# PepSlide® Analyzer 2.0

## Quick Start Guide

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PepSlide® Analyzer is only intended for research and not intended or approved for diagnosis of disease in humans or animals.



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# 1 Introduction

*PepSlide® Analyzer* (*PSA*) facilitates microarray image analysis and data quantification. The software is particularly useful for peptide array users; you can benefit from analyses that directly support peptide array applications such as epitope mapping, biomarker discovery, and characterization or optimization of peptides.

This Quick Start Guide describes basic commands to enable immediate access to the functionalities of the software solution. Please find more details in the User's Guide.

## 1.1 Installation

PSA is natively supported on Windows and Mac OS X platforms, thus the software is able to run with high performance and native look. Installation of PSA requires rights of a system administrator.

### *Hardware Requirement*

Minimum hardware: 1.5 GHz Processor, 1GB RAM.

Recommended hardware: 2.0 GHz Dual-Core or faster Processor, 2 GB or more RAM.

### *Windows Platforms*

PSA works on Windows XP, Windows 7, and Windows 8. Simply run the PSA setup. If the current account is not administrator, you will be asked to input an administrative account and password.

### *Mac OS X platforms*

The software runs on Mac OS X 10.7 and 10.8. Unzip the package and double-click on the *.pkg* file to launch the installer. During installation you will be prompted to provide a system administrator's account and password. Upon completion, PSA is installed in folder */Applications/PepSlide/Analyzer*.

## 1.2 Product Activation

After installing PSA® on Windows, you need to activate the software with a *trial serial number* obtained from the software provider or its distributor. This enables the use of PSA® with full functionality for 14 days. The trial use for PSA® on Mac OS X platforms is handled automatically and does not require this step.

When the free trial time has expired, you can buy a software license to continue using PSA®. Upon the purchase, you receive a serial number and use it to activate the license. Please refer to the product activation steps here.

# 2 Software User Interface

Related software controls are grouped in labeled components as shown in Fig. 1. We refer to a software component using the name listed in Table 1.

