The TXM-XANES Wizard

The TXM-XANES Wizard is part of the TXM-Wizard software package developed for data analysis of data collected with the Xradia Transmisson X-ray Microscope (TXM) installed at beamline 6-2 at the Stanford Synchrotron Radiation Lightsource (SSRL).

Please make sure to check the section 'Known bugs and pitfalls' before evaluating your data!

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The GUI

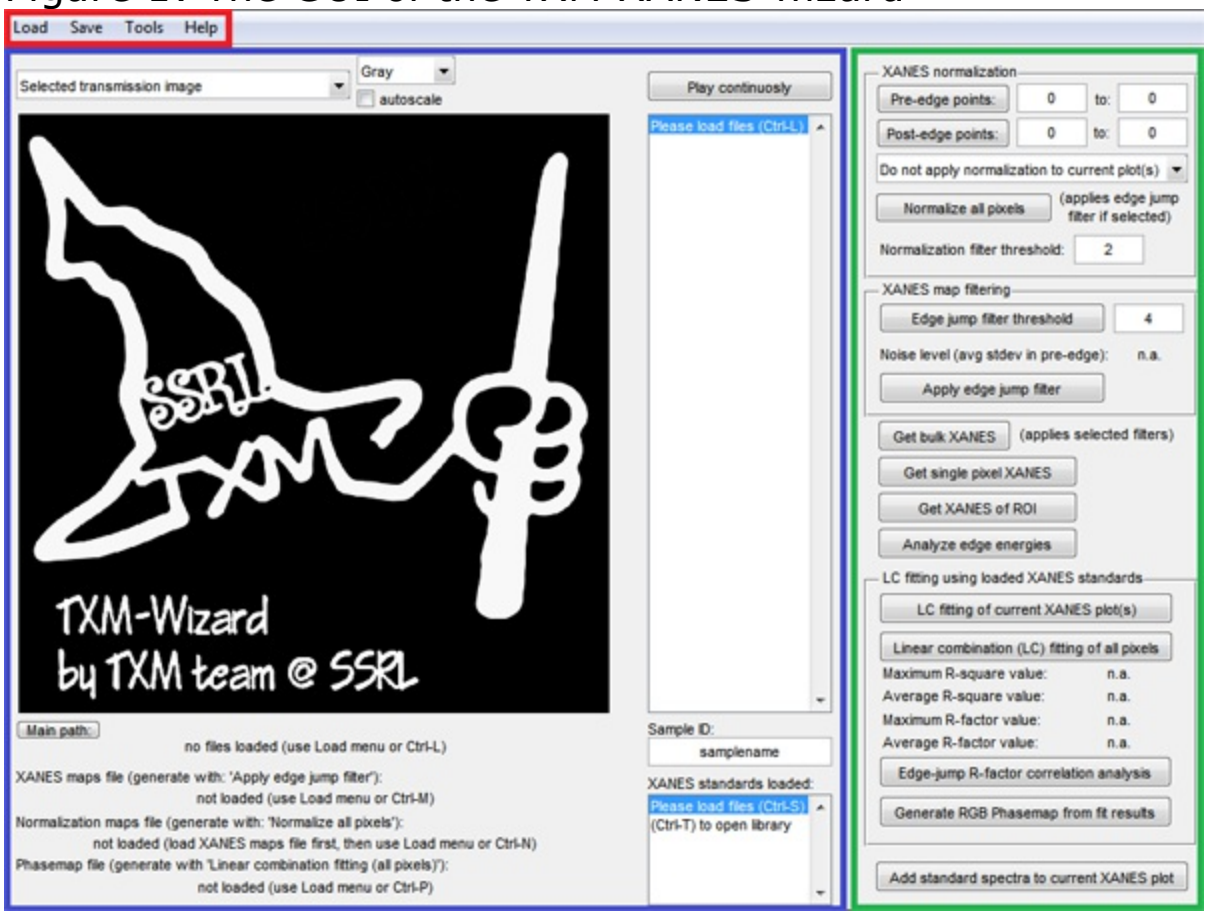
The Graphical User Interface (GUI) of the TXM-XANES wizard consists of three areas:

The Menu Bar (red)

The Information Area (blue)

The Processing Options (green)

Figure 1: The GUI of the TXM-XANES wizard



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The Menu Bar

The menu bar offers the following options:

Load Save Tools Help

The Load menu

- Load image stack (Ctrl-L)
  loads a dataset of TXM images recorded during an energy scan. The loaded files are displayed in the dataset information area of the GUI and the directory containing the files is set as main path in the workspace information area of the GUI.
- Load XANES maps (Ctrl-M)
  loads a .mat file containing parameters and data of Analysis step 1: The Edge-jump Filter previously saved in the main path directory.
- Load normalization maps (Ctrl-N)
  loads a .mat file containing parameters and data of Analysis step 2: Normalizing the Data previously saved in the main path directory.
- Load XANES standards (Ctrl-S)
  loads plaintext (ASCII) files containing normalized XANES spectra to be used as standards in Analysis step 4: Fitting the Data.
- Load XANES standard library (Ctrl-T)
  loads a previously saved .mat file containing multiple normalized XANES standard spectra to be used in Analysis step 4: Fitting the Data.
- Load LC-fit results file (Ctrl-P)
  loads a .mat file containing parameters and data of Analysis step 4: Fitting the Data previously saved in the main path directory.
- Load external cluster map(s)
  loads any image file(s) (.jpg, .tif, .png, .gif, or .bmp) which have to contain a binary image (pixel values either 0 or 1). The map(s) will be used to filter pixels of the currently loaded image stack (keep only pixels of value 1) and display the average XANES of non-filtered pixels for each cluster map.

The Save menu

- Save image(s)

opens a dialog showing all currently loaded images or maps. The selected image(s) are saved at the location specified by main path and using the text specified under Sample ID. Selected filters are applied.

- Save all open figures
  saves all currently open figures at the location specified by main path using the text specified under Sample ID.
- Save all open XANES
  saves all currently displayed XANES spectra either as individual plaintext (ASCII) files or in a single plaintext (ASCII) file at the location specified by main path using the text specified under Sample ID.
- Save XANES standard library
  saves all currently loaded normalized XANES standard spectra to a .mat XANES standard library file at the location specified by main path.
- Save each transmission image
  saves each currently loaded TXM transmission image in the sub-folder ..\transmission-images at the location specified by main path using the text specified under Sample ID and the selected color map. Selected filters are applied.
- Save RGB phasemap
  opens the same GUI as the button 'Generate RGB Phasemap from fit results' where the parameters necessary to create a RGB phasemap from the fit results can be entered. Then the RGB image can be saved either as .png or a binary file (.mat file) containing the image and metadata necessary for processing multiple RGB phasemaps (this feature will be implemented in future versions of the XANES wizard).
- Save movie of transmission images
  saves an .avi movie file sequentially playing all currently loaded TXM transmission images from lowest to highest energy applying the selected color map. Filename and location can be chosen. Selected filters are applied.
- Create Powerpoint report
  saves a .ppt file containing an analysis report at the location specified by main path using the text specified under Sample ID. The analysis report is generated on the basis of the data saved in the currently loaded parameter files (.mat files: XANES maps file, normalization maps file, LC-fit results file).
- Add open figures to Powerpoint report (Ctrl-A)
  adds all currently open figures to a previously created Powerpoint analysis report (.ppt file).

The Tools menu

- Crop image(s)
  opens a figure displaying the currently selected image the image display. A rectangular area can be adjusted and selected (double click in the rectangle) and all images will be cropped based on the selection. The cropped images will saved and loaded into the wizard. All parameters will be reset.
- Remove selected file
  removes the currently selected image from the list of loaded images (not in the folder on your disk). All parameters will be reset. This is useful if you want to remove a single bad image from the image stack.
- Advanced XANES plotting
  this will open an additional toolbox offering several options to display the currently displayed XANES plot. See: Advanced XANES Plotting.
- Process multiple evaluations
  opens another GUI designed to process results of multiple data evaluations performed with the TXM-XANES wizard. See: Process Multiple Evaluations.
- PCA and k-means clustering
  starts a PCA and subsequent k-means clustering of the dataset. See: PCA and K-means Clustering.

The Help menu

- Help topics
  opens this documentation.
- About
  displays information about the TXM-XANES wizard version in use.

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The Information Area

The information area of the TXM-XANES wizard GUI consists of three areas:

The Image Display (red)

The Dataset Information Area (blue)

The Workspace Information Area (green)

Figure 1: The information area of the GUI of the TXM-XANES wizard



The Image Display

Displayed Image and selected color map:

The image display shows the image or the map currently selected in the popup menu in the upper left corner using the color map selected in the popup menu on the right. If the entry 'Selected transmission image' is selected the transmission image selected in the dataset information area is displayed. The images and maps available for selection

depend on the currently loaded parameter files (.mat files: XANES maps file, normalization maps file, and LC-fit results file).

The autoscale checkbox: checking this box adjusts the color range of the currently loaded color map; i.e. scales the values of all pixels in the displayed image to 256 colors.

Selected filters: After loading or generating the respective parameter files (.mat files: XANES maps file, normalization maps file, and LC-fit results file), two checkboxes will appear in the upper right corner of the Image Display area. These

checkboxes allow selecting either one of the two filters available: the edge jump filter and the combined filter.

The Dataset Information Area

Loaded transmission images:
The dataset information area contains a list box showing the filenames of all loaded TXM transmission images. Files of .bin, .bim, or .xrm file format can be loaded using the load menu or the shortcut Ctrl-L.

Play continuously:
The button 'Play continuously' plays all currently loaded TXM transmission images sequentially from lower to

higher energies starting with the currently selected image. Pressing the button again stops.

Sample ID:
In the field 'Sample ID' a name identifying the dataset should be entered. The entered text is used as part of the filename when saving files using the save menu.

XANES standards loaded:
This list box lists the currently loaded XANES spectra which are used (or have been used) in Analysis step 4: Least squares linear combination fitting. Files can be loaded in the load menu either as single plaintext (ASCII) files containing normalized

XANES spectra or as a previously saved .mat file containing multiple normalized XANES standard spectra.

The Workspace Information Area

This area displays the
Main path of the working directory:
This path is automatically set when the TXM transmission images are loaded using the load menu or the shortcut Ctrl-L. All parameter files (.mat files: XANES maps file, normalization maps file, and LC-fit results file) have to be saved in this directory. All automatically saved files are saved in this directory or sub-directories.
Pressing the button 'Main path' opens the folder at the main path in Windows explorer.

Filenames of currently loaded parameter files:
(.mat files: XANES maps file, normalization maps file, and Phasemap file (i.e. LC-fit result file)).

