## **SOP: Collaborative Cross Project**

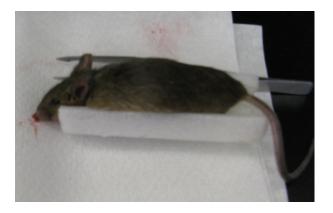
Revised: May 13, 2011

## 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).



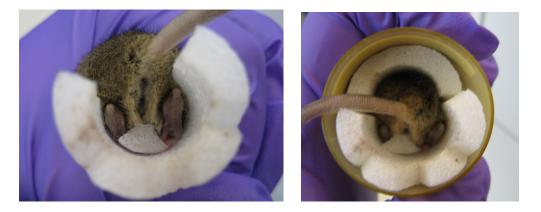
Place the mouse on the bottom piece of foam tubing with the upper limbs folded under the mouse (flexed position) so the hands are near the head.



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.

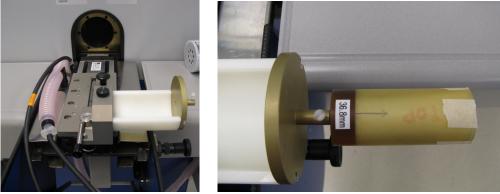


## 2. Scanning

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.



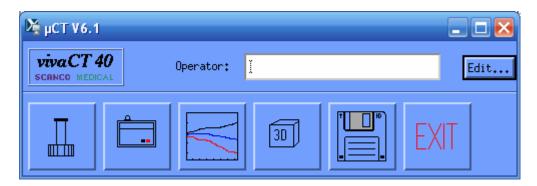
Rotate the animal bed so that it is aligned with the bore of the scanner and slide it along the track to the position closest to the scanner. Lock the animal bed by rotating the lever 90 degrees clockwise.



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  $\rightarrow$  Exit).

	🎽 SCANCO μCT-Samples V1.1	_ 🗆 🔀
	<u>F</u> ile	
	Sample Number: 1 6780	
	Name: BH_CC_GB0182	
GB0182	Date of Birth:	
000102	Remarks:	
	Modified:	
	1. Measurement:	
	j 13001	
	Find Find Next New Update	

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.

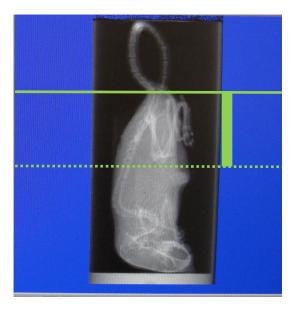
Tomography V5.4C						
TU						
SCHC0 HE3TCH. VIVACT 40						
Messurement Sample: Number: [						
Other Modify New Measurement History:	Die Select San		×			
* = aborted	Enter Name of I	an Number	Help			
			20			
Controlfile: 1: QC1 45KVp 1200 mg HA (weekly) 2: QC1 55KVp 1200 mg HA (weekly) 3: QC1 70KVp 1200 mg HA (weekly)	Ê		15			
4: QC2 70KVp 1200 mg HA (monthly) 5: Tost Scans 6: CARLAND20 8: BatchCarpal 9: DistalRadius 10: SB VALIDATION			10			
Bodify Delete New Look	NI IN		5			
Unload Precalibrate Scon-Vionary Sconary			0 min			

Select the control file to 73: BH\_CC\_360proj

The settings for the scan are 55 kVp, 109  $\mu$ A, 360 projections per 180 degrees, 200 ms integration time, 35.8 mm field of view, 1024x1024 reconstruction matrix. A 1200 mg hydroxyapatite/cm<sup>3</sup> beam hardening correction is applied. The resulting voxel size is 35  $\mu$ m.

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

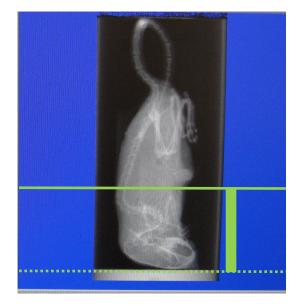
When the scout view is complete, press Reference Line to set the scan region. Use the computer mouse to move the solid green line just above the top of the mouse's foot, then hold down the Shift key while dragging the computer mouse to extend the scan region to just below the mouse's hip. Press the left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



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

μCT Tomography V5	.4C
File LUT	
SCANCOMEDICAL vivaCT 40 X-Ray 142 µA	
Measurement Sample: Number:  5759   Name:  BH_CC_FC0054 Date of Birth:	
Meas. Remarks:	
Other   Modify   New	
1: No.: 15076 719 Slices 12-Hz formatien	
Scout-View	31
30.000 Scout-View Startposition (nm) 108.000 Reference-Line	23
Endposition (mm)	15
Cancel OK Start Batch Scans	7
	0 min

Now press the Reference Line button again and setup a second scan region that will include the upper limbs and skull. Press Batch Measurement and record the measurement number.



Press Start Batch Scans. The scanning program will close.

