SKYSCAN 2214 Workflow

Method note
MCT-142

1. Overview workflow

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2. Workflow in detail

2.1. Sample preparation and mounting on a sample holder

A first important step in acquiring good quality results, is to prepare and mount the sample correctly. Below, four important considerations can be found. For more information we gladly refer Method note *"MCT 064: Sample mounting options"*. *www.brukersupport.com*

i. Different samples require different preparation/mounting techniques
   - Scan dry or wet?
   - Scan at room temperature or use temperature control?
   - Scan in air, water, ethanol…?
   - Mount using polystyrene foam, double sided tape, wax, …?
   - …

ii. If possible, reduce sample size to smallest representative size.

iii. The ideal shape of an object is a cylinder.
   - If this is not possible, try to have an object that is equally large in all three dimensions.

iv. Mount stable
   - Position the sample in the center of sample holder to allow for maximal zooming and to get optimal image quality.
   - Make sure the sample is mounted stable to prevent movement during scanning.

2.2. Sample positioning in the SKYSCAN 2214

i. Fix the sample holder on the sample stage.

ii. Close the door and give object info
   - The system will ask for the sample diameter and sample weight to restrict the sample movement as a safety measure (to prevent collision with the source).
   - Turn on the X-ray source.

iii. Select the X-ray detector (CCD1, CCD2, CCD3 or FP):
   - The SKYSCAN 2214 can be equipped with up to four X-ray detectors: the flat panel for high energy scanning and large samples, the CCD's for higher resolution imaging.
   - To prevent radiation damage to the CCD's the accelerating voltage is limited to 100 kV for CCD1 and 110 kV for CCD2.

iv. Make the volume of interest in the sample rotate completely inside the camera field of view whilst maximizing zoom.
   - The parts of the sample that rotate outside the field of view can't be reconstructed due to missing information.
   - The integrated micro-positioning stage enables to perfectly center the sample. Observe the sample at different rotation angles, and adjust the micro-positioning until the sample does not move anymore. In case the sample cannot be centered nicely for the full 360 degrees, please perform mechanical alignment.
   - When an object is larger than the field of view at a specific pixel size, it is possible to run an offset scan (see step 15).

v. Optional: Mechanical Alignment
   - Perform the mechanical alignment procedure only in case the sample does not stay in the field of view (or sufficiently in the center) for the full 360 degrees of rotation.
   - The mechanical alignment procedure is best performed at the position you will scan, i.e. the pixel size and camera position.

vi. Set the pixel size:

- The pixel size is defined by a combination of:
  - Sample position (FP and CCD's)
  - Camera binning mode (FP and CCD's)
  - Camera position (CCD's). The CCD's move automatically to one of three preset positions (far, middle, close) for optimum scan quality and speed. However, this can also be set manually.
- Note that the same pixel size can sometimes be set using different camera binning modes and zoom (with different size of FOV). In these cases, camera binning is preferable as it allows to reduce scan time and increase the signal-to-noise ratio.
- For high resolution scanning:
  - Use the visual camera as an aid to see how far you are from the source and collimator.
  - Reduce the pixel size in small steps if you want to go closer to the source, e.g. 5µm -> 4 µm → 3 µm → … → 1 µm. Do not immediately go from 5µm to 1µm or you risk hitting the collimator and/or source.

2.3. Optimize transmission through the sample

One key aspect of microCT imaging is partial attenuation of the X-ray beam by the sample. The transmission is 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 an optimal transmission profile through the sample. Ideally, the minimum transmission is not too low (>20%) and simultaneously the overall average transmission not too high (30%-60% is ideal). Too low transparency (<10%) can cause significant reconstruction artefacts, while too high transparency (>70%) may decrease signal-to-noise. Be mindful to take in to account that the attenuation of samples can be different at different angular positions and that enough transmission is needed from all angles.

