TXM Wizard Data Analysis 20140617

(Be sure to also look at the online help for TXM Wizard)

Analyze the data (overview):

(*Note: Steps I-III may be done with* ***preprocessing on the fly*** *– available for data collected with scripts generated using* ***Python version of TXM Wizard only****. Separate steps provided here for data collected with other methods, or for data in which the sample is moving (e.g. under gas flow or heat.*)

Preprocessing:

I. Average sets of multiple reference images
II. Subtract references from sample images
III. Average reference-corrected sets of sample images (and align if necessary, e.g. if sample is moving due to heat etc.)

IV. Stitch together any mosaic images

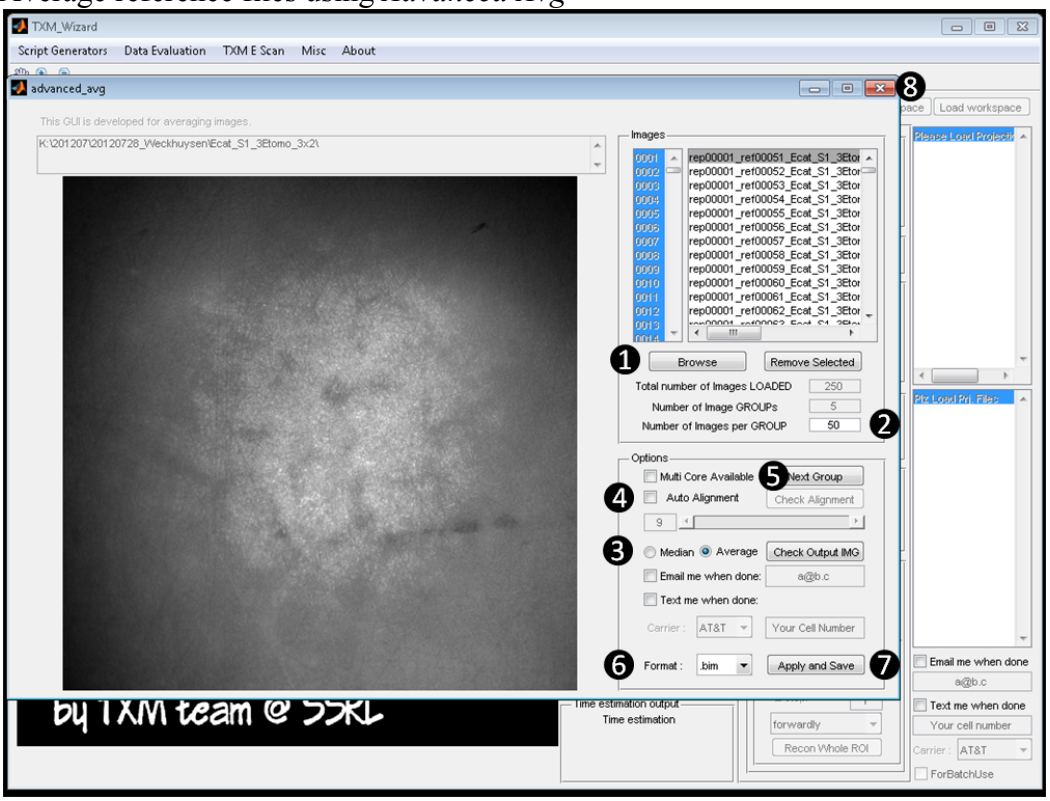
V. Reconstruct tomography
    a. If tomo at multiple energies,
        i. align (click) on highest energy and use that workspace file for other energies
        ii. do magnification correction
    b. Crop/scale projection images
    c. Align tomographic data set / reconstruction
    d. GUI reconstruction

VI. XANES imaging – do magnification correction and alignment of energy stack (Corr for XANES), then XANES analysis with XANES Wizard
VII. 3D Visualization of data (Avizo)
VIII. XANES Imaging

I. Average reference files:

Reference images are taken before and after tomographies, or before and after the energy is changed in a XANES map. Averaging the reference files increases the signal to noise ratio, before you perform a reference correction.

Average reference files using *Advanced Avg*



1. Browse for reference files
2. Adjust number of images per group (equal to number of repeat exposures)
3. Choose average
4. Do not choose “Auto Align” (after all, there is nothing to align here)
5. Choose “Multi Core Available” (utilizes parallel computing capability)
6. Save as .bim file (image and metadata file; header information like the photon energy, motor positions, etc. stored in the metafile).
7. Click “Apply and Save”
8. Close the *Advanced Avg* window

Files will be saved in “Average” folder. Rename this folder “Average_refs”

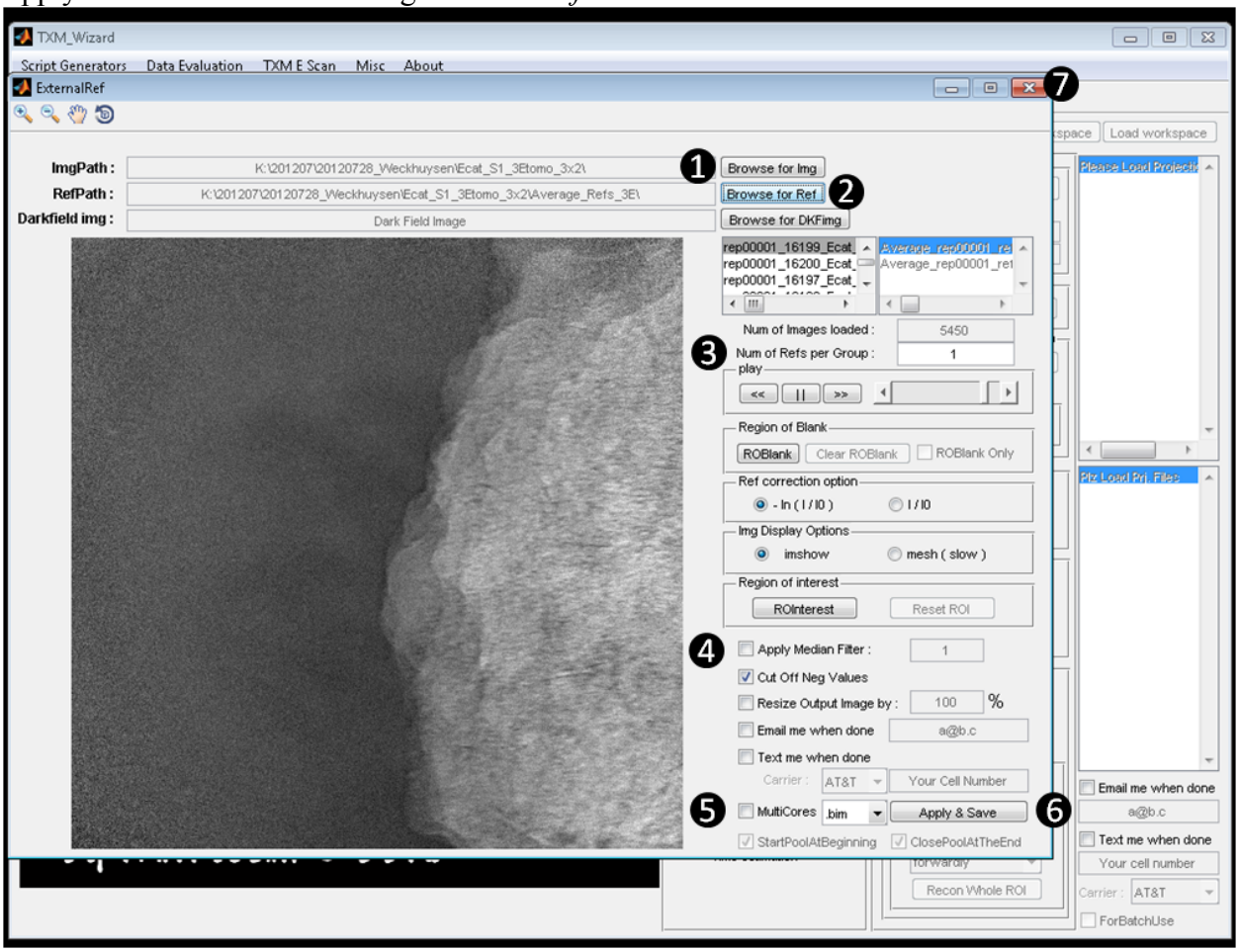
Note: If the program stops responding for any reason (always monitor the performance of the CPU cores in Task Manager), then there is likely to be a corrupted file in the data set. In order to identify which file is corrupted, there are a number of things that you can do:

