

MICROPLOTTER™ II

MANUAL

REVISION 2.1

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INTRODUCTION

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 two software packages that control the dispenser and automate the dispensing of patterns on a surface, SonoGuide and SonoDraw, will be described in Chapters 4 and 5, respectively. Solutions to common problems will be listed in Chapter 6 and the technical specifications will be described in detail in Chapter 7. As appendices, how to refurbish a dispenser will be covered in Chapter 8, and the file format used by SonoGuide and SonoDraw is specified in Chapter 9.

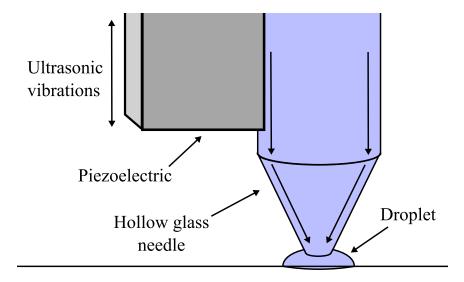
Before that, however, it would be appropriate to describe the fundamental operating principles of a Microplotter instrument.

Ultrasonic fluid dispensing

A Microplotter instrument is capable of applying picoliters of fluid to a surface in order to create features as small as 5 micrometers (microns) wide due to its use of a novel form of fluid ejection based on ultrasonic pumping.

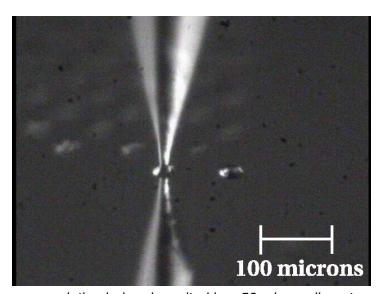
The dispenser at the core of a Microplotter system is composed of a hollow, tapered glass needle attached to a piezoelectric element. When an alternating current is supplied to the piezoelectric, it vibrates. At certain frequencies, the resonant frequencies of the dispenser, a pumping action occurs within 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 crosscontamination.

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 or arcs. 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 or curve, leaving behind a smooth line or arc wherever it passes. Only when the line or arc 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 30 x 9.6 cm volume with a 5 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 designed in the SonoDraw package (featured in Chapter 5).

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

2 Installation

Unpacking and setup

The GIX Microplotter II instrument will come in one large crate containing the system itself, the control electronics, a box of smaller components, and the all-in-one iMac control computer.

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:

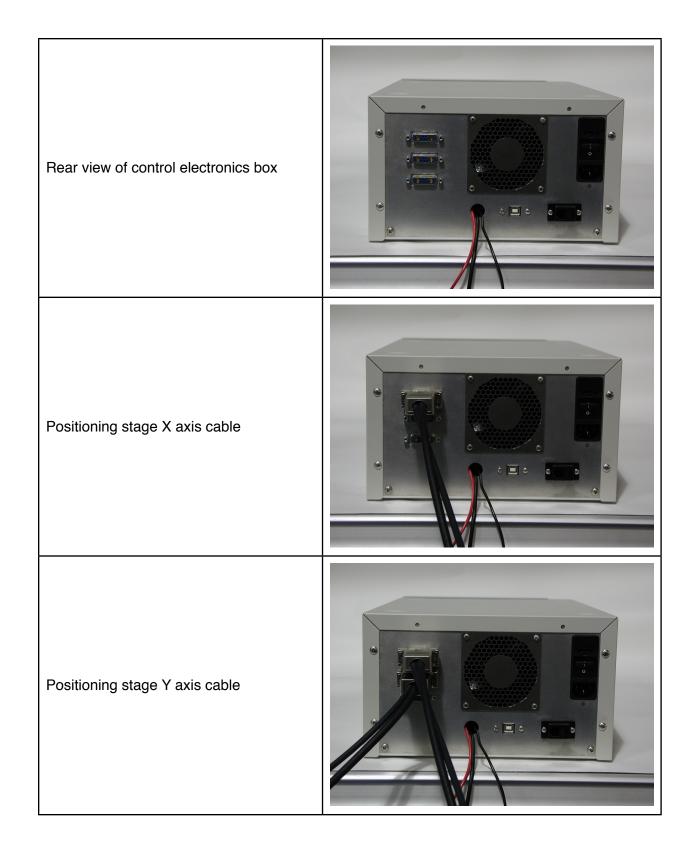
- · Positioning system, with printhead and optics system already mounted
- iMac control computer (with keyboard and mouse)
- Control electronics

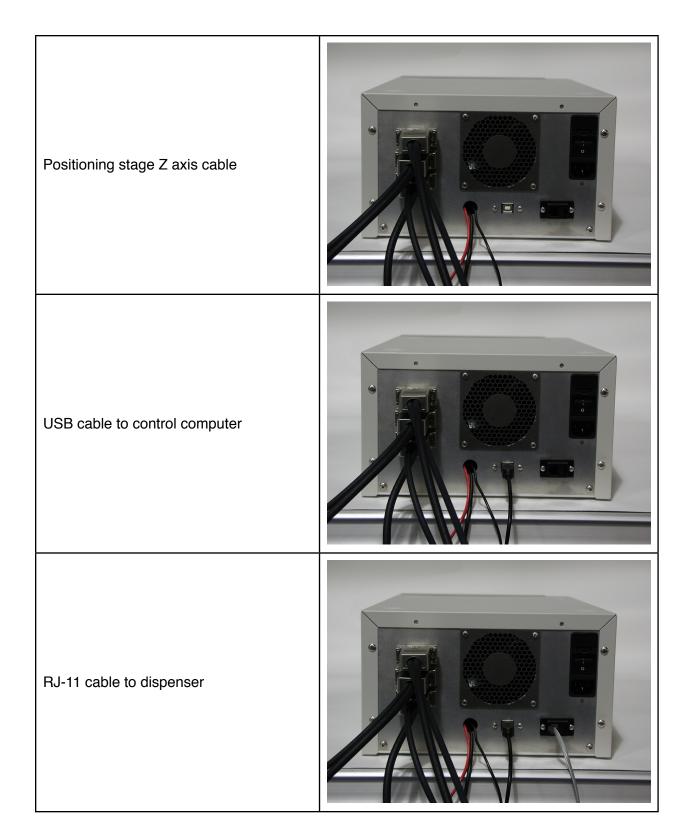
Additionally, there will be a box containing cables for attachment of the various components.

Positioning system

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 computer

Place the computer next to the control electronics. For almost all of the cables going into the computer, there is only one place that they can be plugged into, but the following sections will step you through the process of attaching all of them.

If your system comes with a wireless keyboard and mouse, turn on the keyboard by pressing in the power button on its side.



The wireless mouse can be turned on using the small switch on the upper-right side of its bottom.



Computer cables

Insert the USB cable for connecting to the control electronics.



Insert the USB cable from the CCD camera.



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 iMac and the control electronics.

Redistributing Iubrication

Sometimes during shipment or after lengthy periods of inactivity the positioning stages (in particular the Y axis) can suffer from rougher movement, leading to intermittent positioning errors and failures to home. If you notice that the positioning stages do not move all the way to the rear during initial homing after unpacking, or experience errors during movement, the lubrication in the stages may need to be redistributed.