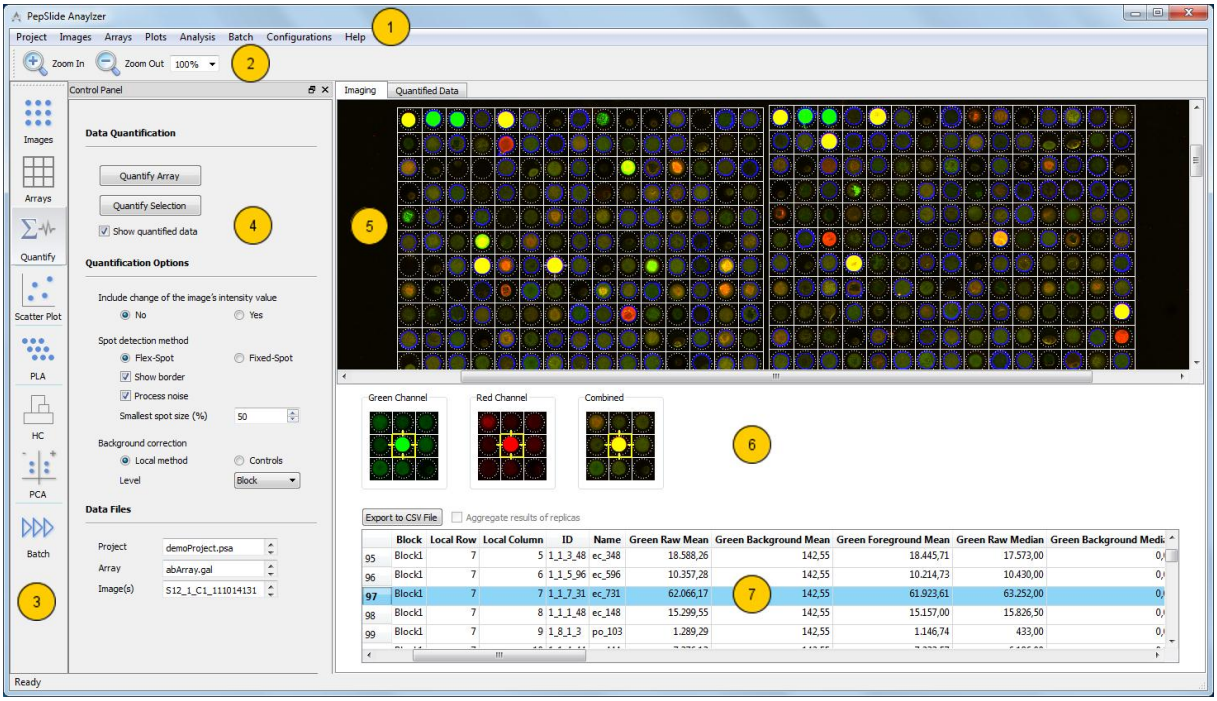



**Fig. 1: The Software User Interface.**

| Area | Component Name |
|---|---|
| 1 | The *menu* |
| 2 | The *canvas toolbar* |
| 3 | The *main toolbar* |
| 4 | The *control panel* |
| 5 | The *canvas* |
| 6 | The *Spot Image* widget |
| 7 | The *table of quantified data* |

**Table 1: Area Names.**

The main toolbar enables quick access to a group of related functions summarized in Table 2. Clicking a button on the main toolbar opens the control panel containing controls for these functions and the sheet that displays the data and the processed results.

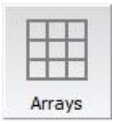
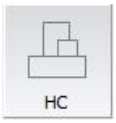
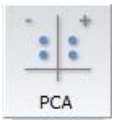
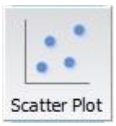
| | | | |
|---|---|---|---|
|  | Select image channel.<br>Change image's intensity.<br>Rotate images. |  | Peptide Library Analysis:<br>Support epitope mapping, peptide optimization, etc. |
|  | View properties of spot families (SF), blocks, and spots.<br>Rotate and move SF or blocks. |  | Hierarchical Clustering Analysis:<br>Show peptides and samples on a heat map with their correlation. |
|  | Quantify the array data and browse the quantified data. |  | Principal Component Analysis:<br>Select important peptides and samples. |
|  | Show spots in a scatter plot according their quantified data. |  | Batch Processing:<br>Automatically process and quantify multiple array images. |

**Table 2: The Main Toolbar and Related Functions.**

# 3 Peptide Array Analysis Diagram

The analysis diagram (Fig. 2) summarizes the steps for analyzing a single peptide array slide or a batch of multiple slides. Please note that in this manual, the term *array* is used to mean both *array* and *microarray*, unless otherwise stated.

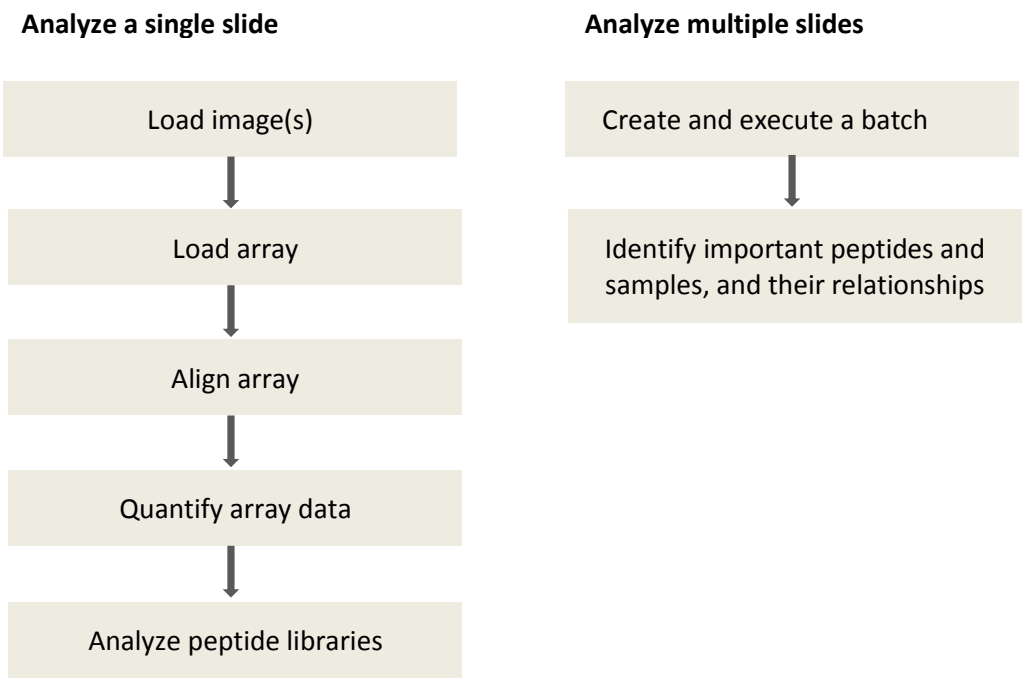



**Fig. 2: The Analysis Diagram**

Section 4 details the steps to *analyze a single slide* if the array file is of *PSF* format (*.psf). If you are using *GAL* files (*.gal), please find these steps in Section 5. The steps to *analyze multiple slides automatically* are described in Section 6.

