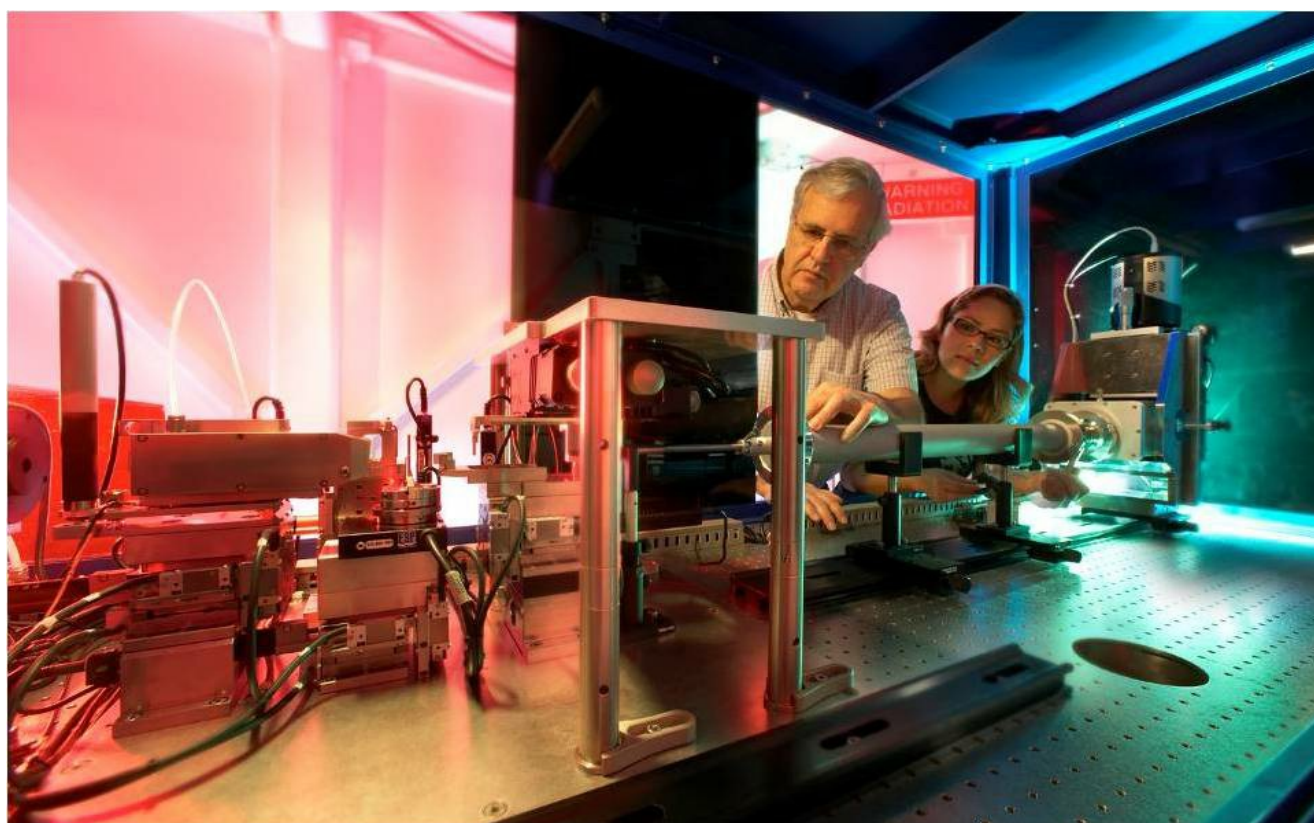



Transmission X-ray Microscopy (TXM, a.k.a. [UltraXRM](#) ) is an advanced and well-recognized technology/instrument developed by [Xradia Inc.](#) ([Pleasanton, CA](#) ). A TXM is typically capable of taking transmission X-ray images over a field of view (FOV) around  $30 \times 30 \text{ micron}^2$  with spatial resolution as good as 30-50 nm. Another important feature of TXM is the capability of non-invasive investigation of 3D inner structure by performing tomographic scan of the sample. Many different research area ranging from bio-medical study to material science have benefit from the availability of this technique at different facilities all over the world. An image of the TXM installed at [beamline 6-2](#) of SSRL is displayed below.



The combination of TXM system and the illumination from a synchrotron radiation source (e.g. [Stanford Synchrotron Radiation Lightsource](#) ) has led to a variety of demands to system control and data evaluation. Two main factors responsible for the variety of imaging techniques, (1) imaging speed and (2) energy tunability are made possible through the use of synchrotron radiation sources. The first enables e.g. 2D imaging of large areas by stitching together multiple fields of view, or the combination of time consuming measurements (such as averaged imaging and imaging at different energies) with tomography. Secondly, the ability to tune the energy of the incident photons with high energy resolution opens the vast field of applications of X-ray absorption spectroscopic ([XAS](#) ) imaging. XAS

Executable files of TXM-Wizard are compiled for Windows operating system ( [64 bit Windows](#) required for the latest releases of TXM-Wizard) and posted at the [project page for TXM-Wizard](#) existing at [SourceForge.net](#) . A screenshot of the project page is displayed below. As shown in the screen shot, new releases of TXM-Wizard are posted in the folders named by the date of the upload. In order to run the compiled executable file of TXM-Wizard, the users need to download and install the latest version of Matlab Compiler Runtime (the [MCRInstaller](#) file), which is also freely available on this project page. The installation of MCR needs to be done only once unless a newer version is posted and/or required. Once the installation of MCR is completed, the user can then download the latest executable file of TXM-Wizard and double click to start.

**sourceforge** Find Open Source Software Browse Blog Support Jobs Resources Yijin Liu Account Log Out











 **TXM-Wizard** by liuyijin

Summary **Files** Reviews Support Develop Tracker Mailing Lists Forums Code Project Admin

Looking for the latest version? [Download TXM-Wizard\\_Win64\\_20120524.exe \(5.4 MB\)](#)

Add File Add Folder

Home

Name	Modified	Size	
 <a href="#">20120524</a>	< 1 hour ago		 
 <a href="#">20120417</a>	2012-04-17		 
<a href="#">UpdatingLog.docx</a>	< 30 mins ago	21.0 kB	 
<a href="#">MCRInstaller_7.15_For64bitWindows.exe</a>	2011-05-12	205.4 MB	 
Totals: 4 Items		205.5 MB	

You should [add a README file](#), to provide release notes. It will be shown right here.

Status Terms Privacy Cookies/Opt Out Advertise About Private Cloud SourceForge.JP © 2012 Geeknet, Inc.

As mentioned in the [introduction](#) section, there are different types of demand for command scripts for TXM system control mainly due to two desires (1) much faster imaging speed and (2) the ability to tune the X-ray energy. As a result, three different script generators for generating script-command files that are suitable for different types of experiments are developed/designed in TXM-Wizard.

