PRINTING WITH THE DERISI ARRAYER

version: 1.5
for ArrayMaker version: 2.5

You will need:
Approximately 500 poly-lysine coated slides, aged at least 2 weeks
384-well plates containing DNA to be printed, resuspended at least 24 hours
A set of printing tips, with springs
500 ml 0.5X SSC, filtered
500 ml 95% EtOH, squeeze bottle
Parafilm for resealing plates
Timer

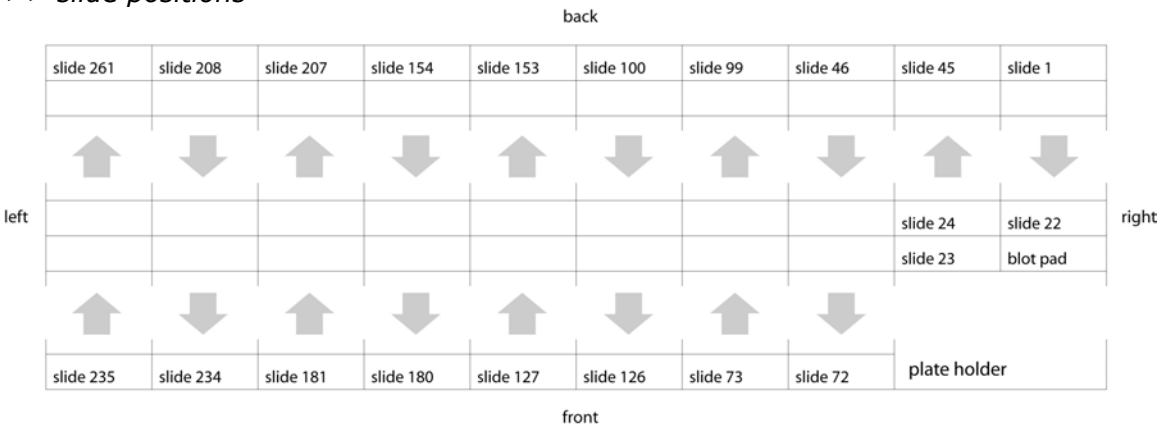
Set Up

Clean up Dust off the slide platter, dustcovers, and horizontal surfaces around the arrayer using 95% EtOH and kimwipes. When cleaning the platter, avoid brushing up against the slide column alignment pins, so as not to rip the kimwipe and scatter lint over the surface. Dust the rear dustcover in place. Use the rows of bolts on the table to correctly position the front dustcover.

Slides Load the poly-lysine coated slides onto the platter. Check the coating of each slide by exhaling gently and observing the appearance of the surface. Slides with dust, obvious residue, scratches, or irregular coatings should be rejected, set aside, and used as blot pads. If enough dust is present to risk clogging the tip, the slide should be discarded entirely.

If you are using slides from different batches of poly-lysine coated slides, be sure to record which batch was used for which slides. The following guide (which is also taped to the arrayer table) indicates the slide numbers for each column.

>> slide positions

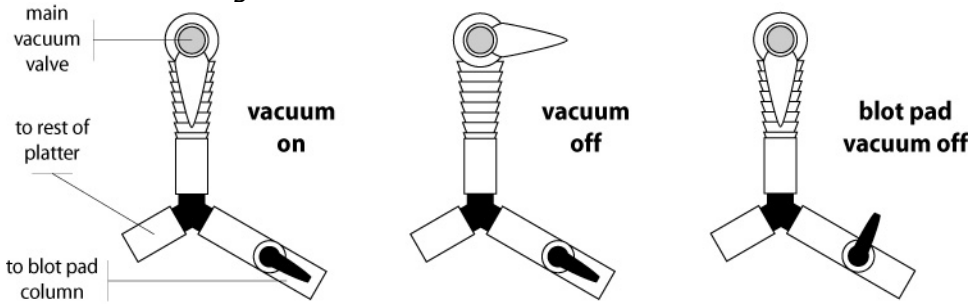


Place the slides onto the platter gently to avoid chipping the glass. Push the slides gently to the back and left so that they are flush against one another and the guide pins along the left side of the column.

Secure slides with vacuum Push the slides gently as you place them to ensure they are aligned along the left edge of the column and that each slide is flush against

its neighbor, leaving no gaps. Once all slides have been placed, apply the vacuum using the valves on the wall behind the arrayer. The first row, slides 1 through 22, and the "blot pad" have a separate vacuum line controlled by the small black valve on the tubing. The vacuum flow is controlled using the silver valve on the wall.

>> vacuum valve settings



Check for leaks Listen carefully for leaks with your ear close to the platter. If there is a good seal, you should hear silence. If you can hear a leak, the most likely cause is a slide that is not flush against the surface of the platter, frequently due to a glass chip underneath the slide. You can locate such a slide by listening carefully at different places on the platter, or by looking for irregularities in the reflection on the surfaces of the slides. To fix a leak like this, you will need to shut off the vacuum, lift the slide using a pair of forceps, and remove any glass chips from the back of the slide and the surface of the platter. Replace the slide, readjust the slides in the column so they are flush against one another, and reapply the vacuum.

Another possible source of leakage is the failure of the slides to cover the last vacuum hole on the platter completely. This results from a batch of slides that are thinner than the average width. In this case, you can swap some of the slides from the problem column with wider slides from other columns. You may also remove the last slide(s) and cover the hole(s) with a piece of tape. If you tape the holes, your print run will be missing the slides in those positions.

Alternatively, you can use a popular method that allows you to print on those slides. Simply slide the column(s) of slides in question towards the front of the platter to cover all the holes and apply the vacuum. Once the vacuum is secured, apply a piece of tape to the slide the back of the column -- the tape should only go a millimeter or two onto the surface of the slide, but should be secured carefully and completely, as it is substituting for the metal alignment pins as the backmost limit.

When the slides are held down by the vacuum and no leaks can be detected, you can secure the front of each column with a small piece of tape, in case the house vacuum should fail during the run. Place the tape in the gap in between the slide columns, so as to cover as little of the surface of the slide as possible. Press it down firmly so it will not touch the print tips. As soon as you are finished, replace the dustcover to prevent dust from gathering on your slides.

Powering up the Arrayer

Start computer first Start up the computer and log on. (un: arrayer, pw: arrayer). Open ArrayMaker v2.5 using the shortcut icon in the middle of the desktop. The program should launch and the **Welcome** tab should be displayed.

Plug in motors Once you have the program running, plug in the Arrayer. There are two plugs, one large round one and a smaller one labeled "Z-stage." Plugging in the cords should cause the amplifiers (located under the Plexiglas box at the back of the arrayer table) to click twice. After the clicks, each amplifier should display a green light on top of its housing. Check for the green lights on top of each amplifier before proceeding.

> **NOTE:** If power should fail during a run, you MUST UNPLUG the two power plugs *IMMEDIATELY*.

Connect Click on the **Connect** tab in ArrayMaker. Then click on **Connect to Controller**. The text box should read "Connected," along with a lot of other data, and the **Home All Axes** button should now be clickable. **(Screen Shot 1)** Before homing, make sure that the slides are properly secured with the vacuum and that the travel length of all axes is clear. The platter will move during the homing process and will jettison your slides off if they are not properly secured. Click **Home All Axes**. The process should take just over a minute. When the homing finishes, the other tabs (**Align**, **Test Print**, etc.) at the top of the window should become available.

Station Calibration

Before you place all of your printing tips in the print head, you will need to align the station positions. There are a few phases to this operation, so you will put the arrayer in quite a few intermediate states. Because many users print on our arrayer, this is a very important step.

Getting Started

Click on the **Align** tab. **(Screen Shot 2)** A list of buttons for each of the stations on the arrayer should be present in the lower left corner of the screen under "Align Station."

Reset Z The bottom-most of these buttons is the "Reset Z" button. Clicking this button will reset the Z axis setting for all of the stations. **Click this button before you do anything else.** By resetting the Z, you ensure that you will not crash your tips into any part of the arrayer. You should do this any time you start up the arrayer.

General Pressing a station button will cause the array to move to that station. To return from the station to the home position, simply click the button again. Once the arrayer is at the station, the position can be adjusted using the axis arrows in the upper-left corner of the screen under "Motion Control." The size of the movement is controlled by the "step size." The move is executed when one of the six direction buttons is clicked.

> **CAUTION:** Do NOT move the print head down in steps larger than **500**. Larger steps risk severe and expensive damage to your tips!

The adjustments for the station are saved by clicking on the "Set" button that appears when the station button is depressed. All of the saved adjustments must then be written to the disk by clicking "Save Alignment to Disk."

It is helpful to have two people for the Alignment steps: one to drive the computer and one to watch the print head.

Any time that you make an adjustment to the X-Y position of the print head, the Z position will reset to the ready height (3000) before the move is executed. You will need to move the head back down to re-check the alignment. If you pay attention to the coordinates in the upper left hand corner of the Align tab, you should be able to return quickly (but carefully) to the desired height, without having to watch the arrayer.