# 4 Analyze a Single Slide with a PSF File

1 Load the image of the peptide array slide and observe the image data:


- Click the *Images* > *Open Image* menu and select the array image file.
- Assign the image signal to either *Red* channel or *Green* channel.

Suppose that the *Red* channel was chosen. The canvas then shows the image with signal as red pixels (Fig. 3a); the stronger the signal, the brighter the pixels.

PSA supports *uncompressed TIFF* images. For image quality we recommend 16-bit grayscale images. (8-bit grayscale or 24-bit color images are also supported.)

1.1 If the slide contains both peptides and controls, and they are separately scanned into *two* TIFF images or a *two-page* TIFF image:

- Assign signal of each image (or each page) to either *Red* channel or *Green* channel.

To show the signal of only peptides, or only controls, or both:

- Open the *Images* control panel.
- In the *Image Channels* section, select *Red*, *Green*, or *Combined* respectively.

1.2 To view the image and the signal at a proper scale:

- Use the *Zoom In* and *Zoom Out* buttons on the canvas toolbar. Alternatively, select or enter a zoom level in the *Zoom* combo-box.

1.3 To increase the signal's visibility:

- Open the *Images* control panel.
- In the *Image Intensity* section, enter a positive contrast number (e.g. 75) or select the *Enhance contrast automatically* option.

2 Load the array file (*.psf) and view the array data:


- Click the *Arrays* > *Open Array* menu and select the array file.

The array is then overlaid on top of the image in the canvas.

The array consists of spot families. A *spot family* (abbreviated *SF*) is a group of spots generated from the same source, such as a *peptide library* or a *control*.
In the canvas, a *spot* is illustrated as a white rectangle (Fig. 3a). Thus an SF is seen as a grid of white rectangles.

2.1 View properties of an SF or a spot:

- Open the *Arrays* control panel.
- To view an SF's properties, click to open the *Spot Family* page in the *Array Object* section. Then hover the mouse over the SF or click on it in the canvas.
- A spot's properties, which include the amino acid sequence, can be seen by opening the *Spot* page and then hovering mouse over the spot or clicking on it in the canvas.

3 Align the array to correctly associate spots in the array with their signal in the image:


- Improve the spots' visibility. (Refer to Step 1.3.)
- Based on the spot pattern, particularly from control spots, you may need to:
  - *Rotate* the image.
  - *Move* the SFs.
  - *Rotate* the SFs.
- Save the aligned position of the array.

3.1 To rotate the image:

- In the *Image Rotation* section:
  - Flip horizontally and/or vertically.
  - Rotate images at angles of 90°, 180°, or 270°.

The rotated image can then be saved with the *Images* > *Save Image As* menu.

You may want to check if the image should be *flipped horizontally*. A hint is to look at the control spots at the top-left corner and their signal in the image.

3.2 To select SFs containing spots that need reposition:

- Open the *Arrays* control panel and then click on the *Spot Family* page.
  - To select individual SFs, click on them while pressing the Ctrl key.
  - Click Ctrl-A to select all SFs in the array.

3.3 To move SFs:

- Select SFs.
- Click on the selection and drag the corresponding SFs to the intended position.

In the image, the spots' signal is shown in red, green, or a color different from white. Reposition the SFs such that for each spot, the inner region bounded by the *dashed rectangle* is as close to the spot's signal as possible (Fig. 3a).

3.4 To rotate SFs:

- Select SFs.
- In the *Array Rotation* section, enter the rotation angle and direction.

If the array slightly deviates from the image at a small angle, it is recommended that the relevant SFs be rotated, since image rotation may change the image data.

3.5 To save the aligned position of the array:

- Choose the *Arrays* > *Save Array* menu to save the aligned array to the current file.
- Save the aligned array to another PSF file using the *Array* > *Save Array As* menu.

4 Quantify the array data:

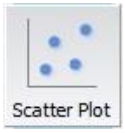

- Make sure the array is correctly aligned with the image.
- Click the *Quantify* button on the main toolbar to start the quantification procedure.
- Upon finishing, the results are updated into the *table of quantified data,* each row showing the intensity values of each spot. Suppose you assigned the peptide signal to the *Red* channel, you may use the following values for finding spots of interest:
  - *Red Foreground Mean* or *Red Foreground Median*
  - If spot replication is employed: *Aggregate Red Foreground Mean (or Median)*

Quantification is a complex process which involves many concepts. If you want detailed information, please check the User's Guide (Section 1.3 - Software Concepts and Section 4 - Quantification of Array Data).