- [Mosaic Tomo Script Generator](#)
- [XANES Imaging Script Generator](#)
- [Multiple E Tomo Script Generator](#)
- [Monitor the Running Script Data Collection](#)

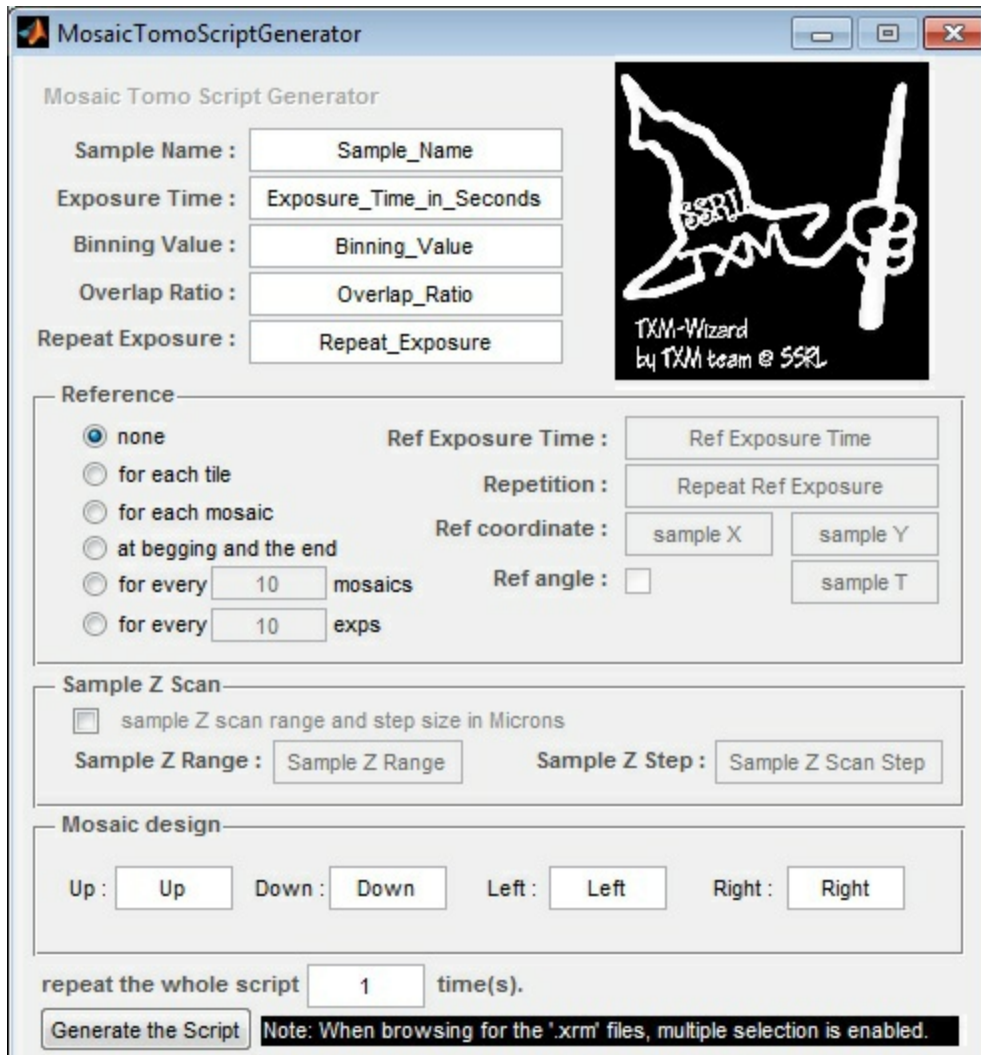
All the 3 script generators can be found under the "Script Generators" menu as shown in the following image. Quick links to "[Mosaic Tomo Script Generator](#)" and "[XANES Imaging Script Generator](#)" are also available in the main GUI.





One of the most common requirements for the TXM system is to perform 2D-mapping and/or tomography over an area that is larger than the available field of view. The Mosaic Script Generator allows the user to define the area of interest, and generates a script to perform raster scanning of the sample with appropriate motor movements. In order to overcome possible motor errors, a certain amount of overlap in the raster scan can be defined, enabling post-measurement fine alignment among single image tiles when stitching them together (see [Mosaic Image Stitcher](#)). Different strategies for reference image collection can be set up, in order to optimize data collection speed and quality of reference correction. The mosaic image collection over the same area can be easily repeated within the script (user-defined number of repetitions) e.g. to achieve better S/N ratio and/or for monitoring of time-dependent processes (e.g. *in-situ* monitoring of chemical reactions).

A screen shot of the "Mosaic Tomo Script Generator" is shown below.



Mosaic Tomo Script Generator

Sample Name :

Exposure Time :

Binning Value :

Overlap Ratio :

Repeat Exposure :

Reference

☒ none      Ref Exposure Time :

☐ for each tile      Repetition :

☐ for each mosaic

☐ at begging and the end      Ref coordinate :

☐ for every  mosaics      Ref angle :

☐ for every  exps

Sample Z Scan

☐ sample Z scan range and step size in Microns

Sample Z Range :       Sample Z Step :

Mosaic design

Up :       Down :       Left :       Right :

repeat the whole script  time(s).

Generate the Script      Note: When browsing for the '.xrm' files, multiple selection is enabled.

Another strong demand for synchrotron-based TXM systems is to collect a set of images across the X-ray absorption edge of a certain element. [X-ray Absorption Near Edge Structure](#) (XANES) analysis is widely used in transmission or fluorescence mode for studying bulk material, and in fluorescence mode with scanning micro probes for better spatial resolution. In the latter case, the final image is built from point-by-point information collected via raster scanning of the sample using a focused X-ray beam. However, when a full-field TXM system is used for XAS, the experiment and data analysis are quite different, mainly because spectra are extracted from the image stack collected, usually resulting in  $\sim 9E5$  generated spectra. The sample area covered by the field of view (FOV) of the TXM (tens of microns) is usually much larger than the area which can be covered by a scanning probe (within reasonable measuring times) when using a beam size offering a comparable spatial resolution (tens of nanometers; the resolution of the TXM).

For XANES imaging, the "XANES Imaging Script Generator" allows the user to define the range and steps of the desired energy scan, and calculates corresponding zone plate coordinates for each energy point based on the initial inputs. When executing the script the incident X-ray energy is changed and the zone plate is moved simultaneously in order to keep the sample in focus during the scan (see 2E Imaging Toolkit). We have also implemented an option to perform an energy scan at different angles of the sample stage, extending this method to XANES tomography. The collection of 3D XANES data can easily be set up within the Script Generator using two different modes: collecting an energy scan at each angle, or performing tomography at each energy. As shown in the screen shot below, all the options for different modes of data collection are on the bottom part of the "XANES Imaging Script Generator".

The users need to manually align/focus the zone plate at two end energy points (not necessarily the end points, but the further away from each other the better for interpolation purpose), and enter the energy value as well as corresponding zone plate stage coordinates. And then define the desired energy scan by editing the "Region Parameters". The users can also use the buttons "Save Parameters" and "Load Parameters" to save/load the user inputs so that it can be reusable for different experiments.

Images taken above and below characteristic X-ray absorption edges can be used to determine the distribution of the corresponding element within a sample (see *2E Imaging* ). Most usually, a fluorescence detector is firstly used to analyze the presence and relative concentration of different elements in the illuminated area (the field of view of the TXM system). And then, the user can choose the "elements of interest" and decide the energy points for performing (tomographic) TXM imaging. Depending on the chosen "elements of interest" the desired energy points can be very far away from each other leading to imprecision if interpolating/extrapolating for the zone plate coordinates.

In this case, the best thing to do is to manually align/focus the zone plate at all the desired energies. The "Multiple E Tomo Script Generator" (see the following screen shot) is designed for this purpose.

