



• User Manual

MICDOTOMOCDADUV

Desk-top X-ray microtomograph

SkyScan 1272

User Manual

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Designed and manufactured by **Bruker microCT**

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1 INTRODUCTION IN X-RAY MICROTOMOGRAPHY

1.1 Introduction

Conventional optical or electron microscopy allows visualizing two-dimensional images of a specimen surface or thin slices. However, in most cases a conclusion about 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 visualized 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 3D information.

An X-ray (radiography) system produces two-dimensional shadow images of complete internal threedimensional structures, but in a single two-dimensional shadow projection the depth information is completely mixed. Only an X-ray tomography system allows us to visualize 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 1 - 2.5 mm, which corresponds to 1 - 10 cubic mm voxel (volume element) size. Computerized X-ray microscopy and microtomography now gives possibilities to improve the spatial resolution by seven to ten orders in the volume terms. The system "SkyScan 1272" allows reaching a spatial resolution in the level of microns, which corresponding to near 1x10⁻⁹ 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 microtomography

An X-ray shadow image represents a two-dimensional projection from a three-dimensional object. In the simplest case, we can describe the X-ray illumination as parallel. In this approximation, each point on the shadow image contains the integration of absorption information inside the three-dimensional object along the path of the corresponding partial X-ray beam.



For parallel geometry one can divide the problem of three-dimensional reconstruction from twodimensional projections into the serial reconstruction of two-dimensional object slices from onedimensional shadow lines.

Let us show the possibility of this reconstruction on a simple example, an object with only one point with significant absorption in an unknown place. In the one-dimensional shadow line a decreasing of intensity of the shadow of absorption in the object area will be seen (see the next picture). Now we can initialize 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 we can 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 the position of the shadow. This operation is named "back-projection". After certain number of rotation steps we can localize the position of the absorption point inside the area of reconstruction. As we increase the number of shadow projections from different views, this localization becomes better 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 point image because it is produced as a superposition of lines with all inclinations. Now that we know what image will be produced from the point object, we can ``pre-correct'' the initial information from the absorption lines to make the resulting image correspond more closely to the real object.



This correction adds some ``negative absorption'' outside the region of the object shadow to eliminate the positive blur in the back-projection process (convolution) which is shown in the figure above.

The same algorithm can produce cross section images not only from a single point object but from any real object. Each object can be represented as a large number of separate absorption voxels and the 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.

However, most X-ray sources are not able to generate parallel beams. In the real case, one will use a point source which produces a cone X-ray beam encompassing the object.



For tomographical reconstruction the solution of this problem can be found by taking into account the cone-beam geometry in the back-projection procedure. In the cone-beam geometry the rays going through the front and the back of the object will not be projected on the same row of the detector. The fastest slice to reconstruct will be the one corresponding to the optical axis because no thickness information has to be used here.

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 an exponential law (Lambert-Beer), the linear absorption information from the shadow image can be restored by taking the logarithm. This is a non-linear operation, and a consequence of this is that any noise in small signal areas can produce significant errors in reconstruction. To eliminate these errors, an averaging of initial data can be used. On the other hand, we can try to improve the signal to noise ratio in the shadow image by optimizing the exposure time in order to obtain the most representative information. A more effective way of noise reduction in the reconstruction process is special selection of a correction or filter 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 behavior becomes very dangerous for noisy initial information. Special selection of a convolution function for correction with spectral limitation by a "Hamming window" prevents noise from increasing during the reconstruction procedure. In X-ray micro -tomography, 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.

During acquisition the object will rotate over 180 or 360 degrees with a fixed rotation step. At each angular position a shadow (or transmission) image will be acquired. The acquisition program will save all these projection images on the disk. The number of files after this acquisition is dependent upon the rotation step selected and the total rotation which is chosen. When the acquisition is finished the reconstruction can be started. The obtained shadow angular projections will be used for the reconstruction of the virtual slices through the object. A raw data cross section is then generated using the reconstruction algorithm. This raw data is not yet an image; it is a matrix of numbers holding the absorption values in the reconstructed cross section. The size of the reconstructed array NxN is defined by the number of pixels in the lines of the angular projection images N. The reconstruction results can be saved directly (typically - by conversion to a 16bit image) or after interactive selection by the operator of a density window, with subsequent conversion to 8bit or 16bit images. During selection of the density window, minimum and maximum values are selected. All values between these will be displayed as half tone image. Depending on the choice of the user, all attenuation values below the minimum will be black while everything above the maximum will then be displayed as white. 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.

2 SKYSCAN 1272 SYSTEM OVERVIEW



The SkyScan 1272 is a compact desk-top micro-CT system for material science, industrial, biomedical and quality control applications. The X-ray scanner contains a microfocus X-ray source, an X-ray detector (camera), an object manipulator for positioning and rotation of the objects during tomographic acquisition and associated electronics to power the X-ray source and camera and for the control of the object manipulator. The scanner supplied with workstation operating under Microsoft Windows with two wide-format LCD monitors. The workstation supplied with all preinstalled software for image acquisition, tomographical reconstruction, 2D/3D numerical analysis of the images and realistic visualization by surface and volume rendering. As an optional part, the scanner can be equipped with automatic sample changer, which can be attached to the front-top part of the specimen chamber.

SPECIFICATIONS

SkyScan 1272 desk-top X-ray microtomograph:

- Microfocus sealed X-ray source 20-100kV, max. 10W, <5um spot size @ 4W.
- X-ray detector 16Mp (4904x3280 pixels) or 11Mp (4032x2688 pixels), 14bit cooled CCD fiberoptically coupled to scintillator.
- Pixel size on the object at maximum magnification [nominal resolution] is 0.35um (16Mp) or 0.45um (11Mp).
- Reconstruction arrays up to 14450x14450x2630pixels (16Mp) or 11840x11840x2150pixels (11Mp) after a single scan.
- Maximum scanning object diameter is 75mm using three camera offset positions, scanning diameter 52mm using two camera offset positions, scanning diameter for fastest scan (central camera position) is 26mm.
- 6-position integrated filter changer, standard filters: no filter, Al 0.25mm, Al 0.5mm, Al 1mm, Al 0.5mm + Cu 0.038mm, Cu 0.11mm.
- Integrated micro-positioning stage, 5...7mm travel (dependent on orientation).
- Radiation safety: <1microSv/h in 10cm from any point of instrument surface.
- System dimensions (excluding control PC): 1160W x 520D x 340H mm (440H mm with sample changer), 150kg.

- Installation requirements Power 100-240V/3A/50-60Hz, 18-25C temperature, <70% humidity, no condensation, pollution degree 2, category (overvoltage category) II, mains supply voltage fluctuations up to +/-10% of the nominal voltage, fuses 2 x T 4AH 250V, vibrations 0.1...100Hz <0.1mm.
- The system is for indoor use.
- Altitude < 2000m

Optional sample changer:

- Automatic sample changer with 16 sample positions,
- Maximum sample size: 25mm diameter, 50 mm length
- Sample mounts with four sizes, 16pcs of each size supplied with the changer.
- Scanned samples can be removed / replaced without interrupting scanning process.
- Typical sample replacement time is 65 sec.
- Powered from SkyScan 1272 scanner.

Control computer configuration:

- DELL Precision T7920 Workstation, Windows 10/64bit:
- Two Intel XEON GOLD 6128, 3.4GHz, 10.4 GT/s processors,
- 128GB 2666MHz DDR4 RAM,
- 512 GB SolidStateDrive for operating system and software,
- 16TB (4x4TB on RAID0) HDD for data storage,
- 8GB NVIDIA graphical card,
- Two 24" UltraSharp LCD monitors, 1920x1200 native resolution,
- DVD/CD RW optical drive, keyboard, mouse.
- IEEE1394 FireWire card.



The necessary installation space (excluding workstation) is shown in the following image:

The scanner should be accessible at the right side for connection to workstation and from the front (at least in the front of black specimen chamber door) for specimen exchange.

3 INSTALLATION PROCEDURE

In the case of damage during transportation or any defect in the scanner operation, please contact your local Bruker microCT distributor (see <u>www.bruker-microct.com</u> for distributors). Alternatively, you can consult directly to Bruker microCT:

BRUKER MICROCT, Kartuizersweg 3B, Kontich B-2550, BELGIUM. Email: <u>info@bruker-microct.com</u>, Tel: +32 3 877 5705, Fax: +32 3 877 5769

Only people trained by Bruker microCT are authorized for service and reparations on the scanner!

The system is not intended to be placed in the public areas. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired. We recommend consulting national authorities to determine any local operational requirements.

Scope of this document

This manual is meant to use as a guideline to unpack, connect and startup the SkyScan-1272 X-ray microtomograph. Always use your common sense, specific onsite difficulties with transportation are not described in this manual. We recommend consulting national authorities to determine any local operational requirements.

3.1 Unpacking procedure

The scanner should be carefully removed from the flightcase.



For lifting and carrying, keep the scanner in the same orientation as in working conditions, and use the side steps on the enclosure plate as handgrips. The weight of the scanner is around 150kg and we recommend to carry or to lift the system with 4 persons. Alternatively, the scanner can be removed from flightcase using forklift and two lifting slings, (available from RS <u>http://uk.rs-online.com</u>, stock number 729-3057). The lifting slings can be installed under the side steps of the instrument enclosure using following four simple steps:





Under the scanner you will find toolkit box, cables to connect scanner to workstation, power cable and factory report.



Keep the flightcase for possible moving of the scanner to another location or for sending it back for service or repair.

If scanner supplied with optional automatic sample changer, you can find it in the second (small) flightcase. We would recommend you to check that sample changer is not damaged in transportation and keep it in the flightcase until scanner will be finally installed and tested. To unpack sample changer, open top part of the case and remove plastic box with sample changer's toolkit:



The changer can be found under separation plate in the bottom compartment of the flightcase.



To remove the changer use two round holes in the plastic foam to put your hands under the neck of the changer in the middle of the case. The changer is relatively light and can be handled by one person.

3.2 Installation of the scanner

Site requirements:

The system needs to be connected to a wall socked with earth connection.

The power cord can only be replaced by H05VV-F 3G 1.5mm2

Don't position the system so it is difficult to manipulate the electrical power cable. To remove the electrical power from the system, unplug the power cable from the appliance coupler.

Before starting the scanner, one should make the connections shown in the figures below. All interconnections with signal cables should be made before connection of the main power plug to the wall socket. Side wall of the scanner contains following connectors:



Connect the scanner to the computer:

- Use a USB cable from the connector on the right side wall of the scanner to any USB port on the back wall of workstation.
- Use a FireWire cable to connect the camera output in the right scanner side wall to the FireWire socket in the back wall of the workstation.

The length of every signal cables is 1.2...2m. Use only the supplied signal cables.

For power connections you will need four power sockets available. All sockets should have ground connections and ground in all sockets should be connected together and to reliable ground line.

After connecting all signal cables, connect your monitors to workstation, connect keyboard and mouse (you can use single USB connection between workstation and hub in the monitor and connect keyboard and mouse to USB-outputs on the back panel of the monitor). Afterwards connect the power cables to the scanner, to your workstation and to both monitors.

In the supplied workstation all software is already pre-installed and configured by the producer or distributor. Back-up CD supplied in the toolkit box contains back-up of all software, electronic version

of all User Manuals and necessary device drivers. Most recent versions of software can be downloaded from the producer website: <u>www.bruker-microct.com/products/downloads.htm</u>

3.3 Supplied toolkits

The toolkit supplied with scanner contains following parts:



Two supplied keys (1) for X-Ray keyswitch can be used for protection against unauthorized access to scanning by the instrument. Two brass sample mounts (2) can be used for medium size samples (8-25mm in diameter). Two brass thin sample mounts (3) can be used for scanning small samples at high magnification. Large samples can be hold by sample holder (4), which can be attached to the scanner's turntable by supplied screws (5) using supplied screwdriver (6). Small and medium size samples can be attached to the sample mounts (2) and (3) by supplied wax (7). For scanning wet or hydrated samples one can use sample mount (8) fitted to plastic containers (9). To scan small samples from all sides they can be hold by transparent for X-ray foam tubes (10) attached to supplied mount (11). For scanning samples in the liquid one can use a standard syringe fitted to the supplied mount (12).

The toolkit also contains alignment pin (13) and spare collet holder for sample mounts.

The toolkit supplied with automatic sample changer contains following parts:



A cable (1) allows connecting power to the changer from the scanner. A USB-cable (mini-USB) connects data socket on the changer to USB2 socket on the back wall of workstation. Supplied landing pad (3) should be attached to the scanner turntable before changer installation using screwdriver supplied in the scanner toolkit. A centring pin (4) used to support the changer exactly in the middle of the scanner door. A screwdriver (5) can be used to tight the screws, which hold the changer on the front support of the scanner. The 64 sample mounts (6) – four types, 16 pieces each – can be used to fit sample changer by samples with different sizes.

3.4 Connection to external interlock

If regulations in the user site require additional protection in access to laboratory during scanning with involvement of X-rays, it may be necessary to connect the system to external interlock switch (typically – switch on the laboratory door) using connection to the socket on the side wall of the scanner. The same socket can be used to control illuminated sign "X-RAY" above laboratory door if necessary.



It necessary, connect "external interlock" connector by following way:

For such connections you can use supplied plug inserted in the socket in delivery of the scanner. If connection to external interlock is not necessary, keep supplied plug inside "external interlock" socket. In factory delivery this plug is wired by such way, which allows starting scanner without external interlock.

4 SCANNER'S CONSTRUCTION

4.1 Main parts of the scanner



The construction of the SkyScan1272 scanner is shown below:

It contains a microfocus X-ray source in the left part of the scanner. The source operates at 20-100kV with maximum power of 10W. It produces X-ray from the very small focal spot to ensure that smallest details inside the objects can be resolve in X-ray image. The system uses natural propagation of X-ray beam between point-shape source and two-dimensional X-ray camera to magnify object image. If object positioned close to the source, the image of the object will be significantly magnified on the camera. The magnification is proportional to ratio of the distances from source to camera and from source to object. In the case of object positioning close to the camera, the image will be projected with relatively small magnification. By moving object between X-ray source and camera the system can adjust necessary magnification.

The X-ray CCD-camera located on the right side of the scanner and combined with motorized 6positions filter changer. There are two camera options available: 16Mp and 11Mp. Both cameras contain large-format CCD sensor connected by fibre optics to thin scintillator layer behind amorphous carbon window to protect CCD against visible light. The X-ray radiation can pass through thin carbon window to be converted at scintillator to the image in visible light. This image pass through fibre optics without any spread and reaches CCD where it converted to electrical signals. Camera electronics digitizes this electrical signal and send it to computer. To keep CCD sensor with stable temperature and avoid recalibration connected to temperature fluctuations, camera contains solid-state cooler, which keeps CCD sensor at stable temperature with very high accuracy (<0.1C).

The camera located in the top of two orthogonal translation stages. First one can move camera across X-ray beam (towards and backward from operator position) to align camera to the central X-ray beam and to acquire images in offset positions. The SkyScan-1272 can obtain images using central camera position as well as two or three offset positions to extend field of view and to scan objects with large physical sizes. Control software automatically connects images acquired in offset positions to form of

one large-format image. Second translation stage can move camera towards X-ray source to change distance between source and camera. This movement is necessary to implement automatically variable geometry, which will be explained in next chapter.

The camera assembly also contains motorized filter changer. It allows covering front window of the camera by selected thin metal filter to make precise energy selection. There are six positions for the filters in the changer. Typically, one of six positions has no filter for detecting by camera lowest energies in the X-ray spectrum for highest sensitivity for materials with low X-ray absorption. Other five positions occupied by metal foils with different materials and thickness. In combination with tuning peak energy by changing voltage in X-ray source, radiation filtering allows making adaptation of acquisition conditions to wide range of object's densities and materials.

Central part of the system is occupying by an object stage. This stage contains a turntable for object rotation during acquisition of tomographic series, a linear stage for object positioning in vertical direction, a linear stage for object assembly alignment by moving across X-ray beam, a long travel linear stage to move object assemble towards X-ray source for changing magnification and a micropositioning stage in the top of turntable for precision XY object positioning.

All positioning stages and motorized door are driven by 9-axis stepping motor electronics, which is working in microstepping mode (with accuracy up to 1/128 step of physical stepping motor) and can provide smooth positioning with programmable acceleration and de-acceleration curves.

