

4. Spotting DNA Arrays with the DeRisi Arrayer

NOTE: You MUST be trained by a qualified user of the arrayer before use

There are two types of arrayers that have been employed by the Fordyce lab. There's the antiquated Cartesian Axis arrayer in the Quake lab in the Clark building and there is also the DeRisi style arrayer in Alway. Traditionally, the Quake arrayer was used for small libraries while the DeRisi is more of a high-throughput machine. The computer controlling the Quake arrayer was fried in a power outage, so the arrayer is no longer controllable.

Preparation for Spotting with the Arrayer

1. Soak and shake four SMT-S75 silicon quill-tip pins in EtOH for several hours. This helps release debris or other contaminants that might adhere to the tip
2. Prepare a 384 well polypropylene plate with your DNA samples of interest. Refer to Protocols 1 - 2
3. Find a set of epoxy-coated glass slides for printing (2x3")

Preparing the Arrayer for Printing

1. Replace water in humidifiers and wash bath. Turn on humidifiers
2. Remove the bronze suppressor from the arrayer platter compressor. Toggle the power switch supporting the compressors and PC. Replace the bronze suppressor on the arrayer platter compressor
3. Place the desired number of glass slides onto the platter and tape remaining open vacuum holes on the platter. Replace printing pins, if needed
4. Confirm that one of the emergency stop buttons is depressed. Power on the arrayer axes by plugging in the transformer box into an outlet. Release emergency stop
5. Power on PC and press "F2" to continue beyond the booting screen, log in without a password, launch ArrayMaker on the desktop
6. Press "Connect to Controller" followed by "Home Axes"

Turning Off the Arrayer After Printing

1. Close the ArrayMaker program and shutdown the PC
2. Toggle off power strip connected to the PC and compressors
3. Press an emergency stop button
4. Remove transformer power supply unit from wall
5. Turn off humidifiers and dispose of wash bath water
6. Confirm that room is locked when leaving

Spotting Sequence in ArrayMaker

Typical components in automated spotting cycle:

1. Wash pins
2. Dry pins
3. Pick up sample from 384 well plate
4. Deposit sample onto glass slide
5. Wash pin
6. Dry pin
7. Repeat 3-7 for all samples
8. Set pin block in safe location

Arrayer Settings for Spotting Slides for MITOMI

On the "Print" tab, begin running a 20 cycle warm up, enter the following settings and start the print after the warm up. Ideal humidity is 65% or more

- 4x1 pins
- Slow Pickup
- Soft Touch Down
- 2 Wash Cycles
- 8000ms Wash
- 5000ms Dry
- 500 ms Load
- Swish Wash
- Tandem Print
- Sector Width: 56
- Spots per Load: (depends on number of plates - 1 full plate = 4)
- Column spacing: 320
- Row Spacing: 643

Removing and Loading the Pin Block

DO NOT replace arrayer pins with every print. Handling the pins as little as possible leads to a longer pin life and makes the print more reproducible between prints and between users. If you suspect that one is broken or are in need of replacing pins, follow this protocol.

1. Detach the tape securing the foam cushion
2. Carefully remove suspect pins with forceps and examine pin underneath a stereoscope. If pin is fractured, discard pin. If the state of pin is uncertain, place pin in tube and label it accordingly along with the date
3. Partially insert replacement into a slot on top of the pin block with forceps
4. Softly nudge the pin with forceps to an upright position

5. Very softly tap the head of the pin until it reaches the second slotted plate
6. Very softly nudge the pin with forceps until it is aligned with both slots. It usually drops a small distance when aligned
7. *CRITICAL* Verify the pin is upright and not diagonal. If it is diagonal, very carefully remove pin from lower plate and retry
8. Very, very softly tap the pin until the head reaches the top plate
9. Return foam and metal bar to block if not recalibrating pin positions

Determining Proper Pin Positions in ArrayMaker

DO NOT reset any positions if the pins have not been removed. Everyone uses common settings and they depend on these positions to be correct. If any positions are reset, it is recommended to redefine positions for all components including glass slide calibration to ensure that the entire system is updated for any changes.

