

Chapter 2

Microarray Spotter and Printing Technologies

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2.1 Introduction

The early stages of drug discovery heavily rely on high-throughput screening (HTS) of compound libraries to identify effective lead compounds. Biochemical and cell-based assays have been commonly performed with the assistance of robotic liquid dispensing systems to rapidly test and identify the potential efficacy and toxicity of drug candidates [1, 2]. In an effort to save costs and reduce expensive resources such as primary human cells and reagents, pharmaceutical industries have focused on miniaturizing HTS assays by using higher density well plates including 384- and 1536-well plates, leading to a reduction in reagent volumes and an increase in the speed of the liquid dispensing [1, 3].

Biological sample printing using microarray spotters is an important advancement in the field of miniaturized assay development and HTS. Compared to traditional liquid dispensing systems, microarray spotters allow one to dispense extremely small volumes (typically 200 pL to 950 nL) of biological samples (including reagents, growth media, compounds, hydrogels, genes, proteins, viruses, and cells) in microtiter plates, on glass slides, or on plastic chips. The process of cell printing in hydrogels on a micropillar/microwell chip platform allows a precise positioning of human cell spots, leading to the creation of more physiological relevance of human cells grown in three dimensions (3D) [4, 5].

In this chapter, we will briefly introduce the advantages of various printing technologies and go over general precautions that have to be taken when printing various biological samples with a microarray spotter. In general, microarray spotting is divided into direct contact printing using pins and non-contact printing using solenoid valves, piezoelectric nozzles, *etc.* [6, 7]. Although we have experience in operating several microarray spotters, including MicroSys and PixSys from DigiLab and Nano-Plotter from GeSim, we will provide detailed protocols on how to operate S+ MicroArrayer from Samsung Electro-Mechanics, Co. (SEMCO). S+ MicroArrayer represents the most advanced microarray spotter for cell printing, which is specifically designed to accommodate the micropillar/microwell chip. The principle of operating and troubleshooting S+ MicroArrayer can be applicable to any solenoid-driven microarray spotters.

2.1.1 Contact Printing Techniques

Contact printing technologies mainly function on the principle of transferring biomaterials via directly contacting tips to the surface of functionalized glass slides. Examples of contact printing technology are microarray spotters with an array of multiple contact pins and atomic force microscopes (AFMs) with scanning tips (also known as dip-pen nanolithography). The contact microarray spotters have a single pin or a pin array to transfer liquids from source plates, which is usually a 96- or 384-well plate, to destination glass slides. A droplet forms at the tip when

the tip comes in direct contact with a liquid. Several types of tips have been developed, depending on the material used and the capillary size inside the tip to manage the volume of the droplet printed [8]. Factors that determine the volume of the droplet are the capillary size, surface tension of the liquid, affinity of the liquid with the glass slide, and the surface chemistry of the glass slide. Pin-based printing is mainly used to print extremely small volumes of proteins and DNA on the surface of glass slides [9, 10]. Although pin-based printing is straightforward and fast, it is difficult to print colloidal suspensions (such as cells), and the droplet size may be inaccurate and inconsistent depending on surface and liquid properties [9]. For dip-pen nanolithography, AFM is used to deposit some biological samples, mostly small molecules on the surface [7, 11]. Although this technology has capable of printing small spots in 25–200 nm resolution, it is not widely used in printing biological samples due to lack of multiplexing capabilities and limited detection methods [7, 9].

2.1.2 Non-contact Printing Techniques

In non-contact printing, microarray spotters with solenoid valves are the most commonly used, which function on the principle of electromagnetic induction. Typical solenoid valves consist of a metal rod that is surrounded by coiled wires that conduct an electric current. When voltage is applied across the coils, a magnetic field is generated that forces the metal rod to act as a shaft that moves up and down. This metal rod acts as a gate while printing biological samples. The setup is enclosed in a nozzle that has constant sample supply and constant pressure maintained using syringe pumps. When the shaft moves up and down, the outlet for printing biological samples opens and closes. This is how the voltage applied to the solenoid valves controls dispensing of liquid samples [6]. Unlike other dispensing systems, the solenoid valves can handle colloidal samples quite well. Typical dispensing volumes are 30–950 nL per droplet. One particular printer, S+ MicroArrayer, is equipped with six solenoid valves for printing six samples simultaneously.

Piezoelectric nozzles that are used in conventional inkjet printers are also commonly used in microarray printing with dispensing volume of 200–600 pL per droplet. Unlike solenoid valves, the dispensing volume is controlled by the inner structure of piezoelectric nozzles. Microarray spotters with piezoelectric nozzles use electric pulses that control the expansion and contraction of a piezoelectric membrane that acts as a pump to push biological samples through the tip [12, 13]. Thus, the dispensing volume can be increased not by changing voltages, but by depositing multiple spots at the same location. Although piezoelectric nozzles are capable of printing biological samples, its use is limited due to inconsistent printing of colloidal samples and difficulty of controlling large dispensing volumes [14]. Piezoelectric nozzles are more commonly used for printing compounds dissolved in DMSO, proteins, and amino/nucleic acids.

Another popular type of bioprinting technology adapted in the field of tissue engineering is laser-assisted bioprinting (LAB), also known as laser-induced forward transfer (LIFT) [15]. Typically, LIFT consists of a pulsed laser beam, a glass slide coated with a biological sample, and a receiving glass slide. The laser beam is focused onto the absorbing layer, from which the heat is transferred to the biological sample where a bubble is generated. The bubble expands until it explodes, and the material in the bubble gets deposited onto the receiving glass slide [16]. However, this type of printing needs delicate control of the laser spotting, intensity, moisture and viscosity of the sample, thickness of the absorbing layer, and the printing speed so that cell viability cannot be compromised [17].

Acoustic printers became popular when single cell-based experiments needed to be carried out and demand for pL-sized droplets increased [18]. Acoustic printing requires a source for ultrasound generation, a pool of a biological sample, and a receiver glass slide. High intensity ultrasound is generated which is focused on air-liquid interface. When the energy exceeds the surface tension of the sample, liquid is ejected from the pool of the sample [19]. The advantage of this technique is its independence of nozzles and tubing. This technology gains in popularity for printing compounds in DMSO. However, high intensity ultrasound in some cases may cause an obstruction [20]. For example, printing delicate primary cells could be problematic because high vibration energy may damage cell membranes and lower cell viability.

2.2 Materials

- Solenoid valve (Lee Company)
- S+ MicroArrayer with six solenoid valves and ceramic tips (Samsung Electro-Mechanics, Co. or SEMCO, Suwon, South Korea)
- Micropillar and microwell chips (SEMCO, Suwon, South Korea)
- Ceramic tips with 150 μm orifice (SEMCO, Suwon, South Korea)
- Reagent alcohol 200 proof ACS grade (VWR)

2.3 Components of S+ MicroArrayer

To better explain the operation of the microarray spotter, we will classify it under mechanical and software components. Although both components work hand-in-hand, their maintenance and parameter setting are different from each other. Mechanical components are divided into the main body, the utility body, and externally connected parts that play an important role in chilling, rinsing, and sample dispensing (Figs. 2.1 and 2.2).

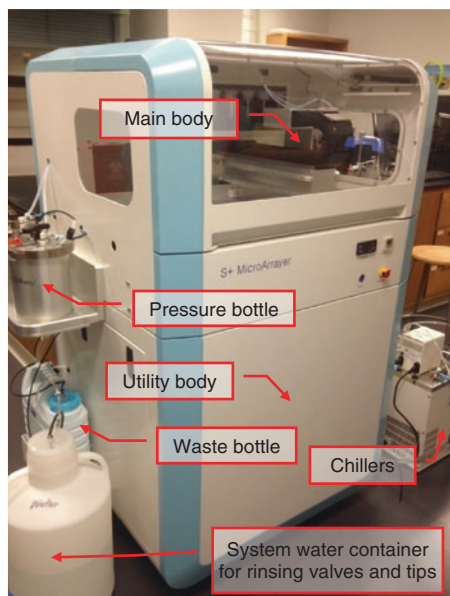


Fig. 2.1 The picture of S+ MicroArrayer

2.3.1 Main Body Components

The main body components are housed in a chamber where biological samples are loaded in a 96-well plate and printed on a micropillar/microwell chip at controlled temperature and humidity. It mainly consists of a robotic arm, a dispensing head with six solenoid valves and ceramic tips, six syringe pumps, a droplet inspection camera, a chip inspection camera, two chip-loading decks, a well plate deck, a vacuum pump, a humidifier, a water bath with a sonicator, and a waste drainage basin (Fig. 2.3).

- **Dispensing head unit**

The dispensing head unit of the microarray spotter is mainly responsible for moving and printing biological samples in accurate volumes and positions. It consists of a chip alignment inspection camera, six solenoid valves connected to syringe pumps through tubing, and six ceramic tips (Fig. 2.4). It is capable of moving X, Y and Z directions, hence aspirating biological samples from 96-well/384-well plates and dispensing in different patterns on the micropillar/microwell chip.

