BIO DOT

AD6000

System Operating Manual

Version 1.2
September 2007

BIO DOT

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1. Purpose

This manual serves as both a tutorial and reference for the BioDot Inc. AD6000 System. The manual covers all of the important areas of equipment use: installation, setup, maintenance and troubleshooting. **It is strongly suggested that the user read this manual in its entirety before operating the equipment.** Specific sections should be referred to as necessary.

2. Product Description

The BioDot AD6000 combines a robotic dispensing platform with one or more BioJet Plus™ dispensers. The system consists of a dispense platform with a wash / vacuum source for the wash station, peristaltic pumps for the wash station, AxSys™ software, and optional hardware or software adaptations such as vision, chilled water recirculator, and plate loading capabilities. The system can be ordered with one of four nest plates and can be configured with 1 BioJet Plus™ pump or up to 96 BioJet Plus™ pumps. Review the conventions section for details.

3. Precautions

- This symbol ⚠ will indicate a **Warning** to the user. This symbol 👉 will alert the user to a **NOTE** in the operator manual.

- Do not drop or expose the equipment to severe mechanical shock. The AD6000 system assembly components are assembled with delicate precision and should be treated with care. The unit is heavy, 348.54Kg (768.4+lbs.) with base and 8 pumps.

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- All power supplied to the unit should be from an approved **EARTH grounded** source. Refer to Equipment Specifications for power requirements. **Warning: Never attempt to disconnect or connect any wiring harness and or connector with the power ON. Doing so may damage internal electrical components**.

- Do not override or tamper with any of the door safety interlocks. Never operate with the guards removed unless you are a skilled professional maintenance mechanic and are servicing the unit. Never reach around guarding or physical injury will occur.

- Clean all chemicals from the AD6000 platform should any be spilled or otherwise brought into contact with the unit. **Warning: Wear lab gloves and safety glasses during any cleaning procedure that could involve contact with hazardous materials or fluids. Dispose of all cleaning materials in accordance with state and local laws**. **Note**: If you are returning a unit to BioDot for repair or an upgrade, you must first complete and return the Decontamination form to BioDot Inc. prior to shipment. The form can be obtained at support@biodot.com.

- **Do not attempt** to disassemble any defective platform components beyond that described in this manual. This will **void all warranty obligations** and may render the unit unserviceable.

- **Warning: Never touch any electrical components with the power ON. Electrical shock could occur. The AD6000 should be plugged into an approved EARTH grounded power source (refer to the Equipment Specifications Section). If power is lost or disconnected the headpiece may drop. Be sure to support the headpiece when powering down the unit.**

B I O DOT
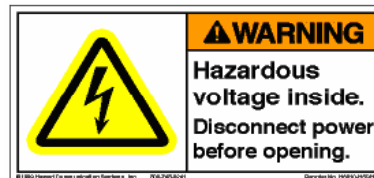
⚠ • Be sure ample clearance (user defined) exist between the dispense head (Z-Axis) and items mounted to the platform nest plate when the X or Y-axes are active. **Warning: Damage to the head assembly and tips can occur if this precaution is not observed**. Damage can be avoided by carefully ensuring that the Z-axis is properly controlled to move the dispense head to a safe travel height before any moves are made in the X and Y dimensions. All new program moves should be tested without the use of tips prior to using the platform for transferring samples.

• Make sure that the unit has enough clearance around it to be able to service the different components of the AD6000. Minimum clearance is 610mm (2 ft). If the unit comes with a supplied computer be sure to leave more room for the computer to be install next to the unit.
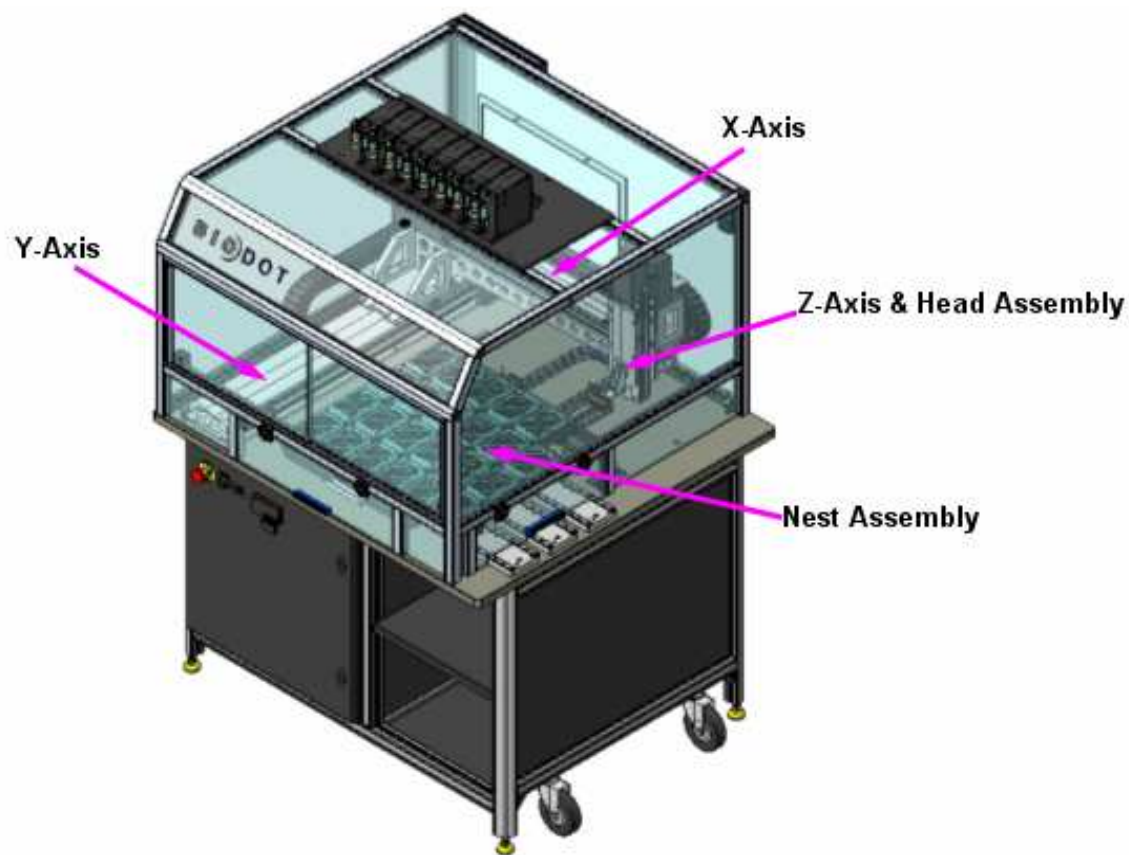
AD6000 Dispensing Platform

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4. Unpacking

The AD6000 comes fully assembled in its own shipping crate. The front panel of the crate doubles as a ramp to aid in the removal of the machine. Retain the crate and all shipping materials until the unit is completely assembled and working properly. Remove the crate front panel screws and gently lower the ramp. Remove any packing or bracing and with help roll the AD6000 out of it crate.
Note: Inspect the unit for any shipping damage. If damage has occurred please contact BioDot Inc. immediately at support@biodot.com or call (949) 440-3685.

Once the unit has been unpacked roll the unit to its final location. There should be a minimum 610mm (2 ft) of clearance around the machine. Remove any padding or tie straps that may be securing the axes. Refer to Equipment Specifications for utility requirements.

5. Equipment Specifications

AD6000 General Specifications

The Following Specifications Are For The AD6000 Operating System	
Utilities	
Electrical	110 VAC, 60Hz, 6.0A
	220 VAC, 50Hz, 3.0A
Fuse Rating	250V, T 3A
AC Cord Set	EN60320 plus local EU plug.
Air (if required)	4.14 – 5.51bars (60-80PSI) Clean dry source.
Vacuum	84.65kPa (25in.Hg) In house, vacuum pump or vacuum generator.
Size	
Length	119.4cm (47.0")
Height	170.1cm (67.0") With 8 pumps
Depth	113.0cm (44.5")
Weight	
Total	363kg + (800lbs. +) With 8 pump. (Weight will depend on number of pumps and added options or customization)
Fluid Pumps	
Type	Three 24VDC peristaltic pumps (2 supplies and 1 take up of spent fluids) 4mm & 2.5mm Tubing.

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	Recirculating Chiller (**Optional**) 120VAC 60Hz BioDot P/N 2026-0001 230VAC 50Hz BioDot P/N 2026-0002
System Operation Requirements	
Operating System	PC based utilizing Windows 2000 / XP for system control using AxSys™ proprietary software.
Platform Motion	
Motion	Stepper Driven Ball Screws.
X-Axis	600mm (23.6 in.)
Y-Axis	600mm (23.6 in.)
Z-Axis	100mm (3.9 in.)
Axis Speed	250mm/s +
Motion Envelope	600mm x 600mm (23.6 in. x 23.6 in.)
BioJet Plus™ Pump	
Power Requirements	24VDC/1A & 24VDC/20mA
Quantity	1-96 Pumps
Dispense Modes	Aspirate/Dispense (source to destination) Continuous (prime through from bulk reservoir)
Dispense Specifications *Based on 20nl drop size, within and between 4 channels, TMB measurement method. Result may vary depending on reagent dispensed.	Range: 20 nanoliters to 1 microliter Accuracy: <+/-10% at >20 nanoliters* Precision: Drop to Drop: <5% CV* Channel to Channel: <8% CV* Speed: Up to 100 drops per second (100 nanoliters).
Aspirate Specifications	Range: 5 to 500 microliters Recovery: 50-90% dependent on process parameters & reagent/reservoir fluid properties.

B I O D O T

Unit Dimensions

[cm]
inches
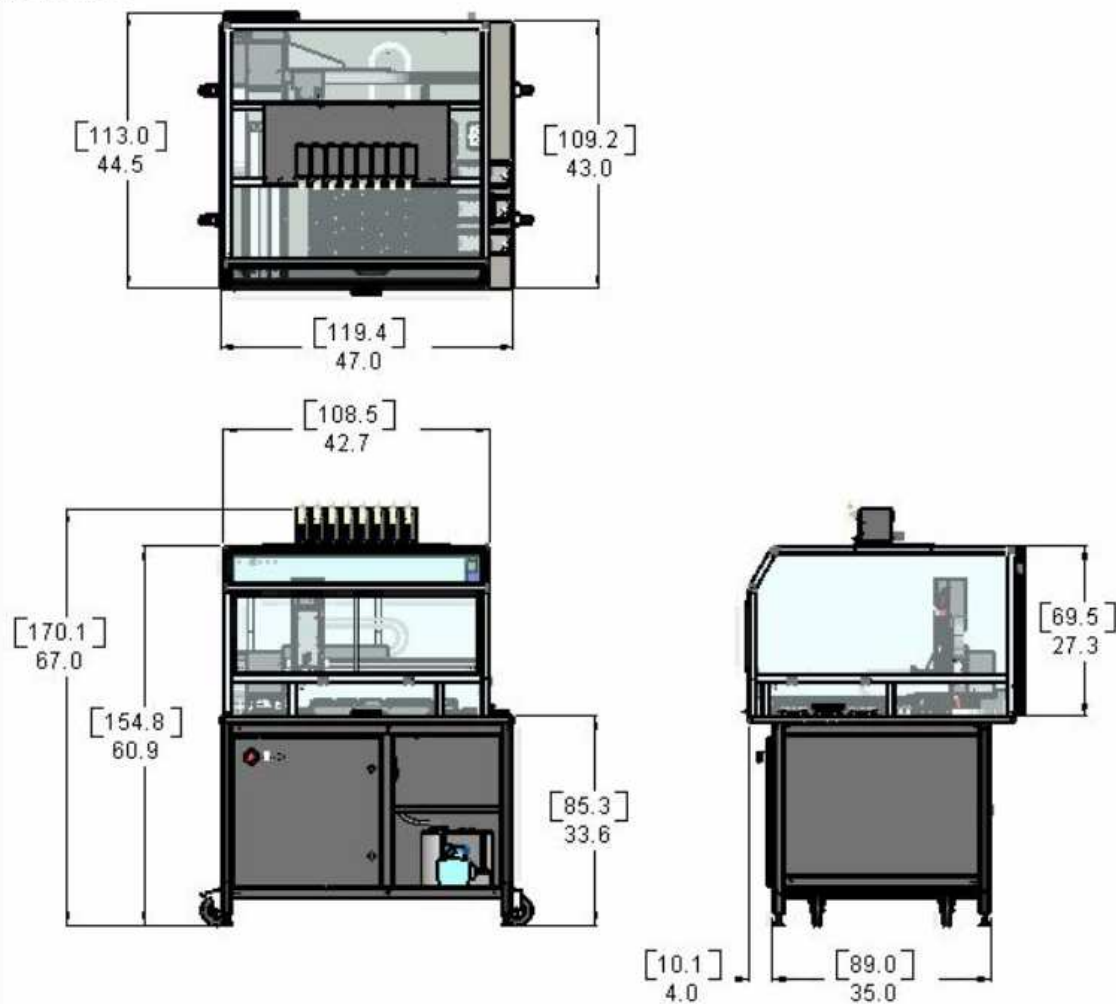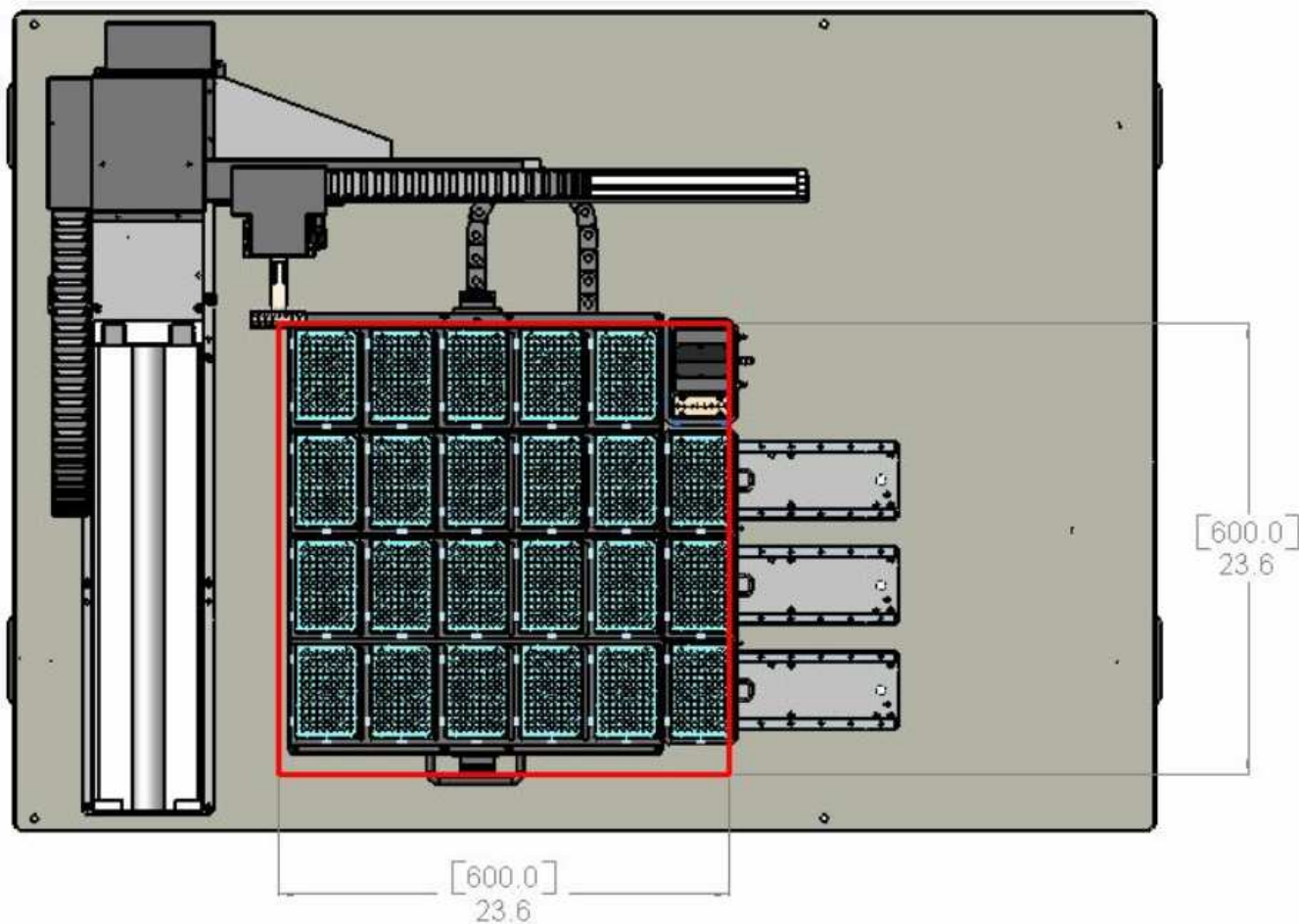
20 MTP Nest, 3 Source & Wash position

[mm]
inches

Wash / Vacuum Station
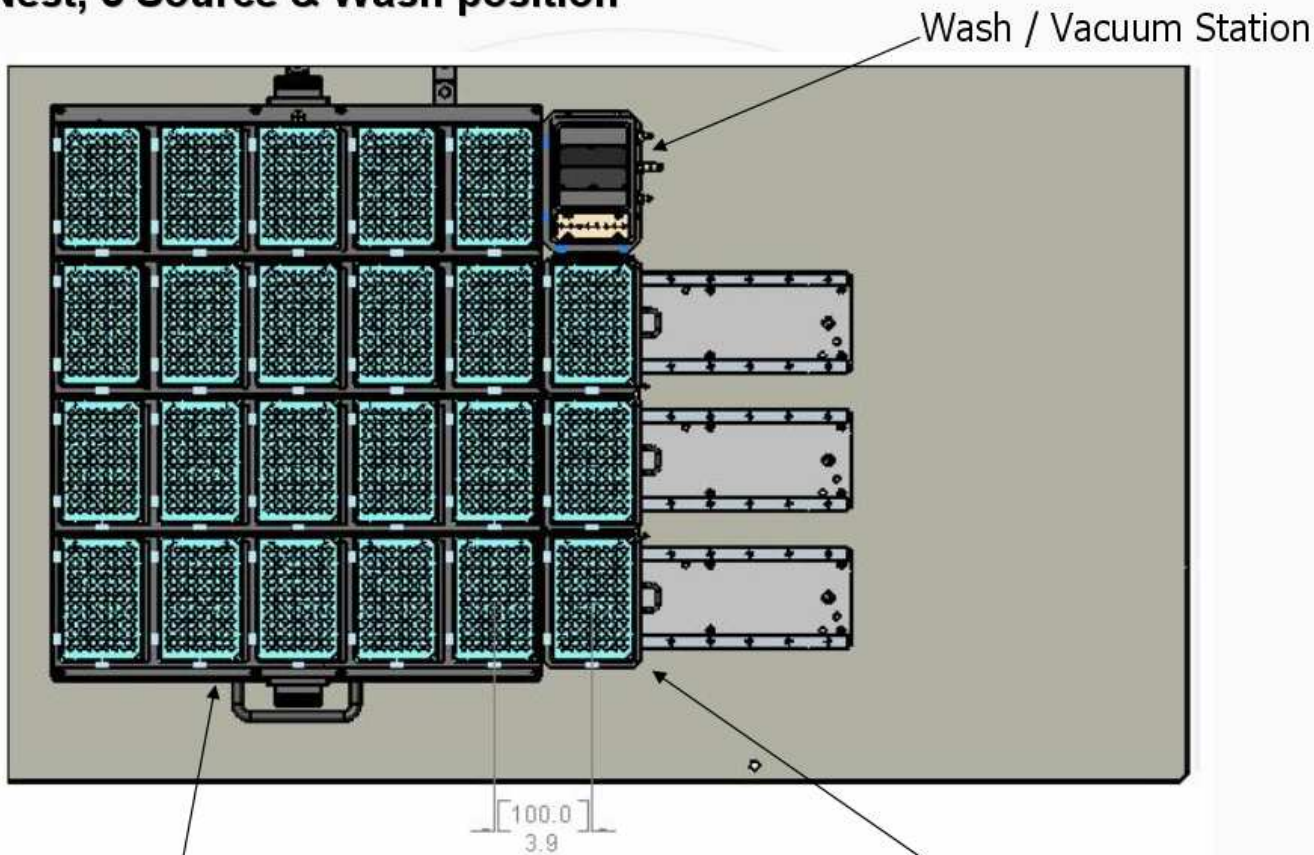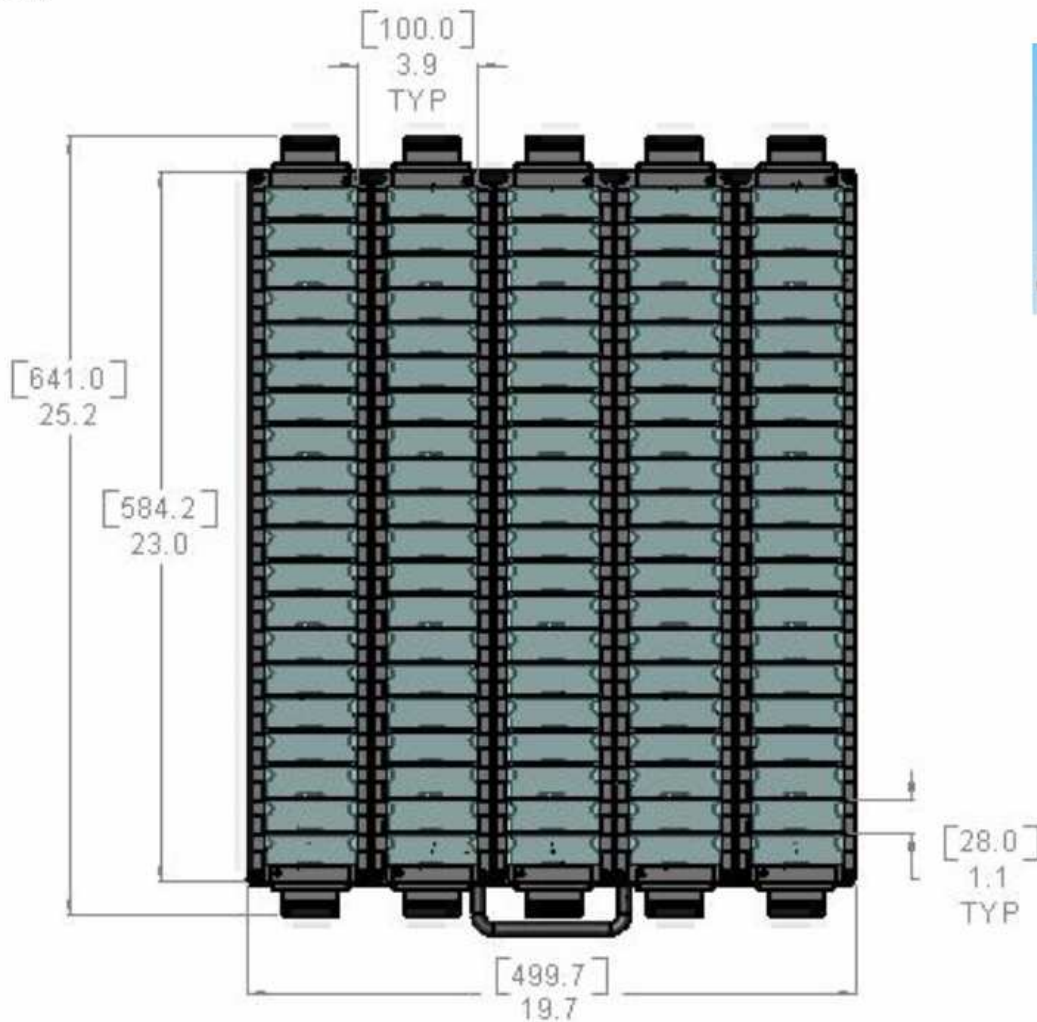
20 MTP Nest
on pull out tray

(Glass Slide and Vacuum/Magnetic Nest
fit on same tray)

3X Source Plate Positions
on manual slide

[100.0]
3.9

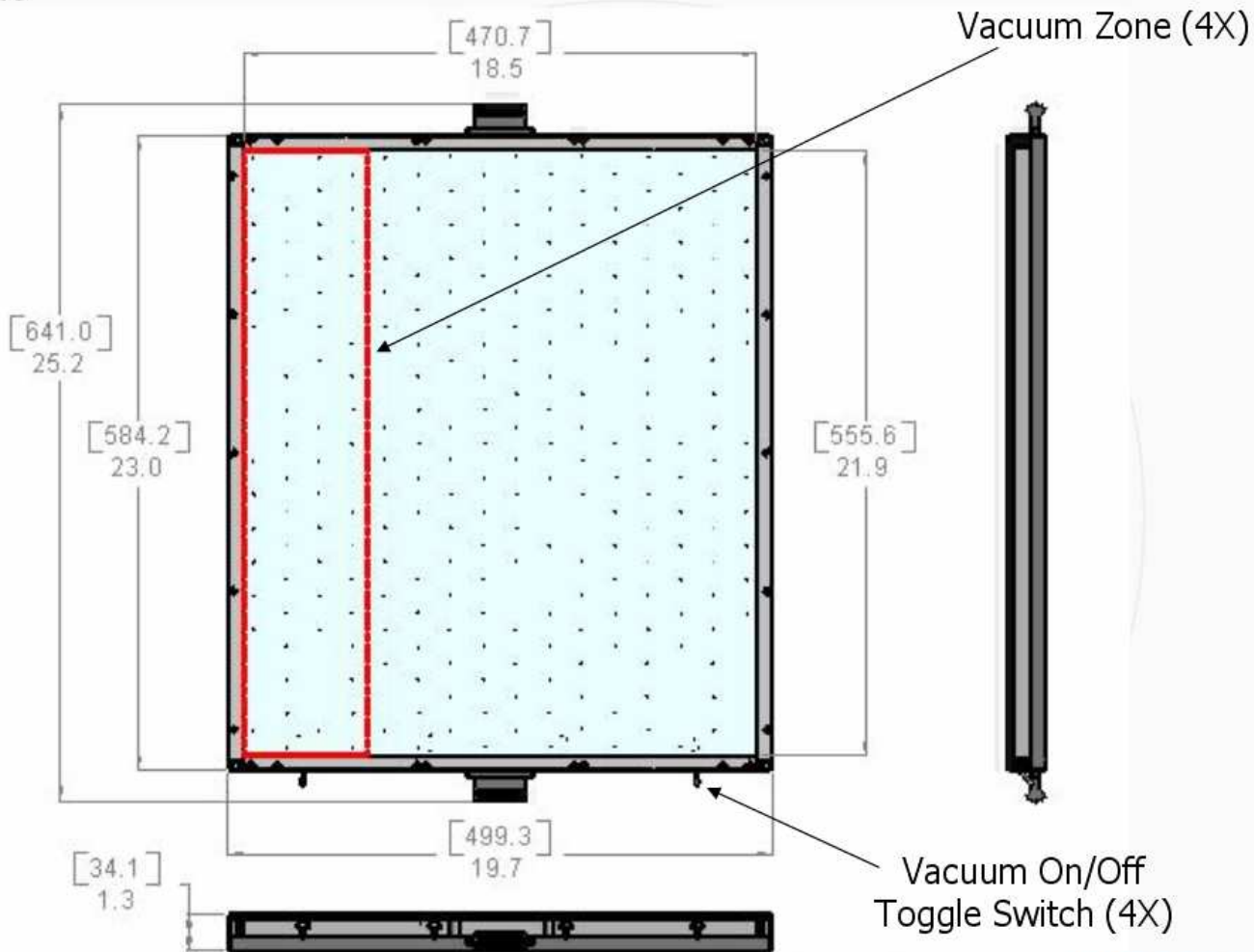## 100 Glass Slide Nest (5X 20 Position Sub Nests)
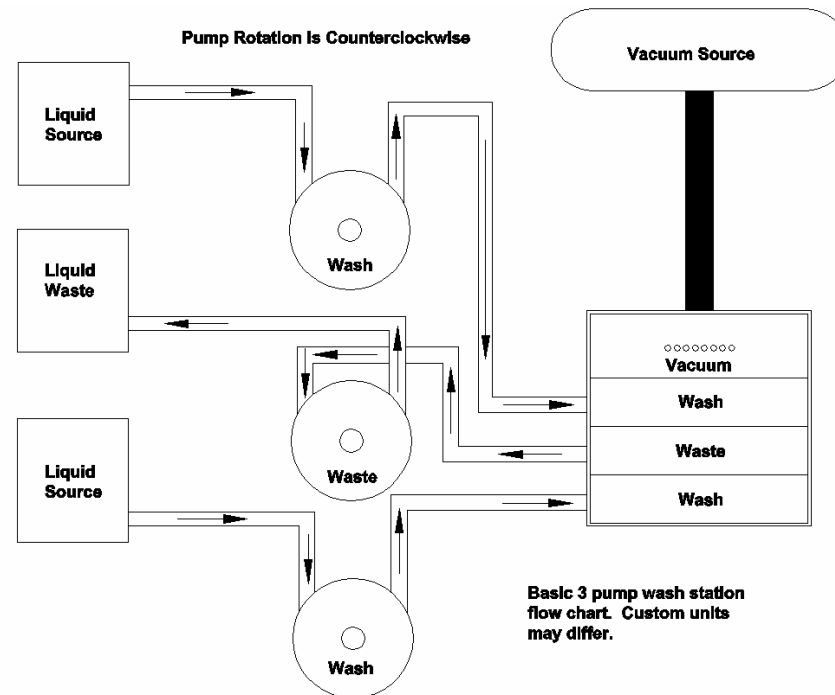### 6048-A012

[mm]
inches
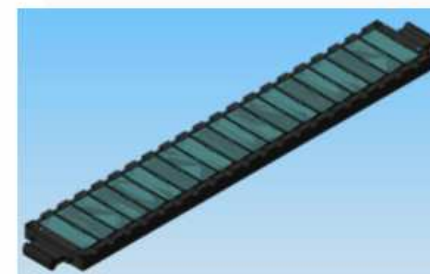
20 Position Sub Nest

Vacuum-Magnetic Nest
6048-A013

6. Installation

This section describes the actions necessary to prepare the equipment for operation. After completing this section, the unit will be ready for operation as a stand-alone unit.

- Position the unit in a suitable location with a minimum of 610mm (24 in) of clearance around the unit and remove any interior shipping tie downs or padding that may have been installed.

- Once the unit is in its final location unscrew the adjustable feet with an adjustable wrench until the wheels are no longer touching the floor. With a bubble level or measuring tape level the platform by adjusting each of the feet until the top plate of the unit is level or has an equal measurement to the floor. Once the platform has been leveled lock each of the adjustable feet in position by tightening the jam nut up against the bottom of the leg.

- Make the utility connections. This would include, but not limited too, clean dry air (if used), vacuum, chilled water recirculator, wash station cleaning solution and waste receptacles and **EARTH grounded** electrical power (Refer to Equipment Specification Section). **Warning: Make sure that the power switch on the side of the unit is in the OFF (O) position before connecting the electrical cord**. Below is a diagram of the peristaltic pump connections.