Reset Z Height

1. After axes have been homed on startup, press the "Align" tab
2. Begin by clicking the "Reset Z" button on the bottom left
3. "Save Alignment to Disk"

Glass Slide Width and Height Calibration

4. Remove tape from all vacuum ports on the platter for the two left-most columns. Remove any residue or debris with 70% EtOH. Place as many dummy glass slides as possible in those columns and tape remaining vacuum holes. Be sure that slides are pressed up against the back edge of the platter
5. Lower calibration arm such that the calibrating pin is as low as possible
6. Click "Print" position button
7. Center calibration pin over back edge of glass slide in upper right corner using manual controls in upper left of the ArrayMaker window
8. Lower the calibration pin with the manual controls until the pin is ~1mm away from the glass slide. It is okay if the pin comes into light contact with the glass slide, back off as necessary
9. Repeat Steps 7-8 as necessary
10. Press "Set " followed by "Save Alignment to Disk"
11. Press the "Calibrate Slides" tab
12. Provide the following settings:
 - Start of Column: 1 {Second column: 12}
 - End of Column: 11 {Second column: 23}
 - Slides per Step: 1
13. Press "Begin"
14. Move the platter forward as backward as needed to center pin at top edge
15. Press "Next" and repeat as necessary
16. Repeat 12 - 15 for the second column

17. Press "Save Alignment"
18. Return to "Align" tab and center the pin above the back right glass slide in the "Print" setting. Lower until ~1mm above glass slide
19. Press "Set" and "Save Alignment to Disk"
20. Return to the "Calibrate Slides" tab
21. Provide the following settings:
 - Start Slide: 2
 - End Slide: 23
 - Step Increment: 20
 - Z-Axis Speed: 200
 - Start Height Offset: 500
 - Distance Limit: 3000
 - Consistency Threshold: 200
22. Press "Start Height Calibration"
23. Press "Consistency Check" to ensure there are no significant anomalies
24. Press "Save Alignment"
25. Raise calibration arm until the pin rests above the printing pin block

Print Position

26. Align the left most pin with the lower left corner of the glass slide in the back right position of the platter. Pins should be near the surface, but not touching the glass with the foam cover removed from the pin block
27. Shift the pins 15000 units to the right and 27000 units towards the back of the platter
28. Slowly lower the pins until a small gap appears between the pin heads and the upper colimiter plate supporting the pins. A gap barely large enough to see light reflecting underneath the pin head
29. "Set Position" and "Save Position"

Well Plate Positions

30. Insert blank 384 well plate into plate holder
31. Press the "Position" tab, select "4 x 1" pin arrangement, press "Well Plate"
32. Gradually lower the pins toward the plate
33. As it nears the plate, confirm that the pins are centered over the well. If the pins are not centered, shift the platter in the appropriate direction and lower once more
34. If the pins have been confirmed to be centered above the wells, slowly lower pins into the well plate until very little contact has been made with the bottom of the plate. Contact can be determined by seeing the pin lift slightly from the upper colimiter plate holding the pins
35. Back off 300 um from contact point
36. "Set Position" and "Save Positions"
37. In the top center portion of the window, press "Move to Last Load"

38. Lower pins and center above last wells of the plate as needed
39. Press "Calculate Skew" followed by "Save Plate"

Wash and Dry Bath Positions

40. Press the "Wash" position button
41. Position pin above the bath port and gradually lower the pin towards the center of the bath. Avoid the edges as the pin block "swishes" when washing
42. Lower the pins until the pin quill reservoir is at least 50% submerged but the water line is at least 5mm below the pin block
43. "Set Position" and "Save Position"
44. Remove aluminum foil cover and tape from dry bath
45. Identify which holes are connected to vacuum and holes that are dead-filled
46. Lower pins until they sit above vacuum holes, check centeredness, and adjust as needed
47. Lower pins into vacuum holes until there is a 2 mm gap between the bottom of the pin block and the top of the dry bath
48. "Set Position" and "Save Position"
49. Move to "Station 3"
50. Smooth out a rectangular piece of aluminum foil and tape to the dry bath so that there are no air leaks
51. Move to "Dry Bath"
52. Move to "Station 3"
53. Repeat 12-13 (3) more times
54. Arrayer is ready to print

Arrayer Tab Images:

(Next few pages - shows tabs and options available)

 Connect to Controller
 Home all axes

```
Message and Debug Window
SP 1000000,1000000,300000
AC 1000000,1000000,1000000
DC 1000000,1000000,1000000
KD 80,70,70
KP 9,6,10
KI 1,1,1
Limit Switch Status: 13, 13, 13
:
Setting home speeds:
SP 100000,150000,3000
The Z-Stage is being reset ...
Z Status Loops per second: 6542
Home: Z-Up Limit Found, X,Y,Z Homing ...
Home Status Loops per Second: 2677
Time to home: 54
All axes at home position.
```

Homing Clock: 54

	X-Axis	Y-Axis	Z-Axis
Motor Stopped			
Motor Running			
Motor at Home Position			

Galil DMC-1832 3 axis controller revision 1.0q

V7s

DeRisi Lab ArrayMaker Version 2.78

Welcome | **Connect** | Align | Test Print | Print Run | Calibrate Slides | Configure | Terminal | Version Notes