1. Check file sizes in the parent folder. The corrupted file will sometimes have a different file size than most of the files in the folder.

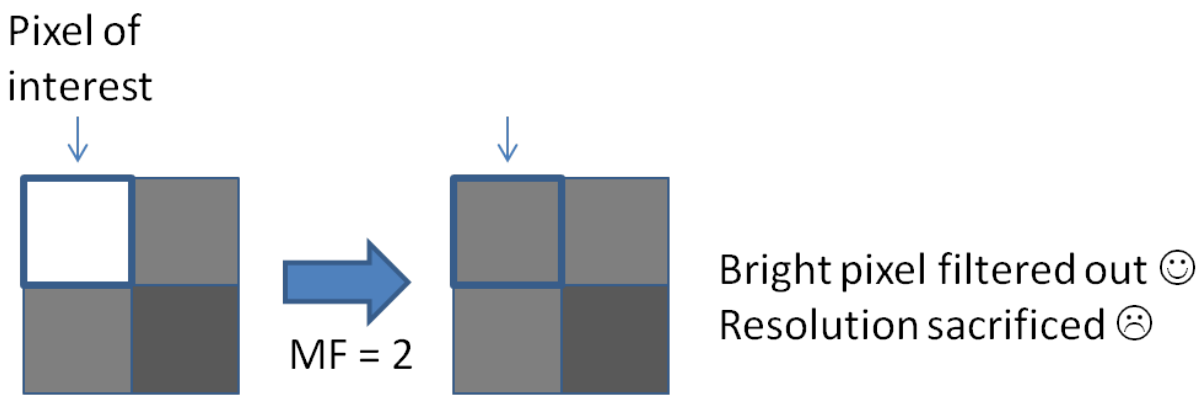
2. Uncheck multiple CPUs available, and run the analysis again. The program will perform its functions on files in a numerical/alphabetical order from a single core. The program will stop at the corrupted file

II. Apply reference correction to projection images:

Apply reference correction using *External Ref*



1. Browse for sample files
2. Browse for reference files (load averaged reference files)
3. Keep number of references per group equal to 1
4. Choose median filtering if bad pixels are present
    a. *(Bright bad pixels from the camera can hurt the contrast of the image that you are viewing. Sometimes the auto-alignment features within TXM wizard will align images based on these bad pixels and not the attributes of your image. You can tell the bad pixels are greatly affecting your contrast by toggling back and forth between a median filtering of 1 and 2).*



5. Choose "Multi Cores"
6. Save as .bim file
7. "Apply and Save"
8. Close the *External Ref* window when the calculation is complete

Files will be saved in "RefCorr" folder

III. Average projection images:

Average corrected sample files using *Advanced Avg*

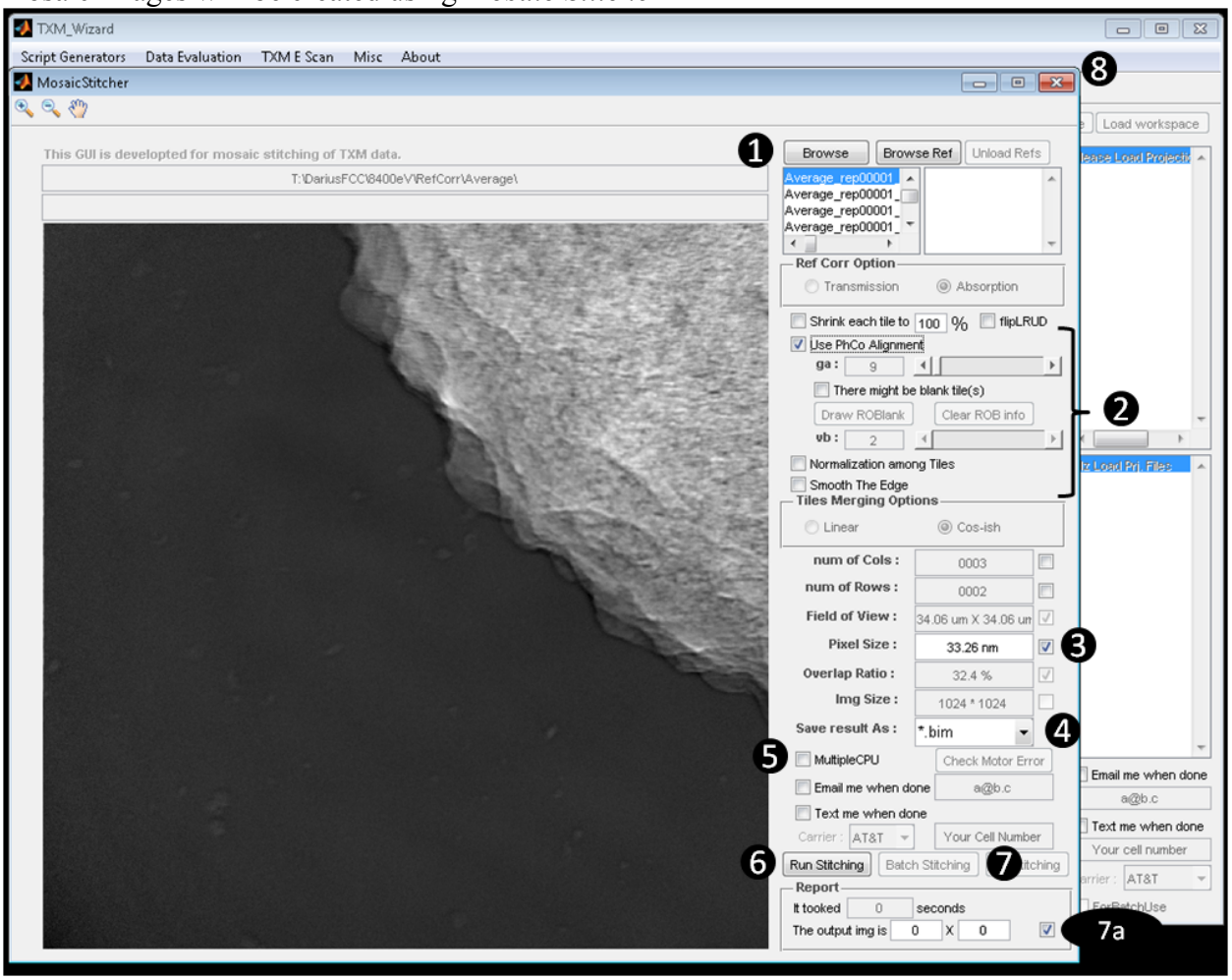
1. Browse for reference corrected files
2. Adjust number of images per group (equal to number of repeat exposures)
3. Choose average
4. Choose "Auto Alignment"
5. Choose "Mulit Core Available" (utilizes parallel computing capability)
6. Save as .bim file (image and meta file; header information like the photon energy, motor positions, etc. stored in the metafile).
7. Click "Apply and Save"
8. Close the *Advanced Avg* window

