

## Data Extraction Tips

NOTE: Using the following guidelines, along with scanner settings that reduce the background as much as possible, you should get very good results (inter-assay and intra-assay CV <15%).

- Scan using Cy5-compatible (red; 635 nm) laser only.
- Most gene microarray laser scanners are compatible with GAL file formats, which define a grid matching the array map.
  - You may request a GAL file from Parallelex Bioassays at no charge.
  - For custom 24-wells design, use the following parameters:

	<b>Blocks</b>	<b>Spots</b>
Number of columns:	3	9
Number of rows:	8	12
Columns spacing (in $\mu\text{m}$ ):	7000	450
Rows spacing (in $\mu\text{m}$ ):	9000	450
Diameter (in $\mu\text{m}$ ):	N/A	150

- If the Excel file provided by Parallelex Bioassays is to be used for data analysis, make sure blocks, rows, and columns are identified according to the following template:

**Blocks**

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18
19	20	21
22	23	24

**Rows & Columns**

1	2	3	4	5	6	7	8	9
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

- If available, use the automatic alignment tool to find all antibody spots. Verify that all antibody spots were well recognized. In some cases, you may need to manually align circles in the super-imposed grid to match the antibody spots on the array. Any existing “comet tails” should be ignored.
- Signal values for data analysis are the median of 635 nm signal minus the median background at 635 nm (also known as F635 – B635).
  - Use MEDIAN signal values, not the total or the mean. This minimizes the influence of “comet tails” and outlier data.
  - Use local background correction (also using Median value).

- The laser power, photomultiplier tube (PMT) or other signal gain settings of the scanner may be used to increase spot signal intensities and/or to reduce background signals. Optimal settings will generate:
  - Strong Positive Control signals
  - Low and even background signals
  - A wide range of signal intensities for antibody spots
- Adjusting the brightness and contrast settings on your data extraction software can improve the quality of the scanned image. Changing these settings only affects the image as seen on your computer monitor and has no net effect on the data that can be extracted from the image.
- For any given analyte, you should only compare fluorescence data generated using the same laser power, PMT and/or signal gain settings for all sub-arrays for which you wish to compare the results. However, you may scan all slides at multiple settings to obtain optimal signal responses for each analyte.
  - For example, you may use data obtained with a higher PMT value for weaker signals and data obtained with a lower PMT for stronger signals.
- For a list of recommended scanners, please visit our Website (<http://www.parallexbio.com/>).
- For a quick and easy data analysis, you can download an Excel file for free using the link in the user manual provided with the SnapChip™.