



Femto Pulse

User Manual



Notices

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In This Guide

Agilent has prepared this manual as a technical reference for the Femto Pulse system.

This document includes system overviews, 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 topic or procedure, please contact your corresponding Agilent Sales/Service Representative.

1 System Overview

This chapter gives an instrument overview.

2 Femto Pulse Software – File Menu

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

3 Femto Pulse Software – Admin Menu

This chapter describes the Femto Pulse System software in more detail on the commands of the Admin menu.

4 Femto Pulse Software – Utilities Menu

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

5 Femto Pulse Software – Help Menu

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

6 Femto Pulse Software – Operation Tab

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

7 Femto Pulse Software – Run Status Tab

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

8 Femto Pulse Capillary Array

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

9 Femto Pulse – Sample Name Entry

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

10 Femto Pulse – Automated Analysis

This chapter explains the procedure for automated analysis using Femto Pulse.

11 Femto Pulse – Using the Bar-Code Reader

This chapter explains how to add and remove solutions to the gel and conditioning solution bottles using the bar-code reader.

12 Appendix

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

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System Overview

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This chapter gives an instrument overview.

About the System

The Femto Pulse 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 μ m 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 Femto Pulse 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 samples are collected in a single experimental run.

Configured Femto Pulse System Dimensions

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

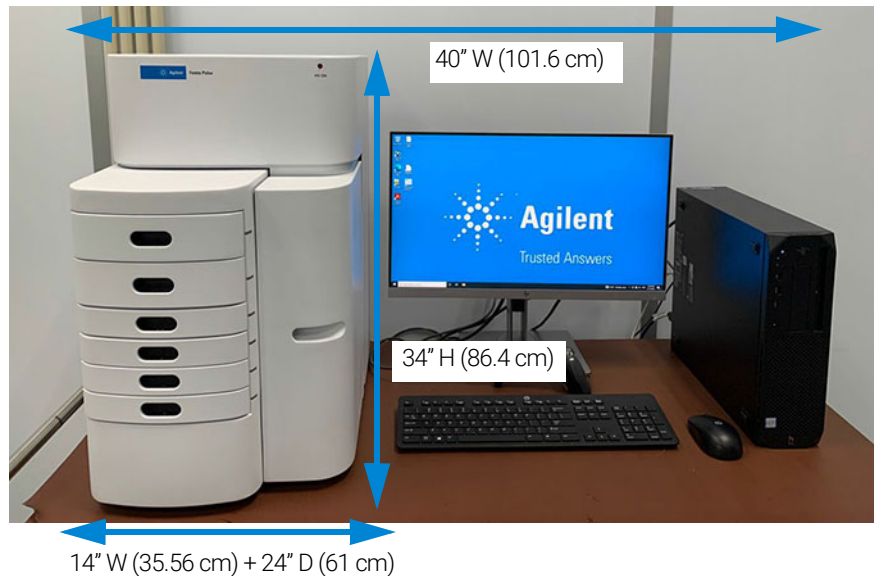


Figure 1 Configured Femto Pulse system with computer workstation

Femto Pulse System Connections

The back of the Femto Pulse instrument 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 your corresponding Agilent Sales/Support 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 Femto Pulse instrument are summarized below:

- From the Femto Pulse System:
 - Two USB cables to PC USB.
 - Power cord to grounded electrical outlet.
- From the PC:
 - Two USB connections to Femto Pulse system.

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

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

AC Power Connection

Labeled USB Connections

Internet Connection

Computer Monitor Connection

Mouse and Keyboard Connections



Figure 2 Back of panel of the computer connections

Labeled USB Cables

AC Power Connection

Power Switch

Fuse Mount

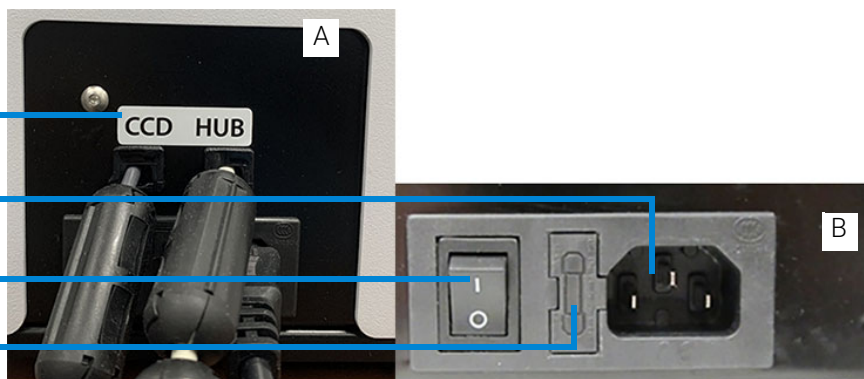


Figure 3 Back of panel of the Femto Pulse instrument connections

Femto Pulse External Cabinet

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

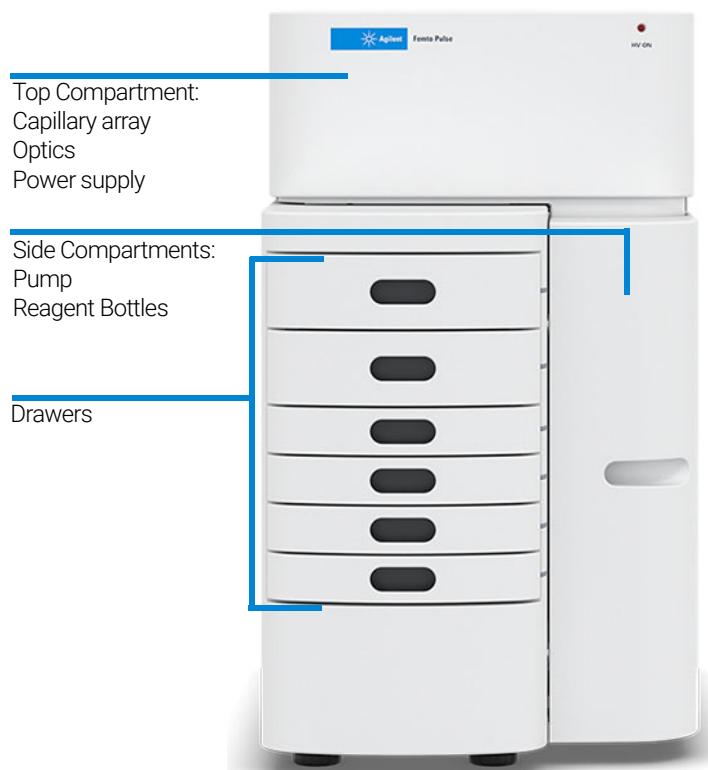


Figure 4 Entry points of the Femto Pulse system

Top Compartment

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

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

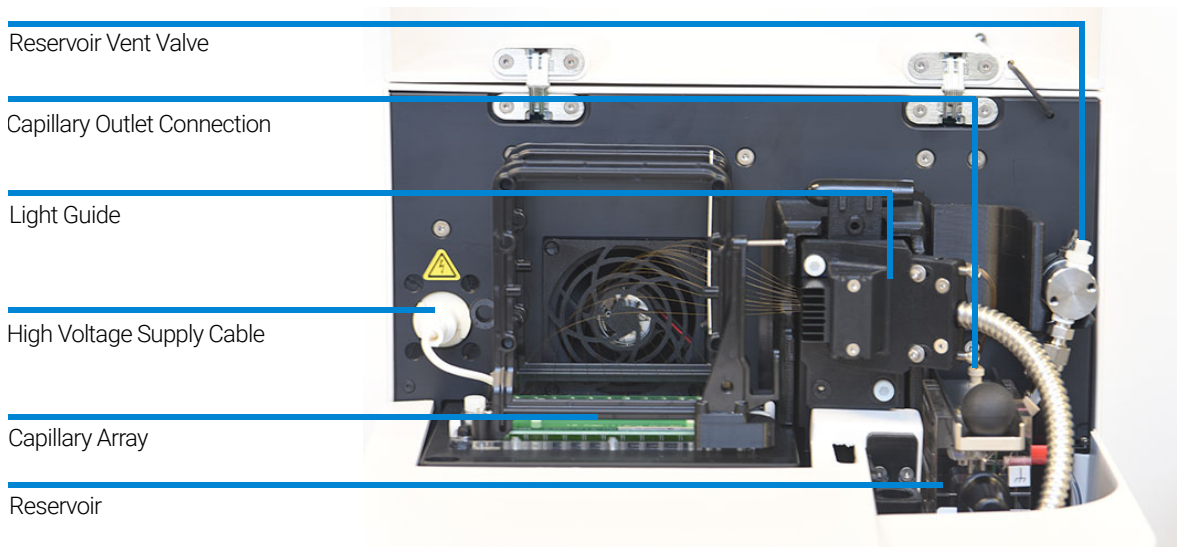


Figure 5 Femto Pulse main unit top compartment opened

Side Compartment

The side compartment allows access to the high-pressure pump, syringe, waste bottle, conditioning solution, and gel solutions (gel 1 and gel 2).

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

The system design enables vacuum injection (hydrodynamic injection) of samples in addition to traditional electrokinetic (voltage) sample injection, which is a feature unique to the Femto Pulse 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.

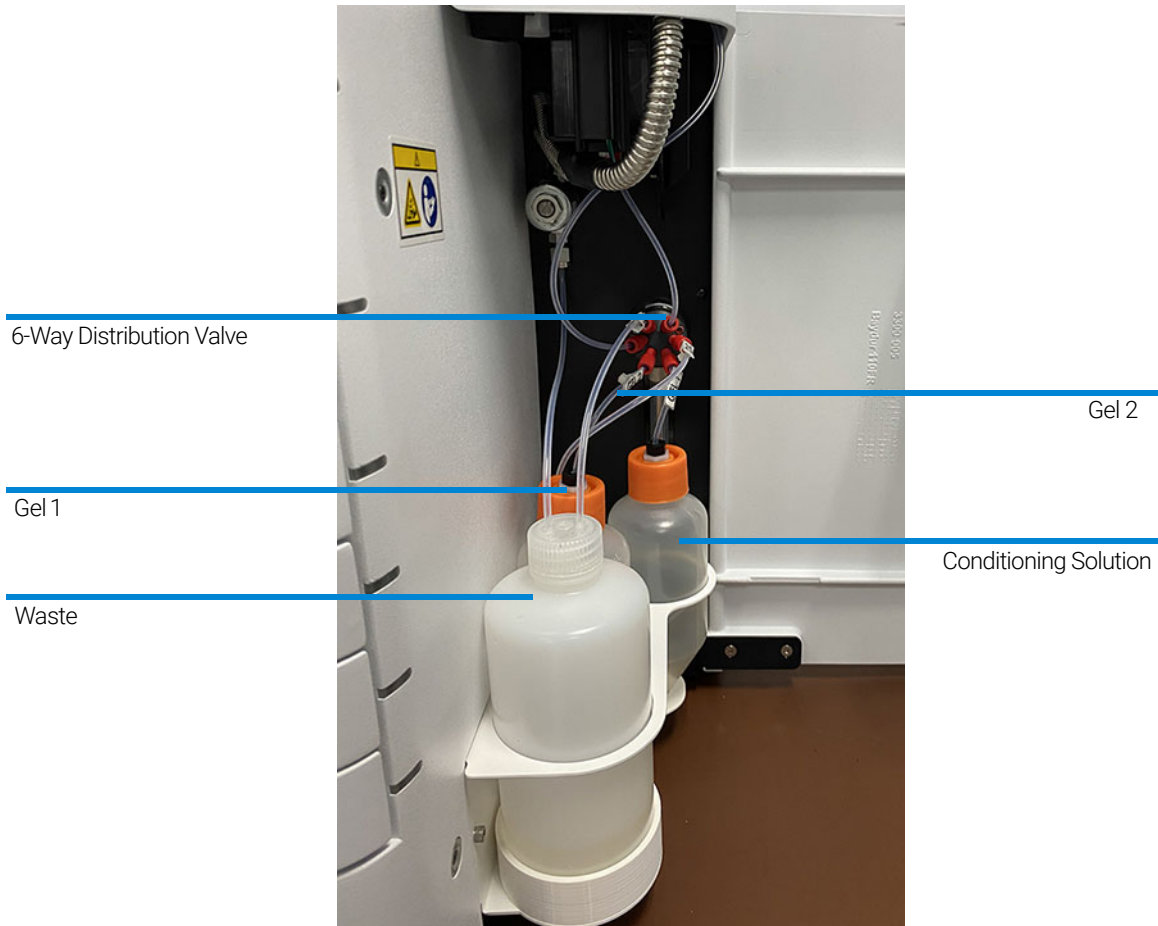


Figure 6 Side door compartment

The four fluid line connections inside the Femto Pulse system are:

- *Gel line* (gel 1 or gel 2) from syringe pump to gel bottle (gel 1 or gel 2)
- *Conditioning fluid* from syringe pump to conditioning fluid
- *Overflow waste line* from syringe pump to waste bottle
- *F-Port line* from syringe pump (6-way valve) to F-Port

Drawers

The Femto Pulse front-panel drawers provide an external interface for loading *Buffer*, *Marker*, and *Sample 96-Well Plates* or *PCR Tubes* into the system.

- Drawer B (top drawer): This location is used for the *Inlet Buffer Tray* used during the CE separation. This position is also used for *Sample Storage Solution* in the 12-capillary instrument.
- Drawer W (second drawer from top): This location is utilized for a *Waste Tray* when the capillary array is flushed.
- Drawer M (third drawer from top): This location is used for loading the *Marker Tray* or *Rinse Buffer*.
- Drawer 1 (fourth drawer from top): This location is utilized for *Sample Plate Number 1*.
- Drawer 2 (fifth drawer from top): This location is utilized for *Sample Plate Number 2*.
- Drawer 3 (sixth drawer from top): This location is utilized for *Sample Plate Number 3*. It is also used for a 96-well plate containing *Sample Storage Solution*.

Drawer Status

Status

Drawers B and W are Interlocked

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

Description

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

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



Figure 7 Instrument drawer positions

Femto Pulse Loading and Orientation of 96-Well Plates

The Femto Pulse system is a multiplexed CE system containing a 12-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.

The capillary array orientation is indexed as follows: capillary #1 corresponds to Well A1, and capillary #12 to Well A12.

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

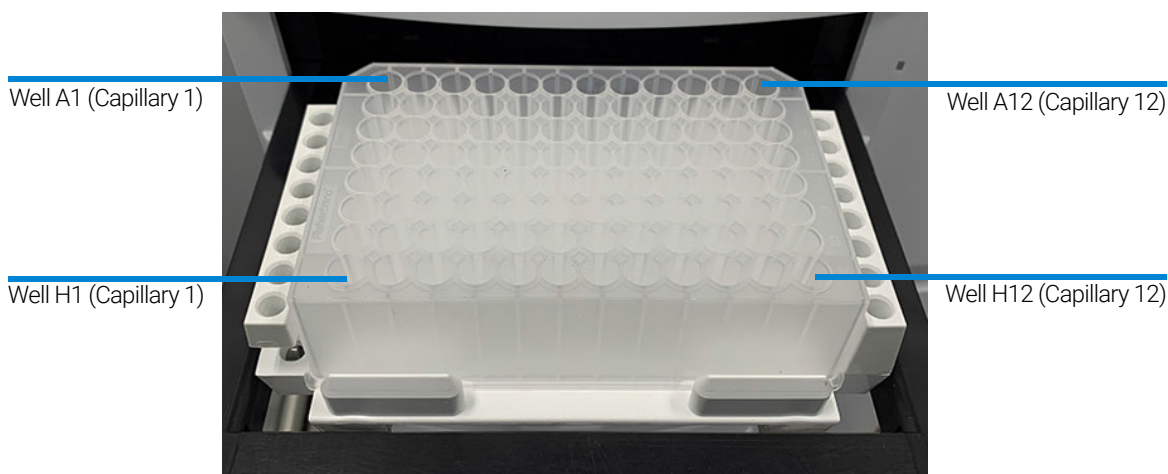


Figure 8 Proper orientation when loading 96-well marker and sample plates for a 12-capillary system

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 Femto Pulse 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, refer to **“Compatible Plates and Tubes”** on page 141.

Femto Pulse Loading Samples

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

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

Ensure the sample has been adequately mixed with the diluent marker or dilution buffer before loading on the instrument.

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

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

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

Individual tips are given in each Kit Guide for reference.

2

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

About the Software

The Femto Pulse system uses proprietary software for operation and data analysis.

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

System Requirements

The software is run using a Microsoft Windows 10 PC with the following requirements (**Table 1**).

Table 1 Minimum computer requirements

Type	Specification
Processor	Intel Core i5 - 8500
SVGA Video	Minimum Resolution 1024 X 768
Memory	4 Gigabytes (1 x 4 GB) DDR4 - 2666
Available Hard Disk Space	500 Gigabytes
USB Serial Ports	6 ports (2 instrument, keyboard, mouse)
Network	If not using a local database, a network connection to the database server host is desired.

System Installation

To install the Femto Pulse software:

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

Opening the Femto Pulse Software

- 1 To log in to the software, select the Femto Pulse software icon.



Figure 9 Femto Pulse icon

There are two levels of users available:

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

- 2 To log in to the Femto Pulse software, type **Administrator** or **User** into the **User ID** field of the login window.
- 3 Enter your password.

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

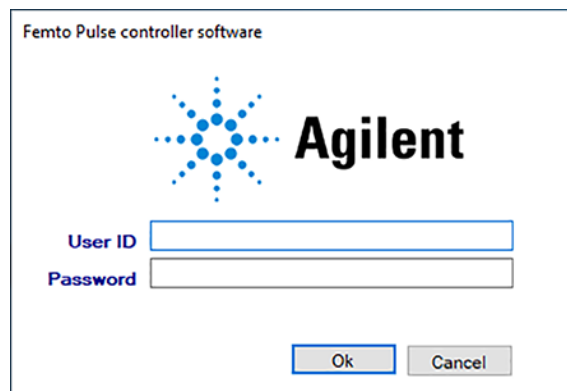


Figure 10 Login menu

Femto Pulse Software – File Menu

Opening the Femto Pulse Software

4 Select **OK**.

The main screen opens.

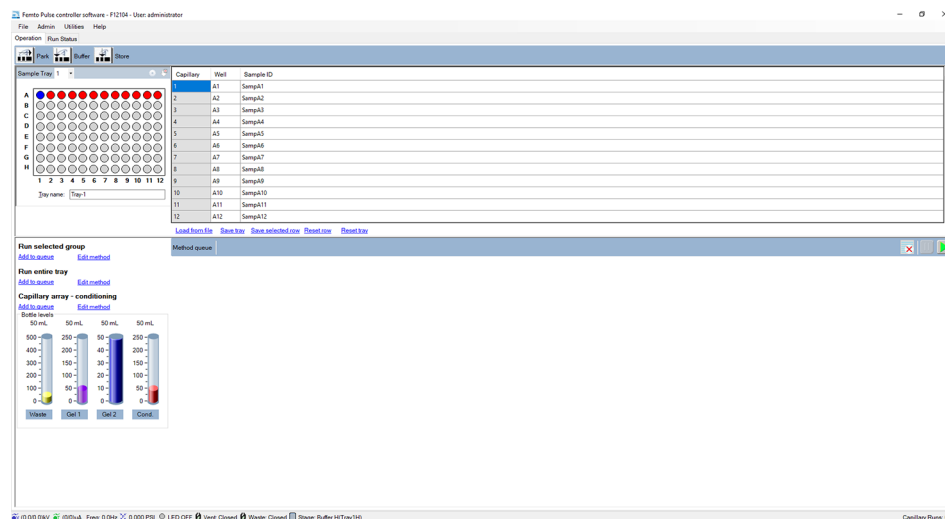


Figure 11 Femto Pulse software main screen window

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

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

Main Screen Toolbar

The main screen toolbar is located at the top of the Femto Pulse main screen as shown in **Figure 11**.

File Menu

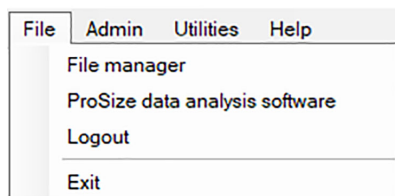


Figure 12 File menu functions

File Manager

The **File Manager** command allows electropherogram data to be examined within the *Femto Pulse* 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** command will open a window allowing the user to navigate to a data file. Once a file is selected, the file manager screen opens (**Figure 13**).

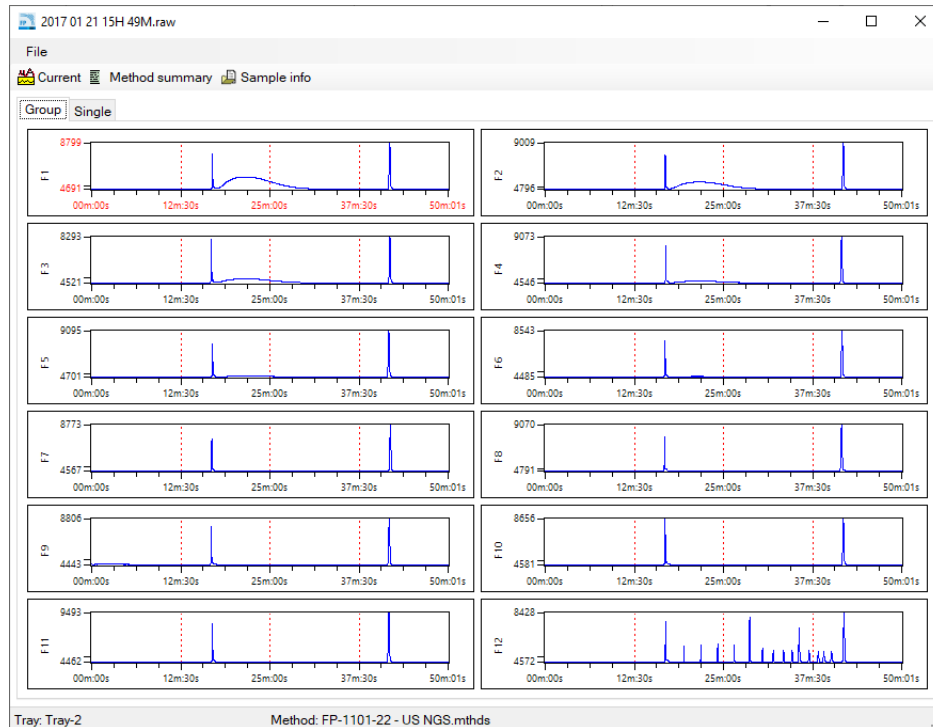


Figure 13 File manager window

The **File** functions of the file manager screen are reviewed in **Table 2**.

