SmartArrayer™ Series

Microarray Spotter

User Manual

Version 4.0

CapitalBio SmartArrayer^{TM} Software End User License Agreement

IMPORTANT-PLEASE READ CAREFULLY:

This End User License Agreement (hereinafter referred to as "Agreement") is a valid and binding agreement between CapitalBio Corporation (hereinafter referred to as "CapitalBio") and you (either an individual or a single entity) for the use of the SmartArrayer^{TM} Software System (hereinafter referred to as "System").

This Agreement is an indispensable part of the System documents. You have the right to require the whole documents from the seller of this System in order to protect your rights and interests.

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CapitalBio warrants that within ninety (90) days after the selling of this System, this System shall not have any material defects or technical defects, subject to the condition that this System is used in its normal manner of operations.

In the event that a defect is confirmed by CapitalBio, CapitalBio only shall be responsible for replacing the defective software, This is the only remedy available for you.

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Subject to the scope of applicable laws specified in this Agreement, in no event shall CapitalBio and its agents and sales persons be liable for any special, incidental, indirect, or consequential damages and/or losses (including but not limited to profit loss, operation interruption, business interruption, business information loss and any other pecuniary loss) arising from the use of this System or inability to use this System.

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This system may only work and function with properly under correct usage.

CapitalBio's total liability to you for any damages and/or losses for any causes whatsoever under the terms of this Agreement will be limited to the actual amount paid by you for this System.

Termination of License:

If you breach any terms or conditions of this Agreement, CapitalBio shall have the right to terminate the license at any time. If CapitalBio decides to terminate this license, you must immediately destroy (no more than three (3) days after the termination) this System and any copies of any documents of this System, or return them to CapitalBio.

Applicable Laws

This System shall be protected by the Copyright Law of the People's Republic of China and relevant international intellectual property protection treaties, which have been acceded to and enforced in the People's Republic of China.

This Agreement shall be governed by the law of the People's Republic of China.

If you have any question with respect to this Agreement, please contact CapitalBio:

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Chapter 0 Preface

0.1 Overview

In this chapter, the function, principles and series of CapitalBio SmartArrayer^{TM} microarray workstations will be introduced. Also, the contents of this manual, abbreviations and alternative names will be listed.

0.2 Contents of This Manual

Chapter	Description
Chapter 1. **Important Safety Information**	Provides crucial information to use the system safely. All SmartArrayer^{TM} users should read this chapter before installing and using the system.
Chapter 2. **Hardware Installation**	Provides SmartArrayer^{TM} hardware specifications and the step-by-step instructions for installation of the system.
Chapter 3. **Software Installation**	Provides instructions on how to install the SmartArrayer^{TM} application software and the device drivers.
Chapter 4. **Tutorial**	Provides instructions on how to prepare a microarray chip. To get detailed information, users can see the following three chapters: **Getting Started, System Configuration, Spotting or Dispensing**.
Chapter 5. **Getting Started**	Provides instructions on how to run the spotting system for a test immediately after the hardware and software installation.
Chapter 6. **System Configuration**	Provides instructions on how to configure the system parameters before spotting or dispensing.
Chapter 7. **Spotting or Dispensing**	Provides instructions on how to successfully do slide spotting or dispensing operations.
Chapter 8. **Additional Software Features**	Provides descriptions of additional software features.
Chapter 9. **Operation& Maintenance**	Provides information on routine maintenance for quality service.
Chapter 10. **Troubleshooting**	Provides answers to frequently asked questions and solutions to frequently encountered problems.

0.3 Functions and Series

0.3.1 Functions

CapitalBio SmartArrayer^{TM} series of microarray workstations are used for fabricating bio-molecular microarrays, spotting or dispensing several to tens of thousands of kinds of bio-chemical samples precisely onto set positions on preformed microarray chips.

CapitalBio SmartArrayer^{TM} series of microarray workstations, utilizing precise robotic motion

control technology to control the movement of X, Y, Z (and W) axes and move the printing pins or dispensing head to set positions to spot or dispense sample droplets and meet the requirements of high precision and high velocity printing for microarray fabrication.

Both Telechem pin printing technology and CapitalBio non-contact dispensing technology (patents pending) are able to be used in the CapitalBio SmartArrayer^{TM} series microarray workstations. This flexible technology enables CapitalBio SmartArrayer^{TM} series microarray workstation users to have access to more applications than other brands. These applications include: the ability to fabricate microarrays of nucleic acids, proteins, antigens and other biomolecules on preformed chip surfaces of glass, plastic or membranes.

Our highly efficient combinatorial cleaning of dispensing pins consists of sonication, rinsing and drying can decrease or prevent most potential cross-contamination among samples. The humidifying unit and HEPA filter help to improve the fabrication quality of the microarrays.

0.3.2 Series

CapitalBio has developed two types of microarray workstations, the CapitalBio SmartArrayer^{TM} 48 and the CapitalBio SmartArrayer^{TM} 136 with throughputs of 48 and 136 slides, respectively.

All CapitalBio SmartArrayer^{TM} series microarray workstations can adopt both pin printing technology and non-contact droplet dispensing technology.

SmartArrayer^{TM} 4.0 is the updated application software for CapitalBio SmartArrayer^{TM} series microarray workstations. There are corresponding user interfaces for different workstation types and work applications.

0.4 Conventions

Table I below lists the abbreviations and alternative names used frequently in the user manual.

Table I Abbreviations and alternative names

Microarray workstation	Alternative name of CapitalBio SmartArrayer^{TM} 48 and CapitalBio SmartArrayer^{TM} 136
48S	CapitalBio SmartArrayer^{TM} 48 equipped with spotting unit(pin printing)
48D	CapitalBio SmartArrayer^{TM} 48 equipped with non- contact dispensing unit
136S	CapitalBio SmartArrayer^{TM} 136 equipped with spotting unit(pin printing)
136D	CapitalBio SmartArrayer^{TM} 136 equipped with non- contact dispensing unit
NDM	Non-contact Nano- Dispense Module, NDM
Dispensing	Non-contact droplet dispensing
Spotting	Contact-printing or pin printing

0.5 Where to get help

Our staff at CapitalBio Corporation wants to help with any questions or comments you might have regarding the system, its software and other components. Feel free to contact CapitalBio Corporation in the following ways:

Address: CapitalBio Corporation
　　　　　 18 Life Science Parkway
　　　　　 Changping District
　　　　　 Beijing 102206
　　　　　 P.R. China
Tel: +86-10-80725793
Fax: +86-10-80726782
Website: http://www.capitalbio.com
Email: techsupport@capitalbio.com
　　　　　 globalsales@capitalbio.com

Please be prepared with the following information for the optimum technical support:

⊙ SERIAL NUMBER of your CapitalBio SmartArrayer^{TM} (at the back of the microarray workstation)
⊙ Version number of the application software
⊙ Nature of the problem (Please refer to the Troubleshooting Chapter)
⊙ Steps you have taken to solve the problem
⊙ Your phone number, fax number, and email address

In addition, convenient access to your CapitalBio SmartArrayer^{TM} during the duration of a technical support call would facilitate the process.

Additional information can be found in the following:

● Documentation provided with the computer workstation and its software

Chapter 1 Important Safety Information

Warning:

Use of controls or adjustments or performance of procedures other than those specified in installation guide and user manual may result in damage to the instrument, loss of data, invalid data, hazardous radiation exposure, laser light, hazardous voltage, moving parts, or even cause fire. Exposure to the hazards may cause severe or fatal injury.

1.1 Certification, Identification and Power Supply Label
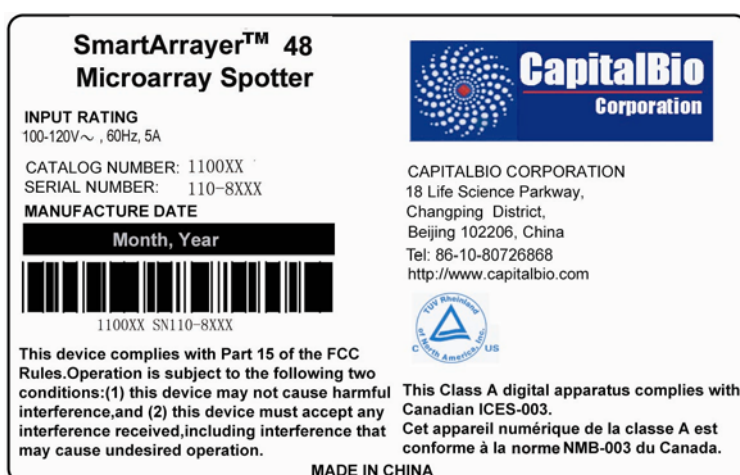
Figure 1-1 The identification label of the *48S*

Figure 1-2 The identification label of the *136S*

Figure 1-3 The identification label of the *NDM*

1.2 The protective housing (Cover or Enclosure)

The protective housing is designed to protect users from exposure to hazardous voltage, or moving parts. Users are not allowed to access the interior of the CapitalBio SmartArrayer[TM] There is no component in the interior of the CapitalBio SmartArrayer[TM] that requires maintenance work to be performed by the user. Users are not allowed to open or remove the cover of the CapitalBio SmartArrayer[TM] during operation; otherwise, warranty on the product will be voided with immediate effect.

	Warning: Users are not allowed to gain access to the interior of the CapitalBio SmartArrayer™ through any other openings except that is needed to perform the function of the CapitalBio SmartArrayer™. Removing the housing may damage the CapitalBio SmartArrayer™ components and result in hazardous exposure to hazardous voltage, or moving parts. If the protective housing is damaged, users are instructed to halt all use until a certified technician has inspected the instrument.

1.3 Power Supply Cable (Cord)

The manufacturer of the CapitalBio SmartArrayer™ provides the user with the power supply cable (cord). The cord is PVC insulated with an area of $0.75mm^2$, the voltages of which are up to and including 450V/750V. Users are not allowed to replace the power supply cable with different rating, or it may result in electric shock, even cause fire and damage the arrayer.

	Warning: We suggest that users do not replace the power supply cord provided by the manufacturer. If needed, contact the technical support team for the CapitalBio SmartArrayer™ Replacing the power supply cable with another cable of a different rating may result in electric shock or even cause fire and damage the arrayer.

1.4 Fuses

See Chapter 2.2.3.

	Warning: Replace only with same type and rating fuses, otherwise it may result in electrical hazard or even dangerous fire. Turn off electrical power at source before attempting to remove or replace the fuses.

1.5 Fan

There is a fan in the CapitalBio SmartArrayer™ for the purpose of ventilation. Users *must not* block or cover the vent of the fans. Blockage will cause the temperature to rise and may result in damage to the arrayer; possibly causing electric shock and fire.

	Warning: Users are not allowed to cover the vent of the fan in the platen of the CapitalBio SmartArrayer™. Otherwise the temperature will rise and result in damage to the arrayer; possibly causing electric shock and fire.

1.6 Moving Parts

The CapitalBio SmartArrayer™ contains moving parts which may lead to a mechanical hazard if users do not follow the instructions manufacturer has provided, or moving parts may cause injury to users. The protective housing is designed to protect users from exposure to the moving parts during operation.

	Warning:
⚠️	Users are not allowed to gain access to the interior of the arrayer during operation. The protective housing of the arrayer is designed to protect users from the hazardous moving parts under operation.

1.7 Water

The CapitalBio SmartArrayer^{TM} has some components，such as the *Sonicator*, *Humidifier,* and the *Working Water Tanks*, which for operations must be filled with some water. For details, see Section 9.3.

1.8 Sample Requirements

Applications to fabricate nucleic acid or protein microarrays on CapitalBio SmartArrayer^{TM} are possible with either the contact pin printing module or the non-contact dispensing module.

The properties of the sample are an important factor influencing the production of the microarray and the quality of microarray chips. To fulfill the quality specification, samples should comply with the following requirements.

	Note:
⚠️	All of the samples and buffer must be water-soluble to ensure that they could be rinsed out completely with the distilled water. No corrosive components should be present in the sample.
	The pre-treatment procedures, such as filtration and purification, are indispensable to eliminate large particles in the sample and avoid circumstances of high viscosity or clotting.
	The viscosity of the sample solution should be at a low or moderate level to avoid undesirable consequence, such as jammed nozzle, blocked pin and cross-talk caused by incomplete cleaning. A level of viscosity lower than 40% glycerol is recommended. For protein, the concentration should not be higher than 5mg/mL.
⚠️	**Note**:
	When an innovative sample and buffer system is about to be applied, exploratory tests for the better printing or dispensing conditions are compulsory.

1.9 System Cleaning

When an innovative sample and buffer system is about to be applied, exploratory tests for the most effective cleaning protocol are compulsory. It is recommended to use fluorescent-labeled reagent in such experiments to evaluate the effectiveness of the cleaning procedures.

When using the spotted array protocols, the pins should be passed through no less than 2 cleaning cycles prior to switching to printing a new sample source, and the number of washing cycles must be increased more when printing samples of higher viscosity or higher

concentration.

⚠	**Note:** Stand-alone cleaning protocols should be completed before shutting down the system, to ensure complete cleanliness for subsequent use.

1.10 Emergency stop button

The EMS (Emergency Stop) button can be found on the front panel of the 136S and the 136D model microarray workstations. In the *48S* and the *48D* models, the EMS button located in the both sides of the body of the machine.

⚠	**Warning**: Press the EMS button if the microarray workstation functions incorrectly or needs to be shut down prematurely. The EMS button needs to be reset before the microarray workstation can be restarted.

1.11 Protection locks

The protection locks locate on both sides of the equipment; It provides safety protection for operators.

⚠	**Warning**: The locks are necessary for the operators. Do not unlock them while the arrayer is running. If you do need to do it, please pause or halt the arrayer first.

1.12 Switch between pin-printing and dispensing system

When switching pin-printing and dispensing systems, users should confirm that the application software is compatible with the hardware subsystem. Then users must newly configure the system parameters and redo all position calibrations. For detailed instructions of each operation, please see Section 2.7 and Section 6.3.

1.13 Service

Service is conducted by the technical support team of CapitalBio Corporation or authorized agencies only. Consult CapitalBio Corporation or authorized agencies for repair instructions. See "Where to Get Help" in the Preface for more information.

Chapter 2 Hardware Installation

2.1 Summary

The chapter will introduce hardware installation requirements and installation procedures and inspection details, debugging, switching of dispensing module and spotting module, and hardware mark conventions.

2.2 Requirements

2.2.1 Environmental requirements

The SmartArrayer[TM] should be placed in a clean room that meets the environmental conditions (Spotting or Dispensing environmental requirements) described in the following table:

Table II Environmental Requirements

Site Requirement	Indoor Use
Cleanliness degree	10000 class (meets or exceeds GMP requirements)
Temperature Range	5-30 ^{o}C
Relative Humidity Range	Up to 50%
Altitude	Up to 2000m

Because the microarray workstation is a precision instrument, please keep the environment as clean as possible. Install the microarray workstation away from sunlight, ventilation ducts and any other devices that may cause significant change in temperature and humidity.

2.2.2 Space requirements

Table III Minimal dimensions of the instruments

Models / Dimensions	48S	136S	NDM
Length (mm)	980	1190	610
Width (mm)	1250	1080	620
Height (mm)	1500	1600	710

⚠	**Warning**: The SmartArrayer[TM] series microarray workstation should be placed in a room which has appropriate space to operate conveniently. Place the computer at the left side of the microarray workstation, and leave enough space between them.

2.2.3 Power requirements

Make sure the power supply meets the requirements appropriate for the relevant model (*48S*, *48D*, *136S*, *136D*). The requirements of each model are listed in the following table.

Table IV Electrical input ratings

For the series of EU

Parameter Model	*48S*	*136S*	*NDM*
Frequency (Hz)	50	50	50
Rating voltage (V)	230	230	230
Maximum input current (A)	3.0	3.5	0.2
Rating power (kW)	0.69	0.805	0.046
Fuse (A)	6.3	6.3	0.315

For the series of NA

Parameter Model	*48S*	*136S*	*NDM*
Frequency (Hz)	60	60	60
Rating voltage (V)	100-120	100-120	100-120
Maximum input current (A)	5.0	5.5	0.4
Rating power (kW)	0.55	0.605	0.044
Fuse (A)	8	8	1.0

Comment: the electricity rating for the *48D* and *136D* respectively is the sum of the power supply of the *48S* and *136S* and the rating input electricity of the *NDM*.

To avoid or reduce losses caused by an accidental power cut, we advice users to equip the microarray workstation with a UPS constant voltage power source. The recommended voltage parameter values for the UPS for all models are listed in the following table.

Table V Recommended UPS specifications

Parameter Model	*48S*	*48D*	*136S*	*136D*
UPS electrical source power (kW)	1.5		1.5	
UPS power supply time after power cut (minutes)	15			

2.2.4 Electromagnetic compatibility (EMC) requirements

Warning:

A good EMC environment is critical for operation of the microarray workstation, as electromagnetic fields may lead to unpredictable results. The

Electromagnetic property of microarray workstation must be according with the requirements of EMC directive about CE ,the FCC Part15 and ICE-003 directive.

The following cautions should be considered:

- Keep the microarray workstation away from high electromagnetic discharge equipment, such as pacemakers, electric welding equipment, etc

- Site the microarray workstation away from equipment such as refrigerators, centrifuges, etc, that frequently consume high power upon starting-up

- Keep the microarray workstation away from any strong magnetic fields

- Do not connect too many power cables to the same junction box to which the arrayer is connected

- Do not plug-in or pull-out any other equipment connections to the same junction box while the arrayer is running

2.2.5 Computer requirements

Computer requirements:

Table VI Computer requirements

CPU	Pentium 4 2.0GHz or higher
Memory	512M or more
Disk space	40G or more
Display system	17 inch colored, with resolution of 1024×768 pixels or more
CD-ROM	40× speed or faster
PCI slot	1
Serial port	1
Operating system	Windows$^{®}$ 2000 (with Service Pack 4) or Windows$^{®}$ XP (with Service Pack 2)

⚠	**Warning**: Please open the computer shell, and plug the control card firmly into the PCI slot of the computer. In order to prevent computer errors, please avoid sharing slots with other PCI cards on the same computer.

2.2.6 Pressure Supply requirements

In the *136D*/*48D* models, a gas bottle filled with high purity compressed nitrogen gas is required as a gas-pressure supply. The compression resistance pressure of gas bottle should be no less than 14MPa and inner volume of the bottle should be no less than 30L.

Between the gas tank and the "Pressure Inlet" of *NDM*, there should be a pressure regulator, which can be operated manually. The output pressure range of the regulator must be 0.2~0.4 MPa. The regulator should be equipped with suitable connectors linked to the *NDM* and the gas bottle, while the outer diameter of the tubing connecting *NDM*, regulator and gas tank must be 8 mm and some adaptors may be needed to interconnect different gas bottles, gas regulators and the tubing.

⚠	**Warning**: The output pressure range of the regulator Must be 0.2~0.4 MPa. Make sure the output pressure of the outer pressure source is lower than 0.5MPa.

2.3 Receiving Inspection

2.3.1 Initial Inspection upon receipt of equipment

Please check if the number of packages is correct and whether they are in good condition or not. Please refer to the following table.

Table VII Packed box listing

	48S	136S	NDM
Wood packing box	1	3	1
Cardboard packing box	1	1	

2.3.2 Unpacking

It is important to avoid shaking and dropping the equipment while transporting it or while opening their packing boxes. Please check if the components are all present after opening the packing boxes. Please contact CapitalBio if you have any questions or concerns.

2.3.3 Components List

Please refer to packing list in the package.

2.4 Hardware Installation

2.4.1 Hardware Installation

The hardware installation should be carried out by specialized technicians. End users must have a thorough knowledge of the microarray workstation components in order to operate the workstation correctly and efficiently. It is essential that end users fully understand the operations required to switch between the *136D* and *136S* functions or to switch between the *48S* and the *48D* functions.

2.4.2 Description and Components

Upper enclosure

Workbench

Safety protection

Switch pannel
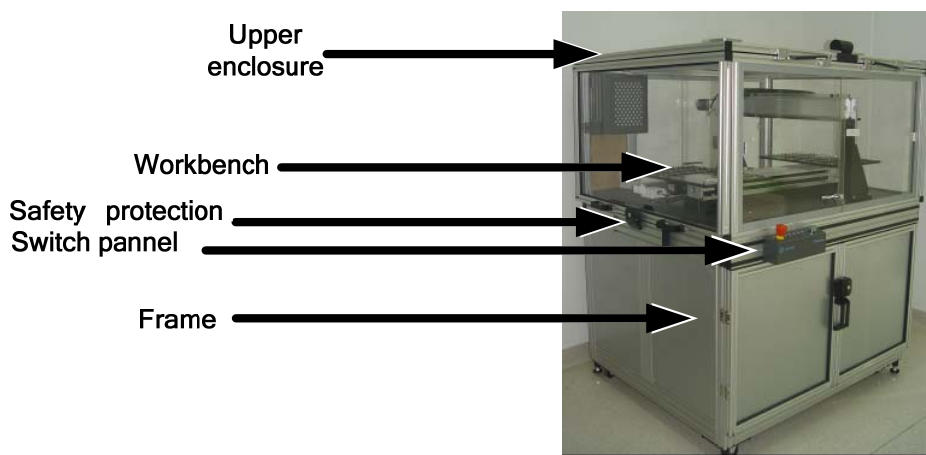
Frame

Figure 2-1 The *136S* instrument components

The *136S* microarray workstation instrument components include: the frame, the body, the enclosure, the control panel and attachments such as the humidifier and the vacuum pump as shown in the above Figure 2-1. The *136D* is a combination of the *136S* and the *NDM* while the spotting module is replaced by the dispensing module and the cables and tubing are connected.

The frame forms the base of the workstation and stably supports the total weight of the equipment.The humidifier, vacuum pump, and water tanks shoulde be placed behind the frame.The body includes four axes fixed on to the workbench. The enclosure covers the body to form a closed and clean environment for microarray fabrication. There is control panel at the front of the instrument with the startup buttons for the workstation, five status indicator lights and an EMS button.

Humidifier   Sonicator   Peristaltic pump

Figure 2-2 Accessories

Fuller details of the instrument body are shown in the following figures. The upper platen is connected with the slide block of the X-axis and the lower platen is connected with the slide block of the W-axis and they move in the left and right directions. The pins or dispensers in the spotting/dispensing units move up and down. It is assembled on the slide block of the Z-axis. The X- and W- axes are fixed on the workbench and the Z- axis is fixed on the Y-axis, which moves forward and backward. The axes are controlled by the application software via a motion control card which should be inserted into the PCI-slot in the main board of the computer. The card is connected to the workstation via a 100-pin cable.
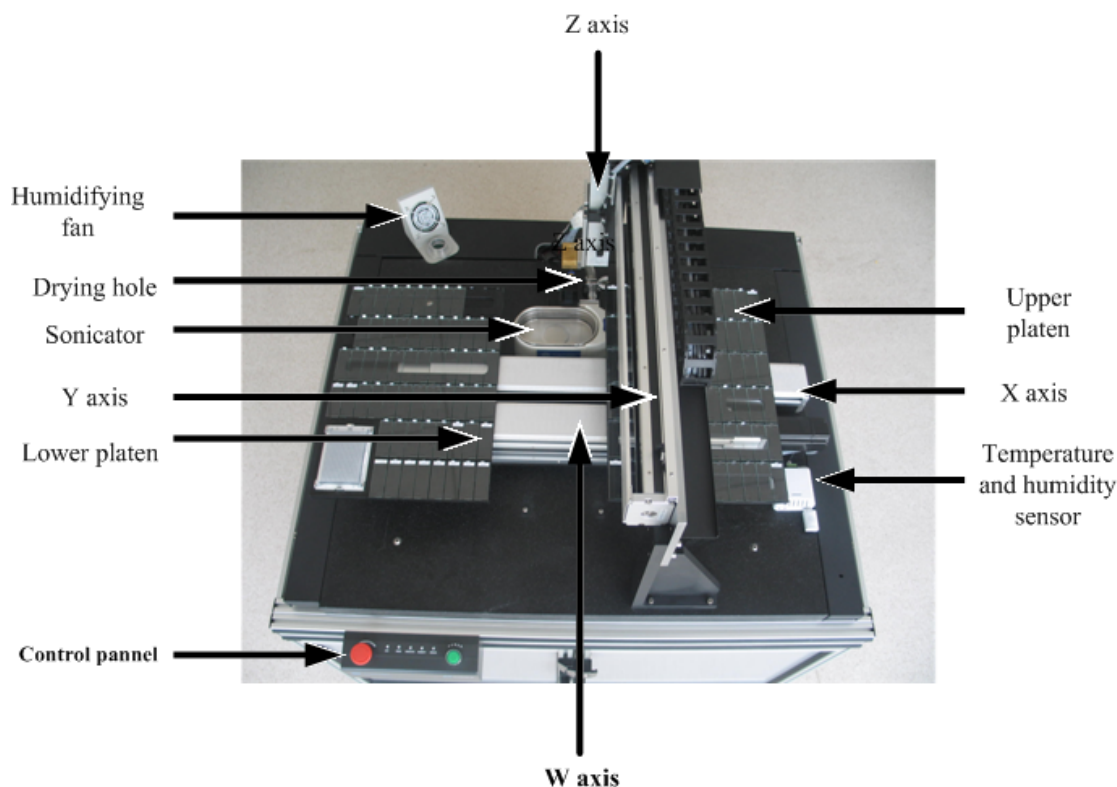
Figure 2-3 Top view of the Workbench

Other cleaning unit attachments such as the rinsing unit and the vacuum drying unit (the wash/dry stations) and the sonicator are fixed on different sides of the X-/W- axes. For the *136D*, the basin of the sonicator is used as a reservoir for system liquid such as distilled water.