Pump Rotation Is Counterclockwise

Vacuum Source

Liquid Source

Liquid Waste

Liquid Source

Wash

Waste

Wash

Vacuum

Wash

Waste

Wash

Basic 3 pump wash station flow chart. Custom units may differ.

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- The pumps and harness should already be installed, if not, install the BioJet Plus™ pumps to the mounting plate and install on top of the AD6000 enclosure. **Note**: Refer to the OEM pump manual for setup of the BioJet Plus™ pumps. Use the supplied hardware to attach the pumps to the platform mounting plate as shown. Remove the blue valve cover (if installed) and install the syringes into the bottom port on the 3-port valve and tighten. Pull the piston end down and install it into the hole in the lifter arm. Secure the end of the piston with the thumbscrew. Repeat for any other pumps. Attach the harness to the rear of the pumps and secure with a flat blade screwdriver. Connect the other end to the appropriate connection on the rear of the machine. The cable marked COM will go to one of the PC's communication ports.
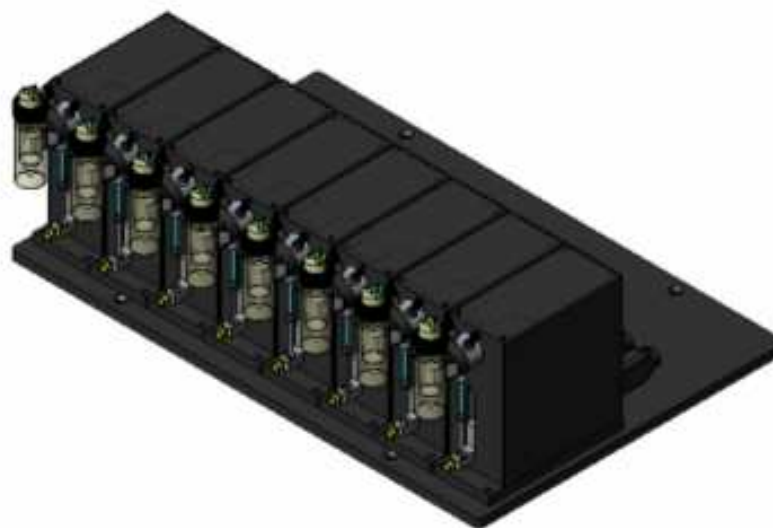
- If the reagent bottle brackets have been removed for shipment, then you will need to reinstall them on the face of the 3 port valves. Install the reagent bottle bracket to the front of the pump 3-port valve by removing the two screws holding the 3 port valve to the pump face and mounting the bracket to the face of the valve as shown. This bracket may already be installed or another device may be provided to hold the supply bottles. Connect the reagent line to the source bottle and as shown and then attach the other end to the left side of the 3-port valve. Place the bottle in the bottle rack or holder. Repeat the process for any additional bottles. Install the dispense line fitting in the right side of the 3-port valve and secure. **Note**: Make sure that the # 1 dispense line is attached to the right hand port on pump # 1 and that the other end is attached to BioJet valve # 1 on the dispense head.   Repeat for any additional pumps. **Warning: Do not cross thread the fittings in the three port valves. Doing so will render the 3 port valve useless due to leaks**.

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Attach Bottle Holder & Clip To 3-Port Valve

#1 To #1

- Connect one of the supplied DB9 interface cables to the **COM 1** port on the upper left rear side of the rear panel of the electrical enclosure. Connect the other end of the DB9 cable to **COM1** on the rear of the PC. Attach the other DB9 cable to **COM 2** (PC) and attach the other end to the BioJet Plus™ pump harness **COM** connector. **Note**: If you are using a CAN-BUS configuration you will need to install the CAN-BUS card in your computer (if not already installed) and install communication cable #6017-C060. Attach the DB9 connector to the PC CAN-BUS card and insert the other end into the rear lower port of the first BioJet Plus Pump™ as shown.

COM From Harness
COM From Computer
CAN-BUS Connection

- Connect the dispense line(s) to the BioJet(s) and install them in their respective positions in the dispense head. Example standard head shown below. Attach the BioJet leads coming from the e-chain to their corresponding positions. **Warning**: **Make sure that all numbers match. Pump #1 to Dispense Line #1 to BioJet #1 etc. Damage to the BioJets could occur if the lines are crossed**. Install the ceramic tip to the end of the BioJet. Make sure it is pushed up all the way on to the end of the BioJet. **Note**: A small piece of thin rubber pad is useful in twisting the tip into place.

Dispense Line
Spring Retainer
Ceramic Tip
BioJet Lead
BioJet Valve
Dispense Head Mounting Bracket

B I O)D O T
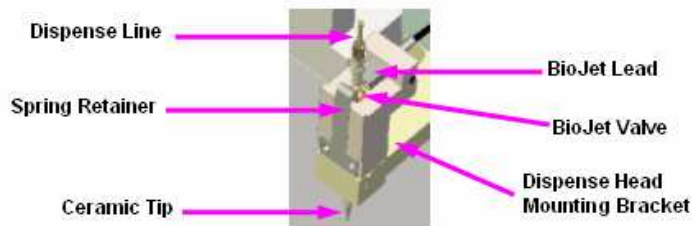
- If your unit is equipped with humidity you will need to unpack the humidifier and connect the Tygon™ tubing to the upper rear connector on the rear of the unit enclosure.   Attach the other end of the hose to the adapter on top of the humidifier. If your AD6000 is a 115VAC unit then all you have to do is plug the humidifier into the plug on the rear of the platform electrical enclosure marked HUMIDIFIER. If your unit is a 230VAC unit then you will need to plug the humidifier into the power converter supplied with your system. Plug the 100W power converter into the outlet on the rear of the unit marked HUMIDIFIER and select the incoming voltage on the rear of the converter. Plug the humidifier into the front of the converter. Refer to Manufacturer's instructions below.

- **Instructions For Step-Up / Down Voltage Converter**

1. Check the wattage requirement of your appliance from the product label or manual to determine the correct Step-up/down voltage converter for your appliance.
2. Set input voltage and select output socket/jack.

   A. For use in Europe or other 220-240V countries:
      - Set the "Input Voltage Selection Switch" at 220V position.
      - Connect your appliance to any socket/s on front panel for 110/120V output with total loaded capacity not exceeding the wattage of this converter.
   B. For use in North America or other 110-120V countries:
      - Set the "Input Voltage Selection Switch" at 110V position.
      - Connect your appliance to any socket/s on front panel for 220/240V output with total loaded capacity not exceeding the wattage of this converter.
   C. Check if the input plug provided on this converter is suitable for your country. If not, simply add an appropriate adaptor plug to it.

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⚠️ **Warning: Incorrect selection of input voltage, output socket/jack or overload may cause bodily injury and permanent damage to your converter and appliances and the MANUFACTURER/SUPPLIER of this unit assumes no responsibly for improper use**.

3. Plug the AC power cord of the converter to an approved **EARTH grounded** outlet and turn the ON/OFF switch to "ON" position. Now you may operate your appliances as usual.

4. If there is any vibration, immediately turn "OFF" the converter and disconnect the AC power cord from the electrical outlet and review the instructions to ensure all steps are followed properly.

5. Input 60Hz or 50Hz just pass through, no change.

6. When not in use turn "OFF" and unplug the converter from the electrical outlet and keep away from children.

- **Chiller Water Recirculator (optional equipment)**

    BioDot Part number 2026-0001 (120VAC 60Hz unit) and 2026-0002 (230VAC 50Hz unit)

👉 **Note**: Review the supplied manufacturer's literature for installation and use of their equipment.

1. Remove the recirculating chiller from its container and retain the shipping carton until the unit is completely assembled and working properly.

2. Attach the two provided lengths of Tygon™ tubing to the inlet and outlet ports on the rear of the unit.

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3. Fill the unit to just under the filler neck with the proper fluid. This would be distilled water for applications above 15°C and for applications below 15°C you should protect the unit with an antifreeze solution. Ethylene Glycol (laboratory grade) and distilled water in a 50/50 mixture is satisfactory. Refer to manufacturer's manual page 5 section 2.2.

4. Run the power cord through the lower opening in the center panel of the AD6000 base and lift the unit into the right side opening of the base assembly. Attach the other ends of the Tygon™ tubing to the inlet and outlet fittings of the chilled source position. Attach a provided length of small surgical tubing to the barbed fitting on the bottom of the chilled source position and place the other end into a small lab beaker. This will catch the condensation coming from the chilled source position.

5. Remove the filler cap and plug the chiller into the proper AC outlet (refer to manufacturer's manual page 6 section 3.1). Press the power switch ON. The chiller will begin to pump fluid through the system. Check for any possible leaks. Set the system to the desired temperature.
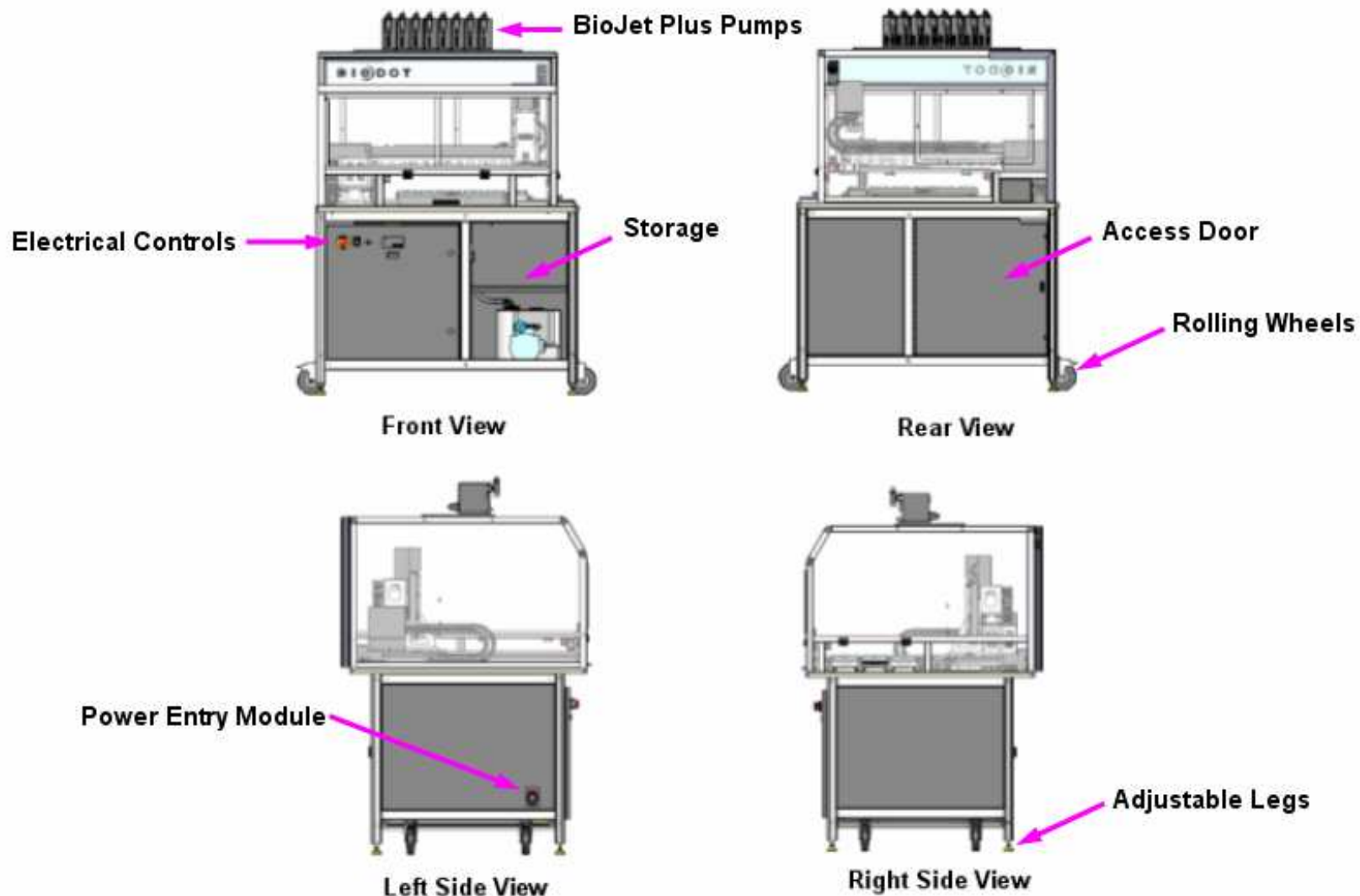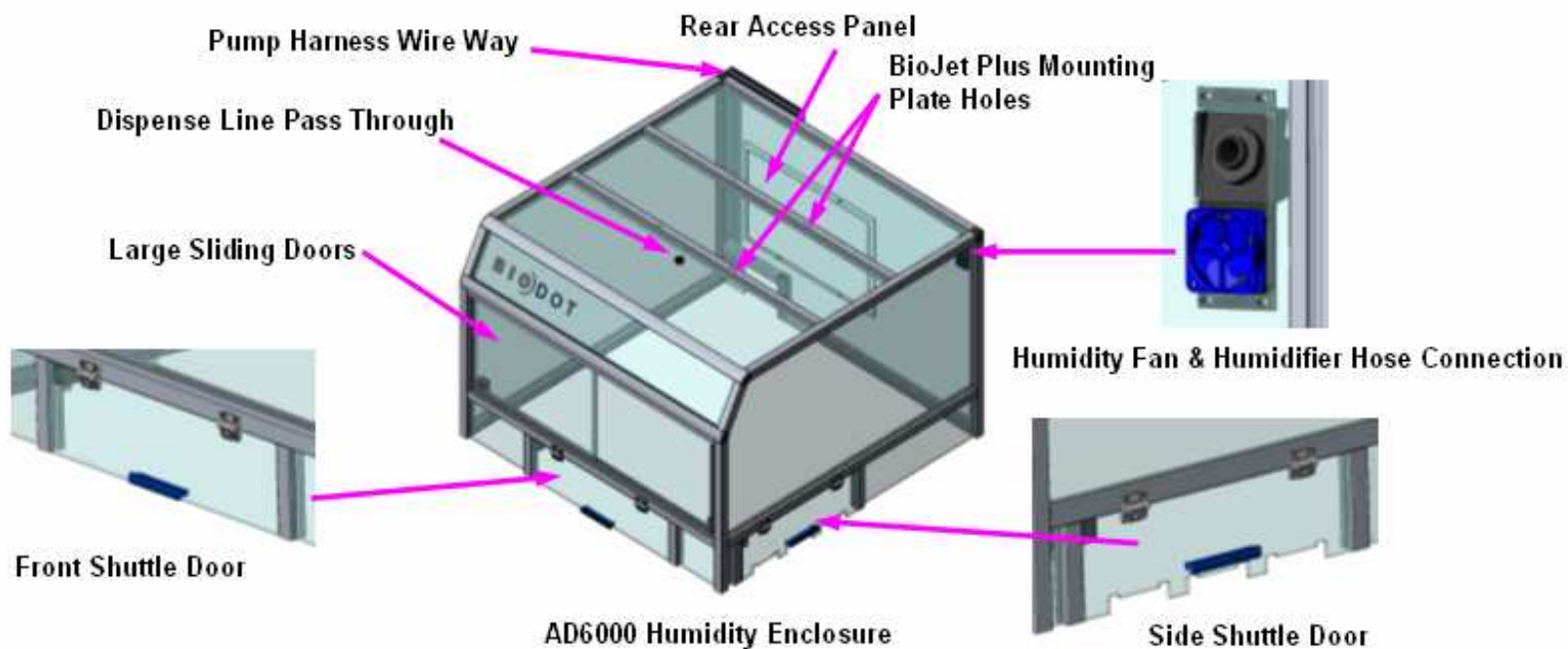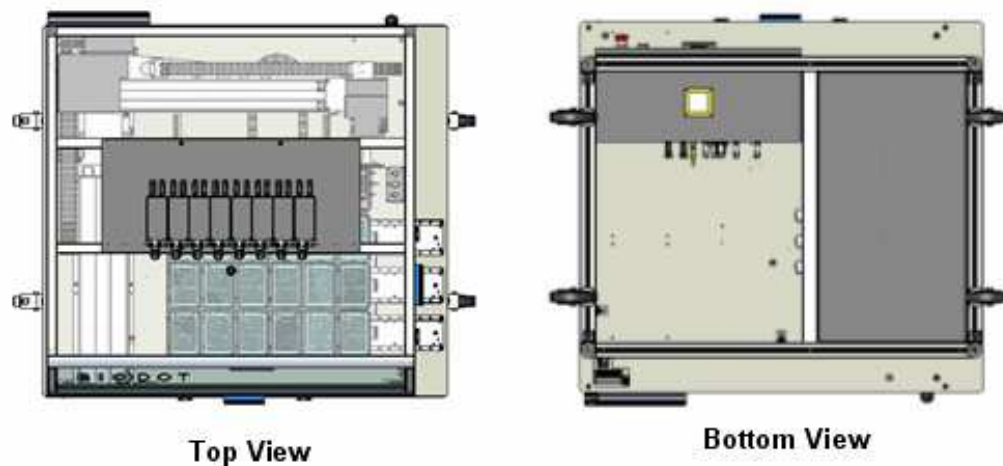

B I O)D O T
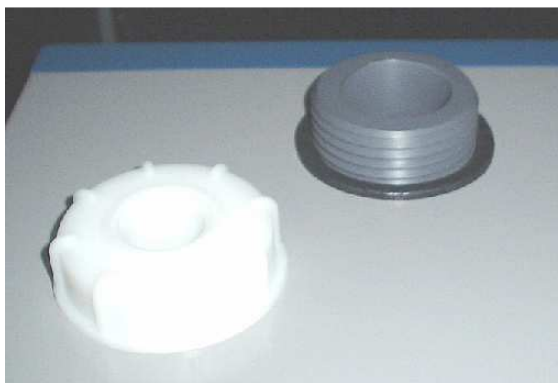
6. With the pump running, the fluid level in the reservoir will decrease as the closed system begins to fill. Add fluid as needed until the level in the reservoir stops going down. This means that your system is filled and the air has been purged from it. Replace the filler cap and turn it clockwise to lock it.
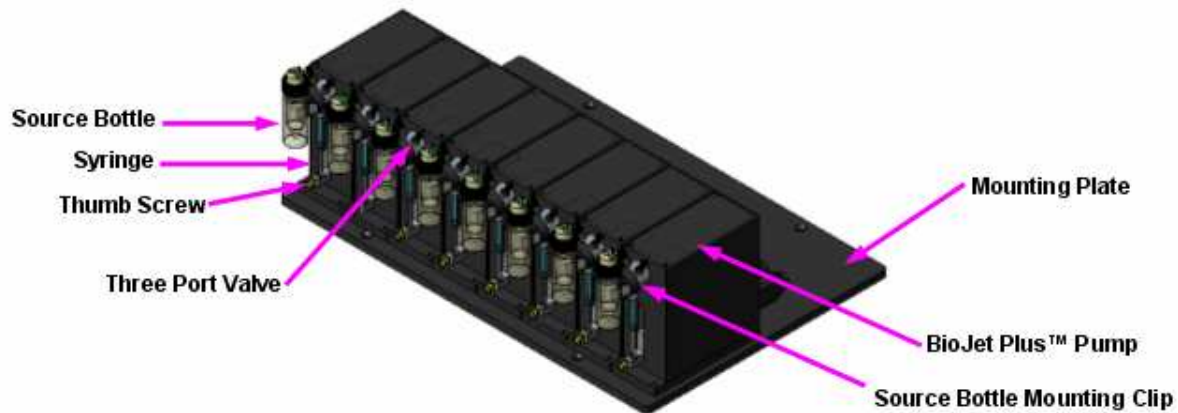

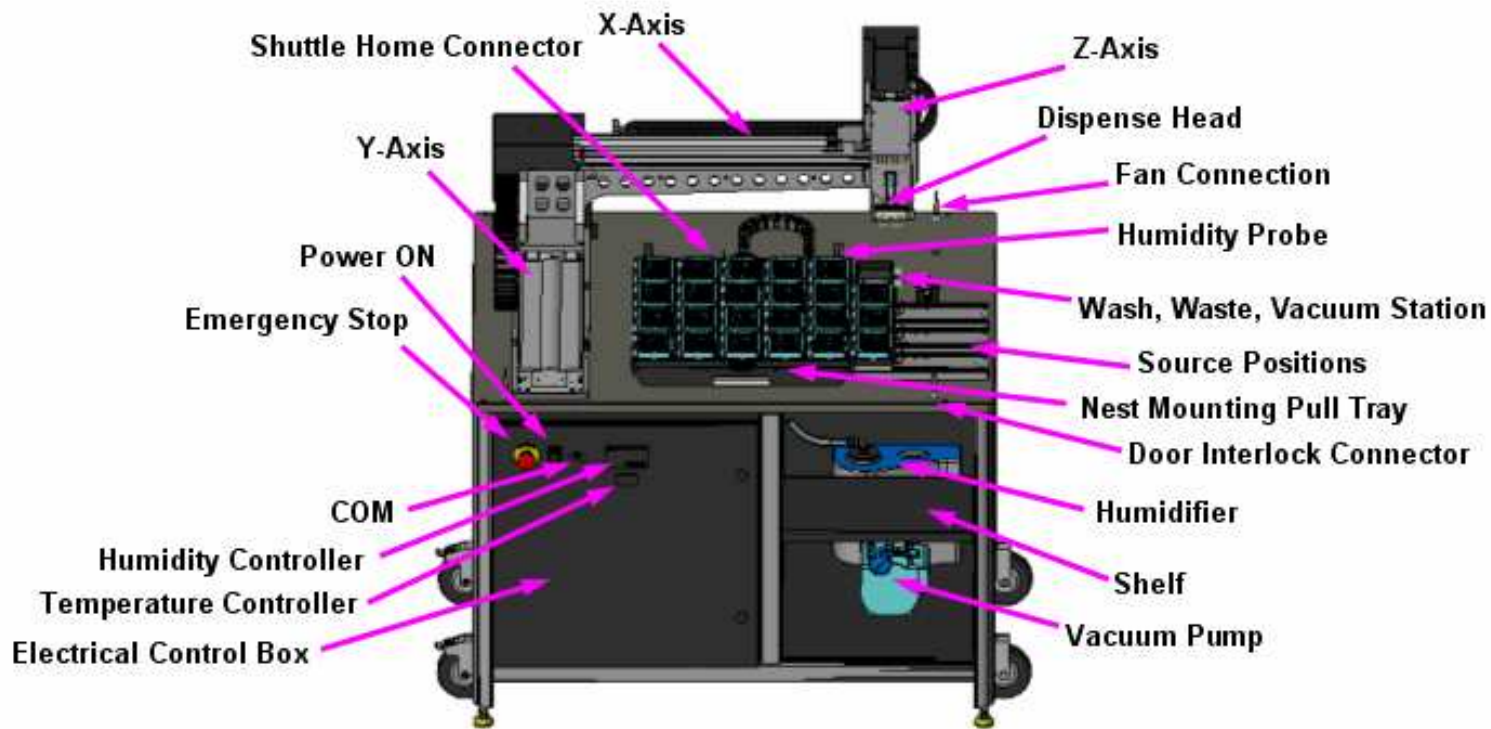**B I O** D O T

7. Conventions

The following conventions pertain to the AD6000 operating system. Custom nest and dispense head configurations can be incorporated.
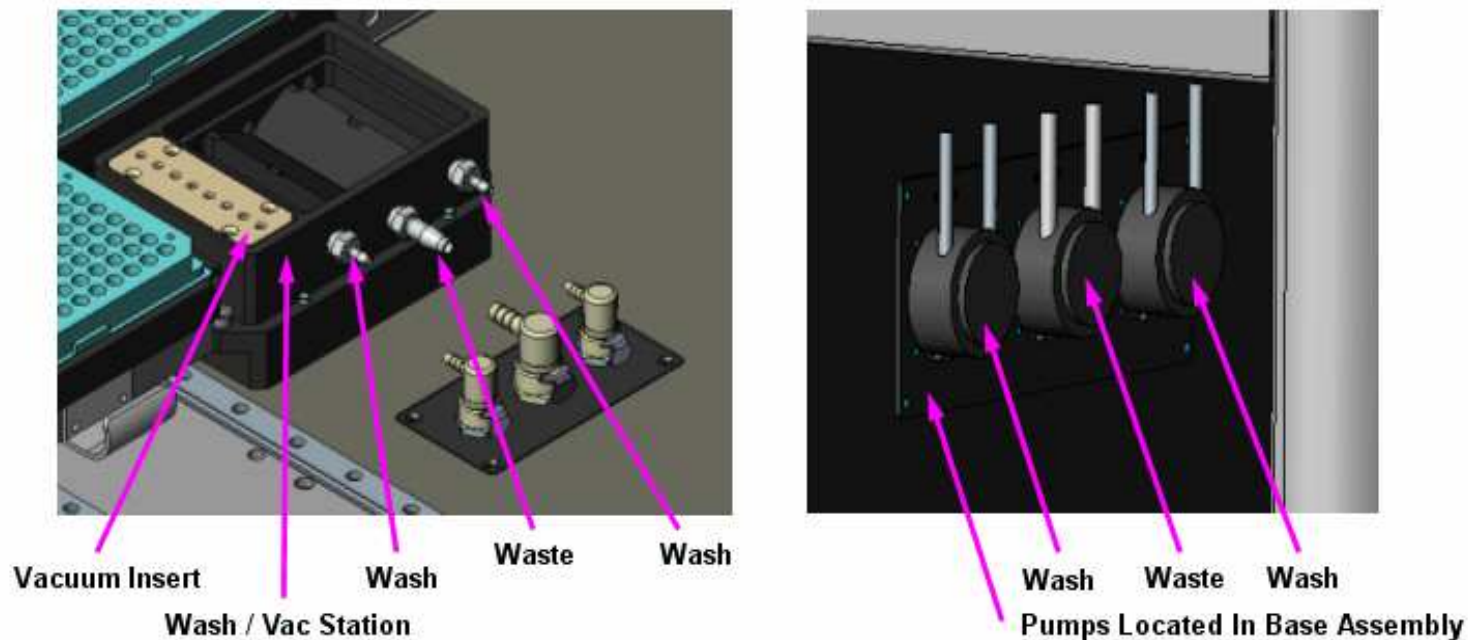

BioJet Plus Pumps

Electrical Controls

Storage

Access Door

Rolling Wheels

Front View

Rear View

Power Entry Module

Adjustable Legs

Left Side View

Right Side View

B I O D O T

Top View

Bottom View

Pump Harness Wire Way

Rear Access Panel

BioJet Plus Mounting Plate Holes

Dispense Line Pass Through

Large Sliding Doors

Humidity Fan & Humidifier Hose Connection

Front Shuttle Door

AD6000 Humidity Enclosure

Side Shuttle Door

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Note: Refer to the BioJet Plus™ OEM Pump Manual for setup and addressing of the BioJet Plus™ Pumps.

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Vacuum Insert   Wash   Waste   Wash
Wash / Vac Station

Wash   Waste   Wash
Pumps Located In Base Assembly

**Note**: Peristaltic pumps rotate counter clockwise.

The following components are accessed through the rear door of the base unit.

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The power entry module is located on the lower left side panel and has serviceable fuses. If replacing the fuses use the same type and size.

Supply Power ON / OFF
Serviceable Fuses & Voltage Selector
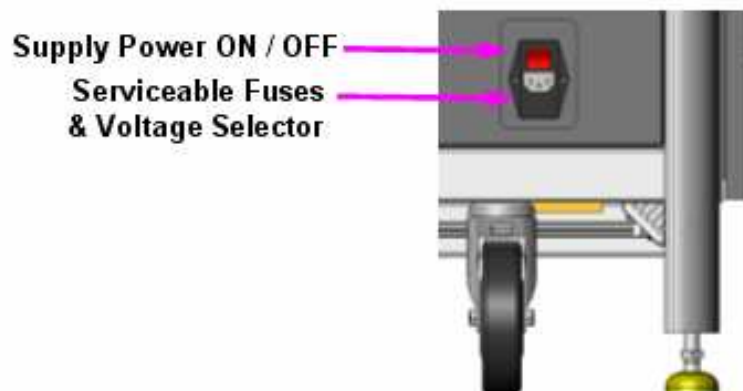
Note: All nest assemblies that utilize the pull tray feature will be of the modular type. This allows for quick nest changes with no alignment required. All nests of this type will have bullet nose liner locaters. Simply install the nest on the bullet pins and press down to install a nest assembly. This feature also allows for mixing of single nest assemblies such as 2 plate nests and 3 glass slide nests or any combination of the two assemblies.
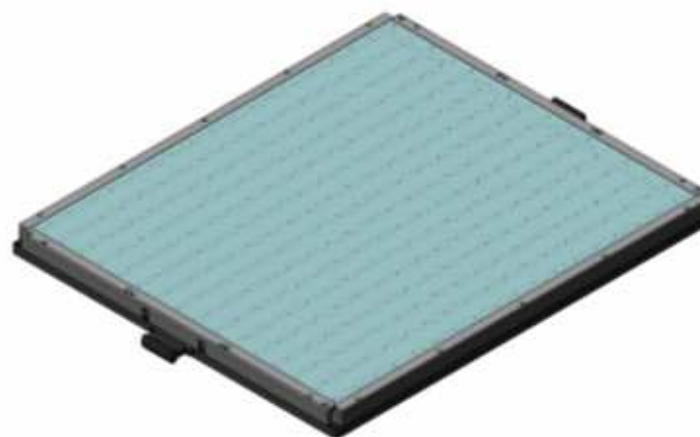

Locators
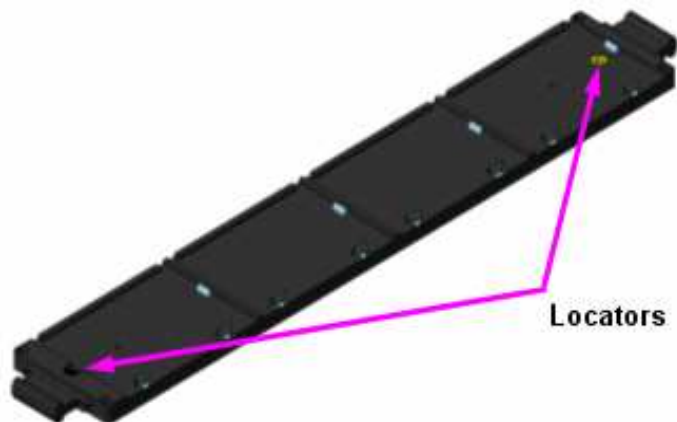
6048-A010B
4 Position Microtiter Plate Nest Assembly
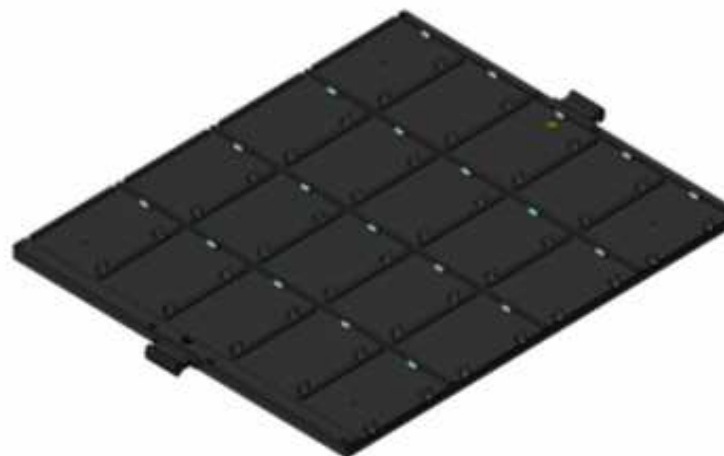
6048-A011B
20 Position Microtiter Plate Nest Assembly

BIODOT
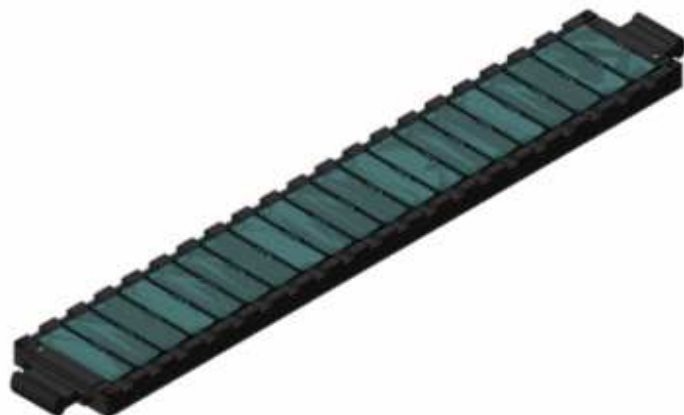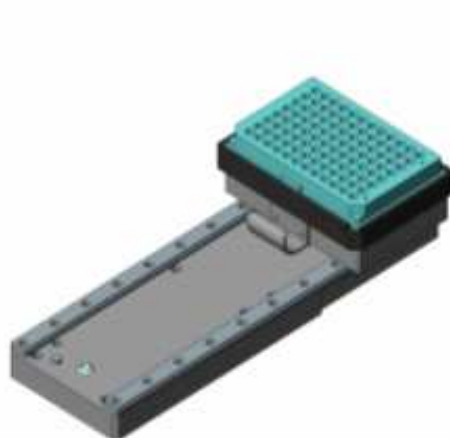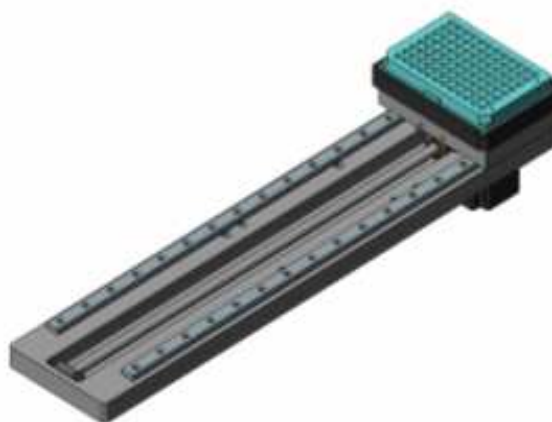
6048-A012B
20 Position Glass Slide Nest Assembly

6048-A013B
Vacuum-Magnetic Nest Assembly

The AD6000 has three source options manual slide, motorized slide and temperature controlled. Custom sources can be incorporated depending on customer applications.

