User's Guide

mPrep™ ASP-1000™

Automated Specimen Processor
for Electron Microscopy Applications

VERSION 1.0

MICROSCOPY ™
INNOVATIONS LLC

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ORIGIN

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EDITIONS

Version 1.0, November 5, 2020

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CHAPTER 1.
Getting Started

Welcome to the mPrep™ ASP-1000™, your automated biological specimen processor for electron microscopy applications. In addition to freeing lab technicians to work on other projects, the ASP-1000

- enables one-touch specimen handling that maintains biological specimen integrity.

- decreases specimen processing time.

- provides walk-away automation.

- decreases the amount of technician exposure time to toxic chemicals.

- ensures consistency and quality in processing.

ASP-1000 Component Identification & Specifications

Fume Enclosure

Z -arm

Pump Module, Syringes
(power switch - back, upper right)

HEPA Filter Port

Laptop Controller running ASP-1000 Software

Exhaust Port

Base Unit
(power switch - back, upper right)

Status Indicator

8- channel Head

Exhaust Fan

Deck, 6 Plate Positions

mPrep ASP-1000 Specifications	
Base Unit	Approx. dimensions with fume enclosure (W x D x H): 48.3 cm x 61 cm x 63.5 cm (19" x 24" x 25")
	Weight: 34 kg (75 lbs.)
	6 ANSI/SLAS format microplate-style reservoir capacity
	Integrated fume enclosure
	8-channel pipettor-style head
Pump Module	Dimensions (W x D x H): 20.3 cm x 20.3 cm x 30.5 cm (8" x 8" x 12")
	Weight: 7.25 kg (16 lbs.)
Exhaust Fan	Dimensions (W x D x H): 20.3 cm x 25.4 cm x 20.3 cm (8" X 10" x 8")
Laptop Controller	Dimensions (W x D x H): 25.4 cm x 35.6 cm x 2.54 cm (10" x 14" x 1")
	Weight: 1.6 kg (3.5 lbs.)
	Windows 10, solid-state drive
	ASP-1000 controller software
Power Requirements	100-240 VAC, 47-63 Hz
Net Weight	43.1 kg (95 lbs.)

Labware & Accessories

The instructions in this guide refer to mPrep capsules, filter couplers and reagent reservoir plates. This labware is required for operation of the ASP-1000. You can find these items and other common accessories in the table below. For the most up-to-date product list, see www.microscopyinnovations.com.

Filter Couplers	Unit	Cat. No.
mPrep/f30 Standard Filter Couplers, 16/pk	Pk	31500
Capsules for Specimen Processing		
mPrep/s Capsule Pack *8 capsules, 12 screens, 8 blank label sets, in storage box*	Pk	22200
mPrep/s Capsules - 96-count *96 capsules, screens & blank label sets*	Bag	22500
Capsules for Grid Processing		
mPrep/g Capsule Pack *16 capsules, with blank label sets, in storage box*	Pk	21300
mPrep/g Capsules - 96-count *96 capsules, screens & blank label sets*	Bag	21500

Reagent Reservoir Plates	Unit	Cat. No.
12-channel Reagent Reservoir Plate, polypropylene, single-pack	Ea	52501
12-channel Reagent Reservoir Plates, polypropylene, 5/sleeve	Slv	52503
12-channel Reagent Reservoir Plates, polypropylene, 25/case	Cs	52502
96-well Square-well Plates, 1.2ml, polypropylene,10/sleeve	Slv	51010
96-well Square-well Plates, 1.2ml, polypropylene,100/case	Cs	51011
96-well Plates, 500uL, polypropylene, 10/sleeve	Slv	51001
Plate Seals		
X-Pierce Cross-cut Vinyl Sheets, self-adhesive, pierceable, 100/box	Box	53010
Pierce Heat Seals*, foil heat sealing sheets, pierceable, 100/pk	Pk	53070
Accessories		
mPrep/Bench™ 96-well Silicone Rack	Ea	34000
mPrep/s Insertion Tool	Ea	32010
mPrep/s Workstation	Ea	42100

* For applying Pierce Heat Seal sheets, we recommend the 4titude 4s3™ Semi-Automatic Sheet Heat Sealer.

Equipment Placement & Installation

- The ASP-1000 is designed with an integrated fume enclosure for venting vapors safely. Place the ASP-1000 in a location where the venting apparatus can be attached to a fume removal system to duct vapors outside.

- If you decide to locate the ASP-1000 within a fume hood, it is acceptable to have an ASP-1000 field service engineer remove the ASP's fume enclosure.

- Position the ASP-1000 on a level surface indoors.

- Ensure grounded 110V power supply and power switches are accessible.

- Allow 10 cm (~4") space on each side of the ASP-1000 for air flow.

- If your lab is susceptible to power disruptions, use an uninterruptible power supply (UPS) to plug in all devices associated with the ASP-1000.

- The ASP-1000 controller software will require minimal access to your institution's public wi-fi network to log on as a guest and install the most recent version.

ASP-1000 Software

The ASP-1000 software installs files in the following locations:

Component	Location *
ASP-1000 Software	C:\Program Files\mPrep
Protocols	C:\Users\LAPTOP_NAME\mPrep\mPrep Protocol Files
Macros	C:\Users\LAPTOP_NAME\mPrep\mPrep Protocol Files
Operators	C:\Users LAPTOP_NAME\mPrep\mPrep Operators.txt
Tray Files	C:\Users\LAPTOP_NAME\mPrep\mPrep Tray Files
Protocol Releases	C:\Users\LAPTOP_NAME\mPrep\mPrep Releases
Preferences	C:\Users\LAPTOP_NAME\mPrep\mPrep Preferences.txt
Log Files	C:\Users\LAPTOP_NAME\mPrep\mPrep Log Files

where LAPTOP_NAME is defined during installation by the ASP-1000 field service engineer.

Note: The ASP-1000 software will not allow you to save files elsewhere.

ASP-1000 Workflow

In general, the broad categories of tasks below represent the workflow to incorporate the ASP-1000 into a lab's EM biological specimen preparation process. However, depending on the particular application (for example TEM, immunolabeling, or 3DEM) and your lab's best practices, the workflow may vary.

Start the ASP-1000 and Select Your Protocol

Power on the ASP-1000. For more information, see Starting the ASP-1000 in Chapter 2.

Log in to the laptop controller, and select and review the protocol you want to run. For more information, see Selecting a Protocol in Chapter 2.

Load Your Reagents

Load the correct reagents into the appropriate microplate or reservoir position per the tray map in the selected protocol. For more information, see Loading Reagents in Chapter 2.

Place the reagent plates onto the ASP-1000 deck, sliding them into the correct positions.

Load Your Specimen Capsules

Load your specimen into the mPrep capsule appropriate for your application. For more information, see Appendix C: Loading mPrep Capsules.

Store loaded capsules short-term in a mPrep/Bench™ 96-well silicone rack. Once you begin running a protocol, the software will prompt you to mount the capsules on the 8-channel head.

Run the ASP-1000

Click the Run button in the ASP-1000 software on the laptop controller. For more information, see Running a Protocol in Chapter 2.

Protocol Information and Safety

- Read ASP-1000 User's Guide before use of the instrument.

- Use eye protection at all times.

- Wear gloves and take standard precautions as you handle samples.

- Observe chemical safety requirements when using this instrument

- To ensure that the exhaust fan is working, check that the telltale flag hanging from the exhaust port is pulled in the direction of the airflow.

- Do not use aluminum plate sheets with osmium, as a reaction will occur.

- Do not use adhesive plate seals with acetone or ethanol, as these chemicals will dissolve the adhesive.

- Dispose of used reagents per your institution's safety protocols.

Online Help

The ASP-1000 software has built-in hover tips for real-time help. Place the cursor over an item within the software window to display a help box describing its function.

Technical Support

Microscopy Innovations technical support scientists have the necessary laboratory and analytical experience to respond to your inquiries.

Telephone   (715) 384-3292
                 Monday - Friday, 9:00am to 5:00pm US Central Time

Email   info@microscopyinnovations.com

CHAPTER 2.
Basic Instructions: Operator

This chapter describes how to use the mPrep ASP-1000 system to prepare samples for electron microscopy applications. The ASP-1000 has two modes: developer and operator. Developer mode is reserved for your on-site instrument programmer and is covered in Chapter 4. One person is delegated as developer. For day-to-day running of established protocols on the ASP-1000, you will use Operator mode. Multiple users can be set up as operators.

