

MSA plugin for Gatan DigitalMicrograph Help

Version 1.6

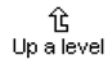
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MSA

Welcome to MSA

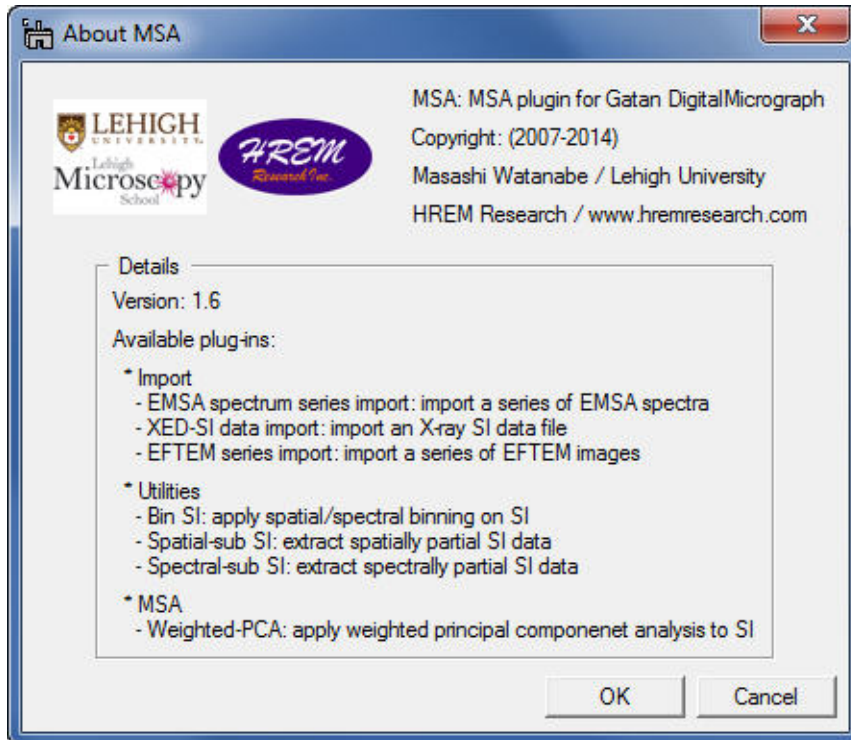
Welcome to MSA, the multivariate statistical analysis (MSA) plugin package for Gatan DigitalMicrograph.

The current package version is **1.6**, which consists of following functions:

- . [Import](#)
 - [EMSA spectrum series import](#)
 - [XED-SI data import](#)
 - [EFTEM image series import](#)
- . [Utilities](#)
 - [Bin SI](#)
 - [Spatial sub SI](#)
 - [Spectral sub SI](#)
- . [MSA](#)
 - [Weighted-PCA](#)
- . [Release notes](#)

These plugins should work in GMS version 1.7.1 or higher.

Information of the package version and available functions can be seen by selecting MSA>[About MSA](#)



The detailed description of each function can be also found by clicking the above link.



MSA

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](#)



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MSA>Import>EMSA spectrum file series

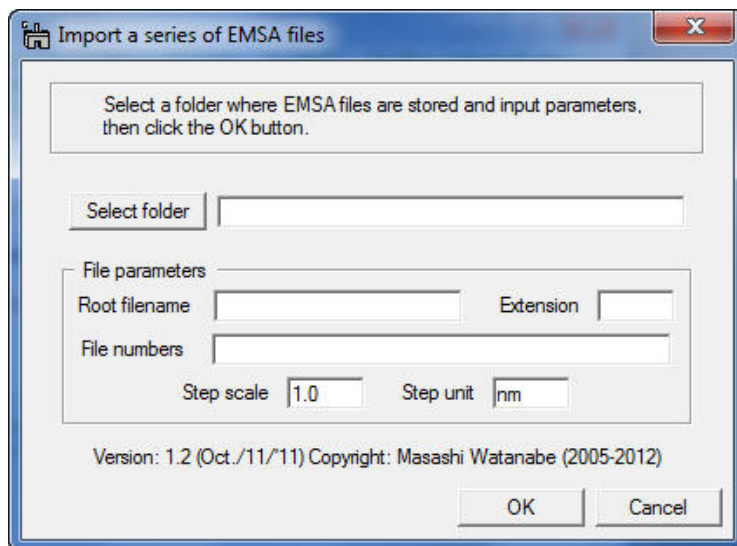
Current Function Version: 1.1 (Sep./18/'11)

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 spece 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.

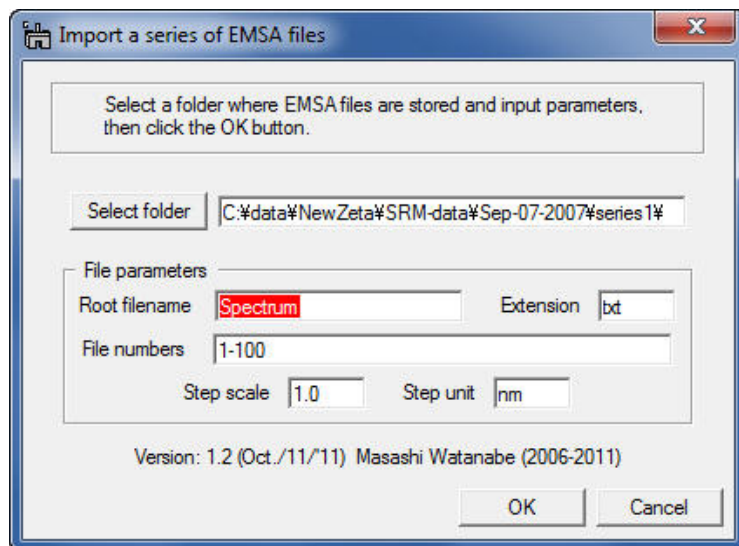
Then, input the Step scale nad Step Unit in the fields. For example, input 5.0 and nm into the fields respectively if you measured the line profile with 5 nm

step.

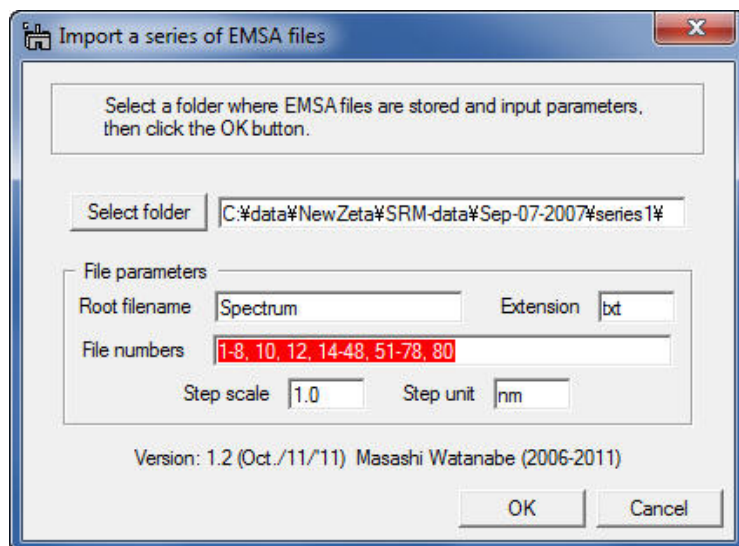
Finally, click the **Import** button.


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

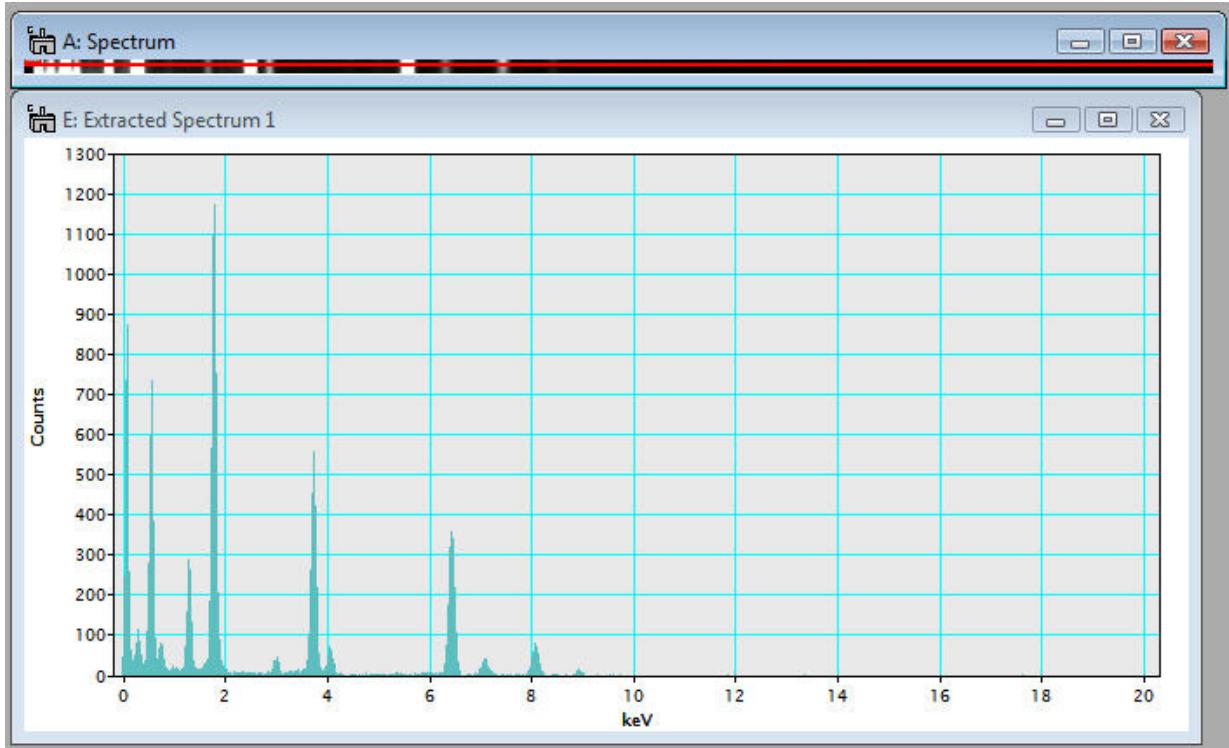
Example for **case 1**:



Example for **case 2**:



The imported file can be treated as a 2D spectrum image. So, individual spectra can be displayed via SpectrumPicker tool  in the tool bar (after DigitalMicrograph version 3.11.1) or in the BasicTools window (before version 3.11.1).



