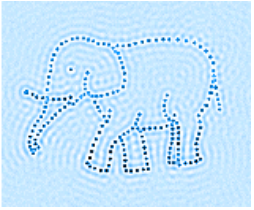
EMBO Practical Course on Image Processing for Cryo EM
1-11 September 2015



Practical 4: Tomography with IMOD (v 4.7.15) – Reconstructing a dual axis tomogram from a freeze-substituted HeLa cell section, focusing on the Golgi apparatus

Introduction

In this practical, you will learn how to use IMOD to align and reconstruct a tomogram from a tilt series, to get a 3D reconstruction of a HeLa cell section. Electron tomography is closely analogous to CT scanning in medical imaging. No averaging is involved, but the cost is a high electron dose, since the whole tilt series must be recorded from the same area. Also, it is not possible in EM to tilt up to 90º, so that there is always missing data in the direction of the optical axis. Dual axis tomography, in which two tilt series are recorded in orthogonal directions, helps to fill in some of the missing data. You will also learn how to align dual axis data.

Getting started

IMOD was developed and is maintained by the EM group at Colorado University Boulder. They have extensive web help that contains installation instructions, tutorials and a tomography guide (http://bio3d.colorado.edu/imod/). This practical is taken from the IMOD tutorial with only a few minor alterations (http://bio3d.colorado.edu/imod/doc/tomoguide.html).
You can access detailed information about each of the processing steps during the practical by clicking on the **Help** tab on the **eTomo Main Window** and selecting **Tomography guide**

Log into the server using the instructions provided. Open a terminal window and in your home directory.

> cd PRAC-4

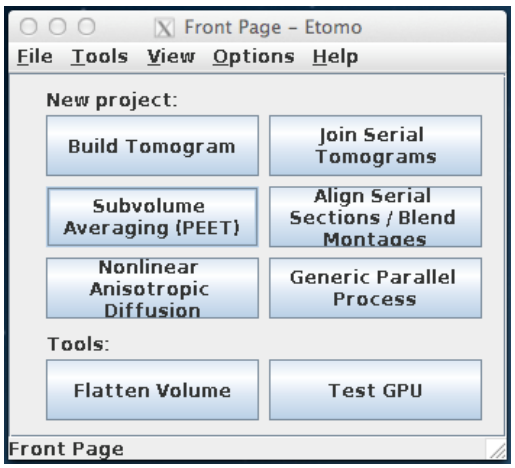
For this practical, go to the HELA directory;

> cd HELA

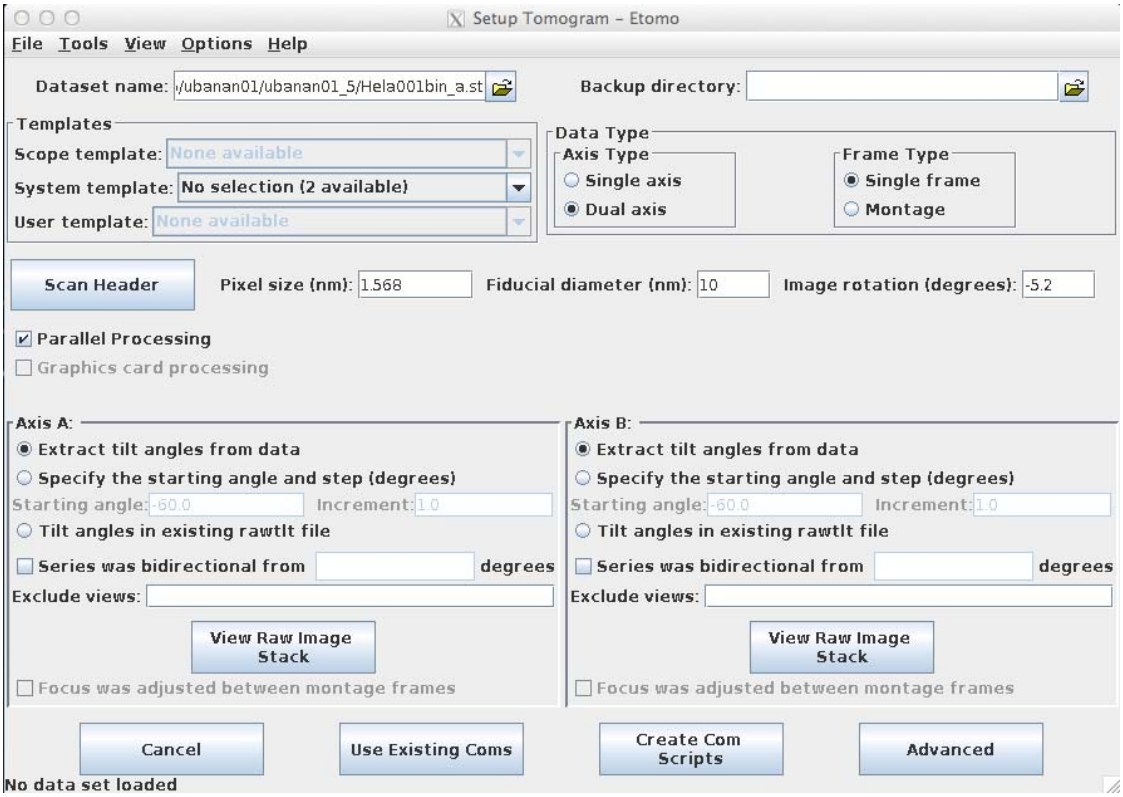
1) To start eTomo, at the command line type:

> etomo

When eTomo is first started, a **Front Page** panel will come up (shown below), allowing you to select which operation you want to perform with eTomo.



Press **Build Tomogram** and the **Setup Tomogram** panel will come up (shown below).



2) To start working with the data set, the following fields must be filled out (see below). The **Dataset name** is the name of the file containing the raw tilt series (or the root name if a dual axis data set was collected, followed by lower case a for axis 1 and b for axis 2). You can enter the **Dataset name** by clicking on the yellow file selection dialogue box associated with the **Dataset name** field and selecting Hela001bin_a.st. Alternatively, type the directory path and file name directly in the field. The **Backup directory** is an optional field to save small working files every time you run a procedure and can be left blank.

Our data set is dual axis so make sure **Dual axis** and **Single Frame** are selected. The next fields specify pixel size (nm), the size of gold fiducials (nm) and Image rotation (degrees). **Pixel size** (in nm) depends on the microscope, camera, and magnification. The **Image rotation** (tilt axis angle from vertical, in degrees) will also vary with the microscope and magnification. Selecting **Scan Header** will retrieve the **Pixel size** and **Image rotation** values from the MRC file header. For this data set the pixel size is 1.568 nm and the tilt axis rotation is -5.2°. We used 10 nm gold as our fiducial marker and this must be manually entered into the **Fiducial diameter** field. Make sure the **Parallel Processing** checkbox is enabled. If the option is greyed out you will need to go to the options menu, select settings and turn parallel processing on and set the CPU's to 2. Hit **Apply**. Then you will have to exit etomo and restart it. For our sample data, the tilt angles are stored in the extended headers of the raw tilt series and so the default, **Extract tilt angles from data,** should be used for both Axis A and Axis B. The tilt angles for the Hela001bin_a.st are -54° to 60° and for the b stack they are -50° to 60° with a 2° step for both tilt series.

You can also optionally specify individual views in the tilt series to exclude from the processing steps. The syntax for this exclude list is a comma separated list of ranges (i.e. 1, 4-5, 60-70). To determine if you have views that have poor image quality (poor focus, etc.), open the raw stack(s) by pressing the **View Raw Image Stack** button(s). You can movie through the raw tilt series images by clicking the middle mouse button in the 3dmod (ZaP) window or you can step through them by pressing the page up and down buttons on the keyboard. In our data set, there are no images to exclude. Close the 3dmod windows after you have viewed each of the raw image stacks.

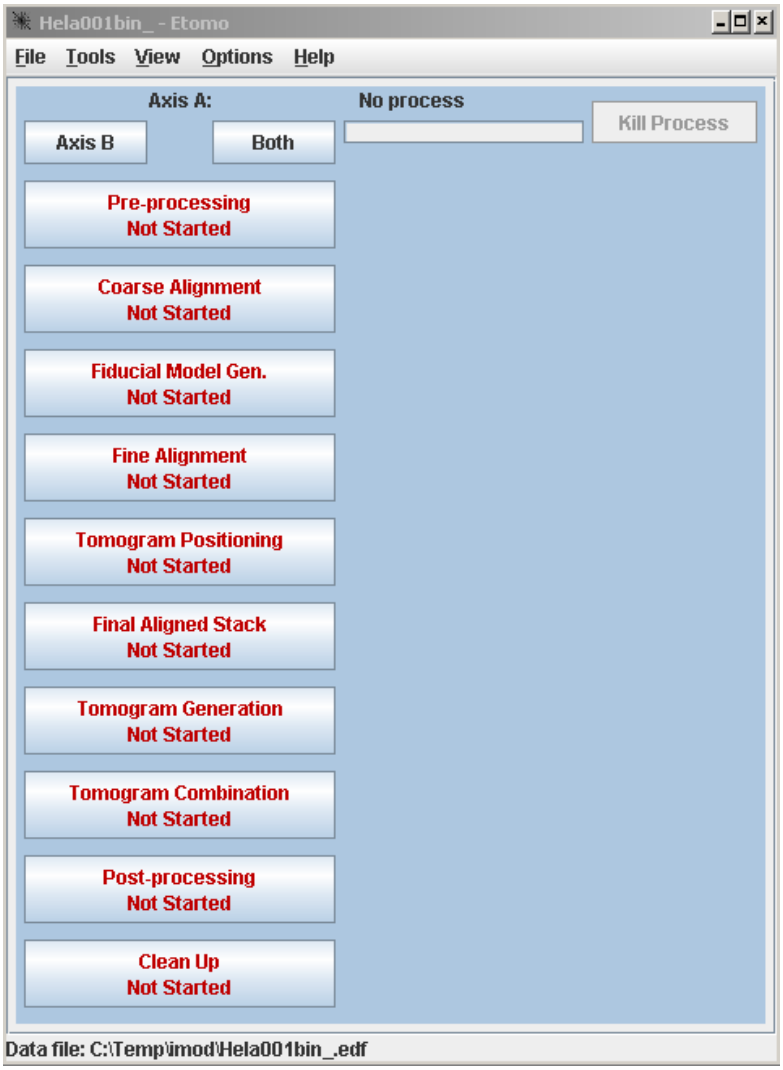
Press the **Create Com Scripts** button to start generating the tomograms. This creates the *.com files used during the different processing stages and can be seen in your directory.