The ASP-1000 is designed to be another pair of hands in your lab. Once trained, lab personnel will find the ASP-1000 straightforward to use to ensure consistent processing and eliminate technician-to-technician variability.

Starting the ASP-1000

Note: Information on the location of power switches can be found in ASP-1000 Component Identification & Specifications in Chapter 1.

➲ **To start the ASP:**

1. Flip the power switch on the ASP-1000 base unit to the ON position. The Z-arm will home itself (0, 0, 0), if necessary, and the instrument status indicator light will illuminate blue.

2. Flip the power switch on the pump assembly to the ON position.

3. On the laptop controller, press the power button to start the computer.

4. Depending on your ASP-1000 model, you may need to flip the power switch on other ASP-1000 components to the ON position (for example, the exhaust fan).

5. Start the ASP-1000 software by clicking the program icon ⬤, located on the desktop and/or in the taskbar of the laptop controller. The ASP-1000 Dashboard window displays.

6. Click the Operator button, select your name from the drop-down list, and click the OK button. The ASP-1000 Operator window opens, displaying your name in the upper left corner under Operator Name.

Getting to Know the ASP-1000 Operator Window

Operator Name

Displays a drop-down list of all operators authorized to use the ASP-1000. Your on-site ASP developer has set up authorized operators previously within developer mode.

Select Your Protocol

Displays a drop-down list of all protocols released by the developer to the individual selected under Operator Name.

Show in Browser

Displays the Tray Map for the selected protocol in your default browser window. This action is useful when you want to print a Tray Map as a visual aid to ensure and record proper reagent location on the ASP-1000's deck.

Software Status Indicator

Displays a configurable light indicating the current status of the software. Under default programming conventions:

Red   =   Manual intervention needed
Green   =   Running
Blue   =   Standby

Action Commands

Run: Starts the ASP-1000 at the currently selected step within the protocol listing.

Pause: Pauses the ASP-1000 at the conclusion of the currently selected protocol step. If the current step includes repeats, the ASP-1000 will complete all before pausing and displaying the window:

Note: The software status indicator light retains the color associated with "Run" mode (by default, green). To continue running the protocol from the currently selected step, click the OK button. To stop immediately, click Stop, but do so after an aspiration step to ensure the specimen does not dry out.

Stop: Stops the ASP-1000 immediately, regardless of where it is within a step, and returns to the beginning of the protocol.

Duration Indicator

End of Step: Estimates the time in seconds that the current step will finish.

End of Protocol: Estimates the time in seconds that the entire protocol will finish.

Instrument Status Indicator

Displays a configurable light indicating the current status of the ASP-1000 instrument. This indicator color mirrors the color of the status indicator on the ASP-1000 itself (see ASP-1000 Component Identification & Specifications in Chapter 1). Under default programming conventions:

Red = Manual intervention needed
Green = Running
Blue = Standby

Tray Map

Displays the 6 reagent plates on the ASP-1000's deck and each one's contents for the selected protocol.

Manual Controls

Displays commands available to the operator should manual intervention be required mid-protocol. For example, you may need to stop the ASP-1000 when it is already running, if you forgot to fill a reagent plate or placed a plate on the deck in the wrong location. The manual controls enable you to correct an error mid-protocol, including moving the Z-arm out of the way to better access the ASP's deck.

Step Commands

Displays each step within the protocol.

Tip: If a step is visually truncated on the right side, hover the cursor over it to display the entire step.

```
8) Dialog Popup: Attach mPrep capsules to pi ▲
9) LED Color - Green
10) Select Plate 5 Row 1 z=100.000 mm
11) Pump Dispense speed 12 vol 100 ul hold 0.
12) Buffer  (sq-well plate) M-A-D Plate 5 Row 1 speed 15 vol 100 ul asp 0.000s dsp 0.000s rpt 75
13) Buffer 2 M-A-D Plate 5 Row 2 speed 15 vol
14) Buffer 3 M-A-D Plate 5 Row 3 speed 15 vol
15) Pump Dispense speed 0 vol 50 ul hold 0.50
16) Osmium (trough) M-A-D Plate 2 Row 3 spe
17) Pump Dispense speed 0 vol 50 ul hold 0.50 ▼
```

Selecting a Protocol

The ASP-1000 software requires your on-site developer to release a protocol to a specified operator before s/he can select it from the protocol list. Depending on an operator's training and experience, a developer may access only to select protocols. If a protocol is not available in your operator list, see your on-site ASP-1000 developer.

➲ **To select a protocol:**

1. In the Operator window, check the Operator Name in the upper left corner to ensure you are using the correct operator account. If not, click within the field to display a drop-down list and select the correct name.

2. Click the Select Your Protocol drop-down list and select the protocol you want to run.

```
Select Your Protocol
✓ 3D EM
  Immuno gold labeling
  kidney TEM
  liver TEM
  muscle TEM
  nerve TEM
  Startup
```

The tray map displays the reagent layout, and the protocol listing panel displays each step within the selected protocol.

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Loading Reagents

After you have selected a protocol in the ASP-1000 Operator window, the software displays the tray map, which indicates the reagents required per plate to perform the protocol and their location on the ASP-1000's deck. The ASP-1000 allows up to six SBS-format reservoir plates on its deck. Plate columns and rows are numbered as follows:

96 Round-well Plate

96 Square-well Plate

12-Column Reservoir Plate

When placing a reagent tray on the deck, 1A is always in the upper left.

➲ **To load reagents:**

1. Navigate to the ASP-1000 Operator window, and check the Operator Name in the upper left to ensure you are using the correct operator account. If not, click within the field to display a drop-down list and select the correct name.

2. Select the protocol of interest. For more information, see Selecting a Protocol earlier in this chapter.

3. Print the Tray Map for the protocol.

   a. Click the Show in Browser button in the ASP-1000 Operator window.

      The Tray Map displays on a new tab in your default internet browser window.

   b. Print from your internet browser window, or save as a PDF to a USB drive and print elsewhere.

   Note: Alternatively, you can use the layout provided in Appendix B.
   96-well Reagent Plate Template to hand write the reagents per column.

4. Locate or make the required reagents.

⚠ Warning: Use appropriate precautions when handling all reagents.

5. Using an alcohol-resistant marker or label maker, label the front of each plate with the required reagents prior to dispensing.

6. Dispense the required volumes of reagents into the plates, per the Tray Map.

We recommend sealing pre-filled 96 square-well (51011) and 12-column reservoir plates (52502) with Pierce Seal aluminum heat seals (catalog no. 53070) and 96 round-well plates (used for IEM; 51001) with X-Pierce adhesive vinyl plate seals (53010).

7. Slide the prefilled plates onto the deck of the ASP-1000, under the clips that hold each plate, into the correct positions per the tray map. For example, slide plate 1 into the upper left position (i.e., closest to the back panel of the base unit, left side) and plate 2 in the upper right position. Refer to the placement guide below:

8. Double-check that the reagent reservoir positions on the deck match the ones on the Tray Map.

Loading Specimens in Capsules

Note: The fixation method used to preserve biological specimens prior to EM specimen preparation is a key factor in the quality of the resulting EM images. We recommend Karnovsky's Fixative.

mPrep capsules are required for preparing specimens on the ASP-1000 and help prevent loss, misidentification, and damage. Two types of mPrep capsules are available, and which one you use depends on your application:

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mPrep/s (Specimen) Capsules

Securely hold biological specimens up to 3 mm^3 for TEM, SEM, and 3DEM specimen preparation and other applications. Capsules hold specimens through fixation, en bloc staining, dehydration, infiltration, embedding, and sectioning.

mPrep/g (Grid) Capsules

Holds grid for TEM grid staining, on-grid immunolabeling, or negative staining of macromolecules (DNA, RNA, protein).

➲ **To load specimens into the mPrep/s or mPrep/g capsules, see Appendix C: Loading mPrep Capsules.**

Running a Protocol

The ASP-1000 integrates seamlessly into your existing workflow. When the instrument is installed at your site, the ASP-1000 field service engineer will work with your on-site ASP developer to write or optimize protocols to align with your lab's practices (see Creating or Modifying Protocols in Chapter 4.)

We encourage users to share ASP protocols. For more information, see Sharing Protocols Between Labs in Chapter 4.)

➲ **To run a protocol on the ASP-1000:**

1. Make sure you have followed the actions described earlier in this chapter:

    a. See Starting the ASP-1000 in this chapter.

    b. See Selecting a Protocol in this chapter.

    c. See Loading Specimens in this chapter.

    d. See Loading Reagents in this chapter.