Files will be saved in "Average" folder

IV. Stitch mosaics from single shot projection images (if necessary):

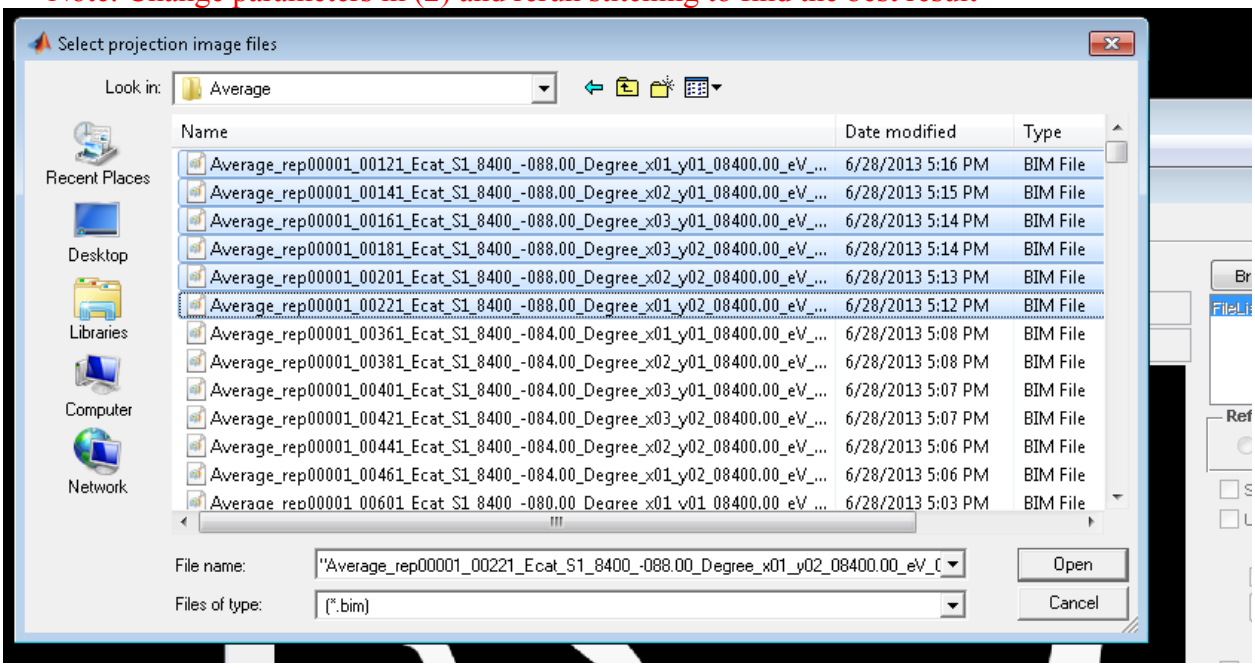
If the sample is larger than the single-shot FOV, then mosaic imaging is performed. Mosaic stitching will use a phase correlation algorithm to align overlapping areas from consecutive single-shot images in the mosaic. After applying the appropriate parameters for stitching to one projection mosaic, the same parameters can be applied to the other projections using batch stitching.

Mosaic images will be created using *Mosaic Stitcher*



1. Browse for averaged projection images (load only the files from a single projection angle; see below)
2. Choose mosaic stitching parameters (See paper for more details Y. Liu et al., J. Synchrotron Rad 2012, 19, 281-287.)
    a. PhCo Alignment: applied a phase correlation parameter for aligning mosaics
    b. Normalization among tiles
    c. Smooth the edge
3. Fill in the exact pixel size for the energy of this mosaic.
4. Save as .bim file
5. Choose “multiple CPU”
6. Choose Run Stitching to apply these parameters and create the mosaic image

Note: Change parameters in (2) and rerun stitching to find the best result



7. Once the appropriate parameters have been chosen for the first projection image, the same parameters can be used for mosaics at the other projection angles for that same energy, by choosing Batch Stitching. A window will open up to choose all of the files for stitching, including the first one you did. Choose "Multiple CPU" for this.
    a. Batch stitching applies the image size for the first mosaic to all other angles. If the file is smaller, zeroes will be added to increase the file size to the checked value.
8. Repeat for the other energies, with the correct pixel size for those energies.
    a. Choose the exact same output image size for all angles. (Click check mark to change the size at 7a).
    b. All files created should be the same size.
9. Close the *Mosaic Sticher* window

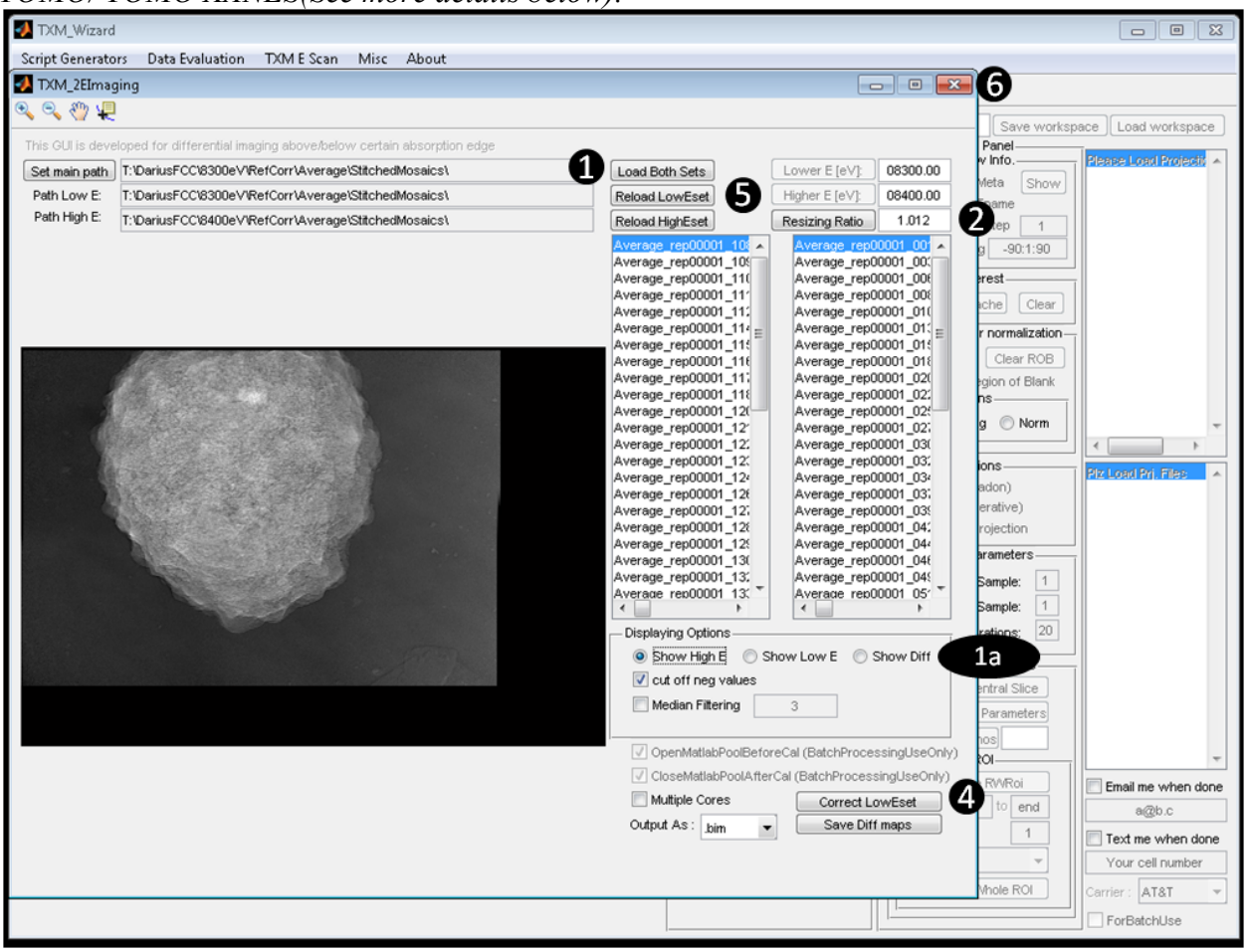
Files will be saved in "StitchedMosaics" folder

