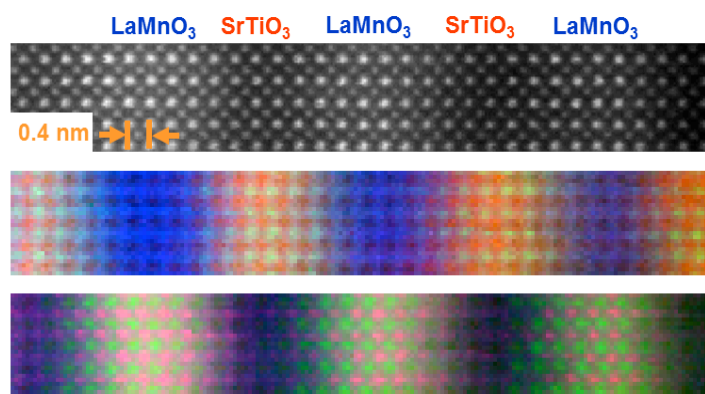


2018/March

MSA for DigitalMicrograph

Multivariate Statistical Analysis

Advanced Manipulation Tools for Spectrum Images (SIs)



MSA User Manual v2.3

HREM Research Inc.

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Conventions

The typographic conventions used in this manual are described below.

Convention	Description
Bold	Used to denote specified elements of the user interface such as buttons, field names, menus, and menu options. For example, the New button.
Menu...MenuOption	Select the Menu from the Menu bar then select the Menu Option from the Menu. For example, File...Open would mean select the File Menu and then the Open Option.
CAPS	Used to denote the name of a key on the keyboard. For example, the ENTER key.
<i>Italics</i>	Used to denote emphasis, captions and the result of an action in a procedure.

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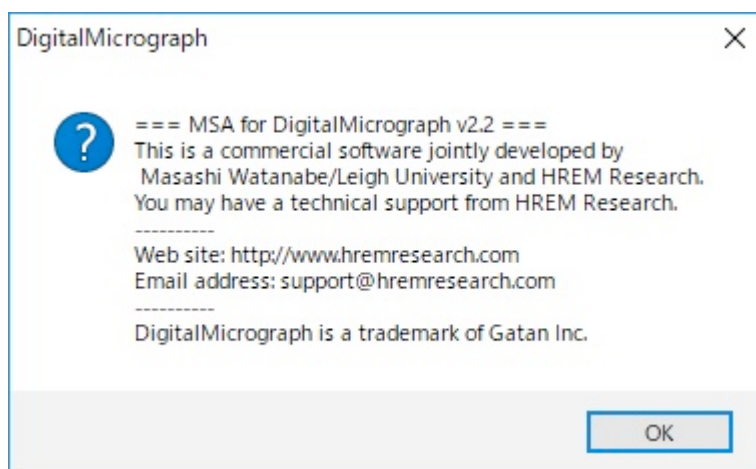
Welcome to MSA

Welcome to MSA, the multivariate statistical analysis (MSA) plugin package for Gatan DigitalMicrograph.

The current package version is 2.2, which consists of following functions:

- PCA
 - Weighted-PCA: apply weighted principal component analysis on SI
 - Local wPCA: apply weighted PCA on local regions of SI data
- Import
 - EMSA spectrum series import: import a series of EMSA spectra
 - XED-SI data import: import an x-ray SI data file
 - EFTEM image series import: import a series of EFTEM images
- Utilities
 - Bin SI: apply spatial/spectral binning on SI data
 - Spatial sub SI: extract spatially partial SI data
 - Spectral sub SI: extract spectrally partial SI data
 - Extract component(s): extract a component(s) from a stacked component(s)
- Release notes

These plugins should work in GMS version 1.7.1 or higher.



Software requirements

The following is a list of the software requirements necessary to run the MSA plug-in:

- DigitalMicrograph (GatanTM)
- USB Key Driver
- IPU Plug-in (freeware downloadable from www.hremresearch.com)

Software Installation

Installing USB Key Driver

The user key driver should be installed by following the instructions given by the key driver installer. You can find the key driver installer at the plug-in page on HREM Research web site.

Installing DigitalMicrograph Plug-ins

The DigitalMicrograph Plug-in will be provided as Gatan compiled script file(s) (.gtk) and dynamic link library file(s) (.dll). All these files can be installed by copy-and-paste to the folder “PlugIns.” (The PlugIns folder should exist under a normal installation of the DigitalMicrograph).

When the DigitalMicrograph is launched after placing the plug-ins into the PlugIns folder, the PlugIns will appear as a new menu or an addition to the standard tools.

Installing IPU Plug-in

The MSA plug-in uses some functions based on the Intel’ MKL (Math Kernel Library) provided by the IPU plug-in. All the files relating the IPU plug-in can be installed by copy-and-paste copy. The IPU plug-in will appear as under *IPU* menu. Please consult the ReadMe file that comes with the IPU plug-in.

Installing MSA

DMMSA32(64).dll, DMMSAUtills32(64).dll and DMMSA32(64).gtk can be installed by drag-and-copy to the folder “PlugIns” (The PlugIns folder should exist under a normal installation of the DigitalMicrograph.)

When the DigitalMicrograph is launched after placing the plug-ins into the PlugIns folder, MSA menu commands will appear.



New features of version 2

MSA 2.x is a major upgrade that includes local PCA analysis, which will reduce some artifacts and substantially extend an area of application of the PCA.

MSA - Getting Started

Brief Description

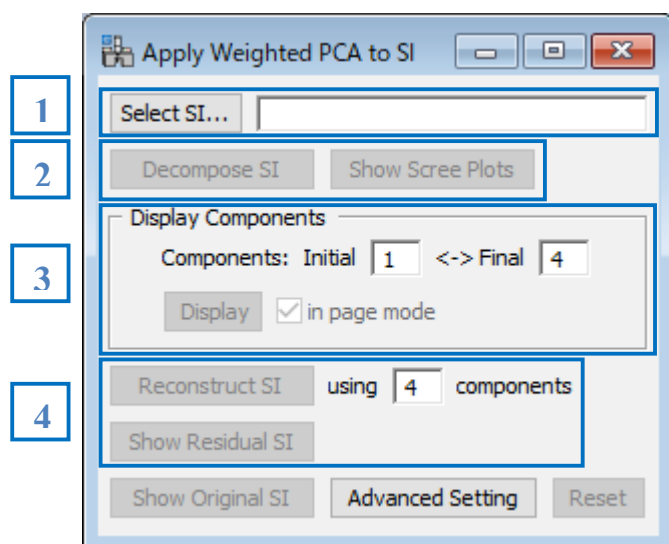
MSA performs principal component analysis (PCA) to a 2D or 3D spectrum image (SI) with proper weighting based on Poisson statistics. Since this function creates many result files from a single SI file, it would be better to create a dedicated folder before you apply this function. After decomposing the SI file, you can extract each component: a pair of a loading spectrum (spectral feature) and a score image (spatial amplitude). Then, you can reconstruct a noise-free (or noise-reduced) dataset from the original SI. In addition, you can also check the PCA application from a residual data (difference between the original and reconstructed SIs).

Before using MSA

As mentioned above, you should create a dedicated folder before you apply PCA to your SI. This technique requires a lot of memory. So, it is strongly recommended to close all files in DigitalMicrograph prior to applying MSA. This function tries to use only available physical memory. Even if the available physical memory is limited or smaller than the targeting SI, you can still complete PCA as long as the SI can be opened in DigitalMicrograph. However, if the available physical memory is smaller, it would take much longer for processing.

Weighted PCA Usage

After closing all the opened files, select **MSA...Weighted PCA...PCA** from the menu bar. Then, the following dialog appears.

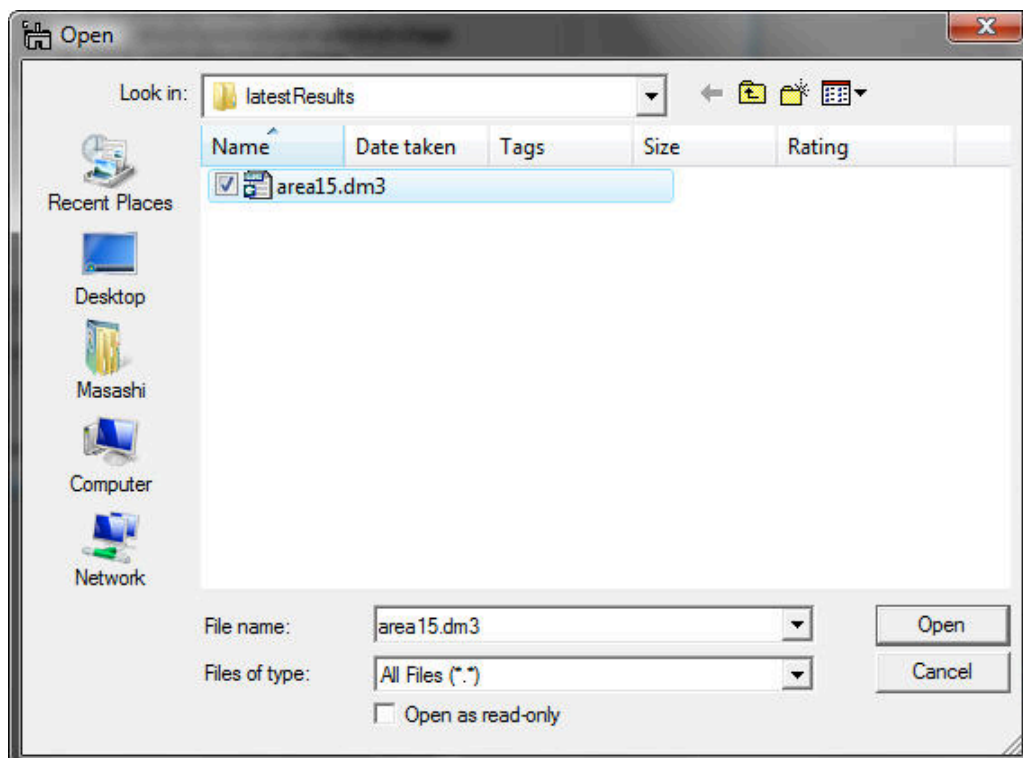


The numbers in the blue boxes indicate the step you should follow in order to apply PCA. Namely,

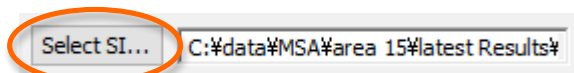
1. Select a 2D or 3D spectrum image in the Gatan dm3 (dm4 for GMS 2.x) format
2. Apply matrix decomposition to the SI
3. Evaluate components
4. Reconstruct the noise-reduced spectrum image

Each step is explained as follows:

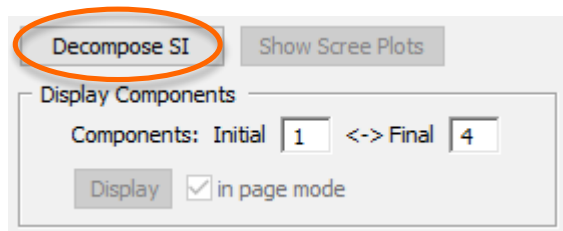
1. First, click the **Select SI** button in the *region 1* of the dialog, then the dialog for file open appears.



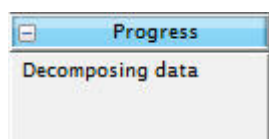
You can select the desired SI file and click the **Open** button. The selected file name appears in the string field with the absolute path



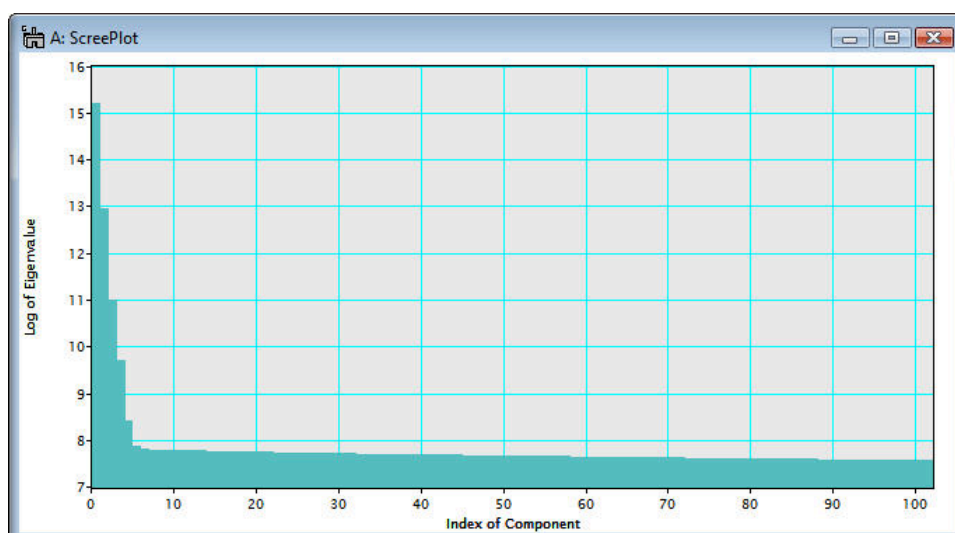
2. Once the data is selected, the **Decompose SI** button is activated in the *region 2* of the dialog.



Then, click the **Decompose SI** button. This process would take a few seconds to a few minutes depending on your CPU speed and the data size. As long as the progress window displays the following message, your PC is NOT frozen.



