

SOP: Collaborative Cross Project

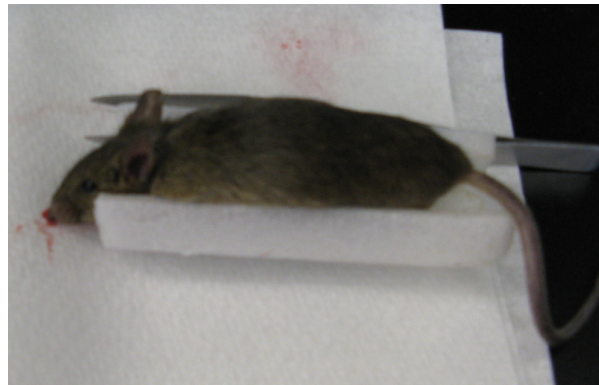
Revised: May 13, 2011

1. Sample Preparation and Setup

Thaw the mice the night before in the refrigerator. Roughly 6 to 7 mice can be scanned per day (i.e., 6 to 7 hours of scanning).



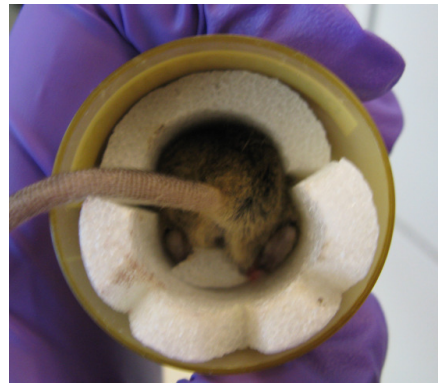
Place the mouse on the bottom piece of foam tubing with the upper limbs folded under the mouse (flexed position) so the hands are near the head.



Separate the upper limbs with a piece of foam so that they will be separated in the scan. Push the foam piece down slightly so that there is room for the mouse's head once it is in the micro-CT sample holder.



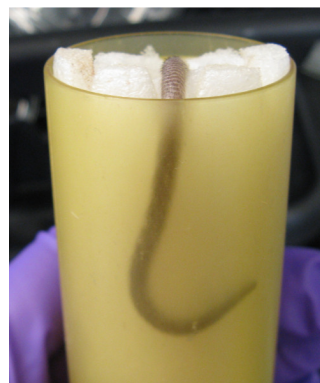
Tuck the lower limbs under the mouse so that they are folded up to the chest (flexed position) and place a foam piece in between them. Place the top piece of the foam tubing on top of the mouse and insert the mouse into one of the 37 mm yellow micro-CT sample holders so that the spine is aligned with the arrow drawn on the holder. If the mouse is large and will not fit in the sample holder, then remove the top piece of foam tubing. The mouse must be fully contained in the micro-CT sample holder.



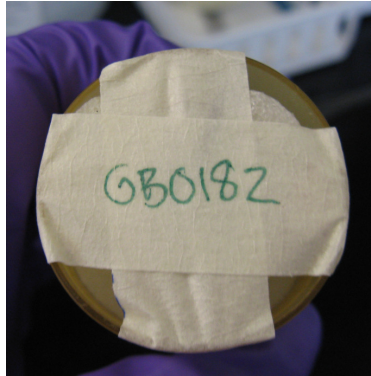
Add additional foam around the mouse's tail to prevent movement of the sample during scanning.



Tuck the tail into the micro-CT holder on the outside of the foam.



Tape over the end of the micro-CT sample holder to ensure that nothing protrudes from the sample holder and label the tape with the mouse ID written on the Falcon tube that the mouse was stored in. Flatten the tape on the sample holder as much as possible.

