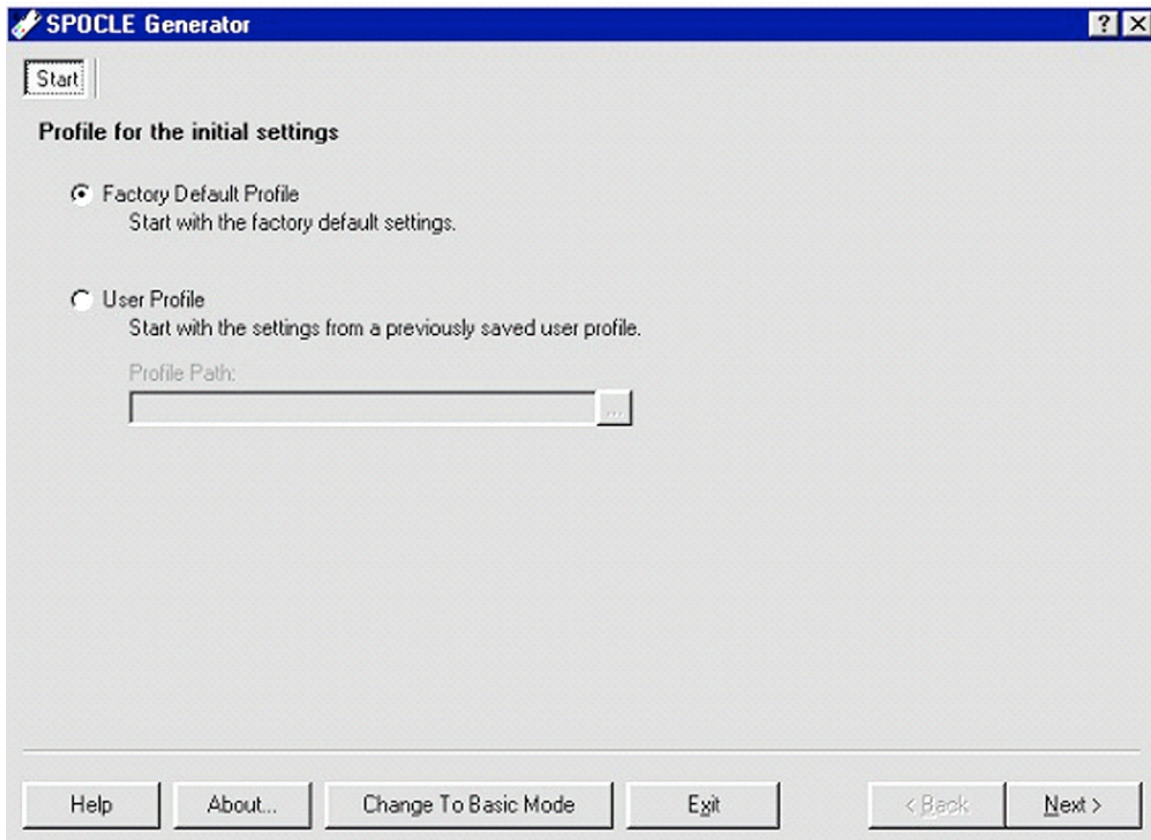# arrayit

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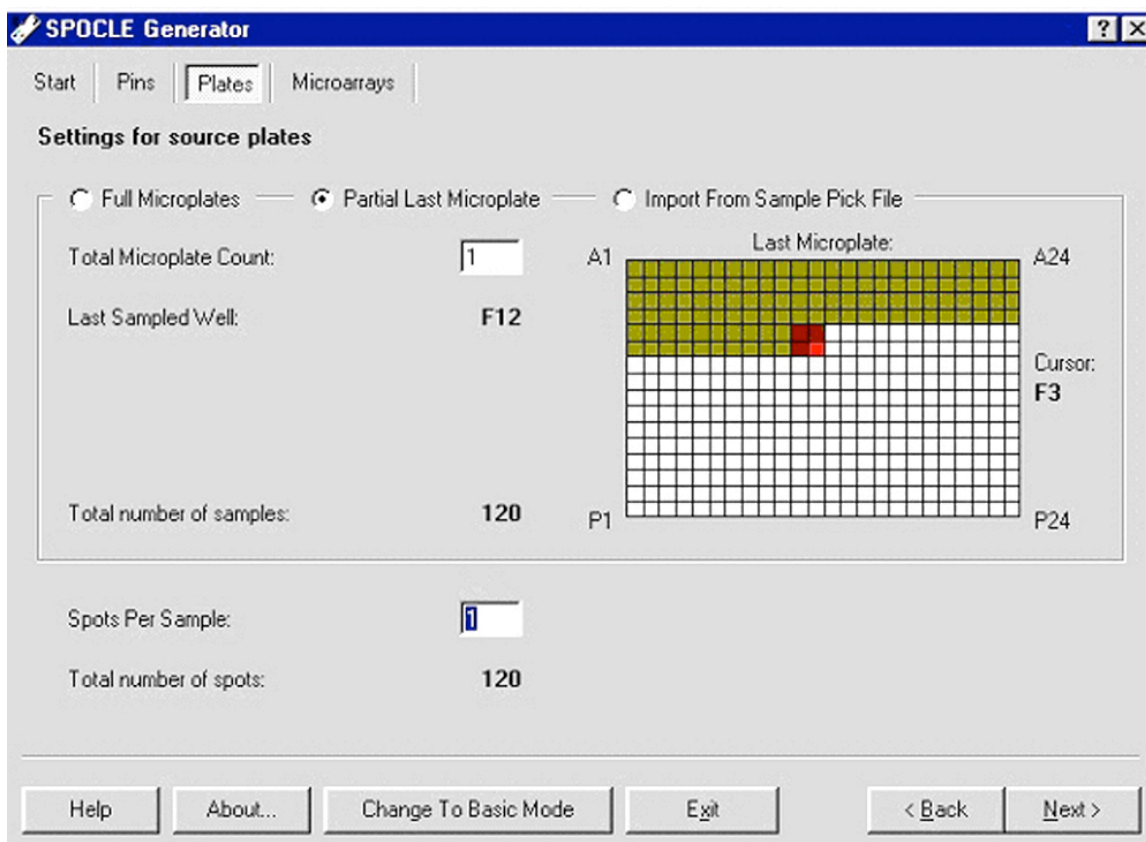

Step 1. This is the first dialog window of the SPOCLE Generator. SPOCLE stands for SpotBot® Control Language and the SPOCLE Generator allows the user to design custom software programs. Click on "Factory Default Profile" to start a new program. Click "User Profile" and select file path to edit an existing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

As you move through the next set of windows make the desired selections to create a file called "Test Run".
