

#### SpotBot® 2 Biacore Surface Plasmon Resonance Microarray Printing Software for SpotBot® 2 Personal Microarrayer Desktop Microarray Manufacturing

This program is designed specifically to print microarrays for the Biacore FlexChip SPR system.

III Biacore Edition SPOCLE Generator 1.1.02	
Start Pins Plates	
Profile for initial settings	
<ul> <li>Factory Default profile</li> </ul>	
Start with the factory defalt settings	
🔿 User Profile	
Start with the settings from a previously saved user profile	
Profile path	
Open profile	
Help About Change to Basic Exit Back Ne	xt

Screen Shot 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.



Biacore Edition SPOCLE Generator 1.1.02	
Start Pins Plates	
Micro Spotting Pin Setup	
○ SMP       946MP2       ✓         ● 946       946MP3       ✓         946NS3       946MS4       946NS4         946NS4       946NS5       946NS5         946NS5       946NS6       ✓	Pin Configuration legend IX1 Front of the SpotBot
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Screen Shot 2. Select a Micro Spotting Pin type from the drop down menu. The Biacore Edition software prints microarrays with only 1 pin, to use more than one Pin, use other SPOCLE generating programs available. See <u>www.spotsupport.com</u>. Selecting a Pin will provide a suggested center-to-center spot spacing later in the software.

III Biacore Edition SPOCLE Generator 1.1.02	
Start Pins Plates Microarray:	
Source Plate Settings	
<ul> <li>Full Microplate</li> <li>Partial Microplate</li> <li>Import from sample pi</li> </ul>	ck file
A1	A24
	Lursor
	B3
C:\Program Files\Array.	
Load File Save file	
P1	P24
Spots per sample 3 Total number of spots per cell -72	
Sample Reloading Reload pins after 100 Printed Spots	
Wash/Dry Pins between reloading	
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Screen Shot 3. The software can accommodate up to 1 full 384 well plate. By selecting "Partial Microplate". The configuration of partial plates is designated using the computer mouse to denote the wells that contain samples. It is also possible to create custom sample pick files (sample shown above), to "cherry pick" samples from each well in any order. Define this pattern and order of pickup by clicking on wells. The selected spots are printed according to their normal order (as they are printed on full microplate option). Enter the number of spot replicates for each well desired into the dialog box "Spots Per Sample". Selecting 3 Spots Per Sample, for example, would print each sample in triplicate at the spot spacing defined by the user (see next screen). The Micro Spotting Pin can be reloaded after a user-defined amount of spots with one load. Washing and drying micro spotting pins is optional by clicking the box Wash/Dry Pin between reloading.



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Screen Shot 4. Select spot spacing, lateral and vertical start and stop position of the microarrays in each FlexChip micro-fluidic cell and define the numbers of columns and rows for the microarray. Each microarray on each Biacore FlexChip will be identical; up to 14 chips can be printed. This version of software does not allow printing outside the fluidic cell, use other SPOCLE generators if this function is needed.



=	Biacore	Edition	SPOCLE	Generat	or 1.1.0	2			
	Start	Pin	s	Plates	Micro	array:	Substrate	es Motion	
	Settin	gs for 9	oubstrat	es					
								Preprint spots per sample	5
		Unused	Unused	Unused	Unused	Unused	Unused		
٢	Help		About		hange to	Basic		Exit Back	Next

Screen Shot 5. The SpotBot holds 14 slides or substrates of 25 x 76 x 1mm (1" x 3"). By double clicking on a location a slide can be printed, unused for used as a pre-print slide. Pre-printing is used to remove excess sample from a Micro Spotting Pin if the volume in the source plate used to load the samples into the pin is too high. See catalog ID MMP384 on the arrayit.com website for more information.

III Biacore Edition SPOCLE Ge	nerator 1.1.02	
Start Pins P	ates Microarray: Substrates	Motion
Settings for motion cor	trol	
Sample loading	3.0	
Pre-printing	0.0	
Printing	0.0	
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Screen Shot 6. 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 pin rests 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 surfaces.

Biacore Edition SPOCLE Genera Start Pins Plates	tor 1.1.02 Microarrays	Substrates Motion Was	h / Dry	
Settings for printing pin Cle During printing	ansing routine	s 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	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	
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Screen Shot 7. The 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. For best washing and drying results, use the Megasonic Wash Station Hardware.

III Biacore Edition SPOCLE Generator 1.1.02
Start Pins Plates Microarray: Substrates Motion Wash / Dry Finish
Settings for SPO file generation
SpoCLe Microarray printing file path <pre>nerator\BESPOCLE_2006Y6M8D12h35m.spo</pre> 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. In order to generate a content map file, be sure to 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.