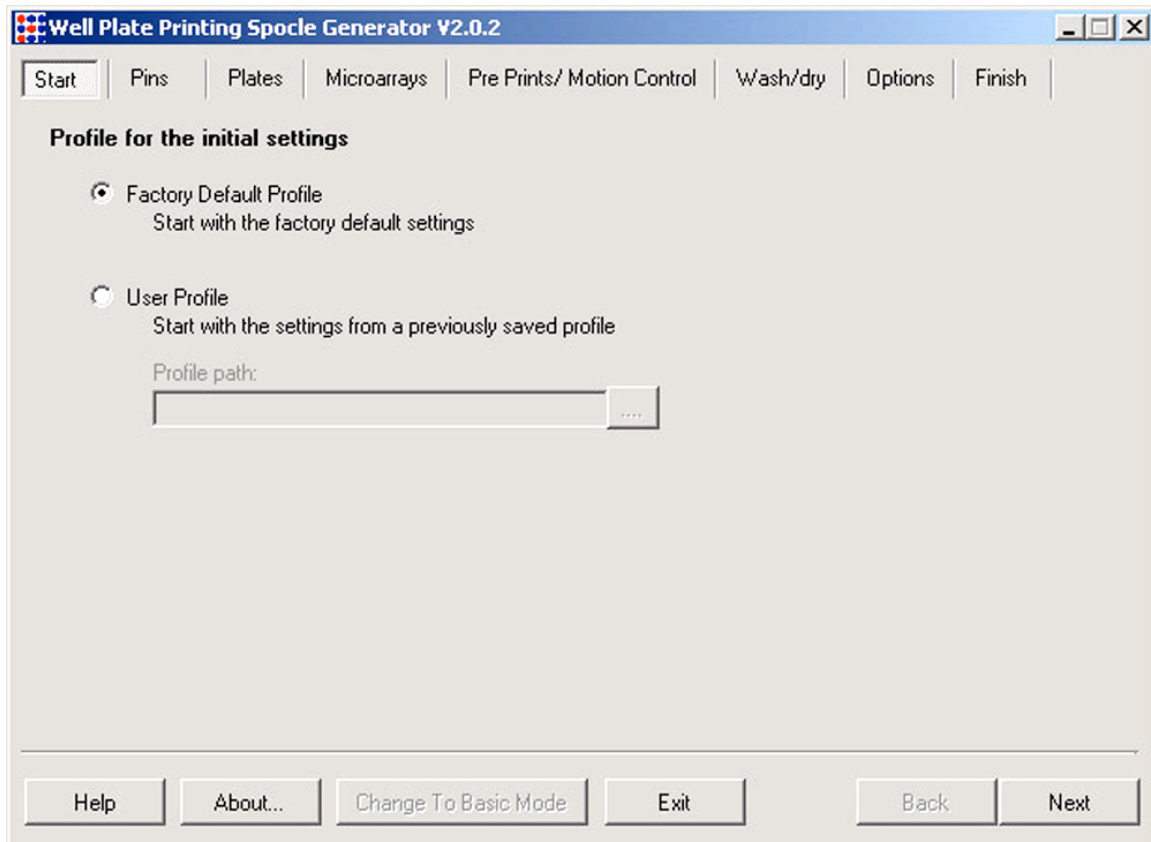


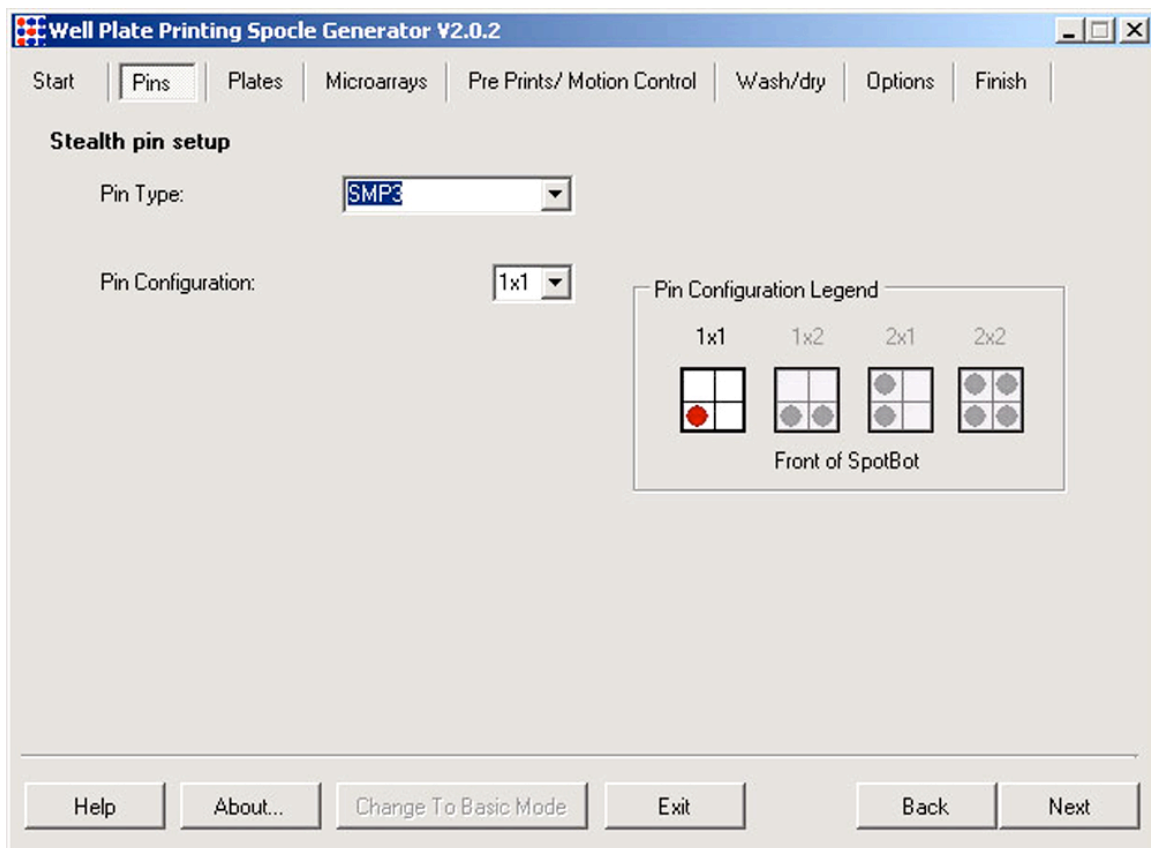


## SpotBot® 2 96-Well and 384-Well Microplate Microarray Printing using SpotBot® 2 Desktop Personal Microarrayers

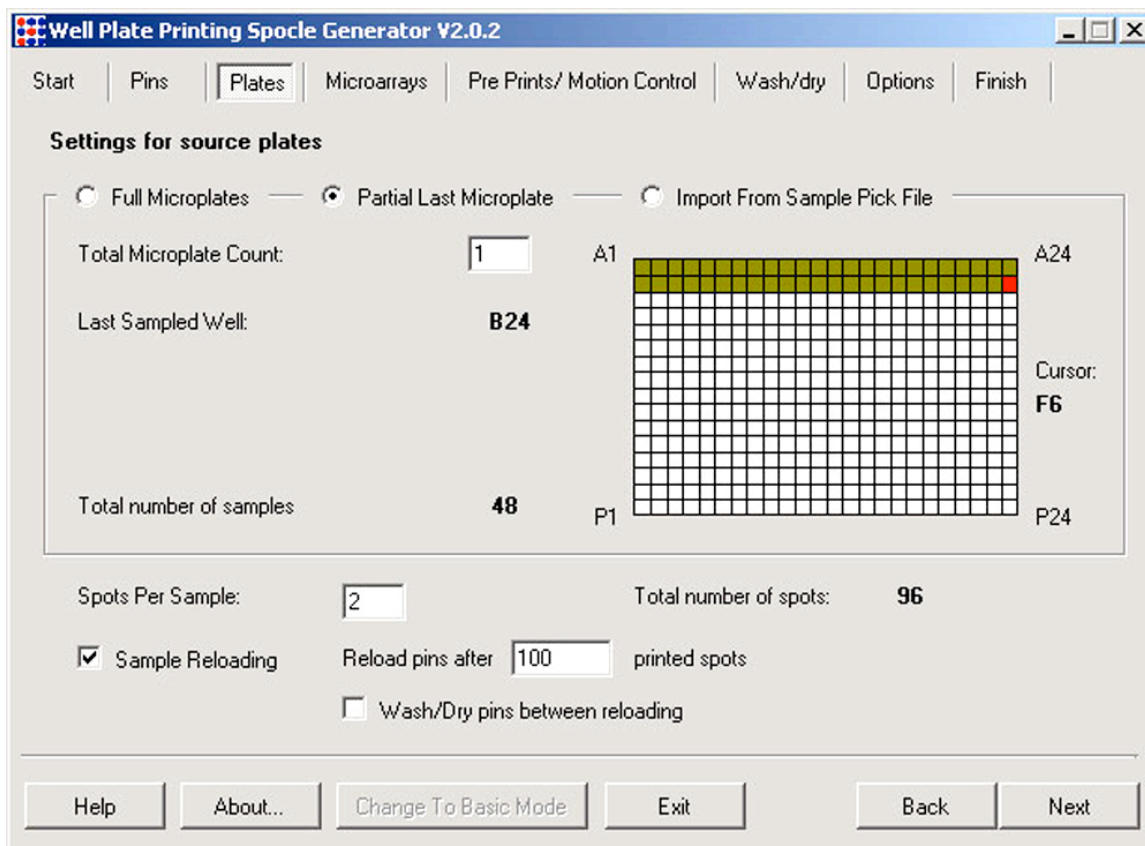