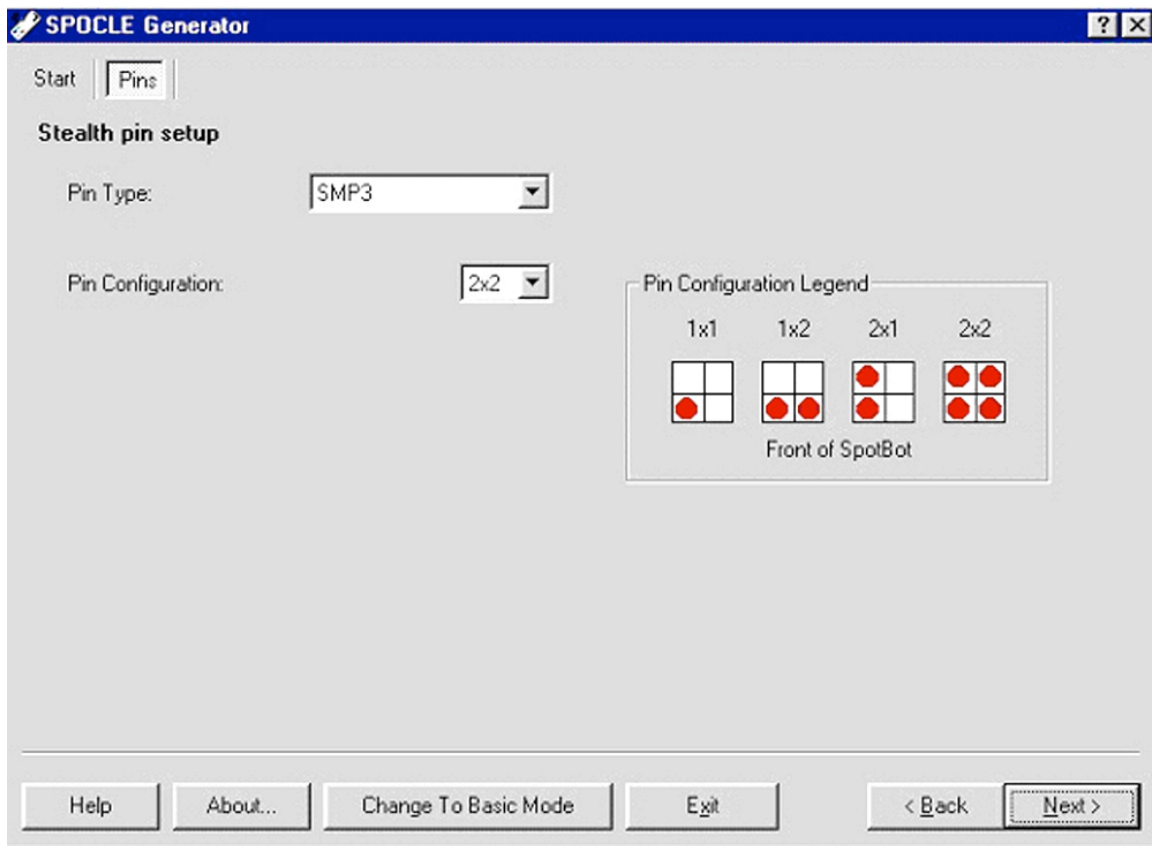
Step 2. This is the second dialog window of the SPOCLE Generator. Use these drop down menus to choose the Stealth Micro Spotting Pin (SMP) Type and the Pin Configuration (e.g. 2 x 2) you plan to use. Use the "Pin Configuration Legend" to assist in defining the desired pin set. The SPOCLE Generator will 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.
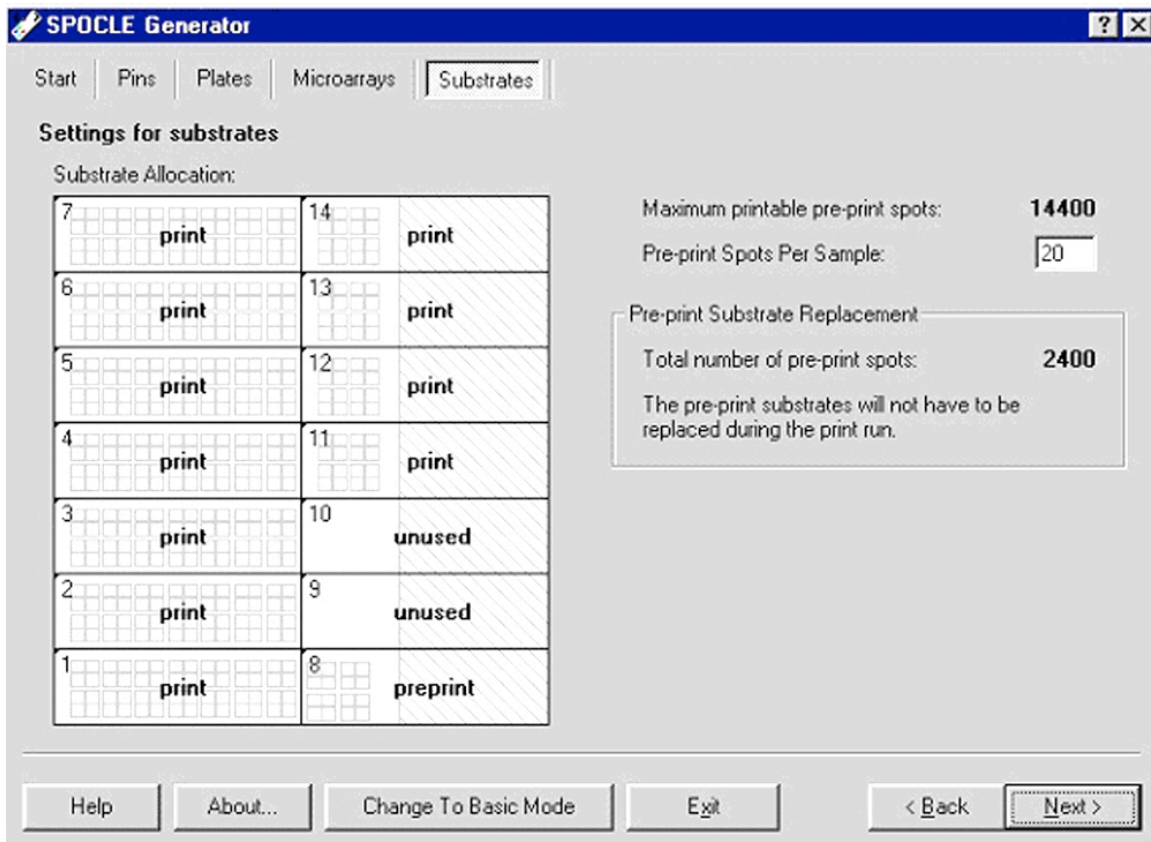
