

Tomato User Manual

Tomato IDL tomography tools version 3.6, 10. February 2011

(c) L. Houben, www.er-c.org

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1 Getting Started

1.1. Installation

System Requirements

The compiled version of the tomato program relies on the presence of the IDL virtual machine v8.0 or higher. The IDL virtual machine itself is freely available from the [IDL website](#). IDL is a cross-platform application and can be installed on the following operating systems: MAC OS X, Windows, PC-Unix (Linux) and Unix.

Download and Installation

Visit the tomato download page and get the latest tomato build. Unpack and copy the downloaded folder into an appropriate installation directory. On Windows this is preferably the the system-wide 'Program Files' folder, on Mac OS X the system-wide 'Applications' folder.

On other Unix variants there are a few more steps to take: copy the tomato folder to an installation directory of your choice (e.g. '/usr/local' and Create a script named "tomato" containing the following line:

```
idl -vm=/INSTDIR/tomato/tomato.sav
```

where INSTDIR is the installation path for the tomato.sav

Make the script executable ('sudo chmod a+x tomato') and copy it to a binary directory, e.g. '/usr/local/bin'.

Program Start

On windows systems: Double-click on the file tomato.sav.

On Mac OS X: Double-click on the supplied application icon (tomato slice image). You may want to drag the icon onto the dock to keep it in the dock.

On Other Unix systems: first check the DISPLAY environment variable and the access permissions to the client X-server, then type 'tomato' on the command line.

1.2 User Interface

tomato offers an easy-to-use graphical user interface. The user interface consists of a command menu, control-windows and dialog windows (Fig. 1.3.1):

The tomato command menu or *menu bar* is used to call a command in order to perform an action on a data set.

The menu items are sorted according to the following considerations:

- File - Input/Output operations for the import and the export of data sets.
- Transformations - Geometrical transformations, like scaling, rotating or cutting to a smaller size. Simple data normalization operations or filter operations.
- Alignment - Alignment operations for the tilt series images in a data set.
- 3D Transforms - Three dimensional transformations from Radon to cartesian space vice versa.
- Windows - Access to GUI control windows.
- Utilities - Miscellaneous useful routines.

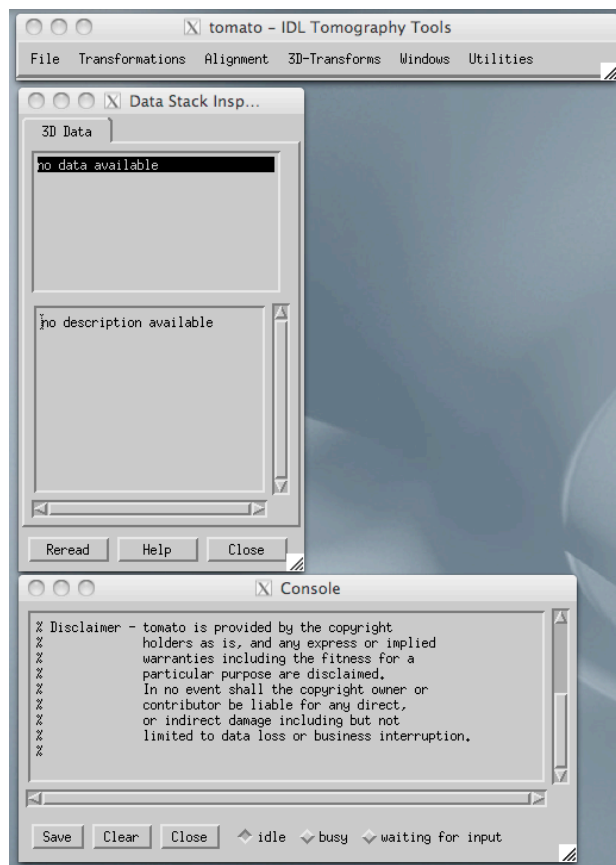


Fig. 1.3.1: GUI windows. Menu bar, Data Inspector and Console.

An important control window is the 'Data Inspector' that is used for the administration of the list of data sets. Context based menus are available for changing e.g. display options.

With the 'Data Inspector' you take control over the list of currently managed data sets. The 'Data Inspector' window is currently organized in a single tab named '3D Data'. The tab "Images"

The tab contains two scrollable frames. The upper frame lists all entries in the list of data sets that are available. This upper frame is mainly used in order to set the focus onto a certain data set on which any command subsequently selected will be performed: A left mouse button click on a label string in will highlight the string and focus this data set for operations that you select from the command menu. (Note that you cannot focus to a data set just by clicking on the graphical output window associated with the data set because there is no interaction between the window manager and the list manager.) The lower frame in the '3D Data' tab presents informational text about the selected data set.

The upper frame of the '3D Data' tab is associated with a context sensitive menu. A simple click on the right mouse button will activate this menu.

The third GUI window, the 'Console' window, shows informational program output, that can be saved as a text file.

Additional dialog window are used by processing routines to interact with the user and to read parameters. They are displayed when a user invokes a function from the menu bar or from a contextual menu in the 'Data Inspector'.

1.3 License

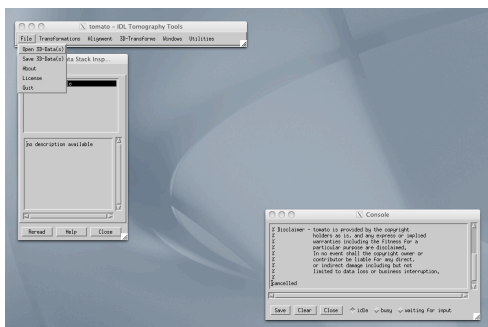
tomato uses a machine based license consisting of two hexadecimal codes. License codes are available from the author. Start tomato on the installation computer and note the MAC address displayed. Send this address in an e-mail with the subject 'tomato license request' to L.houben@fz-juelich.de.

Once you have the license code and number create an empty file called 'tomato.pref' in the installation directory (Unix systems: in your home directory) and start tomato. Enter the license information in the '**File → License**' dialog

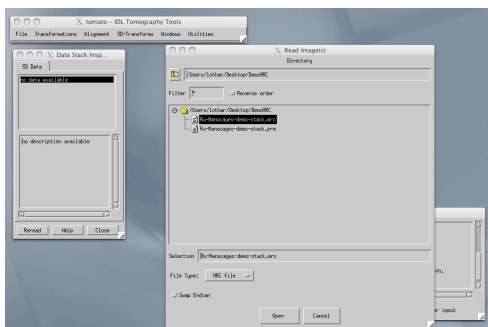
1.3 A Quick Demonstration for the Impatient

Load the package with the demonstration data from www.er-c.org by clicking [here](#). Unpack the zip file. It contains a folder 'DemoMRC' and two files, an mrc file with a tilt series and a parameter file.

Go to '**File → Open 3D-Data**' ..



