# SkyScan 1176

# In vivo X-Ray Microtomograph

# Instruction Manual



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# 1. INTRODUCTION TO X-RAY MICROSCOPY AND MICRO-CT

## 1.1 Introduction

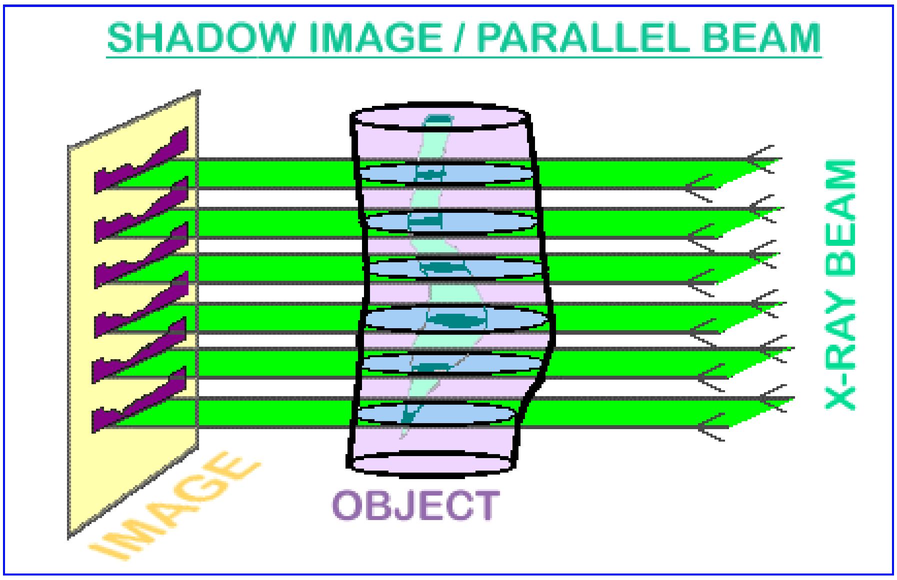
Any conventional optical or electron microscope allows visualisation only of two-dimensional images of a specimen surface or of thin slices. However, in most cases a conclusion about the original three-dimensional object structures cannot be made on the base of two-dimensional information.

One can obtain the three-dimensional information of object structures by cutting them into very thin slices, which can then be visualised in the light microscope, and interpolate the two-dimensional information into a three-dimensional structure model. This method however is not only very cumbersome but also not very reliable since the object structure itself can be altered by the preparation technique and the distance between the slices is usually too coarse to avoid loss of 3-D information.

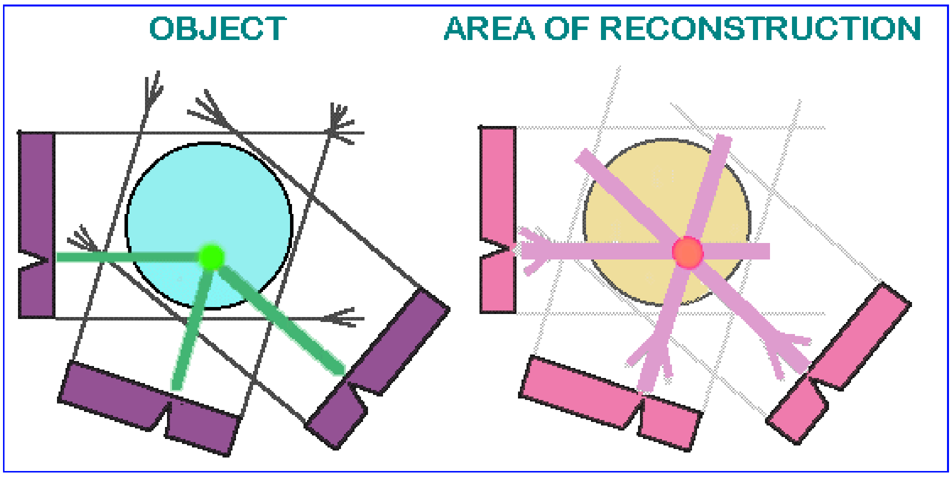
An x-ray (radiography) system produces two-dimensional shadow images of complete internal three-dimensional structures, but in a single two-dimensional shadow projection the depth information is completely mixed. Only an x-ray tomography system allows us to visualise and measure complete three-dimensional object structures without sample preparation or chemical fixation. Typically, the spatial resolution of conventional medical CT-scanners is in the range of 0.5-1.5 mm, which corresponds to 0.1-5 cubic mm voxel (volume element) size. Computerised x-ray microscopy and microtomography now makes possible the improvement of spatial resolution by seven orders of magnitude in the volume terms. The system "SkyScan 1176" allows a spatial resolution of 14 µm corresponding to about $3x10^{-6}$ cubic mm voxel size. As in the "macro" CT-scanners, the internal structure can be reconstructed and analysed fully non-destructively.

# 1.2. Basic principles of micro-computed tomography ("micro-CT")

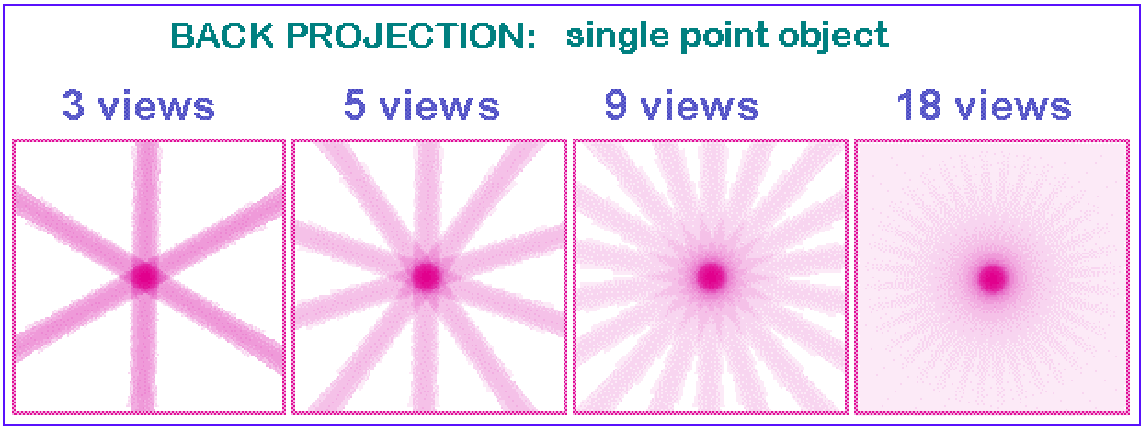
Any x-ray shadow image corresponds to a two-dimensional projection from the three-dimensional object. In the simplest case, we can describe it as a parallel x-ray illumination. In this approximation, each point on the shadow image contains the integration of absorption information inside the three-dimensional object in the corresponding partial x-ray beam.



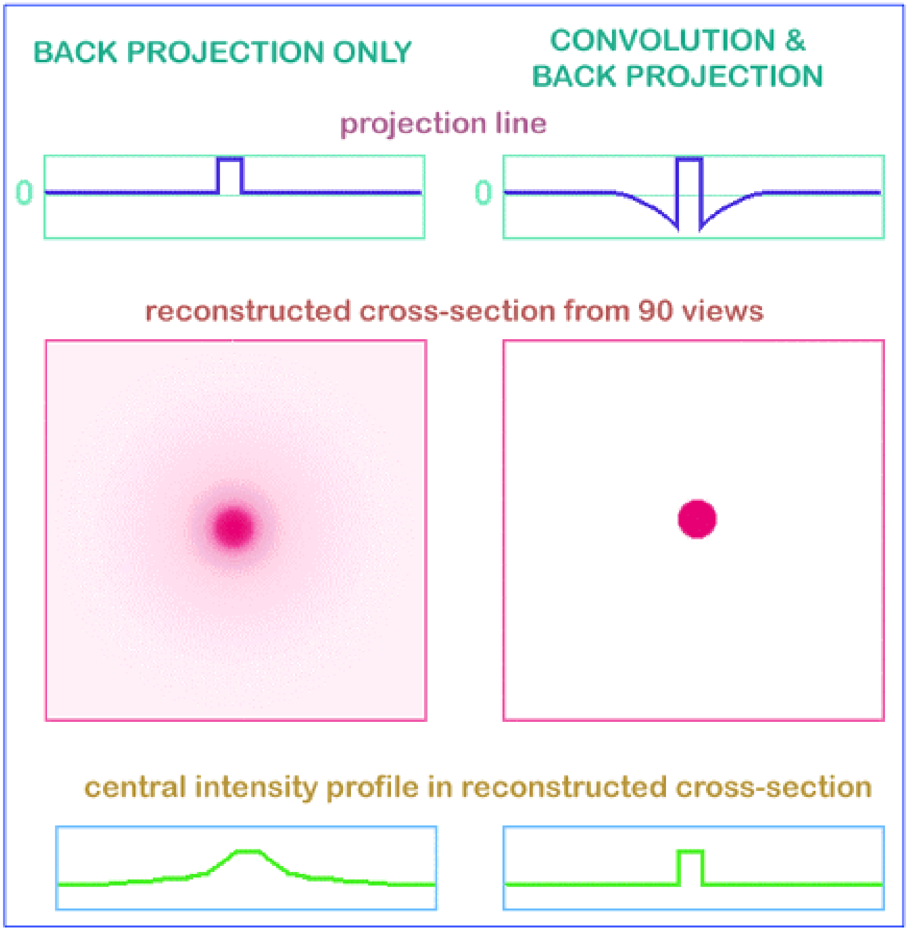

For parallel geometry one can divide the problem of a three-dimensional reconstruction from two-dimensional projections into the serial reconstruction of two-dimensional object slices from one-dimensional shadow lines. Let's show a simple example of this reconstruction of an object with only one point with significant absorption, in an unknown place. In the one-dimensional shadow line we will have a decreasing of intensity of the shadow of absorption in the object area, see the figure on the next page. Now we can initialise in the computer memory an empty array of pixels (picture elements) corresponding to possible object displacement. Of course, one must be sure that all parts of the reconstructed object will be inside the field of view. Because we have the position of the shadow from the absorption points of the object, we can mark on the area of reconstruction in the computer memory all possible positions of absorption points inside the object as lines.




Now let's rotate our object and repeat this operation. In each new rotation position of the object, we will add to the area of reconstruction the lines of possible object positions corresponding to position of shadow. This operation is named "back-projection". After several rotations we can localise the position of the absorption point inside the area of reconstruction. By increasing the number of shadow projections from different views this localisation become more and more defined.

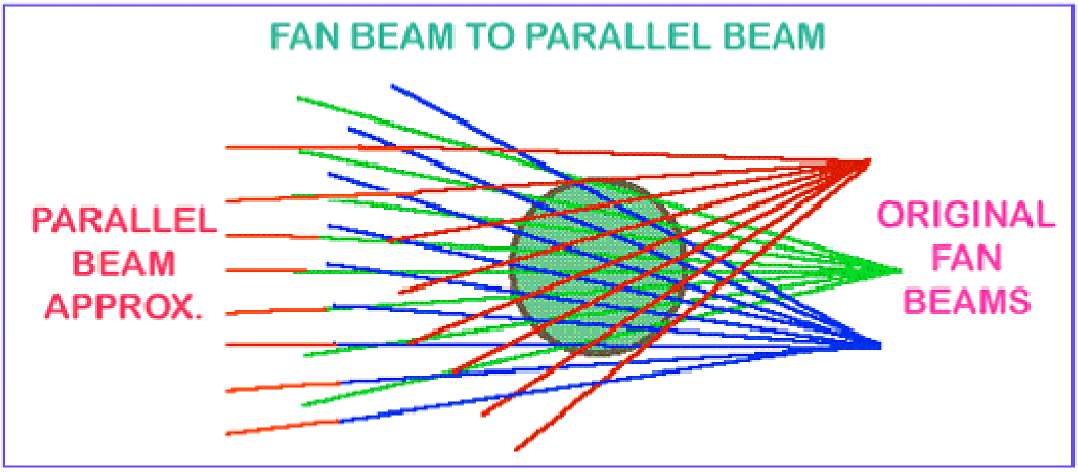



In the case of reconstruction from an infinite number of projections one can get an image with a good definition of the absorption area position inside the initial object. At the same time a blur area will accompany the pointer image because it is produced as a superposition of lines with all inclinations. Now we know what image will be produced from the pointer object and we can "pre-correct" the initial information in absorption lines to make the resulting image correspond more closely to the real object. This correction and some "negative absorption" outside the point of the object shadows, is called “convolution”, and eliminates the positive blur in the back projection process.




The same algorithm can produce the cross-section image not only from the single point object. Any real object can be represented as a big number of separate absorption voxels and linear absorption in any x-ray beam corresponds to the sum of all absorption from all voxels inside this beam. In this way the two-dimensional cross-sections of the object can be reconstructed from the one-dimensional shadow lines in different views.

Unfortunately practically no laboratory x-ray sources can generate parallel beams. In a real case, one will use a point source and fan / cone x-ray beam in the object area. For tomography reconstruction we can find the solution of this problem by the reordering of the shadow information. New pseudo-parallel beams can be constructed from the parts of several fan beams with different views and the same reconstruction method for fan-beam x-ray sources can be used.




In the case of x-ray acquisition, the image contains information about the intensity reduction inside the three-dimensional object. Because the x-ray absorption corresponds to exponential law, we can restore the linear absorption information from the shadow image by logarithmisation.

This operation is very non-linear and any noise in the small signal areas can produce significant errors in reconstruction. To eliminate these errors an averaging of the initial data and the results of logarithmisation can be used. On the other hand we can try to improve the signal to noise ratio in the shadow image to reach the most representative information.

One more effective way of noise reduction in the reconstruction process is a special selection of a correction function for convolution prior to back projection. In the simplest case (described above) the correction function produces two "negative absorption" reactions around any signal or noise peak in the shadow line and this behaviour becomes very problematic for noisy initial information.

Special selection of convolution function for correction with spectral limitation by the “Hamming window” provides a solution to this problem. In x-ray microtomography image information from a voxel with a very small physical size should be detected and the right choice of parameters for noise reduction becomes very important.

# 1.3 Reconstruction to image

## 1.3.1. Acquisition, creation of acquisition data

During the acquisition the source-detector pair will rotate over 180[1] or 360 degrees. At each position the shadow image or transmission image will be acquired.

Cone beam acquisitions saves all of these projection images as 16 bit TIF files. The data set after scanning consists of a set of images, all of them are normal transmission X-ray images. For each position over the 180 degrees rotation a full 16 bit shadow image will be stored on disk. The number of files after this acquisition is thus depending upon the rotation step selected. For a typical step of 0.7 degree, there will be 257 images plus a small number to start the re-sampling of the images for horizontal or fan compensation of the x-ray beam.

## 1.3.2. Start of the reconstruction

After the acquisition is finished we have to start the reconstruction. We will use the 16-bit TIF shadow images for the reconstruction. We will now generate from this, by using the reconstruction algorithm, a raw data reconstructed cross-section.  This is not yet an image, it is a float point matrix holding absorption values in the reconstructed cross-section.

| | 1 | 2 | 3 | | n-2 | n-1 | n |
|---|---|---|---|---|---|---|---|
| 1 | 0.022 | 0.024 | 0.013 | ………… | 0.910 | 0.990 | 0.950 |
| 2 | 0.023 | 0.026 | 0.012 | ………… | 0.870 | 0.900 | 0.890 |
| 3 | 0.028 | 0.027 | 0.019 | ………… | 0.800 | 0.810 | 0.780 |
| 4 | 0.026 | 0.210 | 0.020 | ………… | 0.820 | 0.830 | 0.840 |
| ⋮ | ⋮ | ⋮ | ⋮ | | ⋮ | ⋮ | ⋮ |
| n-5 | 0.030 | 0.031 | 0.034 | ………… | 0.710 | 0.720 | 0.740 |
| n-4 | 0.031 | 0.034 | 0.390 | ………… | 0.700 | 0.730 | 0.760 |
| n-3 | 0.034 | 0.035 | 0.042 | ………… | 0.730 | 0.790 | 0.780 |
| n-2 | 0.036 | 0.036 | 0.041 | ………… | 0.740 | 0.780 | 0.770 |
| n-1 | 0.040 | 0.037 | 0.040 | ………… | 0.750 | 0.770 | 0.720 |

The size of the matrix is like the number of pixels inside a cross-section or in a line on the CCD array (n is the number of pixels in a line of the shadow image or the CCD array).

We can save the reconstructed cross-section as a floating point matrix holding the attenuation values after the reconstruction or, as explained in the next section, transform it to an image with the 256 grey values (8bit).

[1] In practice a “180 degree” scan will take images over more than 180 degrees, the total rotation being equal to 180 degrees plus the cone angle of the x-ray source-camera geometry.

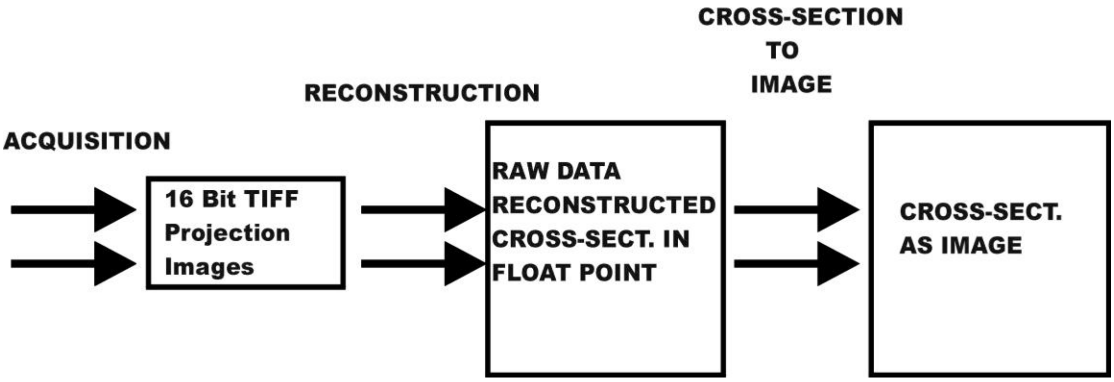
### 1.3.3. Reconstruction contrast limits ("cross-section to image")

After creating the cross-section reconstructed from the raw data, we have to generate an image. An 8-bit image uses 256 gray scales. Therefore, we have to find a way to convert the 12 bits or more, depending upon the camera, into an 8-bit gray scale image. Note – optionally, reconstruction to 16-bit (65536 gray scales) is available.

Minimum and maximum values are selected. All values between these will be displayed as half tone. In a normal image, all attenuation values below the minimum will be white everything above the maximum will be displayed as black.

The reconstructed array will be shown as a half-tone image of cross- section with linear conversion to 256-grades of gray inside selected density interval.
In the SkyScan systems, using a Windows environment, the final image generated can be exported to BMP, JPG, TIF (16 bit), RAW 16Bit or TXT -files.

The following image summarizes all action and steps to generate the cross sectional data.

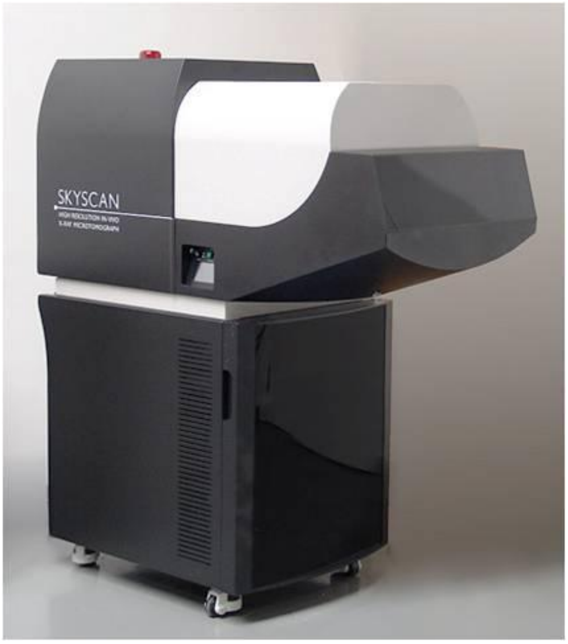


# 2. SKYSCAN 1176 SYSTEM OVERVIEW

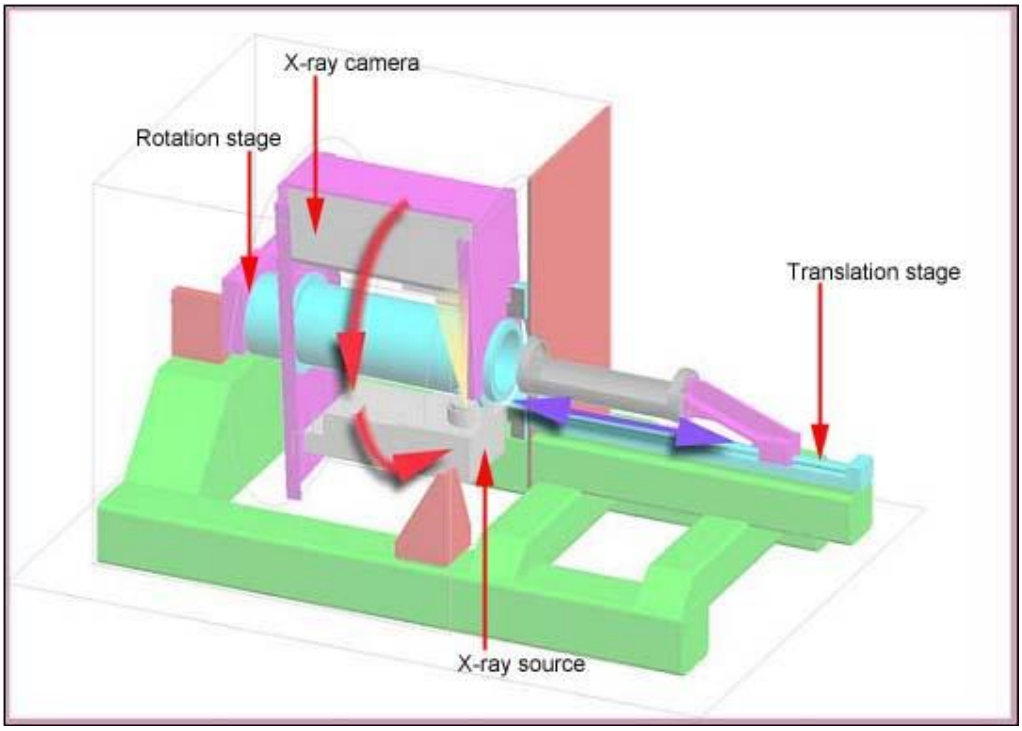
SPECIFICATIONS:

| | |
|---|---|
| Maximum object size | 68mm(D) x 200mm(L) for rats or 35mm(D) x 200mm(L) for mice, 20mm(L) per single scan |
| X-ray source | 20-90kV, 25W, air cooled sealed type, 6-positions automatic filter changer for energy selection |
| X-ray detector | 11 Megapixel (4000x2672x12bit) cooled digital X-Ray camera with fibre-optic coupling to scintillator |
| Spatial resolution | User selectable pixel size 9µm / 18µm / 35µm (isotropic), 15µm low-contrast resolution (10% MTF) |
| Projection / cross-section Image size and formats | 1000x668…8000x2672pixels projection images (16-bit TIFF format) 1000x1000…8000x8000 pixels cross-section (BMP 8bit, TIFF 16-bit, JPEG 8 bit) |
| X-ray loading to the animal | 0.1-0.3 Gy per scan typical |
| Scanning system | source-detector pair rotation with 0.02 deg. min. step size, 6um object positioning accuracy with 400mm travel, 50mm camera positioning/alignment with 2.5um accuracy, <10 microns overall stability during scanning |
| Software package | scanner control, preview (35x200mm scan), acquisition for reconstruction, volumetric (cone-beam) reconstruction of one / several / all cross sections, ROI-reconstruction, local density measurements in HU, 3D-surface and volume rendering, virtual manipulation with reconstructed object, morphological analysis in 2D and 3D |
| Reconstruction algorithm | Modified Feldkamp: multi-slice volumetric (cone-beam) reconstruction. Up to 2000 slices can be reconstructed after one scan. Full image mode, partial reconstruction mode, possibility for detail local reconstruction with object bigger than field of view. |
| Radiation safety | <1uSv/h average during full scan at 10cm from the instrument surface |
| Installation requirements | Power 100-240V/10A/50-60Hz, 18-25C temperature, <85% humidity, no condensation, vibrations 0.1…100Hz <0.1mm |
| Size/Weight | 1700mm(H) x 750mm(D) x 1450mm(W), 475Kg with single PC; 600Kg with 4 PC cluster |

The "SkyScan-1176" is a high-resolution low-dose X-ray scanner for in-vivo 3D-reconstruction with spatial resolution of up to 9 microns inside the small laboratory animals (rats, mice, etc.). It consists of the combination of Micro-CT system and a computer with system control software and reconstruction software. This system allows reconstructing non-invasively any cross-section through the animal body with possibilities to convert the reconstructed dataset into a realistic 3D-image and calculate internal morphological parameters.




The equipment contains an X-ray micro-focus tube with high-voltage power supply, a rotation stage with overall accuracy of <10μm, a translation stage, a two-dimensional X-ray CCD-camera connected to the frame-grabber and a Dual-Quad Core computer with LCD monitor. From 1 to 5 high-performance PC computers of the rack mounted server type are accommodated in the base section below the scanner unit.



For the "SkyScan-1176" the X-ray micro-focus tube operates at 20-90kV, 25W. The special X-ray CCD-camera is based on 11 Megapixel (4000x2672 pixels) cooled CCD-sensor with fibre optic coupling to X-ray scintillator.

The X-ray shadow projections are digitised as 1000x668 to 8000x2672pixels with 4096 brightness gradations (12 bit). The reconstructed cross-sections have a 1000x1000 to 8000x8000 pixels (float point) format and 9 /18 /35µm pixel size in any place of the scanning area. The scanning area is 68mmx200mm or 35mm x 200mm (two carbon-composite beds supplied, plus one carbon bed attached to a tray to hold large animals).

For the reconstruction one can use a volumetric (cone-beam) reconstruction of one / several / all cross-sections or a ROI-reconstruction. After the serial reconstruction, one can display the cross-sections onto the screen as well as construct a realistic 3D-image with possibilities to "rotate" and "cut" the object model. On this model, one can calculate the internal morphological parameters.

