



ProteoAnalyzer

System Manual



Notices

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CAUTION

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In This Guide

Agilent has prepared this manual as a technical reference for the ProteoAnalyzer systems.

This document includes system overviews, installation procedures, analytical methods, maintenance procedures, software operation, troubleshooting guide, and instrument shutdown procedures. Additional information includes instrument specification and utility requirements, parts and supply lists, and product specification sheets.

This document is intended for use by technical personnel that are proficient with analytical instrumentation operation and upkeep. A certain level of training and expertise is assumed and fundamentals are not addressed herein. Procedures are presented in a step-by-step format using photos and screen captures. If questions remain after reviewing a given procedure, please do not hesitate to contact your corresponding Agilent Sales/Service Representative.

1 System Overview

This chapter gives an instrument overview.

2 ProteoAnalyzer Software – File Menu

This chapter describes the ProteoAnalyzer software in more detail on the commands of the File menu.

3 ProteoAnalyzer Software – Admin Menu

This chapter describes the ProteoAnalyzer software in more detail on the commands of the Admin menu.

4 ProteoAnalyzer Software – Utilities Menu

This chapter describes the ProteoAnalyzer software in more detail on the commands of the Utilities menu.

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6 ProteoAnalyzer Software – Operation Tab

This chapter describes the ProteoAnalyzer software in more detail on the Operation tab.

7 ProteoAnalyzer Software – Run Status Tab

This chapter describes the ProteoAnalyzer software in more detail on the Run Status tab.

8 ProteoAnalyzer Capillary Array

This chapter explains the unpacking, installation, and storage of the capillary array.

9 ProteoAnalyzer – Sample Name Entry

This chapter provides information on how to enter the sample names in the ProteoAnalyzer software.

10 ProteoAnalyzer – Automated Analysis

This chapter explains the procedure for automated analysis using the ProteoAnalyzer.

11 Appendix

This chapter provides additional information on part numbers, maintenance procedures, and system settings.

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1

System Overview

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About the System

The ProteoAnalyzer system is a multiplexed capillary electrophoresis (CE) instrument for performing automated, high throughput assessment of size, purity, and composition of protein mixtures. Separation is achieved by applying an electric field through a narrow bore (50 μm i.d.) fused silica capillary array filled with various conductive gel matrices designed to sieve protein molecules of a specific size range. When a high voltage is applied to the capillary array, injected protein migrates through the gel matrix as a function of protein size, with smaller sized proteins eluting faster than larger sized proteins.

At a point toward the far end of the capillary array, detection of the separated protein is achieved by fluorescence of a sensitive dye present in the sample. The ProteoAnalyzer system utilizes a high intensity light emitting diode (LED) excitation light source that is focused across the capillary array detection window and imaged onto a sensitive, two-dimensional charge-coupled device (CCD) detector. By monitoring the relative fluorescence unit (RFU) intensity as a function of time during the CE separation, digital electropherograms representative of the protein content of up to 12 samples are collected in a single experimental run.

Configured ProteoAnalyzer System Dimensions

This chapter provides a basic overview of the ProteoAnalyzer system hardware and operation. **Figure 1** shows an external view of a fully configured ProteoAnalyzer system, which has a compact footprint of 40" on the bench top with a weight of 82 lbs (37 kg).

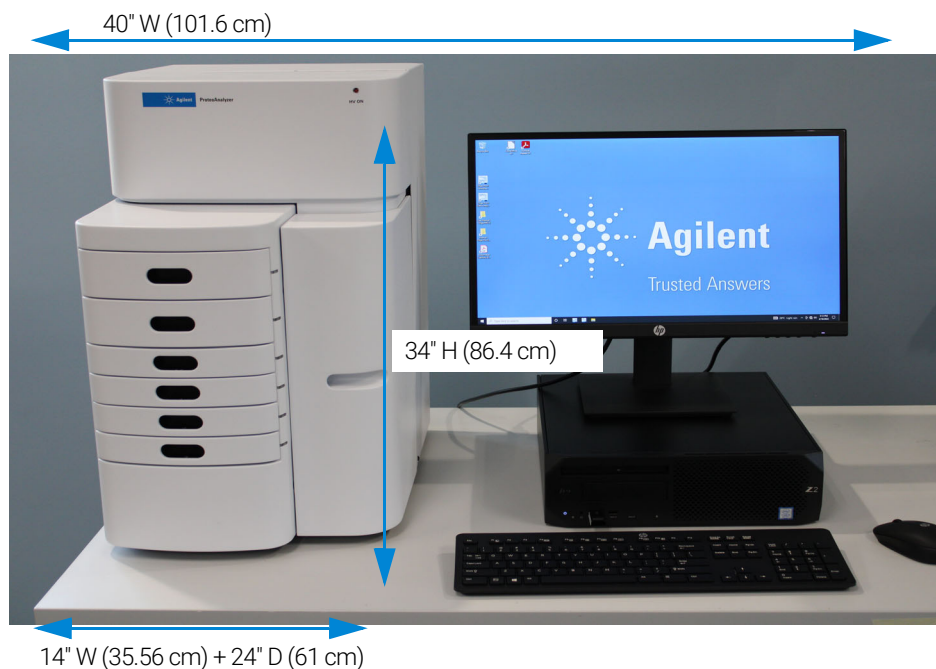


Figure 1 Configured ProteoAnalyzer system with computer workstation

ProteoAnalyzer System Connections

The back of the ProteoAnalyzer system contains the Communications Panel where necessary connections are made to the Instrument Computer and Electrical Outlet for operation (**Figure 2** and **Figure 3**).

The use of a double-conversion surge protection or uninterruptible power supply (UPS) device is highly recommended. Contact the corresponding Agilent Sales/Service Representative for specific recommended models.

A minimum of three standard electrical wall outlets should be available to connect the instrument, computer and accessories, although a power strip can be used in place of separate wall outlets if needed.

Each connection is labeled on the PC. The various connections of the ProteoAnalyzer system are summarized below:

- Connection option 1: From the ProteoAnalyzer system
 - Two USB cables to PC USB
 - Power cord to grounded electrical outlet
- Connection option 2: From the PC
 - Two USB connections to ProteoAnalyzer system

The order/location of connections is critical, and the locations have been identified on both the computer and the ProteoAnalyzer.

- Power cord to grounded electrical outlet
- Connection to monitor, keyboard, mouse, etc.

System Overview

ProteoAnalyzer System Connections

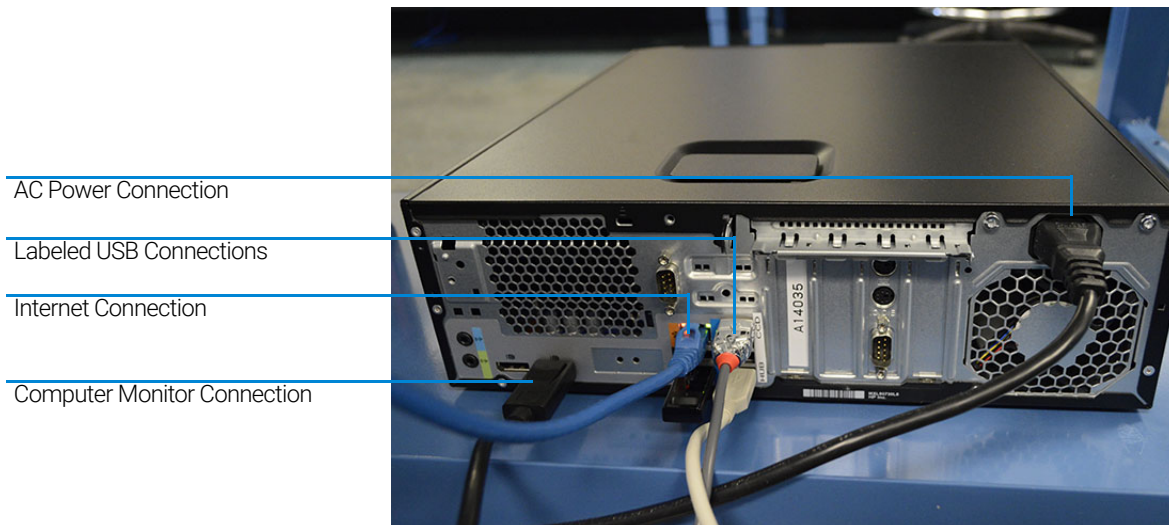


Figure 2 Back of panel of the ProteoAnalyzer computer connections

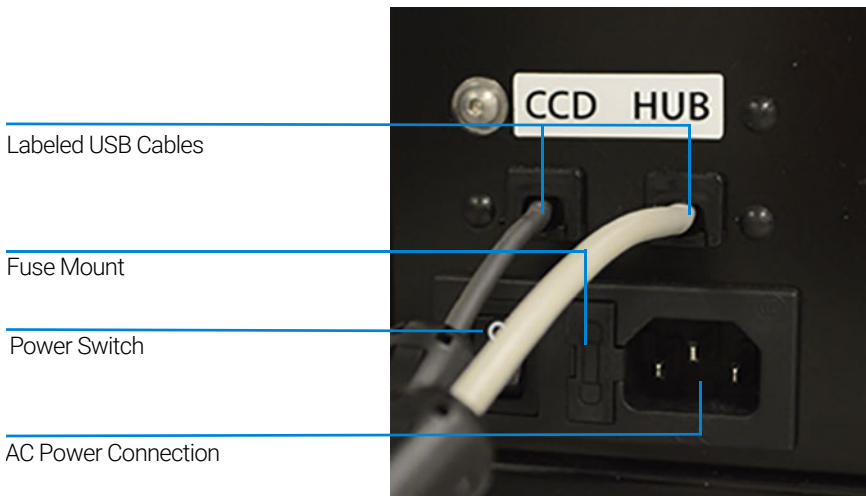


Figure 3 Back of panel of the ProteoAnalyzer instrument connections

Physical Specification

Table 1 Physical specifications

Type	Specification
Weight	39 kg (86 lbs)
Dimensions (w x d x h)	101.6 x 61 x 86.4 cm (40 x 24 x 34 inches)
Line voltage	100 – 200 VAC (200 – 230 available)
Line frequency	50 – 60 Hz
Max. power consumption	110 VA / 90 W
Interface	USB (2 instrument, keyboard, mouse)
Ambient operating temperature	15 – 35 °C (59 – 95 °F)
Operating humidity	<80 % (non-condensing)
Storage temperature	-40 – 70 °C (-40 – 158 °F)
Operating altitude	Up to 4000 meters
Safety standards	IEC, EN, CSA, UL, Overvoltage category II, Pollution degree 2 For indoor use only
ISM Classification	ISM Group 1 Class A According to Cisp11
Sound pressure	<70 dB (A) According to ISO 7779:1988/EN 27779/1991
Assay specific temperature range	18 – 25 °C (64 – 77 °F) for Protein Broad Range Kit

NOTE

This is an ISM Group 1 Class A product intended for use in industrial environment. In a domestic environment, this product may cause radio interference, in which case the user may be required to take adequate measures.

ProteoAnalyzer External Cabinet

There are three primary points of access to the inside of the ProteoAnalyzer system: the top compartment, the side compartment access door and the drawers (6 total) (**Figure 4**).

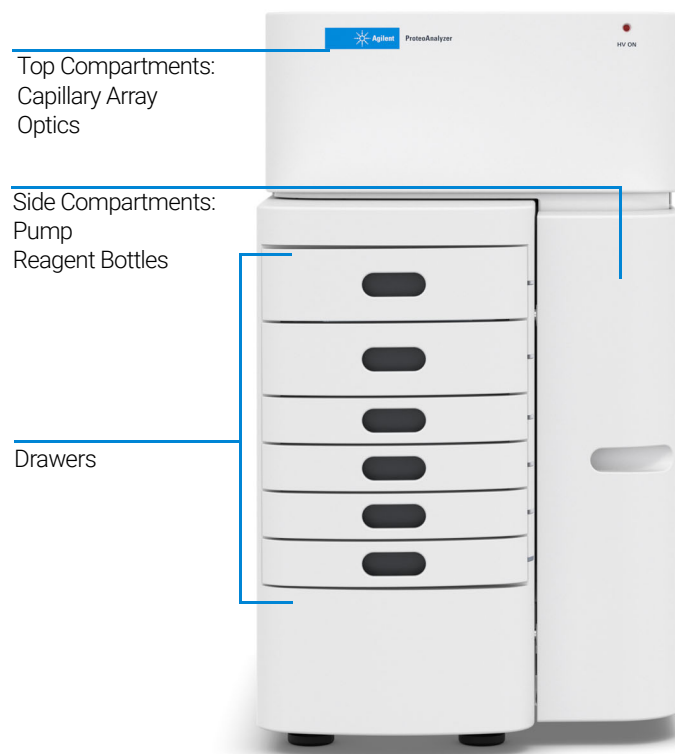


Figure 4 Entry points of the ProteoAnalyzer system

Top Compartment

The *Top Compartment* provides access to the Optical detection platform and a 12-capillary array cartridge. A non-accessible compartment on the back of the instrument contains the high voltage power supply and electronics that are connected to the array cartridge and safety interlock system. The safety interlock system shuts off the high voltage in case the top compartment is opened while the instrument is running.

The *12-Capillary Array Cartridge* is a replaceable, modular component of the ProteoAnalyzer system. The user can easily exchange the capillary array cartridge (for more information, refer to **Chapter 4**, “ProteoAnalyzer Software – Utilities Menu” and **Chapter 1** “ProteoAnalyzer Capillary Array”).

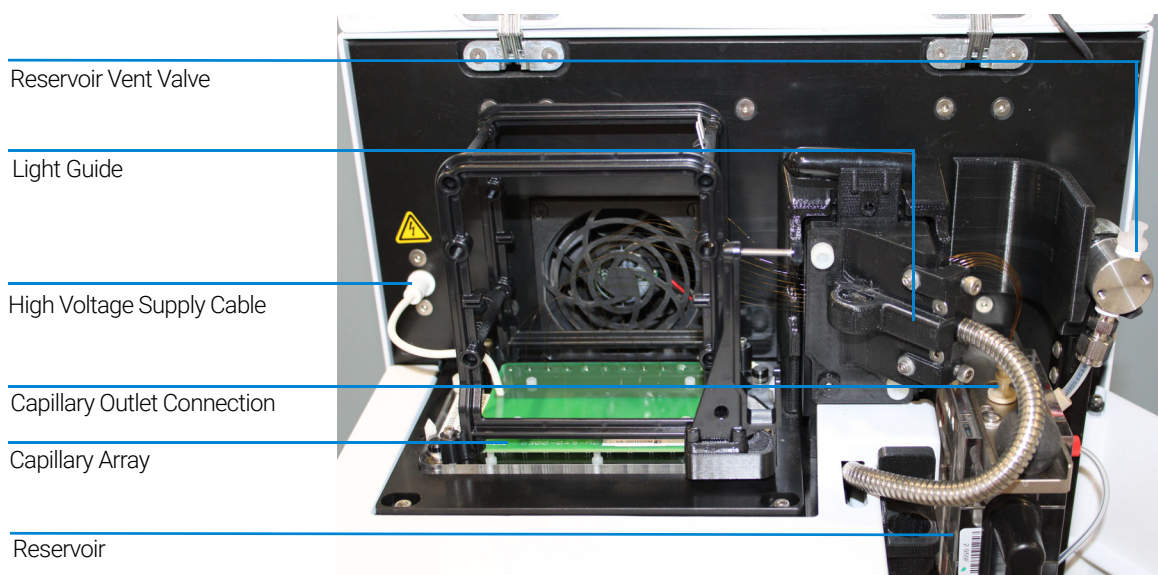


Figure 5 ProteoAnalyzer sub-components of the top compartment

Side Compartment

The *Side Compartment* allows access to the high pressure pump, syringe, waste bottle, conditioning solution, and gel solutions (gel 1 and gel 2).

The High Pressure Syringe Pumping System provides automated flushing and filling of the capillary array with conditioning solution and separation gel between experimental CE runs, providing pressurization of the capillaries up to 280 psi.

Three different solutions are fed to and pumped through the capillary array during routine operation:

- **1 M NaOH solution** (Gel 2 line)
- **Protein Conditioning Solution** (Conditioning line)
- **Protein Gel** (Gel 1 line)

The appropriate solution is selected for pumping by way of a 6-way distribution valve.

The system also contains a Waste Bottle, which collects solutions pumped via the Waste Line from the capillary array reservoir during the filling process.

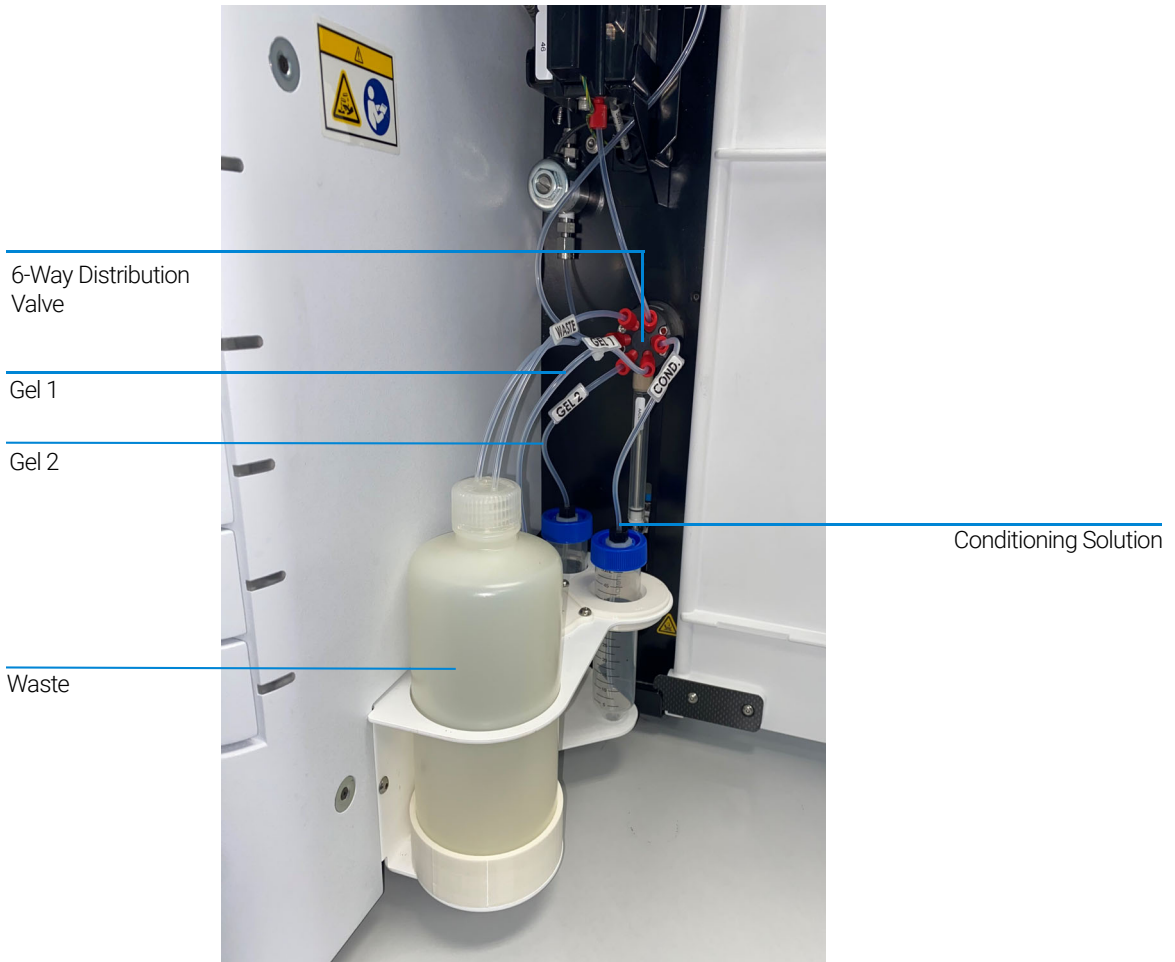


Figure 6 Side Door Compartment

The seven fluid line connections inside the ProteoAnalyzer system are:

- *Gel 1* line from bottle to 6-way valve
- *Gel 2* line from bottle to 6-way valve
- *Conditioning Fluid* line from bottle to 6-way valve
- *Supply* line from 6-way valve to reservoir
- *F-Port* line from reservoir to F-port of 6-way valve
- *Waste* line from 6-way valve to waste bottle
- *Overflow waste* line from reservoir main valve to waste bottle

Drawers

The ProteoAnalyzer front-panel *Drawers* provide an external interface for loading *Buffer, Waste, Marker, and Sample 96-Well Plates* into the system.

- Drawer B (top drawer): This location is used for the *Inlet Buffer Tray* used during the CE separation. This position is also used for *Sample Storage Solution* and the Rinse row.
- Drawer W (second drawer from top): This location is utilized for a *Waste Tray* when the capillary array is flushed.
- Drawer M (third drawer from top): This location is left empty.
- Drawer 1 (fourth drawer from top): This location is utilized for *Sample Plate Number 1*.
- Drawer 2 (fifth drawer from top): This location is utilized for *Sample Plate Number 2*.
- Drawer 3 (sixth drawer from top): This location is utilized for *Sample Plate Number 3*.

Drawer status

Status

Drawers B and W are interlocked

Drawers M, 1, 2, and 3 are not interlocked

Description

When any of the top two drawers are open, the high-voltage (for electrophoresis) will automatically shut off.

Sample trays can be exchanged while the instrument is in operation.

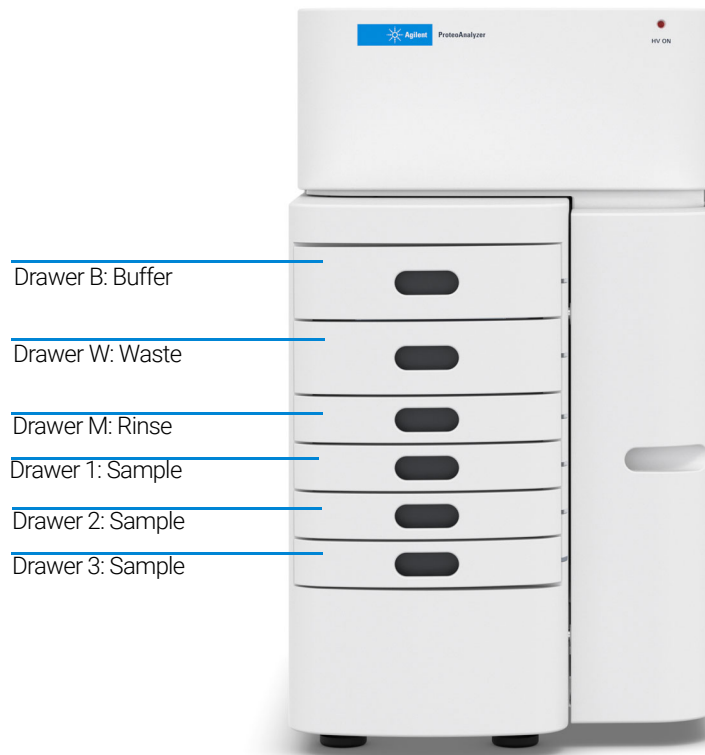


Figure 7 Instrument drawer positions

ProteoAnalyzer Loading and Orientation of 96-Well Plates

The ProteoAnalyzer system is a multiplexed CE system containing a 12-capillary array, which is designed to interface directly with a single row or a standard 96-well plate footprint. Each capillary of the array corresponds to a specific well for a given row in the 96-well sample plate. For example: The capillary array orientation is indexed such that Capillary #1 corresponds to Well A1 and Capillary #12 = A12.

Well A1 of the 96-well plate should always be oriented to the back left location of the instrument drawer to ensure that the sample well location is correctly assigned and reported in the software.

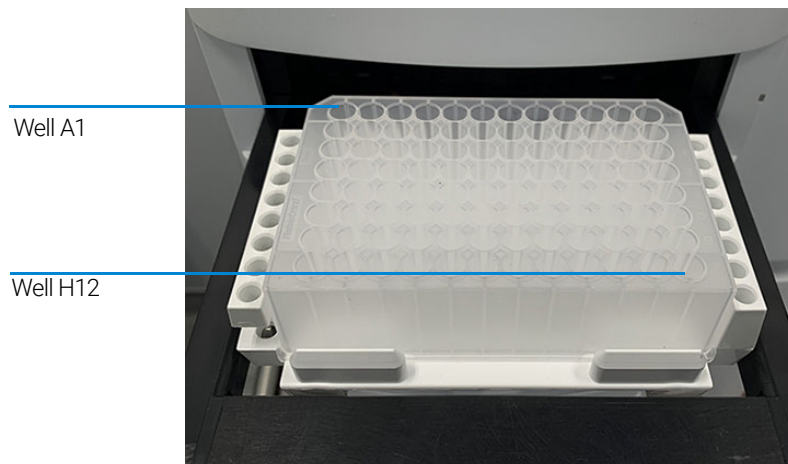


Figure 8 Proper orientation when loading 96-well marker and sample plates

Each drawer location houses a tray carrier containing alignment pins for ensuring proper alignment of the 96-well plate when placed against the capillary array.

The ProteoAnalyzer system has been designed to operate using specific dimensions and styles of plates.

For a list of compatible 96-well plates please refer to the Appendix (see **“Compatible Plates for the ProteoAnalyzer System”** on page 129).

ProteoAnalyzer Loading Samples

The ProteoAnalyzer system requires a minimum volume of 20 μL /well in the sample plate for proper injection.