Open the file 'Ru-Nanocages-demo-stack.mrc'



[illegible]

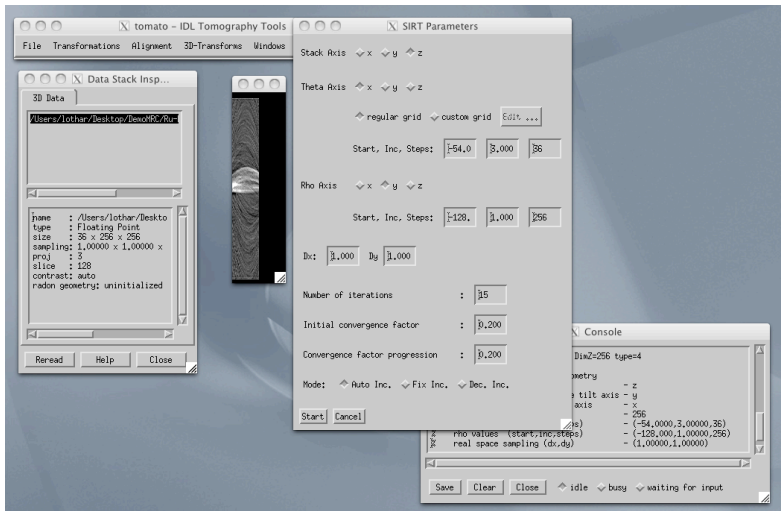
The screenshot displays the IDL Tomography Tool interface with two windows open:

- 3D Transform**: This window shows the file path `/Users/lothar/Desktop/BendPC/for` and a list of parameters:
 - name : /Users/lothar/Desktop
 - type : Floating Point
 - size : 36 x 256 x 256
 - sampling : 1,00000 x 1,00000 x
 - proj : 1
 - slice : 128
 - contrast: auto
 - radon geometry: uninitialized
- Read Images(s)**: This window shows a directory tree with the following structure:
 - /Users/lothar/Desktop/BendPC
 - Ru-Nanocages-demo-stack.wo
 - Ru-Nanocages-demo-stack.pr
 The `Ru-Nanocages-demo-stack.pr` file is selected. The **Selection** field shows `Ru-Nanocages-demo-stack.pr` and the **File Type** is set to `Parameter Object File`.

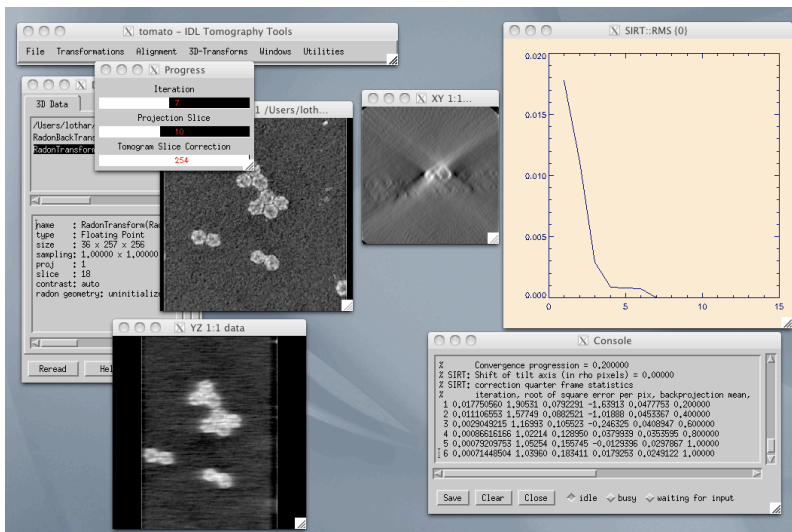
At the bottom of the interface, there are buttons for `Open`, `Cancel`, `Save`, `Clear`, `Close`, and a status bar indicating `> Idle ~ busy ~ waiting for input`.

[illegible]

Simply press 'Start' in the SIRT Parameters dialog ...



Watch the reconstruction process ...



2 Handling Data Stacks

2.1 Data Import/Export

Load an MRC-file or a group of Tietz-formatted images via the '**File → Open 3D-Data**' dialog (Fig. 2.1.1). Endian detection is automatic except for the raw data format.

A new list entry with the filename will appear in the Data Stack Inspector. A display window will open, showing a slice of the data. Information about the data type, the array dimensions, the viewing direction and the currently displayed array slice is given in the lower part of the Data Stack Inspector.

Multiple data stacks can be loaded. The current data stack active for manipulation is highlighted in the Data Stack Inspector. You can choose the active data stack with a left mouse button click on the list in the Data Stack Inspector.

Data Stacks can be exported using the '**File → Save 3D-Data**' dialog. You can choose between raw binary data format, standard MRC format with a 1024 Byte header or 'extended MRC' format. The latter has a header size of 1024 x 1024 bytes which corresponds to the header size of the FEI XPlore3D mrc format.

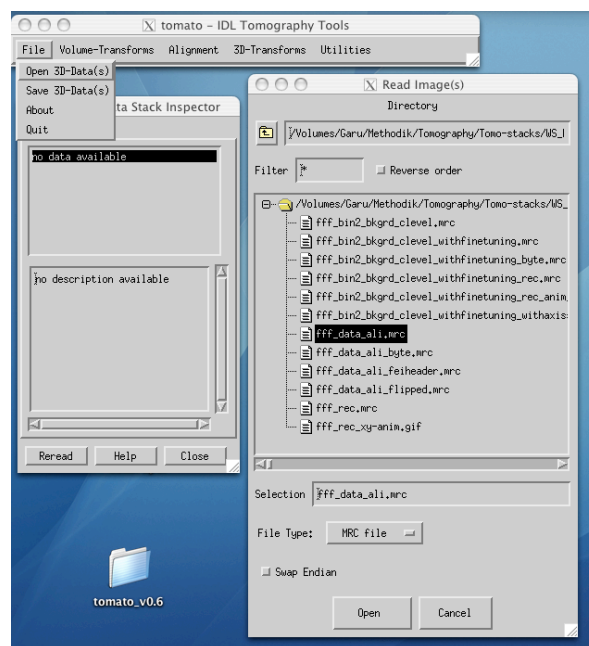


Fig. 2.1.1: Input/Output dialog window.

2.2 Data Stack Manipulation

Activate the context menu in the Data Stack Inspector by pressing the right mouse button while the mouse pointer is on the list entry. All manipulations will apply to the highlighted data stack.

Refresh - Update the display in the data stack view window

Zoom - Change the magnification for the display of the stack on the screen.

Projection - choose viewing direction

Animate - Animation tool to slice through the data stack in the viewing direction.

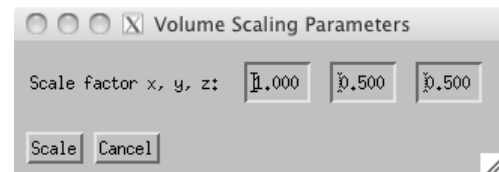
Flip Axes - Use this dialog to swap two axes of the 3D volume.

Delete - Delete the array data from the memory and the display window.

2.3 Data Stack Transformation

A number of functions are available for the manipulation of the 3D-volume data under **Transformations** in the menu bar.

Transformations -> **Scale** is useful in order to resample the 3D volume. A new 3D data array will be produced containing the scaled data. Choose the scaling factors in all three dimensions. Any floating point number is applicable. Note that scaling may not be precise because the scaled image will have integer pixel dimensions. The output in the console window will tell you the actual scaling values and the scaling error relative to the desired values.



The **Transformations** -> **Normalization** menu offers a number of functions for the normalization of the 3D volume data. Currently available are the subtraction of the 3D mean value, the division by the 3D mean value and the division by the 3D standard deviation.

The **Transformations** -> **Geometric Transformation** menu provides routines for translation or rotation of the volume data.

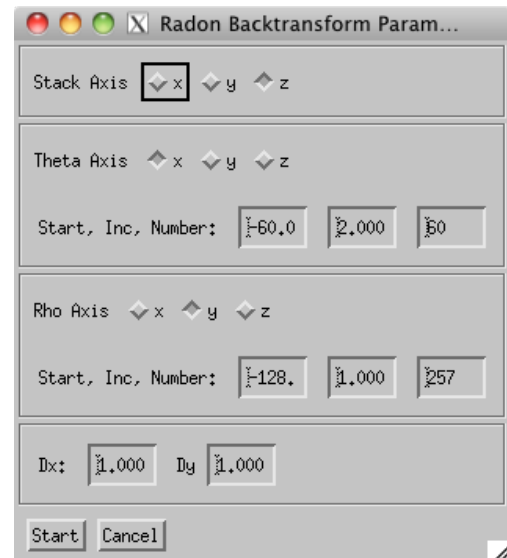
Transformations -> **Projections** can be used to calculate a tilt series of 2D projection images from 3D volume data.

