Spotxel® Microarray 3.6

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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1 Introduction

Spotxel® Microarray, previously known as Spotxel® Microarray Image and Data Analysis Software, provides intuitive and user-friendly tools for microarray image and data analysis. The software includes features for microarray image analysis and the automatic processing of multiple microarray images. Additionally, it offers tools for replicate processing, data filtering, and data normalization, which can significantly enhance the quality of your microarray data. The data mining tools enable you to identify key features and samples in your microarray study and explore their relationships.

This Quick Start Guide offers an overview of basic commands for immediate access to the software's functionalities. For more detailed information, please refer to the User's Guide.

1.1 Installation

The software runs natively on both Windows and Mac OS X platforms. Depending on the installation directory, installation of the software may require system administrator rights.

Windows Platforms

Spotxel® Microarray is compatible with 64-bit versions of Windows 7, Windows 8, Windows 10, and Windows 11. To install the software:

- Run the installer.
- If the current Windows account is not an administrator, you will be prompted to input an administrative account and password.

Mac OS X platforms

The software is compatible with Mac OS X 10.7 and later versions. To install the software:

- Double-click on the installer to initiate the setup program.
- Confirm the installation directory when prompted; by default, this is set to *$HOME/SpotxelMicroarray*.

Once the installation is complete, navigate to the installation folder and click on the *Spotxel* app to launch the software.

1.2 Product Activation

After installing the software on Windows, you may want to activate it with a *trial serial number*. This allows you to use premium functionalities such as data quantification, automatic array alignment, and batch processing of multiple images. The trial use for the software on Mac OS X platforms is automatically managed and does not require this step.

It is important to note the GAL Array Editor module, along with the functionality for handling microarray images, is entirely **free** and become accessible immediately upon software installation, with **no license** required.

When the free trial period expires, you can purchase a software license for continued use of premium functions. Upon purchase, you will receive a serial number to activate the license.

1.3 Upgrade

Simply run the installer for the new version to upgrade. The software configuration will be handled automatically. You do not need to activate the software again if it has already been activated.

1.4 Software User Interface

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

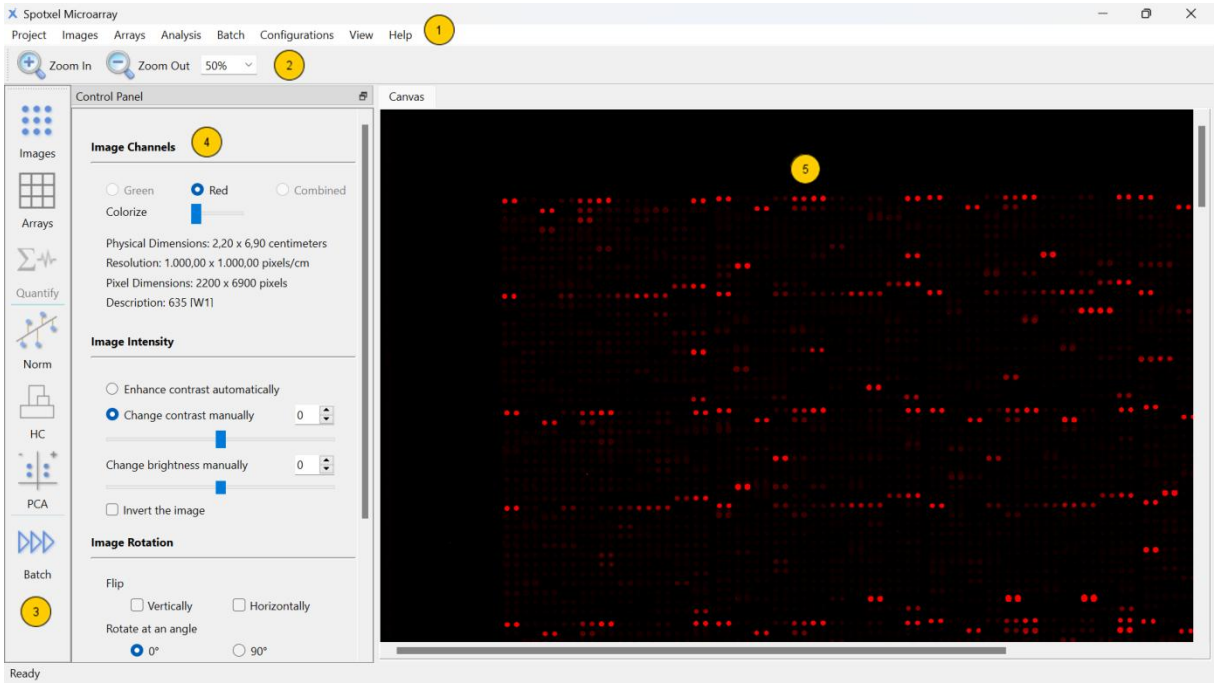

Fig. 1: User Interface.

