Microarray Scanner Calibration Slide

User's Guide

Part Number: DS 01

FULL MOON
BioSystems

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Precautions

Handling

It is highly recommended to wear gloves when handling the slide. Handle the slide by holding the slide edges or the area with the bar-code label. Do not touch the slide surface. Do not attempt to clean or wash the slide with any chemicals. If dust particles are found on the slide surface, clean the surface by blowing a gentle stream of compressed nitrogen (industrial grade or above) with a pressure of 20psi or less. Do not use canned compressed air. Avoid exposing the slide to light. When finished with each scan, keep the slide in a container to protect it from light and dusts.

Storage

The slide is packaged in an air and moisture resistant foil bag. Once opened, the slide should be kept away from light. When not in use, please store the slide desiccated in the dark.

Disclaimer

This product is intended to be used as a reference tool only and should be not used as an absolute standard. Verification of results using other methods is strongly recommended. Full Moon BioSystems is not responsible for any claims made by users of this product.

Product Description

The Microarray Scanner Calibration Slide has been developed for users to perform quantitative evaluations of their microarray scanners. It is designed for determining the dynamic range, limit of detection, and uniformity of microarray scanners. It is also an excellent tool for detecting laser channel-to-channel cross-talk and laser stability.

This slide contains two separate blocks of arrays in dilution series of Cy™3 and Cy™5 fluorescent dyes. Each block consists of 28 sets of two-fold dilutions of Cy™3 or Cy™5, coupled with 3 sets of blanks and one set of position markers. Each column contains 12 repeats of each sample. See page 13 for Array Layout.

Specification

* Slide size: 3" x 1" x 1 mm
* Array dimensions: 2 blocks; each block contains 32 columns x 12 rows
* Array Layout: Page 13
* Spot center-to-center distance: 350 μm

Applications

* Analyze and calculate microarray scanners' dynamic range and limit of detection
* Detect and analyze performance variations among different microarray scanner units
* Detect channel-to-channel cross-talk
* Verify laser alignment and focus

General Operating Instruction

1. Carefully remove the slide from the slide box. The arrays are to be found on the same side of the slide where the bar-code label is located.
2. Load the slide on a microarray scanner's slide holder.
3. Perform a quick scan to locate the arrays on the slide.
4. Adjust appropriate settings and parameters on the scanner, and proceed to scan the arrays.
5. Using data provided in Table 1.1 (Appendix B), perform necessary analyses.
6. Follow the detailed protocols provided in this booklet for suggested applications, or use the device accordingly for applications of your interests.

Suggested Applications

A. Limit of detection

Typically, the limit of detection (LOD) is defined as the minimum detectable signals for which the signal-to-noise ratio (SNR) is 3. The sensitivity of a scanner is inversely related to the limit of detection — the detection sensitivity increases as the limit of detection decreases. Knowing the limit of detection before starting a microarray experiment allows researchers to determine the minimum sample concentration needed for the experiment, in turn maximize research efficiency.

To determine SNR, the following formula is commonly used:

$$\text{SNR} = \frac{\text{Average signal intensity - Average background intensity}}{\text{Standard deviation of background}}$$

Protocol:

1) Scan a Scanner Calibration Slide.
2) Optimize PMT, laser power, and gain settings so that saturated signals are observed for the spots in the last 4 to 6 columns on the far right side of the array, where the CyDye™ concentrations are high.
3) Scan the arrays with both red and green lasers.
4) Analyze the image and calculate the signal-to-noise ration (SNR) for each column of the arrays.
5) Identify the corresponding column for which the SNR value is equal to or larger than 3.
6) Using Table 1.1 (Appendix B), determine the number of fluorophores/um^2 for the column identified in Step 5. This number is the limit of detection for the scanner.

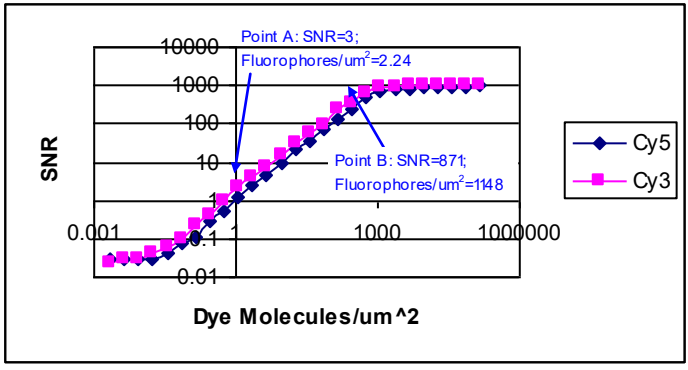
B. Dynamic Range

A scanner's dynamic range is referred to as the linear response range of a system, which helps researchers to optimize sample concentrations in a microarray experiment.

Protocol:

1) Scan a Scanner Calibration Slide.
2) Optimize PMT, laser power, and gain settings so that saturated signals are observed for the spots in the last 4 to 6 columns on the far right side of the array, where the CyDye^{TM} concentrations are high.
3) Analyze the image and calculate the signal-to-noise ratio (SNR) for each array column.
4) Plot, in logarithm scale, the SNR data against the number of fluorophores/um^2 for each column, data provided in Table 1.1 (Appendix B).
5) Using the graph, locate Point A, which has a SNR value of 3, and Point B, which is the end point of the plotted line's linear portion, i.e., the point at which the line begins to plateau. Find the corresponding number of fluorophores/ um^2 for the two points. These two numbers constitute the two end points of the linear dynamic response range of your system.

Note: An example graph plot is provided below.
The linear dynamic ranges for common biological experiments are approximately 3 to 3.5.