The screenshot shows a software window titled "MultipleETomo\_ScriptGenerator". The main title is "Script Generator for (Mosaic) Tomography at Multiple Energy Points". The interface includes several input fields and buttons:

- SampleName: [SampleName]
- ExposureTime: [ExposureTime]
- PixelBinning: [1]
- ScriptName: [ScriptName]
- RepeatExposure: [RepExposure]
- DummyMotor: [Yes]

Below these fields are four buttons: "Add E P", "Edit E P", "Remove E P", and "Sort E P". To the right of these buttons is a large empty list box. Below the list box is a section titled "Settings for Reference Collection" with radio buttons and input fields:

- ☒ No Ref
- ☐ For Each Tile
- ☐ For Each Mosaic
- ☐ Before & After
- ☐ Every 10 Mosaics
- ☐ Every 10 Exposures

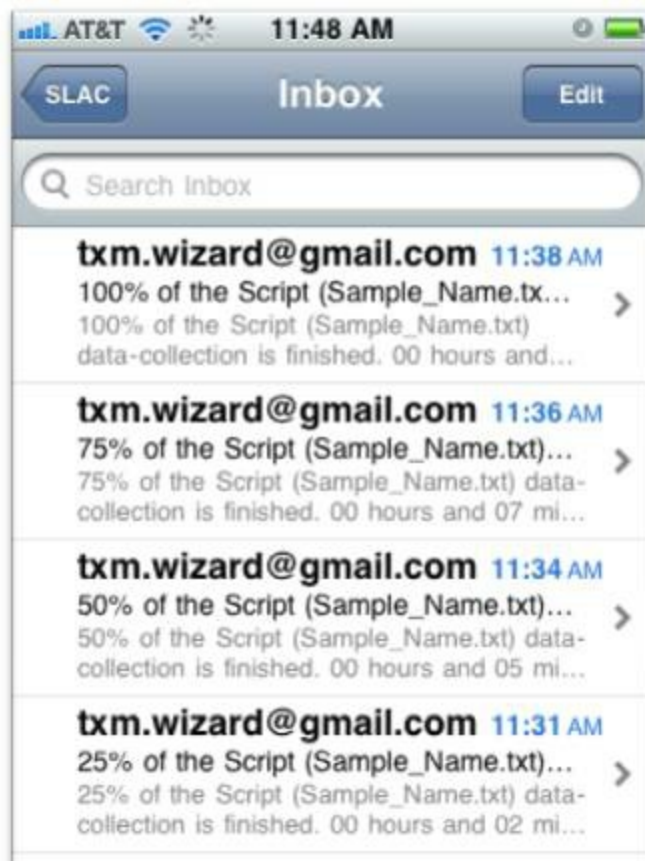
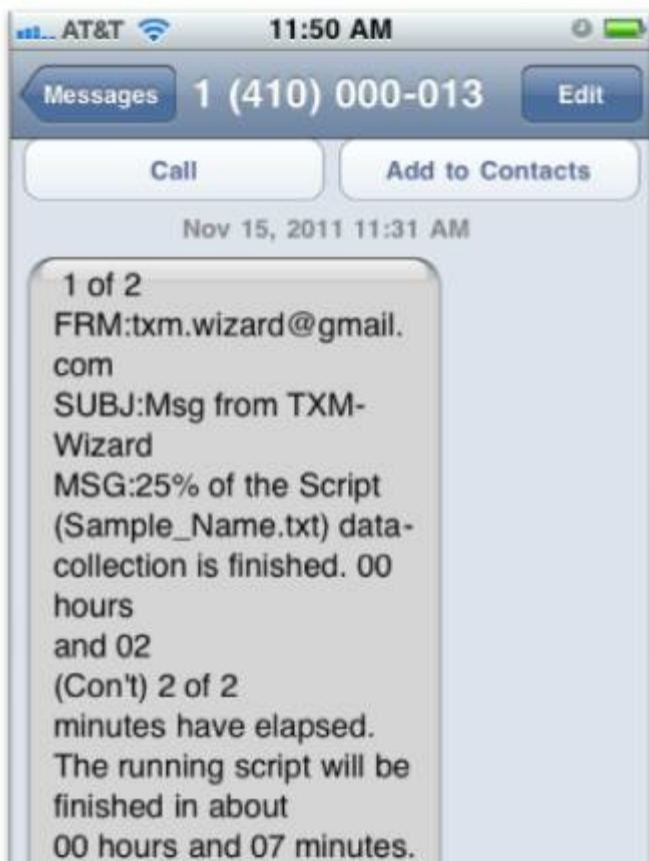
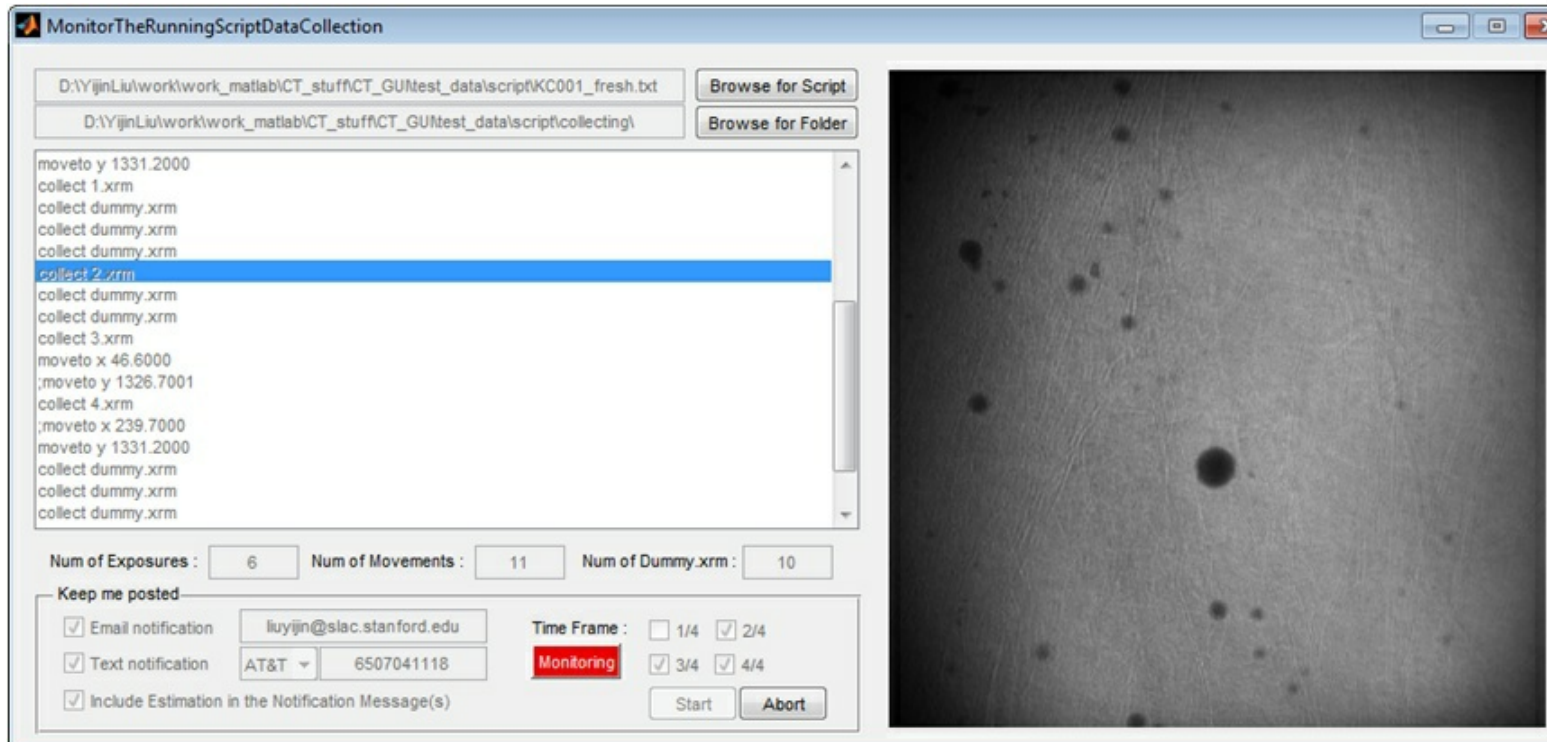
Reference collection settings include:

- RefExpTime: [ExposureTime]
- RepRefExp: [Rep Ref Exp]
- Ref Binning: [1]
- Ref X: [RefX]
- Ref Y: [RefY]

At the bottom right is a logo for "TXM-Wizard by TXM team @ SSRL" and a "Generate the Script" button.

Note (1): It typically takes about tens of minutes to manually fine focus the zone plate at different energies. However, it totally worth it since your script will run for several hours (even overnight) and you certainly want to optimize your script before start the collection.

