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Arrayit SpotBot® 3 Personal Microarrayers
Advanced Vision System
Technical Manual
v11.01.05

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Introduction

This technical manual details the steps required to utilize Arrayit's SpotBot® 3 Personal Microarrayers Advanced Vision System (SBV). SBV allows users to print on custom substrates such as microarrays, semi-conductor chips, microfluidic chambers, biochips, biosensors and other types of custom substrates for biochemisty, bioengineering, nanotechnology and aerospace applications.

SpotBot® 3 Advanced Vision System Components

- SpotBot® 3 Advanced Vision System Software (pre-installed on computer)
- SpotBot® 3 door sensor override (pre-installed on SpotBot® 3)
- High-resolution digital camera
- High-magnification lens with custom optics
- Camera joy stick and base
- Camera power supply
- USB cable (3.3 m)
- LED 12-light illumination source

I. Installation

Carefully unpack and place the SpotBot® 3 Personal Microarrayer Advanced Vision System components on a certified laboratory bench as shown here. The SpotBot® 3 instrument can be placed at a 45° angle to facilitate camera accessibility. Alternatively, the camera can be placed on a laboratory cart to conserve bench space. The LED light source should be placed near substrate position 8, which is the printing position for the SpotBot® 3 Advanced Vision System. The camera should be placed approximately 1.0 cm from the instrument case at an approximate 45° angle as shown.

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It is important to leave room for the camera on the right side of the instrument for side-viewing as shown, which may be useful for visualizing certain printing substrates. Subtle changes to the camera angle can be made by depressing the spring-loaded gray hand lever on the front side of the camera "joy stick". The SpotBot® 3 accessories including the air compressor, peristaltic pump and megasonic power supply should be connected correctly and positioned at the rear of the instrument as shown here. The LED 12-light illumination source may be adjusted or re-positioned as necessary to achieve optimal visual contrast on the computer screen.

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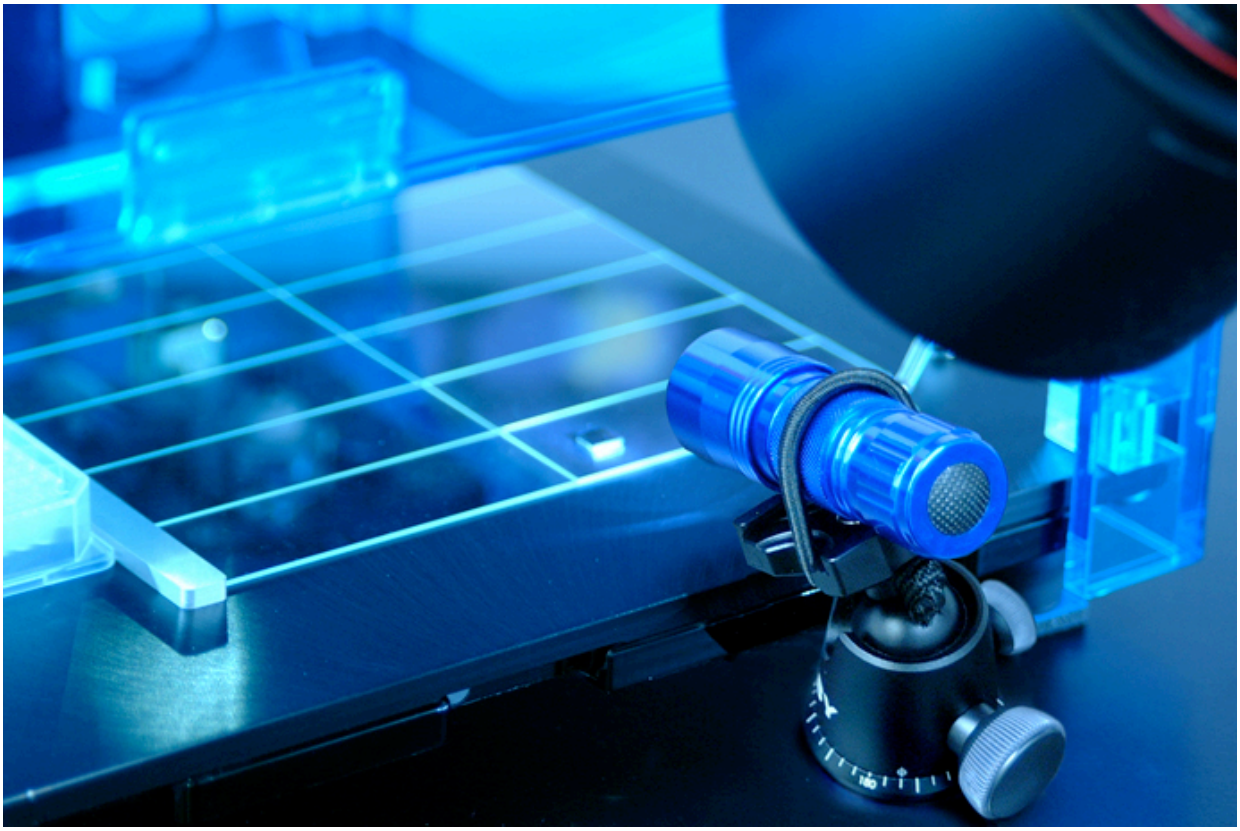
The LED 12-light illumination source is configured on a ball head, allowing fine angle adjustments in all three axes (x, y and z). Adjustments can be made by loosening the tensioner knobs located in the base of the ball head, and then re-tighteniing the tensioner knobs to fix the angle. The LED beam should be focused on the printing substrate so as to maximize the visual contrast on the computer screen. The LED illumination source power can be turned on and off using the power switch located at the back of the light source. The light source should be turned off after each use to conserve the batteries. Batteries can be changed by removing the light source from the ball head. The light source can be removed from the ball head by stretching the flexible band fastener and sliding the light source off the ball head. Unscrew the base of the light source to remove the battery pack. The light source uses three triple A batteries, which should be replaced at regular intervals to maintain maximum beam intensity.



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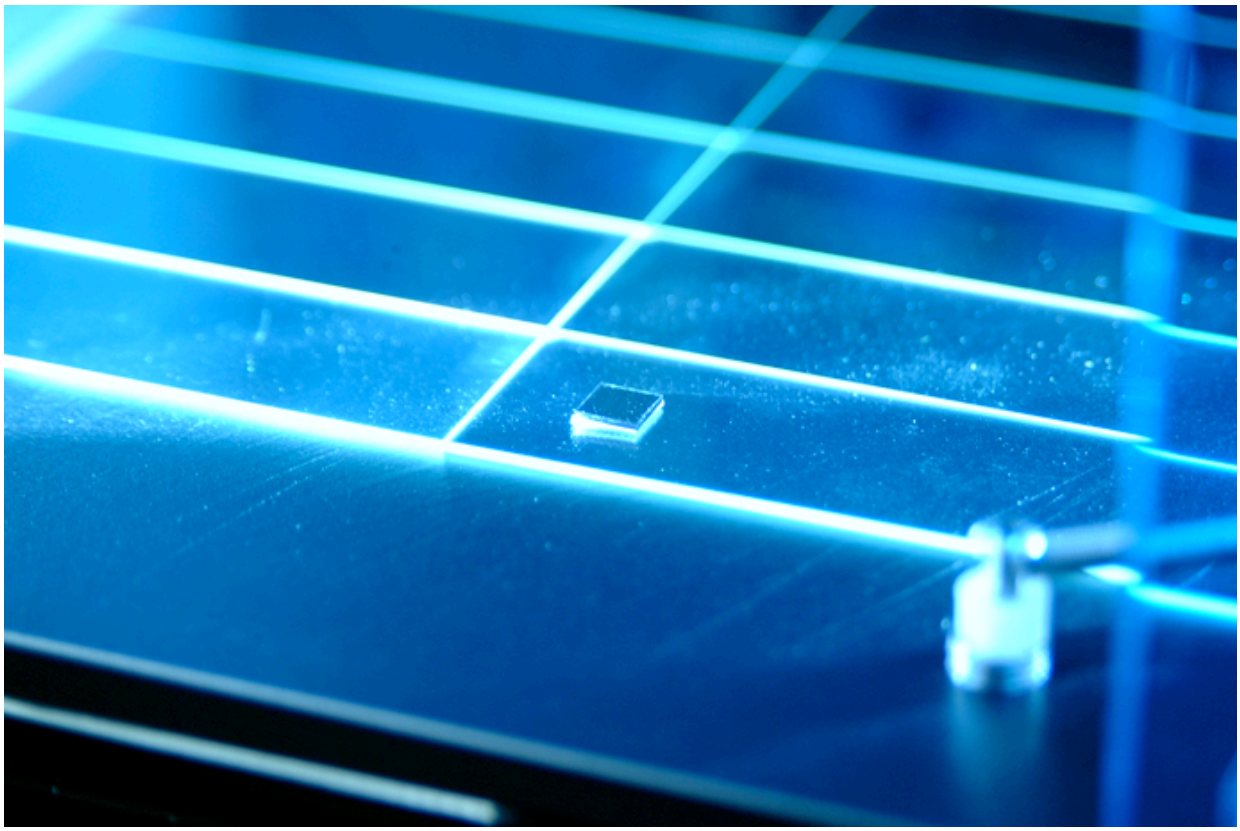
The custom printing substrate such as a microarray, microfluidic, biochip, or biosensor should be placed at position 8 on the printing platen as shown. Double-backed adhesive tape can be used to secure the printing substrate to the glass substrates below. Glass substrates provide a convenient way to identify printing position 8, allow attachment of the custom substrate and increase the effectivenss of the LED light source. The L-bracket substrate holder can be removed as shown here to improve visualization of the custom printing substrate.



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The USB cable should be connected to the BioBlue Computer on one end and to the SpotBot® 3 Advanced Vision System camera as shown here. The USB cable transmits digital information from the camera to the computer.

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The power supply should be connected to to an appropriate surge protected 110-volt or 220-volt power source on one end and to the SpotBot® 3 Advanced Vision System camera as shown here. The power supply allows continuous use of the digital camera without depleting the camera's batteries.

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The SpotBot® 3 Advanced Vision System camera should be set on manual mode with the power switch in the "on" position as shown here. If objects fail to appear on the computer screen, turn the power switch off and then on to remedy this issue.

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The SpotBot® 3 Advanced Vision System camera is connected to the camera base using the switching connector shown here. To remove the camera from the base, flip the gray switch to the left and gently remove the camera by moving it upward. In addition to its use in the Vision System, the camera can be used for a wide range of other digital camera applications including product photographs, technical movies and other laboratory purposes.

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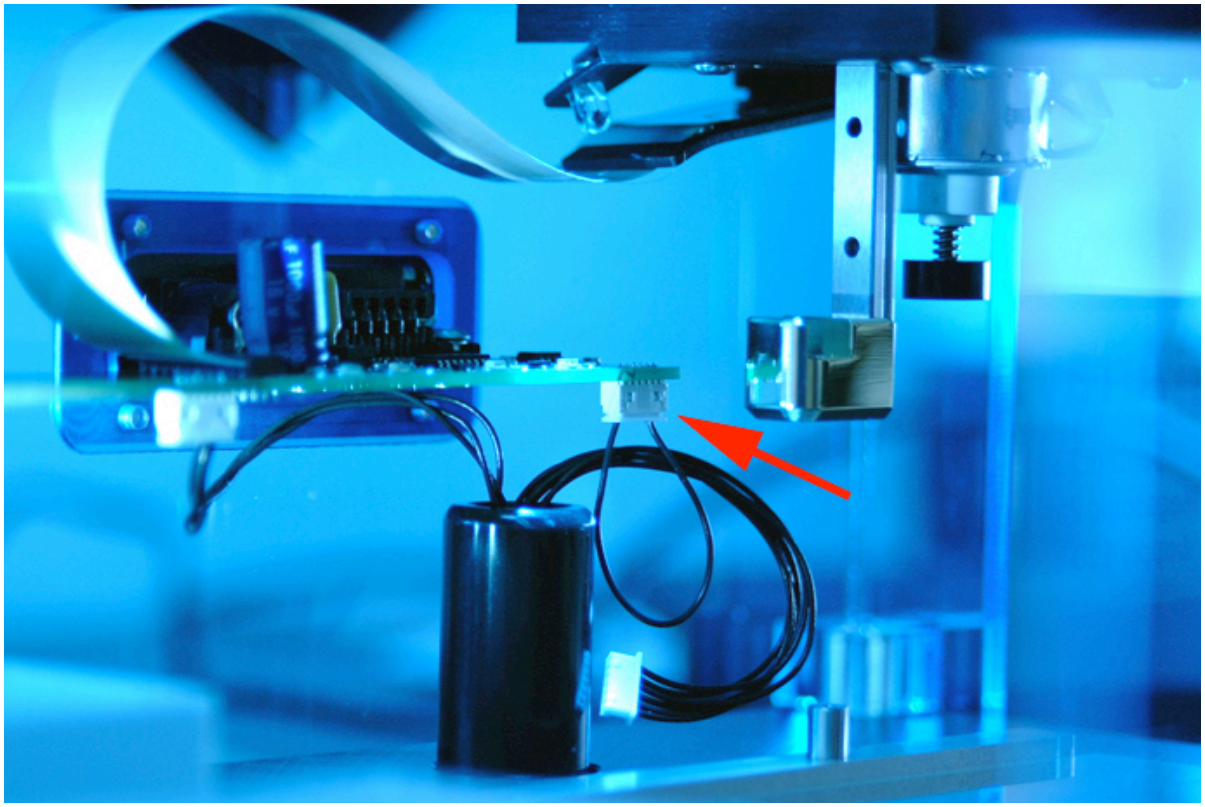
SpotBot® 3 Personal Microarrayers configured for the Advanced Vision System are equipped with a door sensor override to allow the instrument to be operated with the doors open. The capacity to run the system with doors in the open position improves Vision System functionality by enhancing the quality of the digital viewing process. The door sensor override inserts into the main circuit board as shown here (red arrow). Extra care must be taken to ensure the proper location of microplates, substrates, pins and other objects on the printing deck prior to operation because robot movements can only be halted in software (rather than by opening the instrument doors as is the case if the door sensors are operational). Please do not attempt to install or remove the door sensor override without technical assistance from Arrayit (arrayit@arrayit.com).



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II. Creating Vision System Printing Routines

Launch the SpotBot® 3 Vision System EOS Utility on the desktop as shown here. The software interface will connect the digital camera to the computer via the USB cable.