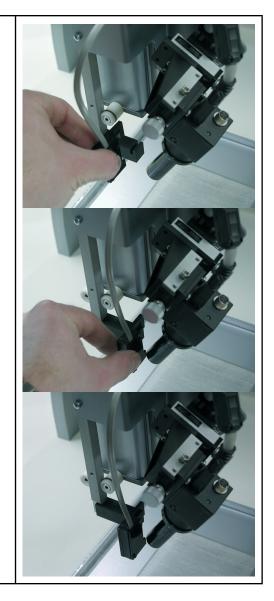
To do this, exit out of SonoGuide and turn off the control electronics box. Grab the overhanging X axis and gently move the positioning stages to the rear and to the front limits four or five times. This should be enough to smooth out the lubrication and prevent these errors.

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

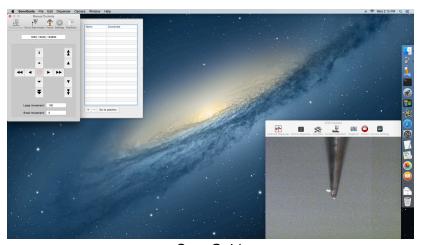
Once all components have been assembled and the computer and control electronics are plugged in, it is possible to power on the system and start dispensing. To turn on the computer, press the power button located on its back (on the left side as you face the display). Likewise, flip the power switch 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 will be illuminated on the front of the box.

Assuming the positioner and control electronics have been turned on, and the computer has booted into Mac OS, a user will automatically be logged in and you will be presented with a desktop similar to the following (the specific arrangement of the desktop may vary slightly, depending on system model).



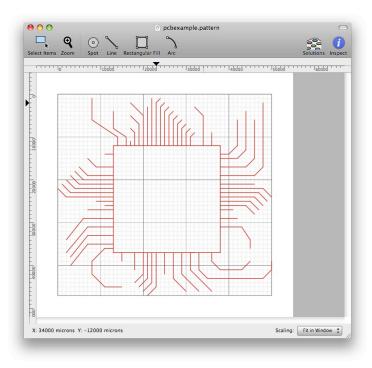
Mac OS X desktop

To the right is the Dock, a bar containing commonly used applications. Two of the icons are for programs installed as part of your GIX Microplotter II instrument: SonoGuide and SonoDraw. 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

SonoDraw, the focus of Chapter 5, is a vector-based drawing program that lets you create and edit the patterns dispensed through the SonoGuide software.



SonoDraw

If you wish to install new software, or edit properties of the system, you may need to log in as or provide the password for the primary user of the computer. From the factory, that user's settings are:

Username: microplotter Password: sonoplot

You may change these values using the System Preferences panel, which can be accessed through the Apple menu in the upper-left-hand portion of the screen.

Help about the functioning of the Mac OS X operating system can be found in the Help menu of the Finder.



Fully installed Microplotter II system

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SAMPLE APPLICATIONS

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.

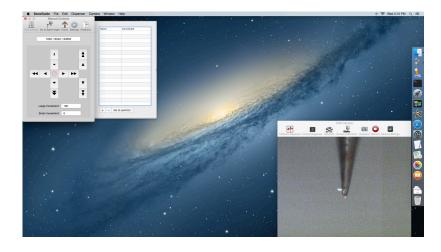
These examples assume that the system has been properly installed as described in Chapter 2.

One-component oligonucleotide microarray

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 icon in the Dock on the right-hand side of the screen.

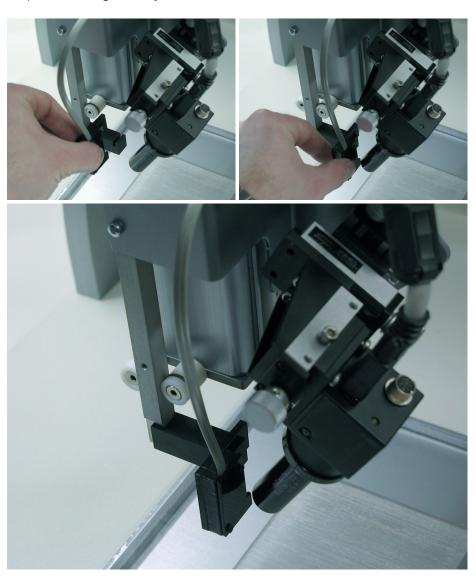
This will start up the SonoGuide control software, your main interface to the Microplotter system. Because the positioning robot has just been turned on, it will need to home itself to establish a starting position. The software should inform you of this, ask you to clear the movement area of the robotic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

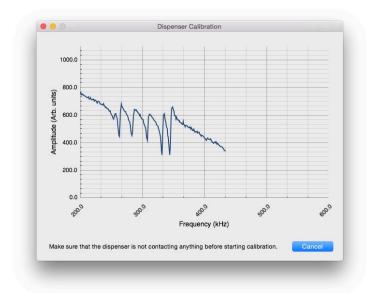
After loading the slide, clip into place a new dispenser if one is not already in the system. Connect its telephone wiring to the jack on the Z-axis.



As soon as the new dispenser's wiring is connected, a little status message will appear onscreen to indicate that the dispenser has been detected.



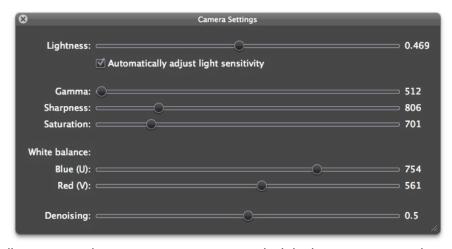
The software will then calibrate this dispenser, using a window like the following:



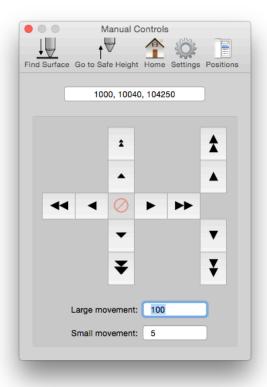
This should take approximately two minutes, after which a notification will appear that confirms the calibration of the dispenser.



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 either by selecting the menu item Camera | Show camera settings or clicking on the toolbar item Camera settings and adjusting the sliders in the dialog that will appear. Close this window when finished.



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 movement and Small movement text input boxes. All values are in microns. 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.

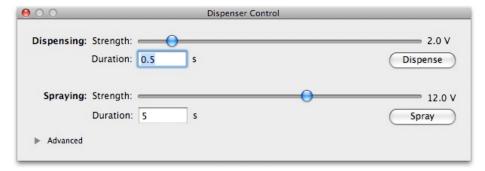
Change the large step size to 1000 microns (one millimeter) and more finely position the dispenser relative to the surface of the microscope slide. Click on the Find surface button above the manual controls to have the dispenser move down slowly and detect the height of the surface. If this stops before 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.

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



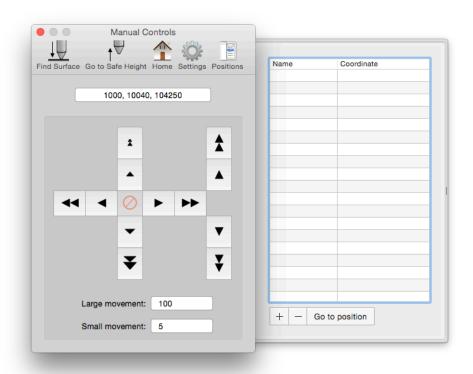
Under the Distance to calibrate group,set the distance to calibrate along the X axis to 20000 microns (20 mm is just a little less than the full 25 mm width of a typical microscope slide) and set the distance to calibrate along the Y axis to 70000 microns (again, 70 mm is just shorter than the full 75 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 <code>Dispenser</code> | Show <code>dispenser</code> controls or click on the <code>Control</code> dispenser toolbar item to bring up the screen that controls the dispenser settings.