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The Processing Options

The processing options of the TXM-XANES wizard GUI can be grouped into five parts:

Figure 1: The Processing Options - XANES normalization

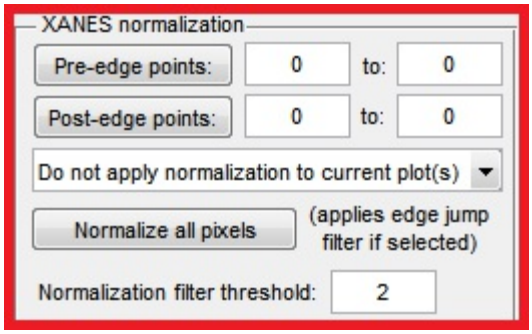


Figure 2: The Processing Options - Edge-jump filtering

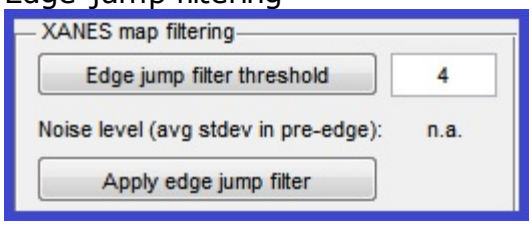


Figure 3: The Processing Options - XANES and edge energy analysis

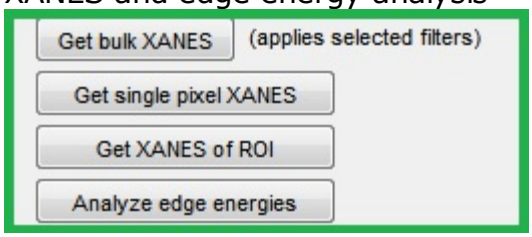


Figure 4: The Processing Options - Linear combination (LC) fitting

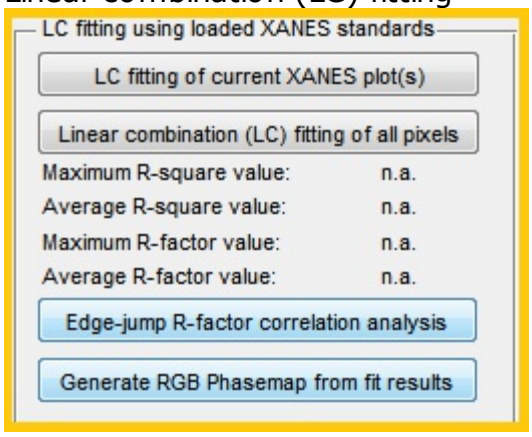
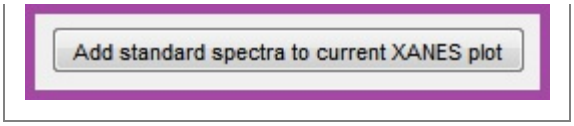


Figure 5: The Processing Options - Adding standard spectra to the current XANES plot

XANES normalization (red):
In this area the parameters necessary for automated XANES normalization and filtering are entered either directly or by pressing the buttons 'Pre-edge points' and 'Post-edge points'. The popup menu XANES normalization offers three ways of displaying the currently plotted XANES spectra. The button 'Normalize all pixels' applies the normalization to each pixel of the loaded dataset. The normalization filter threshold determines the strength of the normalization filtering.

Edge jump filtering (blue):
Here, the edge jump filter threshold, which determines the strength of the edge-jump filtering can be set. Pressing the button 'Edge jump filter threshold' displays all filters using a threshold from 1 to the value entered. The Noise level value shows the average standard deviation of the XANES pre-edge region for the entered edge jump filter threshold. Pressing the button 'Apply edge jump filter' applies the edge jump filter to the whole dataset.

XANES and edge energy analysis (green):
Pressing the button 'Get bulk XANES' calculates the average XANES of all (filtered) pixels. Pressing the button 'Get single pixel XANES' opens a GUI which allows selecting pixels of interest and displays their XANES spectra. Pressing the button 'Get XANES' of ROI allows selecting a Region Of Interest (ROI) in the image



display and calculates the average XANES of the selected and the non-selected pixels. The button 'Analyze edge energies' starts a GUI for analyzing the edge energy distribution of the dataset (i.e. the edge energies calculated for each pixel).

Linear combination (LC) fitting (yellow):
The button 'Linear combination (LC) fitting of all pixels' starts the least squares linear combination fit of all loaded XANES standards to each (non-filtered) single pixel XANES of the dataset. Maximum and average R-square and R-factors calculated from the fit results are displayed after fitting. The button 'Edge-jump R-factor correlation analysis' starts a GUI which allows analyzing the correlation plot of R-factor vs. Edge-jump values. The button 'Generate RGB Phasemap from fit results' opens a GUI where the parameters necessary to create a RGB phasemap from the fit results can be entered.

Add standard spectra to current XANES plot (purple):
Pressing this button adds a plot of all currently loaded XANES standards to the currently open XANES plot.

XANES normalization

Normalization parameters:
The range of energy points defining the pre- and post edge region of the XANES spectra can be entered either manually or by pressing the respective button left from the text fields (for more details see: Analysis step 1: The Edge-jump Filter).

The buttons 'Pre-edge points' and 'Post-edge points':
If a figure displaying a XANES is present, the two regions can be set by clicking on the desired energy points (for more details see: Analysis step 1: The Edge-jump Filter).

The popup menu 'XANES normalization':
The three options of the popup menu allow to display the currently plotted XANES spectra
a) without normalization
b) without normalization but showing pre- and post-edge lines used for normalization
c) the normalized spectra
The selected option is applied to all currently displayed XANES spectra.

Normalize all pixels:
The button 'Normalize all pixels' applies the normalization (using the range of energy points selected) to each pixel in the image (any selected filters are applied) and saves parameters and resulting maps to the normalization maps parameter file (.mat) (for more details see: Analysis step 2: Normalizing the Data).

Normalization filter threshold:
The normalization filter threshold determines the strength of the normalization filtering (for more details see: Analysis step 2: Normalizing the Data).

Edge-Jump filtering

Edge jump filtering uses the standard deviation in the pre-edge region of each single pixel XANES to filter pixels with edge jumps too small for further analysis. Pixels showing an edge jumps lower than their pre-edge standard deviation multiplied by the edge jump filter threshold are filtered (for more details see: Analysis step 1: The Edge Jump Filter).

The button 'Edge jump filter threshold':
After pressing this button the edge jump filter for each integer edge jump filter threshold value ranging from 1 to the value entered is calculated and displayed to show the effects of different threshold values (for more details see: Analysis step 1: The Edge Jump Filter).

The Noise level value:
This value is the average standard deviation of the XANES pre-edge region of all pixels, calculated using the entered edge jump filter threshold.

Apply edge jump filter:
The button 'Apply edge jump filter' applies the edge jump filter (calculated using the value entered for the edge jump filter threshold) to the whole dataset and saves parameters and resulting maps to the XANES maps

parameter file (.mat) (for more details see: Analysis step 1: The Edge Jump Filter).

XANES and edge energy analysis

The options in this area are related to data analysis without previous LC fitting. Data can be analyzed with or without filtering, and, in the case of 'Bulk XANES' and 'Single pixel XANES' analysis even without normalizing all pixels. The option 'Analyze edge energies' needs the results from the 'Normalize all pixels' procedure where the edge-energies of all single pixel XANES spectra are calculated. (for more details see: Analysis step 3: Exploring the Data)

The button 'Get bulk XANES':
After pressing this button the average XANES of all pixels in the dataset is determined and displayed. Selected filters are applied, i.e. pixels which are filtered are not considered in the average. The average XANES shows the mean absorbance of all pixels at each energy of the energy scan.

The button 'Get single pixel XANES':
After pressing this button the GUI:'Get single pixel XANES' opens, which allows selecting a user-define number of pixels. For all of these pixels the corresponding XANES are determined and displayed in the same figure. Furthermore, for each selected pixel the surrounding area of 100x100 pixels is displayed in (a) separate figure(s).

The button 'Get XANES of ROI':
After pressing the button draw a region around the pixels you want to group (press and hold the left mouse button) in the image display. The region will be closed automatically once you release the left mouse button. You can then move the whole region if necessary. Double click in the region to accept the selection. Then 3 figures will be generated:
1: The (binary) cluster masks based on your selection
2: The segmented image (based on your selection)
3: The average XANES of each cluster (i.e. the average XANES of all pixels in the cluster)

The button 'Analyze edge energies':
Pressing this button starts the GUI: 'Analyze edge energies'. This GUI shows the distribution of the edge energies (i.e. the edge position of the XANES of each pixel on the energy axis) as a histogram of edge energy vs. number of pixels. The user can cluster different edge energies and the pooled pixels corresponding to each cluster are displayed.

Linear combination (LC) fitting

Linear combination (LC) fitting of the data is done by performing a least squares linear combination fit of all loaded standard XANES spectra to each single pixel XANES of the dataset. This results in a percentage value for each used standard between 0% and 100% (i.e. a value between 0 and 1). The fit is forced to sum to 1. For more details about LC fitting see: Analysis step 4: Fitting the Data

The button 'Linear combination (LC) fitting of all pixels':
Applies the combined filter (see: Analysis step 2: Normalizing the Data) and starts the least squares linear combination fit of all loaded standard XANES spectra to each single pixel XANES of the dataset. Then the parameters and resulting maps are saved to the LC fit results parameter file (.mat) (for more details see: Analysis step 4: Fitting the Data).

The button 'Edge-jump R-factor correlation analysis':
Pressing this button starts the GUI: 'Edge-jump R-factor correlation analysis' which allows analyzing the correlation plot of R-factor vs. Edge-jump values. Usually, edge-jump and R-factor should be anti-correlated i.e. high edge jump means low R-factor and vice versa). This plot is useful to identify pixels for which this is not the case indicating that the set of standards used for LC fitting is not sufficient to model the whole dataset (e.g. if a standard is missing) (for more details see: the GUI: 'Edge-jump R-factor correlation analysis' and Analysis step 4: Fitting the Data).

The button 'Generate RGB Phasemap from fit results':
Opens the GUI: 'Generate RGB Phasemap from fit results' which is used to assign the three colors red (R), green (G) and blue (B) to the standards used for LC fitting. If only two standards were used one color is skipped or used for 50:50 mixtures; if more than three standards were used two standards are represented by two colors while the third color is assigned to the sum of all other standards. In this way the final phasemap is always an RGB image where the three colors of each pixel are values between 0 and 1 which sum to 1 and therefore represent the LC fit result (for more details see: the GUI: 'Generate RGB Phasemap from fit results').