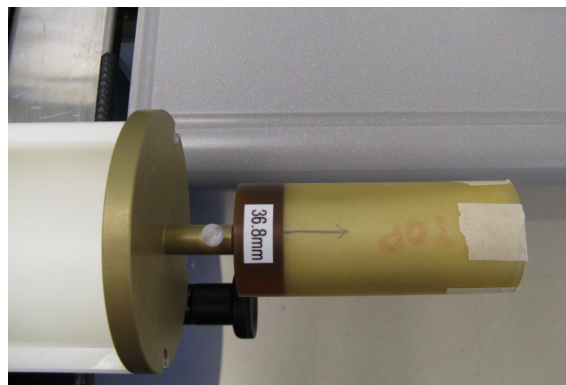
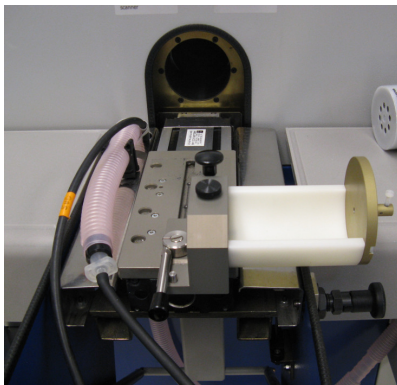


2. Scanning

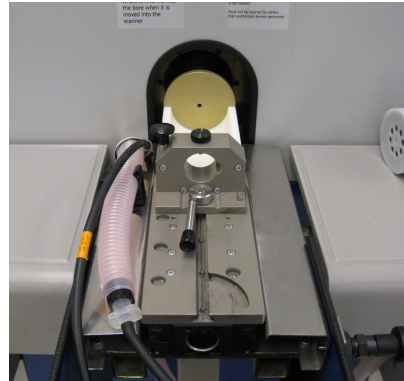
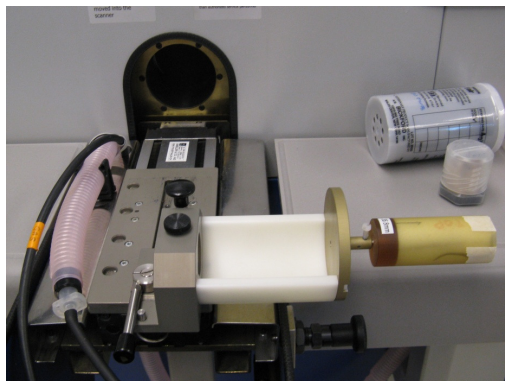
Open the door to the vivaCT 40 scanner.



Attach the sample holder adapter to the small animal bed. Slide the animal bed onto the track of the scanner and rotate it 90 degrees clockwise to facilitate attaching the micro-CT sample holder. Loosen the set screw on the adapter plate, insert the metal spindle of the micro-CT sample holder and finger tighten the plastic set screw. The spine of the mouse should be facing up.



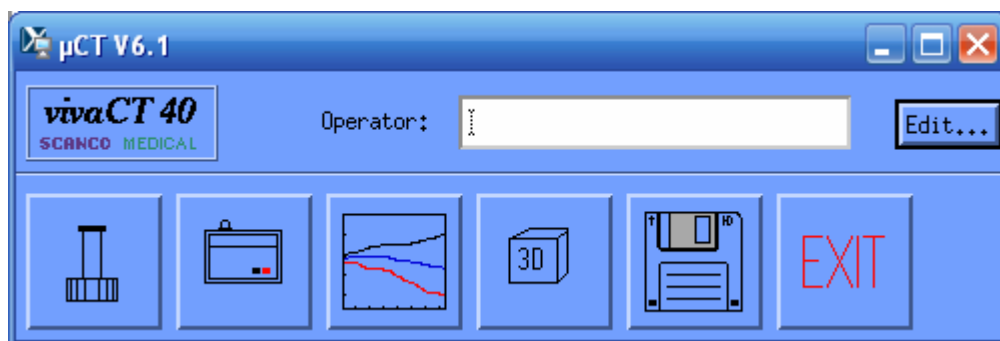
Rotate the animal bed so that it is aligned with the bore of the scanner and slide it along the track to the position closest to the scanner. Lock the animal bed by rotating the lever 90 degrees clockwise.



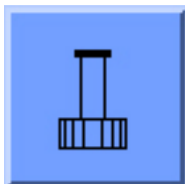
Close the door to the scanner.

Login to the vivaCT computer (username: vivact).

Enter an operator into the micro-CT control panel. To create a new operator with your name, press the edit button, input your information, then press save. You will be assigned an operator number, which can be entered into the micro-CT control panel in place of your name.