2. On the laptop controller, make sure the first step is selected in the Protocol Listing panel and click the Run Now button.

    The ASP-1000 will perform its homing routine, prepare to run the protocol, and prompt you to mount mPrep capsules preloaded with specimens.

3. Mount 1 to 8 capsules preloaded with specimens on the 8-channel head. To do so, push the open end of an mPrep capsule up onto a channel head and rotate counter-clockwise slightly until it fits securely.

Note the locations of A – H when mounting your capsules. Location A on the 8-channel head is closest to the back of the ASP-1000 unit, and location H is closest to the front.

Location A

Location H

Note: You can run the ASP-1000 with fewer than 8 capsules.

4. Click OK.

The ASP-1000 will run the selected protocol, prompting for user input or action as required.

Upon completion of the protocol, the software will prompt you to click OK to raise the Z-arm to make room to remove the capsules.

5. Click OK. The software will prompt you to remove the capsules.

6. Use your thumbnail to apply a gentle downward force on the lip of each capsule to pry it from the channel head, so it will fall into the mPrep/Bench silicone rack.

7. When all the capsules are removed, click OK. The ASP-1000 will finish running the protocol and return to the beginning, and the Z-arm will return to the home (0, 0, 0) position.

8. Remove the silicone rack from the ASP's deck.

9. For polymerization, choose one of the following:

    - If specific specimen orientation is required, polymerize per your existing SOP, for example in a flat mold or BEEM® capsule.

    - If polymerizing directly in the mPrep/s capsule, add resin to top off each capsule.

10. Place the flat mold or silicone rack in an oven at a temperature and time appropriate for your resin (e.g., 60°C for Embed 812).

Monitoring When a Protocol or Step Will Complete

➲ **To determine when a protocol or step will complete:**

On the laptop controller, open the ASP Operator window running a protocol.

The duration indicators specify the time the current step will complete as well as when the entire protocol will finish.

Pausing or Stopping the ASP-1000

You may need to pause or stop the ASP-1000 mid-protocol. For example, perhaps you forgot to fill a reagent plate or placed one in the wrong location on the ASP-1000 deck. The ASP-1000 allows users to pause the equipment temporarily and then restart it at its current location within a protocol. Or you may want to stop the ASP-1000 from running the remainder of the protocol altogether.

The ASP-1000 provides manual controls that enable you to correct an error mid-protocol, including moving the Z-arm and 8-channel head out of the way to better access the ASP's deck. To locate the manual controls, see Getting to Know the ASP-1000 Operator Window in this chapter.

Note: You can use the manual controls only when the ASP-1000 is stopped. These controls are not available if the ASP-1000 is paused.

⮕ To pause the ASP-1000 and correct an error on its deck, choose one of the following:

- If the Z-arm is **not** obstructing your access to reagent plates on the ASP-1000 deck:

  1. Click the Pause button.

     The ASP-1000 finishes the current step before pausing; for example, if a step has 500 repeats, the ASP-1000 will perform all 500 actions prior to pausing. The Z-arm will raise but stay in its current x and y planes, and the software will display a window requesting action to continue to the next step.

  2. Make adjustments on the ASP-1000 deck as necessary.

  3. When ready, click the OK button in the software window to continue running the protocol from the point where it left off.

- If the Z-arm is obstructing your access to reagent plates on the ASP-1000 deck:

  1. Wait for an aspiration step to complete to ensure the specimen does not dry out, then click the Stop button. The ASP-1000 immediately stops, and the Z-arm returns to home (0, 0, 0) position.

2. Use the manual controls as necessary to correct the error:

Zero Z
Moves the Z-arm to the 0 (top) z coordinate.

Home Stage
Moves the Z-arm to the home position (back left, or 0, 0, 0).

Return Z
Returns the Z-arm and the 8-channel head to its position within the protocol step where you stopped the program. You will select this command prior to continuing on with the protocol.

Aspirate
Draws in the amount of reagent you indicate in the Amount slider.

Dispense
Expels the amount of reagent you indicate in the Amount slider.

Switch to Input
Moves the 8 pump valves to ambient air.

Switch to Output
Moves the 8 pump valves into position to aspirate or dispense from the 8-channel head.

3. After fixing the error, click the Return Z button. This action brings the Z-arm back to the location where the ASP-1000 was stopped initially.

4. Click the Run Now button. The ASP-1000 will continue running from the protocol step where it had stopped.

➲ **To stop the ASP-1000:**

Click the Stop button in the Operator window. The ASP-1000 stops running immediately, regardless of the step or number of repeats, and returns to the beginning of the protocol. The Z-arm returns to the home (0, 0, 0) position.

Note: You may want to wait for an aspiration step to complete before stopping the ASP-1000 to ensure the specimens do not dry out.

Cleaning Up and Shutting Down

The ASP-1000 offers fast and easy clean-up following specimen preparation.

➲ **To clean up the ASP-1000:**

1. Dispose of leftover reagents and plates on the ASP's deck per your lab's standard SOP.

   ⚠ Warning: Use the appropriate precautions when handling all reagents.

2. After all plates are removed, wipe down the ASP's deck using a damp cloth.

3. Turn off the ASP-1000 base unit, pump assembly, and exhaust fan (see ASP-1000 Component Identification & Specifications in Chapter 1).

4. On the laptop controller, select File -> Exit.

The ASP-1000 software application closes.

5. Shut down your laptop using on-screen prompts.

   Note: The power button, screensaver, and sleep functions have been disabled to prevent inadvertent power down of the ASP-1000.

CHAPTER 3.
Global Preferences & Settings

You can configure several mPrep ASP-1000 settings as preferences, which are then applied when running any protocol. These preferences, configured within developer mode, set up default settings and include:

- Authorized operators trained to use the ASP-1000

- Access to specific protocols

- Aspirate/dispense parameters

- Developer connectivity credentials

- Default tray file

- Default z reagent tray settings, which configure the z coordinate (depth) of reagent 12-column plates, 96 round-well plates, and 96 square-well plates.

Note: Some of these preferences, such as the z coordinate and aspirate and dispense settings, can be individually adjusted when writing or editing a protocol.

Setting Global Z Coordinates

The ASP-1000 field service engineer defines the x and y coordinates for your ASP during installation, which become fixed parameters you will not need to adjust under normal circumstances. However, you may need to modify the z coordinate (depth), which is plate specific. The z coordinate is measured in millimeters (mm), with 0 being at the top and 106 as the max depth.

➲ To set global z coordinates:

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in Chapter 4.

2. Click the Edit Tray Files button. The Tray File Editor window opens, displaying the currently loaded (default) tray file, the location for loading and unloading capsules as well as the default x, y, and z coordinates for plates 1 through 6.

Note: Do not modify the x or y coordinates. These values have been set and tested by the ASP-1000 field service engineer upon the ASP-1000 installation.

3. Edit the z coordinate values per your plate and reagent. The z dimension runs vertically, up from and down toward the deck of the ASP-1000 and range from 0 (top) to 106 (bottom). Typical depths are:

   12-column plate: 106
   96 square-well plate: 100
   96 round-well plate: Reagent level dependent

4. Choose one of the following and follow the on-screen prompts:

   • Click the Save button to save your changes to the current filename.

   • Click the Save As button to save the file under a new name.

   The file with your updated z coordinates is saved in the following directory: C:\Users\LAPTOP_NAME\mPrep\mPrep Tray Files.

Adding or Removing an Operator

Because it takes skill and training to do quality sample preparation for electron microscopy, the ASP-1000 provides control over who can use the instrument. The on-site ASP developer can add and remove ASP operators. The ASP-1000 provides further control by enabling the on-site ASP developer to specify which protocols have been released to an operator, ensuring the user has been appropriately trained before using the instrument. For more information, see Granting or Removing Access to a Protocol Program in this chapter).

⮕ **To add a new operator to run protocols on the ASP:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in Chapter 4.

2. Click the Edit Operators button. The Operator Editor window displays.

3. For each individual trained to operate the ASP-1000, complete the following fields:

    Operator Name
    Enter the name of the individual granted access to run the ASP-1000.

    TLA
    Enter an abbreviation up to three letters for the operator. This abbreviation will be used within the log file.

    SMS Number
    (optional) Enter the operator's 10-digit cell phone number, using no spaces, symbols, or punctuation. For example, 9876543210 is correct, but (987) 654-3210 is not. The ASP-1000 will use this number to send alerts via text message, if programmed as a step within a protocol. To enable this feature, the laptop controller must be connected to your institution's wi-fi.

