RayBiotech
The protein array pioneer

Data Extraction Tips For Glass Chip Arrays

NOTE: By 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.
- 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, 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 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 consult this document.