3 Arranging the Data Stack

Important note:

tomato is in general free in the choice of the direction of the tilt axis, it may be “x”, “y” or “z”. The program routines are, however, tested only for a certain configuration. The correct operation is guaranteed only for this default configuration. The default configuration is explained below.

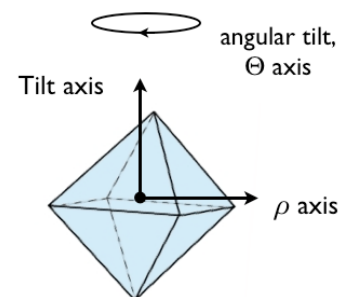
Simply use the ‘[Flip Axis](#)’ function to adjust the axes of your data stack to the default axis configuration.



3.1 Coordinate System

Stack Axis (default=z):

Each of the images along the stack axis represents a two-dimensional array $A(\text{theta}, \text{rho})$ in Radon space of size M by N to be transformed. The stack axis corresponds to coordinate parallel to the tilt axis.

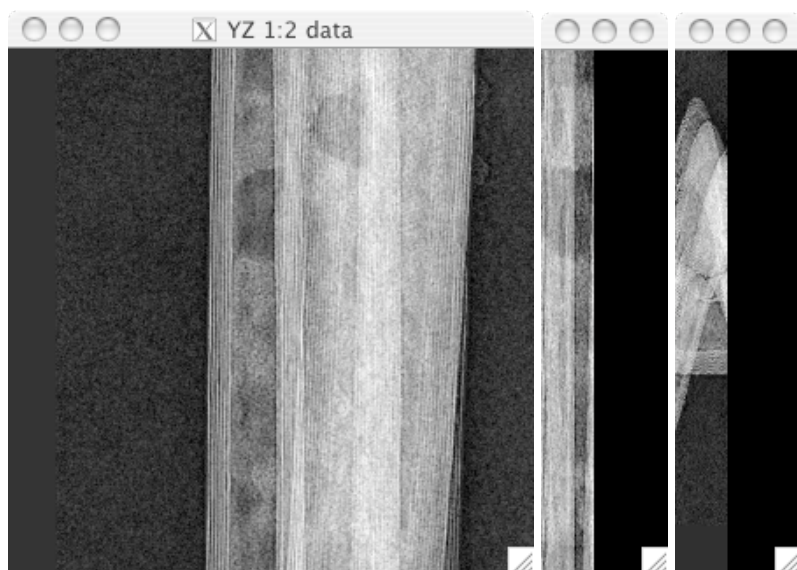


Theta Axis (default=x):

Each slice in the direction of the theta axis correspond to one tilt angle. The theta axis corresponds to the axis on which the different tilt images are aligned. I.e. the stack axis in an mrc-file. Specify the angular coordinates. Number should be the same as the z-Dimension of your stack.

Rho Axis (default=y):

Rho is the coordinate orthogonal to the tilt axis. It is the coordinate orthogonal to the tilt axis in a single image of the tilt series.

Example: Inorganic Nanotube Bundle

The figure shows the projections of the image stack flipped to the default coordinate system for back-projection, from left to right: yz-, xz-, xy-projection

4 Alignment

4.1 Interactive Alignment Tools

4.1.1 Tilt Axis Shift and Rotation Alignment

The function ‘**Alignment → Tilt Axis Shift and Rotation Alignment**’ is helpful when trying to find the position and rotation of the tilt axis in the two-dimensional image space of each single image in the tilt series. The function creates a tentative two-dimensional back-projection of a single slice on the stack axis.

The first step of the ‘Tilt Axis Alignment’ function is to **set up the back-projection parameters**. The following dialog will appear:

‘Stack Axis’, ‘Theta Axis’ and ‘Rho Axis’ are explained in the section ‘Coordinate System’.

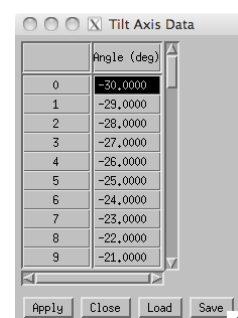
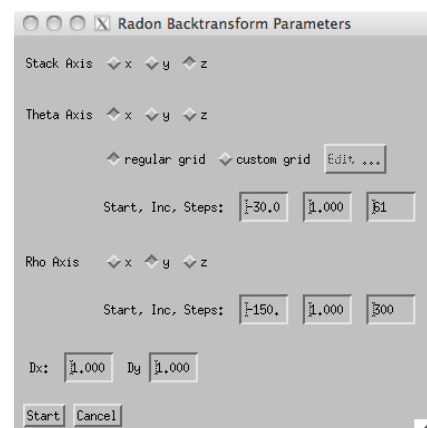
The stack axis is the coordinate parallel to the tilt axis.

For the theta axis - meaning the tilt angles - you can choose a regular grid with equidistant angular step or a custom grid. When choosing a regular grid the theta axis Start, Inc and Steps parameters specify the tilt series start angle, angular increment and number of tilt increments, respectively.

Choosing ‘custom grid needs’ activates the ‘Edit ...’ button. Clicking on the edit button will open a spreadsheet with a table of image numbers and tilt angles. The preset values will be either taken from the regular grid data or previously defined angular parameters. From the spreadsheet there is an option to load or save the data to a file.

The ‘Rho Axis’ parameters specify the rho-axis coordinates of the input images. The number of rho coordinates has to match the number NRho of pixels in the direction orthogonal to the tilt axis in single image of the tilt series. The default setting is: $\text{Start} = -\text{FLOOR}((\text{NRho} * \text{Inc}) / 2)$, $\text{Inc} = 1$, $\text{Number} = \text{NRho}$.

The rho axis coordinates should be in increasing order. Placing the origin



($\rho=0$) not in the centre by adding an offset to start corresponds to a shift of the tilt axis along the ρ coordinate by the according amount (pixels if $\text{Inc}=1$).

D_x and D_y are equal to the scalar spacing (in pixel units) between the horizontal and vertical y coordinates in real space. The default is 1.

The second step of the ‘Tilt Axis Alignment’ is the interactive dialog that allows you to **readjust the position of the tilt axis**. Besides the ‘Tilt Axis Alignment Tool’ dialog window there will be two more windows on your screen. One window shows a certain slice of a backprojection of the shifted and rotated data stack, the other window shows the sinogram that corresponds to this slice.

The ‘Tilt Axis Alignment Tool’ dialog provides the following interactive adjustment tools:

Slice: Select the position along the tilt axis (z), for which the two-dimensional back-projection is displayed.