    SMS Carrier
    Enter the service provider for the operator's cell phone, for example AT&T, Verizon, Sprint, etc.

4. Click the Save button. This action saves the file in the following directory: C:\Users\ LAPTOP_NAME\mPrep.

Granting or Removing Access to a Protocol Program

The ASP-1000 ensures data quality and reproducibility by eliminating variability among laboratory technicians. Because technicians still require training to use the ASP-1000 with specific protocols, the ASP-1000 provides control over which protocols are made available to each operator.

⊃ To grant or remove access to a protocol program:

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in Chapter 4.

2. Click the Edit Releases button. The Release Editor window opens.

    Predefined users are listed as column headings. If you do not see a particular operator listed, see Adding or Removing an Operator in this chapter.

    Predefined protocols are listed as rows. If you do not see a particular protocol listed, make sure all protocols are saved to the directory C:\Users\LAPTOP_NAME\mPrep\mPrep Protocol Files and have a *.txt extension.

3. Click the corresponding cell of each protocol to select or deselect it as being available to a predefined operator. Selected cells will highlight in green.

4. Click the OK button to save and exit the window.

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Setting Default Aspirate and Dispense Preferences

A common, frequently used step in any ASP-1000 protocol is the "Move – Aspirate – Dispense" (MAD) command. You can set default values for each of the actions in this step using the Edit Preferences window.

Note: A developer can override these default settings in a particular protocol within the Edit tab. For more information, see Creating or Modifying Protocols in this chapter.

⮌ **To set default aspirate and dispense preferences:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in Chapter 4.

2. Click the Edit Preferences button. The Preferences Editor window opens.

3. Modify the pump parameters as necessary:

    Speed
    Sets the pump speed. Values range from 0 to 40, where 40 is the slowest. The default value is 12.

    Volume (ul)
    Sets the default volume of reagent to be aspirated or dispensed.

    Asp. Hold (s)
    Sets the default hold time, in seconds, after aspiration.

    Disp. Hold (s)
    Sets the default hold time, in seconds, after dispensing.

    Repeats
    Sets the default number of times the reagent is aspirated and dispensed during that step.

4. Click the Save button to save your default parameter changes. The Save to Preference window displays.

5. Click OK to confirm and close the Save Preferences window.

    Your preferences are saved in C:\Users\ LAPTOP_NAME\mPrep\mPrep Preferences.txt.

Viewing Developer Account Preferences

The ASP-1000 can send text messages to an operator, for example to monitor the system for tracking purposes or at points in a protocol where manual intervention may be required. To do so, during installation the ASP-1000 field service engineer will set up a specific ASP-1000 administrative email account and password. The ASP-1000 uses this email account to send SMS messages to an operator's phone, if programmed as a step within a protocol. To enable this feature, the laptop controller must be connected to your institution's wi-fi.

➲ **To view developer account preferences:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in Chapter 4.

2. Click the Edit Preferences button. The Preferences Editor window opens, displaying the email account name. The password remains hidden to prevent inadvertently changing it.

⚠ Warning: Do not modify this account information or password, and do not log into another Gmail account on the ASP laptop controller. If you do, the ASP-1000 text messaging capability will not work.

Selecting a Default Tray File for Use with Protocols

Different protocols may require different reagent plates, for example 12-column plates versus 96-well plates, or a combination of the two. If so, the z coordinate will change according to the depth of the reservoir per the different plates. To control the Z-arm and then save your changes as a tray file to use at a different time, see Setting Global Z Coordinates in this chapter.

The default tray file is specified within Developer mode. The ASP-1000 uses the default tray file with all protocols until you select a different tray file within the developer's Edit Preferences window.

⮞ **To select the default tray file:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in Chapter 4.

2. Click the Edit Preferences button. The Preferences Editor window opens.

3. Click within the Tray File Name field. The File Picker window opens.

4. Click the Pick a File from the List drop-down menu, select a file, and click the OK button.

The selected tray filename appears in the Preferences Editor window.

5. Click the Save button and confirm the preference change.

6. Click the OK button to exit the Preferences Editor window.

CHAPTER 4.
Advanced Operation: Developer

The mPrep ASP-1000 gives labs total control over their automated methods used to prepare samples for electron microscopy. At installation, the ASP-1000 field service engineer will help you implement your lab's protocols on the ASP. The ASP provides the flexibility to easily modify protocols as necessary to conform to best practices in your lab or to program your own protocols from scratch. In either scenario, the ASP-1000 frees lab technicians to work on other projects while it performs repetitive yet critical steps for quality EM sample preparation.

One individual at a site is designated as the ASP-1000 developer. The developer has advanced ASP privileges, and this chapter describes how to use this mode to create new or modify existing protocols, define the operators who can use the ASP, and designate which protocols an operator has access to run.

Accessing Developer Controls

➲ **To access ASP-1000 developer controls:**

1. Start the ASP-1000 software by clicking the program icon ⬤, located on the desktop and/or in the taskbar at the bottom of the laptop controller. The ASP-1000 Dashboard window opens.

2. Click the Developer button. The ASP-1000 Developer window opens.

Getting to Know the ASP-1000 Developer Window

The ASP-1000 developer window enables you to set ASP-1000 global parameters and provides a developer sandbox for creating, editing, and testing protocols before releasing them to designated operators for use in your lab.

DEVELOPER TABS

You will find three tabs that enable you to perform developer actions:

- Edit: Use this tab to write new and edit existing protocol methods within the sandbox.

- Scratchpad: Use this tab to program or test a single step in isolation. For example, you may want to test a particular step prior to adding it to an existing protocol.

- Run: Use this tab to verify the reagent tray setup on the ASP-1000 deck. As the software cycles through each protocol step, it highlights the reagent column in use.

GLOBAL PARAMETERS

The global parameter buttons appear at the top of the Developer window and are visible regardless of what developer tab is selected. These buttons enable you to define who can operate the ASP-1000, which protocols s/he can access, default pump values, and the x, y, and z coordinates that may vary between different reagent plates. For more information, see Chapter 3. Global Preferences & Settings.

DEVELOPER SANDBOX

The developer sandbox commands and parameters are available in both the Edit and Scratchpad tabs. These commands enable you to create and modify protocols. For more information on specific commands, see Appendix D. Step Commands & Parameter Settings.

Protocol Commands

Step Commands: Displays a drop-down list of available commands for controlling the ASP-1000. For a description of each command, see Appendix D. Step Commands & Parameter Settings.

Step Comment: Attaches an optional comment to display with the current step. The Step Comment is most useful for the Move-Asp-Disp action to list the reagent being used on the tray map.

Command Parameters: Only those parameters specific to the selected Step Command are made available for modification. Those parameters not applicable appear grayed out.

Duration Indicators

Step: Displays a progress bar visually indicating the length of time remaining in the current step to finish.

Step (s): Displays the number of seconds remaining for the current step to finish.

End of Step: Displays the time at which the current step will finish.

Protocol: Displays a progress bar visually indicating the length of time remaining for the protocol to finish.

Protocol (s): Displays the number of seconds remaining for the entire protocol to finish.

End of Protocol: Displays the time at which the protocol will finish.

Current Protocol File:

Displays the file name of the current protocol loaded in the Developer window.

Show Protocol in Browser:

> Displays the current protocol in your default browser window for saving and/or printing.

Instrument Status Indicator

> Displays a programmable colored light indicating the current state of the equipment. This indicator color mirrors the color of the Status Indicator on the ASP-1000 itself (see ASP-1000 Component Identification & Specifications in Chapter 1).
>
> As a developer, you can customize the colors the ASP-1000 displays when running a protocol. Under default programming conventions:
>
> Red   =   Manual intervention needed
> Green   =   Running
> Blue   =   Standby

File Actions

> **New**: Opens a new, empty protocol file.
>
> **Open**: Displays a file selection window, from which you can select and open an existing protocol file.
>
> **Save**: Saves the current protocol
>
> **Save As**: Displays a file selection window, from which you can save the current protocol under a different filename.

Edit Actions

> **Copy Step**: Copies the currently selected step.
>
> **Insert Step**: Inserts a new step below the currently selected step. The software inserts a No-Operation step if the clipboard is empty, or if you previously copied a step to your clipboard, inserts that step.
>
> **Delete Step**: Deletes the currently selected step.

Override Repeats: Displays the Override Repeats wiindow, which allows the developer temporarily to specify a different number of repeats for a step. This action is useful when you want to test a protocol, using a reduced number of repeats to save time. As a visual reminder, the Override Repeats button turns yellow when it is on; to turn it off, click the Override Repeats button again.