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The GUI: 'Get single pixel XANES'

This GUI which allows selecting pixels of interest and displays their XANES spectra.

the tutorial 'XANES normalization': Link 1 (browser) Link 2 (help file)
NOTE: If you are using a browser use link 1, if you are running the help file open link 2 and make sure the tutorial is saved in the subfolder ../movies of the folder where you run the help file.

After pressing the button 'Get single pixel XANES' in the processing options, a window opens showing the image currently displayed in the image display.
Now, one of four actions is possible in

Selection mode

A. **Closing the figure** (press X in the figure panel): the figure is closed and no further action is performed.
B. **Left mouse click** on the image: The clicked pixel is selected and marked by a small black cross-hair
C. **Middle/wheel mouse click** on the image: The zoom mode is turned on
D. **Right mouse click:** The options menu is displayed
   a. 'Use pixels selected': Ends selection mode and creates the output.
   b. 'Remove last selected pixel': removes the last selected pixel(and the corresponding cross-hair in the map)
   c. 'Reset pixel selection': removes all selected pixels (and the corresponding cross-hairs in the map)
   d. 'Turn on zoom mode': ends the selection mode and turns on the zoom mode

and one of six actions is possible in

The zoom mode

To enable accurate selection of a pixel of interest in the displayed image the zoom mode can be turned on by a middel/wheel mouse click or by selecting 'Turn on zoom mode' in the options menu. When the zoom mode is on the pointer symbol changes to a magnifying glass and the following actions are possible:

A. **Closing the figure** (press X in the figure panel): the figure is closed and no further action is performed.

B. **Left click:** zooms in by a factor of 2; the position of the mouse cursor is the center of the plot

C. **Upward rotation of the mouse scroll wheel:** zooms in by a factor of 2

D. **Downward rotation of the mouse scroll wheel:** zooms out by a factor of 2

E. **Selecting a zoom area:** draw a rectangle to select the area to zoom in

F. **Right click:** opens the options menu:

   a. 'End zoom mode': ends the zoom mode and returns to selection mode

   b. 'Reset to original view': resets any zoom

   c. 'Zoom out': zooms out by a factor of 2

The output

After pressing 'Use pixels selected' during selection mode a series of figures is created. First a figure is created for every four pixels of the list of selected pixels showing the image used for selection and one to four subplots displaying a 100x100 pixels area surrounding the selected pixel. Second, a plot showing all XANES of the selected pixels is created.

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The GUI: 'Analyze edge energies'

This GUI helps to analyze the distribution of edge-energies found during XANES normalization of all pixels (i.e. pressing the button 'Normalize all pixels'). For more details see: Analysis step 2: Normalizing the Data).

Figure 1: The GUI 'Analyze edge energies' - the Histogram analyzer

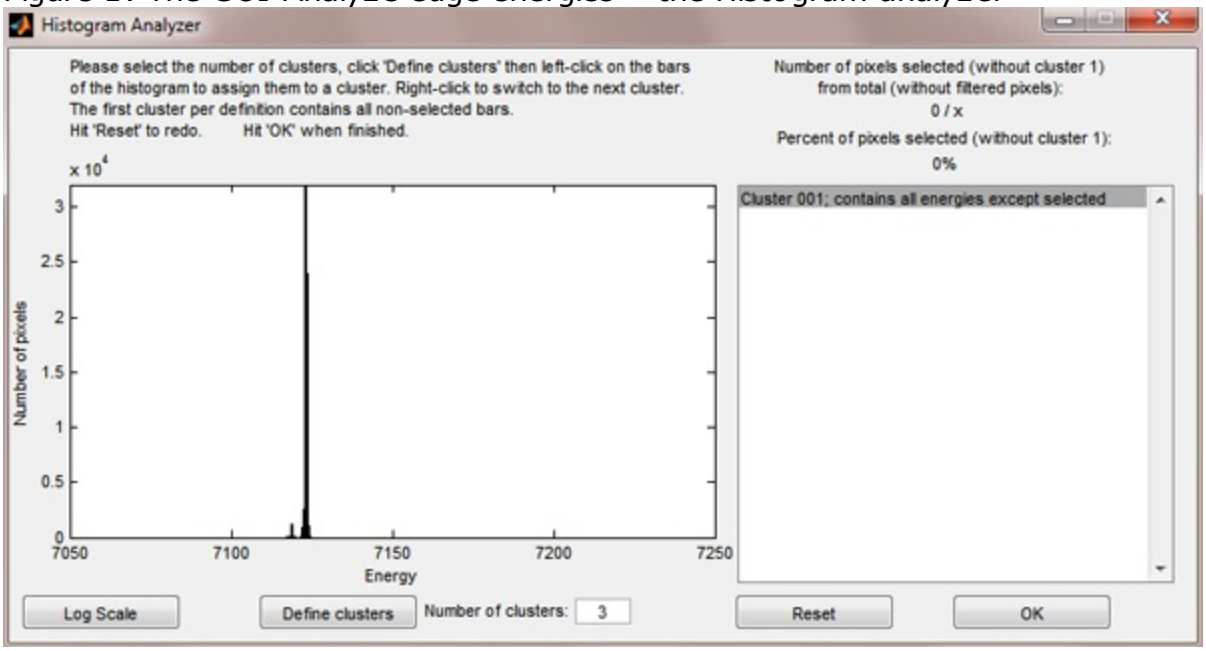


Figure 2: The Histogram analyzer - LOG scale and zoomed

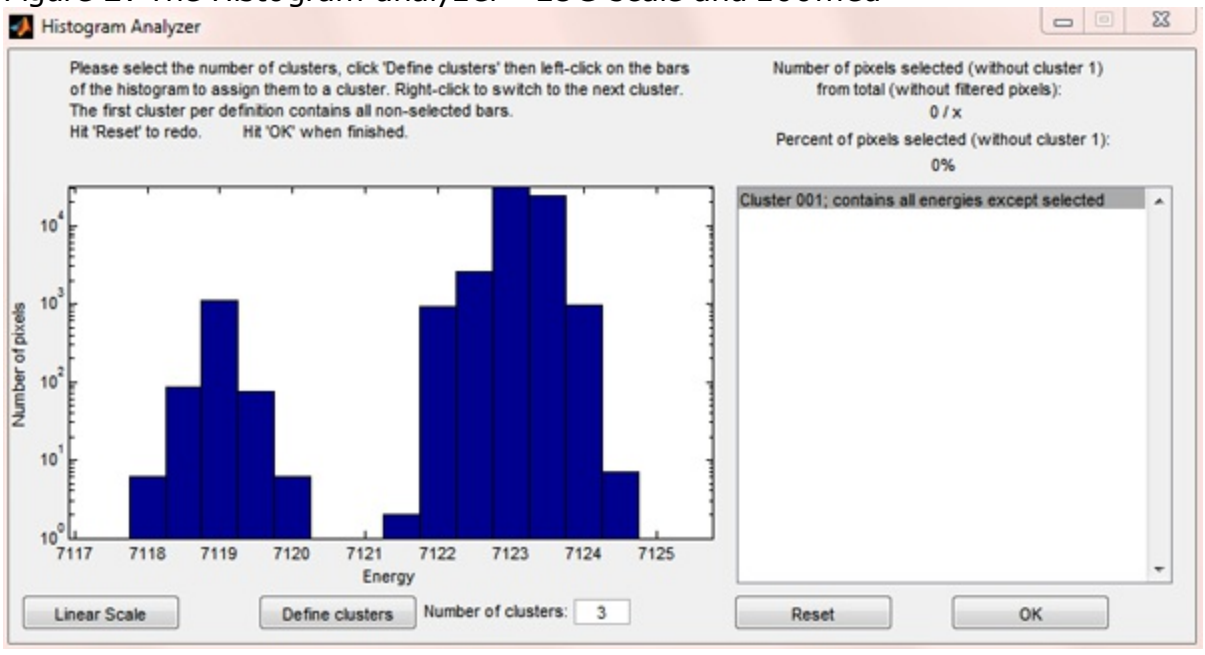
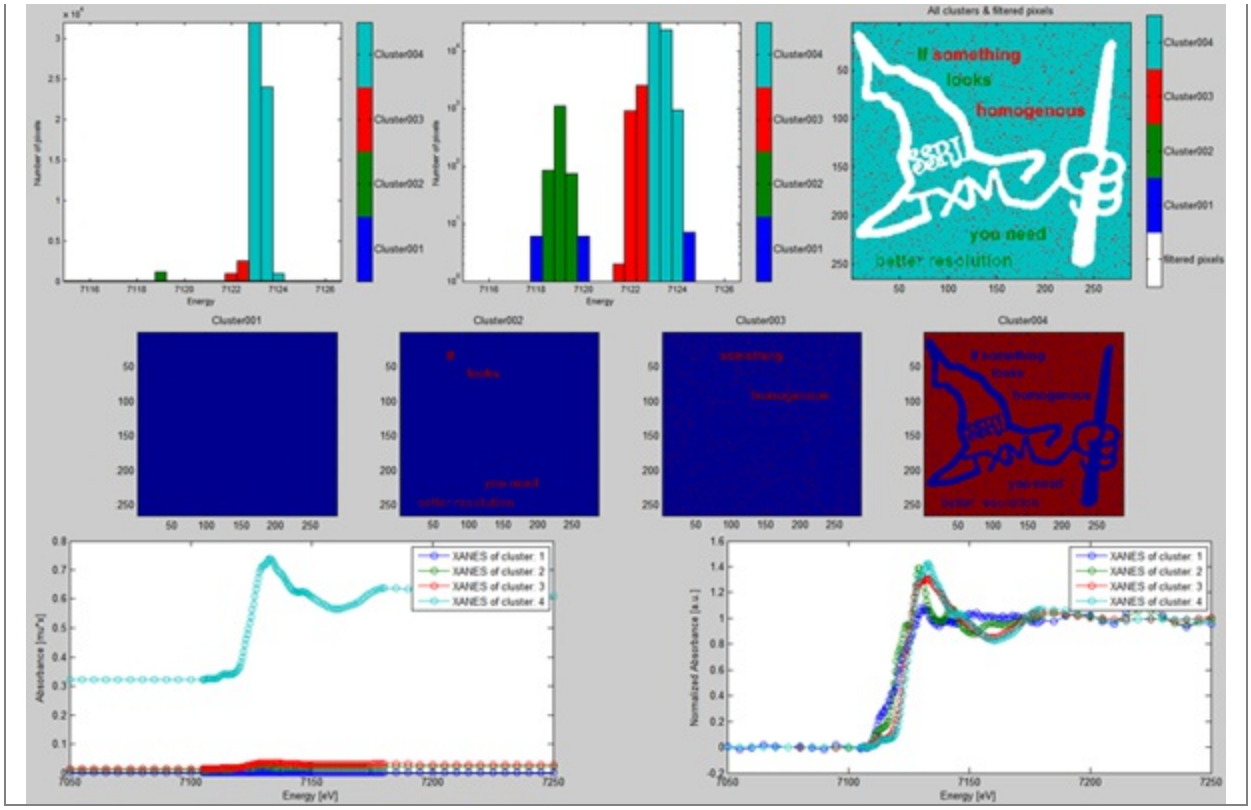


Figure 3: Results of the edge-energy analysis
The figure shows results achieved using the artificial XANES dataset.