When you are trying to align any station, you will need to close one eye. Otherwise, the parallax in your vision will mess up your alignment. You can ensure that you are looking straight along an axis if you can get the tip closest to you to obscure the tip or tips directly behind it.

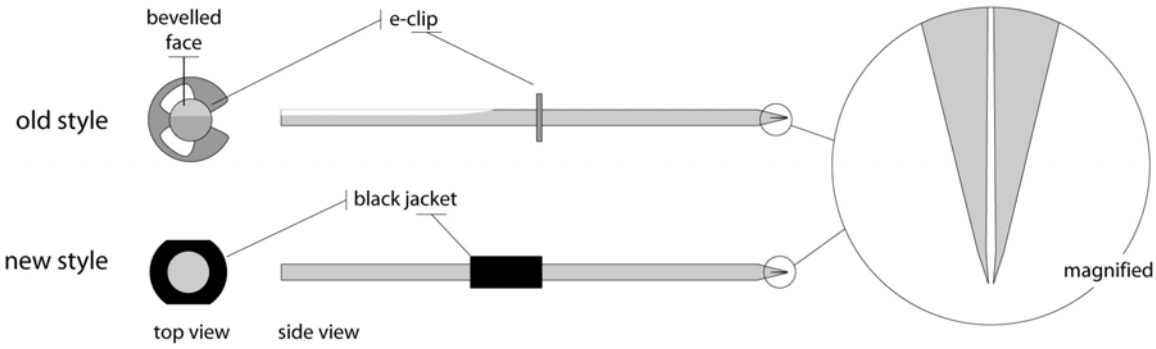
Go through the following positions/processes in order.

Map the Platter Heights

This step will generate a **relative** height map of the slide platter. There is substantial variation over the surface that must be accounted for. This step is largely automated, using a switch to detect the height of each slide individually.

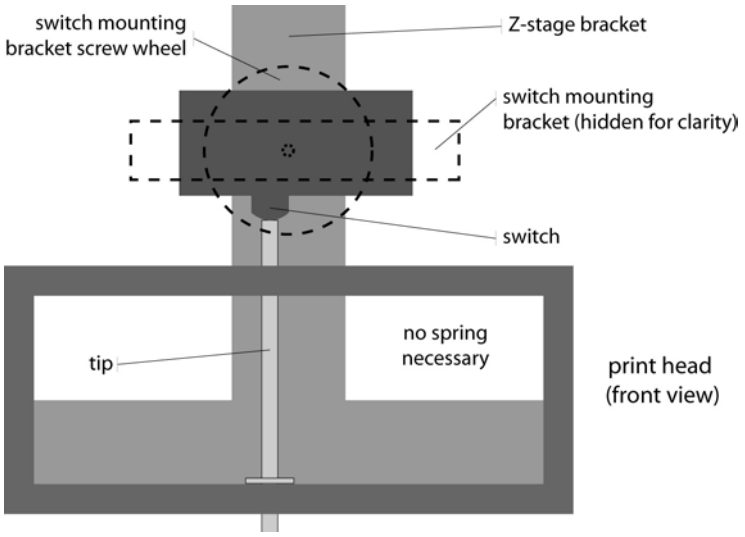
Insert one old printing tip Remove the lid from the print head by unscrewing the two screws and removing the washers. These screws should never be more than hand-tight, so they should come out easily. Insert the tip into the back-most row of the print head in line with the Z-stage bracket and replace the lid -- you do not need a spring at this stage.

>> microarray printing tips



Mount the switch on the Z-stage bracket Place the height-detection switch on top of the tip as shown in the diagram below. Close the gate of the switch bracket behind the Z-stage bracket and tighten the screw wheel securely. The switch button itself should be centered over the tip and with the top of the tip touching the switch. Be certain that the screw wheel is tightened securely.

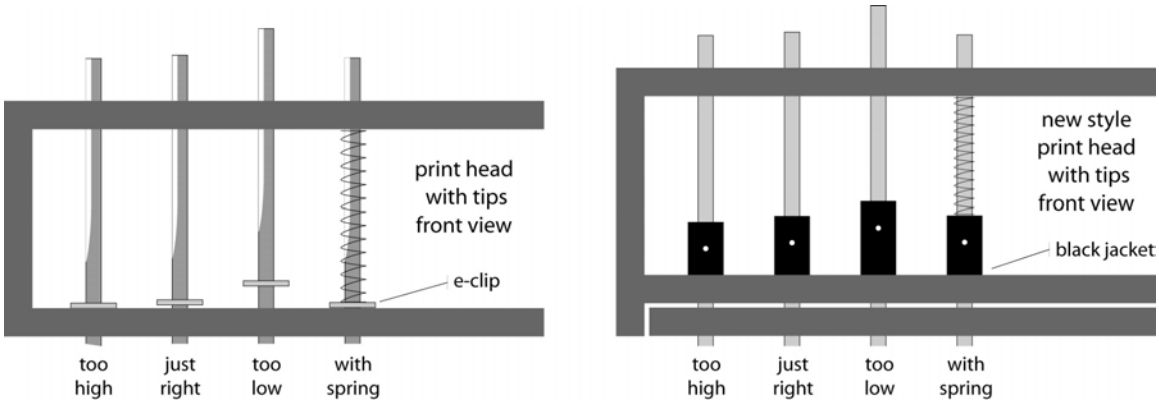
>> Height Detector Mounting



Align the Print station You will align this station three times. The first time will be an alignment that will permit you to calibrate the heights of the platter. The second time will allow you to calibrate the slide positions in the X axis (front to back). The third time will be the actual print position. This station is simply slide 1, the back-most, right-most slide on the platter. All other numbered slide positions on the platter are calculated from this one.

Move the print head down in steps of 500 until you come close to the surface of the glass. Then switch to steps of 100, so that you do not hit the surface too hard. Stop once the tip is just barely touching the surface (see diagram below). You use the e-clips, or the black jackets of the new tips, to determine if the tip is touching -- it is very difficult to see by looking directly at the glass.

>> e-Clips, black jackets -- and determining the proper height of the print head



For the first alignment, the tip should be more or less centered on the first slide. Because the slide widths are not yet calibrated, a central location is best as it ensures that the height will be mapped on the correct slide. The tip should just barely be touching the surface of the slide, as judged using the e-clip. When the alignment is satisfactory, Set and Save.

Start the Alignment When moving between tabs, arrayer will move to its ready position -- to prevent surprising moves, it is good to be in the habit of moving the arrayer out of the station you were working with when you are finished aligning it. In order to do this step, you will need to leave the **Align** tab and go the **Calibrate Slides** tab. **(Screen Shot 4)**

On the right side of the screen are the controls for the height mapping. Select the slide range for which you have placed glass, usually a full platter of 261 slides. The starting slide is always 2, as Slide 1 is the Print positions, which you have already aligned. The remaining default settings should not be altered. They control the range and speed of the detection.

Click Start Height Mapping to begin. The print head will move to the Print position (Slide 1) and measure the travel in the switch. It will then proceed in turn to each slide and measure its height relative to the first slide. Doing the whole platter should take about 25 minutes. Keep an eye on the machine to make certain the cable to the switch does not get stuck under the print head or drag across the slides.

At any point during the alignment you can press the stop button and the arrayer will return to the ready position. Any work done so far can be saved by clicking Save Alignment to Disk. If you wish to do each column individually, that works, too -- the software will only save the changes measured without altering the values for slides not measured. When you are finished, click "Save Alignment to Disk" and remove the switch and tip from the print head.

Align the Rinse, Dry, Plate, and Print stations

Reset Z The only station you have set so far is the print position, and this will be changed again. To ensure that your tips are not damaged moving into the low setting used to calibrate the platter heights, it is a good idea to click "Reset Z" at this point.

Insert four printing tips Select four tips, either your own or from the public set, and insert them into the print head using the left-most holes first. Place the tips in the corners for the tip format (4, 16, 32 or 48) that you will be using for your print. Using only four tips at this stage decreases the financial consequences of an alignment accident.

When placing tips in the head, be sure to insert the tip directly into the hole and do not drag the point over the interior surface of the print head. Place a spring on the shaft of each tip, orient the tips so that the beveled side faces the back of the arrayer, and replace the lid of the print head. All the beveled surfaces of the tip need to be aligned properly to enable the placement of the lid. It is useful to use a pair of pliers or forceps to orient the tips.

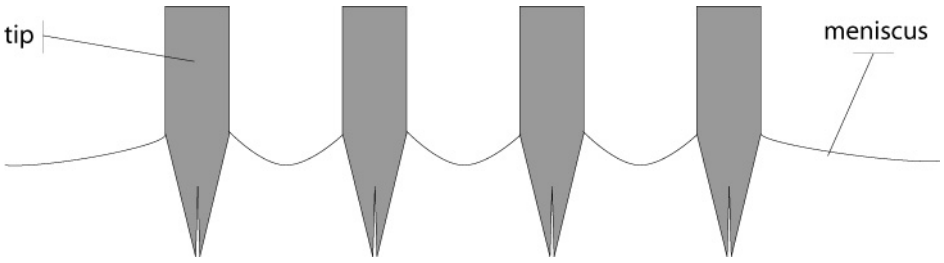
If you are using the new style of tips, they do not have beveled faces, but they do have a small white dot on the black jacket. It is useful to orient this dot to face the front of the arrayer.

