

TUTORIAL

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All computations performed for this tutorial on a DELL LATITUDE D620, cpu intel core 2 duo T7200 at 2GHz and 2 Gb of RAM.

If you use TomoJ please cite it as follow:

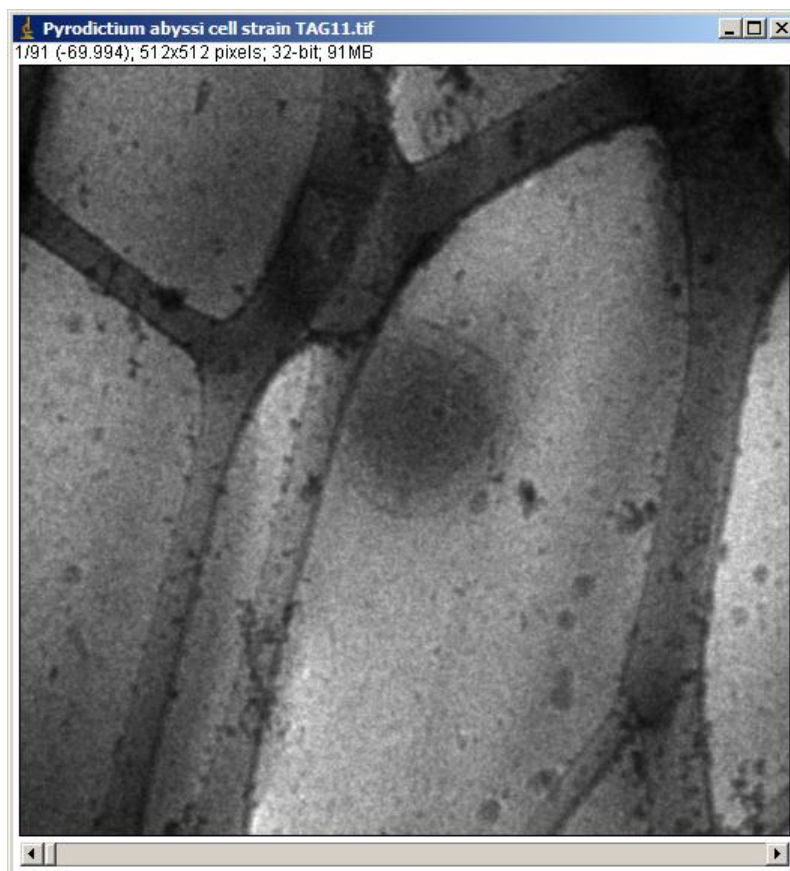
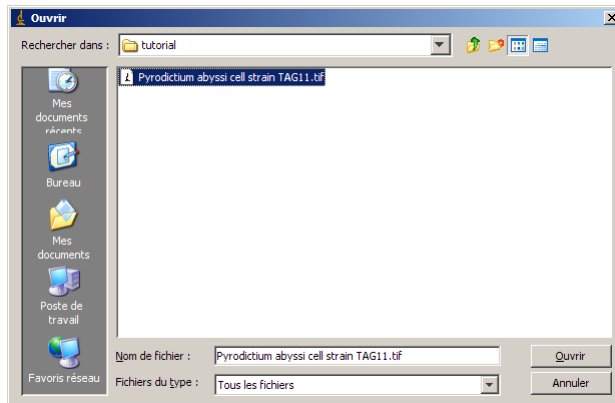
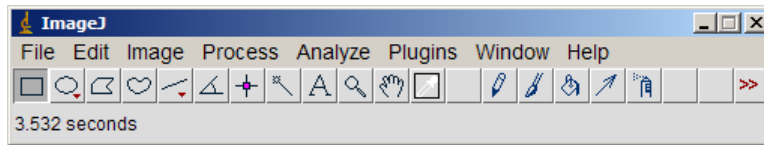
BMC Bioinformatics. 2007 Aug 6;8:288. "TomoJ: tomography software for three-dimensional reconstruction in transmission electron microscopy." Messaoudi C, Boudier T, Sanchez Sorzano CO, Marco S.

If you use the alignment as described in this tutorial please cite also:

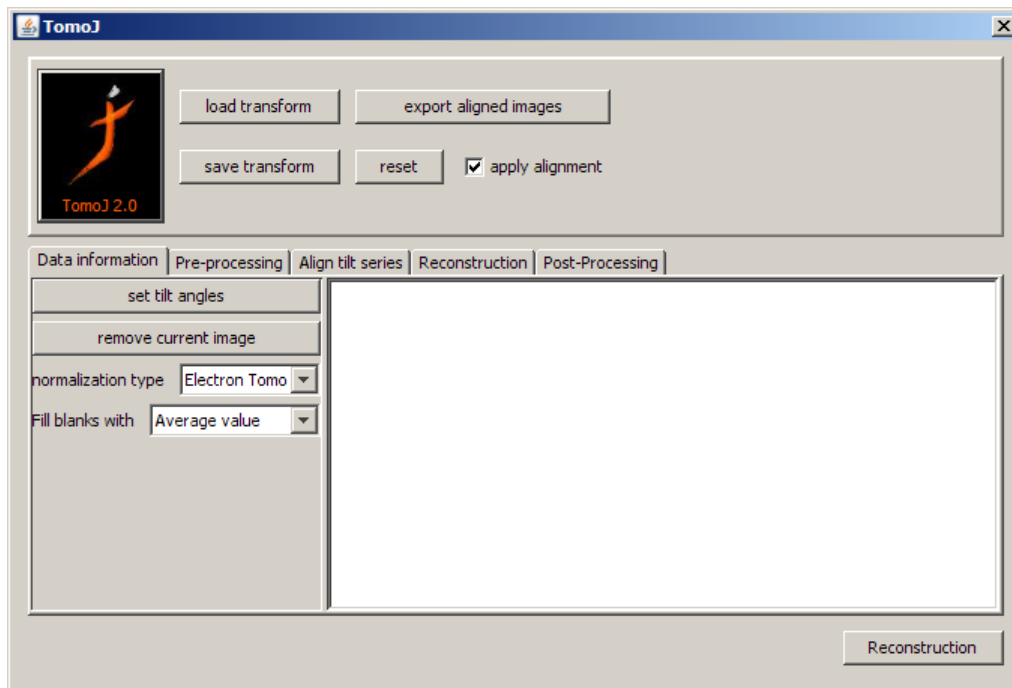
BMC Bioinformatics. 2009 Apr 27;10:124. "Marker-free image registration of electron tomography tilt-series." Sorzano CO, Messaoudi C, Eibauer M, Bilbao-Castro JR, Hegerl R, Nickell S, Marco S, Carazo JM.

OPENING FILES

Open your tilt series using ImageJ menu File>Open. Any format known to ImageJ is Ok for working with TomoJ. Select Pyrodictium abyssi cell strain TAG11.tif

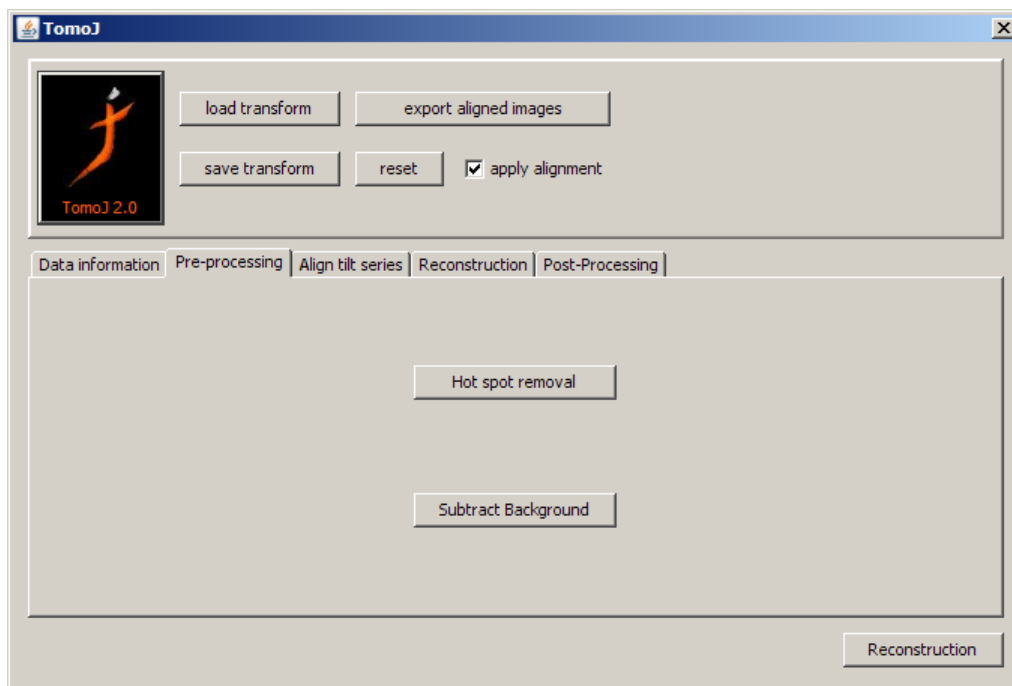


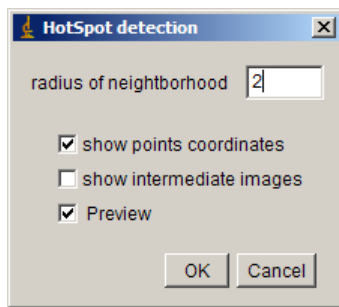
Execute TomoJ using Plugins>TomoJ>TomoJ. As the tilt angles are defined in the slice label, no window appears asking for tilt angles, but directly the TomoJ interface.



