

Silicon Microarray™ Technology



Silicon Pins and Printhead

User Manual

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These printing tools are for research use only and should not be used for medical or diagnostic purposes.

I. PRECAUTIONS

A. Mechanical Precautions

1. Please pay attention during unpacking the pins as the lids on the centrifuge tube should be pulled straight up while the tube shaft itself remains unbent. Bending the centrifuge tubes during unpacking the pins could damage/break the pins.
2. Do not allow any object or force to contact the pins in the x or y axes (lateral directions), which could break the pin shafts. The pins should never be dragged across or smashed into solid surfaces.
3. All the motion control movements, printing parameters and hardware should be completely checked before loading the silicon pins into the printhead.
4. Test printing with a single pin is very strongly recommended before loading all the pins. Use only one pin for programming the motion control system and aligning the printhead.
5. Any of the z -axis printing parameters should be changed with utmost care. (See below for detailed printing parameters)
6. Load the pins only after attaching the printhead to the robot arm of the motion control system.
7. The thinned region of all pins in the printhead must face the same side. See pin loading section (Sec. 4) for detailed instructions.
8. Before loading the pins into the printhead practice handling the silicon pins by loading and unloading a practice pin (included in the package) into the printhead several times until you get used to handling the silicon pins.

B. Humidity

9. The entire printing apparatus should be enclosed in a chamber with humidity controller to maintain an adequate and constant humidity.
10. Always set the relative humidity (RH) at 60% or higher. Since pins imbibe a very small volume of liquid ($\sim 100\text{nL}$), the higher humidity levels are critical to prevent evaporation and obtain successful printing results.

C. Cleanliness

11. Do not touch the pin tip at any time.
12. Do not handle the pins with bare hands. Always wear oil/powder free gloves to handle the pins.
13. Maintain an oil free environment inside the printing chamber. No vapors/plasticizers should be allowed to come in to contact with the pin surface.
14. Never dry the pin tips with canned "air" or other aerosol propellants. Use only dry, oil-free and particulate-free air.
15. If any of the pins are jammed in the collimator holes due to drying of the wash solution or the printing buffer solution on the pin shaft, soak the pins in distilled water by flooding the metal holder with distilled water for a few minutes and gently pick up the pins with the tweezers. Do not forcibly remove the pins at any time.

16. Do not use an ultrasonic cleaner to wash the pins. The pins can be cleaned well in still/flowing water without the use of an ultrasonic cleaner.

D. Printing Substrates and Printing Solutions

17. Make sure that the printing buffer solution is fresh and is filtered free from any solid particulates.
18. The substrate slides have a tendency to become non functional abruptly. Make sure they are stored under proper conditions and never use the slides after their expiration date.

E. Pin Washing

19. The bottom of the printhead should be at least 6-7mm above the level of wash solution in the wash bath.

II. USER INSTRUCTIONS

This manual will help you to become acquainted with Parallel's Silicon Microarray™ Technology and allow you to quickly begin printing the highest quality microarrays possible.

The instructions in the following sections will permit you to adapt the silicon printing technology to your existing microarray spotting system and provides instructions to avoid improper handling of the spotting pins.

This section contains instructions on:

1. Unpacking
2. Handling
3. Assembly and disassembly of the printhead
4. Loading pins
5. Suggested printing parameters
6. Washing and drying
7. Deep cleaning procedure
8. Unloading pins from the printhead

1. Unpacking the pins

All pins are individually packed and shipped in microcentrifuge tubes as shown in Fig. 1. Carefully open the centrifuge tube cap and pick up the head of the pin with the enclosed tweezers as shown in the Fig. 2 and load the pins into the printhead as described below. Please pay close attention during unpacking the pins as the lids on the centrifuge tube should be pulled straight up while the tube shaft itself remains unbent. Bending the centrifuge tubes during unpacking the pins could damage/break the pins.



Fig. 1 A silicon pin packed in a microcentrifuge tube.



Fig. 2 Unpacking the pins.

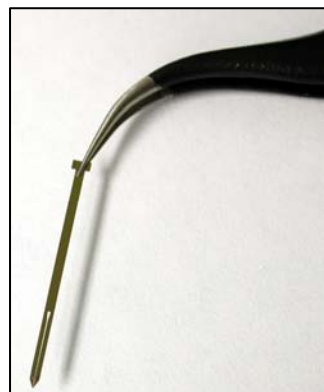


Fig. 3 Handling the pins.

2. Handling the silicon pins

The silicon pins should be handled using the enclosed curved extra fine stainless steel tweezers. Always hold the pin head of the silicon pin using the tweezers as shown in Fig. 3. Avoid holding the pins by their edges or corners as it may chip the sharp edges of the pins.

Do not handle the pins with bare hands. Always wear oil free and powder free gloves to handle the pins. Contact of the pins with any of the following common laboratory contaminants, or any especially oily or hydrophobic materials, could render the pins unable to print aqueous solutions properly and will require cleaning of the pins:

- Canned “air” or other aerosol propellants
- Bare hands
- Lipids, oily or greasy materials
- Surfactants

3. Assembly and disassembly of the printhead

The printhead should be mounted to the robot arm of the spotting machine prior to inserting the pins into the printhead. The printhead will be shipped assembled and should last indefinitely with proper care.

The disassembled view of the printhead is shown in Fig. 4, which consists of

- a metal holder embedded with rare earth magnets on the bottom and alignment pins for precise mounting of the silicon collimators,
- two silicon collimator plates with high tolerance, easy loading holes on a 1536 format. Fig. 5 shows the cross sectional view of the holes in the collimator. The top part of the hole is tapered into a funnel shape for easy loading of the pins, while high tolerances ($5\mu\text{m}$) are retained in the vertical bottom part of the hole to minimize any lateral movement of the pins in the printhead.
- a magnetic stainless steel bottom plate to hold the bottom collimator to the metal holder,

- a piece of die cut elastomeric foam, which provides the proper compliance and pressure for optimum printing, and
- Tygon[®] tubing which secures the top collimation plate from moving during printing (Fig. 6).