When the tips are loaded, replace the washers and hand-tighten the screws to secure the lid.

Rinse Before aligning, rinse out the sonicator with water and fill with 0.5x SSC. The liquid height should ideally be within a few mm of the top of the vertical part of the interior of the sonicator.

Click on the "Rinse" button and the print head should move over to the sonicator, but not down. Use the "Motion Control" to move the print head down. Select a step size of 500 and click on the "Down" button. Move the print pins down until the beveled part of the tip is completely submerged, as shown below.

>> proper liquid level in the sonicator



It should not be necessary to adjust the left/right position at all, if the sonicator is properly placed within its ring of bolts. Obviously, you cannot adjust the front-to-back position with the software, as that moves the platter, not the print head.

When the print head is at the correct height, click on the "Save" button. It is not a bad habit to click the "Save Alignment to Disk" every time you save an individual station position -- it will prevent you from forgetting to do it if you just go back and change one position.

With the head in the rinse station, cut four pieces of parafilm and tape them over the top of the sonicator, leaving a few cm on each side of the tips. This will slow evaporation of the SSC from the sonicator. You want to leave enough space on the sides to permit checking the liquid level on the tips.

Dry The Dry station is a more challenging alignment than the Rinse station, simply because there is not much margin for error when the tips enter the holes in the aluminum block -- so be CAUTIOUS. This is one of the reasons it is important to Click "Reset Z" before proceeding with aligning the stations.

Click on "Dry" and the print head should move over to the Dry station, but not down. Move down in steps of 500 until the tips come close to the surface of the aluminum block. Then, move down in steps of 100 until the tips are just about to enter the holes. At this point, check the alignment to make sure that the tips will enter the holes when you move them further down. If they will not, make the necessary adjustments to the left-right position using the software. If the position needs front-to-back adjustment, you will need to move the aluminum block itself. This should not be a common problem.

If the tips are not going to hit the surface of the block, continue moving down in steps of 500. The tips will be much easier to align if only the cylindrical portion of them is visible (i.e. the conical section is hidden in the holes). Double-check the alignment and make any needed adjustments.

Set the correct height for the Dry station by moving down in steps of 500 until the e-clips lift off the bottom of the print head (see below). You will probably want to raise the print head a little and then adjust the height in steps of 100 to get it just right.

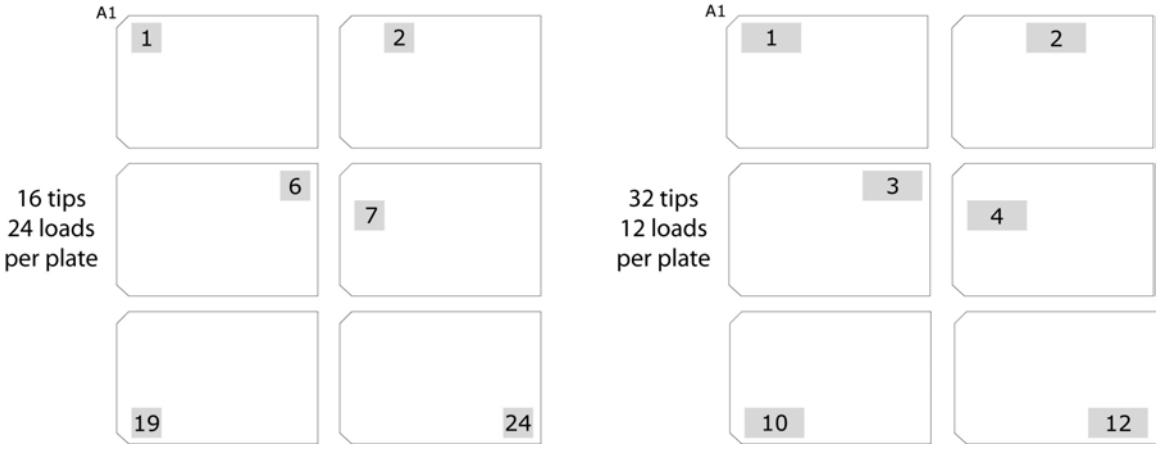
The vacuum will not draw properly through the print tips unless the height is sufficiently low. Later, when you have all the tips you will use for your run in the print head, use the buttons under "Station Control" in the upper right of the **Align** tab to switch the vacuum pump on. Adjust the height in steps of 100 until you can hear that a good seal has been made. Then, Set and Save.

Station 3 This station is not currently in use.

Plate Setting the Plate station is very similar to setting the Dry station. You will set the position of the first load (the back- and left-most wells, e.g. A1-D4 if you are using 16 tips), and the rest of the load positions will be calculated from this position.

First, place an empty 384-well plate in the plate holder. Then, move the print head down carefully to make certain the tips are going to go into the wells. Then move the tips further into the wells so only the cylindrical portion is visible and check the X-Y alignment. Make any necessary X-Y adjustments. Finally, adjust the height so that the tips are not quite touching the bottom of the well. Move the head down until the e-clips move, then back it off in steps of 100 so that it is no longer touching the bottom. Set and Save.

>> first and last load positions



Calibrate Plate The "Calibrate Plate" section in the upper, middle section of the **Align** tab allows you to account for variations in the layout of different plates. **(Screen Shot 2)**

Once you have set the Plate station, you can use this to check how well the tips go into the last load position. The software will use any adjustments you make to help calculate the precise location of the intervening load positions.

Select the radio button for how many tips you'll be printing with -- if you fail to do this correctly, the next step could cause the tips to collide with the surface of the plate. Click on "Move to last load." The print head will move to the last load position, but not down into the plate.

Lower the print head as you did to set the Plate position, taking care that the tips are going into the wells. With the tips far enough into the wells that only the cylindrical section is visible, determine if the tips are properly aligned in the wells. Make any required X-Y adjustments and recheck the alignment. When you are satisfied, click on "Calculate correction," and then "Save Correction."

You do not need to place the tips all the way down at the bottom of the well for this step. The correction only changes the X-Y settings -- the Z setting is not altered.

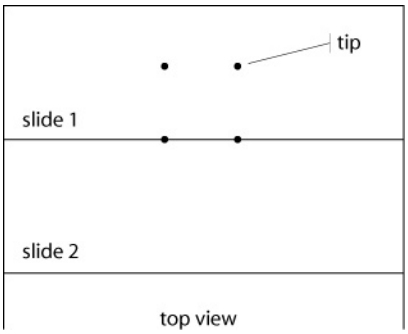
Print Align this station for the second time. This time, you will align it to facilitate the calibration of the slide widths.

Move the print head down in steps of 500 until you come close to the surface of the glass. Then switch to steps of 100, so that you do not hit the surface too hard. Once the tips are just barely touching the surface (see diagram above), you will be able to determine accurately the X-Y location of the tips on the glass.

> Advanced Tip: You can easily return to the same Z position after the print head resets to its home position after an X-Y move. Take note of the Z value displayed under "Motion Control" when the print head is at the desired height. Move in steps of 500 to within 500 of this value, then move in steps of 100. This will save you the hassle of inspecting the e-clips after each move.

For this alignment, you want the front-most tips to be aligned on the front edge of the first slide, as shown below. For the purposes of this calibration, be careful to set the height so that the tips are barely touching so as not to damage the glass or the tips. Set and Save.

>> Print position alignment for calibrating slides



Having the front tips aligned with the edge will simplify the process of calibrating the slides by providing a reference line. Do NOT forget to set this position again as described below, after completing the Calibrate Slides step.

Calibrate Slide Widths

This step enables you to account for the small differences in slide width that can accumulate in a column and make the calculated slide positions different from the actual slide positions. This step is MUCH faster with two people than with just one. One person should watch the print head, while the other controls the computer: the Spotter and the Driver.

Start the alignment Go to the **Calibrate Slides** tab **(Screen Shot 4)**. On the left side of the screen are the controls for the width calibration. This procedure will move several slides at a time down the column, moving to the calculated Print position for each slide. Under "Calibrate Setup," check to see that the software has correctly entered the start and end slides for the first column. The "Slides per Step" default is 5 -- the differences will be averaged across those five slides. Start calibrating the first column by pressing "Begin."

The first slide should be set properly, so press "Next." The Spotter then inspects the location of the front-most tips with respect to the front edge of the slide. If the tips are not directly on the edge, the Spotter tells the Driver to move forward or backward in steps of 0.25, 0.5, 1, or 2 mm. The Driver clicks the appropriate button, and the arrayer will pull the print head up, move over, and then move back down. The Spotter can then make a further adjustment or say "Next." Continue to the end of the column.