6046-A037B
Manual Source Nest Assembly

6046-A038A
Actuated Source Nest Assembly

6048-A019-XX
Chiller Source Plate Assembly

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The AD6000 can utilize any number of different BioDot head configurations, and can also be customized and equipped with different readers and vision applications (possible examples below). The system can utilize anything from a single BioJet dispense head to a stacked 96 BioJet dispense head. It can be equipped to do powder dispensing, pin arraying, barcode reading and have vision applications incorporated to check for drop inspection or visual inspection.

Stacked BioJet Head   **Powder Dispense Head**   **Drop Inspection**   **Vertical Inspection**

B I O D O T

8. Glossary

- **E-Stop**: The red button head switch located on the left side of the front cover will stop "O" the unit immediately when depressed.

- **Start Switch**: This is the green momentary start "I" push button located on the left side of the front cover. This switch when depressed will power up the AD6000.

- **BioJet™ Valve**: Is an electronically controlled micro solenoid valve that precisely aspirates & dispenses fluid to a substrate or microwell plate. Shown with BioJet Plus™ fitting.


- **Command**: A unit of information used by the AxSys™ software to instruct the platform to complete a simple task. The user supplies the parameters of a command.

- **Dispense Envelope**: The portion of the platform that can be reached by the dispense head.

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- **Foundation Function**: A function that is used in all methods. These include (but are not limited to) functions that carry out such operations as tip washing or vacuuming tips.

- **Function Library**: Each method contains a list of functions that are available for use within the method. This library of functions exists independently of any functions that may be found in other methods, regardless of name or other identifiers. Libraries may be exported from and imported into methods.

- **Function**: A user determined list of commands that can be used as a macro when programming. Often, simpler functions are used to create more complex functions.

- **BioJet Plus™ Pump** (Optional): Is a pump with the ability to synchronize the pump syringe and the platform motion with the BioJet™ valves. Multiple BioJet Plus™ pumps can be ordered to accommodate most standard and custom applications.

- **Icons**: Graphical representations of the AxSys™ program commands. Those found on the tool bar as small buttons serve as a short cut for insertion of the command into the program list. Those found in the program list serve as a short cut to the command-editing window. (Example is of the **GO** command).

- **Method**: A set of commands created with the AxSys™ software that performs a specific series of tasks. They exist as individual AxSys™ files. This term is used interchangeably with the term "program".

- **Peristaltic Pump(s)**: Supply fluids to and from the wash station(s). These are externally mounted and may also be supplied with a heat source depending on the application.

B I O D O T
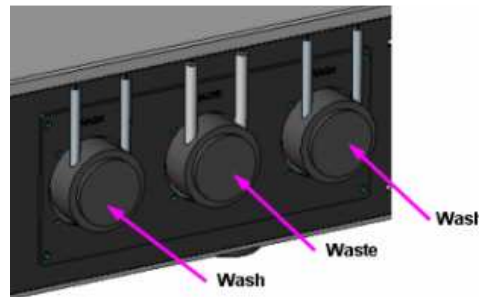
- **Pre-dispensing**: The process of dispensing sample to the waste area of the wash station or back into the sample solution prior to transfer to achieve a steady state. Pre-dispensing is done in order to ensure the quality of drops dispensed in the transfer.

- **Program**: See "Method".

- **Program List**: The operator determined list of commands that the computer will follow in a program. The operator can add, remove, or edit commands as necessary.

- **Programming**: The act of entering data via the computer to define operation parameters.

- **Set Up**: Preparing the equipment to transfer samples.

- **Substrate**: This may include but not limited to microwell plates, glass slides, membranes, or other targets and printed materials.

- **Source Plate**: The microwell plate-containing sample that is to be transferred to arrays or other targets.

- **Troubleshooting**: Identifying and resolving problems with the operation of the platform or the AxSys™ software.

- **Wash Station**: An assembly consisting of a wash solution bath (heated or ambient) and vacuum drying apparatus for cleaning tips. Wash stations can be ordered for custom applications.
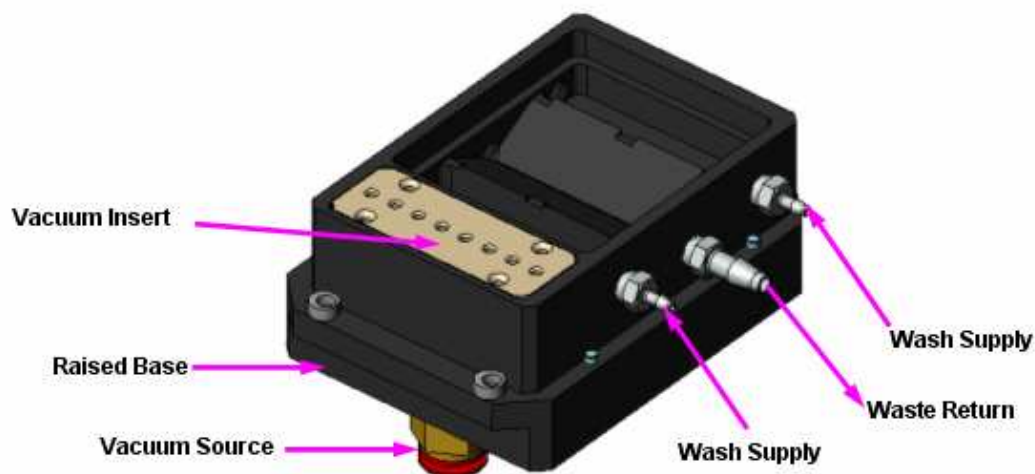
B I O)D O T

9. Software Installation

9.1.   Software Installation

The BioDot AD6000 should be shipped already loaded with operating software. If it is not or if a repair has been made in the field, then follow the steps below to load the operating software. Always back up your NVSRAM to disk when receiving a new piece of equipment from BioDot Inc. If there is an installer present please ask them to save your NVSRAM for you.

• Insert the CD into your disk drive.

• Click the Windows 2000/XP "Start" button. Select the control panel option, and then choose "Add/Remove Programs". Next select Install and follow the Windows prompts, or open up the drive and select the setup icon.

• Before opening the AxSys™ software program, be sure your AD6000 unit is connected to your computer. The interface cable should be connected to the TERMINAL or COM 1 port, located on the back of the AD6000 unit. The other end of the interface cable should be plugged into one of the computer's COM ports. This is normally COM 1; however you may use any of the computer's COM ports. Make sure the connector is screwed in completely. If you have BioJet Plus™ pumps connect the harness com cable to COM 2 on the computer. If you are using the CAN-BUS configuration disconnect the pump harness COM cable and connect the CAN-BUS cable to the lower port on the rear of the first pump as shown below and attach the other end to the installed CAN-BUS card.

PC Connection   CAN-BUS Pump Connection

B I O D O T
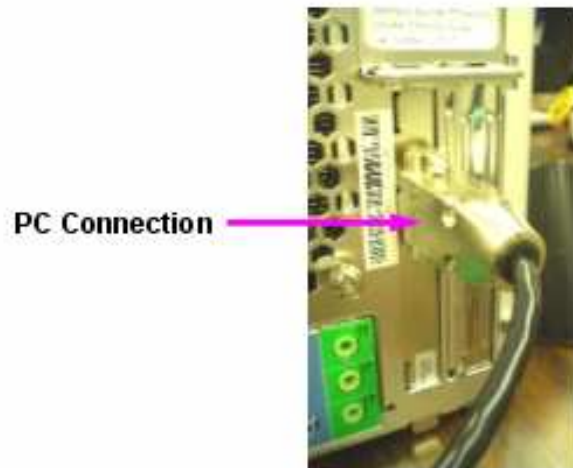
- Turn **ON** the switch on the side of the machine. Release the emergency stop and start the unit by depressing the start button.

- Open the AxSys™ software program. The software will search for the unit connection on COM port 1 & 2. If there is a connection, then the software will display a green connection in the lower right hand corner of the screen (refer to diagram below). If it is not connected you will receive an error message. Select "Retry" and choose the COM port into which the interface cables are connected. Select the COM port by clicking on the arrow next to the blank labeled "Switch To:" within the "Communications Settings" window. The software will try to connect again. If you receive an error message again, close the AxSys™ software program and turn OFF your AD6000 unit. Wait 10 seconds, turn ON your AD6000 unit, wait an additional five seconds, and run AxSys™. If a connection cannot be established, check all of your connections and try again. If you still cannot connect your configuration may not be correct. Go to tool → options → configuration → edit and select the components. Once you have configured the system then try to connect again. If you are still having problems please contact BioDot support at support@biodot.com, or telephone (949) 440-3685 for assistance.

DISCONNECTED

CONNECTED

B I O D O T
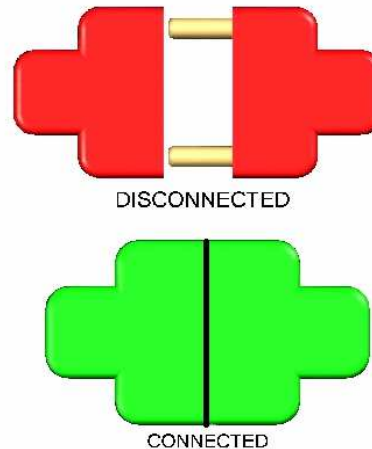
10. Operation

10.1.   Basic Operation

Follow the steps below to activate the AD6000 platform. **Note**: Operation may differ depending on application requirements and configurations of the platform. Also make sure that the installation has been completed. All pumps, lines and BioJets must be flushed and primed before operating the platform. Refer to the Addendum at the rear of this manual for first time startup and care of the dispenser system. Other references are the BioDot OEM Pump and Dispenser manuals.

- Make sure you have your source and target materials in place. Turn **ON** the switch on the side of the machine.
- Turn **ON** the air and vacuum supply sources if applicable.
- Release the Emergency stop button and press the Start button.
- Open the AxSys™ software and configure the platform under the Tools pull down menu. Cycle the power and then try to connect to the unit under the Tools pull down menu. Once connected, under Tools, Manual Mode, go to the speed tab and enter the values of 10, 50 and 1000 respectively, apply. Then go to the axis tab and select all three axes and select OK to home the unit. Once the unit has been homed go to the other tabs set the dispense speed select all of this type, then set the speed for the pumps to 10, 50 and 1000 and. Go to prime and cycle the pumps. Go to wash pump tab and prime the wash pumps until liquid is circulating through the system.
- If the machine has been setup and is ready to start running programs, then load or create your program and press the GO icon.
- The machine will now home and then start implementing commands. **Warning: If you see the head assembly coming too close to objects on the nest when moving to various positions, or that the program parameters have been altered select the STOP button and review the program settings before proceeding. You should always maintain a safe Z-Axis height to prevent damage to components**.
- Once you have completed your run flush your system, refer to Methods – Flushing in the rear of this manual, and shut down your system by pressing the E-stop. Turn **OFF** the power to the unit on the left side panel.
- Turn **OFF** any air or vacuum supplies.
- Examine the AD6000 for any spilled reagents or accumulations of dust. Using a moist, lint free cloth removes any dust or spilled reagents.

Wear gloves and safety glasses during any cleaning procedure that could involve contact with hazardous materials or fluids.

B I O D O T

10.2.   Humidity & Temperature Controller

If you need to change the parameters on the humidity controller follow the procedure below. **Note**: Always refer to any manufacturer's literature for more detailed information and operation of their products.

OPERATION:
The upper display may be RH, Temperature or Dew point readings depending on your Reading Configuration selections. Factory defaults are shown. The Dual Display allows the user to observe the Relative Humidity or Dew point (upper display) and Temperature Value (lower display), at the same time.

1	Output 1 / Setpoint 1 / Alarm 1 Indicator
2	Output 2 / Setpoint 2 / Alarm 2 Indicator
°C	°C Unit Indicator For Temperature or Dew point
°F	°F Unit Indicator For Temperature or Dew point
%RH	Display Shows The Percent Relative Humidity
D	Display Shows The Dew point
⊙	Changes Display To Configuration Mode And Advances Through Menu Items. If Unit Is Equipped With Option.
⬆	Used In Program Mode
⬇	Used In Program Mode
↵	Accesses Submenus In Configuration Mode And Stores Selected Values. If Unit Is Equipped With Option.

Note: Refer To Manufacturer's Literature For Configuration Flow Chart.

⊙ Menu	• To enter the Menu, the user must first press this button. • Use this button to advance/navigate to the next menu item. The user can navigate through all the top level menus by pressing this button. • While a parameter is being modified, press this button to escape without saving the parameter.
⬆ Up	• Press the up button to scroll through "flashing" selections. When a numerical value is displayed press this key to increase value of parameter that is currently being modified. • Pressing the up button for approximately 3 seconds will speed up the rate at which the set point value increments. • In the Run Mode, pressing the up button changes display form RH readings to Temperature readings.
⬇	• Press the down button to go back to a previous Top Level Menu item. • Press this button twice to reset the controller to the Run Mode.

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Down	• When a numerical value is flashing (except set point value) press down to scroll digits from left to right allowing the user to select the desired digit to modify. • When a Setpoint value is displayed press down to decrease value of a Setpoint that is currently being modified. Pressing the down button for approximately 3 seconds will speed up the rate at which the Setpoint value is decremented. • In the Run Mode, press the down button changes from RH readings to Dew point readings.
⏎ Enter	• Press the enter button to access the submenus from a Top Level Menu item. • Press enter to store a submenu selection or after entering a value – the display will flash an **StRd** message to confirm your selection. • In the Run Mode, press enter twice to enable Standby Mode with flashing **Stby**.

SPECIFICATION
SENSOR SPECIFICATIONS
Relative Humidity Accuracy/Range:
±2% for 10 to 90% RH
±3% for 0 to 10%RH and 90 to 100%RH
Non-linearity: ±3%
Response Time:
4 sec (63% slowly moving air)
Repeatability: ±0.1%
Resolution: 0.03%, 12bit
Temperature Accuracy/Range:
±1°C (±2°F) for -40 to 0°C and 80 to
123.8°C (-40 to 32°F and 176 to 254°F)
±0.5°C (±1°F) for 0 to 80°C (32 to 176°F)
Response Time:
5 sec (63% slowly moving air)
Repeatability: ±0.1°C
Resolution: 0.01°C, 14 bit
METER SPECIFICATIONS
Display:
4-digit, 9-segment LED,
• 10.2 mm (0.40"): (Dual Vertical)
• 10.2 mm (0.40") and 21 mm (0.83")
(Dual Horizontal)
Red, green, and amber programmable colors for setpoint and temperature units.

B I O)D O T

Output 1:	
Relay 250 Vac @ 3 A Resistive Load,	
SSR, Pulse, Analog Voltage and Current	
Output 2:	
Relay 250 Vac @ 3 A Resistive Load,	
SSR, Pulse	
Options: Communication	
RS-232 / RS-485 or 10BaseT or Excitation: 24 Vdc @ 25 mA	
Not available for Low Power Option	
Line Voltage/Power:	
90 - 240 Vac ±10%, 50 - 400 Hz*, or 110 - 375 Vdc, **5 W**	
***No CE compliance above 60 Hz**	
Low Voltage Power Option:	
12 - 36 Vdc, **3 W***	
****Units can be powered safely with 24 VAC but No Certification for CE/UL is claimed.**	
Dimensions:	
Dual Horizontal: 48H x 96W x 127D mm	
(1.89 x 3.78 x 5")	
Dual Vertical: 48W x 96H x 127D mm	
(1.89 x 3.78 x 5")	
Weight:	
295 g (0.65 lb)	
Approvals:	
CE per EN50081-1,	
EN50082-2, EN61010-1	

Humidity Probe
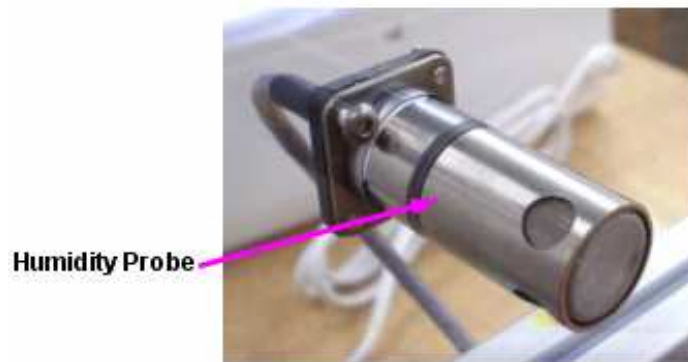
B I O DOT

Procedure:

Quick Reference Key Strokes for Setting or Changing SP1 Temp, °F to °C and PID. Refer to definitions above and flow chart below.
BioDot factory settings are **C°**, **60.00** and **PID**.

⟳ = MENU   ▼ = ↓ DOWN ARROW

▲ = ↑ UP ARROW   ⏎ = ENTER

1. To set the value for SP1 select the following (BioDot factory setting is 060.0):

MENU, ENTER, ↑↓ to change value to 060.0, ENTER, MENU, MENU.

⟳ ⏎ ▲ ▼ TO VALUE ⏎ ⟳ ⟳

2. To set the temp degree °F or °C select the following (BioDot factory setting is °C):

MENU, MENU, MENU, ENTER, ENTER, MENU, ENTER, ↑ to toggle between °F & °C, ENTER, MENU x 10, ENTER, MENU.

⟳ ⟳ ⟳ ⏎ ⏎ ⟳ ⏎ ▲ °F OR°C ⏎ ⟳ X 10 ⏎ ⟳

3. To set the "Dead Value" for Output 1 select the following (BioDot uses PID but Dead Value would be 001.0):

MENU, MENU, MENU, ENTER, MENU x 4, ENTER, MENU x 5, ENTER, ↓ to move ←→ ↑ to change value, ENTER, MENU x 5,
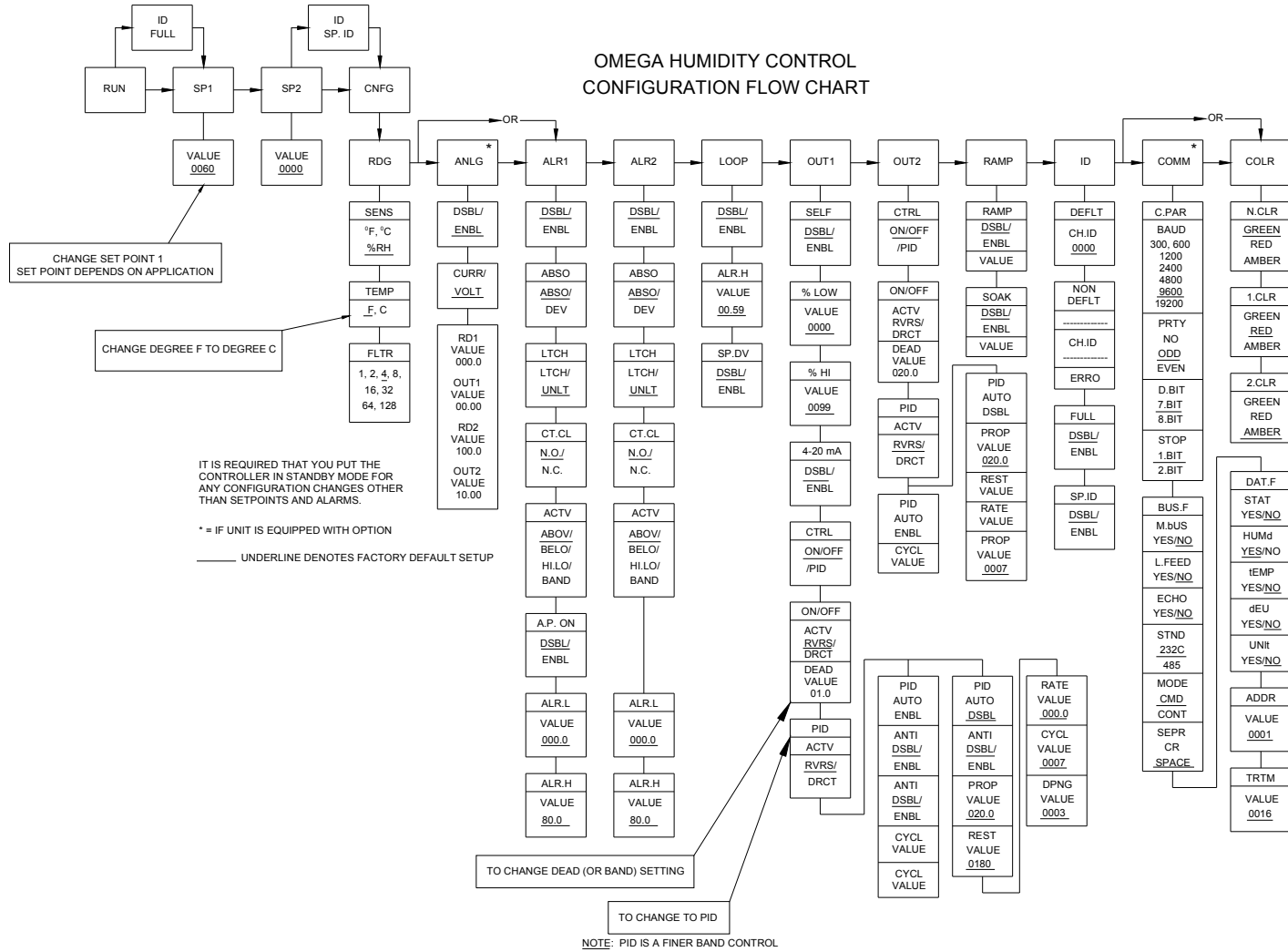ENTER, MENU.

⟳ ⟳ ⟳ ⏎ ⟳ X 4 ⏎ ⟳ X 5 ⏎ ▼ TO MOVE SIDE TO SIDE ▲ TO CHANGE VALUE ⏎ ⟳ X 5 ⏎ ⟳

4. You may choose to use PID instead of Dead Value. PID keeps a tighter band width than Dead Value. To set PID select the following:

MENU, MENU, MENU, ENTER, MENU x 4, ENTER, MENU x 3, ENTER, ↑, ENTER, MENU x 13.

⟳ ⟳ ⟳ ⏎ ⟳ x 4 ⏎ ⟳ x 3 ⏎ ▲ ⏎ ⟳ x13

B I O ⟩ D O T

OMEGA HUMIDITY CONTROL
CONFIGURATION FLOW CHART

Top row boxes:
- ID FULL
- ID SP. ID
- RUN — SP1 — SP2 — CNFG

SP1: VALUE 0060
SP2: VALUE 0000

CHANGE SET POINT 1
SET POINT DEPENDS ON APPLICATION

CHANGE DEGREE F TO DEGREE C

Main column headers: RDG — ANLG * — ALR1 — ALR2 — LOOP — OUT1 — OUT2 — RAMP — ID — COMM * — COLR

RDG
- SENS: °F, °C / %RH
- TEMP: F, C
- FLTR: 1, 2, 4, 8, 16, 32, 64, 128

ANLG *
- DSBL/ ENBL
- CURR/ VOLT
- RD1 VALUE 000.0
- OUT1 VALUE 00.00
- RD2 VALUE 100.0
- OUT2 VALUE 10.00

IT IS REQUIRED THAT YOU PUT THE CONTROLLER IN STANDBY MODE FOR ANY CONFIGURATION CHANGES OTHER THAN SETPOINTS AND ALARMS.

* = IF UNIT IS EQUIPPED WITH OPTION

_____ UNDERLINE DENOTES FACTORY DEFAULT SETUP

ALR1
- DSBL/ ENBL
- ABSO: ABSO/ DEV
- LTCH: LTCH/ UNLT
- CT.CL: N.O./ N.C.
- ACTV: ABOV/ BELO/ HI.LO/ BAND
- A.P. ON: DSBL/ ENBL
- ALR.L VALUE 000.0
- ALR.H VALUE 80.0

ALR2
- DSBL/ ENBL
- ABSO: ABSO/ DEV
- LTCH: LTCH/ UNLT
- CT.CL: N.O./ N.C.
- ACTV: ABOV/ BELO/ HI.LO/ BAND
- ALR.L VALUE 000.0
- ALR.H VALUE 80.0

LOOP
- DSBL/ ENBL
- ALR.H VALUE 00.59
- SP.DV: DSBL/ ENBL

OUT1
- SELF: DSBL/ ENBL
- % LOW VALUE 0000
- % HI VALUE 0099
- 4-20 mA: DSBL/ ENBL
- CTRL: ON/OFF /PID
- ON/OFF: ACTV RVRS/ DRCT / DEAD VALUE 01.0
- PID: ACTV RVRS/ DRCT

OUT2
- CTRL: ON/OFF /PID
- ON/OFF: ACTV RVRS/ DRCT / DEAD VALUE 020.0
- PID: ACTV RVRS/ DRCT
- PID AUTO ENBL
- CYCL VALUE

RAMP
- RAMP: DSBL/ ENBL / VALUE
- SOAK: DSBL/ ENBL / VALUE
- PID AUTO DSBL
- PROP VALUE 020.0
- REST VALUE
- RATE VALUE
- PROP VALUE 0007

ID
- DEFLT
- CH.ID 0000
- NON DEFLT
- CH.ID ----------
- ERRO
- FULL: DSBL/ ENBL
- SP.ID: DSBL/ ENBL

COMM *
- C.PAR
- BAUD: 300, 600 1200 2400 4800 9600 19200
- PRTY: NO ODD EVEN
- D.BIT: 7.BIT 8.BIT
- STOP: 1.BIT 2.BIT
- BUS.F
- M.bUS: YES/NO
- L.FEED: YES/NO
- ECHO: YES/NO
- STND: 232C 485
- MODE: CMD CONT
- SEPR: CR SPACE

COLR
- N.CLR: GREEN RED AMBER
- 1.CLR: GREEN RED AMBER
- 2.CLR: GREEN RED AMBER
- DAT.F
- STAT: YES/NO
- HUMd: YES/NO
- tEMP: YES/NO
- dEU: YES/NO
- UNlt: YES/NO
- ADDR VALUE 0001
- TRTM VALUE 0016

Lower PID boxes:
- PID AUTO ENBL / ANTI DSBL/ ENBL / ANTI DSBL/ ENBL / ANTI DSBL/ ENBL / CYCL VALUE / CYCL VALUE
- PID AUTO DSBL / ANTI DSBL/ ENBL / PROP VALUE 020.0 / REST VALUE 0180
- RATE VALUE 000.0 / CYCL VALUE 0007 / DPNG VALUE 0003

TO CHANGE DEAD (OR BAND) SETTING

TO CHANGE TO PID

NOTE: PID IS A FINER BAND CONTROL

Note: All settings are factory settings. The only settings changed at BioDot will be SP1 (60.0), Temp from °F to °C and the band width changed to PID (Recommended) instead of Dead Value.

BIO DOT

## 11. Theory & Practice of Dispensing

11.1. Theory of Dispensing

This section describes the background, theories and calculations of the Aspirate/ Dispense system. After completing this section, the operator will gain a better knowledge of the unit's function.

11.1.1. Background

The BioJet Plus™ technology combines the high-resolution displacement capabilities of a syringe pump with a high-speed BioJet Plus™ valve. This combination permits the non-contact dispensing of nanoliter volumes. This section will examine the theory of this technology as well as explain some the practical aspects in using the technology.

The dispensing system has the syringe pump connected to the backside of the valve, as shown below.

