5200, 5300, and 5400 Fragment Analyzer

System Manual

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In This Guide

Agilent has prepared this manual as a technical reference for the 5200/5300/5400 Fragment Analyzer systems.

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

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 Fragment Analyzer Software – File Menu

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

3 Fragment Analyzer Software – Admin Menu

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

4 Fragment Analyzer Software – Utilities Menu

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5 Fragment Analyzer Software - Help Menu

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

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1　System Overview

This chapter gives an instrument overview.

About the System

The Fragment Analyzer system is a multiplexed capillary electrophoresis (CE) instrument for performing automated, high throughput separation and quantification of double stranded nucleic acids (DNA and/or RNA). 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 DNA/RNA molecules of a specific size range. When a high voltage is applied to the capillary array, injected DNA/RNA migrates through the gel matrix as a function of length or size, with smaller sized fragments eluting faster than larger sized fragments.

At a point toward the far end of the capillary array, detection of the separated DNA/RNA is achieved by fluorescence of a sensitive intercalating dye present in the separation gel matrix, which fluoresces when bound to double stranded DNA or RNA molecules. The Fragment Analyzer 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 DNA/RNA content of 12-, 48-, or 96-samples are collected in a single experimental run.

Configured Fragment Analyzer System Dimensions

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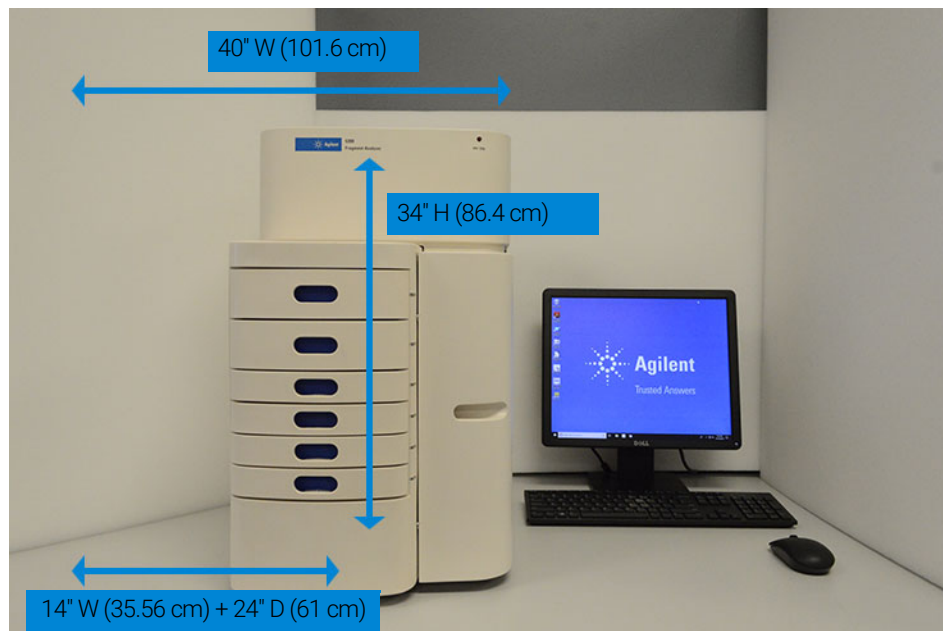
Figure 1   Configured Fragment Analyzer system with computer workstation

Fragment Analyzer System Connections

The back of the Fragment Analyzer 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 uninterrupted 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 between the system and the Fragment Analyzer system are summarized below:

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

    The order/location of connections is critical, and the locations have been identified on both the computer and the Fragment Analyzer.
  - Power cord to grounded electrical outlet
  - Connection to monitor, keyboard, mouse, etc.

AC Power Connection

Labeled USB Connections

Internet Connection

Computer Monitor Connection

Figure 2   Back of panel of the Fragment Analyzer computer connections

Labeled USB Cables

Fuse Mount

Power Switch

AC Power Connection
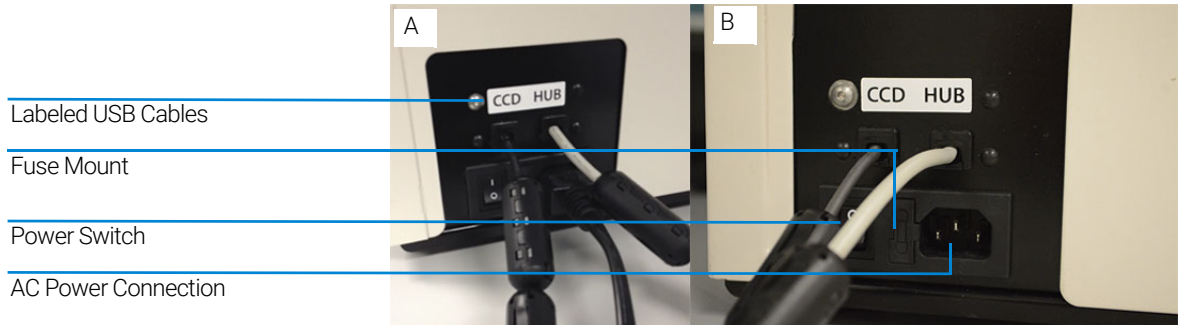
Figure 3   Back of panel of the Fragment Analyzer instrument connections

Fragment Analyzer External Cabinet

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

Top Compartments:
Capillary Array
Optics

Side Compartments:
Pump
Reagent Bottles

Drawers
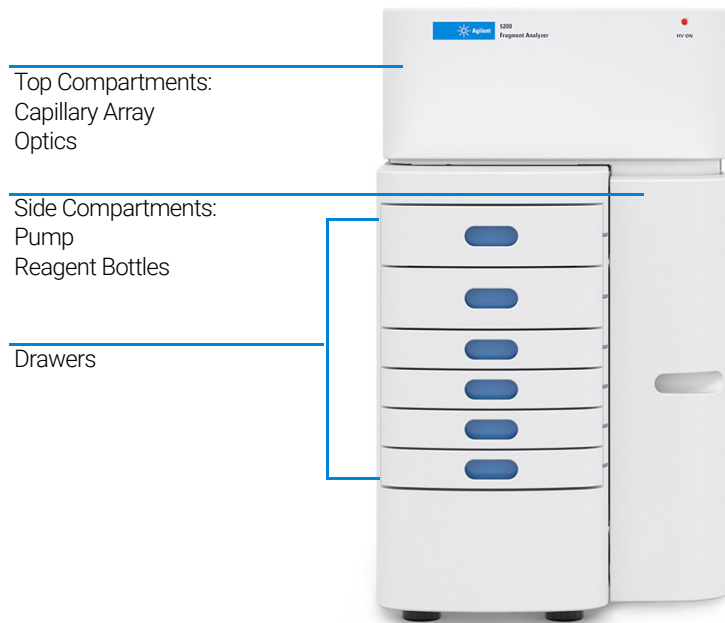
Figure 4   Entry points of the Fragment Analyzer system

Top Compartment

The *Top Compartment* provides access to the Optical detection platform and a 12-, 48-, or 96-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 this door is opened while the instrument is running.

The *12-, 48-, or 96-Capillary Array Cartridge* is a replaceable, modular component of the Fragment Analyzer system. The user can easily exchange the capillary array cartridge (for more information, refer to **Chapter 4**, "Fragment Analyzer Software – Utilities Menu").

NOTE   Note that 96- or 48-capillary arrays cannot be placed onto a 12-capillary system.

Reservoir Vent Valve

Light Guide

High Voltage Supply Cable

Capillary Outlet Connection

Capillary Array

Reservoir
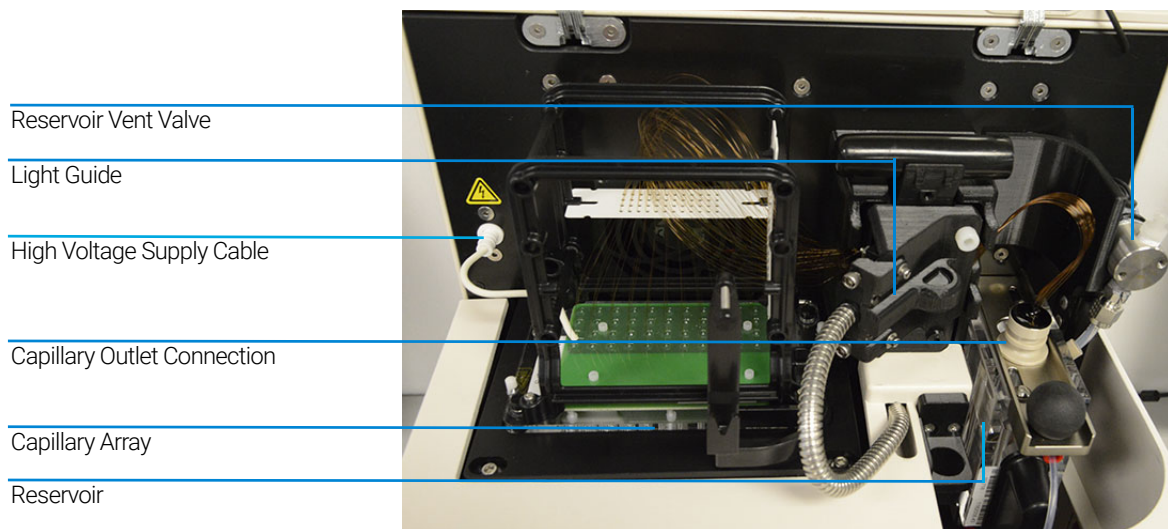
Figure 5   Fragment Analyzer main unit top compartment opened

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.

The system design enables vacuum injection (hydrodynamic injection) of samples in addition to traditional electrokinetic (voltage) sample injection, which is a feature unique to the Fragment Analyzer platform and advantageous when working with samples containing high salt matrices.

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

- **Capillary Conditioning Solution**
- **Separation Gel** (Gel 1 or Gel 2)

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.

6-Way Distribution Valve

Gel 2

Conditioning Solution

Gel 1

Waste
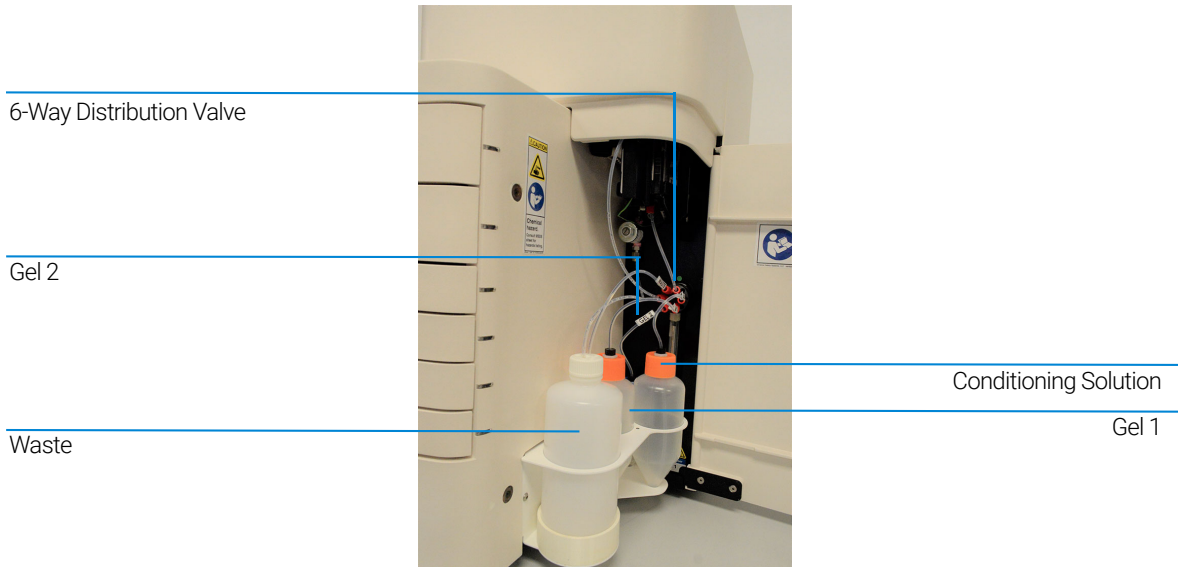
Figure 6   Side Door Compartment

The four fluid line connections inside the Fragment Analyzer system are:

• *Gel Line* (Gel 1 or Gel 2) from Syringe Pump to Gel Bottle (Gel 1 or Gel 2)
• *Conditioning Fluid* from Syringe Pump to Conditioning Fluid
• *Overflow Waste Line* from Syringe Pump to Waste Bottle
• *F-Port Line* from Syringe Pump (6-way valve) to F-Port

Drawers

The Fragment Analyzer front-panel drawers provide an external interface for loading *Buffer*, *Marker*, and *Sample 96-Well Plates* or *PCR Tubes* 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 in the 12 capillary instrument.

- 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 used for loading the *Marker Tray* or *Rinse Buffer*.

- 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*. It is also used for a 96-well plate containing *Sample Storage Solution*.

Drawer status

Status	Description
Drawers B and W are interlocked	When any of the top two drawers are open, the high-voltage (for electrophoresis) will automatically shut off.
Drawers M, 1, 2, and 3 are not interlocked	Sample trays can be exchanged while the instrument is in operation.

Drawer B: Buffer
Drawer W: Waste
Drawer M: Marker/Rinse
Drawer 1: Sample
Drawer 2: Sample
Drawer 3: Sample

Figure 7   Instrument drawer positions

Fragment Analyzer Loading and Orientation of 96-Well Plates

The Fragment Analyzer system is a multiplexed CE system containing a 12-, 48-, or 96-capillary array, which is designed to interface directly with a single row or entire plate of 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.

Well A1

Well H12
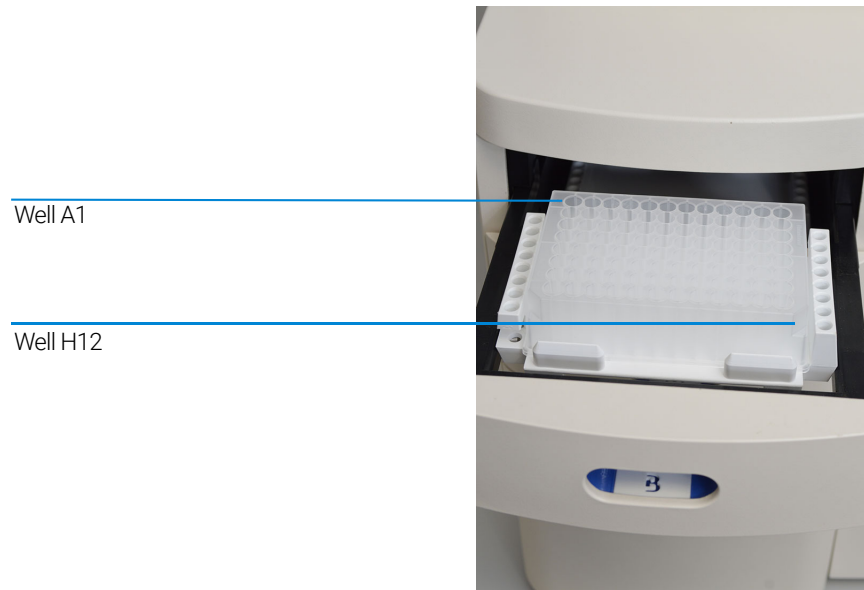
Figure 8   Proper orientation when loading 96-well marker and sample plates for a 12-capillary system

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 Fragment Analyzer system has been designed to operate using specific dimensions and styles of plates.

Plates with similar dimensions may be used, but capillary damage may occur with the use of poor-quality PCR plates.

For a list of compatible PCR plates please refer to **"Compatible Plates and Tubes for Fragment Analyzer Systems"** on page 141.

Fragment Analyzer Loading Samples

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

When preparing lower/upper DNA marker plates for repeated use, a volume of 30 µL/well with a 20 µL mineral oil overlay is recommended.

Ensure the sample has been adequately mixed with the diluent marker or dilution buffer 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.

2 Fragment Analyzer Software – File Menu

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

About the Software

The Fragment Analyzer 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 1**).

Table 1   Minimum computer requirements

Type	Specification
Processor	Intel Core i5 – 8500
SVGA Video	Minimum Resolution 1024 X 768
Memory	4.0 Gigabytes (1 x 4 GB) DDR4-2666
Available Hard Disk Space	500.0 Gigabytes
USB Serial Ports	6 ports (2 instrument, keyboard, mouse)
Network	If not using a local database, A network connection to the database server host is desired.

System Installation

To install the Fragment Analyzer software:

1   Locate the Fragment Analyzer 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\Fragment Analyzer.

Opening the Fragment Analyzer Software

1 To login to the software, select the Fragment Analyzer software icon.

Figure 9   Fragment Analyzer icon

There are two levels of users available:

- **Administrator**: The administrator login has enhanced access to functions such as allowing the user to edit separation methods.
- **User**: The user login has restricted access that allows only routine operation of the instrument.

2 To login to the Fragment Analyzer software, type **Administrator** or **User** into the **User ID** field of the login window.

3 Enter your password.

The first time you login, the password information is left blank.
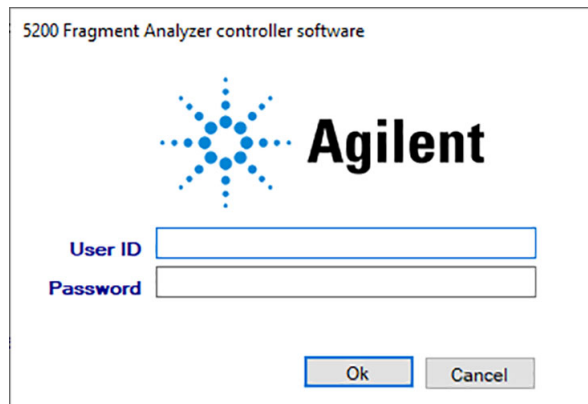
Figure 10   Login menu. Example shows login for 5200 Fragment Analyzer instrument

4   Select **OK**.

The main screen opens.
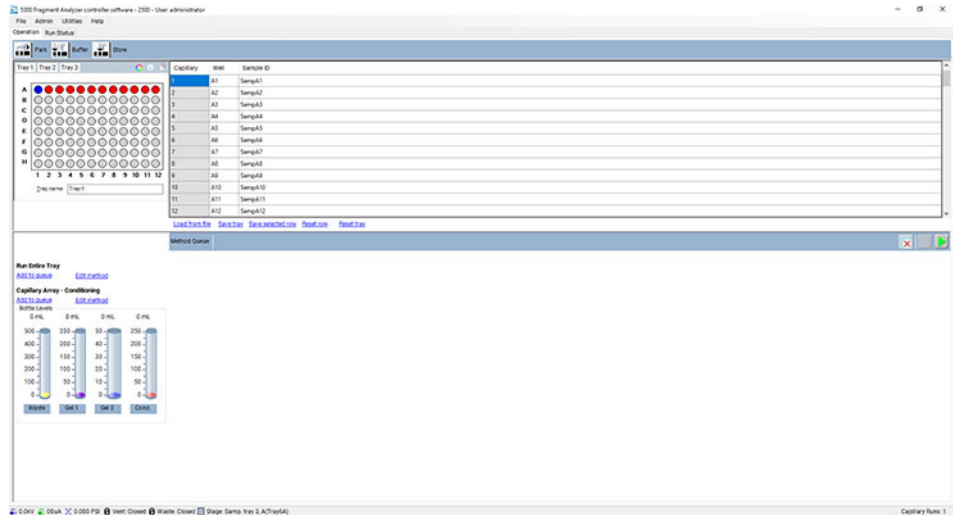
Figure 11   Fragment Analyzer software main screen window (example shows the software for a 5300
Fragment Analyzer instrument)

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 Fragment Analyzer main screen as seen in **Figure 11**.

File Menu
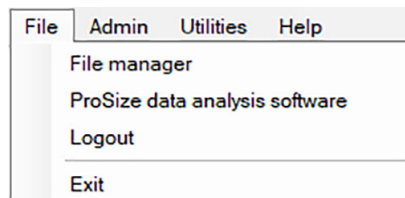
Figure 12   File menu commands

File Manager

The file manager function allows electropherogram data to be examined within the *Fragment Analyzer* 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 2**.

Table 2   File manager – file functions.

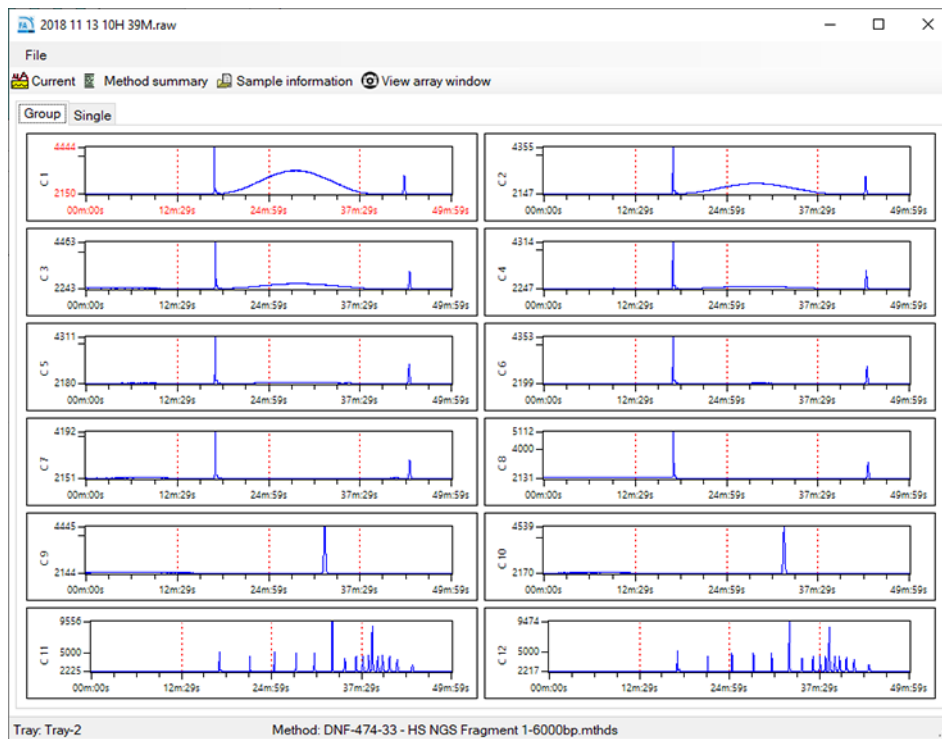| Field	Description
Open	Opens a Windows dialogue box to navigate to desired data file.
Cap. Alignment	Allows the user to view and manipulate the capillary alignment for the data file opened only. Capillary alignment from a file is discussed in the capillary alignment chapter.
Merge Files	Available for users running an entire 96 well tray on a system with less than 96 capillaries. 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 **Current**, **Method Summary**, and **Sample Info** toolbar functions are discussed in Table 3.

Table 3   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. A page selection is located at the bottom of the screen allowing for navigation of all rows in a plate (assuming 96 capillary array data is chosen).