3.1 Assembly

The top and bottom collimators are precisely aligned to one another by mounting them onto the alignment pins in the metal holder as shown in Fig 7. The two alignment holes in each collimator for mounting onto the alignment pins of the metal holder have high tolerances (10 μ m) and align the collimators to the nearest 10 μ m in *x* and *y* direction. See below for a step by step procedure on how to assemble the printhead.

- a) Make sure the surfaces of the metal holder onto which the collimators will seat are clean and free of any dirt, oil or particulates.
- b) Wearing powder and oil free gloves mount the bottom collimator onto the alignment pins at the bottom of the metal holder and make sure the tapered side of the collimator holes faces toward what will be the top of the printhead when mounted to the motion control arm. The collimator must be kept parallel to the holder surface as it is placed onto the two alignment pins to avoid chipping the high precision alignment holes in the collimator (see Fig. 7). In order to retain the high tolerances of the alignment holes and still make them easy to mount, one of the alignment holes is oval shaped (see Fig. 8). This provides extra room for the high tolerance collimator holes while mounting and eases the collimator mounting procedure as compared to two circular alignment holes. After mounting the bottom collimator gently place the magnetic stainless steel plate on top of the collimator.
- c) Flip the holder over and mount the top collimator onto the alignment pins. Again the collimator must be kept parallel to the holder surface as it is placed onto the two alignment pins. First carefully position the round hole of the top collimator onto one of the alignment pins as shown in Fig. 9, and then carefully align the oval hole onto the other alignment pin. After aligning drop the collimator onto the alignment pins. The funnel-shaped (tapered) holes of both the collimators should now be facing up (Fig. 5).
- d) Place the enclosed Tygon[®] tubing (1/16" OD) onto the alignment pins and gently press them down to secure the top collimator to the metal holder (Fig. 6).
- e) Mount the printhead onto the motion control system.
- f) Load the pins into the printhead on either a 384 or 1536 format (*see Section 4*).
- g) Place the foam on top of the pins and secure the lid to the metal holder as shown in Fig. 4
- h) The printhead is now ready to start printing.

3.2 Disassembly

- a) Unload the pins as described in section 7 and store them in the microcentrifuge tubes as shown in Fig.1.
- b) Unfasten the printhead from the motion control system.
- c) Disassemble rest of the parts of the printhead in the order opposite to that mentioned in section 3.1 (i.e., 3.1.d \rightarrow 3.1.a).

(Note: Proper care should be taken while dismounting the top collimator. After removing the lid and the foam, loosen the top collimator by removing the Tygon[®] tubing. Put the foam on top of the top collimator and flip the metal holder over. Now gently lift the metal holder vertically up leaving behind the foam and the top collimator.)

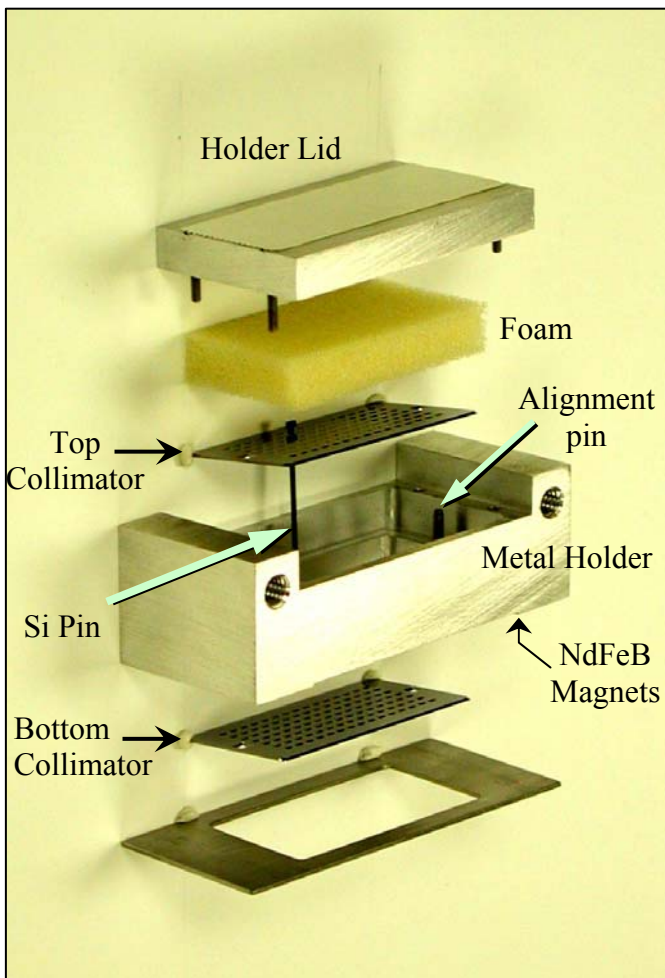


Fig. 4 Disassembled view of the silicon pins and printhead

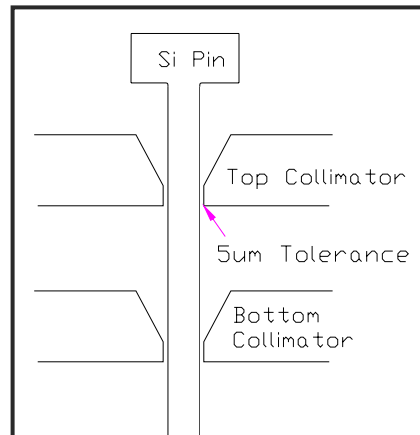


Fig. 5 Cross sectional view of the holes in the collimation plates and a silicon pin.

