Spotxel® Microarray 4.1

Microarray Image and Data Analysis Software

User's Guide

07 September 2026 - Rev. 1

Spotxel® Microarray is designed solely for research purposes. It is not intended for, nor approved for, the diagnosis of disease in humans or animals.

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CHAPTER 01

Introduction

Product overview, installation, user interface, core terminology, and analysis workflow.

Spotxel® Microarray provides easy-to-use and intuitive tools for microarray image and data analysis. The software supports image analysis, automated processing of multiple images, replicate processing, data filtering, and data normalization. These capabilities help improve data quality and reliability and support the identification of relevant features and samples and the exploration of their relationships using data mining tools.

Spotxel® Microarray is compatible with both Windows and macOS.

Free features available after installation

- **Microarray Image Handling:** Load, view, and work with microarray images.
- **GAL Array Editor:** Create and edit array design and layout files.

Premium features

Premium features include data quantification, automated array alignment, high-throughput batch processing of multiple images, replicate processing, data filtering, data normalization, and data mining. These features require Full access.

Installation

Spotxel® Microarray 4.1 is currently available for Windows through the Microsoft Store. The Mac App Store release is in preparation.

Microsoft Store

https://apps.microsoft.com/detail/9PGC0PZSLJKH

Updates

Software updates are distributed through the Microsoft Store or the Mac App Store. Depending on the Store settings on your system, available updates may be installed automatically or manually through the Store application.

Using Premium Features

Premium features require **Full access**, which is available through either an active trial or a purchased license.

Licensing and purchasing options vary by platform and Store. See **Purchases and Licenses**.

Software User Interface

Associated software controls are grouped in labeled components (Figure 1). This guide refers to each component by the name listed in Table 1.

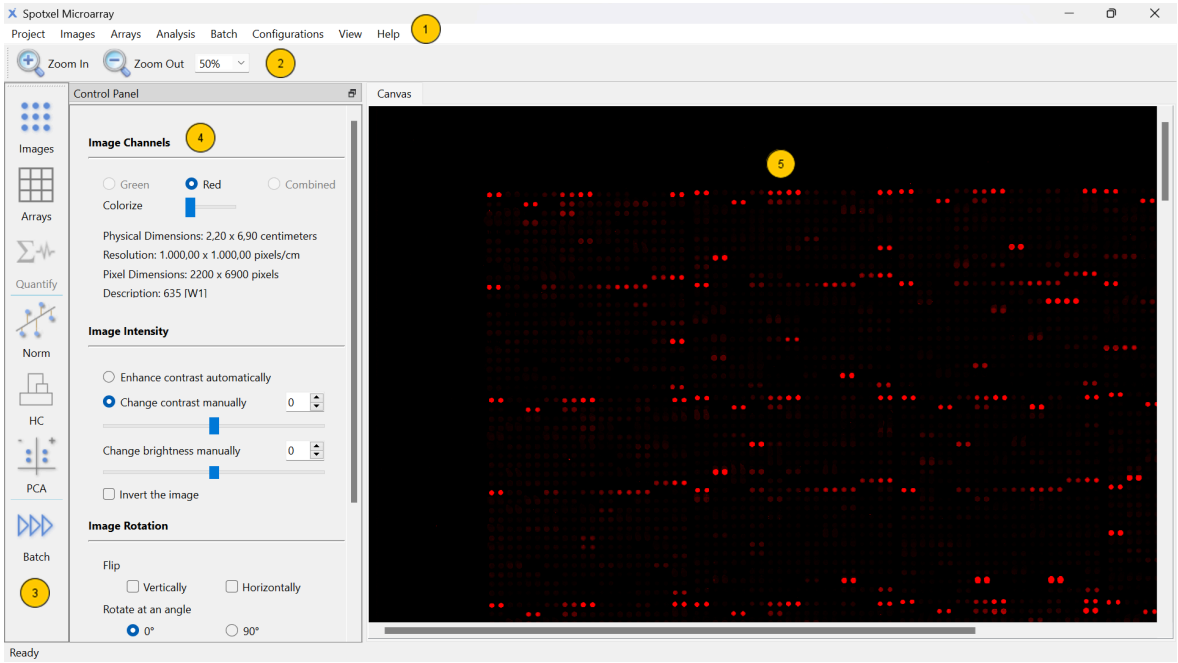


Figure 1. The Software User Interface.

Component	Component Name
1	The menu
2	The canvas toolbar
3	The main toolbar
4	The control panel
5	The canvas

Table 1. Software Components.

The main toolbar provides quick access to groups of related functions, summarized in Table 2. Clicking a toolbar button opens the corresponding control panel. Data and analysis results are displayed in the workspace to the right of the control panel.

Tool	Functions	Tool	Functions
	Select the image channel, change image intensity, and rotate images.		Process replicates, filter data, and normalize data.

Tool	Functions	Tool	Functions
	Create and edit GAL blocks; view spot and spot-family properties; position and rotate GAL blocks or PSF spot families.		Perform Hierarchical Clustering Analysis and display features and samples in a heat map.
	Quantify array data and browse quantified results.		Perform Principal Component Analysis and select important features and samples.
	Automatically process and quantify multiple microarray images.		

Table 2. The Main Toolbar and Related Functions.

Terms and Concepts

Array: In this guide, an array is the spot layout plus its annotation. The layout can be stored as either a GenePix Array List (GAL) file (`*.gal`) or a PepSlide Designer file (PSF) (`*.psf`).

Microarray image: In a microarray assay, binding events at designated spots produce signals when the slide is exposed to a sample. A scanner captures these signals as a digital microarray image - a matrix of grayscale pixels whose intensity represents signal strength. For easier visual interpretation, Spotxel® Microarray can display the grayscale intensities in red or green.

GAL Arrays - Spot and Block

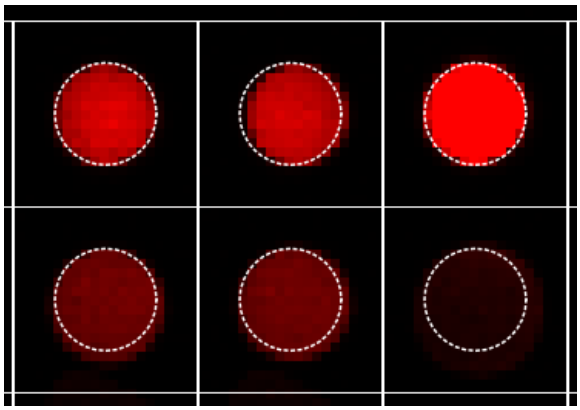


Figure 2. A rectangular block with 6 spots.

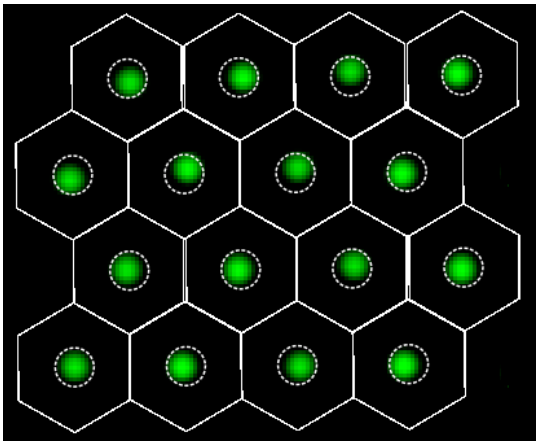


Figure 3. A hexagonal grid with 16 spots.

Spot Appearance: A spot is represented as a white square (Figure 2) in a rectangular block, or a white hexagon (Figure 3) in a hexagonal block, commonly referred to as orange packing.

Spotted Region: Within each spot, the spotted region is enclosed by a dashed circle that indicates where true binding is expected.

Block: An array is composed of blocks, where each block is a group of adjacent spots arranged in a grid.

> **Tip**
>
> Working exclusively with GAL arrays? You can skip the PSF-specific section below.

PSF Arrays - Spot and Spot Family

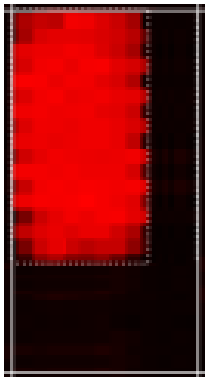


Figure 4. Rectangular Spot.

Spot Appearance: A spot in a PSF array is displayed as a white rectangle (Figure 4).

Spotted Region: Within each spot, the spotted region is enclosed by a dashed rectangle, defining the region of interest for quantification.

Spot Family (SF): Spots produced from the same source and located next to each other are grouped as a spot family. In all analysis steps, an SF functions the same way as a block in a GAL array.

Analysis Workflow (Common to GAL and PSF)

Quantification

Quantification estimates the binding signal at each spot by calculating statistical measurements of pixel intensity.

Method

- Determine the mean and median of all pixels within the spot.

Quality factors

- **Spot detection:** Accurate spot finding determines which pixels in the array image belong to a spot.
- **Background correction:** Estimates and subtracts background signal.

Signal definitions

- **Raw:** The original intensity value measured for the spot.
- **Background:** The estimated background intensity for the spot.
- **Foreground:** The background-corrected spot signal, calculated as the raw intensity minus the background intensity.

Array Alignment

Array alignment maps each spot in the array layout to its corresponding signal region in the image. The software adjusts the positions of blocks in GAL arrays or spot families in PSF arrays so that their spotted regions align with the detected signal.

Key Tasks in Microarray Image and Data Analysis

Typical tasks in microarray image and data analysis include:

1. **Quantification of microarray data**
    - Load the scanned images and the array file.
    - Process the image and array if necessary.
    - Align the array to the images.
    - Quantify the microarray data.
2. **Batch processing of multiple microarray images, if necessary**
    - Automate the steps in task 1 for multiple images.
3. **Preprocessing the data**
    - Use data filtering, replicate processing, and data normalization tools.
4. **Discovery of key features and samples**
    - Explore patterns and relationships among features and samples using the data mining tools.

The following chapters explain how to accomplish these tasks using the software.

CHAPTER 02

Setting Up for Microarray Image and Data Analysis

Load images and GAL or PSF layouts, align the array, and save a project.

Loading Data

To analyze microarray data, two types of input data are required:

- Scanned microarray images in TIFF format.
- An array file in either GenePix Array List (`*.gal`) or PepSlide Designer (`*.psf`) format.

Spotxel® Microarray supports 8-bit, 16-bit, and 24-bit grayscale images, as well as 20-bit Extended Dynamic Range (XDR) images. For most microarray workflows, 16-bit grayscale TIFF images are recommended. XDR images can be used when produced by compatible scanners.

Loading Images

- Select *Images* > *Open Image* and choose the image file or files.
- Spotxel® Microarray supports single-color and dual-color analysis. Assign each loaded image or image page to either the *Red* or *Green* channel.

For single-page images, the *Channel Assignment* dialog (Figure 5) is displayed. If only one image is selected, only one channel assignment is required.

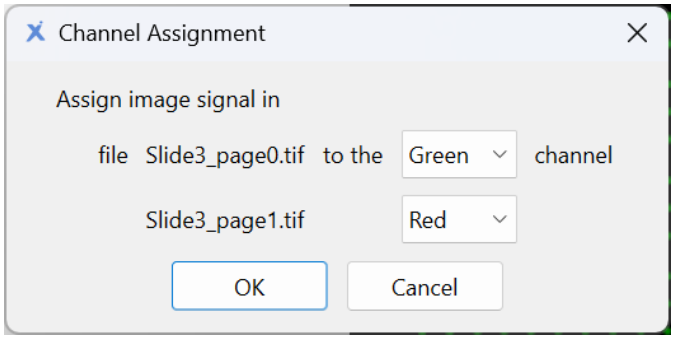


Figure 5. Loading single-page images.

In dual-color mode, load either two single-page images or two pages from a multi-page image. The two images or pages must have the same resolution. Spotxel® Microarray detects whether the selected file is single-page or multi-page and displays the appropriate channel-assignment options.

When a multi-page image contains two pages with the same resolution (Figure 6), you can assign them to different color channels. If the pages have different resolutions (Figure 7), for example when one page is only a preview, only the main page can be selected for single-color analysis.

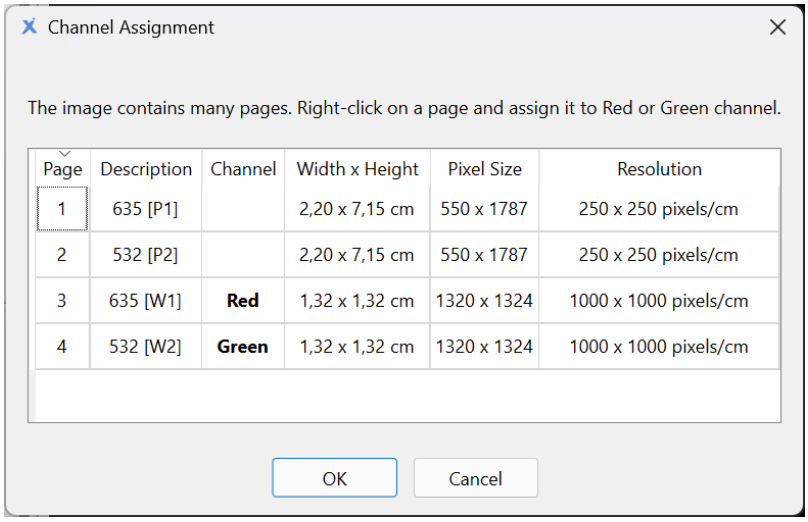


Figure 6. A multi-page image with two pages having the same resolution.

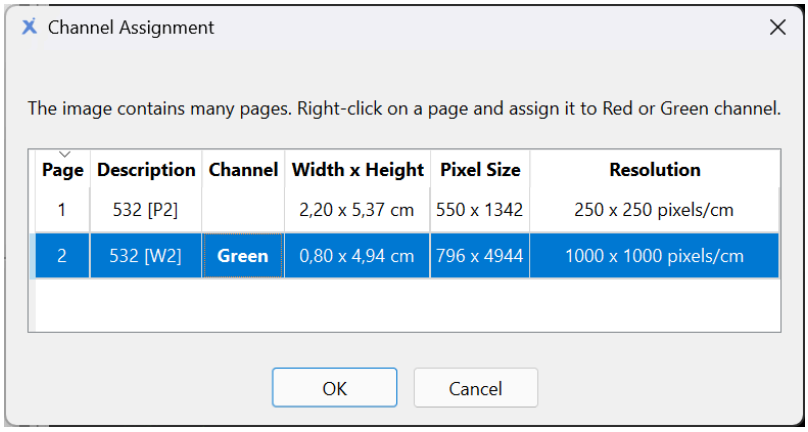


Figure 7. A multi-page image with two pages having different resolutions.

Images without Resolution Information

TIFF images usually contain resolution information in pixels per inch or pixels per centimeter, which the software reads automatically. If this information is unavailable, Spotxel® Microarray displays the *Image Resolution Options* dialog (Figure 8). You can also open it from *Configurations* > *Image Resolution Options*.

If you are uncertain about the resolution, use the default values shown in Figure 8. If the grids appear too small relative to the spots, increase *Resolution X* and *Resolution Y* by a factor of 10 - for example, to 10,000 pixels per centimeter. To reuse the setting for other images, select *Use these options for images without resolution information*.

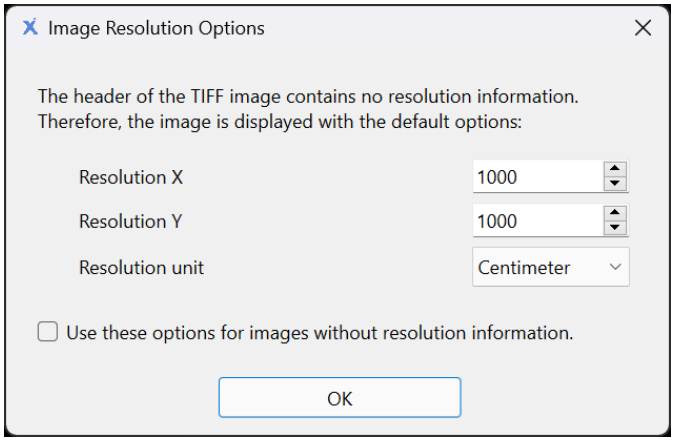
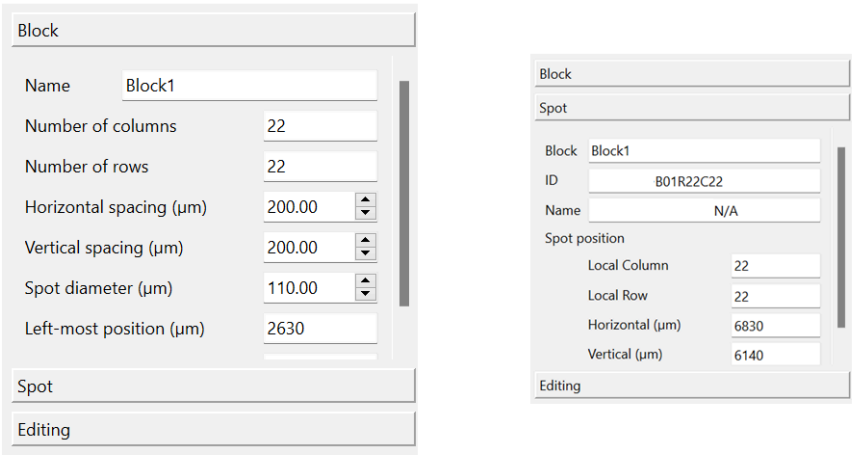


Figure 8. Setting image resolution.

Loading and Viewing GAL Arrays



(a) Block Properties (b) Spot Properties

Figure 9. Properties of a GAL Array Object.

Loading a GAL Array

- Select *Arrays* > *Open Array* and choose the GAL array file (`*.gal`).
- The scanned images and GAL array are displayed on the canvas.
- Adjust the view with *Zoom In*, *Zoom Out*, or the zoom value on the canvas toolbar.

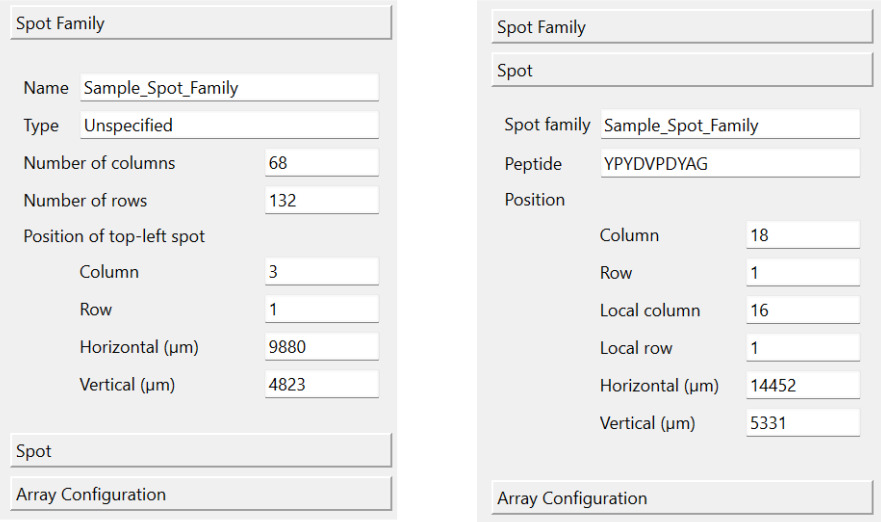
Viewing GAL Array Data

In the *Array File* section of the *Arrays* control panel:

- **Block Properties**
  - Open the *Block* page.
  - Hover over or click a block on the canvas to view its properties (Figure 9-a).
- **Spot Properties**
  - Open the *Spot* page.
  - Hover over or click a spot on the canvas to view its properties (Figure 9-b).

For instructions on creating and editing GAL arrays, see **Managing GAL Array Data with GAL Array Editor**. If you already have a GAL file and only need to use it, continue with **Array Alignment** below.

Loading and Viewing PSF Arrays



(a) Spot Family Properties (b) Spot Properties

Figure 10. Properties of a PSF Array Object.

Loading a PSF Array

- Select *Arrays* > *Open Array* and choose the PSF array file (`*.psf`).
- The scanned images and PSF array are displayed on the canvas.
- Adjust the view with *Zoom In*, *Zoom Out*, or the zoom value on the canvas toolbar.

Viewing PSF Array Data

In the *Array File* section of the *Arrays* control panel:

- **Spot Family Properties**
  - Open the *Spot Family* page.
  - Hover over or click a spot family on the canvas to view its properties (Figure 10-a).
- **Spot Properties**
  - Open the *Spot* page.
  - Hover over or click a spot on the canvas to view its properties (Figure 10-b).

PSF arrays can be edited with PepSlide Designer, available at https://www.sicasys.de/pepslide.

Array Alignment

The array must be aligned with the image before quantification. Alignment can be performed automatically or manually.

Automatic alignment

- Click *Align* in the *Arrays* control panel.
- Use the options described in **Image and Array Processing** to optimize automatic alignment.

Manual alignment

- Select, move, and rotate blocks or spot families as described in **Managing GAL Array Data with GAL Array Editor** and **Image and Array Processing**.

To preserve the original array file, use *Arrays* > *Save Array As* to save the aligned array as a copy. Use *Arrays* > *Save Array* only when you intend to overwrite the current array file.

Spotxel® Microarray Project File

Save the analysis for each microarray image as a Spotxel® Microarray project file (`*.spotxelproj`) by selecting *Project* > *Save Project*. The project stores the image reference, aligned array, quantified data, and other analysis results. Open it with *Project* > *Open Project* to restore the analysis.

Project files can also be used directly to create datasets for further processing and data mining. The paths to the microarray image, array file, and project file are shown in the *Data Files* section of the *Images*, *Arrays*, and *Quantification* control panels.

CHAPTER 03

Managing GAL Array Data with GAL Array Editor

Create and extend GAL arrays, edit spot data, position blocks, and use keyboard shortcuts.

To use the GAL Array Editor, first load a TIFF microarray image. TIFF images typically contain resolution information in pixels per inch or pixels per centimeter. Spotxel® Microarray uses this information to scale the array layout, whose dimensions are specified in micrometers.

If you already have a GAL file and do not need to edit it, skip this chapter and continue with **Array Alignment**.

Creating Blocks

To create an array from scratch, open *Arrays* > *Array File* and click *Create*. Configure the following options:

- **Block type:** Select *Rectangular* for a conventional grid (Figure 12), or select a hexagonal layout such as *Hexagon (orange packing) even row* for a more compact arrangement (Figure 13).
- **Horizontal orientation:** Define the number of blocks arranged horizontally and the horizontal spacing between blocks.
- **Vertical orientation:** Define the number of blocks arranged vertically and the vertical spacing between blocks.
- **Block properties:** Define the number of spot rows and columns, the spot spacing, and the spot diameter.

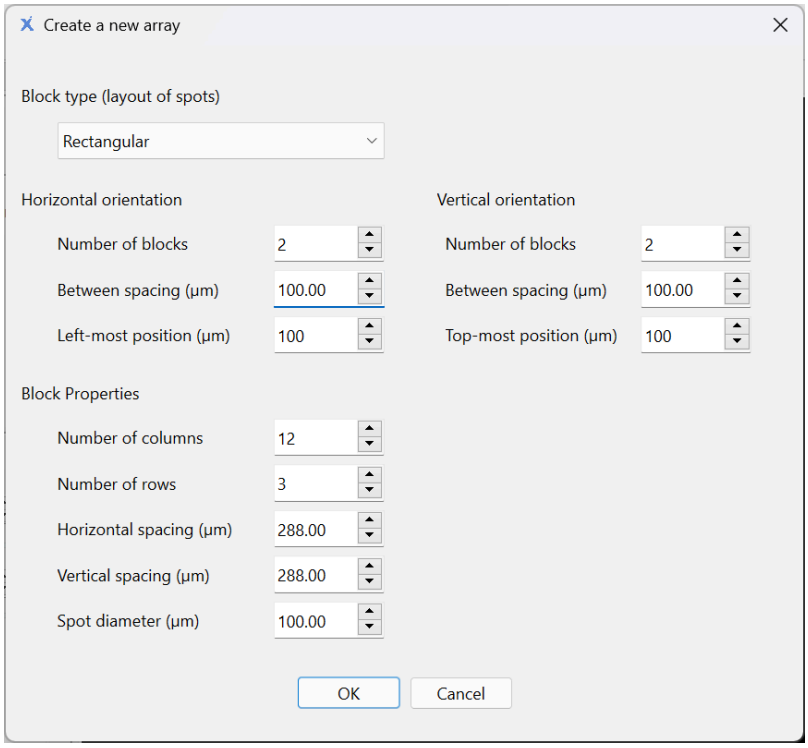


Figure 11. Creating a New Array.

Spacing and diameter values may be fractional, enabling more precise alignment with the image. Click *OK* to add the blocks to the canvas. Figure 11 shows an example configuration, and Figure 12 shows the resulting rectangular blocks.

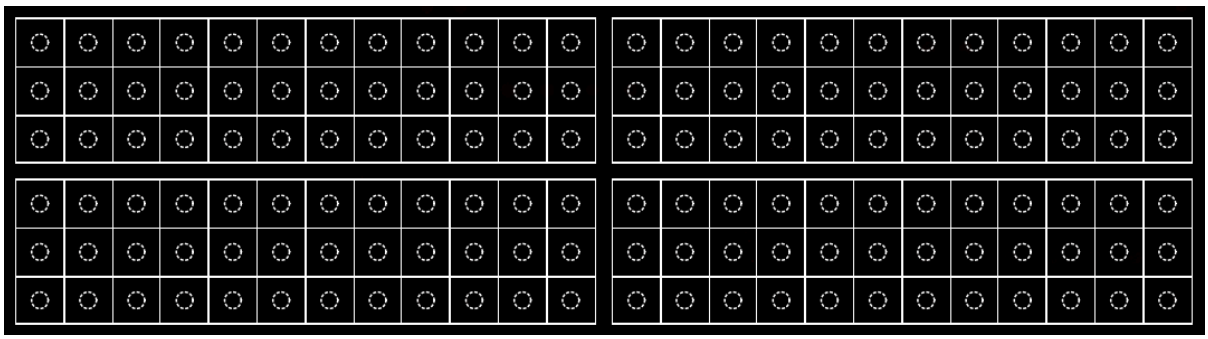


Figure 12. Rectangular Blocks.

Figure 13 shows two hexagonal even-row blocks, each containing 6 rows and 12 columns. The horizontal and vertical spot spacings are 288 µm and 249 µm, respectively.

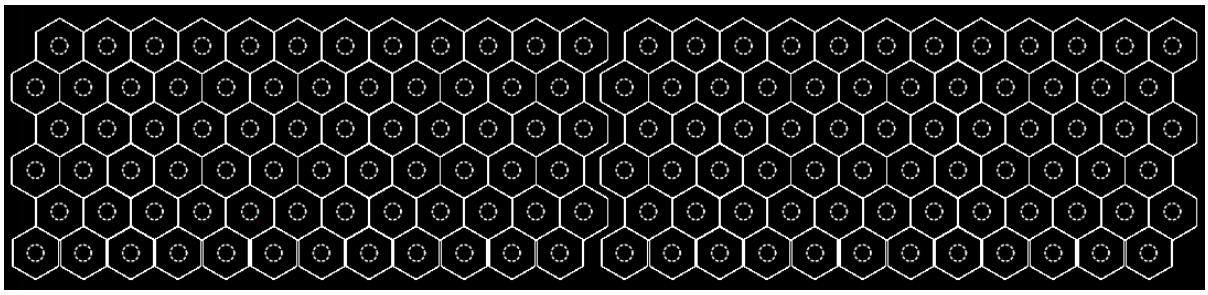


Figure 13. Hexagonal (Orange Packing) Blocks.

Save the array regularly to reduce the risk of data loss. Open *Arrays* > *Array File* and click *Save*. The first time the array is saved, choose its file name and location. The status bar confirms each successful save.

Extending an Array

To extend an existing array, open *Arrays* > *Array File* > *Editing* and click *Add*. The same configuration dialog used to create a new array appears (Figure 11), and the new blocks are appended to the existing array.

Editing Spot Names and IDs

You can modify spot names and IDs using a spreadsheet-like interface:

- Open *Arrays* > *Array File* > *Editing* and click *Edit*. The *Array Spot Data* window (Figure 14) displays one row for each spot.
- Edit the corresponding *Name* or *ID* cell and press **Enter**.
- Navigate with the mouse or arrow keys. Use **Ctrl+Home** and **Ctrl+End** to move to the first and last rows. Save the array after editing.
- The table and graphical canvas are synchronized. Selecting a row highlights the corresponding spot on the canvas, and selecting a spot highlights its table row.
- Click a column header to sort by that column. Double-click any column header to clear sorting.

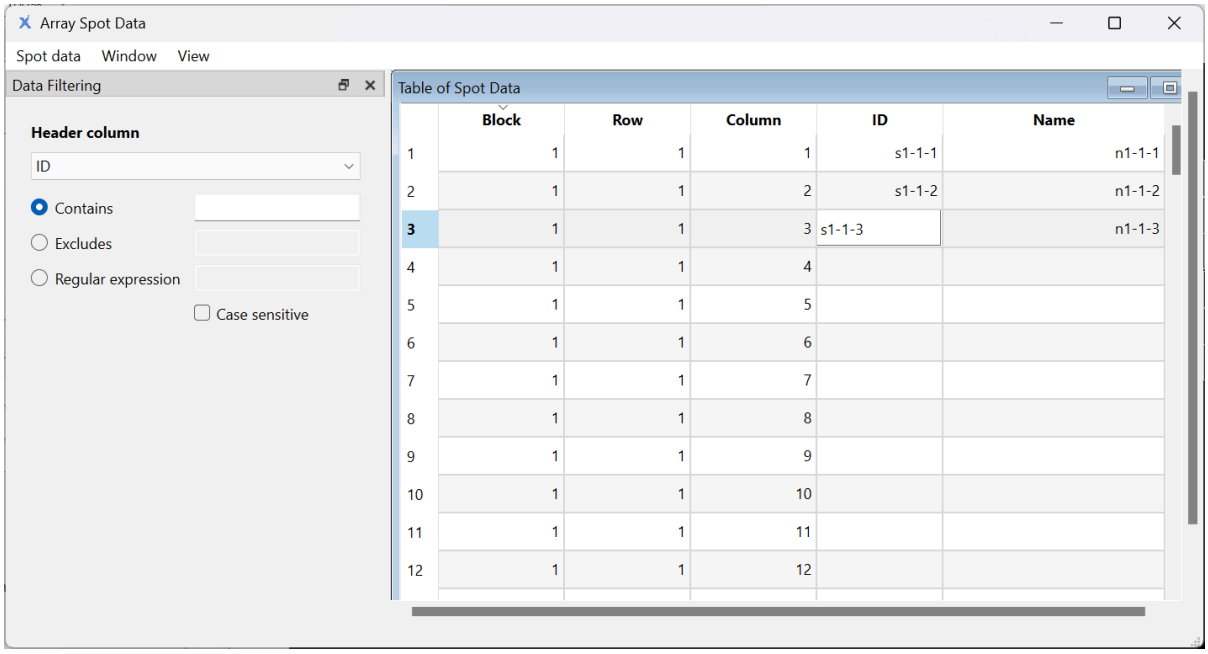


Figure 14. Table of Spot Data.

The *Data Filtering* panel can filter the table by *Block*, *Row*, *Column*, *ID*, or *Name*. Use *Contains*, *Excludes*, or *Regular expression* to define the match. Hover over an input field to view guidance and examples.

On a multi-monitor system, place the graphical canvas on one screen and the synchronized table on another for more convenient array editing.

Selecting and Editing Blocks

Click a block to select it. To select multiple blocks, hold **Ctrl** while clicking. Press **Ctrl+A** to select all blocks. To clear the selection, click an empty area of the canvas.

To change the spot size and spot spacing:

1. Select the blocks.
2. Open *Arrays* > *Array File* > *Block*.
3. Adjust the horizontal spacing, vertical spacing, and spot diameter.
4. Press **Enter** after changing a parameter.

Positioning Blocks

Align blocks with their corresponding spots by selecting the blocks and dragging them to the desired position.

Fine-tune selected block positions with the keyboard. Hold **Ctrl** and press an arrow key to move the blocks by one position-tuning increment. Repeat as needed.

Each increment corresponds to 1 µm and is converted to pixels using the image resolution. At 1000 × 1000 pixels per centimeter, for example, 1 µm corresponds to 0.1 pixels. The minimum on-screen increment is 0.1 pixels.

Rotating Blocks

Rotate selected blocks using *Arrays* > *Array Rotation*. Choose the rotation center and direction, enter the angle, and press **Enter**.

Undo and Redo

You can undo or redo the following block edits:

- Position changes made by dragging
- Rotation
- Spot spacing and diameter changes

Use *Undo* or *Redo* in *Arrays* > *Array File* > *Editing*. Adding blocks and fine-position shifts made with **Ctrl+Arrow** cannot be undone or redone.

Shortcuts and Keyboard Support

Use either the controls in the *Arrays* panel or the shortcuts in Table 3. For shortcuts beginning with **Ctrl**, hold the **Ctrl** key while pressing the indicated key.

Shortcut	Function
Ctrl+N	Create a new array
Ctrl+S	Save the array
Ctrl+O	Open an array
Ctrl+L	Align the array
Ctrl+T	Edit the table of spot data
Ctrl+Z	Undo the last change
Ctrl+Y	Redo the last change
Ctrl+I	Add new blocks to the array
Ctrl+Home	Move to the first block in the array
Ctrl+End	Move to the last block in the array
Ctrl+A	Select all blocks in the array

Shortcut	Function
Ctrl+Right Arrow	Shift right
Ctrl+Left Arrow	Shift left
Ctrl+Up Arrow	Shift up
Ctrl+Down Arrow	Shift down

Table 3. Shortcuts for Editing Arrays.

> **Note**
>
> In Table 3, **Shift** means moving the selected blocks by one position-tuning increment in the indicated direction.

CHAPTER 04

Image and Array Processing

Adjust image display, rotate images and arrays, and configure automatic alignment.

Image Processing

Image Properties

In *Images* > *Image Channels* (Figure 15), select the *Red*, *Green*, or *Combined* view for the canvas. This section also shows the image dimensions, resolution, pixel dimensions, and TIFF description. The TIFF description often includes the scanning wavelength.

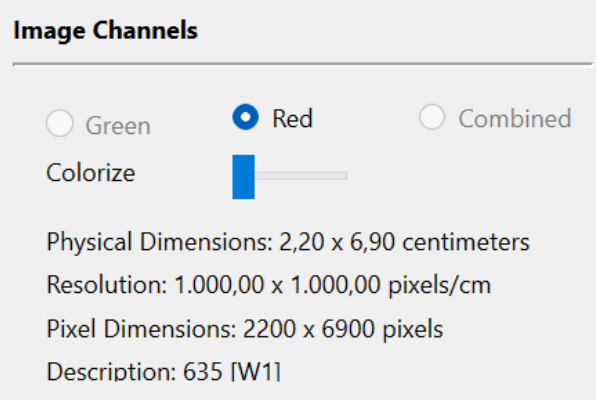


Figure 15. Image Properties.

Adjusting Contrast and Brightness

Adjusting image brightness and contrast can make weak spots easier to see and assist array alignment. Use the controls in *Images* > *Image Intensity* (Figure 16).

- Select *Enhance contrast automatically* to maximize spot visibility.
- For manual adjustment, move the brightness and contrast sliders or enter values from -99 to 99.
- For some images, setting contrast to 99 and brightness to -99 produces an effect similar to automatic enhancement while amplifying less noise.

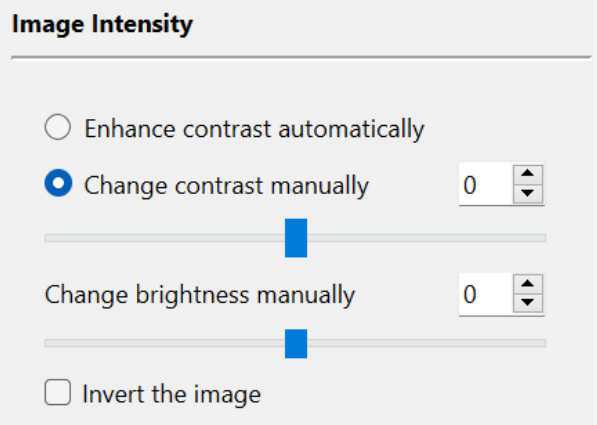
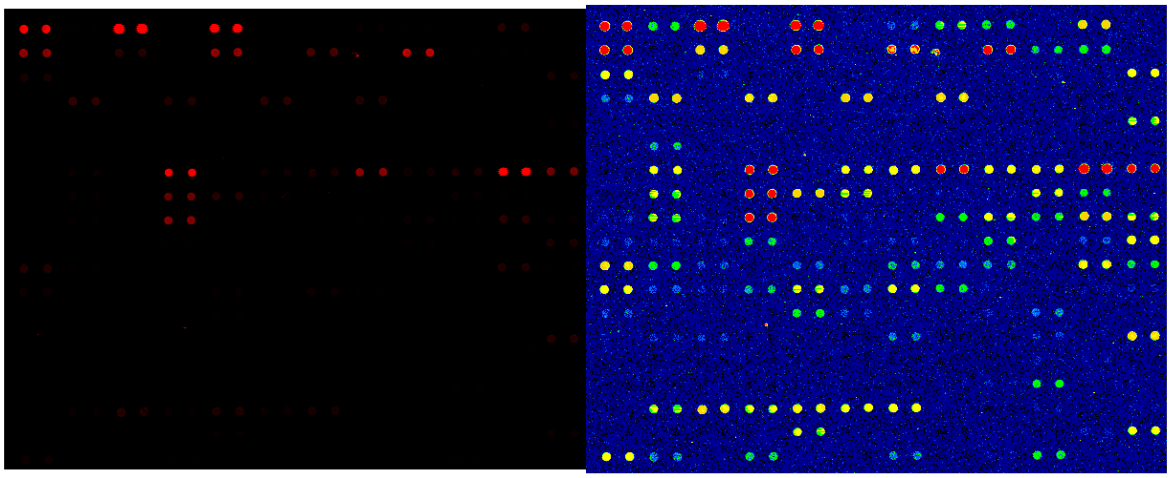


Figure 16. Image Processing.

Colorization

The colorization tool maps grayscale intensity ranges to colors, making weak signal patterns easier to distinguish. Figure 17 compares the original and colorized views.

Colorization is available for 16-bit grayscale images only.



(a) Original image (b) Image after colorization

Figure 17. Colorization.

Turn colorization on or off with the *Colorize* switch in *Images* > *Image Channels*. Select a single channel, either *Green* or *Red*, before enabling the switch.

For each pixel, the software determines the intensity range to which the pixel belongs and displays the corresponding color. The number of colors is fixed, but the intensity ranges can be adjusted from *Configurations* > *Colorization Setting*.

Image Inversion

Select *Invert the image* to display a negative image.

Image and Array Rotation

Rotating Images

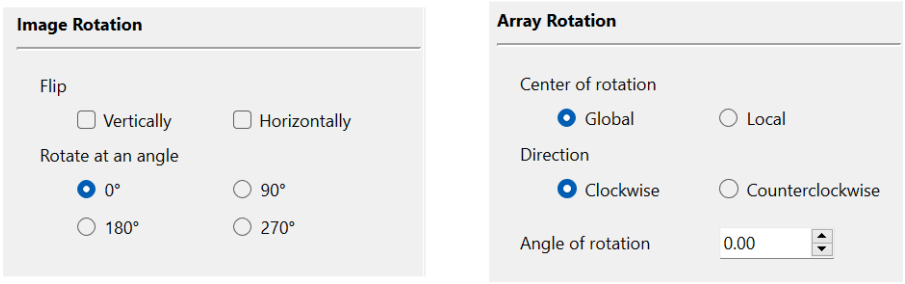
Use *Images* > *Image Rotation* to flip an image vertically or horizontally, or rotate it by 90°, 180°, or 270°.

Rotating the Array

When only a small angular correction is needed, rotate the array rather than the image because rotating the image changes its pixel data. Select GAL blocks or PSF spot families and use *Arrays* > *Array Rotation*. The rotation angle can be adjusted in increments as small as 0.01°.

Rotate selected blocks or spot families clockwise or counterclockwise around one of the following centers:

- **Global:** The top-left corner of the image.
- **Local:** The top-left corner of each selected block or spot family.



(a) Image Rotation (b) Array Rotation

Figure 18. Rotation of Images and Arrays.

Automatic Array Alignment for GAL Arrays

Automatic alignment positions the GAL blocks over their corresponding spot signals in three stages:

1. **Rotation detection:** The software estimates and compensates for image rotation. Disable this step when rotation is negligible, for example below 0.1°.
2. **Initial positioning:** The software estimates block positions using the relative distances stored in the GAL file. This limits the search area and improves processing efficiency.
3. **Block refinement:** Each block position is optimized against the detected spot signals while allowing for configured offsets.

Relative Block Positions

The relative block positions stored in the GAL file should match the printed array design. Accurate positions allow the software to initialize and refine the alignment reliably.

If you experience misalignment:

- Confirm that the GAL file block positions correspond to the printed array design.
- Adjust the permitted offsets between blocks as described below.
- Run the alignment again.

Alignment Options

The default settings work for many arrays, but variations in image and spot configuration may require adjustment. Select *Configurations* > *Array Alignment Options* to configure the signal, optional controls, rotation detection, and permitted block offsets.

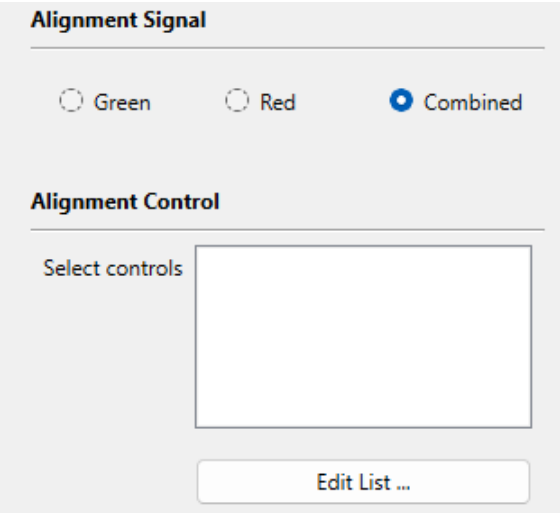


Figure 19. Alignment Options - Signal and Controls.

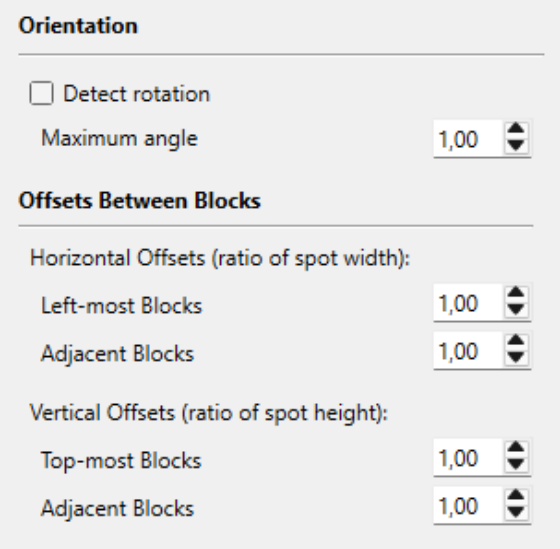


Figure 20. Alignment Options - Orientation and Offsets Between Blocks.

Alignment Signal

For dual-color images, select the *Red*, *Green*, or *Combined* alignment signal. *Combined*, the default, uses the stronger signal from the two channels. Single-color analysis uses the loaded channel.

Alignment Controls

Optional alignment controls identify spots with known IDs that should guide alignment. Select one or more saved controls in the *Alignment Control* list, or click *Edit List* to add or modify controls. Spots with matching IDs are used as positional references.

Detect Rotation

Select *Detect rotation* in the *Orientation* section to estimate image rotation and compensate for it during alignment.

Use *Maximum angle* to define the rotation search range expected from the acquisition setup. At the default value of 1.0°, the software searches from -1.0° to 1.0°. A wider range increases processing time.

Offset of Blocks' Position

Normally, adjacent blocks should have approximately the same horizontal (X) or vertical (Y) position. Printed blocks, however, may be offset from their planned positions. Figure 21 illustrates horizontal and vertical offsets among four blocks.

Horizontal Offset (ratio to spot width)

- **Left-most Blocks:** Maximum difference in X-position between left-most blocks, such as blocks 1 and 3 in Figure 21.
- **Adjacent Blocks:** Maximum difference in X-position between adjacent blocks that are not left-most, such as blocks 2 and 4.

Vertical Offset (ratio to spot height)

- **Top-most Blocks:** Maximum difference in Y-position between top-most blocks, such as blocks 1 and 2.
- **Adjacent Blocks:** Maximum difference in Y-position between adjacent blocks that are not top-most, such as blocks 3 and 4.

The offset values are ratios of spot width or height and define how far blocks may deviate from their planned positions:

- The default value for all offsets is 1.0.
- A common alternative is 0.75 for all offsets.
- Smaller gaps between adjacent blocks or spot families often correspond to values such as 0.5 or 0.75.
- For larger gaps, consider values of 1.5 or 2.0 times the spot size.

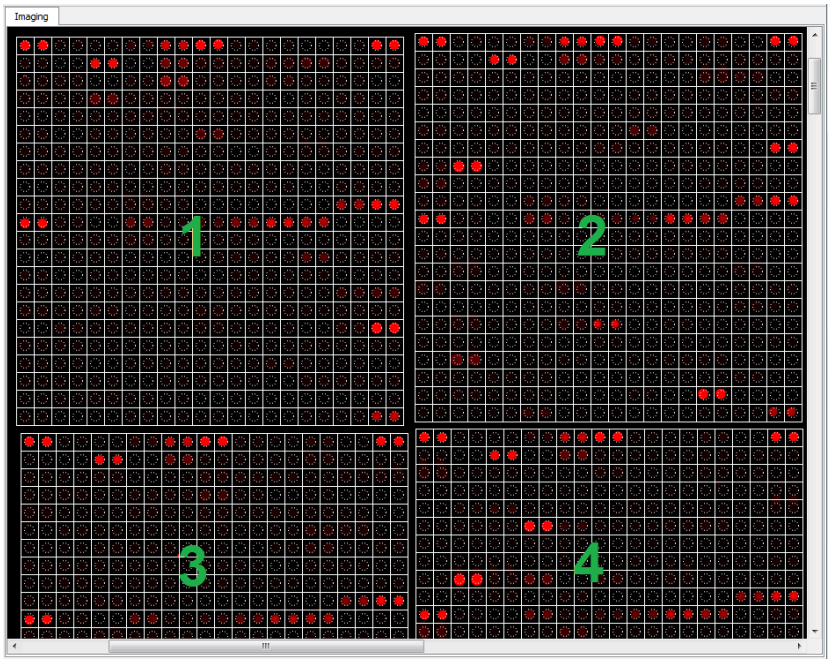


Figure 21. Offset of Blocks.

Automatic Array Alignment for PSF Arrays

PSF arrays typically contain a single large spot family and designated positional control spots. Their alignment behaves as follows:

- It relies on the alignment signal and, when specified, positional controls.
- It does not use the orientation or offset parameters; those settings apply only to GAL arrays.
- Rotation is always included when aligning a PSF array.

Alignment Signal

- For dual-color images, select the channel used for alignment. The default *Combined* option uses the stronger signal from the two channels.
- For single-color images, alignment uses the loaded channel.

Alignment Controls

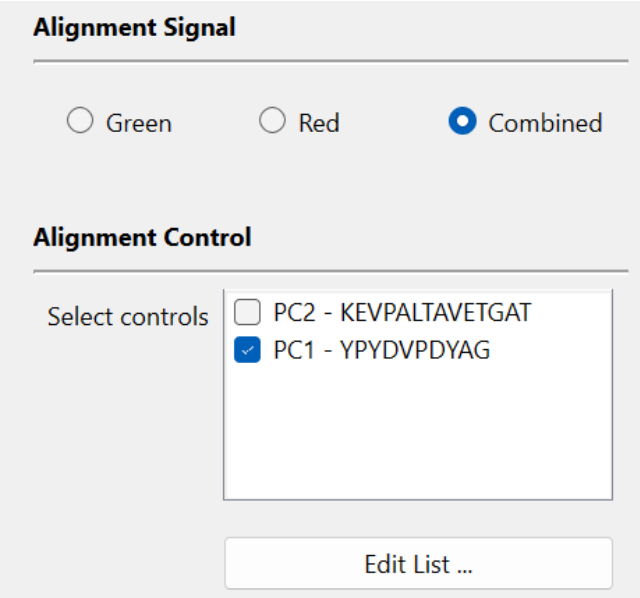


Figure 22. Alignment Options - Signal and Positional Controls for PSF Arrays.

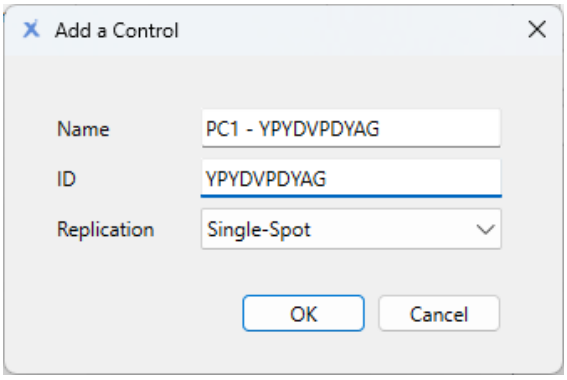


Figure 23. Add an Alignment Control.

To improve precision, assign positional controls as follows:

- Select the checkbox next to each control to use. In Figure 22, control *PC1* with ID `YPYDVPDYAG` is selected.
- Click *Edit List* to open all defined controls.
- To define a new control, click *Add*, then enter its name, ID, and replication mode.
- To edit an existing control, click *Change*.
- Created controls are saved and available for future projects.

During alignment, spots with a matching ID are used as positional references. The array layout is adjusted to align with these controls.

CHAPTER 05

Quantification of Microarray Data

Quantify spots, inspect results, select detection methods, and process noise and defects.

Quantifying Microarray Data

Click *Quantify* on the main toolbar to open the *Quantification* control panel.

- To quantify the entire array, click *Quantify Array*.
- To quantify selected blocks, select them and click *Quantify Selection*.

A new window displays the quantified data table. Detected spots on the canvas are outlined in blue by default (Figure 24).

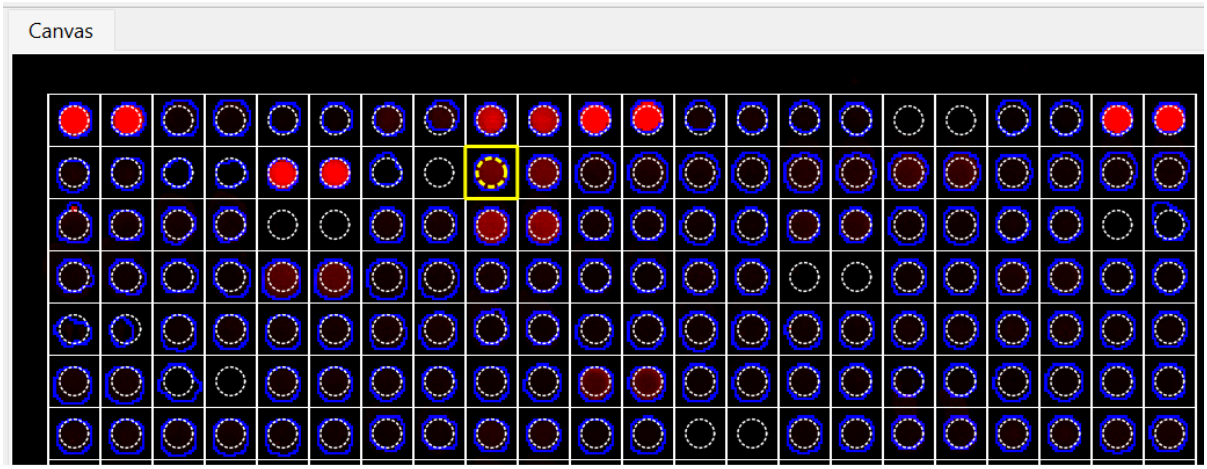


Figure 24. Highlighted Spots on the Canvas.

The canvas and quantified data table are synchronized. Selecting a spot on the canvas highlights the corresponding table row, and selecting a row highlights the corresponding spot. On a multi-monitor system, you can place the canvas on one screen and the table on another.

Quantification time depends on image resolution, array size, and the enabled processing options. Processing is multithreaded, and you can continue navigating the image and array while quantification is in progress.

The Table of Quantified Data

For each loaded channel, the table calculates the mean and median of each spot's raw, background, and foreground values. If a spot's raw value is smaller than its background value, the spot is flagged as an error and its foreground value is set to zero.

Visible Data Columns

By default, the table displays six values per channel: the mean and median of the raw, background, and foreground signals. You can choose which values are visible:

1. Select *Configurations* > *Quantified Data Table Columns Options*.
2. In the dialog, select or clear the required columns.

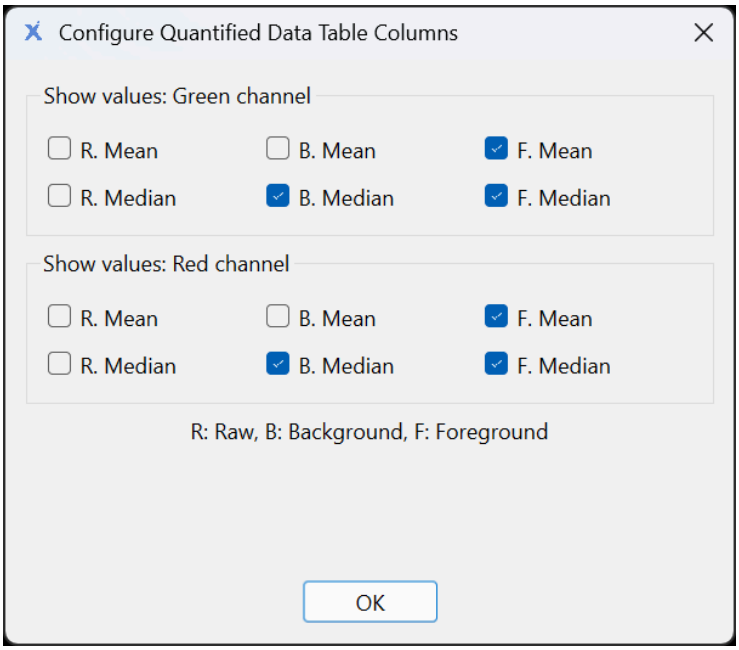
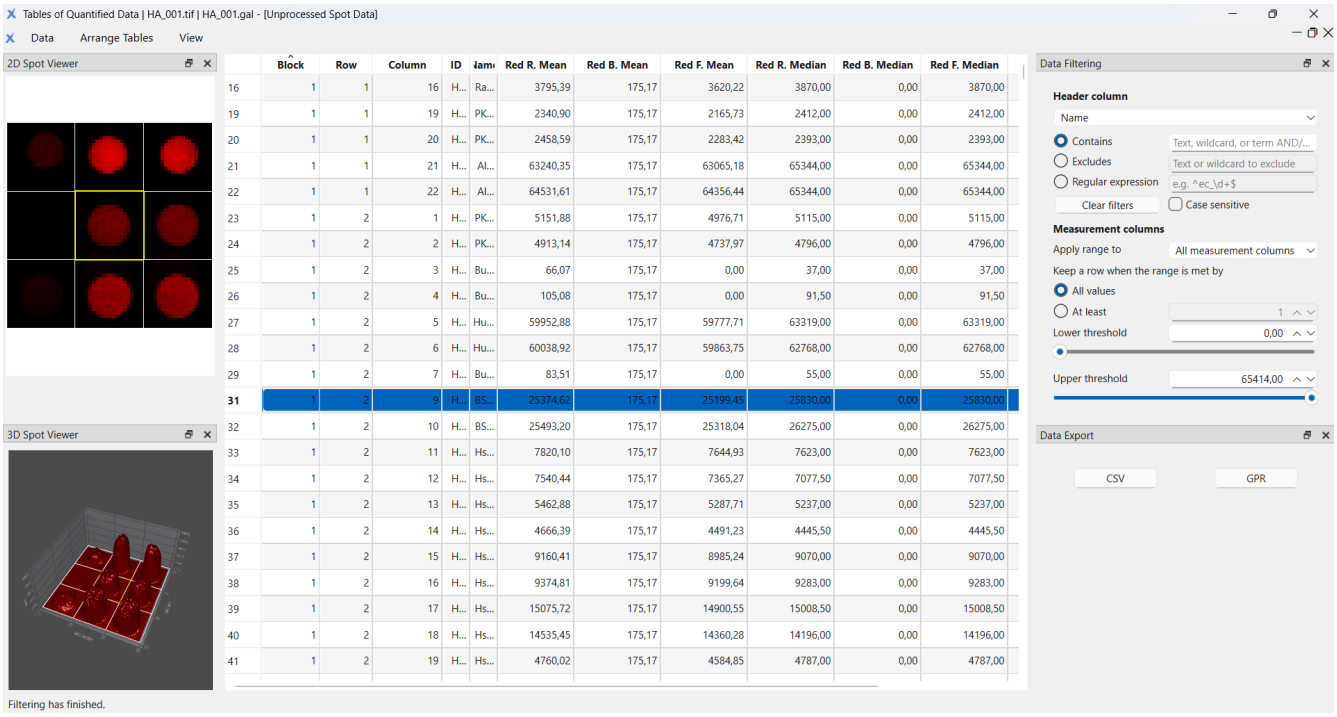


Figure 25. Configure Visible Data Columns.

> **Note**
>
> CSV and GenePix Result exports always contain all six values per spot, regardless of the visible-column settings.



	Block	Row	Column	ID	Red R. Mean	Red B. Mean	Red F. Mean	Red R. Median	Red B. Median	Red F. Median
16	1	1	16	H...	3795,39	175,17	3620,22	3870,00	0,00	3870,00
19	1	1	19	H...	2340,90	175,17	2165,73	2412,00	0,00	2412,00
20	1	1	20	H...	2458,59	175,17	2283,42	2393,00	0,00	2393,00
21	1	1	21	H...	63240,35	175,17	63065,18	65344,00	0,00	65344,00
22	1	1	22	H...	64531,61	175,17	64356,44	65344,00	0,00	65344,00
23	1	2	1	H...	5151,88	175,17	4976,71	5115,00	0,00	5115,00
24	1	2	2	H...	4913,14	175,17	4737,97	4796,00	0,00	4796,00
25	1	2	3	H...	66,07	175,17	0,00	37,00	0,00	37,00
26	1	2	4	H...	105,08	175,17	0,00	91,50	0,00	91,50
27	1	2	5	H...	59952,88	175,17	59777,71	63319,00	0,00	63319,00
28	1	2	6	H...	60038,92	175,17	59863,75	62768,00	0,00	62768,00
29	1	2	7	H...	83,51	175,17	0,00	55,00	0,00	55,00
31	1	2	9	H...	25374,62	175,17	25199,45	25830,00	0,00	25830,00
32	1	2	10	H...	25493,20	175,17	25318,04	26275,00	0,00	26275,00
33	1	2	11	H...	7820,10	175,17	7644,93	7623,00	0,00	7623,00
34	1	2	12	H...	7540,44	175,17	7365,27	7077,50	0,00	7077,50
35	1	2	13	H...	5462,88	175,17	5287,71	5237,00	0,00	5237,00
36	1	2	14	H...	4666,39	175,17	4491,23	4445,50	0,00	4445,50
37	1	2	15	H...	9160,41	175,17	8985,24	9070,00	0,00	9070,00
38	1	2	16	H...	9374,81	175,17	9199,64	9283,00	0,00	9283,00
39	1	2	17	H...	15075,72	175,17	14900,55	15008,50	0,00	15008,50
40	1	2	18	H...	14535,45	175,17	14360,28	14196,00	0,00	14196,00
41	1	2	19	H...	4760,02	175,17	4584,85	4787,00	0,00	4787,00

Figure 26. Table of Quantified Data.

2D Spot Viewer

The 2D Spot Viewer displays the selected spot, its neighbors, and their quantified data simultaneously. It shows the image region corresponding to the selected spot and its neighbors.

3D Spot Viewer

The 3D Spot Viewer displays a three-dimensional representation of the selected spot and its neighbors. Both the 2D and 3D viewers use the contrast-adjusted intensity shown on the canvas.

- To change the viewing angle, hold the right mouse button and drag.
- To zoom in or out, use the mouse wheel.

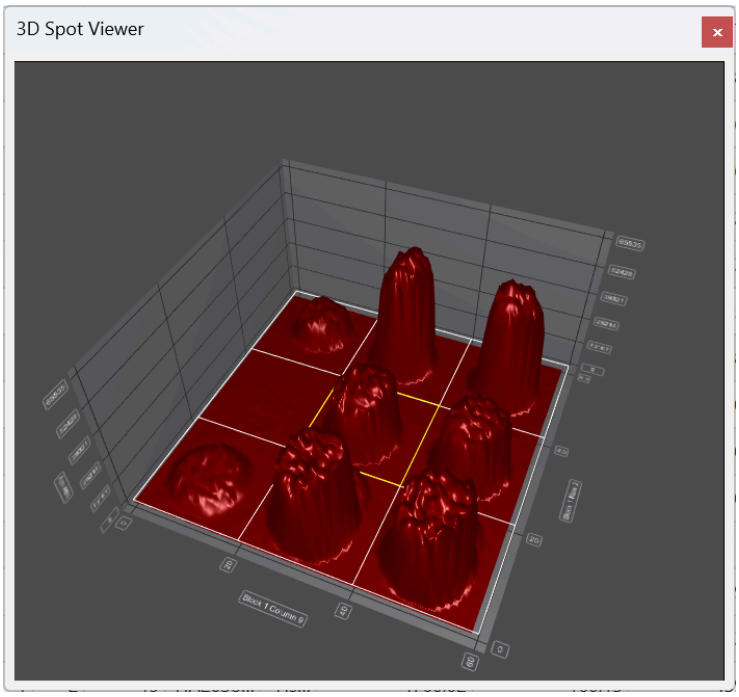


Figure 27. 3D Spot Viewer.

Data Filtering, Replicate Processing, and Data Normalization

Use the corresponding panels to filter spots, process replicates, or normalize the quantified data for the current image. Hover over a control, such as the input field for *Data Filtering* > *Contains*, to display its tooltip. These tools are described in **Data Preprocessing and Normalization**.

Exporting Quantified Array Data

Use the *Data Export* panel to export quantified data to a CSV file (`*.csv`) or GenePix Result file (`*.gpr`).

Docking and Undocking Panels

Double-click a panel header to undock the 2D or 3D Spot Viewer. The viewer becomes an independent, resizable window that can be moved to another screen. Double-click its header again to dock it. The same behavior applies to the *Data Filtering*, *Replicate Processing*, and *Data Normalization* panels.

Showing and Hiding Panels

Use the *View* menu on the quantified data table toolbar to show or hide its panels and information views.

Quantification Options

Select *Show quantification options* in the *Quantification* control panel. Spotxel® Microarray provides three spot detection methods: **Flex-Spot**, **LoG-Spot**, and **Fixed-Spot**. Available detection settings depend on the selected method.

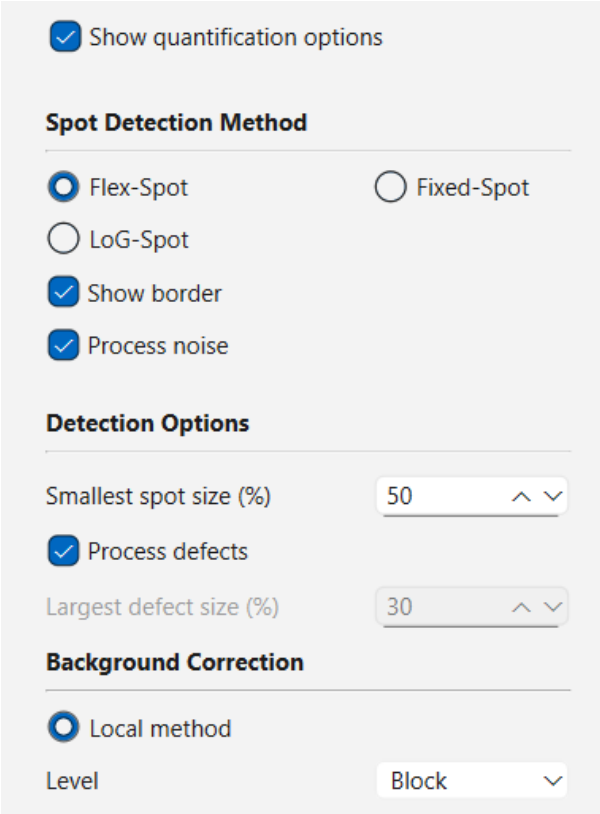


Figure 28. Flex-Spot Options.

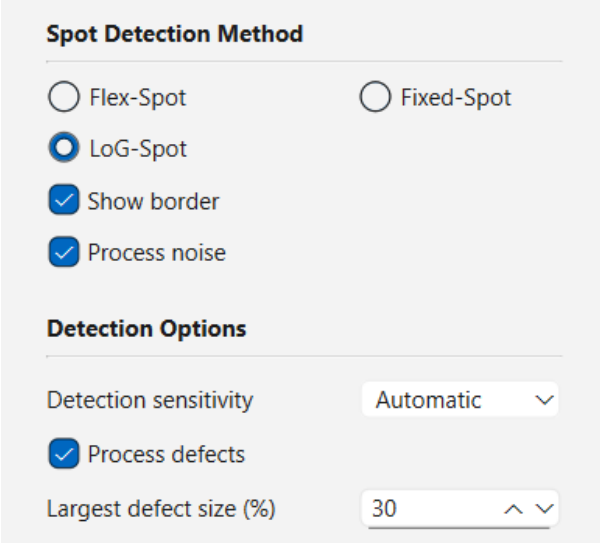


Figure 29. LoG-Spot Options.

Flex-Spot

Flex-Spot detects the actual foreground signal of each spot, allowing the detected region to adapt to variations in shape, size, and position relative to the predefined spot region. The detected border is shown in blue.

The raw value is calculated from pixels within the detected border. The background value is calculated from the remaining background pixels within the spot area.

If Flex-Spot cannot reliably detect a spot because of size limitations, weak signal, or image noise, Spotxel® Microarray falls back to Fixed-Spot for that spot. No detected blue border is displayed when Fixed-Spot is used.

LoG-Spot

LoG-Spot complements Flex-Spot with a different detection approach. While Flex-Spot follows the observed foreground signal and can detect irregular shapes, LoG-Spot searches the local image region for the most likely approximately circular spot and determines its center and diameter.

LoG-Spot uses the expected spot center and diameter from the array layout as reference information. If a reliable center is found but the diameter cannot be determined reliably, the expected spot diameter is used. If a reliable center cannot be found, the method falls back to Fixed-Spot for that spot.

> **Note**
> LoG-Spot is available for GAL arrays only. PSF arrays support Flex-Spot and Fixed-Spot.

Fixed-Spot

Fixed-Spot calculates signal values using the predefined spot region without attempting to detect the actual foreground shape. Use it when Flex-Spot or LoG-Spot cannot detect spots reliably, or when quantification based strictly on the predefined geometry is required.

- **Raw Value:** Calculated from pixels within the predefined spot region:
  - a dashed circle for GAL arrays; or
  - a dashed rectangle for PSF arrays.
- **Background Value:** Calculated from the remaining background pixels within the spot area but outside the predefined spot region.

Show Border

For spots detected with Flex-Spot or LoG-Spot, use *Show border* to show or hide detected borders. Border information is stored in the Spotxel® Microarray project file (`*.spotxelproj`) and restored when the project is reopened. This is particularly useful for reviewing batch-processing results.

Default Settings and Tooltips

Figure 28 and Figure 29 show the default settings. Hover over a control to display additional guidance in its tooltip.

Detection Options

Flex-Spot Options

Smallest Spot Size

Use *Smallest spot size (%)* to specify the minimum size of a valid detected spot. Foreground regions smaller than the specified percentage of the configured spot dimensions are rejected.

At the default value of 50%, Flex-Spot rejects detected regions that fit within a square whose side length is 50% of the configured spot diameter. Valid values range from 25% to 100%.

LoG-Spot Options

Detection Sensitivity

Use *Detection sensitivity* to control how broadly LoG-Spot searches for a valid spot:

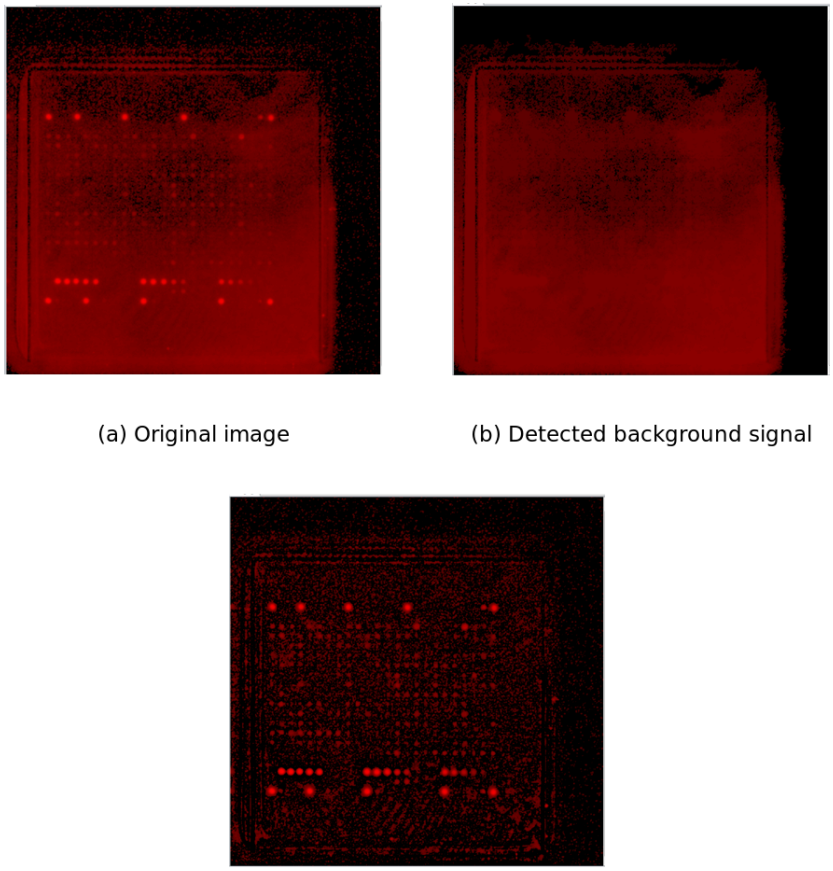
- **Conservative:** Uses a more restricted center and size search with stricter acceptance criteria. Recommended for noisy images or when minimizing false detections is particularly important.
- **Automatic:** Starts with a balanced search and expands it when the spot center or size cannot be determined reliably. Recommended for most arrays.
- **Sensitive:** Uses a wider and more permissive search from the beginning. This can improve detection of weak, displaced, or unusually sized spots, but may increase sensitivity to image artifacts.

Noise Processing

Select *Process noise* to apply background-noise, foreground-noise, and smearing processing during quantification.

Background Noise

Non-uniform background signal can interfere with spot detection and lead to inaccurate estimation of the foreground signal. Figure 30 shows an original image, the detected background component, and the resulting raw signal after the background component has been removed.



(a) Original image

(b) Detected background signal

(c) Raw signal after background removal

Figure 30. Background Noise Processing.

Foreground Noise

Figure 31 contains foreground noise, including two large red bands caused by non-specific binding. Spotxel® Microarray distinguishes valid spots from such artifacts based on their shape and size. Valid detected spots are outlined in blue.

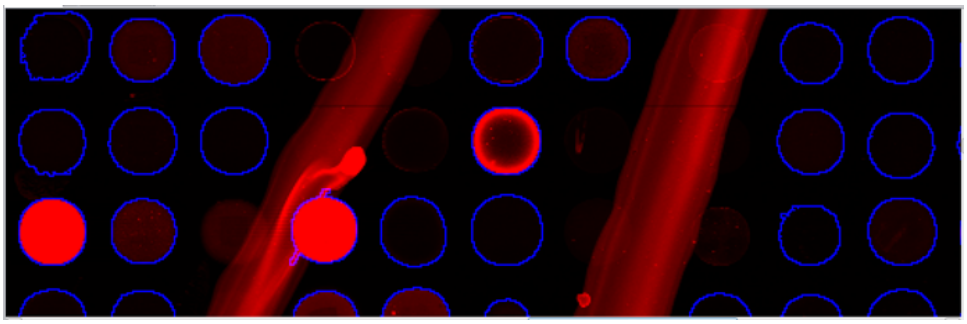


Figure 31. Foreground Noise Processing.

Smearing Signal

Figure 32 shows non-specific signal caused by smearing between neighboring spots. The image contrast has been increased for illustration. Spotxel® Microarray identifies the local spot signal associated with each spot while excluding surrounding smeared signal.

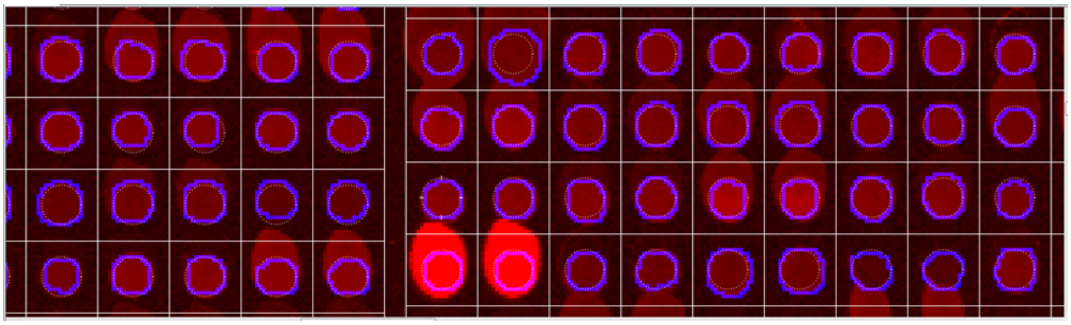


Figure 32. Smearing Signal Processing.

Defect Processing

Largest Defect Size

Select *Process defects* to enable removal of small bright defects during spot detection.

Use *Largest defect size (%)* to specify the maximum size of a structure that may be treated as a defect. The value ranges from 0% to 50% of the spot diameter, with 30% as the default.

Increasing the value allows progressively larger bright structures to be removed. Excessively large values, however, increase the risk that a genuine weak, partial, irregular, or undersized spot will be treated as a defect. This setting applies only when *Process defects* is selected.

Background Correction

Spotxel® Microarray supports the *Local* background correction method, with several levels determining how background values are calculated and shared among spots:

- **Block Level:** Default for GAL arrays. All spots within a block use the same background value, derived from the background pixels of that block.
- **Spot Family Level:** Default for PSF arrays. All spots in the same spot family use the same background value, derived from that family's background pixels.
- **Global Level:** All spots in the array use a single background value derived from the background pixels of the entire array.
- **Spot Level:** Each spot has its own background value, calculated from the background pixels associated with that spot.

CHAPTER 06

Batch Processing

Apply a common template and processing options to multiple microarray images.

Batch Processing automates alignment and quantification for multiple microarray images that share the same array layout. The common layout, called the **template array**, can be either:

- a GenePix Array List (`*.gal`); or
- a PepSlide Designer file (`*.psf`).

The scanner or image-acquisition system produces one TIFF image for each slide. In the Batch Processing tool, the same template and processing options can be applied to all selected images. Depending on the selected tasks, the batch can:

- align the template array with each image;
- save the aligned array; and
- quantify the image and save the analysis results.

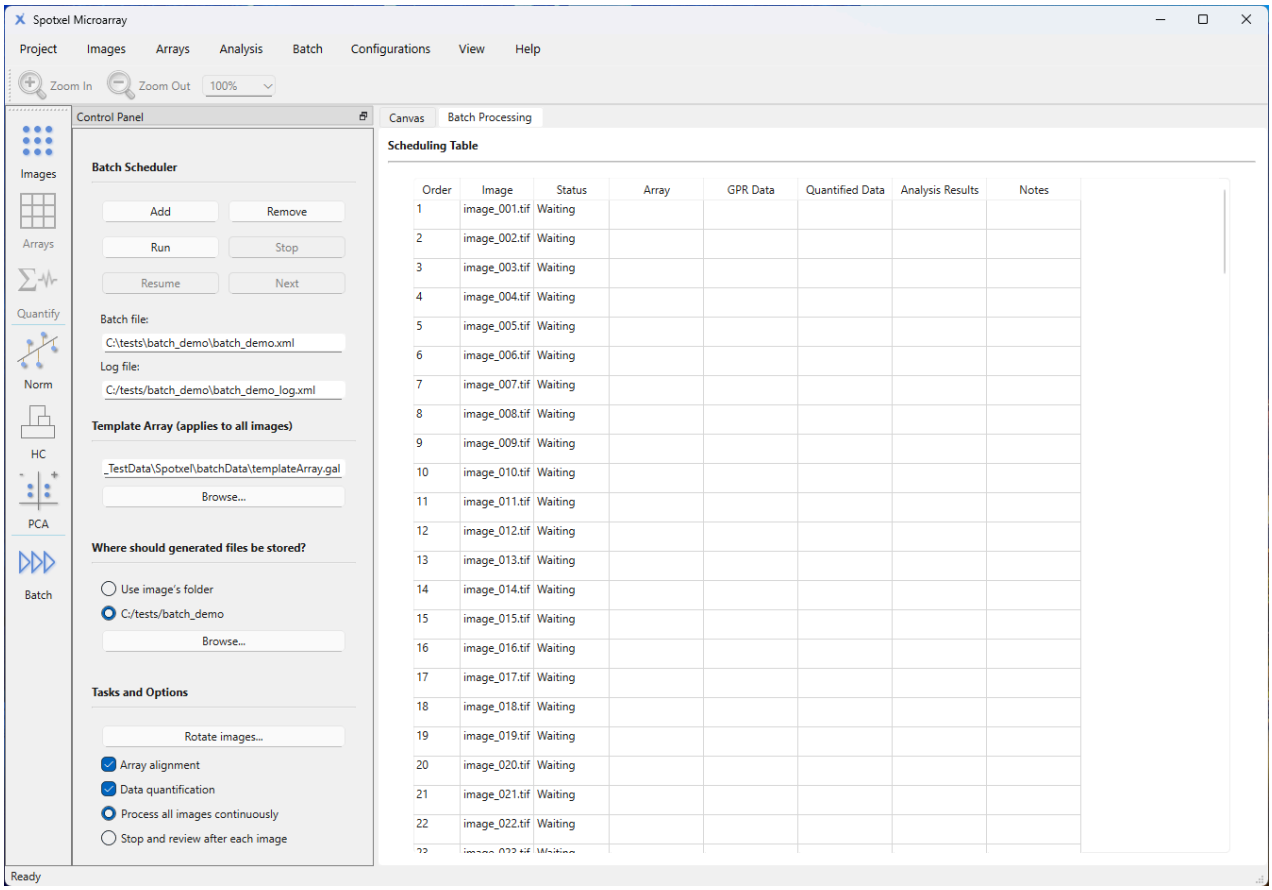


Figure 33. Batch Setup.

Creating and Configuring a Batch

1. **Open the Batch Processing tool**
   - Click *Batch* on the main toolbar.
2. **Add images**
   - Click *Add* and select the TIFF images to process.
3. **Select the template array**
   - Under *Template Array (applies to all images)*, click *Browse* and select the `*.gal` or `*.psf` file.
4. **Choose the output folder**
   - Under *Where should generated files be stored?*, use the folder containing the images or click *Browse...* to choose another folder.
5. **Optionally rotate the input images**
   - Click *Rotate Images* and configure the required flip or rotation by 90°, 180°, or 270°.
6. **Save the batch setup**
   - Select *Batch* > *Save Batch*.
   - A log file with the same base name is created automatically.

> **Tip**
> Use a separate folder for each batch to keep its inputs and outputs organized. Avoid periods in folder and file names except for the final file extension.

Running the Batch

- Click *Run* to start the batch.
- Choose a running mode:
  - **Process all images continuously:** Processes the complete image list without pausing. Click *Stop* to pause and *Resume* to continue.
  - **Stop and review after each image:** Pauses after each image so you can inspect the alignment and results before continuing.
- Monitor the scheduling table. It updates the status and generated files for each image during processing.

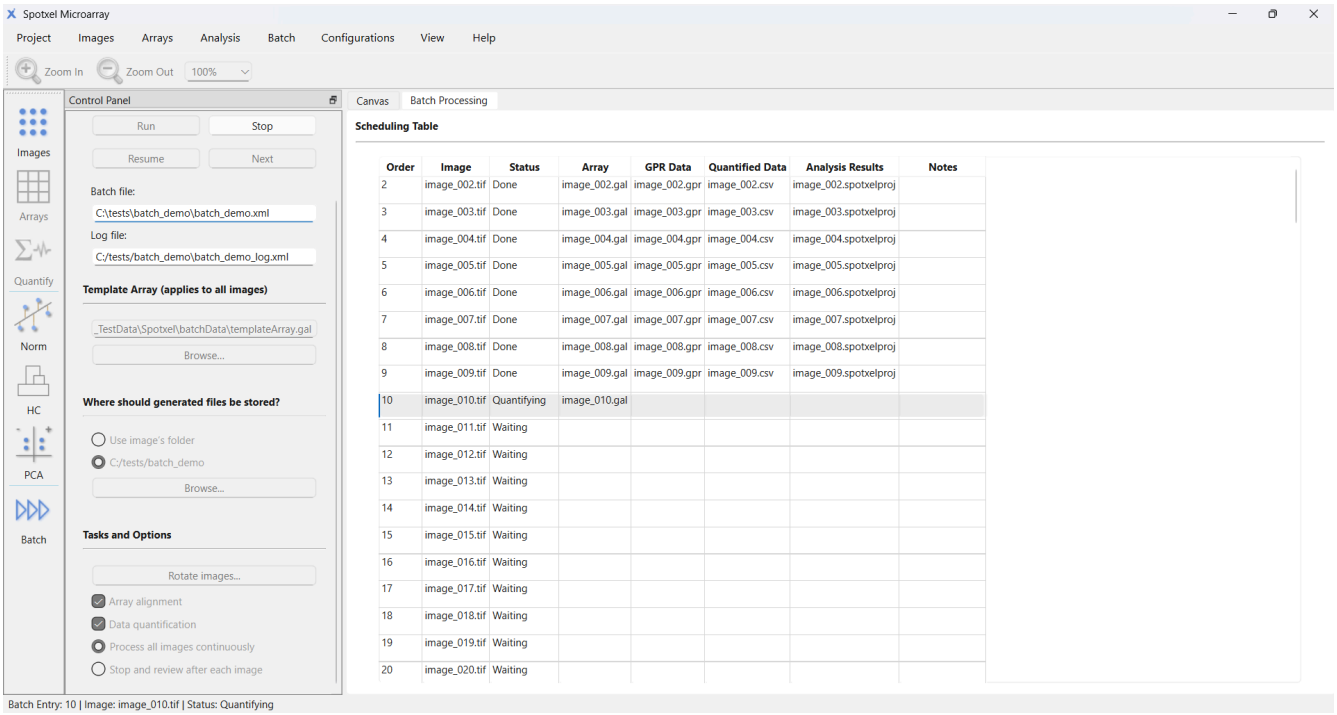


Figure 34. Batch Execution.

Generated Output Files

Depending on the selected tasks, an input image such as `sample001.tif` can produce:

- `sample001.gal` or `sample001.psf`: the aligned array layout for the image;
- `sample001.csv`: quantified data in CSV format;
- `sample001.gpr`: quantified data in GenePix Result format; and
- `sample001.spotxelproj`: the Spotxel® Microarray project containing the complete analysis results.

When project output is enabled, processing *N* images creates *N* `*.spotxelproj` files that can be combined into a dataset for downstream analysis.

CHAPTER 07

Data Preprocessing and Normalization

Create datasets, filter measurements, consolidate replicates, and normalize values.

The *Data Preprocessing and Normalization* tool provides tools for preparing quantified microarray data for further analysis.

- **Data Filtering** selects features of interest based on header information and measurement values.
- **Replicate Processing** consolidates replicate features into a single representative value using their mean or median.
- **Data Normalization** transforms measurement values to improve comparability between features and samples and provides the Z-factor quality metric for evaluating assay performance.

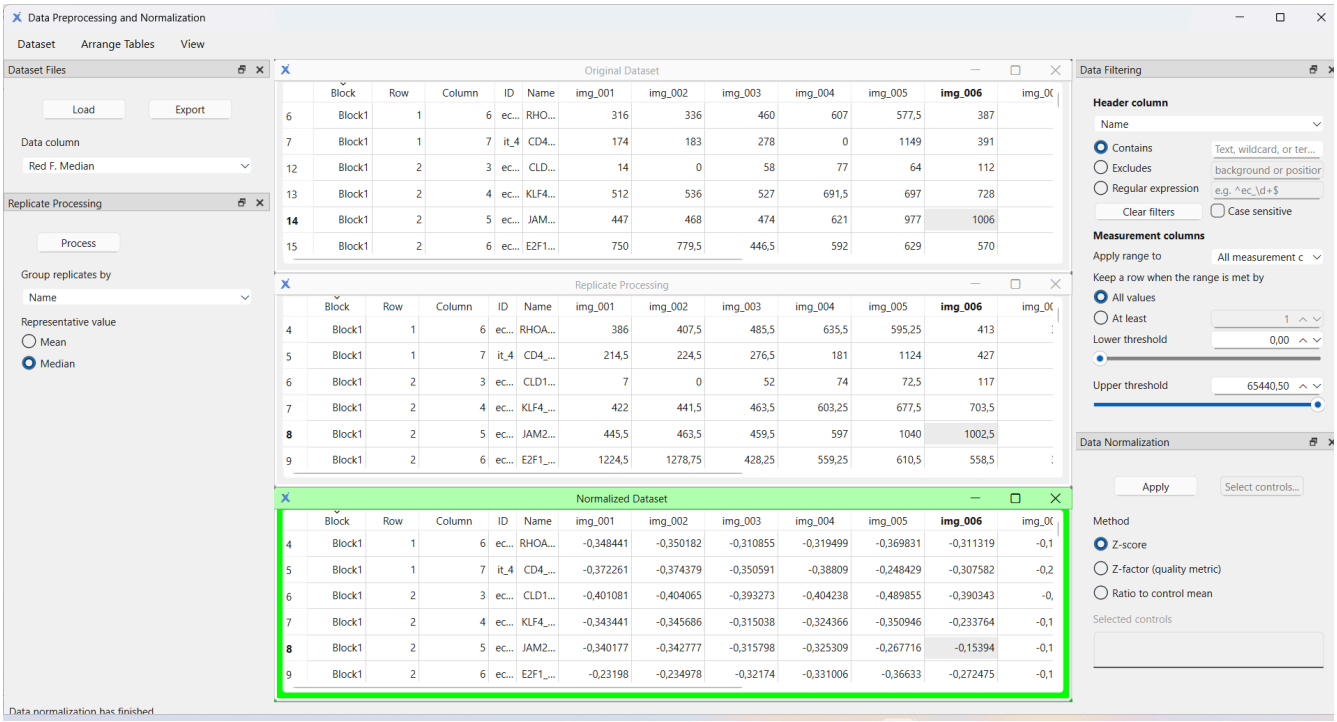


Figure 35. Replicate Processing, Data Filtering, and Data Normalization.

Click *Norm* on the main toolbar to open the tool. It operates on datasets containing quantified feature values.

Datasets

A dataset can be created from multiple Spotxel® Microarray project files.

Consider a protein microarray screened with *k* samples. After batch processing, *k* Spotxel® Microarray project files are available, each containing quantified data for one sample. These files can be combined into a dataset such as Table 4.

In the example, V_{1k} represents the measurement value of *Feature 1* for *Sample k*. The measurement can be selected from the available quantified data columns, such as *Red F. Median*.

The first five columns contain feature information defined in the GAL file. The remaining columns contain measurement values for individual samples.

Block	Row	Column	ID	Name	Sample 1	Sample 2	...	Sample k
				Feature 1	V_{11}	V_{12}	...	V_{1k}
...	...	...	...	...	...	...	...	...
				Feature n	V_{n1}	V_{n2}	...	V_{nk}

Table 4. A Sample Dataset.

A dataset can also be created from multiple GenePix Result (`*.gpr`) files. Select the GPR files and choose the measurement column to import, for example `F635Mean - B635`.

Alternatively, load an existing CSV dataset. The first five columns must be *Block*, *Row*, *Column*, *ID*, and *Name*. Subsequent columns contain numeric measurement values.

To create a dataset from project or GPR files:

1. Click *Load* in the *Dataset Files* panel.
2. Select the files to import.
3. Select the measurement from *Data column*.

To load an existing dataset, select its CSV file instead. The result is displayed in the *Original Dataset* table.

Any original, processed, filtered, or normalized dataset can be exported to CSV. Select the corresponding table and click *Export* in the *Dataset Files* panel. The selected table is highlighted.

Data Filtering

The *Data Filtering* panel selects rows based on header information and numeric measurement values.

Header Filtering

Select a column such as *Name* or *ID* from *Header column*, then choose a method:

- **Contains:** Keeps rows whose selected header contains the specified text or terms. Combine multiple terms with uppercase `AND` or `OR`. For example, `protein OR positive` keeps rows containing either term.
- **Excludes:** Removes rows whose selected header contains the specified text or terms.
- **Regular expression:** Filters rows using a regular-expression search pattern for more flexible matching.

Select *Case sensitive* when uppercase and lowercase characters must be treated differently. Click *Clear filters* to remove the current header filters.

Measurement Filtering

The *Measurement columns* options filter rows according to numeric values.

- Use *Apply range to* to apply the range to all measurement columns or one selected column.
- Set the allowed range with *Lower threshold* and *Upper threshold*.
- Under *Keep a row when the range is met by*, choose:
    - **All values:** Keep the row only when all applicable measurements are within the range.
    - **At least:** Keep the row when at least the specified number of applicable measurements are within the range.

Header and measurement filters can be used together to progressively narrow the dataset.

Replicate Processing

Microarray features are often printed in duplicate, triplicate, or more to improve measurement reliability. Because replicate spots normally produce slightly different values, they can be consolidated before further analysis.

In the *Replicate Processing* panel:

1. Select the header used to identify replicates from *Group replicates by*, for example *Name* or *ID*.
2. Under *Representative value*, select *Mean* or *Median*.
3. Click *Process*.

Rows with the same selected header value are grouped into a single row. For each measurement column, the new value is calculated as the mean or median of the corresponding replicate values. The result is displayed in the *Replicate Processing* table.

Data Normalization

Technical variation can make absolute signal values difficult to compare directly between samples or experiments. The *Data Normalization* panel provides methods for transforming measurements and evaluating assay quality.

- **Z-score:** Expresses each measurement relative to the mean in units of standard deviation. Positive values are above the mean, negative values are below the mean, and values near zero are close to the mean.
- **Z-factor (quality metric):** Calculates the Z-factor from the means and standard deviations of the measured features and selected negative controls. It assesses assay quality rather than expressing measurements on a normalized scale.
- **Ratio to control mean:** Divides each measurement by the mean of selected controls. This is useful when control features provide a reference for comparison.

For methods requiring control features, click *Select controls...*. The *Control Selection* dialog displays the features in the selected dataset. Choose one or more controls.

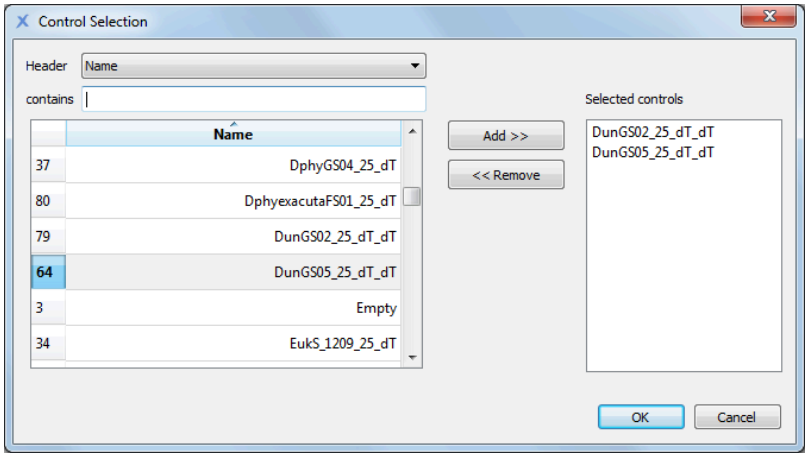


Figure 36. Control Selection for Data Normalization.

Select the required method and controls, then click *Apply*. The result is displayed in the *Normalized Dataset* table.

Reference: Zhang, J.-H., Chung, T. D. Y., and Oldenburg, K. R. (1999). *A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays*. Journal of Biomolecular Screening, 4(2), 67-73.

Arranging Tables

When multiple dataset tables are open, use *Arrange Tables* to tile them vertically or cascade them.

CHAPTER 08

Data Mining Tools

Explore quantified datasets with PCA and hierarchical clustering analysis.

The data mining tools help you explore patterns and relationships in quantified microarray datasets.

- **Principal Component Analysis (PCA)** provides lower-dimensional views of the data and can create a simplified dataset.
- **Hierarchical Clustering Analysis (HCA)** groups features and samples by similarity and displays clustering trees and a heat map.

Spotxel® Microarray project files generated by batch processing can be loaded directly. These tools also accept datasets prepared from GPR or CSV files.

Principal Component Analysis (PCA)

PCA transforms the dataset into principal components that summarize major patterns of variation. Spotxel® Microarray displays pairwise two-dimensional projections of the first three components and can generate a simplified dataset containing three features or three samples.

Click *PCA* on the main toolbar, then:

1. Click *Load Data* and select a dataset.
2. Select the measurement to analyze from *Data column*.
3. Under *Simplify the dataset to three*, select *Features* or *Samples*.
4. Click *Start Analysis*.

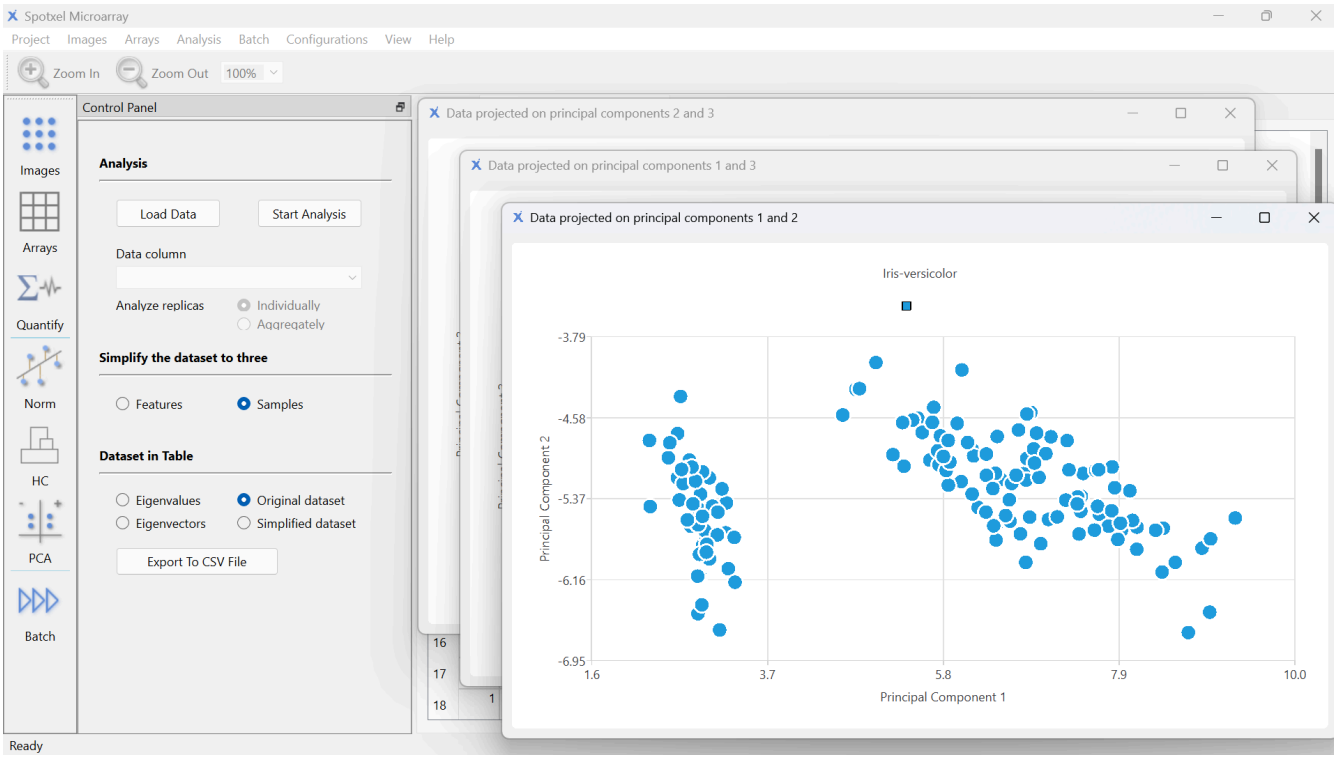


Figure 37. Principal Component Analysis in 2D View.

When *Samples* is selected, the simplified dataset contains three samples. When *Features* is selected, it contains three features. Use the option that matches the dimension you want to reduce.

The three charts show pairwise projections of the first three principal components. Drag across a region to zoom in, and double-click a chart to restore its original view.

In *Dataset in Table*, select the original or simplified dataset and export it to CSV when further analysis is required.

Hierarchical Clustering Analysis (HCA)

HCA groups features or samples according to the similarity of their measurement profiles. Click *HC* on the main toolbar, then:

1. Click *Load Data* and select a dataset.
2. Select the measurement to analyze from *Data column*.
3. Choose whether to cluster *Features*, *Samples*, or *Both*.
4. Select the distance metric and linkage method, or keep the default settings.
5. Click *Start Analysis*.

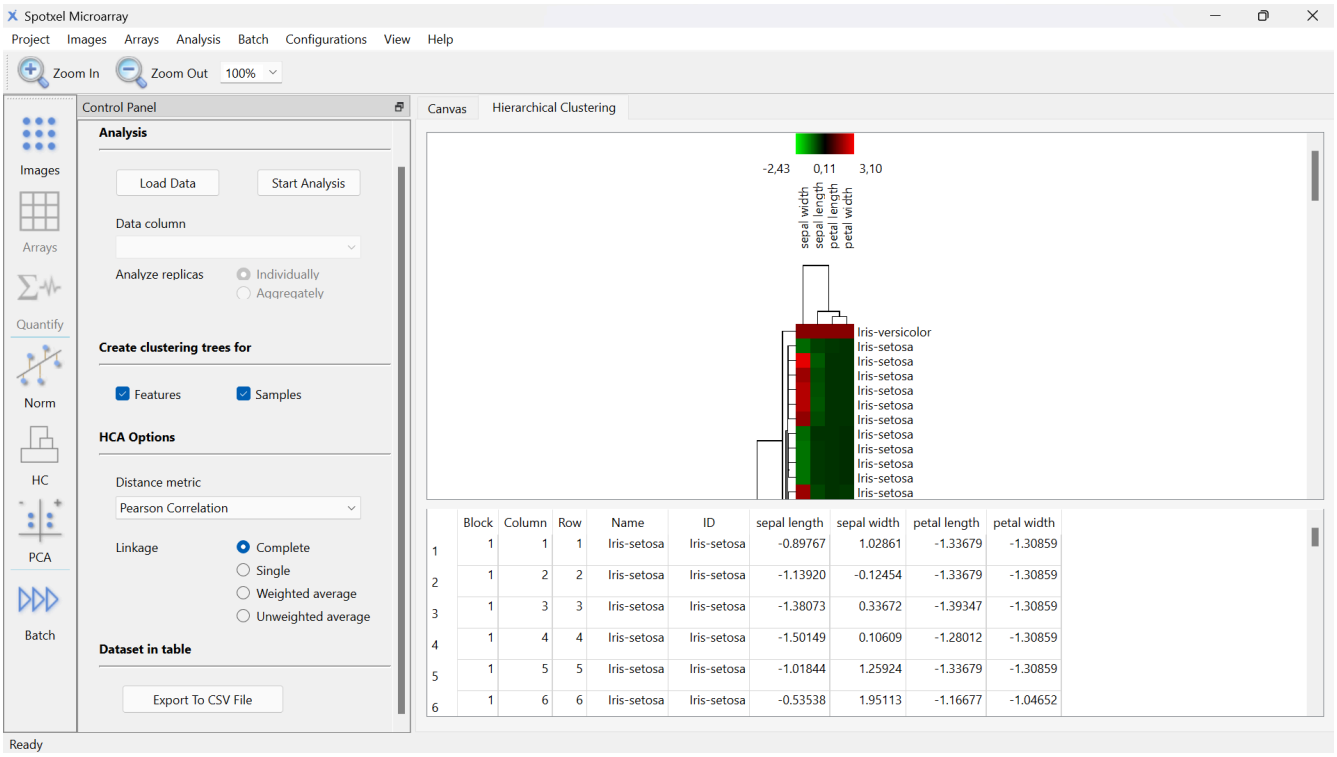


Figure 38. Hierarchical Clustering Analysis.

Spotxel® Microarray constructs the selected clustering tree or trees. Closely related features or samples are joined into clusters, and those clusters are progressively combined to form the hierarchy.

The heat map represents each feature-sample measurement by color alongside the clustering trees. To save the complete visualization, use the *Export to Image* context-menu command.

CHAPTER 09

Purchases and Licenses

Start trials, purchase Full access, manage subscriptions, and restore Microsoft Store licenses.

Premium features require **Full access**, which can be obtained through one of the following products:

- **Spotxel® Microarray 3-Month Subscription**
- **Spotxel® Microarray 1-Year Subscription**
- **Spotxel® Microarray Perpetual License**

Spotxel® Microarray is distributed through the Microsoft Store on Windows and the Mac App Store on macOS. Purchases, subscriptions, trials, and license entitlements are managed by the Store through which the product was obtained.

Licenses purchased previously through the SICASYS Online Store are not transferred automatically to the Microsoft Store or Mac App Store.

Products, prices, and trial terms shown in *Purchases and License* are provided by the Store and may vary by country, region, or time.

Select *Help* > *Purchases and License* to view, purchase, or restore a license.

Microsoft Store

Spotxel® Microarray purchases on Windows are associated with the Microsoft account used for the purchase. Before purchasing or restoring a license, sign in to the Microsoft Store with the appropriate Microsoft account.

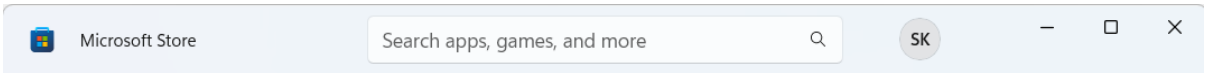


Figure 39. Microsoft Store with a Signed-In Account.

Subscriptions and Free Trials

On Windows, a free trial is available through either subscription plan:

- **3-Month Subscription:** 1-week free trial
- **1-Year Subscription:** 1-month free trial

To start a trial, select the desired subscription in *Purchases and License* and click *Start plan*. The Microsoft Store handles the subscription and may require payment information before the trial begins.

The subscription becomes active immediately, and its access-expiration date is shown under *Your license*. Figure 40 shows an active 3-Month Subscription as an example.

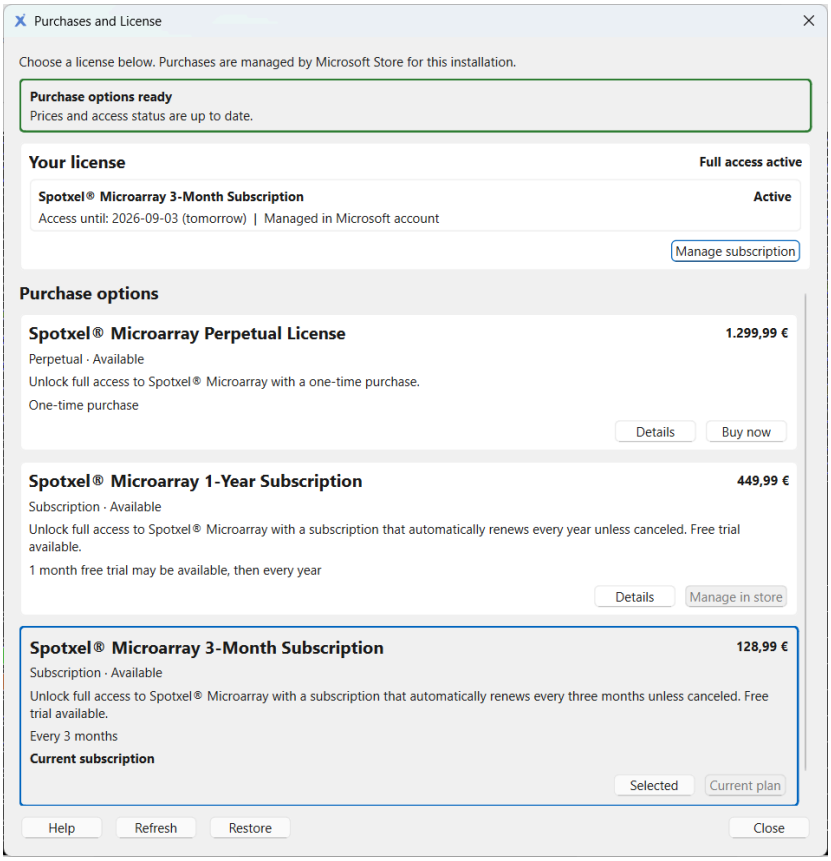


Figure 40. Active 3-Month Subscription.

Subscription plans renew automatically unless recurring billing is turned off or the subscription is canceled through Microsoft. To avoid continuing with a paid subscription after a trial, manage it before the trial ends.

Click *Manage subscription* under *Your license* to open the subscription-management page in your Microsoft account.

Managing or Canceling a Subscription

Click *Manage subscription* under *Your license*. The Microsoft account page provides recurring-billing and cancellation options.

Turning off recurring billing prevents future renewal while allowing the subscription to remain usable until its expiration date.

Perpetual License

The **Perpetual License** provides Full access without a recurring subscription.

Select *Spotxel® Microarray Perpetual License* in *Purchases and License* and click *Buy now*. The Microsoft Store handles the transaction.

> ⚠ **Important**
>
> Purchasing a Perpetual License does not cancel an existing subscription or trial automatically. If the subscription is no longer needed, click *Manage subscription* and cancel it or turn off recurring billing through your Microsoft account.

Figure 41 shows an example in which a Perpetual License and a 1-Year Subscription trial are both active.

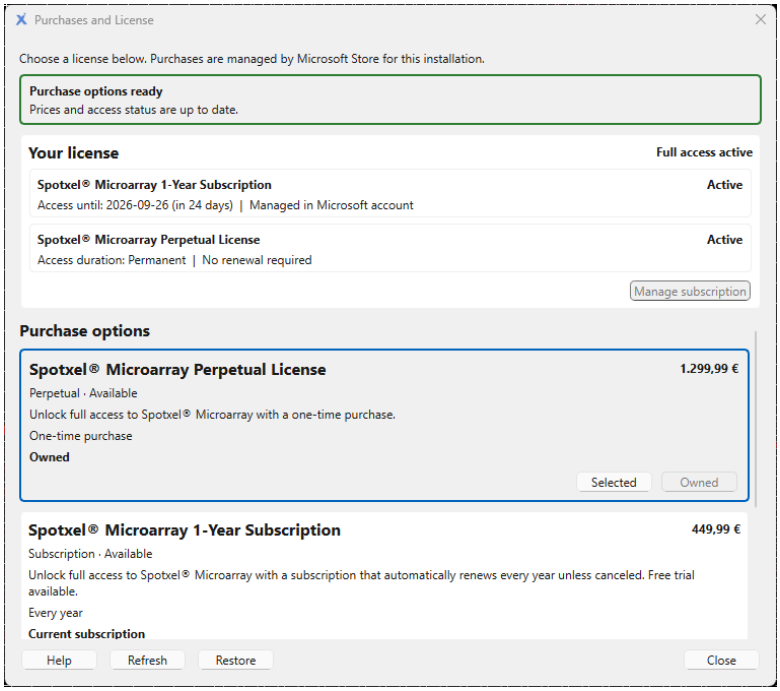


Figure 41. Active Perpetual License and 1-Year Subscription Trial.

Restoring a License

If you own a license but Full access is not active, for example after installing Spotxel® Microarray on another eligible device:

1. Connect the computer to the Internet.
2. Open the Microsoft Store and sign in with the Microsoft account used to purchase the license.
3. Open *Help* > *Purchases and License* in Spotxel® Microarray.
4. Click *Refresh* to update purchase and access information.
5. Click *Restore* to restore available license entitlements.

If restoration still fails, reset the Microsoft Store cache:

1. Close Spotxel® Microarray and the Microsoft Store.
2. Press **Windows+R**, enter `wsreset.exe`, and click **OK**.
3. After the Microsoft Store reopens, restart Windows.
4. Open the Microsoft Store and verify that the correct account is signed in.
5. Launch Spotxel® Microarray and try *Refresh* and *Restore* again.

If necessary, uninstall Spotxel® Microarray and reinstall it from the Microsoft Store, then repeat the restore procedure. If the license still cannot be restored, contact SICASYS for support.

Mac App Store

To be updated.

CHAPTER 10

End-User License Terms

License terms governing the use of Spotxel® Microarray.

Spotxel® Microarray is licensed, not sold. The license is granted by SICASYS Software GmbH and is governed by the applicable standard end-user license terms of the Store through which the software was obtained.

For installations obtained from the Microsoft Store, the Microsoft Standard Application License Terms apply.

For installations obtained from the Mac App Store, the Apple Licensed Application End User License Agreement (Standard EULA) applies.

CHAPTER 11

Third-Party Software Licenses and Notices

License and notice information for third-party open-source components.

Spotxel® Microarray uses third-party open-source software components, both directly and through other software included with the application.

License information for the main open-source components is available from *Help* > *Third-Party Licenses*. Complete third-party open-source license and notice information is provided in `THIRD_PARTY_LICENSES.txt`, which is distributed with Spotxel® Microarray. To view the complete notice file, click *View Complete Notices...* in the *Third-Party Software Licenses* dialog.

CHAPTER 12

Revision History

Review the documented changes in this and earlier guide revisions.

Revision	Date	Changes
4.1 Rev 1	07 Sep 2026	**New and improved:** Support for 20-bit Extended Dynamic Range (XDR) images; LoG-Spot detection and sensitivity options; Store-based purchasing, trials, subscriptions, perpetual licensing, and restoration; updated Data Preprocessing and Normalization interface; updated Batch Processing and Data Mining guidance; revised end-user license terms and third-party notices.
Rev 10	20 Jun 2025	**New functions:** Support for PepSlide Designer (`*.psf`) arrays; Batch Processing with image rotation; configurable visible columns in the quantified data table. **Updated:** Terms and concepts; Fixed-Spot method; Batch Processing.
Rev 9	03 Jan 2025	**New function:** 3D Spot Viewer. **Updated:** Automatic array alignment.
Rev 8	14 Aug 2024	**New functions:** GAL file editor; feature-based spot finding. **Removed:** Background controls; Scatter Plot and K-Means Clustering; *Include change of the images' intensity value*; *Image Intensity > Filter noise*.

CHAPTER 13

Subject Index

Find key topics by subject and the chapters in which they are explained.

Use this subject index to find topics in the User's Guide. Select a chapter reference to jump to it in the PDF.

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