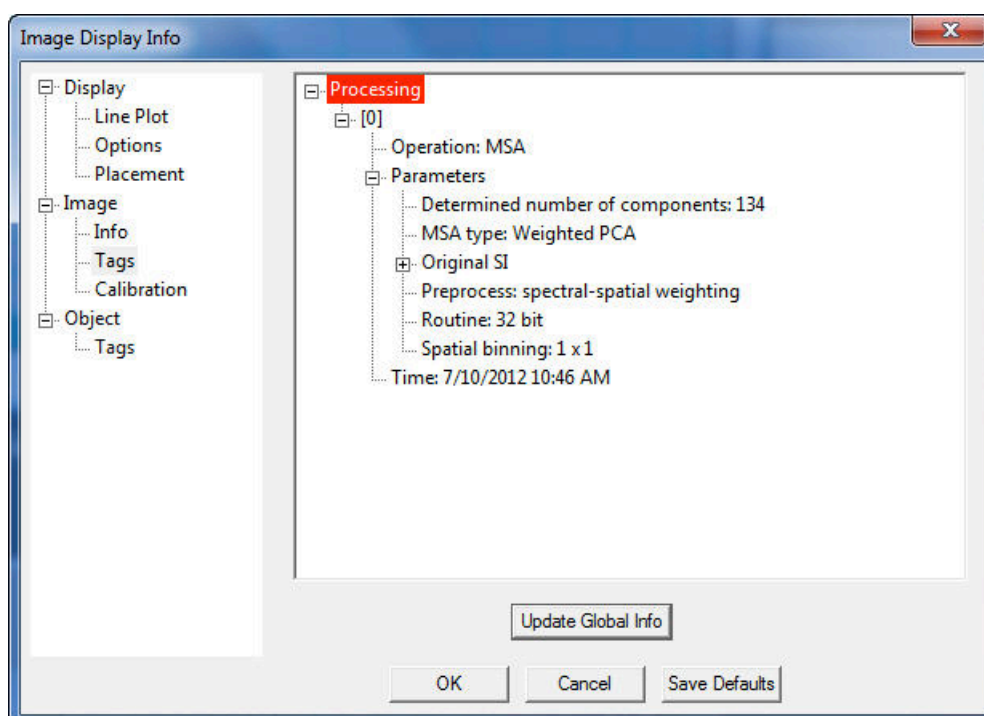
Just wait until the scree plot is displayed as shown below:



Note that the scree plot is logarithm of the eigenvalues graphically plotted against the index of component. A number of principal components is usually evaluated based on the scree plot. In the above example, a number of the principal components distinguished from the noise components can be 6 or 7 from the primary principle component.

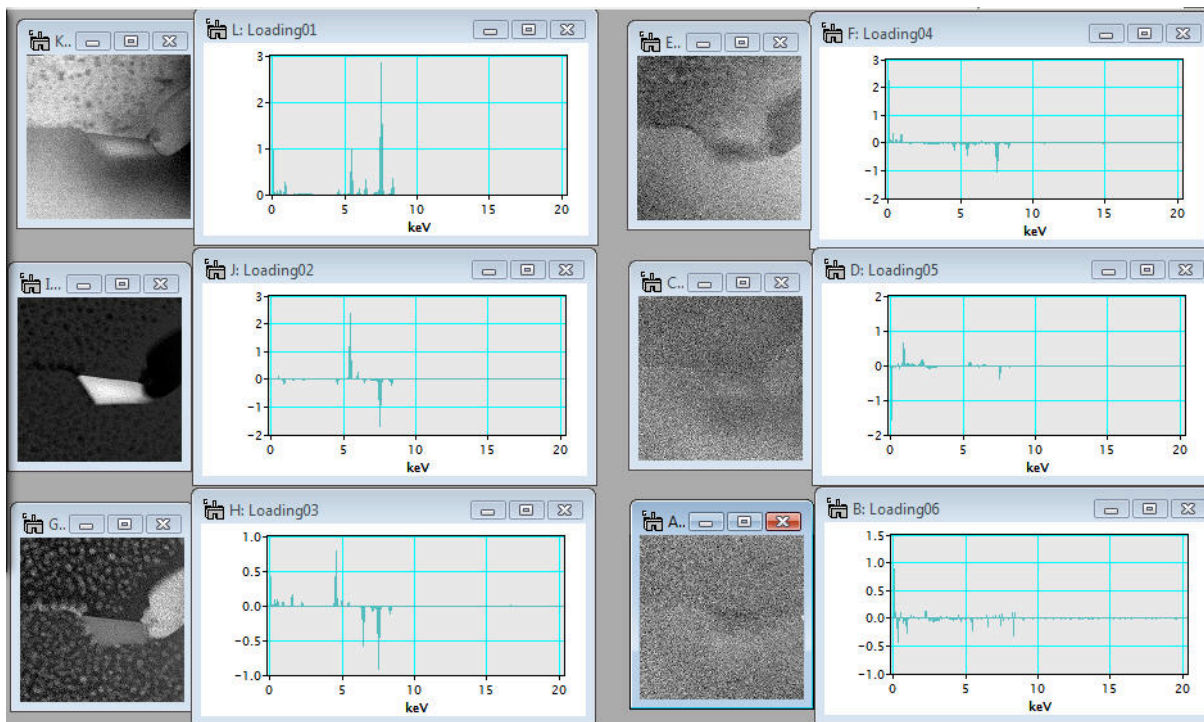
Once the data has been decomposed, the **Show Scree plot** button is activated in the *region 2*. When you have closed the scree plot window, you can open again the window clicking the **Show Scree plot** button.

Tips: Detailed information of this Weighted PCA process can be found in **Operation: MSA** tag under the **Processing** Tag of the Scree Plot, available through **Image Display Info**.

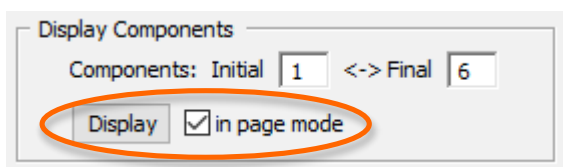


It is essential to evaluate each component in terms of a pair of a loading spectrum (spectral feature) and a score image (spatial amplitude) in order to distinguish the principal components from noise. The function of displaying components is one of main features of this plugin.

In the example below, the spectral feature and spatial amplitude of each component are displayed individually (Note: in this example, the components are rearranged manually to show all six of them).

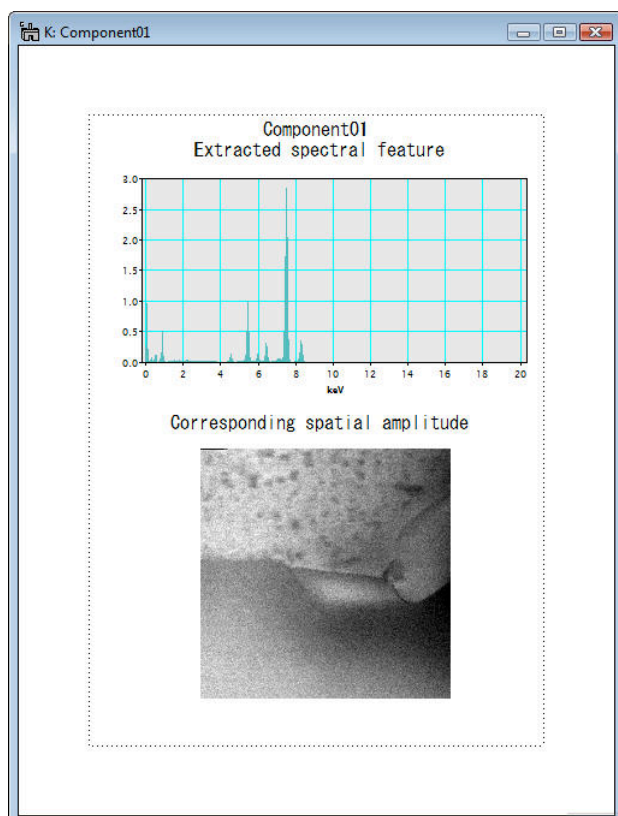


- After the data is decomposed, the **Display** button in *Display components* is activated in the **region 3** of the dialog.

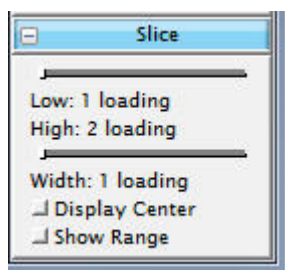


To view components, input the component range you want to evaluate in the Initial and Final boxes and click the **Display** button. When the check box **Display in page mode** is checked in the **region 3**, the spectral feature (loading) and spatial amplitude (score) can be displayed together in the page mode.

As in the example below, the spectral feature and spatial amplitude of each component are displayed as an image stack (Slice). Here, the spectral feature and spatial amplitude of the first component (Component 1) are displayed in one page (page mode).



In order to check other extracted components, you can use the slice tool, which is used to manipulate 3D data cubes such as spectrum images. Since the spectral feature and spatial amplitude are displayed as an image stack, the slice tool becomes active, when you select the spectral feature or spatial amplitude in the page mode.

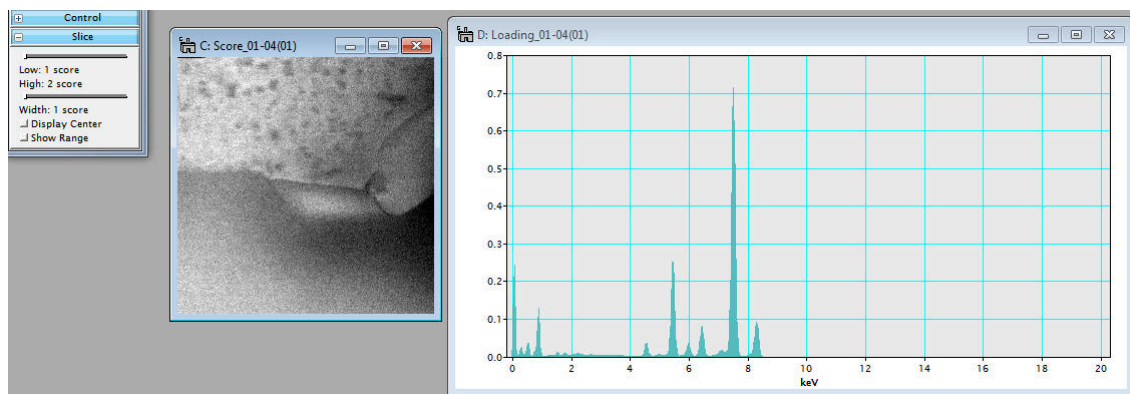


By sliding the index of component in the slice-tool pane, a desired component can be displayed.

Tips: To extract a displayed component, select **MSA...Extract Component(s)...Current Component** from the menu bar. Then, the displayed component (both the loading spectrum and score image) is extracted.

If you want to extract all the components currently contained in the page mode stack, select **MSA...Extract Component(s)...Extract All** from the menu bar.

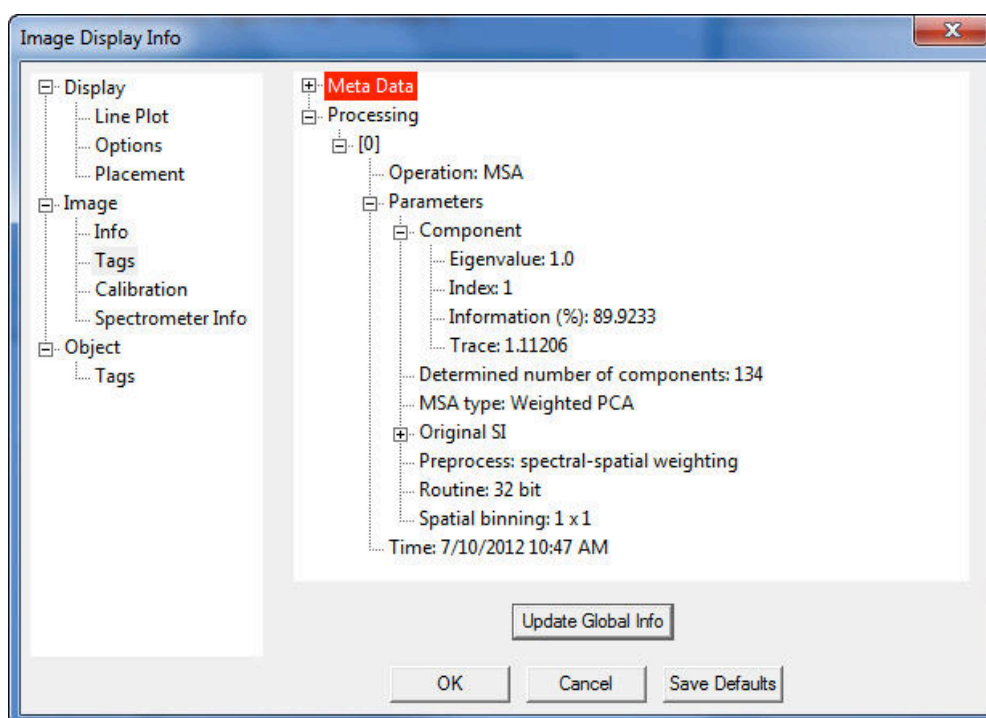
Alternatively, the image stacks of spectral features (loadings) and spatial amplitudes (scores) can also be displayed separately (not a page mode), if the **Display in page mode** is unchecked.



Again, using the slice tool, an individual loading and score of the components can be evaluated. (The slices of the loading and score changes simultaneously, when you change one of them by using the slice tool.)

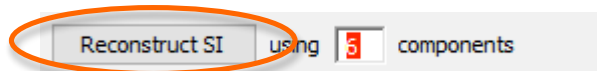
In order to extract individual or all components, select **MSA...Extract...Component(s)...** **Current Component** or **MSA...Extract Component(s)...Extract All** from the menu bar.

Again, detailed information of the individual components including this weighted PCA process can be found in **Operation: MSA** tag under the **Processing** Tag of the Loading spectra or Score images, available through **Image Display Info**.

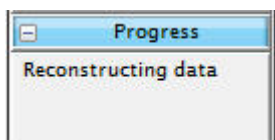


It may be noted that the **information (%)** tag under the Component tag indicates the fraction of information that this particular component (index 1 in the above example) contains in this SI data.