Ensure the sample has been adequately mixed before loading on the instrument.

Vortexing sample is the best way to ensure adequate mixing before analysis.

Check the wells of the sample plate/s after pipetting to ensure that there are no air bubbles trapped in the bottom of the wells. The presence of trapped air bubbles can lead to injection failures.

Air bubbles can be removed from the plates by introducing a brief centrifugation step prior to placing the plates into the tray carrier.

Individual tips are given in each kit guide for reference.



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This chapter describes the ProteoAnalyzer software in more detail on the commands of the File menu.

About the Software

The ProteoAnalyzer system employs proprietary software for operation and data analysis.

This software is preloaded on the instrument and checked prior to shipment as part of the instrument qualification.

System Requirements

The software is run using a Microsoft Windows 10 PC with the following requirements (**Table 2**).

Table 2 Minimum computer requirements

Type	Specification
Processor	Intel Core i5
Display resolution	Minimum Resolution 1024 X 1280
Memory	4 Gigabytes
Available Hard Disk Space	500 Gigabytes
USB Serial Ports	4 ports (2 instrument, keyboard, mouse)
Network	If not using a local database, A network connection to the database server host is desired.

System Installation

If the instrument software needs to be reinstalled or updated, install the ProteoAnalyzer software:

- 1** Navigate to the ProteoAnalyzer installer on the Agilent website. Download the installer and double-click on setup.exe.
- 2** Follow the setup instructions provided by the installation wizard. The default installation directory is C:\Agilent Technologies\ProteoAnalyzer.

Opening the ProteoAnalyzer Software

- 1 To login to the software, select the ProteoAnalyzer software icon.



Figure 9 ProteoAnalyzer icon

There are two levels of users available:

- **Administrator:** The administrator login has enhanced access to functions such as allowing editing of separation methods.
 - **User:** The user login has restricted access that allows only routine operation of the instrument.
- 2 To log into the ProteoAnalyzer software, type **Administrator** or **User** into the **User ID** field of the login window.
 - 3 Enter your password.

The first time you log in, the password information is left blank.

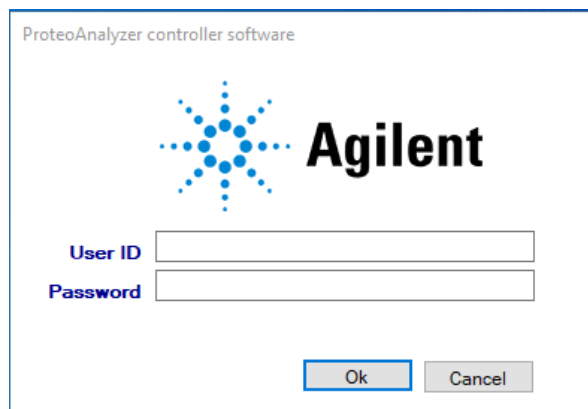


Figure 10 Login menu

4 Select **OK**.

The main screen opens.

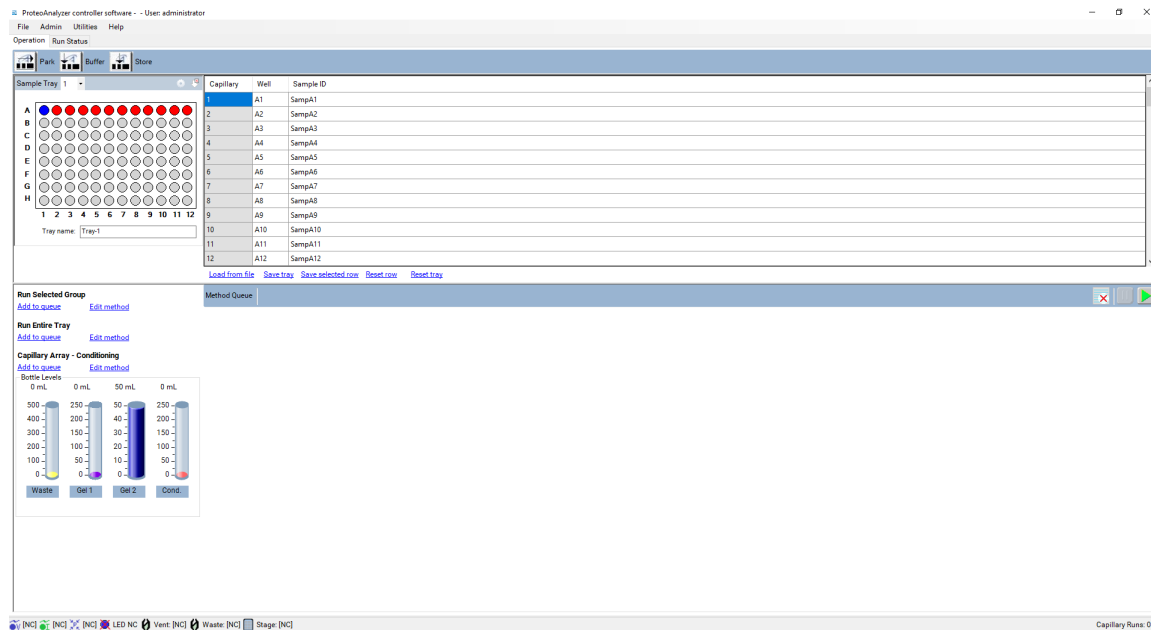


Figure 11. ProteoAnalyzer software main screen

More information about the **User** and **Administrator** functions within the software will be discussed in a later chapter.

A password can be set for the system during the Agilent training and installation period at your facility, or by using the **Change Password** command of the **Admin** menu described in section “**Change Password**” on page 34.

Main Screen Toolbar

The Main Screen Toolbar is located at the top of the ProteoAnalyzer main screen as seen in **Figure 11**.

File Menu

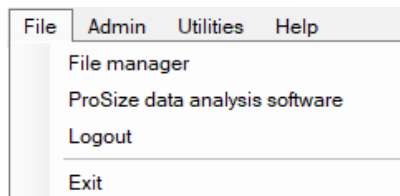


Figure 12 File menu commands

File Manager

The file manager function allows electropherogram data to be examined within the *ProteoAnalyzer* program environment.

Files are normally analyzed using the ProSize Data Analysis Software, which is covered in the *ProSize software User Manual*.

The File Manager also enables one to correct the capillary alignment for an individual data file.

Selecting the **File Manager** function will open a window allowing the user to navigate to a data file. Once a file is selected, the file manager screen will appear (**Figure 13**).

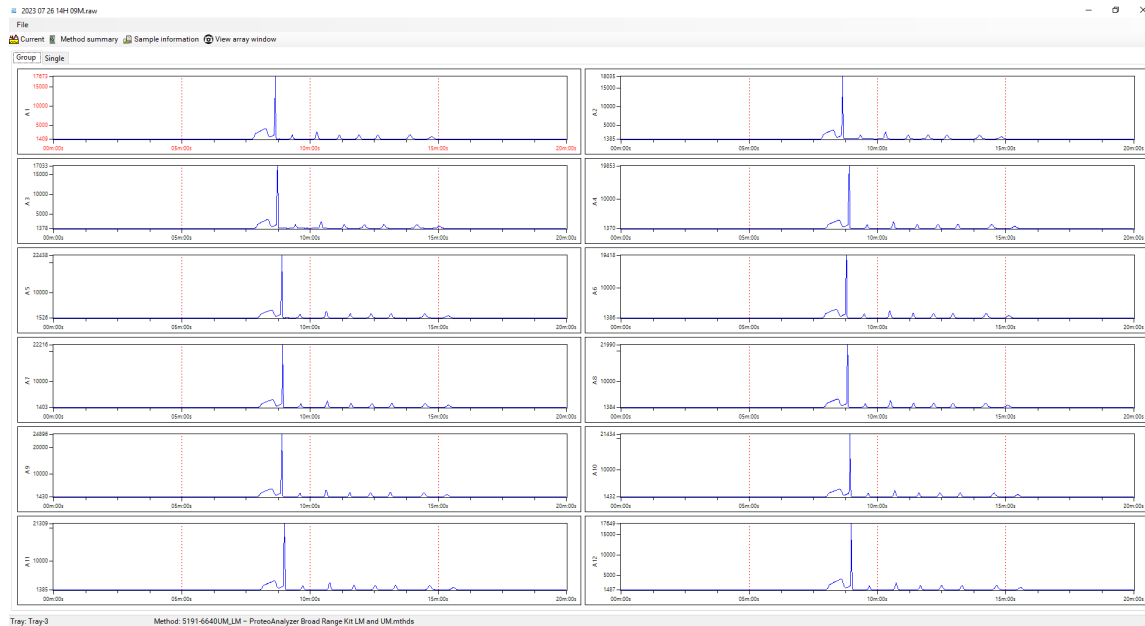


Figure 13 File manager window

The **File** functions of the file manager screen are reviewed in **Table 3**.

Table 3 File manager – file functions.

Field	Description
Open	Opens a Windows dialogue box to navigate to desired data file.
Cap. Alignment	Allows the user to view and manipulate the capillary alignment for the data file opened only. Capillary alignment from a file is discussed in the capillary alignment chapter.
Merge Files	Available for users running an entire 96 well tray. This will generate: file with a single sample name, a single raw data file, a single method file.
Print	Allows the user to print twelve electropherograms to a page.
Exit	Closes the file manager window.

The file manager toolbar functions are discussed in Table 3.

Table 4 File manager toolbar options.

Field	Description
Current	Selecting current allows user to view the current of the separation during the analysis.
Method Summary	Selecting the method summary option shows a summary of the method that was used for the separation.
Sample Information	Selecting the sample Information option shows the user the sample names input for the separation file.
View Array Window	Selecting the View Array Window option shows the camera image of the capillary array window.

Once the data file is opened in file manager the data can be viewed in groups of 12 (by row) when the **Group** tab is selected.

To view a single electropherogram at a time, either double left-click on the desired well or select the **Single** tab. A page and well selection is located at the bottom of the screen allowing for navigation of all rows and wells in the plate.

Electropherogram data can be panned, zoomed, or zoomed out by right-clicking on the chart and selecting the function of interest.

ProSize Data Analysis Software

Selecting this option will open the ProSize data analysis software.

Logout

The Logout command allows the user to logout of the ProteoAnalyzer program and login as a different user.

The login menu (**Figure 10**) will be presented for the user to login.

Exit

The Exit command closes the ProteoAnalyzer program. Alternatively, the user can exit the program by selecting the red **X** on the top right corner of the main screen.



3

ProteoAnalyzer Software – Admin Menu

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This chapter describes the ProteoAnalyzer software in more detail on the commands of the Admin menu.

Admin Menu

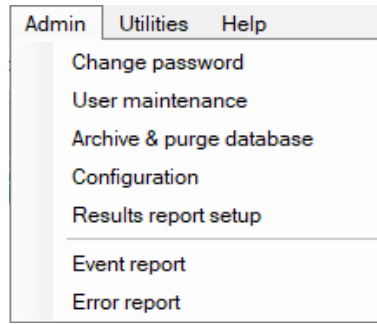


Figure 14 Admin menu commands

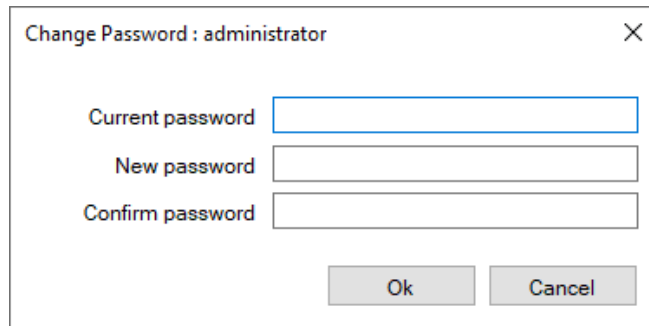
Change Password

The command **Change password** opens the window shown in **Figure 15**.

Changing the password is only accessible to users with administrator privileges.

Password requirements:

- Maximum password length is 40.
- Password can contain letters or numbers.
- Passwords are case insensitive.





The image shows a dialog box titled "Change Password : administrator" with a close button (X) in the top right corner. Inside the dialog, there are three text input fields stacked vertically, labeled "Current password", "New password", and "Confirm password". Below these fields are two buttons: "Ok" and "Cancel".

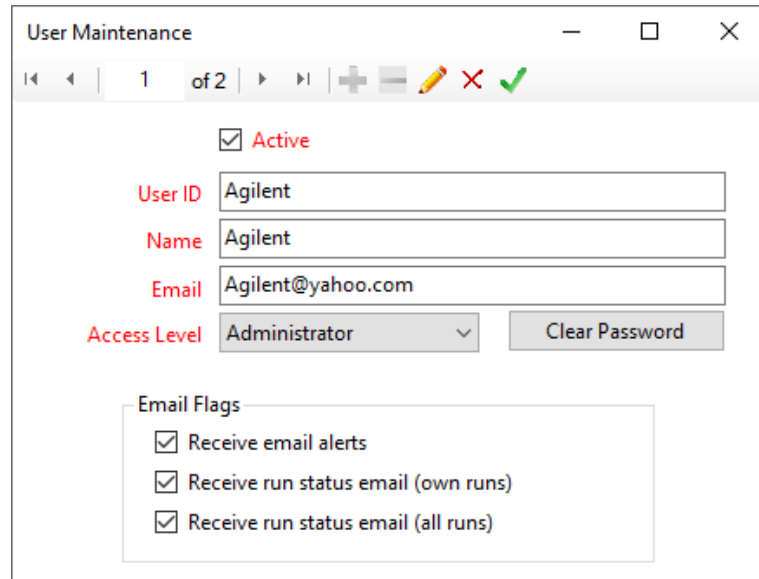
Figure 15 Change Password popup menu

User Maintenance

The command **User maintenance** opens the **User Maintenance** window (Figure 16).

In this window, the administrator can add, delete, or make modifications to all users that can access the ProteoAnalyzer program.

- 1 To edit the settings, select the pencil icon .
- 2 After editing, and if all entries are acceptable to the user, select the check mark .



The screenshot shows a 'User Maintenance' window with a toolbar at the top containing navigation and action icons. The main area displays a user entry for 'Agilent' with the following details:

- Active
- User ID: Agilent
- Name: Agilent
- Email: Agilent@yahoo.com
- Access Level: Administrator (dropdown menu)
- Clear Password button
- Email Flags:
 - Receive email alerts
 - Receive run status email (own runs)
 - Receive run status email (all runs)

Figure 16 User Maintenance popup window

A summary of the parameters in the **User Maintenance** window is illustrated in Table 5.

Table 5 User maintenance Window parameters

Field	Description
User ID	User ID for login or signature.
Name	An optional description of the User ID or the user name
Email	(Optional) The e-mail address for receiving e-mails on the run status and instrument status
Access Level	Set the user access level. User or Administrator.
Email Flags	Select the type of e-mails the user wants upon completion of a run.
Active	If checked: The selected user is active and the User ID will work. If inactive: The User ID cannot be used.
Clear Password	Sets the Users login password to blank. If a minimum password length has been set, the user will need to change their password on login.

NOTE

All these parameters can be changed by selecting the pencil icon.

NOTE

The e-mail preferences (sender, host, etc.) are set up in the **Configuration Settings**, as discussed further below.

Archive and Purge Database

The option **Archive & purge database** is used to maintain the event and error log database.

Event and error logs are saved in the database and can be retrieved for advanced troubleshooting.

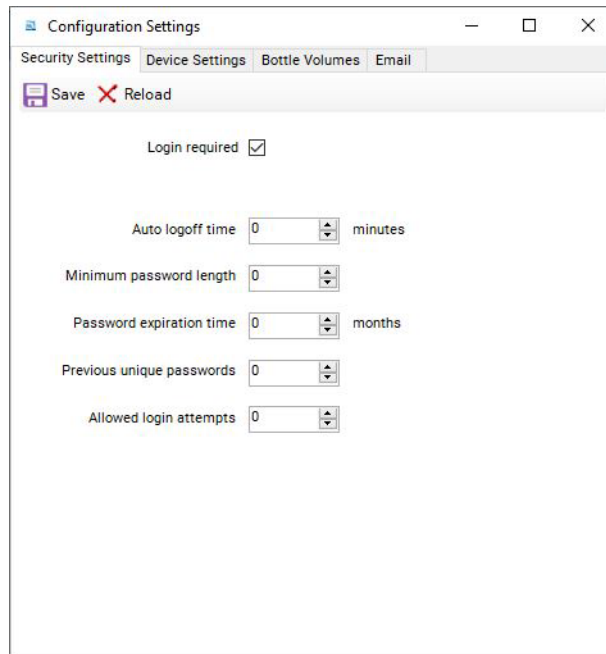
This function allows the user with administrative privileges to back-up the data for future use in a different location or on an external storage device.

Configuration

Selecting the command **Configuration** opens the **Configuration Settings** window where the administrator can modify **Security Settings**, **Device Settings**, **Bottle Volumes** and **Email** parameters for the system.

The **Security Settings** tab allows the administrator to modify the Login requirements for all users (**Figure 17**).

A summary of the configuration options in the **Security Settings** tab is provided in **Table 6**.



The screenshot shows a window titled "Configuration Settings" with four tabs: "Security Settings", "Device Settings", "Bottle Volumes", and "Email". The "Security Settings" tab is active. Below the tabs is a toolbar with a "Save" icon and a "Reload" icon. The main area contains the following settings:

- Login required
- Auto logoff time: 0 minutes
- Minimum password length: 0
- Password expiration time: 0 months
- Previous unique passwords: 0
- Allowed login attempts: 0

Figure 17 Configuration – Security Settings tab

Configuration Options

Table 6 Configuration – Security Settings tab functions

Configuration Option	Range	Description
Login Required	True or False	If True: User must login to the application. If False: No login is required for user level access.
Auto Logoff Time	0 to 30 Minutes	If the application is left unattended for length of time, the current user will be logged off. If set to zero, there is no auto logoff.
Minimum Password Length	0 to 12	The password must exceed this number of characters
Password Expiration Time	0 to 36 Months	A password (Login ID and Signature) will expire after the set number of months. If set to zero, there is no password expiration.
Previous Unique Passwords	0 to 4	When a user changes their password, they may not select from this number of previously used passwords. When set to zero, there is no previous used password restriction.
Allowed Login Attempts	0 to 12	If a user attempts to login with an invalid password after this many attempts: <ul style="list-style-type: none"> • That user ID will be made inactive and the error logged. • The failed login attempt is recorded in the event log • The application is shut down If set to zero, there is no limit to the number of login attempts.
Save	n.a.	Saves the chosen settings.
Reload	n.a.	Reloads the previously saved settings.

The **Device Settings** tab allows modification of the device settings (**Figure 18**).

The settings should be updated whenever a new capillary array cartridge is installed.

A summary of the configuration options in the **Device Settings** tab is provided in the **Table 7**.

The screenshot shows the 'Configuration Settings' window with the 'Device Settings' tab selected. The window has a title bar with standard minimize, maximize, and close buttons. Below the title bar are four tabs: 'Security Settings', 'Device Settings', 'Bottle Volumes', and 'Email'. A toolbar contains a 'Save' icon and a 'Reload' icon with a red 'X' over it. The main area contains the following settings:

- Instrument type:** ProteoAnalyzer (dropdown menu)
- Instrument serial number:** [Empty text box] with a gear icon to its right.
- Number of capillaries:** 12 (dropdown menu)
- Capillary length:** 22 (dropdown menu)
- Capillary array serial number:** [Empty text box] - [Empty text box] USPA (dropdown menu)
- Buffer tray:** Buffer (dropdown menu) Row A (dropdown menu)
- Storage solution tray:** Buffer (dropdown menu) Row H (dropdown menu)
- Set tray name to folder prefix:**
- Reset tray info when queuing:**
- Automated report application:** C:\ProSize data analysis software\Auto Data Processing.exe (text box) with a search icon to its right.
- ProSize data analysis software path:** C:\ProSize data analysis software\ProSize data analysis software.exe (text box) with a search icon to its right.

Figure 18 Configuration – Device Settings tab

Table 7 Configuration - Device Settings tab functions

Parameter	Access Level	Description
Number of Capillaries	Administrator	Values: 12
Capillary Length	Administrator	22 (Effective length in cm)
Capillary Array Serial Number	Administrator	The format must be XXXXXX-XX USPA
Buffer Tray	Administrator	Default selection is locked.
Storage Solution Tray	User	Allows for the selection of tray and row for the storage solution tray.
Set Tray Name to Folder Prefix	Administrator	Sets the tray name to the folder prefix used when loading sample trays.
Reset Tray Info when Queuing	Administrator	Resets tray info for each new tray that is loaded.
Automated report application	Administrator	Allows for changing the file path used for the automated report application.
ProSize data analysis software path	Administrator	Allows for changing the file path used to open the ProSize data analysis software.
Instrument type	Locked	Instrument device name
Instrument serial number	Administrator	Set at factory
Save	Administrator	Saves the chosen settings.
Reload	Administrator	Reloads the previously saved settings.

The **Bottle Volumes** tab allows modification of the reagent bottle volume size used (**Figure 19**).

The gel 1, gel 2, conditioning, and waste bottles can be set from 50 mL to 5000 mL by entering the appropriate bottle volumes. Larger volumes may be used if the system is configured with larger containers.

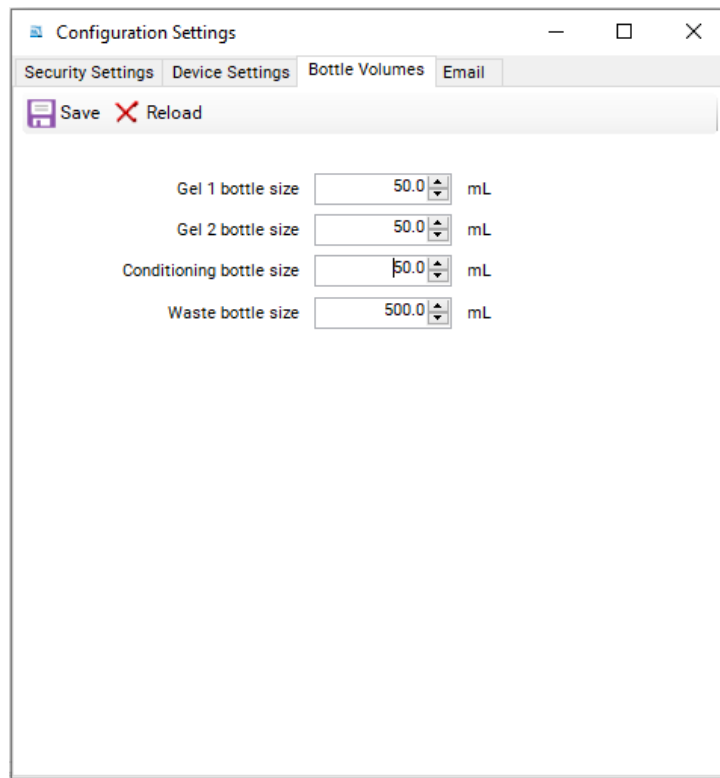
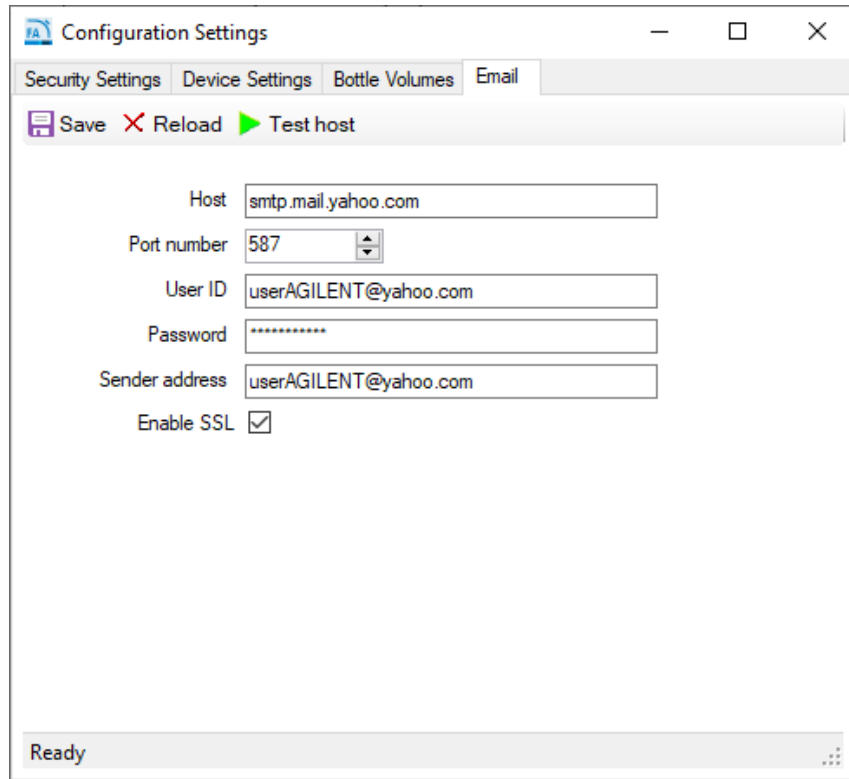


Figure 19 Configuration – Bottle Volumes tab

The **Email** tab allows the administrator to set up e-mail settings (**Figure 20**).



The screenshot shows a web browser window titled "Configuration Settings" with a tab for "Email". The window contains a toolbar with "Save", "Reload", and "Test host" buttons. Below the toolbar are several input fields: "Host" (smtp.mail.yahoo.com), "Port number" (587), "User ID" (userAGILENT@yahoo.com), "Password" (masked with asterisks), and "Sender address" (userAGILENT@yahoo.com). There is also a checked checkbox for "Enable SSL". A status bar at the bottom of the window displays "Ready".