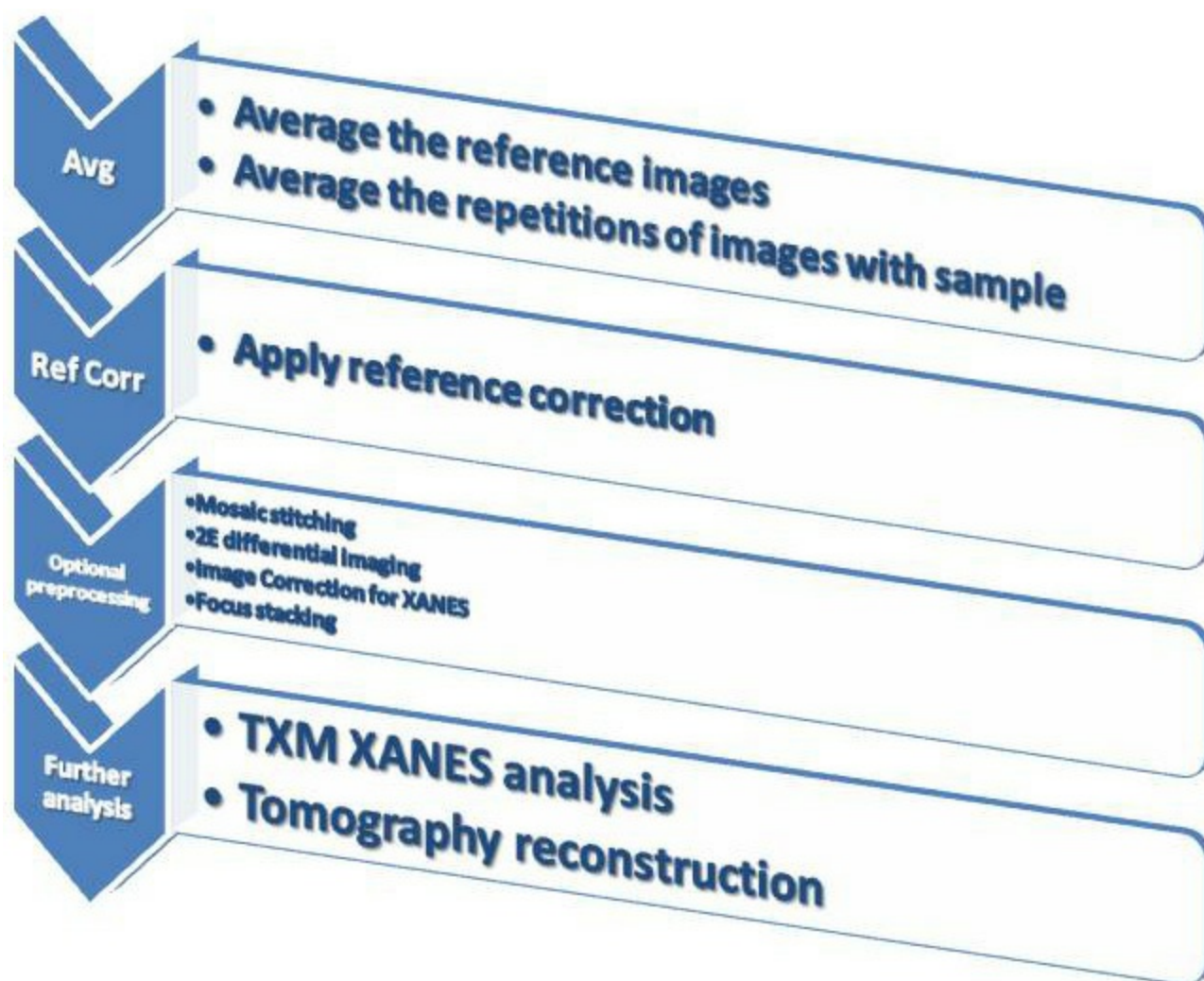
TXM Wizard allows you to monitor the running script through email and/or text message. It will keep an eye on the destination folder and will send message to your mobile device at requested time frame(s).





The work flow of data processing depends on the experimental design and also on the characteristic of the sample under investigation.

Usually, we design the procedure of data collection in such a way that (1) a certain amount of repetitions of images are taken with exposures on the sample and then (2) a few reference images are collected with the sample moved out of the field of view, after that, (3) necessary motor movements (motors on the sample stage, zone plate stage, and monochromator *et. al.* ) take place before repeating the step (1). A typical work flow of data processing for handling the data set collected in the procedure described above can be described by the flow chart shown below.



Several sub GUIs are designed for accomplishing the data processing steps shown in the flow chart including: "[Advanced Avg](#) ", "[External Ref](#) ", "[Mosaic Stitcher](#) ", "[2E Imaging](#) ", "[Image Corr 4 XANES](#) ", and *et al.* Links to these GUIs can be found in the menu bar of the main window.



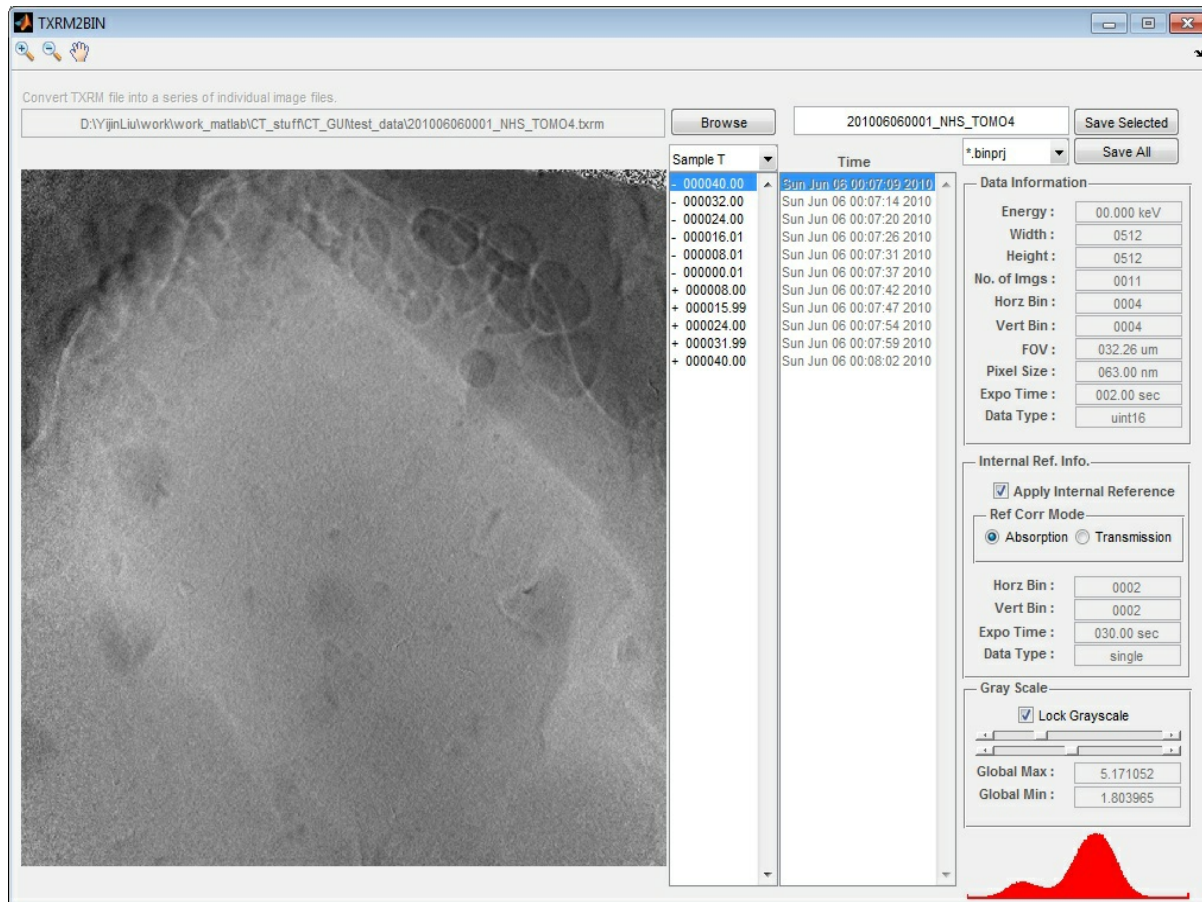
"TXM-Wizard" provides a series of tools for data pre-processing prior to [further analysis](#) ([tomography reconstruction](#) and/or [XANES imaging analysis](#) ).