# 3. INSTALLATION OF THE SKYSCAN 1176

## 3.1. Scope of this document

This manual is meant to use as a guideline to unpack, connect and startup the SkyScan-1176 X-ray microtomograph. Always use your common sense, specific onsite difficulties with transportation are not described in this manual. Make sure to know local safety rules.
This is what we will do in a nutshell:

- Unpack the scanner
- Connect the scanner to the pc
- Start up the system

## 3.2. Preparing the installation

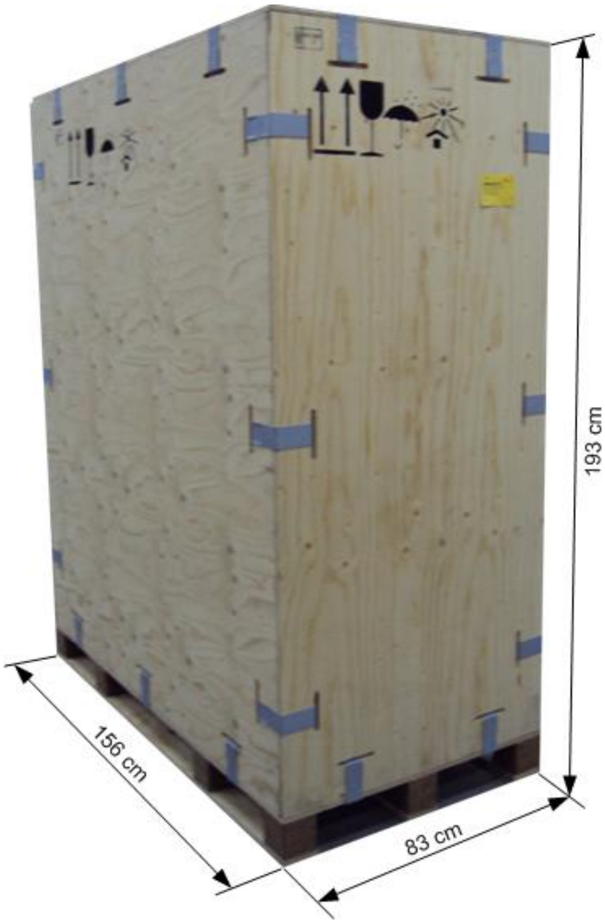
- Have enough people, we recommend 6 persons.
- One of these should be a certified SkyScan service engineer.
- A local IT-engineer can be use full for the connection from the pc to a network but is not necessary for the functionality from the System itself.
- Common tools for service engineers.
- Know the customer site and be sure all facilities are present.
- Etc…

## 3.3. Site requirements

The wooden transport box is mounted pallet. it is lift able with a normal pallet lifter but be aware, this box is top heavy.

### 3.3.1. Crate dimensions

Weight crate with system = 670Kg
Weight crate with system + cluster = 770Kg




### 3.3.2. Route to the room

Make sure that all doors that need to be passed are wide enough and that you have enough room to make turns where this is needed.

Make sure you do not need to pass stairs or other obstacle with a height difference.

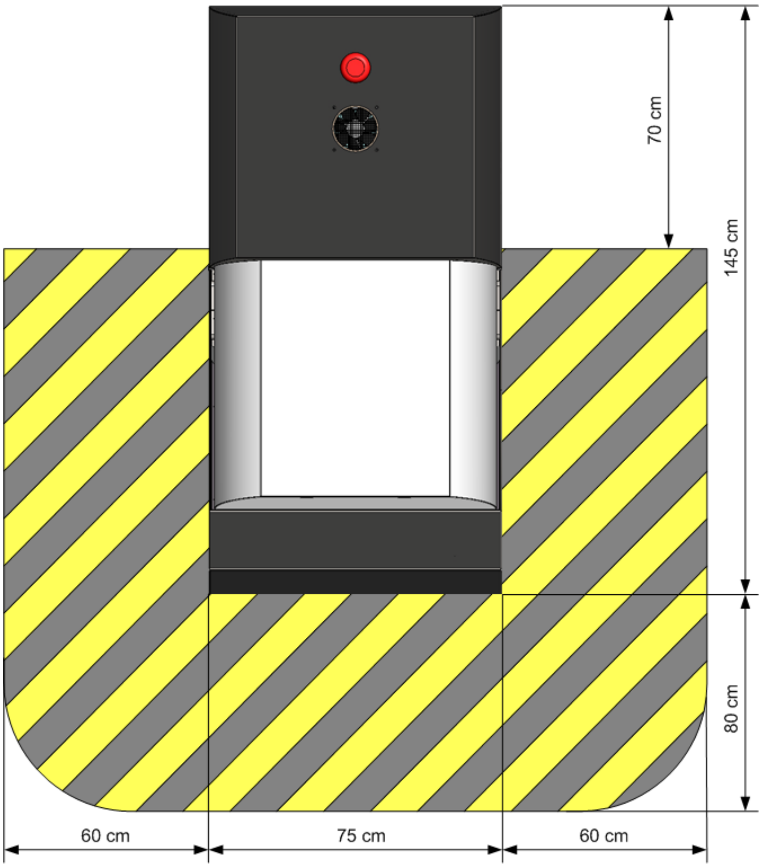
### 3.3.3. Room requirements

Two normal power plugs (scanner, monitor) need to be present.

One network connection can be useful but is not needed for the functionality from the scanner.

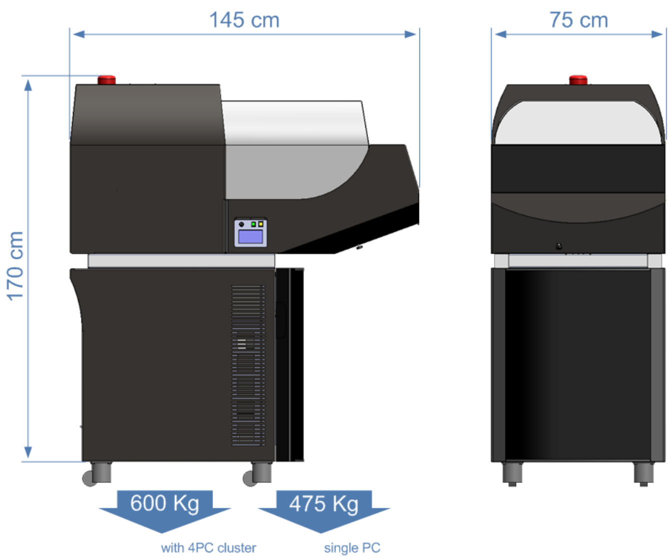
A working desk for keyboard/mouse, monitor and sample preparation need to be present in the room

Floor space:




Be mindful of the shaded space on the picture, this is the minimum empty space around the scanner needed to access the scanner during normal operation.

## 3.3.4. Scanner dimensions



# 3.4. Unpack the crate

The crate as it should arrive:

Check the chock- & tilt watches

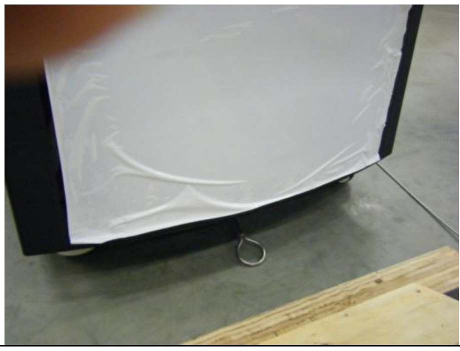
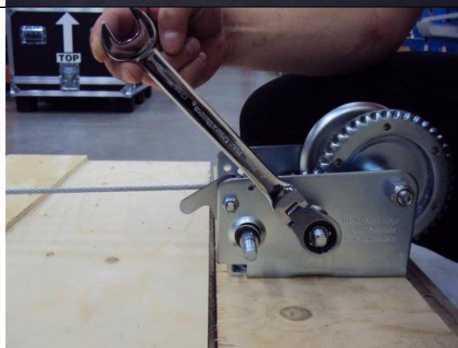


Both should be OK, if not accept the crate but make a remark about this on the delivery document from the courier.

Open the clips, if you do not have a special tool you can lift this clips also out with a big flat screw driver.

| | |
|---|---|
| The crate with the side walls removed: |  |
| In the compartment box under the nose from the 1176 you can find the scanner accessories.<br><br>Empty this compartment. |  |
| Remove the wooden compartment box, it is attached on the pallet from the side with some Philips screws drilled in the pallet base. |  |

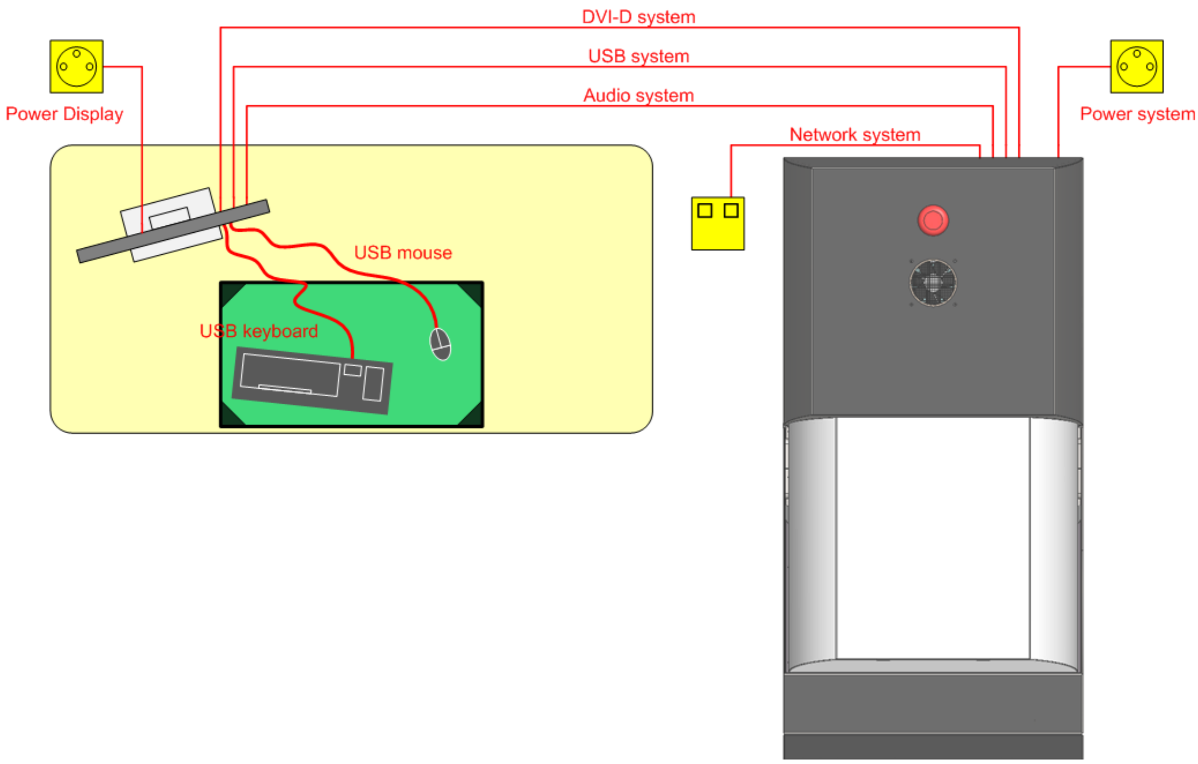
| | |
|---|---|
| Remove the wooden stopper, be care full, the system can wheel from the pallet. |  |
| Prepare the ramp:<br><br>Position the 2 trapezium shaped supports, that are located in the wooden compartment box that you just have removed, like on the picture.<br><br>Use the roof from the crate as the base from the ramp. |  |
| Make sure that the roof is hooked over the edge. Secure the roof in the pallet with the screws that were used to attach the compartment box to the pallet. |  |
| Add the rails on top off the ramp and secure them with screws.<br><br> |  |

| | |
|---|---|
| Add this part to smooth the edge from the ramp and secure this part with screws |  |
| The result should look like this. |  |
| Operate the winch:<br>With the pal you can select the operation direction from the winch, select the downwards position.<br>The rachtelet spanner can be found in the gray supplies box.<br>The hook is used to attach the cable to the system, it fits in a hole in the bottom plate from the system.<br> | <br> |
| Lower the system from the ramp:<br><br>**PUSH THE PAL DOWNWARDS WHILE WINCHING THE SYSTEM FROM THE RAMP, IT HAS THE TENDENCY TO JUMP OVER THE TEETH.** | |

# 3.5. Connect the system


4 cables emerge from the rear of the 1176: (from left to right)

- Audio
- Network
- USB
- DVI-d

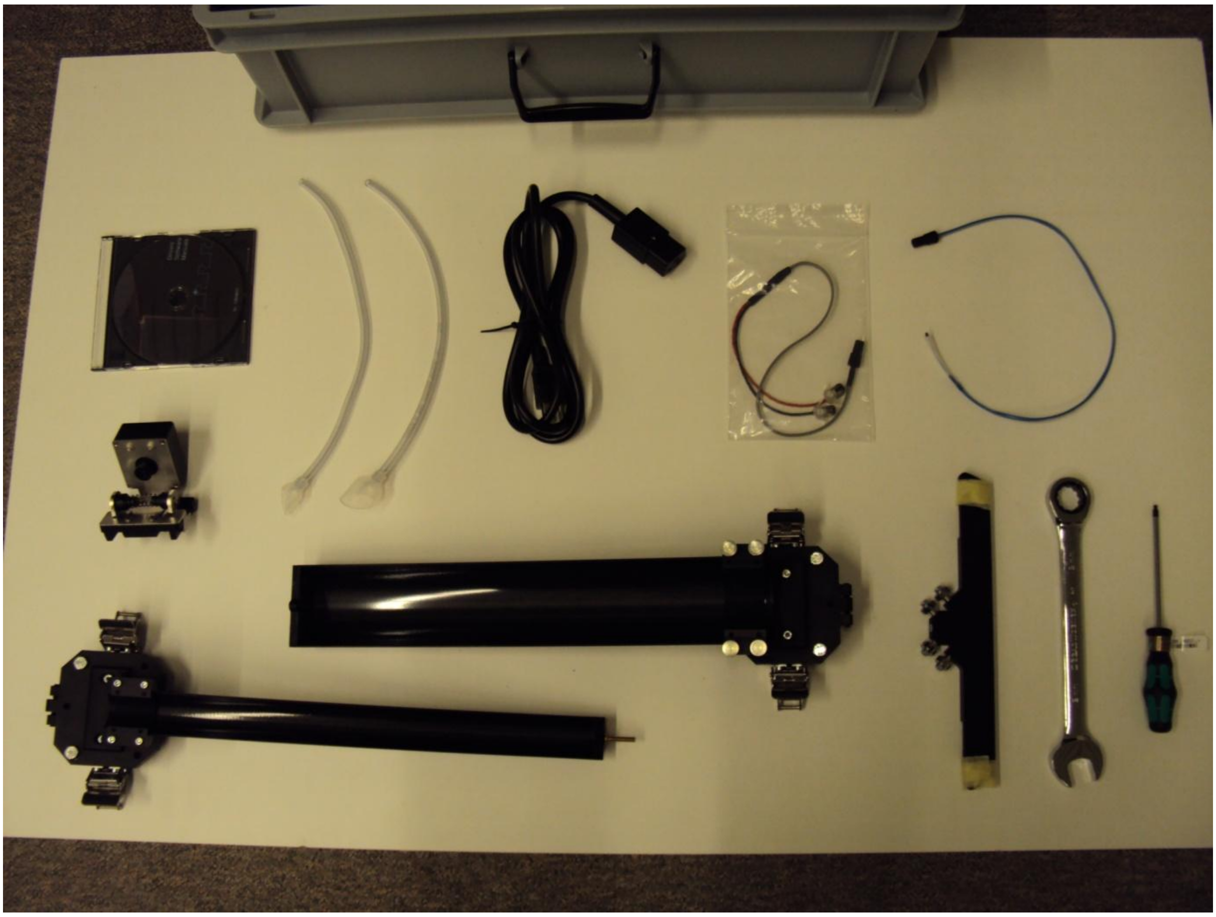



- Connect the mouse and keyboard to the display
- Connect the dvi-d cable and USB from the 1176 also on the monitor
- Connect the 1176 and the display to the power wall socket

*Optional:*

- Connect the audio to the sound bar (if present).
- Connect the RJ45 network cable from the system to the local network

# 3.6. Contents of the supplies box



Top row from left to right:

- Backup DVD
- Breathing masks
- Power cable
- ECG-clamps
- Temp sensor

Below the DVD is the visual camera

- The mouse bed
- The rat bed
- Bed supports
- Ratchlet spanner
- Hex key nr3

# 4. SYSTEM CONTROL SOFTWARE

## 4.1.Introduction

Welcome to the SkyScan High performance *in vivo* micro-CT scanner.

The SkyScan 1176 *in vivo* micro-CT scanner is a complete self-contained unit, movable on wheels. The scanner unit integrates together the scanner hardware and computers for scanner control and reconstruction, with a high degree of automation. The aim is to make the operation of the scanner straightforward and user-friendly. The control computer and (optionally) from one to four reconstruction computers are located in the base of the scanner as rack-mounted servers:

The top rack-mounted computer is the scanner control computer. Up to four reconstruction servers are located lower down. A table or desk next to the scanner is required only for the computer monitor and keyboard.

# 4.2. Starting the scanner

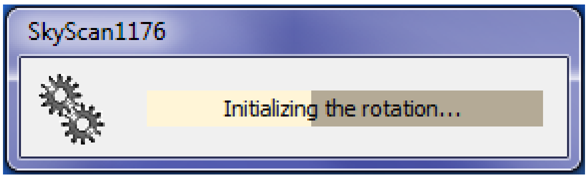
To start the scanner, locate the panel on the left side of the scanner with the touch screen (see image below). First check that the key labelled “x-ray” is in the vertical “ON” position.

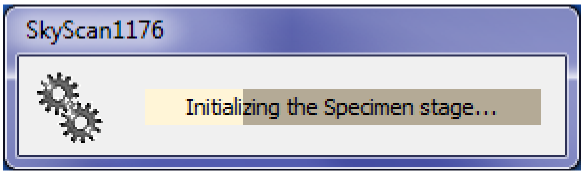

Open the plastic cover of the right, orange button labelled “computer” and press it. The control computer will start.

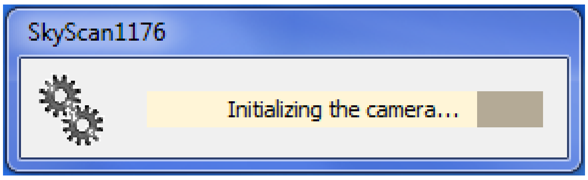
Note that below the key and scanner / computer buttons there is a blue touch screen on which you can change the imaging x-ray filter setting by touching the corresponding filter icon. This is described further below (section ...).

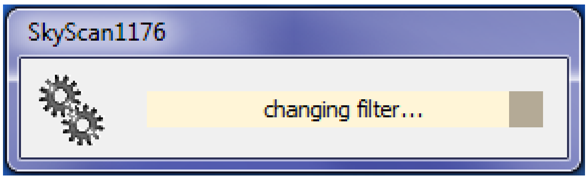
Once the control computer (with Windows 7, 64 bit version) has started, launch the 1176 scanner control software by pressing this desktop icon:


While the scanner initialises for about a minute, several hardware initialisations are performed, and corresponding progress bars appear on screen:





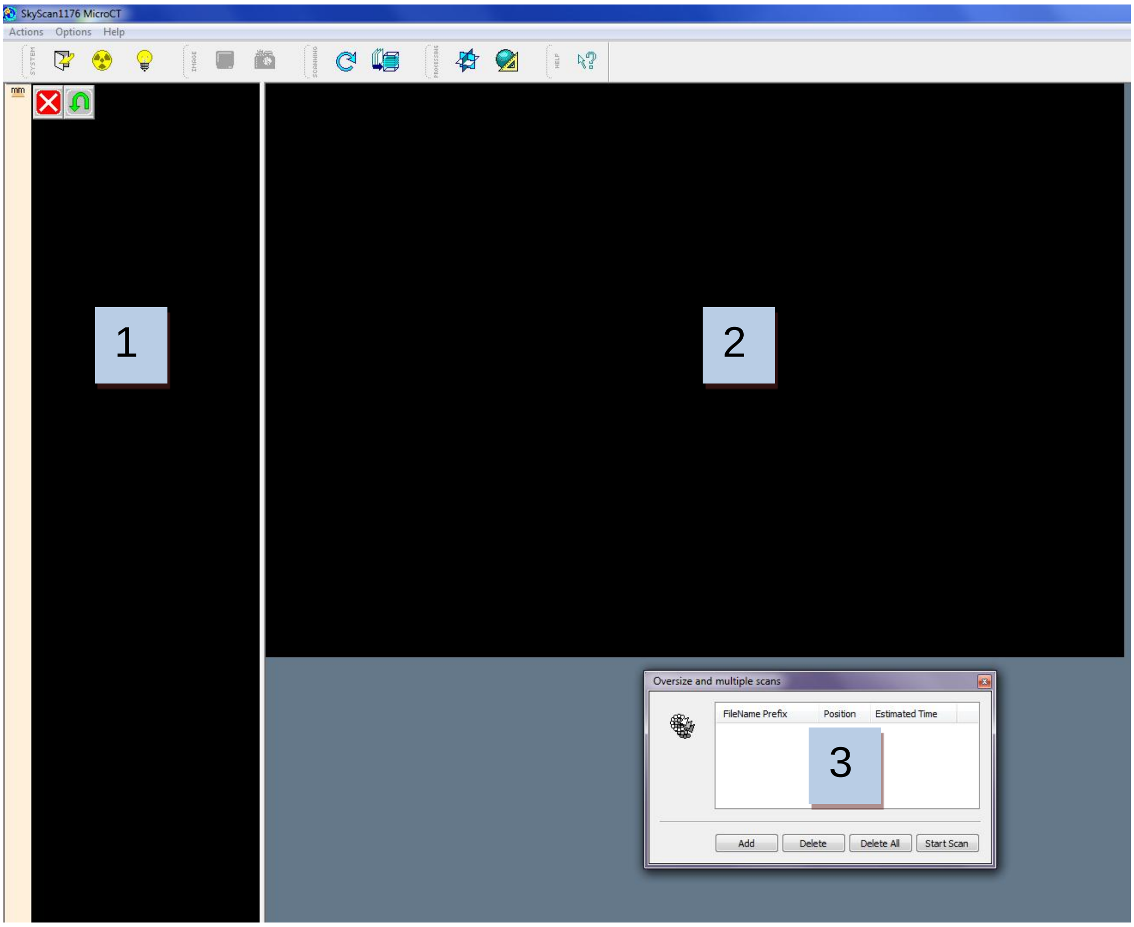




At the end of initialisation, the sample bed inside the scanner is set at the position furthest away from (out of) the scanning chamber – this position is called the “start position”. With any of the 1176 animal / sample beds in the start position, the scanner imaging field of view (FOV) is empty; the bed and end-pin are out of the FOV.

This is important since to take a flat field correction (see below) the FOV must be empty.

The control software window you will see is shown below (note it will vary depending on some view options):




The parts of the control screen labelled 1-3 are:

1. The scout window
2. The image window
3. The batch scanning window

Note – please wait for the scanner initialisation to fully complete before proceeding to operate the 1176 scanner.

# 4.3. The top button bar

The top button bar of the SkyScan 1176 control software is shown below:

*(The numbering is added for annotation – not visible in the software window.)*

The function of these buttons as numbered is given below:

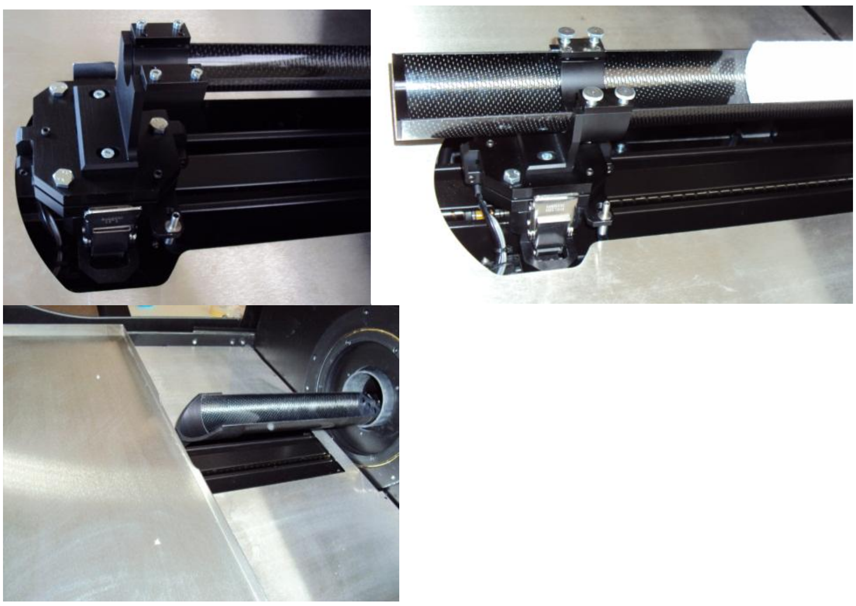
1. Open the scanner animal / sample chamber door
2. Turn on or off the x-ray source
3. Turn on or off the video camera for real time viewing of the animal and physiological monitoring
4. Take x-ray images in continuous video-mode
5. Take a single x-ray image
6. Open the scan window to set up a scan
7. After a scan: start the NRecon reconstruction software
8. After a scan: start the DataViewer scan viewing software
9. After a scan: start the CT-Analyser analysis program
10. Help pointer

Please note that only buttons 1-6 are involved in scanner operation. Buttons 7-9 are optional links to external programs (they can be started separately without these buttons). Button 10 is the help pointer.

## 4.4. Fitting and changing of the animal / sample beds in the SkyScan 1176 scanner

The SkyScan 1176 in vivo scanner has three interchangeable animal / sample stages, to allow mounting for scanning of mice, rats, and rabbits, and other similarly sized animals or non-living samples, for micro-CT imaging.

The beds on all three stages are half tubes (180°) of carbon fiber, for strength combined with x-ray translucency.

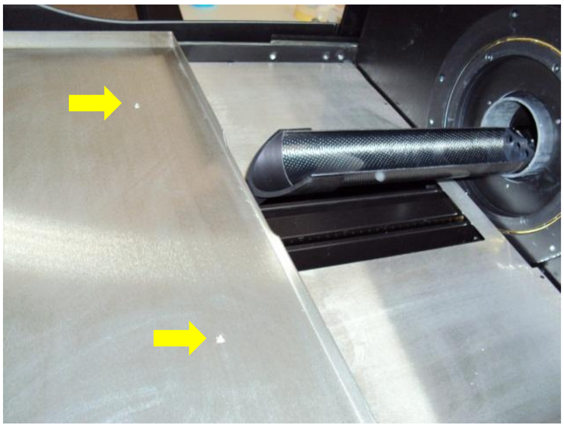


(a) (b) (c)

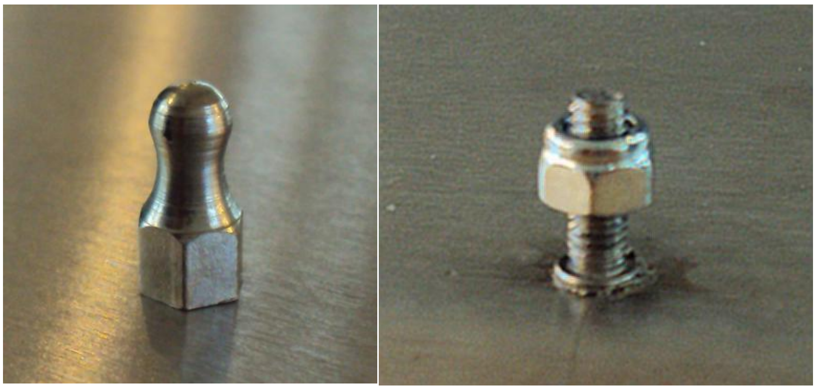
The mouse bed attached to the mounting block (a), the rat bed (b) and the bed with the large steel tray allowing accommodation of a rabbit (c) or similar sized animal, for limb extremity scanning.

### 4.4.1. Fitting and removing of the rabbit bed and tray

When the scanner is first installed it is fitted with the largest of the available beds, the rabbit bed and tray.