Figure 20 Configuration – Email tab

Information on the **Host**, **Port number**, etc. may be found at the e-mail source or with the local site information technology administrator. For example, yahoo.com offers an e-mail settings page, as shown in **Figure 21**.

Important: After inputting all the desired e-mail settings, be sure to select the **Test host** (Green Arrow) to ensure a positive test. If the test is not positive or passed, then the parameters are not set correctly.

Important: After passing the **Test host**, select **Save**.

Find Outgoing Mail settings (Example: Yahoo)



The screenshot shows the Yahoo Mail help page for POP server settings. The page title is "POP server settings for Yahoo Mail". Below the title, there is a paragraph explaining POP. The page is divided into two sections: "Incoming Mail (POP) Server" and "Outgoing Mail (SMTP) Server". The "Outgoing Mail (SMTP) Server" section is highlighted with a blue box, and a blue arrow points from the text "Use 'Outgoing' Mail properties" to this section.

YAHOO! HELP UK & IRELAND

Help Central > Article

POP server settings for Yahoo Mail

POP lets you connect your Yahoo Mail account to a desktop mail client or mobile app. It uses 1-way synching, which downloads your email as a copy into the app, allowing you to move and delete them in the app without affecting the original emails. Here are the settings you'll need to configure your mail client or app.

Incoming Mail (POP) Server

- Server - *pop.mail.yahoo.com*
- Port - *995*
- Requires SSL - *Yes*

Outgoing Mail (SMTP) Server

- Server - *smtp.mail.yahoo.com*
- Port - *465 or 587*
- Requires SSL - *Yes*
- Requires TLS - *Yes (if available)*
- Requires authentication - *Yes*

Use "Outgoing" Mail properties

Figure 21 Example outgoing mail settings

Results Report Setup

The option **Results report setup** opens the **Automated Report Settings** window (Figure 22).

The settings allows the administrator:

- to enable auto processing, and
- to select the types of reports generated upon the act of auto processing.

For more information about auto-processing, refer to **Chapter 10**, “ProteoAnalyzer – Automated Analysis”.

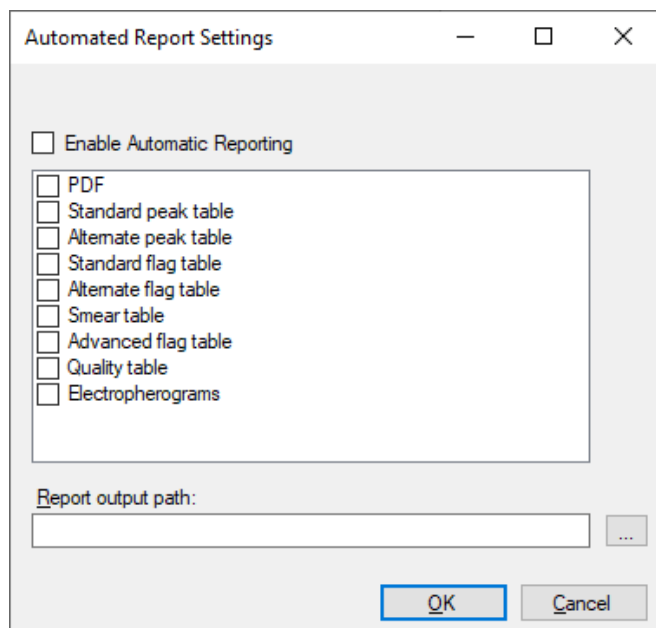


Figure 22 The Results Report Setup screen

Checking the **Enable Automatic Reporting** turns the auto-processing function on/off. When the auto-processing function is selected, the program will call a ProSize executable, process the data, and then export the desired results (PDF, standard peak table, etc.). For a complete description of each of these data types, refer to the ProSize data analysis software manual, or to **Chapter 10**, “ProteoAnalyzer – Automated Analysis” which gives a detailed description of auto processing.

NOTE

In order for auto-processing to work correctly, the name of the ProteoAnalyzer method must exactly match the name of the ProSize configuration file. For more details, please refer to **Chapter 10**, “ProteoAnalyzer – Automated Analysis”.

Event Report

The command **Event Report** provides a tabular report of the audit trail of the events that have occurred in the ProteoAnalyzer program.

Selecting the command **Event Report** from the **Admin** menu opens the **Select Date Range** window where the user can **Use all dates** or **Use selected date range** (**Figure 23**).

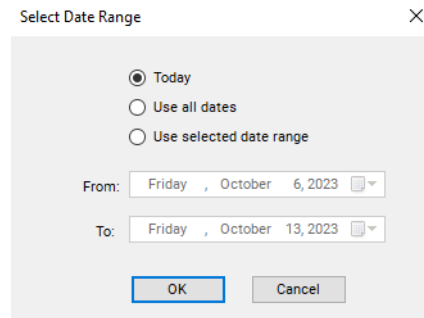


Figure 23 Event Report popup window

Users with both administrator and user privileges can view the **Event Report**.

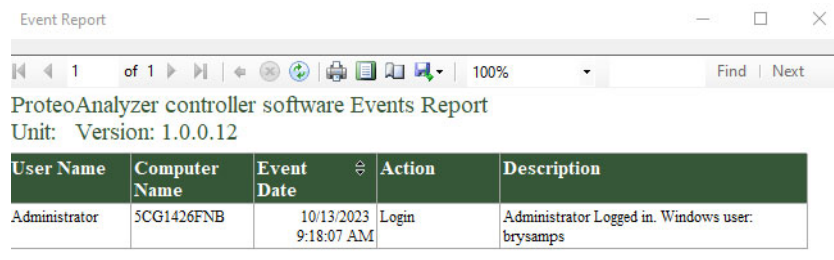
The event report contains the following information for each event log item:

- User name - User who was logged in.
- Computer name - Network name of the computer where the event occurred.
- Event date
- Event code action
- Description

After selecting the appropriate date range in the **Select Date Range** window and selecting **OK**, an Event Report is generated (**Figure 24**).

ProteoAnalyzer Software – Admin Menu

Event Report



User Name	Computer Name	Event Date	Action	Description
Administrator	5CG1426FNB	10/13/2023 9:18:07 AM	Login	Administrator Logged in. Windows user: brysamps

Figure 24 Event Report example

The icons along the top of the **Event Report** follow standard Windows function nomenclature and are summarized in **Table 8**.

Table 8 Event Report icons and descriptions

Icon	Description
	Page Selection
	Back to Parent Report
	Stop Rendering (i.e. Stop Report Generation)
	Refresh
	Print
	Print Layout
	Page Setup
	Save
	Zoom

Error Report

The command **Error Report** is used for advanced troubleshooting.

Selecting the command **Error Report** from the *Admin menu* opens the **Select Date Range** window where the user can **Use all dates** or **Use selected date range** (Figure 25).

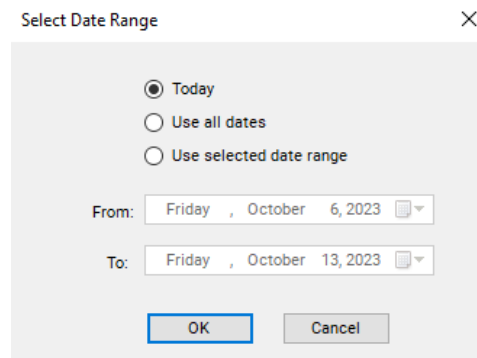


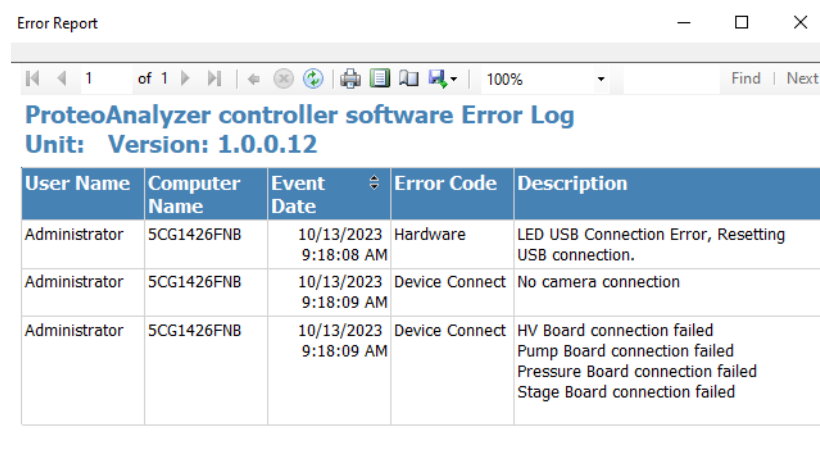
Figure 25 Error Report popup window

The error report captures the following information:

- Software exceptions and Hardware errors detectable by the software
- User Name - The user who was logged in when the error occurred
- Computer Name - Network name of the computer where the error occurred
- Event Date
- Error Code
- Description

After selecting the appropriate date range in the **Select Date Range** window and selecting **OK** an **Error Report** is generated (**Figure 26**).

The icons along the top of the report follow standard Windows function nomenclature and are summarized in **Table 8**.



User Name	Computer Name	Event Date	Error Code	Description
Administrator	5CG1426FNB	10/13/2023 9:18:08 AM	Hardware	LED USB Connection Error, Resetting USB connection.
Administrator	5CG1426FNB	10/13/2023 9:18:09 AM	Device Connect	No camera connection
Administrator	5CG1426FNB	10/13/2023 9:18:09 AM	Device Connect	HV Board connection failed Pump Board connection failed Pressure Board connection failed Stage Board connection failed

Figure 26 Error Report example



4

ProteoAnalyzer Software – Utilities Menu

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- Capillary Alignment 52
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- Prime 61
- Solution Levels 62
- Clean Reservoir Vent Valve 63
- Results Dashboard 64

This chapter describes the ProteoAnalyzer software in more detail on the commands of the Utilities menu.

Utilities menu

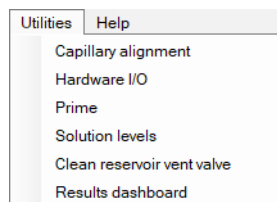


Figure 27 Utilities menu commands

Capillary Alignment

The menu command **Capillary alignment** is required when a new capillary array is installed. It may also be performed to address issues as part of a troubleshooting exercise.

There are two ways to perform a capillary alignment, though Method A is the recommended and easiest method of alignment:

- A) Alignment from a file
- B) Alignment without dye

NOTE

When capillary alignment is selected from the Utilities menu, the **Capillary Alignment Prep** window will open a prompt with the option to fill the capillaries with dye (**Figure 28**). This message is a carryover from the QC process and is not relevant to customer alignment methods. To continue with the capillary alignment, click no.

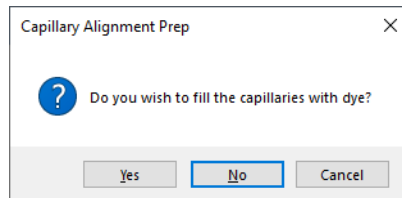


Figure 28 Capillary Alignment Prep window.

Method A – Capillary Alignment from a File

- 1 Select **No** when prompted from the **Capillary Alignment Prep** window.
This will open the real time view of the *Capillary Alignment* window (Figure 29).

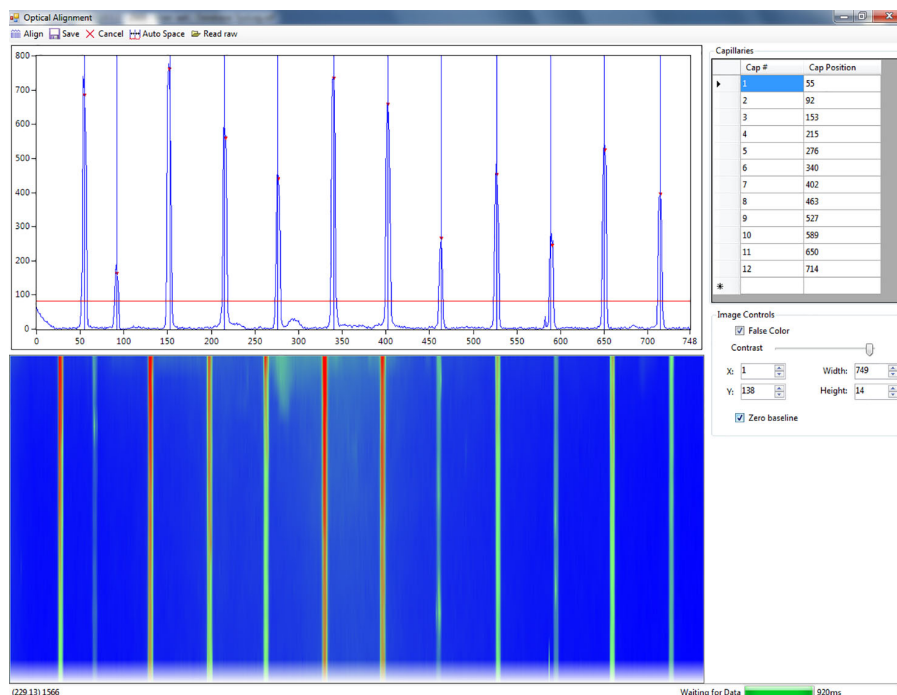


Figure 29 Real time capillary alignment popup window (example shows 12-capillaries)

- 2 If the capillary window needs to be redrawn, please refer to steps 2-6 of the Method B procedure later in this document.

NOTE

Skip to step 5 if the window does not need to be changed and a run has already completed with the currently installed capillary array.

- 3 Once the window has been drawn, proceed to click **Align** and then **Auto Space** to ensure all blue vertical lines are evenly spaced between the first and last capillary peaks.
- 4 Click **Save**, which will close the capillary alignment screen. Perform a test separation with lower marker and dye in each well. The run needs a peak to show up in each capillary.
This file will be used for the alignment.

- 5 From the top menu bar of the *Capillary Alignment* window, select **Read raw**.
- 6 Navigate to the raw file saved location using the Windows prompts.

The default saved location of raw data is:

C:/Agilent Technologies/Data/(Date: YYYY MM DD)/(Time: XXH XXM).

- a Select the latest raw file (i.e., the last run file).

The *Align from File* window will open (**Figure 30**). It allows you to align the capillaries from the selected run file. The toolbar of the *Align from File* windows is described in **Table 9**.

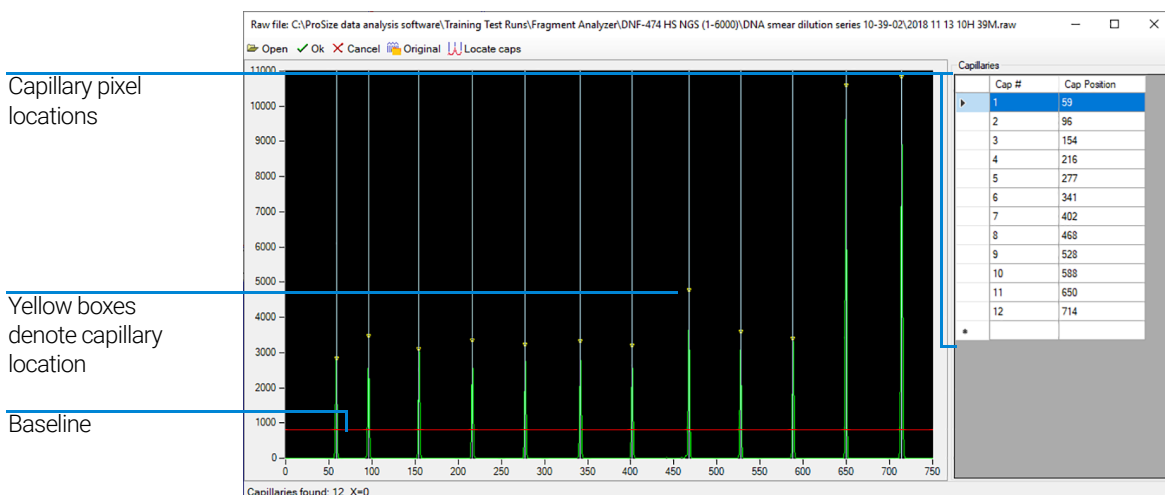


Figure 30 Align from file popup window for 12-capillary system

Table 9 Align from file toolbar functions

Icon	Description
	Opens a new file.
	Accepts changes to the file (i.e., capillary locations).
	Cancels any actions and closes the file.
	Locates the original capillary positions used when the selected file ran.
	Locates the capillaries based on peak positions in the selected open file. Note: Move the red baseline up so that only the peaks of interest are integrated and not noise from the baseline.

- 7 Left-click the red baseline and draw it upwards off the bottom of the graph but not above the top of capillary peaks, as shown in **Figure 30**.

- 8 Select **Locate caps** from the toolbar of the *Align from file* window.

The capillary peaks will be located and a yellow box is placed at the apex of the selected capillaries denoting the capillary pixel location.

The bottom left corner of the window states the number of capillaries found. This should be equal to 12.

If necessary, adjust the capillary positions:

- To manually adjust a capillary position, left-click on the white line showing the capillary position and drag it left or right to the desired location.
 - To zoom-in for desired resolution, right-click and select **Zoom** (and dragging the appropriate area).
 - Should the number of capillaries be off due to too many or too few capillary positions chosen, adjust the red baseline and repeat the steps above.
 - To insert or delete a capillary position, right-click on the black area of the graph or the capillary pixel location table to the right of the graph.
- 9 Once the desired number of capillaries is located, select **OK** from the *Align from File* toolbar. This will save any changes made to the capillary alignment and close the *Align from File* window, returning you to the *Capillary Alignment* window.
- 10 Select **Save** from the *Capillary Alignment* window.

From this point forward the instrument will use these saved pixel locations for all future runs.

Method B – Capillary Alignment without Dye

NOTE

An optical alignment can only be performed without dye for a 12- capillary array.

- 1 Select **No** when from the **Capillary Alignment Prep** window (see **Figure 28** on page 52).

This will open the real time view of the *Capillary Alignment* window.

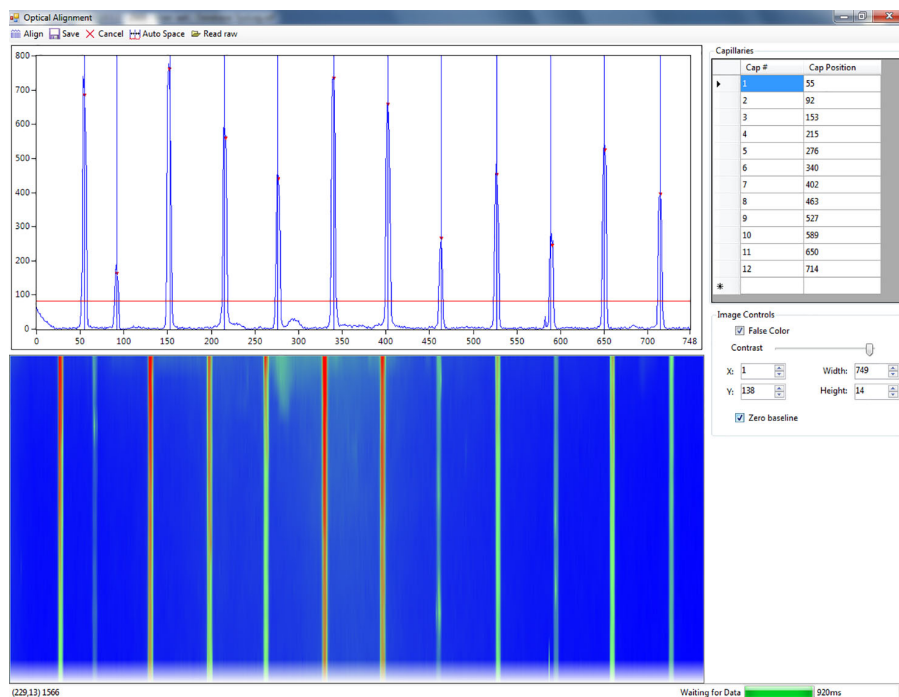


Figure 31 Real time capillary alignment popup window (example shows 12-capillaries)

- 2 Right-click on the blue display area and select **Reset All** to reset the camera array window.
- 3 Adjust the contrast slide bar to the left to brighten the display (**Figure 32**).

- 4 Draw a box around the capillary array display area. Left-click and drag the appropriate area (**Figure 32**).

NOTE

Avoid the Top Red CCD Camera Reference Area and the Capillary Alignment References.

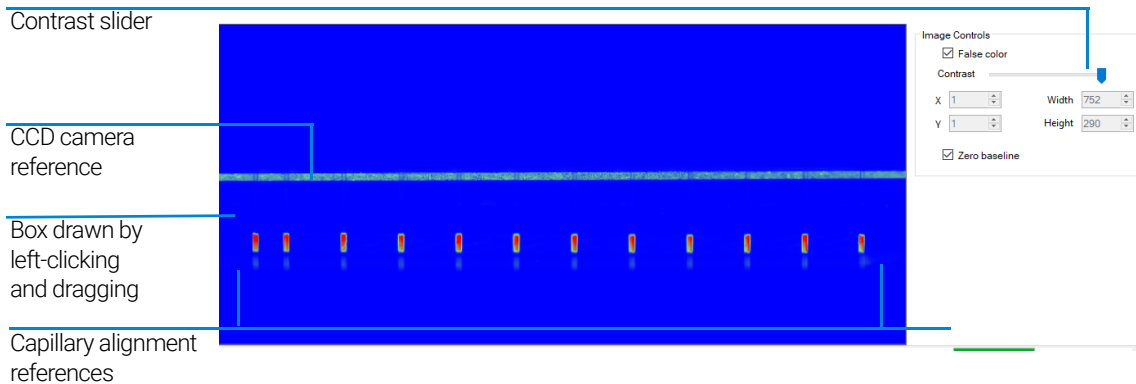







Figure 32 Capillary alignment display – window reset

- 5 After the box is drawn, right-click and select **Set Camera Window**.
- 6 Adjust the height to 14.

Table 10 Capillary alignment display menu options

Icon	Description
	Aligns cursors to peaks.
	Saves changes to the alignment and exits the window.
	Cancel any actions and closes the file.
	Auto locates the capillary positions based off the first capillary position. Positions will need manual adjustment.
	Opens the Align from File window allowing the user to complete the capillary alignment using a previously run file.

- 7 Adjust the red baseline seen in **Figure 29** until a red dot is observed above each capillary peak. This determines which capillary peaks are selected. It is important to ensure this red line is above the baseline.

Capillary Alignment

- 8 Select **Align** from the menu of the top capillary alignment display area. A blue vertical line will be placed through the center of each capillary. If the blue lines are not in the exact center of each peak, adjust the lines by left-clicking and dragging to the desired location.
- 9 Select **Align** every time the red baseline is moved. This ensures that the instrument has selected the peak for integration and places the blue vertical alignment line in the middle of each peak (corresponding to where the red dots are present).
- 10 Select **Save** from the menu of the top capillary alignment display area to save the capillary locations and close the *Capillary Alignment* window.

Hardware I/O

The command **Hardware I/O** is available to users with administrator privileges and is used for troubleshooting the instrument.

Selecting the **Hardware I/O** command from the **Utilities** menu opens the **Hardware Testing Screen** (Figure 33).

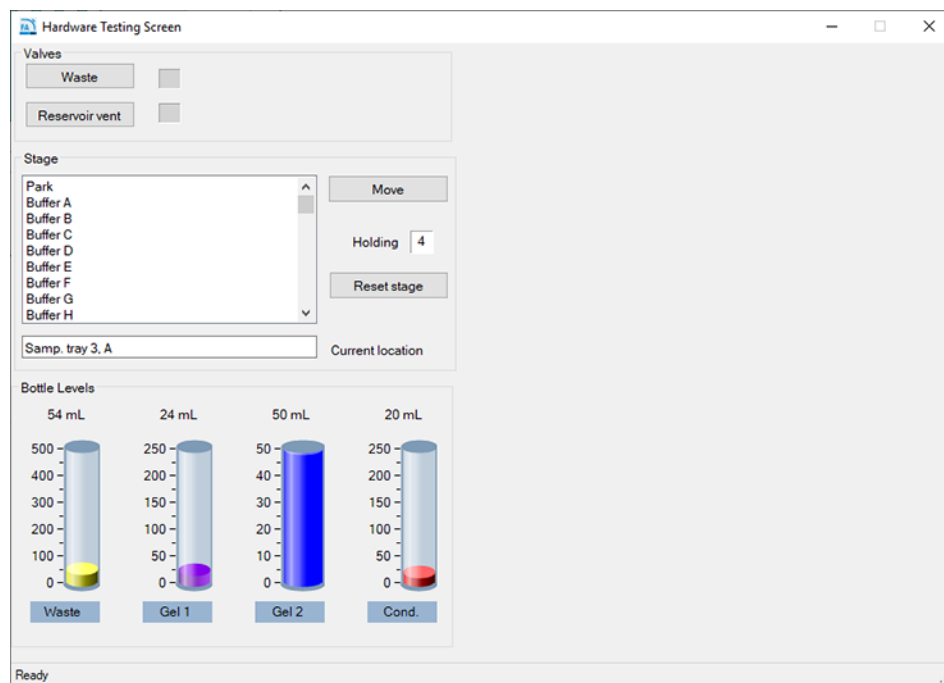


Figure 33 Hardware Testing Screen

An overview of the functions available in the Hardware Testing Screen is listed in **Table 11**.

