An introduction to MICRO CT SCAN

1.1 Introduction

The conventional optical or electron microscopes allow to visualise only two-dimensional images of a specimen surface or of thin slices. In most cases a conclusion about the original three-dimensional object structures cannot be made on the base of this two-dimensional information.

One can obtain the three-dimensional information of objects by cutting them into very thin slices. These can then be visualised in the light microscope and the 2-D information can be interpolated into a 3-D structure model.
This method 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.

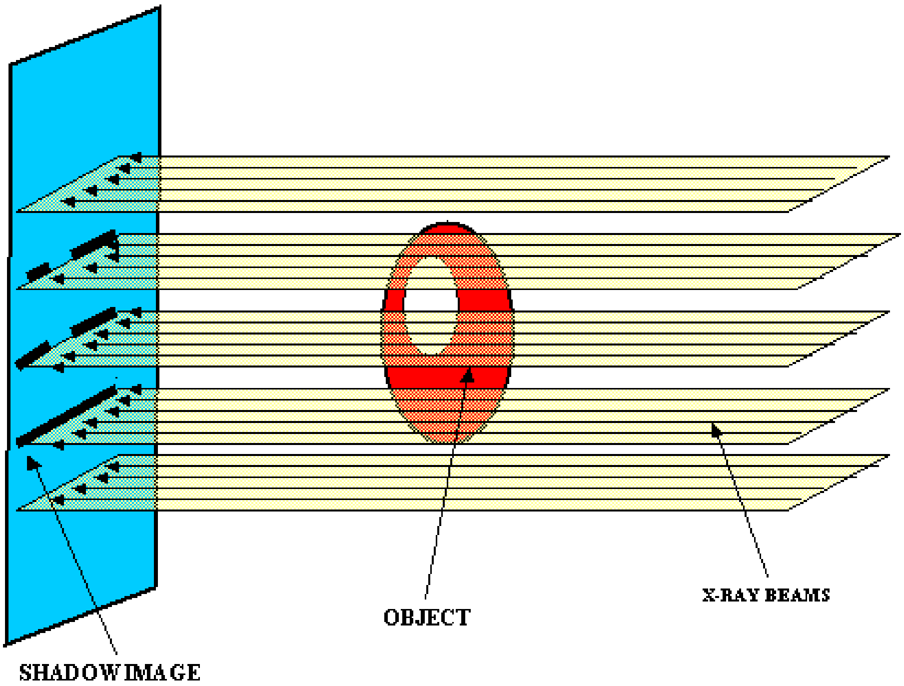
Only an X-ray tomography system allows us to visualise and measure the interior of opaque objects. With X-ray CT we obtain digital information on the 3D-geometries and properties of these objects, without sample preparation or chemical fixation.
Typically, the spatial resolution of conventional medical CT-scanners is in the range of 1-2.5 mm, which corresponds to a 1-10 cubic mm voxel (volume element) size.
Computerised X-ray microscopy and microtomography now gives possibilities to improve the spatial resolution by seven to eight orders in the volume terms.

The system "SkyScan 1076" allows to reach a spatial resolution of 15 µm corresponding to near $3x10^{-6}$ 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 microtomogaphy

“Tomos” is the Greek word for “cut” or “section”, and tomography is a technique for digitally cutting a specimen open using X-rays to reveal its interior details. A CT image is typically called a *slice*, as it corresponds to a slice from a loaf of bread. This analogy is apt, because just as a slice of bread has a thickness, a CT slice corresponds to a certain thickness of the object being scanned. Therefore, whereas a typical digital image is composed of *pixels* (picture elements), a CT slice image is composed of *voxels* (volume elements).

An 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.