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 software.

Logout

The Logout function allows the user to logout of the Fragment Analyzer program and login as a different user.

The login menu (**Figure 11**) will be presented for the user to login.

Exit

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

3 Fragment Analyzer Software – Admin Menu

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

Admin Menu

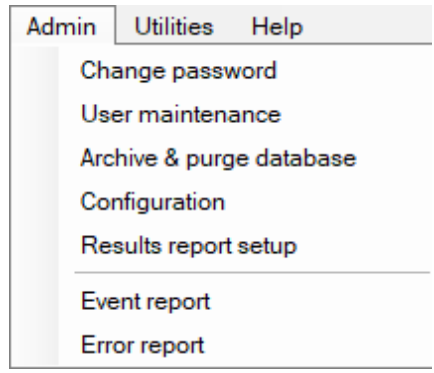**Figure 14**   Admin menu commands
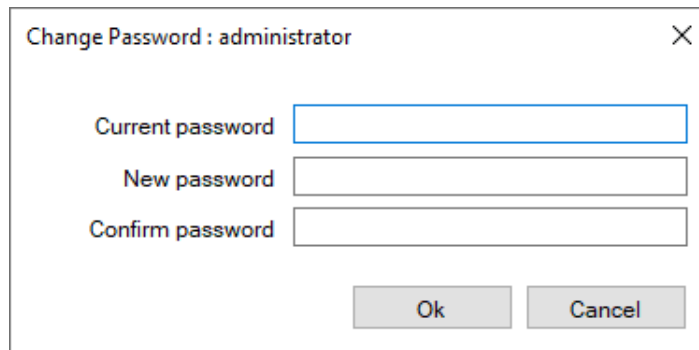
Change Password

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.
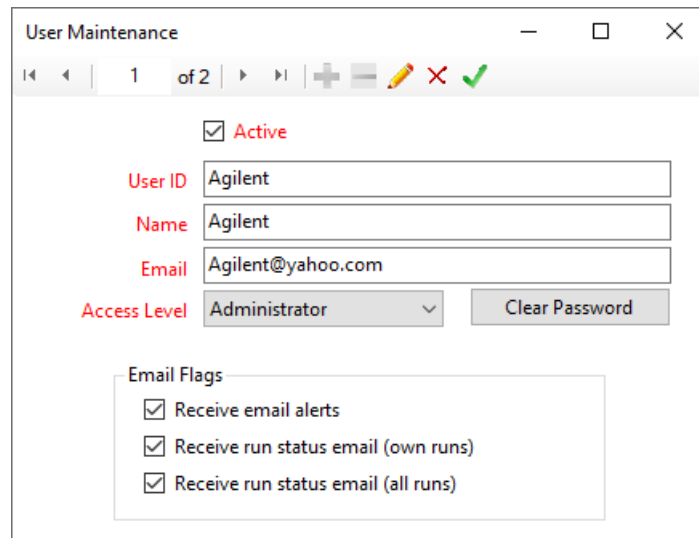
Figure 15   Change Password popup menu

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 Fragment Analyzer 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 ✔ .

Figure 16   User Maintenance popup window

A summary of the parameters in the **User Maintenance** window is illustrated in **Table 4**.

Table 4   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**).

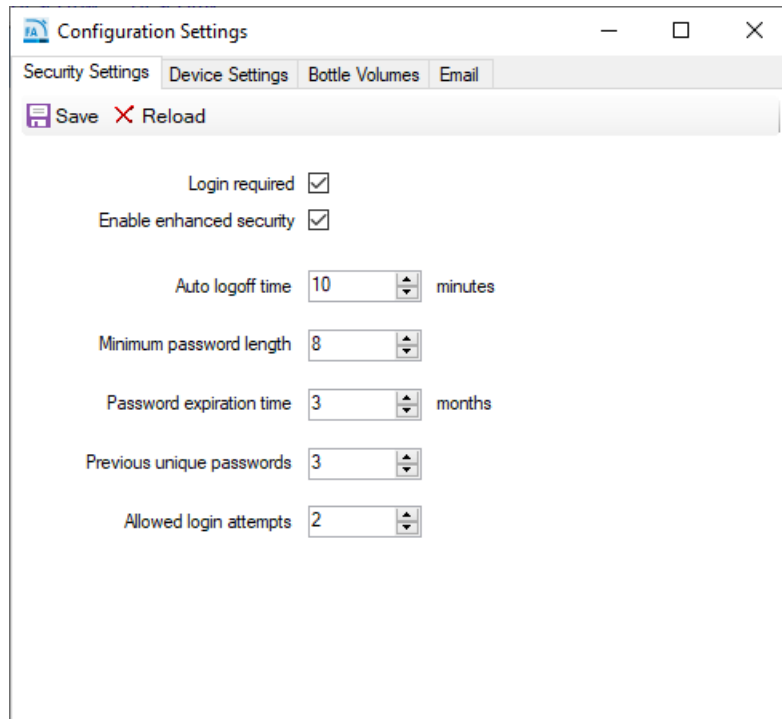A summary of the configuration options in the **Security Settings** tab is provided in **Table 5**.

Figure 17   Configuration – Security Settings tab popup window

Configuration Options
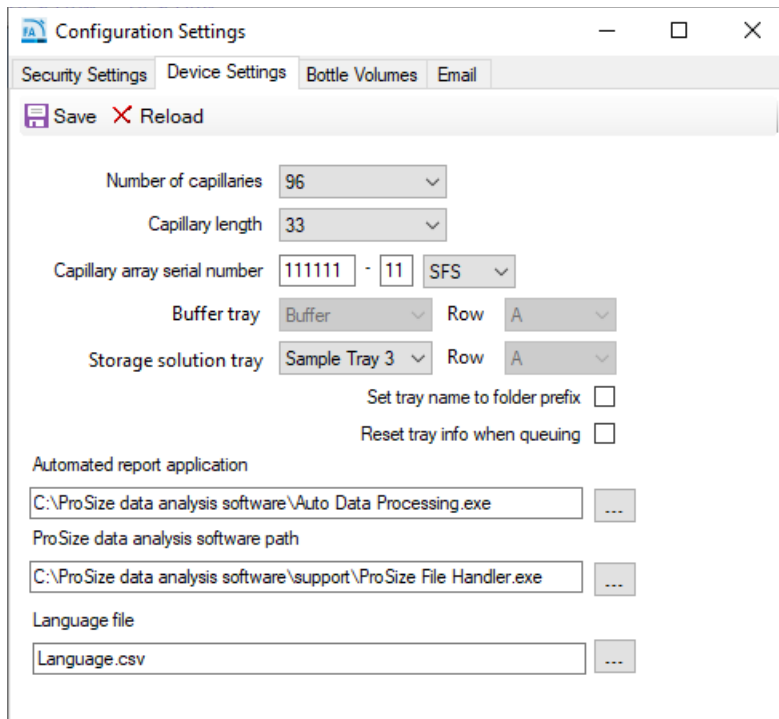
Table 5   Configuration – Security Settings tab functions

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.
Electronic Records	True or False	If True: Enhanced security features is enabled. If False: Enhanced security features is NOT enabled
Minimum Password Length	0 to 12	The password must exceed this number of characters
Allowed Login Attemps	0 to 12	If a user attempts to login with an invalid password after this many attempts: • 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.
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.
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.
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.

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 6**.

Figure 18   Configuration – Device Settings tab popup window

Table 6   Configuration - Device Settings tab functions

Parameter	Access Level	Description
Number of Capillaries	Administrator	Values: 12, 48, or 96 Note: Selecting 12 or 48 when a 96 capillary array is installed may cause hardware issues and ruin the array.
Capillary Length	Administrator	22, 33, or 55 Note: Refers to the effective length of the capillaries in use. Selecting a capillary length will force the system to use those methods only. 22 cm effective capillaries are only available with 12 capillary instruments.
Capillary Array Serial Number	Administrator	The format must be xxxxxx-xx-xxxx.
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.
Language File	User	Enables user to change language of the application by selecting the appropriate (.csv) language file (Example: Chinese, English and German).
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.
Save	Administrator	Saves the chosen settings.
Reload	Administrator	Reloads the previously saved settings.

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

The gel 1, gel 2, conditioning, and waste bottles can be set from 50 mL to 5000 mL by entering the appropriate volumes. These settings depend on the types of containers used in the system. For example, most 12-capillary systems use 50 mL centrifuge tubes for gel 1 and gel 2, with a 250 mL centrifuge for the conditioning solution. 96-capillary systems may use 250 mL for gel 1, 250 mL for conditioning, and 50 mL for gel 2. Larger volumes may be used if the system is configured with larger containers.
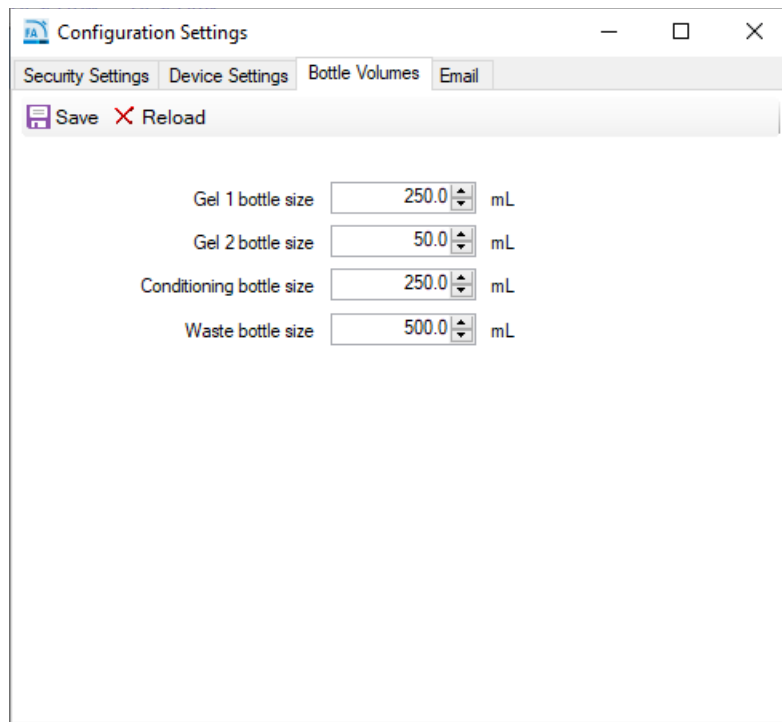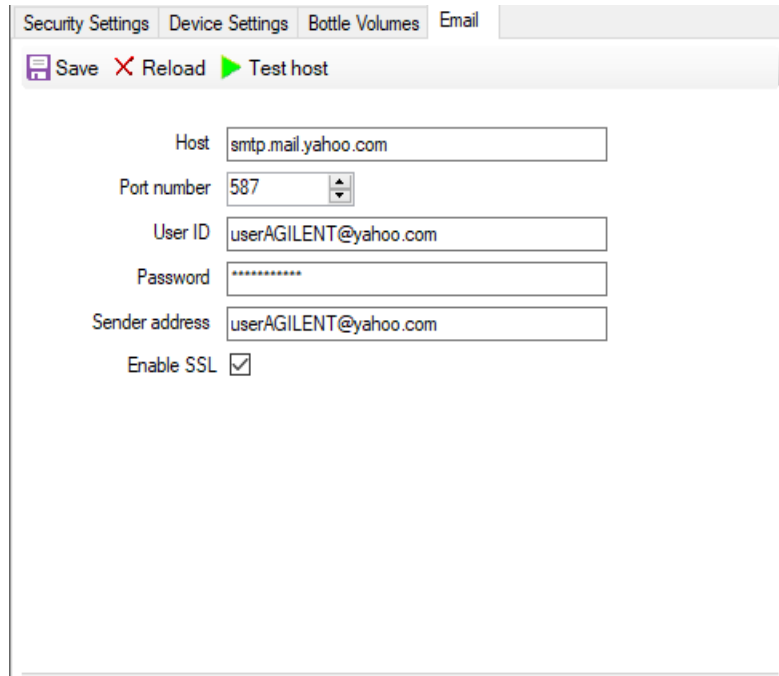
Figure 19   Configuration – Bottle Volumes tab popup window

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

Figure 20   Configuration – Email popup window

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**.

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**, "Fragment Analyzer - Automated Analysis".
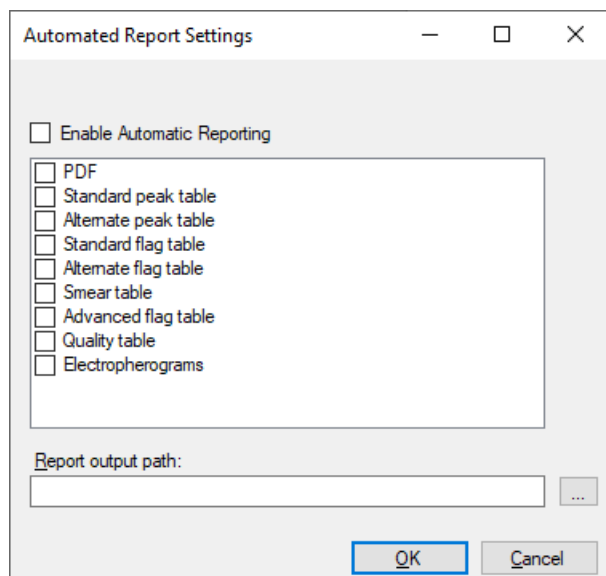
Figure 22   The Results Report Setup screen

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**, "Fragment Analyzer - Automated Analysis" which gives a detailed description of auto processing.

NOTE   In order for auto-processing to work correctly, the name of the Fragment Analyzer method must exactly match the name of the ProSize configuration file. For more details, please refer to **Chapter 10**, "Fragment Analyzer - Automated Analysis".

Event Report

The command **Event Report** provides a tabular report of the audit trail of the events that have occurred in the Fragment Analyzer 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**).
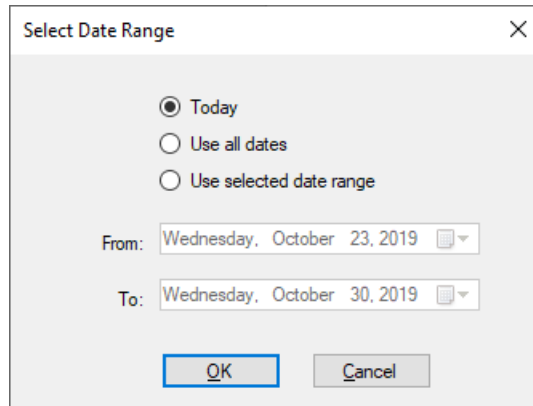
Figure 23   Event Report popup window

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**).

Figure 24   Event Report example

The icons along the top of the **Event Report** follow standard Windows function nomenclature and are summarized in **Table 7**.

Table 7   Event Report icons and descriptions

Icon	Description
◁◁ 1 of 10 ▷	Page Selection
⬅	Back to Parent Report
⊗	Stop Rendering (i.e. Stop Report Generation)
↻	Refresh
🖶	Print
▣	Print Layout
📖	Page Setup
🖫▾	Save
100% ▾	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**).
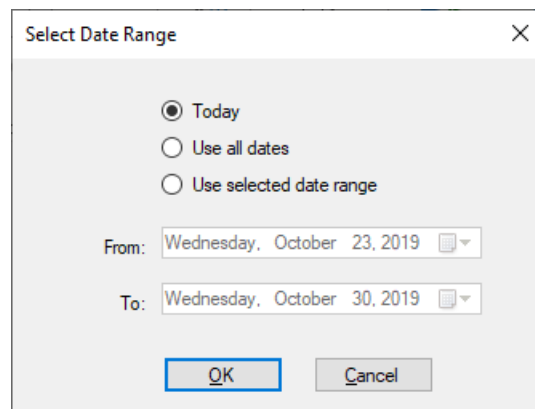
Figure 25   Error Report popup window

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 7**.

Figure 26   Error Report example

4 Fragment Analyzer Software – Utilities Menu

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

Utilities menu
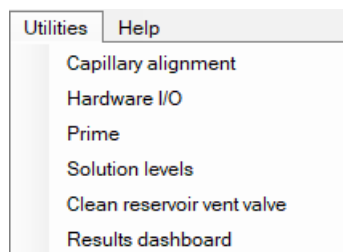
Figure 27   Utilities menu commands

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 – best used when installing new 96-cap array or fine-tuning an alignment without dye.

> **B)** Alignment without dye (12-capillary and 48-capillary only)

The methods discussed in this chapter will first be illustrated with images from a 12-capillary array. Images from a 96-capillary array will be discussed at the end of this chapter.

All of the method steps outlined for performing capillary alignments will be the same for a 96–capillary array unless otherwise noted.

NOTE   When capillary alignment is selected from the Utilities menu, the **Capillary Alignment Prep** window will open prompting the user with the option to fill the capillaries with dye. This message is a carryover from the QC process and is not relevant to customer alignment methods. (**Figure 28**).
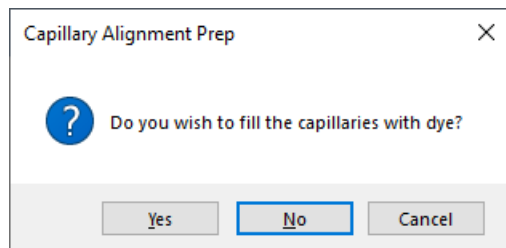
Figure 28   Capillary Alignment Prep window.

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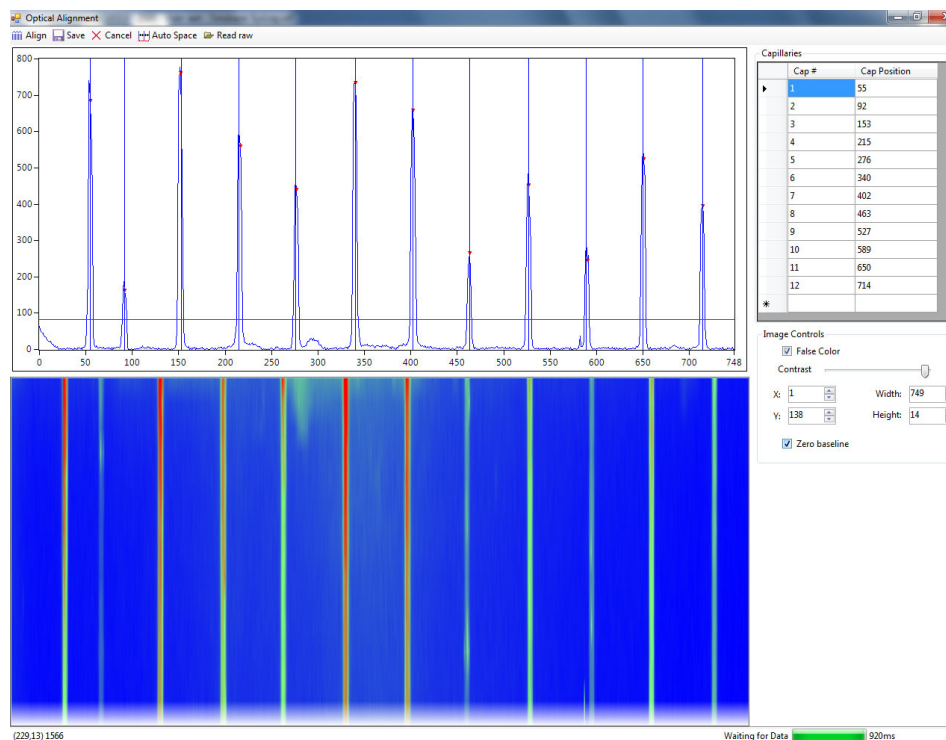
Figure 29   Real time capillary alignment popup window (example shows 12-capillaries)

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 the **Align** button and then the **Auto Space** button to ensure all blue vertical lines are evenly spaced between the first and last capillary peaks.

4 Click the **Save** button which will close the capillary alignment screen. Perform a test separation with blank solution or diluent marker 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** shows an example for a 12-Capillary and **Figure 31** for a 96-Capillary) allows you to align the capillaries from the selected run file. The toolbar of the *Align from File* windows is described in **Table 8**.
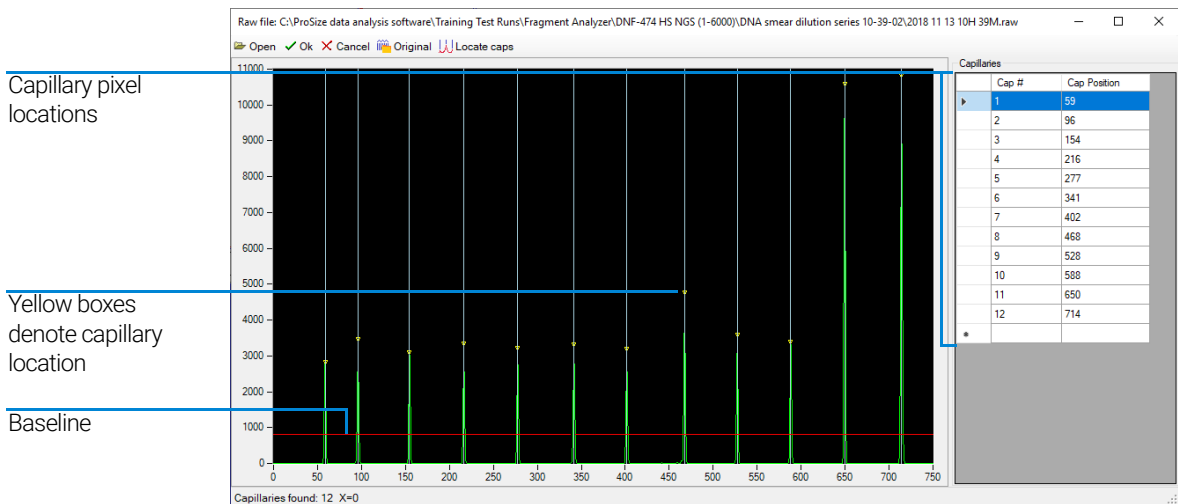
Figure 30   Align from file popup window for 12-capillary system

Capillary pixel locations

Yellow boxes denote capillary location

Baseline
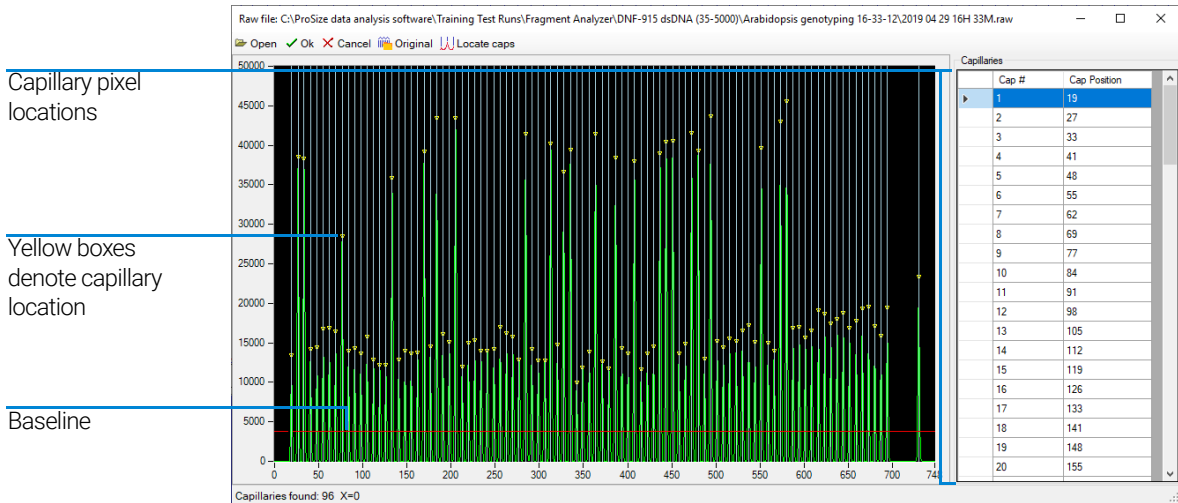
Figure 31   Align from file popup window for 96-capillary system

Table 8   Align from file toolbar functions

Icon	Description
Open	Opens a new file.
Ok	Accepts changes to the file (i.e., capillary locations).
Cancel	Cancels any actions and closes the file.
Original	Locates the original capillary positions used when the selected file ran.
Locate caps	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 on 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** and **Figure 31**.