At the front edge of this tray, on the underside, two pins fitting into sockets hold the tray down (see below, left). These correspond to the two bolt heads seen in the above image (arrows). Below right is shown one of the pair of bolts at the rear under edge of the steel tray, which fit in half brackets.



(a) (b)

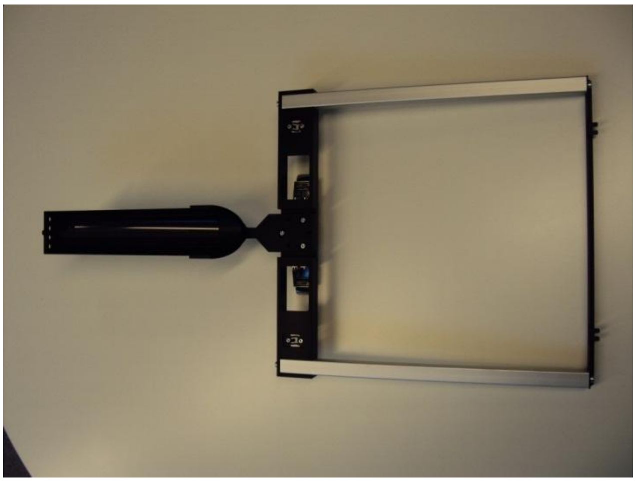
The metal pin on the tray underside at the front edge (a) for attachment to the frame, and (b) the bolts at the rear underside of the tray

To remove this tray, in order to change to either the rat or mouse beds, first lift the tray off at its “front” edge – the edge connecting to the carbon bed, as shown in the image below.

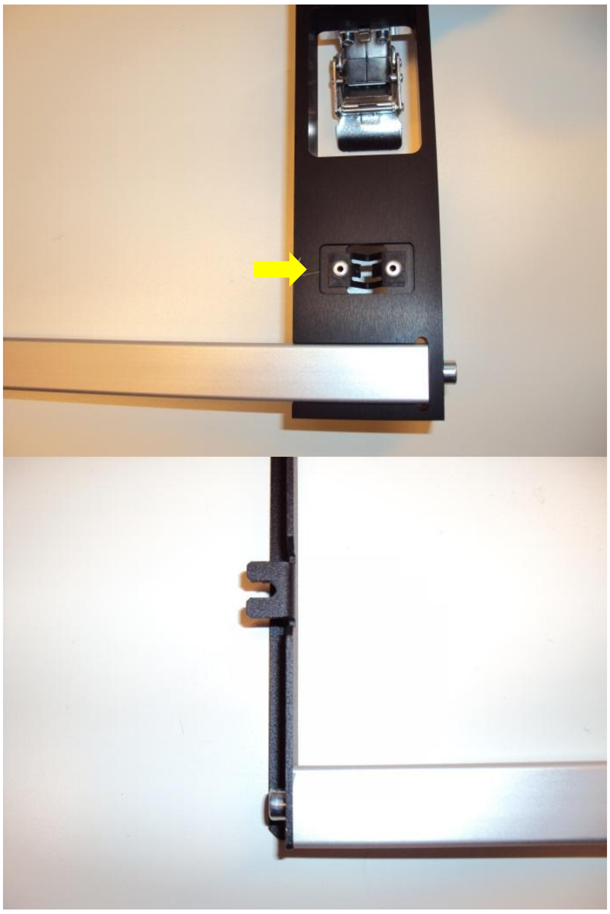
With modest lifting force the pins will pull out of their sockets (reversibly) and the tray will lift up.

At the rear end of the tray (furthest from the scanner chamber) two bolts are held in place by metal brackets, from the front end only. The bolts are not fully enclosed by the brackets, which are open at the rear. Therefore, after lifting the front end of the tray up as shown above, you then move the tray carefully in a rearwards direction, to move the bolts out of their brackets. After this, the tray is free to be lifted up and out of the scanner.

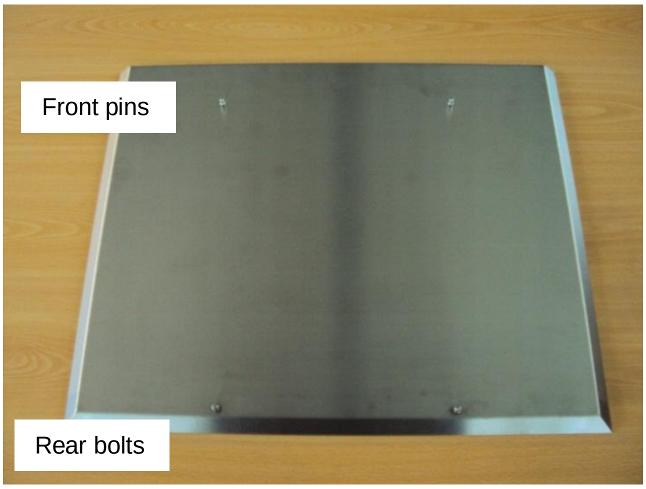
The image to the right shows the frame of the rabbit tray, with the attached 6cm carbon bed, which allows the rabbit bed, as well as mounting rabbit limbs for scanning, also to mouse rats, mice – with polystyrene inserts – and other samples.



At the front end of the frame are the sockets for the pins at the front edge of the steel tray (image below left, yellow arrow) and at the rear of the frame the half-bracket for holding the bolts at the rear underside of the steel tray (image below right, orange arrow).



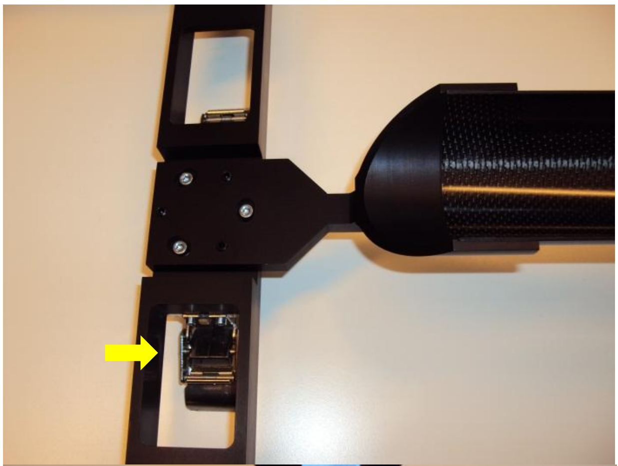
The entire underside of the metal tray, with paired pins at the front edge and bolts at the rear edge, is shown to the right:



## 4.4.2. The clip system for securely mounting the three interchangeable beds in the SkyScan 1176.

Two side clips hold each of the three animal / sample beds (rabbit, rat and mouse bones) securely to the sub-stage mounting.

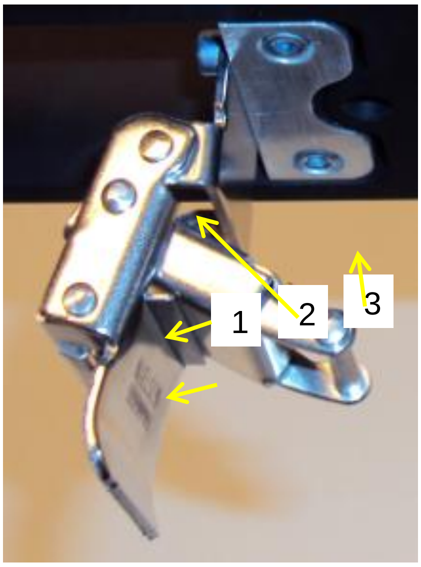
The images below show these clips on the rabbit bed, on either side of the central mounting block.



The clips hang below the frame of the rabbit bed at the front edge. They are accessible through gaps in the frame as shown.

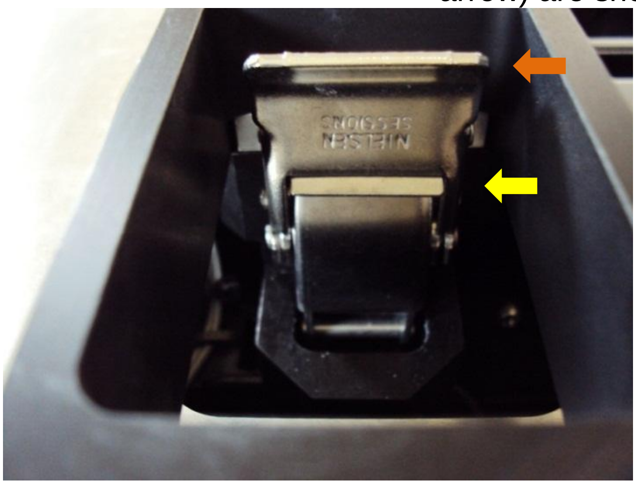
## 4.4.3. Removing the clips to remove a bed: the release plate

The design and structure of the clip should be understood, in order to facilitate attachment and removal of the three beds of the SkyScan 1176.
The bed side clip has a flange at the top, and a release plate just below it. The **release plate** is of first importance in the release of the clip to remove the bed. You can’t remove the clip without pressing the release plate. At the bottom of

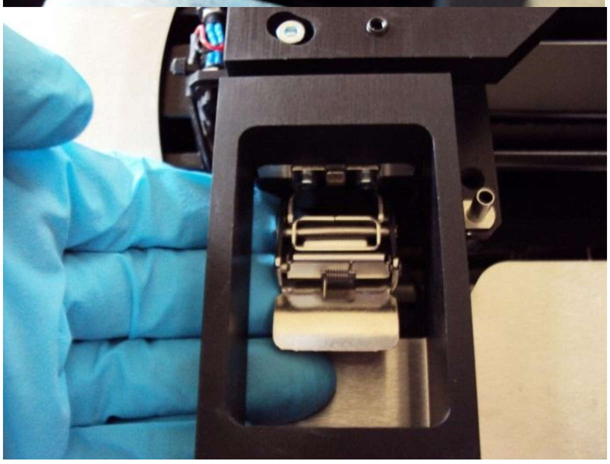


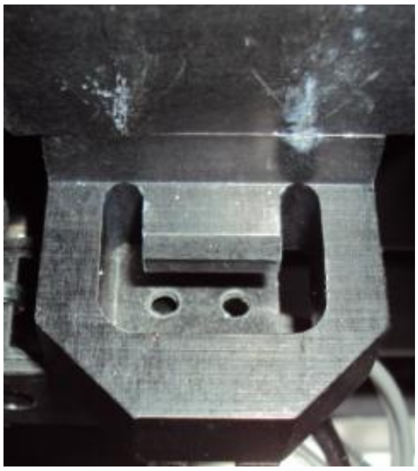

the clip assembly is the bottom bar. How to release the clip and remove the plate is shown below for the example of the large rabbit bed. However the method is the same for all three beds.

*Left:* The clip mechanism for bed attachment. The three parts show by arrows are (1) the top flange, (2) the release plate and (3) the bottom bar.
The image below shows the clip closed with the rabbit bed attached to the bed mounting – visible through a gap in the frame under the rabbit bed. The flange at the top (orange arrow) and the release plate on the side of the clip (yellow arrow) are shown.



To release the clip, press in the release plate, and then the top flange can be pushed downwards and sideways, as shown in the images below:



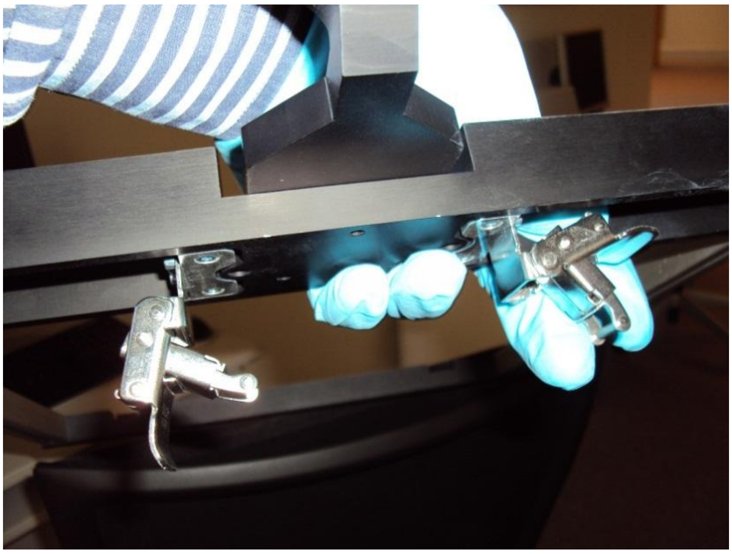


With the top flange released and hanging down, the bottom bar of the clip can be removed from the bottom bracket.

The image to the right shows the bottom bracket under which the bottom bar under the clip is held.

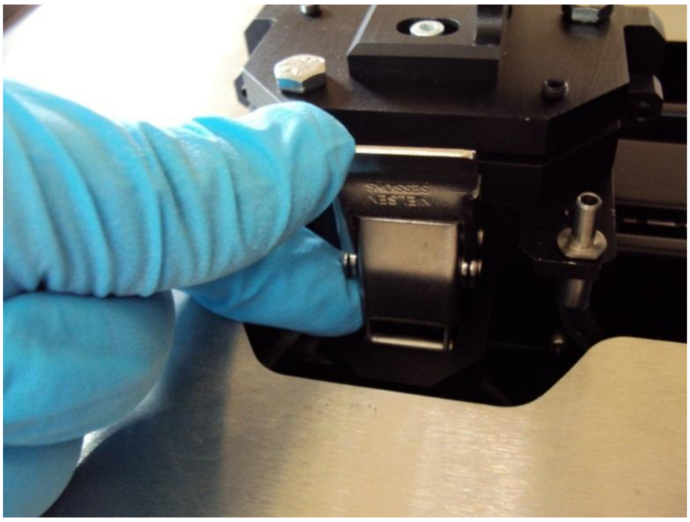
With the clips released on both sides of the rabbit bed mounting, as described above, the frame of the rabbit bed can be removed:

Please note that when replacing the rabbit bed, as you lower the frame into place on the bed mounting, you need to guide the two clips, which hang below the frame, into the gaps on the chamber base plate where the clips are attached.



### 4.4.4. A note on the release of the smaller rat and mouse beds.

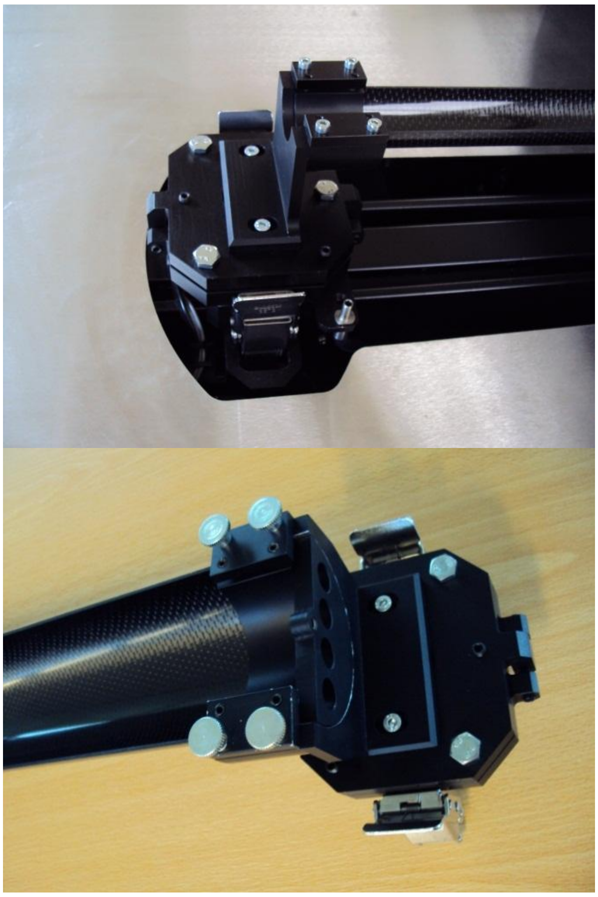
The method is essentially the same for removing the smaller rat and mouse beds. First release the side clips by first pressing the release bar. As you move the top flange down, also release the bottom bar of the clip out from under the bottom bracket.



After both the side clips of the smaller beds have been released from the bed mounting (bottom bar removed from the bottom bracket), the bed still fits quite tightly to the mounting. Grab it firmly as shown and lift it, applying a moderate vertical twist to release the bed if necessary (see image below).

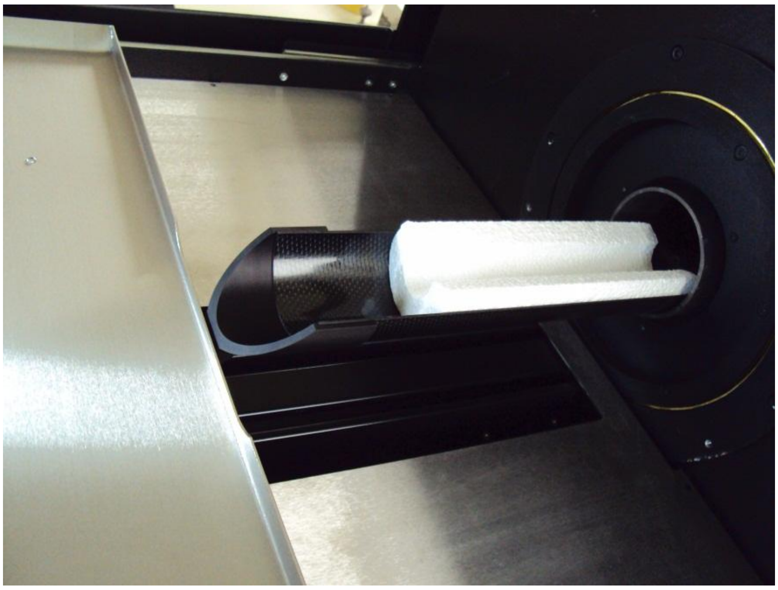
## 4.4.5. The attachment of the rat and mouse beds: the position of the carbon fiber bed insert is *variable*

Attachment of the carbon fiber half-tube beds to the mouse and the rat stages is by means of an anodised aluminium half ring insert into the bed, secured from above by aluminium plates with screws or rotatable nuts, as shown in the image below:

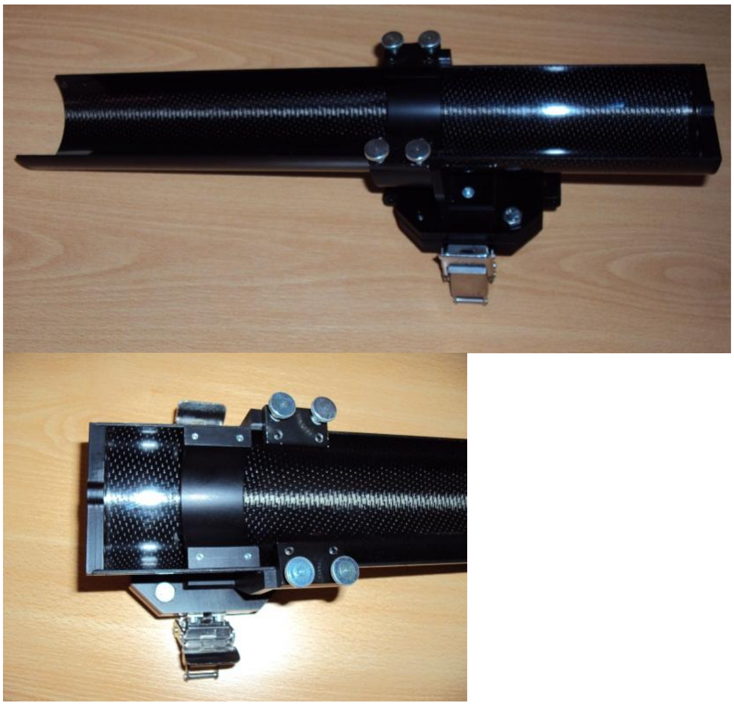


The attachment of the carbon bed to the block by ring insert and upper plates, in the mouse stage (left) and rat stage (right).

Note that in the rabbit stage, the bed is permanently fixed to the bed frame, without screws or bolts:



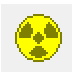
The position of the carbon bed in the mounting blocks, in the case of the mouse and rat beds, is variable, as shown in the image below. Loosening the metal screws allows the carbon bed to slide rearward. Note the metal ring insert in the right photo, which holds the bed to the plate with screws.



This movement of the bed attachment position allows polystyrene inserts to be used, and to extend beyond the end of the carbon bed. This is useful for scanning soft animal tissues or low density samples, to exclude the x-ray absorption of the carbon bed.

Examples of available polystyrene foam tubes are shown below. Tubes are available with outer diameters of 6cm for the rat bed (left), and 3 cm for the mouse bed (center), and the 3cm tubes can fit inside the 6 cm tubes (right) – to assist for instance in holding the limb of an animal for scanning.

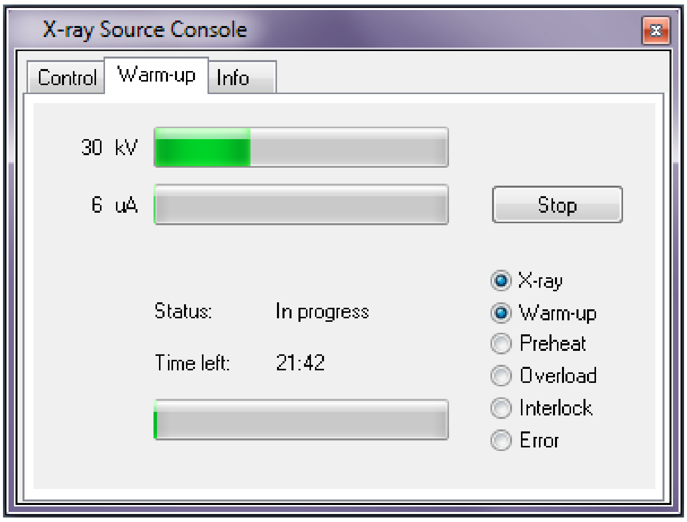
# 4.5. Turning on the x-ray source

Press the x-ray button  to start the x-ray source.

## 4.5.1. Management of your x-ray source

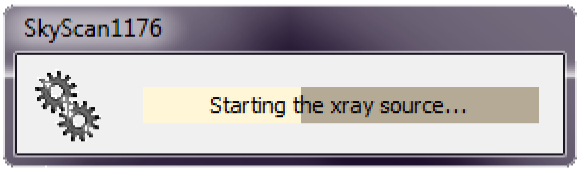
It is important that as the user of the SkyScan 1176 you understand the essentials of successful management of the scanner's micro-focus x-ray source.

Periodically, the x-ray source will undergo a process of conditioning, otherwise known as "ageing". This means that after pressing the yellow x-ray button, the x-ray source will increase in power from zero to maximum gradually, over 22 minutes. The x-ray source control window will open and display the progress of the ageing:




Ageing is performed automatically at intervals of 3 days. If you are turning on the x-ray source for the first time in the day, after a delay of 3 days or more since the previous ageing took place, then the ageing will be performed. This is automatic and the user cannot change this schedule.

The ageing is only performed once every three days when the scanner is in daily use. After ageing, subsequent start-ups of the source, for instance between scans, happen much more quickly – over several seconds only. The progress bar of source start-up without ageing is shown below:

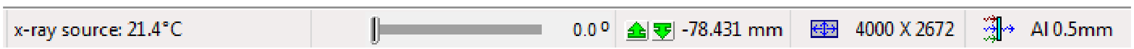



With the x-ray source on, the x-ray imaging buttons (number 4 and 5 above) will become active, and x-ray imaging and scanning of the animal or sample are possible.

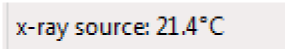
Please note: it is recommended that the x-ray source in the 1176 scanner is turned on and "aged" in the way described above, *at least once every week,* including during periods in which the scanner is not being used. This will prolong the lifetime of the source.

# 4.4. The bottom status bar buttons

In the bottom right corner of the 1176 control software window, there are a number of control items along the status bar:

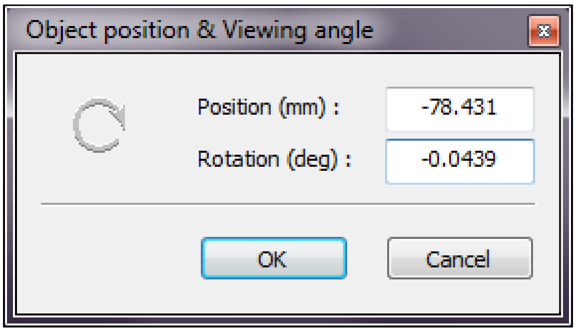


From left to right:

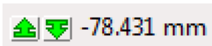



First, there is a notification of the current x-ray source temperature.


Second, there is a slider control of the rotation angle of the camera-source gantry. Rotating this to different angles can be useful to assess the positioning of an animal or sample for scanning. The angular position is also shown in degrees. Mouse-clicking on this rotation angle number will open a dialog box entitled “object position and viewing angle”:




As an alternative to changing angle with the slider, any chosen rotation angle over 0-360 degrees can be entered in the “rotation (deg)” box.




Third, there is the control of longitudinal position of the animal / sample bed. The green arrows adjust the location of the image field of view (FOV) by moving the bed into and out of the scan chamber on its rail mounting. Beyond the green arrows is a numerical display of the longitudinal position of the bed. The “start position” of the bed at its maximum distance out of the scanner chamber is as shown in the above image, about -78.4 mm. (This value will vary slightly between scanners.) As with the numeric display of rotation position, clicking on the numeric bed position will open the “object position and viewing angle” dialog box shown above, allowing text entry of the required longitudinal bed position.

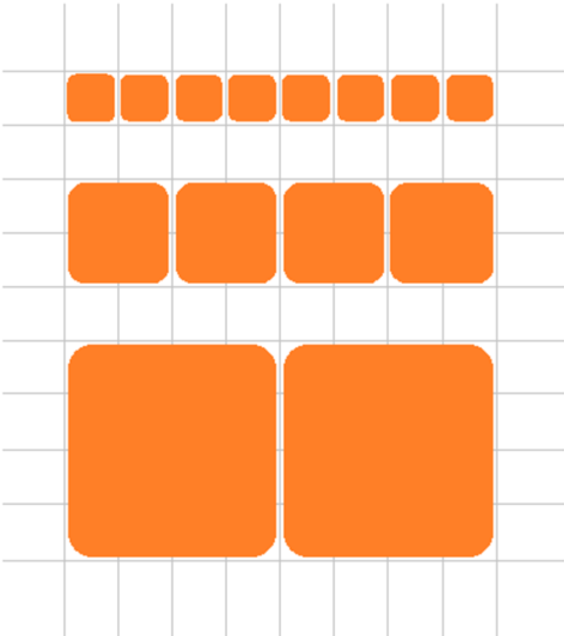

Fourth, is the control of the resolution setting. The three levels of resolution available are: 9 micron pixel (4000 pixels across image), 18 micron pixel (2000 pixels across image) and 35 micron pixel (1000 pixels across image). The image magnification in fixed, as is FOV width (35mm). The different resolution levels are achieved by pixel binning in the camera as shown in the image below.

The choice of resolution level is a balance between spatial resolution and speed / duration of the scan.

At 9 micron pixel, the spatial resolution is maximum. But to register enough x-ray photons on each individual un-binned pixel (physical size 11 microns wide) requires long exposure time of several seconds.

At 35 microns, the effective area of each pixel is 16 times larger, so camera exposure times are correspondingly 16 times shorter – a few tens of milliseconds. So very fast scans – but at the minimum spatial resolution.

18 micron pixel represents an intermediate level of both scan time/speed and image resolution.