3) eTomo Main Window

If you need to exit eTomo before finishing this practical, you can continue where you left off by going to your directory, which now contains your dataset, and typing: etomo Hela001bin_.edf

The Main Window consists of several areas: on the left is a column of buttons (Process Control Buttons) that allow you to select a particular stage of tomogram computation to work on. On the top is a Process Monitor that informs you of the status of the current process or the last process completed. To the left of the

Process Monitor are the Axis Buttons which allow you to move between Axis A and Axis B. The Main Window is currently open to Axis A.



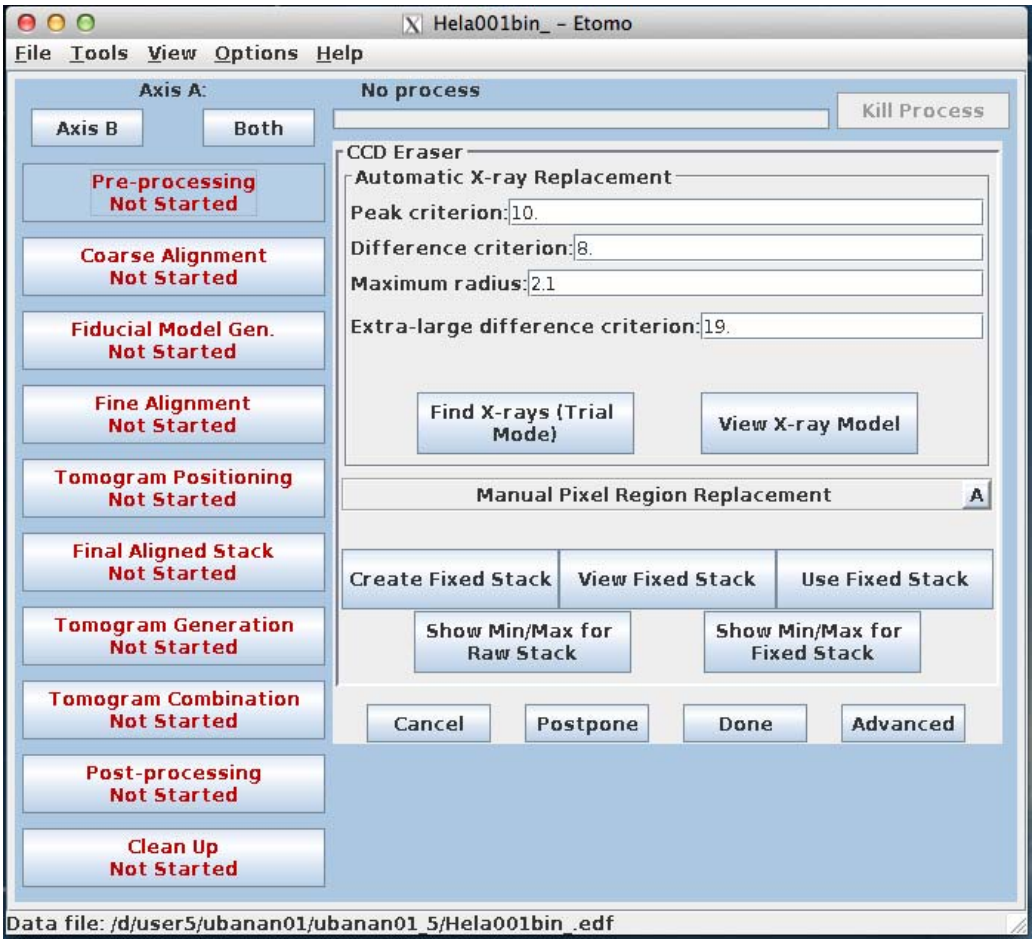
The Process Control Buttons are arranged in the suggested order of processing from top to bottom. The buttons are color coded to signify the stage of the process, where red indicates that the process has not been started, magenta indicates that the process is currently in progress, and green indicates that the process has been completed. When one of the buttons is selected, the right side of the window will fill in with information and fields associated with a specific process. These forms are referred to as Process Panels and allow you to modify the necessary parameters and execute specific programs required by that processing step. The parameters and buttons on each Process Panel are typically laid out from top to bottom in the order they should be executed, much like a flow chart. When you execute a process (by pressing a button on one of the Process Panels) the Process Monitor will indicate what the process is doing and when it is complete.

4) Pre-Processing

CCD images can be affected by random X-rays recorded during collection of the initial dark reference or the individual images, which causes extreme high or low

pixel values in your data file. As a result, these extreme values compress the data contrast and can cause artifacts in the reconstruction. In this practical you will not need to pre-process the images so you can press the **Done** button, but the procedure is listed below for your reference.

Press **Pre-processing** button to open this panel:



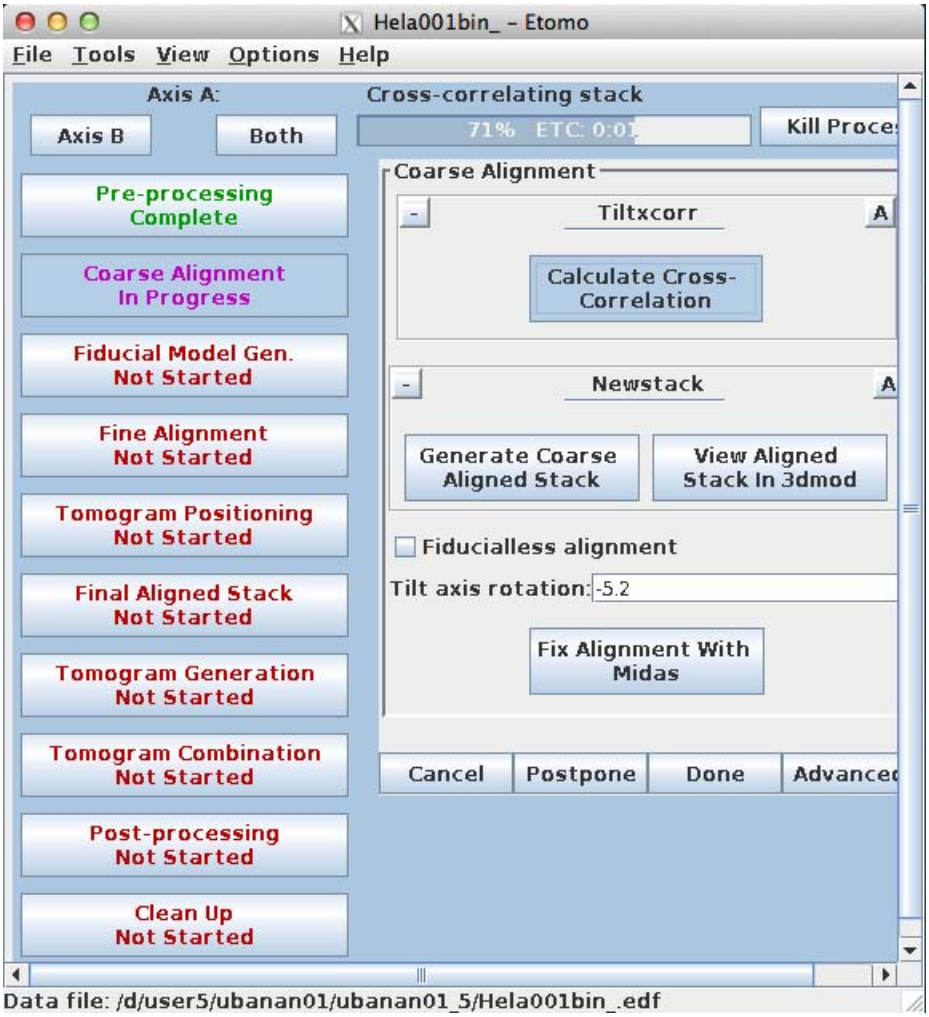
The basic steps involved in pre-processing (again, not needed for the tutorial data set) are:

- Press the **Show Min/Max for Raw Stack** button to run **clip stats**, a program which displays the minimum and maximum densities for each section, on the raw stack.
- Press the **Create Fixed Stack** button to create a second stack with X-rays removed.
- Press the **View Fixed Stack** button to view that stack, and press the **Show Min/Max for Fixed Stack** button to run **clips stats** on the fixed stack.
- If the contrast appears good in the display, and the outliers from the raw stack **clip stats** output are gone from the fixed stack output, then press the **Use Fixed Stack** button.

Press **Done** at the bottom of the page.

5) Coarse alignment

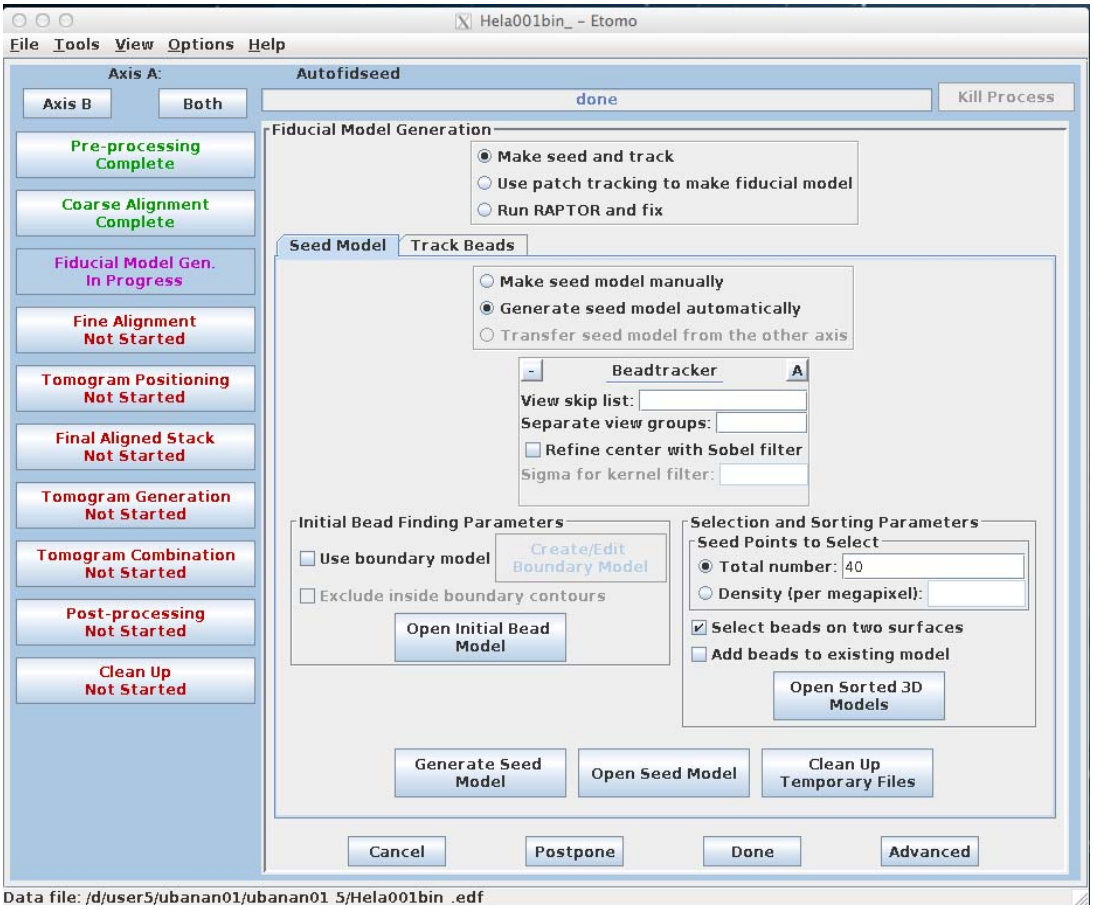
Press the **Coarse Alignment** Process Control Button to proceed with creating a coarse-aligned stack.



