

MICROPLOTTER[™] PROTO

MANUAL

REVISION 1.4

SonoPlot, Inc. 3030 Laura Lane, Suite 120 Middleton, WI 53562 (608) 824-9311 Contact@sonoplot.com

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Congratulations on your purchase of a SonoPlot Microplotter instrument! A Microplotter system is a leading-edge tool for the fabrication of biological microarrays, polymer electronics, or any other application that requires precise and accurate dispensing of extremely small liquid volumes. It can dispense picoliters of a wide variety of solutions onto almost any planar surface in discrete spots or continuous traces such as lines or arcs.

This document will guide you through the installation and setup process for your Microplotter system in Chapter 2, followed by walkthroughs of simple dispensing applications in Chapter 3. The software package that controls the dispenser and automate the dispensing of patterns on a surface, SonoGuide, will be described in Chapters 4. Solutions to common problems will be listed in Chapter 5 and the technical specifications will be described in Chapter 7.

Before that, however, it would be appropriate to describe the fundamental operating principles of a Microplotter instrument.

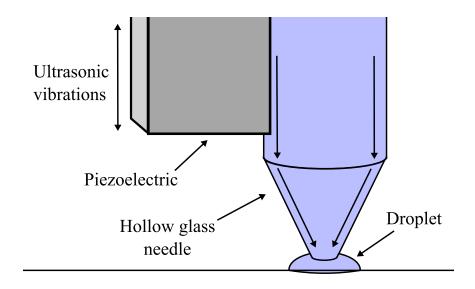
Ultrasonic fluid dispensing

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A Microplotter instrument is capable of applying picoliters of fluid to a surface in order to create features as small as 20 micrometers (microns) wide due to its use of a novel form of fluid ejection based on ultrasonic pumping.

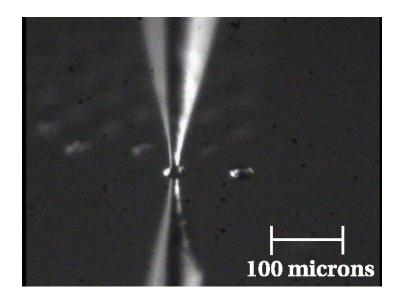
The dispenser at the core of a Microplotter system is composed of a hollow, tapered glass needle attached to a piezoelectric element. When an alternating current is supplied to the piezoelectric, it vibrates. At certain frequencies, the resonant frequencies of the dispenser, a pumping action occurs within the needle or, if the amplitudes of vibration are high enough, fluid is sprayed out of the end of the needle. This electronically controlled pumping action makes it possible for microscale fluid features to be put down by a Microplotter instrument. The spraying action allows for rapid switching between different solutions while minimizing cross-contamination.

To dispense a spot of fluid on a surface, a Microplotter dispense head is first loaded with fluid and then brought near the surface. The ultrasonic pumping action is activated by the controlling computer either automatically when running a preset pattern or manually by the user. This pumping causes the fluid meniscus at the tip of the dispenser to bow outward. When the dispenser is brought close enough to the surface, a droplet will be touched off. The ultrasonics are then deactivated and the dispenser is retracted from the surface.



Fluid deposition via ultrasonic pumping

A Microplotter system also has the unique capability of dispensing continuous lines. This is accomplished by starting as if to create a spot on a surface, but not deactivating the dispenser after fluid contact has been made with the surface. Instead, the dispenser is moved along the surface in a straight line, leaving behind a smooth line wherever it passes. Only when the line is finished is the dispenser deactivated and retracted from the surface.



Aqueous solution being deposited in a 50 micron diameter spot

Surface height sensing

The detection of the surface height is handled by an electronic feedback system that uses changes in the vibrational properties of the dispenser to detect whether or not it has contacted a physical object, no matter how gently. This surface sensing capability allows for the cant of a surface to be calibrated, so that the control software can maintain a consistent height of the dispenser above the surface for reliable dispensing.

Precision robotic positioner

The Microplotter picoliter dispenser would not be of much use without a precision positioning system to place that dispenser at select locations, and do so in an automated fashion. The robotic positioner of the Microplotter system occupies most of its physical volume, and can move the dispenser over a 31 x 31 x 7 cm volume with a 10 micron resolution in the X, Y, and Z axes. All of this movement is controlled through the SonoGuide software package (described in Chapter 4) either manually by the user or automatically through the running of a pattern.

As you read through the following chapters, you will learn how to install, configure, and operate your Microplotter Proto instrument in order to take advantage of its unique capabilities in your line of research or development.



Unpacking and setup

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The Microplotter Proto instrument will come in one large crate containing the system itself, the control electronics, and a box of smaller components.

Warning: The positioning system is heavy, and should be lifted by more than one person. When lifting the system, do not pull on the positioning arm, as this may damage the stages.

Once unpacked, the system consists of the following components:

- Positioning system, with printhead and optics system already mounted
- Control electronics
- · A box containing cables for attachment of the various components

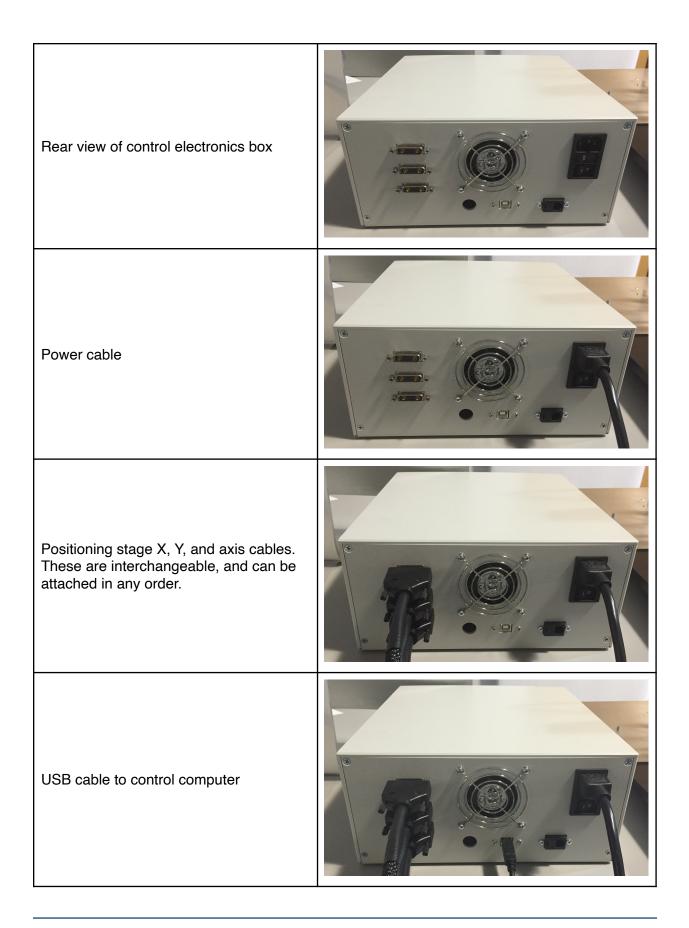
Additionally, SonoGuide, the primary interface for your Microplotter system, will need to be installed to a controlling computer.

Positioning system

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After unpacking, the first step in the setup process is to move the positioning system into place. Two people should be able to lift the system from its base and position it so that its feet are firmly on a table.

Next, place the control electronics box on either side of the system, and connect the cables to the electronics as follows:



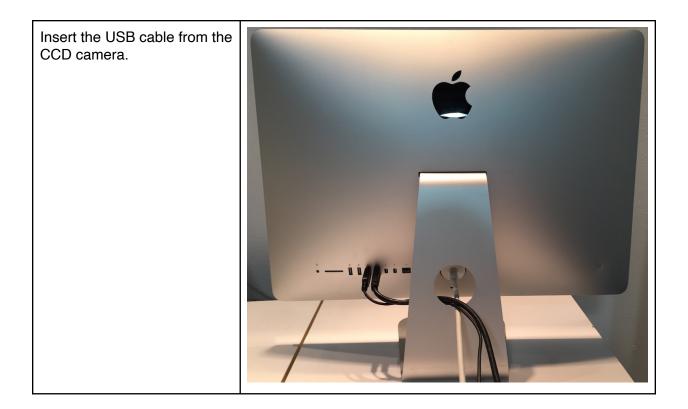


Connecting the system to a computer

Once the cables have been connected to the electronics box, insert the USB cable from the electronics box into a USB port on your computer and also insert the USB cable from the CCD camera to a different USB port on your computer. This step is shown below on a Mac computer. The process is the same for a Windows computer.

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If your system comes with a relay controller within the control electronics box (an optional accessory for triggering external devices), connect the USB cable for that to the computer and connect the power leads to the appropriate terminals on the device to be controlled. Finally, plug in the power connectors to the control electronics.

Installing the SonoGuide software

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The SonoGuide software is available on both the MacOS platform and Windows platform. Follow the steps below to install the SonoGuide software on your computer.

MacOS

Go to <u>www.sonoplot.com/downloads</u> and click on the **SonoGuide for Mac** link.

