

Considerations for a Specimen Micro-CT Scan:

Voxel Size:

Voxel size is the size of a 3D pixel in the rendered image, which is also termed nominal resolution. This is a setting that can be chosen by the investigator prior to the scan. The MicroCT 100 can achieve nominal isotropic voxel sizes between 5.0 μm – 200 μm . Using the smallest voxel size possible (i.e. higher nominal resolution) will allow for more accurate measurements, but smaller voxel sizes are coupled with longer scan times and large data sets. When considering all of these outcomes, a *minimum* voxels to object size ratio of 2:1 is a good guideline to follow (Bouxsein *et al*, 2010, JBMR).

The following table depicts the diameter and length of available sample holders and the possible voxel sizes that can be achieved. Red denotes voxel sizes that are not possible with the associated sample holder, and yellow denotes voxel sizes that can be achieved by acquiring a field of view smaller than the diameter of that sample holder. Voxel sizes are not limited to the values listed. Binning pixels can allow for any voxel size within the range that is possible for that sample holder. For example, although not listed, a voxel size of 15.0 μm with a full field of view can be achieved with the 9.0 mm, 14.0 mm, and 19.0 mm diameter sample holders. Therefore, these values are meant to serve as a guideline when designing a scanning protocol.

Sample Holder Diameter (mm)	Sample Holder Length (mm)	Field of View (mm)	High Resolution Voxel Size (μm)	Medium Resolution Voxel Size (μm)
9.0	78.0	10.2	5.0	10.0
		15.2	7.4	14.8
		20.5	10.0	20.0
		35.2	17.2	34.4
		50.4	24.6	49.2
		75.4	36.8	73.6
		90.1	44.0	88.0
		105.1	51.3	102.6
14.0	92.0	10.2	N/A	N/A
		15.2	7.4	14.8
		20.5	10.0	20.0
		35.2	17.2	34.4
		50.4	24.6	49.2
		75.4	36.8	73.6
		90.1	44.0	88.0

		105.1	51.3	102.6
19.0	84.0	10.2	N/A	N/A
		15.2	7.4	14.8
		20.5	10.0	20.0
		35.2	17.2	34.4
		50.4	24.6	49.2
		75.4	36.8	73.6
		90.1	44.0	88.0
		105.1	51.3	102.6
34.0	110.0	10.2	N/A	N/A
		15.2	N/A	N/A
		20.5	10.0	20.0
		35.2	17.2	34.4
		50.4	24.6	49.2
		75.4	36.8	73.6
		90.1	44.0	88.0
		105.1	51.3	102.6
48.0	110.0	10.2	N/A	N/A
		15.2	N/A	N/A
		20.5	N/A	N/A
		35.2	17.2	34.4
		50.4	24.6	49.2
		75.4	36.8	73.6
		90.1	44.0	88.0
		105.1	51.3	102.6
73.0	130.0	10.2	N/A	N/A
		15.2	N/A	N/A
		20.5	N/A	N/A
		35.2	17.2	34.4
		50.4	24.6	49.2
		75.4	36.8	73.6
		90.1	44.0	88.0
		105.1	51.3	102.6
88.0	130.0	10.2	N/A	N/A
		15.2	N/A	N/A
		20.5	N/A	N/A
		35.2	17.2	34.4
		50.4	24.6	49.2

		75.4	36.8	73.6
		90.1	44.0	88.0
		105.1	51.3	102.6
103.0	130.0	10.2	N/A	N/A
		15.2	N/A	N/A
		20.5	N/A	N/A
		35.2	17.2	34.4
		50.4	24.6	49.2
		75.4	36.8	73.6
		90.1	44.0	88.0
		105.1	51.3	102.6

Additionally, voxel sizes between 1.25 μm – 5.0 μm can be achieved with custom protocols; however, depending on the sample, overall image quality may be compromised due to increased noise and limitations in adjusting scan settings. If a user is interested in obtaining images of their sample with voxel sizes smaller than 5.0 μm , it is highly suggested they meet with the micro-CT technician prior to developing a protocol.