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

Table 1: Software Components.

The main toolbar provides 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 displays 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.
	Add, edit, rotate and move blocks. View and edit spots' ID and name.		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.
	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, an **array** is the spot layout plus its annotation. The layout can be stored as either a GenePix Array List (GAL) (*.gal) or a PepSlide Designer file (PSF) (*.psf).

In a microarray assay, binding events at designated spots generate signals when the slide is exposed to a sample. These signals are captured by a scanner and converted into a digital **array image** - a matrix of grayscale pixels whose brightness reflects the signal intensity. For enhanced visual interpretation, our software maps these grayscale intensities to red or green hues.

1.5.1 GAL arrays – Spot and Block

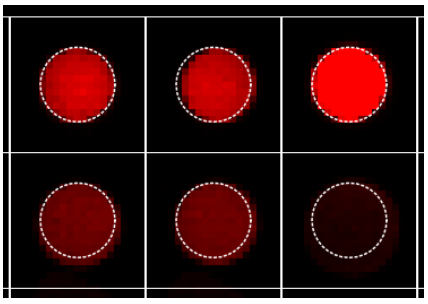

Fig. 2: A rectangular block with 6 spots.

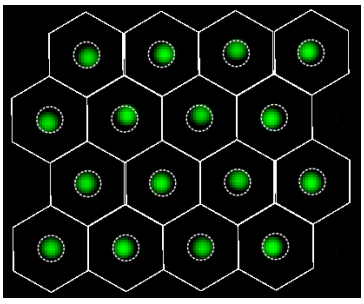

Fig. 3: A hexagonal grid with 16 spots.

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

Within each spot, the *spotted region* is enclosed by the *dashed circle*, indicating the region where true binding is expected. The array composes of blocks. A **block** is a group of spots located next to each other, appearing as a grid of white squares or hexagons.

Working exclusively with GAL? You can skip ahead to section 1.5.3.

1.5.2 PSF arrays – Spot and Spot Family

A **spot** is displayed as a *white rectangle* (Fig. 4). Within each spot, the *spotted region* is enclosed by a *dashed rectangle*, marking the region of interest for quantification.

Fig. 4: Rectangular Spot

A **spot family** (abbreviated SF) consists of adjacent spots produced from the same source. In all analysis steps, an SF functions the same way as a block in a GAL array.

1.5.3 Analysis Workflow (common to GAL & PSF)

Quantification estimates the true binding signal of each spot, based on statistical measurements of the pixel intensities within that spot.

Array alignment links layout spots to image signals. Because the spot signal is assumed to reflect true binding, the software adjusts block or SF positions to maximize alignment between the spotted region and the signal.