Table 11 Functions of the Hardware Testing Screen

Function	Description
Valve > Waste	Activates (toggles) the valve open (open circle) or closed (dark circle).
Valve > Reservoir Vent	Activates (toggles) the valve open (open circle) or closed (dark circle).
Function of LED	Turns the LED lamp off and on.
Stage > Move	Move tray to the selected position.
Stage > Reset Stage	Allows the user to reset the stage position should a drawer be opened before the stage finishes its movement.
Bottle Levels	Gives a visual indication (simulation based on calculated usage) of the amount of reagents available in the system.

Prime

The **Prime** command allows the user to prime any of the three available reagent bottle lines. This is useful when a user wants to purge a line containing an old gel or fluid with a new gel or fluid (should a new solution be added to the instrument). Another reason for priming is to remove air bubbles that may be found in the reagent lines after extended periods of sitting idle.

Selecting the **Prime** command from the **Utilities** menu opens the **Prime** window (Figure 34). The prime functions are discussed in Table 12.

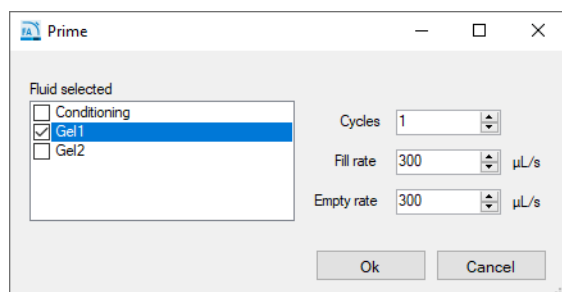


Figure 34 Prime window

Table 12 Functions of the Prime window

Function	Description
Fluid selected	Allows the user to select which reagent line to prime.
Cycles	Refers to number of cycles (1-10) of the syringe to complete. 1 cycle is generally sufficient.
Fill rate	Allows the user to adjust the syringe fill rate up and down 0-1000, the default setting is 300 $\mu\text{L/s}$.
Empty rate	Allows the user to adjust the syringe empty rate up and down 0-1000, the default setting is 300 $\mu\text{L/s}$.

Solution Levels

The command **Solution levels** allows the user to adjust the volumes added to the reagent bottles and adjust the waste bottle level when emptied.

The ProteoAnalyzer software tracks the solution levels as the instrument is used. This ensures that the instrument has enough fluids for all of the planned runs.

If the solution levels are low, the program will issue a warning and ask the user to adjust the Solution Levels before it can proceed with a separation.

Selecting the **Solution levels** command from the **Utilities** menu opens the **Check Solution Volumes** window (Figure 35).

	Volume (mL)	Solution name
Gel 1	50.0	Protein Gel
Gel 2	50.0	1.0 M NaOH
Conditioning	50.0	
Waste	0.0	

Figure 35 Check Solutions Volumes window

- When solutions are re-filled, open this window and enter the correct solution levels (mL) for each container:
 - Use the up and down arrows or type the solution level in each entry field to adjust solution levels.
 - To save the changes to solution levels, select **OK**.

CAUTION

Incorrect solution levels.

For the program to run correctly (i.e., to issue the correct warning), the solution level in the software has to accurately reflect the level of solution in the instrument.

- ✓ Enter the correct solution levels into the program each time that new solutions are placed onto the instrument.

Clean Reservoir Vent Valve

The command **Clean reservoir vent valve** allows the user to clean the reservoir vent valve manually.

Selecting this command from the **Utilities** menu opens the reservoir vent valve and the waste valve and displays the **Clean Reservoir Vent Valve** window (Figure 36).

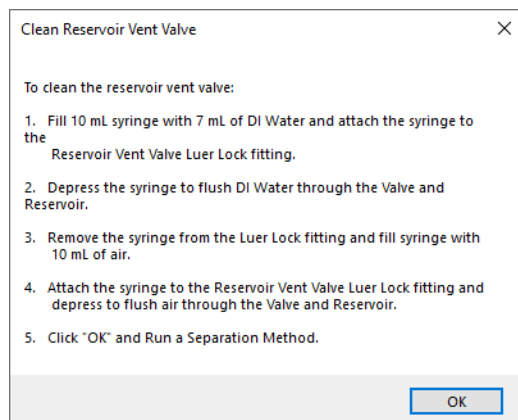


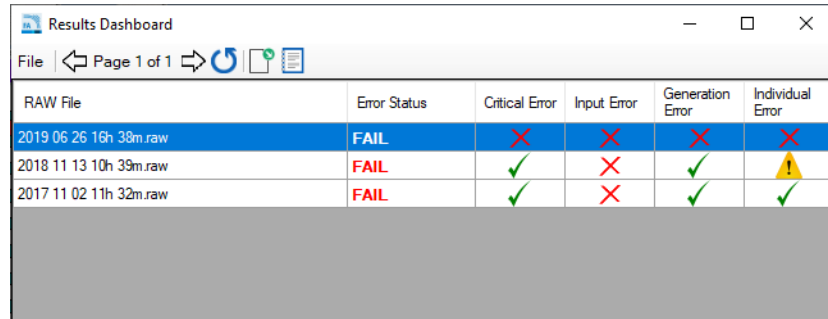
Figure 36 Clean reservoir vent valve screen

Follow the steps outlined in **Figure 36** to clean the reservoir vent valve.

Results Dashboard

The command **Results dashboard** allows the user to quickly view the status of auto-processed data.

Figure 37 shows an example in the **Results Dashboard** window.



The screenshot shows a window titled "Results Dashboard" with a toolbar containing icons for File, navigation, refresh, and help. Below the toolbar is a table with the following data:

RAW File	Error Status	Critical Error	Input Error	Generation Error	Individual Error
2019 06 26 16h 38m.raw	FAIL	✗	✗	✗	✗
2018 11 13 10h 39m.raw	FAIL	✓	✗	✓	⚠
2017 11 02 11h 32m.raw	FAIL	✓	✗	✓	✓

Figure 37 Results dashboard output

For more information about the **Results dashboard** and the window toolbar, refer to **Chapter 10**, "ProteoAnalyzer – Automated Analysis".

5

ProteoAnalyzer Software – Help Menu

Help Menu 66

User Manual 66

About 66

About Firmware 66

License Agreements 67

This chapter describes the ProteoAnalyzer software in more detail on the commands of the Help menu.

Help Menu



Figure 38 Help menu commands

User Manual

Navigating to the command **User manual** will provide access to the system user manual.

About

The **About** command opens an **About ProteoAnalyzer** window displaying the version number of software, hardware serial number and copyright information.

About Firmware

The **About firmware** command opens an **About Firmware** window displaying the version numbers of the High Voltage Board, Pump Board, and Motion Control Board.

License Agreements

The **License Agreements** command opens the End User License Agreement (EULA), outlining the terms of the software agreed by using the software. This agreement also contains links to Agilent's privacy policy.

The **Open Source Notice** menu item displays a notice regarding the use of third party software made available under open source licenses within this software, with any relevant links to those terms.



6 ProteoAnalyzer Software – Operation Tab

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Tray Selection and Sample ID 71

Experimental Run Controls and Adding to Queue 73

Method Queue 80

This chapter describes the ProteoAnalyzer software in more detail on the Operation tab.

Operation Tab Overview

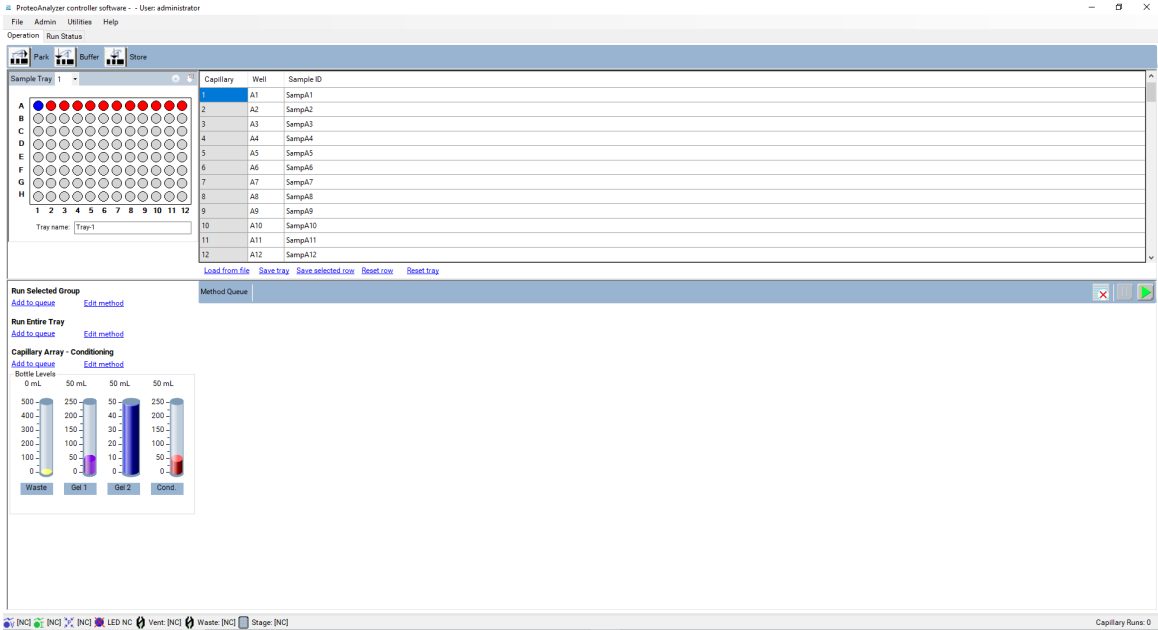





Figure 39 ProteoAnalyzer main screen – Operation tab

Hotel Position Icons

There are three hotel positioning icons located at the top of the **Operation** tab, as seen in **Figure 39**. The icons and their function are discussed in **Table 13**.


Table 13 Hotel position icon functions

Icon	Description
 Park	This command is used to place the existing tray being held by the stage robot back into its respective drawer and move the stage platform to the bottom of the instrument.
 Buffer	This command is used to pick up the buffer tray from the buffer drawer and move it up against the capillary array.
 Store	This command is used to place the existing tray being held by the stage robot back into its respective drawer and then pick up the storage solution tray to move it up against the capillary array.

Tray Selection and Sample ID

Select the sample tray to be used from either the **Sample Tray** drop-down list or the colored tab tray selection, depending which configuration is set (**Figure 40**).

NOTE

The configuration can be set by selecting the  icon located in the upper right corner of the window shown in **Figure 40**.

Configure the Visual Style of Tray Selection Window

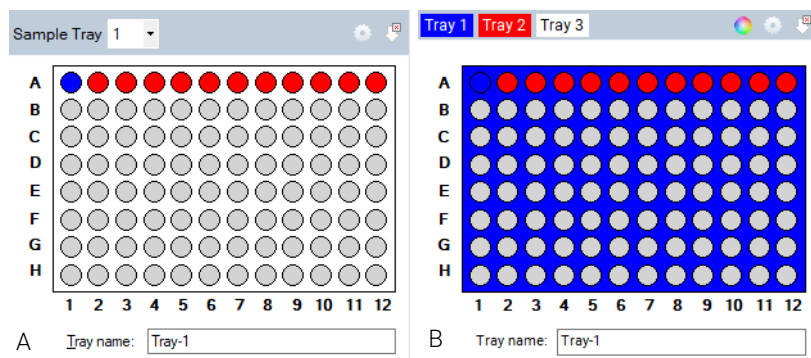


Figure 40 Classic drop-down tray selection (left), and colored tab tray selection (right).

- 1 In the tray window, select .

The **Visual preferences dialog** window opens (**Figure 41**).

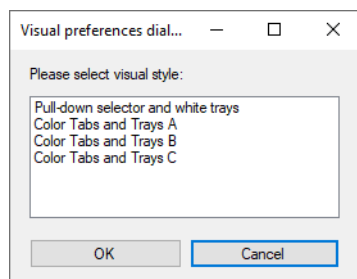



Figure 41 Visual preferences dialog window.

- 2 Choose between the sample tray drop-down list or the colored tab tray selection as shown in **Figure 40**.
- 3 If you use the tab tray selection window, select  to change the color of each sample tray in the **Color selection** window.

4 To select a row from the 96-well plate depicted in the sample/sample tray window, left-click once in that row (**Figure 40**). To select a new row left-click on another row.

5 To clear a row selection, select . (**Figure 40**)

The **Tray name** dialog box allows you to input a name for the tray being run (**Figure 40**). Alternatively, select this dialog box and use a barcode scanner to import sample names for the plate being run (for more information, refer to **Chapter 9**, “ProteoAnalyzer – Sample Name Entry”).

Enter sample information in the **Sample ID** section of the main screen. (**Figure 42**)

Sample names and information can also be saved or loaded using .txt or .csv files. These functions are discussed in **Table 14**.

Capillary	Well	Sample ID
1	A1	SampA1
2	A2	SampA2
3	A3	SampA3
4	A4	SampA4
5	A5	SampA5
6	A6	SampA6
7	A7	SampA7
8	A8	SampA8
9	A9	SampA9
10	A10	SampA10
11	A11	SampA11
12	A12	SampA12

[Load from file](#)
[Save tray](#)
[Save selected row](#)
[Reset row](#)
[Reset tray](#)

Figure 42 Sample information editor

Table 14 Sample information editor functions

Item	Description
Load From File	Allows the ability to load sample names from a .txt or .csv based file. See Chapter 9 for further information.
Save Tray	Allows the user to save the information entered for an entire sample tray.
Save Selected Row	Allows the user to save the information entered for the selected row of a sample tray.
Reset Row	Resets the selected row to the default sample ID setting.
Reset Tray	Resets the entire sample tray to the default sample ID settings.

Experimental Run Controls and Adding to Queue

The ProteoAnalyzer software provides pre-loaded methods for both Capillary Array Conditioning and Experimental Methods for each Analysis Kit offered by Agilent.

The *Experimental Run Controls* shown in **Figure 43** shows the settings to **Run Selected Group**, **Run Entire Tray**, and **Capillary Array – Conditioning**.

Reagent Levels of the bottles are also shown.

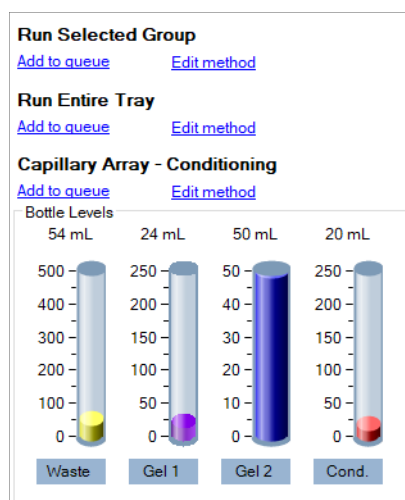


Figure 43 Experimental run controls

Run Selected Group or Run Entire Tray – Edit Method

Selection of the **Edit method** option will show the method editor pop-up window exhibited in **Figure 44**. When logged in under user level access, the edit method window will not be available. It will be replaced with **View method** as the user level does not have access to change method parameters. Only logins under administrator level can edit methods.

ProteoAnalyzer Software – Operation Tab

Experimental Run Controls and Adding to Queue

Separation Method: 5191-6640LM – ProteoAnalyzer Broad Range Kit LM only.mthds

Full conditioning Gel prime to buffer Gel selection: Gel 1

Gel prime

Prerun Voltage: 5.0 kV Time: 30 sec.

Rinse Tray: Buffer Row: B Dip count: 2

Marker injection Row: A

Voltage: 0.00 kV Time: 20 sec.

Rinse Tray: Buffer Row: A Dip count: 1

Sample injection Voltage: 7.00 kV Time: 10 sec.

Separation Voltage: 9.0 kV Time: 20.00 min.

OK
Cancel

Figure 44 Run selected group and run entire tray edit method editor window

The Method editor window allows for customization of the run parameters for a CE separation.

Full conditioning, the gel-prime, and gel prime to buffer are not editable and cannot be disabled.

Selecting the check box next to the individual parameter can enable different steps and parameters. The individual parameters are discussed in [Table 15](#).

Optimum capillary conditioning values are preloaded and defined for each method. Refer to each method Kit Guide of interest for further definition of these values.

Table 15 Method editor window functions.

Item	Description
Gel selection	Using the drop-down menu, the user can select the Gel 1 or the Gel 2 reagent bottle position. This assigns which gel bottle position is used to fill the capillaries with gel for the CE separation.
Prerun	When enabled, this option will perform a voltage pre-run from the buffer tray location. A short pre-run is recommended to normalize and condition the gel inside the capillaries.
Rinse	The rinse option allows the user to dip the capillary tips into the selected position, which rinses both the capillary tips and electrodes between the pre-run and sample or marker injection. The tray position for sample rinse (row) and # dips (Dip count) can be altered as well.
Marker injection	The user has the option to change the Voltage, Pressure, and Time parameters.
Rinse	The rinse option allows the user to dip the capillary tips into the selected position, which rinses both the capillary tips and electrodes between marker injection and sample injection (or, if marker injection is not selected, this step is a second rinse between pre-run and sample injection). The tray position for sample rinse (row) and # dips (Dip count) can be altered as well.
Sample injection	Selection of a Voltage, Pressure, and Time for the voltage or vacuum injection.
Separation	Allows the entry of Voltage and Time of the CE separation.

Administrator level users can **Load** a new method, **Save as** a new method with a unique name, select **Save** to accept the changes and close the window, or **Cancel** to close the method editor window without accepting any changes made.

NOTE

When creating a new method with a unique name the user will need to make a corresponding Global Configuration in ProSize data analysis software with a matching name, please see the ProSize software user manual for more complete instructions.

Run Selected Group or Run Entire Tray – Add to Queue

Selecting the **Add to queue** option opens the **Separation Setup** window as seen in **Figure 45**.

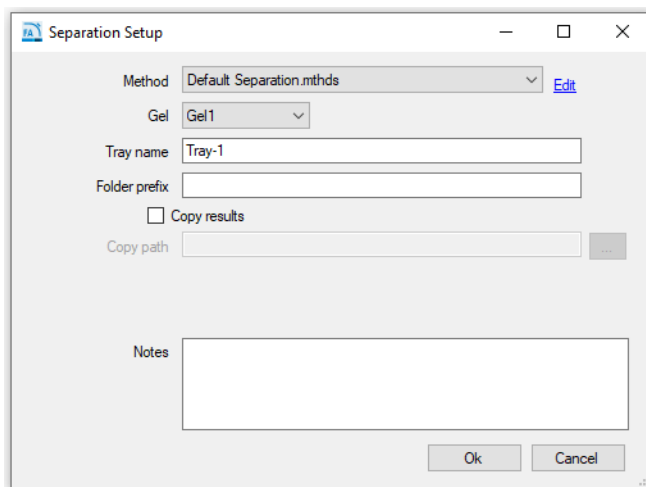


Figure 45 Separation Setup window

The settings of the **Separation Setup** window are discussed in **Table 16**.

Table 16 Separation setup window functions

Item	Description
Method	Methods can be selected from the drop-down menu. A user with administrator privileges can also select Edit to change any parameters of the method by opening the method editor window in Figure 44 . User level access only allows the user to View the method file selected.
Gel	The user can toggle the gel bottle location to the desired bottle to use for the separation method without having to alter a predefined method.
Tray name	The tray name appears as input by the user on the main screen or the default name appears. The user may edit this field by typing in the provided box.
Folder prefix	The folder prefix allows the user to add a prefix to the folder name where the results files will be written.
Copy results / Copy path	The default directory location for the data is C:\Agilent Technologies\Data. The user may select the Copy Results option and choose to copy the saved data to a different location by selecting the [...] option.

Table 16 Separation setup window functions

Item	Description
Create Size Calibration File	This is used for automated analysis (Chapter 10 , "ProteoAnalyzer – Automated Analysis"). When selected (and auto-processing is enabled), the run will be used to create a size calibration file, which will be used to calibrate the size of fragments in subsequent files. Upon completion of the run, a size calibration file will be named and placed into a file directory as defined in the "Size cal file" section. Note: If both Create Size Calibration File and Use Size Calibration File boxes are unchecked, the system will assume the ladder is present in A12 as defined in the kit manuals.
Use Size Calibration File	This is used for automated analysis (Chapter 10 , "ProteoAnalyzer – Automated Analysis"). When checked (and auto-processing is enabled) the program will use the size calibration file defined in the Size cal file section to define the sizes.
Size Cal. File	This is used for automated analysis (Chapter 10 , "ProteoAnalyzer – Automated Analysis"). The user defines a name and file location of the size calibration file. When Create Size Calibration File is checked, the program will write a .SCAL file to the defined name and location of the file. When Use Size Calibration File is checked, the program will import and use the .SCAL file at the defined location.
Notes	The section allows for the addition of any additional information the user may require for a set of samples.
Merge rows (Run Entire Tray option only)	When selected, will merge 8-rows of 12-capillary "runs" into a single run file. The original non-merged rows are also available for data processing. This function is useful when running 8 rows of 12, and the user wants to view the data file as a single 96-well file.

After the appropriate method is chosen from the drop-down menu select **OK** to add the chosen method to the **Method Queue**, or **Cancel** to close the window.

Capillary Array - Conditioning

The ProteoAnalyzer software provides preloaded capillary conditioning methods for cleaning the capillary array.

Administrator level logins can also select to create a method of their choosing by selecting the **Edit Method** option seen in **Figure 46**. User level logins can only view conditioning methods in the software.

Selecting the check box next to the individual step can enable different bottles to be used for the conditioning. The individual parameters are discussed in **Table 17**.

Conditioning Method: Default Conditioning.mthdc

<input checked="" type="checkbox"/> Step #1	Solution	Conditioning			
Fill pressure	280	PSI	Time	3.0	min.
Flow rate	200	μL/s	Tray	Waste	Row A
<input checked="" type="checkbox"/> Step #2	Solution	Gel 1			
Fill pressure	280	PSI	Time	3.0	min.
Flow rate	200	μL/s	Tray	Waste	Row A
<input type="checkbox"/> Step #3	Solution	Conditioning			
Fill pressure	0	PSI	Time	1.0	min.
Flow rate	1	uL/s	Tray	Waste	Row A

Figure 46 Conditioning method editor

Table 17 Conditioning method setup window functions

Item	Description
Step #1, 2, or 3	Enables/disables the step to be used.
Solution	Allows selection of the Conditioning solution, Gel 1 , or Gel 2 reagent bottles for use.
Fill Pressure	Default value is set to 280 psi. This can be changed from 1-300 psi.
Flow Rate	Default value is set to 200 uL/s. This can be adjusted from 1-1000 uL/s .
Time	This is set in minutes from 1 – 240.
Tray	Allows the user to select the tray and row (12 capillary unit only) to pump into when conducting the conditioning (the default is the waste tray—and is the best option for most users).

The user can **Load** a new method, **Save as** a new method with a unique name, select **Save** to accept the method and close the window, or **Cancel** to close the method editor window and discard the changes.

Selecting the **Add to queue** function will open the **Select Conditioning Method** window (**Figure 47**).

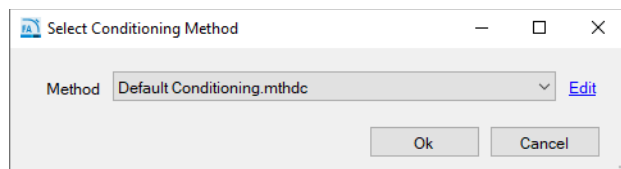


Figure 47 Select Conditioning Method window

A previously saved method can be chosen from the drop-down menu or a user with Administrator privileges can select **Edit** to view the conditioning method editor window seen in **Figure 46**. Users with user level access can only select a previously used method and **View** the method, but not edit a method.

After the appropriate method is chosen from the drop-down menu, select **OK** to add the chosen method to the **Method Queue**, or **Cancel** to close the window.

Method Queue

Once a conditioning method or separation method for a sample tray or row (12-capillary unit only) has been selected and added to the queue, the method name and tray location selected for injection will be shown in the **Method Queue** (Figure 48).

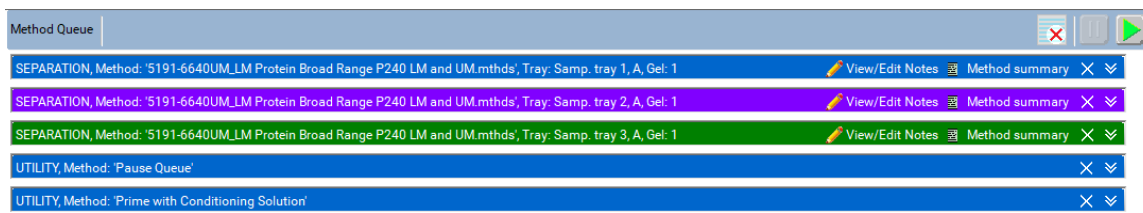


Figure 48 Method Queue

Figure 48 shows three sample separation methods ("runs") chosen from sample trays 1, 2, and 3 followed by a pause in the method queue and a priming method.

A **Pause** or **Prime** can be inserted into the method queue by right clicking in the Method Queue area of the screen. When **Insert Prime** is selected, the **Select Solution** window will appear prompting the user to choose the Priming fluid from a drop-down menu (Figure 49).

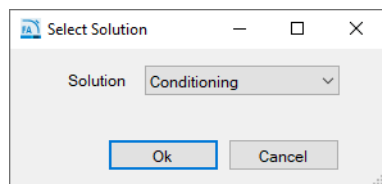


Figure 49 Select Solution popup window

Methods loaded into the method queue can be moved up or down based on the user's needs by left-clicking on the method and dragging it to the desired location in the queue.