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 12, 48, or 96 depending on the configuration of the instrument and the type of array in use.

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- or 48-capillary array.

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

This will open the real time view of the *Capillary Alignment* window (see **Figure 29**).

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.

Contrast slider

CCD camera reference

Box drawn by left-clicking and dragging

Capillary alignment references
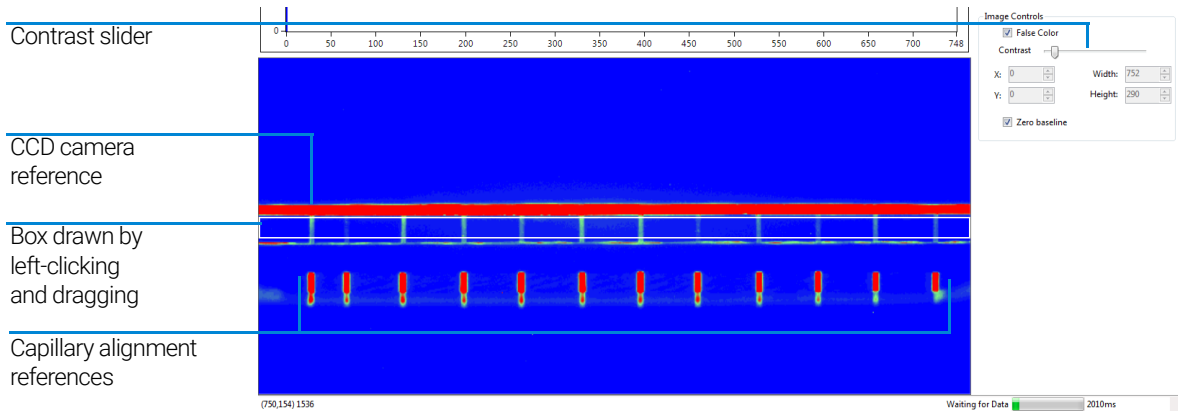
Figure 32   Capillary alignment display – window reset

5 After the box is drawn, right-click and select **Set Camera Window**.

6 Adjust the height to 14.

Table 9   Capillary alignment display menu options

Icon	Description
Align	Aligns cursors to peaks.
Save	Saves changes to the alignment and exits the window.
Cancel	Cancels any actions and closes the file.
Auto Space	Auto locates the capillary positions based off the first capillary position. Positions will need manual adjustment.
Read raw	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.

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**).
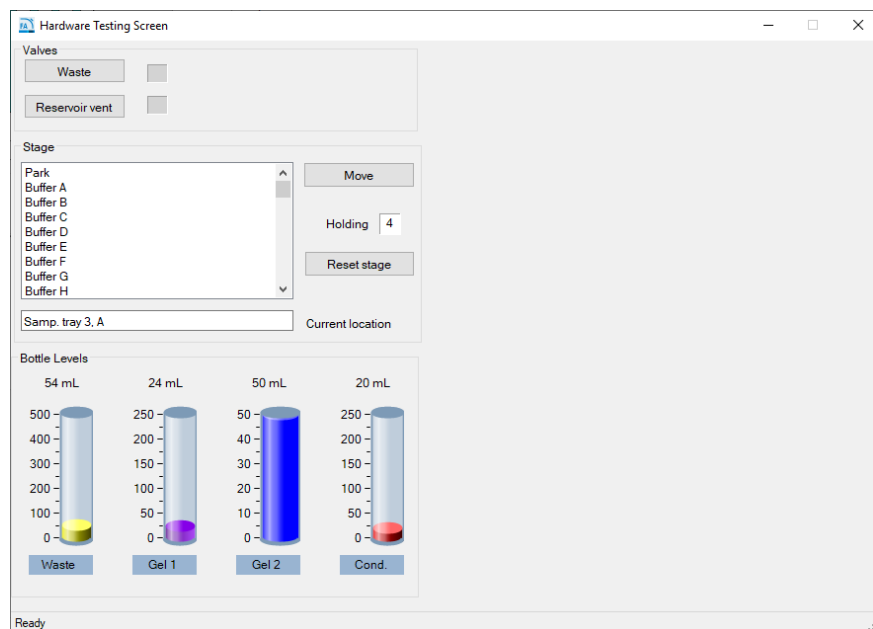
Figure 33   Hardware Testing Screen

An overview of the functions available in the Hardware Testing Screen is listed in **Table 10**.

Table 10   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).
Stage > Move	Move tray to the selected position.

Function	Description
Stage > Reset Stage	Allows the user to reset the stage position should a drawer be opened before the stage finishes its movement. Available on instrument serial numbers 2600 and above.
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). For example, if a user is switching between RNA gel and NGS gel, a gel prime can be used to purge of the old fluid prior to beginning a run. 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 11**.
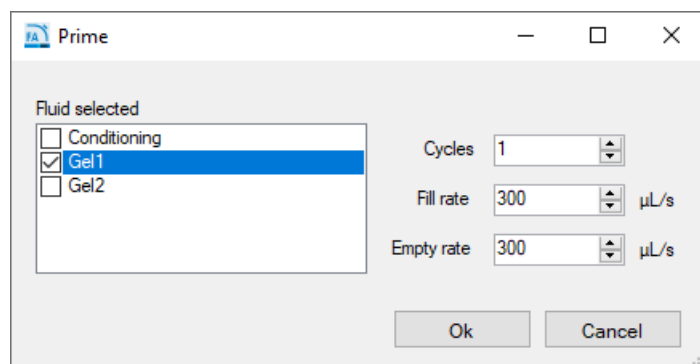
Figure 34   Prime window

Table 11   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 fill rate up and down 0-1000, the default setting is 300 uL/s.
Empty rate	Allows the user to adjust the fill rate up and down 0-1000, the default setting is 300 uL/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 Fragment Analyzer 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**).

Figure 35   Check Solutions Volumes window

1   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**.

For the program to run correctly (i.e., to issue the correct warning), it is important that the solution levels be entered 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**).
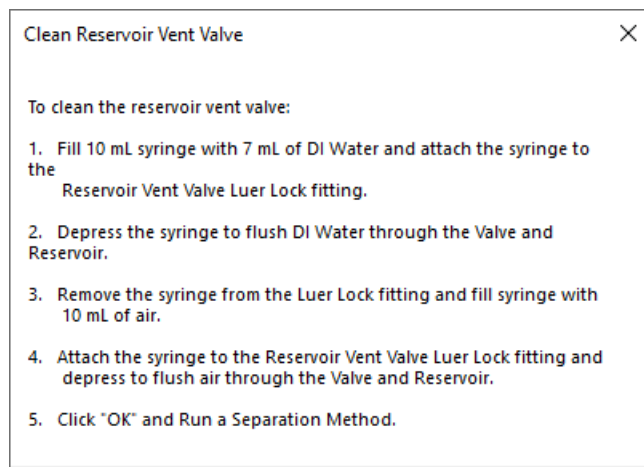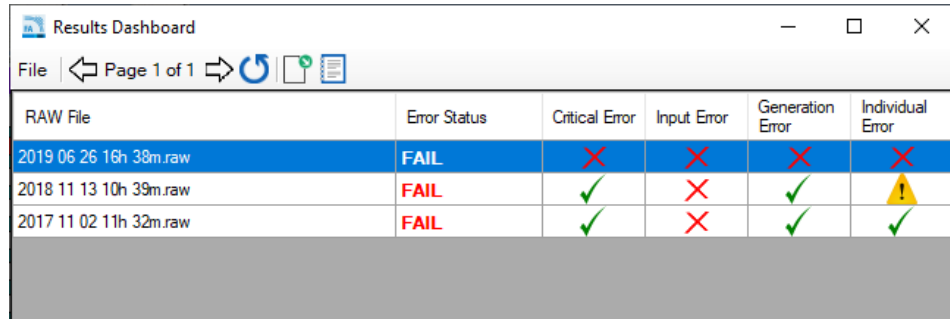
Figure 36   Clean reservoir vent valve screen

Follow the steps outlined in **Figure 36** to clean the reservoir vent valve. Should you have an older system without the reservoir vent valve luer lock fitting and syringe please contact your Agilent Sales Representative for information on how to acquire them.

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.

Figure 37　Results dashboard output

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

5 Fragment Analyzer Software - Help Menu

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

Help Menu
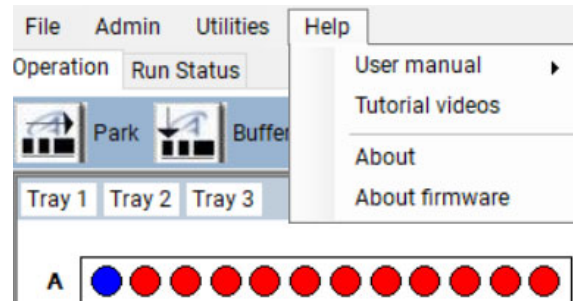
Figure 38   Help menu commands

User Manual

Navigating to the command **Users manual** will provide a drop-down list of every chapter of the user manual.

Tutorial Videos

Selection of this command will open a folder containing videos made to help the user with such talks as *Placing an array on the Fragment Analyzer* and *Performing a Capillary Alignment*.

About

The **About** command opens an **About Fragment Analyzer** 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.

6 Fragment Analyzer Software – Operation Tab

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

Operation Tab Overview
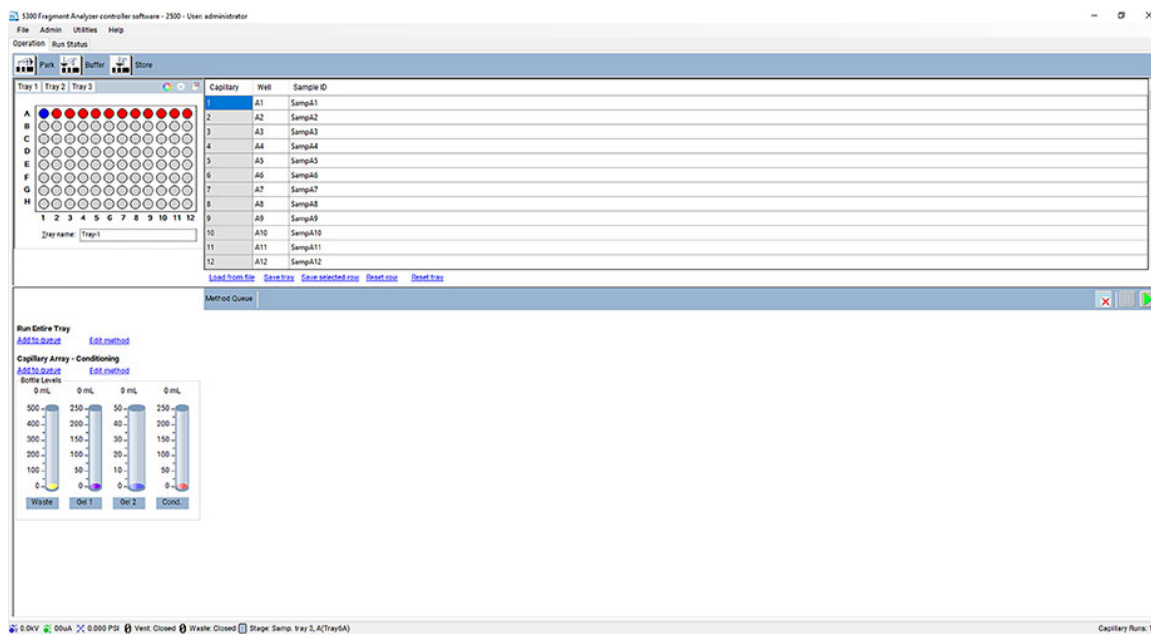
Figure 39   Fragment Analyzer main screen

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 12**.

Table 12   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
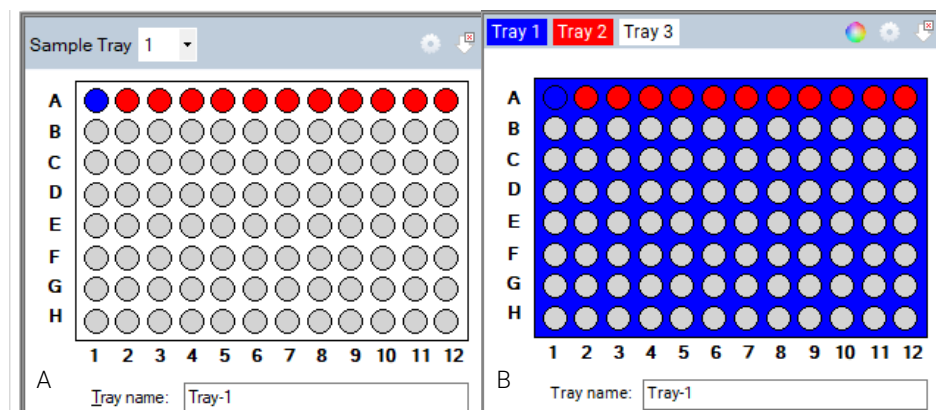
Figure 40   Classic drop-down tray selection (left), and colored tab tray selection (right).

1   In the tray window, select ⚙.

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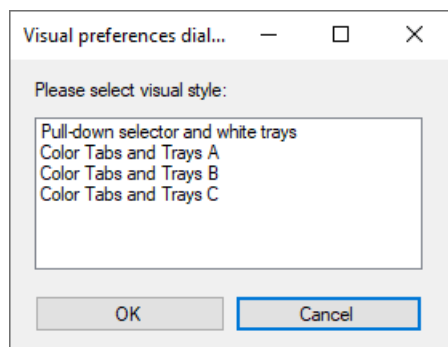
Figure 41   Visual preferences dialog window.

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**, "Fragment Analyzer - Sample Name Entry").

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

For a 96-cap system, you must select each row individually to manually enter data (rows A-H). Sample names and information can also be saved or loaded using .txt or .csv files. These functions are discussed in **Table 13**.

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 13   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 10 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.
Tray	Resets the entire sample tray to the default sample ID settings.

Experimental Run Controls and Adding to Queue

The Fragment Analyzer 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 Row** (12-cap system only), **Run Entire Tray**, and **Capillary Array – Conditioning**. The **Run Selected Group** option is only available for a 12-capillary Fragment Analyzer and will be hidden for 96-capillary instruments.

Reagent Levels of the bottles are also shown.
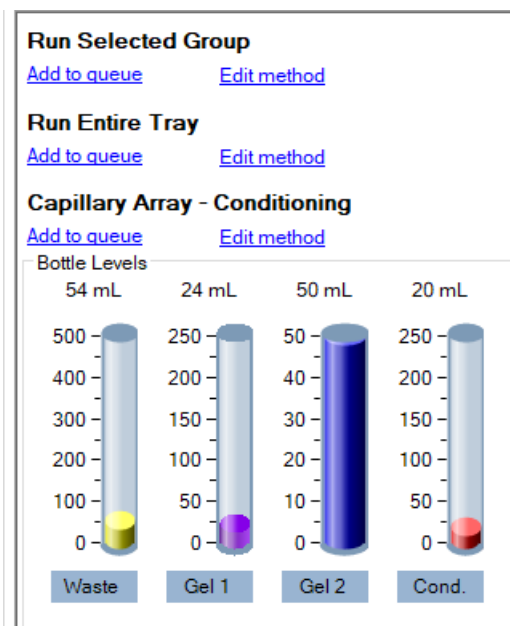
Figure 43   Experimental run controls

Run Selected Group or Run Entire Tray – Edit Method

Selection of the **Edit method** will show the method editor popup 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.

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 14**.

Optimum capillary conditioning values are preloaded and defined for each method. Refer to each method Kit Guide of interest (for example, NGS, genomic DNA, etc.) for further definition of these values.

Table 14   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.
Prerun	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	Marker injection is enabled when using the Qualitative Kits. The user has the option of a voltage or vacuum injection with selection of **Voltage**, **Pressure**, and **Time** parameters. On a 12-capillary unit, the user is able to select the **Row** to use for marker injection.
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 to enter the **Voltage** and **Time** of the CE separation.

The user 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**.
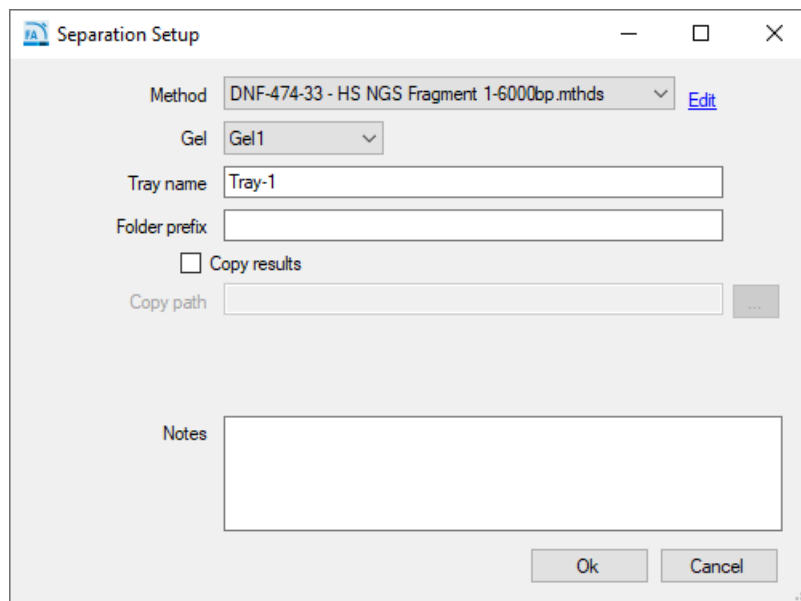
Figure 45   Separation Setup window

The settings of the **Separation Setup** window are discussed in **Table 15**.

Table 15   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.

Table 15   Separation setup window functions

Item	Description
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.
Create Size Calibration File	This is used for automated analysis (**Chapter 10**, "Fragment Analyzer - 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 director 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 or H12, as defined in the kit manuals.
Use Size Calibration File	This is used for automated analysis (**Chapter 10**, "Fragment Analyzer - 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 of DNA fragments.
Size Cal. File	This is used for automated analysis (**Chapter 10**, "Fragment Analyzer - 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.

Table 15　Separation setup window functions

Item	Description
Notes	The section allows for the addition of any additional information the user may require for a set of samples.
Merge rows	When selected, will merge 8-rows of 12 or 2 sets of 48-cap "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 Fragment Analyzer software provides a preloaded capillary conditioning method for cleaning the capillary array.

The user can also select to create a method of their choosing by selecting the **Edit Method** option seen in **Figure 46**.

Selecting the check box next to the individual parameter can enable different bottles to be used for the conditioning. The individual parameters are discussed in **Table 16**.

Figure 46   Conditioning method editor

Table 16   Separation 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 **OK** 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**).
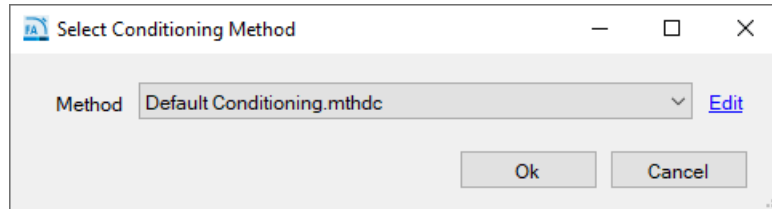
Figure 47   Select Conditioning Method window

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 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**).
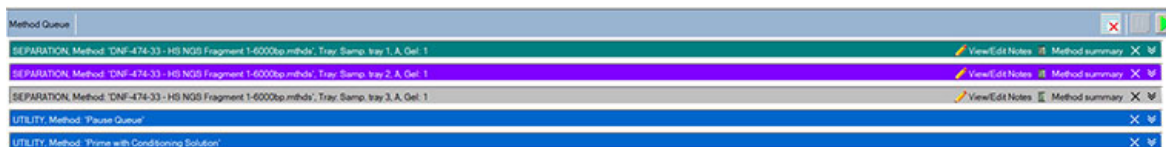
Figure 48	Method Queue

Figure 48 shows three sample 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**).
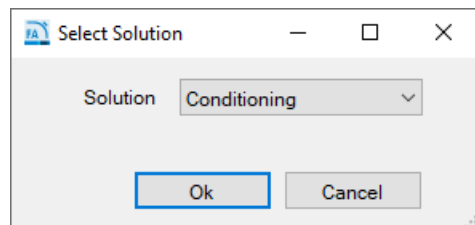
Figure 49	Select Solution popup window

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**.
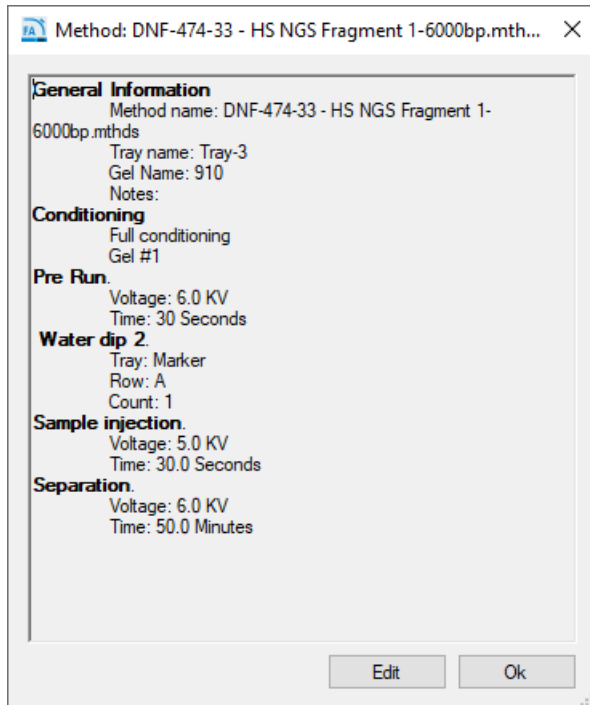
Figure 50   Method summary popup window

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

To delete a specific item on the queue, select the **X** icon next to the Separation Method. To delete all items in the queue, select [x] **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 Separation Method.