Run Actions

Run Now: Runs the protocol from the selected step onward.

Pause: Pauses the ASP-1000 at the conclusion of the current protocol step. If the current step includes repeats, the ASP-1000 will complete them all before pausing and displaying the window:

To continue running the protocol from the current step, click the OK button.

Stop: Stops the ASP-1000 immediately and returns to the beginning of the protocol.

Software Status Indicator

Lights are programmable. Under default programming conventions:

Green   =   Running
Blue   =   Standby

Creating or Modifying Protocols

During the installation of your ASP-1000, the ASP-1000 field service engineer will work with you to either write new protocols to match your existing SOP or to modify any protocols the company provides to correspond to your lab's best practices and procedures. This section details how to use the developer sandbox within the Edit and Scratchpad tabs to create or modify a protocol.

Note: It is often easier to modify an existing protocol than to start completely from scratch to program a new protocol. See Tips for Creating a New Protocol in this chapter.

To create a new protocol:

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in this chapter.

   The Developer window displays with the Edit developer sandbox tab open.

2. Click the New button. The Protocol Listing displays the step 0) No-Operation.

3. With No-Operation selected in the Protocol Listing, click the Step Command drop-down list, select an action, and specify the action's parameters. For more information, see Appendix D. Step Commands & Parameter Settings.

4. Repeat step 3 to build the steps of the protocol.

   Note: To create a macro for a series of steps that may be repeated within a single protocol or between protocols, see Creating and Using Macros in this chapter.

5. (Optional) To test a single step, a macro, or an entire protocol, see Testing Steps, Macros, and Protocols in this chapter.

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6. At any point in your progress, click the Save As button to save the new protocol under a new filename. A file selection window opens.

7. Type a new name for the protocol in the File Name text box and click the OK button.

   Note: The software will allow you to save the file only in C:\Users\ *LAPTOP*_NAME\mPrep\mPrep Protocol Files.

➲ **To edit an existing protocol:**

1. Click the Open button. A file selection window opens, listing all available protocol files.

   Note: By default, the ASP-1000 recognizes only those protocols saved in C:\Users\ *LAPTOP*_NAME\mPrep\mPrep Protocol Files with a *.txt extension.

2. Select the protocol of interest and click the OK button. The selected protocol loads in the Edit tab of the developer sandbox, displaying the protocol name in the Current Protocol File field in the lower left corner. The steps of the protocol display in the Protocol Listing panel.

3. Choose one of the following:

   • To insert a new step:

     a. Select the step within the Protocol Listing panel after which you want to insert a new step.

     b. Click the Insert Step button. The software inserts a No-Operation step if the clipboard is empty, or if you previously copied a step to your clipboard, it inserts that step.

     c. With the step selected, click the Command drop-down list, select a new ASP command, and edit its parameters as necessary. For more information, see Appendix D.

- To copy a step:

  a. Select the step within the Protocol Listing panel that you want to copy, and click the Copy Step button.

  b. Select the step within the Protocol Listing panel after which you want to insert the copied step.

  c. Click the Insert Step button. The step is inserted into the Protocol Listing.

- To modify a step:

  a. Select the step within the Protocol Listing panel that you want to modify.

  b. Click the Step Command drop-down list and select a new command.

  c. Adjust the new command's parameters as necessary.

- To delete a step:

  a. Select the step you want to delete within the Protocol Listing panel.

  b. Click the Delete Step button.

4. At any point, save the protocol file. Choose one of the following:

- Click the Save button to save the protocol under the existing filename.

- Click the Save As button to save the protocol under a new filename, and follow the system prompts to do so.

Note: By default, the software allows you to save protocol files only in C:\Users\ LAPTOP_NAME\mPrep\mPrep Protocol Files.

TIPS FOR CREATING A NEW PROTOCOL

- Start all protocols by homing the stage and the pump. These actions place the Z-arm into the correct starting position and center the pistons in the syringes. For example:

  0) LED Color - Green
  1) Home Stage
  2) Home Pump

- Use the Pump Valve Input and Pump Valve Output parameter settings to move the plungers into the correct positions (syringe volume is 1 ml, thus aspirate 500 μl to position the piston in the middle of the syringe). The piston should be in the center of the syringe. For example:

  3) Pump Valve Input
  4) Pump Aspirate speed 0 vol 500 μl hold 0.00 s
  5) Pump Valve Output

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- For the ASP-1000 to alert you to load and unload the mPrep capsules, you must program those steps within the protocol. For example:

  6) Load/Unload X 0.000 mm Y 255.000 mm Z 9.000 mm
  7) LED Color – Red
  8) Dialog Popup: Attach mPrep Capsules to pipettor shaft and press 'Enter'.
  9) LED color - Green

- For each Move-Asp-Disp step in the protocol, type the reagent name or its use in the Step Comment text box. This text will appear in the Tray Map, which can be accessed in the Run tab within the Developer window or via the Show in Browser button in the Operator window.

Viewing a Protocol

The ASP-1000 makes it easy to view and read through a protocol in its entirety, as the steps are written as you see them in the Protocol Listing panel. To view a protocol, display it in a web browser window.

➲ **To view a protocol:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in this chapter.

   The Developer window displays with the Edit developer sandbox tab open.

2. Click the Open button. A file selection window opens, listing all available protocol files.

   Note: By default, the ASP-1000 recognizes only those protocols saved in C:\Users\LAPTOP_NAME\mPrep\mPrep Protocol Files with a *.txt extension.

3. Select the protocol of interest and click the OK button.

   The selected protocol loads in the Edit tab, displaying the protocol name in the Current Protocol File field in the lower left corner. The steps of the protocol display in the Protocol Listing panel.

4. Click the Show Protocol in Browser button. The protocol displays in your default web browser window within a new tab for easy viewing and printing.

LVtemp20200831162320_0_0.htm   +

← → C   ⓘ File   C:/Users/jolee/AppData/Local/Temp/LVtemp20200...

ASP-1000 Protocol Listing: kidney TEM

0) LED Color - Green
1) Home Stage
2) Home Pump
3) Load/Unload
4) LED Color - Red
5) Dialog Popup: Load capsules & specimens onto the 8-channel head
6) No-Operation
7) LED Color - Green
8) No-Operation
9) Buffer (sq-well plate) M-A-D Plate 5 Row 1 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 75
10) Buffer 2 M-A-D Plate 5 Row 2 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 60
11) Buffer 3 M-A-D Plate 5 Row 3 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 60
12) Send SMS: Josie Smith (9871234567, Verizon) ()
13) No-Operation
14) Osmium (trough) M-A-D Plate 2 Row 3 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 750
15) No-Operation
16) OS Rinse 1 (water) M-A-D Plate 5 Row 4 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 75
17) OS Rinse 2 (water) M-A-D Plate 5 Row 5 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 60
18) OS Rinse 3 (water) M-A-D Plate 5 Row 6 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 60
19) No-Operation
20) UA 2% (trough) M-A-D Plate 2 Row 10 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 750
21) UA rinse 1 (water) M-A-D Plate 5 Row 7 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 75
22) UA rinse 2 (water) M-A-D Plate 5 Row 8 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 60
23) UA rinse 3 (water) M-A-D Plate 5 Row 9 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 60
24) No-Operation
25) 50% Acetone (sq. well plate) M-A-D Plate 3 Row 1 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 100
26) 70% Acetone M-A-D Plate 3 Row 2 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 100
27) 80% Acetone M-A-D Plate 3 Row 3 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 100
28) 95% Acetone M-A-D Plate 3 Row 4 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 100
29) No-Operation
30) 100% Ace 1 M-A-D Plate 3 Row 5 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 120
31) No-Operation
32) 100% Ace 2 M-A-D Plate 3 Row 6 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 120
33) No-Operation
34) 100% Ace 3 M-A-D Plate 3 Row 7 visc. 15 vol 100 ul asp 0.000s disp 0.000s rpt 120
35) No-Operation
36) 25% resin M-A-D Plate 6 Row 1 visc. 25 vol 100 ul asp 0.000s disp 0.000s rpt 30

Creating and Using Macros

You may find there are multi-step, repeated actions within a protocol or between protocols. Instead of adding each action as a separate step, you can save these actions together as a macro, which you then can insert within a protocol as a single step. Macros are like mini-protocols and can aid in creating consistency both within and across lengthier protocols.