i. Select the appropriate tube focus mode (small – middle - large) for the desired resolution, taking into account the pixel size you have selected. Note that there is some overlap in the advised pixel sizes below.
   - Large focal mode: smallest spot-size 3-4 µm, at low power. To be used if pixel size > 8 µm.
   - Middle focal spot mode: smallest spot-size 2 µm (W), 0.8 µm (LaB6). To be used if pixel size is between 1 and 10 µm.
   - Small focal spot mode: smallest spot-size 0.8 µm (W), 0.5 µm (LaB6). To be used if pixel size < 1.5 µm.

ii. Set the correct filter
   - Filters are used to change the energy profile of the X-ray beam by reducing the number of low energy photons. As the average photon energy increases, the overall transmission will also increase. This allows having a more efficient detector use (higher signal-to-noise) and significantly reduces beam hardening artifacts.
   - The SKYSCAN 2214 has:
     - 6 default filter options for the CCD's (no filter; 0.25 mm Al; 0.5 mm Al; 1 mm Al, 1 mm Al + 0.075 mm Cu; 0.5 mm Cu). With each filter

option a corresponding voltage is associated in the scanning modes menu as a factory default.

- 4 default filter options for the FP (no filter; 1mm Al; 1 mm Cu; 1mm Al + 1 mm Cu)

iii. Adjust the applied voltage if needed

- 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.
- Guidelines for the combination of filter and voltage for the default filters

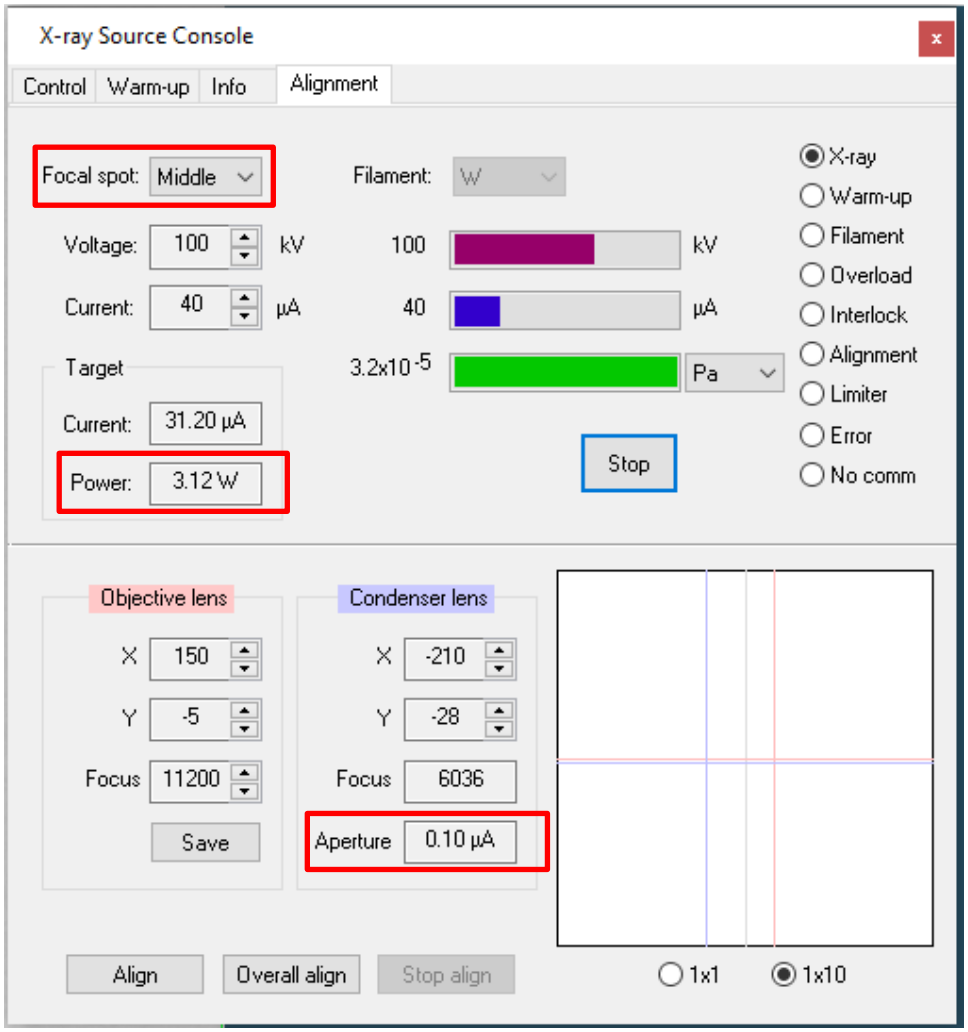
Filter (changer)	**Voltage (kV)**
No	20-60
0.25 mm Al	50-80
0.5 mm Al	70-90
1 mm Al	70-110
1mm Al + 0.075 mm Cu	90-120
0.5 Cu	100-140
0.5 Cu + 1mm Al	130 - 160