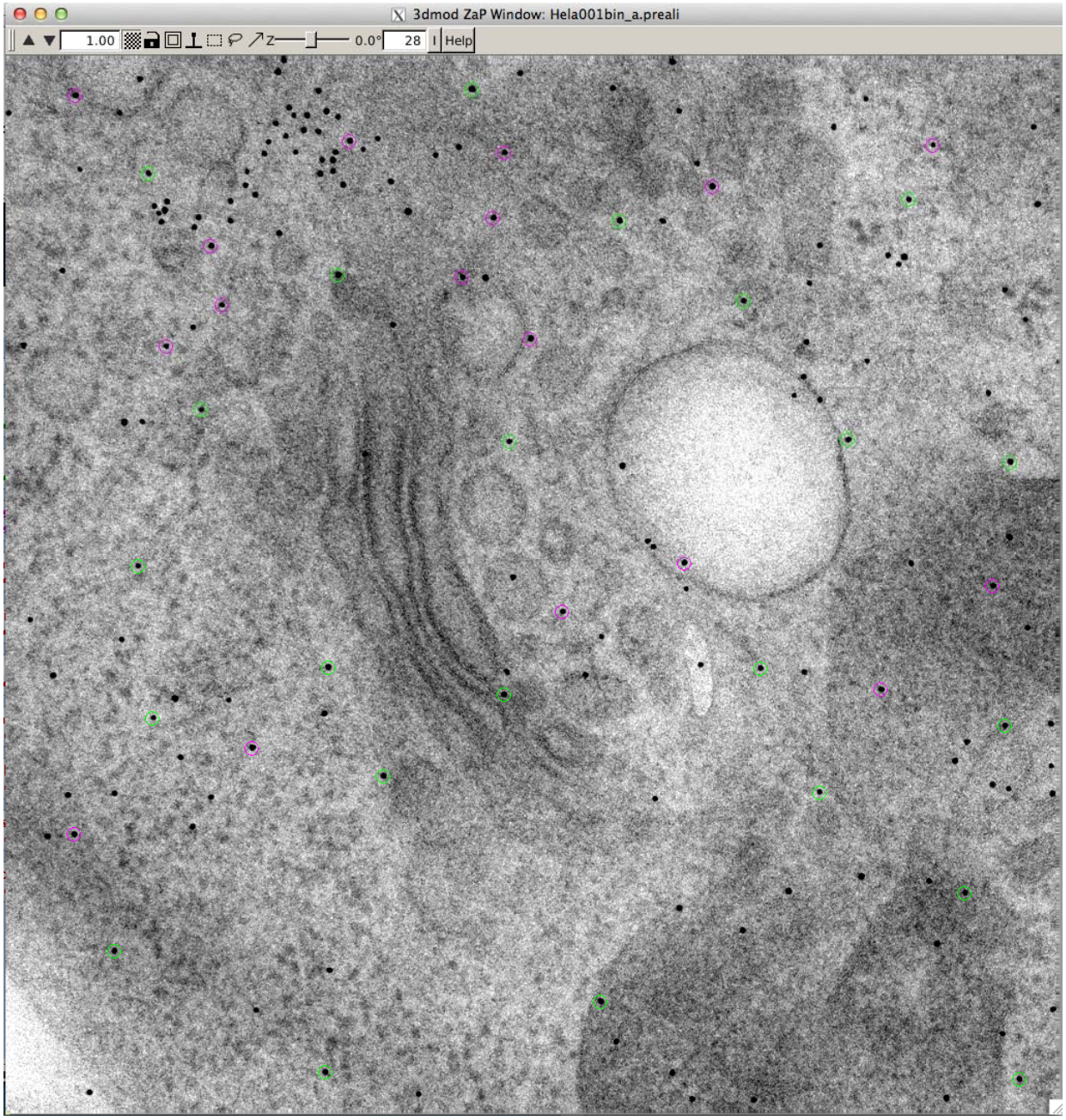
The default parameters are sufficient for aligning the tilt series in this practical. Pressing the **Calculate Cross-Correlation** button runs the program Tiltxcorr. The program uses cross-correlation to find an initial translational alignment between successive images of a tilt series (i.e. just shifts in x and y). The output file, Hela001bin_a.prexf, contains a list of transforms (or recommended shifts) that will be applied to the image data in the next step.

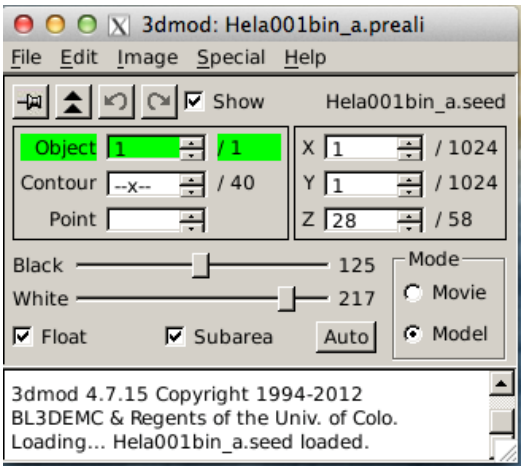
Pressing the **Generate Coarse Aligned Stack** button will run 2 programs. Xftoxg takes the transforms created by Tiltxcorr to obtain a single consistent or 'global' set of alignments. These new transforms are then applied to the image data using the program Newstack. The output file created is Hela001bin_a.preali. To view the prealigned stack, press the **View Aligned Stack in 3dmod** button. Large image shifts can be edited manually using the interactive program, Midas. This is not an issue with this data set. The **Tilt axis rotation** entry is only used if Midas is run, because Midas rotates images to make the tilt axis vertical. If you are satisfied with the prealigned stack, press the **Done** button to proceed to the next step.

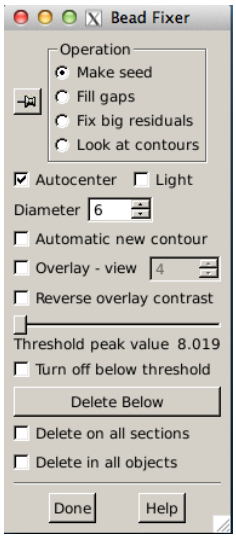
6) Creating a fiducial model based on the position of gold particles



Make seed and track should be selected at the very top of the page. The **Seed Model** tab should be visible. Cell sections tend to have high contrast and gold fiducials can usually be detected automatically. Select **Generate seed model automatically.** In the **Selection and Sorting Parameters** window, for **Seed Points to Select** next to **Total number**, enter 40. Make sure **Select beads on two surfaces** is checked. Click **Generate Seed Model** to automatically detect 40 representative gold particles on the section. When it's finished, click **Open Seed Model** to display the tilt series with the gold particles selected. Scroll to the 0° tilt image using the scroll bar at the top of the window or by using the page up or page down keys. You can also go to the central view of your tilt series by pressing the Insert key on your keyboard (normally 0° tilt image, but not in this data set). Note that 3dmod is now in "model" rather than "movie" mode (see this on the main dialogue box opened with the 3dmod ZaP window, below). Gold particles will be marked by either green or purple circles. These colours indicate whether the particles originate from the top or bottom surface of the section.

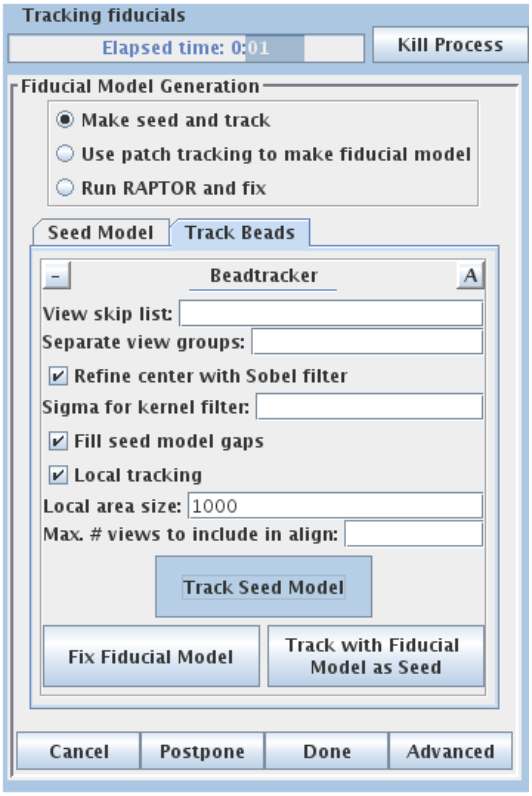

3dmod ZaP Window: Hela001bin_a.preali
1.00
0.0°
28
Help


3dmod: Hela001bin_a.preali
File Edit Image Special Help
Show
Hela001bin_a.seed
Object 1 / 1
Contour --x-- / 40
Point
X 1 / 1024
Y 1 / 1024
Z 28 / 58
Black 125
White 217
Mode
Movie
Model
Float
Subarea
Auto
3dmod 4.7.15 Copyright 1994-2012
BL3DEMC & Regents of the Univ. of Colo.
Loading... Hela001bin_a.seed loaded.

