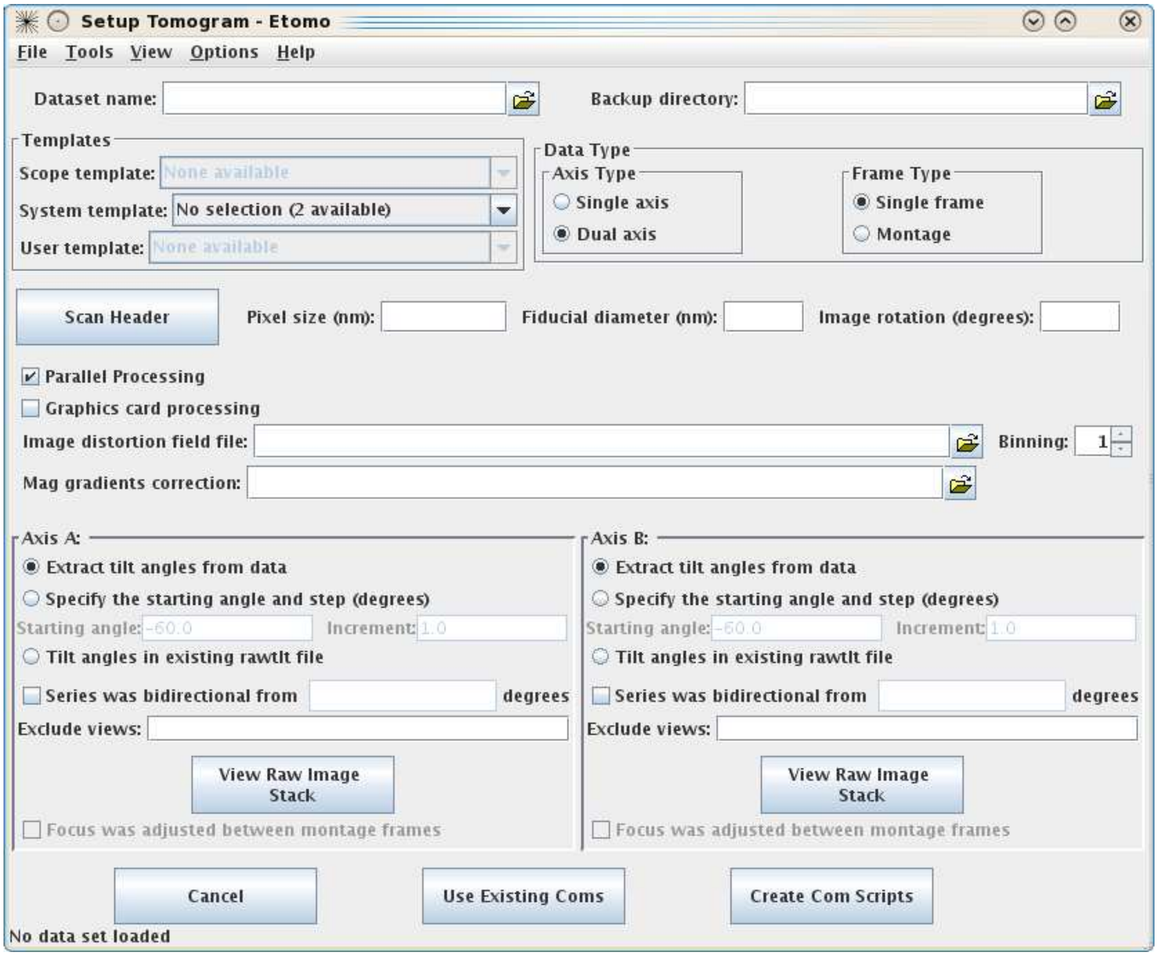
Image processing: manual/semi-automatic alignment using ETOMO

VERSION used: 4.6.3
ETOMO: setup tomogram
http://bio3d.colorado.edu/imod/doc/etomoTutorial.html



Dataset name:
Backup directory:

Templates
Scope template: None available
System template: No selection (2 available)
User template: None available

Data Type
Axis Type: ○ Single axis ● Dual axis
Frame Type: ● Single frame ○ Montage

Pixel size (nm):
Fiducial diameter (nm):
Image rotation (degrees):

☑ Parallel Processing
☐ Graphics card processing
Image distortion field file:
Binning: 1
Mag gradients correction:

Axis A:
● Extract tilt angles from data
○ Specify the starting angle and step (degrees)
Starting angle: -60.0 Increment: 1.0
○ Tilt angles in existing rawtlt file
☐ Series was bidirectional from degrees
Exclude views:
☐ Focus was adjusted between montage frames

Axis B:
● Extract tilt angles from data
○ Specify the starting angle and step (degrees)
Starting angle: -60.0 Increment: 1.0
○ Tilt angles in existing rawtlt file
☐ Series was bidirectional from degrees
Exclude views:
☐ Focus was adjusted between montage frames

No data set loaded

Settings:

Data set name: File with extension “.st” (is an mrc file but with another name, you can just copy your name.mrc file to name.st; the angle file has to be renamed with the same name of the .st and with extension .rawtlt)

single axis
single frame
set: pixel size, fiducial diameter, image rotation: 0º (ALBA)

specify starting angle and step in degree

➔ Create Com Scripts

1) Pre-processing: CCD eraser

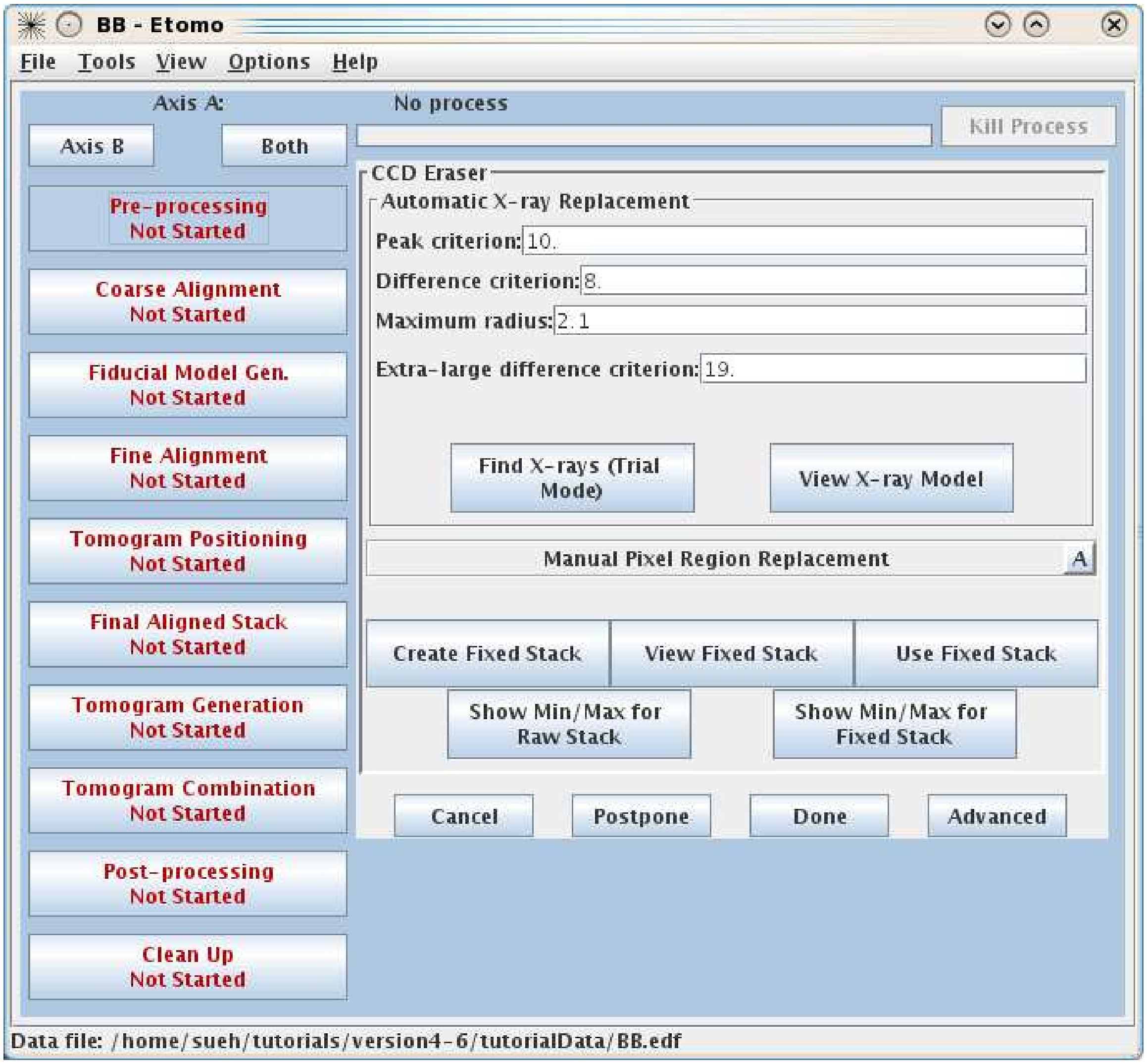
To erase the hot pixels or the dark pixels: it looks for "peaks", or pixels whose intensity deviate from the surrounding pixels by a certain number of standard deviations, which is specified in the **Peak criterion** text box. It also looks for pixel-to-pixel differences that exceed background by a certain number of SDs, specified in the **Difference criterion** text box.

Standard settings: Peak criterion 10.0
Difference criterion: 8.0

Maximum radius: 2.1

find ⟶ view (if it is ok*) ⟶ create com scripts⟶ view fixed stack ⟶ use fixed stack ⟶ Done

*If nothing else is detected lower the peak criterion to 8 and the difference criterion to 6.