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 17**, below.

Table 17   Method queue run controls

Item	Description
	Clear: Selection of this icon will clear all separation 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 Fragment Analyzer Software - Run Status Tab

This chapter describes the Fragment Analyzer 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 82), 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 Fragment Analyzer stage is moving to/from to give the user real time view of what is happening.
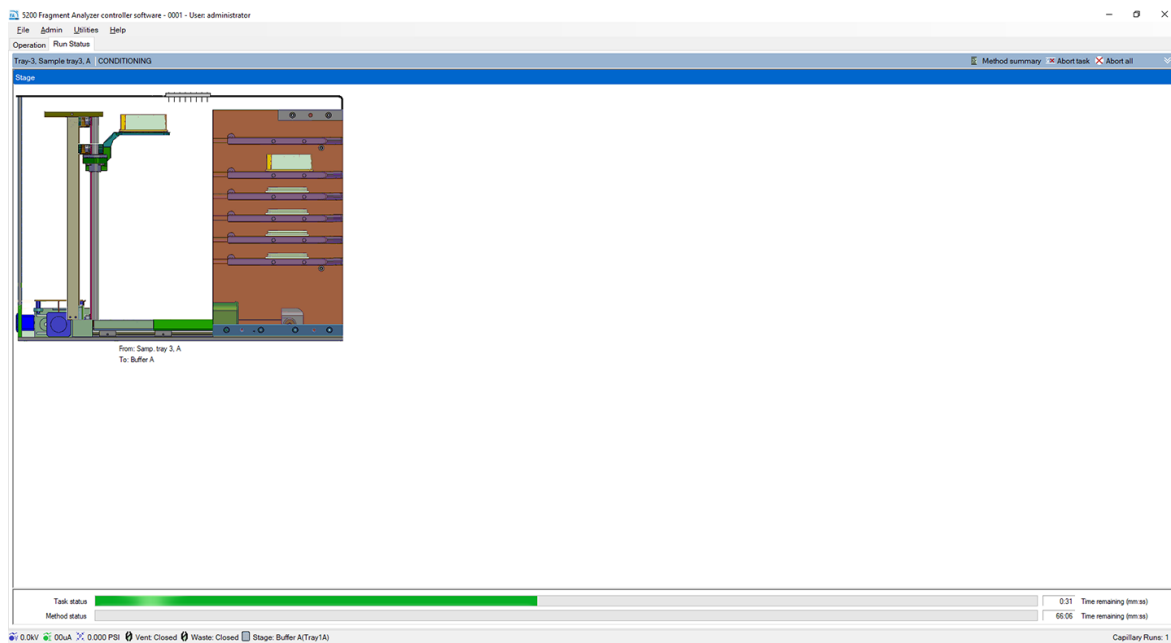
Figure 51 Stage movement animation

Conditioning Animation

When the Fragment Analyzer instrument is pumping conditioning solution or gel, 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.).
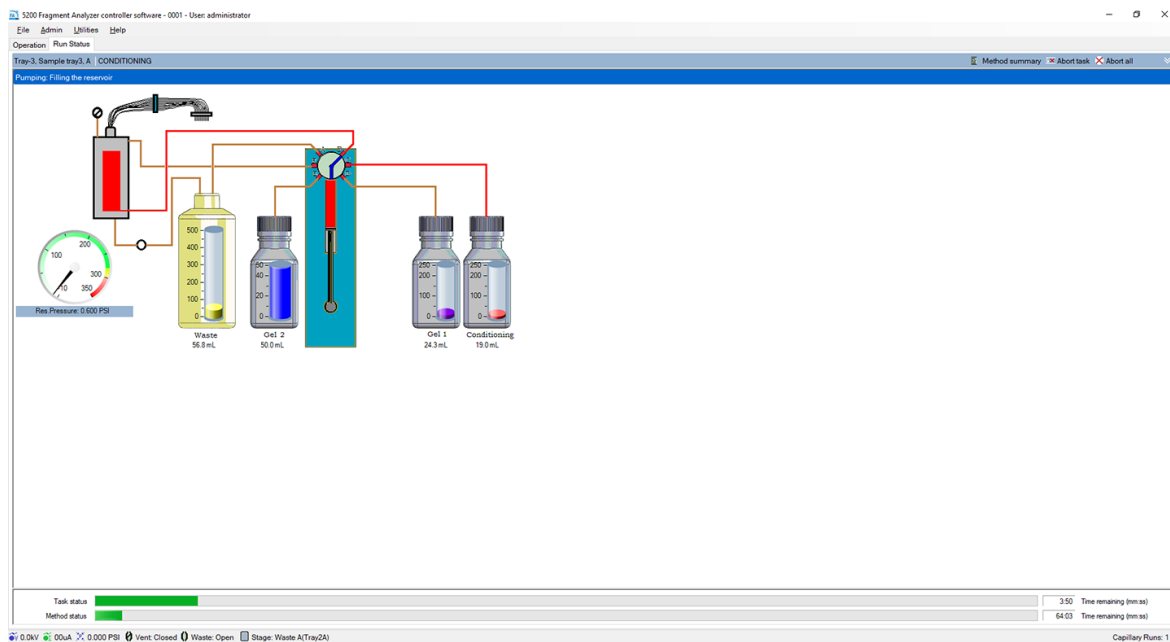
Figure 52 Conditioning animation

Pre-Run / Injection View

When the Fragment Analyzer system is completing a pre-run or injection, the screen as shown in **Figure 53** will appear.
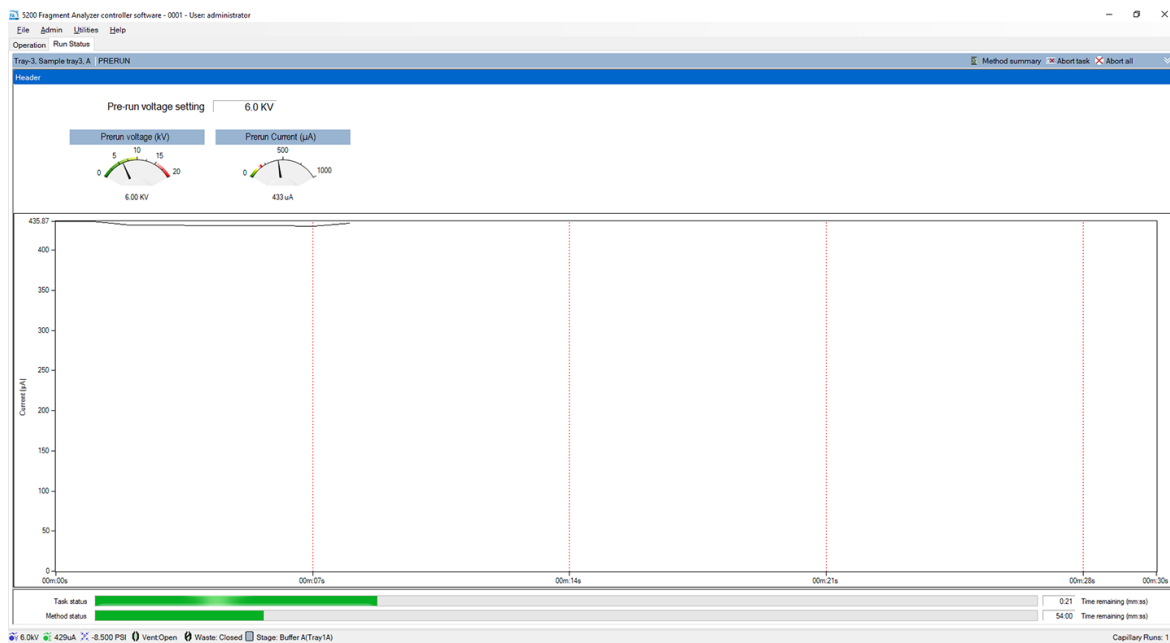
Figure 53 Pre-Run/Injection screen

Real-time Separation View

When the Fragment Analyzer system performs a separation, the screen in
Figure 54 appears, which shows a real-time view of the separation.
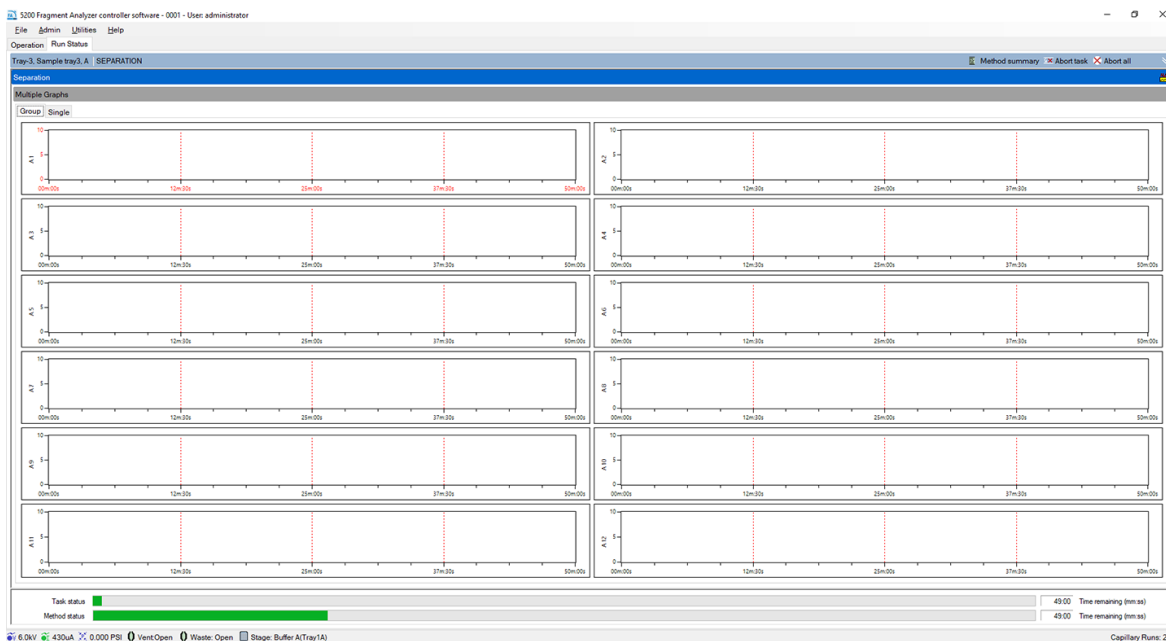
Figure 54 Real-time separation window

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. When a 96-capillary system is running, the user can select
between groups of 12 or single electropherograms by selecting the appropriate
row/capillary with the **Group** or **Single** tabs.

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**, "Fragment Analyzer Software – Utilities Menu".

Other options available from the **Run Status** tab are discussed in **Table 18**.
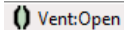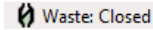
Table 18 Run Status Tab options

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 Fragment Analyzer software shows a real time status bar containing important information about the instrument status. The icons and their function are discussed in **Table 19**.

Table 19 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 Fragment Analyzer Capillary Array

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

Capillary Array Parts

The Fragment Analyzer instrument capillary array allows for direct parallel injection and separation of 12-, 48-, or 96-samples at once.

Capillary array bundle

Capillary array frame

Allignment pin

Capillary tips and electrodes
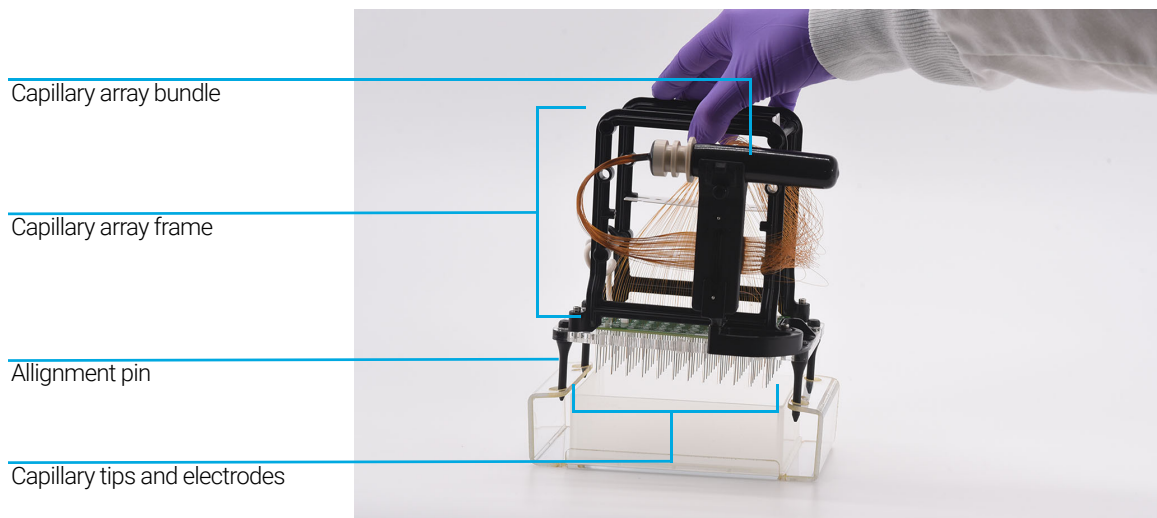
Figure 55   Capillary array parts (96-capillary array shown)

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 Fragment Analyzer instrument.

Before proceeding with capillary array removal, select the **park** icon from the main screen to place the tray being help 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   Fragment Analyzer instrument

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

Holder with high voltage plug

High voltage cable connection
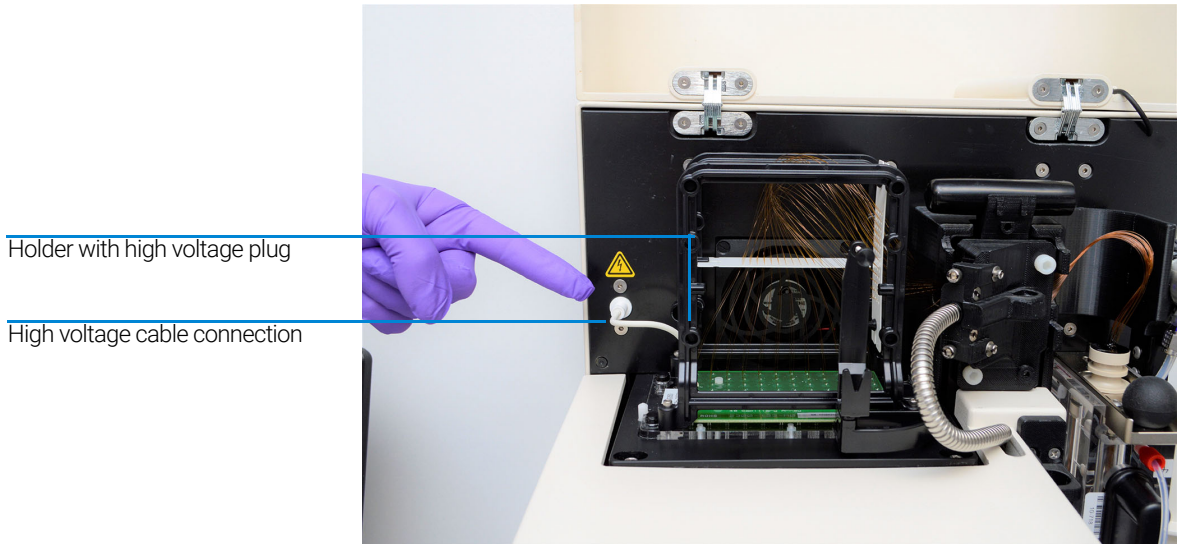
Figure 57   Instrument top compartment – high voltage supply cable

3 Use the provided allen wrench to remove the two white screws that secure the light guide to the array window.
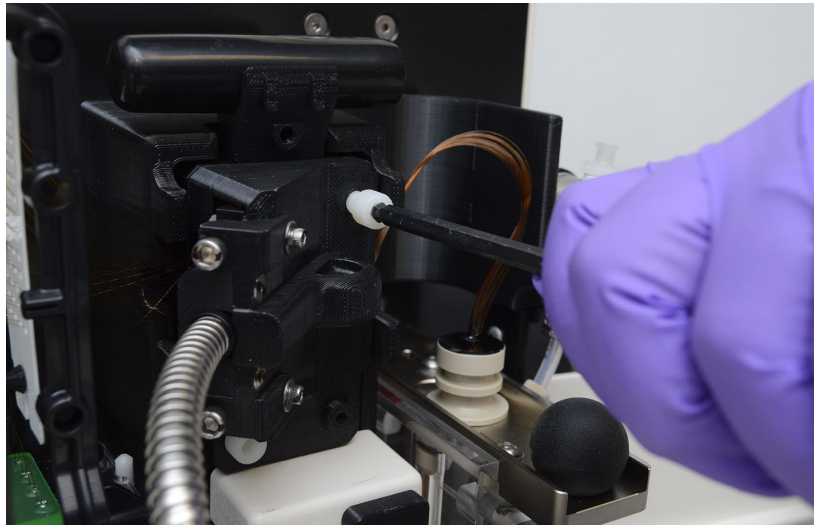
Figure 58   Instrument top compartment – unscrew light guide

4 Remove the light guide.
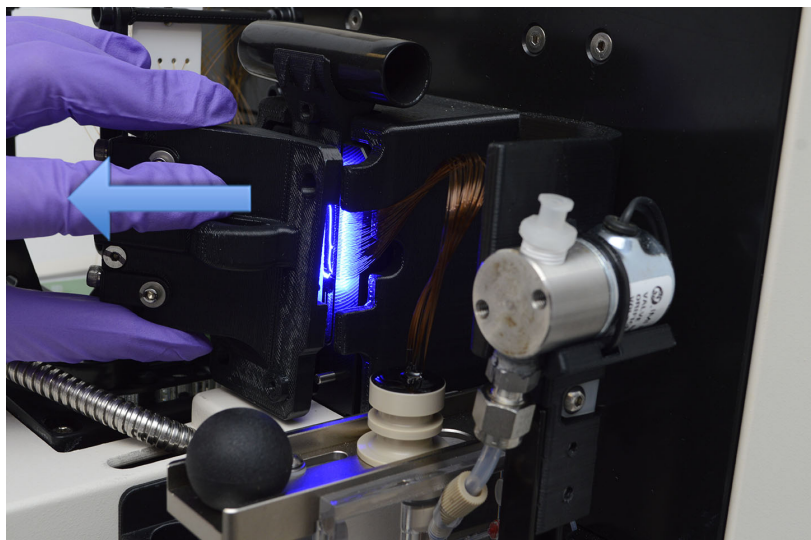
Figure 59   Instrument top compartment – light guide removal

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.

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 to loosen the capillary array bundle by prying up on the bundle.
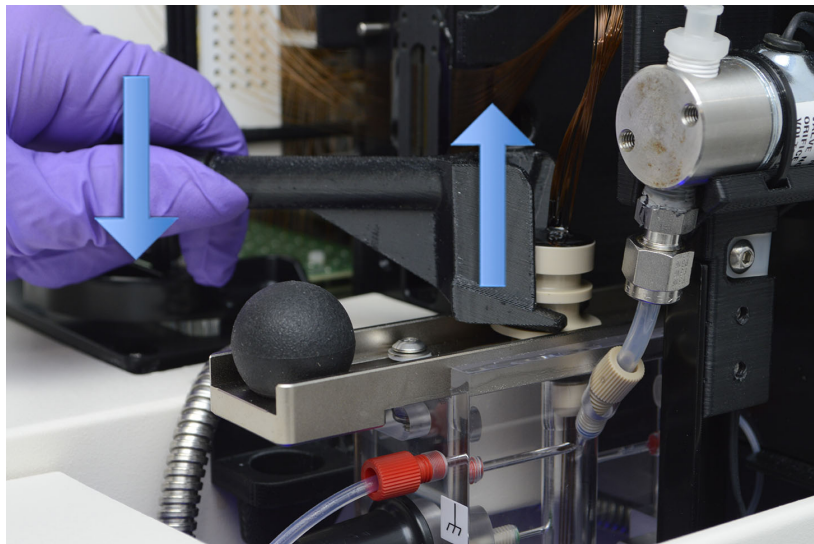
Figure 61   Instrument top compartment – capillary reservoir connector tool

7 Remove the capillary array bundle by pulling up gently.

NOTE   Avoid pulling up hard as to not break any capillaries.
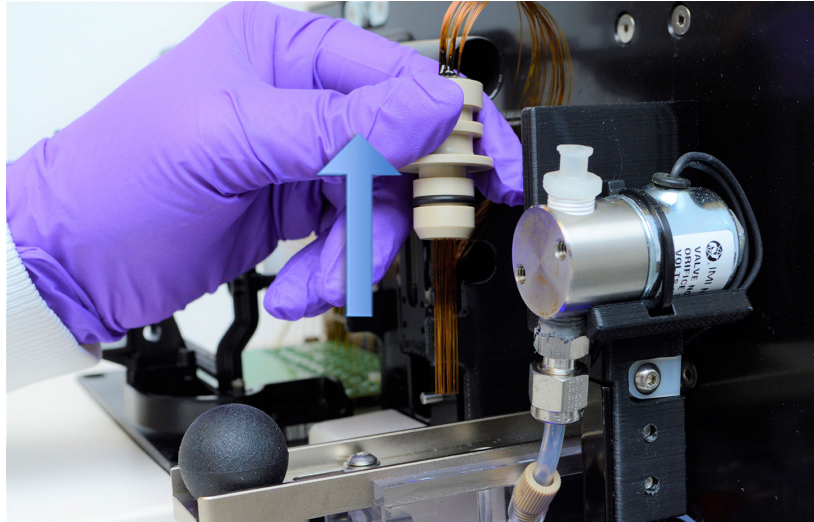
Figure 62   Instrument top compartment – capillary array bundle removal

8 Carefully insert the protective cover over the capillary bundle.
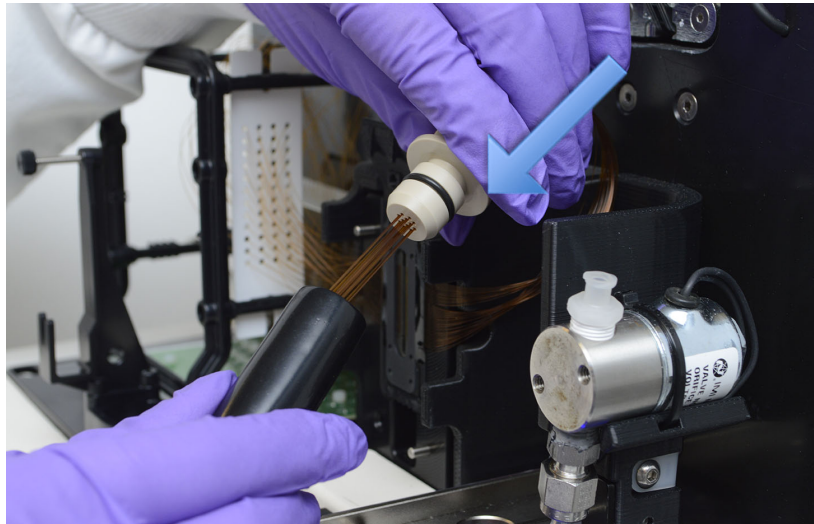
Figure 63   Instrument top compartment – installing protective cover

