Agilent

5200, 5300, and 5400 Fragment Analyzer

System Manual

Notices

Document Information

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Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

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.

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This chapter gives an instrument overview.

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This chapter provides additional information on safety.

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13 Maintenance and Troubleshooting

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

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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.

Intended use of the Fragment Analyzer System

The Fragment Analyzer System (M53XAA) separates nucleic acids by means of capillary electrophoresis.

The system is designed to detect:

- Fluorescently stained double-stranded DNA including genomic DNA and cfDNA
- Fluorescently stained total RNA (Eukaryotic and Prokaryotic)

The Fragment Analyzer system is designed for professional use to exclusively run Agilent Fragment Analyzer reagent kits and specified consumables.

For Research Use Only. Not for use in diagnostic procedures.

Do not use this product in any manner not specified by the manufacturer. The protective features of this product may be impaired if it is used in a manner not specified in the operation instructions.

CAUTION

Unapproved, non-recommended or altered reagents

Altering any reagents and/or use of unapproved or non-recommended reagents may materially alter the performance of the instrument such that the instrument no longer performs to Agilent specifications.

Any work performed by Agilent to bring the instrument back into compliance with Agilent specifications will be performed at the customer's expense.

- ✔ **Always use the recommended and approved reagents to ensure proper instrument performance according to Agilent specifications.**
- ✔ **Do not use reagents that are altered, damaged or not properly labeled.**
- ✔ **Ensure correct handling, storage and disposal of reagents.**

Physical Specifications

Table 1 Physical specifications

Type	Specification
Weight	39.0 kg (86.0 lbs)
Dimensions (w×d×h)	101.6 × 61 × 86.4 cm (40.0 × 24.0 × 34 inches)
Line voltage	100 – 200 VAC
Line frequency	50 – 60 Hz (200 – 230 VAC; 50 – 60 Hz available)
Power consumption	~110 VA / 90 W
Ambient operating temperature*	15 – 25° C (59 – 77° F)
Operating humidity*	< 80 % (non-condensing)
Safety standards	IEC, EN, CSA, UL, Overvoltage category II, Pollution degree 2 for indoor use only
ISM classification	ISM Group 1 Class A According to CISPR 11
Sound pressure	< 70 dB (A) According to ISO 7779, 1988/EN 27779/1991

* The given operating range is for the instrument itself. Many sample migration speeds will be significantly slowed at temperatures below 20° C

Installation

This chapter provides a basic overview of the Fragment Analyzer system hardware installation 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 86 lbs (39 kg).

Figure 1 Configured Fragment Analyzer system with computer workstation

Fragment Analyzer System installation should only be performed by licensed Agilent representatives and approved channel partners. Installations are not to be completed directly by customers.

Ensure the lab space meets the criteria specified in the Instrument Site Preparation Checklist (5200/5300 Site Prep Guide (D0029169) or 5400 Site Prep Guide (D0029173)). Spacing, environmental conditions, power consumption, and miscellaneous required operating supplies are included in this checklist.

WARNING

Heavy weight

The instrument is heavy.

- ✔ **Avoid back strain and injury by following all precautions for lifting heavy objects.**
- ✔ **Ensure that the load is as close to your body as possible.**
- ✔ **Ensure that you can cope with the weight of your load.**

If an instrument needs to be relocated to a new location, please contact your local support representatives for instrument-specific guidance.

PC Management

The software is run using a PC with Microsoft Windows 10 or higher, with the following requirements (**Table 1**):

Table 2 Minimum computer requirements

Type	Specification
Processor	Intel i5 or above
SVGA Video	Display Resolution 1280 x 1024 or 1280 x 800
Memory	8 Gigabytes
Available Storage Space	500 Gigabytes
USB Serial Ports	6 ports (2 instrument, keyboard, mouse)

NOTE

Use of non-Agilent PCs are permitted though they are "use at own risk". Desktop PCs are recommended, laptops should not be used.

PC setup

CAUTION

Wrong settings

The recommended PC to use comes bundled with the Fragment Analyzer.

If the Fragment Analyzer computer settings do not match those below communication issues to the instrument are possible, resulting in lost time and productivity.

✔ **If a different PC is to be used or if any changes are made to the existing PC, check the following settings and enabled/disabled the PC settings to match the recommended setup.**

Date and Time Settings

1 Go to **Date and Time Settings** > **Set to Local Date & Time**.

2 Go to **Settings** > **Time & Language** > **Date & Time** and turn off the option **Adjust for daylight saving time automatically**.

USB Power Management Settings

1 Go to **Device Manager** > **Universal Serial Bus Controllers** > **USB Root Hub (USB 3.0)**.

2 Right-click **Properties** > **Power Management** and deselect the **Allow the computer to turn off this device to save power** check box.

To ensure best operating conditions it is recommended to not have any other applications open and running while using the data acquisition software.

USB Power Management Settings

1. Go to **Device Manager** > **Universal Serial Bus Controllers** > **Intel® USB 3.1 extensible Host Controller**.
2. Right-click **Properties** > **Power Management** and deselect the **Allow the computer to turn off this device to save power** check box
3. Go to **Settings** > **System** > **Power & Sleep** and select **Never**.
4. Go to **Additional Power Setting** and select **High Performance**.
5. Go to **Settings** > **System** > **Notification** and set all to **Off**.
6. Go to **Settings** > **Gaming** and turn off the Xbox Game Bar:
   a. Deselect **Open Xbox Game Bar**.
   b. Set **Game Mode** to **Off**.
7. Go to **Settings** > **Privacy** and set all to **Off**.
8. Go to **Settings** > **Updates** and set all to **Off**.
9. Go to **Security** > **Windows Security** > **Virus & Threat Protection Settings** and set all to **Off**.
10. Go to **Settings** > **Windows Update** > and select **Check Now/Refresh** to update to the latest available version.

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.

No licenses are required for this software and the latest version installer is made freely available on the Agilent website.

https://www.agilent.com

Software Installation

To install the Fragment Analyzer software:

1. Navigate to 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.

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 **Figure 2**: Rear of PC
  - Two USB connections to Fragment Analyzer system

    The order/location of these cables can be in any configuration though we do recommend to connect these cables into non-adjacent ports as a preventative measure against communication drops.
  - Power cord to grounded electrical outlet
  - Connection to monitor, keyboard, mouse, etc.
  - (Optional) ethernet cable

Connection to monitor, keyboard, mouse, etc.

- Connection **Figure 3**: From the Fragment Analyzer system
  - Two USB cables to PC USB
  - Power cord to grounded electrical outlet

NOTE A wired mouse and keyboard can lead to connection issues. It is always recommended to use a wireless mouse and keyboard like the ones that come with the instrument bundle.

NOTE When power-cycling the instrument and PC, disconnect the instrument USB cables from the PC as several boards/components receive power through these cables.

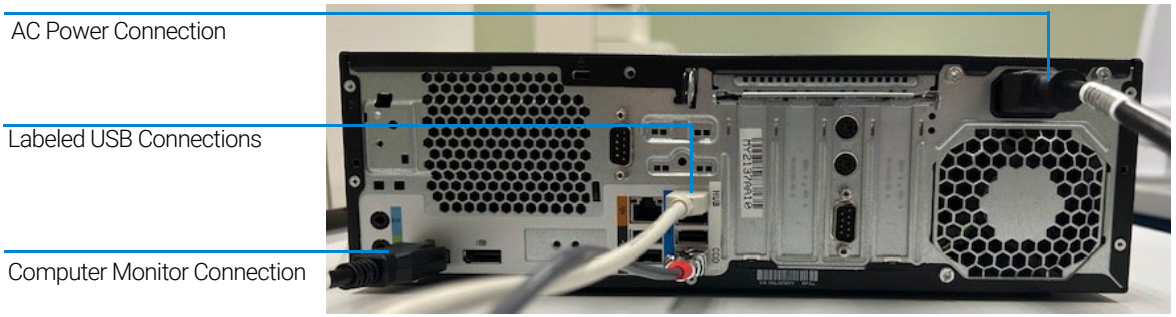

Figure 2 Rear of PC showing all electrical connections

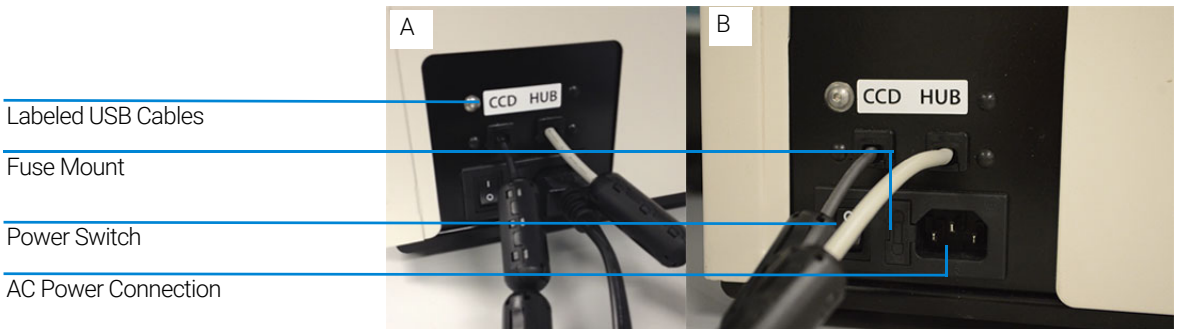

Figure 3 Rear of instrument showing all electrical connections

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 (six total) (**Figure 4**).

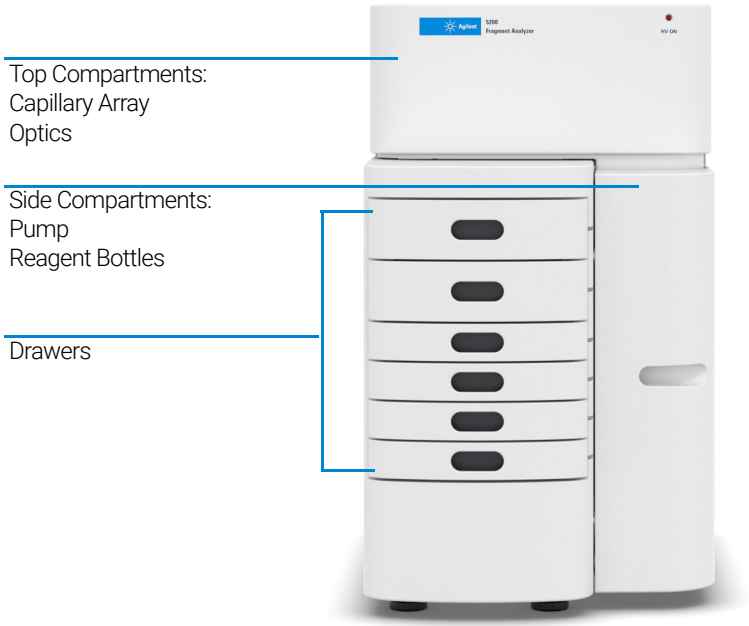

Figure 4 Entry points of the Fragment Analyzer system

Top Compartment

Figure 5 Top compartment

CAUTION

Interrupted operation

Opening the top compartment while the HV ON light is illuminated will abort the active operation, stop the method queue, and result in data loss.

✔ **Ensure that the light is on for any action involving the high voltage power supply. This includes voltage injections and separations.**

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

NOTE

The 5200 Fragment Analyzer is only compatible with 12-capillary array cartridges. The 5300 Fragment Analyzer can be compatible with 48 and 96-capillary array cartridges.

The 5400 Fragment Analyzer is only compatible with 96-capillary array cartridges.

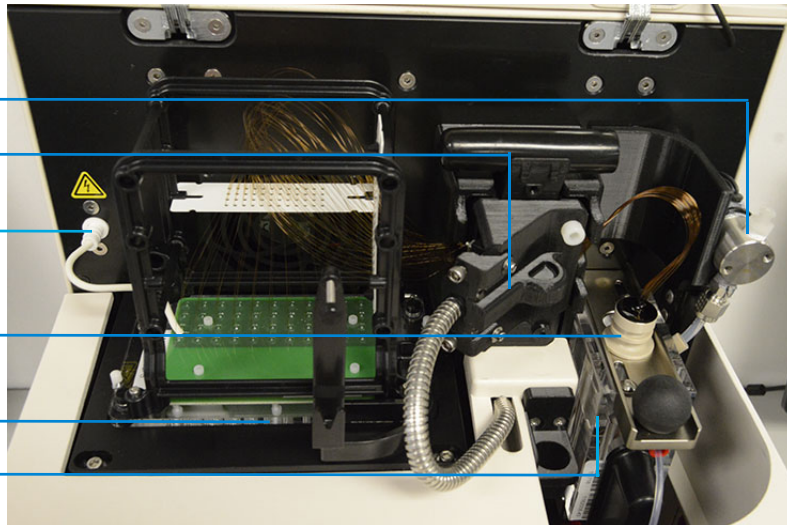

Figure 6 Fragment Analyzer main unit top compartment opened

WARNING

High voltage

The Fragment Analyzer contains a high voltage supply cable. It is marked by a hazardous voltage sticker. This cable sends electricity to the capillaries during any actions that use high voltage (pre-run, injections, separation). If the top compartment is not shut properly, the high voltage power supply will not be delivering any power to the cable.

✔ **Ensure that the cover is properly shut before operating the instrument.**

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, pressurizing 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.

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

- ✔ **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- ✔ **The volume of substances should be reduced to the minimum required for the analysis.**
- ✔ **Do not operate the instrument in an explosive atmosphere.**

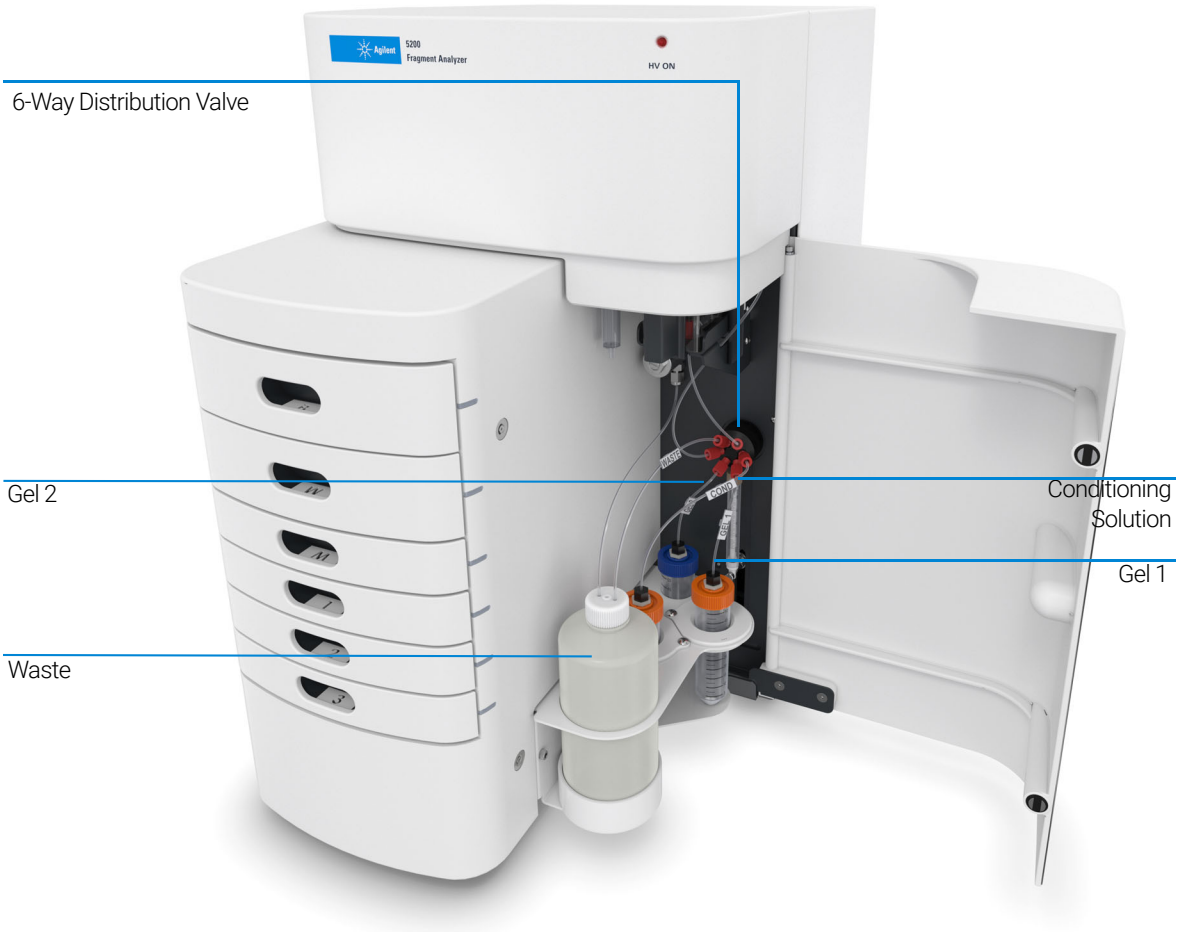

Figure 7 Side Door Compartment

The six fluid line connections inside the Fragment Analyzer system as they connect to the 6-way multivalve:

- Line to waste bottle – valve position A
- Reservoir filling line – valve position B
- Conditioning line – valve position C
- Gel 1 line – valve position D
- Gel 2 line – valve position E
- F-port line – valve position F

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 drawer position is also used for *capillary 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 the default location for a 96-well plate containing *capillary storage solution*.

NOTE

The marker drawer (drawer M) is used for a separate marker injection when running a qualitative kit. It is used for a TE rinse step (capillary tip dip) for any of the quantitative kits.

Drawer status

Status	Description
Drawers B and W are interlocked	When either of the top two drawers are open, the high-voltage (for electrophoresis) will automatically shut off. This voltage should only be enabled during a pre-run voltage check, voltage injection steps, and the separation itself. This will be indicated by the illumination of a LED in the upper right corner of the top compartment hood.
Drawers M, 1, 2, and 3 are not interlocked	Sample trays can be exchanged while the instrument is in operation.

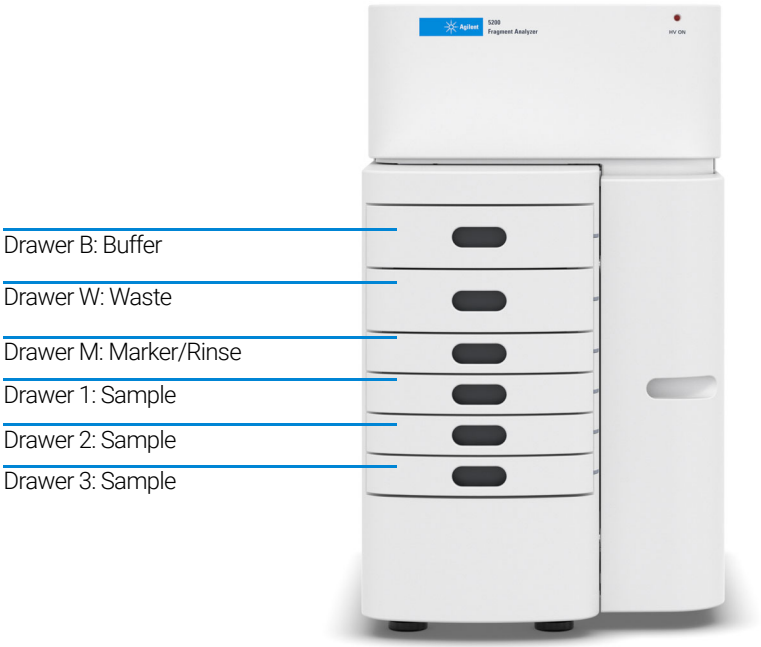

Figure 8 Instrument drawer positions

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.

Figure 9 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 132.

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.

NOTE

If you use the mineral oil dropper bottle supplied with some Fragment Analyzer reagent kits, one drop from the bottle is adequate.

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 recommendations are given in each kit guide for reference.

2 Safety

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General Safety Information 29

This chapter provides additional information on safety.

General Safety Guide

The general safety guide can be found on **https://www.agilent.com** through the general search function.

The general safety guide contains all symbols, warnings, etc., as well as any information on how to locate the Declarations of Conformity.
The table below is a relisting of all Fragment Analyzer Systems symbols from the general safety guide.

Safety Symbols

Table 3 Symbols

Symbol	Location	Description
	Top Compartment	Indicates hazardous voltages.
	Syringe Pump	Indicates a pinching hazard.
	Reservoir	Indicates frame or chassis terminal, which is bonded to conductive parts of an equipment for safety purposes.
	Serial Number Tag Top Compartment	Adhere to specific voltages indicated. instrument is equipped with high voltage interlocks for user safety. For proper operation top cover should be closed. Interlocks should never be defeated.
	Inside Reagent Door	Warns of the use of and exposure to hazardous and/or corrosive chemicals. Consult reagent kit guides and SDS sheets for listing of precautions and handling information.
	Serial Number Tag	Confirms that a manufactured product complies with all applicable European Community directives. The European Declaration of Conformity is available at: **http://regulations.corporate.agilent.com/DoC/search.htm**
	Power Supply Switch	Power symbol indicates ON.
	Power Supply Switch	Power symbol indicates OFF. The apparatus is not completely disconnected from the MAINS when the power switch is in the OFF position.

General Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

WARNING

Heavy weight

The instrument is heavy.

- ✔ **Avoid back strain or injury by following all precautions for lifting heavy objects.**
- ✔ **Ensure that the load is as close to your body as possible.**
- ✔ **Ensure that you can cope with the weight of your load.**

WARNING

Unintended use of power cords

Using power cords for unintended purposes can lead to personal injury or damage of electronic equipment.

- ✔ **Never use a power cord other than the one that Agilent shipped with this instrument.**
- ✔ **Never use the power cords that Agilent Technologies supplies with this instrument for any other equipment.**
- ✔ **Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.**

WARNING

Reagents

Toxic and hazardous reagents and flammable liquids. The handling of reagents can hold health risks.

- ✔ **When working with reagents observe appropriate safety procedures (for example: goggles, safety gloves and protective clothing) as described in the safety data sheet supplied for the reagent kits, especially when toxic or hazardous solvents and flammable liquids are used.**

3 Legal and Regulatory

Sound Emission 31

Waste Electrical and Electronic Equipment (WEEE) Directive 32

This chapter provides additional information on legal and regulatory aspects.

Sound Emission

Manufacturer's Declaration

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

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

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

NOTE

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

Waste Electrical and Electronic Equipment (WEEE) Directive

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

NOTE

Do not dispose of in domestic household waste.

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

4 Fragment Analyzer Software – File Menu

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Exit 39

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

Opening the Fragment Analyzer Software

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

Figure 10 Fragment Analyzer icon

The main screen opens.

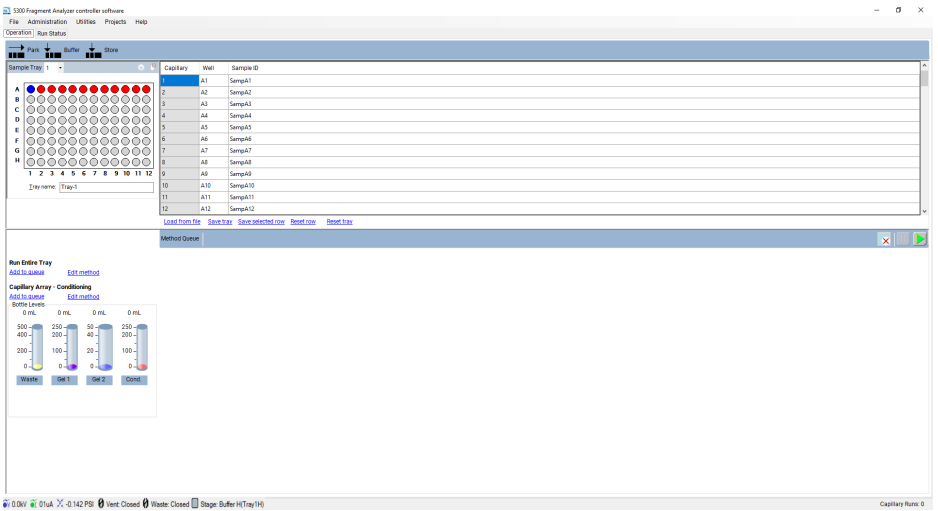

Figure 11 Fragment Analyzer software main screen window (example shows the software for a 5300 Fragment Analyzer instrument)

NOTE

Unless you download and set up the standalone Agilent Administration software, there will not be a login prompt for the Fragment Analyzer controller software with version 5.0 and later.

More information about the different permissions and access to the software is available in the Agilent Administration software manual.

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

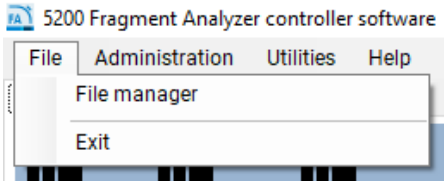

Figure 12 File menu commands

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 4**.

Table 4 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 5 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 48- or 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.

Exit

The Exit command 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.

5 Fragment Analyzer Software – Administration Menu

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Error Report 48

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

Administration Menu

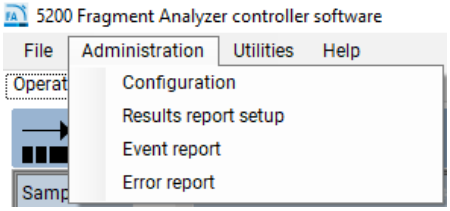


Figure 14 Admin menu commands

Configuration

Selecting the **Configuration** option from the drop-down menu opens the **Configuration Settings** window. You can modify the **Device Settings** and **Bottle Volumes** for the system here.

The **Device Settings** tab allows modification of the device settings (**Figure 15**).

Update the settings whenever you install a new capillary array cartridge.

Update the capillary array serial number field whenever you install a new capillary array cartridge.

Ensure that the instrument serial number field accurately reflects the stickered number on the physical instrument.

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

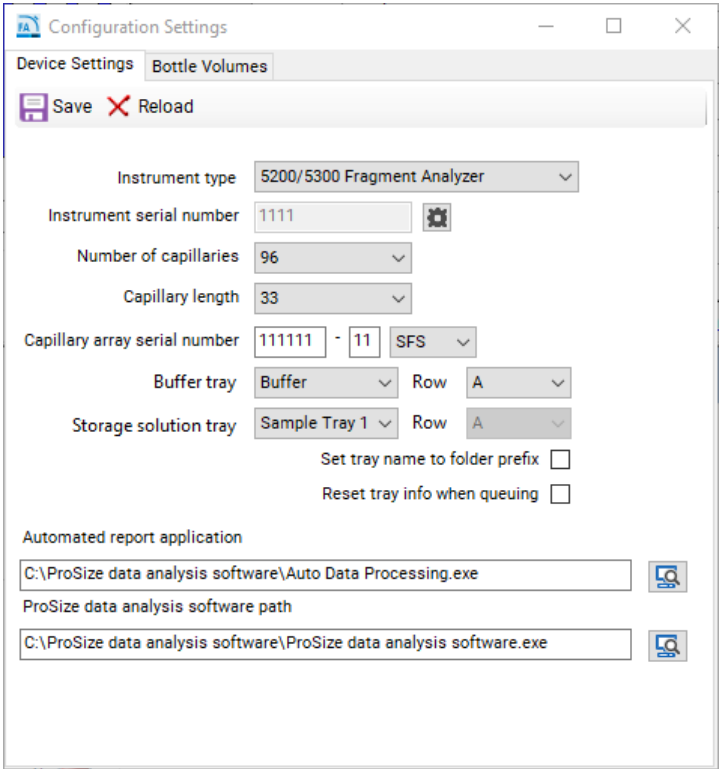


Figure 15 Configuration – Device Settings tab

Configuration Options

Table 6 Configuration – Device Settings tab functions

Parameter	Description
Number of Capillaries	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	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	The format must be xxxxxx-xx-xxxx.
Buffer Tray	Default selection is locked.
Storage Solution Tray	Allows for the selection of tray and row for the storage solution tray.
Set Tray Name to Folder Prefix	Sets the tray name to the folder prefix used when loading sample trays.
Reset Tray Info when Queuing	Resets tray info for each new tray that is loaded.
Automated report application	Allows for changing the file path used for the automated report application.
ProSize data analysis software path	Allows for changing the file path used to open the ProSize data analysis software.
Save	Saves the chosen settings.
Reload	Reloads the previously saved settings.

The **Bottle Volumes** tab allows modification of the reagent bottle volumes (**Figure 16**).

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.

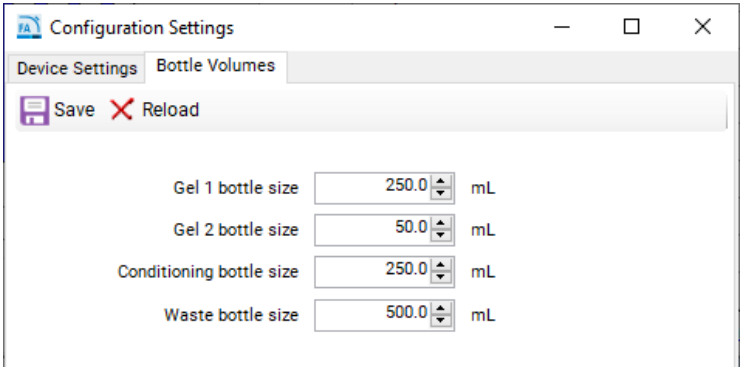


Figure 16 Configuration – Bottle Volumes tab

Results Report Setup

The option **Results report setup** opens the **Automated Report Settings** window (**Figure 17**).

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 12**, “Fragment Analyzer – Automated Analysis”.

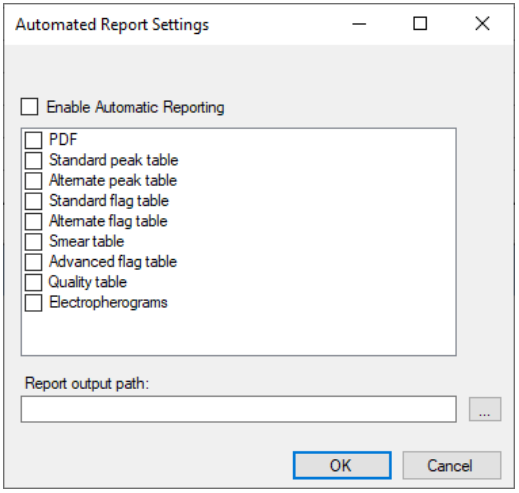


Figure 17 The Results Report Setup screen

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 12**, “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 12**, “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 **Administration** menu opens the **Select Date Range** window where the user can **Use all dates** or **Use selected date range** (Figure 18).

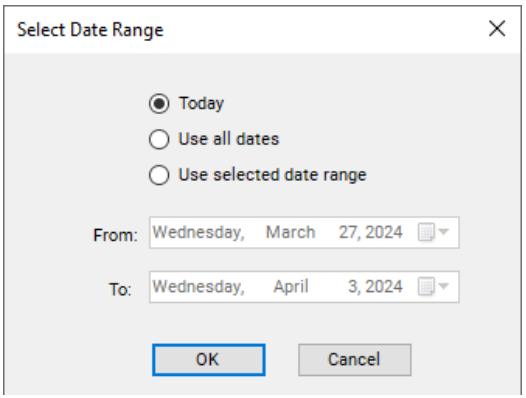


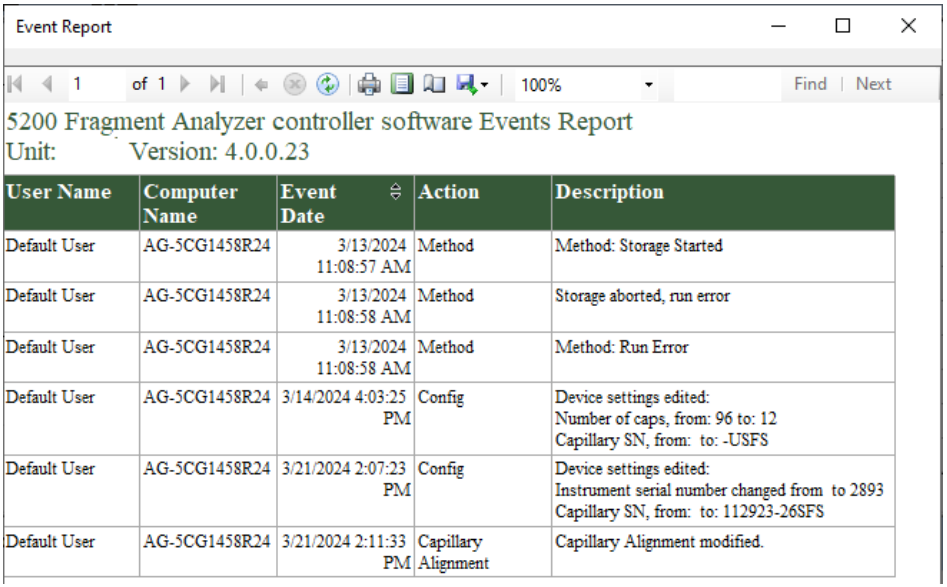
Figure 18 Event Report popup window

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 19).



User Name	Computer Name	Event Date	Action	Description
Default User	AG-5CG1458R24	3/13/2024 11:08:57 AM	Method	Method: Storage Started
Default User	AG-5CG1458R24	3/13/2024 11:08:58 AM	Method	Storage aborted, run error
Default User	AG-5CG1458R24	3/13/2024 11:08:58 AM	Method	Method: Run Error
Default User	AG-5CG1458R24	3/14/2024 4:03:25 PM	Config	Device settings edited: Number of caps, from: 96 to: 12 Capillary SN, from: to: -USFS
Default User	AG-5CG1458R24	3/21/2024 2:07:23 PM	Config	Device settings edited: Instrument serial number changed from to 2893 Capillary SN, from: to: 112923-26SFS
Default User	AG-5CG1458R24	3/21/2024 2:11:33 PM	Capillary Alignment	Capillary Alignment modified.

Figure 19 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
Find | Next	Search

Error Report

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

Selecting the command **Error Report** from the Administration menu opens the **Select Date Range** window where the user can **Use all dates** or **Use selected date range** (Figure 20).

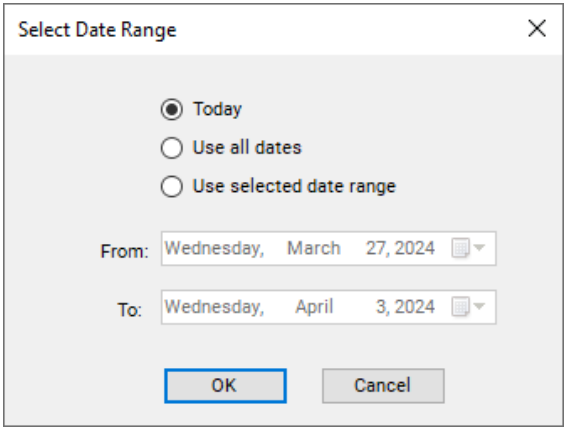


Figure 20 Error Report popup window

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 21).

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

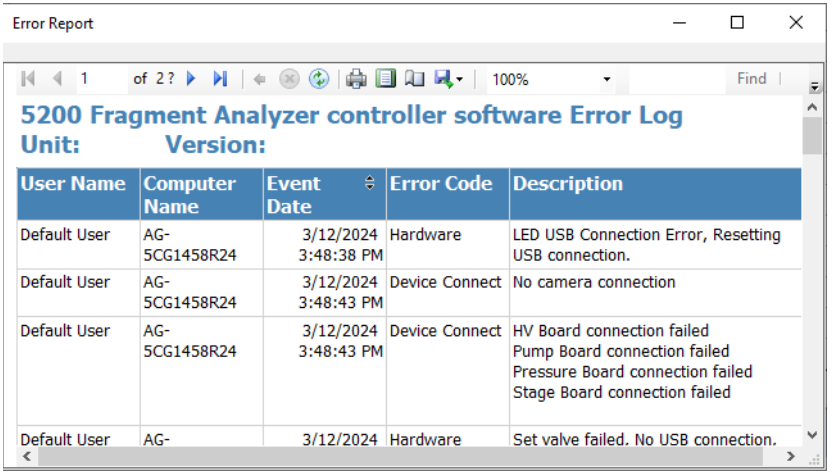


Figure 21 Error Report example

6 Fragment Analyzer Software – Utilities Menu

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Hardware Testing Screen 59
Prime 61
Solution Levels 62
Clean Reservoir Vent Valve 63
Results Dashboard 64

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

Utilities menu

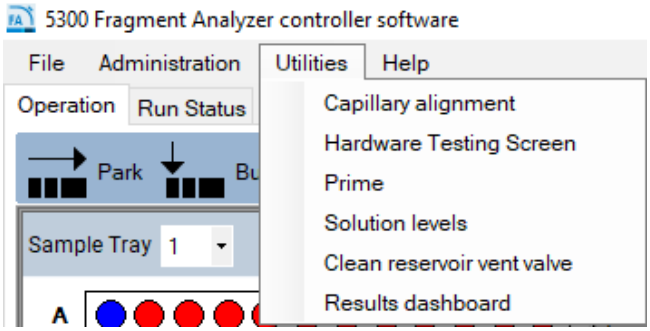

Figure 22 Utilities menu commands

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 considered the most accurate. Method B can be the quickest:

A) Alignment from a file – best used when installing new 96-capillary array or fine-tuning an alignment without dye.

B) Alignment without a file (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.

Method A – Capillary Alignment from a File

1 Select **Capillary Alignment** from the **Utilities** drop-down window.

The *Capillary Alignment* window opens (see **Figure 23**).

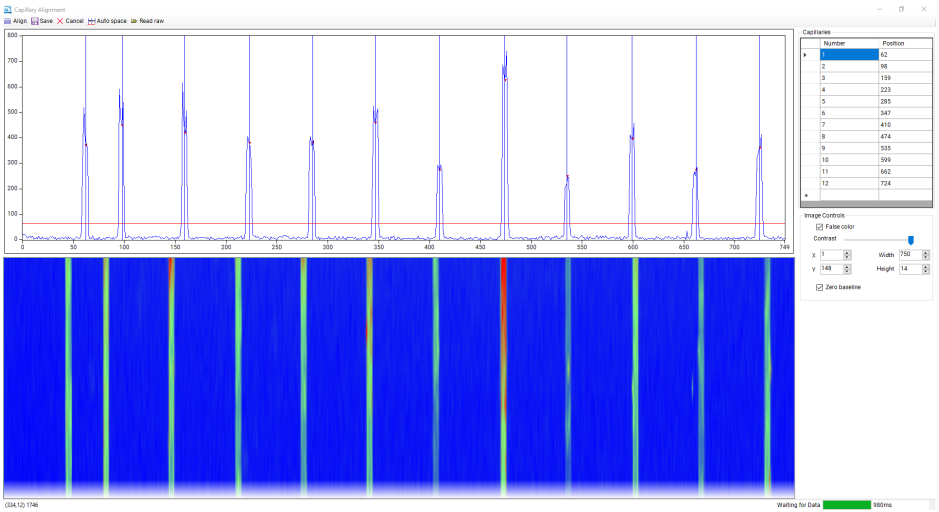

Figure 23 Real time capillary alignment popup window (example shows 12-capillaries)

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

NOTE

If a window is already drawn as the example shows, it will only need to be redrawn upon request by Agilent support or if some of the physical capillaries are not captured in the viewing window. We always recommend drawing this viewing window as wide as possible as shown in Method B.

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 a window has been drawn, proceed to raise the red horizontal line above the baseline noise and click **Align**.

4 Click **Auto Space** to ensure all blue vertical lines are evenly spaced between the first and last capillary peak signals.

NOTE

It is normal for the blue vertical lines to not match with the capillary peaks at this step. The **Auto Space** function just serves to ensure all vertical lines have a unique X-coordinate. If any of these lines have the same **Cap Position** in the table in the upper right, an error message will appear when you attempt to save.

5 Click **Save**, 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.

6 From the top menu bar of the *Capillary Alignment* window, select **Read raw**.

7 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).

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

The *Align from File* window will open (**Figure 24** shows an example for a 12-capillary and **Figure 25** 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**.