Step 3. This is the third dialog window of the SPOCLE Generator. Use these dialog boxes to select the number of 384-well microplates and the number of printing replicates. Enter the number of 384-well microplates in the "Total Microplate Count" dialog box. The software can accommodate as many as 50 full 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\ArrayIt\ SpotBot® in the file "SamplePickList_Example.txt".
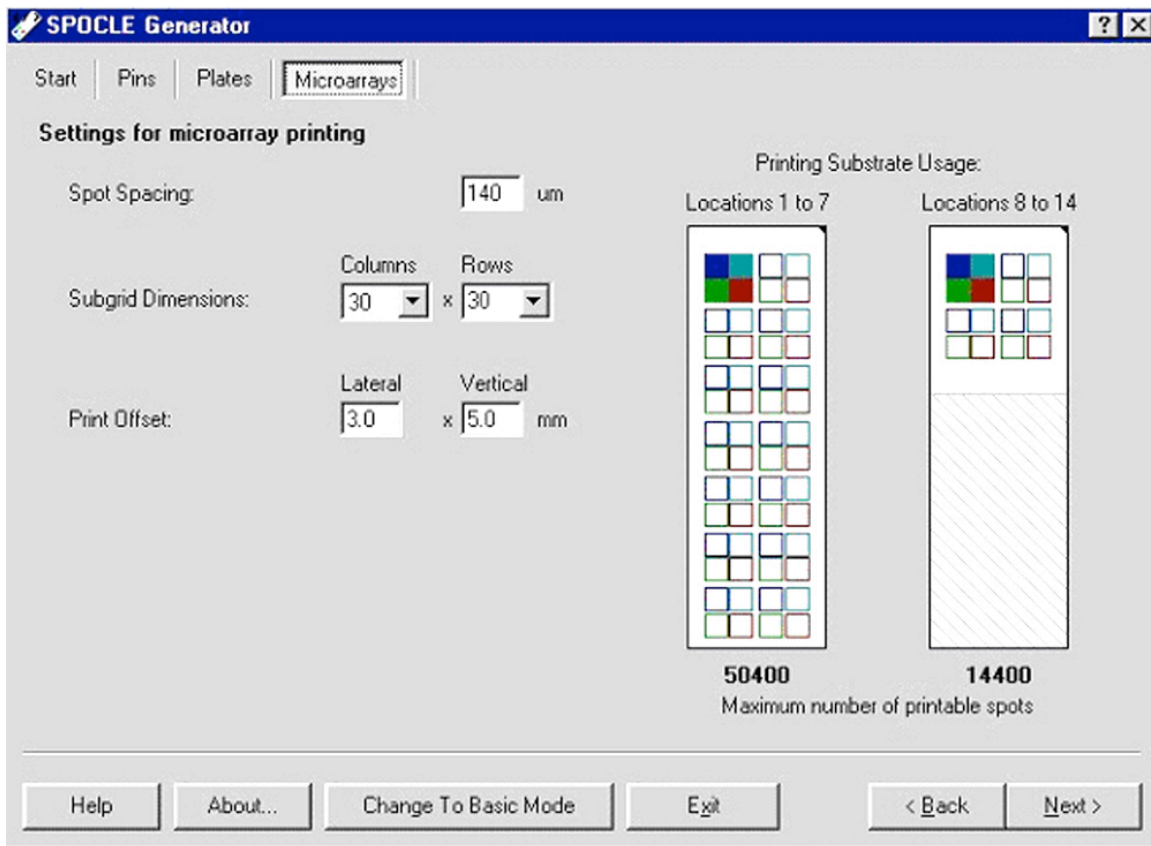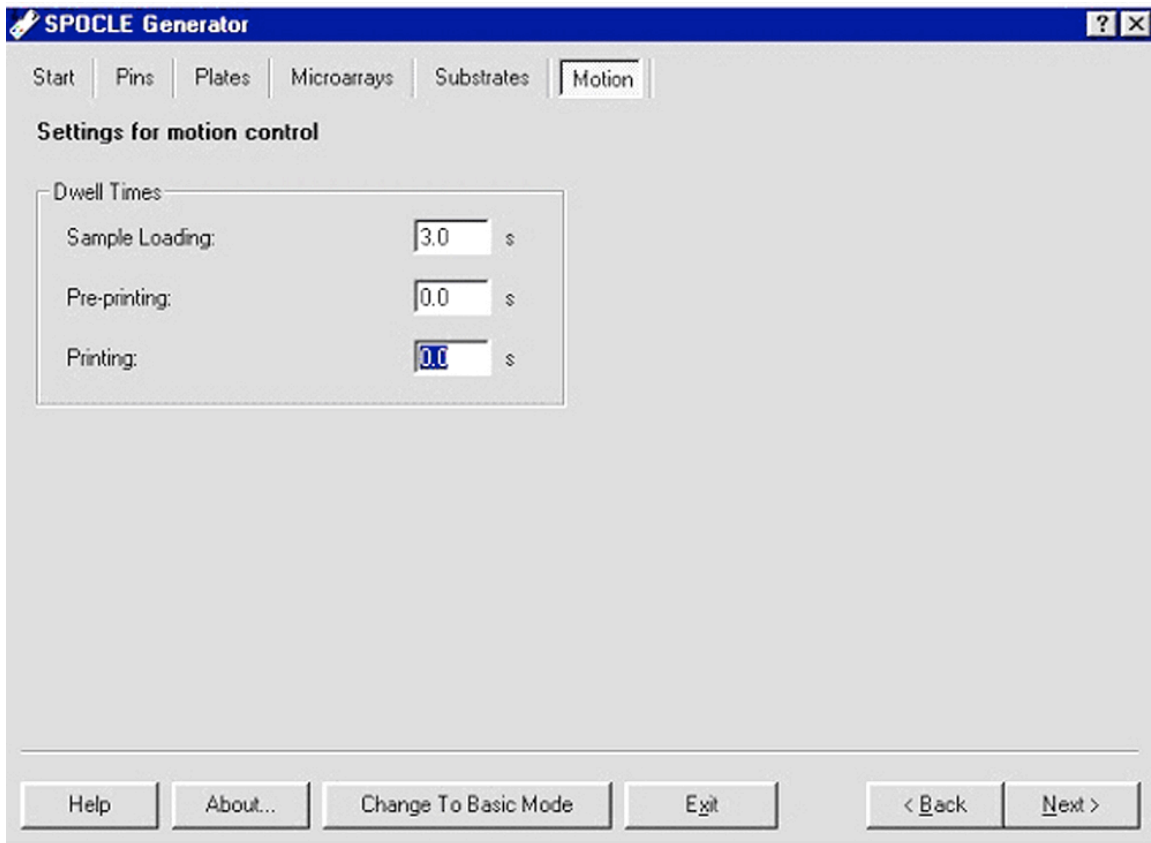
Step 4. This is the fourth dialog window of the SPOCLE Generator. Use this dialog window to define the spacing, configuration and location of the spots on the printing substrates (slides). Specify the center-to-center spacing (in microns, μm) in the "Spot Spacing" dialog box. Specify the number of Columns 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
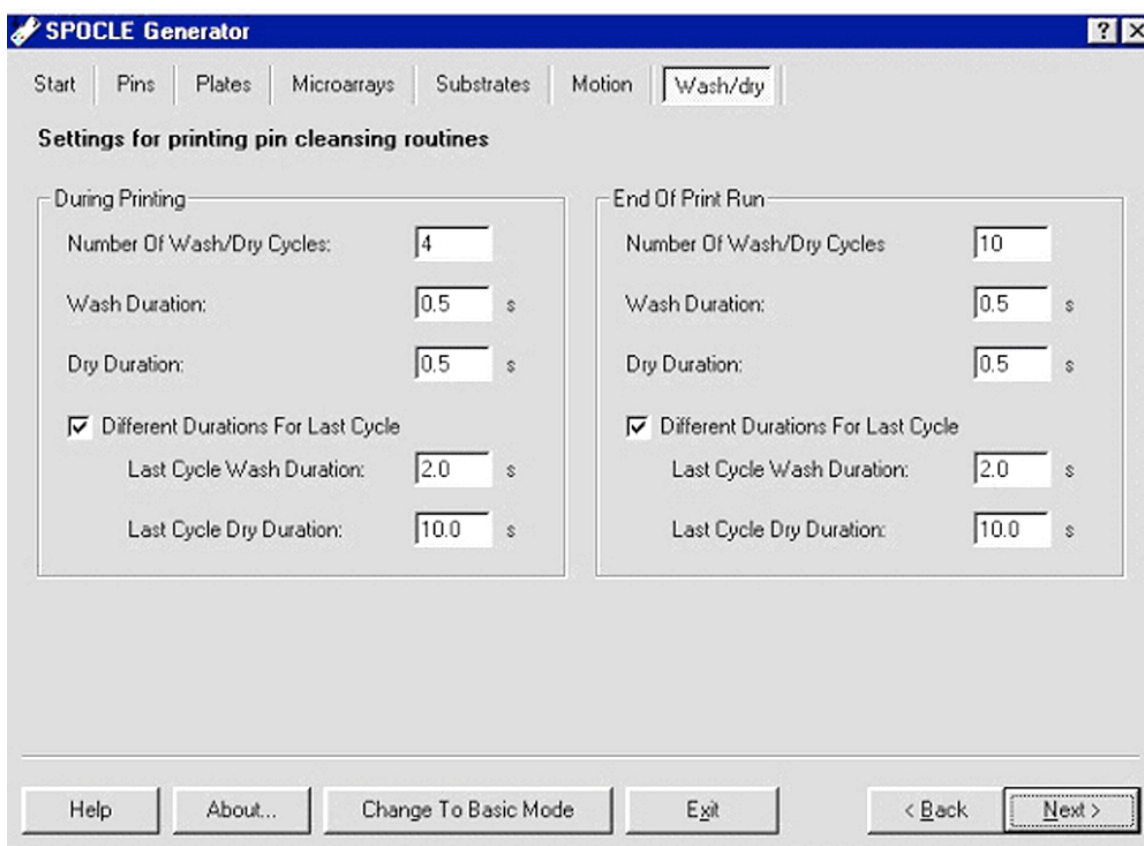
across the subarray and the subarray spacing will be 300um, the minimum possible value (15 x 300 = 4500, only 300um remains for spacing). If the spot spacing is set at 301um, 14 spots will be printed across the subarray and the subarray spacing will be 586um (301 x 14 = 4214, 286um + 300um remains). Changing the Lateral and Vertical Print Offset will determine the number of microarrays that will fit on the substrate.

