SkyScan 1276 CMOS Edition

How to set up a scan?

Method note

MCT-147

Overview workflow

1. Anesthesia and preparation of the animal
2. Animal positioning in the SkyScan 1276 CMOS
    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 CMOS can be used to scan both living animals as well as samples *ex vivo*.

In vivo scans:

i. The animal must 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 1276 CMOS

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:
   - The parts of the sample that rotate outside the field of view can't be reconstructed due to missing data.

- 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:

- The pixel size is defined by a combination of the camera position ('zoom') and the camera binning mode.
- 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.
- Note that for a pixel size below 4µm 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 20 and 40% (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 1276 CMOS 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.

The following steps can be followed for the easiest way to update the flat field:

i. Select 'update flat-field for current mode' from the options menu.

ii. Select the options needed for the flat-field update, depending on your scan set-up.

- 'Update only central camera position' if only using a single field of view (not an offset or double width scan)
- 'Adjust exposure automatically' if exposure time is not set manually (see below) and not updated recently
- Keep x-ray on after FF update' to continue setting up the scan immediately afterwards
- 'Move animal bed out of field of view' to ensure an empty field of view for the flat-field update.

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.

There is an additional way of updating the flat-field using the scanning modes.

i. Remove the sample from the field of view by repositioning.

ii. Inactivate the flat-field correction by double clicking in the top left corner of the field of view (indicated by 'ff' or 'flat-field correction off').

iii. Adjust the exposure time and current in the 'scanning modes' menu to get an average transmission in air between 45 and 55% without flat-field correction (aim for 50%). This can also be done automatically by selecting 'adjust exposure automatically' when updating the flat-field. The 'scanning modes' menu is activated by pressing the "Ctrl+Alt+Shift+S" key combination.

iv. In case the voltage/current are changed, update the numbers in the 'scanning modes' window accordingly.

v. Update the flat-field correction in the 'scanning modes' menu.

vi. Activate flat-field again by double clicking in the top left corner of the field of view (indicated by 'ff' or 'flat-field correction off').

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
0.5K mode	≤ 0.8°
1K mode	≤ 0.6°
2K mode	≤ 0.4°
4K mode	≤ 0.2°

- Note that in parallel to step and shoot scans where a rotation step is defined, the SkyScan 1276 CMOS 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)

Binning	Frames
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 the optimum scan result and 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.
- Retrospective gating: set the number of frames by selecting the list mode option ‘Time-resolve scan, Nimg:’.

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

8. Start scan