To view the parameters for the separation method in the method queue select the **Method Summary** icon next to the separation method. A summary of the method will appear, as shown **Figure 50**.

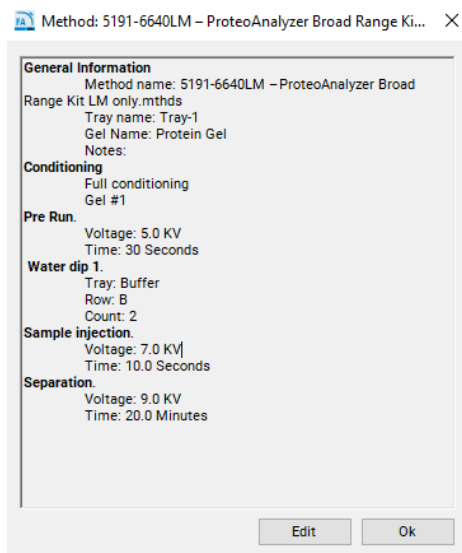



Figure 50 Method summary popup window




Selection of the **Edit** option from the **Method Summary** window allows the user to make final changes to the method if desired.

The **View/Edit Notes** option enables the user to make final changes or additions to the notes associated with and saved with the method. To delete a specific item on the queue, select the **X** icon next to the method summary. To delete all items in the queue, select  **Clear** from the Method Queue menu bar.

To show a detailed summary of the run parameters associated with a method on an item in the queue, select  **Down Arrows** next to the method summary.

There are three Run Controls for the Method Queue, **Clear the Method Queue**, **Pause the Method Queue**, and **Start the Method Queue**. These Run Controls are described in [Table 18](#), below.

Table 18 Method queue run controls

Item	Description
	Clear: Selection of this icon will clear all separation and conditioning methods, pauses, and primes from the queue.
	Pause: Selection of this icon will pause the method queue. The current method running will still complete. To restart the queue, select Start (below).
	Start: Selection of this icon will start the method queue. Once started the top method will disappear and the screen will switch to the Run Status tab. The next method will move up in the queue. Note: When you add a method or item to the queue, you must select Start to begin operation of the instrument.




7

ProteoAnalyzer Software – Run Status Tab

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This chapter describes the ProteoAnalyzer software in more detail on the Run Status tab.

Run Status Tab Overview

Once a Start command  has been selected (for more information, refer to section **Method Queue** on page 80), the display will switch from the **Operation** tab to the **Run Status** tab. The **Run Status** tab has several features, as shown below.

Stage Movement Animation

Whenever the stage moves from one position to another, the animation shown in **Figure 51** will show where the ProteoAnalyzer stage is moving to/from to give the user real-time view of what is happening.

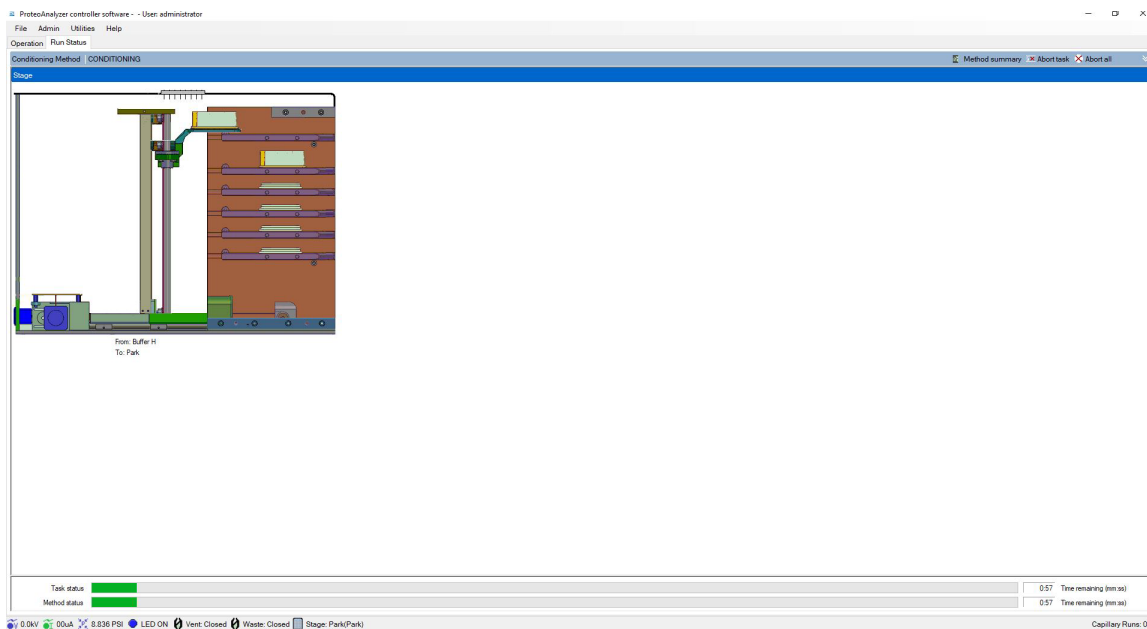


Figure 51 Stage movement animation

Conditioning Animation

When the ProteoAnalyzer instrument is performing a capillary array conditioning method, the following animation is shown (**Figure 52**). The animation gives a real-time view of what the instrument is doing during a conditioning sequence (including fluid flows, valve switches, etc.).

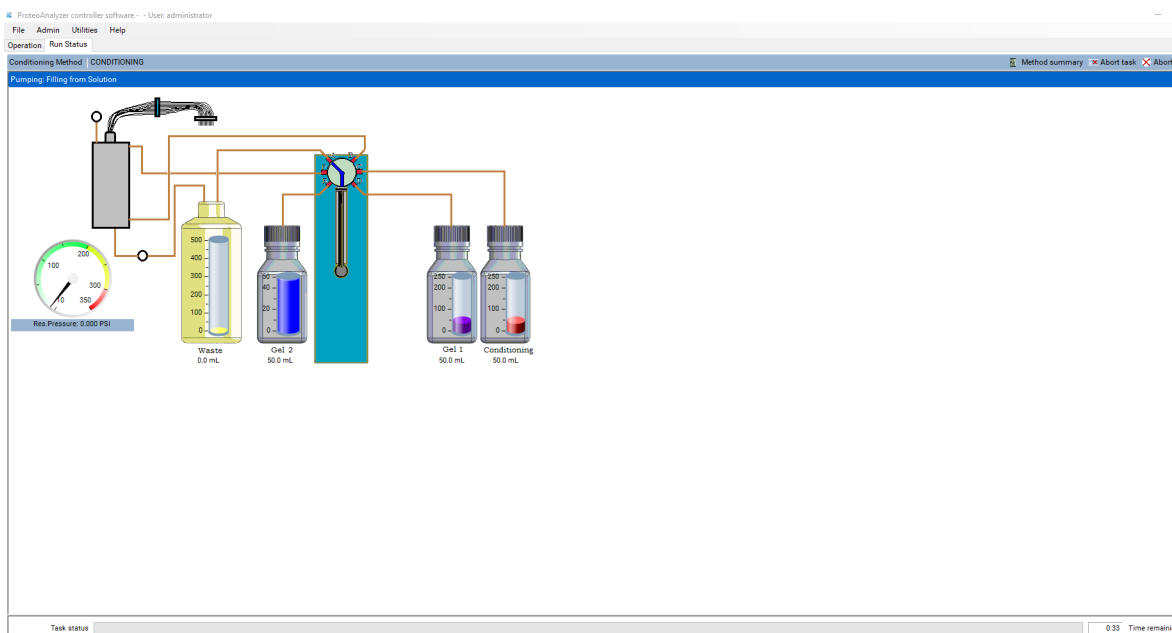


Figure 52 Conditioning animation

Pre-Run / Injection View

When the ProteoAnalyzer system is completing a voltage pre-run or injection, the screen as shown in **Figure 53** will appear. The screen displays the real-time voltage and current readings along with the voltage setting.

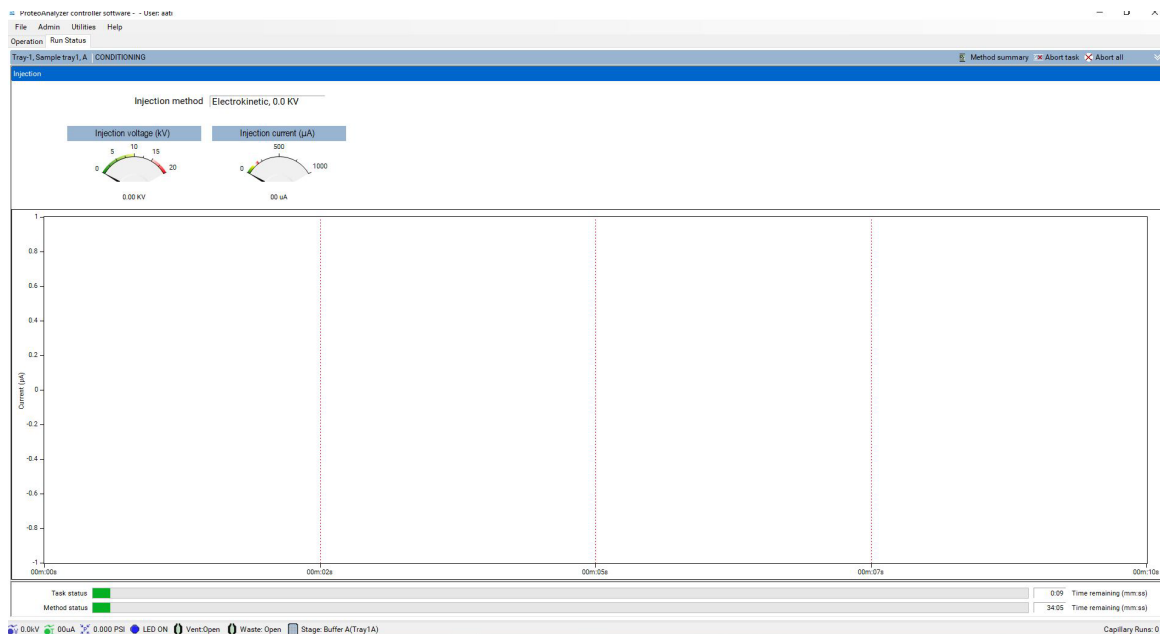


Figure 53 Pre-Run/Injection screen

Real-time Separation View

When the ProteoAnalyzer system performs a separation, the screen in **Figure 54** appears, which shows a real-time view of the separation.

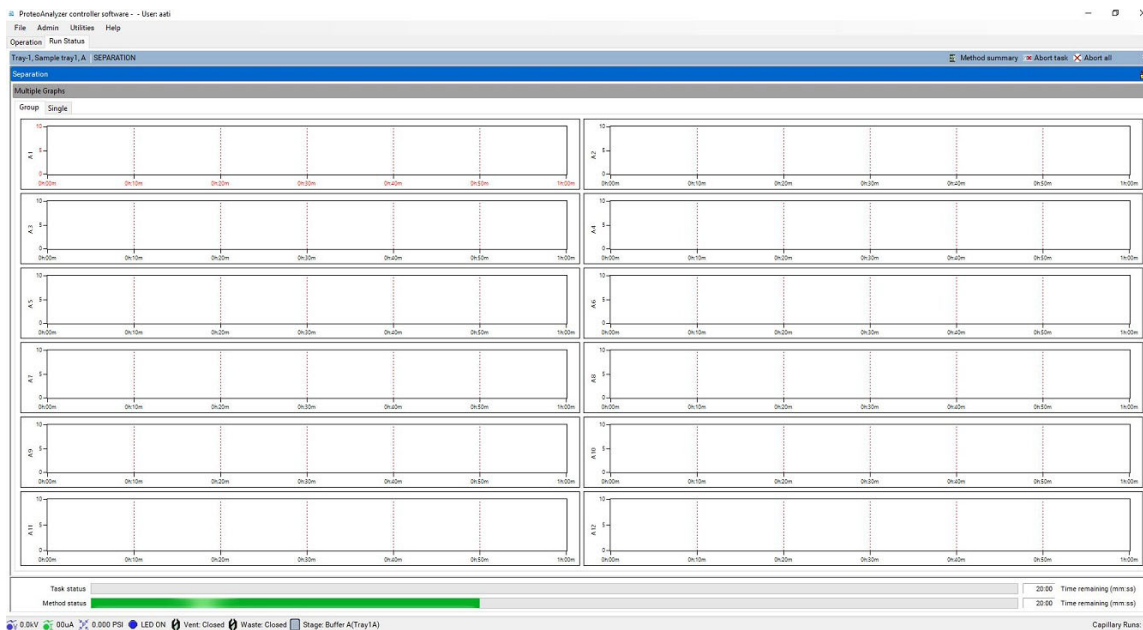


Figure 54 Real-time separation window





The user can view the run in a group of 12 electropherograms (as shown in **Figure 54**), or view individual electropherograms by selecting the **Single** tab located at the top.

NOTE

In order to correctly view the real-time separation data, the capillary array must be aligned prior to starting the separation. For instructions on aligning the capillary array, refer to **Chapter 1**, “ProteoAnalyzer Software – Utilities Menu”.

Other options available from the **Run Status** tab are discussed in **Table 19**.







Table 19 Run Status Tab options

Icon	Description
 Method summary	Opens a popup window showing the method summary for the current method being run.
 Abort Task	Aborts only the individual task being done, i.e., stage movement, pumping, or injection.
 Abort All	Aborts the entire Method being run and begins the next method in the queue. If no methods are found, it returns to the storage position. When selected the user will be presented with a popup screen asking to verify they want to Abort the current run.
	Shows the current for the separation being performed.
Task Status	Shows the status bar and time left for each individual task being accomplished, i.e., stage movement, pumping, or injection.
Method Status	Shows the status bar and time left for the entire method to complete.

Status Bar

The bottom bar of the ProteoAnalyzer software shows a real-time status bar containing important information about the instrument status. The icons and their function are discussed in **Table 20**.

Table 20 Instrument status information

Icon	Description
 6.0kV	Left-clicking on this icon will show the voltage level for the last 5 minutes.
 44uA	Left-clicking on this icon will show the current level for the last 5 minutes.
 0.0 PSI	Left-clicking on this icon will show the pressure level for the last 5 minutes.
 Vent: Open	Denotes if the Reservoir vent valve is open or closed.
 Waste: Closed	Denotes if the Waste valve is open or closed.
 Stage: Buffer A(Tray1A)	Denotes the location of the stage at that point in time.



8 ProteoAnalyzer Capillary Array

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Capillary Array Installation 104

Using the Capillary Array Wet Station for Storage 112

This chapter explains the essential operational parameters of the capillary array.

Capillary Array Parts

The ProteoAnalyzer instrument capillary array allows for direct parallel injection and separation of 12-samples at once. The capillary array is a user-replaceable hardware consumable, consisting of the frame, baseplate with alignment pins and circuit board containing the capillary inlets, HV lead and corresponding electrodes, the capillary array window, and capillary outlet bundle, as shown in **Figure 55**.

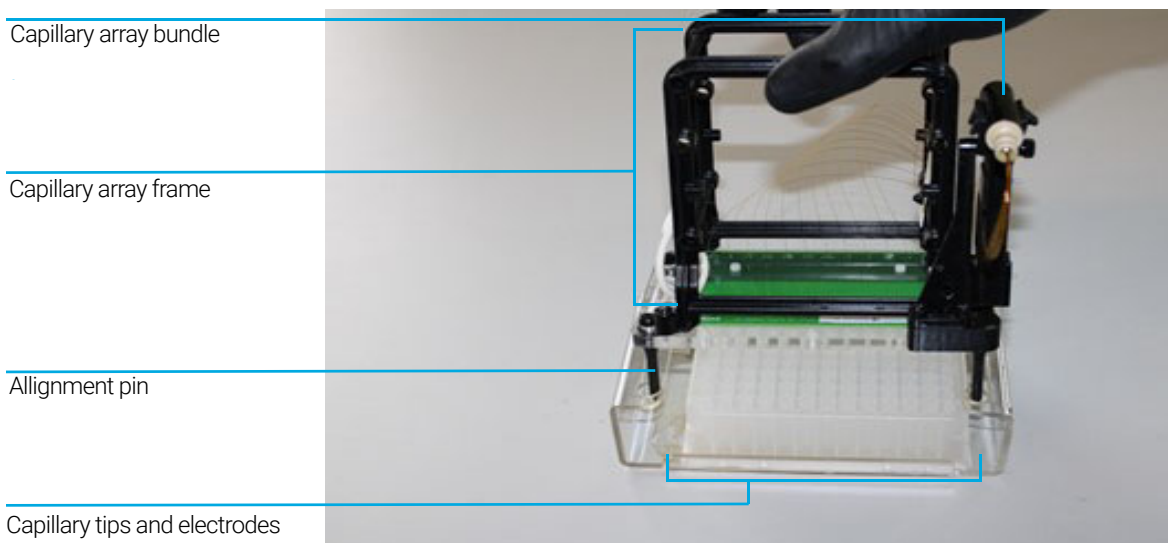


Figure 55 Capillary array parts

Removal of the Capillary Array

This section will provide a pictorial guide of the steps required to physically remove a capillary array cartridge from the ProteoAnalyzer instrument.

Before proceeding with capillary array removal, select the **Park** icon from the main screen to place the tray being held back into its drawer, and move the stage into a resting position.

- 1 Open the reagent door and top hood of the instrument:

First, open the reagent door to the side.

When the reagent door is open, the top hood flips upwards.



Figure 56 ProteoAnalyzer instrument

ProteoAnalyzer Capillary Array

Removal of the Capillary Array

- 2 Unplug the white high voltage supply cable from the top front panel, and place in the holder of the capillary array frame.

Holder with high voltage plug

High voltage cable connection

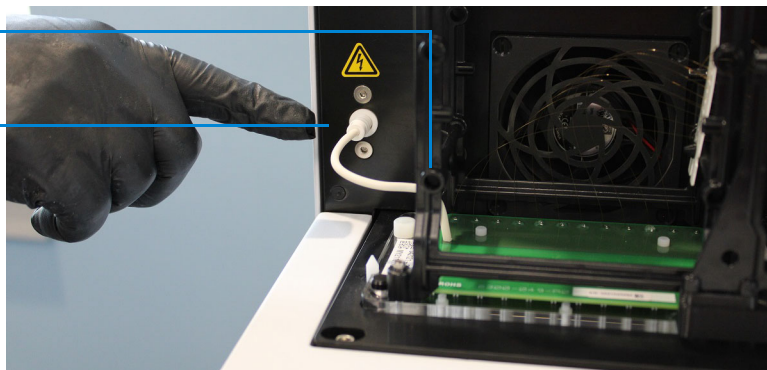


Figure 57 Instrument top compartment – high voltage supply cable

- 3 Use the allen wrench (supplied with the instrument accessory kit) to remove the two white screws that secure the light guide to the array window.

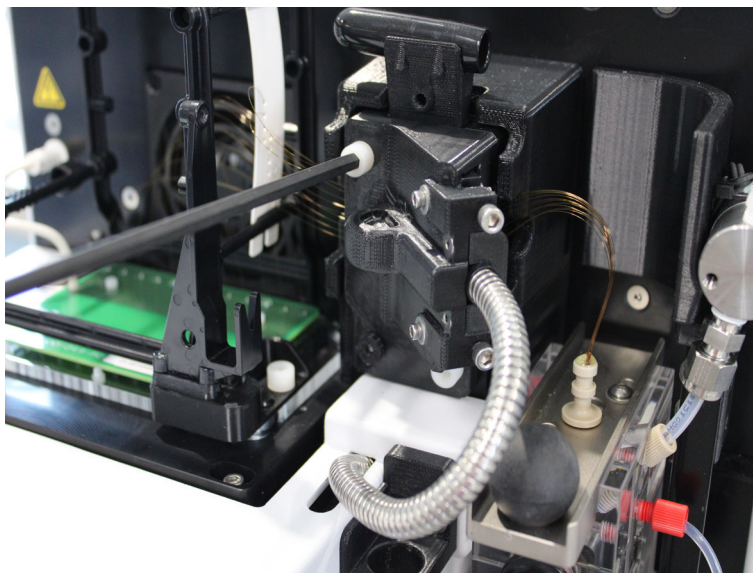


Figure 58 Instrument top compartment – unscrew light guide

- 4 Remove the light guide from the array window by carefully pulling out towards you.

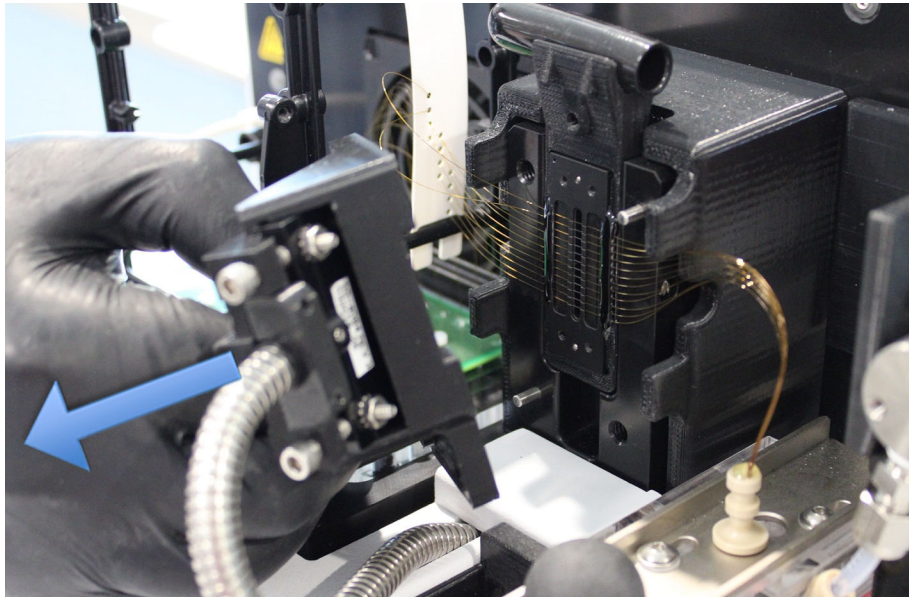


Figure 59 Instrument top compartment – light guide removal

Once the light guide has been removed, it can hang straight down into the reagent door compartment. It is recommended to close the reagent door to minimize light shining in any eyes.

NOTE

Avoid looking directly at the LED light.

ProteoAnalyzer Capillary Array

Removal of the Capillary Array

- 5 Pull back on the capillary reservoir connector slide.

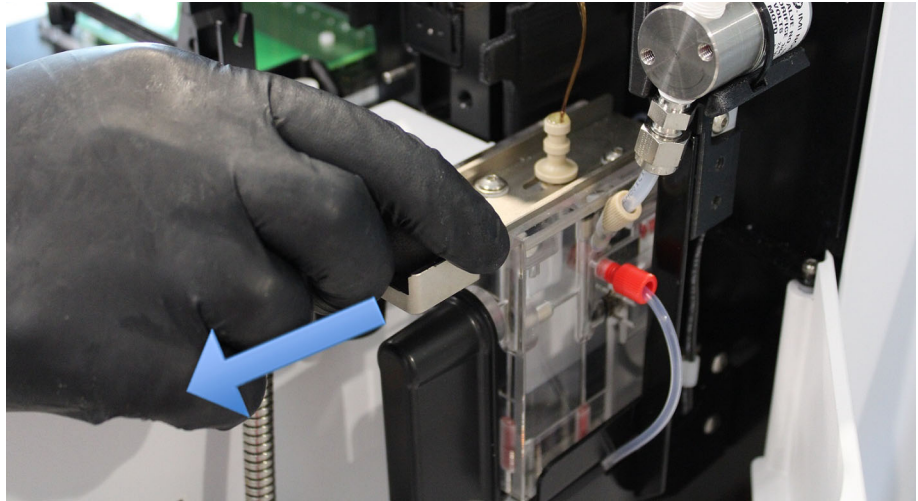


Figure 60 Instrument top compartment – capillary reservoir connector slide

- 6 Use the capillary reservoir connector tool (supplied with the instrument accessory kit) to loosen the capillary array bundle by prying up on the bundle.

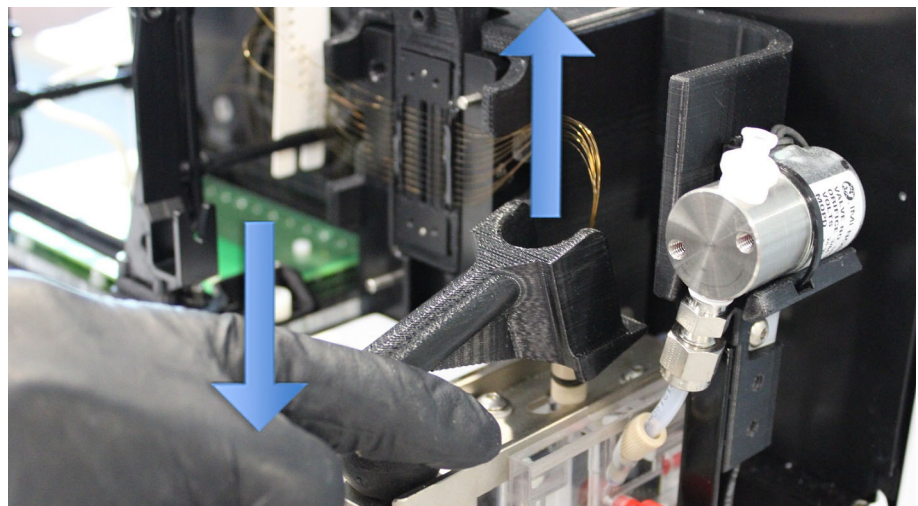


Figure 61 Instrument top compartment – capillary reservoir connector tool

ProteoAnalyzer Capillary Array

Removal of the Capillary Array

7 Remove the capillary array bundle by pulling up gently.

NOTE

Avoid pulling up hard as to not break any capillaries.

