Image processing: manual/semi-automatic alignment using ETOMO

VERSION used: 4.6.3 ETOMO: setup tomogram

http://bio3d.colorado.edu/imod/doc/etomoTutorial.html

💥 🕞 Setup Tomogram - Etomo				\odot	
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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

BB - Etomo	_ = ×
Eile Options Help	
Axis A:	No process
Axis B Both	Kill Process
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)ata file: /home/sueh/tutorials;	version3-14/tutorialData/BB.edf

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-topixel 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

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

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

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Tomogram Combination Not Started	Cancel Postpone	Done Advanced
Post-processing Not Started		
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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) \rightarrow generate coarse alignment (converts all translations in

translations that will bring all images into alignment) \rightarrow view aligned stack in 3dmod \rightarrow 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".



3) Fiducial model generation

Make seed and track \rightarrow 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).

dmod window:	Bead Fixer window:
3dmod: 20140529_S1_tomo_08.p 📼 💷 🔀	Bead Fixer (📼 💷 🐹
Eile Edit Image Special Help 20140529_S1_tomo_08.seed Image Image<	Operation

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

ile <u>T</u> ools <u>V</u> iew <u>Options</u>	Help
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Pre-processing Complete	Fiducial Model Generation
Coarse Alignment Complete	 Make seed and track Use patch tracking to make fiducial model
Fiducial Model Gen. In Progress	© Run RAPTOR and fix
Fine Alignment Complete	View Seed Model - Beadtracker A
Tomogram Positioning In Progress	View skip list:
Final Aligned Stack Complete	Fill seed model gaps Local tracking
Tomogram Generation Not Started	Local area size: 1000 Max. # views to include in align:
Post-processing Not Started	Track Seed Model
Clean Up Not Started	Fix Fiducial Model Model as Seed

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 \rightarrow Seed Points to Select \rightarrow Total number"

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



Data file: ...7-II/2017022084-dluque/DATA/RECON_S30/tomo14/20170922_tomo14_edf

4) Fine alignment

e <u>T</u> ools ⊻iew <u>O</u> ptions	Help		
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In Progress	Neighboring views		
Final Aligned Stack	-Analysis of Surface Angles		
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Not Started			
	Volume Position Parameters		
Post-processing Not Started	Total tilt angle offset: 0.0		
	Int axis z shift: 0.0		
Clean Up Not Started			
Hot Starten	Local Alignment Parameters		
	Enable local alignments		
	-Local Patch Layout		
	Target patch size (x,y): 700,700 Min. # of fiducials (total, each surface): 8,3		
	\bigcirc # of local patches (x,y): 5.5		
	Compute Alignment View/Edit Fiducial Model		
	View Residual		
	View SD Wodel Vectors		
	Cancel Postpone Done Advanced		

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:

 \rightarrow 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*:

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

 \rightarrow go to the next big residual \rightarrow move point by residual (do it for all necessary points) In the 3dmod window: File \rightarrow 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

 \rightarrow 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.

 \rightarrow Done

5) Tomography positioning

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

ile Tools View Options	Help
	Aligning stack
Pre-processing	done Kill Process
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Fiducial Model Gen. Complete	Fiducialless alignment
Fine Alignment Complete	Tilt axis rotation: 0.0 Use whole tomogram Binning
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Final Aligned Stack Complete	Create Boundary Model
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Post-processing Not Started	Final Alignment
Clean Up Not Started	Angle offset: Total: 0.0 Z shift: Total: 0.0
	Create Final Alignment
	Tilt Parameters
	Final Tomogram Thickness: 100
	Cancel Postpone Done Advanced

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

 \rightarrow Create Sample Tomograms (it will reconstruct regions "top/middle/bottom")

 \rightarrow 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).

- \rightarrow Compute z shift and Pitch Angles
- \rightarrow Create Final Alignment
- \rightarrow Done

6) Final aligned stack

le <u>T</u> ools <u>V</u> iew <u>O</u> ptions	<u>H</u> elp			
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Tomogram Generation Not Started	Cancel	Postpone	Done	Advanced
Post-processing Not Started				
Clean Up				

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

 \rightarrow 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:

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SIRT reconstruction:

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Cancel Postpone Done Advanced		Cancel Postpone Done Advanced

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:

Front	Page - Etomo		×
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	New Nonlinear Anisotropic Diffusion	New Generic Parallel Process]
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