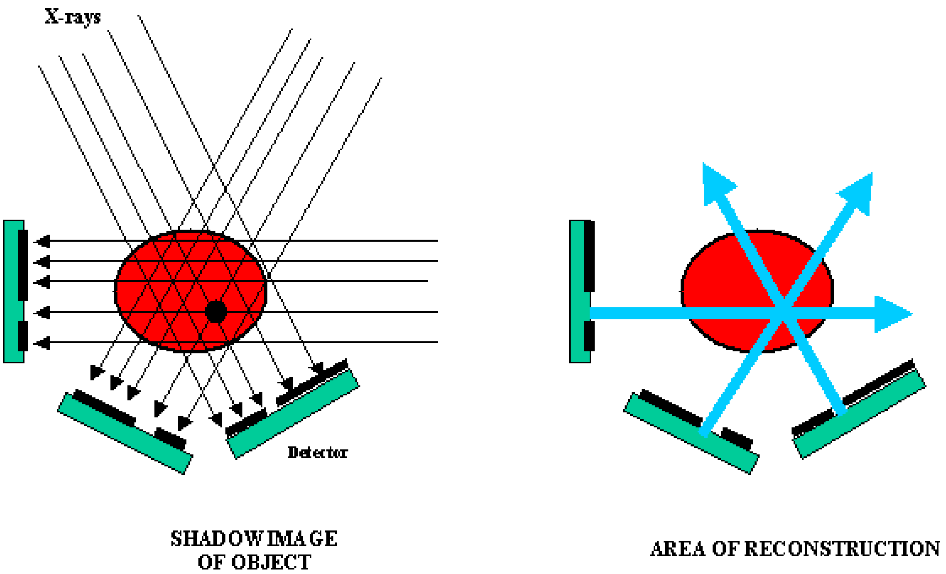


The X-rays that pass through the object they are scattered and/or absorbed
The gray levels in a CT slice correspond to X-ray attenuation, which reflects the proportion of X-rays scattered or absorbed as they pass through each voxel. X-ray attenuation is primarily a function of X-ray energy and the density and atomic number of the material being imaged. A CT image is created by directing X-rays through the slice plane from multiple orientations and measuring their resultant decrease in intensity. A specialized algorithm is then used to reconstruct the distribution of X-ray attenuation in the slice plane. By acquiring a stacked, contiguous series of CT images, data describing an entire volume can be obtained, in much the same way as a loaf of bread can be reconstructed by stacking all of its slices.

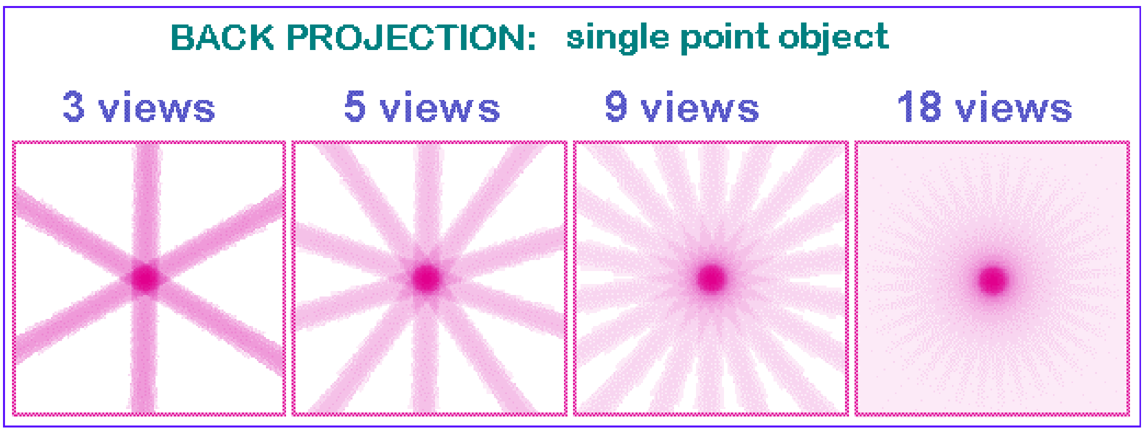
A special algorithm is used to reconstruct the distribution of X-ray attenuation in the slice plane.