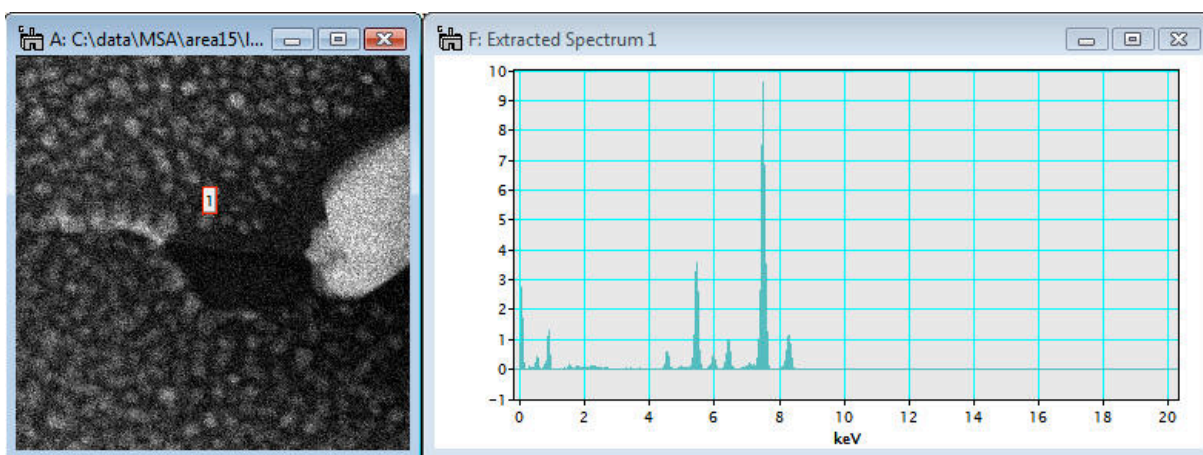
4. After the data is decomposed, the **Reconstruct SI** button is also activated in the *region 4* of the dialog



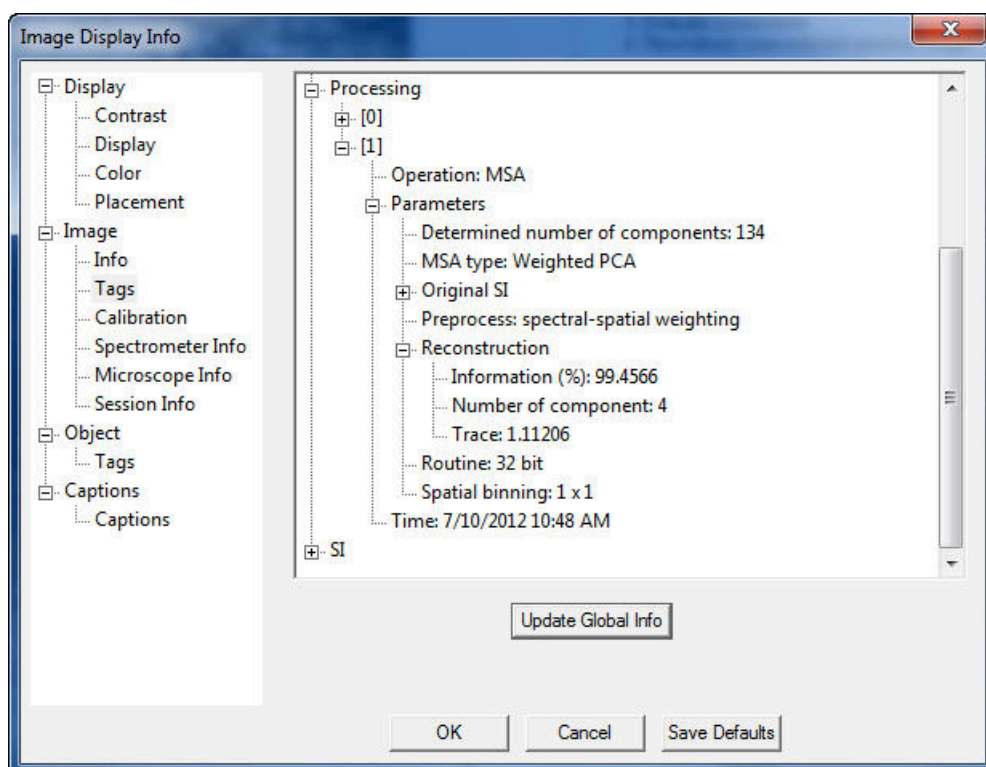
By evaluating of individual components, principal components can be distinguished from noise components and it is possible to reconstruct the spectrum image data with reduced noise. Input the last index of principal component in the box and click the **Reconstruct SI** button. Then, data reconstruction process starts. This process could take longer depending on the using PC and data size. However, if the following message appears in the progress window, the process is progressing.



After the reconstruction process is completed, the reconstructed spectrum image is displayed.

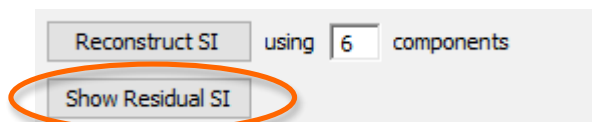


Detailed information of the reconstruction process can also be found in in **Operation: MSA ... Reconstruction** tag under the **Processing** Tag of the Reconstructed SI file, available through **Image Display Info**.



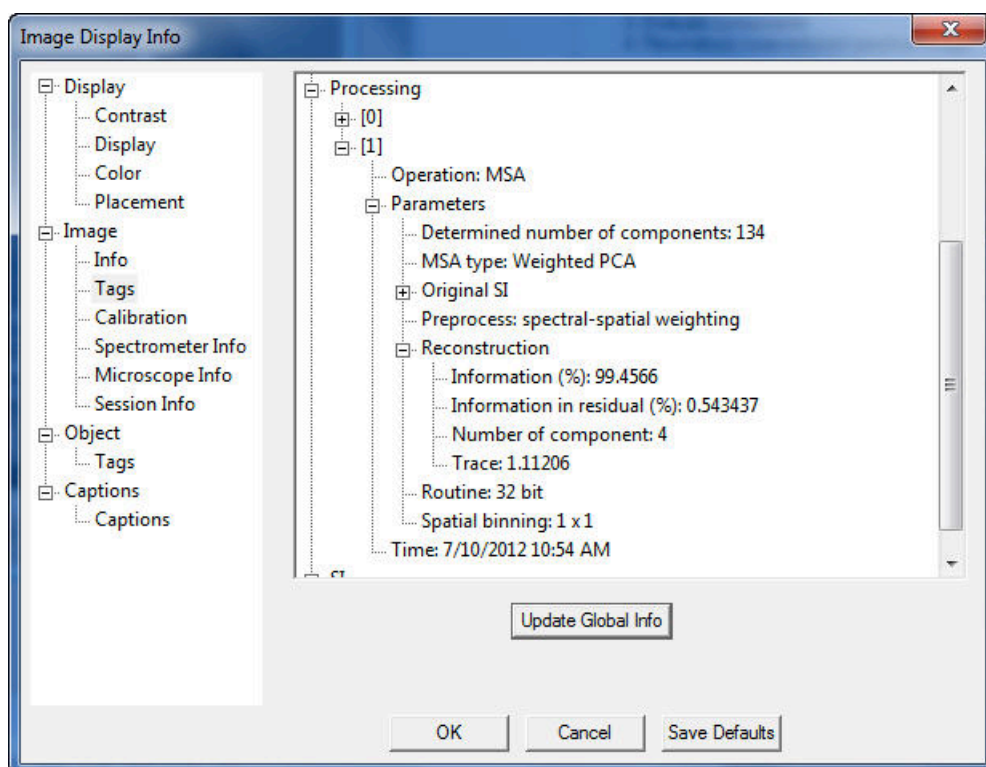
The fraction of information contained in the reconstructed SI file over the original SI file can be found in **Information (%)** tag under the Reconstruction tag.

5. Now, the **Show Residual SI** button is finally activated in the *region 5* of the dialog.



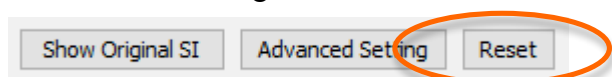
The residual SI is the difference between the original and reconstructed SIs. Therefore, the validity of reconstruction can be confirmed by comparing the residual SI with the original and reconstructed SIs.

Detailed information of the reconstruction process can also be found in in **Operation: MSA ... Reconstruction** Tag under the **Processing** Tag of the Reconstructed SI file, available through **Image Display Info**.



The fraction of information contained in the residual SI file over the original SI file can be found in **Information in residual (%)** tag under the **Reconstruction** tag.

Note that you can reset the parameters in this dialog by clicking the **Reset** button at the bottom of the dialog



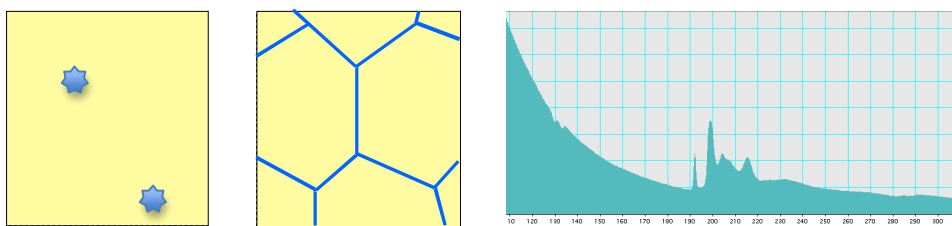
The summary of each step described above can be seen in the **Result** window.



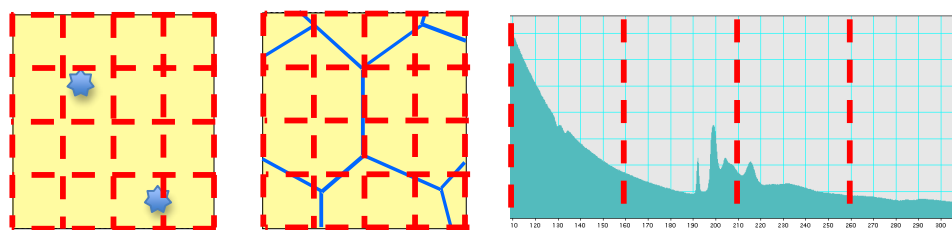
Local PCA

Brief Description

PCA (Principal Component Analysis) of the MSA plug-in is briefly explained above. The PCA approach tries to explain the data variation (variance) as much as possible using a small number of components. If the variance in random surpasses any fluctuation of true signals, the PCA may not successfully extract the signals as components. In the worst case, PCA might induce unexpected artefacts. Such situations might arise when you are analysing SI datasets containing minor or trace-level impurities in matrix, interface components, or weak signals in a wide variation of the background:



In general, there may be two possible approaches to improve the PCA sensitivity: one is to reduce random noise components in the SI datasets and other is to enhance true variations. The former requires modifications in experimental conditions, e.g. higher currents and/or a longer acquisition time to enhance signals (remember, more signal counts are always beneficial even for PCA!). Conversely, the latter can be achieved by applying PCA treatment to small segments divided from an original SI dataset. We provide this functionality in this MSA plug-ins, which is now called the local PCA approach. The segments can be achieved spatially and spectrally as shown below.



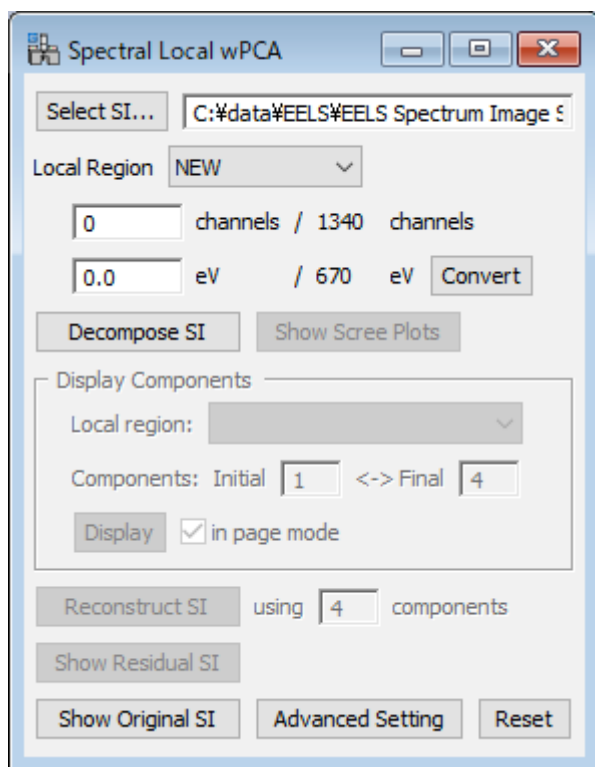
The spatially local PCA approach might enhance moderate signals, which are not repeated very frequently in the SI datasets. For example, signals from interface segregant elements on grain boundaries with relatively large grain components (Note: in this case, we also recommend to extract a part of the SI dataset in the vicinity of the grain boundaries).

The spectrally local PCA is useful to enhance weak signals in a SI dataset, in which higher signal intensities are also contained, e.g. characteristic edge-signals at a high energy-loss region with intense signals and background at lower energy-loss regions (i.e. most of EELS SIs!).

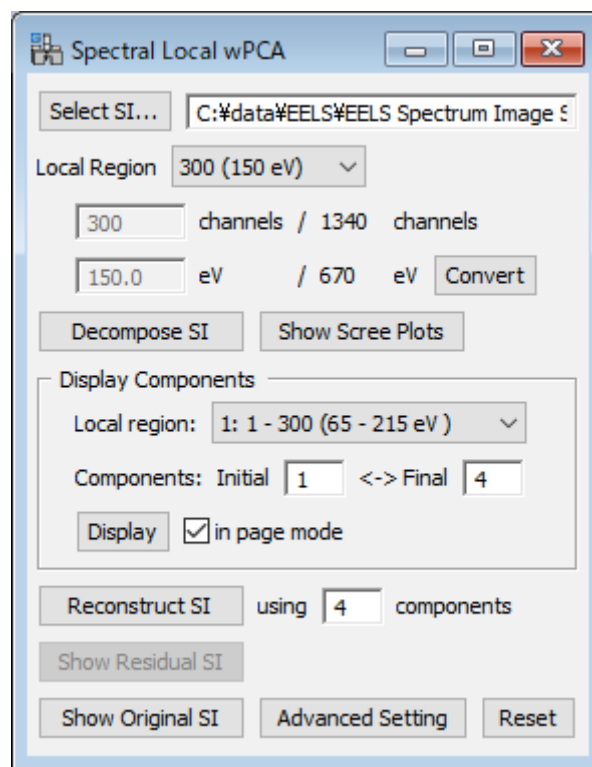
Both local PCA approaches are available in the MSA plug-in. Users can decide the division direction (spatially or spectrally) and a number of divided segments prior to applying the local PCA method. Then, all the local segments are processed sequentially, and component evaluation and following data reconstruction of all the segments can be carried out together.

Spectrally Local wPCA

The dialog below corresponds to Spectrally Local weighted PCA. Most of the components of this dialog are essentially similar to those in the regular weighted PCA dialog.



When you start new processing.