- **Solenoid valves:** They are the main component behind microarray bioprinting technology, which function on the principle of electromagnetic induction. The voltage applied to the valves, creates a magnetic field that forces the gate to open and close. The syringe pumps maintaining the pressure help the samples to flow when the gate is open. Each solenoid valve can be controlled individually.

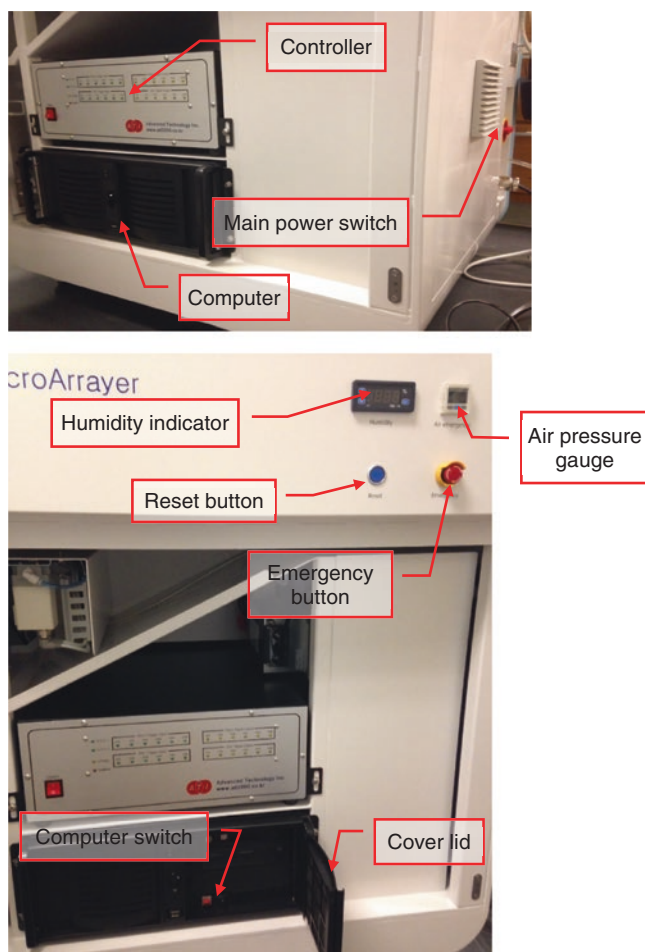


Fig. 2.2 The utility body components of S+ MicroArrayer

- **Ceramic tips:** They are necessary to dispense small droplets of biological samples when the gate is open. They are made of an inert material for rinsing with alcohol and sonication, hence preventing contamination while several biological samples are printed.
- **Chip alignment inspection camera:** It checks the position of micropillar/microwell chip on the chilled chip-loading deck for accurate sample dispensing.
- **Syringe pumps:** The main role of the six syringe pumps is to maintain pressure when the samples need to be aspirated or dispensed. When the gates of the six solenoid valves open to dispense the samples, the positive pressure main-

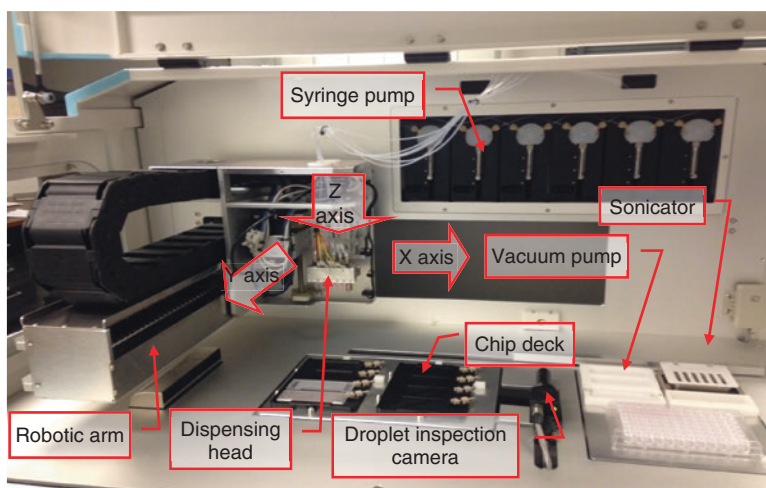


Fig. 2.3 The main body components of S+ MicroArrayer

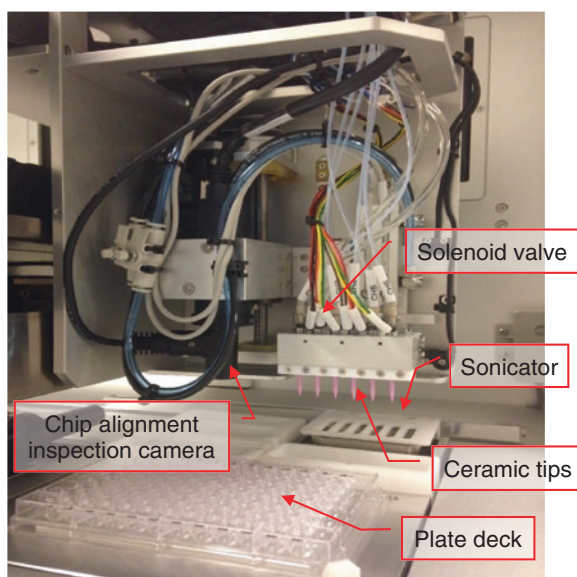


Fig. 2.4 The dispensing head unit of S+ MicroArrayer

tained by the syringe pumps forces the samples out on the micropillar and microwell chips.

- **Droplet inspection camera:** There are two cameras in the main body of the microarray spotter. The chip alignment inspection camera is installed in the dispensing head, and the droplet inspection camera is placed beside the chip-loading deck, which is used to optimize dispensing parameters such as air

pressure and solenoid valve open time. When sample droplets are dispensed on a hydrophobic plastic strip, the droplet inspection camera takes pictures of the droplets and calculate dispensing volumes at the setting by measuring the height and diameter of the droplet. The dispensing setting and actual dispensing volume can be compared for parameter optimization.

- **Water bath with a sonicator and a vacuum pump:** These two components are sequentially used to clean the tips and tubing that hold the samples between aspiration and dispensing of samples. The water bath with a sonicator is responsible for rinsing the surface of the tips and breaking down any bigger particles that tend to settle and clog the tips using ultrasound. The vacuum pump is responsible for drying the tips to prevent sample carry-over.

2.3.2 Externally Connected Components

- **Pressure bottles:** One pressure bottle contains water for sample printing and the other bottle holds 70 % ethanol for rinsing and sterilization (Fig. 2.5). The tubing has to be filled with water to transfer energy from the syringe pumps and push the samples to flow for printing. The bottles are pressurized with air to supply water and alcohol.
- **Chillers:** There are two chillers equipped in the microarray spotter, one for the dispensing head for printing temperature-sensitive samples and the other for the chip-loading decks for preventing spot drying, the 96-well plate deck, and the two pressure bottles (Fig. 2.6).
- **Waste bottle:** It holds wastewater generated by rinsing and washing the solenoid valves, the ceramic tips, and the tubing.

Fig. 2.5 The picture of the two pressure bottles containing distilled water and 70 % ethanol

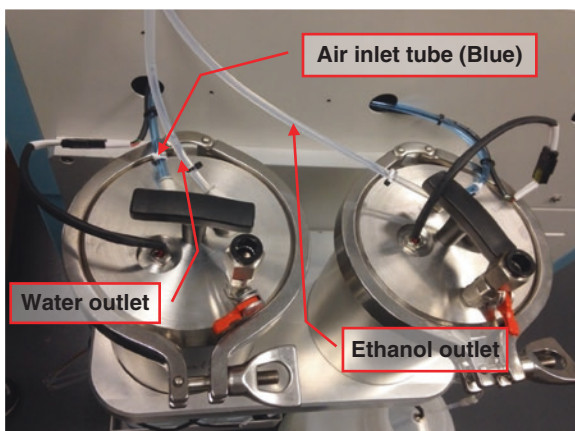
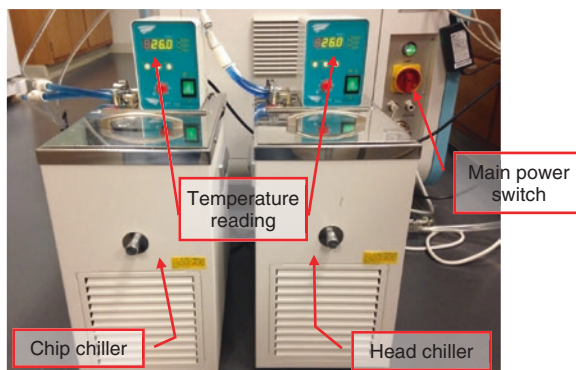


Fig. 2.6 The picture of the two chillers for preventing water evaporation on the chip and printing temperature-sensitive samples



