

TomoJ user manual

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1- Installation

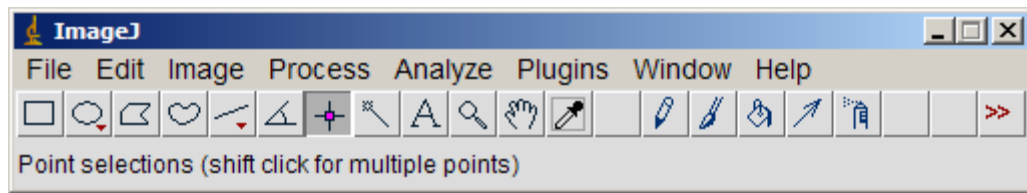


Figure 1: ImageJ window

First install ImageJ from <http://rsb.info.nih.gov/ij/download.html> (java 1.6 or newer is required by TomoJ) and update it to last version. If you are working under windows system, it would be good to edit the ImageJ.cfg file inside the ImageJ directory. Change the second line to use java.exe instead of javaw.exe, it will display the command line window where extra information is displayed.

Please, extend the memory available in ImageJ at the maximum capability of your computer (see http://imagejdocu.tudor.lu/doku.php?id=faq:technical:how_do_i_increase_the_memory_in_imagej).

Download TomoJ from <http://u759.curie.u-psud.fr/software/u759.html>

Unzip it to the plugins directory of ImageJ

Start ImageJ (or restart if already started). TomoJ will now be accessible in the plugins menu.

To allow the import/export of images in MRC, SPIDER and SEL file format you will need to download the U759_InputOutput.jar to the plugins directory of ImageJ and remove the HandleExtraFileType.class from the Input-Output directory of ImageJ.

To import .dm3 files, please go to the plug-ins web page of ImageJ (<http://rsb.info.nih.gov/ij/plugins/index.html>) where you will find a plug-in called DM3_Reader. Follow the installation's instructions of this plug-in.

2- Citing TomoJ In scientific papers

The publication corresponding to TomoJ is the following:

BMC BIOINFORMATICS. 2007 AUG 6;8:288. "TOMOJ: TOMOGRAPHY SOFTWARE FOR THREE-DIMENSIONAL RECONSTRUCTION IN TRANSMISSION ELECTRON MICROSCOPY." MESSAOUDI C, BOUDIER T, SANCHEZ SORZANO CO, MARCO S.

If you use the alignment with landmarks please also cite:

BMC BIOINFORMATICS. 2009 APR 27;10:124. "MARKER-FREE IMAGE REGISTRATION OF ELECTRON TOMOGRAPHY TILT-SERIES." SORZANO CO, MESSAOUDI C, EIBAUER M, BILBAO-CASTRO JR, HEGERL R, NICKELL S, MARCO S, CARAZO JM.

If you use the BqART reconstruction algorithm please cite :

MICROSC MICROANAL. 2013 DEC;1669-77. "THREE-DIMENSIONAL CHEMICAL MAPPING BY EFTEM-TOMOJ INCLUDING IMPROVEMENT OF SNR BY PCA AND ART RECONSTRUCTION OF VOLUME BY NOISE SUPPRESSION." MESSAOUDI C, ASCHMAN N, CUNHA M, OIKAWA T, SORZANO CO, MARCO S.

As TomoJ is running as a plug-in of ImageJ, it would be good to also cite ImageJ as described on ImageJ website:

Here are two possible ways to reference ImageJ:

1. Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2009.

2. Abramoff, M.D., Magelhaes, P.J., Ram, S.J. "Image Processing with ImageJ". Biophotonics International, volume 11, issue 7, pp. 36-42, 2004.

3- Description of Interface

To use TomoJ you need first to open your tilt series as a stack and then click in the menu Plugins>TomoJ>TomoJ. A window will appear asking for tilt angles when tilt angles are not in slice names. If you check the “load from file” and OK, then a new window will appear asking for the file where the angles are defined. This file can be rawltlt as given by FEI acquisition or recParam.txt as given by Jeol acquisition.

Now the slice names will contain tilt angles and the TomoJ window will appear. It is divided in many parts:

- The top part containing some sort of menu
- The bottom part contain tabs corresponding to different processing steps
 - General information and modification of tilt series
 - Pre-processing of tilt series images
 - Registration
 - Reconstruction
 - Post-processing
- The right part contains the history of the processing.

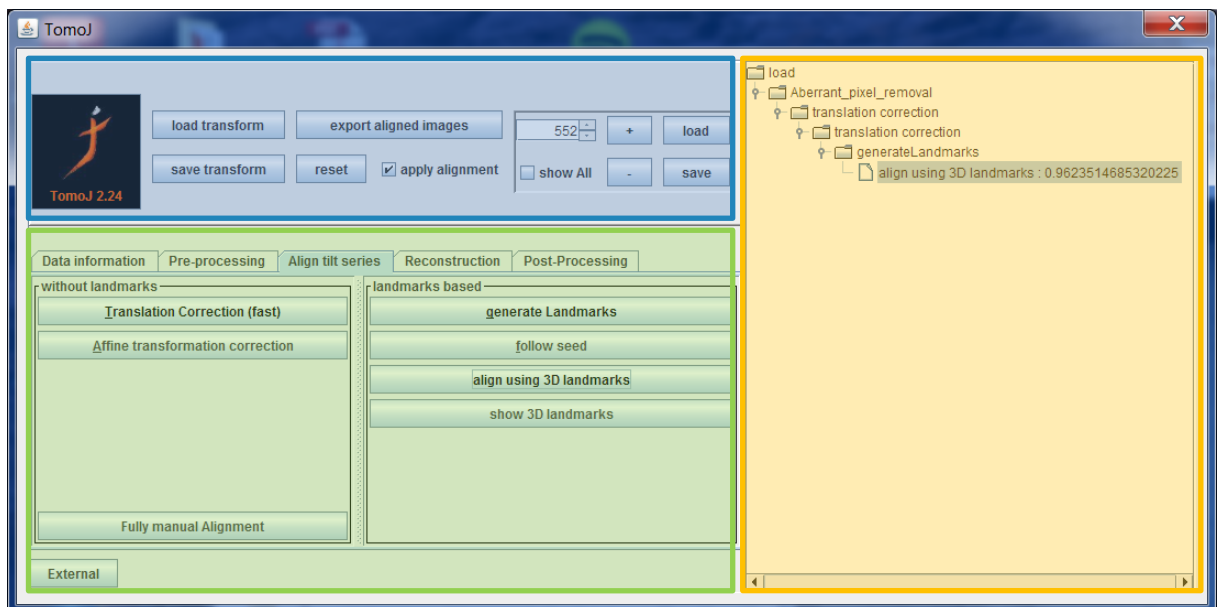


Figure 2: TomoJ interface. it is divided in 3 main parts : in blue the menu panel, in green the processing panel and in orange the history panel

3.1 Menu Panel

The top panel contains general options that may be needed at any time.

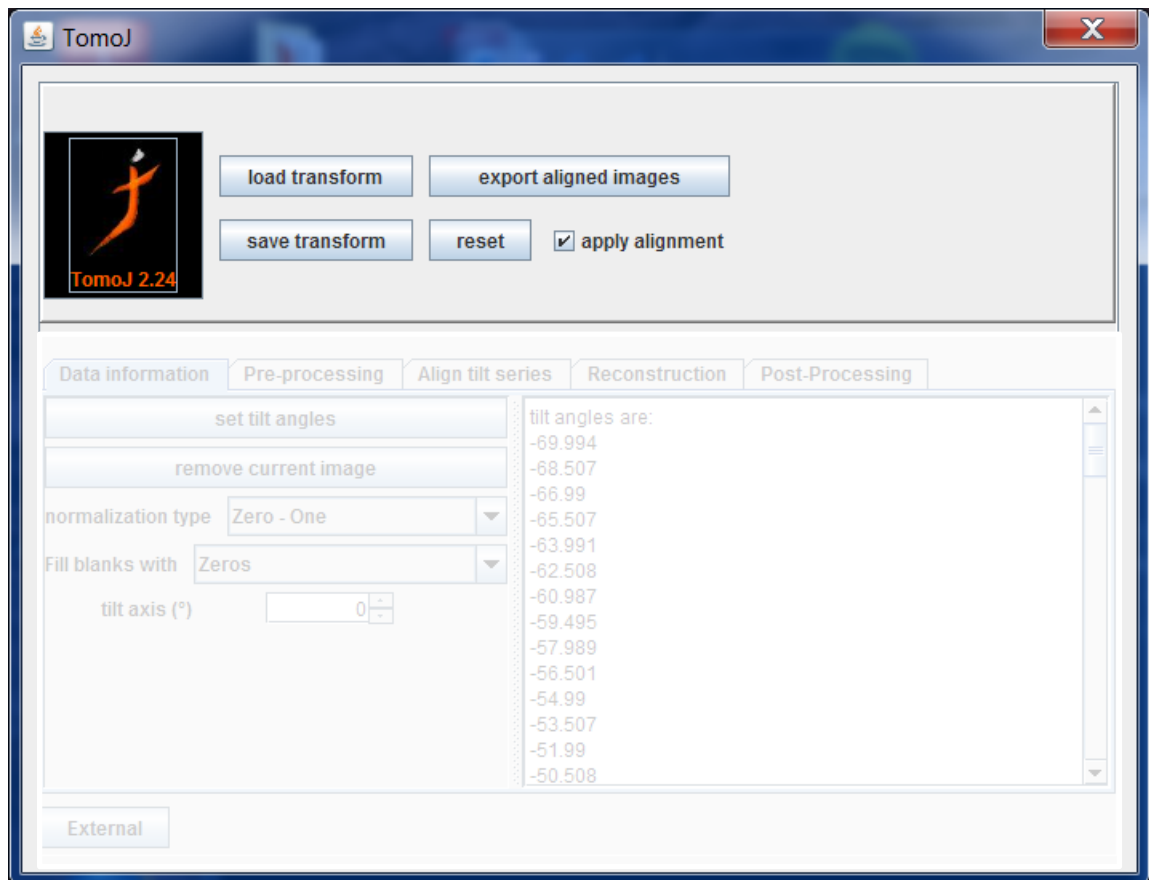


Figure 3: menu panel is highlighted

TomoJ logo

It shows the version of TomoJ running. A click on the logo will open the web page of TomoJ in your browser. On this webpage you have the possibility to check new versions of the program or download this manual.