➲ **To create a macro, follow the steps in Creating or Modifying Protocols in this chapter.**

You create and save macros just as you do a regular protocol. To avoid confusion, you may want to devise a file naming convention to separate macros from full protocols, as they are saved in the same directory. For example, you may want to include "macro" in the filename, i.e. Startup_Macro.txt.

Note: By default, the software allows you to save macro files only in C:\Users\ LAPTOP_NAME\mPrep\mPrep Protocol Files.

➲ **To use a macro file within a protocol:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in this chapter.

2. Choose Select Macro File from the Step Command drop-down list. A file selection window displays.

3. Select the correct macro filename and click the OK button. The macro filename appears as a single step within the protocol listing.

Note: The software will display all files with a *.txt extension located in the C:\Users\LAPTOP_NAME\mPrep\mPrep Protocol Files directory, which will include previously saved macro files as well as full protocol files. If you do not select a filename, the software automatically creates a file called Startup.txt, which contains no content, to use within that step of the protocol.

Configuring Notices of Required Operator Input

While the ASP-1000 is automated, there may be points within a protocol where manual intervention is needed. The ASP-1000 can notify an operator in four ways.

- Status indicator light on the ASP-1000. By default, the color is blue.

- Sound. The laptop controller will sound an audible alert.

- Software popup alert. The ASP-1000 software will pause and display a window notifying the operator of the type of input required.

- Text message. The ASP-1000 can send a text message to any operator's cell phone with a notification of the required operator input. To enable this feature, the laptop controller must be connected to your institution's wi-fi.

➲ **To configure notices of required operator input:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in this chapter.

   The Developer window displays with the Edit developer tab open.

2. Click the Open button. A file selection window opens, listing all protocol files.

3. Select the protocol of interest and click the OK button. The selected protocol loads in the Protocol Listing panel of the Edit tab.

4. Click on the step after which you want to insert a notice of required user input and click the Insert Step button. The No-Operation command is inserted.

5. Select No-Operation and choose one of the following:

- **Instrument Status Indicator light**

    a. Select the LED Color action in the Step Command drop-down list. A color picker window displays.

The Instrument Status Indicator matches the LED color in the developer sandbox, which can be customized in the color selection window.

    b. Place the cursor in any of the three color bars. As you move the cursor, the rectangle in the lower left corner of the window displays the color at the cursor point.

    Note: The specific RGB values of the color at the cursor point display at the bottom of the color picker window.

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c. To select a color, choose one of the following:

Note: Some colors work better on the LED than others. Be sure to observe how they look to your eye on the LED indicator light and do not rely on the color on your laptop screen.

- Click within one of the three color bars. The window closes and the color selected displays as the LED Color parameter.

- Select a color used previously by clicking a swatch under History. The window closes and the color selected displays as the LED Color parameter.

- To select a specific color palette, RGB (red, green, blue), or HSL (hue, saturation, luminosity) color:

    i. Click the painter's palette button [🎨...] in the lower right corner of the color picker window. The Color window displays.

    ii. Choose one of the following:

        - Select an existing swatch under Basic Colors.

        - Click and move the cursor within the rainbow canvas square to find a specific color.

        - Type the specific RBG or HSL values of your choice.

        The large Color|Solid swatch displays the color selected.

    iii. (Optional) Click the Add to Custom Colors button to add the color to the Custom Color swatches.

    iv. Click the OK button. The Color window closes and the selected color is displayed as the LED Color parameter.

- **Sound.** Select Play Sound from the Step Command drop-down list.

Note: You can adjust the volume using F2 and F3 on the laptop controller.

- **Software Popup Alert**

    a. Select Dialog Popup from the Step Command drop-down list.

    b. Type the message you want to display in the Dialog Text parameter text box.

- **Text Message**

    a. Select Send SMS from the Step Command drop-down list. The SMS Picker window displays.

    b. Click Pick an Operator from the List to display a drop-down list and select an operator. For more information on entering default operator information, see Adding or Removing an Operator in Chapter 3.

    c. Click the OK button. The selected operator and his/her information automatically displays in the Operator, SMS Number, and SMS Carrier parameter text boxes.

    d. Type a message in the Dialog Text parameter text box to be sent to the operator's phone.

    e. Click the Save button.

Testing Steps, Macros, and Protocols

As you create new protocols and macros or edit existing ones, you may want to test the procedures to ensure they work as intended. Your testing may include:

- an individual protocol step or macro

- a portion of or an entire protocol, ignoring repeats

- the layout of reagents on the ASP-1000 deck

➲ **To test an individual protocol step:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in this chapter.

    The Developer window displays with the Edit developer sandbox tab open.

2. Click the Open button. A file selection window opens, listing all protocol files.

3. Select the protocol of interest and click the OK button. The selected protocol loads in the Edit tab of the developer sandbox.

4. Select the step you want to test within the Protocol Listing panel.

5. Click the Copy Step button.

6. Select the Scratchpad tab.

7. Click the Paste button. The copied command and its parameters are pasted into the Scratchpad Step panel.

8. Click the Run button to test the step and observe the ASP-1000.

9. (Optional) Modify the parameters as necessary. For more information, see Appendix D.

10. Repeat steps 8 and 9 until you are satisfied with the performance.

11. Click the Copy button.

12. Select the Edit tab to return to the full set of steps within the Protocol Listing panel.

13. Select the step within the Protocol Listing panel after which you want to insert the new step and click the Paste button. The copied step displays within the Protocol Listing panel.

14. Delete the step you replaced. To do so, select the step and click the Delete Step button.

➲ **To test a protocol and the layout of reagents on the ASP-1000 deck:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in this chapter.

    The Developer window displays with the Edit tab open in the developer sandbox.

2. Click the Open button. A file selection window opens, listing all available protocol files.

3. Select the protocol of interest and click the OK button. The selected protocol loads in the Protocol Listing panel of the Edit tab.

4. Select the step with which you want to start the testing.

5.   (Optional) Click the Override Repeats button. This button enables you to decrease the number of aspirate-dispense repeat actions, which take the most time when running a protocol. The Override Repeats window displays.

a.   In the Override Repeat Counts text box, specify the number of repeat actions you want applied to every aspirate and dispense action in the protocol listing panel.

b.   Click the OK button to close the window and return to the Edit tab. The Override Repeats button visually remains selected.

6.   Select the Run tab. The Run tab displays the layout of reagents on the 6 plates on the ASP's deck and the Protocol Listing panel.

7.   Click the Run button. The software runs through the protocol, highlighting in green the column of the reagent plate used in the respective steps of the protocol.

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Sharing Protocols Between Labs

The ASP-1000 enables researchers to easily share protocols between labs. You can do so via email, downloading from a cloud location, or copying from a physical storage device, such as a USB drive.

⮑ **To share a protocol:**

1. From the ASP-1000 laptop controller's desktop, navigate to the default protocol directory: C:\Users\ LAPTOP_NAME\ASP-1000\ASP-1000 Protocol Files

   *Note: The ASP-1000 recognizes only those protocols saved in this location with a *.txt extension.*

2. Choose from the following:

   - If you are sharing a protocol, copy the file from the ASP-1000 Protocol Files directory to a location from which you can share it, for example a USB drive or cloud storage, and email it to a colleague.

   - If a protocol is being shared with you, copy the file to the ASP-1000 Protocol Files directory.

     The ASP-1000 will recognize and display the *.txt file the next time the Open file selection window displays.

   To view the full protocol, see Viewing a Protocol in this chapter.

   To modify the protocol, see Creating or Modifying Protocols in this chapter.

Notes

CHAPTER 5.
Maintenance & Troubleshooting

The mPrep ASP-1000 should be cleaned on a regular basis and follow a routine maintenance schedule as laid out in this chapter.

Routine Maintenance

Normal standards of laboratory safety and routine maintenance procedures are necessary to keep the ASP-1000 in good, serviceable condition. Inspect the ASP-1000 for obvious damage whenever you clean or use it.

Always wipe up spills immediately. Take the necessary GLP safety precautions when you clean to protect yourself against the effects of chemicals.