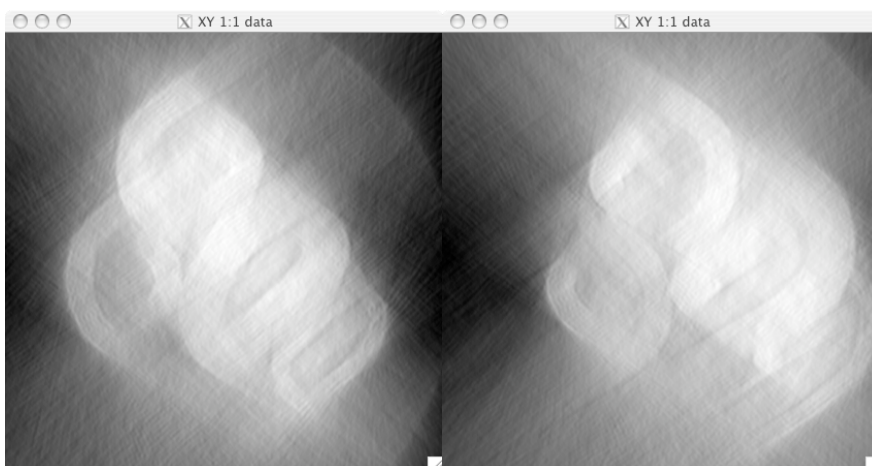
Shift: Shift the tilt axis along the ρ coordinate.

Rotation: rotate the tilt axis in the (ρ, z)-plane.

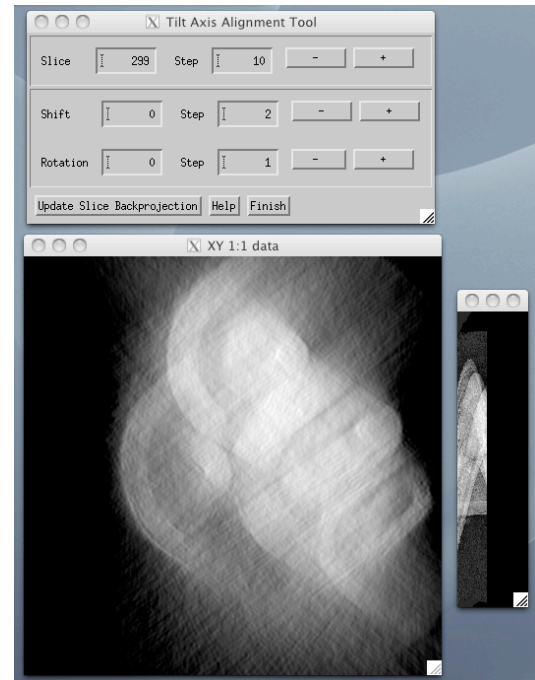
When finished, press the ‘Finish’ button. A new stack with the applied changes will appear in the data stack list. Use this stack for further processing.

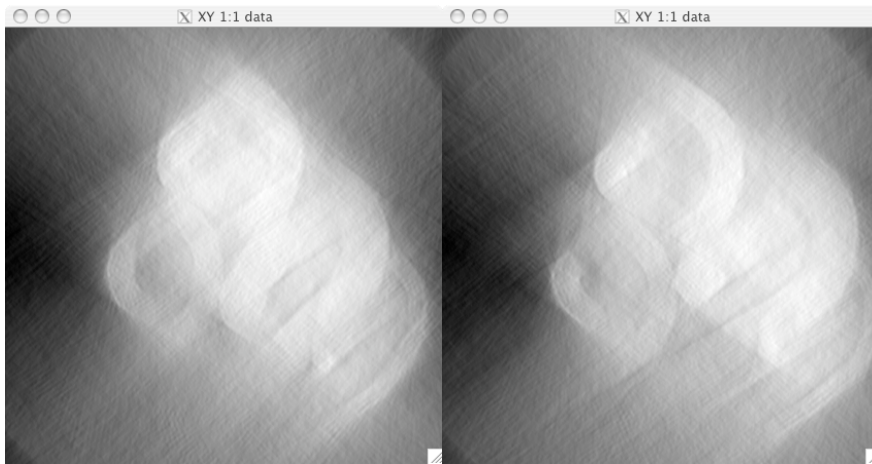
Guidelines for a proper alignment:

Rotation needs correction - backprojection artifact **bows change direction** between top and bottom of the stack (left: slice 50, right: slice 550).

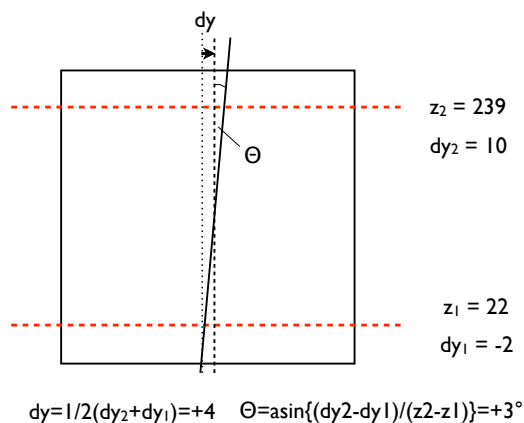


Shift needs correction - **bows do not change direction** between top and bottom of the stack (left: slice 50, right: slice 550)





The rotation can be estimated by a shift optimization on two different z-slices at the top and bottom of the stack. The figure demonstrates how to calculate the effective shift and the rotation angle.



The sketch to the left shows how to calculate the effective shift and the rotation angle from the shift values of two slices at the top and the bottom of the data stack.

4.1.2 Tilt Axis Drift

The 'Tilt Axis Drift' dialog provides the following interactive adjustment tools:

Slice: Select the position along the tilt axis (z), for which the two-dimensional back-projection is displayed.

Shift: Shift the tilt axis along the rho coordinate.

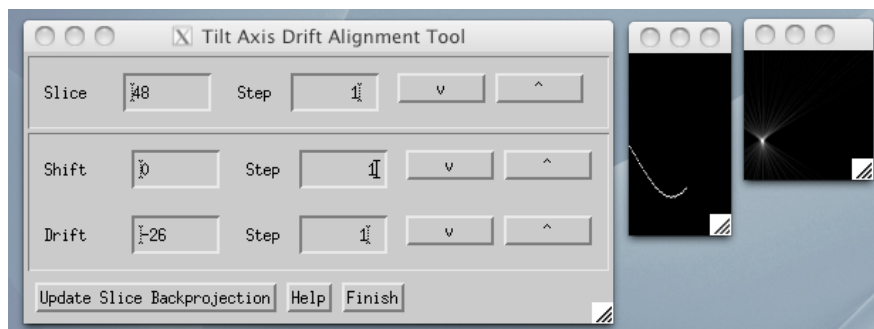
Drift: Correct displacement of the tilt axis orthogonal to the tilt axis that is linear with the tilt angle.

When finished, press the 'Finish' button. A new stack with the applied changes will appear in the data stack list. Use this stack for further processing.

Guidelines for a proper alignment:

Shift needs correction - backprojection artifact **bows** show at the top and bottom of the stack.

Drift needs correction - backprojection artefact **bows** turn into triangular features at the top and bottom of the stack. Note that large pixel drift values are required in general to be able to observe a substantial effect, about 1 pixel per tilt increment.

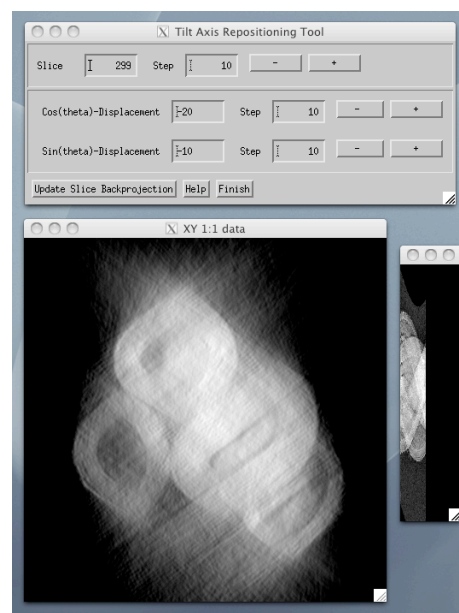


More general objects will tend to be flattened on one side and stretched on the other side, along the limiting angles of the missing wedge.

