Fragment Analyzer™ User Manual

ADVANCED ANALYTICAL

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ABOUT THIS MANUAL

Advanced Analytical Technologies, Inc. (Advanced Analytical) has prepared this manual as a technical reference for the *Fragment Analyzer* ™ system.

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

This document is intended for use by technical personnel that are proficient with analytical instrumentation operation and upkeep. A certain level of training and expertise is assumed and fundamentals are not addressed herein. Procedures are presented in a step-by-step format using photos and screen captures. If questions remain after reviewing a given procedure, please do not hesitate to contact the Advanced Analytical Technical Support staff at 515-296-6600.

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This manual is subject to change without notice. This manual may be revised and updated periodically as components and/or maintenance procedures are modified. These updates will be provided to all manual holders on record. Contact Advanced Analytical if you have questions regarding availability of updates. Please take care to keep your contact information current with Advanced Analytical so that revisions and updates can be provided in a timely manner.

A Revision history is listed in the Appendix of the User Manual

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Safety Information

In This Chapter

- Warnings and Precautions – Before Getting Started
- *FRAGMENT ANALYZER*™ System Requirements
  - ✓ Power Requirements
  - ✓ Serial Cable (USB) Connections
  - ✓ Instrument LED Lights
  - ✓ Interlock System Checking
- *FRAGMENT ANALYZER*™ Cleaning Instructions
- Table 1-1. Warning Symbols
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Warnings and Precautions – Before You Get Started

The *FRAGMENT ANALYZER*™ system has been designed, built, and tested to ensure maximum user safety.

Strict conformance to the safety guidelines discussed in this chapter and the operating procedures described in this manual will ensure user safety and help provide optimal instrument performance.

Please read and understand the safety guidelines carefully prior to operating this equipment.

When working with the *FRAGMENT ANALYZER*™ system, observe the following warnings:

- High voltage is present in the power supplies. There are no user-serviceable parts inside these devices.
- Do NOT attempt to remove any instrument covers.
- Due to High Voltage within the instrument - DO NOT REMOVE DRAWERS OR REACH HANDS INTO THE INSTRUMENT WITHOUT FIRST UNPLUGGING THE SYSTEM.
- Do NOT touch any open cable connections on the unit while the power is turned ON.
- The power supply must be grounded with a 3-prong plug. Test the electrical outlet for proper grounding before plugging in the unit.

- Install the *FRAGMENT ANALYZER™* system in an area free from excessive dust and dirt.

- Protect the instrument from extreme temperatures and humidity. Extremes in temperature and humidity can affect the performance and safety of the instrument.

- Place the system on a level surface.

- If the equipment appears damaged or operates abnormally, protective features may be impaired - Do NOT attempt to operate the unit. When in doubt, contact AATI technical support prior to operation.

- WARNING. This equipment is not intended for operation in wet locations. Miscellaneous liquids on or inside of the instrument could cause hazardous conditions.

- WARNING. Do not use the equipment in a manner not specified in this manual as serious injury could result.

Power Requirements

The unit is factory wired for the supply voltage in your region.

The *FRAGMENT ANALYZER*™ system requires:

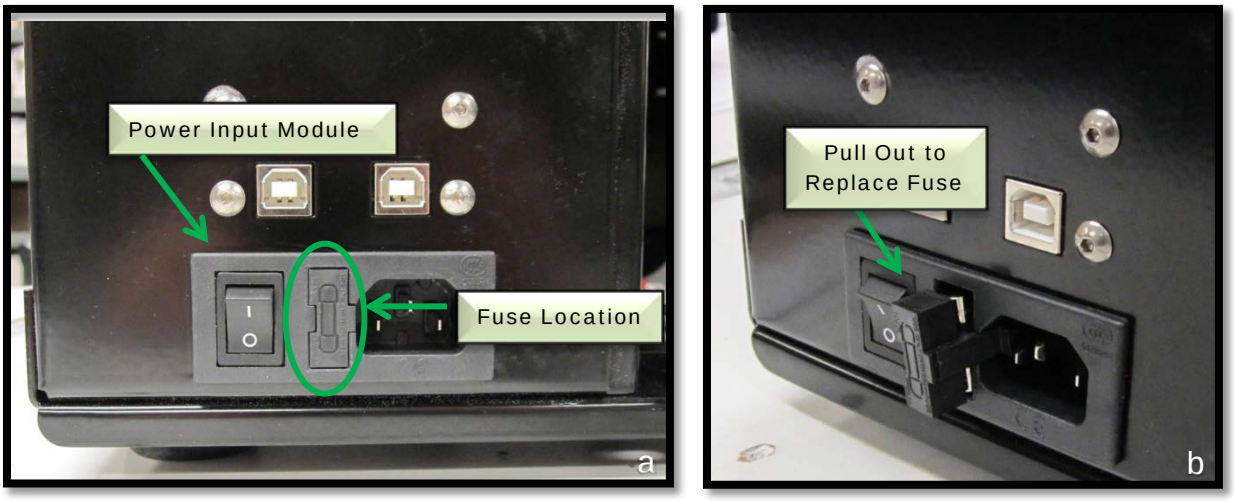
- 100-240V 50/60Hz 5.0VA

When **connecting** the system to the **supply voltage**, the **outlet MUST have a ground** for the instrument to function properly. The internal frame of the instrument must be grounded through the grounding conductor of the power cord.

The instrument utilizes controlled over-voltage techniques that require the equipment to be grounded whenever normal mode or common mode AC voltages or transient voltages may occur.

When necessary, **replace fuses** in the **Power Input Module** located on the back of the instrument (Fig. XYZ) with:

- 100 – 240v: 2A 250v Fuses



Figures XYZa and b. Back of *FRAGMENT ANALYZER*™ Instrument.
Power Input Module Fuse Placement

Warnings and Precautions – Powering the Instrument

- IMPORTANT – DO NOT attempt to rewire the instrument for different voltages. Tampering with the wiring and fuses to allow for a different voltage will void all precautions set forth by this manual, resulting in an increased risk to the safety of yourself and others.

- AC POWER SOURCE - The equipment is intended to operate from an AC power source that will not apply more than 264 V AC between the supply conductors or between the supply conductor and ground. A protective ground connection by way of the grounding conductor in the power cord is required for safe operation.

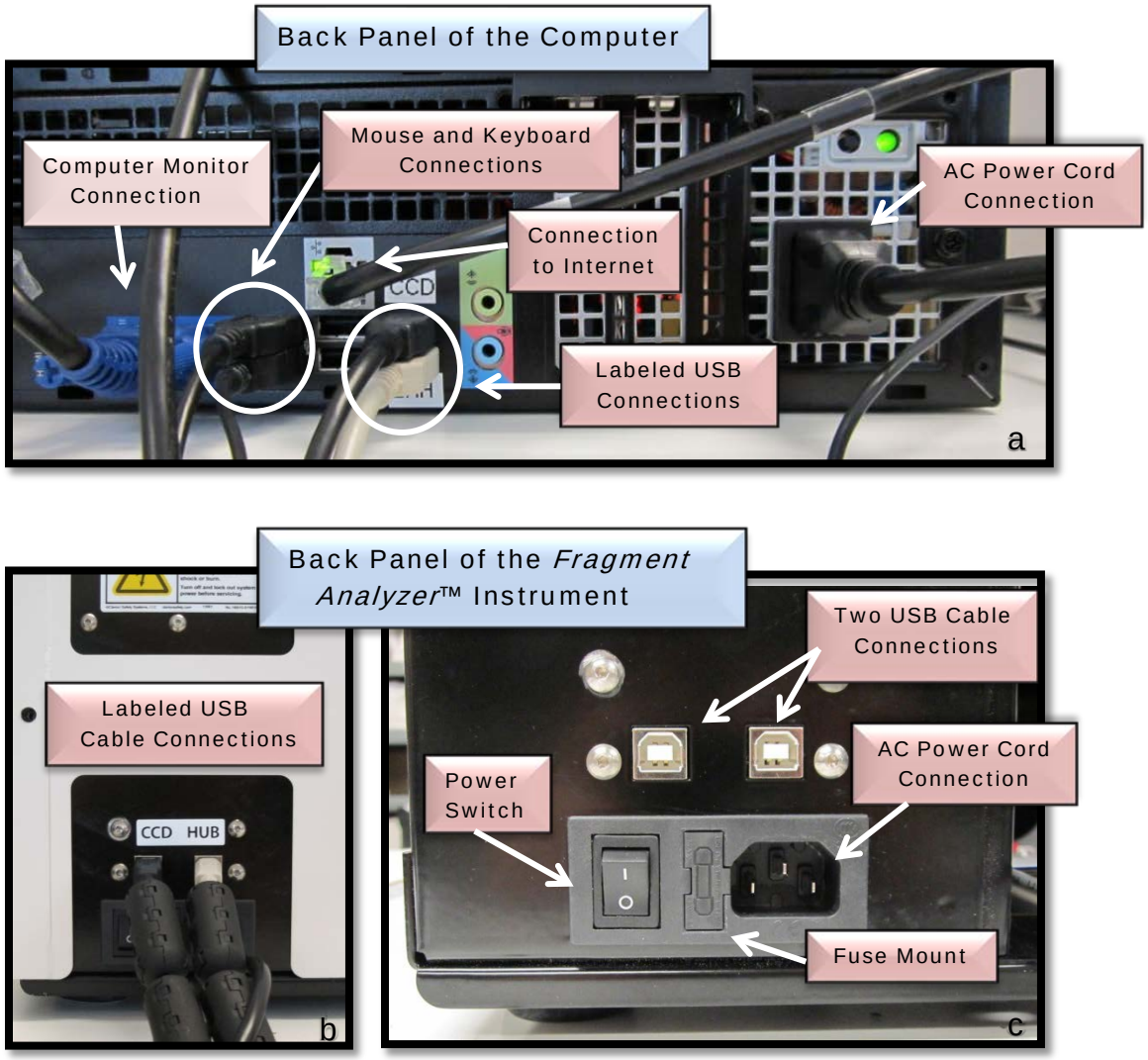
- WARNING - The power switch on the back panel IS NOT the way to ensure that power is not going to the instrument.

- TO SAFELY ISOLATE THIS MACHINE FROM THE POWER SOURCE - Turn off the power switch on the back of the instrument AND disconnect the power supply cord from the source outlet. In the event of an emergency that requires immediate disconnection, exercise maximum precaution and safety by leaving enough room to allow easy disconnection of the power cord and remaining connections.

Fragment Analyzer™ System Connections

Figures XYZ and XYZ show the basic connections necessary for operation of the *FRAGMENT ANALYZER*™ system.

- Be sure to have a grounded electrical outlet for each of the parts in the system.



Figures XYZa, b and c. Back of *FRAGMENT ANALYZER*™ Instrument. Power Input Module Fuse Placement

Serial Cable (USB) Connections

The *FRAGMENT ANALYZER*™ has 2 USB connections from the computer to the instrument. These connections are labeled on the back of the instrument.

- The order of the 2 USB connections to the computer is CRITICAL. Please refer to Figure XYZ below for the correct attachment of the USB cable connections.

Electricity is supplied to the instrument and the computer through the grounded electrical wall outlet and the power entry connections on the instrument and the computer.

- Use only the power cord and connector appropriate for the voltage and plug configuration in your country. Use only a power cord that is in good condition. Refer questions about cord and connector changes to qualified service personnel.

LED Lamp Precautions *(FRAGMENT ANALYZER™* Systems*)*

On *FRAGMENT ANALYZER*™ systems, when the top door is in the open position stray light may be encountered from the LED (light emitting diode).

Light from the LED can also be encountered when performing a capillary array replacement or removal. These activities are explained in detail in later chapters.

- IMPORTANT – Avoid looking directly into the LED when the instrument hood is open and when replacing or removing a Capillary Array Cartridge.

Front Instrument LED Lights

The High Voltage LED Light is located on the front panel of the instrument. This LED emits a red light when HIGH VOLTAGE is applied to the system. Figure XYZ.

Figure XYZ. The *FRAGMENT ANALYZER™* Instrument.
Red LED indicating HIGH VOLTAGE

Interlock System Checking

The Interlock System is a safety feature that stops the flow of electricity to the instrument in the event that the top compartment or either of the top two drawers (Drawer B; Drawer W) are opened when high voltage is supplied during a CE separation.

This safety feature is checked by opening the top cover of the instrument or either of the top two drawers (Drawer B; Drawer W) when a high voltage is being supplied to the instrument.

When one of the top two drawers or the top cover is open, the red High Voltage LED WILL NOT light up when a High Voltage is applied.

- This feature is checked during the installation & qualification of the instrument.

Cleaning Instructions

To clean the instrument, use a damp, clean cloth. Mixed detergent may be used.

Instrument Cleaning - Warnings and Precautions

- IMPORTANT – Keep all areas in and around the instrument dry to avoid electrical shock and to prevent damage to the system.

- NEVER apply solvents to the instrument housing.

- Do NOT use aromatic or hydrocarbon-based solvents, chlorinated solvents, or alcohol-based fluids for instrument cleaning.

- If liquid reagents are spilled in or around the instrument, clean up immediately using an absorbent cloth. Dispose of the any absorbed reagent according to the MSDS instructions. It is the user's responsibility to properly adhere to all local and state laws regarding waste disposal procedures.

Fragment Analyzer™ Instrument Warning Symbols and Labels

The tables that follow provide an overview of the warning symbols and labels found on the instrument, what they represent and their location/s.

Table 1-3. Warning Symbols

Symbol	Purpose and Meaning	Location
	**General Warning** • Potential danger • Please see manual for additional information	Inside Right Front Door
	**High Voltage** • Power Source or High Voltage wires exist near this location	Top Compartment Back Panel

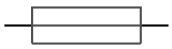
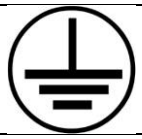
Symbol	Purpose and Meaning	Location
	**Pinch Point** • Possible pinching or catching of fingers and/or hands • Proceed with extreme caution keeping your fingers out of danger	Inside Right Front Door.
	**Heavy Object** • Object is heavy • Only multiple people should lift this object	Manual Only
~	**Alternating Current** • This symbol is used to show that the instrument uses an alternating current.	Above PWR Entry Fuse Labels (Back panel)
	**Line Fuse, Other Fuse, Proper Fuse** • AC PWR Distribution Fuse 1. 100 – 240v: 2A 250v	See Figure 1-1
o	**Off (Supply)** • The power supply is off.	Power Supply Switch (Back Panel)
|	**On (Supply)** • The power supply is on.	Power Supply Switch (Back Panel)
	**Protective conductor terminal** • This symbol indicates the protective ground (earth) terminal.	HV PWR Supply (Inside instrument—not user accessible).
WARNING	The WARNING notice denotes a hazard. It calls attention to a procedure, practice, or the like, that, if not correctly performed or adhered to, could result in personal injury. Do not proceed beyond a WARNING notice until indicated conditions are fully understood and met.	Manual Only
CAUTION	The CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or the like, which if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.	Manual Only

Table 1-4. Warning Labels

Label	Purpose and Meaning	Location
WARNING **HAZARDOUS VOLTAGES MAY BE PRESENT IF INTERLOCKS ARE DEFEATED**	**High Voltage Warning** • Alerts of a high voltage risk if interlocks are defeated.	Inside Right Panel Door, Inside Upper hood.
CAUTION **To Avoid Instrument Damage** • **Open and close hood and doors slowly.** • **Remove any spilled liquid located in and around the instrument.** • **Do not over fill containers.**	**Instrument Damage** • Explains proper environment and how to manage the *Fragment Analyzer*™ doors.	Instrument Right Panel Door.
	**Chemical Hazard Warning** • Warns of the use and exposure to potentially hazardous chemicals. Consult reagent MSDS sheets for listing of precautions and handling information.	Inside right panel door.
100/240V ~ Use Fast Acting **2.0A 250 VAC 5x20mm Fuse**	**Fuses Input Module** • Specifies fuse to be used.	Back panel of instrument

NOTICE

The information contained in this document is subject to change without notice.

AATI, Inc. makes no warranty of any kind with regard to this material, including but not limited to, the implied warranties of merchantability and fitness for a particular purpose. AATI, Inc. shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Looking over the *Fragment Analyzer™* System

In This Chapter

- Overview of system components and connections
- Loading and orientation of 96-well plates
- Preparing samples
- Instrument Compatible 96-well plates

The *Fragment Analyzer*™ system is a multiplexed capillary electrophoresis (CE) instrument for performing automated, high throughput separation and quantification of double stranded nucleic acids (DNA and/or RNA). Separation is achieved by applying an electric field through a narrow bore (50 μm i.d.) fused silica capillary array filled with various conductive gel matrices designed to sieve DNA/RNA molecules of a specific size range. When a high voltage is applied to the capillary array, injected DNA/RNA migrates differentially 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 separation gel matrix, which fluoresces when bound to double stranded DNA or RNA molecules. The *Fragment Analyzer*™ system utilizes a high intensity light emitting diode (LED) excitation light source that is focused across the capillary array detection window and imaged onto a sensitive, two-dimensional charge-coupled device (CCD) detector. By monitoring the relative fluorescence unit (RFU) intensity as a function of time during the CE separation, digital electropherogram traces representative of the DNA/RNA content of an entire row of 12 samples, or plate of 96 samples can be collected in a single experimental run (as shown in the Figure below)

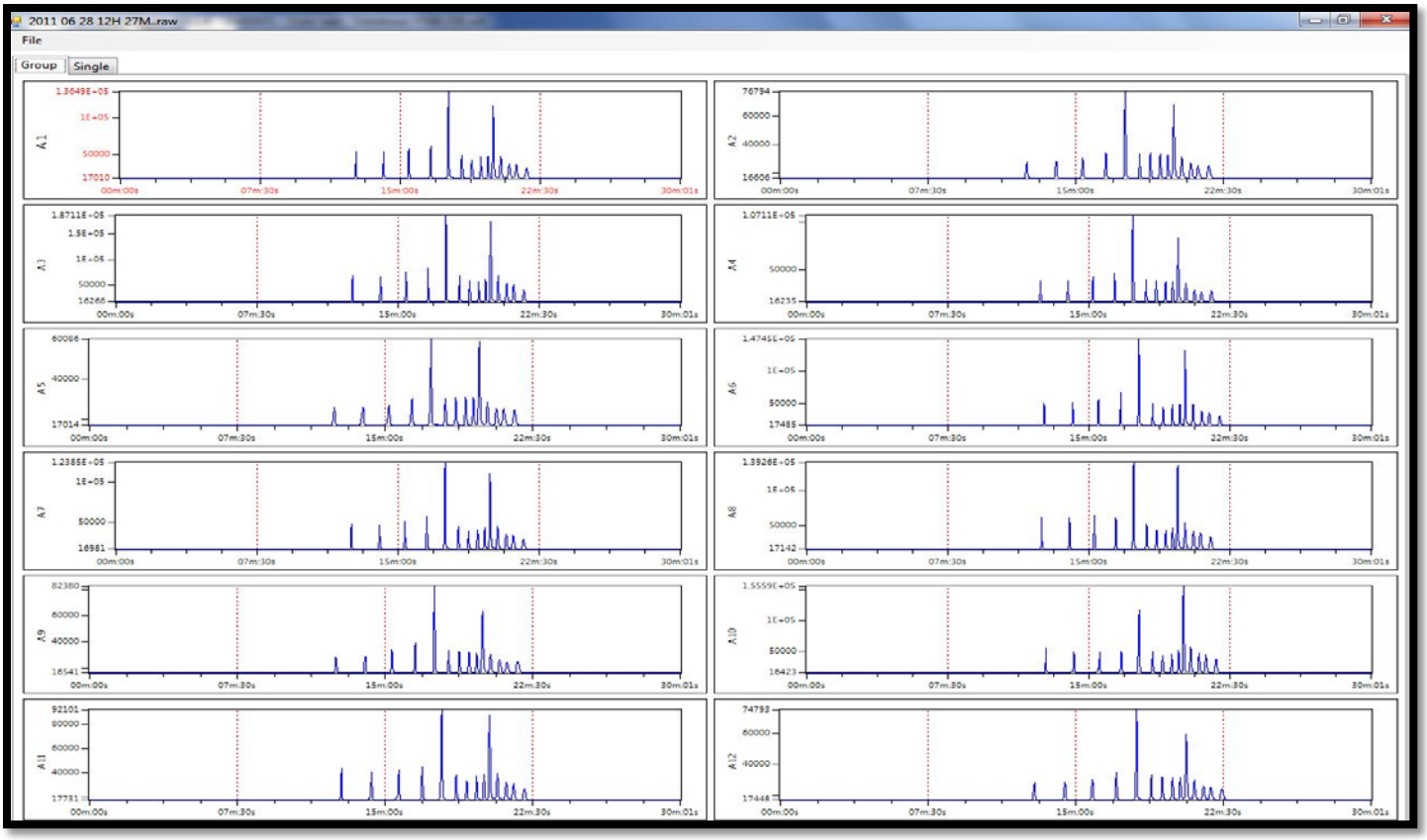


Fig 2-1. The File Manager Screen with run data from 12-Capillaries

Configured *Fragment Analyzer™* System Dimensions

This chapter provides a basic overview of the *Fragment Analyzer*™ system hardware and operation. The figure below shows an external view of a fully configured *Fragment Analyzer*™ system, which has a compact footprint of 40" on the bench top and it weighs 82 lbs (37 kg).

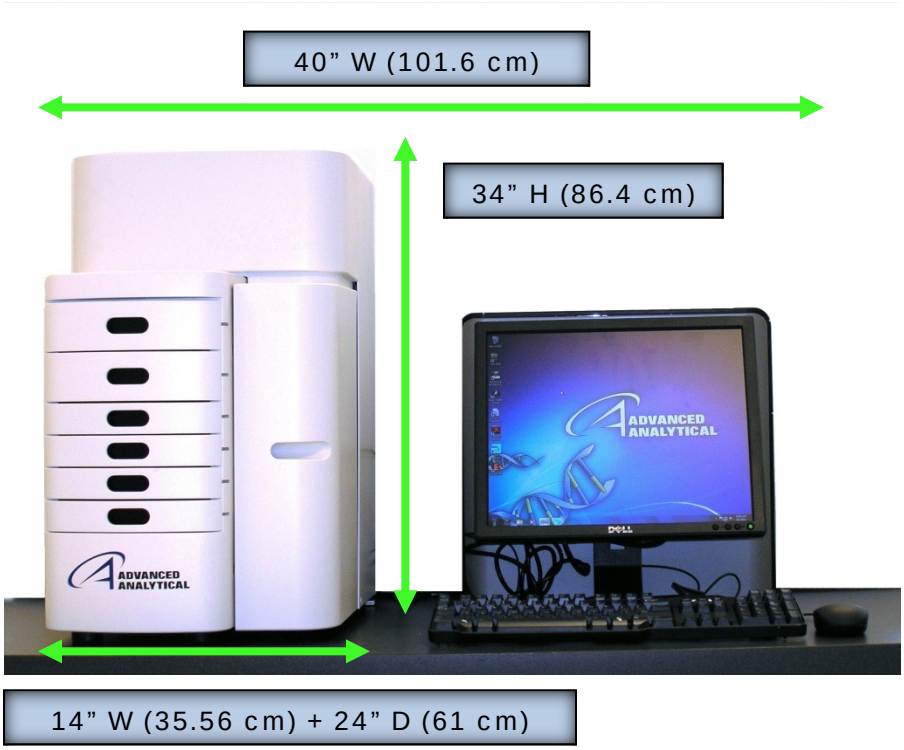


Figure 2-2: Configured *Fragment Analyzer™* system with computer workstation

Fragment Analyzer™ System Connections

The back of the **Fragment Analyzer™ instrument** contains the **Communications Panel** where the necessary connections are made to the **Instrument Computer (PC)** and **Electrical Outlet** for operation. (Fig. **2-3**)

The instrument utilizes a **standard 120V, 20 Amp, 3-prong outlet.**

⚠ **The use of a surge protection device is highly recommended.**

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

Each connection is labeled on the PC. The various connections between the system and the *Fragment Analyzer*™ instrument are summarized below:

1. From the *Fragment Analyzer*™ System:
    a. Two USB connections to PC USB.

    ⚠ The order/ location of connections is critical and they are labeled.

    b. Power cord to grounded electrical outlet

2. From the PC:
    a. Two USB connections to *Fragment Analyzer*™ system.

    ⚠ The order/location of connections is critical and the locations have been identified on both the computer and the *Fragment Analyzer*™.

    b. Power cord to grounded electrical outlet

    c. Connection to monitor, keyboard, mouse etc.

Fragment Analyzer™ System Connections

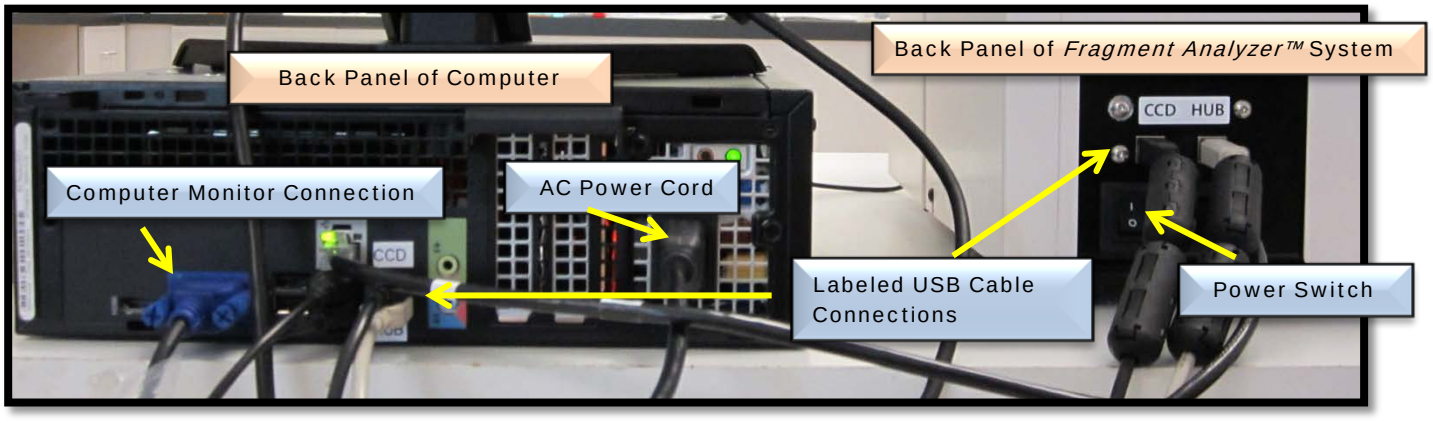


Figure 2-3. *Fragment Analyzer*™ System Connections (with computer)

Fragment Analyzer™ External Cabinet

There are three primary points of access to the inside of the *Fragment Analyzer*™ system: the **Top Compartment**, the **Side Compartment Access Door** and the **Drawers (6 total)**.

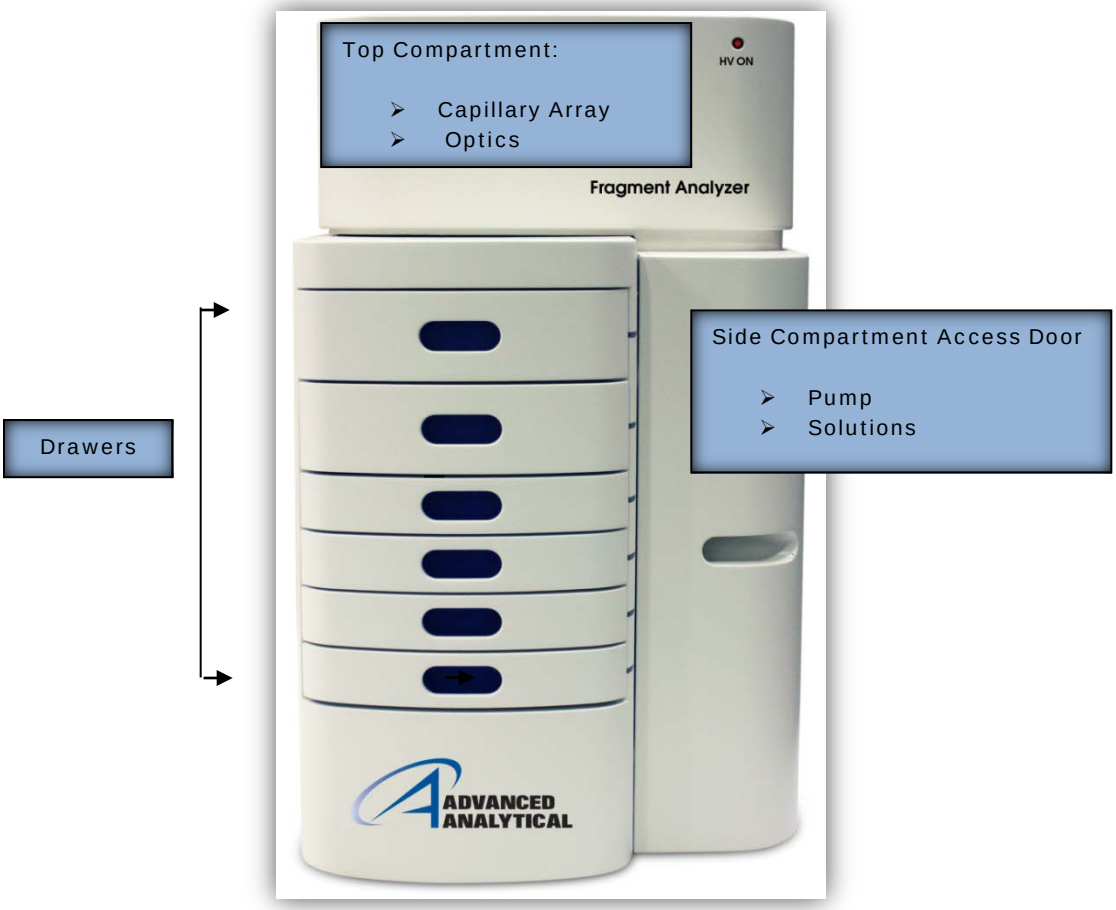


Figure 2-4. Entry points of the *Fragment Analyzer™ System*

Top Compartment (Fig 2-5): Provides access to the **Optical Detection Platform** and a **12 or 96-Capillary Array Cartridge.** A non-accessible top 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 or 96-Capillary Array Cartridge is a replaceable, modular component of the *Fragment Analyzer*™ system. The cartridge can be easily exchanged by the user and this process is explained in **Chapter 5: Capillary Array Replacement**. The top compartment should only be opened when the user changes the capillary array cartridge or replaces an array cartridge at every 700-1000 injections, or as needed due to data quality.

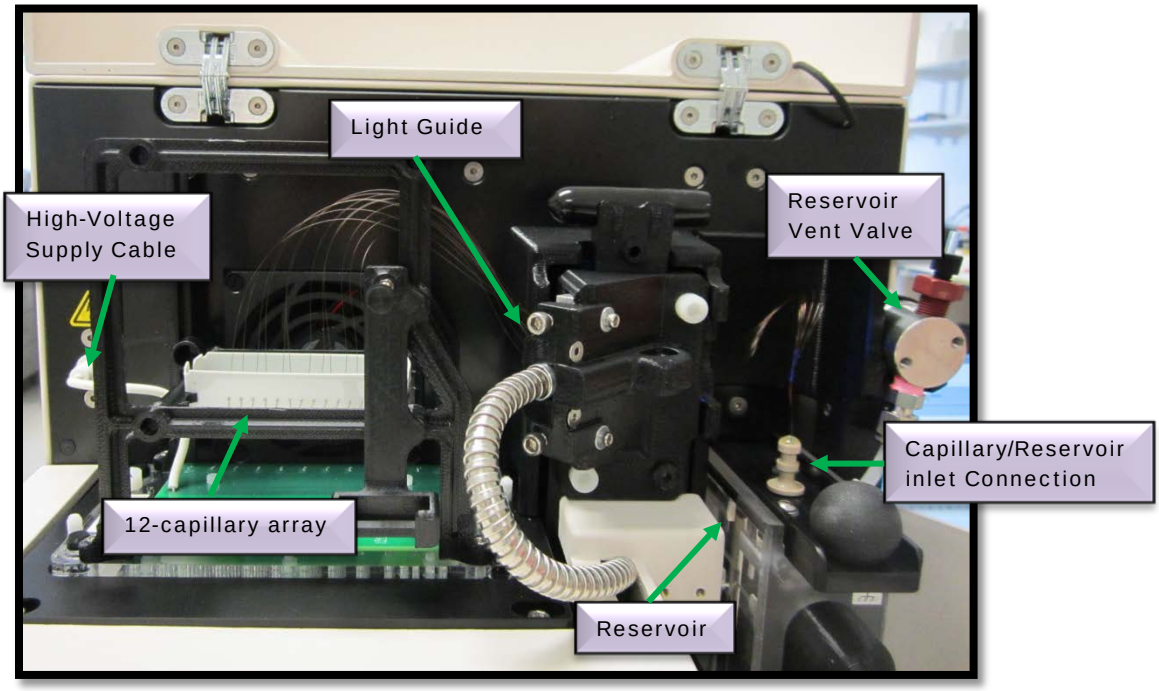


Figure 2-5. *Fragment Analyzer™* Main Unit Top Compartment (Capillary Array Cartridge installed)

Side Compartment Access Door (Fig 2-6): Allows access to the **High Pressure Pump's Syringe, Waste Bottle, Conditioning Solution** and **Gel Solutions (Gel 1 and Gel 2)**.

The Internal High Pressure Syringe Pumping System provides automated flushing and filling of the capillary array with separation gel matrix between experimental CE runs, providing pressurization of the capillaries up to 300 psi and it is rated up to 500 psi. It also pumps fluid through the capillary walls at periodic intervals to maintain maximum separation resolution.

The system design enables vacuum injection (hydrodynamic injection) of samples in addition to traditional electrokinetic (voltage) sample injection - a feature unique to the *Fragment Analyzer*™

platform and advantageous when working with samples containing high salt matrices.

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

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

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

The system also contains a Waste Bottle, which collects Separation Gel or Capillary Conditioning Solution pumped via the Waste Line from the capillary array reservoir during the filling process.

Fragment Analyzer™ Side (Reagent) Compartment

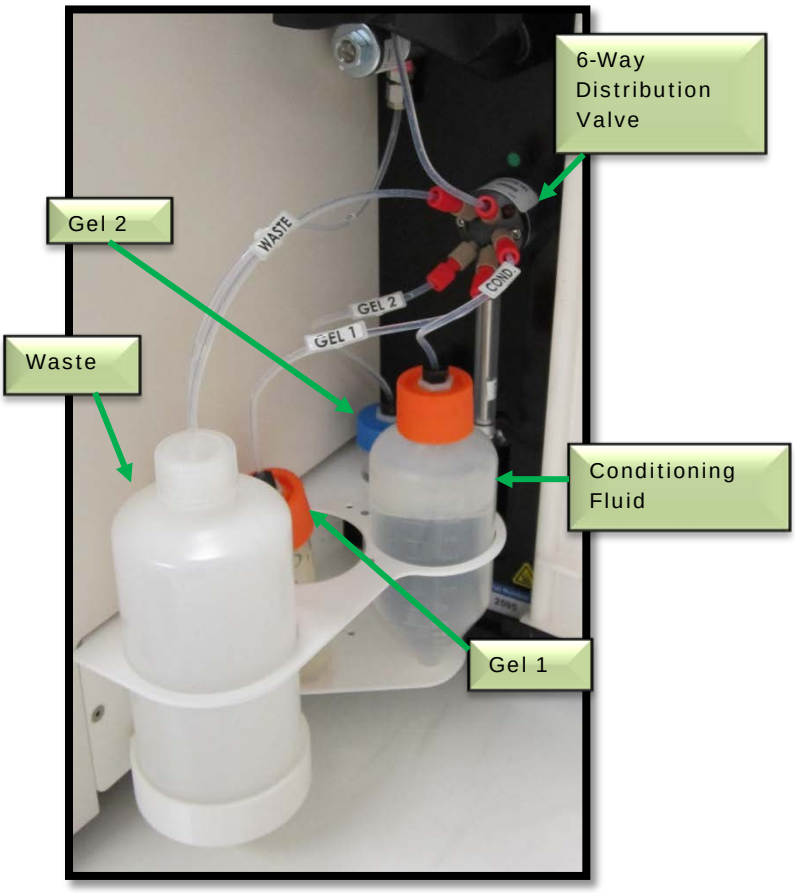


Figure 2-6. Side Door Compartment

The three fluid line connections inside the *Fragment Analyzer*™ system are:

- Gel Line (Gel 1 or Gel 2) from Syringe Pump to Gel Bottle (Gel 1 or Gel 2)
- Conditioning Fluid from Syringe Pump to Conditioning Fluid
- Overflow Waste Line from Syringe Pump to Waste Bottle

Fragment Analyzer™ Drawers

The Drawers (Fig 2-7): The *Fragment Analyzer*™ front-panel drawers provide an external interface for loading Buffer, Marker and Sample 96-Well Plates into the system.

- Drawer B (Top Drawer): This location is used for the Inlet Buffer Tray used during the CE separation. This position is also used for a Rinse/Water – Dip and Sample Storage Solution in the 12 capillary instrument.
- Drawer W (Second Drawer from top): This location is utilized for a Waste Tray when performing a capillary conditioning method where the capillary array is flushed with Capillary Conditioning Solution **or** Gel. This tray is also used for an Array Conditioning between runs.
- Drawer M (Third Drawer from top): This location is used for loading the Lower/Upper DNA Marker Tray.
- 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.

Drawers B and W are Interlocked - Which means that when any of the top two drawers are open, the high-voltage will automatically shut off.

Drawers M, 1, 2, and 3 are NOT interlocked - Which means that sample trays can be exchanged while the instrument is in operation.