Area	Item	Frequency	Action
Base Unit	Deck	After each processing run	Clear the deck of all plates and gently wipe with a soft, damp cloth. Do not use solvents.
	8-Channel Head	After each processing run	Gently wipe with a soft, damp cloth. Do not use solvents.
	Fume Enclosure	Monthly	Use cleaner provided. Avoid cleaners containing ammonia, e.g., Windex®, which may damage the surface.
	Filter Coupler	Approx. every 5 runs, depending on use	Replace when visibly black from osmium or showing other signs of contamination.
Pump Assembly	High-grade FEP Tubing	Monthly	Visually inspect. If damage found, replace.
	Syringes	Monthly	Visually inspect. If damage found, replace.
Deck	Silicone Rack	Quarterly	Visually inspect. Discoloration is indicative the rack is becoming brittle. Replace.
Laptop Controller	Monitor	Monthly	Gently wipe with a soft, damp microfiber cloth. Do not use solvents.
	Keyboard	Monthly	Unplug and turn off the laptop. With the laptop open, turn it upside down and tap gently or shake to dislodge debris. Set down right side up and use a microfiber cloth lightly dampened with water to wipe keyboard. To further remove debris from between keys, use a can of compressed air.

Annual Maintenance

To help keep your ASP-1000 in peak condition and running smoothly, we recommend scheduled maintenance at least once per year. Microscopy Innovations offers a service contract that is intended to help you avoid unnecessary downtime, reduce strain on your laboratory staff, and substantially extend the life of your ASP-1000. For more information, contact Microscopy Innovations.

ASP-1000 Software Installation & Updates

New versions of the ASP-1000 software are periodically released. Your on-site ASP developer will receive an email with instructions and a link to download and install any required software upgrades.

Adjusting the Z Height of the 8-Channel Head

When the ASP-1000 is initially installed at your site, the field service engineer will calibrate the Z-arm to align precisely with the x, y, and z coordinates of the six plate locations on the ASP-1000 deck. We recommend adjusting the ASP-1000's z height under the following circumstances:

- If you move the ASP-1000 to a different location.

- As part of an annual maintenance.

- If you use different types of reagent plates. For example, a 12-column plate, 96 round-well plate, and 96 square-well plate will each have a different z parameter.

➲ **To calibrate the Z-arm:**

1. Navigate to the ASP-1000 Developer window. For more information, see Accessing Developer Controls in Chapter 4.

2. Click the Open button to display a file selection window.

3. Select the filename of a protocol for which you want to calibrate the Z-arm to a particular set of trays and click the OK button. The selected protocol opens in the Developer window, displaying each step in the Protocol Listing panel.

4. Click the Edit Tray Files button. The Tray File Editor window opens, displaying the position for loading and unloading capsules as well as the default x, y, and z coordinates for plates 1 through 6.

5. Edit the default x, y, and z values as necessary.

The x dimension starts in the back left corner of the ASP-1000 deck and runs to the right.

The y dimension starts in the back left corner of the ASP-1000 deck and run to the front edge of the deck.

The z dimension starts at the top of the ASP-1000 and runs downward toward the deck.

6. Choose one of the following:

    - Click the Save button to save your changes to the current filename.

    - Click the Save As button to save the file under a new name. Follow the on-screen prompts.

    The Tray File Editor window closes, returning you to the Edit tab in the Developer window.

7. Select a step in the protocol listing at a point where you can observe the movement of the Z-arm and click the Run Now button.

8. Repeat steps 4 through 7 as needed.

9. Save your changes. Choose one of the following:

   - Click the Save button to save your changes to the current filename.

   - Click the Save As button to save the file under a new name. Follow the on-screen prompts.

   The file with your updated x, y, and z coordinates is saved in the following directory: C:\Users\ LAPTOP_NAME\mPrep\mPrep Tray Files

Changing Filter Couplers

Filter couplers protect the ASP-1000's 8-channel head and the pump module from damaging fumes associated with some of the reagents, osmium tetroxide in particular.

You need to change the filter couplers based on visual cues indicating contamination. Exposure to osmium makes filter coupler surfaces turn black; the more exposure, the sooner the color change. When the bottom of the filter is black and the middle is brown, it is time to change the filter.

1. Move the Z-arm to the position for loading and unloading capsules.

2. Slip the power switch to the ASP-1000 base unit to the OFF position.

3. Gently, manually raise the Z-arm to the top position.

4. Place an empty 96-well plate, in portrait orientation, under the "shelf" created by the Z-arm.

5. Holding the 96-well plate in place with your right hand, use your left hand to push down on the white plastic block to de-compress the springs holding the U-bracket, which holds the filter couplers in place.

Step 4

Step 5

Step 6

Step 8

6. Once the U-bracket clears the tip shafts, slide it to the left to remove it.

7. Replace the filter couplers with new ones.

8. Re-install the U-bracket by reversing the process.

9. Remove the 96-well plate.

10. With the fresh filter couplers mounted on the shafts, give each one a slight turn counter-clockwise to confirm a tight seal.

Troubleshooting

LOG FILES

Each time the ASP-1000 runs, it automatically creates a log file capturing the operator name, protocol name, time spent on each step, and amounts and names of reagent used. You can find log files stored in C:\Users\LAPTOP_NAME\mPrep\mPrep Log Files. These files are human readable and designed to be accessible for other departments, such as HR or Accounting, to analyze and assign costs, monitor project progress, or maintain appropriate reagent inventory.

ERROR MESSAGES

Error	Possible Cause	Corrective Action
Didn't find the stage! Cycle the power, check connections, and try again.	Robot power is off. USB-to-square-USB cable is not connected or has failed.	Cycle power on ASP-1000. Check the connections on both ends of the USB-to-Square-USB cable. Try again. Replace cable if still not communicating. (Many printers use the same cable.)
Error:005 Illegal Command	May occur upon startup after reconnecting laptop controller.	Press "OK" and system will recover automatically.
Error:016 Home movement required	Movement stopped by blockage of an axis. Most likely cause is a stalled z-axis from entering the wrong z-depth.	Cycle power to return head to the home position. Check method and decrease z-depth. For more information, see Setting Global Z Coordinates in Chapter 3.
Error:018 Passthrough command pending	Pump communication is lost.	Cycle power on the pump. Check both ends of the cable with D-shaped 9-pin RS-232 serial port connectors: one end connects to the "COM2" port on the back of the ASP-1000, and the other end connects with the connector on the back of the pump housing.

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APPENDIX A.

Microscopy Innovations Warranty
mPrep™ ASP-1000™ Automated Specimen Processor

Coverage & Term

Microscopy Innovations, LLC ("MI") warrants the mPrep ASP-1000 Automated Specimen Processor ("Equipment") as supplied by Microscopy Innovations and its authorized distributors to be free from defects in materials and workmanship for one (1) year from the date of shipment by Microscopy Innovations to the end-user customer. The Equipment covered by this warranty includes:

- BASE UNIT (fluid handling platform, 8-channel head assembly, deck platform assembly, and fume enclosure)

- PUMP MODULE (8-channel syringe pump)

Remedies & Service

Microscopy Innovations shall, at its discretion, repair or replace Equipment found in its sole determination to be defective; provided Microscopy Innovations has been notified in writing of such defect within the warranty period. The repair or replacement of defective Equipment shall constitute buyer's sole remedy and Microscopy Innovation's sole obligation hereunder. Warranty service shall include:

- Telephone support;
- Parts and labor;
- Travel costs for on-site repairs;
- Shipping costs for return-to-factory/depot repairs.

LIMITATIONS AND RESTRICTIONS

The following limitations and restrictions apply to this warranty:

1. The Warranty covers all parts manufactured or purchased by Microscopy Innovations and incorporated into the Equipment except the following:

    a. LAPTOP CONTROLLER (computer) provided by MI is covered only for a period of 30 days against defects in materials and workmanship; Windows operating system and any software other than ASP-1000-specific software is not warranted by MI.

    b. Exhaust Fan and additional accessories used for installation are not warranted.

2. The Warranty covers all ASP-1000-specific software included in/with the Equipment and licensed by Microscopy Innovations for Equipment operation. The software shall perform according to the criteria set forth in the ASP-1000 User's Guide. The software is intended solely for use in the operation of the Equipment; the Warranty does not cover use of the software for functions other than those identified and described in the User's Guide; use of the software with other software not licensed by Microscopy Innovations; and use of the software in equipment not supplied by Microscopy Innovations. Modification of the software voids the Warranty.

3. The Warranty does not cover wear and tear resulting from normal laboratory use; including discoloration or deterioration of external frame, housing and other component finishes resulting from installation in damp or hot environments, exposure to chemical reagents or corrosive cleaners, and use of high-pressure washers.

