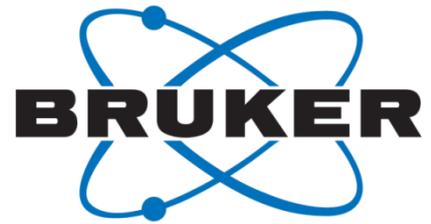


Bruker **microCT**



# **SkyScan 1276**

## **How to set up a scan?**

Method note

### Overview workflow

1. Anesthesia and preparation of the animal
2. Animal positioning in the SkyScan 1276
  - i. Close the door
  - ii. Switch on the x-ray source
  - iii. Take a scout scan
  - iv. Position region of interest animal in the camera field of view
  - v. Set the pixel size
3. Optimize transmission through the sample
  - i. Set the correct filter
  - ii. Adjust voltage
4. Update the flat-field correction
  - i. Remove the animal bed from the field of view
  - ii. Select 'update flat-field for current mode' from the options menu
5. Reposition the animal bed (cfr step 2)
6. Evaluate image
7. Set acquisition settings in 'acquisition' menu
  - i. Rotation step
  - ii. Frame averaging
  - iii. Random movement
  - iv. 180/360° scan
  - v. Partial width/offset scan
  - vi. Synchronized scans
8. Start scan

## Workflow in detail

### 1. Anesthesia and preparation of the animal

The *in vivo* SkyScan 1276 can be used to scan both living animals as well as samples *ex vivo*.

*In vivo* scans:

- i. The animal has to be anesthetized. Several options are available including gas anesthesia (preferable) and injection anesthesia.
- ii. Position the animal in the center of rotation to avoid misalignment artifacts.
- iii. Preferentially put animals on their back when scanning the thorax to limit body movement due to breathing.
- iv. When scanning a hindlimb, extend the hindlimb through a polystyrene tube to position the hindlimb in the center of rotation and avoid x-ray exposure of the body.

*Ex vivo* scans:

- i. Different samples require different preparation/mounting techniques:
  - o Scan dry or wet?
  - o Scan at room temperature or keep the sample cool/frozen?
  - o Scan in air, water, ethanol...?
  - o Prevent movement of the sample!
  - o Mount using polystyrene foam, double sided tape, wax, ...?
  - o ...
- ii. If possible resize your sample but make sure the size is still large enough to give representable data.
- iii. The ideal shape of an object is a cylinder.
- iv. Position the sample in the center of rotation to avoid misalignment artifacts

## **2. Bed positioning in the SkyScan 1276**

- i. Close the door.
- ii. Switch on the x-ray source.
- iii. Take a scout scan.
- iv. Position the ROI in the field of view.
- v. If possible make the sample rotate completely inside the field of view:
  - o The parts of the sample that rotate outside the field of view can't be reconstructed due to missing data.
  - o When an object is larger than a field of view on a specific pixel size, one still has the option to run an offset scan (point 6).
- vi. Define a pixel size:
  - o The pixel size is defined by a combination of the camera position ('zoom') and the camera binning mode.
  - o Note that the same pixel size can sometimes be set using different camera binning modes. In these cases, camera binning will reduce the scan time and increase the signal to noise ratio.
  - o Note that for a pixel size below 4 $\mu$ m the ex vivo sample beds should be installed.

## **3. Optimize transmission through the sample**

One key aspect of microCT imaging is partial absorption of X-rays in the sample. Too much transmission will reduce the contrast between different densities, while a low transmission will increase the noise level in the images. The transmission should be evaluated by inspecting the profile line in the acquisition software (activated by a single right click on the projection image). Adjust filter and voltage settings to get a minimum transmission between 10 and 50% (aim for 30% if possible). Note that these parameters should be adjusted for the highest dense part/angle in/of the sample.

- i. Set the correct filter
  - o A filter absorbs X-rays below a certain energy level and thus increases the average energy of the X-ray beam. As a result, applying a (thicker) filter will increase the transmission through the sample and reduce beam hardening artifacts.