Print microarrays into the standard SBS microplate format using this software with our SpotBot. Well Plate Printing SpoCLE (SpotBot Control Language) software is designed to use with 96-well and 384-well flat bottom microplates and microplate sized glass.



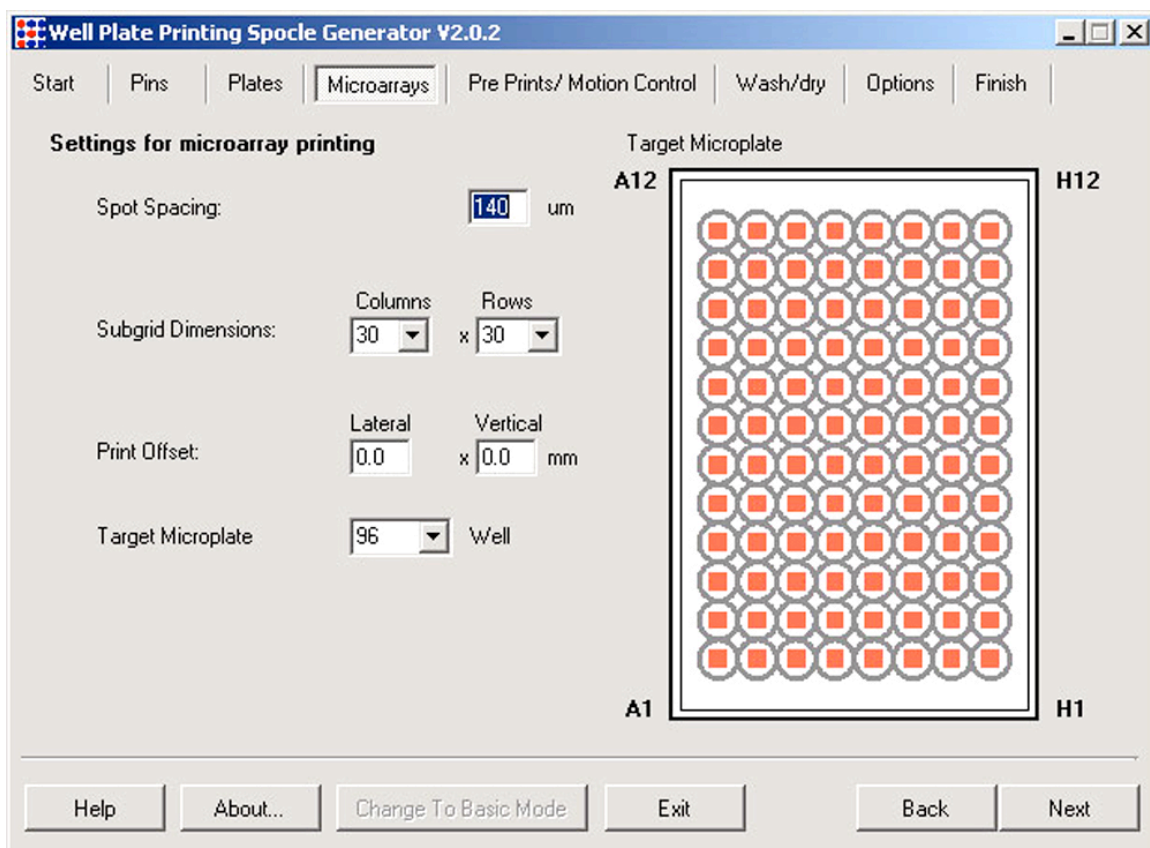
Screen Shot 1: Start with the factory default to start a new program, or select an existing SpoCLE file to edit. Click Next.