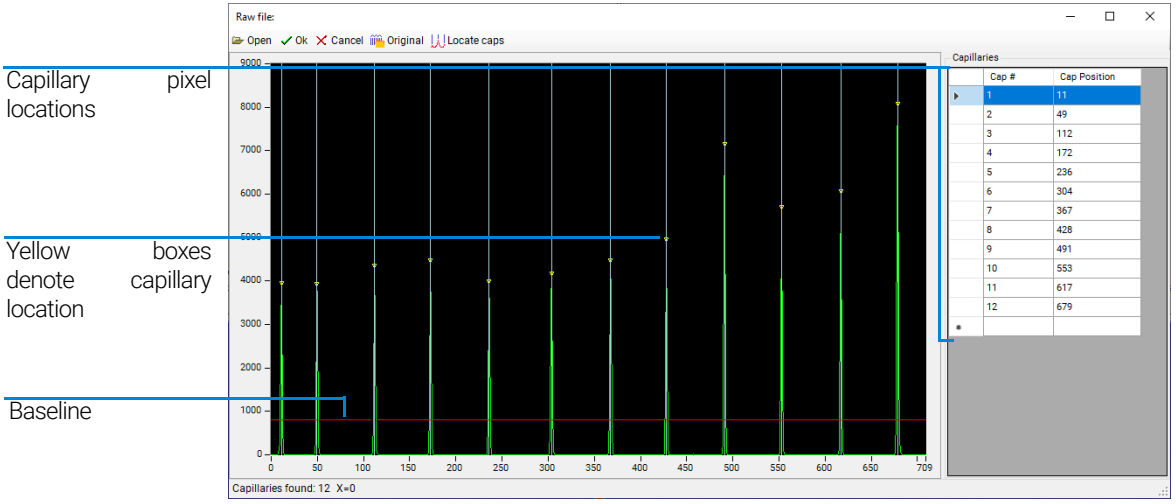

Figure 24 Align from file popup window for 12-capillary system

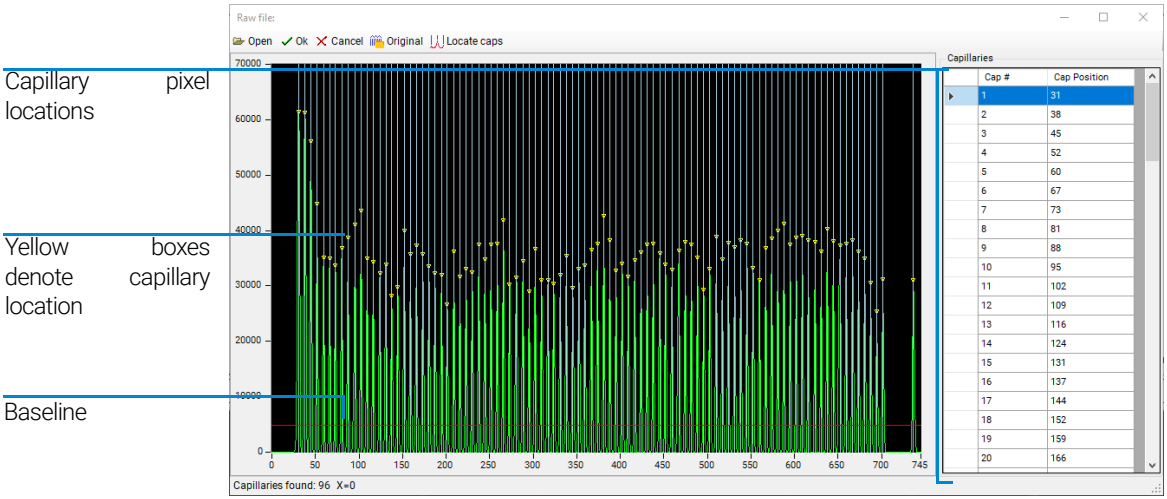

Figure 25 Align from file popup window for 96-capillary system

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.

9 Left click on the red baseline and drag it upwards off the bottom of the graph but not above the top of capillary peaks, as shown in **Figure 24** and **Figure 25**.

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

The capillary peaks are 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 drag 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.

11 Once the correct number of capillaries are located, select **OK** from the *Align from File* toolbar. This will save changes and close the current window returning you to the live *Capillary Alignment* window. The blue vertical lines should now match up with each capillary peak location.

12 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 a file

NOTE

A capillary alignment without a file can only be performed for a 12-capillary or 48-capillary array. The 96-capillary array does not have enough physical space between capillaries to reliably perform this alignment procedure in the software.

1 Select **Capillary Alignment** from the **Utilities** drop-down menu.

The real time view *Capillary Alignment* window opens (see **Figure 23**).

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 26**).

4 Draw a box around the capillary array display area. Left-click and drag the appropriate area. (**Figure 26**).

NOTE

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

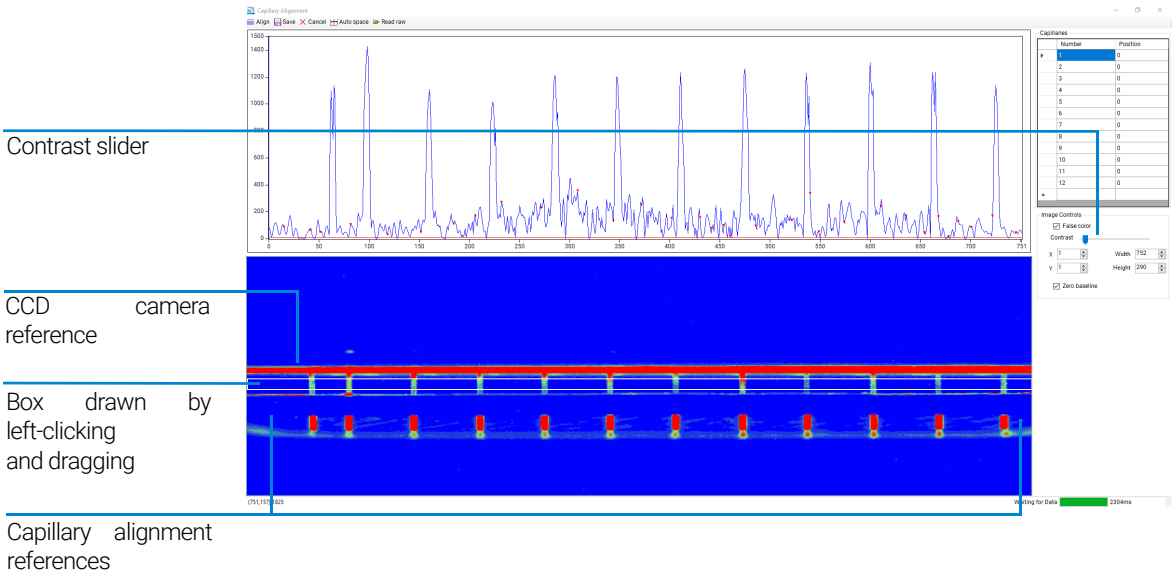

Figure 26 Capillary alignment display – window reset

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 Click and drag the red baseline seen in **Figure 26** until a red triangle is observed on each capillary peak. This triangle determines where the blue vertical lines will be assigned in the next step. 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).

NOTE

Every time you select **Align**, it will shift the blue vertical lines to the first set of peaks where the red triangles are present. For example: If the software is looking for 12 capillaries, it will place 12 vertical lines on the alignment screen where the first 12 red triangles are present from the left-hand side of the screen.

10 Select **Save** from the menu at the top of the *Capillary Alignment* window to save the current capillary locations and close the window.

Hardware Testing Screen

The command **Hardware Testing Screen** is used for troubleshooting the instrument.

Selecting the **Hardware Testing Screen** command from the **Utilities** menu opens the **Hardware Testing Screen** (Figure 27).

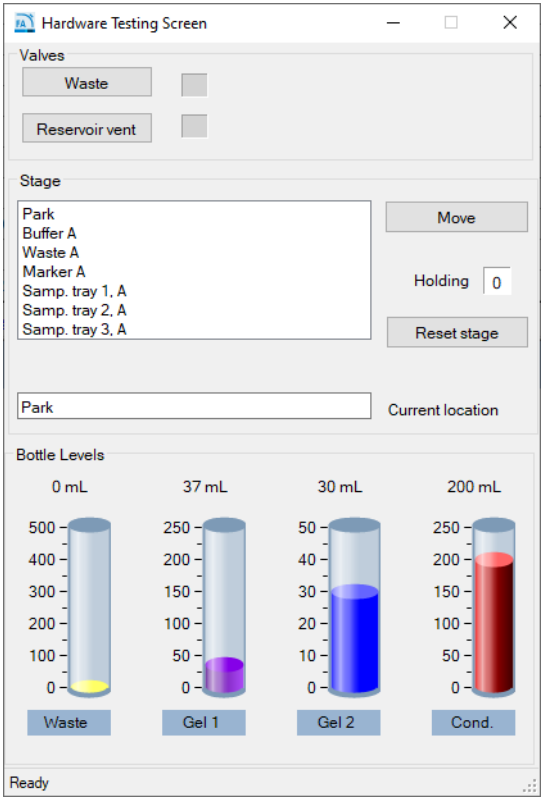

Figure 27 Hardware Testing Screen

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.
Stage > Reset Stage	Allows the user to tell the software that the stage is in the park location (forces the Holding number to '0'). This does not move the physical stage. Should only be used if the digital and physical stage positions do not match up – can be a user-generated error if a drawer is opened before the instrument has registered that a plate and adapter has returned to its corresponding drawer location.
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 28**). The prime functions are discussed in **Table 11**.

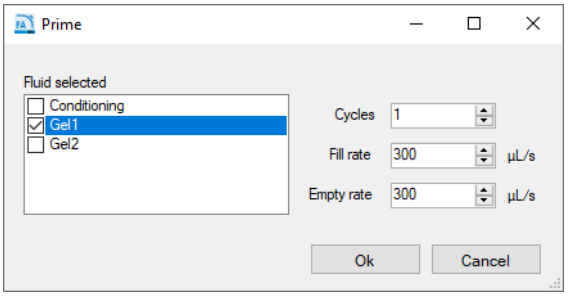

Figure 28 Prime window

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.

NOTE

A prime function does not involve the reservoir/capillaries. It is simply a single 2.5 mL syringe pull from the chosen liquid line pushed directly into the waste line.

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 29**).

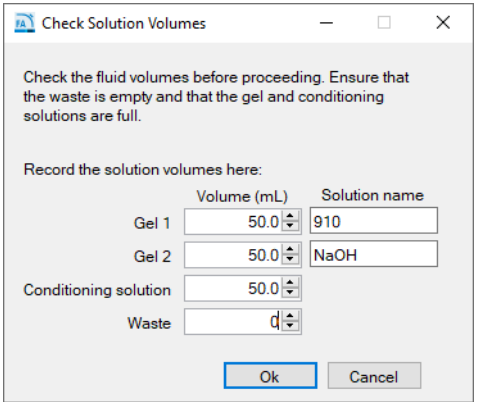

Figure 29 Check Solutions Volumes window

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 30**).

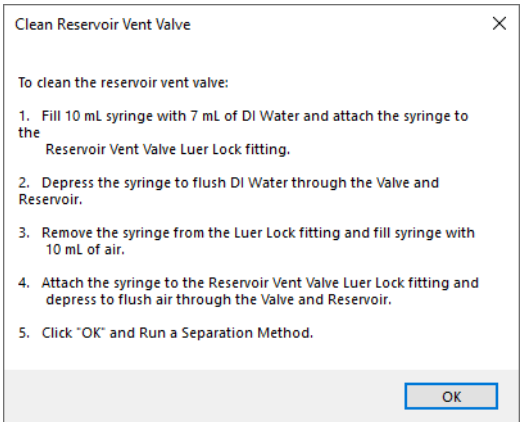

Figure 30 Clean reservoir vent valve screen

Follow the steps outlined in **Figure 30** 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 31 shows an example in the **Results Dashboard** window.

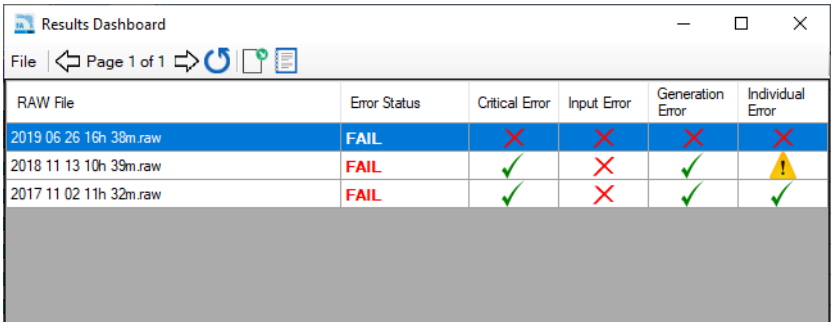

Figure 31 Results dashboard output

For more information about the **Results dashboard** and the window toolbar, refer to **Chapter 12**, “Fragment Analyzer – Automated Analysis”.

7 Fragment Analyzer Software – Help Menu

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User Manual 66
About 66
About Firmware 66

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

Help Menu

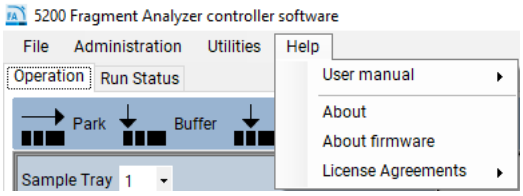

Figure 32 Help menu commands

User Manual

Navigating to the command **Users manual** will provide a link to the full user manual in PDF form.

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 a window displaying the version numbers of the firmware present on the High voltage board, Pump control board, Pressure board, and stage motion control board.

8 Fragment Analyzer Software – Operation Tab

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

Operation Tab Overview

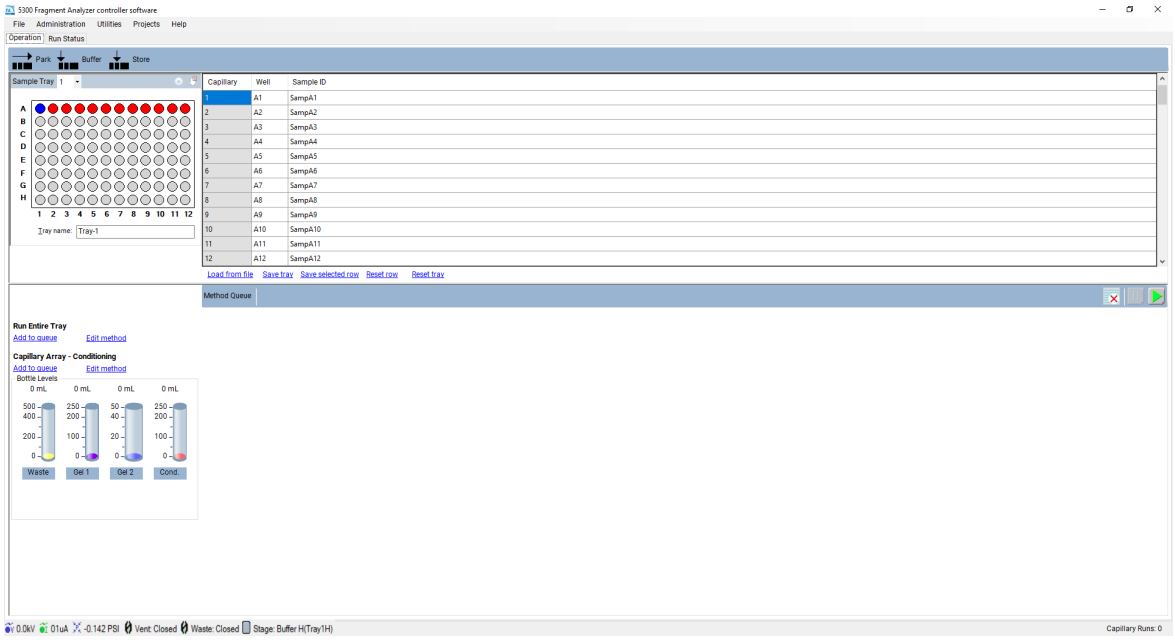

Figure 33 Fragment Analyzer main screen

Hotel Position Icons

There are three hotel positioning icons located at the top of the **Operation** tab, as seen in **Figure 33**. The icons and their function are discussed in **Table 12**.

Table 12 Hotel position icon functions

Icon	Description
	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.
	This command is used to pick up the buffer tray from the buffer drawer and move it up against the capillary array.
	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 34**).

NOTE

The configuration can be set by selecting the icon located in the upper right corner of the window shown in **Figure 34**.

Configure the Visual Style of Tray Selection Window

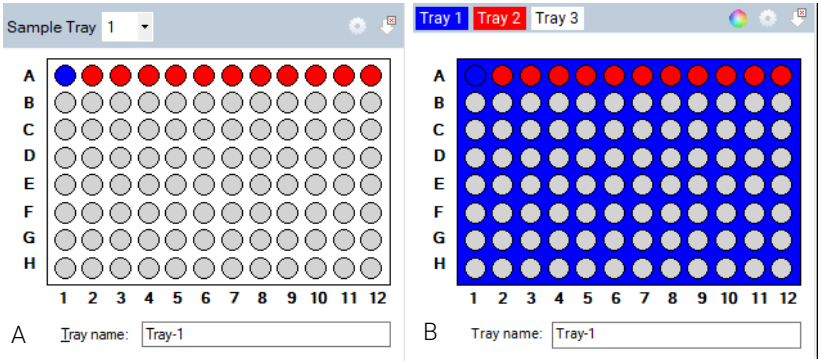

Figure 34 Classic drop-down tray selection (left), and colored tab tray selection (right).

1 In the tray window, select .

The **Visual preferences dialog** window opens (**Figure 35**).

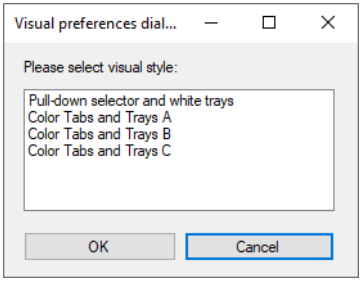

Figure 35 Visual preferences dialog window.

2 Choose between the sample tray drop-down list or the colored tab tray selection as shown in **Figure 34**.

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 34**). To select a new row left-click on another row.

5 To clear a row selection, select  . (**Figure 34**)

The **Tray name** dialog box allows you to input a name for the tray being run (**Figure 34**). 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 11**, "Fragment Analyzer – Sample Name Entry").

Enter sample information in the **Sample ID** section of the main screen. (**Figure 36**)

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 36 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.

Adding Methods to the Queue

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

The *Separation Run Controls* shown in **Figure 37** 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 5200 Fragment Analyzer (12-capillary) or 5300 Fragment Analyzer (48-capillary configuration). It will be hidden for both the 5300 Fragment Analyzer (96-capillary configuration) and the 5400 Fragment Analyzer.

Reagent Levels of the bottles are also shown.

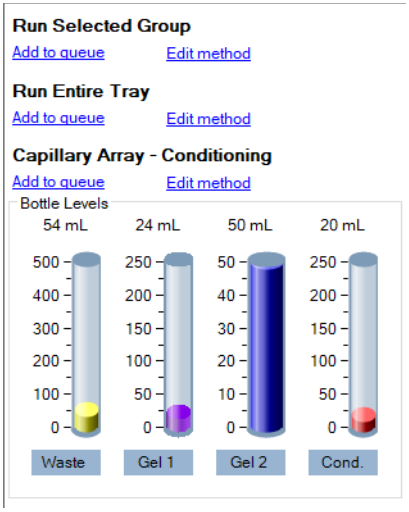

Figure 37 Separation run controls

Run Selected Group or Run Entire Tray – Edit Method

Selecting **Edit method** shows the method editor pop-up window for both separation methods **Figure 38**.

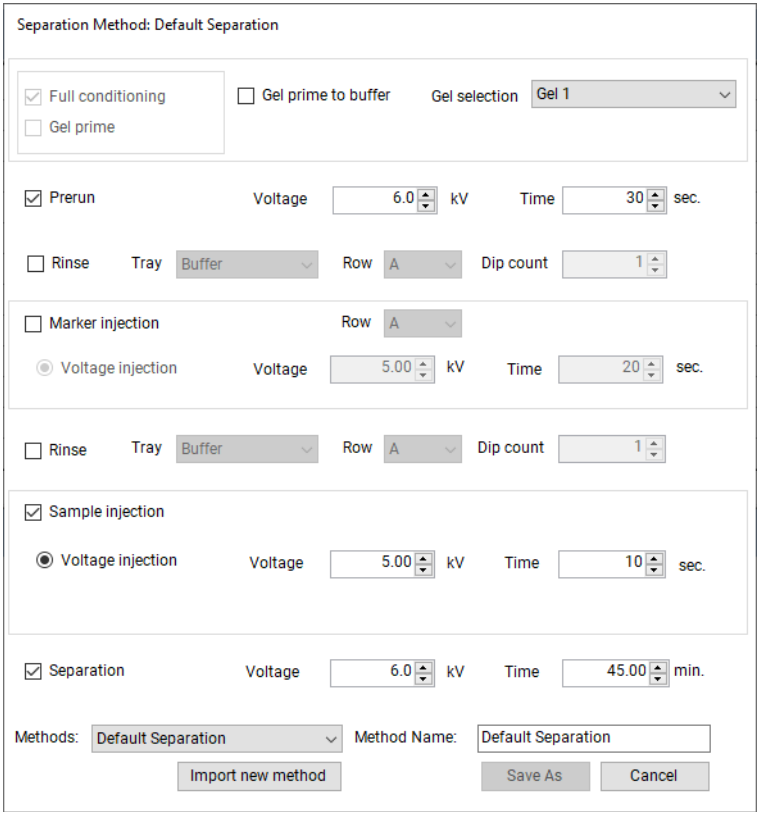

Figure 38 Separation method editor

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 39**.