2.4 General Precautions for Operating S+ MicroArrayer

- The S+ MicroArrayer uses surface cooling and condensation so that it requires low humidity setting. In particular, water condensation on the moving part (not on the chip-loading deck) or the circuit board would be detrimental. **Do not increase relative humidity higher than 60 % at room temperature.** In addition, maintain the temperature of the chip-loading deck between 4–10 °C to retard evaporation of water in spots on the chip. **It is extremely important to avoid excess water condensation on the bottom layer (e.g., BaCl₂/PLL spots) to prevent spot detachment.**
- Unlike MicroSys and PixSys microarray spotters, there is no vacuum applied to hold the chips on the S+ MicroArrayer. **Before spotting, make sure that all chips lie flat on the spring-loaded chip deck and no obstacles are on the work place.** It is the main cause of the ceramic tips crashed.
- **All ceramic tips should be positioned inside of 96-wells when aspirating samples.** If any one of the tips left outside of the 96-well plates when aspirating due to wrong programming, then they will be broken. **Always use the same kind of the 96-well plates to avoid tip crashing due to Z height difference in 96-wells.**
- Washing solenoid valves and ceramic tips with alcohol is essential to remove air bubbles prior to sample dispensing. **After alcohol washing, thorough rinsing solenoid valves and ceramic tips with water is necessary to avoid enzyme deactivation or cell death due to remaining alcohol.**
- **Do not change Z levels randomly to prevent the ceramic tips crashed into the chip surface.** The typical distance between the chip surface and the tip is approximately 0.7 mm.
- Always check a streamline of water while rinsing solenoid valves. If the streamline is deflected from the vertical or water is beaded up at the ceramic tip end, clean the ceramic tip with sonication. It is highly important to stop working with a clogged tip immediately.

- Solenoid valves can be used to print viscous solutions, but may not be suitable for spotting organic solvents except alcohols because of incompatible plastic parts in the solenoid valve. Aspirating DMSO with solenoid valves for a short period of time will be okay. When compounds in DMSO is diluted with solenoid valves, use a 384-well plate, and do not aspirate more than 20 μL of samples.
- **Do not attempt to print spontaneously gelling materials in any case.** In case of printing cells in Matrigel, always maintain the dispensing head at low temperature (4 $^{\circ}\text{C}$) to prevent the gelation of Matrigel inside of the solenoid valves while printing. **An extensive rinse of tubes and solenoid valves with ice-cold water is critical before and after printing Matrigel solution.**
- Keep in mind that there is no “space” allowed when saving a file name, and do not use special characters as well.
- If you have a new operational file made, test it without ceramic tips installed first. Do not install the expensive tips for testing unknown work files.

2.5 Daily Operations of S+ MicroArrayer

Now that we understand the mechanical components of the microarray spotter, it is important to know how to control these components and make them work as a whole. ezAOI is a customized software developed to operate S+ MicroArrayer (Fig. 2.7). This user interface helps us to control the microarray spotter and print

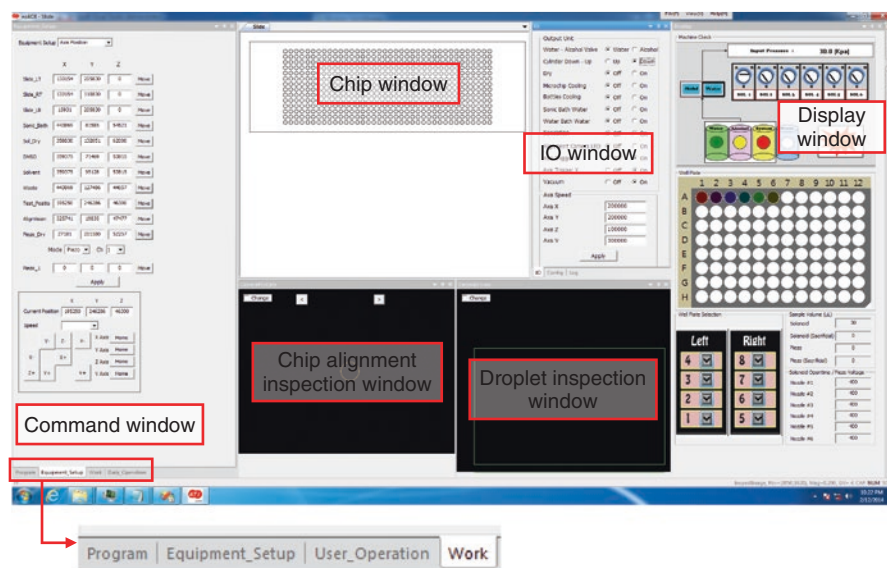


Fig. 2.7 The main screen of ezAOI. The Command window has four groups—Program, Equipment setup, User operation, and Work

samples on the micropillar and microwell chips in distinctive patterns. The main screen that pops up displays six windows, including command window, slide window, IO window, display window, chip alignment inspection window, and droplet inspection window. The command window in itself has four tabs, including program, equipment setup, user operation, and work. Each of these tabs has different functions, which allow us to program the microarray spotter more efficiently. For daily operation of the microarray spotter, follow the protocols provided below.

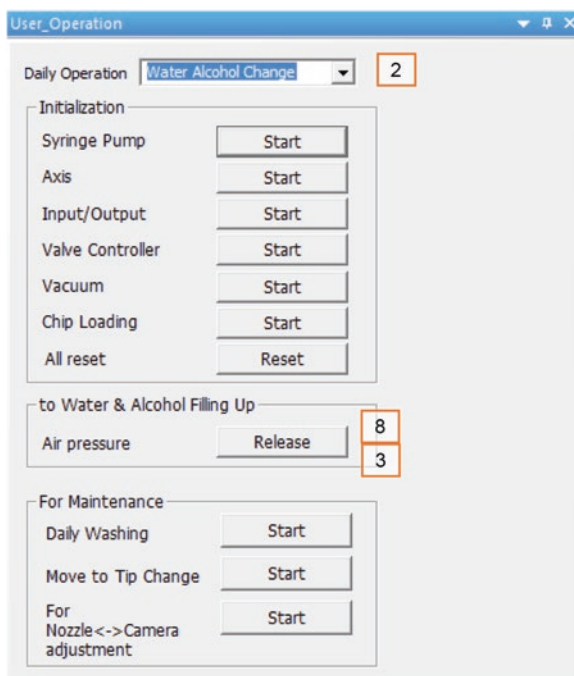
2.5.1 Turning ‘ON’ the System

1. Open the air cylinder (or in-house air valve) and maintain the pressure of compressed air at 100 psi.
2. Turn on the chiller. ***Note: For most of applications, there is no need to turn on the humidifier because of surface cooling. Maintain the temperature of the chip-loading deck between 4–10 °C to reduce evaporation of water in spots on the chip. It is extremely important to avoid excess water condensation on the bottom layer to prevent spot detachment.***
3. Turn on the external switch in the utility body.
4. Turn on the computer and the monitor.
5. Prior to running the ‘ezAOI’, push ‘Reset button’ and reset XYZ coordinates. ***Note: This step is essential to avoid malfunctioning of the microarray spotter. Do not skip this step!***
6. Run the ‘ezAOI’ software. The software will initialize the system automatically when running. ***Note: Make sure that no obstacles are on the work place to avoid the robotic arm crashing.***

2.5.2 Refilling the Pressure Bottles

1. Select the ‘User Operation’ window.
2. Go to ‘Water Alcohol Change’ in the ‘Daily Operation’ box (Fig. 2.8).
3. Click ‘Release’ in the ‘Air pressure’ box to release air pressure in the pressure bottles.
4. Turn the orange knob connected to the pressure bottles from ‘S’ to ‘O’ position, which releases the pressure inside.
5. Open the lid of the pressure bottles and fill the pressure bottles with distilled water and 70 % ethanol. ***Note: Make sure to have no precipitates at the bottom of the pressure bottles. Dust and precipitates can be the source of solenoid valve and tip clogging. Occasionally, the pressure bottles have to be cleaned and sterilized. To minimize microbial contamination, distilled water with Clear Bath® or sterilized distilled water can be used.***

Fig. 2.8 The screen of 'User Operation' > 'Water Alcohol Change'