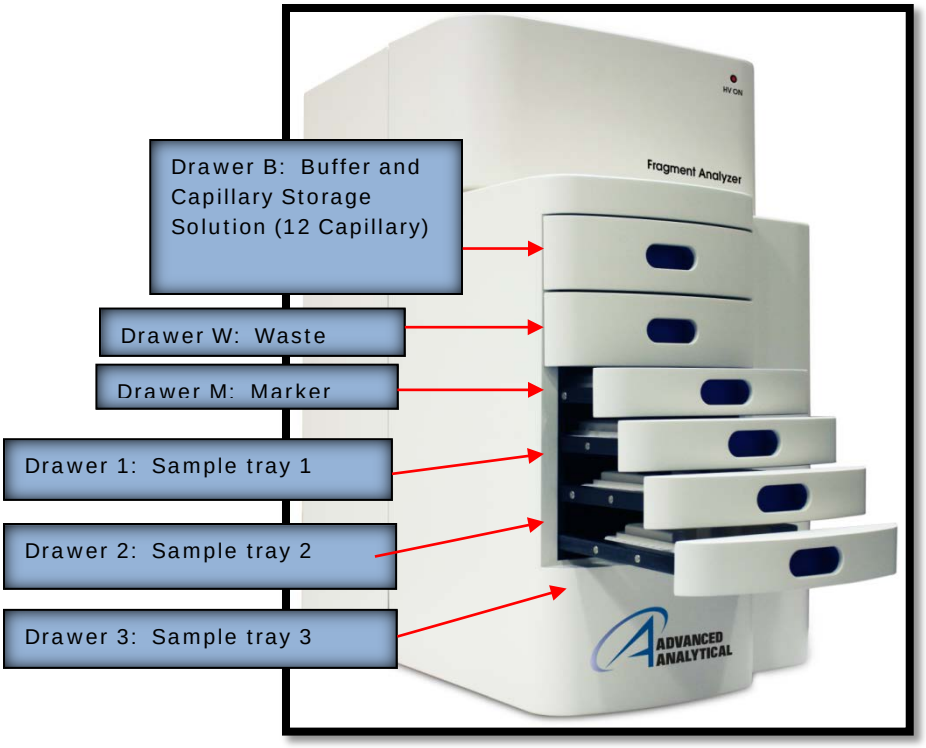


Figure 2-7. Instrument Drawer Positions

Fragment Analyzer™ Loading and Orientation of 96-Well Plates

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

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

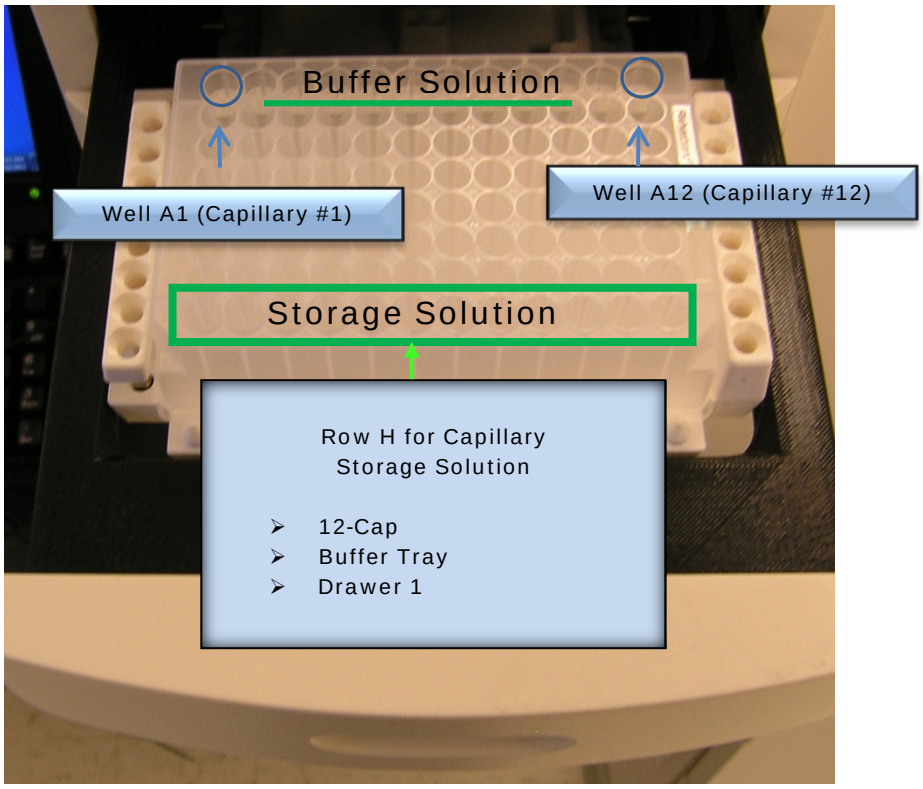


Figure 2-8. Proper orientation when loading 96-well marker and sample plates for a 12-capillary system.

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.

When loading a **1.2 ml deep 96-well plate (31 mm height),** the plate is directly placed in **Drawer B or Drawer W** (Fig **2-9**).

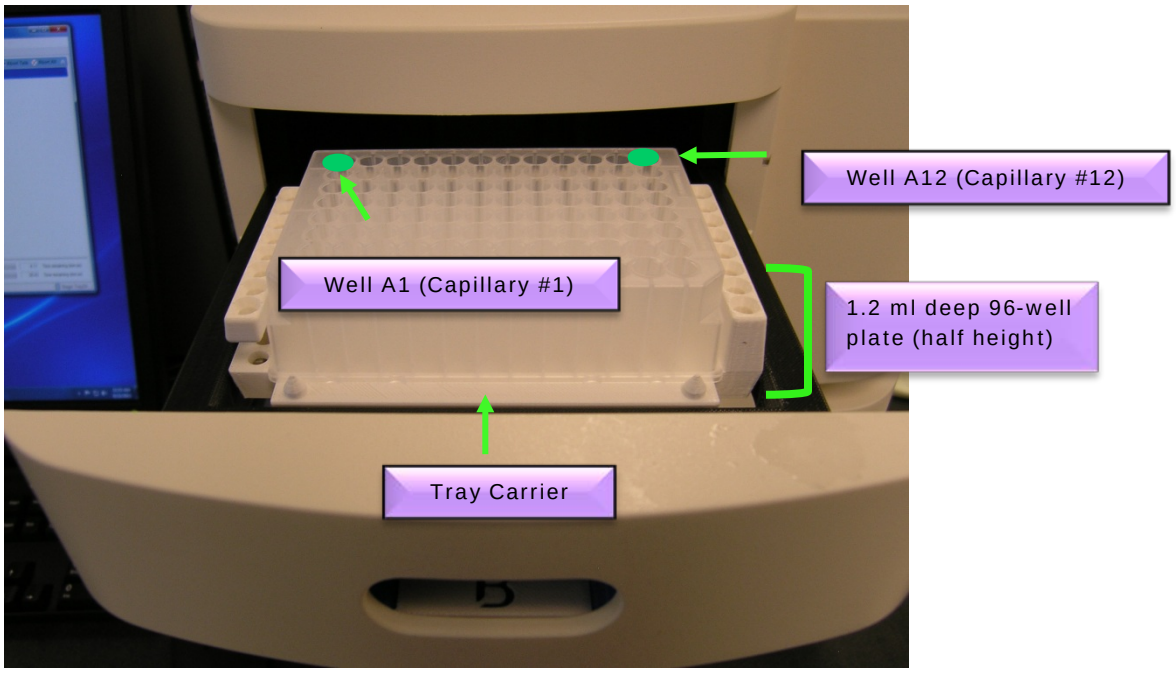


Figure 2-9. Proper loading of a 1.2 ml deep 96-well plate on the instrument stage (Drawer B)

- When loading 96-Well Sample **or** Marker Plates into Drawers M, 1, 2, and 3, a Non-Skirted or Semi-Skirted PCR Plate is used (Fig **2-10**).

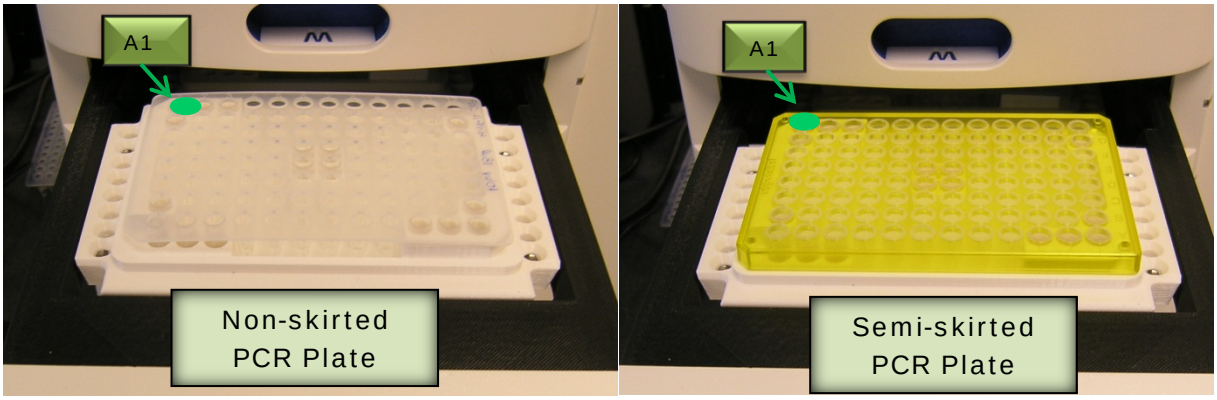


Figure 2-10. Proper loading of a non-skirted (left) or semi-skirted (right) 96-well plate on the tray carrier (Drawers M, 1, 2 and 3)

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

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

For a list of compatible PCR plates please refer to the last section of this chapter. Contact AATI if a different vendor or style of PCR plate is to be used in order to verify compatibility.

Fragment Analyzer™ Loading Samples

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

When preparing Lower/Upper DNA Marker Plates for repeated use, a volume of 30 µl /well with a 20 µl mineral oil overlay is recommended.

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

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

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

Fragment Analyzer™ Compatible 96-Well Plates

The *Fragment Analyzer*™ system has been designed to operate using specific dimensioned non-skirted or semi skirted PCR plates and deep 96-wel plates. A list of approved non-skirted or semi-skirted PCR plates are provided in Table 2-1.

⚠ Contact AATI regarding the compatibility of any PCR plate NOT listed in the table in order to verify compatibility. The use of PCR plates with different dimensions could potentially damage the tips of the capillary array cartridge.

The instrument uses a specific Deep 96-Well Plate (31 mm height) supplied by Fisher Scientific (Part # 12-566-120) for the buffer and waste plate. The same specified buffer/waste plate is also available directly from AATI, in the event these plates cannot be obtained directly from the manufacturer. Table 2-2

⚠ This specific plate MUST be used with the instrument and two plates are supplied upon installation. Standard 1 mL deep well, half height or square well 1 mL 96-well plates should NOT be used as buffer /waste plates with the *Fragment Analyzer*™ system as damage to the capillary array will occur.

Item	Approved Vendor/Part Number	Description
Sample/Marker PCR plates (non-skirted)	**Axygen # PCR-96-FLT-C**	Axygen® 96 Well Clear, Flat Top Non-Skirted PCR Microplate
	VWR # 82006-636	VWR® 96-Well PCR Plates, Flat Plates, Natural
Sample/Marker PCR Plates (semi skirted)	**VWR # 83007-374**	VWR® 96-Well PCR Plates, Half-Skirted Plates, Natural
	Eppendorf # 951020303 (various colors)	Eppendorf® 96-Well Twin.tec PCR Plates, Semi Skirted
	MidSci Pryme #AVRT1	Pryme® PCR Ergonomic Plates, 96 x 0.2mL, Semi-Skirted, Natural
	BioRad Hard-Shell # HSS-9601	BioRad Hard-Shell® Full-Height 96-Well Semi Skirted PCR Plates

Table 2-1. Sample/Marker PCR Plate List

Item	Recommended Vendor/Part Number	Description
Buffer/Waste Deep 96-Well Plates	**Fisherbrand # 12-566-120**	Fisherbrand® 96-Well DeepWell Polypropylene Microplates; Well Capacity: 1 mL
	AATI # P60-20	Fragment Analyzer 96-Well Buffer/Waste Tray, Case of 50

Table 2-2. Buffer/Waste Deep 96-Well Plate List

Getting Started - *Fragment Analyzer™* Software

In This Chapter

- System Requirements and Installation
- Operation Tab Overview
    - Instrument Status Information
    - Hotel Positioning Icons
    - Capillary Conditioning
        - Viewing and Editing a Capillary Conditioning Method
        - Running a Capillary Conditioning Method

The *FRAGMENT ANALYZER*™ system employs proprietary software for operation and data analysis.

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

System Requirements

The software is run under a Microsoft® Windows XP or Windows 7 PC with the following requirements (Table **3-1**).

Processor	2 GHz Intel Pentium or equivalent
SVGA Video	Minimum resolution 1024 X 768
Memory	2.0 Gigabytes
Available hard disk space	40.0 Gigabytes
USB Serial Ports	4 ports (2 instrument, keyboard, mouse)
Network	If not using a local database, A network connection to the database server host if desired

Table 3-1: Minimum Computer Requirements

System Installation

To install the *FRAGMENT ANALYZER*™ software:

1. Place the CD in the CD drive. Navigate to *FRAGMENT ANALYZER*™ Installer →setup.exe and double-click on setup.exe.

2. Follow the setup instructions provided by the installation wizard. The default installation directory is C:\AATI\Fragment Analyzer.

Opening the *FRAGMENT ANALYZER™* software

To login to the software, select the *FRAGMENT ANALYZER*™ software icon (Figure **3-1**).

Figure 3-1: Fragment Analyzer Icon

Type '**User**' or '**Administrator**' into the **User ID Field** of the **Login Window** (Figure **3-2**). Click the **[OK]** button. The first time you login, the password information is left blank.

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

A password can be set for the system during the AATI training and installation period at your facility or by using the **Admin-Change Password Function**, described in a later chapter.

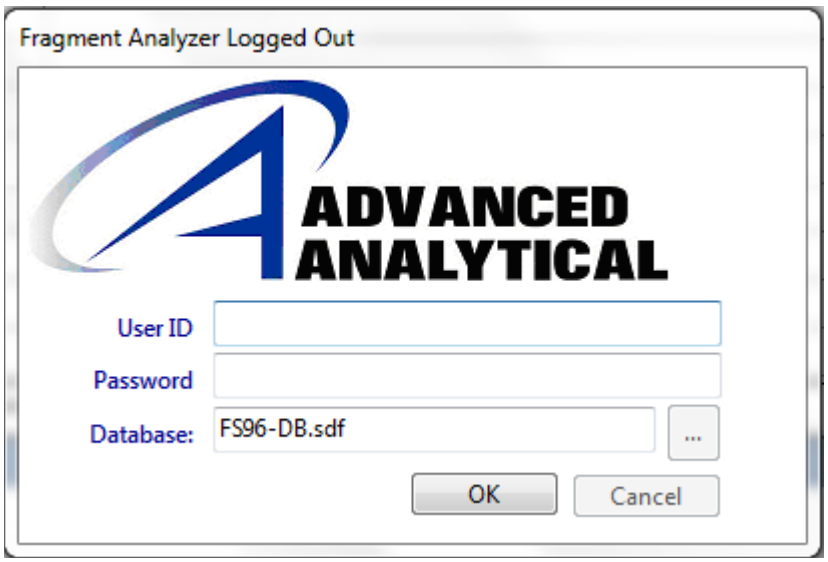


Figure 3-2: Login Form

The first time a user logs into the *FRAGMENT ANALYZER*™ software, a database is displayed indicating where the application is connected.

The database connection can be changed prior to logging on by clicking the browse button next to the database field (Figure **3-2**).

- The login information is used in event and error logging to aid in controlling access to the system, tracking usage and monitoring changes to the system.

Every time you log into the *FRAGMENT ANALYZER*™ software, you begin on the Main Screen (Fig. **3-3**).

Navigating the Main Screen

Use of the *FRAGMENT ANALYZER™* software is accessed from the **Operations Tab** on the **Main Screen Window** as shown in Fig. **3-3**.

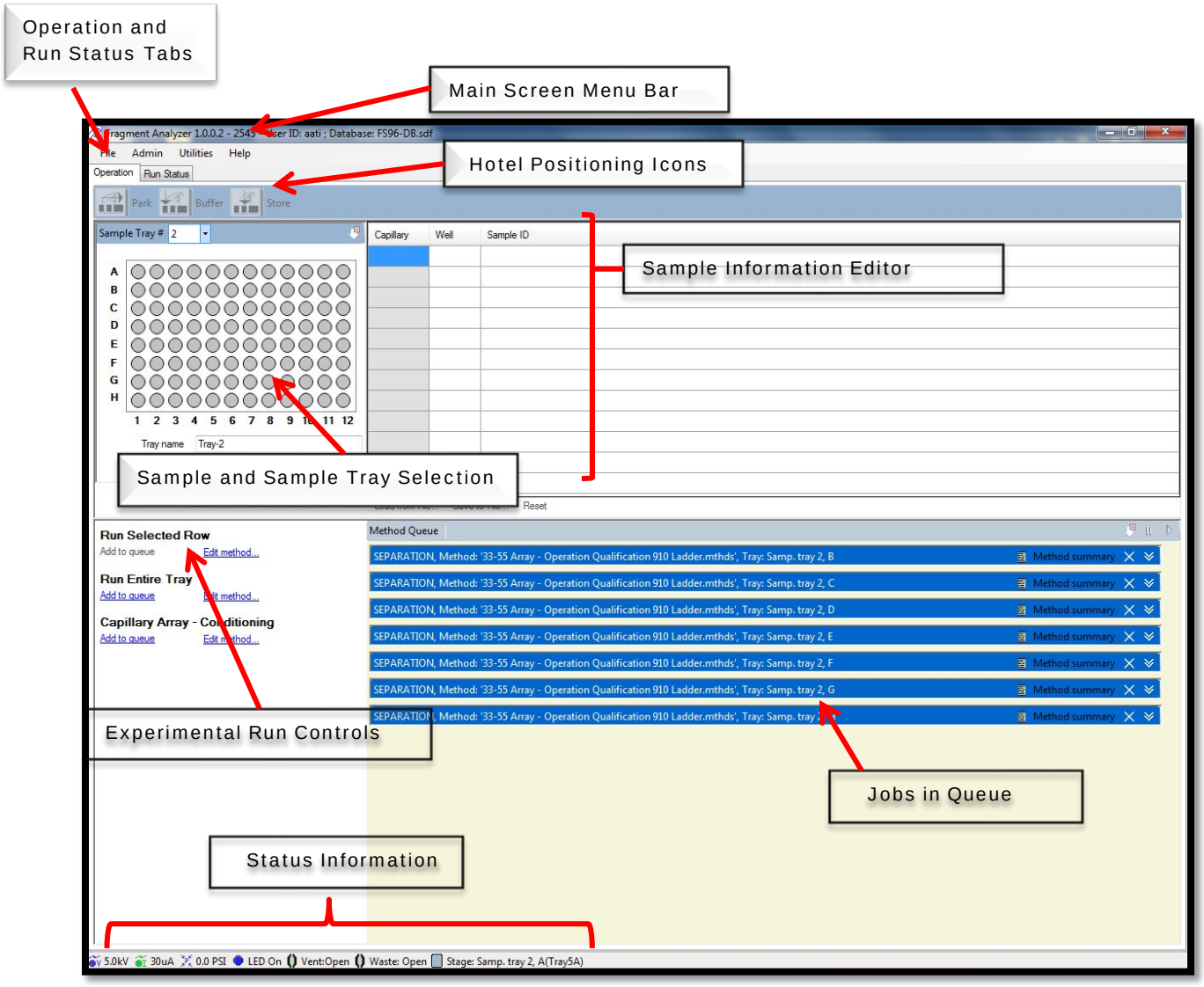


Figure 3-3. *FRAGMENT ANALYZER™* Software Main Screen Window.

This view contains the **Hotel Positioning Icons, Experimental Run Controls, Sample and Sample Tray Selection Interface, Sample Information Editor, Status Information, Operations and Run Status Tabs and Main Screen Menu.**

Status Information

The bottom, left side of the **Main Screen Window** contains important **Instrument Status Information**. Fig.3-4

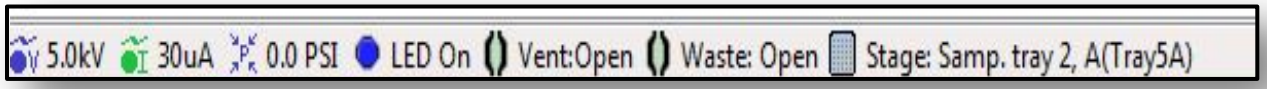


Figure 3-4: Instrument Status Information Bar

The **Stage Icon** shows the **current tray** positioned **underneath the capillary array.** Fig. 3-5

Prior to making changes or **opening** the **Top, Side or Drawers of the Instrument, check** the **Stage Icon** to verify whether a tray is positioned underneath the capillary array as the **positions of trays** and the **Instrument Stage** can **impact** system **operation.**

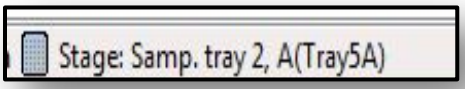


Figure 3-5: Main Screen Window
Current Tray Position Indicator

The Voltage, Current and Pressure Icons show the real-time status of key operational parameters. Fig. 3-6

Figure 3-6: Main Screen Window
Operational Parameter Indicators

The LED, Vent and Waste Icons show whether the machine is on and the status of the vent and waste valves (open or closed). Fig. 3-7

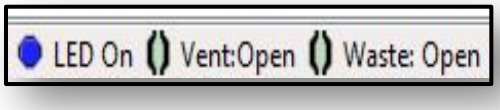


Figure 3-7: Main Screen Window
LED, Vent and Waste Icons

Hotel Positioning Icons

The **Hotel Position Icons** are shown in Fig **3-8**. Selecting these icons will place **Buffer** or **Storage Solution Trays** in specific locations.

Figure 3-8. The Hotel Position Icons.

The Park Icon

This icon is ***frequently*** utilized.

This command **places all Sample, Waste, and Buffer Trays in their drawers.** It also moves the **Sample Stage** to the bottom of the instrument (Fig **3-9**).

- This command is also used to **position the Buffer Tray in Drawer B** (top drawer) to allow the user to **replace or re-fill the Buffer/Sample Storage Solution.**

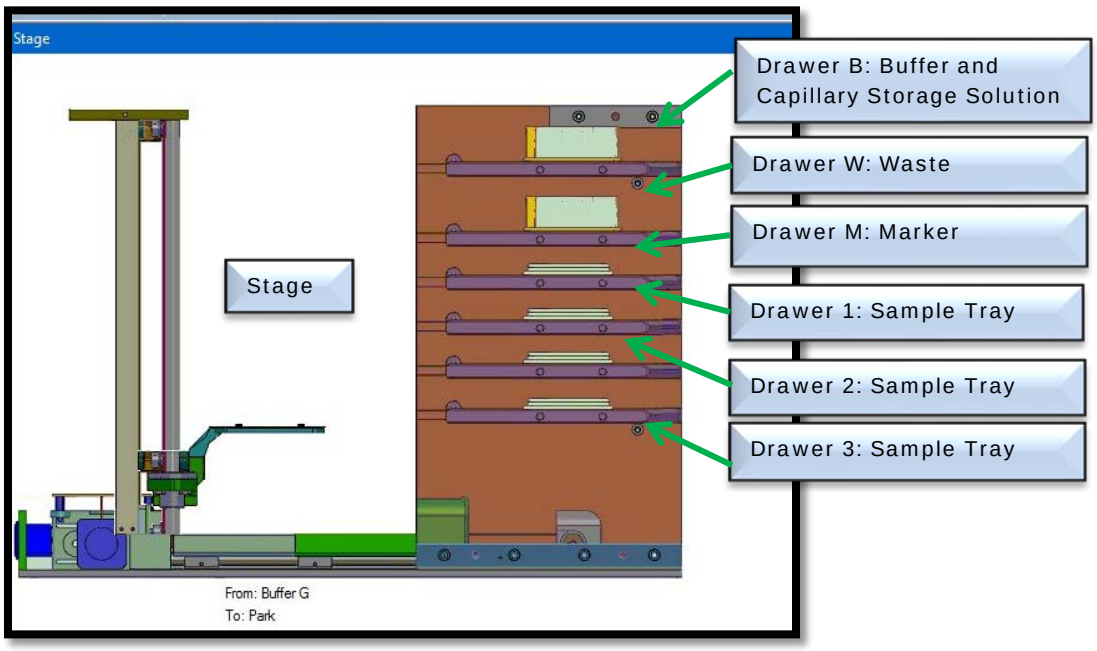


Figure 3-9. The "Park" Position. The stage is located at the bottom of the instrument and all samples/buffers are in tray positions.

The Buffer Icon

This command **places the Buffer Tray underneath the Capillary Array,** as shown in Figure **3-10**.

This icon is ***frequently*** used - during normal operation the program automatically positions the buffer tray underneath the array. It is used ***daily*** when operating the instrument with a **12-Capillary Array**.

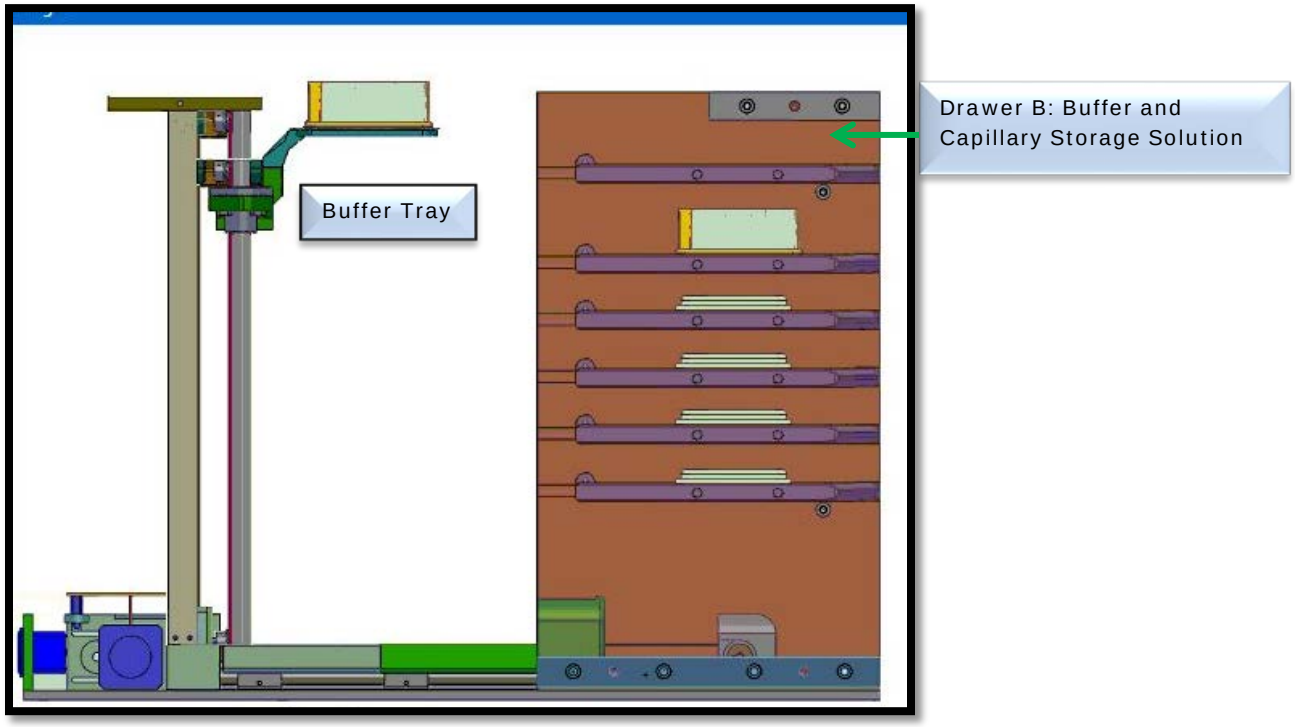


Figure 3-10. The "Buffer" and the "Store" Position. The buffer tray is positioned underneath the capillary array. This also represents the **Store Position** for a 12-cap system - Row H of the Buffer Tray.

The Store Icon

This command places the Capillary Storage Solution underneath the Capillary Array, as shown in Fig **3-10**.

For a 12-capillary array, this corresponds to Row H of the Buffer Tray.


This icon is also used to place the Storage Solution under the Array after changing out the Buffer Solution.

This icon is *rarely* used - during normal operation the program automatically positions the storage solution tray underneath the array.

Experimental Run Controls

The Experimental Run Controls are shown in Fig **3-11**.

These controls contain the Run Selected Row, Run Entire Tray and the Capillary Array-Conditioning commands.

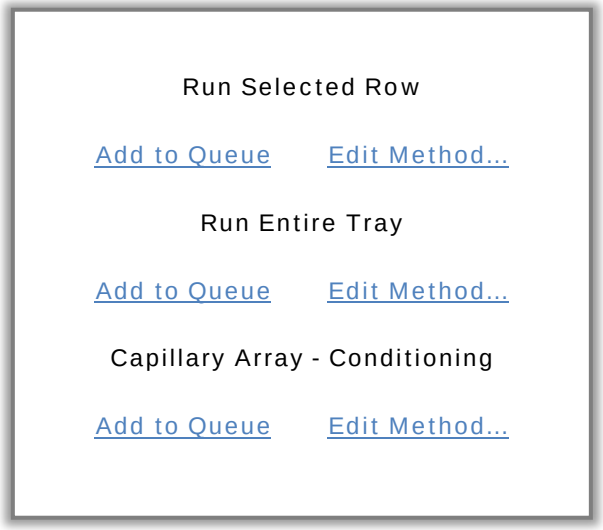


Figure 3-11. Main Screen Window - Experimental Run Controls

Capillary Conditioning

The *Fragment Analyzer*™ instrument software provides **Pre-Loaded Capillary Conditioning Methods**.

Through these **Capillary Conditioning Methods**, the software directs the pumping system that provides automated flushing and filling of the capillary array with **Separation Gel** or **Capillary Conditioning Solution** between experimental CE runs.

Conditioning of the capillary walls is also maintained by the system software at periodic intervals to maintain maximum separation resolution.

- **Capillary Conditioning** ensures that the system is ready to run samples for analysis.

Capillary conditioning does not typically require user intervention, however there are two circumstances that require that capillary conditioning be performed outside of an experimental run cycle.

- When a Capillary Array Cartridge is replaced the array will have to be conditioned using a capillary conditioning procedure. Cartridges are replaced approximately every 1000 injections (12,000 samples) or (96,000 samples) for a 96-capillary cartridge.

- In rare cases a capillary may become clogged or the capillaries show poor performance which may require a conditioning step.

The Capillary Conditioning Process is comprised of six steps which are outlined in Appendix A01. Some or all of the steps are performed depending upon the method employed.

Capillary Conditioning Commands -
View or Edit a Capillary Conditioning Method

A **Capillary Conditioning Method** is accessed via the **Capillary Conditioning Commands** under the **Operations Tab** from the **Main Screen Window**.

Select either **[Add to Queue]** or **[Edit Method…]** under the **Capillary Array-Conditioning Commands** to view or edit the parameters of a conditioning method. (Fig.**3-12**)

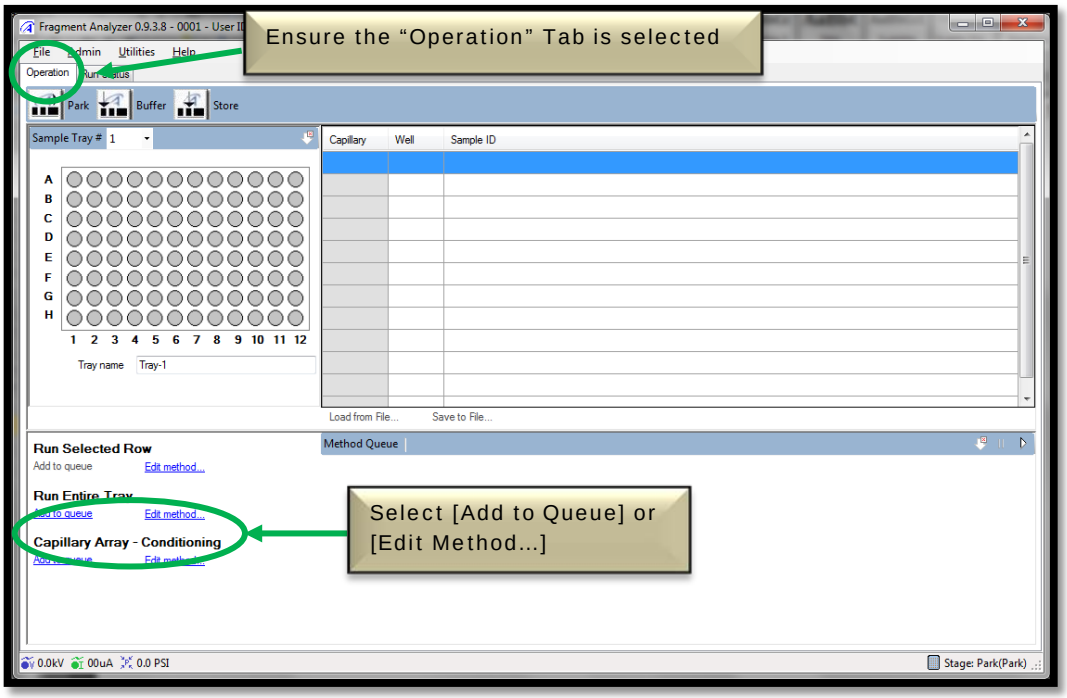


Figure 3-12. Main Screen Window: Capillary Array – Conditioning Commands

To edit a **Capillary Array Conditioning Method**, select [**Edit Method…**].

From the **Conditioning Method Edit Window**, each step of a **Capillary Conditioning Method** can be modified. (Fig. **3-13**)

In the **Conditioning Method Edit Window** the user can adjust:

- Fill Pressure (psi)
- Time (min)
- Gel Selection (Conditioning Solution, Gel 1, or Gel 2)
- Flow Rate (ml/min)

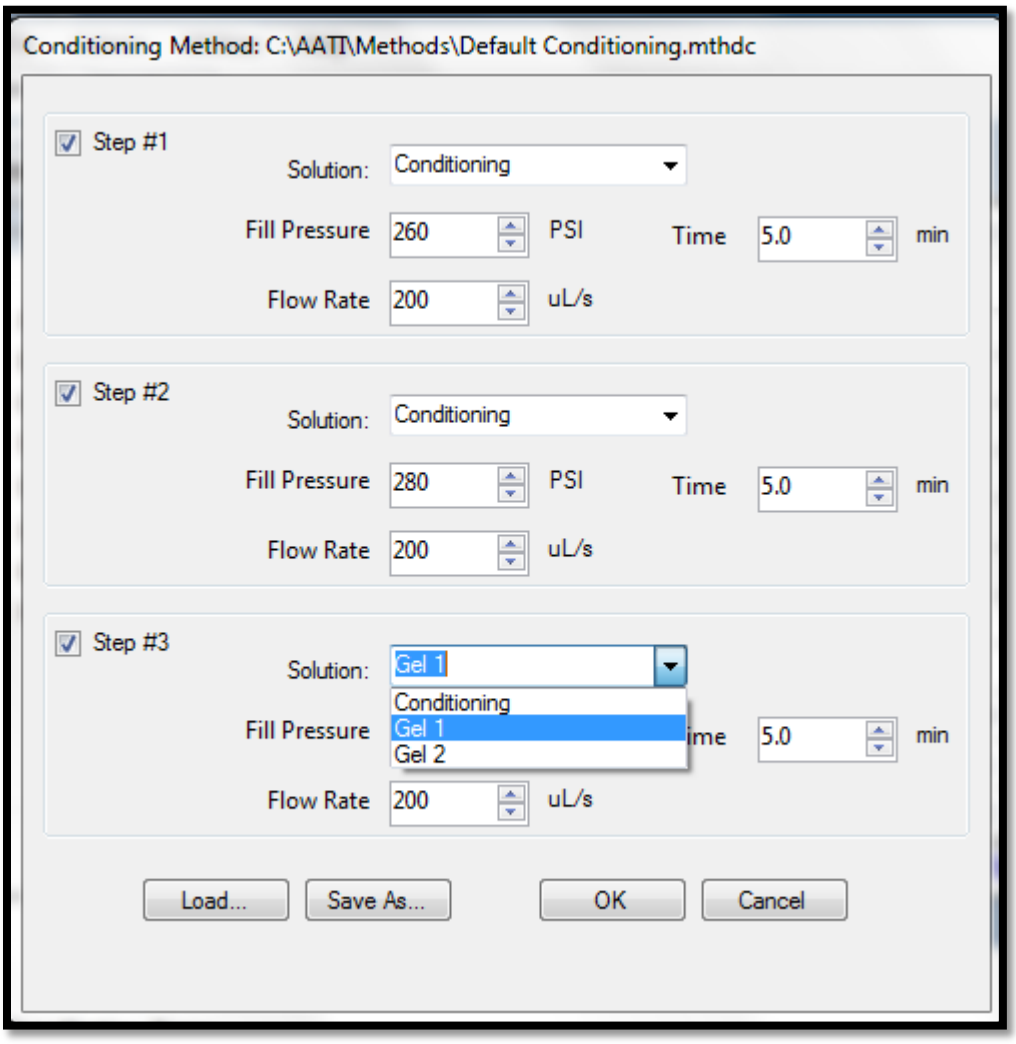


Figure 3-13: Edit Conditioning Method Screen

The default capillary conditioning process values are provided in the table below:

Fill Pressure (psi)	Time (minutes)	Gel Selection	Flow Rate (ml/min)
280 psi	3 minutes	Cond, Gel 1 or Gel 2	100 µL/s

Table 3-2: Default Capillary Conditioning Parameters

- Optimum capillary conditioning values are defined for each method. Refer to each Kit Method Manual of interest (i.e. NGS, genomic DNA, etc.) for further definition of these values.

Capillary conditioning parameters can be adjusted with the up and down arrows located to the right in each data entry field. For each step, the Solution Selection Data Field is populated via a drop down menu.

To save changes to a conditioning method select [Save As...]. A window will prompt you to enter a name for the method. Once a name is entered select [Save]. The new method will be saved in a *.mthds* format and it will be added to the [Load...] dropdown menu.

To load a previously saved Capillary Conditioning Method, select the [Load...] button from the Conditioning Method Edit Window and from the dropdown menu, navigate to the appropriate method file (in a *.mthds* format).

To accept the current capillary conditioning method and return to the Main Screen Window, press [OK].

To cancel out of the Conditioning Method Edit Window press the Cancel button.

Running a Capillary Conditioning Method

Prior to performing a capillary conditioning:

- Check the **Waste Tray in Drawer W** (2nd drawer) to ensure that it is empty.
- Check the **Conditioning Solution, Gel 1, or Gel 3** solution levels to ensure that there is sufficient volume for the operation (located in the instrument **Side Compartment**).

⚠ The minimum solution volume required to run the default Capillary **Conditioning Method is ≥ 5 ml** for a 12 Capillary Array.

Once a capillary conditioning method has been selected, start the method from the **Main Screen Window** by selecting **[Add to queue]** under **Capillary Array-Conditioning**.

To start capillary conditioning, left mouse click the **[Start Arrow]** on the **Method Queue Status Bar** as shown in Fig. **3-14**.

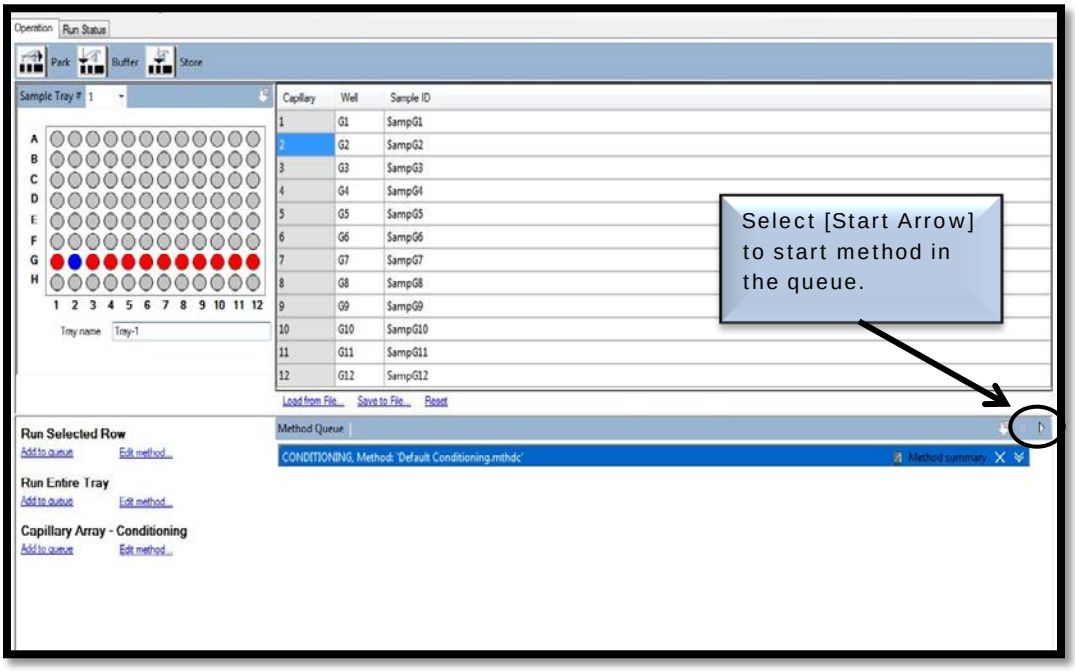


Figure 3-14: Starting a Capillary Conditioning Run.