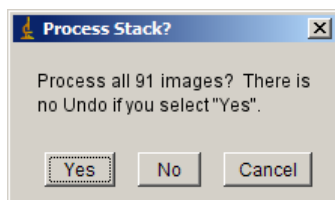
PRE-PROCESSING OF DATA

Go to the **Pre-processing** tab and use **remove hot spots**. This step is recommended but is not mandatory. You won't see much effect on the tutorial data as there is really few hot spots on it.

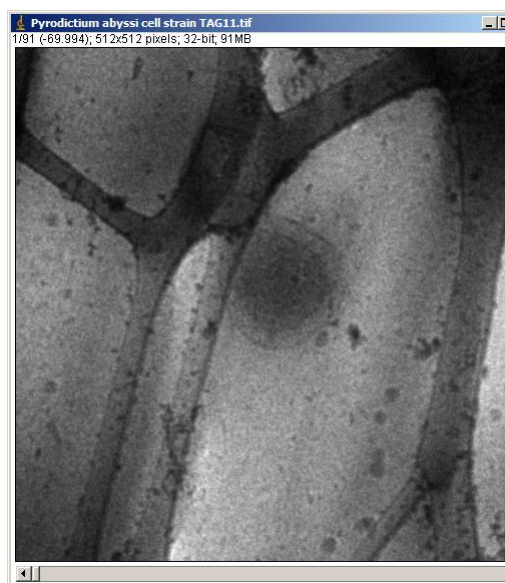




Select a radius of neighborhood of 2, the computing is a little slower but it will detect better hot spots. Higher value would need a more and more uniform image.



Select yes to perform the removal of hot spot in all images. If you click no, the change on the current image won't be kept when changing the displayed image.



before

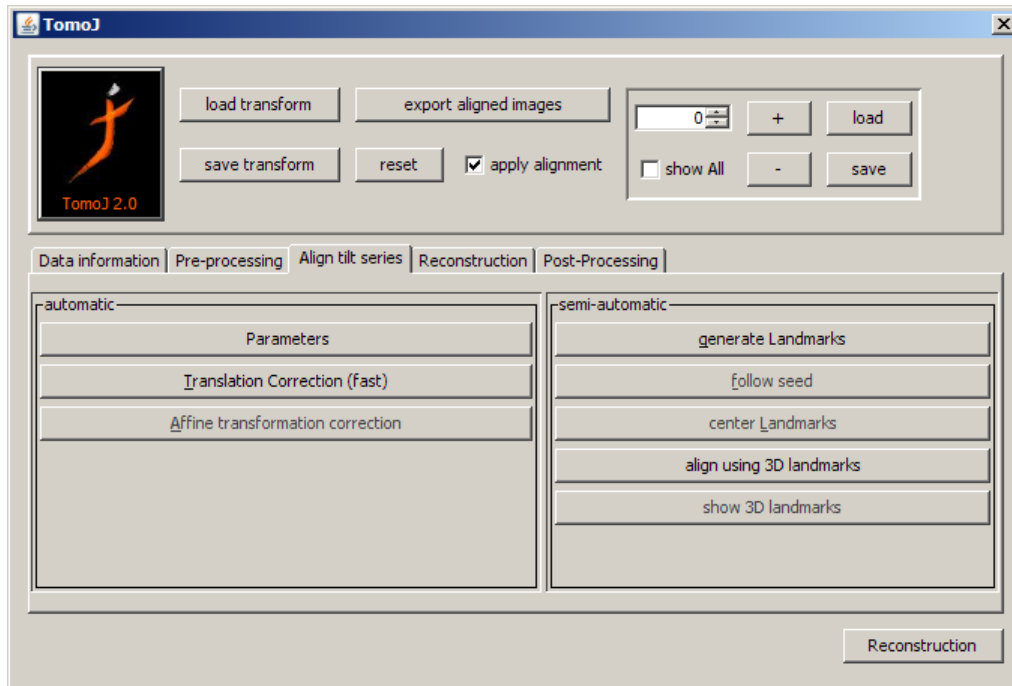


after

As said before the variations are very small and this data set has very few hot spots, so no detectable difference is seen on these screen shots.

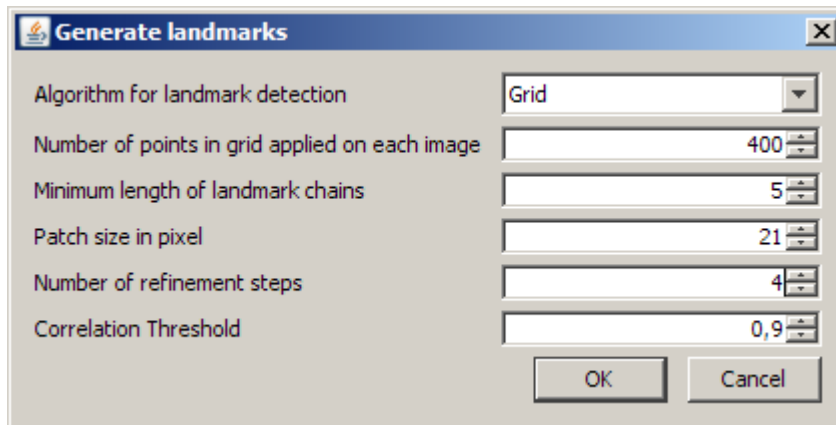
ALIGNMENT OF TILT SERIES

Go to the **Align tilt series** tab. Click first on **Translation Correction (fast)**. It will perform an alignment by cross-correlation in Fourier space.