> NOTE: It can be difficult to see this alignment without getting close to the print head. If you lean in close, be very careful when reaching the end of a column! The arrayer will return quickly to the Home position when the Driver clicks "Next" for the last slide. Don't be the second person to be injured by this arrayer!

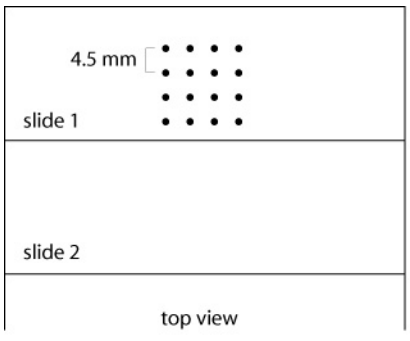
When the first column is complete, use the slide map on the first page of this protocol to enter the first and last slides in a column. Always enter the lower number into the "Start" box and the higher number into the "End" box. Click "Begin" to start the selected column. When all columns have been completed, click "Save Alignment to Disk" in the lower right corner of the window.

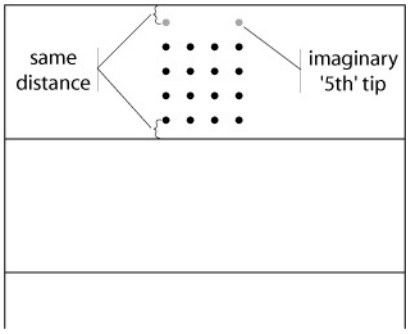
Set Print and Blot Pad positions

Now that the slides are calibrated, you need to set the Print station to a location suitable for printing. Return to the **Align** tab.

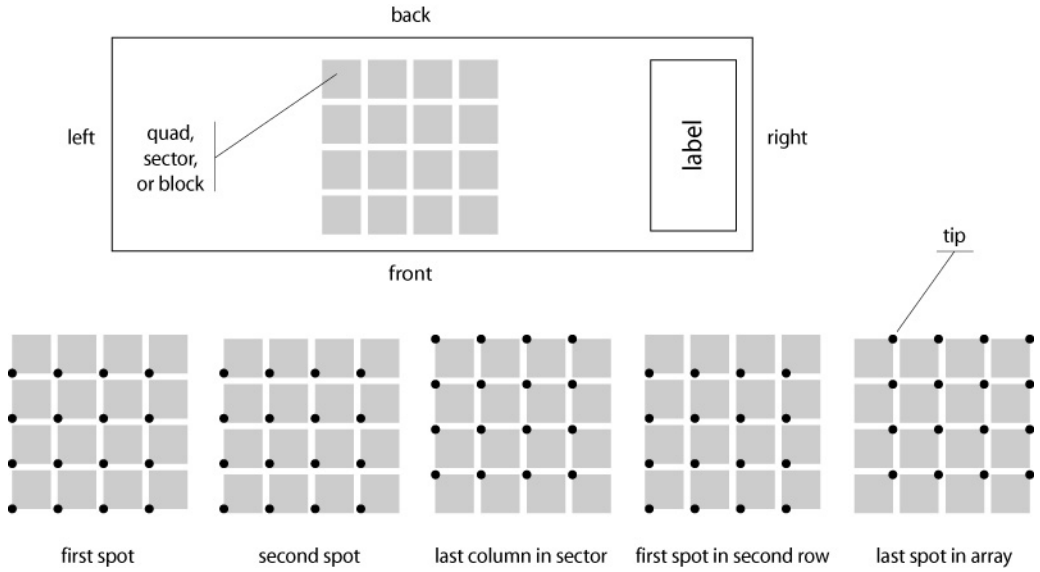
Now you want to align the tips so they are about 3.5 mm from the front edge of the slide. During the run, the arrays will form from the Print position towards the back and right of the arrayer, as shown below. One method for finding the appropriate alignment is to imagine that there is a fifth tip towards the back of the print head. The first tip and the "fifth" tip should be the same distance from the edge of the slide they are closest to. The correct distance from the edge is usually 3500 motor steps.

>> print position for print run





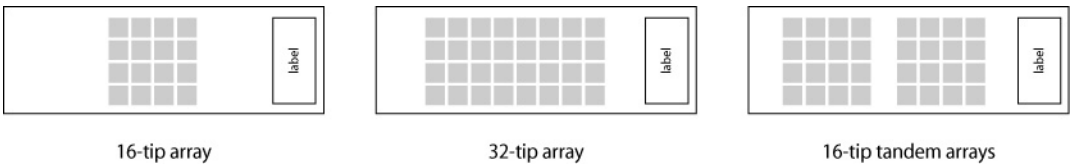
>> array formation



You will also need to reset the height so that the tips just barely touch the surface of the glass. Watch the e-clips in the print head, as described before.

You should also consider the left-right alignment at this point. If you are printing with 16 tips, you will not have difficulty fitting the array onto the slide. However, if you are printing with 32 or 48 tips, or if you are printing 16-tip tandem arrays, you will need to set the Print positions sufficiently far to the left to allow for the formation of the array.

>> types of arrays



You will have an opportunity to test the alignment by doing test prints, so get it as close as you can and then proceed. Set and Save.

Blot Pad The Blot Pad, where excess liquid is removed from the printing tips during a print run, can be a confusing concept. There is a station called "Blot Pad," which is the front-most slide in the right-most column -- it's position is set independently and is not calculated from the Print position. The software can now use numbered slides as a series of blot pads during a run. The Blot Pad station is now most useful for doing test prints as the slide can be easily removed through the hatch in the dust cover.

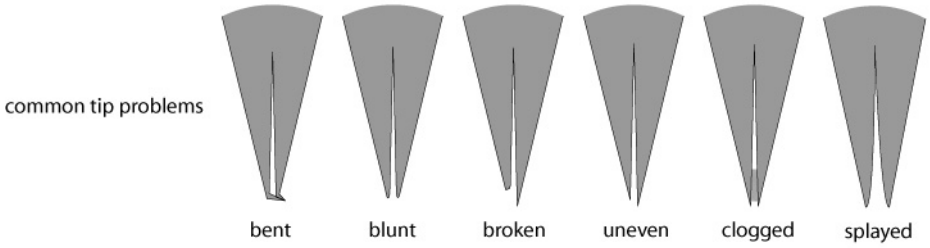
Align the Blot Pad station just as you did for the Print position when you did it for the third and final time, so that the array that forms will be centered on the slide and the tips are just barely touching the slide.

Test Print

Select and insert all printing tips

Visually inspect your tips under the microscope to ensure they will all print effectively. You can clean the channel with water and ethanol or with a thin piece of foil. You can sometimes reshape them using the foil or a razor blade on the outside surface of the tip. Be very very gentle when attempting to repair or reshape a tip.

>> microarray printing tip, common problems



Insert the tips into the print head starting from the left side of the print head. Place a spring over the shaft of each tip. Use the pliers to align the beveled faces of the tips to all face the back of the arrayer. Place the cover on the print head and hand-tighten the screws.

Check to see that each tip slides freely in the print head. You can use the pliers to get a grip on the tip of the shaft that extends from the top of the print head.

Clean tips

Go to the **Test Print** tab. **(Screen Shot 3)**
In the lower right-hand corner under "Macros," click on "Wash and Dry." Repeat a few times to ensure that you start off with clean tips.

Prepare a plate for Test Print

To make the test print an accurate simulation of your print conditions, you cannot print with 3X SSC alone. Instead, use a 150 ng/µl solution of salmon sperm DNA in 3X SSC. Set up a test plate with salmon sperm DNA in each well of the first load position. Use the same volume for each well that are in the wells of your print plates.

Test Print settings

The settings found in the **Test Print** tab are very useful for checking your Print alignment and ensuring that the tips you have selected are printing. It can also be useful and flexible for doing different kinds of prints than you can specify in the **Print Run** tab. **(Screen Shot 5)**

Slide and Plate Setup

"Load Number"

The plate load position that will be used for the test print. You can use any one, but it is easiest to use the first or the last.

"Loads per plate"

Automatically calculated based the number of tips you select below.

"Slide Number"

The slide on which the test print will be performed. Again, you may pick any one you like, but 1 is a good place to start. This entry will not be used if you check "Print on blot pad" below.

"Number of Slides"

The number of replicate test prints that will be performed. In general, you will only ever need one.

"Print on blot pad"

If you check this box, the test print will be performed on the Blot Pad. It is best to do your first test prints on the Blot Pad, as it is easy to remove, inspect and replace. You will also want to do a test print on a numbered slide as well, to check the accuracy of your alignment.

"Auto advance load"

If you want to do more than one load, checking this box will make the arrayer go to a different load position each time. If you do not check this box, the arrayer will return to the same load position each time, as specified in "Load Number" above.

Print Setup

"Total number of taps"

You want to set number so that you get a test print comparable in size to your array. About 15 times your sector width is a good value (around 300). Note: this is not the same as the number of spots that will be put down -- the arrayer touching the glass counts as a single tap, regardless of the number of tips in the head.