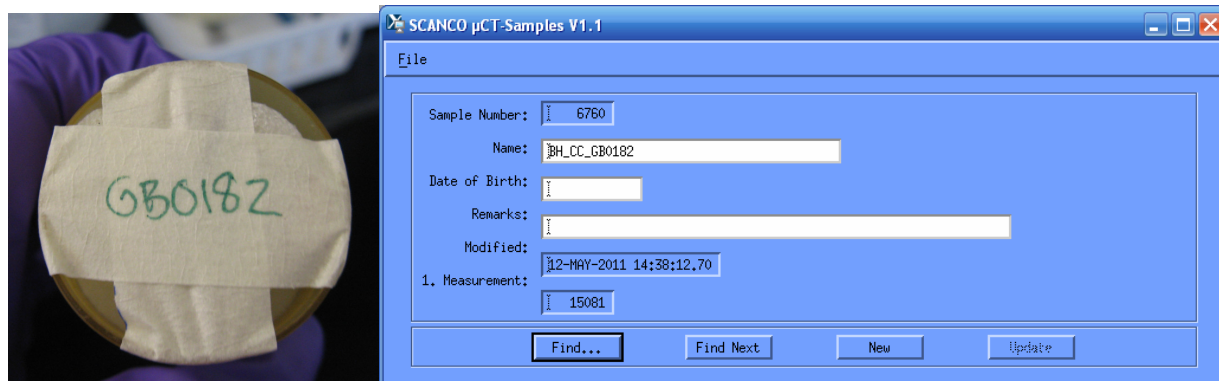


Register the sample about to be scanned by pressing the left-most button in the control panel.

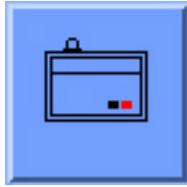


The sample naming convention is as follows: BH_CC_MouseID

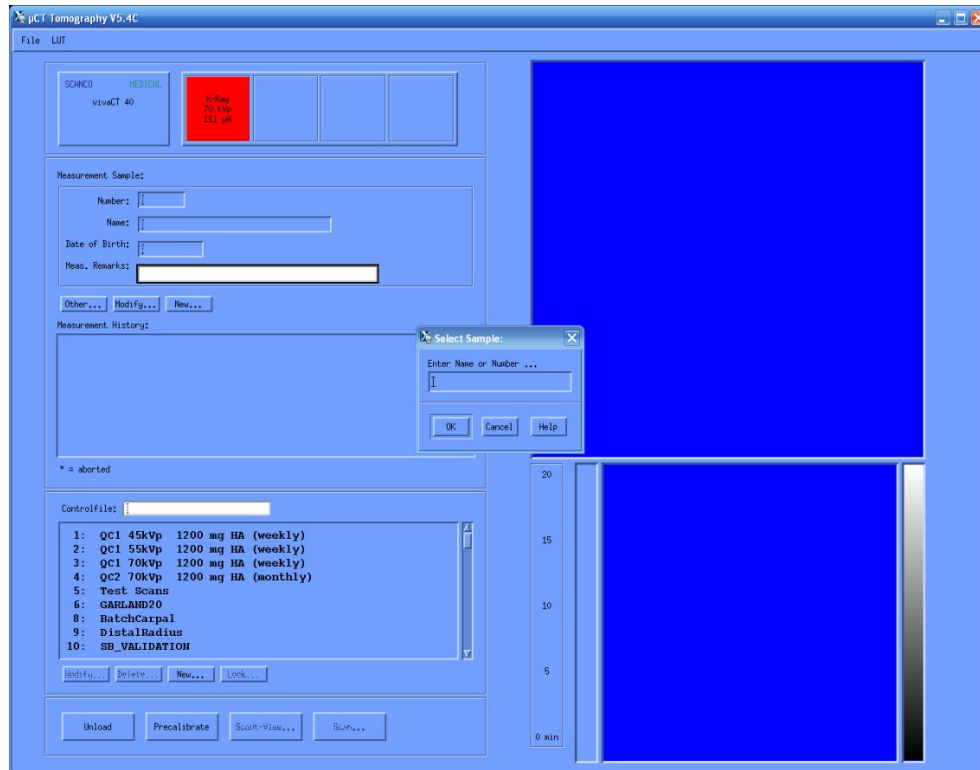
The Mouse ID is found on the Falcon tube and should be registered in the micro-CT system as a six digit identifier (two letters followed by four numbers). Input the name of the sample and press Save. The sample is assigned a Sample Number; record this on the log sheet. Exit the program (File→Exit).