iv. Beam Alignment

- To maximize the power on the target, the electron beam impinging on your target should be well aligned. In the Source-menu, go to the “Alignment”-tab, and select “Align”. The automatic procedure will run.
- Repeat this if you are unsure whether the power would be maximized.

v. Adjust the emission current

- Most of the time, you will want to maximize the output of your source. Increase the emission current to the point where the target power (= voltage x target current) stops increasing significantly. This behavior is non-linear, different at every voltage and for every new filament. Higher emission current thus not always means higher target current.
- For the optimal results try to optimize the scan parameters with Target Power and Aperture current within the following ranges *(These parameters can be applied to both the Tungsten (W) and LaB6 filament. However it is recommend that the LaB6 Filament is only used for the highest resolution scans with Small Focal Spot Mode)*
    - Target Power
        - Small Focal Spot: 0.3 W
        - Middle Focal Spot: 3 W
        - Large Focal Spot: 13 W
    - Aperture Current
        - 0.1 uA for all Focal Spot sizes


X-ray Source Console
Control
Warm-up
Info
Alignment
Focal spot:
Middle
Filament:
W
Voltage:
100
kV
100
kV
Current:
40
µA
40
µA
Target
3.2x10 -5
Pa
Current:
31.20 µA
Power:
3.12 W
Stop
X-ray
Warm-up
Filament
Overload
Interlock
Alignment
Limiter
Error
No comm
Objective lens
Condenser lens
X
150
X
-210
Y
-5
Y
-28
Focus
11200
Focus
6036
Save
Aperture
0.10 µA
Align
Overall align
Stop align
1x1
1x10

2.4. Update the flat-field correction

The flat-field correction is a two-component intensity correction applied to each individual pixel. The dark reference field compensates for signal acquired without X-ray illumination (camera and cable signal noise). The bright reference field compensates for inhomogeneity in response to a given X-ray illumination (the signal through air). As such the flat-fields ensure a homogeneous pixel-per-pixel response, a normalized background signal and overall image quality (e.g. reduction of ring artefacts). It is possible to toggle between Flat Field Correction ON and OFF mode. At the top left corner of the camera view double click on the yellow "FF" to switch between Flat Field Correction on and OFF.

Always update the flat-field correction upon changes in the following settings: filter, voltage, current, focus mode, camera binning mode and camera position (far-middle-close) or offset mode.

i. Automatic method
   - Ensure that the sample is not too high, otherwise it may remain in the field of view of the camera when the stage lowered. Remove the sample from the scanner if it were too high.
   - Go to "options" → "Update flatfield for current mode"
     - Deselect "acquire bright flatfield only"
     - Select "adjust exposure times automatically"
     - Select other options if needed.

ii. Manual method
   - Remove the sample from the camera field of view (either by lowering the sample or taking it out of the scanner).
   - De-activate the flat-field correction in the 'Preferences' menu.
   - If necessary, adjust the exposure time via the "Scanning Modes"-menu to get an average transmission in air around 60% (between 50% and 70%) without flat-field correction.