"Number of loads"

Just one load is more than enough solution for putting down at least 500 taps.

"Sector Width"

This is the number of spots that will be printed front to back before the arrayer returns to the front and moves right to begin the next row. In a scanned array image, this is the number of columns in each quad.

"Spacing"

The distance in microns between spots, both between rows and between columns. A distance smaller than 180 will risk running your spots together. A distance above 200 is preferred.

"Suggested Spacing"

This value is calculated based on your entered Sector Width. You can simply enter this value into Spacing. If you want a little extra space between quads, you can enter a value smaller than the one calculated here.

Wash and Load Settings

Wash Cycles	2	
Wash time	3000 msec	
Dry time	8000 msec	
Load time	500 msec	
Bounce Dry	NO	
Swish Wash	YES	(useful for disrupting standing waves in Rinse)

Slow Pick Up YES

The Slow Pick Up will use a slower acceleration setting when leaving the print plate, resulting in less liquid clinging to the outside of the tip. In most cases, this should mean you don't need to use a blot pad at all during your print run. See below in Print Run.

Tip and Blot Setup

"Tips"

Select the radio button for the number of tips you are using. If you get this wrong, you risk crashing the tips. Your selection here determines the value for "Loads per plate."

"Blot Pad Taps"

The number of blot pad taps that will be performed before proceeding to the actual test print.

"Use Blot Pad"

If you want to use a blot pad to remove excess liquid from the tips before proceeding to the test print, you must check this box. You cannot use the Blot Pad as a blot pad if you are also doing your test print on the Blot Pad.

Station Control

These controls are useful for cleaning or soaking the tips and checking the liquid level in the Rinse station sonicator. They work just like the controls in the **Align** tab. Click on the button to move the arrayer to the position and click it again to return home.

Control Panel

When all of the values are correctly set, click the big green "Start" button to begin the test print. The tips will go through a wash cycle, perform the print, and then go through a finishing wash cycle. If you do not want to wash the tips in between test prints, set "Wash Cycles" to zero.

Macros

Shortcuts for cleaning the tips. You can do the same thing manually in "Station Control." The macros use the wash settings you have entered above.

Inspecting your Test Print

If you have printed on the Blot Pad, you can break the vacuum to the first column and remove the test print. Replace it with a fresh poly-lysine slide and reapply the vacuum.

The first thing to check is that every tip is printing. You should have a complete quad produced by each tip. Be watchful for tips that stop printing halfway through a quad.

If a tip fails to print, check to see if it is stuck in an "up" position, where it would be unable to contact the glass when the rest of the tips do. If it slides freely and is not stuck, check to see if its spring has worked its way underneath the e-clip. If this doesn't correct the problem, remove it, inspect it visually, and clean or replace it if necessary. If nothing is visibly wrong with it, it might be too short to touch the array -- try swapping it with a tip on the other side of the print head and repeat the test print.

The first few rows of each quad are bound to be pretty messy due to excess liquid on the outside of the tip. This is not a problem -- you can fix it two ways. You can use a blot pad during the actual print run or you can use the Slow Pick Up feature.

You may observe that the spots are not evenly spaced. This seems to occur when the arrayer is repetitively printing many spots in close proximity to one another. It does not appear to be a problem during actual print runs.

Once you have a couple of good Test prints in a row, try the test print on slide 1. If that goes well, it is time to begin your run!

Print Run

Click on the **Print Run** tab and enter the settings. **(Screen Shot 5)**

Print Run Settings These settings are very similar to the ones found in the **Test Print** tab, but provide added control for switching plates.

Print Setup

"Total Number of Plates"
: Simply the total number of 384-well plates in your print run. If you are printing individual plates more than once, count each printing as a separate plate.

"Tips"
: The number of tips you are using.

"Spots per load"
: This controls the printing of duplicate (or more) spots. If you set this higher than one, each array will get that number of identical spots next to each other. This is distinct from "Tandem Print," described below.

"Entire Platter"
: Checking this box is a simple way to enter the maximum number of slides in the "End on Slide" box below.

"Re-Fill"
: Selecting this option will cause the arrayer to return to reload the tips with DNA (from the same load position) halfway through the platter. This should generally not be necessary -- one load should be good for more than 500 taps.

"Slow Pick Up"
: Checking this box will slow the arrayer down as it leaves the liquid during the load step, decreasing the excess liquid left on the outside of the tip. There are two settings in the configure tab that control this; the Slow Pickup Height should be 2500 and the Slow Pickup Speed should be 2000. When using the Slow Pick Up option, blot pad tapping should not be necessary. This will NOT result in an appreciable increase in total print run time! In fact, by skipping the blot pad features, it will probably be faster.

Station Settings

These settings and their optimal values are listed in the **Test Print** section above.

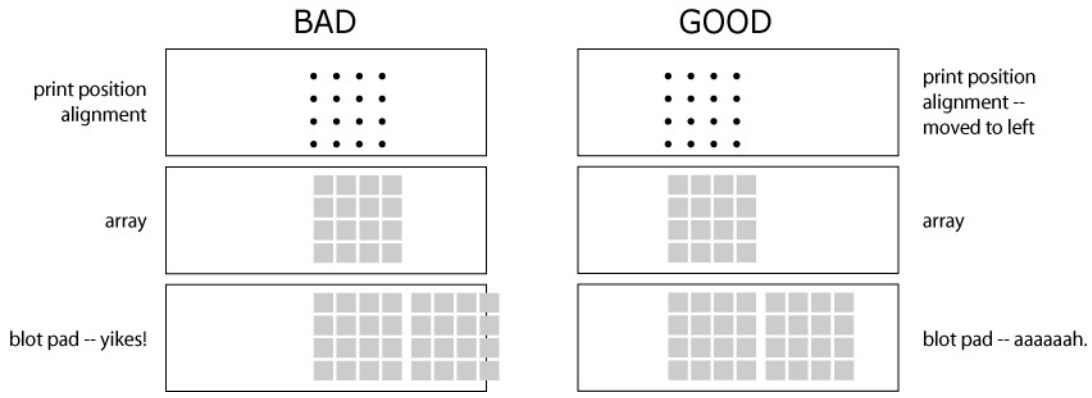
Start Settings

If you need to restart a stopped print, you will use these settings to start at the appropriate location. The "Plate" and "Load" positions entered here describe specific

locations on the arrays that will not change if you do not alter the saved alignment of the Print station.

"Start from Plate"
At the beginning set to 1. If you are restarting a print, set it to be the number of the current plate.
"Start from Load"
Again, set to 1 at the beginning. If you are restarting a print, you can look in the "Load" section of the **Control Panel** to determine what load the print was stopped on.
"Tandem Print Offset"
This is the distance in microns that will be used to separate the tandem arrays. It should be at least 20000 for 16-tip tandem arrays.
"Tandem Print"
A tandem print is two physically separated, identical arrays printed on a single slide. This can be useful for doing two experiments on one array. The largest number of tips you can use in producing tandem arrays is 16. Be sure to set the "Tandem Print Offset."
"Use Blot Pad"
Blot pads can be used to remove excess liquid from the outside of the tips -- this excess liquid can spoil the quality of the first arrays by causing spots to run together. This feature is not necessary when using the Slow Pick Up feature.
"Standard Blot Rows"
This will double the spacing between rows of blot pad taps, which can increase the effectiveness of blotting and eliminate any possibility of cross-contamination between loads. Checking this box will also constrain you to one "Sector Width" of blot pad taps! If you are printing with **16 tips**, you will need to set the Print alignment far enough to the left to accomodate the second array of blot pad taps that will form to the right of the first.

>> blot pad and print position when using Standard Blot Rows



Print Configuration Settings
These values control the speed of the run by determining how high the print head moves in between slides (Z Print Height) and how quickly it moves (Z Print Acceleration). These values are displayed here so that they may be readily checked. They are actually set in the **Configure** tab. **(Screen Shot 6)**
The safest settings are 450 for the Height and 400000 for the Acceleration.

Slide Setup

"Begin on Slide"

The first, non-blot pad slide on which you will begin printing arrays -- usually around slide 23 if using the Blot Pad feature. You can begin from slide 1 if you are using the Slow Pick Up feature.

"End on Slide"

The last slide that you will print on -- usually slide 261.

"Sector Width"

Just like the test print value: the number of spots printed front-to-back, corresponding to the number of columns seen in a scanned array.

"Spacing" and "Suggested Spacing"

These behave just as they did in Test Print -- a value of around 200 is ideal.

Station Control

These controls are useful for cleaning or soaking the tips and checking the liquid level in the Rinse station sonicator. They work just like the controls in the **Align** tab. Click on the button to move the arrayer to the position and click it again to return home.

Control Panel

The master control buttons for the Print run. Also displays the Plate, Load, Sector Row, and Sector Column values for the current pass, as well as the slide currently being printed. It will also calculate and display the time to next plate, time remaining and slides/minute rate after the first load is completed.