9 Place the capillary array bundle on the top holder of the capillary array window.
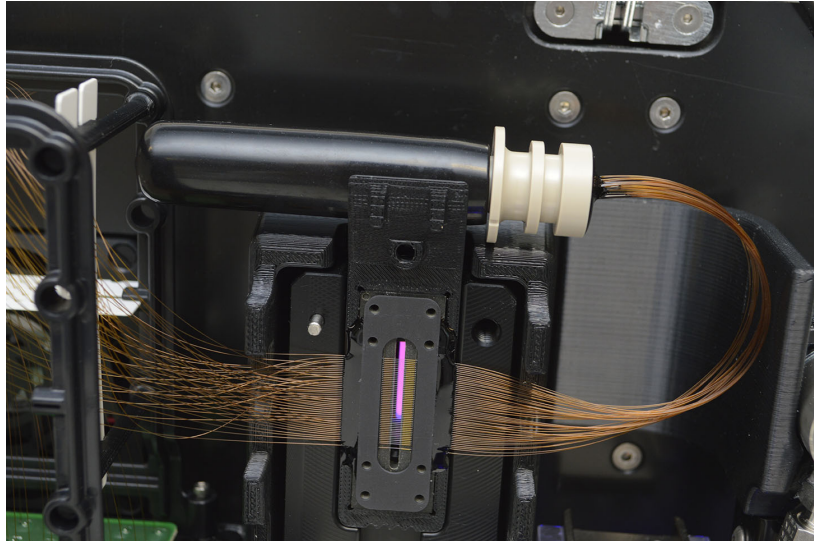
Figure 64   Instrument top compartment – storing covered capillary array bundle

10 Remove the capillary array window from the window holder. 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.
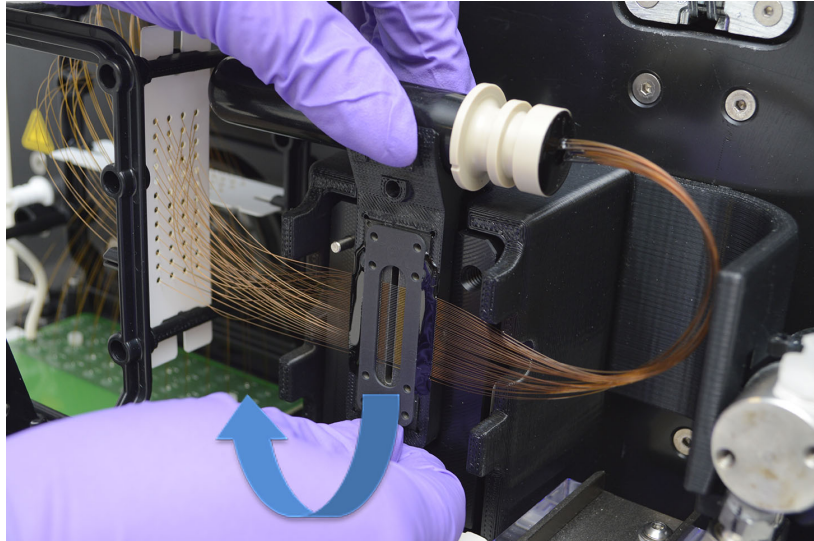
Figure 65   Instrument top compartment – remove capillary array window

11 Attach the array window to the capillary array frame using the attachment screw.
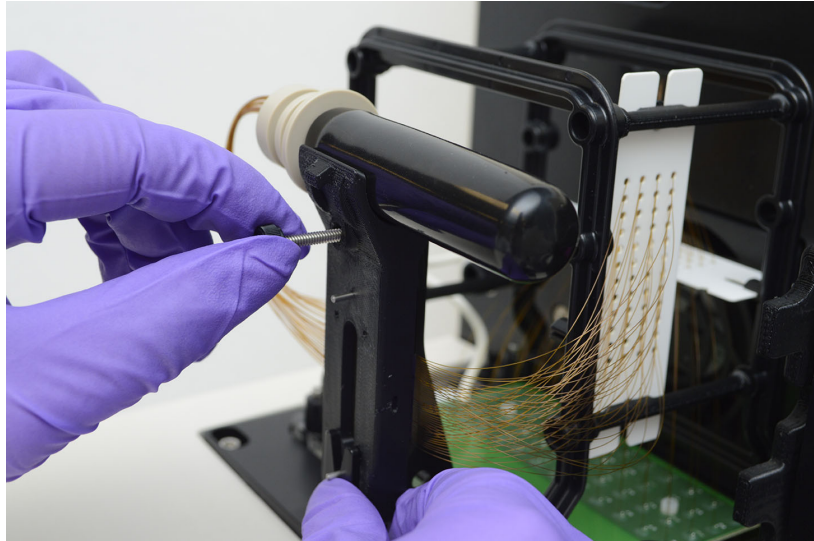
Figure 66   Instrument top compartment – attach array window to capillary array frame

12 Use the provided allen wrench to remove the two white screws holding the capillary array in place.
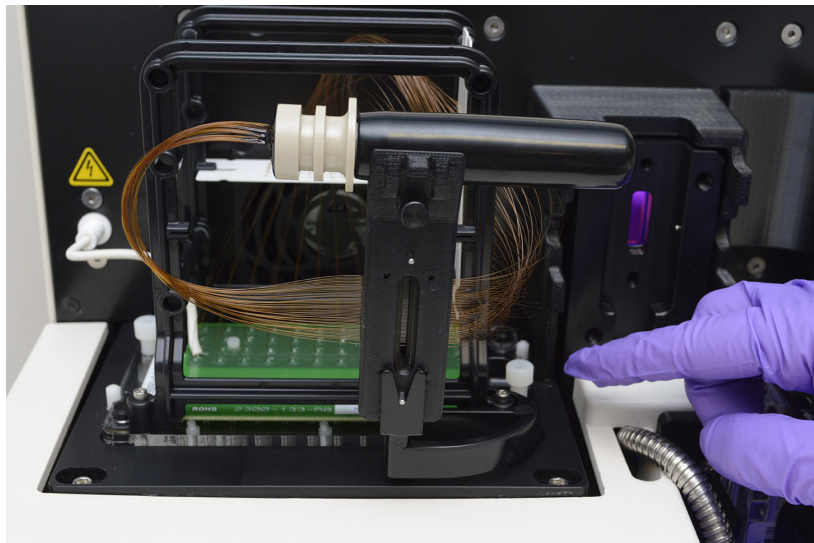
Figure 67   Instrument top compartment – array attachment screw removal

13 Carefully lift the array straight up to remove it from the Fragment Analyzer instrument.
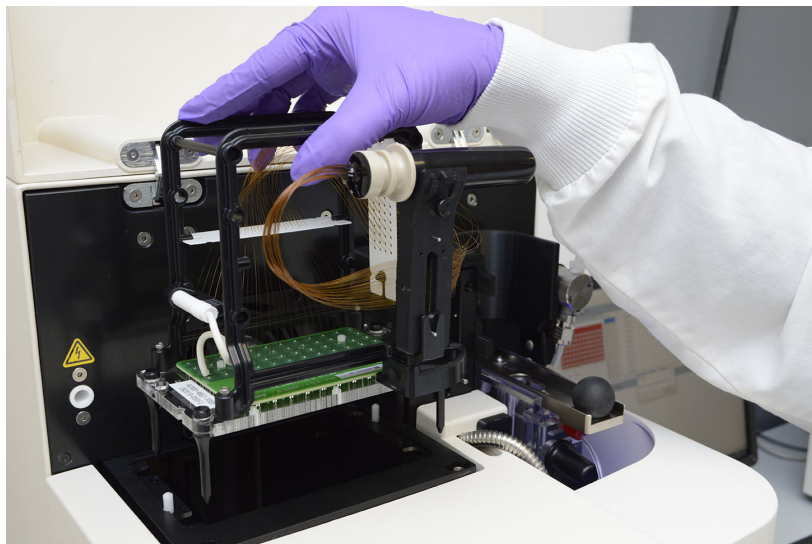
Figure 68   Instrument top compartment – capillary array removal

Once removed from the instrument, the capillary array cartridge is ready for disposal or storage in the Fragment Analyzer wet station (**"Long Term Capillary Array Storage"** on page 155).

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

   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.

2 Remove the capillary array bundle-securing rubber band.

Figure 70   Remove capillary array bundle-securing rubber band.

3 Remove the two white nylon screws that secure the array to the shipment frame.
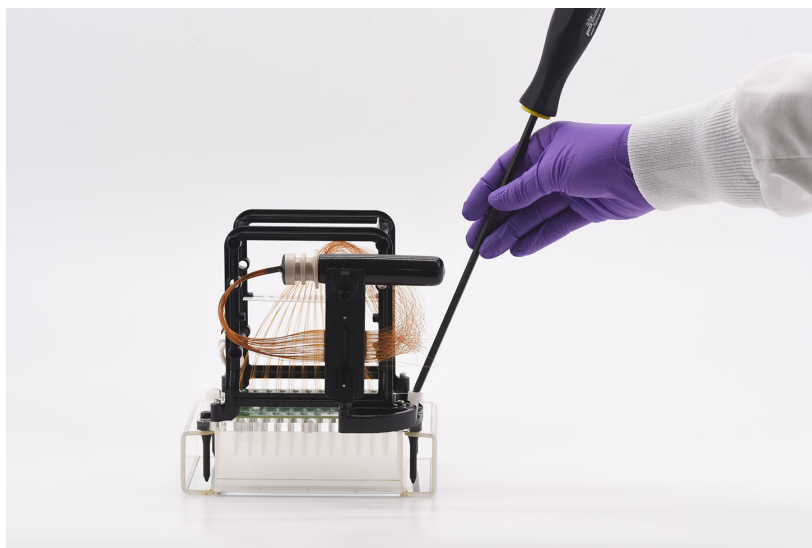
Figure 71   Capillary array shipping box

4 Carefully lift the array straight up to remove it from the shipment frame.
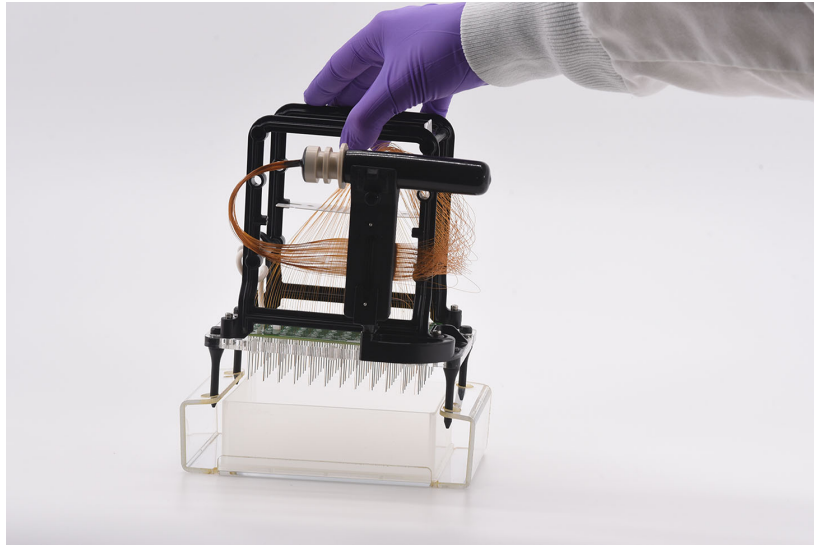
Figure 72   Capillary array shipping box – removal of array from shipment frame

Capillary Array Installation

This section will provide a pictorial guide of the steps required to physically install a capillary array cartridge into the Fragment Analyzer 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 help back into its drawer and move the Stage into resting position.

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

   a First, open the reagent door to the side.

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

Figure 73   Fragment Analyzer instrument

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.
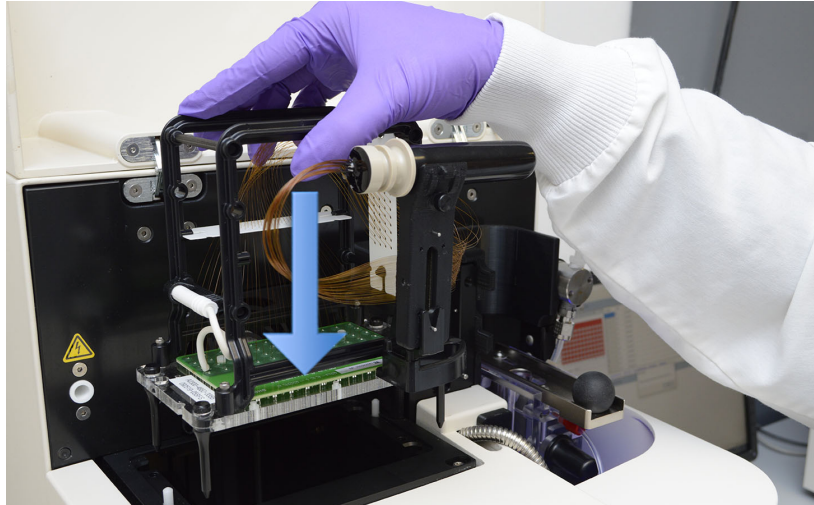
Figure 74   Instrument top compartment - capillary array installation: a 48 capillary is used in the example

3 Use the provided allen wrench to install the two white screws holding the capillary array in place.
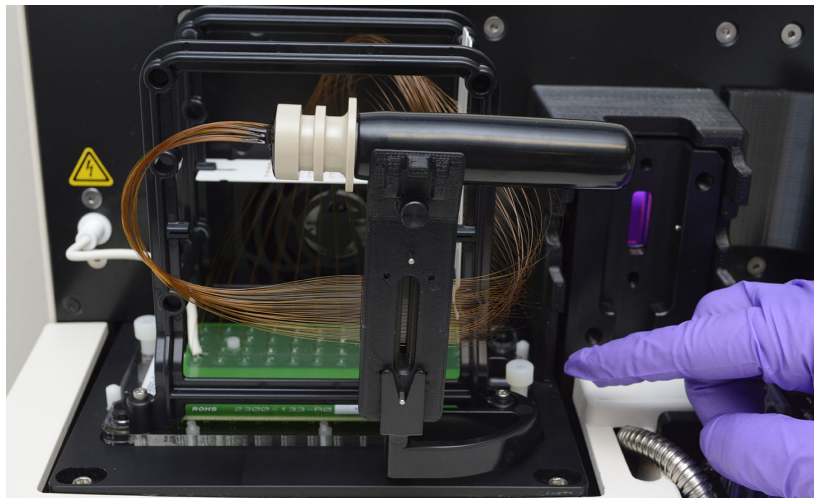
Figure 75   Instrument top compartment - array attachment screw installation, picture indicates one of these screws

4 Remove the array window attachment screw.
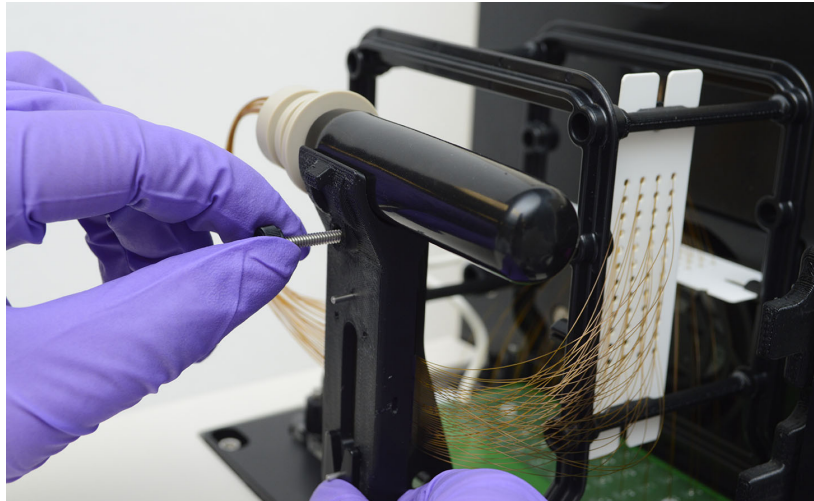
Figure 76　　Instrument top compartment – remove array window

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 and firmly press it into place.

Do not press on or touch the capillaries.
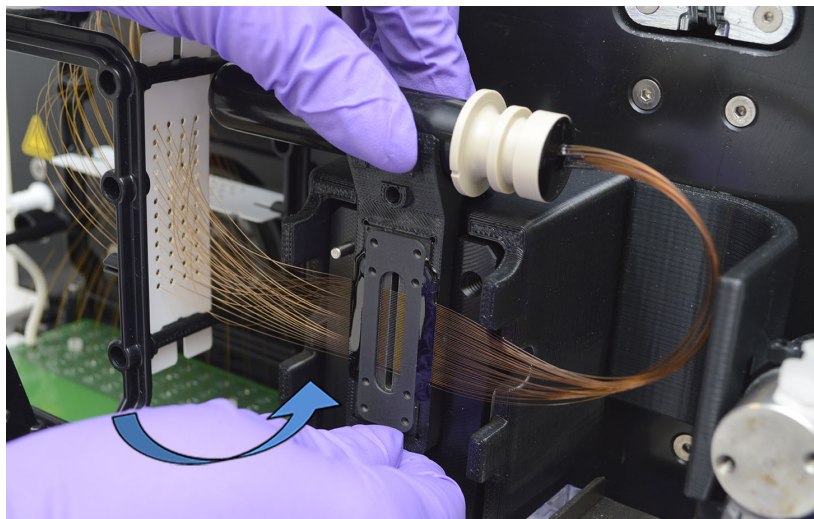
Figure 77　　Instrument top compartment – array window installation

6 Remove the capillary array bundle from the top holder of the capillary array window.
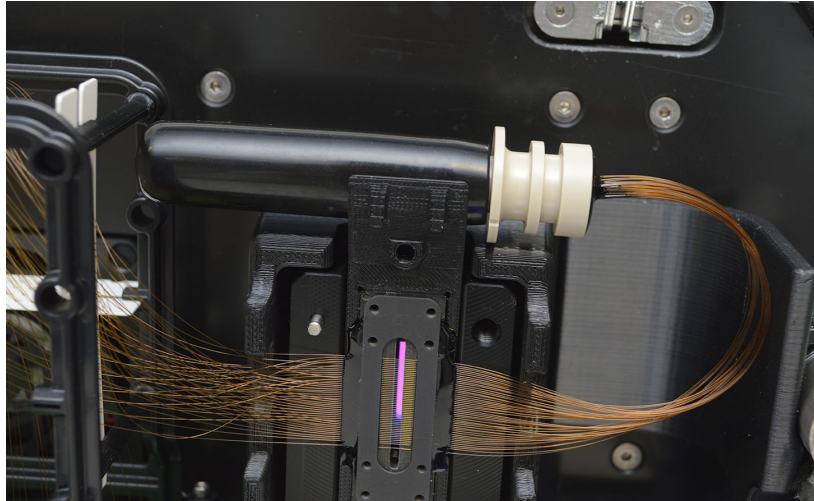
Figure 78 Instrument top compartment – capillary array window installed, and bundle shown here

7 Carefully remove the protective cover from the capillary bundle and place it back on the holder on top of the window.
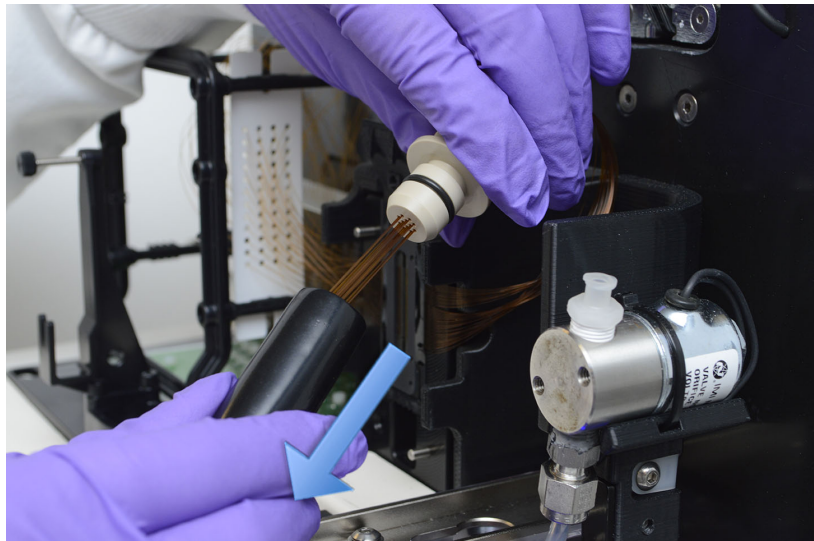
Figure 79 Instrument top compartment – removing protective cover

8 Install the capillary array bundle by firmly pushing the capillary array bundle into the reservoir opening until a distinct click is heard.
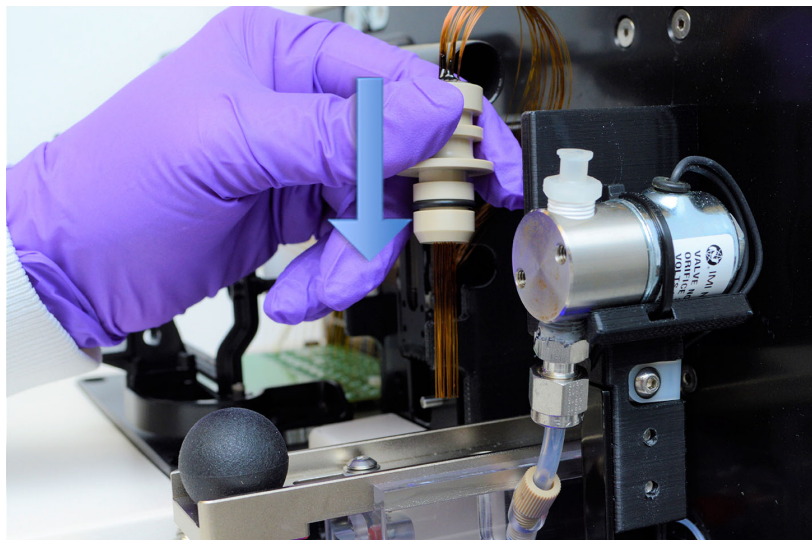
Figure 80   Instrument top compartment – capillary array bundle installation

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

If this step is not followed, the capillary array bundle will be damaged upon pressurization.

