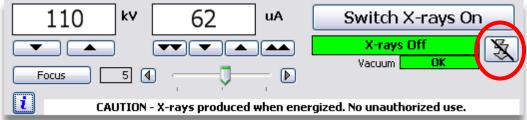
Operating Procedures for MICROCT1 Nikon XTH 225 ST

Ensuring System is Ready (go through to ensure all windows and tasks below have been completed – either by you or someone else – prior to mounting and scanning your sample

- □ Log usage into the SMIF web site
- □ Ensure "X-ray On" light is not currently lighted on the front of the cabinet
- □ Ensure key interlock on control station is set to "on"
- □ Run Inspect-X program
 - Load wanted configuration i.e. Reflection-Front
 - Enter password hp23.4jx
 - Click Yes to login as "Supervisor"
 - Screen should have properties icon if logged in correctly
- □ Home axis before anything is placed in chamber
 - To Home, click down-arrow next to and select Mag
 - Mag axis will then initialize, once it's finished move stage all the way forward
 - Then, click down-arrow next to and select All Axes
 - Stage will then initialize all axes and Absolute coordinates will go from red to black when stage is ready
- □ If you are the first user of MICROCT1 today, you must follow these steps otherwise continue onto next section:
 - Click the circled button (red circle) below from the main screen



- On the menu that pops-up (Intelligent Auto-Conditioning), click start
- Monitor "Time stable at maximum kV", when greater than 15:00 -> Switch Xrays Off
- If conditioning takes longer than 1hr. or value of vacuum is higher than 40 for long periods of time, please STOP conditioning and contact SMIF staff

Mounting Specimen

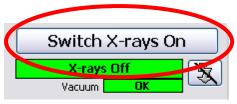
1) Secure sample in appropriate material of minimal attenuation (i.e. florist foam, batting, light/non-thick plastic, etc) while ensuring rigidity

MICROCT1

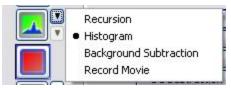
- 2) Ensure sample or region of interest in sample is placed along a vertical axis (center of rotation) within container to promote best resolution
- 3) Place sample and container onto turntable with region of interest placed as directly over center of rotation as possible
- 4) If known or needed, insert filter
- 5) Close door and ensure interlock is engaged
- 6) Continue onto next section

CT Scan Setup (Do For Every New Scan, See Appendix A for Batch Scanning)

- 1) In Inspect-X software, **Set kV** & **W** to reasonable values (**130kV** and **12W**) are good starting points for most samples)
- 2) Switch X-rays On



3) Live Image On with Histogram selected



4) (Properties) -> Imaging Tab -> Imaging Set-Up and change Exposure to desired value, Save (354ms or 500ms is a good starting point)

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X-ray controller	Imaging Manipulator CT
Application	
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Camera Controls	
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	1
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Exposure	
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Accumulation	▶ 1X
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Digital Gain 🔳	V 1 X
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dur 🕒	, y u u u
Brightness	.0.0%
Status	
Preset	Remove Save
Bad Pixel Correction	Collect
	Revert Save Close

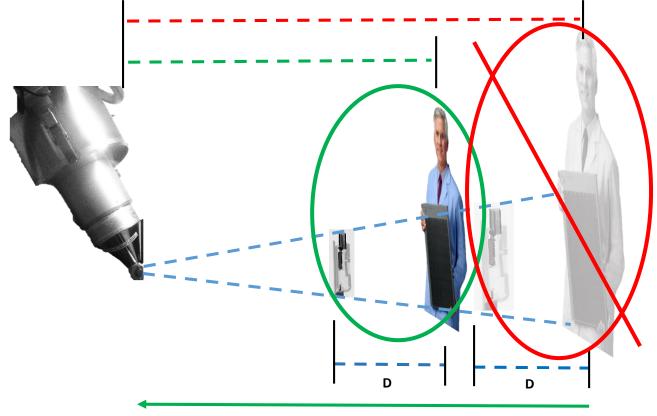
5) **Corrections** tab of **Properties->Imaging** window, Shading Correction -> **Disable**, **Save** and close window

erties		
X-ray controller	Imaging Manipula	ator CT
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aparal Topa Control (nnotation Imaging Set-Up Corre	ections
image Corrections	ninocación i Inaging Sec-op	
image corrections		
	Shading Correction	Disabled
		Restore Disable
	Valid time for reference images	3 🚔 hours
	Time for X-rays to die down	10 💭 seconds
-		
Time t	o collect standard correction images	seconds
System Calibration		
	System Ima	ging Calibration Calibrate
	System and	ging coloración Calibrace
	Revert	Save Close