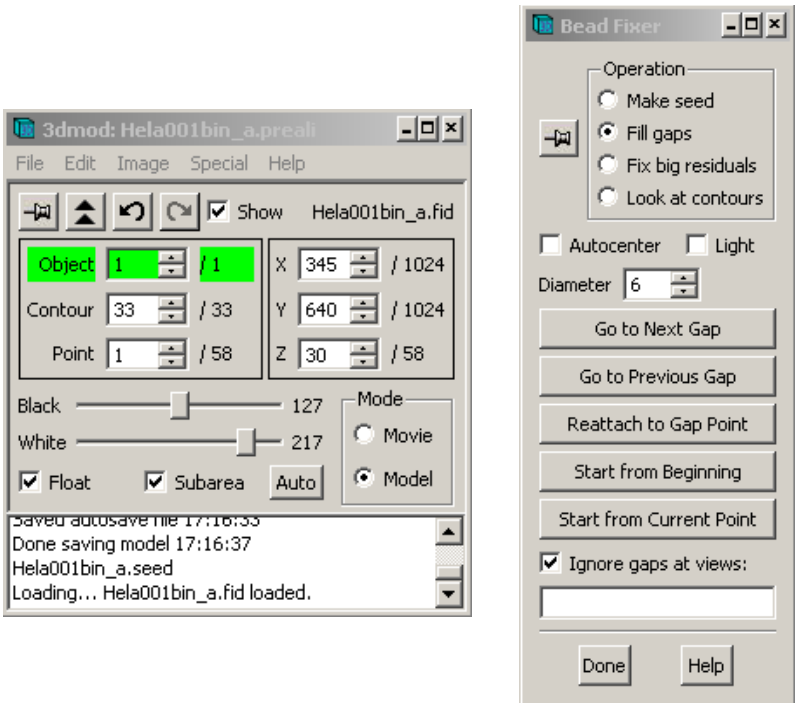

Bead Fixer
Operation
Make seed
Fill gaps
Fix big residuals
Look at contours
Autocenter
Light
Diameter 6
Automatic new contour
Overlay - view 4
Reverse overlay contrast
Threshold peak value 8.019
Turn off below threshold
Delete Below
Delete on all sections
Delete in all objects
Done
Help

Our data set contains gold beads on both sides, which helps with correcting image distortions in the later alignment steps. Ideally, there should be equal numbers of gold beads from both surfaces that are also evenly distributed over the whole view, but sometimes one surface will have less gold beads than the other.

Switch over to the **Track Beads** tab and click **Track Seed Model**. This will run the Beadtrack program to find the gold on all other views. The output file created by tracka.com is Hela001bin_a.fid, which is the completed fiducial model. This computer-generated model is not perfect, and so the next procedure involves an iterative process to edit this fiducial model. If bead tracker is unable to find any of the beads through the tilt series they will be listed in the etomo Project log file. If you have any gaps, before pressing **Fix Fiducial Model**, you can try pressing the **Track with Fiducial Model as Seed** as this may automatically fill in some of the gaps**.**



Press the **'Fix Fiducial Model'** process button (Even if you have no gaps you should still use this option)**.** This procedure will display green or purple circles on all the picked gold beads through the tilt series. You can movie through the tilt series to check that Beadtrack has done its job, but you must turn 3dmod back into movie mode from model mode before you click the middle mouse button.



The Bead Fixer dialog box will come up in **Fill gaps** mode. Hit **'Go to Next Gap'** by clicking using the left mouse button, or by using the spacebar as a hot key. This will attach to a point (highlighted with a yellow circle in the ZaP window) that has a missing model point on an adjacent section. Use the Page Up key (when an up arrow appears above the point) or the Page Down key (when a down arrow appears above the point) to find the view with the missing point and use the middle mouse button to add the point in the center of the gold particle. It is useful to increase the magnification of the image with the '+' key and adjust the contrast on the sections, especially at high tilt. Repeat **'Go to Next Gap'** until the message, '**no more gaps are found**' comes up in the main 3dmod window. Save the model file, close 3dmod and hit the **Done** button to advance to the fine alignment step.

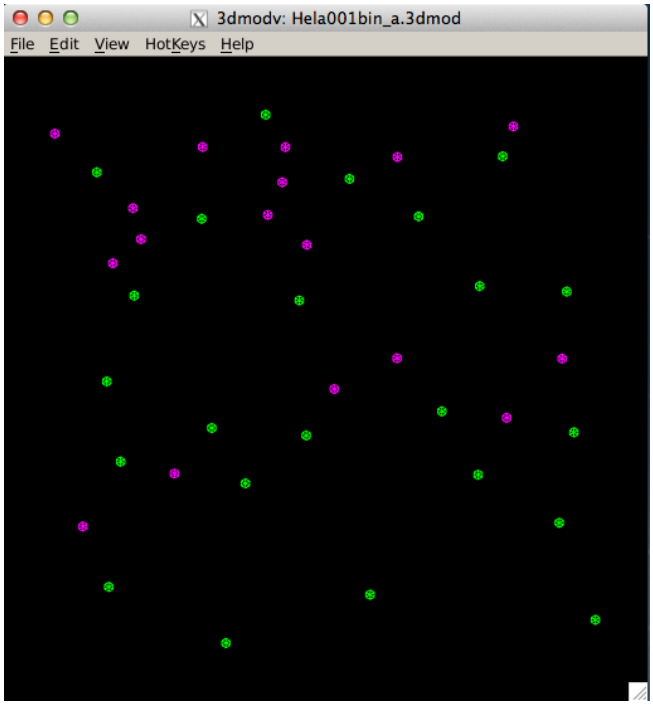
7) Fine alignment

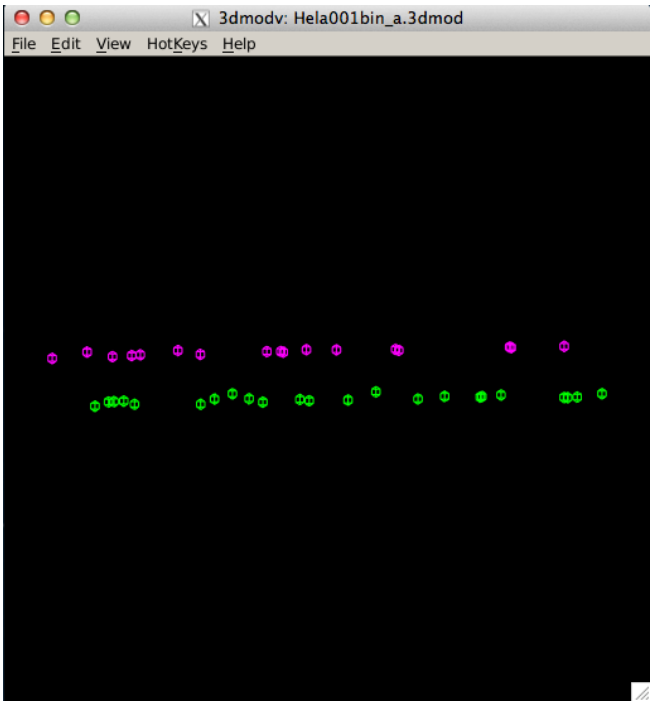
The **Fine Alignment** panel is organized with a set of three tabs to solve for various alignment parameters. Ensure that the **Assume fiducials on 2 surfaces for analysis** option is selected in the Analysis of Surface Angles menu box. A general alignment is done when you press the **Compute alignment** button at the bottom of the **Fine Alignment** box.

This command file runs the program Tiltalign to solve for the displacements, rotations, tilts and magnification differences in the tilted views. The program uses the position of the gold particles in the fiducial model and a variable metric minimization approach to find the best fit (the program generates a log file which can accessed by

right clicking in the eTomo window and selecting **Align axis:a log file**). The residual error and the standard deviation for the alignment solution are also displayed in the eTomo Project Log file (this log file will open as soon as you start eTomo).

The goal of the fine alignment step is to reduce the mean residual error to 0.2-0.6. The Tiltalign program also creates two model files that provide useful information about the fiducial model. The first (Hela001bin_a.3dmod) displays a 3-D model of the fiducials based on their solved positions. Fiducials are represented as purple spheres or green spheres according to which surface they are found on. Examine this model by hitting the **View 3D Model** button on the bottom of the **Fine Alignment** box:

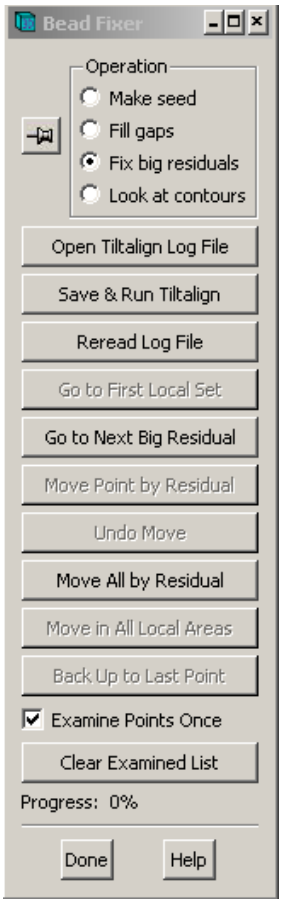


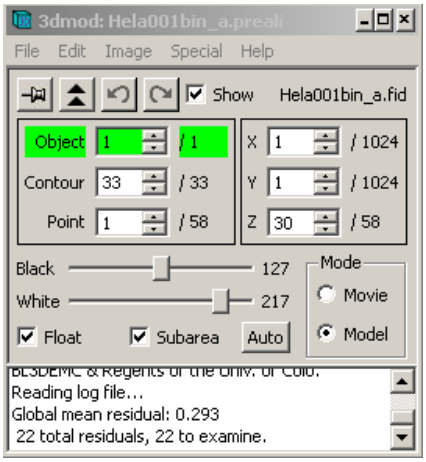


You should see a nice distribution of purple and green spheres across the field of view. Rotate the model to view edge-on by pressing the F and T hot keys. You will see the separation of the two surfaces with this view. Avoid using models that have a cluster of fiducials in any particular region because this will skew the alignment. Close the 3dmodv window.

If the distribution of the gold beads is fairly even you can go to the Global Variables page (second tab) and select the **Full solution** for Distortion Solution Type (see below). This option will allow the program to correct for both X-axis stretch and Skew distortions. Once you have done this go back to the general page and click the **Compute alignment** button again. If the distribution of fiducials is not even over both surfaces, then skip the distortion solution steps.