Download the SonoGuide.DMG file.

Once downloaded, double click on the DMG file. The SonoGuide Installer window will appear on the screen:



Drag the SonoGuide icon over to the Applications folder.

SonoGuide has now been installed to your MacOS computer.

Windows

Go to <u>www.sonoplot.com/downloads</u> and click on the **SonoGuide for Windows** link.

Download the SonoGuideInstaller.exe.

Once downloaded, run the installer. Select the Installation Folder.

		Setup - SonoGuide	
Setup - SonoGuide	^	Welcome to the SonoGuide Setup Wizard.	
Installation Folder			
Select Components			
License Agreement			
Start Menu shortcuts			
Ready to Install			

		Installation Folder		
Setup - SonoGuide	^	Please specify the directory where SonoGuide will be installed.		
nstallation Folder		C:\Program Files\SonoGuide	E	rowse
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Select the components to install.

SonoGuide Installer	Setup		
		Select Components	
Setup - SonoGuide	^	Please select the components you want to install.	
Installation Folder		Default Select All Deselect All	
Select Components		SonoGuide	SonoGuide Application
License Agreement			
Start Menu shortcuts			
Ready to Install			This component will occupy
	~		approximately 182.58 MB on your hard disk drive.

Select where you would like to create SonoGuide's shortcut.

			×
← SonoGuide Installer Se	etup		
		Start Menu shortcuts	
Setup - SonoGuide	^	Select the Start Menu in which you would like to create the program's shortcuts. You can also enter a	aname
Installation Folder		to create a new directory.	
Select Components		SonoGuide	
License Agreement		Accessibility Accessories	^
Start Menu shortcuts		Administrative Tools Anaconda3 (64-bit)	
Ready to Install		GitHub, Inc Maintenance	
	~	OriginLab Origin 2020	*
		Next Car	ncel

SonoGuide is ready to be installed.

	×
← SonoGuide Installer Setup	
Satura SanaGuida	Ready to Install
Setup - SonoGuide	Setup is now ready to begin installing SonoGuide on your computer. Installation will use 182.58 MB of disk space.
Select Components	
License Agreement	
Start Menu shortcuts	
Ready to Install	
	Install Cancel

Click Install to start installing SonoGuide.

		×
 SonoGuide Installer Setup 		
,	Installing SonoGuide	
Installation Folder		36%
Select Components	Installing component SonoGuide	
License Agreement	Show Details	_
Start Menu shortcuts		
Ready to Install		_
Installing		_
	Install Can	cel

Once the installer has finished installing SonoGuide, click on Finish.

SonoGuide has been installed and is ready to use.

Loading a dispenser cartridge

Warning: Avoid contacting the exposed glass needle to any surface, as even a slight touch can shatter the fragile tip.

To load a dispenser cartridge, carefully place the notched side of the dispenser onto the printhead, as shown.

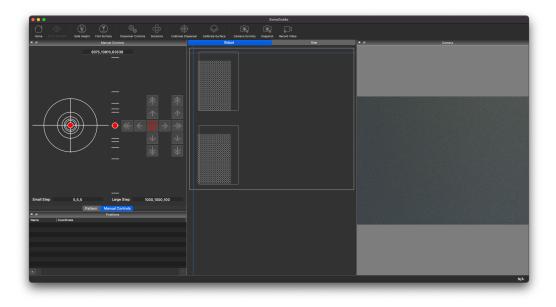
Plug the RJ-11 cable into the RJ11 adapter.



Powering on the system

Once all components have been assembled, the control electronics are plugged in, and the SonoGuide software has been installed, it is possible to power on the system and start dispensing. To turn on the control electronics flip the power switch for the control electronics on the rear left as you face the front of the control box. When the system is powered on, the green power light and orange COMM light will be illuminated on the front of the box.

Double click on the SonoGuide icon to open the controlling software. SonoGuide, described in detail in Chapter 4, is your primary interface with a Microplotter system, allowing you to manually move the positioner, automatically dispense complex patterns, control the dispenser, and observe and record a digital video feed from a high-magnification CCD that tracks the dispenser.



SonoGuide



Fully installed Microplotter Proto system

SAMPLE APPLICATION

In this chapter, two sample applications of a Microplotter system will be used to illustrate its capabilities and the basic functions of its hardware and software. The first, a deposition of a one-component oligonucleotide (DNA) microarray, serves as an introduction to the operation of a Microplotter instrument and its software while providing an example of a common application for this technology within the biological sciences. The second application, dispensing conductive traces from nanometallic inks, expands upon the first and demonstrates the ability of a Microplotter instrument to lay down continuous lines.

These examples assume that the system has been properly installed as described in Chapter 2.

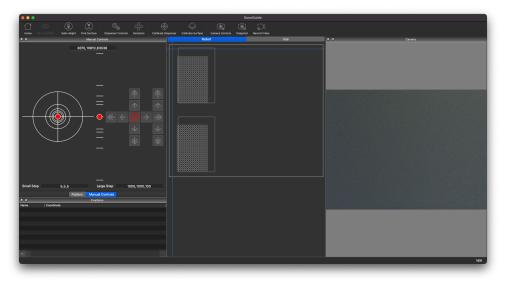
One-component oligonucleotide microarray

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To begin, turn on the control computer, the positioning system, and the controlling electronics. Once the computer has booted itself to the desktop, click once on the SonoGuide.

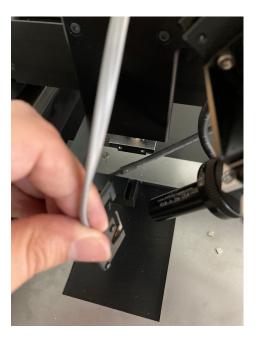
This will start up the SonoGuide control software, your main interface to the Microplotter system. Because the positioning robot has just been turned on, it will need to home itself to establish a starting position. The software should inform you of this, ask you to clear the movement area of the robotic arm, and press OK when ready.

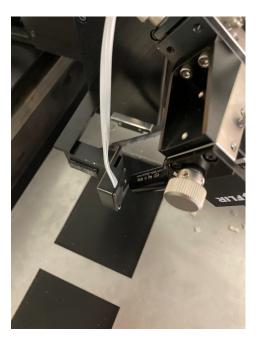
Once homing is complete, you will be presented with SonoGuide's main control interface.



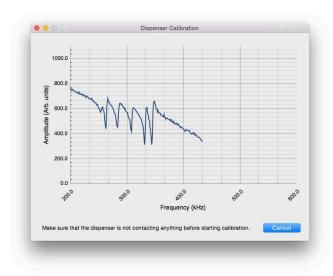
Before you start the dispensing process, you will need to calibrate the cant (level) of the surface to be dispensed onto. Place a treated glass microscope slide in an appropriate slot on the deck (or platen) of the Microplotter system. If your deck does not support microscope slides, either replace the deck with one that does or use a different substrate material.

After loading the slide, clip into place a new dispenser if one is not already in the system. Connect its telephone wiring to the jack on the Z-axis.





As soon as the new dispenser's wiring is connected, select Calibrate Dispenser from SonoGuide's main control interface. The software will then calibrate this dispenser, using a window like the following:



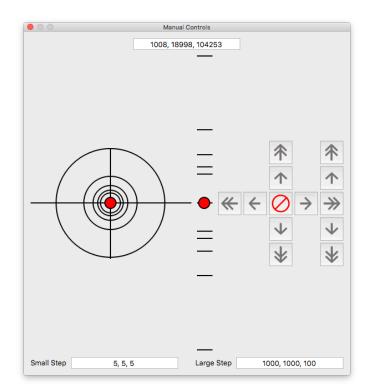
This should take a few seconds, after which a notification will appear that confirms the calibration of the dispenser.



The camera may not be focused on the tip of the new dispenser. Adjust the three thumbscrews on the camera mount to bring the tip of the dispenser into focus. If the contrast of the image is poor, the image is too bright or dark, or the hue is wrong, you can adjust the settings of the camera by opening the Camera Controls from the windows options and adjusting the sliders in the dialog that will appear. Close this window when finished.

8	Camera Settings	
Lightness:	✓ Automatically adjust light sensitivity	⊃ 0.469
Sharpness:	00	⇒ 512 ⇒ 806
Saturation: White balance:	O	⇒ 701
	0	⊃ 754
Red (V):	O	561
Denoising:	сО	⇒ 0.5 //.

Now that the dispenser and camera are set up properly, it is time to measure the topography of the microscope slide. To do this, the dispenser will need to be moved close to the surface. Use the directional buttons on the upper-left portion of the main SonoGuide interface to move the robotic positioning arm in a stepwise fashion over its three axes. The buttons with single arrows move the positioner in small steps, while the ones with two arrows move in large steps.



The sizes of the large and small steps are set by the Large Step and Small Step text input boxes. All values are in microns and can be set differently for each axis. Set the large step to 10000 microns (one centimeter), to move one centimeter at a time, and grossly position the robot above the upper-left corner of the microscope slide.