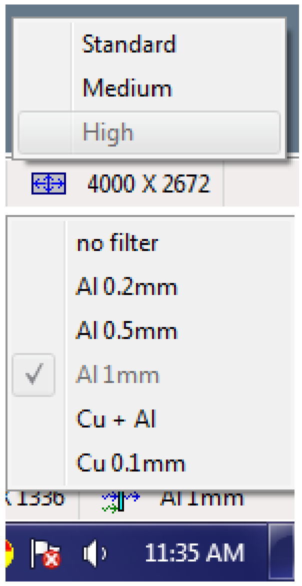


9 micron (4000) pixel setting: pixels are un-binned

18 micron (2000) pixel setting: pixels are binned 2x2

35 micron (1000) pixel setting: pixels are binned 4x4

Clicking on the numeric display of resolution level opens a selection menu between the three resolution levels:

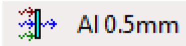



Here are some examples of recommended choices of scan resolution levels.

*9 micron pixel resolution:* mouse hindlimb *in vivo*, mouse or rat bones *ex vivo*, small samples of biological tissue (e.g. lung, other tissue) *ex vivo* or scaffold and biomaterial samples.

*18 micron pixel resolution:* mouse body for lung, bone and soft tissue (higher resolution) *in vivo*, mouse head, rat head, larger bone samples *ex vivo* (rabbit, sheep, etc.)

*35 micron pixel resolution:* lung and cardiac synchronised scans (very short exposure time needed), mouse body for soft tissue e.g. fat, whole body mouse scan, rat body scan (offset mode), large and dense samples *ex vivo*.



Fifth, the filter is selectable from the status bar filter button. (Alternatively filter can be changed by pressing the filter buttons on the touch screen on the scanner.)

Here are some examples of recommended choices of scan resolution levels:

*No filter:* Non calcified biological material *ex vivo* (lung, aorta in paraffin wax, etc.) Low density biomaterial scaffolds e.g. collagen, PLA, etc., soft tissues with light staining with contrast agent (*ex vivo*)

*0.25 mm Aluminium:* Soft tissue containing limited calcification (e.g. bmp-induced calcifications), very small bones e.g. neonatal mouse bones or tissue with low calcification; soft tissues with medium staining with contrast agent (*ex vivo*).

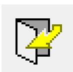
*0.5mm Aluminium:* Mouse bones *in vivo* and *ex vivo*, mouse body scans, calcified biomaterials and scaffolds, soft tissues with heavy staining with contrast agent (*ex vivo*).

*1mm Aluminium:* Rat hindlimb bones *in vivo*, rat and rabbit bones *ex vivo*.

*Cu (0.04mm) + Al (0.5mm):* Rat body *in vivo*, large bones (sheep, dog etc.), bone containing metal implant.

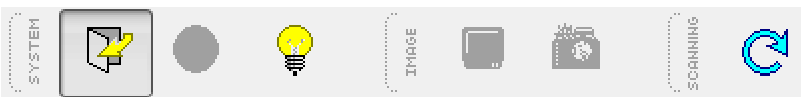
*0.11 mm Cu:* Very large rat body *in vivo*, large dense bone *ex vivo*, bone containing large metal implant.

# 4.6. Mounting an animal or sample for scanning

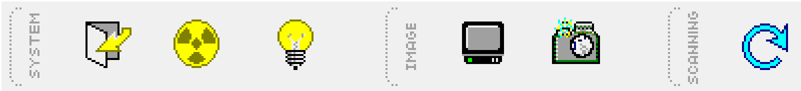
If the x-ray source is on, turn it off to allow the scanner door to be opened. Then click on the  button to open the scanner chamber door. The door will slide open automatically driven by an electric motor. Wait for the door to fully open.

Prior to opening the door, the top button bar icons for the x-ray source and the two imaging buttons are greyed out. With the door open, a safety system disconnects the x-ray source power supply so that it cannot be turned on (a regulatory requirement for any laboratory x-ray device).
After closing the door, these icons become active. The button bar appearance with the door open and closed is shown below:

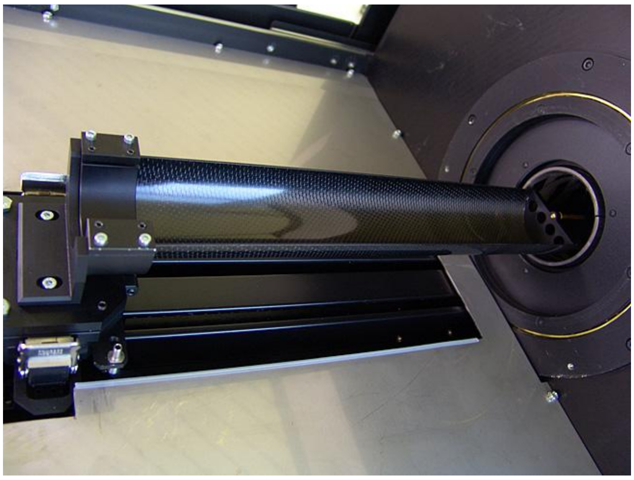
Door open:



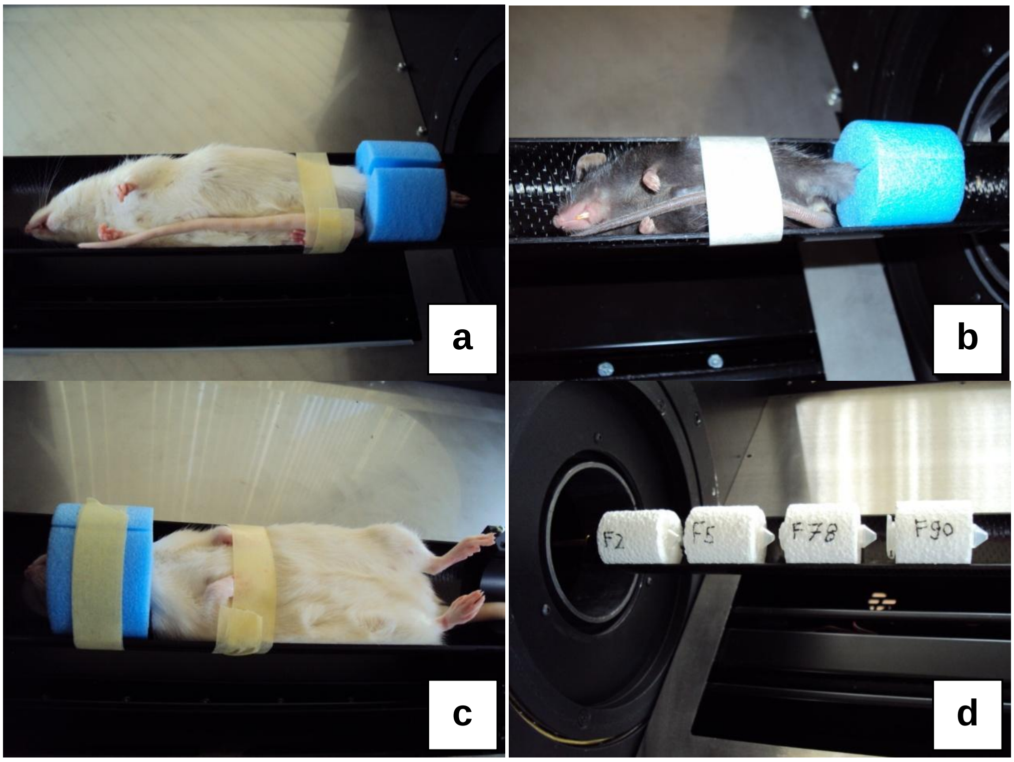
Door closed:




With the door open, the scanner interior will appear as shown in the image below – depending on which of the several interchangeable beds is currently fitted:



In this example it is the larger (6cm width) of the two carbon fiber beds that is fitted – the bed for rats (the smaller 3cm width carbon fiber bed is for mice).

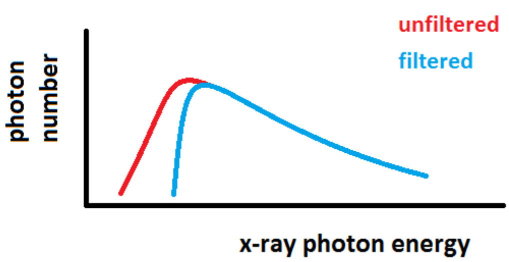


The above photos show options for mounting animals and samples for scanning. (a) A rat, in the 6cm bed, under injection anaesthesia with its hindlimb held for knee or ankle imaging by a foam split tube; (b) a mouse in the 3cm bed, with its hindlimb similarly mounted for scanning; (c) a rat with its head in a foam split tube for centred positioning and stability during a head scan; (d) ex-vivo rat bone samples mounted in polystyrene tubes for batch scanning.

Animal and sample mounting and animal anaesthesia will not be described in detail here. Anaesthesia can be carried out by injection or by gas – x-ray shielded ducts are provided to allow access of the necessary tubing from outside the scanner. The important principles of all micro-CT scanning apply: (i) the sample should be held as close as possible to the central axis of scan rotation (the axis of the cylindrical beds) and (ii) the sample must be held securely to prevent movement or slipping during the scan. The latter does not include breathing movements which cannot be prevented and are addressed by synchronised scanning that is described in other manuals and documents.

# 4.7. Selecting the filter setting

A “filter” is used to modify the x-ray energy. The x-rays generated in the micro-focus source have a wide energy spectrum. Filtration (a sheet of metal in front of the source) removes a part of the low energy end of the spectrum, thus increasing average x-ray energy – see the image below:

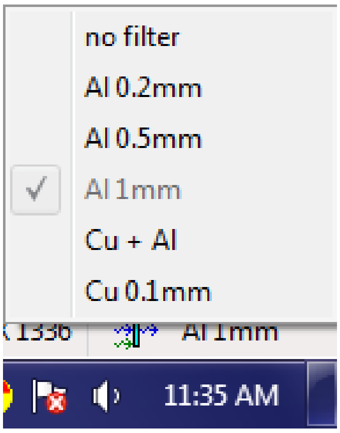



How to choose the correct filter? Aim for a medium shade of grey intensity of the image. If x-ray energy is too low, too few x-rays transmit through the sample, and the image is too dark. Conversely, with x-ray energy too high, too many x-rays are transmitted, and the image is too pale. An example is given the image table on the next page. This table compares the transmission image of a rat femur, scanned *ex vivo*, with all the filter settings.

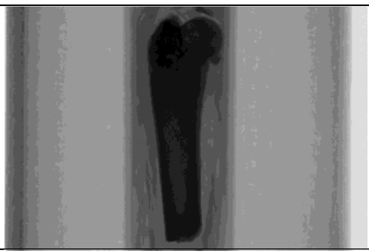
Filter changing can be done in two ways. The touch screen on the left side of the scanner allows any filter setting to be selected by a single touch of the corresponding button:


Alternatively, filter can be changed by the filter display in the status bar at the bottom of the 1176 control software window.

Please note that when the filter is changed by either method, other settings such as voltage, current and exposure time are also changed automatically. No adjustment of these settings by the user is required. However after changing filter it is recommended to take a fresh flat field correction (see section 4.5 below)



This table compares the transmission image of a rat femur, scanned *ex vivo*, with all the filter settings.

| | | |
|---|---|---|
| No filter |  | X-ray energy too low (minimum transmission 10.2%) |
| 0.25mm Al filter |  | X-ray energy too low (minimum transmission 18%) |
| 0.5mm Al filter |  | Acceptable range (minimum transmission 24%) |
| 1 mm Al filter |  | Acceptable range (minimum transmission 37%) |
| Cu+Al filter |  | X-ray energy too high (minimum transmission 40%) |
| 0.11 mm Cu filter |  | X-ray energy too high (minimum transmission 56%) |

Note – in different samples (e.g. live animals) density distributions can be very different, so do not regard the minimum transmission values above as strict targets – the overall transmission is more important.

Some examples of recommended settings are given below. These settings are present in the scanner and will thus require no adjustment by the user – just selection of the filter.

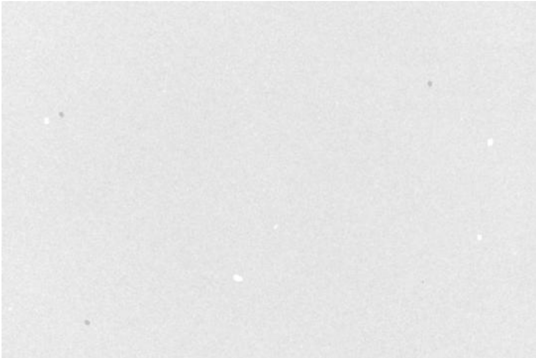
| Filter | Applied voltage | Type of sample |
|---|---|---|
| No filter | 40 | Low density *ex vivo*, e.g. biological non-mineralised, dried. 30 kV for very low density materials and to reduce beam hardening |
| 0.25mm Al | 45 | Partly mineralised biological soft tissue, very small bones from embryo or neonatal mice / rats |
| 0.5 mm Al | 50 | All mouse scans *in vivo* (bone or soft tissue), mouse bone *ex vivo* |
| 1 mm Al | 65 | Rat scans *in vivo* (small – medium size rat), rat bones *ex vivo* |
| Cu+Al | 80 | Rat scans *in vivo* (large rat), larger bones *ex vivo* |
| 0.11 mm Cu | 90 | Metal containing bones, very large rat. |

# 4.8. Taking the flat field correction

The flat field correction re-sets the image background. On selecting this function, the scanner automatically takes x-ray images with an empty field of view (FOV) and uses these images to generate a smooth background in scan projection images.

**It is a good idea to take a flat field correction at the start of a day's scanning, for each filter setting used. At a minimum the flat field should never be more than one week out of date.**

The images below illustrate and out of date (left) and an up to date (right) background image (empty, no object or bed). Signs of an out of date flat field include white or dark dots or blotches in the image. Non-uniformity or change in the background brightness also indicates that the flat field needs refreshing.


Flat field out of date


Flat field up to date

### 4.8.1. The flat field needs two images: the bright and dark fields

The flat field correction involves taking two images of empty space (sample bed moved out) in the scanner: one with the x-ray source on, and one with it off. These are the “bright field” and “dark field” respectively.

The bright field characterises the un-attenuated ambient x-ray intensity, while the dark field characterises camera electronic noise. For each image pixel acquired during a scan, the flat field (*ff*) corrected intensity is calculated as:

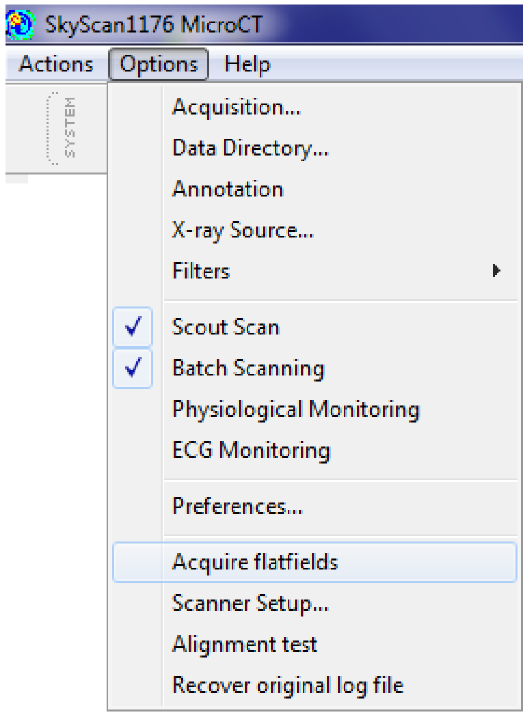
$$Intensity\ (ff\ corrected) = \frac{image\ value - dark\ field\ value}{bright\ field\ value - dark\ field\ value}$$

### 4.8.2. Flat field correction of the current scan mode

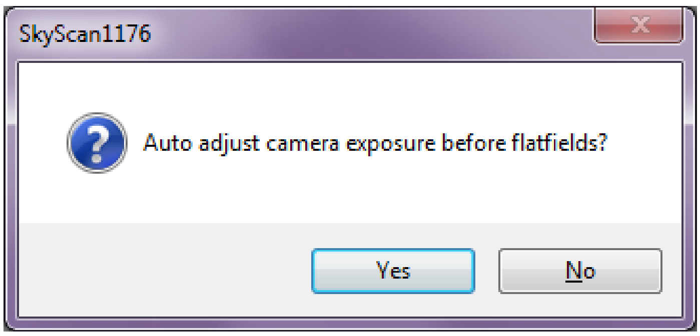
The “current scan mode” means the currently selected filter and resolution level.

An easy way to take a flat field correction before a day’s scanning, is to first select the required scan mode, then run the flat field correction of that mode only from the options menu.

To take a flat field for the current mode, go to the options menu and select the “acquire flat fields” item, as shown below:




A window will pop up asking if you wish to perform auto-exposure time adjustment:

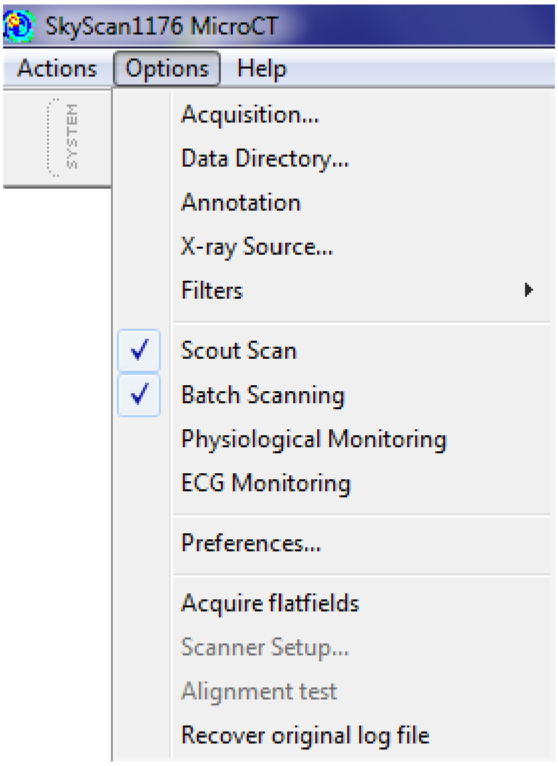



For most routine updating of the flat field, choose “no”. At intervals of several months, and in between experimental studies, the “yes” option can be selected to ensure that the exposure time is optimal.

Please note that the “acquire flat fields” function in the options menu corrects only the central camera position, regardless of whether the “use camera central position only” option is selected in the scanner setup window.

## 4.8.3. Flat field correction from the scanner setup window

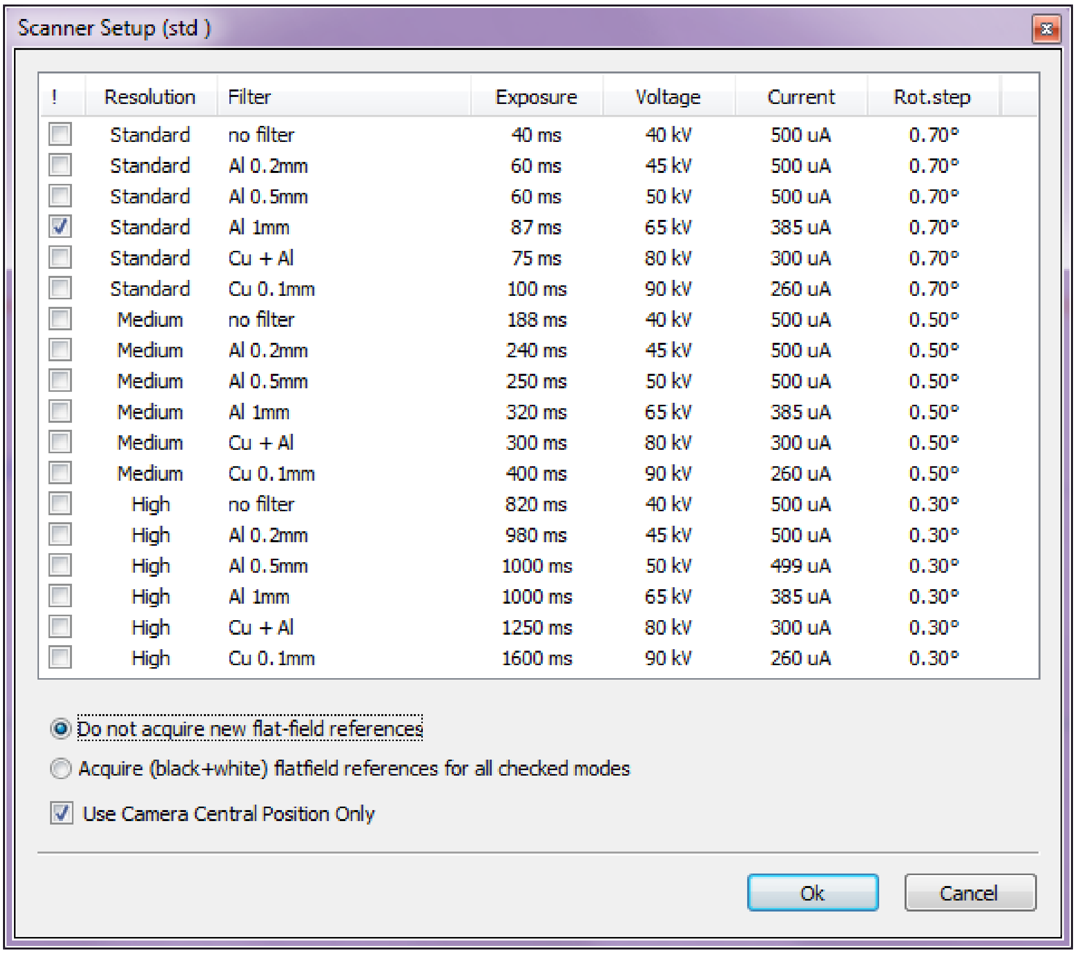
When the options menu is first clicked after starting the scanner and the source, two items are greyed out, the “scanner setup” and the “alignment test” items:




To activate these items and allow high-level access to the scanner control software, press the following keyboard key combination:

Shift + control + alt + S

After this, those two buttons become available. The scanner setup window is opened from the options menu:




| ! | Resolution | Filter | Exposure | Voltage | Current | Rot.step |
|---|---|---|---|---|---|---|
| ☐ | Standard | no filter | 40 ms | 40 kV | 500 uA | 0.70° |
| ☐ | Standard | Al 0.2mm | 60 ms | 45 kV | 500 uA | 0.70° |
| ☐ | Standard | Al 0.5mm | 60 ms | 50 kV | 500 uA | 0.70° |
| ☑ | Standard | Al 1mm | 87 ms | 65 kV | 385 uA | 0.70° |
| ☐ | Standard | Cu + Al | 75 ms | 80 kV | 300 uA | 0.70° |
| ☐ | Standard | Cu 0.1mm | 100 ms | 90 kV | 260 uA | 0.70° |
| ☐ | Medium | no filter | 188 ms | 40 kV | 500 uA | 0.50° |
| ☐ | Medium | Al 0.2mm | 240 ms | 45 kV | 500 uA | 0.50° |
| ☐ | Medium | Al 0.5mm | 250 ms | 50 kV | 500 uA | 0.50° |
| ☐ | Medium | Al 1mm | 320 ms | 65 kV | 385 uA | 0.50° |
| ☐ | Medium | Cu + Al | 300 ms | 80 kV | 300 uA | 0.50° |
| ☐ | Medium | Cu 0.1mm | 400 ms | 90 kV | 260 uA | 0.50° |
| ☐ | High | no filter | 820 ms | 40 kV | 500 uA | 0.30° |
| ☐ | High | Al 0.2mm | 980 ms | 45 kV | 500 uA | 0.30° |
| ☐ | High | Al 0.5mm | 1000 ms | 50 kV | 499 uA | 0.30° |
| ☐ | High | Al 1mm | 1000 ms | 65 kV | 385 uA | 0.30° |
| ☐ | High | Cu + Al | 1250 ms | 80 kV | 300 uA | 0.30° |
| ☐ | High | Cu 0.1mm | 1600 ms | 90 kV | 260 uA | 0.30° |



At the bottom of the setup window is the option to “acquire (black and white) flat field references for all checked modes”.

The scan “mode” is the combination of the resolution level and the filter. With three resolution levels “standard” (1000 pixels or 35 microns), “medium” (2000 pixels or 18 microns) and “high” (4000 pixels or 9 microns), as well as six filter options, this makes a total of 18 scanning modes. These are all listed in the scanner setup window, and each is selectable with a check box.

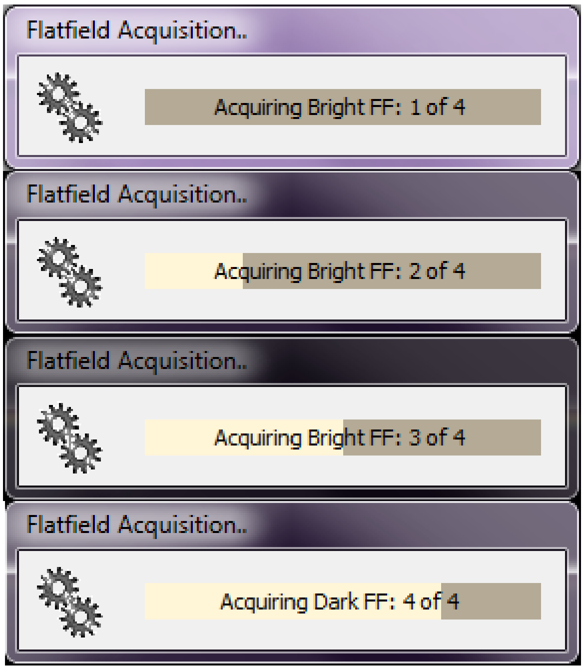
When the “acquire flat field references...” option is selected followed by clicking “OK”, then flat field correction is performed on any selected modes.

## 4.8.4. Correction of the three lateral camera positions

The standard FOV width in the 1176 scanner is 35 mm; however the FOV can be extended to 68 mm by “offset” scanning in which the x-ray camera acquires images from a left and a right position, and connects these images horizontally. Therefore there are three camera lateral positions – left, right and center. In a standard scan, the camera acquires images from the central position only. In an offset scan, the camera acquires images from both left and right positions (the scan thus takes two times longer to run).

Thus a full flat field correction of a scanning mode involves acquiring background images for all three camera positions – left, right and center. However if the user is planning to perform only standard scans, and no offset scans, then the option exists to performed a shortened flat field correction of the central camera position only. This option is selectable by the item at the bottom of the scanner setup window, "use camera central position only".