If not already set at this value, change the Dispensing strength to be 2 V and the Spraying strength to be 14 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 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 drawer to the right side of the manual controls window.

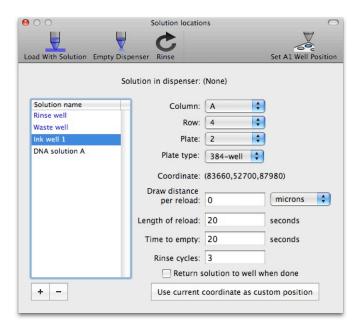


This will create a new position and leave you able to edit its name. Type in Above slide and hit Return when you are done. The new position is added to the list of saved positions, meaning that it will be easy to return here in the future.

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).

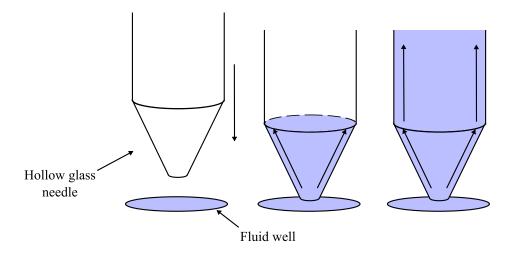
Warning: Be careful when moving the dispenser near a solid object, such as the wells. If the glass tip comes in contact with the side or bottom of the well, it can shatter.

To save the location of this well, select the Dispenser | Show solution locations... menu item or click on the Solutions toolbar item. This will bring up a window for setting solution locations.



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. By default, it will set this up as a custom position, using the current coordinates.

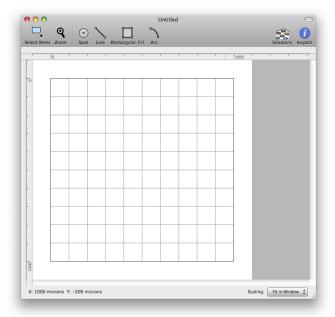
When the glass tip of the dispenser dips into solution, that solution should start rising up the inside of the dispenser through capillary action, as shown in the following illustration:



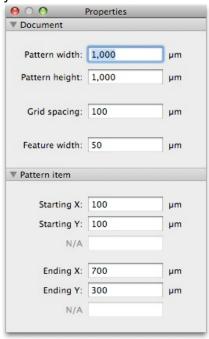
If it does not, the glass tip may be clogged with a small dust particle. To clear this, open the dispenser control window again by selecting the menu item <code>Dispenser</code> | Show <code>dispenser</code> controls. Click first on the <code>Dispense</code> button to drive the dispenser at its more gentle settings. Wait for a few seconds. If that has not caused fluid to rise up within the dispenser, click on <code>Spray</code>. 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 <code>Cancel</code> 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 pattern to be dispensed on the surface. A pattern is a representation of the fluid features (spots, lines, etc.) that will be drawn onto the surface by the Microplotter system. Patterns are created and edited in a separate drawing program, called SonoDraw.

Start that program now by clicking on the SonoDraw icon in the Dock on the right-hand side of the screen. You will be presented with the SonoDraw menu at the top of the screen and an empty pattern.



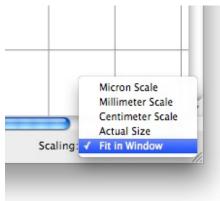
This pattern has a grid as a drawing aid with a spacing of 100 microns. To change that, click on the toolbar item labeled Inspect. This will bring up an inspector window containing settings for the current document. Change the spacing on the grid to 150 microns, a typical center-to-center spacing for spots on a microarray.



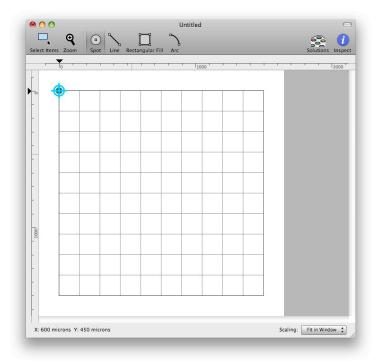
Change the pattern width and height to 1500 microns each. The grid and document will resize themselves after you hit Return in each field or click away to another one.

Set the feature width to be 50 microns. This last setting only helps to preview the pattern as it will be drawn, it does not change any dispenser settings. When these settings have been entered, close this window.

With the larger working grid and document area, you may wish to zoom out to see the entire document. You can manually zoom out by pressing -, or selecting View | Zoom Out. To automatically zoom the document to fit the window, either choose View | Zoom To Fit or select the menu option Fit in Window in the lower-right-hand corner of the document window.

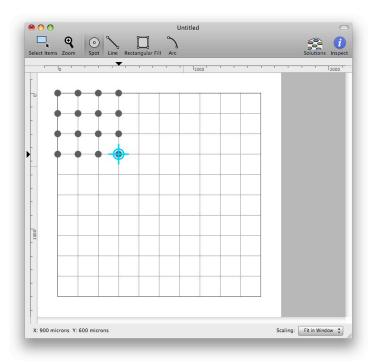


The pattern you will construct will be a 4 x 4 array of spots. In order to draw the spots, you will first need to select the spot tool by clicking on the toolbar item named Spot. Click once on the upper-left-hand corner of the layout grid to draw a spot.



Complete the rest of the array by drawing one spot after another, from left to right and top to bottom. If a spot is drawn in a wrong location, either select Edit | Undo or change to the selection tool by clicking on the Select Items toolbar item, click on the bad spot, and choose

Edit | Delete or hit the Delete key. Afterwards, be sure to reselect the spot drawing tool. When done, you should be presented with an array that looks like the following.



To save this pattern to disk so that it can be loaded by SonoGuide, choose File | Save As... and select a location and file name. For this example, let's pick the Documents folder and name the file SpotGrid. SonoDraw will automatically append the proper .pattern file extension when saving the pattern.

Now that drawing of the new pattern is finished, you can close out of SonoDraw using SonoDraw | Quit SonoDraw. 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 upper-left corner 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, SpotGrid.pattern, that was saved in Documents. A preview of the pattern will be shown, along with options for assigning solution wells to layers within the pattern and other settings.

Click on Print to start the printing process. The controlling computer will automate the dispensing process from beginning to end. A graphical display will show the items being printed as they are deposited, and a progress bar will fill in the lower right corner of this window. Buttons in the lower-right will also allow for the pattern printing to be paused or cancelled.

When the pattern has finished, open the list of solutions through the <code>Dispenser</code> | Show solution locations... menu item or Solution toolbar item. Click on the <code>Empty dispenser</code> 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

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.

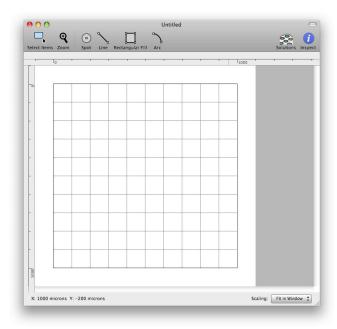
Next, an appropriate ink needs to be loaded in a solution well. Which well to load this ink in will be determined by the ink itself.

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.