Note: if the Mosaic Stitcher GUI does not respond, then there isn't enough overlap between 2 single-shot images due to motor error. Thus, the PhCo alignment parameter cannot find the appropriate conditions for aligning the 2 images. To check whether this is the case, uncheck PhCo Alignment and run stitching again. If the GUI produces an image (the resulting image might have terrible alignment), then there is indeed an issue with the image overlap and PhCo parameter. To attempt to fix this issue, adjust the overlap by increasing the pixel size, while using the PhCo parameter.

V. a. Apply magnification correction (and align) to lower energy tomography sets:

During the experiment the focal length of the zone plate is determined by the x-ray energy. Thus the zone plate must move for proper focusing of the image, thus affecting the magnification of each image (M = q/p = [(ZP to CCD)/(sample to ZP)]). The image size must match for subtraction of difference maps. This GUI adjusts the magnification of the lower energy sets to match that of the higher energy set. This GUI also aligns the images of the lower energy sets to the image in the highest energy set.

The magnification correction will be applied using *2 E Imaging* – or batch analysis *XANES TOMO/ TOMO XANES(See more details below).*



1. Load both energy sets (When you click "Load Both Sets" select the lower E data first, then select the higher E data)
   Note: The resizing ratio is calculated for the energy sets loaded. Toggle from Show High E to Show Low E (1a). The image magnification and misalignment can be seen.
2. Keep all default values
3. Choose multiple cores
4. Select Correct LowEset (If you save difference maps and try to reconstruct them, the signal/noise ratio suffers. See Liu et al. SPIE paper 2013 for a comparison).

Files will be saved in "Corrected_[Lower energy]_eV" folder

5. In order to view the magnification correction and alignment, you must Reload Low Eset with the magnification corrected energy set (Corrected_[energy]_eV). Toggle from Show High E to Show Low E (pretty!). Make sure that the images are aligned properly, because you will perform feature alignment on one set of data (the highest energy data set; saved in workspace.txt) and apply the same alignment/workspace file to the other sets.
    a. If you want to check alignment, load the files from the lower energy data set, load the workspace.txt file created by clicking to align the higher energy file, and scroll through the images to be sure they are aligned properly.

   Note: Do not save the Diff maps. You will create your difference maps after reconstructing the mosaic (the difference map will get rid of features that you would like to align on).
6. Close the *2 E Imaging* window.

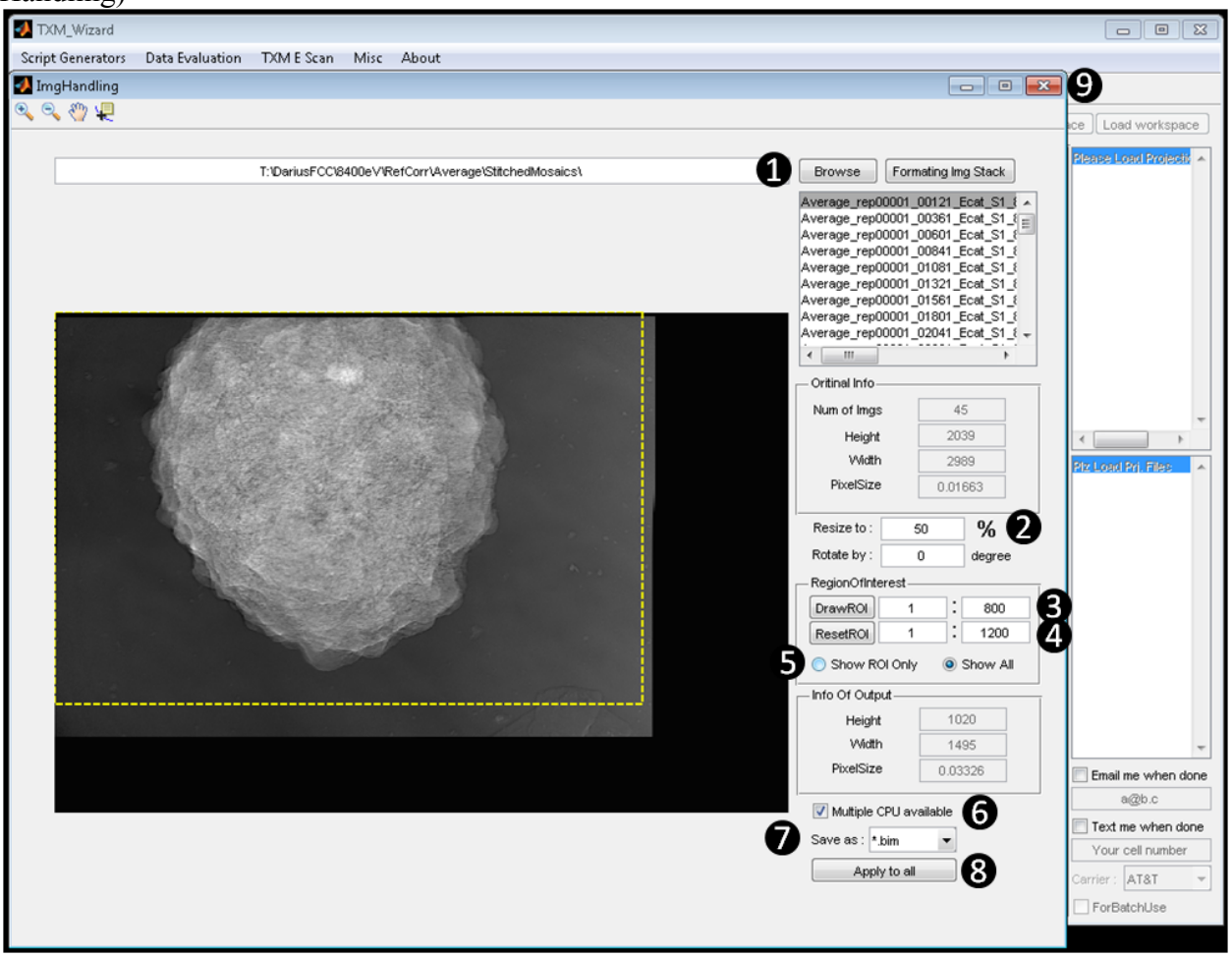
7. The higher and lower energy data sets will be reconstructed separately. (Magnification correction and reconstruction can be done for multiple energy data sets, including XANES tomography, using XANES TOMO/ TOMO XANES analysis. More details follow the *SinoReconGPU* section).