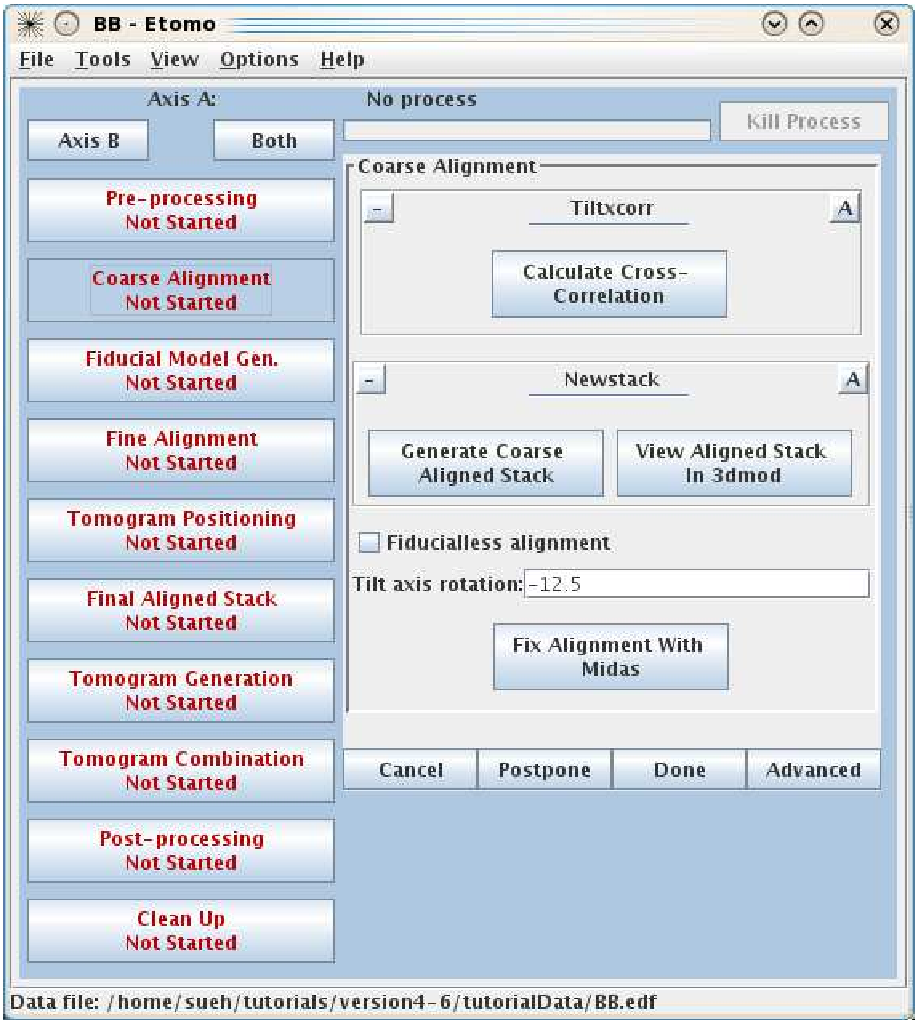
Peak criterion: 10.
Difference criterion: 8.
Maximum radius: 2.1
Extra-large difference criterion: 19.
Data file: /home/sueh/tutorials/version4-6/tutorialData/BB.edf

2) Coarse alignment

The main purpose of this step is to get images aligned well enough for automatic tracking of fiducials.

Calculate cross-correlation (determine X and Y shifts of one image with the previous one) ⟶ generate coarse alignment (converts all translations in translations that will bring all images into alignment) → view aligned stack in 3dmod → Done

Usually alignment is not good enough and you need to manually click on a certain number of features/contours: you do this with the next step "fiducial model generation".



☐ Fiducialless alignment

Tilt axis rotation: -12.5

Data file: /home/sueh/tutorials/version4-6/tutorialData/BB.edf

3) Fiducial model generation

Make seed and track → View Seed Model

Here you have several options:

1- Manual seed
2- Generate seed model automatically

1- Manual seed:

The 3dmod, ZAP window and Bead Fixer window will appear:

In the 3dmod window:

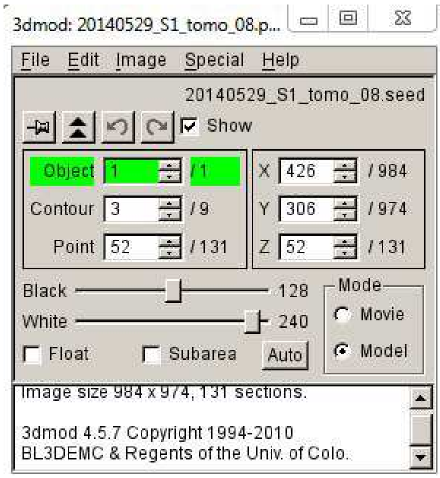
- Object: it will be one object.
- Contour: minimum 3, 7-8 will be enough. To generate a new contour use “N”.
- Point: for each contour you will follow a feature along the tilt series in the ZAP window creating “point” for each projection. The best is to choose details that you can follow along the whole series and in such a way to cover the entire field of view (some details in the center, some details in the corners).

De-select the “Autocenter” option in the Bead Fixer window. To create a point on the chosen detail uses the central mouse button. To go from one projection to another one, use “forward page/ rear page”. To move a selected point (and improve the positioning of your selection) use the arrows. To delete a selected point use the “delete” button. To select a point use the left mouse button (in the selected point a small yellow circle will appear). Change the contrast in the image using the 3dmod window.

The goal is to click on some fiducials and then let IMOD complete the tracking in the next step (track seed model etc.)

Save your model from time to time and when you finish (in the 3dmod window File->Save Model).

3dmod window:



3dmod: 20140529_S1_tomo_08.p...

20140529_S1_tomo_08.seed

☑ Show

Object 1 /1

Contour 3 /9

Point 52 /131

X 426 /984

Y 306 /974

Z 52 /131

Black 128

White 240

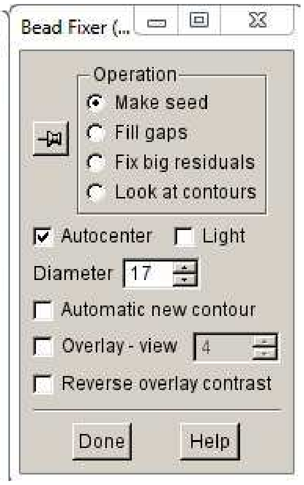
☐ Float ☐ Subarea

Mode ○ Movie ● Model

Image size 984 x 974, 131 sections.

3dmod 4.5.7 Copyright 1994-2010
BL3DEMC & Regents of the Univ. of Colo.

Bead Fixer window:



Bead Fixer (...