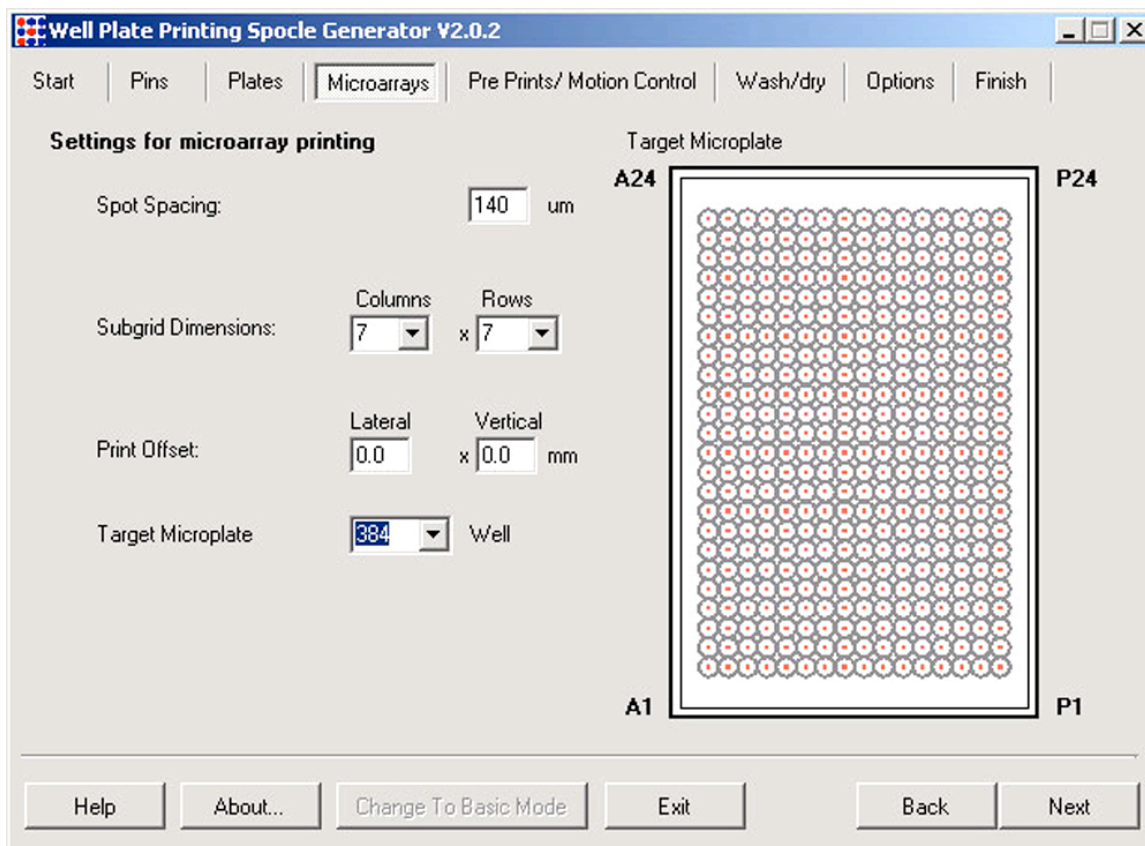
Screen Shot 2: Select your pin type from the drop down menu. This will provide a suggested center-to-center spot spacing later in the software. Note: the number in the catalog ID designates the size of the tip of the pin and suggested center to center spacing. See pin documentation for more details. This software interface uses 1 Pin, since the locations on the slides will be at 9mm spacing and the Pin spacing on the SpotBot Printhead is at 4.5 mm spacing.