Status indicator lights

Figure 2-4 The control panel

There are two buttons on the control panel of the *136S*. The red elevated button on the left is the Emergency Stop (EMS). The green button on the right is the unit Startup button and it is used to start up the microarray workstation only after the power switch on the back of the workstation is turned ON, but it is unable to shut down the workstation. Located between the two buttons are five status lights that indicate the operation of the vacuum drying unit, the rinser and washing unit, the sonicator unit, the humidifying unit and a Function Error notice, respectively. The power switch and the coupling connectors of the *136S* are at the back of the instrument and are shown below.

Power switch

Power input jack

Vacuum pump power
jack

Humidifier power
jack

Dispensing control
jack

Dispensing tubing
tie-in

Vacuum tubing
tie-in
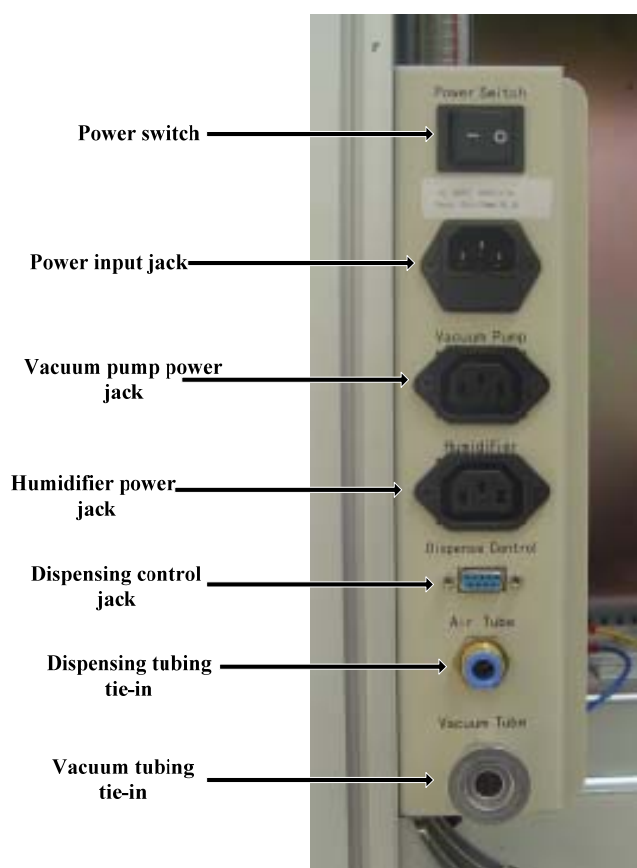
Figure 2-5   The connection plate at the back of the frame

The *48S* includes the frame, body and enclosure, with similar attachments to the *136S*. One of the major differences between the *48S* and the *136S* is that the *48S* has only one printing roller with 48 slide capacity, while the *136S* has two printing rollers with 136 slide capacity. The second difference is the shape of the control panels, which for the *48S* is fixed on the outside of the frame/body at the top front right side. The shapes of the EMS button and the Startup button are also different.

Similarly, the *48D* is a combination of the *48S* and the *NDM* while the spotting module is replaced by the dispensing unit and the cables and tubing are connected.
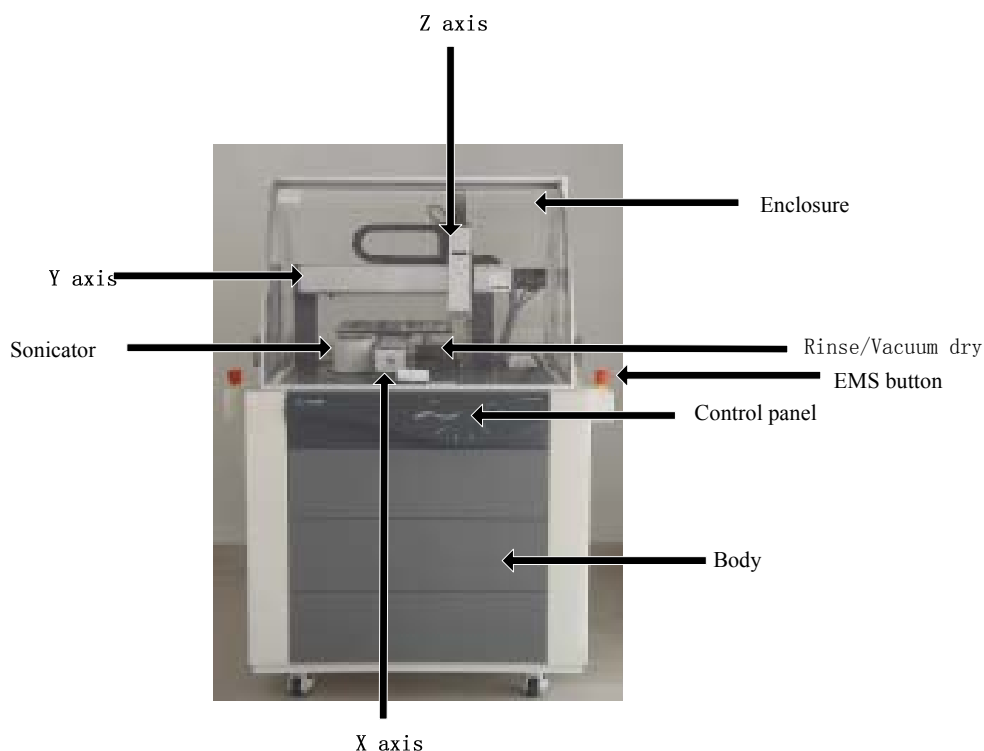
Figure 2-6   The *48S* instrument components

2.5 Attention Items of Installation

⚠	**Warning**: Make sure the power input is the same as the rated power requirements (Refer to Section 2.2.3).

● UPS (Uninterrupted Power Supply) is recommended

⚠	**Warning**: Connect the cables and tubing according to the requirements stated in the user manual and notices shown on the equipment. Distinguish among the pressure inlet from the outlet and the power input from the output. Connect the pneumatic tubing, connectors and/or adaptors in clean surroundings and make sure there is no pressure leakage in the pneumatic systems. Make sure the output pressure of the outer pressure source is lower than 0.5Mpa. Ensure that the work bench/ platen is completely level using a spirit level to align the instrument. Once the dispensing module or the spotting module has been installed, its position must be completely level (using a spirit-level) to assure perfect motion between the pin/dispenser tips and the glass slide surface. The calibration methods can be referred to in Section 6.3.

- Check each equipment module and unit and make sure the connectors and terminals are connected, inserted and fastened correctly
- Ensure that no objects obstruct the motion pathway of the printing heads

⚠	**Warning**: Make sure the equipment is correctly electrically grounded.

- Make sure all the power switches are turned off
- Do not clean the inner parts of the axis. If necessary, please use a cotton cloth to clean the outer parts of the axis
- Avoid entry of dust into the tubing by loading the dispenser enclosure onto the dispensing unit during the installation process
- After installation:
  - Wipe off the particles and fragments from the platen and clean the platen, enclosure and workbench
  - Clean the humidifier, sonicator and water tanks
  - Remove any liquid spills from the workbench surface

2.6 Debugging

- Before starting up the system:
  - Check the cable and tubing connections again
  - Make sure that the photoelectric stop slide stay is away from the motion limit sensor

⚠	**Warning**: Check the workbench and make sure there are no obstacles to obstruct the movement of the printing head. Clean the wash water tank and sonicator wash tank and refill distilled water into distilled water tank.

- Test the instrument step by step
  - Install debugger software
  - Startup the microarray workstation following the instructions in Section 5.2.2 and startup the debugger software

⚠	**Warning**: Set the velocity for each axis at 20mm/s and the acceleration at $100mm/s^2$. Check the forward and backward movement of each axis.

  - Listen to whether there are any abnormal noises produced by the axes, or by other equipment while moving or stopping
  - Test the rinse, sonicate wash, vacuum dry, drain and EMS functions separately
- Test the system
  - Install application software, startup the hardware and software
  - Calibrate the key positions and prepare the samples and slides, The calibration methods can be found in Section 6.3
  - Run a protocol and make a microarray
  - If there are problems, please refer to other chapters, including the trouble shooting chapter

2.7 Switching between Spotting and Dispensing Function

To switch to the *136D* /*48D* from the *136S* /*48S*, the *NDM* has to be linked through the corresponding cable connectors and the tubing connectors, in addition to replacing the spotting module with the dispensing module. Notice that there are tubing and cables that need to connect with the dispensing unit, and the connection plate for the *48S.* The serial port (labeled "Serial") of the *NDM* should be connected with the serial port of PC and the dispense control port should be connected to the same labeled port on the *136D* /*48D*. See **Table IX**.

The *NDM* has two tubing connectors namely "Pressure Inlet" and "Pressure Outlet", with the "Pressure Outlet" tubing connector connecting with the "Air tubing" connector of the *136S/48S*, and the "Pressure Inlet" tubing connector connecting with the pressure supply.
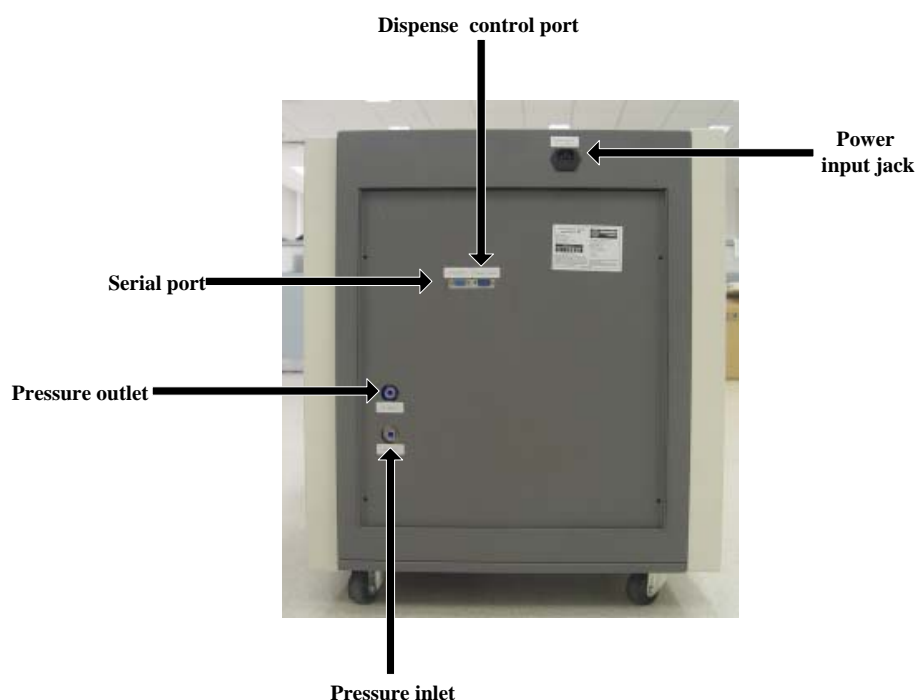
Figure 2-7 The rear panel of *NDM*
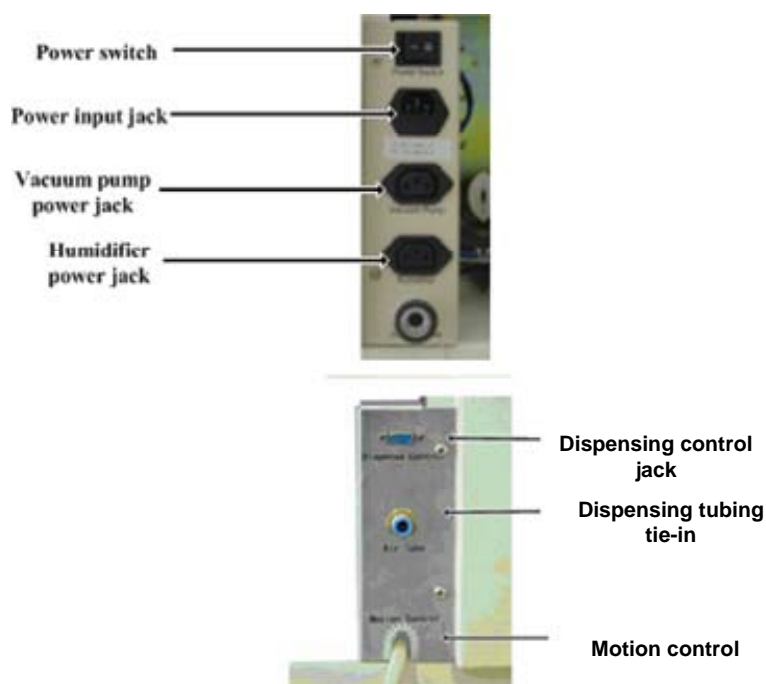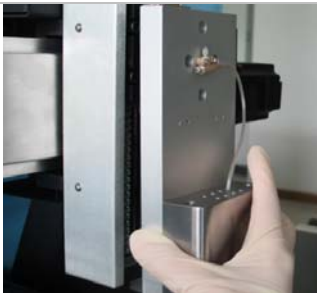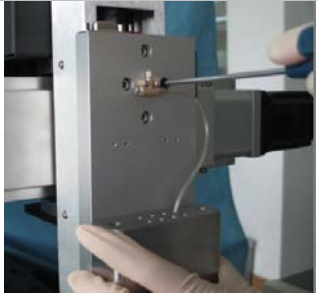
Figure 2-8 The rear panel of *48S*

The following Table shows how to exchange the pin-head module with the dispenser-head module.

Table VIII Procedure for changing from spotting to dispensing modules

Step No.	Schematic Drawing	Description
1		Move the Z-axis to home position
2		To unload the pins from the pin holder, the operator should use the tweezers to lift the pin up by one hand, and then slip the pin out from the pin hole slowly and with care. Store the pins carefully.
3		The operator should use the screwdriver to unload the pin-head module from the Z-axis.

4		Insert the dispensing head module
5		Fasten the dispensing module
6		Adjust the dispensing head to level
7		Plug in the control cable
8		Screw fasten the control cable
9		Loosen the connector from the dispensing unit

10		Connect the tubing to the connector
11		Link other cables and tubing, see the above content in this section

Once the dispensing module has been replaced by the spotting module, the position of the dispensing/spotting module has to be adjusted slightly to be completely level by using a spirit-level to assure perfect motion between the pin/dispenser tip and the glass slide surface. The calibration methods can be referred to in Section 6.3.

	Warning: Key position calibration must be done before running any protocol.

2.8 Control Card Driver Installation

After the installation of the workstation hardware, the next step is to install the driver for the motion control card. The driver can be found on the SmartArrayer^{TM} 4.0 software disk.

Place the SmartArrayer^{TM} 4.0 software disk into the CD-ROM or DVD-ROM and then browse the driver folder to find the setup.exe file. Run the setup.exe and follow the instructions.

After successfully installing the driver, shut down or restart the computer.

2.9 Hardware Mark Conventions

In general, the top right hole (shown in the following figure) is referred to as the 1st hole for the pin in the software setting and the hardware installation. Accordingly, the top right corner of the slide is referred to as (0,0) position when you hold the slide longitudinally near the end with the barcode.

The layout and definitions of these conventions for the *136S* and the *136D* are shown in the following figures – Figure 2-7 and 2-9.   The layout and definitions of these conventions for the *48S* and the *48D* are shown in Figure 2-8.

In the view at the left side of the *136S* and the *136D* workstation (Fig. 2-7), the slide at the top left corner of the upper platen is the 1st slide, and the slide at the top left corner of the lower platen is the 136th slide.

The source microplate should be fixed into its bracket on the lower platen with the number. P24 well at the bottom right corner of the bracket, and the number A24 well should correspond to the 1st pin. Accordingly, the top right hole of the vacuum drying unit is assumed to be underneath the 1st pin.
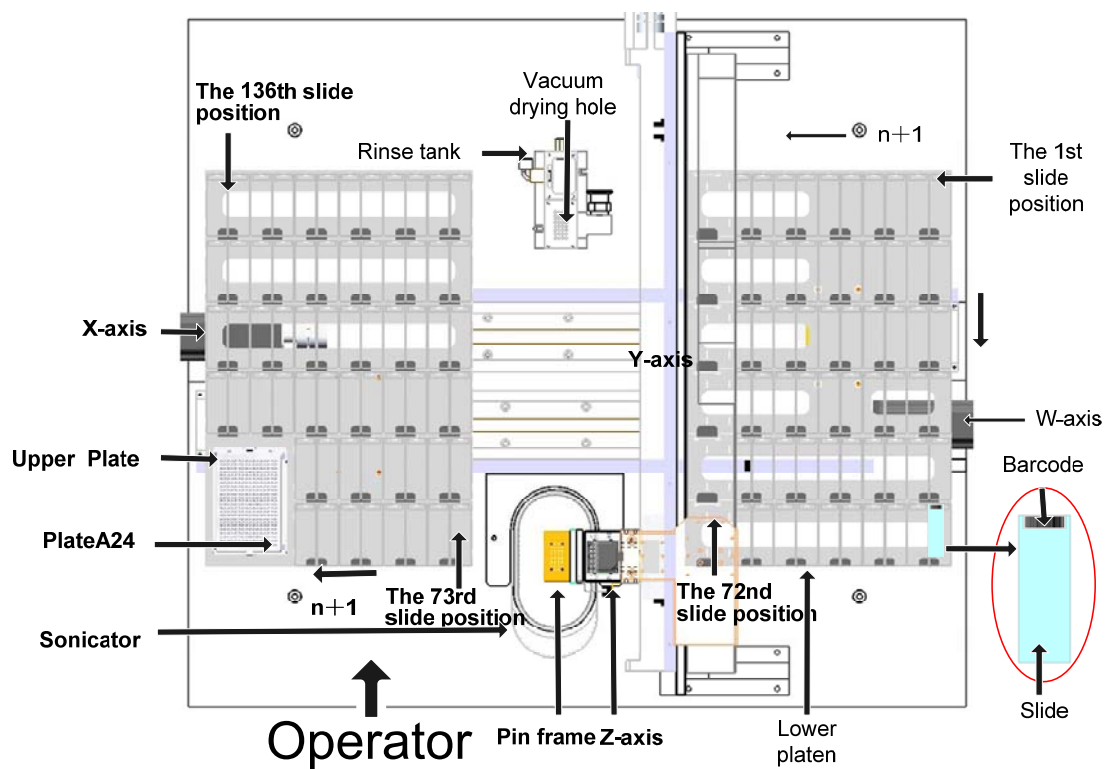
Figure 2-9 The *136S* position information (Top view)
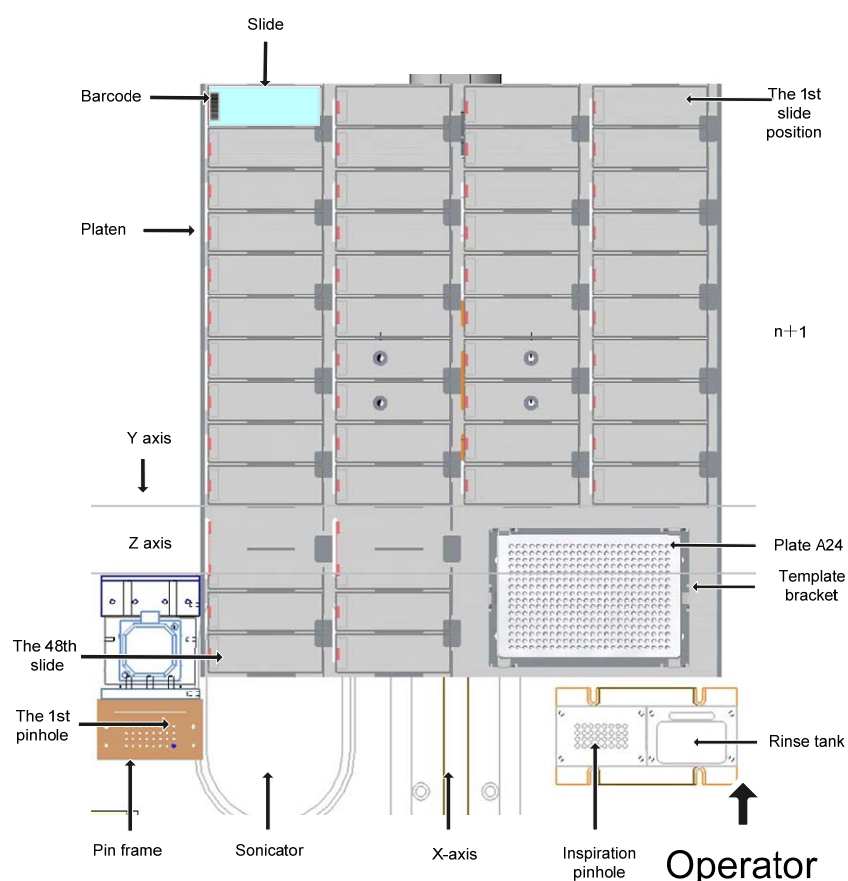
Figure 2-10 The *48S* position information (Top view)

In the front view of the *48S*, the slide at the top right corner of the platen is referred to as the 1st slide while the slide at the bottom left of the platen is the 48th slide. The microplate should be fixed into its bracket on the platen with the number P24 well at the bottom right corner of the bracket, and the number A24 well is assumed to be underneath the 1st pin. Accordingly, the top right hole of the vacuum drying unit is assumed to be underneath the 1st pin.
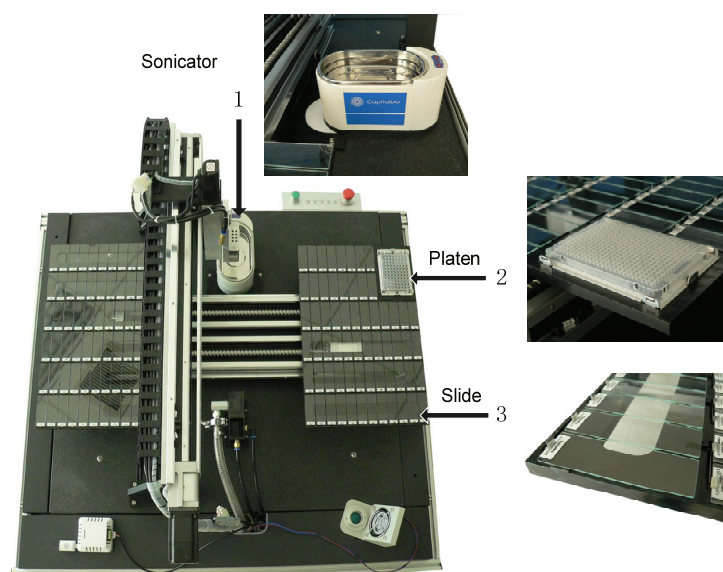
Figure 2-11 Calibration of the coordinate positions of 136S

Chapter 3 Software Installation

3.1 Overview

The SmartArrayer[TM] CD provided contains the setup program of the application software of the SmartArrayer[TM] series, which supports four different models (**48S**, **48D**, **136S**, **136D**). Please confirm that the software configuration is compatible with the instrument model when you setup the application software.