These sub-GUIs are:

- 1) [TXRM2Bin](#) , the program for file format conversion;
- 2) [ExternalRef](#) , the program for reference correction;
- 3) [AdvancedAvg](#) , the program for average a image stack;
- 4) [MosaicStitcher](#) , the program for stitching image tiles together for a larger FOV;
- 5) [2EImaging](#) , the program for generating differential images that corresponds to the distribution of selected element.
- 6) [ImageCorr4XANES](#) , the program for magnification correction and image stack alignment for further analysis using [TXM-XANES-Wizard](#) .

".TXRM" is a standard Xradia file format which is usually an image stack taken with a certain motor scan (e.g. focus series, and/or tomography data). A reference image may be included in the file. A header containing the information about the motor positions and etc. is also included in the ".txrm" file.

A screen shot of of the "TXRM2BIN" GUI is shown below.

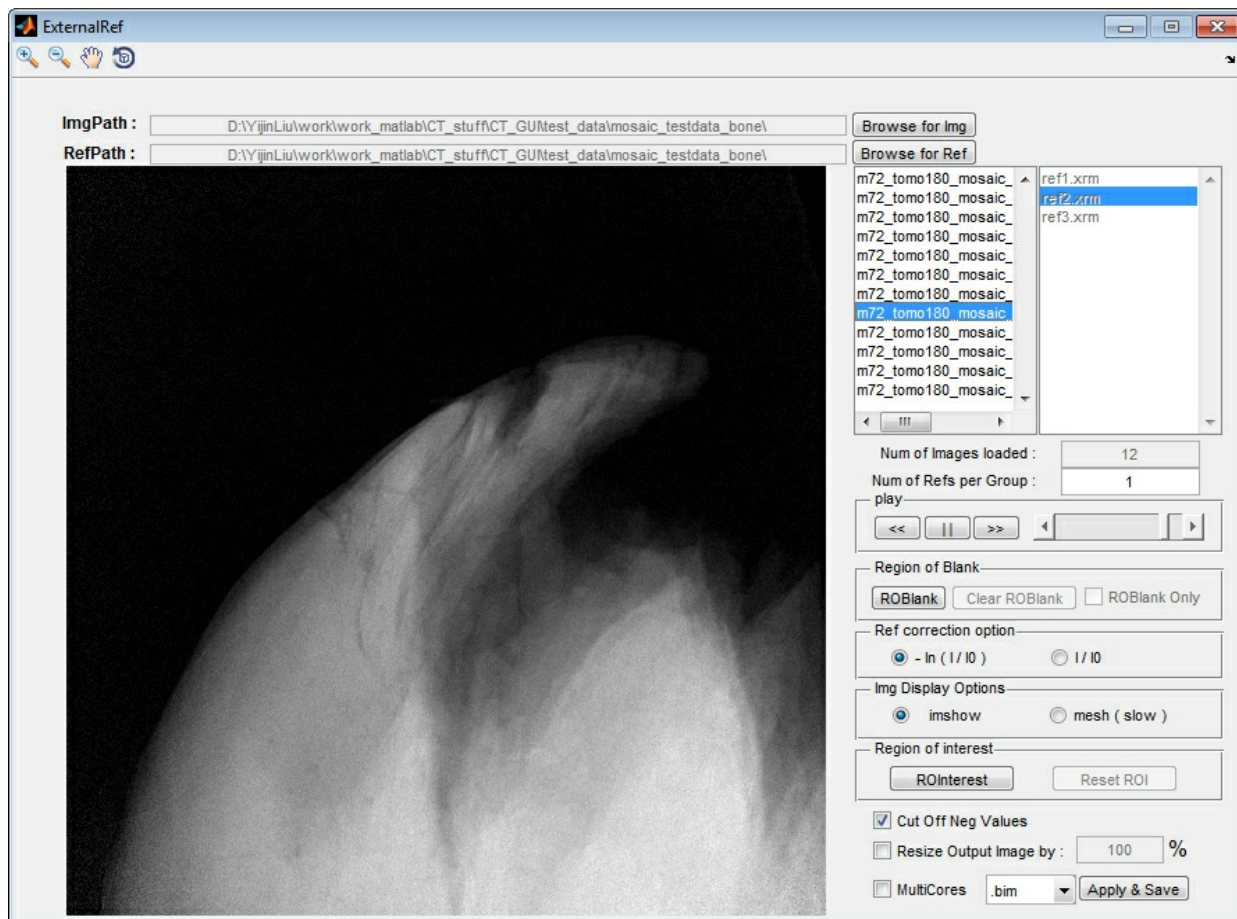


"TXRM2BIN" is designed for performing file formats (including standard image formats, such as "jpg/bmp/tif/png", [ASCII format](#) "\*.prj", and binary formats "\*.bim" / ".binprj") conversion so that the data can be exported for different data analysis softwares. The source matlab code for Read/Write functions is posted on our sourceforge web page.

Note (1): When the reference image is included in the ".txrm" file, the "TXRM2BIN" will not apply the reference correction, instead, it will save the reference image as a seperated file in the output folder. More sophisticated reference subtraction can be done using ["ExternalRef"](#).

When performing TXM imaging, reference images are collected as well as the images with samples in position. By applying [Beer-Lambert law](#) to the data, the projection of the distribution of [absorption coefficient](#) can be retrieved. This is what we do using the GUI "ExternalRef" (see the screen shot below).

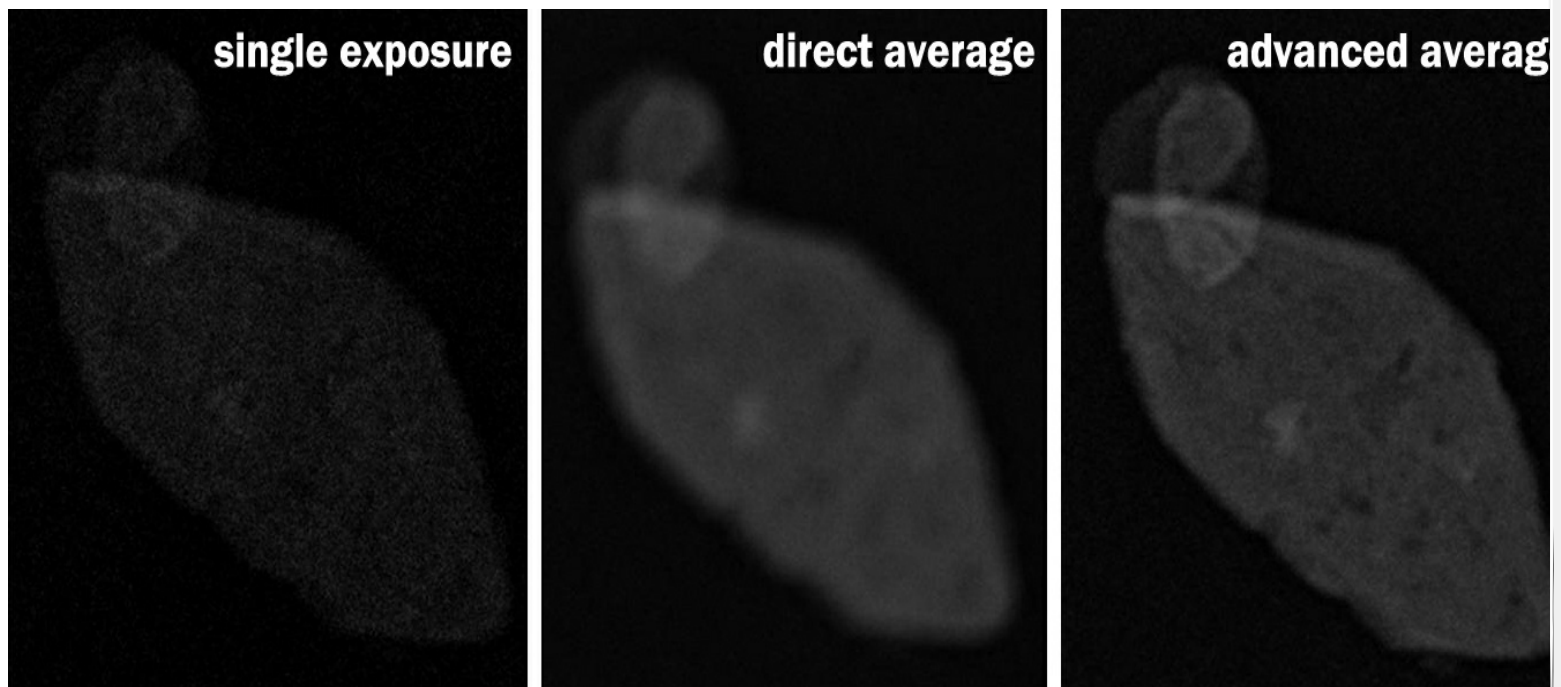
Due to the stability characteristic of the synchrotron light source and our imaging system, a reference image is usually good for 20-30 minutes. As a result, the number of reference images is usually different from the number of images with samples in a typical scan. The "ExternalRef" figures out which reference image to apply and supports different output file formats. In case of that multiple exposures were taken for each reference collection, the edit box "Num of Refs per Group" allows the user to separate the reference images in to groups, which will be averaged prior to reference correction.