Save transform

It saves the current transformations to file. This file is a simple text format with a line for each image containing tilt angle, tilt axis and affine Transformation as defined in [http://java.sun.com/javase/6/docs/api/java/awt/geom/AffineTransform.html#getMatrix\(double\[\]\)](http://java.sun.com/javase/6/docs/api/java/awt/geom/AffineTransform.html#getMatrix(double[])). This transformation is the one needed to align each image to the previous one. A last line is containing only the transformation (no tilt angle and tilt axis) is used for the all images. If CTRL key is down it is the final transform applied to each image that is saved. By default the word final is added to the proposed name for the file.

See section 6.1 of the manual for more information about transforms in TomoJ.

Load transform

This button allow to load already computed transformations saved in a file. The file format may be the transformation file created by TomoJ or .prexg, .prexf, .xf format created by other programs such as Imod.

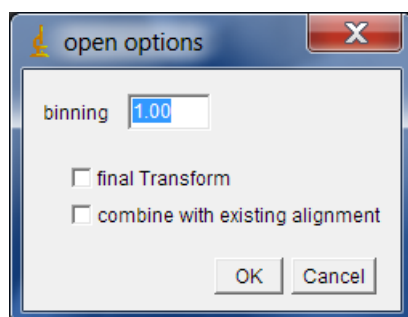


Figure 4: load transforms options window

If CTRL key is down while clicking on the button, a window will appear to ask some other loading options:

binning :

if the transformations were computed on a differently binned version of the tilt series. The value to give is the ratio between size of current tilt series and size of the tilt series used to produce the transformation files. For example, if the transformations were computed on a binned by 4 version of the tilt series, put 4 as value. If you have binned the tilt series by 2 to compute the reconstruction but did the alignment on original sized tilt series, put a 0.5.

final transforms:

the file in TomoJ format was saved with final transforms instead of consecutive transforms. See section 6.1 for more details about transforms in TomoJ

combine with existing alignment.

If for some reason you want to add the loaded transforms to existing alignment instead of overwriting them, please check this option.

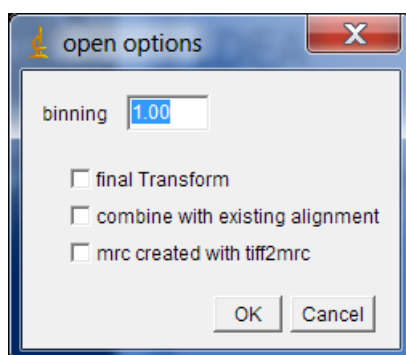


Figure 5: load transform option window when the file format is prexg, prexf or xf

Mrc created with tiff2mrc

If the file opened is in the imod's format prexf, prexg or xf, an additional option appears asking if mrc file used in Imod was created using tiff2mrc. If it is the case there is a minus in the translation that needs to be put. Checking this option corrects automatically this behavior.

Export aligned images

Due to data handling in TomoJ use of ImageJ File>save as, to save the aligned tilt series do not work as expected : it only save the unaligned tilt series. This button allows you to export the aligned tilt series. If the name finishes with .mrc, .spi, .xmp or .sel the file will be saved in the corresponding format. If another extension is given it will be saved in tiff format.

Reset

Resets the transformations currently in memory.

Apply alignment

Allows to switch between display of aligned images and unaligned ones.

3.2 Processing Panel

The processing panel is divided in 5 tabs: data information, pre-processing, align tilt series, reconstruction and post-processing.

3.2.1 Data Information

This tab contains general information about display and allows some modifications of tilt series.

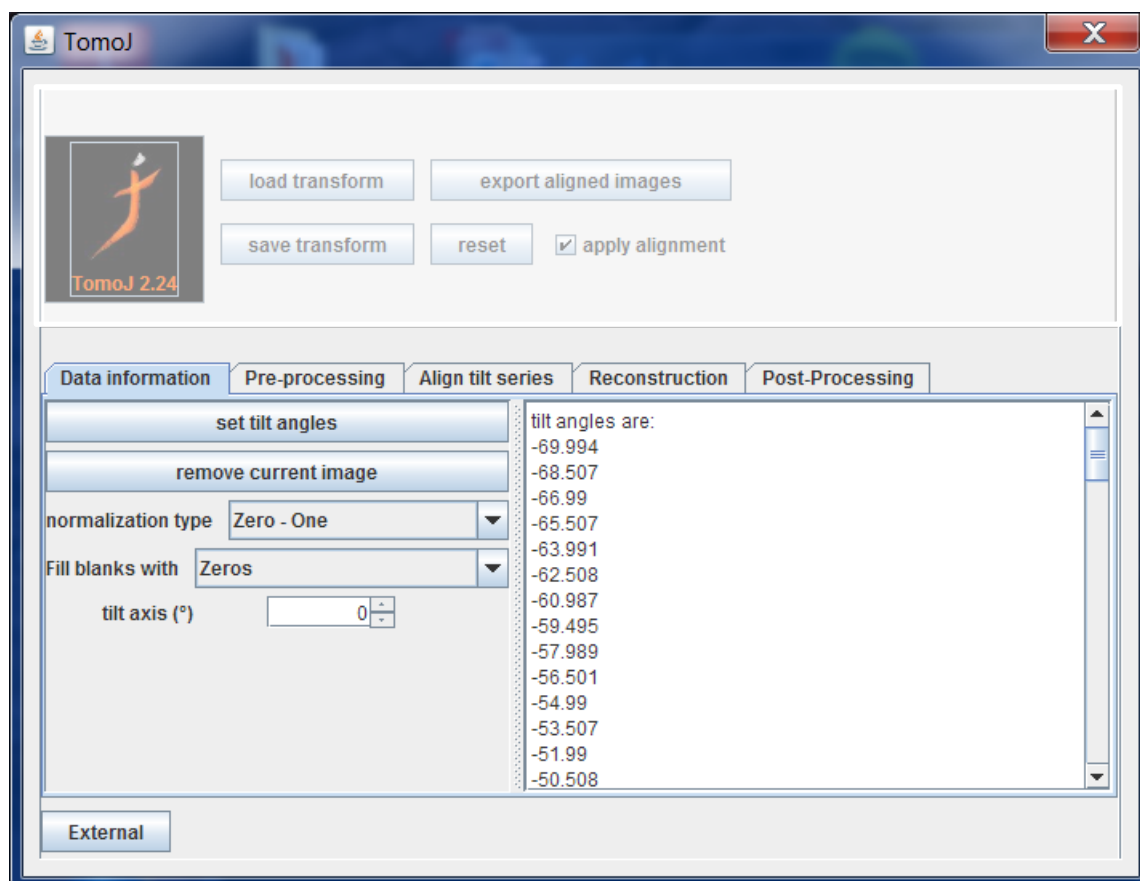


Figure 6: Data information panel

Set tilt angles

Allows changing the tilt angles starting from current image. The tilt angles are given in degree.

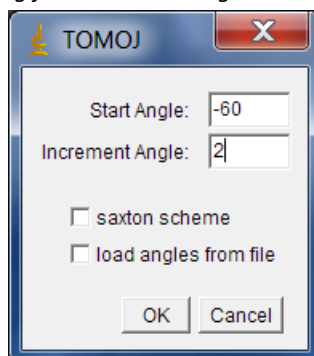


Figure 7: window to define angles of tilt series.

A dialog appears asking for the starting angle and the increment. The starting angle will be given to the image at index currently displayed, so if you want to change tilt angles to all images, check that

you are displaying first image of the tilt series. If angles are available in a text file check this option, a new windows will appear asking for the file. This file format can be rawtlt as given by FEI acquisition or recParam.txt as given by Jeol acquisition.

Remove current image

Removes the image currently displayed and all data associated.

If CTRL key is pressed while clicking on this button, the alignments of all other images are left unchanged.

Normalization type

For iterative reconstruction there is need of normalization 2 types are allowed:

- Zero-One: puts all images with zero mean and standard deviation of 1
- Zero-One (common field): puts all images with zero mean and standard deviation of 1 but this time mean and standard deviation are computed only in the field of view common in all images of the tilt series.
- Electron tomography: puts all images mean to zero, standard deviation is varying as a function of cosine of tilt angle
- None: no normalization applied, this is useful when images are already normalized before execution of TomoJ (by TomoJ or any other way)

Fill blanks with

Fills the blanks created by alignment by different ways

- Zeros: puts zeros in the blanks
- Mean: puts the mean of image in the blanks (if images are normalized, equivalent to zeros)
- NaN: puts Not a Number value. For reconstruction with ART/SIRT these values (positions) are not use in the reconstruction process. WBP will change this to mean automatically as NaN values are not compatible with FFT computing.

Tilt axis

The value of tilt axis used for reconstruction. You may modify it by entering directly the value or by clicking on the up and down arrows. A line showing the tilt axis is displayed on the tilt series.