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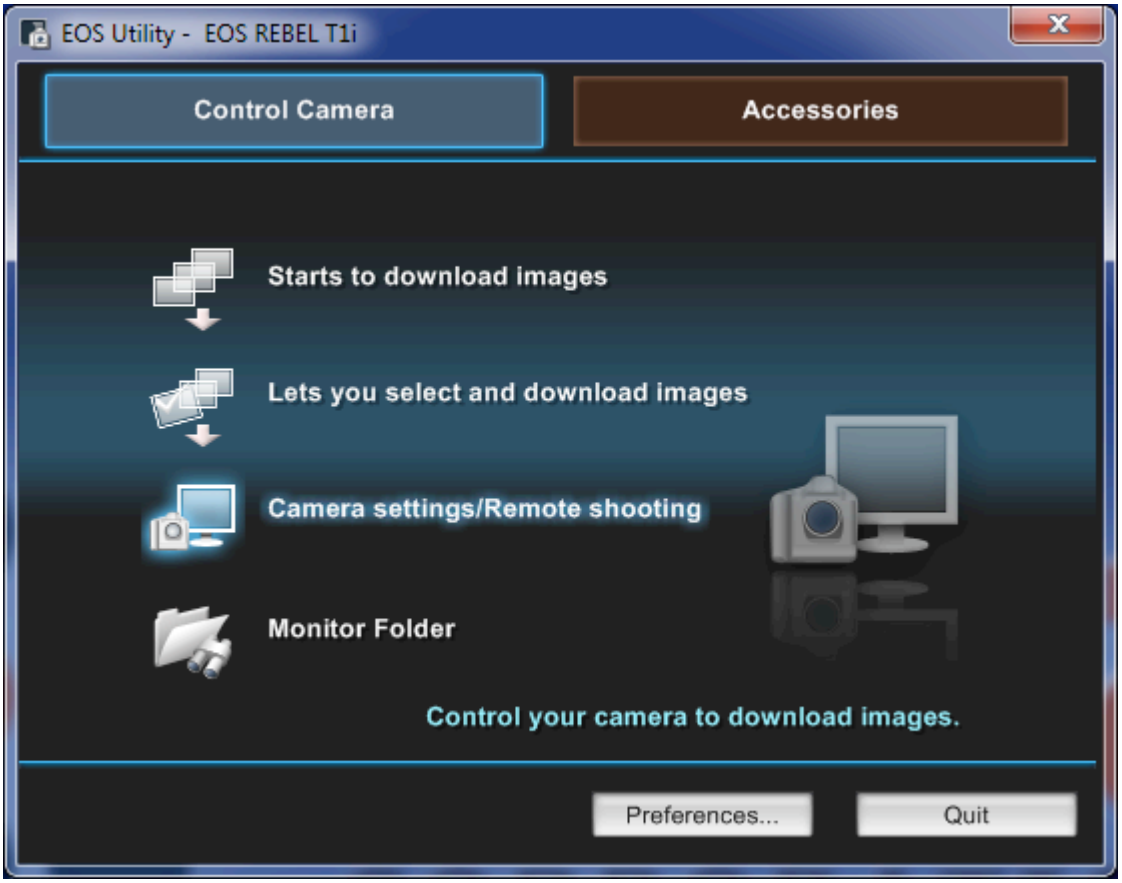
After the Vision System software launches, click on "Camera settings/Remote shooting" as shown here. If this software window does not appear, power the camera off and then back on using the power switch located on the top right side of the camera.



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Adjust the digital camera settings to a shutter speed of 2.5 secs, F32 and ISO 800 as shown here, and click "Live View shooting" at the bottom of the software window to begin the viewing process.

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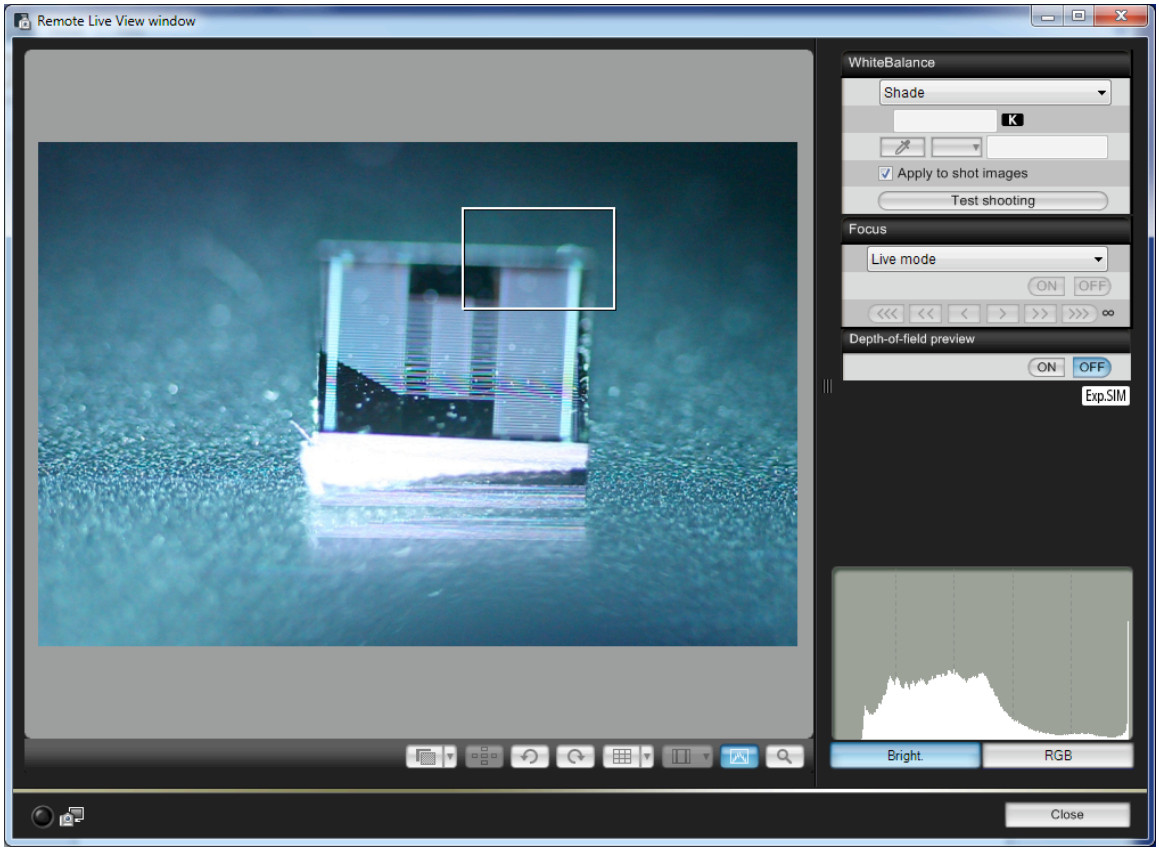
Adjust the LED illumination source and the manual camera focus until the printing substrate appears in the Live View Window as shown here. Manual focusing is achieved by turning the focus portion of the lens by hand as shown in the next photograph.



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The digital camera of the SpotBot® 3 Vision System is focused manually by turning the focus ring gently clockwise and counterclockwise as shown here until the printing substrate appears in sharp focus on the computer screen.

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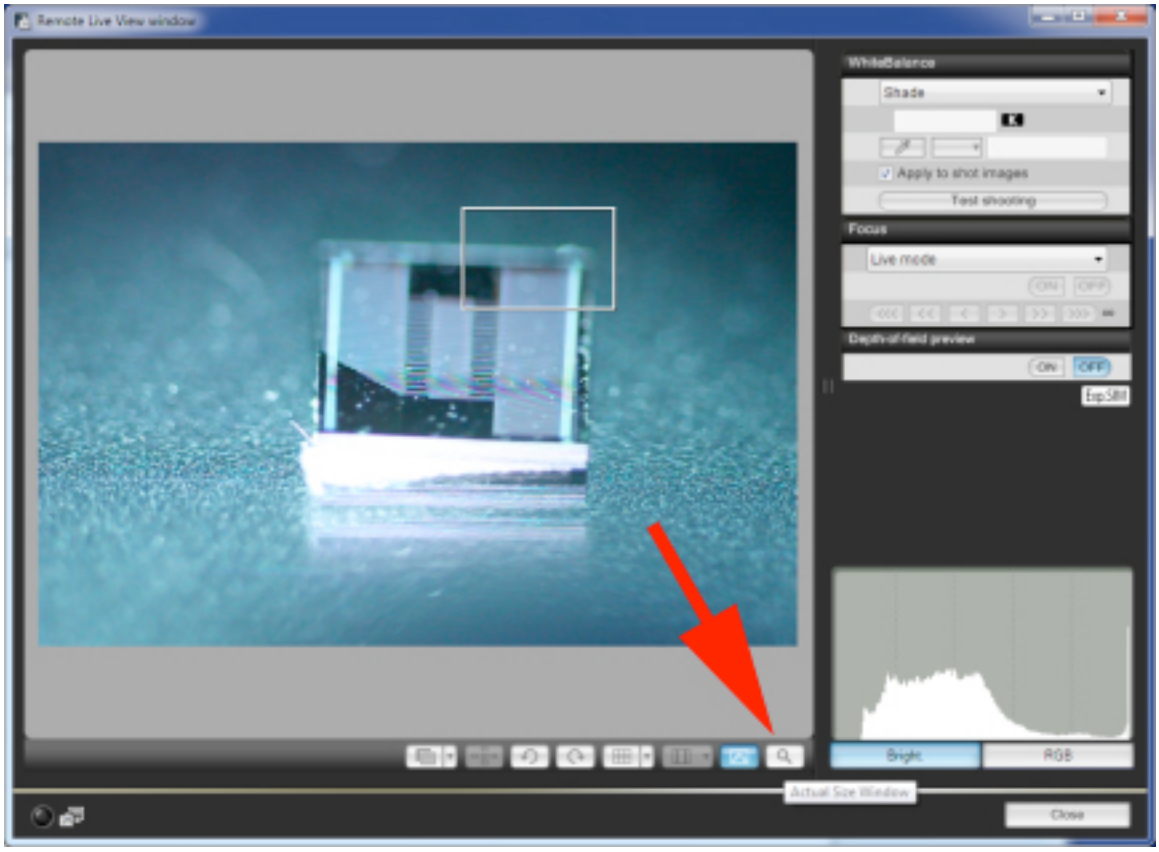
Once the printing substrate appears in sharp focus, click on the magnifying glass icon to magnify the image. The magnifying glass icon is the right-most of the eight icons located in the bottom center of the Live View Window shown here (red arrow).



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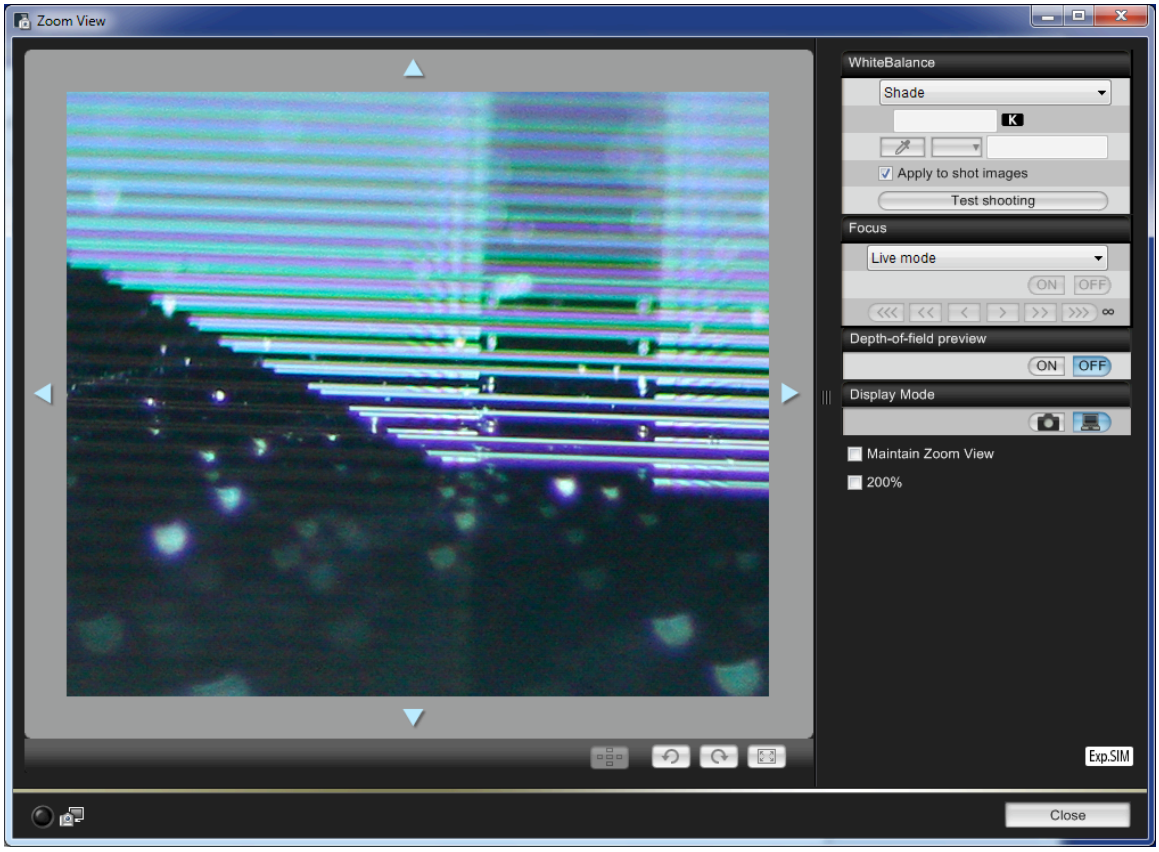
Use the manual focus ring on the digital camera to make minor focus adjustments until the front edge of the printing substrate appears in sharp focus in the magnified view as shown here.



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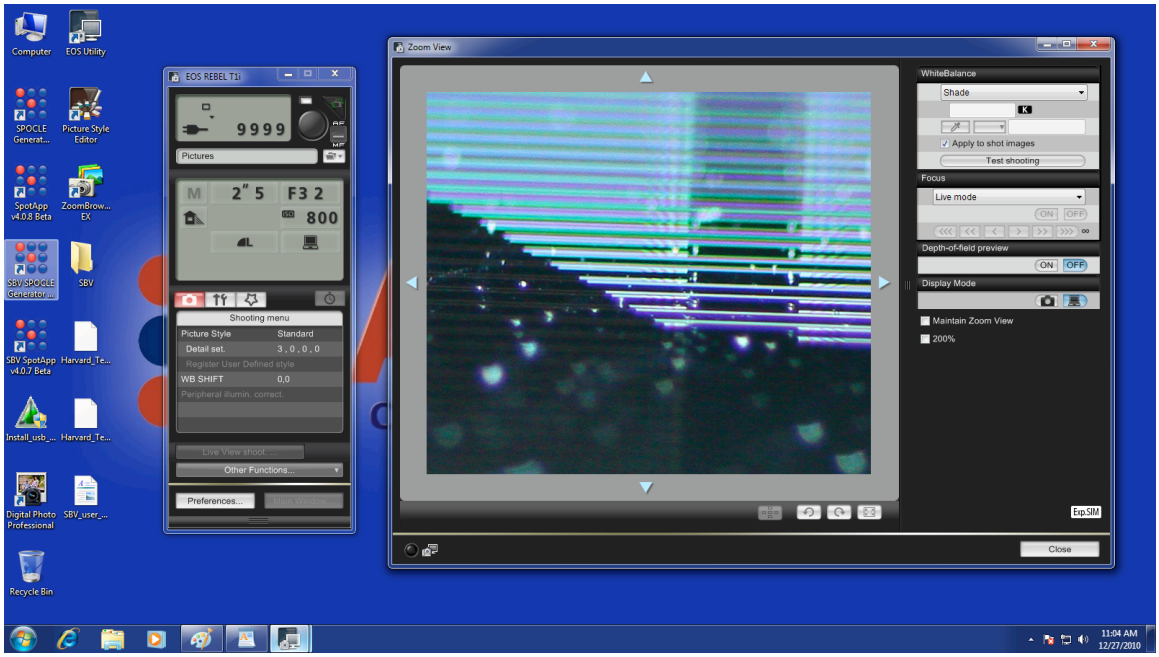
Launch the SBV SPOCLE Generator by clicking on the desktop icon as shown here. The SBV SPOCLE Generator allows the user to create custom printing routines using the SpotBot® 3 Vision System to define the printing locations in absolute space.



