#### SpotBot® 2 SPOCLE Generator Software to Create Printing Routines for SpotBot® 2 Desktop Personal Microarray Printers

SPOCLE Generator	? ×
Start	
Profile for the initial settings	
<ul> <li>Factory Default Profile</li> <li>Start with the factory default settings.</li> </ul>	
User Profile Start with the settings from a previously saved user profile.	
Profile Path:	
Help About Change To Basic Mode Exit <	Rack <u>N</u> ext>

Step 1. This is the first dialog window of the SPOCLE Generator. SPOCLE stands for SpotBot<sup>®</sup> Control Language and the SPOCLE Generator allows the user to design custom software programs. Click on "Factory Default Profile" to start a new program. Click "User Profile" and select file path to edit an existing SPOCLE SpotBot<sup>®</sup> printing routine.

As you move through the next set of windows make the desired selections to create a file called "Test Run".

Pins		
alth pin setup Pin Type:	SMP3	
Pin Configuration:	2x2 _	Pin Configuration Legend
		Front of SpotBot

Step 2. This is the second dialog window of the SPOCLE Generator. Use these drop down menus to choose the Stealth Micro Spotting Pin (SMP) Type and the Pin Configuration (e.g. 2 x 2) you plan to use. Use the "Pin Configuration Legend" to assist in defining the desired pin set. The SPOCLE Generator will suggest the user automatically in selecting a center-to-center spacing and a microarray configuration based on the Pin Type and Pin Configuration chosen. Spot spacing and microarray configuration are shown in the "Microarrays" dialog window.

1 (*1	ficroarrays				
ttings for source plate	s				
C Full Microplates	Partial Last Microplate	— C Impo	ort From Sample Pic	k File	
Total Microplate Count:	1	A1	Last Microp	late:	A24
Last Sampled Well:	F12				
					Cursor F3
Total number of samples:	120	P1 HH			⊞ <sub>P24</sub>
Spots Per Sample:					
Total number of spots:	120				

Step 3. This is the third dialog window of the SPOCLE Generator. Use these dialog boxes to select the number of 384-well microplates and the number of printing replicates. Enter the number of 384-well microplates in the "Total Microplate Count" dialog box. The software can accommodate as many as 50 full plates, and partial plates can be used by selecting "Partial Last Microplate". The configuration of partial plates is designated using the computer mouse to denote the wells that contain samples, as shown by the yellow and red shading under "Last Microplate". The last set of samples and last printed sample are denoted in dark red and bright red, respectively. Enter the number of spot replicates desired into the dialog box "Spots Per Sample" from 1-5. Selecting 3 Spots Per Sample, for example, would print each sample in triplicate at the spot spacing defined by the user (see next page). Select "Import From Sample Pick File" to import your plate set list as a comma-delimited (.txt) file. An example of the delimited format required is provided at C:\Program Files\Arraylt\ SpotBot® in the file "SamplePickList\_Example.txt".

POCLE Generator			342. Semanan er en merer en anne
t Pins Plates	Microarrays		
ttings for microarray (	printing		
o	110	Printing Subs	trate Usage:
Spot Spacing:	140 um	Locations 1 to 7	Locations 8 to 14
	Columns Rows		
Subgrid Dimensions:	30 💌 × 30 💌		
	Lateral Vertical		
Print Offset:	3.0 x 5.0 mm		
		50400 Maximum numbe	14400 r of printable spots
		Meximum numbe	r or printable spots
1 AL .		r.) [	
Help About	Change To Basic Mode	E <u>x</u> it	< <u>B</u> ack <u>N</u> ext

Step 4. This is the fourth dialog window of the SPOCLE Generator. Use this dialog window to define the spacing, configuration and location of the spots on the printing substrates (slides). Specify the center-to-center spacing (in microns, µm) in the "Spot Spacing" dialog box. Specify the number of Columns and Rows in the "Subgrid Dimensions" drop-down menus. Specify the location of the first printed spot on the substrate using the "Print Offset" dialog boxes. Lateral and Vertical offset values of 3.0 mm and 5.0 mm, respectively, will place the first printed spot 3.0 mm from the left edge and 5.0 mm from the top edge of the substrate as shown in the "Printing Substrate Usage" icons. The "Printing Substrate Usage" icons also show the number and location of multiple subgrids. The SpotBot® will print onto the entire substrate of the first column of substrates (1-7) and on the upper portion of the second column of substrates (1-8). The travel distance of the x and y-axes allow printing onto 14 substrates, while maintaining a small robot footprint and high positional accuracy.