3.2.2 Pre-processing

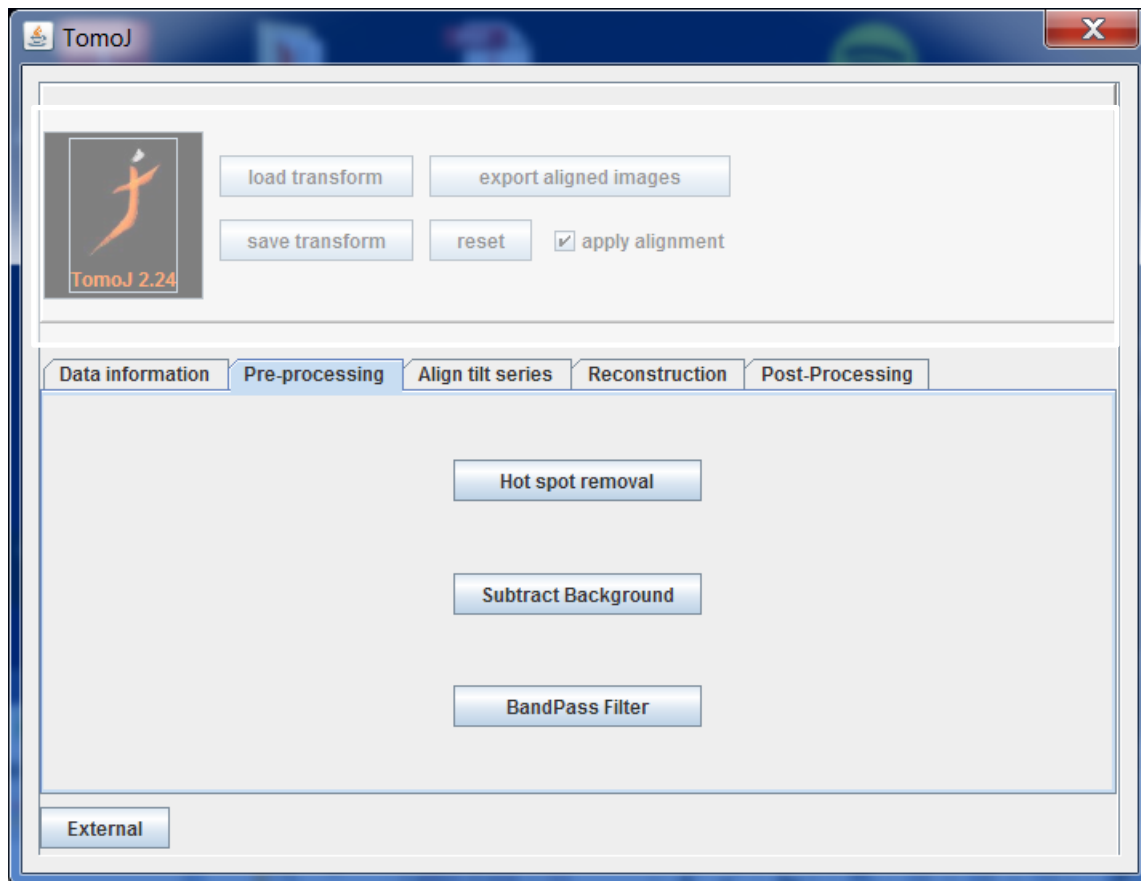


Figure 8: pre-processing panel

Three pre-processing possibilities exist in the pre-processing tab. These processing modifies the original unaligned images

Hot spot removal

It is a tool to remove aberrant pixels (white or black) due to X rays or dead pixels in CCD for example. It is computing for each pixel the squared difference with its neighborhood and then detects aberrant pixels as the one having the biggest value of difference. It replaces the pixels with the average of the neighborhood (without detected aberrant pixels of course). This tool is also available outside of TomoJ. Radius of neighborhood: a value of 1 or 2 is sufficient to remove aberrant pixels. The other parameters are not needed from inside TomoJ.

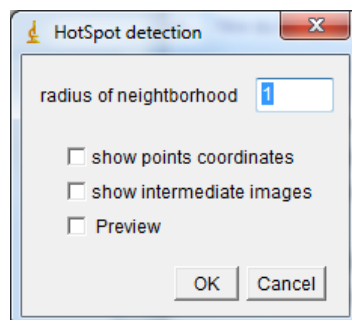


Figure 9: hotspot removal option window

Subtract background

It is the tools proposed by ImageJ, it allows the removal of variation of beam intensity. See ImageJ documentation for more details

http://imagejdocu.tudor.lu/doku.php?id=gui:process:subtract_background

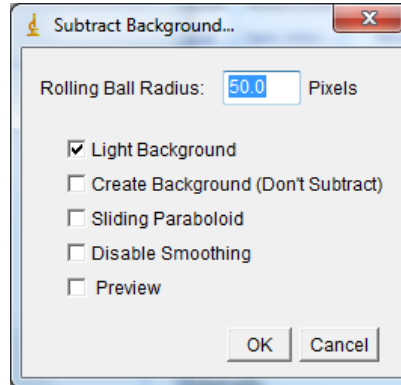


Figure 10: subtract background option window

Band pass filter

It allows filtering in Fourier space.

- Low cut is the minimum radius below which all frequency values are put to zero and above which the signal is multiplied by a cosine function to go from zero to the signal level.
- Low pass is the minimum radius above which the signal is kept intact.
- High pass is the maximum radius above which the signal is kept intact.
- High cut is the maximum radius above which all frequency values are put to zero and below which the signal is multiplied by a cosine function to go from zero to the signal level.

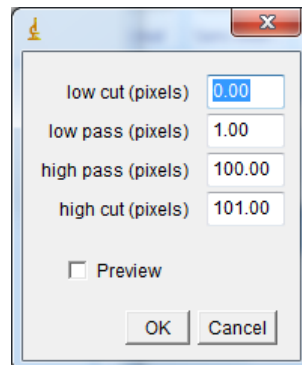


Figure 11: bandpass option window

3.2.3 Alignment of Tilt Series

The alignment panel is divided mainly in two parts. The first one called without landmarks contains the alignment procedure that needs no landmark. The second one called landmarks based contains all the alignment procedure using landmarks. To allow the definition and modification of landmarks a new menu appears in the top part (menu) of TomoJ interface.

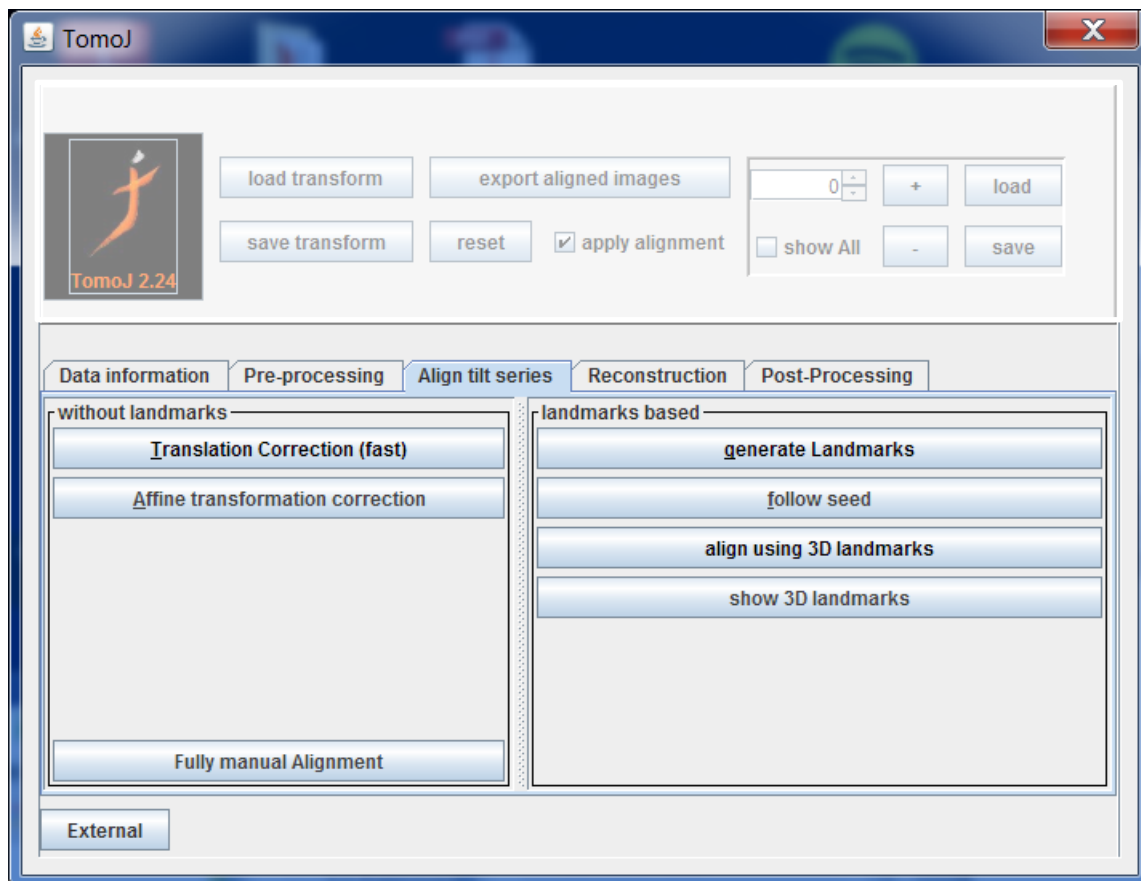


Figure 12: align tilt series panel

3.2.3.1 alignment WITHOUT Landmarks

parameter window

A parameter window will appear when clicking translation correction or affine transformation correction: it allows you to put some options for automatic alignments procedure. A preview window allows you to see in live the effect of parameters

Roi centered

It allows using only the central part of the image of the defined size. (size of roi given on original size of images, not binned one if binning enabled)

Bandpass filter:

if checked computation use a band pass filter in Fourier Space with the 4 parameters (in pixel)
The first one is the minimum radius below which all frequency values are put to zero and above which the signal is multiplied by a cosine function to go from zero to the signal level.
The second one is the minimum radius above which the signal is kept intact.
The third one is the maximum radius above which the signal is kept intact.
The last one is the maximum radius above which all frequency values are put to zero and below which the signal is multiplied by a cosine function to go from zero to the signal level.

Downsampling:

If checked computation use a binned version of the data. The binning is 2, 4 or 8. The binning is performed in Fourier space.

Variance filter:

If checked computation use this filter that will mainly give contours. The spinner allows putting the radius in pixel of the (square) neighborhood used.

Integer translation:

If checked, the result of computation will be integer translation; uncheck to get floating point translation.

Expand images:

If checked it will expand the images in the direction perpendicular to tilt axis by a factor dependent on tilt angles

Cumulative reference (for translation only):

If checked, instead of aligning images 2 by 2, it will take the image at zero tilt as the first reference to align the image at the next most negative tilt. It will then add this aligned image to the reference to align the next image. At each tilt, the reference will then be the sum of images that have already been aligned. When the most negative tilt angle is reached, the procedure is repeated from the zero-tilt view to more positive tilt angles.