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

This is what the scan log sheet looks like:

PROJECT: Collabo SCAN REGION: mc		pper limbs, and s	skull	vivaCT 40: 55 kVp	SETTINGS : 73, BH 109 µA, 200 ms IT, ousto g HA/om <sup>3</sup> beam hardening	m resolution (102		atrix, 360 proj/	180°), 35.8 m	m FOV, 35 µ	im voxels		LOG SH	ieet ni	JMBER:	
SAMPLE	sample no	lower meas no	upper meas no	weight [g]	ecan notee	RAW	IMA tape		D-CT EVALL			RVER CH			NDMARK	
BH_CC_		ineas no	meas no			саре	tape	Iowel #20	upper #21	SKUII #22	IOWEI	upper	skull	IOWEI	upper	skui
BH_CC_																
вн сс																
BH_CC_																
BH_CC_																
вн_сс_																
BH_CC_																
BH_CC_																
вн_сс_																
BH_CC_																
BH_CC_																
вн_сс_																
BH_CC_																
вн_сс_																
BH_CC_																
вн_сс_																
BH_CC_								-								
BH_CC_																
BH_CC_								-				-				
BH_CC_																
BH_CC_									-			-				
вн_сс_																
вн_сс_																
вн_сс_						-										
вн_сс_	_															-
вн_сс_									-							
вн_сс_	_											1				-
вн_сс_																-
BH_CC_	-					-										-
вн_сс_																
BH_CC_					1			1								1

## 3. Scan Processing

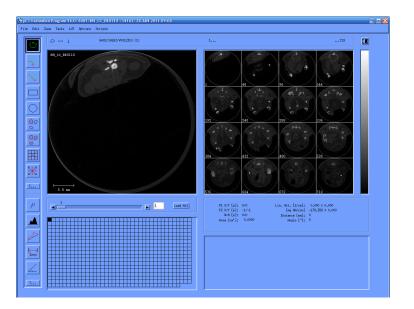
Scans can be processed from any of the micro-CT workstations. Login to the workstation (username: vivact).

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

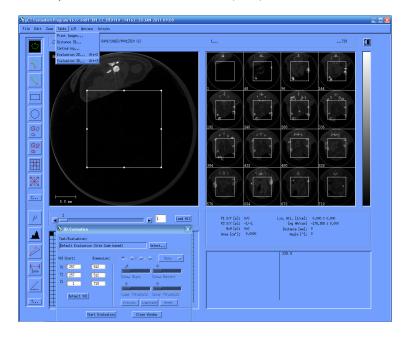
In the sample selection window, enter BH\_CC in the file filter to show only the Collaborative Cross project scans. Choose a scan to be processed. For each sample there are two measurements (scans). The first measurement listed is for the scan of the lower limbs and the second measurement listed is for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

🏹 Select Sample and Measurement		$\mathbf{X}$
Sample: Filter: BH_CQ	Measurement:	
6401: BH_CC_BE0110 6403: BH_CC_HE0016 6405: BH_CC_BE0099 6407: BH_CC_BE0094 6412: BH_CC_BE0101 6414: BH_CC_CE0134 6416: BH_CC_BF0134 6417: BH_CC_GB0152 6419: BH_CC_BE0097 6425: BH_CC_BE0109	14162: 28-JAN-2011 09:08 CU 719/CU + 14163: 28-JAN-2011 09:38 CU 658/CU +	
	* = aborted o = archived + = evaluated	
OK	Cancel	

A scan of the lower limbs:



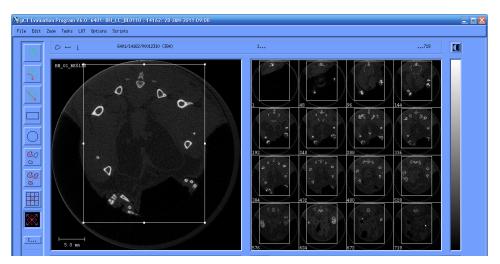
Click either the T... button in the bottom left corner of the program or select the Tasks drop down menu from the top of the window and then Evaluation 3D. This will open the 3D Evaluation box as well as place a volume of interest (VOI) box on the slices.



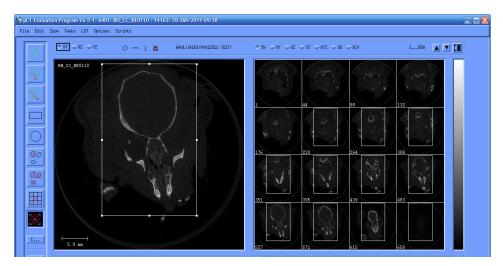
The VOI box shows the region of the scan that will be processed. You can move the VOI box using one of the handles (small white squares) on the edge of the box using the right mouse button and resize it using the middle mouse button.

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 Selection		
Filter:	20]		
Items			
20: COLLABO	RATIVE CROSS_PROJECT	: LOWER LIMB	
) Selection			
	DRATIVE CROSS_PROJEC	T: LOWER LIMBĮ	
	DRATIVE CROSS_PROJEC	T: LOWER LIMB [	
20: COLLAB	DRATIVE CROSS_PROJEC		Helo
	DRATIVE CROSS_PROJEC	T: LOWER LIMB [	Help
20: COLLAB	DRATIVE CROSS_PROJEC		Help

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.

🏹 3D-Evaluati	on	×
Task/Evaluation	n: TVE_CROSS_PROJECT:	LOWER LIMB Select
VOI Start:	Dimension:	◆: ◆2 ◆ ł ◆ ł None =
X: 173 Y: 25	( 656 ( 852	.0 0 Gauss Signe Gauss Support
Z: 1 Default V	719	0 0 Lower Threshold Upper Threshold
		Firevie- Grayscale Eeset
	Start Evaluation	Close Window

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