Acknowledgements

Thanks to Bernhard Schaffer (FELMI/ZFE, Graz University of Technology) for providing me the request to skip spectra.

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MSA>Import>XED-SI data

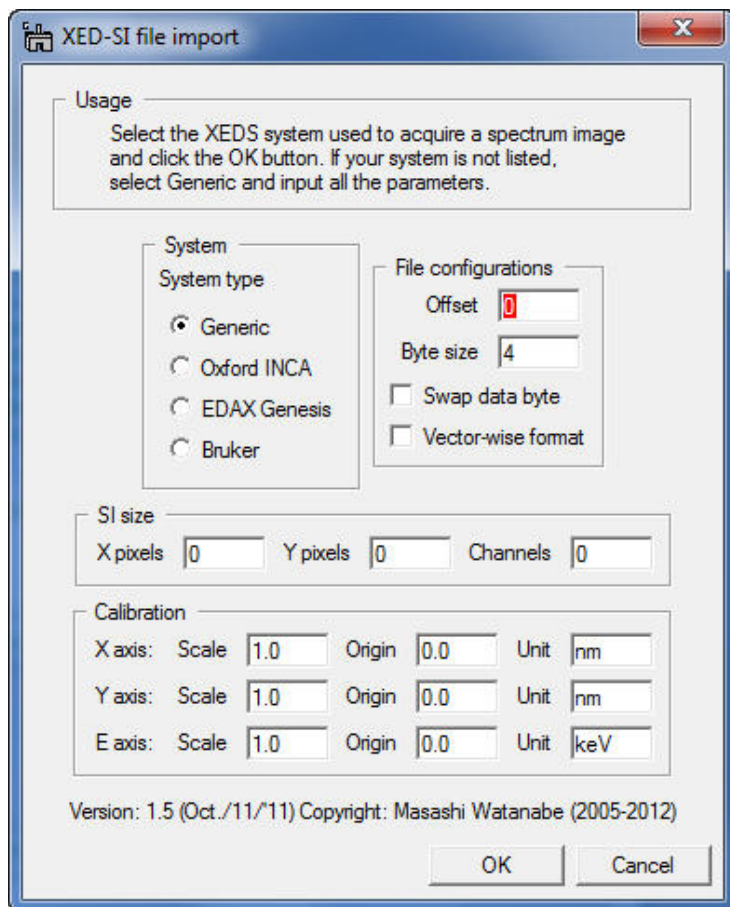
Current Function Version: 1.4 (Sep./18/'11)

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, "**foo.spd**" files from **EDAX Genesis** system and "**foo.raw**" files from **Bruker** (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.

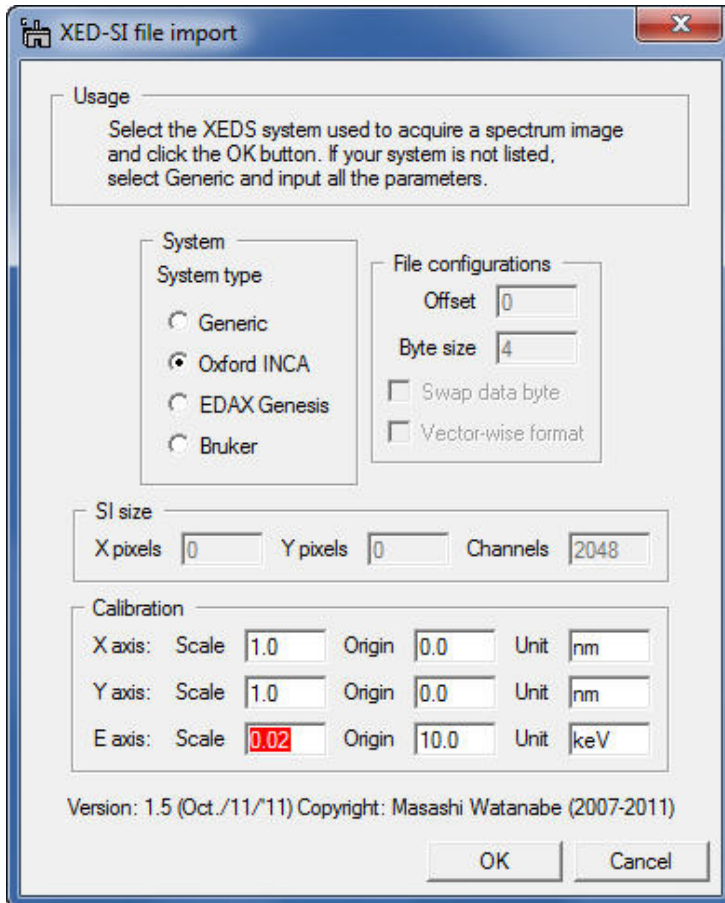
Usage

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

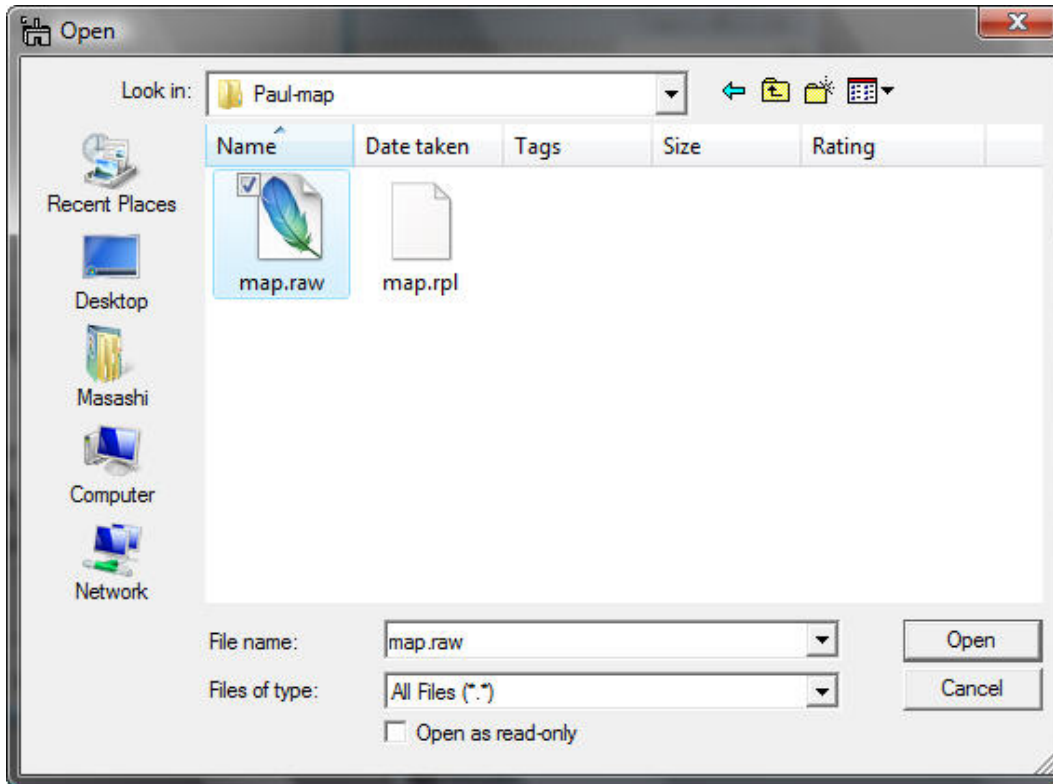


First, should select your system for X-ray analysis by clicking the radio button.

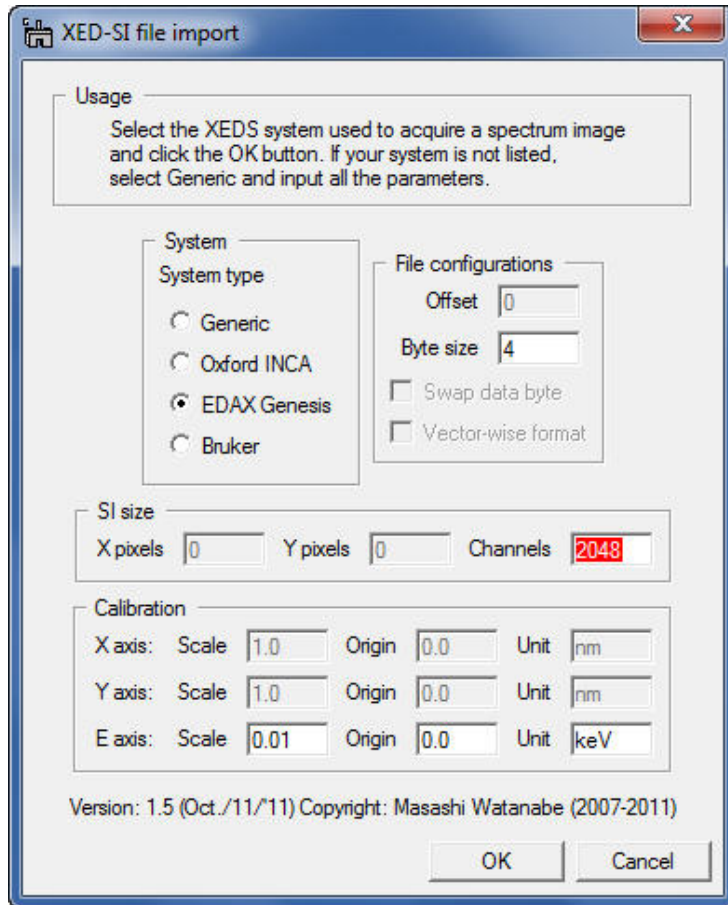
Case 1: if you select **Oxford INCA**, several fields become gray out.