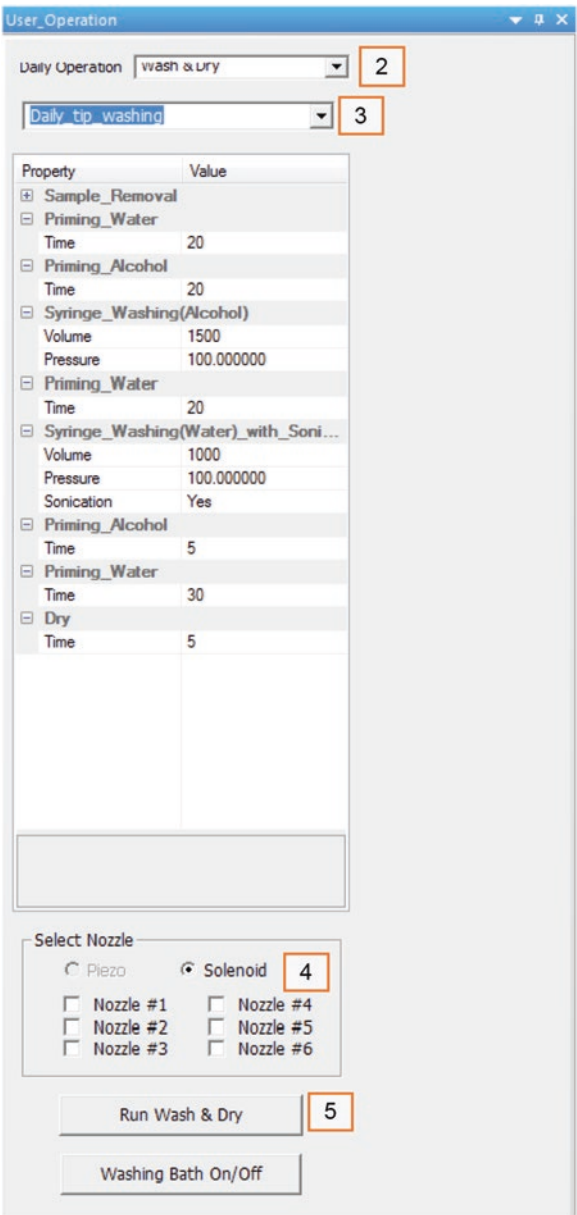


6. Close the lid of the pressure bottles carefully. ***Note: Make sure that the 'O' ring is properly placed before placing the lid.***
7. Turn the orange knob of the pressure bottles from 'O' to 'S' position, which close the pressure bottles completely.
8. Click 'Initialize' of the 'Air pressure' box in the 'Water & Alcohol Filling Up' box to apply air pressure in the bottles.

2.5.3 Washing Tubes, Solenoid Valves, and Ceramic Tips with Ethanol and Water

1. Select the 'User Operation' window.
2. Select 'Wash & Dry' in the 'Daily Operation' box (Fig. 2.9).
3. Select the wash and dry sequence you generated.
4. Select the number of solenoid valves you want to wash and dry in the 'Select Nozzle' box.
5. By clicking the 'Run Wash & Dry' button, run the wash and dry sequence. ***Note: Washing tubes and solenoid valves with alcohol is essential to remove air bubbles prior to sample dispensing. After alcohol washing, thorough rinsing tubes and solenoid valves with water is necessary to avoid enzyme deactivation or cell death due to remaining alcohol. An extensive rinse of tubes***

Fig. 2.9 The screen of ‘User Operation’ > ‘Wash & Dry’

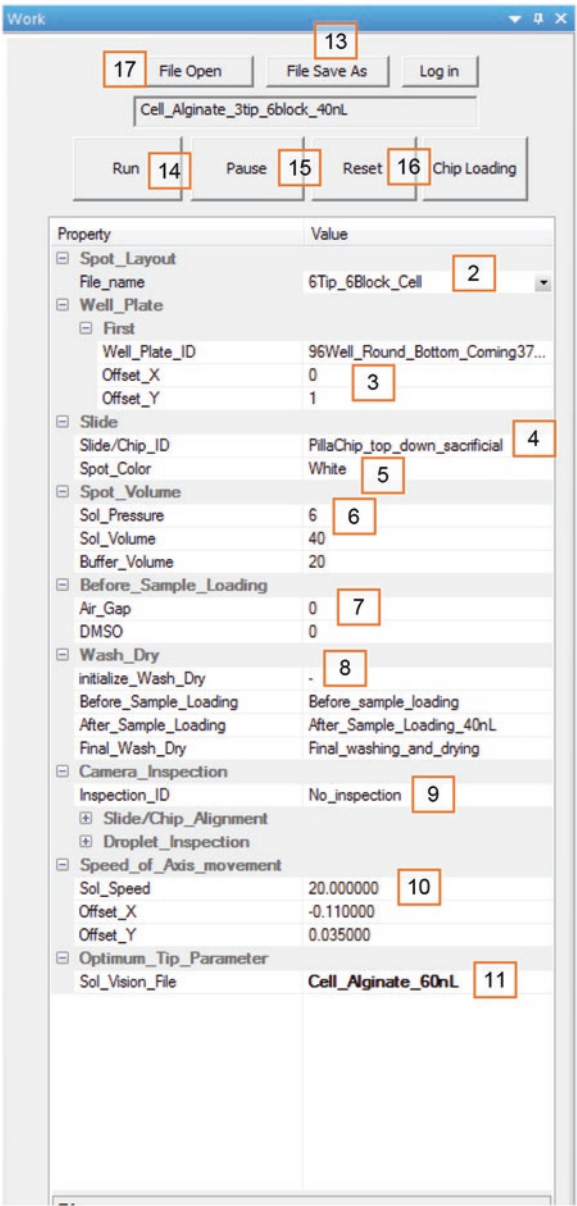


- and solenoid valves with ice-cold water is critical before and after printing Matrigel solutions.*
6. Check a streamline of water while washing. *Note: If the streamline is deflected from the vertical or water is beaded up at the tip end, clean the ceramic tip with sonication.*

2.5.4 Dispensing Samples on the Micropillar/Microwell Chips with a Work File

- 1. Select the ‘Work’ window (Fig. 2.10).
- 2. Select the layout of spots on the chip in ‘Spot Layout’.

Fig. 2.10 The screen of ‘Work’ window



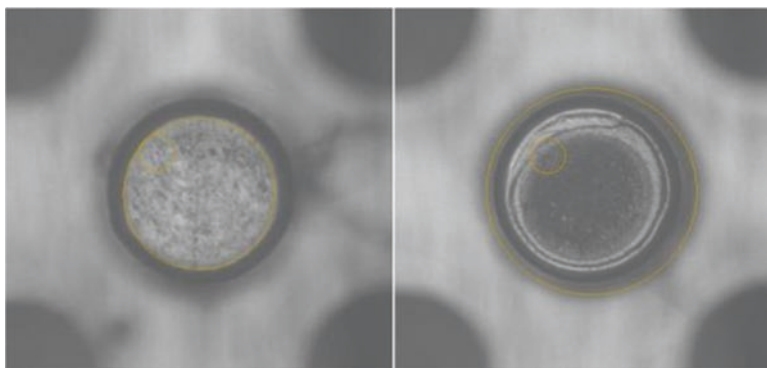


Fig. 2.11 The images of the original micropillar shown as “white spot” (left) and the micropillar with the BaCl_2 -PLL bottom layer shown as “black spot” (right)

3. Select the well plate used for sample loading and aspiration in ‘Well Plate ID’. *Note: It is extremely important to use the same kind of the 96-well plates always to avoid tip crashing due to Z height difference in 96-wells.* Enter the offset values in the X and Y directions to designate the location of samples in 96-wells. The exact location of sample wells will be appeared in the schematic of the well plate in the ‘Display’ window.
4. Select the type of a chip in ‘Slide/Chip ID’ onto which samples are dispensed. The schematic of the chip selected will be appeared in the ‘Chip’ window.
5. Select the color of spots on the chip under the camera in ‘Spot Color’. Typically, it is white when nothing is dispensed on the chip or black when BaCl_2 /PLL is dispensed (Fig. 2.11). *Note: This color selection is necessary to automatically identify the location of the chip with the chip alignment inspection camera.*
6. Enter the air pressure used for sample spotting and the desired droplet volume for solenoid valves in ‘Spot Volume’. *Note: The typical air pressure used is 6 kilopascal (kPa) for 40–950 nL droplets.*
7. Enter the air gap between the sample and water. *Note: The typical air gap used is 0 μL for 40–150 nL droplets and 20 μL for 700–950 nL droplets. For accurate sample spotting, small or no air gap is allowed for small dispensing volumes.*
8. Select the wash and dry sequences you want before and after sample dispensing in ‘Wash Dry’.
9. Select ‘No inspection’ in ‘Camera Inspection ID’. *Note: For rapid sample dispensing, we typically skip camera inspection of droplets. Camera inspection of sample spotting is necessary to optimize dispensing parameters at first.*
10. Enter the speed of the axis movement for solenoid valves in ‘Speed of Axis Movement’. It is typically 10–20 mm/s.
11. Open the optimum dispensing parameters of solenoid valves obtained from the vision inspection in ‘Optimum Tip Parameter’. *Note: Manually change the open time, if needed. Optimum dispensing parameters will not be changed for each sample and droplet volume used, unless the solenoid valves are broken*

Table 2.1 Typical dispensing parameters for solenoid valves

Samples printed	Spot volume (nL)	Air pressure (kPa)	Air gap (μL)	Open time (μs)
BaCl ₂ /PLL	60	6	0	600
Cells in alginate	60	6	0	600
Enzymes in Matrigel	100	6	0	600
Virus in growth media	320	6	5	4500
Growth media	950	6	20	12,000

or contaminated. Use predetermined parameters without droplet inspection to reduce the dispensing time. See Table 2.1 for typical dispensing parameters, including air pressures, air gaps, and solenoid valve open times.