V. b. Crop/scale projection images:

Now that all of the images are the same size and aligned, you should crop and scale your data before reconstructing to avoid unnecessarily large sizes, which can lead to large reconstruction slices and longer reconstruction times.

Cropping can be done on the main TXM Wizard GUI, in the Region of Interest box. Click "ROI" and then make multiple clicks around the area you want to include in reconstruction. Save the workspace.txt file.

Or: cropping and scaling can also be done using *Image Handling* (Data Evaluation>Image Handling)

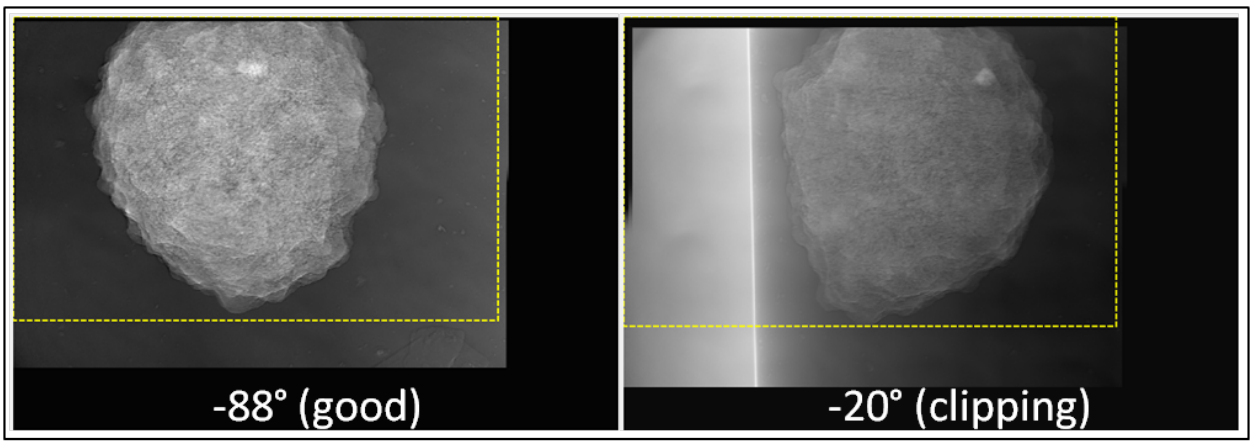


1. Browse and load the highest energy data set (this data set has the highest contrast)
2. Resize energy set if needed

   Note: reducing your file size will reduce your resolution. Similar to median filtering?
3. Adjust the vertical limits of the region of interest (ROI)

   Note: Adjust the vertical ROI such that the image remains in the ROI. Make sure that the ROI that is chosen encloses the image in all projection angles.
4. Adjust the horizontal limits of the region of interest (ROI)

   Note: Adjust the horizontal ROI such that the "filler" voxels (black voxels) are no longer in the ROI (these voxels add unnecessary size to the resulting reconstructed image).

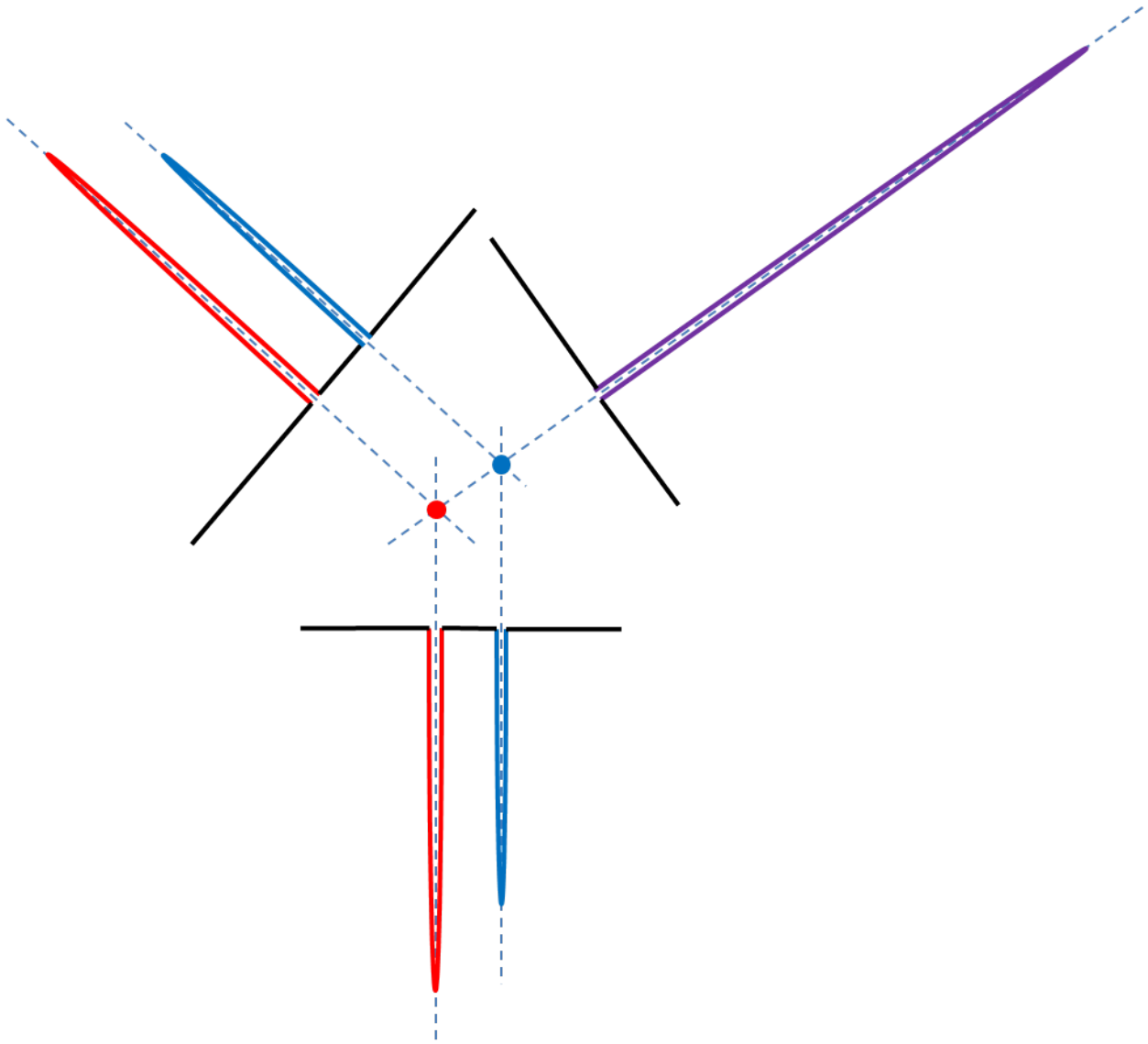


5. Choose “Show ROI Only”
6. Choose “Multiple CPUs available”
7. Save as .bim
8. Choose “Apply to all”
9. Close *ImgHandling* window