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 **Import SI** button and select a desired **"foo.raw"** file.

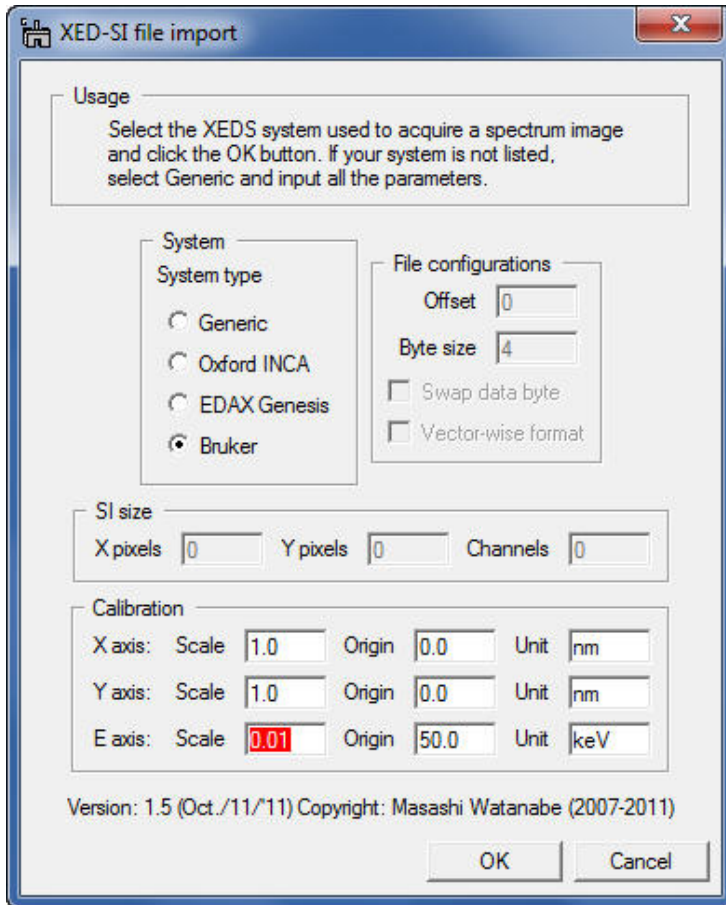


Case 2: if you select **EDAX Genesis**, again several fields are gray out.



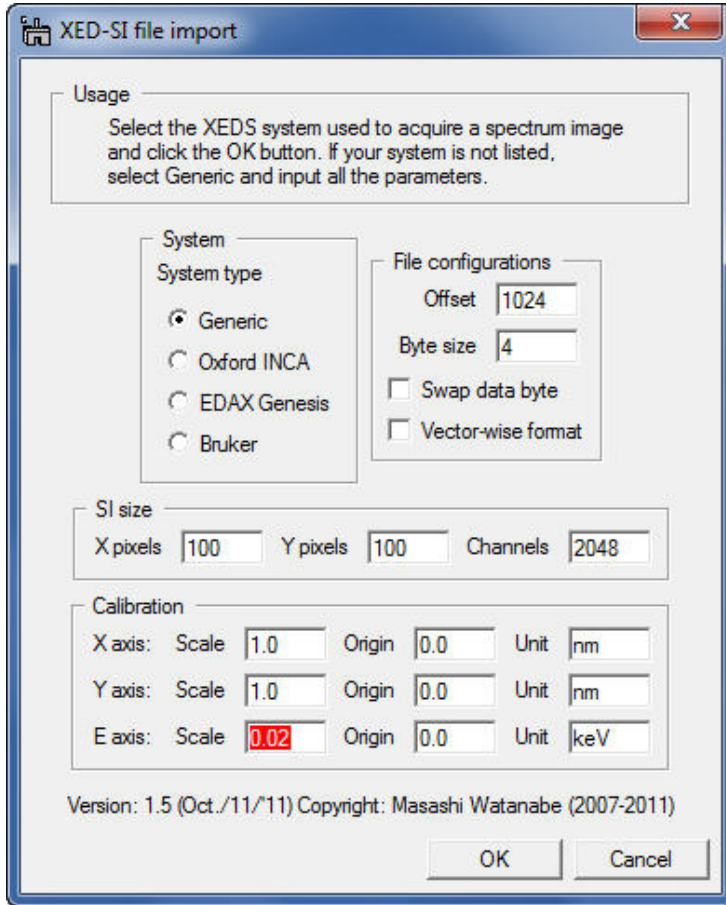
To obtain the parameters for a Genesis spectrum image, this function looks for **"foo_SE1.txt"** file. When X-ray spectrum image was acquired in EDAX genesis system, an electron image (such as bright-field STEM, annular dark-field STEM, or secondary electron image etc.), you should save the image as 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 **Import SI** button and select a desired **"foo.spd"** file.

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

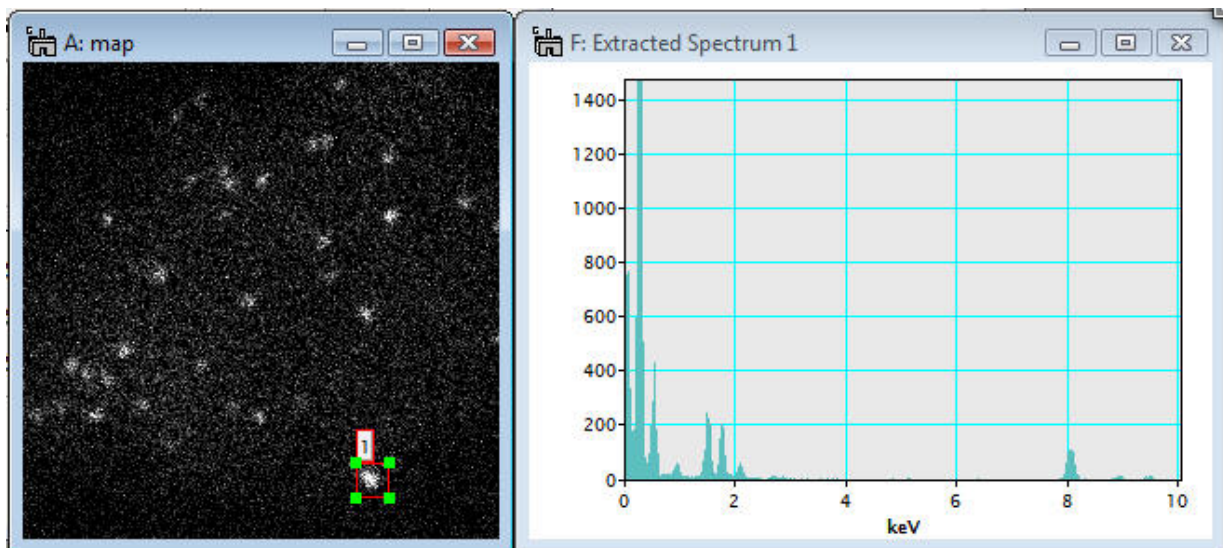


You do not fill the gray fields since this function will obtain the parameters from **"foo.rpl"** file, which is saved with **"foo.raw"** file in Bruker software (so, you must locate the **"foo.rpl"** file with the **"foo.raw"** file in the same folder). Now, the most important thing is to input the 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 **Import SI** button and select a desired **"foo.raw"** file.

Case 4: if you do not have parameter files (**"foo.rpl"** file for **Oxford INCA** or **"foo_SE1.txt"** file for **EDAX Genesis**), 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.



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MSA>Import>EFTEM image series

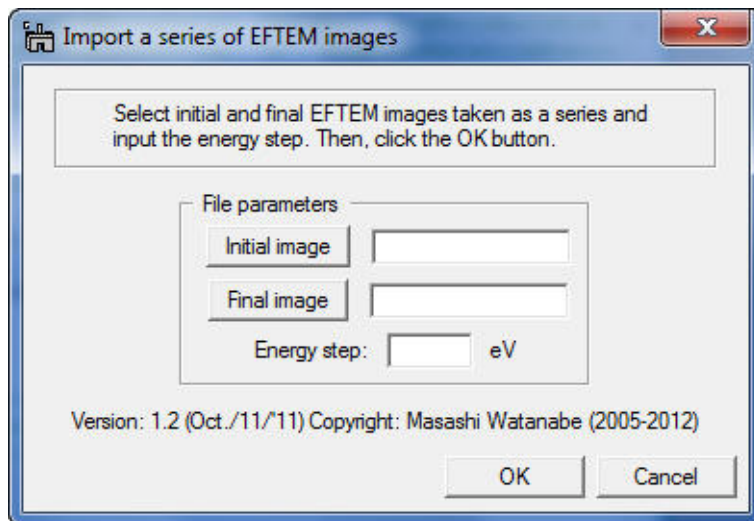
Current Function Version: 1.1 (Sep./18/'11)

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).

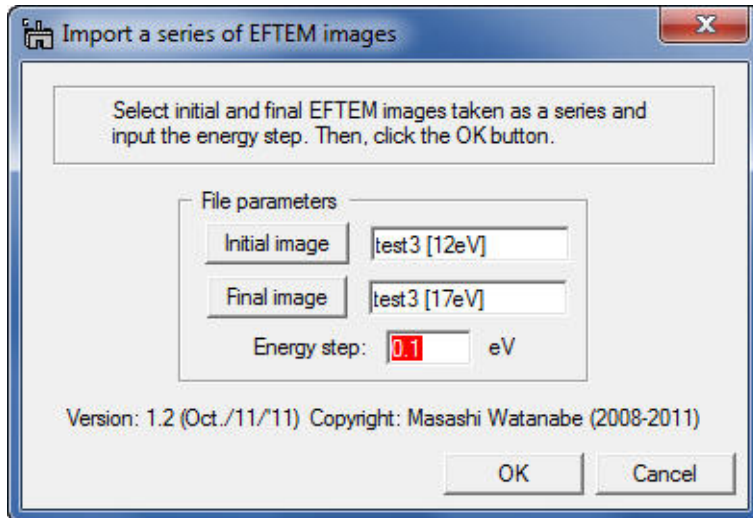
Usage

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 **Convert** button.