4.1.3 Tilt Axis Repositioning

This function is useful if you want to place the tilt axis. During the manual alignment of images and the consecutive cross-correlation you actually define a certain image feature which stays in place. This also defines the position of the tilt axis. However, in some cases it is desired to move the tilt axis to a different place. The displacement of the tilt axis involves a cosine and sine transformation of the tilt images, which can be performed with this function.

Besides the 'Tilt Axis Repositioning Tool' dialog window there will be two more windows on your screen. One window shows a certain slice of a backprojection. It shows the result of the repositioning of the tilt axis. In essence, the objects will be shifted within the 3D volume, since the center of the 3D volume is coincident with the tilt axis. The other window shows the sinogram that corresponds to the slice.



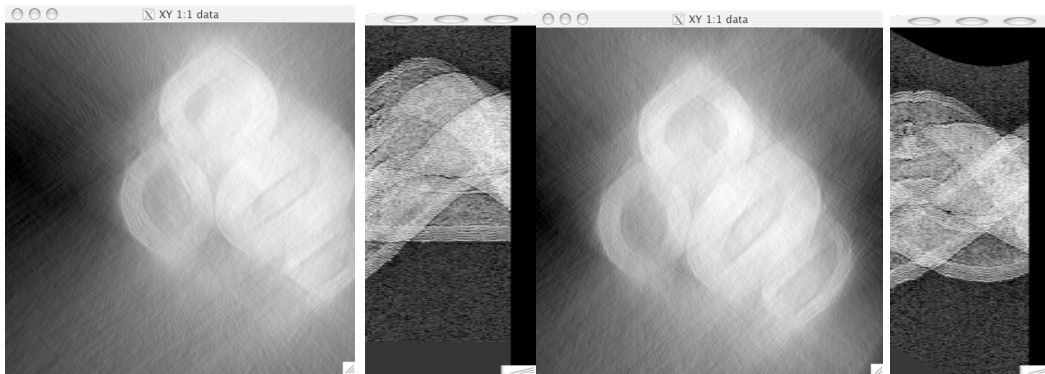
The 'Tilt Axis Repositioning Tool' dialog provides the following interactive adjustment tools:

Slice: Select the position along the tilt axis (z), for which the two-dimensional back-projection is displayed.

Cos(theta), Sin(theta)-Displacement: shift the position of the tilt-axis with respect to the reconstructed volume, i.e. centre the reconstructed volume.

When finished, press the 'Finish' button. A new stack with the applied changes will appear in the data stack list. Use this stack for further processing.

Example: Displacement of the tilt axis for a tilt series of a nanotube bundle (left: before, right, after)



4.1.4 Sinogram Alignment

to be written

4.2 Cross-Correlation Alignment Tools

4.2.1 Sequential Cross-Correlation

The sequential cross-correlation aligns subsequent images in the tilt series. Taking the first image as the reference the second image is aligned to it, then the second image is taken as the reference for the alignment of the third image and so forth. The algorithms implemented in tomato are based on the calculation of the cross-power spectrum in the Fourier domain.

Preparation: make sure that the data stack follows the conventions (stack axis='z', theta axis='x', rho axis='y').

Calling '**Alignment → Sequential Cross-Correlation**' will first open a 'Correlation parameters' dialog window that allows you to specify parameters for the cross-correlation algorithm:

Normalization: Before cross-correlation, the image data may be normalized. The default normalization is with respect to the image contrast. Choose 'sdev' in this case. Normalization to the amplitude means that only the phase of the frequency components of the images will have an effect on image registration, this normalization will therefore provide phase correlation. You may also choose no normalization at all, this is however of very little practical use. The default nor-

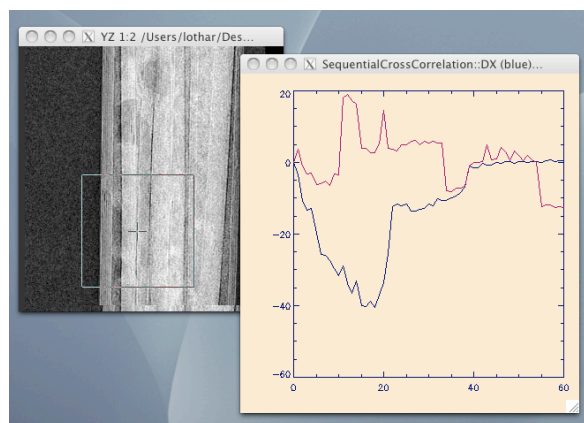
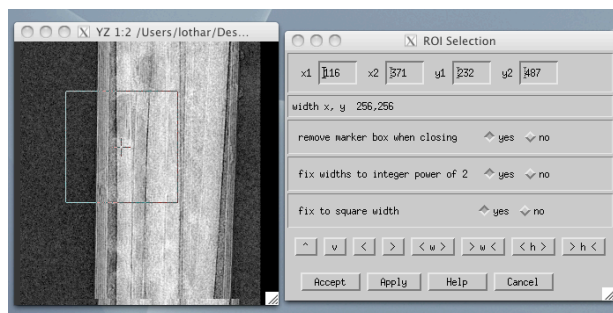


malization is contrast normalization.

Accuracy: You may choose pixel or sub-pixel accuracy. The default is sub-pixel accuracy, in this case the maximum of the cross-power spectrum will be optimized by a parabolic fit and subsequent image shifts are performed with sub-pixel steps.

Continuing with the procedure you will be asked to choose the region of interest for the correlation analysis. Note that so far only frame sizes equal to a power of 2 and of square size are allowed. Pressing on Accept will start the cross-correlation analysis.

The results of the correlation analysis are displayed in a separate plot window. Horizontal shifts between an image and its predecessor are plotted in blue, vertical shifts in red.

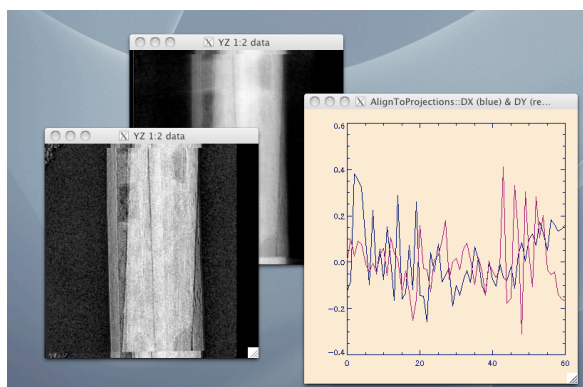
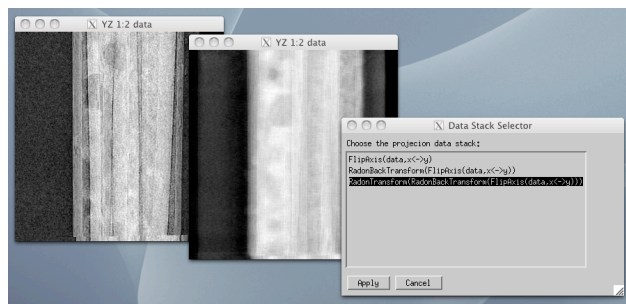


4.2 Alignment to Projections

Activate 'Macros → Tomography → Align to Tomogram Projections'. Choose then the cross-correlation settings. Then choose the tilt series and the reprojection data.

Afterwards select the region of interest for the cross-correlation analysis. Don't make it too big, because the reprojection data does not cover the full rho coordinates.

