

Adult mouse vertebrae nanoCT imaging protocol

Please reference the nanoCT Core (RRID: SCR_026772) when using this protocol. Current as of 7/1/26. 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 vertebrae nanoCT imaging protocol

Depending on the imaging system you use, you may need to alter this protocol. Our protocols are optimized for our Phoenix Nanotom M. This protocol is our standard for wet scanning of mouse vertebrae in a fixture with an integrated calibration standard (air, water, and 1.69mg/cc hydroxyapatite). We use a custom printed acrylic holder with 5 sample spaces in each of 3 carousels (Figure 1). If needed, additional carousels with larger sample spaces are available.

Materials:

Specimen holders
Water bath
Forceps
Small pieces of soft foam

System Settings:

Voltage: 80kV
Current: 400 μ A
Mode: 0
Exposure time: 500ms
Images averaged: 3
Images skipped: 1
Number of images: 1000
Target type: Diamond-coated Tungsten
Voxel size: 8 μ m (specimen position 48mm, detector position 600mm)
Filter: 0.381mm thick Aluminum
Detector shift: On
Automatic Scan Optimizer (pre-scan): On
Notes: The detector calibration is done with air, and with 50 images averaged

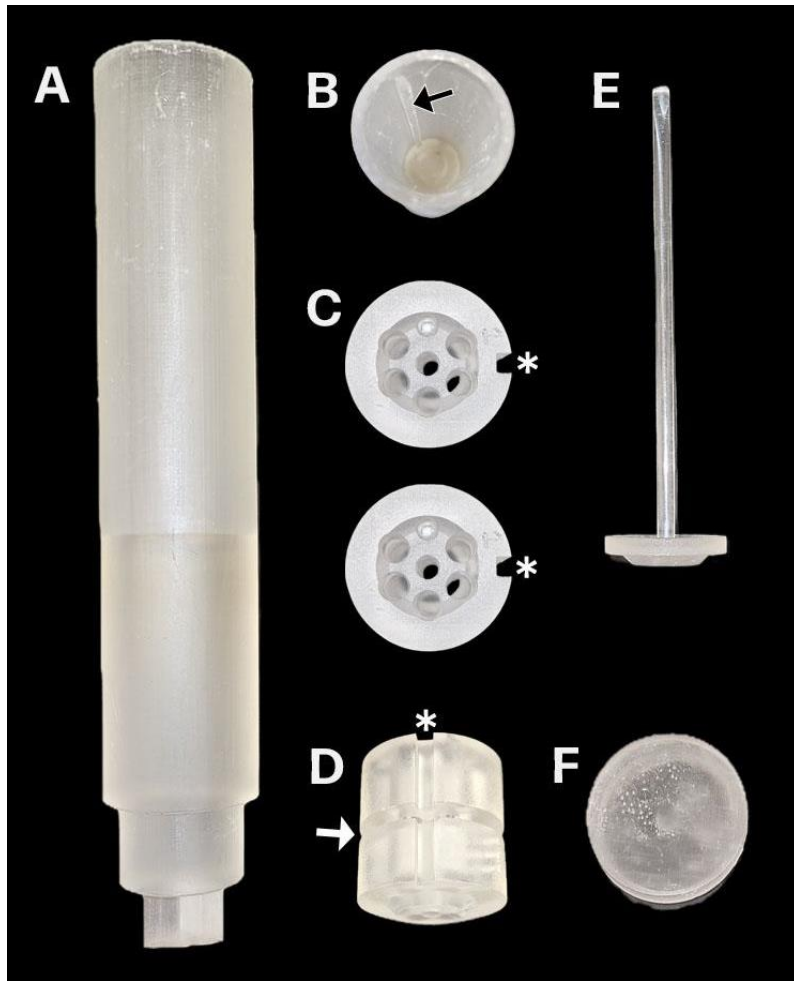


Figure 1. Custom printed acrylic specimen holder. A) specimen tube; B) specimen tube top view, showing ridge (arrow); C&D) three 5-well specimen holders with embedded hydroxyapatite and air calibration standards, showing groove (C&D, *) and horizontal in-cut for rubber bands (D, Arrow); E) post; F) plug style cap.

Specimen set-up:

Submerge all acrylic pieces in a water bath. All preparation is done under water to avoid introducing air bubbles into the tube and specimen holders. Tap the specimen holders to remove air bubbles, then insert bones while under water, starting with holder 1. Our convention is to insert samples in a clockwise direction starting from the calibration standard. Holders are labeled 1-3, and each one has the corresponding number of slivers of hydroxyapatite inserted in holes next to the embedded air standard, which function to eliminate ambiguity in sample identification. After gently pushing bones to the bottom of the sample wells, add small pieces of foam (saturated with water to eliminate air bubbles) to secure the vertebrae in the bottom of the well. Depending on the size of the bones, you may need to use the specimen holders with larger holes. If the vertebrae are sufficiently small, you may be able to add a second layer of bones on top of the foam; the second layer should also be secured with a layer of foam. After all 3 specimen holders have been loaded, pass the post through the

center of each holder, stacking them with #1 on the top and #3 on the bottom. Insert the stack of holders on the post into the tube, aligning the ridge and the groove on each holder to enable them to slide in. Once they are inserted, press the plug/cap into place. Remove loaded tube from the water and dry. The tube is marked for orientation while scanning.