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



Note that the EFTEM image series must be named as

`foo [jjj]eV].dm3`

where `foo` is the rootfile name, `jjj` is an energy-loss value of the particular image. For example, `test 3 [12.4eV].dm3`.

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



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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](#)



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MSA>Utilities>Bin SI

Current Function Version: 1.5 (July/10/'12)

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 DMS (ver. 1.7.1 or higher). This function is only for users who still use previous versions of DMS.

Usage

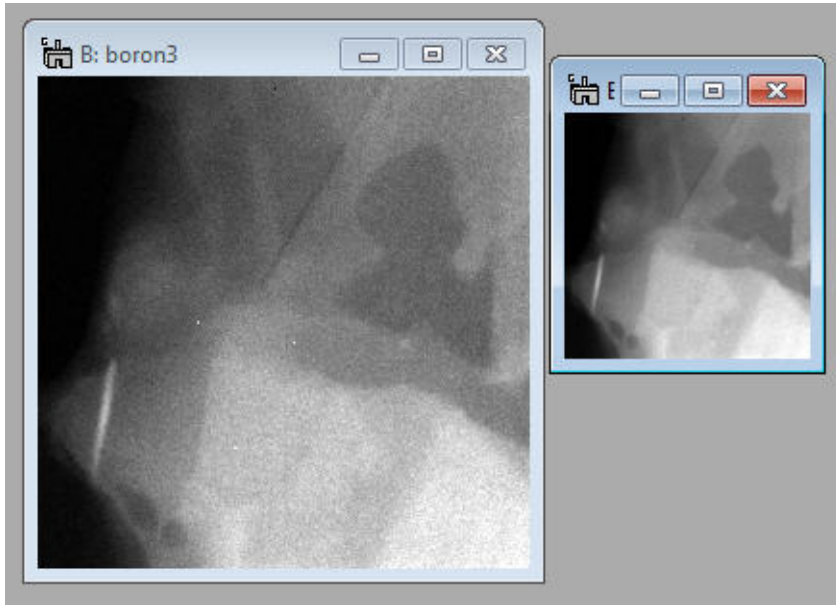
Open a 2D or 3D spectrum image, first. Make sure that the spectrum image is the front image, and select **MSA>Utilities>Bin SI** from the menu bar. Then, following dialog appears.



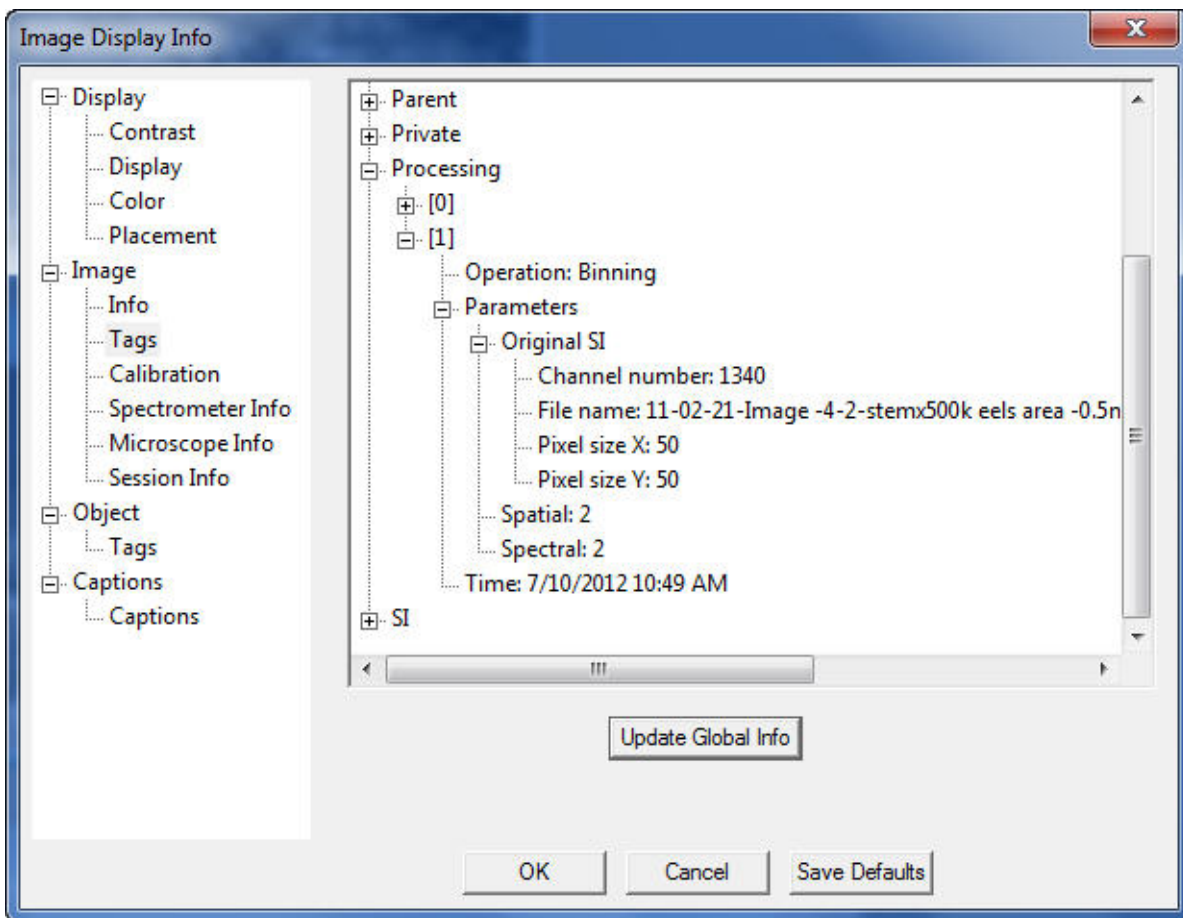
Select binning 2 or 4 in the spatial and/or spectral popup fields.



Then, click the **Binning** 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.



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MSA>Utilities>Spatial sub SI


Current Function Version: 1.2 (July/10/'12)

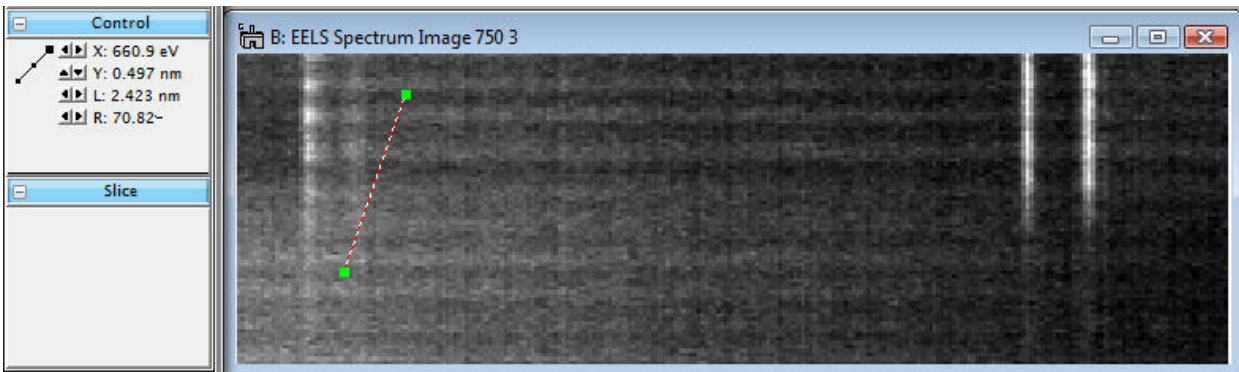
Brief Description

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.

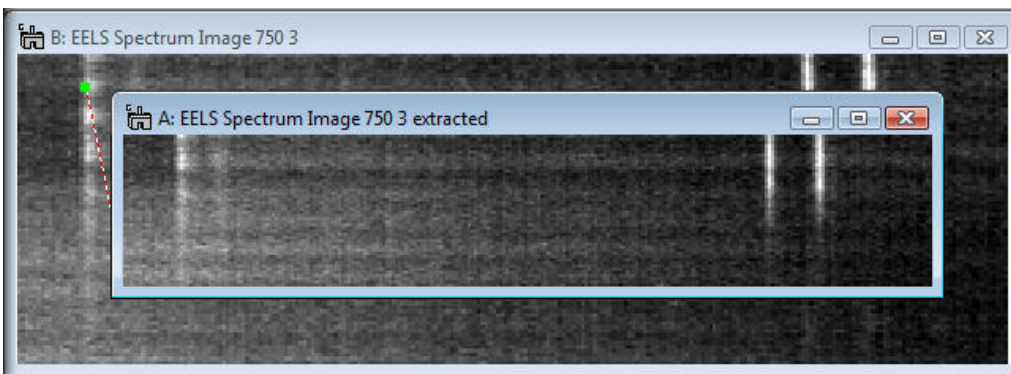
Usage

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)



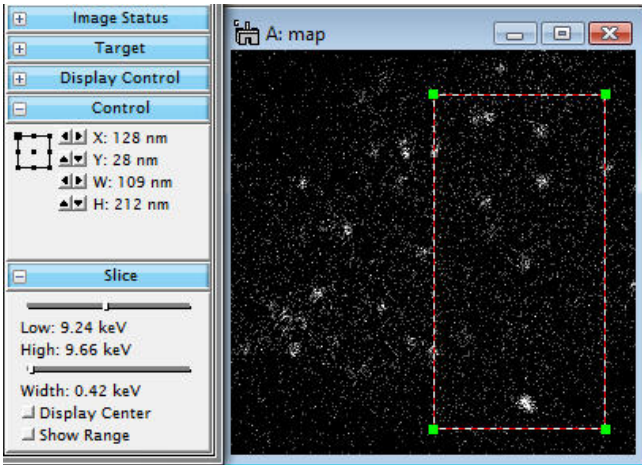
Then, select **MSA>Utilities>Spatial sub SI** from the menu bar. A new 2D spectrum image appears.