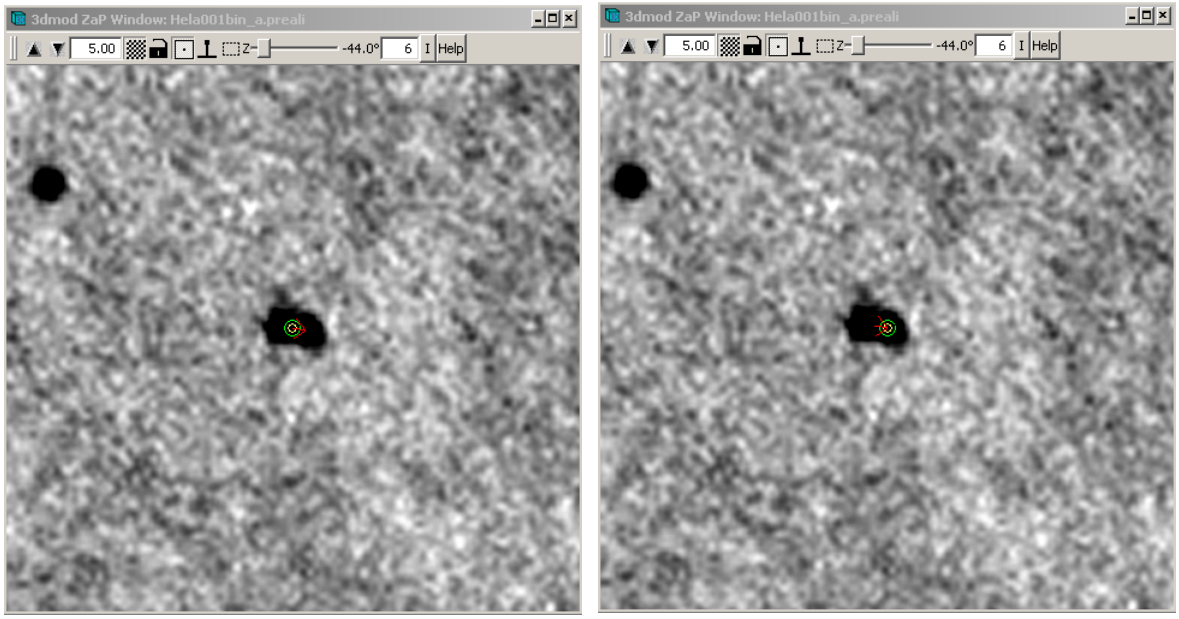
Leave 3dmod open for the next several steps and increase the magnification so that you can clearly see the gold beads. Press the **'View/Edit Fiducial Model'** button to reload the fiducial model for editing. This will bring up the Bead Fixer dialog box in **Fix big residuals** mode (see below) and load the relevant details from the aligna.log file (reported in the 3dmod dialogue box below).



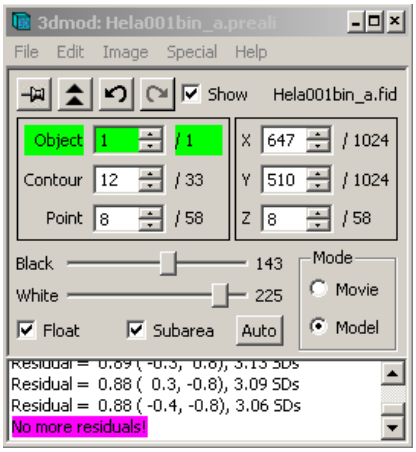


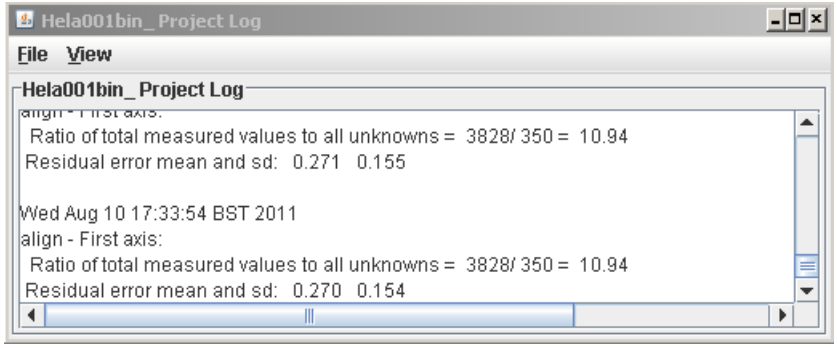
The following iterative steps involve fixing fiducial points with large residuals:

Zoom in with the ZaP window to 5 so that the individual gold beads can be clearly visualized. Click, '**Go to Next Big Residual**', in the **Bead Fixer** dialog box. The model point that had the biggest residual will have a red arrow pointing in the direction of the recommended move. You'll probably be able to see that the model point is not centered properly on the gold bead. If you click '**Move Point by Residual**' in the **Bead Fixer** dialog box, it will move the model point by the recommended amount. This works most of the time, but if the recommendation looks wrong, you can move it by manually by centering the cursor over the middle of the gold bead and then clicking the right mouse button.



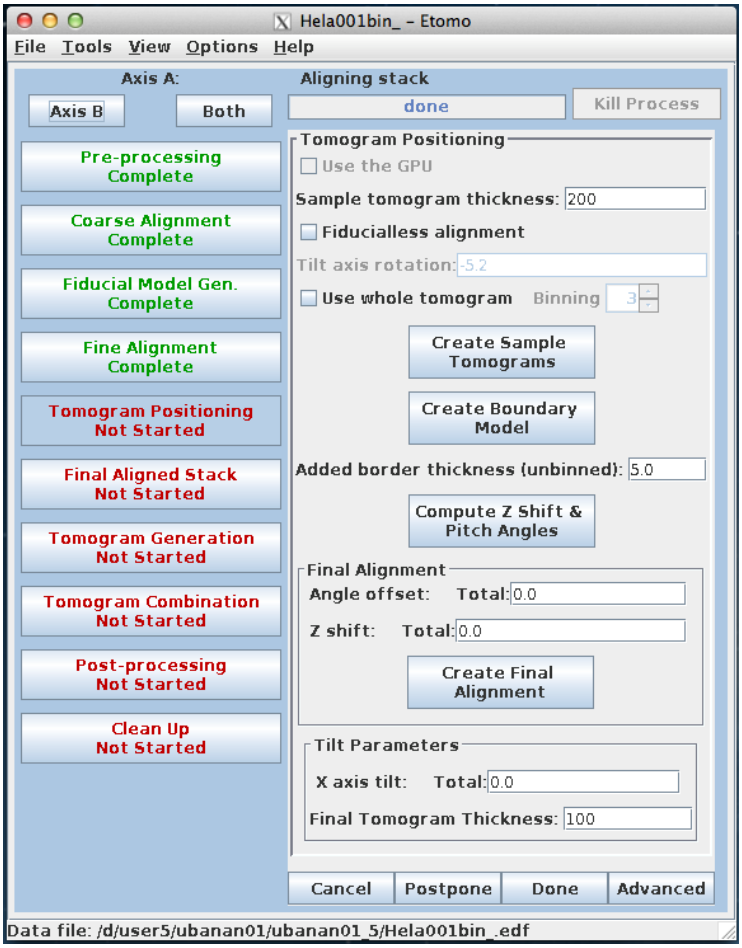
Repeat selecting '**Go to Next Big Residual**' and '**Move Point by Residual**' until no residuals are found. The hot key **'** will cycle to the next residual and the hot key **;** will move the point by the residual. Once you have been through all the residuals you need to press the **Save and run tilt align button** on the Bead fixer dialogue box. If you check the eTomo log file you should see that the residual and standard deviation have decreased. Repeat this process until there are no more residuals or until the mean residual error does not decrease any further. Save the model and close 3dmod. Then press the **Done** button, if eTomo asks you if you want to save the model select yes.





8) Tomogram positioning; sampling the data set to create 3 small reconstructions

The goal of the next step is to shift and rotate your reconstruction so that it is aligned with the coordinate system axes. This is done by sampling three regions of the tomogram from near the top, middle, and bottom of the tilt series. If these samples are not adequate, you can do this instead with a whole tomogram (binned down). The sample tomogram thickness can remain at 200 in this practical, to create a reconstruction that is much thicker than the original section. Then press **Create Sample Tomograms** button.

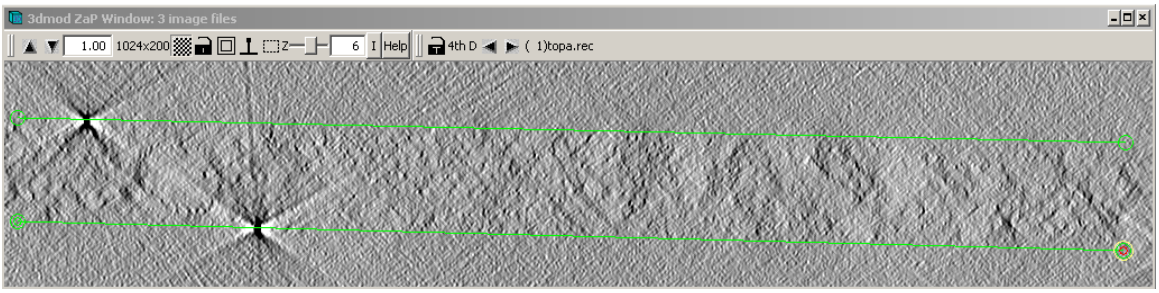


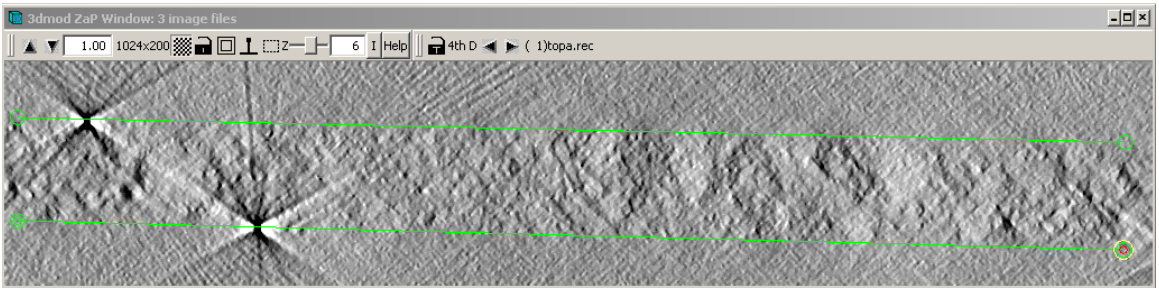
This command file first extracts and aligns a 60 pixel sliver from the top, middle, and bottom of the image stack. These output files are named topa.rec, mida.rec, bota.rec.

Press **Create Boundary Models** button.

This will open 3dmod which then reads in all three reconstructions at once, with the topa.rec displayed first and viewed edge on (X-Z plane). 3dmod will also start with an empty model, named tomopitcha.mod (see below). The top bar of the Zap window has a feature '4th D' and a backward and forward arrow. If you click the forward arrow, you can cycle through to the mida.rec and bota.rec reconstructions, respectively. Start with the topa.rec. Use the contrast sliders to adjust contrast. If the contrast is too low you can filter the images using a median filter. To do this go to the edit menu (on the 3dmod dialogue box) click on image and then select process. This

will open the process dialogue box in which you can select the median filter option. Then press Apply to filter the image (you need to do this for each of the three images). Notice the material in the center of the volume with a mottled appearance. This is the part of the reconstruction with biological material. Using the middle mouse button, place a model point on the left side of the top surface that defines the region containing the biological material, and a second model point at the right side of the top surface. A line will connect the two points. Model the bottom surface of the section with 2 points on the left and right sides, respectively. Toggle to the mid.rec and bot.rec file by hitting the arrow button to the right of '4th D' at the top of the 3dmod zap window. Repeat modeling the top and bottom surfaces of the other two reconstructions. The final model should have **1 object** and **6 contours**, and each contour should have **2 model points**. Save this model file and close 3dmod.





