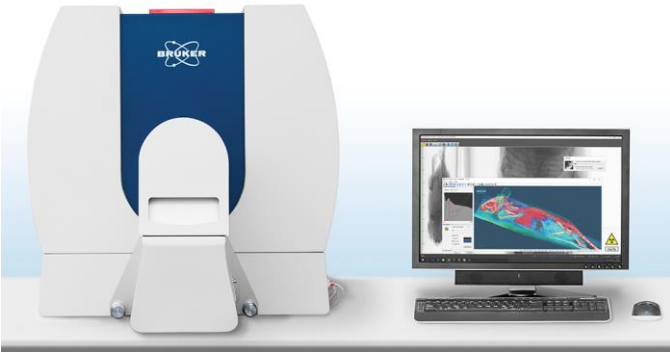
Figure 5. Left: Trabecular bone ROI delineated by automatic image analysis in CTAn, by an algorithm that excludes peripheral metaphyseal extending remnants of growth plate-associated primary spongiosa. Right: a hollow cortical ROI.

**ROI delineation for cortical bone:** This is simpler than for trabecular bone. Hollow ring ROIs at either end of the selected range allow accurate separation of cortical bone along the standardized diaphyseal segment (Fig.5).

**Segmentation (thresholding) and 3D analysis:** Typically threshold values are lower for trabecular bone than for cortical due to the thinness of trabecular structures. Advanced segmentation techniques such as SkyScan CTAn's local adaptive thresholding can improve thresholding by minimizing the partial volume effect. With optimal VOI selection and segmentation, full morphometric 3D analysis of trabecular and cortical bone can then proceed, either singly or in batch mode.



● Bruker microCT News

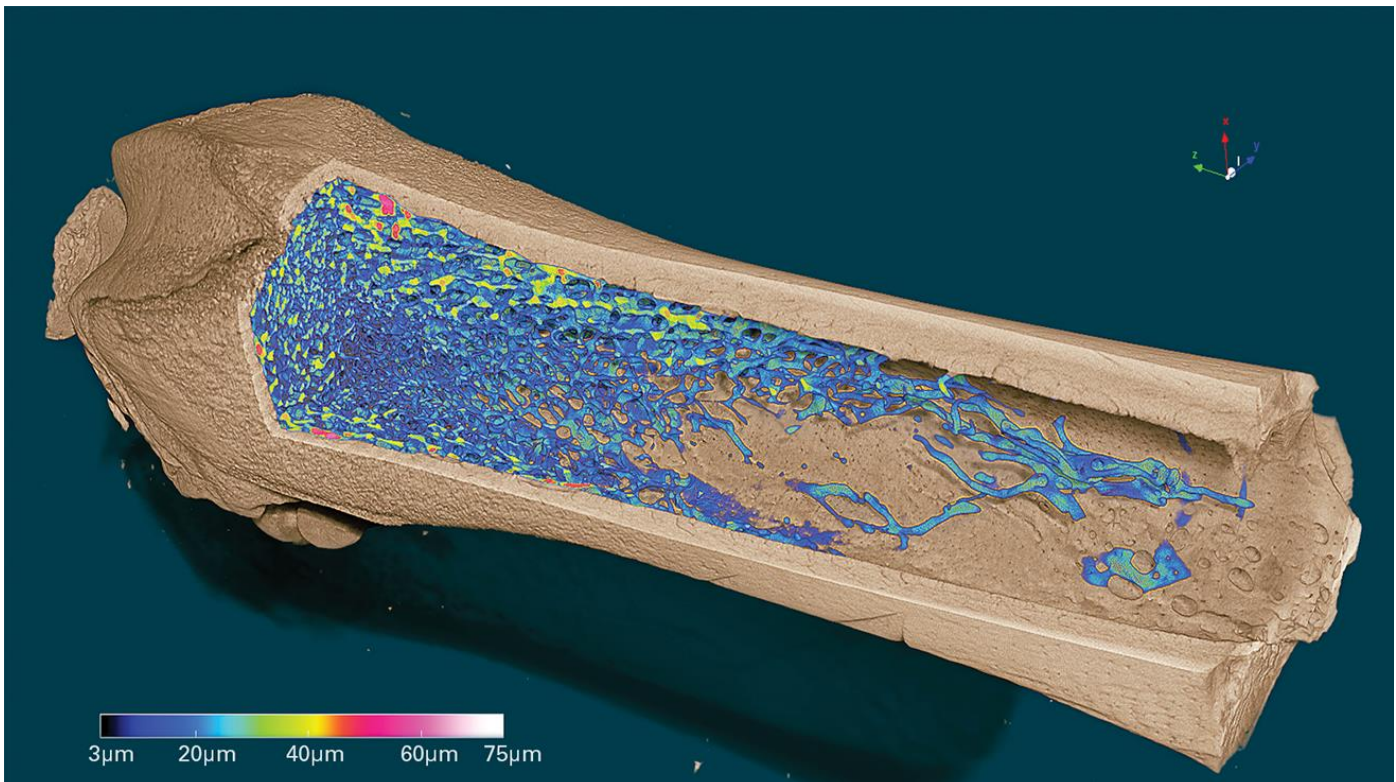


Bruker announces the arrival of the SkyScan1276, a new benchmark for *in vivo* microCT for preclinical research: 2.8 micron pixel size, 3.9 second scan cycle, continuous image zooming, helical scans, high-speed 4D gated scanning. See more new features at [webinar!](#)

● Image of the month

3D volume rendering of a rat bone with color coded local trabecular thickness.

The scan was done with the new SkyScan 1276 at 2.8  $\mu\text{m}$  voxel size, 80 kV, Al 1 mm filter; 4032x4032x7693 pixels reconstructed volume.



● Upcoming events

Bruker microCT will participate with an exhibit in the forthcoming conferences. Please click the link below for more information. We hope to see you there!

2016:

- [Interpore](#) Oct. 07 Venlo, the Netherlands
- [IASLC](#) Dec. 04 – 07 Vienna, Austria
- [Zoology](#) Dec. 16 – 17 Antwerp, Belgium

2017:

- [iCT](#) Feb. 07 – 09 Leuven, Belgium
- [ORS](#) Mar. 19 – 22 San Diego, USA
- [IADR](#) Mar. 22 – 25 San Francisco, USA
- [AACR](#) Apr. 01 – 05 Washington DC, USA
- [EMIM](#) Apr. 05 – 07 Cologne, Germany