

# User Manual

## Microarray evaluation using a .gal-File with JPT's Microarray Feature Viewer

Allocation of peptide sequences to spot positions on peptide microarrays

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## 1 Introduction

A peptide microarray is a planar slide with peptides spotted onto it. These peptides are spotted onto the microarray surface in small droplets. The peptides within the droplets react with the functional groups present on the slide surface resulting in a stable covalent bond between peptide and slide. The slide area where the droplet was printed is called a spot. A spot is defined by its diameter and is distinguishable from the surrounding slide surface thus enabling a reliable identification of each spot with its associated peptide sequence.

### 1.1 Software Modules & Files Required

You need:

- Image of your peptide microarray scanned by a high resolution scanner system capable of detecting signals excited by peptide spots which were treated according to protocol (for fluorescence-based readout JPT recommends scanning systems of Molecular Devices/Axon like Genepix microarray scanners).
- .gal-file (GenepixArray-List) containing all peptide sequence and layout information (provided by JPT).
- Software tool capable of opening .tif-pictures (tagged image file) to display your image of the scanned peptide microarray (JPT recommends the software tool GenepixPro 6.0 (or higher) for evaluation and analysis of microarray data). JPT also provides an HTML file for each .gal-file which allows inspection of the slide layout and can be used for simple applications (enclosed with each microarray shipment).
- Optional: Software modules processing tab-separated text files, such as Microsoft Editor (Notepad) or Microsoft Excel.

## 2 Design of a .gal File

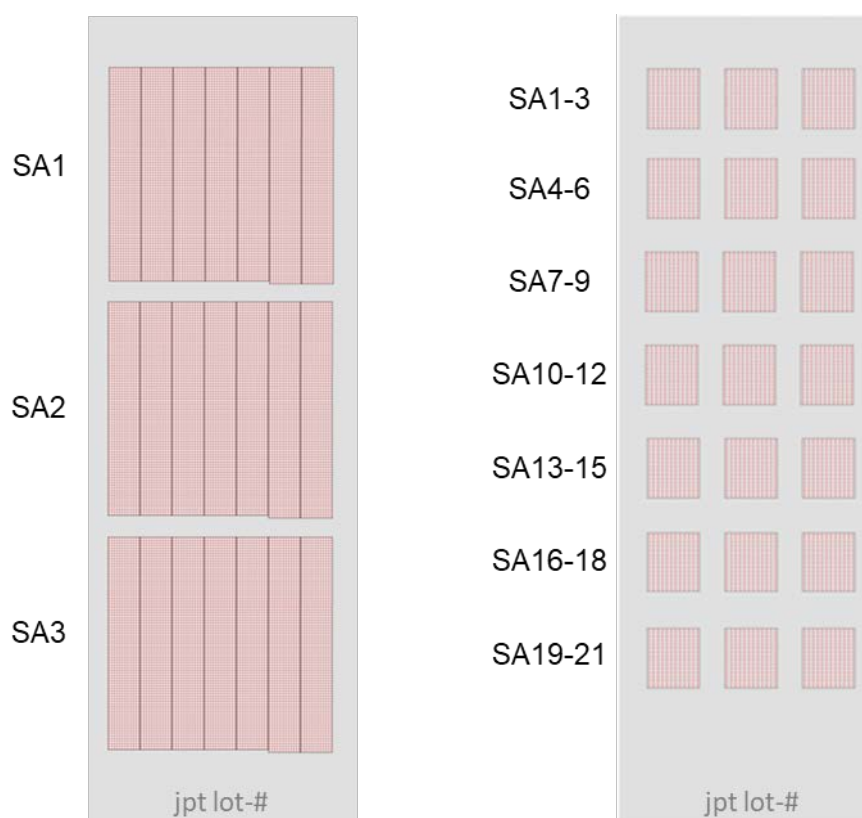
After scanning of the dried microarray slides, the scanner records a 16-bit (or 8-bit) numeric image in tagged image file format (\*.tif). The .tif-image can be opened by a software-tool enabling interpretation and quantification of grayscales representing the data in each fluorescent spot on the scanned microarray slide. These quantitative data are the basis for performing statistical analysis on measured binding events or peptide modifications on the microarray slide. For evaluation and interpretation of detected signals an allocation of peptide spot (visible in the image) and its corresponding peptide sequence has to be performed. The data for allocation are saved in the GenePix Array List (.gal) file.