Getting Started - *Fragment Analyzer™* Software

In This Chapter

- Main Screen Interface Overview
  - ✓ Sample Selection Controls
    - Selecting Samples for an Experimental Run
    - Adding Samples to the Queue
    - Entering Sample Information into the Sample Information Editor
    - Sample Information Parameters
  - ✓ Capillary Electrophoresis (CE) Setup
  - ✓ Editing an Experimental Method
    - Selecting and Editing an Experimental Method
    - Experimental Method Editor Window
    - Experimental Method Steps

In this chapter, we will continue exploring the *FRAGMENT ANALYZER*™ system software and the operations that are accessed from the Main Screen Window.

The next sections will cover the Selection of Samples, Setup of a CE Run, Selection and Editing of an Experimental CE Method.

Sample Selection Controls

Selecting Samples for an Experimental Run

To perform a CE separation on **one to eight rows of a 96-well sample plate**, open the **Main Screen Window** of the *Fragment Analyzer*™ instrument software. (Fig. **4-1**.)

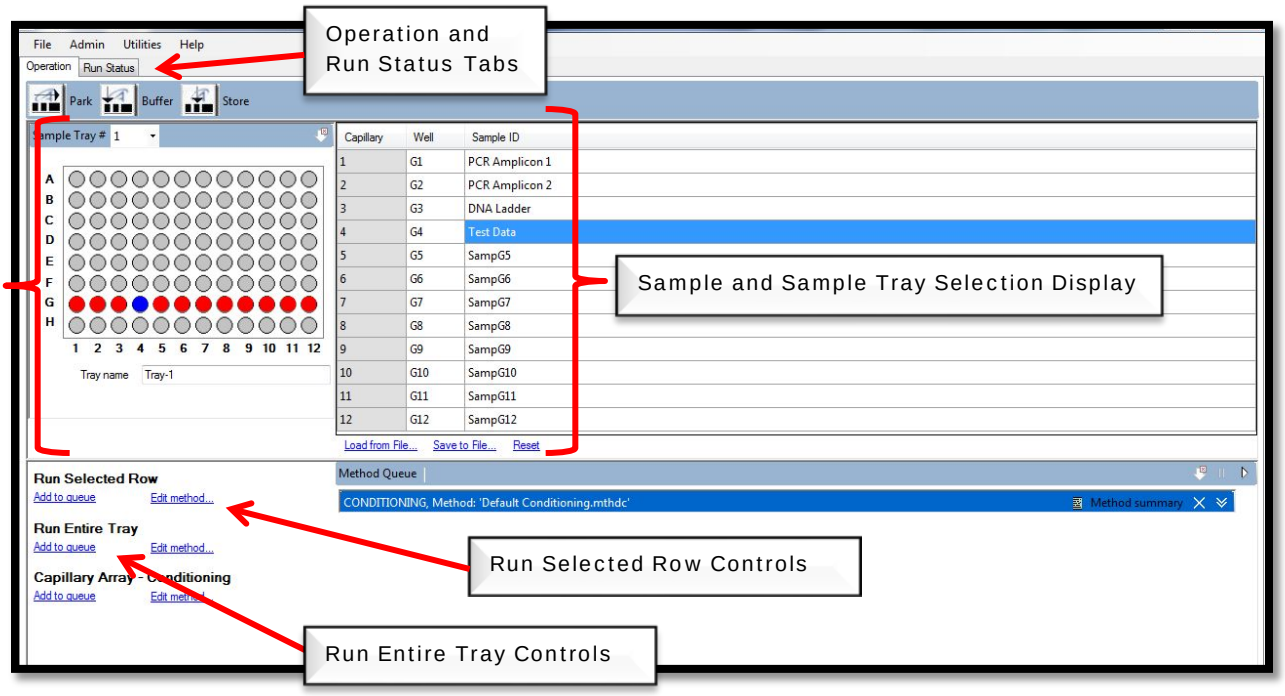


Figure 4-1. Main Screen Window: Sample Selection Controls

From the Operations Tab on the Main Screen Window use the dropdown menu in the Sample Selection Control Bar to choose the Sample Tray Location (Drawer 1, 2 or 3) that the instrument will use for the CE run. (Fig. **4-2**.)

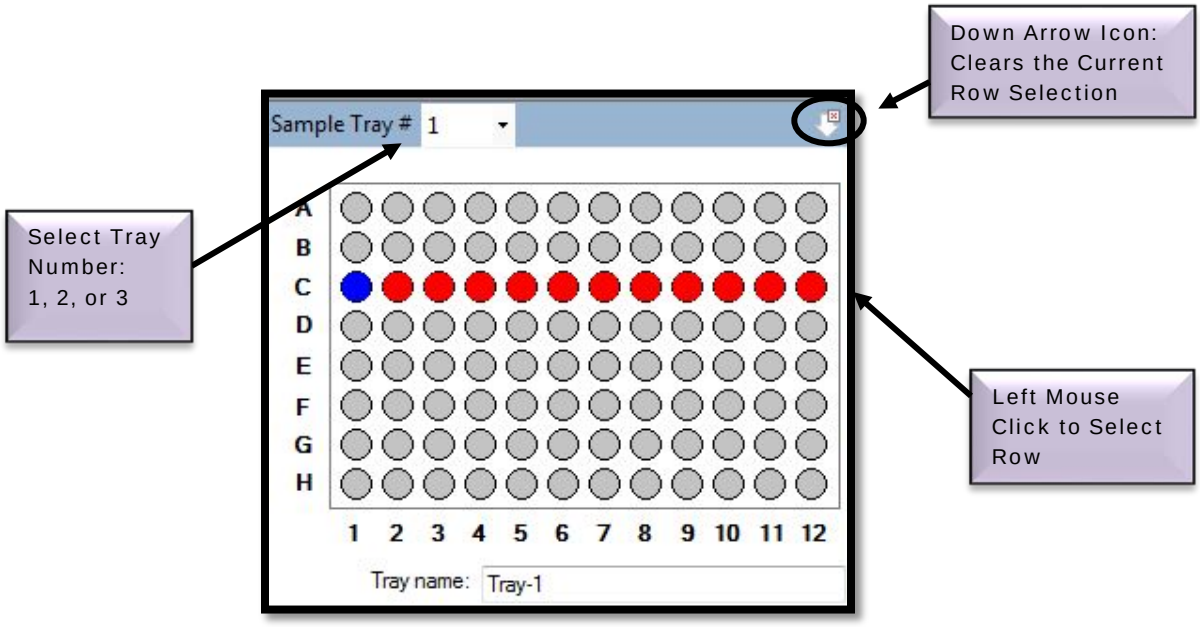


Figure 4-2: The Sample and Sample Tray Selection Display: Row C of Tray 1 Highlighted

To select a row from the 96-well plate depicted in the Sample and Sample Tray Selection Display left mouse click once in that row. (Fig **4-2.)**

The Down Arrow Icon in the right corner of the Sample Selection Control Bar can be used to clear a row selection. (Fig. **4-2**)

Adding Samples to the Queue

To perform and experimental run on one or more rows highlighted in the **Sample and Sample Tray Selection Display**, select **[Add to Queue]** from the **Run Selected Row Menu**.

An entire sample tray can be added to the queue by selecting **[Add to queue]** under **Run Entire Tray. (**Fig **4-3**)

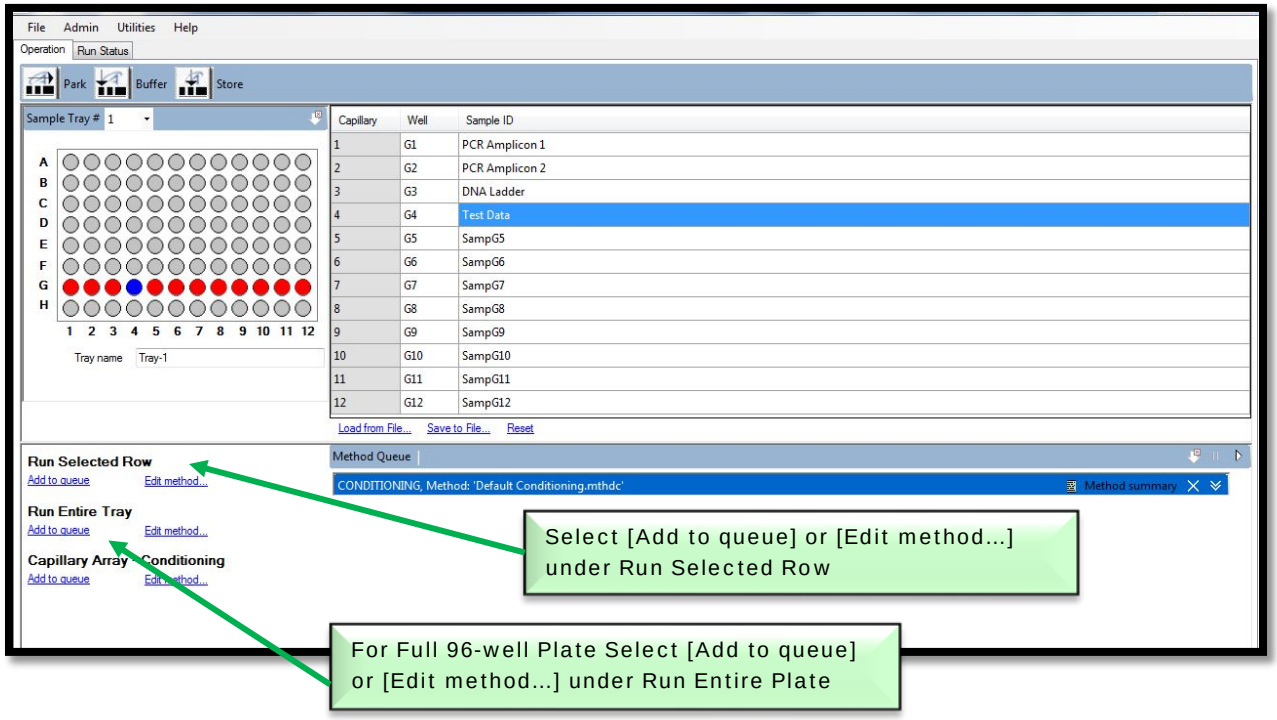


Figure 4-3. Main Screen Window : Sample Selection – Adding Samples to Queue

Entering Sample Information into the Sample Information Editor

Once samples have been selected, information for each sample well can be added in the **Sample Information Editor Window** located to the right of the **Sample and Sample Tray Selection Display Window**. (Fig **4-4**)

Sample information can be typed in or loaded from a .csv or .txt file using the **[Load from File…]** command at the bottom left of the table.

Sample information can also be saved as a .csv or .txt file using **[Save to File…]**. This is useful for when the same set of samples is repeatedly analyzed.

If no information is entered or loaded from a pre-existing file for the samples, a **Default Sample ID of 'Samp#'** will be assigned for each sample where '**Samp#**' **equals the associated capillary number (Example: SampA1)**..

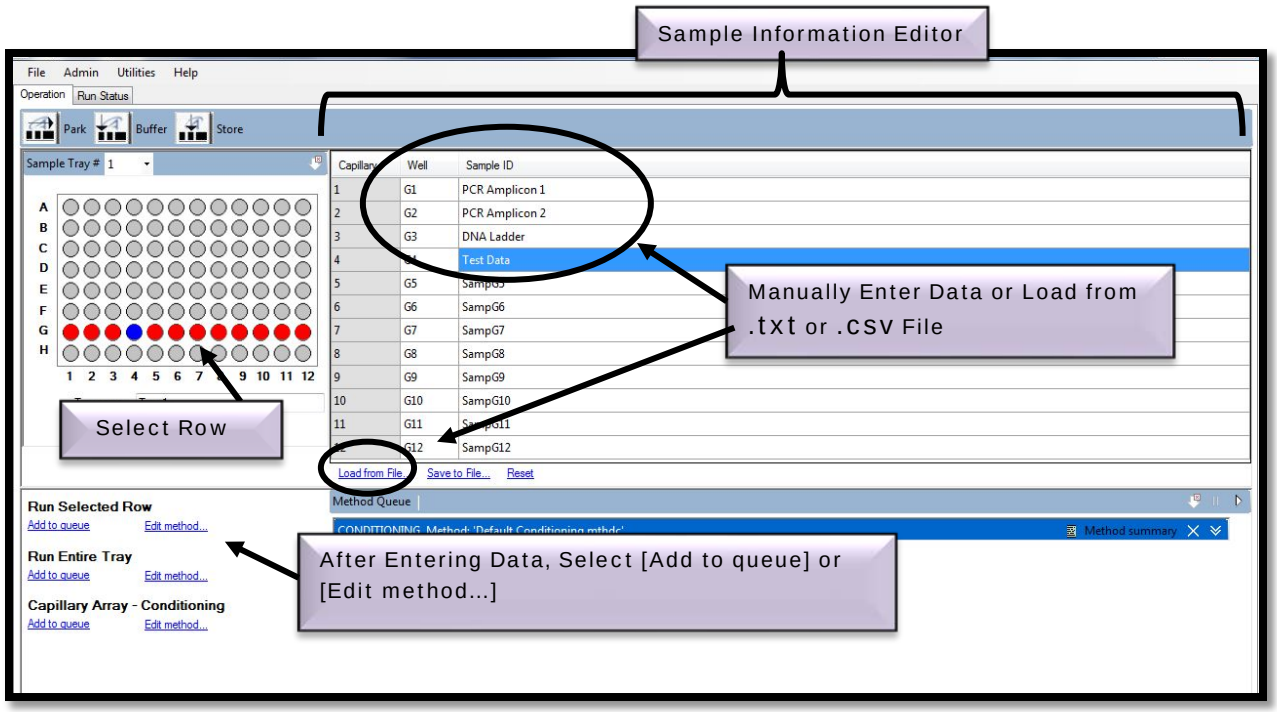


Figure 4-4. Main Screen Window:
The Sample Information Editor -
Selecting a Row and Entering Sample Data

Sample Information Parameters

The parameters for describing samples in the **Sample Information Editor Window** are outlined below.

In the example shown on Page 5, Figure **4-4**; Sample information is entered for wells G1 through G4.

Capillary	Well	Sample ID
Displays the capillary number 1 through 12.	**The sample well position 1 through 12 for the selected row A through H.**	**A descriptive name for a sample. Should be less than 40 characters in length.**

Table 4-1. Sample Information Editor Parameters

After entering sample data, select **[Add to Queue]** under the **Run Selected Row Menu.** Fig **4-4**.

For a 96-cap system, sample naming is similar. Since 96-well trays are analyzed, the user must select each row to manually enter data (in this case, rows A-H) (Fig. **4-4**). Sample names and information can also be saved or loaded using .txt or .csv files, as explained above for the 12-capillary system.

CE Separation Setup

The Separation Setup Window

After adding samples to the queue under the **Run Individual Rows Menu** or the **Run Entire Plate Menu,** you will observe the **Separation Setup Screen** shown below in Figure **4-5**.

The **Separation Setup Window** allows the user to:

- Verify, choose or make additional changes to a selected method
- Add a folder prefix
- Modify where CE separation data will be uploaded
- Add additional sample information and notes

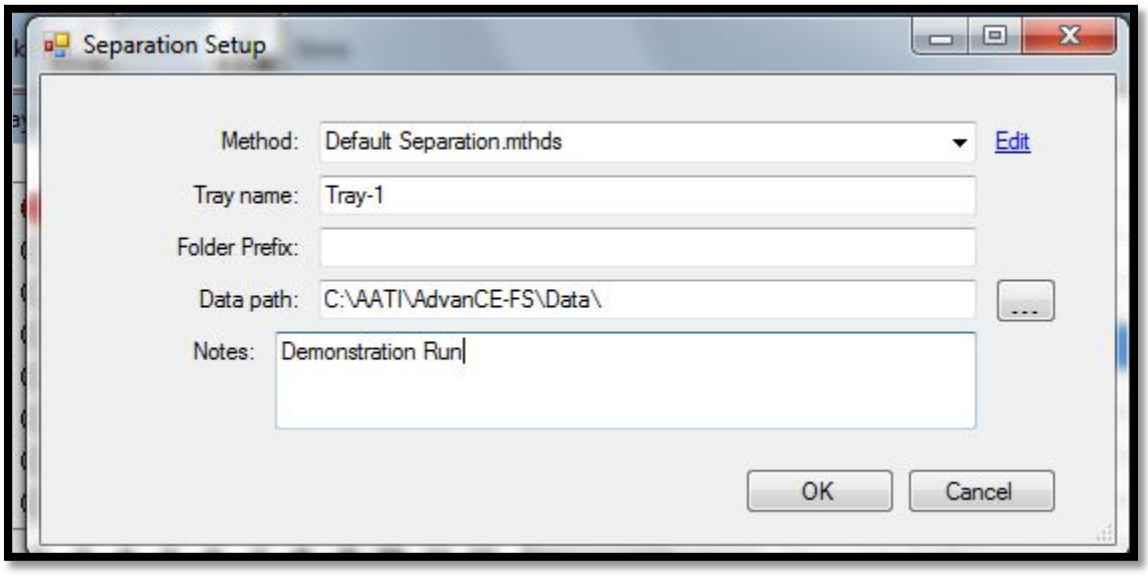


Figure 4-5: The Separation Setup Window

Selecting an Experimental Method

To select a CE Separation Method from the Separation Setup Window, click the arrow in the Method Field. (Fig **4-6**)

⚠ Every time the Separations Setup Screen is opened it will default to the method 'Default Separation'. To run a method other than the 'Default Separation Method', it must be selected from the Drop Down Menu.

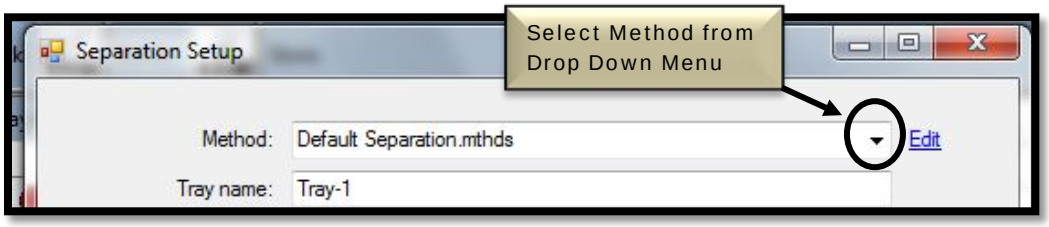


Figure 4-6: The Separation Setup Window:
Select Method Command

To Edit a CE Separation Method from the Separation Setup Window, select the [Edit] command located to the right of the Method Field. This will open the Method Editor Window. Fig. 4-**12**, Pg. 14

◆ Editing a method can also be performed by selecting the [Edit Method...] function located under the Run Selected Row Menu or the Run Entire Tray Menu from the Main Screen Window. Editing a method will be covered in greater detail later in this chapter.

Entering the Method Editor Window:

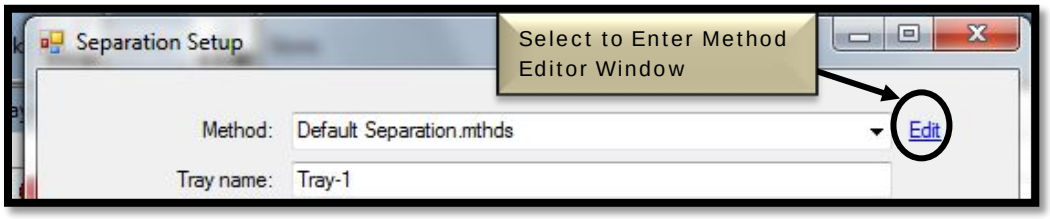


Figure 4-7: The Separation Setup Window:
Enter Method Editor Window Command

Default Directory Location for CE Separation Data

The Default Directory Location for saving CE separation data is C:\AATI\Data with a subfolder and data file name automatically created according to the date and time the experiment is performed. Fig. **4-8**.

The File Name within the directory is saved as the Date and Time of the experiment.

For example, an experiment performed on July 10, 2010 at 1:30:10 PM will be saved to the directory C:\AATI\Data\2010 07 10 with a filename of [Folder Prefix]*13-30-10.raw*. If the [Folder Prefix] is left blank the sample file will be named 13-30-10.raw, as an example.

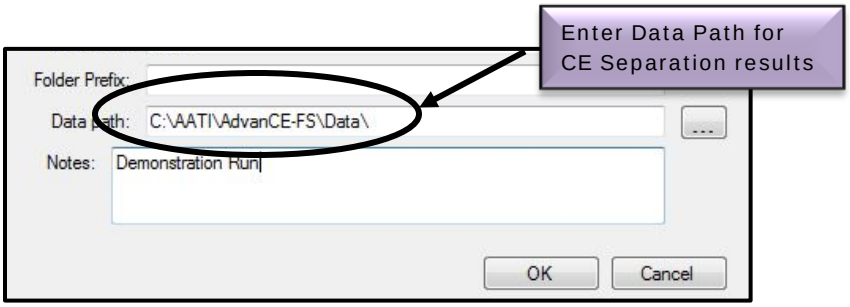


Figure 4-8. The Separation Setup Window:
Default Directory Data Path

Entering Tray Name, Folder Prefix and Notes

The **Default Tray Name** selected on the **Sample and Sample Tray Selection Display Window** will populate the **Tray Name Field**. Fig. 4-9.

This field can be edited from the **Separation Setup Window**.

The **Folder Prefix** allows the user to add a prefix to the folder name where the results files will be written. This allows users to add useful information on the nature of the sample. For example, if running a 100 bp ladder, the user may want to put "100 bp ladder" as the Folder Prefix.

The **Notes** section of the **Separation Setup Window** allows for the addition of any additional information the user may require for a set of samples.

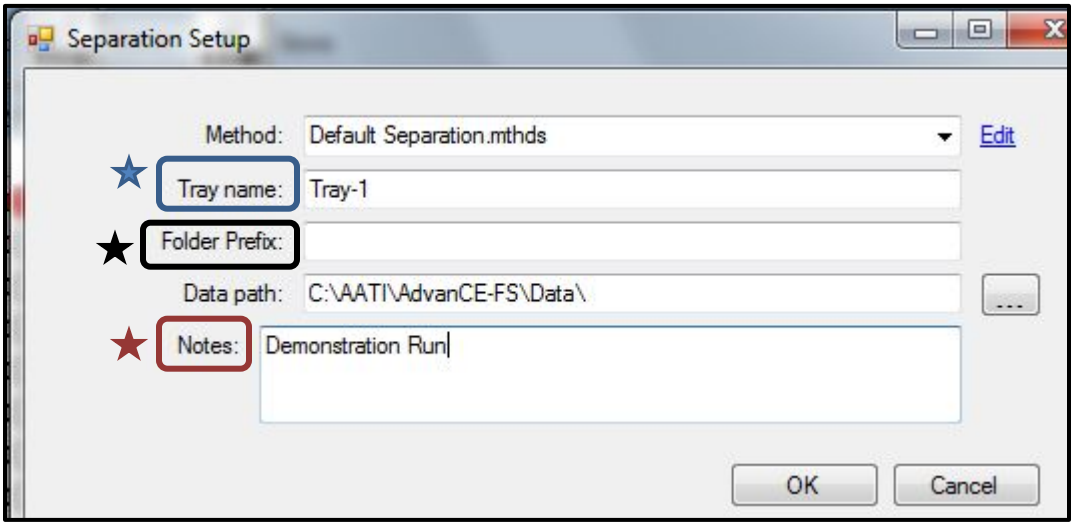


Figure 4-9. The Separation Setup Window:
Tray Name, Folder Prefix and Notes

Separation Setup Window Parameters

A summary of the **Separation Setup Window Parameters** is illustrated in the table below.

Task	Description
Method	**The Separation Method is shown in a drop down list control. The user is able to select any available separation methods from this list. An edit link next to the method field brings up the Method Editor for that method.**
Tray name	**The default tray name appears as it was selected by the User on the main form. The operator may edit this field.**
Folder prefix	**The folder prefix allows the User to add a prefix to the folder name where the results files will be written.**
Data path	**The data path specifies the location where the generated output files are stored. The User can browse for the desired location using the browse button.**
Notes	**The User may add notes to this separation.**
OK	**This function adds the separation to the queue.**
Cancel	**Cancels the operation.**

Table 4-2: The Separation Setup Window Parameters

Saving Separation Setup Window Parameters

After modifying the **Separation Setup Parameters**, select [OK]. The **Method Queue** on the **Main Interface Screen** will be populated with the method entered for the samples placed in either the **Run Selected Row** or **Run Entire Tray** queue. Fig. 4-10

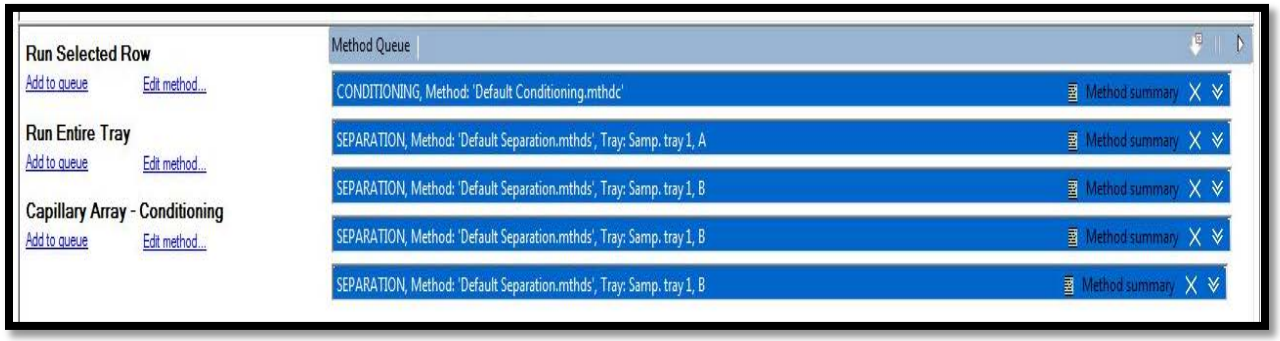


Figure 4-10. Main Interface Window: Populated Method Queue

The next sections will go over the **Steps for Editing a Separation Method** using the **Method Editor Window**.

The **Method Editor Window** can be accessed from either the **Separation Setup Window** as described in the previous section or from the **Main Interface Window** under the **Run Selected Row** or **Run Entire Tray Menu**.

- If you would like to skip the **Method Editor Overview** and continue on to **Running a CE Separation**, please proceed to later sections.

Selecting and Editing an Experimental Method

During normal operation, the user will select pre-defined methods. Several pre-defined methods have been pre-loaded onto your Fragment Analyzer™ system, including NGS, RNA, gDNA, 910 kit, 930 kit, etc.

However, there may be cases in which a user has to create a new, unique method, or modify an existing method. The following section explains how to create a new method or modify an existing method.

Before a CE separation can be initiated for a set of samples, an **Experimental Method** must be either selected or created.

The **Method Editor Window** will allow you to **View**, **Edit**, **Load** or **Save** an **Experimental Method** prior to running samples.

The **Method Editor Window** is accessed from the **Main Screen Window** by selecting the **[Edit Method…]** function located under the **Run Selected Row Menu** or the **Run Entire Tray Menu**. Fig. **4-11**.

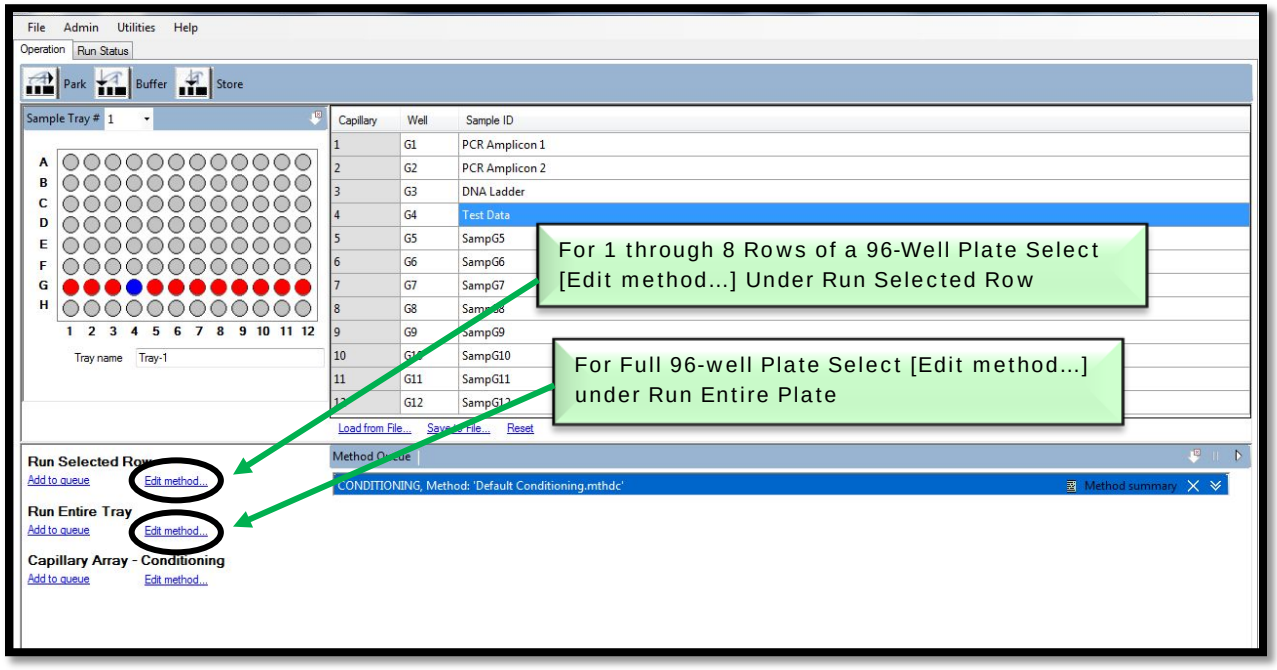


Figure 4-11. Main Screen: Opening the Method Editor Window

Experimental Method Editor

The **Method Editor Window** allows you to customize run parameters for a CE separation. Fig. **4-12**.

To enable steps within the **Method Editor Window**, click the checkbox next to that step and modify the parameters as needed.

⚠ The **Gel-Prime** and **Conditioning Steps** are hard coded and cannot be disabled..

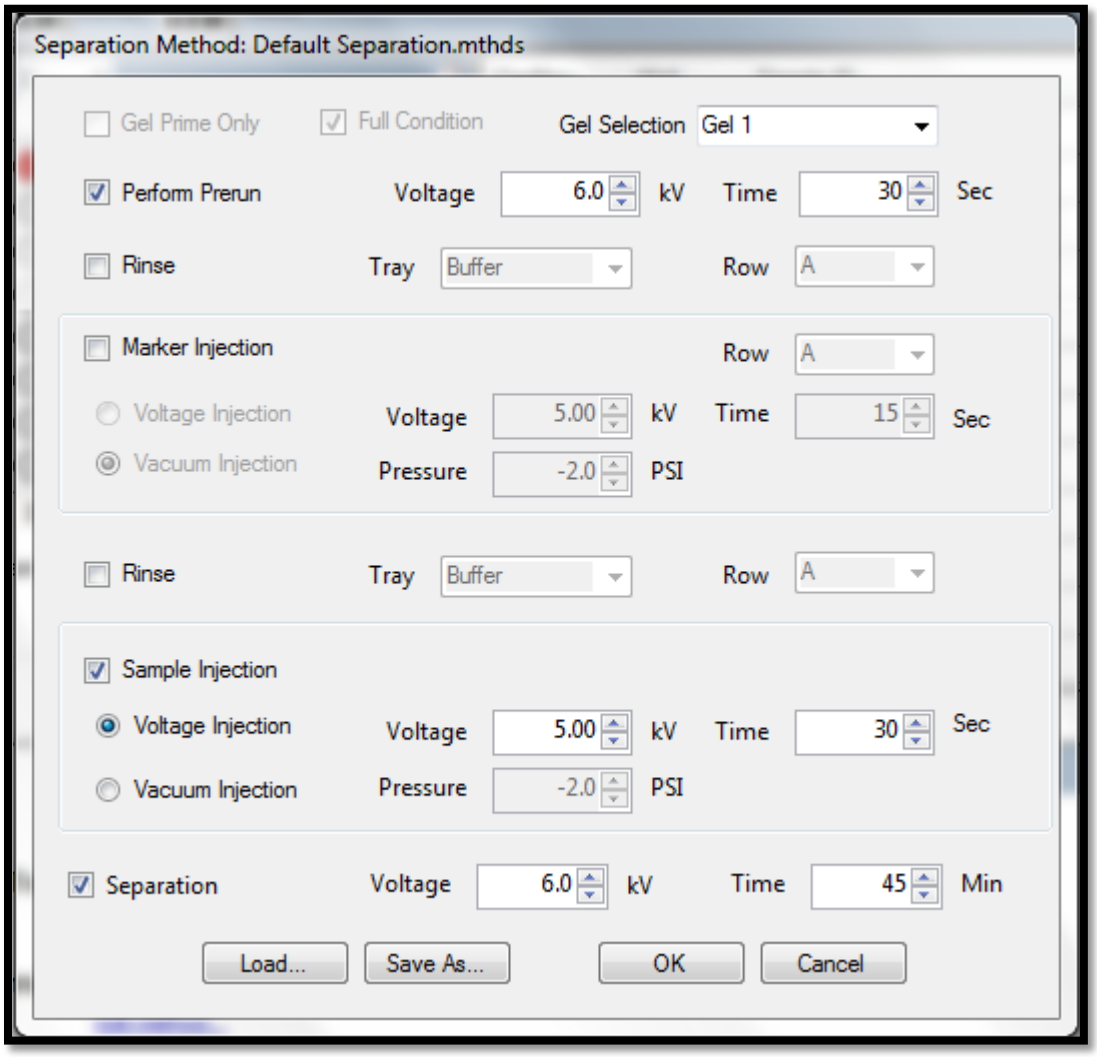


Figure 4-12. Method Editor Window (These conditions are hypothetical – please refer to the Kit Manual for Method Conditions)

Experimental Method Steps

In this section, we will explore each of the steps that make up an **Experimental CE Method**. The parameters for these steps can be modified through the **Method Editor Window**. Fig **4-12**.

Gel Prime/Conditioning Step

This is a hard-coded feature that cannot be disabled. The instrument performs a full conditioning and gel prime step prior to each run.

This ensures fresh gel is used for each run and ensures that there are no cross-contamination issues between runs.

The conditions for the **Gel Prime Step** can be defined in the **Method Editor** under the **Conditioning step**. The **Gel Selection** dropdown menu allows the user to change the gel type used for the **Gel Prime/Conditioning Step**. Fig. **4-13**.

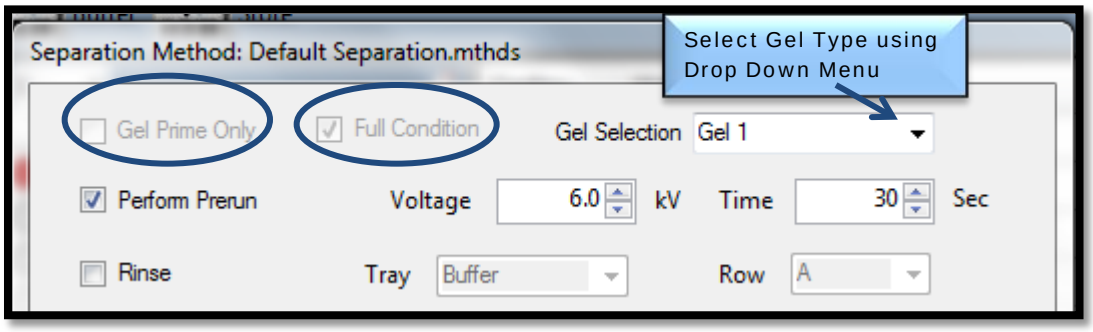


Figure 4-13. Method Editor Window:
Gel Prime and Conditioning Steps

Perform Pre-run Step

The **Voltage Pre-run** will electrochemically condition the **Separation Gel Matrix** in the capillaries prior to a CE separation.

Both the **Voltage** and **Time** can be adjusted for the **Separation Pre-run**. Fig. 4-14.

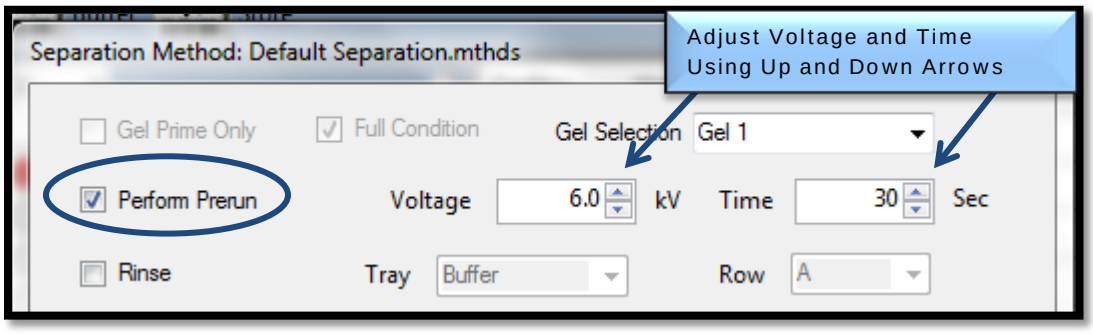


Figure 4-14. Method Editor Window:
Perform Pre-run Step Controls

The system performs a **Voltage Pre-run** with the capillary array tied to the **Inlet Buffer Tray** in **Drawer B** (Top Drawer).

For a **12-Capillary Array** the **Voltage Pre-run** will be conducted using **Inlet Buffer** placed in **Row A of a 96-Well Plate** located in **Drawer B**.

For a **96-Capillary Array** the **Voltage Pre-run** will be conducted using **Inlet Buffer** placed in **each well** of a **96-Well Plate** located in **Drawer B**.

Pre-run Parameters

The parameter limits for the **Perform Pre-run Step** are defined below in Table **4-3**.

✳ A typical pre-run is 6 kV for 10 to 30 seconds.

Parameters	Range
Voltage	**0 to 15 kV**
Time	**0 to 1200 sec**

Table 4-3: Pre-run Parameter limits

Rinse Step

The **Rinse** function is sometimes used for obtaining good-quality CE data. The use of a rinse step may (or may not) be included in the instruction manual for each kit. The purpose of this step is to clean the capillary tips prior to injection. Fig. 4-15.

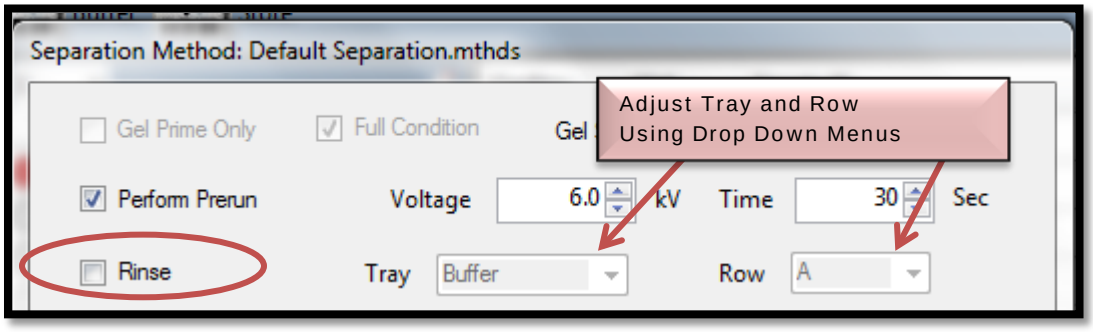


Figure 4-15. Method Editor Window:
Rinse Step Controls

The Rinse Step occurs after the instrument performs a Gel-Purge Step and before the Marker Injection Step and/or Sample Injection Step.

This step takes approximately 1 minute and can prevent contamination between the marker and sample plates.