The procedure will then calculate the cross-correlation in the region of interest. The results are printed in the console window and plotted in graphical form.



4.3 Grid-Search Alignment

A grid-search alignment may be used to refine an initial coarse image alignment. The grid search algorithm shifts an individual image or a set of images by a certain vector. This creates a new set of input images for the backprojection. The refinement algorithms then calculate the backprojection and a certain figure of merit for the tomogram. The optimum set of shift vectors inside the search grid of vectors is then determined by the optimum figure of merit.

The figure of merit implemented in 'tomato' so far is the contrast in a sub-volume of the tomogram. Choose the sub-volume carefully, since contrast enhancement will occur e.g. in a vacuum part when artifacts are present. Therefore it is best to choose a sub-volume with a structural feature as clear as possible. The bigger the sub-volume is, the better, since averaging provides better precision.

Input parameters for the routines are parameters for the search grid, and parameters for the sub-volume over which the figure of merit is calculated.

Several optimizations can be done this way:

- Sub-pixel accurate shift of the tilt axis common to all images in the tilt series (see 4.2.1).
- Compensation of a linear drift of the tilt axis, in other words the drift of the tilt axis from one side of the frame of view in the beginning of the tilt series to the other side at the end of the tilt series (see 4.2.2).
- Sequential sub-pixel accurate shift of single images in a certain range of tilt angles (see 4.2.3).

All grid search optimization procedures work in a very similar way. They require one preparation step in order to choose appropriate tomogram sub-volumes for contrast optimization. There is yet no three-dimensional sub-volume selection tool, therefore the selection is done in two-dimensional rectangular regions for a range of tomogram slices in a certain viewing direction which is more or less free to choose.

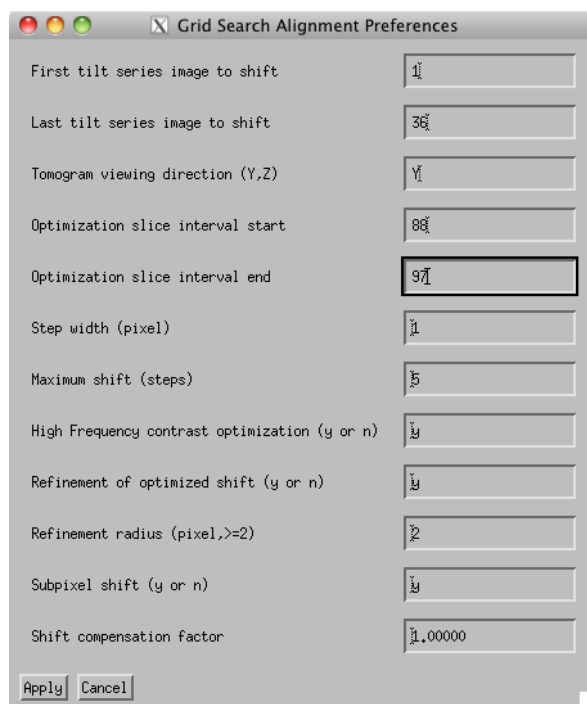
Preparation step for the selection of tomogram slices: Make sure that the data stack follows the conventions (stack axis='z', theta axis='x', rho axis='y'). For preparation of the sub-volume coordinates it is a good idea to display the data stack in the 'X (YZ-plane)' projection and choose the slices along the tilt axis which will be used for contrast optimization. To do this go to '**Utilities → ROI Selection**'. Mark a contrastive feature and memorize the z-coordinates of the selection box. They are displayed (oddly enough) as y1, y2 in the console output.

4.3.1 Grid-Search Tilt Axis Shift

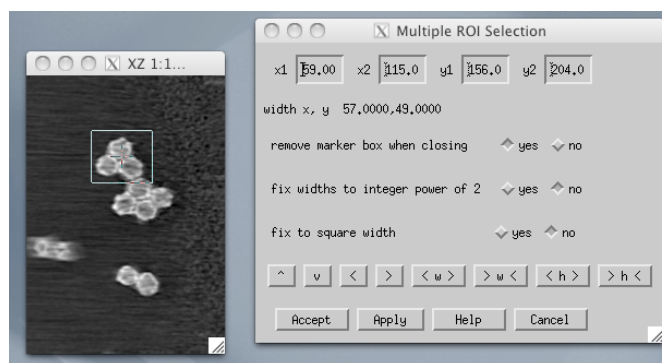
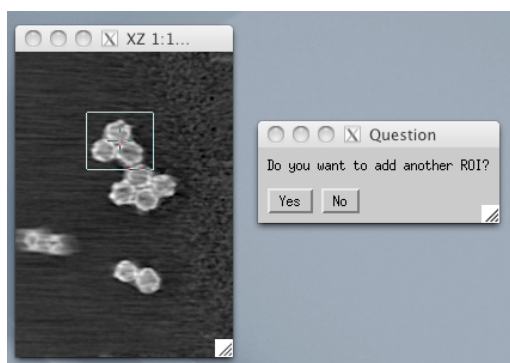
This function can be used to optimize the in-plane offset of the tilt axis. The offset is with respect to the centre of the tilt images and equal in magnitude for each of the images.

Activate '**Alignment → Grid Search Tilt Axis Shift**'. Choose the reconstruction geometry settings in the 'Radon Backtransform Parameters' dialog window. Then you need to adjust a few parameters for the grid search alignment in the 'Grid Search Alignment Preferences' dialog:

A range of images in the tilt series will be shifted in the same way. By default the range starts with the first image. If you want to shift a sub-range of images then adjust the range with 'First tilt series image to shift' and 'Last tilt series image to shift'. Images are numbered starting from 1. The tomogram viewing direction specifies which slices are examined. Y and Z are valid viewing directions for the tilt axis shift analysis. The contrast will be analyzed in slices along this viewing direction starting from 'Optimization slice interval start' to 'Optimization slice interval end'. The more slices are chosen, the smaller is the uncertainty of the contrast values in general. 'Step width' and 'Maximum Shift' define the grid step width and search range. 'High frequency contrast optimization' is in general appropriate in order to enhance the weight of the fine detail information. If 'Refinement of the optimized shift' is chosen, then the figure of merit will be approximated by a parabolic function over the radius of grid data points given under 'Refinement radius'. If the refinement is switched off then only full step increment accurate maximum determination of the figure of merit will be applied. Put 'Subpixel shift' to 'y' if you want to activate sub-pixel accurate alignment. The 'Shift compensation factor' is a multiplier for the measured optimum shift. Use a conservative value of 0.5 if you are iterating e.g. with the alignment of the rotation axis.



The next step is to choose the subregions of interest for the evaluation of the figure of merit. Note that the projection slices have been chosen before in the 'Grid Search Alignment Preferences' dialog, so that regions of interest are only two-dimensional. Choose areas with features and particularly edges. Do not include too much support or vacuum, since there the contrast increases when a tomogram reconstruction suffers from alignment artifacts. You may include a number of regions by adding more

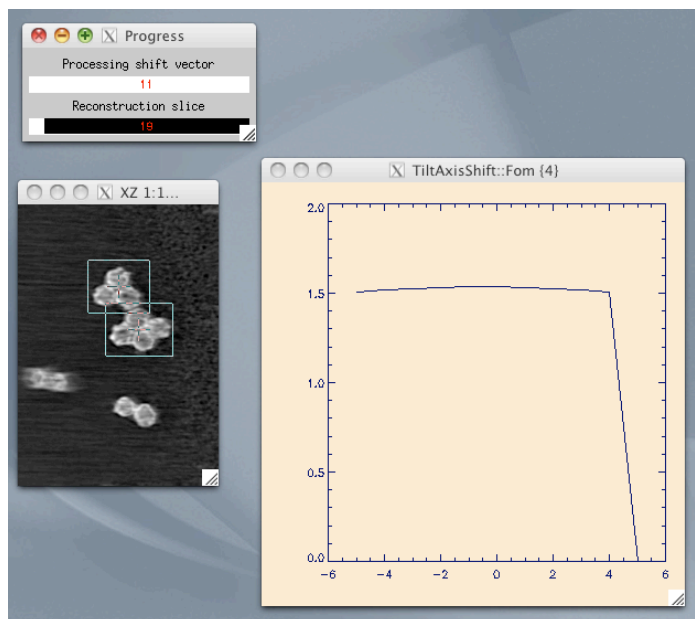


than one region.