Warning: Avoid bringing the dispenser close to the surface using this step size, as overshooting the surface too far will damage the glass needle.

Change the large step size to 1000 microns (one millimeter) and more finely position the dispenser relative to the surface of the microscope slide. Click on the Find surface button above the manual controls to have the dispenser move down slowly and detect the height of the surface. If this stops before the dispenser is visibly contacting the surface, or overshoots and needs to be cancelled manually (using the abort button in the center of the manual controls), consult Chapter 6, Troubleshooting. Once on the surface, move the dispenser up by 500 microns.

Width 5000 µm 0	
Height 5000 µm 🗘	
Depth 1000 µm 🗘	
Top-Left N/A Use current position	
Top-Right N/A Use current position	
Rotation angle 0°	
X slope 0	
Y slope 0	
Calibrate Surface Cant Reset	

Open the surface calibration window by clicking on the Calibrate Surface button.

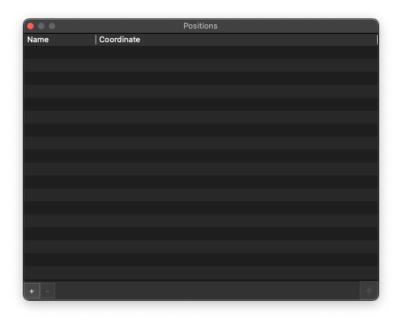
Under the Distance to calibrate group, set the distance to calibrate along the X axis to 20000 microns (20 mm is just a little less than the full 25 mm width of a typical microscope slide) and set the distance to calibrate along the Y axis to 70000 microns (again, 70 mm is just shorter than the full 75 mm height of a microscope slide). Once these values have been entered, press the Calibrate surface cant button to begin the surface topography measurement. The dispenser will move down until it contacts the surface, the position will be measured, and then the dispenser will pick up and move to a new location. The software measures 5 points in a line along both the X and Y axes to determine the cant of the surface. This process should take a few minutes to complete.

Now that all calibration has been taken care of, back the positioner away from the surface by 1000 microns. Select the menu item Dispenser Controls toolbar item to bring up the screen that controls the dispenser settings.

000	Dispenser Cor	ntrol
Dispensing:	Strength: Ouration: 0.5 s	2.0 V Dispense
Spraying:	Strength: Duration: 5 s	12.0 V
Advanced		

If not already set at this value, change the Dispensing strength to be 1 V and the Spraying strength to be 12 V. These settings regulate the ultrasonic pumping action within the dispenser while dispensing fluid and emptying the glass needle, respectively. The values listed should be good defaults for aqueous solutions within a dispenser having a tip inside diameter of between 10 and 50 microns. If you later find that the spot deposition on your surface is inconsistent, try decreasing or increasing the Dispensing strength value. Likewise, if your dispenser is not completely emptying itself during rinse cycles, increase the Spraying strength. When done, close this window and return to the main interface.

Save the current position of the dispenser so that it will be easy to return to later. To do this, click on the + button in the Positions window.



This will create a new position and leave you able to edit its name. Type in Above slide and hit Return when you are done. The new position is added to the list of saved positions, meaning that it will be easy to return here in the future.

It is now time to load solution into the dispenser. Place approximately 50 microliters of an oligonucleotide solution in a well within a 384- or 1536- well microtiter plate on the deck or use a custom well within the reach of the positioner. Manually move the dispenser above the well (using gross positioning of 10000 micron steps, then finer positioning using 1000 micron ones) and then down into the well (again, starting with 10000 micron steps, followed by 1000 micron ones).

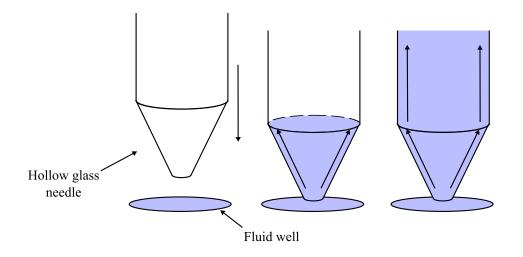
Warning: Be careful when moving the dispenser near a solid object, such as the wells. If the glass tip comes in contact with the side or bottom of the well, it can shatter.

To save the location of this well, open the Solutions toolbar item and the Solutions Settings window. This will bring up a windows for setting solution locations.

80		Solutions	
	Well Plate	Coordinate	
Solution 1			
			_
+ -			-\$
80		Solution Settings	
Name	Solution 1		
_			
Plate		0	
Well			
Coordinate			
Draw distance		Unlimited ᅌ	
Draw distance		Unimited 😜	
Reload time	10.0	© seconds	
Empty time	20.0	© seconds	
Waste well			
Waste well	Return to Solution Wel		
Rinse well	Return to Solution Wel	0	
Rinse Cycles	3		
	Set Custom Position		

Click the + button to add a new solution to the list. Enter in a name of Solution 1 and hit Return when you are done. Click Set Custom Position to set this up as a custom position, using the current coordinates.

When the glass tip of the dispenser dips into solution, that solution should start rising up the inside of the dispenser through capillary action, as shown in the following illustration:



If it does not, the glass tip may be clogged with a small dust particle. To clear this, open the dispenser control window again by selecting the menu item Dispenser | Show dispenser controls. Click first on the Dispense button to drive the dispenser at its more gentle settings. Wait for a few seconds. If that has not caused fluid to rise up within the dispenser, click on Spray. This will cause severe agitation within the fluid well and should dislodge any material stuck in the dispenser. Once fluid starts to flow, click on the Cancel button to turn off the ultrasonics. It should take 30 seconds to one minute to load a few microliters of solution into the dispenser.

While solution is being loaded into the dispenser, there is time to set up the DXF pattern to be dispensed on the surface. A DXF pattern is a representation of the fluid features (spots, lines, etc.) that will be drawn onto the surface by the Microplotter system. DXF patterns can be created and edited in a separate drawing programs.

Now that drawing of the new DXF pattern is finished, click on the SonoGuide window to switch back to that application.

Before starting to dispense the pattern that was drawn, the dispenser will need to be moved to the center of where this pattern is to be drawn. Lift the dispenser out of the fluid well and move it to the previously saved location above the microscope slide by choosing Above slide from the list of saved positions and clicking on the button Go to position. This should retract the dispenser and move it over the slide. Move the dispenser 2-4 millimeters to your right and towards you to better center the pattern on the slide.

With the addition of fluid to the dispenser, there is the possibility that its vibrational properties have changed, so it's recommended that you recalibrate it before dispensing. To do this, click on the Calibrate dispenser toolbar item, followed by clicking on the Scan button in the calibration window that pops up. When the calibration process has finished, close out of that window.

The dispenser should be approximately 1000 microns (1 mm) above the surface, so you should be able to activate the surface height sensing to determine the exact starting position of the array. Click on Find Surface to begin the surface sensing process. This should end when the dispenser detects the surface and stops. As mentioned previously, if this stops before the dispenser is visibly contacting the surface, or overshoots and needs to be cancelled manually (using the abort button in the center of the manual controls), consult Chapter 6, Troubleshooting.

You can also move the dispenser in manual steps toward the surface, if you do not wish to use the surface sensing or if you have moved the dispenser a ways away from the surface. Use a step size of 1000 microns if you are starting far from the surface, switch to 100 micron steps as the dispenser becomes very close to the surface, and finally 10 micron steps when the surface can be seen from the camera. Approach until fluid contact is made.

Warning: Be very careful when moving the dispenser towards the surface. Until you are familiar with how the positioner behaves, err on the side of caution when moving the dispenser in order to avoid overshooting the surface and shattering the glass tip.

Once fluid contact has been made, it is time to start the pattern. Choose the menu option File | Open Pattern... and pick the pattern you drew earlier. Open the Pattern window and assign the solutions saved to the associated layer. Repeat until all solutions in the pattern are accounted for. You may choose to not assign some or all of the solutions, in which case the solution within the dispenser *will not* be switched out before the pattern items with the unmatched solutions are printed. Choose the menu option File | Print... to start the printing process.

The controlling computer will automate the dispensing process from beginning to end. A progress bar will be displayed giving an indication of the pattern completion percentage, and providing a button should you wish to cancel the pattern mid-deposition.

When the pattern has finished, open the list of solutions through the Dispenser | Show solution locations... menu item or Solution toolbar item. Click on the Empty dispenser toolbar item. This should lead to a forcible ejection of all the fluid within the dispenser in under a minute over a previously configured waste well. This last fluid ejection step is unnecessary, but might be required if you wished to work with a different solution or if the dispenser would be sitting unused for some time.

Finally, click on the Home toolbar item to return the positioner to its safe resting location and remove the finished microarray from the deck of the Microplotter system.

Wires from nanometallic inks

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A typical microelectronic application of a Microplotter instrument is in the dispensing of nanometallic inks to form conductive wires, pads, and to repair open circuits. These nanometallic inks vary in composition, but some have many of the same properties of an aqueous oligonucleotide solution. In this example, many of the steps will be the same or very similar to those described in the previous section, so you will be referred to that section for more details. Only the steps that differ will be elaborated on here.

