Spotxel® 1.7

Microarray Image and Data Analysis Software

Quick Start Guide

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

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1 Introduction

Spotxel® provides easy-to-use microarray image and data analysis software tools. These include microarray image analysis and automatic batch processing of many microarray images. The quality of microarray data can be improved with replicate processing, data filtering, and data normalization tools. You can then discover features and samples that influence the microarray study and their relationship with data mining tools.

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

1.1 Installation

Spotxel® runs natively on Windows and Mac OS X platforms. Installation of the software requires rights of a system administrator.

Windows Platforms

Spotxel® works on Windows XP, Windows 7, Windows 8, and Windows 10. We recommend the 64-bit version of Spotxel for 64-bit Windows computers. Check your Windows version as described here.

Simply run the Spotxel® installer. If the current Windows account is not an 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 later versions. Unzip the package and double-click on the .pkg file to launch the installer. During the installation you will be prompted to provide a system administrator's account and password. Upon completion, Spotxel® is installed in the */Applications/Spotxel* folder.

1.2 Product Activation

After installing Spotxel® on Windows, you need to activate the software with a *trial serial number*. This enables the use of Spotxel® with full functionality. The trial use for Spotxel® 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 for further using Spotxel®. Upon the purchase, you receive a serial number and use it to activate the license.

1.3 Upgrade

Suppose the current version of Spotxel® on your computer is 1.4.7 and a new version 1.5.5 is available. Simply run the installer of Spotxel® 1.5.5 to upgrade to the new version; the software

configuration will be handled automatically. You do not need to activate the software again if it has been activated.

1.4 Software User Interface

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

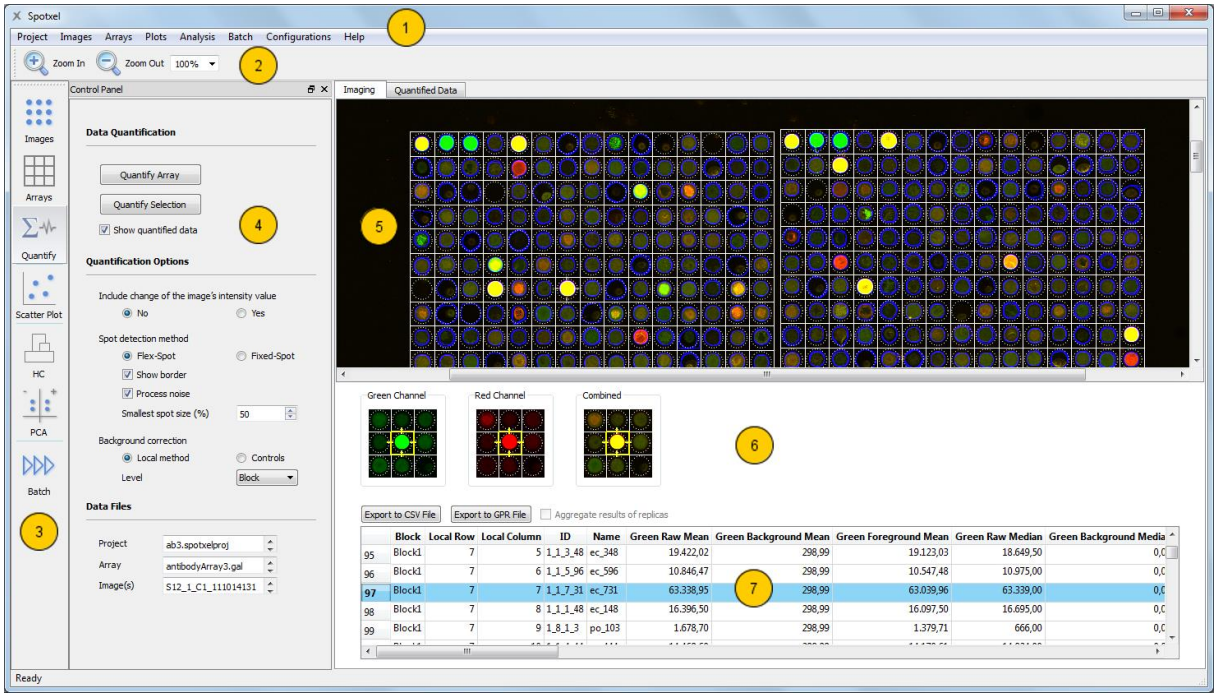


Fig. 1: User Interface.

Component	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: Software Components.

The main toolbar enables quick access to a group of related functions. They are described in Table 2. Clicking on a button on the main toolbar opens the control panel for the function group. The software shows the data and the analysis results in a sheet on the right of the control panel.

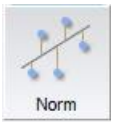
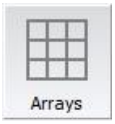
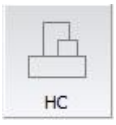
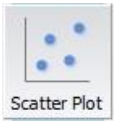
	Select image channel. Change image's intensity. Rotate images.		Process replicates, filtering data, and normalize data.
	View properties of blocks and spots. Rotate and move blocks.		Hierarchical Clustering Analysis: Show features and samples on a heat map with their correlation.
	Quantify the array data and browse the quantified data.		Principal Component Analysis: Select important features and samples.
	Show spots in a scatter plot according their quantified data.		Batch Processing: Automatically process and quantify many microarray images.

Table 2: The Main Toolbar and Related Functions.

1.5 Terms and Concepts

In this manual the term *array* is used to refer to the spot layout and annotation of a microarray. We assume that the array is saved as a *GenePix Array List (GAL) file*.

(a) Image Signal in Red Channel (b) Combined Image Signal From Two Channels

Fig. 2: A rectangular block with 6 spots in 2 rows and 3 columns.

The binding signal of a microarray slide tested with a sample is converted by a scanner into a digital *array image* containing a matrix of *pixels*. In the canvas, the pixels are shown in red, green, or a color different from white; the stronger the signal, the brighter the pixels (Fig. 2).

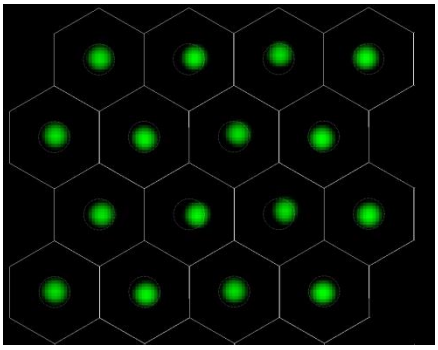


Fig. 3: A hexagonal block with 4 rows and 4 columns.

A *spot* is illustrated as a *white square* (Fig. 2) in a *rectangular block*, or a *white hexagon* (Fig. 3) in a *hexagonal block* (also called *orange-packing*).

Within each spot, the *spotted region* is enclosed by the *dashed circle*, defining the region in which true binding is expected. The *array* consists of blocks. A *block* is a group of spots located next to each other. Thus a block is seen as a grid of white squares or hexagons.

Q*uantification* is the procedure that estimates the true binding signal for each spot and represents its signal value in terms of statistic measurement of pixel intensities within that spot. *Array alignment* is the process of associating spots in the array with their signal in the image. The spot's signal is presumably due to the true binding. Therefore, before quantification we will reallocate the array such that the spotted regions are as close to the spots' signal as possible.

2 Microarray Data Analysis Diagram

The analysis diagram (Fig. 4) summarizes the steps for analyzing a single microarray slide or a batch of many slides.

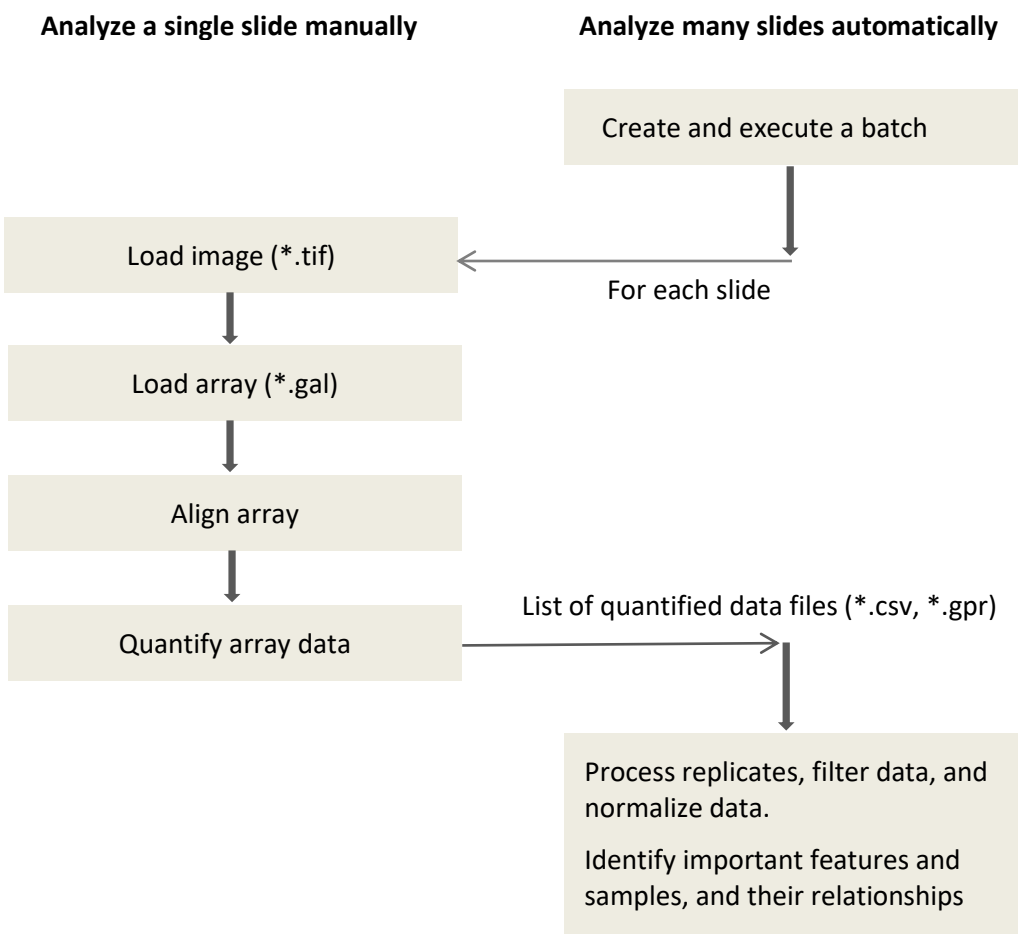


Fig. 4: The Analysis Diagram.

Section 3 details the steps to *analyze a single slide*. The process to *analyze many slides automatically* is described in Section 4.

3 Analyze a Single Slide

1 Load the image of the microarray slide and observe the image data:

- Click the *Images > Open Image* menu and select the 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. 2-a).

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

1.1 In the case *two* TIFF images or a *two-page* TIFF image are loaded:

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

To show the signal of only one channel or both (Fig. 2-b):

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

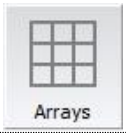
1.2 To view the image and the signal at a proper scale:

- 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.
- Alternatively, in the *Image Channels* section, select either *Red* or *Green*, and then click on the right side of the *Colorize* switch.

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



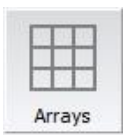
- Click the *Arrays > Open Array* menu and select the array file.

The array (the white grid in Fig. 2) is then overlaid on top of the image in the canvas.

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 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 (green or red pixels, Fig. 2-b). 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 be saved to another file 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. 2-b).

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:

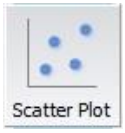
- Make sure the array is correctly aligned with the image.
- Click the *Quantify* button on the main toolbar to start the quantification procedure.
- Upon finishing, the results are updated into the *table of quantified data,* each row showing the intensity values of each spot. Suppose you assigned the image 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 would like detailed information, please check the User's Guide (Section 1.4 - Terms and Concepts and Section 4 - Quantification of Microarray 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 a 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 *hits* which are spots whose intensity value is between the bars' value. Their data are then updated into the table below the chart.
- Clicking on a hit in the chart highlights its row of data in the table. Conversely, select a row in the table to highlight the corresponding spot.

4.3 Save the quantified data to a CSV file (*.csv) or GenePix Array Result file (*.gpr):

- Click the *Export to CSV File* button or the *Export to GPR File* button, respectively.

4.4 Save the analysis results:

- Choose the *Project > Save Project* menu to save the analysis results to a Spotxel® project file (*.spotxelproj).

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

4 Analyze many slides automatically

Suppose that the study is to screen a protein microarray with *k* samples. This employs *k* slides of the protein microarray 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 images are either one-page or two-page grayscale TIFF file. For correct array alignment, 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, the 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 and 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:

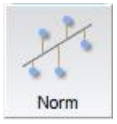
- Click the *Run* button to start the batch. During execution, the status of the currently processed images will be updated.

Suppose that *sample001.tif* is an image in the batch. The following 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,
- *sample001.gpr*: the file containing quantified data in GenePix Result format, and
- *sample001.spotxelproj*: the project file containing the analysis data for the image.

As a result, *k* project files (*.spotxelproj) will be generated for a batch of *k* images. They can be used for further processing described in the next steps.

2 Process replicates, filtering data, and normalize data:



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

2.1 To consolidate replicates' signal value with the *Replicate Processing* panel:

- At the *Unique header* list-box, select a suitable header such as Name.
- Click the *Process* button.
- The processed dataset is then shown in the *Replicate Processing* table.

2.2 To filter a dataset using keywords and thresholds with the *Data Filtering* panel:

- First, select the dataset by clicking on its table. If there is more than one table, the selected one is highlighted with green border.
- Select *Contains*, *Excludes*, or *Regular expression*. Enter one or more keywords, or the search pattern, into the corresponding textbox. Press Enter. The data is directly in the currently selected table.
- Use the sliders or enter a number directly to set the lower and upper thresholds.

2.3 To normalize the data with the *Data Normalization* panel:

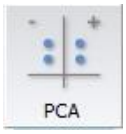
- First, select the dataset by clicking on its table.
- Choose to normalize with *Z-Score*, *Z-Factor*, or *ratio to mean value of controls*. In the two latter cases, click the *Select Controls* button and specify the controls.
- Click the *Normalize* button. The normalized data is then shown in the table titled *Normalized Dataset*.

2.4 Iteratively optimize the dataset:

- Replicate processing (2.1), data filtering (2.2), and data normalization (2.3) can be applied many times. Simply select the table containing the dataset first.
- Use the *Export* button in *Dataset Files* panel to export the selected dataset to a CSV file, e.g., for PCA or HCA analysis.
- Use the *Window > Tile* or *Window > Cascade* menu to arrange the datasets.

In addition to Spotxel® project files (*.spotxelproj), the 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 – Datasets - and Section 7 - Data Preprocessing and Normalization - of the User's Guide.

3 Identify important proteins 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 (*.spotxelproj).
  - Select a screening value in the *Data Column* list-box, e.g. *Red Foreground Mean* or *Red Foreground Median*.

3.1 To find *proteins* that influence the variance of the study:

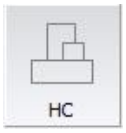
- Choose *Simplify the dataset to three Samples*.
- Click the *Start Analysis* button.

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

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

For detailed steps on the dataset and the PCA tool, refer to Section 7.1 – Datasets - and Section 8.1 - Principal Component Analysis - of the User's Guide.

4 Discover relationships between proteins 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 (*.spotxelproj).
  - 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. proteins), 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 proteins and columns representing samples. Consider the box at row *p* and column *s*, its color represents the screening value of protein *p* against sample *s*.
In addition, samples that are related (e.g. those that response similarly to a certain group of proteins) are grouped into a *cluster*. Their relationship is illustrated by a line connecting them. The clusters of proteins are similarly depicted.

4.1 To review the screening result of protein *p* against sample *s*:

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

4.2 To identify the relationship between proteins or between samples:

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

For detailed steps on the dataset and the HCA tool, refer to Section 7.1 – Datasets - and Section 8.2 - Hierarchical Clustering Analysis - of the User's Guide.