In the case that this option is not selected, then the flat field correction for each selected mode will run four times – three times for bright field acquisition (x-ray on) of the left, right and central camera positions, and once for the dark field (x-ray off). Corresponding progress bars will appear during the flat field acquisition:

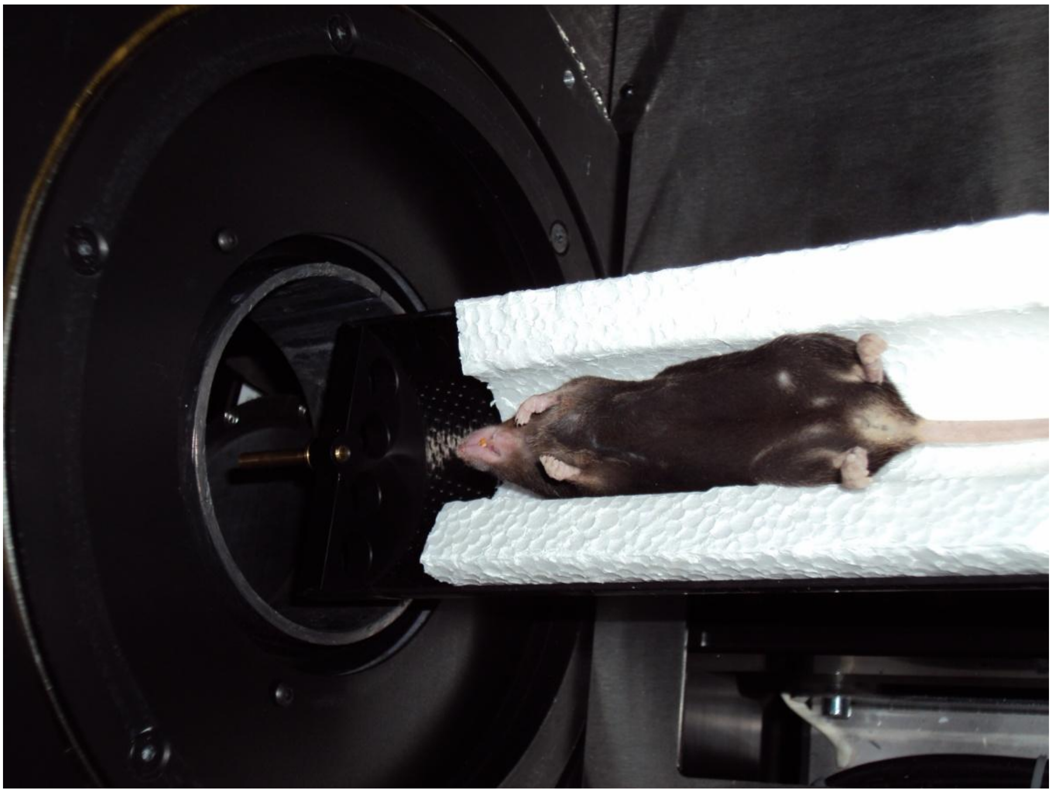



# 4.9. Doing a scan

With the animal or samples in place, and the door closed, the x-ray source on, and the flat field correction up to date, you are ready to proceed with a micro-CT scan.

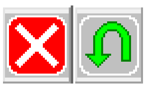
## 4.9.1. Summary description of a single mouse scan, standard FOV (no offset)

The example will be given of scanning a mouse thorax. Place the mouse under anaesthesia in the scanner sample bed. Then select the scanning mode – that is, the filter and resolution level. For example, if the intention is to perform a fast scan of the mouse's soft tissues such as lung and fat, or the regional BMD of one or more skeletal sites, then an appropriate setting could be the 0.5mm Al filter and the 35 micron "standard" resolution level (1000 pixel FOV width).

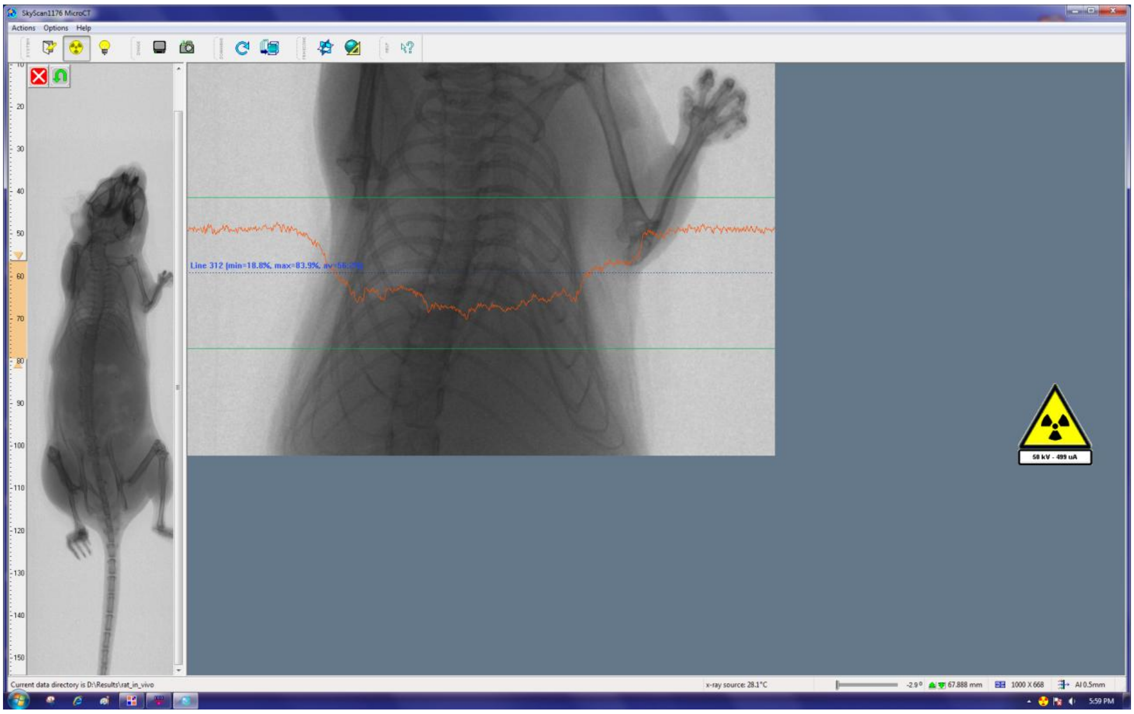


If it is the first scan today with this filter and resolution, take the flat field correction (section 4.6 above – for example the quick flat field from the options menu).

First click on the green arrow button which launches the scout view:



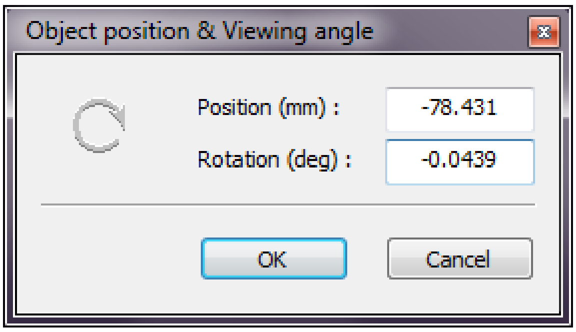
The scout view can be aborted once the whole of the mouse – or other object you wish to scan – is imaged within the scout view. The appearance of the control software following the scout view of the mouse is shown below:



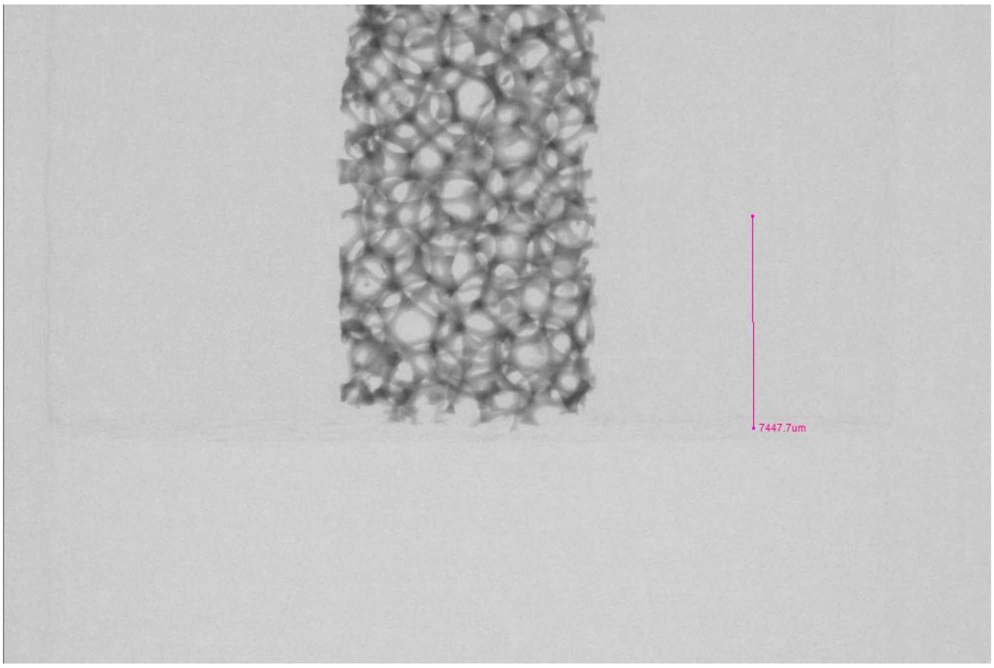
### 4.9.1.1. Positioning the field of view (FOV) for scanning

Double-click with the left mouse button within the scout view window, and the bed will move such that the scan field of view (FOV) will become centred on the location of the mouse cursor.

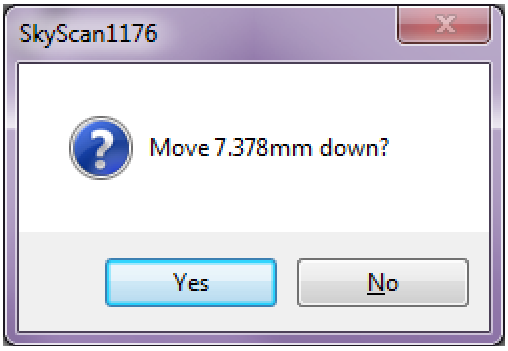
Other options in the scanner software for moving the animal / sample bed include the status bar position control , using either the green in / out arrows or entering an exact bed position in the control window as a distance in mm:



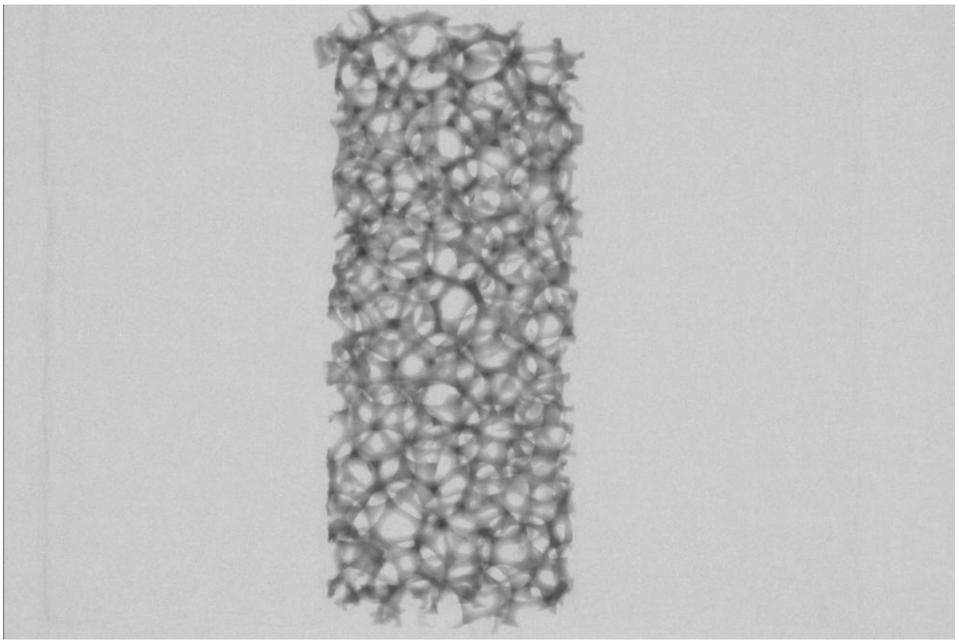

A further option is to use left mouse button drag and drop in the image, using vertical drawing of a line in an upward or downward direction. The measurement pink line will appear. With the line still displayed on the image, double click the left mouse button: the bed and FOV will then move by the distance drawn, and in the direction that the line was drawn (either upward or downward from the viewer's perspective).




*Step 1*: draw a measure line with left mouse button drag and drop – drawing from the top downwards will result in a downward movement of the sample as viewed.




*Step 2*: immediately on completion of the line drawing, double click with the left mouse button: a dialog will open asking confirmation of the requested movement – click yes.



*Step 3*: The bed will move; after the movement, click on the image button (either video or single) and the new position of the sample will be displayed.

Once the field of view (FOV) position is correct, click on the button to open the scan dialog (see the image below).

The currently selected pixel resolution is displayed at the top – here 35 microns.

Next the scan filename prefix is entered: this string of text will be followed by a 4-digit number in the set of scan projection images, and subsequently in the reconstructed crossectional dataset.

The data directory is next specified: clicking on “browse” opens a file manager dialog box allowing set up of a destination folder for the scan projection images.

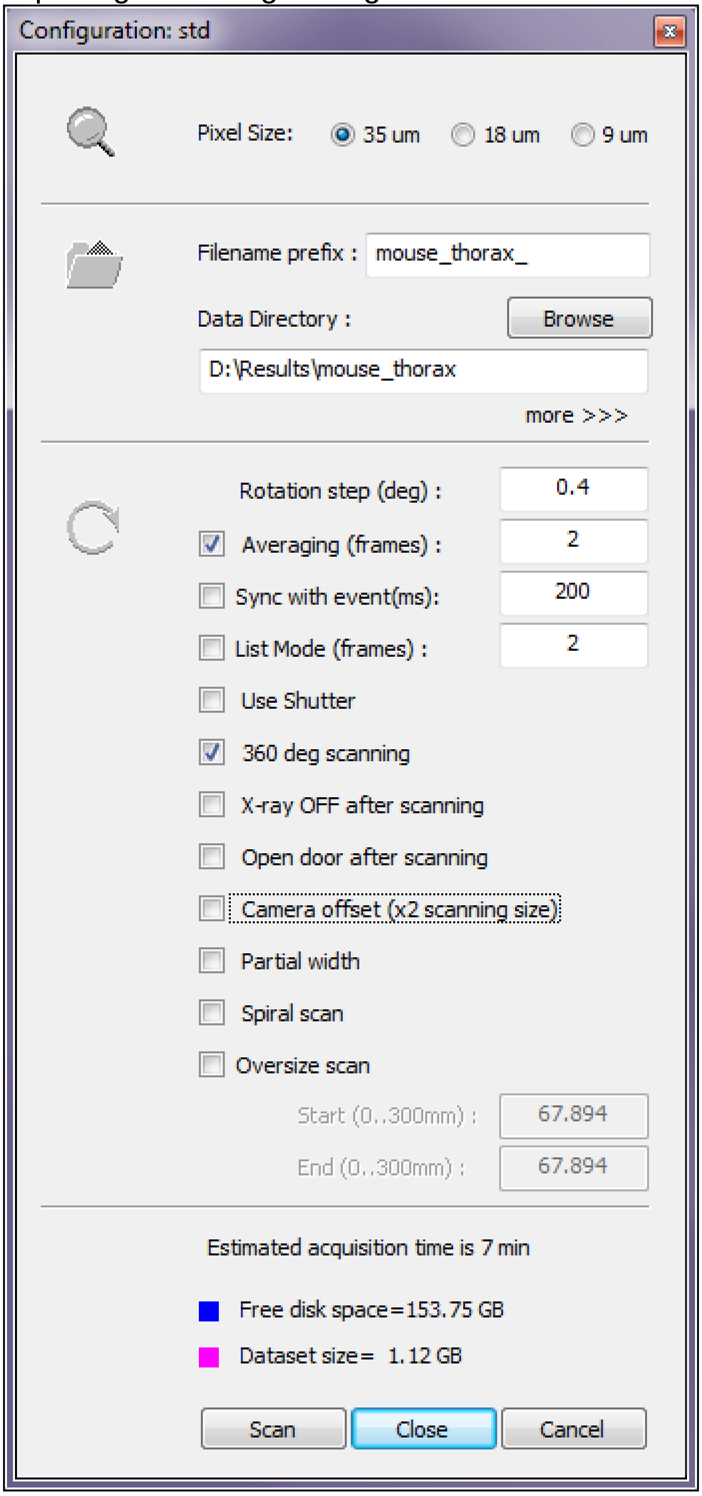
Below this is the full list of scan options. (These are listed and described in detail in section 5.) In this example a rotation step of 0.4 degrees and frame averaging of 2 are selected. The scan is over 360 degrees. At 35 micron resolution this gives a scan time of 7 minutes.

Note that at the bottom of the scan dialog there are three buttons:

Scan: Starts the scan;

Close: Closes the scan dialog but remembers all entered settings – so they reappear unchanged on re-opening the dialog;

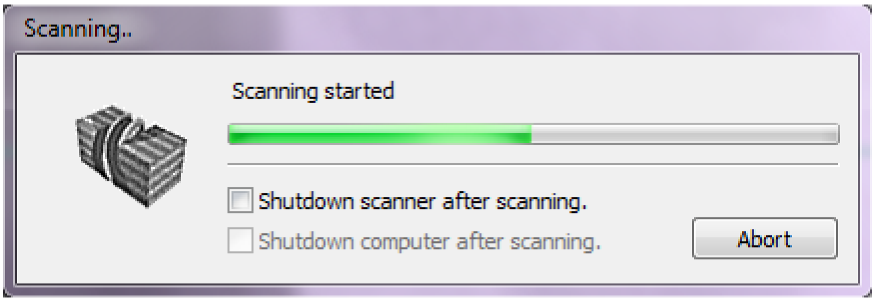
Cancel: Closes the scan dialog and discards the entered settings – so on re-opening the dialog settings have to be re-entered.




To view the mouse by real time video during the scan, click on this button at the top:

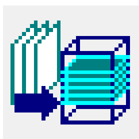
Providing that the video camera gantry is connected (see section 5) a window will open showing the mouse:


In the above example a small block of polystyrene foam is fixed onto the mouse's chest to facilitate viewing of the mouse's breathing. During the scan a progress bar is displayed:

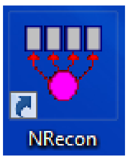



### 4.9.1.2. Launching the reconstruction software NRecon after the scan

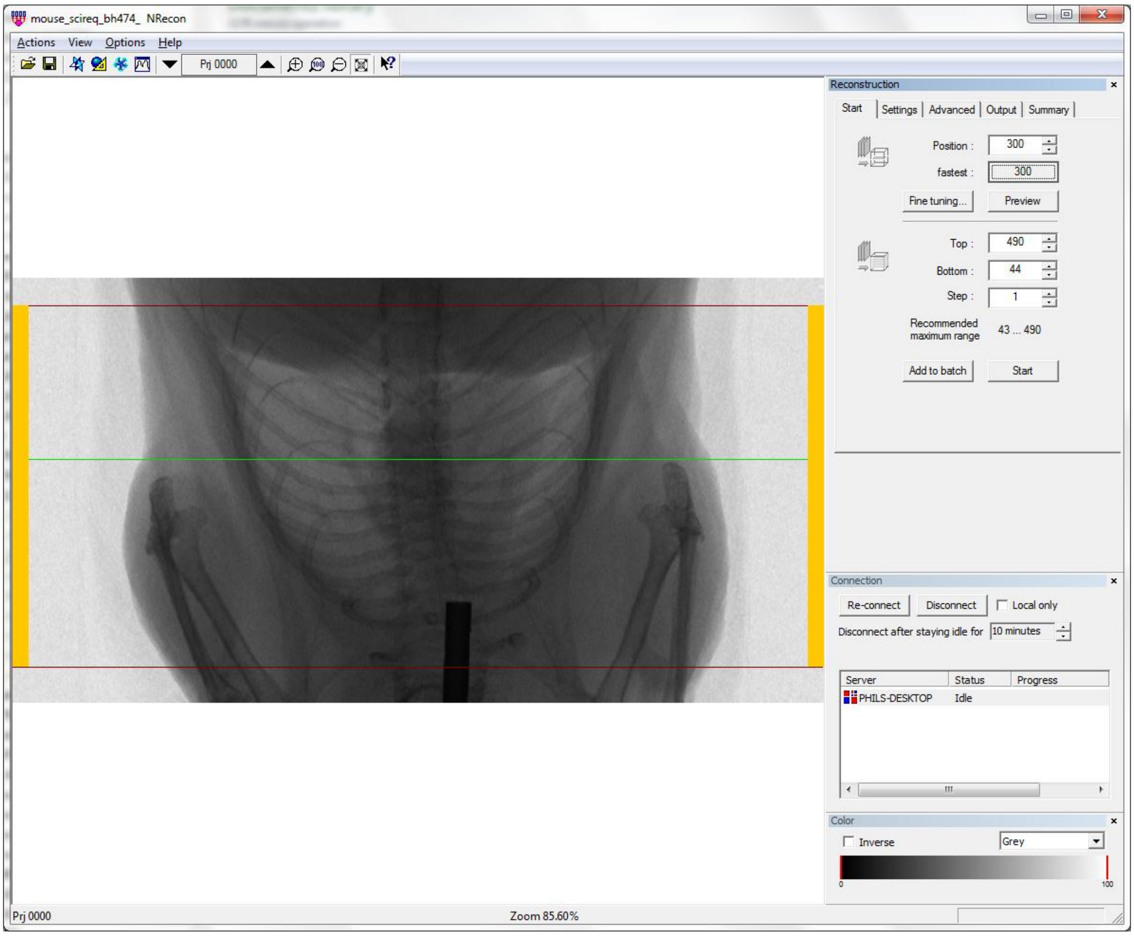
When the scan is complete, clicking the top button for reconstruction will open the program SkyScan NRecon:



NRecon can alternatively be started from the desktop by its shortcut icon:

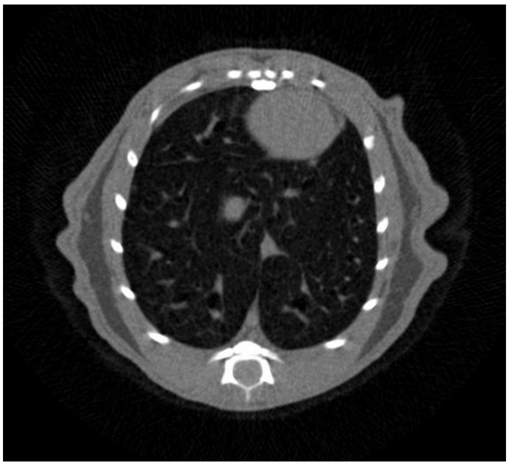



The NRecon window after the mouse thorax scan will appear as shown:




The process of dataset reconstruction will not be described in detail here. However some of the reconstruction parameters recommended for the scan of a mouse body (thorax or abdomen) at 35 or 18 micron pixel, are given below:

Smoothing: 4
Post-alignment correction: use calculated value
Ring reduction: 2-10, use minimum needed to suppress rings
Beam hardening correction: 30%

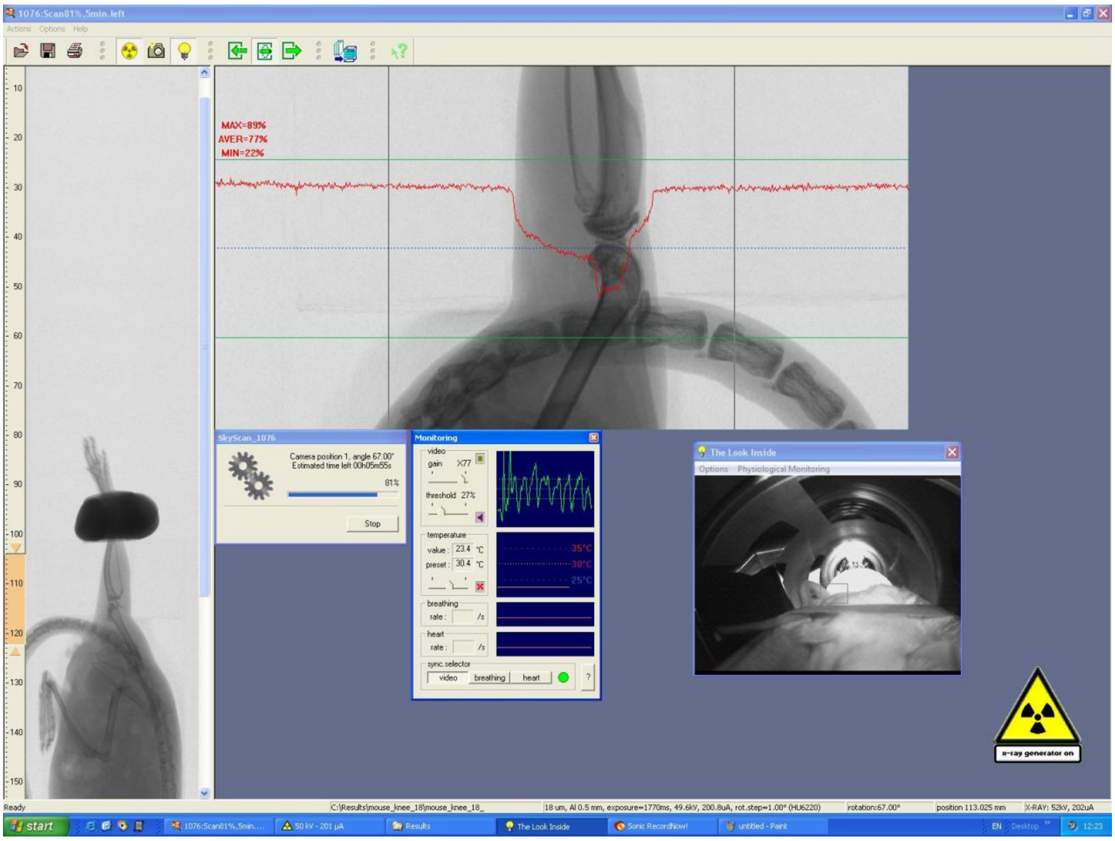


A reconstructed crossection of a mouse thorax scan.

## 4.9.2. Video inspection and physiological monitoring during a scan.

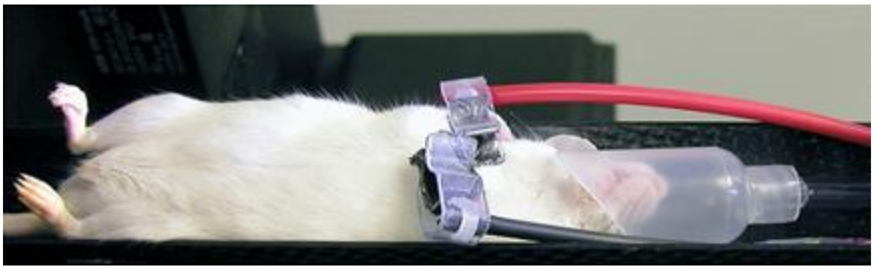
After the scout scan of a live mouse or rat, click on the “look inside” light bulb button to open the inspection video window. This window can be set at three size settings (actions menu), and gain and offset control allow image adjustment.

With the “look inside” activated, start the physiological monitoring window (PMW) by pressing on the “physiological monitoring” button. The upper box in the PMW shows a movement trace taken from a selected square in the video window – a square green button close to the top of the PMW activates this mouse-controllable box on the video image. The moving trace shows amount of movement within the selected square, so positioning the square on a part of the mouse with the most visible breathing movements gives a trace of breathing on the monitor. Adjusting gain and threshold controls the delivery of a breathing signal from the video monitor. (Clicking the small speaker button turns on a sound indication of breathing.)




Next down in the PMW is “temperature”. This control allows the animal to be kept sufficiently warm during a scan. “Value” gives the measured temperature in the chamber, “pre-set” allows you to set a target temperature – e.g. 30°. To turn on heating of chamber airflow, click on the red fan button. Then the airflow heating will automatically try to move the actual temperature toward selected temperature. Note: position the temperature sensor wire close to the animal but not touching it, to measure air temperature (with the aid of tape if necessary).