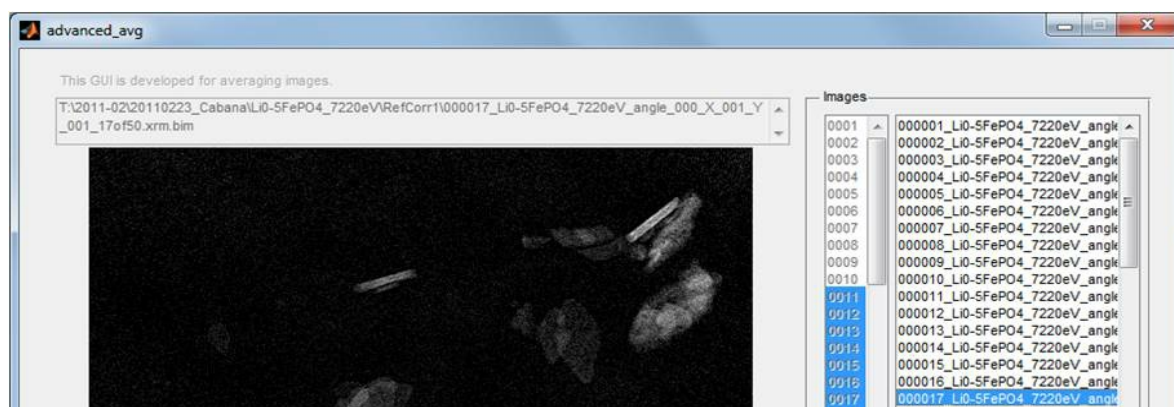
Note (1): the "Region of Blank" is a useful tool for performing normalization between images with sample and the references. The "Blank area" of the image with sample is suppose to be of the same intensity of the



When investigating low contrast samples (due to the size/shape or the material of the sample), multiple exposures over the same area is the most common way to improve the S/N ratio of a image. However, the task of averaging the image stack becomes complicated in reality due to the stability issue of the whole system (stability of the sample, motor accuracy, et. al.) Basically, the frames in the image stack to be averaged are of (random) shifts and even distortion. Direct averaging will result in a blurred out-put image (see the figure below).

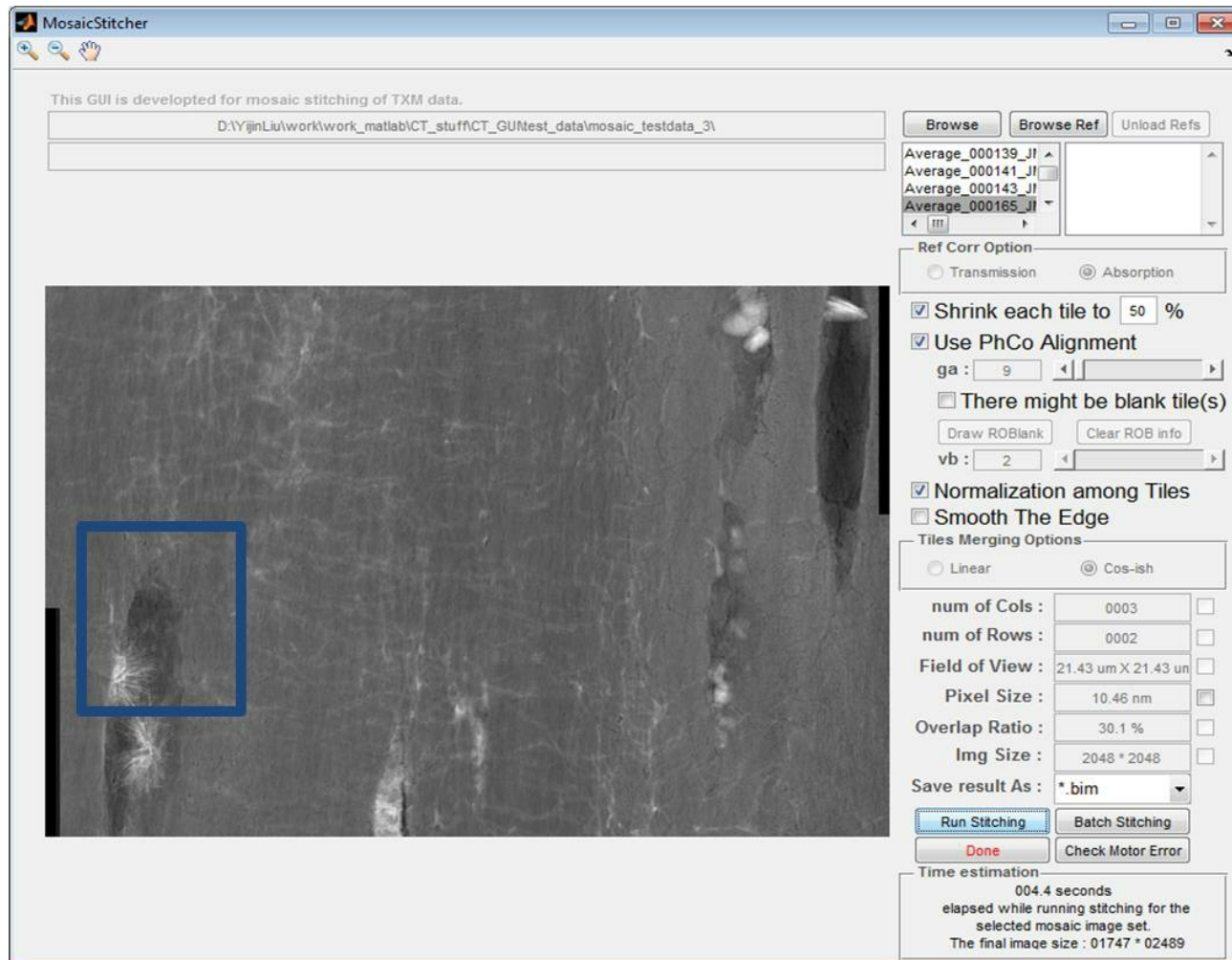


"AdvancedAvg" allows the user to load multiple images and define the "group size". In order to solve the problem mentioned above, [phase correlation](#) algorithm can be applied to align the image stack of each group prior to averaging, thus, to yield better our-put image for further analysis. A screen shot of "AdvancedAvg" shown below.