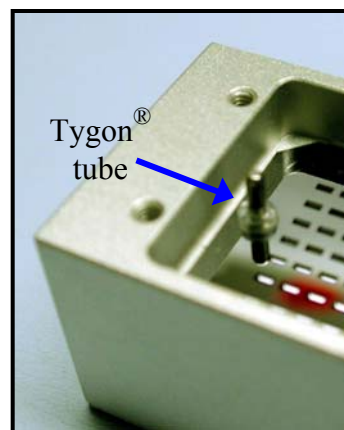


Fig. 6 Securing the top collimator to the metal holder with Tygon® tubing.

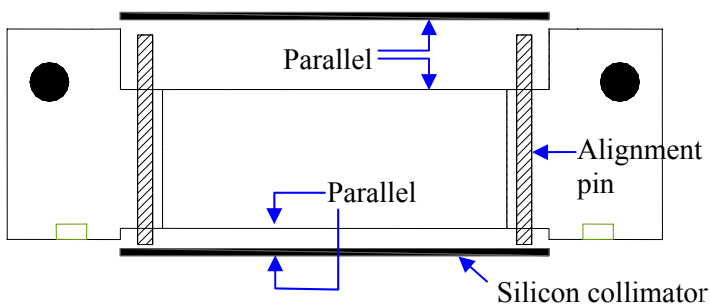


Fig. 7 Sectional view of the metal holder and the collimators

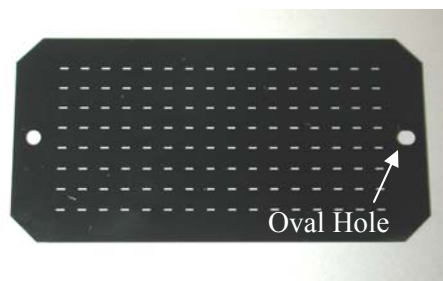


Fig. 8 Silicon collimator

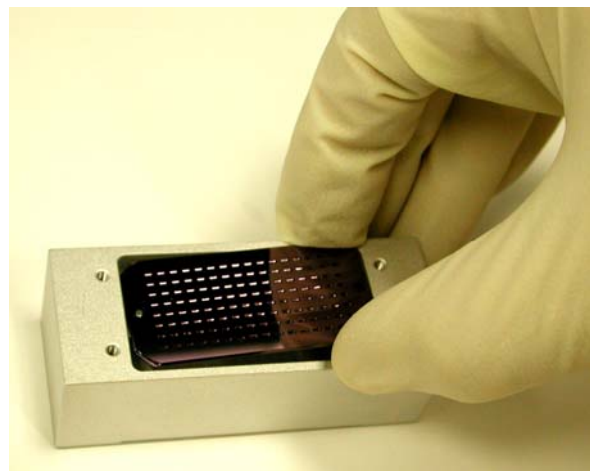


Fig. 9 Mounting the top collimator onto the alignment pins in the metal holder

4. Loading Pins

Test printing with a single pin to validate the entire printing procedure is strongly recommended before loading all the pins. All the motion control movements, printing parameters and hardware should be completely tested and verified before loading the silicon pins into the printhead. The silicon collimators in the printhead can accommodate pins on either 384 well (4.5mm spacing) or 1536 well (2.25mm spacing) format. Load pins in every other hole in the collimator, as indicated, for printing from 384 well plates. Load pins in all the holes to print directly from 1536 well plates (Note: the ID of the wells of the source plate must be at least 1.1 mm for the pins to access the fluid in the wells). Parallel

has designed and offers a pin loading funnel to minimize pin damage while loading the pins into the high tolerance holes in the collimator. See below for detailed instructions on how to load the pins with and without a pin loading funnel (***it is strongly recommended that the pins be loaded with the pin loading funnel to minimize the possibility of pin damage.***)

4.1 Loading the pins with the funnel: The pins can be easily loaded using the pin loading funnel. The pin loading holes in the funnel are spaced on 4.5mm (384 well format) providing access to every other hole in the collimator. A photomicrograph of the funnel is shown in Fig. 10. It is a two part assembly which serves as a funnel for loading the pins when the two parts are assembled while the bottom part serves as an unloading funnel when the two parts are disassembled. See the Sec. 8 for detailed instructions regarding unloading the pins.

Mount the funnel onto the alignment pins in the metal holder so that the funnel rests on top of the Tygon[®] tubing. The sectional view of a loading hole in the funnel and a Si pin is shown in Fig. 11. As a Si pin is dropped into one of the funnel holes the long cylindrical hole aligns the pin vertically ($>89^\circ$) to the surface of the collimator. The combination of the slot and the cone shaped ending of the cylindrical hole at the bottom of the funnel guides the pin tip into the high tolerance holes of the collimator (Fig. 12). The features of the funnel are designed in such a way that the pin tip doesn't touch the funnel walls and remains undamaged while loading.

Note that the pin should be dropped into the funnel with its wider head dimension parallel to the direction of the slot and the collimator hole (Fig. 13C). If it is dropped perpendicular to the slot (Fig. 13B) the pin rests on top of the cone shaped hole as shown in Fig. 11B. In this case, the pin should be lifted up by holding the pin head with tweezers and dropped again after orienting it to the slot. ***If any of the pins stop halfway from falling through the funnel hole, gently tap on the funnel until the pins fall through to the bottom of the funnel. Or the pins can be individually pushed to the bottom of the funnel by placing a small weight (2-5 gm) on top of the pins that are trapped halfway in the funnel hole (eg. using a thin metal rod).*** Once all the pins are loaded the funnel should be gently removed from the printhead. ***Always remove the funnel very gently by pulling it in the vertical direction.*** Currently the funnel can be used only

to load pins on 384 well format. Please contact Parallel if you need a funnel for loading pins on 1536 format.

