

# Dual energy microCT imaging of bone and vasculature inside bone

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## Aims

Bone development and repair requires a normal vascular system to supply the bone with oxygen and essential nutrients. To study the interaction between the vasculature inside the bone and the trabecular bone itself, accurate information on their 3D structure is required. As it is not possible to discern blood from soft tissue by CT imaging, the attenuation of blood vessels is altered by injecting a contrast agent, often based on iodine, barium or lead. Unfortunately these contrast agents have attenuation coefficients similar to bone, making it hard to discriminate them from bone. We investigate whether dual energy microCT scanning can be used to obtain 3D images of both bone and the vasculature inside the bone of mice.

## Method

**Animal preparation.** Two mice were anaesthetised with a ketamin-xylazin-heparin mixture (100 mg/kg ketamin, 15 mg/kg xylazin and 1000 I.E./kg heparin) and laid on their back. Incisions extending across the thorax and abdomen were made and the heart was exposed. Subsequently, a winged needle (Microflex, Vygon) was inserted into the left ventricle and the right atrium was cut open. Mice were first perfused with  $\pm 10$  ml of heparinized saline (100 I.E./ml) to remove the blood, then with  $\pm 10$  ml of a 10 % neutral-buffered formalin solution to fix the tissue and afterward with  $\pm 10$  ml of saline to remove the formalin. Finally, animals were perfused with  $\pm 5$  ml of a preheated 30% m/v barium sulfate solution (Micropaque, Guerbet) containing 2 % gelatin. After perfusion, animals were placed on ice for at least 1 hour to allow the gelatin to solidify, before removing the hind legs for microCT analysis.

**MicroCT acquisition.** The principle of dual energy imaging lies in the exploitation of the energy dependence of the linear attenuation coefficient. Contrast agents have a sharp jump in their attenuation values at the binding energy of their K-shell electron, this is called the K-edge (figure 1). By taking two microCT scans with effective beam energy below and above this K-edge energy, the attenuation of the contrast will differ significantly. The attenuation of bone and soft tissue will however remain largely the same, allowing extraction of the contrast agent. The effective energy of the beam was manipulated to be below and above the K-edge by adjusting the maximum tube voltage and the filtration. The perfused bone samples were first scanned with the low energy beam, obtained by setting the peak voltage to 50kV and filtering the beam with 0.5 mm of aluminum. For the high energy scan, the settings were altered to a maximal tube voltage of 100 kV and filtering with 0.5 mm of aluminum and 0.038 mm of copper. Both scans were performed with an image pixel size of 5  $\mu\text{m}$  in the SkyScan 1172 microCT system and both filters are standard installed on this scanner. To reduce noise, both scans were performed over 360 degrees with a rotation step size of 0.4 degrees while 7 frames were averaged for the lower energy beam and 20 for the higher energy beam, resulting in scan durations of 1h20m and 3h30m respectively.

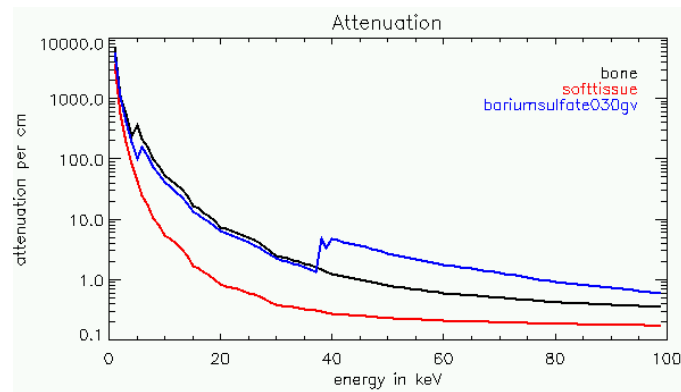


Figure 1: The attenuation coefficients of soft tissue, bone and 30% m/v barium sulfate.

