

J-Express Pro

tutorial documents

Loading Image Quantitation data
from GenePix, Scanalyze, Agilent etc.

Getting Started

Start the J-Express application and open the SpotPix Suite by selecting *load raw data (SpotPix Suite)* from the *File* menu. This is the main framework for loading and preparing data directly from image analysis systems. See also documentation available by clicking *F1* while the SpotPix Suite window is activated.


For GenePix files, you can also add the Jpeg image output files corresponding to the gpr data files. This information can be used to extract spot images from processed and analyzed data or for quality control.

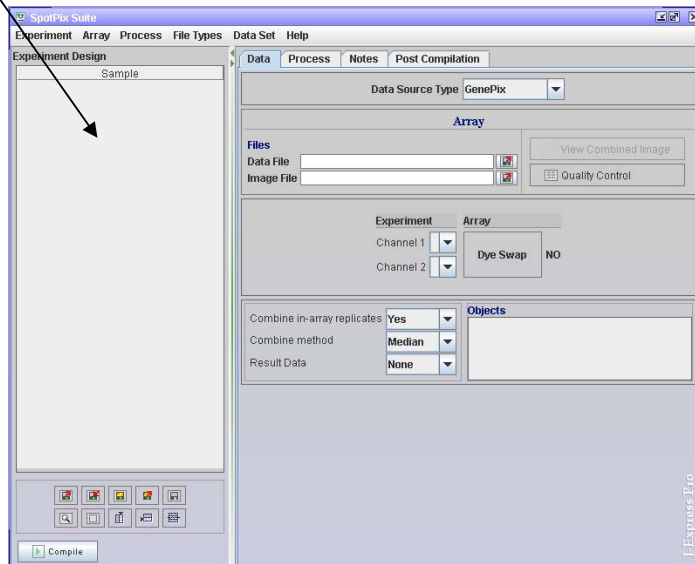
Setting up the experiment

There are several ways of setting up the experiment select one of the following:

1. Drag and Drop: Drag the files from your file explorer onto the experiment table. Double-click in the first column to change the names of the samples. If your experiment is a GenePix experiment and you have jpg files for the arrays,

Drop here

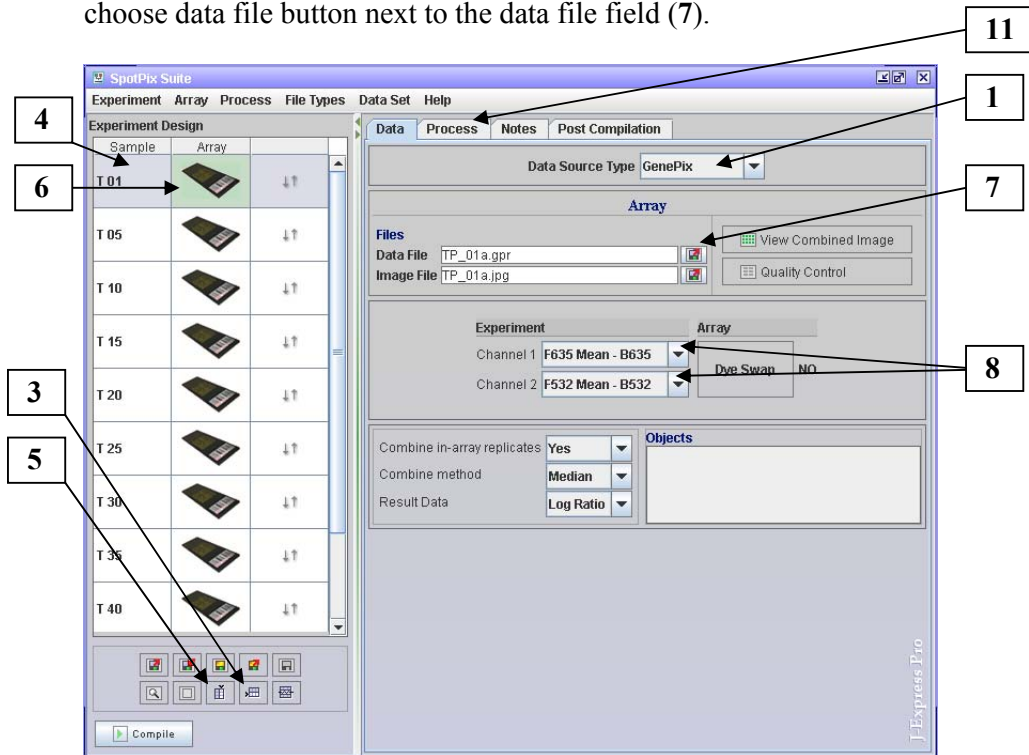
you can drag these files onto the array icons ().