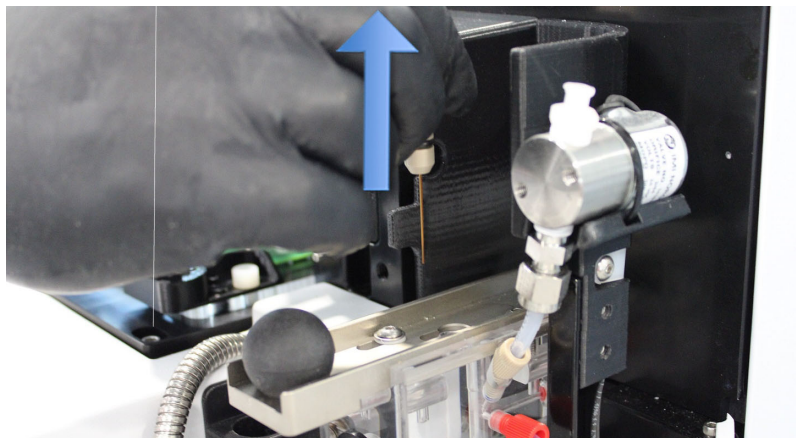


Figure 62 Instrument top compartment – capillary array bundle removal

8 Carefully insert the protective cover over the capillary bundle.

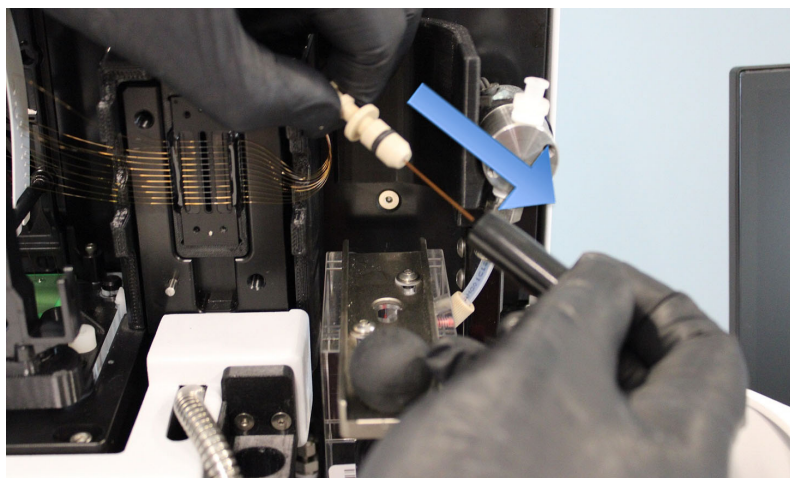


Figure 63 Instrument top compartment – installing capillary bundle protective cover

NOTE

Hold the capillary bundle straight while installing the protective cover. Take care to not scrape the capillaries along the inside of the cover.

ProteoAnalyzer Capillary Array

Removal of the Capillary Array

- 9 Place the capillary array bundle on the top holder of the capillary array window.

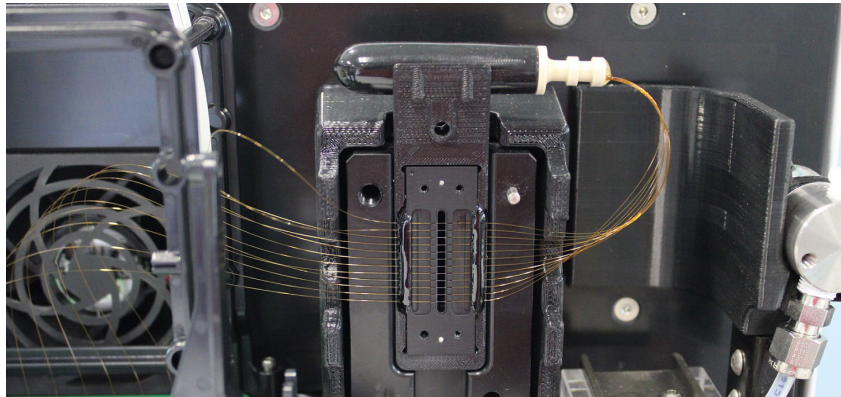


Figure 64 Instrument top compartment – storing covered capillary array bundle

- 10 Remove the capillary array window from the window holder, gently rocking the window back and forth to loosen it from the holder. Do not press on or touch the capillaries.

Flip the array window after removal so that the capillary array bundle goes from the right to the left side of the array frame.

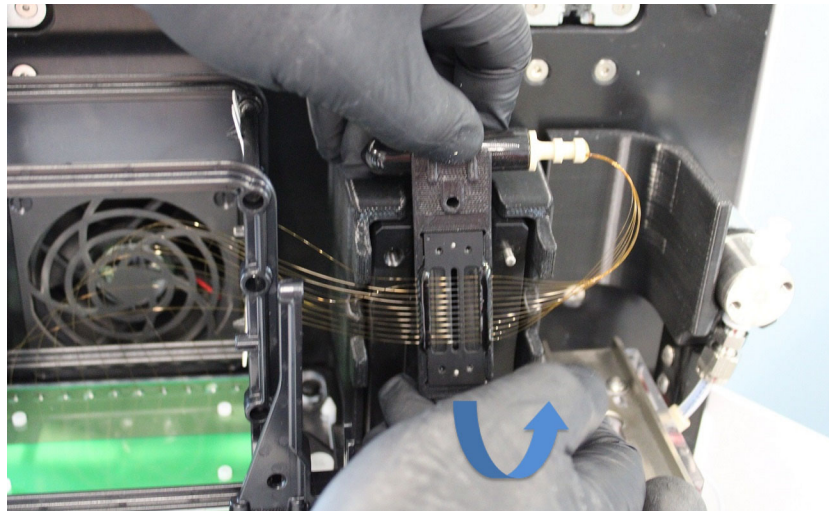


Figure 65 Instrument top compartment – remove capillary array window

ProteoAnalyzer Capillary Array

Removal of the Capillary Array

- 11 Attach the array window to the capillary array frame using the attachment screw.



Figure 66 Instrument top compartment – attach array window to capillary array frame

- 12 Use the provided allen wrench to remove the two white screws holding the capillary array in place.

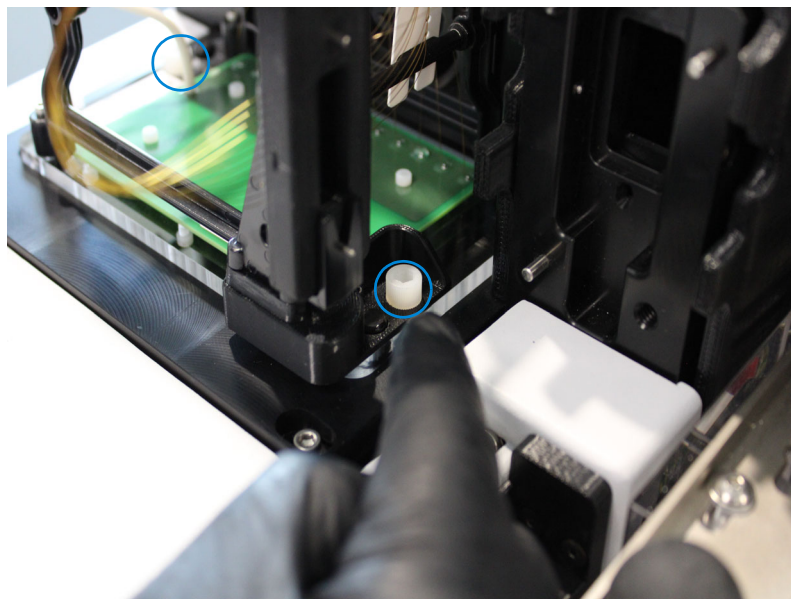


Figure 67 Instrument top compartment – array attachment screw removal

ProteoAnalyzer Capillary Array

Removal of the Capillary Array

- 13 Carefully lift the array straight up to remove it from the ProteoAnalyzer instrument.

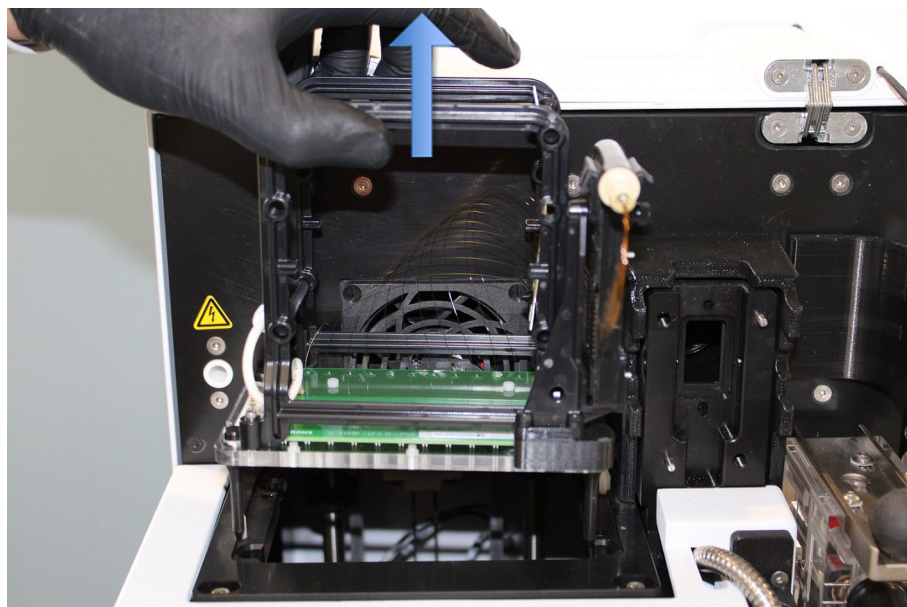


Figure 68 Instrument top compartment – capillary array removal

Once removed from the instrument, the capillary array cartridge is ready for disposal or storage in the ProteoAnalyzer wet station (see **“Long Term Capillary Array Storage”** on page 143).

Unpacking a New Capillary Array

This section will provide a pictorial guide of the steps required to physically unpack a new capillary array from the shipping container and packaging.

- 1** Unpack the new capillary array:
 - a** Open box. Take care not to damage the contents if you use sharp tools.
 - b** Remove foam cover.
 - c** Lift array out of the packaging.
 - d** Remove array from the plastic bag.



Figure 69 Capillary array shipping box

Take care not to break capillaries or touch the array window when removing packaging. Hold the array by the black plastic frame when handling.

ProteoAnalyzer Capillary Array

Unpacking a New Capillary Array

- 2 Remove the capillary array bundle-securing rubber band.

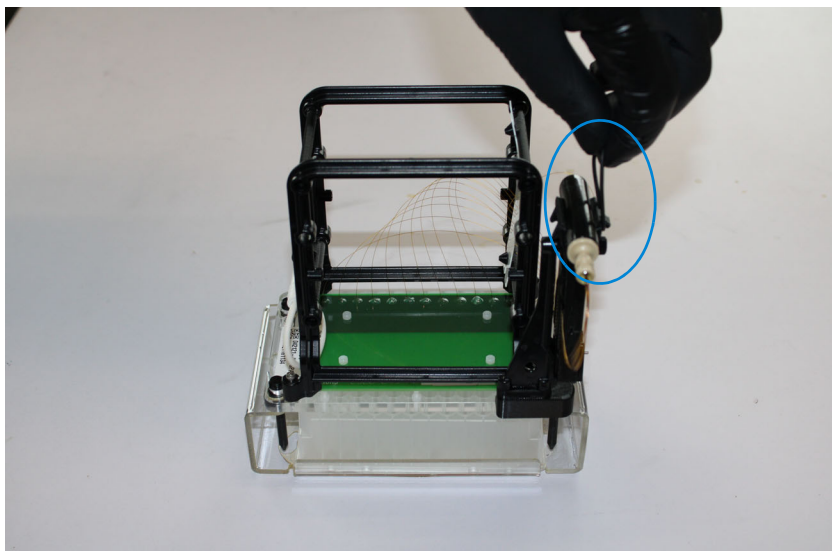


Figure 70 Capillary array rubber band

- 3 Remove the two white nylon screws that secure the array to the shipment frame, using the Allen wrench supplied with the instrument accessory kit.

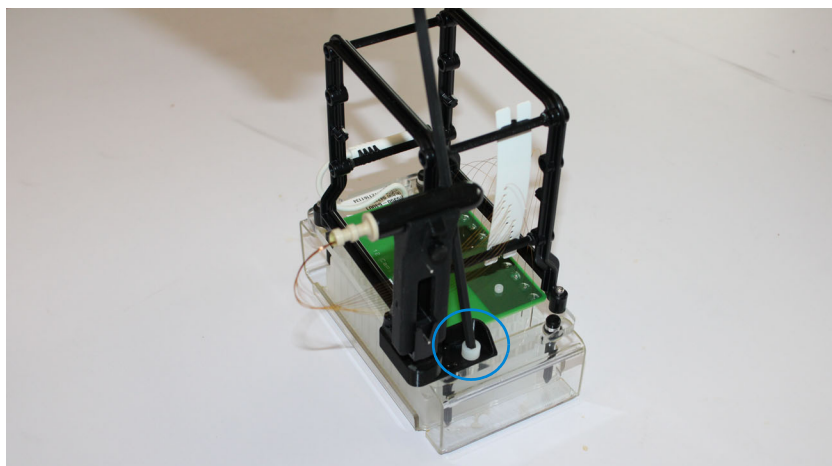


Figure 71 Capillary array nylon screw

ProteoAnalyzer Capillary Array

Unpacking a New Capillary Array

- 4 Carefully lift the array straight up to remove it from the shipment frame.

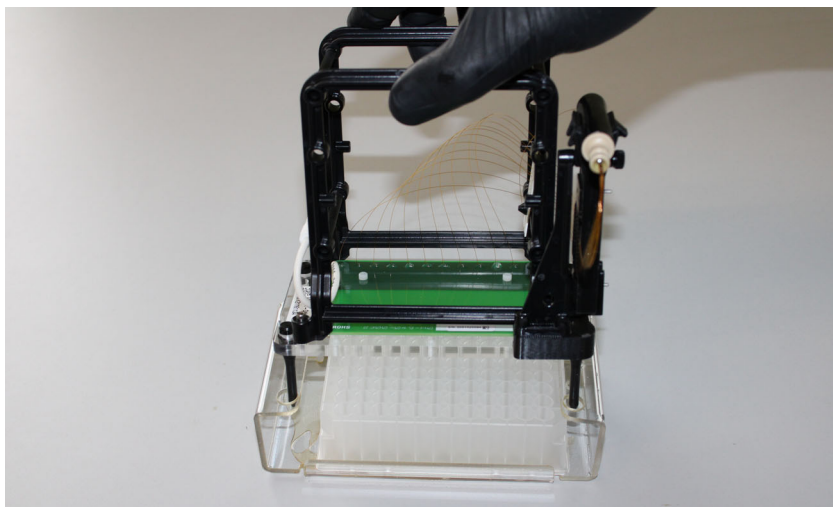


Figure 72 Capillary array – removal of array from shipment frame

Capillary Array Installation

This section will provide a pictorial guide of the steps required to physically install a capillary array cartridge into the ProteoAnalyzer instrument.

Before proceeding with Installation ensure the instrument is in the **Park** position. If it is not in the **Park** position, select the **Park** icon from the main screen to place the tray being held back into its drawer and move the Stage into resting position.

- 1 Open the reagent door and top hood of the instrument:

First, open the reagent door to the side.

When the reagent door is open, the top hood flips upwards.



Figure 73 ProteoAnalyzer instrument

ProteoAnalyzer Capillary Array Capillary Array Installation

- 2 Carefully place the capillary array into the top compartment of the instrument with the array window facing out.

The four alignment pins should align with the alignment holes in the instrument.

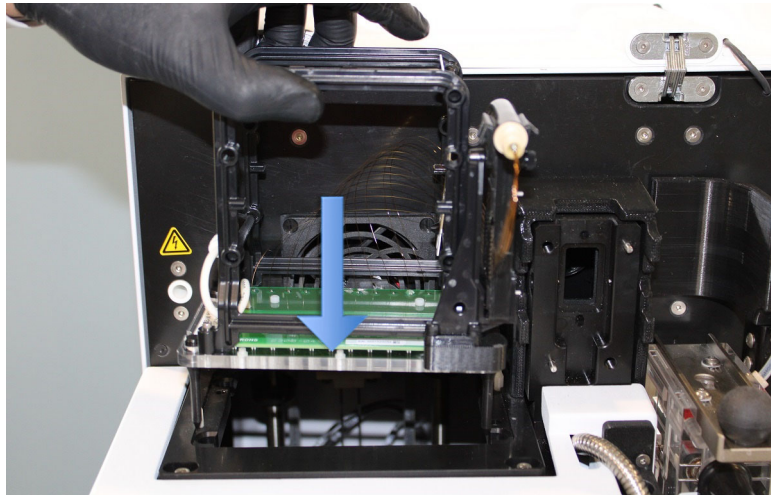


Figure 74 Instrument top compartment - capillary array installation

- 3 Use the allen wrench (supplied with the instrument accessory kit) to install the two white screws holding the capillary array in place.

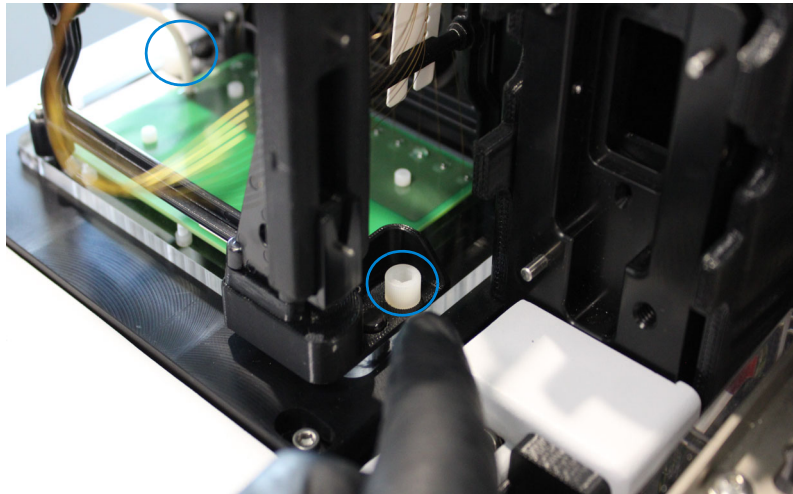


Figure 75 Instrument top compartment - array attachment screw installation

- 4 Remove the array window attachment screw.



Figure 76 Instrument top compartment – array window attachment screw

- 5 Carefully flip the array window so that the capillary array bundle goes from the left to the right side of the instrument.

Position the capillary array window into the holder, inserting the alignment pins to the provided holes, and firmly press it into place.

Do not press on or touch the capillaries.

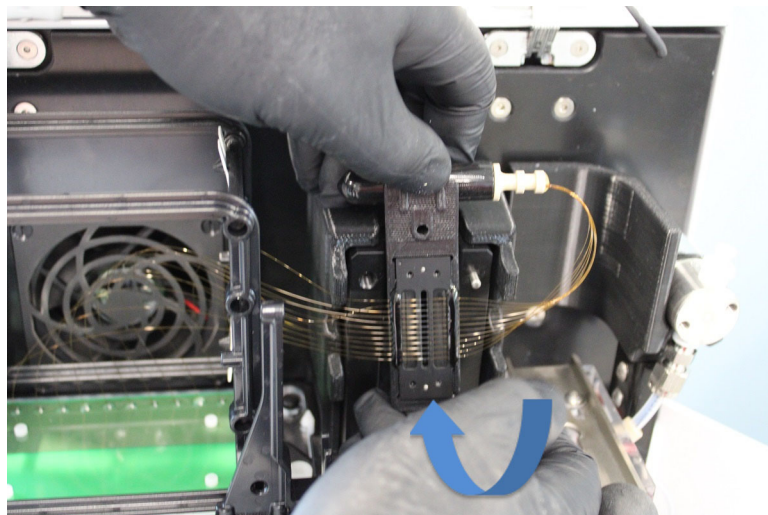


Figure 77 Instrument top compartment – array window installation

ProteoAnalyzer Capillary Array

Capillary Array Installation

- 6 Remove the capillary array bundle with the protective cover from the top holder of the capillary array window.

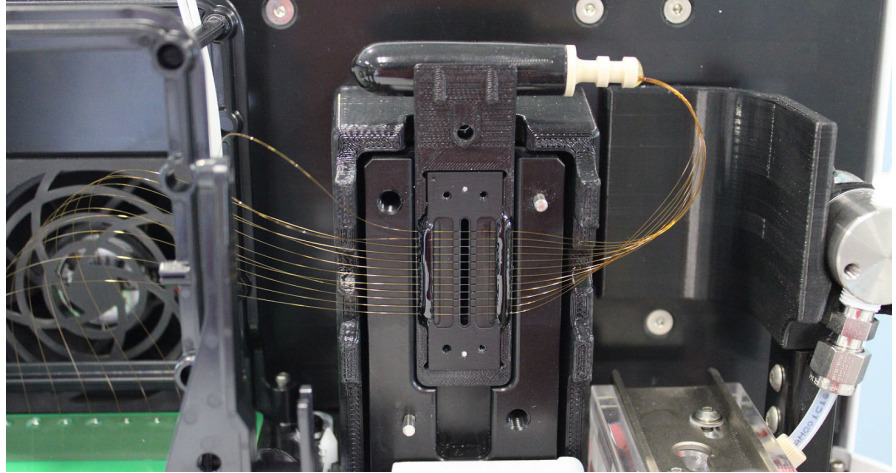


Figure 78 Instrument top compartment – capillary array window installed, with capillary array bundle on top

- 7 Carefully remove the protective cover from the capillary bundle by pulling it straight off without touching or rubbing against the capillaries. Place the cover back on the holder on top of the window.

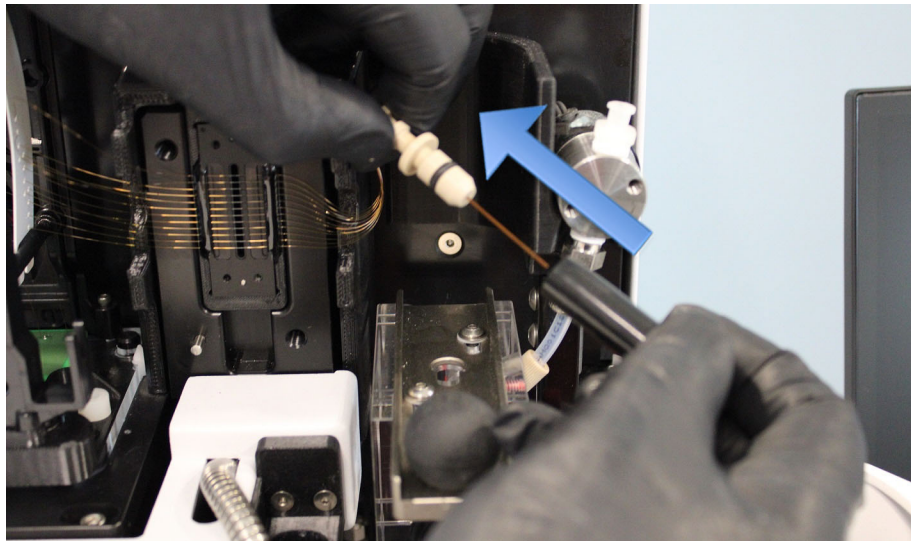


Figure 79 Instrument top compartment – removing protective cover

- 8 Install the capillary array bundle by firmly pushing the capillary array bundle into the reservoir opening until a distinct click is heard.

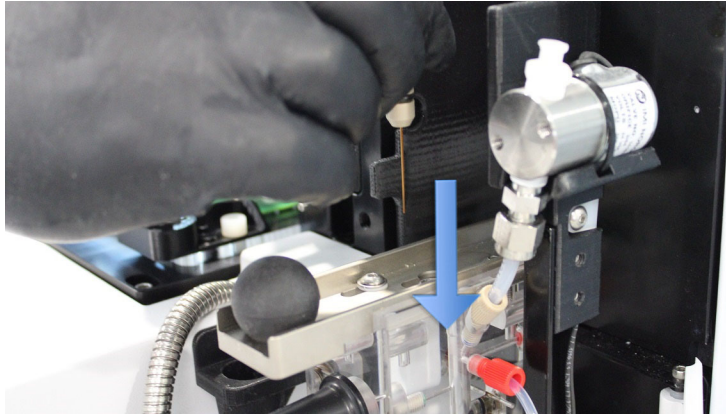


Figure 80 Instrument top compartment – capillary array bundle installation

- 9 Push in the capillary reservoir connector slide to secure the capillary array bundle.

CAUTION

Unsecured capillary array bundle.

If the capillary array bundle is not properly secured, it will be damaged upon pressurization.

- ✓ Push in the capillary reservoir connector slide properly.