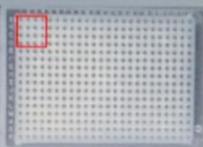
Motion Control

X -422322 Y -520039 Z -8500

Back Up
Left Right
Down Forward

20 100 500 2K 20K 40K
step size

Calibrate Plate



Move to last load

Calculate correction

Calculated Spacing
X: 00000
Y: 00000

Correction Factor
X: 00000
Y: 00000

Tips
 1 8x1 32
 2x2 12 48
 4x1 16

Save Correction Cancel

Station Control

Sonicator
 ON OFF

Vacuum Pump
 ON OFF

Station 3
 ON OFF

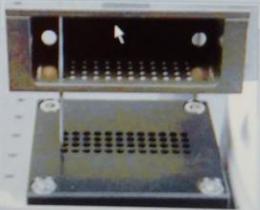
Align Station

Rinse	422322
	453690
	3018
Dry	422322
	473957
	2332
	422322
	70000
	0
Print	-120941
	-120950
	12361
Plate	209452
	-101907
	14162
Blot/Pack	-120920
	-124405
	12127

Soft touch down ON

Set

Map Slide Patter



Go to slide 2

Set Height

2 thru 3

Offset: 00000

Save Alignment to Disk

Welcome to ArrayMaker

No Error Messages

Plate Orientation: Forward

31 Slides Placed

JTX

7s

DeRisi Lab ArrayMaker Version 2.78

Welcome | Connect | Align | Test Print | Print Run | **Calibrate Slides** | Configure | Terminal | Version Notes

Calibrate Width

Start of Column	End of Column	Slides per Step
1	22	5

Begin

Calibrate Wizard

Move Platter Back (mm)

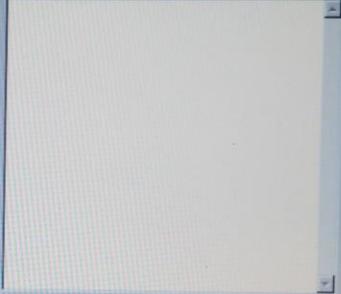
2
1
0.5
0.25
-0.25
-0.5
-1
-2

Move Platter Forward (mm)

Next >>

Slide 000 Offset 00000

Calibrate Height



Start Slide	2
End Slide	261
Step Increment	20
Z-Axis Speed	200
Start Height Offset	1000
Distance Limit	1000
Consistency Threshold	100

Start Height Calibration

Stop Height Calibration

Consistency Check

Save Alignment

Welcome to ArrayMaker No Error Messages Plate Orientation: Parallel X Stage: Flipped

JTX

V7s

DeFisi Lab ArrayMaker Version 2.78

Welcome | Connect | Align | Test Print | **Print Run** | Calibrate Slides | Configure | Terminal | Version Notes

Print Setup

Total Number of Plates: 1

Tips: 1 8x1 32
 2x2 12 48
 4x1 16

Orange Pack
 Entire Platter
 1x Re-Fill
 2x Re-Fill
 Slow Pick Up
 Soft Touch Down

Station Settings

Wash Cycles: 2 Wash time: 3000 msec
Dry time: 8000 msec Load time: 500 msec

Bounce Dry
 Swish Wash

Start Settings

Start from Plate: 1 Start from Load: 1
Tandem Offset: 27000 um

Tandem Print
 Auto Advance Plate

Slide Setup

Begin on Slide: 1
End on Slide: 261
Sector Width: 24 spots
Spots per load: 1

Spacing: 183 microns (column), 183 microns (row)

Sequential Arrays after row: 5

Suggested Spacing: 183 microns

Print Configuration Settings

Z Print Height: 400 um Slow Pick Up Speed: 2000
Z Print Accel/Decel: 1000000 / 350000

Station Control

Rinse: ON OFF
Dry: ON OFF
Station 1: ON OFF
Plate: ON OFF
Soft touch down: ON

Control Panel

Plate: 00 Load: 000 Slide: 000
Sector Row: 00 Sector Column: 00

Time to Next Plate: 00 min Total Time Remaining: 0000 hrs Slides / Minute: 000

START **STOP**

Macros

Wash and Dry
Rinse
Warm Up (1 cycles)

Plate Control

Last Plate: ? Current Plate: ?
Plate ID: _____
 Use Plate IDs

Save ?

Blot Pad Settings

Blot Pad Slide Range: Begin: 1, End: 22, Taps: 20

Standard Blot Rows
 Use Blot Pad

Welcome to ArrayMaker. No Error Messages Plate Orientation: Parallel X Stage: Flipped

JTX

<FUNCTION> - ADJUST +