BioJet Plus Dispensing System
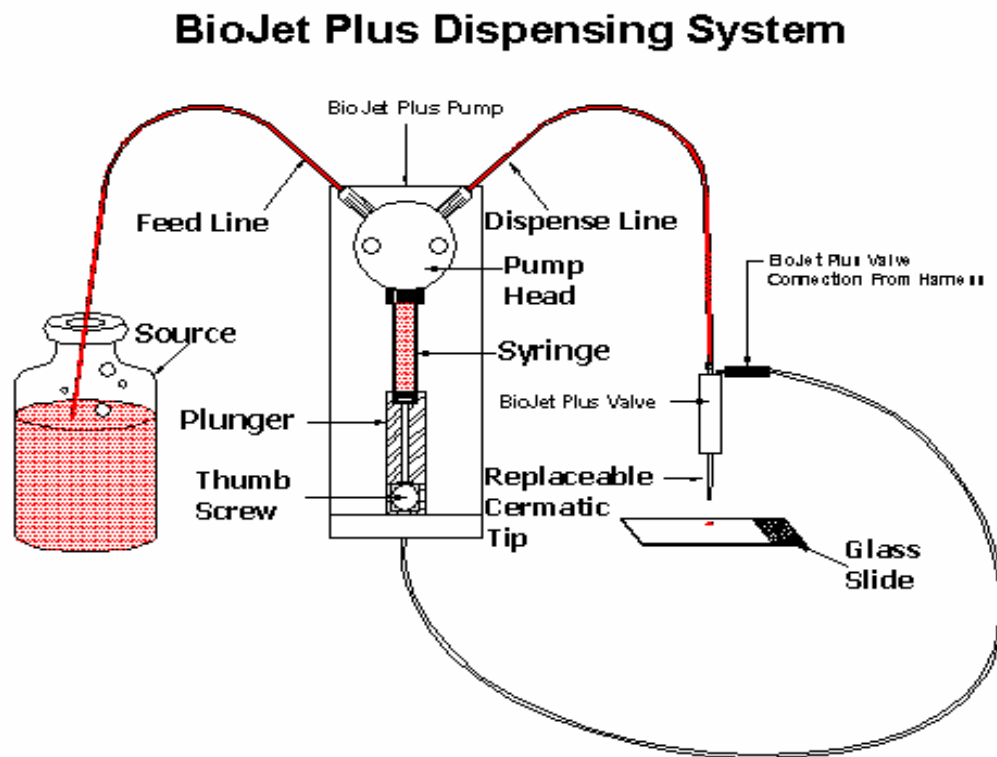
For a typical dispensing system, 4 to 8 of these syringe/solenoid channels are placed together. On the AD6000 you may order from 1 to 96 channels. Two modes of liquid handling are possible: Continuous (bulk) dispensing and aspirate/dispense.

Continuous dispensing involves pulling reagent or solvent from a reservoir into the syringe and then dispensing it through the micro solenoid valve. Filling the system with a system or backing fluid, dipping the tip of the valve into a sample, withdrawing the syringe to aspirate the sample, and then dispensing the aspirated sample accomplishes Aspirate/dispense.

B I O D O T

12. Theory & Practice of BioJet Plus™ Dot Dispensing

12.1. The Steady-State Pressure (SSP)

The BioJet Plus™ dot dispensing system is a hydraulically driven system and requires a fluid medium to be present from the syringe to the microsolenoid BioJet Plus™ valve. The dispensing process involves the following steps: 1) the syringe is displaced a given amount, 2) the valve is opened for a short period of time (milliseconds), 3) fluid is released from the valve and travels to the tip, 4) the fluid increases its linear velocity as it passes through the tip orifice and ejects as drop (or stream if the amount of fluid is large). One valve actuation results in one drop.

The key to BioJet Plus™ dot dispensing the proper volume is the steady-state pressure (SSP) in the dispensing system. This pressure has several important features:

- It is achieved by the displacement of fluid by the syringe pump
- The SSP is displacement (drop size) dependent, increasing with increasing displacement (drop size)
- The SSP is determined by the system compliance, which is dominated by entrapped air bubbles. Since the entrapped air is system variable the SSP is not unique for a given displacement
- Once the SSP is established, the amount of fluid displaced by the syringe pump will equal the amount dispensed

The BioJet Plus™ dot dispensing system can be modeled as an electrical circuit with the pressure acting as the voltage, the flow rate as the current, the system compliance as capacitive elements, the valve, tip, and feed lines as resistive elements, and the valve as a switch. This model shows the syringe pump as a current source, which provides an advantage over a pressure source (e.g. gas pressure) in that any changes in resistance will not affect the flow rate. In contrast a pressure source will be affected by changes in resistance in the system.

12.1.1. Factors Affecting the Dispense Volume

The model described above shows the fluidic circuit possessing a feedback loop, which can be used to achieve the SSP. Once the SSP has been achieved, the volume displaced will equal the volume dispensed.

Achieving the SSP is accomplished by first pre-pressurizing the system: displacing an experimentally determined volume using the syringe pump. Usually this pressure is slightly above the required pressure and requires several pre-dispenses (dispenses at the desired volume) to reach steady state, and thus the desired dispense volume.

However, several factors influence the achievement and maintenance of the SSP and thus achieving the desired dispense volume:

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12.1.1.1. Priming

When the dispense system is primed, several hundred micro liters of fluid are dispensed as a stream. The resistance to flow caused by the valve and tip orifice causes the pressure within the system to become higher than desired for SSP. To achieve SSP, one must first vent the valves, which involves opening the valves without displacing fluid. This brings the system to ambient (zero) pressure and from this point; the SSP pressure can be achieved.

12.1.1.2. Aspiration

When sample is aspirated, the syringe pump draws fluidic through the tip orifice. The resistance to flow from the tip and valve creates a negative pressure, which must first be overcome to achieve a steady state pressure. As with priming, venting the system can bring the system to a known (zero/ambient) pressure, from which the SSP can be applied (Note: venting is typically done with tips in the sample to prevent the introduction of air).

The SSP during a prime/aspirate/dispense cycle is shown schematically below:
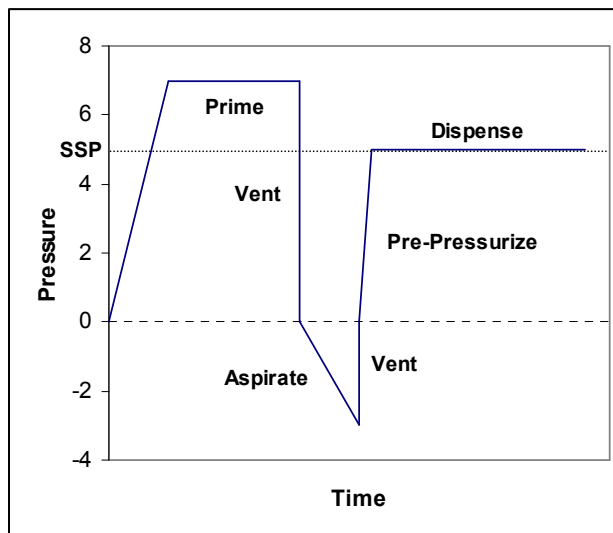
12.1.1.3. Gas Bubbles

Gas bubbles can occur in the dispensing system due insufficient removal of bubbles from the system, especially the solenoid valve or from outgassing/precipitation of gas from the solution. The major effect of bubbles is to change the system compliance, which can affect drop formation. Input will still equal output but the fluid will collect on the dispense tip rather than eject as a full drop. This can cause variation in dispensed volumes and eventually cessation of dispensing. Bubbles can be removed by purging the system with isopropanol and using degassed solvents as well as the system fluid or a vacuum degasser shown below.

B I O D O T

BioDot Vacuum Degasser

12.1.1.4. Tip Effects

Condensation of liquid on the dispense tips can also lead to loss of control of dispense volumes do to the interaction of ejected drops with resident fluids near the tip orifice. This effect can be reduced and/or eliminated by vacuum drying of the tips at appropriate times during the dispense cycle. Vacuum drying the tips is especially important when dispensing for long periods of time.

12.1.1.5. Syringe Speed

Syringe speed is the speed of displacement of the fluid in the syringe pump. For dispensing, the syringe speed has little if any affect on the SSP. In the limit of extremely slow speeds, the syringe may not be finished displacing during the dispense time. For priming, the syringe speed will effect the pressure build-up and in the limit of very high speeds, may cause too much pressure and result in leaking. For aspiration, slow speeds are best to prevent excessive negative pressures being generated, which could lead to out-gassing of the fluids.

12.1.1.6. Valve Open Time

The microsolenoid BioJet Plus™ valve open-time is one of the most important parameters for achieving and maintaining the SSP. This is the time the valve opens to release displaced fluid and ejects the drop. In previous versions of software the open time is set in % duty cycle. Duty cycle gives the percent of time the valve is open for one valve actuation and the open-time is the actual amount of time that the valve is open in one valve actuation.

For a dispense system at SSP, the proper open time will result in the displaced volume equal to the dispensed volume. If the open time is too short, then over time pressure will build beyond the SSP. If the open time is too long, the SSP will be dissipated eventually resulting in drops not being ejected from the tip.

B I O)D O T

The open time is the approximate time required for the fluid to move though the valve. Thus open time increases with increasing drop size and increasing viscosity.

A list of appropriate on-times is given in Section 12.2.3 below.

12.1.2. Drop Mode

The BioJet Plus™ system can still be used for step and repeat drop dispensing. In this case, the dispense positions are programmed as coordinates. The drops are programmed in terms of drop volume and open time. One can also dispense a burst of drops at a given frequency. For example, one could dispense 1 uL as a single drop or as 10 x 100 nL drops. The BioJet Plus™ technology increases the step and repeat speed for groups made by the dispenser head. In this case, step and repeat times in the order of 0.03 seconds can be achieved using a 1 x 8 dispense head to dispense into a 1536 format (2.25 mm centers). For old technology in a normal 1 x 8 configuration, the step and repeat time is more in the range of 1.5 seconds.

12.2. Description of BioJet Plus™ Fluidic Actions

The BioJet Plus™ technology can be used in several different actions: Prime, Aspirate, and Dispense). The AxSys™ software can be used for programming each of these modes.

12.2.1. Prime

Prime is used to initialize the syringe pumps and fill the syringe pumps, microsolenoid BioJet Plus™ valves of the dispense head, and connecting tubing with fluid from the reservoir(s). The reservoir fluid is either system fluid for aspirate/dispense of reagent, or sample fluid for continuous (line or dot) dispensing. There are two profiles in the prime function. The "fill" profile draws fluid from the system reservoir. The "dispense" profile displaces the fluid from the syringe into the feed lines which lead to the dispense.   A series of primes using a 70 % IPA solution as reservoir source is recommended at the start of a new day or whenever there appears to be air in the lines. A vent is also recommended after a prime to eliminate any pressure that builds during the prime. See Section 14.4.5. for programming the Vent.

12.2.2. Aspirate

The aspirate action draws sample from a reservoir, usually a microwell plate, into the tip of the dispense head. To perform an aspirate, several parameters must be set.   These parameters are set by using Syringe Speed and Channel Parameter actions. The syringe speed controls the speed of the syringe pump dispensers. In general, slow syringe speeds are used for aspiration to prevent a large vacuum from being developed which can result in outgassing of the fluid in the system. Typical syringe speeds for aspiration are as follows:

B I O D O T

	Syringe Size		
	50uL	100uL	250uL
Syringe Speed			
Start (µL/sec)	1	1	1
Top (µL/sec)	2.1	2.1	2.1
Stop (µL/sec)	1	1	1
Acceleration (µL/sec^2)	100	400	1000

The Channel Control option sets open-time of the valve. (Formerly set as duty cycle) for each channel. The open-time for aspiration will be 10,000 µsec. The aspiration volume is set in the Aspiration action.

During the normal aspiration process there is a slight negative pressure that is produced, as described above. This negative pressure is relieved by performing a vent, a function found in the Foundation Function. The vent opens the microsolenoid valve without displacing any fluid. See Section 13.4.5 for programming the Vent.

12.2.3. Dispense
The dispense causes the syringe pump to displace fluid and the BioJet Plus™ valve to open resulting in drop or series of drops of the programmed volume. To dispense, several parameters must be set using the Syringe Speed and Channel Control actions (these actions only need to be issued once for a series of dispenses; they will remain current until new parameters are set). Typical Syringe Speed parameters are shown below:

	Syringe Size		
	50uL	100uL	250uL
Syringe Speed			
Start (µL/sec)	1	1	1
Top (µL/sec)	20	20	20
Stop (µL/sec)	1	1	1
Acceleration (µL/sec^2)	100	400	1000

In general, changes in the syringe speed do not affect the dispense volume.

The Channel Parameter action is used to select which channels are to be dispensed and set the time the valve will be open, the "open-time". The open-time plays a large role in the quality and quantity of the dispensed fluid. The open-time should be matched to the dispense volume. If it is too short, then the volume dispensed will be less than expected. If it is too long, then the drop will not

B I O D O T

properly eject from the tip. Typical open-times are shown below (for reference, duty cycle, used with older versions of software, is shown). The dispense volume is set in the Dispense action.

Drop Volume (nL)	Open Time Optimal (μsec)	Open Time Range (μsec)	Freq (Hz)	% Duty Cycle
4.16	300	200-500	100	3
8.33	400	300-600	100	4
12.5	400	300-600	100	4
16.7	400	300-600	100	4
20.8	500	400-600	100	5
25	500	400-600	100	5
29.2	500	400-700	100	5
33.3	600	400-800	100	6
37.5	600	400-800	100	6
41.7	600	400-900	100	6
50	800	500-1000	100	8
62.5	1000	700-1400	100	10
75	1200	800-1800	100	12
100	1500	800-2200	100	15
250	2500	1500-4000	100	25
375	2700	1500-4200	100	27
500	3000	1500-4500	100	30
625	3200	1700-4500	100	32
750	3350	1800-4500	100	33.5
1000	3500	2000-4500	100	35
5000	4000	2000-7000	100	40
10000	5000	3000-7000	100	50

BIO DOT

12.2.3.1. Preparing to Dispense
Before dispensing can be accomplished, three procedures must be preformed, pre-pressurization, pre-dispensing, and vacuum drying.

12.2.3.2. Pre-Pressurization
In order to achieve the programmed volume, the dispense system must have the appropriate steady-state pressure (SSP, Theory of BioJet Plus™ Dot Dispensing). To achieve this, the syringe pump is displaced without opening the BioJet Plus™ valve.   The appropriate amount of displacement depends on the volume and composition of the sample being dispensed.   Some general guidelines are given below:

Dispense Volume (nL)	Pre-Pressurization Volume (µL)
10	.500
50	.400
100	.300
250	.200
1000	.050

A pre-pressurization function is provided in the Foundation Library of the AxSys™ software.

12.2.3.3. Pre-Dispense
After pre-pressurization, several pre-dispenses at the programmed volume should be preformed for fine-tuning the SSP (The pre-pressurization brings the system very close to the desired SSP where as the pre-dispense brings the pressure the appropriate level for the programmed volume). The pre-dispense is usually done over the waste section of the wash station but in cases where sample is to be conserved (e.g. aspiration from microwell plate), the pre-dispense can be done back into the source location.
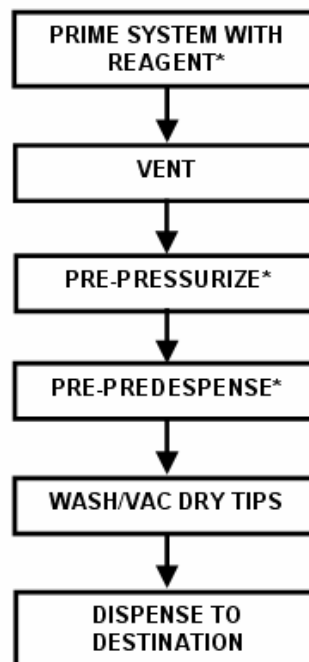
12.2.3.4. Tip Cleaning
After pre-dispensing, it is recommended that the tips of the dispense head be cleaned by dipping them into the wash tank and vacuum drying. The vacuum drying removes any liquid from the outside of the tips which could affect the dispense.

12.3. Process Flow for Different Liquid Handling Modes
This section will show how the fluidic actions described above are used in the two basic liquid handling modes: Continuous (Bulk) Dispense and Aspirate/Dispense (Transfer).

B I O)D O T
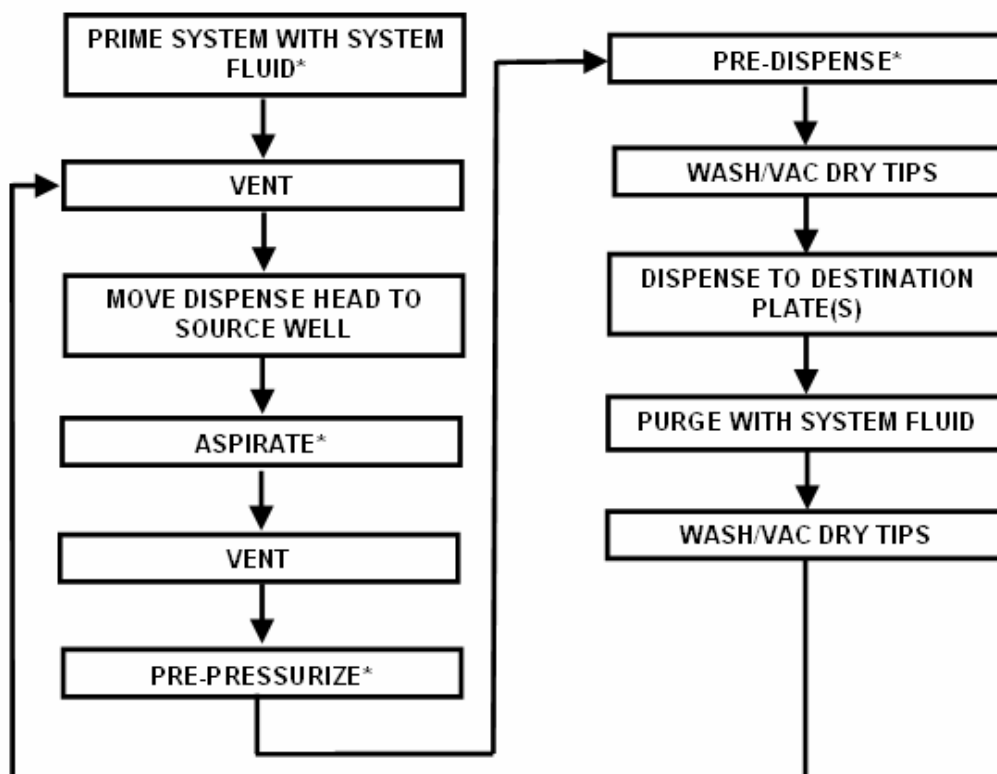
12.3.1. Continuous (Bulk) Dispense

Continuous dispense involves filling the system with a reagent which will be dispenses onto a substrate. This mode encompasses applications such as plate filling and line dispensing. The process is shown in shown in the flow chart below:

```
        ┌─────────────────────┐
        │  PRIME SYSTEM WITH  │
        │      REAGENT*       │
        └─────────────────────┘
                   │
                   ▼
        ┌─────────────────────┐
        │        VENT         │
        └─────────────────────┘
                   │
                   ▼
        ┌─────────────────────┐
        │   PRE-PRESSURIZE*   │
        └─────────────────────┘
                   │
                   ▼
        ┌─────────────────────┐
        │   PRE-PREDESPENSE*  │
        └─────────────────────┘
                   │
                   ▼
        ┌─────────────────────┐
        │  WASH/VAC DRY TIPS  │
        └─────────────────────┘
                   │
                   ▼
        ┌─────────────────────┐
        │     DISPENSE TO     │
        │     DESTINATION     │
        └─────────────────────┘
```

Each box of the flow chart is a function found either in the Foundation Library or the example programs. Steps marked with an asterisk (*) require parameters to be set (e.g. syringe speed, channel parameter).

12.3.2. Aspirate/Dispense (Transfer)

The Aspirate/Dispense mode involves the aspiration of sample from a source (e.g. a microwell plate) and dispenses to a destination (e.g. a slide, membrane, or microwell plate). The process is shown below:

B I O D O T

```
  ┌──────────────────────────┐                    ┌──────────────────────────┐
  │ PRIME SYSTEM WITH SYSTEM │───────────────────▶│      PRE-DISPENSE*        │
  │          FLUID*          │                    └──────────────────────────┘
  └──────────────────────────┘                                 │
               │                                               ▼
               ▼                                  ┌──────────────────────────┐
  ┌──────────────────────────┐                    │    WASH/VAC DRY TIPS     │
  │           VENT           │                    └──────────────────────────┘
  └──────────────────────────┘                                 │
               │                                               ▼
               ▼                                  ┌──────────────────────────┐
  ┌──────────────────────────┐                    │  DISPENSE TO DESTINATION │
  │  MOVE DISPENSE HEAD TO    │                    │         PLATE(S)         │
  │      SOURCE WELL          │                    └──────────────────────────┘
  └──────────────────────────┘                                 │
               │                                               ▼
               ▼                                  ┌──────────────────────────┐
  ┌──────────────────────────┐                    │  PURGE WITH SYSTEM FLUID │
  │        ASPIRATE*          │                    └──────────────────────────┘
  └──────────────────────────┘                                 │
               │                                               ▼
               ▼                                  ┌──────────────────────────┐
  ┌──────────────────────────┐                    │    WASH/VAC DRY TIPS     │
  │           VENT           │                    └──────────────────────────┘
  └──────────────────────────┘
               │
               ▼
  ┌──────────────────────────┐
  │      PRE-PRESSURIZE*      │
  └──────────────────────────┘
```

Each box of the flow chart is a function found either in the Foundation Library or the example programs. Steps marked with an asterisk (*) require parameters to be set (e.g. syringe speed, channel parameter).

B I O D O T

13. BioJet Plus™ Line Dispensing

13.1. Theory of BioJet Plus™ Dispensing

This section describes the background and theories of the BioJet Plus™ dispense system. After completing this section, the reader will have a better knowledge of the unit's function and practical uses.

13.1.1. Background

BioJet Plus™ technology adds the synchronization of the XYZ motion control system with the synchronization of the syringe pump motion and the opening and closing of the BioJet Plus™ valve. This was accomplished by integrating the solenoid, syringe and motion control driver electronics with a set of printed circuit boards, which are packaged within the syringe pump housing. Thus it looks and an act like a conventional syringe pump, but it provides the ability to synchronize the syringe and table motion with the solenoid valve. With this level of control, the dispense can be accomplished "on the fly" resulting in ultra-fast dispensing speeds. Using the BioJet Plus™ technology allows for the increase in the pump resolution by a factor of 8.   Example: The syringe step resolution for a 50uL syringe is reduced from 2.07nL to 0.26nL.

13.1.2. Line Mode

The real power of BioJet Plus™ is to dispense using the line mode. This mode allows lines to be printed with the precise positioning of the drops both relative and absolute.   Syringe sizes vary from 50 to 5000uL and are preset in the system configuration.   Lower volume syringes are used for dispensing small drop volumes in the 50 to 100nL ranges. The high end, are used for dispensing large drop volumes in the 0.1-10uL range.

13.1.3. Applications

The basic trend in HTS is to reduce the assay volume into the 10uL range or below. To accomplish this, low volume, high-density plates such as 384-, 1536-, or 3456-well plates are being used. Once the compound has been delivered to the plate, several common reagents are dispensed into the wells. The common reagents include substrate and enzymes with dispense volumes in the range of 50-500nL, and buffer with dispense volumes in the range of 1-10uL. Typical Open Times and Axes Speeds are listed below.

B I O D O T

250 uL Syringes

Start Speed	Top Speed	Acceleration	Volume	Optimal Open Time	Open Time Range
mm/sec	mm/sec	mm/sec^2	nL	usec	usec
3	3	800	5000	43000	35000-43000
5	5	800	4000	30000	25000-38000
5	12	800	2000	22000	17000-27000
5	30	800	1000	15000	12000-18000
10	20	2000	750	10000	6000-13000
10	30	3000	500	5000	3500-6000
10	30	3000	200	3000	2000-4000
10	80	3000	100	1500	1000-3000
10	80	3000	50	1200	600-1400

5000 uL Syringes

Start Speed	Top Speed	Acceleration	Volume	Optimal Open Time	Open Time Range
mm/sec	mm/sec	mm/sec^2	nL	usec	usec
5	20	800	5000	43000	35000-43000
5	30	800	2000	22000	17000-27000
5	30	800	1000	15000	12000-18000
5	30	2000	500	5000	3500-6000
10	30	3000	200	3000	2000-4000

B I O D O T

13.2. Advantages of BioJet Plus™

- **Speed-**
  There are two distinct speed advantages of using the BioJet Plus™ relative to conventional dispensers. The first is dispensing "on the fly". This eliminates the step and repeat process.   The other advantage is the non-contact nature of the dispense mechanism. Because the tips never touch the reagents in the well (compound plus previous reagents), the tips do not have to be washed or replaced. Typical dispense fill times are on the order of 30 to 40 seconds to fill a 1536 well plate with 1-8uL.

- **Elimination of Trapped Air-**
  Because the dispense occurs from a small orifice (typically 190uM), and is centered on the well, the well fills from the bottom up with no trapped air bubbles.

- **Enhanced mixing-**
  The fluidic inertia (i.e. mass of the fluid moving at a high speed) of the dispensed reagent enhances mixing of the fluid in the wells.

- **Multiple Reagents-**
  The dispense head is composed of either 4 or 8 independent channels, each of which can be used to dispense a different reagent at different volumes.

- **Multiple dispense modes-**
  Reagent can be dispensed by either aspirate/dispense (source to destination), or continuous dispense from a reagent reservoir.

- **Wide dispense volume range-**
  Volumes from 50nL to 8uL can be programmed for dispensing.

- **Variety of reagents-**
  To date, wide varieties of reagents have been successfully dispensed. These include:
  - SDS/Protein Solutions
  - Mammalian Cells
  - SPA beads

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14. Using the AxSys™ Software

This section covers the basic items found within the AxSys™ software program. After reading this section, the operator will understand the process of programming the AD6000 unit via the PC and Windows Software for dispensing.

14.1. Installing AxSys™ Software

- Insert the CD into your disk drive.

- Click the Windows Start button. Select the control panel option, and then choose "Add/Remove Programs". Next select Install and follow the Windows prompts.

- Before opening the AxSys™ software program, be sure your AD6000 unit is connected to your computer. The interface cable connector should be connected to the COM 1 port, located on the back of the AD6000 electrical enclosure unit. The other end connects to one of the computer's COM ports. This is normally COM 1; however you may use any of the computer's COM ports. Make sure the connector is screwed in completely.

- Turn on the unit(s).

- For BioJet Plus™ units follow these directions for first time connection:
  Connect the supplied DB9 cable to BioJet Plus™ COM cable and connect the other end to COM 2 on the rear of the PC.
  Open the AxSys™ software application. Because the configuration is only set up for the "Main" module the user will need to configure the system for both "Main" module and "BioJet Plus™" pumps.

   To configure select:
  - Tools/Options/Configuration/Edit
  - Once under the edit mode, check the boxes beside "Main Module box" and External Dispenser: "BioJet Plus™ .
  - Next, fill in Number of BioJet Plus™ dispensers. There will generally 1-4 dispensers on an AD6000.
  - Finally, the syringe volume will need to be entered. The figures will need to be entered manually if the volumes are different for each pump. If the volumes are all the same then use the Auto Configure feature.
  - After configuring syringes, select next and then select Finished. The unit(s) will then be placed off line and the power will need to be cycled.
  - Upon cycling the power, select Tools and then open Connection. The window will have a picture of a plug that should be red to indicate that the unit is not connected. Before clicking "OK" to activate connection, check the Module Box and Com # to make

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sure they match up to the connection on the backside of the computer. Make the necessary adjustments by clicking on the Com pull down arrow.

- Upon verifying selections, click the "OK"button and the unit will connect to the PC. If a connection error occurs, review the previous settings to ensure they are correct, and retry. It may be necessary to cycle the power on the units as well as restarting the AxSys™ application.

14.2. The Program List

The Program List is where the list of operator determined commands reside. These instructions are carried out in sequential order. Below is a graphic of a Program List:
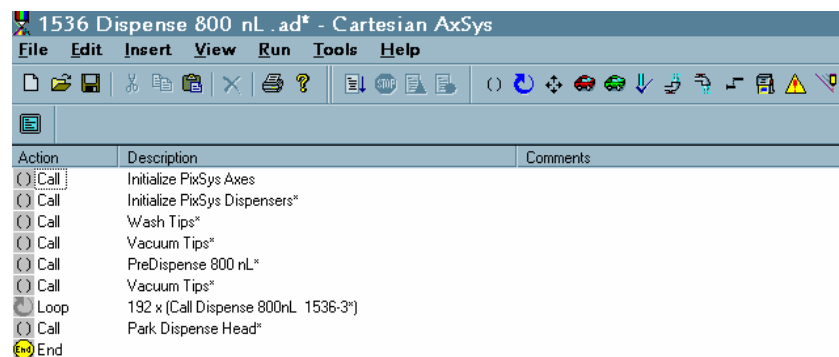
14.3. Tools
The following toolbar tools are available in AxSys™. Most of them function in a manner typical of Windows based applications.

14.3.1. New
Selecting NEW allows the user to create a new program. A blank page will appear and only the END function will be listed. The user can now begin inserting instructions.

14.3.2. Open
This icon opens a list of programs that the user has programmed and saved.

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14.3.3. Save
Selecting "Save" will save the program to the location instructed by the user.

14.3.4. Cut
This button cuts out any instruction that has been highlighted using the mouse. To highlight instructions, click the left mouse button and drag it over the instructions you wish to cut out. First highlight your selection and then click the CUT button. The information will be removed from the list once the CUT button has been selected. Cut items are stored in the "clipboard" and can be pasted (see below).

14.3.5. Copy
This button copies any information that has been highlighted using the mouse, to the clipboard. To highlight information click the left mouse button and drag it over the instructions you wish to copy. First highlight your selection and then move the pointer so that it clicks on the COPY button. The selected information will now be copied onto the clipboard. This copied information can be pasted (see below).

14.3.6. Paste
Selecting this button will paste any information on the clipboard to the current location in the information list. If an instruction is highlighted then the item on the clipboard will take its place. If you do not wish to remove the instruction yet wish to put the paste below the instruction, click to the side of the instruction. The instruction should now have a small dotted box around it. You will not lose the old instruction and the new information pasted will be inserted below it.

14.3.7. Delete
This button deletes any highlighted information in the list of instructions. To delete more than one instruction, hold down the left mouse button and drag it until all of the instructions to be deleted are highlighted. Lift up on the left button and place the pointer on the Delete button. Now click the left mouse button and the information will be erased completely. Note: There is no "Undo" button.

14.3.8. Print
This icon will print the information within the program list. The information printed is a condensed version and all details regarding each instruction will not be printed.

14.3.9. About
This button displays the current software version.

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14.3.10. Go

This button begins the programmed list of instruction. Once this button is clicked the instructions will begin in the order that they are listed.