2 Microarray Data Analysis Diagram

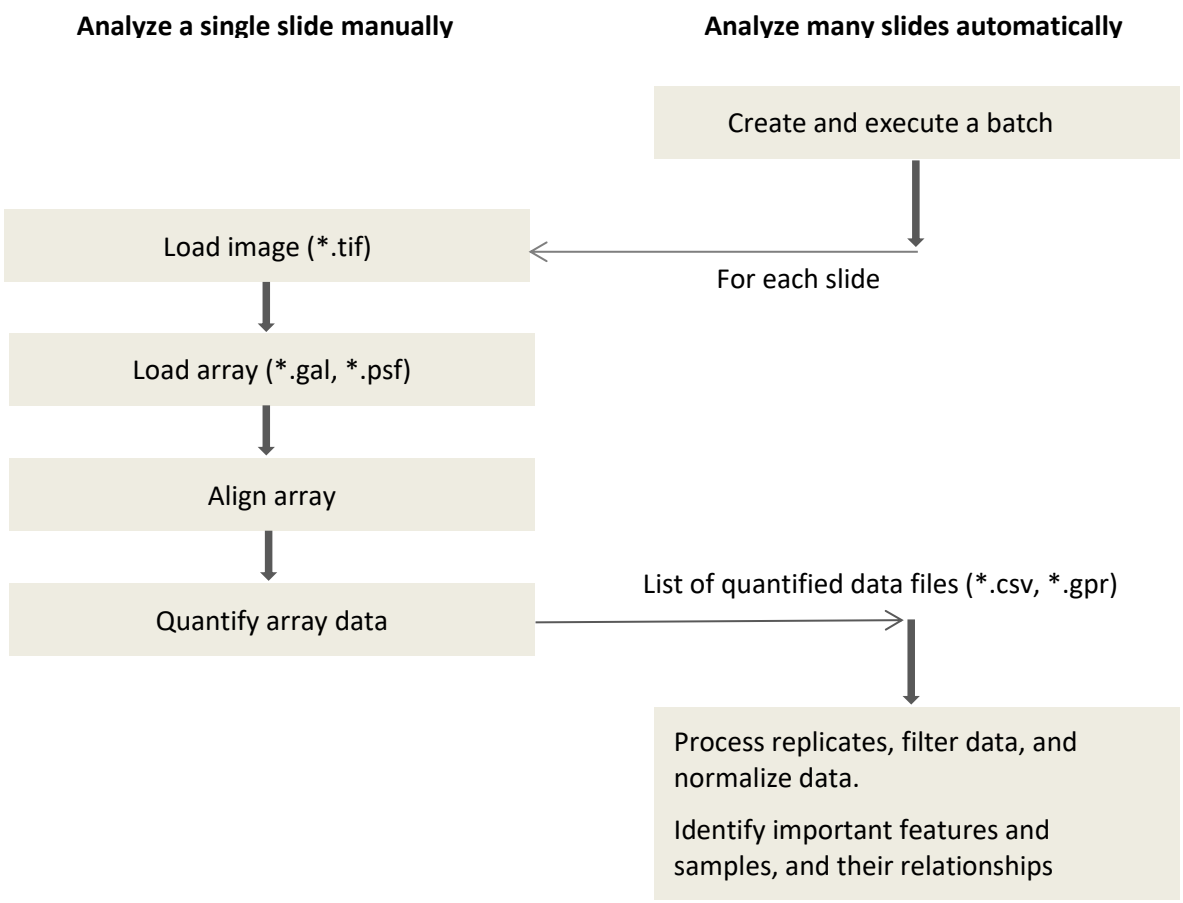

Fig. 5: The Analysis Diagram.

The **analysis diagram** (Fig. 5) provides a visual summary of the two primary workflows supported by the software:

- **Single-slide analysis**, covered in Section 3, and
- **Batch processing** of multiple slides, detailed in Section 4.

3 Analyze a Single Slide

1 **Load the Microarray Image and Inspect the Signal**

- Go to **Images > Open Image** and select the TIFF file of the slide.
- Assign the image signal to either the **Red** or **Green** channel.

For example, if you assign the signal to the Red channel, the canvas displays red pixels to represent signal intensity (Fig. 2).

The software supports 8-bit, 16-bit, and 24-bit grayscale TIFF images. For optimal quality and performance, use 16-bit grayscale TIFFs.

1.1 **If using two TIFF images or a multi-page TIFF:**

- Assign each image (or each page) to the **Red** or **Green** channel.
- To view individual or combined channels:
  - Open the **Images** control panel.
  - In the **Image Channels** section, select **Red**, **Green**, or **Combined**.

1.2 **Adjust the View Scale**

- Use **Zoom In** and **Zoom Out** on the canvas toolbar.
- Or, choose a specific zoom level from the **Zoom** dropdown.

1.3 **Enhance Signal Visibility**

- Open the **Images** control panel.
- In the **Image Intensity** section:
  - Enter a positive contrast value (e.g., 75), or
  - Select **Enhance contrast automatically**.

Alternatively:

- In the **Image Channels** section, choose **Red** or **Green**, then toggle the **Colorize** switch for better contrast.

2 **Load the Array File (*.gal or *.psf) and View Layout**

- Open the **Arrays** control panel.
- Click **Open** and select the layout file.
- The white array grid (see Fig. 2 and Fig. 3) is then overlaid on the image in the canvas.

2.1 **View Block and Spot Properties (for GAL arrays)**

- To view **block properties**:
  - Select the **Block** tab in the **Array File** section.
  - Hover over or click a block in the canvas.
- To view **spot properties**:
  - Select the **Spot** tab and hover over or click any spot.