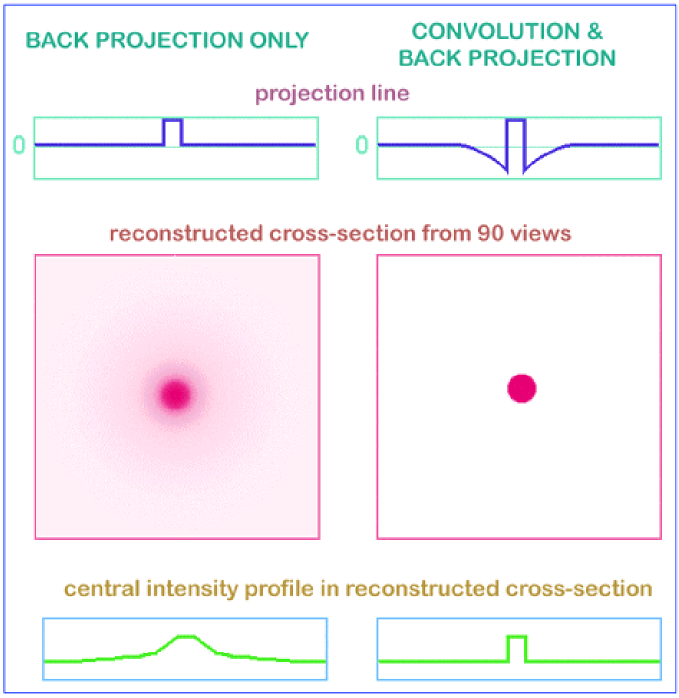
How this reconstruction is done can be shown with a very simple example, namely an object consisting of only one point with significant absorption in an unknown place.
In the one-dimensional shadow line we will have a decrease of intensity of the shadow of absorption in the object area. (see figure on the next page)
In the computer memory we now can initialise 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 in 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 ;
Each new rotation position of the object will add to the area of reconstruction the lines of possible object positions corresponding to the position of the shadow.
This operation is called **"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 becomes more and more defined.

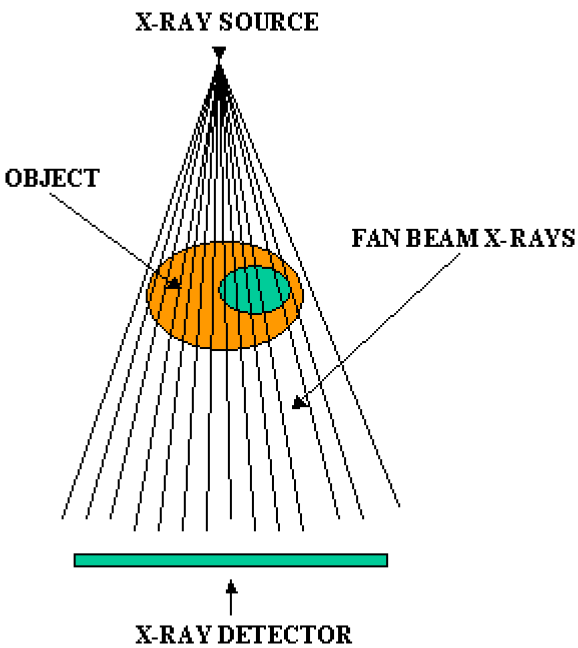




In the case of reconstruction from an infinite number of projections one can get an image with a good definition of the absorption area position inside the initial object.
At the same time a blur area will accompany the pointer image because it is produced as a superposition of lines with all inclinations.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

Any real object can be represented as a big number of separate absorption voxels and linear absorption in any X-ray beam is corresponding to the sum of all absorption from all voxels inside this beam. Thus the same algorithm can be used to produce the cross-section image from a real object.

In this way the two-dimensional cross-sections of the object can be reconstructed from the one-dimensional shadow lines in different views.



Unfortunately X-ray sources do not generate parallel beams.
In a real case, one will use a pointer source and a fan 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 can be used for fan-beam X-ray sources.
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 is

corresponding 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 initial data and 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 correction function for convolution before 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 dangerous for noisy initial information.
Special selection of convolution function for correction with spectral limitation by “Hamming window” allows solving this problem.

In X-ray microtomography information from voxels with very small physical size should be detected and the right choice of parameters for noise reduction becomes very important.