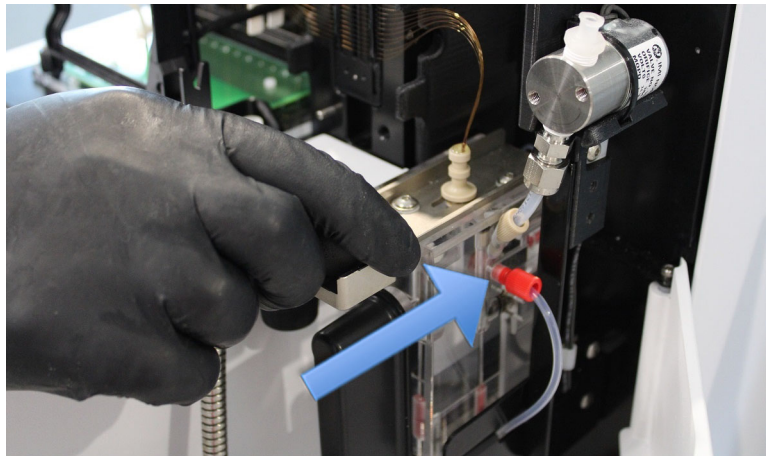


Figure 81 Instrument top compartment – capillary reservoir connector slide

- 10** Place the light guide over the array window using the two alignment pins. The finger hold should be facing the right side of the instrument. The steel optical cable should be on the left.

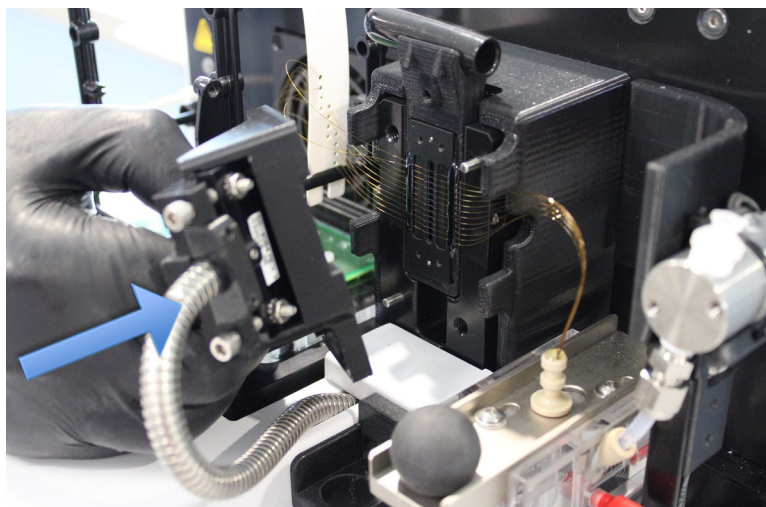


Figure 82 Instrument top compartment – light guide placement

ProteoAnalyzer Capillary Array Capillary Array Installation

- 11 Use the provided allen wrench to install the two white nylon screws that secure the light guide to the array window.

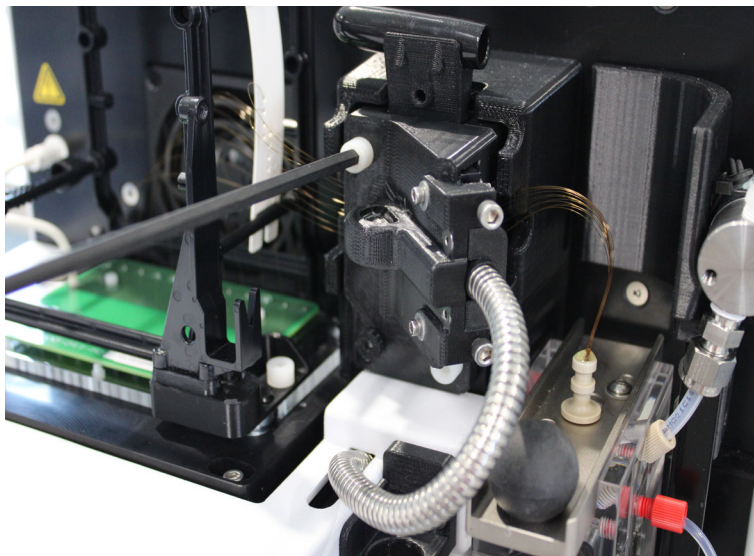
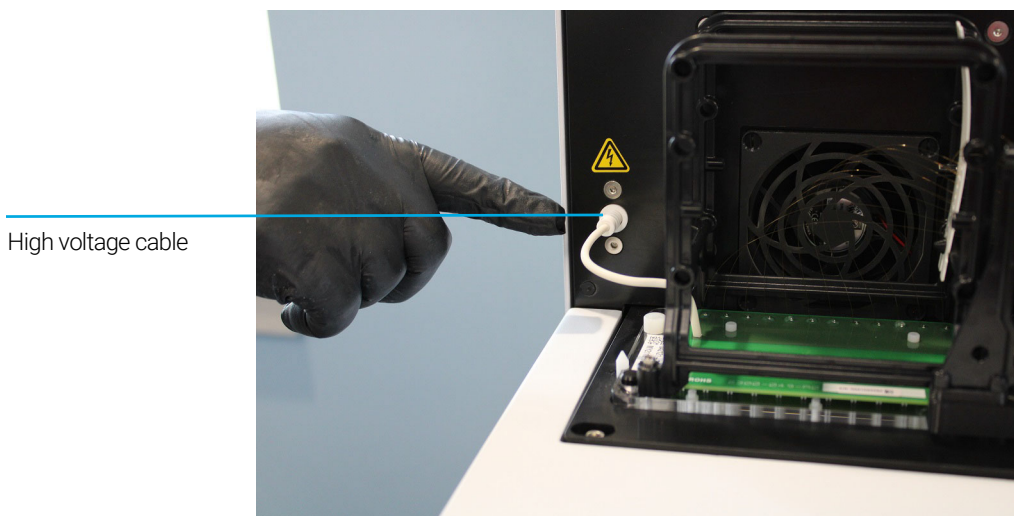


Figure 83 Instrument top compartment – light guide installation

- 12 Remove the high voltage cable from the array frame holder and firmly push it into the high voltage cable connection.



High voltage cable

Figure 84 Instrument top compartment – fully installed array shown

ProteoAnalyzer Capillary Array Capillary Array Installation

13 Double check all of the installation points on the capillary array:

- ✓ Array base secured with two nylon screws
- ✓ Array window installation
- ✓ Light guide installed with two nylon screws
- ✓ Capillary bundle installed in reservoir
- ✓ Reservoir slide in locked position
- ✓ HV cable installed

14 Close the reagent door and top hood of the instrument.



Figure 85 ProteoAnalyzer instrument

After installation of an array, the ProteoAnalyzer will require a capillary alignment as described in **Chapter 4**, "ProteoAnalyzer Software – Utilities Menu".

Using the Capillary Array Wet Station for Storage

For information about capillary array storage, refer to section “**Long Term Capillary Array Storage**” on page 143.



9

ProteoAnalyzer – Sample Name Entry

Sample Name Entry 114

Entering Sample Names Manually 114

Importing Sample Names 115

Importing Sample Names Using a Bar-Code Reader 117

This chapter provides information on how to enter the sample names in the ProteoAnalyzer software.

Sample Name Entry

Entering Sample Names Manually

- 1 From the **Operation** tab, select the tray number, the desired row, and the sample cell.
- 2 In the field **Sample ID**, enter the desired sample names.
- 3 Select the **Save tray** or **Save selected row** to save the file as a .txt or .csv (Figure 86).

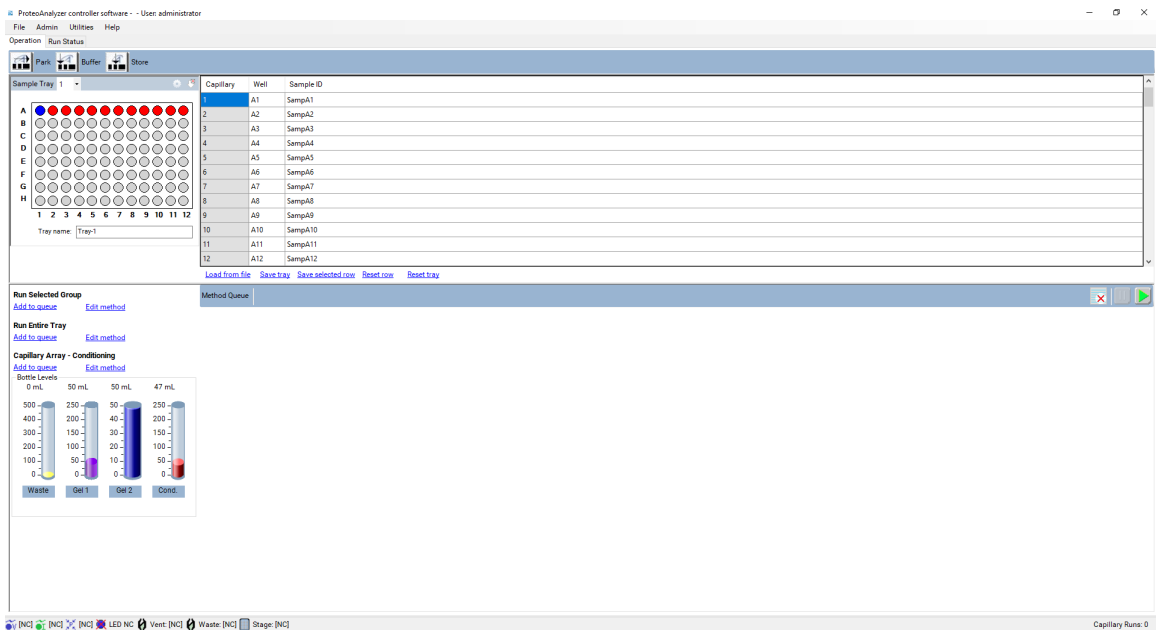


Figure 86 Adding samples names manually

Importing Sample Names

- ✓ The files must be available in .txt or .csv file format.
 - ✓ The data format must comply with the format described below in order for the system to read the files correctly.
- 1 In the **Operation** tab, select **Load from file** to load a set of saved or previously created sample names.
 - For a .txt file, the sample names must be arranged in a single column (**Figure 87**).

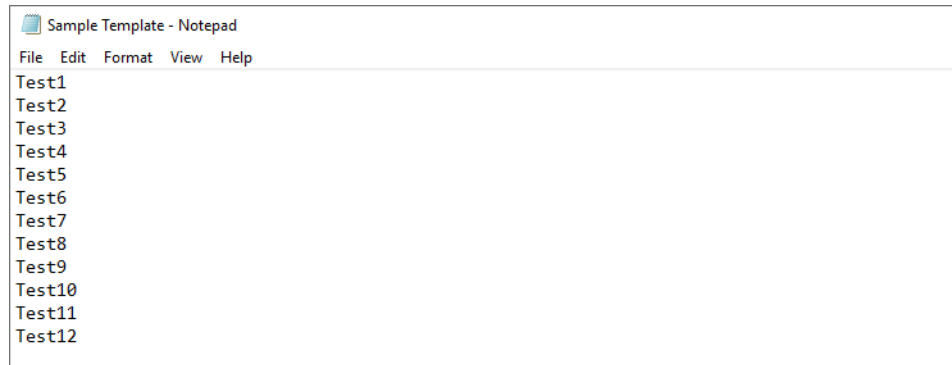
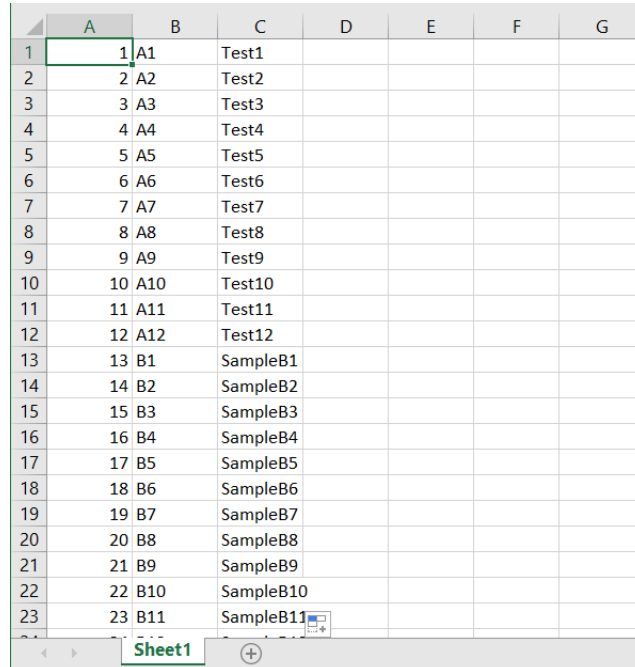


Figure 87 .txt file format (single row of names—no well numbers or row numbers).

ProteoAnalyzer – Sample Name Entry

Sample Name Entry

- For a .csv file, the column format is sample number (1-12 for single row, 1-96 for entire sample plate), well number, and sample name (**Figure 88**).



	A	B	C	D	E	F	G
1	1	A1	Test1				
2	2	A2	Test2				
3	3	A3	Test3				
4	4	A4	Test4				
5	5	A5	Test5				
6	6	A6	Test6				
7	7	A7	Test7				
8	8	A8	Test8				
9	9	A9	Test9				
10	10	A10	Test10				
11	11	A11	Test11				
12	12	A12	Test12				
13	13	B1	SampleB1				
14	14	B2	SampleB2				
15	15	B3	SampleB3				
16	16	B4	SampleB4				
17	17	B5	SampleB5				
18	18	B6	SampleB6				
19	19	B7	SampleB7				
20	20	B8	SampleB8				
21	21	B9	SampleB9				
22	22	B10	SampleB10				
23	23	B11	SampleB11				

Figure 88 .csv file format: sample number, well number, and sample name

Importing Sample Names Using a Bar-Code Reader

For the purposes of sample name import, a bar-code reader is equivalent to a keyboard. When a bar-code is read, the program searches the *Samples* folder for a name that is identical to the bar-code. If a name is found, then the file (and the corresponding sample names) is imported.

NOTE

No bar-code scanner is provided with the ProteoAnalyzer system.

- 1 Place the sample name files into the C:\Agilent Technologies\Samples folder (**Figure 89**). If a folder does not exist, create a new *Samples* folder. The sample name file can be either a .txt file or .csv file (using the formats described in section “**Importing Sample Names**” on page 115).

The sample name files can be created by a user, or automatically by a LIMS system.

Name	Type
Data	File folder
ProteoAnalyzer	File folder
Methods	File folder
Samples	File folder
User Manual	File folder

Figure 89 Samples folder

It is critical that the name of the file is identical to what is read by the bar-code reader.

Example:

In **Figure 90**, the name associated with the bar-code is 00060065.

ProteAnalyzer – Sample Name Entry

Sample Name Entry



Figure 90 Bar-code name 00060065

Thus, the .csv file or .txt file must be given the file name *00060065* and located in the *Samples* folder (Figure 91).

Name	Type
Sample Names Template - CSV File - Enter Names in ...	Microsoft Excel Comma...
Sample Names Template - txt File	Text Document
00060065.txt	Text Document

Figure 91 File name

- In the field **Tray name** of the **Operation** tab, highlight the tray name with the mouse cursor (Figure 92).

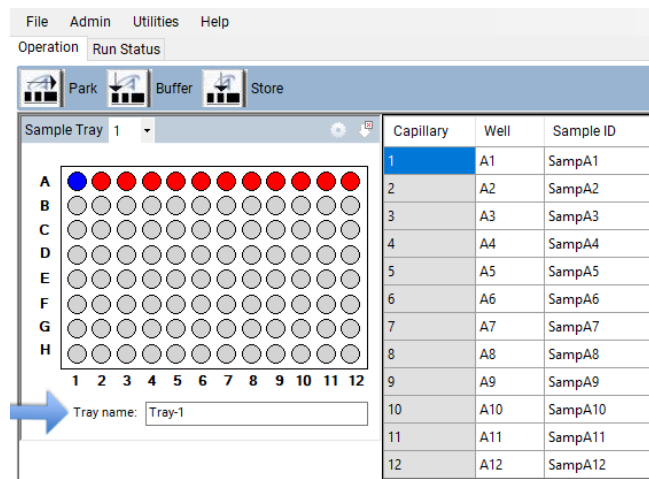


Figure 92 Highlight of the tray name

ProteoAnalyzer – Sample Name Entry

Sample Name Entry

- Use the bar-code reader to scan the bar-code on the plate.

The file name and the sample names will be automatically imported from the .txt or .csv file of the *Samples* folder (**Figure 93**).

The screenshot displays the ProteoAnalyzer software interface. At the top, there is a menu bar with 'File', 'Admin', 'Utilities', and 'Help'. Below the menu bar, there are tabs for 'Operation' and 'Run Status'. A toolbar contains icons for 'Park', 'Buffer', and 'Store'. The main area is divided into two sections: a sample tray layout on the left and a table of imported sample names on the right.

The sample tray layout shows a grid of wells labeled A through H (rows) and 1 through 12 (columns). The wells in row A are filled with red circles, and the well in column 2, row A is filled with a blue circle. A blue arrow points to the well in column 2, row A. Below the tray layout, there is a text field labeled 'Tray name:' with the value '00060065'. A blue arrow points to this text field.

The table of imported sample names has the following columns: 'Capillary', 'Well', and 'Sample ID'. The data is as follows:

Capillary	Well	Sample ID
1	A1	AA001
2	A2	AA002
3	A3	AA003
4	A4	AA004
5	A5	AA005
6	A6	AA006
7	A7	AA007
8	A8	AA008
9	A9	AA009
10	A10	AA010
11	A11	AA011
12	A12	AA012

Blue arrows point to the 'Sample ID' column in the table, specifically to the cells containing 'AA001' and 'AA002'.

Figure 93 Imported sample names



10

ProteoAnalyzer – Automated Analysis

Automated Analysis Using the ProteoAnalyzer 121

Enabling Automated Analysis 122

Monitoring the Status of the Automated Processed Data 125

This chapter explains the procedure for automated analysis using the ProteoAnalyzer.

Automated Analysis Using the ProteoAnalyzer

Automated analysis is performed by the ProteoAnalyzer software at the end of a run using ProSize. Instead of manually opening a file and exporting the results (for example, pdf, peak table, smear table, etc.) this is done automatically at the end of each run.

Automated analysis is applicable to labs that always run the same type of sample.

Automated analysis is ideally suited for linking the ProteoAnalyzer to a LIMS system. Sample names can be generated by the LIMS system and imported via plate bar coding (refer to **Chapter 9**, “ProteoAnalyzer – Sample Name Entry”). Sample results are automatically exported via automated analysis. Error logs on automated analysis are located in .txt files that can be monitored by the LIMS system.

Automated analysis should not be done in sample matrices where results are unpredictable (broad, messy peaks, complex mixtures, low sample quantity, etc.).

Enabling Automated Analysis

- 1 From the **Admin** drop-down menu, select **Results Report Setup** (Figure 94).

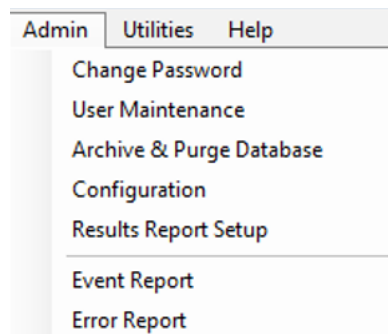


Figure 94 Admin menu

This will open the **Automated Report Settings** window (Figure 95).

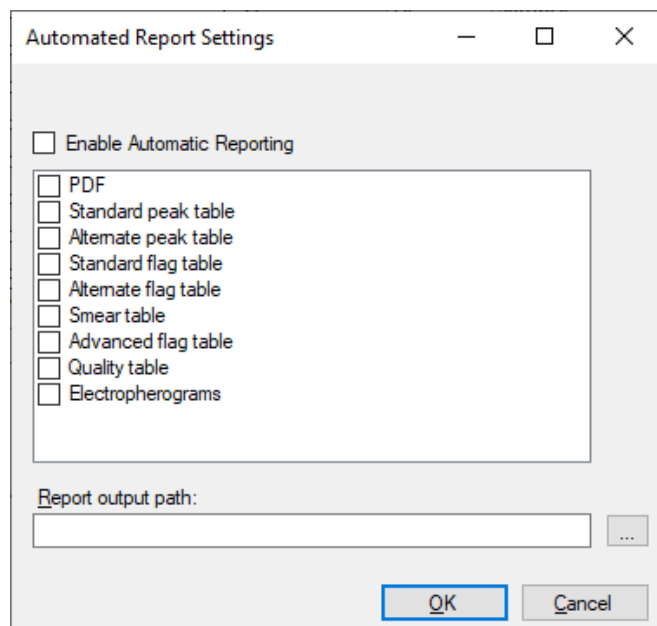


Figure 95 Automated Report Settings window

- 2 To enable automated analysis, select **Enable Automatic Reporting**.
- 3 Select the desired export options (PDF, etc.).

Each of the export options (PDF, Standard peak table, etc.) are described in Chapter 7, “Exporting Data from ProSize”, and Chapter 8, “Generating Reports from ProSize” of the *ProSize Data Analysis Software User Manual*.

The **Report output path** defines where the exported data is placed. If this field is left empty, the exported data will be placed into the original data folder. Create an output folder in a desired location other than the data folder, if desired.

For automated analysis to work correctly there are two main criteria that must be met:

- The name of the method in the ProteoAnalyzer system (used to acquire the data) must exactly match the name of the configuration file in ProSize.

For example, **Figure 96** shows a protein ladder where the peak height setting is too high, resulting in incorrect picking of all the ladder peaks. This will result in an automated analysis run failure.

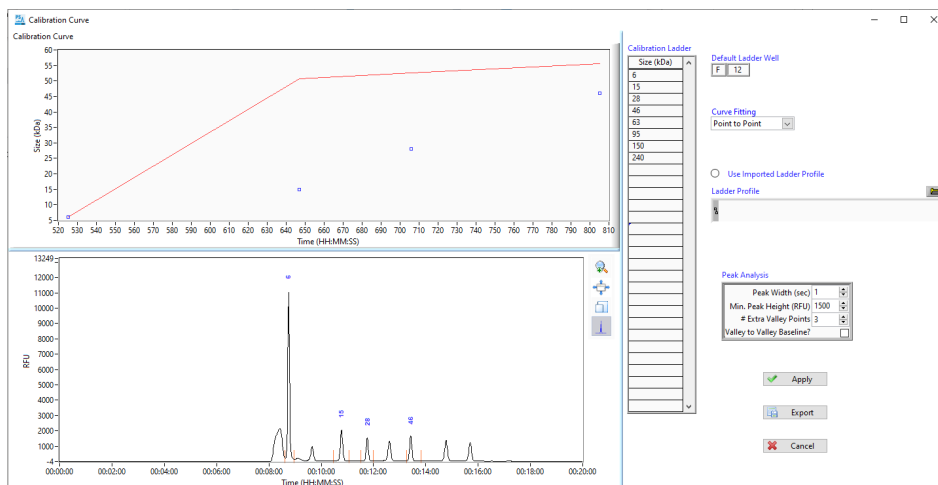


Figure 96 ProSize calibration curve setup

If the configuration file is set with a minimum peak height of 500, then the ladder is processed correctly by ProSize, and all the ladder elements are recognized (**Figure 97**).

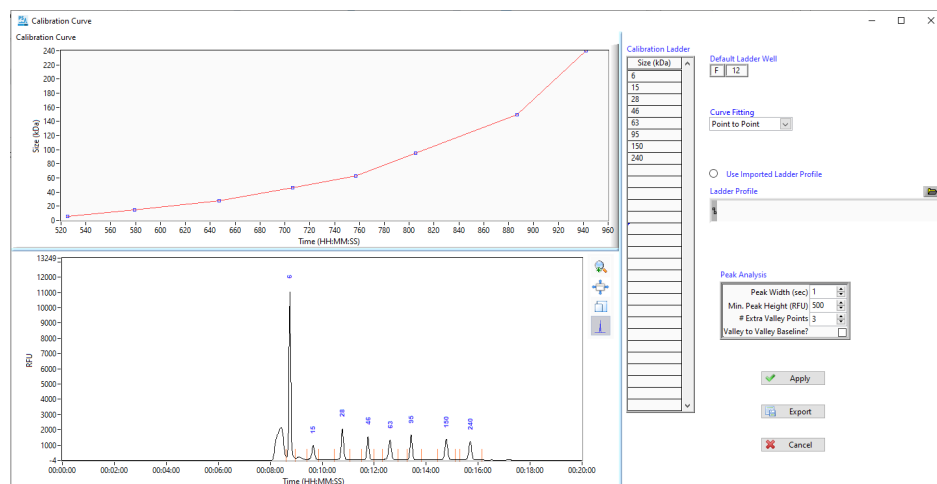


Figure 97 ProteoAnalyzer calibration curve setup

Importing a Ladder File for Automated Analysis

The ProteoAnalyzer system uses ProSize to perform automated processing. Thus, you must utilize ProSize to modify configuration files, which defines how the data is processed. In the example above, you would change (and save) the *minimum peak height* from 1500 to 500 in the configuration file using ProSize.

Both ProSize and the ProteoAnalyzer software give you the option of using an imported ladder file. For batch or automated processing, the use of imported ladders has several advantages:

- You can use all 12-wells of the sample plate, without having to reserve well A12 for the ladder.
- A high-quality, saved ladder file allows you to process many subsequent files without the need for re-calibration.
- A high-quality ladder file eliminates the chance of a bad auto-processed file due to a poor quality of a sample plate ladder (i.e., a ladder well that has poor signal, missing, or poorly resolved peaks).