The object stage also equipped with a colour optical camera, which allows operator to see all sample movements inside the shielded specimen chamber and to avoid collision of large objects with X-ray source.

The system has no power switch. The power of the system can be switched ON and OFF by commands from control software sent through USB port. In every start control software wakes-up the scanner and in every closing of control software the scanner will be switched OFF automatically. The software also supports automatic shut-down of scanner and (if selected) control PC after long scanning and after time consuming procedures of scanner calibration and stability testing.

4.2 Automatically variable acquisition geometry

The SkyScan-1272 utilizes automatically variable acquisition geometry invented by Bruker microCT to reduce scanning time and increase scanning quality in microtomography systems with large-format cameras. All other micro-CT systems on the market use static acquisition geometry where the X-ray source and X-ray detector are separated by a fixed distance and image magnification is adjusted by object movement between them. The limited opening angle of emission from the X-ray source and attempts to extend the magnification range by increasing the source-detector distance reduces quadratically the intensity of the X-ray beam at the detector, with a corresponding steep increase in the scan time. To escape this dilemma the SkyScan-1272 uses automatically variable scanning geometry. Following picture explains how it works:



Using an X-ray source with a big beam opening angle and a large format detector, the maximum distance between the source and the detector, required for maximum and minimum magnifications, can be reduced with a corresponding increase in detected X-ray intensity. At intermediate image magnifications, both object and detector are moved towards the source until they reach the most compressed geometry possible while collecting all X-ray photons emitted from the source. Such adaptive scanning geometry applied to all magnifications allows improvement in quality or reducing of scan time by a factor of 2...5 compared to traditional fixed scanning geometry. To avoid infinite number of camera-source distances which will require infinite number of calibrations, number of possible camera positions limited to three and for all three positions control software stored calibration in advance. The repositioning of object stage and camera during changing magnification doesn't require any operator's command. The control software automatically optimizes the scan geometry to the image magnification selected by operator.

4.3 Tuning of X-Ray energy according to absorption in an object

The SkyScan-1272 includes a 6-position automatic filter changer and is equipped with an X-ray source designed in such a way that adjustment of X-ray peak energy always keeps the same output power. The flexibility for precise tuning of maximum X-ray energy emitted by the source and the minimum

energy cut-off selected by numerous filters allows operator to select the most optimal energy window for any particular object. For object containing transparent for X-ray materials energy window can be pushed down to low values for getting enough significant adsorption from object's smallest details. In the case of objects, which contain materials with high X-ray absorption, X-ray energy window can be pushed up to get enough transmission through the object body. Typical energy windows with six combinations of filters and peak energies of X-ray source are shown on this picture:



Peak energy in the source and energy filter on the camera side can be selected independently by operator. The control software can also select best possible combination of X-ray peak energy and one of six installed filters automatically according to X-ray opacity of every particular object.

4.4 Software components and dataflow between them

The system supplied with number of software components, which are linked by dataflow of files saved on the hard disk in conventional formats.



The dataflow between the supplied programs is shown in the following image:

The control program acquires images from the X-ray camera, controls all positioning and X-ray source in the scanner. It can save separate X-ray images or complete acquired set of angular projections for following tomographical reconstruction. Every set of projections is accompanying by log-file. During acquisition the control program saves all angular projections as 16-bit TIFF images with filenames "prefixNNNNNNNN.tif", where "prefix" corresponds to the selected name for the dataset and "NNNNNNN" indicates the number of angular projection in an 8-digit format. The set of acquired projection images can be opened and shown as a movie by several supplied programs, such as DataViewer. Any individual projection can be opened by double click to the corresponding file or by imaging program, which supports 16-bit TIFF encoding, for example "Adobe Photoshop".

Once the acquisition of the angular projections is completed, the control program will save a "prefix.log" file, where "prefix" corresponds to the selected dataset filename. This text file contains all the necessary information about the scanner and all settings used during the acquisition process. The reconstruction program uses this file to extract the acquisition geometry for a particular scanner, and the object position and image format.

The reconstruction is performed by the NRecon program, which uses either a single computer or a computer cluster with several identical or different PCs connected by a Gigabit network and can execute reconstruction process using only CPU (by linked NReconServer program) or accelerated by GPU (using linked GPUReconProgram). The program loads the angular projections in 16-bit TIFF format and numerical information from the corresponding "*.log" file. The NRecon program works using floating-point data values for internal calculations during reconstruction and afterwards allows the operator to define the density window as a range of the reconstructed values, which will be saved as a grayscale image to the output files. Reconstructed images can be saved in 16-bit TIFF format or in 8-bit BMP, PNG or JPEG formats.

Filenames of the reconstructed cross section images contain the same prefix as the angular projections extended by "_rec" and a four or eight-digit index, which indicates now the vertical position of the reconstructed cross section inside the three-dimensional reconstructed space. 0000 corresponds to the bottom of the reconstructed space and the bottom line in the projection images. The reconstruction program updates the dataset's "*.log" file by adding the program version and all reconstruction settings. The full set of reconstruction results can be opened by any one of the listed programs for dataset visualization.

All reconstruction results can be used by number of programs. A DataViewer allows visualizing reconstructed results as a slice-by-slice movie or as a set of three orthogonal slices crossed at any point of reconstructed volume. It also supports resampling of reconstructed results in any orientation and making simple measurements such as distance measurements in 2D and 3D, etc.

A program for volume rendering – CTvox – also uses reconstructed dataset. This program creates realistic visualization of reconstructed object by volume rendering. The results of rendering can be saved as BMP or JPG images. The program also supports automated creation of movies, which can be saved as the *.avi files with all compressions supported by operation system. The program can export dataset through any file transfer service, such as DropBox – to mobile devices. Special version of CTvox for mobiles makes volume rendering on iPhone or iPad.

The CTAn program for 2D/3D processing and analysis can also load reconstructed datasets in TIFF, BMP, PNG or JPG formats, and save the results of processing and analysis into text files and tables compatible for export to Microsoft Excel. It also accepts results from many other micro-CT scanners (XRadia, Scanco, etc.) and can work with datasets in DICOM-format, which is commonly exported from 3D scanners and clinical CT systems. During rendering the CTAn program can save 3D-models of reconstructed objects in standard STL format for 3D printers and rapid prototyping as well as in "*.ply" or internal "*.p3g" formats for sending 3D models to the CTvol program for realistic visualization by surface rendering. The CTvol program can manipulate 3D models in listed formats. Results of rendering can be saved as BMP images as well as an animated "movie" in compressed "*.avi" file.

Software package also includes number of utilities for conversion between image formats (FormatConverter) and for exporting datasets (DICOMconverter).

5 GETTING STARTED

The software package for system control has the same operational principles as any other Windowsbased program. One can select any function from the menu/submenu in the top line, and also change modes and parameters using the corresponding control buttons or sliders. To start the SkyScan1272 control software double click to the corresponding icon on the desktop:



After starting the program, the system will be switched on automatically and an initialization window will be shown on the screen. During initialization the program will establish connection to all nine drives in positioning system, to X-ray source and two cameras (visual and X-ray). If software cannot be connected to X-ray source, make sure that the key at the right side of the black base of the scanner is turned to the "ON" position.

5.1 Acquiring images and collecting datasets

The start image on the screen contains the main menu bar, the toolbar in the top of the screen, and the status bar at the bottom.

SkyScan1272				- • ×
Actions Options Help	ИENU			
C C C	🏮 🛞 🗳 🗹 🔊	TOOLBAR		
	STATUS	BAR		
	2.1um 🕑 📲	57.6 °	∰ 1224 x 820	No Eile

Dependent on last system usage it can also contains scout view bar on the left and batch scanning list in the middle.

To insert an object, to start the X-ray source and obtain an on-screen projection image, you can use any one of the first three buttons at the left side of the toolbar:



Open / Close specimen chamber

X-Ray ON / OFF



Grab X-ray image continuously



Snap single X-ray image

The "Grab Image" function will first start the X-ray source (if it is selected in Menu => Options => Preferences) and then begin acquisition of projection images. Clicking the "Grab Image" button once more will stop continuous acquisition. The "Snap Image" function will also turn on the X-ray source first and acquire a single image, keeping the X-rays ON. Selecting "Open specimen chamber" will automatically switch OFF the X-ray source.

When the source is ON an indicator appears on the screen:



Note that the indication under the X-ray sign can be changed by double click on the corresponding small notification icon in the right bottom corner of the screen.

If you start the X-ray source more than 8 hours after the last usage, an "aging" procedure will be executed. Aging means the warming up of the source by gradually increasing the voltage and current. This aging procedure is important for the life time of the source. After 8 hours of inactivity the source aging lasts for 15 minutes. After longer interrupts in usage, aging process can take longer time. The maximum possible aging time is 2h.

After opening-closing specimen chamber, the X-ray source will need "preheating", which can take up to one minute and executed automatically with indication in balloon in the right bottom corner of the screen.

To put an object inside the specimen chamber, click to first (leftmost) button in the toolbar. Use one of the supplied brass specimen mounts to support the object. You can use supplied wax to secure your object on the top of the brass mounts. Put the base of the mount in the central hole of the collet in object manipulator and hold it by turning the brass ring in a clockwise direction. Don't apply significant torsion to the collet – it stays on the high precision and accurate XY micropositioning stage, which can be damaged by applying force.



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For large objects one can use a supplied steel sample mount. This mount has a chuck with four independently adjusted jaws to hold the objects with complex shape and to adjust their proper centring:



To install steel sample mount use supplied screwdriver. Firstly select angular position of the mount to be sure that connector on the turntable targeted towards milled cavity in the bottom of the mount and afterwards put it coaxial to turntable and secure by two supplied screws:





Close the specimen chamber using the leftmost button on the toolbar:



Start X-ray and continuous imaging using "Grab Image" button:



To change magnification, click to new position on the left slider on the status bar (at the bottom of program window). By moving mouse cursor across the slider without clicking you will see expected pixel size in the tooltips. Don't drag the slider by mouse: just click to new position.

The second slider in the status bar will activate the rotation of the object. Click to new position to turn the sample.

The next field in the status bar controls movement of the object up and down (Z position) by mouse "pushing" on one of the two small buttons with green arrows.

All three listed movements can be adjusted using numerical values by clicking to numerical indication on the right side of any status bar pane and filling new position(s) in the following dialog.

You can click by right mouse button in the imaging area to show a signal intensity profile along the line where the mouse pointer is situated. (a second right-click will remove the profile from the image). A ruler for measurement of distances and sizes is activated with left mouse button drag-and-drop. Two small yellow lines in the top and in the bottom of the image indicate central line of the image. By selecting magnification try to fit the projection of your object inside the field of view for all angular positions. If object, which placed on the brass mounts and connected to micropositioning stage, is wobbling during rotation, you can centre it by following way: firstly find angular position with object image most offset from the central line; then move object to the middle of the image using micropositioning stage. You can activate micropositioning control by Menu -> Options -> MicroPositioning or simply drag central line of the object to the middle of the imaging area by left mouse button keeping Ctrl on the keyboard (length indication will be switched to arrow) and after releasing mouse button the object will be moved by selected distance.

The second group of buttons in the toolbar starts the scan:



Start Scanning

Click the "Start Scanning" button. The control program will open the "Scanning Options" dialog to adjust scanning parameters and the location for storage for the projection image files:

Scanning Opt	ions 💌			
	Filename prefix: newscan			
	Data Directory : browse			
	F:\Results\test			
\sim	Rotation step (deg) : 0.3			
	V Averaging (frames) : 2			
	Random movement (pix) : 30			
	360 deg scanning			
Select filter automatically				
	X-ray OFF after scanning			
Open specimen chamber after sca				
	$\overline{\ensuremath{\mathbb V}}$ Send e-mail after scanning to address :			
	myname@mycompany.com			
	send test e-mail now			
	Pixel Size :			
Q	○ 5.4um ○ 10.8um ○ 16.2um ○ 21.6um			
	Partial width : 72%			
 Standard scan in central camera position 				
	Offset scan with two camera positions			
	snap wide image now			
	scan position: 30.000 47.699			
	Estimated scanning time: 45 min			
	Dataset size= 0.82 GB Free disk space=4446.67 GB			
	OK Cancel			

Select filename prefix and target folder for file location. For first attempt use Averaging (frames) as 2-4, all other checkboxes unchecked, use largest pixel size, 100% Partial width and Standard scan in central camera position. You can make selection of scanning parameter in advance by Menu -> Options -> Acquisition. In this case the Scanning Options dialog will contain only OK and Cancel button like it shown on the image above. When the same dialog appears before beginning of the scan, it will contain also Scan button in the bottom. Click to Scan button to start scanning process.

After beginning of the scan the control software will show progress dialog with following information:



During acquisition all angular projections will be saved to the hard disk of your computer in the selected folder. After finishing scanning this dialog will disappear from the screen and you can start reconstruction of virtual slices across your object using next button on the toolbar:



Start Reconstruction

If more than one dataset exists in the current folder, the control software will show you a list of available datasets for reconstruction. Click on the name of your dataset and then the OK button. If only one dataset exists in the current datafolder, the reconstruction will start immediately.

5.2 3D Reconstruction

The reconstruction process will be executed by separate program, NRecon. Dependent on the security settings in your computer, during first start of this program it may ask you to unlock access – do so.

The reconstruction program NRecon will show you the first acquired angular projection image on the left side and several control panels on the right side (select "Start" tab). Click the "Preview" button to reconstruct one central cross sectional slice through your object. The reconstructed slice will then be displayed in the "output options" panel. This panel allows you to select the top and bottom of the density window by moving the colour vertical lines in the histogram, to obtain optimal contrast of the materials inside the object. Then switch the control panel back to the "Start" tab and select the top and bottom (vertical range) of the reconstruction space by the numerical position of the first and last slices or by moving the top and bottom horizontal lines in the shadow image with the mouse. You can also select a step between reconstruction parameters such as smoothing, ring artefact reduction and beam hardening correction, are set in the second "parameters" panel. Finally when all reconstruction parameters are correctly set, reconstruction can either be started immediately by the "Start" button, or placed in the batch manager by pressing "Add to batch" to execute reconstruction later. After reconstruction, all reconstructed slices will be saved to the same folder on your hard disk (or in subfolder if it selected in Options -> Preferences).

5.3 Visualization of reconstructed results

To show the reconstruction results you can start the DataViewer program directly from the reconstruction program by using following button in the toolbar:



The same visualization program can be started from identical toolbar button in the control program. This will start the DataViewer program which displays the reconstructed cross sections as a slice-by-slice animation. Second button in the toolbar of DataViewer starts / stops such animation. You can

also load the full reconstructed 3D space into DataViewer and visualize three orthogonal slices crossing at any selected point inside the object using third button in the toolbar. The next toolbar button opens a "3D Navigator" display to show the spatial position of three selected slices:

<u>A</u> ctions	View	Options				
2			K,	D \oplus	Θ	
Load	for	3D vie	wina			
			3	D Mai	Jan	A.F

You can select any position for the crossing point of the three virtual slices by moving the crossbars in the images or the slice planes in the 3D Navigation window by the left mouse button. Right mouse button drag-and-drop inside the 3D Navigation window allows rotation of the spatial model. Moving mouse with left mouse button in any of three slices with keeping Ctrl on keyboard allows rotating reconstruction volume to any orientation. You can create new dataset in alternative orientation by saving it after rotation using Menu -> Actions -> Save command.

5.4 Realistic visualization by volume rendering

To start volume rendering program from the reconstruction program or from the main control program use following button in the toolbar:



It will start CTvox program, which can perform realistic 3D visualization of reconstructed object using volume rendering. Once a dataset has been loaded to CTvox, the transfer function plot area (in the *Transfer Function* pane, in the upper left corner) will display a histogram for the data. Likewise, these low-intensity contributions (which dominate the periphery of reconstructed images) tend to obscure the core of the volume data. Therefore, it is usually useful to suppress these low intensities in the rendering. The purple "opacity" curve (initially a straight line) shows conversion between intensity in reconstructed slices (low-left, high – right) and shown on the image opacity of corresponding object's details.



Add a knot to the opacity curve by clicking in the lower left side of the purple curve on transfer function window. Move the knot down to get low-intensity clouds around the object more transparent.



Two modes exist for navigating the scene:

- *Object movement*: the orientation (rotation) and position (translation) of the object can be adjusted using the right and left mouse button respectively; the camera can be moved closer to or farther from the object using the mouse wheel.
- *Camera movement*: the object is held in place; the camera can be rotated around the object (right mouse button) and the camera viewing angle can be adjusted (left mouse button); again, the mouse wheel moves the camera forward or backward.