4.1 View the quantified data:

- In the canvas, clicking on a spot shows the row in the table of quantified data that contains the spot's intensity values.
- Alternatively, selecting a row in the table also highlights the associated spot in the canvas. You can thus conveniently browse the quantified data with navigation keys such as *Up*, *Down*, *Page Up*, and *Page Down*.

4.2 Observe hits with the scatter plot:




- Click the *Scatter Plot* button on the main toolbar to display spots in the 2D-chart according to their quantified data.
- Select an SF in the *Spot families* box in the *Scatter Plot* control panel to show only spots of that SF.
- Use the blue bars to select positive spots. Their data, including the peptide, is then updated into the table below the chart and can be exported to a CSV file.

4.3 Save the quantified data to a CSV file:

- Click the *Export to CSV File* button.

4.4 Save the analysis results:

- Choose the *Project* > *Save Project* menu to save the analysis results to a PepSlide® Analyzer project file (*.psa).

The project file, e.g. named s1.psa, contains the path to the image, the aligned array, and the quantified data. When later opening s1.psa with the *Project* > *Open Project* menu, the image, the aligned array, as well as the quantified data will be reloaded.

5 Analyze peptide libraries:

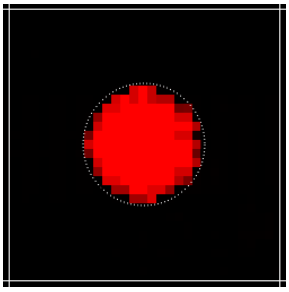

- Make sure the quantified data is available. (Refer to Step 4.)
- Click the *PLA* button on the main toolbar to start the analysis. Upon finishing, the results are shown in the *Peptide Library Analysis* sheet.

This tool facilitates analyses with respect to the peptide array application. If e.g. an antigen is translated into overlapping peptides for epitope mapping, the software may suggest a list of peptides which represent an epitope. In the case a peptide's variants are generated by substituting its residues with desired amino acids, you can observe all variants and their intensity value simultaneously, and easily select those of interest.

This analysis thus depends on how a peptide library is generated. For detailed steps, please check the User's Guide (Section 5 - Analysis of Peptide Libraries).

If you encounter the warning message: "*Please specify the peptide library design for the following spot families*", it means that from the array file, the software does not know how the peptides are generated. Please contact the peptide array provider for further information.

(a) Rectangular Spot



(b) Circular Spot

**Fig. 3: Spots in PepSlide® Analyzer.**

# 5 Analyze a Single Slide with a GAL File

1 Load the image of the peptide array slide and observe the image data:


- Click the *Images > Open Image* menu and select the array image file.
- Assign the image signal to either *Red* channel or *Green* channel.

Suppose that the *Red* channel was chosen. The canvas then shows the image with signal as red pixels (Fig. 3b); the stronger the signal, the brighter the pixels.

PSA supports *uncompressed TIFF* images. For image quality we recommend 16-bit grayscale images. (8-bit grayscale or 24-bit color images are also supported.)

1.1 If the slide contains both peptides and controls, and they are separately scanned into *two* TIFF images or a *two-page* TIFF image:

- Assign signal of each image (or each page) to either *Red* channel or *Green* channel.

To show the signal of only peptides, or only controls, or both:

- Open the *Images* control panel.
- In the *Image Channels* section, select *Red*, *Green*, or *Combined* respectively.

1.2 To view the image and the signal at a proper scale:

- Use the *Zoom In* and *Zoom Out* buttons on the canvas toolbar. Alternatively, select or enter a zoom level in the *Zoom* combo-box.

1.3 To increase the signal's visibility:

- Open the *Images* control panel.
- In the *Image Intensity* section, enter a positive contrast number (e.g. 75) or select the *Enhance contrast automatically* option.

2 Load the array file (*.gal) and view the array data:


- Click the *Arrays > Open Array* menu and select the array file.

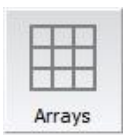
The array is then overlaid on top of the image in the canvas.

The array consists of blocks. A *block* is a group of spots located next to each other. In the canvas, a *spot* is illustrated as a white square (Fig. 3b). Thus a block is seen as a grid of white squares.