Blot Pad Settings

"Blot Pad Range Start"

"Blot Pad Range End"

Enter the range of slides to be used as the blot pads. Each plate will take one slide. Remember to skip slides that you have already used as test prints. If you need more blot pads than will fit in the first column, simply enter the values for the first column (1 through 22). The software will prompt you when it is out of blot pads to remove them and refill the column with fresh poly-lysine slides.

"Blot Pad Taps"

Determine this number empirically by observing your test prints. You want to see how many spots must be put down before the spots stop running together with the previous spot. Do not set this value to more than the Sector Width, if you can help it -- the print head will move to the right to put down the excess taps, and you run the risk of the tips being off the edge of the slide where they can collide with the alignment pins. Usually 20 taps is sufficient. The tip should contain enough DNA solution for more than 500 taps, so running out is not a concern.

> **NOTE:** If you are using the Slow Pick Up feature, it should not be necessary to perform any blot pad taps. Leave the "Use Blot Pad" box unchecked.

Macros

"Wash and Dry"

"Rinse"

These macros use the settings you entered in **Station Settings.**

"Warm Up"

This is a useful feature that improves the regularity of spacing early in the run. The platter movement will cause the motors to heat up and behave slightly differently. By running 10 warm up cycles IMMEDIATELY before you press the "Start" button to begin the run will prevent irregular spacing in your arrays.

Plate Control
This feature is ideal for a print run done by many people (or one tired person), because it encourages the user to double check that he or she is placing the correct plate on the arrayer. Check "Use Plate IDs" to activate this function. This will require the user to enter a name for the plate currently in use. The actual text is NOT checked against a master, but it is recorded and provides an opportunity to double check that the correct plate is being used.

File Operations
"Record Plate List" will save the list of plates for the run that have been entered into **Plate Control**. Do this when the run is complete.

Begin the Run Double check all of your settings. Spin down the first 2 384-well plates. A brief spin of up to 1000 rpm and then back down in a GS-6 is sufficient. This helps recover a little bit of the condensation that forms while the plates are at 4°C.

If you are ready to go, run the arrayer through 10 "Warm Up" cycles. Then, place the first 384-well plate on the plate holder. Click Start and you are rolling.

If at any time during the run, you want to stop the arrayer, wait until the print head is in the Rinse or Dry station, then click on the Stop button. It will finish its Wash cycle and then stop. You can restart it (after adjusting the start settings!) by clicking the Start button.

If at any time, you need to stop the arrayer in an emergency, use the red EMERGENCY STOP button located on the table. To switch the arrayer back on, simply twist the button to let it pop up.

Plate Changing Checklist

It is generally very difficult to see much of anything on the arrays before the first plate is finished. Also, it is very hard to see if there is anything slightly wrong while the arrayer is moving. Thus, it is very important that you check everything out thoroughly when the arrayer stops in between plates.

- ☐ Spin down 2 plates about 5 minutes before the current plate is finished.
- ☐ Remove the current plate when it is finished.
- ☐ Remove the parafilm from the new plate and place on the arrayer.
- ☐ Inspect the tips visually for hairs or other foreign objects.
- ☐ Inspect the last blot pad for tips that are not printing.
- ☐ Inspect the arrays using an eyepiece for incomplete quads and/or tips that have stopped printing -- fix any that are having problems (see below).
- ☐ Move the tips into the sonicator and check the liquid level. Add liquid if necessary.
- ☐ Enter the new plate number into **Plate Control**.
- ☐ Double check that you have done everything.
- ☐ Click the Start button.
- ☐ Cover and store the previous plate.

If a tip stops printing during the run…

Check to see if it is stuck in the "up" position. If so, remove the print head lid and replace the spring with one of the stronger ones.

Check for an obvious clog in the tip (i.e. a hair). If possible, remove it with forceps.

If neither of these explanations seems correct, remove the tip from the print head and inspect it under the microscope for clogs or bending or breaking of the tip. If necessary, clean, reshape (gently!), or replace with a new tip. If using a new tip, you should check its printing ability by returning to the **Test Print** tab and doing a test print. Remember to enter the appropriate start settings when you return to the **Print Run** tab!

Taking the Run Down

Save the Plate List by clicking on "Record Plate List" under **Plate Control**. **(Screen Shot 5)**

Use the Brother Label Maker to print out sequentially numbered labels for the arrays. Print them out in long strips so they can be quickly placed on the arrays. Use spaces to control the spacing between the entries. Give the print run a unique and informative name. (You can prepare the labels during the run to save time).

Break the vacuum and remove the dustcover. Apply the labels to the first column of slides. The standard convention is to put the labels on the RIGHT side of the arrays -- this means that the orientation of the label text with respect to the array will be different for every other column.

Cut the label with a razor to separate the slides: make two cuts on either side of the text, ON TOP of the slide, not in between them. Be very careful to avoid scratching

the platter with the razor -- if it becomes damaged, it may not hold a vacuum well. Remove the arrays one at a time and place them in a clean slide box.

Label and remove the remaining arrays.

Dispose of blot pads -- if used.

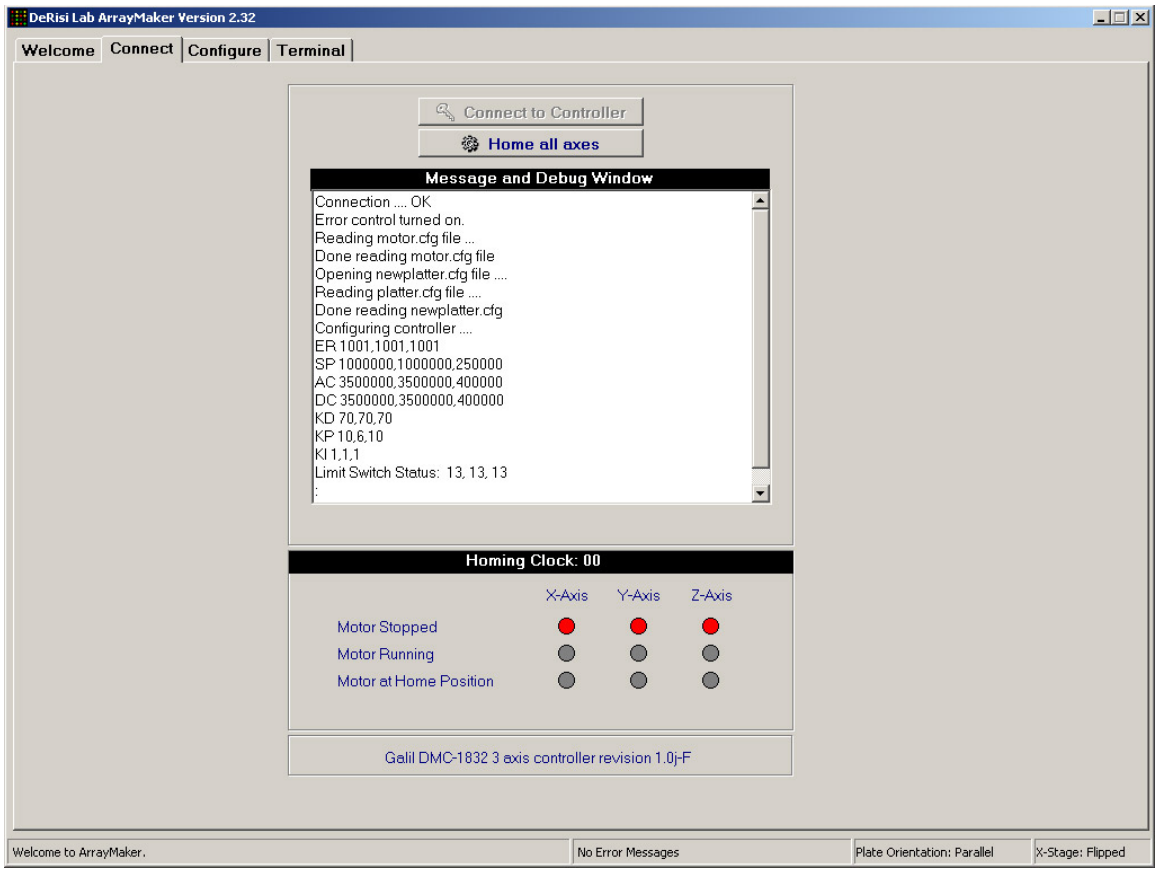
Dry down the 384-well plates in a SpeedVac and store them at -20°C.