The right mouse button controls rotation in both modes. In object movement mode, the left button controls the translation *in the viewing plane*. *Out-of-plane* translation (i.e. perpendicular to the viewing plane) is performed using the middle mouse button. In camera movement mode, the left button controls the *camera viewing angle* (acting as a zoom, but introducing perspective distortion when the camera is close to the object; the viewing angle can also be set in the *View Options* pane.) By holding the **Shift** key down while left clicking, the face of the clipping box under the mouse pointer can be 'grabbed' and subsequently dragged to clip a portion of the object. Holding the **Ctrl** key during rotation or translation (in both modes) will keep the object in place and move only the clipping box, allowing positioning a clipping plane appropriately. The mouse wheel controls the distance between camera and object in both modes.

Object's colour can be adjusted by manipulating partial colour transfer functions (red, green and blue) after corresponding selection in drop-down list "Channel".

The Flight Recorder functionality can be used to produce fly-through animations for your 3D data. Start *Flight Recorder* dialog by selecting **Actions > Flight Recorder** command or click on the toolbar

button: 躗

The idea is to set up the scene and to tag it as a *key frame* by clicking the *Add* button. After preparing next scene, click *Add* again and so on. When creating the movie, the program will generate the specified number of intermediate frames by appropriately interpolating these key frames.

When ready, the movie can be generated by clicking Save movie. The output is either an AVI file or a series of bitmaps (BMP, JPG or PNG).

More details on CTvox functions can be found in "Quick Start Guide" available for downloading from Bruker microCT website.

5.5 2D / 3D image analysis and surface rendering

The next button in the toolbar of the SkyScan1272 control program and in the toolbar of reconstruction program starts the software package named CTan for 2D/3D image analysis of the reconstructed slices and for realistic 3D visualization:



The software package for image analysis and visualization of reconstructed datasets practically contains two programs: CTAn for 2D/3D analysis and CTvol for realistic 3D visualization by surface rendering. To use the two programs together, for direct export of the 3D object models for visualization, connect CTvol to CTAn: in CTAn select "File"->"Preferences"->"Tools" and define CTvol.exe as the 3D visualization program.

If CTAn is starting from the other program, the selected dataset will be opened automatically. Alternatively you can open any set of micro-CT results by the 🖻 button in the toolbar of CTAn. The shadow image (if present) and list of files will be shown in the top part of the program window and one of the cross sections in the bottom part. You can animate through the selected dataset levels by pushing 🕨 or using the slider on the left side of the image files list. Right mouse click in the cross section image to open a submenu for image zoom. Drag and drop the left mouse button in the cross section image or the projection image to measure distances. Different colour look-up tables can be selected in the "Palette". Click the 🕥 sign to invert the image colour scheme.

After loading a dataset, several buttons will appear in the main toolbar:



original image view,



selection of region or volume of interest,



conversion of images to binary for analysis,

*

(appearing after conversion to binary) image analysis.

If you wish to select a region/volume of interest, click the solution and draw with left mouse button held any shape of the region directly on the cross sectional image. This shape can be copied to all cross sections in the dataset by the solution. To select a 3D volume of interest you can draw different regions in different cross sections, and the software will interpolate them automatically through all intermediate cross sections. Use the toolbar button select regular shapes for the region-of-interest. Volume-of-interest can be limited in the vertical direction by two sliders to the right of the files list. Double-click on the blue bar between these sliders to enter upper and lower limits numerically. One can reset all regions-of-interests by the 🚇 button to "start again" with VOI selection.

Go to conversion to binary images with the 📓 button. If "Histogram" pane cannot be seen, open it by dragging down the top border of the "Palette" window by the mouse:



Select the upper and lower global threshold levels by sliders above and under the histogram. The white part of the binary images represents the objects selected for the subsequent 2D and 3D analysis.

Use the 🖻 button in the main toolbar for image analysis. Distributions of size, perimeter, form, orientation and internal porosity from the current 2D-image are shown as colour-coded histograms in the "Analysis" part of the program window. Colours or brightness (dependent of selected "Palette") of the histogram's bars match the colours of the objects in the image with corresponding 2D parameter values. To enhance this colour coding of the 2D histogram select "colour" or "colour2" from the "Palette" drop-menu and use sliders under the colour scale in the "Palette" pane . To see the results from another cross section, select it in the list of available files. Use the local toolbar or right mouse button in the "Analysis" area to start full individual 2D object analysis from the current image (🖾 button) and to start full 3D analysis (😢 button) from the selected volume of interest. A further 2D analysis giving integrated 2D parameters for all selected dataset images is possible by selecting the button, which will create a tabular comma-delineated text file, which can be imported to Microsoft Excel.

To send the selected volume of interest to CTvol for realistic visualization, go back to the binary images by the button in the main toolbar and then push the button in the local toolbar. We recommend that you select a small volume of interest in the central part of the object for your first visualization attempt, to avoid memory limitation and slow reaction for the object movements in 3Dspace. Select "Launch associated program to show model" in the "Create 3D Model" dialog to start visualization program after model creation. If the model will be used only for visualization, select the "p3g" file format, which creates smaller file sizes. CTAn will export a 3D-model of the selected part of the object into the CTvol program for visualization. The CTvol program starts in a separate window with its own controls as explained below. It can also be started independently.

From the same function in CTan program any 3D model canbe saved in STL format. This type of files are using for 3D printers, can be imported to most CAD packages and used in number of programs for finite element analysis.

The CTvol toolbar contains buttons to select object movement modes and object(s)/space properties. Object and camera movement functions have behaviour similar to corresponding functions in described above CTvox program.





show/hide light properties dialog.

Object and camera can be moved by the mouse. By holding the right mouse button one can rotate the object or camera around the object(s) depending on the selected mode in the toolbar. The mouse wheel always changes the distance from the camera to the object. The left mouse button can shift the object in the case of selection for object movement, or zoom in / zoom out in the case of camera movement being selected. Double click on either mouse button to switch the program between object movement and camera movement modes.

The object properties dialog allows adjustment for the selected object its surface colour and other visual properties, and the object can be set as movable/unmovable and visible/invisible. One predefined object – the plate – is generated automatically and can be used to cut the selected object using "cut by plane" button.

The stage properties dialog allows adjustment of the camera viewing angle and selection of the background: one specific colour, a 3D colour box or a landscape image. It allows also display the object in stereo mode for red-cyan or red-green glasses (use in the toolbar for quick ON/OFF stereo) and the adding of fog effect with adjustable density to the scene.

The light properties dialog allows adjustment of the colour and direction of the light for object illumination.

Making movies is similar to corresponding function in CTvox with slightly limited number of options. The toolbar button 😰 opens the "Flight recorder" window. This option stores a number of scenes (keyframes) with subsequent smooth interpolation in between. The resulting movie can be shown directly on the screen and saved as a number of BMP or JPG files as well as compressed and uncompressed movie in AVI format.

5.6 How to get help

Every one of described programs contains context help function. Just click to "Context help" button in toolbar:

₿?

The mouse cursor will be converted from arrow to question mark. Now you can click to any function in Menu, to any button in toolbar or to any other control elements of the program workspace. In the popped-up window you will get detailed explanation for selected function or item.

For main control program all functions summarized in next chapter of this manual. All other programs supported by complete User Manual or by "Getting Started" guide, which can be found in the "Manuals" subfolder in the program folder of the computer supplied with the scanner. Most recent versions of the User Manuals can be downloaded from http://bruker-microct.com website.

The documents available in the moment of writing this Manual are:

NRecon User Manual: <u>http://www.bruker-microct.com/next/NReconUserGuide.pdf</u> CTvox Quick Start Guide: <u>http://www.bruker-microct.com/next/CTvox_QuickStartGuide.pdf</u> CTan/CTvol Getting Started: <u>http://www.bruker-microct.com/next/CTan_CTvol_getting_started.pdf</u> CTan User Manual: <u>http://www.bruker-microct.com/next/CTan_UserManual.pdf</u> CTvol User Manual: <u>http://www.bruker-microct.com/next/CTvol_UserManual.pdf</u> CTan List of Functions: <u>http://www.bruker-microct.com/next/CTAn02.pdf</u> CTan Description of Measured Parameters: <u>http://www.bruker-microct.com/next/CTAn03.pdf</u> CTan Creating User Plug-Ins: <u>http://www.bruker-microct.com/next/CTanPlugIns.pdf</u>

Most recent versions of all application programs can be downloaded from: <u>http://www.bruker-microct.com/products/downloads.htm</u>

To close the control program for the SkyScan1272, double click on the icon in the top left corner of the program window or click once on the red box with a cross in the top right corner. Note that closing the program will automatically stop the X-ray source if it is running and shut down the scanner. This can take a certain time.
6 PRACTICAL USAGE OF THE SCANNER

This paragraph contains explanations of functions, actions and checks that should be done either routinely or before starting a scan with the SkyScan 1272 micro-CT scanner. Remember that the micro-CT scanner is a sensitive high-precision instrument, and treat it accordingly. Insert and remove the sample holders at the stage with the minimum force possible, and never subject the scanner to jolts or strong vibrations. Some functions listed below are accessible only in Service Mode. If mentioned functions are greyed, push Ctrl+Alt+Shift+s to activate Service Mode.

6.1 Scanner Alignment

Three main components of the scanner – the x-ray source, the object stage and the camera – should stay aligned by such way that at any magnification rotation axis of the object will be projected to the middle column of the camera's image. To check and adjust alignment use alignment pin supplied in the toolbox with the scanner.



Close specimen chamber door, start X-ray and continuous imaging using "Grab Image" button in toolbar. Using micropositioning stage control adjust position of the pin with minimum wobble. This position is not necessary central position on the screen. It is an aim of alignment to get rotation around central column of the camera for all magnifications.

Remember that you can control position of te sample by micropositioning stage using dialog available by Menu -> Options -> Micropositioning or by drawing line in horizontal direction using left mouse button on the imaging area with pushed Ctrl on keyboard.

If image of alignment pin is far from the central column (which can be found by yellow marks in the top and in the bottom of the image) after minimizing wobble in rotation, push Ctrl+Alt+Shift+c to get camera close to the central position.

Try to increase magnification and check up to what magnification (using lowest image format in the camera) the image of alignment pin is staying inside field of view for all angular positions. If necessary, adjust position of the pin by micropositioning stage to get minimum wobble during rotation.

Alignment test can be activated by Menu -> Options -> Alignment Test. Appeared dialog will give you choice in one of two possible options: single point test in the current position or two-point test. Single point test at low magnification will detect mainly camera misalignment and will try to correct it. Single point test at high magnification will detect mainly object stage misalignment and will try to correct it. Because of camera either object stage movement change position of the image of the pin for the camera, the tests are not independent and should be repeated itteratively until both of them will show 1 pixel or less misalignment values.

Two-point alignment test uses misalignment measurements at two magnification positions to calculate proper movements for camera and for object stage to get them aligned to the central beam without iterations. If image of the alignment pin always stays inside field of view for 5 micron pixel size (use camera format with smallest number of pixels), you can go directly to two points alignment test. Otherwize you have to make iterative alignment by single points tests using minimum magnification to correct camera position and maximum magnification with pin always inside field of view during rotation for object stage position correction.

In the end of misalignment procedure control software will show results of alignment test. For single point alignment it will show necessary displacement, which should be applied to the camera or to the object stage to compensate misalignment. In the case of two-point alignment tests, results will be shown for camera and object stage together. The results are shown in pixels on the image and in microns with values in microns in editable areas. After clicking OK button shown movements will be applied to corresponding components.

In the bottom of dialog with misalignment results one can find angular misalignment value, which shows angular difference between vertical columns in the camera and direction of rotation axis. If following checkbox is checked, this value will be stored in Scanner Settings and will be applied for rotation of every image coming from the camera to get proper orientation of the image agaist rotation axis. This correction can be switched ON and OFF in Menu -> Options -> Preferences as a "geometrical correction" applied to the image. Normal acquisition for reconstruction requires this correction to be switched ON. The "geometrical correction" can also include individual pixel-by-pixel distortion mapping for some types of the cameras. This map stored in "camera.dst" file, which can be created (if necessary) in factory calibrating procedure and shouldn't be updated or removed during lifetime of the system.

How oftet you have to repeat alignment test? In the case of intensive usage of te scanner we would recomment you to check alignment every month. You have to repeat alignment test also in the case of big misalignment values detected by reconstruction program in the case of using pixel size above 2 microns.

6.2 Flat-field correction

A flat-field correction procedure applied to every image between acquiring it from the camera and applying any other correction and displaying on the screed and saving it to the disk. The procedure based on two reference images for every camera format, which can be acquired in advance and stored on hard drive. First one, named as a dark reference field, acquired without X-ray illumination to the camera. It contains distribution of thermal current ("dark current") in every pixel of the image

accumulated by CCD during selected exposure time. Second reference image, named as a bright reference field, acquired with X-ray illumination to the camera without any object in the field of view. This image reflects intensity distribution, which can vary pixel-by-pixel. The camera contains CCD connected by fiber-optic plate to scintillator, which converts X-ray to visible light. The fiber optics plate contains different types of fiber glass packed to periodic structure by such way that produce minimum cross-talk between individual fibers. The scintillator contains small grains and local efficiency can vary dependent on packaging density of the grain powder. It is why uncorrected image from the camera, shown below, clearly displays periodic structure from fiber-optics and random brightness variation from scintillator grainy structure.

Uncorrected image from the camera (magnified small part):



Based on the saved dark and bright reference maps, flat-field correction procedure calculates pixel-bypixel intensity by following way: dark field intensity in every particular pixel considered as 0% and bright-field intensity in the same pixel considered as 100%. Intensity of the signal coming from the camera for every pixel normalized to such local 0...100% range to compensate any differences in dark current, scintillator efficiency and local transparency of fiber optics.

The same part of the image as in the previous picture, but after applying flat-field correction:



The SkyScan1272 scanner keeps flat-field references for all image formats, all camera positions against X-ray source and all filters. For offset camera positions we need only bright fields because in offset positions X-ray illumination from the source may be difference, but dark field will be identical because all offset images are taken with the same exposure. Totally control software keeps:

4 image formats x 7 possible camera positions x 6 filters = 168 bright reference maps and

4 image formats x 3 position with different distance to the source x 6 filters = 72 dark reference maps

All listed maps are pre-acquired and saved to the files in "FFreferences" subfolder. The filenames contains "white" for bright reference fields, "black" for dark reference field with following three digit indexes, which indicates image format, filter and camera position. Full set of flat-field references

requires significant disk space – 1.7GB for scanners with 11Mp cameras and 2.5GB for scanners with 16Mp cameras. Every time when user saves specific configuration, all flat-field references will be duplicated to "configurations" subfolder. Keeping big number of user-specific configurations may require a lot of disk space.

During normal operation control software updates flat-field reference fields used for image correction in every change of image format, filter or camera position. All updates executed completely automatically and don't require any operator's actions.

To refresh flat-field references one can use Menu -> Options -> Scanning Modes dialog. Dependent on selection in the bottom part, it will update some or all flat-field reference files. Be sure that there is no object in the field of view during updating flat-field references.

Dark-field references are relatively stable because of precise temperature stabilization of CCD in the camera. You have to update dark field references only in the case of modifying camera exposure. Bright-field references are less stable because of slow changes of local scintillator and fiber-optic properties during long acquisitions. They should be updated also in the case of changing exposure, but also periodically every 1-2 months.

Applying flat-field correction can be switched ON and OFF in Menu -> Options -> Preferences of by mouse click to "ff" or to the beginning of the "flat-field correction OFF" shown in yellow at left top corner of the imaging area.

6.3 How to select energy filters and X-ray source settings

Standard settings for every energy filter include specific voltage and current for X-ray source, which adjusted automatically during filter changing. Factory pre-set chosen by such way that filters with high cut-off energies will be used with high peak energy in X-ray source. Typical factory settings include 50kV peak energy (voltage) in X-ray source for "no filter" position of the filter wheel with increase by 10kV for following filters up to 100kV for most dense Cu filter.