Table 2 File manager – File functions

Field	Description
Open	Opens a Windows dialog 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 Chapter 8 , "Femto Pulse Capillary Array".
Create Time shifted file	Allows the user to manually create a time-shifted file from the raw 2D file.
Print	Allows the user to print twelve electropherograms to a page.
Exit	Closes the file manager window.

The **Current**, **Method Summary**, and **Sample Info** toolbar functions are discussed in **Table 3**.

Table 3 File manager toolbar options

Field	Description
Current	Selecting current allows user to view the current of the separation during the analysis.
Method summary	Selecting the method summary option shows a summary of the method that was used for the separation.
Sample info	Selecting the sample info option shows the user the sample names input for the separation file.

Once the data file is opened in file manager, the data can be viewed in groups of 12 (by row) when the **Group** tab is selected. A page selection is located at the bottom of the screen allowing for navigation of all rows in a plate (assuming 96-capillary array data is chosen).

To view a single electropherogram at a time, either double left-click 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 the chart and selecting the function of interest.

ProSize Data Analysis Software

Selecting this command will open the ProSize software.

Logout

The **Logout** command allows the user to log out of the Femto Pulse software and to log in as a different user.

After logout the login menu opens (**Figure 10**).

Exit

The **Exit** command closes the Femto Pulse software. Alternatively, exit the program by selecting the red **X** on the top right corner of the main screen.

3

Femto Pulse Software – Admin Menu

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

Admin Menu

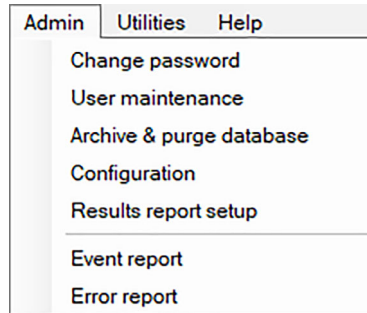


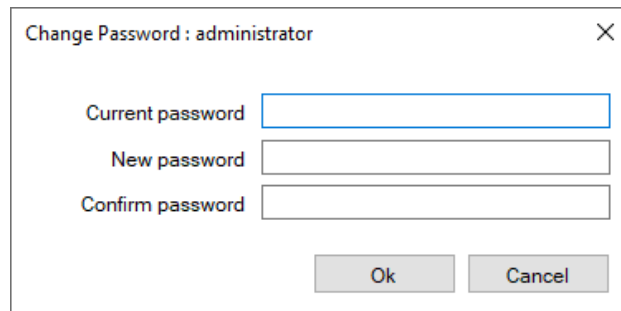
Figure 14 Admin menu commands

Change Password

Selecting the **Change password** command opens the window shown in **Figure 15**. Changing the password is only accessible to users with administrator privileges.

Password requirements:

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





A screenshot of a 'Change Password' dialog box. The title bar reads 'Change Password : administrator' with a close button (X) on the right. The dialog contains three text input fields: 'Current password', 'New password', and 'Confirm password'. Below the fields are two buttons: 'Ok' and 'Cancel'.

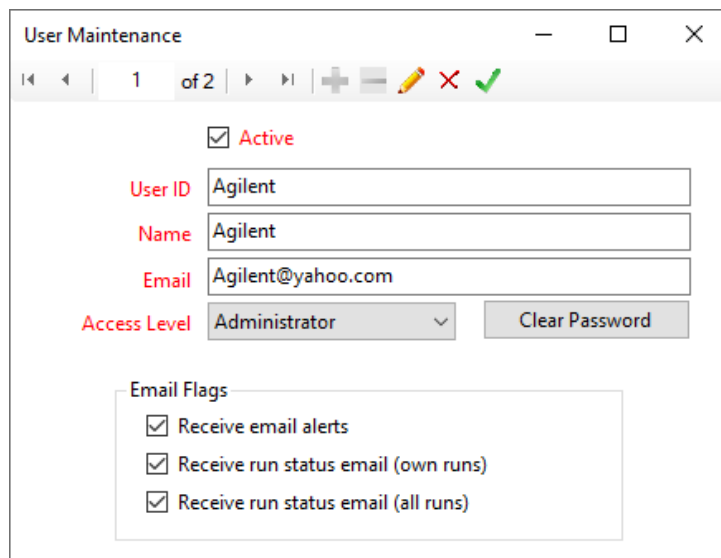
Figure 15 Change password menu

User Maintenance

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

In this window, the administrator can add, delete, or make modifications to all users that can access the Femto Pulse software.

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



The screenshot shows the 'User Maintenance' window. At the top, there's a title bar with the text 'User Maintenance' and standard window controls. Below the title bar is a toolbar with navigation icons (back, forward, search), a list indicator '1 of 2', and action icons (add, delete, edit, cancel, confirm). The main content area has a section for 'Active' users, indicated by a checked checkbox. Below this, there are input fields for 'User ID' (containing 'Agilent'), 'Name' (containing 'Agilent'), and 'Email' (containing 'Agilent@yahoo.com'). There is also a dropdown menu for 'Access Level' set to 'Administrator' and a 'Clear Password' button. At the bottom, there is a section titled 'Email Flags' with three checked checkboxes: 'Receive email alerts', 'Receive run status email (own runs)', and 'Receive run status email (all runs)'.

Figure 16 User maintenance window

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

Table 4 User maintenance window parameters

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

NOTE

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

NOTE

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

Archive and Purge Database

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

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

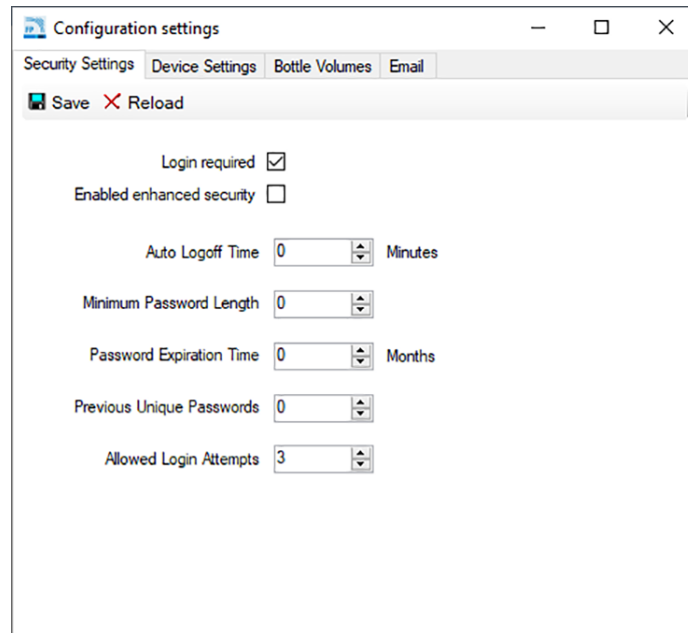
This function allows the user with administrative privileges to back-up the data for future use in a different location or on an external storage device.

Configuration

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

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

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



The screenshot shows a window titled "Configuration settings" with four tabs: "Security Settings", "Device Settings", "Bottle Volumes", and "Email". The "Security Settings" tab is active. Below the tabs is a bar with a "Save" button (with a floppy disk icon) and a "Reload" button (with a red 'X' icon). The main area contains the following settings:

- Login required: ☒
- Enabled enhanced security: ☐
- Auto Logoff Time: 0 Minutes (with a spinner box)
- Minimum Password Length: 0 (with a spinner box)
- Password Expiration Time: 0 Months (with a spinner box)
- Previous Unique Passwords: 0 (with a spinner box)
- Allowed Login Attempts: 3 (with a spinner box)

Figure 17 Configuration – Security Settings tab

Configuration Options

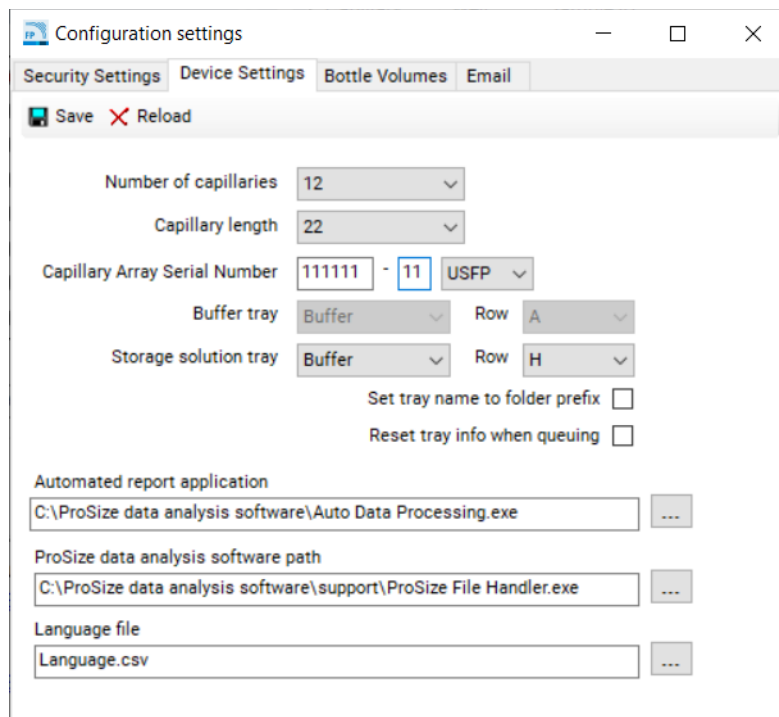
Table 5 Configuration – Security Settings tab functions

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

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

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

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



The screenshot shows the 'Configuration settings' window with the 'Device Settings' tab selected. The window has a title bar with standard Windows controls and a tab bar with 'Security Settings', 'Device Settings', 'Bottle Volumes', and 'Email'. Below the tabs is a 'Save' button and a 'Reload' button. The main area contains the following settings:

- Number of capillaries:** A dropdown menu set to '12'.
- Capillary length:** A dropdown menu set to '22'.
- Capillary Array Serial Number:** A text field containing '111111', followed by a hyphen and a small box containing '11', and a dropdown menu set to 'USFP'.
- Buffer tray:** A dropdown menu set to 'Buffer'.
- Row:** A dropdown menu set to 'A'.
- Storage solution tray:** A dropdown menu set to 'Buffer'.
- Row:** A dropdown menu set to 'H'.
- Set tray name to folder prefix:** An unchecked checkbox.
- Reset tray info when queuing:** An unchecked checkbox.
- Automated report application:** A text field containing 'C:\ProSize data analysis software\Auto Data Processing.exe' and a browse button ('...').
- ProSize data analysis software path:** A text field containing 'C:\ProSize data analysis software\support\ProSize File Handler.exe' and a browse button ('...').
- Language file:** A text field containing 'Language.csv' and a browse button ('...').

Figure 18 Configuration – Device Settings tab

Table 6 Configuration – Device Settings Tab Functions

Parameter	Access Level	Description
Number of capillaries	Administrator	Value: 12
Capillary length	Administrator	Value: 22 Note that this setting refers to the effective length of the capillaries in use.
Capillary Array Serial Number	Administrator	The format must be xxxxxx-xx-xxxx.
Buffer tray	Administrator	Default selection is locked.
Storage solution tray	User	Allows the selection of Tray and Row for the Storage Solution Tray.
Set tray name to folder prefix	Administrator	Sets the tray name to the folder prefix used when loading sample trays.
Reset tray info when queuing	Administrator	Resets tray info for each new tray that is loaded.
Language file	User	Enables the user to change the language of the application by selecting the appropriate (.csv) language file. Example: Chinese, English, and German
Automated report application	Administrator	Allows for changing the file path used for the automated report application.
ProSize data analysis software path	Administrator	Allows for changing the file path used to open the ProSize data analysis software.
Save	Administrator	Saves the chosen settings.
Reload	Administrator	Reloads the previously saved settings.

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

The gel 1, gel 2, conditioning, and waste bottles can be set from 50 mL to 5000 mL by entering the appropriate volumes. These settings depend on the types of containers used in the system. By default, 12-capillary systems use 50 mL centrifuge tubes for gel 1 and gel 2, with a 250 mL centrifuge for the conditioning solution. Larger volumes may be used if the system is configured with larger containers.

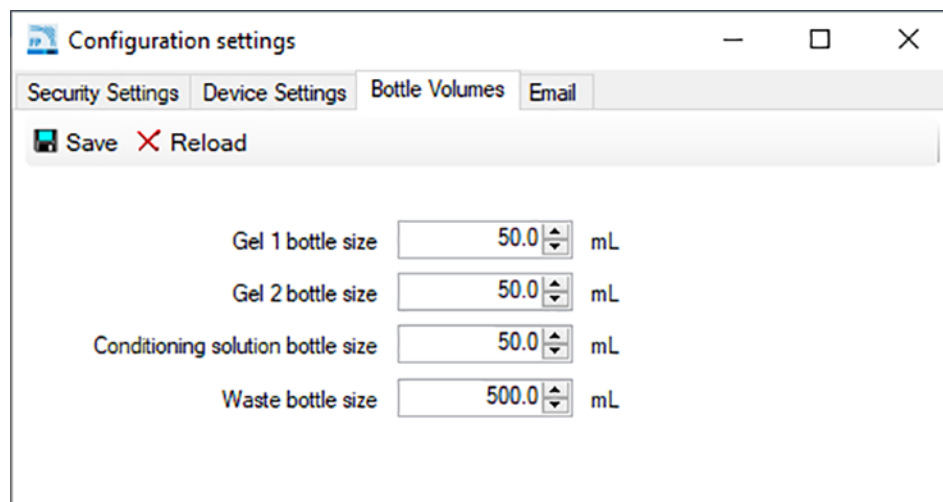
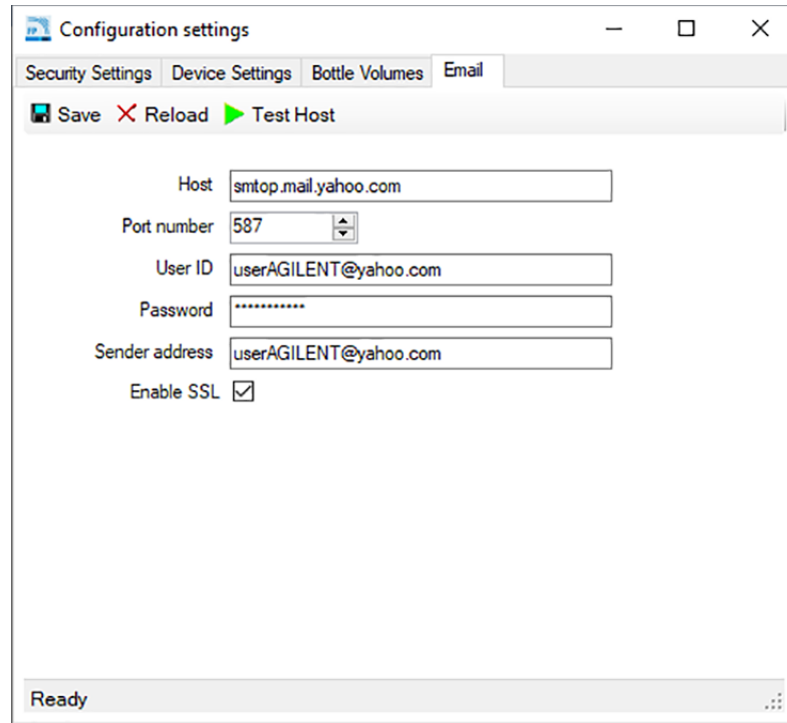


Figure 19 Configuration settings – Bottle Volumes tab

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



Configuration settings

Security Settings Device Settings Bottle Volumes **Email**

Save Reload Test Host

Host smtp.mail.yahoo.com

Port number 587

User ID userAGILENT@yahoo.com

Password *****

Sender address userAGILENT@yahoo.com

Enable SSL ☒

Ready

Figure 20 Configuration settings – **Email** tab

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

NOTE

After inputting all the desired e-mail settings, select **Test Host** (green arrow) to ensure a positive test. If the test is not positive or passed, then the parameters are not set correctly.

NOTE

After passing the **Test Host**, select **Save**.

Find Outgoing Mail settings (Example: Yahoo)

YAHOO! HELP
UK & IRELAND

Search Help Search web

Help Central > Article

POP server settings for Yahoo Mail

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

Incoming Mail (POP) Server

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

Outgoing Mail (SMTP) Server

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

Use "Outgoing" Mail properties

Figure 21 Example outgoing mail settings

Results Report Setup

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

The settings allow:

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

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

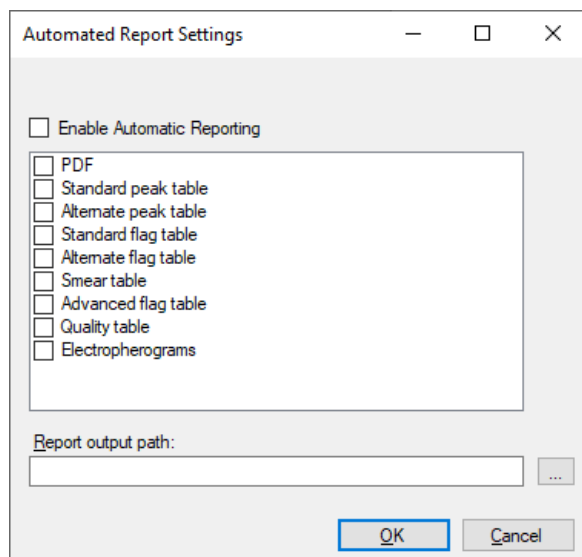


Figure 22 The results report setup window

Selecting **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 manual, or to **Chapter 10**, “Femto Pulse – Automated Analysis”, which gives a detailed description of auto-processing.

NOTE

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

Event Report

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

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

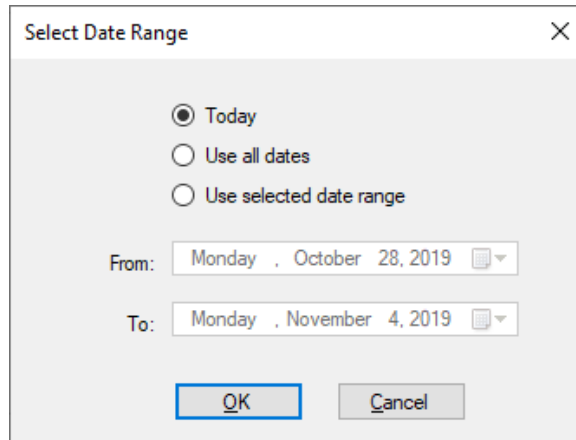
A screenshot of a 'Select Date Range' dialog box. The dialog has a title bar with a close button (X). Inside, there are three radio buttons: 'Today' (selected), 'Use all dates', and 'Use selected date range'. Below the radio buttons, there are two date pickers. The 'From:' date is 'Monday, October 28, 2019' and the 'To:' date is 'Monday, November 4, 2019'. At the bottom, there are 'OK' and 'Cancel' buttons. The 'OK' button is highlighted with a blue border.

Figure 23 Event Report window

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

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

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

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

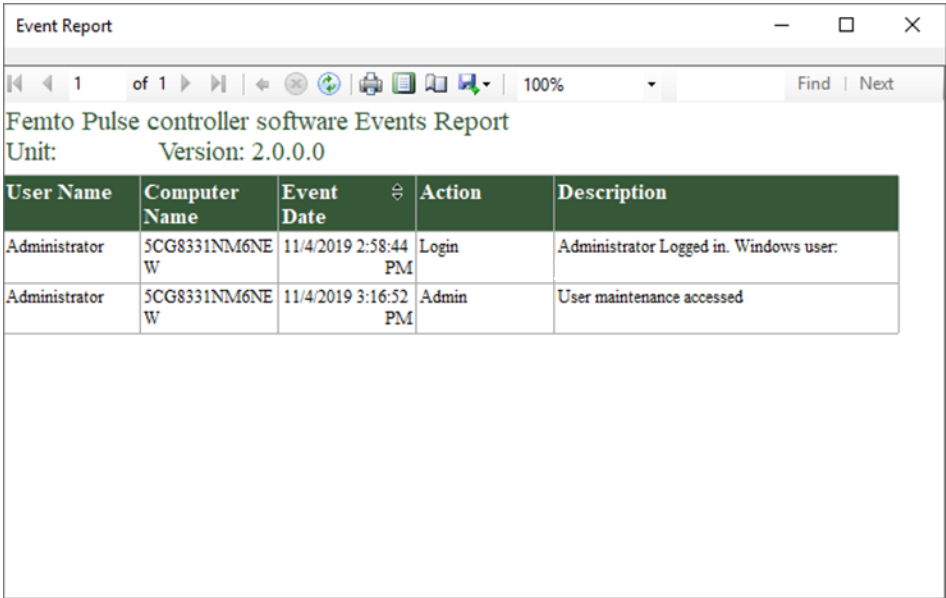


Figure 24 Event Report example

The icons along the top of the Event Report follow standard Windows function nomenclature and are summarized in [Table 7](#).

