Data Selection Panel

<table>
<thead>
<tr>
<th>Name of Image Matrix</th>
<th>Name of Variable Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Select Data</td>
<td>Select Variables</td>
</tr>
</tbody>
</table>

This tutorial contains navigation buttons that enable you to move throughout the tutorial.

Please use the navigation buttons and not the page up/page down or arrow keys to navigate through the tutorials.

This is the 'Next' button. It takes you to the next frame or stop point.

This is the 'Previous' button. It takes you to the previous frame or stop point.

This is the 'Go to frame' button. It takes you to a specified frame.

This is the 'Go to URL' button. It takes you to a website link.

Press the 'Next' button below to start this tutorial.
This tutorial covers how to run MCR using the Imagegui.

NOTE: I did not write the code for the implementation of MCR used in the Imagegui. I am using the MCR-ALS toolbox developed by Roma Tauler and Anna de Juan from the University of Barcelona.

The MCR-ALS toolbox can be downloaded here: http://mcrals.info
This must be installed before you can use the MCR panel in the Imagegui.

I do not use all of the options available in the MCR-ALS toolbox. If you need access to those, you will need to use the MCR-ALS toolbox directly.

My implementation of the MCR-ALS toolbox uses the following defaults:
- Non negativity for components and spectra
- Fast non linear least squares (fnlsls)
- No closure

See the MCR-ALS website for more information on those settings.
MCR requires an initial guess in order to find a solution. It is typical to use PCA scores as the initial guess.

It should be noted that MCR results are completely dependent on the initial guess. Meaning that there are an infinite number of MCR solutions depending on the initial guess and settings used. It is up to the user to determine if the solution obtained makes sense.

I find MCR most useful to display results from data after using PCA to interpret the data. Meaning I only use MCR after using PCA to figure out what I think the data is showing me and getting an idea of how many components there may be on a sample.

For this tutorial we will use PCA scores as an initial guess for MCR. To start we need to run PCA on our data.
A detailed tutorial on running PCA can be found in tutorial 06. Here we will just cover the basics required in order to use MCR.

Select the data you want to use from the menus above and press the 'Load Selected Data' button.
Next choose the type of pre-processing you would like to do on the data. You need to run MCR on the data that has been processed in the same way as the data used for PCA.

Here we will choose to Poisson scale and then mean center the data.
When you choose a pre-processing option in the image PCA panel, a new matrix is output to the Matlab workspace. We will need this later when running MCR.
Data Selection Panel

Name of Image Matrix  Name of Variable Matrix
imagedata_e2n_1   exactmass_e2n_1

Load Selected Data

Image: imagedata_e2n_1
Variables: exactmass_e2n_1

Data Preprocessing
Poisson Scaling & Mean Center

Run PCA

Press the 'Run PCA' button

PCA Summary
PC#  %Var  %Vartotal

Name for scores matrix

Name for loadings matrix

Name for Variance matrix

# of PCs to keep in model

Save PCA Data

Close Panel
Now let's look through the PCA results to see if we can determine how many components appear to be present in the sample.

Select the first PC and press 'Plot Scores and Loads'.

Image: imagedata_e2n_1
Variables: exactmass_e2n_1
From the PC1 scores plot it is apparent that there is a central spot, a bright background and some white spots. This would suggest maybe 3 components.

The negative loadings are indicative of DNA which should be on the spot (dark areas)
From the PC2 scores plot we see the spots again and the background. The loadings show the dark areas are showing peaks indicative of PEG (negative loadings) and the background is showing peaks typical of the silane linker on the substrate (positive loadings).
PC3 shows a gradient in the scores plot and some of the same peaks seen previously in PC2. Other PCs show noise.
Based off of this information I think the guess of 3 components is a good one. So we need to save the PCA information and keep 3 PCs.

Fill in the boxes here and press the 'Save PCA Data' button.
At this point we can close the PCA panel.
Now we need to load the preprocessed data matrix that was created when we ran PCA.

Select 'Import From Workspace' from the 'Data Pre Processing' -> 'Import Data' menu.
Data Selection Panel

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Import Data From Workspace

Press the "Get Variables" button to see a list of all variables in the workspace. Then select a variable and then press the appropriate button to load it into the proper list menu in the "Data Selection Panel".

Press 'Get Variables' to list the available data.
## Data Selection Panel

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## Import Data From Workspace

Press the "Get Variables" button to see a list of all variables in the workspace. Then select a variable and then press the appropriate button to load it into the proper list menu in the "Data Selection Panel".

![Get Variables button]

### Data
- Add to Image List
- Add to Variable List
- Add to Image Overlay List

### MVA Data
- Add to PCA Scores List
- Add to PCA Loadings List
- Add to PCA Variance List
- Add to MAF Scores List
- Add to MAF Loadings List
- Add to MAF Variance List

Select the pre processed data matrix and press 'Add to Image List'.
Import Data From Workspace

Press the "Get Variables" button to see a list of all variables in the workspace. Then select a variable and then press the appropriate button to load it into the proper list menu in the "Data Selection Panel".

We don't need the Total counts or Sum images so check this box.
Data Selection Panel

Name of Image Matrix
imagedata_e2n...

Name of Variable Matrix
exactmass_e2n_1

Import Data From Workspace

Press the "Get Variables" button to see a list of all variables in the workspace. Then select a variable and then press the appropriate button to load it into the proper list menu in the "Data Selection Panel".

Please fill in the information below

Name of Total counts matrix
Choose one...

Name of sum of selected matrix
Choose one...