**Tissue decomposition.** We use a post reconstruction approach to combine the two datasets and to obtain a tissue decomposition. We reconstructed both datasets with NRecon (SkyScan) and affinely registered them to each other to make sure that corresponding voxels in both images contained the same tissue. The mean intensities for bone and soft tissue were determined in the two reconstructions and the intensities of the low energy reconstruction were linearly transformed to match those of the high energy reconstruction. After smoothing and subtraction of the low from the high energy reconstruction, the matched bone and soft tissue voxels have very small intensities, while voxels containing contrast agent have higher intensities due to the attenuation jump. This image was thresholded to obtain the vasculature segmentation. The bone segmentation was acquired by thresholding the bone and vasculature out of one of the reconstructions and removing the calculated vasculature from it.

## Results

A detailed view of two reconstructions at the different energies can be seen in figure 2. The arrow indicates a typical blood vessel that is not easy to distinguish on these conventional microCT scans. Applying the tissue decomposition we obtain the difference image of the two scaled reconstructions on the left in Figure 3. The resulting vasculature map can be shown overlaid on the high energy reconstruction as seen on the right of figure 3. A 3D rendering of this blood vessel is shown in figure 4, while a larger region is shown in figure 5.

Some vessels did not exactly coincide in both scans, leading to different tissues in corresponding voxels and segmentation errors. The bottom vessel in figure 2 is such a vessel.

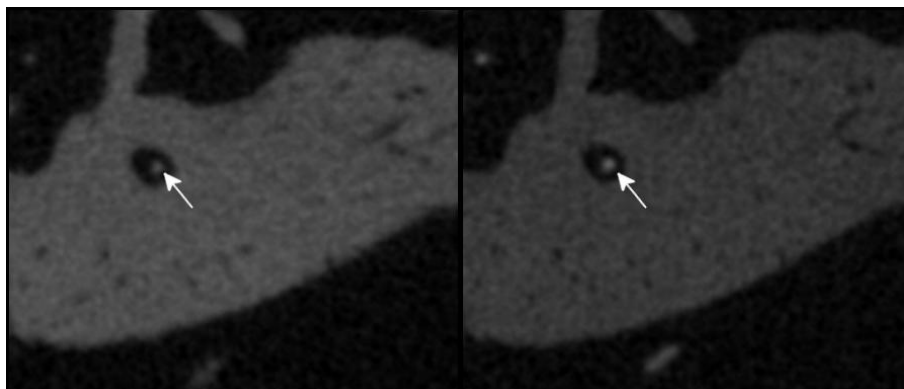


Figure 2: Details of a low energy (left) and a high energy (right) microCT image of a perfused femur. The arrow indicates a blood vessel.

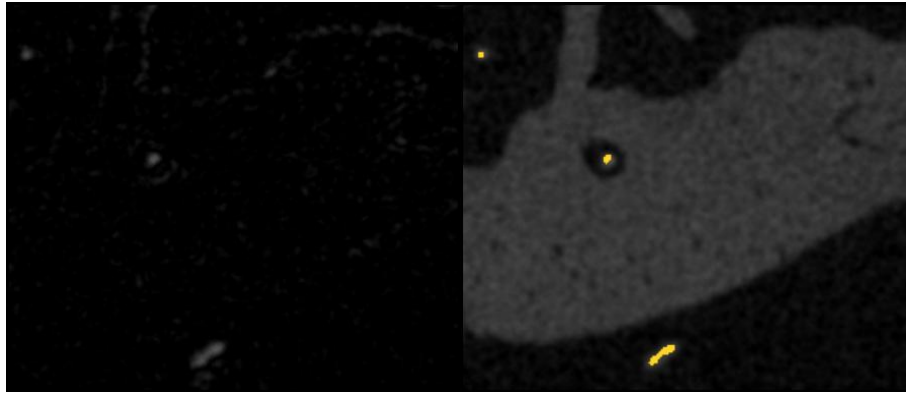


Figure 3: The attenuation difference map of the region shown in figure 2 (left) and the vasculature segmentation overlaid on the initial image (right).