The .gal-file is a simply text file giving specific information about position and size of the blocks, the layout of spot-indicators in the blocks, and the identity of each peptide sequence printed onto the microarray slide. The .gal-file can be opened using microarray evaluation software-modules capable of evaluating high-density microarray slides. Since .gal-files are tab-separated text files, they can also be accessed with text-processing software such as Microsoft Editor (Notepad), Notepad++ or Microsoft Excel.

For qualitative analysis the provided HTML file can be used. This file can be opened with any common web browser. No internet connection is required, however, JavaScript must be enabled for the page to be displayed properly. Simple highlight and export options allow qualitative evaluation of low-density microarray images.

## 2.1 Layout of Microarray

JPT's standard peptide microarrays are composed of 3 or 21 subarrays that hold one or several blocks of peptide spots. A schematic and exemplary layout of both peptide microarray formats is shown in Figure 1.



*Figure 1: Schematic layout of JPT's standard peptide microarray layouts (SA=subarray). Peptides are displayed at the same side as the engraved lot-#. For custom-made peptide microarrays, total dimensions, layout details and distances might vary according to customers' needs.*

As shown in Figure 1 JPT's peptide microarrays are printed in 3 or 21 identical subarrays (SA). This enables efficient intra-chip-reproducibility tests using scatter plots or correlation functions. Each peptide subarray (SA) is printed in individual blocks (see Figure 2). An exemplary subarray and block layout is shown in Figure 2 and Figure 3.

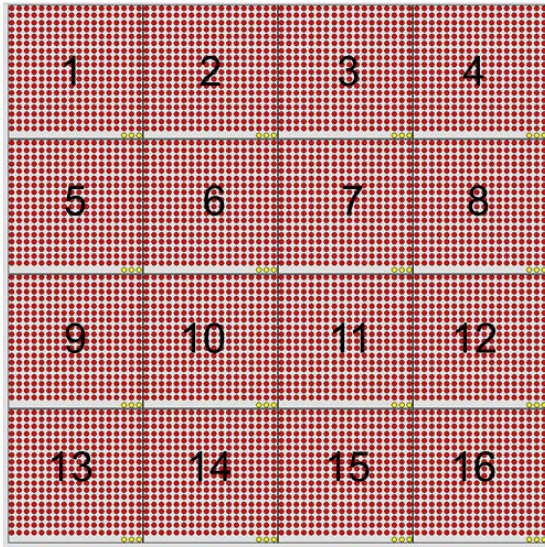


Figure 2: Exemplary subarray consisting of 16 individual blocks. Each block is calculated by number of rows vs. number of columns.

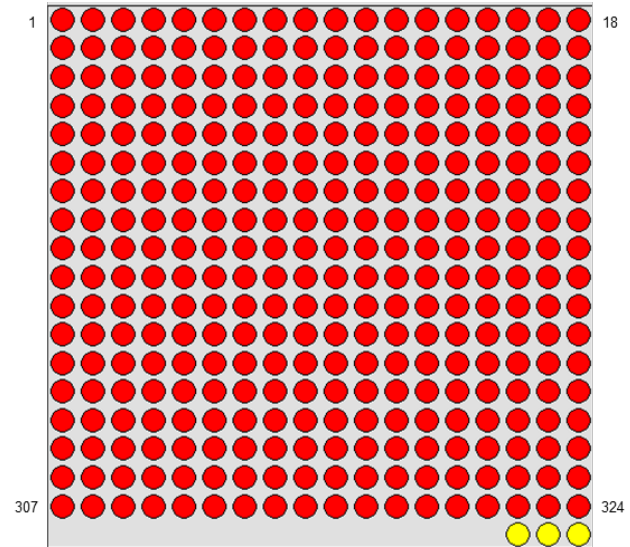


Figure 3: Exemplary block layout, yellow colored spots represents protein controls.