3.2 Preparation

Power on the computer workstation linked to the microarray workstation, login in the operating system, insert the compact disc labeled with the SmartArrayer[TM] V4.0 into the CD/DVD ROM, browse the catalog of the CD and find the setup executive file named "Setup.exe".   Double click this file to launch the installation wizard.

3.3 Installation Wizard

Step1: Greeting page, click the "Next" button to proceed.
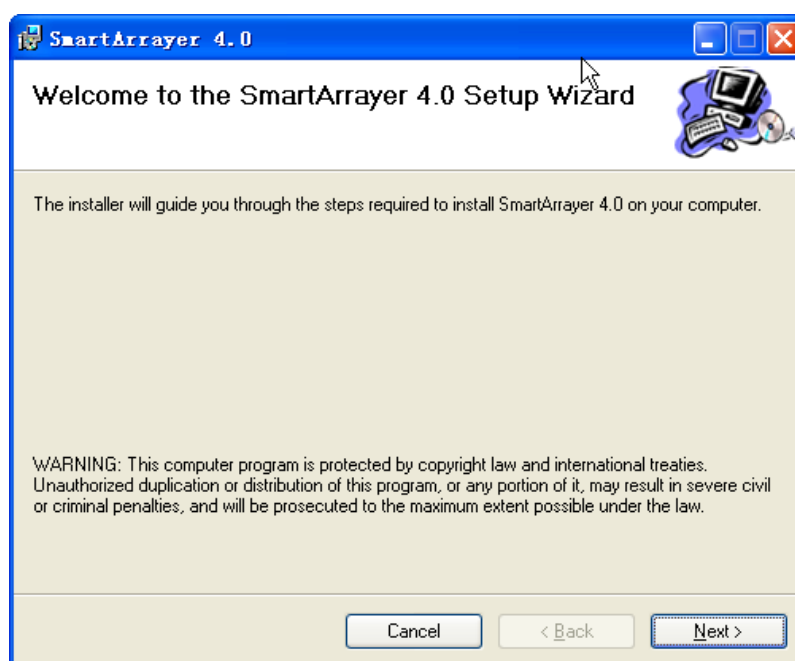
Figure 3-1 Installation welcome interface

Step 2: Choose the hardware model.
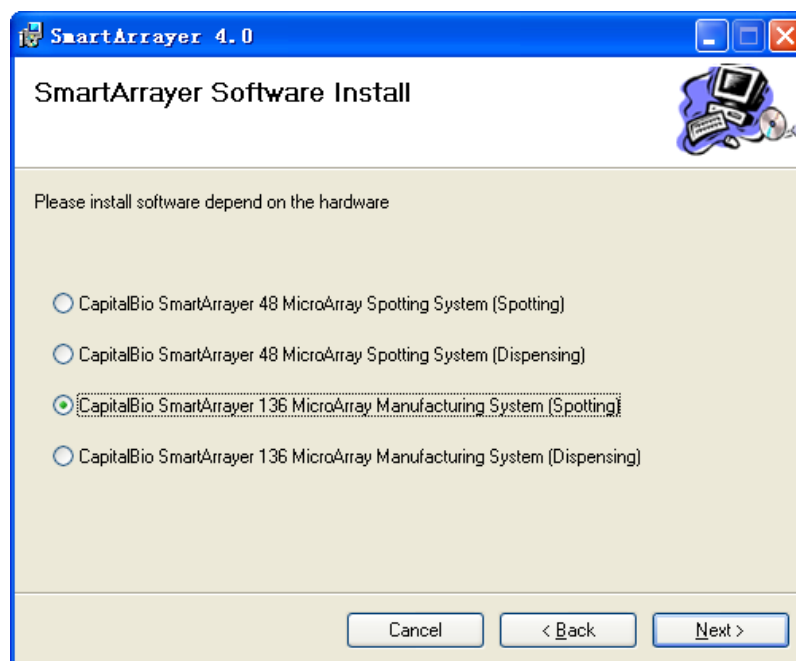
Figure 3-2 Software Control System Choice Interface

	Warning:
⚠	The interface allows you to choose the appropriate software for your instrument, you must choose the right software to install. Otherwise, it may cause damage to the instrument.

Step 3: Choose the target folder to install.
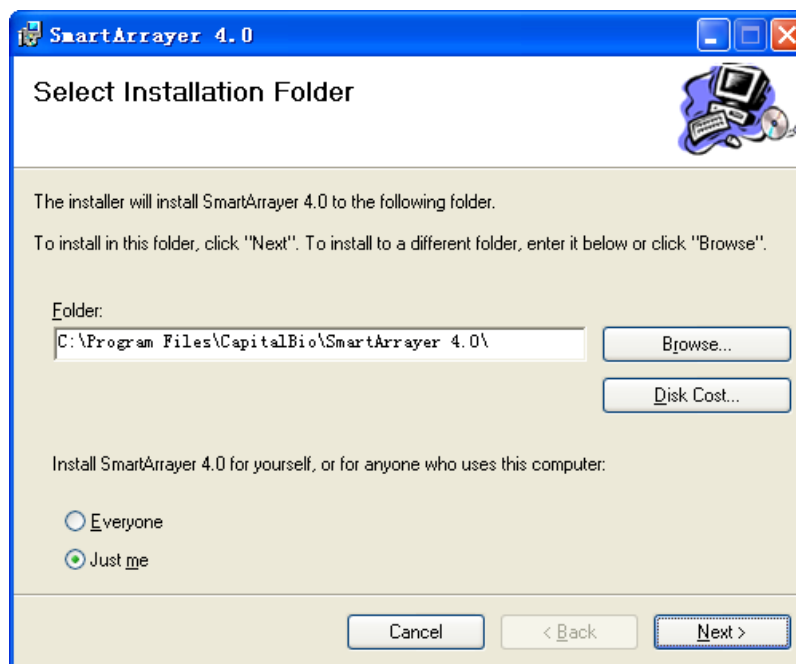
Figure 3-3 Target folder interface

Step 4: Confirm to install the software.
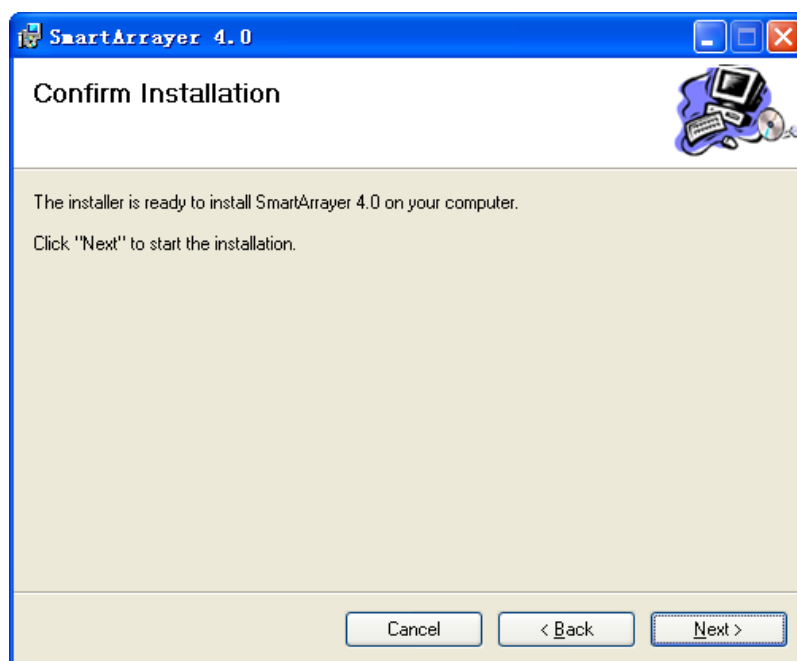
Figure 3-4 Confirm installation interface

Step 5: An animated progress bar depicts the installation process.
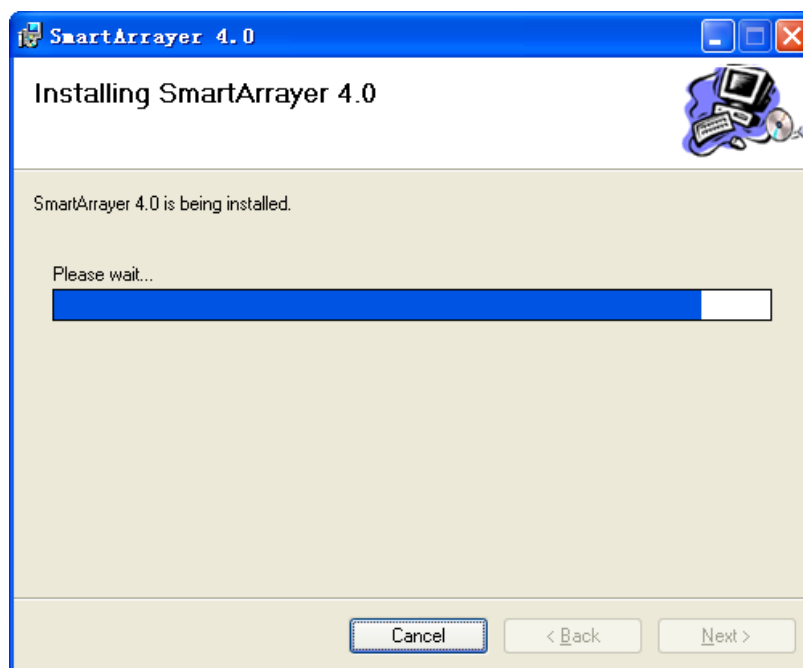
Figure 3-5 Installation interface

Step6: Installation successful, click the "Close" button to exit the installation guide.
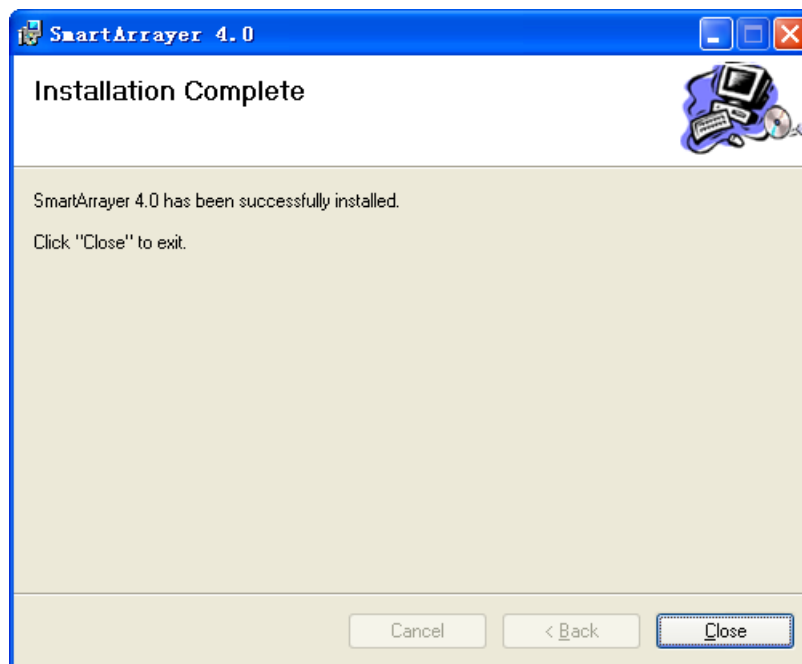
Figure 3-6 Installation complete interface

⚠	**Note**: Within the installation wizard, you can click the "*Back*" button to return to the previous step to modify the configuration, or click the "*Cancel*" button to exit the installation wizard**.**

Chapter 4 Tutorial

4.1 Overview

In this chapter, we will guide you to spot or dispense a microarray chip. This way, you can quickly grasp the basic skills to use the software and accomplish basic spotting/dispensing tasks.

⚠	**Note**: To get detail information of all operations, users can read the following three chapters: **Getting Started, System Configuration, Spotting or Dispensing**.
⚠	**Warning**: Whenever a new system is installed, or the system is moved to a new place, or some changes are made to the hardware or the software, it is mandatory that you to do a spotter/dispenser calibration to ensure that the system functions correctly.

4.2 Launch the System

Step 1	Turn on the vacuum pump
Step 2	Turn on the switch which is on the rear panel of the microarray workstation.
Step 3	Turn on the microarray workstation (press the device startup button) prior to turning on the linked computer
Step 4	Turn on the control box power if using the **NDM** (for **136D/48D**)
Step 5	Then turn on the computer linked to the microarray workstation
Step 6	Launch the microarray workstation application software

4.3 Configuration

For safety, Users should configure the system *immediately* after installation or switching the spotting module to the dispensing module (or *vice versa*). Current configurations are then stored and will be used for routine spotting or dispensing operations. The system does not need to be configured again for subsequent routine operations. Users can go directly to normal running operations (preparation) when the machine is used again. If the spotting or dispensing operations at some later stage become inaccurate, the system should be re-configured.

Step 1	Access system configuration, user can click **System configuration** button on the right top toolbar of main window and input the password to access the configuration.		
Step 2	Position Calibration	Rinsing Position Calibration, see Section 6.3.1	
		Drying Position Calibration, see Section 6.3.2	
		136S/48S	Sonicator Position Calibration, see Section 6.3.3
		136D/48D	System Liquid Position Calibration, see Section 6.3.3
		Slide	**136S/136D(136 slides)**, see Section 6.3.4

		Position Calibration	*48S/48D(48 slides)*, see Section 6.3.4
			Microplate Position Calibration, see Section 6.3.5
		136D/48D	Pre-dispensing Position Calibration, see Section 6.3.6
Step 3	Motion setting, see Section 6.4		
Step 4	*136D/48D*	Pressure parameters setting, see Section 6.5	
		Dispensing parameters setting, see Section 6.6	
Step 5	Slide Parameters setting, see Section 6.7		
	Sample Selection Priority setting, see Section 6.7		

4.4 Preparation

You can access the preparation panel by clicking on the *Prepare* button on the left.

Step 1	Cleaning	Circulate, see Section 7.3.1.1
		Rinse, see Section 7.3.1.2
		Vacuum, see Section 7.3.1.3
		Dry, see Section 7.3.1.4
		Excite, see Section 7.3.1.5
		Sonicate, see Section 7.3.1.6
Step 2	Load slide, see Section 7.3.2	
Step 3	Home, see Section 7.3.3	
Step 4	Humidify, see Section 7.3.4	

4.5 Protocol

Click the *Protocol* button on the left to gain access to the microarray setting, sample sequence (Virtual plate) and the spotting/dispensing page process.

Step 1	Array setting (*see* Section 7.4.1)	Sub step 1: Specify pin/dispenser pattern
		Sub step 2: Slide Settings
		Sub step 3: Slides
		Sub step 4: Array Settings
		Sub step 5: Sample Replicates
		Sub step 6: Array Replication
Step 2	Sample Sequence (*see* Section 7.4.2)	Sub step 1: Select sample wells on sample plate
		Sub step 2: Select Plates
		Sub step 3: Add to sequence list
		Sub step 4: Adjust sequence list
Step 3	*136S/48S* (*see* Section 7.4.3)	Sub step 1: Cleaning
		Sub step 2: Sample Uptake
		Sub step 3: Pre-spotting Settings
		Sub step 4: Z-Motion Settings of Spotting
		Sub step 5: Minimum Relative Humidity
	136D/48D (*see* Section 7.4.4)	Sub step 1: Cleaning
		Sub step 2: Sample Uptake
		Sub step 3: Pre-dispensing Setting
		Sub step 4: Dispense time
		Sub step 5: Minimum Relative Humidity

4.6 Preview

Click on the *Preview* button on the left and access the spotting preview panel, you can access the *Save* and *Load* buttons on the top toolbar to backup and restore the editing protocols.

Step 1	Slide Preview: the simulation of the spotting pattern on the slide will be displayed to help users confirm whether the protocol and configuration are correct
Step 2	Sample Preview: it provides a visual aid for users to estimate the sample spot distribution on each slide, according to the current microarray settings and sample sequence

4.7 Run

Click the *Run* button on the left to access the run page, which implements two functions: Status notification and spotting process control.

Start	Check the protocol and save it. Check that the sample plate and pre-spotting slides are placed in the proper positions. Click the *Start* button on the run page to perform spotting.
Pause	Click the *Pause* button to pop up a dialogue for users to select the next action to take. The arrayer will pause temporarily AFTER the current action is completed. Select *Pause* after the cleaning option to halt the system after the wash operation.
Resume	Select the *Continue* option to resume spotting/dispensing at the current position.
Stop	Select the *Exit* option to abort the current run of spotting/dispensing.

4.8 Shutdown the System

Step 1	Close main window of the microarray workstation application software
Step 2	Turn off the computer linked to the microarray workstation
Step 3	Turn off the power switch which is on the rear panel of the microarray workstation
Step 4	Turn off the control box power if using the *NDM*
Step 5	Turn off the vacuum pump

Chapter 5 Getting Started

5.1 Overview

It is essential to run the spotting system for a test immediately after the hardware and software installation. Please check again whether the cables are linked correctly before you startup the system, then switch on the power of the instrument and startup the application software to run the whole system.

5.2 System Startup and Shutdown Procedures

5.2.1 Important Issues before Startup

⚠️	**Warning**: Make sure the workbench is clean and has no obstacles for the platform movement. Make sure there is enough water in the humidifier. Make sure there is enough water in the tank Replace or refill water in the sonicator basin. Make sure the EMS button is released (red light is off). Make sure the photoelectric stop slide does not touch the limit position sensor.

5.2.2 System Startup Steps

- Turn on the vacuum pump
- Turn on the switch which is on the rear panel of the microarray workstation.
- Turn on the microarray workstation (press the device startup button) prior to the linked computer
- Turn on the control box power if using the *NDM*
- Then turn on the computer linked to the microarray workstation
- Launch the microarray workstation application software

5.2.3 Shutdown System Steps

- Turn off the computer linked to the microarray workstation
- Turn off the power switch which is on the rear panel of the microarray workstation
- Turn off the control box power if using the NDM
- Turn off the vacuum pump

5.3 System Startup

Click the "*Start*" (at the left bottom corner on the desktop) menu of the Windows operating system. Select *Program>CapitalBio>SmartArrayer4.0>SmartArrayer4.0*, or click the shortcut "*SmartArrayer4.0*" to start the microarray workstation application software.

After system startup, the following action is required for a self test, which checks the system control card and the serial port communication.

Figure 5-1 System self test interface

Once the self test is successfully completed, the main interface of the software will appear.

⚠️	**Warning**: If a message box "?" appears, please shut down the microarray workstation and contact the technical support engineer to handle it.

5.4 Software Main Interface

The software main interface varies with different microarray workstation models. Here the main interface of the *136D* is shown in Figure 5-2.
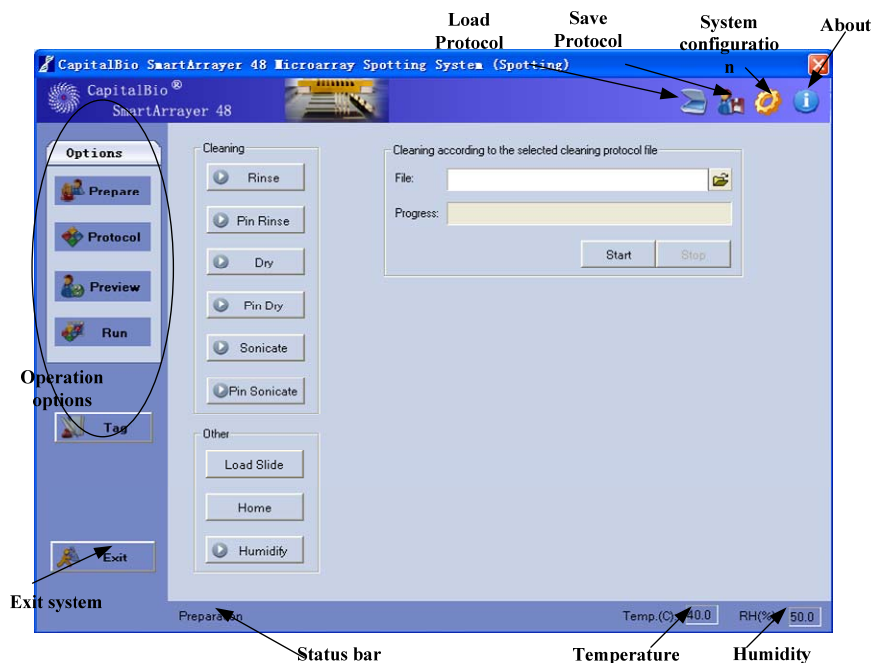
Figure 5-2 The Main interface (*136D*)

⚠️	**Warning**: Please check that all the axes have already moved to the home position.

Chapter 6 System Configuration

	Note:
⚠️	**When to do this:** 1) Immediately after installation. 2) After changing the spotting module to dispensing module, or vice versa. 2) Whenever the instrument is moved, struck or jarred. 3) Whenever pins or rings have been replaced. 4) Whenever you change the type of slides used. 5) Whenever you change the type of microplates used. 6) When you change samples. 7) When the spotting or dispensing is regularly inconsistent. 8) At least once a year.

6.1 Overview

If you do not configure the system after installation, the microarray workstation software will remind you to configure it, as shown in Figure 6-1. This is separated from normal functions such as spotting or dispensing.
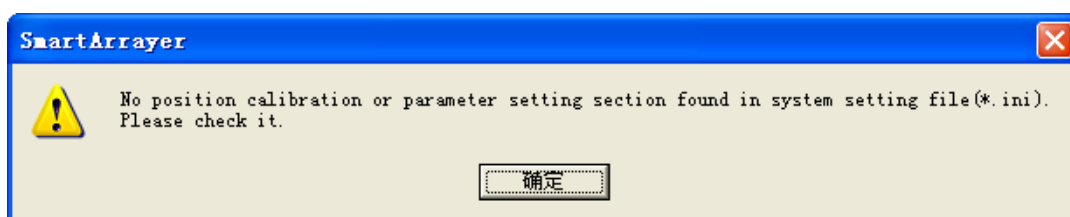
Figure 6-1 The "system setting required" note

This chapter below describes the following procedures: position calibration, axis velocity and acceleration setting, slide configuration, pressure and dispenser setting (only for the dispensing module), other option settings, and backing-up and restoring settings.

6.2 Access System Configuration

	Warning:
⚠️	The parameters described below are crucial. Incorrect Revision or incorrect alteration could cause disastrous outcomes and damage to the instrument.

It is recommended that only a qualified and authorized administrator gains access to modify these settings and parameters. In order to ensure that the settings and parameters are protected, password protection is available. The correct password should be filled in the text box Password: as shown in Figure 6-2, and then click on the button *OK* to access the configuration panel. Immediately after installation, the initial password is "*arrayer*". It is strongly recommend resetting it with a new one as shown in Figure 6-3.
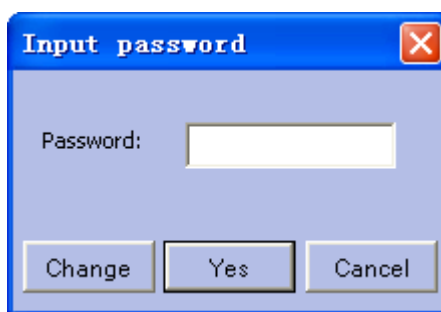
Figure 6-2 Input the password interface
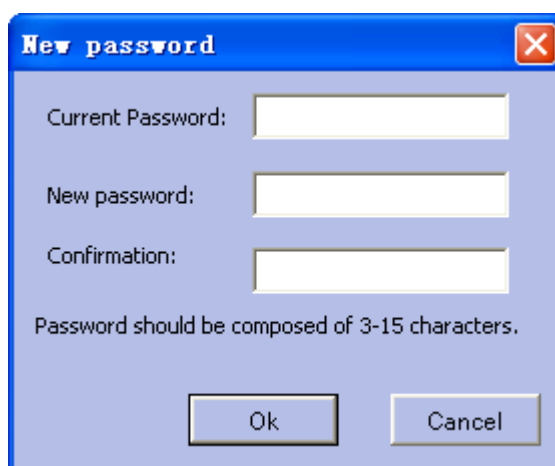
Figure 6-3 Setting the password interface

⚠️	**Warning**: After any modification on the configuration panel, please click on the *OK* button to validate your change.