12. Place the chips on the chip-loading deck and select the location of chips in the ‘Display’ window onto which samples are dispensed. **Note: It is extremely important not to leave the chip unattended long time on the chilling chip deck because excess water condensation on the bottom layer (e.g., BaCl₂/PLL spot) on the chip will facilitate cell spot detachment.**
13. Click the ‘File Save’ button and enter a file name to save all information. **Note: There is no space and special character allowed in the file name.**
14. Add proper amounts of samples in the designated 96-wells shown in the ‘Display’ window, place the 96-well plate on the deck, and then dispense samples on the chips by clicking the ‘Run’ button.
15. Click the ‘Pause’ button to pause the work process. **Note: In case of emergency such as robotic arm and tip crashing, push the red ‘Emergency’ button to stop the process (Fig. 2.2).**
16. Click the ‘Reset’ button to reset the work process after clicking the ‘Pause’ button.
17. When dispensing samples with a saved work file, click the ‘File Open’ button, select the work file, place the chips on the chip-loading deck, select the location of desired chips to print in the ‘Display’ window, add proper amounts of samples in the designated 96-wells, place the 96-well plate on the plate deck, and then click the ‘Run’ button to print the samples on the chips.

2.5.5 Replacing Solenoid Valves and Ceramic Tips

In case of ceramic tips clogging and solenoid valves malfunctioning, ceramic tips and solenoid valves have to be removed and replaced. Prior to removing tips and solenoid valves, run ‘Solenoid Fix’, force to dispense large droplets multiple times (typically 500 times), and rinse solenoid valves with water and sonication.

1. Select the ‘User Operation’ window.
2. Select ‘Wash & Dry’ under Daily Operation options (Fig. 2.12).
3. Select ‘Solenoid Fix’ and select all the nozzles from 1 through 6.

Fig. 2.12 The screen of 'User Operation' > 'Wash & Dry'

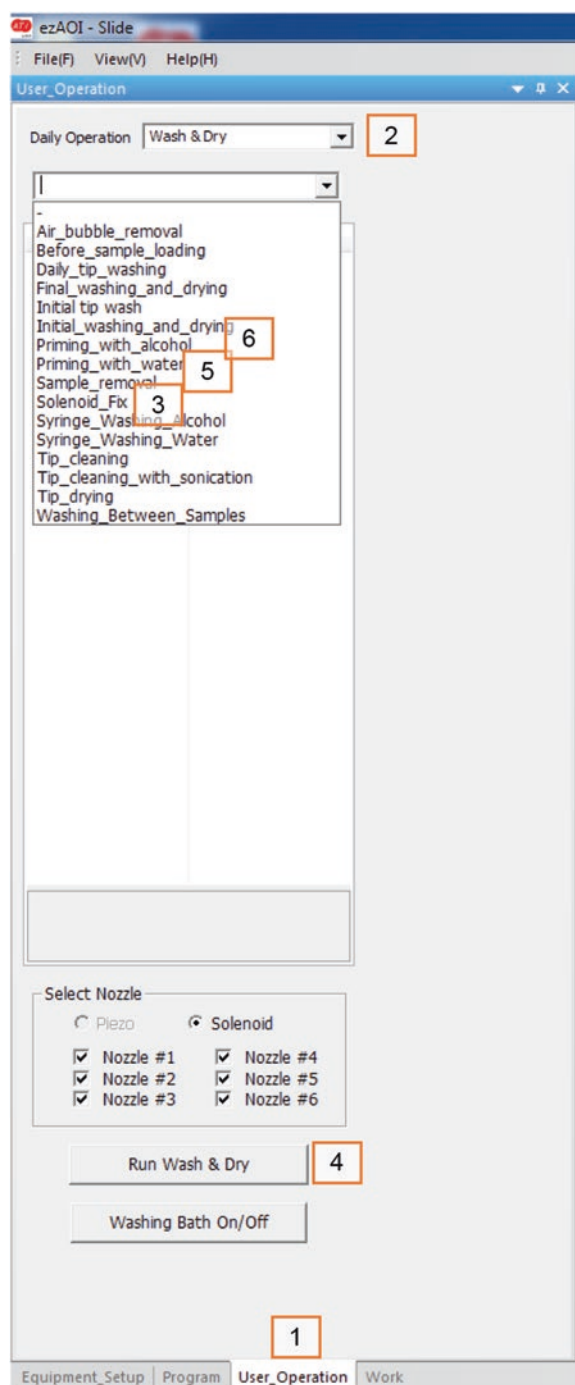
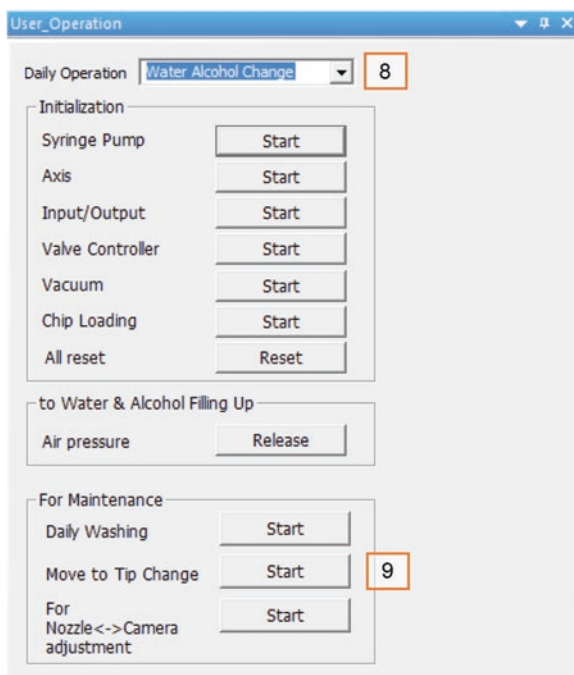


Fig. 2.13 The screen of 'User_Operation' > 'Move to Tip Change'



4. Execute 'Run Wash & Dry'.
5. Remove dust by running 'Priming with water'.
6. In case of compound precipitates clogging solenoid valves, run 'Priming with alcohol' and then execute 'Run Wash and Dry'.
7. Repeat Steps 3–6 to remove the clogs from ceramic tips and/or solenoid valves.
8. If the clogging problem persists, select the 'User Operation' window and select 'Water Alcohol Change'.
9. Select 'Move to tip change' under the 'For maintenance' tab (Fig. 2.13). The robotic arm moves forward so that the dispensing head with ceramic tips and solenoid valves is accessible for repair.
10. Unscrew the two screws that hold the clogged solenoid valve or the clogged tip between them (Fig. 2.14).
11. Carefully pull out the malfunctioning solenoid valve with the ceramic tip from the metal block by holding its connecting wires.
12. Separate the ceramic tip from the solenoid valve by gently pulling the tip.
13. Hold the opening of the ceramic tip across a spray bottle with ethanol and flush the tip with ethanol to see if ethanol can pass through the orifice without any obstruction.
14. In case of experiencing a clog, sonicate the ceramic tip until dust is removed. If the clogging problem persists, replace the ceramic tip.

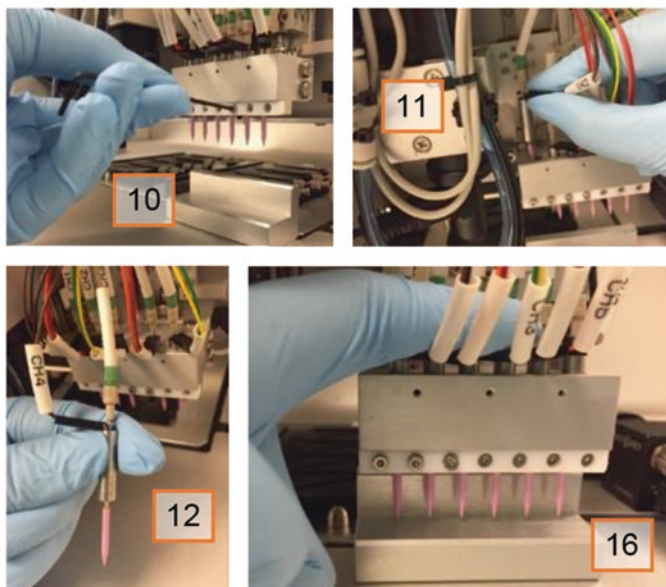


Fig. 2.14 The pictures of the dispensing head for replacing ceramic tips and solenoid valves

15. Attach a new ceramic tip to the solenoid valve, place them accordingly, and connect the wire back in.
16. Before putting the screws in, make sure that the ceramic tips are leveled on the metal block.
17. If the new ceramic tip still doesn't print samples properly, the solenoid valve is clogged, thus replacing it with a new one. ***Note: Run 'Daily Washing' after replacing solenoid valves and before printing samples.***

2.5.6 Turning 'OFF' the System

1. To avoid potential contamination issues, rinse tubes and solenoid valves with ethanol and water thoroughly by running 'Daily Washing'.
2. Close 'ezAOI' software.
3. Turn off the computer and the monitor.
4. Turn off the external power switch in the utility body.
5. Turn off the chillers. ***Note: Make sure to turn off the chillers to avoid excess water condensation on the chip-loading deck and the dispensing head, causing a short circuit by water.***
6. Close the air cylinder (or in-house air valve). ***Note: Do not close the air cylinder while 'ezAOI' is on.***

2.6 Detailed Programming for Normal Operation

These sections are prepared for advanced users who want to know how to program the operation of S+ MicroArrayer.

