Bruker microCT

SkyScan 1278
How to set up a scan?

Method note

Overview workflow

1. Anesthesia and preparation of the animal
2. Animal positioning in the SkyScan 1278
    i. Close the door
    ii. Switch on the x-ray source
    iii. Position region of interest animal in the camera field of view
    iv. 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 1278 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:
    - Scan dry or wet?
    - Scan at room temperature or keep the sample cool/frozen?
    - Scan in air, water, ethanol…?
    - Prevent movement of the sample!
    - Mount using polystyrene foam, double sided tape, wax, …?
    - …
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 1278

i. Close the door.
ii. Switch on the x-ray source.
iii. Position the ROI in the field of view.
iv. If possible make the sample rotate completely inside the field of view:
    - The parts of the sample that rotate outside the field of view can't be reconstructed due to missing data.
v. Define a pixel size:
    - The pixel size is defined by the camera binning mode. One has the choice between 50µm, 100µm and 200µm pixel size.

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
    - 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 1278 has 4 filter options: no filter, 0.5mm Al, 1mm Al filter 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
0.5 mm Al filter	50 kV
1 mm Al filter	65 kV
Low dose Cu filter	50 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 scanner.
ii. Close the door and activate the X-ray source again.
iii. 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

Binning	Rotation step
50µm pixel size	≤ 0.5°
100µm pixel size	≤ 0.75°
200µm pixel size	≤ 1°

- Note that in parallel to step and shoot scans where a rotation step is defined, the SkyScan 1278 also allows for continuous rotation for faster scanning. In this case no rotation step is defined. Instead the scan time will be specified using a slider in the acquisition menu.

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)

Binning	Frames
50µm pixel size	<4
100µm pixel size	<3
200µm pixel size	<2

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.

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