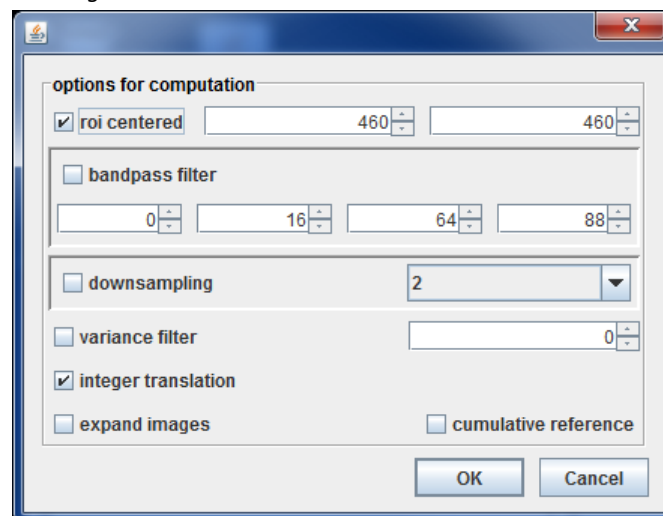


Figure 13: automatic alignment option window

Translation correction:

It allows correcting translation using cross correlation in Fourier space. If CTRL key is pressed while clicking, the computation will be done with a loop until no more translation is found.

Affine transformation:

It allows correcting translation, rotation and adding some deformations in the images. The result **SHOULD NOT** be used for reconstruction; it may just help for landmarks detection.

Fully manual Alignment:

A new window appears showing the image that is currently aligned (top left) and the previous image that serve as reference (top right and bottom left). A superposition of the two is shown at the bottom right, when the two images are well aligned you should see only grays (magenta and green should disappear or be really at minimum).

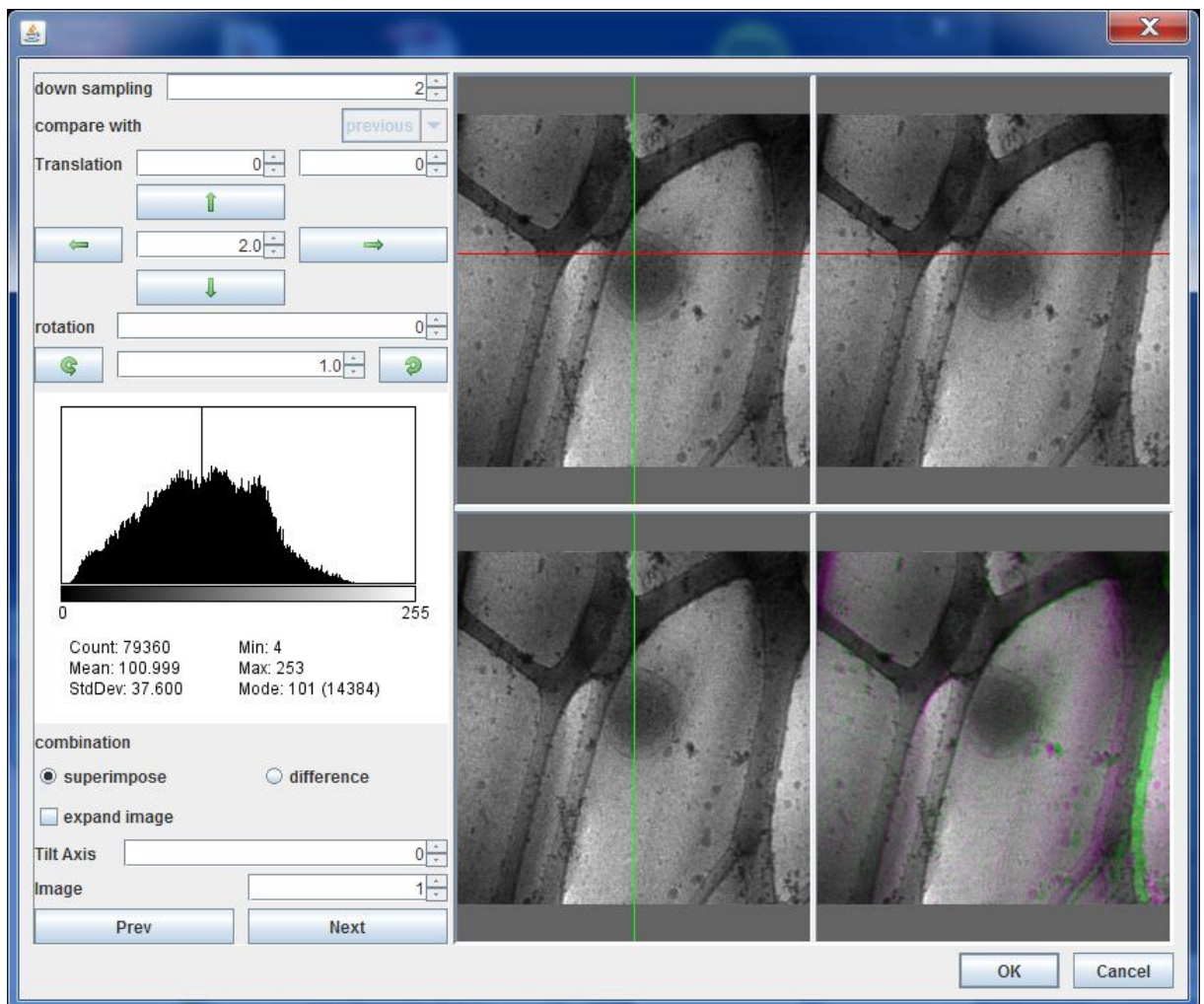


Figure 14: manual alignment window

To translate:

- *Using the mouse, click with left button on the image (top left) or the superposition (bottom right) and drag the mouse to translate.*
- *Use the spinners in the left panel where the values of translation are shown*
- *Use the arrows (up, down, left, right). The value between the arrows is the increment used when clicking the arrows or during the mouse dragging.*

To rotate:

- *Using the mouse, click with right button on the image (top left) or the superposition (bottom right) and drag the mouse to translate.*
- *Use the spinner in the left panel where the value of rotation is shown*
- *Use the curved arrows (left, right). The value between the arrows is the increment used when clicking the arrows or during the mouse dragging.*

Function helping alignment:

- *You may change the size of image (zooming in or out) using the down sampling option (top left)*
- *Red and green line between previous image (reference) and current image*
- *You may change the combination of the two images with the choice of superimposition (magenta and green) or difference.*
- *The histogram of combined image is displayed : when combination is difference the mean value should be minimal.*
- *Image expanding: the image is expanded in the direction perpendicular to tilt axis to compensate the difference of tilt angle between images. Beware that the tilt axis angle should be known. The value used for tilt axis is displayed in the tilt axis spinner, it may be modified interactively.*

Changing image to align:

- *Using mouse wheel*
- *Using buttons next and prev (bottom left)*
- *Changing the value of the image currently aligned (spinner image – bottom left)*

Finishing working with manual alignment

When all images are aligned you may go back to TomoJ with this alignment by clicking OK button. Using cancel button will allow you to go back in TomoJ discarding the manual alignment.

3.2.3.2 Landmark menu

When getting to Align Tilt Series tab, a menu appears in the top panel. It is for landmarks creation, deletion, modifying. The landmarks are given as point ROI of ImageJ (figure 7). There should be only one point at the same time on the image for it to be considered as landmark. When changing displayed image or activating many functions, the landmark coordinates are stored.

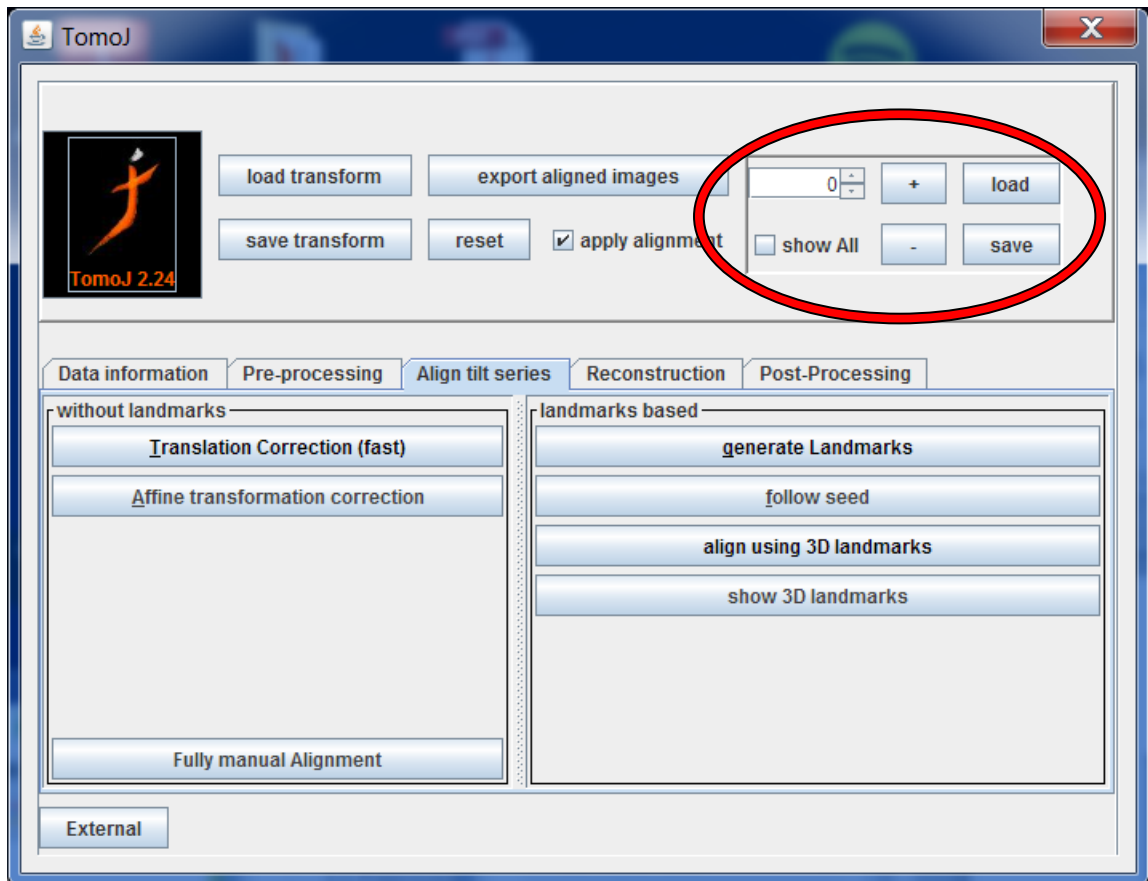


Figure 15: TomoJ window with alignment tab selected. Inside red circle are the new functionalities associated with landmarks.