For imaging in low-dense materials or in materials with small average atomic number, use low energy and "no filter" or thin Al filters. To transmit through dense materials or through the samples with high average atomic number, use Al/Cu or Cu filters with associated high energies from X-ray source. By clicking right mouse button on the imaging area you can show/hide horizontal intensity profile through selected by mouse position. The profile contains straight lines for indication minimum and maximum values and numerical information with minimum, maximum and average signal intensity in the profile. By choosing filters try to adjust object's transparency (or minimum intensity in signal profile) between 30 and 60% if possible. Transparency under 10% may create significant artefacts on the reconstructed images. Transparency more than 70-80% may increase noise on the reconstructed slices.

In most cases control software can adjust proper filter and X-ray source voltage automatically according to X-ray transparency of the object. After getting image of the object on the screen call Menu -> Options -> Filters -> Select Automatically or use similar selection in pop-up menu after clicking to filter indication area in the toolbar. The software will start from "no filter" position of the filter wheel and corresponding low peak energy in X-ray source and gradually increase voltage and filter density/thickness with measuring object transparency for every setting. The procedure will be stopped as soon as object transparency will reach 30-40% or in the highest X-ray energy and most dense filter.

6.4 How to select scanning parameters

Scanning Options dialog shown before every scan contains number of parameters, which can influent to quality of acquired information and results of reconstructions. In this paragraph we will explain what particular influence of every parameter selected.

Scanning Opt	tions	
	Filename prefix: newscan	
	Data Directory :	browse
	F:\Results\test	
\sim	Rotation step (deg) :	0.3
	Averaging (frames) :	2
	Random movement (pix) :	30
	360 deg scanning	
	Select filter automatically	
	X-ray OFF after scanning	
	Open specimen chamber af	ter scanning
	Send e-mail after scanning	to address :
	myname@my	company.com
	send test e	e-mail now
	Pixel Size :	
	© 5.4um ⊚ 10.8um ⊚ 16.2	um 🔘 21.6um
÷	Partial width : 72%	
	Standard scan in central car	mera position
	Offset scan with two camer	a positions
	Offset scan with three came	era positions
	snap wide	image now
	scan position: 30.000	47.699
	Estimated scanning time: 45 mi	n
	Dataset size = 0.82 GB	LCP.
	Free usk space=4446.67	00
	ОК	Cancel

Rotation step is mainly dependent on image format. In most cases good quality can be obtained by choosing rotation step as 360 degrees divided by number of pixels in the line. For faster scanning rotation step can be 2-4 times bigger than this value. For scanning using several camera offset positions, rotation step should be reduced proportionally to number of offset positions. Minimum rotation step is 0.01 degree. Any selection of rotation step smaller than that value will command to control software for acquiring set of images without object rotation. It can be used for investigation of dynamic changes in the object. In this case operator can select maximum number of images to be acquired and time interval between acquisitions in following dialog.

Averaging of several frames in each angular position allows reducing noise, but increases acquisition time. For objects with very high or very low absorptions, number of frames should be higher because useful part of camera dynamic range between signal inside the object and minimum or maximum level will be small and to extract this small signal from the noise one have to use big number of frames in averaging.

Random movement of the object, which applied during rotation to next angular position, with corresponding movement of readout area from the camera allows reducing ring artifacts in the reconstructed slices. Random movement maximum amplitude

can be adjusted in number of pixels for vertical image shift. Corresponding physical movement of the object will be calculated according to magnification. In the case of low magnification and small number of frames in averaging, moving object up and down can take significant time, which will be added to the scanning time. We would not recommend using this option for the scans with pixel size smaller than 3-5 microns because limited mechanical accuracy in vertical movement can add unsharpness in small details on reconstructed slices.

The next option allows switching from standard scan using 180 degree +opening of the cone beam rotation range to 360 degree rotation range. In relatively rare cases it can reduce artifacts around small dense inclusions or in other special cases, but requires near twice scanning time.

The option "Select filter automatically" will initialize automatic filter selection described in the previous chapter before starting scanning.

The next option allows automatically switching OFF X-ray source in the end of the scan. The source will be also switched off if you will choose to stop scanner and / or shut down computer in the end of the scan. Such options are available in the progress dialog, which will appear on the screen after beginning of the scan.

The next option will command software to "Open specimen chamber after scanning".

Last option in this section of the dialog will activate e-mail reporting in the end of the scan. E-mail will be sending to selected address and will contain direct link to the datafolder with results, which allows inspecting the results from computer where you will receive this e-mail. This option is activated only if mail server already defined in the Menu -> Options -> Scanner Settings. After selecting your particular e-mail address first time check this mail reporting by "send test e-mail now" button. If e-mail from scanner's mailservice will not be found in your Inbox, check that your e-mail defined correctly and try to find it other folders connected to your Inbox, for example – in Junk E-mail in the case if it moved by spam-filter.

The next part of the Scanning Options dialog allows selecting image format for scanning with indication of corresponding pixel sizes.

Following part of the dialog provides control over size of the acquired images. It contains slider for adjustment of partial horizontal size, which will be indicated as a dotted frame on the screen, and selection for three options of offset scans: standard scan with only central camera position, scanning using two or three offset camera position with automatic connection of all partial images to wide-format images in the end of the scan. The button in the bottom part allows acquire image using two or three camera offset positions to check that object fits to the field of view.

Last control section of Scanning Options dialog indicates scan position for single scan without possibility to change values, but for oversize scan explained in the next paragraph this part will contain editable fields to adjust top and bottom of the scanning area by numerical values.

Bottom part of the dialog contains scanning time estimation and indication of available and required disk space. If current location is too small for required dataset, try to find better location using "browse" button above "Data Directory" indication in the top of dialog.

Dependent on scanning time and setting in Options -> Preferences dialog named "Add drift compensation scan if main scan longer than ... min" the main scan may be accompanied by relatively short post-scan with small number of angular positions, which will be used by reconstruction program as a reference for compensation of possible drift during long scan. The slider, which defines threshold time for executing short post-scan in the Preferences dialog can be pushed to leftmost or rightmost position, which will switch this option to "always" and "never" independent of duration of main scan.

6.5 Scanning long samples and batch scanning

The control software provides possibility to scan long sample using multiple partial scans with automatic connection to one set of results during reconstruction. It also allows creating list of scanning for multiple samples (part or all of them can require multiple partial scans) with following automatic

execution in batch mode. For every sample in the list all scanning parameters can be adjusted individually.

To activate batch/oversize scanning mode go to Menu -> Options -> Oversize and Batch Scanning. It will add vertical narrow view to the left side of the screen and (if selected in Options -> Preferences) will start scout view with collecting overlapped images using vertical stepping starting from the topmost position of the object stage. The images will be combined together to continuous image, which will be updated after every arrival of partial image. The scout scan can be interrupted any time by using Cancel button in progress dialog.



To scale up or down particular view, you can move separator between images left and right. Draw line by left mouse button on the scout view to measure distances. Use right mouse button to open submenu, which allows activating scout scan again (for example – after changing image magnification), Close Scout View and Show/Hide Batch Dialog. The Batch Dialog wills pop-up on the screen as soon as you will select first part for scanning on the scout view. You can do it by drawing line on the scout view using left mouse button and keeping Ctrl on keyboard. In this case distance measurement will be accompanied by indication for number of partial scans, which will be used to scan selected object's length. After releasing left mouse button, control software will suggest to add this scan to the batch and (if you confirm it) will allow selecting scanning parameters and afterwards will add selected scan to Batch and Oversize Scanning dialog. You can select as many parts on the scout view as you want. Every part can be scanned with individual scanning settings. To edit scanning settings for particular scan, click twice to corresponding line in the list inside Batch and Oversize Scanning dialog. You can also add single scan selected by previously explained way (not from scout view) as well as delete one selected or all scans from the list. To move object to particular position, click twice to this position on the scout view.

After completing the list of required scans, start execution of in the batch mode by "Start Scan" button. After starting scanning all editing buttons in this dialog will be greyed and "Start Scan" button will be converted to "Abort Scan", which will allow interrupting scanning sequence.

6.6 Using shortcuts to simplify scanner control

There is number of shortcuts, which can simplify and accelerate scanner control by operator. Most of them can be seen at Menu -> Help -> Shortcuts. You can also print list of shortcuts from the file "shortcuts.txt" in the folder with control program or keep this list on the screen by opening it by any text editor. This paragraph will explain how to work with most useful shortcuts and to access different functions by simplified way.

If X-ray is ON and yellow "X-RAY" sign can be seen on the screen, double mouse click to this sign will open X-Ray Control Panel by the same way as Menu -> Options -> X-Ray Source.

Use left mouse button on the imaging area to measure distances or right mouse clock to show/hide intensity profile.

To move object up or down draw vertical line by left mouse button with Ctrl on keyboard from current position to new one. The object will be moved up or down by selected distance (for any magnification).

For centering object by micropositioning stage find angular position when image of the object most offsets from the central line and draw horizontal line using left mouse button with Ctrl on keyboard from central point of the object to central marks (small yellow lines) on the image. After releasing mouse button object will be moved to required distance. Remember that micropositioning stage works only with objects on the brass sample mounts installed to the collet in the middle of turntable and cannot move samples installed to the large steel mount or on the mounts from automatic sample changer.

Flat-field correction can be switched ON or OFF by double click to yellow text in the left top corner of the image.

Any click to numerical values in the status bar opens dialog for adjustments by exact numerical values. Any click to filter or image format indication in status bar allows selecting new filter or image format from pop-up submenu.

For testing field of view in offset scan with two or three camera positions we would recommend to stay in image format with smallest number of pixels to accelerate acquisition and connection of partial scans. Use Ctrl+w to start offset scan using two camera positions. Use Ctrl+Alt+w to start offset scan with three camera positions.

Double click by left mouse button to particular place on the scout view will quickly move object to selected vertical position.

Image from visual camera can be placed anywhere on the screen including areas outside main window and on the different monitor. This image can be scaled separately by dragging sides of the image window.

7 CONTROL PROGRAM FUNCTIONS

7.1 General layout

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 in *Service Mode* press Ctrl+Alt+Shift+s on the keyboard. The same combination of keys pressed again will cancel *Service Mode* and restore the access protection.

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



Under Windows 7 you can also maximize program window by dragging title bar to the top of the screen and restore window size by dragging it out. Docking window to the side of the screen will resize it to fit vertical screen dimension.

7.2 Toolbar



The toolbar is displayed across the top of the control program window. It provides quick mouse access to most usable function of the control program.



7.3 Status bar

)
• [•] 2	21.6um 🗘 🌓	0.0 ° 主 😨 30.000 mm	₩ 1224 x 820	🤹 → AI 0.25mm	cam:far

The status bar is displayed at the bottom of control program window. It contains a slider to adjust magnification, a slider for object rotation, control pane for object elevation, control pane to select image format, control pane for selection of filters in x-ray beam and information pane to display current camera position of CCD sensor temperature.

First two sliders can be controlled by clicking mouse to selected final designation (not by sliding to new value). Expected new angular position or image magnification can be seen in tooltip during moving mouse cursor over the slider. Clicking to numerical value allows adjusting corresponding parameter by introducing exact value to the following dialog.

Third pane allows moving object stage up or down by pushing to button with arrow. Like in previous controls, clicking to numerical value allows vertical object positioning by direct numerical selection. In the case of using arrow buttons, elevation speed will be adjusted according to image magnification.

Forth field shows current image format. To change it one can click to numerical indication and select new format from pop-up submenu. Particular list of formats can be different from shown below because it is camera dependent.

	8 2	1224 x 820	
	\checkmark	1224 x 820	
		1632 x 1092	
		2452 x 1640	
		4904 x 3280	
_			

Fifth field allows changing energy filters by clicking to description of currently used filter with following selection of new one in pop-up submenu:



"Select automatically" mode is available only if X-ray is ON and scanner not in the sample scanning process. In this mode the software will try to find most optimal combination of energy filter and peak energy of X-ray source for your particular object.

The information pane on the right hand side of the status bar shows current camera position against X-ray source. There are three possible positions: close, middle and far. The position is automatically selected by software according to required magnification to get shortest possible scanning time. The same information pane will show cooling process for CCD in X-ray detector until it will reach stable temperature to ensure high imaging quality. You can acquire images and even start scanning without waiting to the moment when information pane will be switched from temperature indication to camera position indication, but acquiring flat-field reference fields recommended only after temperature stabilization, i.e. starting from the moment when information pane will display camera position instead of CCD temperature.

During moving mouse cursor across toolbar buttons, left corner of status bar will display context help for corresponding controls.

7.4 Actions menu



Specimen Chamber Open/Close opens or closes specimen chamber door to install or replace the specimens.

X-Ray Start/Stop starts or stops X-ray source.

Grab Image starts or stops continuous acquisition of X-ray images with displaying on the screen.

Snap Image acquires single X-ray image and displays it on the screen.

Snap Double Width acquires X-ray image using two camera offset position to double horizontal coverage of the object.

Snap Triple Width acquires X-ray image using three camera offset position to triple horizontal coverage of the object.

Open Image reads image from the disk and displays it on the screen.

Save Image writes currently displayed image to the disk.

Delete Dataset removes full dataset from the disk by selecting dataset prefix.

Start Scanning initializes scanning process after selecting scanning parameters.

Start Reconstruction starts 3D reconstruction program with sending last scanned dataset to reconstruction.

Start Volume Rendering starts external program named CTvox for realistic visualization of reconstructed results by volume rendering.

Start DataViewer starts external program named DataViewer to show reconstructed results as sliceby-slice movie or as three orthogonal slices crossed in any point of reconstructed space.

Start 2D/3D Image Analysis starts external program named CTan for obtaining numerical characteristics of reconstructed structure wit possibility to send surface rendered 3D models to CTvol program for realistic visualization by surface rendering.

Exit command closes control program and shut down the scanner.

7.4.1 Open / Close Specimen Chamber



Use this command to open or to close specimen chamber's door to install or replace your specimen.

There are several sample mounts supplied with the system. To install small and medium size brass sample mounts insert cylindrical core of the mount in the central hole of the sample stage and tight it by turning brass nut clockwise. To remove the mount, turn this brass nut counterclockwise. The brass nut connected to very delicate and precise micropositioning drives: don't apply any significant force or torque.



For large and/or asymmetrical samples one can use steel sample holder with 4-jaw chuck. Firstly find such orientation of the holder which will get black connector on the object stage positioned against milled space on the holder:



Then attach the holder to the specimen stage and tighten the two screws by supplied screwdriver:



Before closing specimen chamber check that no objects are left on the black plate in the bottom of the chamber. Any object left on this plate will damage your scanner during specimen chamber closing.

7.4.2 X-Ray ON / OFF



This command starts or stops X-ray source. In the case of more than 8 hours after last usage of X-ray source, this command will start preheating process, which takes typically 15 minutes and will keep X-ray OFF afterwards. After opening-closing specimen chamber and after starting the scanner, short preheating may be also executed with keeping X-Ray source in selected position afterwards.

If "Start X-ray for imaging modes" selected in "Options" >>> "Preferences", you can start image acquisition by Grab, Snap and other imaging commands without prior switching X-ray ON because in this case the X-ray source will be started automatically when necessary.

7.4.3 Grab Image Continuously



This command starts / stops continuous acquisition and displaying of the images from X-ray camera. If option "Start X-ray for imaging modes" selected in "Options" >>> "Preferences" and X-ray source is not started yet, it will be started automatically during activation of image acquisition.

During image acquisition and displaying one can measure linear dimensions of the objects using left mouse button on the imaging area or display horizontal intensity profile through selected position by right mouse button. In the case of selection "Fit the window" in "Options" >>> "Preferences", the images acquired in any format will always be displaying completely, but large format images will be probably downscaled. If this option is unchecked, the images will be displayed in native format with possibility to scroll across large format images by side scrollbars.

7.4.4 Snap Single Image



Use this command to snap single x-ray image using with averaging of number of frames defined in "Options" >>> "Acquisition". It will interrupt continuous image acquisition if Grab command has been previously activated. The image can be saved by "Actins" >>> "Save image" command. If option "Start X-ray for imaging modes" selected in "Options" >>> "Preferences" and X-ray source is not started yet, it will be started automatically before snapping image.