6) Make sure you are on the Image Tools -> Image Tone Taba. See below image

ols CT		
Image Tone	Measure Range	PTH Fill
	Erode/ Dilate	Package Void
Pseudo-Colour	Thresholding	Bond Wires
BG Subtraction	BGA Analysis	Wire Sweep
Measure	BGA Ball Void	Drill Offset
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Centre Cross 🔽 🛛	Grid G	45 🔿
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Average	1 Std Dev	9.20
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Tone Curve Cust	om	~

- 7) Set X coordinate -> 0 and position sample for optimal resolution and detector to source distance (see image below) using digital joysticks in GUI. Remember, sample must fit within FOV across all angles of stage rotation
 - When detector is closer to the source, images are brighter and sharper
 - When detector is too close to the source, maximum resolution for sample may not be achieved or sample may not fit into FOV of the detector
 - When detector is too close to the source the image may oversaturate and so detector exposure or source current/watts can be reduced
 - Best practice is to optimize above for resolution vs. image clarity vs. scan time

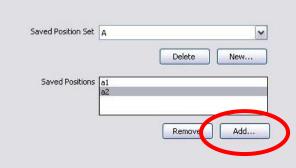


Increased Image Brightness

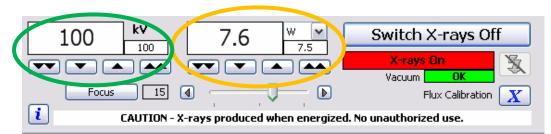
D is the length which defines magnification (resolution of scan). The greater distance D between your sample and the detector, the smaller the voxel size your scan will have (i.e. higher resolution). This image shows that the same resolution can be achieved by moving both sample and detector closer to the source while maintaining their respective distance, D. Therefore, the green circle would be a quicker scan, sharper, and brighter image than that of the red-crossed circle while maintaining the resolution.

- 8) Rotate to 0 and save position(s) by clicking Add under the Saved Position menu and labelling appropriately
 - a. For Batch Scan, repeat 7, saving all batch positions at Rotate = 0

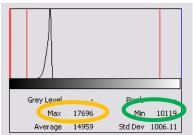
b. Note, if scanning 1 continuous item across multiple scans, make sure the only coordinate that changes value b/w the scans is Y, otherwise scans will not be able to be seamlessly combined



- 9) Reset Image Tone and Delete All measurements, then rotate sample to darkest imagea. Darkest Image = The angle at which the sample is deepest/thickest in the image
- 10) Adjust kV and W until max gray value = 60000 at brightest projection
 - a. Higher kV helps to penetrate denser areas, but reduces contrast in low density areas and can blur sample edges
 - b. Higher W helps to heighten dynamic range, lower scan time and deafen noise, but can cause image blurriness and over-saturation if set to high



- 11) Now, optimize width of histogram by adjusting W & Detector Exposure
 - a. Ensure image is not over-saturated and gray values are b/w 10,000 min and 60,000 max
 - b. Above 15W and the source will auto-defocus which doesn't affect image sharpness for voxel sizes above 30um but may for smaller sizes
 - c. The difference b/w Max and Min gray values will be the dynamic range of your image(s) and data
 - d. The image blurs as a function of W. As a rule, keep W smaller than the voxel size (W to um).