Below temperature are boxes for breathing and heart beat rate. These require extra monitoring equipment (provided by SkyScan with your system). The breathing monitor requires that the plastic air tube with face mask (sized for rat or mouse) is fitted to the animal as shown in the figure below, and the tube routed out of the sample chamber either using the “caterpillar” type movable black duct casing, or out through the gap at the back of the sample chamber. The tube fits to one of the two tube attachments on the breathing signal box. The other attachment should be closed with the cap provided. This breathing signal can give a good quality regular breathing signal. Exercise caution if injection anaesthesia is used – breathing can become weaker as a scan progresses and anaesthesia deepens.



The ECG electrodes also connect to a corresponding signal box. The electrodes – shown above in red and black, have electric wrist contacts constructed of carbon (graphite) to avoid high density artefacts associated with metal parts. Note that electrical contact with the mouse or rat’s wrists is improved if some lubricating gel such as Vaseline is applied lightly to the wrists. The ECG connecting cable should be routed out of the sample chamber to the signal box as for the breathing monitor tube.

Finally at the window bottom are three buttons to select which monitoring signal will be used if synchronised acquisition is selected in the scan dialog. “Video” selects the video camera movement signal, “breathing” selects the signal from the air tube and pressure sensor, and “heart” selects the ECG signal.

To apply synchronisation with a video (breathing), air pressure (breathing) or ECG signal during a scan, set up the video image and the physiological monitoring before starting the scan. Then in the scan acquisition dialog (left) select “synch scanning with event”. When this is selected an additional slider bar will appear controlling “delay”; this means the delay between a received signal and the start of each image acquisition.

#### 4.9.2.1. Measurement of animal breathing by real time video motion sensing

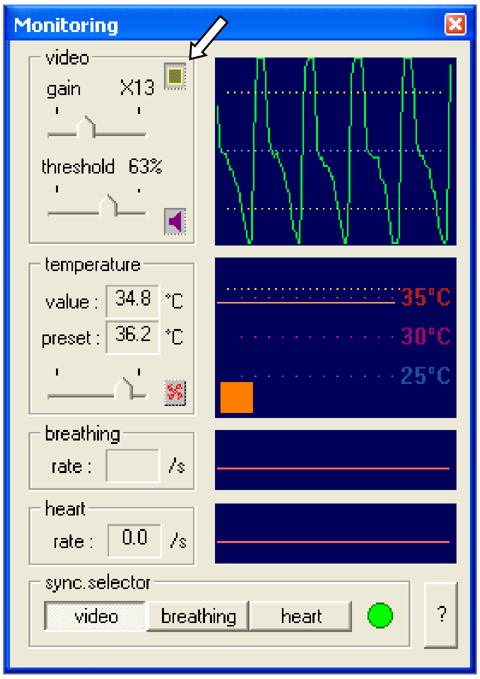
If breathing is monitored by image movement detection, then it helps to fix an object such as a block of polystyrene foam on the animal’s midrif; the vertical movement of this object provides a clear moving edge for the image analysis software to detect (see image below).

Turn on the video camera by pressing the light-bulb button. Then click on "physiological monitoring". The monitoring window shown below will appear. There are five panes from top to bottom:

1. Video
2. Temperature
3. Breathing
4. Heart
5. Synch selector

*Physiological monitoring: 1, The video pane*

In the top, video pane, click on the green box (arrow). This activates a dotted line square in the video image which can be positioned by the computer mouse (left button). Position this square along the upper border of the polystyrene block on the animal's body, as shown. This will result in a strong movement video signal from breathing.



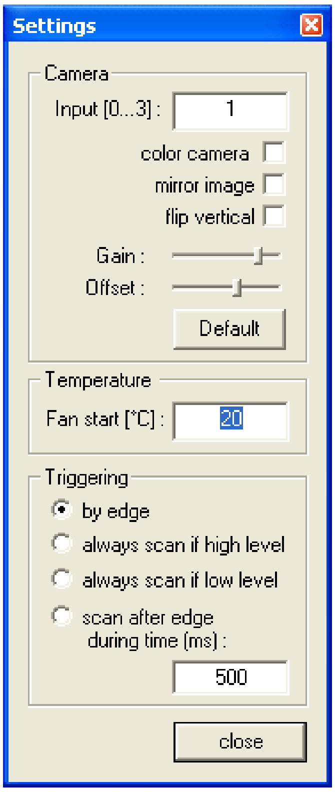


Note that the video output has sliders for controllable gain and threshold.

Gain controls amplification of the movement trace. Increase the gain until it is just sufficient to make the breathing trace reach the top and bottom of the display.

The threshold lines can be moved closer to the centre or further toward the top and bottom. A breathing time-point signal will be output every time the movement trace crosses both top and bottom threshold lines (dotted yellow). Position the threshold lines to be clear of noise fluctuation near the central region (vertically) of the display. Clicking on the small pink speaker icon activates an audible signal with each recorded breathing time-point.

*Physiological monitoring: 2, The temperature pane*




The temperature sensor wire with the black tip (arrow, left), containing a thermistor, should be plugged in to the video camera gantry. Secure the wire and tip close to the animal, for instance by taping it to the polystyrene bed wall. With this in place, the temperature monitoring is functional (second pane). Set the temperature pre-set to 28-30 degrees to maintain a warm environment for the animal in the scanner (otherwise mice in particular can suffer hypothermia during scans). If the measured temperature falls below the pre-set temperature, the orange square appears indicating that airflow heating is on. (If heating does not turn on, go to the settings menu of the video image – see image right – and decrease the “fan start” temperature to 20 degrees C or lower).

*Physiological monitoring: 3, The breathing pane*

This pane relates to the breathing detection using the air pressure sensor outlet, which is at the base of the bed mounting block on the right side. An associated air outlet is shown in the image below (next page) on the physiological monitoring panel on the right side of the scanner, under the “breathing” box and with the red cover. Remove the red cover when using this pressure breathing system. A tube and facemask (supplied with scanner) are attached to this outlet, and breathing measured as change in pressure of air in the mask and tube.

There are thus two ways of measuring breathing – by the video movement detection method described above (with polystyrene object) and this pressure sensing method. It is recommended that you start by trying the video based method first. The pressure sensing method is not compatible with gas anaesthesia.

When measuring breathing by this pressure sensing method, take care not to fit the mask too closely to the animal’s face so that breathing is not obstructed. Abnormal breathing pattern detected by this method can indicate partly obstructed breathing.

Adjust the sensitivity dial to obtain a strong noise-free signal from the breathing pressure sensing.


*Physiological monitoring: 4, The heart pane*

The heartbeat of a small animal can be measured by ECG. Carbon fiber based electrodes (no metal parts) are provided for both mouse and rat as shown in the image at the top of this section. The ECH cable is attached at the base of the bed mounting block on the right side.

It is advisable when fitting ECG leads to apply to the hands and feet of mice / rats a conductive gel to improve electrical conduction. Substances that work well include ultrasound gel, specialist ECG gels or “hand creams” of the gel type such as Neutrogena “crème mains” (hand cream) which is a clear moisturising gel.

Adjust the sensitivity dial to obtain a strong noise-free signal from the heart (ECG).

*Physiological monitoring: 5, The synch selector*

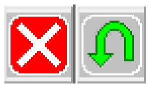
If you are using synchronisation during the scan, either prospective (selecting “synch with event” in the scan window) or retrospective (ListMode, *without* “synch with event” selected) then a synchronising signal must be selected. In this “synch selector” pane, select the appropriate signal. With the correct channel selected, the green circle will flash red with each detected cycle.

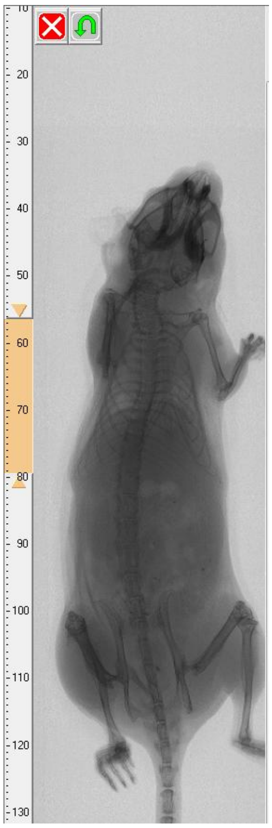
**Important**: Unless the correct synch signal is selected, the synchronised scan might appear to be going normally but NO SYNCHRONISATION WILL TAKE PLACE.

## 4.9.3. Summary description of an “oversize” multi-part scan of a whole mouse

The previous section described a single scan of the mouse thorax (the FOV height in the SkyScan 1176 is 22mm). If you wish to scan the whole mouse, a multi-part auto-connected scan is required. This is referred to as an “oversize scan”

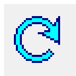
Start the scan by running the scout view, as previously, by pressing the green button at the top of the scout window:






The scout view will show the connected image of the mouse.

Next launch the scan dialog with this button:

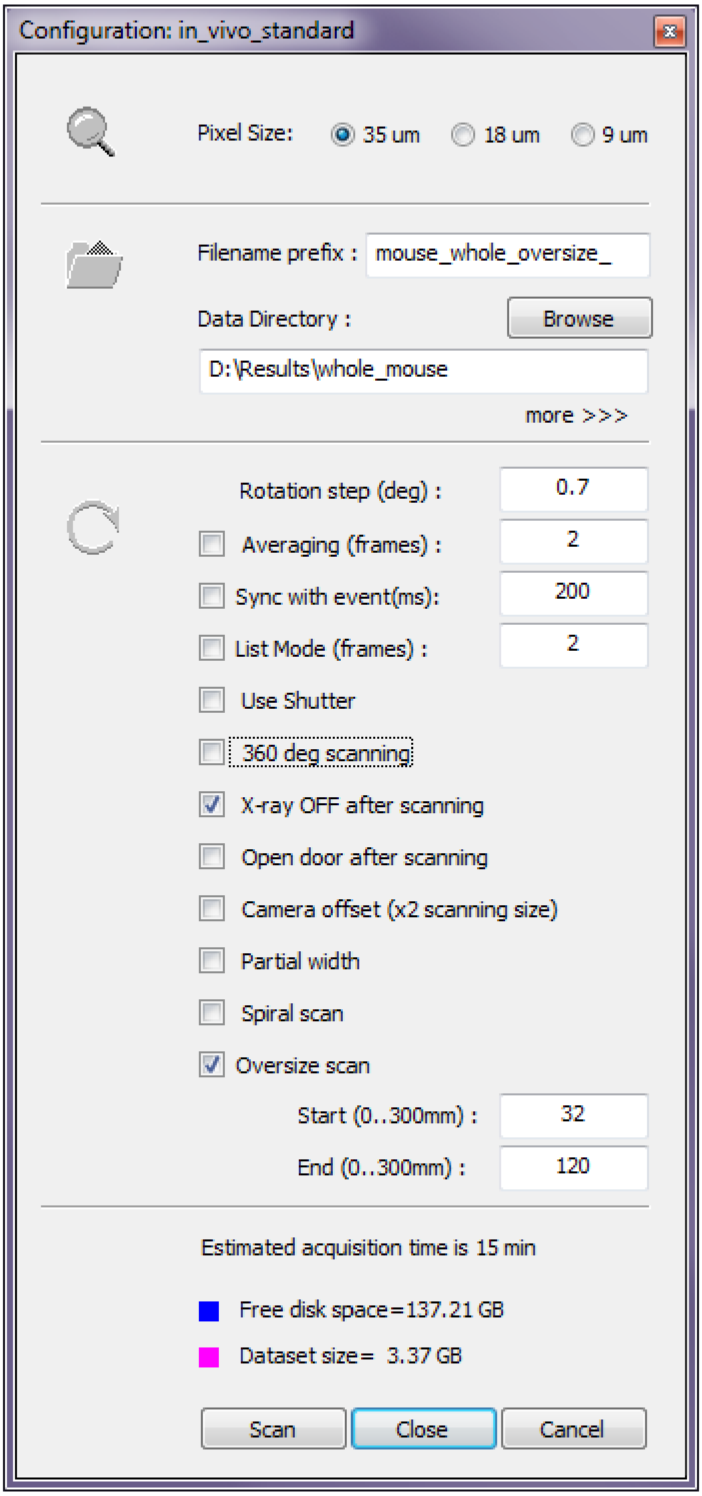


Fill in the filename prefix and destination folder.

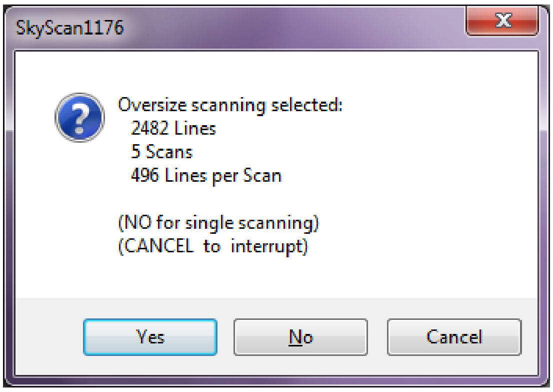
Under the scan options, select the item “oversize scan”. Two boxes will be activated where you need to enter the start and end position of the scan. You determine the star and end position by looking at the ruler alongside the mouse image in the scout view. In the example here, the start and end positions are approximately 32mm and 120 mm (the tail is excluded).

The completed oversize scan dialog window is shown below:

Note that with scan parameters of 0.7 degree step, no frame averaging and 180 degree rotation, a full scan of the whole mouse takes about 15 minutes.



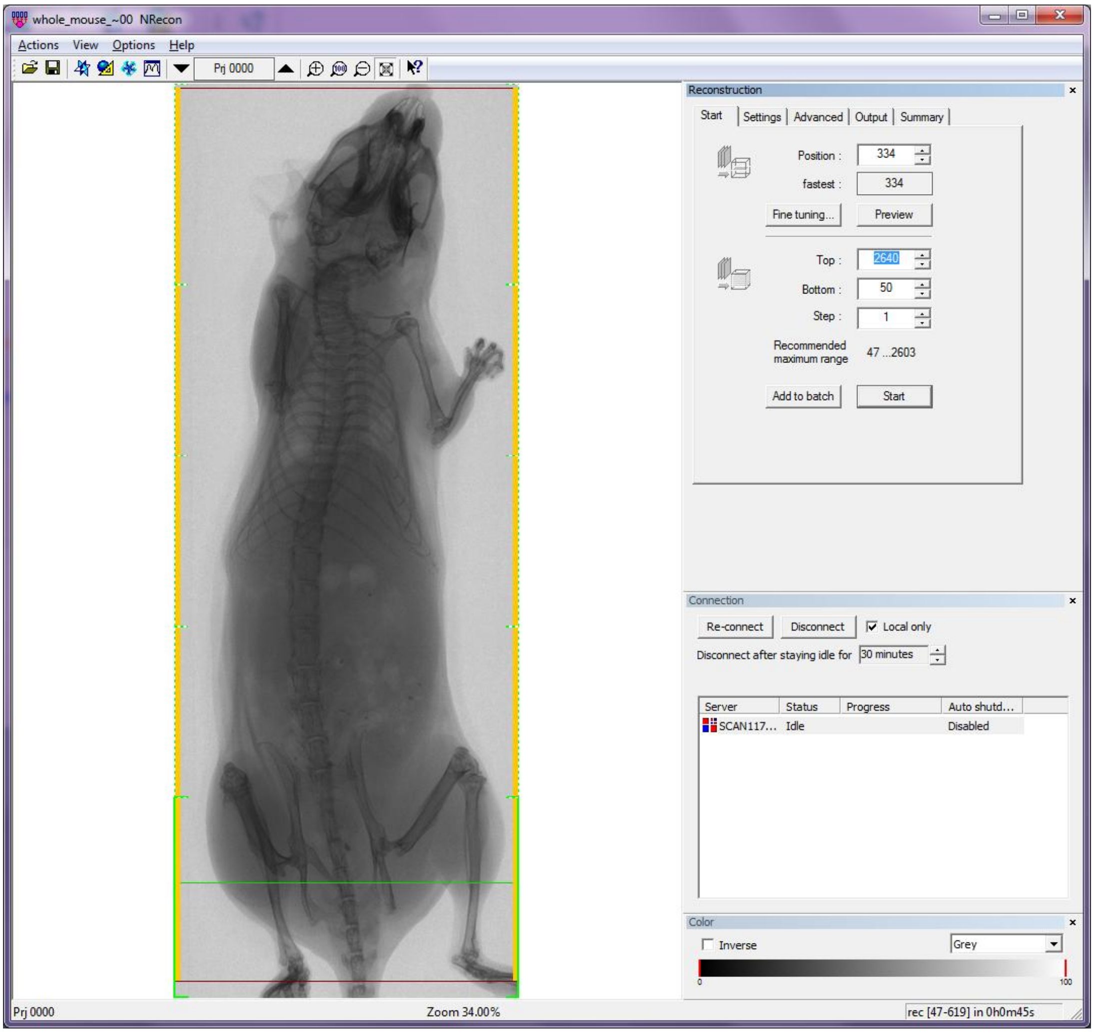

When the “scan” button is pressed, a dialog box opens stating the number of auto-connected scans that will be carried out; confirm the oversize scan be clicking “Yes”.



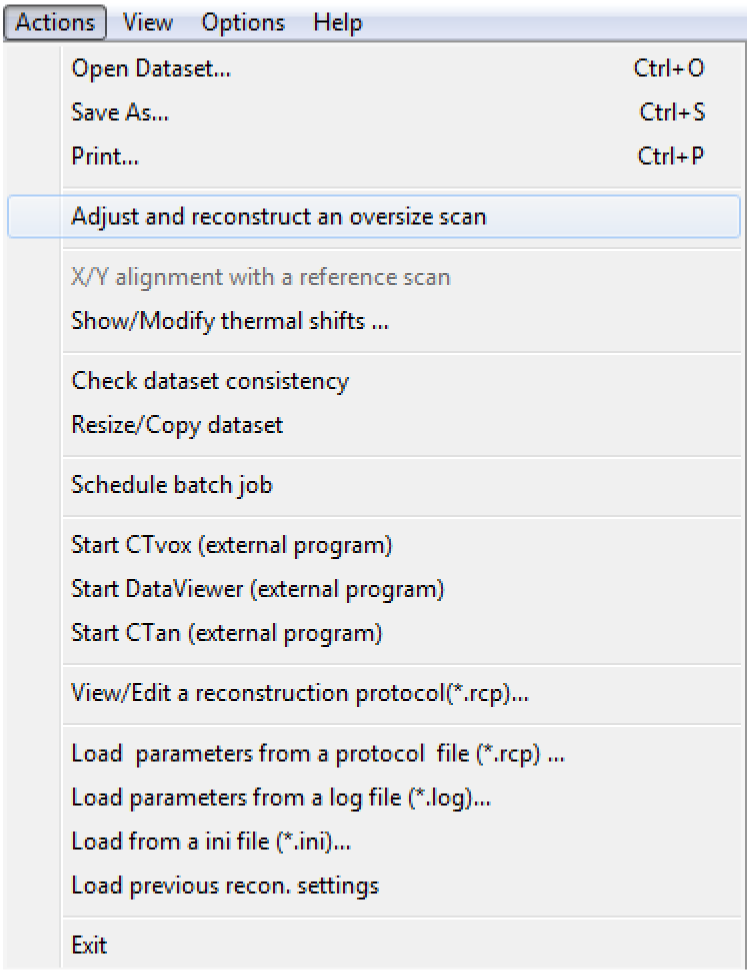

The oversize scan will now run. At the end of each scan the bed will move the mouse to a new position and the next scan will start. At the end of all the connected scans (in this example, 5) the scan will stop as normal and if the option has been selected for shut-down of the source, this will take place (the source will not shut down between connected scans).

### 4.9.3.1. Notes on reconstruction of an oversize scan

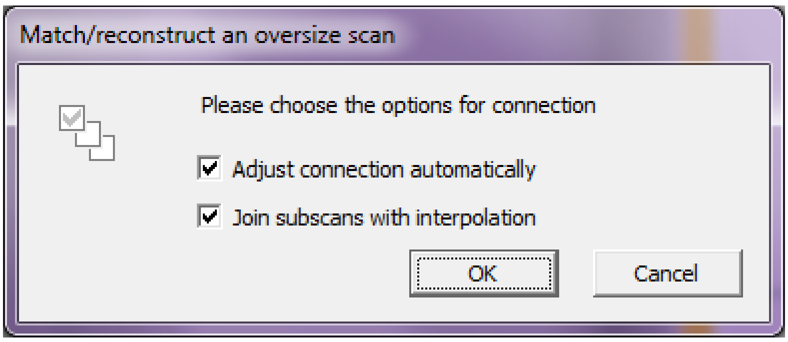
On opening the 5-part oversize scan in NRecon for reconstruction, all five scan parts will be loaded sequentially. Once loaded, a composite projection image made up from 5 connected parts is shown in the first, “start” tab:



Reconstruction will not be described in detail here. However, there is a feature in NRecon for automatic connection of the parts of an oversize scan. This is launched from the 'Actions" menu of NRecon:




Clicking on this item, the following small dialog will open with two tick-box options:

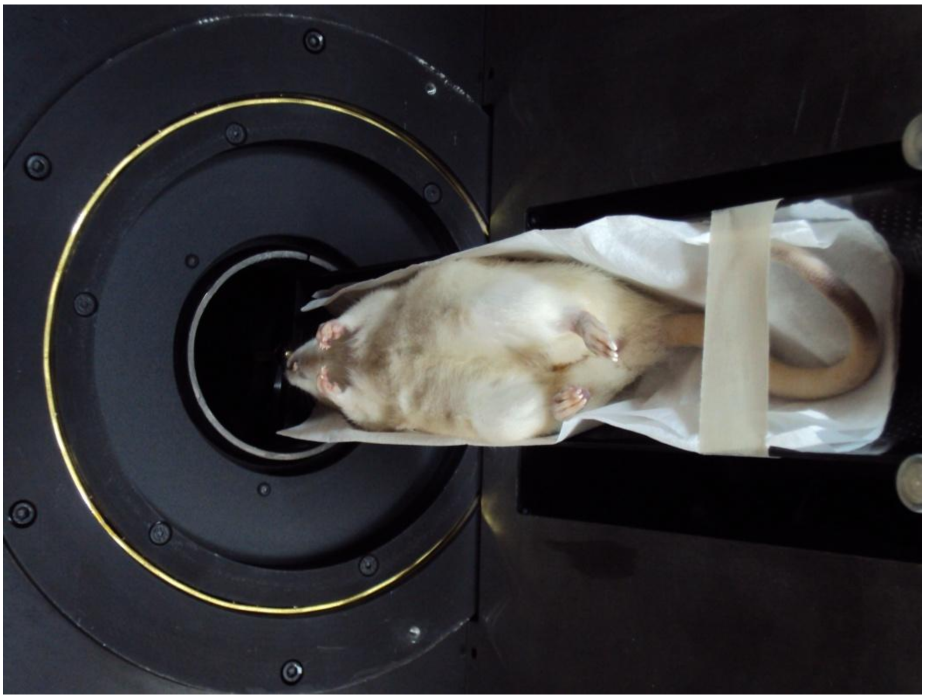



Select both "adjust connection automatically" and "join sub-scans with interpolation". This will result in a smooth reconstruction of the multi-part dataset with no discontinuities at the scan borders.

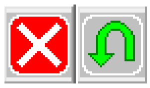
## 4.9.4. Summary description of an "offset" scan of a wide object such as a rat

While the term "oversize" scan described above refers to scans of objects longer than the FOV in the vertical direction, the "offset" scan is for objects wider than the FOV in the horizontal direction.
The field of view (FOV) with the standard single camera image is 35 mm. An extended FOV width of 68 mm is achieved by an offset scan which takes right and left hand images and connects them together for each rotation step. This for example allows the scanning of the rat body which is typically 4-6 cm in diameter.

Mount the rat in the larger of the carbon fiber animal beds in the SkyScan 1176 scanner:

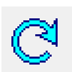


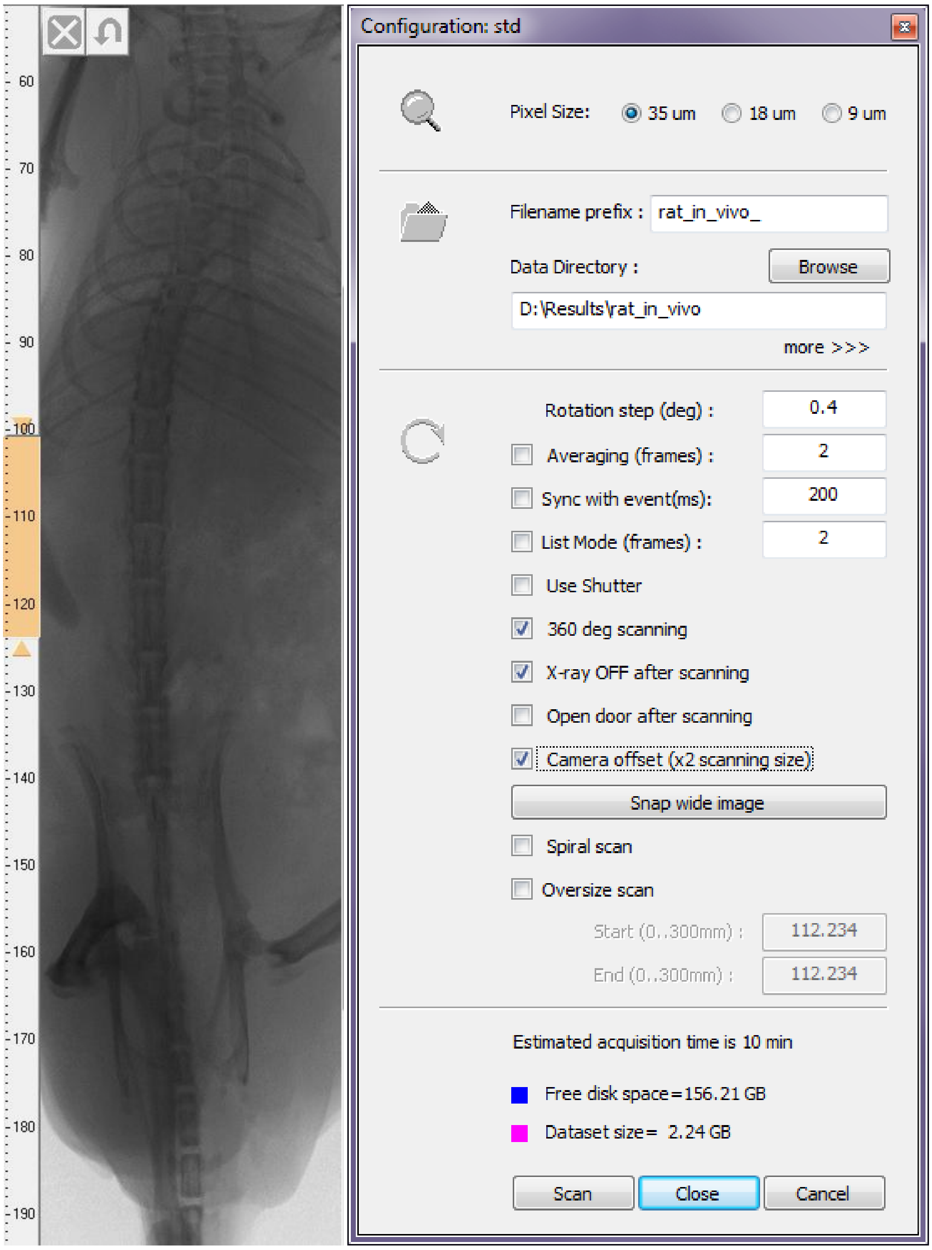
To start the scan, run the scout scan as usual from the green button.