Fig. 10 Loading-unloading funnel

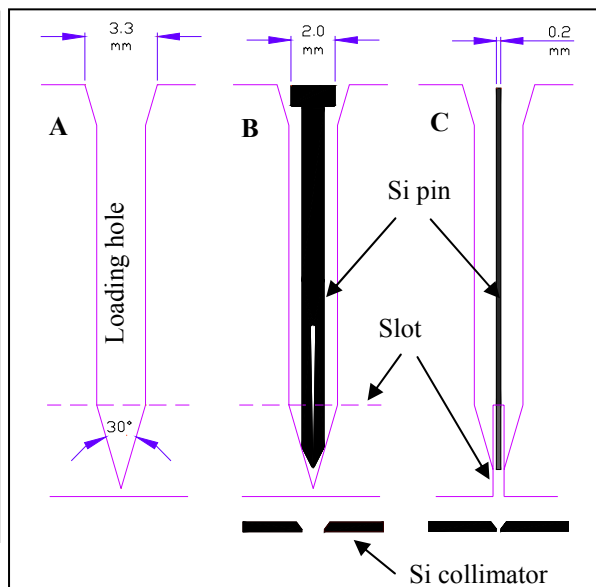
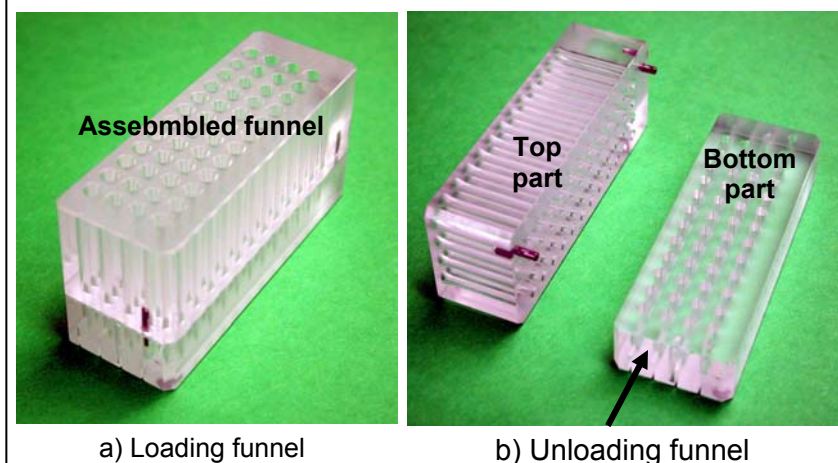
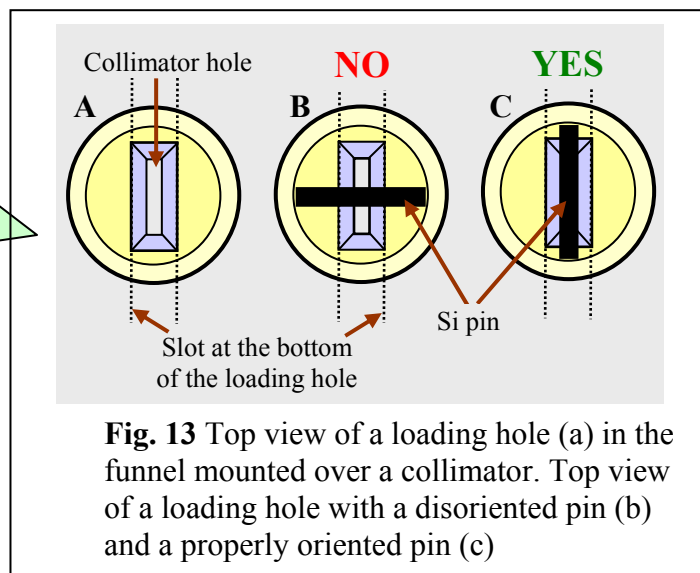
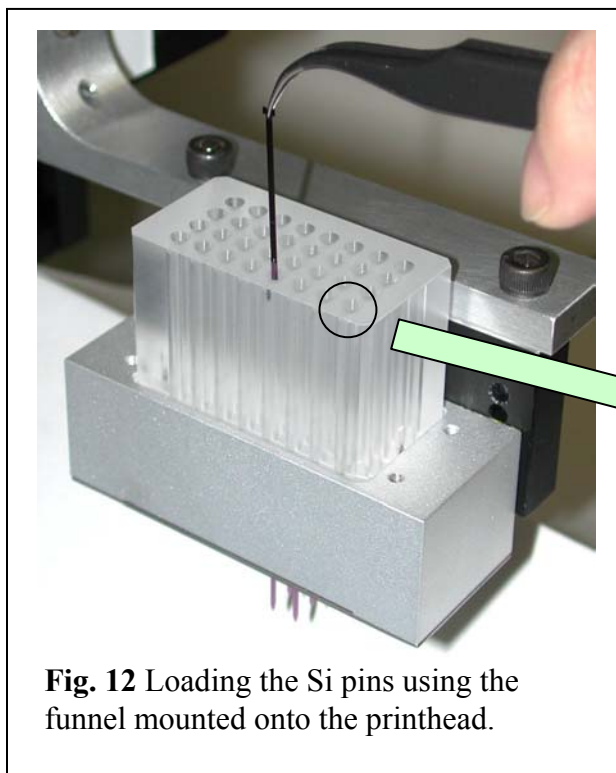