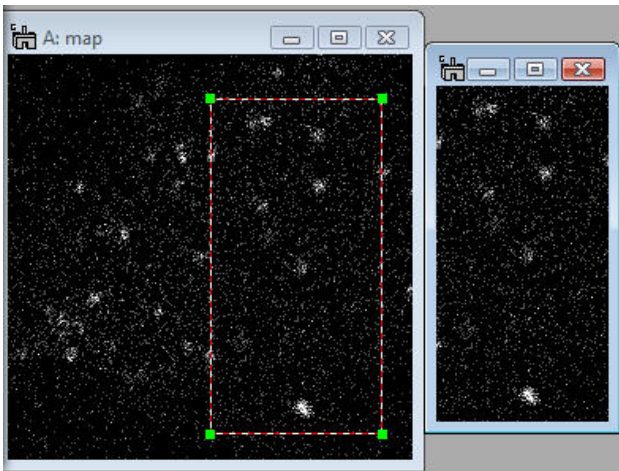
Case 2: 3D spectrum image

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



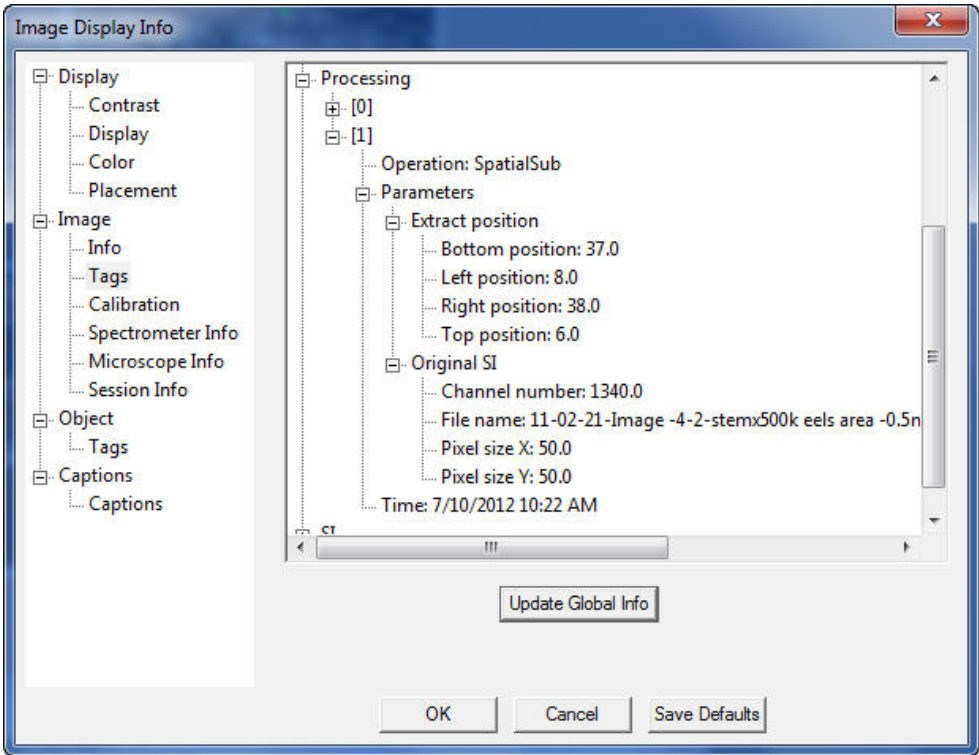


Then, select **MSA>Utilities>Spatial sub SI** from the menu bar. A new 3D spectrum image appears.



Detailed information of this

processing via the Spatial sub SI plugin can be found in Spatial sub tag under the Processing Tag of the extracted SI file, available through Image Display Info.



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MSA>Utilities>Spectral sub SI

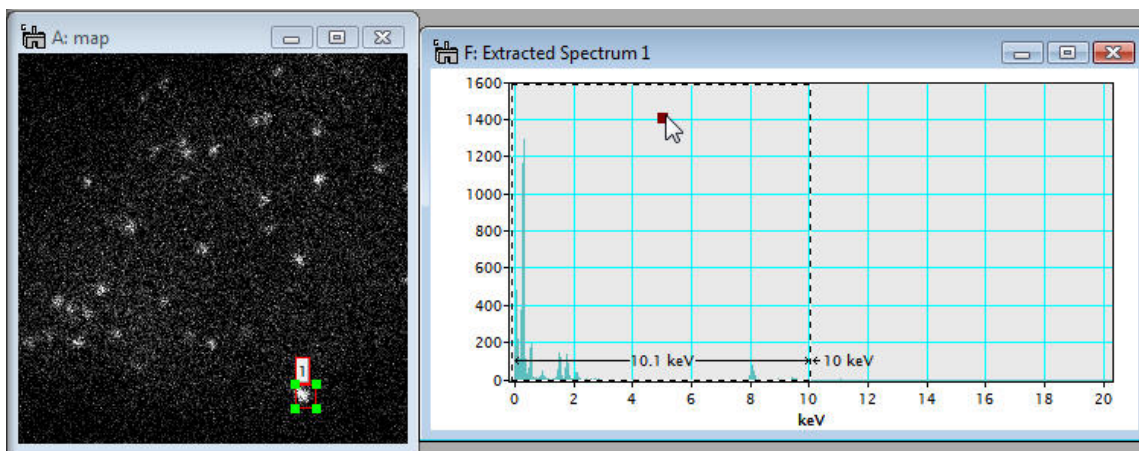
Current Function Version: 1.2 (July/10/'12)

Brief Description

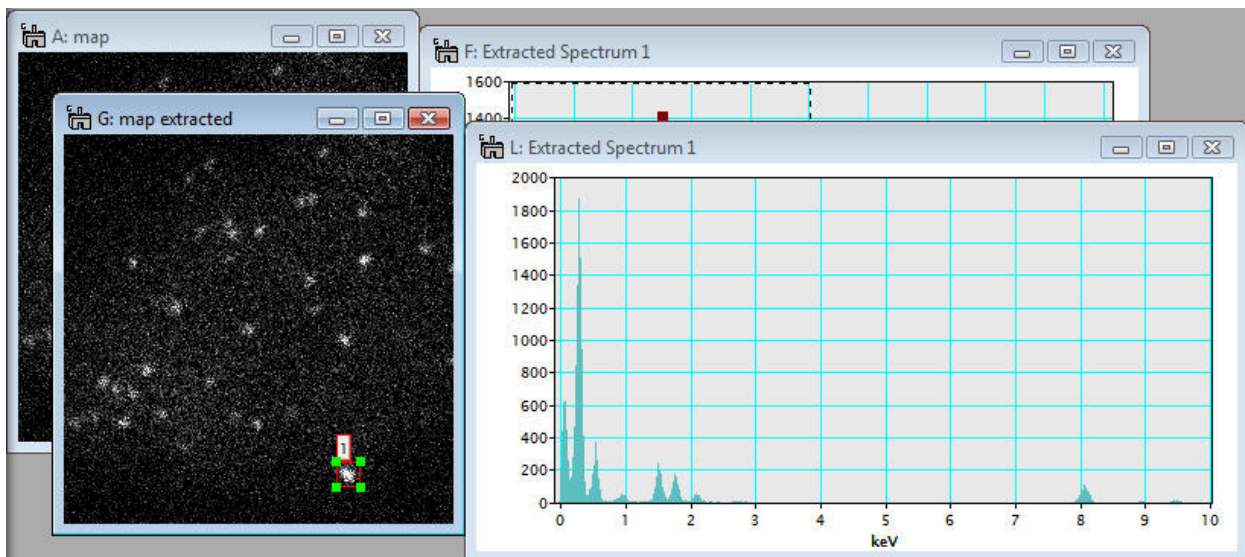
This function extracts a selected energy region from an existing 2D or 3D spectrum image and shows a new spectrum image with the selected energy region.

Usage

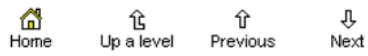
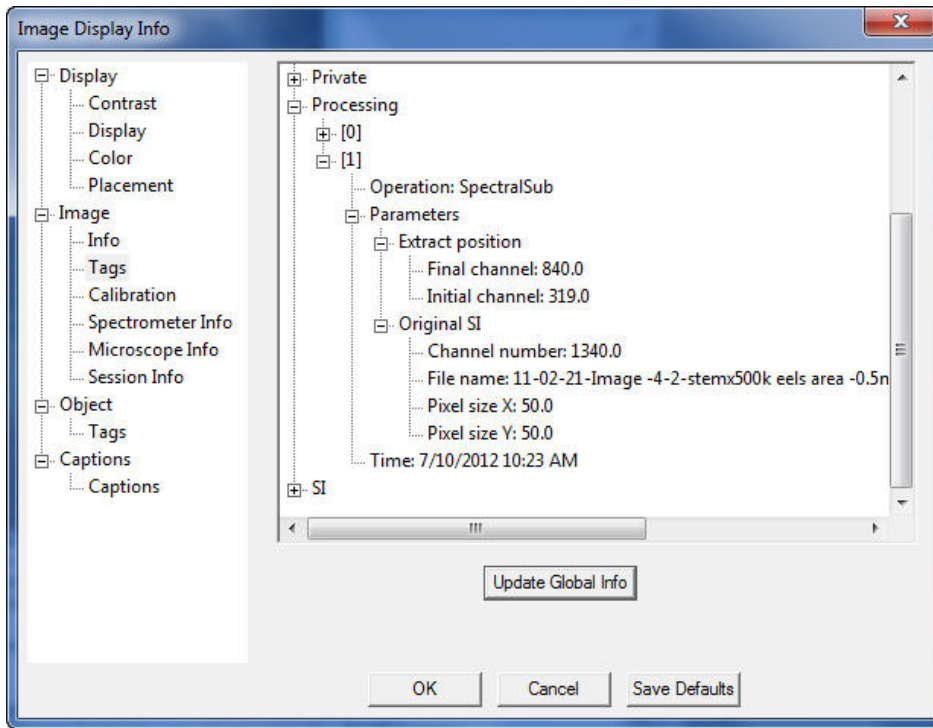
Open a 2D or 3D spectrum image and show a spectrum from the spectrum image using the SpectrumPicker tool  in the tool bar. Next, select a energy range you want to extract from the existing spectrum image.