- The SkyScan 1276 has 6 filter options: no filter, 0.25mm Al, 0.5mm Al, 1mm Al filter, AlCu and low dose filter.
- ii. Adjust voltage
- Changing the applied voltage will change the average energy of the X-ray spectrum. Increasing (decreasing) the applied voltage will increase (decrease) the average energy of the X-ray beam and thus increase (decrease) the transmission through the sample.
  - Note that a filter will also reduce the radiation dose to the animal. It is recommended not to perform *in vivo* scans without filter.
  - Guidelines for the combination of filter and voltage (presets):

Filter	Voltage
No filter	40kV
0.2 mm Al filter	50 kV
0.5 mm Al filter	65 kV
1 mm Al filter	80 kV
AlCu filter	90 kV
Low dose Cu filter	100 kV

Note, the filter-voltage combinations can be changed at any time by the user in the 'scanning modes' menu if needed.

#### 4. Update the flat-field correction

The flat-field correction is a background correction that will make sure the background is always represented in the same grey level and will level out interpixel intensity variations that would otherwise result in ring artifacts. As such the flat-field correction is essential when a comparison is needed between multiple scans generated on different time points.

One should always update the flat-field correction upon changes in the following settings: filter, voltage, current, power of the source and camera binning mode.

- i. Remove the sample/bed from the field of view (move the bed out).
- ii. Select 'update flat-field for current mode' from the options menu.

Note that this flat-field correction can be saved and re-used for similar samples. We recommend updating the flat-field correction at the start of the day, and when the above described settings are changed.

### 5. *Reposition the sample/bed*

Cfr. step 2.

### 6. *Evaluate image*

Evaluate the transmission through the sample (cfr step 3). If the transmission is fine, proceed to step 7. If the transmission is too low or too high, repeat steps 3 and 4.

### 7. *Set acquisition settings in 'acquisition' menu*

- i. Rotation step
  - A smaller rotation step will increase the signal to noise ratio. Preferentially lower the rotation step (instead of increasing frame averaging) for low dense samples when the signal to noise ratio is too low.
  - Guidelines for choosing the rotation step

<b>Binning</b>	<b>Rotation step</b>
0.5K mode	$\leq 0.8^\circ$
1K mode	$\leq 0.6^\circ$
2K mode	$\leq 0.4^\circ$
4K mode	$\leq 0.2^\circ$

- Note that in parallel to step and shoot scans where a rotation step is defined, the SkyScan 1276 also allows for continuous rotation for faster scanning at 0.5K or 1K. In this case no rotation step is defined. Instead the scan time will be specified using a slider in the acquisition menu. For 2K and 4K binning modes continuous rotation is not recommended.

ii. Frame averaging

- A higher number for frame averaging will increase the signal to noise ratio. Preferentially increase the frame averaging (instead of decreasing the rotation step) for high dense samples when the signal to noise ratio is too low.
- Guidelines for choosing the frame averaging for EX VIVO scans (use low to no frame averaging for in vivo scans to limit the scan time)

<b>Binning</b>	<b>Frames</b>
0.5K mode	1-2
1K mode	1-3
2K mode	2-4
4K mode	3-5

iii. 180/360° scan

- 360° scans should be selected for samples consisting of a combination of high dense materials inside low dense materials to avoid depletion artifacts.

iv. Partial width/offset scan

- By activating the partial width, the width of the projection imaged is cropped. Make sure the sample rotates within the new field of view (boundaries) at all angles. By activating the partial width, the rotation step can be slightly increased.
- The offset scan mode will double the width of the field of view by doing 2 scans subsequently side by side (change of the camera position). To preserve the signal to noise ratio, make sure you also decrease the rotation step with a factor 2.

v. Synchronized scans

- Make sure the physiological monitoring is activated and the right gating signal is selected in the physiological monitoring window (option to use movement, ECG signal or pressure signal as trigger).

- Prospective gating: specify the lag time by selecting the 'sync with event' mode. (Note – this mode will be available soon)
- Retrospective gating: set the number of frames by selecting the 'list mode' option.

Note: for synchronized scanning and image sorting we also refer to more dedicated method notes on these specific topics.

## **8. Start scan**