Once you are finished with the selection of sub-volumes the program will start shifting images, calculating the tomogram and displaying the contrast in the selected tomogram regions. The progress will be displayed on the screen. Check the selection of the regions of interest carefully. If no clear maximum can be observed try first with a different selection or search range.

The 'Console' window presents informational output. First there is information about the parameter choice. Finally there is a result line that states the image range of shifted tilt series images, the extremum grid position, the refined extremum position obtained from the parabola fit and the corresponding uncertainty as well as the applied shift in pixel:

```
% image range shift refined error compensated
1:36 0.00000 -0.366196 0.0100011 -0.366196
```

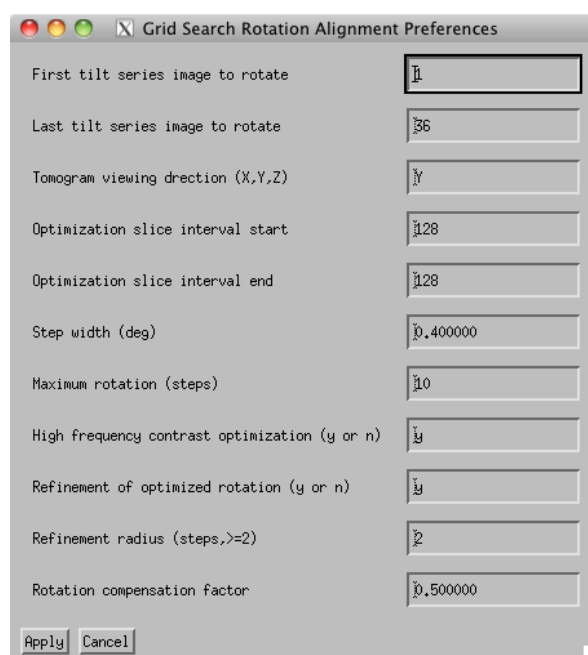


4.3.2 Grid-Search Tilt Axis Rotation

This function can be used to optimize the in-plane rotation of the tilt axis. The rotation is with respect to the centre of the tilt images and equal in magnitude for each of the images.

Activate '**Alignment → Grid Search Tilt Axis Rotation**'. Choose the reconstruction geometry settings in the 'Radon Backtransform Parameters' dialog window. Then you need to adjust a few parameters for the grid search alignment in the 'Grid Search Rotation Alignment Preferences' dialog:

A range of images in the tilt series will be rotated at once. By default the range starts with the first image. If you want to shift a sub-range of images then adjust the range with 'First tilt series image to rotate' and 'Last tilt series image to rotate'. Images are numbered starting from 1. The tomogram viewing direction specifies which slices are examined. X, Y and Z are valid viewing directions for the tilt axis rotation analysis. The contrast will be analyzed in slices along this viewing direction starting from 'Optimization slice interval start' to 'Optimization slice interval end'. 'Step width' and 'Maximum rotation' define the grid step width and search range. 'High frequency contrast optimization' is in general appropriate in order to enhance the weight of the fine detail information. If 'Refinement of the opti-



mized rotation' is chosen, then the figure of merit will be approximated by a parabolic function over the radius of grid data points given under 'Refinement radius'. If the refinement is switched off then only full step increment accurate maximum determination of the figure of merit will be applied. The 'Rotation compensation factor' is a multiplier for the measured optimum rotation. Use a conservative value of 0.5 if you are iterating e.g. with the tilt axis shift.

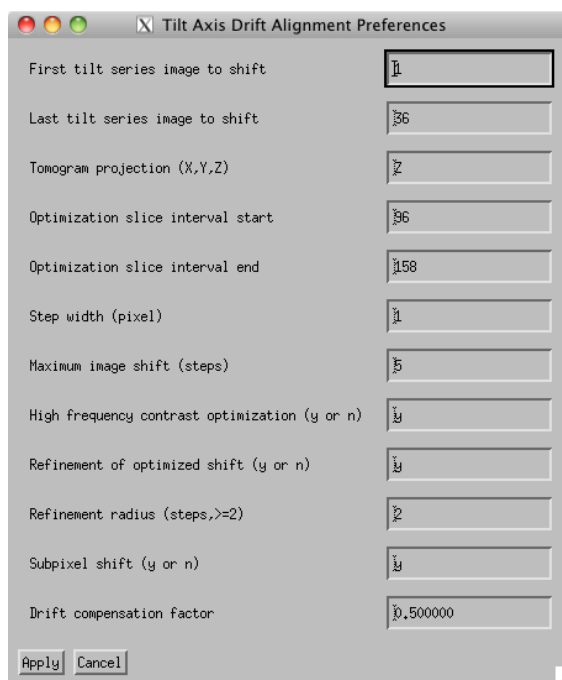
The further procedure is identical to the 'Grid Search Tilt Axis Shift' Alignment. Read in 4.3.1 for more information.

4.3.3 Grid-Search Tilt Axis Drift

This function can be used to optimize the in-plane drift of the tilt axis orthogonal to the direction of the tilt axis. The image displacement is assumed to be symmetric with respect to the central image in the tilt series and scales linearly with the angular increment with respect to the central image.

Activate '**Alignment → Grid Search Tilt Axis Drift**'. Choose the reconstruction geometry settings in the 'Radon Backtransform Parameters' dialog window. Then you need to adjust a few parameters for the grid search alignment in the 'Tilt Axis Drift Alignment Preferences' dialog:

A range of images in the tilt series will be shifted in an affine linear way at once. By default the range starts with the first image. If you want to shift a sub-range of images then adjust the range with 'First tilt series image to shift' and 'Last tilt series image to shift'. Images are numbered starting from 1. The tomogram viewing direction specifies which slices will be examined for their contrast. X, Y and Z are choosable viewing directions for the tilt axis drift analysis. The contrast will be analyzed in slices along this viewing direction starting from 'Optimization slice interval start' to 'Optimization slice interval end'. 'Step width' and 'Maximum image shift' define the grid step width and search range. 'High frequency contrast optimization' is in general appropriate in order to enhance the weight of the fine detail information. If 'Refinement of the optimized rotation' is chosen, then the figure of merit will be approximated by a parabolic function over the radius of grid data points given under 'Refinement radius'. If the refinement is switched off then only full step increment accurate maximum determination of the figure of merit will be applied.