1.3. From reconstruction to image

1.3.1. Acquisition, creation of acquisition data

During the acquisition the source-detector pair will rotate over 180 degrees.
At each position the shadow image or transmission image will be acquired.
Cone beam acquisitions save all of these projection images as 16 bit TIFF 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 TIFF shadow images for the reconstruction. By using the reconstruction algorithm we now generate a raw data reconstructed cross-section.
This is not yet an image, it is a floating 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

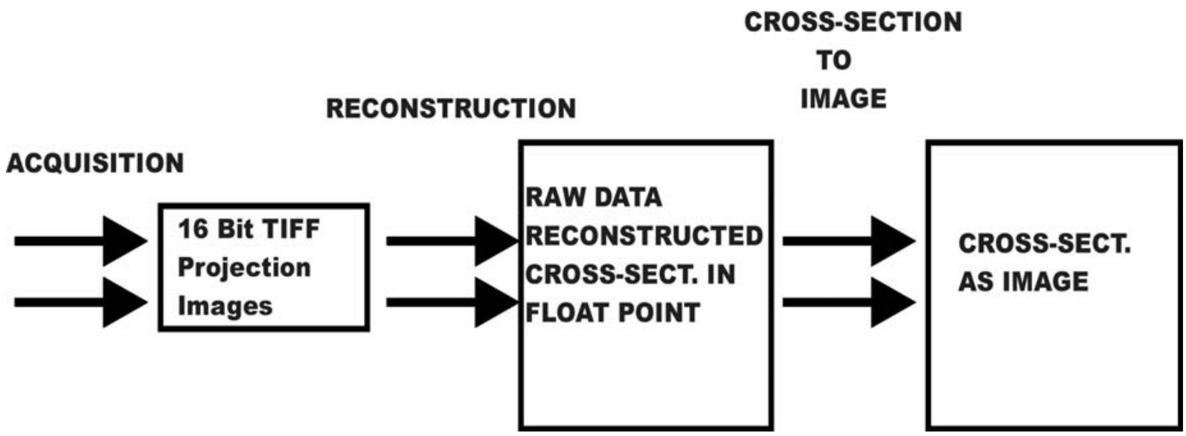
The size of the matrix is equal to the number of pixels inside a cross-section or in a line on the CCD array (n is the number of pixels in a line of the shadow image or the CCD array).
We can save the reconstructed cross-section as a floating point matrix holding the attenuation values after the reconstruction or as explained in the next section transform it into an image with 256 grey values (8bit).

1.3.3. Cross-section to image

After creating the reconstructed cross-section, we can generate an image.
The images are reproduced in 256 gray scales. Therefore, we have to find a way to convert the 12-bits or more information - depending upon the camera - into a gray scale image.
First we select minimum and maximum values. All values between these minimum and maximum will be displayed as half tone. In a normal image, all attenuation values below the minimum will be white and everything above the maximum will be displayed as black.
The reconstructed array will be shown as a half-tone image of the cross- section with linear conversion to 256-grades of gray inside the selected density interval.

The Skyscan systems use a Windows environment and the final images can be exported as BMP, RAW 16bit or TXT -files.

Following image summarizes all actions and steps to generate the cross sectional data.