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.

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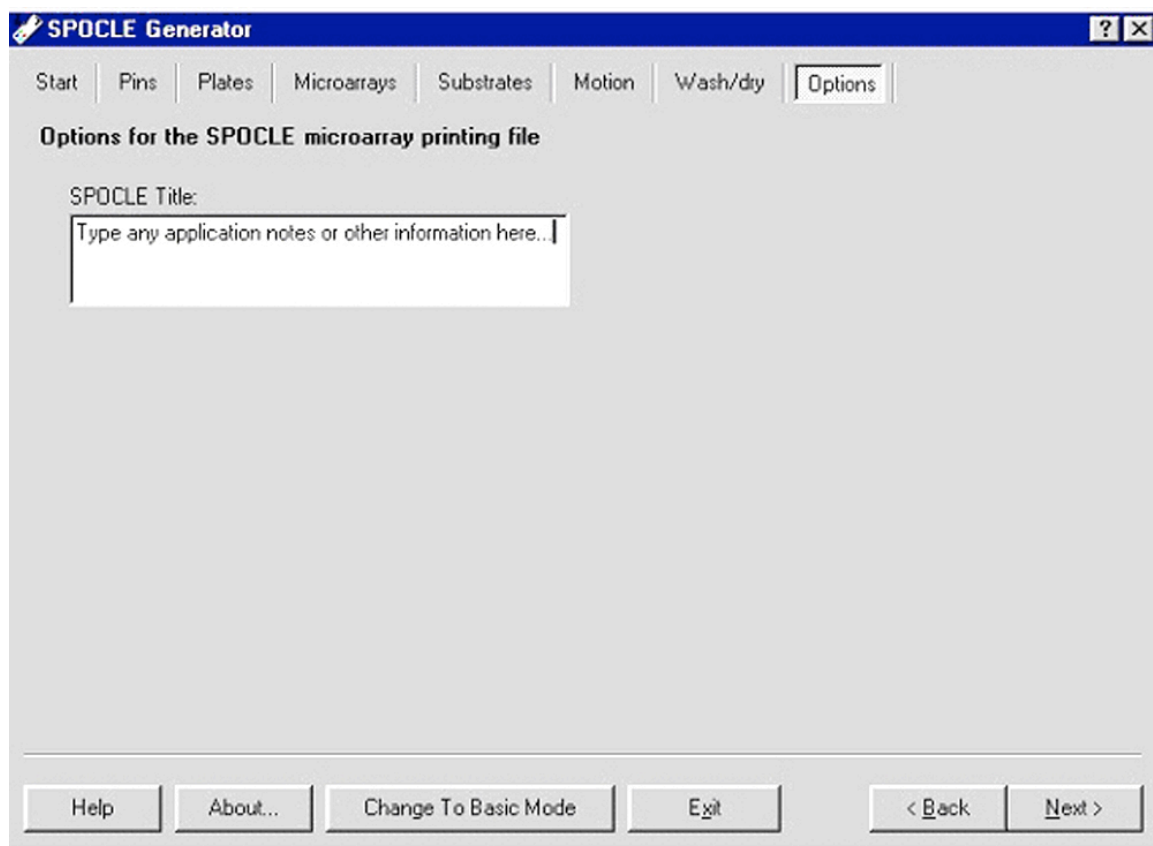
Step 7. This is the seventh dialog window of the SPOCLE Generator. Use these dialog boxes to adjustment the wash and dry procedures during printing and at the end of a print run. The factory-recommended settings are shown in the "Settings for printing pin cleansing routines" dialog boxes above. A minimum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

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.