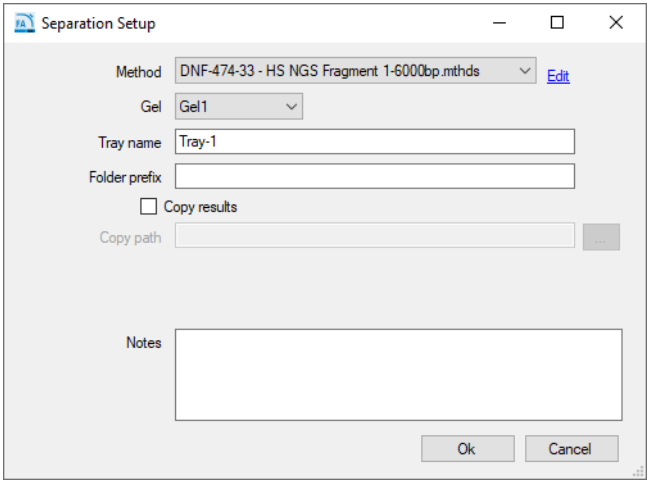

Figure 39 Separation Setup window

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 38**. User level access only allows the user to **View** the method file selected.
Gel	The user can toggle the gel bottle location to the desired bottle to use for the separation method without having to alter a predefined method.
Tray name	The tray name appears as input by the user on the main screen or the default name appears. The user may edit this field by typing in the provided box.
Folder prefix	The folder prefix allows the user to add a prefix to the folder name where the results files will be written.
Copy results / Copy path	The default directory location for the data is C:\Agilent Technologies\Data. The user may select the **Copy Results** option and choose to copy the saved data to a different location by selecting the **[...]** option.

Table 15 Separation setup window functions

Item	Description
Create Size Calibration File	This is used for automated analysis (**Chapter 12**, "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 12**, "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 12**, "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. Note: The size calibration settings will only be visible on the Separation setup window when the Enable Automatic Reporting checkbox is selected in the Automated Report Settings window (**Figure 23**).
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. Note: The **Merge rows** function will only become available if you select **Run Entire Tray** > **Add to queue selection** for a 5200 Fragment Analyzer or for a 48-capillary configured 5300 Fragment Analyzer.

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 several programmed capillary conditioning methods for cleaning and maintaining the capillary array.

The user can also select to create a method of their choosing by selecting the **Edit Method** option seen in **Figure 40**.

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**.

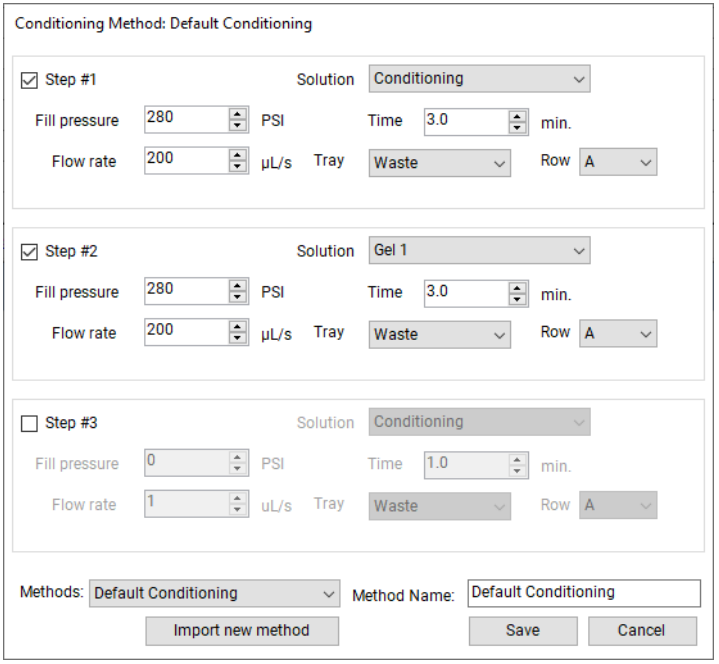

Figure 40 Conditioning method editor

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 41**).

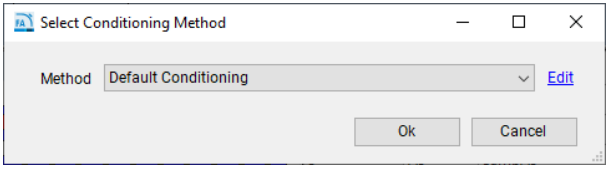

Figure 41 Select Conditioning Method window

You can select an already created method from the drop-down menu or click **Edit** to view the conditioning method editor window as seen in **Figure 40**.

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.

NOTE

These standalone conditioning methods can help with maintenance and basic troubleshooting. This is explained in more detail in **"Capillary Array Cleaning"** on page 137.

Method Queue

Once a sample tray or row/group has been selected and added to the queue, the method name and tray location is shown (**Figure 42**).

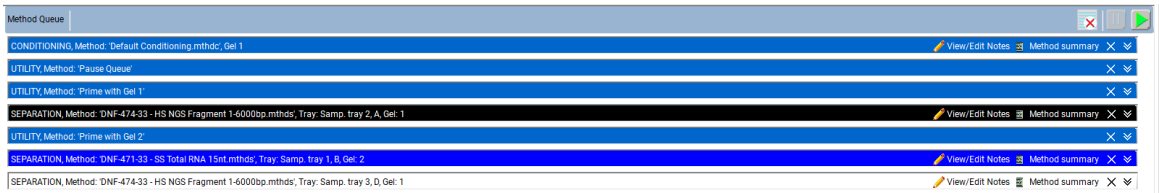

Figure 42 Method Queue

Figure 42 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 43**).

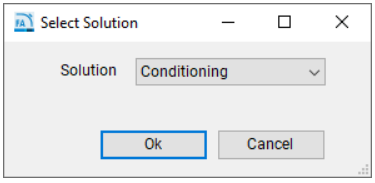

Figure 43 Select Solution popup window

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 44**.

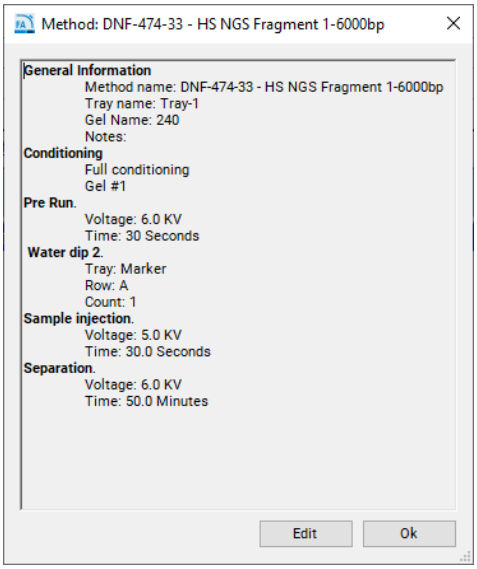

Figure 44 Method summary popup window

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 **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.

Permissible Characters

The following tables show which characters are permissible (**Table 18**) and non-permissible (**Table 19**) for a file name.

Table 18 Permissible characters for a file name

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

Table 19 Non-permissible characters for a file name

Characters	
*|	|
\	:
"	'
<	>
?	/

9 Fragment Analyzer Software – Run Status Tab

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Status Bar 90

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 79), 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 45** will show where the Fragment Analyzer stage is moving to/from to give the user real time view of what is happening.

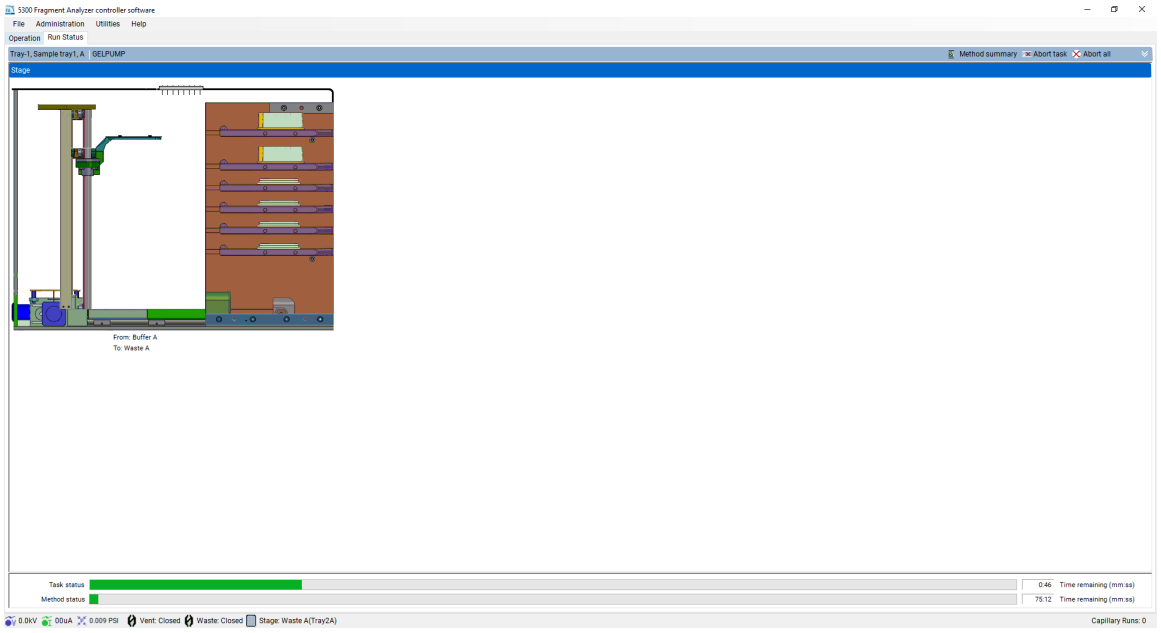


Figure 45 Stage movement animation

NOTE

The animation does not time perfectly to the physical stage movements. We recommend to confirm all stage movements are finished by checking the drawer indicator lights. If you open a drawer before a stage movement has finished, the run is aborted.

Conditioning Animation

When the Fragment Analyzer instrument is pumping conditioning solution or gel, the following animation is shown (**Figure 46**). The animation gives a real-time view of what the instrument is doing during a conditioning sequence (including fluid flows, valve switches, etc.).

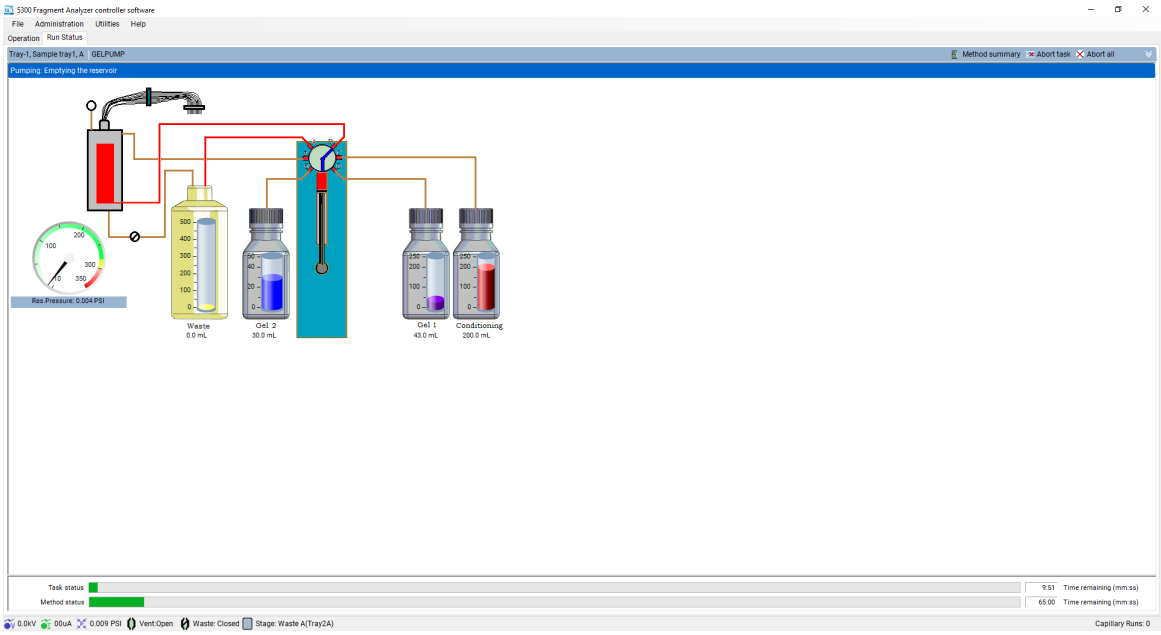


Figure 46 Conditioning animation

Pre-Run / Injection View

When the Fragment Analyzer system is completing a pre-run or injection, the screen as shown in **Figure 47** will appear.

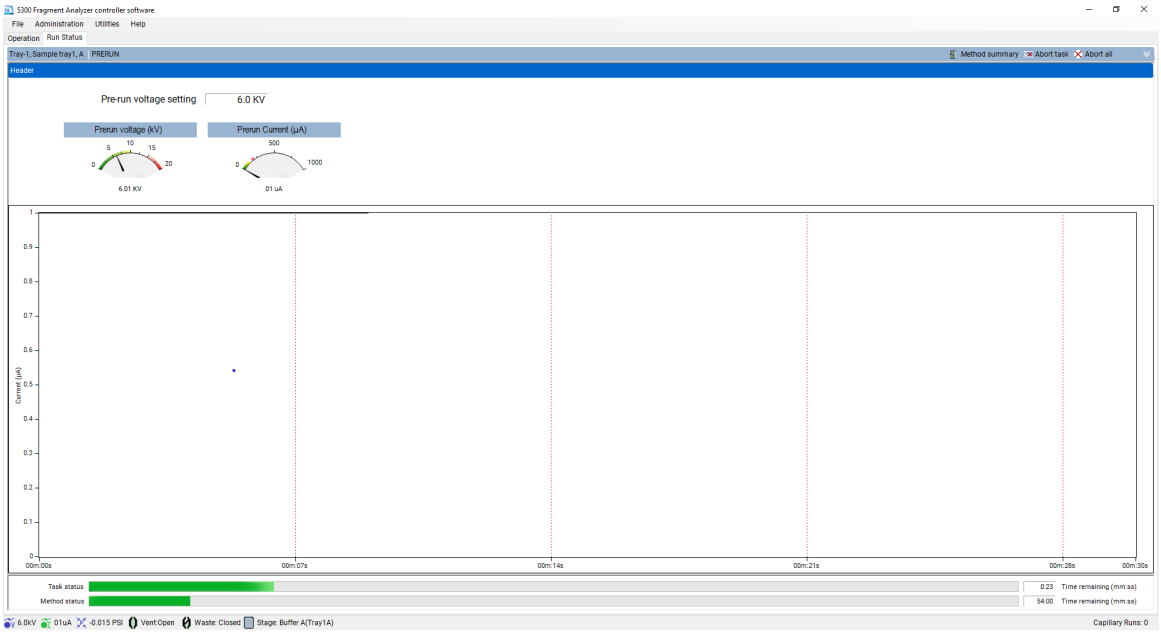


Figure 47 Pre-Run/Injection screen

Real-time Separation View

When the Fragment Analyzer system performs a separation, the screen in **Figure 48** appears, which shows a real-time view of the separation.

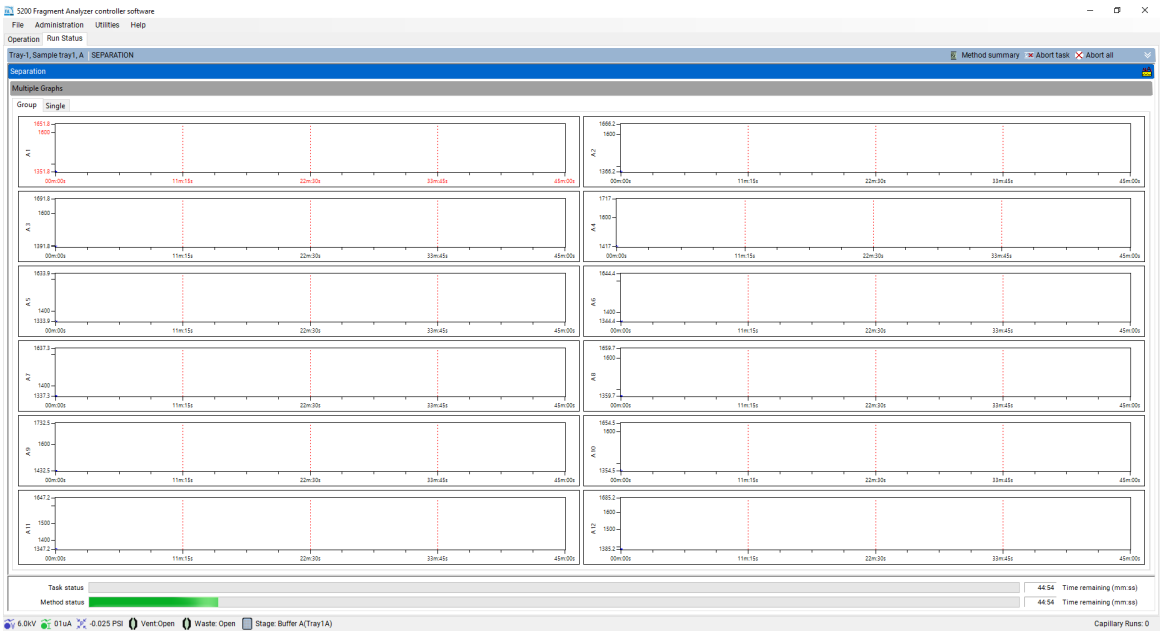


Figure 48 Real-time separation window

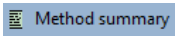
The user can view the run in a group of 12 electropherograms (as shown in **Figure 48**), or view individual electropherograms by selecting the **Single** tab located at the top. When a 96-capillary system is running, the user can select between groups of 12 or single electropherograms by selecting the 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 7**, “Fragment Analyzer Software – Utilities Menu”.

Other options available from the **Run Status** tab are described in **Table 20**.

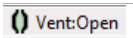
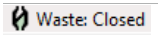
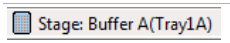
Table 20 Run Status Tab options

Icon	Description
	Opens a popup window showing the method summary for the current method being run.
	Aborts only the individual task being done, i.e., stage movement, pumping, or injection.
	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 21**.

Table 21 Instrument status information

Icon	Description
	Left-clicking on this icon will show the voltage level for the last 5 minutes.
	Left-clicking on this icon will show the current level for the last 5 minutes.
	Left-clicking on this icon will show the pressure level for the last 5 minutes.
	Denotes if the Reservoir vent valve is open or closed.
	Denotes if the Waste valve is open or closed.
	Denotes the location of the stage at that point in time.

10 Fragment Analyzer Capillary Array

Capillary Array Parts 92

Removal of the Capillary Array 93

Unpacking a New Capillary Array 103

Capillary Array Installation 106

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.

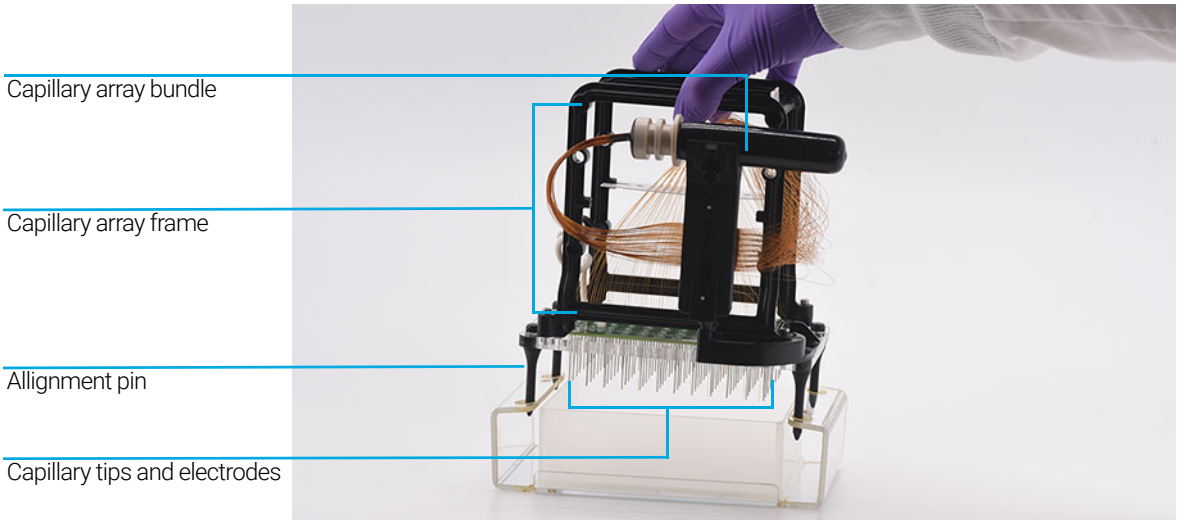


Figure 49 Capillary array parts (96-capillary array shown)

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 50 Fragment Analyzer instrument

WARNING

High voltage

The Fragment Analyzer contains a high voltage supply cable. It is marked by a hazardous voltage sticker. This cable sends electricity to the capillaries during any actions that use high voltage (pre-run, injections, separation). If the top compartment is not shut properly, the high voltage power supply will not be delivering any power to the cable.

