Adult mouse vertebrae nanoCT imaging protocol

Please reference the Jepsen laboratory when using this protocol. Current as of 11/5/13. This protocol is subject to change. We hold no responsibility for errors in the protocol or user error that lead to personal or property damage.

Safety considerations
Please follow all federal, local, and institutional rules and regulations when using this protocol.

Mouse femora nanoCT imaging protocol
Depending on the imaging system you use, you may need to alter this protocol. We use a nanotom S from GE. This protocol is our standard for wet scanning of ten mouse lumbar spine segments (L1-L3) and a calibration standard (air, water and 1.69mg/cc hydroxyapatite). We use a custom acrylic holder (Figure 1).

Figure 1. Custom acrylic specimen holder. a) specimen tube b&c) 6 well specimen holder with an incut for a latex rubber band d) calibration standard with embedded hydroxyapatite sized to fit in a well of the specimen holder. e) empty calibration standard, top small whole is air.

Materials:
Specimen holders
Water bath
Forceps
Additional tube filled with water
Equilibration bath (i.e. beam flattener)
Latex hair bands
**Specimen set-up:** Submerge all acrylic pieces in a water bath. Insert spine segments while under water to avoid introduction of bubbles (cause artifacts). We typically place the calibration standard in the first well (arrow above it) in Figure 1 moving counter-clockwise to well 2 and so on. It is important to embed a directional standard next to the hydroxyapatite in the calibration standard. We use a small sliver of hydroxyapatite indicating the direction that is counter-clockwise to ensure specimen identification in the image. The rubber band is then placed around the center of the specimen holder to keep the bones from moving during the scan. Depending on the size of the vertebrae being scan the use of the rubber band may not be necessary. The sample holder is then screwed tightly into the tube, again all this is done under water to avoid introducing bubbles. After the lid in placed on the tube and the tube is dried off, the whole thing is vortexed by hand for about 20 seconds to ensure that the bones have settled into place. The full tube is usually left to stand for 20 minutes prior to scanning. These steps are important because the system is very sensitive to movement. The scan is done with the equilibration block in place.

**System settings:**

Voltage: 85kV  
Current: 220µA  
Mode: 0  
Exposure time: 2000ms  
Images averaged: 3  
Images skipped: 1  
Total scan time: 135 minutes  
Target type: Diamond-coated tungsten  
Voxel size: 8.0µm (specimen position at 80.0mm, detector position at 500mm)  
Filter: 0.3mm thick Aluminum  
Calibration low: 85kV, 70µA, 100 images averaged  
Calibration middle: 85kV, 145µA, 100 images averaged  
Calibration high: 85kV, 220µA, 100 images averaged  
Virtual sensor: 1  
Detector shift: On  
Number of images: 1000  
Notes: The calibration is done with a water filled tube in place of the specimen tube as well as the equilibration block.