2. Load Experiment From File List: select this option from the *experiment* menu. This opens a file browser and enables the selection of multiple files. Hold the

ctrl button down on your keyboard to select more than one file. Double-click in the first column to change the names of the samples.

- Manually set up the experiment by first choosing the *Tabular* or *GenePix* format in the Data Source Type Combo-box (1). If tabular, Set the File Type to your type of data file (If you can not find your data type in this box, see the documentation on how to add new file types). Start adding the data files by clicking the *Add Sample* (3) button or select from the *Experiment* menu. Double click in the first column (4) to set the names of the samples in your experiment. Click the *Add Replicate Column* button (5) or select from the *Experiment* menu. Create an abstract array by right-clicking in a row on the column called *Array* and selecting *Add Array* (6). Set the file reference by clicking on the array-icon (6) and opening the file chooser by clicking on the choose data file button next to the data file field (7).



repeat this procedure for all your data files.

Setting up the experiment

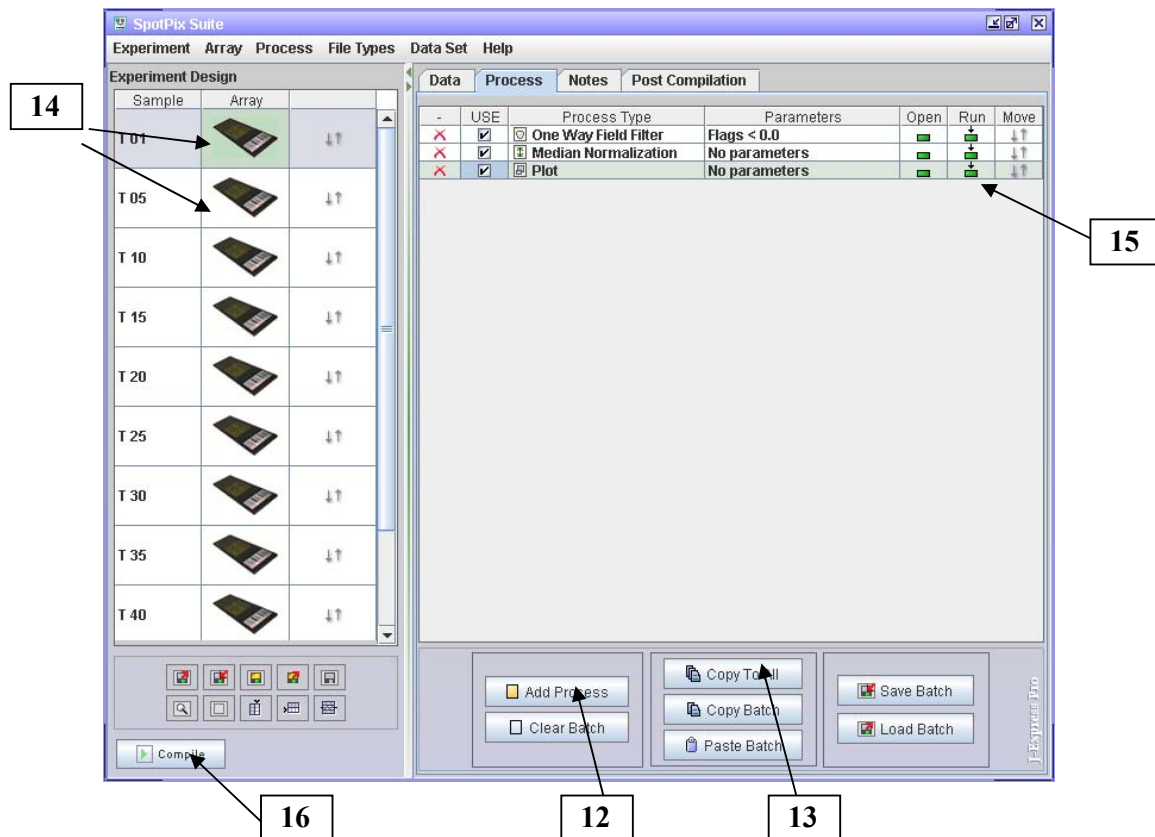
Set the correct channels by clicking the *channel 1* combobox (8). Click the Single Channel checkbox if this is a tabular one-channel experiment, and select a base sample (to normalize against).

