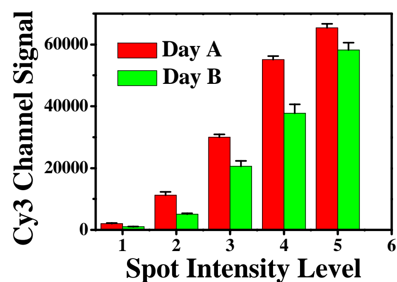

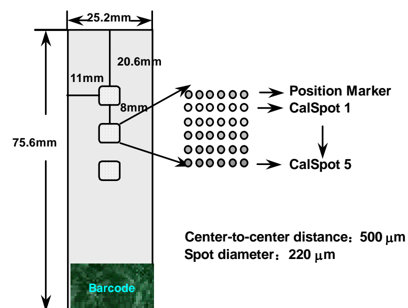

Fig.1 Comparison of daily scanning data with original reference data of the same CalSlide™-I

Fig. 2 CalSlide™-I layout

2. Data comparison between different scanners

Similar to the above application, by comparing scanning data of the same calibration slide obtained from different scanners, experimental data from different sources could be normalized and compared, which makes it possible for reliable microarray data comparison.

Protocol

1) Perform a scan of the calibration slide after each scanning experiment with specific scanning parameter settings;
2) Undertake data analysis and calculate the average signal intensity and coefficient variation of each level of the calibration spots and list as an attachment of each experimental report.

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CapitalBio CalSlide™ I

Cat. No. 410011

User Manual

For Laboratory Research Use Only

Not for Diagnostic Purposes

CapitalBio Corporation

General Introduction

CapitalBio's CalSlide™ is a novel fluorescence calibration tool based on CapitalBio's proprietary Nanobrite™ technology. It is specifically designed for daily maintenance of fluorescence microarray scanners and comparison with scanning data obtained from different scanners. Patterned spots of the Nanobrite™ inorganic phosphorescent material are arranged on the surface of a standard microscope slide, which can be detected at both Cy3 (($\lambda_{ex}/\lambda_{em}$=532 nm/570 nm) and Cy5 (($\lambda_{ex}/\lambda_{em}$=635 nm/670 nm) channels. The spots are of different fluorescence intensities to ensure its use when scanned at different scanning parameters. The unique anti-photobleaching feature of Nanobrite™ guarantees the stability of fluorescence emission intensity of the calibration spots after continuous exposure to 110 mW laser for 9 hours.

Storage and Handling

Keep the slide sealed in the original box when it is not in use. It is recommended to wear powder-free gloves and hold the slide edge or part with barcode label when handling. To remove dust from the slide surface, a low-rate nitrogen blow or air blow may be used. If properly stored, the slide should be stable for over twelve months.

Operation Instruction

1. Switch on the microarray scanner, turn on the laser and wait until ready to use.
2. Take out the calibration slide and load it onto the slide holder with the barcode slide facing up.
3. Perform a quick scan to locate the array blocks on the slide.
4. Adjust the scan area and focus distance to get the optimal fluorescence signal.
5. For the first time user, adjust laser power and PMT setting in a stepwise manner from lower to higher to perform scans. If laser power and PMT setting are continuously adjustable, frequently used settings should be selected. Scan files are stored in .tif format.
   For daily maintenance, appropriate scanning parameters should be selected to perform the scans.

Data Analysis

1. Scanning images are first transferred into data files;
2. Analyze the features using median values of the signals to calculate fluorescence signals. Then calculate the average signal intensity of each level of calibration spot (except for the first row in each block) within blocks, and then compare these data to get the average signal intensity and coefficient variation of each level of calibration spot among the three blocks;
3. For the first time user, results obtained at different laser power and PMT settings are collected in a table as an original reference; for daily maintenance, results are compared with the original reference data obtained at the same scanning parameters.

Applications

1. Performance evaluation of individual scanners

As the laser emission from the source of each microarray scanner inevitably fluctuates or even declines during operation, and the confocal plane may also shift due to optical system malfunction, scanning accuracy should be checked constantly to assure best performance. By comparing scanning data of the calibration slide collected at different times and by normalization of experimental scanning data, any instrument instability can be compensated.

Protocol

1) For semi-quantitative microarray analyses, performing a scan of the calibration slide and data comparison with the original reference data are highly recommended, just before performing new scanning experiments.
2) If the average signal intensity of the calibration spots is 20% higher or lower than the correspondent original reference data, experimental data should be normalized accordingly (Fig 1).