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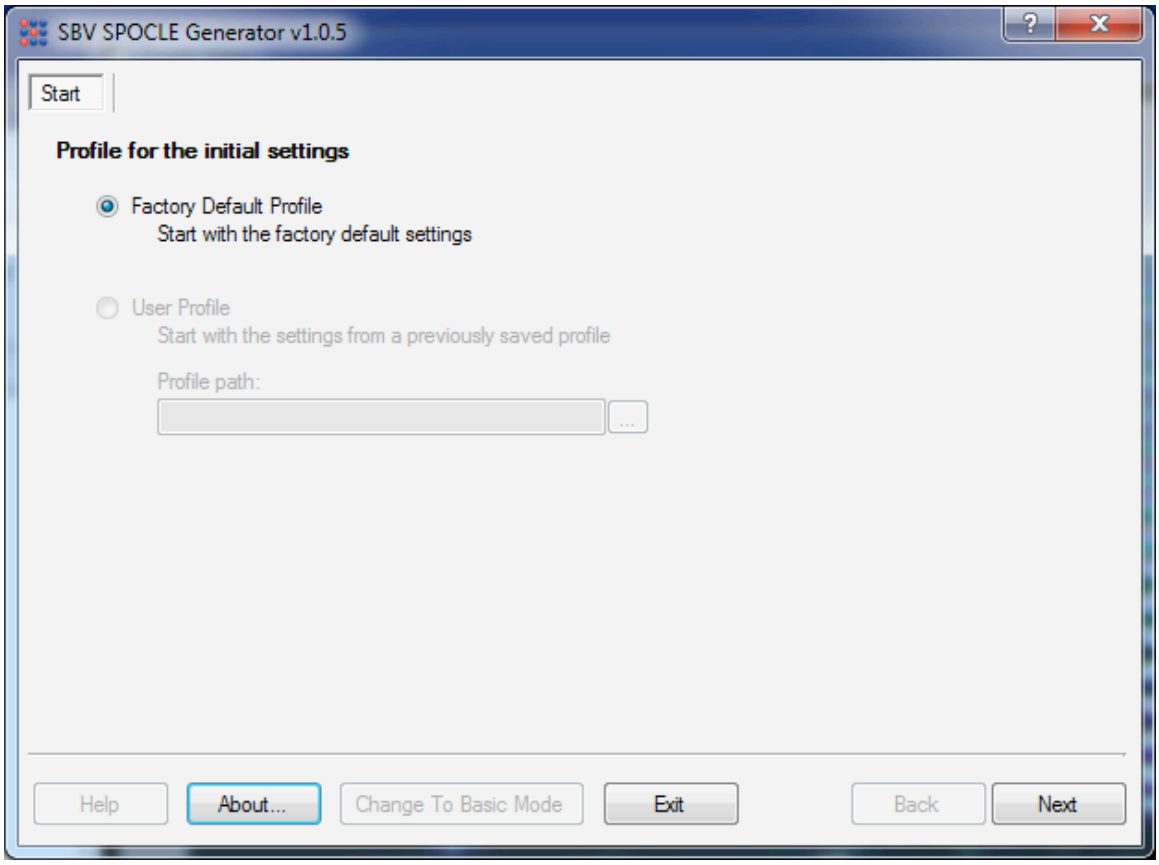
Select the Factory Default Profile in the SBV SPOCLE Generator Start menu as shown here. Click Next.



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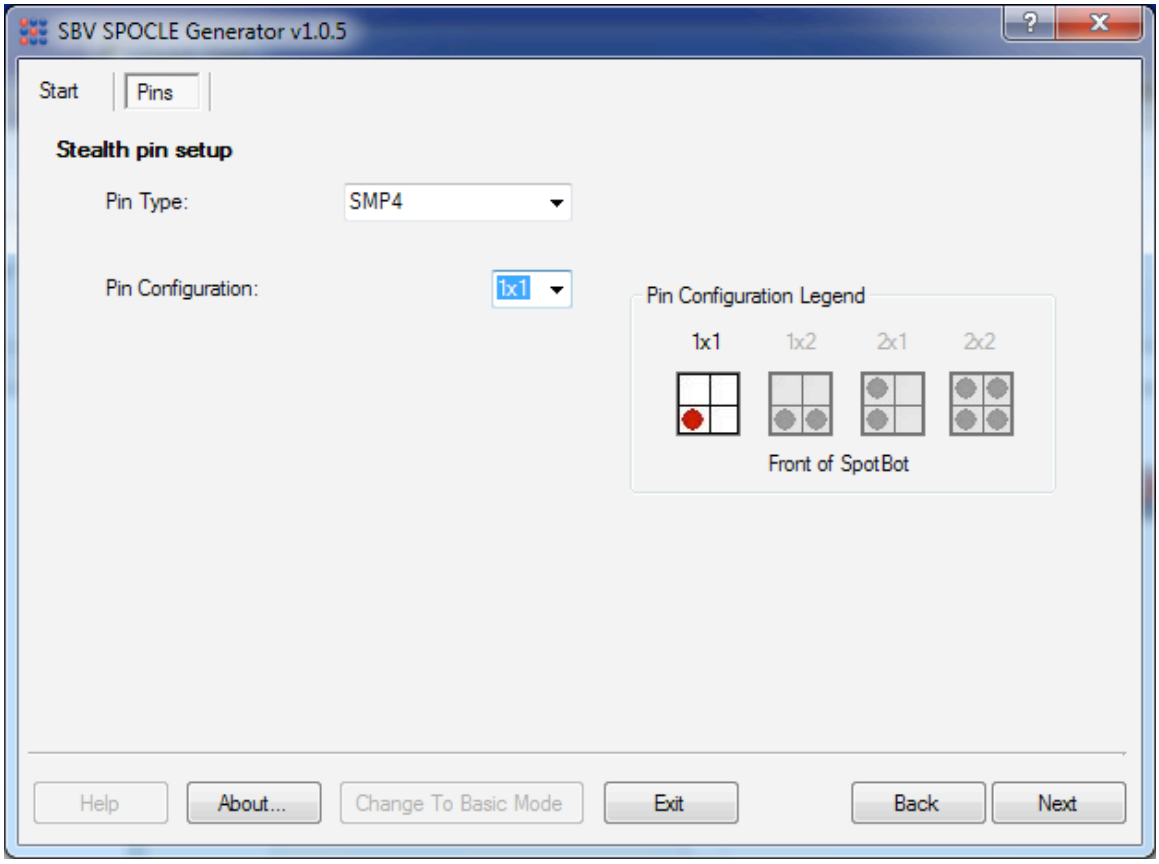
Select the Pin Type and and Pin Configuration in the SBV SPOCLE Generator Pins menu as shown here. The Pin Configuration requires the selection of a single pin only as shown. Click Next.



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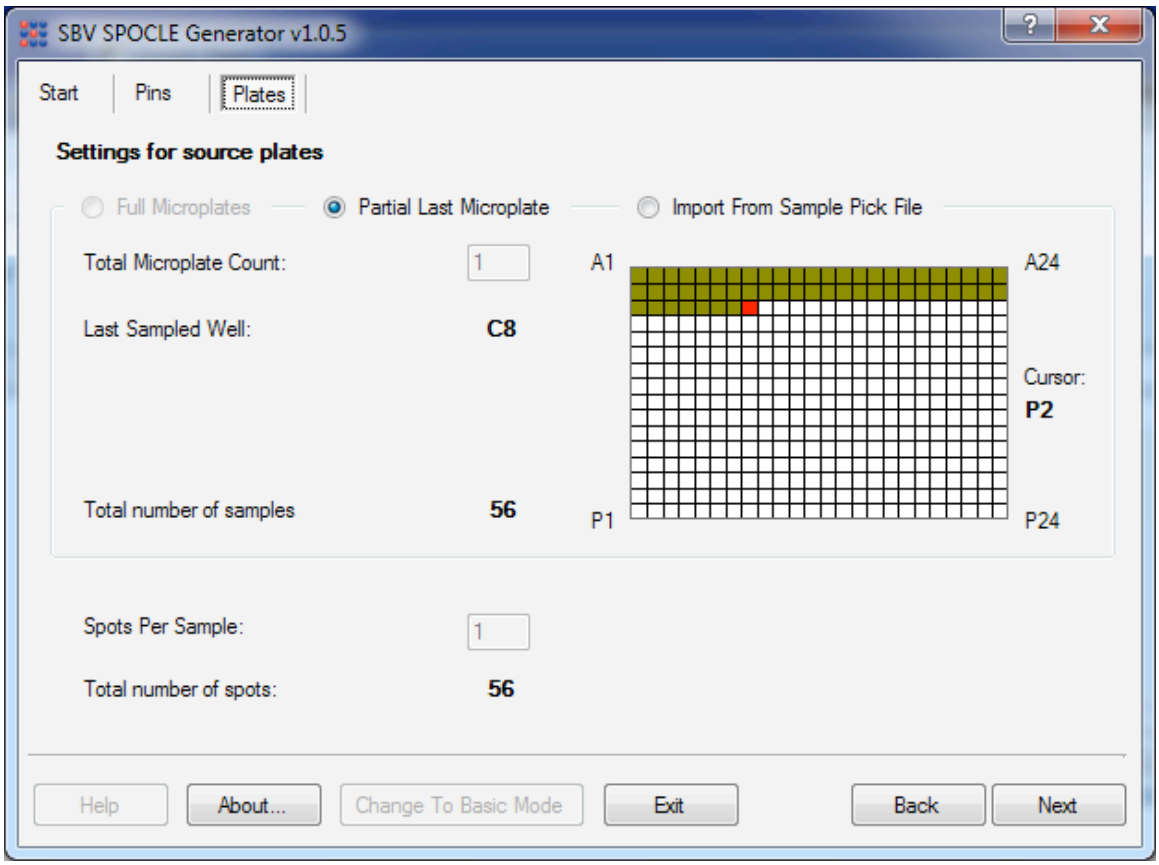
Select the number of samples in the SBV SPOCLE Generator Plates menu as shown here. The number of samples should correspond precisely to the number of single pin loadings from the 384-well microplate that are required to print at every location on the printing substrate. A total of 56 samples (14 columns x 4 rows = 56 samples) will be printed using the Plates selection shown here. Click Next.



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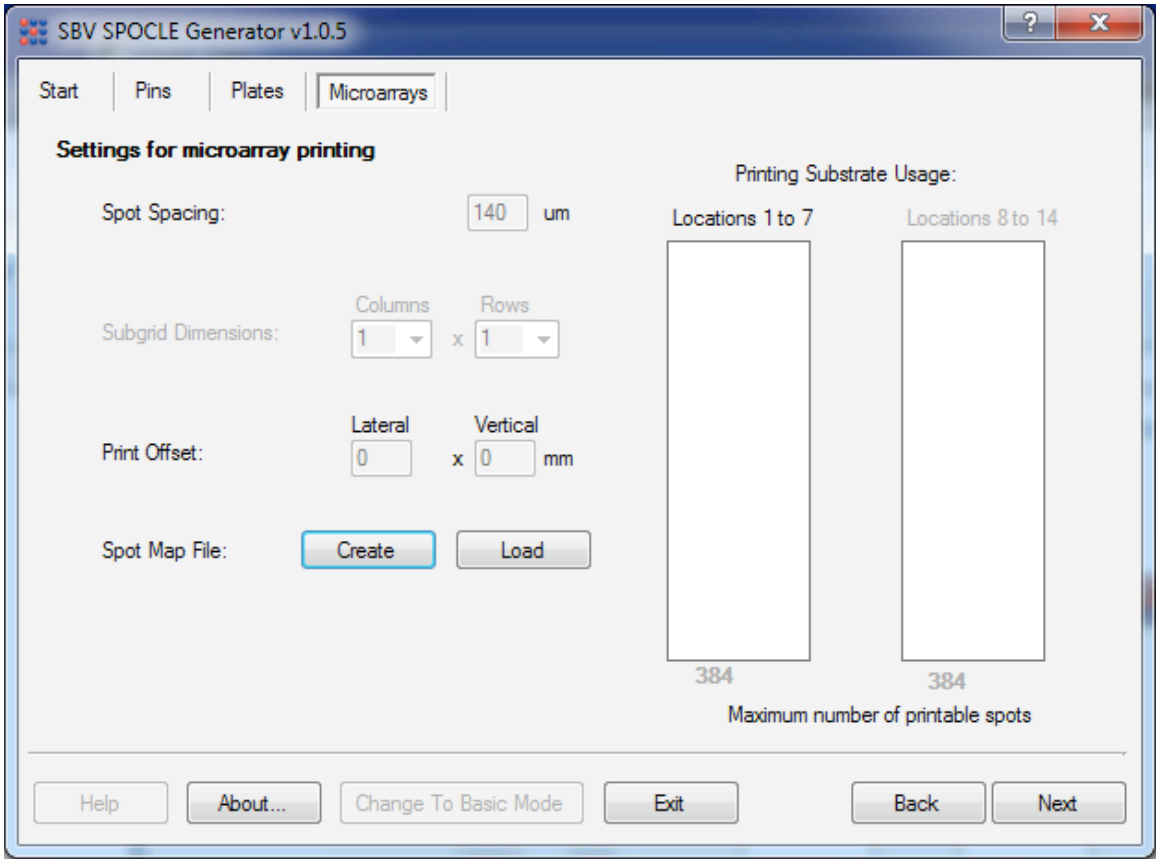
Create a Spot Map File by clicking Create in the SBV SPOCLE Generator Microarrays menu as shown here. The Spot Map File defines the location, number, and spacing of elements on the printing substrate.



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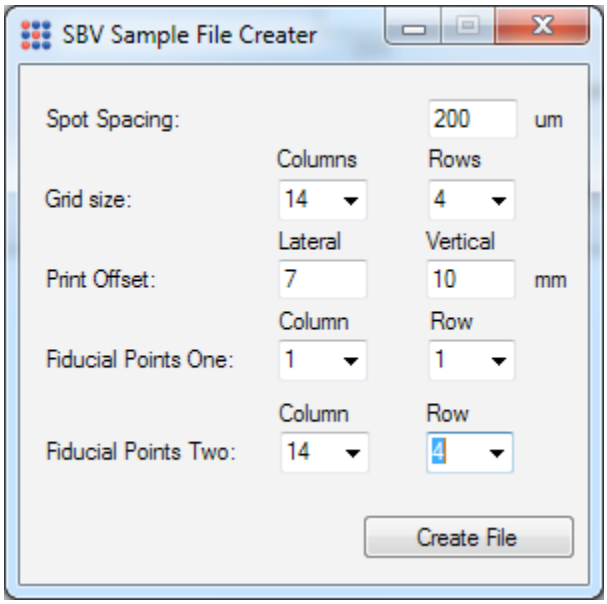
Use the SBV Sample File Creator to create a printing routine that matches the location, number and spacing of elements on the printing substrate. The printing substrate shown here has 14 columns and 4 rows of elements spaced at 200 micron centers, with the substrate located 7 mm from the leading (front) edge of substrate 8, and 10 mm down from the top (left) edge of substrate 8. Select the first and last printed spots as Fiducial Point One and Two, respectively. For the example shown here, Fiducial Point One is the printing location defined by Column 1 and Row 1, and Fiducial Point Two is the printing location defined by Column 14 and Row 4. SpotBot® 3 Vision System software defines "columns" as the printed elements running along the Y axis from the front to the back of the instrument, and "rows" as the printed elements running along the X axis from the left side to the right side of the instrument. Click Create File.



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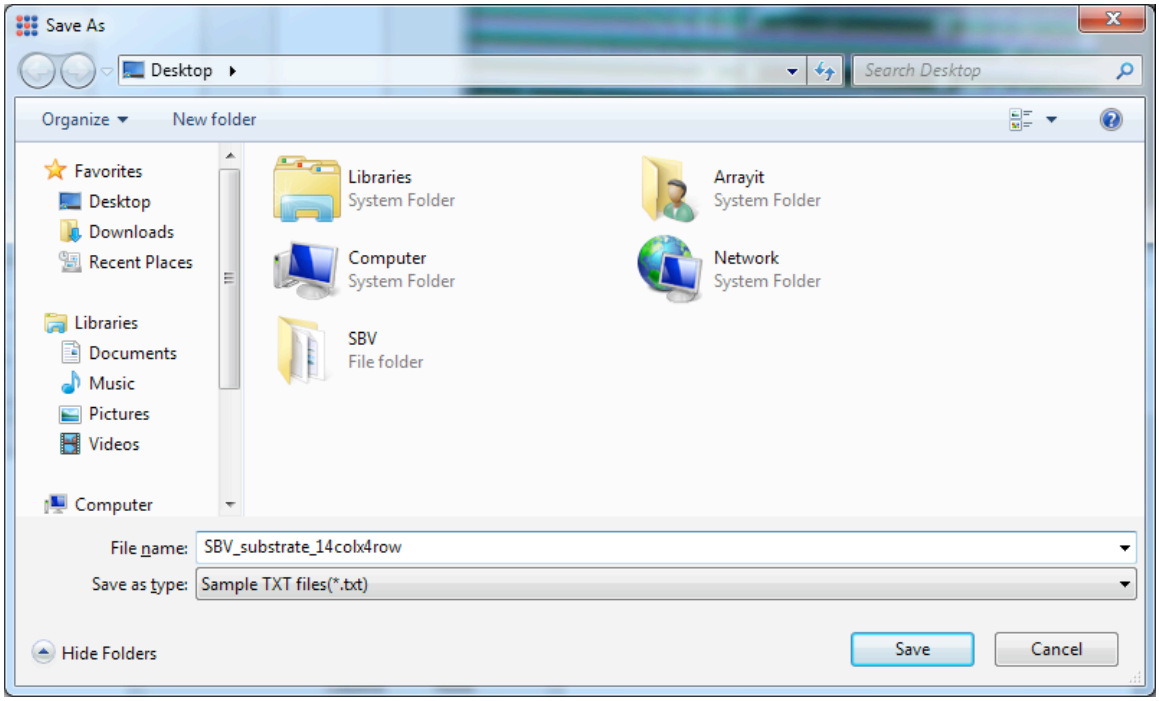
Save the Spot Map File using a descriptive file name as shown here. Click Save.