Screen Shot 3: The software can accommodate 384 well plates (MMP384 recommended). By selecting "Partial Microplate" specific wells that contain samples can be selected, as shown by the green and red shading. The last sample and last printed sample is red. Enter the number of spot replicates for each well desired into the dialog box "Spots Per Sample". Selecting 2 Spots Per Sample, for example, would print each sample in duplicate at the spot spacing defined by the user (see next dialog box). 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.

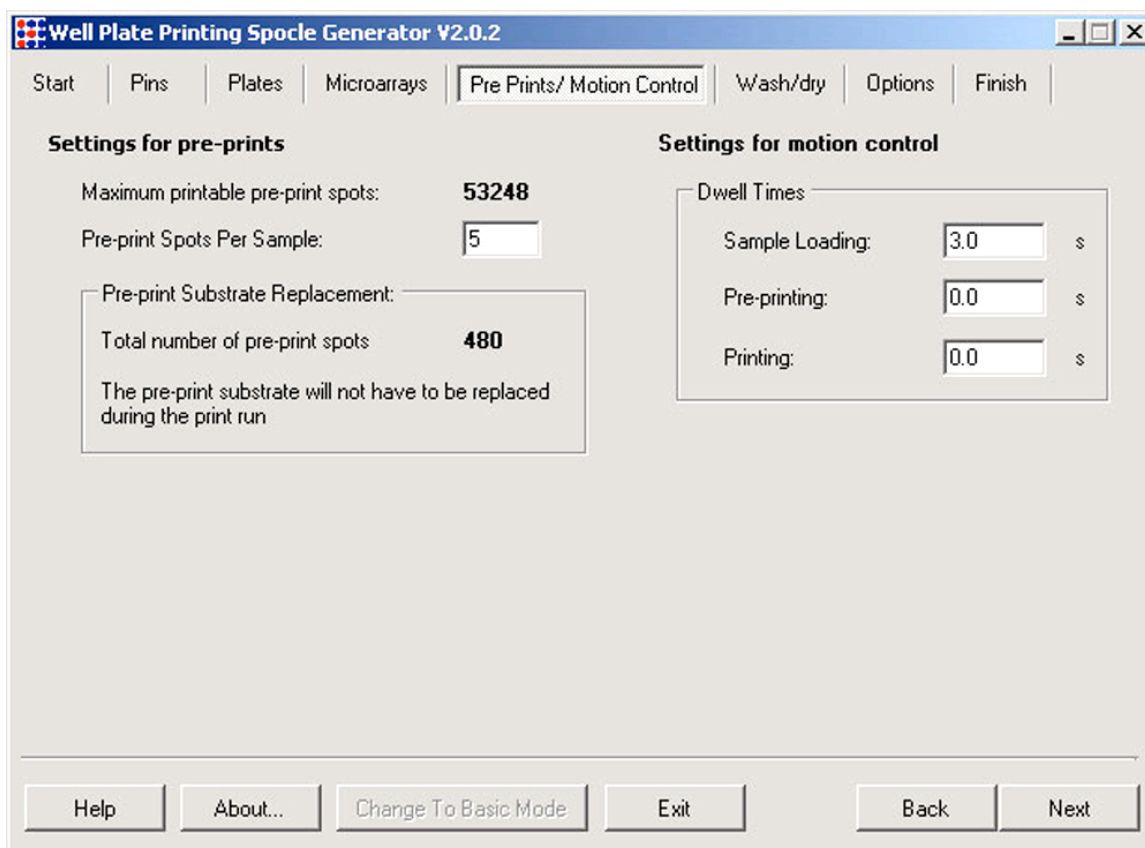


Screen Shot 4A.