Start the scanning program by pressing the scanner button (second from the left):



In the Select Sample window, enter the sample number and press OK.

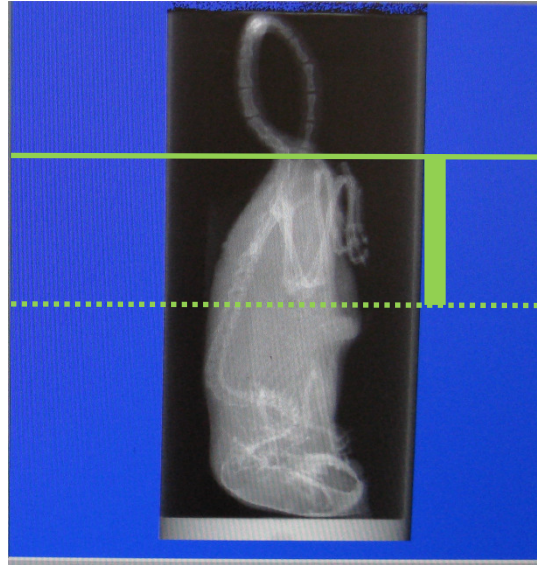


Select the control file to 73: BH_CC_360proj

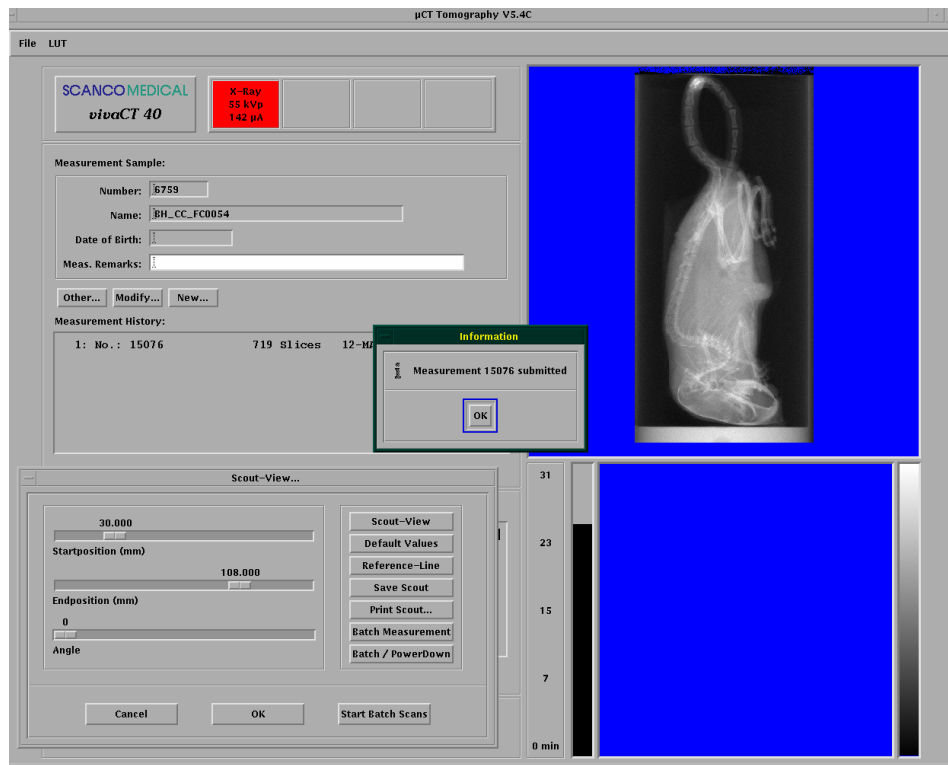
The settings for the scan are 55 kVp, 109 μ A, 360 projections per 180 degrees, 200 ms integration time, 35.8 mm field of view, 1024x1024 reconstruction matrix. A 1200 mg hydroxyapatite/cm³ beam hardening correction is applied. The resulting voxel size is 35 μ m.

Press Scout View to open the scout view window, then press Scout View in the new window.