Rinse Parameters

The Drop Down Menu Options for the Rinse Step are defined below in Table **4-4**.

Parameters	Drop Down Menu Options
Tray	**User Selectable: Any of the 6 tray locations**
Row	**A through H (12 cap) All rows for 96 cap.**

Table 4-4: Rinse Drop Down Menu Options

Marker Plate Injection Step

Injection of the **Lower/Upper Markers** is **required** **with every CE separation** in order to normalize migration times across the capillary array and calibrate the size of the separated DNA/RNA fragments.

Markers can be injected in two ways, depending on the kit used:

1) From a marker plate, using a separate injection. In this case, the "Marker Plate" injection step must be used.

2) From markers added directly to the sample. In this case, **there is no need** for the "Marker Plate" injection step.

The **Lower/Upper DNA Markers** can be injected by either **Voltage (Electrokinetic)** or **Vacuum mode**. Fig. **4-16.**

When **Injecting by Voltage (Electrokinetic)**, the applied **Voltage** and **Time** can be adjusted.

When **Injecting by Vacuum**, the **Pressure** and **Time** is entered. A typical pressure is from about -1 to -3 psi, with a time ranging from 5 sec to 30 sec. Actual times and pressures will depend on the observed peak magnitudes.

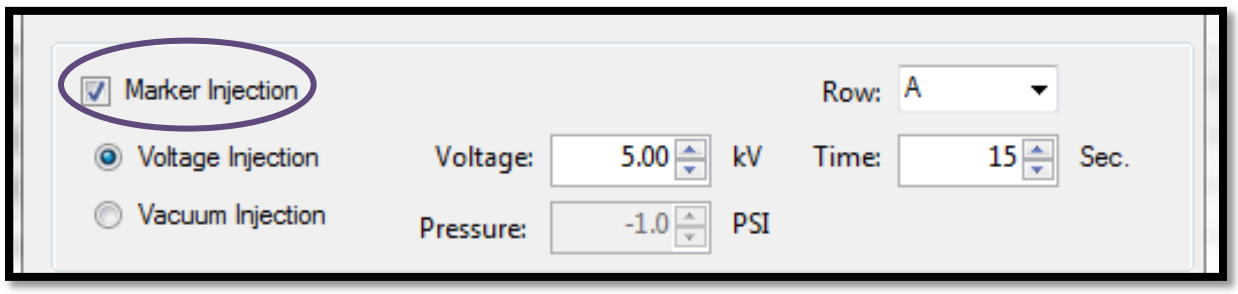


Figure 4-16. Method Editor Window:
Marker Injection Controls.

When the Marker Plate Injection Box is checked, following the optional Pre-run and optional Rinse Step, the instrument will move the tray located in Drawer M (third drawer from the top) to the capillary array and inject the Lower/Upper DNA Markers.

- For instructions on how to set up the Lower/Upper DNA Marker Plate for a12-Capillary or a 96-Capillary System please refer to Appendix C.

Marker Plate Injection Parameters

The parameter limits for the Lower/Upper DNA Marker Plate are defined below in Table 4-5.

The typical values for a Voltage Injection of the Lower/Upper DNA Marker Plate are 5 kV for 10 sec. The user may increase times and/or voltages to obtain higher peak magnitudes, or use lower voltages and/or times to obtain lower peak magnitudes.

The typical values for a Vacuum Injection of the Lower/Upper DNA Marker Plate are -2 psi for 10 sec. The user may increase times and/or decrease pressures to obtain higher peak magnitudes, or use higher pressures and/or lower times to obtain lower peak magnitudes.

Options(enabled)	Voltage	Pressure	Time
Voltage Injection	**0 to 100kV**	**N/A**	**0 to 240 sec**
Vacuum	**N/A**	**-5 to 0 PSI**	**0 to 240 sec**

Table 4-5: Marker Plate Injection Parameter Limits

2nd Rinse Step

The 2nd Rinse is an optional step and allows for the rinse of the capillary tips between the Lower/Upper Marker Plate Injection and the Sample Injections. (Fig. **4-17**)

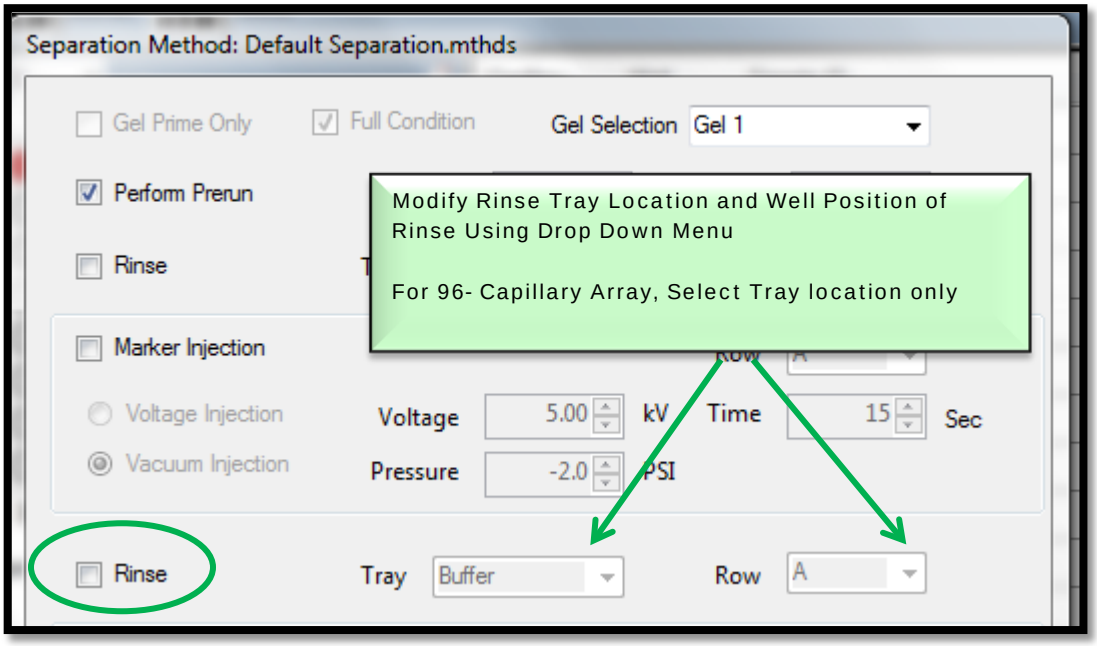


Figure 4-17. Method Editor Window:
2nd Rinse Step Controls

The Drop Down Menu Options for the 2nd Rinse Step are defined below in Table **4-6**.

Parameters	Drop Down Menu Options
Tray	**Sample Tray 3,**
Row	**A through H (12-cap only)**

Table 4-6: Rinse Drop Down Menu Options

Sample Injection Step

When enabled, this will move the Selected Sample Tray in Drawer Position 1, 2, **or** 3 to the capillary array for injection. (Fig **4-18).**

Just like the DNA markers, samples can be injected by either Voltage (electrokinetic) or Vacuum Mode.

- When injecting by Voltage, the applied Voltage and Time can be adjusted.

- When injecting by Vacuum the Time and pressure are entered.

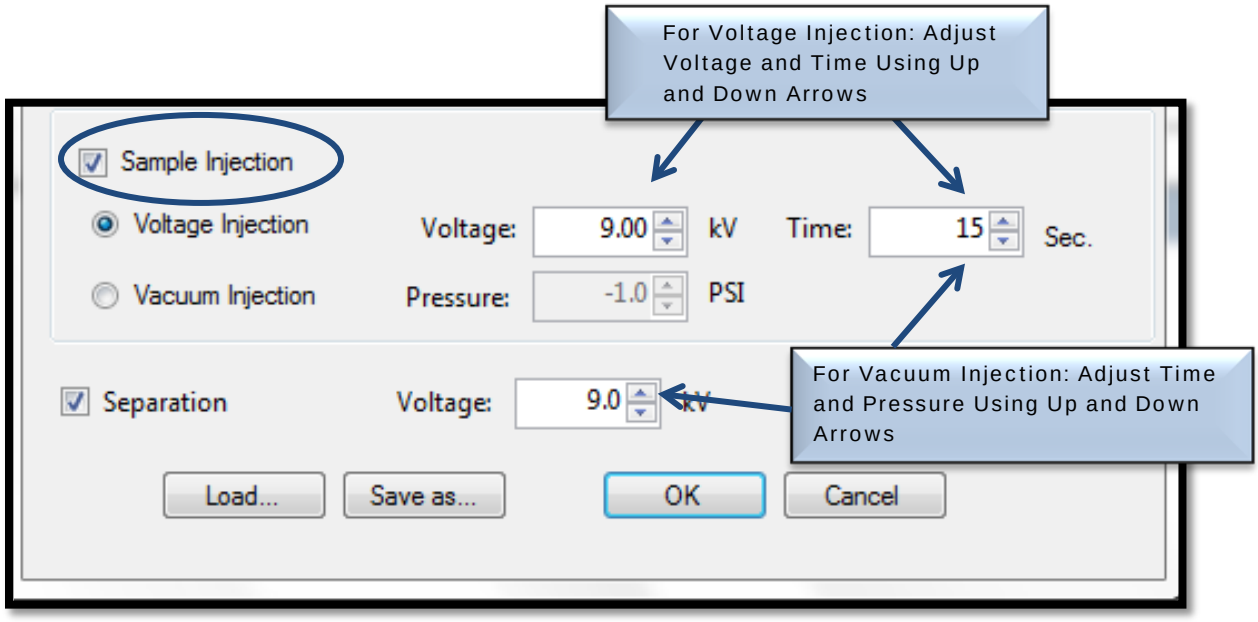


Figure 4-18. Method Editor Window:
Sample Injection Controls

Sample Injection Parameters

The parameter limits for the **Sample Injection** are defined below in Table **4-7**.

The typical values for a **Voltage Injection** of the **Sample Plate** are 3-10 sec at 5 kV. Specific injection conditions are given in separate kit manuals (for example, the NGS kit). The user may increase times and/or voltage to obtain higher peak magnitudes, or decrease voltage and/or lower times to obtain lower peak magnitudes.

The typical values for a **Vacuum Injection** of the **Sample Plate** are -2 psi for 10-20 sec. The user may increase times and/or decrease pressures to obtain higher peak magnitudes, or use higher pressures and/or lower times to obtain lower peak magnitudes.

Options(enabled)	Voltage	Pressure	Time
Voltage Injection	**0 to 100kV**	**N/A**	**0 to 240 sec**
Vacuum	**N/A**	**-5 to 0 PSI**	**0 to 240 sec**

Table 4-7: Sample Injection Parameter Limits

Sample Run Step

In this step the run parameters for the **Electrophoresis Step** are defined. (Fig. **4-19**).

The instrument will move the tray in **Drawer B (The Buffer Tray)** under the **capillary array** and **apply high voltage to perform the CE separation.**

Both the **Voltage** and **Time** can be adjusted for this step.

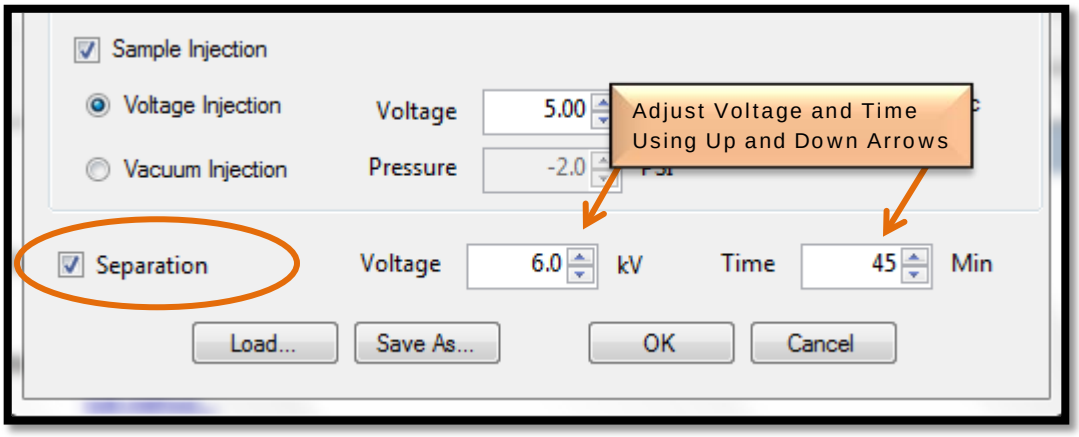


Figure 4-19. Method Editor Window:
Sample Injection Controls

CE Electrophoresis Run Parameters

The Parameter Limits for performing a CE Electrophoresis are defined below in Table **4-8**.

The typical values for a CE Electrophoresis are 6 kV for 50 minutes. Specific kits may have different times. The user may adjust voltages and times to optimize separations.

Parameters	Range
Voltage	0 to 15 kV
Time	0 to 1200 min

Table 4-8: CE Electrophoresis Parameter Limits

Saving and Applying an Experimental Method

After an experimental method has been modified in the **Method Editor Window**, the parameters can be saved using the [Save As…] button. Fig **4-20**.

A window will open, prompting you to provide a name for the new method.

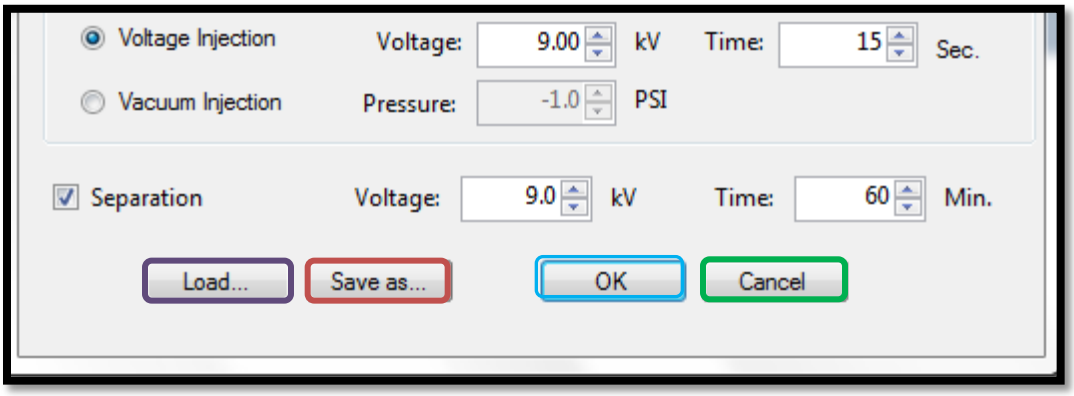


Figure 4-20. Method Editor Window:
[Load…], [Save As…], [OK] and [Cancel] commands

This newly created and saved method will now become part of the [Load…] command drop down menu in the **Method Editor Window**.

- A newly created and saved method will also be available for selection from the **drop down menu** of the **Method Field** located in the **Separation Setup Window**.

To load a previously saved CE method, select the [Load...] command from the Method Editor Window and navigate the drop down menu to the method file of choice.

To accept the current method without editing in the Method Editor Window press [OK] to return to the Main Screen.

To cancel out of the Method Editor Window and return to the Main Screen press the [Cancel] button.

- If a method is edited and saved in the Method Editor Window it will become part of the Drop-Down Menu under the [Load...] command in the Method Editor Window. It will also become part of the [Method] field Drop Down Menu in the Separation Screen.

- Any edited and saved method will need to be added to the Method Queue via the Separation Screen Window in order for it to be used for a CE separation.

Getting Started - *Fragment Analyzer™* Software

In This Chapter

- Main Screen Interface Overview

  - ✓ Performing a CE Separation

    - Method Queue
    - Verifying and Adjusting Solution Levels
    - Starting the CE Run

  - ✓ Separation Status Display Window

    - Group and Single Capillary Real-time Display
    - Saving CE Results

  - ✓ Examining Data with the File Manager

    - Viewing Electropherograms
    - Adjusting Capillary Alignment

In this chapter, we will continue exploring the *FRAGMENT ANALYZER*™ system software and the operations that are accessed from the Main Screen Window.

The next sections will cover Performing a CE Separation, the Separation Display Window and Examining Data with the File Manager.

Performing a CE Separation Method

Method Queue

The **Method Queue** is located in the lower right quarter of the **Operation Tab** accessed from the **Main Screen Window**. Fig. **5-1**.

Prior to performing a CE separation, one or more methods must be placed in the **Method Queue**.

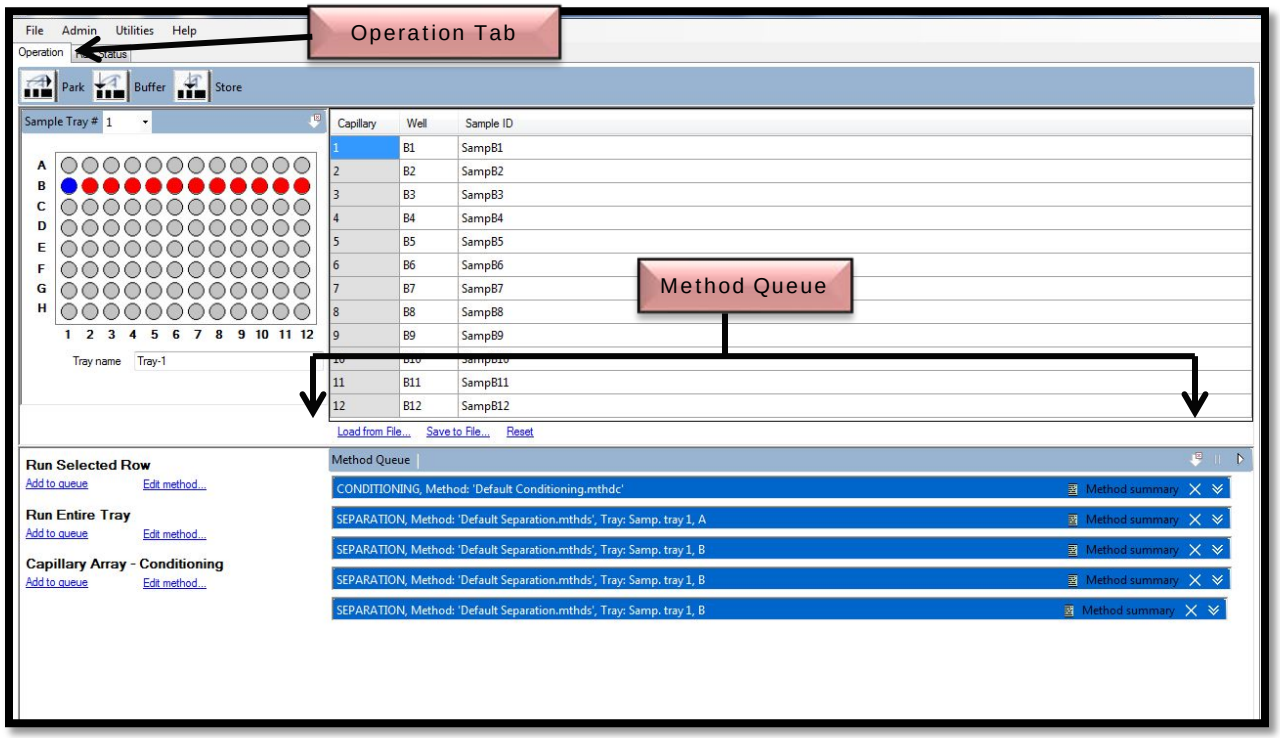


Figure 5-1. Main Screen Window: Method Queue

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Placing Methods in the Method Queue

CE separation methods can be added to the **Method Queue** from the **Method Editor Window** under the **[Load…]** command. Chapter 4, Pgs. 26-27.

Methods can also be added to the **Method Queue** from the **[Method]** field in the **Separation Setup Screen**. Chapter 4, Pgs. 8-9.

Each method placed in the **Method Queue** will be run sequentially from the top down and the job on the top of the list will be removed once it is completed.

Method Queue Limits

The **Method Queue** is limited by the available consumables. When a method is added to the **Method Queue**, a counter is checked to see how many runs have been made since the last fluid level check.

A message box instructing the operator to check the fluid levels is presented if the counter indicates the limit has been reached.

- For **50 ml bottles** the estimated gel, conditioning and waste **capacity supports 8 methods or jobs added to the queue.** This is **equal to eight rows or one 96-well plate.**

    - With the use of **250 mL bottles**, approximately **three 96-well plates or 24 rows** can be run without user intervention.

⚠ Due to the parameters listed above, the *FRAGMENT ANALYZER™* software will accept **no more than eight methods or one 96-well plate** between consumable checks for **50 ml bottles** and only methods to support **three 96-well plates** at a time for the **250ml bottles**.

Method Queue Tool Bar

The **Method Queue Tool Bar** is located along the top of the **Method Queue** in the lower right quarter of the **Operation Tab** accessed from the **Main Screen Window**. Fig. **5-2**

To **start** the **methods** in the **Method Queue**, Click the  **Icon**. This icon is also referred to as the **[Run Control Arrow]**.

When the queue is in the '**Running State**', all of the queued jobs will run until the queue is emptied, or until the user presses the  **Icon**. This icon is also referred to as the **[Pause Button]**.

Select the  **Icon** to **remove all** of the **methods** from the **Method Queue**.

When the list of methods in the **Method Queue** is empty, the instrument will return the storage solution after finishing the final run.

Jobs may be added to the queue at any time and will be added to the bottom of the list in the **Method Queue**.

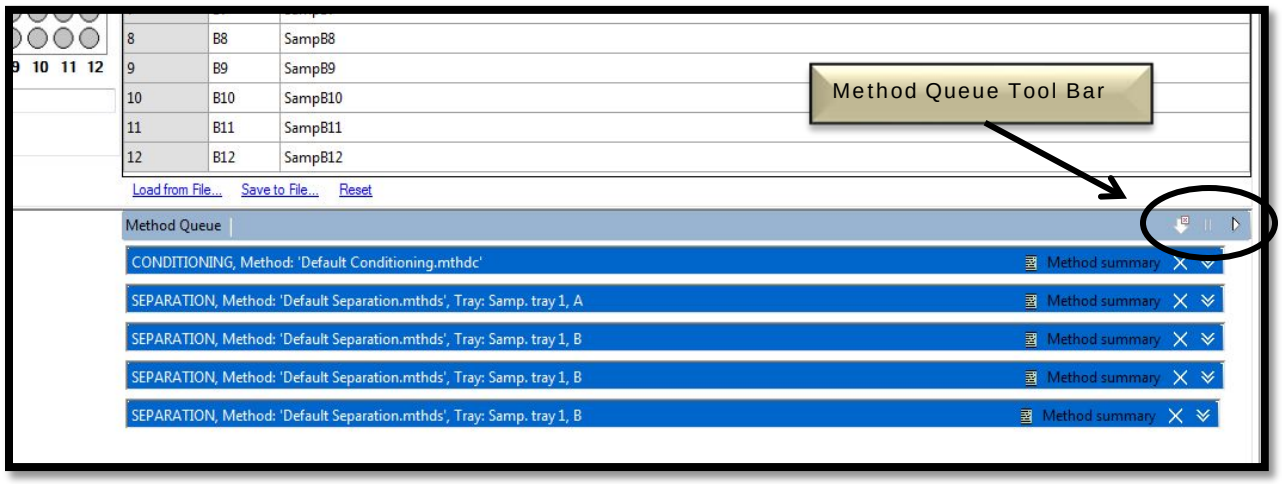


Figure 5-2: Method Queue Tool Bar

A summary of the Method Queue Tool Bar Icons is presented in Table **5-1** below.

Tool	Description
 **Clear Queue**	**Deletes all jobs in the queue.**
 **Pause**	**Pauses the queue after the currently running job.**
 **Run**	**Resume the run after a pause.**

Table 5-1: The Method Queue Toolbar Commands

Method Queue Commands

After one or more methods have been added to the **Method Queue**, you can start a method by clicking the **Run Control Arrow Icon** on the **Method Queue Tool Bar**. Fig. 5-3

To view information for a CE separation, select the **Double-Down Arrow Icon** or select **[Method Summary]**.

To remove a method from the **Method Queue**, select **[X]**.

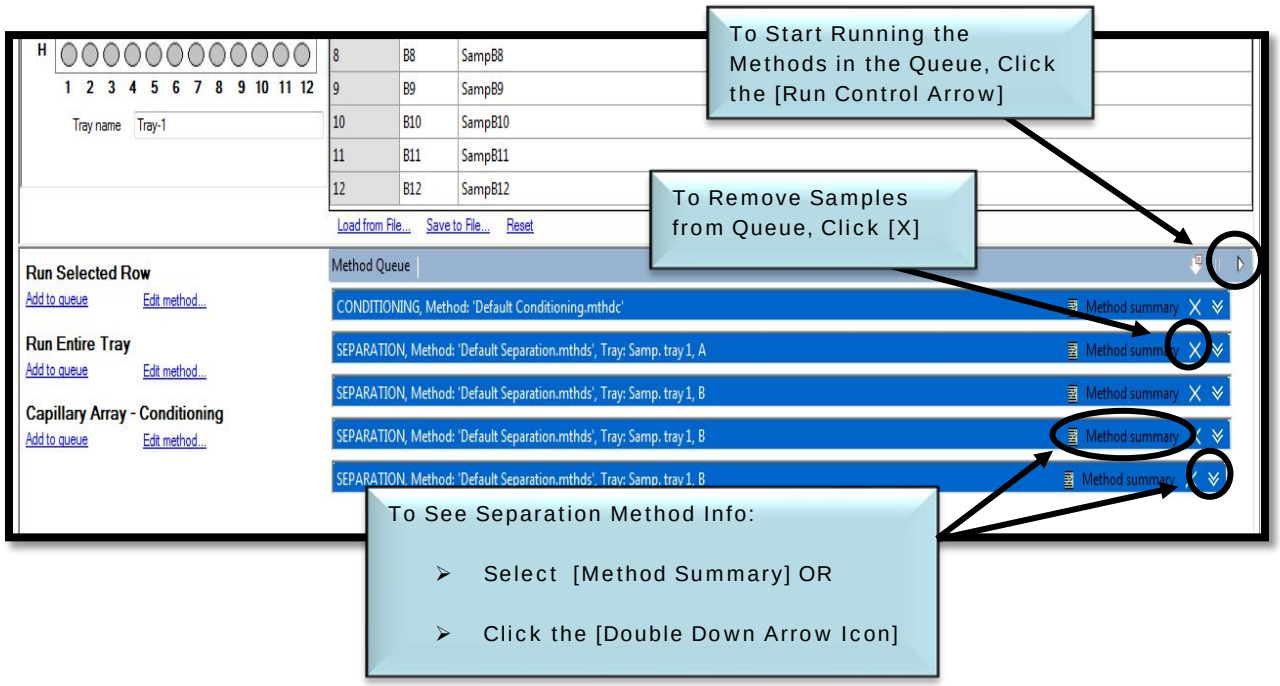


Figure 5-3: Main Screen Window: Method Queue Commands

Verifying and Adjusting Solution Levels

Before a CE separation is performed the software will verify the **Solutions** and the **Solution Levels** to ensure there are enough reagents for the job.

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

 If the solution levels are low, the program will issue a warning.

The CE separation solutions are located in the **Side Compartment** of the instrument. For a detailed description refer to Chapter 2, pgs.8 -9.

 For the instrument to run correctly it is important that the solution levels be entered into the *FRAGMENT ANALYZER*™ software **each time** solutions are changed or placed in the **Side Compartment**.

FRAGMENT ANALYZER™ Side Access Door

The instrument solutions are accessed by opening the Side Compartment Access Door. Fig **5-4**.

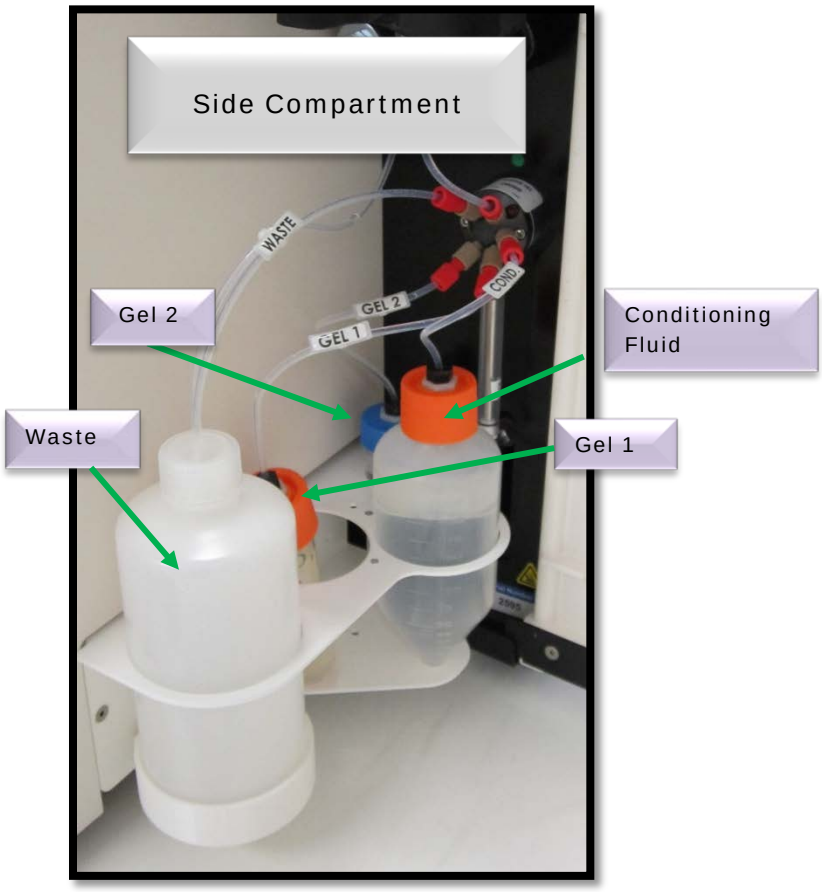


Figure 5-4. Solutions Housed in the Side Door Compartment

Small Container Solution Replacement

When replacing or changing solutions in the Side Compartment, fill the 50 ml Conical Tubes to the following levels:

- 50 mL for the Conditioning Fluid
- 50 mL for Gel 1
- 50 mL for Gel 2
- Empty the Waste Container

Large Container Solution Replacement

The container size for solutions can be increased to 250 ml Centrifuge Bottles. In this instance, the following fluid levels are used:

- 250 mL for the Conditioning Fluid
- 250 mL for Gel 1
- 50 mL for Gel 2
- Empty the Waste Container

Entering Solution Levels in the *FRAGMENT ANALYZER™* Software

To change the fluid levels in the software, from the **Main Screen Menu** select **[Utilities]**. Fig. **5-5**

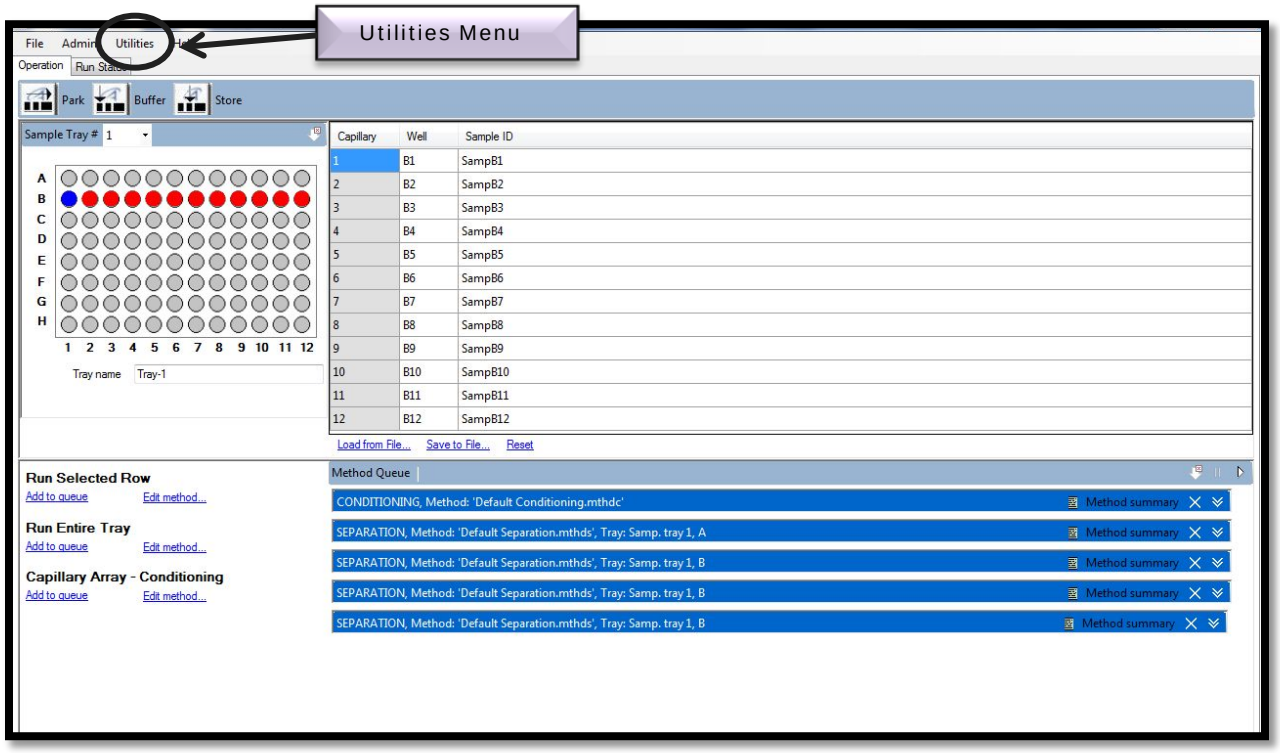


Figure 5-5. Main Screen Window: Utilities Menu

From the Utilities Menu select [Solution Levels]. Fig. **5-6**

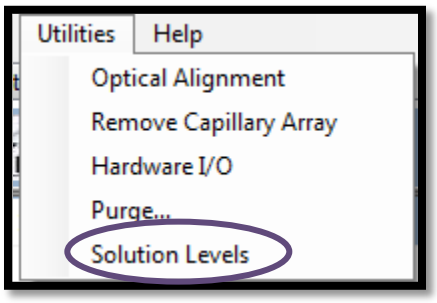


Figure 5-6. Utilities Menu

Selecting [Solution Levels] opens the Solution Levels Data Entry Window, Fig. **5-7**.

The solution levels can be modified for each component with the up and down arrows located in each data entry field.

Once the solutions levels have been modified select [Ok].

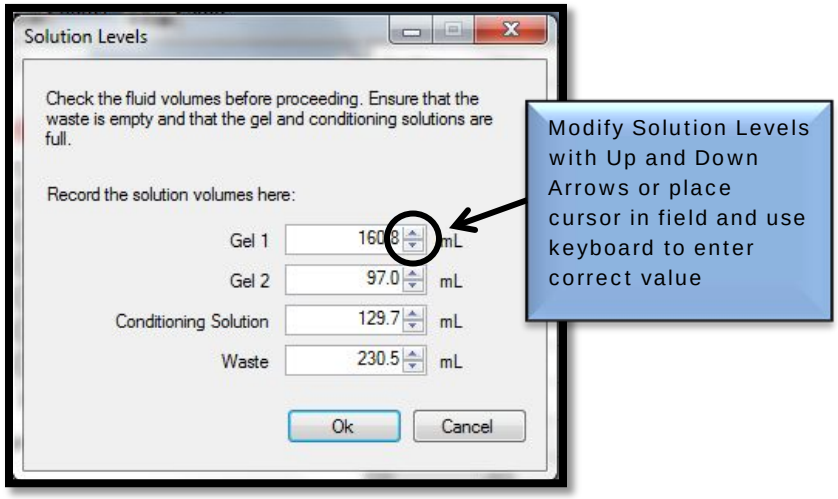


Figure 5-7. Solution Levels Data Entry Window:
Values Prior to Entry of Updated Solution Levels

Starting the CE Run

Once solution levels have been verified in the instrument and the software, a **CE Separation Method is started** by clicking the **Run Control Arrow Icon** on the **Method Queue Tool Bar**. Fig. **5-8**.

The **Method Queue Tool Bar** accessed from the **Operations tab** on the **Main Screen Window**.

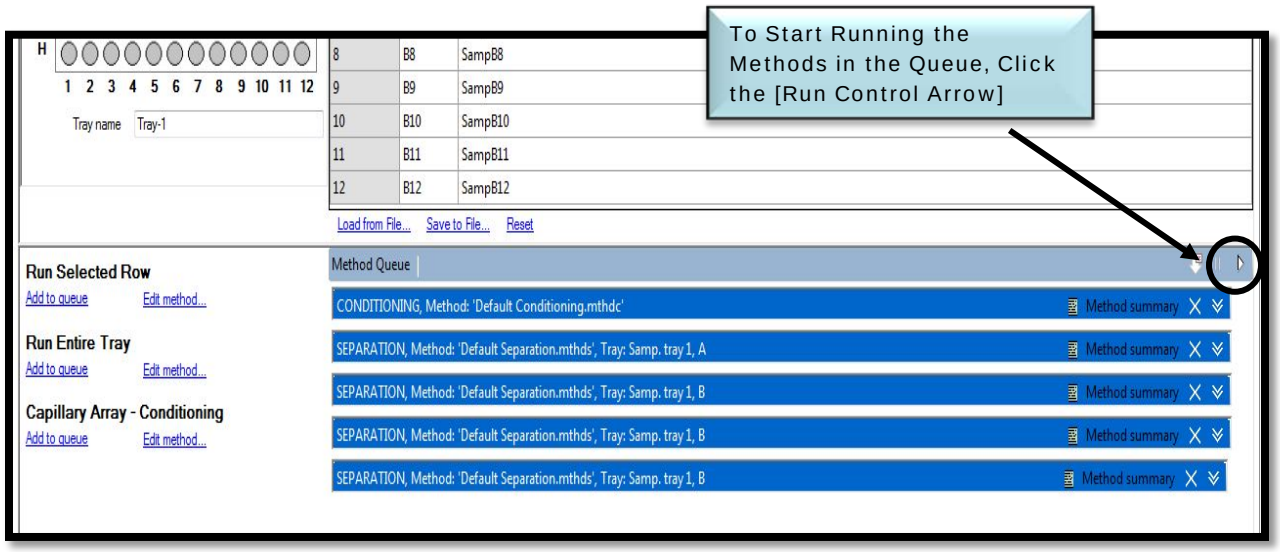


Figure 5-8: Main Screen Window: Method Queue

Status of the CE Separation

Once a CE separation is in progress, a window with the real time progress of the separation can be viewed by selecting the [Run Status] Tab, which is accessed from the Main Screen Window located next to the Operations Tab. Fig. **5-9**

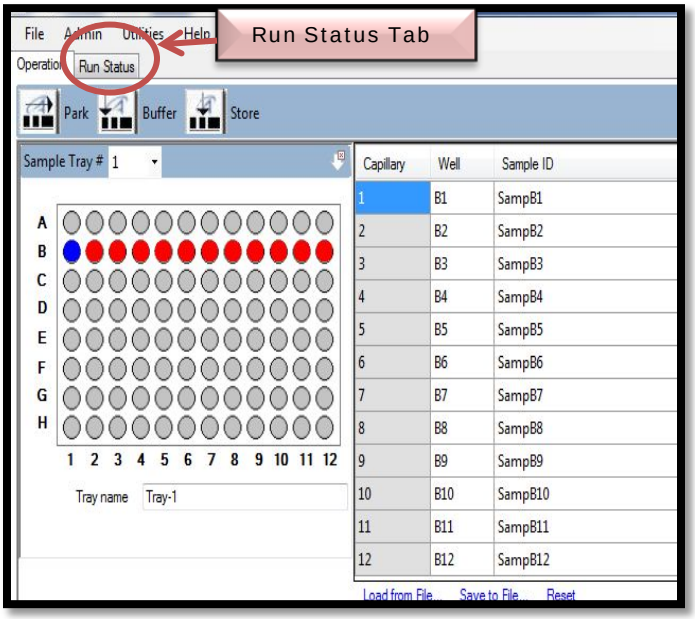


Figure 5-9: Main Screen Window: Select Run Status

Selecting the Run Status Tab during the CE separation opens a Real Time Separation Display Window where electropherograms are displayed for each capillary.