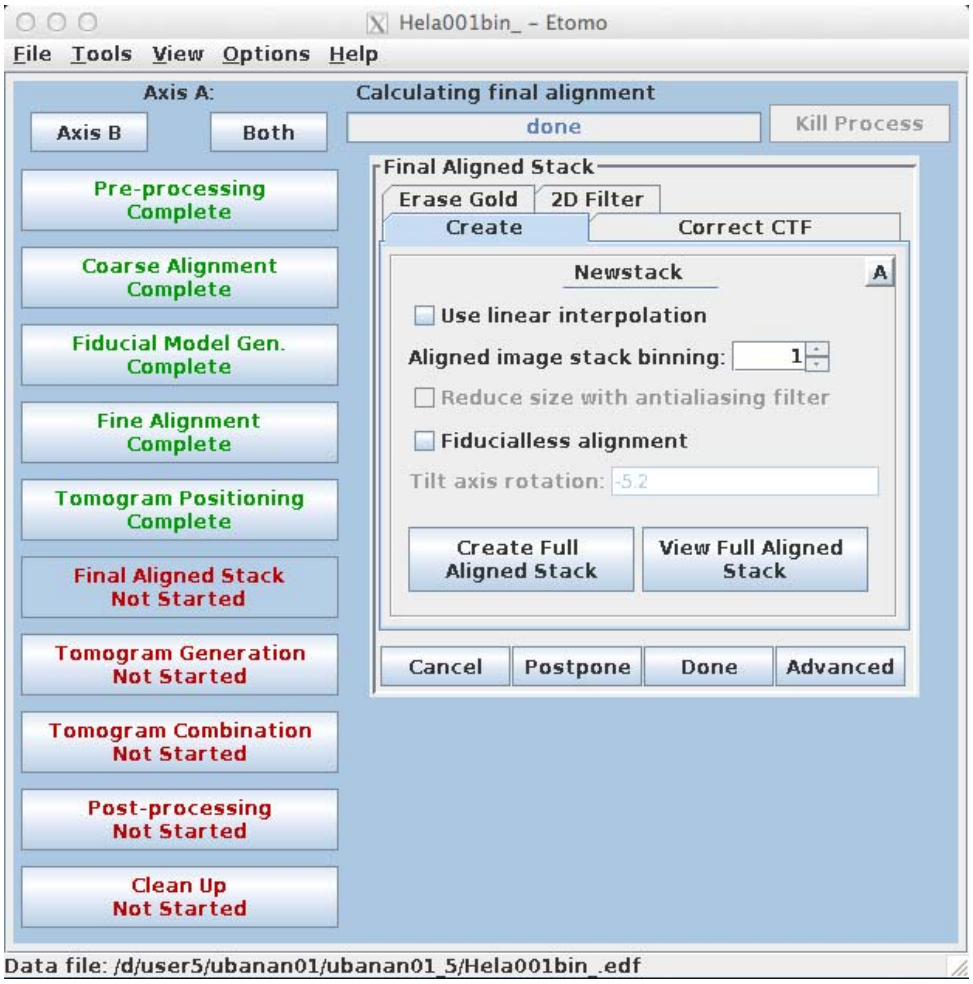
It is generally helpful for the thickness of the final tomogram to be about 10 to 20 pixels greater than the actual distance between the lines that you draw. You can adjust the entry in **Added border thickness** in the eTomo main window to accomplish this. The default value of 5 will make the tomogram 10 pixels thicker; change it to 10 to make it 20 pixels thicker.

Then press **'Compute Z Shift and Pitch Angles'** button**.** This will fill in the tilt angle offset box which will correct for tilt in Y, the X axis box which corrects for tilt in X, the Z shift box that centres the volume in Z and in the Final tomogram thickness box. Then press **'Create final alignment'** which will apply these values to the alignment solution**.**

Finally press **Done.**

9) Create the final aligned stack

Press **'Create Full Aligned Stack'** button**.** This command will apply the alignment transforms to the full-sized image for the final, aligned stack. The output file is named Hela001bin_a.ali.

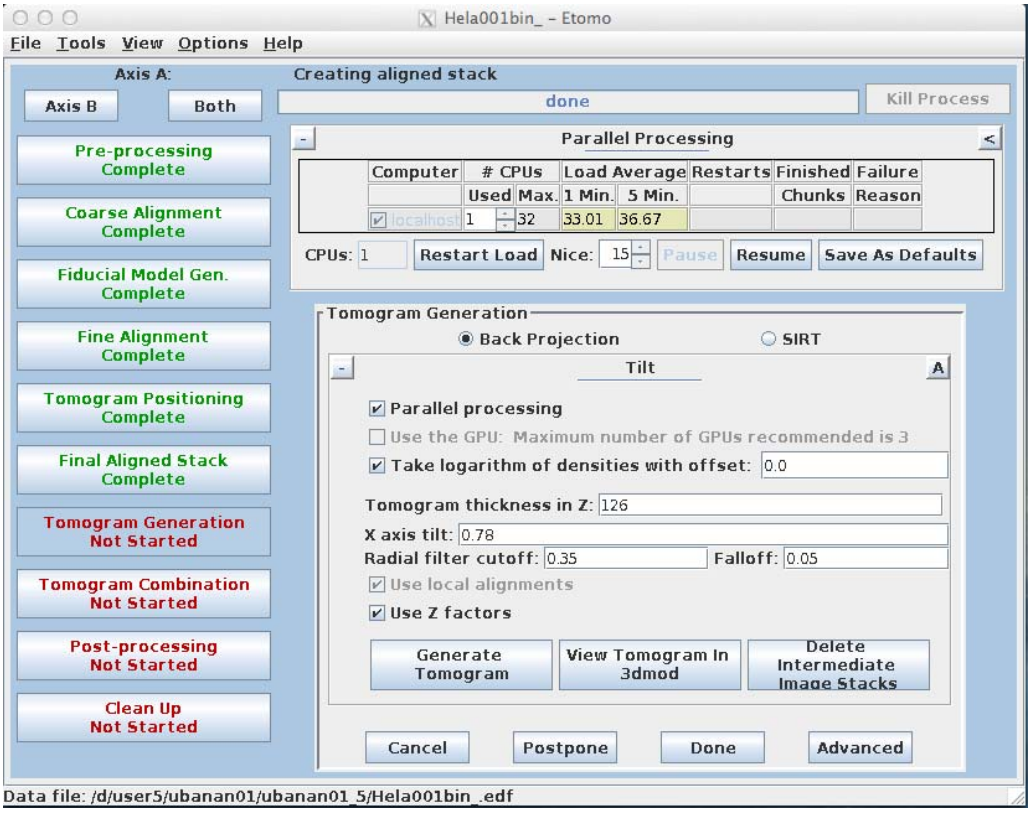


The full aligned stack may be viewed by pressing **View Full Aligned Stack**, although this is not essential. There are optional steps for CTF correction, erasing fiducials, and filtering the aligned stack, which are also not needed here. Press the **Done** button to advance to the next step.

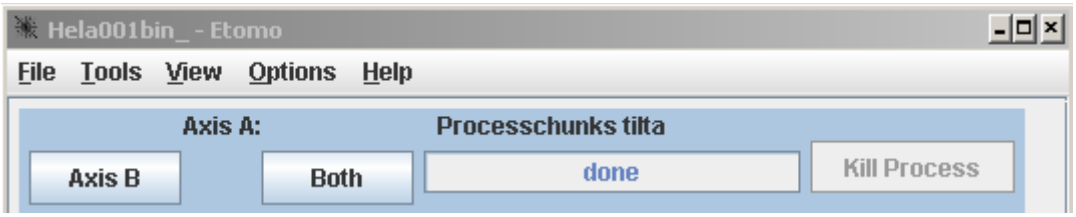
10) Generate the final tomogram for Axis A

If the parallel processing option has been selected you will need to select the local host and make sure both processors are being used**.** Press the 'up' arrow to change the number of CPU's used to 2. **Press 'Generate Tomogram'** button**.** When the tomogram is computed, examine it by pressing **View Tomogram in 3dmod**. The default parameters for generating the tomogram are sufficient for the sample tilt series provided. You can Movie through the reconstruction by clicking the middle mouse button.

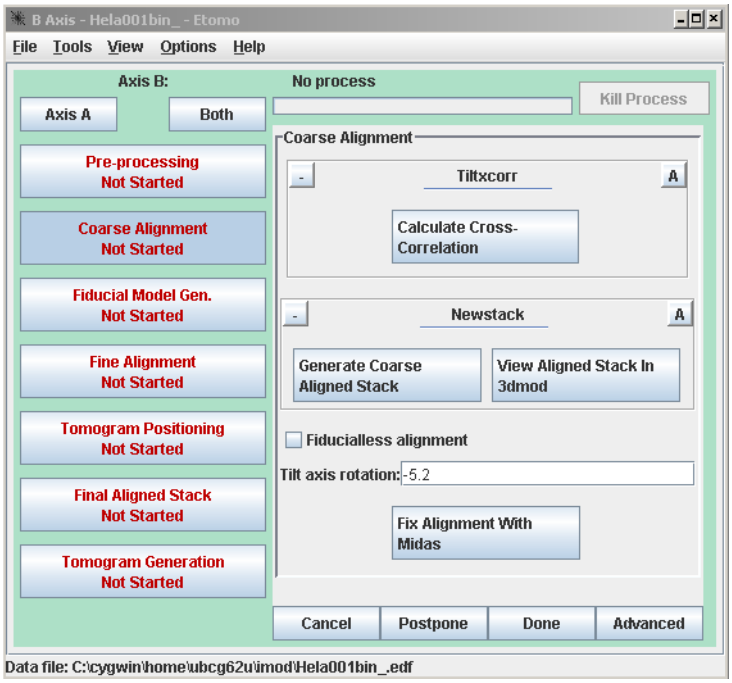
Then press the **Done** button.