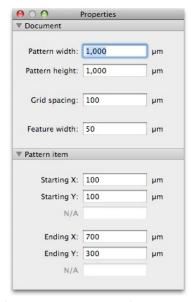
Nanometallic inks using ethanol or water as a solvent can safely be loaded in the wells of a standard microtiter plate.

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. If you wish to use an existing pattern, these series of instructions can be ignored. To start, open SonoDraw as described in the previous section. As mentioned there, you will be presented with the SonoDraw menu at the top of the screen and a blank pattern.



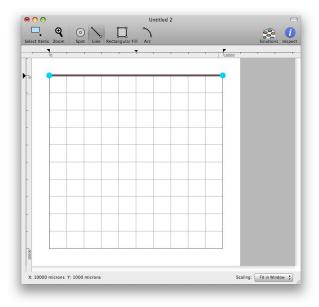
For this pattern, you will want to change the grid spacing, so bring up the document inspector by clicking on the Inspect toolbar button.



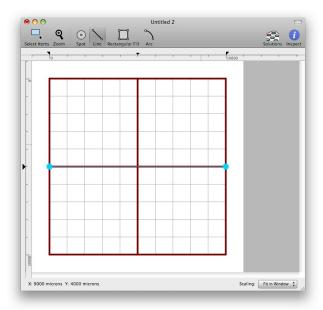
This will bring up the settings for the current pattern document. Change the spacing on the grid to 1000 microns, the X and Y sizes to 10000 microns each, and the feature size to 100 microns. When these settings have been entered, close this window.

Zoom out the document so that it fits the window by choosing the Fit in Window menu option in the lower-right-hand corner of the document window or by selecting the menu item View | Zoom To Fit.

The pattern you will construct will be a grid of lines. In order to draw the lines, you will first need to select the line tool by clicking on the Line toolbar item. Click and drag to draw a line.



Draw the remaining lines in the pattern. An example of a drawn grid of lines is shown in the following figure.



Save this pattern to disk using File | Save As... For this example, let's pick the Documents folder and name the file LinePattern. The proper .pattern file extension should be added automatically.

Now that drawing of the new pattern is finished, you can close out of SonoDraw using SonoDraw | Quit SonoDraw. 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.

4

SONOGUIDE

Overview

SonoGuide is the main control interface for the Microplotter II 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.

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 communicate with the positioner. In that case, check all connections, turn all equipment on, and click OK to the warning dialog that appears:



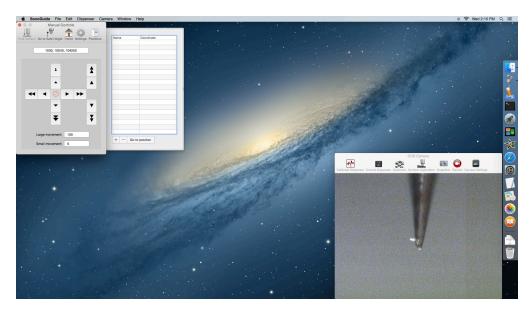
The software should reconnect to the positioner automatically. If it does not, exit out of SonoGuide and restart the software.

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.



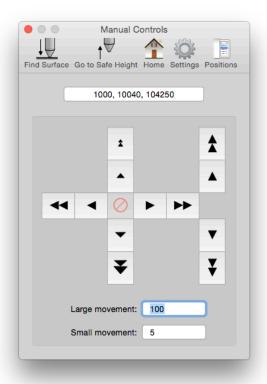
The graphical interface has several sections, including:

- · Directional controls
- · Saved positions
- CCD camera display window

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

Directional controls

The panel for the directional controls appears as follows:



These controls allow you to move the positioner manually in a stepwise fashion. There are two sets of buttons, those with a single arrow and those with two arrows. 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 movement and Small movement text boxes. For reference, 1000microns = 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.

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 this control window are several toolbar items. The first 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.

After that is 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.

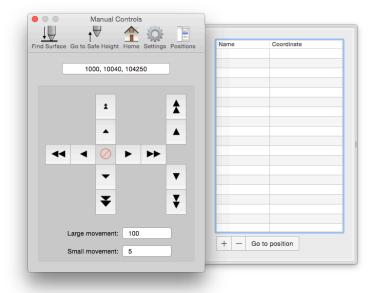
Warning: The home move travels over a large distance and can be very fast, so make sure that all objects and personnel are clear of the positioner's working range before clicking on this button.

The next item is labeled Settings and clicking on it will open a window that allows you to adjust motor speeds, as well as other positioner preferences (see the related section for more).

Finally, the Positions toolbar item gives the ability to open and close the saved positions drawer to the right of the manual controls window.

Saving positions

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



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

- +
- _
- Go to position

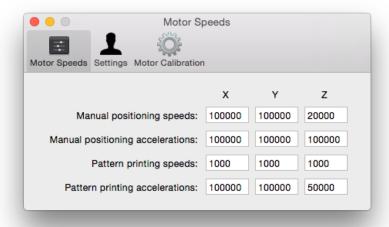
To move to a previously saved position, highlight a position by clicking on it and press Go to position. This will cause the dispenser to pick up to a set height (the setting of which will be explained in the next section), move over to the correct X, Y coordinate, and move the print head back down to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

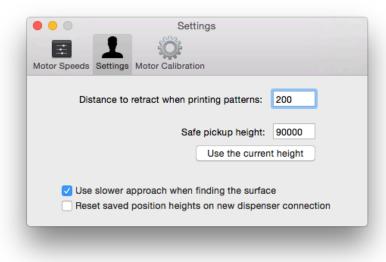
Robotic positioner settings

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 | Preferences menu option or Settings toolbar item.



This window is split into three sections. The first 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 have clicked elsewhere in the window or hit Enter to confirm.

The next section lets you set various customization options for the operation of the system:



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 Distance to retract when printing patterns 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.

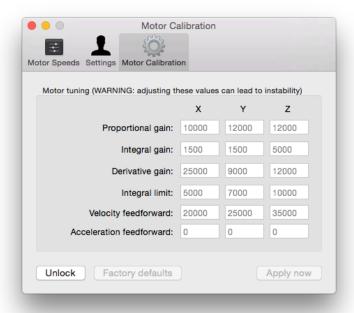
In the main interface, when you select to move to a previously saved position, the positioning robot lifts the dispenser head up to a safe height, moves it to the X and Y coordinate of the new position, and brings the dispenser down to the proper Z coordinate. That safe pickup height is set in this window, 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.

The remaining checkbox is a safety option that can be enabled so that the Z heights of saved positions are reset to the safe pickup height when a new dispenser is connected.

The last section of this window 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.



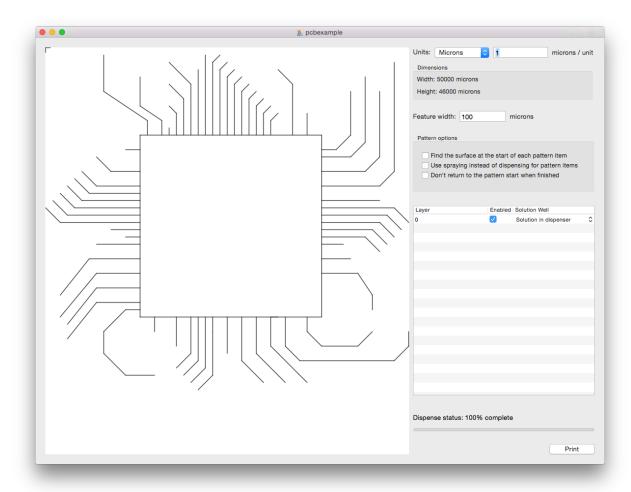
If you do need to adjust these settings, you need to first unlock them by clicking the Unlock button. You will then be asked for the password for the primary user account of this system. The default login information is contained within Chapter 2. Once unlocked, each value can be edited, and the new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

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 following window:



On the left will be the pattern as it is to be printed. A crosshair indicates the current position of the dispenser, relative to the rest of the pattern, and its location is based on the (0,0) coordinate in the pattern or DXF file. This usually will be in one of the corners of the preview.