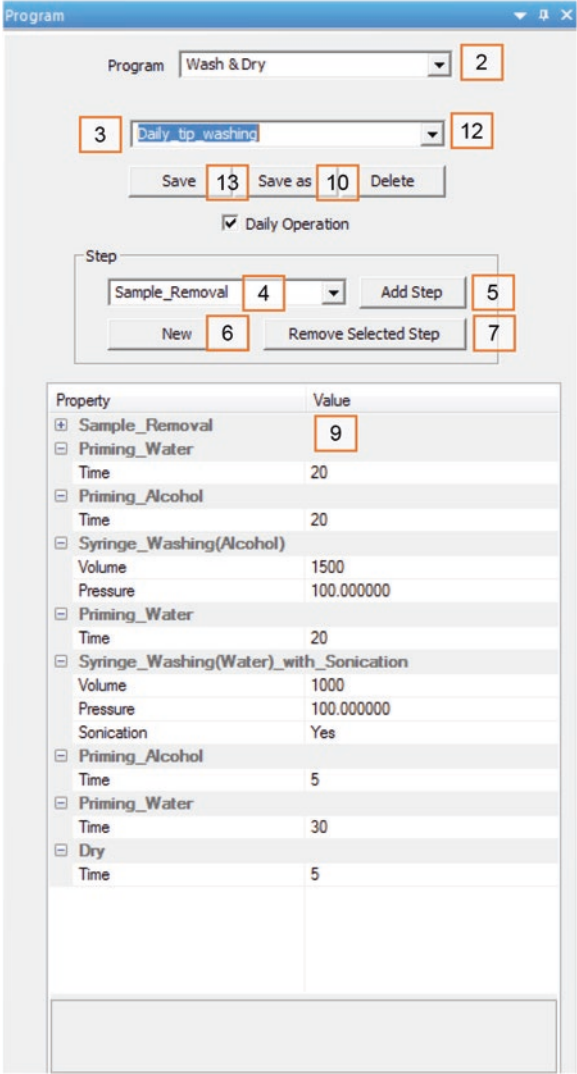
2.6.1 *Generating Wash and Dry Sequences*

1. Select the 'Program' window.
2. Select 'Wash & Dry' in the 'Program' box (Fig. 2.15).
3. In the second tab select the wash and dry sequence you made in the 'Daily Operation' window.
4. Select the process you want to add in the 'Step' box.
5. Click 'Add Step' in the 'Step' box.
6. If you want to start all over, click 'New' in the 'Step' box.
7. If you want to delete one of many wash and dry processes you generated, select the process you want to delete, and then click 'Remove Selected Step'.
8. Repeat Steps 4 and 5 according to the wash and dry sequences you want to generate.
9. Change numbers and options in the 'Value' column as you need (Fig. 2.15 & Table 2.2).
10. Click the 'Save as' button to generate the wash and dry sequence.
11. Enter a file name. ***Note: There is no space and special character allowed in the file name.***
12. If you want to modify the wash and dry sequence you generated, select the file name in the box above the 'Save as' button and repeat Steps 8 and 9 as you need.
13. Click the 'Save' button to overwrite the changes in the same file.

2.6.2 *Defining Well Plates*

1. Select the 'Program' window.
2. Select 'Well Plate' in the 'Program' box.
3. Select the type of a well plate, 96 or 384 (Fig. 2.16).
4. Enter the well layout such as the number of wells and the well-to-well distance (in mm) in X and Y directions. Refer to the 96-well plate figure for correct X and Y directions (Fig. 2.17).
5. Select the 'Equipment Setup' window.
6. Select 'Axis Position' in the 'Equipment Setup' box (Fig. 2.18).
7. Move X, Y and Z axes to locate the first solenoid valve/ceramic tip at the bottom of the A1 well (i.e., the tip locating approximately 3 mm above from the bottom of the well) and then read the current position of X, Y and Z axes.

Fig. 2.15 The screen of 'Program' > 'Wash & Dry'



- Note: This will be the position of the first ceramic tip for aspirating samples. Do not change Z levels randomly to prevent the tips crashed into the well surface.*
8. Select 'Well Plate' in the 'Program' box again.
 9. Enter 'Well A1 Position' determined from Step 7.
 10. Enter minimum and maximum sample volumes allowed in either 96 or 384 wells.
 11. Click 'Save as' button to register well plate information.
 12. Enter a file name. *Note: There is no space and special character allowed in the file name.*

Table 2.2 Processes for the wash and dry sequences

Process	Description	Property	Unit
Sample removal	Syringe pumps are moving up to the zero position so that all samples are removed from the solenoid valves	–	–
Priming water	Tubes and solenoid valves are rinsed with water in the pressure bottle	Time	s
Priming alcohol	Tubes and solenoid valves are rinsed with 70 % ethanol in the pressure bottle	Time	s
Priming system water	Tubes and solenoid valves are rinsed with system water in the big container. This step is applied for solenoid valves only	Time	s
Syringe washing	Tubes and solenoid valves are rinsed with water by operating syringe pumps	Volume	μL
Syringe washing with sonication	Tubes and solenoid valves are rinsed with water by operating syringe pumps while sonicating tips immersed in the water bath	Volume	μL
		Sonication	Yes/No
Sonication	The outside of ceramic tips are cleaned by sonication in the water bath	Time	s
		Sonication	Yes/No
Dry	Ceramic tips are dried by vacuum	Time	s
Solvent wash	Tubes and valves are washed with a solvent in the solvent bath. Make sure not to use hydrophobic solvents. Only alcohols and DMSO are allowed	Volume	μL
Pre-dispensing	Prior to sample dispensing on the chip, aspirated samples are pre-dispensed in the waste drainage basin to equilibrate pressure difference	Droplet	Number

Fig. 2.16 The screen of ‘Program’ > ‘Well Plate’

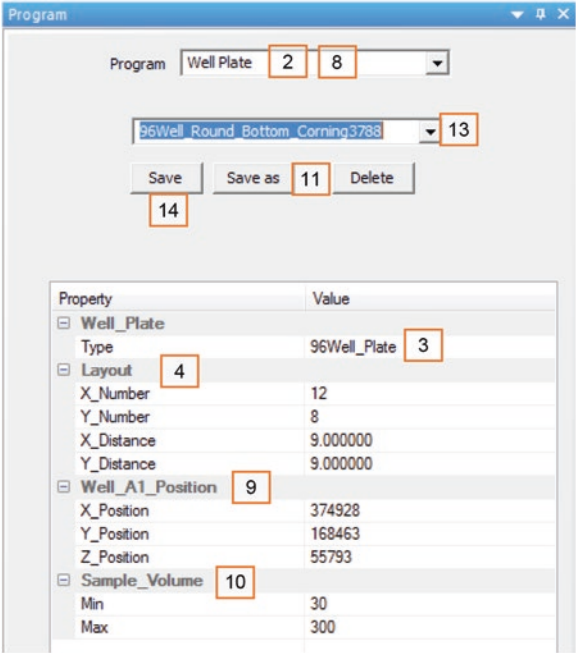
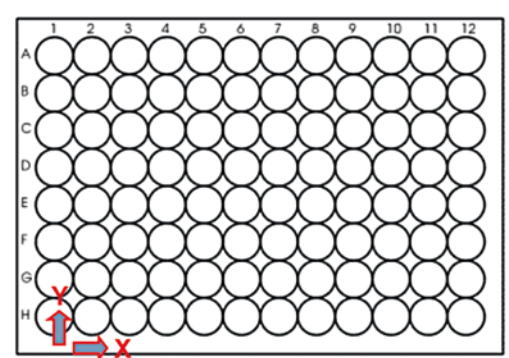