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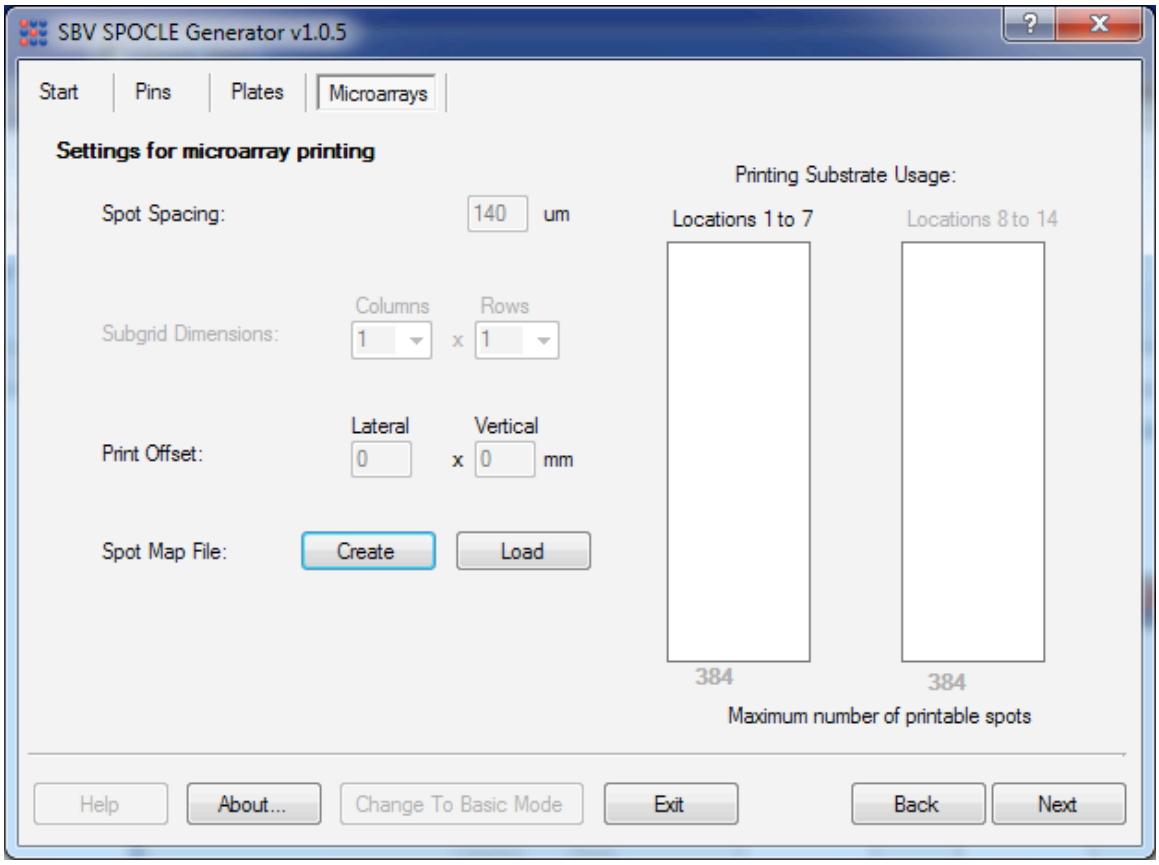
Click Load in the SBV SPOCLE Generator Microarrays menu to select the recently-saved Spot Map File.



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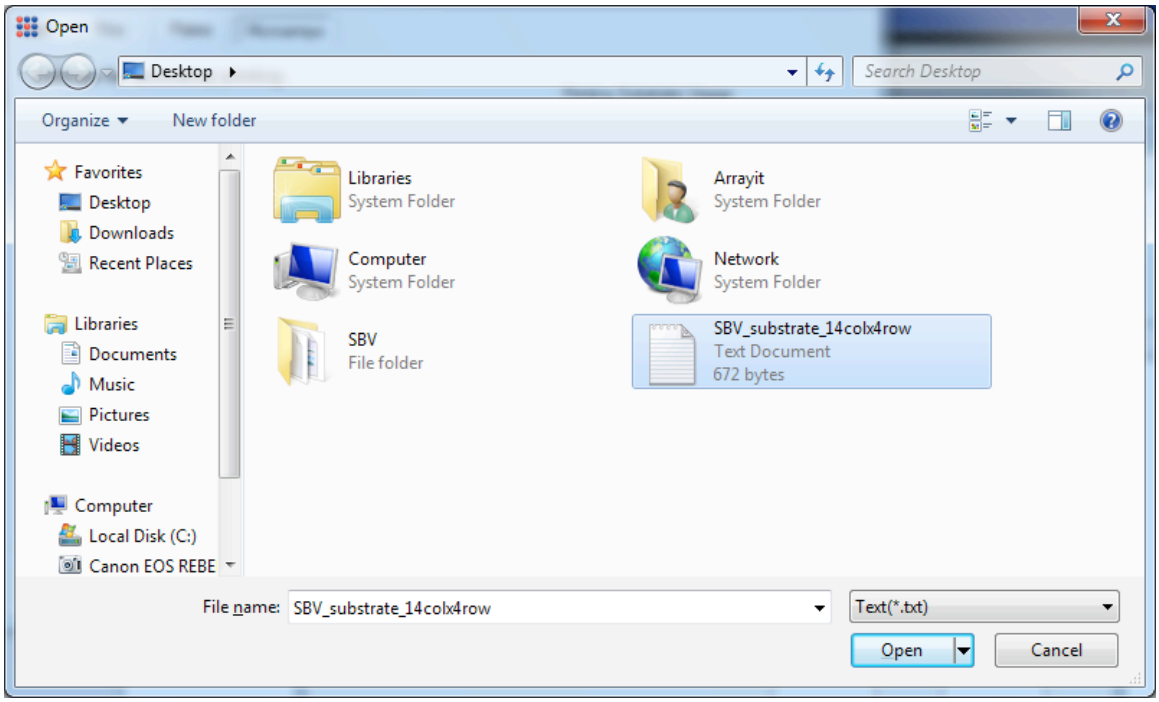
Click on the Spot Map File to highlight the file and Click Open to open the file as shown here.



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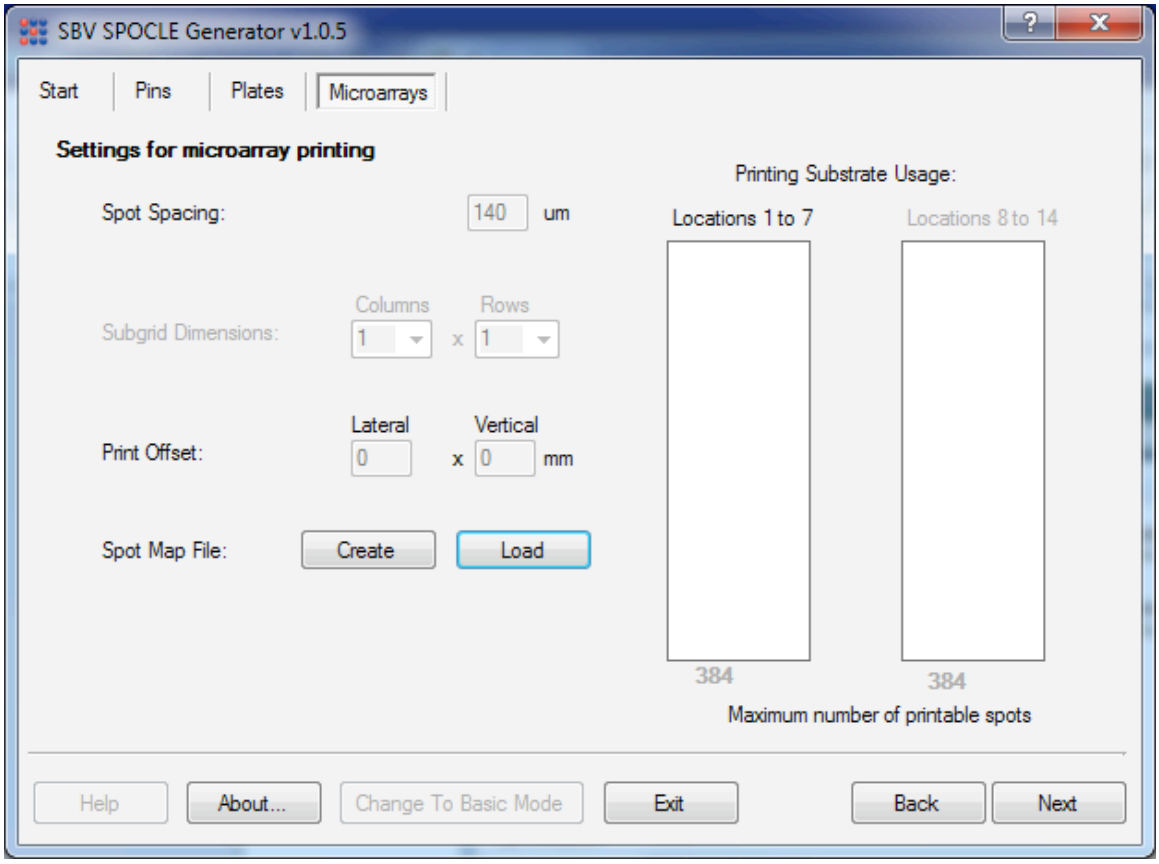
Click Next in the SBV SPOCLE Generator Microarrays menu to proceed to the next menu.



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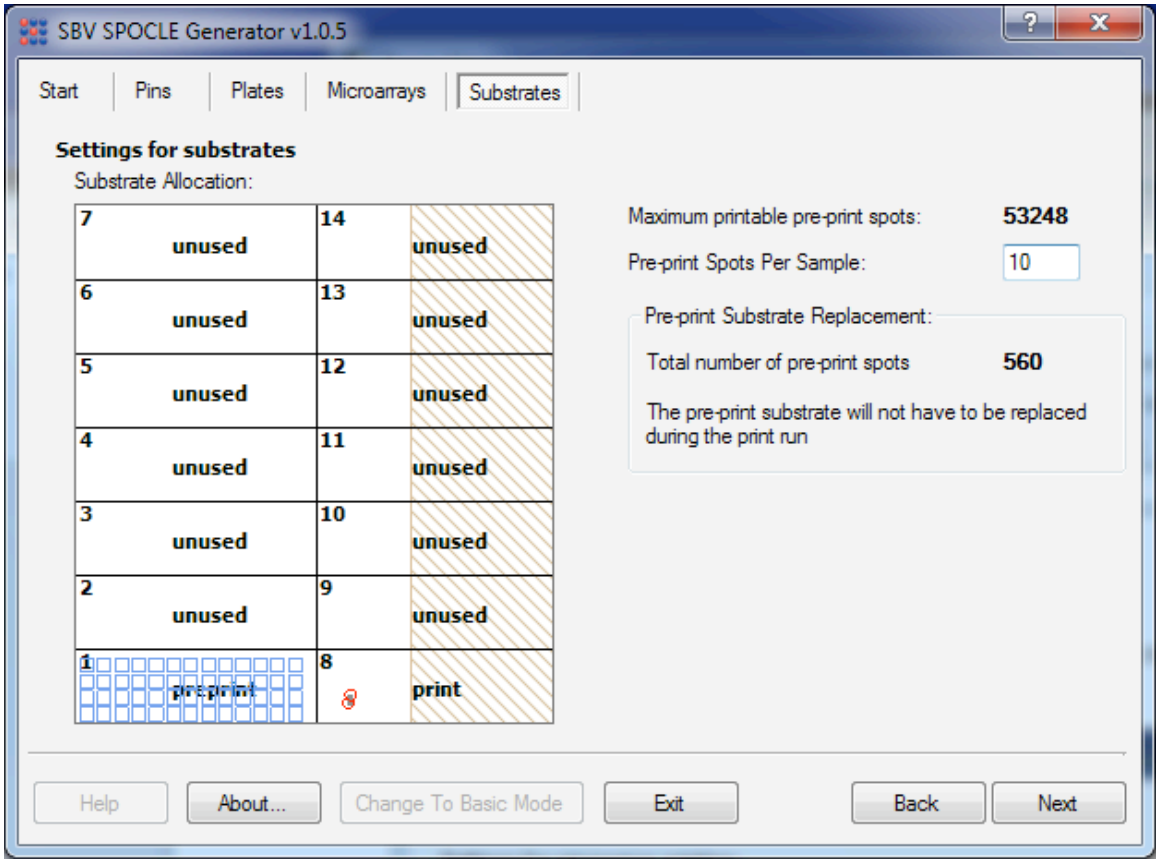
Select Substrate 1 as the preprint substrate, 8 as the print substrate, and 10 as the number of Pre-print Spots Per Sample in the SBV SPOCLE Generator Substrates menu as shown here. Please note that the position of the printing substrate on the SpotBot® 3 deck is shown as a red icon on substrate 8. Click Next.



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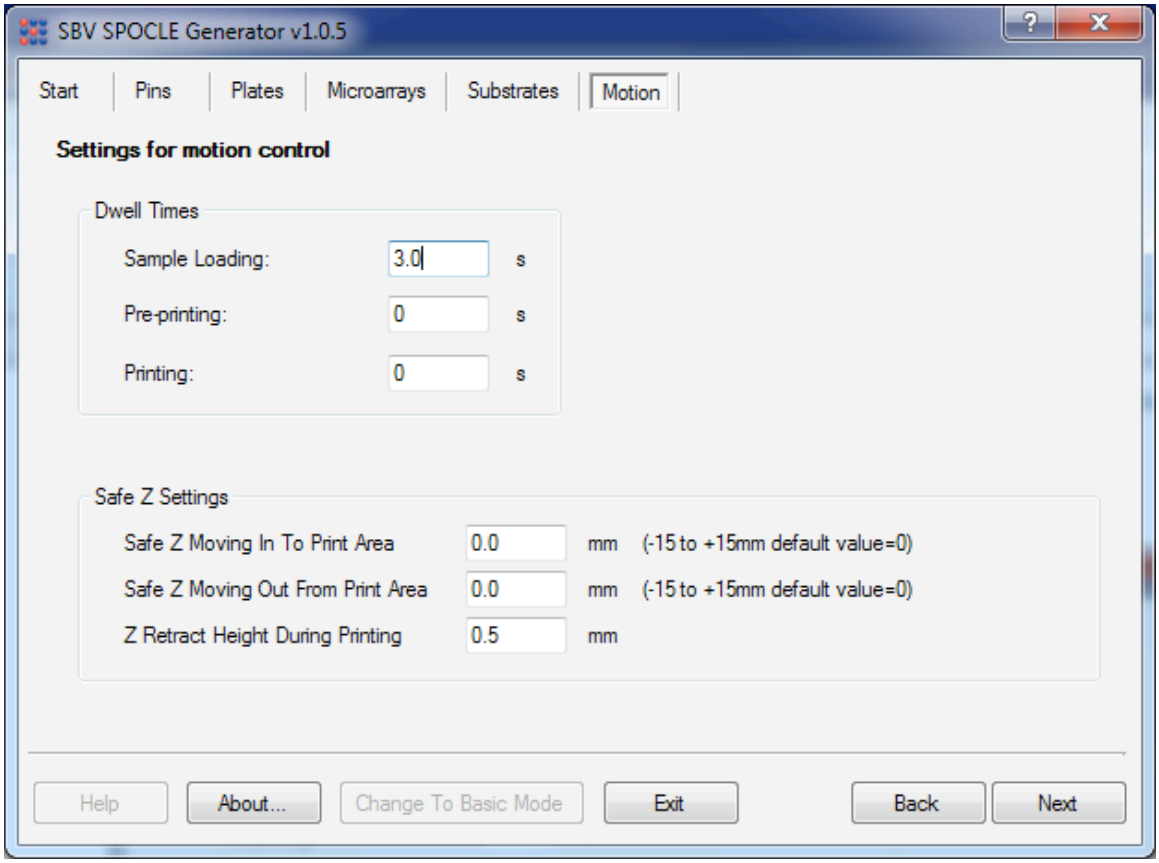
Enter 3.0 seconds in the Sample Loading Dwell Times, and use the default settings for the remaining SBV SPOCLE Generator Motion menu options as shown here. Please do not change the Safe Z Settings without technical assistance from Arrayit (arrayit@arrayit.com), as such changes may cause permanent pin and instrument damage. Click Next.