Real-time Separation Display

The figure below shows the open **Group Tab** accessed through the **Run Status Tab** with twelve electropherograms displayed. Fig. **5-10**.

The electropherogram plots are updated each second as data is received from the CCD (Charge Coupled Device) camera.

During **Conditioning Steps** and during **Injection**, the **Real-time Separation Display** shows the **status** of the **pump** and **plate positions**.

Figure 5-10: Run Status Tab
Group Real-time Display

Selecting the [Single] Tab located to the right of the Group Tab opens a window showing a single electropherogram for a selected capillary. (Fig. **5-11**).

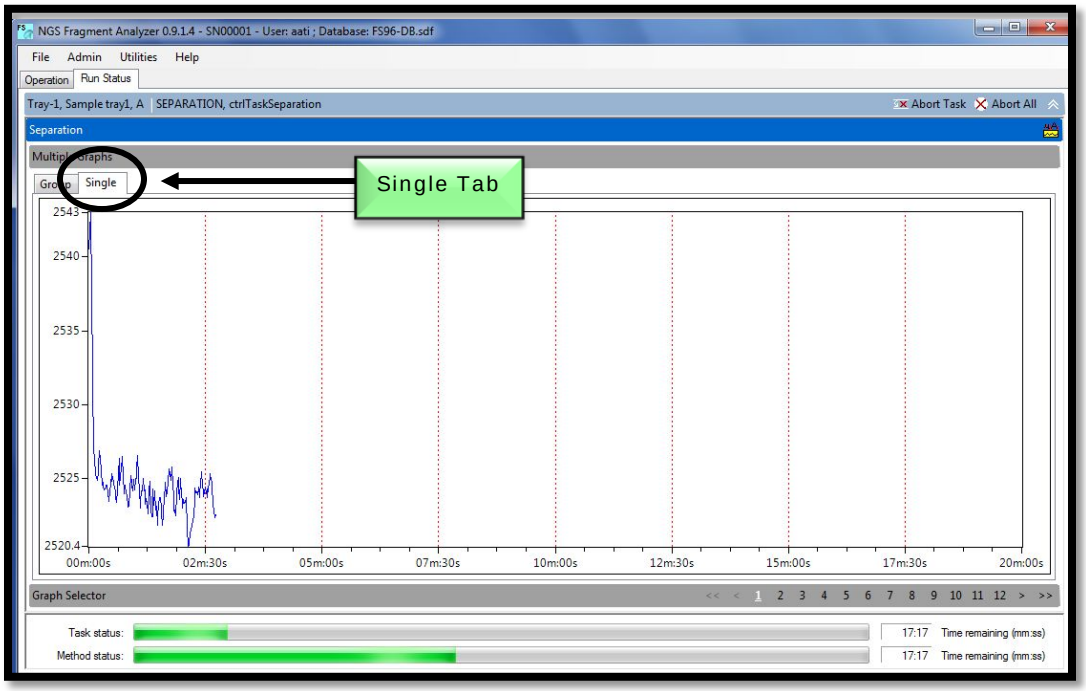


Figure 5-11: Run Status Tab
Single Capillary Real-time Display

Saving CE Separation Results

After a CE run is completed, the data is saved into several output files, as shown in Table **5-2** and Figure **5-12**, below:

- The default location of the saved files is C:\AATI\AdvanCE-FS\Data.

- The electropherogram data is contained in a folder with the format:

  - Year (XXXX): Month(XX): Day(XX).
    - Then a subfolder
  - Hour (XX) – Minute(XX)-Second(XX).

File extension type	Description
***.current**	This is a Text File which has data recorded for Current, Voltage and Pressure.
***.raw**	This is an Image File which has the Raw Intensity Data.
***.raw2D(optional)**	This is a 2Dimensional Image File which has Raw Data Output.
***.txt**	This is a Summary Text File containing the sample information.
method.mthd	This is a method file which contains all the information about the separation method used in the run.

Table 5-2: File Extension Types

CE Separation output file example:

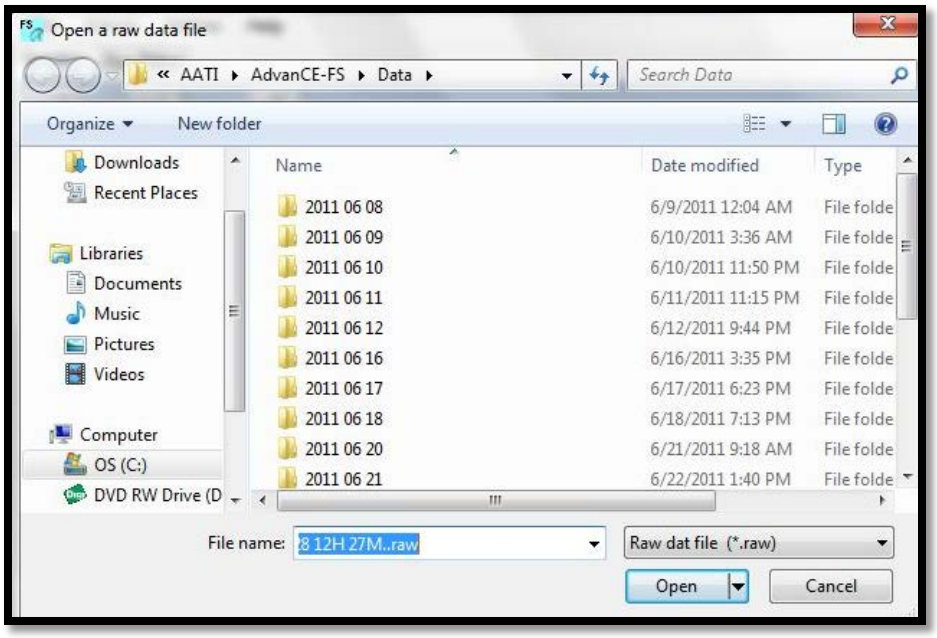


Figure 5-12: Example of Raw Data Files, as they appear in the "Open File" dialog box of the Fragment Analyzer™ program.

Troubleshooting Database

Some data is also written to a Troubleshooting Database, as shown below in Table **5-3**. This database is currently used only for troubleshooting and does not contain any electropherogram data.

Field	Description
Operator	The operator who was logged in when the method was placed on the queue.
Run Date	The date and time that the run was completed.
Capillary Number	The capillary (1 through 12)
Sample ID	The sample ID text from the sample information. See section **Error! Reference source not found.**
Sample Type	The sample type text from the sample information. See section **Error! Reference source not found.**
Description	The description from the sample information. See section **Error! Reference source not found.**

Table 5-3: Information Stored on Troubleshooting Database

Examining Data with the File Manager

From the ***Fragment Analyzer™*** software, the **File Manager** allows electropherograms to be examined within the program environment.

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

Files are normally analyzed using the PROSize™ Software, which is covered in a separate manual.

To access the **File Manager**, from the **Main Screen Window** go to the **[File] menu** and select **[File Manager]**. Fig. **5-13**.

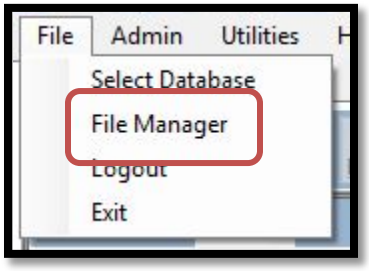


Figure 5-13. Main Screen File Menu:
Selecting File Manager

Viewing a Group of Electropherograms

To view CE separation data within the File Manager, open a file in the C:\AATI\AdvanCE-FS\Data folder with a .raw extension.

Once the data file is opened in the File Manager, the data can be viewed for a group of capillaries by selecting the [Group] tab. Fig. **5-14**

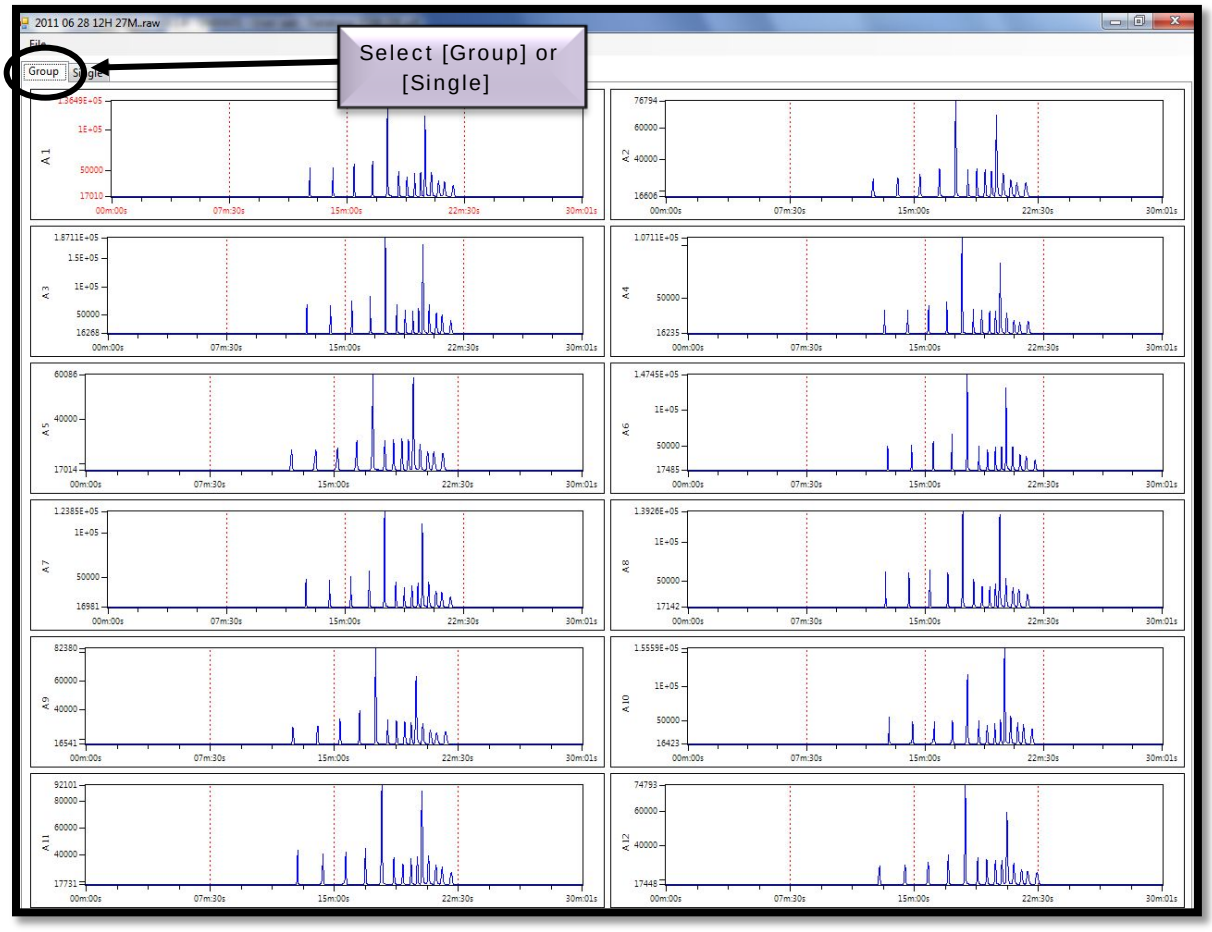


Figure 5-14. The File Manager Screen:
Display of Run Data from 12-Capillaries

Viewing a Single Electropherogram

To view a single electropherogram, select it with the mouse and then hit the [Single] tab. Fig **5-15**

From this view, the user can toggle between electropherograms by selecting the capillary number in the Lower Menu Bar of the File Manger Window.

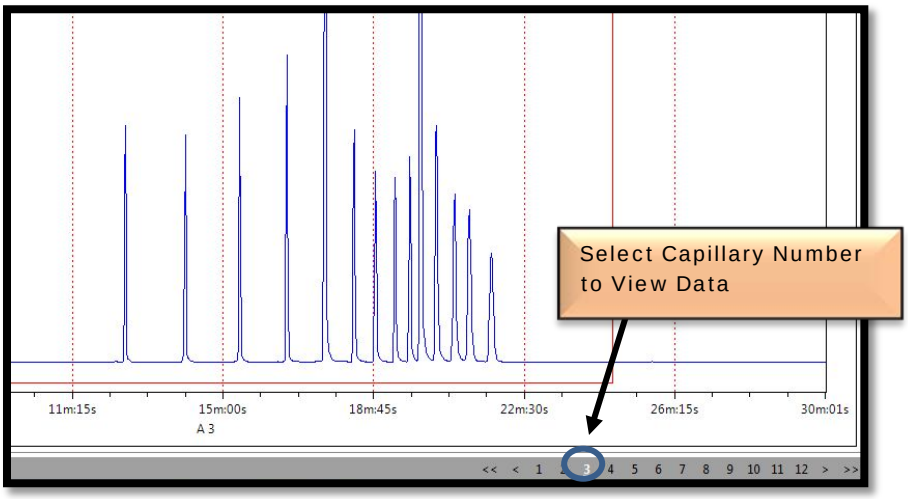


Figure 5-15. The File Manager Screen:
Display of Data from Single Capillary

Zoom in and Zoom out

Electropherogram data can be panned, zoomed, or zoomed out by right-clicking on the chart and selecting the function of interest. Zooming, unzooming, or panning is often desired to take a closer look at specific regions of an electropherogram.

To zoom, right click and select "Zoom" from the menu (Fig **5-16**)

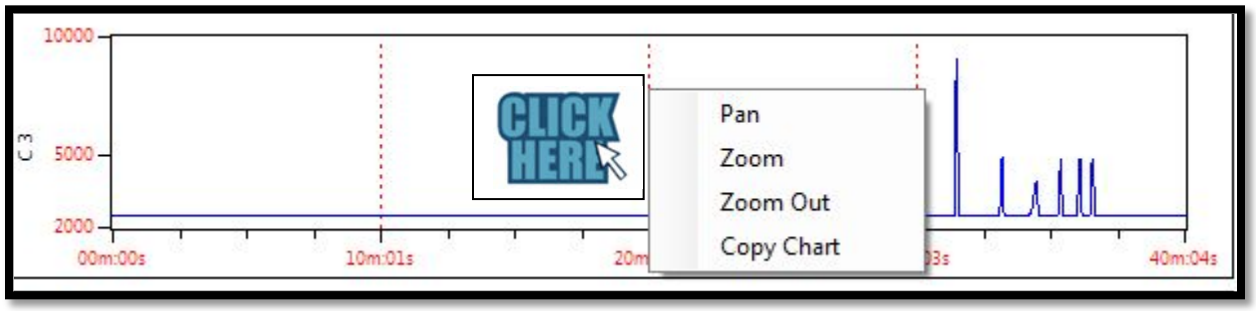


Figure 5-16: The right-click menu for electropherogram view.

Drag the mouse from the upper left-hand corner to the lower right-hand corner of a desired zoom range (Fig **5-17**).

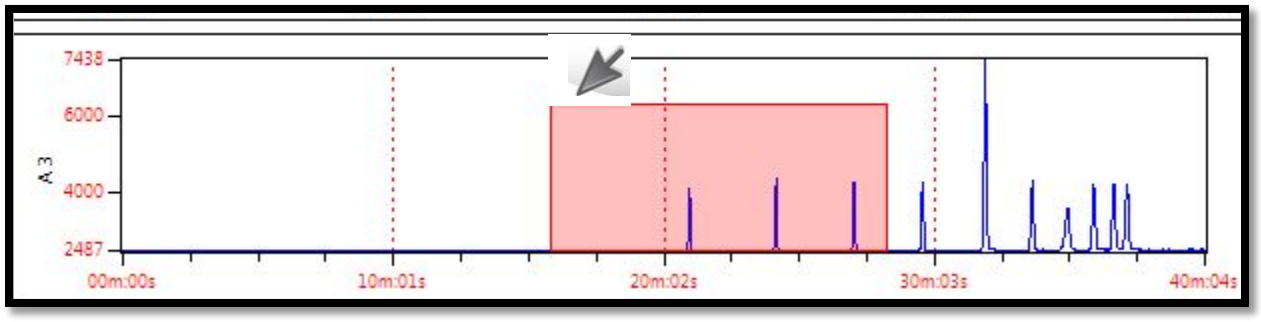


Figure 5-17: Zoom area (dragging mouse to define).

The image will now be zoomed (Figure **5-18**).

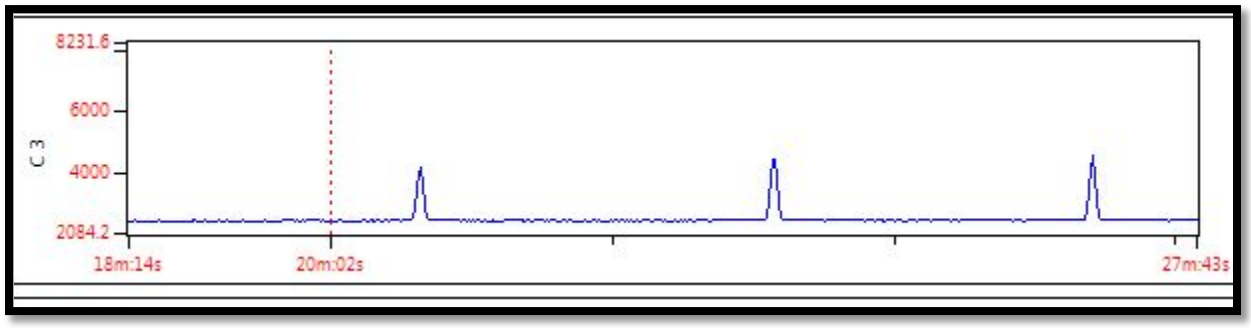


Figure 5-18: Zoomed Data

To pan the data (move the electropherogram around), right-click and select "pan" (Fig. **5-16**). A "mouse hand" will appear that enables you to move the image around. An example of a moved image is given below in Figure **5-19**.

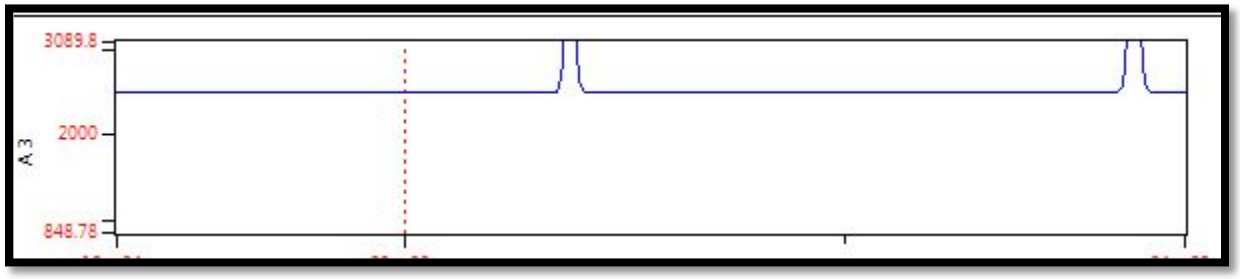


Figure 5-19: Panned Area

To un-zoom data, right-click and select "unzoom" (Fig **5-16**).

Adjusting Capillary Alignment

The File Manager can also be used to adjust the capillary alignment.

Within the File Manager, the Capillary Location Features are used in the rare case in which data was collected with the capillary array out of alignment.

- This feature allows data to be recovered with the correct alignment, without re-running the sample. The sample results will be corrected and accurate.

To apply the correct alignment to sample, in the File Manager Window select [File] then [Cap. Alignment]. Fig **5-20**

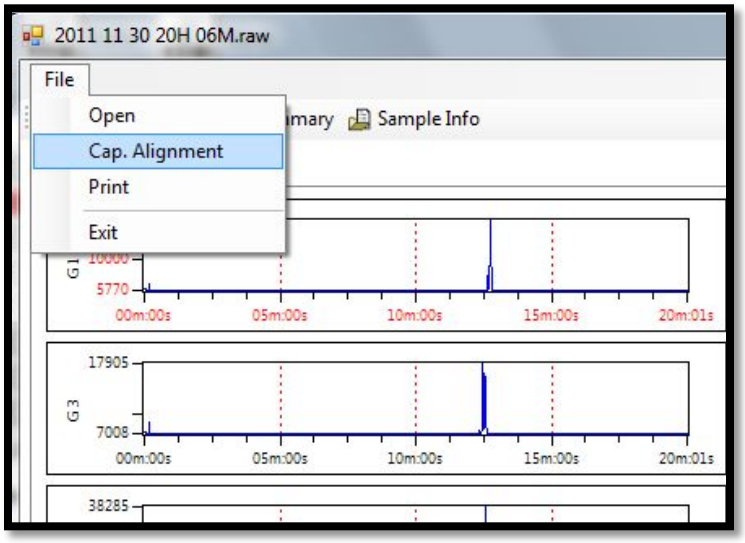


Figure 5-20. File Manager Window:

File Menu - Capillary Alignment

Capillary Alignment Window

Selecting [Cap. Alignment] opens the Capillary Alignment Window. Fig. **5-21**.

In this window, the red line selects where integration or peak picking will occur. The software will evaluate everything above the red line and nothing below it. This helps remove background noise, but as long as the red line intersects each peak (in green), sample results will not be harmed. This line is initially unseen at the very bottom of the screen and must be moved up with the mouse.

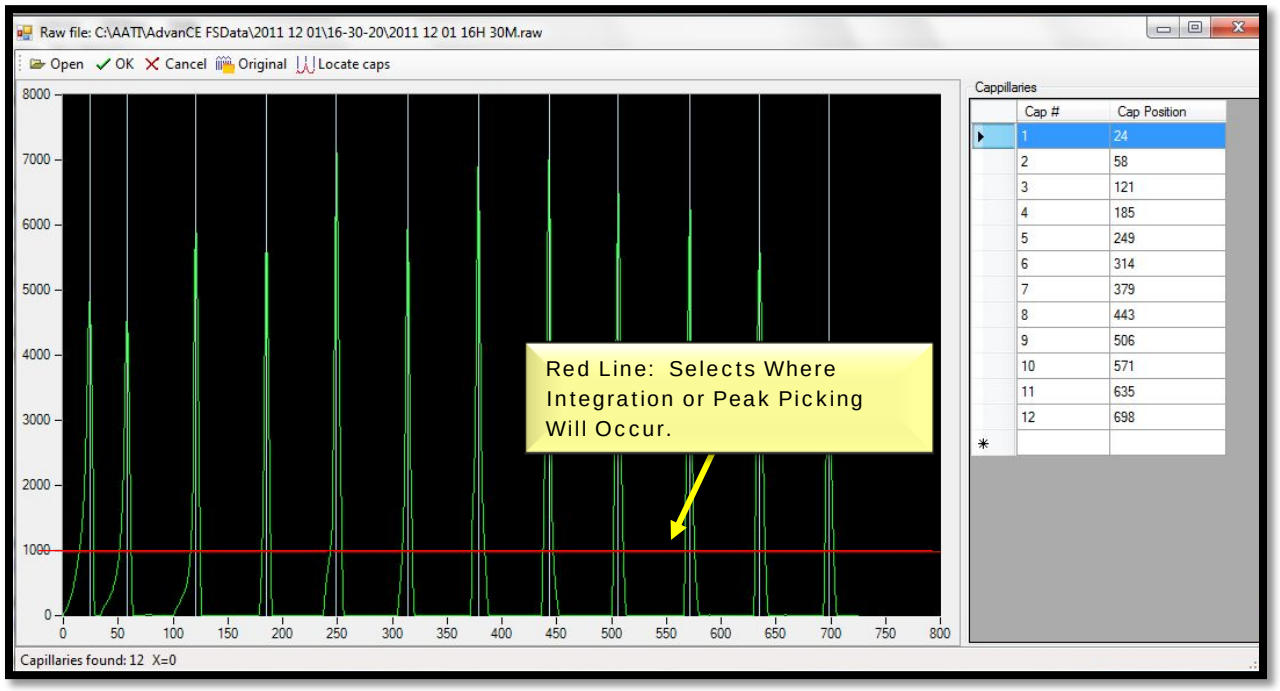


Figure 5-21: File Manager – Capillary Alignment Window

Capillary Alignment Window – Menu Bar Commands

The menu bar commands are located in the upper right corner of the **Capillary Alignment Window**. Fig. 5-22, Table 5-4

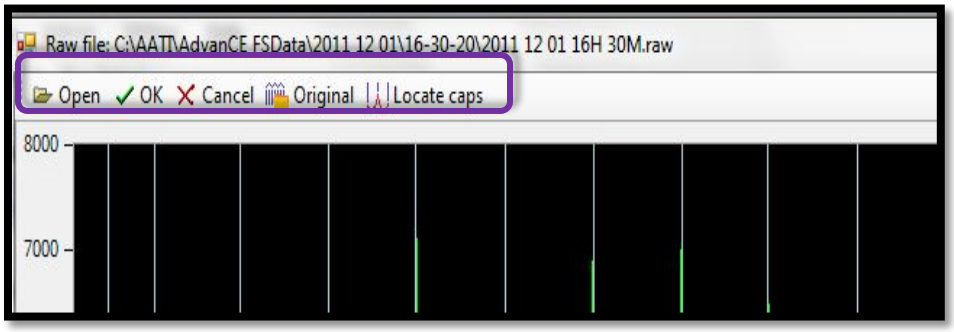


Figure 5-22. Capillary Alignment Window: Menu Bar Commands

Summary of Menu Bar Commands:

Command	Function
Open	**Opens** a new file
OK	**Accepts Changes** to the file (i.e. capillary locations)
Cancel	**Cancels any actions** and **close**s the file
Original	**Locates** the **Original Capillary positions**
Locate caps	**Locates capillaries based on peak positions** in the open file (note: move red line up so that only the peaks of interest are integrated)

Table 5-4. Capillary Alignment Menu Bar Commands

Capillary Alignment Window – Right Click Commands

The following **right click** options are available from within the **Capillary Alignment Window**. Fig. **5-23**, Table **5-5.**

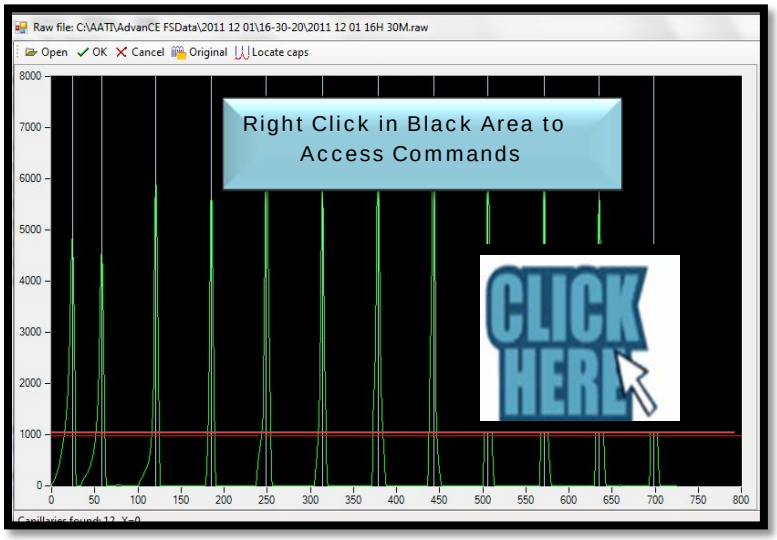


Figure 5-23: Capillary Alignment Screen
Right Click Commands

Right Click Command	Function
Pan	**Allows the electropherogram image to be moved.** To pan, right-click. A "hand icon" appears, enabling the movement of the image.
Zoom	**Zoom a select rectangle.** Drag and select.
Zoom Out	**Un-Zooms the image**. To Zoom in, drag and select the area of interest
Copy Chart	**Copies the chart into the clipboard**
Zoom XY	**Enables the XY zoom mode**. Drag and select area of interest.
Zoom X	**Enables X-axis zoom mode**. Drag and select area of interest. Useful for selecting a small set of capillaries when viewing 96 capillaries.
Insert Capillary	**Inserts a capillary location at the current mouse pointer location**
Deletes Capillary	**Deletes a capillary location closest to the current mouse pointer location.**

Table 5-5: Capillary Alignment Screen
Right Click Commands

 In the next section, a step by step example will be given for how to correct a capillary alignment using the Capillary Alignment Window Menu Bar and Right Click Commands.

Adjusting Capillary Alignment –Example

As an example of a capillary alignment problem, assume you open a data file, and a capillary appears to show no data - In this case, capillary B12. Fig. **5-24**

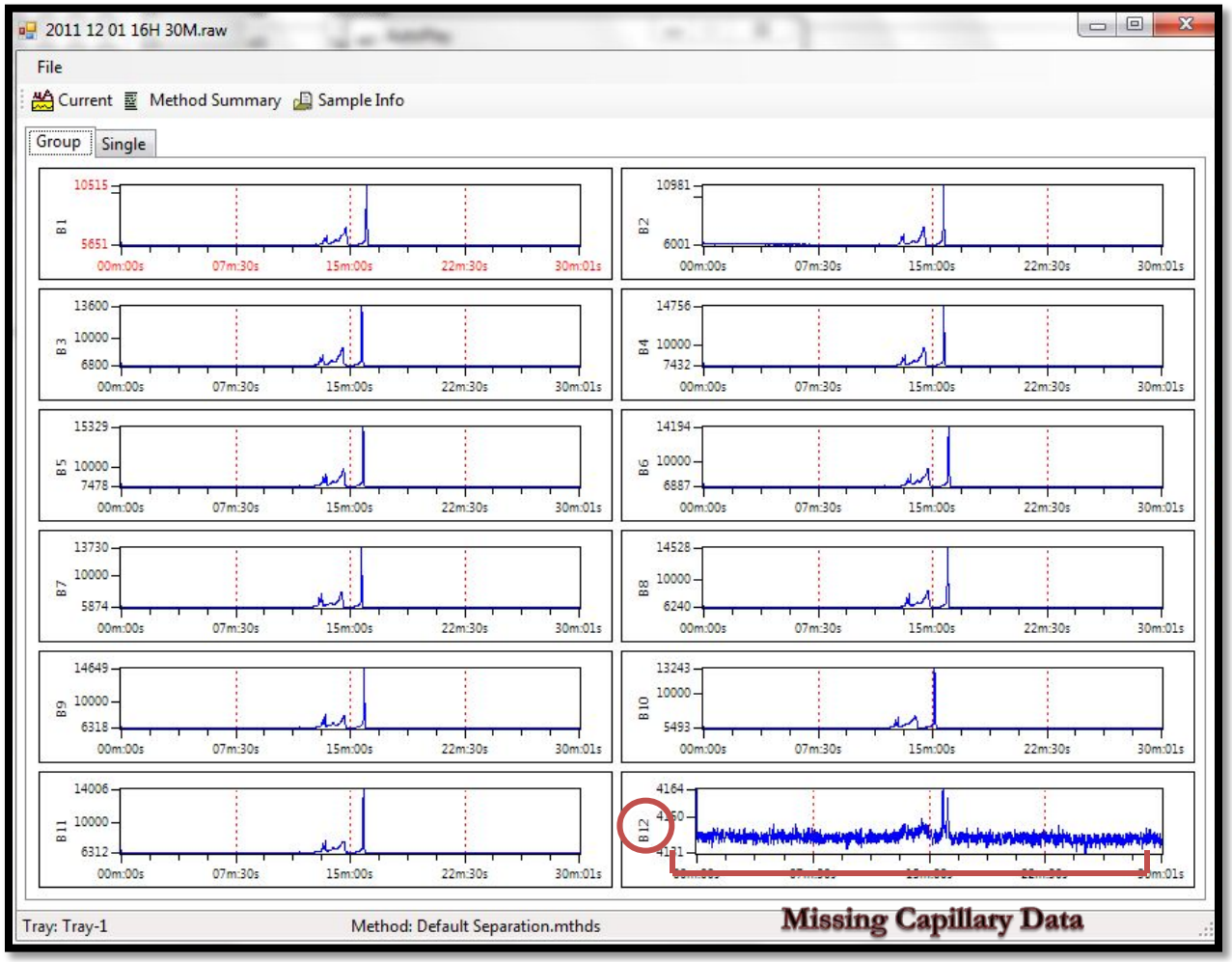
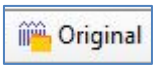


Figure 5-24. Capillary Alignment Window: B12 Capillary Missing Data

When the Missing Capillary Phenomenon is observed, check the alignment by opening the File Manager Window.

To access the File Manager, from the Main Screen Window go to the [File] menu and select [File Manager]. Then select [File] from the File Manager Menu and [Cap. Alignment].

Select the 

Icon. The capillary alignment window shows that the alignment for capillary 12 is not correct. The blue line should be aligned with the green peak. (Fig. **5-25**.)

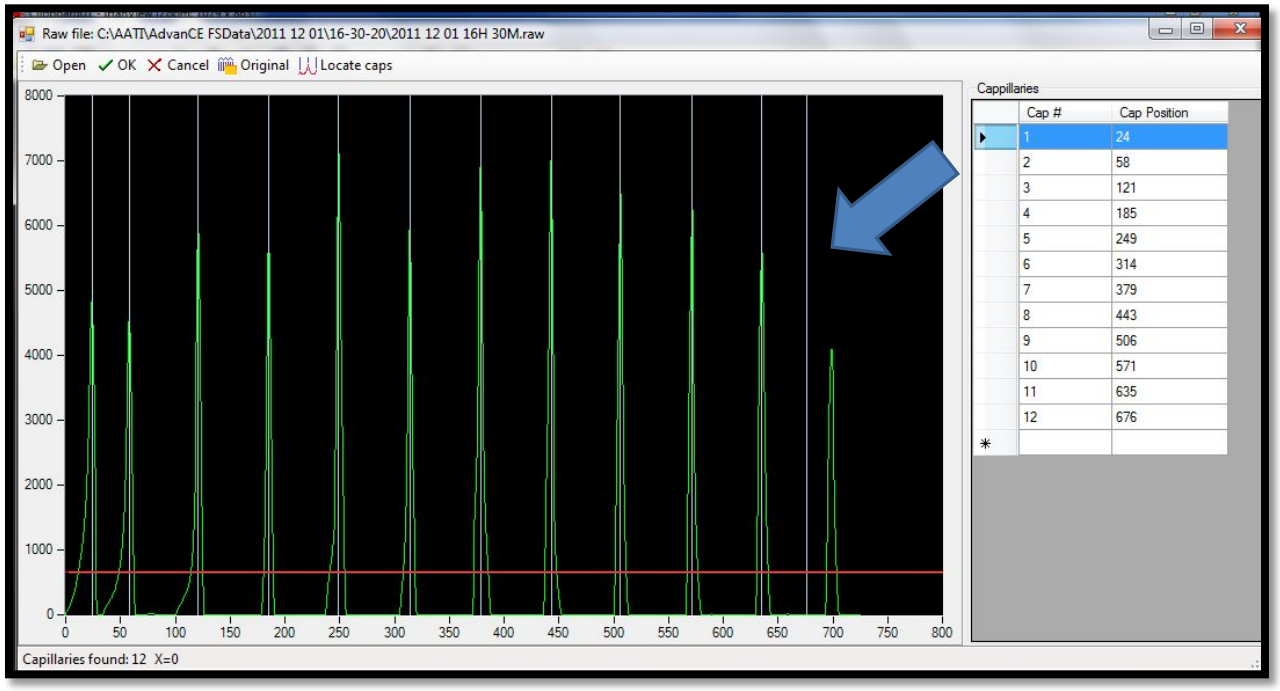
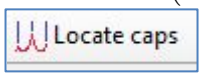


Figure 5-25. Capillary Alignment Window:
Incorrect Alignment for Capillary 12

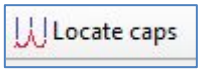
To correct the alignment:

- Move the red line at the very bottom of the screen (which originally will be at the "0" of the y-axis) and select the 

Icon.

OR

- Manually move the blue vertical line with the mouse. Select [OK] to save the results.

The figure below shows the correct alignment after selecting 

or moving the vertical blue line with mouse. (Fig. **5-26**.)

⚠ Be Sure to Select [OK] After Obtaining the Correct Alignment.

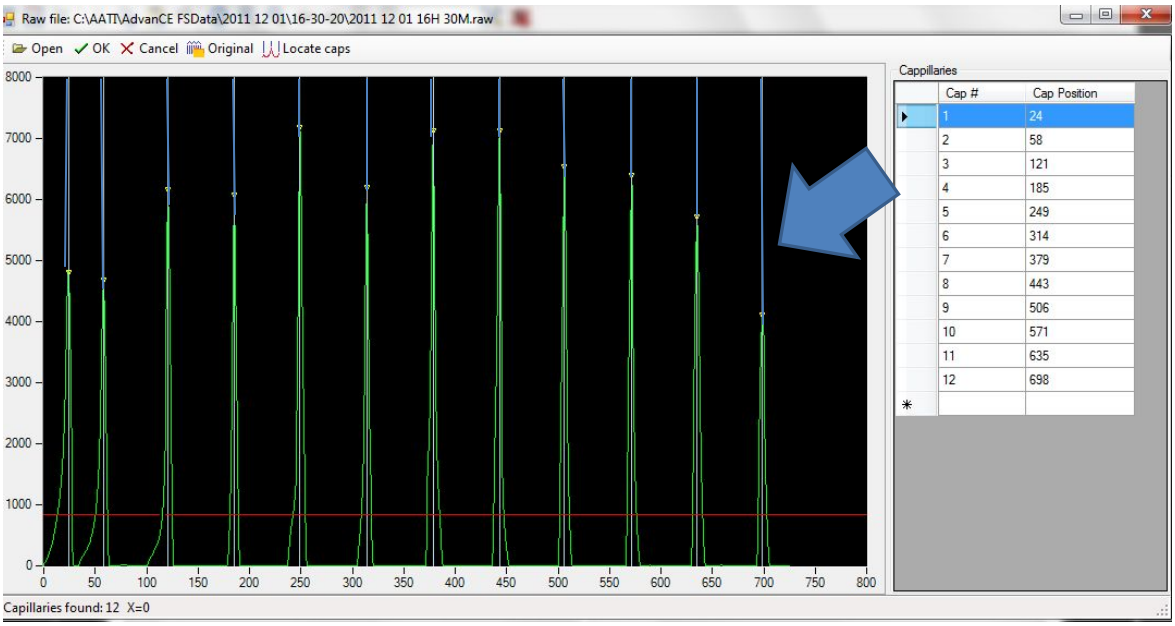


Figure 5-26. Capillary Alignment Window:
Correct Alignment for Capillary 12

The data will now be correct, as shown on the next page in the corrected view of capillary B12.

Figure 5-27. Capillary Alignment Window: Correct Alignment for Capillary 12

Getting Started - *Fragment Analyzer™* Software

In This Chapter

- Main Screen Interface Overview
    - ✓ Menu Bar Commands
        - File Menu Functions
        - Admin Menu Functions
        - Help Menu Functions

In this chapter, we will continue exploring the *FRAGMENT ANALYZER*™ system software and the operations that are accessed from the Main Screen Window.

Menu Bar Commands

File Menu Functions

The File Menu is located in the Main Screen Toolbar in the upper left corner of the Main Screen Window. The commands accessed from the File Menu are shown below. (Fig. **6-1**).