Block numbers are assigned starting from top leftmost block in subarray 1 (then row-wise from left to right, see Figure 2). Therefore, in this case subarray one is represented by blocks 1-16, subarray two is represented by blocks 17-32 and subarray 3 by blocks 33-48. Each subarray will contain at least one block. The number of blocks and arrangement might differ, depending on total number of peptides and technology used. Spot numbering within each block starts in the top left corner increasing from left to right and from top to bottom (see Figure 3). All spots are uniquely identified by block, column and row number.

## 2.2 Description & Content of a .fal File

All .gal-files consist of two sections: Header and data records. The header contains all structural and positional information about the blocks. The data records contain all information on the printed peptide sequences and identifier information for each spot.

## 2.21 Description of Header Records

The header section describes basic file information and all the block properties. Each line is explained below:

<b>ATF 1</b>	First line of an Axon Text File (ATF) file; the same in all GAL files: File format (ATF) and version (1.0)														
<b>7 5</b>	Second line of an ATF file: 7 number of header records 5 number of data columns (in the data record section).														
<b>Type=GenePix Array List V1.0</b>	Type of file; the same in all GAL files.														
<b>BlockCount=3</b>	Number of blocks described in the file.														
<b>BlockType=0</b>	Type of block described: 0 = rectangular. 1 = orange-packing #1. 2 = orange-packing #2.														
<b>"Block<math>n</math>="</b>	The position and dimensions of each block. There is one record for each block, and each record contains 7 fields. Each field is separated by a comma followed by a space.														
	<table> <tr> <td><i>xOrigin</i></td> <td>X position of center of top leftmost feature of current block (in <math>\mu\text{m}</math>).</td> </tr> <tr> <td><i>yOrigin</i></td> <td>Y position of center of top leftmost feature of current block (in <math>\mu\text{m}</math>).</td> </tr> <tr> <td><i>FeatureDiameter</i></td> <td>Diameter of features within the current block (in <math>\mu\text{m}</math>).</td> </tr> <tr> <td><i>xFeatures</i></td> <td>Number of columns of features in current block.</td> </tr> <tr> <td><i>xSpacing</i></td> <td>Column spacing of current block (in <math>\mu\text{m}</math>).</td> </tr> <tr> <td><i>yFeatures</i></td> <td>Number of rows of features in current block.</td> </tr> <tr> <td><i>ySpacing</i></td> <td>Row spacing of current block (in <math>\mu\text{m}</math>).</td> </tr> </table> <p><b>Note:</b> Positions on arrays are measured in microns with respect to the origin, which is the top left corner of the array.</p>	<i>xOrigin</i>	X position of center of top leftmost feature of current block (in $\mu\text{m}$ ).	<i>yOrigin</i>	Y position of center of top leftmost feature of current block (in $\mu\text{m}$ ).	<i>FeatureDiameter</i>	Diameter of features within the current block (in $\mu\text{m}$ ).	<i>xFeatures</i>	Number of columns of features in current block.	<i>xSpacing</i>	Column spacing of current block (in $\mu\text{m}$ ).	<i>yFeatures</i>	Number of rows of features in current block.	<i>ySpacing</i>	Row spacing of current block (in $\mu\text{m}$ ).
<i>xOrigin</i>	X position of center of top leftmost feature of current block (in $\mu\text{m}$ ).														
<i>yOrigin</i>	Y position of center of top leftmost feature of current block (in $\mu\text{m}$ ).														
<i>FeatureDiameter</i>	Diameter of features within the current block (in $\mu\text{m}$ ).														
<i>xFeatures</i>	Number of columns of features in current block.														
<i>xSpacing</i>	Column spacing of current block (in $\mu\text{m}$ ).														
<i>yFeatures</i>	Number of rows of features in current block.														
<i>ySpacing</i>	Row spacing of current block (in $\mu\text{m}$ ).														
<b>"Block" "Row" "Column" "Name" "ID"</b>	Last line of the header, containing column titles for the data records.														