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 plugin can be found in Spectral sub tag under the Processing Tag of the extracted SI file, available through Image Display Info.



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This is the core function of this plugin package, which applies one of multivariate statistical analyses to spectrum-image data.

- [Weighted-PCA](#)



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MSA>MSA>Weighted PCA

Current Function Version: 1.6 (Sep./11/'13)

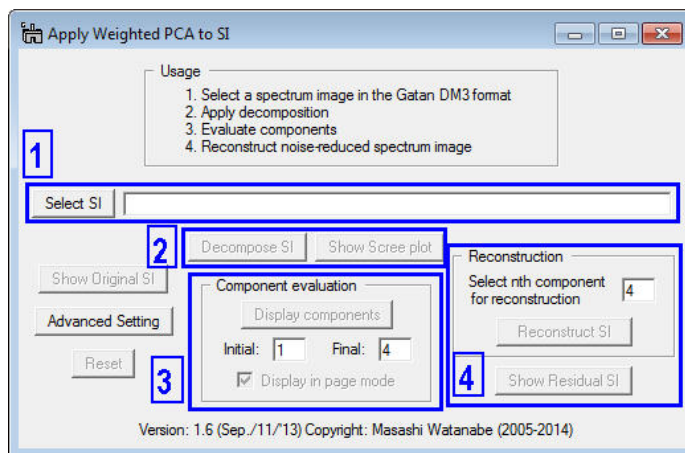
Brief Description

This function 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).

Usage

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. 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.

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

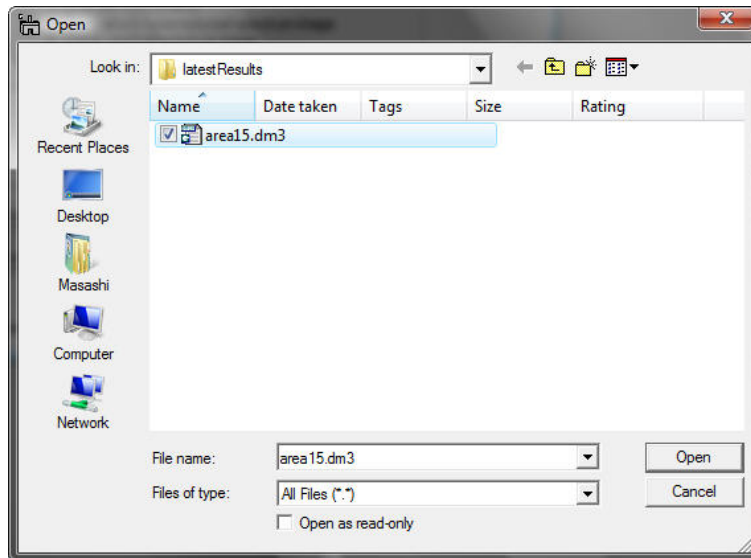


The numbers in the blue boxes indicate the step you should follow in order to apply PCA. Namely,

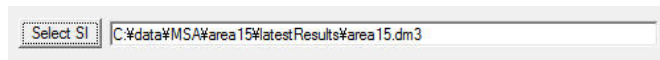
1. Select a 2D or 3D spectrum image in the Gatan DM3 format
2. Apply matrix decomposition to the SI
3. Evaluate componenets
4. Reconstruct the noise-reduced spectrum image

Each step is explained as follows:

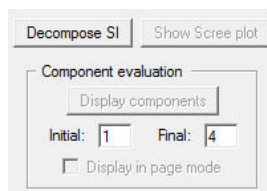
1. First, click the **Select SI** button in the **region 1** of the dialog, then the dialog for file open appears.



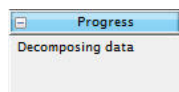
You can select the desired SI file and click the **Open** button. The selected file name appears in the string field with the absolute path.



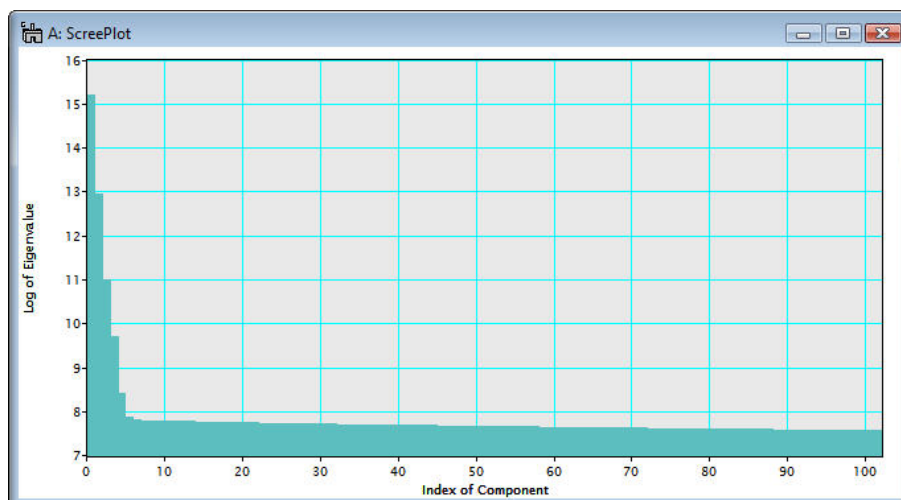
- Once the data is selected, the **Decompose SI** button is activated in the **region 2**.



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.

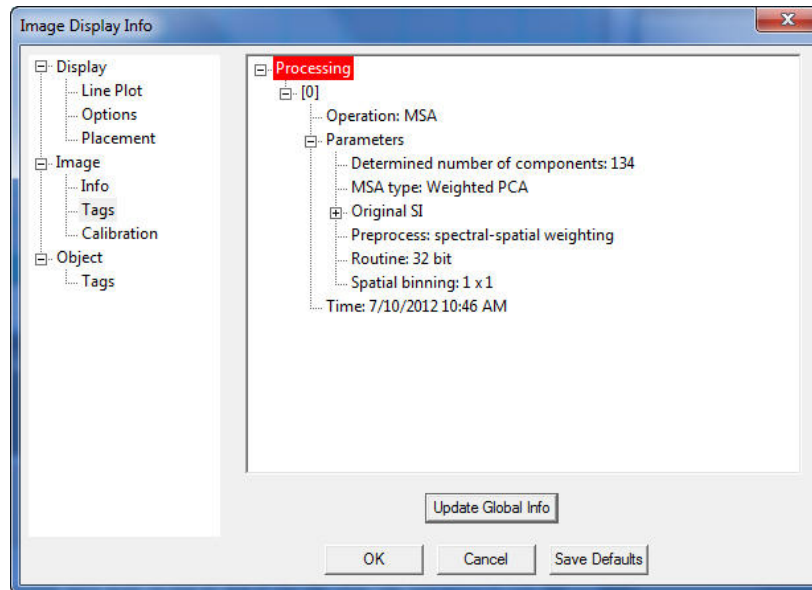


Just wait until the scree plot is displayed.

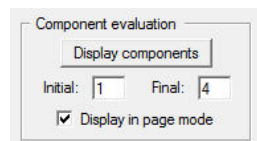


Note that the scree plot is logarithm of the eigenvalues graphically plotted against the index of component. A number of principal components is usually evaluated based on the scree plot. In the above example, a number of the principal components distinguished from the noise components can be 6 or 7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

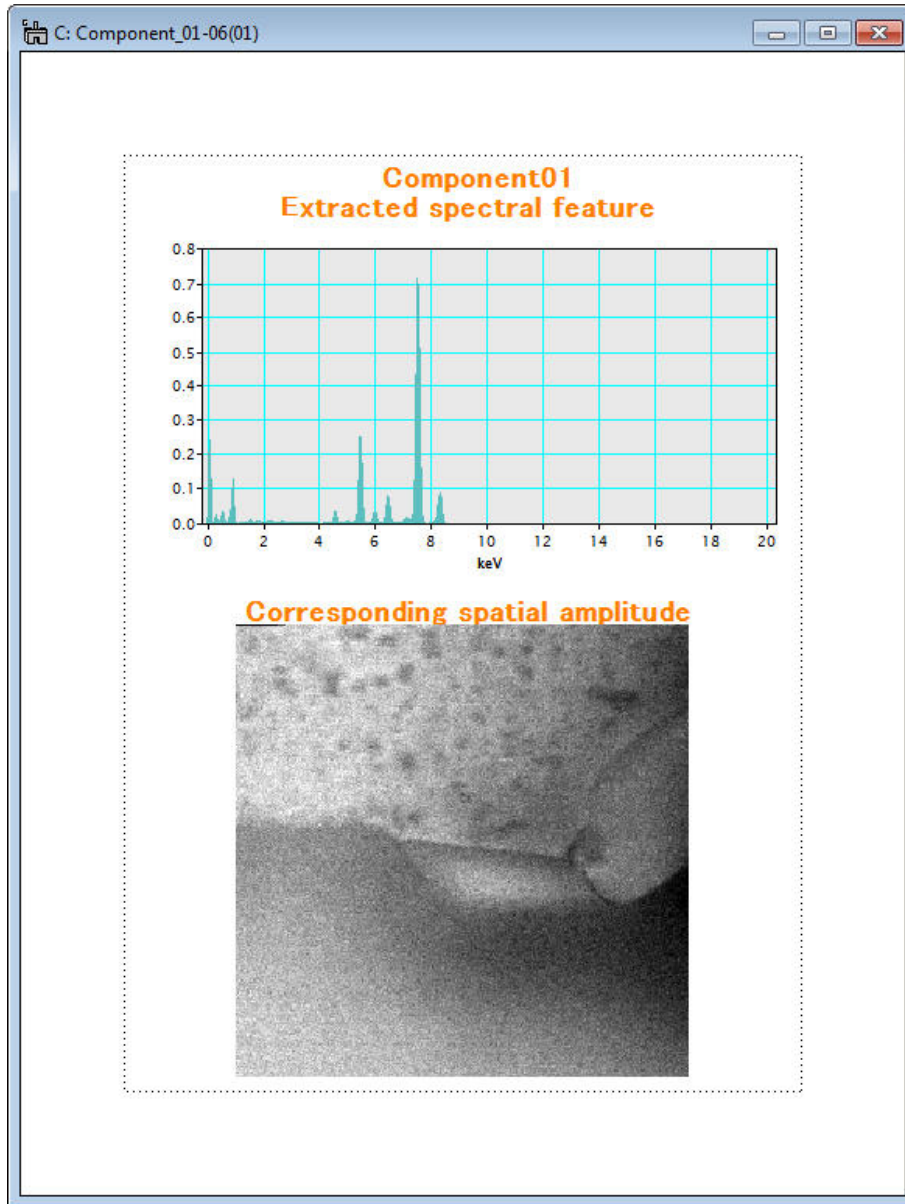
Detailed information of this Weighted PCA process can be found in MSA tag under the Processing Tag of the Scree Plot, available through Image Display Info.