Table 7 Event Report icons and descriptions

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

Error Report

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

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

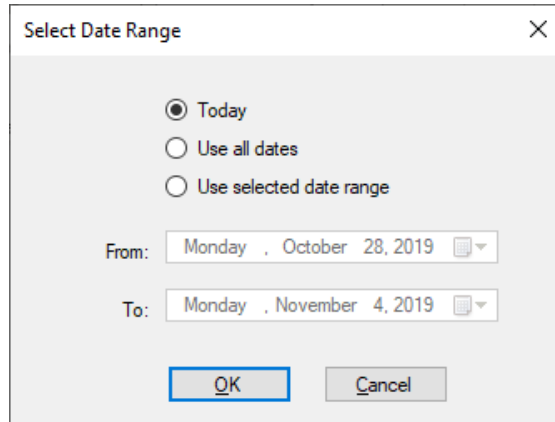


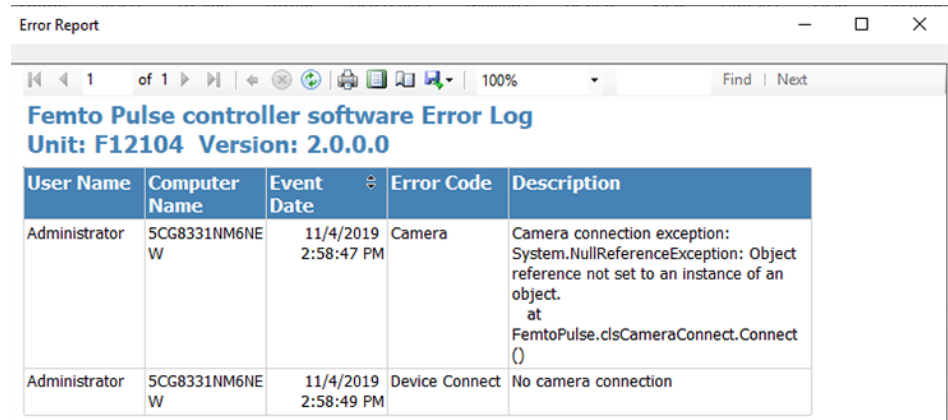
Figure 25 Error Report window

The Error Report captures the following information:

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

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

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



Femto Pulse controller software Error Log
Unit: F12104 Version: 2.0.0.0

User Name	Computer Name	Event Date	Error Code	Description
Administrator	SCG8331NM6NEW	11/4/2019 2:58:47 PM	Camera	Camera connection exception: System.NullReferenceException: Object reference not set to an instance of an object. at FemtoPulse.clsCameraConnect.Connect()
Administrator	SCG8331NM6NEW	11/4/2019 2:58:49 PM	Device Connect	No camera connection

Figure 26 Error Report example

4

Femto Pulse Software – Utilities Menu

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

Utilities Menu

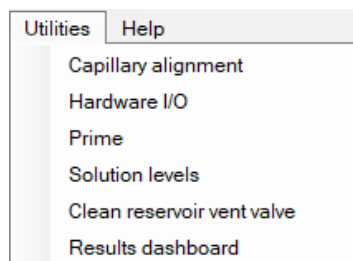


Figure 27 Utilities menu commands

Capillary Alignment

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

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

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

The methods discussed in this chapter will be illustrated with images from a Femto Pulse 12-capillary array.

NOTE

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

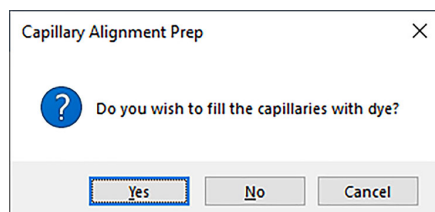


Figure 28 Capillary Alignment Prep window

Method A – Capillary Alignment from a File

- 1 Select **No** when prompted from the **Capillary Alignment Prep** window.

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

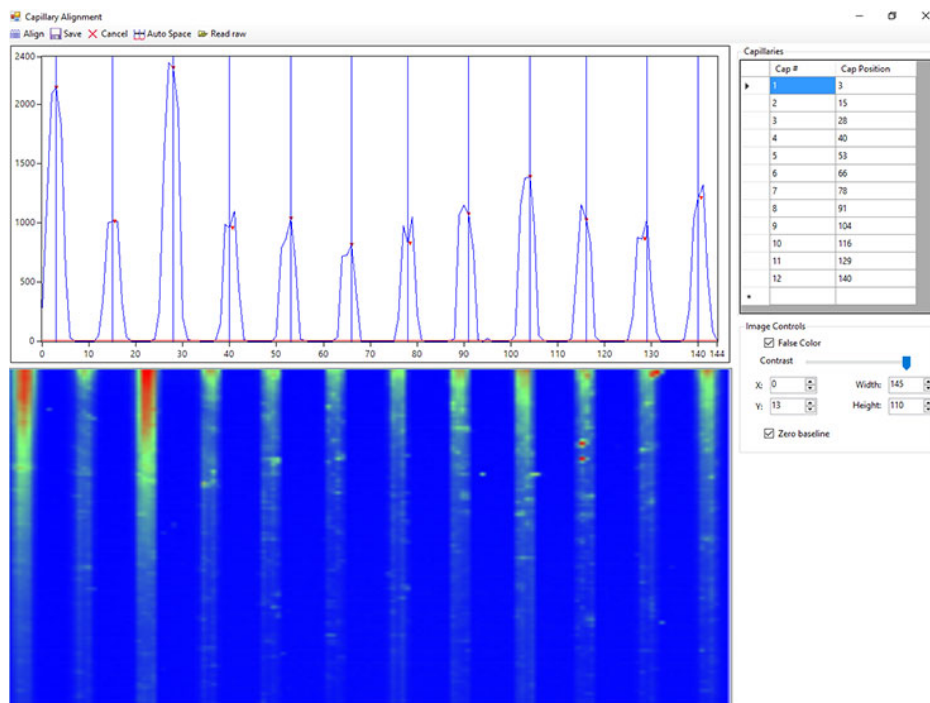


Figure 29 Real time Capillary Alignment window

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

NOTE

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

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

This file will be used for the alignment.

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

The default saved location of raw data is:

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

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

The *Align From File* window opens (**Figure 30** shows an example for a 12-capillary), allowing the user to align the capillaries from the selected run file. The toolbar of the *Align from File* window is described in **Table 8**.

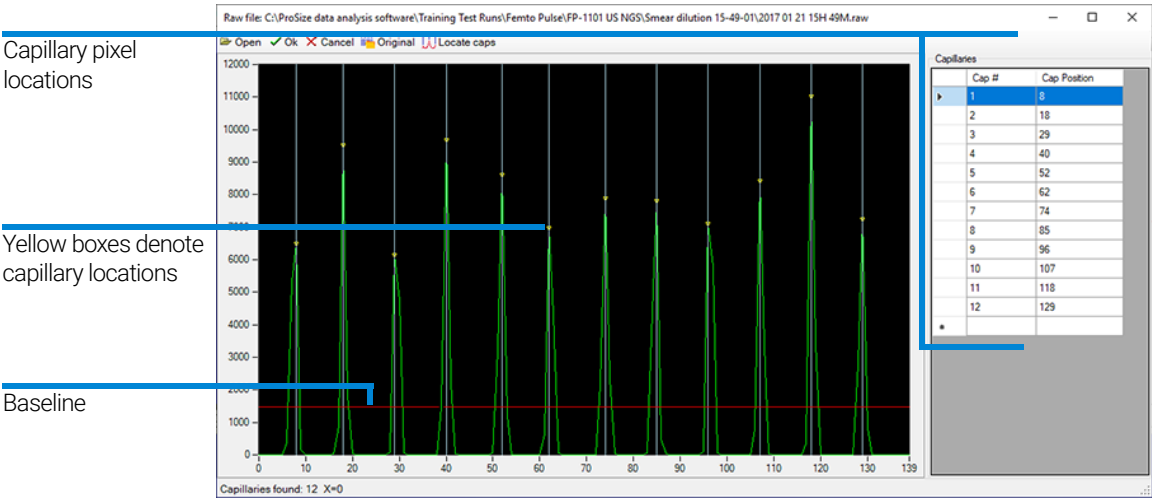


Figure 30 Align from file window for 12-capillary system

Table 8 Align from file toolbar functions

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

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

8 Select **Locate Caps** from the toolbar of the *Align from File* window.

The capillary peaks 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.

If necessary, adjust the capillary positions:

- To manually adjust a capillary position, left-click on the white line showing the capillary position and drag it left or right to the desired location.
- To zoom-in for desired resolution, right-click and select **Zoom** (and dragging the appropriate area).
- Should the number of capillaries be off due to too many or too few capillary positions chosen, adjust the red baseline and repeat the steps above.
- To insert or delete a capillary position, right-click on the black area of the graph or the capillary pixel location table to the right of the graph.

9 Once the desired number of capillaries is located, select **OK** from the *Align from File* toolbar. This will save any changes made to the capillary alignment and close the *Align from File* window, returning you to the **Capillary Alignment** window.

10 Select **Save** from the Capillary Alignment window.

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

Method B – Capillary Alignment without Dye

1 Select **No** from the **Capillary Alignment Prep** window.

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

2 Right-click on the blue 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 31**).

4 Draw a box around the capillary array display area. Left-click and drag the appropriate area (**Figure 31**).

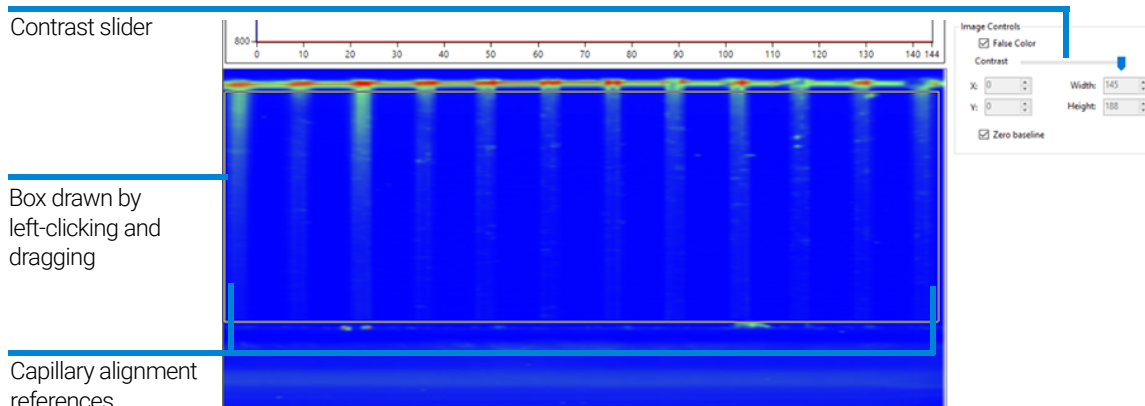


Figure 31 Capillary Alignment display – window reset

- 5 After the box is drawn, right-click, and select **Set Camera Window**.
- 6 Adjust the height setting to 100.

Table 9 Capillary Alignment display menu bar functions

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

- 7 Adjust the red baseline seen in **Figure 29** until a red dot is observed above each capillary peak. This determines which capillary peaks are selected. It is important to ensure this red line is above the baseline.
- 8 Select **Align** from the menu of the top capillary alignment display area. A blue vertical line is placed through the center of each capillary. If the blue lines are not in the exact center of each peak, adjust the lines by left-clicking and dragging to the desired location.
- 9 Select **Align** every time the red baseline is moved. This ensures that the instrument has selected the peak for integration and places the blue vertical

alignment line in the middle of each peak (corresponding to where the red dots are present).

- 10 Select **Save** from the menu of the top capillary alignment display area to save the capillary locations and close the **Capillary Alignment** window.

Hardware I/O

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

Selecting the **Hardware I/O** command from the **Utilities** menu opens the **Hardware testing screen** as seen in [Figure 32](#).

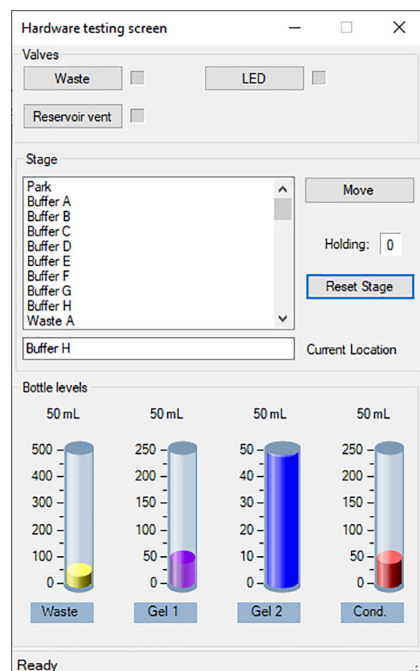


Figure 32 Hardware testing screen

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
Valves > Waste	Select to open the valve. Clear to close the valve. The status of the valve is indicated by an open/filled circle in the status bar, respectively.
Valves > Reservoir vent	Select to open the valve. Clear to close the valve. The status of the valve is indicated by an open/filled circle in the status bar, respectively.
Stage > Move	Select to move tray to the selected position.
Stage > Reset Stage	Allows the user to reset the stage position should a drawer be opened before the stage finishes its movement. Available on instrument serial numbers 2600 and higher.
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 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 33). The Prime functions are discussed in Table 11.

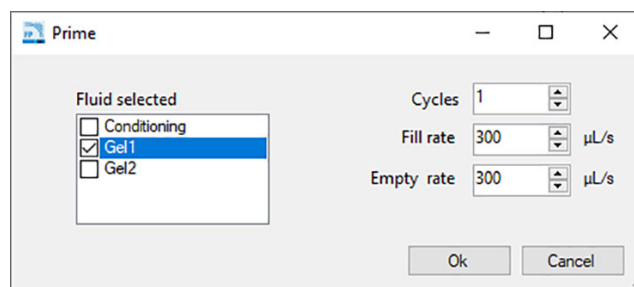


Figure 33 Prime window

Table 11 Functions of the Prime window

Function	Description
Fluid Selected	Allows the user to select which reagent line or to prime.
Cycles	Refers to number of cycles (1-10) of the syringe to complete. One cycle is generally sufficient.
Fill Rate	Allows the user to adjust the fill rate up and down 0-1000, the default setting is 300 uL/s.
Empty Rate	Allows the user to adjust the fill rate up and down 0-1000, the default setting is 300 uL/s.

Solution Levels

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

The Femto Pulse 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 34).

	Volume (mL)	Solution name
Gel 1	50.0	FP 5001
Gel 2	50.0	NaOH
Conditioning solution	50.0	
Waste	50.0	

Figure 34 Check solutions volumes screen

- 1 When solutions are re-filled, open this window and enter the correct solution volumes (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 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 **Clean reservoir vent valve** command allows the user to clean the reservoir vent valve manually.

Selecting the command from the **Utilities** menu opens the reservoir vent valve and the waste valve. The **Clean Reservoir Vent Valve** window opens as seen in **Figure 35**.

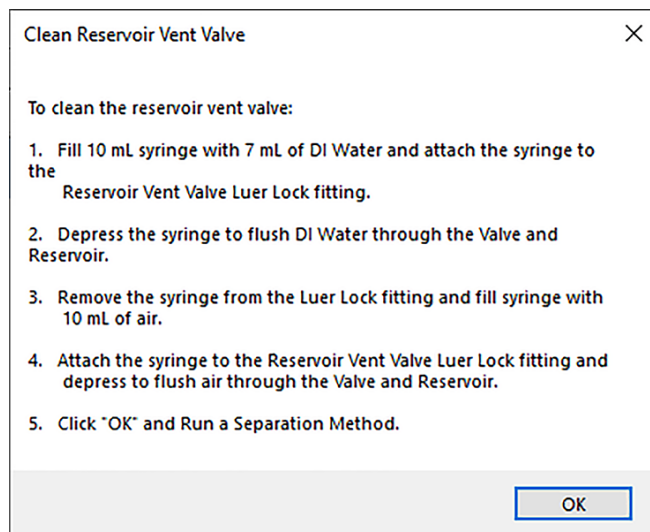


Figure 35 Clean Reservoir Vent Valve window

Follow the steps outlined in **Figure 35** 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 **Results dashboard** command allows to quickly view the status of auto-processed data.

Figure 36 shows the **Results Dashboard** window.

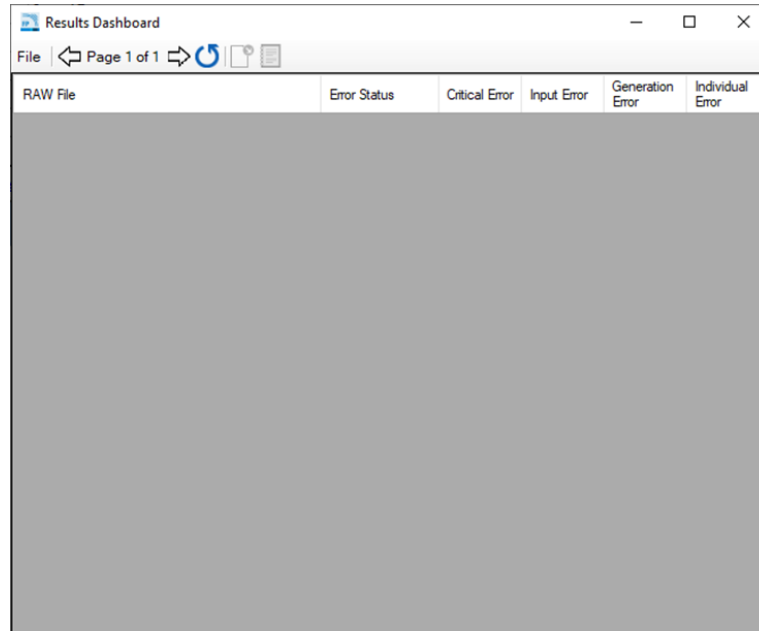


Figure 36 Results Dashboard window

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

5

Femto Pulse Software – Help Menu

Help Menu 64

User Manual 64

About 64

About Firmware 64

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

Help Menu

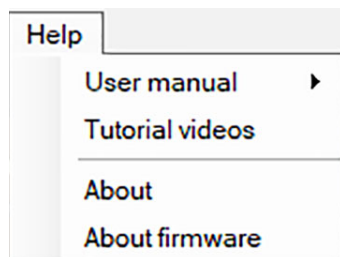


Figure 37 Help menu commands

User Manual

Navigating to the command **User manual** provides a drop-down list of every chapter of the system manual.

About

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

About Firmware

The **About firmware** command opens an **About Firmware** window displaying the version numbers of the high voltage board, pump board, and motion control board.

6

Femto Pulse Software – Operation Tab

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Hotel Position Icons 67

Tray Selection and Sample ID 68

Experimental Run Controls and Adding to Queue 71

Method Queue 78

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

Operation Tab Overview

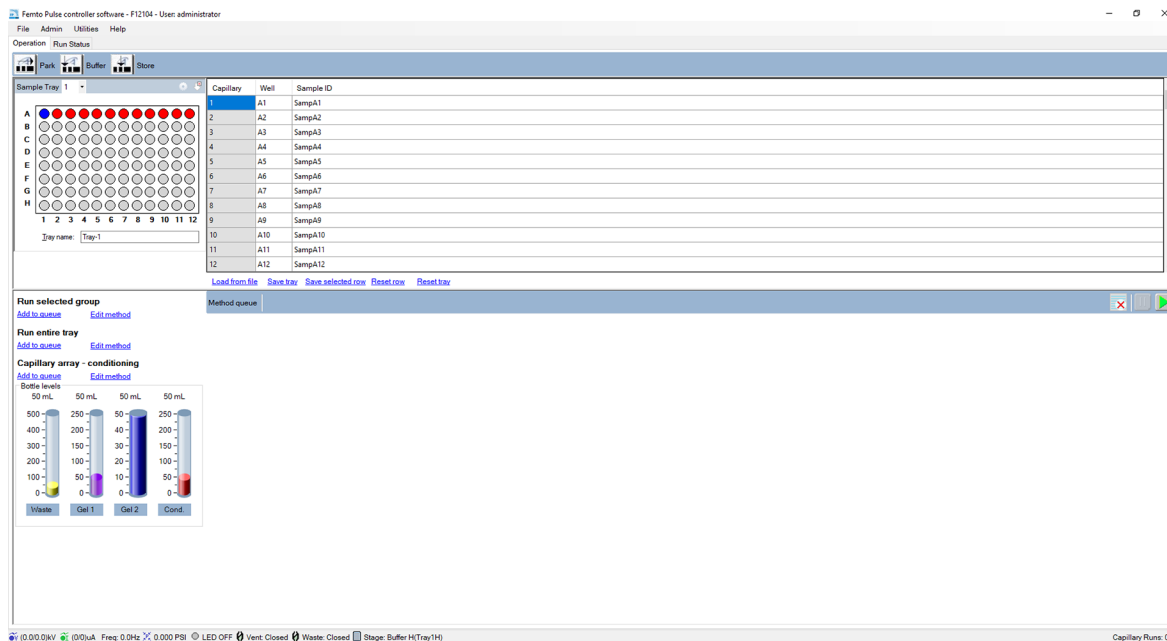





Figure 38 Femto Pulse main screen

Hotel Position Icons

There are three hotel positioning icons located at the top of the **Operation** tab, as seen in **Figure 38**. The icons and their functions are discussed in **Table 12**.