The histogram analyzer plots the number of pixels versus the edge-energy found for each pixel. This is informative as it provides a first hint about how many groups of spectra with similar edge-energies are present in the data set. Each column in the plot represents the group of pixels having edge-energies in the respective energy interval (the energy resolution, i.e. the column width, is set to 0.5eV).

The GUI allows selecting columns in order to group them. The total



number of clusters has to be entered in the box 'Number of clusters'. A color will be assigned to each cluster and in the following to each pixel in the cluster generating a cluster map of the data.

A typical work-flow would be as follows:

Scale and Zoom:
-) Choose logarithmic or linear scale by pressing the button 'Log Scale'/'Linear Scale'
-) Zoom the energy region in which you want to perform the clustering

Set number of total clusters:
-) Enter the number of cluster to use in the box 'Number of clusters'.

NOTE: all

columns which have not been selected, will be put in the first cluster i.e. this cluster contains all energies except the selected.

Select columns:
-) Click 'Define clusters'
-) Left-click on all columns which should be in the cluster.
-) Right-click to switch to the next cluster.

NOTE: The first cluster per definition contains all non-selected columns.

Press RESET or OK:
-) If you are not happy with your selection or if anything went wrong click 'Reset'
-) If everything went well, click OK.

Inspect the results (figure 3, left):
Five figures are generated:
1-2: Your selection in the histogram will be plotted in logarithmic and linear scale in separate figures.
3: The clustered data (image) are plotted.
4: The clusters will be plotted in one figure, each in a separate plot.
5: The average XANES of each cluster (i.e. the average XANES of all pixels in the cluster) is plotted.

NOTE: Figure 3 shows results achieved using the artificial XANES dataset. Select 'Apply normalization to current plot(s)' in the popup menu 'XANES normalization' in the main TXM-XANES Wizard GUI to normalize the

XANES as shown in the lower right corner of figure 3.

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The GUI: 'Edge-jump - R-factor correlation analysis'

The edge-jump - R-factor correlation analysis is a powerful tool to inspect the quality of your fitting results.

Figure 1: The GUI 'Edge-jump vs R-factor correlation analysis'

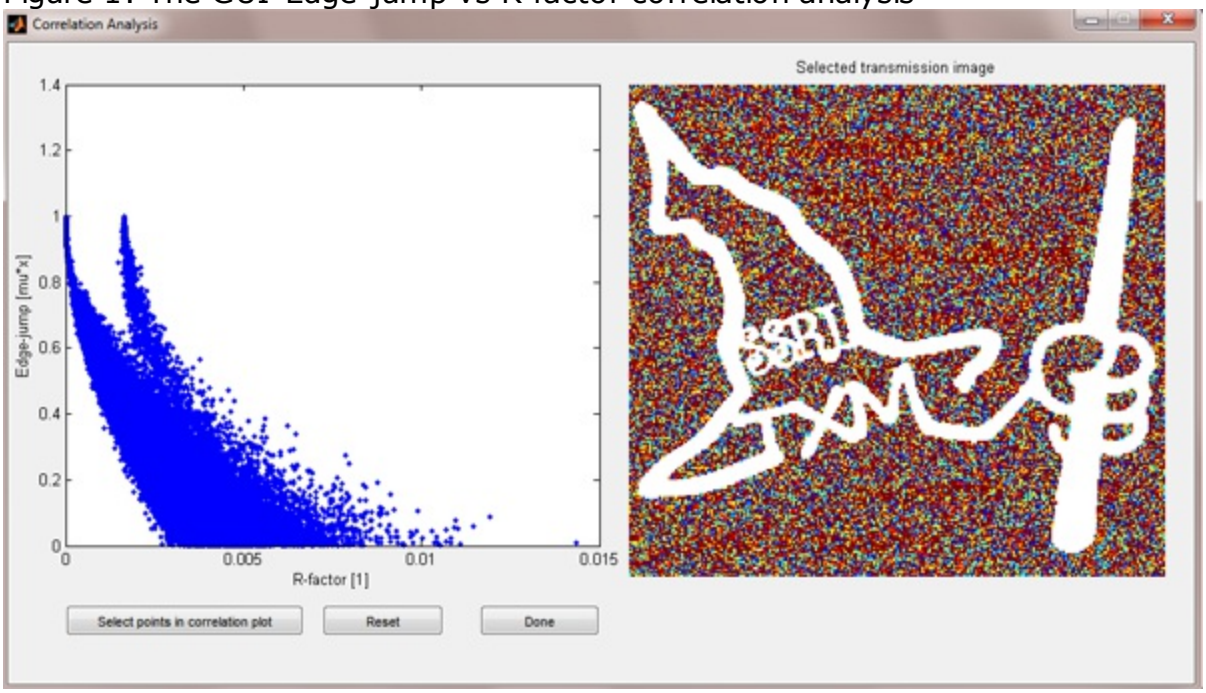


Figure 2: The clustered correlation plot and segmented image

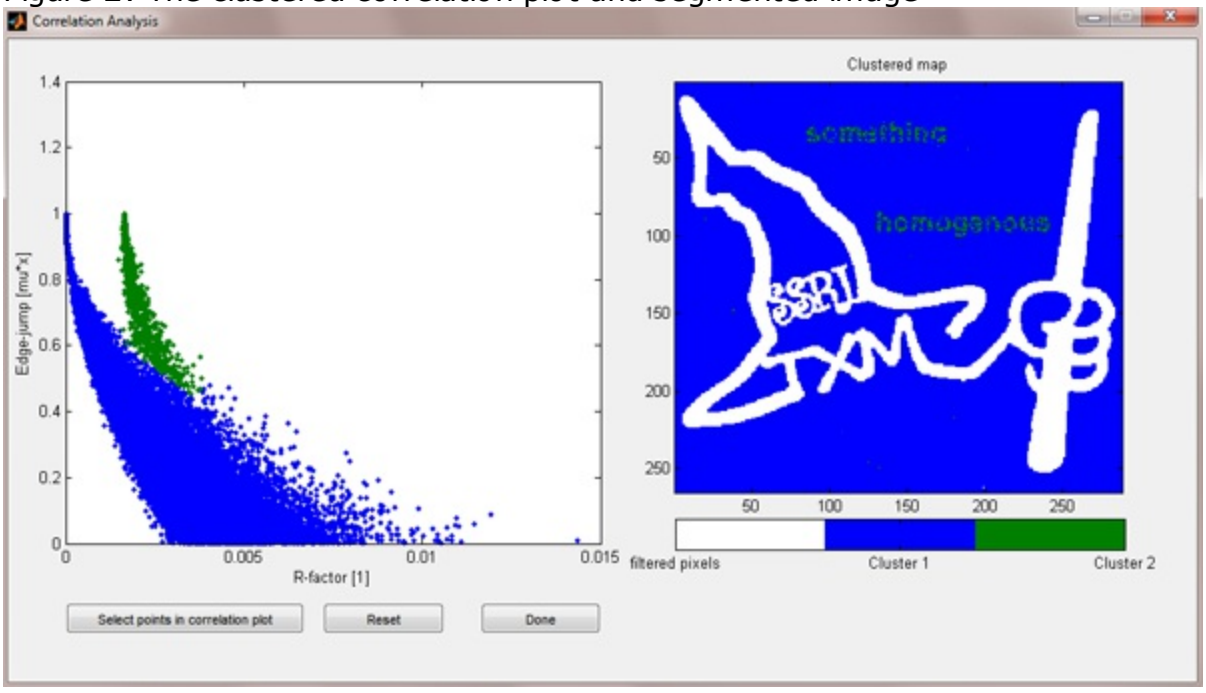
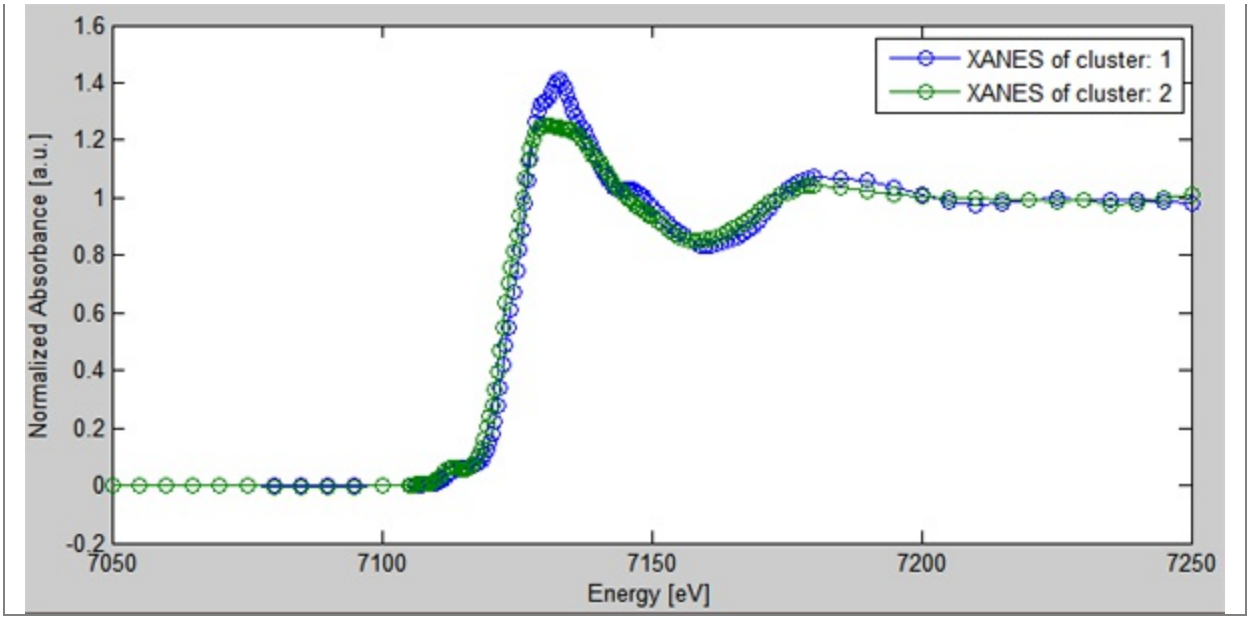


Figure 3: Results of Edge-jump vs R-factor correlation analysis
The figure shows results achieved using the artificial XANES dataset.
The XANES have been normalized using the popup menu 'XANES normalization'

The GUI: 'Edge-jump vs R-factor correlation analysis plots the image selected in the image display on the right and the edge-jump value versus the R-factor for each pixel on the left. Assuming a correct fitting model one would expect a negative correlation between the edge-jump value and the quality of fit parameter R-factor as a larger edge-jump means less noise in the XANES resulting in a better fit (lower R-factor).