The preparatory steps for dispensing a nanometallic ink pattern are the same as those described above: load a substrate material onto the deck of the Microplotter system, load a dispenser cartridge and calibrate it if necessary, and perform the steps to calibrate the surface cant. Depending on the particular application, dispensers with varying aperture sizes can be chosen, 1 micron inside diameter dispensers may be appropriate for very fine features, such as those in LCD backplane wires, and 30 micron inside diameter dispensers may be suited to macroelectronic applications, such as RFID antennae.

Next, an appropriate ink needs to be loaded in a solution well. Which well to load this ink in will be determined by the ink itself.

Warning: Solutions with organic solvents such as acetone, toluene, hexane, or chlorobenzene may dissolve the walls of common 96- or 384-well microtiter plates or

similar wells. Check the solvent resistance of your well material first before using these solvents.

Nanometallic inks using ethanol or water as a solvent can safely be loaded in the wells of a standard microtiter plate.

Another concern regarding inks is their volatility. While it can work with a wider range of solutions than inkjets or similar competing technologies, a Microplotter instrument still has difficulty with highly volatile solvents, such as acetone. These solvents evaporate so quickly at the microscale that a Microplotter system can only dispense inks based on them for a short time before they completely dry up. If a solvent can be selected for the particular ink of interest, it is best to use the one with the lowest volatility. Most solvents with a volatility less than that of toluene have been found to work well in Microplotter instruments.

After choosing an ink, loading it in a well, and setting the location of that well, the next step is to draw a simple line-based pattern for dispensing the ink onto the substrate surface.

Once the drawing of the new pattern is finished, click on the SonoGuide window to switch back to that application.

Manually move the dispenser over to the well containing the ink to be deposited and down into the ink until the tip is below the fill level of the well. The ink should start filling the dispenser through capillary action. If not, please refer to the previous section or Chapter 6. You may also set the position of this well as described above.

Move the dispenser up to a safe height above the surface and then over to the upper-left corner of the area on the substrate you wish to dispense on. As described in the previous section, recalibrate the fluid-laden dispenser and use the Find Surface toolbar item to move down until fluid contact is made with the surface. Load the pattern you drew by choosing File | Open pattern... and loading LinePattern.pattern, saved in Documents. The pattern should begin dispensing immediately.

Once dispensing is finished, empty the fluid from the dispenser as described above. Again, this last fluid ejection step is unnecessary, but might be required if you wished to work with a different solution or if the dispenser would be sitting unused for some time.

Finally, click on the Home toolbar item to return the positioner to its safe resting location and remove the printed substrate from the deck of the Microplotter system.



Overview

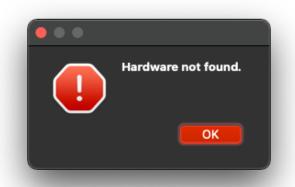
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SonoGuide is the main control interface for the Microplotter Proto instrument. Movement of the positioning system, calibration of substrates, and operation of the dispensers are all controlled through this piece of software. It was designed with the intention of providing the maximum amount of flexibility to the end user in order to enable many novel applications.

SonoGuide has a modular user interface with the ability to open and close windows and arrange these windows in any fashion the user finds useful.

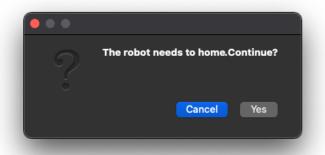
Main interface

Upon starting SonoGuide, it will check with the robotic positioner of the Microplotter system to determine its status. If either the positioner or the controlling electronics are not turned on, or the cables between the computer, the electronics, and the positioner are disconnected, SonoGuide will warn that it is unable to find the hardware. In that case, check all connections, turn all equipment on, and click OK to the warning dialog that appears:



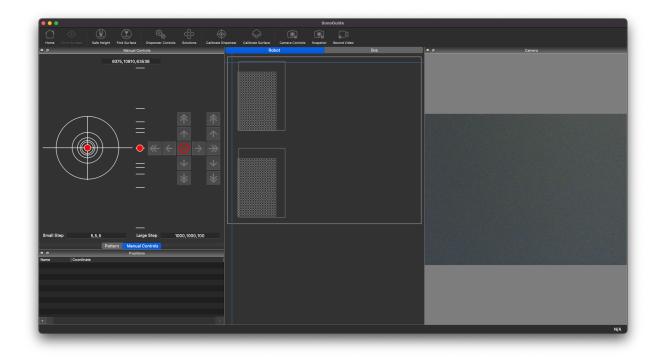
The software should reconnect to the positioner automatically. If it does not, exit out of SonoGuide and restart the software.

Once SonoGuide establishes communication with the positioner, it will next check to determine whether the positioner has been homed. When the positioner has been turned on, its motors need to seek to a standard home position before they can be commanded to move anywhere. This is intended to provide a constant starting location for the absolute positioning of the dispenser head. If SonoGuide detects that the positioner has not yet been homed, it will prompt you that it is about to do so.



Warning: Clear the working area of the Microplotter system and make sure that no one is near the volume covered by the robotic arm, as the positioner will move quickly to home itself. Physical injury may result if a person is near the arm while it is moving and any obstructions to the path of the robotic arm may damage the robotics, the dispenser cartridge, the optics assembly, or the objects in its path.

Click OK to begin the home process. Once the positioner has been homed, the main interface to SonoGuide will appear and the buttons to move the positioner will be enabled.



The graphical user interface has several windows initially, including:

- Directional controls
- Saved positions
- CCD camera display window
- · Working area of the positioner

These will be described in order, starting with the directional controls.

Directional controls

.....

The window for the directional controls appears as follows:



These controls allow you to move the positioner manually in a stepwise fashion or a continuous fashion. There are two sets of buttons, those with a single arrow and those with two arrows. These allow the you to move the positioner in a stepwise fashion. The single-arrow buttons

move the positioner in small steps, while the double-arrow ones move the positioner in large steps. The step sizes are specified, in microns, by the Large step and Small step text boxes. For reference, 1000 microns = 1 mm and 10000 microns = 1 cm. Unless otherwise indicated, all distance inputs in the control software are specified in microns.

The buttons in the left crosshairs control movement in the X axis (horizontal) and in the Y axis (vertical). The vertical buttons to the right of this crosshairs provide movement in the Z axis. If a movement step cannot be made in a direction because it would go past the limits of the positioner, the button for that movement will be greyed out and inactive.

To move the positioner in a more continuous fashion, use the trackball on the left hand side of the window. The positioner will move faster as you drag the ball closer to the outer edge of the circle. The movement will scale up or down with the step sizes specified in the Large step.

These controls will be disabled as certain times, such as when a movement is in progress, a pattern is being dispensed, or a calibration run is being performed.

Toolbar items

.....

At the top of the main interface there are several toolbar items. The first the Home item, which will cause the positioner to lift the Z axis to its home position, then rapidly move the X and Y axes back to their home points.

Warning: The home move travels over a large distance and can be very fast, so make sure that all objects and personnel are clear of the positioner's working range before clicking on this button.

The next item is a Find surface item. The electronic surface sensing technology within the Microplotter dispense head can be used to detect the exact surface height, a very useful function for exactly aligning the dispenser above the surface quickly. This automatic surface height determination can be activated using the Find surface item, but that item will remain greyed out until a dispenser has been attached and calibrated by following the instructions in the later Surface height sensing and calibration section. Once Find surface has been clicked, the positioning system will slowly move the dispenser closer and closer to the surface until contact has been registered. Should this take too long, if it is clear that the dispenser was improperly calibrated, or if the dispenser is overshooting the surface for some other reason, clicking on the Abort button (the one that has the red circle with a slash through it, at the center of the standard movement controls) will cut short the process.

The next is an option to quickly lift up the dispenser, convenient for replacing dispensers or substrates without worrying about accidentally touching the dispenser tip on a surface. This movement is activated by clicking on the Go to safe height item.

There are a few more toolbar items at the top of interface that will be covered in the next few pages

Saving positions

It is often useful to be able to save positions that can be returned to quickly at a later time. A window of the interface is provided to allow you to do this.

•••	Positions	
Name	Coordinate	

This panel presents a list of previously saved positions, their associated coordinates, and a set of 3 buttons. These are

• +

• _

Crosshairs

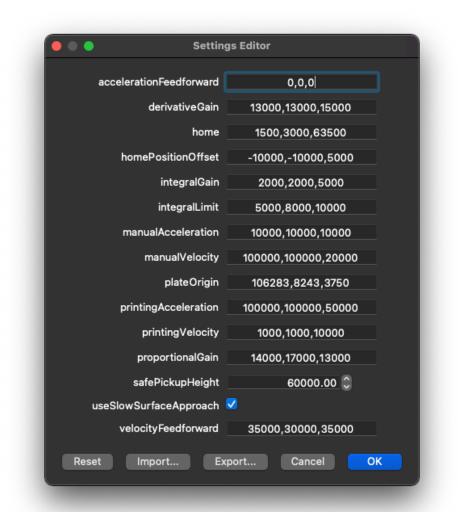
To move to a previously saved position, highlight a position by clicking on it and press Crosshairs. This will cause the dispenser to pick up to a set height (the setting of which will be explained in the next section), move over to the correct X, Y coordinate, and move the print head back down to the necessary height.