6.3 Position Calibration

Position calibration refers to determining and recording the locations of several components of the microarray workstation, which are critical to different steps in spotting or dispensing operations. Position calibration should be carried out after any instrumentation changes or maintenance to ensure that precise location is determined and avoids any potential problems.

- Position calibration consists of determining the following coordinates for pin movements relative to the positions of the slides, microplate, rinsing, drying and sonicator modules.
- For the *48S*, the position of 1st slide should be calibrated; while for the *136S*, the positions of both the 1st and the 73rd slide (which is the 1st slide on the second platen) should be calibrated.
- For the *136D* or *48D*, the system liquid position should be set as the same as the sonicating position in the *136S* or *48S*.
- For the *136D* or *48D*, the pre-dispensing position should be set, and the sample source could be either the 96-well plate or the 384-well plate.
- For the *136S*, the position calibration page of the configuration panel is as shown in Figure 6-4.
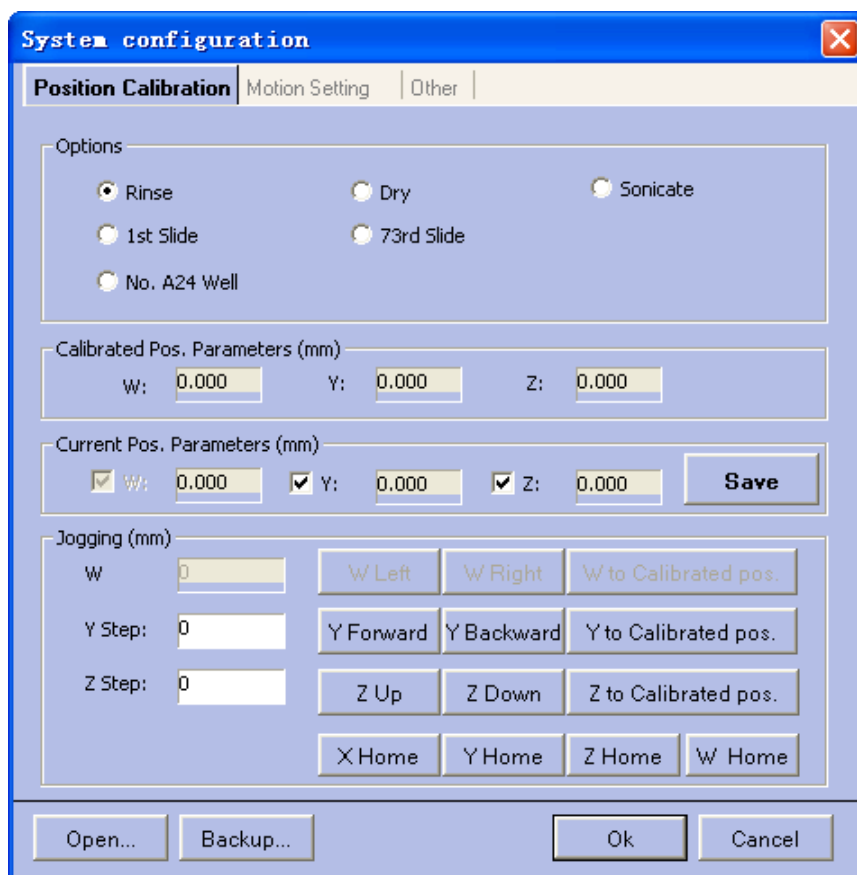
Figure 6-4 The *136S* position calibration interface

For the *48D*, the position calibration page of configuration panel is shown as Figure 6-5.

Figure 6-5 Interface for position calibration of the *48D*

⚠	**Warning**: Axis velocity and acceleration are fixed when calibrating. For axes X(W) and Y velocity is 20mm/s and acceleration is 200mm/s^2; for axis Z velocity is 10mm/s and acceleration is 100mm/s^2.

6.3.1 Rinsing Position Calibration

Rinsing Position Calibration involves the Y and Z coordinates but not the X coordinate. It ensures that all 32 pins (for the *136S* or *48S*, or 8 dispensers for the *136D* or *48D*) are immersed simultaneously in the rinsing solution held by the rinse/dry station and are rinsed thoroughly by flowing distilled water.

The position of the rinse/dry station is described in Section 2.9.

Step 1: Choosing Rinse Location

Choose "*Rinse*" in the location section, then calibrate the current position parameters for the Y-axis and Z-axis but not the X(W)-axis coordinates. If you are setting the Rinse coordinates for the first time, the values will all be set at 0.

Step 2: Initialization of the Axes

Confirm that the X-axis (W-axis also if using the *136S/D*) and Z-axis are in the home position. If they are not in the home position, place them in the home position by clicking on the **X Home** (or **W Home**) or **Z Home** buttons.

Step 3: Adjustments

Move the head along the Y-axis and the Z-axis slowly and carefully watch the head and the axes until the pins or dispensers would be rinsed thoroughly.

⚠	**Warning**: When moving the axes, always be aware of the position of the print-head and pins/dispensers. Make sure that the planned routes of all the moving components are clear of any obstacles before you apply actual movement, to avoid any potentially disastrous collisions.

Step 4: Calibration

In the Current Position Parameters, click box section, confirm that the checked boxes in front of the Y and Z-axis coordinates are *ticked*, and that the ones in front of the X and W-axis coordinates are **not** *ticked*.

Click on the *Calibrate* button and confirm calibration when notified to save the positions to the hard disk. The corresponding coordinates in the calibrated position parameters section will be refreshed at this time. The Z-axis will move to the home position.

6.3.2 Drying Position Calibration

Drying Calibration Position applies to the Y-axis and Z-axis coordinates but not the X-axis coordinate. It ensures that all 32 pins (for the *136S* or *48S*, or 8 dispensers for the *136D* or *48D*) are inserted into the very center of the inhalant holes held by the rinse/dry station at a consistent and sufficient depth to enable efficient simultaneous vacuum-drying of all pins. The location of the inhalant holes is depicted in Section 2.9.

Step 1: Choosing Drying Location

Select the "*Dry*" Location in the location section, then calibrate the current position parameters for the Y-axis and Z-axis, but not the X(W)-axis coordinates. The coordinates will be displayed in the corresponding text box. If the drying location selection is being preformed for the first time, the initial values are all set to 0.

Step 2: Initialization of the Axes

Confirm that the X-axis (W-axis also if using the *136S/D*) and the Z-axis are in their home positions. If not, send the axes to the home position by clicking on the **X Home** (**W Home**) or **Z Home** buttons.

Step 3：Adjustments

Move the Y-axis and the Z-axis by slowly and carefully, watching until the pins or dispensers can be dried properly. It is necessary to immerse the pins or dispensers in the inhalant holes a bit deeper than in the rinsing solution to make sure they are thoroughly dried.

⚠	**Warning**: When moving the axes, always be aware of the position of the print-head and pins/dispensers. Make sure that the planned routes of all the moving components are clear of any obstacles before you apply actual movement, to avoid any potentially disastrous outcomes.

Step 4: Calibration

In the current position parameters section, confirm that the checked boxes in front of the Y and Z-axis coordinates are selected (*ticked*), but that the ones in front of the X and W-axis coordinates are *not ticked*.

Click on the **Calibrate** button and confirm calibration when notified to save the position to the hard disk. The corresponding coordinates in the calibrated position parameters section will be refreshed by the time the Z-axis moves to the home position.

6.3.3 Sonicator (System Liquid) Position Calibration

This section applies to position calibration of the sonicator basin for the pin-based system, and for the dispenser-based system.   For both cases, only the Y-axis and Z-axis coordinates are used and the X-axis (W-axis) coordinate is not used.

The sonicator position calibration ensures that all 32 pins (for the *136S* or *48S*, or 8 dispensers for the *136D* or *48D*) are immersed simultaneously into the sonicator bath. For the dispenser-based system, the sonicator basin is used simply as a reservoir filled with system liquid for uptake into the dispenser.

The location of the sonicator bath is shown in Section 2.9.

Step 1: Choosing Sonicate Location

Choose "*Sonicate*" in the location section, then calibrate the current position parameters for the Y-axis and the Z-axis, but not the X(W)-axis coordinates. The position parameters will be displayed in a corresponding text box. If it is the initial calibration, the initial values are all set to 0.

Step 2: Initialization of the Axes

Confirm that the X-axis (W-axis also if using the *136S/D*) and the Z-axis are in the home position. If not, send them to the home position by clicking on the **X Home** (**W Home**) or **Z Home** button.

Step 3: Adjustments

Move the Y-axis and the Z-axis gradually step by step and carefully watch the axes until the pins are sonicated thoroughly in the sonicator bath, or that the dispensers take in the system liquid properly. It is necessary to immerse the pins in the sonicating solvent or the dispensers in the system liquid a bit deeper than into the sample to ensure completely cleaning the surface area in contact with sample.

⚠	**Warning**: When moving the axes, always be aware of the position of the print-head and pins/dispensers. Make sure that the planned routes of all the moving components are clear of any obstacles before you apply actual movement, to avoid any potentially disastrous outcomes.

Step 4: Calibration

In the Current Position Parameters section, confirm that the checked boxes in front of the Y and Z-axis coordinates are selected, but the ones in front of the X and W-axis coordinates are not ticked.

Click on the **Calibrate** button and confirm the calibration when notified to save the position to the hard disk. The corresponding coordinates in the Calibrated Position Parameters section will be updated by the time the Z-axis moves to the home position.

6.3.4 Slide Position Calibration

Slide Position Calibration applies to the X-axis (W-axis), the Y-axis and the Z-axis coordinates. The X-, Y-, Z-axis coordinates of the 1st slide are required for all models, in addition, the coordinates of the 73rd slide are only required for the *136S/D* models.

The location of the slides is depicted in Section 2.9.

The X-axis (W-axis) and Y-axis coordinates of the upper left corner should be used for calibration. The calibration of the Z-axis coordinates should also be performed. For the pin-based system, the Z-axis coordinate is justified for calibration when the pinhead contacts the slide; while for the dispenser-based system the desired position is when the dispenser pin hovers at 1~2mm above the slide.

⚠	**Warning**: When performing slide position calibration, solo pin or solo dispenser mode is required.

Step 1: Choosing location of the 1st slide

Choose the "1st Slide" in the location section, and then calibrate the current Position Parameters for the X-axis, Y-axis and Z-axis coordinates. The coordinates will be displayed in a corresponding text box. If it is the initial calibration, the initial values are all set to 0.

Step 2: Initialization of the Z-axis

Confirm that the Z-axis is in the home position. If not, send it to the home position by clicking on the Z Home button.

Step 3: Adjustments of the X- and Y- position

Move the X-axis and Y-axis by gradually moving step by step and carefully watch until the pin head or dispenser is located just over the upper left corner of the slide.

| | **Warning**:

When moving the axes, always be aware of the position of the print-head and pins/dispensers. Make sure that the planned routines of all the moving components are clear of any obstacle before you apply actual movement, to avoid potentially disastrous outcomes. |
|---|---|

Step 4: Calibration of the X- and Y- position

In the Current Position Parameters section, confirm that the checked boxes in front of the X and Y-axis coordinates are selected, but the one in front of the Z-axis coordinate is not ticked.

Click on the **Calibrate** button and confirm calibration when notified to save the position to the hard disk. The corresponding coordinates in the Calibrated Position Parameters section will be updated by the time the Z-axis has moved to the home position.

Step 5: Adjustment of the Z- position

Firstly move the X and Y- axes to the center of the pin/dispenser over the first slide.

Move the Z-axis down slowly in incremental movements, and carefully watch until the pin head or dispenser reaches the desired location above (dispenser-based system) or just contacting (spotting system) the slide surface.

Step 6: Calibration the Z- position

In the Current Position Parameters section, confirm that the checked box in front of the Z-axis coordinates is selected, but the ones in front of the X and Y-axis coordinates are not ticked.

Click on the **Calibrate** button and confirm the calibration position when notified to save it to the hard disk. The corresponding coordinates in the Calibrated Position Parameters section will be updated by the time the Z-axis has moved to the home position.

Step 7: Calibration of other slide positions (as required)

For the *136S/D* models, the calibration of the 73rd slide is also required. When performing these calibrations, steps 1 to 6 are to be repeated with all the operations performed on the X-axis substituted with operations on the W-axis.

6.3.5 Microplate Position Calibration

The microplate position refers to the X, Y, Z-axis coordinates when the pin or dispenser drops into the wells of the microplate to uptake sample. The Microplate Position Calibration ensures that the uptake volume of sample is adequate and consistent among the all the pinheads or dispensers. The coordinates of the well A24 for the 384-well plate or A12 for the 96-well plate should be used for calibration.

The location of the microplate is depicted in Section 2.9.

| | **Warning**:

When performing microplate position calibration, solo pin or solo dispenser mode is required. |
|---|---|

Step 1: Choosing Location Well Number

In the Location Section, choose "Well A24" for the *136S/48S*, or choose "Well A24 / Well A12" for the *136D/48D* according to the different type of microplates because the *136D/48D* supports both the 384-well plate and the 96-well plates, then calibrate the Current Position Parameters. The X(W)-axis, Y-axis and Z-axis coordinates will be displayed in the corresponding text box. If it is the initial time, the initial values are all set to 0.

Step 2: Initialization of the Axes

Confirm that the Z-axis (X-axis also if *136S/D*) is in the home position. If it is not in the home position, send it to the home position by clicking on the **Z Home** button (X Home also if using the *136S/D* microarray workstation).

Step 3: Adjustments

Move the X-axis (W-axis instead if the *136S/D*), Y-axis and Z-axis by slowly moving step by step and carefully watching until the pin or dispenser is located at well A12 or A24 on the microplate, thereby ensuring proper sample uptake.

⚠	**Warning**: When moving the axes, always be aware of the position of the print-head and pins/dispensers. Make sure that the planned routines of all the moving components are clear of any obstacle before you apply actual movement, to avoid potentially disastrous collisions.

Step 4: Calibration

In the Current Position Parameters section, confirm that the checked boxes in front of the X-axis (or W-axis if *136S/D*), Y-axis and Z-axis coordinates are *all ticked*.

Click on the **Calibrate** button and confirm calibration when notified to save the axes position to the hard disk. The corresponding coordinates in the Calibrated Position Parameters section will be updated, and then the Z-axis has moved to the home position.

6.3.6 Pre-dispensing Position Calibration

The Pre-dispensing Position Calibration Position applies only to the *136D/48D*. It refers to determining and recording the Z-axis coordinate when pre-dispensing just over the well holding the sample liquid immediately after taking up the sample. Only the Z-axis coordinate is concerned.

Step 1: Choosing the Pre-dispensing Location

In the Location Section, choose "Pre-dispense", and then calibrate the Current Position Parameters. The Z-axis coordinates will be displayed in the corresponding text box. The X(W)-axis and Y-axis coordinates are invalid. If it is the initial calibration, the initial values are all set to 0.

Step 2: Initialization of Axes

Confirm that the Z-axis (X-axis also if *136S/D*) is at the home position. If not, send the Z-axis to the home position by clicking on the **Z Home** (X Home also if *136S/D*) button.

Step 3: Adjustments

Move the Z-axis in slow, incremental moves step by step and carefully watching until the desired location is achieved.

	Warning: It is strongly recommended that you carry out this pre-dispensing position calibration just after you complete the microplate position calibration with out moving the X-axis(or W-axis) and Y-axis, and keep the microplate in position for reference. When moving the axes, always be aware of the position of the print-head and pins/dispensers. Make sure that the planned routines of all the moving components are clear of any obstacle before you apply actual movement, in case of potential disastrous collisions.

Step 4: Calibration

In the Current Position Parameters section, confirm that the checked box in front of the Z-axis coordinate is *ticked*.

Click on the Calibrate button and confirm it when notified to save the calibration position to the hard disk. The corresponding coordinates in the Calibrated Position Parameters section will be updated by the time the Z-axis has moved to the home position.

6.4 Motion Setting

The velocity and acceleration parameters control long-distance movement of each axis. These parameters influence the time and the cost of the spotting procedure, as well as the performance.

It is recommended to set the acceleration to 5-10 times of velocity. The acceleration to velocity ratio should be smaller with higher velocity settings and greater with lower velocity settings.

Operation Guide:
- Access the Motion Setting page on the Configuration Panel, as shown in Figure 6-6
- Drag the scrollbar to adjust the velocity or acceleration of the corresponding axis
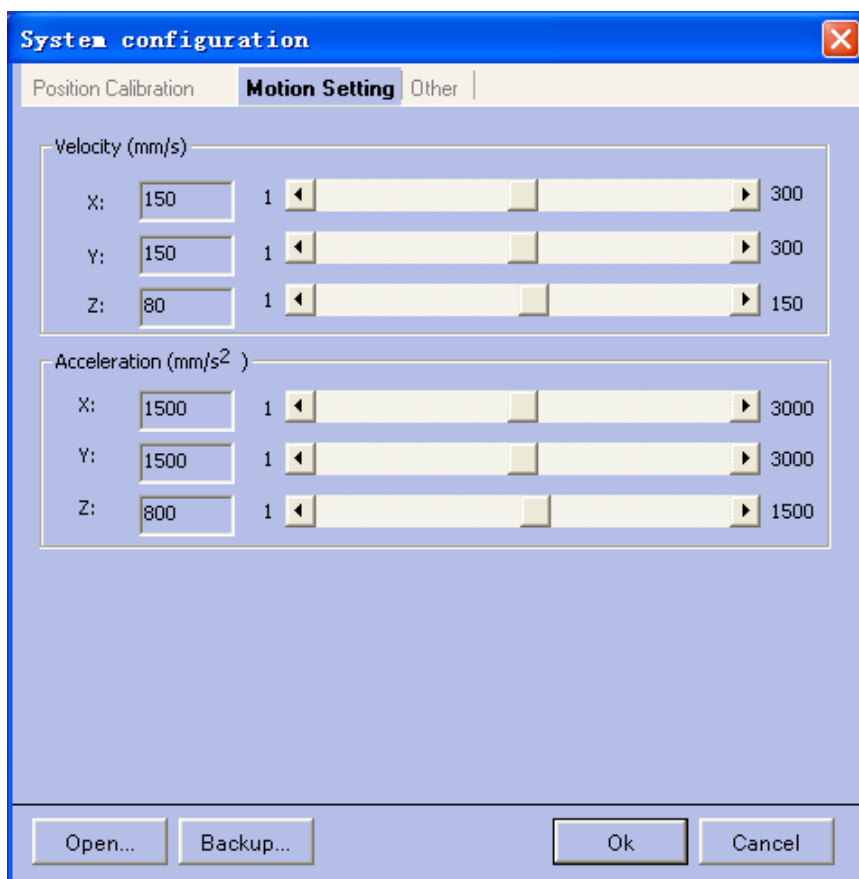
Figure 6-6 Velocity parameters setting interface

6.5 Pressure parameters setting

The Pressure Parameters page applies only to the *136D/48D*. These parameters are used by the control cassette to generate the proper pressure. They are already set by the manufacturer before packing.

Operation Guide:
- Access the Pressure Parameters page on the Configuration Panel, as shown in Figure 6-7
- Specify the positive and negative pressure respectively in the corresponding text boxes. You can always restore them to the default setting of the manufacturer by clicking on the **Default** button
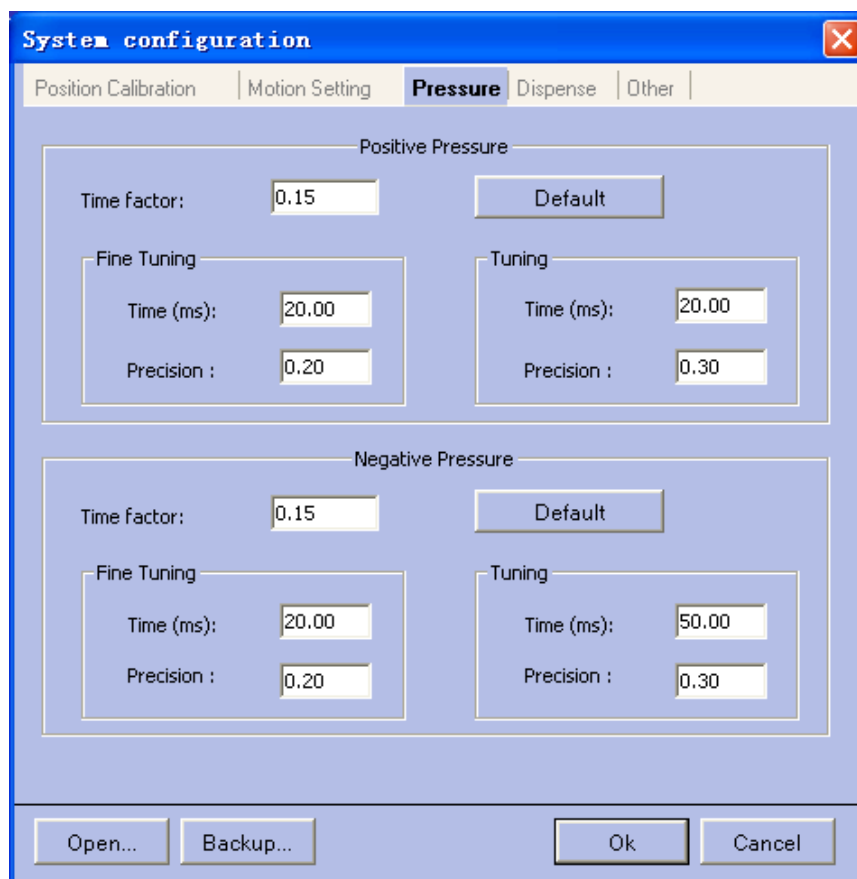
Figure 6-7 Dialogue box for setting Pressure parameters

6.6 Dispensing parameters setting

The dispensing parameters page applies only to the *136D/48D*. There are parameters for pressure and time that are required for different stages of the work flow. The parameters are already set by the manufacturer before packing.

The dispensing parameters consist of two groups of parameters used during the cleaning and dispensing procedures.

There are 3 consecutive steps in the *Cleaning Procedure*: pumping in/out circulating water through the channel. *Rinsing*: washing out liquid droplets that adhere to the inner side of the channel with high flows of water, *Draining*: blowing out liquid droplets that adhere to the inner surface of the dispenser by high pressure air, and *Drying*: drying the inner surfaces by negative air pressure. The first group of parameters applied to these steps in the cleaning procedure.

The second group of parameters applied during the dispensing procedure. They are as follows:

Aspiration pressure: refers to the pressure applied to take up the sample or the system liquid preparations.
Aspiration pressure for air: refers to the pressure applied to inhale air to separate sample and system liquid.
Dispensing pressure: refers to the pressure applied to dispense the sample through the dispenser orifice onto the slide surface.
Aspiration time for sample: refers to the time required to take up the sample.
Aspiration time for system liquid: refers to the time required to take up the system liquid.

Aspiration time for air: refers to the time required to inhale air.

Drying time: refers to the time required for vacuum-drying after taking up sample or system liquid.

⚠	**Warning**: It is recommended to modify the aspiration time and dispensing pressure, according to the viscosity and surface tension characteristics of the sample. When all dispensers are equipped, the dispensing pressure should be no less than 3kPa and the dispensing time no less than 0.25ms.