Therefore, an inspection of the correlation plot



can reveal problems in the fitting model. Figure 1 (left) shows the correlation plot after fitting the artificial XANES dataset using only 3 instead of 4 XANES standards. It is obvious that some pixels have large edge-jump values (low noise XANES) but relatively large R-factors (bad fit). These pixels form a 'second peak' in the correlation plot at R-factors around 0.002. To identify these pixels in the original image press:

'Select points in correlation plot'
and draw a region around the pixels you want to group (press and hold the left mouse button). The region will be closed

automatically once you release the left mouse button. You can then move the whole region if necessary.
Double click in the region to accept the selection.
The image on the left of the GUI will be updated showing the clustered image based on your selection.

In our example (figure 2, left) the second peak was encircled and it becomes obvious that the green pixels belong to a phase which was used to create part of the text in the image and which was not included in the fitting model (see figure 3).

If you want to refine your selection press:

'Reset'
which will reset all selections

and the image displayed in the right panel.

In case you are happy with your image segmentation press:

'Done'
This will close the GUI and generate 2 figures:
1: The correlation plot with clustered pixels and the segmented image
2: The average XANES of each cluster (i.e. the average XANES of all pixels in the cluster)

NOTE: Figure 3 shows results achieved using the artificial XANES dataset. Select 'Apply normalization to current plot(s)' in the popup menu 'XANES normalization' in the main TXM-XANES Wizard GUI to normalize the

XANES as shown in figure 3.

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The GUI: 'Generate RGB Phasemap from fit results'

This small user interface enables the user to select colors for each phase in the phasemap.

Figure 1: The GUI 'Generate RGB Phasemap' - 3 standards

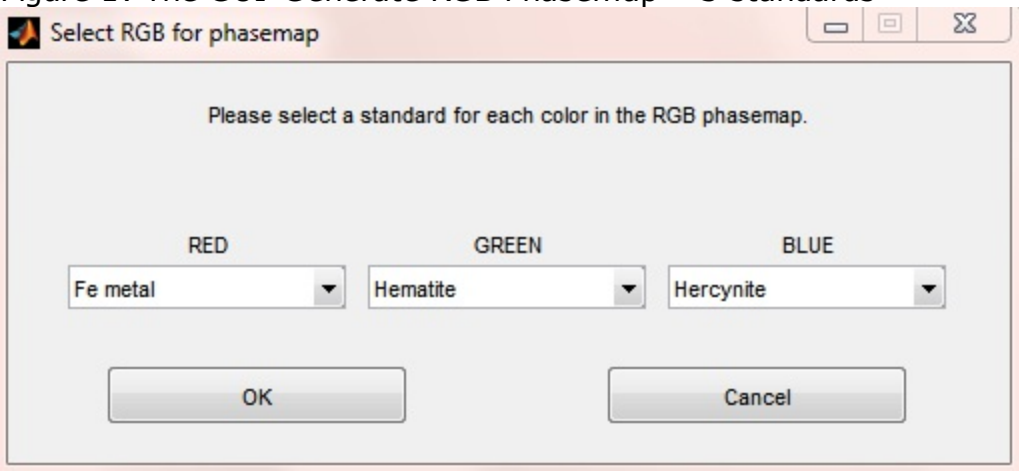


Figure 2: The GUI 'Generate RGB Phasemap' - 2 standards

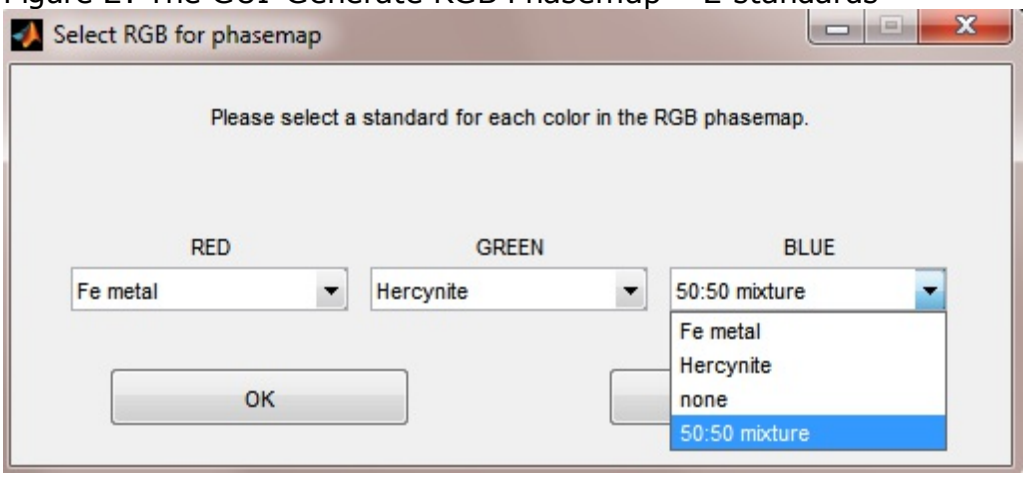
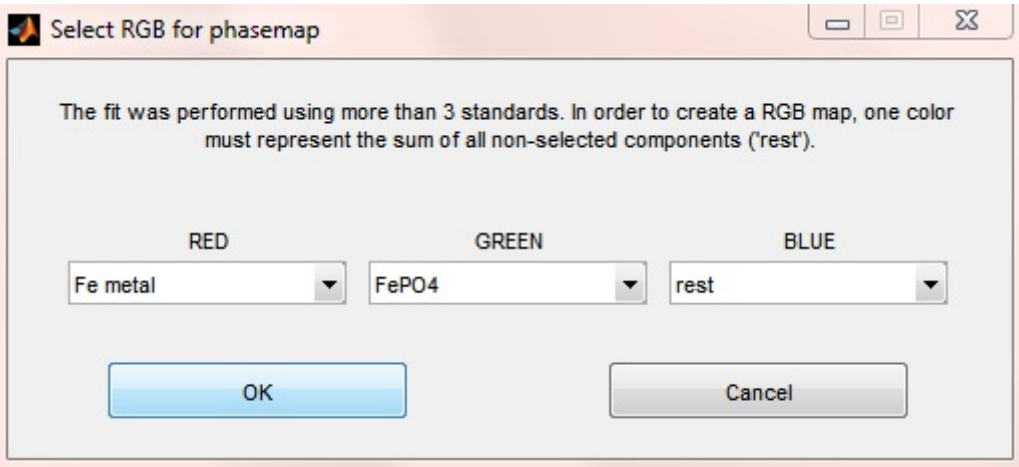


Figure 3: The GUI 'Generate RGB Phasemap' - more than 3 standards



Depending on the number of standards used in the LC fitting (see: Analysis step 4: Least squares linear combination fitting) 3 cases for generating a RGB phasemap are possible:

3 standards
A color is assigned to each standard - phase mixtures are represented by the ratio of the red, green, and blue values of each pixel.

2 standards
a) Skip third color: (selection 'none')
A color is assigned to each standard - phase mixtures are represented by the ratio of the two color values of each pixel. The third color does not exist.
b) Use third color for 50:50 mixture: (selection '50:50 mixture')
A color is assigned to each standard,

additionally the third color is assigned to the 50:50 mixture of the the two phases. This highlights the 50:50 phase front ('interface' between the 2 phases) in the data.

more than 3 standards
In the case of more than 3 phases it is not possible to assign a color to each phase. Therefore two colors can be assigned to two phases while the third color represents all other phases (selection 'rest').
In this way one can generate multiple RGB phasemaps each highlighting two of the phases in the data.

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Advanced XANES Plotting

Topic under construction - coming soon

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Processing Multiple Evaluations

Topic under construction - coming soon

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Principal Component Analysis and K-means Clustering

Topic under construction - coming soon

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Analyzing the data

A typical FOV of the TXM contains 1024 x 1024 pixels, resulting in up to 1E6 XANES spectra to be processed. Spectroscopic data sets consisting of such large numbers of spectra are unprecedented, and manual processing of single XANES spectra (as commonly used for bulk XANES) is no longer feasible.
Extracting scientifically meaningful information from these large data sets is a computational challenge, and requires fast and robust procedures for data processing.
The TXM XANES Wizard provides a series of automated processing options, briefly described in the following sections:

Analysis step 1: The Edge-jump Filter

Determination of the edge jump and noise filtering

Analysis step 2: Normalizing the Data

Normalization and determination of edge energy

Analysis step 3: Exploring the Data

Bulk XANES, single pixel XANES, XANES of ROI, analysis of edge energy distribution, Principle Component Analysis

Analysis step 4: Fitting the Data

Least squares linear combination fitting

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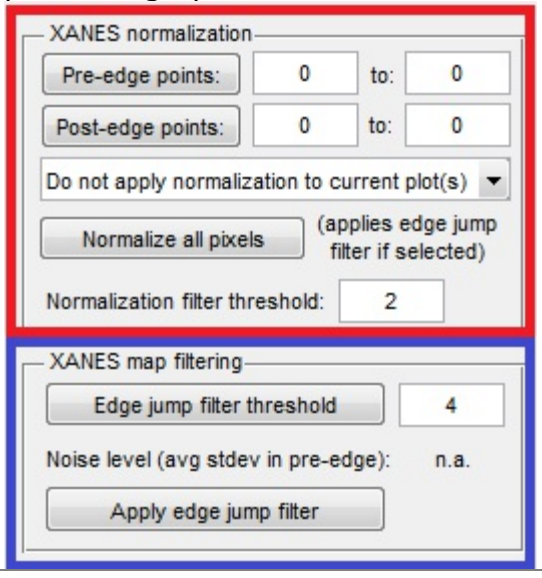
Analysis step 1: The Edge-jump Filter

