SpotBot® 2 SpoCLE Generator Software for Printing Multiple Microarrays on a Single Glass Microarray Substrate Slide

SpotBot[®] 2 Personal Microarrayers can be used to print multiple microarrays per substrate slide using the Multiple Microarray Format SpoCLe (SpotBot Control Language) software option. Multiple microarrays are fully supported by Arrayit hardware including catalog numbers MMH4X24, MMH4X16, AHC1X24 and AHC1X16 Hybridization Chambers and Cassettes. This software option can also be used to print on substrates and slides different from the standard 25 x 76 mm format.

Software Instructions

Follow these screen shots to assist in using the SpotBot[®] 2 Personal Microarrayer Multiple Microarray Format SpoCLe (SpotBot Control Language).

| III Multiple Microarray Format SpoCLe Generator 1.1.03 | |
|--|------|
| Start Pins | |
| Profile for initial settings | |
| Factory Default profile | |
| Start with the factory defalt settings | |
| O User Profile | |
| Start with the settings from a previously saved user profile | |
| Open profile | |
| Help About Change to Basic Exit Back M | lext |

Screen shot 1. Select the factory default to start a new program, or select an existing SpoCLe file to edit. Click Next.

| III Multiple Microarray I | ormat SpoCLe Generator 1 | .1.03 | |
|-----------------------------------|--|--|------|
| Start Pins | | | |
| Micro Spotting Pin Pin Type | Setup | | |
| ○ SMP 946 Pin Configuration | 946MP2 946NS2.5 946MP3 946MP3B 946NS3 946MP4B 946NS4 946MP5 | Pin Configuration legend IX1 Front of the SpotBot | |
| Help Abou | t Change to Basic | Exit Back | Next |

Screen shot 2. Select the pin type form the drop down menu. The software will automatically provide a suggested center-to-center spot spacing later in the software. This software interface uses a single pin in position 1 in the printhead as shown in the graphic. Multiple microarrays are printed at 9 mm spacing on the substrate slides for every printing routine.



Screen shot 3. Select "Full Microplate" to load every sample from the 384-well source micrplate. By selecting the "Partial Microplate" option, it is possible to select a subset of wells from the 384-well microplate using the computer mouse as shown above by the green and red shading. The last last printed sample is shown in red. Enter the number of "Spots per sample" into the dialog box to print 1-5 replicates in succession for each sample. The pin may be reloaded by checking "Sample Reloading" after a user selected number of printed spots (200 is shown here). Pin washing and drying is optional by clicking the box "Wash/Dry Pins between reloading".

| Hultiple Microarray Format SpoCLe Generator 1.1. | 03 |
|--|----------------|
| Start Pins Plates Microarray: | |
| Settings for microarray printing | |
| Well Pattern 🛛 🛛 🔽 | |
| Spot Spacing 4 8 12 | |
| Print Offset from the Well corner 24 | |
| Lateral Vertical 0.6 2 mm | |
| Subgrid Dimensions | |
| Columns Rows | |
| 36 🗸 X 24 🔽 | |
| Help About Change to Basic | Exit Back Next |

Screen shot 4A. The user can select the number of microarrays per substrate slide using the "Well Pattern" drop down menu (24 is shown here). Lateral and Vertical Print Offsets are then selected to specify the starting point within each 9 x 9 mm region (0.6 mm Lateral and 2 mm Vertical is shown here). The number of Columns and Rows are also specified (36 Columns and 24 Rows) are shown here. Click Next.

| III Multiple Microarray For | mat SpoCLe Generator 1.1.03 | |
|-----------------------------|-----------------------------|------|
| Start Pins | Plates Microarray: | |
| Settings for microar | ray printing | |
| Well Pattern | | |
| Spot Spacing | 150 um | |
| Print Offset from the We | ell corner | |
| La | iteral Vertical | |
| 0. | .6 2 mm | |
| Subgrid Dimensions | | |
| Co | olumns Rows | |
| 36 | 6 💌 X 24 💌 | |
| | | |
| | | |
| Help About | Change to Basic Exit Back | Next |
| | | |

Screen shot 4B. A total of four microarrays per substrate slide is specified in this graphic.

| III Multiple Microarray | Format SpoCLe Generator 1.1.03 | |
|-------------------------|--------------------------------|------|
| Start Pins | Plates Microarray: | |
| Settings for micro | parray printing | |
| Well Pattern | | |
| Spot Spacing | 150 um | |
| Print Offset from the | Well corner | |
| | Lateral Vertical | |
| | 0.6 2 mm | |
| Subgrid Dimensions | | |
| | Columns Rows | |
| | 36 🖌 X 24 🖌 | |
| | | |
| | | |
| Help Abou | ut Change to Basic Exit Back | Next |

Screen shot 4C. A total of eight microarrays per substrate slide is specified in this graphic.

| III Multiple Microarray Format SpoC | Le Generator 1.1.03 | |
|-------------------------------------|---------------------|-----------|
| Start Pins Plates | Microarray: | |
| Settings for microarray print | ing | |
| Well Pattern | | |
| Spot Spacing | 150 um | |
| Print Offset from the Well corner | | |
| Lateral | Vertical | |
| 0.6 | 2 mm | |
| Subgrid Dimensions | Powe | |
| 36 🗸 > | < 24 ♥ | |
| | | |
| | | |
| Help About Ch | ange to Basic Exit | Back Next |
| | | |

Screen shot 4D. A total of twelve microarrays per substrate slide is specified in this graphic.