Operation Guide:

● Access the Dispensing Parameters page on the Configuration Panel, as shown in Figure 6-8

● Specify the parameters applying to the cleaning or the dispensing procedures respectively in the corresponding text boxes. You can always restore the parameters to the manufacturer's default setting by clicking on the **Default** button
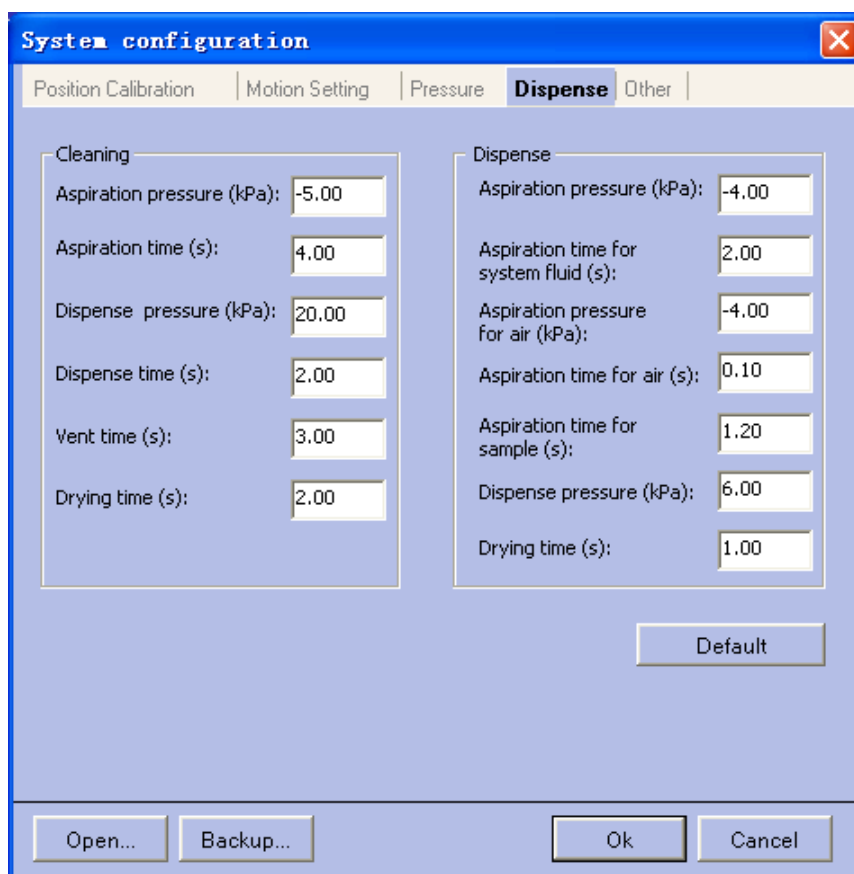
Figure 6-8 The interface for setting Dispensing parameters

6.7 Other Options

Other pages provide access to other important parameters as following (*see* Figure 6-9).

Slide Parameters Section: this involves dimensional information about the slide, the margins preserved from spotting, etc.

Sample Selection Priority Section: applies to virtual sample plate panel (*see* Section 7.4.2),

when editing sample list. These parameters are used to control sample arrangement manner on the plate, and can be customized by users to their specifications.
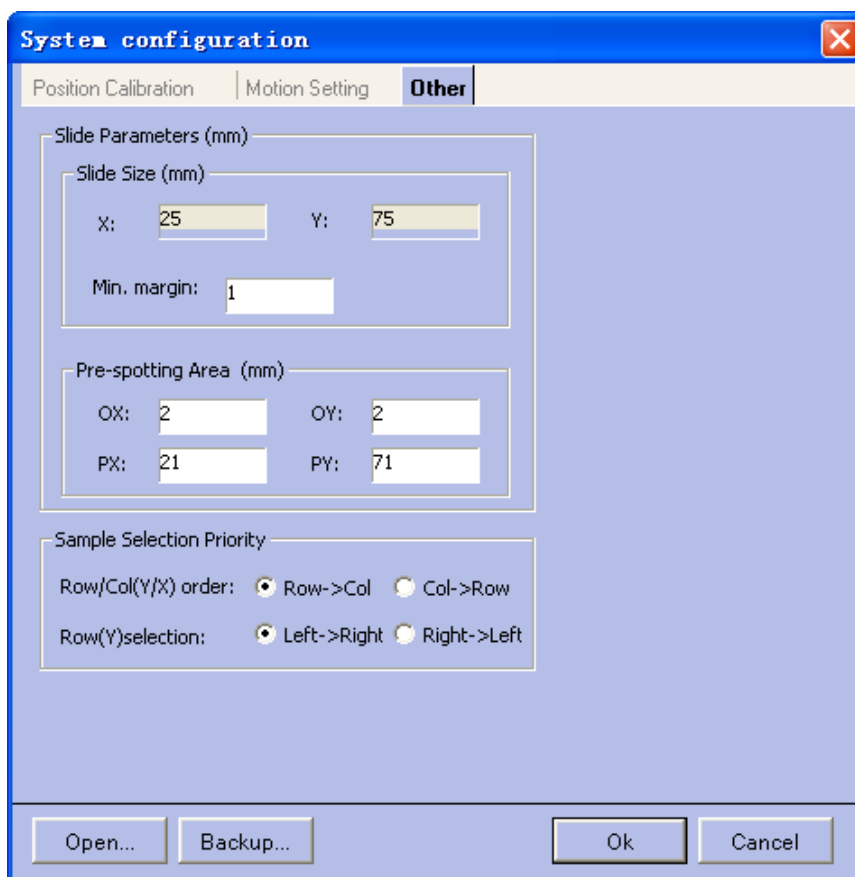
Figure 6-9 The interface for setting Other Options

6.8 Backup and Restore

Confirm that all necessary modifications are put on the configuration panel and click on the *OK* button at the bottom of the panel to save the modifications into the system configuration file and then exit the configuration panel. The settings will automatically be applied when the system starts up next time.

To avoid accidentally misplacing data in the system configuration file, the Save and Load functions are provided. You can click the *Backup* button to save data as a system configuration file (*.sas), and click the *Open* button and confirm when notified to restore settings previously saved by selecting a *.sas file.

Chapter 7 Spotting or Dispensing

7.1 Overview

The most common spotting/dispensing procedure is described in this chapter to guide you to successfully do slide spotting/dispensing operations.

⚠️	**Warning**: Most discussion in this chapter uses the *136S/D* system for examples. For the *NDM*, the same operations are required to a great extent, except some description of 'spotting' operations are substituted with 'printing' and some additional settings are unique to *NDM*.

7.2 System Startup

- Power the system according to the instructions depicted in Section 5.2
- Launch the software system according to instructions depicted in Section 5.2, and access the main User Interface.

7.3 Prepare

You can access the preparation panel by clicking on the **Prepare** button on the left. It is the default, first page displayed immediately after the software system is launched as shown in Figure 7-1. The instructions on this page are common to both the *136S/48S* systems.
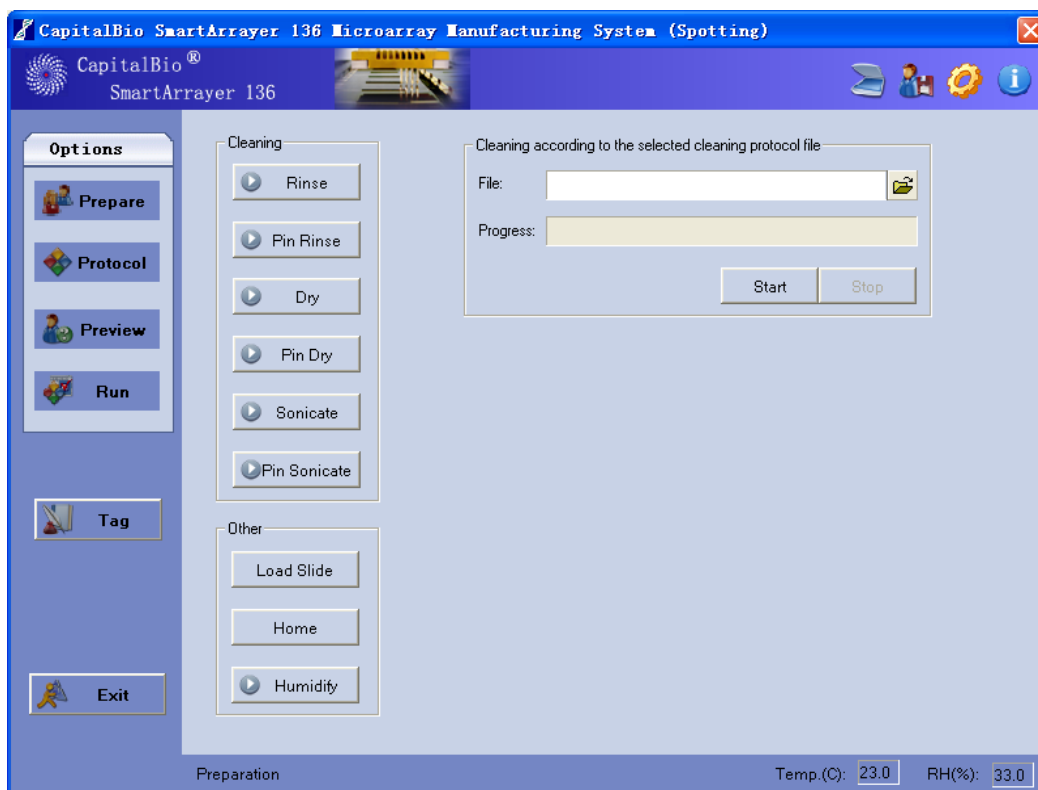

Figure 7-1 Preparation before spotting

7.3.1 Cleaning Section

In the cleaning section you can carry out operations including: *Circulate* (the water held in the rinsing basin), *Rinse* (the pin or dispenser), *Vacuum* (by vacuum pump), *Dry* (the pins or dispensers by inserting them into the inhalant holes on the wash/dry station), *Excite* (water held in the basin by turning on the sonicator) and *Sonicate* (the pins by inserting them into the basin). For the ***NDM***, *Excite* and *Sonicate* will be replaced with *Drain* (the channel inside the dispenser).

7.3.1.1 Circulate

Click on the ***Rinse*** button to start the rinsing program. The pump starts working prior to spotting with the microarray workstation in order to drive a water flow through the basin and make sure that there is enough distilled water in the basin during the subsequent rinse operations.

Operation Guide:
- Click on the ***Rinse*** button and push it down to a depressed appearance for "***Rinse***" to start circulating. Meanwhile the rinse indicator light will light up
- Click on the ***Rinse*** button again to pop it up to a released appearance, and stop the water circulating. Meanwhile the rinse indicator light will turn off

7.3.1.2 Pin Rinse

Move the pins or dispensers to the location indicated by the rinsing position calibration record and rinse them in the basin prior to commencing spotting.

Operation Guide:
- Click the ***Pin Rinse*** button and push it down to a depressed appearance as "***Pin Rinse***". The Y-axis and Z-axis are moved to a rinsing position and rinsing starts. Meanwhile the rinse indicator continues to be illuminated
- Click on the ***Pin Rinse*** button again to pop it up to a released appearance. The pins or dispensers are lifted along the Z-axis and rinsing stops. Meanwhile the rinse indicator light turns off

7.3.1.3 Vacuum

Vacuum refers to the operation to power up the pump prior to spotting.

Operation Guide:
- Click the ***Dry*** button and push it down to a depressed appearance to begin "***Drying***" and start the vacuuming. Meanwhile the dry indicator light is lit up
- Click on the ***Dry*** button again to pop it up in a released appearance and stop vacuuming. Meanwhile the dry indicator light turns off

7.3.1.4 Dry

Move the pins or dispensers to the location indicated by the drying position calibration record and dry them in the inhalant holes on the wash/dry station prior to spotting.

Operation Guide:
- Click the ***Pin Dry*** button and push it down in a depressed appearance for "***Pin Dry***". The Y-axis and Z-axis are moved to the drying position and drying starts. Meanwhile the dry indicator continues to be illuminated
- Click on the ***Pin Dry*** button again to pop it up to a released appearance. The pins or

dispensers are lifted along the Z-axis and the drying stops. Meanwhile the dry indicator turns off

7.3.1.5 Excite

Excite refers to the operation to power up the sonicator.

Operation Guide:
- Click the *Sonicate* button and push it down to a depressed appearance for "*Sonicate*" and the sonicator starts working. Meanwhile the sonicator indicator light is on
- Click on the *Sonicate* button again to pop it up to a released appearance and the sonicator stops working. Meanwhile the sonicator indicator light is off

7.3.1.6 Sonicate

Move the pins or dispensers to locations indicated by the sonicating position calibration record and sonicate them in the basin prior to spotting.

Operation Guide:
- Click the *Pin Sonicate* button and push it down to a depressed appearance for "*Pin Sonicate*". The Y-axis and Z-axis are moved to the drying position and sonicating starts. Meanwhile the sonicate indicator light continues to be on
- Click on the *Pin Sonicate* button again to pop it up to a released appearance. The pins or dispensers are lifted along the Z-axis and sonicating stops. Meanwhile the sonicate indicator light is turned off

7.3.2 Load Slide

Click on **Load Slide** button and the platen moves out automatically to facilitate slide loading for the user.

The platen of the *48S/D* moves closer to the user. The platens of the *136S/D* move laterally to the left and the right sides.

7.3.3 Home

Click the **Home** button to send the X-axis (W-axis also if *136S/D*), Y-axis and Z-axis to the home position quickly.

The **Home** button is usually used after slide loading or abnormal termination during spotting operation.

7.3.4 Humidify

Humidify refers to operations to power up the humidifier to achieve the required humidity within the enclosure of microarray workstation.

Operation Guide:
- Click *Humidify* button and push it down to a depressed appearance for "*Humidify*" and start humidifying. Meanwhile the humidify indicator light is lit up
- Watch over whether the desired relative humidity is achieved by reading the display in the status bar
- Click on the humidify button again to pop it up to a released appearance and stop humidifying. Meanwhile the humidify indicator light is turned off

7.4 Protocol

Click the ***Protocol*** button on the left to gain access to the microarray setting, sample sequence (Virtual plate) and the spotting/dispensing page process. The microarray setting page is the default first page to display, as shown in Figure 7-2.

7.4.1 Array Setting

Figure 7-2 The Pin array setting interface

Step 1: Specify pin/dispenser pattern

Select the correct pattern in the combo box which reflects the pattern of the pins/dispensers actually installed on the bracket. The pattern of pins can differ from 1*1，1*2，1*4, 2*1, 2*2, 2*4，4*1，4*2，4*4；8*1，8*2 to 8*4, representing 12 different patterns.

Whenever you change the pin/dispenser pattern, parameters relevant to arrays previously set *will be invalid*.

For instance, a pattern of 2*4 pin configuration is depicted in Figure 7-3.
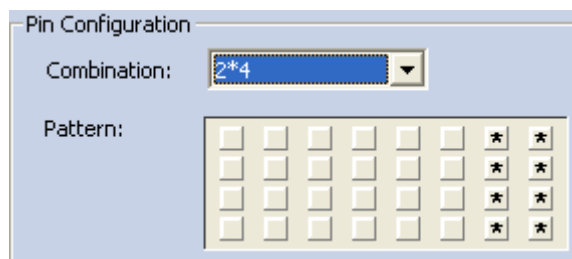
Figure 7-3 Pin configuration

If the *NDM* is concerned, the layout of the dispensers could be one of following six patterns: 1*1, 1*2, 2*1, 2*2, 4*1, 4*2 and should be compliant to the actual situation of the print head.

If a 384-well sample plate is to be used, then pattern 1*1 is the only choice for you can make.

For instance, the pattern of 2*2 dispenser configuration is depicted in Figure 7-4.
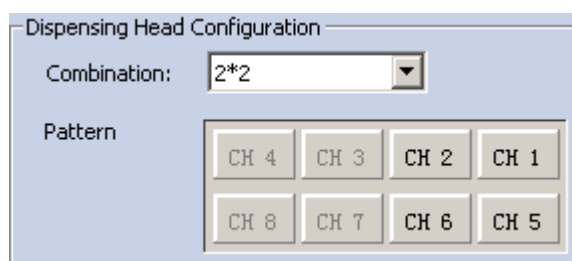
Figure 7-4 The 2*2 Dispensing head configuration

Step 2: Slide Settings

Slide parameters OX, OY, PX, PY, LX, and LY are described in Figure 7-5. Parameters OX, OY, PX, PY determine the area actually used for spotting.
- Confined to the scanner specifications, OX and OY should be no less than the Minimum Margin specified on the configuration panel, and the dimensions of the barcode on the slide should be taken into account when determining PY
- Modifying OX might reduce PX automatically, likewise OY to PY
- The upper bound of PX or PY should not exceed the slide dimension, while the lower bound relates to pin/dispenser patterns
- Modification of PX or PY might affect array settings
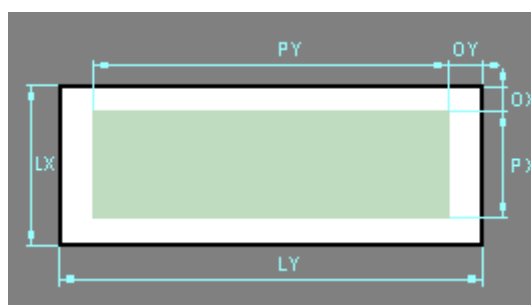
Figure 7-5 Slide parameters

Step 3: Slides

In this section you can specify the number of the slide to start at, the number of the slide to finish at, and the sum of slides to be spotted on. At least one slide must be reserved for pre-spotting by the *136S/48S*. For example, using the *48S*, if you desired 40 spotted slides and

needed 3 prespotting slides, the required total number of slides on the arrayer platen is 43.

Step 4: Array Settings

One spot constitutes one feature on the slide. Parameters such as spot distance, sum of spots, array spacing, and sum of arrays provide information about the dimension and layout of features on the slide.

**Spot distance**: refers to the displacement of the same pin/dispenser between successive spotting actions. It should be modified according to spot size, and will usually be 180-400μm.

Sum of spots: Array spacing: refers to spacing between borders of neighboring arrays.

Sum of arrays: should be no greater than the Maximum sum of arrays. The latter could be calculated according to array spacing, array size and spotting area (determined by PX, PY).

Step 5: Sample Replicates

Sample Replicates define how many spots will be done with the same sample in an array. The maximum could be greater than the product of the X- and Y sum of spots, namely the total sum of spots in an array.

Step 6: Array Replication

Array replication is a switch indicating whether the sample sequences of arrays are consistent.

7.4.2 Sample Sequence

In a *Sample sequence* tab you can plan the sampling method from microplates and arrange the sequence, as shown in Figure 7-6.
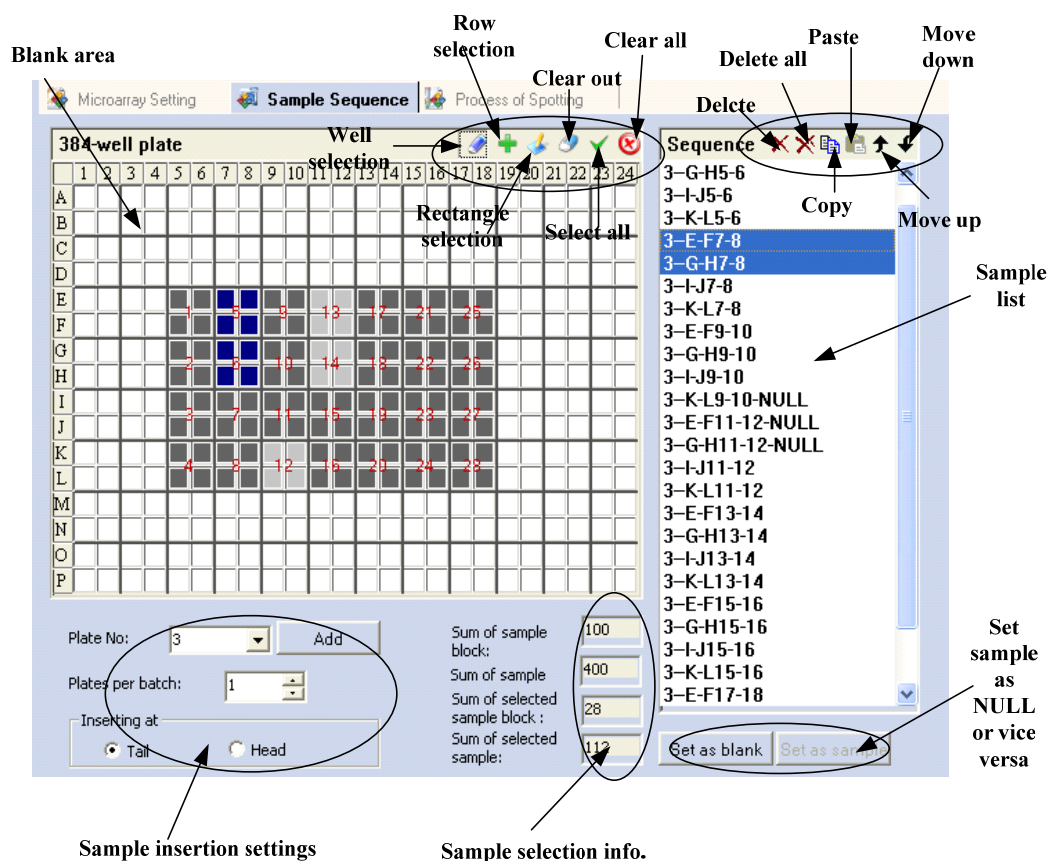

Figure 7-6 Sample setting

Step 1: Select sample wells on sample plate

Use the tool buttons on the upper right corner to select sample wells in a certain order. The tools provided are as following:

Select: You have four ways to select the sample well into the sampling list. Whatever method you apply, the selection operation is based on a sample group determined by the pin/dispenser pattern. That means a sample group (1*1, 2*2 and so on) is the smallest unit that can be selected or deselected.

Single select: Click on the corresponding well and select one sample group at one time.

Row/Column Select: When you click on a row/column head, as many as possible sample groups in that row/column are selected; when you click on the inner area, as many as possible sample groups in either the row or column are selected, according to Sample Selection priority setting on the configuration panel. The sample groups selected by row/column will also be arranged in the order indicated by the sample selection priority setting too. However, if some sample groups in that row/column have been already selected previously, they will not be re-ordered by the new selection.

Rectangle Select: Press the left mouse button and drag to draw a rectangle and sample groups within the rectangle will be selected and arranged in the order indicated by the sample selection priority setting. However, if some sample groups in that rectangle have been already selected previously, they will not be re-ordered by the new selection.

Select All: Select as many sample groups as possible on the plate and REARRANGE THEM ALL according to the sample selection priority setting.

Clear: Click to remove a single sample group or drag and draw a rectangle to remove multiple sample groups from the selection.

Clear all: Remove all the selected sample groups from the plate.

Step 2: Select Plates

In the section just beneath the virtual plate, you can:
- Input or select the sequence number of the current plate
- If several successive plates share the same sequence pattern as the current editing virtual plate, you can specify a number greater than 1 in the plates per batch combo to insert them into a list with one click. The sequence number of the successive plates is automatically increased from the current one
- You can determine whether to insert the current selection before or after the selected item (if there is one) in the sequence list on the right hand side, or to insert the current selection at the tail (default) or head of the sequence list

Step 3: Add to list

Click the *Add* button to insert the current selection into the existing sequence list.