Operation
● Make seed
○ Fill gaps
○ Fix big residuals
○ Look at contours

☑ Autocenter ☐ Light

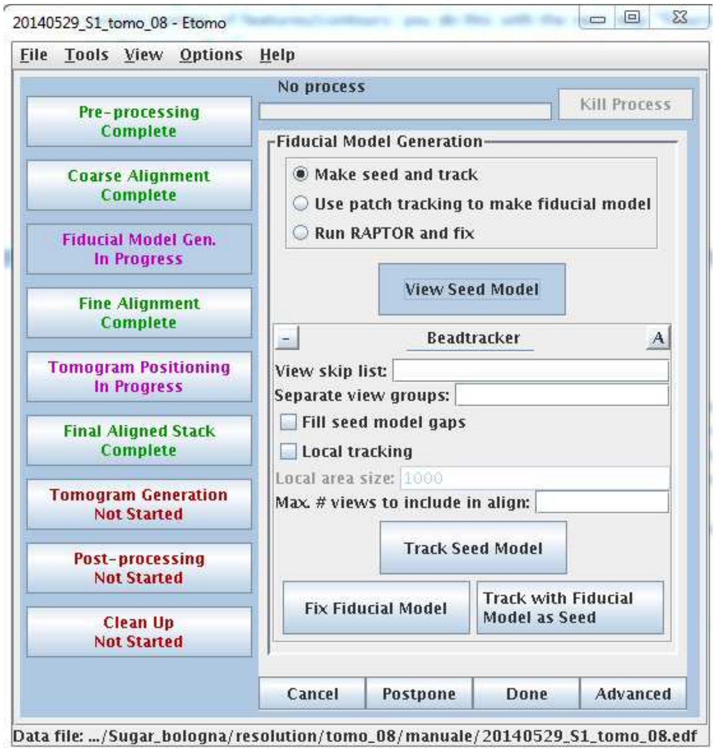
Diameter 17

☐ Automatic new contour

☐ Overlay - view 4

☐ Reverse overlay contrast

Then: → Track Seed Model → Fix Fiducial Model → Track with Fiducial Model as Seed (deselect the option “Fill seed model gaps” and “Local tracking”). Once finished click “Done”.



● Make seed and track

☐ Fill seed model gaps

☐ Local tracking

Local area size: 1000

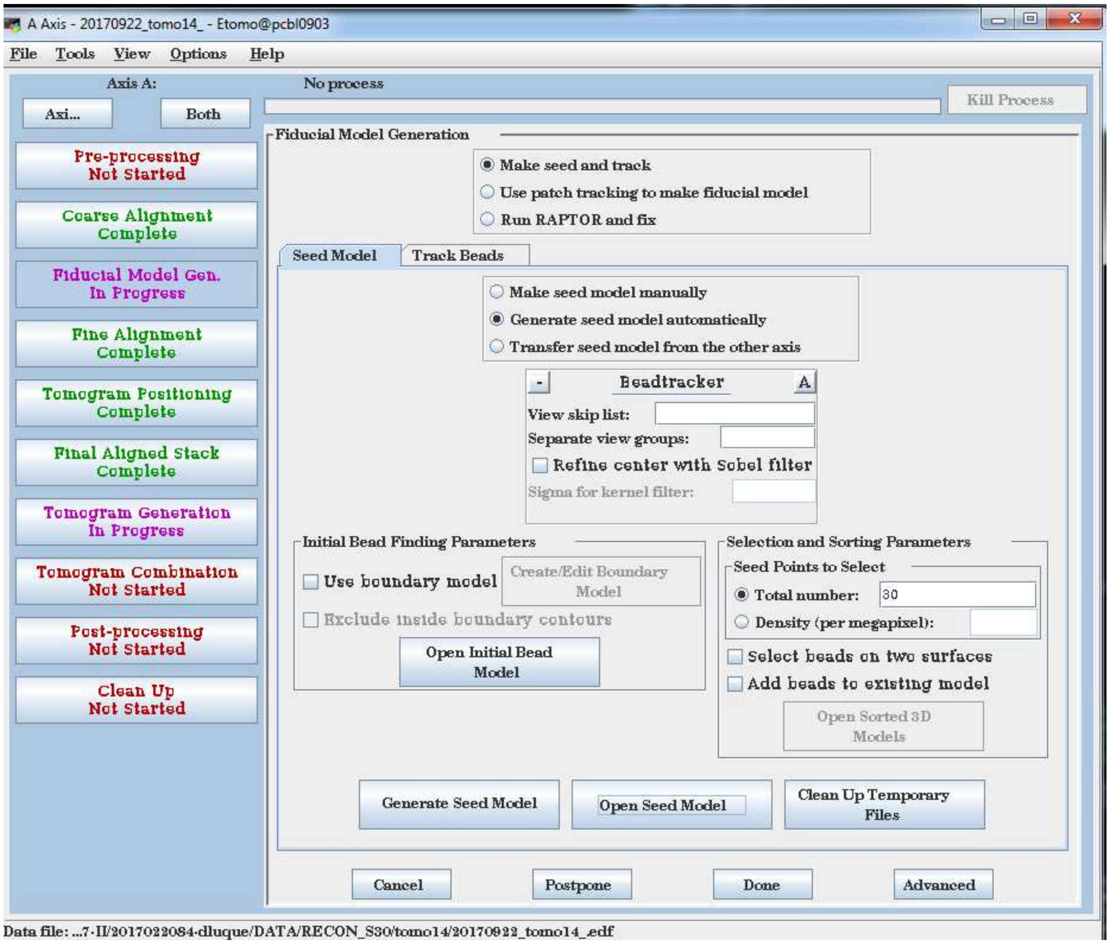
Data file: .../Sugar_bologna/resolution/tomo_08/manuale/20140529_S1_tomo_08.edf

2- Generate seed model automatically

In this case Imod will generate a seed model automatically based on fiducials. To use this approach you will need to have a good fiducial marker distribution. In any case you can try.