✔ **Ensure that the cover is properly shut before operating the instrument.**

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

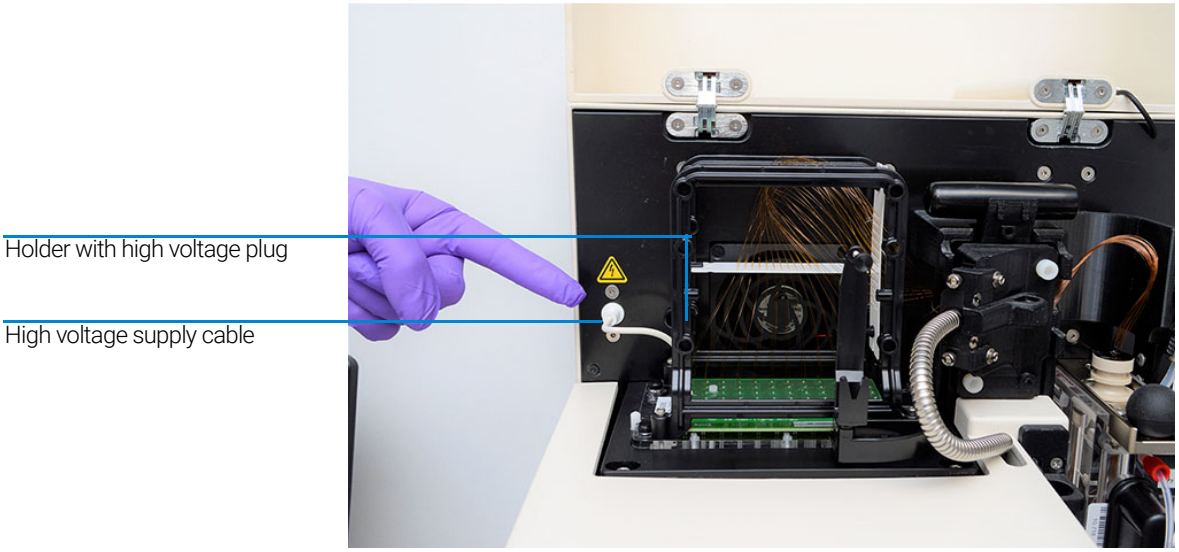


Figure 51 Instrument top compartment – high voltage supply cable

3 Use the provided allen wrench to remove the two white screws that secure the light guide to the array window.

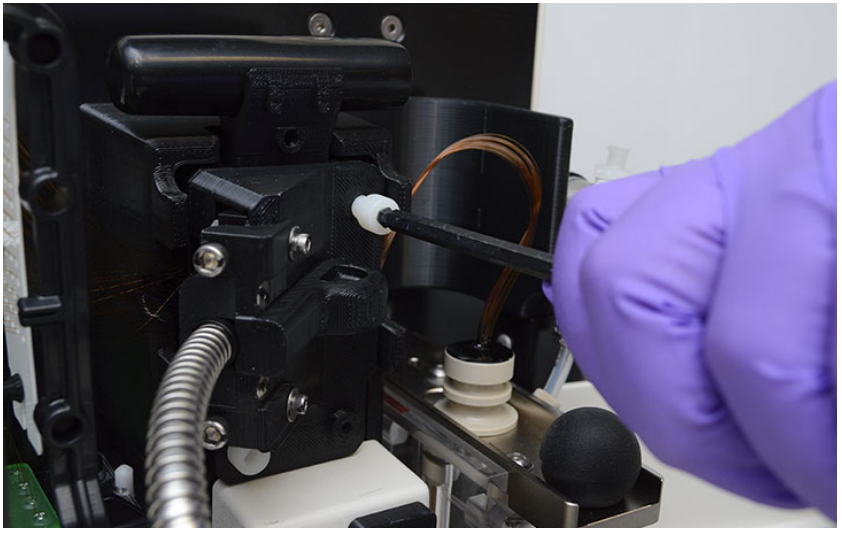


Figure 52 Instrument top compartment – unscrew light guide

4 Remove the light guide.

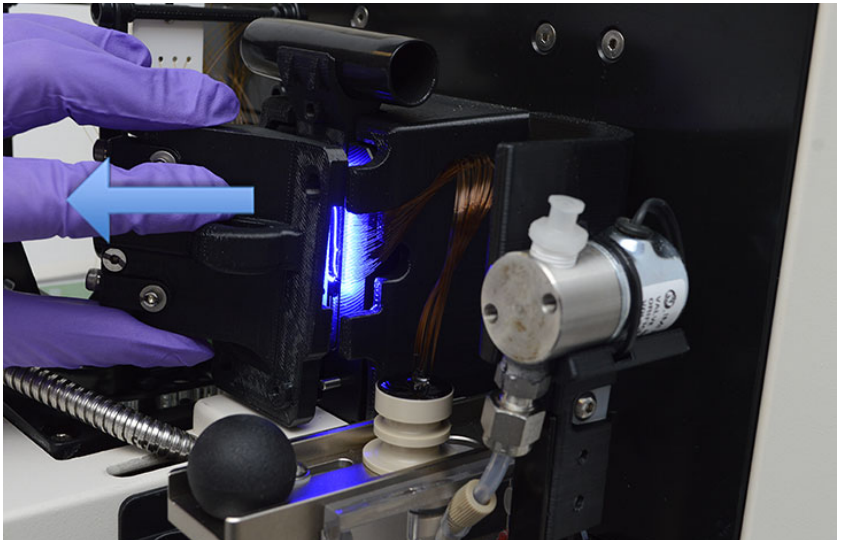


Figure 53 Instrument top compartment – light guide removal

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 54 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.

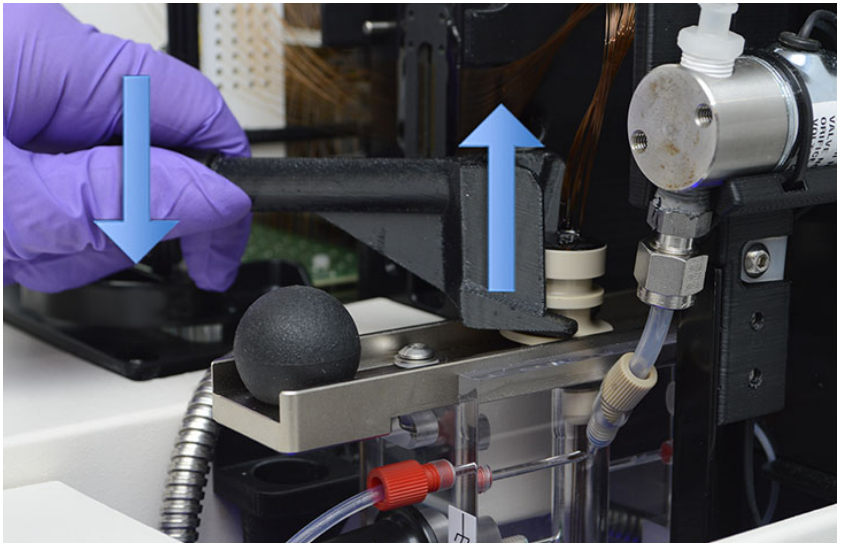


Figure 55 Instrument top compartment – capillary reservoir connector tool

7 Remove the capillary array bundle by pulling up gently.

NOTE

Avoid pulling up hard as to not break any capillaries.

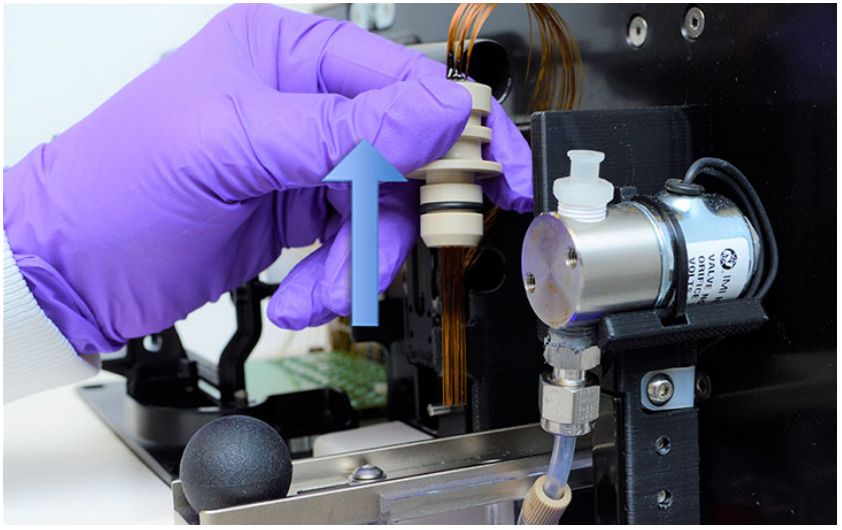


Figure 56 Instrument top compartment – capillary array bundle removal

8 Carefully insert the protective cover over the capillary bundle.

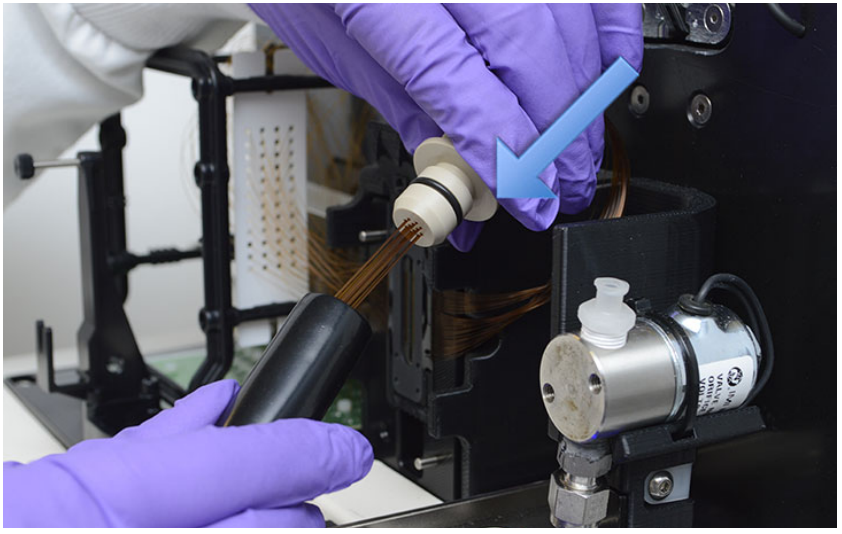


Figure 57 Instrument top compartment – installing protective cover

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

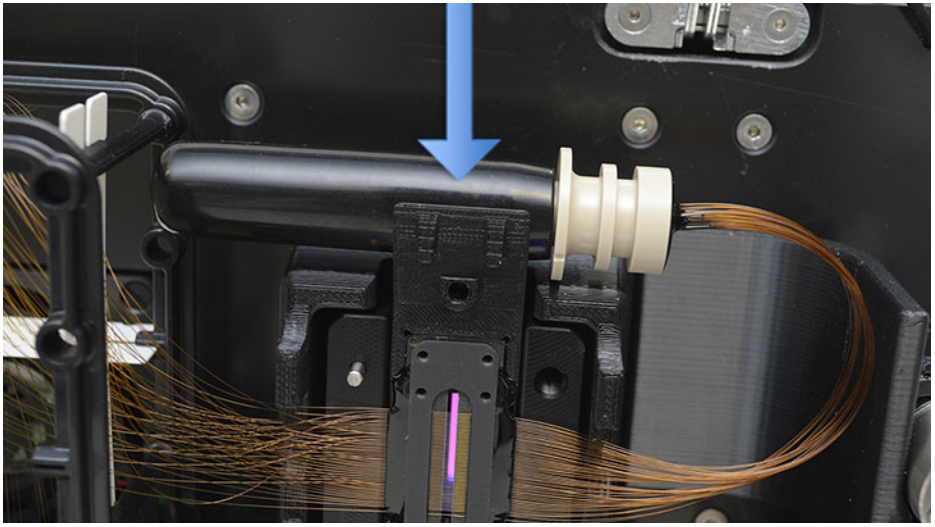


Figure 58 Instrument top compartment – storing covered capillary array bundle

CAUTION

Excessive force

Capillary breakage can occur when removing or installing the capillary array window.

✔ **Take extra care not to use too much force when handling the capillary array window.**

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.

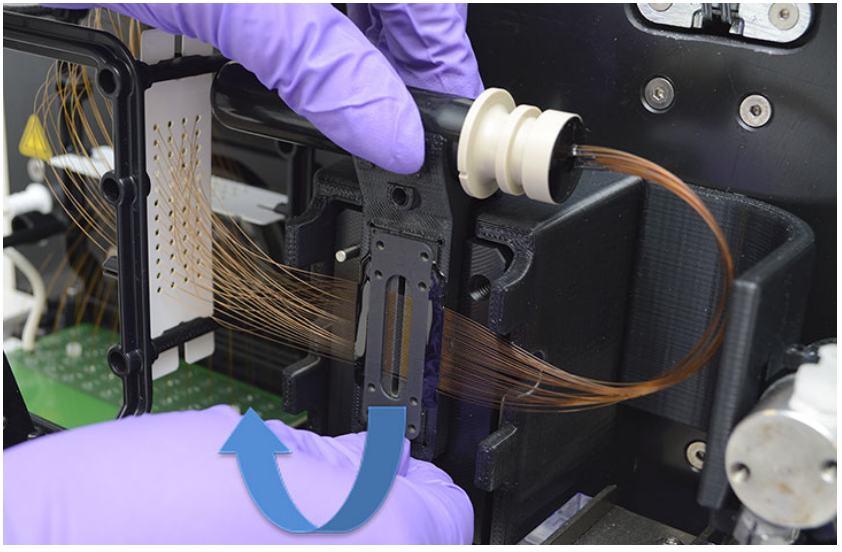


Figure 59 Instrument top compartment – remove capillary array window

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

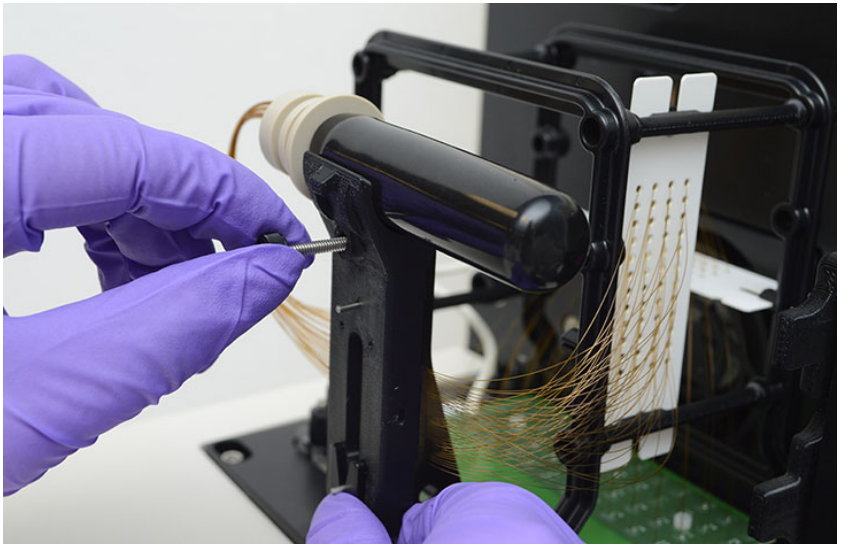


Figure 60 Instrument top compartment – attach array window to capillary array frame

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

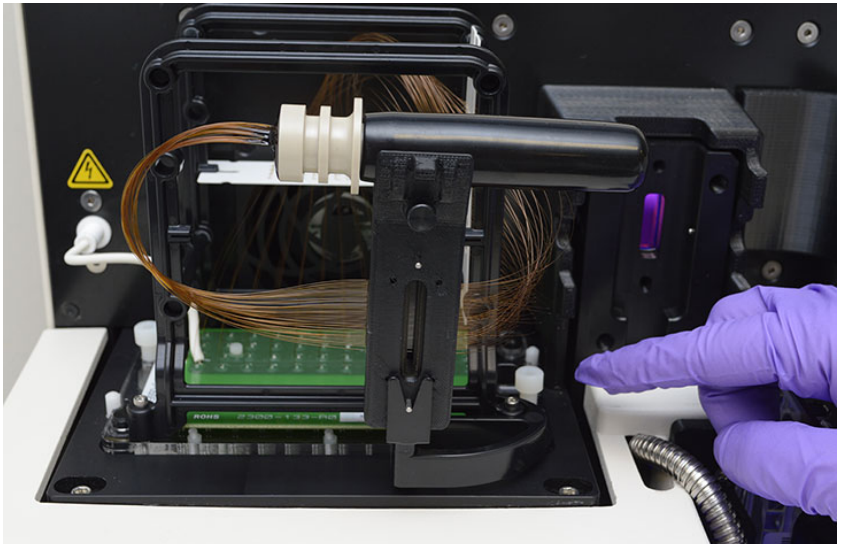


Figure 61 Instrument top compartment – array attachment screw removal

13 Carefully lift the array straight up to remove it from the Fragment Analyzer instrument.

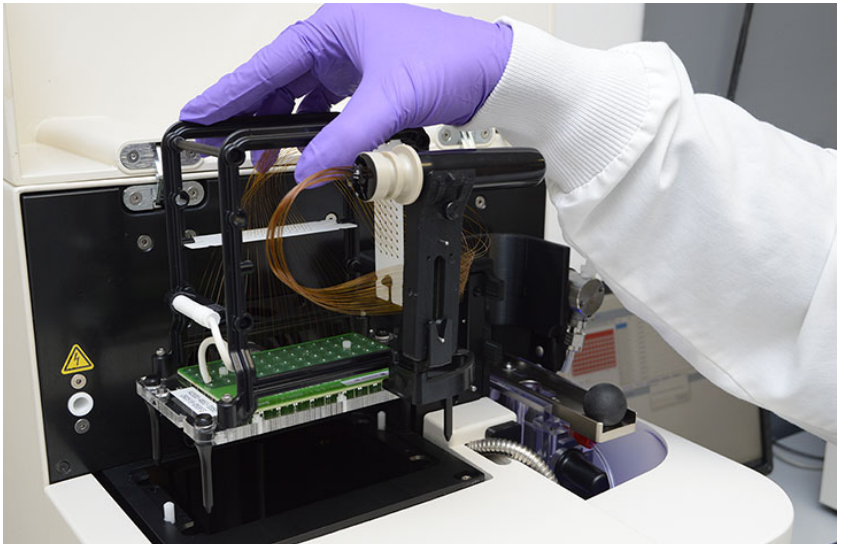


Figure 62 Instrument top compartment – capillary array removal

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 150).

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 63 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.

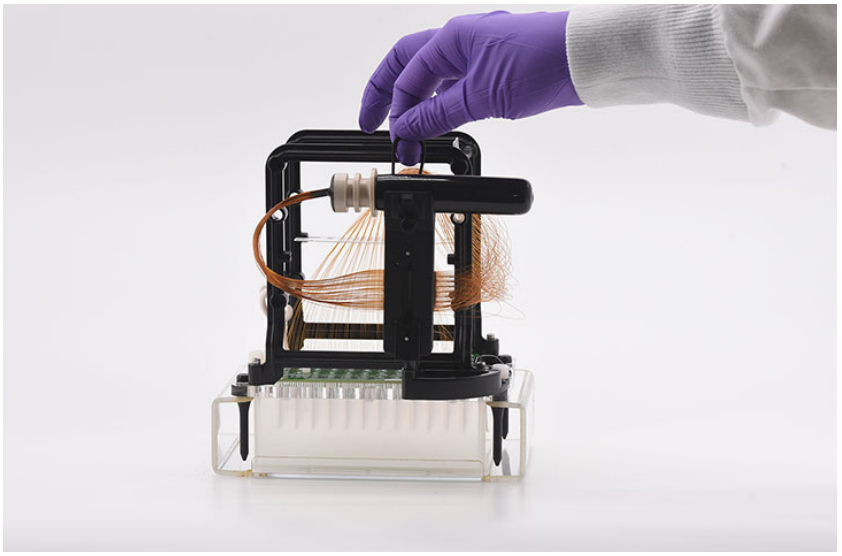


Figure 64 Remove capillary array bundle-securing rubber band.