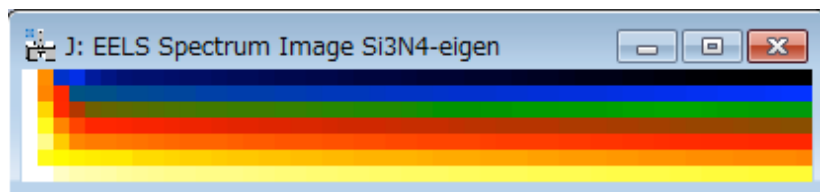
When you re-analyse a previously processed dataset.


At first, select a 2D or 3D spectrum image (SI dataset) in the Gatan dm3 or dm4 format in the folder that is exclusive to the data file to be processed. All the data files of local PCA will be saved to the same folder. Therefore, it is mandatory to select a folder for the specific SI dataset.

Then, select “NEW” from the Local Region pull-down, define a segment size by specifying either a number of channels or an energy width in eV. In either case, you can convert one to other by clicking the **Convert** button. The odd channels will be included in the last segment. In the example above (see the right figure), if the original SI dataset (1340 channels) is divided spectrally into the segments of 300 channels, then the last segment contains 440 channels.

Simply by clicking the **Decompose SI** button, you can decompose all the local segments one-by-one automatically.

After decomposition process is completed, you can check all Scree plots of individual segments by using **Show Scree Plots** button, similar to the regular PCA. Since the scree plot is independent at each local segment, you can obtain scree plots as many as the number of divided segments. So, **Show Scree Plots** displays all the scree plots of local regions as a 2D SI file shown below:



Each horizontal line from top to bottom corresponds to the scree plot obtained for a local energy-loss region from low to high. You can use the *Spectrum Picker* tool  to select a specific scree plot, similar to a 2D SI file.

Note: You may see that a number of the scree plots is larger than a number of segments defined in the dialog. This is because the local segments are shifted halfway and overlapped with neighbour segments in processing to avoid a disruption at the boundary of each segment.

You can evaluate loading and score of components of each local segment by selecting the region from **Local region** pull-down, and specifying the components to be displayed, and then click **Display** button in the similar way to the regular PCA.

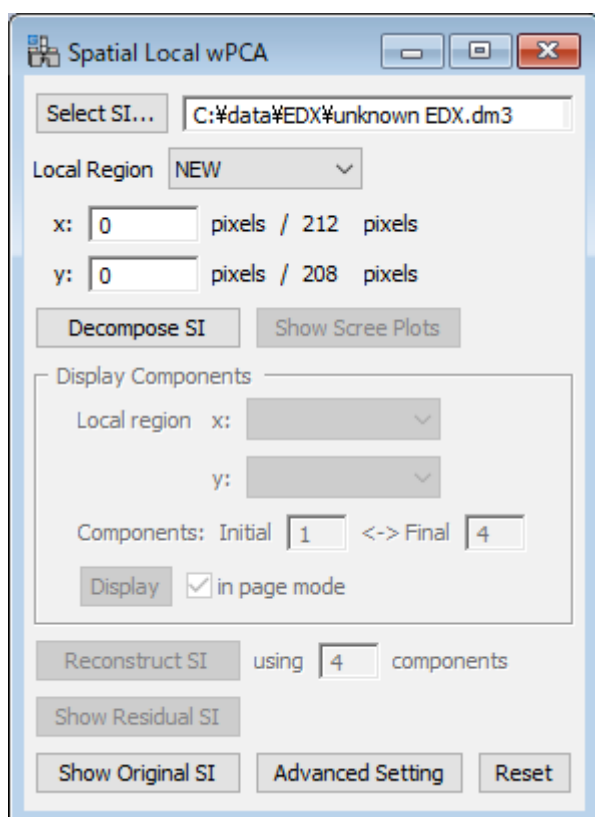
Tips: From the pull-down to select a specific region you may find that the local segments shifted halfway are also processed.

Using **Reconstruct SI** you can reconstruct the *whole* SI data using the specified number of the components. In the current version, the selected number of the components is used for reconstruction at all the local segments, i.e. reconstruction is performed with the same number of the components for each segment

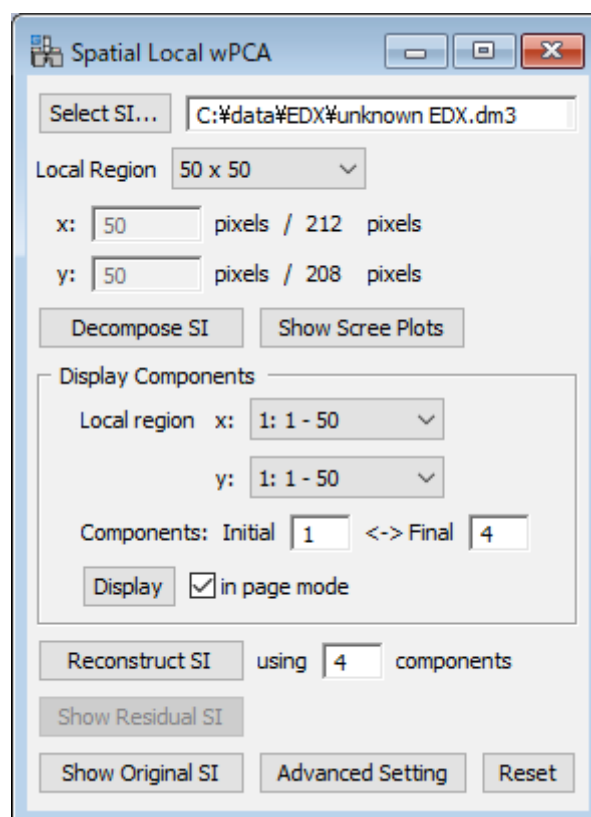
When you have processed the data, you can analyse the results at any time by selecting the processed SI data and then selecting the region from the Local Region pull-down as shown in the right Spectral Local wPCA dialog.

Spatially Local wPCA

The dialog below corresponds to Spatially Local weighted PCA. Most of the components of this dialog are essentially similar to those of the regular PCA dialog.



When you start new processing.



When you re-analyse a previously processed dataset.

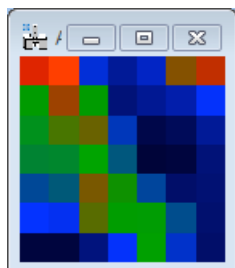
At first, select a 2D or 3D spectrum image (SI dataset) in the Gatan dm3 or dm4 format in the folder that is exclusive to the data file to be processed. All the data files of local PCA will be saved to the same folder. Therefore, it is mandatory to select a folder for the specific SI dataset.

When you select “NEW” from the Local Region pull-down, you can define a segment area size by selecting a number of pixels. The odd pixels will be included in the last segment. In the example above (see the right figure), if the original SI dataset (212 by 208 pixels) is divided spatially into the segments of 50 by 50 pixels, then the segments at the right edge contain 62 by 50 pixels, and the segments at the bottom edge contain 50 by 58 pixels except the right-bottom segment that contains 62 by 58 pixels.

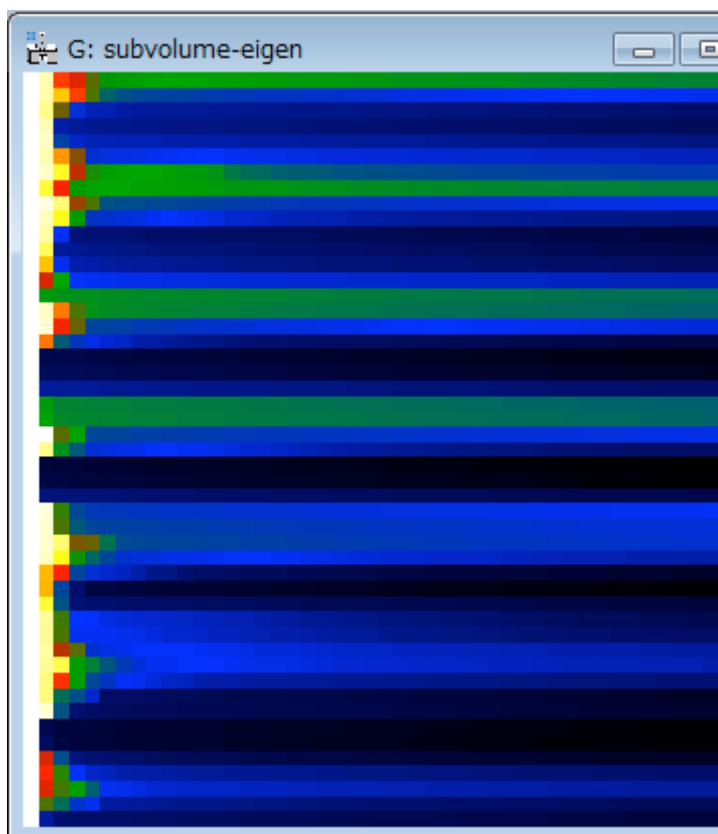
Simply by clicking the **Decompose SI** button, you can decompose all the local segments one-by-one automatically.


After decomposition process is completed, you can check all Scree plots of individual segments by using **Show Scree Plots** button, similar to the regular PCA. Since the scree plot is independent at each local segment, you can obtain scree plots as many as the number of segments. So, **Show Scree Plots** displays all the scree plots of local regions in two different formats: 3D and 2D SI files shown below:


3D scree plot



2D scree plot



In the 3D Scree Plot (left), each pixel corresponds to a spatially arranged single local region, and the direction of the scree plot (i.e. the index of component) is arranged along the z-direction. Namely, a certain position in the 3D scree-plot indicates the scree plot of the specific segment. You can use *Spectrum Picker* tool  to select a specific scree plot, similar to a 3D SI file.

In the 2D scree plot file (right), each horizontal line corresponds to an individual scree plot of a single divided region. Each horizontal line from top to bottom of the 2D scree plot corresponds to the 3D scree plot from top-left to bottom-right. Thus, we can see all the scree plot at the same time. You can use *Spectrum Picker* tool  to select a specific scree plot, similar to a 2D SI file.

Tips: Since the eigenvalues are arranged side-by-side, a magnitude of a certain eigenvalue can be directly compared with other eigenvalues from adjacent segments. It should be noted that both the 2D and 3D scree-plots represent the same result, but only the display style is different. For example, in this example, the 3D scree plot shows the forth slice of the 3D data, which corresponds to the forth pixels of each 2D scree plot.

Note: You may see that a number of the scree plots is larger than a number of segments defined in the dialog. This is because the local segments are shifted halfway and overlapped with neighbour segments in processing to avoid a disruption at the boundary of each segment.

You can evaluate loading and score of components of each local segment by selecting the region from **Local region** pull-down, and specifying the components to be displayed, and then click **Display** button in the similar way to the regular PCA.

Tips: From the pull-down to select a specific region you may find that the local segments shifted halfway are also processed.

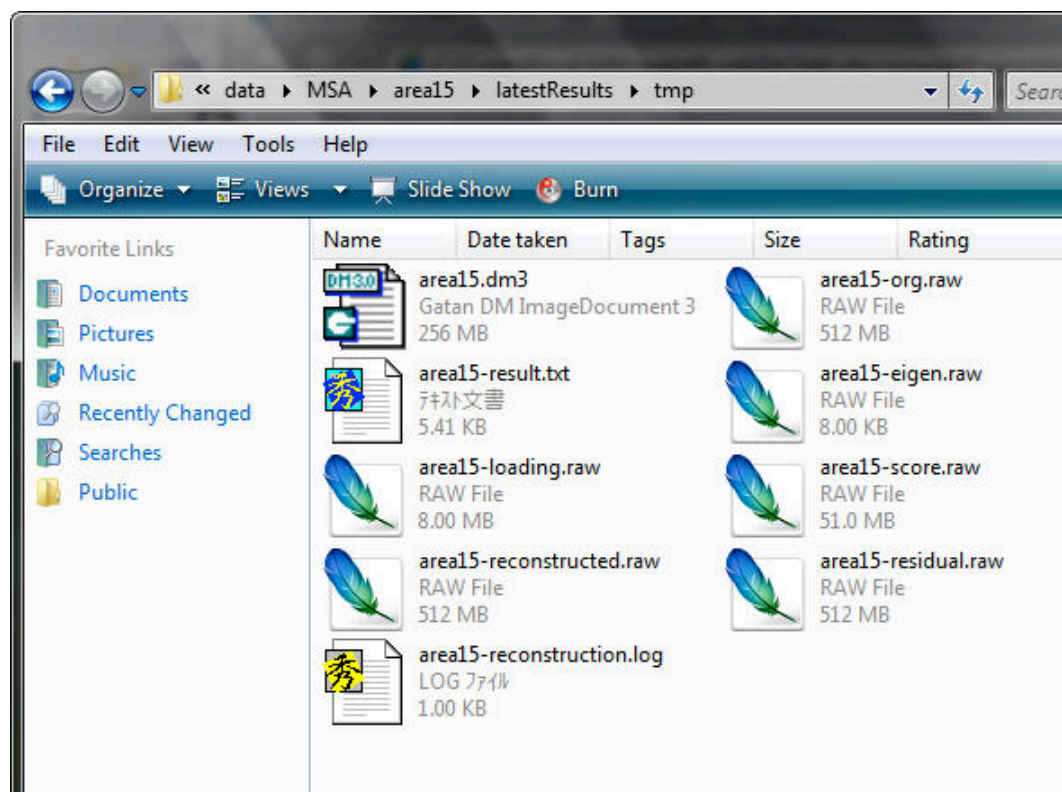
Using **Reconstruct SI** you can reconstruct the *whole* SI data using the specified number of the components. In the current version, the selected number of the components is used for reconstruction at all the local segments, i.e. reconstruction is performed with the same number of the components for each segment.

When you have processed the data, you can analyse the results at any time by selecting the processed SI data and then selecting the region from the Local Region pull-down as shown in the right Spectral Local wPCA dialog.