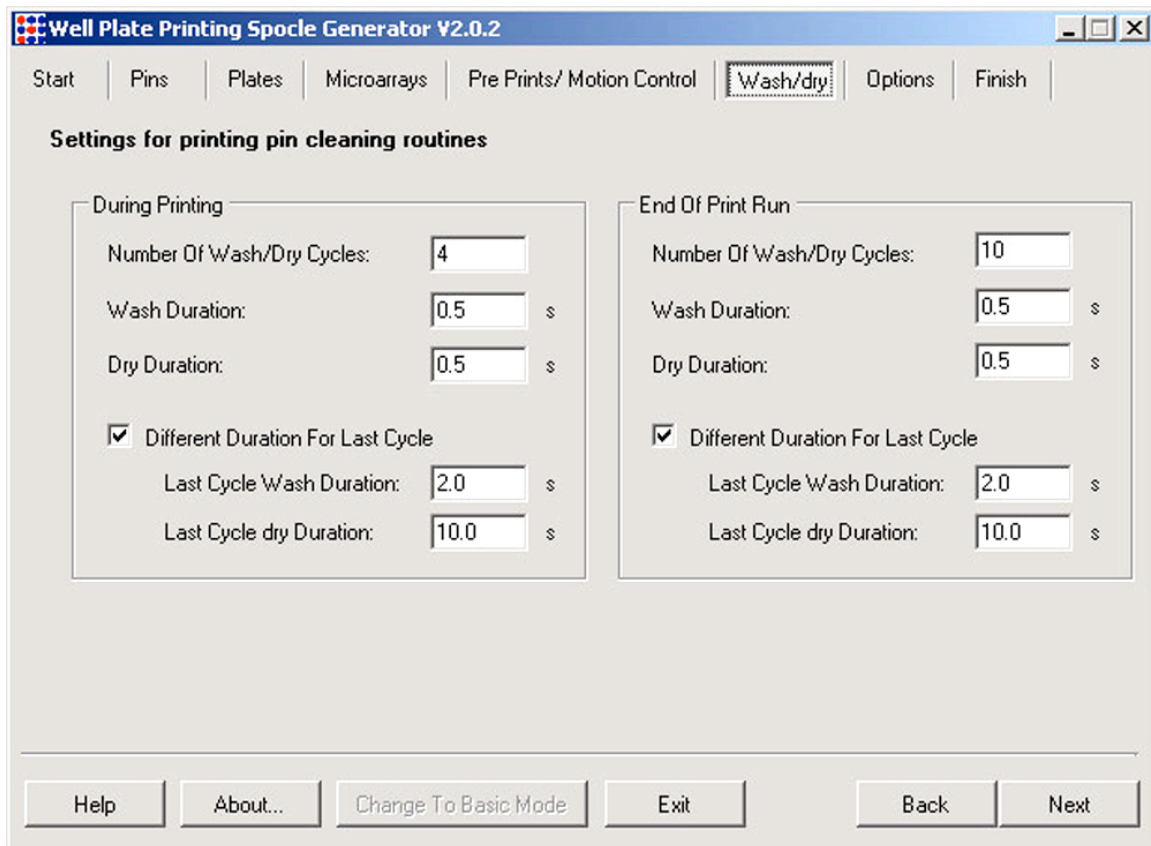
Screen Shot 4B

Screen Shot 4A & B, Description: Select well pattern, spot spacing, lateral and vertical start and stop position of the microarrays in each "well", and define the numbers of columns and row for the microarray. Each microarray in each well will be identical. Plate is loaded onto the SpotBot as shown, with well A1 being closest to the front door of the SpotBot, Pre-print slides are loaded in front of the microplate, a special bracket is sold to hold both the target microplate and pre-print slides in place.