Determination of the edge jump and noise filtering

In the first step for XANES imaging analysis pixels showing weak absorption (i.e. poor signal to noise (S/N) ratios of the XANES) can be filtered using the absorption edge jump, defined as the difference between the average intensity value in the post-edge region and the average intensity value in the pre-edge region. Both energy regions have to be defined by the user. Pixels with edge jumps smaller than a certain threshold are removed (set to zero intensity at all energies). The threshold is calculated for each pixel as the pre-edge standard deviation of that pixel (representing the XANES noise level) multiplied by a user defined factor. Relevant information about the samples is obtained during this step because the magnitude of the edge jump is proportional to the concentration of the element of interest.
The first two parts of the processing options of the TXM-XANES wizard GUI are used during step 1 of the analysis, namely XANES normalization (red, see figure 1) and Edge-jump filtering (blue, see figure 1):

Figure 1: First two parts of the processing options area



Defining the pre- and post edge regions

The most fundamental part of the TXM-XANES analysis is setting the points which define the pre- and the post-edge regions. These regions should be chosen carefully because all following calculations (edge-jump magnitude, edge-energy position, least-squares fitting, ...) use these parameters. The points defining pre- and post-edge region can be set in the part 'XANES normalization' of the processing options area either by entering the first and last point of the region or by pressing the buttons 'Pre-edge points' and 'Post-edge points'.
NOTE: Points are numbered consecutively from lowest to highest energy, e.g. 1 -146 if 146 energy points were recorded.

the tutorial 'XANES normalization': Link 1 (browser) Link 2 (help file)
NOTE: If you are using a browser use link 1, if you are running the help file open link 2 and

make sure the tutorial is saved in the subfolder ../movies of the folder where you run the help file.

Setting the edge-jump filter threshold

The edge-jump filter threshold value (EJFT) is used to filter XANES with insufficient edge jump magnitude (EJ), i.e. the edge jump is too small in comparison to the noise level. The noise level is defined as the standard deviation in the pre-edge region (SD_{Pre}). The filter works by removing pixels (index i) with an EJ smaller than the SD_{Pre} multiplied by the EJFT:

$$EJ_i < SD_{Pre,i} * EJFT$$

This means the larger the EJFT, the stronger the filtering which is based on the individual noise level of each single pixel XANES.
(NOTE: using an EJFT value of 0 will therefore only filter pixels showing 'negative edge jumps'...)

The 'Noise level' value will be displayed after pressing the button 'Edge jump filter threshold' or the button 'Apply edge jump filter' and is calculated as the average over all pixels/XANES standard deviations in the pre-edge region - it can therefore be interpreted as the experimental error of the dataset.

Applying the edge jump filter

Once the normalization parameters are set you can check the effectiveness of the filter before applying it to the data by pressing the button 'Edge jump filter threshold'. This will generate a plot for each integer value between 1 and the value you entered showing the filter mask when using this value.
Example: EJFT is set to 8. Pressing 'Edge jump filter threshold' generates 2 figures: Figure 1 contains 8 plots, each displaying the filter mask calculated using EJFT values of 1-8. Figure 2 shows all filter masks in one plot each with a different color to highlight differences between them.
Once the EJFT value is adjusted press the button 'Apply edge jump filter' - this will apply the filter using the current EJFT value and save all parameters, including the list of file names loaded (image stack) in a .mat parameter file called 'XANES maps file'. If you want to continue work later, all you will need to do is re-loading this file using the the load menu.

Some background information

Performed calculations

During the edge-jump determination the user defined pre- and post-edge regions are used to calculate the following values for each pixel of the XANES dataset:

- The average absorption value in the pre-edge region (AVG_{Pre})
- The standard deviation in the pre-edge region (SD_{Pre})
- The average absorption value in the post-edge region (AVG_{Post})
- The standard deviation in the post-edge region (SD_{Post})
- The edge jump magnitude (EJ) as: $EJ = AVG_{Post} - AVG_{Pre}$

All these values can be visualized as maps by selecting the respective entry in the popup menu in the the image display

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Analysis step 2: Normalizing the Data

Normalization and determination of edge energy

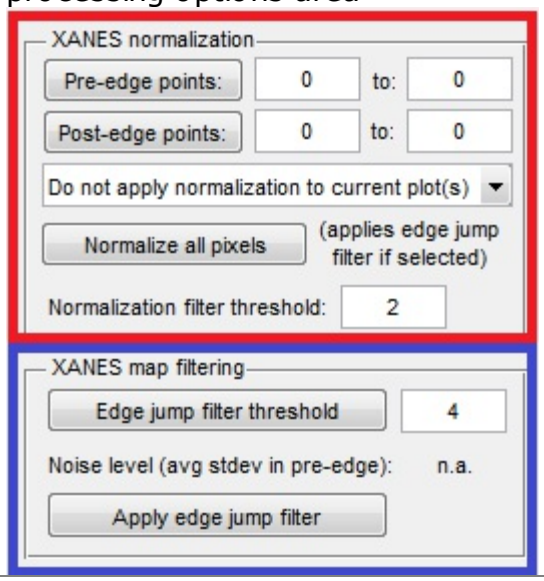
The second step in data analysis using the TXM-XANES Wizard after determining the normalization parameters and noise filtering (see: Analysis step 1: The Edge-jump Filter) is to normalize all single pixel XANES of the data set. During this process the energy position of the absorption edge of each single pixel XANES (called 'edge energy' in following) is calculated and additional filtering can be applied.

Because of the large number of pixels and the fact that some single-pixel XANES can show relatively high noise levels (when compared with bulk techniques), rapid, effective and automated normalization of each XANES is required. The 'traditional' approach of using the first maximum of the first derivative of the XANES to identify the absorption edge is not feasible.
Furthermore, because all XANES are recorded during the same energy scan, differences between XANES of different pixels (e.g. distortions in the pre- and/or post-edge region owing to different slopes) are exclusively caused by the sample itself. The normalization procedure therefore uses simple linear regression in the pre- and the post-edge region to normalize the XANES to 0 (pre-edge) and 1 (post-edge).
Automated normalization must be capable of processing up to 1E6 spectra within reasonable times, and cannot account for single 'problematic' pixels for which a more sophisticated manual normalization could be necessary. Therefore, pixels which could not be normalized sufficiently by the algorithm (i.e. pixels in which the calculated pre- or post-edge lines show very high or very small slopes) are filtered (set to zero intensity at all energies). The filter threshold for unreasonable slope values can be adjusted by the user. Usually, if the threshold for noise filtering was chosen reasonably well (see: Analysis step 1: The Edge-jump Filter), the number of additionally filtered pixels owing to poor normalization is much less than 1%.

After normalization and normalization filtering, the energy position of the absorption edge can be determined using an approach *(Koningsberger et al., 2000)* which is more robust against noise than using the derivative of the XANES: the energy position is interpolated (linearly) for each of the filtered pixels as the energy where the absorption values of the XANES are half the edge-jump intensity (as defined in Analysis step 1: The Edge-jump Filter). The XANES edge position contains valuable information about, for example, the

valence state of the element of interest, and a map of the edge jump (available in the popup menu in the image display after normalization of all pixels) therefore provides initial information about the chemistry of the sample.

Figure 1: First two parts of the processing options area



The normalization filter threshold

The normalization filter threshold value can be set by the user and defines the strength of the filtering (see The normalization filter explained). Typical values are between 0 (no filtering) and 4.

Normalize all pixels

Pressing the button 'Normalize all pixels' normalizes all single pixel XANES, applies the normalization filter using the current threshold value, and save all parameters in the .mat parameter file 'Normalization maps file'. (NOTE: normalizing all XANES of the dataset may take a while - please be patient!)

If you want to continue work later at this point of the analysis, all you will need to do is re-loading the 'XANES maps file' and the 'normalization maps file' using the load menu.

After normalizing all pixels 6 additional maps will become available in the popup menu in the image display:

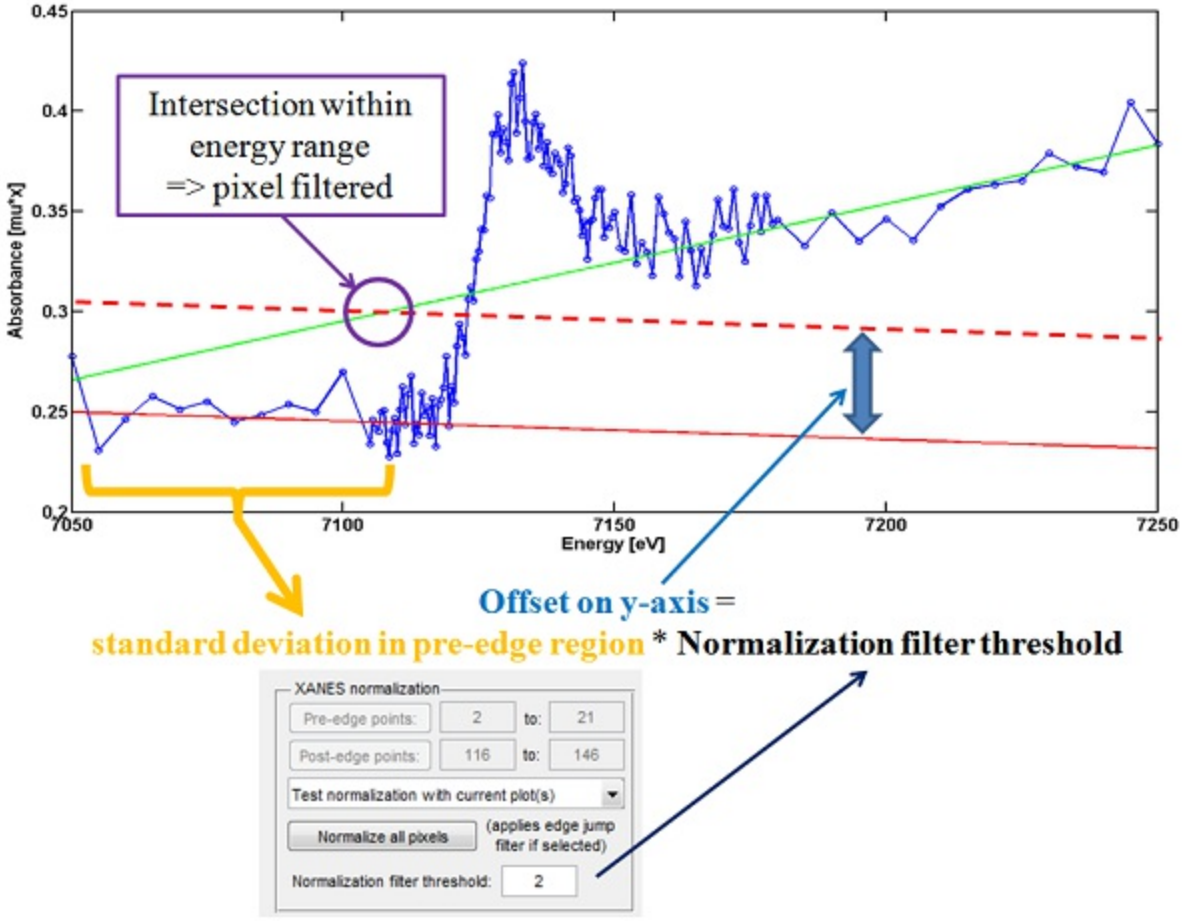
1: The combined filter (i.e. the combination of edge-jump and normalization filter) 2: The slope value of the pre-edge line
3: The slope value of the post-edge line
4: The Y-intercept of the pre-edge line
5: The Y-intercept of the post-edge line
6: The edge energy (i.e. the energy position of the absorption edge of each single pixel XANES)

NOTE: in the current version of the Wizard two additional plots are produced, showing a) the correlation between the slope in the pre-edge region and the slope in the post-edge region and b) the correlation between the edge-jump and the slope in the post-edge region. The author is not sure if these plots add valuable information to the analysis and welcomes any suggestions

whether to keep or remove these plots in future versions.

