

Protocol

Microarray evaluation using a .gal-File with JPT's software Galviewer

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 moistened by the droplet is called spot. A spot is defined by its diameter and is well-defined to its surrounding slide surface thus realizing a reliable identification of each spot and peptide sequence.

2 Software modules and 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). If no such software tool is available, JPT's software Galviewer can be used for simple applications (enclosed with each microarray shipment or available at www.jpt.com)
- Optional: Software modules processing tab-separated text files, such as Microsoft Editor (notepad) or Microsoft Excel.

3 Design of a .gal-file

After scanning the dried and fluorescently labeled microarray slides, the scanner records a 16-bit (or 8-bit) numeric image in tagged image file format (*.tif). The .tif-image is opened by a software-tool enabling interpretation and quantification of grayscales representing the data in each fluorescent spot on the scanned microarray slide. This quantitative data is 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 the peptide sequence has to be performed. The data for allocation is 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 processed with software modules such as Microsoft Editor (Notepad) or Microsoft Excel.

For qualitative analysis our software module Galviewer.exe can be used. It works as stand-alone module for visualization of sequence allocation defined by the gal-file. Simple export options allow qualitative evaluation of low-density microarray images.

3.1 Layout of a microarray

JPT's standard peptide microarray is composed of three subarrays that hold one or several blocks of peptide spots. A schematic and exemplaric layout of the peptide microarray is shown in Figure 1.

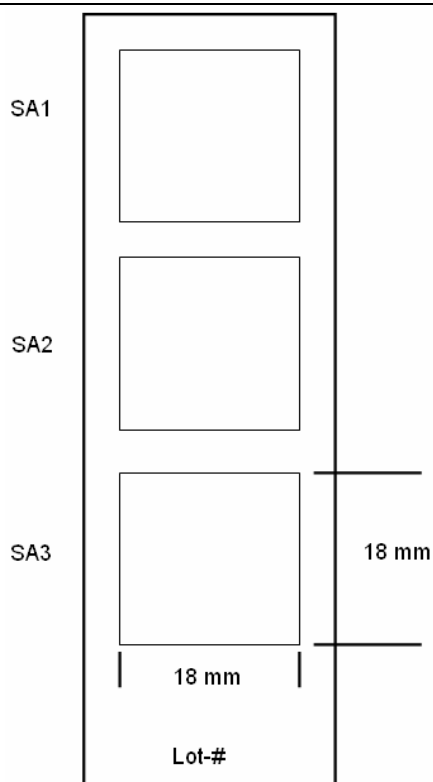


Figure 1: Schematic layout of peptide microarray (SA=subarray).

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 three 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 exemplaric subarray and block layout is shown in Figure 2 and Figure 3.

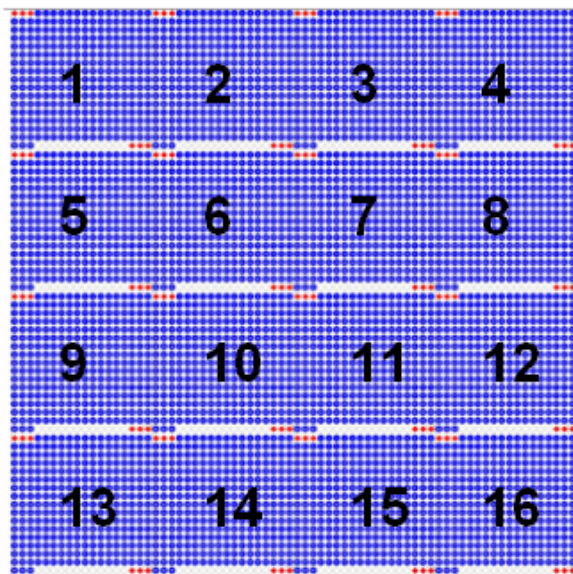


Figure 2: Exemplar subarray consisting of 16 individual blocks. Each block is calculated by number of rows vs. number of columns.