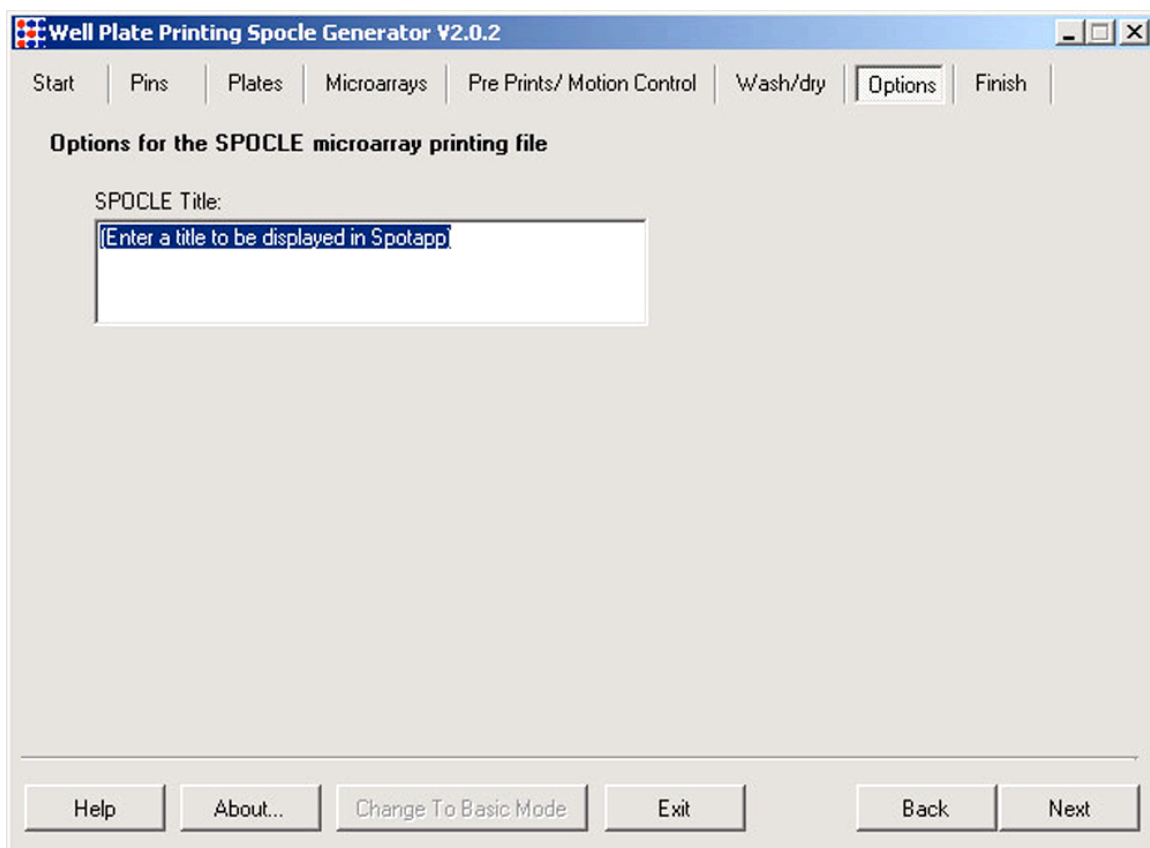


Screen Shot 6: Use these dialog boxes to adjust the number of desired pre-print spots prior to pins moving to print into the target well plate. Also in this dialog box is Pin resting or “dwell” time during sample loading, pre-printing and printing. The dwell time corresponds to the length of time the 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.