For that you have to estimate the number of fiducials and include it in the parameter “Selection and Sorting Parameters → Seed Points to Select → Total number”

Once the seed model is generated you proceed as in the other option (Track Beads).



● Make seed and track
○ Use patch tracking to make fiducial model
○ Run RAPTOR and fix

○ Make seed model manually
● Generate seed model automatically
○ Transfer seed model from the other axis

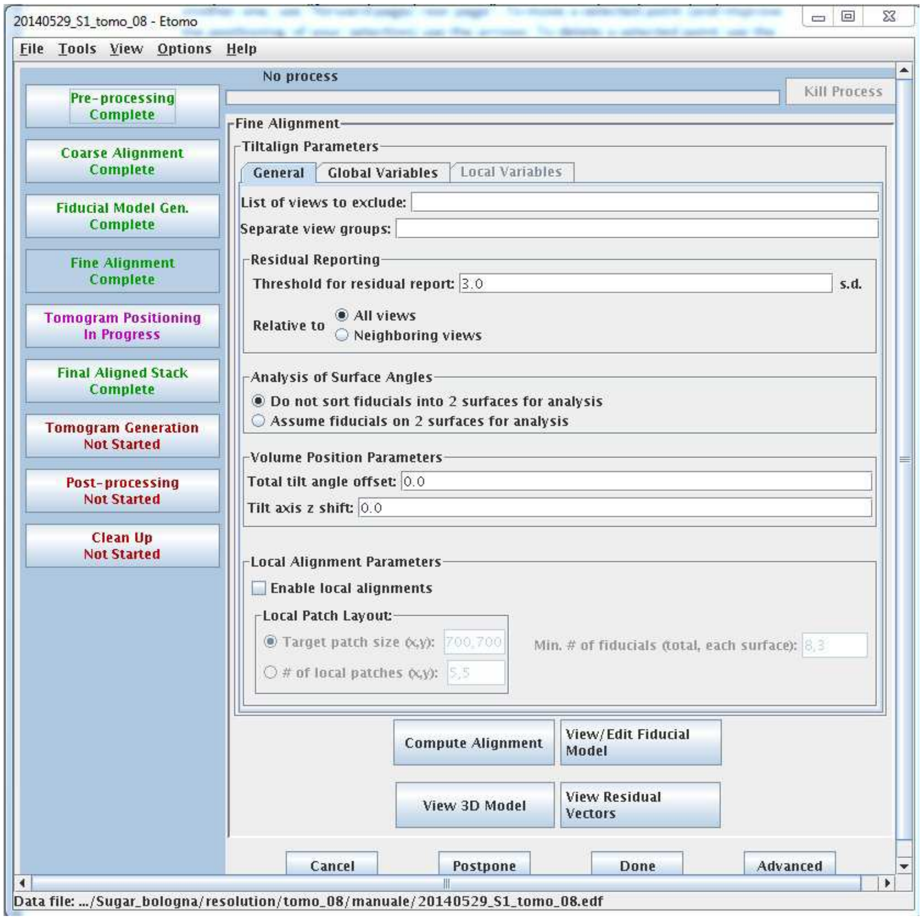
View skip list:
Separate view groups:
☐ Refine center with Sobel filter
Sigma for kernel filter:

☐ Use boundary model
☐ Exclude inside boundary contours

● Total number: 30
○ Density (per megapixel):
☐ Select beads on two surfaces
☐ Add beads to existing model

Data file: ...7-II/20170922084-dluque/DATA/RECON_S30/tomo14/20170922_tomo14_.edf

4) Fine alignment



Fine Alignment

Tiltalign Parameters

List of views to exclude:

Separate view groups:

Residual Reporting

Threshold for residual report: 3.0 s.d.

Relative to ● All views ○ Neighboring views

Analysis of Surface Angles

● Do not sort fiducials into 2 surfaces for analysis

○ Assume fiducials on 2 surfaces for analysis

Volume Position Parameters

Total tilt angle offset: 0.0

Tilt axis z shift: 0.0

Local Alignment Parameters

☐ Enable local alignments

Local Patch Layout:

● Target patch size (x,y): 700,700

○ # of local patches (x,y): 5,5

Min. # of fiducials (total, each surface): 8,3

Data file: .../Sugar_bologna/resolution/tomo_08/manuale/20140529_S1_tomo_08.edf

Basic – starting options (corresponding to minimum degrees of freedom to create the aligned stack):

General: All views

Do not sort fiducials into 2 surfaces for analysis

Global variables:
- One rotation
- Fixed magnification
- Fixed tilt angles
- Distortion solution: Disable

Then:

→Compute alignment. Look at the Log window (if it is not already open: view -> show Log window) and check the two number "Residual error mean and sd".

Then*:

→ View/Edit Fiducial Model. In the Bead Fixer window select "Fix big residuals".