Step 4: Adjust sequence list (optional)

The *Delete*, *Delete all*, *Copy*, *Paste*, *Move up* and *Move down* tool buttons are provided in the sequence list box for your convenience to adjust the content of the existing sequence list.
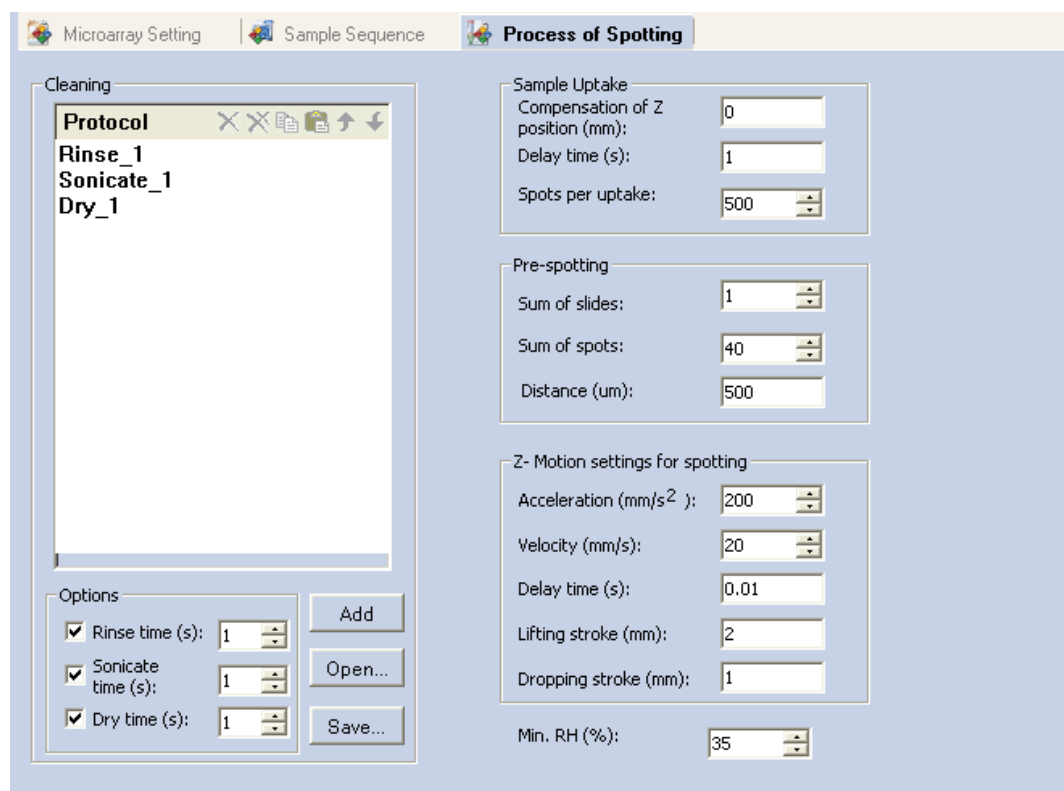
Step 5: Set some samples as blank (optional)

	Note: For samples whose dispensing properties are greatly different, the user can divide the samples into several groups and dispense them separately using different protocols, including aspiration time, aspiration pressure, dispensing time and dispensing pressure. To do this, users can insert all samples in the array and set those groups of sample which have different protocols as blank.

	Note: If certain wells on the plate actually hold no sample, though corresponding sample groups are inserted into the sequence list, you can select the item and click "*Set As Blank*" button to skip operations such as sample taking and spotting to these very sample groups.

7.4.3 Spotting Process

Spotting Process tab only applies to the *136S/48S* as shown in Figure 7-7.

Figure 7-7 The spotting parameters setting interface

Step 1: Cleaning

The *Cleaning* Section describes the cleaning function of the system.

The cleaning protocol has four steps: *Rinse-Sonicate-Rinse-Dry*. The number of cycles of this

iteration is dependent on the sample characteristics. Samples with high viscosity require more cycles and an elongated time span. It is recommended to optimize the cleaning protocol to save time by conducting a test for remnant fluorescence test prior to full-state usage.

- Specify *Rinse Time*, *Sonicate Time* and *Dry Time* respectively, and confirm that the check box in front of the desired operation is selected
- Click the *Add* button to insert the selected operations into the protocol
- Adjust the protocol with the tools provided in the list box, such as *Delete*, *Delete all*, *Copy*, *Paste*, *Move up* and *Move down*
- You can save and load protocols for different sample types with the aid of the *Save* and *Open* Buttons

Step 2: Sample Uptake

The parameters of sample uptake are corresponding to different sample preparations.

In case the sample phase boundary in the plate well deviates from the record of the Z-axis position calibration, it is necessary to compensate for the sample-uptake descending stroke of the pin. The positive values represent deeper deviations, while negative values indicate the opposite.

Delay time: refers to time spent on keeping the pin head immersed into the sample preparation. Its optimum differs from sample to sample and could be customized.

Spots per uptake: refers to the maximum number of spots that should be done per uptake. It differs from sample to sample.

Step 3: Pre-spotting Settings

The pre-spotting refers to the procedure to deposit certain excess amounts of spotting solution onto the pre-spotting slide immediately after initial up-take of samples, for the purpose of spot uniformity. In this section, the parameters indicate how many slides, how many spots and how much distance (spacing) between pre-spots should be used in the pre-spotting operation.

Sum of slides: refers to the amount of slides that will be used for pre-spotting. In the *Slide Preview* tab (see Section 7.5.1), you will be informed about how many pre-spotting slides will be needed. If you do not have many slides to spot, more slides could be set as pre-spotting slides.

Sum of spots: it is dependent on the sample characteristics.

Distance: refers to the displacement between two neighboring pre-spots. Pre-spots are larger than working spots, so the distance should be greater. It is also dependent on the sample characteristics. It should not be too small otherwise contamination between samples could be brought into the pre-spotting procedure.

Step 4: Z-Motion Settings of Spotting

Parameters herein should be optimized according to sample characteristics to ensure the proper performance.

Delay time: refers to the time required while the pins contact the slide when spotting.

Lifting stroke: refers to the distance the pin moves upward relative to the record of the Z-axis position calibration when spotting.

Dropping stroke: refers to the distance the pin moves downward relative to the record of the Z-axis position calibration when spotting.

Step 5: Minimum Relative Humidity

Min. RH: refers to the minimum relative humidity that should be used within the microarray workstation. It is dependent on the characteristics of the sample.

7.4.4 Dispensing Process

Process of Dispensing tab only applies to *136D/48D,* as shown in Figure 7-8.

Figure 7-8 The dispensing parameters setting interface

Step 1: Cleaning

The cleaning section describes the cleaning method of the system.

The cleaning protocol is the iteration of five steps: *Rinse-Drain-Rinse-Drain-Dry*. The number of cycles of this iteration is dependent on the sample characteristics. Sample with high viscosity requires more cycles and extended cleaning times. It is recommended to optimize the cleaning protocol to compress the time required by conducting a remnant fluorescence test prior to full-state usage.

● Specify *Rinse time*, *Drain time* and *Dry time* respectively, and confirm that the check box in front of the desired operation is selected
● Click the *Add* button to insert the selected operations into the protocol
● Adjust the protocol with the tools provided in the list box, such as *Delete, Delete All, Copy, Paste, Move Up* and *Move Down*

You can save and load the protocols for different sample types with the aids of the *Save* and *Open* Buttons.

Rinse times: refers to the recurrence of Rinse operations, which gives the pin head a flush with circulating distilled water. Each Rinse operation lasts 1 second.

Drain times: refers to the recurrence of Drain operations, which wipe out liquid droplets out from the outer side of the dispenser channel with positive pressure. Each Drain operation lasts 1 second.

Dry times: refers to the recurrence of Dry operations, which dry the dispensers with a negative vacuuming pressure. Each Dry operation lasts 1 second.

Step 2: Sample Uptake

Select proper sampling mode for sample for different sample preparation.

Mode: refers to sampling mode for sample and can be selected from two alternatives: Full Sample mode and Partial Sample mode. In *Full Sample* mode, only sample preparation is taken into the dispenser channel, so the sample uptake can be put back into the sample plate. In *Partial Sample* mode, system liquid (such as distilled water) is first taken into the dispenser channel, then some air and actual sample preparation is taken, while the sample uptake cannot be put back into the sample plate.

Advantages and disadvantages of the two modes respectively are listed in the Table X below.

Table IX Sample uptaking mode

Mode	Full sample	Partial sample
Uptake sequence	Sample only	System liquid-Air-Sample
Sample uptake	>35μL	>5μL
Sample reutilization	Yes	No
Maximum spots per uptake	>5000	>200

Extraction Times: refers to the recurrence of Dry operations after taking in system liquid or sample preparation. Each Dry operation lasts 1 second. The recurrence is related to the sample characteristics.

Step 3: Pre-dispensing Setting

Pre-dispensing refers to the procedure and method to dispense a certain amount of sample preparation at a specified location immediately after taking up sample, for the purpose of spots uniformity.

Times: refers to the repeat of pre-dispensing operations after taking up sample.

Dispense Time: refers to the duration of each pre-dispensing operation measured in micro seconds.

Step 4: Dispense time

Allows one to differentiate the dispense time period for each channel when multiple channels are used in the application.

Dispense time: refers to the duration of each Dispense operation measured in micro seconds.

Step 5: Minimum Relative Humidity

Min. RH: refers to the minimum relative humidity that should be used in the microarray workstation. It is dependent on the sample.

7.4.5 Backup & Load Setting File

You can access the *Save* and *Load* buttons on the top toolbar to backup and restore the editing protocols.

	Warning:
⚠️	It is recommended that you save the protocols before you run spotting application.

7.5 Spotting Preview

Click on the ***Preview*** button on the left and access the spotting preview panel. There are two tabs: Slide preview and sample preview. Slide preview is the first to display.

7.5.1 Slide Preview

Slide Preview provides a visual aid for users, as shown in Figure 7-9. In this section, according to the protocol planned, the simulation of the spotting pattern on the slide will be displayed to help users confirm whether the protocol and configuration are correct, thus reducing the possibility of mistakes. In addition, you can adjust the slide sum placed one time (see Section 7.4.3).

Total spots per slide: refers to the total sum of spots per slide, calculated from microarray settings.

Sum of pre-spotting slides: refers to an estimation based on the microarray settings and only applies to the ***136S/48S***.

Slide View: depicts the distribution of the spots on the slide.

Slide info: slides for spotting, slides for pre-spotting, and unused slides are each labeled with different colors as well as text descriptions. There is no pre-spotting for pre-spotting in the ***136D/48D***.

Figure 7-9 The slide preview interface

7.5.2 Sample Preview

Sample Preview help users to estimate the sample spots distribution on each slide, according to the current microarray settings and sample sequence, as shown in Figure 7-10. In addition, tools for exporting GAL files are accessible on this page.

Save Info…

Click the "*Save Info…*" button to save the distribution information of spots on the slide as an Excel file. A dialog will pop up for the user to customize the file name and path.

Tag Sample...

Click the *Tag Sample* button to import an Excel file holding record of the sample ID and tag pairs to prepare sample information for exporting GAL files. The imported Excel file can be edited and arranged in a special format with stand-alone Microsoft Excel application or generated by Tag module of this software system (see Figure 7-10). A dialog will pop up for user to select the proper Excel file.

"*Save Tagging Info…*" button will be eventually enabled on successful retrieval of sample information from the file mentioned above.

Save Tagging Info…

Click the "*Save Tagging Info…*" button to save the combination of spot distribution and sample tag information as a GAL file, which can be recognized by GenePix software for data analysis. A dialog will pop up for users to customize the file name and path.
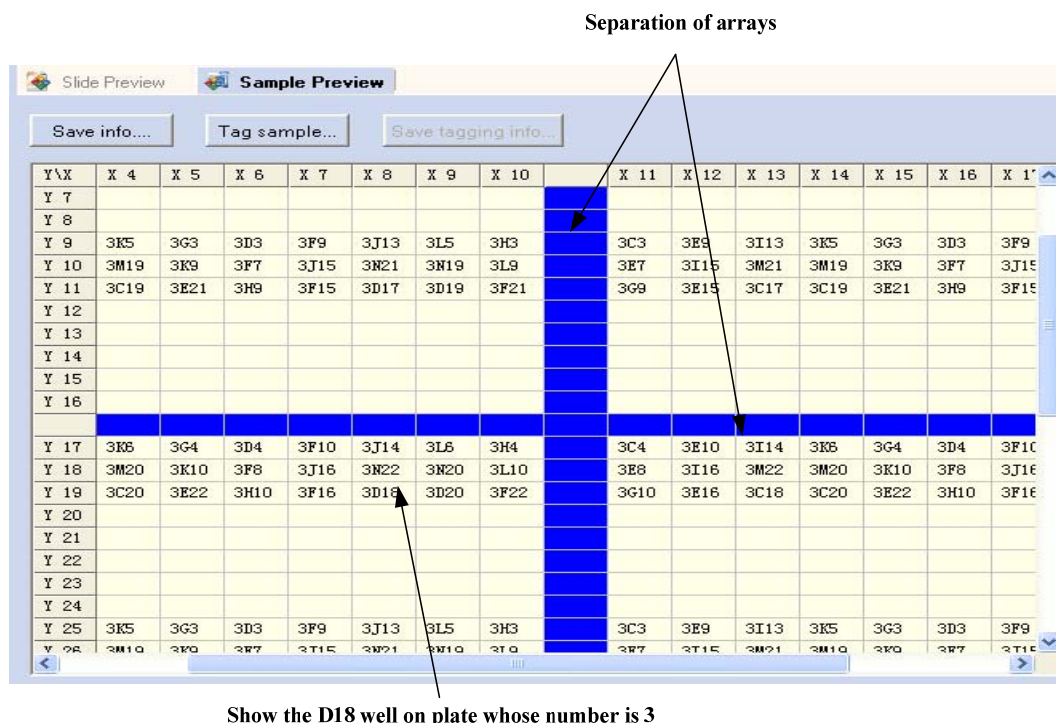

Figure 7-10 The sample preview interface

7.6 Start Spotting

Click the *Run* button on the left to access the run page, which implements two functions: Status notification and spotting process control.

7.6.1 Start

Step 1: Check the protocol and save it.

Step 2: Check that the microplate and pre-spotting slides are placed in the proper positions.

Click the *Start* button on the run page to perform spotting.

Once the spotting procedure is initiated, the slide information, plate information and progress bar will notice users of the status of the operation in time. The *Pause* button will be enabled in need operation halt (see Section 7.6.3 for details).

⚠	**Warning**: If the *Start* button remains in a disabled state while the microarray workstation is fully functional, it is possible that you have not performed the configuration settings, especially the position calibration after an installation or hardware change.

7.6.2 Running Status Prompt

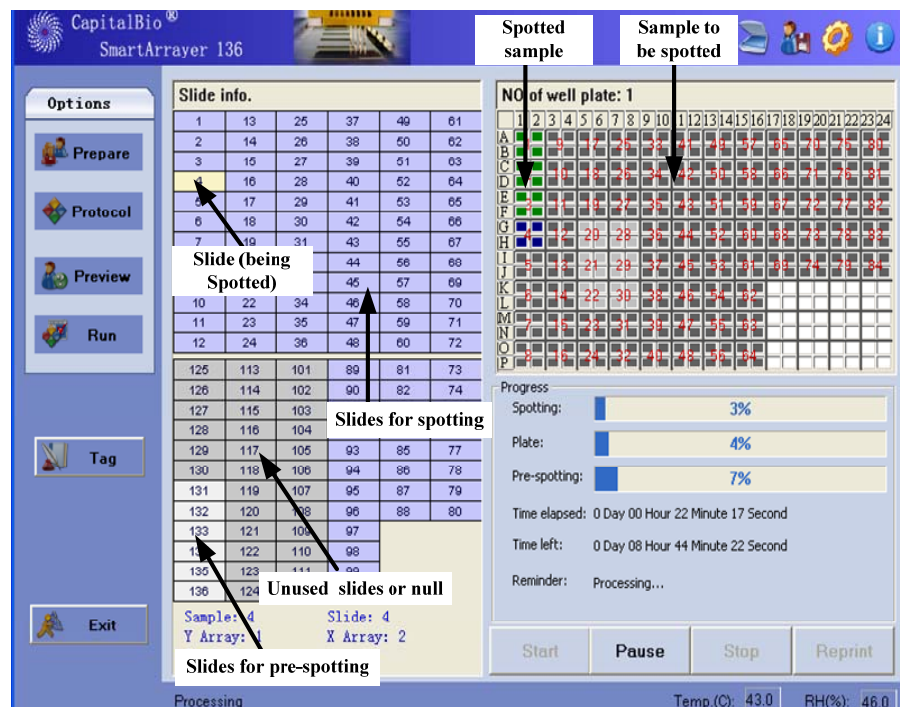Status Notification consists of Slide information, Plate information and Progress section as shown in Figure 7-11.

Figure 7-11 The running state interface

7.6.3 Pause and Resume

The Pause and Resume functions are supported to handle unexpected emergency events, particularly during spotting applications.

Step 1: Click the *Pause* button to pop up a dialog for users to select the next action to take. The microarray workstation will pause temporarily AFTER the current action is completed.

An action is a unit of operation or a series of operation which cannot be broken into individually controllable ones, For example, a sample taking procedure is composed of successively but inseparable operations of moving the X and Y axis and moving the Z axis downward, delaying, moving the Z axis upward.

Step 2: Select *Next Action*. Five options are provided to handle different circumstances. Users can select one solution to resume normal status as shown in Figure 7-12.
- Select the *Continue* option to resume spotting/dispensing at the current position

If the Wash checkbox is selected, the wash operation will be executed first and then the sample will be taken up

If the Re-uptake Sample checkbox is selected, the sample will be taken up again directly
- Select the *Exit* option to break away from the current loop of spotting/dispensing

If the spotter is in the home position after the washing checkbox is selected, the wash operation will be executed and then the axes will be taken to the home position before exiting
- Select *Pause* after the cleaning option to halt the system after the wash operation

If the Cleaning First checkbox is selected, the wash operation will be executed prior to the Continue operation launched by users
- Select *Backward* option to go back to a certain position and continue the spotting/dispensing

- ⊙ Input the index of a sample group within the sample list in the Sample textbox to appoint which sample group the spotting/dispensing procedure should be brought back to
- ⊙ Input the Slide Index in the Slide textbox to designate which slide the spotting/dispensing procedure should be brought back to
- ⊙ Input the index of the Y-direction vector in the Y Direction textbox to designate from which vector the spotting/dispensing procedure should be brought back to. The index is related to the sample display and slide textbox. If the array is not replicated, the Y direction textbox is gray and disabled
- ⊙ Input the index of the X-direction vector in the X direction textbox to designate from which vector the spotting/dispensing procedure should be brought back to. The index is related to the sample display slide and the Y direction textbox. If the array is not replicated, the X direction textbox is gray and disabled
- ⊙ Input the index of the replicate spot within the replicate list to indicate from which replicate spot the spotting/dispensing procedure should be brought back to. The index is related to the sample display, Slide and Y and X direction textbox
- ● Select the *Forward* to *Last Position* option to move to the last position where it used to be before the last operation, and then continue spotting or dispensing. This function only applies just after the Back operation

Step 3: Click on the *Yes* button and take action. If users select *Pause* after the cleaning option to halt the system after completion of the wash operation, the Pause button will change to the *Resume* button and the *Stop* button will be enabled.

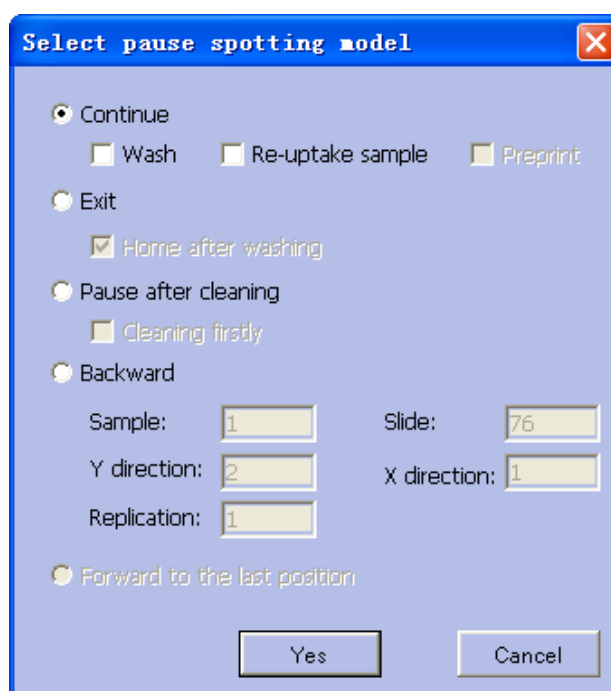**Step 4: While the system is halted, click the *Resume* button to resume spotting or dispensing.**

Figure 7-12 The continue option after pause

7.6.4 Stop

You can either select the *Exit* option when prompted for next action, or click the *Stop* button when the system is halted.

Chapter 8 Additional Software Features

8.1 Overview

There are some additional features in the software: reprinting, tagging sample, etc.

Reprinting (also called selective spotting/dispensing), means to re-spot at missing spot positions. The information for sample groups, slides, arrays and replicates involved in the spotting/dispensing procedure base in the microarray settings defined previously is needed to be specified. Or you can also use Reprinting to refine your spotting task just before the procedure starts to do part of the plan and leave the remaining spots temporarily incomplete.

The *Tag* function is a complement to the protocol. It provides facilities to generate GAL files to save information for sample sequence, tag and location distribution on the slide.

8.2 Steps of Reprinting

Step 1: On the run page, click the Reprint button to access the reprinting pop up dialog as shown in Figure 8-1.

Step 2: Set the range to the reprinted.
- Select starting and ending sample indexes from the Sample lists to specify the sub sample sequence list for reprinting between the selected ranges
- Select starting and ending slide indexes from the Slide list to specify the sub slide sequence list for reprinting between the selected ranges
- Select indexes of starting and ending Y-direction vectors from the Y-Direction drop lists to specify the sub-array for reprinting between the selected ranges. The index selection only applies to circumstances when arrays are replicated.
- Select indexes of starting and ending X-direction vectors from the X-Direction list to specify the sub array for reprinting between the selected ranges. The index selection only applies to circumstances when arrays are replicated
- Select starting and ending spot replicate indexes from the Replication (Replicates) list to specify the sub replication list for reprinting between the selected ranges

Step 3: Click on the *Start* button to perform the reprinting operation. The following operation applies to a normal run.
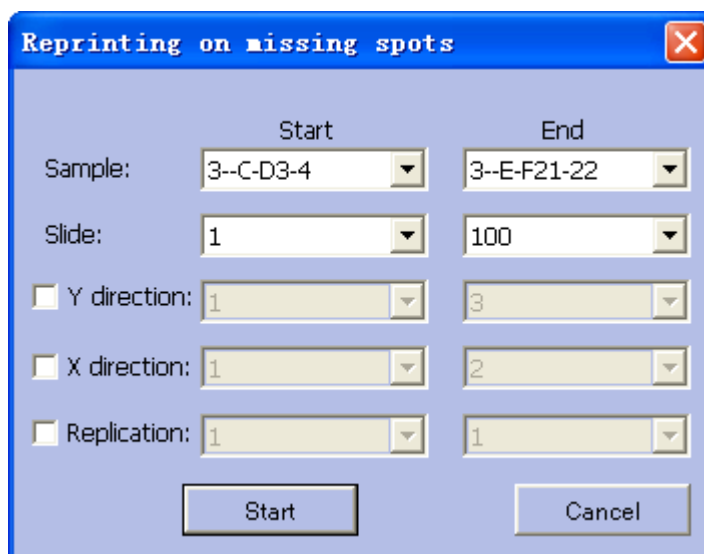
Figure 8-1 The reprinting on missing spots setting interface

8.3 Tagging Sample

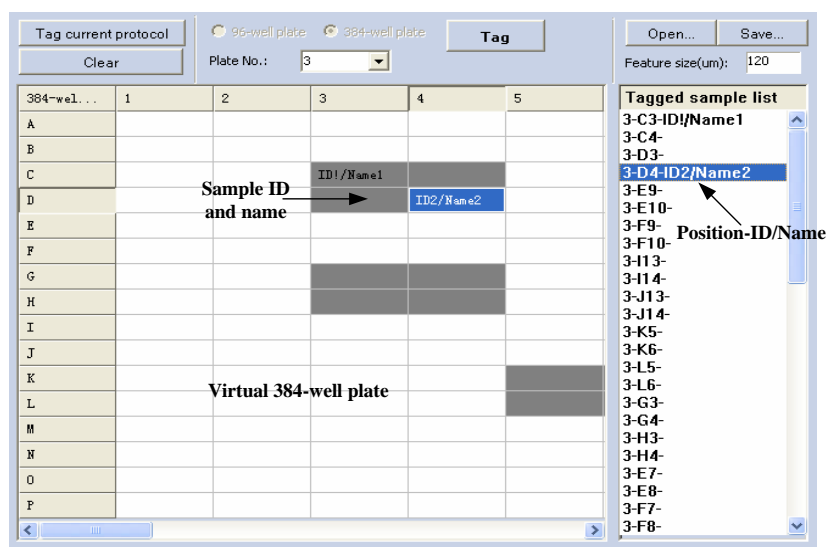Click the *Tag* button on the left to access the tag page as shown in Figure 8-2.