2.2 **View Spot Family and Spot Properties (for PSF arrays)**

- To view **spot family properties**:

- Select the **Spot Family** tab in the **Array File** section.
- Hover over or click an SF in the canvas.

- To view **spot properties**:
  - Select the **Spot** tab and hover over or click any spot.

3 **Align the Array**

- **Enhance spot visibility** (Refer to Step 1.3.)
- **Assess the spot pattern.** Use control spots to judge whether rotation or block (SF) repositioning is needed.
- **Save the alignment**.

If signal quality is sufficient, you can attempt automatic alignment. Otherwise, follow Step 3.3 to align the array manually.

Tip: Always back up the original array file. For each image, use **Arrays > Save Array As** to create and align a copy.

3.1 **Rotate the Image**

- In the **Image Rotation** section:
  - Flip horizontally or vertically.
  - Rotate by 90°, 180°, or 270°.

3.2 **Align the Array Automatically**

- In the **Array File** section, click **Align**.

3.3 **Reposition Blocks and Spot Families**

For GAL arrays

- Open **Arrays > Block** tab.
- Select blocks individually (Ctrl + Click) or all blocks (Ctrl + A).
- Drag blocks to align the dashed circle with the signal (shown in red, green, or another non-white color; see Fig. 2 and Fig. 3).

For PSF arrays

- Open **Arrays > Spot Family**.
- Select and drag spot families (SFs) the same way as blocks.

3.4 **Save the Alignment**

- Use the **Arrays > Save Array** menu to overwrite the current file, or
- Use **Save Array As** to store it in a new GAL file.

4 **Quantify the Array Data**

- **Ensure alignment**. Confirm that the array is correctly aligned with the image.
- Open the **Data Quantification** control panel.
  - Click ***Quantify* Array**.
  - Once complete, the quantified data table is updated - each row shows intensity values for a spot.
  - To identify spots of interest, consider using intensity values such as **Red F. Mean**, **Red F. Median**, **Green F. Mean**, or **Green F. Median**.

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 image.

- Use the navigation keys (*Up, Down, Page Up, Page Down*) to browse data interactively.

4.2 **Save Quantification Results**

Export data:

- In the *Data Export* widget, click **CSV** or **GPR** to save the quantified data to a .csv or .gpr file.
- If the widget is not visible, enable it via **View > Data Export**.

Save the project:

- Go to **Project > Save Project** to store the full analysis in a Spotxel project file (*.spotxelproj).

The project file (e.g., s1.spotxelproj) keeps links to the image, aligned array, and quantified data. Reopen it via **Project > Open Project** to resume the analysis at any time.

4 Analyze many slides automatically

In high-throughput studies, you may need to screen a microarray with **N samples** using **N slides**. These slides share the same layout annotation - known as the **template array** - which can be either:

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

Processing generates **N grayscale TIFF images**, one for each slide. You can create and run a **batch** to automatically analyze all N images and extract their quantified data.

Tip: Ensure each image has sufficient spot signal for automatic array alignment.

1 **Create and Execute the Batch**

- Click the **Batch** button on the main toolbar.
- In the **Batch Scheduler** control panel, specify the images, the template array, and processing options.
- Click the **Run** button to execute the batch.

1.1 **Specify Parameters and Options**

- Click **Add** and select the images to process.
- In ***Template Array (applied to all images)***
  - Click **Browse** and select the .gal or .psf file.
- In **Where should generated files be stored?**
  - use the default (image's folder), or
  - Click **Browse** to choose a custom location.
- If rotation is needed:
  - Click **Rotate Images** and choose Flip and/or Rotate (90°, 180°, or 270°).
- To save the batch setup:
  - Go to **Batch > Save Batch**.
  - A log file will be created automatically with the same name.

Tip: Use a separate folder for each batch to keep files organized.

1.2 **Execute the Batch**

- Click **Run** to begin processing.
- The status of each image will update live as it is processed.

For an input file like sample001.tif, the following output files are generated:

- sample001.gal or sample001.psf: array file linking layout to signal in the image
- sample001.csv: quantified data in CSV format
- sample001.gpr: quantified data in GenePix Result format
- sample001.spotxelproj: project file containing full analysis results

Running the batch for **N images** will generate **N** .spotxelproj files for downstream analysis.