→ go to the next big residual → move point by residual (do it for all necessary points)

In the 3dmod window: File → save model

You can also delete points that have been tracked not correctly, as well as, modify the position of the points proposed.

some tips:
To go from one contour to another: use the middle button mouse
To move the selected contour to another position: use the right button on the mouse
The square brackets allow you to go from one projection to the other with the same contour selected
coma goes to next residual
to change size of the contour (green circle) go to edit- object-type to get the size bar

****

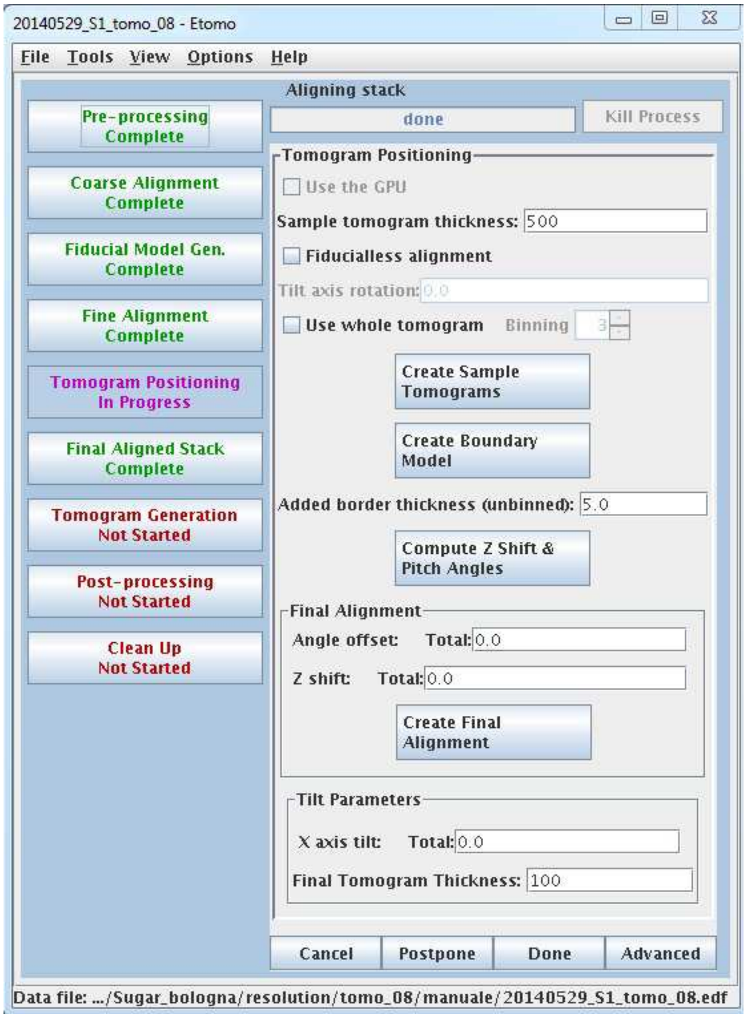
→ Compute alignment. Look at the Log window. The two number "Residual error mean and sd" have to be smaller than before.

Repeat from * until the Big Residuals will finish.

→ Done

5) Tomography positioning

Correct if the region you want to reconstruct is above or below the rotation axis.



20140529_S1_tomo_08 - Etomo

Aligning stack: done

Tomogram Positioning

☐ Use the GPU

Sample tomogram thickness: 500

☐ Fiducialless alignment

Tilt axis rotation: 0.0

☐ Use whole tomogram Binning 3

Added border thickness (unbinned): 5.0

Final Alignment

Angle offset: Total: 0.0

Z shift: Total: 0.0

Tilt Parameters

X axis tilt: Total: 0.0

Final Tomogram Thickness: 100

Data file: .../Sugar_bologna/resolution/tomo_08/manuale/20140529_S1_tomo_08.edf

Sample tomogram thickness: 1200 slices (the important is that it has to be bigger than the actual sample thickness).

→ Create Sample Tomograms (it will reconstruct regions “top/middle/bottom”)

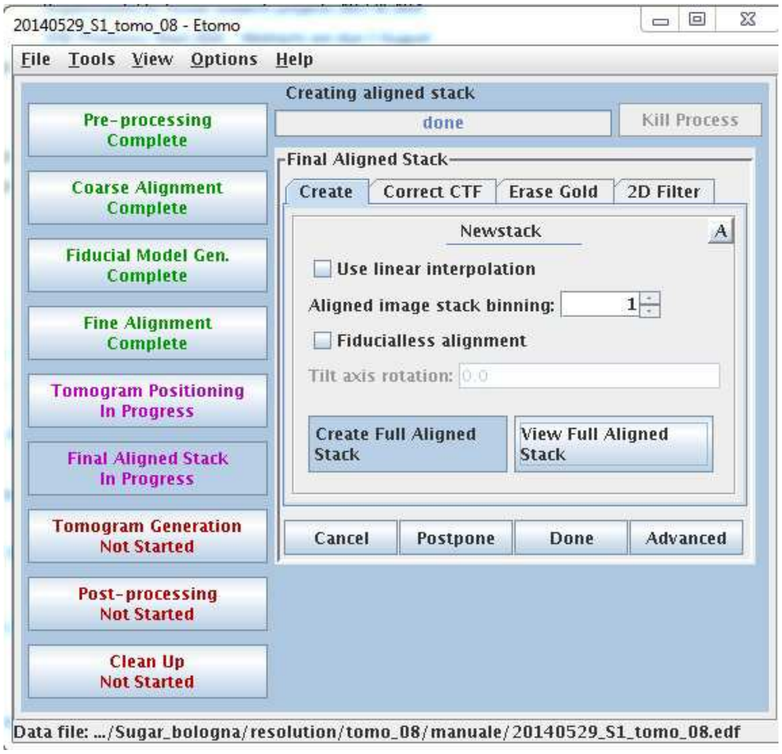
→ Create Boundary Model. The ZAP window will open. For the 3 regions top/middle/bottom, define two parallel* lines containing the sample (organic material).

*they are supposed to be also parallel to the quantifoil.

Save the model (in the 3dmod window File->Save Model).