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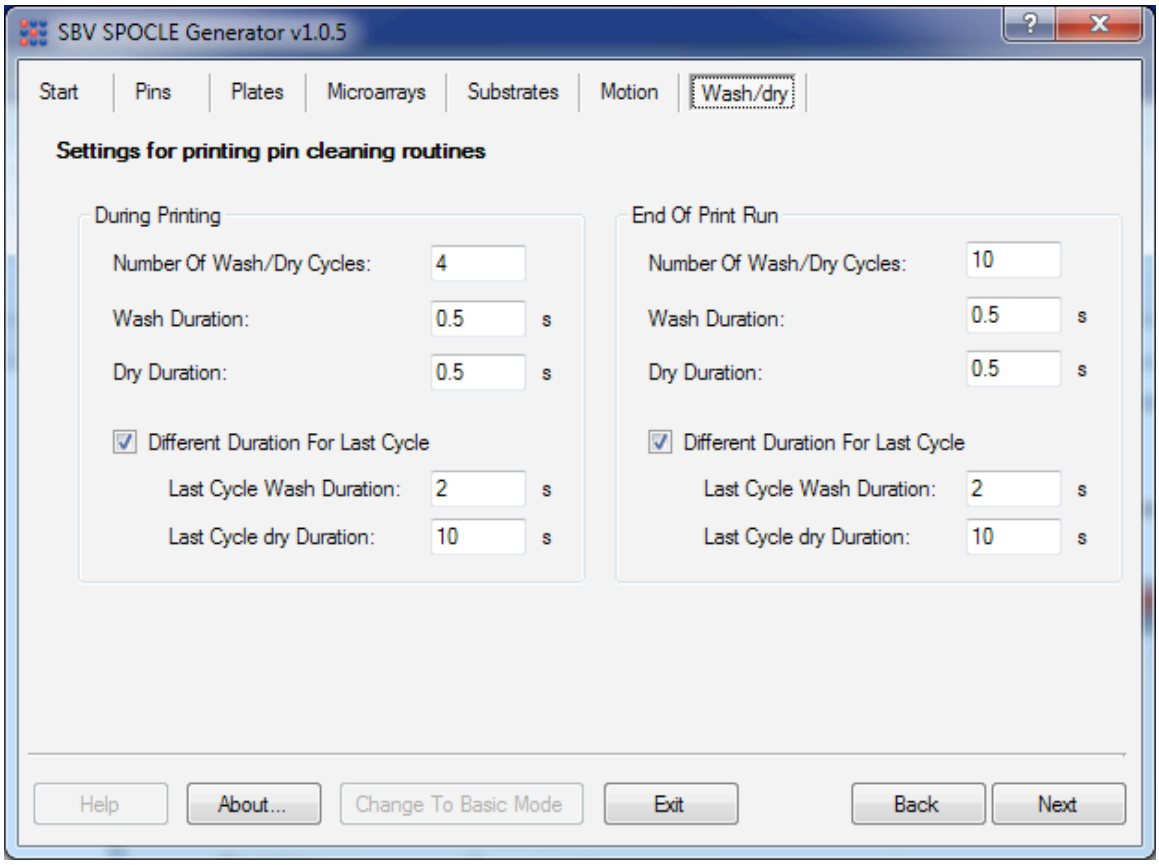
Use the default parameters in the SBV SPOCLE Generator Wash/dry menu as shown here. Click Next.



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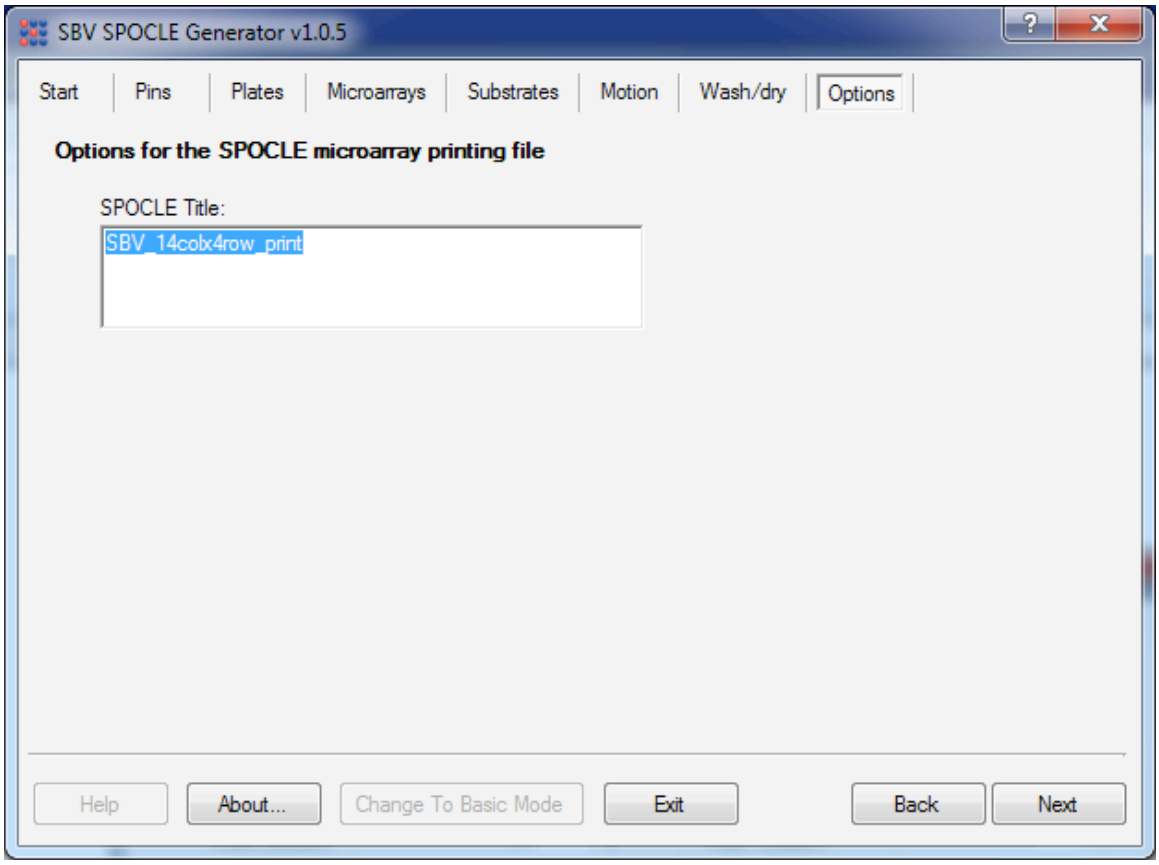
Enter a descriptive SPOCLE Title into the SBV SPOCLE Generator Options menu dialog box as shown here. Click Next.



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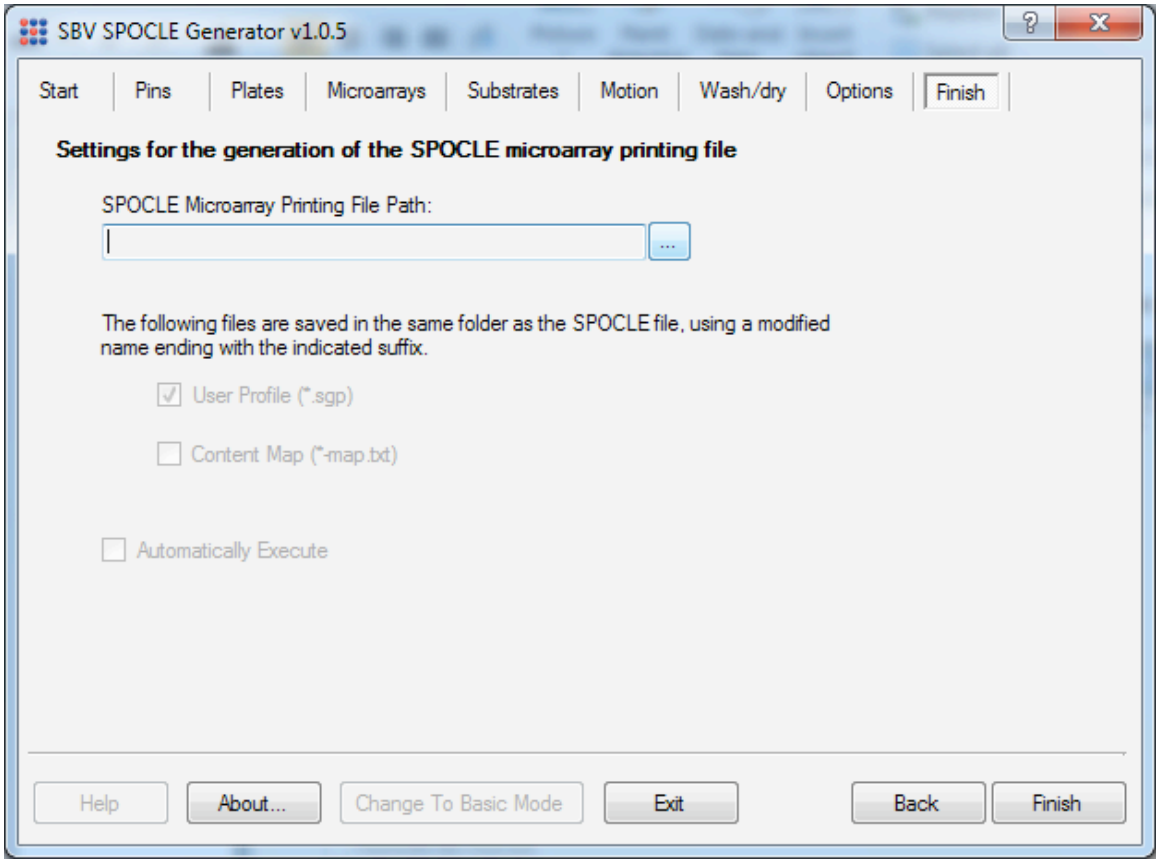
Enter a file name into the SPOCLE Microarray Printing File Path dialog box in the SBV SPOCLE Generator Finish menu as shown here. Save the file in an appropriate location on the computer as shown.



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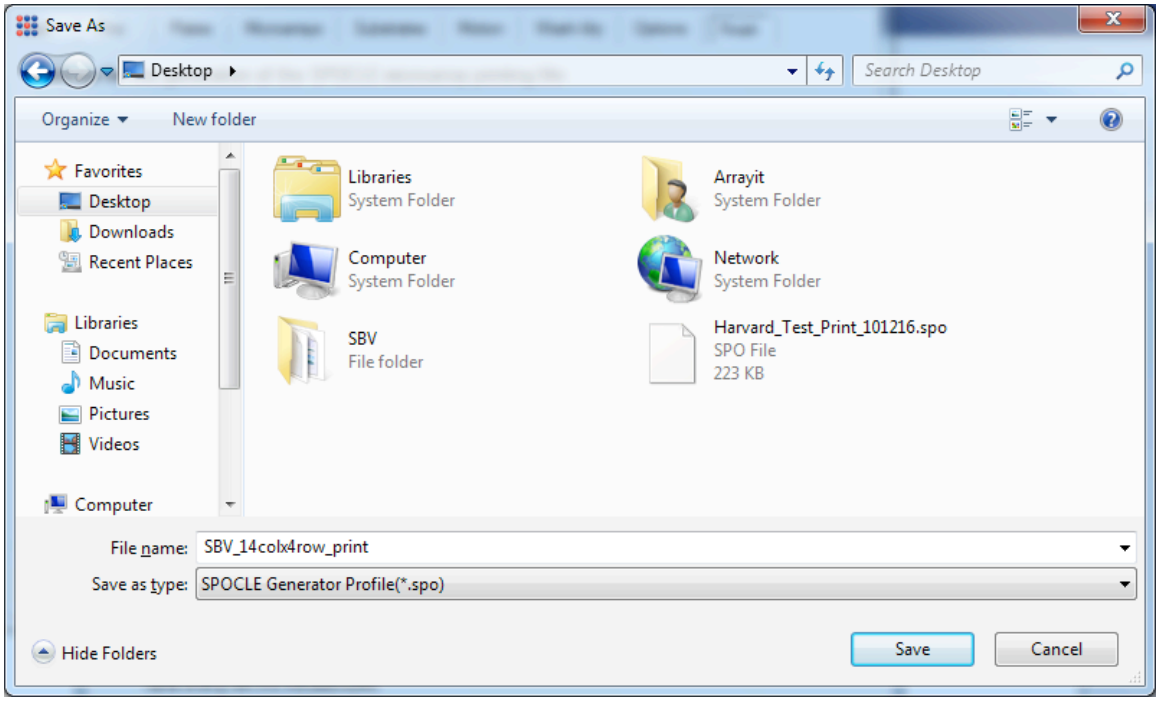
Enter the file name for the printing routine and click Save.