To the right are options for how the pattern will be printed. Because DXF files do not have native units, a conversion needs to be applied to size them appropriately for printing. A pull-down list lets you specify one of a few standard unit types, or the number of microns per drawing unit can be manually entered in the box to the right of that. The overall size of the pattern after this scaling is applied can be found in the box right below this, to verify that the units are correct.

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.

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.

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.

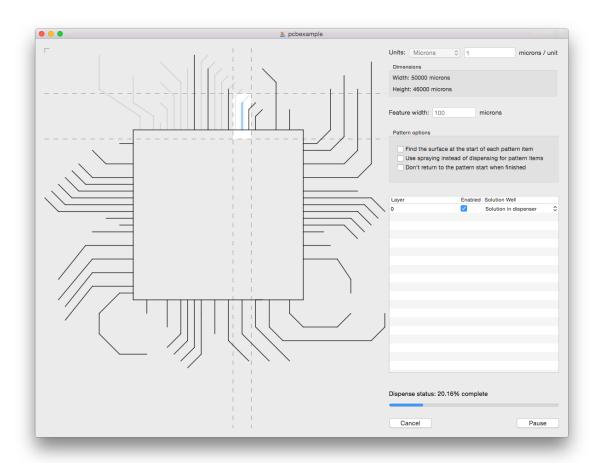
The next item tells the system to use the spraying action instead of the more gentle dispensing actuation when printing. This is occasionally used to coat larger areas with a less controlled spray of material.

Finally, the system can 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.

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, you can click on the Print button. Closing the window will abort the print.

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.



Also present are options to pause or cancel the printing of the pattern. Clicking Cancel will terminate the printing of the pattern and return the dispenser to the starting point. Choosing Pause will instead pause the pattern and let you make adjustments.

This lets you manually reload solution in the dispenser, adjust the camera or other settings, or move the dispenser around to relocate the remainder of the pattern. If you choose Resume, the pattern will resume printing from the location it left off and ignore any positioning adjustments you made. If you choose Resume From Here, the pattern will resume printing from the current location and will preserve any positioning adjustments. Cancel will once again abort the pattern.

Camera display

A final portion of the 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.



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 (http://www.videolan.org/vlc/), 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 camera display window are a series of 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.

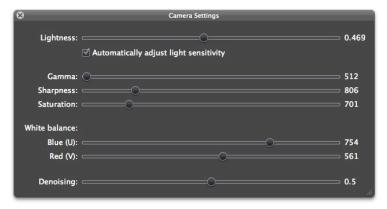
The second is the Control dispenser item, which will bring up the window that lets you control the dispenser.

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.

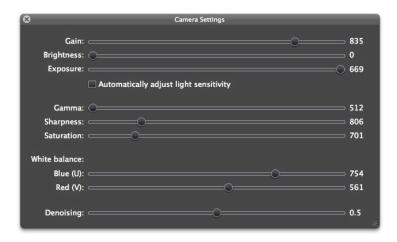
Camera settings

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.



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:



The settings that can be controlled include:

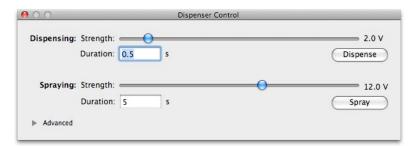
- **Gain** The CCD's gain setting controls the degree of amplification of the video input. A high gain is necessary to see poorly lit objects, but will introduce camera noise. As stated above, this should be used with Exposure to tune the camera input for different lighting conditions.
- **Brightness**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



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

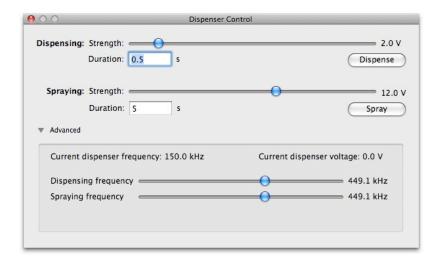
variabilities for droplets dispensed on chemically uniform surfaces. However, it can lead to missing droplets.

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.



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.

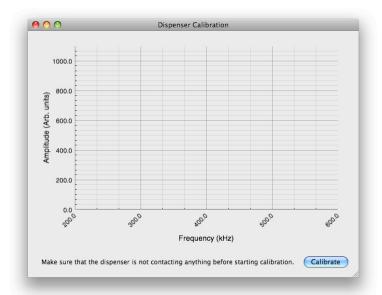
Dispenser calibration

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.

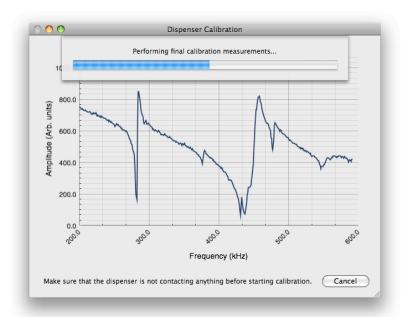
The connection of a dispenser to the system is automatically detected, and will cause the software to display a notification.



This will then bring up the dispenser calibration window.



If a dispenser has just been connected, the calibration process will start automatically. A recalibration can also be triggered manually 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.



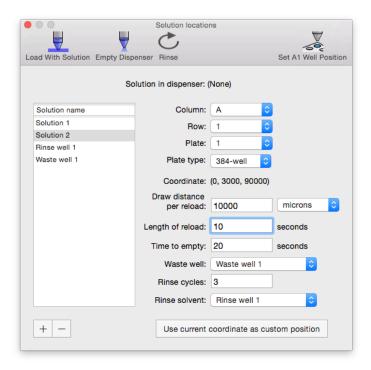
To return to this calibration dialog, either choose the menu item Dispenser | Perform Dispenser Calibration... or the Calibrate dispenser toolbar item.

Solution well locations

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 the rinse solvent and waste wells, locations are specified in the following window:



The very top of the window shows which solution is currently loaded into the dispenser.

A list of solutions is present at the left of this window. 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.

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.

When a solution has been selected, its properties will appear on the right of the window. The easiest way to set up many solutions is to place those solutions within a 96- or 384-well microtiter plate. If you have done this, then all you need to do to specify their position is to select the appropriate plate type from the pull-down list, and then choose the column and row for that solution on the plate.

If your solutions are not in a microtiter 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. Additionally, sturdy metal pins in the configuration of a dispenser can also be used to sense surface positions and calibrate the cant of a deck to further prevent damage to the dispensers. 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.

Open the surface cant calibration window by selecting the menu item Dispenser | Perform Surface Cant Calibration... or selecting the Surface calibration toolbar item.



In the Distance to calibrate group, enter in 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 move the pin or dispenser slightly to the right and down so that it is just over the sample.

