Lesson 2: Reconstructing a Cryo Tomogram using BRT

In this exercise, you will build a single tomogram to learn how to use the BatchRunTomo interface in Etomo.

1) `cd $WORKSHOP_HOME/IMOD_Labs/cryo-BRT`

2) `etomo`

3) Press **Batch Tomograms** on the **Front Page**. The interface opens with the Batch Setup tab. The initial radio buttons allow stacks to be moved from elsewhere into automatically created data set directories. Your **stacks are already in dataset directories**, so there is no need to change the default selection.

   Select *cryoSample.adoc* as the **System Template**. This template turns on specific settings that work well for most cryo data sets.

   At the bottom, two fields allow one to change the root name that will be used as a prefix for all the batch processing files, and to select a different Location in which various batch project files will be kept. Again, there is no need to change the defaults here.

4) Switch to the **Stacks** tab. Press **Add Stack(s)**. Select *cryo.st* and press **Open**. Notice how the program automatically detects this is not Dual Axis or a Montage. Press the **3D** icon under **Open Stack A**. Here is where you check to see if you need a Boundary Model or to Exclude Views. In this simple case, neither option is needed.

5) Switch to the **Dataset Values** tab. We need to choose the **Image distortion File** *27.5kGIF2007-03-24.idf*. Select **Remove X-Rays**. Enter **15** for **Bead size (nm)** and **15** for **Target number of beads**. There are only 12 beads in this case, but it doesn't hurt to estimate a little high. Select **Do positioning for Cryo sample**. Enter a tomogram thickness of **250**. In the Reconstruction section, the selection of the **Calculated thickness (unbinned pixels)** is appropriate because it will be doing positioning, and an entry for **With fallback** is required with this choice in case the positioning comes out with too low a thickness. Enter **250** here. Also enter **16** for **Plus (optional)**. This will allow some extra thickness that can be trimmed away in the postprocessing step, which we will do manually once the tomogram is built automatically.

6) Switch to the **Run** Tab. Increase the **Resource** table # **CPUs Used** to **4**, then press **Save As Defaults**. Press **Run**. Directive files will be written for each data set and the processing will begin. Watch the **Status** output in the table at the bottom. Pausing, killing, and resuming are possible but more complicated than for ordinary processing; see Running the Data Sets in the Batch Guide for details.

   Essential items like mean residual values and warnings appear in the **Project Log** during the processing. At any time after a set begins being processed, you can see the full output from the batch program by pressing the button in the Open Log column. Once a set has finished or reached a stopping point, it can be opened in the Etomo reconstruction interface with the button in the Open Set or Open Rec column. You can see that if you turned on Stop after, the default choice would be to stop after the gold detection, which might be useful if you were erasing gold. All data sets would run to that point—regardless of whether those steps were actually run for a particular data set. When you restart, both sets would restart after that point, unless an earlier point were selected.

7) Once the **Status** of the tomogram has changed to **Done**, click on **Open Set**. This will open the Etomo interface. Etomo has no a priori knowledge about what has happened during batch processing, so all status values show Not Started. Go to the **Post-processing** section and press **3dmod Full Volume**. Page through the tomogram and choose the lowest and highest slices you would like to keep. Put them into the **Volume Trimming** table under **Z min** and **Z max**. Use the rubber band tool and the **Lo** and **Hi** boxes to select a small region in X, Y, and Z for **Scaling**. Press **Get XYZ Sub-Area from 3dmod**. Press **Trim Volume** and when done, **3dmod Trimmed Volume**. Press **Done**.

8) On the Clean Up section, you will notice that you cannot Archive Original Stack. This is because the stack gets archived automatically after X-ray removal in BatchRunTomo. Instead, select all intermediate files and press **Delete Selected** and then **Done**. Exit Etomo.