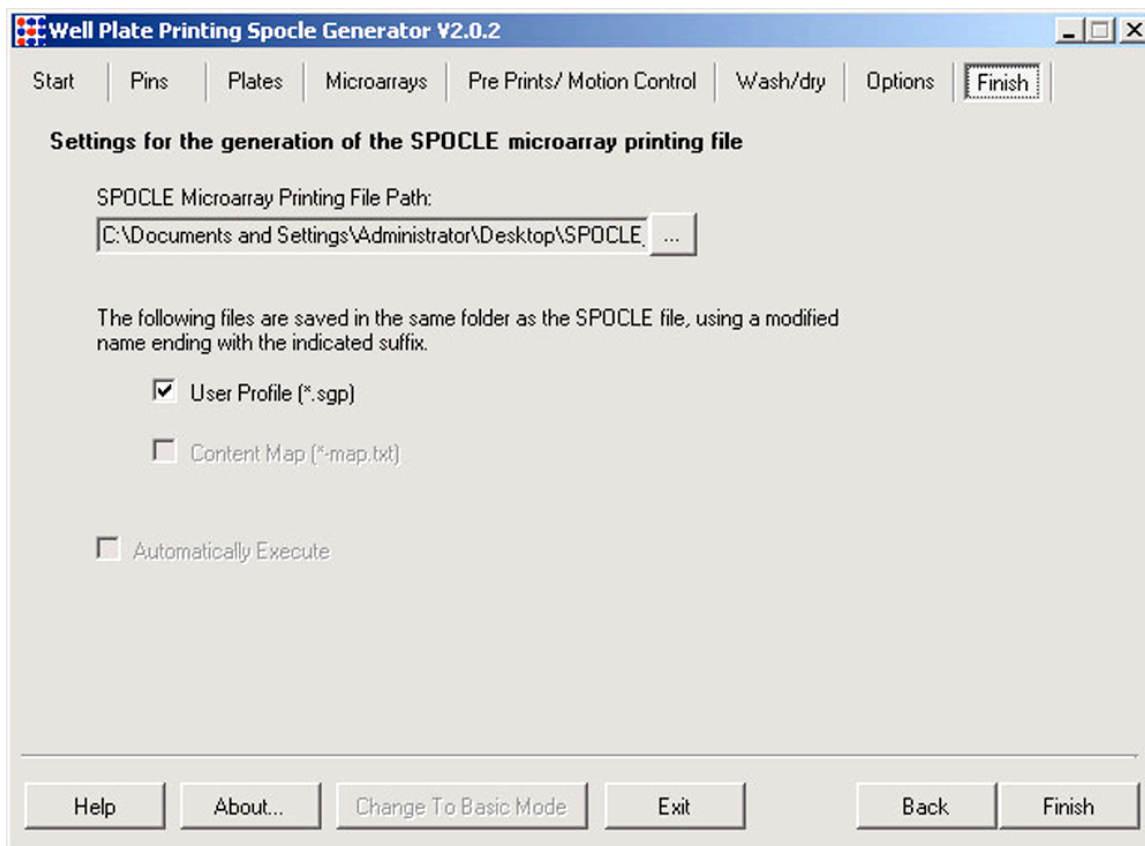


Screen Shot 7: The recommended settings are shown in the “Settings for printing pin cleansing routines” dialog boxes above. A minimum of 4 Wash/dry cycles is recommended to remove samples from the previous printing cycle. Using fewer than 4 Wash/Dry cycles may result in sample “carry over” into the subsequent 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.

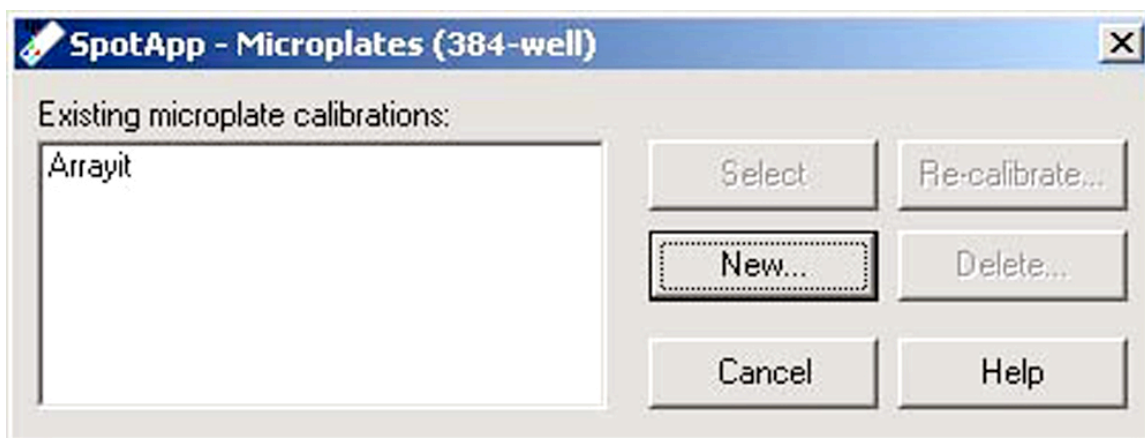


Screen Shot 8: Use this dialog box to save short notes or title about the file and experiment this program is being used for. This information will be displayed when the program is running. This is not the file name for the program.





Screen Shot 9: Use this dialog box 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.



Screen Shot 10: Use this dialog box to create and calibrate a new 384-well microplate.