To save the current position in order to return to it quickly at a later time, click on +. This will insert a new position into the list for the current coordinate and give you the ability to edit its name.

To delete a position, highlight it in the list and click on -.

Robotic positioner settings

The robotic positioner can be moved manually via the controls in the main interface, but it is possible to have even finer control of movement properties by using the Positioner configuration window accessed under the SonoGuide | Tools | Motor Settings menu option.



This window lets you set the speeds and accelerations for movement in all three axes. All speeds are in microns/s and all accelerations are in microns/s². These values can be set for two conditions: when the positioner is being moved manually, and when the movement is controlled by the computer during the automatic deposition of a pattern. For example, it might be desirable to have faster speeds and accelerations set for manual positioning than for automatic positioning so that you can quickly move the dispenser over a large area in order to align it above a specific position, and then have it move much more slowly over the small scale at which the pattern is being dispensed so as to produce the smoothest, most accurate movements. Each speed or acceleration will take effect once you have finished inputting the new value in its respective box and clicked elsewhere in the window to move the focus out of that box.

This window also lets you set various customization options for the operation of the system:

In the main interface, when you select to move to a previously saved position, the positioning robot lifts the dispenser head up to a safe height, moves it to the X and Y coordinate of the new position, and brings the dispenser down to the proper Z coordinate. That safe pickup height is set in this window, under Safe pickup height. The position is recorded in microns as an absolute Z coordinate. To set this, move the positioner to a height such that the dispenser safely clears all obstacles, then click the Use the current height button.

Warning: Be careful in setting the pickup height for the positioner. Leaving this too low can lead to damage to dispensers, the print head, or the optics assembly if obstacles are encountered while moving to a saved position.

At the bottom of the window is a checkbox that enables a more gentle surface sensing process. This process is appropriate when working with dispensers that have inside diameters of 10 microns and less, because they may be too fragile to use with the standard surface finding routines. By enabling this option, the system will cause the dispenser to approach the surface in smaller steps and use a more sensitive detection routine. The downside to this is that the approach to the surface will be much slower than the standard technique.

This window also contains settings for the proportional-integral-derivative (PID) control loop of the system motors. Unless instructed to do so, these values should never need to be adjusted.

If you do need to adjust these settings, you need to first unlock them by clicking the Unlock button. You will then be asked for the password for the primary user account of this system. The default login information is contained within Chapter 2. Once unlocked, each value can be edited, and the new tuning parameters can be applied by clicking the Apply now button.

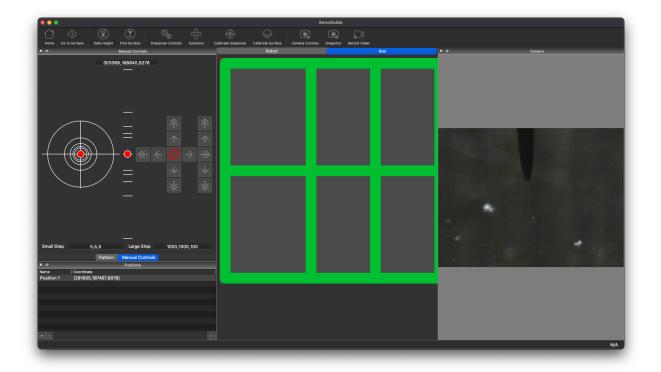
If a mistake has been made in setting these values and you want to return to the factory defaults, click the Reset button and the original settings will be restored.

Warning: Changing the tuning values can lead to positioner inaccuracy or complete instability of the positioning system. Only alter them if necessary to correct positioning problems or when instructed by SonoPlot personnel.

Additionally, this window allows you to import motor settings and export these settings which may be necessary if using a different computer.

Printing patterns

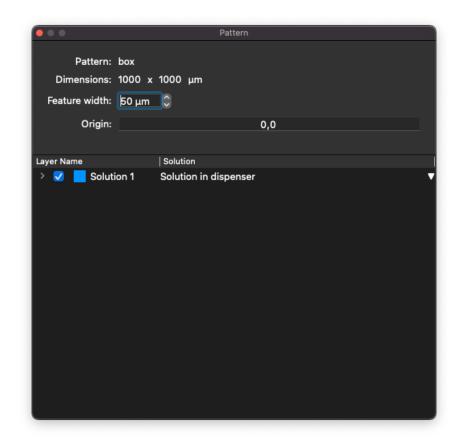
Once the dispenser is positioned in fluid contact with the surface, you can initiate the printing of patterns by choosing the File | Open Pattern... menu item. You can also open recently printed patterns from the File | Open Recent menu. Both SonoPlot's .pattern files and AutoCAD DXF format files can be loaded in this manner. Once a pattern has been loaded, it will be previewed in the middle of the main interface:



A crosshair indicates the current position of the dispenser, relative to the rest of the pattern, and its location will always be in the middle of the pattern or DXF file.

If a portion of the pattern cannot be printed because it extends outside of the working area of the system, that part of the pattern will be colored in red.

More information and pattern settings can be found from Window | Pattern window.



The feature width specified below the pattern dimensions is used to make the preview more accurately reflect what will be printed, and does not control the actual dispensing size. It also is used to set the spacing between lines when drawing a filled rectangle.

Each layer in the pattern or DXF file is listed below these options. Individual layers can be enabled or disabled by using the checkbox next to their name. Only the enabled and visible layers will be printed. These layers can be associated with solution wells that have been laid out in SonoGuide by clicking next to them in the Solution Well column and choosing one of the solution wells. This will associate that layer with that solution, and will cause the dispenser to be loaded with that solution as needed before the layer is printed. See the Solution Well Locations section of this chapter for how to set up solution well locations.

When you are ready to print, go to File | Print or use the keyboard shortcut cmd + p.

This will bring up the following window:

	Print P	attern
	Dispense Mode	Dispense 文
	Curing Mode	
🗸 Retu 🔲 Opti	rn to pattern start mize pattern layou rride droplet dispe	start of each pattern item when finished t for fastest printing nsing time 1000 ms uight: 300 µm 20 µm
		Cancel Print

The first item lets you select the Dispense Mode. Here you can select the system to use the spraying action instead of the more gentle dispensing actuation when printing. This is occasionally used to coat larger areas with a less controlled spray of material.

Then there are three selectable options for how the pattern is to be printed. The first instructs the system to sense out the height of the surface before printing each item. This is potentially useful when printing onto curved or irregular surfaces.

The system can then be instructed to stay at the last location in a pattern when done, as opposed to the normal process of returning to the start on print completion with the second option.

The third option lets the system optimize the pattern printing. Usually in a right to left, top to bottom motion.

This window also allows you to override the dwell time when printing droplets. The units are in milliseconds.

When dispensing a pattern, the control software retracts the positioner a set distance after the completion of a feature. That distance can be set in the Item retraction height box. A larger distance may be required for larger-aperture dispensers or for more irregularly shaped surfaces, while a smaller distance can lead to the faster dispensing of a pattern using a smaller

aperture dispenser. As a rule of thumb, a 200 micron pickup height works well for dispenser apertures up to 50 microns wide and a 1000 micron pickup height works for anything above that.

When you are ready to print, you can click on the Print button.

The controlling computer will automate the dispensing process from beginning to end. A progress bar will be displayed giving an indication of the completion percentage as the pattern prints, and the current or next item to be printed will be highlighted in blue.

Camera display

A final portion of the main interface is a realtime display from the CCD camera pointed at the tip of the fluid dispenser. The high-magnification optics surrounding the CCD have a field of view that is approximately 1200 microns (1.2 mm) across, and can resolve features as small as 5 microns wide. LED illumination is provided to enhance viewing in low-light conditions.

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Aside from providing feedback for the dispense process and enabling entirely manual dispensing for one-off tasks (such as placing polymer solution on a MEMS structure), the live camera feed can be recorded to disk as digital video. To start recording from the camera, select the menu item Start recording under the Camera menu or click on the Record toolbar item. This will bring up a dialog box prompting you to specify where to save the recorded video

file and allowing you to give it a name (the default is temp.mp4). Once you click OK, the video will start recording to disk. When you are finished recording from the camera, click on the Stop recording menu item under the Camera menu or click on the Stop toolbar item.

The video files saved by SonoGuide are recorded into ISO standard MPEG4 files with an MP4 extension. These video files are playable by default in Mac OS and editable in iMovie (bundled on the control computer). They are playable on Windows via the QuickTime player (<u>http://www.apple.com/quicktime/download/</u>), the VLC player (<u>http://www.videolan.org/vlc/</u>), or through the appropriate Windows Media Player plugins.