MSA – Advanced Topics

Output files

Many output files are generated from a single spectrum image by this PCA plugin. Here is the summary:



If a spectrum image file in the dm3 format named foo.dm3 is processed, following files are generated:

- foo-org.raw: original data converted from the original dm3 file (8 byte real, binary)
- foo-result.txt: summary of eigenvalues for scree plot (ascii)

```
# C:\data\MSA\area15\latestResults\area15-result.txt: Results of eigen analysis↓
# Preprocessing: 0 (Spatial and spectral scalings)↓
# Spatial binning: 3 (Spatially 4 x 4)↓
# Routine: 64 bit↓
# dimensions of original spectrum image↓
# nPixelX: 256↓
# nPixelY: 256↓
# nChannel: 1024↓
# dimensions of result vectors by eigen decomposition↓
# Score: 65536x102↓
# Loading: 1024x102↓
# Eigenvalue: 102↓
# trace = 1.3950591↓
# eigenvalue      C ratio      absolute eigenvalue ↓
1 1.00000000      0.71681553      4194304.00000000↓
2 0.10515268      0.79219060      441042.30608604↓
3 0.01474060      0.80275689      61826.54907351↓
4 0.00401008      0.80563138      16819.48802894↓
5 0.00110764      0.80642535      4645.77184136↓
6 0.00064896      0.80689054      2721.94933466↓
7 0.00060927      0.80732727      2555.47619955↓
8 0.00060500      0.80776094      2537.53892223↓
```

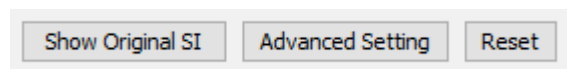
- foo-eigen.raw: full eigenvalues (8 byte real, binary)
- foo-loading.raw: limited loading matrix (8 byte real, binary)

- foo-score.raw: limited score matrix (8 byte real, binary)
- foo-reconstructed.raw: reconstructed data matrix (4 byte real, binary)
- foo-residual.raw: area15-residual.raw: difference between original and reconstructed data matrices (4 byte, binary)
- foo-reconstruction.log: summary of reconstruction (ascii)

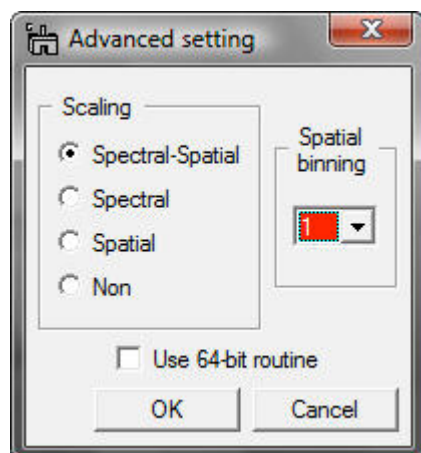
```
# C:\data\MSA\area15\latestResults\area15-reconstruction.log: detail information of data reconstruction via MSA
# dimensions of original spectrum image
# nPixelX: 256
# nPixelY: 256
# nChannel: 1024
# dimensions of result vectors by eigen decomposition
# Score: 65536x1024
# Loading: 1024x1024
# Eigenvalue: 1024
# number of components selected for data reconstruction: 7
# number of non-zero data: 6792197
# chi square value between original and reconstructed SIs: 0.751857
#
# result file names
# - area15-org.raw: original data (8 byte real, binary)
# - area15-loading.raw: limited loading matrix (8 byte real, binary)
# - area15-score.raw: limited score matrix (8 byte real, binary)
# - area15-eigen.raw: full eigenvalues (8 byte real, binary)
# - area15-result.txt: summary of eigenvalues for scree plot (ascii)
# - area15-reconstructed.raw: reconstructed data matrix (4 byte real, binary)
# - area15-residual.raw: difference between original and reconstructed data matrices (4 byte, binary)
#
```

Advanced Setting

Several parameters for pre-processing/processing can be chosen in this plugin. If you prefer to change those settings, click **Advanced Setting** button.



Then, a sub dialog appears.



Caution: *If you do not have any idea, DO NOT CHANGE THESE PARAMETERS (KEEP THE DEFAULT SETTING)!*

Scaling: selection of scaling is extremely important for proper PCA applications. The detail can be seen in literature (R.N. Cochran & F.H. Home, "Statistically weighted principal component analysis of rapid scanning wavelength kinetics experiments", *Anal. Chem.*, 49 (1977) 846-853; M.R. Keenan, P.G. Kotula, "Accounting for Poisson noise in the multivariate analysis of ToF-SIMS spectrum images", *Surf. Interface Anal.*, 36 (2004) 203-212).

Binning: you can apply spatial binning to enhance your spectral features from a spectrum image with many pixels. If the spectrum image has many pixels, you may apply spatial binning of 2 or 4. However, this preprocess does not degrade spatial resolution in the final decomposed data.

Use 64-bit routine (Only for GMS 1.x): the major limit of the 32-bit DigitalMicrograph is memory size to allocate continually (about 2GB). If you use DigitalMicrograph 32bit version under 64-bit Windows, this check box is automatically activated. If this option is checked, this plugin uses a 64-bit PCA routine via a system call. This 64-bit routine can expand the limitation up to whatever your PC has (now 4GB is just a standard in any 64-bit system). Other than that, this 64-bit routine works exactly same as this plugin. If data size is larger than 2 GB, this 64-bit routine can process much faster than the 32-bit routine. If data size is larger than 8 GB, this 64-bit routine is only way to process the data in this plugin. *In the MSA package for GMS 2.x, this option is no longer necessary because GMS 2,x 64bit is available!*

References related to the PCA process

General references related to the PCA process:

1. S.J. Gould, *The Mismeasure of Man*, Norton, New York, NY, (1996).
2. P. Geladi & H. Grahn, *Multivariate Image Analysis*, Wiley, West Sussex, UK, (1996).
3. J.T. Jolliffe, *Principal Component Analysis*, 2nd ed., Springer, New York, NY, (2002).
4. E.R. Malinowski, *Factor Analysis in Chemistry*, 3ed ed., Wiley, New York, NY, (2002).

Specific references of PCA applications for electron microscopy:

1. P. Trebbia & N. Bonnet, "EELS elemental mapping with unconventional methods I. Theoretical basis: Image analysis with multivariate statistics and entropy concepts", *Ultramicrosc.*, 34 (1990) 165-178.
2. J.M. Titchmarsh & S. Dumbill, "Multivariate statistical analysis of FEG-STEM EDX spectra", *J. Microsc.*, 184 (1996) 195-207.
3. N. Bonnet, N. Brun & C. Colliex, "Extracting information from sequences of spatially resolved EELS spectra using multivariate statistical analysis", *Ultramicrosc.*, 77 (1999) 97-112.
4. J.M. Titchmarsh, "Detection of electron energy-loss edge shifts and fine structure variations at grain boundaries and interfaces", *Ultramicrosc.*, 78 (1999) 241-250.
5. P.G. Kotula, M.R. Keenan & J.R. Michael, "Automated analysis of SEM X-ray spectral images: a powerful new microanalysis tool", *Microsc. Microanal.*, 9 (2003) 1-17.
6. M.R. Keenan, P.G. Kotula, "Accounting for Poisson noise in the multivariate analysis of ToF-SIMS spectrum images", *Surf. Interface Anal.*, 36 (2004) 203-212.
7. M. Watanabe, D.B. Williams & M.G. Burke, "Atomic-Level Analytical Electron Microscopy of Diffusional Phase", in *Proc. Inter. Conf. on Solid-Solid Phase Transformations in Inorganic Materials 2005 - Vol. 2*, The Minerals, Metals and Materials Society, Warrendale, PA, pp. 431-442 (2005).
8. M.G. Burke, M. Watanabe, D.B. Williams & J.M. Hyde, "Quantitative characterization of nanoprecipitates in irradiated low alloy steels: advances in the application of FEG-STEM quantitative analysis to real materials", *J. Mater. Sci.*, 41 (2006) 4512.
9. M. Bosman, M. Watanabe, D.T.L. Alexander, and V.J. Keast, "Mapping chemical and bonding information using multivariate analysis of electron energy-loss spectrum images", *Ultramicrosc.*, 106 (2006) 1024.
10. P.G. Kotula, M.R. Keenan, J.R. Michael, "Tomographic spectral imaging with multivariate statistical analysis: comprehensive 3D microanalysis", *Microsc Microanal.*, 12 (2006) 36-48.
11. P.G. Kotula & M.R. Keenan, "Application of Multivariate statistical analysis to STEM X-ray spectral images: interfacial analysis in microelectronics", *Microsc Microanal.*, 12 (2006) 538-544.
12. M. Bosman, V.J. Keast, M. Watanabe, A.I. Maarroof & M.B. Cortie, "Mapping surface plasmons at the nanometre scale with an electron beam", *Nanotechnology*, 18 (2007) 165505 (5pp).
13. T. Yaguchi, M. Konno, T. Kamino & M. Watanabe, "Observation of three-dimensional elemental distributions of a Si-device using a 360-degree-rotation FIB/STEM system", *Ultramicrosc.*, 108 (2008) 1603-1615.

Import Functions

Three import functions are available in this plugin package. These functions import a series of spectra/images and a spectrum image obtained from different software or acquisition systems, and convert them as a single spectrum image in the dm3 format. Currently available functions are as follows:

- EMSA spectrum series import
- XED-SI data import
- EFTEM image series import

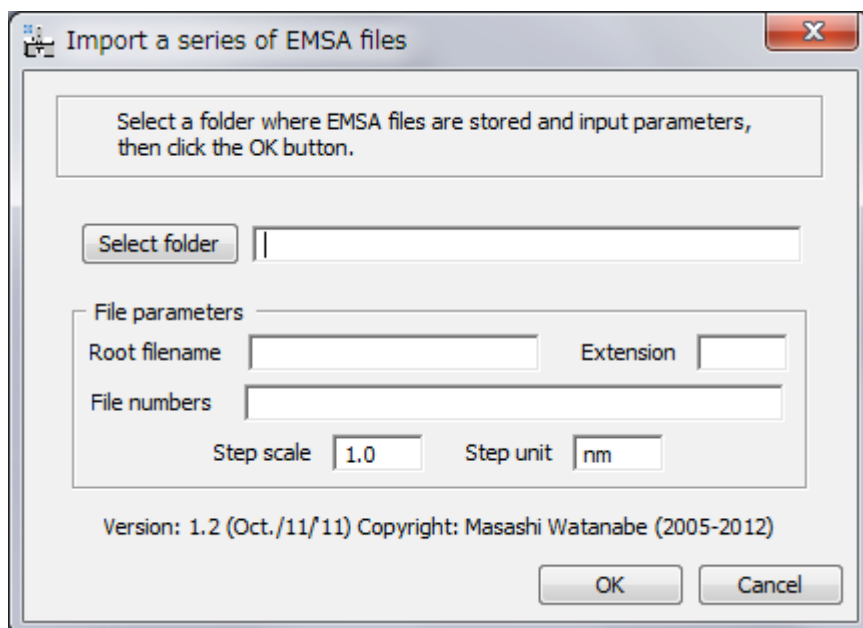
EMSA spectrum file series

Brief Description

This function imports a series of EMSA formatted X-ray spectra (i.e. a line profile) into Gatan DigitalMicrograph as a 2D spectrum image.

Usage

Select **MSA...Import...EMSA** spectrum file series from the menu bar. Then, a dialog appears.



First, you should select a folder, where a series of EMSA formatted X-ray spectra is saved, by clicking the **Select folder** button.

Second, input the rootfile name and extension name into the fields. Note that the EMSA formatted files must be named as **foojjj.ext**, where foo is the rootfile name, jjj is an index number of the series and ext is the extension name. For example, Linescan4.emsa or Spectrum10.txt. If there is a space between the rootfile name and an index number (e.g. default output name from Oxford INCA system), add a space after the root name in the Root filename box.

Third, input list of index numbers in the file numbers field, e.g. case 1: 1-100 (if you want to import 100 spectra (#1 - #100) continuously) or case 2: 1-8, 10, 12, 14, 15-48, 51-78, 80 (if

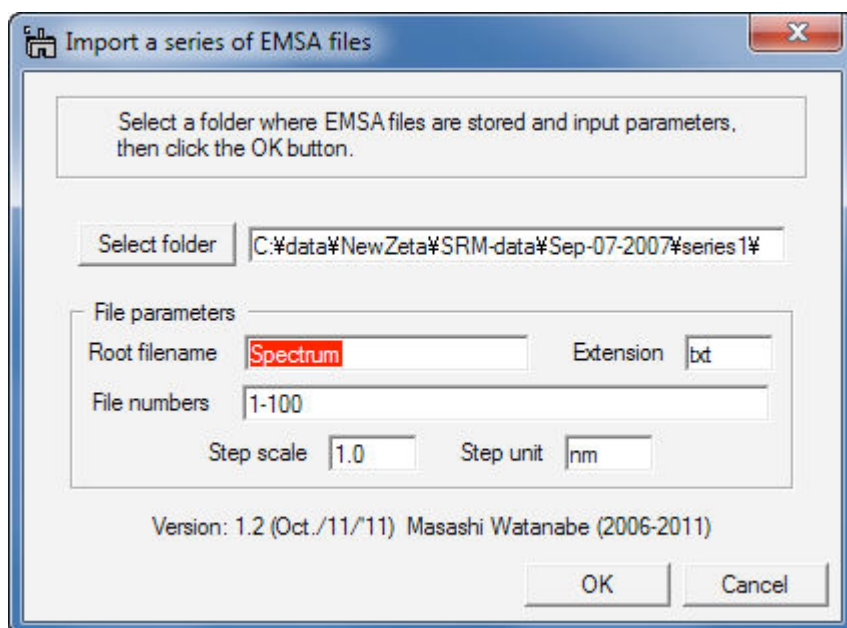
you want to skip spectrum #9, #11, #13, #49, #50 and #79). You can set the range of spectra to be imported just as setting of page range in the general print dialog.

Then, input the Step scale and Step Unit in the fields. For example, input 5.0 and nm into the fields respectively if you measured the line profile with 5 nm step.