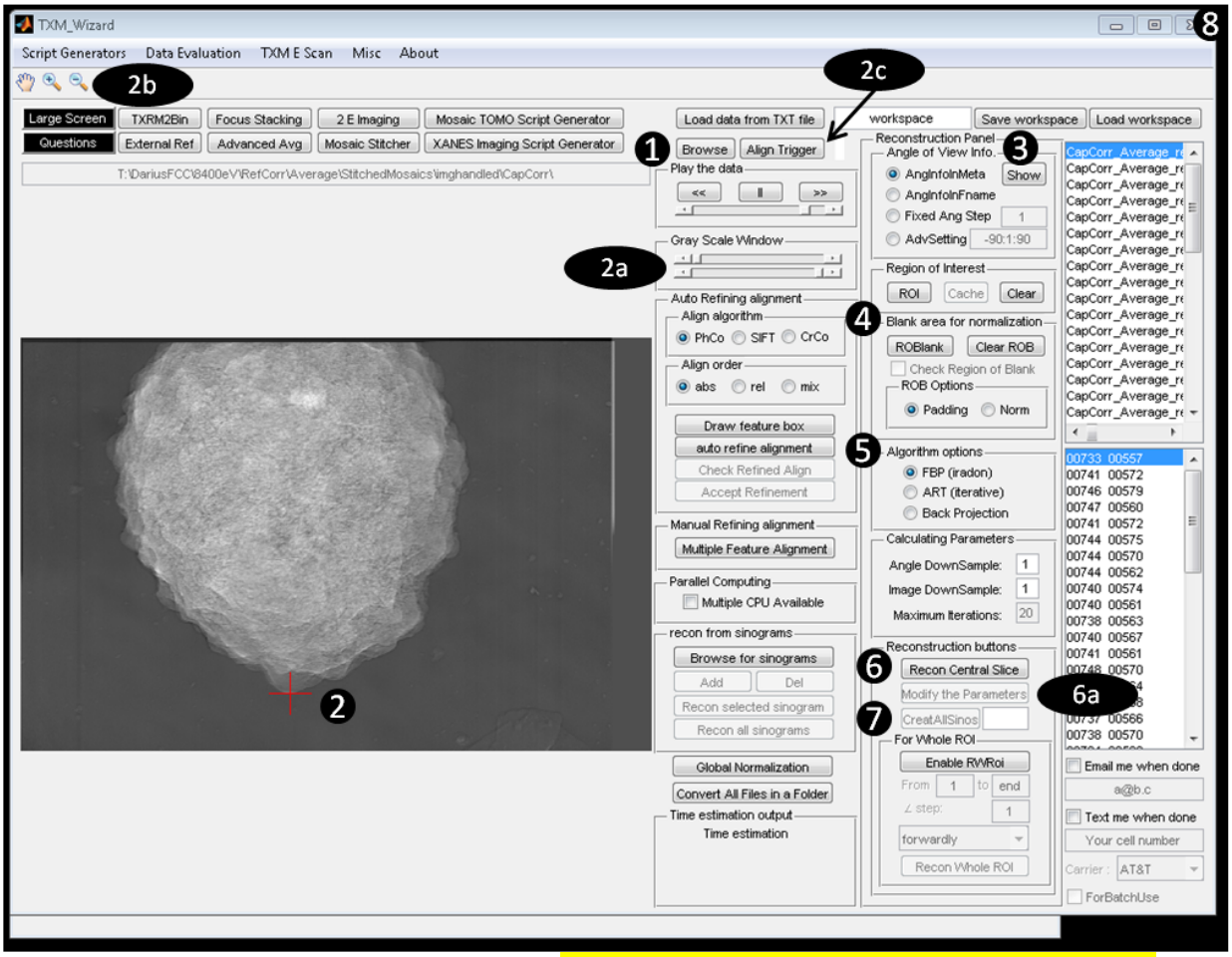
Files will be saved in “imghandled” folder

V. c. Align projection images / tomographic reconstruction:

To reconstruct a 3D tomography from your energy set, you must align a feature that each of the projection images has in common. By projecting the feature of each image into a 3D space, a common feature pixel will be formed in 3D space (see red dot below). Overlapping bright pixels in each of the projection images will form a bright pixel in 3D space (see blue dot below), while overlapping dark pixels in each of the projection image will form a dark pixel in 3D space (empty space below).All of the intensity (graphs below) and phase (projection angle) information for each slice of the 3D tomography is stored in a sinogram. The sinograms are created in this step.



Sinograms and the 3D reconstruction central slice are created on the *TXM-Wizard* main screen



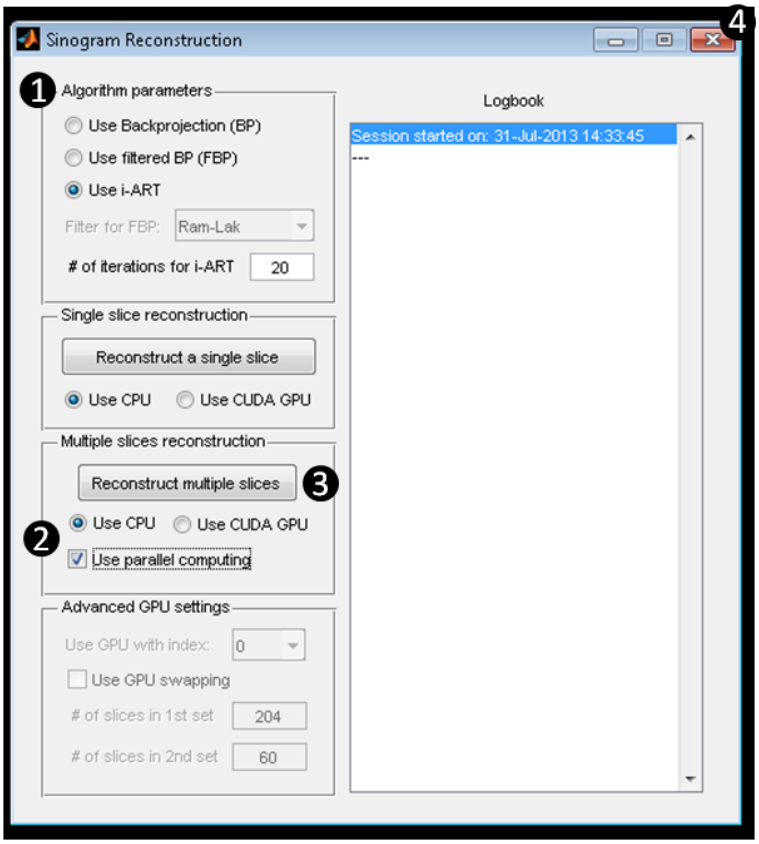
1. Browse and load projection images. If you have tomos at multiple energies, use the highest-energy data set for alignment.
2. **Alignment**: Use mouse pointer to click on the common feature in each of the projection images (a crosshair will form on the image and TXM-Wizard will add the coordinates to the image workspace and move to the next projection image)
   Note: Most of the time the common feature is at the top or bottom of the image. Sometimes it is a gold ball loaded onto the sample during collection. Do not choose a feature that you do not see in all of the projection angles. If you cannot clearly see a feature, try:
   a. Adjusting the contrast using the "Gray Scale Window"
   b. Zoom in/out on the projection images
   c. "Align the trigger" and "Play the data." This will allow you to see whether the feature is moving around your crosshairs
3. Once the feature has been aligned in all of the projection images, save the alignment "**Save Workspace**". You may want to rename the workspace.txt file to give it a unique name, to avoid overwriting it etc. For multiple energies, you will use the workspace file from the highest energy to align tomos from the lower energies ("Load Workspace").