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.
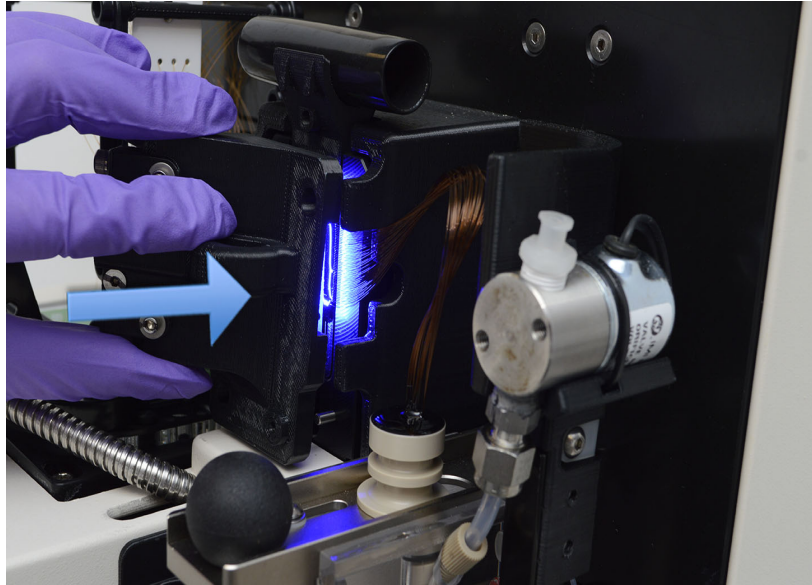
Figure 82   Instrument top compartment – light guide placement

11 Use the provided allen wrench to install the two white nylon screws that secure the light guide to the array window.
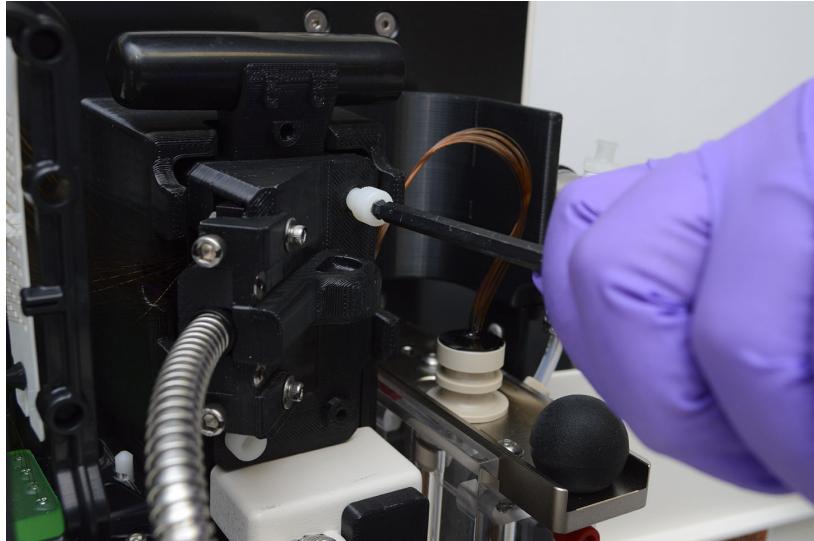
Figure 83   Instrument top compartment – light guide installation

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

High voltage cable
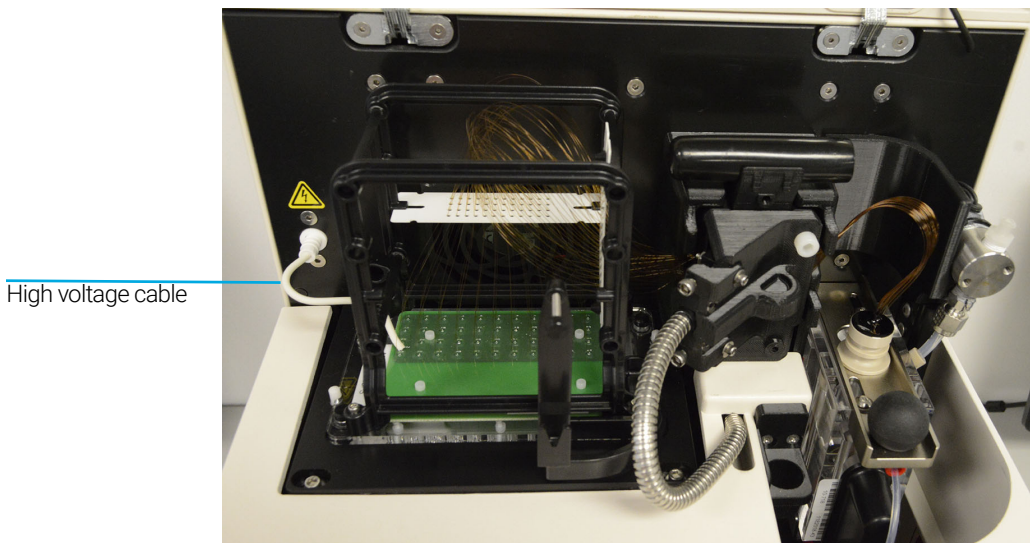
Figure 84   Instrument top compartment –fully installed array 48 capillary array shown here

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   Fragment Analyzer instrument

After installation of an array, the Fragment Analyzer will require a capillary alignment as described in **Chapter 4**, "Fragment Analyzer 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 155.

9 Fragment Analyzer - Sample Name Entry

This chapter provides information on how to enter the sample names in the Fragment Analyzer 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**).
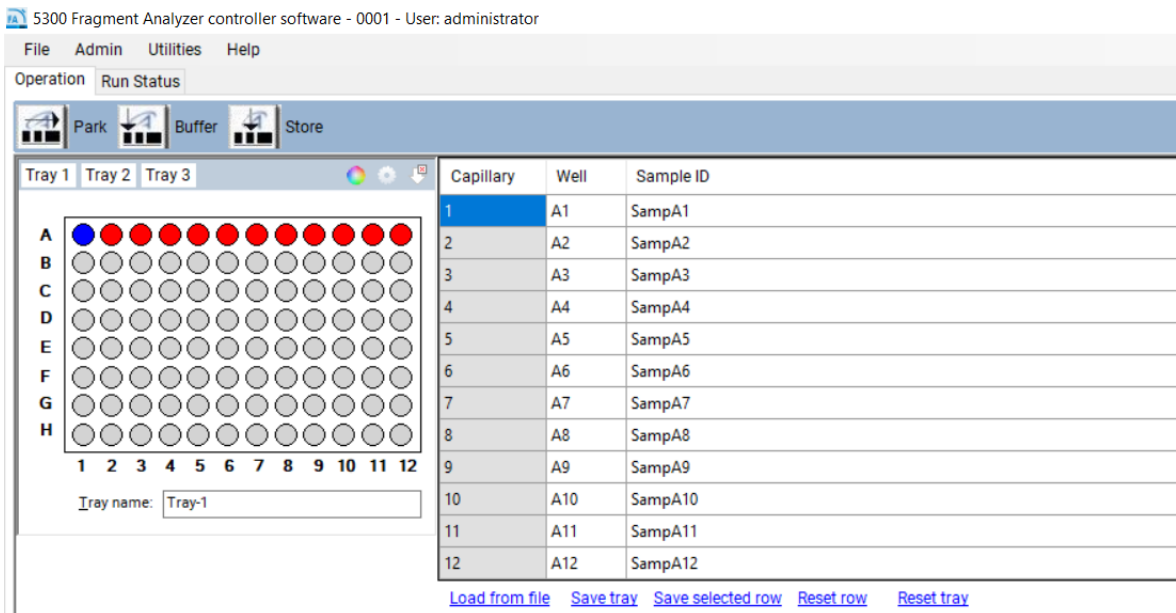
Figure 86   Adding samples names manually

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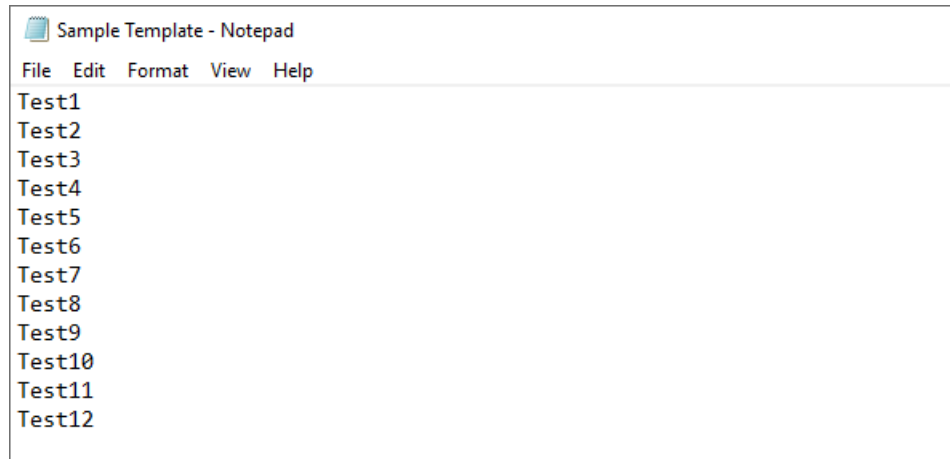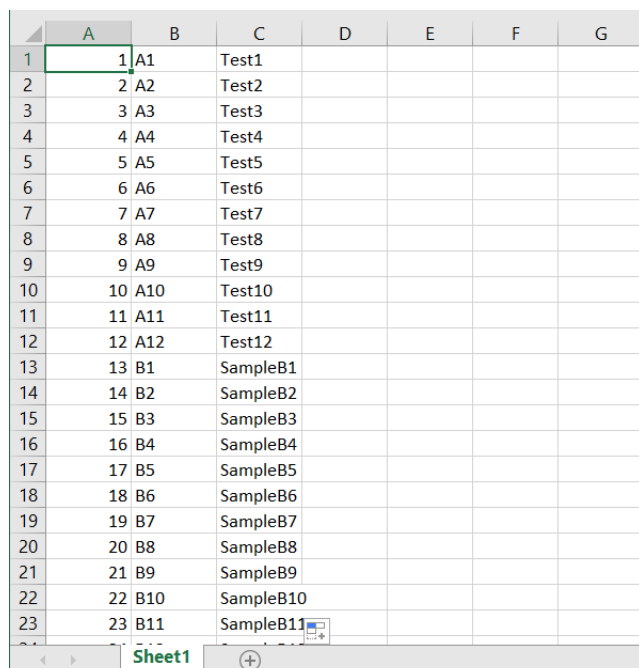
Figure 87   .txt file format (single row of names—no well numbers or row numbers).

• For a .csv file, the column format is row number, well number, and sample name (**Figure 88**).

Figure 88   .csv file format: row 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 Fragment Analyzer 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 119).

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

Name	Type
Data	File folder
Fragment Analyzer	File folder
Methods	File folder
Samples	File folder
Training Videos	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.

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**).

Figure 91   File name

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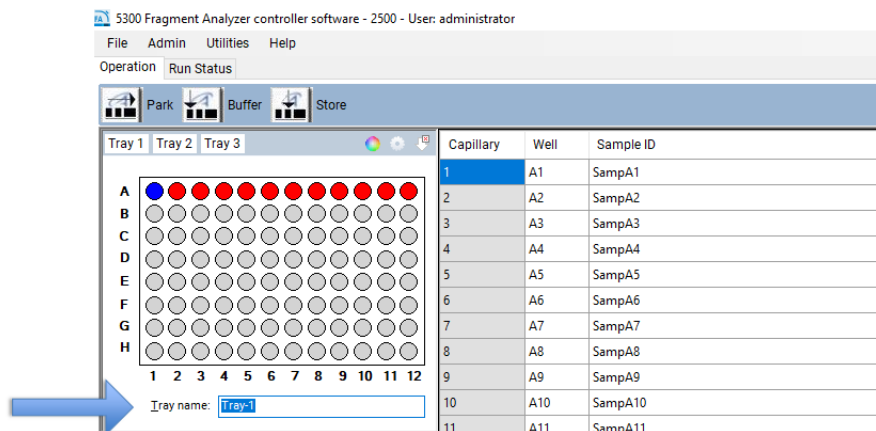
Figure 92   Highlight of the tray name

3 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**).
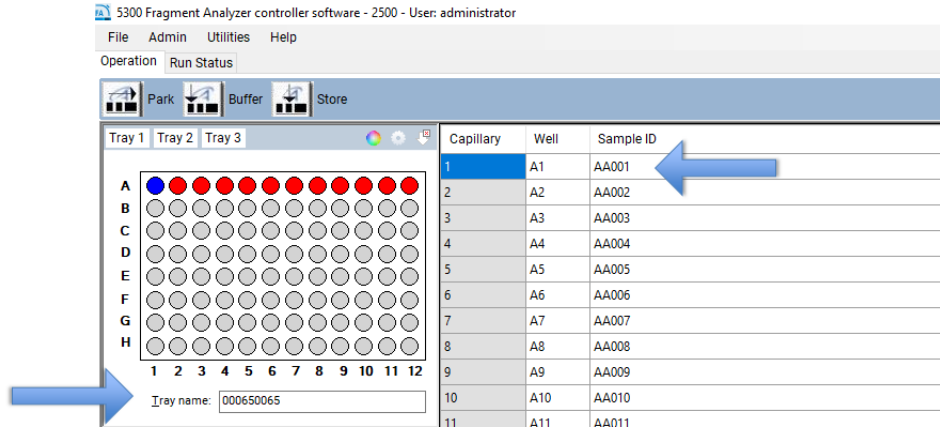
Figure 93   Imported sample names

10 Fragment Analyzer - Automated Analysis

This chapter explains the procedure for automated analysis using the Fragment Analyzer.

Automated Analysis Using the Fragment Analyzer

Automated analysis is performed by the Fragment Analyzer 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 Fragment Analyzer to a LIMS system. Sample names can be generated by the LIMS system and imported via plate bar coding (refer to **Chapter 9**, "Fragment Analyzer - 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**).
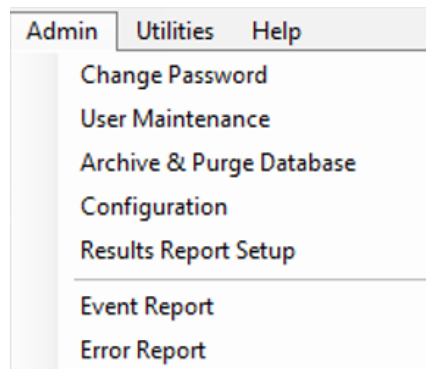
Figure 94 Admin menu

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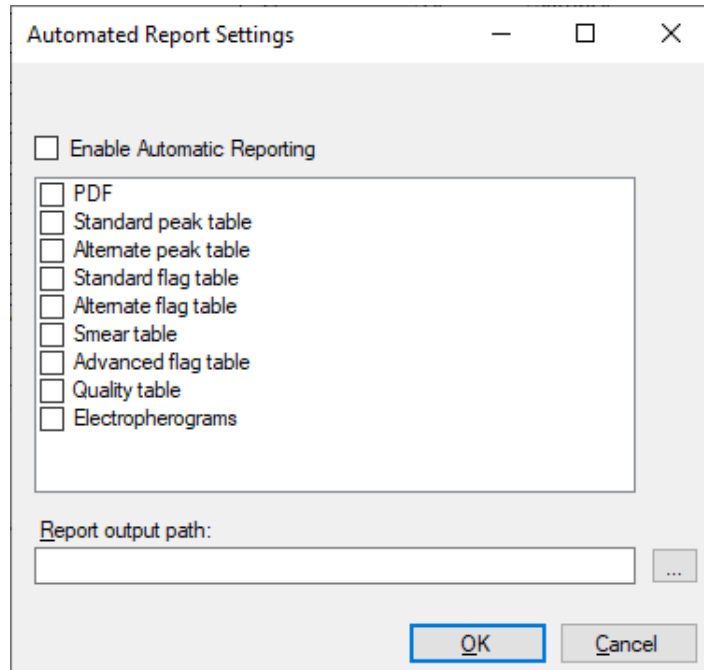
Figure 95 Automated Report Settings window

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 Fragment Analyzer system (used to acquire the data) must exactly match the name of the configuration file in ProSize.

For example, if the method used to run the sample is **DNF-905-33 - DNA 1-500bp**, then the configuration file in ProSize must have the name **DNF-905-33 - DNA 1-500bp**.

• If not using an imported ladder, the ladder well must be able to be processed by ProSize If the ladder well is not read correctly, then the data will not be processed. This means that the configuration file in ProSize must be set correctly—so that the ladder well is correctly read. This also means that ladder well must be of high quality, without anomalous or missing peaks.

For example, assume a 100 bp ladder is used in well H12, but the configuration file in ProSize is set so that the minimum peak height for integration of the ladder is 10.000 units. In this case, the ladder is not read correctly by ProSize (i.e., many ladder elements are missing), and the file will not be auto-processed by the Fragment Analyzer system (**Figure 96**).
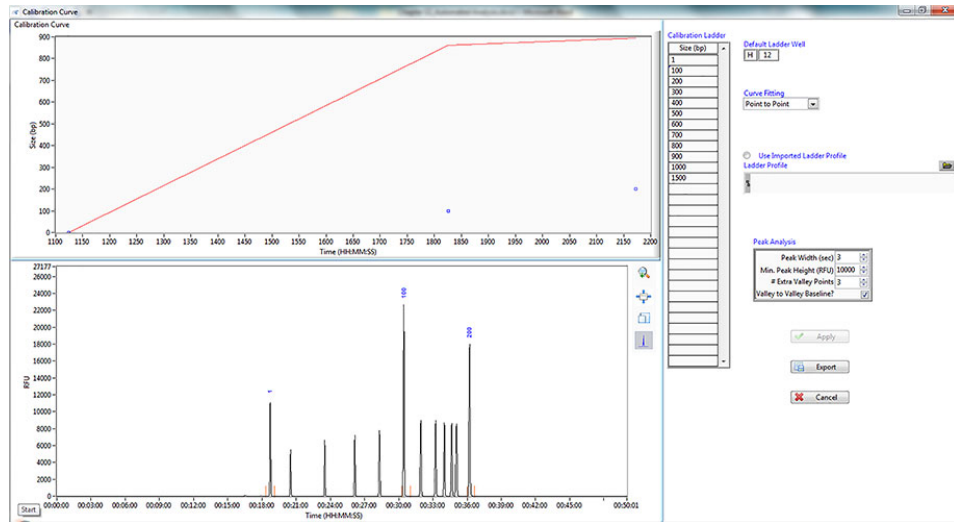
Figure 96   ProSize calibration curve setup

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**).
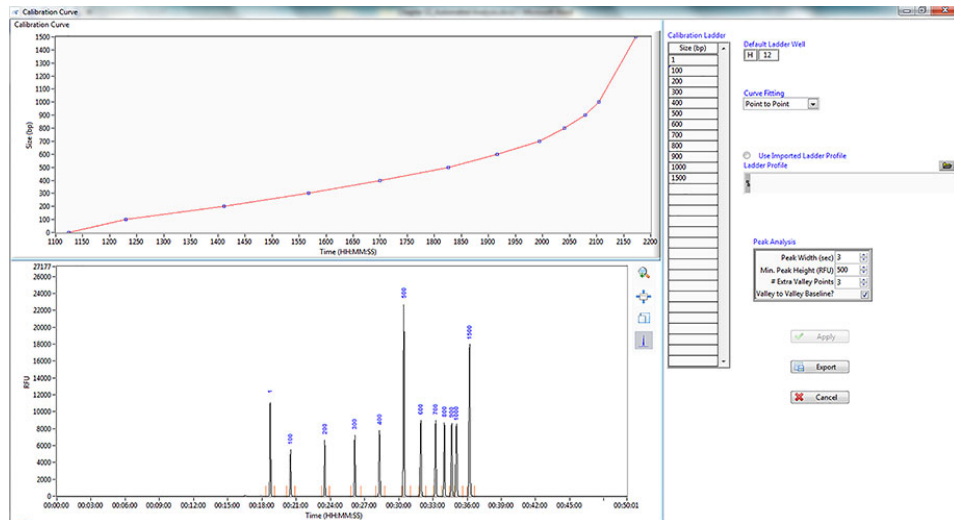
Figure 97   ProSize calibration curve setup

Importing a Ladder File for Automated Analysis

The Fragment Analyzer 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 10.000 to 500 in the configuration file using ProSize.

Both ProSize and the Fragment Analyzer software gives 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 96-wells of the sample plate, without having to reserve well H12 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**).
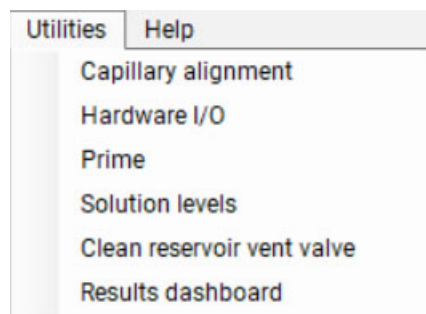
Figure 98   Utilities menu

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

2 Right-click on a file.
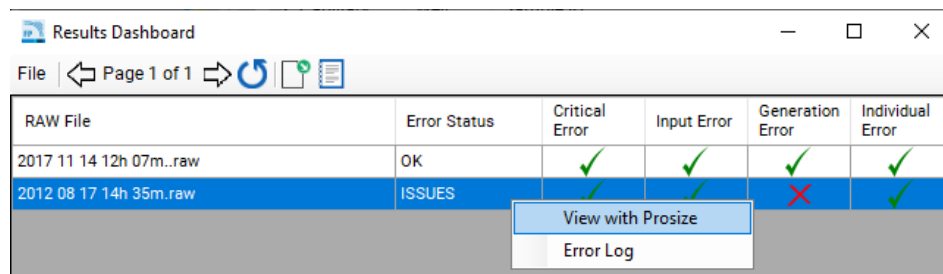
Figure 99   Results Dashboard

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.
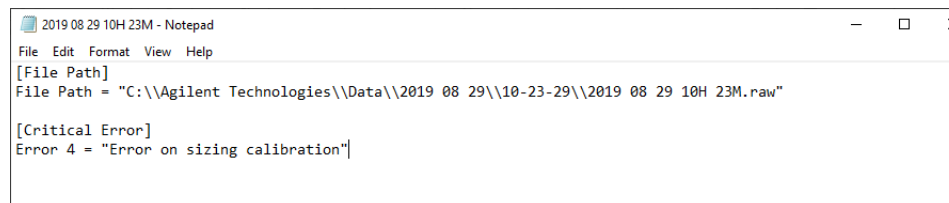
A summary of error messages is given in **Table 20**.

Table 20   Results Dashboard error messages

Message	Description
Error Status	Gives a statement of the status of processing. If there is an issue, *ISSUES* 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.

Figure 100   Example error message

11 Enhanced Security Features

This chapter gives an overview about enhanced security features of the Fragment Analyzer software.

Enhanced Security Features

The Fragment Analyzer version 1.2.0.11, and higher, contains enhanced security features with electronic records tracking and a new run file format, .db3, that supports event tracking and electronic signatures in ProSize and greater.

Enabling Enhanced Security Features

1 To enable enhanced security features in the Fragment Analyzer software, select **Configuration** from the **Admin** menu (**Figure 101**).