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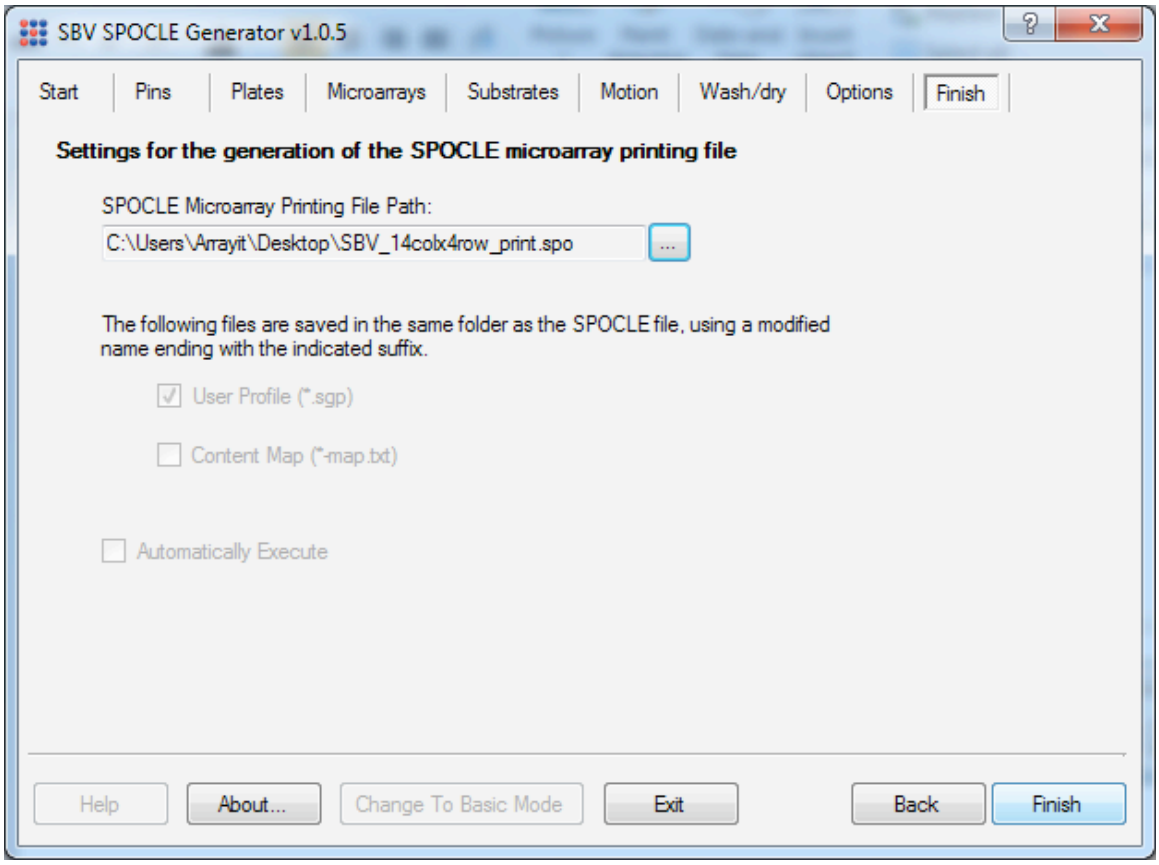
Click Finish in the SBV SPOCLE Generator Finish menu to complete the SPOCLE Generator process as shown here.



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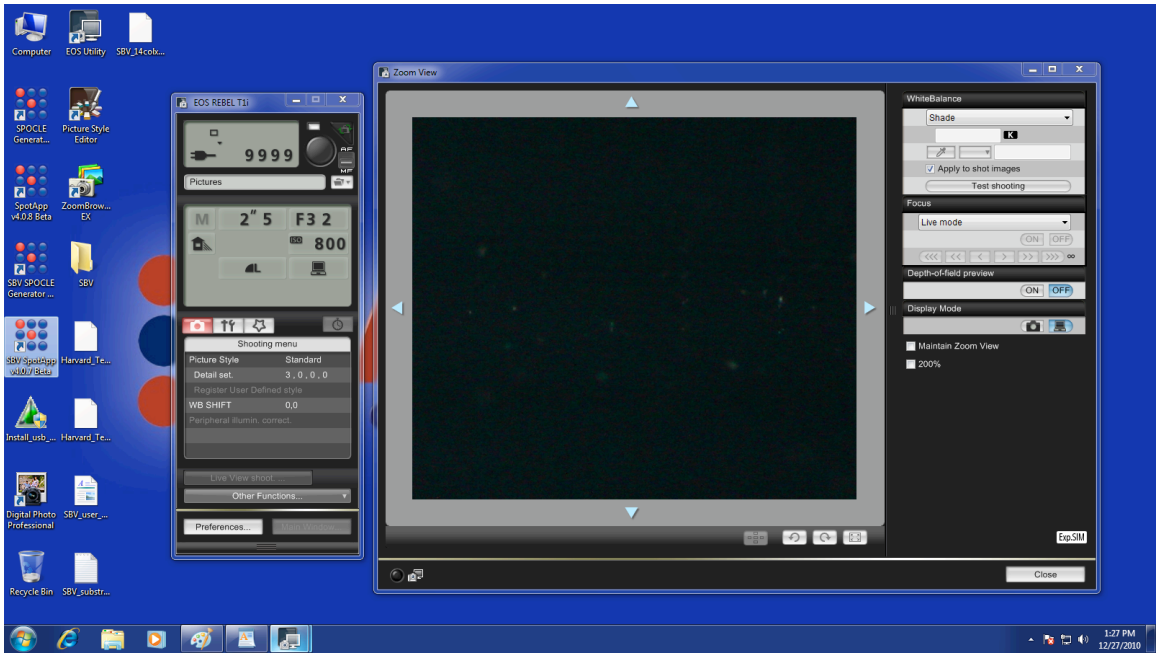
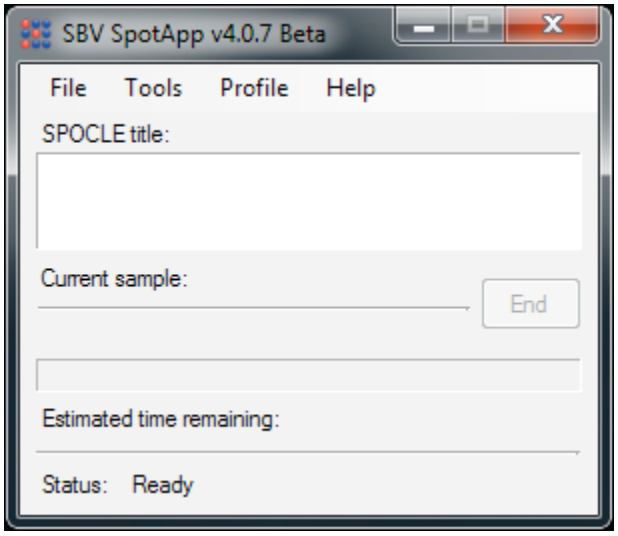
III. Calibration and the Printing Process

Click on the SBV SpotApp icon to launch the calibration and printing routine as shown here.



The SBV SpotApp window will appear and show "Ready" in the Status dialog located in the lower left corner of the window as shown here.



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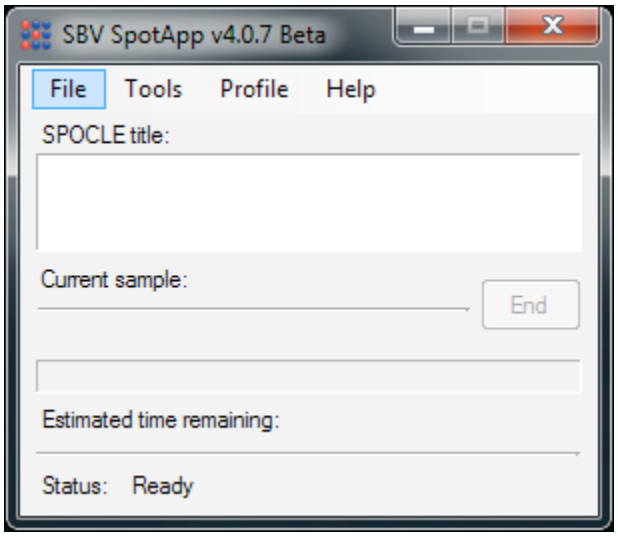
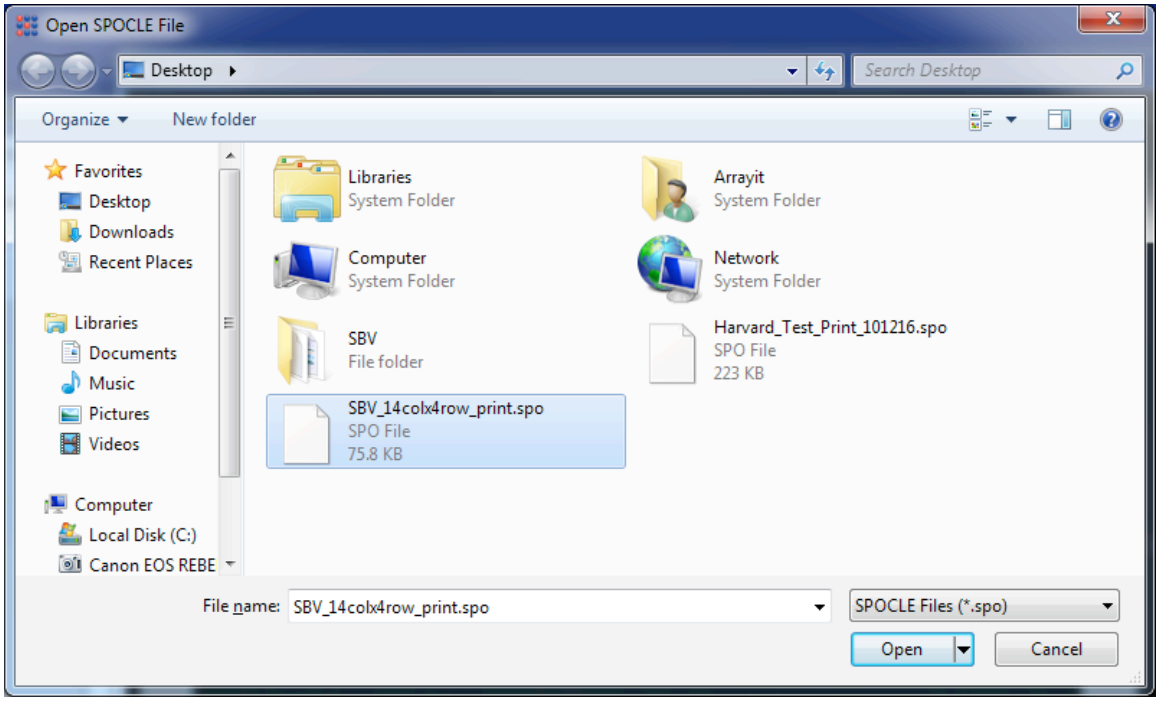
Click on the SBV SpotApp File menu as shown here to load the printing routine created using the SBV SPOCLE Generator.



Click "Open SpoCLe File" in the File menu and select the printing routine of choice as shown here. Click Open.



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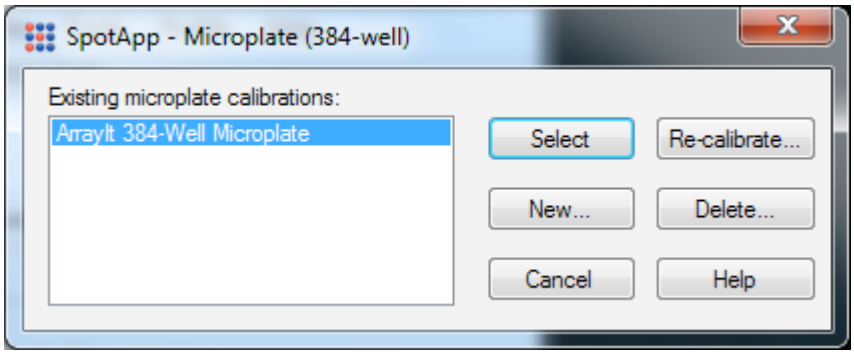
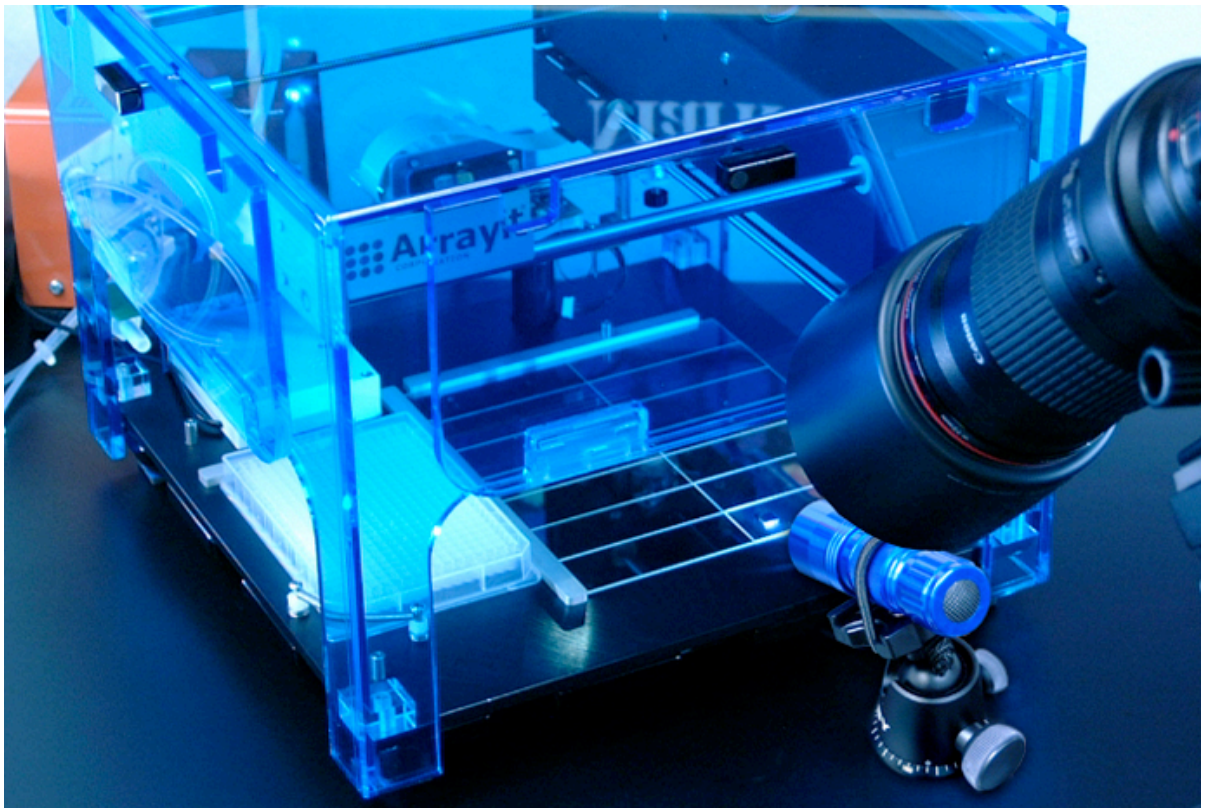
The SpotApp - Microplate (384-well) menu will appear as shown here. Click Select to choose the Arrayit 384-Well Microplate.



Insert the microplate into the proper location on the instrument deck, making sure that the microplate locator spring is pressing against the side of the microplate at well position A1. The microplate should be seated flat against the instrument deck, and firmly against the silver substrate locators at the right and rear sides of the microplate as shown here.



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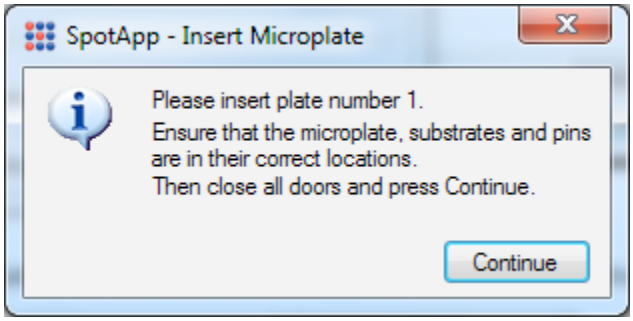
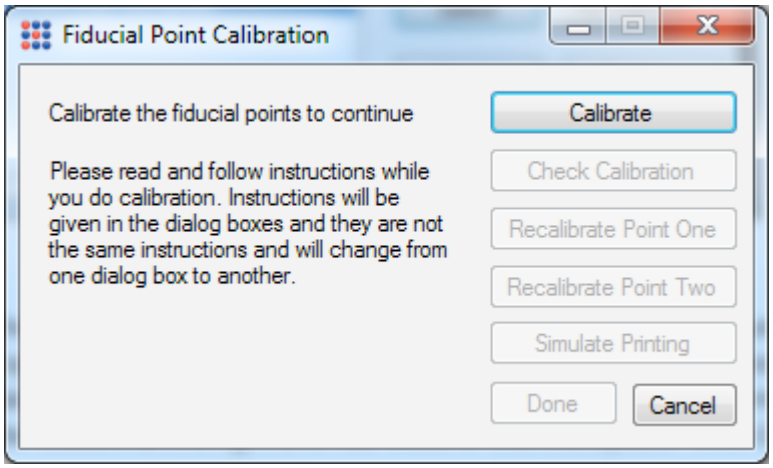
Once the microplate is positioned correctly, Click Continue.