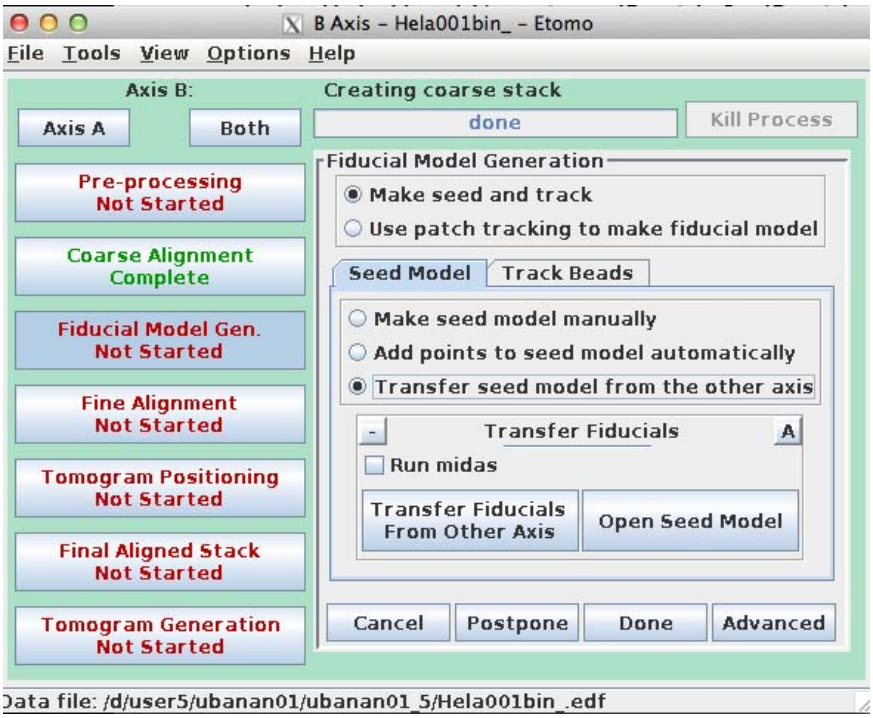
11) Axis B



To see the B Axis, press the **Axis B** button at the top of the eTomo window. This will bring up another set of process buttons which can be used to perform the operations for aligning the Axis B tilt series and calculating its tomogram. To prevent confusion, Axis A has a blue background and Axis B has a green background. On the right-hand side of the eTomo main window are the process buttons to perform the operations for aligning the **Axis B** tilt series and calculating the tomogram. Again, the Pre-processing step is not required for this sample data set. Go to the **Coarse Alignment** step, as described above for **Axis A**.

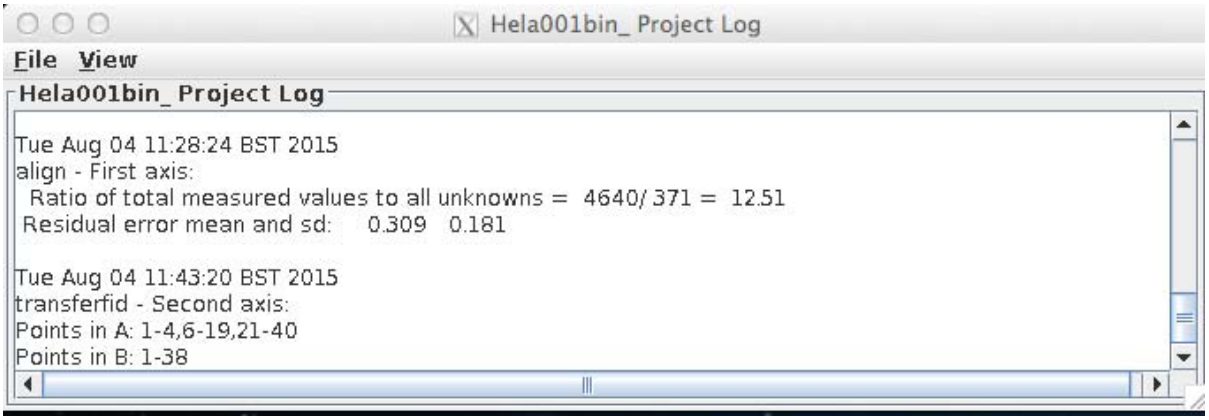


After the coarse-aligned stack has been generated, press **Done** to advance to the next step. In order to combine the tilt series at least some (8-10) of the beads that you track must be the same in the two series. To accomplish this select **Transfer seed model from the other axis**, use the program Transferfid by pressing the **'Transfer Fiducials From Other Axis**.



This program creates a seed model for the second axis based on the fiducial model

from the first axis. The program will search for the pair of views in the two series that correspond best, and then transfer the fiducials from the first series to make the seed model for the second series. At the end, the program indicates how many corresponding fiducials were found in the B axis (shown in the eTomo log file).

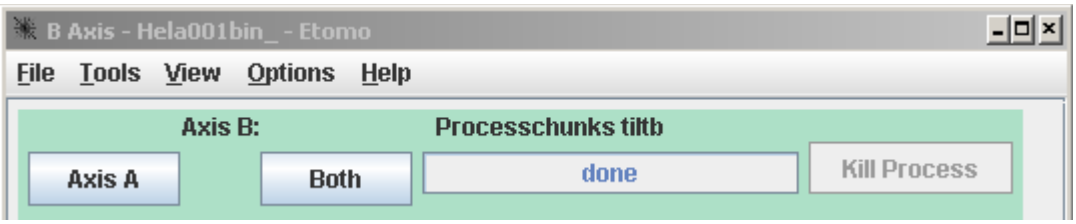


The output file of transferfid is the seed model for the second axis. In this example, the model is named Hela001bin_b.seed. Switch to the **Track Beads** tab and press the **Track Seed Model** in the Axis B window. This will automatically track the fiducials for the 'B' set. Fix the gaps in the Hela001bin_b.fid file by pressing the **Fix Fiducial Model** button in **Bead Fixer** or try the **Track with fiducial model as seed** first. If gaps are still remaining, fill them in as outlined above for the A axis. When all gaps are found press **Done** and proceed to the **Fine Alignment** step. You will now proceed with the same steps following the same procedure as outlined above for the Axis A set. Briefly:

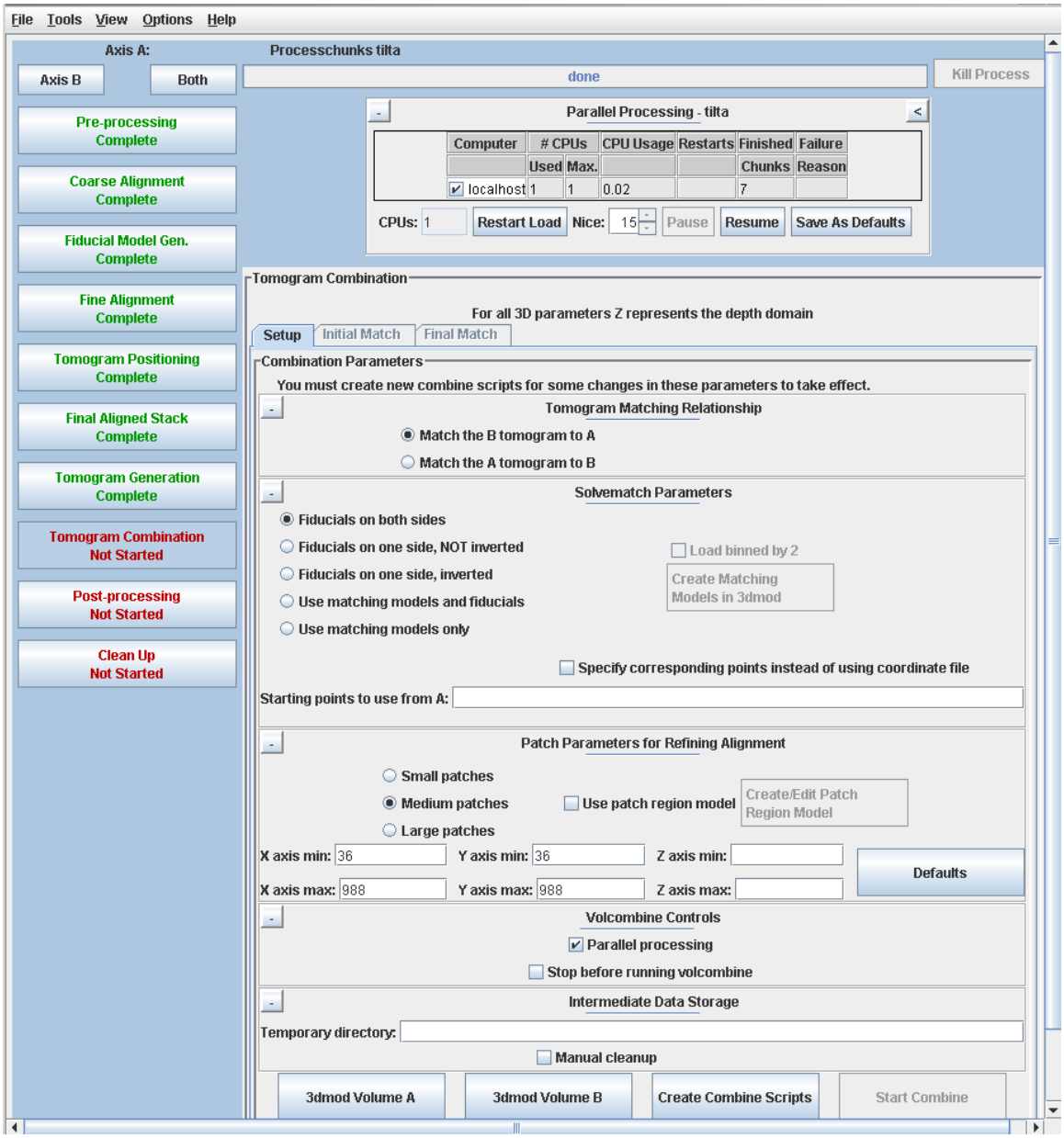
If you were able to select full solution under the global tab for Axis A then select this again before you Press the **'Compute Alignment'** button**.** At this point, you will start the iterative alignment procedure as you did for the 'A' set by editing model points with large residuals, saving the model, and computing the alignment. When the alignment is complete, press **Done**.

Proceed with **Tomogram Positioning, Create the final aligned stack** and **Tomogram Generation** as outlined above for the A Axis**.**

11) Combining the two axes



Go back to the A Axis by pressing the **Axis A** button. To combine the two tomograms press the **Tomogram Combination** process button.



If the parallel processing option has been selected you will need to select the local host and make sure both processors are being used**.** The **Tomogram Combination Panel** is organized with 3 tabs: **Setup**, **Initial Match**, and **Final Match**. The **Setup** window is where information is given about the particular data set. The first section describes the **Tomogram Matching Relationship**. It is most common to match the B tomogram to A. The **Solvematch Parameters** box asks for information on the fiducial marker distribution. In this data the fiducials are on both sides. For this data set, the programs will have no trouble fitting to all of the points at once, so it is not necessary to fill out **Starting points to use from A**.