Note that the scout view only operates with the camera in the standard, central position: so with a rat in the scanner, the scout view will not view the full width of the animal (see image below). However the region to be scanned can be selected by double-clicking in the usual way.

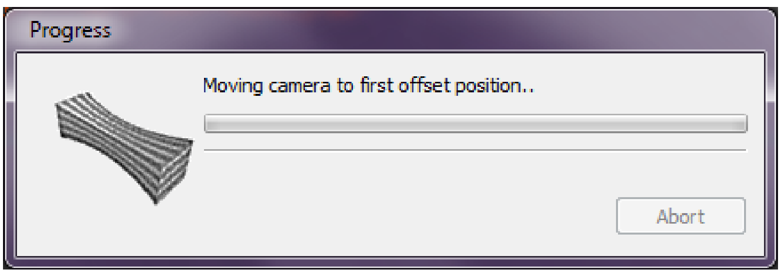
Next launch the scan dialog with this button:



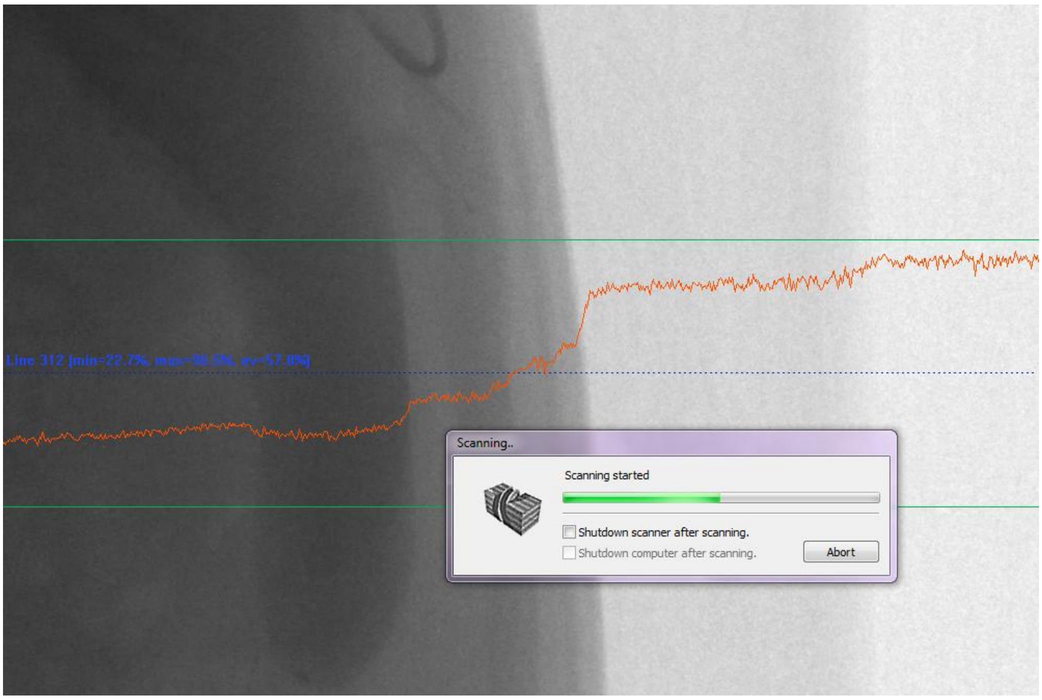



In the scan dialog box, choose the item "camera offset (2x scanning size)". On selecting this option, a new button will appear immediately below entitled "snap wide image". This gives you the option of seeing a full width left-right connected image at any location in the scout view – selected by double clicking in the scout view window as usual.

When the scan starts, the camera moves to one of the offset positions:



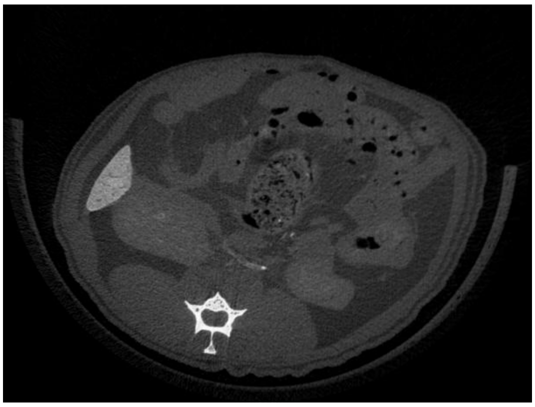
In the image onscreen you will see one side of the rat being imaged:




Note that the camera does not move left to right at each projection step (this would be too slow); instead the full scan rotation is carried out at one offset position, then the camera moves to the other offset and the rotation is repeated. At the end of the scan a dialog will appear showing that the computer is connecting the right and left images at all the rotation steps.

Note that an offset scan will take twice as long as a corresponding standard scan in the central camera position only. For this reason it is probably most practical to scan a rat torso at the fastest 35 micron resolution scanning mode. Remember also that a 35 micron scan in offset mode will have a projection image width close to 2000 pixels, not 1000 as in a standard 35 micron pixel scan.

Loading an offset scan dataset into NRecon and doing reconstruction are however no different from reconstructing a single scan – except for the increased pixel width of the images. An example of a reconstructed offset scan of a rat abdomen is shown below:



# 5. CONTROL PROGRAM MENU FUNCTIONS

## 5.1. General layout

SKYSCAN 1176 MAIN MENU

- Actions menu
- Options menu
- Help

The control program contains a central image area, a main menu at the top, a toolbar for quick access to the most useful functions, and a status bar at the bottom. The main menu gives access to all system functions.

There are three submenus available: Actions menu, Options menu and Help.

Some functions in the submenus are protected. To get access to the protected functions press Ctrl+Alt+Shift+s on the keyboard. The same combination of keys pressed again will restore the access protection if necessary.

The title bar at the top of the control program window contains several buttons:

TITLE BAR

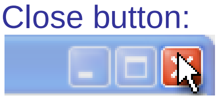
The title bar is located along the top of a window. It contains the name of the application and several buttons.


To move the window, drag the title bar. Note: You can also move dialog boxes by dragging their title bars.

Maximise button:

Minimize button:

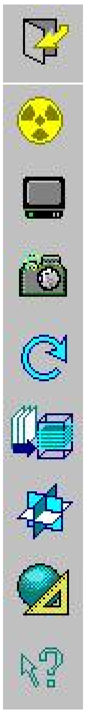
Close button:



5.2. Toolbar

## TOOLBAR

The toolbar is displayed across the top of the application window, below the menu bar. The toolbar provides quick mouse access to many functions of the Control program and also other programs.

- Open specimen chamber.
- On / OFF X-ray source.
- Live acquisition mode.
- Snap single image.
- Start scanning
- Start reconstruction program.
- Start result visualization program
- Start analysis+ realistic 3D visualization.
- Context help.

# 5.3. System group

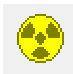
## SYSTEM GROUP


The system group in the toolbar contains several buttons:

**Door Open/Close button**: Use this command to open or close specimen chamber door to place or remove a specimen. If X-ray source emits when specimen chamber should be opened, it will be stopped before opening the door.

The specimen chamber can be opened automatically in the end of scanning cycle in the case of corresponding selection in the Acquisition settings dialog.

In case of stopping scan from touch display, door will be opened and sample will be moved out of the system. In case of pushing start scan button from the touch display, door will be closed and sample will be moved to the last scanning position.



X-ray ON/OFF button: switches the X-ray source on or off. In case of starting the source more than two hours after the last stop, the aging procedure with gradual increasing of voltage and current will be started first. If this X-ray button is pushed, the specimen chamber is locked, if not – the specimen chamber is unlocked and you can change the specimen.

# 5.4. Image group

IMAGE GROUP


The image group in the toolbar contains several buttons:

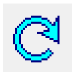
**Video button:** start / stop continuous image acquisition from the X-ray camera. Starting the image acquisition will automatically start the X-ray source (if it is not started yet).

**Single image button**: activates the single-shot acquisition from the X-ray camera. It will also activate the X-ray source (if it is not started yet).

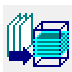
# 5.5. Scan group

SCAN GROUP


The scan group in the toolbar contains two buttons:



Scanning button: starts the acquisition of angular projection images for subsequent reconstruction.

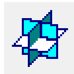


Reconstruction button: starts the reconstruction process to create a set of virtual cross section slices through the sample on the basis of the acquired angular views.

# 5.6. Results group

RESULTS GROUP


The results group in the toolbar contains two buttons:



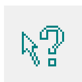
Show results by “DataViewer”: starts the visualization program to see the reconstructed slices. Results can be shown as a slice-by-slice movie or in the form of three orthogonal slices crossing at any selected point inside the reconstructed space.

Analysis and rendering: starts the 2D/3D analysis program “CTAn” to obtain numerical data from the reconstructed internal microstructure. CTAn is connected to the program “CTvol” for rendering and realistic 3D visualization of the object’s internal microstructure.

# 5.7. Help group

HELP GROUP


The help group in the toolbar contains only one button for context help:



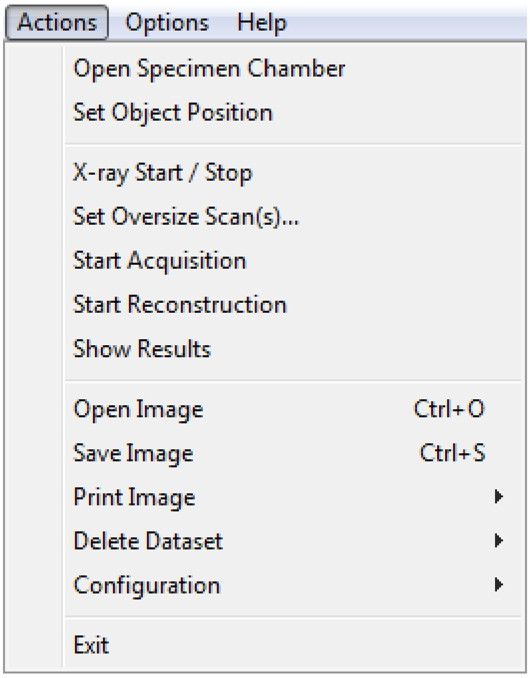
Help button: gives access to contextual help on all items in the menu, toolbar and status bar.

Use the Context Help command to obtain help on any item in the control program. When you choose the Toolbar's Context Help button, the mouse pointer will change to an arrow and question mark. Then click on an object in the program window, such as another Toolbar button.  The Help topic will be shown for the item you clicked.

# 5.8. Actions menu

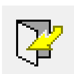
ACTIONS MENU

The Actions menu offers the following commands:




## 5.8.1. Actions menu: Open specimen chamber

OPEN / CLOSE SPECIMEN CHAMBER



Use this command to open or close specimen chamber door to place or remove a specimen. If X-ray source emits when specimen chamber should be opened, it will be stopped before opening the door.

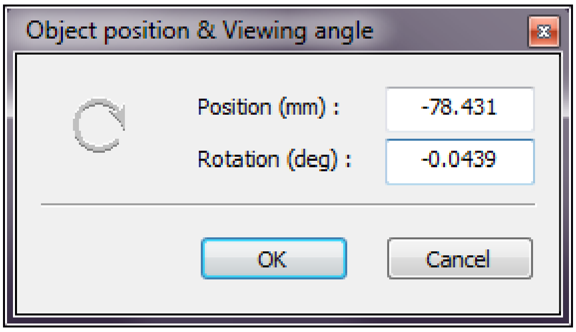
Specimen chamber can be opened automatically in the end of scanning cycle in the case of corresponding selection in the Acquisition settings dialog.

In case of stopping scan from touch display, door will be opened and sample will be moved out of the system. In case of pushing start scan button from the touch display, door will be closed and sample will be moved to the last scanning position.

## 5.8.2. Actions menu: Set object position

SET OBJECT POSITION

Selection in menu “Actions” >>> “Set Object Position” opens following dialog:



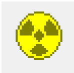

The first parameter defines vertical position of the object in mm (0 corresponds to the bottom position).

The second parameter selects angular position of the object in degrees.

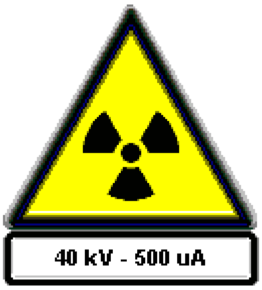
Pressing “OK” button will apply all changed values together by corresponding movement of the object inside the scanner.

## 5.8.3. Actions menu: X-ray start / stop

X-RAY START / STOP



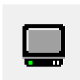
Use this command to switch on or off the x-ray source. After more than 100 hours interrupt in the source emission the aging procedure with gradual increasing of voltage and current will be started first. And following indication will start on the screen:




Using this command once more x-ray emission can be stopped

To open specimen chamber not necessary to push the X-RAY ON/OFF button to stop the emission: the X-ray source will stopped automatically after pressing SPECINEN CHAMBER button:

After closing specimen chamber the x-ray source can be started by pushing X-RAY ON/OFF button or it will be started automatically after selection for any imaging mode:

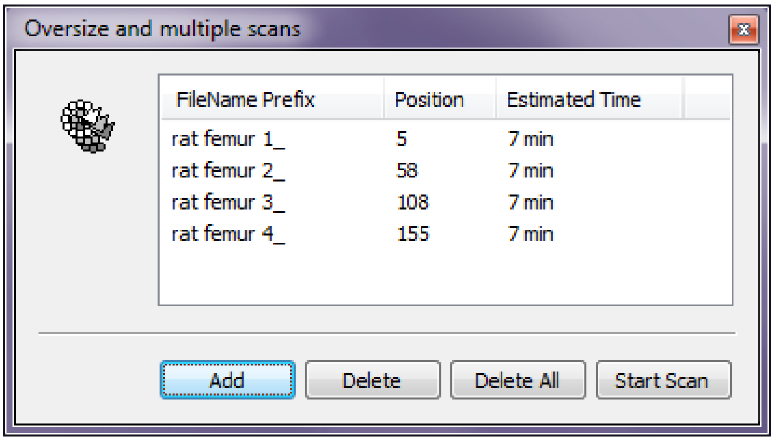
 or 

In the closing program x-ray source will stopped automatically. It can take certain time. Don’t switch off computer before X-ray source will be stopped and control program will be properly closed.

## 5.8.4. Actions menu: Set oversize scan(s)

BATCH SCANNING

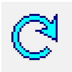
Selection in menu “Actions” >>> “Set Oversize Scan(s)” opens following dialog:



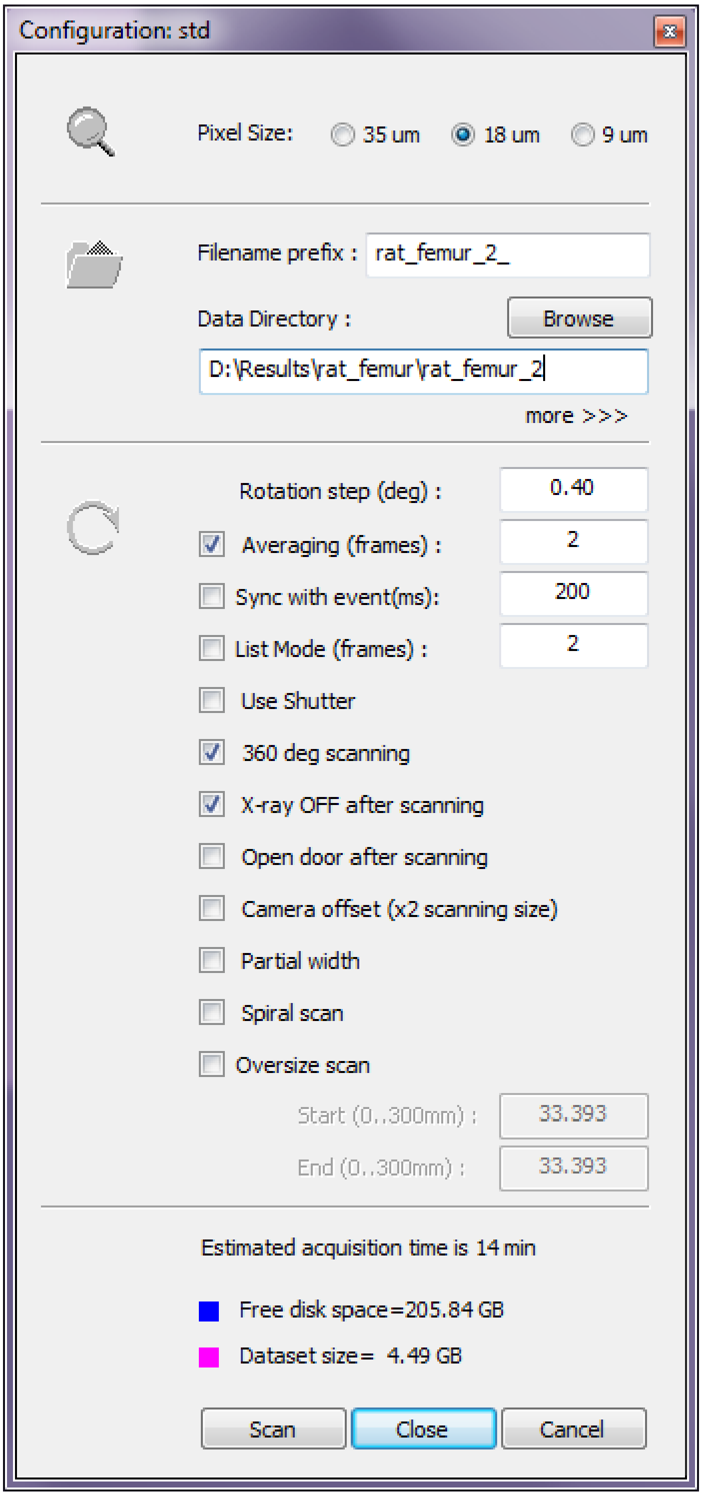

Multiple and oversize scanning is possible by position the sample and then adding the batch entry using above dialog.

## 5.8.5. Actions menu: Start acquisition

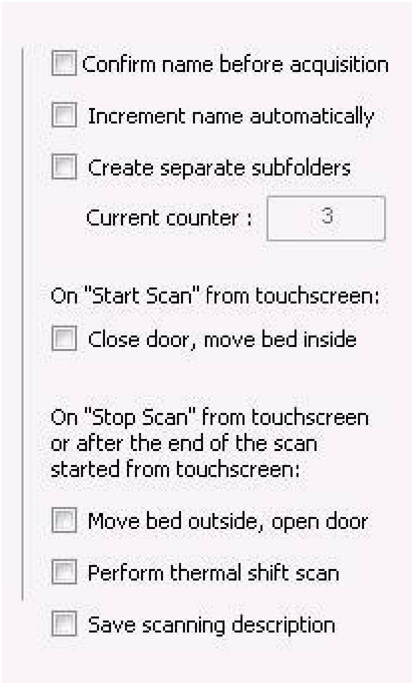
### ACQUISITION SETTINGS



Acquisition settings dialog can be opened through menu “Options” >>> “Acquisition” and also will be shown every time in the beginning of acquisition.



To get access to the advanced features, one can click on the **more>>>** link above.




The first section of the dialog allows adjusting camera resolution different from the previously used one. Numerical values in the end of every line show pixel size for possible camera resolutions.
Next section can be used for selection prefix name for the projection files (during acquisition the program will added four digit number after selected prefix according to number of projection and will save all projection images as 16-bit TIFF files). There is also indication for selected Data directory, which can be changed by  Browse button. Browse button will open the dialog for Data folder selection:

You can click to existing folder in the shown tree or create new one using Make New Folder button. The new folder will be created in the chosen place of the tree and selected as a current data folder for projection files.

The next section of the Acquisition dialog defines acquisition parameters and actions during and after acquisition.

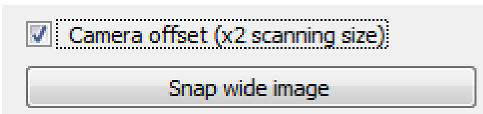
**Rotation step** is originally defined in the “Options” >>> “Scanner Setup” and can be corrected before any particular scan.

**Averaging** (in number of frames with exposure defined in “Options” >>> “Scanner Setup”) allows to improve image quality by averaging of several images in every angular position. Increasing of the number of frames increase quality, but make acquisition longer.

**Use 360 degrees rotation** can reduce asymmetrical artefacts from the dense objects across the surrounded low-absorption materials. This mode will increase acquisition time near twice.

**X-Ray OFF after scanning** defines that X-ray source will be switched off automatically after the end of the acquisition cycle. Open specimen after scanning option will open specimen chamber door after the end of the scanning cycle.

**Camera offset mode** allows to increase horizontal field of view twice by scanning object in two acquisition cycles with offset camera positions. It will take twice scanning time and increase projection file sizes two times. After two acquisition cycles with camera offset in different directions the projection images will be created by combining all pairs of the images from identical projection angles to the wide projection images for following reconstruction. In the case of Camera offset selection, you can check that an object will fit current field of view by  pressing Snap wide image button, which will appear under Camera offset checkbox.




If Camera offset is not selected, next item in the dialog allows to acquire dataset from the central part of the image with adjustable width by selecting Partial width checkbox and adjusting width by the corresponding slider. Scanning area will be marked in the image.

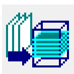

In the case of the scanning with limited width, the rotation step size will be correspondingly corrected to get shorter acquisition cycle without reducing the quality.

The last section of the Acquisition dialog shows estimation for the scanning time, available and needed disk space. In the extended dialog you see some more options.

First part of the extended dialog allows create/increment folder and filename prefix automatically in a sequential manner before start of each scan. Next part gives option to control scanning from the touch display. In case of start of scanning from a command through touch display, door will be closed, sample will be moved to the last scanning position. On the other hand, in case of stopping a scan from touch display, will stop the X-ray, open the door and move the sample to out of the system. The last option if selected save scanning description will append annotation data, input in the Annotation dialog, to the scanning log file and also to the projection files.

### 5.8.6. Actions menu: Start reconstruction

START RECONSTRUCTION



Selection of the menu item "Actions" >>> "Start Reconstruction" or pushing the corresponding button in the toolbar starts the reconstruction program ("NRecon") to create virtual slices from the acquired angular projections.

Select the dataset for reconstruction from those available in the current data directory in the subsequent dialog. Then the control program will start the reconstruction program for the selected dataset.

The reconstruction program works completely independently. After starting the reconstruction you can continue using the control program to scan another sample or for other operations with the scanner.

## 5.8.7. Actions menu: Show results

SHOW RESULTS

Selection in menu “Actions” >>> “Show Results” or clicking to the button in the toolbar will open selection of available reconstruction results in the current data directory and will start an external program “Data Viewer” for visualization of reconstructed results, animation and distance / density profile measurements.

This program also allows cutting the reconstructed set of results in alternative directions. You will see three orthogonal sections through the reconstructed dataset with interactive selection of the viewing point inside three dimensional reconstructed space.

## 5.8.8. Actions menu: Open image

OPEN IMAGE

(Ctrl + O)

Use this command to open an existing image file from the disk. The Open dialog will appear on the screen:

The program can accept bitmap files (such as reconstructed cross sections) or 16-bit TIFF files (such as angular views) according to the type selection in the bottom area of the Open dialog.

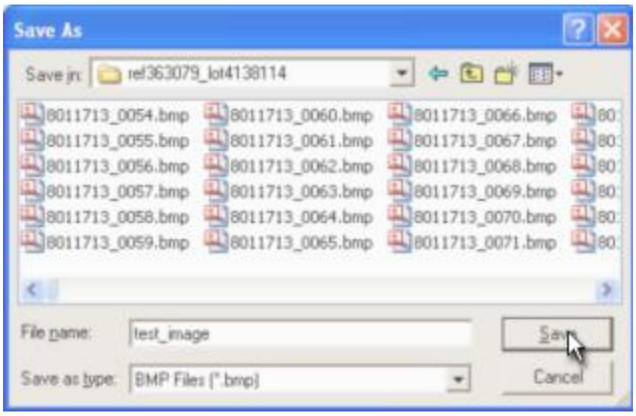
The reconstruction program works completely independently. After starting the reconstruction you can continue using the control program to scan another sample or for other operations with the scanner.

## 5.8.9. Actions menu: Save image

**SAVE IMAGE**

(Ctrl. + S)

Use this command to save the current image to disk. A Save As dialog will appear on the screen:




An image can be saved as bitmap file or 16-bit TIFF file according to the type selection in the bottom area of the Save As dialog.

## 5.8.10. Actions menu: Print image

**PRINT IMAGE**

(Ctrl. + P)

Use this command to print the current image. This command presents a Print dialog box, where you may specify the number of copies, the destination printer, and other printer setup options.

## 5.8.11. Actions menu: Delete dataset

**DELETE DATASET**

Use this function to delete all reconstructed slices in (bitmap, tif, jpeg) format from the dataset with the selected prefix. Most information files associated with the dataset will also be removed.

## 5.8.12. Actions menu: configuration

| CONFIGURATION: SAVE / LOAD / DELETE | Use this command to save, load or delete your scanning configuration. A configuration includes scanner exposure settings for all camera positions / filters / resolution combinations, settings for voltage and current for x-ray source, rotation step sizes and all reference maps for flat-field correction in all acquisition modes.<br><br>Pay attention that saving a configuration requires approximately 755MB of the disk space. To save disk space delete all unnecessary configurations. |
|---|---|

## 5.8.13. Actions menu: Exit

EXIT

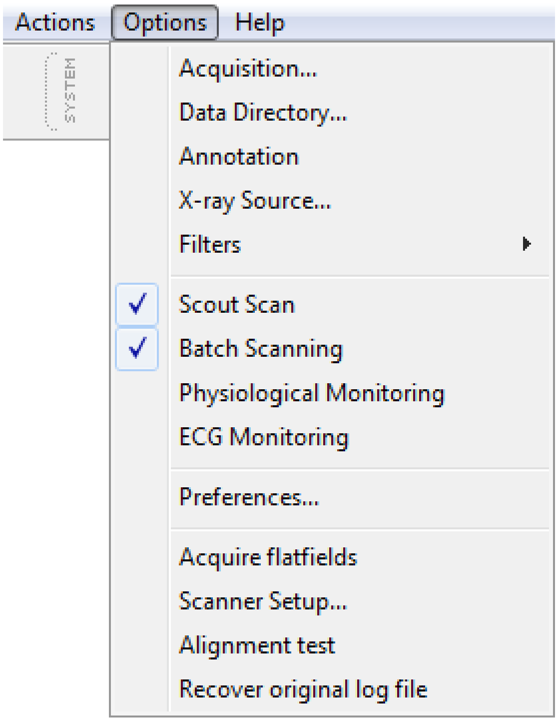
Use this command to exit the Control program. The program will show a waiting message until the scanner is deactivated.
You can also close the program by pressing the exit button in the title bar:

You can also close the program by double-click on the program icon in the title bar.