Admin	Utilities	Help
Change password		
User maintenance		
Archive & purge database		
Configuration		
Results report setup		
Event report		
Error report		
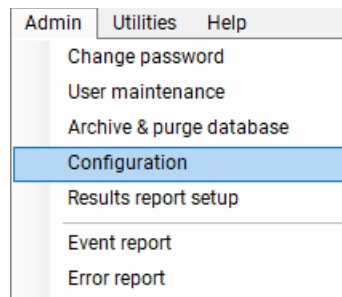
Figure 101 Admin menu

NOTE   To view .DB3 files enhanced security, you must use ProSize 3.0, or higher.

The **Configuration Settings** window will open (**Figure 102**).
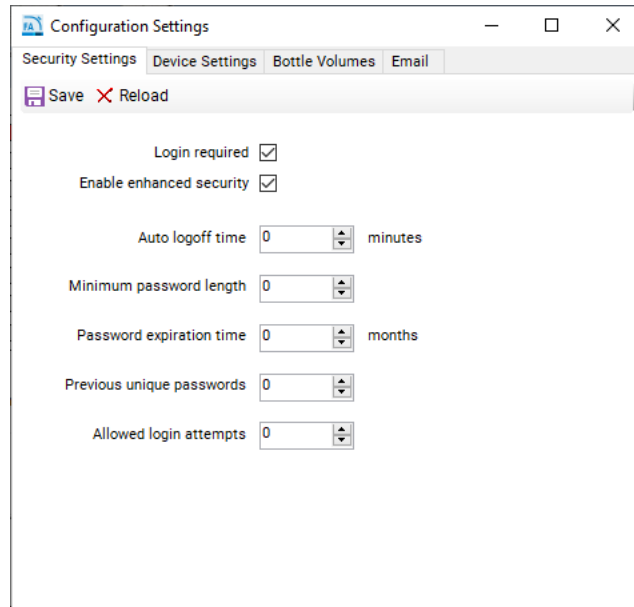
Figure 102 Configuration Settings window

2 Under the **Security Settings** tab, select **Electronic records** to activate enhanced security functionality in the Fragment Analyzer software.

A pop-up message appears informing you about that enabling electronic records will require all results gathered to be analyzed with ProSize 3.0, or higher.
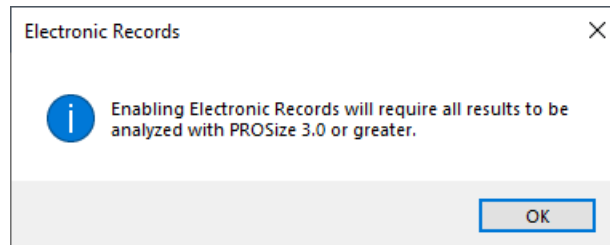
Figure 103 Electronic records message

3 Select **Save** to apply settings.

Results will be reported in .db3 files readable only by enhanced security enabled ProSize 3.0.
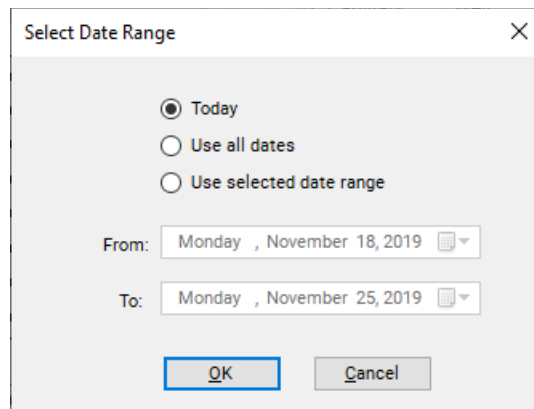
Event Report

The **Event Report** command provides a tabular report of the audit trail of the events that have occurred in the Fragment Analyzer software.

Users with both Administrator privileges and User level access can view the Event Report.

1 From the **Admin** drop-down menu, select **Event Report** (**Figure 101**).

   The **Select Date Range** window opens (**Figure 104**).

Figure 104 Select Date Range window.

2 Select the appropriate date range (you can use **Today**, **Use all dates** or **Use selected date range**).

3 Select **OK** to confirm.

   An Event Report will be generated (**Figure 105**).

Figure 105 Event Report example

With enhanced security enabled, the Event Report tracks all actions by the user including changes to method files, providing a complete record of all actions for audits.

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

The icons along the top of the Event Report follow standard Windows function nomenclature and are summarized in **Table 21**.

Table 21 Event Report icons and descriptions

Icon	Description
◄ ◄ 1 of 10 ►	Page Selection
←	Back to Parent Report
⊗	Stop Rendering (i.e., Stop Report Generation)
↻	Refresh
🖨	Print
▤	Print Layout
📖	Page Setup
💾▾	Save
100% ▾	Zoom

12 Appendix

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

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 and Tubes for Fragment Analyzer Systems

Semi-Skirt Sample/Marker Plates – 5200 and 5300 only

The 5200 and 5300 Fragment Analyzer systems have been designed to operate using specific dimensioned semi-skirted PCR plates and deep 96-well plates. A list of approved semi-skirted PCR plates is shown in **Table 24**.

NOTE   Non-skirted PCR plates are not recommended for use with the Fragment Analyzer systems, as they tend to warp or bow and can therefore interfere with proper sample injection.

Table 24   List of approved PCR plates

Approved Vendor/Part Number	Description
Eppendorf # 951020303 (various colors)	Eppendorf 96-Well twin.tec PCR Plates, Semi-skirted
MidSci Pryme # AVRT1	Pryme PCR Ergonomic Plates, 96x0.2ml, Semi-Skirted,Natural
BioRad Hard-Shell # HSS-9601	Hard-Shell® Full-Height 96-Well Semi-Skirted PCR Plates
Greiner Bio-One # 652280	96W PCR Microplate, Polypropylene, Half-Skirt, Natural, No Lid
4titude Framestar # 4ti-0900, -0950, -0770/C	FrameStar 96 semi-skirted
Scientific Specialty # 3450-00	96-Well "Semi Skirt" UltraFlux PCR Plate
Neptune # 3742.X	Semi-Skirted 96-Well PCR Plates

NOTE   The use of PCR plates with different dimensions to the above recommended plates could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.

0.2 mL Tube Strip – 5200 and 5300 only

The 5200 and 5300 Fragment Analyzer system has been designed to operate with specific tube strips to be used with the **F1300-107 Hotel Drawer Adapter** for tube strips (sold separately). A list of approved tube strips is provided in **Table 25**.

NOTE   The use of PCR tube strips with different dimensions to the below recommended tubes could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.

Table 25   List of approved tube strips

Item	Vendor / Part #	Description
0.2 mL PCR 12-Tube Strip	Fisher Scientific #AB-1113	Thermo Scientific ABgne 0.2 mL Strip Tubes > 12-Tube Strip
0.2 mL PCR 8-Tube Strip	Fisher Scientific #AB-266	Thermo Scientific ABgne 0.2 mL Strip Tubes > 8-Tube Strip

Buffer/Waste Plates – 5200, 5300, and 5400

The 5200, 5300, and 5400 Fragment Analyzer systems 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) (shown in **Table 26**).

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 Fragment Analyzer system, as damage to the capillary array will occur.

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

Table 26   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	Fragment Analyzer 96-Well Buffer/Waste Tray, case of 50

Full Skirt Sample/Marker Plates – 5200, 5300, and 5400

The 5400 Fragment Analyzer system has been designed to work exclusively with full skirted PCR plates. This instrument is shipped with a set of special plate adapters: **M1300-109 - DRAWER ADAPTER-FULL SKIRT 96 WELLPLATES**.

NOTE   The 5400 Fragment Analyzer will not function properly with the semi-skirted plates. However, the 5200 and 5300 Fragment Analyzer has the option to use either of these plate options: full skirted, semi-skirted, provided that the proper drawer adapter insert is present (shown in **Table 27**).

Table 27   List of skirt plates

Item	Vendor / Part #	Description
Eppendorf full skirt plates	Fisher Scientific #E0030129512	Eppendorf twin.tec 96 Well LoBind PCR Plates, Skirted
BioRad Hard Shell plates	BioRad #HSP9601	Hard-Shell 96-Well PCR Plates, low profile, thin wall, skirted, white/clear

NOTE   The use of PCR plates with different dimensions to the above recommended plates could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.

Preventative Maintenance Schedule

Daily Maintenance

- ✔ Empty the waste bottle and waste tray.
- ✔ Replace the inlet buffer in the buffer tray position.
- ✔ Replace rinse buffer solution when applicable.
- ✔ Ensure there is Capillary Conditioning Solution in the conditioning solution bottle location.
- ✔ Ensure there is gel/dye in the gel bottle location.

Monthly Maintenance

- ✔ Replace the buffer and waste plates with new ones.
- ✔ Replace the Capillary Storage Solution and plate.*
- ✔ Replace the gel 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

- ✔ Place 0.6 mL of 0.5N NaOH into the waste tray location and flush the capillary array cartridge with 0.5N NaOH followed by DI water as described in section **"Capillary Array Cleaning"** on page 146.**

* More frequent replacement (i.e., every 1-2 weeks) may be required in low humidity or warmer laboratory environments.
** It is fine to perform this cleaning performance as a part of a regular weekly or biweekly cleaning schedule as well.

Capillary Array Cleaning

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

>   A: Flush with CE grade water
>
>   B: Submerse capillary array tips/electrodes in hot water (150 °F − 200 °F)
>
>   C: Cleaning the capillary tips, electrodes, and capillary walls

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

Method A - Flush with CE Grade Water

When a capillary array is suspected to have any clogged capillaries, the first step is to flush the array with CE grade water.

1 From the operations tab located on the main screen window, select **Add to queue** under the Capillary Array-Conditioning menu.

2 From the **Select Conditioning Method** window, select **Method A Flush - Water - 10 min 200 psi.mthdc** from the drop-down menu.

3 Select **Edit** to ensure that the method matches the parameters shown in **Figure 106**.

Figure 106   Conditioning parameter **Method A Flush - Water - 10 min 200 psi.mthdc**

4 If necessary, adjust the method to that shown in the Figure 1 (on a 96-capillary instrument, the parameter **Row** is not editable).

5 Select **OK**.

6 Select **OK** again to add the method to the method queue.

7 Open the Waste Drawer (second drawer from top) and place an empty 96-Well Deep Well Plate onto the plate holder.

8 Open the Fragment Analyzer systems' side compartment to replace the gel 2 bottle with a bottle containing CE grade water.

Minimum solution volume required to run Method A Flush:

• ≥ 12 mL for a 12-capillary array

• ≥ 25 mL for a 48-capillary array

• ≥ 50 mL for a 96-capillary array

9 Close the door to the instrument side compartment, and select the start icon from 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.

11 Check the volume of water present in each of the wells used for the flush.

For a 10 minute flush there should be ~ 150 µL of CE grade water in each well.

If the waste plate has similar amounts of water present in each well:

1 Empty the 96-well deep well plate and return it to the waste drawer.

2 Open the side compartment of the Fragment Analyzer instrument and replace the bottle containing CE grade water with an empty bottle and perform a capillary conditioning to remove any residual water from the capillaries.

If a well has significantly less or no water present in a well, it is recommended to proceed to Method B or C.

Method B - Submerse Capillary Array Tips/Electrodes in Hot Water (150 °F – 200 °F)

1 Select the **park** icon in the main screen window to place the plate being held back into its respective drawer and move the stage platform to the bottom of the instrument.

2 Fill 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.

  • For a 12-capillary array, fill each well in row A of a 96-well deep well Plate with 1 mL of hot water.

  • For a 48-capillary array, fill each well in row A-D of a 96-well deep well plate with 1 mL of hot water.

  • For a 96-capillary array, fill each well of the 96-well deep well plate with 1 mL of hot water.

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.

7   Select the **park** icon to return the 96-well deep well plate to the buffer drawer and place the stage in a resting position at the bottom of the instrument.

8   Perform Method A as described in this Appendix to check the flow of solution through each capillary or proceed directly to Method C.

Method C - Cleaning the Capillary Tips, Electrodes, and Capillary Walls

WARNING

Hazardous solvent

0.5 N 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.**

Method C − 0.5 N NaOH − 10 min 200 psi.mthdc is the best method for opening clogged capillaries, returning separation to normal as well as ridding the Fragment Analyzer systems of contamination.

1   Clean the Conditioning solution, Gel 1, and Gel 2 bottle caps with isopropanol or EtOH.

2   Open the Fragment Analyzer side compartment and replace the Gel 2 bottle with a bottle containing 0.5N NaOH.

The minimum solution volumes required to run the Method C flush for 10 minutes:

•   ≥ 12 mL for a 12-capillary array

•   ≥ 25 mL for a 48-capillary array

•   ≥ 50 mL for a 96-capillary array

3   From the **Utilities** menu, select **Prime**. Check the boxes next to Conditioning, Gel 1, and Gel 2.

4   Select **OK** to prime all lines with NaOH.

5 Place a new bottle with new conditioning solution in the conditioning solution position.

The minimum solution volumes required to run the Method C flush for 10 minutes:

- ≥ 12 mL for a 12-capillary array
- ≥ 25 mL for a 48-capillary array
- ≥ 50 mL for a 96-capillary array

6 From the **Operation** tab located on the main screen window, select **Add to queue** under the Capillary Array - Conditioning commands menu.

7 From the **Select Conditioning Method** window, select the **Method C Flush - 0.5 N NaOH - 10 min 200 psi.mthdc** from the drop-down menu.

8 Select **Edit** to ensure that the method matches the parameters seen in **Figure 107**.

Figure 107   Conditioning parameter **Method C Flush - 0.5 N NaOH - 10 min 200 psi.mthdc**

9 If necessary, adjust the method to that shown in Figure (Row will not be selectable on a 96-capillary instrument).

10 Select **OK**.

11 Select **OK** again to add the method to the Method Queue.

12 Open the waste drawer (second drawer from top) and place a 96-Well deep well plate filled with 0.6mL per well of 0.5N NaOH in Row A for a 12-capillary array, rows A-D for a 48-capillary array, or all 96 wells for a 96-capillary array onto the plate holder.

13 Close the door to the Instrument side compartment and select the green start icon from the Method Queue to run the capillary conditioning method.

14 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.

15 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

0.5N NaOH is corrosive

0.5N NaOH can damage the capillary array.

✔ **Perform a separation with a Full Conditioning or a flush with separation gel immediately after a 0.5N NaOH flush.**

NOTE

When conditioning flushes are recommended it is important to use conditioning solution at the 1x concentration. Using a reagent aside from conditioning solution to prepare the capillaries can be detrimental to separation quality.

Reservoir Vent Valve Cleaning

Over time the reservoir vent valve may become clogged requiring cleaning. The Fragment Analyzer 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 108**).

Clean Reservoir Vent Valve ✕

To clean the reservoir vent valve:

1. Fill 10 mL syringe with 7 mL of DI Water and attach the syringe to the
        Reservoir Vent Valve Luer Lock fitting.

2. Depress the syringe to flush DI Water through the Valve and Reservoir.

3. Remove the syringe from the Luer Lock fitting and fill syringe with 10 mL of air.

4. Attach the syringe to the Reservoir Vent Valve Luer Lock fitting and depress to flush air through the Valve and Reservoir.

5. Click "OK" and Run a Separation Method.

OK

Figure 108   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. It may be additionally recommended to include 0.5 N NaOH solution in the flush before flushing with water and air for particularly difficult cases.

Capillary Array Window Cleaning

1   Open the side door and hood of the Fragment Analyzer instrument.

2   Remove the light guide from the array window.

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. Place bundle in provided protective cover.

5   Remove the capillary array window from the capillary array window holder. Do not touch the array window.

6   Place a paper tower behind the capillary array window as shown in **Figure 109**.
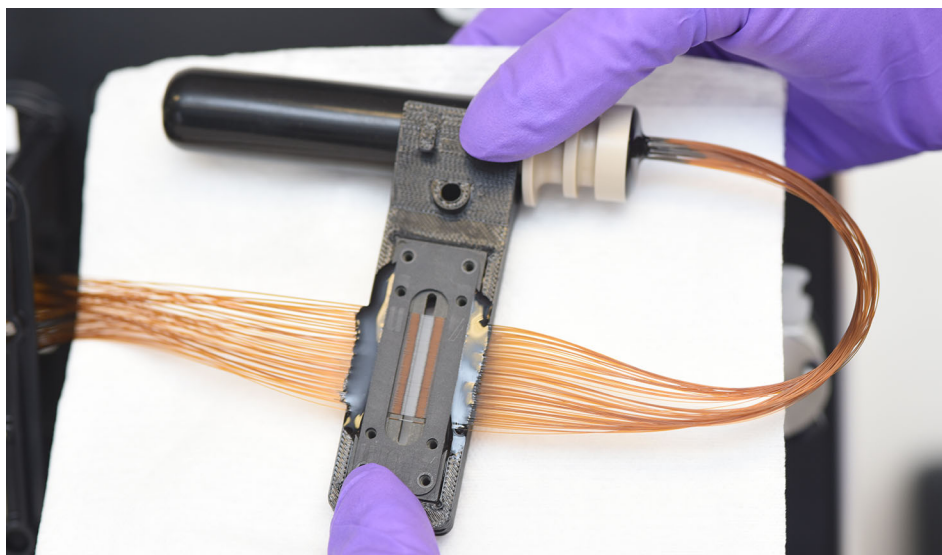
Figure 109   Capillary array window with paper towel behind

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. 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. 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 Fragment Analyzer instrument.

11 Check the alignment of the capillaries when finished by navigating to **Utilities > Capillary alignment**. Realign if necessary.

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 as of 10/20/2015. This requires the array spindle accessory kit part #A1300-910 that shipped with all instruments shipped after 10/20/2015. 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**, "Fragment Analyzer Capillary Array".

2 Place the tray base inside the array docking station as shown in **Figure 110**.
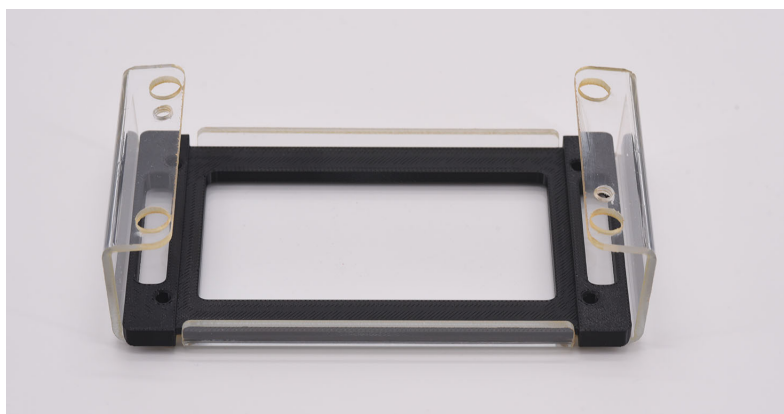
Figure 110   Array docking station with tray base installed

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 111**).

- 12-capillary array – Fill row A only with 1.0 mL storage solution.
- 48-capillary array – Fill rows A-D only with 1.0 mL storage solution.
- 96-capillary array – Fill all wells 1.0 mL storage solution.
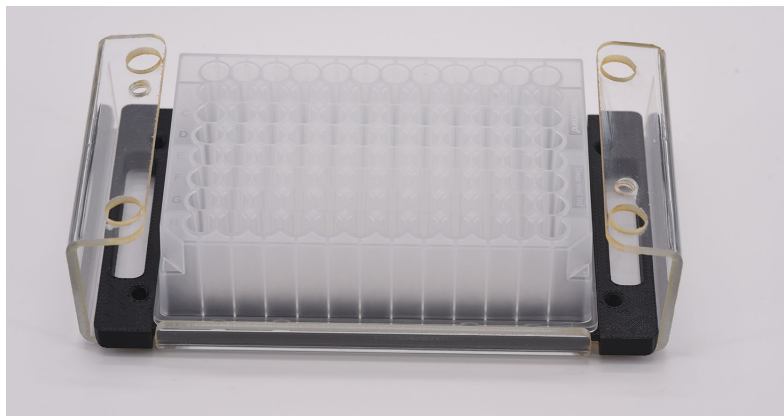
Figure 111   Array docking station with 96-deep well tray

4 Place capillary array into the array docking station using the four leg holes as guides. For 12- and 48-capillary arrays ensure that the capillary tips are in the storage solution side of the tray, not left in open air.

5 Insert the two white screws as shown in **Figure 112** to tighten the capillary array into place.
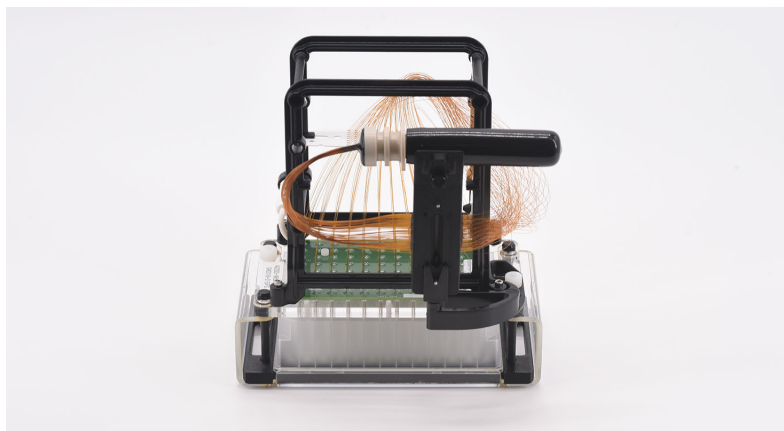
Figure 112   Array docking station with capillary array installed

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

Figure 113   Array spindle storage device, no storage solution in this example bottle

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 114**.