14.3.11. Stop
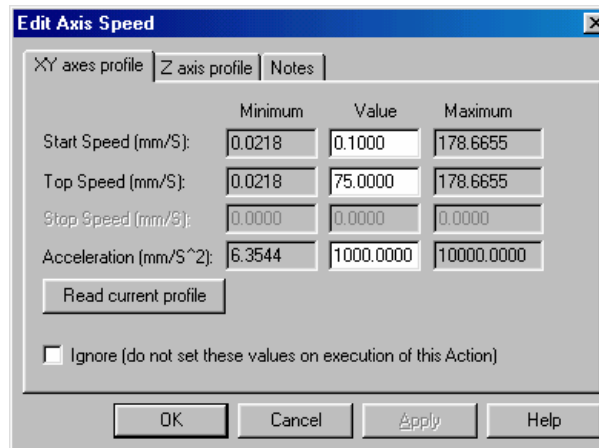
The "Stop" icon appears in the tool bar as a bright red stop sign. This command terminates the program when clicked. The stop icon will only need to be clicked once. The program cannot be restarted from where it was stopped.

14.4.   Programming Commands

Commands are inserted into the Program List by clicking their icons in the toolbar or selecting them from the "Insert" menu. Below is a description of the function of each icon displayed on the toolbar. An inserted command can be edited by double-clicking its icon.

14.4.1. Axes Speed

This instruction sets the speeds of the X, Y, and Z-axes. The "Axis Speed" icon appears in the toolbar as a red automobile. The following information can be edited in the submenus: *XY Axes Profile*, *Z Axis Profile*, and *Notes*. Values for the start speed slew speed, and acceleration can be edited in the sub-menus *XY Axes Profile* and *Z Axis Profile*. The minimum and maximum values are listed for the operator. To move from the *XY Axes Profile* to the *Z Axis Profile* in this sub-menu, simply click the tab using the left mouse button. It is possible to adjust values for the XY axes and Z-axis independently. When the "ignore" feature is checked for the value in question, the program will refer to the most recent previous value programmed for that axis.

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The X, Y, and Z axes operate at their optimum with a very slow start speed, <1.0 mm/sec, particularly when a high acceleration is used. The top speed controls how fast the axes move. This value will depend on what the program needs to accomplish. Set the top speed to a low value, around 40 mm/sec, when first programming.   Top speeds of more than 100 mm/sec will cause the platform to move very quickly. Fast top speeds also require a slow start speed, <1.0 mm/sec. If the top speed is too high the stepper motor will stall and the platform will not move. We recommend 175 mm/sec as a maximum top speed. Keep in mind that any fluid in the Wash Station may splash if fast slew speeds are programmed.

The acceleration rate controls how fast the axes accelerate. This value will also depend on what the program needs to accomplish. Set the acceleration to a low value, 100 mm/sec^2, when first programming. We recommend a maximum acceleration of 1000 mm/sec^2.

If the operator does not reset the "Axis Speeds" after homing, the platform then the original speed values used to home the platform will be used. Doing this will help to maximize the operating efficiency of the AD6000.

The *Notes* tab provides the operator with an area to record any textual information regarding the "Axis Speed" command.

When finished editing the "Axis Speed" command, click the "OK" button. This information can be adjusted by double clicking the "Axis Speed" icon within the function.

14.4.2. Move

This instruction moves the axes. Clicking this button will add it to the list of instructions. To edit this information, place the pointer on top of the move symbol in the list of instructions then double click the left mouse button. The Axis Speed command (see above) must exist somewhere in the program prior to a Move command. All Move commands will be executed according to the most recently encountered Axis Speed command in the program.

The move icon appears in the toolbar as a cross with arrows. The following information can be edited within the sub-menu: *Move*, *Iterate X-Axis*, *Iterate Y-Axis*, and *Notes*.

The *Move* tab lists the movement type, minimum allowable value, actual value and maximum allowable value for each axis. The movement can be one of the following types: None, Home, (+) Home, Absolute, or Relative. Selecting "None" causes the axis to stay in the current position. Selecting "Home" will cause the axis to move to the 0 or minimum position of the platform. Selecting "(+) Home" will cause the axis to move to the maximum position on the platform. For the X-axis the home position is at the far left of the platform. For the Y-axis the home position is at the front of the platform. The Z-axis home is at the highest position for the dispense head.

B I O)D O T
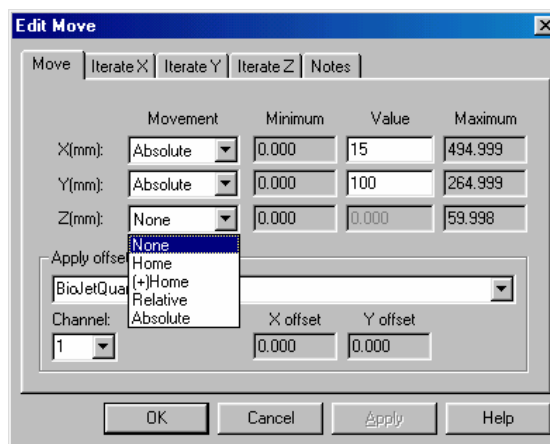
"Absolute" movements cause the axis to move to an exact position relative to home and independent of the current position of the axis. For example, if a value of 100 is entered for an absolute "Move" on the X-axis, then the platform will move to 100mm to the right of home. "Relative" movements cause the axis to move relative to its current location. For example, if the X-axis absolute "Move" mentioned above is followed by a relative "Move" of 50mm, the platform will move to a position 150mm to the right of home. Note that the method will not allow relative movements that extend an axis beyond its limit. The method will stop at the point where an improper relative "Move" is encountered.

The *Move* tab lists minimum and maximum values for the axes. These are the limits of the platform. The actual movement values are entered under the "Value" column. The *Notes* area allows the operator to insert information regarding the "Move" command.

The *Iterate X Axis* and *Iterate Y Axis* tabs allow the operator to make secondary movements offset from the original location when the "Move" command is used in concert with a "Loop" command. "Loop" commands are discussed below. To enable iterations for an axis, click the box named "Enable Iterations" on the appropriate tab. If you wish the position of the move to be tracked throughout the method then the box reading "Global" should be checked. If the "Global" box is not checked the move will reset to its initial value every time the iterate is encountered in either a "Loop" or "Call" command in the program (see below for explanations of these commands).

B I O D O T
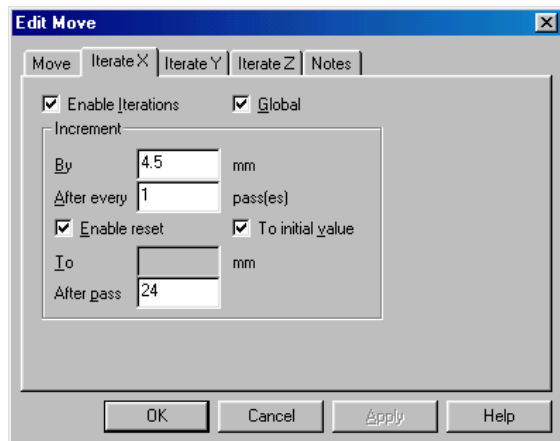
To iterate a move, enter the value that you wish to be added to the original "Move" value. This value will be added after a number of passes specified by the operator in the space labeled "after every # of pass (Es)". This tells the unit when to make the offset. For example, if an increment of 10 mm was programmed with "after every # of passes" set to 2 within the *Iterate X Axis* tab, then the unit will complete the original movement and then offset an additional 10 mm every two passes. After the fourth pass the X-axis would be offset by a total of 20 mm from the original value. It is important to keep in mind that the offset is added after each pass. The 20 mm total offset would be seen on the 5th and 6th pass.

Under the *Iteration* tab the operator may also set the number of passes after which the value of the move is reset. The operator must click in the box labeled "Enable Reset" and then enter a number of passes after which the reset will occur. The AxSys™ software will reset the move value to the initial value by default. In the unusual instance that the user would wish to reset the move value to something other than the initial value the "To initial value" box should be unchecked and the desired value entered. It is possible then to use a single iterated move command to instruct the unit to perform a complex task, such as sequentially taking samples from every well in a source plate.

The use of iterated relative moves is also valuable for accomplishing complex tasks with the unit. To create multiple levels of structure within a program, an iterated absolute move is followed by one or more iterated relative moves. This, in effect, allows a nesting of iterative moves to be associated with the absolute move. To do this the "Move" value for the relative move **must** be set to 0.00 mm for the axis of interest. The iterations should be set to the desired values and the reset value to 0.00 mm (!) after a desired number of passes. This reset affects only the value of the relative move and does not correspond to any absolute coordinate or absolute move command.

The *Notes* tab provides the operator an area to record any textual information regarding the instruction.

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When finished editing a move, click on the "OK" button. The move may be edited at any time by double clicking on the icon within the function list.

14.4.3 Syringe Speed

The use of this instruction sets the speed of the dispenser(s). The "Syringe Speed" icon appears in the toolbar as a green automobile. This instruction should be used each time that the syringe is moved, unless the speed previously set is the correct speed for the next syringe movement. An example is when the syringe speed is set for the pre-dispense of a compound then you do not have to call the syringe speed again when you are ready to dispense.
 The following information can be edited in the sub-menus: Number of dispensers, Set Speed for, Start Speed, Top Speed, Stop Speed, and Acceleration. To activate changes made to Syringe Speed click "OK".
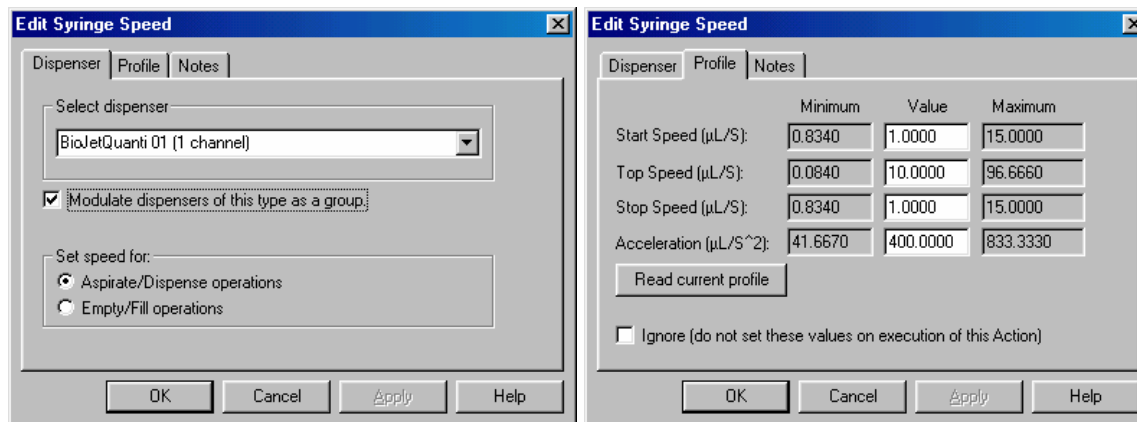
The number of dispensers and set speed for can be edited in the sub-menu *Dispenser*.
The "modulate dispensers of this type of group" box should be checked if all dispensers are going to be used.

The operator can edit values for the Start speed, Top Speed, Stop speed and acceleration. The minimum and maximum values are listed for the operator. To edit these values using the left mouse button, select the sub-menu *Profile*. When the "ignore" feature is checked for the value in question, the program will refer to the most recent previous profile programmed.

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For dispensing, a slow start and stop speed with a quick top and acceleration is recommended. For aspirating, a slow start, top and stop speed and acceleration are recommended. Keep in mind that the speeds need to be appropriate for the On-time that is programmed in a "Dispense" instruction. If the speeds are set too high, then the tubing will disconnect at the pressure fuse connector.

14.4.4. Channel Parameters

This command sets the channel control parameter. This parameter controls the on time of the solenoid valve. In previous versions of software Frequency (Hz) and Duty Cycle (%) controlled the on time. Duty cycle is now shown in μS or "open–time" Editing the open-time in the Channel Parameter command changes the amount of time that the valve is open, which in turn determines the amount of sample dispensed. Lower open-times are used for smaller dispenses and likewise larger volume dispenses use higher open-times. Please refer to the chart (in Section 13.1.3) for selecting the correct parameters for desired volume dispensed.
To activate all of the dispensers the operator must select to modulate as a group under the dispenser profile tab. The operator will also need to enable all of the dispensers to apply the given on time during dispensing. If the previous values are correct then select the box under the channel control tab that says, "Ignore (use previously set values)".
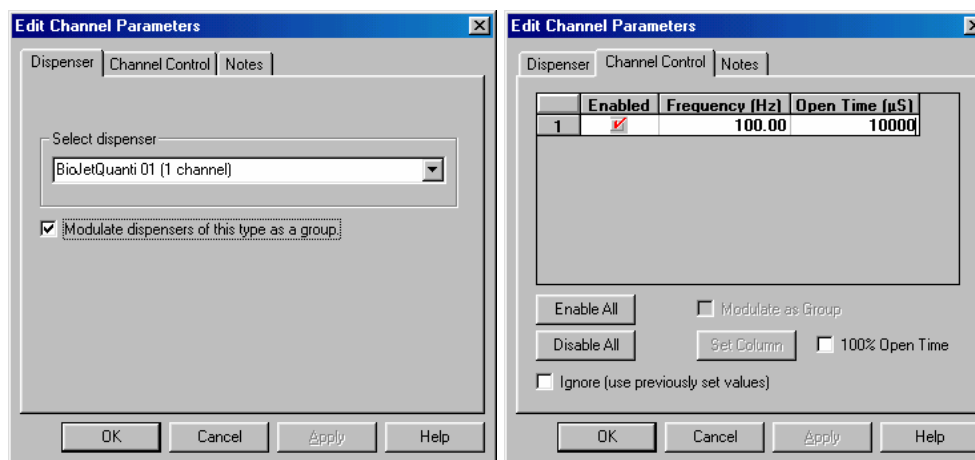
14.4.5. Aspirate

The "Aspirate" instruction appears as an upside down water faucet in the toolbar. There is three profile tabs to edit in this command: Aspirate, Iterations, and Notes. The aspirate instruction controls the drawing up of solution into the lines for dispensing. It also allows the operator to select what channel (BiojetQuanti 1 (channel 1)-8 to aspirate. The channels may also be operated simultaneously for

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like dispenses by selecting "All dispensers of this type" from the "Act on" pull down menu. Different dispenser may also aspirate different amounts by "or delayed and triggered if different volumes will be dispensed.

The iterate tab allows the user to incrementally change the volume aspirated in a program that uses a Loop command.   This is helpful if the amount of sample being dispensed is changing also. The larger the dispense, then the amount aspirated would be larger. Enabling the reset, is much like enabling the reset of a move command, in that after a given number of passes the user would like to have the initial volume aspirated and the iterate would start over again. If for some reason the user would like for the amount aspirated to reset to a value other than the initial value then the "To initial value" box should be unchecked and the amount to reset to should be entered manually.
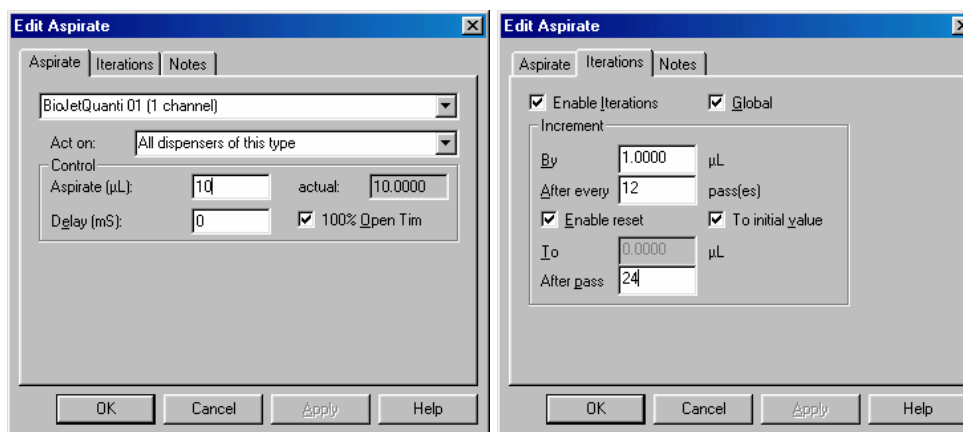
The "Vent" is also programmed in the aspirate tab. To vent, the user should insert the aspirate command into the program with an aspirate volume of 0.000 (uL) and a delay of 1000 (mS).

14.4.6. Dispense

The "Dispense" instruction appears as a water faucet in the toolbar. There is three profile tabs to edit in this command: Dispense, Iterations, and Notes. The dispense instruction controls the drawing up of solution into the lines for dispensing. It also allows the operator to select what channel(s) (BiojetQuanti 1 (channel 1)-8 to dispense. There are four choices in the "Act on" pull down menu. The first is "selected channel". This is used for dispensing a specific channel. The second is "All dispensers of this type". All channels may be operated simultaneously for like dispenses by selecting this command.

B I O) D O T
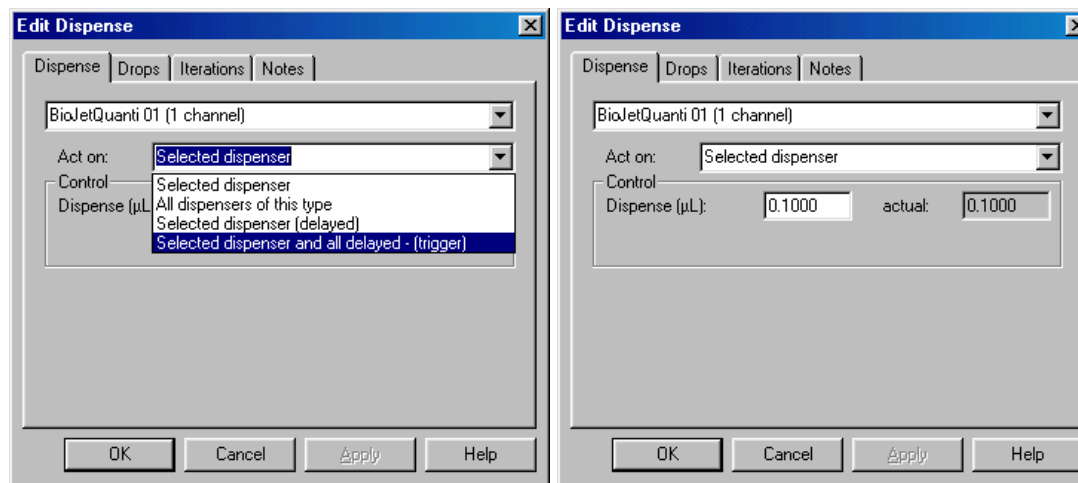
The third option is "Selected dispenser (delayed) ", is used in conjunction with the fourth option. The purpose is to allow each dispenser to dispense different volumes at the same time. Each dispenser should be assigned the proper volume, and "Selected dispenser (delayed)" should be selected for each dispenser. The final dispenser should have an assigned volume and "Selected dispenser and all delayed- (trigger)" should be selected. This will trigger all previous dispensers for simultaneous dispensing.

The "Drops" tab allows the control over the number of solenoid valve openings for each dispense cycle. For example, if "1" is entered in the "Number of drops" field, the solenoid valve will only open once to dispense the desired volume. Total volume, entered under the "Dispense" tab is also displayed. The drop size, which is the total volume divided by the number of drops, is also displayed. This is the size of each individual drop that is dispensed, the total of which makes the total volume. To maintain the quality of dispenses the open-time must be adjusted. The operator can insert open-time parameters or allow the software to determine the open-time by checking the box titled "Use Suggested Open-time".

B I O D O T
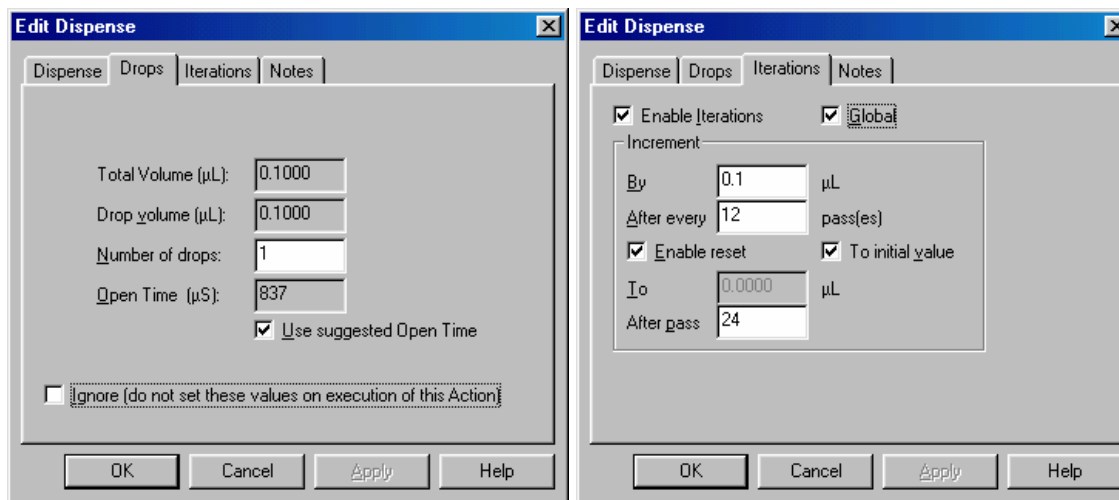
The "Iterations" tab functions similarly to the "Iterations" function previously described for the "Move" command in Section 14.4.2 of the manual, only it adjusts the amount of dispensed fluid with successive passes. To activate this function, check the box titled "Enable Iterations". Enter the volume to be incremented in the "By" field, and choose the number of passes after which the volume should be incremented. For example, using a one-channel system to fill each a 96 well microtitre plate with 1 uL of additional fluid, choose "Increment by 1 uL" and "After every 12 passes". If 5 uL were dispensed into each well of row A, then 6 uL would be dispensed into each well of row B and 7 uL into each well of row C. The dispense volume can be reset to any volume by choosing "Enable reset", selecting a volume and the number of passes after which it will reset.

The Notes tab provides the operator an area to record any textual information regarding the command.

14.4.7. Prime

The "Prime" instruction appears as a set of gears in the toolbar. The prime function serves several different purposes. A prime is necessary at the beginning of each program to initialize (home) the syringes. It is also used to fill the feed lines, syringes, and dispensers before dispensing. This function is also helpful in removing air bubbles trapped within the valves when used in conjunction with Isopropyl Alcohol. It is also used to purge lines and valves of aspirated solutions. "Channel Parameter" should be assigned before a prime. The suggested open-time for priming is 10,000 μsec.

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The following information can be edited: *Prime, Empty/Fill Profile, Aspirate/Dispense Profile*, and *Notes*. The *Prime* tab allows the user to choose the number of dispensers and the number of cycles the prime will complete. To fill the syringe after a prime, check the box to the left of "End with Fill". The initialize box is used to home the syringes. It is suggested that a prime with the "Initialize " box checked be employed to home the syringes during long runs to ensure that the syringe does not lose steps.
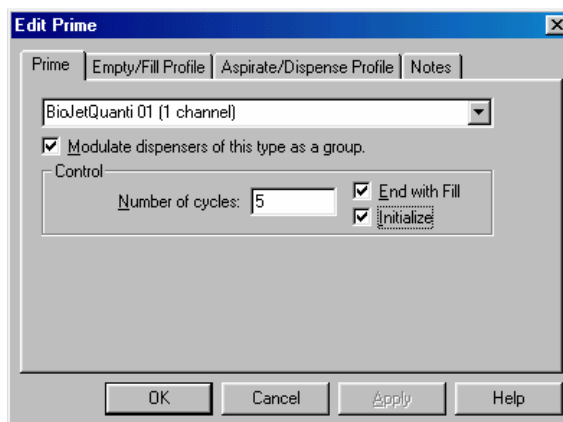
The *Fill/ Empty Profile* tab controls the syringe speeds when filling or drawing from the reagent reservoir. The *Start, Top, Stop* and *Acceleration* speeds can be edited in this tab. The *Aspirate/Dispense Profile* tab controls the syringe parameters when dispensing from the syringes. It is safe to set high syringe speeds for the Fill Profile, but excessive speeds should be avoided for the Dispense Profile to prevent over-pressurization. Refer to the chart in Section 13.1.3. for suggested syringe speeds.
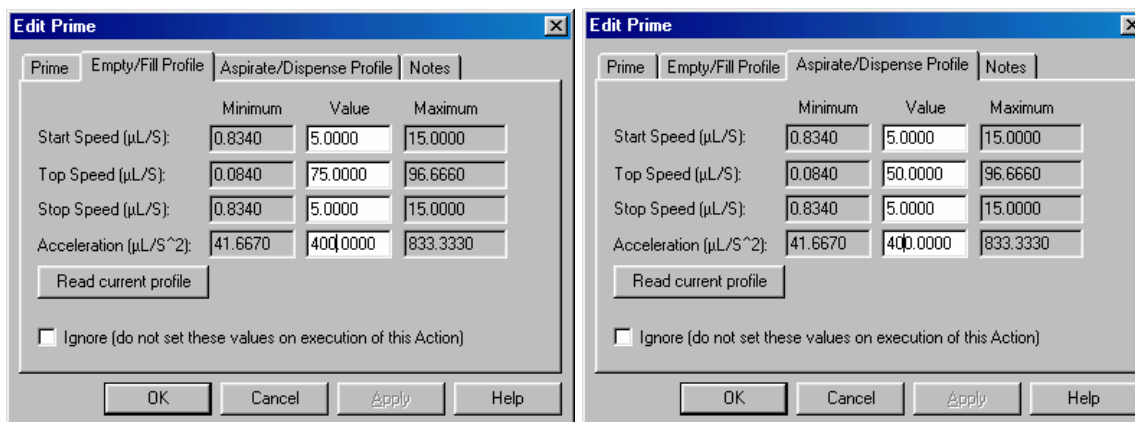
B I O) D O T

The *Notes* tab provides the operator with an area to record any textual information regarding the "Call" command.

14.4.8. Line

This command is used to write functions for the BioJet Plus™ unit only. This command, used in conjunction with a Move command, create a line dispense function.

Note: The line icon on the program list is fully functional with versions 70 or later of the software. If using earlier version, it is highly recommended to request an upgrade in software.

To create a line function from the program list, follow directions below.
- Select Line Icon. (This automatically inserts the command into the program list.)
- Double click on the command once it has been inserted into the program.
- The first window has 4 partitions.

1. Select Dispensers is where channels are selected for dispensers. To select all or consecutive channels (i.e. 1-8 or 2-6), click on the first preferred channel while depressing the "Shift" key on the keyboard, then click on the last preferred channel. To select specific channels (i.e. 1,3,5,7, or 2, 7, and 8), highlight the first required channel and while depressing the "Control" key, then highlight all other channels needed.

2. There are two options to choose from in the "Adjust "box.   This box corresponds directly with the third window (Speeds) of the Line command.   This allows the user to select either the table speed or syringe speed as the variable that AxSys™ will

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calculate. The remaining speed values are entered on the third window of the Line command. For dispensing into microtitre plates, it is best to allow AxSys™ to calculate the syringe speed. A list of Table (Axis) speeds, are listed in section 13.1.3.

3. The XY motion delay is for users that are using their own motion control system. It is not applicable to BioJet Plus™ dispensers.

4. The last box is for Line coordinates. The coordinate value is calculated in the next window. Once the value is coordinated, return to this window and check the location of the value. Although the default is the X location, plate filling is usually is dispensed in the Y direction. The value can be cut and pasted, or deleted and reentered into the Y line coordinate box.
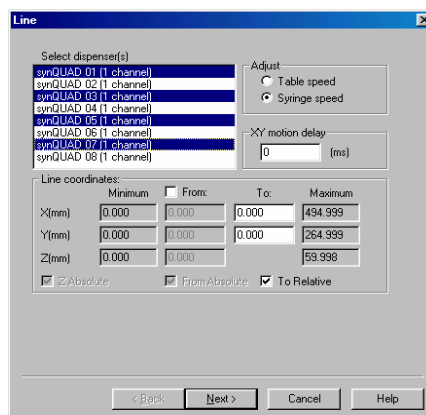
- Proceed to next window by selecting "Next".

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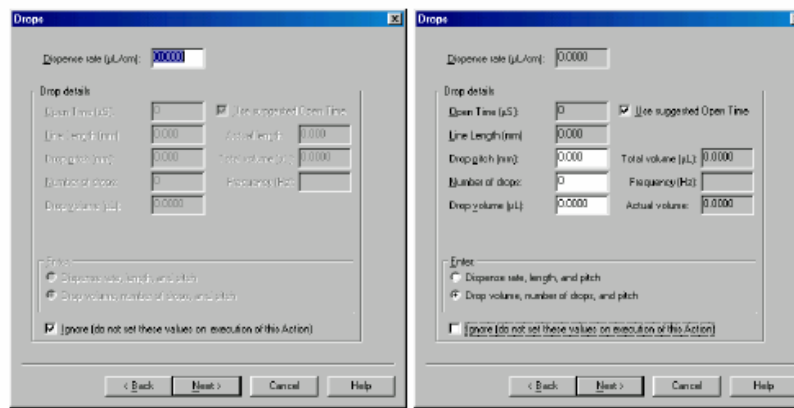
- Upon clicking on this tab, the "Ignore" box will need to be deselected. Once the box has been deselected, there will be two options to select from under the "Enter" window. The first is "Dispense Rate, Length and Pitch, which is primarily used for dispensing lines for bio-diagnostic applications. The second is the "Drop volume, number of drops, and pitch." This is primarily used for dispensing into discreet locations such as microtitre plates.   After selecting the second option, fill in the highlighted fields with the appropriate figures. Below is a description of each field.
  -**Drop pitch**, is the distance desired between each dispense. Example: The pitch for dispensing into a 1536 well plate is 2.25 mm.
  -**Number of Drops**, is the number of dispenses needed for each row. Example: If dispensing into a 1536 well plate, portrait style, the number of drops would be 48. This would fill an entire row of the 1536 well plate.
  -**Drop Volume**, is the size or amount of each drop dispensed. If dispensing nL, drop size will need to be converted to uL.
  -**Open Time**, is the amount of time that the solenoid valve will stay open for each dispense. There are two options when selecting open time. The first is checking the "Use suggested open time" box or referring to the "Axis Speeds and Open Time" table on page 35. It is generally recommended to use the table for volumes over 500nL.

- After completing these fields, refer back to the first window. Examine the value in the "Line to" section, and verify it is in the correct place. The default is the X direction. The value may be moved from the X to Y direction by cutting and pasting or deleting the X value and entering it in for the Y value. Line dispensing is generally done in the Y direction. The type of movement used for plate filling is "Relative", which is the default.

- After adjusting the direction of line coordinates, click the next button two times to advance to the "Speeds" window.

  - The "Speeds" window has both Axes (table) speeds, and Syringe speeds listed. Only one will be adjustable. This window corresponds to the "Adjust" box on the first window of the Line command. As previously stated, it is best to allow the software to adjust the syringe speeds. The table of Axes speeds is in section 13.1.3.

  If any of the values are inappropriate for the line dispense, there will be a warning or error indication to the right of the value's. These should be corrected using the Axes speed table.