## 2.22 Description of Data Records

The data records section contains descriptions of each spot in detail. It includes the block, column and row numbers of all spots with their specific names (origin of peptide sequence, e.g. Protein 1) and ID's (peptide sequence).

There is one line for each spot, containing a field for each of the descriptive columns:

<i>Block</i>	Block number of the spot.
<i>Column</i>	Column location within the block.
<i>Row</i>	Row location within the block.
<i>Name</i>	Name to be displayed for the given spot, name of the origin of the displayed peptide immobilized in the spot.
<i>ID</i>	Peptide sequence for this spot.

**Note:** If there are empty features, 'empty' or 'none' is written.

## 2.23 Exemplary .gal File & Evaluation

The following Figure 4 demonstrates a simple extract of a .gal-file for a microarray with three blocks, each with 12 columns and 5 rows.

```

ATF      1.0
7        6
Type=GenePix ArrayList V1.0
BlockCount=3
BlockType=0
URL=http://|
Block1= 3727,8281,200,14,321,13,346
Block2= 8227,8281,200,14,321,13,346
Block3= 12727,8281,200,14,321,13,346
Block  Row   Column  Name      ID          Annotation
1       1       1       Q04360_Peptide_071  KSTNKDWTLDARMQA 5175_1_1|4850393|10058736_G01_0269|8288_D01_0320
1       1       2       Q04360_Peptide_075  QNAGLCTLVAMLEET 5175_1_1|4850397|10058736_G05_0185|8288_D05_0138
1       1       3       Q04360_Peptide_079  FWLQEITYHGDLPLA 5175_1_1|4850401|10058736_G09_0217|8288_D09_0294
1       1       4       Q04360_Peptide_083  AEDILLACAMSLSKV 5175_1_1|4850405|10058736_H01_0171|8288_D13_0122
1       1       5       Q04360_Peptide_087  LTKLKLAPCFNPNT 5175_1_1|4850409|10058736_H05_0418|8288_D17_0459
1       1       6       Q04360_Peptide_091  DYNFVKQLFYITCAT 5175_1_1|4850413|10058736_H09_0135|8288_D21_0133
1       1       7       Control-Spot      Human_IgG      Human_IgG|Sigma_I4506-10mg|Standard

```

Figure 4: Example of a simple .gal file (header and first rows of data section shown only).

As shown in Figure 4 the .gal-file allocates each spot to a defined position within the microarray. By using the generated microarray grid, assignment of all peptide sequences to a spot (or feature) is easily possible.

For example the features shown in Figure 5 column 16 and 17 yields saturated signals indicating a strong binding event (see yellow arrow) for all three replicates in row 5, 13 and 21.

