A Short Technical Note on MicroArray Printing

There are many important factors that contribute to the production of high quality microarrays. Selections such as the type of slide, the number of pre-print blots, and the overtravel and dwell times are just a few of the parameters that need to be considered prior to the start of microarray production. This technical note offers a summary of several key factors that, when followed properly, will ensure that high quality microarrays are generated. All experiments were conducted using the SpotArray™ 24 and the SpotArray 72 from PerkinElmer Life Sciences, Inc. with four Stealth pins from TeleChem® and a dye plus DNA solution with autofluorescent characteristics unless otherwise specified. Image acquisition and quantification were carried out using ScanArray® Express and QuantArray® 3.0 microanalysis software (both from PerkinElmer).

The areas of microarray printing covered in this technical note are:

- Spot Uniformity
- Accounting for variations in pins and substrates
- Carryover

**Spot Uniformity — The Use of Blotting Slides**

Solid or quill type pins often take up a slight excess of spotting buffer due to the capillary action that helps the buffer load onto the pins. This results in the initial spots being a larger than normal diameter. A small number of pre-prints or blotting will yield uniformly sized spots on each microarray while still retaining enough spotting solution to fulfill the printing needs of the spotter. Plain glass slides are not recommended as blotting slides because inadequate surface tension on the slides causes large irregular diameter spots which could lead to sample contamination.

Experiments were designed and conducted to find the optimum number of blots for several printing buffers and to find the most appropriate type of blotting slide. The images below are from SuperChip™ slides by PerkinElmer. Similar results were found with other coated substrate types.

The following print buffers were studied:

- 1X ArrayIt™ printing buffer (Cat #: MSS-1)
- 3X SSC (saline sodium citrate, app. pH 7.0, Sigma, Cat #: S-6639)
- 1X PCR Buffer (Applied Biosystems, Cat #: N808-0155) and 50% DMSO (dimethyl sulfoxide, Sigma, Cat #: D-8418) in ddH₂O
Using the SuperChip slides or other similarly coated slides for blotting, uniform spots are obtained when either five or ten blots are produced with all print buffers except 50% DMSO. The use of 50% (v/v) DMSO requires more than 25 blots in order to obtain consistent diameter and uniformity of spots. Therefore, the lower number of blots required for uniform spots on substrates such as SuperChip or a similarly treated slide type indicates that these reagents should be used preferentially for blot slides and substrates.

**Post-Wash Carryover Elimination**

The length of the wash cycles that clean each pin during the printing of an array plays an important role in the amount of post-hybridization carryover left on each pin’s surface. Examination and quantification of post-hybridization carryover was conducted using arrays spotted with 50 ug/mL CAT (Calf Thymus) DNA labeled with complimentary labeled cDNA probe. Prior to printing, the pins were washed for four 10-second cycles followed by one 9-second drying cycle to ensure their cleanliness. The layout for the printed array was as follows:

The first row of spots consisted of 3X SSC (saline sodium citrate) only to allow for normalization of the background prior to the introduction of DNA to the pins. The next ten rows each contained twenty-four spots consisting of twelve spots of 50 ug/mL of CAT DNA followed by twelve spots of 3X SSC (i.e. potential carryover). Diagrammatically, the green spots below represent the initial spots of 3X SSC, the yellow circles are the CAT DNA and the blue circles represent the potential carryover.
The arrays were spotted onto slides that were subsequently hybridized at 65°C for 90 minutes with batch-labeled cDNA and MJ Research coverslips. Post-hybridization wash conditions consisted of: one 10-minute wash at 55°C in 2X SSC with 0.1% SDS, followed by one 10-minute wash in 2X SSC and 0.2X SSC at room temperature. Any “questionable” spots, that is, spots inexplicably lacking signal or showing excess background due to non-specific hybridization to the slide, were marked “Ignore” in QuantArray and not used to generate the averages. Post-hybridization carryover was measured by subtracting the mean signal intensity of the intended spots from the mean intensity of the carryover spots. This value was then divided by the average intensity of all DNA spots and multiplied by 100 to obtain a percentage of carryover. Following a series of optimization studies, a four-pin configuration consistently yielded measurable carryover at less than 0.01% following a cycle of one 3-second wash and one 3-second drying period. These settings should be used to insure a contamination-free printing and to optimize spot diameters.

**Overtravel Settings**

Printhead overtravel is the motion of the printhead beyond the contact point of the pins to the substrate. Overtravel is used to account for variations in the pins, substrate or the instrument to ensure contact of the pin with the substrate. Because the pins sit freely in the printhead, overtravel actually only results in the pins contacting the slide for a slightly longer period of time and will not change the pins ability to print millions of spots in its lifetime. One consideration when selecting the overtravel setting is entering the correct slide thickness in the printing protocol. Most manufacturers will list the specification of slide thickness on their product information sheets; however, if you are unsure of a slide’s thickness, measure it with calipers or contact the manufacturer. The following images were printed on PerkinElmer SuperChip slides with the correct slide thickness of 1 mm.

![Images of spotted slides with different overtravel settings](image)

Although there was a slight increase in the CV value for spot uniformity as overtravel decreased, all other measures remained more or less constant. An overtravel setting of 200 µm will ensure that all pins deposit sample evenly onto the substrates.

**Additional Considerations**

TeleChem, the maker of the SMP3 Stealth pins used on this instrument, has several recommendations for printing settings. A printhead speed of 15 mm/sec is recommended for approach and departure settings in contacting the substrate. These settings will ensure uniform distribution of spots. A sample load dwell time of 2,000 msec will ensure that the pins pick up the most viscous sample, such as a glycerol stock, while a shorter sample load time of 200 msec will be adequate for salt-based spotting buffers.
Due to the greater depth of wells and thin walls of PCR plates, samples should be transferred from PCR plates to appropriate polypropylene plates for printing. The SpotArray software currently supports over 30 plate types from various manufacturers. A minimum sample volume of 20–30 µL is recommended for V-bottom 96-well plates and 10 µL is recommended for 384-well plates. Lesser volumes can be used for each plate type; however, sample volumes of less than half of the recommended volume sample may not be adequate for printing the usual complement of spots.

Although some optimization by the user for various plate volume and buffer types may be necessary, the SMP3 pins should be able to produce over 120 duplicate spots with one sample loading.

Summary

Printing high quality microarrays with the SpotArray product line is a very easy task when the proper settings are used. For most print buffers, when using SuperAmine or similarly coated slides, five to ten blots should be made to assure production of uniform arrays. In addition, a 200 µm printing overtravel will ensure that variations in substrate surface and/or printhead irregularities will be accounted for. With the settings for these variables in mind, the SpotArray 24 and the SpotArray 72 require a minimum of optimization.