After image acquisition and displaying one can measure linear dimensions of the objects using left mouse button on the imaging area or display horizontal intensity profile through selected position by right mouse button. In the case of selection "Fit the window" in "Options" >>> "Preferences", the images acquired in any format will always be displaying completely, but large format images will be probably downscaled. If this option is unchecked, the images will be displayed in native format with possibility to scroll across large format images by side scrollbars

7.4.5 Snap Image with Double Width

Use this command to snap x-ray image with double width with automatic combining of two images in camera offset positions. Double width image allows checking proper fit for imaging large objects in particular magnification for following scanning using two camera offset positions. This command will interrupt continuous image acquisition if Grab command has been previously activated. The image can be saved by "Actins" >>> "Save image" command. If option "Start X-ray for imaging modes" selected in "Options" >>> "Preferences" and X-ray source is not started yet, it will be started automatically before snapping image. After image acquisition and displaying one can measure linear dimensions of the objects using left mouse button on the imaging area or display horizontal intensity profile through selected position by right mouse button. In the case of selection "Fit the window" in "Options" >>> "Preferences", the images acquired in any format will always be displaying completely, but large format images will be probably downscaled. If this option is unchecked, the images will be displayed in native format with possibility to scroll across large format images by side scrollbars.

7.4.6 Snap Image with Triple Width

Use this command to snap x-ray image with triple width with automatic combining of three images in camera offset positions. Triple width image allows checking proper fit for imaging large objects in particular magnification for following scanning using three camera offset positions. This command will interrupt continuous image acquisition if Grab command has been previously activated. The image can be saved by "Actins" >>> "Save image" command. If option "Start X-ray for imaging modes" selected in "Options" >>> "Preferences" and X-ray source is not started yet, it will be started automatically before snapping image. After image acquisition and displaying one can measure linear dimensions of the objects using left mouse button on the imaging area or display horizontal intensity profile through selected position by right mouse button. In the case of selection "Fit the window" in "Options" >>> "Preferences", the images acquired in any format will always be displaying completely, but large format images will be probably downscaled. If this option is unchecked, the images will be displayed in native format with possibility to scroll across large format images by side scrollbars.

7.4.7 Open Image

Ctrl+O



You can select a location of the file using left hand side of the "Open" dialog, file format - in the right bottom part and choose file, which you want to open by typing filename in the bottom area or by double click to the file in the list of names or preview. Right mouse button on the central part allows rearrange and change type of visualization in the file list

7.4.8 Save Image

Ctrl+S

Use this command to save image shown on the screen to the file on the disk:



You can select a location of the file using left hand side of the "Save Image" dialog, file format - in the bottom part and choose file name, which you want to use by typing in the corresponding editable area. Right mouse button on the central part allows rearrange list of existing files and change type of visualization in the file list.

7.4.9 Delete Dataset

This function allows deleting complete dataset by choosing filename prefix. Corresponding submenu gives possibility to remove 16-bit TIFF files (typical format for projection images before reconstruction) or 8-bit BMP / JPEG files (typical format for reconstructed slices).



After selecting file type the software will show list of prefixes from all datasets available in current Data Folder. Selecting one of listed prefixes will remove all files from corresponding dataset.

You can switch to another Data Folder by "Options" >>> "Data Folder" command.

7.4.10 Start Scanning



Use this command to start scanning your sample. The software will open *Scanning Settings dialog (see Menu => Options => Acquisition)* for selection for filename prefix, data folder and all scanning parameters. In the bottom of this dialog you will see estimation of scanning time and required/available disk space on the drive selected for dataset. Select "Scan" button if you want to start scanning or "Close" to store selected scanning settings without starting scanning process.

During scanning status bar will show current angular position of the sample and progress dialog display scanning status and measured time till the end of the scan. It is also give possibility to shut down control software and scanner in the end of the scan and shut down computer in the case of long unattended scan. These two selections can be changed any time before the end of the scan and will be taken into account only in the moment when scanning process will be successfully finished.

7.4.11 Start Reconstruction



This command starts reconstruction program NRecon. In the case of only one dataset presents in the current Data Folder, it will be send to reconstruction directly. If multiple datasets exist in the current Data Folder, you will be able to select one, which you want to reconstruct, using following dialog with list of dataset prefixes.

The reconstruction program can work on CPU-only mode with multithreading or in GPUaccelerated mode, which can work 5-30 times faster in the case of using high performance graphical card.

You can download from bruker-microCT website complete User Manual for NRecon reconstruction program as well as GPU-accelerated reconstruction Installation Guide.

7.4.12 Start Volume Rendering



This command starts external program named CTvox for volume rendering from reconstructed cross sections. This program allows getting realistic visualization of reconstructed microstructure including object and viewing point manipulations, creating lighting and shadow effects, making virtual cut by number of shapes and sizes and easy creating of movies with flight around or inside the objects. The program also supports data export to mobile devices, such as IPhon, iPod and iPad, with following volume rendering and manipulations by simple finger touches.

To get more detailed information about CTvox program, download "Quick Start Guide" from Bruker-microCT website.

Latest version of CTvox program can be also downloaded from <u>bruker-microct website</u>. Pay attention that probably you will need to update graphical card driver to latest one available in the website of graphical card producer.

7.4.13 Start DataViewer



This command starts external program named DataViewer, which allows showing reconstruction results saved in BMP or JPG format as slice-by-slice movie or in the form of three orthogonal virtual slices crossed in any point of reconstructed volume. It also allows distance measuring in 3D, rotating and re-slicing dataset in any alternative orientation.

To get more details about menu functions and toolbar buttons, use context help button on the right of toolbar with following click to the item, which you want to clarify.

Most recent version of DataViewer program can be downloaded from *bruker-microct website*.

7.4.14 Start 2D / 3D Image Analysis



Selection in the menu "Actions" >>> "Start 2D/3D Image Analysis" sends reconstruction results to external program named CTan, which can perform 2D and 3D numerical image analysis, build triangulated surface rendering and send it to CTvol program for realistic visualization.

Most recent version of CTan/CTvol programs and complete User Manual can be downloaded from *bruker-microct website*.

7.5 Options Menu



Acquisition opens dialog to choose acquisition parameters, which will be applied during scanning.

Data Folder allows selecting the folder where results of scanning will be placed.

X-Ray Source opens control panel for X-ray source with possibility to adjust accelerating voltage, beam current and get information on source status and parameters.

Filters allows selecting the filter, which will be placed in the X-ray beam by automatic filter changer or allows control program to choose filter automatically;



MicroPositioning opens dialog with access to object movement by micro-positioning stage integrated to the instrument.

Visual Camera ON/OFF opens or hide additional window, which displays visual image of the object inside the specimen chamber.

Oversize and Batch Scanning allows selecting several scans to be executed automatically and/or define position of the scanning of the object longer than vertical field of view by multiple partial scans, which will be afterwards automatically connected.

Preferences opens dialog to select user wishes in how the program will display the images and act in different situations.

Configurations submenu allows to *Save*, *Load* or *Delete* user-defined configurations, load original factory *Preset* or extract scanning settings *from the log-file* associated to one of previous scans:

Configurations •	Save
View >	Load
Sample Changer Settings	Delete
Alignment test	Preset
Scanning Modes	Get Settings from *.log File

View allows changing application look and customizing toolbar.

Sample Changer Settings (available only if sample changer installed and connected) opens dialog with choice of the options and behaviours for automatic sample changer. This item is available only if sample changer connected to the scanner.

Next five items in Options Menu are accessible only in Service Mode

Alignment Test analysis and corrects mechanical misalignment between X-ray source, object stage and X-ray detector.

Scanning Modes shows and allows modifying detector exposures, X-ray source voltage / current and standard scanning rotation steps for all possible camera / source / filters positions and modes. This command also allows collecting/updating flat-field reference maps.

Scanner Settings opens access to calibrating parameters of the scanner.

Trajectory Calibration starts tests for rotation trajectory definition and after corresponding analysis saves trajectory rectification calibration.

Sample Changer Calibration (available only if sample changer installed and connected) allows precise adjusting sample changer movements during sample insertion and removing from the scanner.

7.5.1 Acquisition Options

The function accessible through "Options" >>> "Acquisition" menu and also called every time before starting scanning (with additional button "Scan" in the bottom of the dialog). This command opens following dialog:

Scanning Op	tions 🛛
	Filename prefix: newscan
	Data Directory : browse
	F:\Results\test
	Rotation step (deg) : 0.3
	Averaging (frames): 2
	Random movement (pix) : 30
	360 deg scanning
	Select filter automatically
	X-ray OFF after scanning
	Open specimen chamber after scanning
	$\overline{{oldsymbol V}}$ Send e-mail after scanning to address :
	myname@mycompany.com
	send test e-mail now
Q	Pixel Size :
÷	Partial width : 72%
	Standard scan in central camera position
	Offset scan with two camera positions
	Offset scan with three camera positions
	snap wide image now
	scan position: 30.000 47.699
	Estimated scanning time: 45 min
	Dataset size = 0.82 GB
	Free disk space=4446.67 GB
	OK Cancel

Top part of the dialog allows selecting *Filenames prefix* and *Data directory*. Button *Browse* calls dialog for selecting existing data folder or creating new one.

Next part of the dialog allows choosing scanning parameters, such as *rotation step*, number of frames in *averaging* in every angular position, and others. Averaging and Random movement options can be activated or deactivated without changing parameters. Deactivation of averaging is equal to selecting one image in averaging; deactivation of random movement equal to zero movement amplitude.

Option Send e-mail after scanning is available only if address of your mail server already defined in *Scanner Settings (see Menu -> Options -> Scanner Settings)*. After selecting your e-mail address first time we recommend you to test it by sending *test e-mail* using button under address selection field. Next switch selects camera format with indication which *pixel size* will be used for every format in current magnification.

Partial width selection allows saving disk space in the cases when object takes only part of the width of the image. Be sure that object will not run out of this part during rotation in scanning. During adjustment selected part will be marked on the image.

Following switch allows collecting images in central *camera position* or using two - three offset positions to extend horizontal field of view. A button under this switch will *snap* and show single image using selected number of offset camera positions. Using offset camera positions can be combined with partial width for exact selection of scanning space.

Scan position shown in the next field becomes editable only for oversize scans when this dialog activated by selecting big length of the object for scanning on the scout view.

Bottom part of the dialog indicates *estimated scanning time* (in the batch scanning - for this particular scan only) as well as required and available *disk space* in current location.

7.5.2 Data Folder

Use this command to select or to change current data folder where the software will store your scanning results.

Browse For Fol	lder			x
Select datafo	lder:			
📃 📃 Deskte	op			^
🛛 🖂 🗌 Libr	aries			
🛛 🛛 😹 Skys	scan			
🔺 🖳 Con	nputer			
D 🏭 La	ocal Disk (C:)			=
⊳ 🔐 D\	/D RW Drive (D:)			
a 🔊 Ke	esults (F:)			
Þ 🆺 I	Results			
Þ 퉲 (tmp			
🛛 🖓 Netv	work			
⊳ 👰 Con	trol Panel			
Reco	vcle Rin			Ŧ
<u>F</u> older: F	Results			
Make New	Folder	ОК	Cancel	

You can select one of the existing folders accessible on the local computer or through your network connections or create new one by "Make New Folder" button.

Before every particular scan you can still modify data folder or choose / create different one.

7.5.3 X-Ray Source

This command opens control panel for X-ray source:

X-ray Source Console	
Control Warm-up Info	
100 kV	Stop
Focal spot: Small v x Voltage: 100 k kV Current: 100 k uA	X-ray Warm-up Preheat Overload Interlock Error

The control panel has three tabs: control, warm-up and info. All tabs contain *voltage* and *current* indication, *Stop* button and status indicators in the right hand side.

The *control* tab allows changing source's voltage and current. A manual change of spot size is not accessible for used type of the source. The source adjusts spot size automatically according to selected power to ensure its long lifetime.

The *warm-up* tab indicates progress in warm-up process.

The *info* tab shows source type, connection, power-on time and emission time.

7.5.4 Filters

You can change energy filters by choosing "Options" >>> "Filters" submenu:



You can also select filter using pop-up submenu in status bar, which can be called by mouse click to indication of current filter:



Both submenus are identical and include list of six possible filters installed in automatic filter changer. In the case of customization, the filter names can be edited in "Options"-> "Scanning Modes" dialog.

The option "Select Automatically" is available only if X-ray source is ON. Selecting this option starts automatic procedure, which analyze local absorption of the object in X-ray images using different filters and X-ray energies starting from "No filter" up to the filters with higher absorption. This procedure will be stopped on the filter, which will create X-ray image with 30-45% opacity in the densest parts of the object.

If object is too dense to reach this value, automatic selection will be stopped in the position with maximum energy in X-ray beam and most dense filter.

7.5.5 MicroPositioning

Use this command to open dialog for controlling micropositioning stage (not accessible with sample changer):



Every mouse click to buttons with left or right arrows will command micropositioning stage to move object by such way, that operator will see it as left or right independent of current angular orientation of object stage. Central button moves object to central position. This position will be close to position of rotation axis, but may be not exactly aligned to it.

To get best object positioning for scanning, turn it by small interval to find most offset position. Afterwards move it by micropositioning stage to the central position. With well centered object you can adjust higher magnification and still keep the object fully inside the field of view.

Object micropositioning can be also dove without opening micropositioning dialog. One can drag mouse in horizontal direction (left mouse button) keeping Ctrl key pressed in the moment when mouse button will be released. During dragging you will see magenta line with distance indication. After releasing mouse button with Ctrl pressed on keyboard, object will be moved by micropositioning stage to corresponding distance.

Small vertical yellow lines in the top and bottom of imaging area indicate position of rotation axis and can be used as a reference for object centering.

7.5.6 Visual Camera



This command opens or closes window, which shows the images from the visual camera in the back side of the object stage. Use these images to avoid object collision against X-ray source. If visual camera used during continuous acquisition of X-ray images (Grab mode), the pictures will be updated every time after receiving X-ray image, which can be slow in the case of long X-ray exposures. The update rate can be increased by switching OFF Grab mode for X-ray camera. The Visual Camera window can be scaled by dragging window's corners and sides. The software will always try to fit this window by most detailed image from the camera.

7.5.7 Oversize and Batch Scanning

This command opens scout view in the left part of imaging area and starts (if selected in "Options" >>> "Preferences") scout scanning by combining number of images with object movement in vertical direction:



For objects with limited length, scout scanning can be interrupted any time by "Cancel" button in progress dialog. Otherwise it will continue up to the end of possible vertical travel.

To zoom in / zoom out scout view, one can move the splitter between two views by mouse.

Dragging with left mouse button will show dimensions on the scout view. Dragging with Ctrl keyboard key allows selecting of object height, which will be added to the batch scanning. The scanning length can be larger than single field of view. In this case the scanner will make number of partial scans with some overlap and during reconstruction they will be combined together to complete reconstructed volume. After every particular selection the software will suggest to add selected scan to the batch list:

SkyScan127	72 X
?	Add selected part to batch / oversize scans?
	<u>Y</u> es <u>N</u> o

After confirmation the software will allows selecting scanning parameters using *Scanning Options* dialog with following adding the scan to the batch list:

Batch and Oversize Scanning				
	FileName Prefix foam1 foam2 foam3	Position 20.079-28.945 mm 14.342-16.715 mm 9.648-15.932 mm	Estimated Time 1h:7m 0h:22m 0h:45m	
	foam4	7.562-9.074 mm	0h:8m	
Add Delete Delete All Start Scan				

Any record in the batch list can be edited by double mouse click to select different scanning parameters. Single mouse click will select particular line, which can be deleted by corresponding button in the bottom.

Start Scan button starts execution of all scans listed in the batch dialog. During scanning different lines in the batch list will be highlighted by colour with indication of the current status in the right column:

Batch and Over	size Scanning		Z
	FileName Prefix foam1 foam2 foam3 foam4	Position 20.079-28.945 mm 14.342-16.715 mm 9.648-15.932 mm 7.562-9.074 mm	Estimated Time done scanning 0 h : 45 m 0 h : 8 m
	Add	Delete Delete A	Start Scan

Right mouse click to scout view opens scout submenu:

Rescan Scout View
Close Scout View
Show/Hide Batch Dialog

This submenu allows repeating scout scan, to close scout view and show or hide Batch Dialog.

7.5.8 Preferences

This command opens dialog for setting your preferences in the program usage:



First two checkboxes allows switching *flat-field* and *geometrical correction* ON and OFF. Flat-field correction can be also switch ON or OFF by double mouse click to "ff" (if it is already ON) or to the beginning of "flat-field correction OFF" (if it is currently OFF) shown in the left top corner of the imaging area.

Fit the window option allows rescale images to fit current size of the imaging area in the program window. If this option is unchecked, the images will be shown in their native format with scroll bars along right and bottom side (in the case if image format larger than imaging area).