Table 12 Hotel position icons

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

Tray Selection and Sample ID

Select the sample tray to be used from either the **Sample Tray** drop-down list or the colored tab tray selection, depending which configuration is set (**Figure 39**).

NOTE

The configuration can be set by selecting the  icon located in the upper right corner of the window shown in **Figure 39**.

Configure the Visual Style of Tray Selection Window

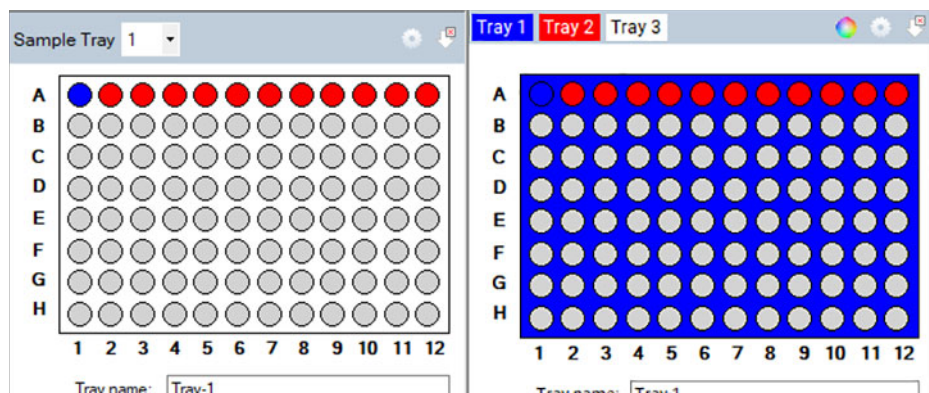


Figure 39 Classic drop-down tray selection (left) or colored tab tray selection (right)

- 1 In the tray window, select .

The **Visual preferences dialog** window opens (**Figure 40**).

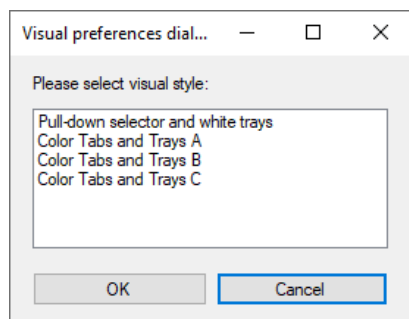




Figure 40 Visual preferences dialog

- 2 Choose between the sample tray drop-down list or the colored tab tray selection as shown in **Figure 39**.

- 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 or sample tray window, left-click once in that row (**Figure 39**). To select a new row left-click on another row.
- 5 To clear a row selection, select  (**Figure 39**).

The **Tray name** dialog box allows you to input a name for the tray being run (**Figure 39**). Alternatively, select this dialog box and use a bar-code scanner to import sample names for the plate being run (for more information, refer to **Chapter 9**, “Femto Pulse – Sample Name Entry”).

Enter sample information in the **Sample ID** section of the main screen (**Figure 41**).

You can also save or load sample names and information 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 41 Sample information editor

Table 13 Sample information editor functions

Item	Description
Load from file	Loads sample names from a .txt or .csv based file. See Chapter 9 , "Femto Pulse – Sample Name Entry" for further information.
Save tray	Saves the information entered for an entire sample tray.
Save selected row	Saves the information entered for the selected row of a sample tray.
Reset row	Resets the selected row to the default Sample ID setting.
Reset tray	Resets the entire sample tray to the default sample ID settings.

Experimental Run Controls and Adding to Queue

The Femto Pulse software provides pre-loaded methods for both capillary array conditioning and experimental methods for each reagent kit offered by Agilent.

The *Experimental Run Controls* shown in **Figure 42** shows the controls to **Run Selected Row**, **Run Entire Tray**, and **Capillary Array – Conditioning**. These options are discussed below.

Reagent levels of the bottles are also shown.

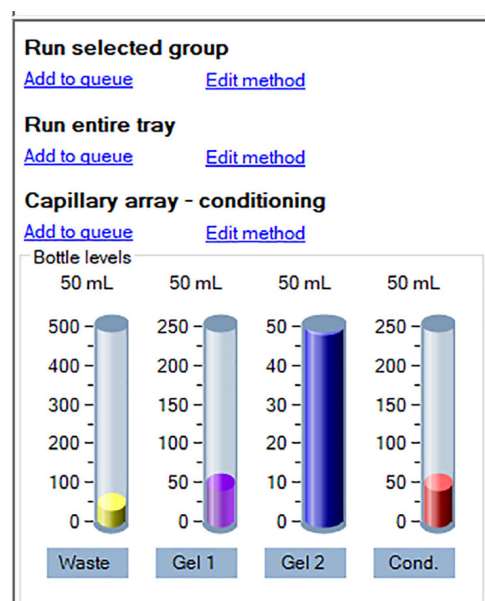


Figure 42 Experimental run controls

Run Selected Row or Run Entire Tray – Edit Method

Selection of the **Edit method** will show the method editor popup window exhibited in **Figure 43**. When logged in under user level access the edit method window is not available, it is replaced with **View method** as the user level does not have access to change method parameters.

Separation Method: Default Separation.mthds

☒ Full conditioning ☐ Gel prime to Gel selection Gel 1

☐ Gel prime

☒ Prerun Voltage 5.5 Time 30 sec.

☐ Rins Tray: Buffer Row A

☐ Marker injection Row A

☒ Voltage injection Voltage 4.50 kV - Time 5 sec.

☐ Vacuum injection Pressure -1.0 PSI

☐ Rinse Tray: Buffer Row A

☒ Sample injection

☒ Voltage injection Voltage 4.50 kV - Time 5 sec.

☐ Vacuum injection Pressure -1.0 PSI

☒ Separation ☐ Pulse field Time 30.00 min. Voltage 5.50 kV -

Figure 43 Run selected row and run entire tray edit method editor window





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

Full conditioning, **Gel prime**, and **Gel prime to** buffer are hard coded 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 (i.e. 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 to use the Gel 1 or the Gel 2 reagent bottle position.
Pre-run	A short pre-run is recommended to normalize and condition the gel inside the capillaries. The pressure setting should always be set to zero.
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) can be altered as well.
Marker injection	Marker injection is enabled when using the qualitative sample 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 can also 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) can be altered as well.
Sample injection	The user has the option of a voltage or vacuum injection with selection of Voltage , Pressure , and Time parameters.
Separation	The user has the option to alter the voltage and time of the CE Separation. Note: For pulse field methods, users cannot change voltage. This is only possible for fixed field methods.
	Save icon for saving results to a file.
	Save as icon for saving results to a new file.
	Open an existing method.
	Cancel (exit out of current screen).

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 Row or Run Entire Tray – Add to Queue

Selecting the **Add to queue** option opens the **Separation setup** window as seen in **Figure 44**.

The image shows a 'Separation setup' dialog box. It has a title bar with a close button. Inside, there's a 'Method' dropdown menu set to 'Default Separation.mthds' with an 'Edit' link. Below it is a 'Gel' dropdown menu set to 'Gel1'. Then a 'Tray name' text box containing 'Tray-1'. A 'Folder prefix' text box is empty. A 'Copy results' checkbox is unchecked. Below it is a 'Copy path' text box with a browse button (...). A large 'Notes' text area is empty. At the bottom, there's a 'Merge rows' checkbox, and 'Ok' and 'Cancel' buttons.

Figure 44 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 level access can also select Edit to change any parameters of the method by opening the method editor window in Figure 43 . 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
Notes	The notes section allows for the addition of any additional information the user may require for a set of samples.
Merge rows	When selected, will merge 8-rows of 12 or 2 sets of 48-cap "runs" into a single run file. The original non-merged rows are also available for data processing. This function is useful when running 8 rows of 12, and the user wants to view the data file as a single 96-well file.

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

Capillary Array - Conditioning

The Femto Pulse software provides a preloaded capillary conditioning method for cleaning the capillary array.

The user can also select to create a method of their choosing by selecting the **Edit Method** option seen in **Figure 45**.

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**.

Conditioning Method: Default Conditioning.mthdc

☒ Step #1 Solution: Conditioning

Fill pressure: 280 PSI Time: 0.0 min

Flow rate: 200 µL/s Tray: Waste Row: A

☒ Step #2 Solution: Gel 1

Fill pressure: 280 PSI Time: 3.0 min

Flow rate: 200 µL/s Tray: Waste Row: A

☐ Step #3 Solution: Conditioning

Fill pressure: 0 PSI Time: 1.0 min

Flow rate: 1 µL/s Tray: Waste Row: A

Figure 45 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).
	Save icon for saving results to a file
	Save as icon for saving results to a new file

Table 16 Separation setup window functions

Item	Description
	Open an existing method
	Cancel (exit out of current screen)

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 without accepting any changes made.

Select the **Add to queue** function to open the **Select Conditioning Method** window (**Figure 46**).

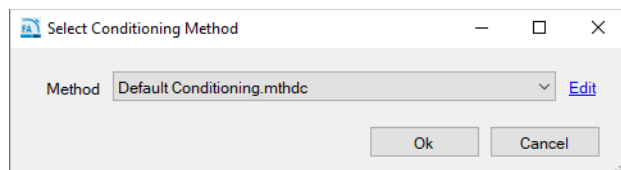


Figure 46 Select conditioning method window

A previously saved method can be chosen from the drop-down menu or a user with administrator privileges can select **Edit** to view the conditioning method editor window seen in **Figure 45**. Users with user level access can only select a previously used method and **View** the method, but not edit a method.

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

Method Queue

Once a sample tray or row (12-capillary unit only) has been selected and added to the queue, the method name and tray location selected for injection will be shown in the **Method queue** (Figure 47).

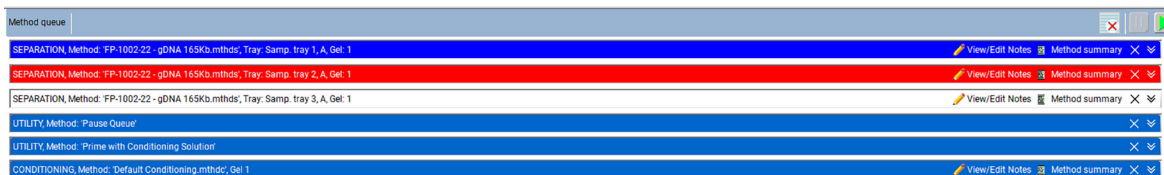


Figure 47 Method queue

Figure 47 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 opens, prompting the user to choose the priming fluid from a drop-down window (Figure 48).

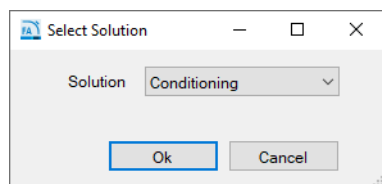


Figure 48 Select solution window

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 49.

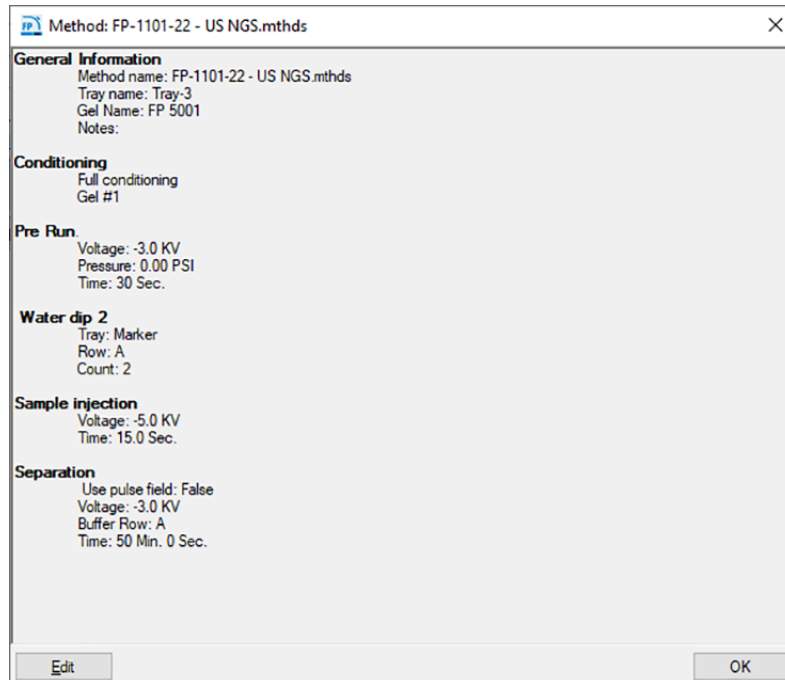



Figure 49 Method summary window




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 the  **Clear** icon 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 the  **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](#).

Table 17 Method queue run controls

Icon	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 disappears and the screen switches to the Run Status tab. The next method will move up in the queue. Note: When you add a method or item to the queue, you must select Start to begin operation of the instrument.

7

Femto Pulse Software – Run Status Tab

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Stage Movement Animation 82

Conditioning Animation 83


Pre-Run / Injection View 84

Real-time Separation View 85

Status Bar 87

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

Run Status Tab Overview

Once **Start**  has been selected (for more information, refer to section “**Method Queue**” on page 78), 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 shows where the Femto Pulse stage is moving to/from (**Figure 50**). This provides a real time view of what is happening.

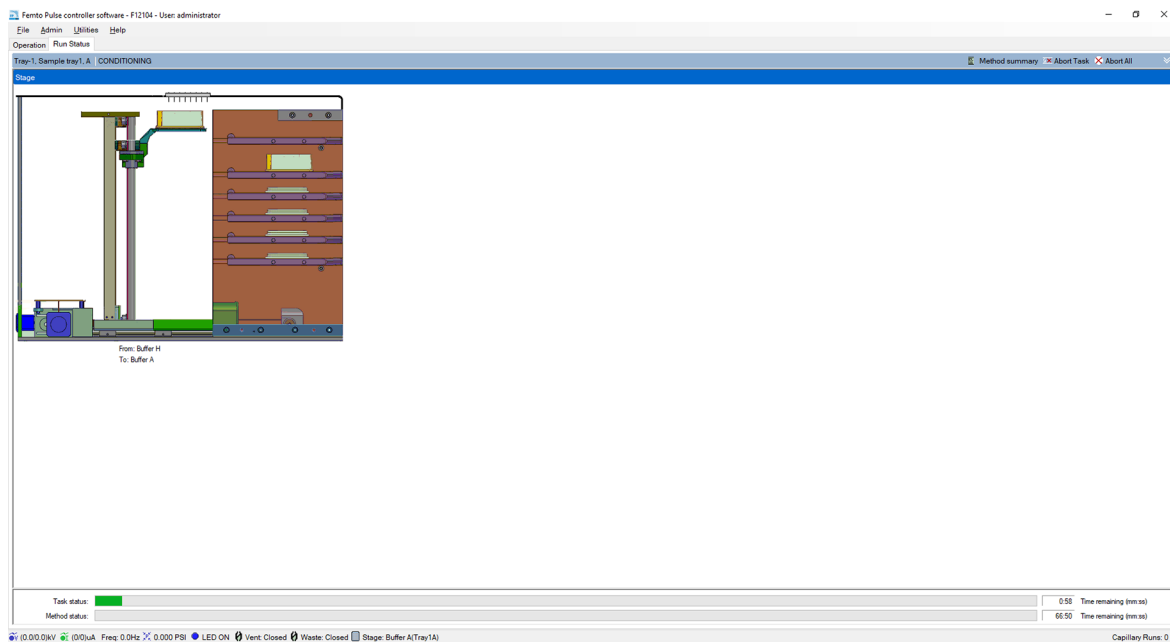


Figure 50 Stage movement animation

Conditioning Animation

When the Femto Pulse instrument is pumping conditioning solution or gel, the animation in **Figure 51** is shown. The animation gives a real-time view of exactly what the instrument is doing during a conditioning sequence.

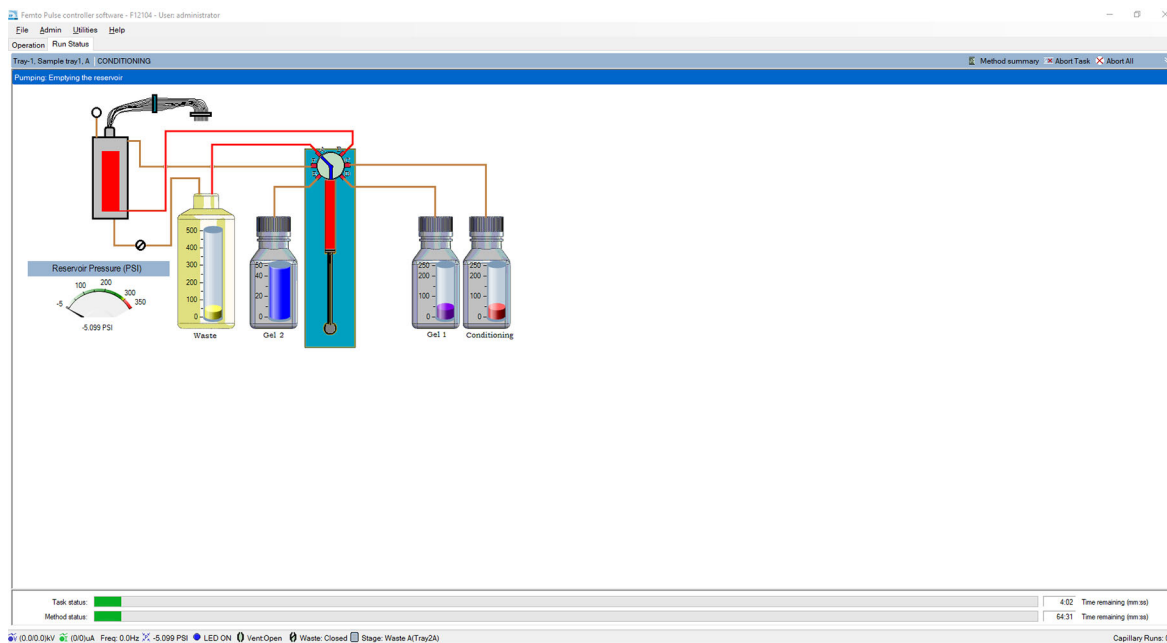


Figure 51 Conditioning animation

Pre-Run / Injection View

When the Femto Pulse instrument is completing a pre-run or injection, the screen in **Figure 52** is shown.

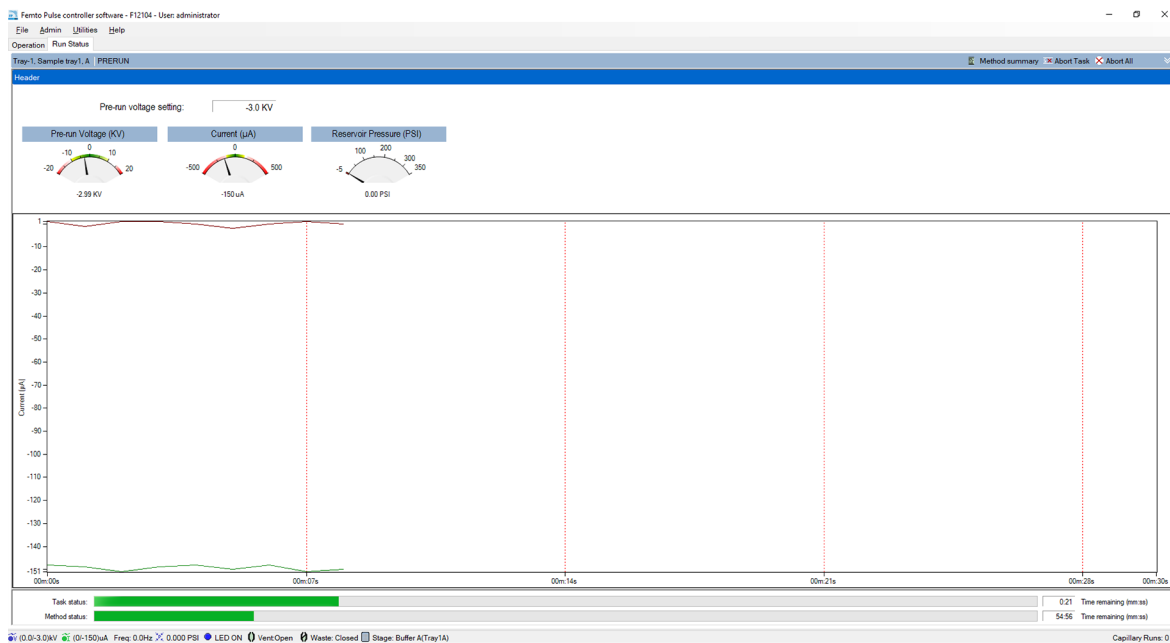


Figure 52 Pre-run/injection screen

Real-time Separation View

When the Femto Pulse instrument starts the separation, the screen shows the real-time view of the separation (**Figure 53**).