Fig. 2.17 The X and Y directions in the 96-well plate

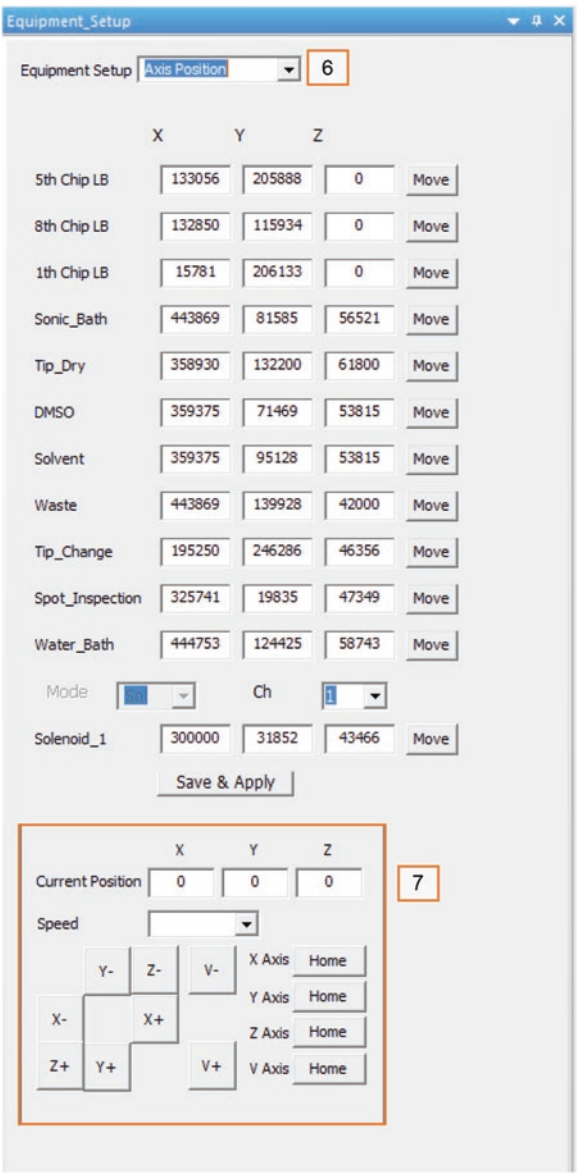


13. If you want to modify the well plate you registered, select the file name in the box above the 'Save as' button and repeat Steps 3, 4, 9, and 10.
14. Click the 'Save' button to overwrite the changes in the same file.

2.6.3 Registering Chips

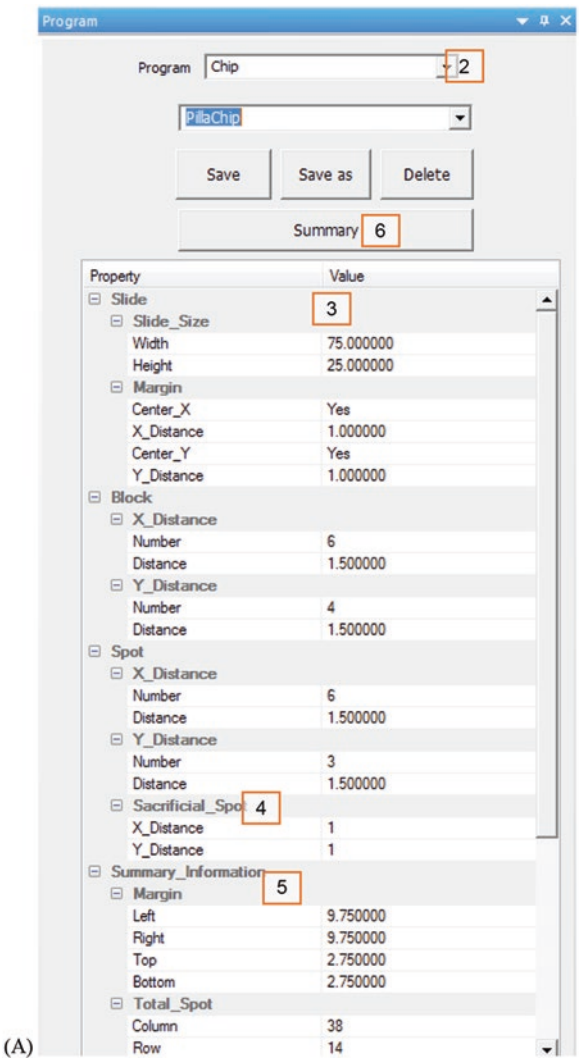
1. Select the 'Program' window.
2. Select 'Chip' in the 'Program' box (Fig. 2.19).
3. Enter the information of the chip such as a width (in mm), a height (mm), a margin (mm) from the top left corner, the number of blocks on the chip, a block-to-block distance (mm), the number of spots in each block, a spot-to-spot distance (mm) in X and Y directions. Refer to the slide specification figure for correct X and Y directions (Fig. 2.20).
4. Enter the number of sacrificial spots. For example, 'X distance' 1 means that there is each one of the sacrificial column inserted on the left and right side of the chip, whereas 'Y distance' 1 indicates that there is each one of the sacrificial row inserted on the top and bottom side of the chip.
5. Check the 'Summary information' of the chip such as the chip margin and the number of spots.
6. Click 'Summary' button to see the schematics of the chip you generated.
7. Select the 'Equipment Setup' window.
8. Select 'Axis Position' in the 'Equipment Setup' box (Fig. 2.21).
9. Move X, Y and Z axes to locate the ceramic tip approximately 0.7 mm above the chip surface and then read the current position of the Z axis.
10. Select 'Chip Inspection Camera' in the 'Equipment Setup' box.
11. Select the box next to 'Camera Live' in the 'Camera Inspection' box (Fig. 2.21).
12. Move X and Y axes to locate the align inspection camera above the chip surface. Move the Z axis to bring the camera into focus and then read the current position of the Z axis.

Fig. 2.18 The screen of 'Equipment Setup' > 'Axis Position'



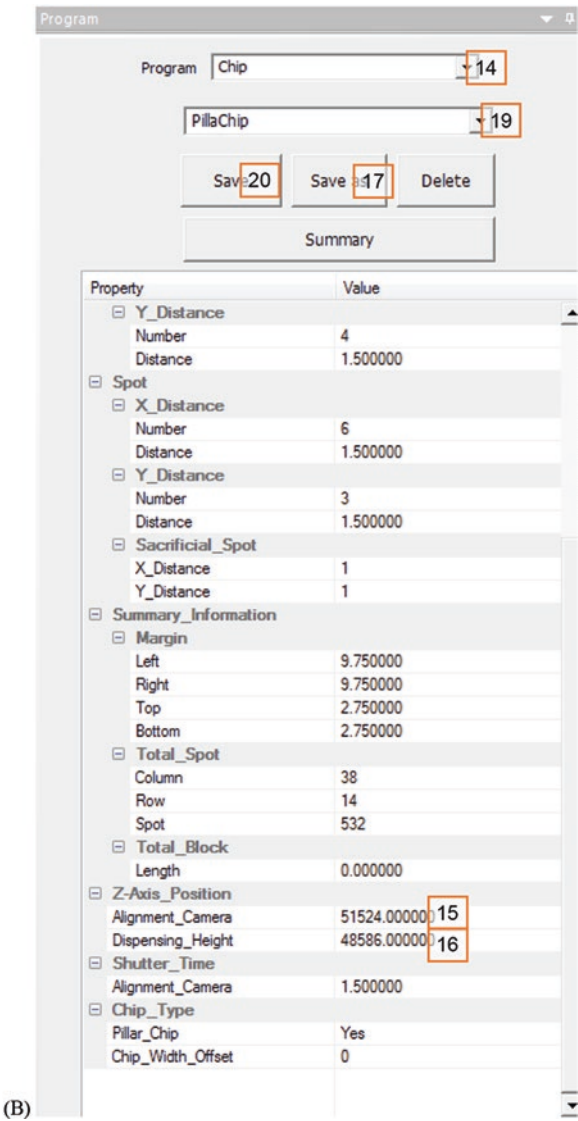
- 13. Deselect 'Camera Live' in the 'Camera Inspect' box.
- 14. Select 'Chip' in the 'Program' box again.
- 15. Enter the Z position of 'Alignment Camera' determined from Step 12 (Fig. 2.19).
- 16. Enter the Z position of 'Dispensing Height' determined from Step 9.

Fig. 2.19 The screen of ‘Program’ > ‘Chip’: (A) top and (B) bottom panel.



17. Click ‘Save as’ button to register the chip.
18. Enter a file name. **Note: There is no space and special character allowed in the file name.**
19. If you want to modify the chip information you registered, select the file name in the box above the ‘Save as’ button and repeat Steps 3, 4, 15, and 16.
20. Click the ‘Save’ button to overwrite the changes in the same file.

Fig. 2.19 (continued)



2.6.4 Registering Spot Layouts

1. Select the 'Program' window.
2. Select 'Spot Layout' in the 'Program' box.
3. Select the name of the chip you registered in the 'Slide/Chip' box (Fig. 2.22).
4. Check the chip information such as a margin, spot numbers, and a nozzle-to-nozzle distance.

Fig. 2.21 The screen of 'Equipment Setup' > 'Chip Inspection Camera'.

Equipment Setup Chip Inspection Camera 8 10

Camera - Nozzle Offset

Mode Sol Ch 1 Check

Offset X 36916 Y -12771

Dispence Height

Height(um) 43000 Update

Undetected error rate

Angle 0 Distance 0

Camera Inspection

☒ Camera Live 11 GV 140

Shutter 1.5

Method White Inspect

Current Position X Y Z

0 0 0 12

Speed

Y- Z- V- X Axis Home

X- X+ Y Axis Home

Z+ Y+ V+ Z Axis Home

V Axis Home

Save & Apply

Note: For safety reasons, the entire well will not be selected when any one of the nozzles is left outside of the well plate for aspiration.