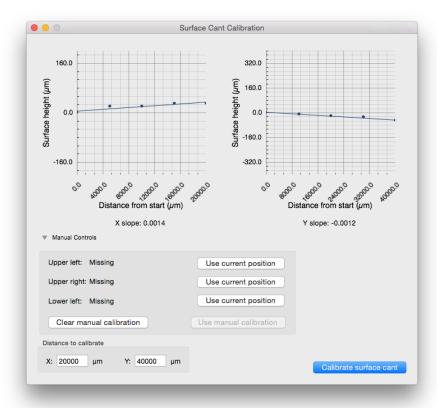
Click on Calibrate surface cant to begin the surface leveling process. The surface height will be electronically sensed at five points in the horizontal and vertical directions. As this sensing is taking place, the display will update with the relative heights of the sensed points in either direction.

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.

As a quality control measure, the fit line for the five points used to determine this slope will be overlaid on the sensed points for the X and Y directions. If there are one or more sensed points that deviate significantly from the fit line, that may indicate that there was an error in the surface calibration process or that an irregular feature like a dust particle on the surface was hit. It is recommended that you re-run the surface cant calibration after moving the dispenser slightly to the right or down.

Additionally, you can manually specify the surface slope to use. This may be needed in some situations, such as for very soft materials that surface sensing fails on or for very large aperture

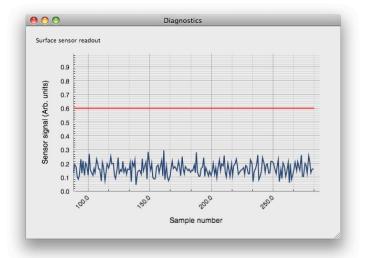
dispensers. To do this, click the arrow to the left of "Manual Controls" to expose the manual settings:



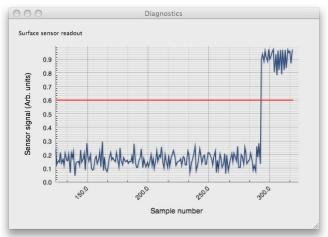
Move the positioner to the upper left corner of the area you want to calibrate, and bring the tip of the dispenser down until it just touches the surface. Click on Use current position to the right of the upper left setting to store that location. Repeat this process for the upper right and lower left points on the surface. When these are ready, click on Use manual calibration to overwrite the previous surface cant calibration with the slopes calculated from these values. If you no longer wish to use these values, click on Clear manual calibration to zero out the X and Y slopes.

Diagnostics

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

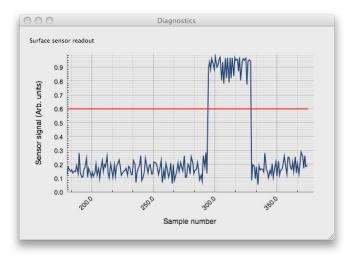


If the dispenser is then moved down until it contacts the surface, the sensor signal will increase in a stepwise fashion.



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.

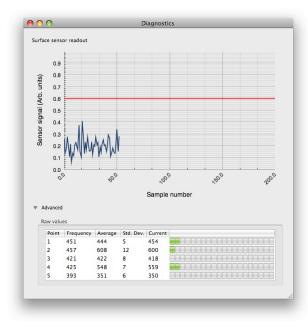
When retracted from the surface, the signal should sharply drop off once again.



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

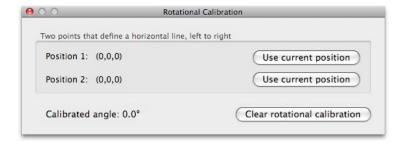
This sensor feedback can be monitored at any time while working with the dispenser, and can be useful when moving the dispenser around manually in order to verify that contact has been made with a surface when dispensing.

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.



Rotational calibration

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 by choosing the Dispenser | Perform Rotational Calibration... menu item. A dialog box similar to the following will be shown.



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 and click the Use current position button next to the first position in the dialog box. Move the positioner to the second point 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.

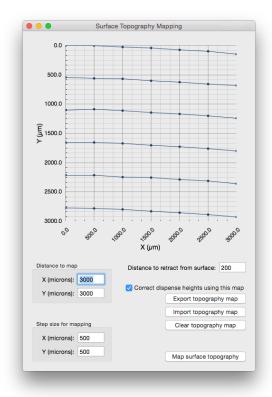
This rotational calibration will be used for every pattern printed after this point until you clear the calibration. To clear the calibration, click on the Clear rotational calibration button at the bottom of the dialog box.

Topography mapping

As an experimental capability, SonoGuide can map the 3-D topography of a surface and use that topography map to print over an irregular or curved surfaces. Typically, this is most useful for large-scale curvature on a surface.

The surface topography mapping uses the surface sensing process to sample surface heights on a rectangular grid. From this grid, the software can interpolate between heights to estimate the surface location at each point within this rectangular area. The less dense these points are, the more likely the interpolated heights are to deviate from the actual surface height at a location.

To access this mapping capability, select the menu item Dispenser | Map Surface Topography.... This will being up a window that looks like the following.



The physical limits of the grid can be set here, as well as the spacing between grid points. A more dense grid will more accurately represent a curved surface, but will take much longer to measure.

Once a topography map has been sampled, it can be exported for later use. Previously captured topography maps can be loaded for reuse. The map itself can be cleared or disabled, if you no longer wish to use it for surface height correction during pattern printing.

5

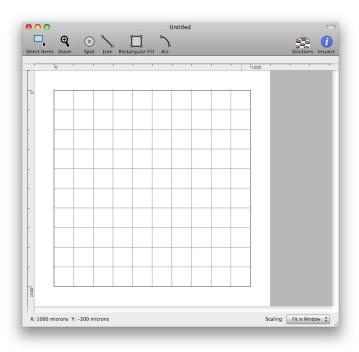
SONO DRAW

Overview

SonoDraw is a vector-based drawing program for laying out the automated patterns a Microplotter instrument can dispense. The fluid dispensing functions of a Microplotter system are closer to those used in blueprint-drawing pen plotters than inkjet printers, so the standard raster images (photos, bitmaps) used as inputs for inkjets are not optimal for a Microplotter system. Raster images are composed of a grid of colored pixels, where vector graphics specify each feature in terms of what it represents (lines, points, arcs, etc.). The unique Microplotter capability of dispensing true continuous lines or arcs makes vector graphics a more natural fit for specifying what to dispense and where.

File functions

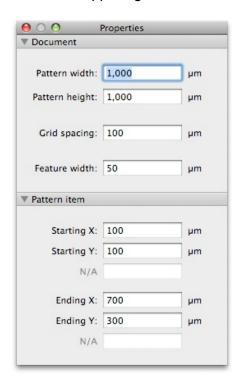
Creating a new pattern, opening an existing one, or saving a new pattern to disk are all handled through the File menu. File | New will create a new window containing a fresh pattern. This pattern will be blank and by default will display a grid to which drawn objects will be snapped.



When you have finished a pattern or just wish to save your changes, choose File | Save and pick a location and file name. SonoDraw patterns are stored as XML files that can also be edited using a standard text editor, and they use the <code>.pattern</code> file extension. The specific structure of this file format is described in a later chapter.

SonoDraw provides a couple of starting templates for commonly-used designs. These include grids and inductor coils. To create a pattern based on one of these, choose the menu item File | New From Template | Grid or File | New From Template | Coil. Each will pop up a dialog where you can set the starting parameters for this template, and then SonoDraw will generate a starting pattern based on that. After that point, the pattern can be edited like any other.

Various document settings can be changed using the inspector window, which is accessed by choosing the Inspect toolbar item in the upper-right-hand corner of the document window.