3 Remove the two white nylon screws that secure the array to the shipment frame.

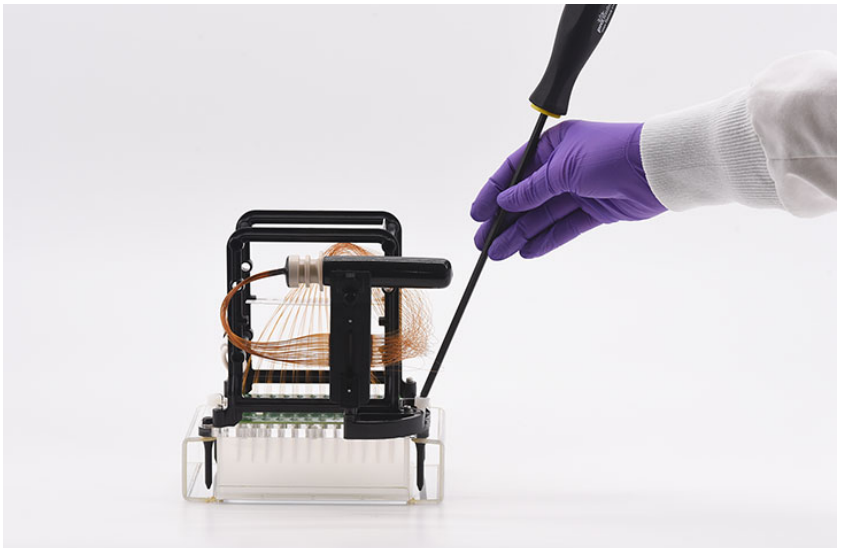


Figure 65 Capillary array shipping box

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

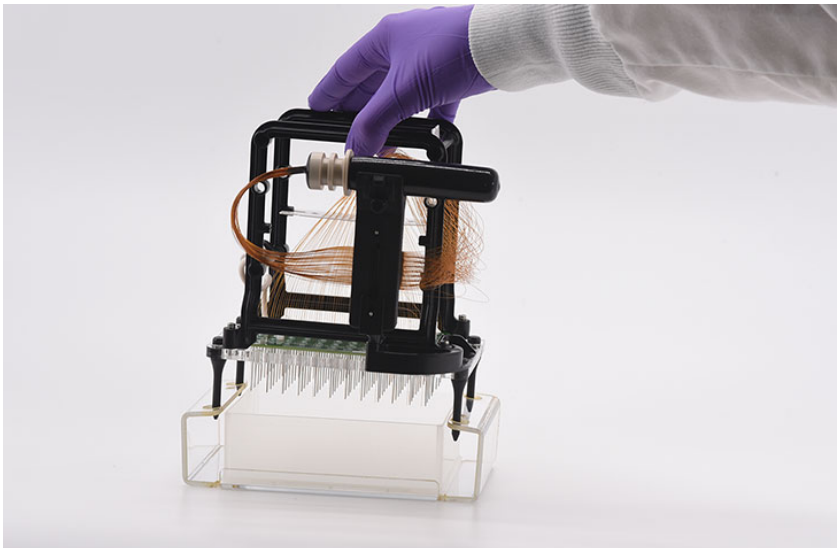


Figure 66 Capillary array shipping box – removal of array from shipment frame

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:

First, open the reagent door to the side.

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

Figure 67 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.

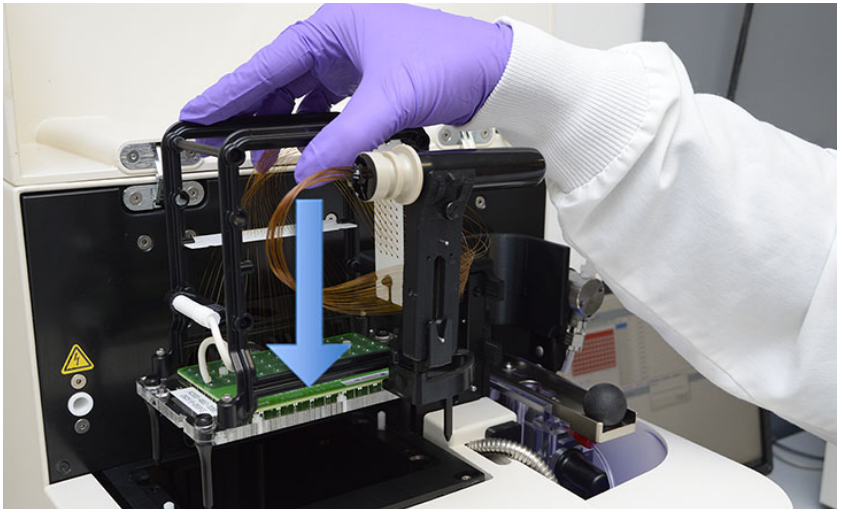


Figure 68 Instrument top compartment - capillary array installation: a 48 capillary is used in the example

3 Use the provided allen wrench to install the two white screws holding the capillary array in place.

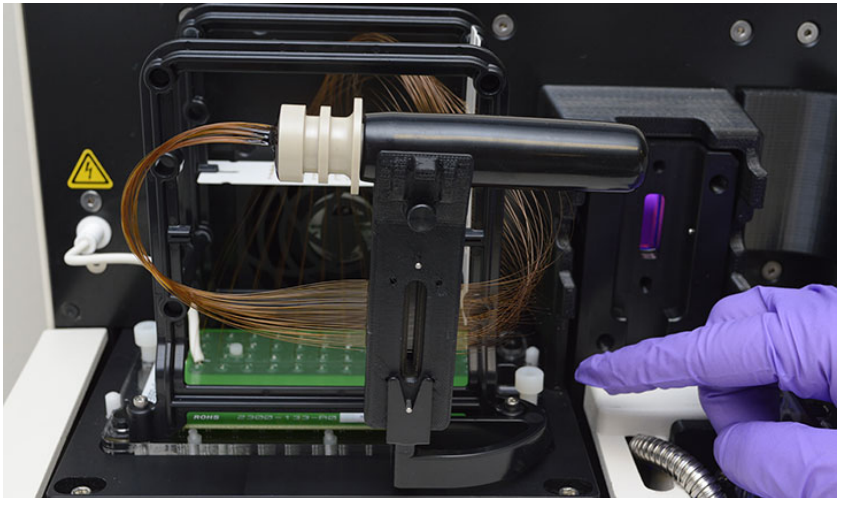


Figure 69 Instrument top compartment - array attachment screw installation, picture indicates one of these screws

4 Remove the array window attachment screw.

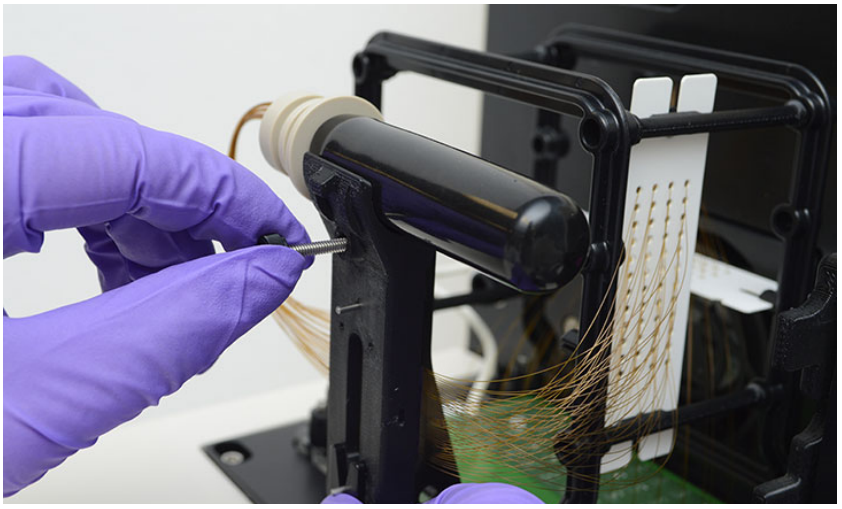


Figure 70 Instrument top compartment – remove array window

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.

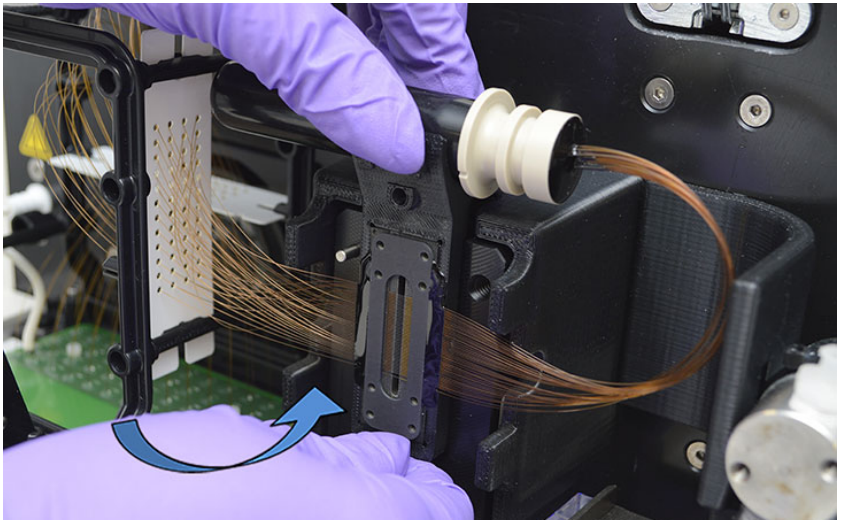


Figure 71 Instrument top compartment – array window installation

6 Remove the capillary array bundle from the top holder of the capillary array window.

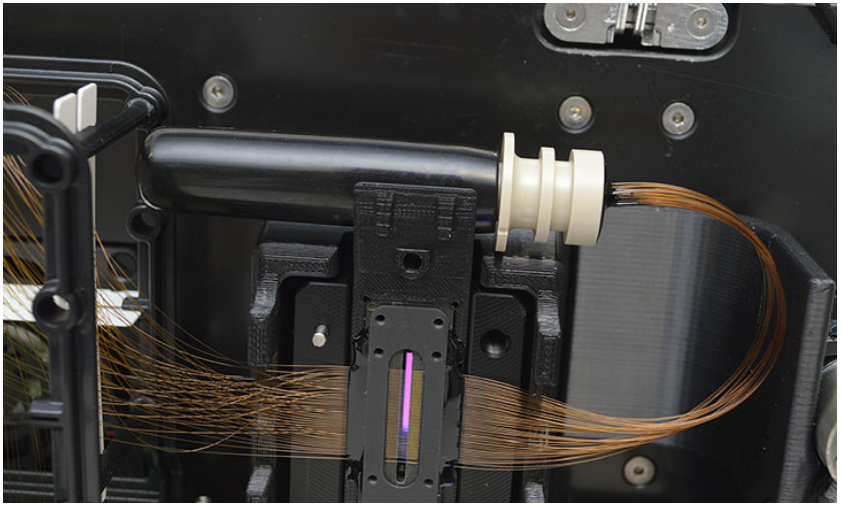


Figure 72 Instrument top compartment – capillary array window installed, and bundle shown here

7 Carefully remove the protective cover from the capillary bundle and place it back on the holder on top of the window.

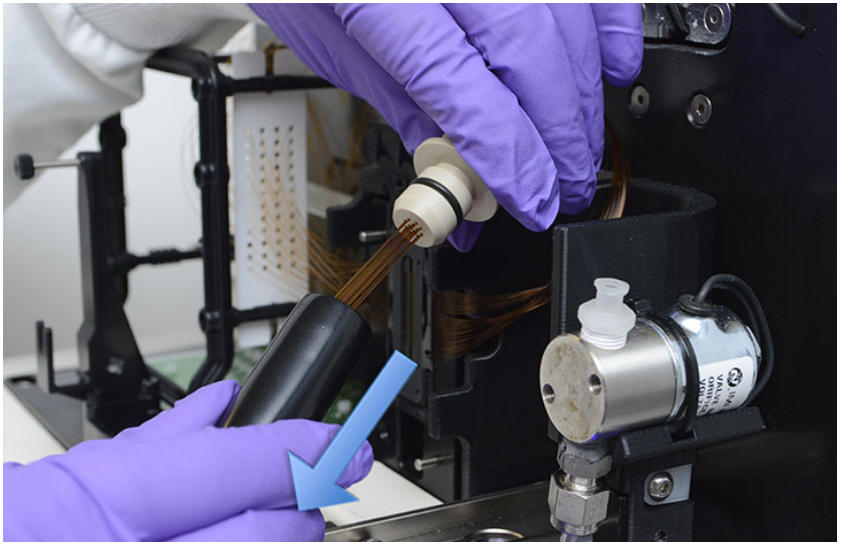


Figure 73 Instrument top compartment – removing protective cover

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

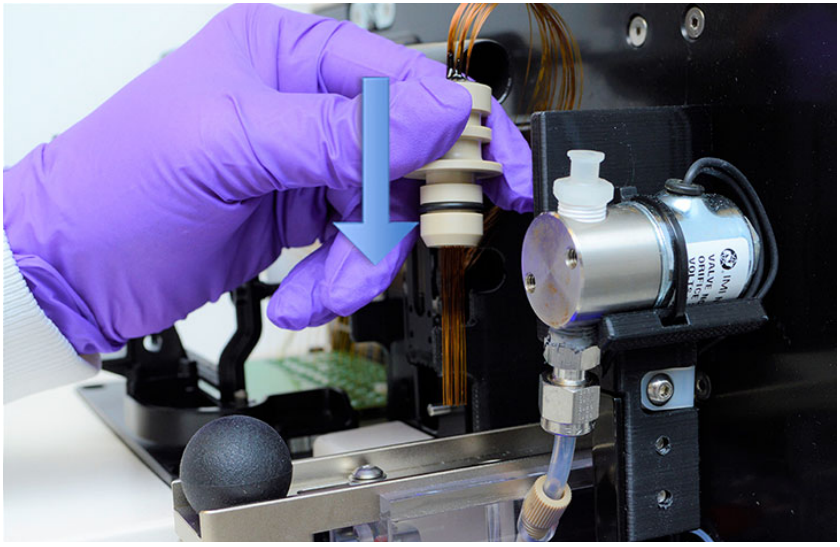


Figure 74 Instrument top compartment – capillary array bundle installation

CAUTION

Capillary array bundle improperly secured

If the capillary array bundle is unsecured, it will be damaged upon pressurization.

✔ **Check that the capillary array bundle is secured.**

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

Figure 75 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.

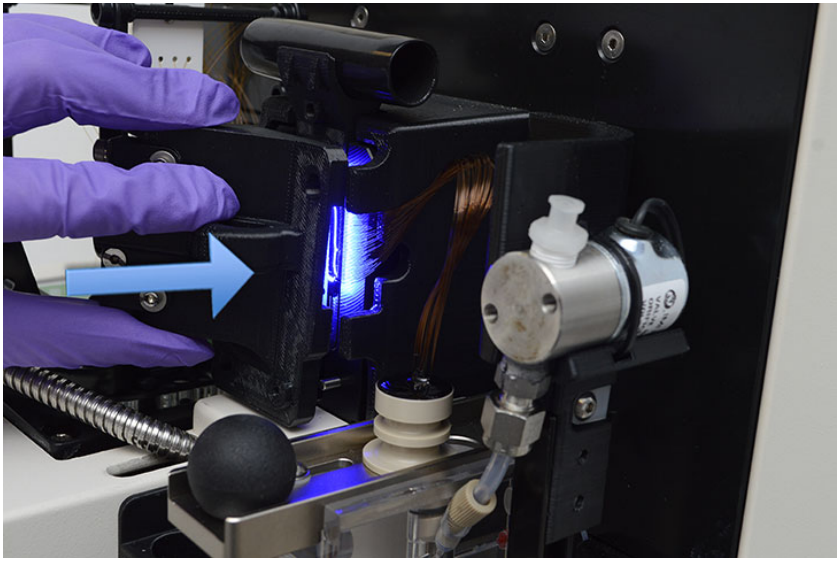


Figure 76 Instrument top compartment – light guide placement

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

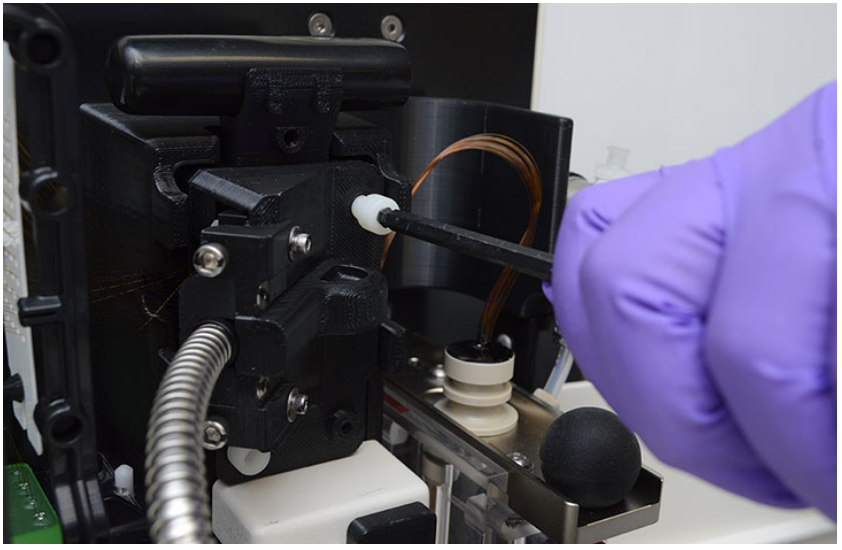


Figure 77 Instrument top compartment – light guide installation

CAUTION

Loss of time and reagents

If the capillary high voltage supply cable is disconnected from the instrument, the conditioning and gel steps will still be completed before the software aborts the method and signals an error.

✔ **Ensure that the high voltage supply cable is connected before starting a method.**

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

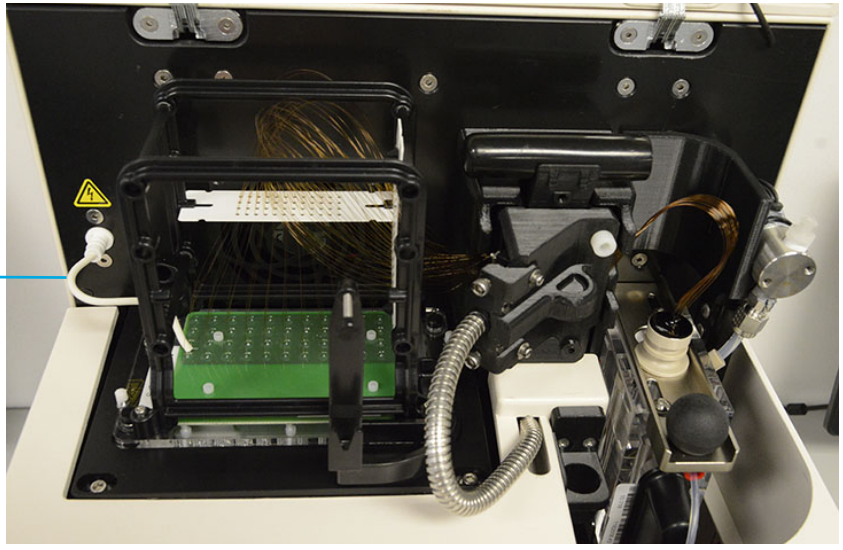


Figure 78 Instrument top compartment –fully installed array 48 capillary array shown here

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 79 Fragment Analyzer instrument

After installation of an array, the Fragment Analyzer will require a capillary alignment as described in **Chapter 6**, “Fragment Analyzer Software – Utilities Menu”.

11 Fragment Analyzer – Sample Name Entry

Sample Name Entry 117
Entering Sample Names Manually 117
Importing Sample Names 118
Importing Sample Names Using a Bar-Code Reader 120

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 80**).

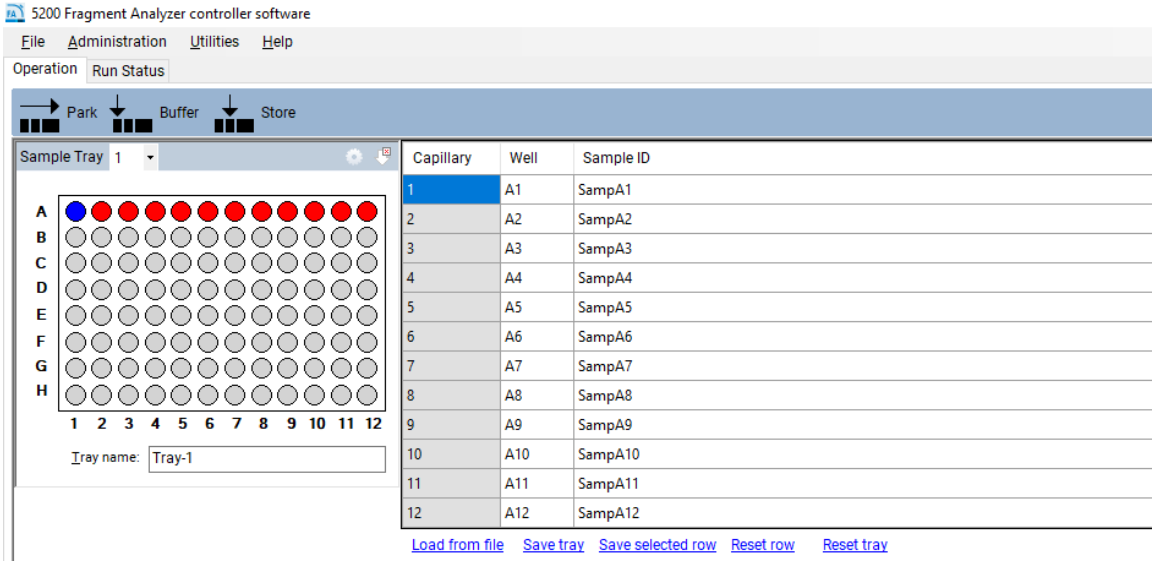


Figure 80 Adding samples names manually

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 81**).