- 12) **Check** all projection angles for over-saturation
 - a. Rotate the sample 360⁰ ensuring **Max** gray value doesn't exceed 62,000

13) Go to **CT Tab**

- 14) **Sample Setup** -> enter number of projections needed based on how much your sample
 - fills the screen from left to right as your rotate it about a full circle
 - a. A sample that spans the image requires 1800 projections
 - b. A sample which is very narrow would require ~1200projections
 - c. For something of moderate width, estimate between 1200-1800 based on how wide it is
 - d. Enter number of **Projections** into the field shown below

Image Tools	ст			
Sample Setup	Shading Correction	Centre of Rotation	Reconstruction Setup	Acquisition
Sample Set	tup			
	F	rojections 220) 📄 🗋 Optimi	se
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	ne sample is pene Rotate button	trated at all ang	les by	Rotate
	are acquired with the image. Press ample.			Move
Flux Norma	lisation			
	Apply flux no	ormalisation 🗹		
Crop an area	a on the image in	which no sampl	e is in view at all an	gles.
CT Profile			_	
				Load

- 15) Set **Frames per Projection** according to image noise level and amount of dark areas in sample. 1-10 is a reasonable range of values
 - a. More frames will multiply scan time by value used i.e. 4 = 4x longer scan
 - b. For weak attenuating material, it is better to have higher Frames per projection than a longer exposure time
 - c. For dense, metallic or samples with beam-hardening prone pockets, it is also better to increase the Frames per projection and keep the exposure time shorter
- 16) Ensure Flux Normalization is unchecked
- 17) Shading Correction tab
 - a. Click New

b. Enter name 1,2,3,etc for name

Setup	Shading Correction	Centre of Rotation	Reconstruction Setup	Acquisition
nading Co	rrection			
			set, that you use a	
rrection wi ames,	nose images nave	obeen acquired	with a minimum nu	mber or
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		Disable st	adia generation	
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		New	Update	Load
-rav condit	ions	kV	130 uA 5	
ray condic	10/13	IV	130 GH 3	

- c. If shown, select **Current** for exposure time as opposed to **Previous**
- d. Create
- e. Move sample out of field of view using digital joysticks

Shading Correction				
	Disable shading correction			
Na	me 110kV.136uA.708ms			
Delete Create	Done << Update Load			
X-ray conditions	kV - uA -			
Use multiple X-ray filters				
Reference Images				
Frames to average	e 16 💌			
White Image				
Filter	r None 🔽 0.000 🗸 mm			
	White target 60000			
	Points >>			
	Generate			

f. Change White Value to 60,000 (Blue Circle)

g. Click Generate

- i. Filters can be left in despite the message.
- ii. Errors of oversaturation of the detector may occur if the image was set up with a filter and it was removed prior to the calibration

h. Number of reference images should be left at 3 -> Click Ok



- i. Wait for corrections to finish (can take multiple minutes)
- 18) For automated reconstruction, Centre of Rotation tab-> Calculate automatically -> Checked -> Acquire otherwise skip to step 17
 - a. Ensure resultant line on left image crosses through a portion of the sample
- 19) <u>For automated reconstruction</u>, **Reconstruction Setup** tab and enter information you would like used
 - a. Leave **Reconstruction quality** at **100%** and enter known number preset for **Beam** hardening and Noise reduction
 - b. Under Volume of Interest click Acquire
 - c. Drag red box edges to crop out unwanted material on resultant 2 images on the left monitor leave a good amount of space from sample edge
 - d. Note the changes in file size under **Disk space for volume**

20) Acquisition tab

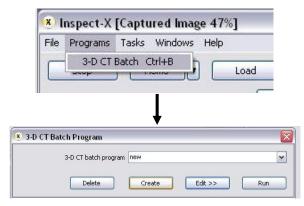
Image Tools	ст				
Sample Setup	Shading Correction	Centre of Rotation	Reconstruction Setup	Acquisition	
Acquisitio	n				
	Reconstruction	computer 192	.168.1.71	~	
Data set name Boyer_UCMP14_omomyid_tarsal					
X-ray filter			Copper		
		0.12	25 🔽 mm		
	Re	construct 🔽			
	Minimise ring	artefacts 🔽			
	Add M	ultiple A	dd to Batch	Acquire	
	Minimum time	to acquire 00:	28:36 Hours:Mins:S	iecs	

- 21) Select appropriate Reconstruction computer (.71 = RECO1, .72 = RECO2) for saving
 - a. Ensure there is sufficient space on that computer
- 22) Fill in Dataset name beginning with your last name (surname or family name) and separate words with underscores "_" rather than spaces
 - a. See image above for example