C. Channel cross-talk

Channel cross talk is a phenomenon where signals from one channel leak into another channel. Because cross talk phenomenon may be concealed in a dual-color image, the effect is less noticeable for users when they scan arrays hybridized with both dyes, with red and green lasers simultaneously.

Protocol:

1) Scan a Scanner Calibration Slide.
2) Optimize PMT, laser power, and gain settings.
3) Activate the red laser only. Scan both sets of arrays. Save the acquired image as Image A.
4) Turn off the red laser, and turn on the green laser. Scan both sets of arrays, and save the acquired image as Image B.
5) Analyze Image A and Image B, calculate the intensity of Cy3 and Cy5 signals from both scans, and determine the percentage of the channel cross talk.

D. Alignment and focus

When scanning arrays with both red and green channels, weak signals or blurry spots are sometimes observed. In this situation, scanner's alignment and focus may require further attention. Typically, alignment problems occur when the mechanical stages fail to correctly return to homing positions, or when the lasers' alignment is not optimized.

Protocol:

1) Scan a Scanner Calibration Slide.
2) Optimize PMT, laser power, and gain settings
3) Scan the arrays with both red and green channels.
4) Examine the acquired image. The Calibration Slide contains arrays of Cy™3 and Cy™5 fluorescent dyes in dilution series. Distinctive and well-defined round spots should be observed when laser focus and alignment are in optimal conditions.

E. PMT effect

Because the scanner's performance may vary at different PMT voltages, PMT settings are important for acquiring adequate images and obtaining reliable data. Using this Scanner Calibration slide, determine the optimal PMT settings for a set of experiments to maximize experimental output.

Protocol:

1) Set the PMT voltage at 100V.
2) Scan the Scanner Calibration Slide with both red and green channels.
3) Record and analyze the image. Calculate the signal-to-noise ratio (SNR) for each array column.
4) Increase the PMT setting by 50V, and repeat the process until the voltage setting reaches 1000V.
5) Plot a graph with SNR against the concentrations of Cy3 or Cy5 at each voltage setting.
6) Determine the dynamic range for each voltage setting.
7) Use this graph as a guide for experiment optimization.

F. Variations among different scanner units

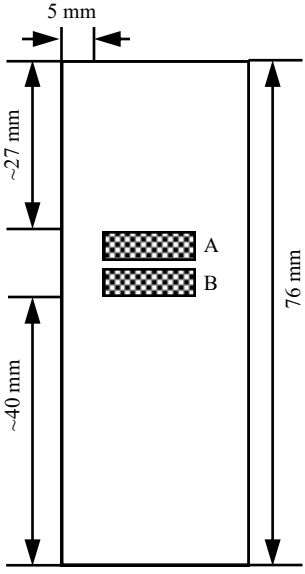
The properties and performance of a microarray scanner unit may be different from another unit. The Scanner Calibration Slide may be used to compare performance between units.

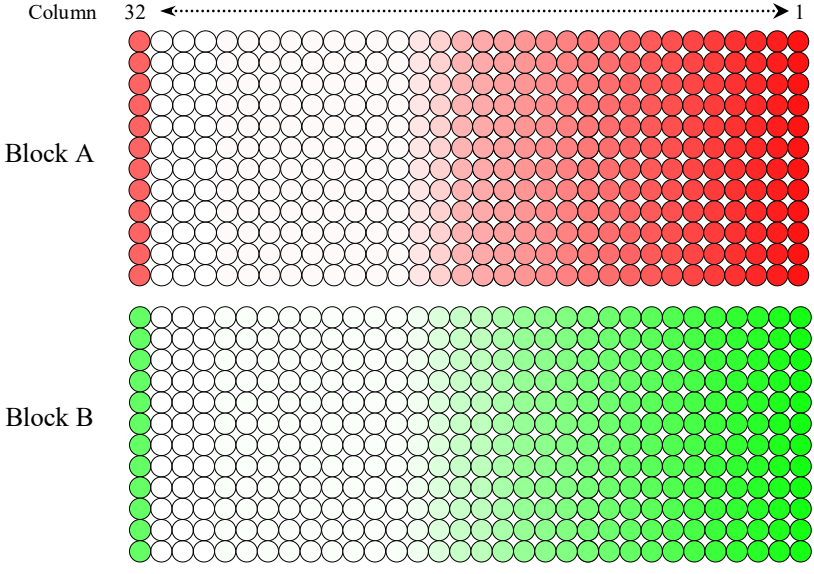
Protocol:

1) Load a Scanner Calibration Slide on a scanner designated as Unit A.
2) Adjust appropriate settings, scan the arrays, save and analyze the image.
3) On a second scanner, Unit B, scan the arrays at the same settings. Save and analyze the image.
4) Compare the two sets of images and data obtained.

Appendix A

Array Layout





Appendix B

Table 1.1 — Fluorophore Density

Series (Column No.)	Fluorophores /um^2		Series (Column No.)	Fluorophores /um^2
1	1.47E+05		17	2.24E+00
2	7.35E+04		18	1.12E+00
3	3.68E+04		19	5.61E-01
4	1.84E+04		20	2.80E-01
5	9.19E+03		21	1.40E-01
6	4.59E+03		22	7.01E-02
7	2.30E+03		23	3.50E-02
8	1.15E+03		24	1.75E-02
9	5.74E+02		25	8.76E-03
10	2.87E+02		26	4.38E-03
11	1.44E+02		27	2.19E-03
12	7.18E+01		28	Buffer
13	3.59E+01		29	0
14	1.79E+01		30	0
15	8.97E+00		31	0
16	4.49E+00		32	Position Marker

www.fullmoonbio.com

754 North Pastoria Avenue

Sunnyvale, CA 94085, U.S.A.

Tel: 408.737.2875

Fax: 408.732.7230

info@fullmoonbio.com