

3607 Parkway Lane, Suite 200 Norcross, GA 30092 Tel: 770-729-2992, 1-888-494-8555 Fax: 770-206-2393 Website: www.raybiotech.com Email: info@raybiotech.com

Data Extraction Tips For Glass Chip Arrays:

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%).

- 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 RayBiotech at no charge.
- Scan using Cy3-compatible (green; 532 nm) laser only.
- Define the area for signal capture for all spots as a circle with 110-120 micron diameter, ignoring any "comet tails".
- In some cases, you may need to manually align circles in the super-imposed grid to match the antibody spots on the array.
- <u>Use MEDIAN signal values, not the total or the mean</u>. 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, where POS1>POS2>POS3
 - 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 <u>only</u> 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 (<u>http://www.raybiotech.com/resources.asp</u>).