The options available in the landmark menu are:

The chain displayed

Changing the value let you see the corresponding landmark if it is defined on the currently displayed image

“+”

allows you to add a new empty landmark chain. Putting a new point Roi on the image will allow you to define the landmark.

“-”

allows you to remove the currently displayed chain. If CTRL key is down when clicking on this button, all landmarks will be removed

“load” and “save”

Allows you saving and loading landmarks to/from file. If CTRL key is down, a window will appear asking the binning (if landmarks were created on a different binned version of the tilt series).

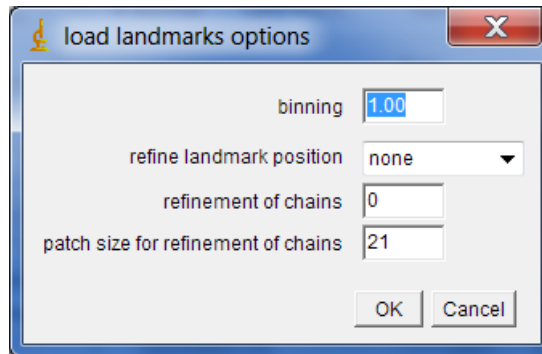


Figure 16: load landmarks option window

Binning:

Use if landmarks were created with tilt series with different binning. It is a ratio between current size and size of tilt series used for previous computation.

Refine landmark position:

When binning is different from 1, refine position of the landmarks by looking at local minima or maxima in the neighborhood.

Refinement of chains:

When binning is different from 1, refine position of landmarks in the chains as described in semi-automatic alignment.

Patch size for refinement of chains:

When binning is different from 1, patch size to refine chains as described in semi-automatic alignment.

“show all”

Makes all the landmarks in current image visible. A number appears at the side of each landmark, it has no significance. It is not possible to modify the position of a landmark in this mode.

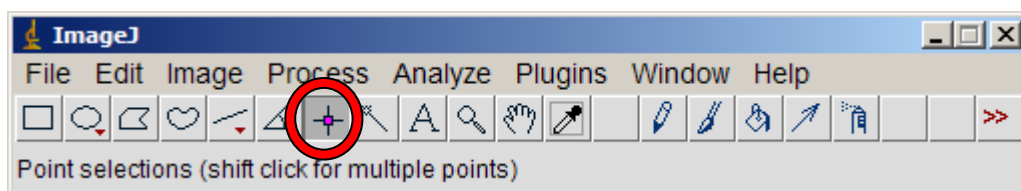


Figure 7: ImageJ window. Inside red circle is the type of ROI used to define landmarks.

3.2.3.3 Semi-automatic alignment

Generate landmarks:

It creates automatically landmarks see¹. In addition to the published algorithm, a version is also available where the regular grid is replaced by the 2% minimum values corresponding to local minima, the chains have also a fixed length and only the number of landmarks decided by user is kept. Two preview windows appear, the first one allow you to check the patch size used, the second one (for critical point) allows the tuning of the critical points detection. Using arrows key or F1 and F2 keys it is possible to change the index of the preview image.

Grid version options:

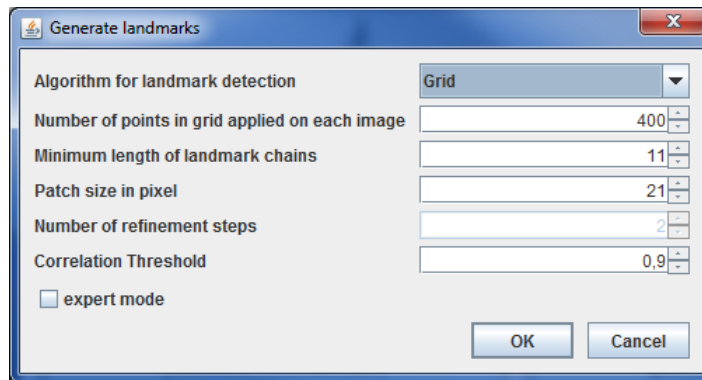


Figure 17: generate landmarks option window (grid version)

Number of points:

the number of points defining the grid (400 corresponds to 20x20 grid)

Minimum landmark chain length:

the minimum size of landmark chains (5 or more is good)

Patch size:

size of the patch used for local refinement. A preview window allows you to check the size of this patch.

Number of refinement step:

the chains are refined in the process. Values of 2-4 are good. You need to go to expert mode to change this value (checkbox at bottom)

Correlation threshold:

minimum correlation score that define as well maximum length of landmark chains and final selection. A value around 0.9 should be used.

¹ BMC Bioinformatics. 2009 Apr 27;10:124. "Marker-free image registration of electron tomography tilt-series." Sorzano CO, Messaoudi C, Eibauer M, Bilbao-Castro JR, Hegerl R, Nickell S, Marco S, Carazo JM.

Critical points version (using local minima or maxima) options:

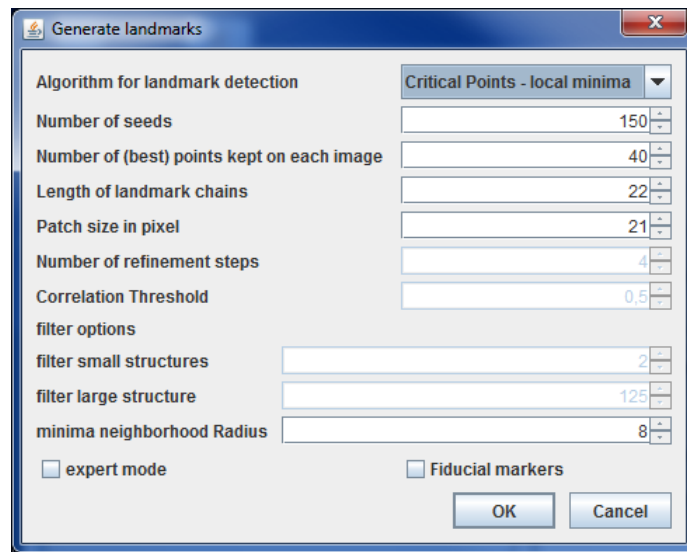


Figure 18: generate landmarks option window (critical point version)

Number of seed:

the maximum number of critical point to try follow

Number of best points kept on each image :

the number of kept landmark chains starting from each image

Length of Landmark chains:

fixed length of the landmark chains

Patch Size:

width of the square patch used during refinement.

Minima neighborhood radius:

to modify the radius used to test if critical point is a local minima (or maxima).

Fiducial markers:

if you check this option, it will apply symmetry to provide a better reference for fiducials and not be distracted by contours in images.

Next options are available only if you check the expert mode.

Number of refinement step:

the chains are refined in the process. Values of 2-4 are good.

Correlation threshold:

minimum correlation score for final selection. It is just to remove really bad chains so a value of 0.5 is enough.

Filter options:

A band pass filter is used (adapted from the one of ImageJ) for critical points detection, so the corresponding options are asked.

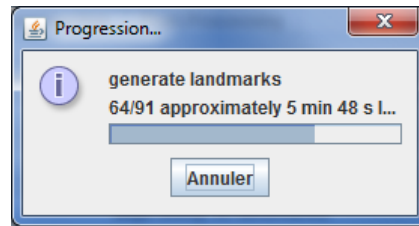


Figure 19: progression window allowing you to cancel computation.

```
C:\Program Files\Java\jre7\bin\java.exe
there are 400 seeds on image 75
there are 400 seeds on image 74
there are 400 seeds on image 87
there are 400 seeds on image 86
there are 400 seeds on image 85
there are 400 seeds on image 84
there are 400 seeds on image 83
there are 400 seeds on image 82
there are 400 seeds on image 81
there are 400 seeds on image 90
there are 400 seeds on image 89
there are 400 seeds on image 88
now follow and refine all seeds
validate chains
landmarks length : 48.132637075718016 (11, 91)
landmarks correlation : 0.9245855028081111 (0.9000390354914025, 0.96745717829382
93)
try to fuse landmarks : before 1916 landmarks
try to fuse landmarks : after 769 landmarks
landmarks created are seen on 31.94928478543563 images (11, 91)
total computation time 2 min 22 s
generate landmarks finished in 2 min 22 s
tp number of points 769
generate landmark finished
```

Figure 20: information displayed by "generate landmarks". The seeds are created for each image and then the chains are detected and refined. The average length of chains and correlation score is shown (in parenthesis the minimum and maximum) to help you to determine quality of detection. The landmarks are fused when possible, the numbers of landmarks chains before and after fusion are displayed. Final average length of chain is also displayed (minimum and maximum between parenthesis).

Follow seed:

It tries to follow a landmark visible on current image on preceding and following images. You need a tilt series that is already pre-aligned for it to work properly.

Patch Size:

width of the square patch used during refinement. A preview window allows you to check the size of this patch.

Length of Landmark chains:

fixed length of the landmark chains

Gold bead:

if you are trying to track a gold bead, please check this option. It will apply symmetry to provide a better reference and not be distracted by any contours in images.

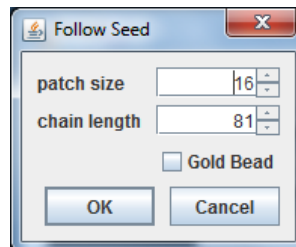


Figure 21: follow seed option window

Align using 3D landmarks:

It refines the alignment of images by determination of 3D position of each landmark and by making it punctual¹

Maximum in plane rotation allowed:

The algorithm allows the rotations of the different images in the tilt series. You have here the possibility to put the range in degrees in which it is allowed to search. The default value is zero as this kind of rotation is somehow not so normal: rotation of grid in holder (?) or instability in image formation. In any case this value should be kept small.