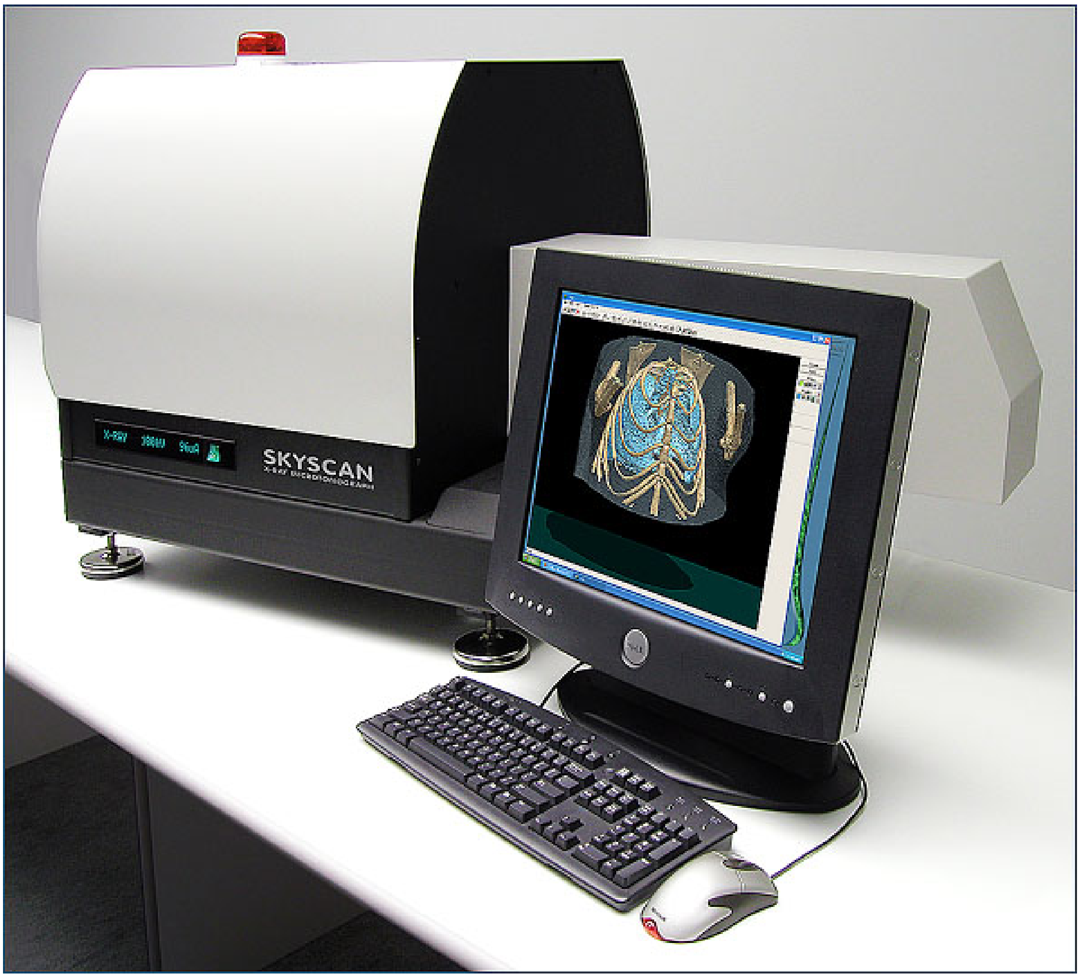


THE SKYSCAN 1076 SYSTEM OVERVIEW

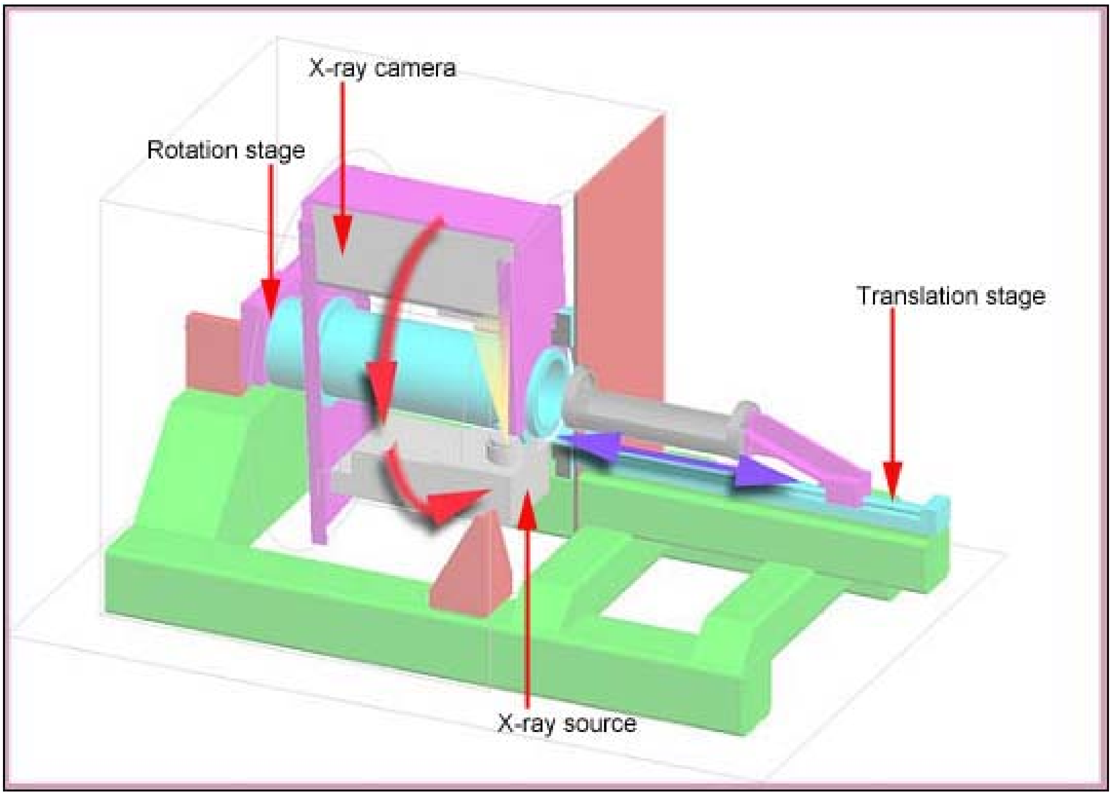
SPECIFICATIONS:

Maximum object size	68mm(D) x 200mm(L) for rats or 35mm(D) x 200mm(L) for mice, 17mm(L) per single scan
X-ray source	20-100kV, 10W, <5um spot size (@4W), >10000h estimated lifetime, air cooled sealed type, 4-positions automatic filter changer for energy selection
X-ray detector	10 Megapixel (4000x2300x12bit) 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	1000x520…8000x2000 pixels projection images (16-bit TIFF format) 1000x1000…8000x8000 pixels cross-section (BMP, RAW 16-bit, TXT formats, converter to JPEG)
X-ray loading to the animal	0.1-0.5 Gy per scan typical
Scanning system	source-detector pair rotation with 0.02 deg. min. step size, 50um object positioning accuracy with 400mm travel, 50mm camera positioning/alignment with 1um 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-rendering, virtual manipulation with reconstructed object, morphological analysis in 2D and 3D
Reconstruction algorithm	Modified Feldkamp: multislice 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 20cm from the instrument surface
Installation requirements	Power 100-130V/5A/50-60Hz or 200-240V/3A/50-60Hz, 18-28C temperature, <85% humidity, no condensation, vibrations 0.1…100Hz <40 microns
Size/Weight	Desk top instrument 750mm(H) x 650mm(D) x 2200mm(W), 150Kg + computer, monitor, keyboard, mouse

The spatial resolution in a CT image is determined principally by the size and number of detector elements, the size of the X-ray focal spot, and the source-object-detector distances.
The "SkyScan-1076" is a high- resolution low-dose X-ray scanner for in-vivo 3D-reconstruction with a spatial resolution of up to 15 microns inside small laboratory animals (rats, mice, etc.).
It consists of the combination of a 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 to calculate internal morphological parameters.



The equipment contains an X-ray microfocus 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 Intel Xeon computer with LCD monitor. All subsystems of the X-ray Microtomograph are inside a steel desktop case.



For "SkyScan-1076" the X-ray microfocus tube with 5 micron focal spot size operates at 20-100kV / 0-250µA.
The special X-ray CCD-camera is based on 10 Megapixel (4000x2300 pixels) cooled CCD-sensor with fibre optic coupling to an X-ray scintillator

The X-ray shadow projections are digitised as 1000x520 to 8000x2000 pixels with 4096 brightness gradations (12 bit).
The reconstructed cross-sections have a 1000x1000 to 8000x8000 pixels (floating point) format and 9 /18 /35µm pixel size in any place of the scanning area.
The scanning area is 68mm x200mm or 35mm x 200mm (two carbon-composite beds supplied)
For the reconstruction one can use a volumetric (cone-beam) reconstruction of one / several / all cross-sections or a ROI-reconstruction. (ROI = Region of Interest)

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.