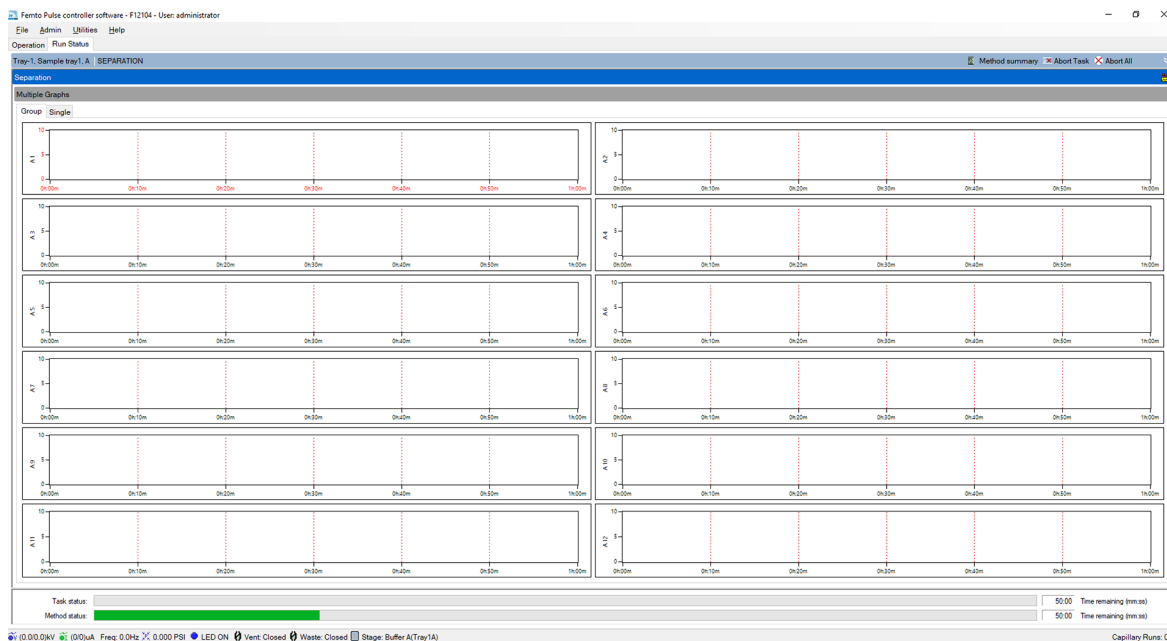


Figure 53 Real-time separation screen





The **Group** tab at the top shows the run in a group of 12 electrophorograms. The **Single** tab shows individual electrophorograms.

NOTE

In order to correctly view the real-time separation data the capillary array must be aligned prior to starting the separation. Refer to **Chapter 4**, “Femto Pulse Software – Utilities Menu” for instructions on aligning the capillary array within the Femto Pulse controller software.

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








Table 18 **Run Status Tab options**

Icon	Description
 Method summary	Opens a popup window showing the method summary for the current method being run.
 Abort Task	Aborts only the individual task being done, i.e. stage movement, pumping, or injection.
 Abort All	Aborts the entire method being run and begins the next method in the queue, if no methods are found returns to the Storage position. When selected the user will be presented with a popup screen asking to verify if they want to abort.
	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 Femto Pulse software screen shows a real time status bar containing important information about the instrument status. These functions are shown in **Table 19**.

Table 19 Instrument status information

Icon	Description
 6.0kV	Left-clicking on this icon will show the voltage level for the last 5 minutes.
 44uA	Left-clicking on this icon will show the current level for the last 5 minutes.
 0.0 PSI	Left-clicking on this icon will show the pressure level for the last 5 minutes.
 LED ON	The blue circle denotes high voltage present and states that the LED is ON. If the circle is gray, the message will read LED OFF.
 Vent: Open	Denotes if the reservoir vent valve is open or closed.
 Waste: Closed	Denotes if the waste valve is open or closed.
 Stage: Buffer A(Tray1A)	Denotes the location of the stage at that point in time.

8

Femto Pulse Capillary Array

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Capillary Array Installation 105

Using the Capillary Array Wet Station for Storage 114

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

Capillary Array Parts

The Femto Pulse instrument capillary array allows for direct parallel injection and separation of 12 or less samples at once.

The capillary array cartridge is located in the upper compartment of the instrument.

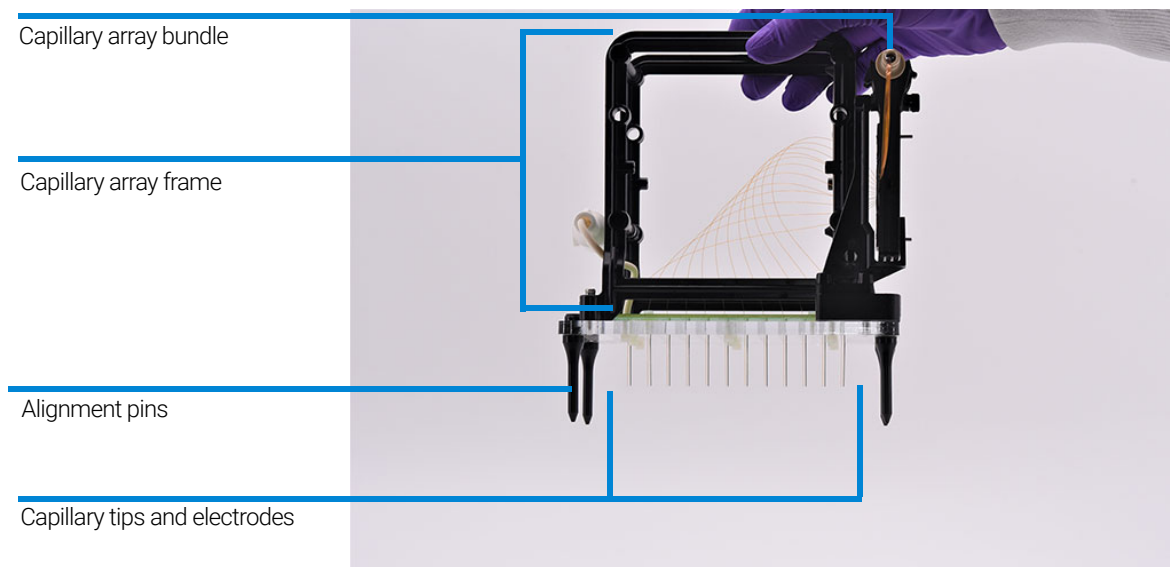


Figure 54 Capillary array parts (12-capillary array shown)

Removal of the Capillary Array

This section provides a guideline to remove the capillary array cartridge from the Femto Pulse 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 55 Femto Pulse instrument

Femto Pulse Capillary Array

Removal of the Capillary Array

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

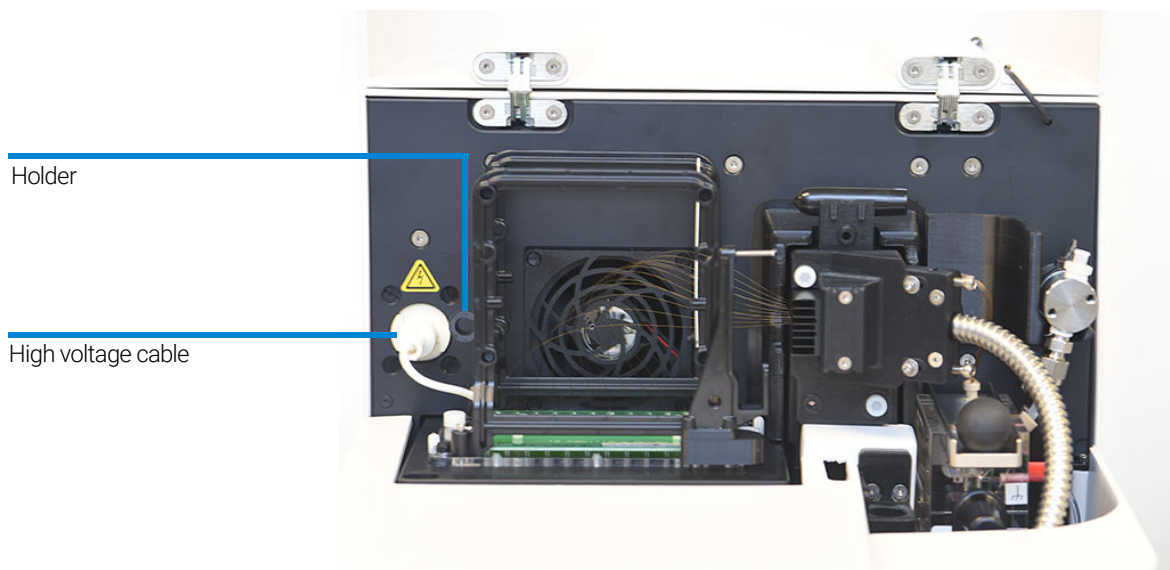


Figure 56 Instrument top compartment – high voltage supply cable

Femto Pulse Capillary Array

Removal of the Capillary Array

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

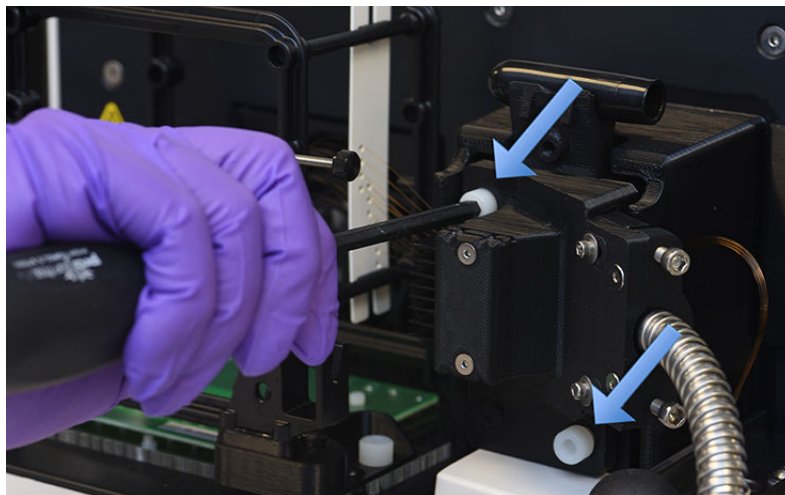


Figure 57 Instrument top compartment – unscrew light guide

- 4 Remove the light guide.

NOTE

Avoid looking directly at the LED light.

Femto Pulse Capillary Array

Removal of the Capillary Array

- 5 Pull back on the reservoir locking slide.

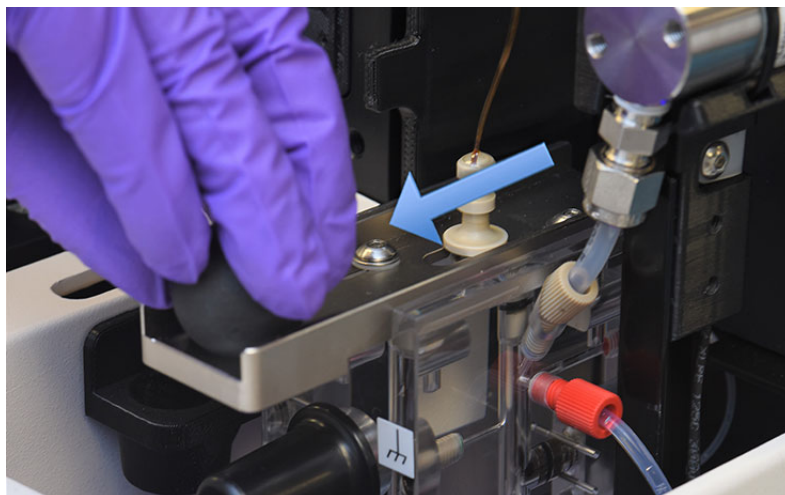


Figure 58 Instrument top compartment – capillary reservoir locking slide

- 6 Use the spindle removal tool to loosen the capillary spindle.

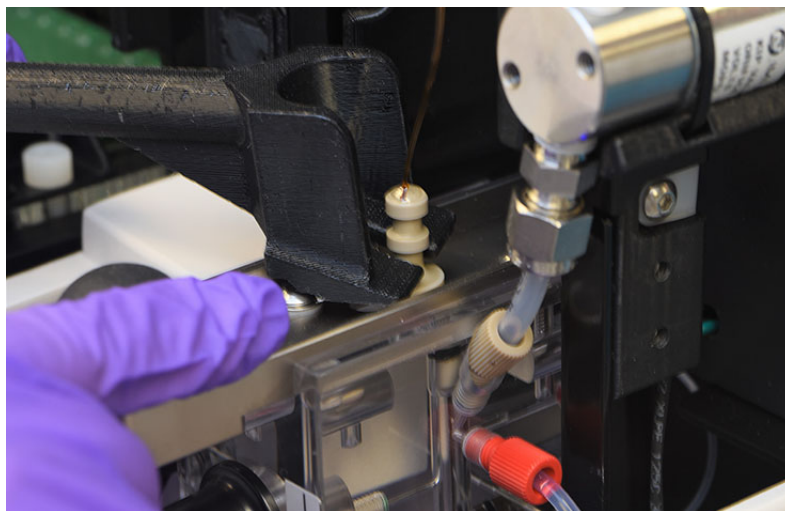


Figure 59 Capillary spindle removal tool

Femto Pulse Capillary Array

Removal of the Capillary Array

- 7 Once the capillary spindle is loosened, pull it up and remove it from the reservoir.

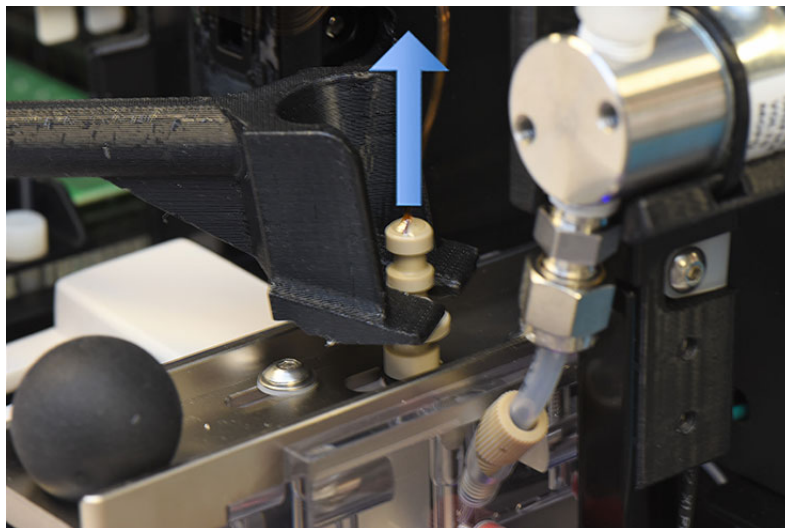


Figure 60 Instrument top compartment – removing capillary spindle

- 8 Carefully insert the protective cover over the capillary spindle.

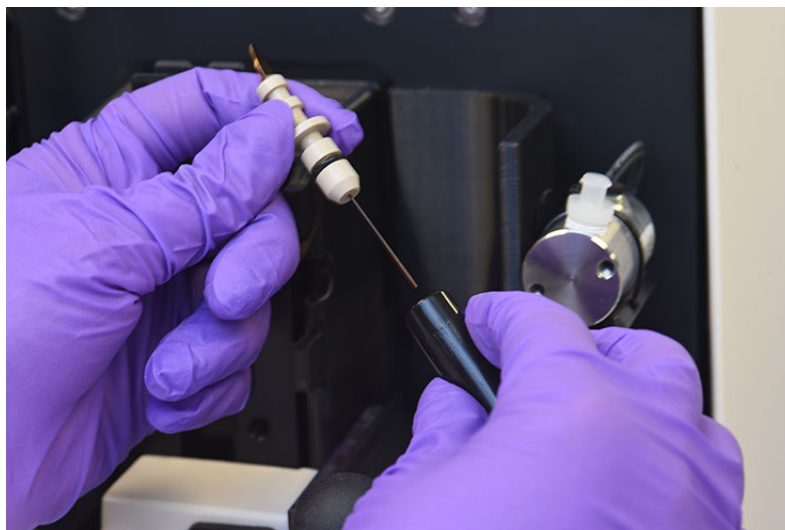


Figure 61 Instrument top compartment – inserting spindle into protective cover

Femto Pulse Capillary Array

Removal of the Capillary Array

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

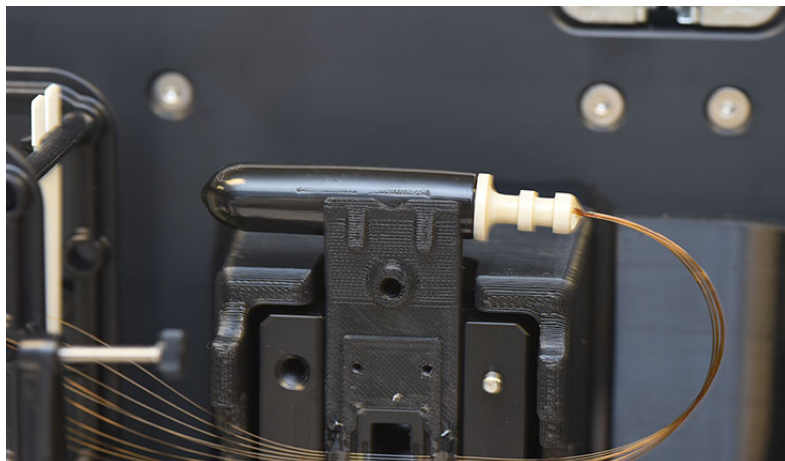


Figure 62 Instrument top compartment – spindle on top of window holder

Femto Pulse Capillary Array

Removal of the Capillary Array

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 spindle goes from the right to the left side of the array frame.

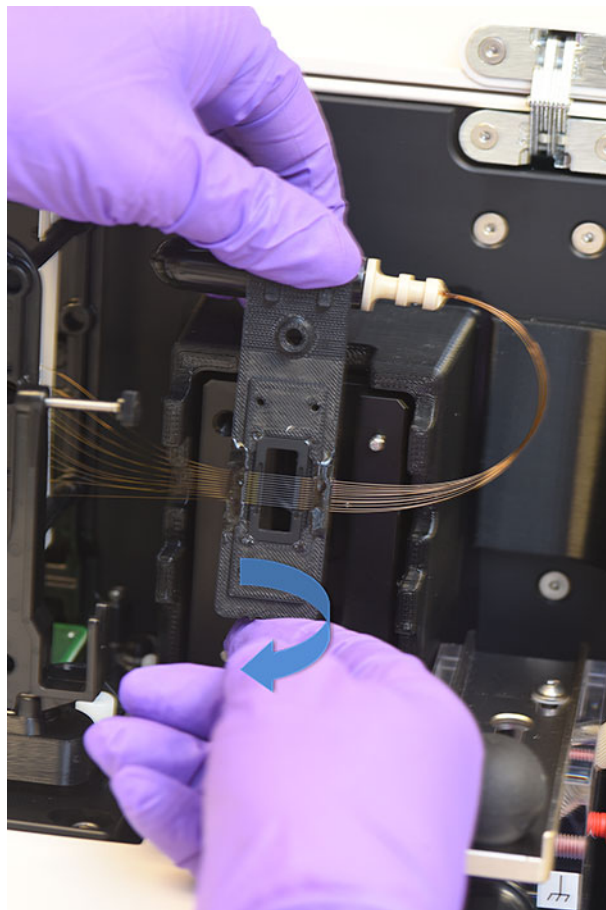


Figure 63 Instrument top compartment – remove capillary array window

Femto Pulse Capillary Array

Removal of the Capillary Array

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

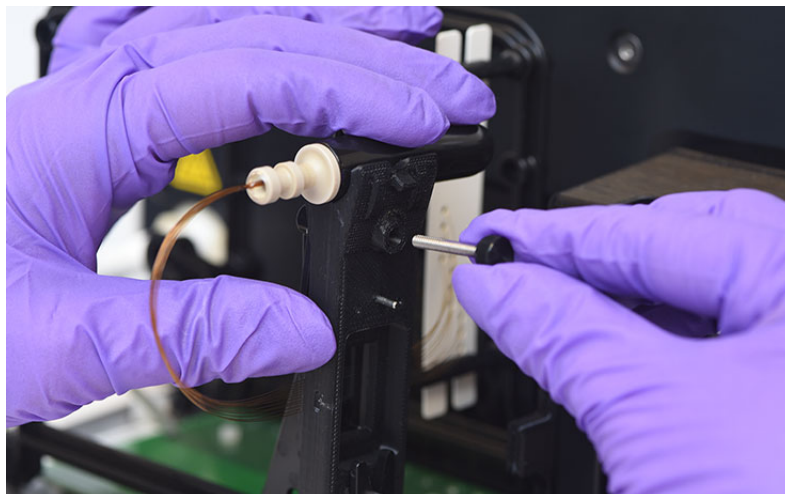


Figure 64 Instrument top compartment – attach array window to capillary array frame

Femto Pulse Capillary Array

Removal of the Capillary Array

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



Figure 65 Instrument top compartment – remove screws that secure array base

Femto Pulse Capillary Array

Removal of the Capillary Array

- 13** Carefully lift the array straight up to remove it from the Femto Pulse instrument.

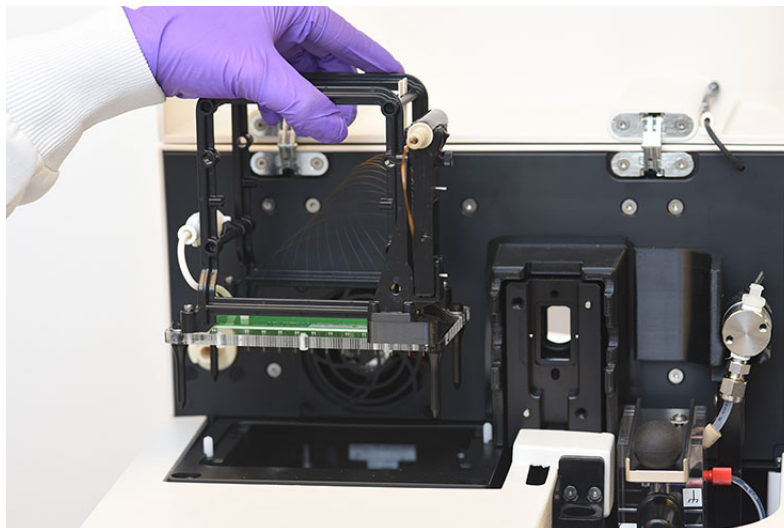


Figure 66 Instrument top compartment – capillary array removal

Once removed from the instrument, the capillary array cartridge is ready for disposal or storage in the Femto Pulse wet station ("**Long Term Capillary Array Storage**" on page 153).