2.1 View properties of a block or a spot:

- Open the *Arrays* control panel.
- To view a block's properties, click to open the *Block* page in the *Array Object* section. Then hover the mouse over the block or click on it in the canvas.
- A spot's properties can be seen by opening the *Spot* page and then hovering mouse over the spot or clicking on it in the canvas.

3 Align the array to correctly associate spots in the array with their signal in the image:




- Improve the spots' visibility. (Refer to Step 1.3.)
- Based on the spot pattern, particularly from control spots, you may need to:
  - *Rotate* the image.
  - *Move* the blocks.
- Save the aligned position of the array.

If the *spots' signal is sufficient* the software may be able to align the array *automatically*. If this is not the case, use steps 3.2 to 3.4 to align the array manually.

3.1 To align the array automatically:

- In the *Array Object* section, click the *Align Array* button.

You can check the array alignment result by increasing the signal's visibility (Refer to Step 1.3) and verifying if for all spots, the inner region bounded by the *dashed circle* aligns well with the spot's signal (in green or red pixels, Fig. 3b). If this is the case, go directly to step 3.5 to save the aligned array.

3.2 To rotate the image:

- In the *Image Rotation* section:
  - Flip horizontally and/or vertically.
  - Rotate images at angles of 90°, 180°, or 270°.

The rotated image can then be saved with the *Images* > *Save Image As* menu.

3.3 To select blocks containing spots that need reposition:

- Open the *Arrays* control panel and then click on the *Block* page
  - To select individual blocks, click on them while pressing the Ctrl key.
  - Click Ctrl-A to select the entire array.

3.4 To move blocks:

- Select blocks.
- Click on the selection and drag the corresponding blocks to the intended position.

In the image, the spots' signal is shown in red, green, or a color different from white. Reposition the blocks such that for each spot, the inner region bounded by the *dashed circle* is as close to the spot's signal as possible (Fig. 3b).

3.5 To save the aligned position of the array:

- Choose the *Arrays* > *Save Array* menu to save the aligned array to the current file.
- Save the aligned array to another GAL file using the *Array* > *Save Array As* menu.

4 Quantify the array data:

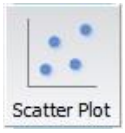

- Make sure the array is correctly aligned with the image.
- Click the *Quantify* button on the main toolbar to start the quantification procedure.
- Upon finishing, the results are updated into the *table of quantified data,* each row showing the intensity values of each spot. Suppose you assigned the peptide signal to the *Red* channel, you may use the following values for finding spots of interest:
  - *Red Foreground Mean* or *Red Foreground Median*
  - If spot replication is employed: *Aggregate Red Foreground Mean (or Median)*

Quantification is a complex process which involves many concepts. If you want detailed information, please check the User's Guide (Section 1.3 - Software Concepts and Section 4 - Quantification of Array Data).

4.1 View the quantified data:

- In the canvas, clicking on a spot shows the row in the table of quantified data that contains the spot's intensity values.
- Alternatively, selecting a row in the table also highlights the associated spot in the canvas. You can thus conveniently browse the quantified data with navigation keys such as *Up*, *Down*, *Page Up*, and *Page Down*.

4.2 Observe hits with the scatter plot:




- Click the *Scatter Plot* button on the main toolbar to display spots in the 2D-chart according to their quantified data.
- Select a block in the *Blocks* box in the *Scatter Plot* control panel to show only spots of that block.
- Use the blue bars to select positive spots. Their data is then updated into the table below the chart and can be exported to a CSV file.

4.3 Save the quantified data to a CSV file:

- Click the *Export to CSV File* button.

4.4 Save the analysis results:

- Choose the *Project > Save Project* menu to save the analysis results to a PepSlide® Analyzer project file (*.psa).

The project file, e.g. named s1.psa, contains the path to the image, the aligned array, and the quantified data. When later opening s1.psa with the *Project > Open Project* menu, the image, the aligned array, as well as the quantified data will be reloaded.

5 Analyze peptide libraries:


- Make sure the quantified data is available. (Refer to Step 4.)
- Specify the peptide design library as described in Section 5.4 - Peptide Library Analysis with GAL Files - of the User's Guide.
- Click the *PLA* button on the main toolbar to start the analysis. Upon finishing, the results are shown in the *Peptide Library Analysis* sheet.

This tool facilitates analyses with respect to the peptide array application. If e.g. an antigen is translated into overlapping peptides for epitope mapping, the software may suggest a list of peptides which represent an epitope. In the case a peptide's variants are generated by substituting its residues with desired amino acids, you can observe all variants and their intensity value simultaneously, and easily select those of interest.