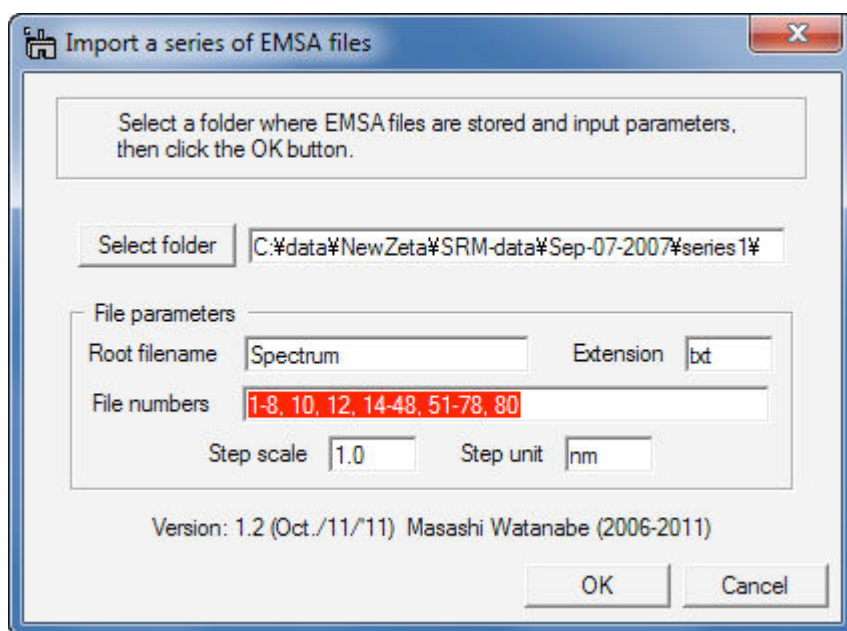
Finally, click the **OK** button.


Here is an example of the dialog just before clicking the Import button.

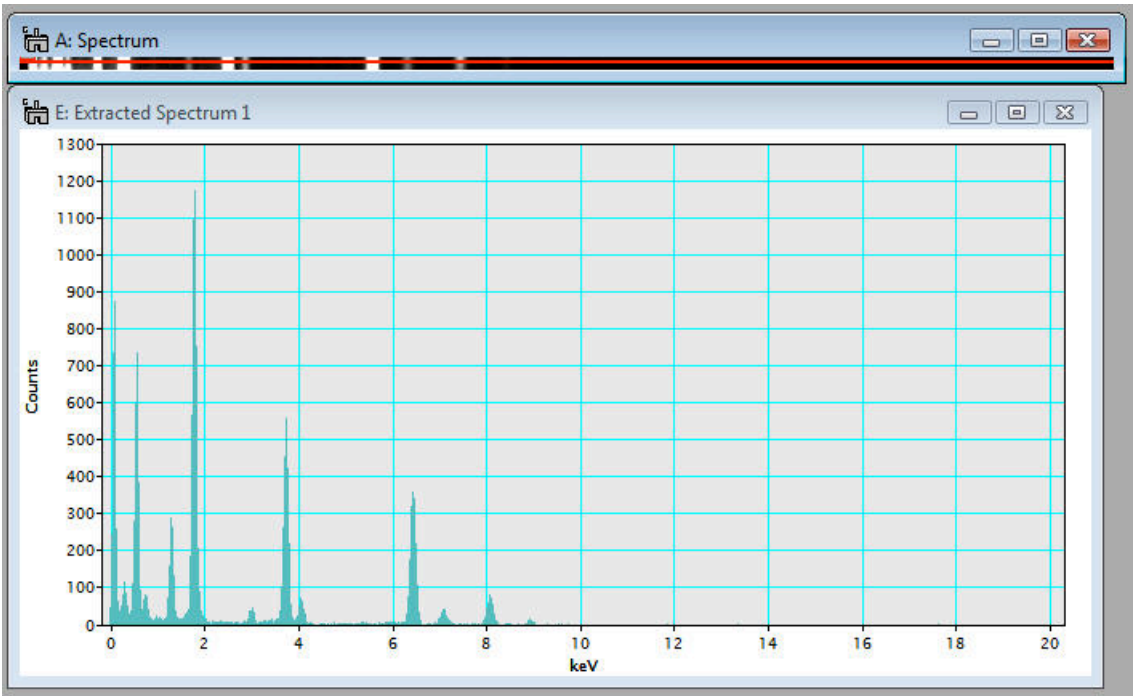
Example for case 1:



Example for case 2:



The imported file can be treated as a 2D spectrum image. So, individual spectra can be displayed via Spectrum Picker tool  in the tool bar (after DigitalMicrograph version 3.11.1) or in the Basic Tools window (before version 3.11.1) as shown below:



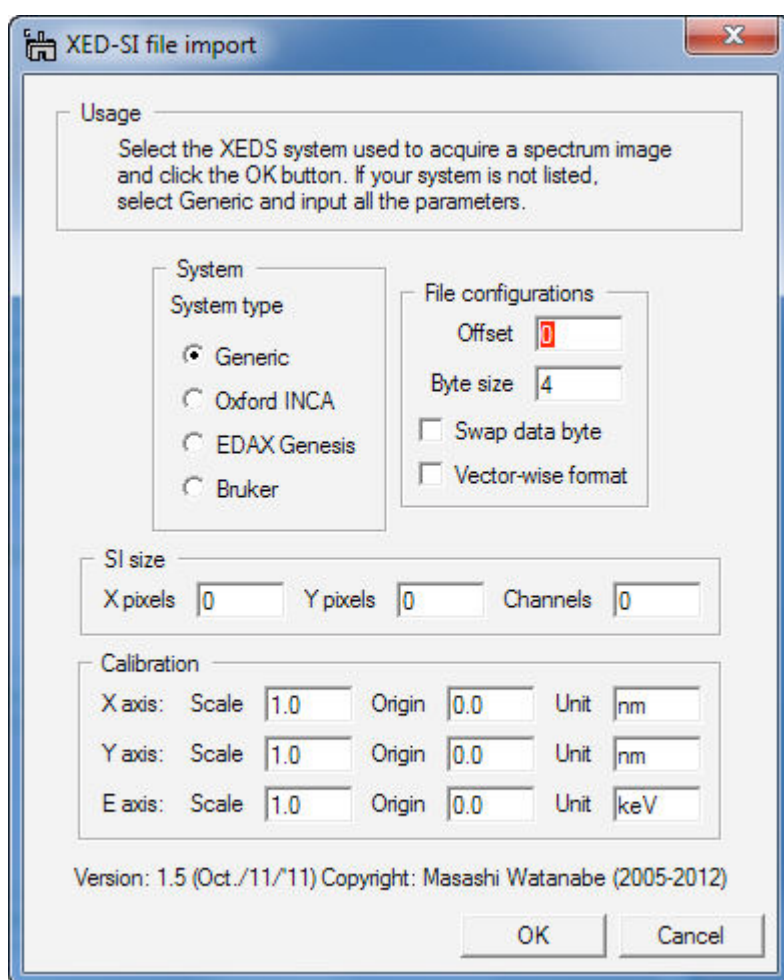
XED-SI data

Brief Description

This function imports an X-ray energy dispersive spectrum-image (XED-SI) saved in a binary format from your software for X-ray acquisition/analysis. Currently, this routine can load "foo.raw" files from Oxford INCA system and "foo.spd" files from EDAX Genesis system (where "foo" means the spectrum image file name). In addition, any "uncompressed" binary 3D data can be loaded if you can figure out the file format. You may need to consult the original software manual or manufacture whether you can save X-ray spectrum images as a regular binary file.

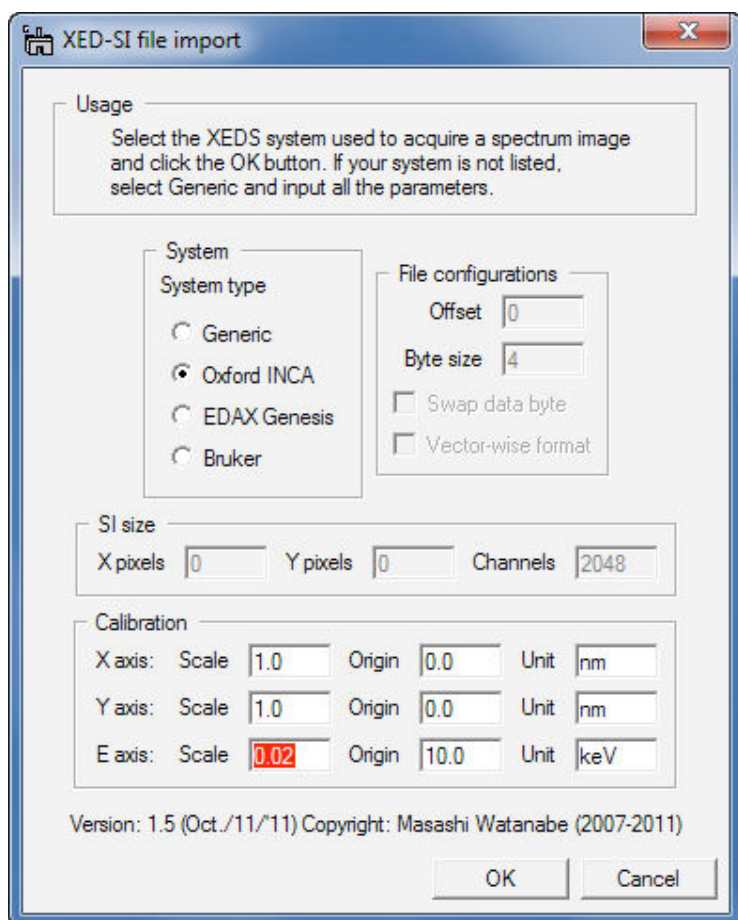
Usage

Select **MSA...Import...XED-SI data** from the menu bar. Then, a dialog appears.

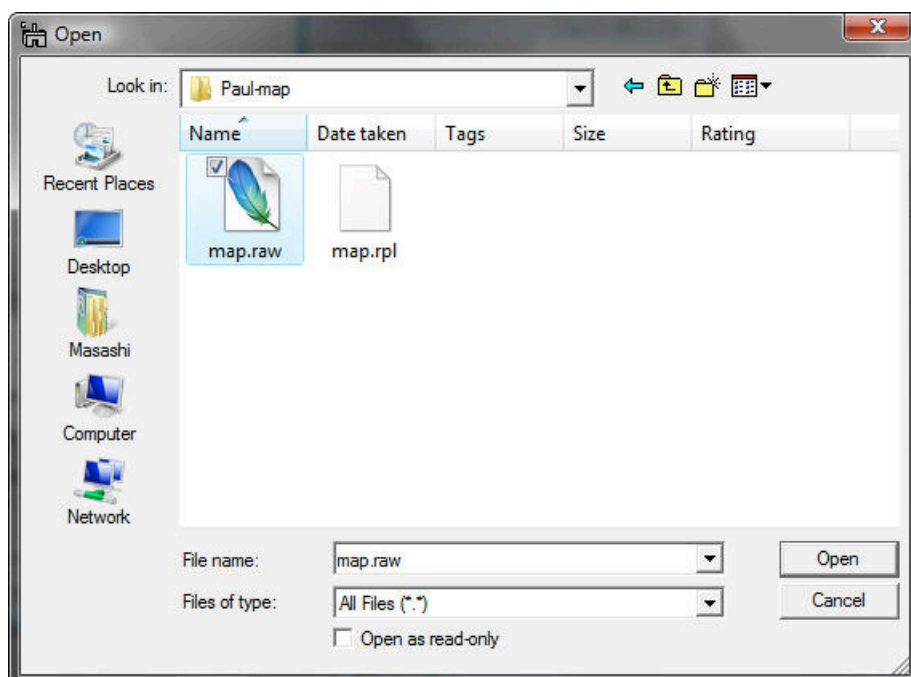


First, should select your system for X-ray analysis by clicking the radio button.

Case 1: if you select **Oxford INCA**, several fields become gray out.



You do not fill the gray fields since this function will obtain the parameters from "foo.rpl" file, which is saved with "foo.raw" file in INCA software (so, you must locate the "foo.rpl" file with the "foo.raw" file in the same folder). Now, the most important thing is to input the E-axis scale, which is the value of channel/keV. This is essential. If you leave this E-axis scale field as 0.0, you will see an error message. Then, click the **OK** button and select a desired "foo.raw" file.



Case 2: if you select **EDAX Genesis**, again several fields are gray out.

XED-SI file import

Usage
Select the XEDS system used to acquire a spectrum image and click the OK button. If your system is not listed, select Generic and input all the parameters.

System
System type
☐ Generic
☐ Oxford INCA
☒ **EDAX Genesis**
☐ Bruker

File configurations
Offset:
Byte size:
☐ Swap data byte
☐ Vector-wise format

SI size
X pixels: Y pixels: Channels:

Calibration
X axis: Scale Origin Unit
Y axis: Scale Origin Unit
E axis: Scale Origin Unit

Version: 1.5 (Oct./11/'11) Copyright: Masashi Watanabe (2007-2011)

OK Cancel

To obtain the parameters for a Genesis spectrum image, this function looks for "foo_SE1.txt" file. When X-ray spectrum image was acquired in EDAX genesis system, an electron image (such as bright-field STEM, annular dark-field STEM, or secondary electron image etc.), you should save the image as a tiff file. The "foo_SE1.txt" file is automatically saved when you save the image as tiff. You must put this "foo_SE1.txt" file into the same folder, where the corresponding "foo.spd" file is saved. Then, click the **OK** button and select a desired "foo.spd" file.

Case 3: if you select **Bruker**, several fields become gray out.

XED-SI file import

Usage
Select the XEDS system used to acquire a spectrum image and click the OK button. If your system is not listed, select Generic and input all the parameters.