[Raster scans](#) with user-defined overlap area can be set up using the [Script Generators](#) described before. The "Mosaic Stitcher" was developed for merging overlapping image tiles in order to generate a full image with larger FOV. Motor errors are corrected with [Phase Correlation](#) auto alignment using the overlapping area between tiles. The edges of image tiles can be corrected using a weighted average over the overlapping area. This feature helps to correct an artifact which is related to the fact that the corners of the images have poorer S/N ratio than the center part of the images.

A screen shot of the "Mosaic Stitcher" is shown in the figure below in which a test data set is used to demonstrate the capability of this program.



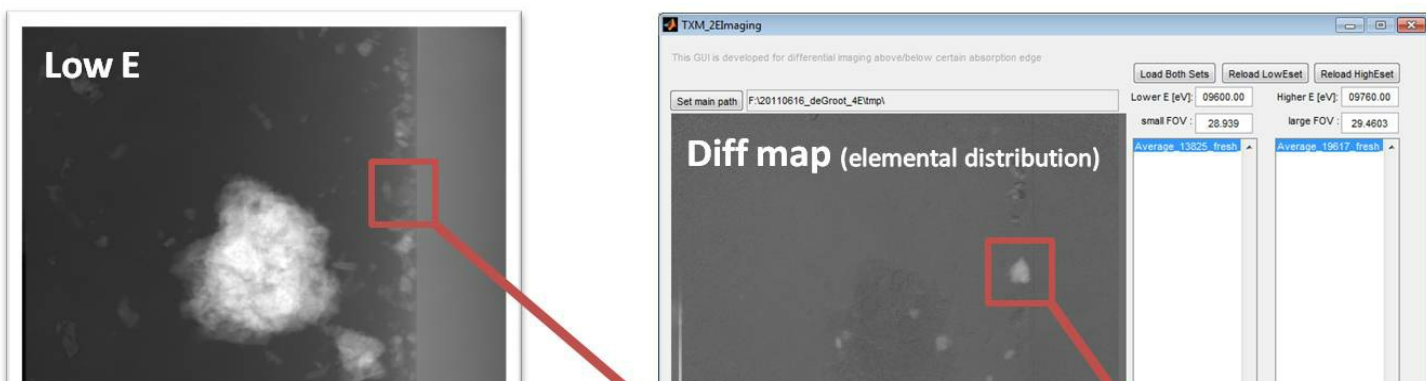
There are several options in "Mosaic Stitcher" including alignment options, normalization options, edge correction options *et. al.* The effect of these options is demonstrated in the following figure using the image from the highlighted area in the figure above.

Images taken above and below characteristic [X-ray absorption edges](#) can be used to determine the distribution of the corresponding element within a sample. A command-line script to acquire images at multiple energies for these experiments can be easily set up using the [Script Generators](#) described in the previous sections. Data evaluation for this experiment requires multiple pretreatment steps including magnification correction and auto image alignment. The first is necessary because the focal length of the objective [Fresnel zone plate](#) is a function of the incident X-ray energy (as given in the formula below), which leads to the necessity of zone plate movement along the beam axis for focusing the sample.

$$f = \frac{2 \cdot R \cdot \Delta_{outermost}}{h \cdot c / E_{incident}}$$

As a result, the magnification factor varies as a function of the illuminating X-ray energy. [Bicubic interpolation](#) is employed to correct the change in magnification in order to obtain consistent image size. Automatic image alignment is necessary, because both misalignment of the zone plate stage and motor movement errors will cause random relative shifts between images recorded at different energies. Therefore, a phase correlation algorithm ([Reddy & Chatterji, 1996](#)) has been implemented for auto image alignment, which can be used to align all magnification-corrected images of the energy stack before further processing. For "2Eimaging", the image taken at lower energy (below the absorption edge of a specific element of interest) is subtracted from the one recorded at higher energy (above the edge).

A screen shot of "2Eimaging" with a test data set loaded is shown below.

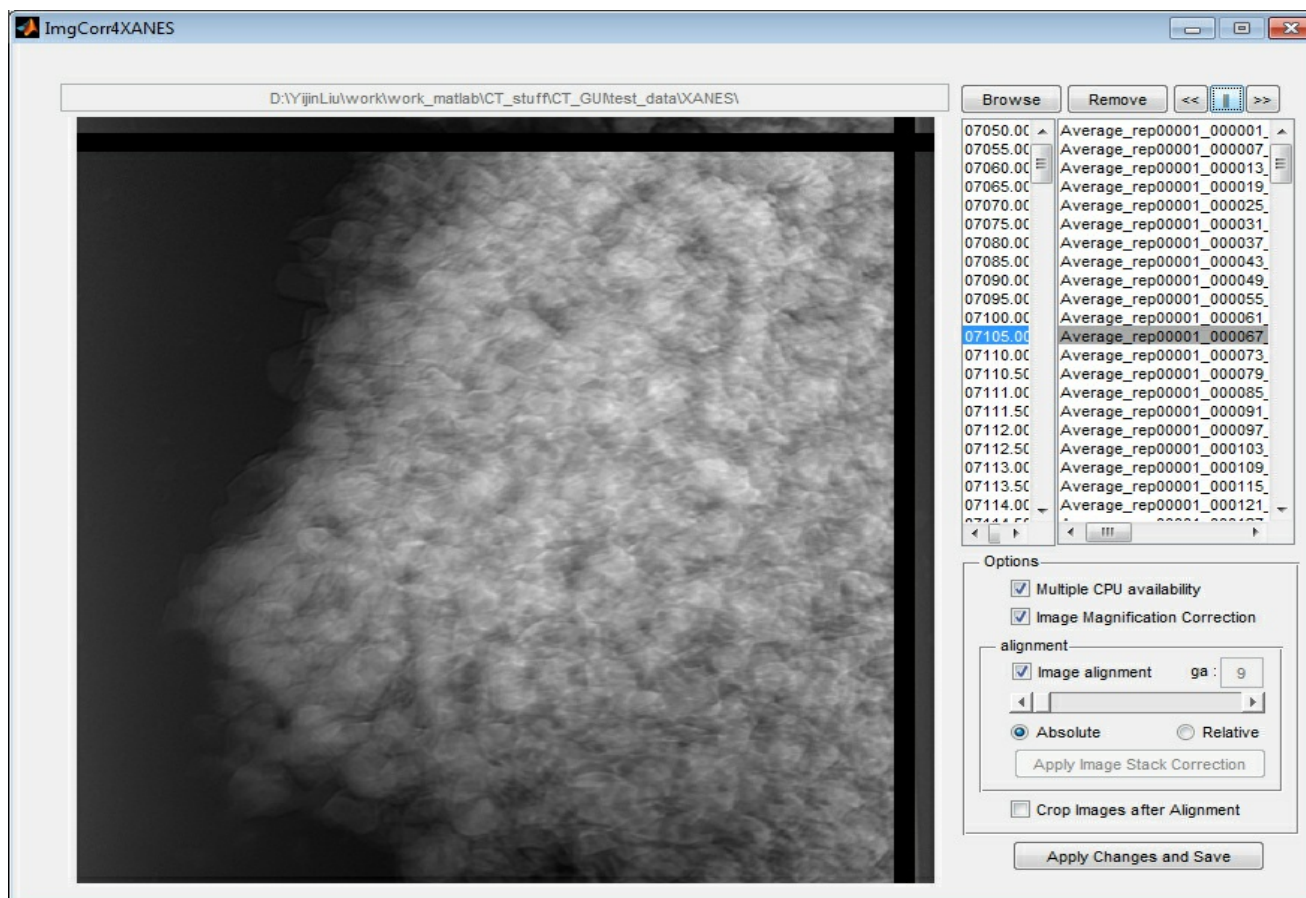




For XANES imaging, the "[XANES Imaging Script Generator](#)" allows the user to define the range and steps of the desired energy scan, and calculates corresponding zone plate coordinates for each energy point based on the initial inputs. When executing the script the incident X-ray energy is changed and the zone plate is moved simultaneously in order to keep the sample in focus during the scan. We have also implemented an option to perform an energy scan at different angles of the sample stage, extending this method to XANES tomography ([Meirer et al., 2011](#) ). The collection of 3D XANES data can easily be set up within the "[XANES Imaging Script Generator](#)" using two different modes: collecting an energy scan at each angle, or performing tomography at each energy.