→ Compute z shift and Pitch Angles
→ Create Final Alignment
→ Done

6) Final aligned stack



Creating aligned stack: done

☐ Use linear interpolation

Aligned image stack binning: 1

☐ Fiducialless alignment

Tilt axis rotation: 0.0

Data file: .../Sugar_bologna/resolution/tomo_08/manuale/20140529_S1_tomo_08.edf

→ Create Full Aligned Stack (create the .ali file* which contain the final aligned stack).
→View Full Aligned Stack (to check it).
→ Done

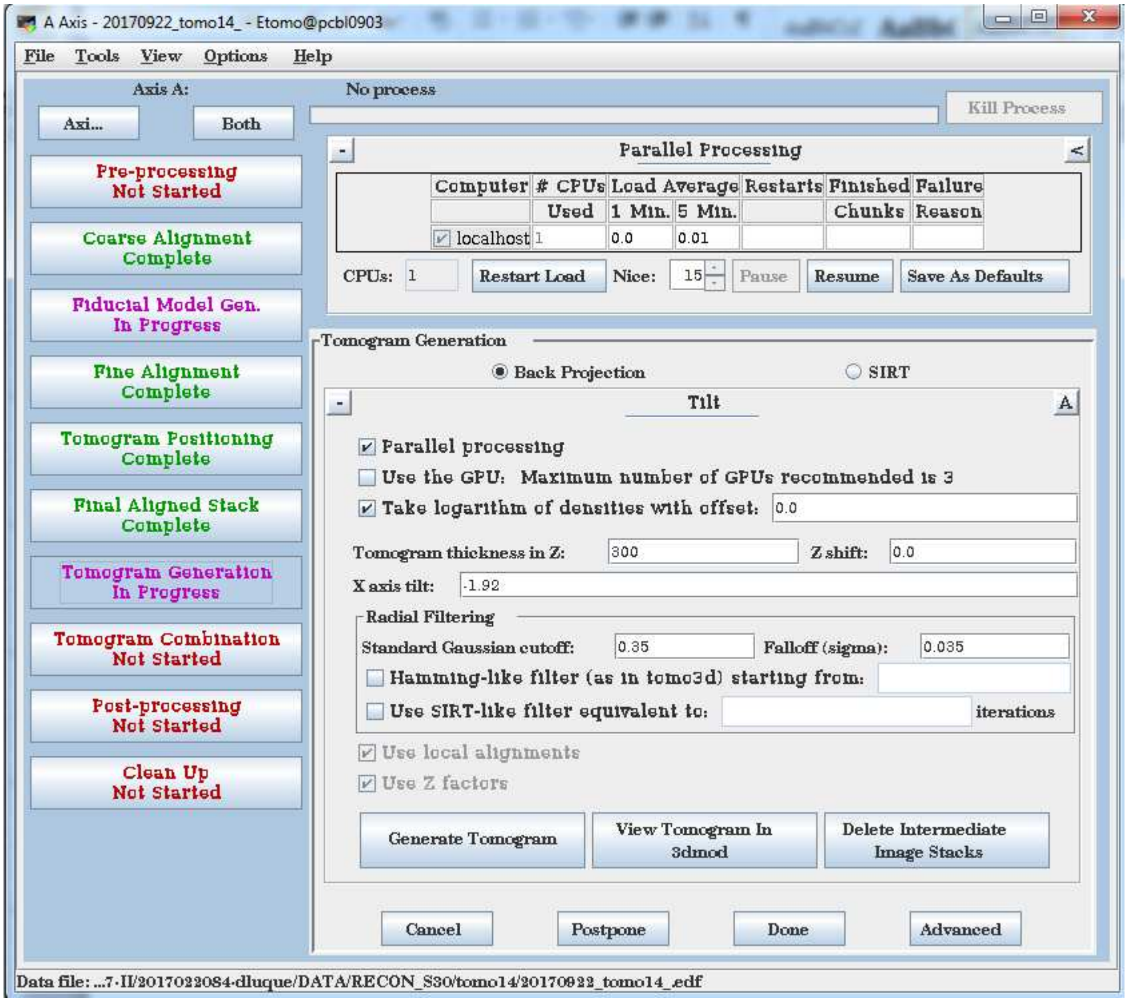
Once this step is complete, you can do the tomogram reconstruction following two procedures:

1- Tomogram generation step in Imod
2- tomo3d

1- Imod Tomogram Generation Step:

You can use two algorithms to do the reconstruction: Back projection or SIRT. The first one is faster but the resulting volume will result in a volume with a lower signal to noise ratio. The SIRT one is an iterative procedure that will result in a volume where features will be more clearly identified.
In both cases the parameter tomogram thickness in Z will be automatically determined by Imod based on the tomogram positioning step. The parameter Standard Gaussian cutoff should be determined empirically, a good starting point is a value of 0.03. You can vary this parameter and look how it is affecting the resulting volume.

Back projection reconstruction:



Computer	# CPUs Used	Load Average 1 Min.	Load Average 5 Min.	Restarts	Finished Chunks	Failure Reason
☑ localhost	1	0.0	0.01			

CPUs: 1

Nice: 15

● Back Projection ○ SIRT

☑ Parallel processing

☐ Use the GPU: Maximum number of GPUs recommended is 3

☑ Take logarithm of densities with offset: 0.0

Tomogram thickness in Z: 300

Z shift: 0.0

X axis tilt: -1.92

Radial Filtering

Standard Gaussian cutoff: 0.35

Falloff (sigma): 0.035

☐ Hamming-like filter (as in tomo3d) starting from:

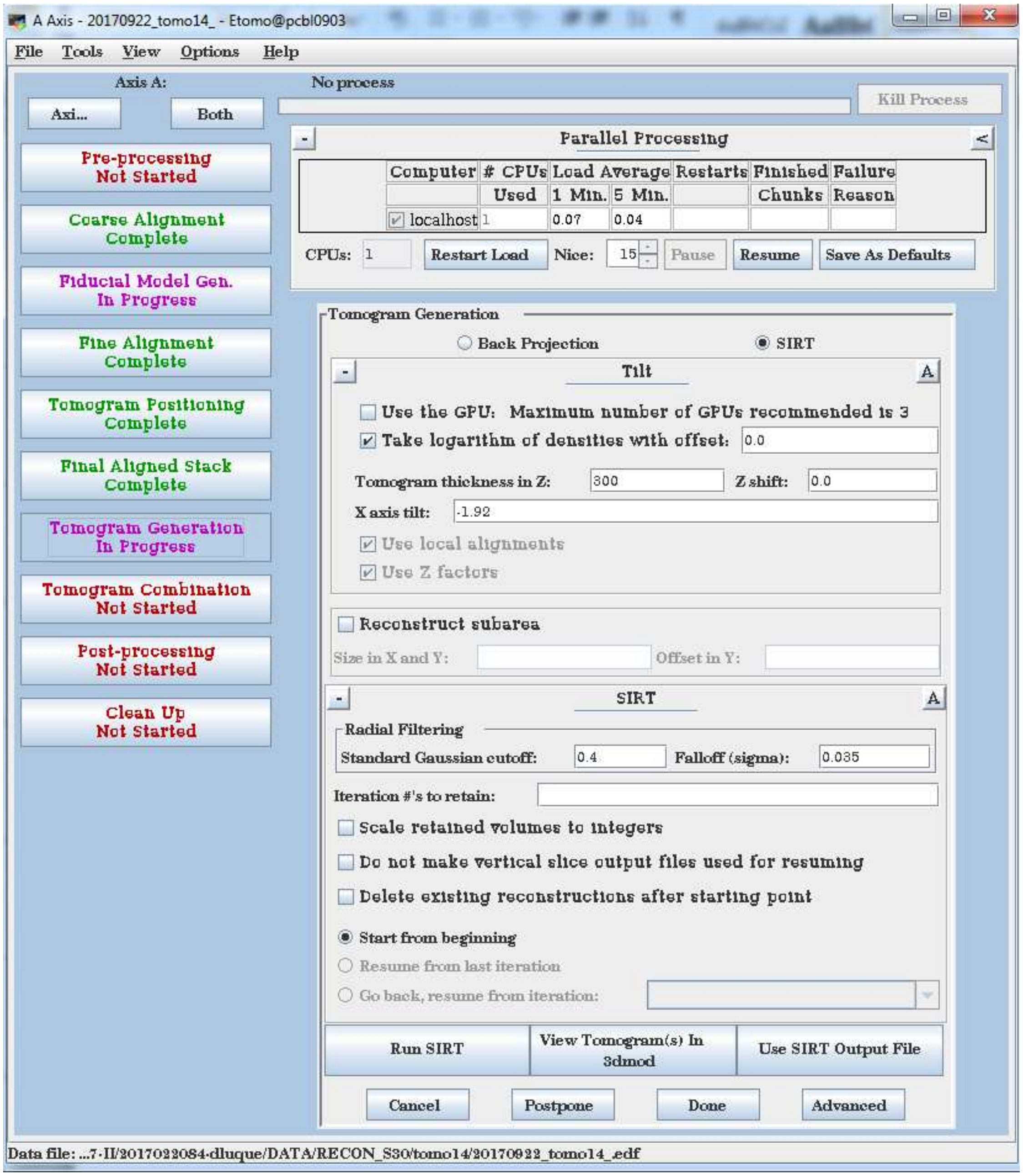
☐ Use SIRT-like filter equivalent to: iterations

☑ Use local alignments

☑ Use Z factors

Data file: ...7-II/2017022084-dluque/DATA/RECON_S30/tomo14/20170922_tomo14_.edf

SIRT reconstruction:



Pre-processing Not Started
Coarse Alignment Complete
Fiducial Model Gen. In Progress
Fine Alignment Complete
Tomogram Positioning Complete
Final Aligned Stack Complete
Tomogram Generation In Progress
Tomogram Combination Not Started
Post-processing Not Started
Clean Up Not Started

Computer	# CPUs	Load Average		Restarts	Finished	Failure
	Used	1 Min.	5 Min.		Chunks	Reason
☑ localhost	1	0.07	0.04			

CPUs: 1
Nice: 15

Tomogram Generation

○ Back Projection ● SIRT

Tilt

☐ Use the GPU: Maximum number of GPUs recommended is 3
☑ Take logarithm of densities with offset: 0.0
Tomogram thickness in Z: 300
Z shift: 0.0
X axis tilt: -1.92
☑ Use local alignments
☑ Use Z factors

☐ Reconstruct subarea
Size in X and Y:
Offset in Y:

SIRT

Radial Filtering
Standard Gaussian cutoff: 0.4
Falloff (sigma): 0.035
Iteration #'s to retain:
☐ Scale retained volumes to integers
☐ Do not make vertical slice output files used for resuming
☐ Delete existing reconstructions after starting point
● Start from beginning
○ Resume from last iteration
○ Go back, resume from iteration:

Data file: ...7-II/2017022084-dluque/DATA/RECON_S30/tomo14/20170922_tomo14_.edf

2- tomo3d
* The .ali file generated in the step final aligned stack is an .mrc file that can be used with TOMO3D (for instance) to obtain the reconstruction.
https://sites.google.com/site/3demimageprocessing/tomo3d

Tomo3D is a very fast reconstruction software that allows to choose between WBP and SIRT (including number of iterations). Do a “-help” to know how to choose one or the other.

Obs: the job can be resumed at any moment by loading the .edf file from the starting window “front page” in File: