



Spotxel® Microarray 4.1

Microarray Image and Data Analysis Software

Quick Start Guide

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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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Introduction

Install Spotxel Microarray, understand Full access, and learn the main interface and concepts.

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, data normalization, and data mining. These capabilities help improve data quality and reliability and support the identification of relevant features and samples and the exploration of their relationships.

This Quick Start Guide introduces the essential workflows for analyzing a single microarray image and processing multiple images in a batch. For complete instructions, see the **Spotxel® Microarray User's Guide** on the [manuals page](#).

Installation

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

Microsoft Store

Install the Windows version from the Microsoft Store:

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

Using Premium Features

Microarray Image Handling and the GAL Array Editor are available immediately after installation and do not require Full access. Premium features, including data quantification, automated array alignment, batch processing, replicate processing, data filtering, data normalization, and data mining, require **Full access**. Full access is provided by either an active trial or a purchased license.

On Windows, select *Help > Purchases and License* to start a trial, purchase a subscription or Perpetual License, or restore an existing license. Purchases and license entitlements are managed by the Microsoft Store.

Updates

Software updates for the Windows version are distributed through the Microsoft Store and may be installed automatically or manually, depending on your Store settings.

Software User Interface

Software controls are grouped into the labeled components shown in [Figure 1](#) and listed in [Table 1](#).

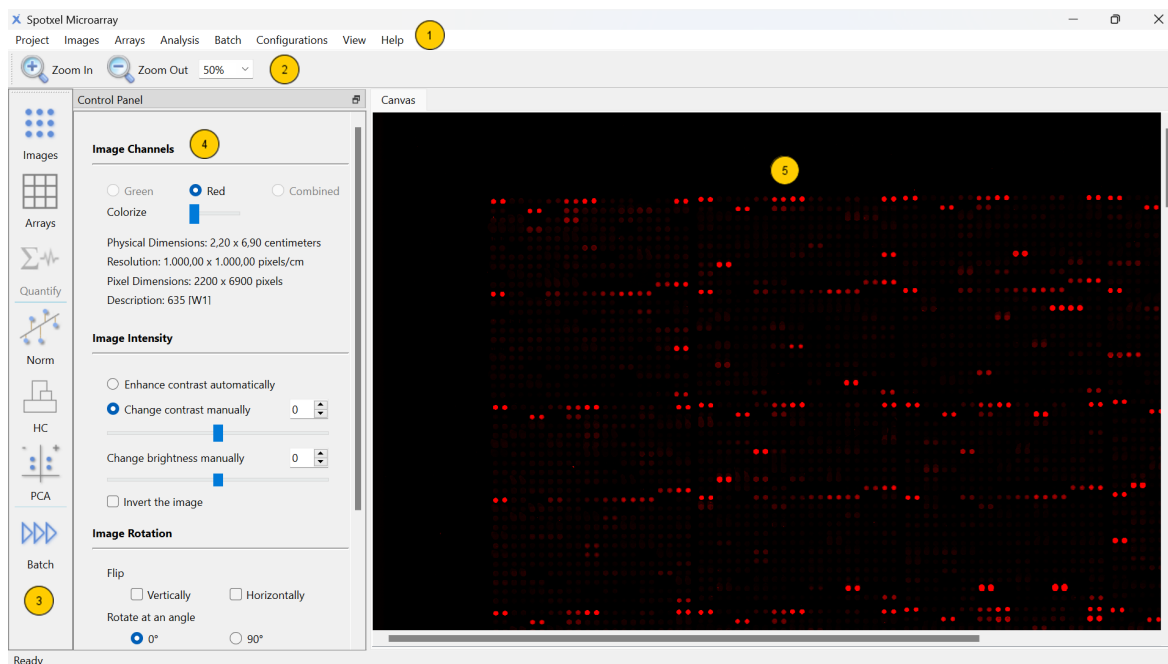


Figure 1. User Interface.

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 related function groups ([Table 2](#)). Clicking a toolbar button opens the corresponding control panel. Data and analysis results are displayed in a sheet to the right of the control panel.

Tool	Functions	Tool	Functions
	Select image channels, adjust image intensity, and rotate images.		Filter data, process replicates, and normalize data.
	Create and edit GAL blocks; view spot and spot-family properties; position and rotate GAL blocks or PSF spot families.		Group related features and samples and display their relationships in a heat map.

Tool	Functions	Tool	Functions
	Quantify array data and review the results.		Explore major patterns among features and samples.
	Automatically align and quantify multiple microarray images.		

Table 2. The Main Toolbar and Related Functions.

Terms and Concepts

Array: An array consists of the spot layout and its annotation. The layout can be stored as a GenePix Array List (*.gal) or PepSlide Designer (*.psf) file.

Microarray image: In a microarray assay, binding events at designated spots generate signals when the slide is exposed to a sample. A scanner captures these signals as a grayscale microarray image whose pixel intensities represent signal strength. Spotxel® Microarray can display the grayscale intensities using red or green colorization.

GAL Arrays - Spot and Block

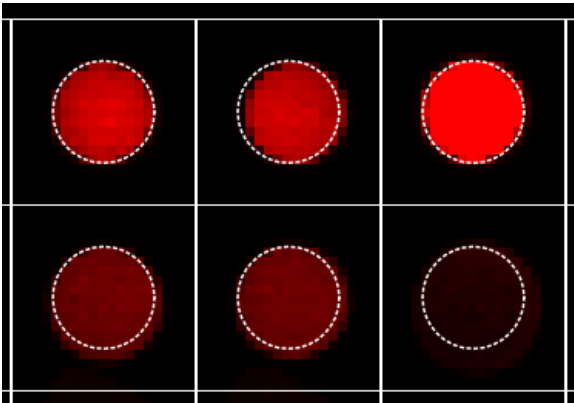


Figure 2. A rectangular block with 6 spots.

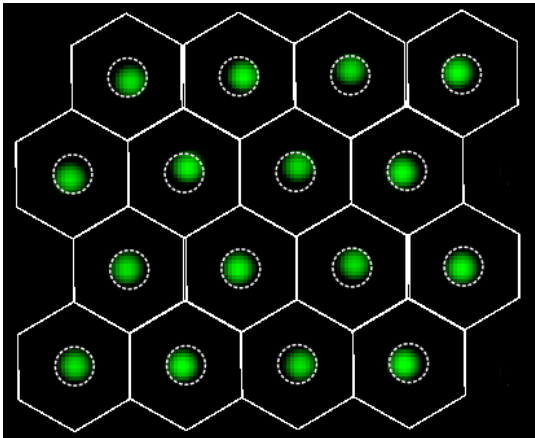


Figure 3. A hexagonal grid with 16 spots.

Spot appearance: In a rectangular block, each spot is displayed as a white square ([Figure 2](#)). In a hexagonal block, each spot is displayed as a white hexagon ([Figure 3](#)), a layout also known as orange packing.

Spotted region: A dashed circle within each spot indicates the region where binding is expected.

Block: A block is a group of adjacent spots arranged in a rectangular or hexagonal grid.



Tip

Working exclusively with GAL arrays? 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: A dashed rectangle marks the region of interest for quantification.

Spot family (SF): Adjacent spots produced from the same source are grouped as a spot family. In the analysis workflow, a spot family serves the same role as a block in a GAL array.

Analysis Workflow (Common to GAL and PSF)

Quantification: Estimates the binding signal at each spot from statistical measurements of pixel intensity.

Array alignment: Links each spot in the layout to its corresponding image signal. The software adjusts the positions of blocks or spot families so that the spotted regions align with the detected signals.

Microarray Data Analysis Workflow

See how single-slide analysis and automated batch processing fit together.

The analysis diagram in [Figure 5](#) summarizes the two primary workflows supported by Spotxel® Microarray:

- **Single-slide analysis**, described in the next chapter; and
- **batch processing** of multiple slides, described in the final chapter.

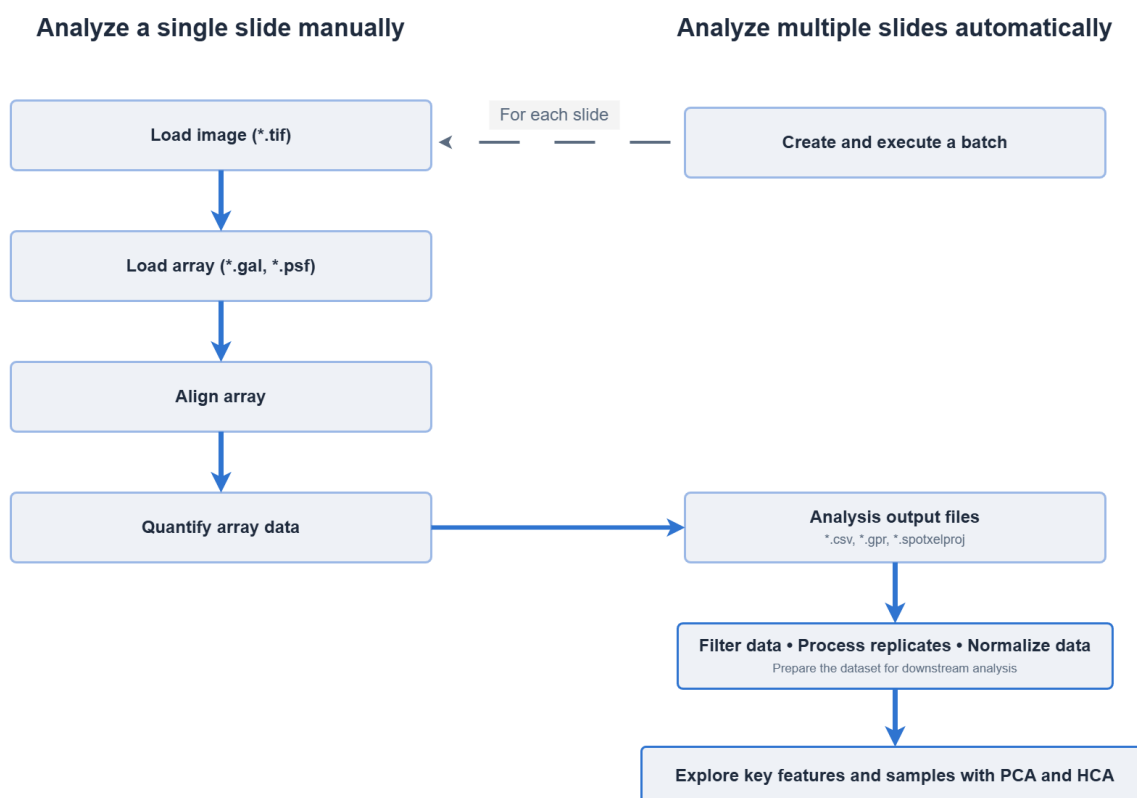


Figure 5. The Analysis Workflow.

Both workflows produce quantified data that can be filtered, consolidated by replicate, normalized, and explored with the data mining tools.

Analyze a Single Slide

Load an image and array, align the layout, quantify spots, and save the results.

1. Load the Microarray Image and Inspect the Signal



Open the image

Select *Images* > *Open Image* and choose the TIFF image. Assign the image signal to either the **Red** or **Green** channel. For example, assigning the signal to the Red channel displays the signal intensity in red on the canvas.



Note

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.

1.1 Use Two Images or a Multi-Page TIFF

- Assign each image or page to the **Red** or **Green** channel.
- Under *Images* > *Image Channels*, select **Red**, **Green**, or **Combined** to change the view.

1.2 Adjust the View Scale

- Use *Zoom In* and *Zoom Out* on the canvas toolbar.
- Alternatively, select a value from the *Zoom* list.

1.3 Enhance Signal Visibility

- Open the *Images* control panel.
- Under *Image Intensity*, select *Enhance contrast automatically* or adjust contrast and brightness manually.
- Alternatively, select one channel under *Image Channels* and use *Colorize*.

2. Load the Array File and View the Layout



Open the array file

Select *Arrays* > *Open Array*, then choose the **GAL** (`*.gal`) or **PSF** (`*.psf`) file. The array layout is overlaid on the image in the canvas.

2.1 View Block and Spot Properties for GAL Arrays

- Open the *Block* page in the *Arrays* control panel, then hover over or click a block to view its properties.
- Open the *Spot* page, then hover over or click a spot to view its properties.

2.2 View Spot Family and Spot Properties for PSF Arrays

- Open the *Spot Family* page in the *Arrays* control panel, then hover over or click a spot family to view its properties.
- Open the *Spot* page, then hover over or click a spot to view its properties.

3. Align the Array



Prepare and align the array

Before quantification:

- **Enhance spot visibility** as described in Step 1.3.
- **Inspect the spot pattern.** Use control spots to determine whether rotation or repositioning is required.
- **Run automatic alignment** when the spot signal is sufficient.

If necessary, use Step 3.3 to adjust the array manually, then save the alignment.



Tip

Preserve the original array file. Use *Arrays > Save Array As* to create an aligned copy for each image.

3.1 Rotate the Image

Under *Images > Image Rotation*:

- flip the image horizontally or vertically; or
- rotate it by 90°, 180°, or 270°.

3.2 Align the Array Automatically

In the *Arrays* control panel, click *Align*.

3.3 Reposition Blocks and Spot Families

For GAL arrays

- Open the *Block* page.
- Select blocks individually with **Ctrl+Click**, or select all blocks with **Ctrl+A**.
- Drag the selected blocks so that the dashed circles align with the spot signals.

For PSF arrays

- Open the *Spot Family* page.
- Select and drag spot families in the same way as GAL blocks.

3.4 Save the Alignment

- select *Arrays > Save Array As* to save an aligned copy (**recommended**); or
- Select *Arrays > Save Array* only if you intend to overwrite the current array.

4. Quantify the Array Data



Configure and run quantification

Confirm that the array is correctly aligned with the image, then open the *Quantification* control panel.

- To choose a detection method, select *Show quantification options* and choose **Flex-Spot**, **LoG-Spot**, or **Fixed-Spot**.
- Click *Quantify Array*.
- When quantification is complete, the quantified data table opens. Each row contains raw, background, and foreground measurements for one spot.
- Use foreground measurements such as *Red F. Mean*, *Red F. Median*, *Green F. Mean*, or *Green F. Median* to inspect and compare spot signal levels.



Note

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

4.1 View Quantified Data

- Click a spot in the canvas to highlight its row in the data table.
- Select a row in the table to highlight the corresponding spot in the canvas.
- Use the navigation keys (*Up*, *Down*, *Page Up*, and *Page Down*) to browse the data.

4.2 Save Quantification Results

- Use *Data Export* to save quantified data as CSV or GPR. If the panel is hidden, select *View > Data Export*.
- Select *Project > Save Project* to create a project file (`*.spotxe1proj`). Use *Project > Open Project* to resume the analysis.

Analyze Multiple Slides Automatically

Configure and run a batch for multiple images that share one template array.

Batch Processing automates array alignment and data quantification for multiple TIFF images that share the same **template array** (`*.gal` or `*.psf`). Each image represents one slide. The batch applies the same template and processing options to all selected images and saves the requested results.



Tip

Automatic alignment requires sufficient spot signal in each image.

1. Create and Configure the Batch



Start batch setup

Click *Batch* on the main toolbar. In the *Batch Processing* control panel, add the images, select the template array, choose where the generated files will be stored, and configure the processing options. Save the batch setup if you want to reuse it later.

1.1 Specify Parameters and Options

1. Click *Add* and select the TIFF images to process.
2. Under *Template Array (applies to all images)*, click *Browse* and select the `*.gal` or `*.psf` file.
3. Under *Where should generated files be stored?*, use the folder containing the images or click *Browse...* to choose another folder.
4. Under *Tasks and Options*, select *Array alignment* and *Data quantification*, then choose the running mode.
5. If needed, click *Rotate images...* and configure the required flip or rotation.
6. To save the setup, select *Batch* > *Save Batch*. A log file with the same base name is created automatically.



Tip

For better organization, click *Browse...* in Step 3, then use a separate folder for each batch. Keep the input images and generated output files together in that folder. Avoid periods in folder names and file base names; use a period only before the file extension.

1.2 Execute the Batch

- Click *Run*. The scheduling table updates each image's status and generated files.
- In continuous mode, use *Stop* and *Resume*. In review mode, inspect each result and click *Next*.
- Depending on the selected tasks, each image can generate an aligned `*.gal` or `*.psf` file, a CSV file, a GPR file, and a `*.spotxe1proj` project file.