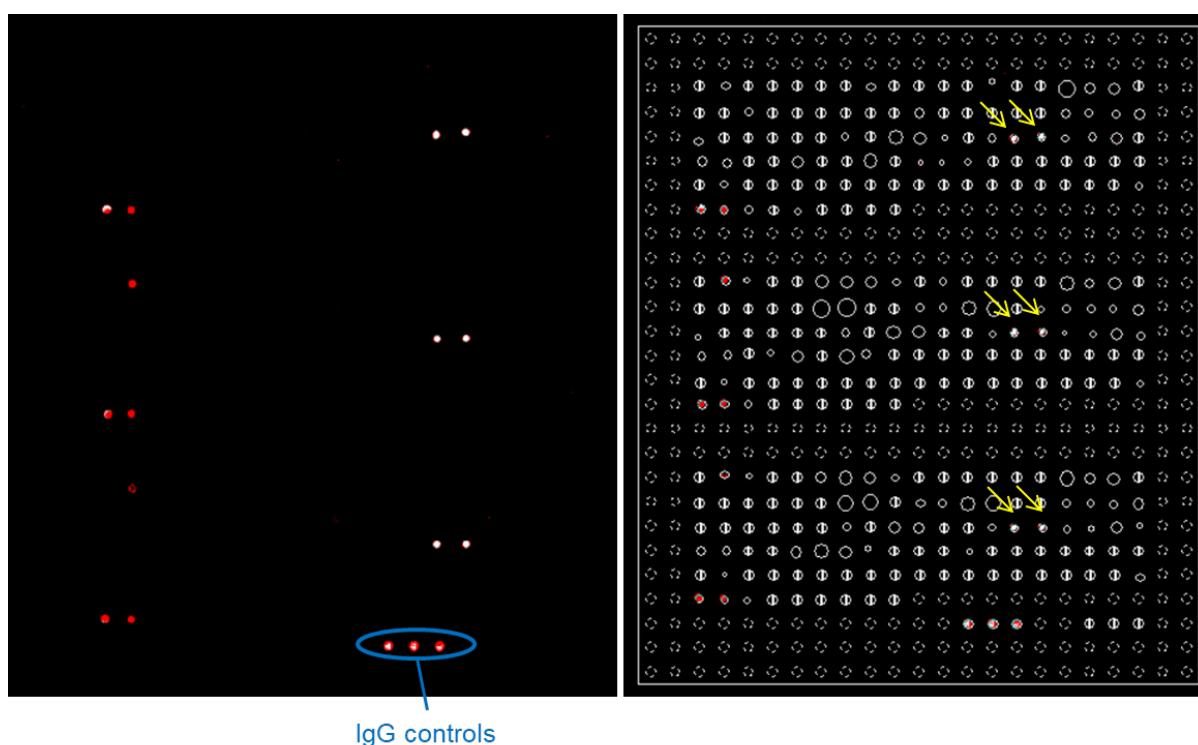


Figure 5: Application of a .gal-file. Left side shows a single block of a scanned microarray image. Right side shows the same block with positions by applying the .gal-file.

For more detailed information, please refer to the homepage of Genepix-software ([www.genepix.com](http://www.genepix.com)).

## 3 Using a .gal File

### 3.1 Image-based Application

By using a software tool (JPT recommends Molecular Devices' GenePix Software) enabling to load the scanned image in .tif format as well as the .gal-file, the quantitative evaluation of the spots is easily performed. After loading the .tif file into the software program, the .gal-file is opened generating a feature grid which can be positioned on the microarray image. For better visualisation of weak signals contrast of the microarray image should be adjusted until the spots are clearly defined. The feature-grid generated by the .gal-file can be placed on top of the corresponding spot within the .tif image orientated by protein controls such as human IgG. If the adjustment is correct, diameter and position of the spot equals the spot in the image, such that the spot in the image is inside the spot position in the grid (see Figure 6). Although the diameter of the spots and the position of each spot in the block might vary by some  $\mu\text{m}$ , all spots should now be properly aligned. The Genepix-software supplies several algorithms to perform an alignment of all features to the most intensive signals visible in the grid (special parameters like "allowed spot movement", "pixel intensity threshold" etc. are dependent on the experiment and should be adjusted by each user). After performing the alignment, all spots should be perfectly circled (see Figure 6).