10. Select the region of spots where the sample will be dispensed with the first solenoid valve/ceramic tip by clicking and dragging on the chip layout.
11. Repeat Steps 9 and 10 according to the spot layout you want to generate.
12. Select the box beside 'Set Sacrificial Spot' to designate a sample well for printing sacrificial regions. **Note:** Always the first solenoid valve/ceramic tip is used for spotting sacrificial regions.
13. Select the sample well for spotting sacrificial regions.
14. Click 'Close' button when done.
15. Click 'Save as' button to register the spot layout.

Fig. 2.22 The screen of
'Program' > 'Spot Layout'

16. Enter a file name. *Note: There is no space and special character allowed in the file name.*
17. If you want to modify the spot layout you registered, click the 'Open' button and select the file name and repeat Steps 3 through 14.
18. Click the 'Save' button to overwrite the changes in the same file.

2.6.5 Optimizing Dispensing Parameters Using Vision Inspection

1. Select the 'User Operation' window.
2. Select 'Volume Check Vision' in the 'Daily Operation' box (Fig. 2.24).
3. Select the number of nozzles you want to inspect with the camera in the 'Nozzle' box.
4. Select the well plate used and indicate the location of a 96 or 384 well containing a test sample in the 'Sample Location' box. The first solenoid nozzle will be located in the well indicated, and the locations of the other solenoid

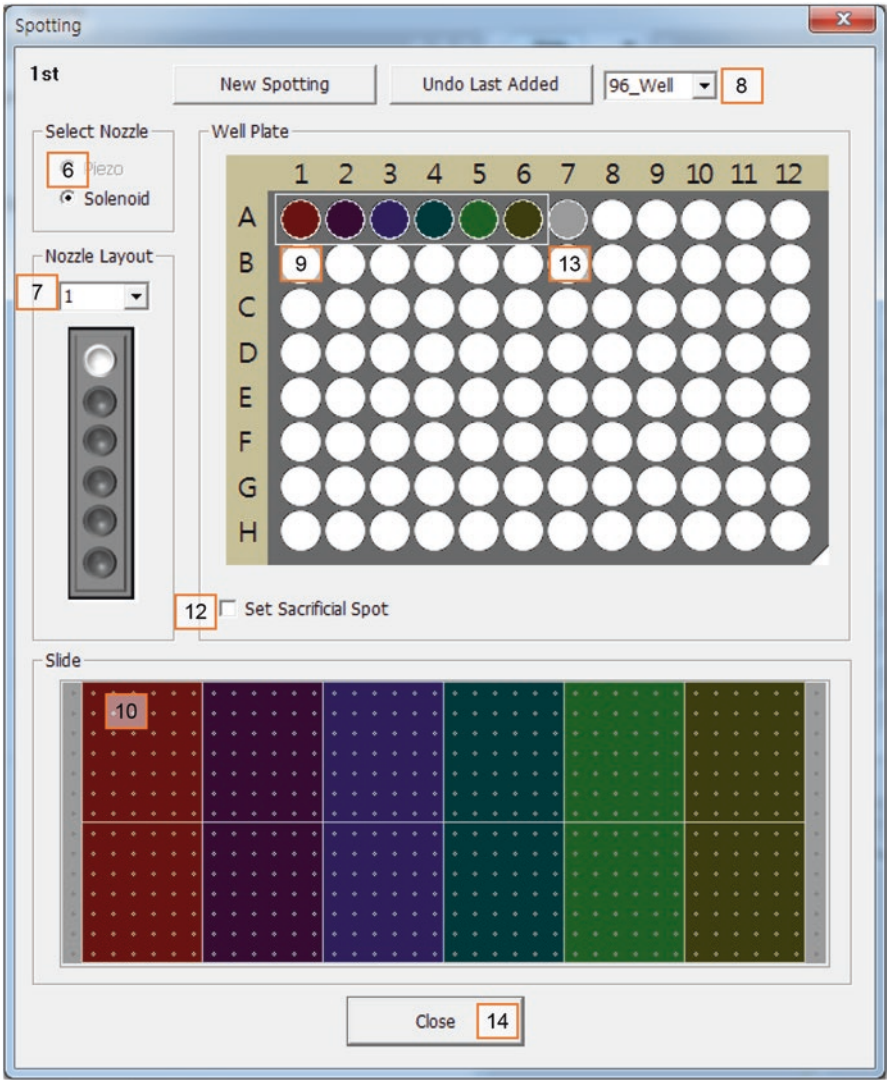


Fig. 2.23 The screen of ‘Program’ > ‘Spot Layout’> ‘1st Spotting Layout’

- valves/ceramic tips will be determined automatically according to the solenoid valves selected.
5. Enter the air pressure used for sample spotting and the volume of the sample loaded for inspection in the ‘Pressure & Sample Aspiration Volume’ box. The typical air pressure used is 6 kPa for 60–950 nL droplet.
 6. Enter aspiration conditions such as air gap and pre-pressurization in ‘Aspiration Condition Parameter’. Refer to Table 2.1.

Fig. 2.24 The screen of ‘User Operation’ > ‘Vision Inspection’

The screenshot shows the 'User Operation' window with the following sections and controls:

- Daily Operation:** A dropdown menu set to 'Volume Check Vision' (callout 2).
- Buttons:** 'Open' (callout 13), 'Save as' (callout 11), and 'Save' (callout 14).
- Solenoid Valve:** A 'Mode' dropdown set to 'Sol' (callout 3) and a 'Valve' section with checkboxes for #1 through #6.
- Sample Location:** A 'Well Plate ID' dropdown set to '4' (callout 4) and 'Location' dropdowns for 'X' and 'Y'.
- Pressure & Sample Aspiration Volume:** A section (callout 5) with 'Pressure' set to 0 (kPa) and 'Sample' set to 0 (uL).
- Aspiration Condition Parameter:** 'Air Gap (uL)' set to 0 (callout 6) and 'Pre-Pressurization (uL)' set to 0.
- Before/After:** 'Before' dropdown set to '7' (callout 7) and an 'After' dropdown.
- Manual Dispensing Setup:** 'Dispence Volume(nl)' set to 0 (callout 8), 'Open Time' set to 0, and 'Number of Spot' set to 0. It includes buttons for 'Set Open Time', 'Dispense' (callout 9), and 'Stop'.
- Stage for Spot Inspection:** 'Spot Distance (mm)' set to 4, 'Max Stage Length (mm)' set to 240, and 'Stage Move' buttons for 'Home' and 'Max Position'.
- Open Time (usec):** A section (callout 10) with a value of 10 and a table for solenoid open times:

Solenoid	Open Time (usec)
Solenoid #1	0
Solenoid #2	0
Solenoid #3	0
Solenoid #4	0
Solenoid #5	0
Solenoid #6	0

- 7. Select tip rinsing conditions in water before and after sample loading prior to sample dispensing in the ‘Before’ and ‘After’ boxes.
- 8. Enter the droplet volume for inspection in ‘Dispensing Volume’, the open time of the solenoid valves selected in ‘Open Time’, and the number of spots dispensed to test the conditions in ‘Number of Spot’ in the ‘Manual Dispensing Setup’ box. *Note: If the disparity between the droplet volume set up and the average volume of the measured droplets at a certain condition is less than the desired CV value (typically 5 %), then the vision inspection will be successfully finished.*

9. After loading test samples, click the ‘Dispense’ button to test the dispensing parameters manually with the camera.
10. The open time of each solenoid valve will be updated when the vision inspection is successfully completed; otherwise 0 will be reported in the ‘Open Time’ box. Repeat Steps 3–9 when failed.
11. After finishing all solenoid valve inspection, click ‘Save as’ button to save the optimum dispensing parameters.
12. Enter a file name. **Note: There is no space and special character allowed in the file name.**
13. If you want to modify the optimum dispensing parameters you saved, click the ‘Open’ button, select the file name, and then repeat Steps 3 through 11.
14. Click the ‘Save’ button to overwrite the changes in the same file.

2.7 Summary

Microarray bioprinting is an important advancement in the field of miniaturized assay development and HTS. In this chapter, we briefly introduced the advantages of solenoid-driven bioprinting over other printing technologies in terms of printability of cells, the range of printing volumes, and printing precision. We also went over general precautions that have to be taken when printing biological samples with a microarray spotter. Finally, detailed protocols on how to operate the S+ MicroArrayer for printing various biological samples and how to program specific functions for advanced users are provided. These protocols will ensure user safety, proper operation of the equipment, and consistent printing results. Although we discussed only the S+ MicroArrayer in this chapter, the principle of operating and troubleshooting S+ MicroArrayer can be applicable to any solenoid-driven microarray spotters.

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Microarray Bioprinting Technology

Fundamentals and Practices

Lee, M.-Y. (Ed.)

2016, XIII, 175 p. 120 illus., 114 illus. in color.,

Hardcover

ISBN: 978-3-319-46803-7