I do not need Total or Sum Images

Data

Add to Image List
Add to Variable List
Add to Image Overlay List

MVA Data

Add to PCA Scores List
Add to MAF Loadings List
Add to MAF Variance List

Close Panel

Close this dialog box.
Data Selection Panel

Name of Image Matrix   Name of Variable Matrix
imagedata_e2n_1        exactmass_e2n_1

Import Data From Workspace

Press the "Get Variables" button to see a list of all variables in the workspace. Then select a variable and then press the appropriate button to load it into the proper list menu in the "Data Selection Panel".

Get Variables

Data

- Add to Image List
- Add to Variable List
- Add to Image Overlay List

MVA Data

- Add to PCA Scores List
- Add to PCA Loadings List
- Add to PCA Variance List
- Add to MAF Scores List
- Add to MAF Loadings List
- Add to MAF Variance List

And close this panel.
Make sure the pre processed data is selected from the 'Name of Image Matrix' drop down menu.
Now open the MCR panel by choosing 'MCR' from the 'MVA' menu.
Press the 'Load Selected Data' button to load the data into the panel.

This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast non-linear least squares (fnlsls)
- No closure

Depending on your data set it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.
Select the scores matrix you saved previously.

This implementation of MCR uses the following defaults:
- Non negativity for components and spectra
- Fast non linear least squares (fnlsl)
- No closure

Depending on your data set it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

Visit MCR-ALS Website

MCR Code written by Roma Tauler and Anna de Juan, 1999
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This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast non-linear least squares (fnls)
- No closure

Depending on your data set, it can take some time to run MCR. Wait until you see a plot of the first MCR component to proceed.

The press the 'Load Selected Scores' button.

You can use any matrix you want as the initial guess. To use a non-scores matrix, enter the name of the matrix in the box below. That matrix must be within the active Matlab workspace.
Enter the desired stopping criteria. The process will stop when one of the two criteria is met.

- # iter = how many iterations to use.
- Tol. = the tolerance value below which the fit is good enough.

More iterations or a smaller tolerance will take longer to find a solution.
This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast non-linear least squares (fnlsls)
- No closure

Depending on your data set, it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

Press the 'Run MCR' button to start MCR processing.
A figure will pop up showing a linear plot of a scores and loadings matrix. I do not find it very useful. You can close it when the processing is done.

NOTE: It can take a long time to find a solution. The process is done when an MCR component image and spectra show up in the MCR window.
While the computer is working on the solution, this information will be output within the workspace. It shows a summary of the fitting procedure.

ITERATION 1
Sum of squares respect PCA reprod. = 403288.0538
Old sigma = 1446.3993 ----- New sigma = 0.20672
Sigma respect experimental data = 0.46946

FITTING IS IMPROVING !!!
Change in sigma (%) = 699583.6453
Fitting error (lack of fit, lof) in % (PCA) = 98.5147
Fitting error (lack of fit, lof) in % (exp) = 99.7084
Percent of variance explained (r2) is 0.58239
This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast non-linear least squares (fnls)
- No closure

Depending on your data set, it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

Once the component image and spectrum are displayed here, the solution has been found within the tolerance and # iterations specified above.
Here we can see that component 1 captures the spots. The peaks in the spectrum are indicative of PEG.
This implementation of MCR uses the following defaults:
- Non negativity for components and spectra
- Fast non linear least squares (fnlsl)
- No closure

Depending on your data set it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

To look at a different component, choose it from this drop down menu.
This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast non-linear least squares (fnlsls)
- No closure

Depending on your data set it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

And press the 'Plot Sel Comp' button.
This component captures the background and the peaks are indicative of the silane linker used on this sample.

Let's select component 3.
This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast non-linear least squares (fnlsls)
- No closure

Depending on your data set it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

And press the 'Plot Sel Comp' button.
Component 3 shows the DNA spot and the peaks are fragments typical of DNA.

So it looks like the assumption of 3 components was logical. However, this may not always be the case. You typically have to look at several solutions in order to determine which one makes the most sense.
This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast nonlinear least squares (fnlsls)
- No closure

Depending on your data set, it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

If you press the 'Plot All Comp' button...
It will create a figure for each component showing the component image and component spectrum.
This implementation of MCR uses the following defaults:
- Non negativity for components and spectra
- Fast non linear least squares (fnlms)
- No closure

Depending on your data set, it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.
Will create a separate Matlab figure window for the selected component image and spectrum.
This implementation of MCR uses the following defaults:
- Non negativity for components and spectra
- Fast non linear least squares (fnlsls)
- No closure

Depending on your data set it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

Pressing the 'Save Fig' button will open dialogs so you can save the component image and spectrum for the selected component.
First you save the component image. Give it a name and press the 'Save' Button.
Then you save the component spectrum. Give it a name and press 'Save'.
Here I have opened the saved images to show they were saved properly.
This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast non-linear least squares (fnls)
- No closure

Depending on your setup, you may need to adjust the number of iterations to run MCR. You can also choose a different initialization point.

Press the 'Save MCR Results' to export the component image and spectrum data into tab-delimited text files. The files contain the data from each component in a separate column. The data can be imported into any program that will accept tab-delimited text files.
First you save the component image. Give it a name and press the 'Save' Button.
Then you save the component spectrum. Give it a name and press 'Save'.
For more information about MCR and the MCR-ALS program, visit the MCR-ALS website. You can access it by pressing the 'Visit MCR-ALS Website' button.

Special thanks to Roma Tauler and Anna de Juan for making their code available for use.
This implementation of MCR uses the following defaults:
- Non-negativity for components and spectra
- Fast non-linear least squares (fnmlls)
- No closure

Depending on your data set it can take some time to run MCR. WAIT until you see a plot of the first MCR component to proceed.

Visit MCR-ALS Website
That's it for this tutorial.

Press the green button on the left to go back to the previous step. Press the button the right to go back to the beginning of the tutorial.