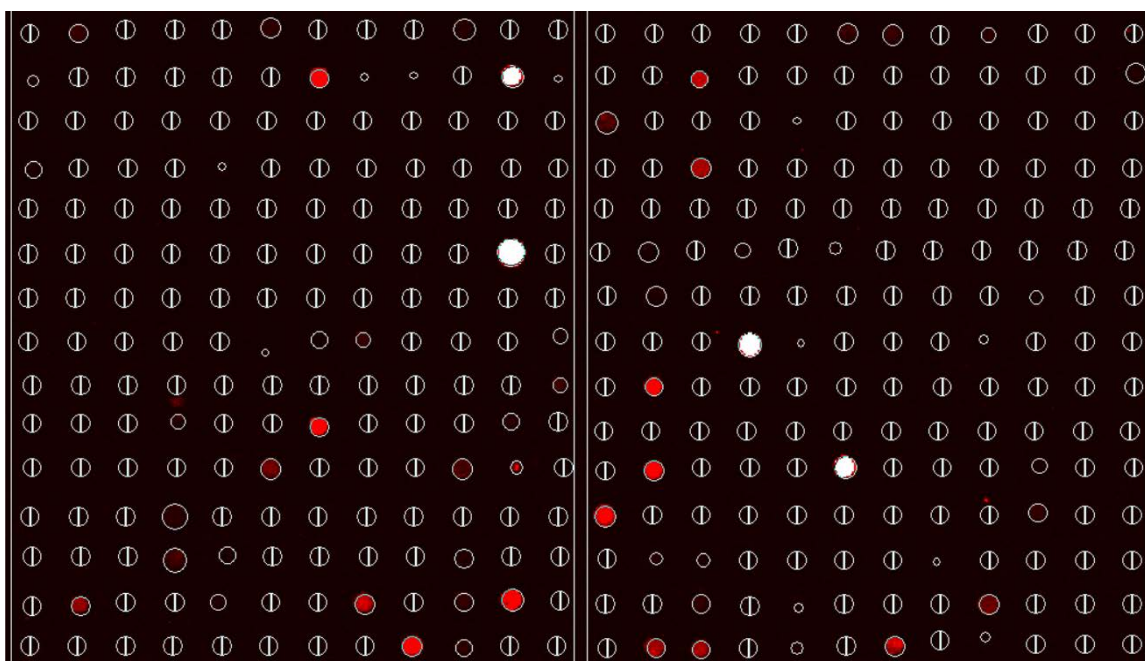


Figure 5: After the alignment, all spots above the pixel threshold are recognized and the diameter is optimized for perfect readout.

Subsequent to alignment all spots can be analyzed, resulting in a table containing all quantitative values of the data recorded, e.g. signal intensity or signal-to-noise-ratio. These GenePix Results data (\*.gpr) are saved as .gpr files, which are in a tab-delimited plain text file format. A results file contains general information about image acquisition and analysis, as well as the data extracted from each individual feature/spot. For further analysis the .gpr-file can be processed in a text interpreting program (Microsoft Excel or Microsoft Editor), database (e.g. Molecular Devices Acuity 4.0) or statistical software packages like R.

## 3.2 .gal File-based Application

### 3.2.1 Open .gal File & Basic Orientation on Layout

If you are reading out the scanned images manually, the spots in the image should be very clearly defined by optimizing the contrast. The .gal-file can be represented visually by loading the HTML file included with any order into a web browser. For each microarray lot a separate HTML file is provided. (User Interface see Figure 7). Any common web browser can be used, however, JavaScript must be enabled to access all functionalities of the HTML file. There is no need to have an Internet connection for displaying the file in the web browser.