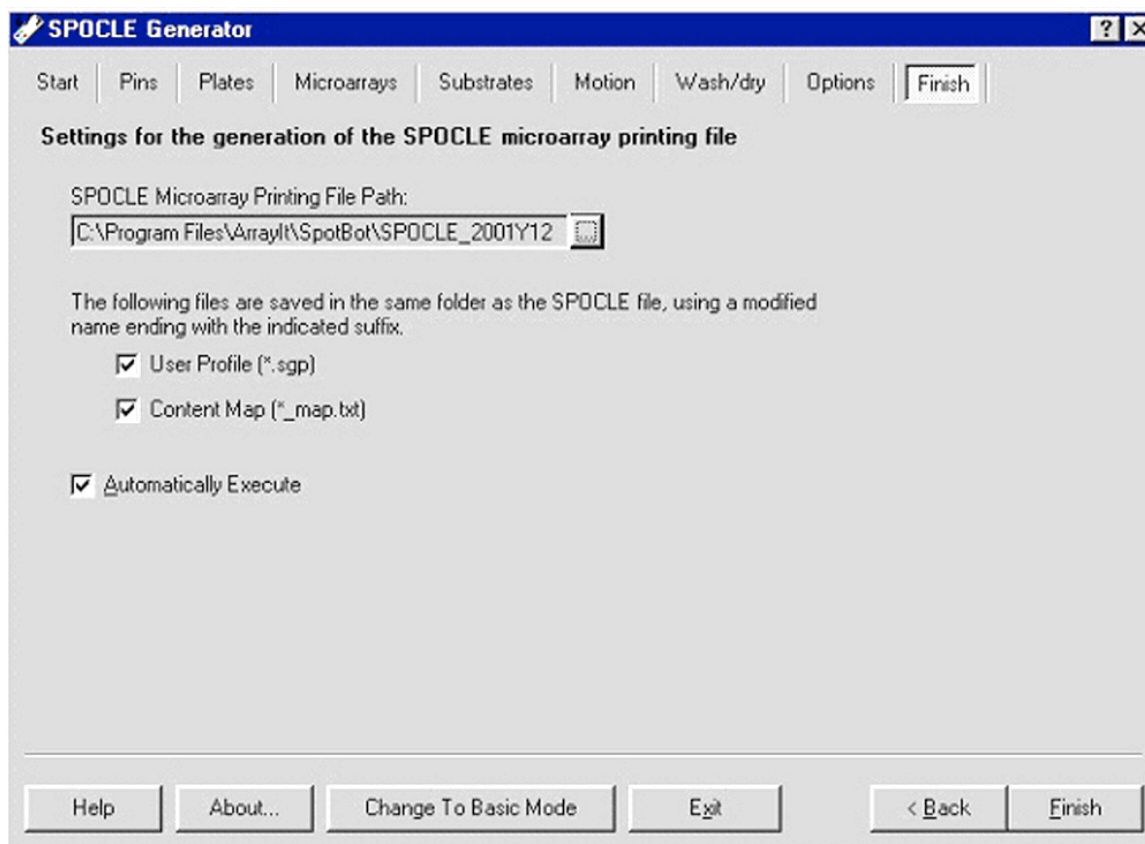
Step 9. This is the ninth dialog window of the SPOCLE Generator. Use these dialog boxes to save the SPOCLE file onto the computer hard drive. The default name of SPOCLE files created by the SPOCLE Generator includes a date and time stamp. To change the file name and/or path of the file, click on the 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 www.spotsupport.com. 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).