- Update the dark and bright flat-field via the “Scanning Modes” menu in “Options”.
- Reposition the sample
  - Cf. step 1 and 2.
- Re-evaluate the image
  - Evaluate the transmission through the sample (cf. step 3), if the transmission is suitable, proceed to step 5 otherwise repeat steps 3 and 4.

2.5. Test X-ray focus current (High resolution scans only)

When setting up a high-resolution scan (i.e. in small or middle focal spot mode) it is important to fine-tune the X-ray focus current.

i. Open autofocus menu:
   - Ensure the X-ray tube is on, and the object is displayed.
   - Enable “Special user mode” (CTRL + ALT + SHIFT + S).
   - Click ‘Start Autofocus’ under the Options tab in the control software.

ii. define the “number of attempts”, “step size” and “amount of averaging” in the pop-up menu that appears.
   - The “number of attempts” is the number of images that are taken with varying focus values, symmetrically spaced around the “current focus value”.
   - “Averaging” is the amount of frame averaging that will be applied for each attempt.
   - “Step between values” is the step size in the focus value between each of the attempts. 3 or 4 can be considered a good start, 2 is accurate enough for a final estimate

iii. evaluate the generated images in terms of sharpness.
   - When in focus, the typical phase contrast enhancement of edges will be most pronounced. The images are save by default in the “D:\\Results\Focus”-folder, but could be saved to another one if you choose. They bear the suffix ‘focus000xxxxx’ with the xxxxx indicating the focus current setting corresponding to that image. The images are recognized as a series by Dataviewer.
   - Evaluate manually the images in Dataviewer to select the best focus-value. Adjust the contrast if needed.

iv. The Control software will also suggest a focus-value based on a 2D fourier transform, which should in most cases correspond with your manual selection.

2.6. Set acquisition settings in 'Acquisition' menu

i. Rotation step

- In the "Scanning modes"-menu, default values are for the rotation step are defined. These default values are suitable upper boundary values for the selected binning mode. For samples with small features, it can be of interest to lower the step, e.g. with a factor of two.
- Guidelines for choosing the rotation step for the different binning modes
- If you have a sample with very low abortion it is recommended to decrease the rotation angle.

Binning mode (flat panel, CCD3)	Rotation step
1x1 Binning	≤ 0.20°
2x2 Binning	≤ 0.40°
4x4 Binning	≤ 0.80°

Binning mode (CCD1 and CCD2)	Rotation step
1x1 Binning	≤ 0.10°
2x2 Binning	≤ 0.20°
4x4 Binning	≤ 0.40°

ii. Frame averaging

- A higher number of frame averaging increases the signal-to-noise ratio. For samples showing very high the number of averaged frames should be increased.
- Guidelines for choosing the frame averaging for the different binning modes

Binning mode	Frames
1x1 Binning	4-8
2x2 Binning	2-6
4x4 Binning	1-4

iii. 180/360° scan

- Typically, 180° is sufficient to obtain good scan results.
- 360° scans can reduce artefacts for samples consisting of a combination of high radio-dense inside low radio-dense material.

iv. Random movement of the camera can be activated under “Preferences” by ticking the box next to “Active Ringartefact suppression”

- Can be activated to reduce ring artifacts.
- It is advisable not to use random movement for highest resolution scans.

v. Optional features:

- Partial width: By activating the partial width option (to reduce the size of the dataset), the width of the projection imaged can be 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 increased linearly according to cropping ratio (e.g. 50% partial width = rotation step can be x2)
- Offset Scanning (only available with CCD cameras): The offset scan mode approximately doubles the width of the field of view by doing 2 scans subsequently side by side (by changing the camera position). To preserve image quality, it is advisable to also decrease the rotation step with a factor 2 from the default setting. The offset scan mode is an advanced feature, more info on the restrictions and possibilities in the user manual

- Spiral or Helical scanning: The spiral scan mode is an advanced feature, more info on the restrictions and possibilities in the user manual and method note 106.
- High aspect ratio scanning: The high-aspect ratio mode is an advanced feature, more info on the restrictions and possibilities in the user manual and method note 123.

2.7. Start scan

Finally, the scan can be started.