In order to format/prepare the XANES imaging data set for the "[TXM XANES Wizard](#)", magnification correction and image alignment are necessary and critical steps to perform. The GUI of "ImgCorr4XANES" is designed to carry out these task in a efficient way.

A screen shot of "ImgCorr4XANES" is shown as following:



[Phase Correlation](#) algorithm is used for alignment of the image stack. This

As mentioned, the previously described GUIs are considered to be data pre-processing tools.

---

Copyright © 2010-2011 TXM Team @ SSRL

## Tomography is one of the most important features of the TXM system.

The reconstruction using the TXM Wizard is implemented in the main window.

Here is a typical work flow for your data pre processing:

1. Use " [TXRM2Bin](#) " to convert a ".txrm" file into ".bim" files (A ".txrm" file contains multiple images in one single file, we just export it into multiple files each contains only one image)
2. Use " [Advanced Avg](#) " to average the reference images (If you have multiple reference images available)
3. Use " [External Ref](#) " to apply reference correction
4. Use " [Advanced Avg](#) " to average the reference corrected images (we do it in this order to make use of the auto alignment feature. If the reference correction is not done prior to averaging, it doesn't make sense to use this feature, because you will be aligning to the scratches on the scintillator crystal).
5. Use " [Mosaic Stitcher](#) " to stitch mosaic tiles together (if applicable)
6. Use " [2EImaging](#) " to generate difference maps (if doing [elemental sensitive TXM imaging](#) )

Assuming that the above mentioned [data pre-processing](#) steps have already been completed, the projection images are now ready to be loaded into TXM-Wizard. Here are detailed steps for performing tomography reconstruction using TXM-Wizard.

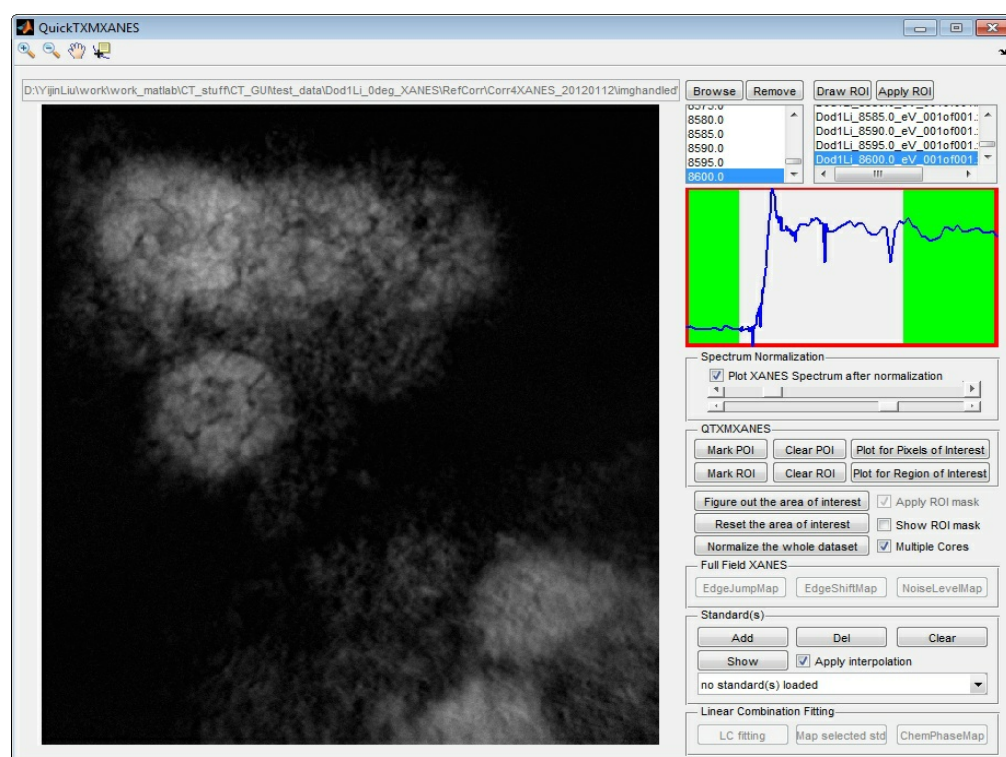
1. "Browse" for the projection images.
2. Find a feature on the image that is visible in all angles. Click on it for aligning the data manually.
  - The reason we have to do this is that we have a rotation stage on top of XYZ stage. When we mount the sample, the region of interest is usually not placed at the rotation center. And we have such a small FOV (30x30 micron<sup>2</sup>) that the sample comes in and out of the FOV as we rotate. In order to be able to see the sample in all angles, what we are doing is rotate + XZ translation to bring the sample back into the FOV and in focus at different angles of view.
3. Refine the alignment automatically by using the "Auto Refining



QuickTXMXANES is a GUI designed to provide fast analysis of the TXM [XANES](#) imaging data (a image stack collected with the scan of the X-ray energy).

It provides the options to view the spectra over selected points/regions of interest, and to perform linear combination fitting of the loaded standard spectrums in order to get the distribution of different chemical species.

The main window of QuickTXMXANES is shown in the following figure.



A typical work flow of using QuickTXMXANES is described as following:

1. Browse for the image stack which should have gone through several data pre-processing steps (averaging, reference correction, ImgCorr4XANES)
2. Use the data cursor (the fourth tool in the toolbar on the upper left corner) to figure out the "bad point(s)" in the averaged plot. So that it can be removed. (Optional)
3. "Draw ROI" and "Apply ROI". (Optional)
4. Adjust the normalization settings (specify the "pre-edge" and "post-edge" regions)
5. Display the spectrum of selected pixel(s)/region(s) of interest
6. "Figure out the area of interest". Basically this button allows users to

## TXM-XANES-Wizard

### 1. get bulk XANES

### 2. XANES normalization : use the buttons to be able to click on the bulk XANES plot

- test normalization with the current bulk XANES
- tweak your pre/post edge points if needed

### 3. XANES map filtering (edge jump filter threshold)

- click button to check filter strength
- apply the filter to save the filter map (loaded map show up on the bottom left)

### 4. Normalize all pixels

- this saves the normalization filter map (loaded map show up on the bottom left)

### 5. Now bulk XANES button only uses unfiltered pictures

- you can remove any energy images which are bad in filtered bulk

**\*\* Note:** changing the normalization parameters will unload your saved normalization map

### 6. Import standards (save loaded standards as a library) and do LC fitting

- does the R factor look bad for any regions? (large edge jump and high R factor) Maybe theres a missing component
- use single pixel XANES (with zooming function) to look at the XANES for this region

### 7. Generate RGB phasemap

### 8. Create a new map file



TXM-Wizard  
by TXM team @ SSRL

TXM-Wizard is developed by TXM team at Stanford Synchrotron Radiation Lightsource.

Please contact the SSRL TXM team ([txm.wizard@gmail.com](mailto:txm.wizard@gmail.com)) if any questions.

Please also see the following publications for reference:

1. [Liu, Y., Meirer, F., Wang, J., Requena, G., Williams, P., Nelson, J., Mehta, A., et al. \(2012\). 3D elemental sensitive imaging using transmission X-ray microscopy. Analytical and Bioanalytical Chemistry, 3-7. doi:10.1007/s00216-012-5242-2](#)