Unpacking a new Capillary Array

This section provides a guideline to unpack a new capillary array from the shipping container and packaging.

NOTE

Save the original packaging when receiving a new capillary array cartridge. This packaging is necessary for proper shipment of a capillary array in the event of a return.

- 1 Unpack the new capillary array:
 - a Open the capillary array shipping box.
 - b Remove the foam cover.
 - c Remove the packaged capillary array from the shipping box.



Figure 67 Capillary array shipping box

Femto Pulse Capillary Array

Unpacking a new Capillary Array

- 2 Remove the plastic shipping cover from the capillary array.
Take care not to break capillaries or touch the array window when removing packaging.

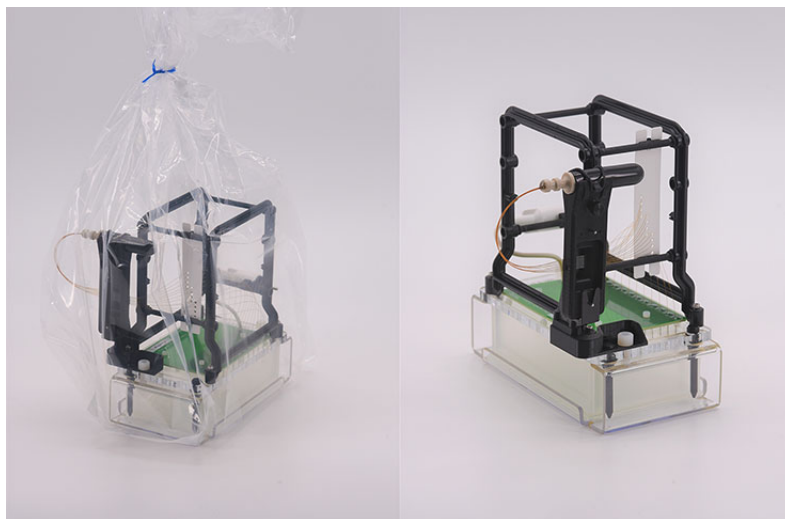


Figure 68 Remove plastic shipping cover

- 3 Remove the tape on the left and right side of the array shipment frame securing the array to the shipment frame.

Femto Pulse Capillary Array

Unpacking a new Capillary Array

- 4 Remove the rubber band securing the array spindle.

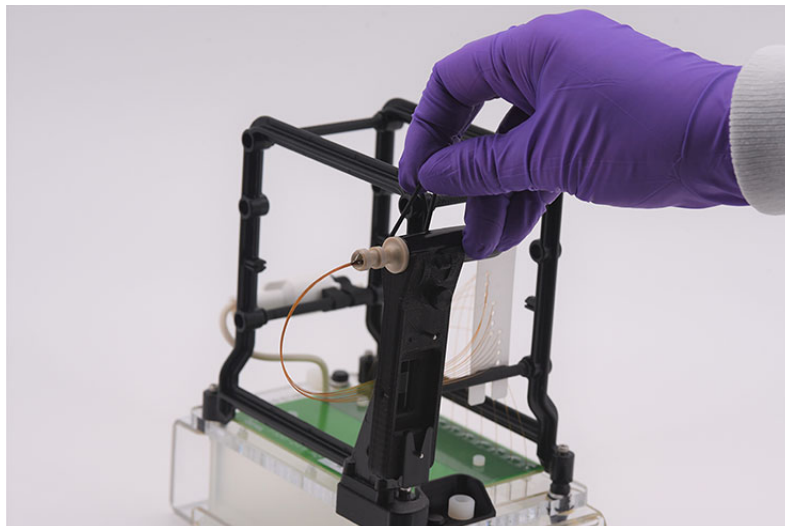


Figure 69 Remove securing rubber band

- 5 Remove the screws that secure the array to the shipment frame.

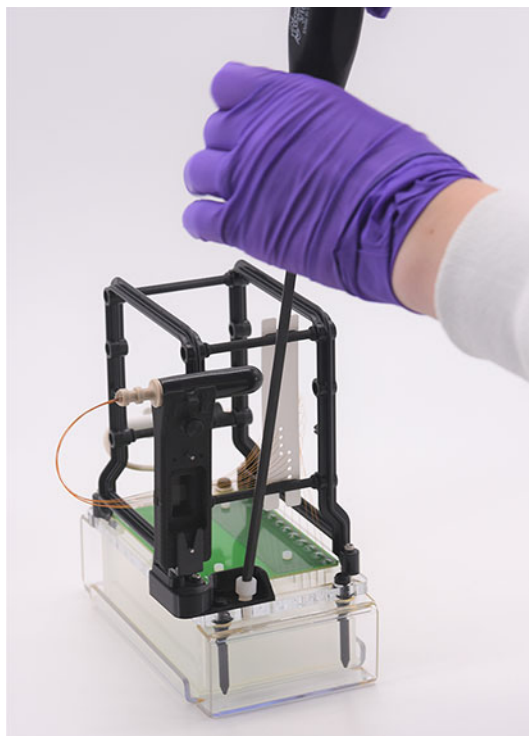


Figure 70 Shipment frame screws

Femto Pulse Capillary Array

Unpacking a new Capillary Array

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

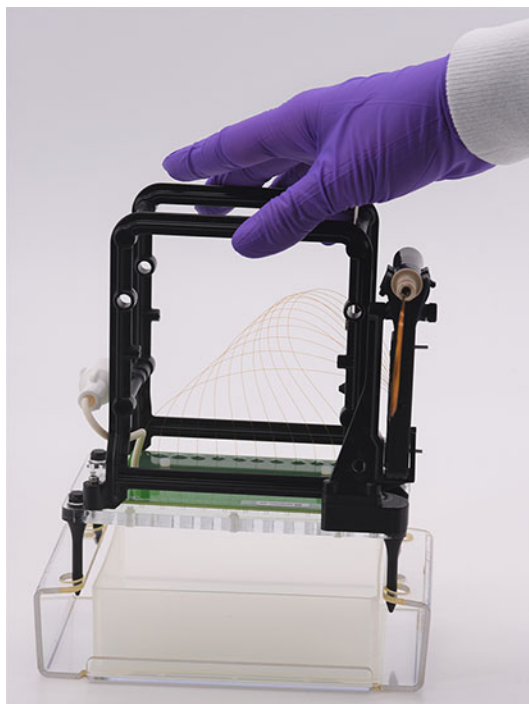


Figure 71 Removal of array from shipment frame

Capillary Array Installation

This section will provide a guideline to install a capillary array cartridge into the Femto Pulse 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 72 Femto Pulse Instrument

Femto Pulse Capillary Array

Capillary Array Installation

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

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

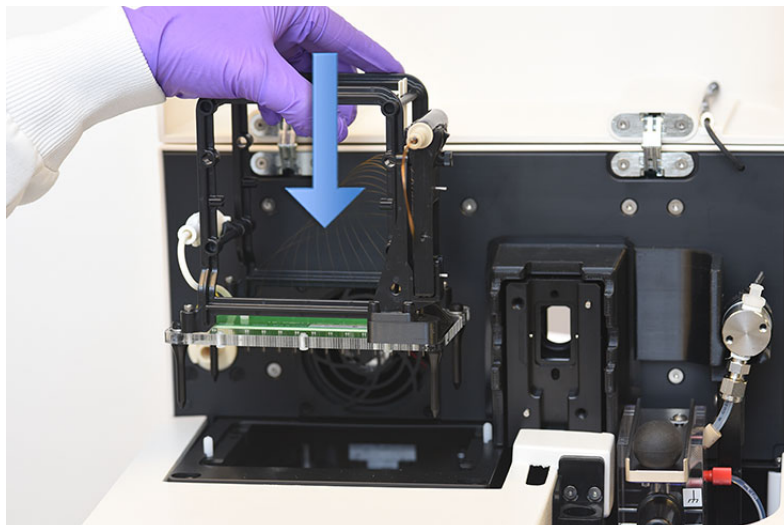


Figure 73 Capillary array installation

Femto Pulse Capillary Array

Capillary Array Installation

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



Figure 74 Array attachment screw installation

- 4 Remove the array window attachment screw.

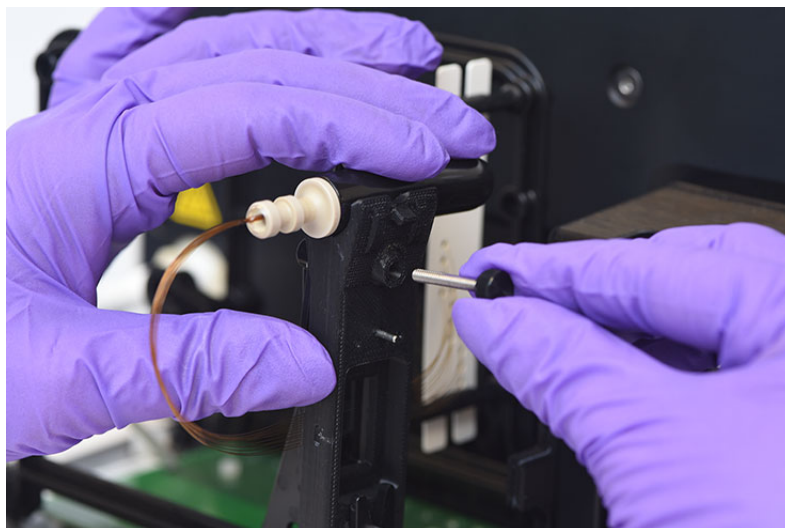


Figure 75 Remove array window

- 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.

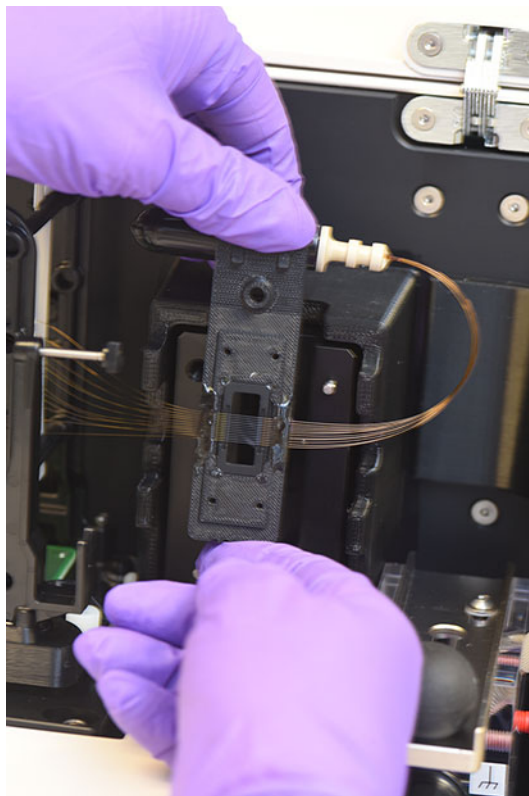


Figure 76 Array window placement

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

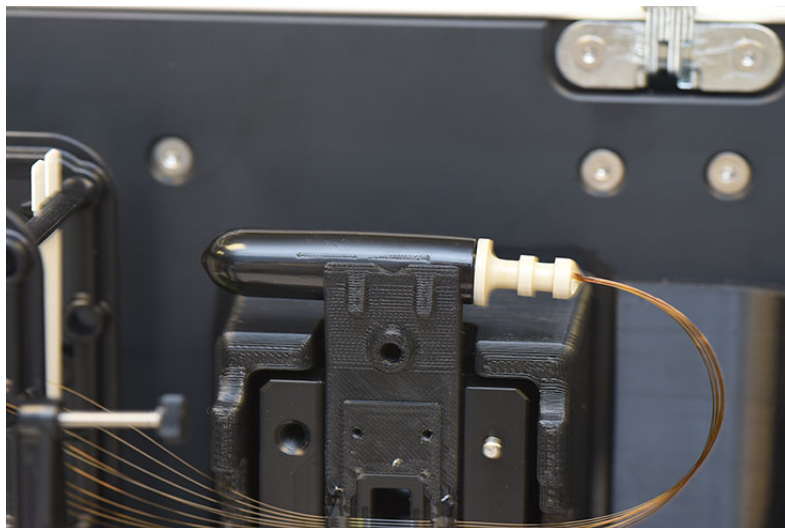


Figure 77 Capillary array bundle placement

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

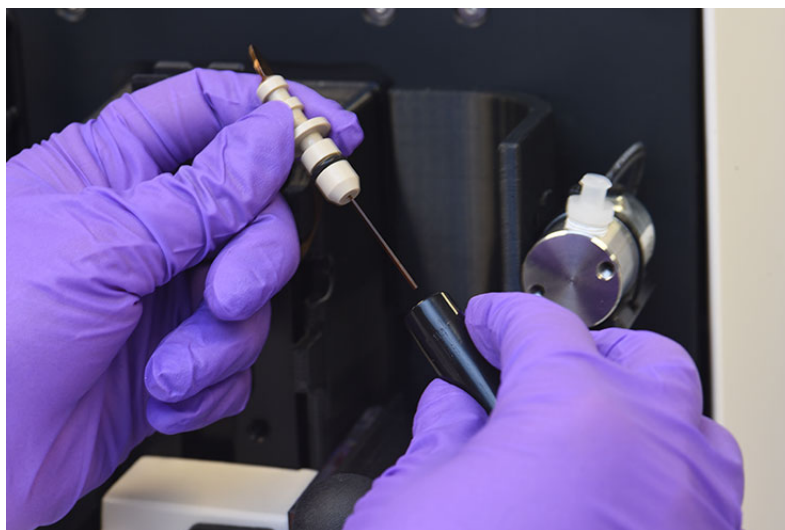


Figure 78 Removal of protective cover

- 8 Install the capillary array bundle by firmly pushing the capillary array bundle into the reservoir opening until a distinct click is heard.
- 9 Push in the capillary reservoir connector slide to secure the capillary array bundle.

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

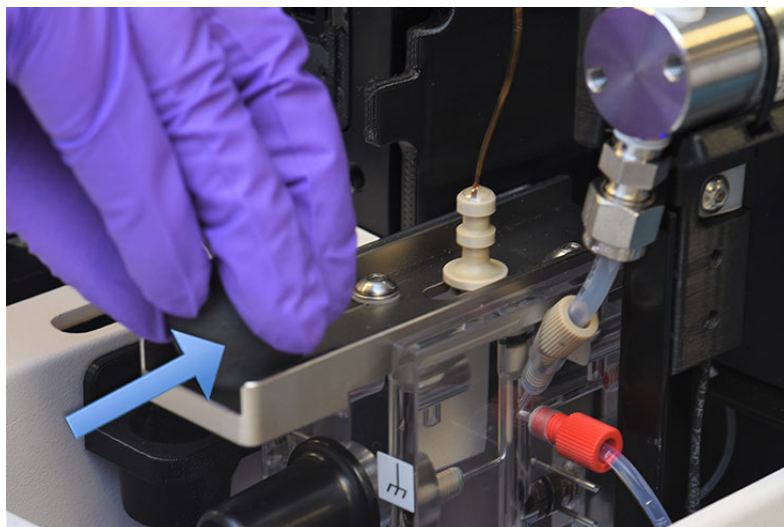


Figure 79 Capillary reservoir connector slide

- 10 Place the light guide over the array window using the two alignment pins. The steel optical cable should be on the right.

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

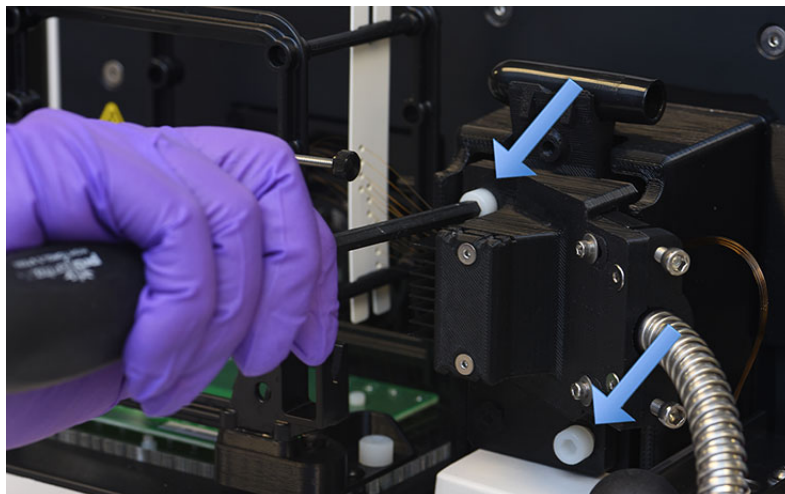


Figure 80 Light guide installation

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

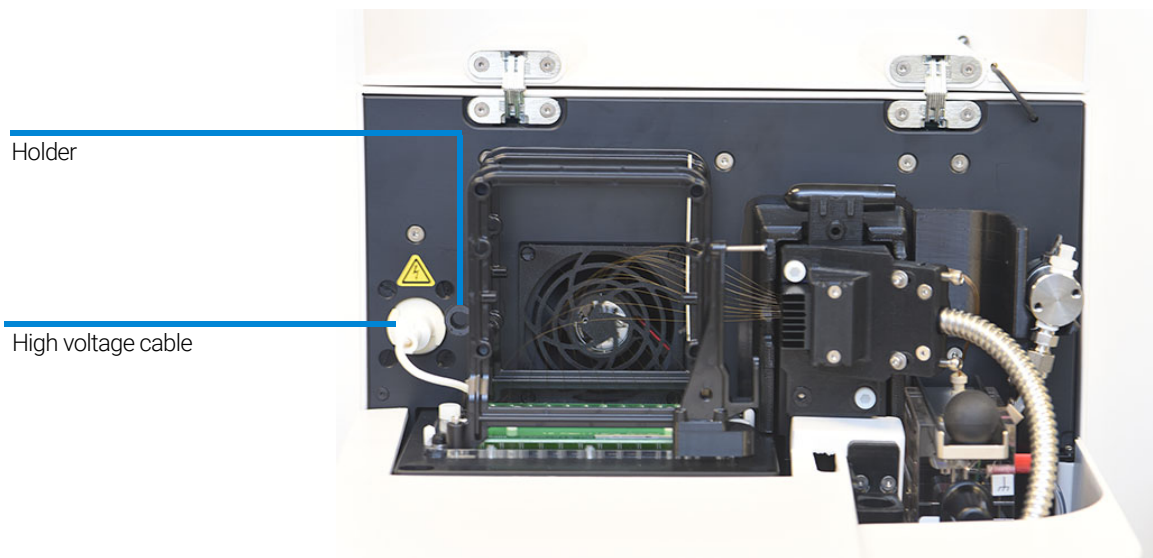


Figure 81 High voltage supply cable

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



Figure 82 Femto Pulse instrument

After installation of the capillary array, the Femto Pulse will require a capillary alignment as described in **Chapter 4**, “Femto Pulse Software – Utilities Menu”.

Using the Capillary Array Wet Station for Storage

For information about capillary array storage, refer to “**Long Term Capillary Array Storage**” on page 153.

9

Femto Pulse – Sample Name Entry

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Importing Sample Names Using a Bar-Code Reader 119

This chapter provides information on how to enter the sample names in the Femto Pulse 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 **Save tray** or **Save selected row** to save the file as .txt or .csv (**Figure 83**).

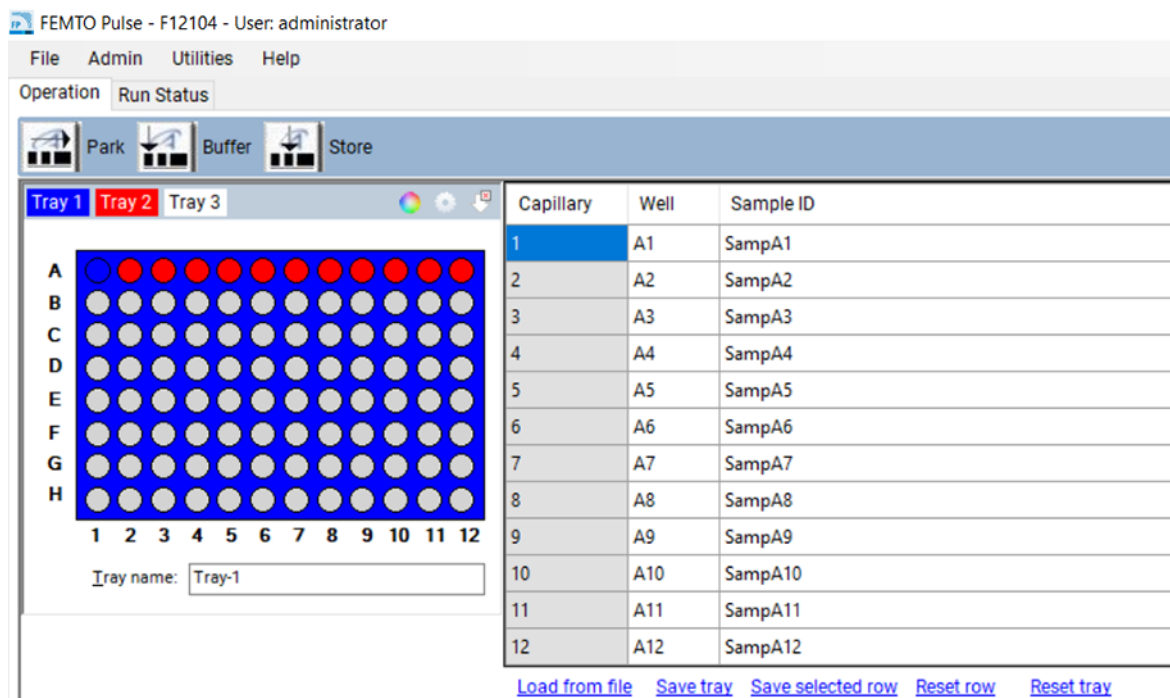


Figure 83 Adding sample names manually

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, a single column of sample names is used (**Figure 84**).