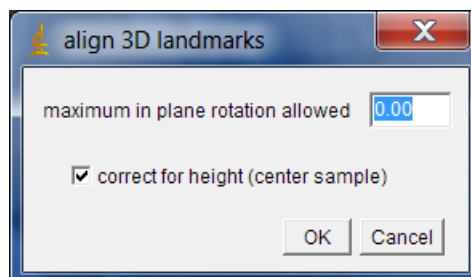


Figure 22: align using 3D landmarks option window

Show 3D landmarks:

It allows after align using 3D landmarks to see the position of the 3D Landmarks computed in align using 3D landmarks

3.2.4 Reconstruction

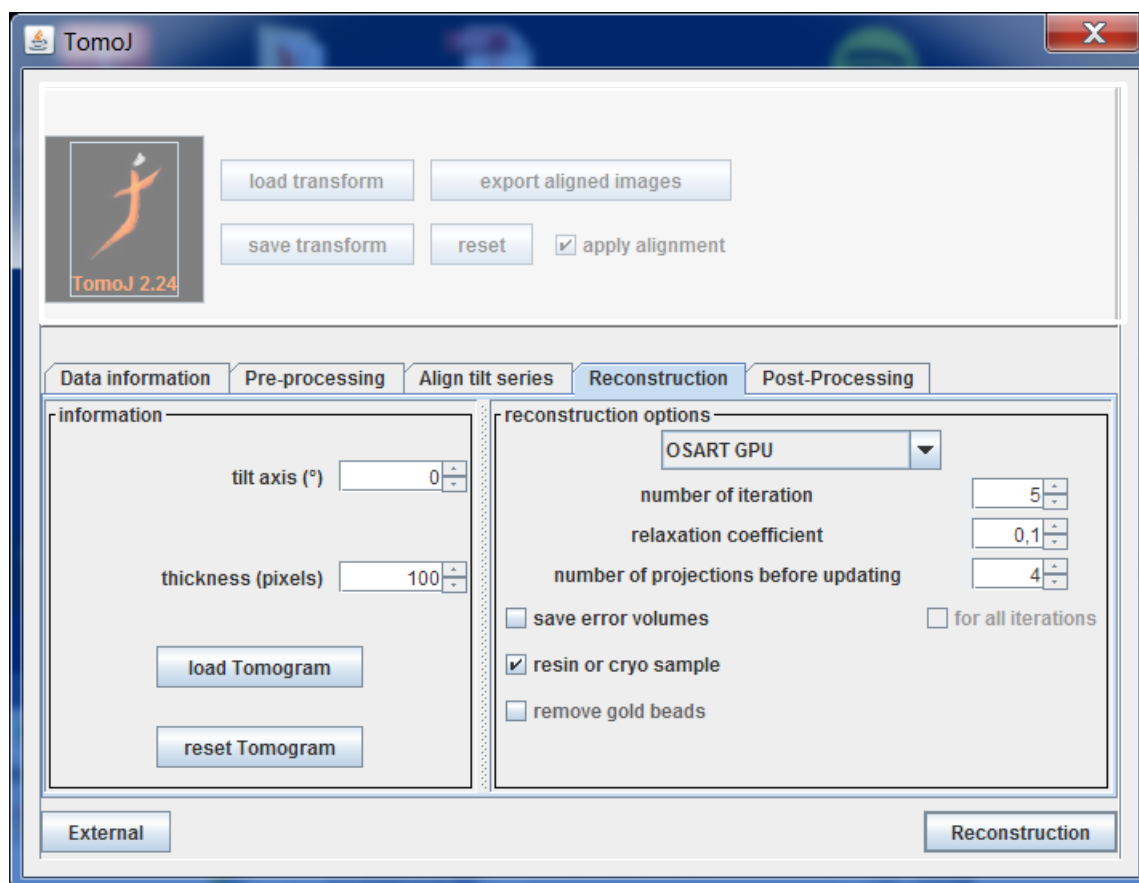


Figure 23: reconstruction tab

The reconstruction tab is divided in two main parts: information and reconstruction options. The first part is for general information for reconstruction; the second one allows the choice of reconstruction algorithms such as back-projection (to avoid), weighted back-projection, ART and SIRT. It also allows access to some parameters of these algorithms.

3.2.4.1 Informations

Tilt axis:

This the value of tilt axis used for reconstruction. You may modify it by entering directly the value or by clicking on the up and down arrows. A line showing the tilt axis is displayed on the tilt series.

- If you click with left button of your mouse on the text "tilt axis" then you will switch between rotating images to have tilt axis vertical in aligned images or not. For automatic alignment procedures, it is strongly recommended to let the images not rotated.
- If you click with right button of your mouse on the text "tilt axis" then you will get the sum of all the aligned images of tilt series. If the CTRL key is down you will get the minimum instead of sum. This functionality is useful for alignment checking. It replace ImageJ's Image>Stacks>Z project that will work on unaligned images.
- If you click with middle button (or wheel) on the text "tilt axis" then you will be able to change the center of tilt axis (by default it is at the center of the images)

Thickness:

Put the thickness in pixel of the reconstruction. The size of the reconstruction will be width x height (of tilt series) x thickness. A good way to estimate the thickness in pixel is to convert the theoretical thickness in nanometer to pixel using the pixel length (voxels in reconstruction are cubic).

Load tomogram:

Load a pre-computed reconstruction to refine using ART/SIRT

Reset tomogram:

Reset the current reconstruction

3.2.4.2 Reconstruction options

Reconstruction method²:

Select the method you want to use for reconstruction.

BackProjection:

This performs a simple back-projection of the tilt series inside the reconstruction. This method is known to have low accuracy due to incorrect sampling of Fourier Space. There is need of weighting of the projections: TomoJ allows one type of weighting³, but if you have previously performed another weighting scheme on your projection you may use this method for reconstruction.

Weighted BackProjection (WBP):

This computes weighted back-projection as has been described in ³.

Weighting diameter: parameter to reduce the diameter of the filter used for weighting. For expert only.

ART⁴:

This performs an iterative algorithm for reconstruction, performing theoretical projections and comparison to experimental projections to maximize the likelihood of the reconstruction with data. Iteration is the use of all projections to refine the volume.

Number of iterations:

ART is an iterative process, so you need to decide the number of iteration you want to spend in the process. Values of 5-15 are good, but you can do even more if you want.

Relaxation coefficient:

This value is used to reduce convergence and be less sensitive to noise. Values are]0,1]. A good estimate can be 1/nbiteration if you don't have too much noise, lower this value if reconstruction is too noisy.

TomoJ developers often use 10 iterations and a relaxation coefficient of 0.1

² BMC Bioinformatics 2007, 8:288. "TomoJ: tomography software for three-dimensional reconstruction in transmission electron microscopy." Messaoudii C, Boudier T, Sanchez Sorzano CO, Marco S.

³ Frank J: "Electron tomography: Three-dimensional imaging with the transmission electron microscope". Plenum Press. New York; 1992.

⁴ J Theor Biol 1973, 42:1-32. "ART: mathematics and applications. A report on the mathematical foundations and on the applicability to real data of the algebraic reconstruction techniques." Herman G.T., Lent A., Rowland S.W.

SIRT⁵:

this performs an iterative algorithm for reconstruction, performing theoretical projections and comparison to experimental projections to maximize the likelihood of the reconstruction with data. Iteration is the use of all projections to refine the volume. The difference with ART is that modification of reconstruction is done only once all comparisons rather than each comparison are finished.

Number of iterations:

SIRT is an iterative process, so you need to decide the number of iteration you want to spend in the process. Values of 30 or more are needed.

Relaxation coefficient:

to reduce convergence and be less sensitive to noise. Values are]0,1]. SIRT is naturally less sensitive to noise than ART, so a value of 1 can be used, lower this value if reconstruction is really too noisy. TomoJ developers at least use 30 iterations and a relaxation coefficient of 1, but prefer 100 iterations.

OSART:

This performs an iterative algorithm for reconstruction, performing theoretical projections and comparison to experimental projections to maximize the likelihood of the reconstruction with data. Iteration is the use of all projections to refine the volume. This is the generalization of ART and SIRT.

Number of iterations:

ART is an iterative process, so you need to decide the number of iteration you want to spend in the process. Values of 5-15 are good, but you can do even more if you want.

Relaxation coefficient:

This value is used to reduce convergence and be less sensitive to noise. Values are]0,1]. A good estimate can be $1/nbiteration$ if you don't have too much noise, lower this value if reconstruction is too noisy.

Number of projections before updating:

ART use a value of 1, SIRT uses a value of number of projection in tilt series. You may choose any value in the interval

OSART GPU:

This performs an iterative algorithm for reconstruction, performing theoretical projections and comparison to experimental projections to maximize the likelihood of the reconstruction with data. Iteration is the use of all projections to refine the volume. This is the generalization of ART and SIRT. The computation is performed on GPU

Number of iterations:

ART is an iterative process, so you need to decide the number of iteration you want to spend in the process. Values of 5-15 are good, but you can do even more if you want.

⁵ J Theor Biol 1972, 36:105-117. "Iterative methods for the three-dimensional reconstruction of an object from projections." Gilbert P.

Relaxation coefficient:

This value is used to reduce convergence and be less sensitive to noise. Values are]0,1]. A good estimate can be $1/n_{\text{iteration}}$ if you don't have too much noise, lower this value if reconstruction is too noisy.

Number of projections before updating:

ART use a value of 1, SIRT uses a value of number of projection in tilt series. You may choose any value in the interval

Save error volume:

Compute an additional volume corresponding to variation in the reconstruction final iteration and save it on disk

For all iterations:

If save error volume is selected, it allows to compute the error volume not only of the last iteration but of all iterations.

Options common for all iterative algorithms

Resin or cryo sample:

check if your sample has constant thickness over an area far larger than the field of view. During reconstruction artifacts due to this kind of samples can be corrected.