Start X-ray for imaging modes will start X-ray source in selection of any command, which requires X-ray imaging, such as Snap, Grab, Start Scanning, etc. Using this option allows avoiding clicking to X-ray Source button before selecting any imaging command.

Sound notification switches ON or OFF playing short soundtracks for indication of beginning or ending some functions and for attention to error messages.

Drift correction scan slider allows adjusting minimum scanning time, which will activate following drift correction scan. Put this slider to leftmost position is you always want to get drift compensations scan or to rightmost if you want that this scan will never be activated.

Start scout scan when oversize or batch scan selected will automatically split imaging area to scout view strip in the left and main imaging area in the right and afterwards start scout scan immediately after selecting "Oversize and Batch Scanning" in "Options" menu. If this box stays unchecked, the imaging area will be split (if it is not split yet), but scout scan will not start automatically. To start or restart scout scan and to close scout view one can use corresponding commands from submenu, which can be called by right mouse click on the scout area.

Keep illumination ON when specimen chamber open allows keeping illumination, which is normally used for object imaging by visual camera, during specimen exchange, which can be helpful in the case of working in dark environment.

7.5.9 Configurations: Load, Save, Delete, Load from log-file

Use *Load* command to load scanner configuration from the one of previously saved on the disk. The software will allow choosing configuration by name with following loading all scanning parameters and all flat-field reference maps.

To load factory preset use "Options" >>> " Configurations" >>> "Preset" command.

Use *Save* command to save current scanner configuration to the disk. The software will allow choosing unique configuration name and will save all scanner settings and flat-field reference maps.

If control software is running in *Service Mode*, be careful not to overwrite "std" configuration name, which contains original factory presets.

Delete command allows deleting previously saved scanner configuration from the disk. Use this command to save disk space because every configuration contains big number of large-format reference files and can occupy up to 3GB disk space.

If control software is running in *Service Mode*, be careful not to remove "std" as a name of configuration, which contains original factory presets.

Get settings from log-file command allows extracting scanner settings from the log-file associated with previously scanned dataset. The dataset should be created by SkyScan-1272 system with the same camera format. The software will extract from the file and set to the scanner corresponding camera mode, filter position, X-ray source voltage/current, partial scanning width, rotation step and other scanning parameters, etc. It will also notify operator about image magnification mentioned in the log-file, but will not adjust it automatically to avoid damaging of the scanner by moving large object too close to X-ray source.

7.5.10 Sample Changer Settings

The command opens following dialog:



A checkbox *Create new subfolder* allows distributing datasets from different scanned objects to subfolders with incremental numbers in folder names. Current number can be edited and reset in the line under this checkbox. Next checkbox control position of specimen chamber door in the end

of scanning all samples in the changer. Next setting allows activating *e-mail* sending in the end of every scan or in the moment when all samples in the changer will be scanned.

Next field controls behaviour of the image from the visual camera, which is displaying in the top of Sample Changer dialog. During sample changing this image allows analysing diameter of the neck on the sample mount for proper vertical positioning, which ensure scanning of the object above the top of the mount. Show profile and mount size option will display intensity profile on the image over the neck of the mount and results of mount diameter analysis on this image. Take new image on click allows updating image to the recent one from the camera by mouse click to the image in the Sample Changer dialog. If this option is not selected, the dialog will keep image of the object after installing until this object will be replaced to next one.

Bottom area adjusts scanning parameters in the case of selecting "Auto" scanning protocol. Object magnification can be selected by one of following ways: *previous*, according to *sample size*, according to *object mount* size and *user defined*. Selection of previous magnification allows scanning all samples with exactly the same magnification. Choosing magnification according to sample size will activate special measuring procedure for every particular sample before the scanning. The measuring procedure will define highest possible magnification where full object will be inside field of view for all angular orientation and will scan every object using such automatically selected magnification. Selection magnification from the object mount size requires selecting such type of object mount for every particular sample, which neck will be larger or equal to sample size. In this case the magnification selected from the diameter of the mount will keep full object inside the field of view. For User Defined magnification exact pixel size can be selected in next line of the dialog.

Z-offset allows selecting scanning position above or below standard scanning position for every particular sample mount. In the case of zero offset, scanning position will be set by such way that top of sample mount will be just below scanning area.

Image format drop-down list allows selecting camera format from four available. Following fields are switching ON or OFF and adjusting parameters as number of frames in *averaging* and *random movement* amplitude applied during scanning.

Next checkbox controls possibility for automatic selection of energy *filter* according to density and chemical composition in particular object. For every object it will execute the procedure similar to Select Automatically procedure in filter selection submenus. Last checkbox controls scanning over *360deg*.

If scanning protocol for particular object selected as Manual, scanning parameter can be adjusted individually using Scanning Protocol dialogs, which will be called for every object in the case of selection as Manual:

Scanning Prot	Scanning Protocol				
<u> </u>	Filename prefix:	F: \Resu	lts		
	Data Directory :		browse		
	08Jan13_02_				
α	Rotation step	(deg) :	0.600		
<u> </u>	🔽 Averaging (fra	ames) :	2		
	Random move	ment :	30		
	📃 360 deg scanr	ning			
\sim	Energy filter :	Al 0.25	imm 👻		
_	Pixel size (um) :		21.59		
Q	Image format :	2452x	1640 👻		
	Partial width :	100%	0		
	Vertical position (n	nm) :	16.874		
	Ok	<	Cancel		

Top two sections of this dialog are identical to corresponding parts of the *Scanning Options* dialog, which appears before every scan. Next drop-down menu adjusts *energy filter*. Bottom field of the dialog allows selecting magnification by defining *Pixel size*, choosing *Image Format* from four available, selecting *Partial width* for scanning if necessary and defining *vertical position* at which you want the object to be scanned.

7.5.11 Alignment Test

Before starting alignment, install alignment pin to the scanner, start grabbing X-ray image and adjust magnification as high as possible for keeping the pin inside field of view for all angular positions. Afterwards try to find angular position wit most offset position of the pin on the screen, turn by 180 deg. (any directions) and notice distance between those two positions. Now move the pin in the middle of two noticed positions using micropositioning stage or by horizontal dragging on the screen using left mouse button with Ctrl on keyboard. Check again that pin can turn now with less wobbling and always stays inside field of view.

Calling alignment function will open Alignment dialog:



Two options in the middle of the dialog allow analyze and compensate misalignment using one or two magnifications.

"Alignment test in current position" in the case of low magnification when object stage is close to the camera will align camera position. In the case of high magnification, when object stage stays close to the source, the alignment procedure will correct relative misalignment between the source and object stage.

Two-point alignment test allows align all parts of the scanner - source, object stage and camera - in one procedure by automatic analysis of pin movements using two different magnifications.

After performing misalignment analysis the software will suggest to compensate misalignment by moving scanner parts to necessary positions. In the dialog with misalignment test results one can also select additional option for image rotation to compensate angular misalignment between x-ray detector and rotation axis.

7.5.12 Scanner Settings

The command opens following dialog:



First line allows defining *serial number* of the scanner, which will be indicated in the log-files and identify this scanner in e-mails send automatically.

Next parameter - Specimen exchange position for object stage counted from home position in the right hand side of travel. In the proper exchange position specimen stage should be in the middle of the hole in the bottom metal plate. If scanner equipped with automatic sample changer, any modification of this position will require sample changer recalibration.

Object to source maximum distance is a parameter, which should be calibrated to get proper measurements of the sample sizes at highest magnification.

Pixel size at minimum magnification is a parameter, which should be calibrated to get proper measurements of the sample sizes at lowest magnification.

Image tilt shows compensating angle to align detector's columns exactly in parallel to rotation axis and can be analyzed and updated using "Options" >>> "Alignment" procedure.

Image shift in elevation measured in microns of horizontal shift per millimeter of object's vertical movement during elevation. This parameter allows proper connection of the partial scans during "oversize scans" for the objects longer than vertical field of view.

Exact pixel size of the camera adjusts proper horizontal connection between left and right images in offset scan; *vertical connection* adjusts proper matching in vertical direction.

Server to send e-mail messages can be define as four digit IP address of SMTP server (contact your system administrator to get this information), for example *192.168.140.21* or as a remote access to mail server, for example *mailhost.mycompany.de* Next two fields allows edition of <From> and <Subject> fields in the case of restrictions on local network.

Save events to log-file will update "SkyScan1272.log" file by indications of activity of control program, which can be useful for problem resolving.

7.5.13 Scanning Modes

The command opens following dialog:

Scanning M	odes								
	Camera format:	4904x32	80 24	52x3280	1632x32	80 122	2 <mark>4</mark> x3280		
R	Rot.Step (deg):	0.10		0.20	0.40		0.60		
~									
	Filter Name :	No Filte	Al 0.25r	Al 0.5m	Al 1mm	Al 0.5 4	Cu 0.25		
	X-Ray Source								
	Voltage, kV :	50	60	70	80	90	100		
	Current, uA :	200	180	160	140	120	100		
	Exposures (ms) for camera position far from the source								
	4904x3280	918	1950	2001	1939	2714	2824		
	2452x3280	971	2292	2902	3943	4083	5315		
	1632x3280	729	1701	2134	2869	3334	3867		
	1224x3280	614	1485	1676	2277	2928	3375		
Exposures (ms) for middle camera position									
	4904x3280	514	1170	1483	1615	1537	1588		
	2452x3280	608	1527	1934	2629	3055	3543		
	1632x3280	420	1134	1409	1894	2223	2578		
	1224x3280	363	990	1118	1518	1706	2046		
Exposures (ms) for camera position close to the source									
	4904x3280	316	715	736	810	948	1344		
	2452x3280	338	942	1085	1589	1847	2142		
	1632x3280	256	700	886	1180	1386	1446		
	1224x3280	218	544	689	917	1185	1237		
	Do not update flat-field references								
	 Update flat-field references for current mode only 								
	O Update flat-field references for all modes with changed exposure								
Update flat-field references for all listed modes									
	\checkmark use only central camera position								
✓ update only bright flat-field reference									
adjust exposure automatically									
					OK				
					UK		ancei		

Top part contains presets for *rotation steps*, which will be used for four possible camera formats. Next table defines camera *exposures*, X-ray source *voltage* and *current* for six possible filters. In the case of filter's customization, the names of the filters can be edited here.

Camera exposures can be set for every filter for every camera format and for three possible camera distances from X-ray source. The current mode will be highlighted.

All exposures can be edited manually or adjusted automatically (recommended) during acquisition of flat-field reference maps.

Bottom part of the dialog controls actions, which will be activated after pressing "Ok" button. One can choose simple exit or *update* for one / several / all *flat-field* reference maps. Following checkboxes clarifies what will be done during every update. One can select update only for *central* camera position (otherwise all possible offset positions will be updated), update *bright* reference only (otherwise white and dark references will be updated) and adjust camera *exposure automatically* in every update.

7.5.14 Trajectory Calibration

Pay attention that calibration profile saved by this function will be automatically applied to all scans. Any mistake in calibration can seriously damage quality of all scans.

Before this calibration scanner should be properly aligned using "Options" >>> "Alignment".

Next adjustment should be also done **before** calling for *Trajectory Calibration*:

Install supplied alignment pin to the scanner, select smallest image formal, no filter and image magnification corresponding to pixel size of 2.4um for systems with 16Mp camera or 3.2um for systems with 11Mp camera. Try to find rotational position where image of the pin will take most offset location from the central vertical line. Using *micropositioning stage control* or by positioning

using horizontal mouse dragging (left button) with Ctrl on keyboard, adjust pin position by such way that it will be inside field of view with small gap from the side of the image:



Turn object by 180 degrees from previous position (any direction) and check that now the object will be close to opposite side of the image, but still with small gap. If object touches image side, move it a little bit close to the middle to see certain gap. Turn by 90 degrees (any direction) and confirm that now the pin can be seen in the middle of field of view. If it not, try to find again angular position with most offset pin image and repeat adjustments.

After finding proper position, select highest camera format and go to "Options" >> "Trajectory Calibration". After warning message it will start calibration procedure, which can take one hour or more. In the end of calibration procedure the software will save binary calibration file into the file with name "SkyScan1272CC.clb" or "SkyScan1272CW.clb" dependent on direction of rotation during scanning selected in "Options" >>> " Scanner Settings". Te software will also save text version of calibration curve to the file with name "1272calibrationCC.log" or "1272calibrationCW.log" correspondingly. It allows checking quality of calibration by opening this file in text editor or importing it to Microsoft Excel.



Calibration curve should be smooth and without sharp jumps or gaps. Any breaks on calibration curve may indicate that calibration procedure has been done with mistakes.

7.5.15 Sample Changer Calibration

The command opens following dialog:

Sample Changer Calibration								
init rob	init robotic arm and all drives							
 Lifting object mour robotic arm : 	 Lifting object mount from sample changer: robotic arm : 							
up d	up down V position: 20.000							
forward bac	kward H posi	tion: 10.002						
carrousei :	ccw R posi	tion: 0.735						
check lifting check return								
STOP								
save lifting pos	save lifting positions load lifting positio							
Landing object mount inside the scanner: robotic arm :								
up d	own V posi	tion:						
object stage -	object stage :							
left r	ight H posi	tion: 0.0						
CW	ccw R posi	tion: 0.000						
check landir	ng che	check removing						
	STOP							
save landing po	sitions load la	load landing positions						
Check specimen exchange procedure :								
install	install remove turn to next object							
	STOP							
		close						

Init button in the top allows reinitialize all drive in the changer when necessary. Next three fields can be activated by selection in corresponding first line.

Selecting *Lifting object mount* field activates all controls in this field and moves robotic arm to the safe position close to the object mount on the carrousel. For calibration, an object mount should be inserted. Clicking to *up, down, forward* or *backward* buttons will start corresponding slow movement, which can be interrupted any time by *STOP* button. The object's carrousel can be aligned to proper angular position by *cw* and *ccw* buttons to start slow rotation, which can be interrupted any corresponding slow not start slow rotation, which can be interrupted by *STOP* button. Proper lifting position is a position of the fork on robotic arm with conical surface aligned with cylindrical neck of the sample mount and top of the fork aligned to the bottom of holding surface of the mount. After adjustments the operator have to check full lifting and return procedure by *check lifting / check return* buttons and, in the case of safe and successful execution, can save this calibration by *save lifting position* button. If sample changer was previously calibrated and you want to check it, load calibration by *load lifting positions* button and check lifting and return afterwards.

Selecting *Landing object mount* field activates all controls in the second area of the dialog. Be sure that landing pad already installed on the specimen stage of the scanner before this calibration:


After selecting this group of functions, robotic arm will be moved to the safe position above landing pad. Using *up*, *down*, *forward* or *backward* movements of robotic arm and *left*, *right* movements and *cw*, *ccw* rotation of the object stage, one have align landing position of object mount to the landing pad coming from the top to get the mount concentric to the landing pad. In vertical direction robotic arm should be in such position that bottom surface of the object mount will be pushed by fork to the top of the landing pad without applying any pressure to the pad. After finding proper position, one should verify it by *check landing* and *check removing* functions. In the case of successful calibration, all landing parameters can be saved by *save landing positions* function. If sample changer was previously calibrated and you want to check landing patterwards.

Third field in this dialog allows verifying full *specimen exchange procedure*. Dependent on presence of sample mounts in the carrousel positions and inside the scanner, use *install* or *remove* functions. ATTENTION! Attempt to install object mount when another object mount is already on the landing pad will damage the sample changer and the scanner.

Turn to next object function will turn carrousel to next position. In the case of problem, the execution can be interrupted by *STOP* button.

Pay attention that only saved parameters from listed calibration procedure will be taken into account during sample changer operation.

7.6 Help Menu



Help Topics opens access to library of help resources with possibility to chose content or search by command name.

About SkyScan1272 opens "about box" with indication of current software version, copyright information and links to producer website and e-mail.

Shortcuts shows list of keyboard shortcuts for quick access to number of functions.

7.6.1 Help Topics

This command will open help window with contents, index and search tabs and selection of help topics by commands in right hand side:



From this screen you can jump to step-by-step instructions for using SkyScan-1272 control program and various types of reference information. Once you open Help, you can click to Contents tab whenever you want to return to the opening Help support.

7.6.2 About SkyScan1272

Use this command to display version number of your control software and copyright notice:



The dialog box also displays current CCD temperature in X-ray detector and allows sending email to producer and connecting to producer website by clicking to corresponding links.

7.6.3 Shortcuts

This command opens list of shortcuts for quick access to number of functions.