The upper portion of this window is devoted to document-wide settings. The first two of these settings allow you to resize the drawing canvas, with units of microns. As with each of the fields in the inspector, you can apply the changes made in these fields by hitting the Return key or by clicking into one of the other fields.

The next option concerns the grid used as a guide to snap objects to when drawing (object snap means that when drawing a feature, the feature coordinates are placed at the nearest grid intersection, not where the pointer is right then). The spacing of this grid can be set in microns via this field.

The size of the feature to be dispensed can also be set here. This does not control the size of feature that the dispenser actually puts down on the surface, as that is dictated by physical factors such as ink, dispenser aperture size, and wettability of the surface, but this option allows

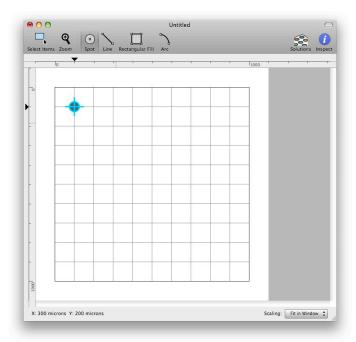
for an accurate preview of what the dispensed pattern should look like when completed. It is best to update this field for a pattern that contains horizontal, vertical, or arc fills before dispensing because the spacing that is used for the over- lapping lines that make up these fills is determined by the specified feature size.

Below the document settings in the inspector are pattern item parameters. If a pattern item is selected, you will be able to edit various aspects of it here. For example, if a line is selected you will be able to edit the starting and ending X and Y coordinates, whereas an arc will present radius, starting angle, ending angle, and center point. As parameters are edited, the pattern item will change to match the values specified.

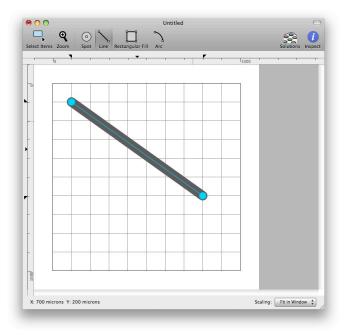
Drawing functions

After starting a new pattern, there are many options for features that can be drawn. These include spots, lines, rectangular area fills made from rastered lines, and arcs. These features can also be edited, as described in more detail in the Editing functions section.

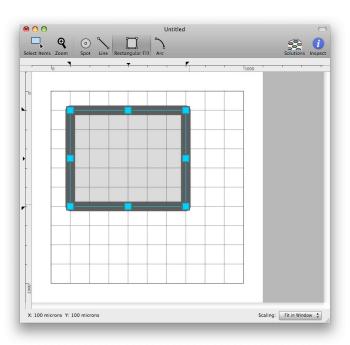
The simplest type of feature that can be drawn is a point, or spot. A spot is simply a round feature drawn by selecting its tool using the Spot toolbar item and then clicking once on a location to place the feature there.



Lines are more complex features than spots, but still are straightforward to draw. The line drawing tool can be selected using the Line toolbar item. To draw a line, click on a starting point for the line, hold the mouse button down, drag the line to the end point, and release the button to leave behind the line.



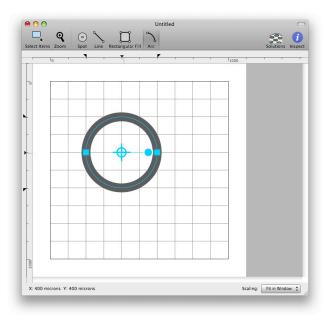
Sometimes it is desired to not just draw individual features, but to coat a specified area with solution. SonoDraw provides a means of drawing a rectangular area to be filled with overlapping lines drawn in either a horizontal or vertical orientation. The edge of line line overlaps with the center of the next to provide a continuous coat of ink over that area. To draw a fill, click on the Rectangular Fill toolbar item, then use the same process as described above for line drawing to click and drag over an area to set the two corners of the fill. The fill will be shown between those two corners, previewed in realtime as you draw.



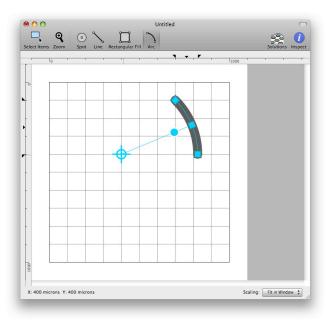
These fills are constructed in such a way as to only coat the area between the two corners, not any- thing beyond. The lines of the fill are moved inward, to avoid overflowing the bounds of the

fill. The amount that the lines are moved inward, in addition to the spacing of the lines themselves, is calculated from the feature size specified as part of the document's settings (for how to set these, consult the previous File functions section). It is therefore important to make sure that the provided feature size accurately matches that of the currently loaded dispenser so that an exact rectangular area can be continuously filled without overflowing.

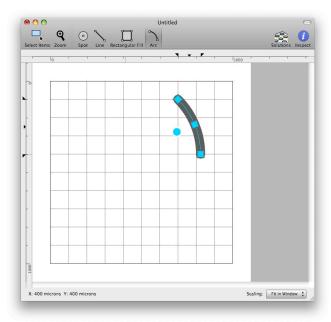
Arcs can also be drawn in SonoDraw. The arc drawing tool can be selected using the Arc toolbar item. To draw an arc, start with the point that will be its center. Click once to set the center.



Move the mouse and click again to set the radius and starting angle.



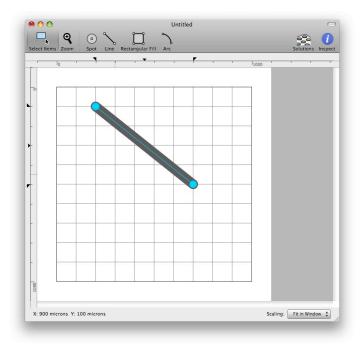
Finish by setting the ending angle of the arc. The arc's two angles and radius can later be edited, as described in the Editing functions section.



Editing functions

After features have been drawn, they can be edited in SonoDraw. Features can be moved, resized, or have their constituent points edited individually.

The first step in editing an item graphically is to select it in the the drawing. To select an item, first switch to selection mode by choosing the Select Items toolbar item. You will then be able to select an item by clicking on it. A selected item will have its control points turn blue, and a small blue wireframe appear within the element. Spots may show a blue crosshairs over each spot, as well.



Multiple items can be selected by clicking and dragging a selection box over the items. Items can also be individually added to or removed from a selection by holding the Select key while clicking on each item.

When items have been selected, you can delete them by either selecting Edit | Delete or hitting the Delete key.

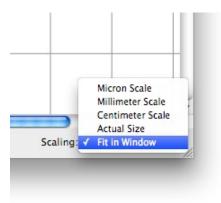
To remove items but store them on the clipboard, where it can be pasted back from at a later time, select it and choose Edit | Cut or press Command-X.

To copy an item to the clipboard, choose Edit | Copy or press Command-C.

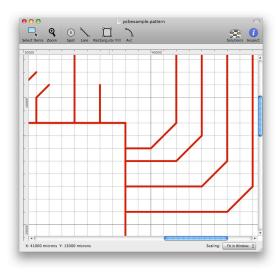
To paste an item from the clipboard to the current position of the cursor, choose Edit | Paste or press Command-V.