Still snapshots can also be captured of the video input from the CCD by either selecting the menu item Camera | Take snapshot or by clicking on the Snapshot toolbar item. A dialog box will appear asking you to select a name and a location to save the snapshot to. Additionally, you can select the image format of the snapshot from a range of different types, with many quality options to choose from.

At the top of the main user interface there are a series of other helpful toolbar items. In addition to the already described camera controls, there are options to bring up other windows for controlling various aspects of the Microplotter system.

The first is an item named Calibrate dispenser, which opens the dispenser calibration window.

The second is the Control dispenser item, which will bring up the window that lets you control the dispenser.

The third item, Solutions, opens the saved solution locations window.

The last item is called Surface calibration and it brings up the surface calibration window.

Camera settings

.....

The CCD camera whose input is displayed in realtime on the main interface can have many of its functions adjusted to provide the best quality picture under various lighting conditions. These functions are accessible through the Camera menu, under the option Camera settings. This will bring up a window similar to the following.

8	Camera Settings	
Lightness:	Automatically adjust light sensitivity	0.469
Gamma:	O	512
Sharpness:	O	806
Saturation:	O	701
White balance:		
Blue (U):	O	754
Red (V):	O	561
Denoising:		0.5
Benoising.		1.

Near the top of this dialog is an option to Automatically adjust light sensitivity. If selected, the software will attempt to analyze the image coming from the camera and adjust the gain, brightness, and exposure settings of the camera to produce an optimal picture under variable lighting conditions.

If this setting is turned off, the camera settings dialog will look similar to the following:

8	Camera Settings	
Gain:	0	835
Brightness:	0	0
Exposure:	O	669
	Automatically adjust light sensitivity	
	0	512
Sharpness:	O	806
Saturation:	O	701
White balance:		
	\sim	754
Red (V):	0	561
200.000		
Denoising:	0	0.5

The settings that can be controlled include:

• **Gain** - The CCD's gain setting controls the degree of amplification of the video input. A high gain is necessary to see poorly lit objects, but will introduce camera noise. As stated above, this should be used with Exposure to tune the camera input for different lighting conditions.

• **Brightness** - The overall brightness of the camera image, too high and it can reduce the contrast of the image, too low and the image may be too dark to see.

• **Exposure** - A value representing the exposure time of the camera CCD, with higher values allowing for the imaging of even dark scenes. This is the primary setting, along with Gain, that needs to be changed with lighting conditions.

• **Gamma** - The gamma provides a nonlinear adjustment of the image, mainly enhancing the contrast. This setting does not cause a significant change on newer camera models shipping with Microplotters.

• **Sharpness** - The sharpness of the image can be modified, with high sharpness values showing clear edges but also amplifying CCD noise and low values blurring the overall picture.

• **Saturation** - The intensity of the color response of the camera can also be controlled using this setting. Low values can lead to a grayscale image and high values can cause color bleeding.

• White balance, Blue / U - The camera uses a color CCD and the color balance of that CCD can be controlled by manipulating the blue and red (U and V) components of the image.

White balance, Red / V - The corresponding red component of the CCD color balance.
Denoising - To improve the clarity of the camera picture, the software will perform processing on the image frames as they come in to remove speckle noise produced by the CCD element. This control adjusts the strength of the denoising filter, from completely off at 0.0 to overwhelming the input image at 1.0. Lower values increase the noise displayed onscreen, where higher values can lead to blurring of the image as the dispenser moves around. A balanced value tends to be 0.5.

When finished, simply close the window.

Dispenser control

.....

The core of a Microplotter instrument is a dispenser that uses applied ultrasonic vibrations to induce a pumping or spraying action within a hollow cavity. Settings for controlling the behavior of the dispenser can be found under Dispenser | Show dispenser controls or the Control dispenser toolbar item.

00	Dispenser Control	
-	Strength: Ouration: 0.5 s	2.0 V Dispense
Spraying:	Strength:	12.0 v
Advanced		

In the default view, the strength of applied ultrasonics when dispensing and when spraying can be modified. These strengths are in terms of the voltage applied to the piezoelectric element within the dispenser. Lower amplitudes of vibration lead to a pumping action at the resonant frequency of the dispenser, with a pumping strength that increases with increasing alternating current voltage. Above a certain threshold, the dispenser stops pumping and starts a less controlled spraying of fluid. Spraying is not typically used for dispensing, but is employed to empty the contents of a dispenser and clean it for use with another solution.

Typically, you will use dispensing voltages between 1-2 Vpp for 30 micron aperture dispensers. This encourages droplets to be consistently dispensed with a wide variety of materials. Higher voltages can sometimes be needed for more viscous liquids, but these higher voltages may lead to irregular dispense volumes with thinner liquids. Additionally, smaller aperture dispensers respond more strongly to the ultrasonics, so lower voltages may be needed with them.

For dispensing, setting the voltage to 0 will cause only a very small pulse to be applied, which can be ideal for dispensing that is primarily driven by surface tension. This can yield very low variabilities for droplets dispensed on chemically uniform surfaces. However, it can lead to missing droplets.

For spraying, a typical range of voltages is from 10-13 Vpp for a 30 micron dispenser.

Warning: Applying alternating current with voltages in excess of 10 Vpp to induce spraying in a dispenser with a tip inside diameter smaller than 10 microns may lead to breakage of the dispenser due to the strength of the spraying action.

From this view, you can manually activate the dispensing or spraying process by clicking on the Dispense or Spray buttons, respectively. The dispensing or spraying action will take place for the duration specified in the text field underneath the appropriate voltage setting.

If you wish to have even finer control over the dispenser settings, you can click on the disclosure arrow to the left of the Advanced label in the dialog box. This will slide down an additional set of controls.

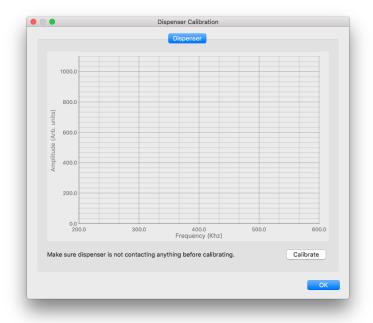
00	Dispen	ser Control	
Dispensing:	Strength: Duration: 0.5 s		2.0 V Dispense
Spraying:	Strength: Duration: 5 s	0	12.0 V Spray
Current o	lispenser frequency: 150.0 kHz	Current disper	nser voltage: 0.0 V
	ng frequency		449.1 kHz 449.1 kHz

These new controls provide a means to adjust the frequency at which spraying and dispensing occur. Normally, the resonant frequency of the dispenser is used for these values, a frequency that is automatically found during the dispenser calibration process. However, a different frequency can be chosen to adjust the behavior of the dispenser.

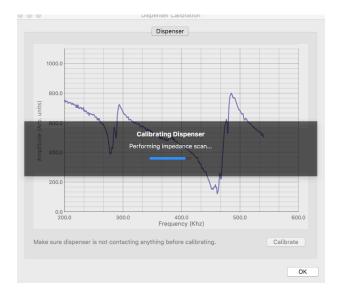
Dispenser calibration

When a new dispenser has been connected to the system, it needs to be calibrated before it can be used to deposit fluid or sense the physical height of a surface. From time to time, the dispenser may need to be recalibrated due to changes in its contents or drift in the system.

The connection of a dispenser to the system is not automatically detected. Open the Calibrate Dispenser toolbar item to begin the calibration process.



A calibration can be triggered by clicking on the Calibrate button. A trace will be displayed as the dispenser is scanned, followed by a brief progress indicator during the final calibration steps.



A sharp dip, followed by a spike, occurs at around 440 kHz. This is a resonance within the dispenser. The sharper the dropoff, in the resonance, the stronger it is. Several smaller resonances can be seen, but those are weaker than the one at around 440 kHz. The exact resonance frequency is the frequency near the bottom of the dip where impedance is at a minimum. After a scan, this frequency is automatically assigned to the Dispense and Spray values in the control window described in the Dispenser control section, because it is the one where pumping and spraying occur most readily.

When done, the window will disappear and all dispenser functions, such as pattern printing, will be enabled. A notification will appear indicating that the dispenser has been calibrated.

To return to this calibration dialog, either choose the menu item Dispenser | Perform Dispenser Calibration... or the Calibrate dispenser toolbar item.

Solution well locations

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The Microplotter has the ability to dispense multiple solutions when printing patterns. To do this, the dispenser will use higher-intensity ultrasonics (the Spray settings seen earlier) to forcibly eject fluid from the dispenser. The procedure for switching solutions consists of the dispenser approaching a waste well (or the original solution well if you wish to conserve material), spraying out its contents, moving to a rinse solvent well and loading solvent, moving back to the waste well and spraying out that solvent, then moving into the well for the next solution. The solvent rinsing process can be performed for multiple cycles before loading the next solution, to minimize cross-contamination.

In order to know where the various solutions are located, as well as rinse solvent and waste wells, locations are specified in the following windows.