Fig. 11 Sectional view of a) a loading hole in the funnel, B) a Si pin in the loading hole and c) side view of a Si pin in the loading hole of the funnel



4.2 Manual loading: Hold the pins with the tweezers such that they are as plumb and vertical (90° to the surface of the collimator) as possible and gently drop them into the holes present in the collimator as shown in Fig. 14. The pins drop right through the holes and until stopping to rest on the pin head. In certain cases when the pins are not loaded vertically (< 88-89°), the pins may not go through the bottom collimator and stand on top of the bottom collimator as shown in Fig. 15. Do not force the pin to go through the bottom collimator as it may damage the pin tip. The pin should be gently picked up with the tweezers and should be loaded again until it goes through both the collimators. *The thinned region of all pins in the printhead must face the same side as shown in Fig. 16.*

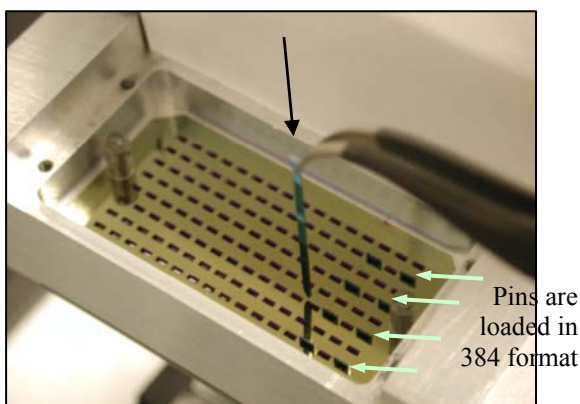


Fig. 14 Manual loading of the pins directly into the collimator holes.

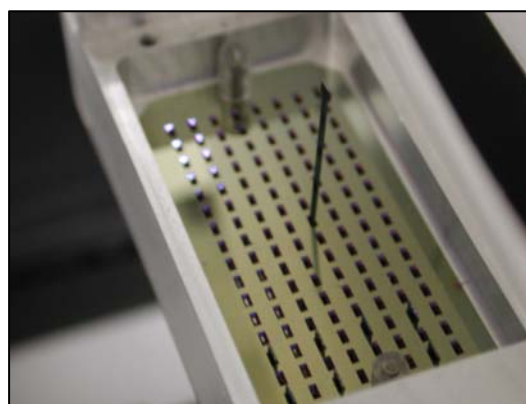


Fig. 15 A misloaded pin.

Once the pins are carefully inserted into the printhead, the pin tips can print millions of spots without minimal tip wear if properly used. The pins should never be dragged across or smashed into solid surfaces. Silicon is a much harder material than stainless steel which prevents the pin tip from wearing, mushrooming and blunting. But it is brittle, hence proper care should be taken such that no object ever contacts the pins in the *x*, *y* directions, which may damage the pins permanently.

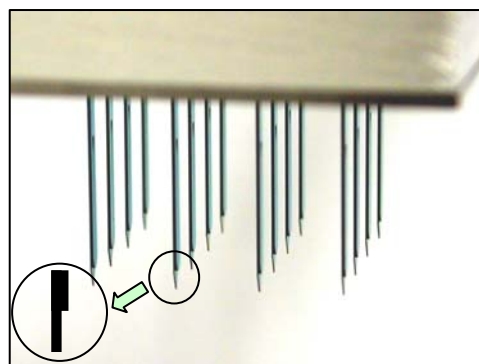


Fig. 16 Printhead loaded with 16 pins with the thinned region all facing in the same direction.

5. Suggested Printing Parameters

- Relative humidity: > 60%
- *z*-axis velocity during printing: 5-50 mm/sec (preferred: 25mm/sec)
- *z*-axis acceleration during printing: 250-500 mm/sec²
- *z*-axis over travel below the surface of the substrate during printing: 0.075-0.20 mm (preferred: 0.15mm)
- *z*-axis surface clearance during printing (above the substrate surface): 1.0-2.0 mm

- Surface dwell time: 50 msec
- Sample uptake time: 2-4 sec
- Pin wash time: 2 cycles at 10 sec/cycle
- Drying time: 5 sec
- Insertion depth of pins in wash solution: 7mm (from the pin tip, see section 6)

All the above parameters except the z-axis parameters are suggested values only, and they can be modified according to one's motion control system and hardware limitations. **All the z-axis parameters should be changed with the utmost care.** Test printing with a single pin is strongly recommended before loading all the pins. All the motion control movements, printing parameters and hardware should be thoroughly and completely checked before loading the silicon pins into the printhead.

6. Washing and Drying

A contaminated or malfunctioning washing/drying station can result in cross contamination of samples and poor printing performance of the pins. Multiple washing and drying cycles are necessary to wash all the residual samples from the pins. At Parallel, extensive testing has repeatedly shown the high value of a good washing system for cleaning. The silicon pins can be easily cleaned in a wash bath with continuous supply of fresh wash solution followed by drying with air using a vacuum dry station. **Do not use an ultrasonic cleaner to wash the pins.** Do not dry the tips with canned "air" or other propellants! Many of these propellants contain extremely hydrophobic hydrocarbon or fluorocarbon polymers which are very difficult to remove from the tip. The following section 6.1 and 6.2 gives instructions on how to adapt your existing washer-dryer stations for effectively cleaning the pins.