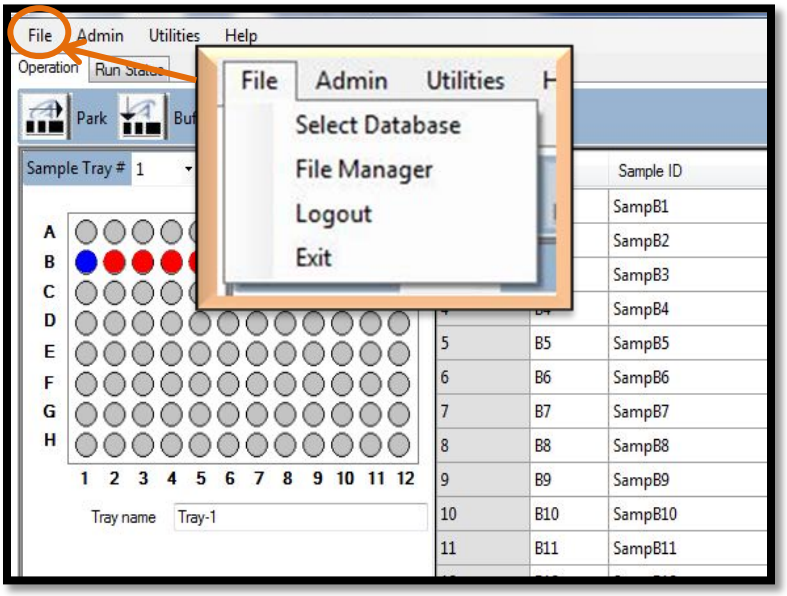


Figure 6-1. Main Screen Window:
File Menu

File Menu — Select Database

The [Select Database] function is only used for advanced troubleshooting. Event and Error Logs can be retrieved using this function in the case of problems.

The Database that contains the Event and Error logs is set up during the installation of the instrument.

If access to a database other than the database set up on the day of installation, the [Select Database] function calls up the window shown in Figure **6-2**, below.

To select a different database, click the ... button and find the appropriate database with a .sdf extension.

Figure 6-2. File Menu:
Select Database Window

File Menu — File Manager

The [File Manager] function is described in Chapter 5, pgs 19-31.

File Menu - Logout

This function allows one to **Log-off the program** and **Log-on as a different user.**

A **login window** is presented when the operator selects [Logout] from the **File Menu**. This screen is also presented when the application is started. (Fig. **6-3**)

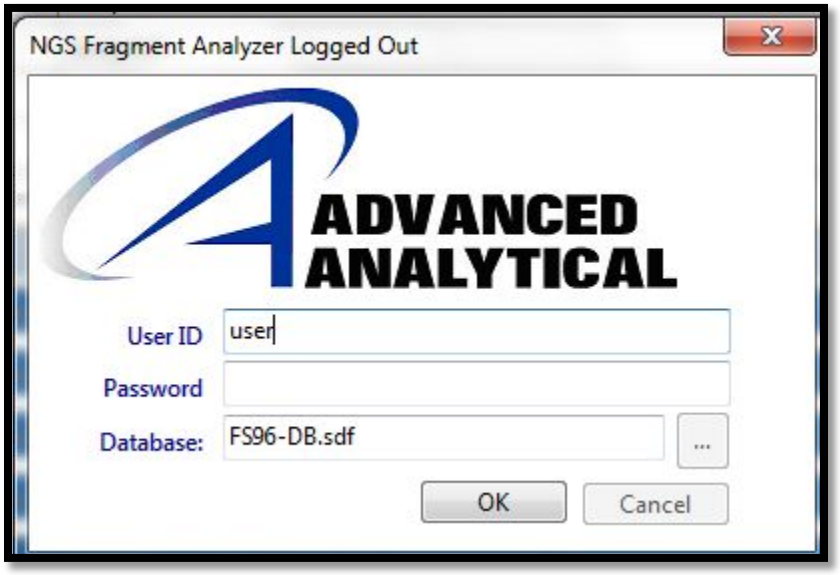


Figure 6-3. Login Window

There are initially two User IDs with which to access the software, defined by the User level:

- User - Has restricted access that allows only routine operation of the instrument and password maintenance.
- Administrator - Has enhanced access to functions such as capillary array change-out

- The initial password for both User and Administrator is blank; i.e the entry field is left blank

Login Window

If the security parameter Login Required (under Admin….Configuration).has been enabled, the operator must enter a valid user ID and password to access the application.

If the login information is correct, but is expired or does not meet the current criteria of the security settings the operator is prompted to change their password at this time. The password must be successfully changed before this user can access the application.

The Login Window shows the database to which the application connected. The operator can change the database connection prior to logging in by clicking the ... button next to the database field.

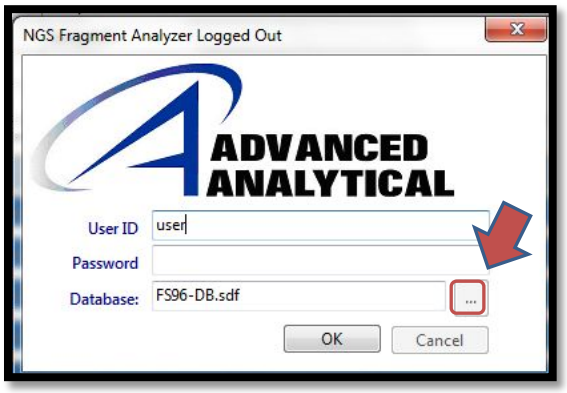


Figure 6-4. Login Window: Select Database Function

- The login information is used in event and error logging to aid an organization in controlling access to the system, as well as tracking usage and changes on the system.

File Menu - Exit

This is a simple function that exits the program. Alternatively, the user can exit the program by clicking on the [X] box in the upper right-hand corner of the program screen.

Admin Menu Functions

The commands accessed from the Admin Menu are shown below. (Fig. **6 -5**).

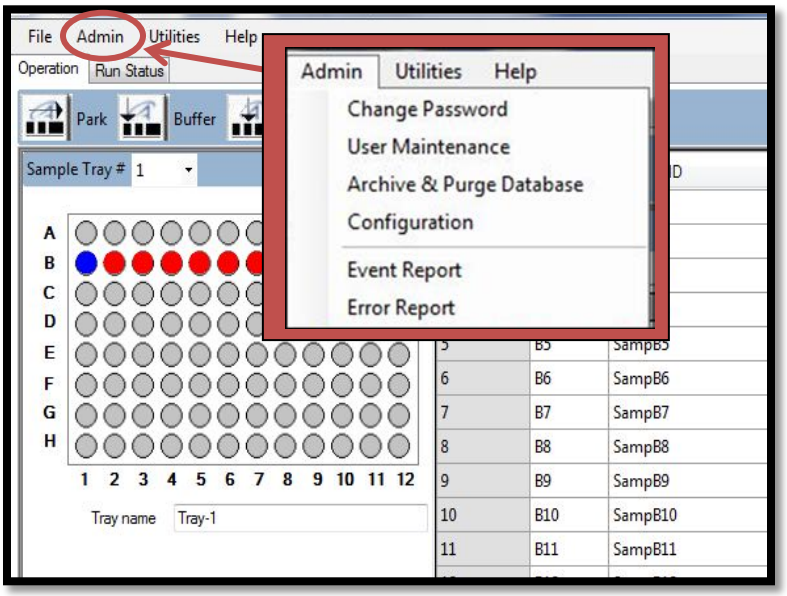


Figure 6-5. Main Screen Window:
Admin Menu

Admin - Change Password

The [Change Password] function brings up the window in Figure **6-6**, below.

Password requirements:

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

Changing the password is accessible to all users.

Figure 6-6. Admin Menu:
Change Password Window

Admin – User Maintenance

Selecting the [User Maintenance] function opens the User Maintenance Window.

From this window, the administrator can Add, Delete or Modify the users that can access this application.

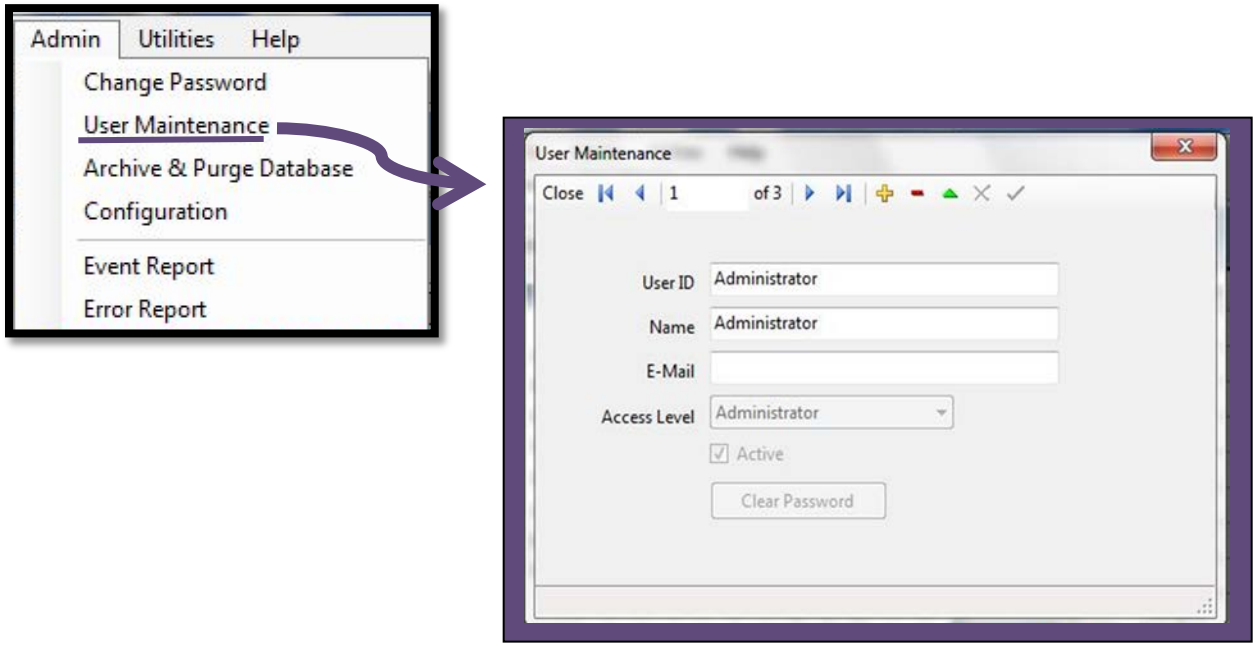


Figure 6-7. User Maintenance Screen

A summary of the User Maintenance Window Parameters is illustrated in the table below.

Field	Description
User ID	User ID for login or signature. **This ID must be unique for the system.**
Access Level	Set the user access level. **User, Administrator or AATI.**
User Name	The full name of the user
Email	Users email address**, optional.**
Active	If Checked: **This user is active and this user ID will work.** If Inactive: **The user ID cannot be used.**
Clear Password	Set this users login password to blank**. If a minimum password length has been set, the user will need to change their password on login.**

Table 6-1: User Maintenance Window Parameters

Admin – Archive and Purge Database

The [Archive and Purge Database] function 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 rights to backup the data for future use in a different location or on an external storage device.

Admin – Configuration

Administrators can select [Configuration] to open the Configuration Settings Window where Security Settings and Device Settings for the system are modified.
(Fig. **6-8**).

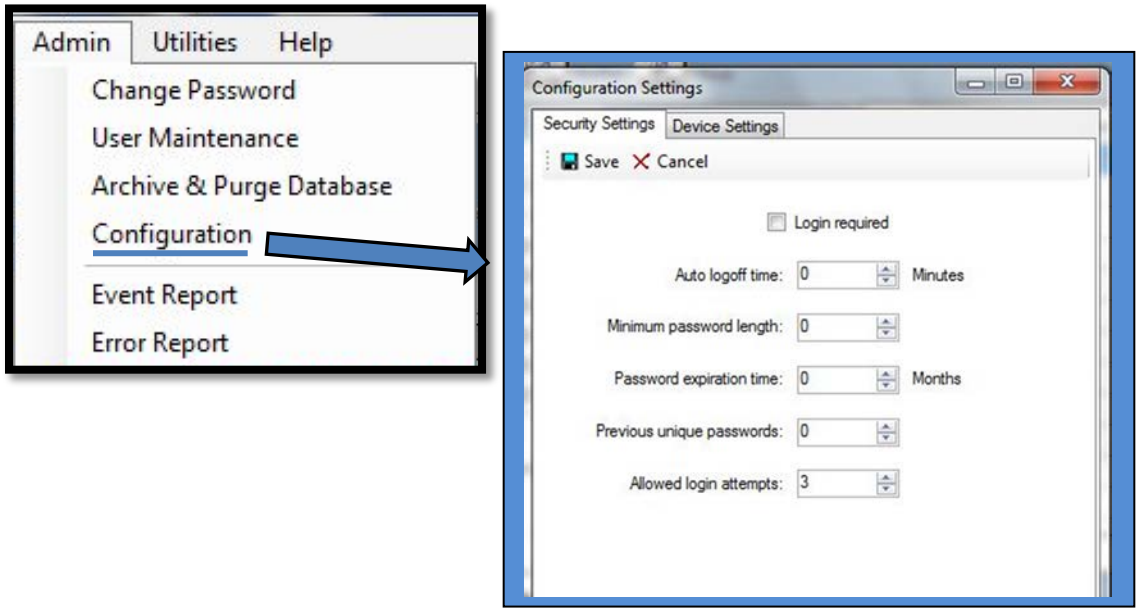


Figure 6-8. The Configuration Settings Window:
Security Settings Tab

A summary of the Configuration Settings - Security Settings Tab Functions is provided in the table below.

Configuration Option	Range	Description
Login Required	True or False	• **If True**: User must login to the application. • **If False:** No login is required for user level access.
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: • That user ID will be made inactive and the error logged • The failed login attempt is recorded in the event log • The application is shut down
Time to change passwords	0 to 36 months	A password (Login ID and Signature) will expire after this number of months
Auto logoff time	0 to 30 minutes	If the application is left unattended for length of time, the current user will be logged off.
Number of previous passwords	0 to 4	When a user changes their password, they may not select from this number of previously used passwords.

Table 6-2. Security Settings Tab Functions

The Configuration Settings Window also allows modification of the system settings for the capillary array under the Device Settings Tab. (Fig. **6-9**).

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

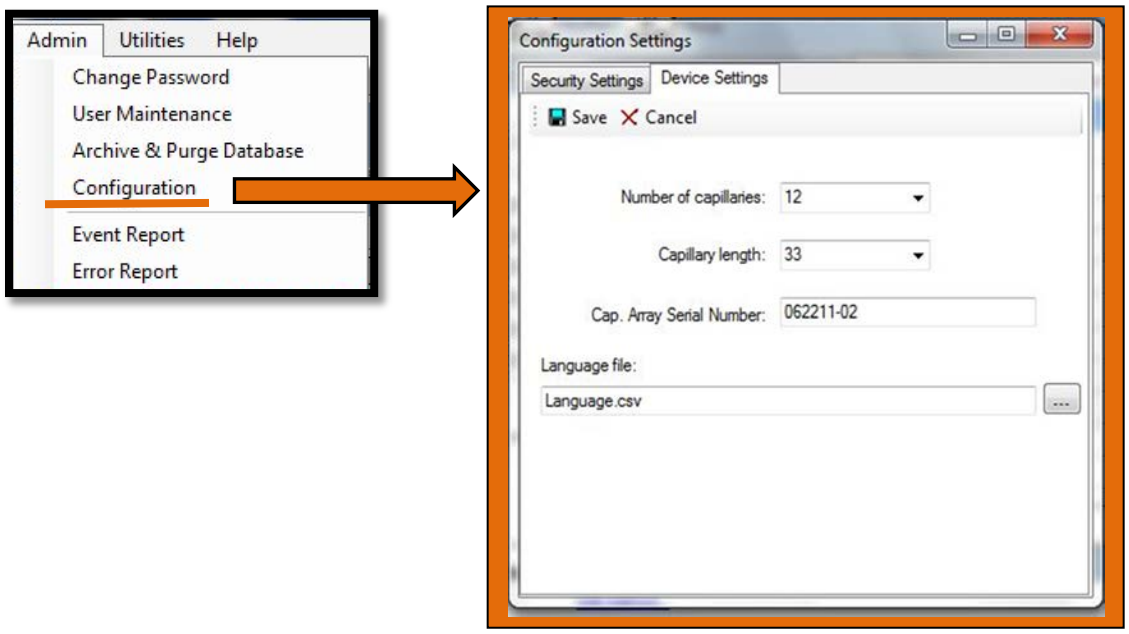


Figure 6-9. The Configuration Settings: Device Settings Screen

A summary of the **Configuration Settings - Device Settings Tab Functions** is provided in the table below.

Parameter	Access Level	Description
Number of Capillaries	Administrator	Values: 12 or 96. Note: Selecting 12 when a 96 capillary array is installed would cause problems. A hardware check verifies the array type.
Capillary length	Administrator	33cm or 55cm.
Capillary Array Serial Number	Administrator	Text field, Maximum length 14 characters.
Language file	Administrator	Enables user to change language of the application by selecting the appropriate (.csv) language file. (Example: Chinese, English and German)
Save	Administrator	Saves the chosen settings.
Cancel	Administrator	Cancels any changes made in the settings.

Table 6-3. Device Settings Tab Functions

Admin - Event Report

The Event Report function provides a tabular report of the audit trail of the events that have occurred in this application.

Both users with administrative access or those with basic access can view the Event Report.

Selecting [Event Report] from the Admin drop down menu opens the Select Date Range Window where the user can Use All Dates or Use a Selected a Date Range. (Fig. **6-10**).

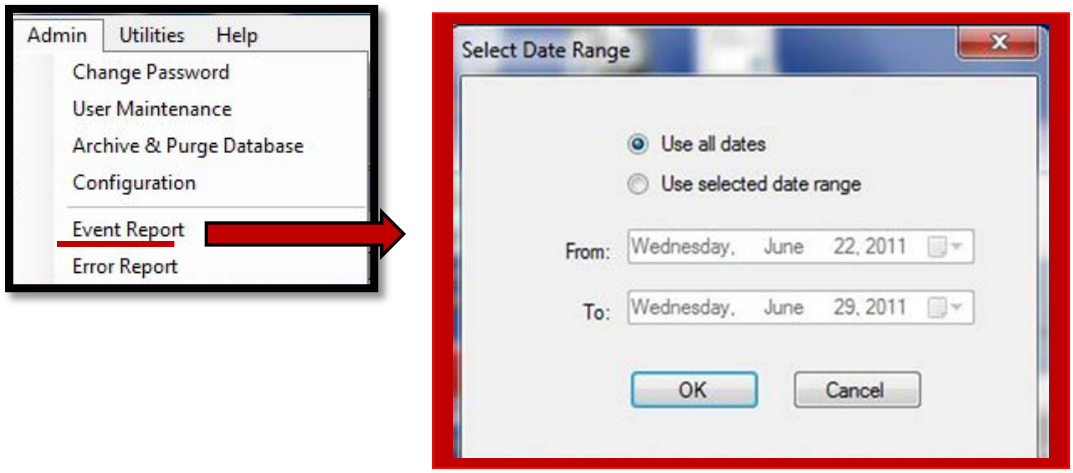


Figure 6-10. Event Report:
Select Date Range Window

The Event Report contains the following information for each event log item:

- User Name - The 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 hitting [OK], an Event Report is generated. (Fig **6-11**).

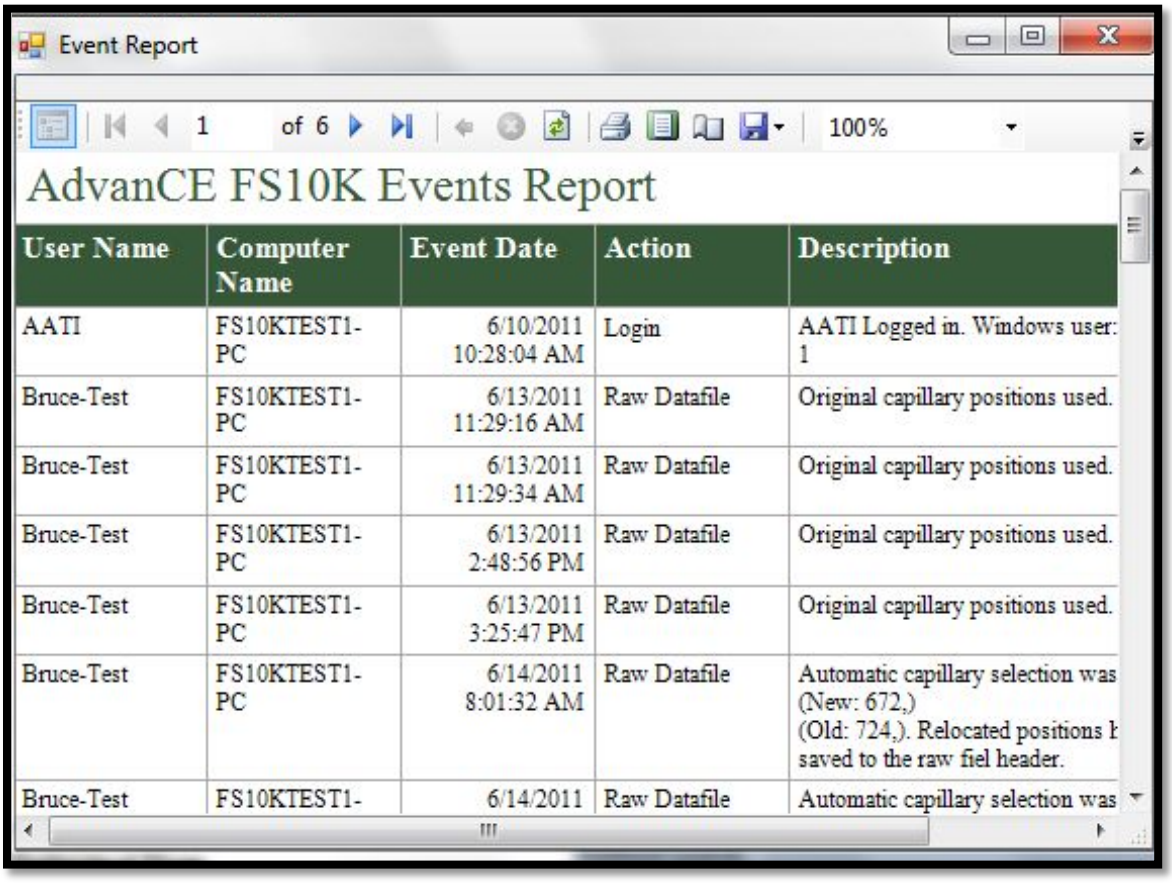


Figure 6-11. The Event Report

The Icons along the top of the event report follow standard Windows™ function nomenclature and are summarized in Table **6-4** below.

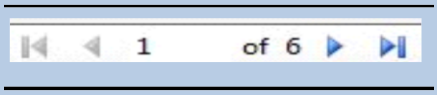
Icon	Description
	Show or hide document map
	Select page
	Back to parent report
	Stop rendering (i.e. stop report generation)
	Refresh
	Print
	Print Layout
	Page Setup
	Save

Table 6-4: Event Report Icons

Admin—Error Report

The **Error Report** is used for advanced troubleshooting. The open **Error Report Window** is shown below in Figure **6-12**.

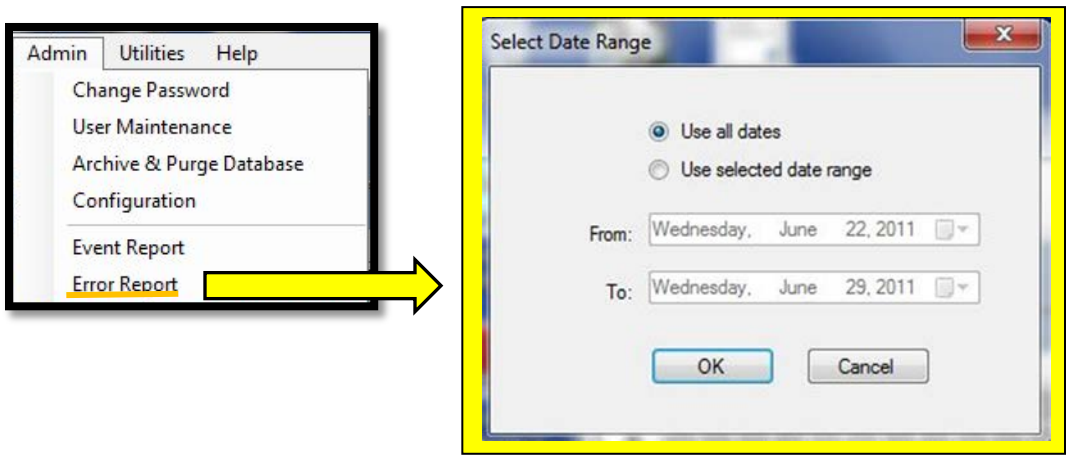


Figure 6-12. Error Report:
Select Date Range Window

The **Error Report** captures the following data:

- **Software exceptions** and **Hardware errors** which are 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 hitting [OK], an Error Report is generated. (Fig. **6-13**).

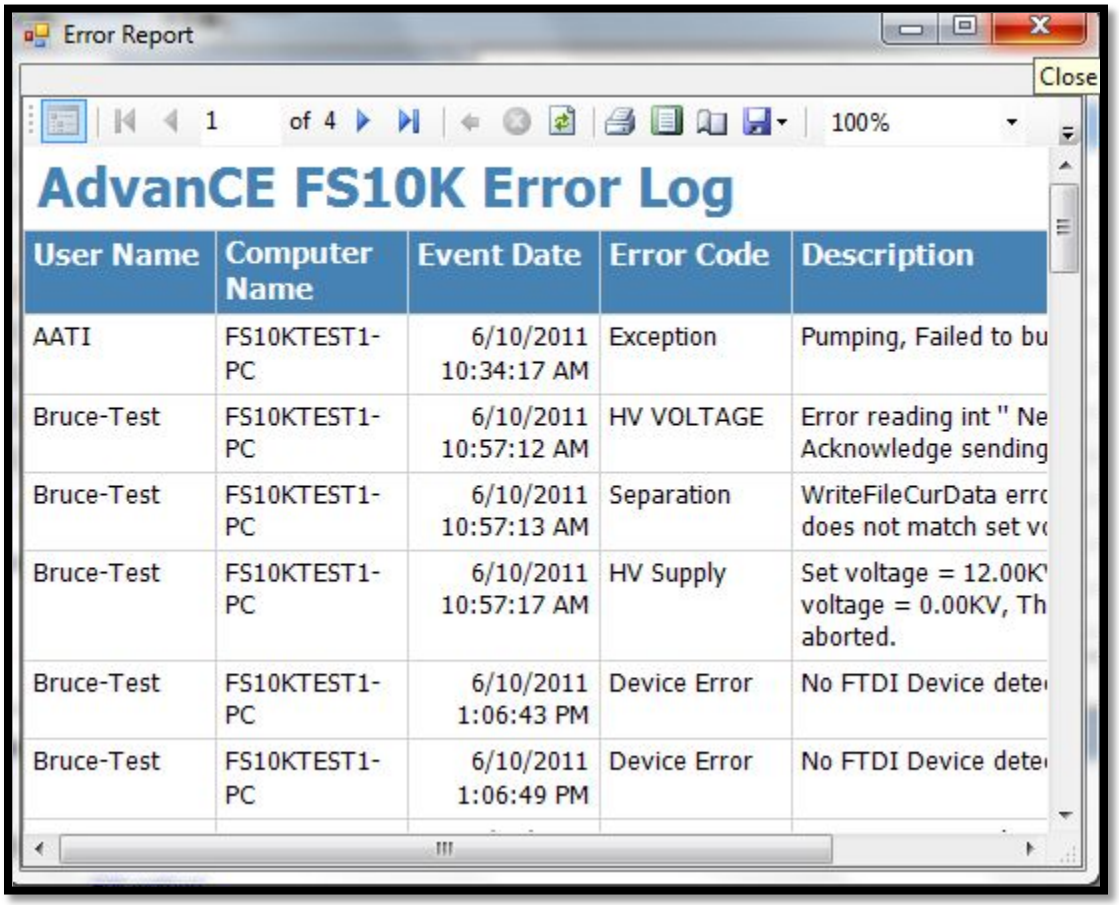


Figure 6-13. The Error Report

Help Menu Functions

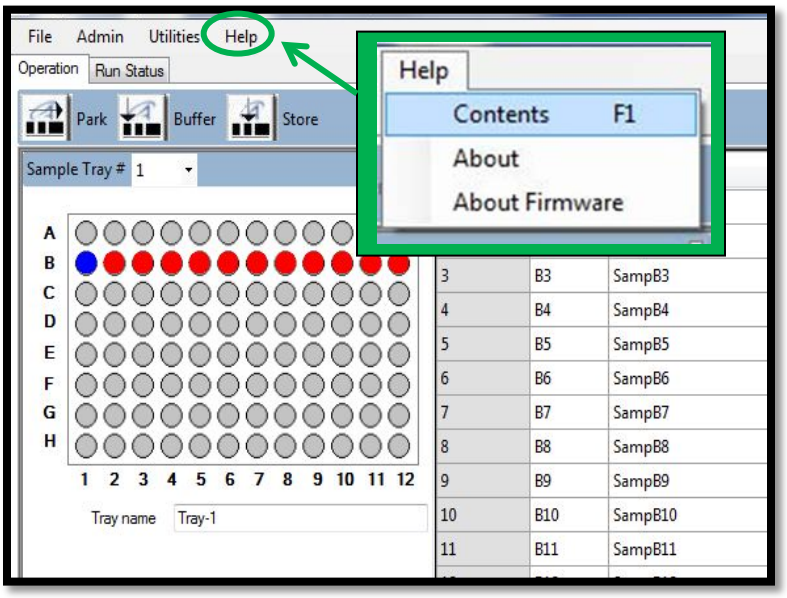


Figure 6-14. Main Screen Window:
Help Menu

Help—Contents

The Contents Function/F1 opens the on-line Help program. The menu is shown in Figure **6-15**, below:

Figure 6-15. The On-line Help Menu

Help – About

The About Function Window displays the most recent version of the system, as shown in Figure **6-16** below:

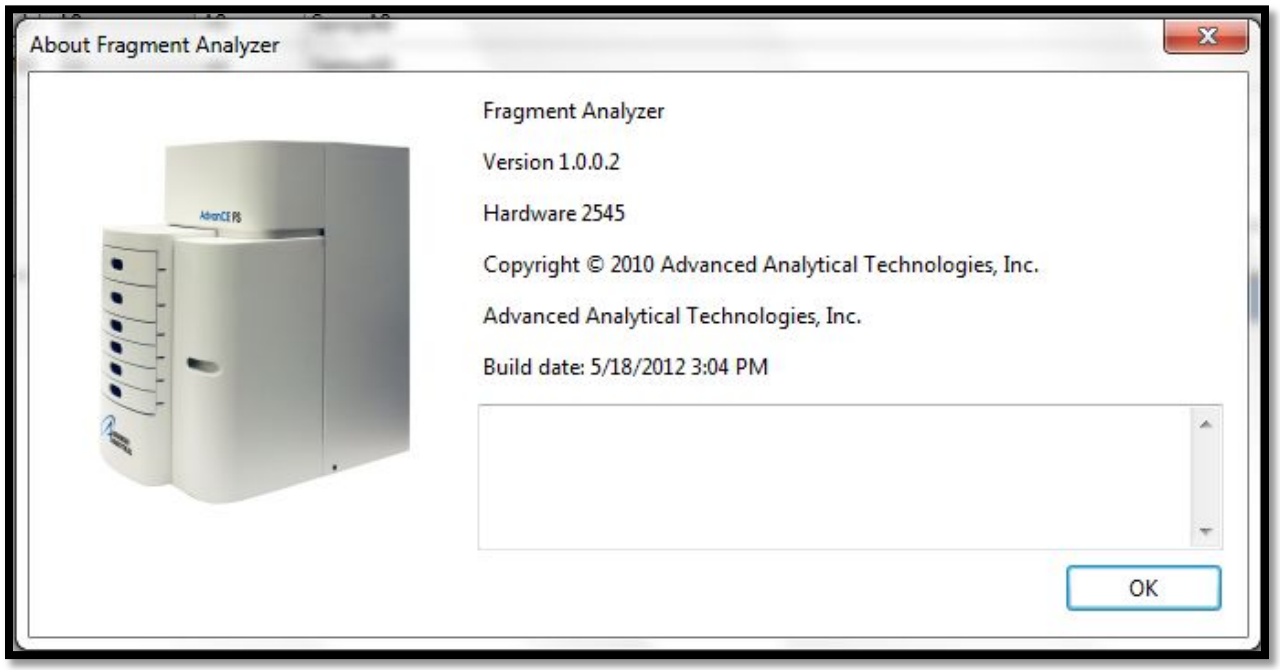


Figure 6-16. The Help Menu
About Function Window

Help—About Firmware

The About Firmware displays the most recent information concerning internal on-board programming, as shown in Figure 6-17, below:

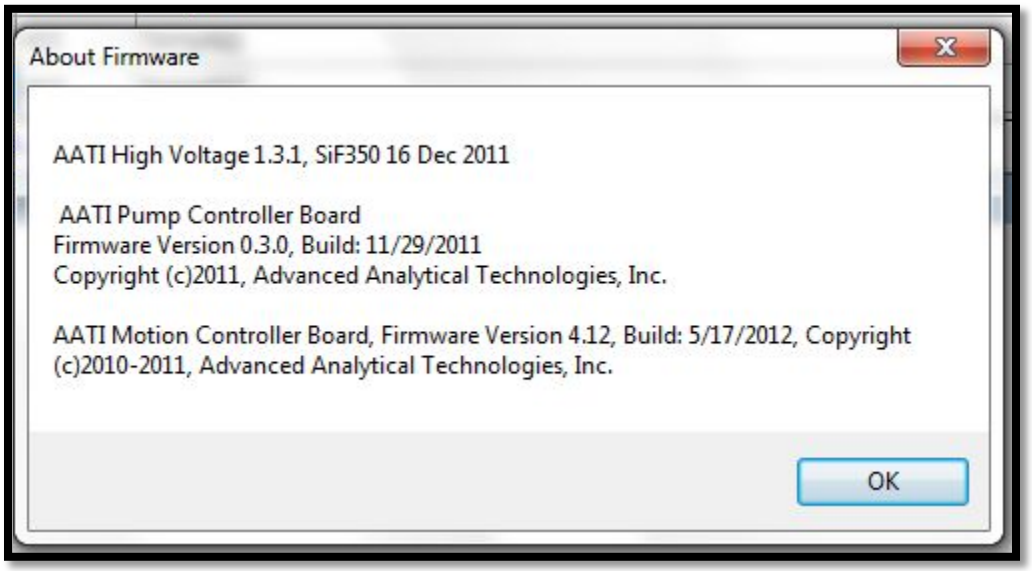


Figure 6-17. The Help "About Firmware" Menu
About Firmware Window

Program Access

When first installed, there are two levels of computer access, user and administrator. User and Administrator Access to functions within the *Fragment Analyzer*™ FS software is defined on the next page in Table **6-5**.

Menu Item	Access Level	Description
File / Select Database	Admin	Allows the administrator to open a database connection
File / File Manager	User	This function provides a user-interface for viewing raw data files. The user can view a file as a single or as a group of 12 electropherograms.
File / Logout	User	Logs the user out of the application to disable unauthorized access of the application.
File / Exit	User	Terminate the application. Prompt the user to ensure that this is their intention.
Admin /Change Password	User	Allow the currently logged in user to change their password. Refer to section **Error! Reference source not found.**.
Admin / User Maintenance	Admin	Add, edit or delete users and access levels for this application. Refer to section **Error! Reference source not found.**.
Admin/Archive & Purge Database	Admin	This function will allow the user with administrative rights to backup the data for future use in a different location or on an external storage device.
Admin / Configuration	Admin	Show the configuration screen. Refer to section **Error! Reference source not found.**.
Admin / Event Report	User	Allow the user to select a data range and show the event log report for the date range in a print preview window.
Admin / Error Report	User	Allow the user to select a data range and show the error log report for the date range in a print preview window.
Utilities / Optical Alignment	User	This menu provides a direct output from the photodiode array detector to facilitate the optical alignment
Utilities / Method Editor	User	Show the configuration screen. Refer to section **Error! Reference source not found.**2.
Utilities / Remove Capillary Array	User	This will initiate a method to prepare the capillary array for removal, i.e. flush gel from the capillaries and home the stage.
Utilities /Hardware I/O	AATI	Enables testing of valves and stage position.
Help / Contents (F1)	User	Advances to the table of contents for the application help.
Help / About	User	View the about box for the application. This display shall contain the copy right and software version number.
Help / About &Controller Firmware	User	View the about box for the FS12 controller board. This display shall contain the firmware version number.

Table 6-5: User access levels for functions within the *Fragment Analyzer*™ FS system software.

Optical Alignment - *Fragment Analyzer™* Software

In This Chapter

- Main Screen Interface - Utilities Menu Commands
  - ✓ Performing an Optical Alignment – 12-Capillary Array
    - Method 1 - Alignment from a File
    - Method 2 - Alignment Without Dye
    - Method 3 - Fluorescein Dye
  - ✓ Hardware I/O
  - ✓ Prime
  - ✓ Solution Levels
  - ✓ Performing an Optical Alignment – 96-Capillary Array
    - Method 1 - Alignment from a File
    - Method 2 - Fluorescein Dye

In this chapter, we will continue exploring the *FRAGMENT ANALYZER*™ system software with an overview of the **Utilities Function** that is accessed from the **Main Screen Window**.

The terms **Optical Alignment** and **Capillary Alignment** are used interchangeably in this chapter.

Menu Bar Commands

Utilities Menu Functions

The **Utilities Menu** is located in the **Main Screen Toolbar** in the upper left corner of the **Main Screen Window**. The commands accessed from the **Utilities Menu** are shown below. Fig. **7-1**

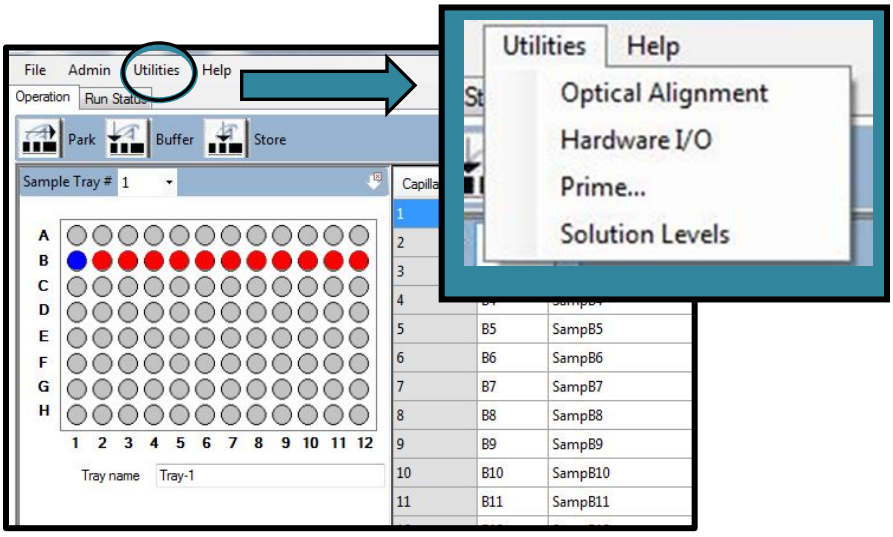


Figure 7-1. Main Screen Window:
Utilities Menu

Utilities Menu – Optical Alignment

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

- The **original capillary array** installed on-site by AATI is **aligned during** the **IQ/OQ process.**

There are three ways to perform an Optical Alignment:

1. Alignment from a File
2. Alignment Without Dye
3. Alignment Using Fluorescein Dye

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

All of the method steps outlined for performing optical alignments will be the same for a 96–Capillary Array unless otherwise noted.