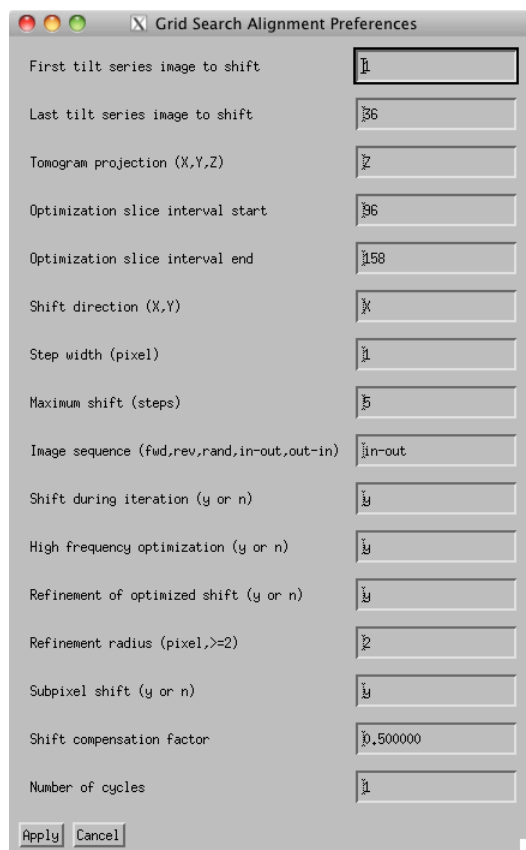
Sub-region selection for contrast evaluation and further processing are identical to the 'Grid Search Tilt Axis Shift' Alignment. Read in 4.3.1 for more information.

4.3.4 Grid-Search Alignment

This function can be used to optimize the in-plane shift of individual images in the tilt series. A sequence of images is processed one after the other and their position is changed during the iteration. Only one shift direction, horizontally (x) or vertically (y) is allowed at a time.

Activate '**Alignment → Grid Search Alignment**'. Choose the reconstruction geometry settings in the 'Radon Backtransform Parameters' dialog window. Then you need to adjust a few parameters for the grid search alignment in the 'Tilt Axis Drift Alignment Preferences' dialog:

A range of images in the tilt series will be shifted in a sequence. By default the range starts with the first image. If you want to shift a sub-range of images then adjust the range with 'First tilt series image to shift' and 'Last tilt series image to shift'. Images are numbered starting from 1. The tomogram viewing direction specifies which slices will be examined for their contrast. X and Y are choosable viewing directions for the analysis. The contrast will be analyzed in slices along this viewing direction starting from 'Optimization slice interval start' to 'Optimization slice interval end'. The 'Shift direction' is either the direction orthogonal to the tilt axis (X, horizontally) or along the tilt axis (Z, vertically). In general the shift optimization along X is more critical and requires more iterations. 'Step width' and 'Maximum shift' define the grid step width and search range. 'Image sequence' is a switch to influence the order in which the images are processed. There are five settings: 'fwd'=forward processing starting with the first image, 'rev'=reverse processing starting with the last image, 'in-out'=processing symmetrically from the inside to the outside of the image range, 'out-in'=processing symmetrically from the outside to the inside of the image range and 'rand'=random sequence.



'High frequency contrast optimization' is in general appropriate in order to enhance the weight of the fine detail information. If 'Refinement of the optimized shift' is chosen, then the figure of merit will be approximated by a parabolic function over the radius of grid data points given under 'Refinement radius'. If the refinement is switched off then only full step increment accurate maximum determination of the figure of merit will be applied. Put 'Subpixel shift' to 'y' if you want to activate sub-pixel accurate alignment. The 'Shift compensation factor' is a multiplier for the measured optimum shift. Using a conservative value of 0.5 is a safe option.

With 'Number of cycles' you can choose to repeat the procedure multiple times.

Sub-region selection for contrast evaluation and further processing are identical to the 'Grid Search Tilt Axis Shift' Alignment. Read in 4.3.1 for more information.

4.3.5 Simulated Annealing Shift Alignment

to be written

5 Radon Space Transforms

5.1 Radon Backtransform

The function '**3D-transforms → Radon Backtransform**' does the back-projection for the full 3D data stack in 2D slices.

Simply follow the instruction for the first step in the tilt axis alignment in the section 'Tilt Axis Alignment' to [set up the backprojection parameters](#).

5.2 Weighted Backprojection

The backprojection produces a reconstruction where low frequency components are more prominent than they should be. The reason is the better sampling of Fourier space for low frequencies compared to high frequencies. In the ideal case a simple weight function proportional to the frequency, i.e. a ramp filter, corrects this backprojection artifact.

In practice, the input images are filtered before backprojection. Use e.g. the function '**Macros → Tomography → Tilt Series FFT Filter**' in iMtools to apply such a ramp filter (see appendix) prior to backprojection.

5.3 Radon Transform

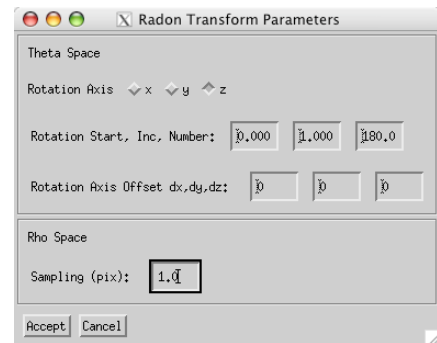
The function '**3D-transforms → Radon Transform**' does the projection for a 3D data stack, e.g. a tomogram, into 2D images.

The following dialog window will open:

The rotation axis correspond to the axis around which the object will be rotated.

The rotation axis Start, Inc and Num parameters specify the tilt series start angle, angular increment and number of tilt increments, respectively.

The rotation offsets dx, dy, dz specify a displacement of the rotation axis from the centre of the 3D data array.



The rho space sampling specifies the spacing between the rho coordinates. The default is one-half of the diagonal distance between pixels, $0.5[(DX^2 + DY^2)]^{1/2}$. Smaller values produce finer resolution, and are useful for zooming in on interesting features. Larger values may result in under-sampling, and are not recommended.

Hint: If you want to preserve the pixel size of the data array upon a combination of projection and backprojection vice versa then make sure that the rho sampling in the Radon Transform dialog and the dx, dy in the Radon Backtransform Dialog are identical

5.4 SIRT Reconstruction

The function '**3D-Transforms → SIRT Reconstruction**' iteratively computes a refined reconstruction of a tilt series. In each of the iteration steps a pixel in the tomogram is corrected by a difference tomogram calculated from the difference of the tilt series data and a reprojection from the previously calculated tomogram.

Activate the tiltseries data stack for reconstruction. Make sure that the tilt axis alignment is correct. You will need the tilt axis offset on the Rho axis as input parameter for the reconstruction.

Follow the instruction for the first step in alignment in the section 'Tilt Axis Alignment' to [set up the backprojection parameters](#) in the SIRT parameter dialog (Fig. 9.1)

The iteration parameters are the number of iterations and the convergence factor. The convergence factor specifies the fraction of the difference tomogram that will be added to the previously calculated tomogram in each of the iterations. A value below 0.5 is usually safe. Typically 15-30 iterations are sufficient. If you choose 'Fix increment' then the convergence factor will be increased after one iteration by the convergence factor progression value until the saturation value of 1 is reached. If you choose 'Auto' then the convergence factor is increased (decreased) by the progression value any time that the difference between images and reprojections gets smaller (bigger). If you choose "Dec. Inc." then the convergence factor will be incremented by decreasing increments according to the formula $\alpha + \beta/k$ where $k > 0$ is the iteration counter and α and β are the initial convergence factor and the convergence progression factor, respectively.

Recommended settings are 'Auto' or 'Dec. Inc.' with a convergence factor of 0.2 and a progression of 0.2.

Pressing on 'Start' will start the iterative reconstruction process. Two windows will open. One of them will show one slice from the centre of the tomogram (Fig. 9.2, lower left). The other window displays the projection of the tomogram from the mid of the range of tilt angles (Fig. 9.2, upper right). The content of these windows will be updated with the iteration progress.