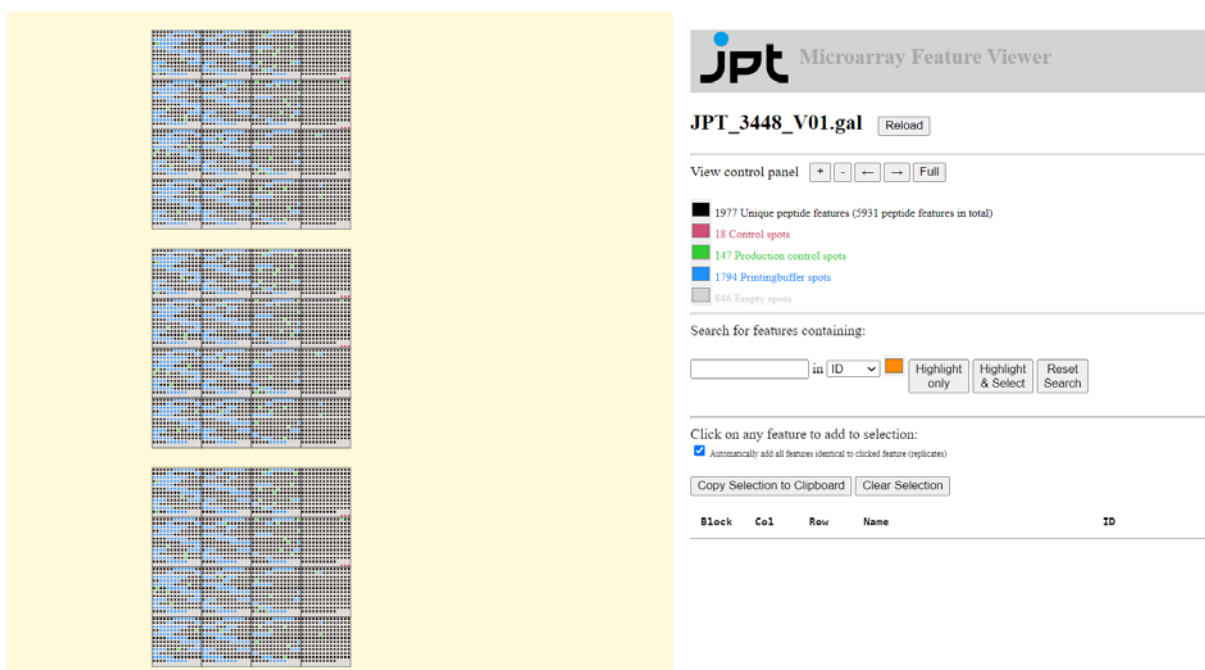


Figure 7: User interface of the Microarray Feature Viewer. Left side shows a .gal-file containing 48 blocks in 3 subarrays. Right side shows several tools for feature selection and highlighting.

All printed spots on the microarray are displayed in the left panel. The right side offers view control buttons as well as some simple tools to highlight and/or select features. The top button row can be used to zoom in and out or pan the display left and right. To reset the view, press the “Full” button. Features are color-coded by their category (peptide spots, control spots etc.). You can change the color of each category by clicking on the color field next to its description.

The orientation of the microarray is always the same. Block one is located in the upper left corner and the last block of your array is located in the lower right corner. Subarray one is on top, subarray 3 or 21 is at the bottom of the page. If necessary, please rotate the scanned image of your microarray to ease manual evaluation (block 1 and subarray 1 are located on top if the engraved label is visible at the bottom of the screen).

### 3.2.2 Select & Export Feature Information

Simple cursor movement over the features on the left side shows detailed information of the corresponding spot while the block that contains the spot changes the color. For manual evaluation, zoom in to the area of interest on both the microarray image and the schematic .gal-file and select spots on the microarray layout by a simple click. All selected spots will change their color to the color of your choice. The selected features are summarized in the selection board on the right side. By default, all features identical to the one selected by clicking on it (“replicates”) are automatically selected with it. You can switch this off by deactivating the checkbox in the selection panel.

Further highlighting or selections are available by the search function (see Figure 8). Here, you can look for any partial or full matches in the peptide name or ID fields of the .gal-file. Type a search term into the text box and select a field name from the dropdown menu next to it. All searches are case-insensitive. You can choose either

to only highlight the features found by the search in the display panel, or to also add the search results to the selection panel. Clear the search terms by pressing the “Reset Search” button.