Now you can see gray bands on borders, this correspond to the displacement of the image, as there is no information uniform bands are in place, the value in these bands may be changed in the tab information>fill blanks with. The choice given are zero, mean value (should be zero if images are normalized) or NaN (see manual).

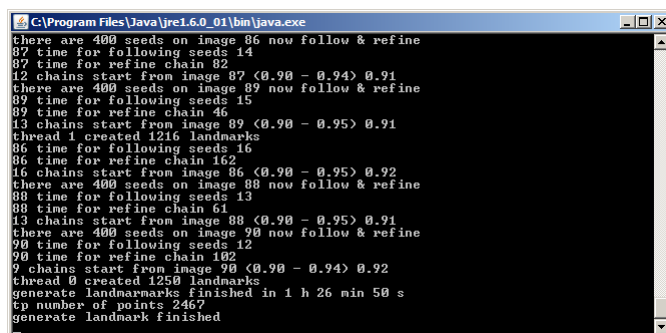
Now click on generate Landmarks



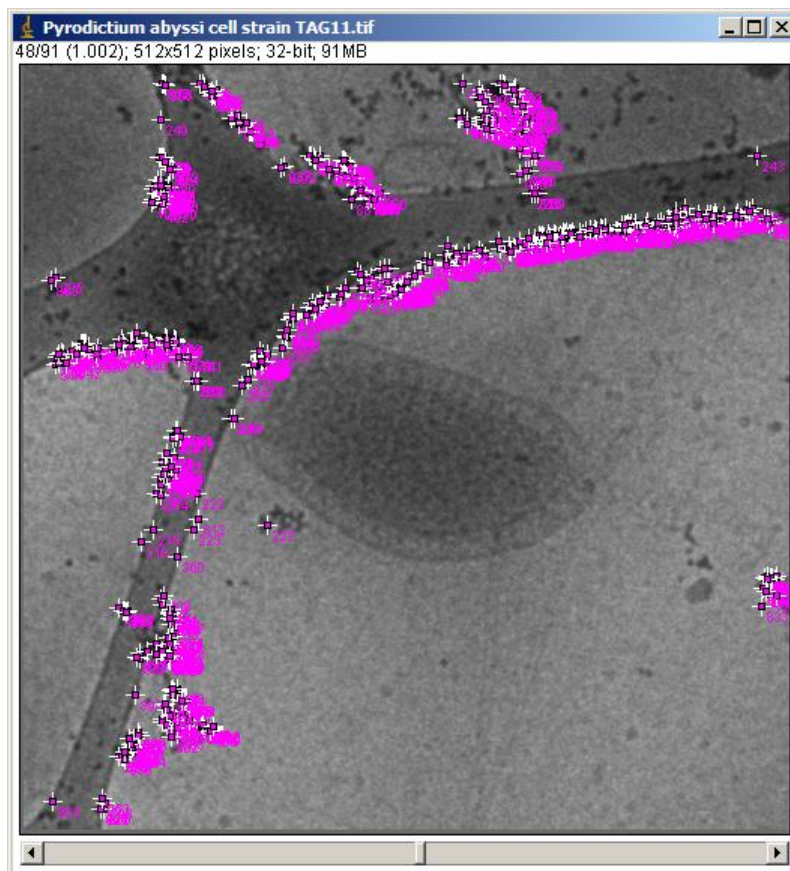
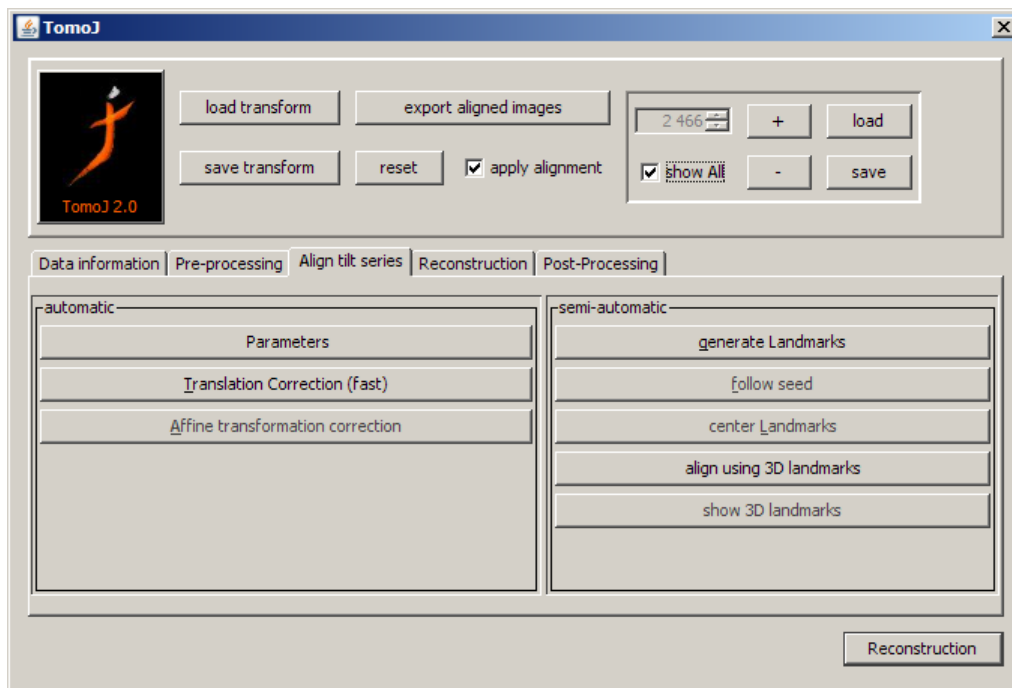
There are 2 different algorithms for generating landmarks: putting a grid of seeds on each image and following as far as possible on previous and next images or looking for local minima (or maxima) on each image and follow on previous and next images for a fixed length. In the case of the tutorial data, the one that is working best is the grid version (check use grid). You will need a big amount of points (400 points corresponds to 20x20 grid), a minimal length quite small (5 is enough). You don't really need to change the patch size for images of this size (512x512) you may go for higher value with bigger images. The number of refinement step is good between 2 and 4. The correlation Threshold is a major parameter as it is this value that will be influent on landmark chains lengths as well as the number kept on each image.