The Fiducial Point Calibration window will appear as shown here. Click Calibrate to calibrate the printing positions on the custom substrate.



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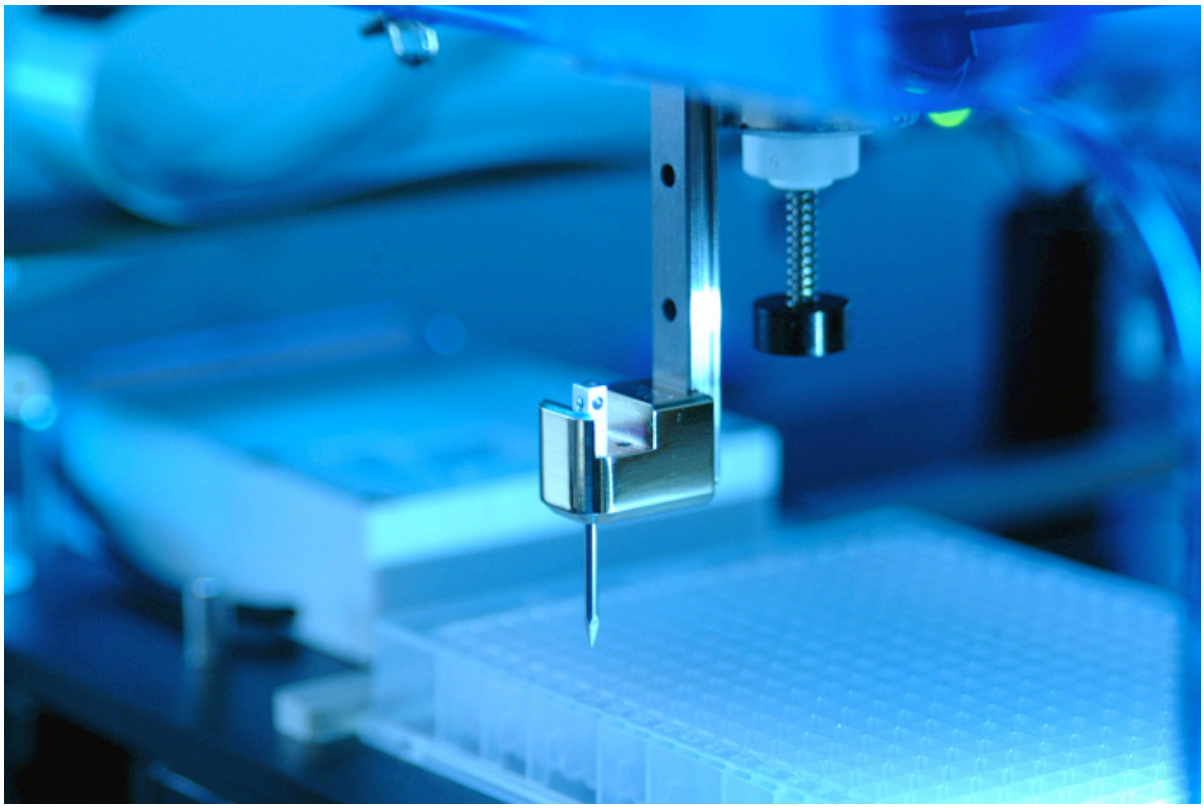
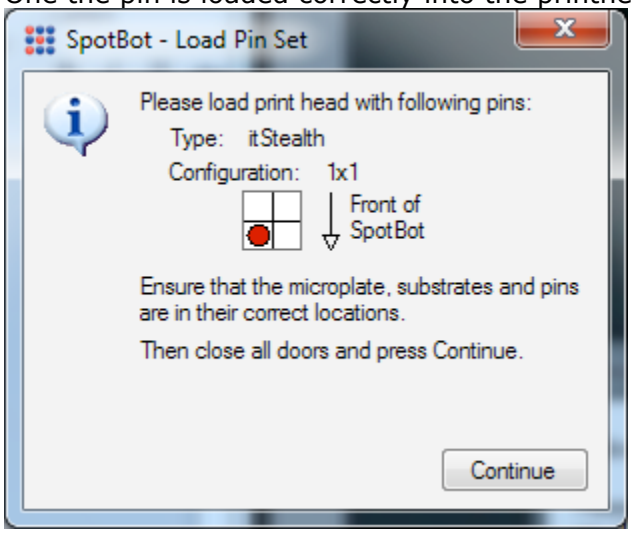
The printhead will move to the left side of the instrument over the microplate to allow pin loading. Load one pin into position 1 in the printhead as shown here.



One the pin is loaded correctly into the printhead, click continue.



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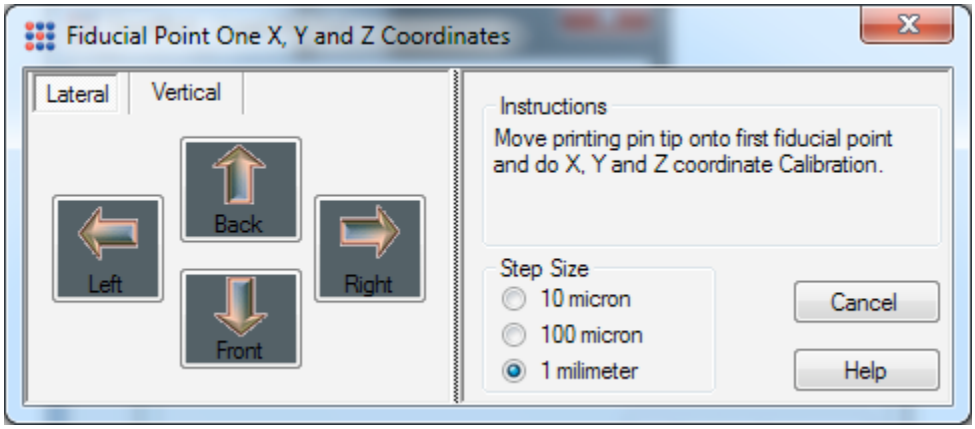
The printhead will now move over substrate position 8, at the front right side of the printing deck. Use the Lateral and Verticle tabs in the Fiducial Point One X, Y and Z Coordinates window to locate the exact printing location of position 1 (spot 1) on the custom printing substrate. Move the pin in 1 millimeter increments and then in 100 micron increments until the exact location is found. When moving the pin laterally, make sure to move the pin tip upward and off the surface of the substrate to avoid damaging the pin.



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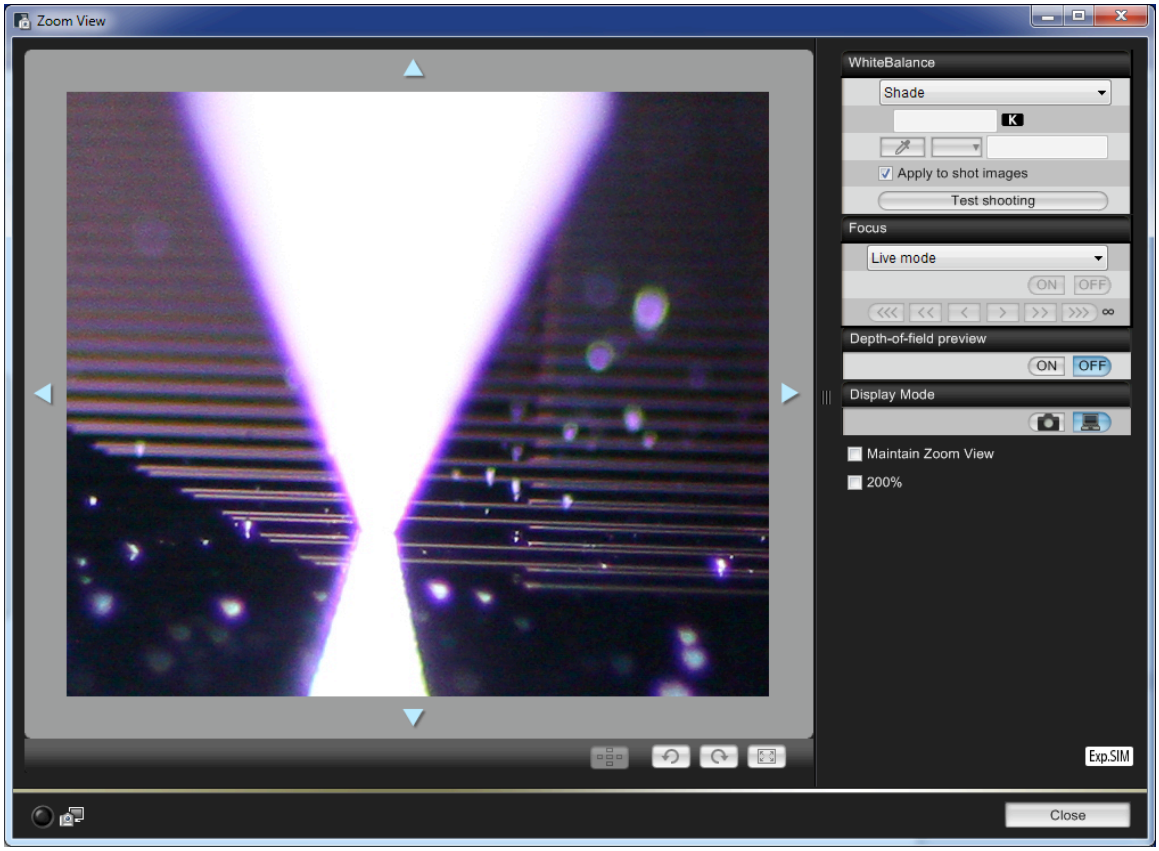
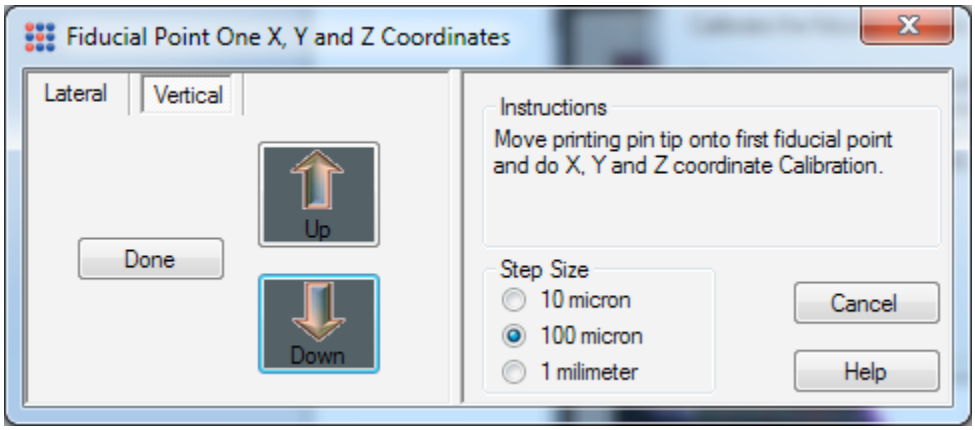
Use the Zoom View in Live View shooting to locate the precise location of the first printing position as shown here. Locate the surface of the printing substrate and then add an additional 200 microns (200 µm) of "overtravel", which will cause the pin to move upward by 200 microns in the printhead during printing.



To achieve the desired 200 µm of overtravel, click Down twice in the Vertical tab with the Step Size set at 100 microns as shown here.



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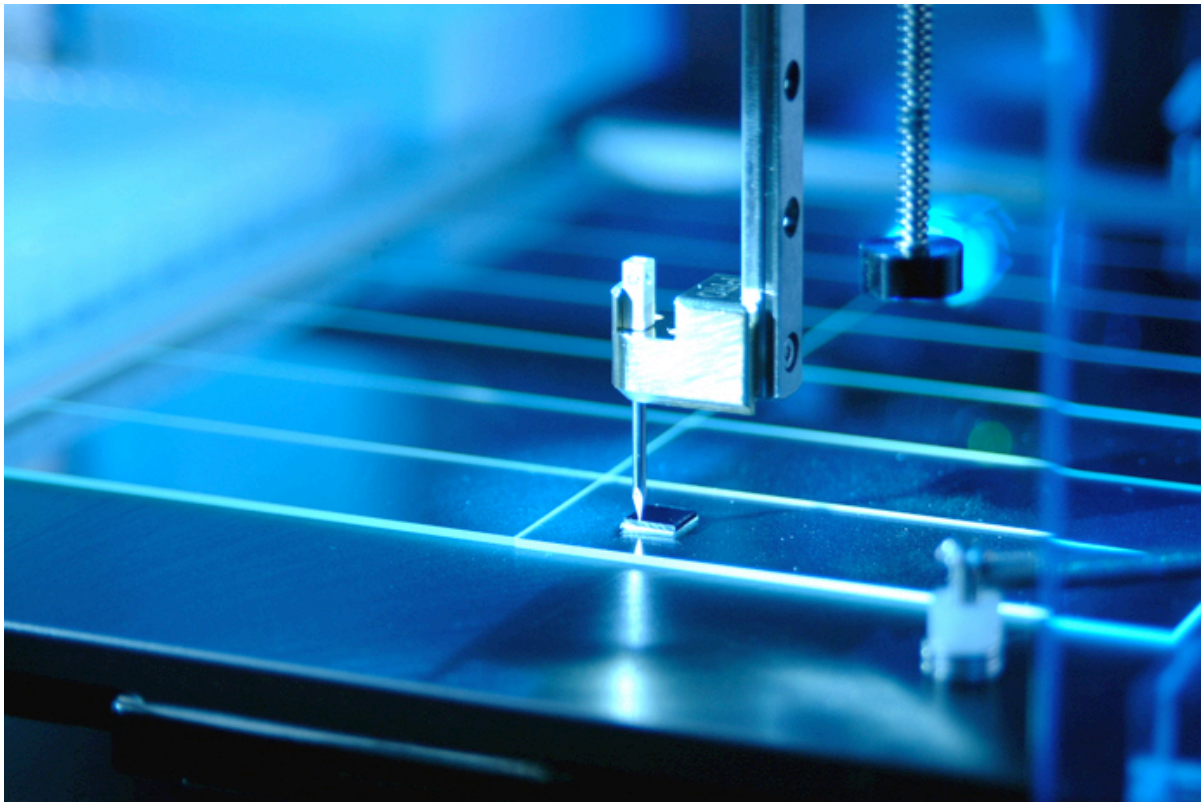
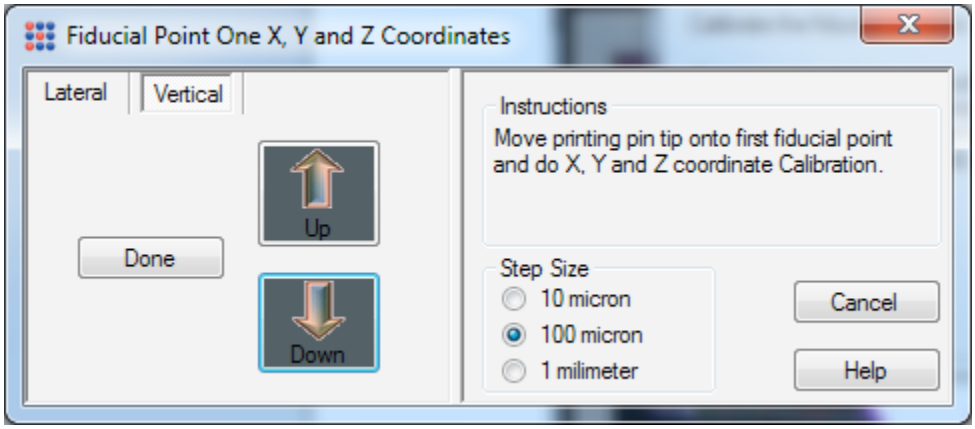
A properly calibrated Fiducial Point One will locate the first printing position exactly in X and Y, and the pin will ride up by 200 microns in the printhead for 200 microns of overtravel as shown here.



When Fiducial Point One has been calibrated properly in all three dimensions (X, Y and Z), click Done.