6.1 Washing

- The pins should be cleaned with distilled water, 1-2 times after each printing cycle (washing time: 4-10 sec per cycle)
- Wash solution should be continually changed during a print run to prevent cross contamination of samples.
- Avoid lowering the pins too low into the wash solution, as the wash solution can be splashed into the bottom of the printhead during washing. If the collimators or printhead becomes wet the printhead must be disassembled, cleaned and dried before reassembly. The printhead should be lowered into the wash bath until the wash solution reaches ~1mm below the tip of the pin's reservoir (Fig. 17b). The tip of the reservoir in the pin is spaced at 7 mm from the bottom of the printhead which provides enough clearance between the printhead and the wash solution (Fig. 17a) during washing.
- Drying of sample residue in the pin channel causes pin clogging and may result in a pin that does not print. Check the wash and dry station for insufficient washing or drying and adjust the washing and/or drying time or the number of washing cycles accordingly.

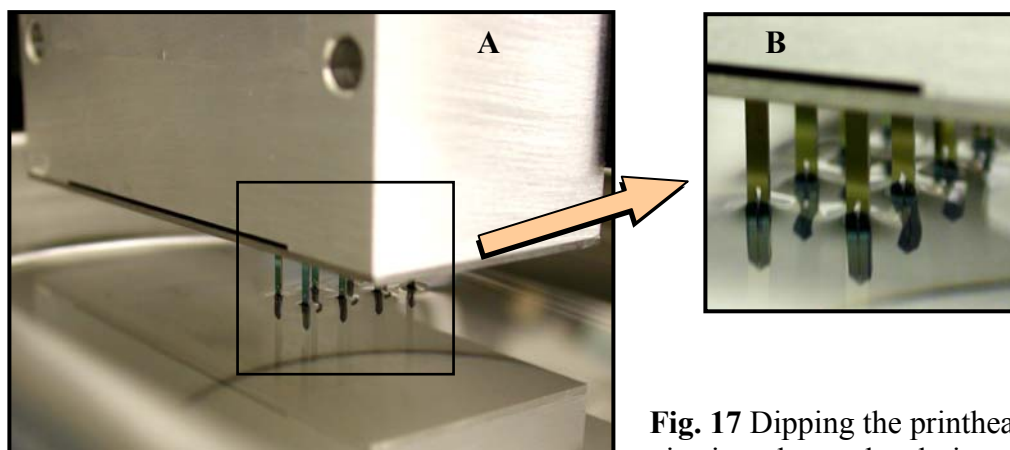


Fig. 17 Dipping the printhead with pins into the wash solution

6.2 Drying and dryer set up

The key to proper drying is to provide sufficient airflow around the pin channel to draw the residual fluid from the pin channel. If there is insufficient airflow the fluid from the pin channel will eventually evaporate precipitating solids and clogging the pin channel.

The silicon pins have a rectangular cross section, whereas the holes in a regular dryer are drilled in a machine shop and have circular cross sections. To provide air flow into the pin channel to effectively dry the pins, the following steps should be followed:

- Align the pins in the printhead with the holes in your existing dryer, by carefully moving the x , y , z stages of the motion control system. Lower the pins into the dryer holes making sure that they are perfectly centered in the holes. Store the x , y , z coordinates for the drying stage using your motion control software. Move the pins up and down in the dryer holes several times to make sure that the pins do not touch the side walls of the dryer. Move the printhead away from the dryer.
- Cut an approximately 1" x 3" strip of the enclosed ultra thin (0.0005" or 12.5 μ m) aluminum foil and swipe both the surfaces of the foil with ethanol on a clean, flat surface.
- Place the cleaned foil on top of the existing dryer covering all of the holes.
- Turn on the vacuum pump connected to the dryer so that the dryer holds the foil in place.
- Attach the foil to the dryer on all four sides with tape (Fig. 18a). **Do not cover the holes with tape.**
- Using the motion control software let the printhead go to the dryer coordinates saved earlier so that the pins puncture through the aluminum foil to form close tolerance holes (Fig. 18b). Make sure that the vacuum is turned on while puncturing the holes.
- The dryer with the perforated aluminum foil dries the pins almost instantaneously.
- If the foil is damaged and has to be changed, recheck and verify the correct alignment of the pins into the dryer holes before puncturing holes into the new foil.

Be extremely careful during puncturing holes in the foil and make sure that the pins do not hit the dryer side walls.

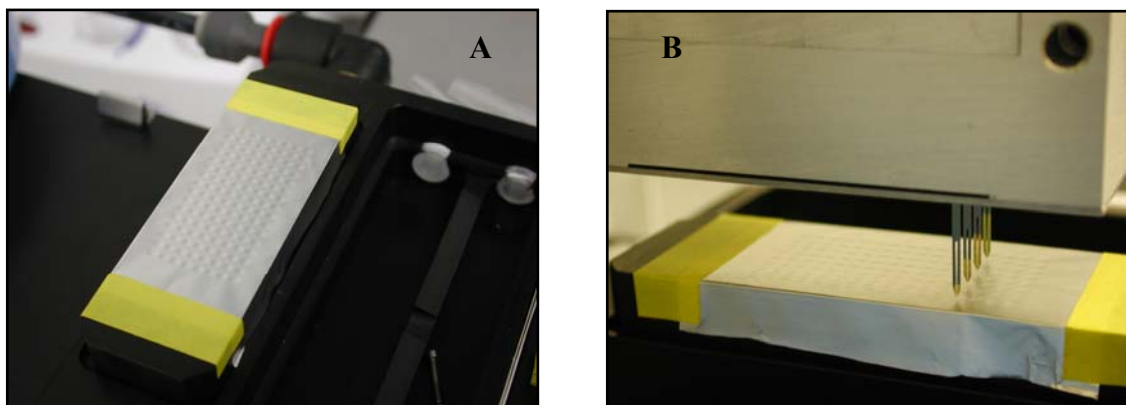


Fig. 18 Adapting an existing drying station to effectively dry the silicon pins using ultra thin aluminum foil (0.0005” thick).