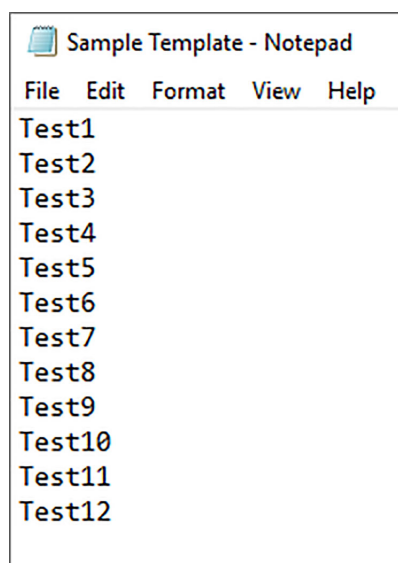


Figure 84 .txt file format (single row of names—no well numbers or row numbers).

- For a .csv file, the format is row number, well number, and sample name (Figure 85).

	A	B	C	D	E	F
1	1	A1	AA001			
2	2	A2	AA002			
3	3	A3	AA003			
4	4	A4	AA004			
5	5	A5	AA005			
6	6	A6	AA006			
7	7	A7	AA007			
8	8	A8	AA008			
9	9	A9	AA009			
10	10	A10	AA010			
11	11	A11	AA011			
12	12	A12	AA012			
13	13	B1	SampleB1			
14	14	B2	SampleB2			
15	15	B3	SampleB3			
16	16	B4	SampleB4			
17	17	B5	SampleB5			
18	18	B6	SampleB6			

Figure 85 .csv file format: row number, well number, 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.

- 1 Place the sample name files into the C:\Agilent Technologies\Samples folder (**Figure 86**). 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 117).

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

Name	Type
Data	File folder
Femto Pulse	File folder
Methods	File folder
Samples	File folder
Training Videos	File folder
User Manual	File folder

Figure 86 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 87**, the name associated with the bar-code is 00060065.



Figure 87 Bar-code name 00060065

Femto Pulse – Sample Name Entry

Importing Sample Names Using a Bar-Code Reader

Thus, the .csv file or .txt file must be given the file name 00060065 and located in the Samples folder (**Figure 88**).

Name	Type	Size
Sample Names Template - CSV File - Enter Names in ...	Microsoft Excel Comma...	1 KB
Sample Names Template - txt File	Text Document	1 KB
00060065.txt	Text Document	0 KB

Figure 88 File name

- 2 In the field **Tray name** of the **Operation** tab, highlight the tray name with the mouse cursor (**Figure 89**).

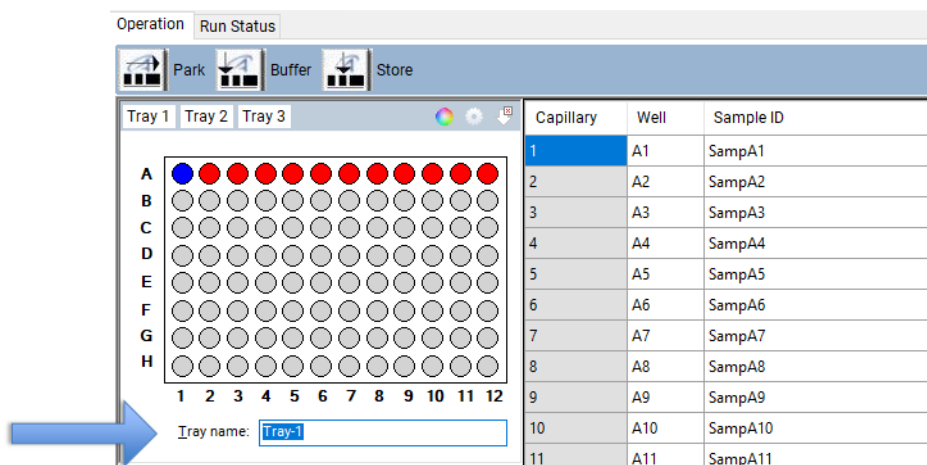


Figure 89 Highlight of the tray name

Femto Pulse – Sample Name Entry

Importing Sample Names Using a Bar-Code Reader

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

The names are automatically imported from the .txt or .csv file in the *Samples* folder (**Figure 90**).

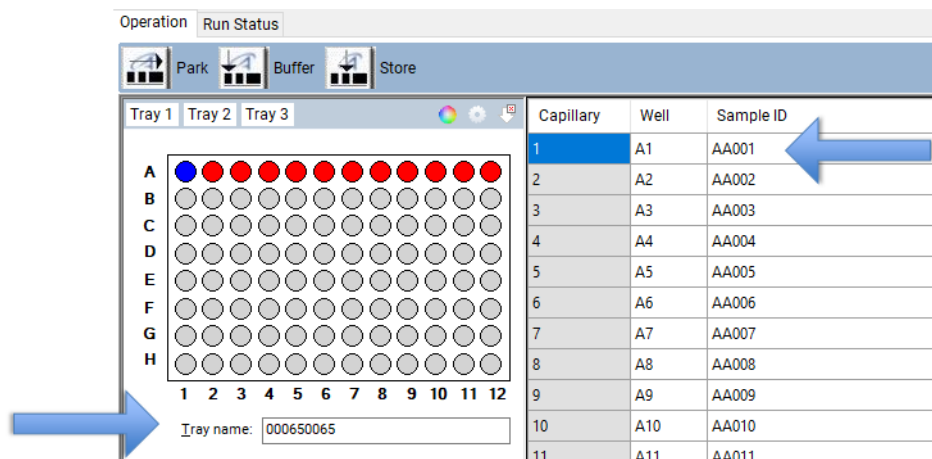


Figure 90 Imported sample names



10

Femto Pulse – Automated Analysis

Automated Analysis Using Femto Pulse 123

Enabling Automated Analysis 124

Monitoring the Status of the Automated Processed Data 130

This chapter explains the procedure for automated analysis using Femto Pulse.

Automated Analysis Using Femto Pulse

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

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

Enabling Automated Analysis

- 1 From the **Admin** drop-down menu, select **Results report setup** (Figure 91).

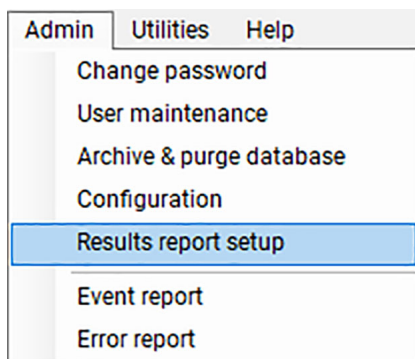


Figure 91 Admin menu

This will open the **Automated Report Settings** window (Figure 92).

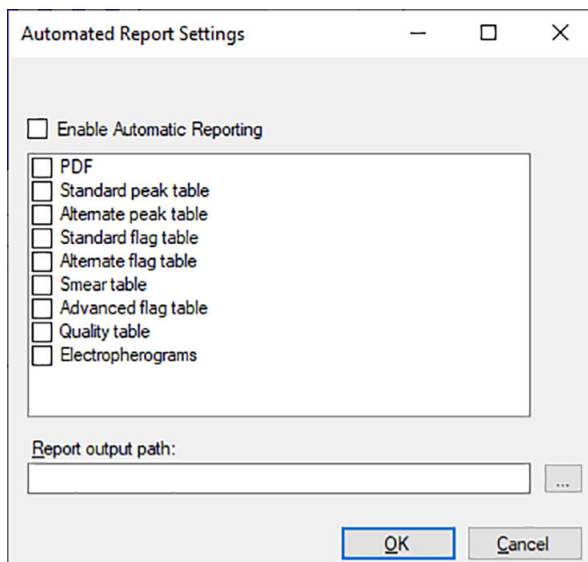


Figure 92 Automated Report Settings window

- 2 To enable automated analysis, select **Enable Automatic Reporting** (Figure 92).

3 Select the desired export options (PDF, etc.).

Each of the export options (PDF, Standard peak table, etc.) are described in the Chapter 7, “Exporting Data from ProSize”, and Chapter 8, “Generating Reports from ProSize” in 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, two main criteria must be met:

- The name of the method in the Femto Pulse 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 **FP-1101-22 – US NGS**, then the configuration file in ProSize must have the name: **FP-1101-22 – US NGS**.

- 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 the ladder well must be of high quality, without anomalous or missing peaks.

For example, assume a 100 bp ladder is used in well A12, but the configuration file in ProSize is set so that the minimum peak height for integration of the ladder is 500 units. In this case, the ladder is not read correctly by ProSize because several ladder elements are missing (**Figure 93**). In this case, the file will not be auto-processed by the Femto Pulse system.

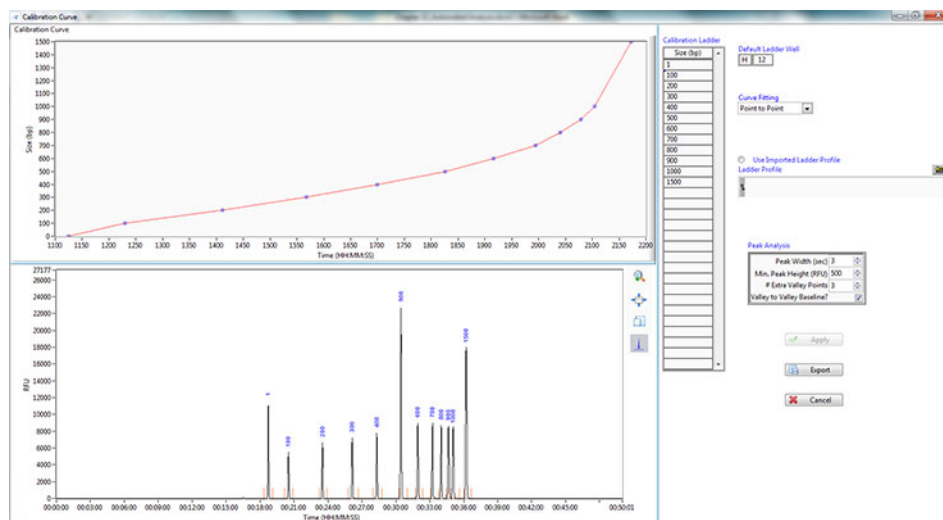


Figure 93 ProSize calibration curve setup

If the configuration file is set with a minimum peak height of 100, then the ladder is processed correctly by ProSize, and all the ladder elements are recognized (**Figure 94**).

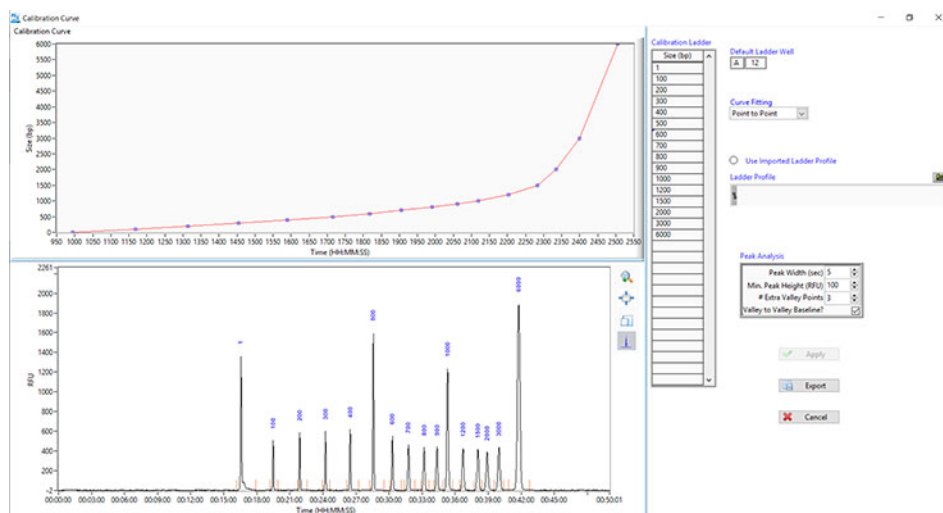


Figure 94 ProSize calibration curve setup

Importing a Ladder File for Automated Analysis

The Femto Pulse 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 500 to 100 in the configuration file using ProSize.

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

- You can use all 12 wells of the sample row, without having to reserve well 12 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).

Using Femto Pulse to Export a Ladder File and Use an Imported Ladder File

- 1 Turn on the auto-processing feature (for details, see “**Enabling Automated Analysis**” on page 124).
- 2 When running a sample, select **Add tray to queue**.
The **Separation setup** window opens (Figure 95).

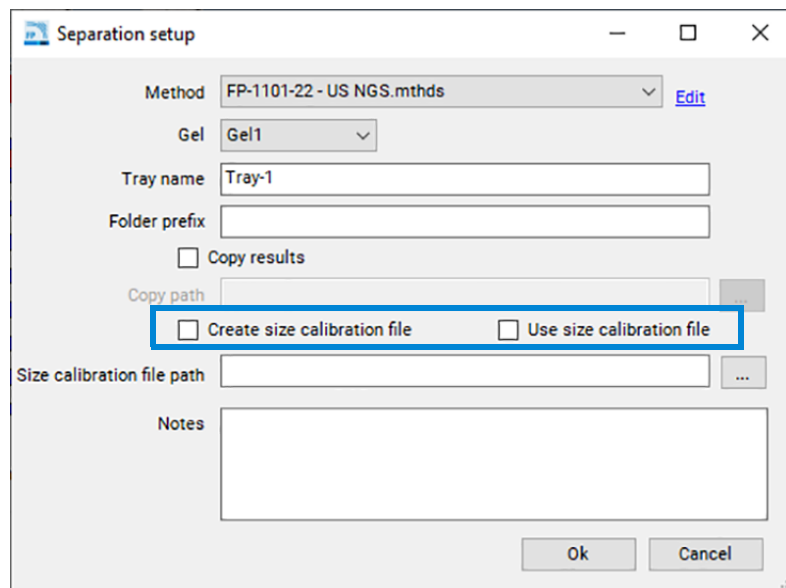


Figure 95 Separation setup window

- 3 Select **Create size calibration file** to create or export a ladder file (.SCAL). At the end of the run, the program automatically uses ProSize to create the file. For the given run, the ladder must be located in well 12 (the last well in the row).

Or

Select **Use size calibration file** to import a previously saved ladder file and apply the .SCAL file to the run. This will automatically process the desired data (users often run a size calibration ladder once per day, and then use the saved, exported calibration ladder for all subsequent runs of the day).

- 4 Under **Size calibration file path**, enter the location you wish to save the calibration file.

For more information on size calibration files and how these are created in ProSize, refer to Chapter 5 “ProSize Size Calibration Screen” in the *ProSize Data Analysis Software User Manual*.

NOTE

If both boxes are unchecked, the program assumes that the ladder well is located in well A12 or H12 (as defined by the Kit manuals). In this case a ladder will not be imported, but instead an internal ladder will be used (which is the normal mode of operation).

Monitoring the Status of the Automated Processed Data

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

- 1 From the **Utilities** drop-down menu, select **Results dashboard** (Figure 96).

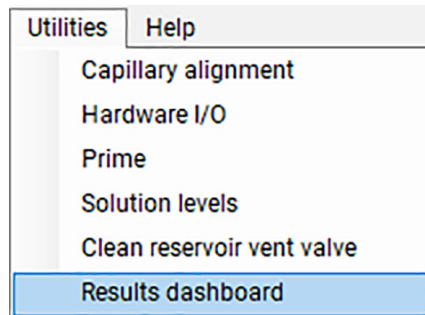


Figure 96 Utilities menu

The **Results Dashboard** window opens. The data files are listed (Figure 97).

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

Figure 97 Results dashboard

- 2 Right-click a file.

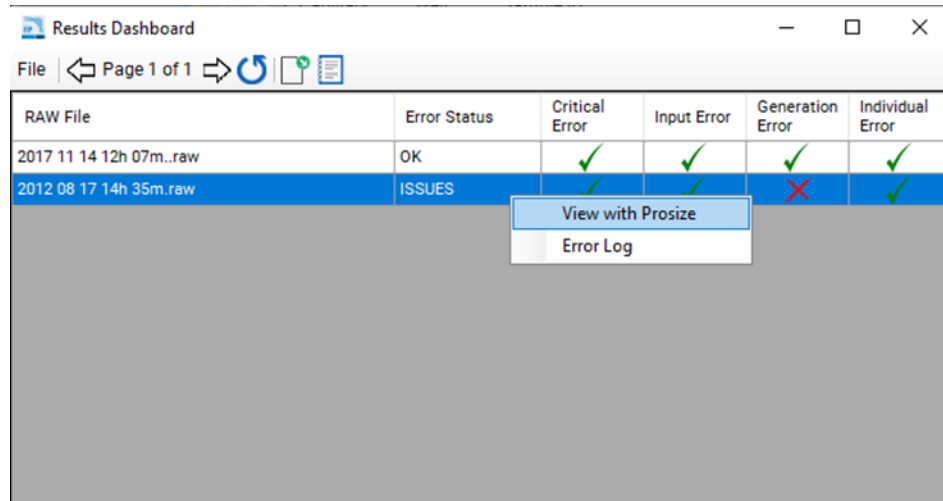


Figure 98 Results Dashboard

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

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

Table 20 Results Dashboard Error Messages

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

The error messages are also recorded in C:\ProSize data analysis software\Error Log. An example error log file is shown in **Figure 99**.

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

A screenshot of a Notepad window titled "2019 08 29 10H 23M - Notepad". The window has a menu bar with "File", "Edit", "Format", "View", and "Help". The text content is as follows:

```
[File Path]
File Path = "C:\Agilent Technologies\Data\2019 08 29\10-23-29\2019 08 29 10H 23M.raw"

[Critical Error]
Error 4 = "Error on sizing calibration"
```

Figure 99 Example error message



11

Femto Pulse – Using the Bar-Code Reader

Changing Levels of Gels 134

Changing Levels of Water or 1N NaOH 137

Changing Levels of Conditioning Solution or Waste 138

This chapter explains how to add and remove solutions to the gel and conditioning solution bottles using the bar-code reader.

Changing Levels of Gels

There are three general circumstances for which gel levels require adjustment:

- You add gel to the system for the first time.
- The system is running low on gel, and the software prompts you to add more gel.
- You switch a gel (remove one gel and replace it with another).

NOTE

When the system is low on gel, the software automatically opens the dialog **Check solution volumes** as shown in **Figure 101**.

- 1 From the **Utilities** drop-down menu, select **Solution levels** (**Figure 100**).

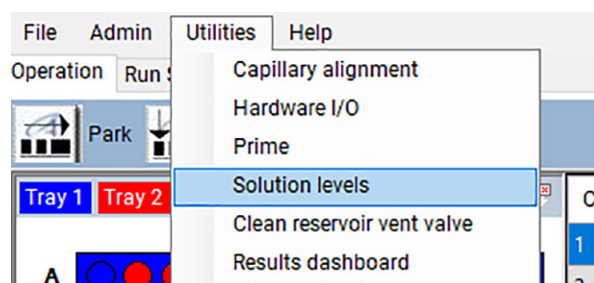
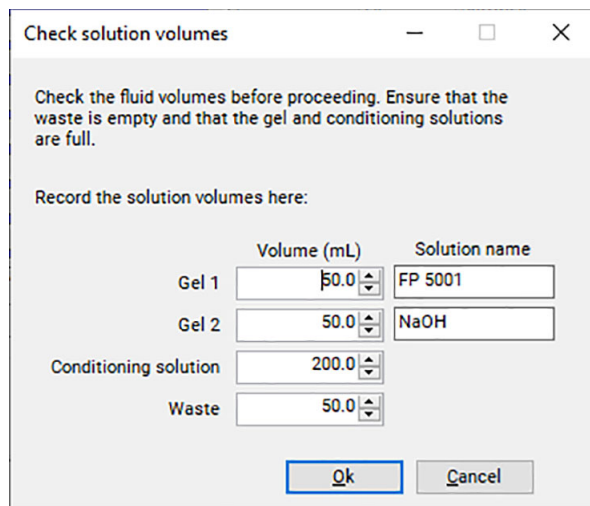


Figure 100 Solution levels utility

The dialog **Check solution volumes** opens.



Check solution volumes

Check the fluid volumes before proceeding. Ensure that the waste is empty and that the gel and conditioning solutions are full.

Record the solution volumes here:

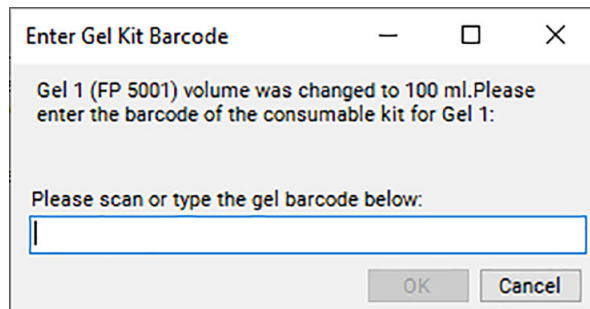
	Volume (mL)	Solution name
Gel 1	50.0	FP 5001
Gel 2	50.0	NaOH
Conditioning solution	200.0	
Waste	50.0	

Ok Cancel

Figure 101 Check solution volumes dialog

- 2 In the dialog, enter the volumes of the gel and conditioning solutions (based on the volume in the gel bottle).

A prompt opens to enter a bar-code (**Figure 102**).