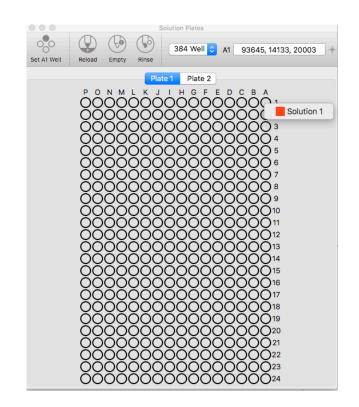
\bigcirc \bigcirc \bigcirc			Solutions	
Name	Well	Plate	Coordinate	
+ -				

The first window is a list of solutions. Each solution can be selected by clicking on its name. New solutions can be added to the list by clicking the plus button, and old solutions can be removed by clicking the minus button. When first adding a solution, you will be given the chance to edit its name. If you want to edit a solution's name after that, click twice on the name in the list.

When a solution has been selected, its properties will appear on the Solution Settings window. In addition to solution wells, locations for rinse solvents and waste wells can be set. These can be shared between solutions, or they can be different for each solution.

30		Solutions	
	Well Plate	Coordinate	
Solution 1			
+ -			
30		Solution Settings	
Name	Solution 1		
- Control - Cont			
Plate			
Well			
Coordinate			
Draw distance		Unlimited ᅌ	
Reload time	100		
	10.0 0	seconds	
Empty time	20.0 0	seconds	
Waste well	Return to Solution Wel ᅌ		
Waste Weil			
	Return to Solution Wel ᅌ		
Rinse well			
	0		
Rinse well Rinse Cycles	3		
	3 \$		

The easiest way to set up many solutions is to place those solutions within a 96- or 384-well micro titer plate. If you have done this, then all you need to do to specify their position in the Solution Plates window. Right click on the desired well and the list of solutions will appear. Select the desired solution to set the well for that solution.



If your solutions are not in a micro titer plate, but in custom wells that you have placed on the deck of the system, you will need to manually define their positions. To set up a custom well location, first move the dispenser into the well so that it is taking up solution. Then click on the Use current coordinate as custom position button to set the location of the well.

Other parameters can be set for the solutions. These include the distance a solution can be drawn before needing to be reloaded into the dispenser, how long (in seconds) to load that solution into the dispenser, the time it takes to spray all of that solution out of the dispenser, and the number of rinse cycles required to fully clean out that solution from the dispenser.

Each solution can be set to either return solution back to its original solution well when done or to eject it into a designated waste well. The waste well can be chosen from any of the solution wells you have added. The same goes for the well to use for rinse solvent.

Along with the solution settings, there are a few toolbar items for manually controlling the solution loading and rinsing process. If you wish to load a solution into the dispenser, select the solution from the list and click on Load With Solution. To empty the dispenser, click on Empty Dispenser. To put the dispenser through the entire rinsing process, click on Rinse.

The last toolbar option is for calibrating the locations of wells within a microtiter plate. If you have changed dispensers, or have switched between 96- and 384-well microtiter plate types, you will need to set the new starting location of the first well in the microtiter plate on the system's deck.

To do this, move to the A1 well position (which should be in the upper-right corner of the plate) on the first microtiter plate (the one towards the rear of the system) and bring the dispenser down into the well. Click on the Set A1 Well Position toolbar item to set the new A1 well location. The positions of all of your specified solutions will be recalculated to match.

Surface cant calibration

The Microplotter ultrasonic dispensers have the capability of electronically sensing when their tips have contacted a physical surface. This means that more reliable dispensing can occur and the lifetime of a dispenser can be prolonged by preventing positioning overshoot that might damage a dispenser's fragile tip. The application of this surface sensing has been described in the Main interface and Robotic positioner settings sections. The ability of a Microplotter dispense head to electronically sense contact with the surface allows the system to calibrate the cant of a flat surface. Every flat surface placed on the substrate platen has some degree of tilt to it and even the slightest cant can cause a difference in surface height great enough that the dispenser either misses the surface completely or overshoots and shatters its tip. In any case, the having a dispenser return to the exact height for each point of a dispensed pattern leads to a more reliable and consistent result.

To electronically determine the cant of a surface, first load a dispenser into the Microplotter system. Next, calibrate the dispenser for sensing using the method described in the section Dispenser measurement.

	Surface Ca	libratio	on
Width		5000) µm 0
Height		5000) µm 🗘
Depth		1000) µm 🗘
Top-Left	N/A		Use current position
Top-Right	N/A		Use current position
Rotation angle		0°	
X slope		0	
Y slope		0	
Calibrate S	urface Cant		Reset

Open the surface cant calibration window by selecting the toolbar item Calibrate Surface.

Enter the width and height of the area that you would like to be calibrated in the X and Y directions (in microns). If calibrating a sample, choose distances that are a little less than the dimensions of the sample, keeping the measurements within the bounds of that sample. Once the proper distances have been set, bring the dispenser to approximately 1 mm over the top left corner of the sample, then click on Use current position next to the Top-Left option. The Top-Left and Top-Right options are for calibrating the rotation of the sample. This will be covered in the next section.

Click on Calibrate surface cant to begin the surface leveling process. The surface height will be electronically sensed at five points in the horizontal and vertical directions. After the sensing run has completed, the cant of the surface in X and Y will be calculated, stored, and reported in this window. This is expressed as a slope (the number of microns to rise for every micron out in that direction). Values of 0.0001 to 0.005 are typical for apparently level surfaces. The calculated cant will be used with automatically dispensed patterns to calculate the correct height the dispenser needs to move to for each feature.

Rotational calibration

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In addition to surface leveling, you may wish to calibrate the rotation of a particular substrate to ensure that your printed features align with prepatterned elements. You can do this in the Calibrate Surface window. A dialog box similar to the following will be shown.

	Surface Calib	bration	
Width	6	5000 μm C	
Height	5	5000 μm 🗘	
Depth	1	1000 μm 🗘	
Top-Left	N/A	Use current position	
Top-Right	N/A	Use current position	
Rotation angle	(0°	
X slope		0	
Y slope		0	
Calibrate S	urface Cant	Reset	

To calibrate the rotation of a prepatterned element, choose two points that make up a horizontal line on that element. Move the positioner to the first point (Top-Left) and click the Use

current position button next to the first position in the dialog box. Move the positioner to the second point (Top-Right) and click on the Use current position button next to the second position in the list.

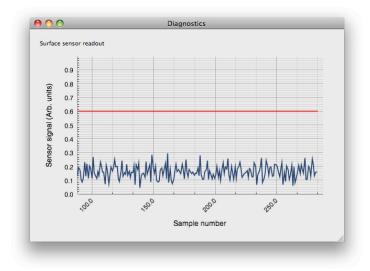
The angle at which the pattern will be rotated, based on these points, is displayed at the bottom of the dialog box. When you go to print a pattern the software will adjust it to match this rotation.

This rotational calibration will be used for every pattern printed after this point until you clear the calibration. To clear the calibration, click on the Clear rotational calibration button at the bottom of the dialog box.

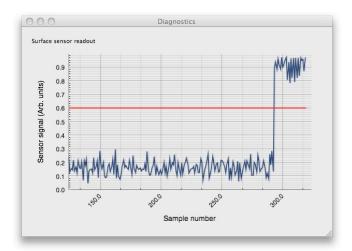
Diagnostics

-

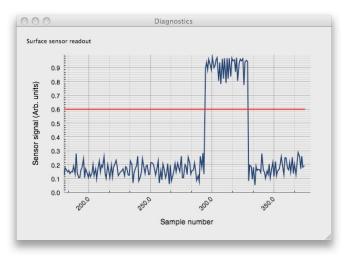
For troubleshooting purposes, the response of the surface sensor can monitored in realtime. To display the feedback from the sensor, choose the Window | Diagnostics menu item. At regular intervals, the surface sensor will be polled and the results displayed in the diagnostics window, as shown.



If the dispenser is then moved down until it contacts the surface, the sensor signal will increase in a stepwise fashion.



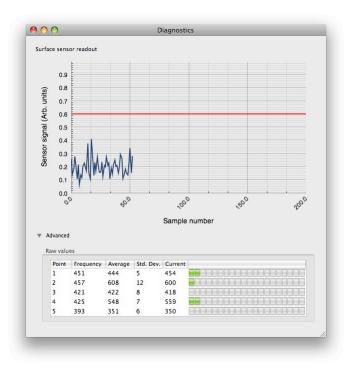
The red line indicates the threshold for surface sensing. If the signal is not strong enough to cross this threshold, the software will not detect contact of the dispenser with the surface. In that case, recalibrate the surface sensor as described in the previous section.



When retracted from the surface, the signal should sharply drop off once again.

If this behavior is not observed, repeat the calibration and try again.

This sensor feedback can be monitored at any time while working with the dispenser, and can be useful when moving the dispenser around manually in order to verify that contact has been made with a surface when dispensing.