Optical Alignment Window

The **Optical Alignment Function** is accessed through the **Utilities Menu** from the **Main Screen Window**. Fig. **7-2**

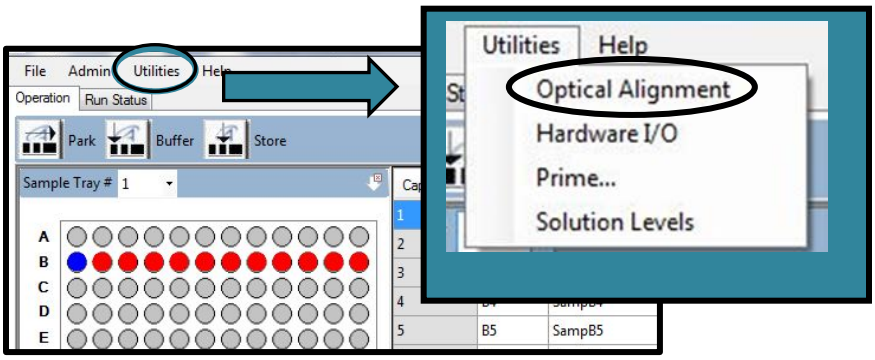


Figure 7-2. Main Screen Window:
Utilities Menu

12-Capillary Array Alignment Method 1 - Alignment from a file

An optical alignment can be performed from a file by selecting **[Optical Alignment]** through the **Utilities Menu** from the **Main Screen Window.**

The **Optical Alignment Prep Window** will open and prompt the user to fill the capillaries with dye. Select **[No]** when asked to fill the capillaries with dye. Fig. 7-3

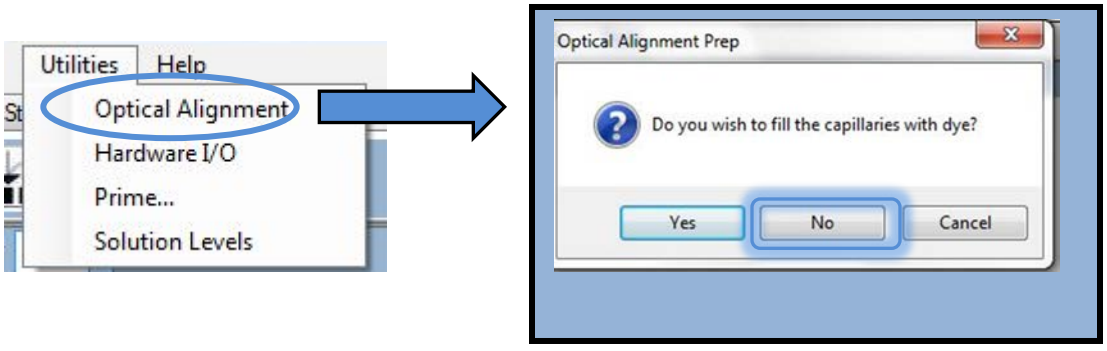


Figure 7-3: The Optical Alignment Prep Window

Select the [Read Raw] icon from the Optical Alignment Menu Bar located at the top of the Optical Alignment Window. Fig. **7-4**.

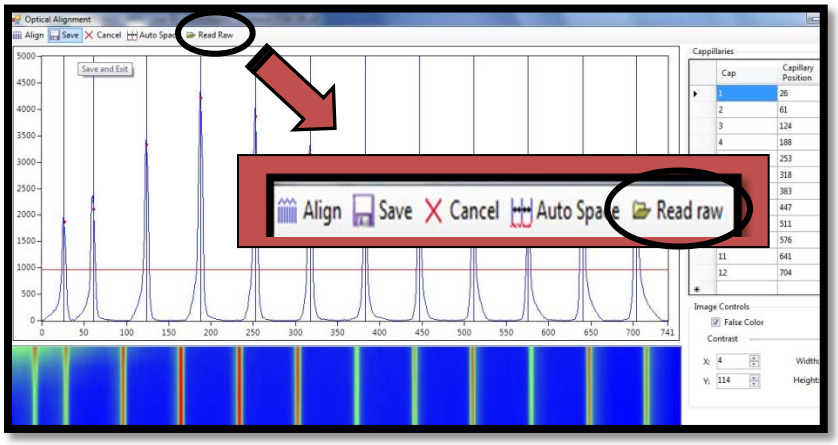


Figure 7-4: The Optical Alignment Menu Bar
Read Raw Command

Open a Raw File using the Windows Prompts. An example of the File Extension for a Raw Data File is displayed below in Fig. **7-5**. The Raw File will be used for the Capillary Alignment.

Figure 7-5: FilePath Example for a Raw Data File

After selecting and opening a Data File, the Align from File Window appears. (Fig.**7-6**)

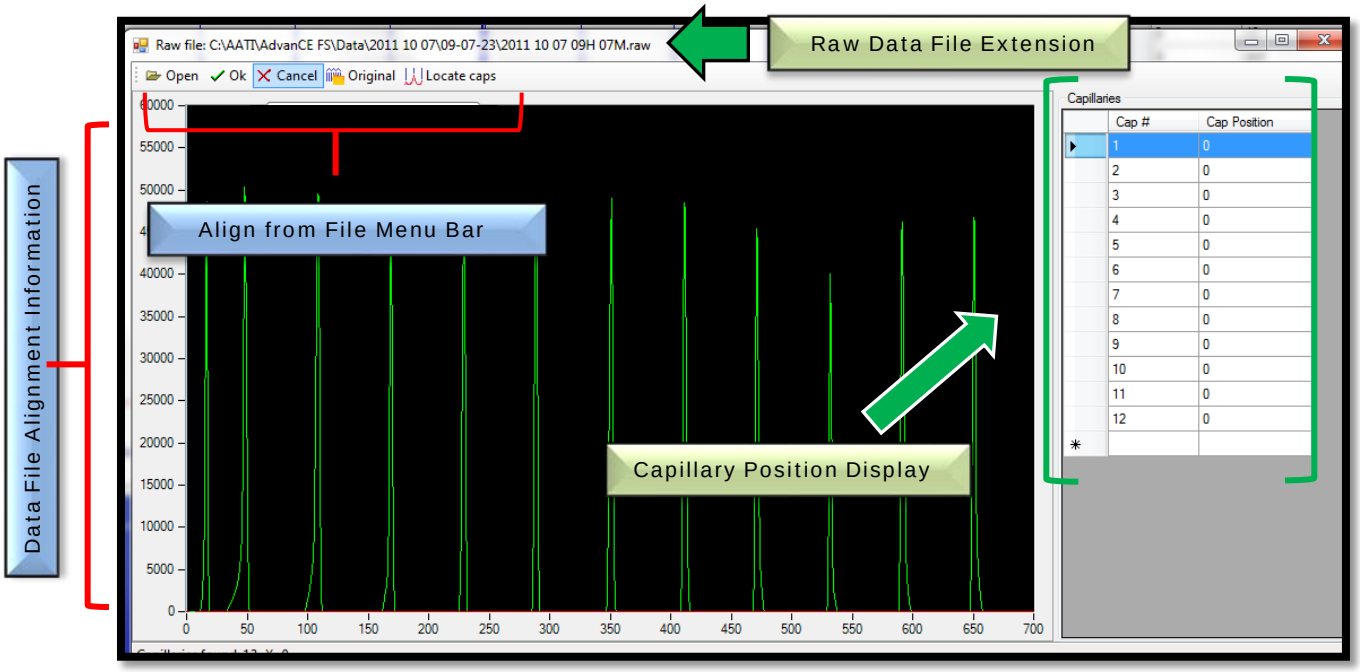


Figure 7-6. Align from File Window.
12-Capillary Array

Select the [Original] tab in the Align from File Menu Bar to show the Original Alignment. (Fig **7-7**). This is the original capillary alignment (from the instrument) that was applied to the data set. Choosing this function allows you to see how well the "original" alignment fits the data.

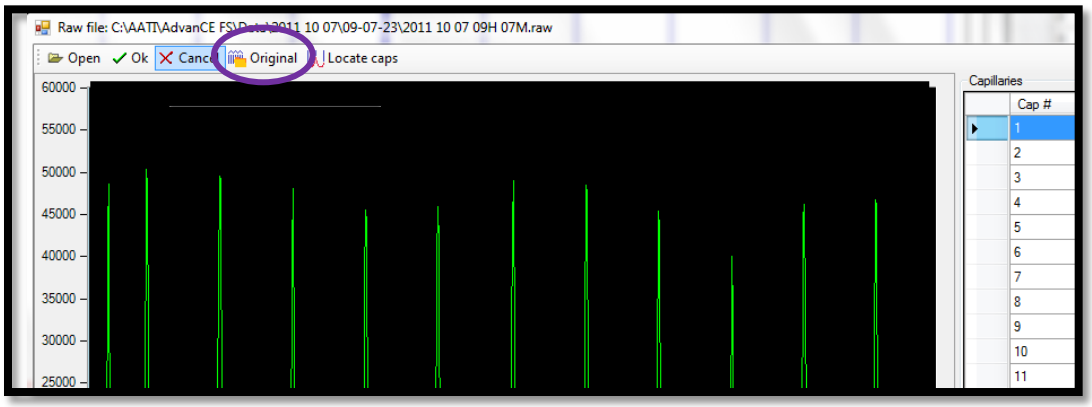


Figure 7-7. Align from File Window.
Original Tab

Selecting the [Original] tab inserts the Original Alignment Data that is depicted in blue into the Align from File Window along with the Alignment Data from the Data File depicted in green. (Fig 7-8.)

In the example below, the blue vertical lines show the Original Alignment, which in this case perfectly overlap the green peaks imported from the Data File.

Select [OK] to accept the alignment (only if the alignment looks good—and the lines exactly intersect the maximum of each peak).

Figure 7-8. Align from File Window
Original Alignment Overlaps the Peaks from Data File

After selecting [OK] and the file is closed, select [Save] in the Optical Alignment Window. Fig. **7-9**.

⚠ If the alignment is not saved from the Optical Alignment Window, it will not be saved to the instrument—and subsequent data files will not be aligned correctly.

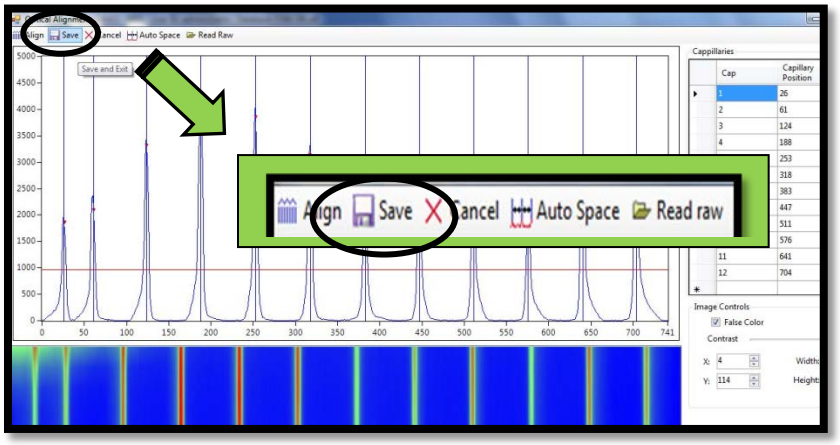


Figure 7-9: The Optical Alignment Menu Bar
Save Alignment after Closing File

If the vertical lines of the Original Alignment and the peaks from the Raw Data File do not line up, adjust the blue vertical lines to fall exactly in the center of each peak.

Adjust the Capillary Alignment by selecting [Locate caps] from the Align from File Window. To correctly align the file, first move the red integration line (located at the very bottom of the plot) with the mouse as shown below in Fig. **7-10**.

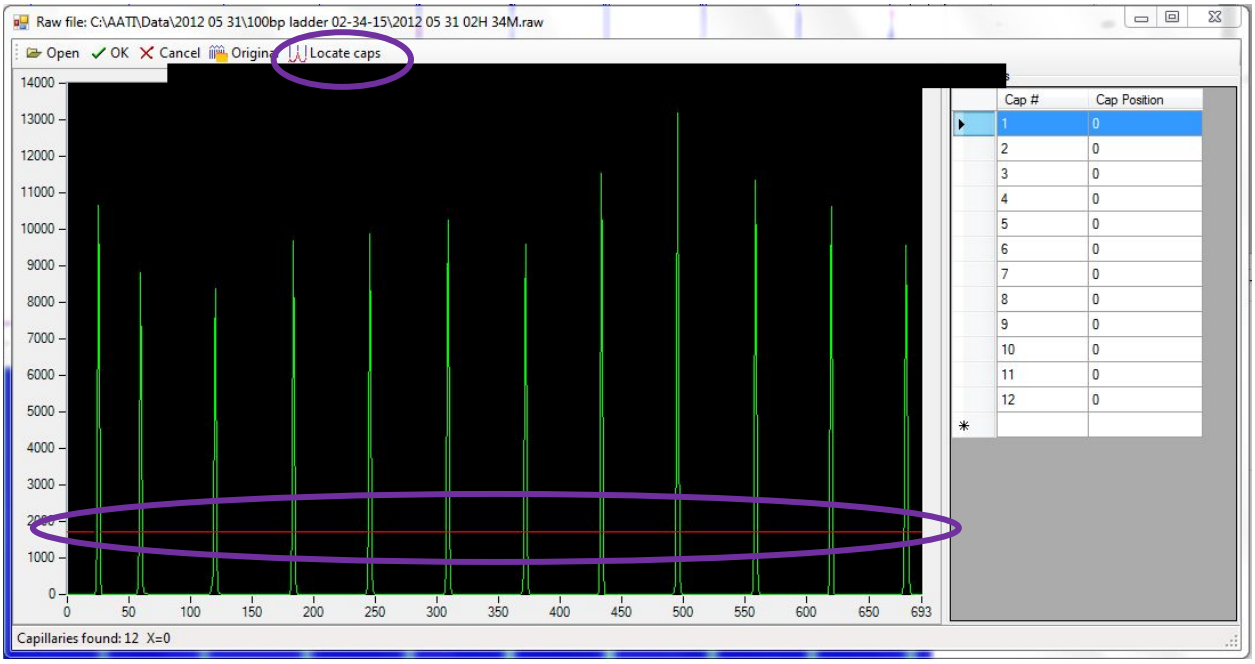


Figure 7-10. Align from File Window.
Move the red line from the very bottom of the plot to a location where the capillaries can be appropriately identified BEFORE selecting "Locate Caps".

The blue vertical lines in the Align from File Window are usually correctly located when the Locate Caps option is selected. However, if necessary, the lines can also be manually moved with the mouse. (Fig. **7-11**) (Note that if the red line is moved appropriately to capture all peaks, the blue alignment lines are always correctly selected with the Locate Caps option is selected).

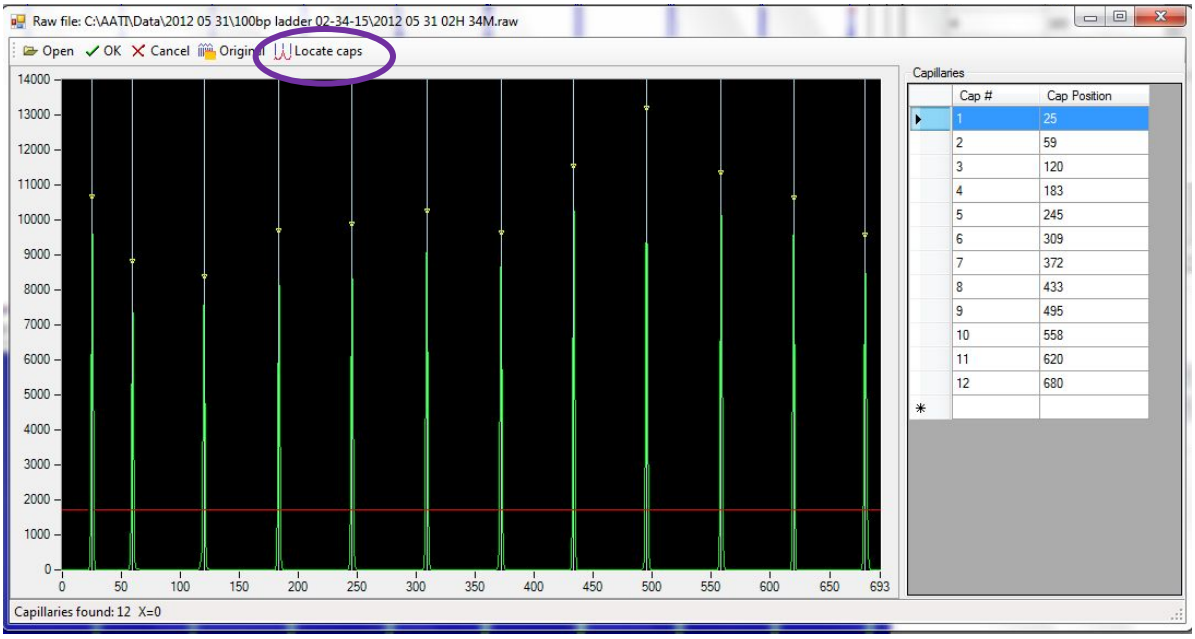


Figure 7-11. Align from File Window with the blue vertical alignment lines shown after the "Locate Caps" option is selected.

To Insert or Delete capillary positions:

- Right click between rows of interest in the table in the Capillary Position Display located to the right of the Align from File Window and select [Insert/Delete Capillary]. (Fig. **7-12**.)

OR

- Right click on the area in the black screen of the Align from File Window and select [Insert/Delete Capillary]. (Fig **7-13**.)

Insert Capillary Position from table in the Capillary Position Display:

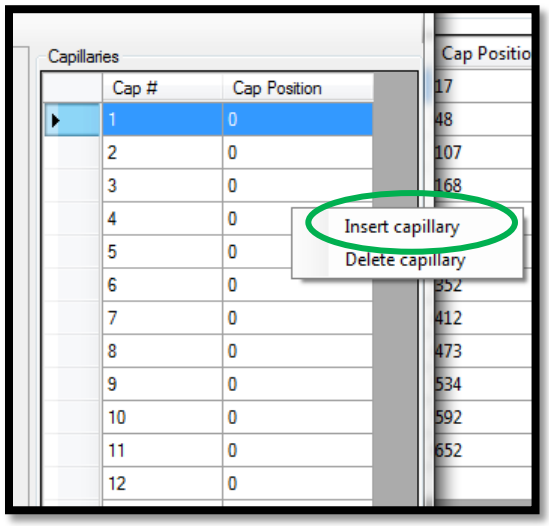


Figure 7-12. Capillary Position Display Table:
Right-Click and Select [Insert Capillary]

Insert Capillary Position in the Align from File Window:

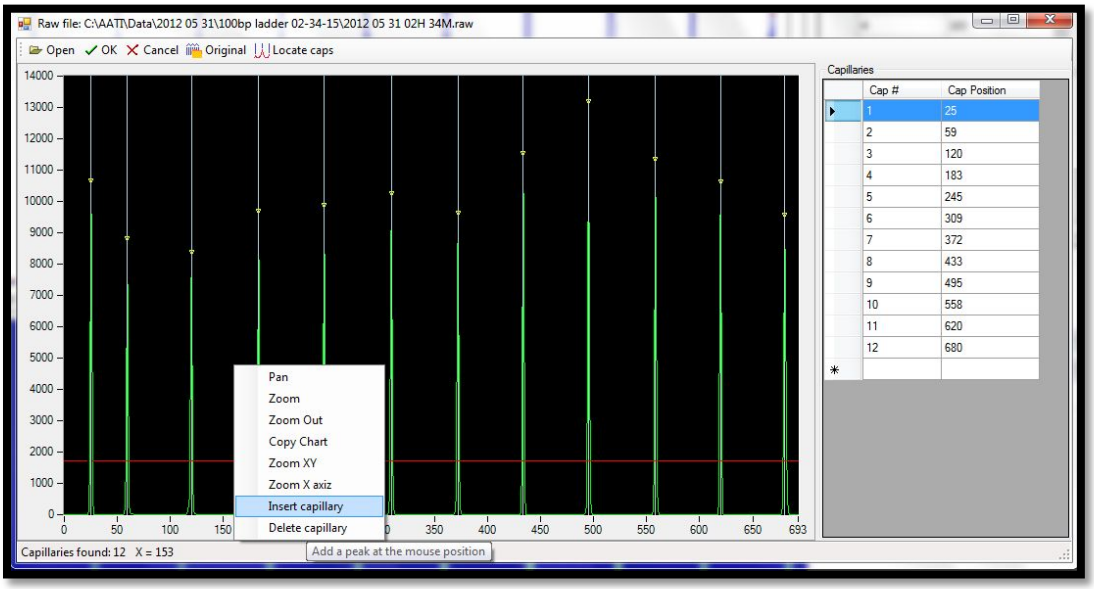


Figure 7-13. Capillary Position Display Window:
Right-Click and Select [Insert Capillary]

After a capillary has been inserted, and additional vertical line is observed in the capillary display window (Fig. **7-14**). Note that in this case it is not necessary to insert a capillary, but in the rare case in which a capillary is not recognized by the program, the user has an option to manually add a capillary.

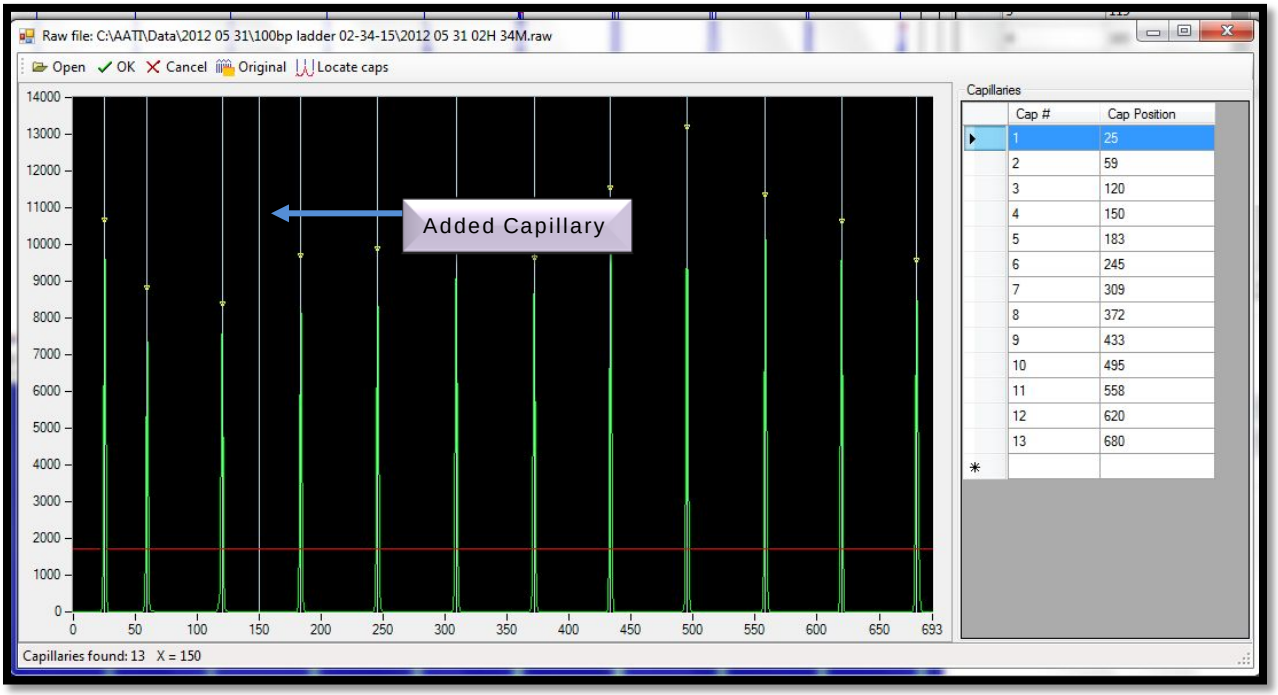


Figure 7-14. Align from File Window.
Added Capillary is observed as a vertical line.

Any blue vertical alignment position can be removed by right-clicking and selecting [Delete Capillary].

Once the capillary positions look good (perfect overlap of blue vertical lines in the center of each green peak), hit [OK] to accept alignment. (Fig. **7-15**.)

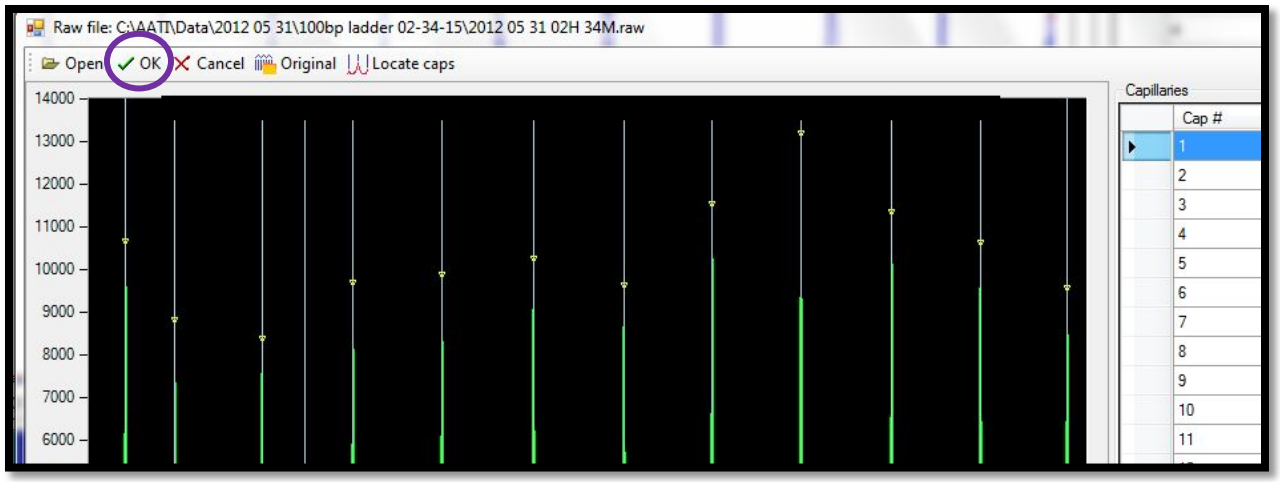


Figure 7-15. Align from File Window.
Select [OK]

After selecting [OK] and the file is closed, select [Save] in the Optical Alignment Window. (Fig. **7-16**.)

⚠ If the alignment is not saved, it will not be saved to the instrument.

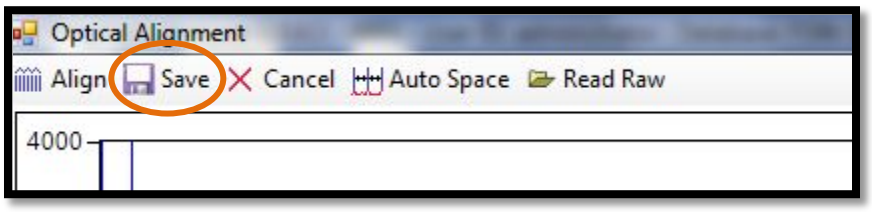


Figure 7-16: The Optical Alignment Menu Bar
Save Alignment after Closing File

The following options are available from the Align from File Window Toolbar. (Table **7-1**.)

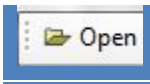
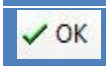
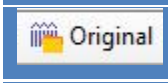
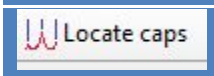
Icon	Description
	**Opens** a new file
	**Accepts Changes** to the file (i.e. capillary locations)
	**Cancels** any actions and closes the file
	**Locates** the **Original Capillary Positions**
	**Locates Capillaries** based on peak positions in the open file (note: move red line up so that only the peaks of interest are integrated)

Table 7-1: The Align from File ToolBar Command Icons

The following Right Click Options are available when within the Align from File Window (black area).

Right Click Commands	Description
Pan	Pops up mouse "hand" which enables user to move image in window
Zoom	**Zooms** image. To Zoom in, drag and select the area of interest
Zoom Out	**Un-Zooms the image.**
Copy Chart	**Copies the chart** into the clipboard
Zoom XY	**Enables the XY zoom mode** (Drag and select area of interest)
Zoom X	**Enables X-axis zoom mode** (Drag and select area of interest). This is useful for selecting a small set of capillaries when viewing 96 capillaries.
Insert Capillary	**Inserts a capillary location** at the current mouse pointer location
Deletes Capillary	**Deletes a capillary location** closest to the current mouse pointer location.

Table 7-2. Align from File Window Right-Click options

12-Capillary Array Alignment Method 2: Alignment without dye

⚠ An optical alignment can only be performed without dye for a 12 –Capillary Array.

To perform an alignment without dye for a 12-Capillary Array, select [Optical Alignment] through the Utilities Menu from the Main Screen Window.

The Optical Alignment Prep Window will open and prompt the user to fill the capillaries with dye. (Figure **7-17**.)

Select [No] when asked to dye fill the capillaries.

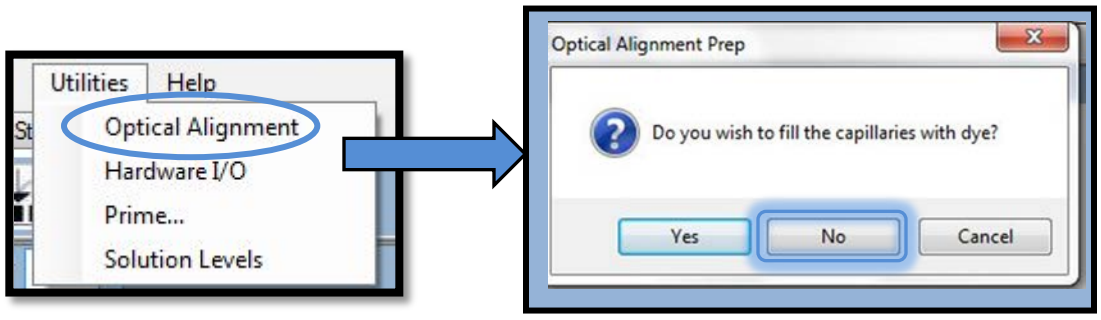


Figure 7-17: The Optical Alignment Prep Window

Next, open the top and side doors of the *Fragment Analyzer*™ instrument to allow ambient light to reflect on the capillaries as shown in Figure **7-18** below.

This step may not be necessary. If you obtain too much light on the capillaries, close the top compartment for this method.

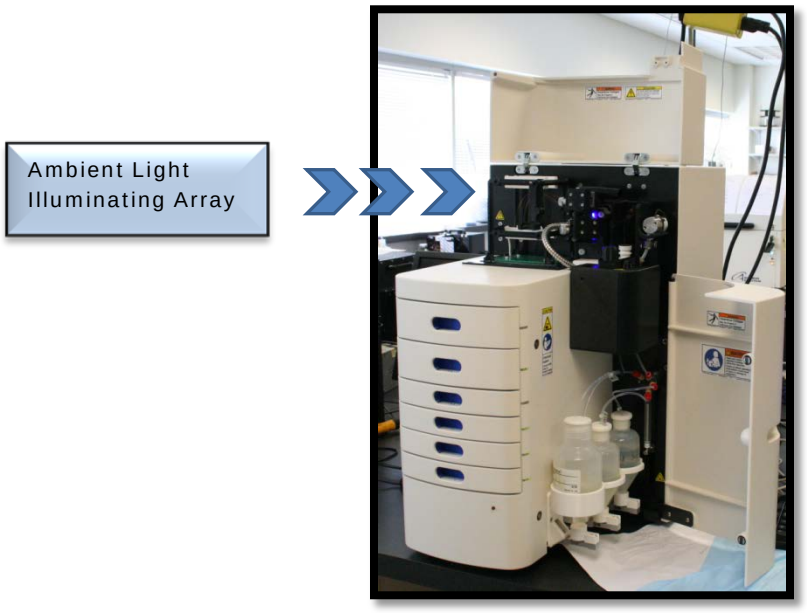


Figure 7-18: Open the Top and Side Doors of the Instrument

After selecting [No] and opening the top and side doors of the instrument, the Optical Alignment Window is displayed. (Fig. **7-19**.)

Right-click in the blue area of the Capillary Alignment Display where the capillaries are visible and select [Reset All].

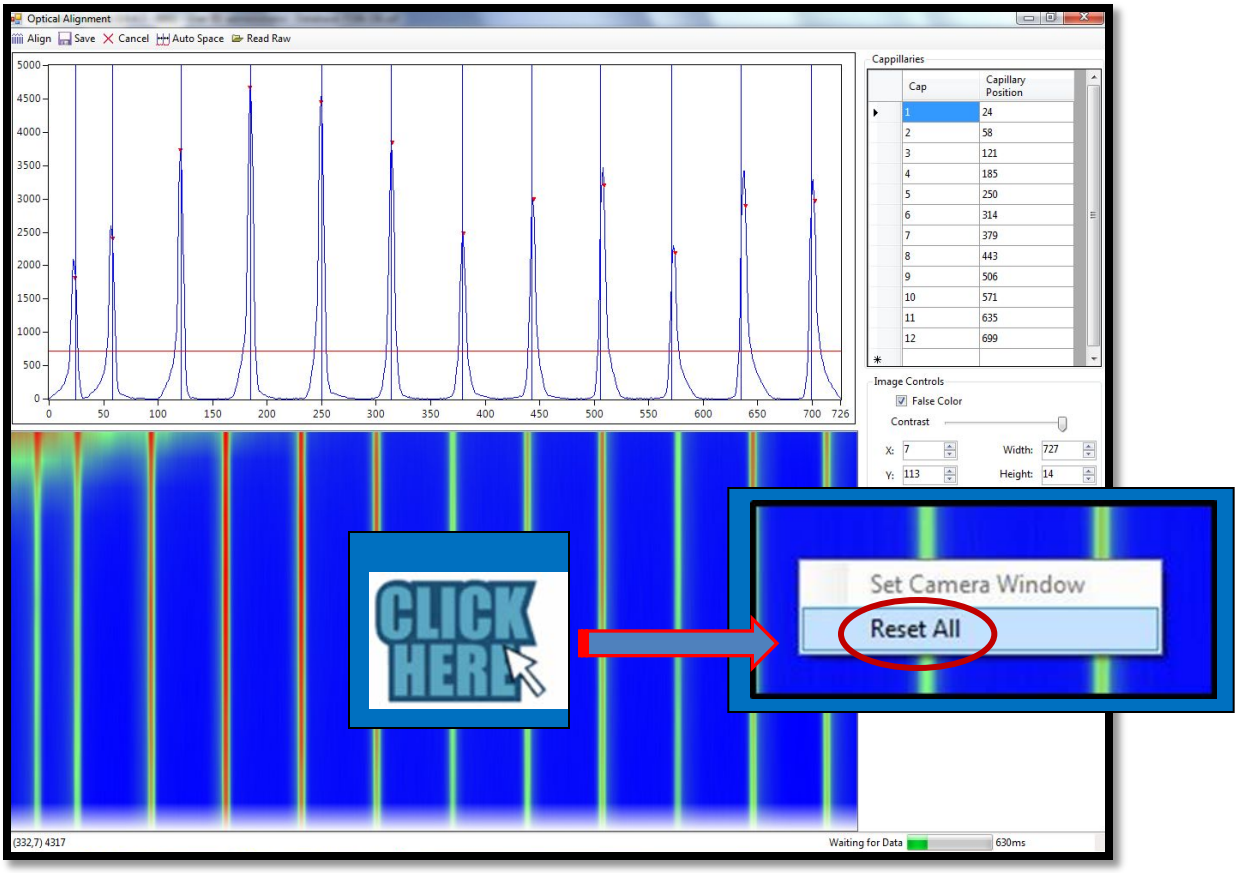


Figure 7-19. Capillary Alignment Display:
Reset All Command

After choosing [Reset All], the Capillary Alignment Display shows the entire area of the CCD Camera Window. (Fig **7-20**.)

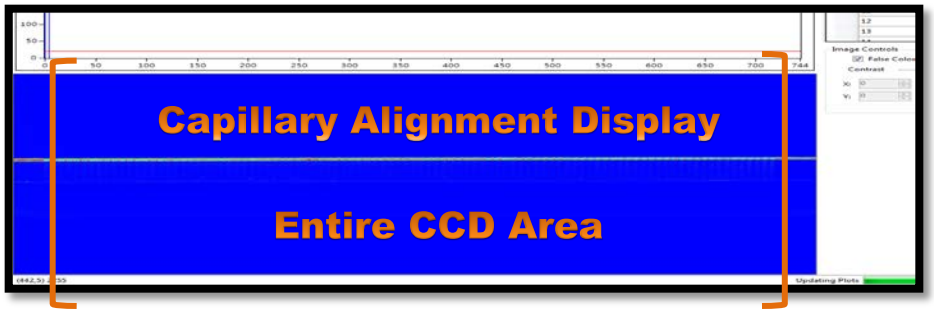


Figure 7-20. Capillary Alignment Display:
CCD Window Area

To set the Capillary Array Camera Window, click and drag a rectangular area that corresponds to the Capillary Array Camera Window Area.

Right-click and select [Set Camera Window] as shown in Fig. **7-21** below.

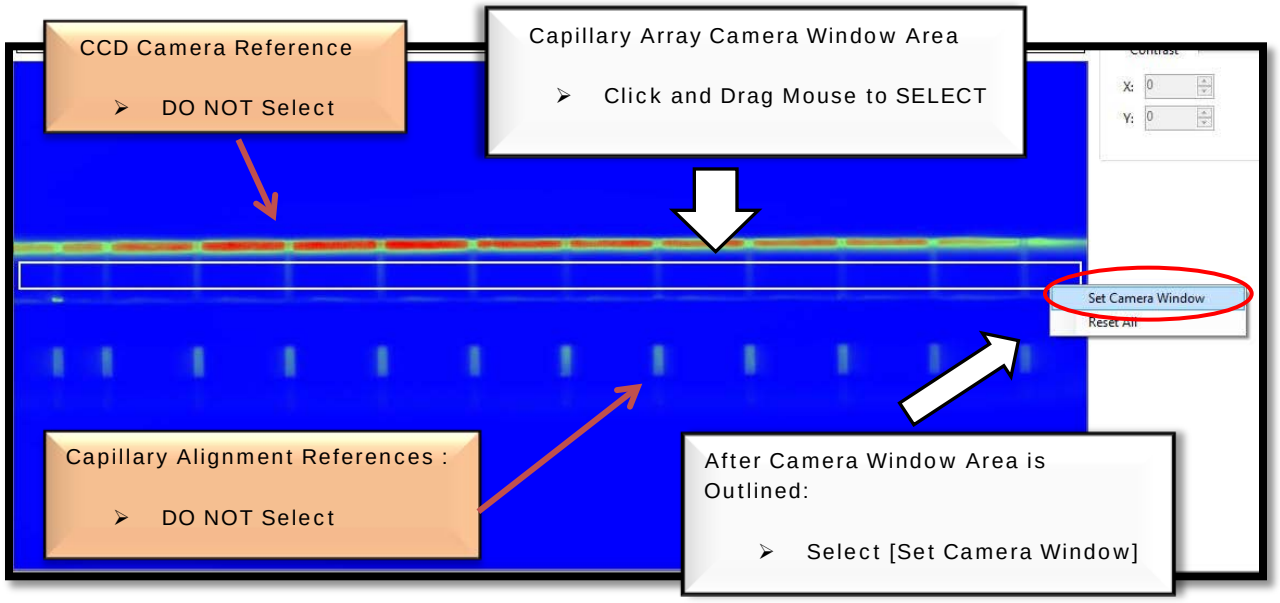


Figure 7-21. Capillary Alignment Display:
Setting Camera Window

After the camera window is set, close the top and side cabinet doors on the *Fragment Analyzer*™ instrument.

Use the [Contrast] Slider Button located below the Capillary Position Display to adjust the image so the capillaries in the Capillary Alignment Display are easily observed. (Fig. **7-22**)

Adjust the [Height] field for the Camera Window so that it is **≥ 14**.