Shortcuts		
	F1 = Help Ctrl + O = Open image from file Ctrl + S = Save image to file Ctrl + Support State Ctrl + W = Snap dowble width image Ctrl + Alt +Shift + C = Move camera to the middle position in the case of positioning problems (requires alignment afterwards) Mouse click to any numerical value on the StatusBar = Set positions numerically Mouse click to image format on the StatusBar = Select different image format	
	Mouse click to filter description on the StatusBar = Select another filter Move mouse with pressed left button on the image = Measure dimensions Right mouse button on the image = Show/hide intensity profile Left mouse button doubleclick to left too corper of the image = switch ON/OFE flat-field correction	- Cose

Some shortcuts available in the Service Mode will be shown only after entering Service Mode.

7.7 Stability Test

It will open following dialog:

Stability test is available only in *Service Mode.* To start – press Ctrl+Alt+Shift+T on keyboard.

	Ŭ		
Stability Te	st		
	filename I	for results :	S

Stability rest		
	filename for results : StabilityTest_001	start test stop and exit
	step#219 [UUn:25m:295], x-ray source: 50kV / 2000A	, intensity=65.2%, CCD: 14.0C
	st	ability estimation: 98%
	after finishing test procedure:	switch off x-ray dose control program shut down computer

One can select filename for the text file where results will be saved. Then click "start test" button. The test procedure will start with X-ray source OFF and after several minutes will switch it ON. During testing software will measure intensity of emission acquired by camera compare to camera signal before emission started and calculate stability value. The results of emission stability will be shown as a plot (yellow curve) in the central part of the dialog with dotted indication of values without emission and with maximum emission. During stability test the software also measures

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stability of CCD temperature stabilization inside the camera. This information is shown as a blue curve with dotted marks for acceptable margins. Information line above the plots shows current position and timing, source voltage/current, emission intensity measured from the camera signal and current CCD temperature.

Full testing cycle can take 70-100 minutes. It can be interrupted any time by "stop and exit" button. The operator also can select behavior of control software in the end of testing by several checkboxes in the bottom of the dialog: "switch off x-ray", "close control program", which will also shut down the scanner and "shut down computer" afterwards. The software will update result's text file with selected filename in every step to be sure that all measured results are saved in the case of interrupting by operator as well as in native end of procedure.

8 AUTOMATIC SAMPLE CHANGER

Automatic sample changer can be supplied together with the scanner or purchased separately. In first case it will arrive pre-calibrated. In second one it will require calibration at user site.

8.1 Installation

All time when changer not installed to the scanner keep it in the transportation box to avoid damaging precise internal manipulator. To remove changer from the box put your hands under central bridge of the changer and lift it up handling under the side walls of the bridge without touching manipulator in the middle.

Before installing sample changer, remove three black screws using screwdriver supplied with the scanner:



The changer will stay connected to the two taped holes on the bottom and supported by centering pin in the top. If changer supplied with the scanner, the centering pin has been already adjusted by height using glued spacers. For changers supplied separately, position of the pin should be adjusted by user using supplied spacers and washers. The pin and necessary spacers can be found in the right side of the changer toolbox. For changers supplied separately, adjust height of the centering pin to get top surface of fully inserted pin in two millimeters under the top surface of the scanner enclosure like it shown on the following picture. Use supplied spacers and washers to adjust the height of the pin. For changers supplied with the scanner, check that this distance is in the range of 1.5 ... 4 mm:



Remove landing pad with two screws from the toolbox and install it to the scanner by following way: firstly adjust angular orientation of landing pad by such way that connector in the object stage inside the scanner will be positioned against milled cavity on the bottom of landing pad:



Afterwards connect landing pad to the object stage. Extend distance between the jaws in the top of the pad to get screws free to move up, center the pad on the object stage of the scanner and secure it on the stage by two screws using screwdriver supplied with the scanner:



Now you can install sample changer to your scanner. Remove the changer from transportation box. Top lid can be packed separately or just stay on the top of the changer: be careful not to drop it down. Take changer by one hand using handle on the back black part of the changer and by other hand on the slop in the bottom of the column of the changer:



Slide it in parallel to the top surface of the scanner towards the back wall to reach connection of the fork under the changer's carrousel with centering pin installed to the scanner. In the proper installation the changer should stay exactly in the middle of the opening of the specimen chamber in parallel to the top surface of the scanner with 2-5mm gap between bottom of the changer carrousel and the top surface of the scanner enclosure:



Be sure that two round extensions on the bottom plate of the changer column fit round milled cavities on the front plate of the scanner. Shake a little bit the column of the changer to get both legs properly inserted:



Afterwards connect the changer to your scanner by two screws using screwdriver supplied with the changer:



Be sure that computer and scanner are switched OFF. Connect your changer to the scanner by supplied power cable and to control computer by supplied USB cable:



Start control computer. Start scanner control software. The software should recognize the changer and show Sample Changer control dialog on the screen (image and data in this dialog may be different from the shown on the picture):



With changer properly recognized by software, the installation procedure is completed.

8.2 How to use

In the first start of the changer without any specimen mount installed all bars in the top of the carrousel will not be illuminated. The changer supplied with four sizes of sample mounts, 16 pieces each. The mounts are different in diameter and length to accept different sizes of samples. Maximum sample diameter should be less than 25mm; maximum sample height should be less than 40mm.



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Sample mount with long thin (5mm in diameter) neck (left on the picture) should be used for small samples to be scanned with highest magnification. We would recommend using this type of mounts for samples under 5mm in size and under 8mm in length. Next one (with neck 10mm in diameter) can be used for small-medium size of samples up to 10mm in diameter. Next medium-large mount with 16mm neck recommended for using with samples up to 16mm in diameter. Right one in the picture with short neck 24mm in diameter can be used for large samples up to 25 in size. As you can see, diameter of the neck of the mount allows identifying recommended sample size.

The neck of all sample mounts painted in white. This part of the mount body will be using to identify particular type of the mount by taking "photo" of the neck by visual camera after inserting mount to the scanner. Don't make any marks on the painted part of the mounts – it can interfere with mount size recognition process. If necessary, use top horizontal surface to write sample identifications by fiber-tip marker. If you want to remove the marks by alcohol, carefully protect painted surface from contact with solvent.



To insert samples on the sample mount into the changer, open top lid of the carrousel. You can insert samples in any order. The sample mounts with different sizes can be randomly mixed in particular installation. If control software for the scanner already started, every newly installed mount with the sample will be indicated by blue colour on illuminated bar in the top of carrousel. Non-illuminated bar indicates absence of a mount in the particular position. During scanning the colours of the illuminated bars will be changed. Scanned samples will be indicated by green colour in the bars, the sample, which is currently under scanning, will be marked by red bar. If scanning cannot be finalized or interrupted by user, corresponding bar will be illuminated in yellow.



Attention! Never install new sample in the empty position, which is indicated in red because automatic procedure to return sample after scanning to this position will damage the sample changer by collision of the sample mounts.

After filling the carrousel by samples on the mounts (you can keep any number of empty positions), close carrousel lid. The lid has special sensor and Sample Changer dialog indicates reaction to this sensor in the bottom part. Second sensor on the sample changer detects door position. If sample changer just installed to your scanner, try to open and to close specimen chamber door and check corresponding indication in the bottom of Sample Changer dialog.

If sample changer received separately from the scanner, in first installation it must be calibrated. The calibration process explained in details in the next paragraph of this Manual. In the case of purchasing scanner together with the scanner, it will arrive already calibrated. After installing calibrated changer to your scanner, check sample insertion / removing process. If checkbox in the bottom of Sample Changer dialog indicates that sample inserted, but you are sure that there is no sample or sample mount on the landing pad inside the scanner (open door for double check), uncheck this box before starting sample insertion test. To see how sample changer will operate inside specimen chamber, you can select "keep illumination ON when specimen chamber open" in Preferences dialog (Menu -> Options -> Preferences).

Click "insert" button to activate sample mount insertion procedure. During this procedure robotic arm inside sample changer should take the sample mount from the carrousel by gently lifting up it by steel fork, afterwards – move it out of the carrousel down to the scanner and land it to the landing pad from the top. After landing the mount, robotic arm will move the fork inside the changer column and lift it to the topmost position. The mount should be hold be jaws on the surface of landing pad. If it is not a case or you can hear some unusual noise during lifting mount up from the carrousel or during landing, repeat calibration procedure described in the next paragraph of this manual.



To check removing procedure, click "remove" button in the Sample Changer dialog. The robotic arm will move the fork to the bottom of the tower and forward up to reaching sample mount from the front. It will open jaws on the landing pad to allow free vertical movement of the mount. Afterwards robotic arm will take sample mount up and around the corner of the carrousel to the topmost position. In the next step the arm will move mount forward and put it down to the pin on the carrousel. Again if the movements are different from described or in the case of unusual noise during lifting mount up from the landing pad or during landing to the carrousel, repeat calibration procedure described in the next paragraph of this manual.

You can repeat testing with next sample by turning carrousel to next populated position by "go to next" button in the Sample Changer dialog.

After every insertion of sample mount, visual camera inside the scanner will take photo of the mount neck to identify mount type and will show it in the top of Sample Changer dialog. You can see identification process by selecting "show profile and mount size" in the dialog for Sample Changer Settings, which can be called by Menu -> Options -> Sample Changer Settings.

The Sample Changer dialog contains table with 16 lines, which corresponds to 16 positions of the samples in the sample changer with identical numbers on the central disk. Every line contains colour bar on the left with indication identical to the indication on the illuminated bars on the changer carrousel. Like in the carrousel's bars, the colour is black when corresponding position is empty, is blue for samples waiting for scanning, is green for samples, which already scanned, is yellow for samples with interrupted scanning and red for sample, which is currently under scanning.

Sample Changer							
0	N.S.						
pos	name	protocol					
01	08Jan13_01_	Auto	-				
02	08Jan13_02_	Manual	-				
03	08Jan13_03_	Previc	Scanning Pro	tocol			x
04	08Jan13_04_	Previc	_		-		
05	08Jan13_05_	Auto		Filename prefix:	F:\Resul	ts	
06	08Jan13_06_	Auto		Data Directory :	ſ	browse	
07	08Jan13_07_	Auto		08Jan 13 02			
08	08Jan13_08_	Auto					
09	08Jan13_09_	Auto	\sim	Rotation step	(dea) :	0.600	
10	08Jan 13_10_	Auto	U.	Augracian (fr		2	-
11	08Jan13_11_	Auto			ames) :	2	-
12	08Jan13_12_	Auto		Random mov	ement :	30	
13	08Jan13_13_	Auto		360 deg scar	ining		
14	08Jan13_14_	Auto	~)				
15	08Jan13_15_	Auto	$\sim \sim$	Energy filter :	Al 0.25	mm	•
16	08Jan13_16_	Auto		Pixel size (um) :	-	21.59	
carr	rousel lid closed		Q	Image format :	2452x1	640	
scal	nner door open				1008/		-0
	insert	go to sample ins		Partial width : Vertical position ((mm) :	16.874	0
	start scannin	19		C	к (Cancel	

Next edited area in every line contains filename prefix for dataset, which will be saved during scanning of corresponding sample. In the first start of control software all "name" fields are populated by combination of current date and position in the changer. You can edit this filename prefix any time for all non-scanned samples.

Right part of every line includes drop-down list for scanning protocol selection. The list contains three possible options: automatic protocol selection, copying scanning protocol from the previously scanned sample and manually selected scanning protocol. In the case of choosing last option, you can select scanning parameters using following dialog shown on the previous picture. It allows direct selection of filename prefix and target data folder, choosing rotation step, number of frames for averaging in every angular position, random movement amplitude and switching to 360 degree rotation cycle instead of standard 180+ degree rotation cycle. Central part of the dialog allows choosing energy filter in the front of the camera. Bottom part defines image magnification through pixel size and image format, add partial width to reduce necessary disk space for scanning samples partially filled to the field of view and adjust individual vertical position for scanning the sample. After pressing OK button selected protocol will be saved for scanning in selected sample from sample changer.

The behaviour during scanning with "Auto" protocol selection and number of other parameters can be adjusted in the "Sample Changer Settings" dialog, which can be called by Menu -> Options -> Sample Changer Settings.

Sample Change	er Settings					
	General :					
E S	create new subfolder for every dataset					
	current index: 2 reset					
	send confirmation by e-mail					
 after scanning every sample in the end of all scans 						
	Image in the top :					
	✓ show profile and mount size					
	take new image on click					
	"Auto" scanning protocol :					
	Magnification :	sample size 🔻				
	Image format :	1224x820 👻				
	🔽 averaging (f	frames) : 2				
	random movem.(pix): 20					
	allow changing filter					
	🔲 use 360 deg					
	ОК	Cancel				

The top part of the dialog provides control for distribution datasets in subfolder with incremental folder names. Control software will continue proper counting of incremental indexes for subfolder names even after closing and opening. You can reset this counter or select particular current number. The datasets will be distributed in subfolders inside the folder selected at Menu -> Options -> Data Folder.

Next control in this dialog activate sending e-mail confirmation after finishing every particular scan or after scanning all samples inserted in sample changer.

Central part controls behaviour of the image in the top of Sample Changer dialog. It can show intensity profile through the neck of the sample mount and recognized mount size during loading the sample (for 1-2 seconds only) and allows updating image to the current view of the sample on mouse click to the image.

Next part defines scanning parameters for the samples, which will be scanned with automatically selected scanning protocol.

Image magnification can be selected in drop-down menu by one of three possible ways: "previous", "mount size" or "sample size". Fist option will use the same magnification like it has been used for

previously scanned sample. Second option will adjust magnification individually according to diameter of the neck of the sample mount in assumption that sample is equal or smaller than mount neck. Last option will adjust magnification individually according to sample position, size and shape. Before starting the scanning sample will be moved to minimum magnification and control software will measure margins between sides of the sample and left/right edges of the image for number of angular positions. Using worst case with minimum margin as a reference it will calculate optimal magnification, which will still fits the sample inside field of view.

Next drop-down menu in this dialog allows selecting image format for scanning. Next two lines allow adjusting of frame averaging and random movement during scanning. Following checkbox can allow automatic filter selection according to X-ray opacity of particular object. If this box stays unchecked, previously used filter will be applied during scanning. Last checkbox allows using 360 degrees rotation cycle instead of standard 180+ degrees rotation.

10 0030113_10_ A010 ▼
carrousel lid closed scanner door open
insert remove go to next sample inside 📝
 start scanning

To start scanning click to "Start scanning" button in the Sample Changer dialog:

The scanning process can be interrupted by cancelling any particular scan by "cancel" button in the scanning progress dialog of by pressing the same button in the Sample Changer dialog in between of scanning. The scanning process will be stopped automatically after scanning all samples installed into the sample changer.

Scanned samples with green indication in the illuminated bars of the carrousel can be removed or replaced any time during scanning process except of the time during sample removing / inserting to the scanner, when carrousel lid should be closed. To replace or remove scanned samples open carrousel lid, remove scanned sample, wait until corresponding illuminated bar will turn to black and insert new sample in the same position. The sample will be recognized as new one with corresponding blue illumination on the carrousel bar. Attention! Newer install any sample into position with red illuminated bar, which indicates that this position will be taken by sample mount returned from the scanner after the scanning.

8.3 Changer calibration

If sample changer purchased separately or installed to the scanner first time after transportation, one particular check should be done before changer calibration. The changer contains sensor for detection of specimen chamber's door position. It installed under the right wall of the bridge between vertical column and carrousel.



The sensor's body has external thread with two nuts: one on the bottom of the bridge (shown on the picture above) and other – on the top (shown on the next pictures). When sensor activated by closed door, a ring on the top of sensor body will be illuminated in yellow color (left picture). For non-activated sensor top part of the body will not be illuminated (right picture):



For reliable activation of this sensor, the distance between bottom sensor surface and the top of the closed specimen chamber's door should be between 4 and 6mm like it shown on the next picture.



The distance can be adjusted by releasing the nuts on the sensor body and tighten them on the proper sensor location.

Following sample changer calibration can be done using Menu -> Options -> sample Changer Calibration function. This function is one of the Service functions: if corresponding line of menu is grayed, push Ctrl+Alt+Shift+s first to activate Service Mode.

Firstly remove all sample mounts from the changer's carrousel and select "keep illumination ON when specimen chamber open" in Preferences dialog (Menu -> Options -> Preferences).