7. Deep Cleaning Procedure

Loss in printing quality may be observed over time, due to accumulation of deposits and other contaminants on the surface of the pins and pin tips. This may be caused due to improper printing conditions, misuse or decomposition of the printing solution or buffers. If a loss of printing quality is observed even after the regular washing-drying procedures as discussed in Section 6, the silicon pins can be cleaned thoroughly by heating them red hot for a few seconds in a butane/propane torch flame (see Fig. 19).

By heating the pins red hot all of the organic deposits on the surface of the pins will burn off rendering the pin surface free of any sort of organic deposits and contaminants. Fig. 20 shows photomicrographs of two silicon pins before and after cleaning them using the butane torch. Torch cleaning should be immediately followed by a regular washing and drying to remove any inorganic deposits left over on the pin surface. All the pins in the printhead can be cleaned directly in the printhead without having to unload the pins (Fig. 21). The foam on top of the pins in the printhead should be removed before heating the pins with the torch as discussed below.

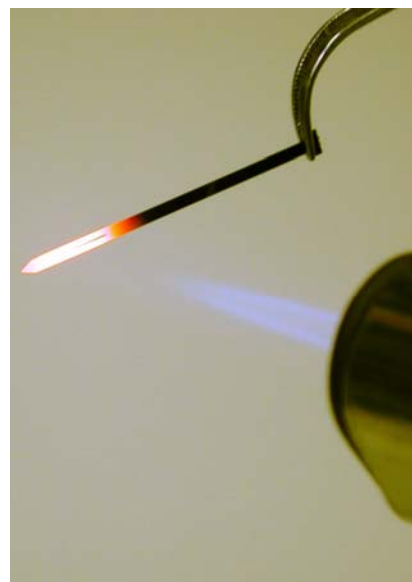


Fig. 19 A silicon pin glowing red hot in a butane torch.

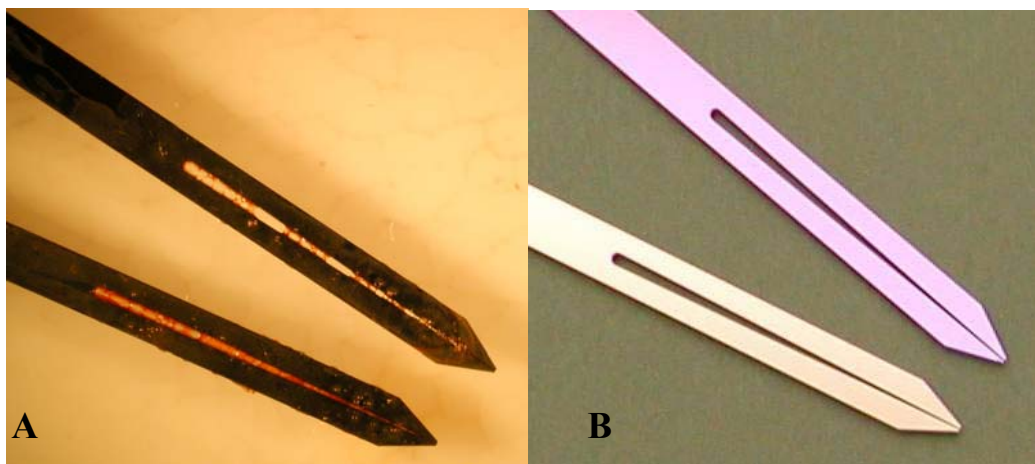


Fig. 20 Picture of two silicon pins soaked in egg white solution and then baked at 150 °C for 30min a) before cleaning and b) after cleaning using butane torch. The pins look brand new after the torch cleaning.

Detailed instructions on how to clean the pins using the torch are given below.

- ***Make absolutely sure there are no flammable materials such as organic solvents inside the chamber or anywhere nearby during the torch cleaning procedure.***
- Remove as much inorganic material (NaCl, phosphates, etc.) as possible from the pins by washing with DI water.
- Move the printhead with the pins to a safe region inside the motion control system.
- Open the lid and remove the foam from the printhead before heating the pins.
- Using a mini butane/propane torch heat the pin tips and reservoir channel as shown in Fig. 21. The pins do not need to be unloaded for this procedure.
- Always heat the pins by keeping the torch flame horizontal as shown in Fig. 21 (keeping it away from the bottom of the printhead). Do not allow the torch to get too close to other parts of the printhead or heat the printhead directly as it may damage the close tolerance parts in the printhead. Do not expose the pins to the torch for long periods of time (< 2-4 sec). If 2-4 sec is insufficient heat the pins several times for short intervals of time instead of continuously heating them.
- While using the torch to clean the pins make sure that the pins stay in the oxidizing region of the flame (as indicated by the green arrow in Fig. 22). This is because when the torch gets too hot or too cool, unwanted compounds can be created and deposited as hydrocarbon soot onto the pin surface.

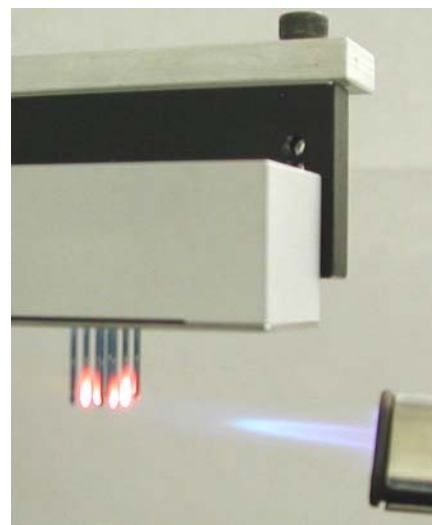


Fig. 21 Simultaneously cleaning all of the silicon pins while in the printhead using butane torch.