Spacing between subarrays can be controlled in this window by changing the Spot Spacing and Subgrid Dimensions parameters. The default subarray spacing is a minimum of the Spot Spacing parameter and a maximum of 2 times the Spot Spacing. The subarray maximum width is 4.5mm and the subarray spacing is adjacent to the subarray area. For example: If the spot spacing is set at 300um, 15 spots will be printed

across the subarray and the subarray spacing will be 300um, the minimum possible value (15 x 300 = 4500, only 300um remains for spacing). If the spot spacing is set at 301um, 14 spots will be printed across the subarray and the subarray spacing will be 586um (301 x 14 = 4214, 286um + 300um remains). Changing the Lateral and Vertical Print Offset will determine the number of microarrays that will fit on the substrate.

iubstri 7	ate Allocation:	14	8	Maximum printable pre-print spots:	14400
EEE	print	BEB EBE	print	Pre-print Spots Per Sample:	20
6-86	print		print	Pre-print Substrate Replacement	
5	print	12	print	Total number of pre-print spots: The pre-print substrates will not have to b	2 <b>400</b>
	print		print	replaced during the print run.	
3-86	print	10	unused		
2	print	9	unused		
1	print	8	preprint		

Step 5. This is the fifth dialog window of the SPOCLE Generator. Use this dialog window to select the number of printing substrates (1-14), the number of pre-printing substrates (0-14), and number pre-prints. All 14 substrates can be designated "print", "preprint", or "unused" under "Settings for substrates". The number of pre-print spots is specified in the "Pre-print Spots Per Sample" dialog box. A normal print run specifies the first 2 substrates (1 and 2) for pre-printing, the remaining 12 substrates (3-14) for printing, and 20 pre-prints. Pre-printing allows the pins to "prime", during which time sample adhering to the outside of the pins is removed to allow consistent sample delivery volume. Not all samples require pre-printing and users can specify "0" pre-prints as long as all 14 substrates are specified as either "print" or "unused" on the "Substrate Allocation" platen map.

1 1 1	roarrays Substrates Motion	
tings for motion control		
vell Times	20	
Sample Loading:	3.0 \$	
Pre-printing:	0.0 \$	
<sup>p</sup> rinting:	S s	

Step 6. This is the sixth dialog window of the SPOCLE Generator. Use these dialog boxes to adjust the pin resting or "dwell" time during sample loading, pre-printing and printing. The dwell time is corresponds to the length of time the pins rest on a particular surface measured in seconds. Specify each value in the "Dwell Times" dialog boxes for "Sample Loading", "Pre-printing" and "Printing". 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 down and upstroke. The factory defaults are 3.0 sec, 0 sec and 0 sec, respectively for the three settings. 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.

uring Printing		-	End Of Print Run	Leo.	
Number Of Wash/Dry Cycles:	<b>]</b> 4		Number Of Wash/Dry Cycles	10	
Wash Duration:	0.5	s	Wash Duration:	0.5	\$
Dry Duration:	0.5	s	Dry Duration:	0.5	s
Different Durations For Last Cycl	le		Different Durations For Last Cyc	;le	
Last Cycle Wash Duration:	2.0	s	Last Cycle Wash Duration:	2.0	s
Last Cycle Dry Duration:	10.0	s	Last Cycle Dry Duration:	10.0	s

Step 7. This is the seventh dialog window of the SPOCLE Generator. Use these dialog boxes to adjustment the wash and dry procedures during printing and at the end of a print run. The factory-recommended settings are shown in the "Settings for printing pin cleansing routines" dialog boxes above. 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.

POCLE Generator	? ×
Start     Pins     Plates     Microarrays     Substrates     Motion     Wash/dry     Options       Options for the SPOCLE microarray printing file       SPOCLE Title:	s
Type any application notes or other information here	
Help About Change To Basic Mode E <u>xit</u>	< <u>B</u> ack <u>N</u> ext>

Step 8. This is the eighth dialog window of the SPOCLE Generator. Use the "SPOCLE Title" dialog box to record any application or program notes.

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art Pins ettings for	Plates Mi	croarrays n of the S	Substrates POCLE micr	Motion oarray prii	Wash/dry nting file	Options	Finish	
-	ficroarray Printing m Files\Arraylt\Sp		CLE_2001Y12	2 0				
The followi	ng files are saved	d in the sam			le, using a moo	lified		
	ser Profile (*.sgp)							
<b>⊽</b> Co	ontent Map (*_ma	ap.txt)						
<b>I</b> Automa	atically Execute							
		4.02						

Step 9. This is the ninth dialog window of the SPOCLE Generator. 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 square box next 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. Content maps are comma-delimited text files that can be imported into spreadsheets and database programs. 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>. Check the "Automatically Execute" box to specify the SpotApp program to open and run a given SPOCLE file automatically. The SpotApp program must be closed and the SpotBot® robot must be connected to the proper power source to execute a SPOCLE file automatically. SPOCLE files (\*.spo) that are stored on the computer hard drive can be opened and run with the SpotApp program using the SpotApp graphical user interface (GUI).