Monitoring the Status of the Automated Processed Data

The **Results Dashboard** allows you to quickly determine the status of post-processed data.

- 1 From the **Utilities** drop-down menu, select **Results dashboard** (Figure 98).

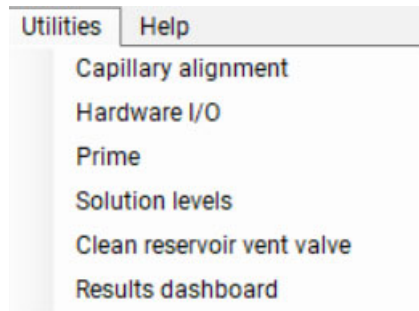


Figure 98 Utilities menu

The **Results Dashboard** window opens. The data files are listed (Figure 99).

- 2 Right-click on a file.

RAW File	Error Status	Critical Error	Input Error	Generation Error	Individual Error
2017 11 14 12h 07m..raw	OK	✓	✓	✓	✓
2012 08 17 14h 35m.raw	ISSUES	✓	✓	✗	✓

Figure 99 Results Dashboard

- a From the menu, select **View with Prosize** to open and review the file in ProSize.
- b Select **Error Log** to view the error messages.

A summary of error messages is given in **Table 21**.

Table 21 Results Dashboard error messages

Message	Description
Error Status	Gives a statement of the status of processing. If there is an issue, <i>ISSUES</i> will appear.
Critical Error	Either a) the method name did not match the configuration file name, or b) the ladder file could not be processed correctly.
Input Error	A user asked for something that could not be generated, such as a flag summary when no flag conditions were set, or a smear table, when the configuration file has no smear conditions.
Generation Error	There was an issue with the generation of a file (.csv, .pdf, or .txt) (usually associated with some operating system error).
Individual Error	There is a problem with an individual capillary, such as a missing upper or lower marker, or unusually broad marker peaks.

The error messages are also recorded under C:\ProSize data analysis software\Error Log. An example error log file is shown in **Figure 100**.

Since this is a .txt file, the error can be monitored by a LIMS system to report the status or accuracy of auto-processing.

```

2019 08 29 10H 23M - Notepad
File Edit Format View Help
[File Path]
File Path = "C:\\Agilent Technologies\\Data\\2019 08 29\\10-23-29\\2019 08 29 10H 23M.raw"
[Critical Error]
Error 4 = "Error on sizing calibration"

```

Figure 100 Example error message

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This chapter provides additional information on part numbers, maintenance procedures, and system troubleshooting.

Permissible Characters

The following tables show which characters are permissible (**Table 22**) and non-permissible (**Table 23**) for a file name.

Table 22 Permissible characters for a file name

Characters	
~	`
!	@
#	\$
%	^
&	(
)	-
-	+
=	{
}	[
]	;
,	.

Table 23 Non-permissible characters for a file name

Characters	
*	
\	:
"	'
<	>
?	/

Compatible Plates for the ProteoAnalyzer System

Semi-Skirt Sample/Marker Plates

The ProteoAnalyzer system has been designed to operate using specific dimensioned semi-skirted 96-well plates and deep 96-well plates.

Approved plates: Eppendorf 96-Well twin.tec PCR Plates, Semi- skirted (Eppendorf # 951020303 (various colors)).

NOTE

Non-skirted 96-well plates are not recommended for use with the ProteoAnalyzer system, as they tend to warp or bow and can therefore interfere with proper sample injection.

CAUTION

96-well plates with wrong dimensions

Using 96-well plates with dimensions other than recommended could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.

- ✓ Only use 96-well plates with the correct dimensions.

Buffer/Waste Plates

The ProteoAnalyzer system uses a specific deep 96-well plate (31 mm height) supplied by Fisher Scientific (Part # 12-566-120) for the buffer and waste plate. This specific plate must be used with the instrument (two plates are supplied upon installation).

CAUTION

96-well plates with wrong dimensions

Standard 1 mL deep well, half height, or square well 1 mL 96-well plates should not be used as buffer/waste plates with the ProteoAnalyzer system, as damage to the capillary array will occur.

✓ **Only use 96-well plates with the correct dimensions.**

The same specified buffer/waste plate is also available directly from Agilent, if these plates cannot be obtained directly from the manufacturer.

Table 24 List of Buffer/Waste Plate

Item	Vendor / Part #	Description
Buffer/ Waste Deep 96-Well Plates	Fisher Scientific #12-566-120	Fisherbrand 96-Well DeepWell Polypropylene Microplates: Well Capacity 1 mL
Buffer/ Waste Deep 96-Well Plates	Agilent #P60-20	ProteoAnalyzer 96-Well Buffer/Waste Tray, case of 50

Preventative Maintenance Schedule

Daily Maintenance

- ✓ Empty the waste bottle and waste tray.
- ✓ Replace the inlet buffer in the buffer tray position.
- ✓ Ensure there is Capillary Conditioning Solution in the conditioning solution bottle location.
- ✓ Ensure there is gel in the gel bottle location.
- ✓ Ensure 1 M NaOH is in the Gel 2 location and perform a Daily Conditioning Flush that day, before running samples.

Monthly Maintenance

- ✓ Replace the buffer and waste plates with new ones.
- ✓ Replace the Capillary Storage Solution and plate.*
- ✓ Replace Gel 1, Gel 2, and conditioning solution bottles with new ones.
- ✓ Clean both gel and conditioning solution lids with IPA or EtOH.
- ✓ Inspect the capillary array vent valve for dried gel, clean if necessary.

As Needed to Restore Separation Performance

- ✓ Perform a Method C flush, or Method B flush. A Method B flush should always be followed by a Method C flush as described in section “**Method C: 1.0 M NaOH Flush**” on page 135.

* More frequent replacement (i.e., every 1-2 weeks) may be required in low humidity or warmer laboratory environments.

Capillary Array Cleaning

There are three methods to clean/flush a capillary array to remove a clog.

A: Submerge capillary array tips/electrodes in hot water (150 °F – 200 °F)

B: 0.1 M HCl Flush

C: 1.0 M NaOH Flush

A combination of two or more of the methods outlined below may be required in some cases.

Method A: Submerge Capillary Array Tips/Electrodes in Hot Water (150 °F – 200 °F)

This method is used for opening clogged capillaries and returning separation to normal.

- 1 Select the park icon in the main screen window. This places the plate being held back into its respective drawer and moves the stage platform to the bottom of the instrument.
- 2 Fill each well in row A of a 96-well deep well plate with 1 mL of hot water (150 °F to 200 °F) for soaking the tips of the capillary array.
- 3 Open the buffer drawer (first drawer from top) and place the hot water filled 96-well deep well plate onto the plate spacer.
- 4 Close the buffer drawer securely.
- 5 From the main screen window, locate the hotel positioning icons under the **Operation** tab. Select the Buffer icon to position the plate underneath the capillary array.
- 6 Allow the capillary array to soak for a minimum of 15 minutes to one hour.

Method B: 0.1 M HCl Flush

WARNING

Hazardous reagents

The handling of reagents can hold health and safety risks.

- ✓ When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.
- ✓ The volume of substances should be reduced to the minimum required for the procedure.

This method is recommended when broad peaks or delayed migration of samples is observed. This flush is meant to clean the capillary walls and improve separation performance.

Following this method, a daily conditioning flush should always be completed (see “**Daily Conditioning Flush**” on page 137). This is needed to ensure that the inner surface of the capillary walls are properly prepared for the conditioning step.

- 1 Open the ProteoAnalyzer side compartment and replace the gel 2 bottle with at least 20 mL of 0.1 M HCl.
- 2 Replace the protein conditioning solution bottle with at least 20 mL of DI water.
- 3 Under capillary array conditioning select **Add to queue**.

Appendix

Capillary Array Cleaning

- Select the HCl method from the drop-down menu. Ensure that the parameters match the ones in **Figure 101**. Once selected, this will add the method to the queue. Select the green play button to start the flush. The total flush time takes approximately 30 minutes.

Conditioning Method: Method B 0.1 M HCl Flush.mthdc

<input checked="" type="checkbox"/> Step #1	Solution	Conditioning	
Fill pressure	280	PSI	Time 5.0 min.
Flow rate	200	μL/s	Tray Waste Row A
<input checked="" type="checkbox"/> Step #2	Solution	Gel 2	
Fill pressure	280	PSI	Time 15.0 min.
Flow rate	200	μL/s	Tray Waste Row A
<input checked="" type="checkbox"/> Step #3	Solution	Conditioning	
Fill pressure	280	PSI	Time 5.0 min.
Flow rate	200	μL/s	Tray Waste Row A

Figure 101 Method B parameters

- Once the flush is completed, place the bottle of 1.0 M NaOH back on the gel 2 location and the protein conditioning solution bottle back on the conditioning solution line.
- It is recommended to run the daily conditioning flush following the Method B: 0.1 M HCl Flush.
- Capillaries should always sit idle with gel in them. We recommend running a protein separation method or a conditioning flush so that gel is placed back in the capillaries while the instrument is sitting idle.

Method C: 1.0 M NaOH Flush

WARNING**Hazardous solvent**

1.0 M NaOH is corrosive and the handling of this solvent can hold health and safety risks. It causes severe eye and skin burns.

- ✓ Avoid contact with eyes, skin, or clothing.
- ✓ Wear eye protection and impervious gloves.
- ✓ Refer to the SDS for all warnings and precautions before proceeding.

This method is the best method for opening clogged capillaries and returning separation to normal.

- 1 Open the ProteoAnalyzer side compartment and replace the Gel 2 bottle with a bottle containing at least 20 mL of 1.0 M NaOH.
- 2 Place at least 20 mL of protein conditioning solution on the conditioning solution position
- 3 From the operation tab located on the main screen, select **Add to Queue** under the **Capillary Array > Conditioning commands** menu.
- 4 From the **Select Conditioning Method** window, select **Method C Flush – 1.0 M NaOH**.

- 5 Select **Edit** to ensure that the method matches the parameters in **Figure 102**.

Conditioning Method: Method C 1.0 M NaOH Flush.mthdc

<input checked="" type="checkbox"/> Step #1	Solution	Gel 2	
Fill pressure	200	PSI	Time 10.0 min.
Flow rate	200	μL/s	Tray Waste Row A
<input checked="" type="checkbox"/> Step #2	Solution	Conditioning	
Fill pressure	200	PSI	Time 10.0 min.
Flow rate	200	μL/s	Tray Waste Row A
<input type="checkbox"/> Step #3	Solution	Conditioning	
Fill pressure	0	PSI	Time 1.0 min.
Flow rate	1	μL/s	Tray Waste Row A

Ok Cancel

Figure 102 Method C parameters

- 6 Select **Ok**.
- 7 Select **Ok** again to add the method to the method queue.
- 8 Open the waste drawer (second drawer from top) and place a 96-well deep well plate filled with 0.6 mL per well of 1.0 M NaOH in Row A.
- 9 Close the door to the instrument side compartment and select the green start icon the method queue to run the capillary conditioning method.
- 10 Once the capillary array conditioning method is complete, open the waste drawer and remove the 96-well deep well plate. Check the volume of solution present in each of the wells. The waste tray wells will be full. Ensure all wells have a similar amount of waste present.
- 11 Empty the 96-well deep well plate in the proper aqueous waste disposal area and return it to the waste drawer (second drawer from the top).

CAUTION

1 M NaOH is corrosive

1 M NaOH can damage the capillary array.

- ✓ Perform a separation with a Full Conditioning or a flush with separation gel immediately after a 1 M NaOH flush.

Daily Conditioning Flush

This flush should be completed before running any samples for the day.

CAUTION

1 M NaOH is corrosive

1 M NaOH can damage the capillary array.

✓ **Perform a separation with a Full Conditioning or a flush with separation gel immediately after a 1 M NaOH flush.**

- 1 Open the ProteoAnalyzer side compartment and replace the gel 2 bottle with a bottle containing at least 20 mL of 1.0 M NaOH.
- 2 Place at least 20 mL of protein conditioning solution on the conditioning dolution position.
- 3 From the operation tab located on the main screen, select **Add to Queue** under the **Capillary Array > Conditioning commands** menu.
- 4 From the **Select Conditioning Method** window, select the **Daily 1.0 M NaOH Flush**.
- 5 Select **Edit** to ensure that the method matches the parameters in **Figure 103**.

Conditioning Method: Daily Conditioning Flush.mthdc

<input checked="" type="checkbox"/> Step #1	Solution	Gel 2			
Fill pressure	280	PSI	Time	10.0	min.
Flow rate	200	μL/s	Tray	Waste	Row A
<input checked="" type="checkbox"/> Step #2	Solution	Conditioning			
Fill pressure	280	PSI	Time	3.0	min.
Flow rate	200	μL/s	Tray	Waste	Row A
<input type="checkbox"/> Step #3	Solution	Conditioning			
Fill pressure	280	PSI	Time	3.0	min.
Flow rate	200	μL/s	Tray	Waste	Row A

Figure 103 Daily flush method parameters

- 6 Select **Ok**.
- 7 Select **Ok** again to add the method to the method queue.
- 8 Close the door to the instrument side compartment and select the green start icon the method queue to run the capillary conditioning method.

New Capillary Array Conditioning

This flush should be run whenever a new array is installed on an instrument.

- 1 Open the ProteoAnalyzer side compartment and replace the Gel 2 bottle with a bottle containing at least 20 mL of 1.0 M NaOH.
- 2 Place at least 50 mL of protein conditioning solution on the conditioning solution position.
- 3 Place at least 20 mL of protein gel on gel line 1.
- 4 From the **Operation** tab located on the main screen, select **Add to Queue** under the **Capillary Array > Conditioning commands** menu.
- 5 From the **Select Conditioning Method** window, select the **New Capillary Array Conditioning**.

- 6 Select **Edit** to ensure that the method matches the parameters in **Figure 104**.

Conditioning Method: New Capillary Array Conditioning Flush.mthdc

<input checked="" type="checkbox"/> Step #1	Solution	Gel 2			
Fill pressure	280	PSI	Time	10.0	min.
Flow rate	200	μL/s	Tray	Waste	Row A

<input checked="" type="checkbox"/> Step #2	Solution	Conditioning			
Fill pressure	280	PSI	Time	45.0	min.
Flow rate	200	μL/s	Tray	Waste	Row A

<input checked="" type="checkbox"/> Step #3	Solution	Gel 1			
Fill pressure	280	PSI	Time	10.0	min.
Flow rate	200	uL/s	Tray	Waste	Row A

Ok Cancel

Figure 104 New capillary array conditioning method parameters

- 7 Select **Ok**.
- 8 Select **Ok** again to add the method to the method queue.
- 9 Open the waste drawer (second drawer from top) and place the waste tough in the tray carrier. If a 96-well deep well plate is used it may overflow and spill.
- 10 Close the door to the instrument side compartment and select the green start icon the method queue to run the capillary conditioning method.

Reservoir Vent Valve Cleaning

Over time the reservoir vent valve may become clogged requiring cleaning. The ProteoAnalyzer instrument has a reservoir vent valve luer lock fitting and syringe, allowing you to flush the valve using the **Clean Reservoir Vent Valve** command from the **Utilities** menu.

- 1 From the **Utilities** menu, select **Clean Reservoir Vent Valve**.

The **Clean Reservoir Vent Valve** window opens (**Figure 105**).

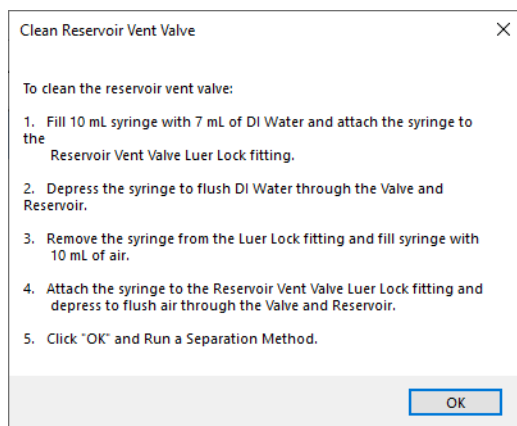


Figure 105 Clean Reservoir Vent Valve window.

- 2 Follow the on-screen instructions to clean the reservoir vent valve.

NOTE

When performing recommended vent valve flushing, it is sometimes required to perform the flush procedure more than once. Filling multiple syringes with water prior to the final flush with air can be conducive to breaking up any debris in the valve.

Capillary Array Window Cleaning

- 1 Open the side door and hood of the ProteoAnalyzer instrument.
- 2 Remove the light guide from the array window, using the Allen wrench supplied in the instrument accessory kit (see **Figure 59** on page 95).
- 3 Use a small nylon paintbrush or Kim-Wipe to gently clean the dust off the window while the window is dry. Brush across the window from left to right or right to left, not up and down.

NOTE

The dust is typically on the capillaries due to static cling and can be removed quite easily with this step. If more intensive cleaning is needed proceed to steps 4-9.

- 4 Remove the bundle end of the capillary array using the capillary array bundle removal tool (supplied in the instrument accessory kit). Place bundle in provided protective cover, taking care to not rub the capillary tips against the inside of the sleeve.
- 5 Remove the capillary array window from the capillary array window holder. Do not touch the array window.
- 6 Place a paper towel behind the capillary array window as shown in **Figure 106**.

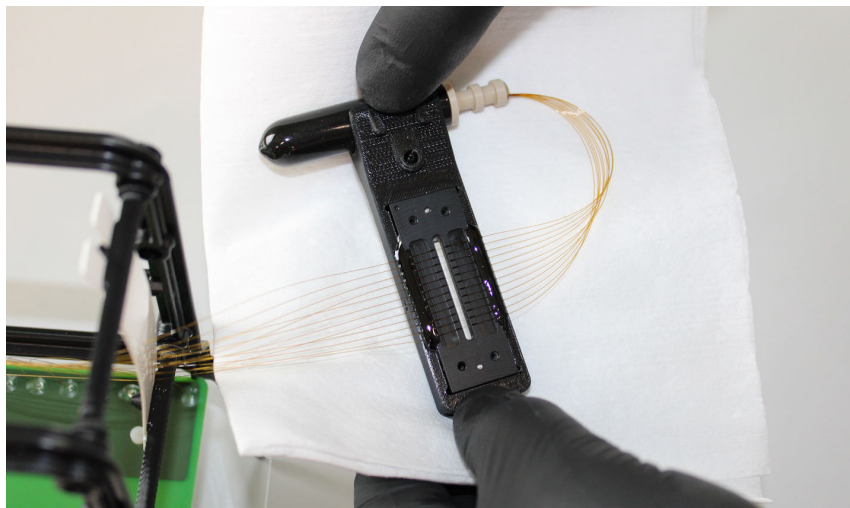


Figure 106 Capillary array window with paper towel behind

Appendix

Capillary Array Window Cleaning

- 7 Using a spray bottle filled with 70% isopropanol or ethanol solution, gently spray the capillary array window.
- 8 Use a small nylon paintbrush to gently brush the capillaries in one direction while they are still wet. Brush across the window from left to right or right to left, not up and down. Alternatively, a Kim-Wipe can be used to blot the array window dry.

NOTE

It is important to let the capillaries air dry *before* reattaching the light guide. If installed before drying, the alcohol solution can be evaporated by the light guide and then condense on the glass filter behind the array window.

- 9 Reinstall the capillary array window, bundle, and light guide.
- 10 Perform a separation on the ProteoAnalyzer instrument.
- 11 Check the alignment of the capillaries when finished by navigating to **Utilities > Capillary alignment**. Realign capillaries (see **“Capillary Alignment”** on page 52).

Long Term Capillary Array Storage

Long term storage is considered longer than 2 weeks without use. There are two methods for storing a capillary array for long term.

- Leave the capillary array installed in the instrument.
Replace the Capillary Storage Solution monthly; in drier climates it may be required to change the Capillary Storage Solution more frequently, i.e., every one to two weeks.
- Use the external array docking station that ships with all new arrays. This requires the array spindle accessory kit part #A1300-910 that is shipped with all instruments. If this part is not on hand, contact your corresponding Agilent Sales Representative to request a quote.

Using the array docking station

- 1 Remove the capillary array from the instrument. For detailed instructions, refer to **Chapter 8**, “ProteoAnalyzer Capillary Array”.
- 2 Place the tray base inside the array docking station as shown in **Figure 107**.



Figure 107 Array docking station with tray base installed

Appendix

Long Term Capillary Array Storage

- 3 Place a 96-deep well tray (Agilent part #P60-20 or Fisher part # 12-566-120) into the array docking station with tray base (**Figure 108**).

Well A1 of the tray should be located in the upper left of the docking station when facing the user, similar to the orientation when placed in the instrument.

Fill row A only with 1.0 mL capillary storage solution.



Figure 108 Array docking station with 96-deep well tray

- 4 Place capillary array into the array docking station using the four leg holes as guides. Ensure that the capillary tips are in the storage solution side of the tray, not left in open air.

Appendix

Long Term Capillary Array Storage

- 5 Insert the two white screws as shown in **Figure 109** to tighten the capillary array into place.

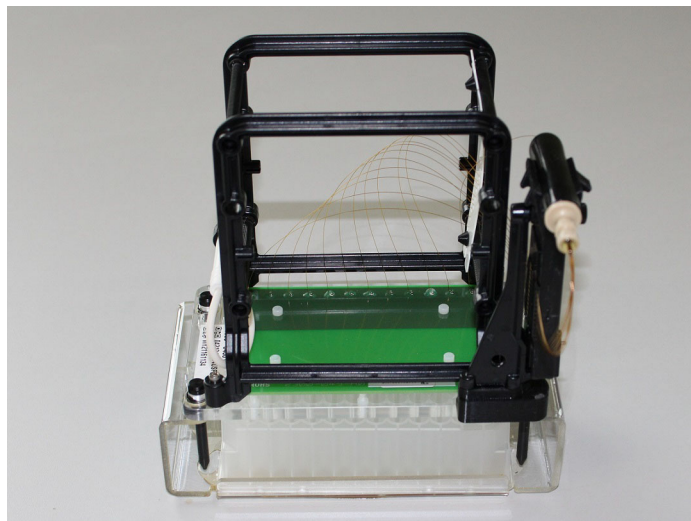


Figure 109 Array docking station with capillary array installed

- 6 Fill the provided glass vial with 20 mL of Capillary Storage Solution and place into the array spindle storage device.



Figure 110 Array spindle storage device, no storage solution in this example bottle

Appendix

Long Term Capillary Array Storage

- 7 Slide the array spindle storage device onto the capillary array side arm, located to the left of the capillary array window, and screw the locking screw into place as shown in **Figure 111**.

To see a full image of the array with the array spindle storage device installed, refer to **Figure 112**.



Figure 111 Array spindle storage device installation

- 8 Remove the capillary array outlet spindle from the black storage plug and place it into the array spindle storage device as shown in **Figure 112**.

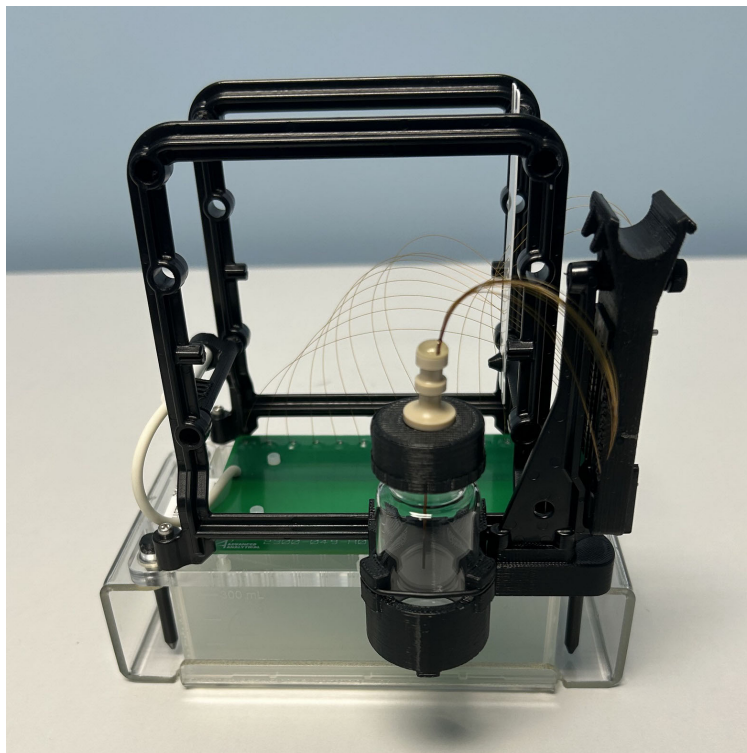


Figure 112 Array docking station with array installed

- 9 Replace the capillary storage solution monthly; in drier climates it may be required to change the storage solution more frequently, i.e., every one to two weeks.

Sound Emission

Manufacturer's Declaration

This statement is provided to comply with the requirements of the German Sound Emission Directive of 18 January 1991.

This product has a sound pressure emission (at the operator position) <70 dB.

- Sound Pressure L_p <70 dB (A)
- At Operator Position
- Normal Operation
- According to ISO 7779:1988/EN 27779/1991 (Type Test)

Waste Electrical and Electronic Equipment (WEEE) Directive

This product complies with the European WEEE Directive marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.



NOTE

Do not dispose of in domestic household waste

To return unwanted products, contact your local Agilent office, or see <https://www.agilent.com> for more information.

In This Book

This manual contains system information about the ProteoAnalyzer.

The manual describes the following:

- system overview,
- software menu commands,
- software tabs,
- capillary array,
- sample name entry,
- automated analysis
- maintenance procedures.

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