System
System type
☐ Generic
☐ Oxford INCA
☐ EDAX Genesis
☒ Bruker

File configurations
Offset:
Byte size:
☐ Swap data byte
☐ Vector-wise format

SI size
X pixels: Y pixels: Channels:

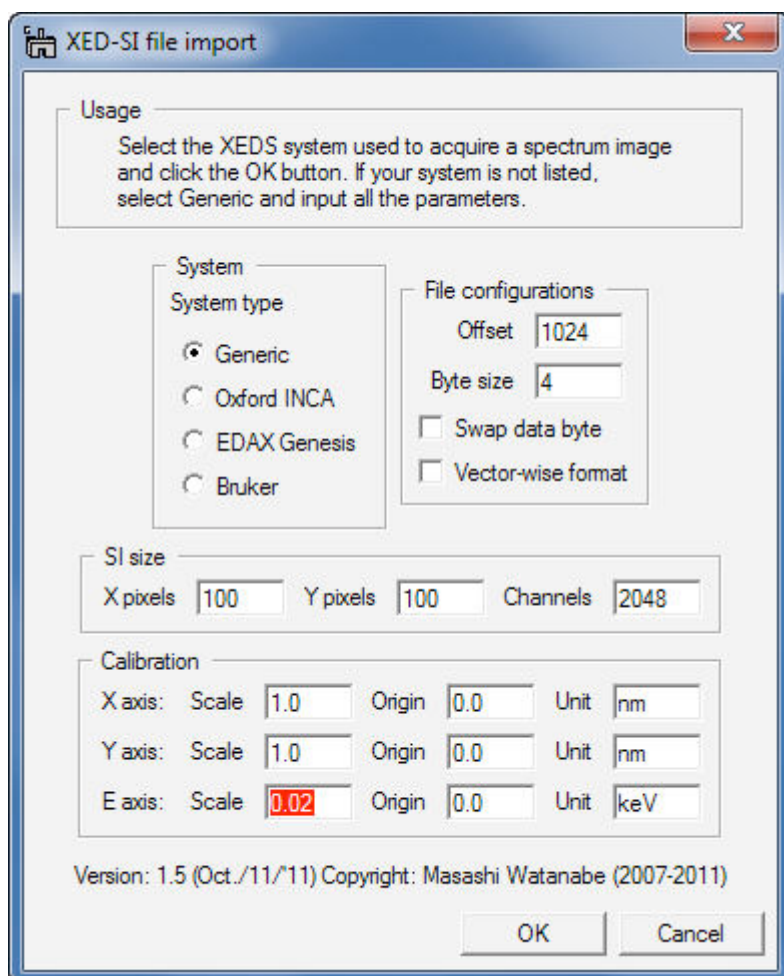
Calibration
X axis: Scale: Origin: Unit:
Y axis: Scale: Origin: Unit:
E axis: Scale: Origin: Unit:

Version: 1.5 (Oct./11/'11) Copyright: Masashi Watanabe (2007-2011)

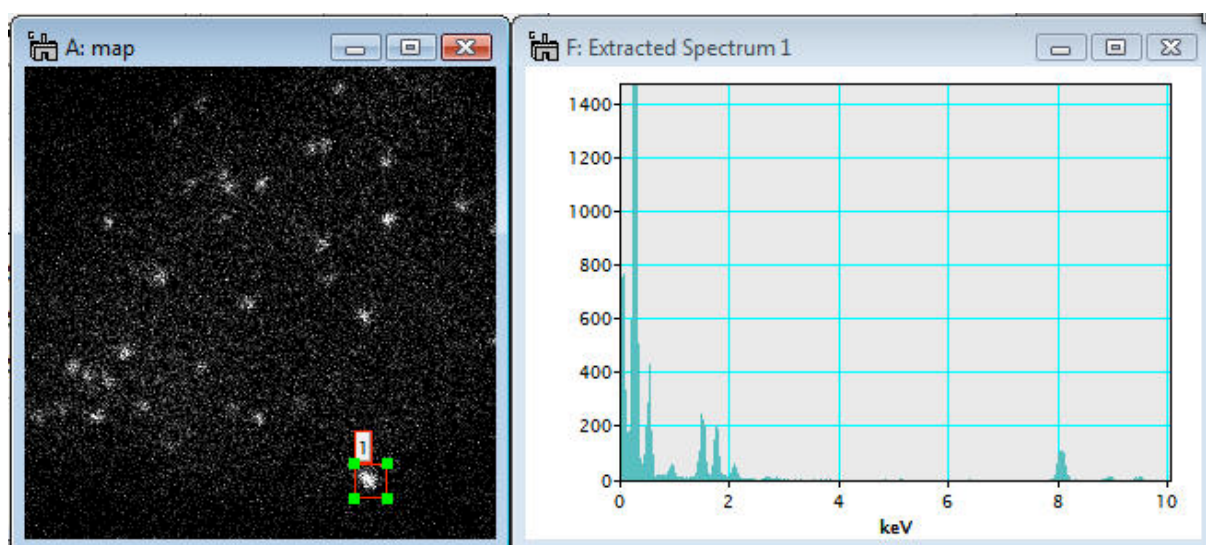
OK Cancel

You do not fill the gray fields since this function will obtain the parameters from **"foo.rpl"** file, which is saved with **"foo.raw"** file in Bruker software (so, you must locate the **"foo.rpl"** file with the **"foo.raw"** file in the same folder). Now, the most important thing is to input the E-axis scale, which is the value of channel/keV. This is essential. If you leave this E-axis scale field as 0.0, you will see an error message. Then, click the **OK** button and select a desired **"foo.raw"** file.

Case 4: if you do not have parameter files (for Oxford INCA or EDAX Genesis or Bruker), you can still import your "uncompressed" binary spectrum-image data by filling all the required parameters. Here is an example for EDAX Genesis "foo.spd" file.



Once you successfully import a binary spectrum-image data, you can treat it as a regular 3D spectrum image.



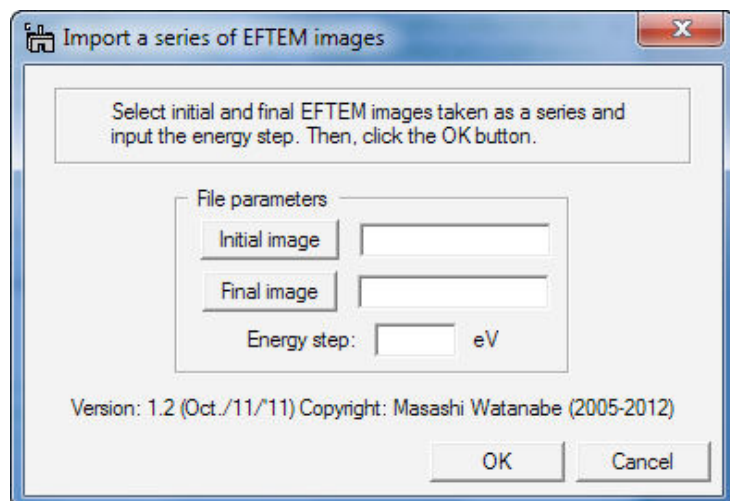
EFTEM image series

Brief Description

This function imports a series of EFTEM images acquired by the Acquire Filtered Series function in Gatan DigitalMicrograph as a spectrum image (data cube).

Usage

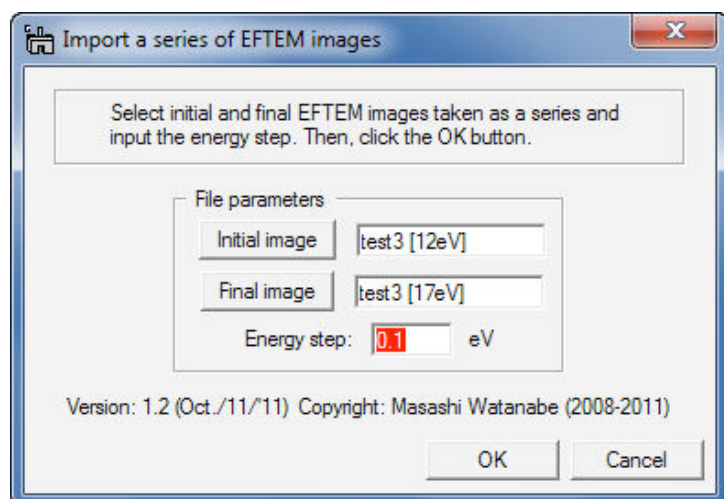
Select **MSA...Import...EFTEM image series** from the menu bar. Then, a dialog appears.



First, you should select the initial and final EFTEM images from a series of acquisition by clicking the **Initial image** and **Final image** buttons.

Second, input the energy step value in eV. Then, click the **OK** button.

Here is an example the dialog just before clicking the Convert button.



Note that the EFTEM image series must be named as **foo [jjjeV].dm3**, where foo is the rootfile name, jjj is an energy-loss value of the particular image. For example, test 3 [12eV].dm3.

The imported file can be treated as a regular 3D spectrum image.

Utilities

These functions in Utilities add additional capabilities to process spectrum images, e.g. pixel/channel binning (which are available in newer version of DM as well) and spatial/spectral data extraction. Details are as follows:

- Bin SI
- Spatial sub SI
- Spectral sub SI

Bin SI

Brief Description

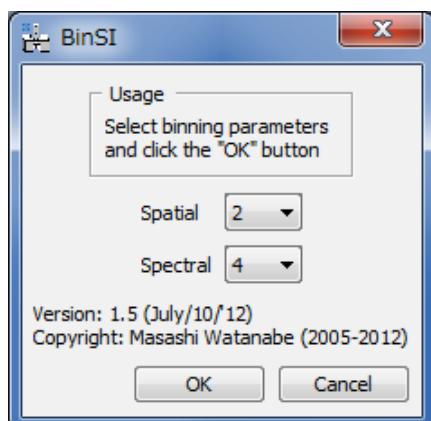
This function applies spatial (x- and/or y-axis) and/or spectral (energy-axis) data binning to an existing 2D or 3D spectrum image. Note that this function is available in recent version of GMS (ver. 1.7.1 or higher). This function is only for users who still use previous versions of GMS.

Usage

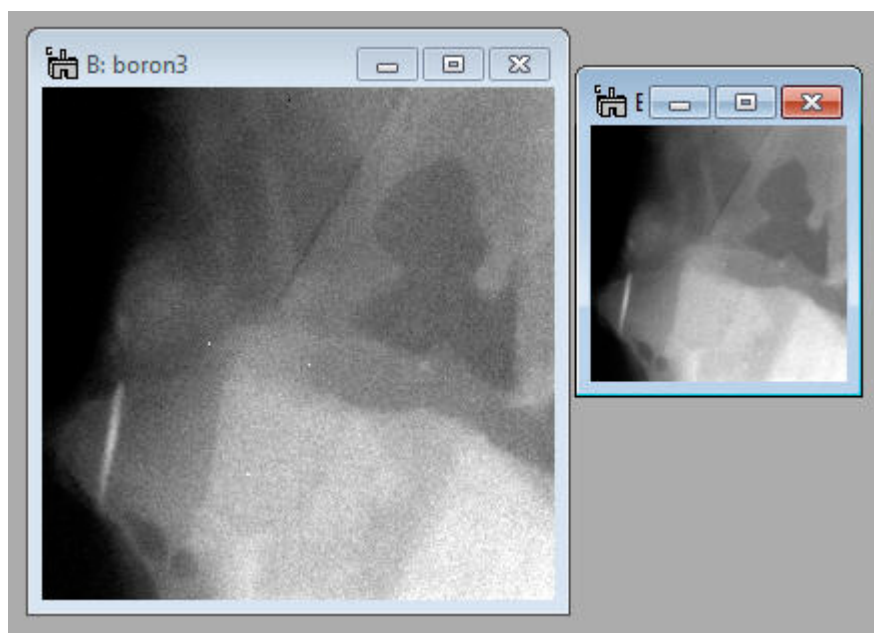
Open a 2D or 3D spectrum image, first. Make sure that the spectrum image is the front image, and select MSA...Utilities...Bin SI from the menu bar. Then, following dialog appears.



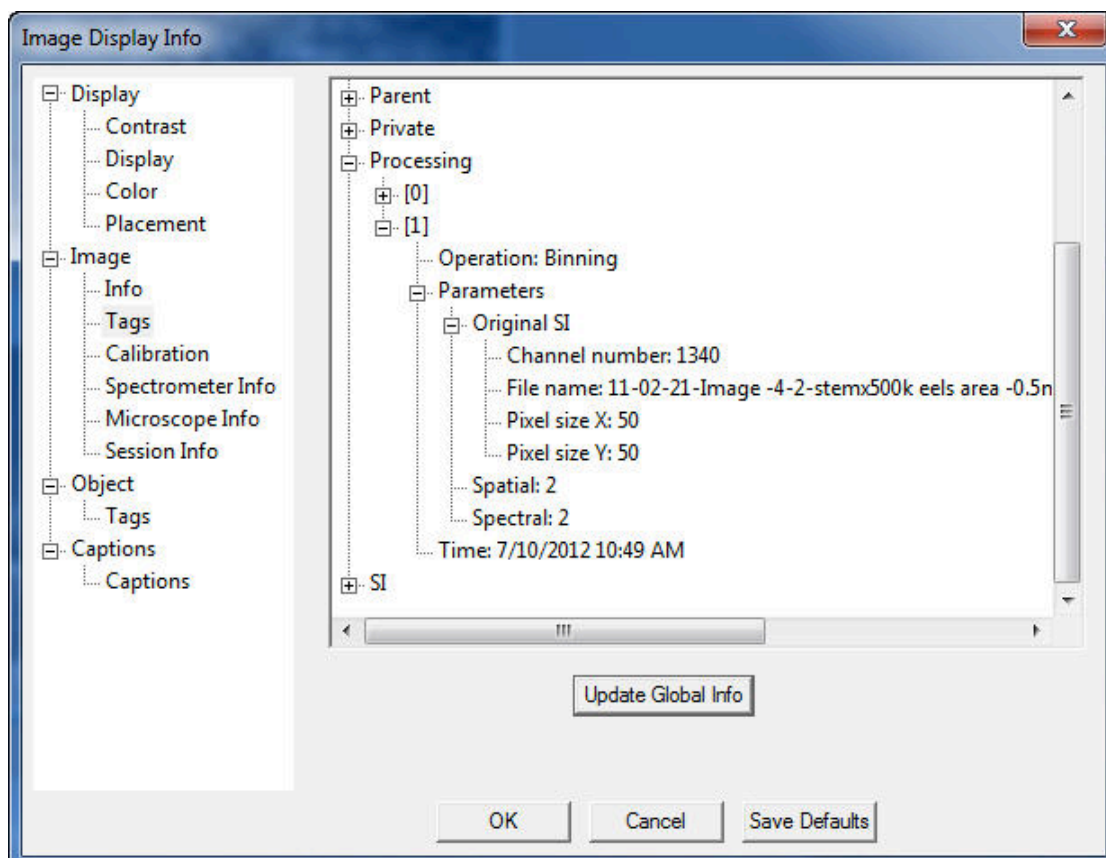
Select binning 2 or 4 in the **spatial** and/or **spectral** popup fields.



Then, click the **Ok** button. The binned spectrum image will appear shortly.



Detailed information of this processing via the Bin SI plugin can be found in Binning tag under the Processing Tag of the binned SI file, available through Image Display Info.