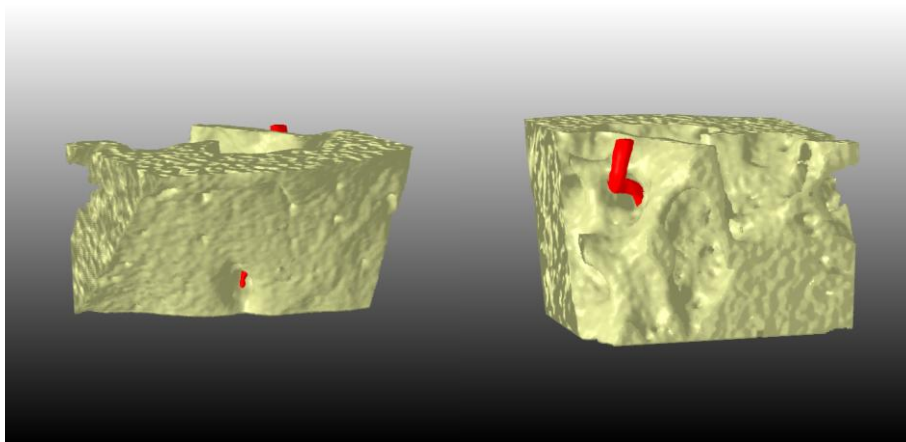


Figure 4: 3D rendering of the blood vessel in figure 2, viewed from outside of the bone (left) and from inside of the bone (right).

## Conclusion

We have shown that it is possible to perform dual energy techniques on a SkyScan microCT scanner without any additional hardware to obtain 3D information of both bone and the vasculature inside the bone. An essential requirement is that an X-ray beam with effective energy above the K-edge of the contrast agent can be achieved, which excludes lead based contrast agents when using an X-ray tube with a maximum tube voltage of 100kV. Scanning time can be reduced by increasing noise robustness with custom tailored filters or a more advanced decomposition technique. Some vessels had slightly different positions in both scans resulting in segmentation errors, which we will attempt to resolve by improved perfusion and registration protocols.

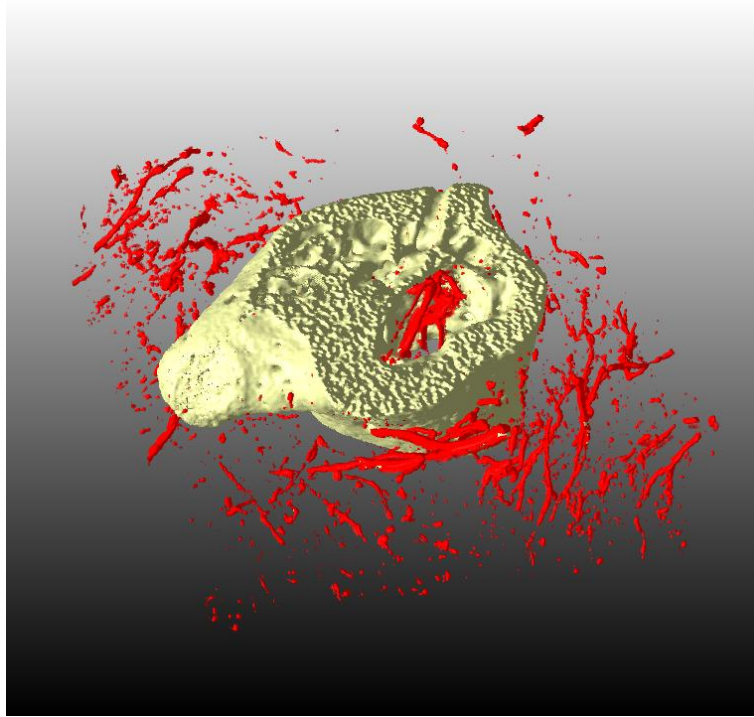


Figure 5: 3D rendering of the vasculature inside and around the femur.