The next section contains information for **Patch Parameters for Refining the Alignment** using local 3D cross-correlations. Select **Medium patches** for this data set. When specifying the limits of the volume from which the patches will be extracted, it is important to look at the A axis tomogram as the B axis tomogram will be matched to it. Using the entire Z axis range will almost never work, and even for the X and Y axes it may not be good to use the defaults. To find the limits, press the

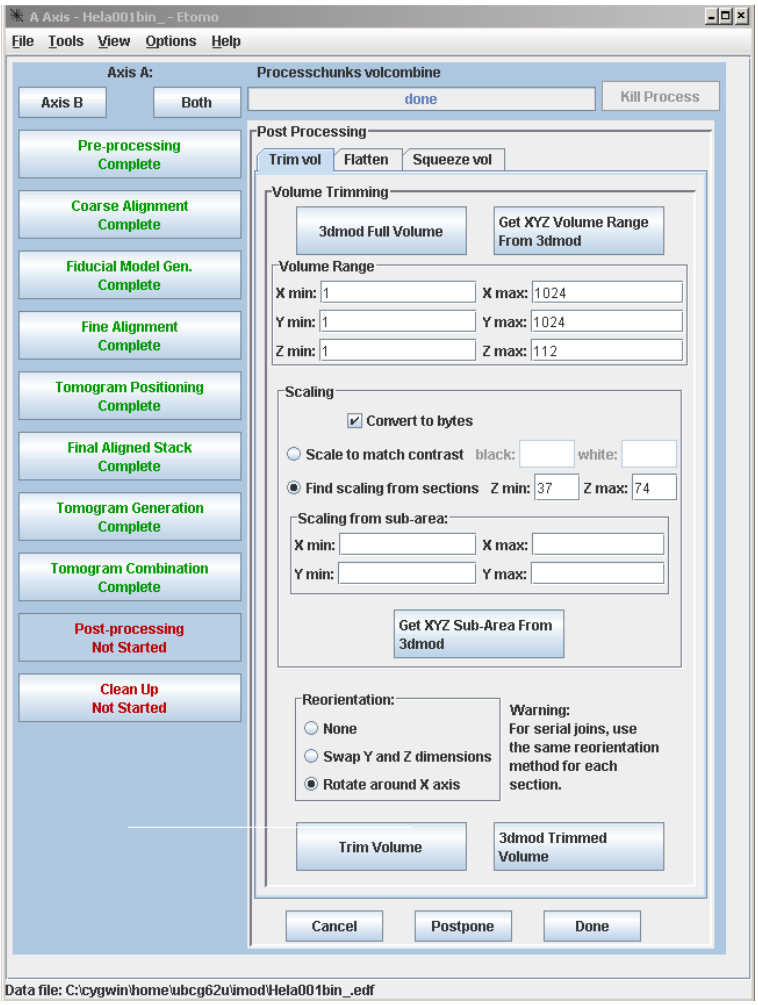
3dmod Volume A button at the bottom of the panel. This will open Hela001bin_a.rec in 3dmod. Step through the images and decide what ranges of X, Y and Z contain useful information for matching up the volumes. In this practical, default parameters can be used for the X and Y axes. The **Z axis min** and **max** need to be entered by you. To do this you will need to check at which z sections the biological material starts and ends in the Hela001bin_a.rec. Close 3dmod.

When the parameters for the **Setup panel** have been entered, press **Create Combine Scripts** to create a series of command files that will run various programs in the combine procedure. Press **Start Combine** to begin the process of dual-axis tomogram combination. eTomo will automatically advance to the **Initial Match** and finally the **Final Match** tabs as various programs are being run. After tomogram combination is complete (around 3 minutes), press **Open Combined Volume** to view the final tomogram.

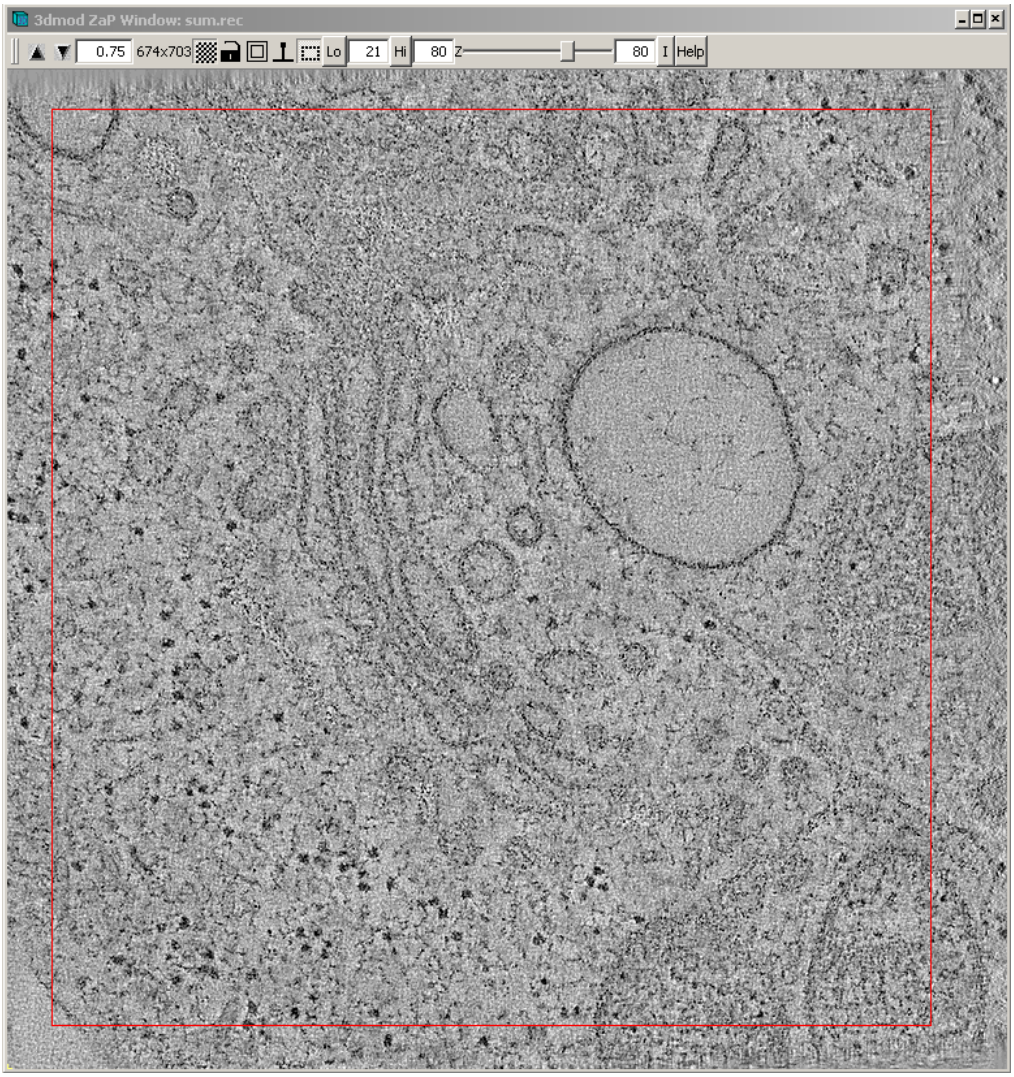
Press **Done** to advance to the final steps.

12) Post-Processing and Clean-up of intermediate files

Post-processing involves a volume trimming and byte scaling step, followed by deletion of intermediate files. There are also options to flatten the volume and to create a squeezed down volume; these can be useful when working with very large data sets, and the flattening is particularly helpful when reconstructing serial sections.



The final reconstruction of the two tilt series combined into one will always be called sum.rec. (If you have a single axis data set, the name of the reconstruction file at this point will be the data set name followed by _full.rec). Open the sum.rec reconstruction by pressing **3dmod Full Volume**. Step through the reconstruction and determine the X,Y and Z ranges for the final volume. A convenient way to set the X and Y range is to turn on the rubberband with the dashed rectangle in the toolbar of the 3dmod ZaP window, press the left mouse button over the upper left corner of the desired area, and drag the mouse to the lower right corner. Z can also be set, if desired; press **Lo** to set the minimum Z and **Hi** to set the maximum Z. When you press **Get XYZ Sub-Area from 3dmod,** eTomo will retrieve the X and Y values of the rubberband (and Z values if they are set) from 3dmod. Finally a scaling range is set, to find the range of slices that exclude the gold beads and allow the grey levels in the tomogram to be stretched over the 8-bit range. Again you can use the rubberband to select a region of tomogram in X, Y and Z for this and press the **Get XYZ Volume Range from 3dmod** button in the middle of the page.



Once you have set both the area you want to cut out and the scaling range, close 3dmod and press the **Trim Volume** button to run Trimvol. Trimvol is a single tool for

trimming a volume and converting it to byte format. Finally, view the final, trimmed volume (named Hela001bin_.rec) by pressing **3dmod Trimmed Volume**.

Press **Done** to proceed to file cleanup.

Cleanup of intermediate files:

Cleaning up of your files is very important! The tomogram generation process creates many large, intermediate files. The **Intermediate file cleanup** box lists what we consider intermediate, nonessential files that can be deleted. Once you are satisfied that the final tomogram is truly final, you can select by highlighting the intermediate files and hitting the **Delete Selected** button.

Press **Done** and close eTomo.

The final, dual-axis tomogram is named Hela001bin_.rec.rec and can be viewed outside of eTomo using the command, 3dmod Hela001bin_.rec. You may also want to filter the reconstruction, which we would normally do by using nonlinear anisotropic diffusion (NAD) option in eTomo. However this can take some time to do so we will use a median filter instead by typing at the command line:

> clip median -3d -n 5 Hela001bin_.rec Hela001bin_median.rec

This takes around 5 minutes to process.

An example of an NAD filtered reconstruction can be found in the DAN directory. This directory also contains a model file in which I have manually modeled (segmented) the cellular material in the reconstruction. To view both the reconstruction and the model go into the DAN directory and at the command line type:

> 3dmod Hela001_bin_nad.rec Model_Binned.mod

To view the model file alone press v in the 3dmod window. This opens the model viewing window. Holding down the middle mouse button allows you to rotate the model.

Refer to the Introduction to 3dmod for information about modeling the many cellular features in the tomogram. This was an easy data set and you will likely encounter more problems with your own data, so it is best to read through the Tomography Guide as you start working on a real data set. Also, you will be able to use eTomo more effectively if you read Using eTomo, which explains features such as accessing help and setting up parallel processing. All of the guides mentioned above can be found on the IMOD website (http://bio3d.colorado.edu/imod/).