If you prefer to use the critical points detection, it is possible on this data but will take longer time. The parameters are then amount of points (around 40) will be the number of chains starting on each image kept at the end of process. The landmarks chain length is the fixed size of landmark chains, a size of 61 is good for this data. For the patch size and the number of refinement step, the remarks are the same as with grid. The threshold is not really important you can put a value low enough (0.5) just to be sure of removing really bad landmark chains (but good enough to be in the N best landmark chains! – reasons could be bad pre-alignment or really noisy data).

You can use local maxima instead of local minima if you have inverted contrast (Dark field or EFTEM images for example).

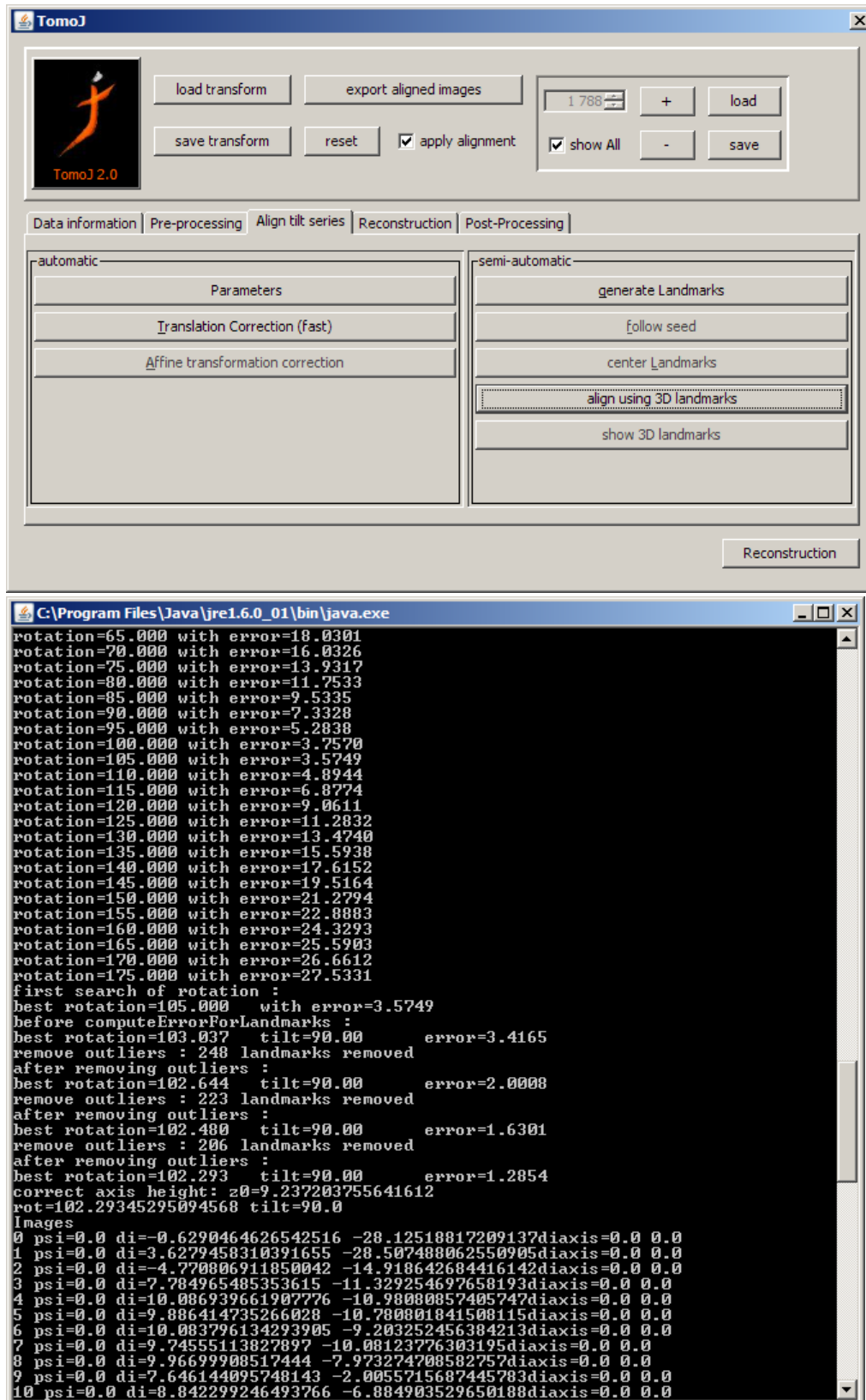


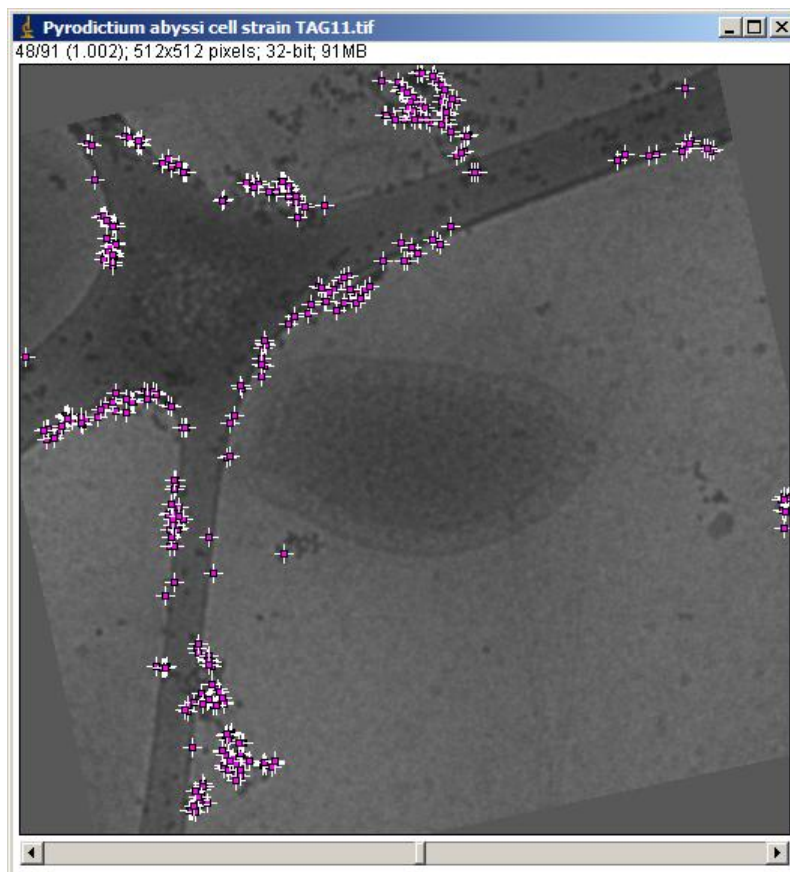
Once the computation is finished, you can check the **show All** button to see all the created landmarks (2467 with the above parameters).



Each cross corresponds to a landmark. The number at the side corresponds to the order of appearance of crosses for each image. To disable this labeling you might configure it in ImageJ Edit>Options>Point Tool: uncheck the label Points check box. Here you can also change the color of the cross if needed.