# 5.9. The Options Menu

## OPTIONS MENU

The options menu offers the following commands:



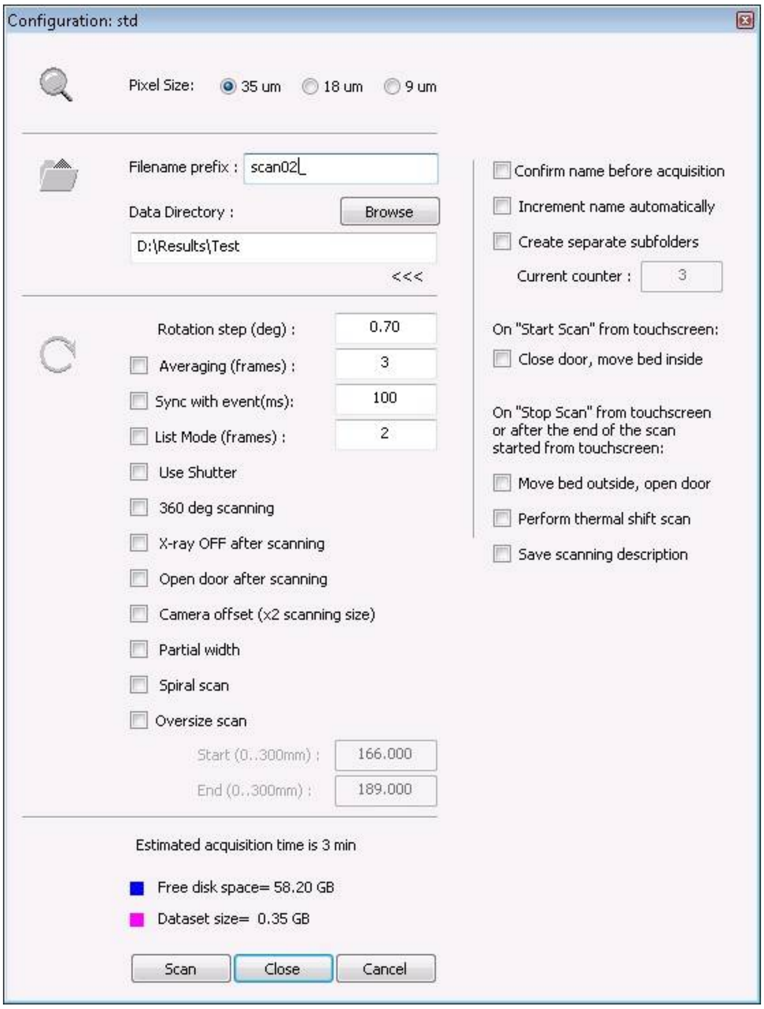

The items in the options menu are listed and described below:

## 5.9.1. Options menu: Acquisition

OPTIONS MENU: ACQUISITION

Acquisition settings dialog can be opened through menu “Options” >>> “Acquisition” and also will be shown every time in the beginning of acquisition.

To get access to the advanced features, one can click on the more>>>link above.

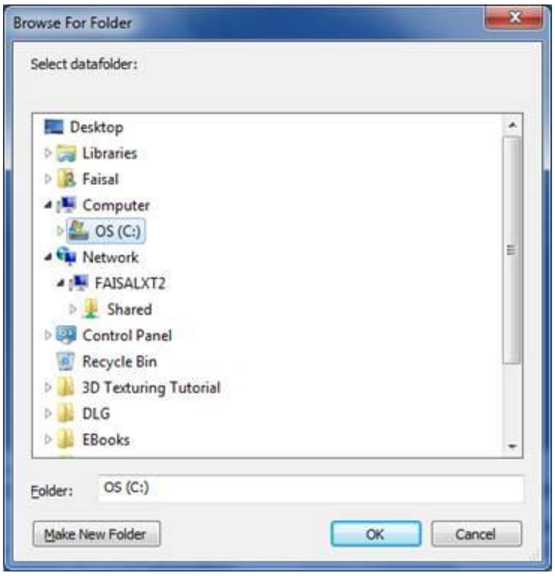



This window has been described above (section 4.9, 5.8.4)

## 5.9.2. Options menu: Data directory

OPTIONS MENU
DATA
DIRECTORY

Data directory selection dialog can be opened through menu “Options” >>> “Data Directory” menu item. It will open following dialog for Data folder selection:

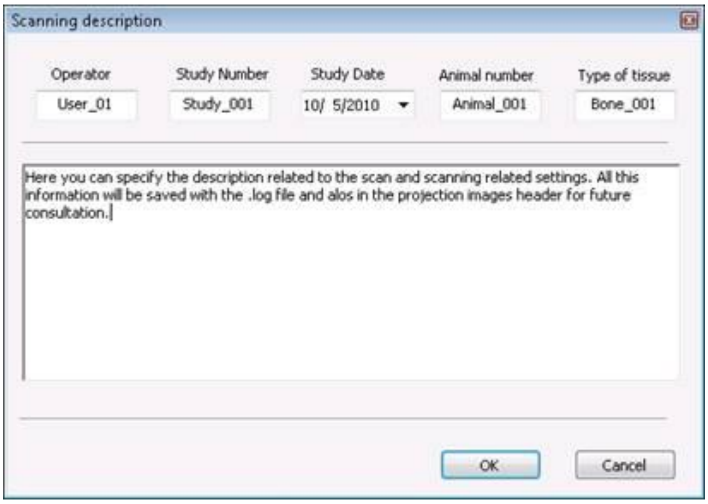



You can click to existing folder in the shown tree or create new one using Make New Folder button. The new folder will be created in the chosen place of the tree and selected as a current data folder for projection files.

### 5.9.3. Options menu: Annotation

OPTIONS MENU: ANNOTATION

"Option>>Annotation" command shows a dialog to input scanning related entries namely Operator name, Study number, study date, Animal number , type of tissue and free text input.



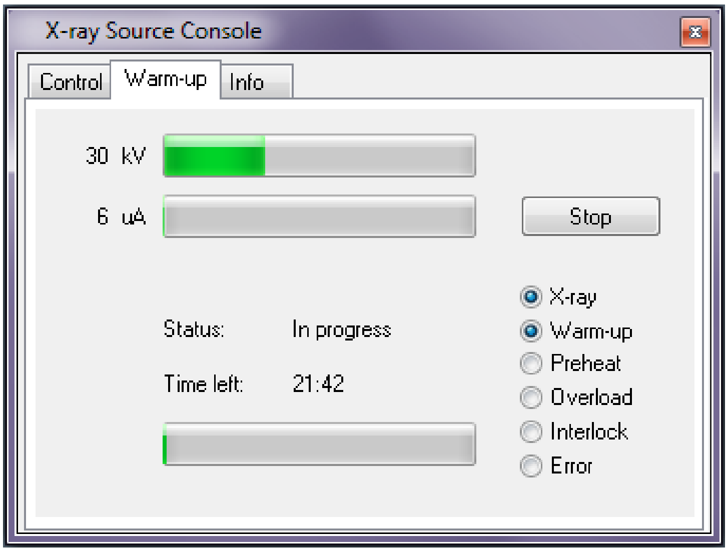

If selected in the acquisition dialog, this information will be appended to the scanning log files and also to the projection images.

## 5.9.4. Options menu: X-ray source

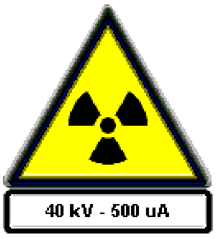
OPTIONS MENU: X-RAY SOURCE

This command can be selected from menu “Options” >>> “X-Ray source” or by double click to the X-Ray sign (if X-Ray source emits). Following dialogs will appear on to the screen:

(1) During ageing (conditioning) which occurs at the first source start-up every 3 days:




(2) After all other source start-ups:



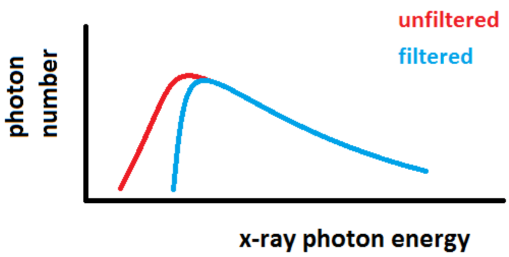

The operation and management of the X-ray source is described in section 4.5.1.

## 5.9.5. Options menu: Filters

OPTIONS MENU: FILTERS

The selected filter will be placed in the front of the camera. It will allow changing camera sensitivity for X-ray radiation with different energies presents in polychromatic X-ray beam from the source. The "no filter" position is typically used for objects with low x-ray absorption (small samples or materials with low average atomic number in composition).

Different metal filters can be used to cut low-energy x-ray radiation to minimise nonlinear x-ray absorption in dense samples named "beam hardening". This phenomenon is connected to the fact that surface layer of the dense samples works as an x-ray filter for the rest part of the sample. Soft (low-energy) x-ray radiation absorbs in the surface layer and remaining high-energy radiation passes through the internal parts of the sample with reduced absorption. This reduces the available information on x-ray absorption from the middle of the dense sample and distorts the reconstructed density distribution – the surface appears more dense than it should be and internal parts less dense.



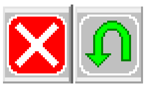

Metal filters in the front of the camera cut out the soft radiation and transmit only the higher energy part, which is absorbed more uniformly in the surface and inside the objects. On the other hand filtering out part of the x-ray radiation reduces the number of the detected photons and is compensated in the scanner settings by longer exposure time and a correspondingly longer scan time.

Operationally there are three ways to select filter: (1) here in the options / filter menu item; (2) clicking on the bottom control bar item for filters, and (3) pressing the filter buttons on the touch screen on the side of the 1176 scanner.

## 5.9.6. Options menu: Scout scan

OPTIONS MENU: SCOUT SCAN

This command shows/hides scout view.

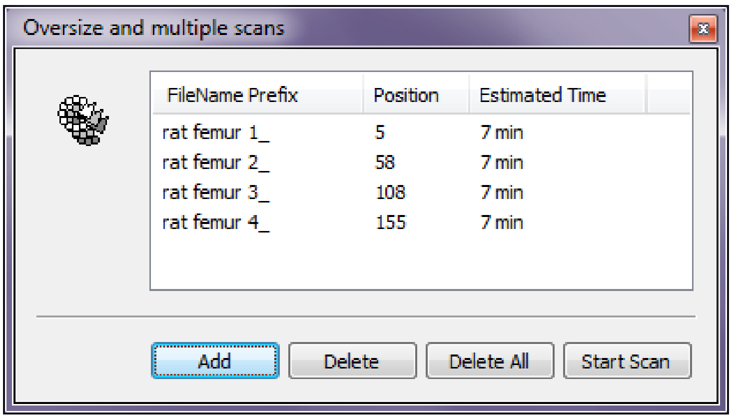


By clicking one of these command buttons one can redo scout view or can close the view.

## 5.9.7. Options menu: Batch scanning

OPTIONS MENU: BATCH SCANNING

This command shows/hides Batch window to setup multiple scans.




Add button opens up Acquisition dialog to setup scanning parameters.

Delete button deletes/removes the selected batch job from the batch list.

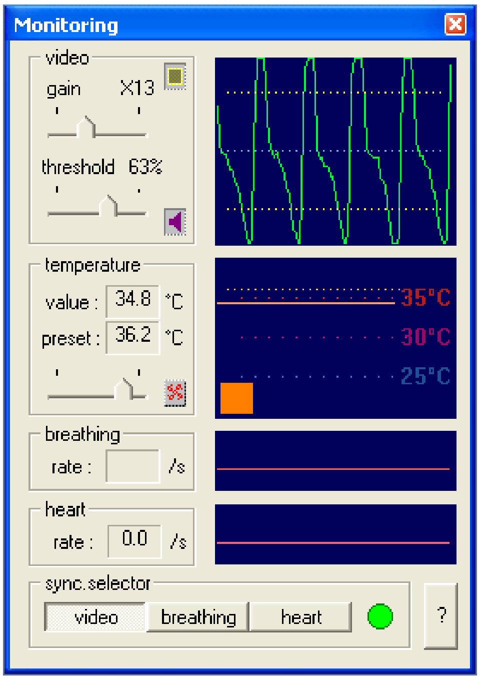
Delete All will clear all the batch entries.
Start Scan command starts scanning in a sequential orderly manner.

It is possible to have scans of all different possible scanning parameters.

## 5.9.8. Options menu: Physiological monitoring

OPTIONS MENU: PHYSIOLOGICAL MONITORING

The physiological monitoring subsystem contains several sensors on the animal bed, with electronics and a software package for imaging, real-time analysis and scan gating. A miniature color USB-camera is mounted on the animal bed and equipped with white LED illumination to produce a real-time image of the animal during the scan.





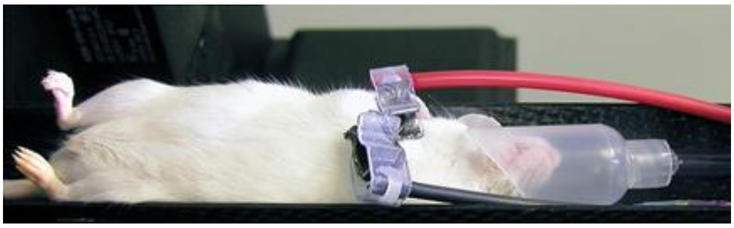
The software analyses the video stream from a user-selected area of the image, which the operator can position on a part of the animal body where breathing movement is visible. These movements are detected and converted into a movement waveform to provide a monitor of breathing and a gating signal for synchronized scanning.

The operation of physiological monitoring, and its use for instance in breathing monitoring and animal temperature stabilisation, is described in section 4.9.2.

### 5.9.9. Options menu: ECG monitoring (optional)

OPTIONS MENU: ECG MONITORING

The ECG monitoring system consists of a sensitive amplifier and specially developed ECG electrodes and wiring, which contain no metal parts to avoid artefacts in the reconstructed slices. The electrodes use advanced carbon-fiber conductive parts with x-ray absorption similar to the animal body.

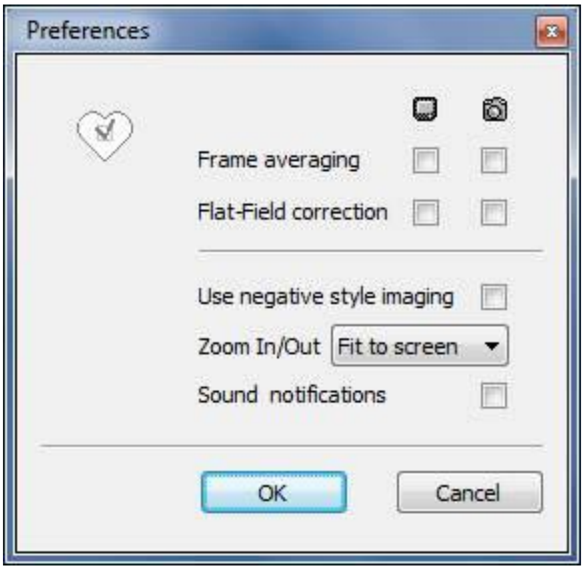


The ECG signal can be used for scan gating and can be seen on screen in real time. Special program can acquire analogue signal through sound card of the computer and show ECG curve in real time on the screen.

## 5.9.10. Options menu: preferences

OPTIONS MENU: PREFERENCES

Selection in menu “Options” >>> “Preferences” opens following dialog:




In the top part of dialog you can select preferences for imaging in continuous acquisition (“Grab”) and single shot (“Snap”) modes. Frame averaging improves image quality, but can delay reaction for the operator actions in the Grab mode. Flat-Field correction compensates spatial irregularities in x-ray emission and camera sensitivity.

**Note**: normally flat field should be selected for all scanning. Temporarily de-selecting flat field, for either image mode (video or snap) allows checking of the intensity level detected by the camera in the uncorrected image; the average intensity should be in the range 60-75% (adjust exposure time if necessary to achieve the correct intensity). Once exposure time is corrected and the flat field taken, re-select the flat field in preferences.

The second part of the dialog allows selecting specific actions and visualization options for control program. Sound notification will add playing sounds in the beginning and the end of long-term processes, such as dataset acquisition. Rescale camera images trying to keep the same image size in the screen for different image formats from the camera.

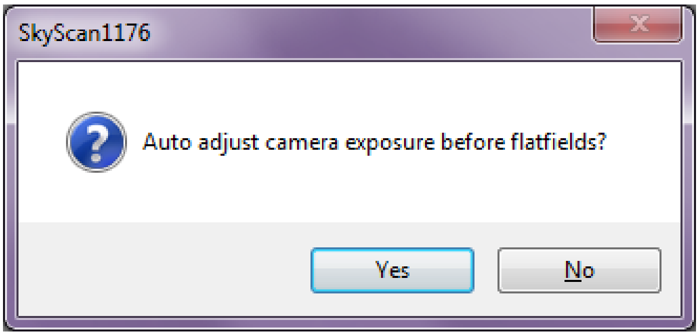
Otherwise the images will be shown in real size with possibility for scrolling across big-format images by scrollbars. "Negative" style for images inverts images in the screen to show bright objects on the black background.

New preferences will be applied after pressing "OK" button.

### 5.9.11. Options menu: acquire flat fields

OPTIONS MENU: ACQUIRE FLAT FIELDS

The "Options>>Acquire flat-fields" command acquires bright and dark flat fields for the current scan mode ("scan mode" means the selected resolution level – 9 / 18 / 35 micron – and filter).



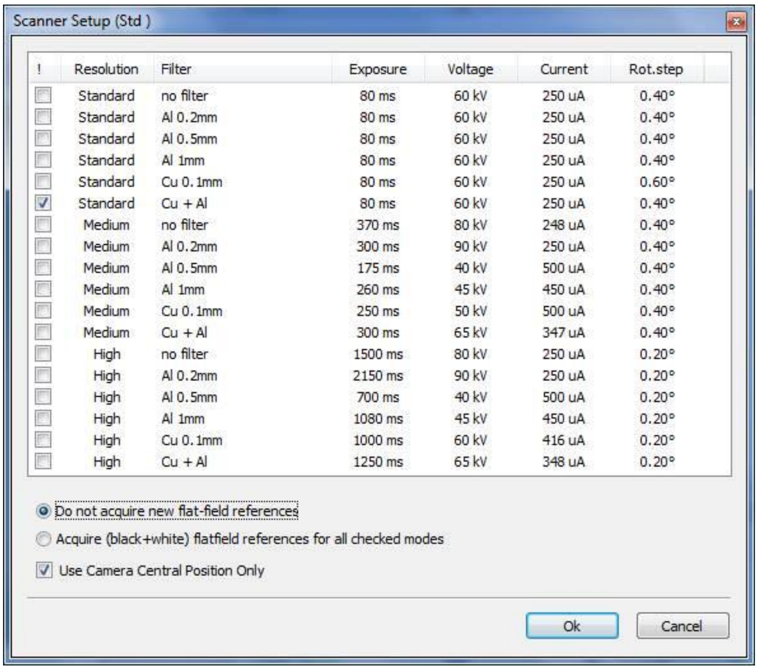

Optionally if selected, camera exposure is adjusted automatically before acquiring flat fields.

## 5.9.12: Options menu: Scanner setup

### OPTIONS MENU: SCANNER SETUP

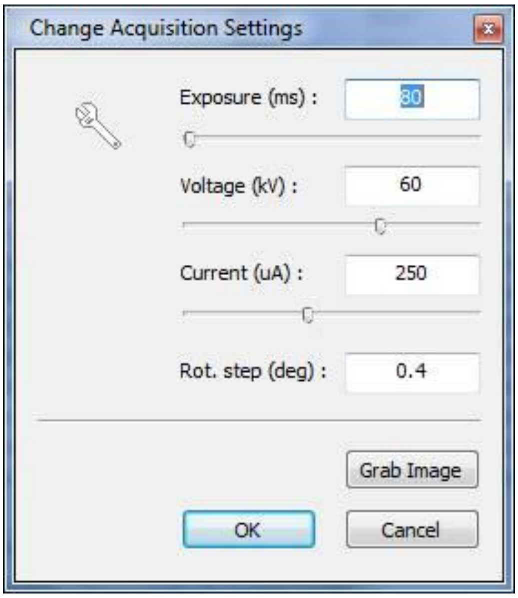
Selection in menu “Options” >>> “Scanner Setup” opens table of “Presets” for exposure times and angular steps for all filters, camera resolutions.

Note: access to this options menu item requires unlocking the security keyboard key (Ctrl+Alt+shift+S). Current acquisition mode is marked in the table:




| ! | Resolution | Filter | Exposure | Voltage | Current | Rot.step |
|---|---|---|---|---|---|---|
| ☐ | Standard | no filter | 80 ms | 60 kV | 250 uA | 0.40° |
| ☐ | Standard | Al 0.2mm | 80 ms | 60 kV | 250 uA | 0.40° |
| ☐ | Standard | Al 0.5mm | 80 ms | 60 kV | 250 uA | 0.40° |
| ☐ | Standard | Al 1mm | 80 ms | 60 kV | 250 uA | 0.40° |
| ☐ | Standard | Cu 0.1mm | 80 ms | 60 kV | 250 uA | 0.60° |
| ☑ | Standard | Cu + Al | 80 ms | 60 kV | 250 uA | 0.40° |
| ☐ | Medium | no filter | 370 ms | 80 kV | 248 uA | 0.40° |
| ☐ | Medium | Al 0.2mm | 300 ms | 90 kV | 250 uA | 0.40° |
| ☐ | Medium | Al 0.5mm | 175 ms | 40 kV | 500 uA | 0.40° |
| ☐ | Medium | Al 1mm | 260 ms | 45 kV | 450 uA | 0.40° |
| ☐ | Medium | Cu 0.1mm | 250 ms | 50 kV | 500 uA | 0.40° |
| ☐ | Medium | Cu + Al | 300 ms | 65 kV | 347 uA | 0.40° |
| ☐ | High | no filter | 1500 ms | 80 kV | 250 uA | 0.20° |
| ☐ | High | Al 0.2mm | 2150 ms | 90 kV | 250 uA | 0.20° |
| ☐ | High | Al 0.5mm | 700 ms | 40 kV | 500 uA | 0.20° |
| ☐ | High | Al 1mm | 1080 ms | 45 kV | 450 uA | 0.20° |
| ☐ | High | Cu 0.1mm | 1000 ms | 60 kV | 416 uA | 0.20° |
| ☐ | High | Cu + Al | 1250 ms | 65 kV | 348 uA | 0.20° |

The column “Resolution” shows three possible binning modes for the camera. The column “Filter” shows six different types of the filters available. By double clicking in any fields will open a new dialog asking for new scanning settings for that particular mode:




At the bottom of the dialog you can select if you want to acquire new flat fields with new settings. If "Use camera Central Position only" checkbox is selected , flat fields will be acquired for central position only (not for offset scan positions).

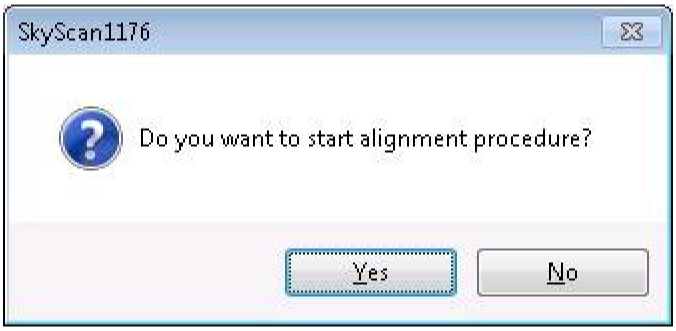
## 5.9.13: Options menu: Alignment test

OPTIONS MENU: ALIGNMENT TEST

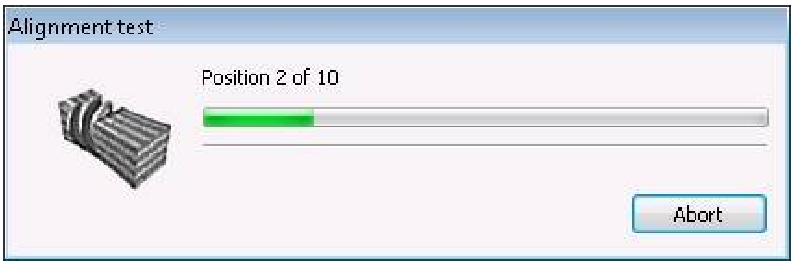
Use this command to check misalignment and compensate it by corresponding movement of the camera stage.

For proper tomographic reconstruction the emission point inside the source, the object rotation axis and the middle column of the camera image should be placed in one straight line parallel to the object stage movement during magnification adjustment. To compensate possible misalignment, the object stage and x-ray camera stage can move those two parts to the straight line along object movement trajectory. To check misalignment put as an object any thin cylindrical wire with significant x-ray absorption (Cu or similar, but not Al). Before starting misalignment test check that your object will stay inside field of view for all angular positions during 360 degrees rotation.

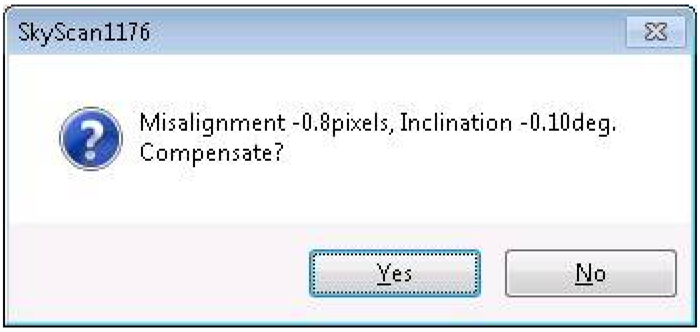
Alignment command will open following dialog:




After confirmation, you progress dialog will show the current status:




During misalignment calculation the software also measure angular misalignment between vertical columns of the camera image and object rotation axes. In the case of selection in corresponding checkbox in the dialog window with results of calculations, angular compensation will be stored and applied to all images during scanning.



# 5.10. The Help menu

## 5.10.1. The Help Menu

**HELP MENU**

The Help menu contains access to Help Index and to information about SkyScan1176 Control Program. Clicking on this link opens the information shown in the next section (5.10.2) below.

Use Help Index to obtain help on all functions of control program. This command will open Help Finder with possibilities to search by contents or by index. You can  also find specific keywords or phrases in help topics.

## 5.10.2. About SkyScan 1176 Control Program

**ABOUT SKYSCAN 1176 CONTROL PROGRAM**

Use this command to open About box with copyright notice, information on current software version and version of firmware in embedded processors:


By clicking to the line with web-site address you can get direct access to the supplier’s web-site for checking available software updates. Clicking to the line with e-mail address will open your standard e-mail service for sending e-mail to supplier.

# 6. SERVICE AND SUPPORT

The SkyScan 1176 scanner is designed as a maintenance-free instrument and doesn't require any periodical service.

New versions of all software packages that are supplied with your scanner may be available for download in the "Products" -> "Software Updates" section of the SkyScan website:
http://www.skyscan.be/products/downloads.htm.

In the case of any problem please contact your local distributor. A list of distributors is available at:
http://www.skyscan.be/company/distributors.htm.

For all users of micro-CT instruments SkyScan periodically organizes Micro-CT User Meetings. Information on previous Meetings and announcement of forthcoming events can be found at the SkyScan website: www.skyscan.be.

If your control computer is connected to internet, you can get access to the SkyScan website by simple click to the corresponding underlined web-address in the "About…" box in the Help part of the Main Menu.