When the scout view is complete, press Reference Line to set the scan region. Use the computer mouse to move the solid green line just above the top of the mouse's foot, then hold down the Shift key while dragging the computer mouse to extend the scan region to just below the mouse's hip. Press the left mouse button to set the reference line.



Press Batch Scan and record the assigned measurement number on the log sheet.



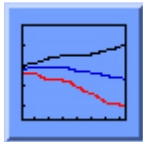
Weigh the mouse either before or after the scan and record its weight on the log sheet.

[illegible][illegible]

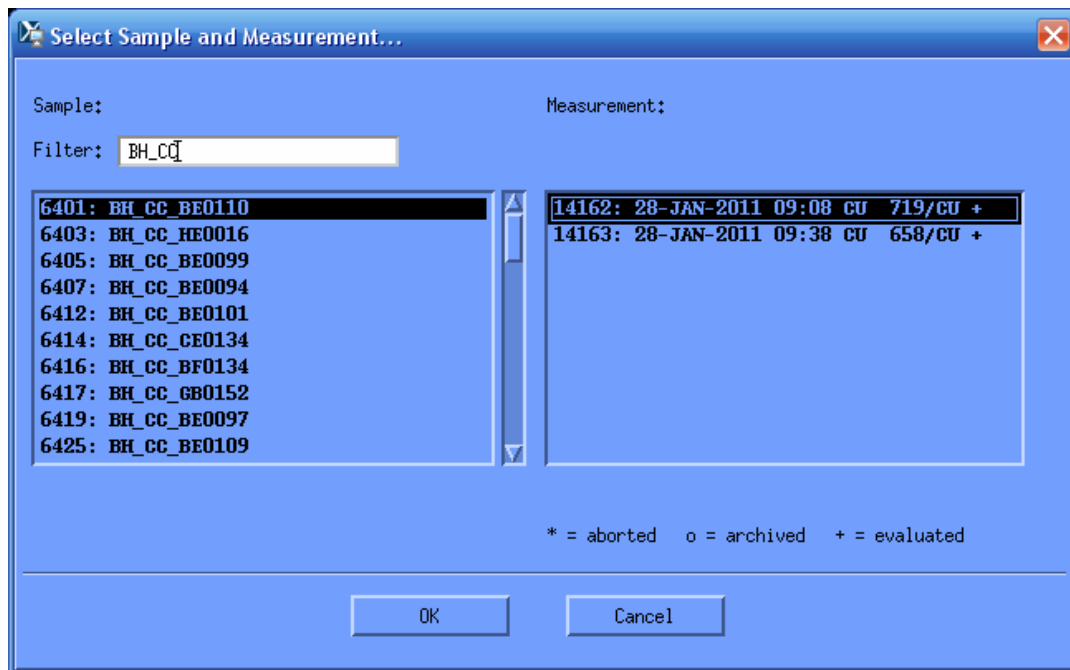
3. Scan Processing

Scans can be processed from any of the micro-CT workstations. Login to the workstation (username: vivact).

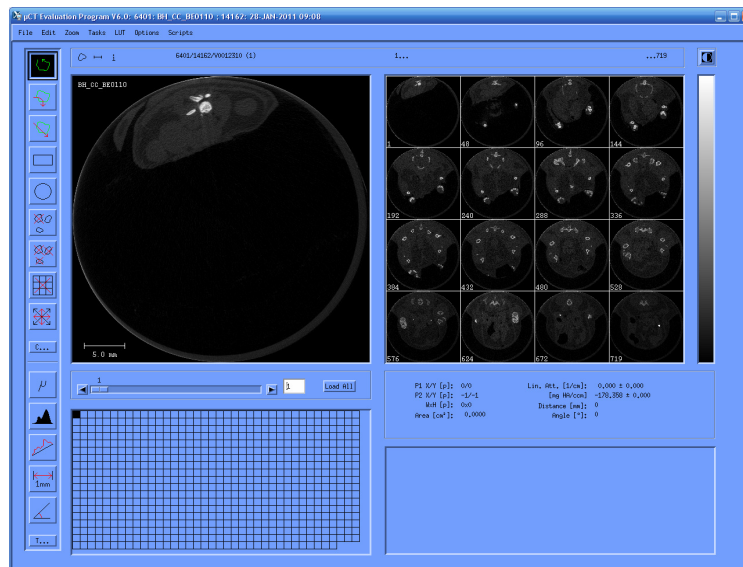
Enter an operator in the micro-CT control panel. Press the button that looks like a graph to start the μ CT Evaluation Program.