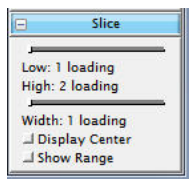
3. After the data is decomposed, the **Display components** button is activated in the [region 3](#).



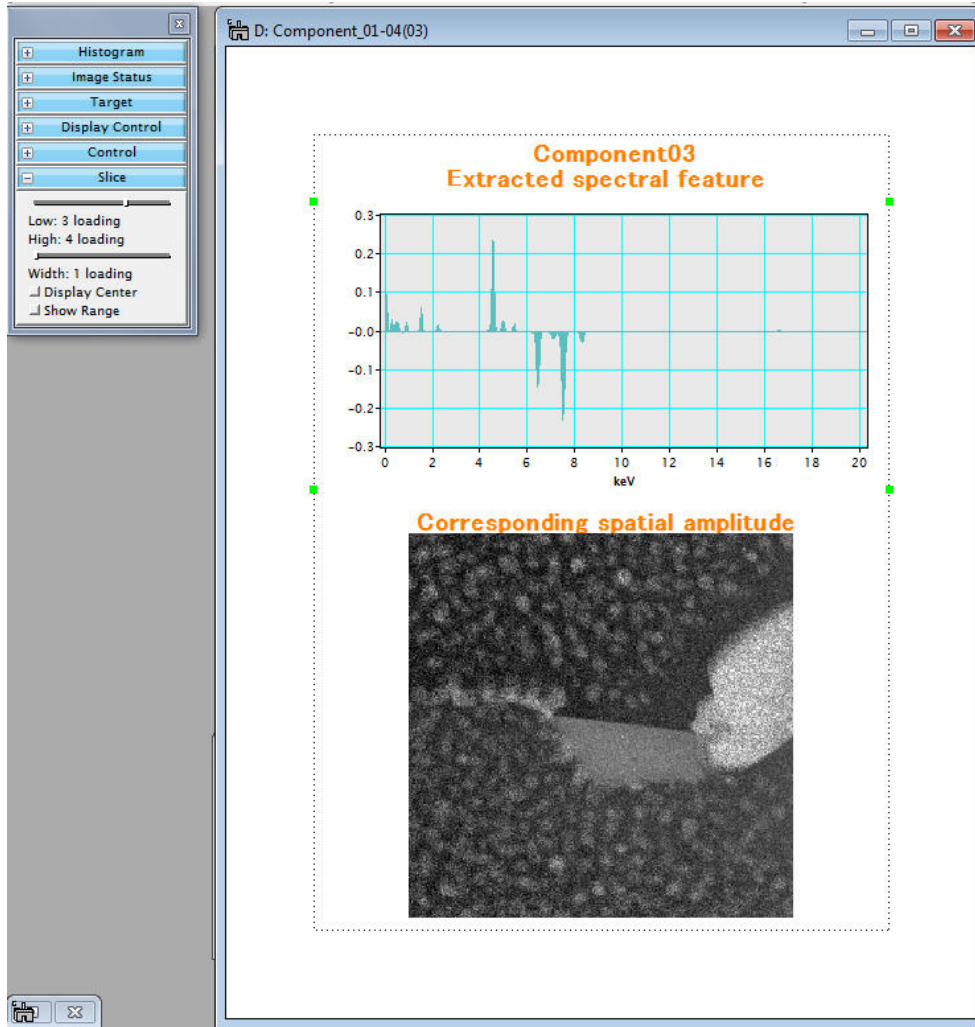
It is essential to evaluate each component in terms of a pair of a loading spectrum (spectral feature) and a score image (spatial amplitude) in order to distinguish the principal components from noise. To view components, input the component range you want to evaluate in the Initial and Final boxes and click the **Display components** button. Then, selected components are displayed. It should be noted that the **page mode** is now default to display components.



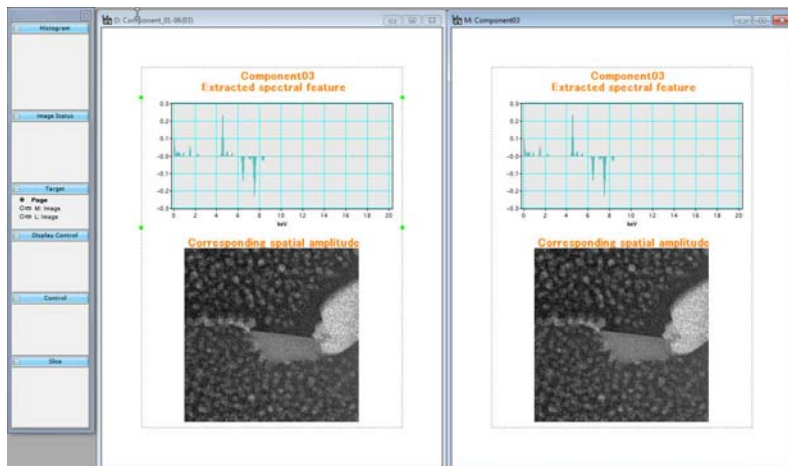
In the above example, a spectral feature (loading) and spatial amplitude (score) of component 1 are displayed in one page. In order to other extracted components, you can use the slice tool, which is used to manipulate 3D data cubes such as spectrum images.



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

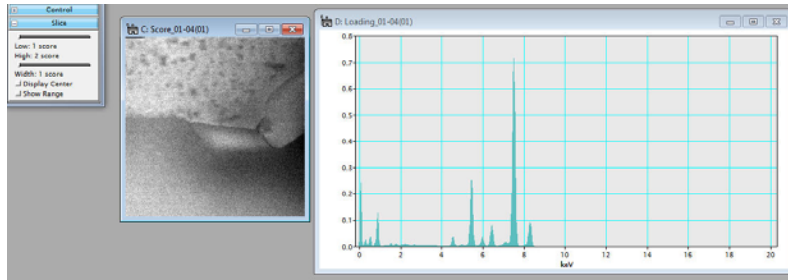


To extract the displayed component, select **MSA>Extract Component (s)>Current Component** from the menu bar. Then, the displayed component (both the loading spectrum and score image) is extracted.

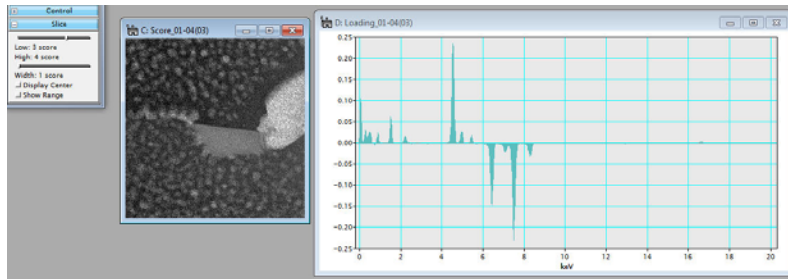


If you want to extract all the components currently contained in the page mode file, select **MSA>Extract Component(s)>Extract All** from the menu bar.

Conversely, components can also be displayed with loading-spectrum and score-image files. If this display mode is desirable, uncheck the display page mode and then click the **Display components** button in the main MSA dialog.



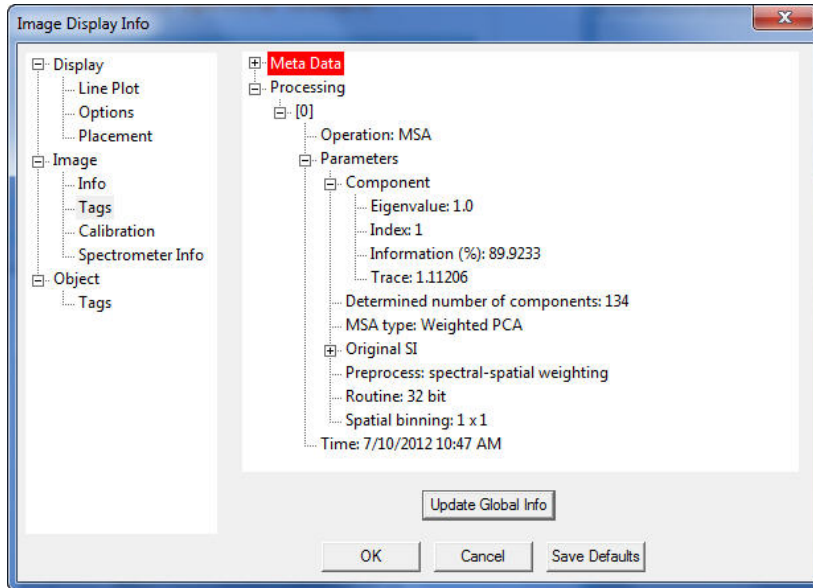
The loading spectrum and score image are displayed separately. Again, using the slice tool, individual components can be seen (corresponding to a selected component, both a loading spectrum and score image are simultaneously loaded).