Additional diagnostic information can be obtained by clicking on the disclosure triangle next to the Advanced label. This will expose a series of realtime readouts from the ultrasonic frequencies used to determine when contact has been made with a surface. This can be used by SonoPlot personnel to help figure out why a given dispenser may not be behaving properly.

Useful Keyboard Shortcuts

The SonoGuide software includes a series of useful keyboard shortcuts:

Find Surface: Ctrl/Cmd + F

Set A1 position: Ctrl/Cmd + 1

Move to desired position: Ctrl/Option + click

Go Home: Fn + Left arrow (MacOS)

Go Home: Home key (Windows)

When using Trackball: Hold SHIFT to move 10X

When using Manual buttons: Hold SHIFT to move 10X, Hold Ctrl/Cmd to move 1/10X

.....

Print: Ctrl/Cmd + P

TROUBLESHOOTING

The dispenser will no longer take up fluid

This may be the result of clogging within the dispenser. To resolve this, move the dispenser back into the source fluid well. Open the dispenser controls by choosing the Dispenser | Show dispenser controls menu item or the Control dispenser toolbar item. First, try agitating the dispenser using gentle ultrasonics by clicking on the Dispense button in that window. If you do not see items within the clogged area starting to move around or loosen up, increase the ultrasonic strength by clicking on the Spray button. If that still isn't working, gradually increase the spraying voltage. If you reach 20 V and the clog has still not cleared, allow the dispenser to sit in the well with the ultrasonics still being applied for at least 10 minutes.

If that still has not cleared the clog, move the dispenser into a well of pure solvent and repeat the same procedure.

Polymer solutions may permanently clog the dispenser if crosslinking or polymerization occurs within or immediately outside of the dispenser's tip. Other polymers may harden to the point where solvent is unable to dissolve the polymer, permanently sealing it shut.

SonoGuide gives the error message "Positioner is not connected"

The control electronics and positioner must be turned on to allow the computer to communicate with the positioner. Turn on the electronics box and positioner, then click OK in the warning dialog box. If you still cannot control the positioner, exit out of SonoGuide and restart the application.

This message may also occur after a crash or other unclean shutdown of the control software. To clear it, exit out of the SonoGuide software and restart.

If the electronics box is on and you still are receiving this message, exit out of the control software, turn the electronics box off and on again, and restart the software.

Software has frozen

This can be caused by several different factors. To exit the software, click on the Finder icon in the Dock (the two-tone blue face) and choose Force Quit... from the Apple menu in the upperleft of the screen. The Force Quit Applications box window will appear. Select SonoGuide from the list and click the Force Quit button. Confirm that you want to Force Quit the application and close this window. Restarting the software should clear the error that caused the software to freeze.

Positioning controls stop responding, but the remainder of the control software still functions

This problem is caused by a communication error with the positioner. Restart the software. It should not be necessary to re-home the positioner.

The positioner does not return to the back left corner when homing, or gets stuck

While rare, it is possible for the motors to experience excessive load when returning to home, or to get stuck at the outer limits of travel if the lubrication has become unevenly distributed. If this happens, make sure no objects are blocking the travel of the mechanical stages, exit out of SonoGuide, and turn off the control electronics box. Manually push the X and Y stages back and to the left by 2-3 centimeters. Restart the control electronics box and restart SonoGuide. This should allow the stages to home properly once again.

Dispenser cracks or shatters

As the dispensers are very fragile, you must be careful to ensure you are not putting too much pressure on the dispensing tip. Sonoplot suggests moving the tip down to a short distance above the surface, then stepping down in 10 micron steps until a fluid bridge is made between the tip and the surface. It will require some experimentation to determine the voltage required to dispense the fluid without spraying. Also be sure that you have calibrated for the cant of the surface, to ensure that the tip does not drag against the surface during your dispense pattern.

The impedance scan shows no strong resonance, or otherwise does not look like the examples in this manual

There are two possible problems here, which can be differentiated by the characteristics of the impedance scan:

If the impedance scan starts at about 980 ohms and decreases linearly, with no major features, the dispenser is not connected to the electronics and an open circuit is being measured. Check that the RJ-11 (telephone) plug at the end of the dispenser is snugly fitted into the black RJ-11 socket on the front of the dispenser. It is also possible that the cable connecting the dispenser to the electronics is unplugged. Check that the gray RJ-11 cable is plugged into both the black RJ-11 socket on the front of the positioner, as well as into the back of the control electronics box.

If the impedance scan is a series of noisy peaks between 0 and 20 ohms, the dispenser is short-circuited. At this time, the only solution is to replace the dispenser.

NOTE: A short circuit is possible if you have been working with a metallic ink, and the ink has formed bridge across both sides of the piezoelectric element (the gray rectangle to which the glass capillary is attached). Try carefully cleaning the piezoelectric with an appropriate solvent for your ink. Be careful to avoid damaging the tip during cleaning.

The dispenser does not accurately locate the surface when using the Find Surface command

The dispenser may be miscalibrated. Move the dispenser so that it is clear of the surface and select the Calibrate dispenser toolbar item. This should bring up the calibration dialog, as described in Chapter 4. Click on Calibrate to begin the calibration process.

To verify that the surface sensor is functioning correctly, you can bring up the sensor diagnostics dialog by choosing the Dispenser | Show Diagnostics menu item (as described in Chapter4). This window displays the realtime readout from the surface sensor. A red line indicates the sensor threshold for when the software will register contact with the surface. When the dispenser is above the surface, the signal should be below this line. Upon contact with the surface, the signal should jump above this threshold. If it does not, and recalibrating the dispenser does not help, the dispenser itself may have a physical problem and may need to be replaced.

When a move command is given, the positioner does not move and the positioning controls stop responding

The positioner is not connected to the control electronics. Ensure the communication cables between the control electronics and the positioner are plugged in at both ends.

The camera view appears as a white square

The CCD camera is not communicating with SonoGuide, most likely because it is not plugged in. Ensure that the USB cable is connected to a USB port on the back of the computer, as well as plugged into the camera. If it is, reseat the cable. Restart SonoGuide.

Feature size	20 <i>µ</i> m - 200 <i>µ</i> m
Feature types	Droplets and contiguous lines
Deposition volume	≥ 1 pL
Deposition variability	As low as 10%
Viscosity	≤ 450 cP
Positioning	31 x 31 x 7 cm (X, Y, Z axes) 10 μ m resolution
Calibration	Automatic surface height calibration
Camera	USB 3.0 with digital video capture
Software	SonoGuide
Dimensions	58.4 x 59.7 x 61 cm (23 x 23.5 x 24 in.)
Weight	91 kg (200 lbs)
Power	3.0 A for 100-200 V or 1.5 A for 220-240 V

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DISPENSER REFURBISHING

The glass micropipette on the dispenser can be replaced by the user. Open the dispenser cartridge assembly by loosening the two black plastic screws on the front of the dispenser assembly. Remove the dispenser cover.

Remove the old glass micropipette by removing the RJ 11 wire assembly and dipping the piezo/

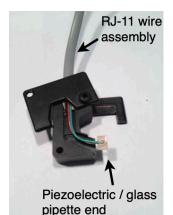
glass pipette end of the RJ 11 wire in acetone until the glue holding the micropipette is dissolved. Be careful to only dip the piezoelectric element in the acetone, and not the gray RJ11 jacket, as the jacket will swell and rip. Attach a new glass micropipette using a cyanoacrylate glue. Use a thin, quick-drying glue and not the gel formulation in order to have the best transmission of ultrasonic vibrations to the glass. Use a fresh tube of glue every day; old glue can result in poor adhesion and improper functioning of the dispenser.

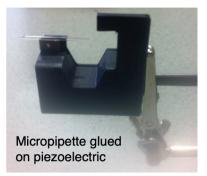
Shorten the glass micropipette from the wide end by scribing the glass and breaking it. Use a scoring wafer such as the Sutter ceramic scoring wafer (<u>www.sutter.com</u>, part number CTS).

Reassemble the dispenser by replacing the RJ11 cable into the cartridge body. Using alligator clips or helping hands, orient the dispenser so that the piezoelectric element is facing up. Apply a very thin bead of glue to the edge of the piezoelectric, ensuring no glue gets on the faces of the piezoelectric. Position the glass micropipette such that the tapered end (tip) does not extend more than 7 to 8 mm below the bottom of the piezo element. Once the new micropipette is attached, and the glue has dried, scribe the wide end of the glass just above the top of the piezo element.

There are several critical parts in the replacement process:

- Ensure correct alignment with the long edge of the piezoelectric element.
- Ensure there is no glue on either face of the piezo.
- Scribe and break the glass cleanly to avoid a jagged end and glass shards in the capillary.
- · Avoid touching the capillary tip. The slightest contact will break the tip









Version 1.3

•Updated SonoGuide chapter to include new version UI/features.

Version 1.2

• Added the <polygon> pattern element to Chapter 9.

Version 1.1

• Revised the SonoGuide chapter to include the new pattern preview window and DXF import capabilities.

Version 1.0

Initial revision