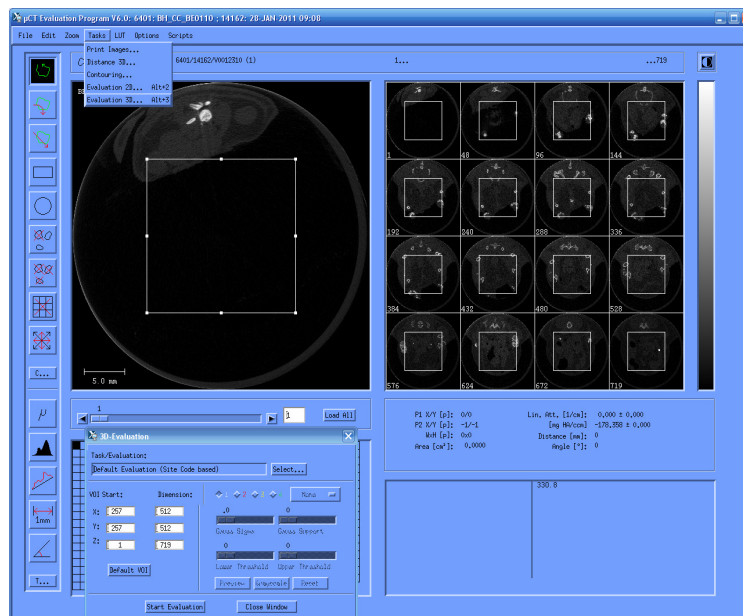
In the sample selection window, enter BH_CC in the file filter to show only the Collaborative Cross project scans. Choose a scan to be processed. For each sample there are two measurements (scans). The first measurement listed is for the scan of the lower limbs and the second measurement listed is for the skull and upper limbs. Select the measurement you wish to process and press **OK**.



A scan of the lower limbs:



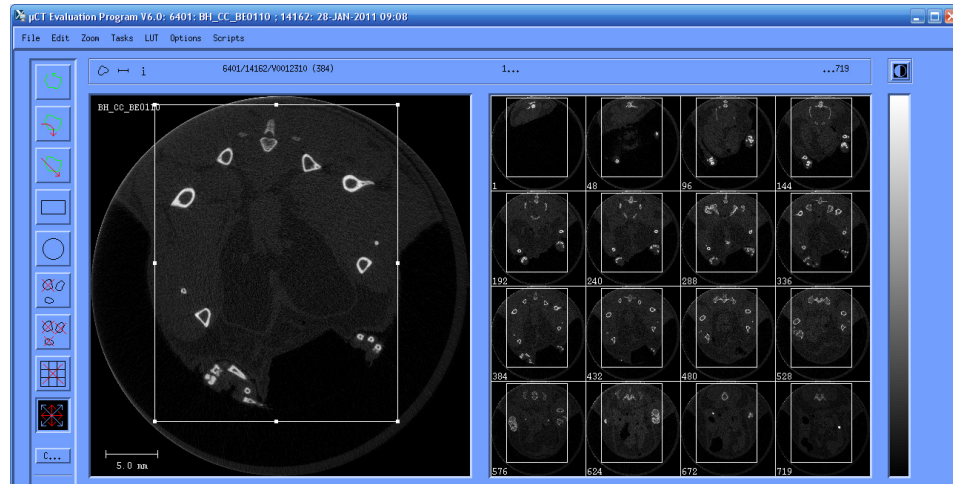
Click either the T... button in the bottom left corner of the program or select the **Tasks** drop down menu from the top of the window and then **Evaluation 3D**. This will open the **3D Evaluation** box as well as place a volume of interest (VOI) box on the slices.