Cleaning Up and Shutting Down

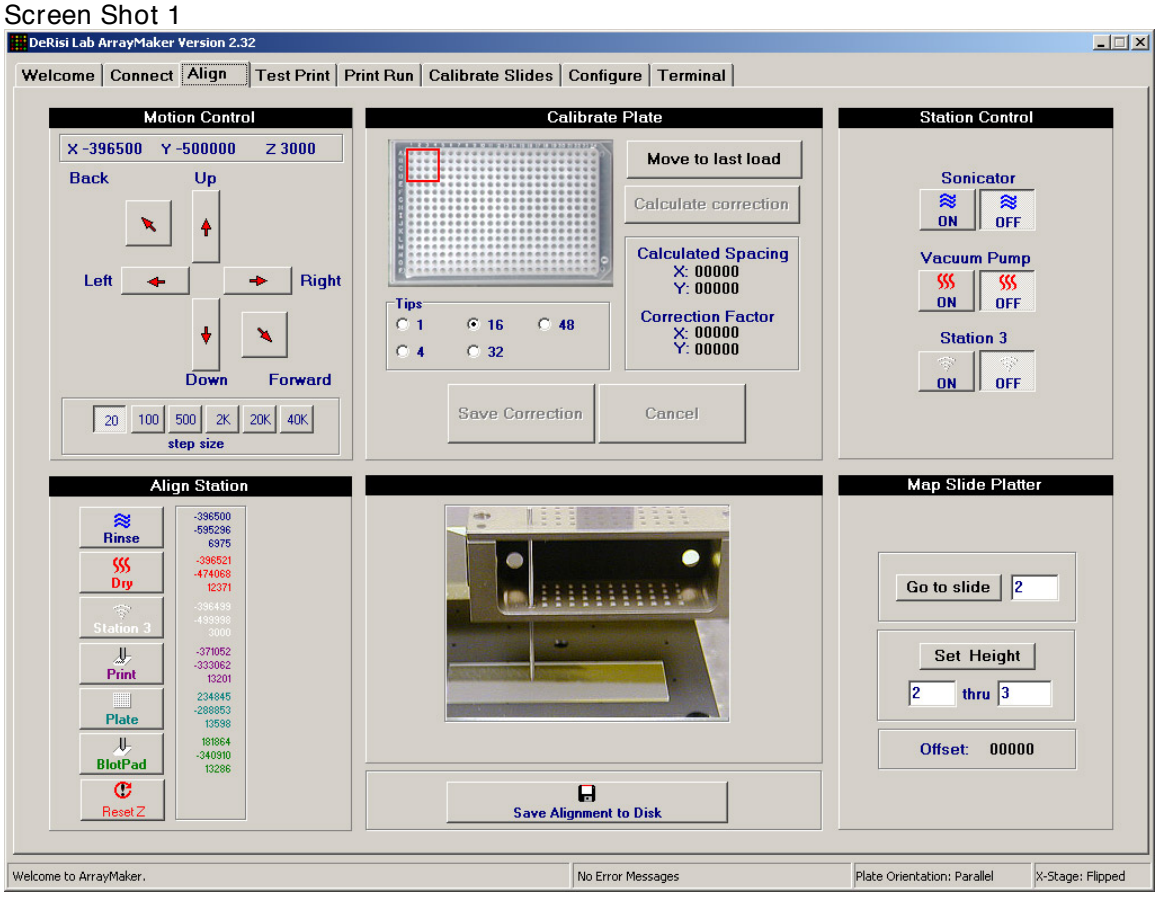
When all the arrays are off the arrayer, replace the dust cover.

Clean the tips with the rinse station. Then, switch the rinse solution to water and clean the salt residue off the tips. Finally, switch the rinse solution to 95% ethanol and clean and dry the tips. Empty the sonicator. Remove the tips from the print head. Replace the print head cover and screws.

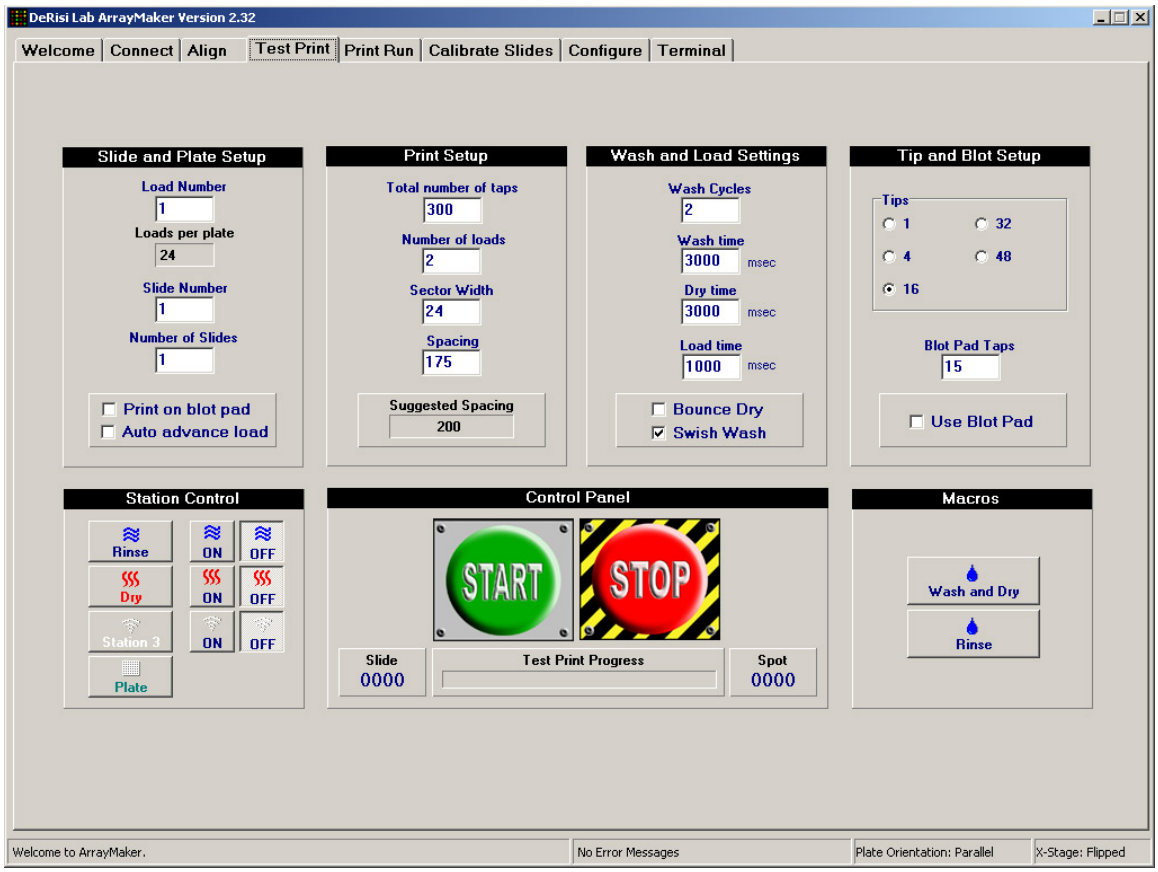
Quit the ArrayMaker program. Switch off the arrayer by unplugging the two plugs.


DeRisi Lab ArrayMaker Version 2.32
Welcome
Connect
Configure
Terminal
Connect to Controller
Home all axes
Message and Debug Window
Connection .... OK
Error control turned on.
Reading motor.cfg file ...
Done reading motor.cfg file
Opening newplatter.cfg file ....
Reading platter.cfg file ....
Done reading newplatter.cfg
Configuring controller ....
ER 1001,1001,1001
SP 1000000,1000000,250000
AC 3500000,3500000,400000
DC 3500000,3500000,400000
KD 70,70,70
KP 10,6,10
KI 1,1,1
Limit Switch Status: 13, 13, 13
:
Homing Clock: 00
X-Axis
Y-Axis
Z-Axis
Motor Stopped
Motor Running
Motor at Home Position
Galil DMC-1832 3 axis controller revision 1.0j-F
Welcome to ArrayMaker.
No Error Messages
Plate Orientation: Parallel
X-Stage: Flipped