Now click on align using 3D landmarks

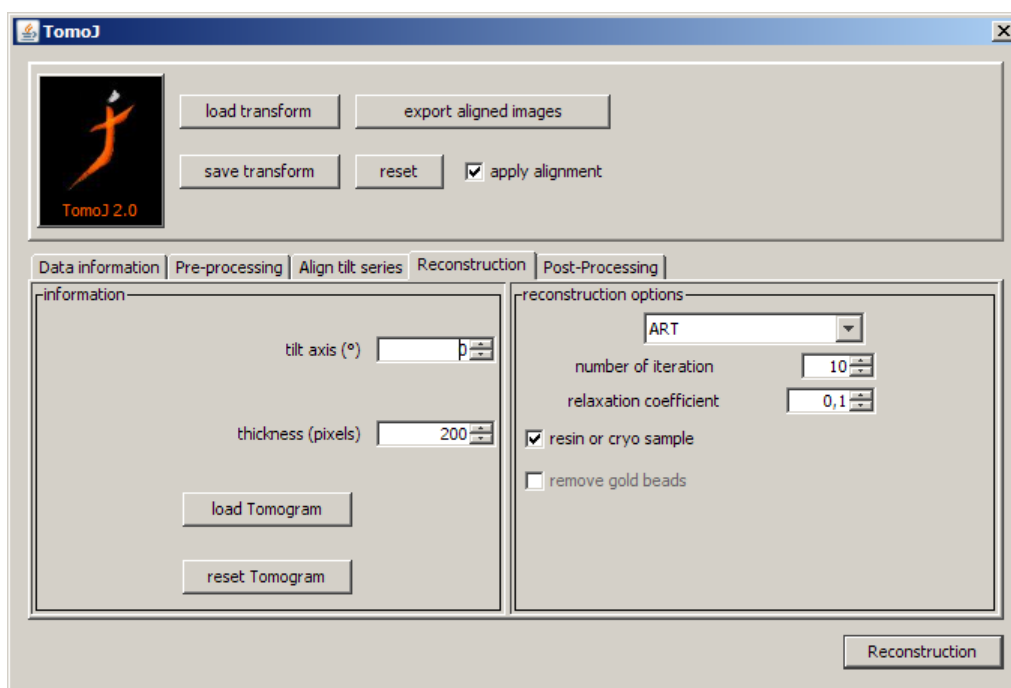




You can see that some landmarks disappeared; they were removed to obtain a better alignment.

RECONSTRUCTION

Go to the Reconstruction tab





As you can see, the points are replaced by a visualization of the tilt axis. Points are not deleted just not displayed. Therefore, going back to the alignment tab, the points will reappear. The tilt axis was put to zero by the alignment procedure (see the rotation of the images), so no need to change its value. The thickness value is quite important for computation time. You need to put it high enough to contain your entire sample or you will get some artifacts. But if it is too high, the computation time will be longer for no good reason. With this sample, a thickness of 150-200 pixels is good.

For reconstruction, use ART or SIRT algorithms, they give far better results than classical weighted back-projection. With ART, 10 iterations and a relaxation coefficient of 0.1 are usually good. With SIRT, you would need something like 30 iterations and a relaxation coefficient of 1.

As this sample was taken in cryo-tomography, check the resin or cryo sample checkbox, it will optimize the reconstruction process for this type of samples.

Click the reconstruction button to start reconstruction.

```

C:\Program Files\Java\jre1.6.0_01\bin\java.exe
iteration #5          maximum : 0.004594358261977144 (image 90.0)
ART : 100% remaining 0 ms total :16 min 26 s
dissimilarity        average : 0.0013724841775037051
                    minimum : 0.727624630393752E-4 (image 44.0)
                    maximum : 0.004621453979617995 (image 90.0)
iteration #6          maximum : 0.001343763676946013
ART : 100% remaining 0 ms total :15 min 45 s
dissimilarity        average : 0.412226407852779E-4 (image 44.0)
                    minimum : 0.004535279806191414 (image 90.0)
iteration #7          maximum : 0.00132213514222505
ART : 100% remaining 0 ms total :15 min 43 s
dissimilarity        average : 0.24497777586841E-4 (image 44.0)
                    minimum : 0.004491673809786314 (image 90.0)
iteration #8          maximum : 0.0012274541415445163
ART : 100% remaining 0 ms total :12 min 0 s
dissimilarity        average : 0.142053156014166E-4 (image 44.0)
                    minimum : 7.544756210200042E-4 (image 44.0)
                    maximum : 0.00458711278518827 (image 90.0)
iteration #9          maximum : 0.00458505294834676 (image 90.0)
ART : 100% remaining 0 ms total :13 min 19 s
dissimilarity        average : 7.769865795549362E-4 (image 44.0)
                    minimum : 0.004469112403402045 (image 90.0)
paint width:230      height:169
paint offsetx:11      offsety:363