Now choose if you would like to combine replicates, select the combine method you like and set the Result data to *Log Ratio* (if this is what you want). *For One channel experiments*, Log vs. Base will give you log ratio values for the sample and the base sample. If you want the absolute values, select NONE in the Result Data combobox.

Preparing the data

Filtering and normalization is normally a very important step of microarray analysis. In this example we have used a simple median normalization method. In some cases, you may prefer the non-linear lowess method instead. Click the *Process* tab (**11**) to create a batch of a simple normalization, a filter and a plot to control the layout of your data:

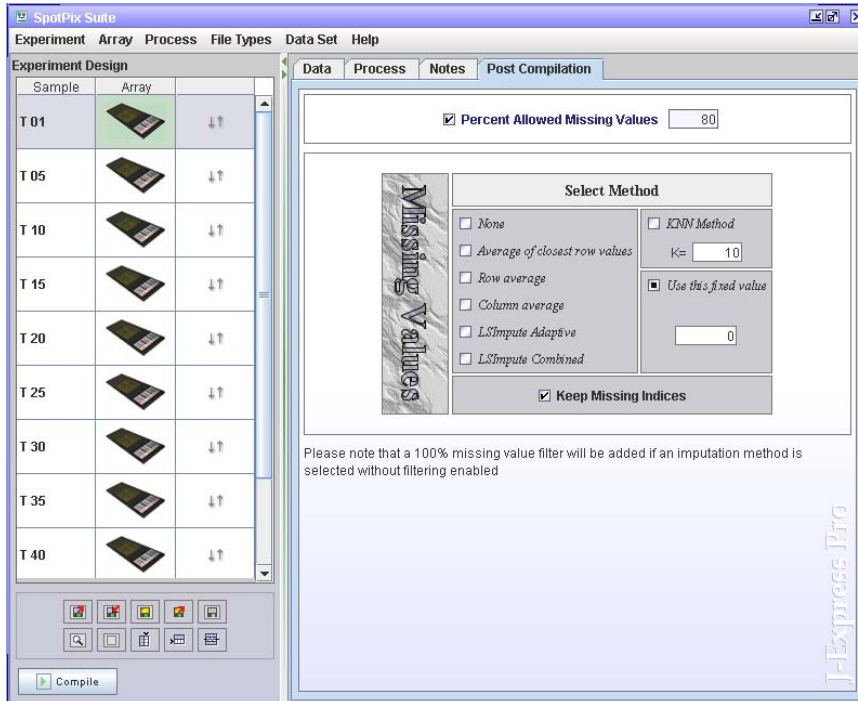
1. Click the *Add Process* button (**12**)
2. If there are flagged spots in your dataset and these have a flag column (GenePix Result Files have this) do the following:
3. Click the One way field filter.
4. Select flags in the field column, set the comparator to “<” and the value to 0 (This will remove all negative flags). Click ok.
5. Click the *Add Process* button (**12**) again.
6. Click the Normalization button to the left.
7. Select the *Global Median* method. Click Plot to see the normalization result (If the selected array is the base array, all spots will be located on the diagonal).
8. Click ok.
9. Click the *Add Process* again.
10. Click on the *Other* button to the left.
11. Select Plot and click OK.
12. Click the *Copy To All* button (**13**) to copy this process batch to all your arrays.
13. Control your arrays by selecting the different arrays (**14**) and clicking the run column on the plot row (**15**) and the *plot* button in the scatter plot window.