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.

It is very important to evaluate individual components and to distinguish statistically significant ones from noise. The function of displaying components is one of main features of this plugin.

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

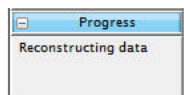


It should be noted that the information (%) tag under the Component tag indicate the fraction of information that this particular component (index 3 in the above example) contains in this SI data.

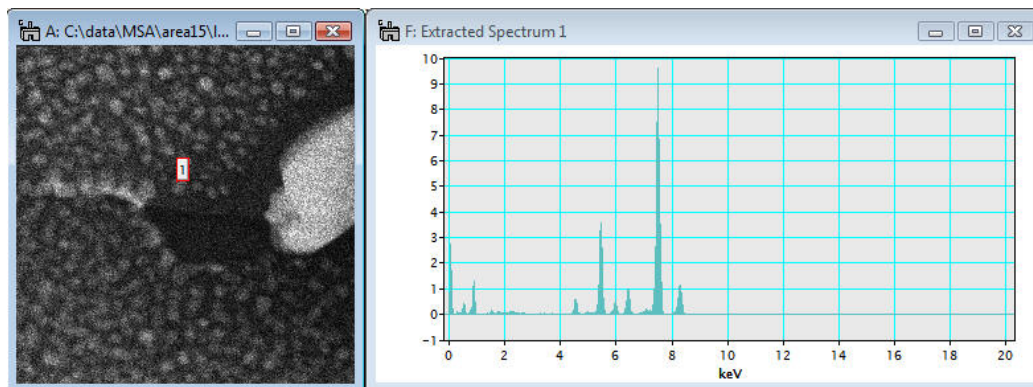
4. After the data is decomposed, the **Reconstruct SI** button is also activated in the [region 4](#).



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.

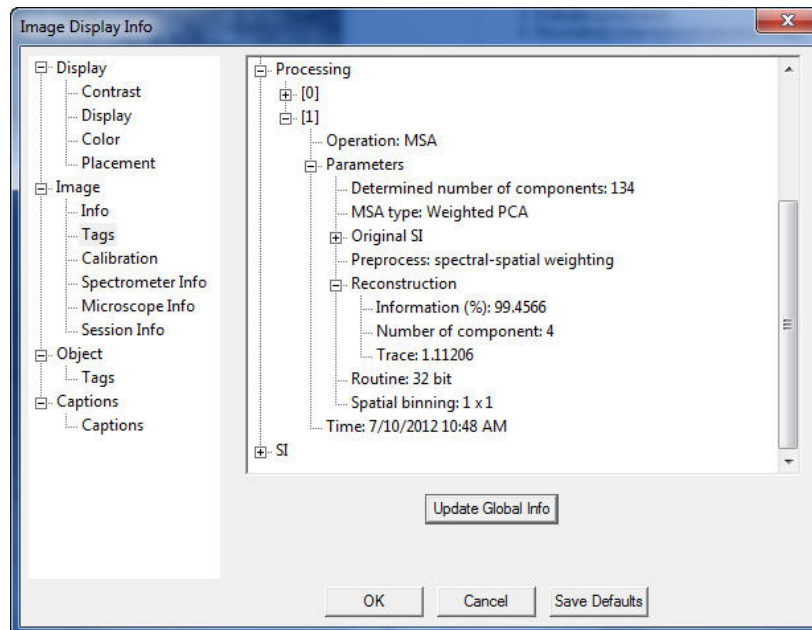


After the reconstruction process is completed, the reconstructed spectrum image is displayed.



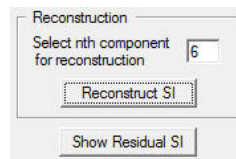
Detailed information of the reconstruction process can also be found in in

MSA > Reconstruction tag under the Processing Tag of the Reconstructed SI file, available through Image Display Info.



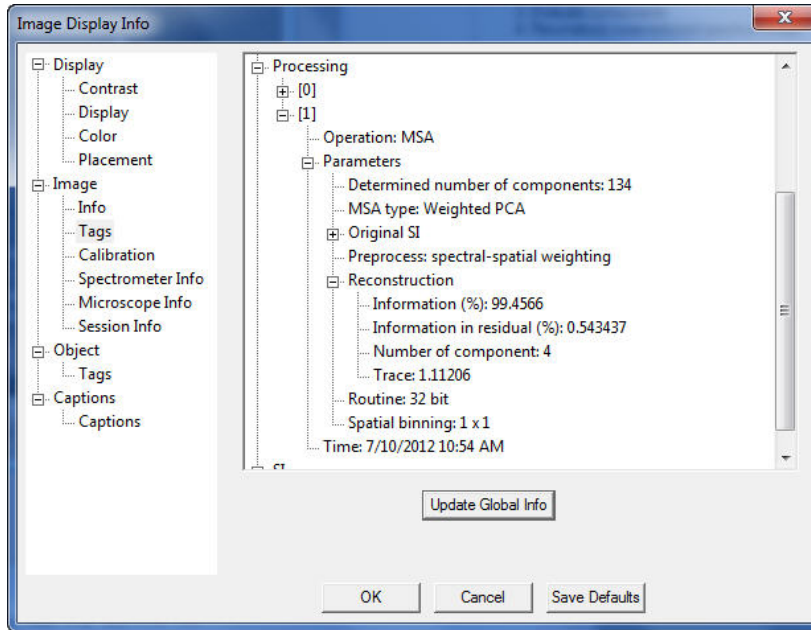
The fraction of information contained in the reconstructed SI file over the original SI file can be found in Information (%) tag under the Reconstruction tag.

Now, the **Show Residual SI** button is finally activated in the [region 5](#).



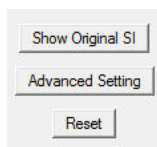
The residual SI is the difference between the original and reconstructed SIs. Therefore, the validity of reconstruction can be confirmed by comparing the residual SI with the original and reconstructed SIs.

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

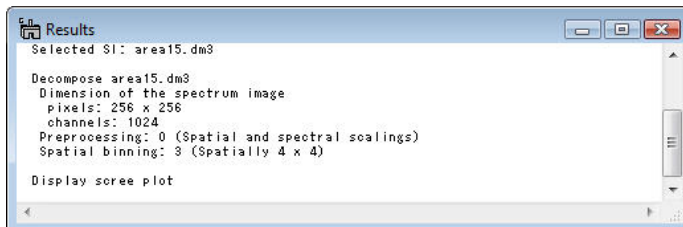


The fraction of information contained in the residual SI file over the original SI file can be found in Information in residual (%) tag under the Reconstruction tag.

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

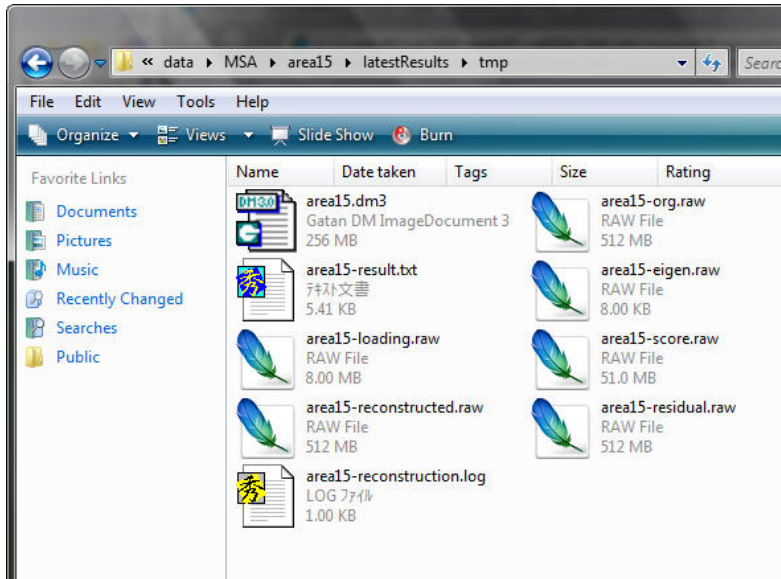


The summary of each step described above can be seen in the Result window.



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)

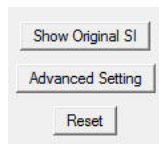
```

# 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: 65536x102↓
# Loading: 1024x102↓
# Eigenvalue: 102↓
# 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)↓
#

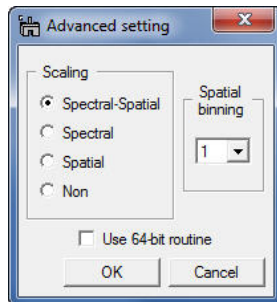
```

Advanced Setting

Several parameters for preprocessing/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 available in the MSA plug-in package for GMS 1.x): if you use DigitalMicrograph (GMS 1.x) under 64-bit Windows (XP x64, 64 bit Vista and maybe 64-bit Windows Servers), this check box is automatically activated. If this option is checked, this plugin calls an independent 64-bit command-line routine via system call. Since DigitalMicrograph (GMS 1.x) is currently 32-bit program, it is impossible to integrate such 64-bit routine under any 32-bit program. However, the major limit of the 32-bit program is memory size to allocate continuously (about

2GB). 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 64-bit GMS is available!**

References related to the PCA process

General references related to the PCA process:

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Specific references of PCA applications for electron microscopy:

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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.
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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.
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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.

MSA

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Release Notes

- **Version 1.6:**
Adjusted to GMS 2.3
Improved a display style for components as a 3D data stack (Jan./16/'14)
- **Version 1.5:**
Modified tag structure (July/10/'12)
- **Version 1.4:**
Modified dialog formats (Oct./11/'11)
- **Version 1.3:**
Adjusted to GMS 2.x and added Tags in resultant files processed by Bin SI, Spatial Sub SI, Spectral Sub SI and Weighted PCA (Sep./18/'11)
- **Version 1.2:**
Added an import option for Bruker system in XED-SI import (Dec./14/'09)
- **Version 1.1:**
Minor bug fix (June/9/'09)
- **Version 1.0:**
First official release version (Oct./30/'08)

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