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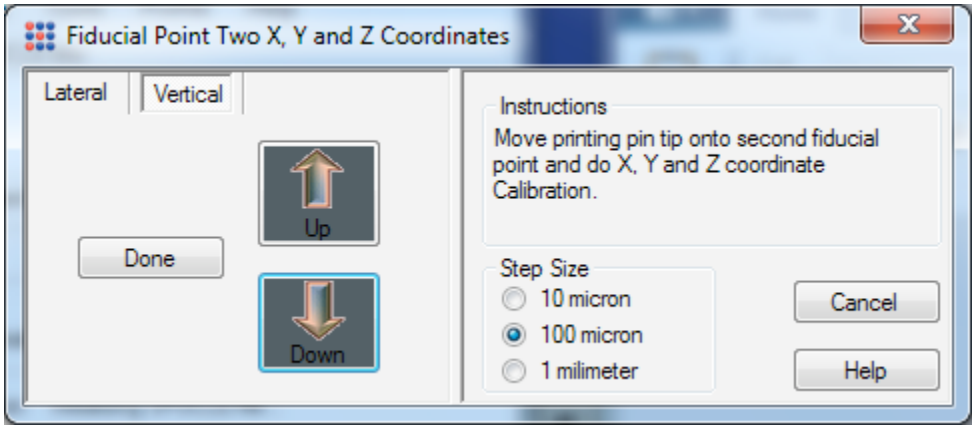
After Fiducial Point One has been calibrated, continue the calibration process to calibrate Fiducial Point Two using the Fiducial Point Two X, Y and Z Coordinates window shown here. Use the Lateral and Vertical tabs, the Up and Down arrows, and the digital image on the computer screen to locate Fiducial Point Two, which will be the last spot to be printed on the custom substrate.



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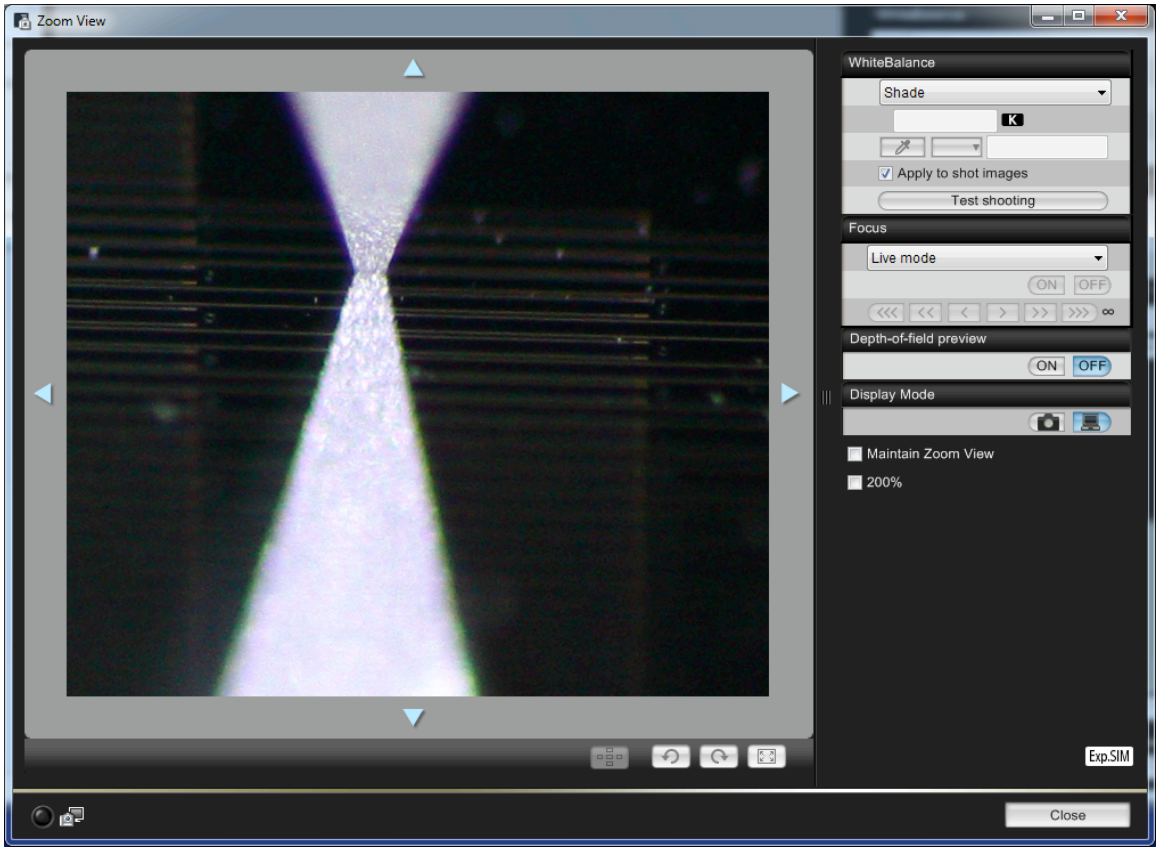
The digital image of the pin point on the computer screen should be used to locate the precise three dimensional location of Fiducial Point Two as shown here. Make sure to add 200 microns of overtravel so that the pin rides up by 200 µm in the printhead as shown in the next photograph.



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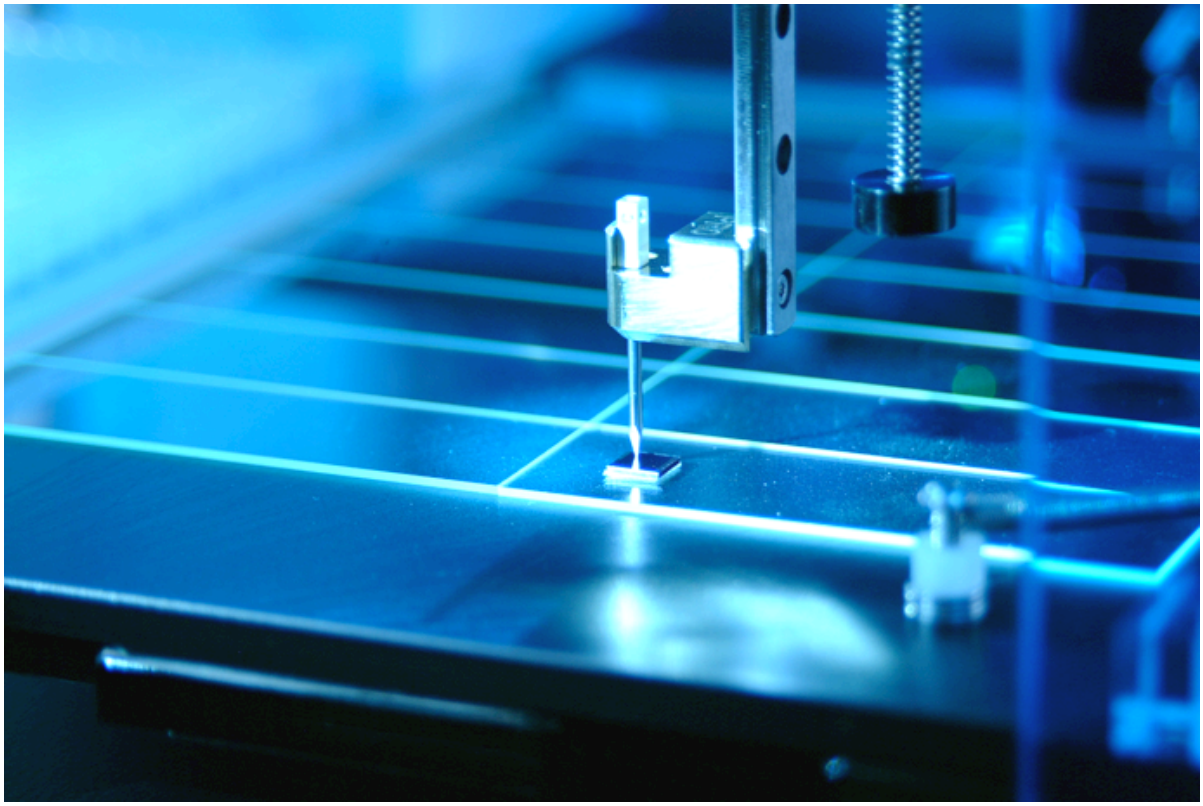
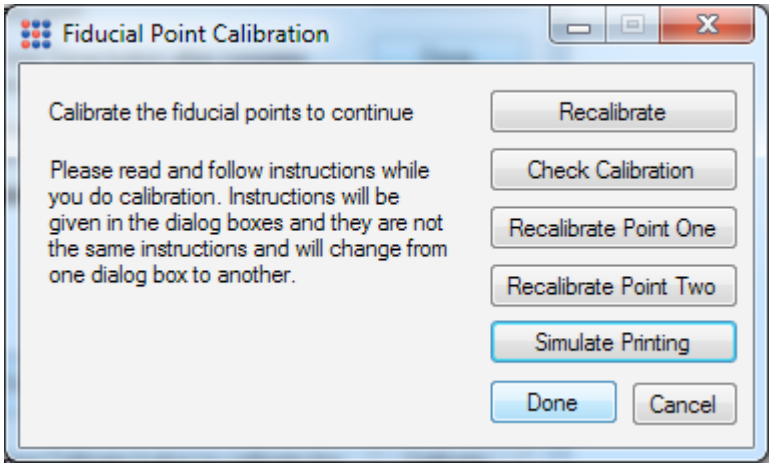
A properly calibrated Fiducial Point Two will locate exactly over the last printing position in X and Y, and the pin will ride up by 200 microns in the printhead for 200 microns of overtravel as shown here.



After both calibration points (Fiducial Points One and Two) have been defined, click on Simulate Printing in the Fiducial Point Calibration window to simulate the printing process prior to printing actual samples on the printing substrate. Use the camera and computer screen to monitor the printing position to ensure that the pins are printing at the correct locations on the custom substrate.



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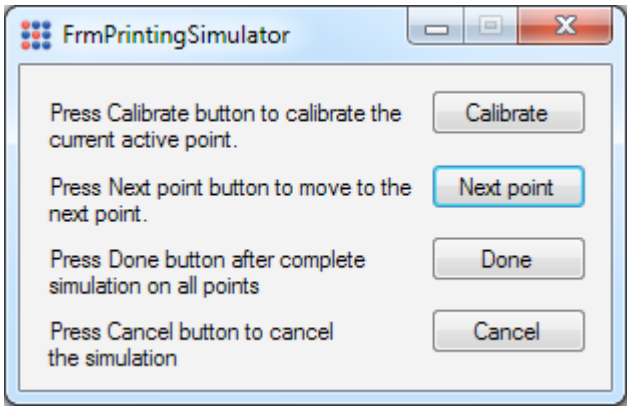
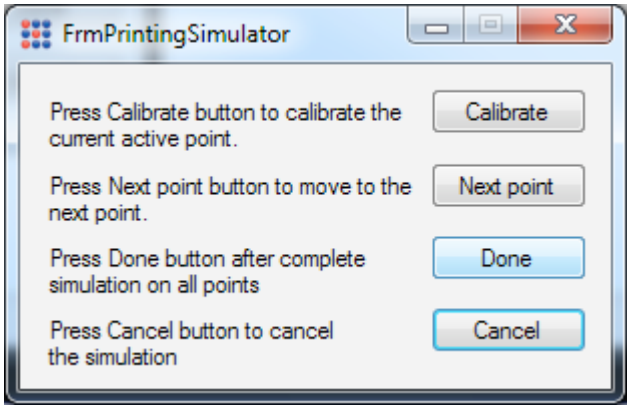
After the pin addresses each printing location, click on Next point in the Frm Printing Simulator to progress through the entire printing routine.



Once the entire printing routine has been simulated and checked for positional accuracy, click Done in the Frm Printing Simulator to complete the printing simulation.



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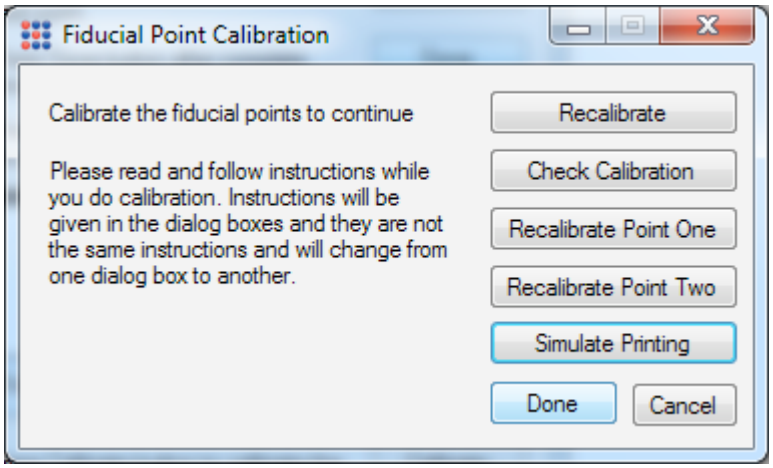
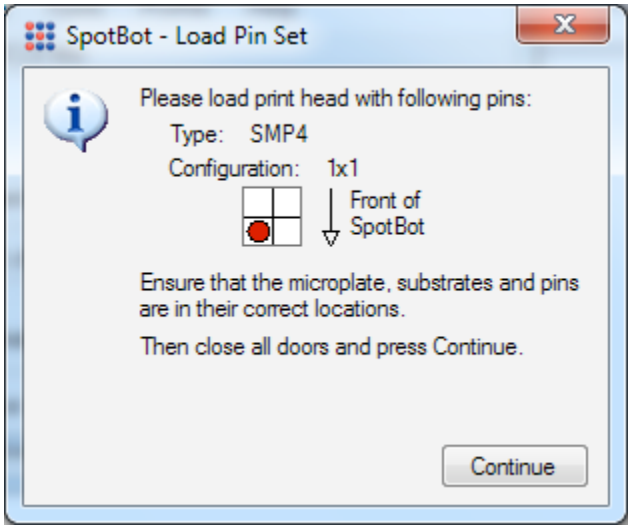
Then click Done in the Fiducial Point Calibration window to proceed to the actual printing process as shown here.



When the SpotBot – Load Pin Set window appears, make sure the printing pin is loaded correctly in the front left pin position as shown here in the SpotBot – Load Pin Set window. Click Continue to begin the printing process.



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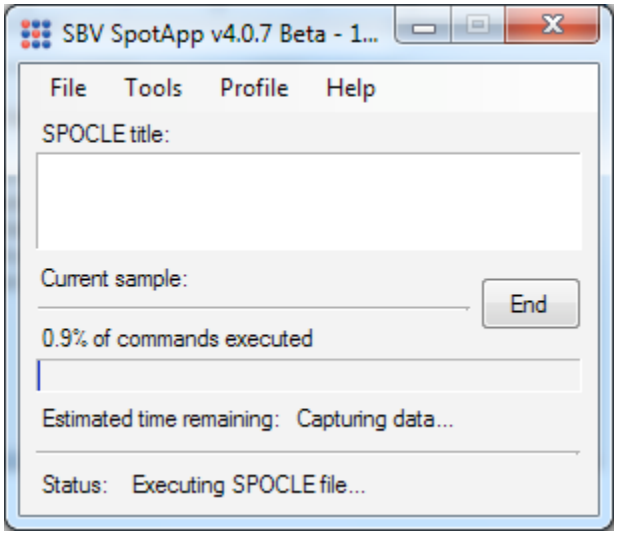
Once printing begins, the SBV SpotApp v4.0.7 Beta window will appear as shown here. The Status bar in the lower left corner of the window displays printing progress in real time. Allow printing to proceed until the entire routine is complete. Upon completion, the printhead will return to the "home" position in the back right corner of the instrument.



Once the printing process is complete, the Status bar will report "SPOCLE completed" as shown here. Click on the red "X" in the upper right corner to close the SBV SpotApp v4.0.7 Beta software. The software should be closed after each printing session to conserver energy and prevent undue instrument wear.