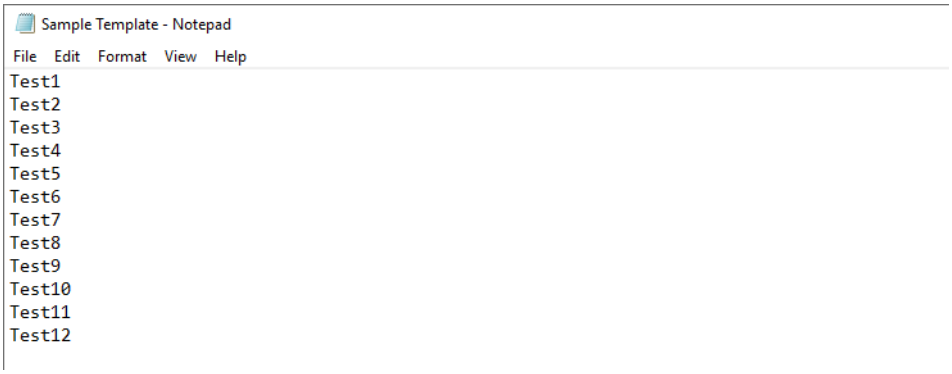
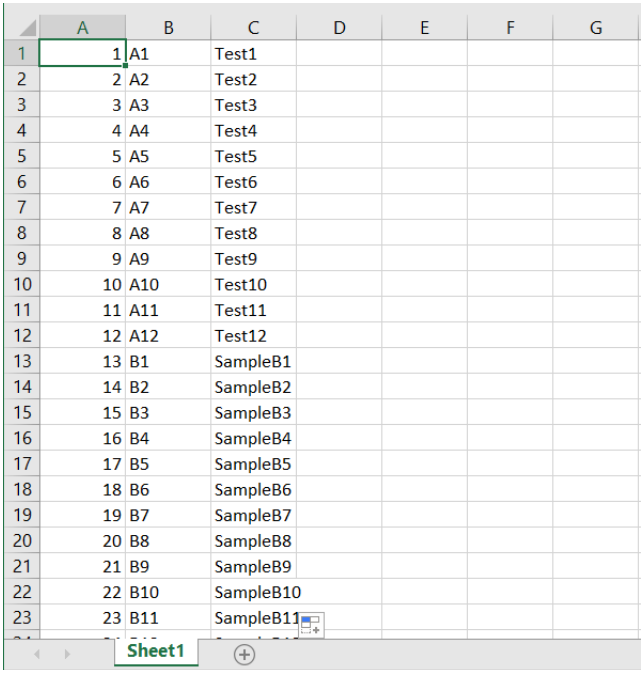


Figure 81 .txt file format (single row of names—no well numbers or row numbers).

- For a .csv file, the column format is row number, well number, and sample name (**Figure 82**).



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

Sheet1

Figure 82 .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 83**). 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 118).

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

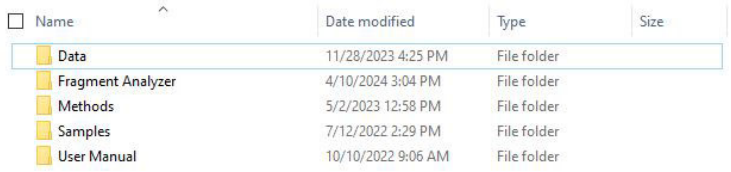


Figure 83 Samples folder

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

Example:

In **Figure 84**, the name associated with the bar-code is 00060065.

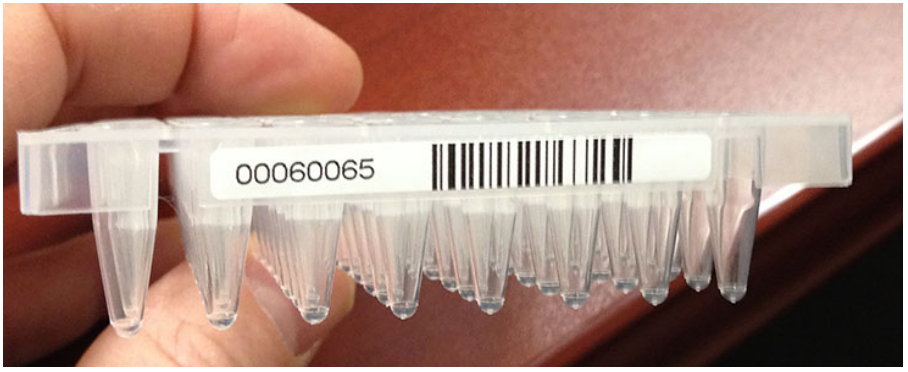


Figure 84 Bar-code name 00060065

Thus, the .csv file or .txt file must be given the file name *00060065* and located in the *Samples* folder (**Figure 85**).

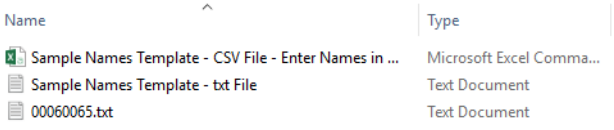


Figure 85 File name

2 In the field **Tray name** of the **Operation** tab, highlight the tray name with the mouse cursor (**Figure 86**).

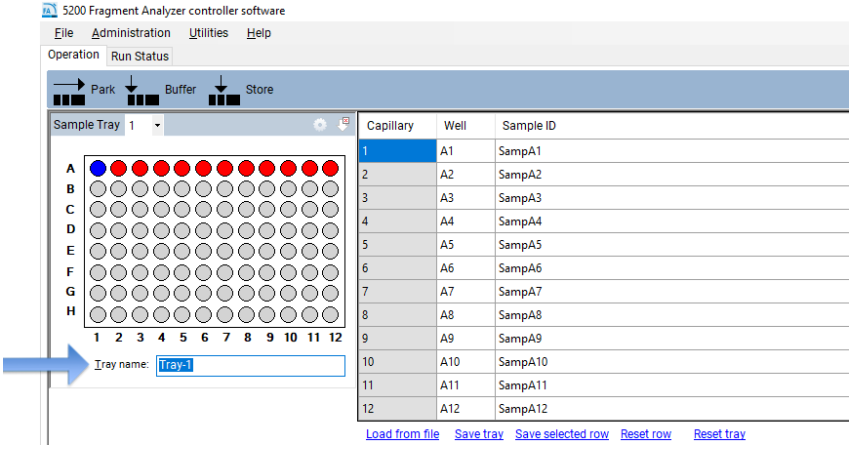


Figure 86 Highlight of the tray name

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 87**).

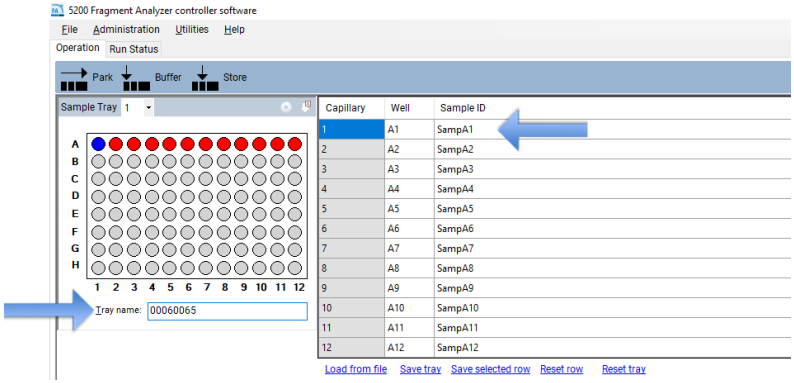


Figure 87 Imported sample names

12 Fragment Analyzer – Automated Analysis

Automated Analysis Using the Fragment Analyzer 124
Enabling Automated Analysis 125
Monitoring the Status of the Automated Processed Data 129

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 11**, "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 **Administrator** drop-down menu, select **Results Report Setup** (**Figure 88**).

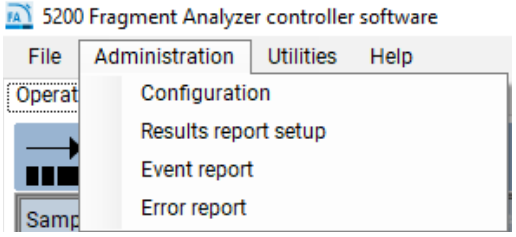


Figure 88 Admin menu

This will open the **Automated Report Settings** window (**Figure 89**).

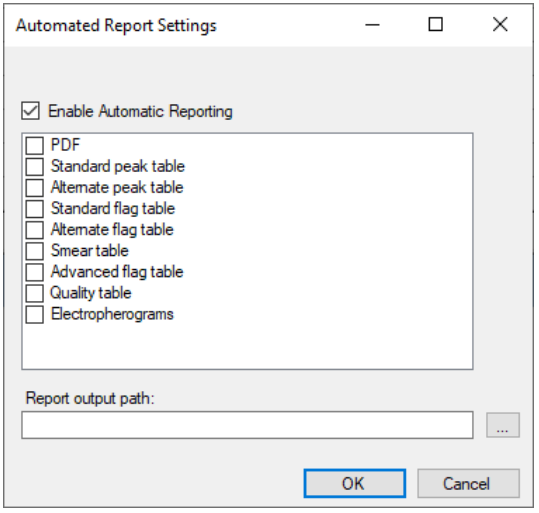


Figure 89 Automated Report Settings window

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 5.000 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 90**).

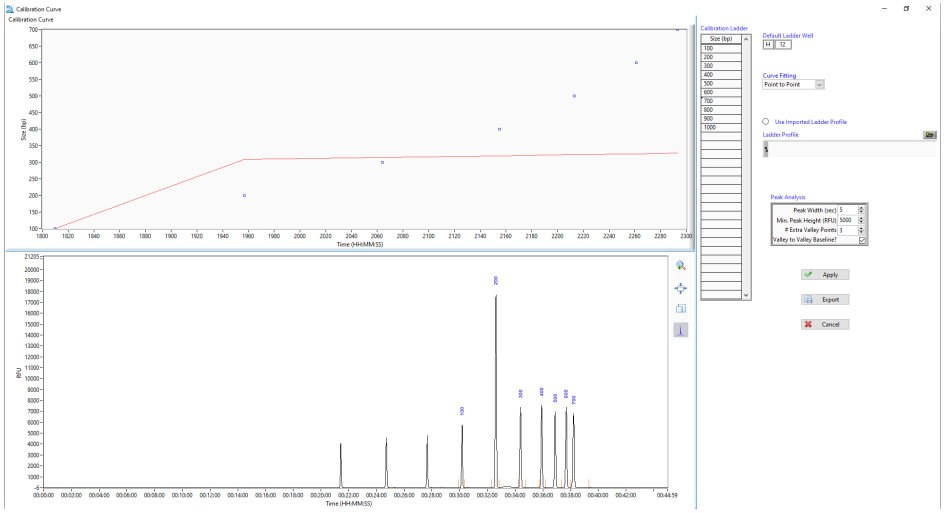


Figure 90 ProSize calibration curve setup

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 91**).

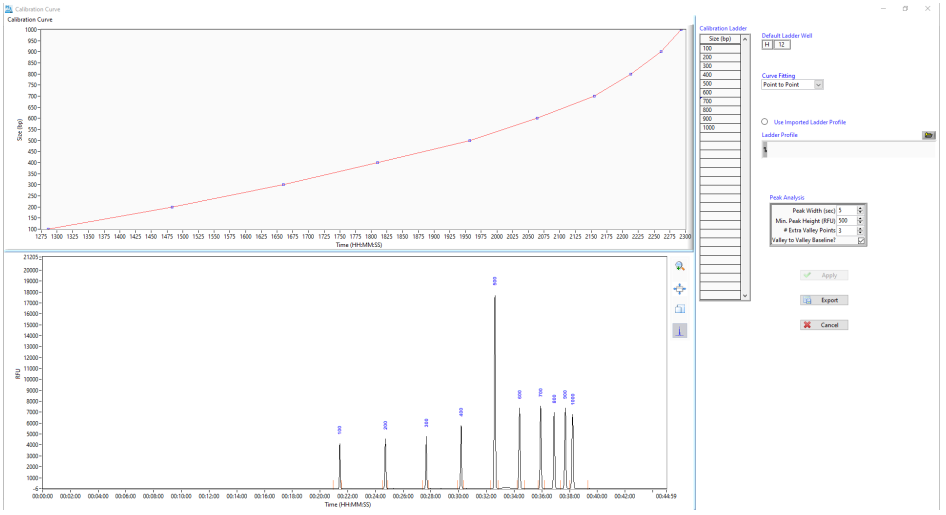


Figure 91 ProSize calibration curve setup

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 5.000 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 92).

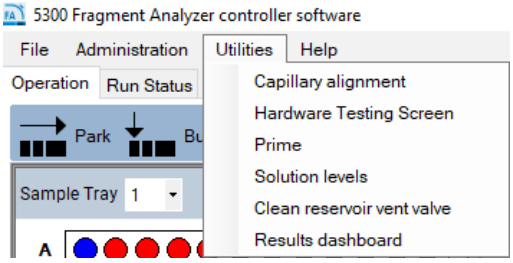


Figure 92 Utilities menu

The **Results Dashboard** window opens. The data files are listed (Figure 93).

2 Right-click on a file.

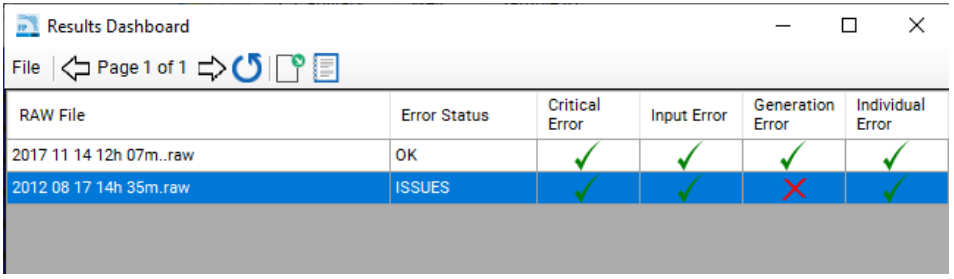


Figure 93 Results Dashboard

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 22.

Table 22 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 94**.

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

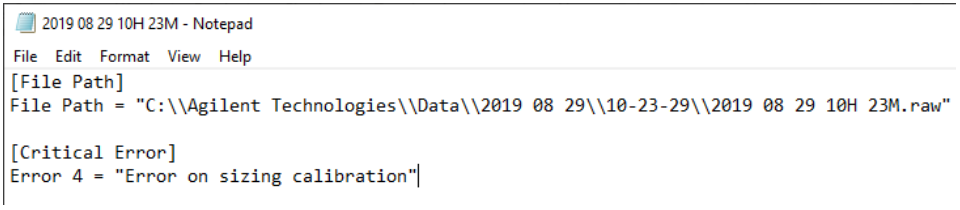


Figure 94 Example error message

13 Maintenance and Troubleshooting

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

NOTE

If you require assistance, contact your local Agilent support representative.

Compatible Plates and Tubes for Fragment Analyzer Systems

NOTE

The plates listed in this section were confirmed compatible when this manual revision was completed. Plate manufactures may make changes, causing incompatibility.

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

The 5200 and 5300 Fragment Analyzer systems operate using specific dimensioned Eppendorf 96-Well twin.tec PCR plates, semi-skirted (Eppendorf #951020303 (various colors)).

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.

CAUTION

Wrong plate dimensions

The Fragment Analyzer requires plates of the following dimensions: 123.7 × 82.2 × 19.7 mm (length × width × height). Semi-skirt platform – 9.1 mm.

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

- ✔ **Use only plates with the correct dimensions.**
- ✔ **If you use plates outside the approved plates listed above, make sure to qualify them.**

0.2 mL Tube Strip – 5200 and 5300 only

The 5200 and 5300 Fragment Analyzer systems have been designed to work with specific strip tubes when using the F1300-107 Tray Carrier – 12 Vial Strip (sold separately). A list of approved tube strips is provided in **Table 23**.

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 23 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 24**).

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 24 List of Buffer/Waste Plate

Item	Vendor / Part #	Description
Buffer/ Waste Deep 96-Well Plates	Fisher Scientific #12-566-120	Fisherbrand 96-Well DeepWell Polypropylene Microplates: Well Capacity 1 mL

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: **F1350-001 – Full Skirt Tray Carrier.**

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**).

If you use the 5200 or 5300, you can purchase full-skirt plate adapters to replace the default semi-skirted adapters that come with the instrument. If you do so, you need to purchase the full set so the instrument only uses one type or the other. The adapter part number is: **M1300-109 – DRAWER ADAPTER-FULL SKIRT 96 WELLPLATES**.

Supported full-skirted plate:

Eppendorf twin.tec 96 Well LoBind PCR plates, skirted (Fisher Scientific #E0030129512)

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 reservoir vent valve for dried gel, clean if necessary.

As Needed to Restore Separation Performance

- ✔ Place 0.6 mL of 0.5N NaOH into each well of a Deep Well plate (Row A for 12 capillaries, Rows A – D for 48 capillaries, all rows for 96 capillaries). Place this plate in the waste tray location and flush the capillary array cartridge with 0.5N NaOH followed by capillary conditioning solution as described in section **"Capillary Array Cleaning"** on page 137.**

* 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

The capillary array may require troubleshooting at times. This troubleshooting can cover several issues including physical clogs, delayed migrations (conditioning deficiencies), and contamination

There are typically four procedures to clean/flush a capillary array to improve performance due to the above mentioned issues.

NOTE

Most clogging will be caused by dried reagents on the capillary tips on the plate side. The tip soaks listed below are the best approved methods to remove these blockages

- Method A: Flow check with CE grade water
- Method B: Tip soak – Submerse capillary array tips/electrodes in hot water (150 °F – 200 °F)
- Method C: 0.5N NaOH flush and soak to clean the capillary tips, electrodes, and capillary walls
- Extended conditioning flush: typically 10 – 20 minute flush of conditioning solution to help coat the capillary walls and facilitate even migrations (this is not a pre-made method in the FA software).

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

Method A – Flow Check 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 95**.

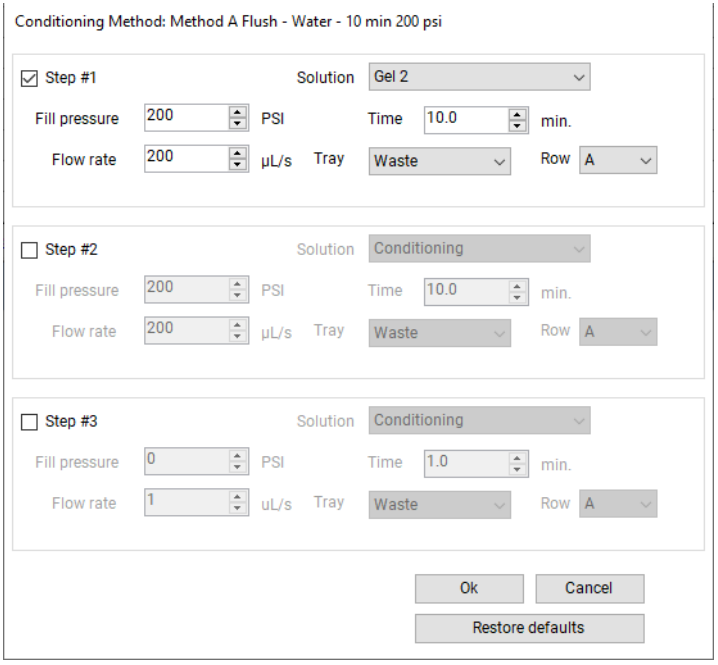

Figure 95 Conditioning parameter **Method A Flush – Water – 10 min 200 psi.mthdc**

4 If necessary, adjust the method to that shown in the **Figure 95** (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
   - ≥ 27 mL for a 48-capillary array
   - ≥ 43 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 a well has significantly less or no water present in a well, it is recommended to proceed to Method B or C.

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

1 Remove the 96-well deepwell plate from the waste drawer and replace it with the open waste trough.

2 Open the side compartment of the Fragment Analyzer instrument and ensure there is conditioning solution and separation gel present to perform a full conditioning method.