Some background information

Figure 2: Working principle of the normalization filter



The normalization filter explained

The normalization filter removes pixels based on the slopes of the regression lines calculated for pre- and post-edge regions of their XANES. A pixel is filtered if the intersection point of pre- and post-edge line lies within the energy range of its XANES. To increase the strength of the filtering the intersection point is determined using an y-axis offset for the pre-edge line (see figure 2, left). If the offset is larger it is more likely that the intersection point lies within the energy range and the

pixel is filtered. The offset value is determined as the standard deviation in the pre-edge region of the XANES, multiplied by the user defined normalization filter threshold value.

References

- Koningsberger, D. C., Mojet, B. L., van Dorssen, G. E. & Ramaker, D. E. (2000). Top. Catal. 10, 143-155.

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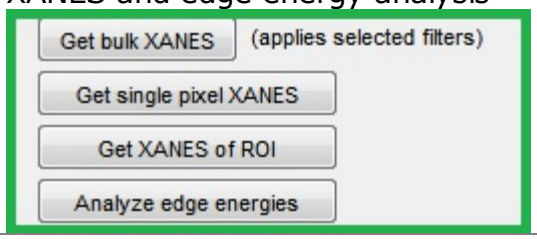
Analysis step 3: Exploring the Data

Bulk XANES, single pixel XANES, XANES of ROI, analysis of edge energy distribution, Principle Component Analysis

The options shown in figure 1 are related to data analysis without previous LC fitting. Data can be analyzed with or without filtering, and, in the case of 'Bulk XANES' and 'Single pixel XANES' analysis even without normalizing all pixels. The option 'Analyze edge energies' needs the results from the 'Normalize all pixels' procedure where the edge-energies of all single pixel XANES spectra are calculated (see: Analysis step 2: Normalizing the Data).
Main purpose of this analysis step is to identify or justify the XANES standards used in the final LC fitting (Analysis step 4: Fitting the Data).

Figure 1: The Processing Options - XANES and edge energy analysis



Get bulk XANES

Calculates the average XANES spectrum of all (filtered) pixels. For more details see: The button 'Get bulk XANES' and
the tutorial 'XANES normalization': Link 1 (browser) Link 2 (help file)
NOTE: If you are using a browser use link 1, if you are running the help file open link 2 and make sure the tutorial is saved in the subfolder ../movies of the folder where you run the help file.

Get single pixel XANES

Opens a GUI which allows selecting pixels of interest and displays their XANES spectra. For more details see: The GUI 'Get single pixel XANES' and The button 'Get bulk XANES' and
the tutorial 'Single pixel XANES': Link 1 (browser) Link 2 (help file)
NOTE: If you are using a browser use link 1, if you are running the help file open link 2 and make sure the tutorial is saved in the subfolder ../movies of the folder where you run the help file.

Get XANES of ROI

Allows selecting a Region Of Interest (ROI) in the image display and calculates the average XANES of the selected and the non-selected pixels. For more details see: The button 'Get XANES' of ROI

Analyze edge energies

A tool to investigate the edge energy distribution of the dataset. Pressing this button starts the GUI: 'Analyze edge energies' showing the distribution of the edge energies (i.e. the edge position of the XANES of each pixel on the energy axis) as a histogram of edge energy vs. number of pixels. The user can cluster different edge energies and the pooled pixels corresponding to each cluster are displayed. For more details see: the GUI: 'Analyze edge energies'

Principle Component Analysis and K-means Clustering

This option is available in the 'Tools' menu of the menu bar and starts a PCA and subsequent k-means clustering of the dataset. For more details see: PCA and K-means Clustering.

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Analysis step 4: Fitting the Data

Least squares linear combination fitting of every (non-filtered) single pixel XANES

To obtain information about the distribution of the chemical phases of the element of interest present in the sample, least-squares linear combination (LC) fitting of each single-pixel XANES can be performed. This step requires a priori knowledge of the phases present in the sample. With the release 2.1.2, the TXM XANES Wizard includes a toolkit for principal component analysis, which can help experienced users in finding unknown or missing phases in the dataset prior to LC fitting.
During LC fitting the loaded set of normalized reference spectra is fit to the XANES of each pixel using a least-squares method. The quality of each LC fit can be checked with the R-factor *(Ravel & Newville, 2005)* commonly used for XANES fitting, defined as

$$R = \frac{\sum(\text{data} - \text{fit})^2}{\sum(\text{data})^2}.$$

Because one R-factor is available for each pixel, an R-factor map can be generated to highlight areas of bad fit results (this map becomes available in the popup menu in the image display after all pixels have been fitted). The R-factor of each pixel can be used to identify problems owing to insufficient or missing references used in the fitting. By plotting the edge-jump value versus the R-factor of each XANES, pixels can be identified which show sufficiently high concentrations of the element of interest (edge-jump height) but a lower quality fit (higher R-factor) than other pixels with similar edge-jump height (see: The GUI: 'Edge-jump - R-factor correlation analysis'). The presence of such pixels can indicate a problem with the fitting model, i.e. missing or incorrect XANES reference(s).

The fitting additionally returns

- the R^2 values (the sum of the squared residuals) for each fit (generating the R-square map).
- the reduced chi-square values for each fit (generating the reduced chi-square map).
- the fit error for each pixel and reference XANES used (generating the fit error maps; one for each standard used).

The last two maps in this list (calculated during the LC fit of all pixels) are currently not available in the popup menu in the image display, but are

displayed after the LC fitting and every time a saved LC-fit parameter file (.mat) is loaded (using the Load menu).
The reduced chi-square and the fit errors are calculated following the approach of the FEFFIT program *(Newville, 1995)* as described in the program documentation *(Newville, 1998)*.

References

- Ravel, B. & Newville, M. (2005). J. Synchrotron Rad. 12, 537-541.
- Newville, M., et al. (1995). Physica B: Condensed Matter, 208-209: p. 154-156.
- Newville, M. (1998). FEFFIT - Using FEFF to model XAFS data, Chapter 5, Goodness of Fit and Uncertainties in the Variables, downloaded April 1st 2012 from http://cars9.uchicago.edu/~newville/feffit/

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Miscellaneous

This section covers topics providing additional or background information about the TXM-XANES Wizard package

Topics

- File Formats
- The Artificial XANES Dataset

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File Formats

The TXM-XANES wizard loads and saves several different file types. In the following a brief list of used file formats is provided.

- TXM transmission images (.xrm, .bin, .bim) These binary files contain the transmission images recorded with the Xradia TXM (.xrm), or pre-processed transmission images using the TXM-Wizard.
- Parameter files (.mat files) These Matlab binary files contain parameters and data saved at different stages of the data analysis. The files are:
  a. *'XANES maps file':*
     Generated after pressing the button 'Apply edge jump filter'; see also: Analysis step 1: The Edge Jump Filter
  b. *'Normalization maps file'*
     Generated after pressing the button 'Normalize all pixels'; see also: Analysis step 2: Normalizing the Data
  c. *'LC fit maps file'*
     Generated after pressing the button 'Linear combination (LC) fitting of all pixels'; see also: Analysis step 4: Fitting the Data
  d. *'PCA results file'*
     Generated after selecting the option 'PCA and k-means clustering' in the 'Tools' menu; see also: Principal Component Analysis and K-means Clustering
  e. *'K-means results file'*
     Generated after pressing the button 'Save clustering result' in the GUI Principal Component Analysis and K-means Clustering

- Plaintext (ASCII) files This file format has to be used to load XANES standards (reference XANES) to be used in the LC fitting (see: Analysis step 4: Fitting the Data).
  The content of these files has to consist of 2 columns separated by whitespace characters. The first column has to contain the energies in eV, the second the normalized absorption. Comments have to be indicated by a leading '#' character. It is highly recommended to use the ATHENA program of the IFEFFIT software package to generate these files. The TXM-XANES Wizard can import .nor and .norm files created by ATHENA (only the first 2 columns will be used).
  NOTE: Make sure the energy range covered by the standards is not shorter

than the one of the TXM data set. Otherwise this will cause problems in the LC fitting - and most likely generate wrong results. In the worst case you need to remove transmission images to trim the energy range of your data set to fit the energy range covered by your reference XANES data.

- Microsoft(TM) Powerpoint files (.ppt)
  Powerpoint files containing a report of the data evaluation are generated by selecting the options 'Create Powerpoint report' or 'Add open figures to Powerpoint report (Ctrl-A)' in the 'Save' menu.

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The Artificial XANES Data Set

Topic under construction - coming soon

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Tutorials

The following lists all available tutorials. If you are using a browser to read the TXM-XANES Wizard help use the first link. To start a tutorial from the compiled help module (.chm file) use link 2 and make sure the corresponding shockwave flash movie file (.swf) is saved in the subfolder ../movies of the folder where you run the help file.

- Tutorial 1: 'XANES normalization' Link 1 (browser) Link 2 (help file)

  Topics covered:

  a. Loading Files
  b. Calculating the 'bulk XANES'
  c. Setting XANES normalization parameters

- Tutorial 2: 'Single pixel XANES' Link 1 (browser) Link 2 (help file)

  Topics covered:

  a. Loading parameter files (.mat)
  b. Selecting single pixel XANES

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Known Bugs and Pitfalls

TXM-XANES Wizard version 2.1.2 - release date: September 2012

- Loading XANES standards
  See: The Load menu and Plaintext (ASCII) files and Analysis step 4: Fitting the Data
  Make sure the energy range covered by the standards is not shorter than the one of the TXM data set. Otherwise this will cause problems in the LC fitting - and most likely generate wrong results. In the worst case you need to remove transmission images to trim the energy range of your data set to fit the energy range covered by your reference XANES data.