Figure 8-2 The interface for tagging sample

Tag Current Protocol

Click on the *Tag Current Protocol* button to load the sample sequence list defined in the protocol to the list on the right hand side of this page, label the listed sample group items tagged, untagged or blank with different marks, and paint the cells on the plate profile with different colors according to corresponding sample group items. These actions will highlight the locations where the tags are to be inserted, as shown in Figure 8-3.
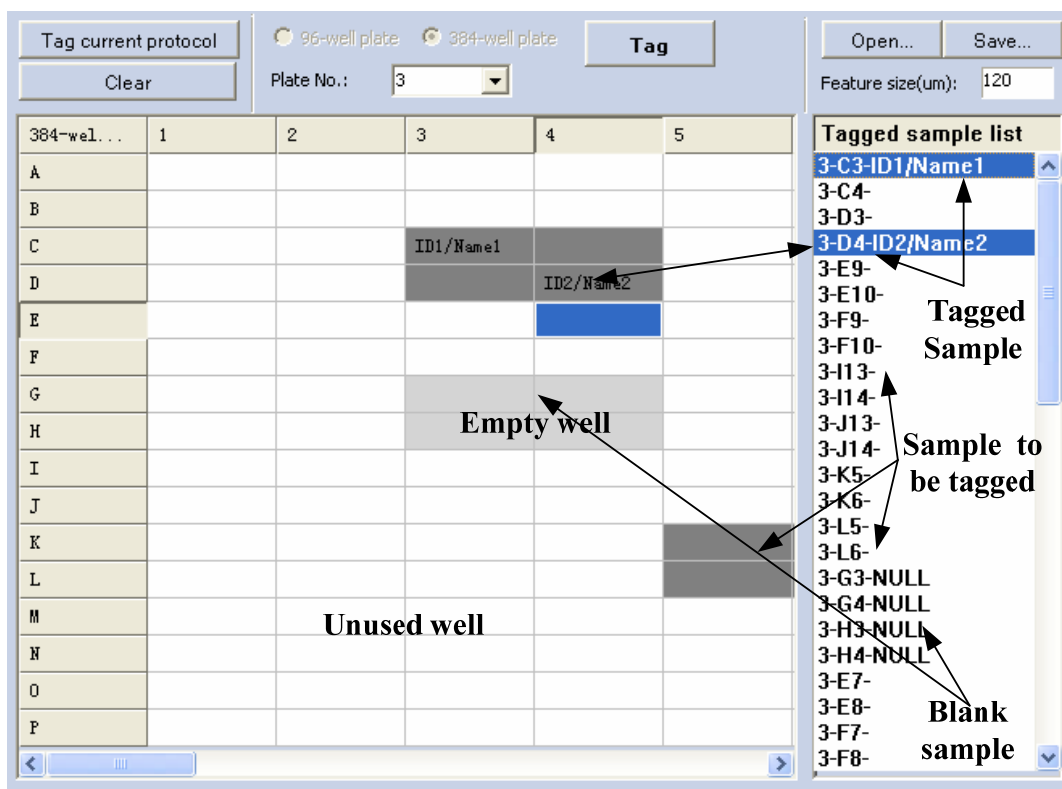
Figure 8-3 Examples of sample attention marks

Input Tags

Here the added Tags must comply with a defined format: first comes sample ID /description. For instance, the tag of a certain sample with ID such as "ID1" and description such as "Name1" should be "ID1/Name1".

Double click on the corresponding cell on the grid to activate editable status, then input the desired tag, and repeat the operation until all required samples are tagged.

Set Microplate

There are two types of microplate supported by the system, the microplate specified here should match to the type used for spotting. When tagging the sample sequence list confined within the current protocol, the microplate will default to the type specified in the protocol and is beyond the user's modifications.

Assign Tags

Click the *Tag* button to assign the tag inputs in the left grid cells to the listed sample sequence items on the right, and then the Save button is enabled which allows files to be stored by describing sample tags, namely the sample flag files(*.sas).

Clear

Click the *Clear* button to remove all the input and settings, and bring the page to the status just after initialization.

Save and Load

Click *Save* or *Open* buttons to save or load the sample flag file in Excel format. The format specifications of the sample flag file are depicted in Figure 8-4.

	A	B	C	D	E	F
1	PlateNo	X Index	Y Index	ID	Name	
2	**Plate** 3	**X** 3	**Y** 3	ID1	Name1	
3	**number** 3	**direction** 1	**direction** 1	ID2	Name2	
4	120	**number** 0	**number** 0	**Sample**	**Sample**	
5				**ID**	**name**	

Feature size (um)

Figure 8-4 The sample marker information file format

Chapter 9 Operation & Maintenance

9.1 Overview

This chapter will introduce operations and safety items, including daily operation and routine maintenance.

Daily operation mainly includes: open and close the upper enclosure of the arrayer, load and unload slides, load and unload pins.

Routine maintenance mainly includes periodically cleaning and replenishing distilled water for the humidifying unit and refreshing system liquid for the sonicator bath.

9.2 Daily operations

Before loading the unit with plates, or slides, or replacing the pins and refreshing the water in the sonicator basin, lift the upper enclosure and place it on the top of the frame.

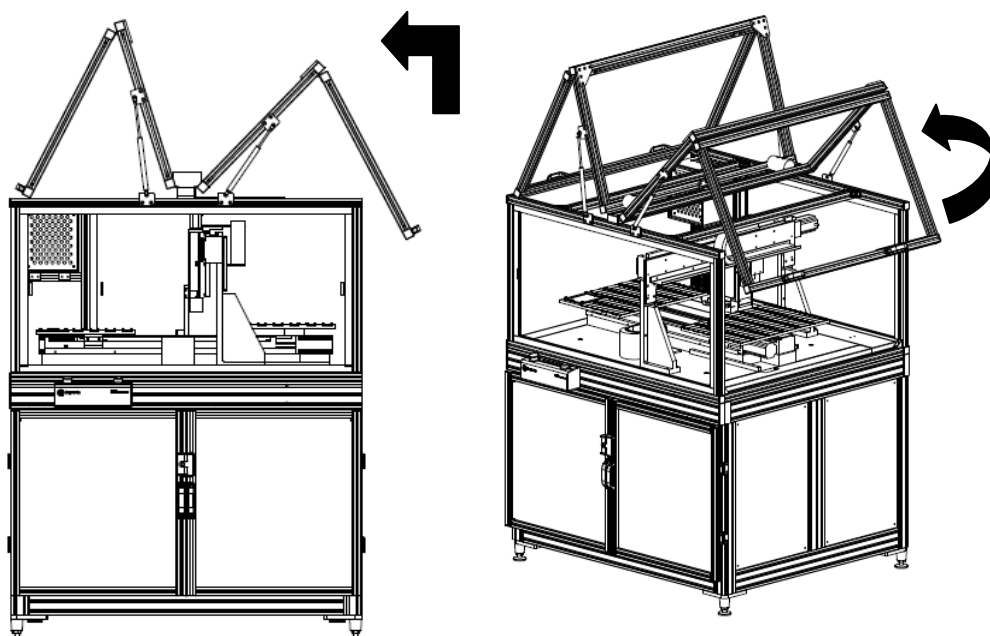| ⚠️	**Note**: Before performing the above operations, make sure the power status of the microarray workstation and computer is set to off.

Figure 9-1 How to open the covers of *136S* and *136D* models

The 384- or 96-well plate surface should be placed in the plate bracket in the lower plate area and pressed downward to fasten the plate via the moveable frame. Be careful to place the plate in the proper direction.
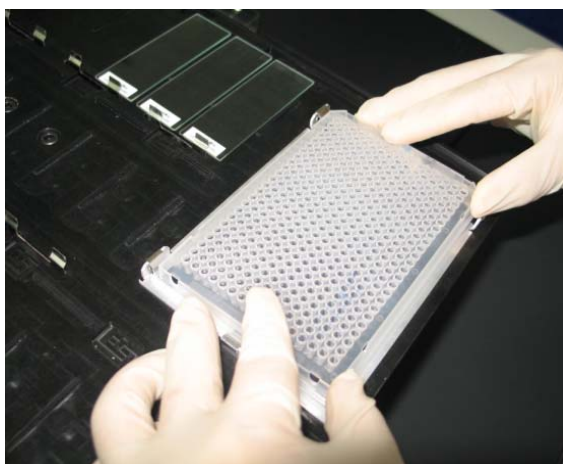
Figure 9-2 Load well plate

To load the slides into the plates, push the moveable frame back and place the glass slide into the concave areas (the barcode of the slides should be placed near the moveable frame) and then the moveable frame should be loosened to attach the slide.
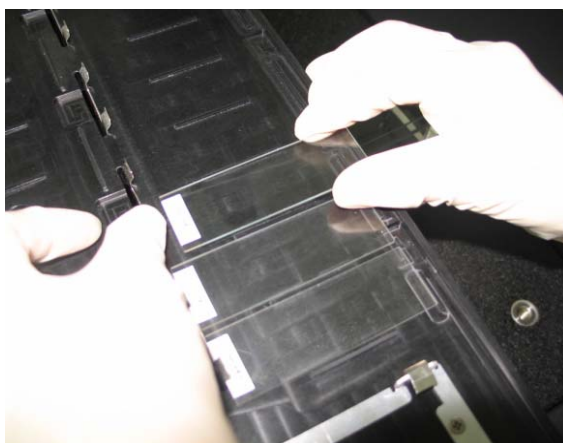

Figure 9-3 Load slide

	Warning:
⚠️	The water volume in the sonicator should be about 2/3 of the inner capacity of the basin. After adding water to the sonicator, the sonicator needs to be placed on a marble workbench and be placed in contact with the positioning plate.
	For models *48S* and *48D* and for *136S* and *136D,* the methods, protocols and notes for installing the microplate, glass slide, sonicator and for loading and unloading of the pins are identical.
	Before loading or replacing pins, ensure that the Z and Y axes are in the home position and then open the upper enclosure of the microarray workstation. When loading, unloading or replacing pins, users must wear lab gloves and try not to touch the tip and stem of the pins by hand in order to avoid contaminating and/or destroying the pin. Users must also protect the pin tip, which can easily become blunt due to collisions with other objects.

To place the pins in the pin holes in the pin holder correctly and safely, the operator should hold the cap of the pin by the right hand and place the tip in the hole in the upright position direction. The user must not shake the pins. After placing the pins, the operator must slide the pin along the pin hole slowly. In succession, with the aid of a tweezers, the operator can rotate the pin in the hole and make sure the cap of the pin enters the slot, which prevents large-angle rotation of the pin while spotting. Use the tweezers to lift the pin up slightly. Then move the tweezers away, and see whether the pin can slip downward along the hole freely or not. If not, the operator must unload the pin from the pin holder and clean the pin and/or the pin holder because dust may adhere to the surface of the pin stem and/or the hole of the pin holder.

To unload the pins from the pin holder, the operator should use the tweezers to lift the pin up by one hand, and then cautiously slip the pin out from the pin hole slightly.

	Warning:
⚠️	Clean the pins and unload them from the pin holder after the microarray fabrication process if they will not be used again within several hours.
	Move the dispenser enclosure onto the dispensing module after the microarray fabrication process if they will not be used again within several hours.
	Do not routinely plug and unplug the connectors, cables and tubing of the microarray workstation in or out.
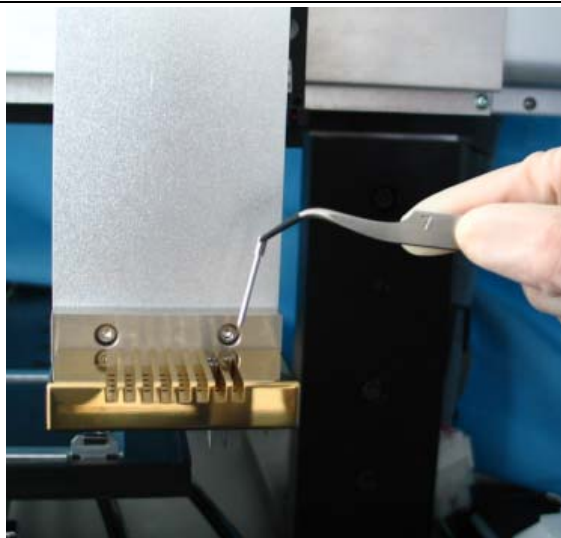
Figure 9-4 Loading or unloading pins

For the *48S* and the *48D*, the method, protocol and notes for installing the microplate, glass slides, sonicator and loading/unloading of the pins are the same as those of the *136S* and the *136D*.

9.3 Maintenance

Sonicator: The distilled water in the basin of the sonicator bath is required to be replaced everyday, or more often. Confirm that microarray workstation is not in any possible motion status before the following steps listed in the table below.

Table X Protocol for replacing water for sonicate wash

Step No.	Schematic Drawing	Description
1		Open the upper enclosure, and unplug the power cable of the sonicator.
2		Carefully take the sonicator out from its positioning plate
3		Empty the water in the basin of the sonicator.
4		Place the sonicator back in the positioning plate, plug in the power cable.
5		Then press it close to the positioning plate.
6		Refill the distilled water into the sonicator bath. Be careful to avoid spilling water on the workbench.

Humidifier: The humidifier should be refilled with distilled water regularly. Confirm the microarray workstation is not in operation, and then unscrew the bolts on the rear plate of the structure with Philips-head screwdriver to open the rear plate. The humid gas tubing and power cable of the humidifier should be unplugged and the upper part of the humidifier should

be pulled out and refilled with more distilled water. After that, place the upper part of the humidifier back in the correct position, plug in the gas tubing and power cable, and then install the rear plate again.

Water Tanks: The distilled water tank and the waste water tank should be refilled or emptied based on actual operational usage. Before undertaking this operation, confirm that the microarray workstation is not in operation and then open the rear plate. Empty the waste water tank and fill distilled water into the distilled water tank. After completion, the tank should be placed in the tank card plate. Please make sure that the connection between the distilled water inlet pipe and the distilled water tank and the connection between the outlet pipe and the waste water tank are in place.

System liquid: For **48D/136D**, regular replacement of system liquid in the sonicator basin is required under partial sample mode. The methods and protocol are similar to those for water in the sonicator.

Chapter 10 Troubleshooting

10.1 Overview

The user can refer to the following table for troubleshooting arrayer problems.

10.2 Troubleshooting

For troubleshooting, check the problem, analyze the cause and review the possible solutions below. Some problem may have multiple causes. The possible causes should be considered and eliminated one by one.

Table XI Troubleshooting (for *136S* and *48S*)

Type	Description	Possible causes	Solutions
Initialization	Abnormally high speed	Unreliable connection between the cable and motion control card	Following the "software installation" instructions, disconnect the cable and motion control card, and then reconnect them and fasten the connection
	Error notice on monitor		
	Error light ON		
	One or more axes exceed the sensor limits position	Sensor Damage, or the sensor light is off	Contact CapitalBio to replace the damaged sensor with a new one
		If head placed manually in the incorrect position beyond limits when power off	Exit software, shut down the PC, shut down the system, and then place the axes between the corresponding Home sensors and the position limit sensors
	One of more axes do not move and stay between the Home sensor and the position limit sensor	Wrong DMC settings	Contact CapitalBio
Running	"?" error pops up by the software	At least one axis exceeds the position limit sensor	Same as "software ini…"—"one or more axes exceed…"
		EMS button was hit	Release **EMS** button, exit software, shutdown computer and system, and then restart the system
	One or more axes moves less than it's set movement	Loose connection between motor and securing screw(s)	Contact CapitalBio
	Restricted motion of pin(s) in their guide hole(s)	Dirty interface of pin and hole due to spillage or other contamination	Remove the any contamination and wash the pins and holders thoroughly; or check if properties of the sample meet the sample requirements
	Water flood at the wash station	Waste water tank is full	Empty the waste water tank

		Tubing connected to the wash station clogs	Lower the height of the waste tubing to remove air bubble and restore flow
	Lots of air bubbles enter the wash station	Lack of water for wash	Refill the wash water tank
Spotting results	Missing spots	Low RH	Increase RH setting
		One or more pins fails to fall after being lifted while last spotting	Same as "System running" — "Restricted motion of pin(s) in their guide hole(s)"
	Irregular spot shapes	High sample viscosity	Decrease sample viscosity
		High motion speed	Reduce motion speed
		Pin holder misaligned	Adjust holder using a spirit-level
	Aggregation of adjacent spots	Insufficient preprinting	Increase number of preprinting spots
	Irregular array	High motion speed and some moving parts have become loose	Contact CapitalBio
	Inconsistent spot sizes after several spots	Insufficient sample	Increase sample uptake time
		High sample viscosity	Reduce the set drop number per sample
	Poor quality of spots	Surrounding environ conditions, slide quality, sample quality, etc	Ensure the temp, RH, environment conditions and high purity of sample requirements are all correct and are met.
File operation	No preview result in saved .xls files	Preview after saving	Preview before saving
Other errors			Contact CapitalBio

Table XII Troubleshooting (for *136D* and *48D*)

Type	Description	Possible causes	Solutions
Initialization	Abnormally high speed	Unreliable connection between the cable and motion control card	Following the "software installation" instructions, disconnect the cable and motion control card, and then reconnect them and fasten the connection
	Error notice on monitor		
	Error light ON		
	One or more axes exceed the sensor limits position	Sensor Damage, or the sensor light is off	Contact CapitalBio to replace the damaged sensor with a new one
		If head placed manually in the incorrect position beyond limits when power off	Exit software, shut down the PC, shut down the system, and then place the axes between the corresponding Home sensors and the position limit sensors
	One of more axes do not move and stay between the Home sensor and the position limit sensor	Wrong DMC settings	Contact CapitalBio
Running	"?" error pops up by the software	At least one axis exceeds the position limit sensor	Same as "software ini…"—"one or more axes exceed…"

		EMS button was hit	Release **EMS** button, exit software, shutdown computer and system, and then restart system
	One or more axes moves less than it's set movement	Loose connection between motor and screw	Contact CapitalBio
	"Invalid index" error pops up on monitor during non-contact dispensing	Loose signal cable linkage between the computer and the micro-dispense module	Check and fasten the two cable connections between the modules
		The power of the micro-dispensing module is off	Set the power to on and restart the software application
	No liquid is seen after sample uptake	Dust blocking the dispenser channel	Wash the channel thoroughly, and check if properties of the sample meet the sample requirements
		Uptake time is too brief	Increase the uptake time
		High sample viscosity	Decrease the sample viscosity
	Air bubbles in the channel after sample uptake	Not enough sample in the source well	Increase the sample volume in the source wells to the required level
		Uptake time is too long	Decrease the sample uptake time
		Air uptake time is too long	Decrease the air uptake time
	Large droplet on nozzle tip while dispensing	Dust on the nozzle tip	Improve surrounding conditions and wash nozzle tip thoroughly
		Dispensing air pressure too low	Increase dispensing air pressure
		Too little sample uptake in dispenser channel	Make sure the sample volume in source plate is adequate
		Nozzle damage	Contact CapitalBio
	Water flood at the wash station	Waste water tank is full	Empty the waste water tank
		Tubing connected to the wash station clogs	Lower the height of the waste tubing to to remove air bubble and restore flow
	Lots of air bubbles enter the wash station	Lack of water for wash	Refill the wash water tank
Dispense results	Satellite drops	Low humidity	Increase the humidity setting
		Dust on the nozzle tip	Wash nozzle thoroughly
		Large gap between nozzle and slide	Adjust the distance between nozzle and slide
		Static charge	Clear static charge
		Low dispensing pressure	Increase the dispensing pressure

		Small air bubbles in channel	Increase the dispense time and increase the number of cycles
		Air bubbles in sample	Centrifuge to remove the bubbles
	Aggregations of adjacent spots	Inadequate pre-dispensing	Increase number of pre-dispensing events
		Improper dispensing settings	Decrease the dispensing pressure or shorten the dispensing period
	Inconsistent spot size after several spots	Small sample uptake	Increase sample uptake time
		Low dispensing pressure	Increase dispensing pressure
		Dispensing time is too brief	Make sure the dispensing time is no less than 0.25ms
	Irregular array	High motion speed and some moving parts are loose	Contact CapitalBio
		Unsuitable slide surface chemistry	Change surface treatment protocol, or change to a different kind of slide
File operation	No preview result in saved .xls files	Preview after saving	Preview before saving
Other errors			Contact CapitalBio

Appendix I   Throughput and Time

Table XIII Test conditions

Parameters	Spotting	Dispensing
Velocity(mm/s)	300，150 (Z axis)	
Acceleration(mm/s^2)	3000,1500 (Z axis)	
Cleaning protocol	Rinse for 3s; Rinse for 3s, sonicate for 3s; Rinse for 3s, sonicate for 3s; Rinse for 3s, sonicate for 3s; Rinse for 3s, dry for 1s; Rinse for 3s, dry for 1s; dry for 2s	Drain 1 times, rinse 1 times, drain 1 times, dry 1 times

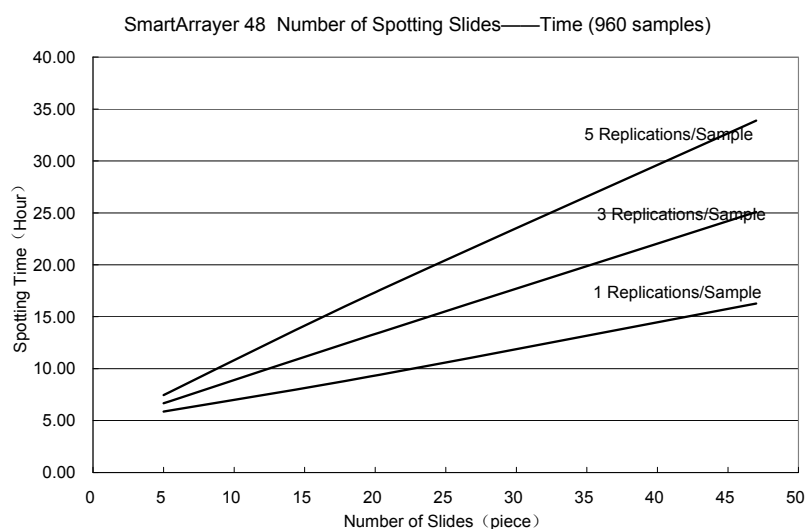**Table XIV SmartArrayer[TM] 48 Number of Spotting Slides—Spotting Time**

SmartArrayer 48 Number of Spotting Slides——Time (960 samples)

Table XV SmartArrayer[TM]48 Number of Slides—Dispensing Time

SmartArrayer 48 Number of Slides——Dispensing Time (960 samples)

Table XVI SmartArrayer^{TM} 136 Number of Slides—Spotting Time

SmartArrayer 136 Number of Slides——Time (960 samples)