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Speeds (left dialog)

XY Axes Profile

	Minimum	Value	Maximum	
Start Speed (mm/S):	0.04035	0.0000	330.5312	*Warning
Top Speed (mm/S):	0.04035	0.0000	330.5312	*Error
Stop Speed (mm/S):	0	0.0000	0.0000	
Acceleration (mm/S^2):	11.76	0.0000	10000.0000	*Error

Syringe Profile

	Minimum	Value	Maximum	
Start Speed (µL/s):	2.315e-006	0.0000	14.9974	*Warning
Top Speed (µL/s):	2.315e-006	0.0000	49.9995	*Error
Stop Speed (µL/s):	2.315e-006	0.0000	0.0000	
Acceleration (µL/s^2):	0	0.0000	822.8661	

Frequency (Hz): 0.00   [Read current profile]   [Fix]

Adjusted Syringe Profile = Table Profile * 0.4444

☐ Ignore (do not set these values on execution of this Action)

[< Back] [Finish] [Cancel] [Help]

Speeds (right dialog)

XY Axes Profile

	Minimum	Value	Maximum
Start Speed (mm/S):	0.04035	1.0000	330.5312
Top Speed (mm/S):	0.04035	25.0000	330.5312
Stop Speed (mm/S):	0	1.0000	0.0000
Acceleration (mm/S^2):	11.76	800.0000	10000.0000

Syringe Profile

	Minimum	Value	Maximum
Start Speed (µL/s):	0.001367	0.4444	14.9974
Top Speed (µL/s):	0.001367	11.1111	49.9995
Stop Speed (µL/s):	0.001367	0.4444	0.0000
Acceleration (µL/s^2):	0	355.5556	822.8661

Frequency (Hz): 11.11   [Read current profile]

Adjusted Syringe Profile = Table Profile * 0.4444

☐ Ignore (do not set these values on execution of this Action)

[< Back] [Finish] [Cancel] [Help]

14.4.8.1. Filling a Plate Using the Line Mode

A line function, as previously discussed, consists of a line command along with a Move command. There is generally a function with an absolute move that precedes the Line function. The line function consists of an Axis Speed, then a series of line commands, and relative move commands.

It is necessary to program the relative move one pitch behind or before the first well dispensed to. Example: The pitch of a 1536 well plate is 2.25 mm. The dispense head should line up 2.25 mm behind well A 1. This adjustment is made to give the axis speed enough time to accelerate to top speed. In essence the drops fall at the same trajectory. The start pitch may need to be increased if the axis speed cannot reach Top speed before the first dispense. Example: 2.25mm may need to be increased to 2.4 mm. This will give the deck ample time to accelerate to top speed. The greater the drop volume the lower the axis speeds will be. An example of a line function is shown below.

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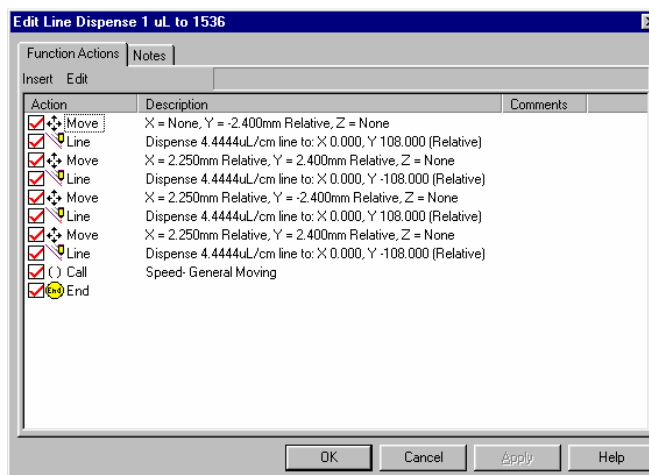
The first action is a relative move that sets the pitch one step behind the first well. The next action is a line command that fills the first row of a 1536 well plate, moving in the positive direction. The third action is a relative move that sets the pitch one step behind the first well as well as moving the dispense head to the next row for dispensing. Since traveling in the positive and then negative direction is the most efficient way to fill a plate, the next action shows a line command filling and traveling in the negative direction. This process is repeated until all wells of the 1536 plate have been filled. The last action is an Axis speed setting that is used for traveling when moving from position to position on the deck.

14.4.9. Calls $[\ ]$ & Functions

The "Call" command inserts a function, or pre-programmed list of commands. The "Call" icon appears as a set of black parentheses in the toolbar. A "Call" instruction inserts a "Function". A function is a list of commands that are grouped together to perform a task. It is suggested that even very simple tasks, those that may involve only one or two commands, be pre-programmed as functions. This can become particularly important for "Move" commands that are to be iterated. Complex functions can be created with a series of "Call" commands for simpler functions. Designing programs using functions as a hierarchical set of sub-routines is efficient and effective.

Functions are created in the Function Editor, which may be accessed by selecting "EDIT", followed by "FUNCTIONS" from the toolbar. Clicking the "New" button will place a new function in the Function List. The new function will be named "Function 1" until renamed by the operator. Double clicking on the function title will allow the operator to modify the list of commands that compose the function. New commands may be inserted into the function list by clicking on "Insert" on the toolbar within the Function Editor window. Unlike

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the main program list, there is no command toolbar within the Function Editor. A *Notes* tab in the function editor provides an area for the operator to record any textual information regarding the function.
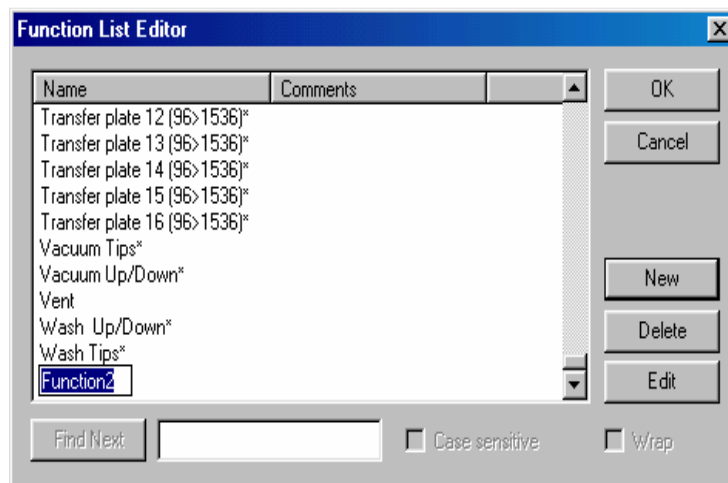
If a function is edited, the changes made are reflected throughout the method. In any place that the function in question is called within a method the newly changed information will be executed. It is important to note that these changes are reflected **only** within the method where the function is edited and **not** in any other method where the same function may be in use. For additional information related to this phenomenon see the section entitled, "Function Libraries".

At least one function must be created before using the "Call" command. To select a function to call, double-click on the "Call" command.   Click on the arrow at the right side of the blank labeled "Select function to call:" and a pull down menu of functions will appear. Highlight the function you wish to call. Clicking the "Edit" button will allow the operator to modify the function contained in the "Select function to call:" area. The function will be displayed in a Function Editor window. Any modifications made to a function by editing it within the "Call" command will be reflected in all other "Call" commands for the same function.
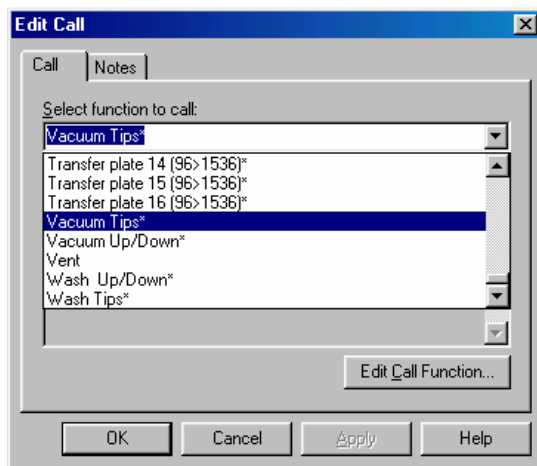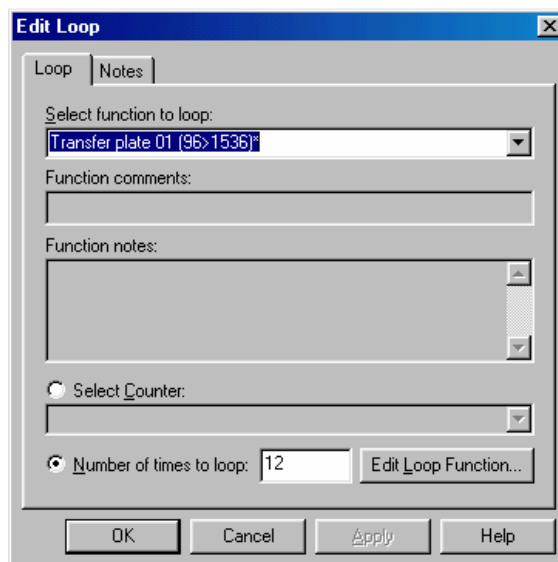
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The *Notes* tab provides the operator with an area to record any textual information regarding the "Call" command.

When finished editing the "Call" command, click the "OK" button. This information can be adjusted by double clicking the "Call" icon within the function.

14.4.10. Loops

The "Loop" icon appears as a circular, blue arrow. The "Loop" command allows a function to be repeated any number of times. Remember that iterated "Moves" that exist within a looped function will be reset to its initial values every time the function is called of looped unless the "Global" box is checked under the iteration tab for the "Move" command.

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The *Notes* tab provides the operator with an area to record any textual information regarding the "Loop" command.

When finished editing the "Loop" command, click the "OK" button. This information can be adjusted by double clicking the "Loop" icon within the function.

14.4.11. Pause

This command pauses the program. The "Pause" icon appears as a red exclamation point within a yellow triangle on the toolbar. This instruction will insert a timed delay or operator message into the program list. The *Delay* and *Notes* may be edited within this command.

The *Delay* tab gives the operator several choices on how the pause will operate. The pause can stop the program for a specified length of time by clicking on the "Fixed Duration" box and then entering a value, measured in milliseconds, in the space provided. The operator may also program the "Pause" instruction to display textual messages when the "Pause" instruction is reached within the method. Click within the "Operator Message" box and type the text. The operator can also have the computer signal with an audible beep when it has reached this point. To enable the beep, click on the "Beep" box. The *Notes* tab provides the operator with an area to record any textual information regarding the "Pause" command.
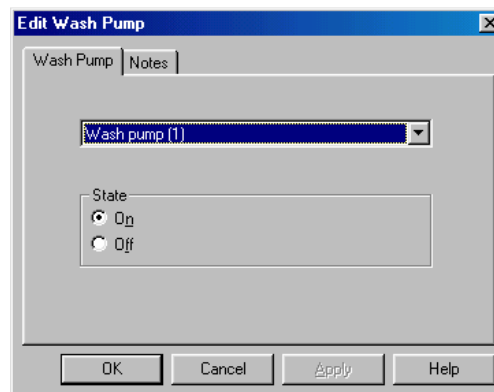
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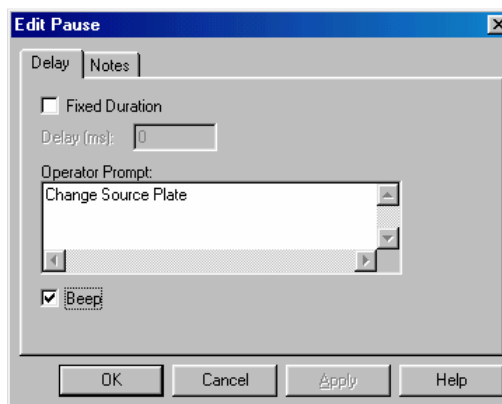
When finished editing the "Pause" command, click the "OK" button. This information may be adjusted by double clicking the "Pause" icon within the function.

14.4.12. Wash Pump

The "Wash Pump" icon appears as a gasoline-dispensing pump in the toolbar. This instruction controls the peristaltic wash pump that is located on the right panel of the AD6000 unit. The *Wash Pump* and *Notes* can be edited within this command. Click the "Wash Pump" icon to insert this command into a function.

The *Wash Pump* tab controls the state of the wash pump: ON or OFF. Individual commands must be inserted turn the pump ON and then OFF. The pump will remain on unless a command is given to turn it off. Typically, the wash pump is operated only during the washing of tips. **Note**: The pumps should turn in a counter clockwise rotation.

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The *Notes* tab provides the operator with an area to record any textual information regarding the "Wash Pump" command.

When finished editing the "Wash Pump" command, click the "OK" button. This information may be adjusted by double clicking the "Wash Pump" icon within the function.

14.4.13. Store

The "Store" command appears in the toolbar as a blue down arrow. The following information can be edited: Store and *Notes* This instruction stores the current X, Y, Z location into memory and allows the dispensers to return to that position easily, with a minimal amount of programming. Storing a position in helpful when the dispensers will need to return to the same location later in the program cycle. This instruction is used with the "Return" command, which returns the dispensers to the location specified by the "Store" command. The "Store" instruction allows the operator to store one or more of the axes positions. Click the box for the axis you wish to store. The operator can also store the position but with an offset. When the "Store Position" instruction is hit, the computer will remember the location and if there is an offset, that offset will be applied.

The *Notes* tab provides the operator an area to record any textual data regarding the instruction.

When you have finished editing the "Store Position" information, click "OK". This information can be adjusted at any time by double-clicking the "Store Position" icon within the program list.

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14.4.14. Return

The "Return" command appears in the toolbar as a blue left-hand arrow. The following information can be edited: *Return* and *Notes.* This command works in tandem with the "Store" instruction and causes the axes to return to the positions that were stored using the "Store" instruction.

The "Return" instruction commands the dispensers to move back to the last "stored" position (see Section 14.4.13). Click the box (es) for the axis you wish to return to.

When you have finished editing" Return Position" information click "OK". This information can be adjusted at any time by double clicking the "Return" icon in the program list.

The *Notes* tab provides the operator an area to record any textual information regarding the command.

14.4.15. Map

The "Map" instruction appears as two blue with a curved arrow in the toolbar. The following information can be edited: Map and Notes. This instruction works in tandem with Functions and assumes that a function as been created. For information on how to create a function, please see Section 14.4.9 on "Call". The "Map" function tells the software two distinct positions and is very helpful when mapping lower density microwell plates (such as a 96 well) into a higher density microwell plate (such as a 384 well).

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The Map includes several areas of information that can be edited. The first area is the "direction". The map function can go forward, which is from smaller density (96 well) to a larger density one (384 well). Or it can go backward, which us from a larger density (384 well) plate to a smaller density (96 well) plate. The "offset current position by" tells the software to move to origin A, but offset a specific amount. The offset can be edited in the X or Y-axes.   The current position refers to origin A. The "Map Parameters" tell the computer where the plates are. The X, Y, and Z-axes can be edited. Origin A and Origin B refer to the first well on the left hand side of each plate.

The "Magnification' tells the software how large or small the plates are in comparison to one another. For example, if an application had a 96 well plate in Origin A and a 384 well plate in Origin B then the "Magnification" would be X: 0.5, and Y 0.5. This is because a 96 well plate has wells 9 mm center – to – center and a 384 well plate has wells 4.5 mm center- to –center (between each well in the X and Y axes). The magnification does not need to be changed when mapping in a backward orientation. The "Mapped position should be" tells the software how to store the positions. If the operator selects "Store on the stack" then the software will use that position similar to a "Store Position" and it is now given priority. For information on "Store Position" please see the above description in Section 13.4.13. If the operator selects "Applied as a move" then the software will treat that position as a "Move" function. For more information on a "Move" command please see Section 14.4.2.
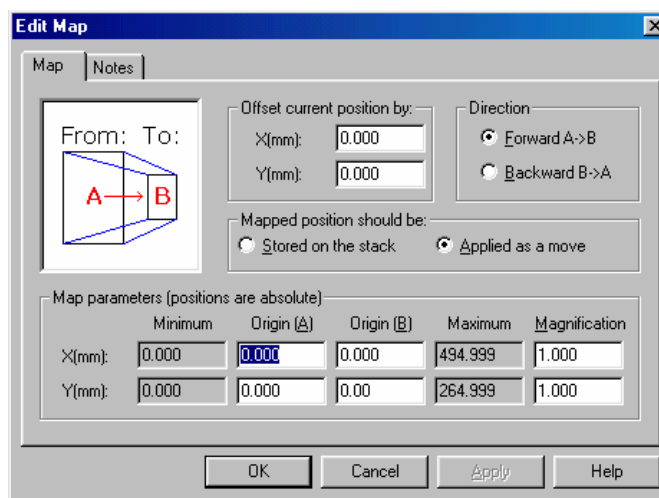
When you have finished editing the "Map" information, click "OK". This information can be adjusted at any time by double clicking the "Map" icon within the program list.

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The Notes tab provides the operator an area to record any textual data regarding the instruction.

14.4.16. End

The "End" instruction remains as a constant icon on the program list. This tells the program where the end of the application is. This command is automatically inserted by the AxSys™ software and cannot be removed from the program list.

The *Notes* tab provided with the "End" icon is a good place to record general notes about the program.

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15. Manual Mode

The manual mode can be utilized by selecting "Tools" from the toolbar, then "Manual Mode". The following instructions are included in the manual mode: Move, Axes Speed, Dispenser, Syringe Speed, Channel Control, Prime, and Wash Pump.

15.3.   Command Tabs

15.1.1. Move

The "Move" tab in the manual mode is useful in calibrating moves to specific positions on the stage, such as the vacuum wash station, source plate, and transfer plates. To do this, select the Move tab under manual mode. The first method used under the "Move "tab is the "Manual Move" box. There are nine buttons in this box. The buttons with arrows move the unit in the X and Y dimensions when pressed. The center button has a "Z" on it. When the center button is activated, the up and down arrows are highlighted and the Dispense head can be moved in the Z dimension. Above the "Manual Move" box is a small window labeled "Profile". This window contains a value that expresses the percent of the Axis Speed value that the operate wishes to use to make moves in the manual mode. Setting the "Profile" value to 1% will allow the user to make very fine adjustments.

The speed at which the platform moves can be slowed even further by entering a lower value for the slew speeds under the Axis Speed tab. After adjusting the Axis Speeds, the "Apply" button must be pushed. (See Axis Speed section to make adjustments.) The second method used for editing the X, Y, or Z-axis is the "Enter Move" box. The "Active" box must be depressed in order to use this section. Upon activating this box the user will be able to assign values to each axis. This option can be used to make final adjustments to the axes positions before inserting them into the program. Once the desire position is achieved in the manual mode, a "Move" command containing the coordinate values may be inserted into the program list. To do this, click the "Active" button in the "Enter Move "box of the Move tab in manual mode. Then press the "Insert" button at the bottom of the screen. Once the "move" command appears in the program list it   can be cut and pasted into the appropriate function.

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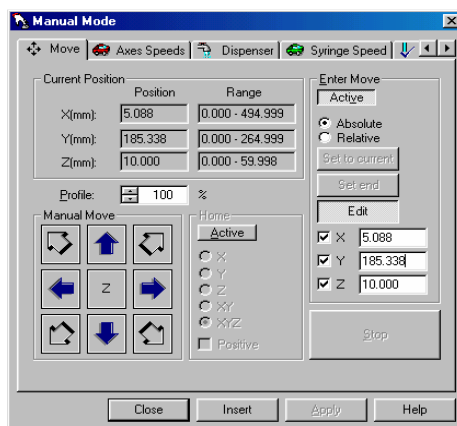
The third section in the "Move" tab is the "Home" box. It will necessary to home the axes before adjusting the X, Y, or Z-axes. To use, depress the "active" button and select the XYZ option. To home the axes the user should depress the "Apply" button.

15.1.2. Axes Speeds

This instruction sets the speeds of the X, Y and Z-axes. The following information can be edited in the manual mode: *XY Axes Profile*, *Z Axis Profile*, and *Notes*. Values for the start speed, slew speed, and acceleration can be edited in the sub-menus *XY Axes Profile* and *Z-Axis Profile*. The minimum and maximum values are listed for the operator. To move from the *XY Axes Profile* to the *Z Axis Profile* in this sub-menu, simply click the tab using the left mouse button. It is possible to adjust values for the XY axes and Z-axis independently. Once the desired speeds are established in the manual mode, an "Axes Speed" command containing the axes speed values may be inserted into the program list. To do this, press the "Insert" button at the bottom of the screen. Once the "Axes Speed" command appears in the program list it can be cut and pasted into the appropriate function.   When initially homing the X, Y, Z-axes, and a slow top speed of approximately 50.00 (mm/s) is suggested. A faster top speed of approximately 125.00
 (mm/s) is recommended.
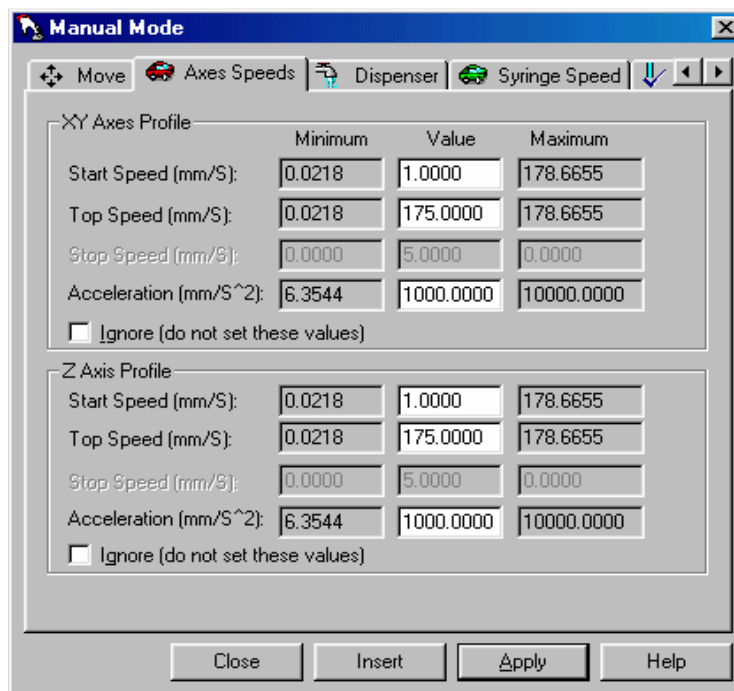
15.1.3. Dispenser
The Dispenser command in "Manual Mode" has multiple features and performs multiple functions. One purpose is to use it to create aspirate or dispense commands to be inserted into a program list. Once inserted into a program list the command can be cut and pasted into a function. To do this, first choose the BiojetQuanti 01 (1channel). The next step is to choose the number of dispensers from the "Act on" pull –down tab. Selected dispenser selects only the channel that is displayed. To aspirate or dispense the same volumes with the same settings, the user should select "All dispensers of this type". If the user wishes to aspirate or dispense different volumes, "Selected dispenser (delayed)" should be chosen for all of the dispensers to be used, except for the last dispenser. "Selected dispenser and all delayed (trigger)" is used in the last aspirate or dispense command. This dispenser will now aspirate or dispense and trigger any previously "delayed" dispensers.
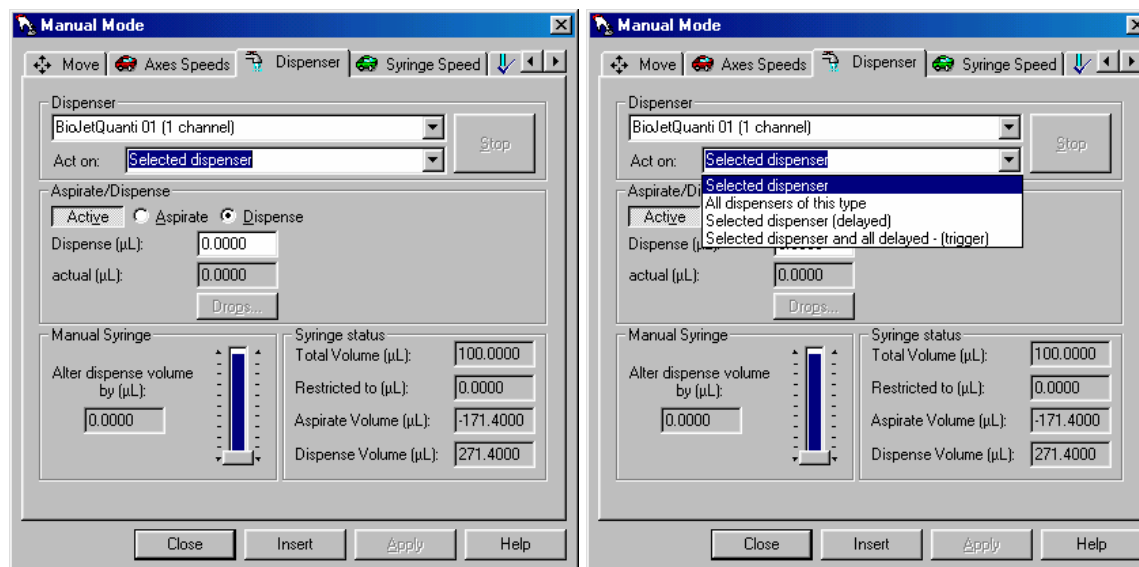
After selecting the number of dispensers, the user should enter the volume to be aspirated or dispensed located in the control box. Once the desired parameters are established in manual mode, an "Aspirate" or "Dispense" command may be inserted into the program list. To do this, press the "Insert" button at the bottom of the screen. This command can also be used to "Aspirate", "Dispense" or set parameters for a "Prime" in the manual mode. To aspirate or dispense the user should select desired parameters then press the apply button at the bottom of the screen. The only parameter needed under "Dispenser" for the "Prime" in manual mode is to select the number of dispensers. It is not necessary to "apply" or "insert" this parameter when setting this for a "Prime" in the "Manual Mode". However, "Syringe speed" and "Channel control" parameters must be set before using the "Dispense".

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15.1.4. Syringe Speed
Please refer to section 14.4.3.

15.1.5. Dispense Parameters
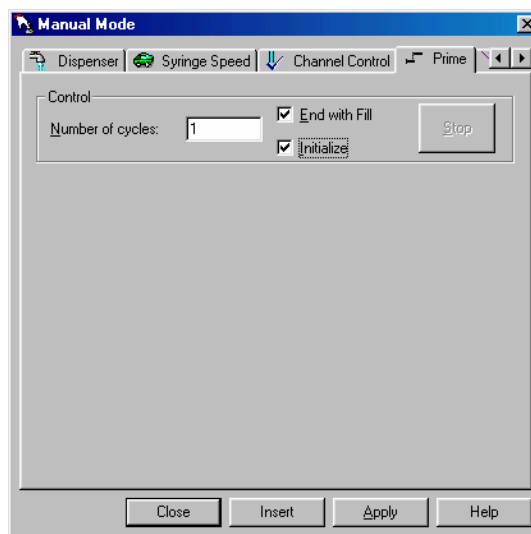Please refer to section 14.4.4.

15.1.6. Prime
The "Prime "function in manual mode is used to initialize (home) the syringes after powering up the unit or to write a prime command to be inserted into a program. It can also be used to fill the system lines with the system fluid.
In order to use this command the number of dispensers must be selected in the "Dispenser" tab. "Syringe speed" and "Channel control" parameters must also be set before using the "Prime".
The "Control" box has 3 options to select. The first option is the number of cycles to run. The second option is whether to fill the syringe after priming. The last option allows the user to initialize (home) the syringes. It is suggested that a prime with the "Initialize " box checked be employed to home the syringes during long runs to ensure that the syringe does not lose steps.

To insert this prime command into a program, press the "Insert" button at the bottom of page. This command can now be cut and pasted into a function in the program.
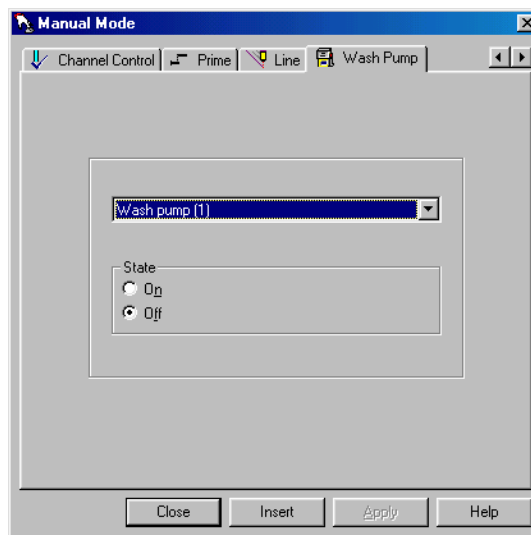

15.1.7. Line
Please refer to section 14.4.8.

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15.1.8. Wash Pump

This command controls the wash pumps. An "ON" and "OFF" must be inserted into the program. To insert a "Wash Pump" command, select the "ON" or "OFF" state and press the "Insert" button at the bottom of the page. It recommended that the wash pumps be used during a prime or a wash command within a program.

15.4.   Function Libraries

A function library is a list of the functions that are associated with a method. Libraries of functions may be imported and exported from method to method. It is strongly suggested that a library of commonly used basic functions (e.g. tip washing and vacuuming tips) be created and used to program all methods. Calibration programs are included with the AxSys™ software and can be used to generate this library. Explanations of the functions in this library are made later.

15.2.1. Exporting and Importing

To export a function library click on the "File" tab and then on "Export Library…" Name the library and click on the "Save" button.

To import a function library click on the "File" tab and then on "Import Library…" Select a library from the directory and click on the "Open" button. The user will be asked if duplicate functions are to be overwritten. Decide which option is desirable and click on the appropriate button.

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15.2.2. Duplicate Functions

Each time a function is newly created in the function library it is assigned a number that is used as a global identifier. After creation, a function may be edited or its name changed but the global identification number remains constant. When a function library is imported into a new or existing program, or when a program is saved under a new name there exist multiple methods that include in their function library functions having the same global identification number. Each method and its associated functions are independent. Though these functions share the same global identification number, they may contain different information because they are stored independently within each program. It is possible then to export a function library from one program and import it into another program that already contains functions with the same global identification number. These functions may or may not have the same name or contain an identical set of commands but they appear as identical entities to the AxSys™ software. These are considered duplicate functions by the AxSys™ software.

When libraries are imported the user may choose either to overwrite existing duplicate functions by the functions being imported or to exclude duplicate functions from import, thereby preserving the existing duplicate functions intact.

It is advisable to minimize the degree to which existing functions are modified. This will allow the user to avoid confusion when deciding whether to overwrite a function or not. It is a good idea to create a new function for new tasks. Commands may be copied from old functions and pasted into new ones. Modified commands pasted into a new function are often preferable to modifying the commands in an existing function.

15.2.3. Importing Portions or Subsets of a Library

There are instances in which a user may wish to selectively import a subset of functions into a program. The best way to do this is to open the AxSys™ method from which the functions are to be exported and delete from the function list all those that the user does not wish to import. The user may then export that subset of functions as a library and import them into the desired method. Be sure **not** to save the method from which the functions have been deleted after exporting the library.