Top part of the Sample Changer Calibration dialog – "initialize robotic arm and all drives" – is always available. Following control become available according to selection in the top of every particular part.

Sample Change	r Calibration	1		
R _	in	it robotic arm	and all drive	5
× •	Lifting objec	t mount from	sample chang	ger:
	up	down	V position:	20.000
	forward	backward	H position:	10.002
	carrousel :			
	CW	CCW	R position:	0.735
	check	lifting	check r	eturn
		ST	OP	
	save lifting	positions	load lifting	positions
_				
0	robotic arm	ct mount ins	ide the scann	er:
	up	down	V position:	
	forward	backward	H position:	
	object stage	:		
	left	right	H position:	0.0
	CW	ccw	R position:	0.000
	check l	anding	check rei	moving
		ST	OP	
	save landin	g positions	load landing	positions
0	Check specir	nen exchang	e procedure :	
	install	remove	turn to nex	kt object
		ST	OP	
				ciose

Top section of the dialog responsible for calibration of lifting object mounts from the carrousel. After selection of this part insert one object mount (without any sample) to the first position of the carrousel. After scanner and sample changer initialization, the carrousel should be in the rotational position with first place for the sample mount oriented towards operator, but not necessary exactly aligned to direction perpendicular to the scanner. After selection "lifting object mount from sample changer" mode the software will move robotic arm to closest safe position of the fork against first position of the mount in the carrousel.

Proper position of lifting sample mount up from the carrousel can be described as following: vertical position of the fork should be chosen by such way that during horizontal movement top surface of the fork will be aligned to the bottom surface of the top wheel in the sample mount; horizontal position should correspond to coaxial relative positions between conical cavity in the top of the fork and conical bottom surface of the top wheel in the sample mount; rotational position of the carrousel should be selected by such way that incoming fork will not push any side of the axle between mount wheels:



If changer was previously calibrated and you want to use calibration function to rectify specimen handling positions, load existing settings by "load lifting position" button. Numerical displays in the right hand side will show end position of robotic arm and angular position of carrousel for lifting up the mount. To check all movements use "check lifting" button. Keep your mouse on the "STOP" button to interrupt lifting process if necessary. If sample mount lifting executed correctly, you can check returning process by "check return" button. Again keep the mouse on the "STOP" button to interrupt execution in the case of problem.

If test of loaded from file positions was not successful, you can change numerical values in "Skyscan1272.ini" file, click to "lifting object mount from sample changer", afterwards "load lifting position" from modified ini-file and try to check lifting and return again. Increasing of value for vertical position corresponds to moving fork down; increasing of value for horizontal position corresponds to moving fork far from the changer column and close to carrousel; increasing value in carrousel rotational angle corresponds to rotation in anticlockwise direction.

If you have to calibrate sample changer first time, for example in the case of purchasing it separately from the scanner, click to "lifting object mount from sample changer" and use "up", "down",

"forward", "backward", "cw" and "ccw" buttons to reach proper position of the fork against sample mount on the carrousel. The fork and the carrousel will move slowly only when corresponding button is pushed. You will see monitoring of all positions in the numerical values on the right side. After reaching proper lifting position for the fork and proper rotational position for carrousel described above, save selected positions to ini-file by pushing to "save lifting positions". Then click to "init robotic arm and all drives", click to "lifting object mount from sample changer" and "load lifting position" and check lifting and return using corresponding buttons. If necessary, correct positions in ini-file and load / test them again.

Attention! Before adjustment for landing of the sample mount to the scanner, remove all sample mounts from the carrousel and push "init robotic arm and all drives" button.

To check and adjust mount landing to the landing pad inside the scanner, use next part of Sample Changer Calibration dialog. Put one object mount directly of the fork of robotic arm (not to the carrousel!). After selection "landing object mount inside the scanner" mode the software will move robotic arm to closest safe position of the fork above landing pad.

The adjustment procedure is very similar to described above, but now we have four degrees of freedom in the movements: robotic arm up/down, robotic arm forward/backward, small movement of object stage inside the scanner left/right and object stage rotational position CW / CCW.

Proper final landing position of the specimen mount is shown in the following pictures. Vertical position of the fork should be adjusted by such way that the bottom surface of the fork will be close (around 0.5mm), but not touch the top horizontal surface of the mount bottom wheel. It can be adjusted by up/down movement of the fork. The fork should land sample mount concentrically to the landing pad, which can be adjusted by forward/backward movement of the fork. The fork should push symmetrically to the both jaws of the landing pad, which can be adjusted by adjusted by adjusted by moving object stage left/right. Side walls of the fork should be aligned to internal surface of the jaws, which can be adjusted by cw/ccw stage rotation.



If changer was previously calibrated, you can "load landing positions" and "check landing" / "check removing" using corresponding buttons. Keep the mouse on the "STOP" button to interrupt movements if necessary. If one or several positions should be modified you can edit corresponding lines in ini-file, "load landing positions" and try again.

In the case of full calibration or calibration of the changer purchased separately from the scanner, after selecting "landing object mount inside the scanner" with following moving of robotic arm close to the landing pad, use "up"/"down", "forward"/"backward", "left"/"right" and "cw"/"ccw" buttons to get proper landing position of the specimen mount on the fork of robotic arm. The fork and the landing pad will move slowly only when corresponding button is pushed. Numerical displays in the right hand side will show current position of robotic arm and angular/linear positions of the landing pad using "check removing" button and try landing again by "check landing" button. If both functions are working properly, you can save landing calibration by "save landing positions" button. In the case of problems, repeat landing calibration.

Before testing of complete specimen exchange procedure, remove sample mount(s) from the carrousel, from the fork of robotic arm and from the landing pad, click "init robotic arm and all drives" button and after initialization select "check specimen exchange procedure" section in the Sample Changer Calibration dialog. Insert several sample mounts randomly to any carrousel positions. If there is no mount in the first position, you can click to "turn to next object" button – carrousel should be turned to get next installed sample mount in the exchange position. Then try to install sample mount using "install" button. Keep the mouse on the "STOP" button to interrupt movements if necessary. Installing procedure will execute lifting up the sample mount from the carrousel and insert it inside the scanner to the landing pad. Afterward try to get sample mount back to carrousel using "remove" button. Check reliability of inserting and removing sample mount several times. In the case of any problem, repeat changer calibration.

After successful calibration "close" Sample Changer calibration dialog.

9 SKYSCAN 1272 WITH SAMPLE CHANGER: SYSTEM CONTROL BY COMMAND FILES

9.1 Overview

Starting from version 1.1.18, 1272 control software allows full control of acquisition and reconstruction processes by text-form command files. It also supports periodically updated status monitoring by text file. Associated NRecon in versions 1.7.4.5 or later supports periodic monitoring of reconstruction program activities by adding corresponding information to the same status file.

All *command files* should be in the folder defined in SkyScan1272.ini-file, [System] section, "Command File Folder" key. The *status file* location defined in the same section at "Status File Folder" key. Both locations should be defined without backslash in the end. The locations can be local, for example – "C\SkyScan1272\command" or network locations started from double backslash \\, for example – "\\server_sv2\results". If selected location cannot be found during execution of the program, it will be created by recursive procedure independent on possible absence of parent directories. If location cannot be created due to absence of server or home drive as well as due to security settings, which prevent access to selected location, corresponding notification will be shown on the screen. In the first start of control program both locations will be defined as subfolders under location of control program. Afterwards, they can be modified. The control program reads the locations only during start-up.

During standard operation of the system with sample changer without control files, the sample changer window shows drop-down lists for scanning protocol selection for every position in sample changer:

Second Change		
sample Chang	er	
0	Ν.	4
	pos name	protocol
	01 15Sep18_01_	Auto 🗸
	02 15Sep 18_02_	Auto 🗸
	03 15Sep 18_03_	Auto 🗸
	04 15Sep 18_04_	Auto 🗸
	05 15Sep 18_05_	Auto 🗸
	06 15Sep 18_06_	Auto 🗸
	07 15Sep 18_07_	Auto ~
	08 15Sep 18_08_	Auto 🗸
	09 15Sep 18_09_	Auto 🗸
	10 15Sep 18_10_	Auto 🗸
	11 15Sep 18_11_	Auto ~
	12 15Sep 18_12_	Auto 🗸
	13 15Sep 18_13_	Auto ~
	14 15Sep 18_14_	Auto 🗸
	15 15Sep 18_15_	Auto ~
	16 15Sep 18_16_	Auto ~
	^ Ctrl + left mouse click to	reset
	carrousel lid closed	
	scanner door closed	
	in the second second	an to mout
	insert remove	go to next
	sa	mpie inside 🔄
	start scanning	

If control program can find command files for some or all changer positions, this window will be modified as following (in this example, control files are found for positions 1,2,3 and 12):

Sample Change		
0	N	. 4
	oos name	protocol
	01 sample 1resu	Its from file
	02 sample2resu	lts from file
	03 sample 3resu	lts from file
	04 15Sep 18_0	4_ Auto ~
	05 15Sep 18_0	5_ Auto ~
	06 15Sep 18_0	6_ Auto ~
	07 15Sep 18_0	7_ Auto ~
	08 15Sep 18_0	8_ Auto ~
	09 15Sep 18_0	9_ Auto ~
	10 15Sep 18_1	0_ Auto ~
	11 15Sep 18_1	1_ Auto ~
	12 sample 12res	ults from file
	13 15Sep 18_1	3_ Auto ~
	14 15Sep 18_1	4_ Auto ~
	15 15Sep 18_1	5_ Auto ~
	16 15Sep 18_1	6_ Auto ~
	^ Ctrl + left mous	e click to reset
	carrousel lid closed	
	scanner door closed	
	insert rem	go to next
		sample inside
	start s	canning

The drop-down lists are replaced to "from file" indications and filename prefix is taken from the command file. Scanner control from command file has highest priority over all other protocol selection modes. Any command file, except of the file currently used for scanning and reconstruction, can be added, removed or replaced any time. The software will scan for command file presences every second and will modify information in "Sample Changer" window according to found command files.

9.2 Command File Structure

Filename of every command file defines position of the sample in the sample changer, which will be controlled by this file. Command file names should be "1272_N.uct" where N is a one or two digits number of sample position in the sample changer, for example, "1272_2.uct" for second sample in the changer or "1272_14.uct" for sample in 14th position.

The command files are text files. Every file contains three sections started from the header in square brackets with following list of keys and parameters. First section should be started from **[scanning]** header and third one – from **[reconstruction]** header. Following table describes all possible keys in every section and default values, which will be used if corresponding key doesn't exist in the command file:

Section / Key	Possible values	Default value
[sample]		
Sample Name	Any text	Unknown
Sample Number	Any text	0
Sample Description	Any text	No description
[scanning]		
Pixel Size in um	Positive float-point value	50.0
Vertical Scanning Position in mm	0.0 60.0	10
Rotation Step in degrees	Positive float-point value	1.0
X-Ray Source Voltage in kV	20100	80
X-Ray Source Current in uA	10250	125
Filter	05	0
Averaging	1100	1
Rotation Cycle 180 or 360	180 or 360	180
Camera Binning	14	2
Data Folder	String shorter than 200 characters	C:\results
Data Prefix	String shorter than 200 characters	unknown
[reconstruction]		
Top Reconstructed Slice	Any positive integer larger than "bottom reconstructed slice" value	200
Bottom Reconstructed Slice	Any positive integer	199
Step Between Slices	1 or more	2
Smoothing	010	3
Ring Artifact Reduction	020	2
Beam Hardening Correction	0100	0
Output Folder	String shorter than 200 characters	C:\results
Output File Format	TIF, JPG, PNG, BMP,	PNG
	TIF+DICOM_N, JPG+DICOM_N,	
	PNG+DICOM_N, BMP+DICOM_N,	
	TIF+DICOM_1, JPG+DICOM_1,	
	PNG+DICOM_1, BMP+DICOM_1,	
Top Grayscale Level	Any float-point value above "bottom	0.1
	grayscale level"	
Bottom Grayscale Level	Any float-point value	0.0

Remarks:

- DICOM_N in output file format means DICOM output will be created as a DICOM header linked to multiple data files. DICOM_1 output file format means DICOM output will be created as a single file (for small datasets only)
- If parameters listed in control file cannot be reached, closest reachable value will be used

Every section of control file contains listed above header with following list of keys. Key name separated from the value by "=" sign, for example:

[sample] Sample Name=MySample Sample Number=123456 Sample Description=sample 1 description [scanning] Pixel Size in um=9.5 Vertical Scanning Position in mm=22.2 Rotation Step in degrees=3.0 X-Ray Source Voltage in kV=80 X-Ray Source Current in uA=100 Filter=0 Averaging=1 Rotation Cycle 180 or 360=180 Camera Binning=2 Data Folder=D:\newtest\recursive\seriesA\scan7 Data Prefix=sample1results [reconstruction] **Top Reconstructed Slice=300** Bottom Reconstructed Slice=150 Step Between Slices=1 Smoothing=1 Ring Artifact Reduction=0 Beam Hardening Correction=0 Output Folder= D:\Results\seriesA\scan7\results Output File Format=PNG Top Grayscale Level=0.1 Bottom Grayscale Level=0

After pressing "Start Scanning" button in the Sample Changer window on the screen, the control software will check presence of command file for next occupied position in sample changer and will read [scanning] section during sample loading to the scanner. Afterwards it will execute sample positioning according to settings in the command file and start acquisition according to acquisition parameters define in the command file. After completing scanning process, the control software will save standard log-file associated with acquired dataset and include in it all information mentioned in the [sample] section of command file. Then, reconstruction will be started with settings taken from [reconstruction] section of command file. Simultaneously with reconstruction process, scanner control software will go to the next sample in the sample changer and, in the case if command file for next occupied position exists, all described above process will be repeated.

Scanned samples in sample changer (indicated by green illuminated bars) can be replaced any time except of time of sample loading/unloading to the scanner. Possibility to move sample changer robotic arm and carrousel when top lid is open connected to safety regulation. It can be overwritten by changing "Ignore lid sensor" key in [Sample Changer] section of SkyScan1272.ini file from 0 to 1.

9.3 Status File Structure

For monitoring activities of control and reconstruction programs, both are periodically updating status file with time marks of last update. The status file name is "SkyScan_status.log". It located in the folder defined by "Status File Folder" key in [System] section of SkyScan1272.ini file. <u>Generation of status file is not connected to presence or absence of control files</u>. It will be always generated or updated during regular execution of control program.

The status file is a text file. It can contain one or two sections dependent on activity of particular program. A [Scanning] section indicates activity of scanner control program. A [Reconstruction] section indicates activity of NRecon. Both sections contain time stamp of last update in the "Time" key. The control software updates status file often than every minute (typically – every 31 seconds). The reconstruction program typically updates status file every 30 seconds.

Example or status file can be found below:

[Scanning] Time=09:12:55 15/09/18 Process=Idle [Reconstruction] Time=17:33:34 14/09/18 Reconstruction=Idle Progress= Input=D:\Results\seriesA\scan9\sample12results Output=D:\Results\seriesA\scan9\results\sample12results_rec0000090.png Finish Status=Succeeded @ 16:01:33 14/09/18. From slice 90 to 810, with step 2.

Process key in the [Scanning] section can be one of following:

Idle StartScanFromChanger Scanning DataDir\Prefix ScanningCompleted SourceStabilityTest SavingConfiguration LoadingConfiguration ScoutScan BatchScanning WaitingForMTS MultipleScanning LoadingFromChanger ReturnSampleToChanger

[Reconstruction section of status file can include information on currently running process in "Reconstruction" key, percentage of reconstruction executed in [Progress] key, data folder for primary

projections in [Input] key, full pass to first reconstructed slice in [Output] key and overview of last executed process in [Finish Status] key.

10 SERVICE AND SUPPORT

The SkyScan 1272 scanner is designed as a maintenance-free instrument and doesn't require any periodical service.

New versions of control software and all associated program packages supplied with your scanner may be available for download in the "Software Updates" section of the Bruker microCT website:

http://www.bruker-microct.com/products/downloads.htm.

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

For all users of micro-CT instruments Bruker microCT organizes annual User Meetings. Information on previous Meetings and announcement of forthcoming events can be found at the Bruker microCT website: <u>http://www.bruker-microct.com</u> (=> Company => User Meetings)

If your control computer is connected to internet, you can get access to the Bruker microCT website by simple click to the corresponding underlined web-address in the "About..." box in the Help part of the Main Menu. In the same place you can find a link to send e-mail to scanner producer.



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