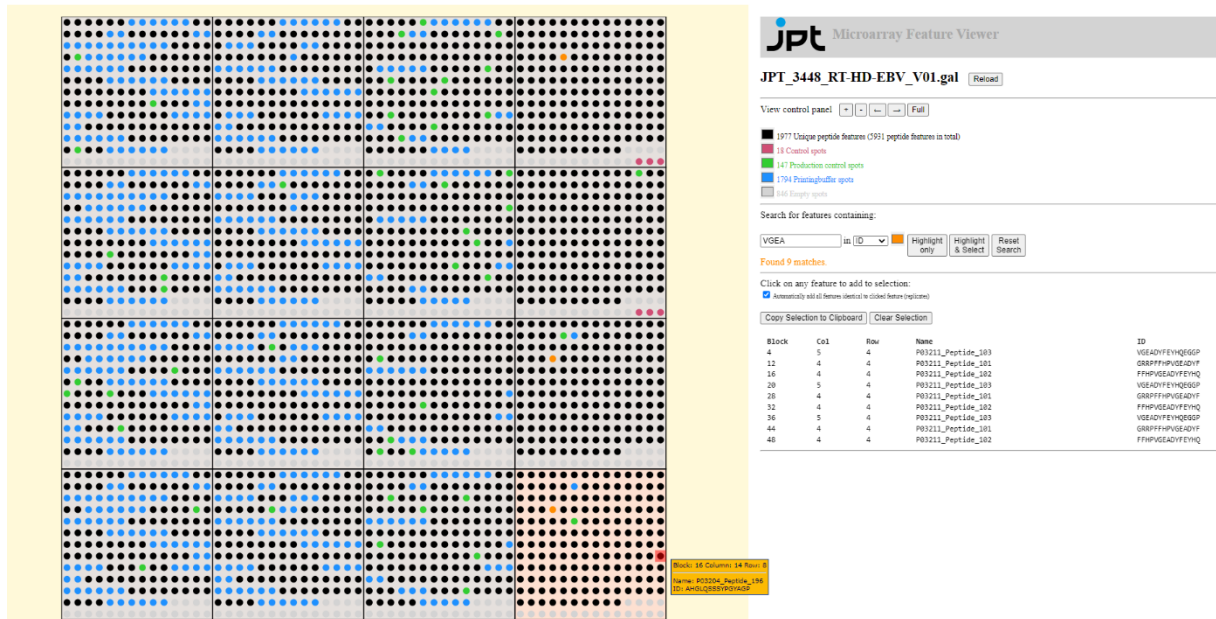


Figure 8: Definition and selection of individual spots on microarray image and .gal-file layout.

Correct allocation can be tricky if you have only low background. Using the control spots (protein spots) immobilized at specific locations on the array, the correct orientation can be easily ascertained.

### 3.2.3 Exporting Selected Feature Information to Text File

Using “Copy Selection to Clipboard” button (see Figure 7) you are able to copy and save information on the selected features as tab-separated textfile (see Figure 9).

Block	Col	Row	Name	ID
4	5	4	P03211_Peptide_103	VGEADYFEYHQEGGP
12	4	4	P03211_Peptide_101	GRRPFHPVGEADYF
16	4	4	P03211_Peptide_102	FFHPVGEADYFEYHQ
20	5	4	P03211_Peptide_103	VGEADYFEYHQEGGP
28	4	4	P03211_Peptide_101	GRRPFHPVGEADYF
32	4	4	P03211_Peptide_102	FFHPVGEADYFEYHQ
36	5	4	P03211_Peptide_103	VGEADYFEYHQEGGP
44	4	4	P03211_Peptide_101	GRRPFHPVGEADYF
48	4	4	P03211_Peptide_102	FFHPVGEADYFEYHQ

Figure 9: Export of selected features.

The locations of the features (block, column, row) alongside the name and sequence information can be copied to the clipboard and pasted into any text-processing software in order to be saved as a textfile. The copied features can also be directly pasted into a spreadsheet program such as Microsoft Excel for further processing. The peptides are exported in the order they were selected. Please use the column and row index for resorting.

However, using this manual readout method, no quantitative value of the data recorded in the .tif-image can be analyzed.

## 4 Contact Us

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Technical Support North America 1.888.JPT.COM0 (1.888.578.2660) <a href="mailto:us-bd@jpt.com">us-bd@jpt.com</a>	

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