15.5.   Foundation Functions

This section contains a list of the functions provided in the Foundation Library, a file included with the AxSys™ software.   An explanation of each function is provided. These functions are intended to serve as a demonstration, foundation and guideline for the creation of a customized set of basic functions that an operator will use to create methods.

A few of the functions in the list are found in many programs but are not included in the Foundation Library. These few functions consist primarily of an iterated "Move" command that provides information unique to the individual arraying patterns of each method. While the structure of the functions remains the same, the array pattern differences will require unique "Move" parameters within the function. Since the Foundation Library consists of functions that will remain unchanged among programs, these variable functions are

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not included. These functions will need to be individually edited within each program that they are used. A description of these functions is included because it is useful to understand how they are used in creating programs.

Each AD6000 unit is likely to have a slightly different home position for each axis. It is important that the operator calibrate the "Move" functions that correspond to places such as the wash station, microwell source plate, and destination plates to account for these differences between units. An asterisk (*) after the function title denotes functions in which calibrations will need to be made.

16.   Example Programs

These programs are intended to serve as a demonstration of the techniques that the operator will use to program task specific methods for the AD6000 series instruments. Contact support for a list of example programs. After carefully reading this document, calibrate the unit using the Calibration program, updating the example programs with the new Foundation Library, and properly editing the moves specific to each example program (described below) the operator may run these programs. If no calibration program is available then contact BioDot Inc. for further assistance. Observation of the unit carrying out the instructions contained in the programs, combined with direct study of the command set, should yield an understanding of the programming techniques.

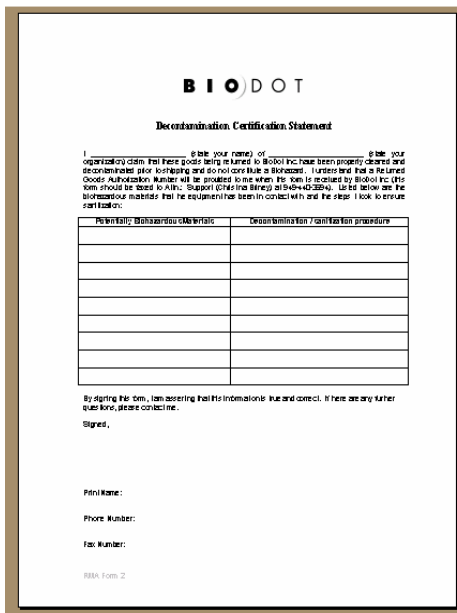### 16.3.   Editing Commands Unique to Each Example Program

There are move commands that are unique to individual programs (that are not a part of the Foundation Library). Many of these commands are in all of the programs but pertain to the specific purpose of the program. The specific differences in the functions are dependent on the amount of the sample to be aspirated or dispensed as well as the type of destination target used. The user may find that the use of many of these functions does not change therefore they can add these functions to the foundation library, but it is important to remember that it is good to make new functions instead.

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17. Maintenance

17.3.   Cleaning The AD6000 Platform

This section will help the user understand how to perform preventative maintenance and cleaning procedures on the AD6000 unit. Refer to the appendix in the rear of this manual for more information on cleaning and decontamination. **Note**: If you are returning any BioDot Inc. platform for an upgrade or repair a decontamination form must be filled out and returned prior to shipment. You can obtain this form by contacting Support at (949) 440-3685 or e-mail support@biodot.com.

17.4.   Routine Cleaning

This section will help the user understand how to perform preventative maintenance and cleaning procedures on the AD6000 unit.

1. Examine the AD6000 for any spilled reagents or accumulations of dust. Use a moist, lint free cloth remove any dust or spilled reagents. Wear safety equipment (lab gloves, safety glasses) when performing any cleaning operation. Dispose of all cleaning materials in accordance with state and local laws. Never spray cleaning liquids directly on to the platform.

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2. Purge the supply lines of all reagents. The operator can save the reagent by placing a container below the dispense tips.

3. Prime the system with isopropanol 6-9 cycles to remove air from system.

4. Clean and refill the supply reservoir bottles with deionized water.

5. Prime the system with the deionized water for 6-9 cycles.

⚠️ **Warning**: **Purge the system and dispensers of all liquid if it will be subjected to freezing**.

Sonication of the tips and the entire BioJet Plus valve housing, when removed from the support arm, may be necessary should the tips or valves become partially or totally clogged.

The reagent reservoirs and feed lines are reusable and may be used for extended periods of time before replacement is necessary.

17.5.   Syringe Seal Periodic Replacement

The syringe seals will require periodic replacement. The frequency of replacement will depend on the type of fluid dispensed, the size of the syringe, and how well the equipment has been maintained. If a syringe seal becomes worn and it is not replaced, the following problems may occur:

• Poor precision and accuracy

• Fluid leaks from bottom of syringe

• Bubbles in liquid column

• The tip of the plunger could break through the seal and scratch the inside of the syringe barrel. If this occurs the entire syringe must be replaced.

17.6.   Installation or Replacement of the Reagent Syringe

1. Prime the dispenser until the syringe plunger shaft is in the lowest position. Refer to photos below.

2. Loosen and set aside the knurled plunger screw on the syringe plunger arm.

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3. Carefully unscrew the syringe barrel from the valve assembly while pulling downward slightly. Then slide the syringe barrel further onto the syringe plunger.

4. The entire syringe barrel and plunger may now be lifted from the syringe plunger arm and removed.

5. Replace the syringe by following the above steps in reverse order.

17.7.   Syringe Seal Replacement - Procedure

1. Remove the syringe from the pump following the above procedure.

2. Remove the syringe plunger.

3. Using a single edged razor or precision knife, carefully slice off the old seal lengthwise and remove it from the plunger. Care must be taken not to damage the plunger.

⚠️   **Warning: 500uL and 1mL syringes have "O" rings beneath the seal. Do not cut the "O" ring. Replace the "O" ring if it is damaged.**

4. Wet the "O" ring (if present) and plunger tip with deionized water.

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5. Place the seal on a flat surface with the open-end facing up. Press the plunger tip firmly into the hole until it snaps into position.

6. Lay the plunger on a flat surface. Position the plunger so the seal (from the "O" ring up) hangs over the edge. Slowly roll the plunger along the surface edge, pressing firmly on the portion of the seal below the "O" ring. Rotate the plunger three complete turns. This is necessary so that the sharp raised edges of plunger bite into the seal for a secure fit.

17.8.   Peristaltic Pump Tubing Replacement

17.8.10.   Pump Removal

1. To remove the peristaltic pumps disconnect all fluid connections to the pump to be removed.
2. Next Pinch the side lock tabs and pull the pump straight out.

17.8.11.   Pump Tubing Replacement

1. To replace the tubing pinch the top clips on the rear cover and with a firm grip pull the front half away.

⚠ **Warning**: **Be careful not to break the top and bottom clips. It will require some force to separate the two halves**.

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2. Once the back of the pump head has been removed simply pull the old tubing out and replace it with a new piece the same length.

Insert Tubing Around Rollers
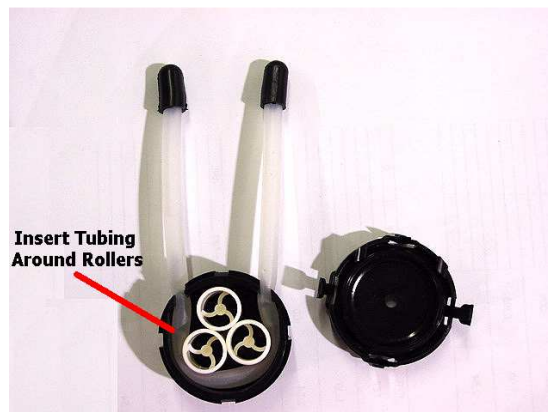
3. Reinstall the rear cover and make sure it has snapped into place.

17.8.12.   Pump Installation

1. To install the pump simply line up the drive shaft and the hole in the rear of the pump head.

Line Up Drive Shaft & Hole In The Rear Of The Pump Head

B I O) D O T
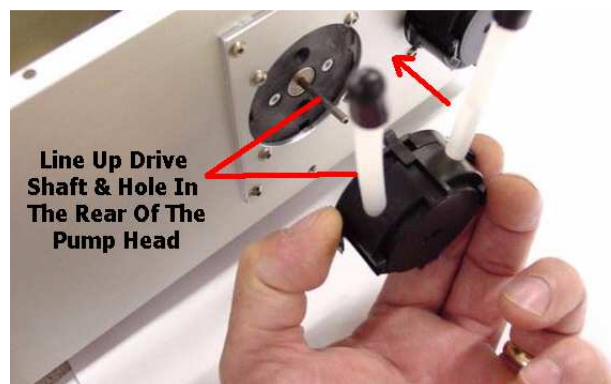
2. Then slide the pump head in until the vertical center clips are started.

3. Once the center clips are seated then depress the side clips and push the pump head in and release the clips until the pump head snaps into place.
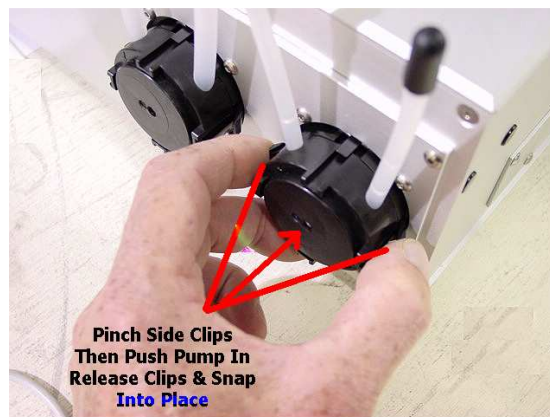
4. Check for proper operation of the pumps then reconnect any fluid lines and fittings.

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5. If further technical assistance is required, please telephone BioDot Inc. at (949) 440-3685 or e-mail support@biodot.com.

17.9. Platform Axis Lubrication

- Please refer to the manufacturer's manual Chapter 6 on axis lubrication and care at:

http://www.daedalpositioning.com/Support/Product_Manuals/404XR_406XR_Manual.pdf

Manual No. 100-5320-01
Rev. 4

**404/406XR Series
Product Manual**

Effective: November 17, 2004
Supersedes: August 22, 2002

**Electromechanical
Positioning Systems**

- Other lubrication points on the AD6000 will be the pull out tray and the manual source slide assembly. These two assemblies will not require a lot of lubrication. The manual source nest assembly, shown below, will require a small amount of SuperLube™ (www.super-lube.com) applied to the each of the rails with a cotton swab. The slide tray assembly will require a small amount of SuperLube™ applied to each of the slide rails as shown below. Do not over grease the assemblies.

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Source Slide Assembly

Remove Old Grease With Lint Free
Cloth. Lubricate Rails With Cotton
Swab & Remove Excess Lube With Lint
Free Cloth

Nest Base Slide Assembly
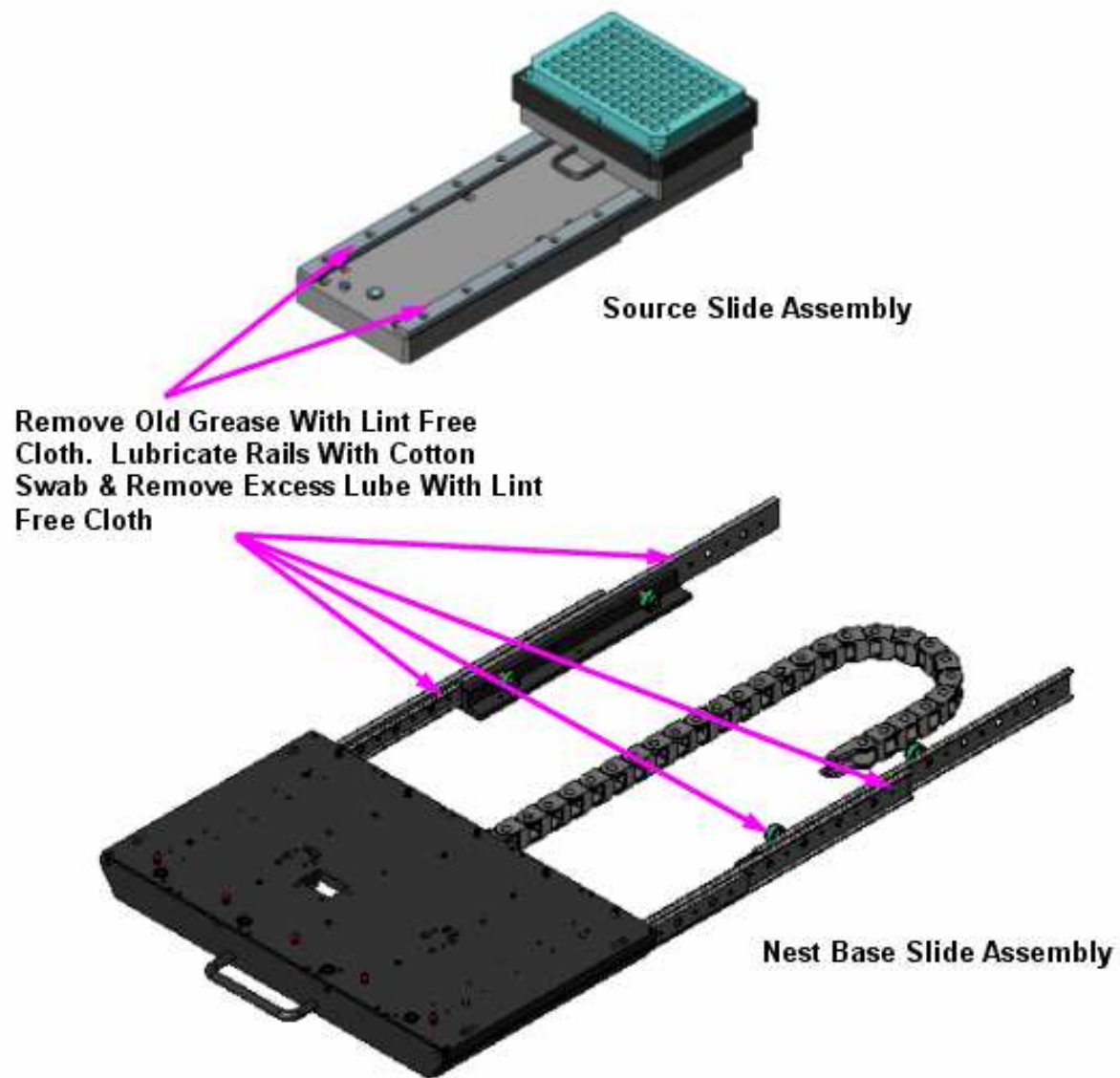
18. Troubleshooting

This section will help the user understand how to identify and clear most errors or problems. If further technical support is required please telephone BioDot Inc. Support (949) 440-3685 or e-mail support@biodot.com.

18.3. Troubleshooting Guide

Symptom	Possible Cause
1. No Power To Unit. Refer to Equipment Specifications Section.	• Power source disconnected. • Wrong power setting. • Check fuses and power entry module. • Incorrect wiring or defective power cord. • Defective power supply. • Low supply voltage.
2. Communication Failure.	• Defective connection at one of the communication terminals. • Check COM port settings. • Configuration not defined. • Defective CPU board. • Unplugging cable with the power ON. • Not plugged into the COM port. • Exit AxSys™ software, turn unit OFF. Wait 5 seconds the turn the unit ON. Enter AxSys™ software again.
3. *AD6000 Axes Not Homing Or Moving. *Occasionally the stepper motors that drive the axes will create a louder than normal hum, this is due to harmonic interactions between two motors or when a single motor is operated at certain speeds. These are normal noises and should not affect the performance of the AD6000 platform.	• No power. • Defective sensor. • Unit needs to be homed. • Wrong speed setting. • An "Axis Speed" instruction has not been placed in the program list. • "Axis Speed" profile parameters are inappropriate for the platform. • Stepper motor defective. • Loose or broken coupler. • Stepper control module defective. • Defective wire connection. • Physical blockage on one of the axes.
4. Peristaltic Pumps Not Running.	• Pump power OFF.

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	• Loose wire connection. • Defective 24VDC power supply. • Pump head not seated properly.
5. The Wash Station Overflows. Refer to Maintenance Section for tubing replacement.	• Tubing in the peristaltic pump(s) has become stuck together. Open the peristaltic pump and massage the tubing to open it. Shift the tubing so that the pump contains a new portion of the tubing. To avoid this problem flush the pumps with water before shutting down. • Observe the wash station and tubing to make sure there are no blockages, kinks or crimps that might impede the flow of wash fluids. The waste pump should remove more liquid that the wash can supply. Insert a flow restrictor if needed.
6. BioJet Plus™ (com/address x) Error Module Bus Time Out.	• No power to the pump. • Address on the rear of the pump set incorrectly. • Shorted pump. • Rear connection defective. • Broken wire in connector. • Defective CPU board.
7. BioJet Plus™ (com/address x) Error Initialization Pump X.	• Defective driver board. • Drive motor defective. • Stepper control board defective. • Loose connection at driver board. • Cable harness wired incorrectly. • Defective BioJet board. • Loose connection inside pump. • Defective CPU board. • Defective home switch / leak detection board.
8. BioJet Valve Not Firing.	• Defective BioJet valve. • Lead not connected to BioJet valve. • Crimped connection or clogged feed line. • Clogged dispense tip or BioJet. • Supply reservoir or line empty. • Improper feed line or electrical connection at BioJet. • Defective syringe pump. • Defective BioJet Plus™ valve driver card. • Defective BioJet Plus™ valve stepper control.
9. All BioJet Plus™ Pumps Do Not Fire At The Same Time.	• Trigger connection broken.

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10. Unequal Volumes Dispensed Between BioJet Plus™ Valves	- Channel parameters are incorrect. - Verify that the reagent and wash solutions have been pre-filtered and degassed. - Broken or clogged tip. - Clogged or defective BioJet valve. - Defective electrical connection. - Crimped or damaged supply or dispense line. - Bubbles in supply / dispense line (check syringe tightness). - Syringe, line and / or fitting leaking. - Syringe speed incorrect. Check dip switches on drive board. - Defective stepper control board (BioJet Plus™).
11. One Or More Syringes Not Traveling At The Same Speed.	In manual mode you must have selected "all dispensers of this type" first before setting the dispenser speeds.
12. Dots Not In Correct Place.	- BioJet valve fixture out of alignment. - Microwell plate not positioned correctly. - Dispense tip partially clogged or improperly installed. - Tip height or positions on X and Y-axes taught incorrectly. - Loss of steps on the X or Y-axes.
13. BioJet Plus™ Pump(s) Not Homing.	- Cables on the rear of the pump not connected properly. - Communication cable not connected properly. - Configuration incorrect. - Pump address incorrectly. - Harness not wired correctly.
14. BioJet Plus™ Pump Bouncing At The Top Of Its Stroke.	- Home switch / leak detection board defective. - Home switch / leak detection board not connected properly.
15. Dispense Tip Drop Forms.	- Clogged tip. - Viscosity of dispended fluid is too high or particulate matter in solution is too large. - The syringe speed is to slow. Select a higher syringe speed. - An air bubble is trapped in the syringe and can't be primed through.
16. Dispense Line Pops Off Or Is Leaking.	- BioJet lead or dispense line installed to wrong valve. - Defective BioJet valve. - Crimped or damaged line. - Dispense parameter is not appropriate. - Dispense Speed parameter is not appropriate.
17. There Is No Suction In The Vacuum Nest	No power to the vacuum pump or the vacuum supply is OFF.

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| | • Holes on the nest are plugged. Periodically verify that the vacuum holes are functional.
• Disconnect vacuum tubing. Ensure that the vacuum supply tubing is connected properly and not kinked.
• Check that the vacuum pump inlet filter is not blocked or clogged. Disconnect the vacuum tubing during this test to avoid possible contamination of the pump. Watch the gage and run the pump with and without the filter in place to see if the filter is interrupting the flow. |

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19.   Replacement Parts

The following replacement parts are available from BioDot Inc. Contact BioDot at (949) 440-3685 or e-mail support at support@biodot.com.

19.3.   Platform Parts AD6000

DESCRIPTION	PART NO.
Cable, RJ11, Coiled (Module Cable Assy)	1005-0021
Ferrule, Flangeless, 1/16 ETFE Blue	4001-0007
Ferrule, Flangeless, 1/8 TEFZEL Yellow	4001-0009
Ferrule, Super Flangeless, W/SS Ring, 1/16 Peek	4001-0010
Fitting, Barbed, Polypropylene, 1/8 NPT, 1/4 Tube	4001-0016
Nut, Flangeless, Headless, 1/16 1/4-28	4001-0055
Fitting, Straight, 3/8NPT, 1/2 Tube	4001-0063
Fitting, Barbed Wash Station Tubing Supply Polypro, Straight, Barbed, 1/8 ID Tube	4001-0119-03
Fitting, Barbed Wash Station Tubing Waste Polypro, Straight, Barbed, 3/16 ID Tube	4001-0119-05
Ftg, barbed, polypropylene, 1/8 NPT, 1/8 tube	4001-0125
Nut, super flangeless, headless, 1/16 tube, 1/4-28 THD	4001-0155
Control, Flow, 10-32, 1/8 Tube	4003-0007
Tubing, Vacuum Dry 1/4-inch OD, Black	4010-0000
Tubing, Silicone, Translucent, 1/8 ID, 1/4 OD (peristaltic pump)	4010-0009
Tubing, Silicone, Translucent, 3/16 x 5/16 OD (peristaltic pump)	4010-0010
Tubing, Vacuum Dry 3/8-inch OD, Black	4010-0016
Tubing, Clamps small 3/8	4010-0021
Tubing, Clamps large 7/8	4010-0022
Fitting, Vacuum Station Tubing Adapter from 1/4 to 3/8 inch	4010-0023
Terminal (Hand Held)	5002-0000
Block, Wash, 4-Pos, 3-Bath	6035-0088-01
Insert, Vacuum, 4-Pos, Miniature	6035-0089-01
Cover, Wash, 4-Pos, 3-Bath	6035-0090-01
Interface Cable, Assembly, DB9M TO RJ-11/12, 6' LG	6090-C003
Cable, CAN BUS COM	6017-C060

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19.4. BioJet Plus™ Dispenser Parts

DESCRIPTION	PART NO.
BioJet Plus™ Interface cable	1005-0005
Ferrule, Flangeless, 1/16 ETFE Blue	4001-0007
Ferrule, Flangeless, 1/8 TEFZEL Yellow	4001-0009
Nut, Flangeless, Headless, 1/16 1/4-28	4001-0055
Tubing Teflon (standard .030 ID, 1/16 OD, per foot) 48"	6002-0327-02
Tip, Ceramic, 190U Orifice, .75 Long	6002-0365
Holder, Installation Tool, BioJet	6002-0427
BioJet Plus Valve, Solenoid, 12 V, 90 Deg Leads Threaded Top	6002-0429
Screw, Adjust, Installation Tool, BioJet	6002-0485
Retainer, Spring, Ceramic Tip, 1-Position	6002-0500-01
Retainer, Spring, Ceramic Tip, 4-Position	6002-0500-04
Retainer, Spring, Ceramic Tip, 8-Position	6002-0500-08
BioJet Plus BioJet Plus™ tubing assembly. Tool #3016-0017 (old) xx=length	6002-A070-xx
Assy, Tool Tip Insert, BioJet	6002-A087
BioJet Plus BioJet Plus™ tubing assembly. Tool # 3016-0027 (new) xx=length	6002-A088-xx
Cap, 3-Port Valve (Blue)	6017-0008
Assy, +Pump, CE, Low-Resolution	6017-A010-11
BioJet Plus™ Assy, Valve, 3-Way,	6017-A030-01

19.5. Syringe Pump Parts

DESCRIPTION	PART NO.
Bottles, 10 mL conical vial & standard cap	0004-0004
Bottles, 1 mL conical vial & standard cap	0004-0005
Syringe Seal 1.0 mL	4008-0004
Syringe Seal 100 uL	4008-0006
Syringe Seal 250 uL	4008-0008
Syringe Seal 5.0 mL	4008-0009
Syringe Seal 2.5 mL	4008-0009-01
Syringe Seal 500 uL	4008-0010
Syringe Seal 50 uL	4008-0011
Syringe 1.0 mL	4008-0013
Syringe 100 uL	4008-0015
Syringe 2.5 mL	4008-0017
Syringe 250 uL	4008-0020
Syringe 5.0 mL	4008-0022

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Syringe 50 uL	4008-0025
Syringe 500 uL	4008-0027
Syringe Valve (3 port, Standard Pump)	4011-0000
Bottles, 10 mL conical vial & modified cap (fitting, plug &cap) Assy	6001-A008-01
Assy, Tubing, 36.0 Long, Bottle To Syringe Pump	6002-A038-03
Assy, .062 Tubing, Syringe to BJQ, Min	6002-A088-72

19.6.   Optional Accessories

DESCRIPTION	PART NO.
Voltage Converter	1023-0003
Vacuum pump 110 VAC, 60 Hz (VP4000) North American	2003-0016
Vacuum pump 230 VAC, 50 Hz (VP4000) Euro (Shuko)	2003-0017
Peristaltic Pump (small tubing) 2.5mm	2012-0011
Peristaltic Pump (large tubing) 4.0mm	2012-0012
Humidifier	2017-0000
Assy, Peristaltic Pump Mtg., Wash Station	6035-A106-01
ILD4000 Vacuum Degasser	6042-A011-XX

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Addendum

TABLE OF CONTENTS

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Section 2: The Dispensing Environment
Section 3: The Fluid
Section 4: The Substrate
Section 5: Programming and Process Design
Section 6: Dispenser Maintenance
Summary
Appendix

B I O D O T

Introduction

Robust dispensing of fluids in low nanoliter volumes is a technically demanding process involving multiple variables. This document is designed to help the user understand how to effectively and robustly dispense low nanoliter volumes using the BioJet Plus dispensing system.

The primary system components contributing to an accurate and reproducible low volume dispense are:

1. The dispenser.
2. The environment.
3. The fluids in the system.
4. The substrate or target to be dispensed onto.
5. Programming and process design factors.
6. Dispenser cleaning and maintenance factors.

Each of these system components will be discussed in detail in this document.

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Section 1: The Dispenser

The BioJet Plus™ technology combines the high-resolution displacement capabilities of a syringe pump with a high-speed micro solenoid valve.   This combination permits the non-contact dispensing of nanoliter volumes.
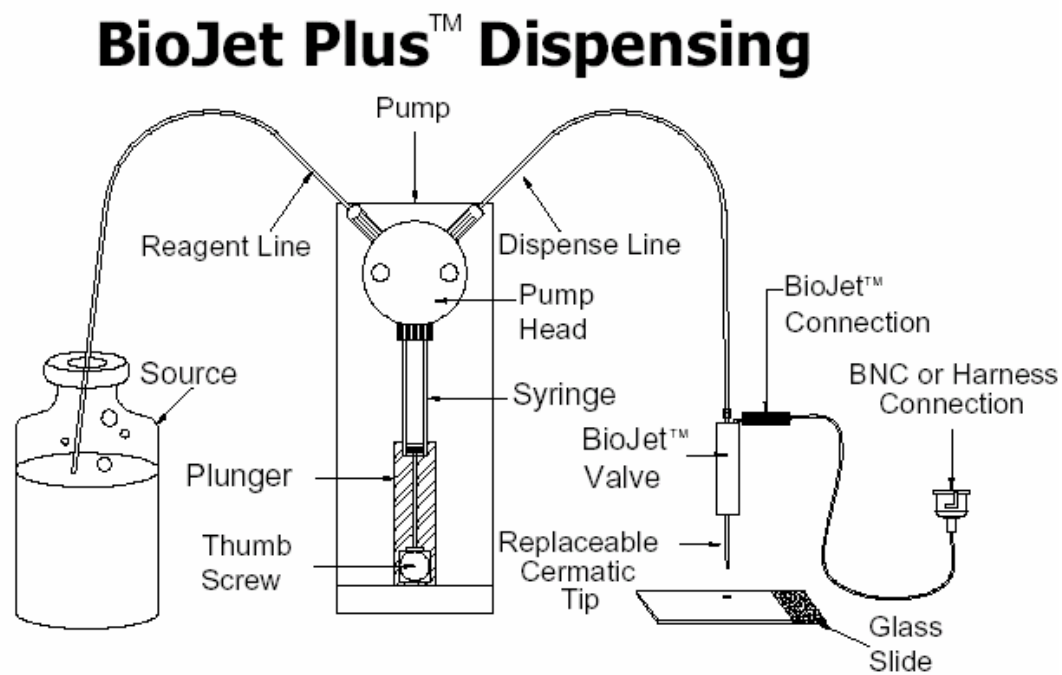
In a typical dispensing system, 4 to 8 of these syringe/solenoid channels are placed together. Two modes of liquid handling are possible: Continuous (bulk) dispensing and aspirate/dispense. The AD6000 has a maximum of 96 channels.

Continuous dispensing involves pulling reagent or solvent from a reservoir into the syringe and then dispensing it through the micro solenoid valve. Filling the system with a backing fluid, dipping the tip of the valve into a sample, withdrawing the syringe to aspirate the sample and then dispensing the aspirated sample accomplishes Aspirate/dispense.

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The BioJet Plus™ dot dispensing system is a hydraulically driven system that requires a fluid medium to be present from the syringe to the microsolenoid BioJet Plus™ valve. The dispensing process involves the following steps:

1) The syringe is displaced a given amount
2) The valve is opened for a short period of time (milliseconds)
3) Fluid is released from the valve and travels to the tip
4) The fluid increases its linear velocity as it passes through the tip orifice and ejects as a drop (or stream if the amount of fluid is large). One valve actuation results in one drop.

The key to a correct volume being dispensed in a given drop from the BioJet Plus™ system is the steady-state pressure (SSP) in the dispensing system. This pressure has several important features:

- It is achieved by the displacement of fluid by the syringe pump
- The SSP is displacement (drop size) dependent, increasing with increasing displacement (drop size)
- The SSP is determined by the system compliance, which is dominated by entrapped air bubbles.
- Once the SSP is established, the amount of fluid displaced by the syringe pump will equal the amount dispensed

The BioJet Plus™ dot dispensing system can be modeled as an electrical circuit with the pressure acting as the voltage, the flow rate as the current, the system compliance as capacitive elements, the valve, tip, and feed lines as resistive elements, and the valve as a switch. This model shows the syringe pump as a current source, which provides an advantage over a pressure source (e.g. gas pressure) in that any changes in resistance will not affect the flow rate. In contrast a pressure source will be affected by changes in resistance in the system.

Factors Affecting the Dispense Volume

The model described above shows the fluidic circuit possessing a feedback loop, which can be used to achieve the SSP. Once the SSP has been achieved, the volume displaced will equal the volume dispensed.

The SSP is achieved by first pre-pressurizing the system by displacing an experimentally determined volume using the syringe pump. Usually this pre-pressure is slightly higher than the required system pressure and requires several pre-dispenses at the desired volume to reach steady state pressure, whereby the desired dispense volume is dispensed.