This analysis thus depends on how a peptide library is generated. For detailed steps, please check the User's Guide (Section 5 - Analysis of Peptide Libraries).

# 6 Analyze multiple slides with a GAL File

Suppose that the study is to screen a peptide array with *k* samples. This employs *k* slides of the peptide array whose annotation is based on *a single GAL file,* the so-called *template array*. The screening of *k* slides results in *k* scanned images. You can then setup a *batch* to automatically process all *k* images and generate their quantified data.

The current version of PepSlide® Analyzer only supports batch processing for *GAL files and circular spots*. The images are either one-page or two-page grayscale TIFF files. In addition, the spots' signal in the images should be sufficient for positioning the array.

1 Create and execute the batch:


- Click the *Batch* button on the main toolbar to create a new batch.
- In the *Batch* control panel, specify the images, and template array, and the options.
- Execute the batch.

1.1 To specify parameters and options of the batch:

- Click the *Add* button and select the images for processing.
- Double-click on the *Template array* edit-box to browse to the template array file.
- Specify the *folder* to *store generated files*.
- Finally, save the batch to a file using the *Batch > Save Batch* menu. The batch log is created automatically and named after the batch file.

For each batch, we recommend using a separate folder to store the batch file and generated data files.

1.2 Execute the batch:

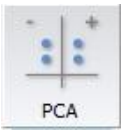
- Click the *Run* button to start the batch. During execution, the status of the currently processed images will be updated.

Suppose that *sample001.tif* is an image in the batch. Three data files are then generated for this image:

- *sample001.gal*: the array file in which the array's spots are associated with their signal in the image sample001.tif,
- *sample001.csv*: the CSV file containing only the quantified data, and
- *sample001.psa*: the project file containing the analysis data for the image.

As a result, *k* project files (*.psa) will be generated for a batch of *k* images. They can be used for discovering important peptides and samples as well as their relationships in Step 2 and Step 3.

2 Identify important peptides and samples with *Principal Component Analysis (PCA)*:




- Click the *PCA* button on the main toolbar.
- In the *PCA* control panel:
  - Click the *Load Data* button and select the *k* project files (*.psa).
  - Select a screening value in the *Data Column* list-box, e.g. *Red Foreground Mean* or *Red Foreground Median*.

2.1 To find *peptides* that influence the variance of the study:

- Choose *Simplify the dataset to three Samples*.
- Click the *Start Analysis* button.

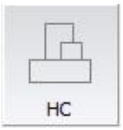
2.2 To find *samples* that influence the variance of the study:

- Choose *Simplify the dataset to three Features*.
- Click the *Start Analysis* button.

In addition to PSA project files (*.psa), the PCA tool also accepts a list of *GenePix Result* files *(*.gpr)* or a CSV file prepared in the suitable dataset format.

For detailed steps on the dataset and the PCA tool, refer to Section 7.1 – Dataset - and Section 7.2 - Principal Component Analysis - of the User's Guide.

3 Discover relationships between peptides and samples with *Hierarchical Clustering Analysis (HCA)*:




- Click the *HC* button on the main toolbar.
- In the *HCA* control panel:
  - Click the *Load Data* button and select the k project files (*.psa).
  - Select a screening value in the *Data Column* list-box, e.g. *Red Foreground Mean* or *Red Foreground Median*.
  - Choose to construct the clustering tree for features (i.e. peptides), or samples, or both of them.
  - Click the *Start Analysis* button.

The *Hierarchical Clustering* sheet then shows a heat map. It is like a matrix of boxes with rows representing peptides and columns representing samples. Consider the box at row *p* and column *s*, its color represents the screening value of peptide *p* against sample *s*.
In addition, samples that are related (e.g. those that response similarly to a certain group of peptides) are grouped into a *cluster*. Their relationship is illustrated by a line connecting them. The relationship between peptides is similarly illustrated.

3.1 To review the screening result of peptide *p* against sample *s*:

- Observe the color of the corresponding box on the heat map.

3.2 To identify the relationship between peptides or between samples:

- Observe the *clusters* and the line connecting them.

In addition to PSA project files (*.psa), the HCA tool also accepts a list of *GenePix Result* files *(*.gpr)* or a CSV file prepared in the suitable dataset format.
For detailed steps on the dataset and the HCA tool, refer to Section 7.1 – Dataset - and Section 7.3 - Hierarchical Clustering Analysis - of the User's Guide.