4. Change the angle information in the Reconstruction Panel (#3 in figure). Choose one of the following:
    a. Fixed angle step (This is the angle step size). -90:1:89 is typical.
    b. AdvSetting (The syntax for using this feature is "[start1:step:end1, start2:step:end2]"). Use this feature if projection images are omitted from the reconstruction
5. If the background intensity in each of your projection images is different, use "Blank area for normalization"
    a. Choose ROBlank and draw a box or circle around an area of the background.
    b. Choose Norm
6. Choose an algorithm option (If iterative, choose the number of iterations)
    a. Compare iterative vs FBP to check central slice
7. Choose Recon Central Slice

    Note: Look at the reconstructed slice ([path]\ croped\sinos\slices\[FBP or ART]. Do you like it? Are the features sharp and well defined? If not, there are a number of things that you can do:
    a. Choose "Modify the Parameters"
    b. Start at step 2 and try to get better alignment of the common feature
    c. Try a different reconstruction algorithm (Iterative can be better than FBP in S/N is low)
8. Once you have a central slice that you like, choose "Create All Sinos"
    a. Use FBP for sinos. If necessary use iterative when reconstructing.
9. Reconstruct as below using SinoReconGPU, or if you have multiple energies use the *TOMO MOSAIC/ MOSAIC TOMO* analysis tool.

V. d. Reconstruct tomography using *SinoReconGPU* GUI:

Note: If you are not able to start the *SinoReconGPU* GUI, make sure that the most current MCR is installed on your computer (Program Files>MATLAB>MATLAB Compiler Runtime). If not, download the MCR (free). Also check MATLAB web site to determine GPUs that will work.



1. Choose Algorithm parameters
2. If you choose i-ART, select "Use parallel computing" and "Use CUDA GPU"
3. Choose Reconstruct multiple slices and load your sinogram files
4. Close *Sinogram Reconstruction* GUI

Files will be saved in "ARTgpu" folder

Note: If you are not able to utilize the CUDA GPU features:

1. Check to see if you have an NVIDIA graphics card (Control panel>Device Manager>Display adapters)
2. If you have an NVIDIA graphics card, then download the CUDA installer from https://developer.nvidia.com/cuda-downloads
3. Follow the instructions for installation and restart your computer.
4. Restart the *SinoReconGPU* GUI

VI. XANES TOMO/TOMO XANES

Found under **TXM EScan/ XANES TOMO/TOMO XANES processing** menu. This analysis tool is used for tomography at multiple energies, as for elemental mapping (+/- absorption edge; can do this with multiple edges/elements), and for 3D XANES (tomography at multiple energies for XANES chemical mapping of a single element).

1. First put averaged, reference-corrected images (or stitched mosaics) for all energies in the same directory.
    a. If you sort them by name, they should be in order of energy. If not (e.g. when scans are restarted, collected at different times, etc.), reorder them (see (b)).
    b. To Sort: Use Misc/SortFiles GUI. Browse for the files from all energies.
        i. Info source: Info in Frame
        ii. Sorting: By Energy. Choose "Sort the loaded Files"
        iii. After checking that order is ok, can rename them (will add leading number). Choose "Multiple Cores" and "Do the job".

2. Open TXM EScan/ XANES TOMO/TOMO XANES processing.
    a. Browse for averaged, reference-corrected images (or stitched mosaics).
    b. If you have not already cropped them, you can do that now using the Region of Interest box. Save the workspace file if you change this.

3. Click "BatchImgRegistration". This corrects magnification of the lower energy data sets to the one with highest energy. It creates separate folders for each, and a file "SubFolderTree.txt", to keep track of them.
    a. Note: you can do the clicking/alignment any time before the Batch Tomo Preparation. There is no need for a particular order before /after BatchImgRegistration.

4. Click "Batch Tomo Preparation". This creates sinograms for all energies, in the separate folders created above.
    a. You will load the workspace.txt file you created by clicking on the highest energy tomo data set. It is fine if you have renamed the file, just load the file you saved.

5. Click "Batch Tomo Recon GPU". This runs reconstruction on each of the tomos at each energy, if computer has appropriate GPU.

6. "Do All" button will do all of the above, just with loading of the workspace file.

7. If you have Tomo XANES data (tomos at multiple E's across a XNES edge, for 3D chemical mapping) "Batch QTXMXANES anal" will take the tomos for 3D XANES and create chemical mapping, by fitting to loaded model compounds.

VII. 3D Visualization of data (Avizo)

After reconstruction of the tomography data, before using Avizo: Use ImgHandling to reduce the number/size of slices loaded into Avizo, and to note the image size for Avizo.

- If the data set is large, remove blank slices above and below the particle. To do this, use Img Handling to convert slices to JPEGs. Look at the JPEGs and remove blank ones. You may leave some slices on the top/bottom.
- Keep all sets in multiple E data sets the same size.
- Crop as needed in ImgHandling. Treat all sets the same (optional, can also be done in Avizo).
- *Find out the image size*: In ImgHandling, open 1 file ("Browse")
    - Note the height and width (Note: these are reversed in Avizo)

Open Avizo. The main window will show the image(s) and the window on the right will show the Tree Structure with the data and displays. The window below that gives further detail on the displays.

Read in the Data:
(You will do the sequence below only the first time you read in a data set. Then you will "Save project" as "Pack and Go" and later access will be faster.)

- Open data
    - If you have multiple energies, read in the highest energy data set first (lower E data sets were corrected to this size/magnification/alignment)
    - Select the reconstructed slices with any edits you did to reduce size of the data set
- The following menus will pop up for reading in data:
    - File Format
        - Choose Raw Data and wait…
    - Out-of-Core data
        - Read complete volume into memory (works if lots of RAM, as on Colfax)
    - Raw Data Parameters
        - Dimensions (From ImgHandling *above*)
        - Data Type – 32 bit float
- If the header reads 8 bytes, the dimensions you entered are correct
        - Voxel Size
- Can leave all as 1 for now, and set the pixel size later when adding a scale bar.
        - Then wait… (red bar will pass several times. Bring something else to amuse yourself…)
        - Units Editor
        - Choose micrometer
- Save Project

Saves converted data
Give it a name
File type: "Pack and Go"

- Right click/object/rename object – give it a clearer name
- Save project

- Rescale data:
    - Right click on the green data set/convert/image type
    - Set to 16 bit-unsigned
    - Scale: 1E4 to 1E8, offset: 0
    - If you see “clip”, the scale is too large
    - Apply and wait….
    - Save project
    - Now you can remove the old data set
    - Save project again.