4. The Warranty does not cover the following items:

   a. User maintainable components and peripherals, as identified in the ASP-1000 User's Guide;

   b. Consumables and supplies;

   c. Accessory products not supplied with the Equipment as shipped and installed;

   d. Used or refurbished equipment not sold, inspected or approved by Microscopy Innovations

5. The Warranty does not cover loss or damage, including loss and corruption of data resulting from:

   a. Modification of the Equipment not approved by Microscopy Innovations;

   b. Inadequate site preparation;

   c. Misuse, abuse, neglect, and inadequate maintenance of the Equipment;

   d. Repair of the Equipment by other than Microscopy Innovations or persons certified by Microscopy Innovations;

   e. Use of equipment for any other purpose than microscopy specimen preparation.

6. The Warranty does not cover, and Microscopy Innovations is not responsible nor will it in any circumstances be liable in any way for any consequential, incidental, indirect and special damages arising out of or in connection with the Equipment and its use, including but not limited to injury and damage to persons or property, and loss of business revenue and profits.

7. The Warranty is for the sole benefit of the original end user of the Equipment and is not transferrable.

8. This Warranty is in lieu of and replaces and supersedes all other warranties, express or implied including, but not limited to warranties of merchantability and fitness for a particular purpose.

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APPENDIX B.
96-well Reagent Plate Template

○ ○ ○ ○ ○ ○ ○ ○								1
○ ○ ○ ○ ○ ○ ○ ○								2
○ ○ ○ ○ ○ ○ ○ ○								3
○ ○ ○ ○ ○ ○ ○ ○								4
○ ○ ○ ○ ○ ○ ○ ○								5
○ ○ ○ ○ ○ ○ ○ ○								6
○ ○ ○ ○ ○ ○ ○ ○								7
○ ○ ○ ○ ○ ○ ○ ○								8
○ ○ ○ ○ ○ ○ ○ ○								9
○ ○ ○ ○ ○ ○ ○ ○								10
○ ○ ○ ○ ○ ○ ○ ○								11
○ ○ ○ ○ ○ ○ ○ ○								12

H   G   F   E   D   C   B   A

Notes

APPENDIX C.
Loading mPrep Capsules

mPrep/s Capsules

UNORIENTED SPECIMENS

1. Remove screen from mPrep/s capsule.

Twist tool
to lock screen

2. Load specimen and replace screen.

Twist tool
to release screen

3. Label capsule and process the specimen on the ASP as directed.

Attach to ASP-1000
8-channel head
and process

ORIENTED SPECIMENS

1. Label capsule and process the specimen on the ASP as directed.

Twist capsule
to lock screen

2. Load and orient specimen.
    A. Compression Method

Orient on the
closed screen

Gently
compress
specimen

Twist capsule
to release
screen

Remove
loaded
capsule

B. Pinch Method

Push lever to
open slot in
screen

Insert
specimen
into slot

Release lever
to close slot

Twist capsule
to release
screen

Remove
loaded
capsule

3. Label capsule and process the specimen on the ASP as directed.

Attach pipettor
and process

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mPrep/g Capsules

1. Use forceps to guide grids into the Grid-Tite™ slots.

   You can insert up to 2 prepared grids into each capsule.

2. Insert each grid to the point of resistance (a) plus ~1mm (b).

   Do NOT insert grid to the bottom of the slots.

3. Label capsule and process the specimen on the ASP as directed.

Notes

APPENDIX D.
Step Commands & Parameter Settings

You access Step Commands, which define the steps in a protocol, in Developer mode within both the Edit and Scratchpad tabs of the sandbox. While some steps for the ASP-1000 are defined within global settings (see Chapter 3. Global Preferences & Settings), you can override these defaults with values defined here.

Step Comment

Inserts an operator comment for an individual step.

Note: It is important to complete the Step Comment field with the "Move-Asp-Disp" Step Command to define reagent plate contents, which will appear on the Tray Map.

No Operation

Default placeholder for a step when you click the Insert Step button.

Move-Asp-Disp (Move-Aspirate-Dispense)

Overrides the default pump parameters specified in the Preferences Editor, which are auto populated here. (For more information, see Setting Default Aspirate and Dispense Preferences in Chapter 3.)

Step Comment: Text entered in this text box appears on the Tray Map in the Edit tab. Type the reagent used in this step.

Plate Number: Values range from plate 1 – 6.

Row Number: Values range from row 1 – 8.

Stage Z (mm): Specifies the depth of the 8-channel head.

Speed: Values range from 0 to 40, with 40 being the slowest.

Vol (µl): Amount of liquid, in microliters.

Asp. Hold (s): Length of time in seconds to hold after aspirating reagent into the capsule.

Disp. Hold (s): Length of time in seconds to hold after dispensing reagent from the capsule.

Repeats: Number of aspirate and dispense repeat actions.

Home Stage
Sets the Z-arm to the home position (0,0,0).

Home Pump
Moves pump to the home position.

Select Plate/Row
Moves the Z-arm to the specified location on the ASP-1000 deck.

Plate Number: Values range from plate 1 – 6.

Row Number: Values range from row 1 – 8.

Stage Z (mm): Specifies the depth of the 8-channel head.

Pump Aspirate
Sets the length of time to hold prior to aspirating reagent from the capsules.

Speed: Values range from 0 to 40, with 40 being the slowest.

Vol (µl): Amount of liquid in microliters to aspirate.

Asp. Hold (s)*:* Sets the length of time in seconds to hold the liquid prior to aspirating.

Pump Dispense
Sets the length of time to hold prior to dispensing reagent from the capsules.

Speed: Values range from 0 to 40, with 40 being the slowest.

Vol (µl): Amount of liquid in microliters to dispense.

Disp. Hold (s)*:* Sets the length of time in seconds to hold the liquid prior to dispensing.

Pump Speed

Sets the pump speed, ranging from 0 to 40, with 40 being the slowest. Note that slower speeds of aspiration and dispensing are gentler on the specimen. As a benchmark, the fastest speed at 100 µl is one second to dispense and one second to aspirate.

Pump Valve Input

Switches valve to input position to draw ambient air into the eight syringes in the pump assembly.

Pump Valve Output

Switches valve to output position, which connects the eight syringes in the pump assembly to the capsules.

Load/Unload

Moves the Z-arm into default position to load capsules onto or unload capsules from the 8-channel head. The default position is defined as a global preference in the Tray Files Editor window. For more information, see Selecting a Default Tray File for Use with Protocols in Chapter 3.

LED Color

Sets the customizable LED color of the Status Indicator on the ASP-1000 instrument as well as the Instrument Status Indicator in the operator's and developer's software windows.

LED Color – Red

Sets the LED color of the Status Indicator on the ASP-1000 instrument as well as the Instrument Status Indicator in the operator's and developer's software windows as red.

LED Color – Green

Sets the LED color of the Status Indicator on the ASP-1000 instrument as well as the Instrument Status Indicator in the operator's and developer's software windows as green.

LED Color – Blue

Sets the LED color of the Status Indicator on the ASP-1000 instrument as well as the Instrument Status Indicator in the operator's and developer's software windows as blue.

Move Abs X

Sets the absolute position, in millimeters, of the *x* axis coordinate for the 8-channel head.

Move Abs Y

Sets the absolute position, in millimeters, of the *y* axis coordinate for the 8-channel head.

Move Abs Z

Sets the absolute position, in millimeters, of the *z* axis coordinate for the 8-channel head.

Move Abs XY

Sets the absolute position, in millimeters, of the *x* and *y* axis coordinates for the 8-channel head.

Move Abs XYZ

Sets the absolute position, in millimeters, of the *x, y,* and *z* axis coordinates for the 8-channel head.

Move Rel X

> Sets the position, in millimeters, of the *x* axis coordinate for the 8-channel head relative to its current position.

Move Rel Y

> Sets the position, in millimeters, of the *y* axis coordinate for the 8-channel head relative to its current position.

Move Rel Z

> Sets the position, in millimeters, of the *z* axis coordinate for the 8-channel head relative to its current position.

Stage Pause

> Adds a short delay between steps.

> **Stage Pause (s)***:* Time in seconds paused between steps.

Perastaltic Pump On

> Controls the peristaltic pump, turning it on.

Peristaltic Pump Off

> Controls the peristaltic pump, turning it off.

Play Sound

> Plays a sound to audibly notify the operator that user input is required.

Dialog Popup

> Displays a window to visually notify the operator of the user input required.

> **Dialog Text**: Type a message in the text box to appear as a popup message on the laptop controller.

Send SMS

> Sends a text message to notify the individual specified that user input is required.

> **Operator Name**: The name of the individual granted access to run the ASP-1000. The only operator names allowed are defined within Adding or Removing an Operator in Chapter 3.

> *Note*: *To enable this feature, the laptop controller must be connected to your institution's wi-fi.*