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The GUI: 'Edge-jump - R-factor correlation analysis'

The edge-jump - R-factor correlation analysis is a powerful tool to inspect the quality of your fitting results.

Figure 1: The GUI 'Edge-jump vs R-factor correlation analysis'

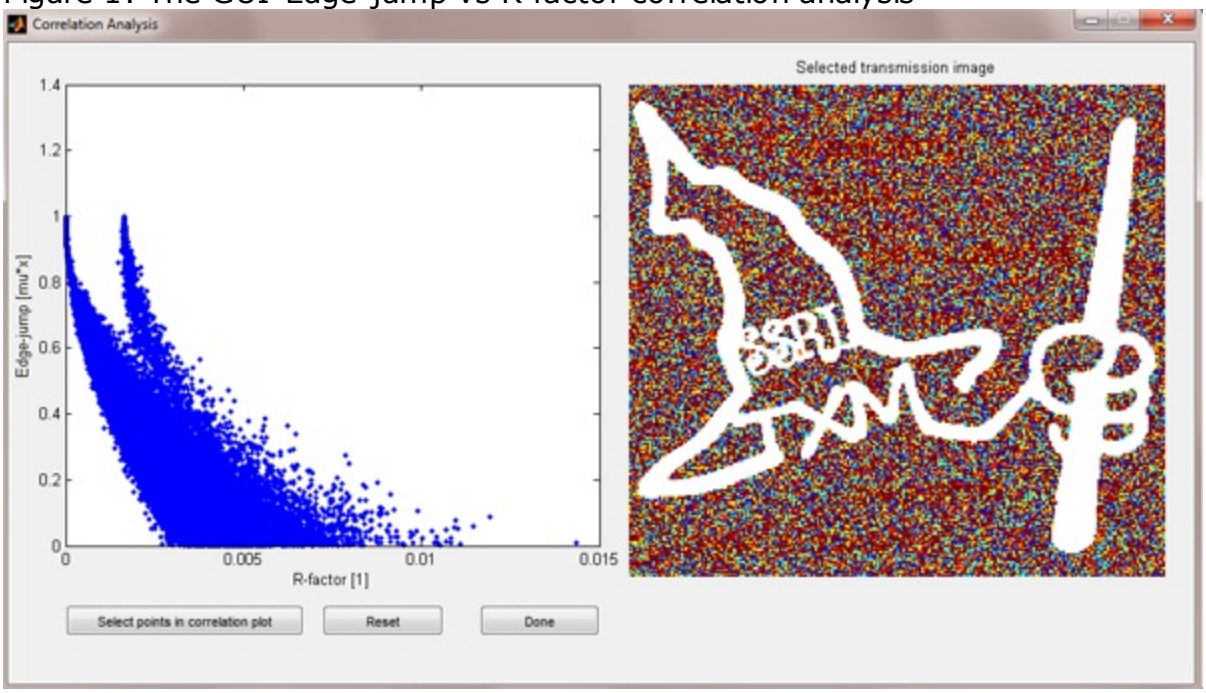


Figure 2: The clustered correlation plot and segmented image

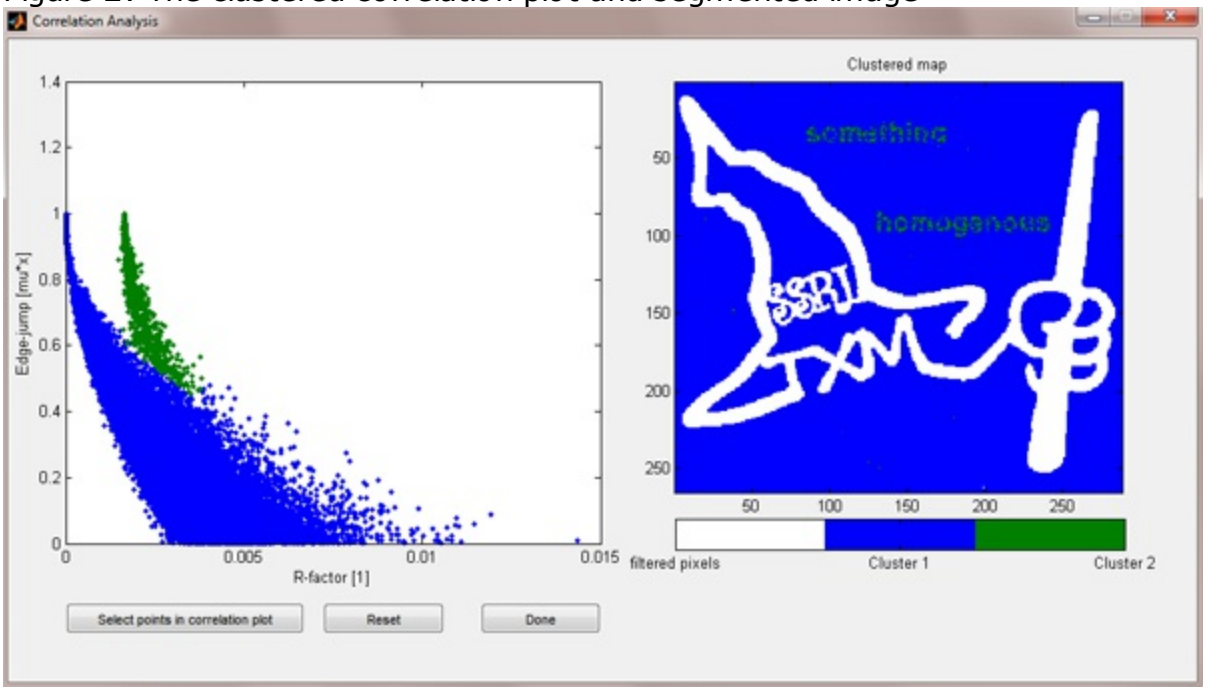
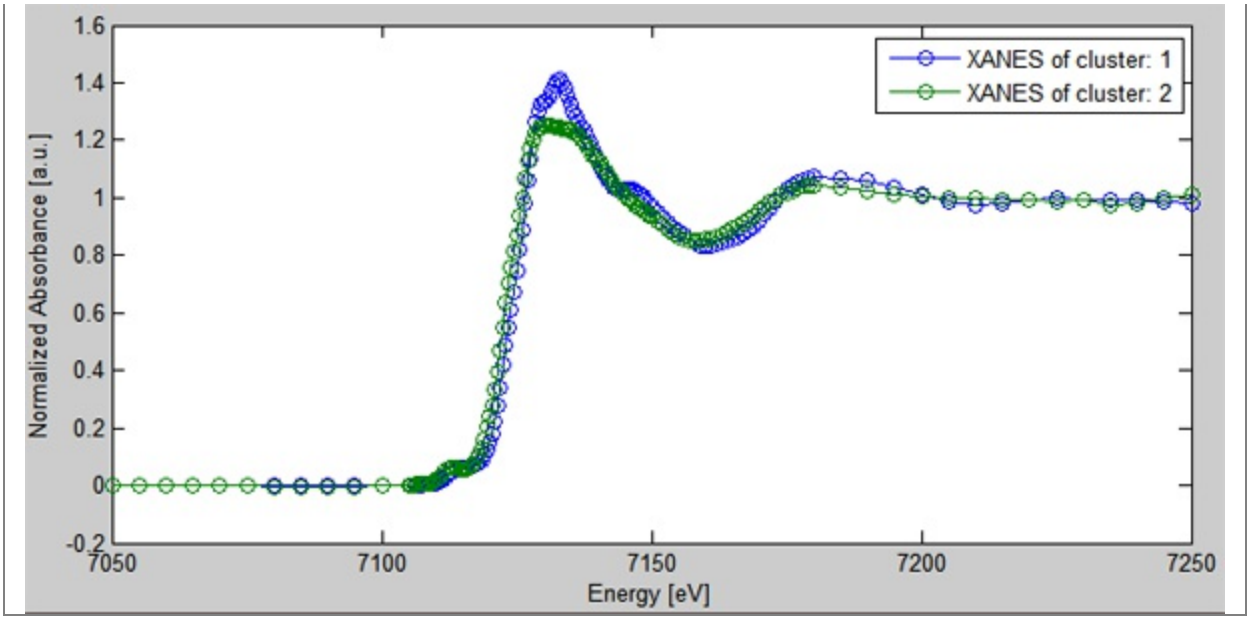


Figure 3: Results of Edge-jump vs R-factor correlation analysis
The figure shows results achieved using the artificial XANES dataset.
The XANES have been normalized using the popup menu 'XANES normalization'

The GUI: 'Edge-jump vs R-factor correlation analysis plots the image selected in the image display on the right and the edge-jump value versus the R-factor for each pixel on the left. Assuming a correct fitting model one would expect a negative correlation between the edge-jump value and the quality of fit parameter R-factor as a larger edge-jump means less noise in the XANES resulting in a better fit (lower R-factor).

Therefore, an inspection of the correlation plot



can reveal problems in the fitting model. Figure 1 (left) shows the correlation plot after fitting the artificial XANES dataset using only 3 instead of 4 XANES standards. It is obvious that some pixels have large edge-jump values (low noise XANES) but relatively large R-factors (bad fit). These pixels form a 'second peak' in the correlation plot at R-factors around 0.002. To identify these pixels in the original image press:

'Select points in correlation plot'
and draw a region around the pixels you want to group (press and hold the left mouse button). The region will be closed

automatically once you release the left mouse button. You can then move the whole region if necessary.
Double click in the region to accept the selection.
The image on the left of the GUI will be updated showing the clustered image based on your selection.

In our example (figure 2, left) the second peak was encircled and it becomes obvious that the green pixels belong to a phase which was used to create part of the text in the image and which was not included in the fitting model (see figure 3).

If you want to refine your selection press:

'Reset'
which will reset all selections

and the image displayed in the right panel.

In case you are happy with your image segmentation press:

'Done'
This will close the GUI and generate 2 figures:
1: The correlation plot with clustered pixels and the segmented image
2: The average XANES of each cluster (i.e. the average XANES of all pixels in the cluster)

NOTE: Figure 3 shows results achieved using the artificial XANES dataset. Select 'Apply normalization to current plot(s)' in the popup menu 'XANES normalization' in the main TXM-XANES Wizard GUI to normalize the

XANES as shown in figure 3.

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