Energy:

Energy is proportional to the frequency of X-ray photons. For the MicroCT 100, X-ray tube potential (peak) is a setting that can be chosen by the user or technician. It refers to the applied peak electric potential of the X-ray tube that accelerates electrons for generating X-ray photons, and is reported in units of kVp. The microCT 100 is capable of scanning at 45kVp, 55kVp, 70kVp, and 90kVp.

Selecting the appropriate energy is important for avoiding undesirable artifacts. Given that the X-rays beam is emitted as a spectrum, one undesirable artifact referred to as beam hardening can occur when lower-energy X-rays are stopped by a sample, while higher energy photons pass through. This results in the appearance of dark streaks in the image. Generally, a thick and/or dense sample will be subject to more beam hardening than a thin and/or less dense sample. For thick or dense objects (e.g. bone), higher energy settings are often chosen in attempt to avoid beam hardening.

Intensity, integration time, and frame averaging:

There are numerous ways to limit noise during scan acquisition, or in other words, increase the signal to noise ratio. If voxel size is to remain unchanged, the most simple and common way to approach this task with the MicroCT 100 is to increase the intensity (i.e. tube current – μA), integration time for each projection (ms), and/or increase the number of times each projection is repeated (frame averaging). Adjusting these parameters simply allows for more photons to be

emitted with no subsequent change in voxel size. However, there are limitations in adjusting these parameters. For example, a high integration time for a particular energy setting may lead to saturation of the detector, and therefore predetermined upper limits for integration time exist for each energy and intensity setting. Furthermore, caution must be taken when adjusting integration time and frame averaging because they directly affect total scan time, which is used to determine the monetary cost for the user (or Supervisor). Total scan time is easily calculated prior to submitting the scan, so adjustments can be made if necessary.

Filters:

The MicroCT 100 conveniently comes equipped with an automatic filter changer, allowing for a wider range of materials that can be scanned. In short, these adjust the shape of the energy spectrum of the x-ray beam. For small and light specimens, using a weak filter enhances the contrast produced by soft materials. Stronger filters, which result in a more concentrated high-energy X-ray beam, improve image quality for more dense materials or larger objects by reducing the effects of beam-hardening. Filter materials are 0.1 mm Aluminum, 0.5 mm Aluminum or 0.1 mm Copper. A fourth filter changer position allows scanning without a filter (information obtained from www.scanco.ch/en/systems-solutions/specimen/specimen-options.html).

Estimating Cost:

The cost of a microCT scan can be estimated prior to scanning the entire sample. For unfamiliar samples (i.e. materials that have not been scanned before at the Centre for High-Throughput Phenogenomics), a test scan may be necessary in order provide a quote. A test scan consists of scanning a small portion of the sample in order fine-tune scanning parameters for the acquisition of high quality images. Once the scanning parameters are determined, the total scan time can be calculated prior to the actual scan occurring. Rates are \$50/hr for scan time and \$50/hr for technician assistance, billed at 15 minute increments. The user will receive their data upon reconstruction of the paid scan.

Accessing Data and Data Formats:

The Centre for High-Throughput Phenogenomics is currently in the process of setting up a network for uploading user data. Eventually, users will be able to sign-in to their individual folders and access their data remotely. In the meantime, data can be acquired by bringing an external hard drive or USB stick to the microCT technician for data transfer. Alternatively, shared sources such as Dropbox can be used as well.

Reconstructed scans come in the form of 2D “slices”, where a stack of these slices can be used to render a 3D image. The pixel dimensions are the

same as the chosen voxel size, and one slice has the thickness of one voxel. Common formats for these slices are .DCM and .TIFF.

Image software is available in the Centre for High-Throughput Pehnogenomics workstation area. Available software includes the Scanco microCT software (manufacturer of microCT-100), ImageJ, MicroView, and Amira. Technicians can provide assistance with this software, but this needs to be discussed and planned prior to booking a workstation.