Remove gold beads:

there is possibility to remove gold bead by hysteresis thresholding from reconstruction by setting 2 thresholds (shown in red on the images)

- *The first one let you see the "seeds" it must be low enough to take ONLY gold beads*
- *The second one is the maximum extent allowed, it must be high enough to take gold beads entirely*

To execute reconstruction click on reconstruction button visible all the time

3.2.5 Post-processing

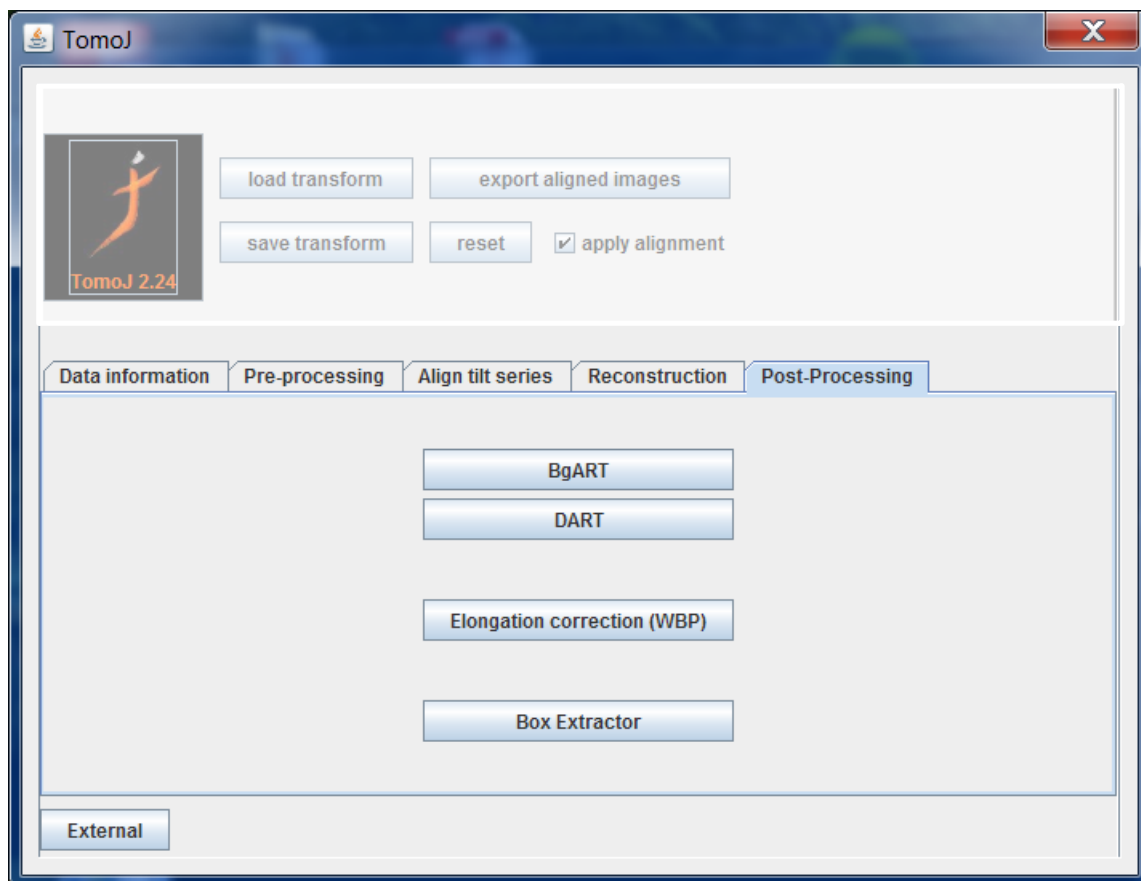


Figure 24: post-processing panel

Four options are available:

BgART :

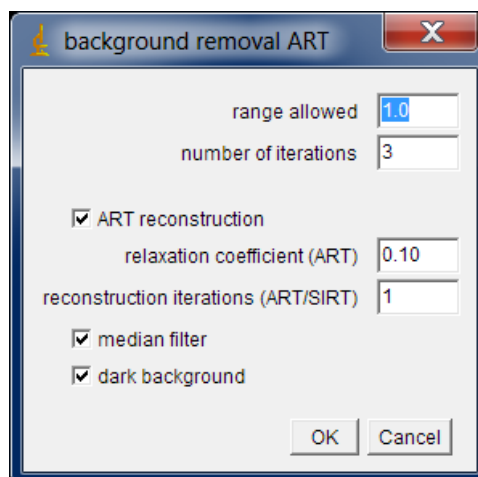


Figure 25: BgART option window

Algorithm described in messaoudi et al 2013⁶. BgART is an iterative reconstruction algorithm that removes the noise, considered as Gaussian white, in the background preserving the object signal. The reconstruction at step n is thresholded using $\text{mean} + k * \text{sigma}$, where mean and sigma are the average voxel value and standard deviation of the reconstruction respectively, and k a user-defined factor describing the level of noise. Values below threshold are put to mean value and values above threshold are kept identical. An iteration of the reconstruction process is then applied. At the end, the background is uniform while the object keeps its intensity values.

Range

It is the factor by which multiply standard deviation to get threshold.

Number of iterations

Number of cycles of filtering + reconstruction

ART reconstruction

Check to do ART, uncheck to do SIRT

Relaxation coefficient

By default use the parameters in the reconstruction panel (see this section for more information)

Reconstruction iterations

Number of iterations for reconstruction process (see reconstruction panel section for more information)

Median filter

Perform a median after filtering to remove salt and pepper noise that is often present after filtering

Dark background

Consider background as black if checked.

DART:

discrete ART reconstruction as described in Batenburg et al 2009⁷ (need a first reconstruction). It will ask how many elements (gray levels) you will have at the end. Then it will ask for some parameters see ⁷ for more details.

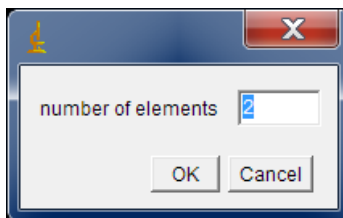


Figure 26: DART first window asking for number of element in the reconstruction

⁶ microsc microanal. 2013 dec;1669-77. "three-dimensional chemical mapping by eftem-tomog including improvement of snr by pca and art reconstruction of volume by noise suppression." messaoudi c, aschman n, cunha m, oikawa t, sorzano co, marco s.

⁷ Ultramicroscopy. 2009 May;109(6):730-40. "3D imaging of nanomaterials by discrete tomography." Batenburg KJ, Bals S, Sijbers J, Kübel C, Midgley PA, Hernandez JC, Kaiser U, Encina ER, Coronado EA, Van Tendeloo G.

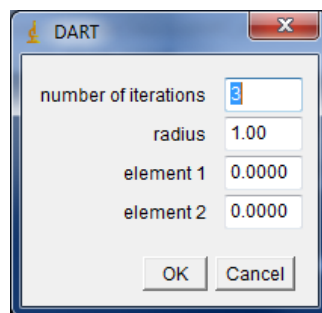


Figure 27: DART option window

Number of iterations :

Number of iteration process of the algorithm.

Radius :

Radius used in border detection.

Element 1 :

Estimated value of first element.

Element 2:

Estimated value of next element.

Elongation correction:

Reconstruction with missing projection, as is the case in electron tomography, produces artifacts. One of them is an elongation in Z axis (perpendicular to plane at 0°); it can be corrected by scaling by a factor along Z axis (ref). This is mainly useful with WBP due to the fact that this method is sensible to missing wedge. ART and SIRT are less sensible and the elongation is lower.



Figure 28: elongation correction option window

Box Extractor:

Allows extracting 3D boxes from the reconstruction. It may be useful to average 3D particles with 3rd party software. You will need to put at least a point selection at the center of the feature you want to extract. You are able to extract more than one box from the same plane by using shift + click (multiple points on image). If you want to extract boxes from different plane, you will have to click "extract all" on the different planes.

Size of box:

Size in pixel of your square box

Preview:

Check this and a preview window will appear (extracted box of first point)

Extract all:

Extracts the boxes having as center the points currently selected on this plane. Boxes are not displayed but directly saved on disk in a “crop” directory at the level of original image.

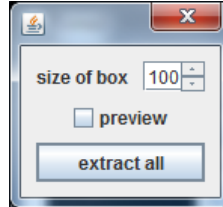


Figure 29: box extractor option window

3.2.6 External Programs

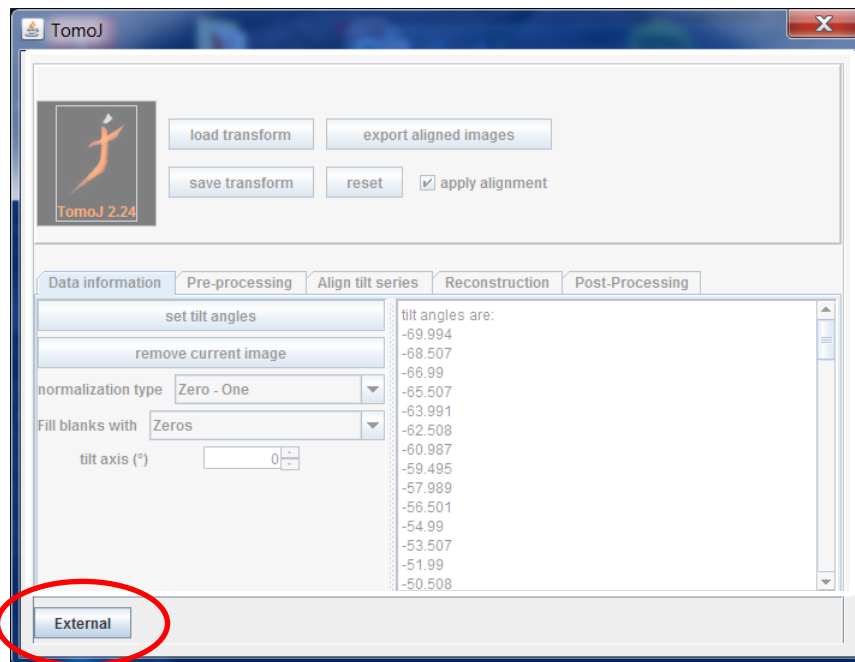


Figure 30: external program button

Clicking on the external button allows you to run external programs from inside TomoJ. It will save the aligned tilt series, run the command and open a result file in a different window.

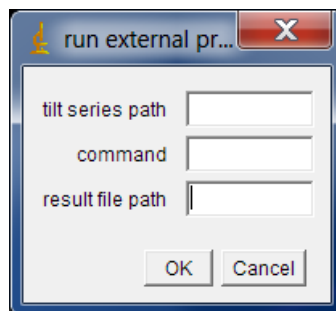


Figure 31: external program parameter window

Tilt series path:

Place where you want to save the tilt series for the external program.

Command:

The command you want to run. The best is to create a batch file to be sure that all environment variables are correctly set.