Enter Gel Kit Barcode

Gel 1 (FP 5001) volume was changed to 100 ml. Please enter the barcode of the consumable kit for Gel 1:

Please scan or type the gel barcode below:

OK Cancel

Figure 102 Prompt to enter bar-code

- 3 Use the bar-code reader to enter the bar-code into the system (**Figure 103**).



Figure 103 Scanning the bar code of the gel

- 4 Once the bar-code is accepted, the software will respond with an acknowledgment that the bar-code was accepted (or rejected) (**Figure 104**).

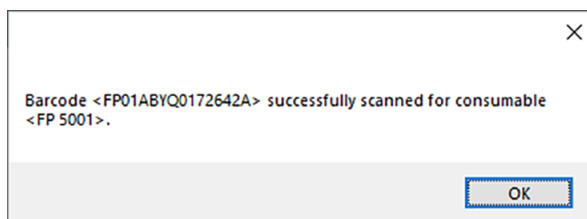


Figure 104 Acknowledgment of bar code entry

The bar-code is only good for the amount of gel sold in the bottle (500 mL). Once 500 mL of gel is exceeded, the bar-code will no longer be accepted by the system. Thus, a rejection of a bar-code is due to the fact that the code is no longer valid, and that the volume allowance for that bar-code has been exceeded.

Changing Levels of Water or 1N NaOH

When **Water** or **NaOH** are selected as the solution type (**Figure 105**), a bar-code is required to change these levels.

The use of the bar-code for **Water** and **NaOH** is not limited to one-time use as with sales bottles. Their bar-codes are located on the inside door of the Femto Pulse instrument (**Figure 105**).

- 1 When prompted for a bar-code, use the bar-code scanner on the bar-codes attached to the inner door.



Figure 105 Bar codes for NaOH and water

Changing Levels of Conditioning Solution or Waste

When changing the level of conditioning solution or waste, simply change the levels in the respective fields (**Conditioning solution, Waste**) as indicated in **Figure 101**, and close the window. No bar-code entry is required for these components.

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Long Term Capillary Array Storage 153

Using the array docking station 153

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

Permissible Characters

The following tables show which characters are permissible (**Table 21**) and non-permissible (**Table 22**) for a file name.

Table 21 Permissible characters for a file name

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

Table 22 Non-permissible characters for a file name

Characters	
*	
\	:
"	'
<	>
?	/

Compatible Plates and Tubes

Sample/Marker Plates

The Femto Pulse system has been designed to operate using specific dimensioned semi-skirted PCR plates and deep 96-well plates. A list of approved semi-skirted PCR plates is provided in **Table 23**.

NOTE

Currently the Femto Pulse is only validated with the below Eppendorf plates.

Table 23 List of approved PCR plates

Approved Vendor/Part Number	Description
Eppendorf # 951020303 (various colors)	Eppendorf 96-Well twin.tec PCR Plates, Semi-skirted

NOTE

Contact your corresponding Agilent Sales/Service Representative if a different vendor or style of PCR plate is to be used in order to verify compatibility. The use of PCR plates with different dimensions than the above recommended plate could possibly damage the tips of the capillary array cartridge.

Buffer/Waste Plates

The Femto Pulse system uses a specific deep 96-well plate (31 mm height) supplied by Fisher Scientific (part #12-566-120) for the buffer and waste plate. This specific plate must be used with the instrument (two plates are supplied upon installation) (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 Femto Pulse system, as damage to the capillary array will occur.

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

Table 24 List of Buffer/Waste Plate

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

Preventative Maintenance Schedule

Daily Maintenance

- ✓ Empty the waste bottle and waste tray.
- ✓ Replace the inlet buffer in the buffer tray position.
- ✓ 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.
- ✓ Inspect the capillary array vent valve for dried gel, clean if necessary.

As Needed to Restore Separation Performance

- ✓ Place 0.6 mL of 0.5N NaOH into the waste tray location and flush the capillary array cartridge with 0.5N NaOH followed by DI water as described in section **“Capillary Array Cleaning”** on page 144.**

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

** It is fine to perform this cleaning performance as a part of a regular weekly or biweekly cleaning schedule as well.

Capillary Array Cleaning

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

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



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

Method A – Flush with CE Grade Water

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

- 1 From the **Operation** tab located on the main screen window, select **Add to queue** under the Capillary array - conditioning menu.
- 2 From the drop-down menu of the **Select Conditioning Method** window, select **Method A Flush - Water - 10 min 200 psi.mthdc**.
- 3 Select **Edit** to ensure that the method matches the parameters shown in **Figure 106**.

Conditioning Method: Method A Flush - Water - 10 min 200 psi.mthdc

✓   ✕

☒ **Step #1** Solution **Gel 2** ▼

Fill pressure **200** ▼ PSI Time **10.0** ▼ min

Flow rate **200** ▼ μL/s Tray **Waste** ▼ Row **A** ▼

☐ **Step #2** Solution **Conditioning** ▼

Fill pressure **200** ▼ PSI Time **10.0** ▼ min

Flow rate **200** ▼ μL/s Tray **Waste** ▼ Row **A** ▼


☐ **Step #3** Solution **Conditioning** ▼

Fill pressure **0** ▼ PSI Time **1.0** ▼ min

Flow rate **1** ▼ μL/s Tray **Waste** ▼ Row **A** ▼

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

- 4 If necessary, adjust the method to that shown in **Figure 106** (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 Femto Pulse side compartment to replace the gel 2 bottle with a bottle containing CE grade water.

Minimum solution volume required to run Method A Flush for 10 minutes is ≥ 12 mL for a 12-capillary array.
- 9 Close the door of the side compartment, and select **Start**  from the method queue to run the capillary conditioning method (for more information, refer to section “**Method Queue**” on page 78).
- 10 Once the capillary array conditioning method is complete, open the waste drawer and remove the 96-well deep well plate.

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

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

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

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

- 1 Empty the 96-Well Deep Well Plate and return it to the waste drawer.
- 2 Open the side compartment of the Femto Pulse instrument and replace the bottle containing CE grade water with an empty bottle and perform a capillary conditioning to remove any residual water from the capillaries.

If a well has significantly less or no water is present in a well, Method A can be repeated, or proceed to Method B or C.

Method B – Submerge 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.
- 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 an 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.

-
- 1 From the **Operation** tab located on the main screen window, select **Add to queue** under the capillary array- conditioning menu.
 - 2 From the drop-down menu of the **Select Conditioning Method** window, select the **Method C Flush - 0.5 N NaOH - 10 min 200 psi.mthdc**.
 - 3 Select **Edit** to ensure that the method matches the parameters shown in **Figure 107**.

Conditioning Method: Method C Flush - 0.5 N NaOH - 10 min 200 psi.mthdc

✓   ✗

☒ Step #1 Solution: Gel 2

Fill pressure: 200 PSI Time: 10.0 min

Flow rate: 200 $\mu\text{L/s}$ Tray: Waste Row: A

☒ Step #2 Solution: Conditioning

Fill pressure: 200 PSI Time: 10.0 min


Flow rate: 200 $\mu\text{L/s}$ Tray: Waste Row: A

☐ Step #3 Solution: Conditioning

Fill pressure: 0 PSI Time: 1.0 min

Flow rate: 1 $\mu\text{L/s}$ Tray: Waste Row: A

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

- 4 If necessary, adjust the method to that shown in the **Figure 107** (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 a 96-Well Deep Well Plate filled with 0.6 mL per well of 0.5N NaOH in Row A for a 12-capillary array.
- 8 Open the Femto Pulse side compartment and replace the gel 2 bottle with a bottle containing 0.5N NaOH. The minimum solution volumes required to run the default capillary conditioning method for 10 minutes is ≥ 10 mL for a 12-capillary array.
- 9 Close the door of the side compartment, and select **Start**  from the method queue to run the capillary conditioning method (for more information, refer to section **"Method Queue"** on page 78).

- 10** Once the capillary array conditioning method is complete, open the waste drawer and remove the 96-Well Deep Well Plate. Check the volume of solution present in each of the wells.

The waste tray wells will be full. Ensure all wells have a similar amount of waste present.

- 11** Empty the 96-Well Deep Well Plate in the proper aqueous waste disposal area and return it to the waste drawer (second drawer from the top).

CAUTION

0.5N NaOH is corrosive

0.5N NaOH can damage the capillary array.

- ✓ Perform a separation with a Full Conditioning or a flush with separation gel immediately after a 0.5N NaOH flush.



Figure 108 Capillary damage caused by NaOH overexposure

Reservoir Vent Valve Cleaning

Over time the reservoir vent valve may become clogged requiring cleaning. The Femto Pulse system 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 109**).

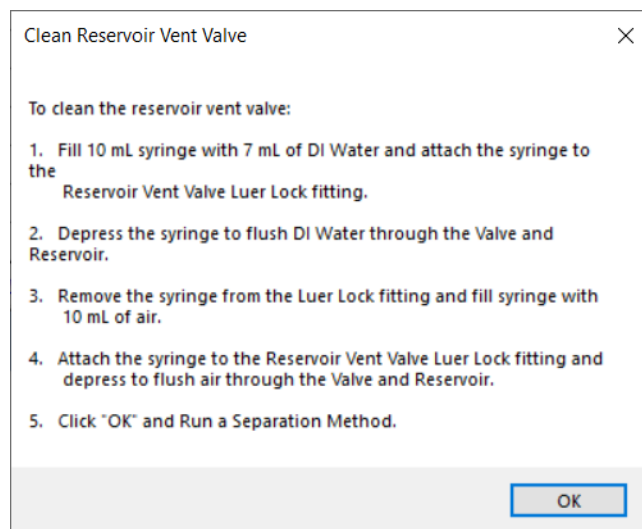


Figure 109 Clean reservoir vent valve window

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

Capillary Array Window Cleaning

- 1 Open the side door and hood of the Femto Pulse 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. The Femto Pulse window is highly fragile!

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 4-8. Otherwise, proceed to step 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 towel behind the capillary array window as shown in **Figure 110**.

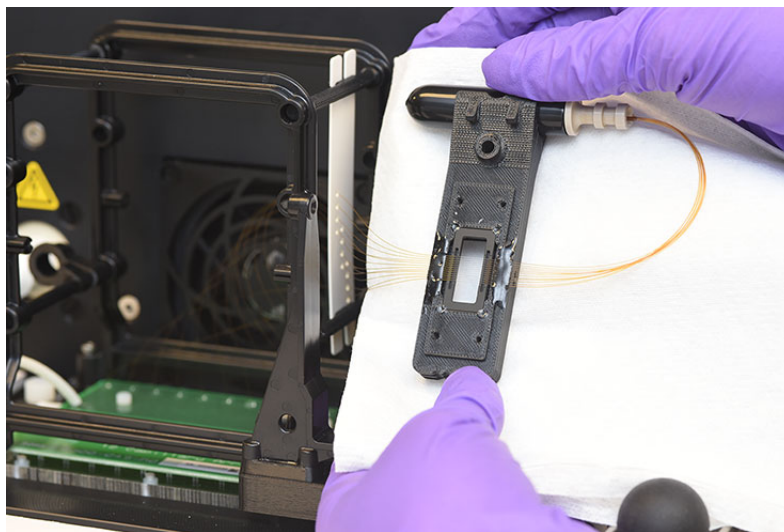


Figure 110 Capillary array window with paper towel behind

- 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 Femto Pulse instrument.
- 11 Check the alignment of the capillaries when finished by navigating to **Utilities > Capillary Alignment**. Realign if necessary.

Long Term Capillary Array Storage

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

- Leave the capillary array installed in the instrument.

Replace the Capillary Storage Solution monthly; in drier climates it may be required to change the Capillary Storage Solution more frequently, i.e., every one to two weeks.

- Use the external array docking station that ships with all new arrays as of 10/20/2015. This requires the array spindle accessory kit part #A1300-910 that shipped with all instruments shipped after 10/20/2015. Contact your corresponding Agilent Sales Representative to request a quote.

Using the array docking station

- 1 Remove the capillary array from the instrument. For detailed instructions, refer to **Chapter 8**, “Femto Pulse Capillary Array”.
- 2 Place the tray base inside the array docking station as shown in **Figure 111**.

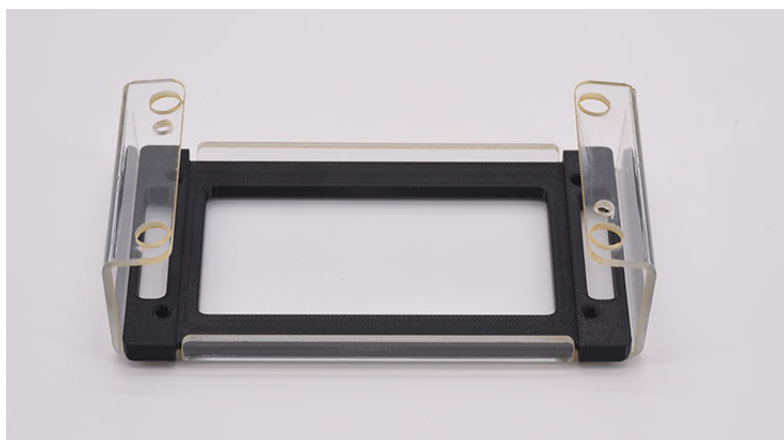


Figure 111 Array docking station with tray base installed

Appendix

Using the array docking station

- 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 112**).

For a 12-capillary array, fill Row A only with 1 mL storage solution.

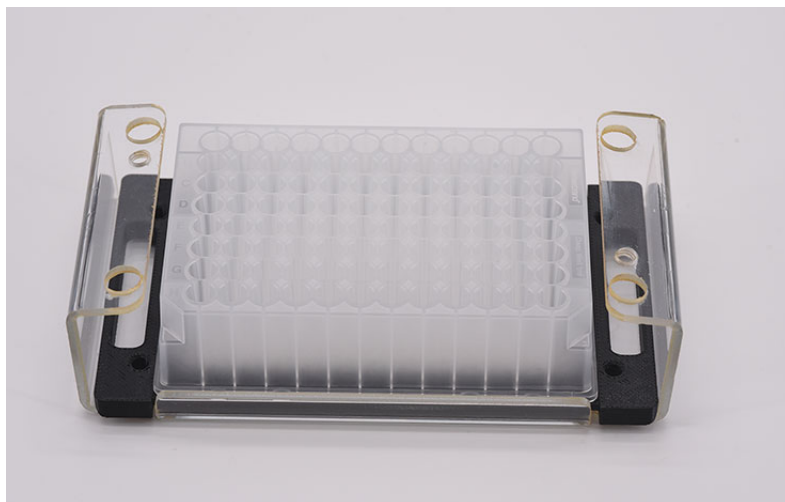


Figure 112 Array docking station with 96-deep well tray

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

- 5 Insert a white screw on each end as shown in **Figure 113** to fasten the capillary array into place.

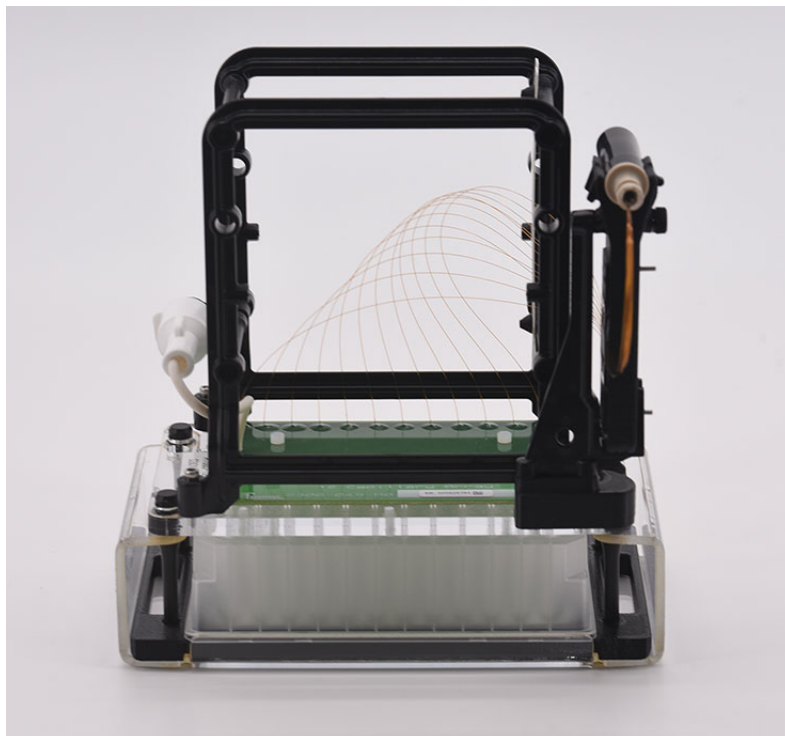


Figure 113 Array docking station with capillary array installed

- 6 Fill the provided glass vial with 20 mL of capillary storage solution and place it into the array spindle storage device.

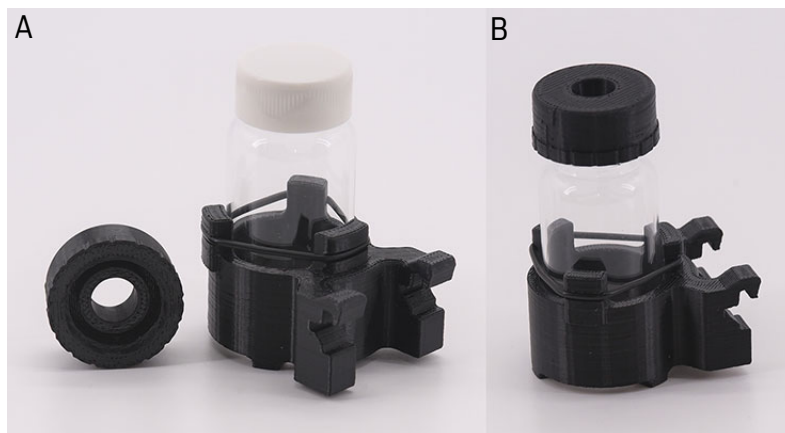


Figure 114 Array spindle storage device before (A) and after (B) attaching the bundle adaptive cap. There is no storage solution in this example figure.

- 7 Slide the array spindle storage device onto the capillary array front rail located to the left of the capillary array window as shown in **Figure 115**.

To see a full image of the array with the array spindle storage device installed, refer to **Figure 116**.

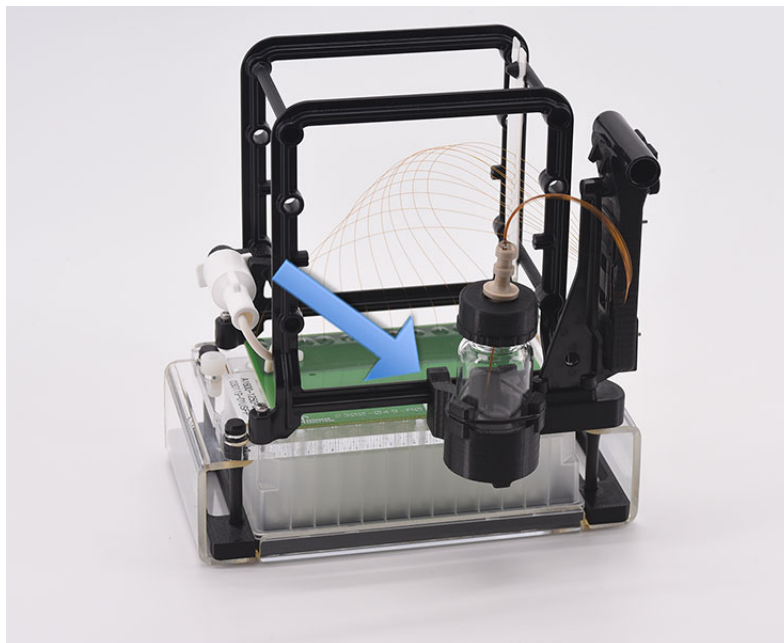


Figure 115 Array spindle storage device installation

- 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 116**.

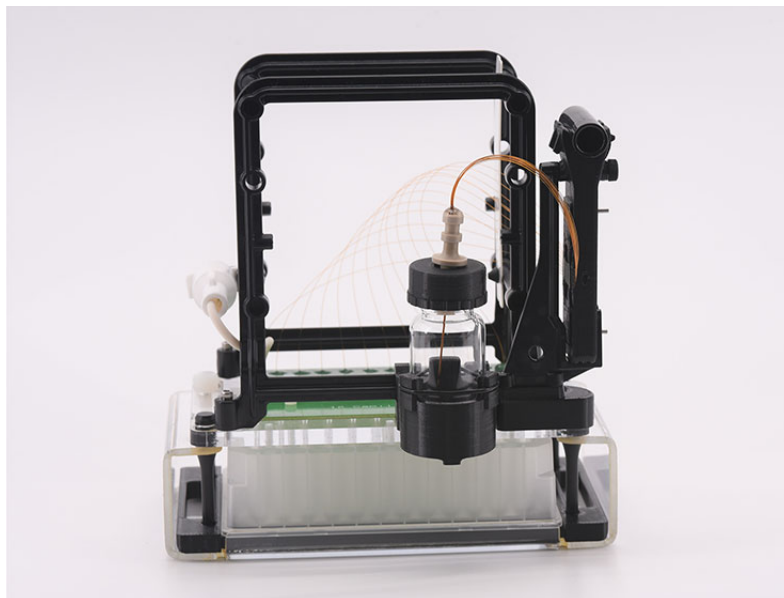


Figure 116 Array docking station and array spindle storage device with array installed

- 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.

In This Book

This user manual contains information about the Femto Pulse software.

The user manual describes the following:

- system overview,
- software menu commands,
- software tabs,
- capillary array,
- sample name entry,
- automated analysis,
- use of the bar-code reader.

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