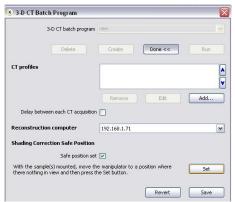
- 23) Select appropriate filter type under X-ray filter and select appropriate known thickness just below, otherwise select None
 - a. See image above for example
- 24) If wanting to have destination computer automatically reconstruct scan upon completion, make sure **Reconstruct** is checked and that steps in **18-19** were followed
 - a. If not automating a reconstruction, uncheck this box
- 25) Minimize ring artifacts can be selected but will extend scan time significantly
 - a. Most scans are fine when this option is not selected
 - b. Disables perpetual movement of stage rotation during scan to reduce angular smearing which causes ring artifacts in reconstructions
- 26) Click Acquire, <u>UNLESS</u> you are collecting a <u>BATCH SCAN</u>, then first follow directions in **Batch Scan Appendix** and at this step select **Add to Batch**
- 27) Scan will begin, note projected scan duration from pop-up
 - a. Do not leave before seeing a displayed time estimate as the scan may never begin
- 28) See RECO Operating Procedure for reconstruction of MICROCT1 data

Appendix A: Batch Scan

- 1) Ensure steps **7-8** under **CT Scan Setup** were completed in full and that all regions/positions of interest were saved
- 2) Programs menu -> Give program a name -> Create



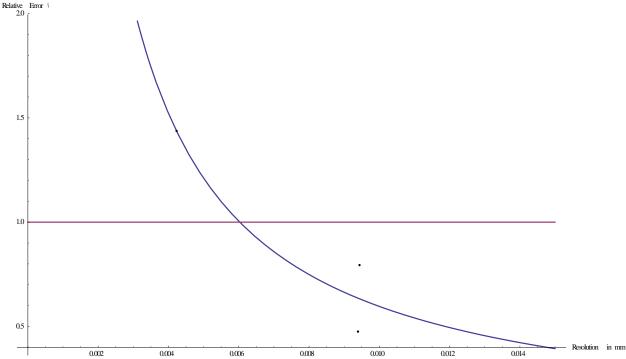
- 3) If batch will run longer than 5 hrs, a shading correction safe position will need to be set
 - a. Make sure to check Safe position set
 - b. Move sample out of view then click Set and Save



- 4) Leave above window open and **repeat steps 14-26** in (CT Scan Set-Up) selecting **Add to Batch** when reaching **step 26** until each scan in your batch appears in the **CT profiles** dialogue
- 5) When Done, Save -> Done<< -> Run
- 6) Batch should begin
- 7) Note projected run time under progress bar from pop-up window

Appendix B: Metrology

- 1) Measurements made on MicroCT become prone to higher error at higher resolutions (see plot below)
 - a. Use of SMIF approved standard or use of a known landmark in the scan to correlate measurements is highly recommended
 - b. For measurements made on samples scanned at resolutions below 15um, error might be >1% and sometimes as great as 25%
 - c. You can choose to scan a standard with your specimen or after your specimen so long as it is done at the same resolution and detector distance
 - i. The y and x stage positions are irrelevant
 - d. Standards scanned alone can be collected at very short exposure time (286ms) and low average (1-2) with needed projections set to 800.
 - i. This scan usually takes 5 minutes



- 2) Once the standard has been scanned at the same resolution as your sample, you will need to open the reconstructed scan data in Volume Graphics VG Studio MAX 2.2 and measure the known dimension in software for correlation between measured vs. actual
 - a. You can use other software as well if you know how to edit the resolution settings of your imported data
- 3) You will then scale the voxel size of your scan by multiplying the ratio produced by $\frac{MEASURED}{ACTUAL}$ of your standard times the voxel size of your scan
 - a. The voxel size of the scanned standard and your sample MUST be the same
 - b. Please speak to Jimmy about this as he has an XLS which makes this very simple
- 4) Measurements now made on your scan data should have a relative error <~0.1%
- 5) For actual procedure to correct data using VG Studio MAX 2.2, see RECO Operating Procedure