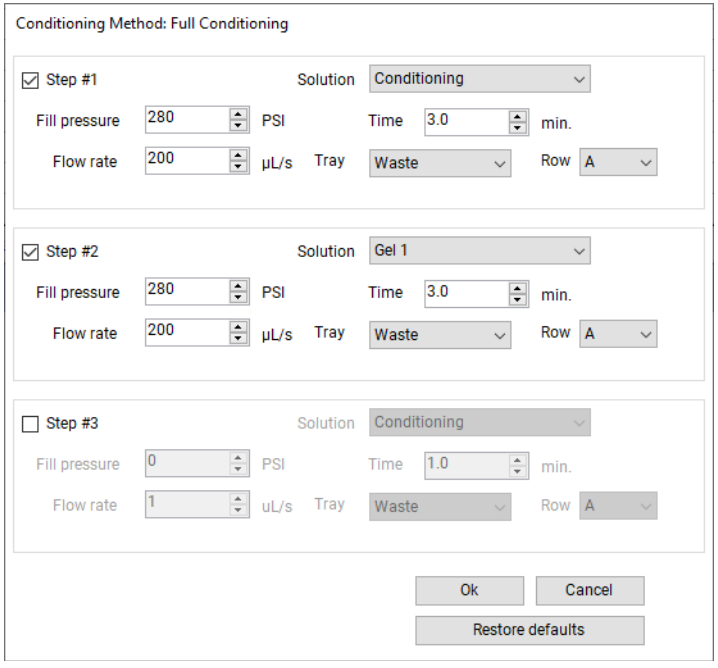

Figure 96 Conditioning method editor

3 Perform a separation run to confirm all capillaries are providing signal.

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.**

NOTE

The Method C cleaning protocol serves several purposes including the removal of clogs, decontamination, and resetting/cleaning of the capillary walls. It is important that the NaOH solution is not in contact with the capillaries for extended periods of time as this can lead to damage to the capillary itself. For this reason, the pre-made Method C flush in the software includes a conditioning flush to follow the NaOH step.

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. The NaOH tip soak portion of this cleaning procedure is very important for removing contamination that is present on the capillary tips – notably RNase contamination which may be present if the user is running both RNA and DNA kits.

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 that are listed below are only for the first step of the Method C flush (a 10-minute pressurization with the NaOH in the Gel 2 line.) If performing the NaOH priming step of the liquid lines, add 2.5 mL for each prime enabled:

   - ≥ 12 mL for a 12-capillary array
   - ≥ 27 mL for a 48-capillary array
   - ≥ 43 mL for a 96-capillary array

NOTE

If there are no contamination concerns and you are using method C to help restore capillary performance, you can skip the priming steps 3 and 4, and proceed immediately with step 5.

3 From the **Utilities** menu, select **Prime**. Select the check boxes next to the lines that are to be primed. For decontamination, we recommend priming all three liquid lines. For any lines that need to be primed with NaOH, you can place each of the lines in single 0.5 N NaOH bottle without screwing the caps on. This will be easier than filling three separate bottles with NaOH.

4 Select **OK** to prime the chosen liquid lines with NaOH.

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

The minimum solution volumes required are only for the second step of the Method C flush (a 10-minute pressurization with the conditioning solution in the cond. line.) Ensure that at least 2.5 mL extra conditioning solution is added to this line if performing a priming step prior to the flush.

Minimum solution volumes:

- ≥ 12 mL for a 12-capillary array
- ≥ 27 mL for a 48-capillary array
- ≥ 43 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 97**.

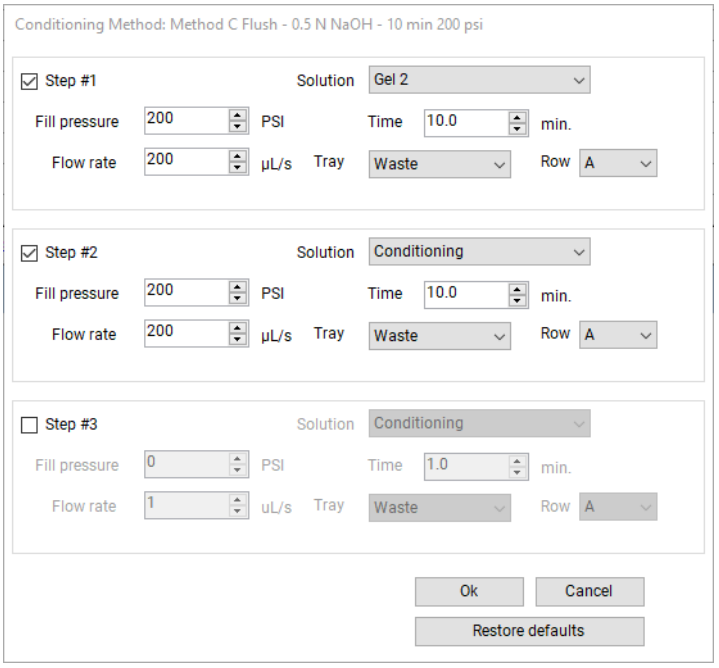

Figure 97 Conditioning parameter **Method C Flush - 0.5 N NaOH - 10 min 200 psi.mthdc**

9 If necessary, adjust the method to that shown in **Figure 97** (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.

NOTE

The NaOH tip soak portion of this cleaning procedure is very important for removing contamination that is present on the capillary tips – notably RNase contamination which may be present if the user is running both RNA and DNA kits.

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.

✔ **Once your cleaning protocols are finished, perform a Full Conditioning flush (with gel as last step) or proceed to the next separation method. Separation gel is the best reagent to be present within the capillaries for long durations.**

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.

Long Conditioning Flush

If there is a delay in migration or smeared migrations, perform a long conditioning flush of 10 – 20 minutes in addition to the Method C flush.

This is not a preset method, but it can be added manually.

1. Go to the **Capilllary Array > Conditioning** *Edit Method* menu.
2. Place a sufficient volume of *Conditioning Solution* in the conditioning solution position.
3. Ensure an empty trough is in the *Waste* position.
4. Add the method with only conditioning solution for 10 – 20 minutes to the queue.
5. Run the method.

NOTE

This process may change the default settings of the conditioning method being edited (Default Conditioning, Method A, etc.).

If the original settings are unknown, click **Restore Defaults** before using these conditioning methods the next time.

Reservoir Vent Valve Cleaning

Over time the reservoir vent valve may become clogged and require 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 98**).

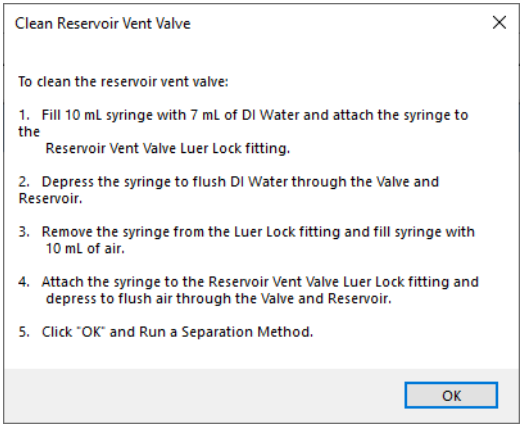

Figure 98 Clean Reservoir Vent Valve window.

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 99**.

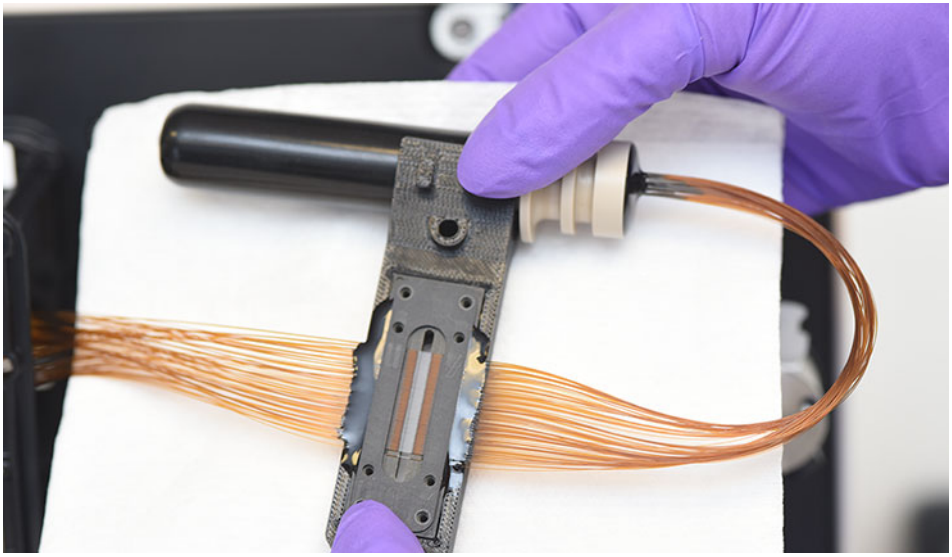

Figure 99 Capillary array window with paper towel behind

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 two weeks without use. There are two methods for storing a capillary array for long term.

- Leave the capillary array installed in the instrument (recommended in most cases).

  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 Fragment Analyzer instruments as part of the instrument accessory kit.

Using the array docking station

1. Remove the capillary array from the instrument. For detailed instructions, refer to **Chapter 10**, “Fragment Analyzer Capillary Array”.
2. Place the tray base inside the array docking station as shown in **Figure 100**.

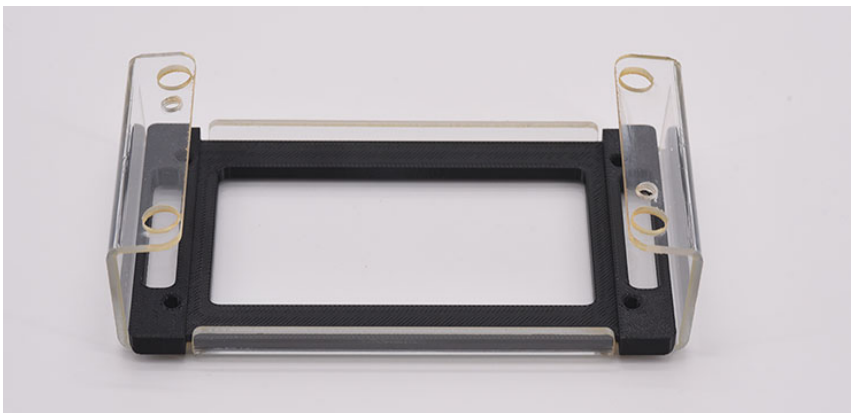

Figure 100 Array docking station with tray base installed

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 101**).

- 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 101 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 102** to tighten the capillary array into place.

Figure 102 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 103 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 104**.

To see a full image of the array with the array spindle storage device installed, refer to **Figure 105**.

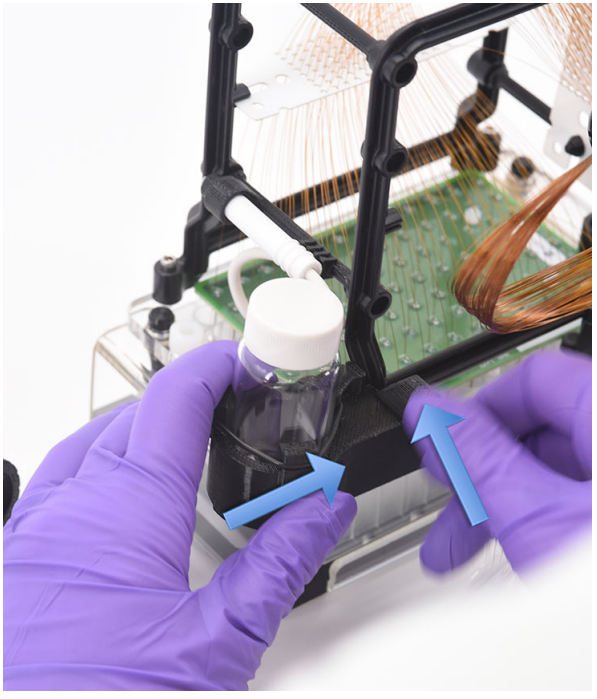

Figure 104 Array spindle storage device installation

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 105**.

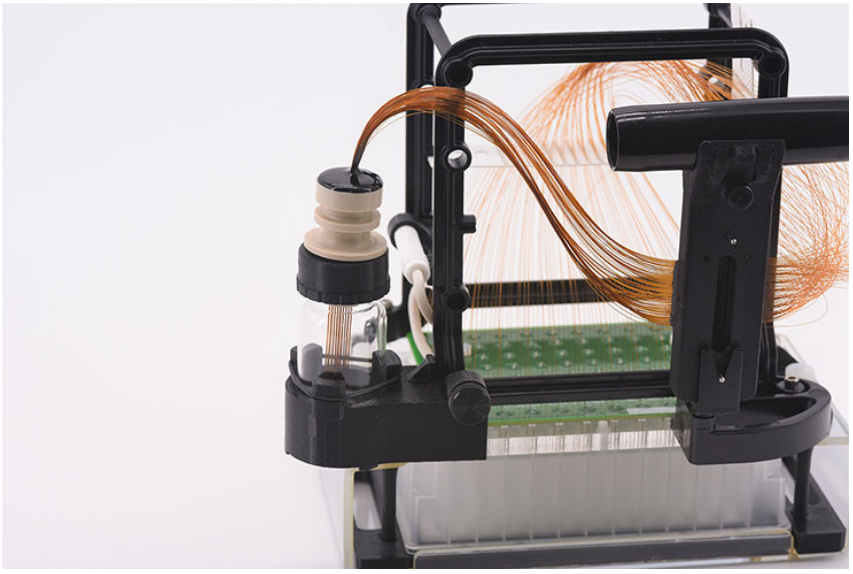

Figure 105 Array docking station with array installed

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.

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NOTE

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

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.

NOTE

The information given in **Maintenance and Troubleshooting** section also applies to this subset of the Fragment Analyzer.

Drawer Assignments

The 5400 Fragment Analyzer system has specific drawer assignments, as shown below in **Figure 106**.

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)

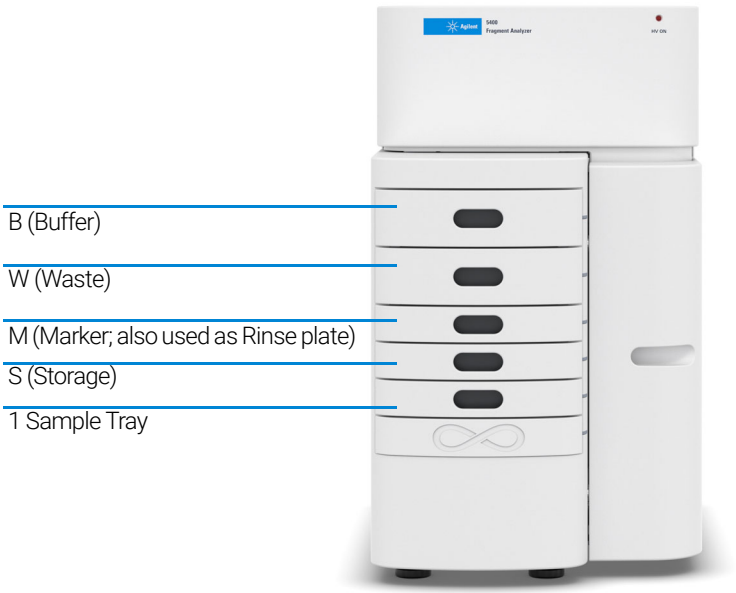

Figure 106 Entry points of the Fragment Analyzer system

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 25 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 26 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 27 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

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 µL/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 µL/well of Capillary Storage Solution, Agilent Part # GP-440-0100. 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 107**).

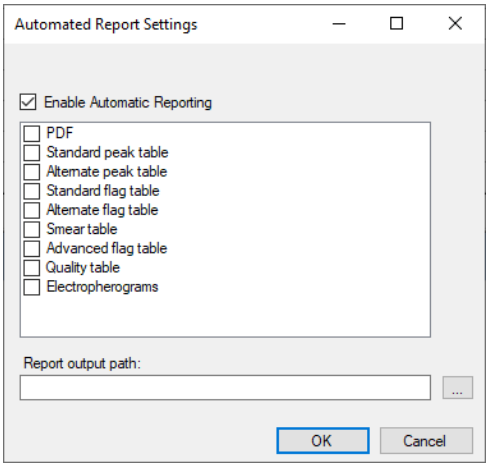

Figure 107 5400 Fragment Analyzer Automated Report Settings window

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 12**, “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 Open the Fragment Analyzer software.

2 Navigate to **Configuration > Device Settings** in the **Administrator** drop-down menu.

3 Ensure that the instrument type listed is the 5400 Fragment Analyzer. If necessary, change to this type and confirm that the serial number matches the instrument sticker.

4 If you changed the instrument type:

a Save any changes.

b Close and re-open the software.

5 From the **Administration** menu, select **Configuration**.

The **Configuration Settings** window will open (**Figure 108**).

6 Select the **Automation** tab.

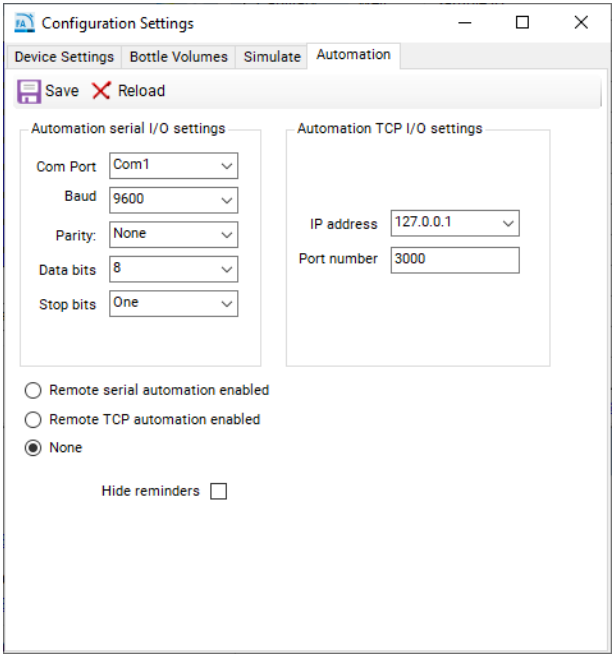

Figure 108 5400 Fragment Analyzer Automation configuration menu

7 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 161, and shown in **Figure 108**.

8 Select the appropriate Com port (usually Com1).

NOTE

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)

9 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.

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

NOTE

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).

11 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 28**.

Table 28 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 11**, "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.
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**.

Command	Function	Example
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 29**.

Table 29 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.
!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. Open the Fragment Analyzer controller software and ensure the instrument type is listed as 5400 Fragment Analyzer.
2. From the **Administration** menu, select **Configuration**.

   The **Configuration Settings** window will open.
3. Select the **Simulate** tab (**Figure 109**).

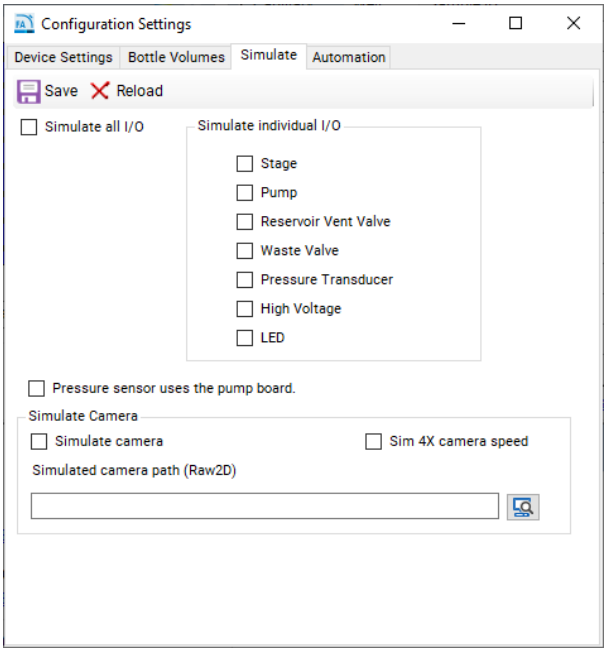

Figure 109 5400 Fragment Analyzer Simulate configuration menu

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 109**, 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 28.

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** Command 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 Agilent sales/service representative.

NOTE

When working on integration with a robotics system, contact your Agilent support representative for the 5400 Fragment Analyzer drawer dimensions.

NOTE

Robotics integration is the sole responsibility of the customer and any 3rd party automation group contracted through the customer. For more information, contact your 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
- Safety
- Legal and regulatory
- Software menu commands
- Software tabs
- Capillary array
- Sample name entry
- Automated analysis
- Maintenance and troubleshooting
- 5400 Fragment Analyzer system

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