To see a full image of the array with the array spindle storage device installed, refer to **Figure 115**.
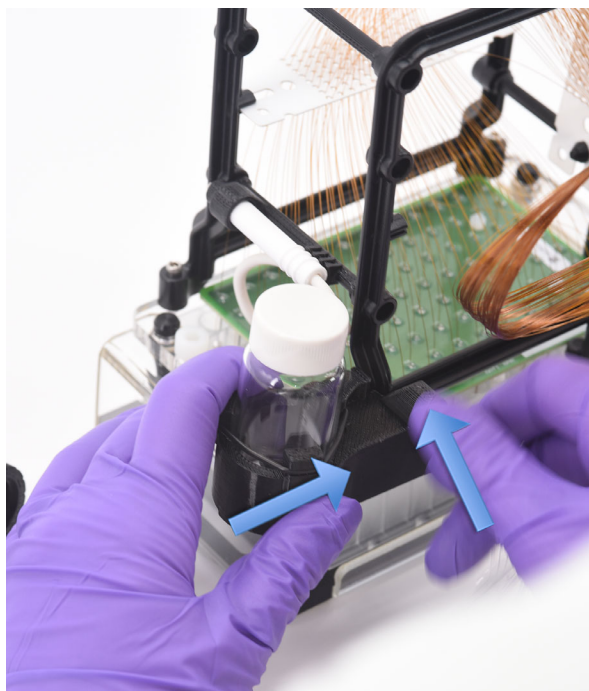
Figure 114   Array spindle storage device installation

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 115**.
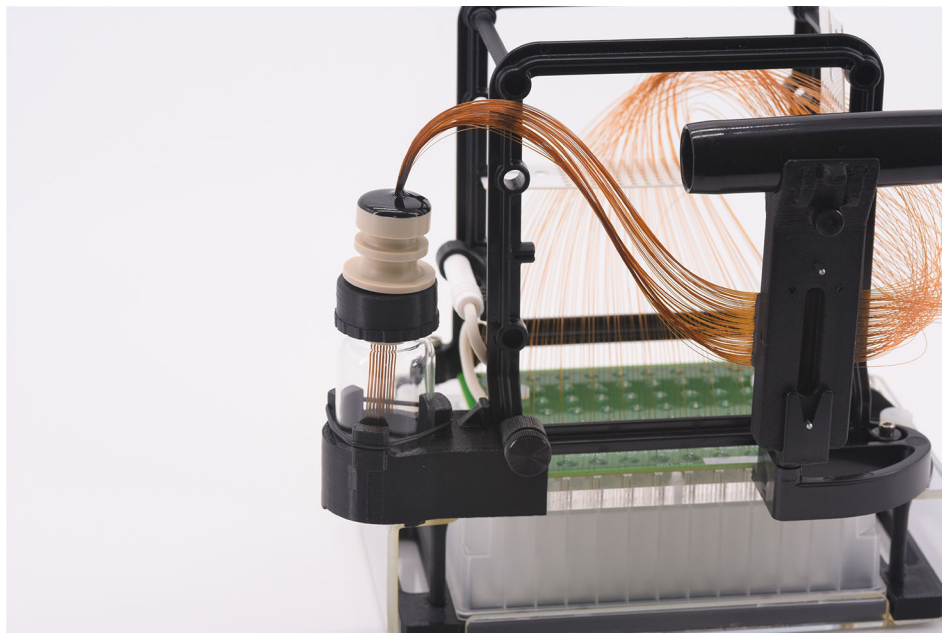
Figure 115   Array docking station with array installed

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.

NOTE   Depending on the capillary array size there are two different cap sizes for the glass vial on the array spindle storage device. The cap with a smaller opening is used for 12-capillary arrays, the cap with the larger opening is for the 48- or 96-capillary arrays.

5400 Fragment Analyzer System

This chapter applies only to customers who have purchased the 5400 Fragment Analyzer system.

The 5400 Fragment Analyzer is designed to allow an external robotic system to interface with the Fragment Analyzer. Each separate drawer of the 5400 Fragment Analyzer system is pushed out or pulled in using remote serial commands. All Fragment Analyzer methods, conditioning steps, and stage movements can be controlled with remote commands, enabling extended walk away use of the instrument.

The 5400 Fragment Analyzer system can also be used as a stand-alone, normal operation Fragment Analyzer. For use in the stand-alone mode, no special set-up or commands are required - just use the system as a standard Fragment Analyzer. Note however that some slight differences exist in the instrument drawer assignments from a standard Fragment Analyzer, as outlined in the following sections.

Drawer Assignments

The 5400 Fragment Analyzer system has specific drawer assignments, as shown below in **Figure 116**.

The drawers are labeled from top to bottom as:

- B (Buffer)
- W (Waste)
- M (Marker; also used as Rinse drawer with quantitative Fragment Analyzer kits)
- S (Storage)
- 1 (Sample Tray 1; sole drawer for loading sample plates)
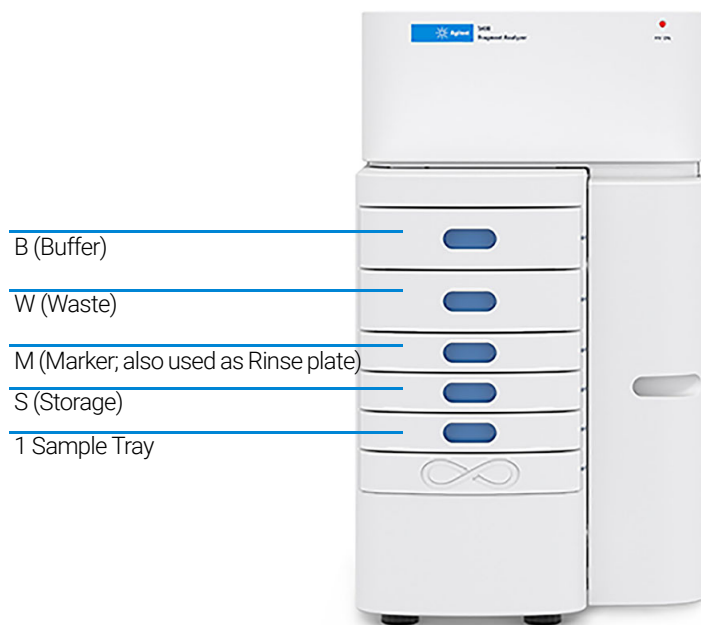
Figure 116   Entry points of the Fragment Analyzer system

Important System and Workflow Considerations

This section covers important aspects of 5400 Fragment Analyzer system operation that should be considered when setting up experimental workflows with the system.

Buffer Plate Information

The 5400 Fragment Analyzer system uses the same specific deep 96-well plate (31 mm height) Buffer plates used on the Fragment Analyzer instrument. The plates can be purchased from either Fisher Scientific or directly from Agilent. This specific plate must be used with the instrument (two plates are supplied upon installation).

Table 28   List of Buffer plates

Item	Approved Vendor / Part Number #	Description
Buffer/ 96-Well Plates	Fisherbrand # 12-566-120	Fisherbrand 96-Well DeepWell Polypropylene Microplates; Well Capacity: 1 mL
	Agilent # P60-20	Fragment Analyzer 96-Well Buffer Tray, case of 50

Waste Plate Information

The 5400 Fragment Analyzer system uses a specific open polypropylene reservoir as the Waste plate. This plate provides a solid wall construction for robotic handling while furnishing sufficient volume for repetitive run cycles prior to emptying/exchange.

Table 29   List of Waste plates

Item	Approved Vendor / Part Number #	Description
Waste Plates	Seahorse Bioscience # 200686-100, or Fisher Scientific # NC0254486	Seahorse single cavity polypropylene reservoir, 170ml, 12 column base geometry, 30.6mm height, case of 25

Sample Plate Information

The 5400 Fragment Analyzer system is configured to work with full-skirt PCR microplates only. The same plates should be used for sample, marker/rinse and storage solution. The current plate options are listed below.

Table 30   List of PCR plates

Item	Approved Vendor / Part Number #	Description
Sample/Marker/Storage PCR Plates (full-skirted)	Eppendorf # 951020401 (various colors)	Eppendorf* 96-Well twin.tec* PCR Plates, Full-skirted
	Bio-Rad # HSP9601 (various colors)	Pkg of 50, white shell/clear well PCR plate, rigid 2-component design

NOTE   The use of PCR plates with different dimensions to the above recommended plates could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.

Buffer, Waste, Marker/Rinse and Storage Plate Replacement Intervals

When operating the 5400 Fragment Analyzer system in unattended robotic control mode, the following plate replacement intervals are recommended (can be done, manually, or with a robot):

- **Buffer Plate – DNA kits/methods**: A volume of 1.0 mL/well should be initially added to the Buffer plate. The buffer in the Buffer tray should be replaced once every 24 hours, or once per 24 runs (whichever comes first).

- **Buffer Plate – RNA kits/methods**: A volume of 1.0 mL/well should be initially added to the Buffer plate. Because all RNA methods pump gel matrix into the Buffer tray, the buffer in the Buffer tray should be replaced once every eight runs.

- **Waste Plate**: The Waste tray should be emptied after every six runs, maximum.

- **Rinse Plate (Quantitative Kits)**: A volume of 200 mL/well should be placed into the Rinse plate. The Rinse plate should be replaced every 24 hours.

- **Marker Plate (Qualitative Kits)**: Refer to the respective Analysis Kit manual for recommendations on plate preparation and usage intervals.

- **Storage Plate**: The Storage tray should be filled with 100 mL/well of Capillary Storage Solution. The Storage plate should be replaced at least once per month; more frequent replacement (one to two weeks) may be required when operating in warm or low humidity environments.

NOTE   The Buffer and Waste plates in particular must be replaced according to the suggested intervals, to prevent potential overflow and damage to the capillary array cartridge or instrument.

5400 Fragment Analyzer Automated Data Analysis

The 5400 Fragment Analyzer software contains tools for performing automated data analysis post run, similar to the Fragment Analyzer system.

Activate the automated data analysis features

1 From the Main menu, select **Admin-Results Report Setup**.

The **Automated Report Settings** window will be displayed (**Figure 117**).
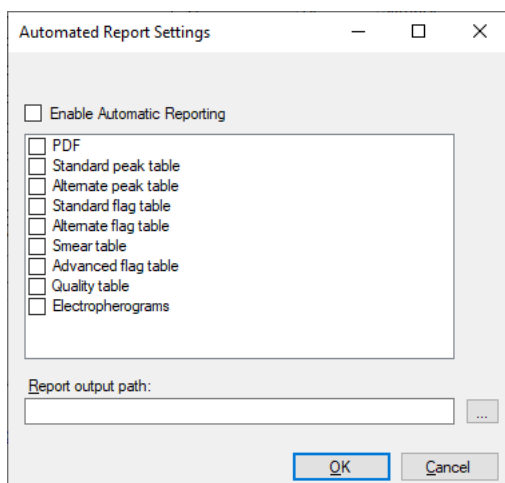
Figure 117 5400 Fragment Analyzer Automated Report Settings window

2 Select **Enable Automatic Reporting** to turn on the automated data analysis feature.

3 Select which types of report are to be generated by selecting the appropriate box in the menu (for example, selecting **PDF** will automatically generate a PDF report post run).

NOTE The report, export fields, or settings used in the **Result output path** will match those last saved in the ProSize data analysis program.

The automated data analysis settings need only be set once in the software during robotic integration process. For further information on performing automated data analysis, refer to **Chapter 10**, "Fragment Analyzer - Automated Analysis".

5400 Fragment Analyzer Communication Specifications

The 5400 Fragment Analyzer system can be interfaced to a robotic plate handling system by a serial port or by a TCP/IP port. The specifications for each are listed below.

Serial Port Communication

The host robotic sample handler communicates with the 5400 Fragment Analyzer computer via a serial communications port using the following serial port settings:

Baud Rate	9600
Data bits	8
Parity	None
Stop bits	1
Flow Control	None

TCP/IP Port Communication

The host robotic sample handler system communicates with the 5400 Fragment Analyzer computer via a TCP/IP communications port using the following settings:

IP Address	Use the 5400 Fragment Analyzer computer's IP address
Port Number	3000 (Recommended)

As the system requires Administrator level login access to set the IP address, the 5400 Fragment Analyzer computer should be setup with a static IP address.

5400 Fragment Analyzer Automation Set-Up

Configure the system for automated operation

1   Log in to the 5400 Fragment Analyzer software with administrator privileges. The default administrator credentials are:

    • User name: **Administrator**

    • No password required

2   From the **Admin** menu, select **Configuration**.

    The **Configuration Settings** window will open (**Figure 118**).

3   Select the **Automation** tab.
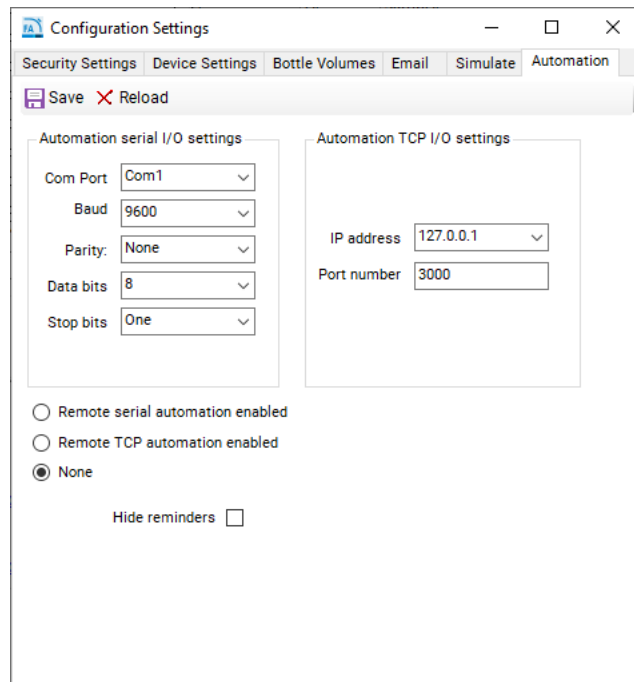
Figure 118   5400 Fragment Analyzer Automation configuration menu

4   To enable serial port communication, select **Remote serial automation enabled** and ensure the parameters are set to those specified in section **"5400 Fragment Analyzer Communication Specifications"** on page 165, and shown in **Figure 118**.

5 Select the appropriate Com port (usually Com1).

<table>
<tr><td>**NOTE**</td><td>The following Com ports should not be used as they are preassigned on the 5400 Fragment Analyzer system:

• Com3 (High Voltage)

• Com4 (Pressure Transducer)

• Com5 (Pump); Com6 (Stage)</td></tr>
</table>

6 If using TCP, select **Remote TCP automation enabled** and set the computer to use a static IP address, as dynamic IP addresses may not allow for consistent communication with the 5400 Fragment Analyzer system.

7 To disable remote automation, select **None**.

<table>
<tr><td>**NOTE**</td><td>Even with the serial automation or TCP automation enabled, the 5400 Fragment Analyzer may be used as a standalone system without a robotic interface (i.e., it can be used as a normal Fragment Analyzer instrument).</td></tr>
</table>

8 Once the appropriate settings have been entered, select **Save** to apply, and close the window.

5400 Automation Commands and Error Messages

Commands in the absence of a robotic system may be tested using any one of several terminal emulator applications, such as Tera Term or Hercules.

• Tera Term: https://ttssh2.osdn.jp/index.html.en

• Hercules: http://www.hw-group.com/products/hercules/index_en.html

All commands are sent as ASCII text and terminated with a carriage return (ASCII 13) or a line feed (ASCII 10). The commands are not case sensitive.

When the Fragment Analyzer receives a valid command, an **acknowledge** shall be returned that consists of an asterisk (*****) followed by the recognized command. When a move command has been carried out, the Fragment Analyzer will return ***COMPLETE**. If the run command failed for any reason **!4, Command failed** will be returned.

The 5400 Fragment Analyzer application recognizes the following commands listed in **Table 31**.

Table 31   5400 Fragment Analyzer commands

Command	Function	Example
STATUS	Fetch the run status. The status may include the following: Ready, Running, and Error	Send: status Receive: *STATUS: Ready
OUT	Push the Sample Tray (Drawer 1) out.	Send: out Receive: *OUT Receive: *COMPLETE **Note**: The Fragment Analyzer will move the Sample Tray (Drawer 1) to the out position, provided it is not running when the command is received.

OUT#	Push the selected tray out, where # is the tray number, numbered from top down:
	1 = Buffer (Drawer B)
	2 = Waste (Drawer W)
	3 = Marker (Drawer M)
	4 = Storage (Drawer S)
	5 = Sample (Drawer 1)
		Send: out1
		Receive: *OUT1
		Receive: *COMPLETE
		Note: The Fragment Analyzer will move the Buffer Tray (Drawer B) to the out position, provided it is not running when the command is received.

STORE	Move the Capillary Storage Solution tray (located in S Drawer) to the capillary array. **Note**: When not in use, the capillaries should always be docked against Capillary Storage Solution to prevent drying of the capillary tips.	Send: store Receive: *STORE Receive: *COMPLETE **Note**: The Fragment Analyzer will move the Storage tray to the capillary array.
TRAY	Specifies the tray name for the next run.	Send: tray agilent0216A Receive: *TRAY **Note**: The Fragment Analyzer will set the tray name to **agilent0216A** and load sample names if a sample names .txt or .csv file is found for that tray name in the C:\AATI\Samples directory. For more information, refer to **Chapter 9**, "Fragment Analyzer - Sample Name Entry".
RUN	Run a specified Fragment Analyzer separation method. The run command must be followed by a space followed by the method name to be run.	Send: run DNF-930-33 - DNA 75-20000bp.mthds Receive: *RUN Receive: *COMPLETE **Note**: The Fragment Analyzer will start running the **DNF-930-33** separation method. A valid separation method file must be present to run.

Command	Function	Example
CAL	Run and create the specified Size Calibration (.scal) file in the ProSize data analysis program. The specified file will be written to the calibration folder.	Send: cal DNF-930-33 - DNA 75-20000bp.mthds, calfile.scal Receive: *CAL Receive: *COMPLETE **Note**: The Fragment Analyzer will run the specified separation method and output the specified .scal file named **calfile.scal**.
LAD-FILE	Use the specified Size Calibration (.scal) ladder file for subsequent runs.	Send: lad-file calfile.scal Receive: *LAD-FILE Note: The specified .scal file will be referenced for the size calibration ladder. Using a previously created size calibration file enables the use of all 96-wells in a sample plate for samples. The same separation method and run parameters should be used for the calibration and for subsequent sample plates to ensure valid sizing results.
ABORT	Used to abort a run.	Send: abort Receive: *ABORT: Running Receive: !10, ABORTED: remote abort command **Note**: The method may take a minute to abort.

The 5400 Fragment Analyzer application will output the following error messages listed in **Table 32**.

Table 32   5400 Fragment Analyzer Error Handling Commands

Command	Description
!1, Invalid command	The command received was not recognized.
!2, No method	The run command was received with no method.
!3, Method not found	The run command specified a method that is not in the appropriate methods directory. Ensure the separation method is present in the C:\Agilent\Methods\33cm directory when using a 33cm array, or in the C:\Agilent\Methods\55cm folder when using a 55cm array.
!4, Command failed	A run command failed for some reason.

Command	Description
!5, Low solution	A run command could not be run due to solution levels – insufficient gel or conditioning solution, or a full waste bottle.
!6, Stage error	The run failed due to a stage position error.
!7, Pump command error	The run failed due to a pump position error.
!8, Pressure error	The run failed due to a failure to build pressure during a pumping operation.
!9, Camera Connection error	The run failed because a camera connection error was encountered.
!10, Other	The run failed due some other error. An error message will accompany this.

5400 Fragment Analyzer Simulation Mode

For testing and demonstration purposes, it is possible to simulate the typical functions of the 5400 Fragment Analyzer system in the absence of an installed capillary array and/or fluids pumped through the system. The software enables simulation of individual components (for example, stage, pump, valves, etc.) or entire instrument operation.

Enable the 5400 system for simulated operation

1 Log in to the 5400 Fragment Analyzer software with administrator privileges. The default administrator credentials are:

   • User name: **Administrator**

   • No password required

2 From the **Admin** menu, select **Configuration**.

   The **Configuration Settings** window will open.
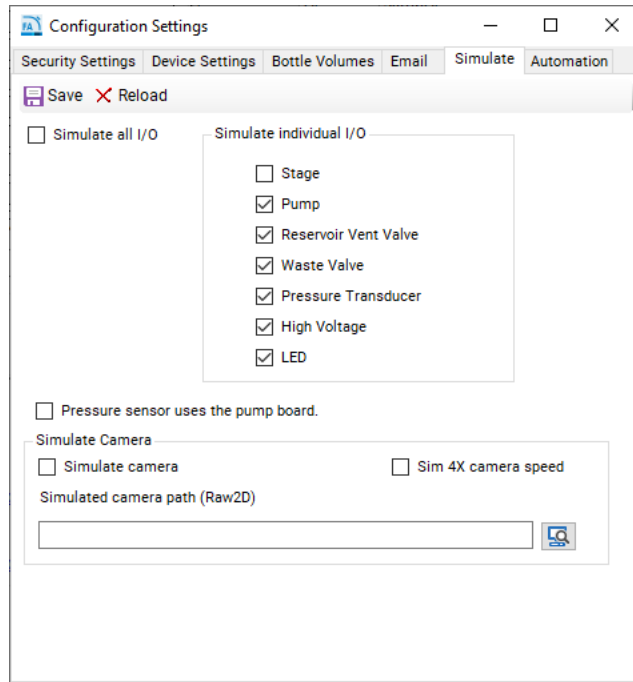
3 Select the **Simulate** tab (**Figure 119**).

Figure 119 5400 Fragment Analyzer Simulate configuration menu

4 To simulate the function of an individual component or components, select the check box next to the respective component(s) under **Simulate Individual I/O**. To enable simulation of all components, select **Simulate all I/O**. To simulate the camera, select **Simulate camera** box. The setting **Pressure sensor uses the pump board** is typically not selected, and only applies to older Fragment Analyzer instruments (Serial Number < 2600).

In **Figure 119**, all individual components except the stage mechanism are simulated, enabling to test stage movement while simulating pumping and high voltage separation methods.

5 Press **Save** to apply your changes.

Example 5400 Fragment Analyzer Operation Sequence

An example sequence of operations is listed below. A multitude of options can be programmed using the list of commands provided in **Table 31**.

Example Sequence for 5400 Fragment Analyzer, Automated Operation

1 Prepared Sample tray is ready (total about 2 seconds)

   c Command to Fragment Analyzer API **Status**

   d Message from Fragment Analyzer ***STATUS: Ready** GOTO Step 2

   e Message from Fragment Analyzer ***STATUS: Busy** GOTO Step 1

2 Run the sample

   a Command to Fragment Analyzer API **Out** (20-40 seconds)

   b Message from Fragment Analyzer ***Complete**

   c Robot places sample tray on stage drawer

   d Command to Fragment Analyzer API **RUN [METHOD NAME]**
     (45 to 85 minutes depending on the method)

     i. Pumping, voltage prerun, sample injection (20-25 minutes)
     ii. Separation (typically 20 to 60 minutes)
     iii. Data analysis and report generation 3 to 15 minutes (depends on report criteria)

   e Message from Fragment Analyzer ***Complete**

   f ommand to Fragment Analyzer API **Status**

   g Message from Fragment Analyzer ***STATUS: Ready** GOTO Step 2i

   h Message from Fragment Analyzer **!<error code>** Notify operator, GOTO Step 2m

   i Command to Fragment Analyzer API **Out** (20-40 seconds)

   j Message from Fragment Analyzer ***Complete**

   k Robot removes sample tray

   l Another Sample? Yes: GOTO Step 2c; No: GOTO Step 2m

   m Command to Fragment Analyzer API **Store** (20-40 seconds)

   n Message from Fragment Analyzer ***Complete**

5400 Fragment Analyzer Technical Support

For questions with the 5400 Fragment Analyzer system operation, contact your corresponding Agilent Sales/Service Representative.

In This Book

This manual contains system information about the 5200/5300/5400 Fragment Analyzer.

The manual describes the following:

- system overview,
- software menu commands,
- software tabs,
- capillary array,
- sample name entry,
- automated analysis,
- enhanced security features.

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