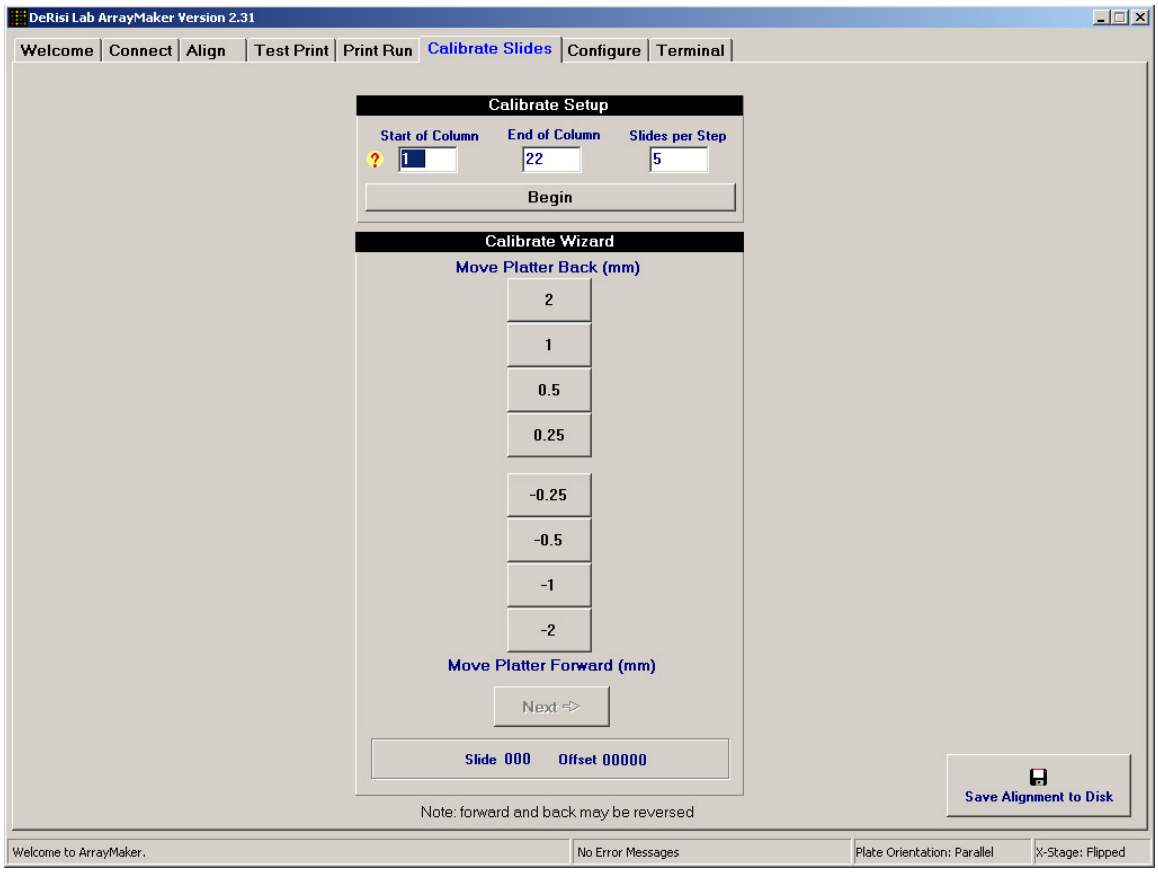
Screen Shot 1



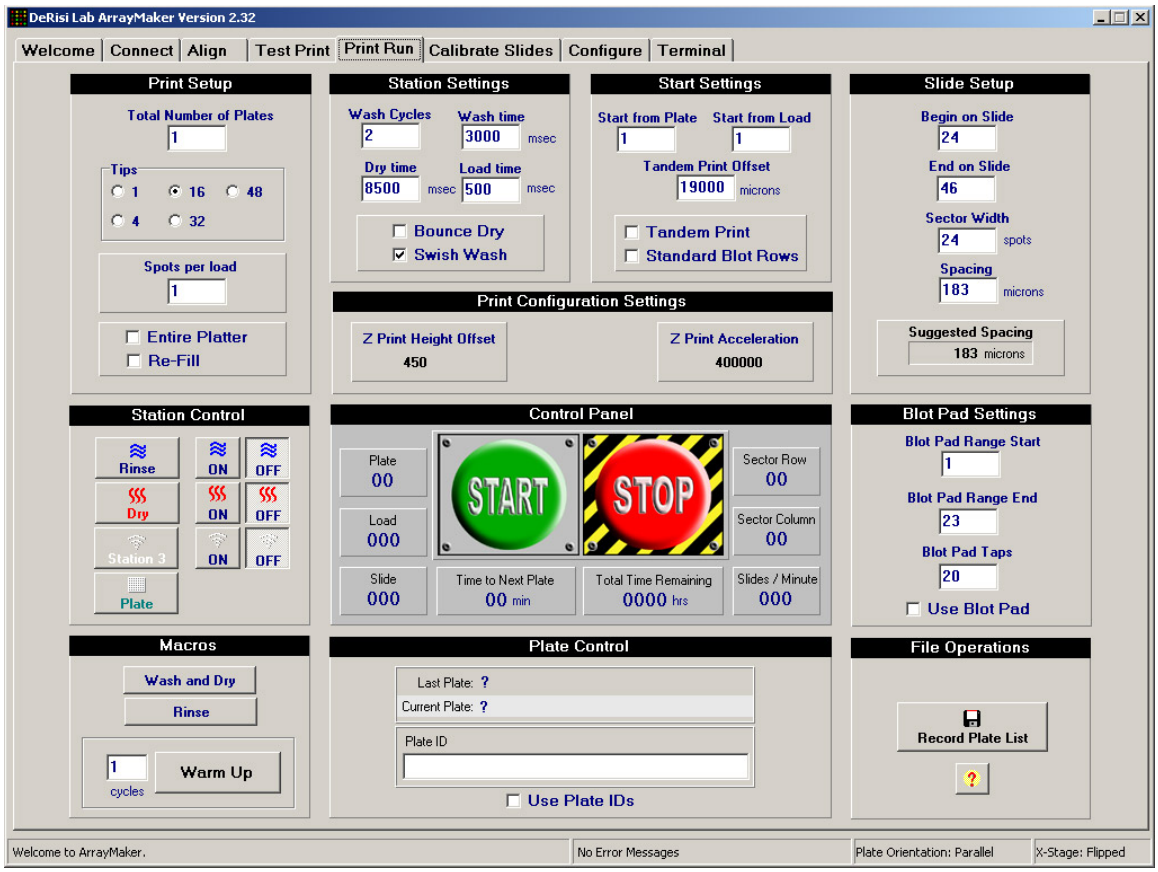
Screen Shot 2



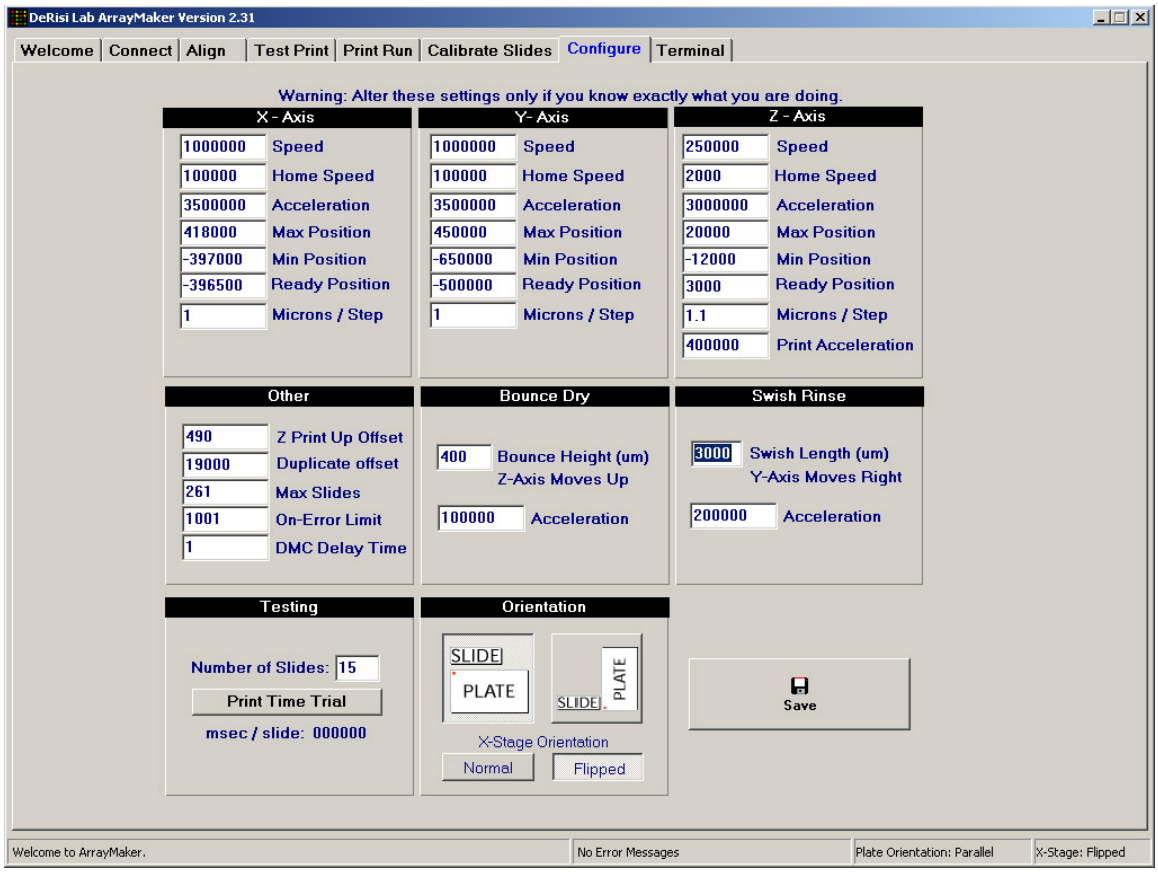
Screen Shot 3



Screen Shot 4



Screen Shot 5



Screen Shot 6