Several factors influence the achievement and maintenance of the SSP and the desired dispense volume:

B I O D O T

Priming

The Prime is used to initialize the syringe pumps and fill the syringe pumps, microsolenoid BioJet Plus™ valves of the dispense head, and connecting tubing with fluid from the reservoir(s). The reservoir fluid is either system fluid for aspirate/dispense of reagent, or sample fluid for continuous (line or dot) dispensing.

When the dispense system is primed, several hundred micro liters of fluid are dispensed as a stream. The resistance to flow caused by the valve and tip orifice causes the pressure within the system to become higher than desired for SSP. To achieve SSP, one must first vent the valves, which involves opening the valves without displacing fluid. This brings the system to ambient (zero) pressure and from this point; the SSP pressure can be achieved.

Aspiration

The aspirate function draws sample from a reservoir, usually a micro-well plate, into the tip of the dispense head. To perform an aspirate, several parameters must be set.   These parameters are set by using Syringe Speed and Channel Parameter actions. The syringe speed controls the speed of the syringe pump dispensers. In general, slow syringe speeds are used for aspiration to prevent a large vacuum from being developed which can result in the development of bubbles in the system.

During the normal aspiration process, a slight negative pressure is produced. This negative pressure is relieved by performing a vent, which opens the microsolenoid valve without displacing any fluid. When sample is aspirated, the syringe pump draws fluid through the tip orifice. The resistance to flow from the tip and valve creates a negative pressure, which must first be overcome to achieve a steady state pressure. As with priming, venting the system can bring the system to a known (zero/ambient) pressure, from which the SSP can be applied (Note: venting is typically done with tips in the sample to prevent the introduction of air).

The SSP during a prime/aspirate/dispense cycle is shown schematically below:

B I O D O T
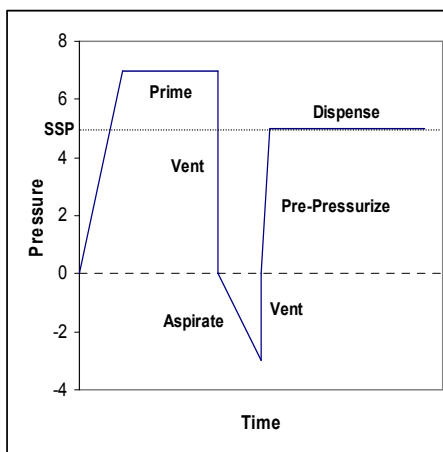
Gas Bubbles

Gas bubbles can occur in the dispensing system for multiple reasons, including primarily leaks and dissolved gases in the fluid. The major effect of bubbles is to change the system compliance, which can affect drop formation. Input will still equal output but the fluid will collect on the dispense tip rather than eject as a full drop. This can cause variation in dispensed volumes and eventually cessation of dispensing. Bubbles can be removed by purging the system with isopropanol and using degassed solvents as well as the system fluid.

Tip Effects

Condensation of liquid on the dispense tips can lead to loss of control of dispense volumes due to the interaction of ejected drops with resident fluids near the tip orifice. This effect can be reduced and/or eliminated by vacuum drying of the tips at appropriate times during the dispense cycle. Vacuum drying the tips is especially important when dispensing for long periods of time. Tip orifice size is also of critical importance. Smaller drop sizes require smaller orifice sizes for proper drop formation. The smaller orifice size must, however, be balanced against the rheological properties of the fluid and the particulate content of the fluid to be dispensed, as smaller orifices clog more easily than larger orifices.

Syringe Speed

Syringe speed is the speed of displacement of the fluid in the syringe pump. For dispensing, the syringe speed has little if any affect on the SSP. For priming, the syringe speed will effect the pressure build-up and at very high speeds, may cause too much pressure and result in leaking. For aspiration, slow speeds are best to prevent excessive negative pressures being generated, which could also lead to out-gassing from the fluids.

Valve Open Time

The microsolenoid BioJet Plus™ valve open-time is one of the most important parameters for achieving and maintaining the SSP. This is the time the valve opens to release displaced fluid and eject the drop. In previous versions of software the open time is set in % duty cycle. Duty cycle gives the percent of time the valve is open for one valve actuation and the open-time is the actual amount of time that the valve is open in one valve actuation.

For a dispense system at SSP, the proper open time will result in a displaced volume equal to the dispensed volume. If the open time is too short, then over time pressure will build beyond the SSP. If the open time is too long, the SSP will be dissipated eventually resulting in drops not being ejected from the tip.

The open time is the approximate time required for the fluid to move though the valve. Thus open time increases with increasing drop size and increasing viscosity. A list of appropriate on-times is contained in the dispenser manual.

Section 2: Dispensing Environment

Many dispensing applications require careful control of the dispensing environment. This can entail control of:

B I O D O T

- Humidity
- Static electricity
- Partial pressure of solvents or noble gases
- Airborne particulates

The use of environmental enclosures around machines can be critical in ensuring proper and continuous operation of the machine. The use of controlled, elevated humidity environments can be important in aspirate and dispense applications where open reservoirs of small volumes of aqueous reagents are used as the source for dispensing. Slightly elevated humidity can also help to reduce the buildup of static electricity, which can be caused by the movement of certain substrates such as nitrocellulose through the unit. Increased partial pressures of noble gases can be used to assist in reduction of oxidation of reagents, as well as to control drying, and increased partial pressures of solvents in the chamber can be used to reduce evaporation of solvent-based reagents within safe operating parameters for the solvents (the possibility of flash must be particularly considered where use of this option is intended).

Critical to the continued trouble-free operation of the machine is minimizing the particulate content of the entire area in which the dispenser operates. Ideally, the unit should be operating in a Class 10,000 or better clean room with temperature and humidity control. At worst the machine should be enclosed to reduce the possibility of airborne contamination of the dispensing area with high levels of particulates, which can serve to disrupt the operation of the BioJet valve.

Section 3: The Fluid, Including Rheology, Dissolved Gases And Particulate Content

Fluid Rheology

Since there are constricted passageways within the AD6000, the flow of liquid is influenced by both the viscosity and surface tension of the dispensed reagent. Fluids of viscosity up to approximately 10cP can be accurately dispensed, however for ideal results, fluid viscosity greater than 4 centipoise should be avoided since drop to drop variations are more likely to occur under these conditions. Highly compressible fluids or fluids demonstrating thixotropic properties must be used with care in the system. In aspirate and dispense applications, it is critical that the backing fluid used in the system be compatible with the fluid to be dispensed. The use of incompatible backing fluids can result in chemical reactions, leading to the formation of particulates, air bubbles, or filaments, any one of which is potentially disastrous to the application and to the BioJet valve. Certain fluids also contract on contact with water, leading to changes in volume that can affect the accuracy and reproducibility of dispensing.

Dissolved Gases

The presence of dissolved gases in the system can lead to inaccuracies in dispense volume or missed dispenses. Dissolved gases can come out of solution at virtually any point in the system that a nucleation site is available, which includes the inlet and outlet lines,

the syringe, the three-port valve and the BioJet valve and tip. These bubbles may be macro-scale and be visible to the eye, or they may be microscopic or in a portion of the fluid pathway that is not visible to the operator, thereby making it extremely difficult to diagnose a dispensing issue when one arises. It is therefore critical to remove as much dissolved gas from the fluid as possible prior to use in the dispenser. This degassing can be achieved in a number of ways. BioDot recommends the use of helium degassing (described elsewhere in this document) but in cases where the required equipment is not available, a less efficient method of vacuum degassing may be used. **Note**: **All fluids that come into contact with the dispenser should be degassed and filtered, including backing fluids.**

Particulate Content

BioJet valves operate in part through the motion of a poppet, which is seated in a rubber seal. When the poppet is in the up position, the fluid pathway is open and fluids pass through the valve opening to the tip under pressure. When the poppet is in the seated position, the valve is closed and no fluid passes through to the tip. Correct seating of the poppet in the EPDM seal is one element of the operation of the system that ensures that the SSP is maintained, that no air enters the system, and that no fluid leaks from the system. If particulates become embedded in the rubber seal, they physically inhibit the proper seating of the poppet in the seal, thereby ensuring that all of the problems just described will occur. The presence of microscopic particulates in the valve seal is the number 1 cause of poor dispense quality and valve failure. Additionally, particulates can cause damage to syringe seals, 3-port valves, and can provide nucleation points for bubble formation in the system.

Note: **It is critical that all fluids used in the system be filtered before use, ideally with a 0.22um filter.** This includes reagents, backing fluids and wash fluids. BioDot can provide in-line filters to reduce the particulate load drawn into the fluid pathway.

Chemical Compatibility

Aqueous Solvents
In general, aqueous solvents are compatible with the BioJet dispensing technology. Buffers with a pH range from 3 to 10 are suitable. Extremes in pH may cause corrosion of the stainless steel or glass materials. In addition, many surfactants and proteins can be used with the dispensing system.

Polar Organic Solvents
In general, polar oganic solvents are compatible with the dispensing system. Although not all polar organic solvents have been tested under long-term conditions, the following have shown good chemical compatibilty with the system:

B I O)D O T

- Dimethyl sulfoxide (DMSO)
- Acetone
- Methyl Ether Ketone (MEK)
- Ethyl acetate

Non-Polar Organic Solvents
Most non-polar organic solvents are incompatible with the BioJet dispensing technology. These solvents cause swelling and/or degradation of the polymeric parts of the microsolenoid valve, especially the EPDM seal.

Overcoming Chemical Incompatibility

In some cases, chemical incompatibilities can be overcome. For example, for samples dissolved in solvents, which are not compatible with the standard BioJet valve, the sample can be aspirated up to but not into the valve (approximately 5 μL maximum aspiration volume). In cases where a larger aspiration volume is needed, a customized extension can be added between the valve and the tip. This results in an increase in the minimum dispense volume from 8 to 10 nL up to 50 to 100 nL. A second option is the use of a more inert valve. BioDot offers a more chemically inert valve, the performance of which can be assessed with the particular solvent of interest on an individual basis.

Section 4: The Substrate

The physical characteristics of the substrate contribute a great deal to the quality and efficiency of a micro-dispense. Characteristics of the substrate that can compromise or contribute to a good quality result include:

- Hydrophobicity
- Planarity
- Reproducibility of modification (additives or treatments)
- Charge

Through careful application of the principles described in this document, the BioJet or BioJet Plus dispenser can deliver a quantifiable, reproducible, programmable volume from the tip of the dispenser. Quite often, what that dispense looks like once it hits the substrate will depend largely on the qualities of the substrate itself.

BioJets have been used to dispense on a wide variety of substrates, from glass slides to nitrocellulose, and a wide variety of plastics, both flexible and rigid. The quality of the dispense achieved can be measured in a variety of ways, and each application has different

B I O D O T

requirements for the aesthetics and volumetric accuracy to be achieved. Micro arrays on glass slides or coated glass slides generally require a final spot with good morphology, perfect roundness and even density of deposition across the spot. The achievement of these characteristics depends on factors such as the hydrophobicity / hydrophilicity of the substrate, the (protein-) binding nature of the substrate, static charge, and wicking characteristics, as well as the rheological and chemical characteristics of the fluid itself being dispensed. While it is relatively simple to optimize the volume of the dispense, it quite often requires more demanding work to optimize the interactions of the reagent, substrate and dispenser to achieve the aesthetic characteristics desired in the final spot. Other applications, such as many biosensor designs, are less demanding in terms of spot morphology after deposition, and demand more in terms of reproducibility of absolute dispense volume or coverage of a defined area at a defined thickness. These applications have their own demands in terms of surface characteristics of the substrate, particularly wicking or spreading characteristics, and tolerances on the placement of printed circuits. Variations in z-axis height on some substrates can cause issues with dispense placement accuracy, as consistent z-axis placement of the tip relative to the surface is important for ensuring accurate drop positioning.

Section 5: Programming & Process Design

Introduction

Great care should be taken during the initial stages of learning the control software for the BioJet and BioJet Plus systems. It is estimated that over 90% of the user-induced damage to BioDot systems occurs within the first week of operation at a customer site. These are sensitive robotic instruments, which, while robust enough to handle demanding manufacturing and R&D environments when handled correctly, can be severely damaged through incorrect operation. Common errors include:

- Crashing of heads through incorrect programming of the compound motion table.
- Damage to BioJet or BioJet Plus valves through incorrect programming of valve operation
- Contamination of the system with particulates or incompatible fluids, leading to errors in dispensing accuracy or damage to valves.

It is strongly recommended that users purchase an Installation and Training package from BioDot, and / or utilize BioDot's Application Laboratory and technical staff to develop their programs and applications at least initially.

The User Manual for the unit should be carefully studied, and operators should pay particular attention to the sections on Cleaning and Programming of the BioJet or BioJet Plus dispensers.

Some basic principles should be applied to process design in order to achieve maximum system performance:

B I O **D O T**

1. Pay particular attention to the geometry of the machine and the dispense head in designing the process in order to minimize process time and to create intelligent process designs
2. Determine early in the process design phase the optimal number of dispensers, balancing throughput, cost and process efficiencies
3. Consider fluid rheology and chemistry in determining backing fluids to be used
4. Consider solvent compatibility with the BioJet valves
5. Determine whether humidity and temperature control are required
6. Determine whether an increased atmospheric partial pressure of a solvent is required
7. Ensure that the backing fluid used is appropriate and compatible with the fluid to be dispensed
8. Think clean. Remove all sources of particulates in fluids, machine, disposables and parts to be dispensed
9. Use the sample programs provided with the machine to establish the basic program that you wish to use, then build complexity from there
10. Use sleep mode programs to prevent the need for continuous machine setup and teardown (see below).

In-Process Cleaning of Tips

Buildup of proteins on tips can lead to inconsistent dispensing due to clogging, and also to carryover of proteins from well to well of a source plate and array (if multiple samples are being aspirated in sequence using the same tip). Appropriate in-process cleaning is crucial to maintaining dispenser function. Depending on the type of protein, hydrophobic interactions can mean that protein adheres quite strongly to the tip and removal of the protein from the tip can be difficult. Each system should be assessed separately; however as a general principal, the use of a buffered solution of MeOH (approximately 20%) plus 100mM NaCl, pH 7.4 (for example in Tris) will be adequate to disrupt binding of proteins to tips. Repeated immersion and aspiration of a cleaning solution such as this one, followed by rinsing with filtered distilled water should be sufficient to remove most proteins from the tips. This can be programmed as a periodic cleaning step in situations where a single sample is being dispensed repeatedly from a tip, or between samples.

Sleep Mode Programs

To maintain optimal system performance, it is advisable to minimize the number of times that the machine has to be shut down and restarted. Every time a shutdown and restart is performed, a cleaning and priming cycle is necessary, which often involves disconnection of syringes, tubing and valves, which in turn increases the opportunity for damage to components and can lead to increased opportunities for contaminants to enter the system. The use of Sleep Mode Programs is one way to minimize the number of setup and shutdown cycles. In this mode of operation, the system is left to idle when not in use, reactivating itself at intervals to dispense small amounts of innocuous fluids, cleaning fluids or backing fluids. The machine can then simply be reactivated when necessary, flushed and set up to run the required program without physical interference with the components of the fluid pathway.

B I O D O T

A methodology for improving system performance through appropriate system setup, teardown, cleaning and programming is outlined below.

- **Purpose**

To outline a procedure for setting up a machine in order to achieve consistent machine performance via de-gassing, filtering, use of helium and sleep mode.

- **Materials**

1. Any BioDot machine containing syringe pumps and BJQ's – configuration will include BioJet Plus™ fittings.
2. Connections and ceramic tips
3. Tank of helium
4. Apparatus/manifold for connecting source reservoir to machine and helium tank concurrently
5. Filter – at least 0.45µm but 0.2µm preferred. If in-line filters on the fluid inlet lines are to be used, a larger filter size (1µm) is recommended in order to minimize effects on the rapid achievement of Steady State Pressure in the system.

B I O D O T

METHODS

First time set-up

The first time a machine is used (OR anytime ANY hardware is changed), it must be assumed that the fluid path (or hardware) contains particulates that may cause the dispensing valve to fail (either mechanically or functionally). It must also be assumed that air is present in the fluid path, which will greatly impede consistent dispensing. Therefore, anything coming into contact with the backing solution must first be cleaned, the backing solution, wash solution and dispensing solution must be filtered and the fluid path cleared of air. These things can be accomplished by cleaning removable parts offline, filtering, and flushing with ethanol, respectively.

- **Cleaning**
  Removable parts should be cleaned off-line (this will include the reservoir bottle, syringe and piston, in-line filter, if being used, and ceramic tip). Once cleaned, reattach all parts.

- **Filtering**
  Filtration of dispense fluids, ethanol, backing fluids and wash fluids can be performed off-line via a bottle-top filter or a syringe tip filter or in-line using BioDot's recommended in-line filter.

- **Flushing**
  Disconnect the fitting at the top of the dispensing valve(s) and position the end of the tubing over a waste basin – repeat this for all channels. Attach a reservoir bottle(s) containing filtered ethanol to the machine. Prime the ethanol through the fluid path until all visible air in the tubing is expelled and at least one more cycle – this will clean the tubing as well as remove any air in the fluid path. Then reattach the fitting to the dispensing valve(s) and make sure the ceramic tip(s) are in place – repeat for all channels. To remove air from the dispensing valve(s) and tip(s), prime through with 2 more cycles of ethanol. The ethanol then needs to be removed from the fluid path which can be done by priming de-gassed backing solution (see below for procedure) through all lines. For most BioDot machines, 10 prime cycles will suffice to remove all the ethanol.

Degassing

All fluids entering the fluid pathway must be degassed. Below are two procedures for de-gassing using vacuum and helium.

B I O)D O T

Vacuum Degassing
- Using BioDot's Reagent Degasser DG950, de-gas the reservoir bottle(s) using sonication for 10 minutes – sonication can be run in 10 second intervals
- If this equipment is not available, apply a vacuum to the reservoir bottle(s) for at least 10 minutes.
- When the de-gassing is complete, **DO NOT** pour the liquid into another container as this will re-dissolve air into the liquid. Cover the bottle with a cap or wrap tightly with parafilm to transfer the bottle to the machine.

Helium Degassing

Note: This procedure must be performed with BioDot's helium degassing system. It is designed for use with fluids to be bulk dispensed (i.e. put through the fluid pathway, not aspirated) or with backing fluids for aspirated fluids.

1. Fill reservoir bottle(s) with desired solution.
2. Apply 15 psig of helium to bottle(s) for 30 minutes. Vent to 0.1 psig.
3. Repeat step 2 twice for a total of 3 fill and vent cycles.
4. Maintain an operating pressure of 0.1-0.2 psig helium for dispensing.

Machine Set-Up (Not First Time)

Once the machine has been set-up according to the above outline, a clean and de-gassed state may be maintained by 1) not changing hardware and 2) always maintaining a constant pressure of helium (i.e. the operating pressure). This means neither the machine nor helium will ever be turned off. When the reservoir needs re-filling, the helium will need to be turned off so the set-up procedure will begin at *Filtering* above.

Sleep Mode

Good quality, consistent dispensing requires great attention to detail when setting the machine up for operation. The factors above, achieving and maintaining a de-gassed backing solution, filtering, cleaning and flushing, must all be addressed each and every time the machine is to be used. In order to minimize the need to perform the above procedure, the machine should be placed in a "sleep mode" when not in use. An example of a Sleep Mode program is outlined below:

- Move to Wash – places tips down into wash water.
- Pause – Fixed duration of 15 minutes (900,000ms)
- Move – lift tips out of wash water

B I O) D O T

- Vacuum Dry Tips
- Move to Waste
- Prime – 1 cycle (be sure to check for sufficient backing solution and to set appropriate   syringe speeds.
- Vacuum Dry Tips
- Move to Waste
- Loop 50X - 100nL dispenses (be sure to include a syringe speed – 10-20-1000- and open time –1500ms)
- Prime – 1 cycle

This entire string should be within 1 function and that function looped according to the following formula → # hours machine will be unused * 60/15 minutes = # of time to loop function (or # of 15 minute cycles in total downtime). The "sleep mode" program then is simply this one function looped x # of times.

Section 6: Dispenser Maintenance

The BioJet and BioJet Plus dispensing systems consist of three major components:

- Controller
- XYZ motion
- Fluid pathway

The maintenance requirements of the controller and XYZ motion system are minimal and these components of the system rarely generate problems in terms of the quality of product produced. The fluid pathway is the portion of the system that requires the most care and attention, constant cleaning, and occasional maintenance and part replacement.

The fluid pathway consists of:

- Fluid reservoir (or source plate)
- Inlet tubing to the 3-port valve
- 3-port valve
- Syringe
- Outlet tubing from the 3-port valve
- BioJet valve
- Tip

BIO DOT

All portions of this pathway are considered to be disposables and are subject to reduced warranty periods, so constant care must be taken to ensure long life and appropriate performance.

In order to maximize life and minimize problems with dispense accuracy; several basic principles should be adhered to:

1. Filter all fluids in the system, ideally with a 0.22um filter before use. This includes backing fluids, wash station fluids and fluids to be dispensed. If possible, use in-line filters for fluids to be bulk-dispensed (i.e. not aspirated).
2. Degas all fluids, ideally using helium degassing, or using vacuum with intermittent agitation or sonication if helium is not available.
3. Flush lines with alcohol regularly to reduce bubble formation
4. Work in a particulate-free environment. Ideally a class 10,000 or better clean room should be used. Remove particulates from reservoirs and machine before use
5. Rigorously and diligently follow all recommended cleaning procedures (see Appendix)
6. Check performance and be prepared to replace ALL fluid pathway components on a regular schedule
7. Use only compatible fluids within the BioJet valve (see Appendix for list of known incompatible fluids). All other fluids should be aspirated and dispensed from the tip and not put through the valve itself
8. Check performance of the system regularly using a calibrated measurement system such as the Artel PCS (see Appendix for details)
9. Utilize "Sleep Mode" programs (see Process Design section. Never turn off the machine if possible
10. Clean tubing separately from BioJet valves. If particulates accumulate in tubing they can be washed into the valve during cleaning of the system. Disconnect the tubing from the BioJet valves before performing cleaning of the tubing. Subsequently reconnect the tubing and clean the valves.

Summary: Considerations for Effective Nanoliter Dispensing

Think clean
Remove all possible sources of particulates in the environment, fluids, machine, disposables and substrates

Work in a particulate-free environment
Where possible a class 10,000 clean room

Remove particulates from reservoirs and machine before use

BIO DOT

Foreign particles can damage both the 3 port valves and the solenoids.

Filter all fluids in the system before use
Including backing fluids, wash station fluids and dispensed fluids. In-line filters can also be used.

Degas all fluids
Ideally use helium degassing, or vacuum with intermittent agitation or sonication.

Follow BioDot's cleaning procedures
Use BioDot-specified cleaning materials and reagents.

Clean tubing separately from valves
Disconnect the tubing from the BioJet valves before cleaning the tubing to prevent contaminants entering the valve.

Flush lines with alcohol regularly
To reduce bubble entrapment in the fluid path.

Check performance of the system regularly using a calibrated measurement system
Use a system such as the Artel PCS or a validated microtiter plate – based method

Replace ALL fluid pathway components on a regular schedule
This includes:
1. Fluid reservoir (or source plate)
2. Inlet tubing to the 3-port valve
3. 3-port valve
4. Syringe
5. Outlet tubing from the 3-port valve
6. BioJet valve
7. Tip

B I O D O T

Use only fluids compatible with the valve
All other fluids should be aspirated and dispensed and not put through the valve itself.

Maintain the stability and uniformity of your reagents
Operate in a temperature-controlled environment or incorporate mixers, heating or cooling plates into the machine design.

Choose source plates or reservoirs appropriate to the application. Consider:
- Protein binding characteristics.
- Aspect ratio.
- Capacity.

Choose the correct backing fluid for your application
Consider fluid rheology and chemistry in determining backing fluids to be used.

Consider the physical characteristics of the substrate, including:
- Hydrophobicity.
- Planarity.
- Uniformity of modification.
- Charge.

Maintain careful control of the dispensing environment, including:
- Humidity.
- Static electricity.
- Partial pressure of solvents or noble gases.
- Airborne particulates.

Take care in programming
Use the sample programs provided with the machine to establish the basic program to be used, and then build complexity from there.

Never turn off the machine if it can be avoided
Utilize "Sleep Mode" programs to minimize teardown and setup, which increase the likelihood of damage, introduction of foreign particles and air into the system.

B I O)D O T

Take care of your dispense head and tips during program development
Crashing of heads during program development is the main source of damage to heads and tips.

Prevent cross contamination of wells in source plates
Clean tips carefully between aspirations. Ensure that the entire tip length exposed to the aspirate is washed before the next aspiration to prevent carry-over of fluids. Use cleaning solutions appropriate to the reagents being handled.

APPENDIX

Cleaning of the BioJet Dispensing System

General Notes:
All cleaning fluids must be filtered and degassed   **The cleaning method outlined here is valid for bulk dispensing of aqueous, proteinaceous reagents. It is a suggested protocol only. Users should validate their own protocol designed for use with their own particular application and reagents.**   **If aspiration of multiple reagents using the same tip is being performed it is critical that the cleaning process be validated to ensure that no carryover of reagent from well to well occurs. It is likely that a solution such as MeOH (approximately 20%) plus 100mM NaCl, pH 7.4 (for example in Tris) will be adequate to disrupt binding of proteins to tips, but depending on the backing solution solvent used, this may require change and validation.**   **The cleaning protocols outlined are suggested for use in systems where Sleep Mode programs are not used.**

Daily Cleaning

To achieve optimum performance and maximum life from the BioJet Quanti3000™ dispenser, it is recommended that the routine cleaning procedure listed below be followed after each period of use (at least once daily).

1. Purge supply lines of reagent.
2. Clean and refill the supply reservoir with deionized water containing 0.05% BioTerge to enhance scrubbing of interior recesses within the BioJet.
3. Prime dispenser for 5 syringe cycles.
4. Repeat steps 1 through 3 with deionized water.

B I O)D O T

The reagent reservoirs and feed lines are reusable and may be used for extended periods of time before replacement is warranted.

Residue Cleaning (Weekly Cleaning during heavy use)

After prolonged dispensing of reagents, some buildup of protein constituents, salts, latex materials, or other particulate matter may occur. It is recommended that the following cleaning steps be followed on a weekly basis to dissolve any accumulated materials. This should be performed in addition to the daily cleaning procedure.

Option 1

1. Purge supply lines of fluids.
2. Clean and refill the supply reservoir with a dilute base (0.1N NaOH). Prime the BioJet for 5 syringe cycles and allow to sit for 10 minutes.
3. Prime using deionized water as in steps 1 and 2 above.
4. Clean and refill the supply reservoir with a dilute acid (0.1N HCl). Prime the BioJet for 5 syringe cycles and allow to sit for 10 minutes.
5. Purge the supply lines and prime for a minimum of 10 cycles using deionized water.

Option 2

For use in systems dispensing proteinaceous fluids or where acid/base use is undesirable

This procedure may be performed as part of the regular weekly cleaning protocol in addition to Option 1 above or as an alternative if the use of acid / base is undesirable in your system. It is of particular value in helping to prevent cross-contamination when dispensing multiple reagents from a single BioJet, or to clean protein buildup from the lines and the valve.

1. Purge supply lines of reagent
2. Prime 3 cycles of deionised water with 0.05%. BioTerge through the system.
3. Prime this solution out of the system.
4. Prime Jetwash into the system and allow to sit in the BioJets for 30 minutes.
5. Prime this solution out of the system.
6. Clean and refill the supply reservoir with deionized water.
7. Prime dispenser for 5-10 syringe cycles.
8. Remove the glass syringe from the syringe pump.
9. Remove the plunger from the syringe and flush deionized water through the open syringe and wash the plunger in deionized water being careful not to damage the plunger seal.
10. Flush deionized water through the ports in the 3-port valve. The valve stem may be turned by hand to switch path positions.

B I O D O T

Cleaning of Tips

Ceramic tips may be removed from the dispenser and washed in 1-10N acid followed by an equal molarity base, in turn followed by flushing with water. Appropriate safety precautions should be observed when handling high molarity acids and bases.

Chemical Compatibility with the BioJet Dispensing Technology

Introduction

The BioJet™ dispensing technology can be used with a wide variety of solvents and reagents. However, there exist a limited number of solvents and reagents that can potentially damage the system. This application note examines the extent of chemical compatibility of a system using the BioJet dispensing technology.

Wetted Materials
The materials which come in contact with fluids in the dispensing system are listed below:

Syringe Pump
- Teflon
- Glass
- Stainless Steel

Microsolenoid Valve
- Stainless Steel (430F, 316SS)
- Polyphenylene Sulfide (PPS)
- Poly Ketone
- Ethylene/Propylene Diene Mono (EPDM)
- Epoxy (minimal wetted area)

B I O D O T

Other Parts
- Teflon (transfer lines)
- PEEK (fittings)
- Polypropylene or Ceramic (dispensing tip)

For continuous dispensing, the fluid wets all of the above materials. For aspirate/dispense, the fluid comes in contact with the dispensing tip (aspiration volume < 6 μL) and the microsolenoid valve (aspiration volume > 6 μL)

Chemical Compatibility

Aqueous Solvents
In general, aqueous solvents are compatible with the BioJet dispensing technology. Buffers with a pH range from 3 to 10 are suitable. Extremes in pH may cause corrosion of the stainless steel or glass materials. In addition, many surfactants and proteins can be used with the dispensing system.

Polar Organic Solvents
In general, polar oganic solvents are compatible with the dispensing system. Although not all polar organic solvents have been tested under long-term conditions, the following have shown good chemical compatibilty with the system:

- Dimethyl sulfoxide (DMSO)
- Acetone
- Methyl Ether Ketone (MEK)
- Ethyl acetate

Non-Polar Organic Solvents
Most non-polar organic solvents are incompatible with the BioJet dispensing technology. These solvents cause swelling and/or degradation of the polymeric parts of the microsolenoid valve, especially the EPDM seal.

Overcoming Chemical Incompatibility

In some cases, chemical incompatibilities can be overcome. For example, for samples dissolved in solvents, which are not compatible with the microsolenoid valve, the sample can be aspirated up to but not into the valve (approximately 5 μL maximum aspiration

B I O D O T

volume). In cases where a larger aspiration volume is needed, a customized extension can be added between the valve and the tip. This results in an increase in the minimum dispense volume from 8 to 10 nL up to 50 to 100 nL.

Summary

The following table shows the compatibility of various solvents and reagents:

SOLVENTS & REAGENTS	COMPATIBLE
Aqueous	
Water	Yes
PBS	Yes
Buffers pH 3 to 10	Yes
BSA	Yes
Surfactants	Yes
Polar Organics	
Acetone	Yes
Ethyl Acetate	Yes
Methyl Ethyl Ketone	Yes
Dimethyl Sulfoxide (DMSO)	Yes
Isopropanol	Yes, Short Term*
Methanol	Yes, Short Term*
Non-Polar Organics	
Hexane	No
Toluene	No
Methylene Chloride	No

*Less than 1 hour. For specific solvents not listed here, please inquire as to possible testing of the solvent.

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