Note: If you save the project and a screen pops up saying you should save a certain dataset first, click “auto save”

Crop your data:

- First visualize your data: right click green window/volume rendering
- Volume Rendering – gives 3D image
- Click grey/orange box to show/hide the 3D data
- Color map: Set 1st higher or the 2nd value lower to get rid of noise, this will show your particle.
- If you have multiple energies: Crop your highest data-set first: Click on the green tree window of the highest data set. Then go the lower right menu and click on the cropping symbol
- To crop: select arrow tool in left upper corner of Avizo window, slide corners to make the box smaller.
- When done cropping, press OK
- To edit out data within the box: Right click on green window/Compute/Volume edit/cut inside. Left click and draw a circle around the part you want to edit out.
- When done cropping, drag the arrow to volume rendering to the new cropped data (x.modif)
- Save project
- Now you can remove the uncropped data set
- Save project again

For multiple energy data sets:

- Load tomo data sets from all energies (one at a time, separately).
- Treat the first data set (the highest) as described above, and then load the subsequent sets. Treat all E-sets the same: same pixel size, voxel size, any rescaling and cropping.
- Right click in the green tree window of the highest (cropped) data/Bounding Box
- Check “text” in properties window to see the coordinates for the corners
- The coordinates in the lower right are “min” values and the upper left are “max” values
- When cropping the other energies, these values can be filled in after clicking on the cropping symbol in the lower right corner.
- Do not change the coordinates of the bounding box for the other energies, you can cut inside the box though, (right click on green window/compute/volume edit/cut inside)
- Do this for all other energies

Difference data:

- Take the difference from the high E and the low E around an X-Ray absorption edge: Right click on the green tree window: Compute/quantification tools/point operation/arithmetic/subtract
- New green data box will appear
- Rename this output file: (right click/object/rename object) e.g. "Co dif" for difference data at Co K-edge.
- Keep the image type at 32-bit signed. (However, to compare high E/low E/difference data make sure all are converted the same with same scale correction e.g. 32-bit float/1e6)

To compare slices: Right click on green/Display/Slice. L click and drag to data windows, for high/low/difference data to view and compare

For 4E, 6E, data sets (>1 elemental mapping): Correlation evaluation can compare elemental distributions. This will be linear if elements are co-located. Make sure data are exact same dimensions.

3D visualization:

- First visualize your difference data: right click green window/volume rendering
- In volume rendering settings you can change the color maps of the data. Choose colors with good contrast.
- To determine the minimum and maximum values for color map, check the histogram:
    - Left click on green data, then go to the properties window bottom right and click histogram.
    - Click: "Show range distribution"
    - The histogram shows two distributions overlapping (noise and signal). Read the x-value of the minimum between the two peaks and choose this as the minimum value for your color map
    - Note: You may have to change this later a little if the noise level is too high, but this should be your starting point
    - The maximum value should be as large as the highest value of the signal histogram.
    - The alpha scale can be used to change the transparency
- Change background color to black (Create – Background)
- Put in a scale bar: Right click on green/Annotate/Scale bars. You can adjust position and length in the properties box
- Put in a demo maker (Create/Animation/Demo/Demomaker)
- In this demo maker you can make a sequence of different actions you like to take place during your movie.
- The movie starts at time "0" and ends at time "1"
- At GUI element: press "Update"
- There will now be a selection of possible actions you can add to your movie.
- At event list, select "New event"

- Now select from the GUI element list an event you like to take place, fill in the start and end value and the start and end time (e.g. from 0 to 0.3) and press “Add”
- You can continue adding, replacing and removing actions
- Under “Create”, you can find certain elements which are correlated with a number of actions. In order to add these options, add these elements to your main panel and then update the GUI element list. The new actions can now be found in the list.
- After the demo has been made, you can create a movie from it: right click on Demo maker/movie maker
- Select Demo maker
- Select MPEG or avi. movies
- Type in a filename (under what name you want to save the movie)
- Select the number of frames. A higher amount of frames makes the movie longer and thus slower
- Select the quality (0.8 is high, 0.5 is low). It is usually wise to make both a high and low quality movie, since some computers can’t handle the high quality movies
- Click apply
- Wait….bring something else to amuse yourself

VIII. XANES Imaging

Work Flow (see online manual for details)

1. **TXM EScan/Image Correct for XANES**. Browse for pre-processed data.
   This does magnification correction and alignment of image stack.
   Check Multiple CPU and Magnification Correction.
   Apply Image Stack Correction
   Check that alignment looks okay – play the data and make sure it stays steady.
   Check "crop images after alignment"
   Apply changes and save. Close window when calculation is done.

2. **TXM EScan/TXM XANES Wizard**
   Load/ Load Image Stack (data from Corr4XANES subfolder)
   Check "Autoscale" and change the colormap to "Jet" or something else

   Get Bulk XANES (from all pixels) to get points for normalization
   (initial data may be very noisy because it includes all pixels, but you will filter them out and it will improve)

   Normalize: On right panel of main window
   click pre-edge points, use cursor to choose first and last points on bulk xanes plot
   click post-edge points, use cursor to choose first and last points on bulk xanes plot
   Choose "test normalization with current plot".
   Red and green lines should be approx. parallel. Adjust pre/post points if not.
   Choose "Apply normalization to current plot".

   Filter:
   Click "Edge jump filter threshold". This compares counts in post and pre edges regions and will throw out those below the threshold. Test with 8, and look at the 8 maps. The best filtering will remove areas with 0 sample, or very thin areas.
   Pick the best threshold and set the number equal to this.
   Apply edge jump filter
   Save XANES maps
   Check "use edge jump filter" box that now appeared (top of main window)
   Get bulk XANES (will now average only filtered data).
   Plot should now be less noisy.
   Choose "Apply normalization to current plots"
   To apply an offset to multiple XANES:
   main Menu/Tools/Advanced XANES plotting
   Choose plots for offset (Ctrl/click)
   Normalize all pixels – second filter is applied (Usually 2-4 is sufficient)
   Save norm maps
   Check "Use combined filter" box on top of main window
   Get Bulk XANES, (both filters are now applied)
   Choose: Apply normalization to these

Get **single pixel XANES**

Click “Get single pixel XANES”

Use cursor to select points

Choose some in center, some near edge

Right click, use pixel selected

Choose “Apply normalization” to these

To apply an offset to multiple XANES: Tools/advanced XANES plotting

Choose plots for offset

Save/write powerpoint report

Save/save all open images

Save/save all open XANES

You may want to rename some of these, particularly if they are models and you will need them later for fitting

Save/Create powerpoint report

Add open figures to powerpoint report

Edit the powerpoint with actual sample name, date, user name etc.

To create chemical maps

Load XANES standards

These can e.g. be bulk XANES from a model compound

Make sure sample is not too thick (can cause self-absorption)

Can use data base standards but at least one model should be run with TXM XANES for calibration to these data

Linear combination fitting

Can also do principal components analysis etc. – see the TXM XANES Wizard Manuals