- Once the silicon pins are deep cleaned using this procedure and cooled place the foam and lid back onto the printhead.
- Using regular washing and drying procedures as discussed in Section 6 wash and dry the pins 2-3 times.
- Make sure the robot is calibrated and functioning properly before resuming printing.

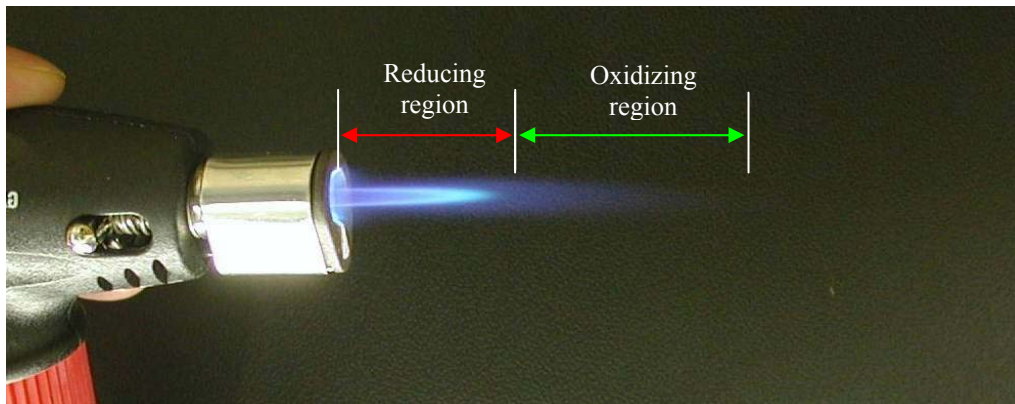


Fig. 22 Oxidizing and reducing regions in a butane/propane torch flame.

8. Unloading Pins from Printhead

The pins can be easily unloaded using the unloading funnel. The unloading funnel looks similar to that of the loading funnel in every aspect except that it is shorter in height. A photomicrograph of the unloading funnel is shown in Fig. 10b. See below for detailed instructions.

- Unscrew the lid from the printhead and remove the foam.
- Place a blank clean microscope slide on the slide platen or on the dryer covering all the holes.
- Make absolutely sure that all the pins are freely moving up and down in the collimator by individually raising the pins ~1-2 mm above the collimator and dropping them back. If any of the pins are jammed in the collimator holes it may damage other pins while you are trying to lower the printhead. See below for instructions if any of the pins are stuck in the holes.
- Align the printhead containing the pins with the blank slide by moving the x and y axes.
- Mount the unloading funnel onto the alignment pins in the printhead in a similar way that the loading funnel is mounted. Please make sure that the orientation of the holes in the unloading funnel matches the holes in which the pins are loaded (there are TWO orientations)!
- Slowly lower the printhead until the pin tips are ~1-2 mm above the slide surface. Now gently lower the printhead so that the pins rise up in the collimator. Once the printhead is completely lowered down, the heads of the pins rise up above the unloading funnel as shown in Fig. 23. Now the pins can be easily picked up with the tweezers as the pins are almost out of the bottom collimator and the unloading funnel prevents the pins from moving side ways while you pick up the pins minimizing any possible pin damage during the unloading operation.

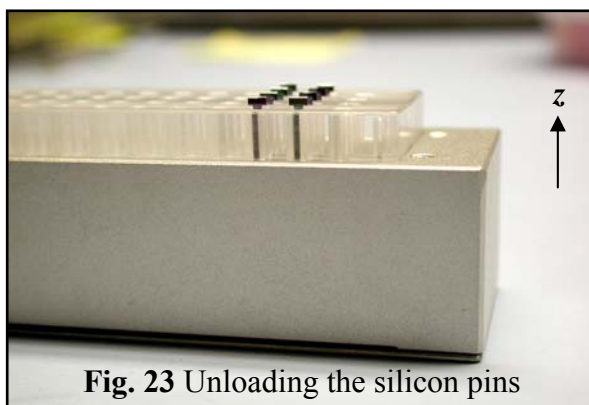


Fig. 23 Unloading the silicon pins

- Remove one pin at a time by holding the pin's head with the tweezers and pull out the pin in the vertical direction (z-axis). If the pin becomes wedged in the collimator holes while pulling out, drop it back into the hole and try again. Do not forcibly pull the pin as it may damage the pin side walls or collimator.

Note: Before lowering the printhead onto the slide make absolutely sure that none of the pins are jammed in the collimator holes. If any of the pins are jammed in the collimator holes due to drying of the wash solution or the printing buffer solution on the pin shaft, soak the pins in distilled water by flooding the metal holder with distilled water for a few minutes and gently pick up the pins with the tweezers. Do not forcibly remove the pins at any time.

III. ACCESSORIES PRICE LIST

The following accessories can be purchased from Parallel at the prices shown below or can be purchased directly from our vendors using the links below:

| Item description | Price (US\$) |
|--|---------------------|
| Ultra fine curved tweezers www.wihatools.com (Cat # 44510) | \$22 (each) |
| Mini butane torch http://www.discount-car-parts-online.com/Autoshop-Tools/Mini-Butane-Torches.htm http://store.breakingnewsproducts.com/bt9000.html | \$25 (each) |
| Ultra thin aluminum foil (0.0005" thick) www.mcmaster.com (Cat # 9060K34) | \$15 (1 Pkg) |
| Printhead leveling tool Starrett Dial Test Indicator (0.005" graduation): Cat # STT - 711HSAZ Starrett Complete Test Indicator Holder, Magnetic Base: Cat # STT - 657AA http://www.mytoolstore.com/starrett/indindx.html | |