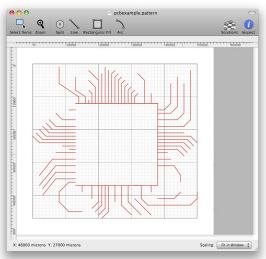
The drawing can be manually zoomed in on using the menu items View | Zoom In and View | Zoom Out or their keyboard equivalents Command = and Command -. Each selection of one of these options zooms in to or out from the drawing by a single step.

The drawing can also be brought to one of a series of zoom factors. All of these can be accessed via a menu at the lower-right-hand corner of the document window, next to the Scaling: label.



By selecting $View \mid Zoom$ to Fit or the Fit in Window menu option in the scaling menu, the scale factor of the document will be adjusted so that the document extents fit within its window.





The zoom factor can also be set so that the document is scaled to specific sizes. Within the scaling menu in the corner of the document window are options to zoom the document to



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 <code>Dispenser</code> | Show <code>dispenser</code> controls menu item or the <code>Control</code> dispenser toolbar item. First, try agitating the dispenser using gentle ultrasonics by clicking on the <code>Dispense</code> 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 <code>Spray</code> 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 upper-left 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.

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 <code>Dispenser</code> | Show <code>Diagnostics</code> 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.

TECHNICAL SPECIFICATIONS

Feature size	size 5 μ m - 200 μ m		
Feature types	Droplets and contiguous lines, arcs, and bends		
Deposition volume	≥ 0.6 pL		
Deposition variability	As low as 10%		
Viscosity	≤ 450 cP		
Positioning	35 x 30 x 7 cm (X, Y, Z axes) 5 μm resolution		
Calibration	Automatic surface height calibration		
Camera	USB 3.0 with digital video capture		
Computer	Included, running Mac OS© X		
Software	SonoGuide and SonoDraw, preinstalled on computer		
Dimensions	86.4 x 71.1 x 48.3 cm (34 x 28 x 19 in.)		
Weight	91 kg (200 lbs)		
Power	3.0 A for 100-200 V or 1.5 A for 220-240 V		

DISPENSER REFURBISHING

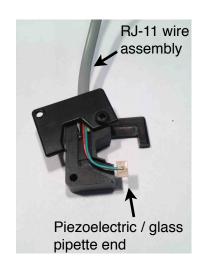
The glass micropipette on the dispenser can be replaced by the user. Open the dispenser cartridge assembly by loosening the two black plastic screws on the front of the dispenser assembly. Remove the dispenser cover.

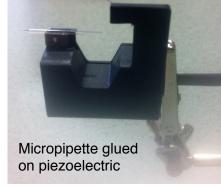
Remove the old glass micropipette by removing the RJ 11 wire assembly and dipping the piezo/glass pipette end of the RJ 11 wire in acetone until the glue holding the micropipette is dissolved. Be careful to only dip the piezoelectric element in the acetone, and not the gray RJ11 jacket, as the jacket 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. A box of glue should have been provided with the replacement glass. Use a fresh tube of glue every day; old glue can result in poor adhesion and improper functioning of the dispenser.

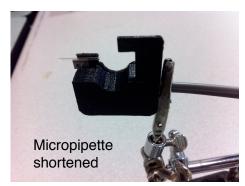
Shorten the glass micropipette from the wide end by scribing the glass and breaking it. Use a scoring wafer such as the Sutter ceramic scoring wafer (www.sutter.com, part number CTS).

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.

PATTERN FILE FORMAT

The .pattern file format saved by SonoDraw and loaded by SonoGuide is a simple XML-based text format. All of these files are editable by a standard text editor, and follow a format similar to this example:

```
<?xml version="1.0" encoding="utf-8"?>
<pattern>
    <xmultiplier>1.00</xmultiplier>
    <ymultiplier>1.00
    <featurewidth>50.00</featurewidth>
    <gridspacing>100</gridspacing>
    <continuous></continuous>
    line>
        < x > 300 < / x >
        < y > 700 < /y >
        < z > 0 < /z >
        <x2>300</x2>
        <y2>200</y2>
        < z2 > 0 < /z2 >
        <solution></solution>
    </line>
</pattern>
```

An XML document consists of a series of tags (the items surrounded in angle brackets) that are grouped in a hierarchy. Starting tags are matched by ending tags, which have a forward slash within them. Anything between these paired tags is contained by that particular element in the document.

At a base level, everything about a pattern is contained within its <pattern> tags, and everything about a line within its <pattern> tags.

The file starts with a series of options that define properties of the overall document. After these properties, the pattern items that make up a design are presented in order. These items will be drawn in the exact order they are listed.

Document options

```
<xmultiplier>, <ymultiplier>
```

These are legacy options that are not used anymore, but are present to maintain backwards compatibility to older versions of the software. Values other than 1.0 will scale the entire pattern up or down.

```
<featurewidth>
```

This specifies the desired feature width for the pattern. It is used for previews in SonoDraw, but its only impact on actual printing is in how far apart the overlapping lines are within a rectangular fill

```
<gridspacing>
```

This dictates the grid spacing used in SonoDraw for the overlaid grid that items are snapped to. It doesn't impact printing in any way.

```
<continuous>
```

When present, pattern items that touch each other are drawn in one continuous motion without lifting off from the surface. If it is missing, even connected items will be drawn separately, with the dispenser picking up from the surface at the end of each item.

```
<noreturn>
```

When present, the dispenser head will not return to the starting point when a pattern is finished. It is specified in a similar manner to the continuous option, with nothing between the opening and closing tags.

```
<startabovesurface>
```

When present, the system assumes that the pattern does not start at the same Z height as the dispenser is when the pattern printing begins. This causes the system to first find the surface in an automated manner before initiating printing. This can occasionally be useful to avoid contaminating the surface by having to touch down at the starting point of the pattern.

```
<enablesprayingforpattern>
```

Instead of normal dispensing, if this option is present the system will use strong spraying at each of the pattern elements. This can lead to uncontrolled deposition of liquid across a large region, but may be useful for specific applications.

Pattern items

```
</line>
```

Points are single droplets, located at a specific x, y, z coordinate. The solution name associated with the item is also specified.

Lines are drawn from one X, Y, Z coordinate to another in a continuous manner. The starting and ending coordinates are specified via the x, y, z and x2, y2, and z2 elements, respectively.

Rectangular fills are denoted by their direction (vertfill or horizfill), which is the orientation of the lines that raster to fill a particular rectangular area. The two pairs of x, y, and z coordinates specify the upper left and lower right corners of the rectangle.

Arcs are defined from their center, a starting angle (in degrees), an ending angle, and a radius.

```
<polygon>
    <vertex>
              < x > 400 < / x >
              <y>3600</y>
              <z>0</z>
    </vertex>
    <vertex>
              < x > 600 < / x >
              <y>3000</y>
              <z>0</z>
    </vertex>
     <vertex>
              <x>1000</x>
              <y>3600</y>
              < z > 0 < /z >
    </vertex>
    <solution>Solution 1</solution>
</polygon>
```

Polygonal filled areas are defined using a series of vertices. The last vertex connects up with the first to close the area. They are drawn in a spiral fashion, from outside to inside, to fill the entire defined area.



REVISION HISTORY

Version 2.2

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

Version 2.1

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

Version 2.0

- Updated to show the USB 3.0 cameras now in use in current Microplotter systems, instead of the previous FireWire cameras.
- Updated figures to reflect latest version of SonoGuide.
- Added dispenser refurbishing instructions.
- · Added command syntax for the .pattern file format.