| III Multiple Microarray Format SpoCL | e Generator 1.1.03 | | |
|--------------------------------------|--------------------|------|------|
| Start Pins Plates | Microarray: | | |
| Settings for microarray printin | ig | | |
| Well Pattern 🚺 | · · | | |
| Spot Spacing | 150 um | | |
| Print Offset from the Well corner | | | |
| Lateral | Vertical | | |
| 0.6 | 2 mm | | |
| Subgrid Dimensions Columns | Rows | | |
| 36 💌 X | 24 🗸 | | |
| | | | |
| | | | |
| Help About Cha | nge to Basic Exit | Back | Next |
| | | | |

Screen shot 4E. A total of sixteen microarrays per substrate slide is being specified in this graphic.

| Hultiple Microarray Format SpoCLe Generator 1.1.03 | |
|--|------|
| Start Pins Plates Microarrays Substrates Settings for Substrates Preprint spots per sample Unused Unused Unused Unused Unused Unused | |
| Help About Change to Basic Exit Back | Next |

Screen shot 5. The SpotBot[®] 2 accommodates 14 standard 25 x 76 mm substrate slides. Each of the 14 positions can be specified a for pre-printing, printing, or non use (Unused). Pre-printing is used to remove excess sample from the pin prior to printing. For each 384-well the sample volume per well should be ideally 5 µl and should never exceed 10 µl. See Cat. MMP384 for more information about Arrayit brand 384-well microplates.

| 🎛 Multiple Microarray Format | SpoCLe Generator 1.1 | .03 | | |
|------------------------------|----------------------|----------------|------|------|
| Start Pins Pla | tes Microarray: S | ubstrates Moti | on | |
| Settings for motion cont | rol | | | |
| Sample loading | 3.0 | | | |
| Pre-printing | 0.0 | | | |
| Printing | 0.0 | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| Help About | Change to Basic | Exit | Back | Next |

Screen shot 6. Use these dialog boxes to specify the Dwell Times (amount of time the pin is at rest at any given location) for the Sample loading (3.0 sec is the default), Preprinting (0 sec is the default), and Printing (0 sec is the default). The "Sample loading" dwell time refers to the length of time the pins rest on the bottom of the 384-well microplate. The "Pre-printing" and "Printing" dwell times denote the length of time the pins "pause" on the printing substrates between the downstroke and the upstroke. The default dwell times produce efficient sample loading and printing in most cases. Viscous samples may require a longer dwell time for efficient loading. A 50 msec (0.05 sec) dwell time for "Printing" can be used to produce larger spots or to increase printing efficiency on highly hydrophobic substrates.

| III Multiple Microarray Format Spo | CLe Generator 1 | .1.03 | |
|--|-----------------|-------------------------------|---------|
| Start Pins Plates | Microarrays | Substrates Motion Was | h / Dry |
| Settings for printing pin Cle During printing | ansing routines | End of run printing | |
| Number of wash dry cycles | 4 | Number of wash dry cycles | 10 |
| Wash duration | 0.5 | Wash duration | 0.5 |
| Dry duration | 0.5 | Dry duration | 0.5 |
| Different duration for last | cycle | V Different duration for last | cycle |
| Last cycle wash duration | 2.0 | Last cycle wash duration | 2.0 |
| Last cycle dry duration | 10.0 | Last cycle dry duration | 10.0 |
| L | | | |
| | | | |
| Help About | Change to Basic | Exit Back | Next |

Screen shot 7. This dialog box shows the default settings for the washing and drying routine. A minimum of 4 Wash/dry cycles is recommended to remove samples from the previous printing cycle. Using fewer than 4 Wash/Dry cycles may result in sample "carry over" into the subsequent printing cycle. For most applications, an extended Wash/Dry cycle (2.0 sec and 10.0 sec) is recommended for the final Wash/Dry step to ensure that residual sample has been removed and that the pins are dry for the subsequent sample-loading step. The parameters governing the final Wash/Dry cycle are specified by checking the "Different Durations for Last Cycle" dialog box. The first 3 Wash/Dry cycles are kept brief (0.5 sec) to prevent sample drying on the pins.

| III Multiple Microarray Format SpoCLe Generator 1.1.03 |
|--|
| Start Pins Plates Microarray: Substrates Motion Wash / Dry Finish |
| Settings for SPO file generation |
| SpoCLe Microarray printing file path ktop/www.spocle_councils.po Save as |
| SpoCLe Title |
| (Enter a Title to be dispalyed in the SpotApp) |
| The following files are saved in the same folder as the SpoCLe file, Using a modified name ending with the indicated suffix Create Content map file (*_map.txt) Save User Profile (*.sgp) |
| Help About Change to Basic Exit Back Finish |

Screen shot 8. Use these dialog boxes to save the SPOCLE file onto the computer hard drive. The default name of SPOCLE files created by the SPOCLE Generator includes a date and time stamp. To change the file name and/or path of the file, click on the "save as" to the "SPOCLE Microarray Printing File Path" drop-down menu, and enter the user-specified information. To generate a content map file, check the Content Map" box. The content map file will be saved in the same folder as the "SPOCLE Microarray Printing File Path", and will provide the location of each sample printed on the substrates. This file can also be used to generate a GAL file using the SpotBot GAL file creator program found at <u>www.spotsupport.com</u>. Content maps are comma-delimited text files that can be imported into spreadsheets and database programs. SPOCLE files (*.spo) that are stored on the computer hard drive can be opened and run with the SpotApp program.