```

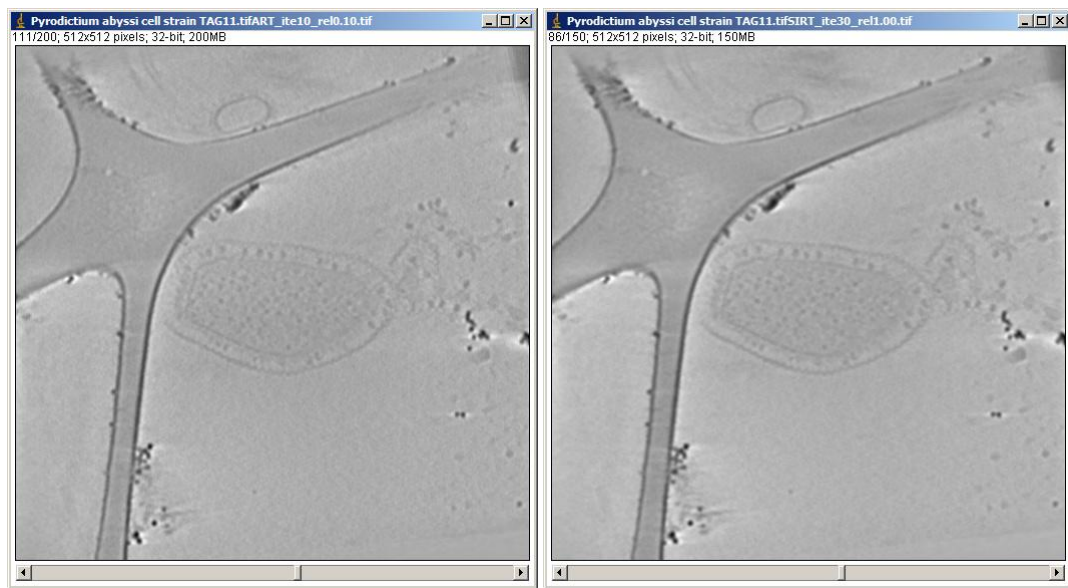
```

C:\Program Files\Java\jre1.6.0_01\bin\java.exe
iteration #26         maximum : 0.00626527626917006 (image 90.0)
number of cpu used 2
SIRT 2 : 100% remaining 0 ms total :13 min 34 s
dissimilarity        average : 0.0019720979915192503
                    minimum : 0.001369547353239556 (image 29.0)
                    maximum : 0.006257773322139684 (image 90.0)
iteration #27         maximum : 0.0013554721464921571
number of cpu used 2
SIRT 2 : 100% remaining 0 ms total :13 min 36 s
dissimilarity        average : 0.0017635425506303798
                    minimum : 0.0013578145770998925 (image 29.0)
                    maximum : 0.006250580804254237 (image 90.0)
iteration #28         maximum : 0.0013467443766954476 (image 29.0)
number of cpu used 2
SIRT 2 : 100% remaining 0 ms total :13 min 36 s
dissimilarity        average : 0.0013362857604763672 (image 29.0)
                    minimum : 0.006243670910523006 (image 90.0)
iteration #29         maximum : 0.0019478517435405194
number of cpu used 2
SIRT 2 : 100% remaining 0 ms total :13 min 34 s
dissimilarity        average : 0.0013263929993487075 (image 29.0)
                    minimum : 0.0062370497676364362 (image 90.0)
iteration #30         maximum : 0.001406425302364100
number of cpu used 2
SIRT 2 : 100% remaining 0 ms total :13 min 37 s
dissimilarity        average : 0.0013263929993487075 (image 29.0)
                    minimum : 0.006230676732661110 (image 90.0)
paint width:230      height:169
paint offsetx:11      offsety:237

```

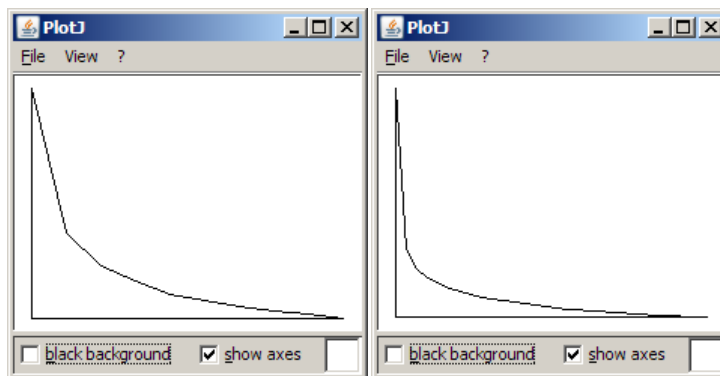
Command line window. Left: after ART reconstruction, 10 iterations, relaxation coefficient of 0.1 and thickness of 200. Right: after SIRT reconstruction, 30 iterations, relaxation coefficient of 1 and thickness of 150.

When the reconstruction is finished you have access to the reconstruction window



Reconstruction window. Left: after ART reconstruction, 10 iterations, relaxation coefficient of 0.1 and thickness of 200. Right: after SIRT reconstruction, 30 iterations, relaxation coefficient of 1 and thickness of 150. You can see that the reconstructions are really similar and information is the same, ART is often more grainy and SIRT more smooth, choose the one that suits you better.

A plot window, where you can see the evolution of difference score, appears. It should stabilize to a constant value.



Plot window. Left: after ART reconstruction, 10 iterations, relaxation coefficient of 0.1 and thickness of 200. Right: after SIRT reconstruction, 30 iterations, relaxation coefficient of 1 and thickness of 150.

Now you can save the reconstruction in tiff format to open it later with ImageJ or other software. ImageJ propose some 3D visualization plug-ins, but they are quite slow and limited for data of this size. If required, to use other 3D visualization software (Amira, Avizo, UCSF Chimera...) you can also export the volume in many format including mrc or Spider. If you want a rendering based on voxel intensity, you will need to invert the reconstruction (ImageJ Edit>Invert) before.