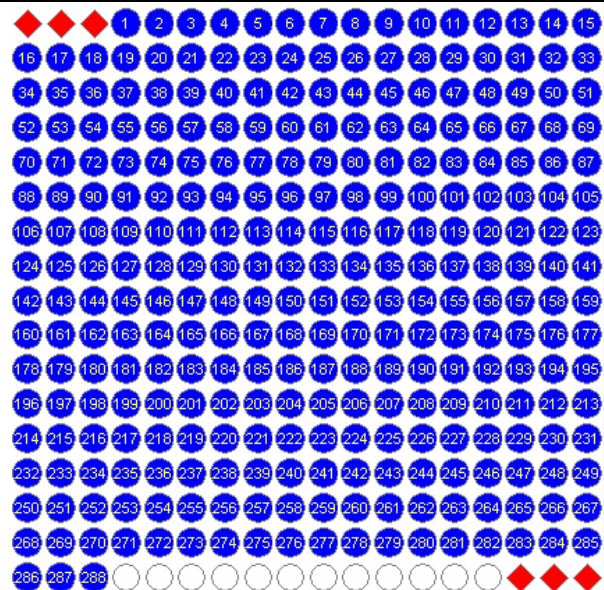


Figure 3: Exemplar block layout, red coloured spots represent fluorescent landmarks, white spots are background controls.

Block numbers are assigned starting from top leftmost block in subarray 1 (then in lines 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. The number of blocks depends on the number of peptides spotted and varies according to the order specifications. Each subarray will contain at least one block.

Spot numbering 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.

3.2 Description/Content of a .gal-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.

3.2.1 Description of header records

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

ATF	1	First line of an Axon Text File (ATF) file; the same in all GAL files: File format (ATF) and version (1.0)
7	5	Second line of an ATF file: 7 number of header records 5 number of data columns (in the data record section).
Type=GenePix Array List V1.0		Type of file; the same in all GAL files.
BlockCount=3		Number of blocks described in the file.
BlockType=0		Type of block described: 0 = rectangular. 1 = orange-packing #1. 2 = orange-packing #2.
"Blockn="		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.
	<i>xOrigin</i>	X position of center of top leftmost feature of current block (in μm).
	<i>yOrigin</i>	Y position of center of top leftmost feature of current block (in μm).
	<i>FeatureDiameter</i>	Diameter of features within the current block (in μm).
	<i>xFeatures</i>	Number of columns of features in current block.
	<i>xSpacing</i>	Column spacing of current block (in μm).
	<i>yFeatures</i>	Number of rows of features in current block.
	<i>ySpacing</i>	Row spacing of current block (in μm).
		Note: Positions on arrays are measured in microns with respect to the origin, which is the top left corner of the array.
"Block" "Row" "Column" "Name" "ID"		Last line of the header, containing column titles for the data records.

3.2.2 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.

3.3 Exemplaric .gal-file and evaluation

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

```

ATF      1
7        5
Type=GenePix ArrayList V1.0
BlockCount=3
BlockType=0
URL=http://
"Block1=4449, 6819, 550, 24, 1211, 25, 1180"
"Block2=21269, 6779, 400, 24, 1201, 25, 1210"
"Block3=38069, 6779, 400, 24, 1203, 25, 1205"
Block Row   Column   Name   ID
1      1      1      Cy3-landmark   Cy3-landmark
1      1      2      Protein 1-aa0001  LLEETKQGNLERECI
1      1      3      Protein 1-aa0004  ETKQGNLERECIEEL
1      1      4      Protein 1-aa0007  QGNLERECIEELCNK

```

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 enabled.

For example the features shown in Figure 5 in row 8 and column 6 yields a saturated signal indicating a strong binding event (see yellow arrow).

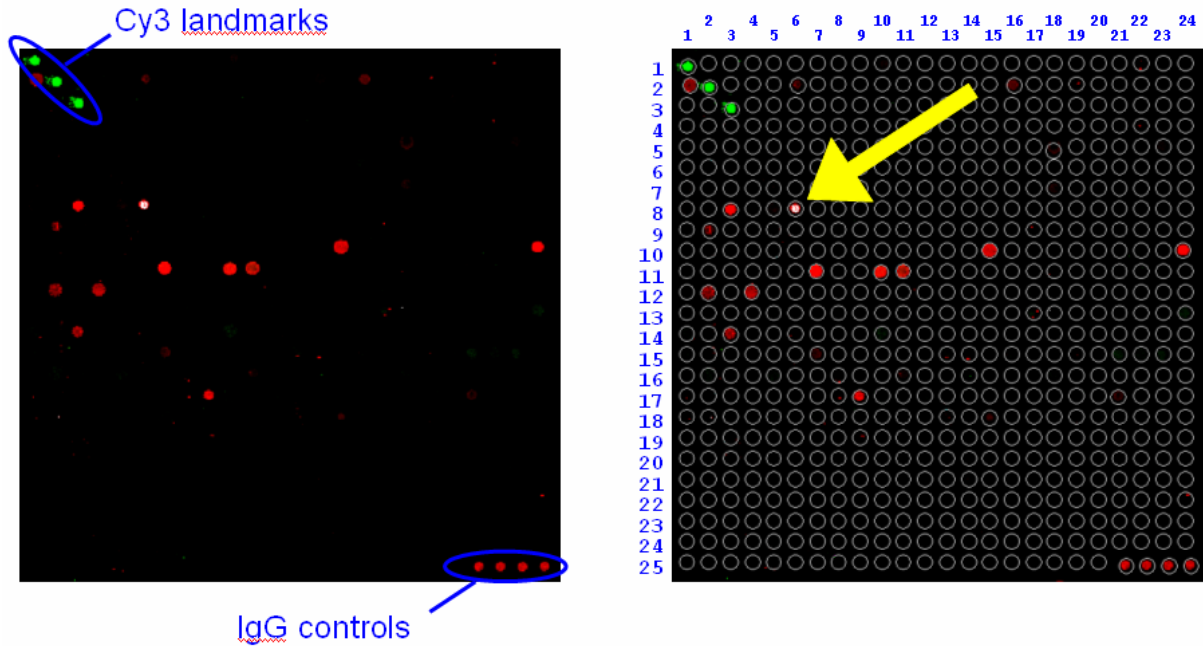


Figure 5: Application of a .gal-file

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).

4 Using a .gal-file

1.1 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. 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 μm , 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 treshhold" etc are individual for each experiment and should be adjusted by each user). After performing the alignment, all spots should be perfectly circled (see **Figure 6**).

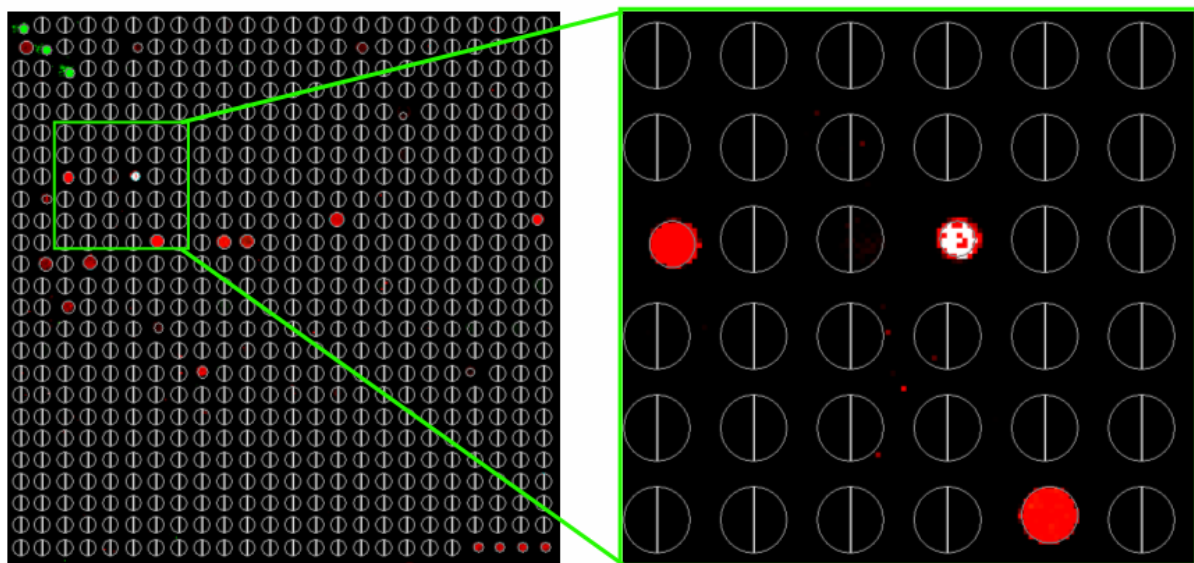


Figure 6: After the alignment, all spots above the pixel treshhold are recognized and the diameter is optimized for perfect readout

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. This 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. Axon's Acuity 4.0) or statistical software packages like R.

4.1 gal-file based application

1.1.1 Open gal file and 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 is loaded to our software Galviewer.exe (User Interface see Figure 7).

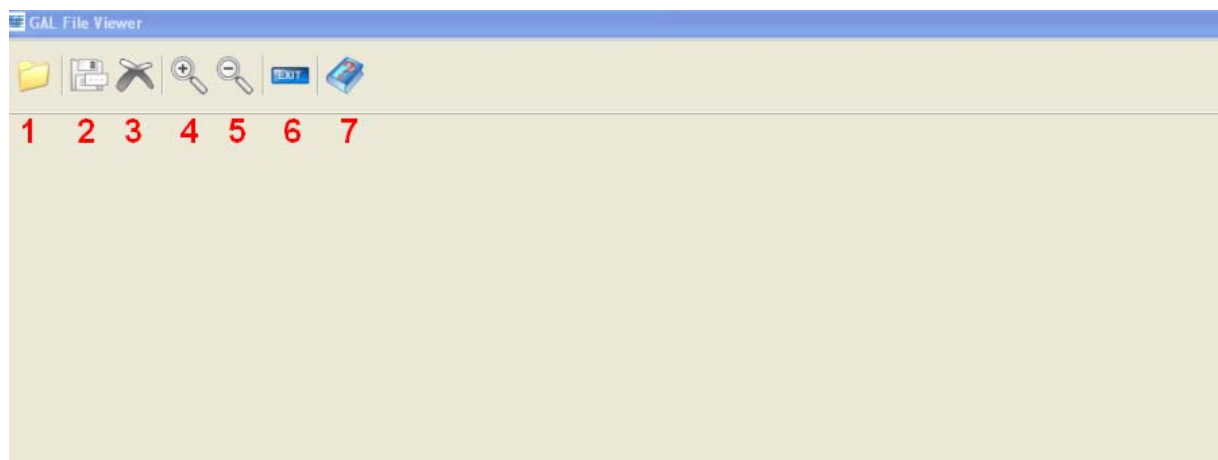


Figure 7: User interface of the Galviewer.

The individual buttons on the upper left corner indicate possible actions:

1. Open a gal-file
2. Export marked features to textfile
3. Delete selection
4. Zoom in
5. Zoom out
6. Close software module
7. Open help / this protocol

If you open a gal-file using button 1, it is loaded and all spots are visualized. In case the resolution of your screen is too low, the individual blocks and subarrays are coloured blue only. By zooming into the image (button 4, click and draw area to zoom in) the spots will become visible (see **Figure 8**).

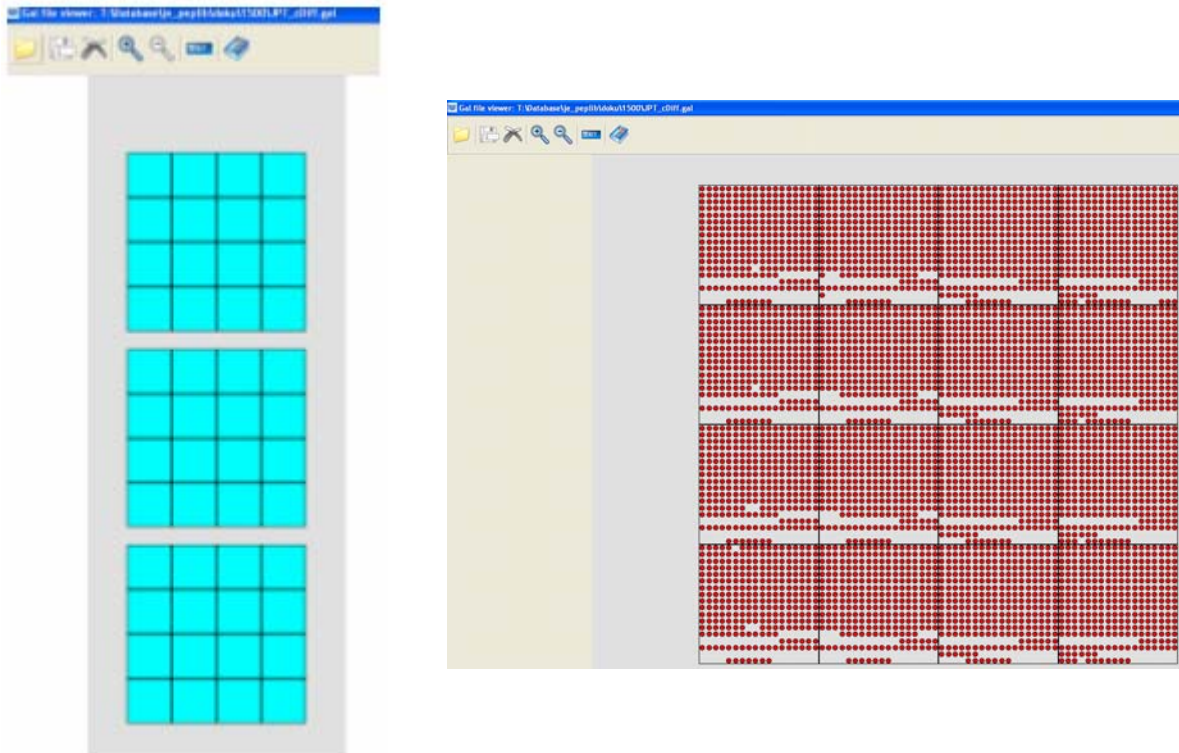


Figure 8: Visualisation of .gal-files in galviewer software

Left side shows a gal-file indicating 48 blocks in 3 subarrays

Right side shows the same array when you zoom into one subarray. Now each feature is shown as red spot

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 is at the bottom of the page. Please rotate the scanned image of your microarray likewise 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).

4.1.1 Select and export feature information

For manual evaluation zoom in to the area of interest on both, the microarray image and the visualized gal-file and select spots on the microarray layout by right-clicking on it. By pressing "CTRL" and selecting Spots, multiple spots can be selected. All selected spots will change colour from red to yellow (see **Figure 9**)

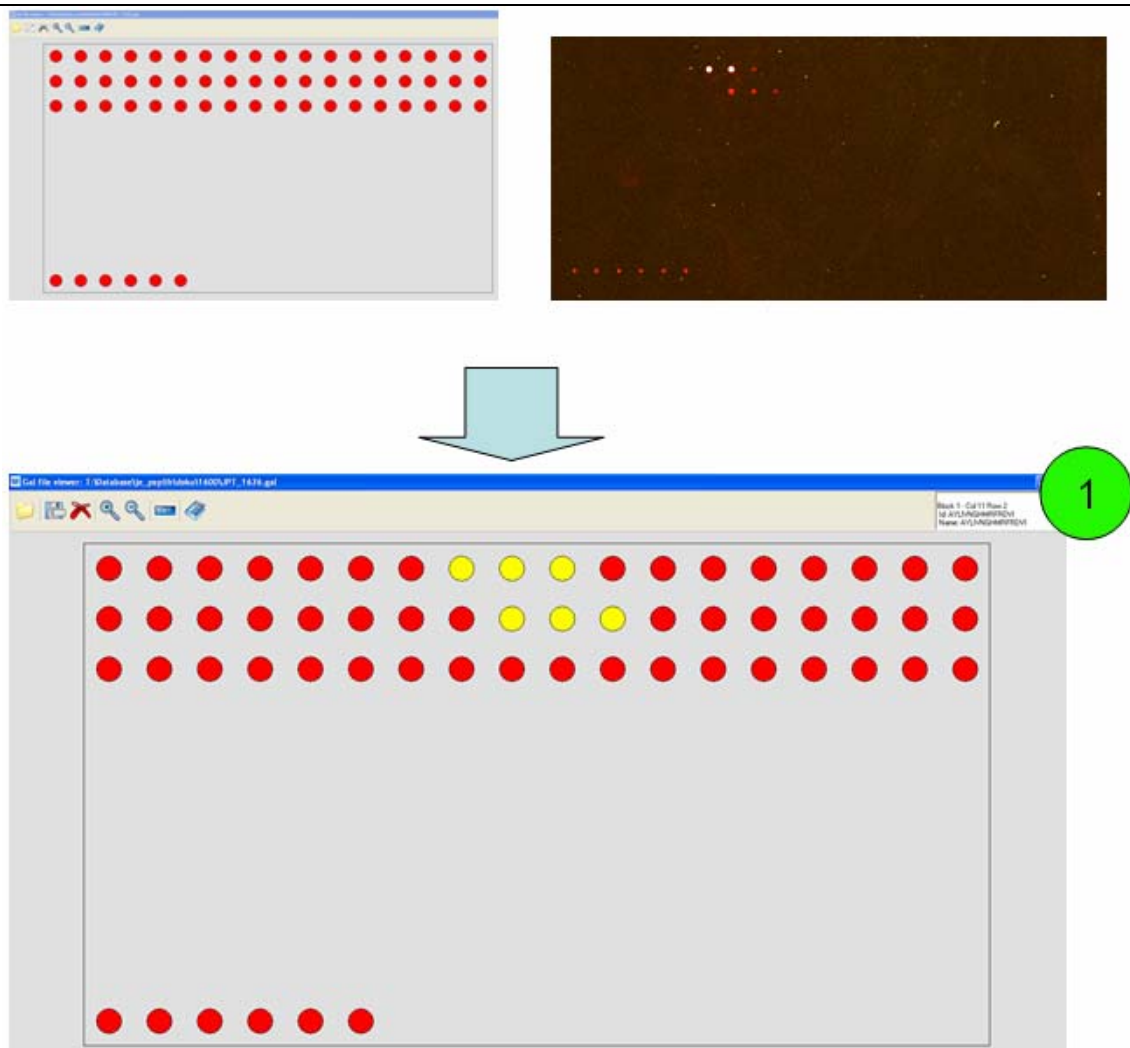


Figure 9: Definition and selection of individual spots on microarray image and gal-file layout

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 eased. Information to selected spots is listed in the comment field (marked with green circled (1) in figure above)

4.1.2 Exporting selected feature information to text-file

Using button 2 (see Figure 7) you are able to save information on the selected features as tab-separated textfile (see **Figure 10**).

Block	Column	Row	ID	Name
1	8	1	peptide-8	protein X
1	9	1	peptide-9	protein X
1	10	1	peptide-10	protein X
1	9	2	peptide-9	protein Y
1	10	2	peptide-10	protein Y
1	11	2	peptide-11	protein Y

Figure 10: Export of selected features

The block number connected to row, column and sequence information is exported into the textfile. 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.