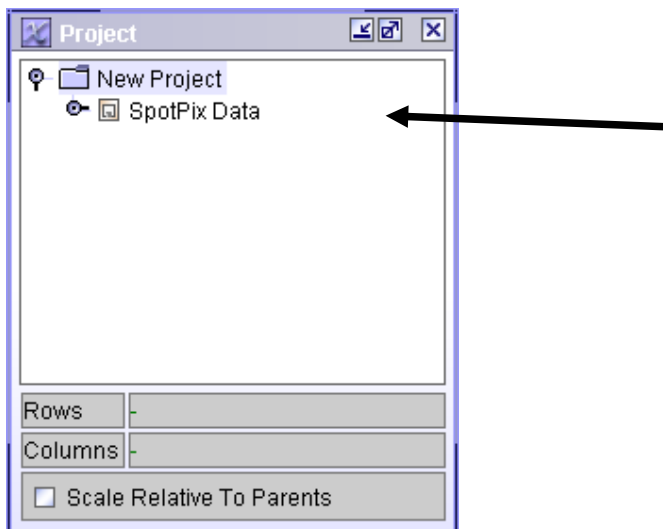
You have now created a simple filter, normalization method and a plot to process your arrays

1. Click the *Post Compilation* tab.
2. Let the Percent allowed missing values be 80 (This will filter all genes having 80% missing values or more.).

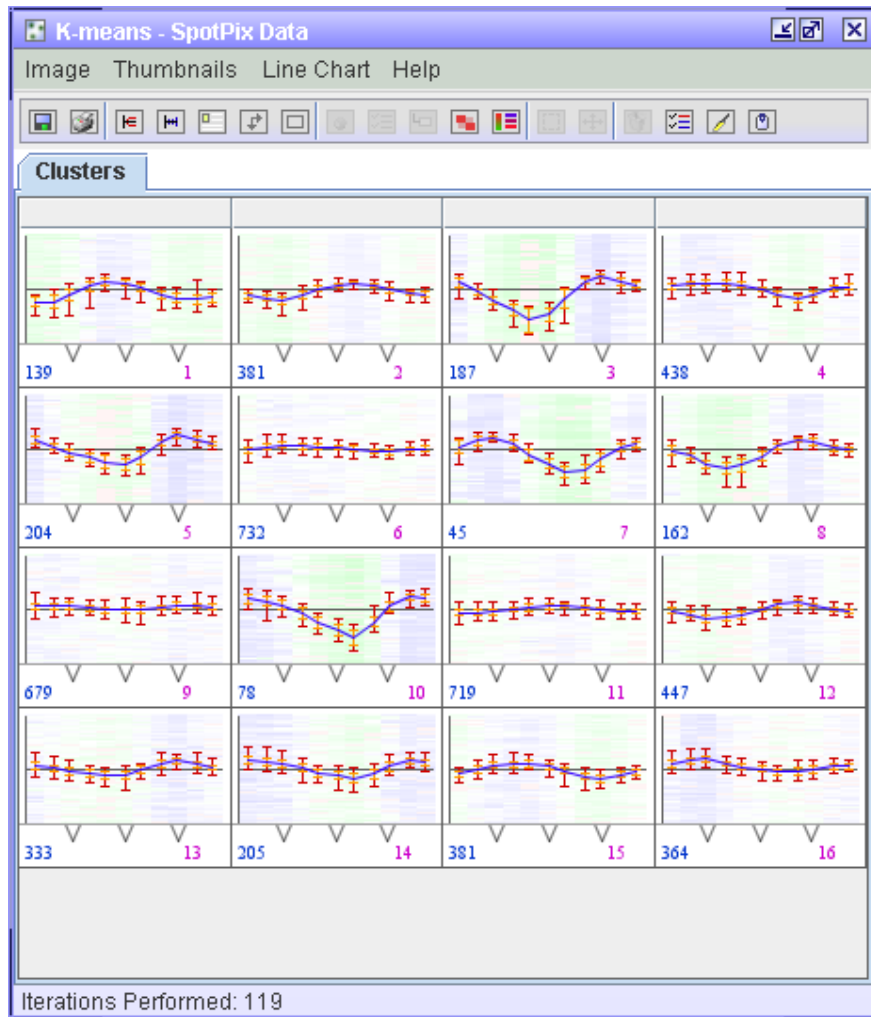
Set the missing values imputation method to be “Use this fixed value” and let the value be 0. Have the keep missing indices selected. This will set all filtered values to 0.0. (See documentation for information about the other imputation methods). The imputed values will be labelled so that you know this in later analysis.



Click the *compile* button (16) to create a gene expression matrix of the experiment.



3. Click on the new dataset (SpotPix Data).
4. Select K-Means from the Methods menu.
5. Click Ok in the K-Means clustering dialog.
6. Click on one of the clusters and select the new tab.



The Data are now grouped in regards to expression profiles using k-mean clustering.

- Continue on your own with downstream analysis. Good luck 😊