Spatial sub SI

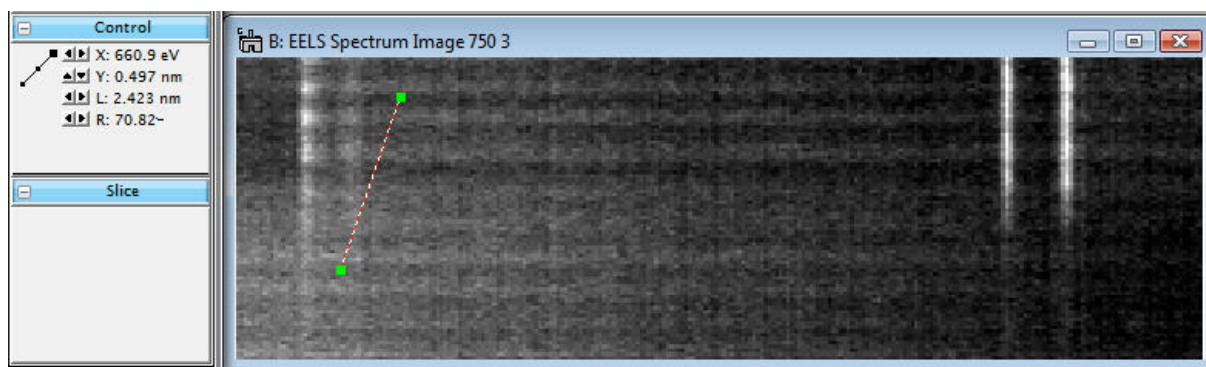
Brief Description

This function extracts a selected area from an existing 2D or 3D spectrum image and shows the selected sub area as a new spectrum image.

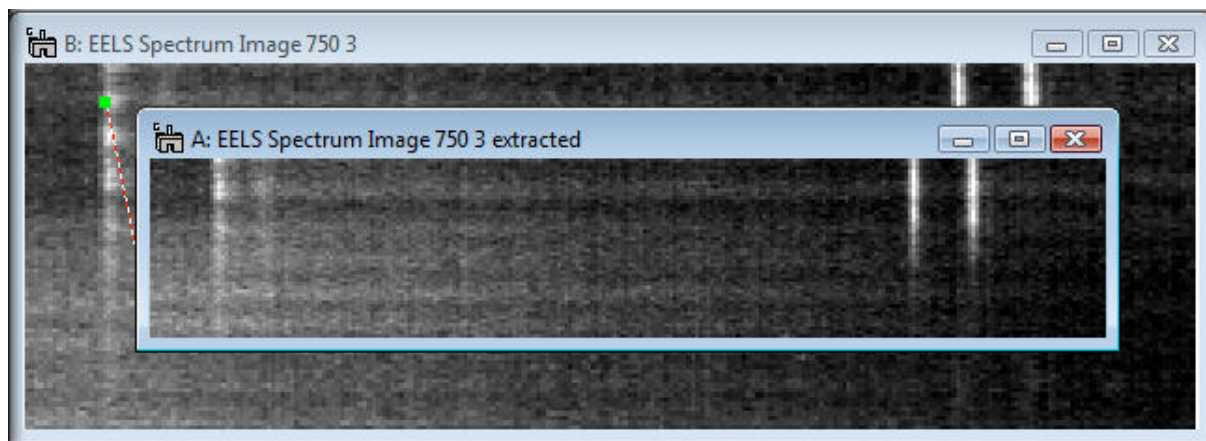
Usage

Case 1: 2D spectrum image

Open a 2D spectrum image and select a sub area to be extracted using the LineROI tool  (note: you do not have to draw a perfectly perpendicular line)

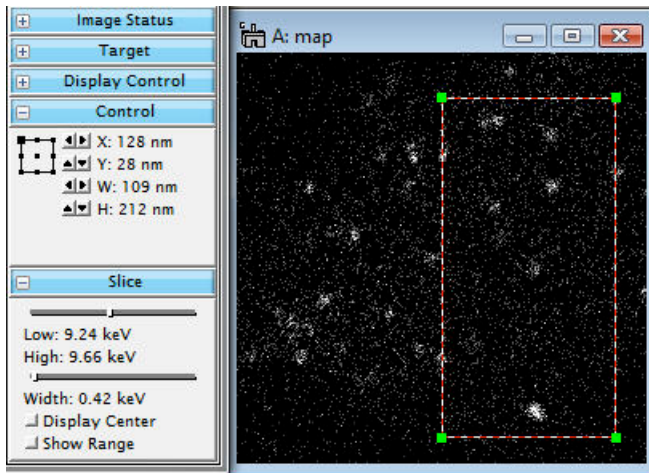


Then, select **MSA...Utilities...Spatial sub SI** from the menu bar. A new 2D spectrum image appears.

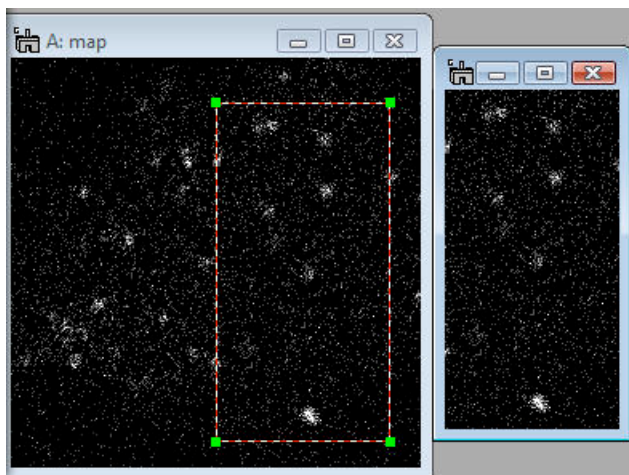


Case 2: 3D spectrum image

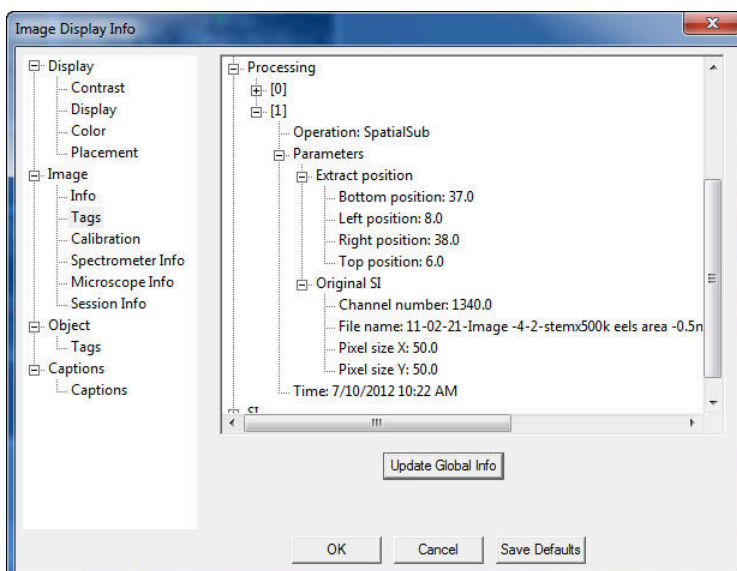
Open a 3D spectrum image and select a sub area using the RectangleROI tool



Then, select **MSA...Utilities...Spatial sub SI** from the menu bar. A new 3D spectrum image appears



Detailed information of this processing via the **Spatial sub SI** command can be found in **Operation: SpatialSub** tag under the **Processing** Tag of the extracted SI file, available through **Image Display Info**.




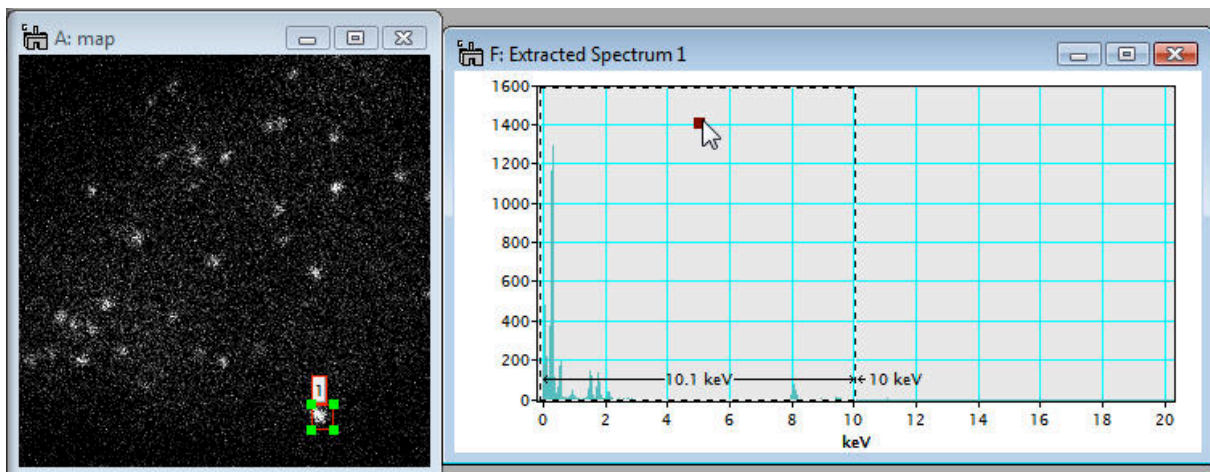
Spectral sub SI

Brief Description

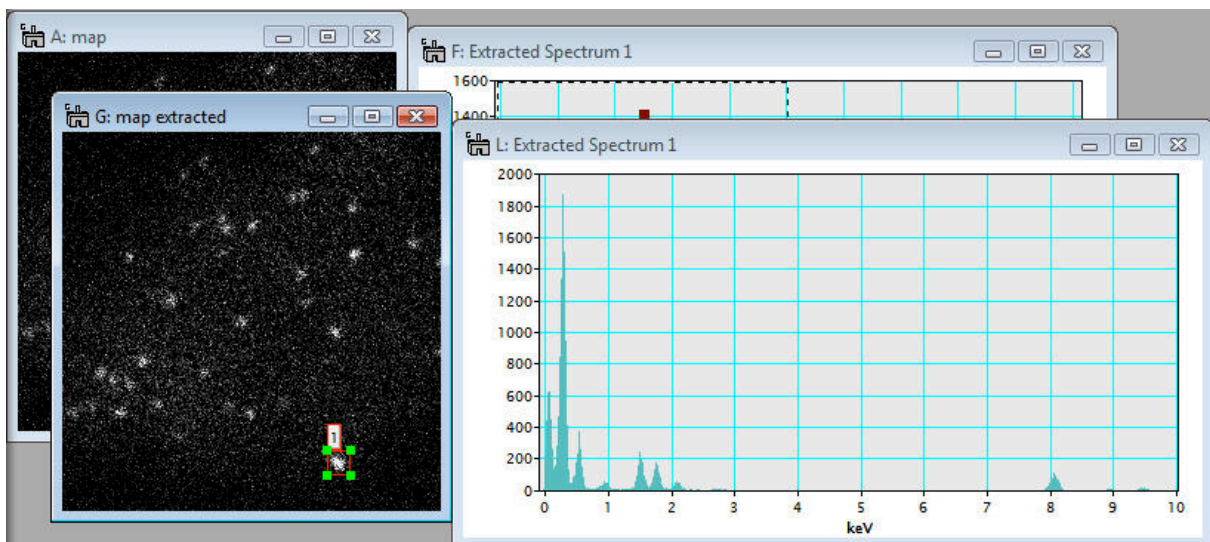
This function extracts a selected energy region from an existing 2D or 3D spectrum image and shows a new spectrum image with the selected energy region.

Usage

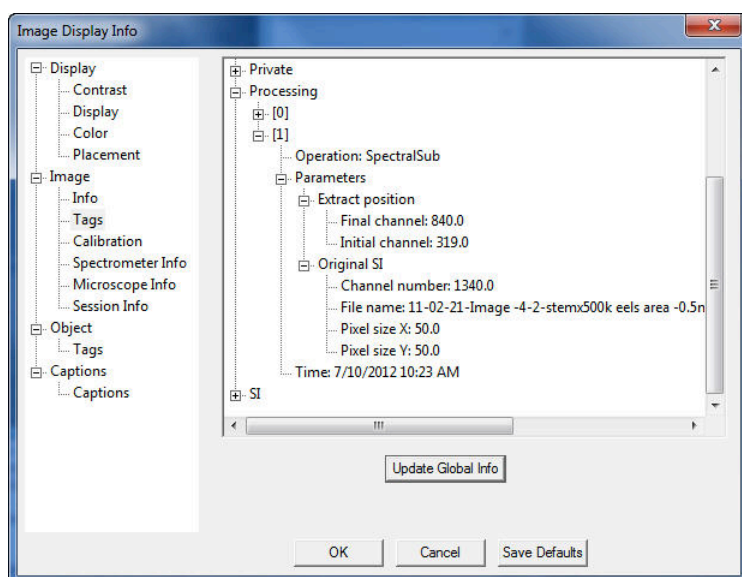
Open a 2D or 3D spectrum image and show a spectrum from the spectrum image using the SpectrumPicker tool  in the tool bar. Next, select an energy range you want to extract from the existing spectrum image.



Then, select **MSA...Utilities...Spectral sub SI** from the menu bar. A new spectrum image with the selected energy range appears.

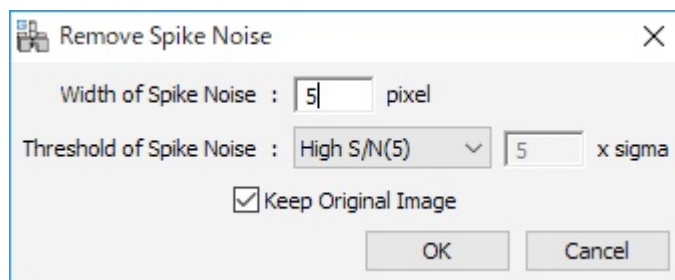


Detailed information of this processing via the **Spectral sub SI** command can be found in **Operation: SpectralSub** tag under the **Processing** Tag of the extracted SI file, available through **Image Display Info**.



Remove Spike Noise

Using this command you can remove a spike noise(s) from a SI data. A spike noise is defined as the data point that shows an abnormal value. Such a point is created, for example, by cosmic rays. The dialog below appears to set processing parameters.



The spike noise is searched within a specified range of pixels (Width of Spike Noise). The data point that exceeds the specified noise level (Threshold of Spike Noise) is recognized as the spike noise, and replaced by the local average.

The number of the removed spike noise will be shown in the dialog, and displayed in the Results (Output) window.