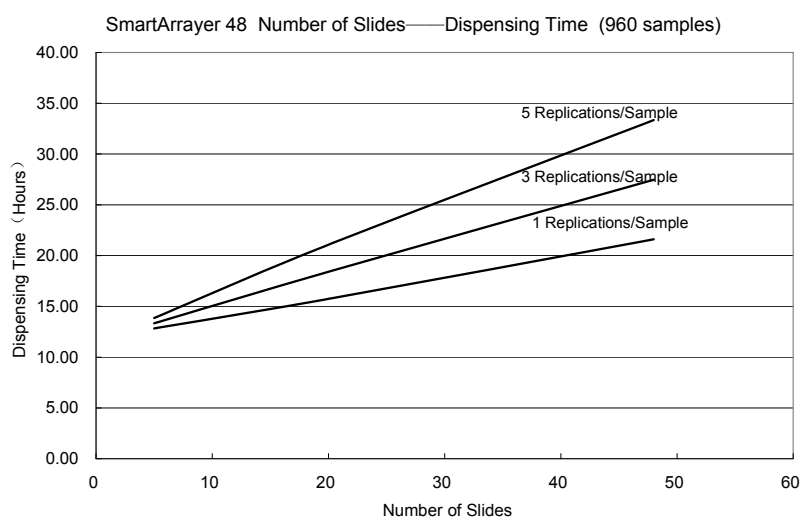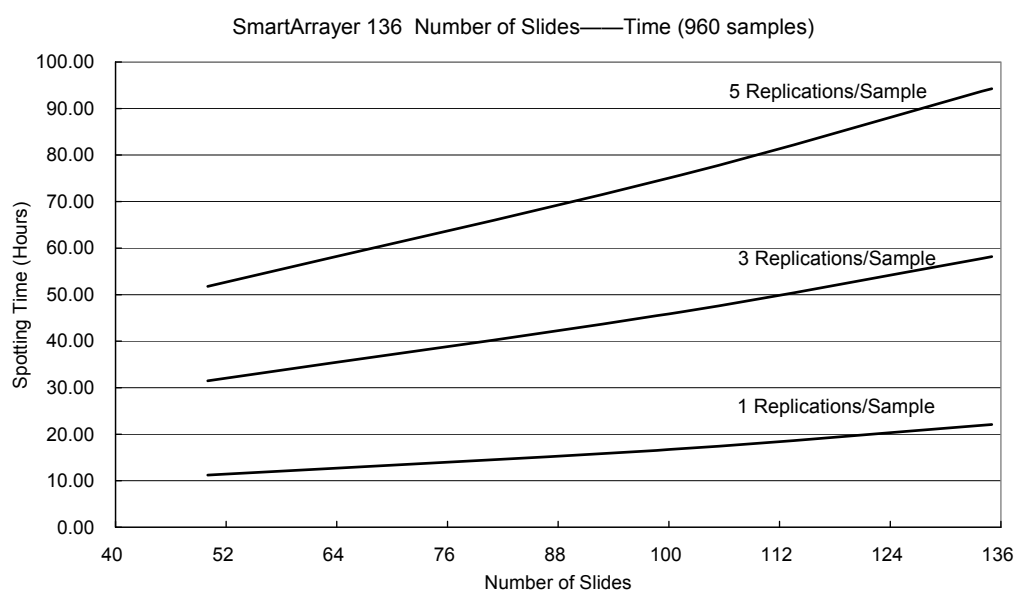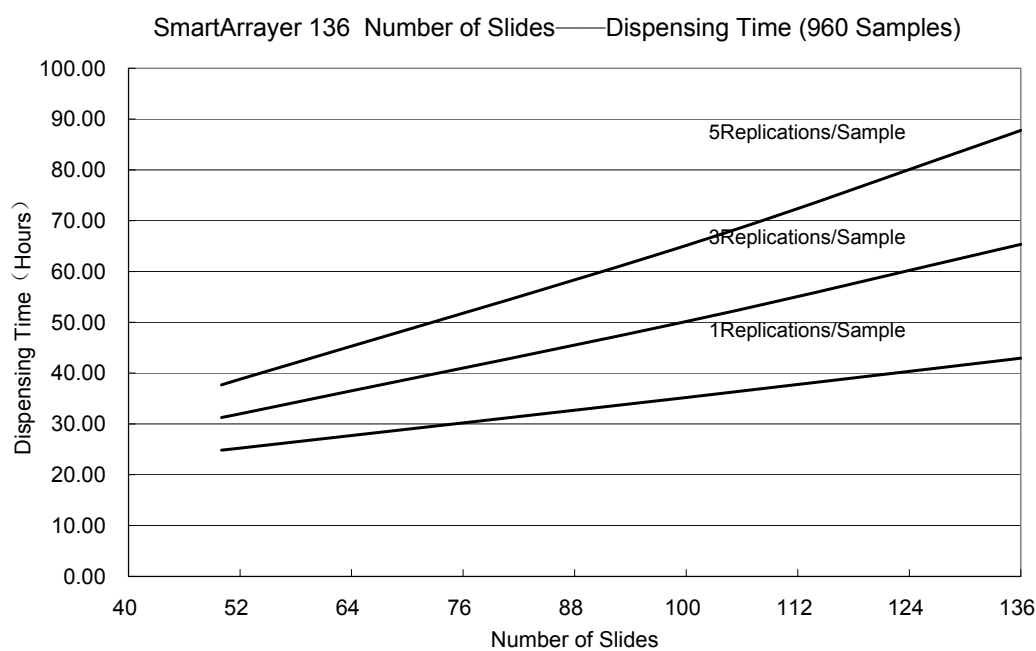**Table XVII SmartArrayer^{TM}136 Number of Slides—Dispensing Time**

SmartArrayer 136 Number of Slides——Dispensing Time (960 Samples)

Table XVIII SmartArrayer^{TM} 48 Number of Sample—Spotting Time
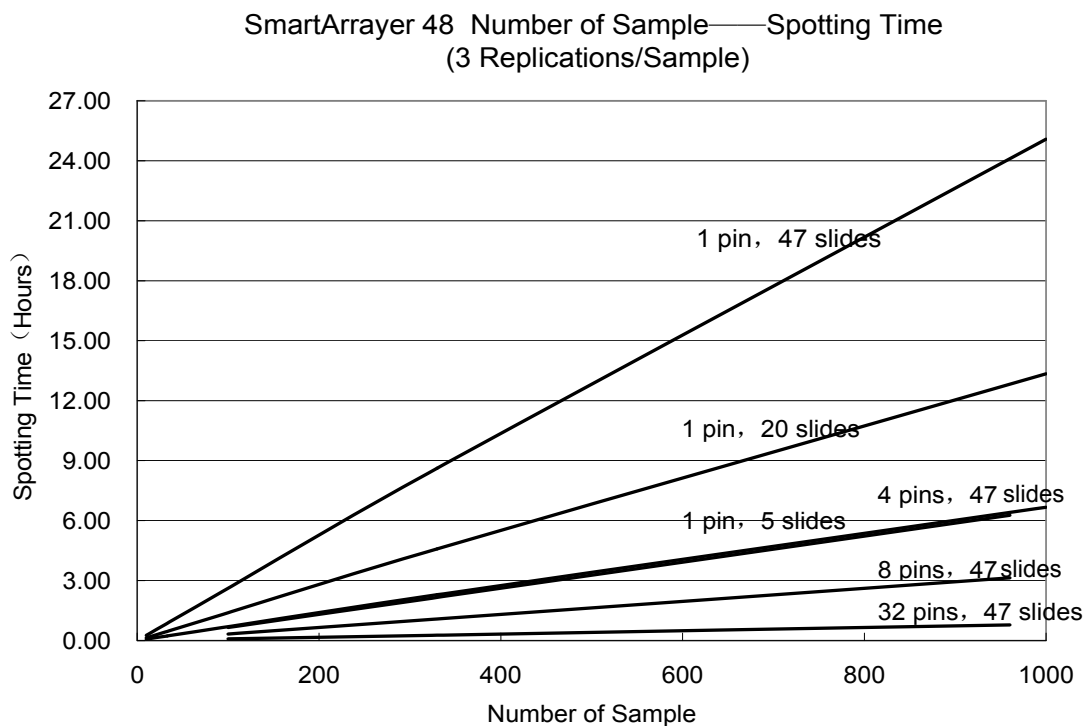
SmartArrayer 48 Number of Sample——Spotting Time
(3 Replications/Sample)

Table XIX SmartArrayer^{TM}48   Number of Sample—Dispensing Time

SmartArrayer 48 Number of Sample ——Dispensing Time
(3Replications/Sample)

Table XX SmartArrayer^{TM} 136 Number of Sample—Spotting Time
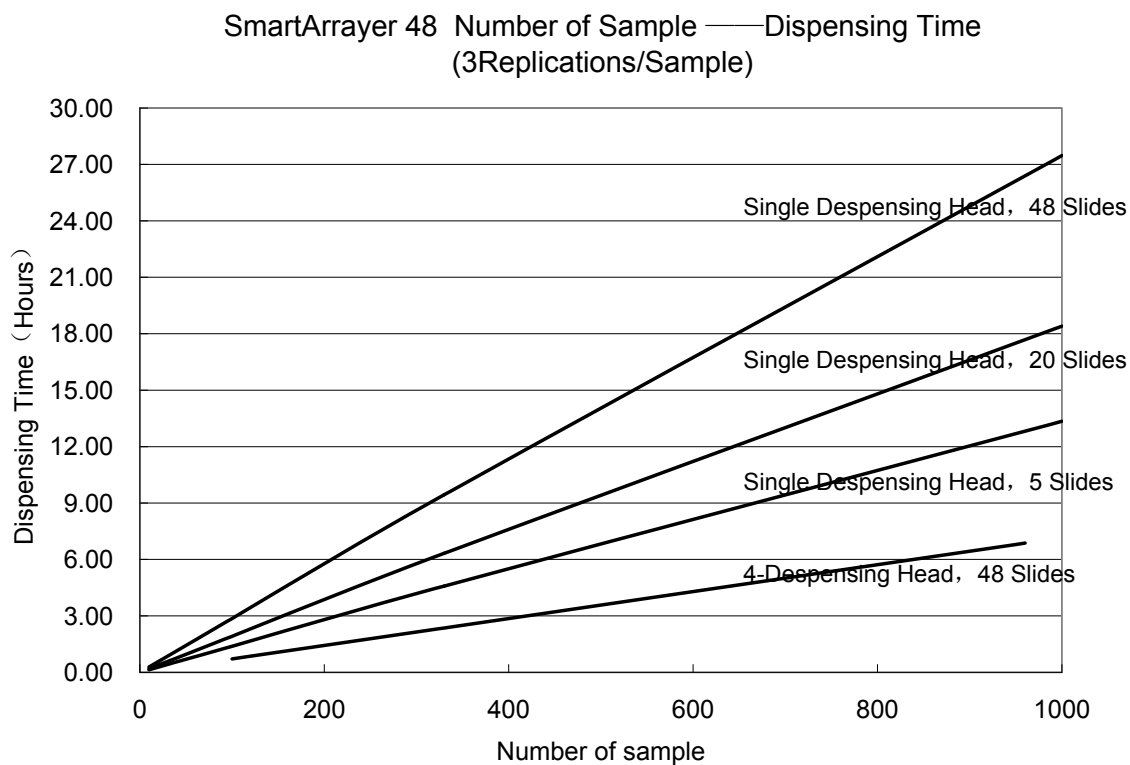
SmartArrayer 136 Number of Sample——Spotting Time Reference
(3Replications /Sample)

Table XXI SmartArrayer^{TM} 136 Number of Sample—Dispensing Time
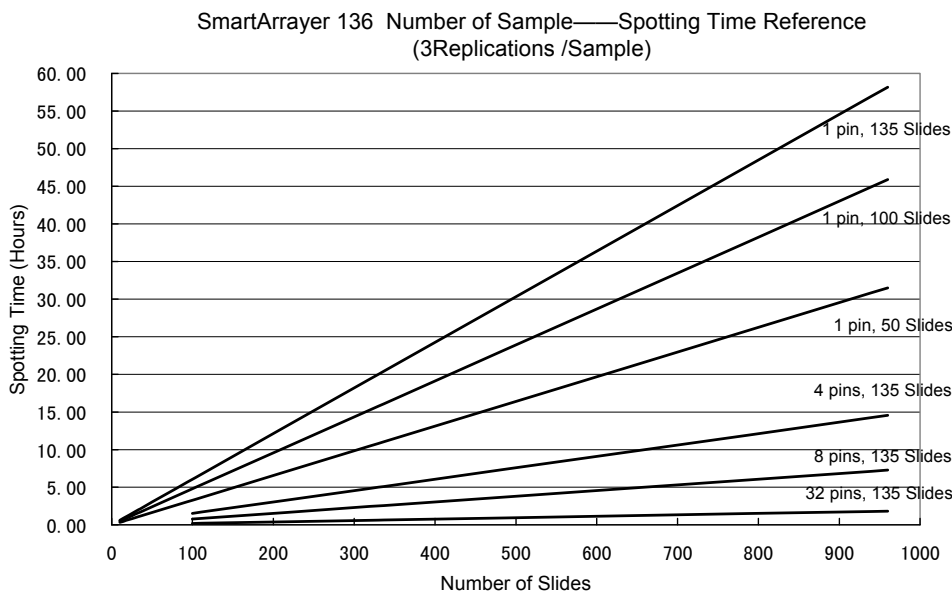
SmartArrayer 136 Number of Sample——Dispensing Time
（3Replications/Sample)
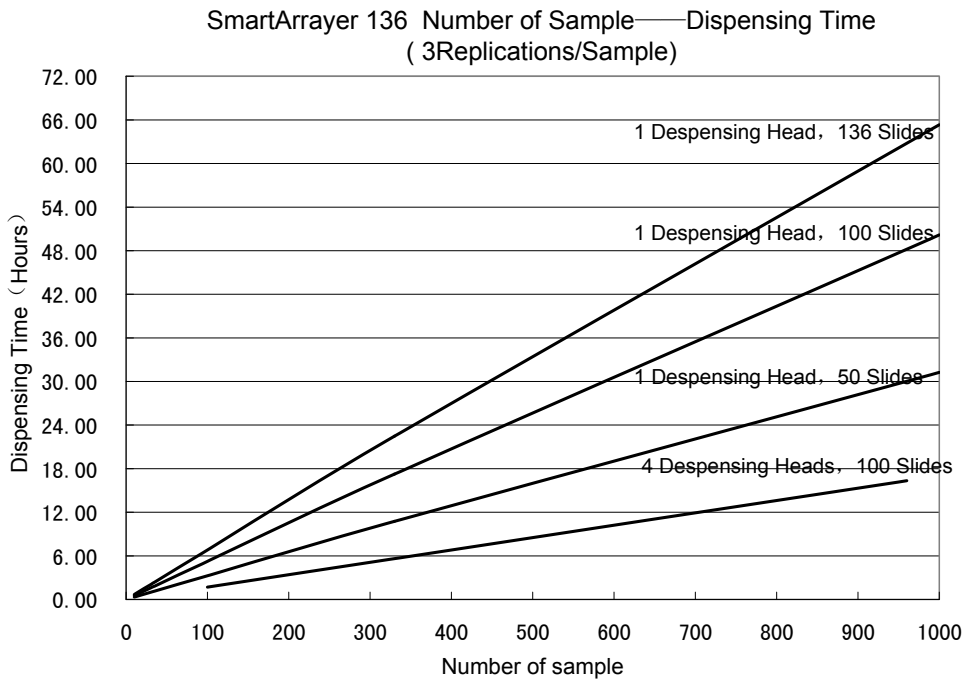
Appendix II   Aspiration Volume and Aspiration Time

Table XXII Aspiration volume of 10％ glycerol under different conditions

Aspiration volume of 10% glycerol under different conditions

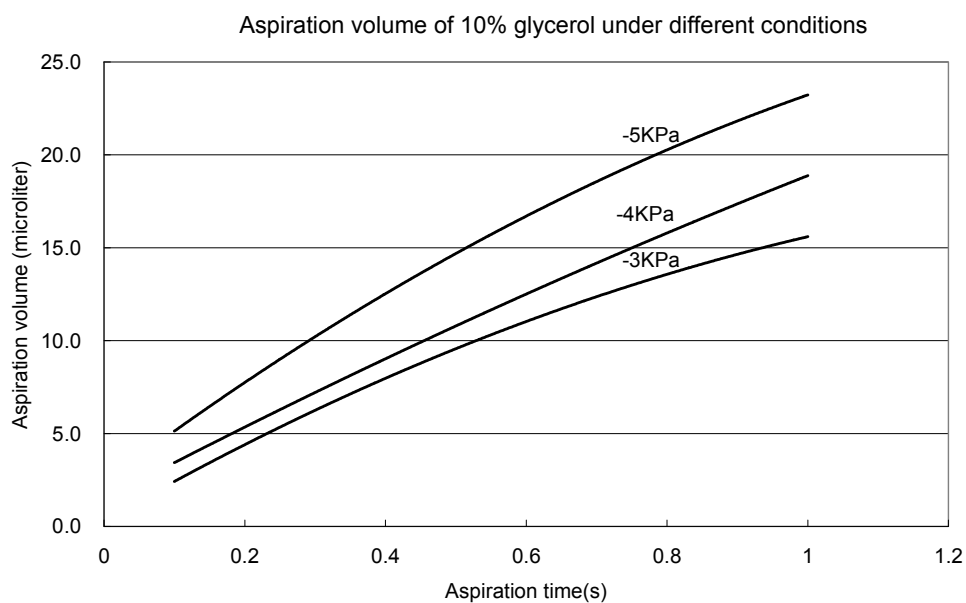**Table XXIII Aspiration volume of 30％ glycerol under different conditions**

Aspiration volume of 30％ glycerol under different conditions

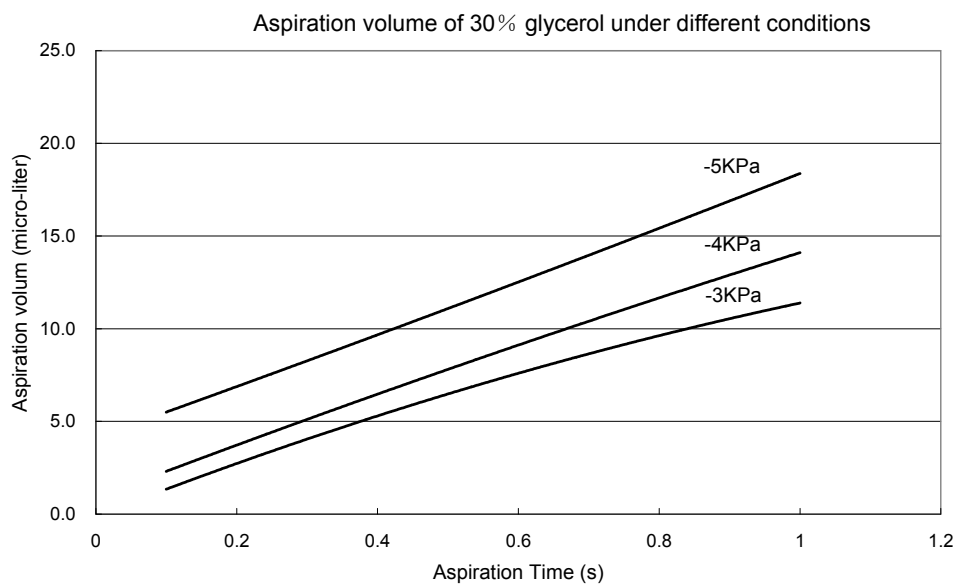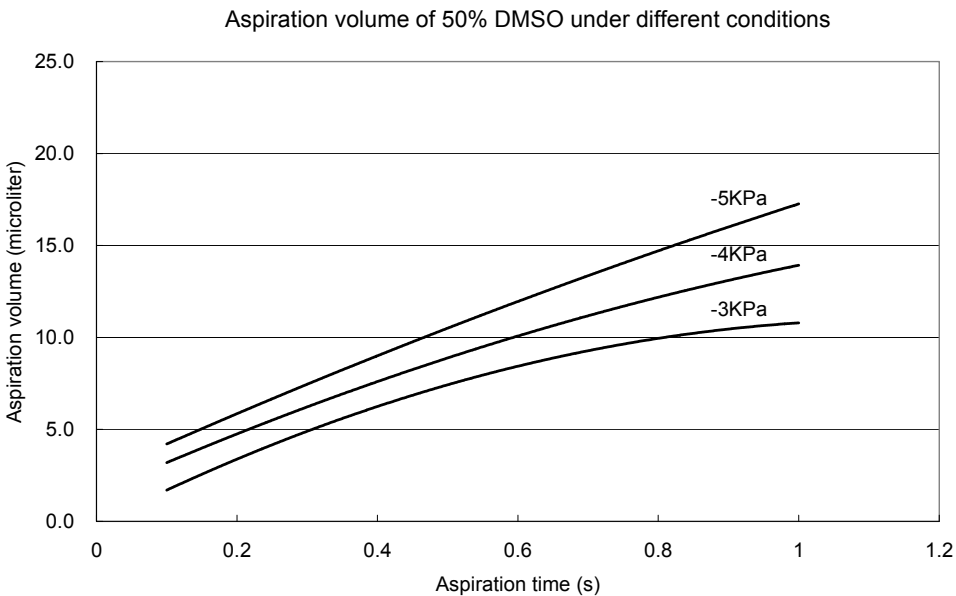**Table XXIV Aspiration volume of 50％ DMSO under different conditions**

Aspiration volume of 50% DMSO under different conditions

Appendix III   Routine Maintenance

III.1 Pre-Run Maintenance

III.1.1   Refresh system liquid for dispensing system, or distilled water in the basin of the sonicator for spotting system.

III.1.2   Make sure there is adequate reservoir in the distilled water tank, and adequate space for drainage in the waste water tank.

III.1.3   Make sure there is plenty of reservoir of distilled water in the humidifier.

III.1.4   Please refer to Chapter 9, Passage 3 for detailed operation instruction about III.1.1~ III.1.3.

III.1.5   Before running any spotting protocol, manually keep the water circulating through the rinsing position for about one minute, and then perform corresponding cleaning operation via the preparation panel from software to wash the pin head or dispenser head.

III.1.6   After being left idle for long time, the instrument should be calibrated again before it is put to work again.

III.2 On-Run Maintenance

If you launch a spotting protocol which lasts long time, more than 24 hours etc, you probably have to check 1.1~1.3 intermittently during the protocol is running.

III.3 Post-Run Maintenance

III.3.1 Perform corresponding cleaning operation via the preparation panel from software to rinse the pin head or dispenser head.

III.3.2 Manually keep the water circulating through the rinsing position for about one minute, to wash away the residuals in the basin/tank.

III.3.3 Check if there is any spillage on the workbench and wipe it away.

III.3.4 Clean the pins and unload them from the pin holder, cover the dispenser head with dispenser enclosure after microarray fabrication processes if the instrument will not be used again within several hours.

Scheduled Maintenance

III.4 Weekly Maintenance

III.4.1 Check if the pin can drop neatly and swimmingly during spotting. If not, please unload it and sonicate it until it is clean from clogging or other contamination.

III.4.2 Check repeatability of dispenser following the instructions below:
        a. Prepare 300μL (600μL for 96-well microplate) aqueous solution of 10%

glycerol, 0.2ug/ml BSA labeled with Cy3 dye.

    b. Pipette the solution into 8 consecutive wells on a microplate, 30µL per well for 384-well plate and 70µL per well for 96-well plate.

    c. Set aspiration time to 0.5s, aspiration time for system fluid to 4s and dispense pressure to 6KPa.

    d. Set up an protocol, set the combination of dispenser to 1*1, sum of slides to 2, sum of spots to 18 * 8(X * Y) , sample replicate(s) to 18, sample uptake mode to "Partial Sample"(utilizing system liquid). Then, run the protocol to dispense 144 spots per slide.

    e. Scan the result, calculate cv(coefficient of variance) value on diameter and intensity of the 144 spots per slide.

    f. If cv>10%, please call CapitalBio or the authorized distributors for technical support.

III.4.3 Check if the pressure of compressed gas source is lower than 1Mpa. If it is, replace the gas source.

III.4.4 When you are replacing the gas source, make sure the new one is clean especially in the proximity of the outlet and wipe away the dust accumulated during transportation, if there is any.

III.5 Monthly Maintenance

III.5.1 Wash the distilled water tank, waste water tank and sonicator basin with disinfectant.

III.6 Seasonal Maintenance

You can choose to perform seasonally maintenance by yourself or call for technical support.

III.6.1 Check if there is any damage on the pin head under microscope, which might affect spotting. More attention should be paid in case of frequent use of the instrument.

III.7 Semiannual Maintenance

Semiannually maintenance should be carried out by professional personnel assigned or authorized by CapitalBio Corporation.

CapitalBio Corporation

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