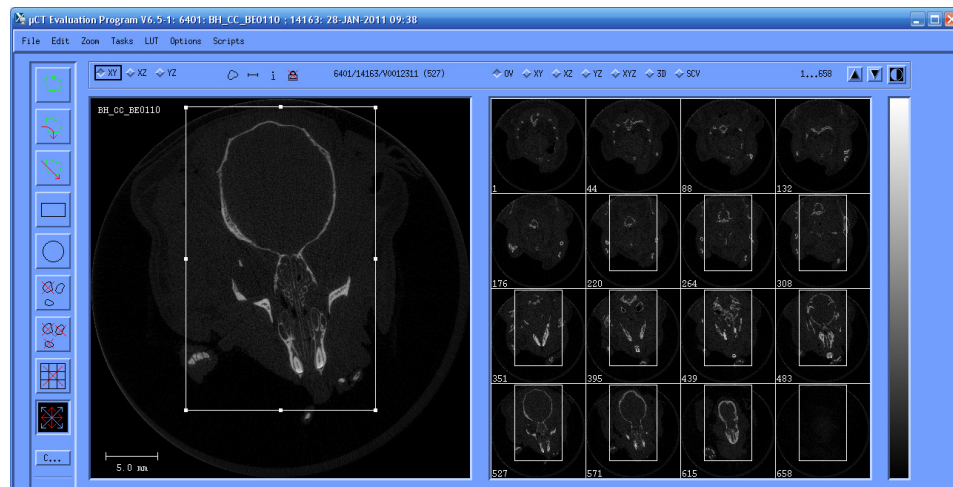
The VOI box shows the region of the scan that will be processed. You can move the VOI box using one of the handles (small white squares) on the edge of the box using the right mouse button and resize it using the middle mouse button.

VOI region notes and evaluation scripts:

- Lower Limbs (script 20): include the feet, tibia, femur and pelvis

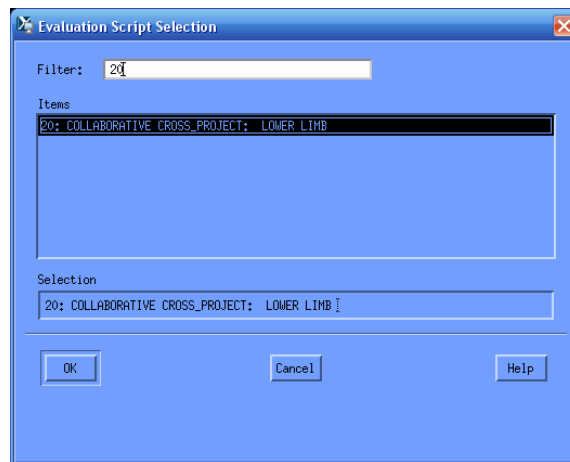


- Upper Limbs (script 21): include the hands, radius, ulna, humerus, shoulder joint and scapula
- Skull (script 22): ensure that all of the skull is included



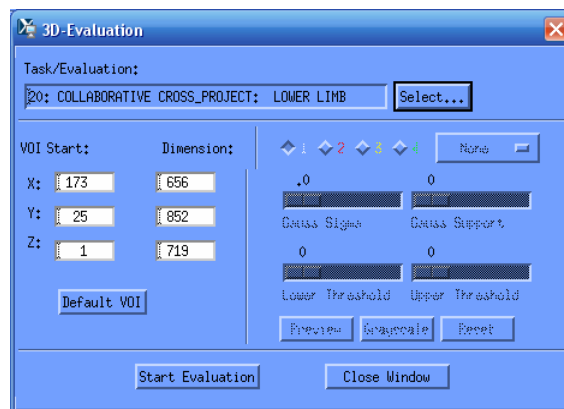
Start by adjusting the VOI box on the first slice and then go through each of the 16 slices shown on the right. Leave a bit of room around the bones to avoid accidentally clipping any of them. Check through additional slices of the scan and extend the VOI box as needed.

In the 3D Evaluation box, press **Select...** This will open the **Evaluation Script Selection** Window where you can either type in the desired script number in the file filter or scroll through them.



Script 20 corresponds to the lower limbs, script 21 corresponds to the upper limbs and script 22 corresponds to the skull. Click on the evaluation script and press **OK**.

In the 3D-Evaluation window press **Start Evaluation**.



On the log sheet, check off the site that was processed (lower limb, upper limb, or skull). Process the three VOIs for each sample.