Result file path:

The path to the result of the program (an image). It will be opened in a different window.

3.3 History Panel

When starting TomoJ a window appears asking for project directory. In this directory TomoJ will save the processing you do. You may choose to create a new directory or use an existing directory. By default the name of the new directory will be date and time. If you cancel this window or select none, processing will not be automatically saved and history panel will not be visible.

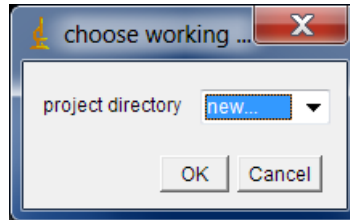


Figure 32: history directory selection window

History tree

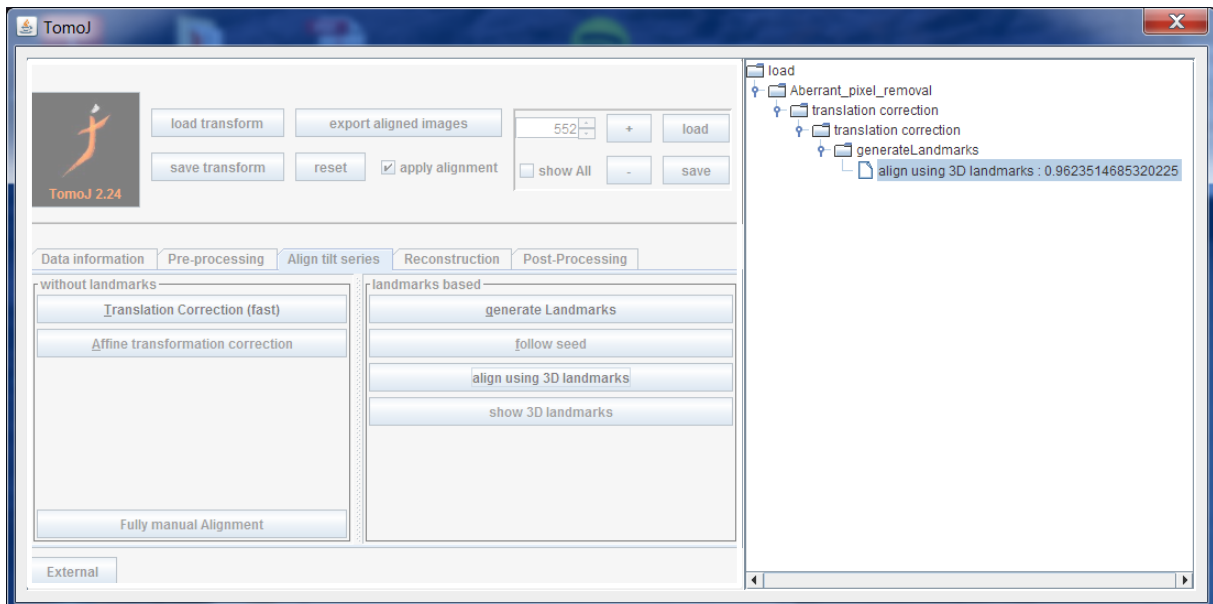


Figure 33: history panel

The history of processing is displayed as a tree. When you do a processing, a leaf is added to the tree at the current branching. By selecting the different node of the tree, you may look at the effect of the different processing.

Going back and forth

By selecting a node, you put back the alignment, landmarks and reconstruction as they were at the end of the processing indicated by the text. The arguments of the processing are displayed in the command line window and in the log window of ImageJ.

Delete dead branching

To delete a dead branching, select the node to remove and press delete. It will remove the node, all children and all files associated.

Copying data from one node to another

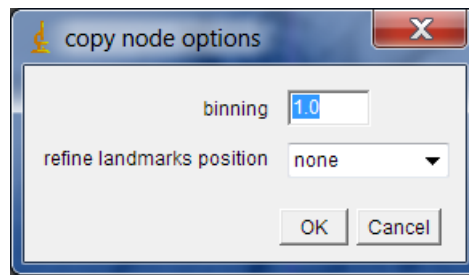


Figure 34: copy node option window

To copy alignment and landmarks from one node to another, select the node you want to copy and then drag'n drop to the location you want to copy to. A window appear asking for binning ratio between node you copy to and node you copy from and also if you want to refine the position of landmarks (when binning is greater than 1). New node is created called "copy from tree" with data associated (and scaled if needed).

4- Classic workflow

The classic way to use TomoJ is quite simple:

- Open your tilt series in ImageJ, execute TomoJ. If tilt angles are not defined in slice labels, a window will appear asking you for tilt angles.
- Then if you have hot spots in your image, go to pre-processing tab and run remove hotspots.
- Then go to alignment tab:
 - Run **correct translation** (you will perhaps have to play a little with the options)
 - run **generate landmarks** (if it does not work you will perhaps have to run affine transformation before)
 - **align using 3D landmarks**
- Check the quality of alignment:
 - Is there no visible “jumps” of the images when displaying them one after the other?
 - Go to reconstruction tab to display tilt axis, does the tilt series seem to turn around it? Is the specimen under the central square (on tilt axis) not moving (or eventually around tilt axis)?
 - Do a sum of your images (see the description of tilt axis to see how). Do you see straight lines perpendicular to tilt axis?
- Then go to reconstruction tab:
 - set the correct **thickness** (high enough to contain all reconstruction)
 - choose the **method of reconstruction** and the associated parameters
 - check the **resin/cryo sample** if needed (important)
- Run **reconstruction**.

If you have difficulties with alignment try the other options available in TomoJ. It is also possible to apply some filters from the many available in ImageJ (see the plug-in web page for more) for alignment, save the transforms, close TomoJ, open your original tilt series, run TomoJ and load the transforms.

For reconstruction with ART and SIRT, if you have a reconstruction that seems “blurry”, first check that the alignment is correct. If alignment is good, then the parameters for reconstruction are not good. Two possibilities arise: you may try more iterations (especially true for SIRT) or your relaxation coefficient is too low (in electron tomography 0.1 is often good enough in ART to reduce the noise).

5- Command line / macro execution

TomoJ is not usable inside macros, but can be executed from a new functionality of ImageJ : java scripting. See <http://rsb.info.nih.gov/ij/developer/javascript.html> for more information about java scripting.

This requires creating a .js file with a line like this:

```
IJ.runPlugin("TomoJ_", "option1 option2 ... optionN");
```

Where options and its parameters might be any of the following (options are applied in given order):

- **Nonnormalisation** : for using no normalization of the images
- **Settiltaxis value**: set the tilt axis to the value. This value should be a real value representing the angle in degree to put tilt axis vertical. By default it is put to zero.
- **center cx cy** : set the tilt series center to (cx,cy). cx and cy are real coordinates in the image. By default this values are put to width/2 and height/2 respectively.
- **settiltanglesregular startangle incrementangle**: set the tilt angles with a starting real angle (in degree) and an increment angle (real value in degree).
- **loadangles tiltAnglesFile** : load the angles from a file. File format can be .rawtlt (FEI, Imod) or recParam.txt (Jeol). Transformation files created by TomoJ include also the tilt angles.
- **loadtransforms transformsFile** : load a transformation file previously created by TomoJ.
- **loadlandmarks landmarksFile** : load landmarks from a file created previously by TomoJ.
- **Xcorr**: perform a cross-correlation alignment.
- **automaticalignment numberOfPointsInGrid minimumSequenceLength localPatchSize correlationThreshold** : perform alignment as described in ⁸ follow : align by cross correlation and affine transformation if no transform file was given, then generate landmarks using grid algorithm and finally align using 3D landmarks. The parameters correspond to the ones needed for the generation of landmarks. Parameters are integer values, only correlationThreshold is a real value.
- **wbp thickness diameter** : executes WBP with the corresponding parameters (see reconstruction tab for more information).
- **art nbIterations relaxationCoefficient thickness resinOrCryoSample** : executes ART reconstruction with the corresponding parameters (see reconstruction tab for more information). nbIterations and thickness (in pixel) are integer values, relaxationCoefficient is a real value in the range]0,1], resinOrCryoSample takes the value 1 is cryo or resin sample, zero otherwise.
- **sirt nbIterations relaxationCoefficient thickness resinOrCryoSample** : executes SIRT reconstruction with the corresponding parameters (see reconstruction tab for more information). nbIterations and thickness (in pixel) are integer values, relaxationCoefficient is a real value in the range]0,1], resinOrCryoSample takes the value 1 is cryo or resin sample, zero otherwise.

For command line execution the best is to create a .js file as described above and then type in the command line:

```
java -jar ij.jar:TomoJ.jar -macro scriptTomoJ.js TiltSeries.tif
```

⁸ BMC Bioinformatics. 2009 Apr 27;10:124. "Marker-free image registration of electron tomography tilt-series." Sorzano CO, Messaoudi C, Eibauer M, Bilbao-Castro JR, Hegerl R, Nickell S, Marco S, Carazo JM.

6- *Tips and tricks*

One way to compute things faster is to resize your data to a smaller size. Then do your alignment, check the quality of it by performing a reconstruction. If you are confident enough you may then use this alignment on your original data. Another option is to use the landmarks generated with downsized data on your original data and refine them to local minima/maxima. Perform then the alignment and the reconstruction.

You may filter drastically your images to improve alignment. When alignment is good enough, you save it and reload it on original data (or copy node in the history tree).

In manual alignment, if the images are black, grey or with awful contrast, please change the down-sampling to lower value. This bug of display appears sometimes when parts of image are undefined.

7- Additional informations about TomoJ

7.1 Handling of Transformations

In TomoJ the images stored in memory are always unaligned ones. The creation of aligned image for display or reconstruction is computed every time it is needed. The transformations used for this purpose are stored in memory as transformation to align each image to the previous one. To create the aligned images it is then needed to combine several transformations to obtain a final transformation. This final transformation is the transformation applied to one image to create the aligned image used in the reconstruction. In this process there is the need to define one image as reference and all images will be aligned to this one. In TomoJ this image is the one whose tilt angle is nearest to zero. As sometime it is needed to apply a transformation to the reference image (rotation to put tilt axis vertical for example) there is an additional transformation for zero tilt image. In the process of creating final transform of one image there is the combination of all the transformations between this image and the zero tilt image and the additional transformation for zero tilt image