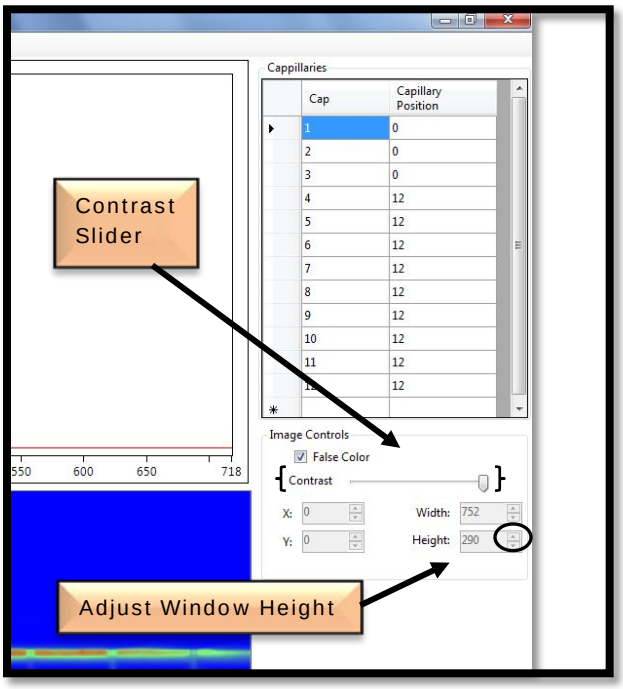


Figure 7-22. Capillary Position Display
Adjust Contrast and Set Height

Select [Align] at the top of the screen in the Optical Alignment Menu Bar. (Fig. **7-23**)

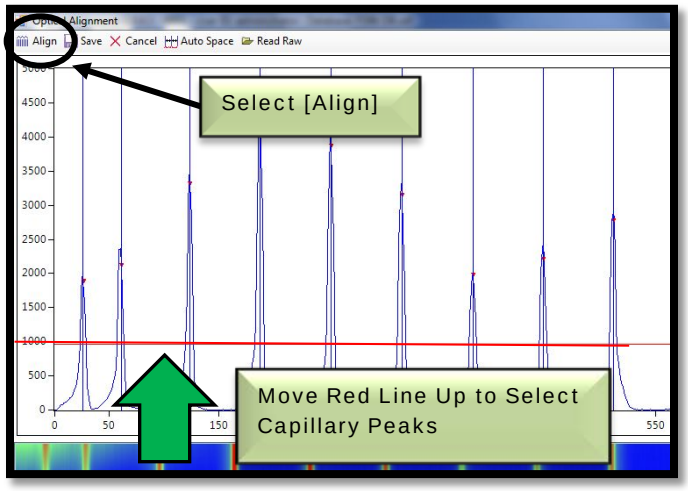


Figure 7-23. Capillary Alignment Processed Image Display: Selecting Capillary Peaks

After [Align] is selected, the red line can be moved up and down until a red dot is observed above each capillary peak. This determines which capillary peaks are selected.

Hit [Align] every time the red line is moved. This ensures that the instrument has selected the peak for integration.

If the blue vertical lines are not in the exact center of each peak, each line can be adjusted using the mouse. This determines the each capillary position.

⚠ Check to make sure the [Height] field for the Camera Window is still **≥ 14**.

Select [Save] at the top of the screen in the Optical Alignment Menu Bar to end the capillary alignment process.

- Ensure that all the capillary peaks have a single red dot and the blue vertical lines are in the exact center of each peak before saving the alignment. (Fig.**7-24)**

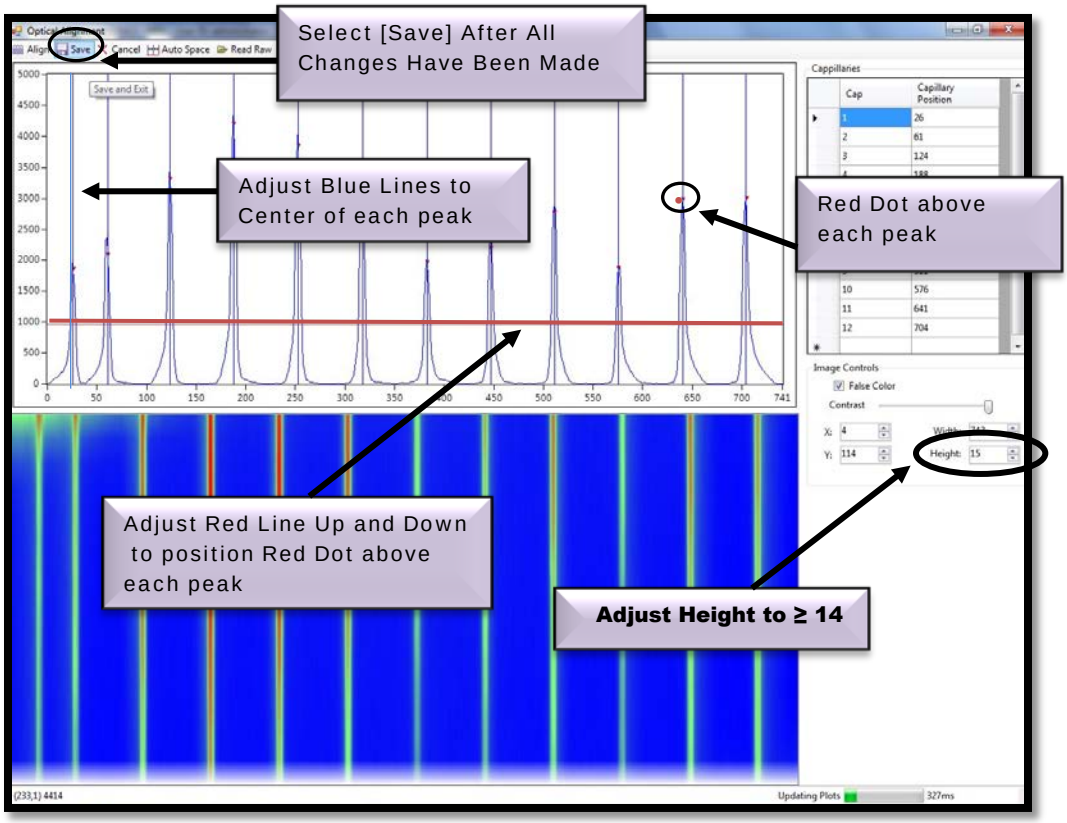


Figure 7-24. Capillary Alignment Processed Image Display: Save Selected Peaks

12-Capillary Array Alignment Method 3: Alignment with Fluorescein Dye

Prior to initiating an **Optical Alignment** with **fluorescein dye** within the *FRAGMENT ANALYZER™* system software, **prepare the fluorescein dye** as described below:

- Prepare a **20 mL volume** of **fluorescein dye** by diluting 20 µL of Fluorescein Sodium Salt CAPS solution (1 milli-Molar o1E-3M, Fluka Number 67834) in 20 mL of de-ionized water.

OR

- Prepare equivalent fluorescein sodium salt dye solution to this concentration (1 micro-Molar 1E-6M) diluting into a volume of 20 ml for 12 cap or 40 ml for 96 cap with de-ionized water.

Once the dye solution is prepared, begin the optical alignment process by selecting **[Optical Alignment]**. This opens the **Optical Alignment Prep Window**. Fig. **7-25**.

From the **Optical Alignment Prep Window**, select **[Yes]** to fill the capillaries with dye.

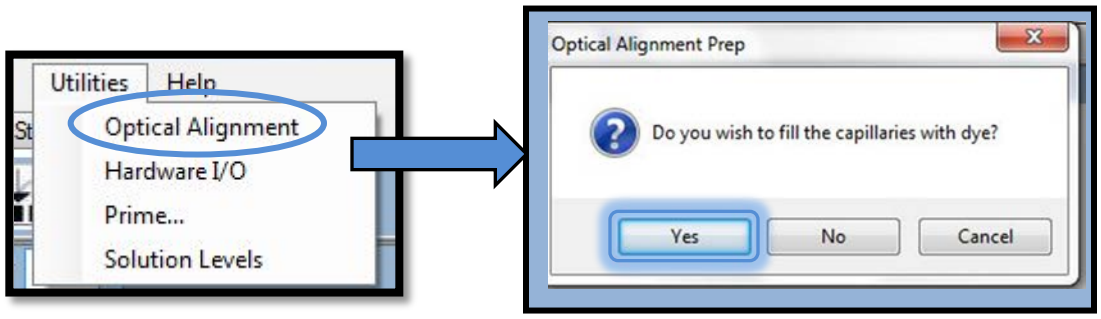


Figure 7-25: The Optical Alignment Prep Window

The software will prompt the user to replace the capillary conditioning bottle in the Instrument Reagent Compartment with a bottle containing 20 mL of the 1 micro-Molar or 1E-6M fluorescein dye solution. (Fig. **7-26**.)

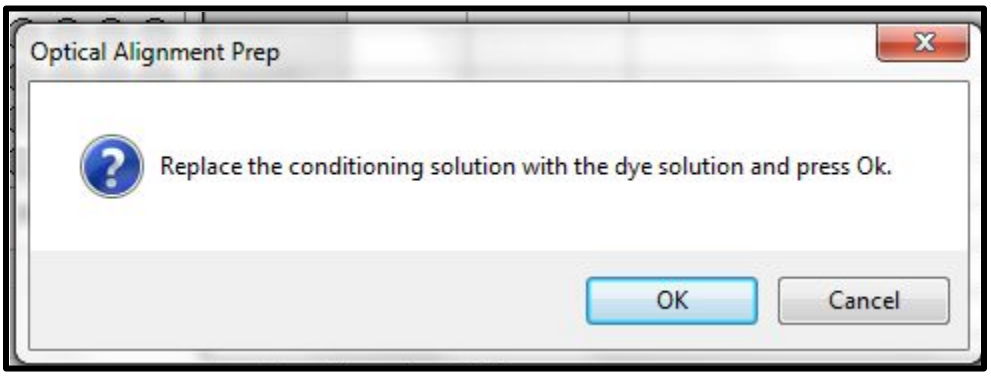


Figure 7-26. Optical Alignment Prep Window
Fluorescein Dye Prompt

After the capillary conditioning bottle is replaced with the 1 micro-Molar or 1E-6M fluorescein dye solution, select [OK].

The instrument will now empty the reservoir and re-fill both the reservoir and capillaries with the 1 micro-Molar or 1E-6M fluorescein solution.

- The re-filling process takes 2 to 3 minutes.

While the instrument is filling the reservoir and capillaries with the fluorescein dye solution, the Optical Alignment Window is displayed. Fig **7-27**.

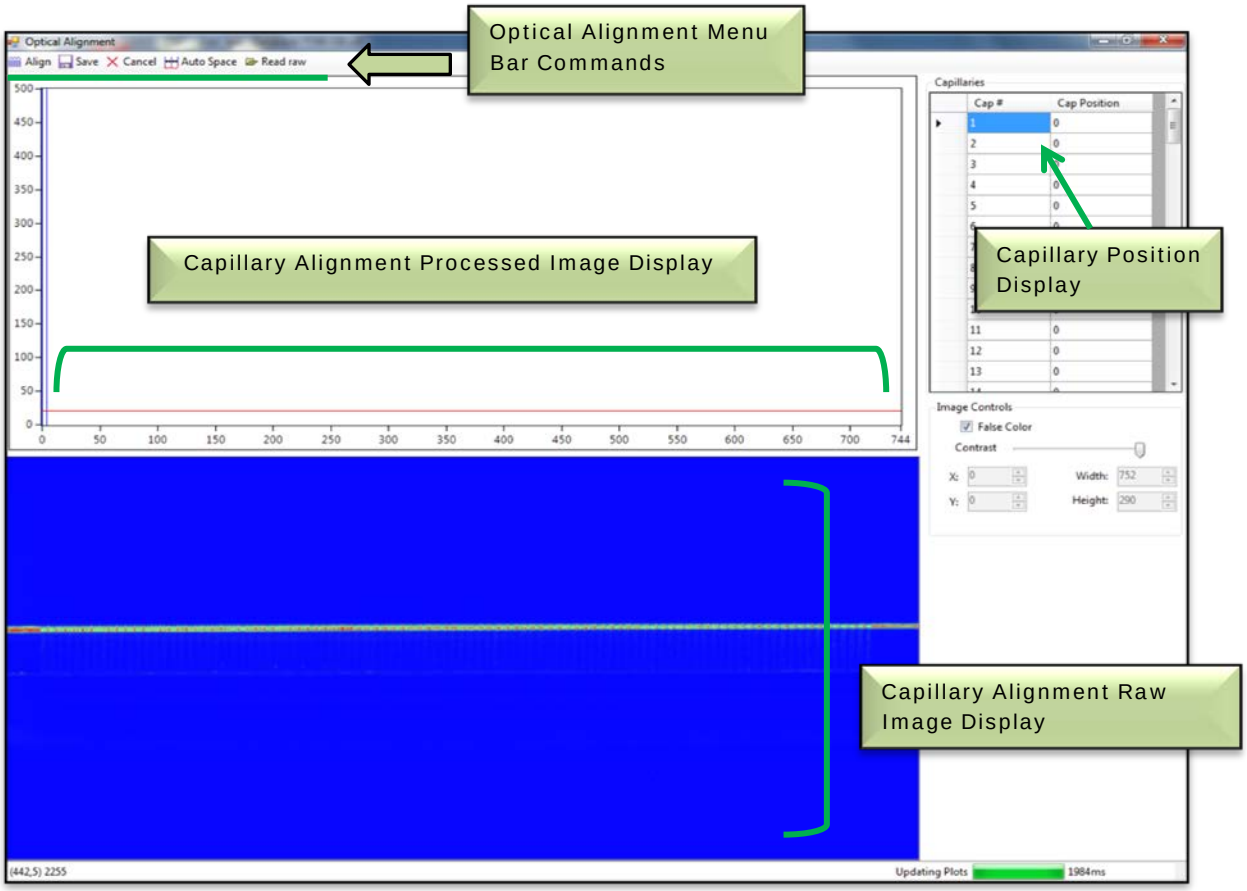


Figure 7-27. Optical Alignment Window

As the capillaries fill with fluorescein dye solution, they will change from transparent to visible in the Capillary Alignment Display, as shown in the time progression below. (Fig.**7-28**.)

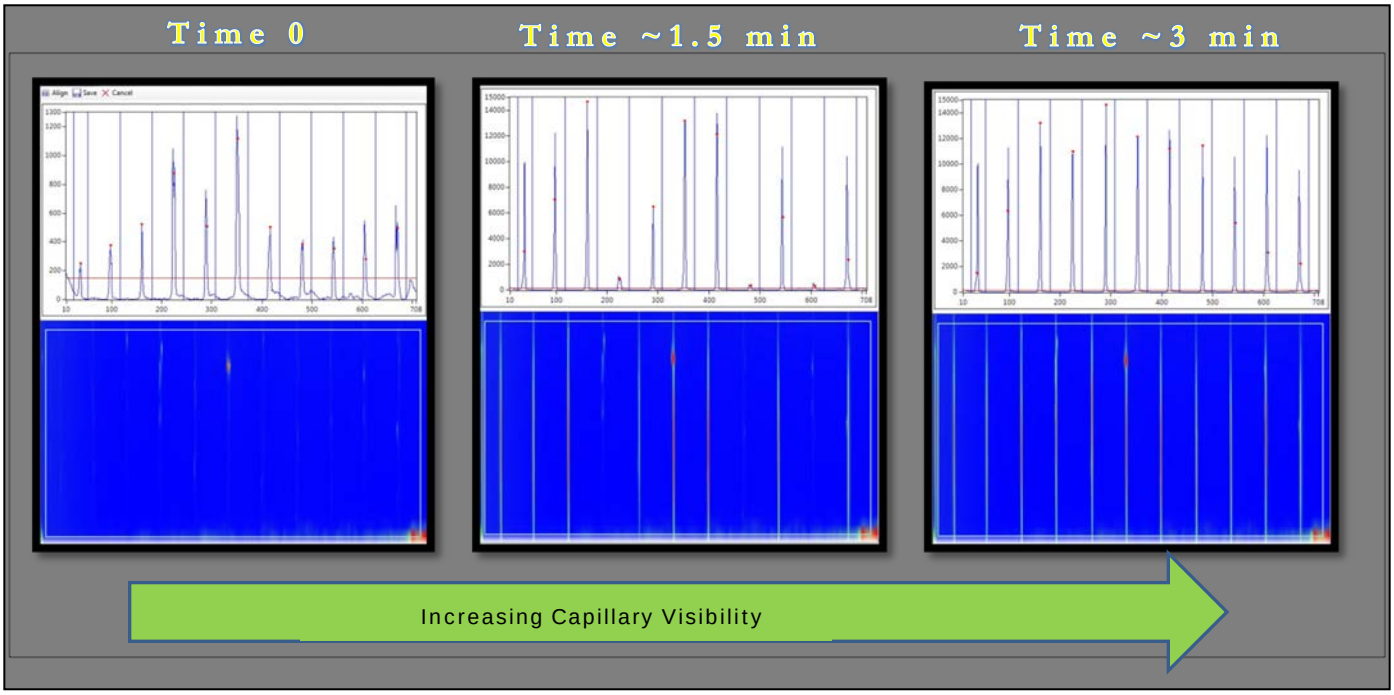


Figure 7-28. Optical Alignment Window Screen Shots
Time Progression - Filling Capillaries with Dye

Once all the capillaries become visible, right-click in the blue area of the Capillary Alignment Display where the capillaries are visible and select [Reset All]. (Fig. **7-29**)

Figure 7-29. Capillary Alignment Display.
Right Click then Select [Reset All]

After choosing [Reset All], the Capillary Alignment Display shows the entire area of the CCD Camera Window. (Fig **7-30**.)

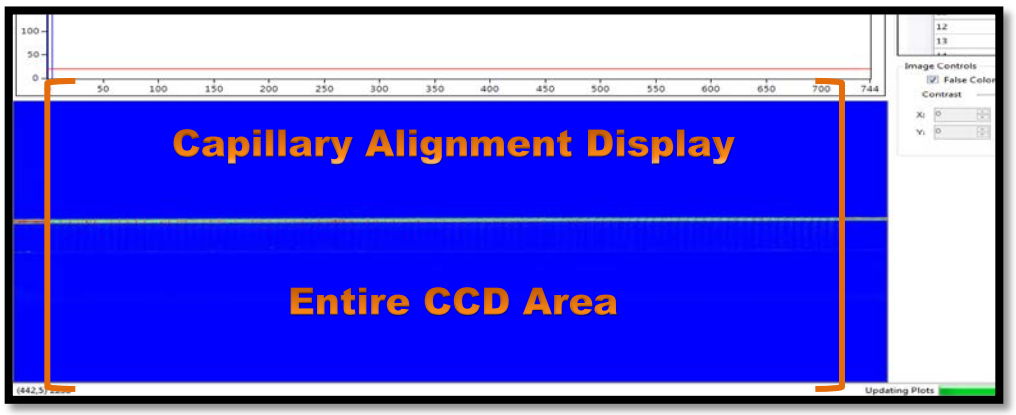


Figure 7-30. Capillary Alignment Display:
CCD Window Area

Only a small fraction of the CCD array area is actually selected and shown by the Capillary Array Camera Window.

The Capillary Array Camera Window is located below the upper edge of the CCD Camera Reference Line and above the poly amide covered Capillary Reference Points. (Fig. 7**-31)**

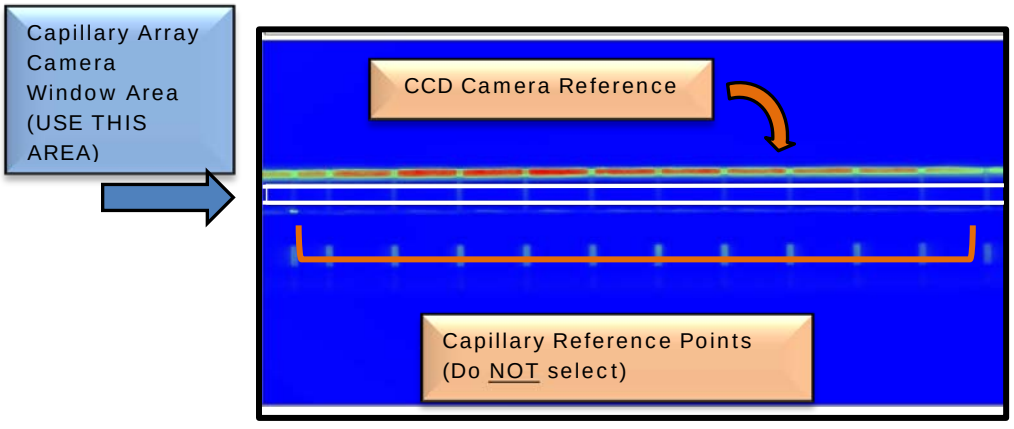


Figure 7-31. Location of Capillary Array Window

To set the Capillary Array Camera Window, click and drag a rectangular area that corresponds to the Capillary Array Camera Window Area.

Right-click and select [Set Camera Window] as shown in Fig. **7-32** below.

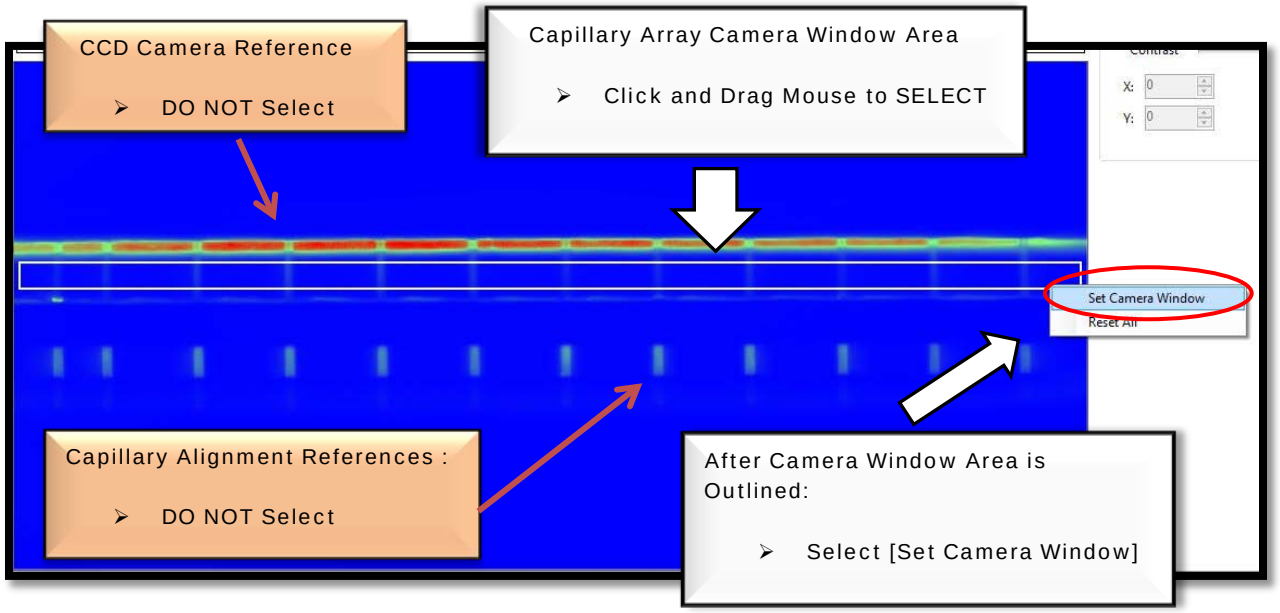


Figure 7-32. Capillary Alignment Display:
Setting Camera Window

After Setting the Camera Window, select [Align] from the Optical Alignment Menu Bar located above the Capillary Alignment Processed Image Display. Fig. **7-25**. Note: Move the red integration line at the bottom of the window, so that only the capillaries are selected (Fig. **7-25**).

This is required to align all of the capillaries with the Camera Window.

Move the horizontal red line up from the very bottom of the y-axis.

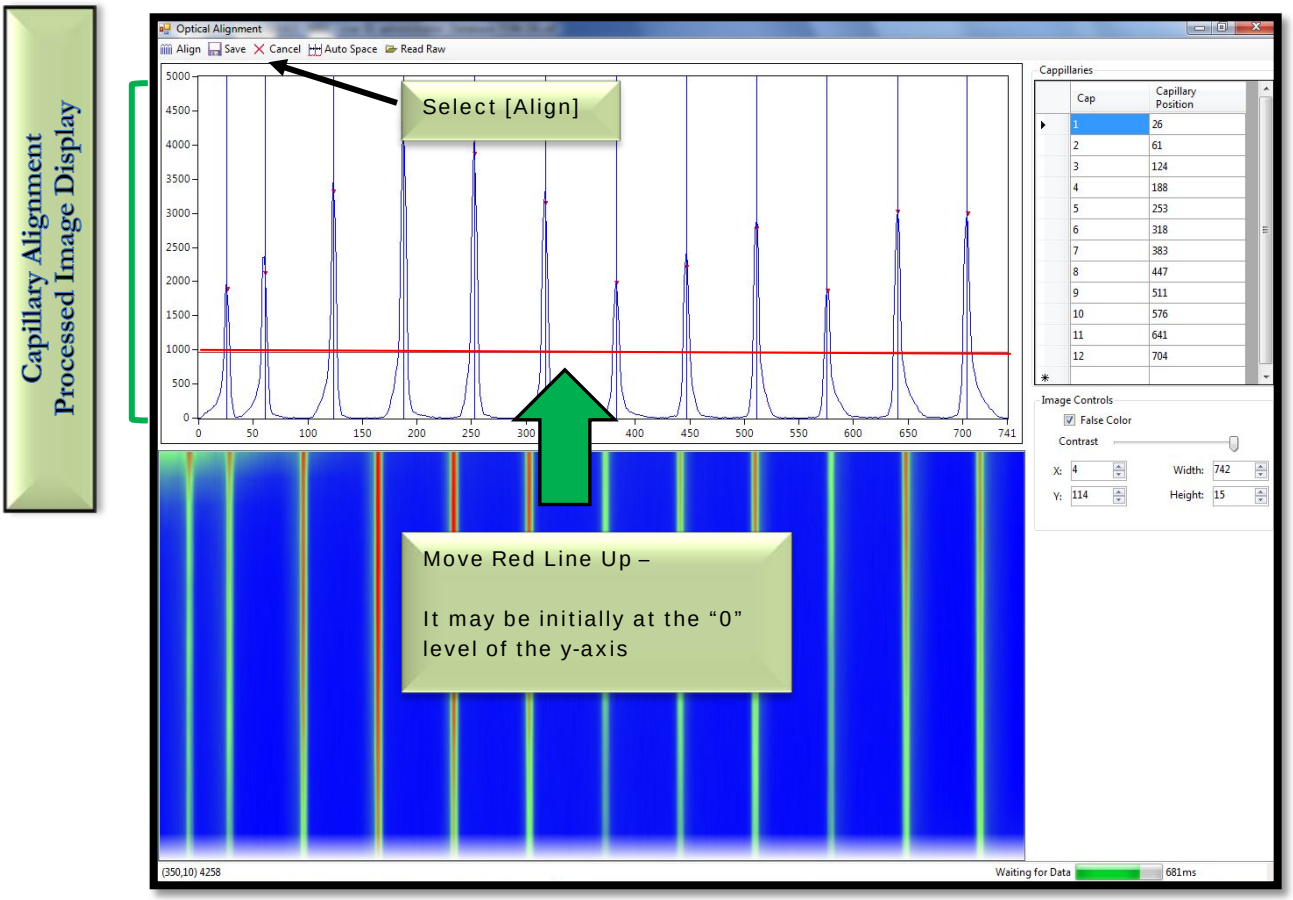


Figure 7-33. Capillary Alignment Processed Image Display:
Move Red Line Up to Select Peaks of Interest

After [Align] is selected, the red line can be moved up and down until a red dot is observed above each capillary peak.

Hit [Align] every time the red line is moved. This ensures that the instrument has selected the peak for integration.

If the blue vertical lines are not in the exact center of each peak, each line can be adjusted using the mouse.

⚠ In the Capillary Position Display, adjust the [Height] field for the Camera Window so that it is **≥ 14**.

Select [Save] at the top of the screen in the Optical Alignment Menu Bar once all the capillary peaks have a single red dot and the blue vertical lines are in the exact center of each peak. (Fig.**7-34**.)

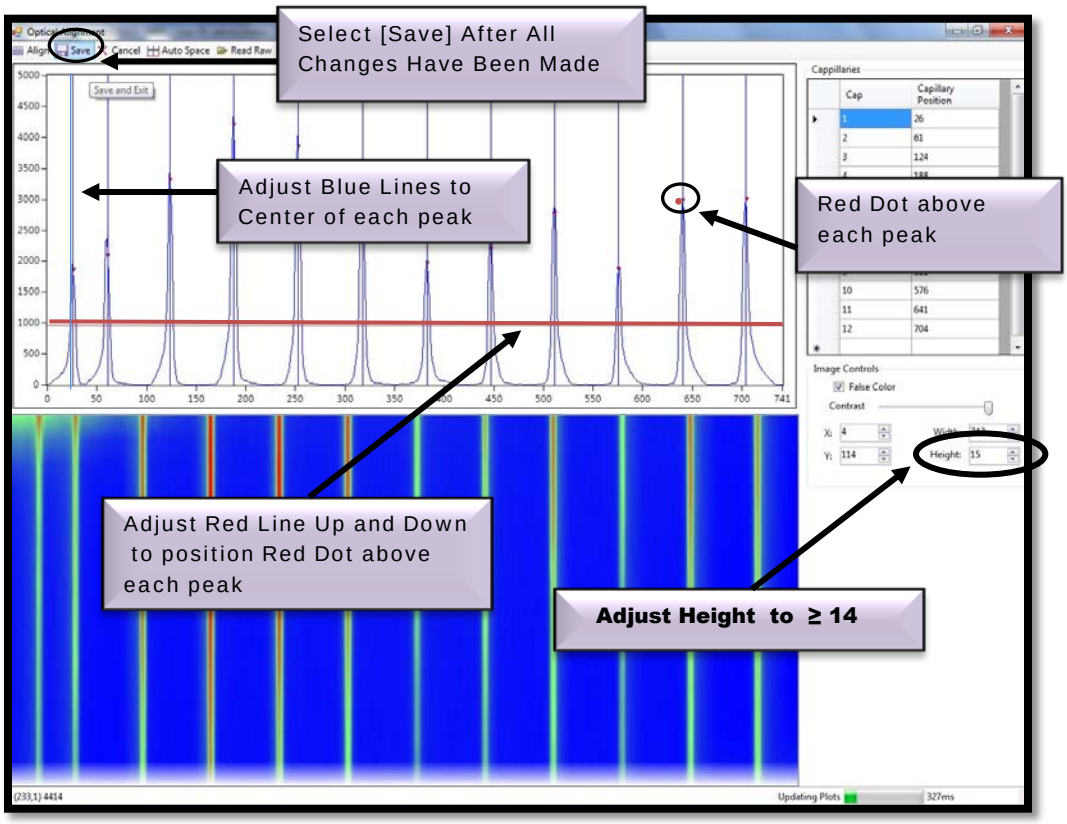


Figure 7-34. Capillary Alignment Processed Image Display: Selecting Peaks of Interest

Utilities Menu—Hardware I/O (Input/Output)

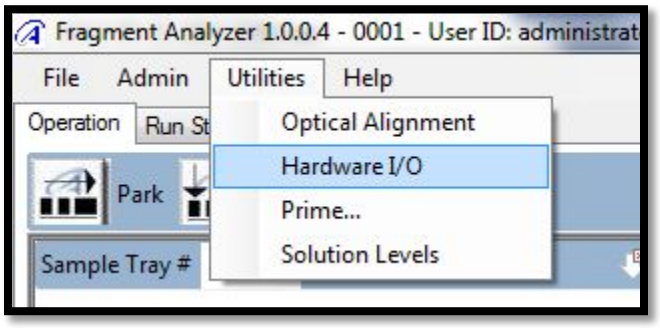


Figure 7-35. Hardware I/O

The Hardware I/O Function is available to User's with Administrator Level Access and is used to troubleshoot the system.

Selecting the [Hardware I/O] function from the Utilities Menu opens the Hardware Test Window. (Fig. **7-36**)

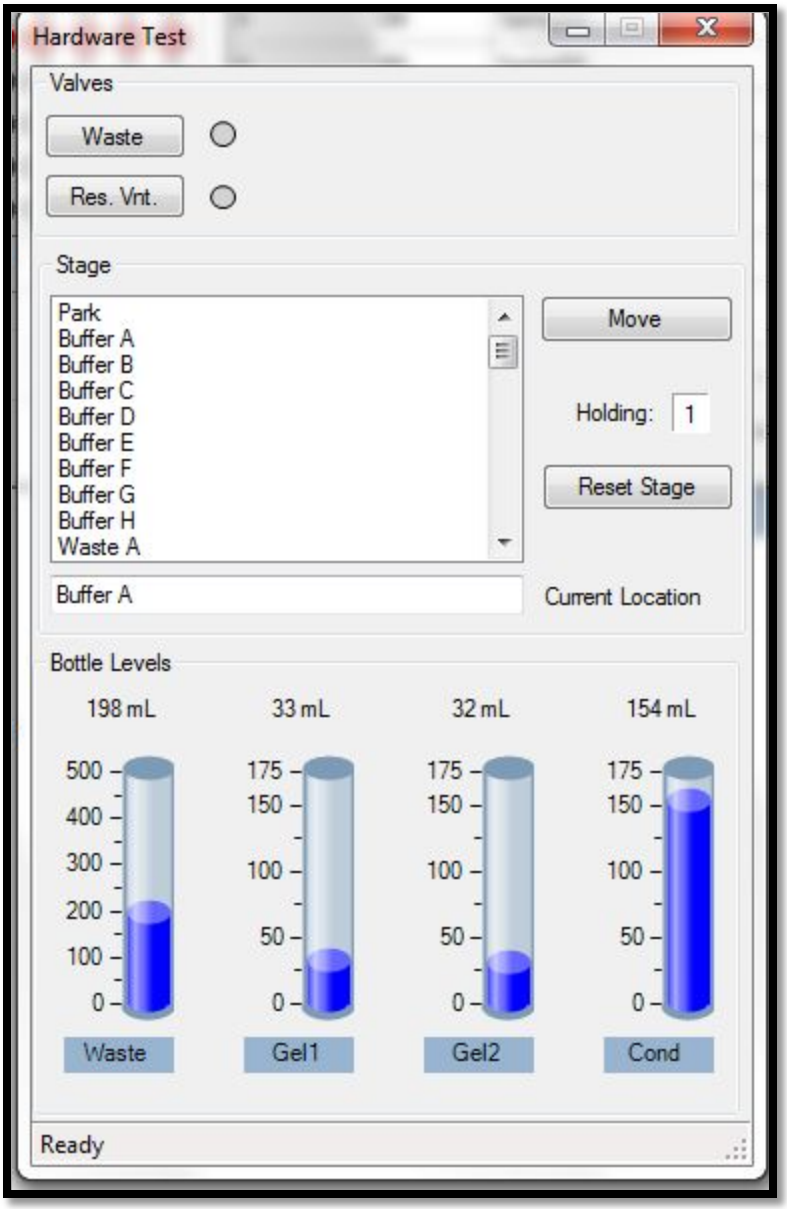


Figure 7-36 Hardware I/O:
Hardware Test Window

An overview of the commands accessed through the Hardware Test Window are listed below:

Function	Description
Valve—Waste	Activates (toggle) valve open (open circle) or closed (dark circle)
Valve--Res. Int	Activates (toggle) valve open (open circle) or closed (dark circle)
Stage	Move tray to the capillary array (A = Buffer tray, B = Waste Tray, C = Marker tray, D = Sample Tray 1, E = Sample Tray 2, F + Sample Tray 3
Bottle Levels	Information only.
Reset Stage	Available on Instrument Serial Numbers 2600 and above Discussed in Troubleshooting section

Table 7: -3 Functions of the Hardware Test Screen.

Utility—Prime

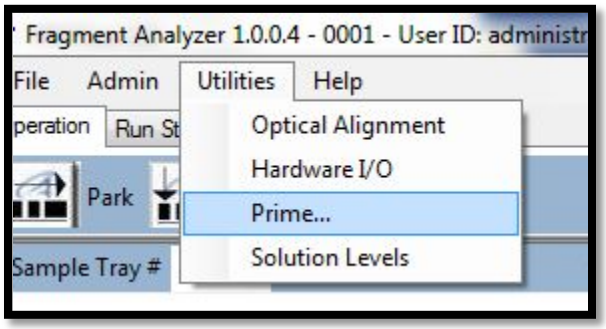


Figure 7-37

Selecting the [Prime...] function from the Utilities Menu opens the Prime Function Window. Fig. 7-38

The Prime Function Window enables the User to set parameters for flushing the syringe pump with either conditioning solution or gel.

- This function is used primarily for troubleshooting purposes.

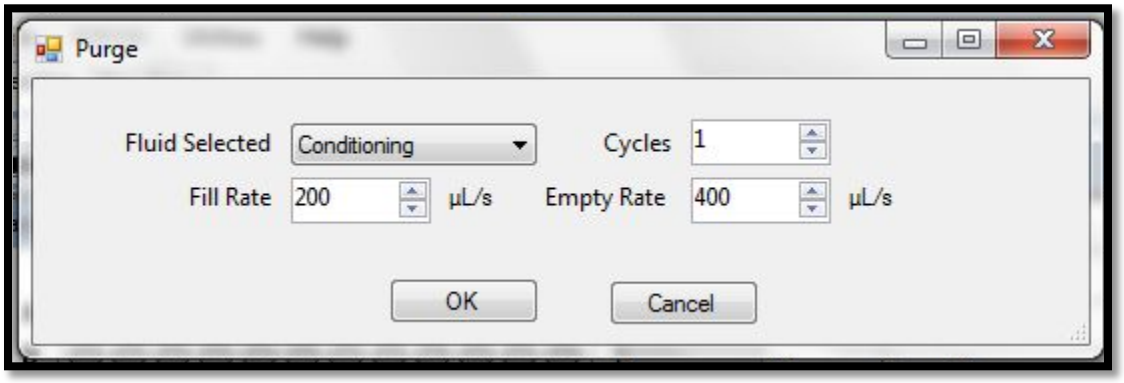


Figure 7-38. Utilities Menu:
Prime Function Window

An overview of the parameters accessed through the Prime Function Window are listed below:

Function	Description
Fluid Selected	Select Conditioning Solution or Gel
Cycles	Number of Cycles (1-100)
Fill Rate	Adjust Fill Rate using the Up and Down Arrows (10-300 uL/s)
Empty Rate	Adjust Rate using the Up and Down Arrows (10-400 uL/s)

Table 7-4: Purge Function Parameters

Utilities—Solution Levels

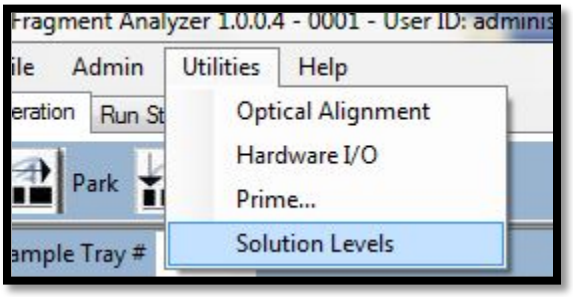


Figure 7-39. Utilities Menu:
Solution Levels

The *FRAGMENT ANALYZER*™ software tracks the solution levels as the instrument is used.

This ensures that the instrument has enough fluids for all of the planned runs.

- If the solution levels are low, the program will issue a warning.

Selecting the [Solution Levels] function from the Utilities Menu opens the Solution Levels Window. (Fig. **7-40**)

When solutions are re-filled, open this window and enter the correct solution levels (mL) for each container. Use the Up and Down Arrows 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.

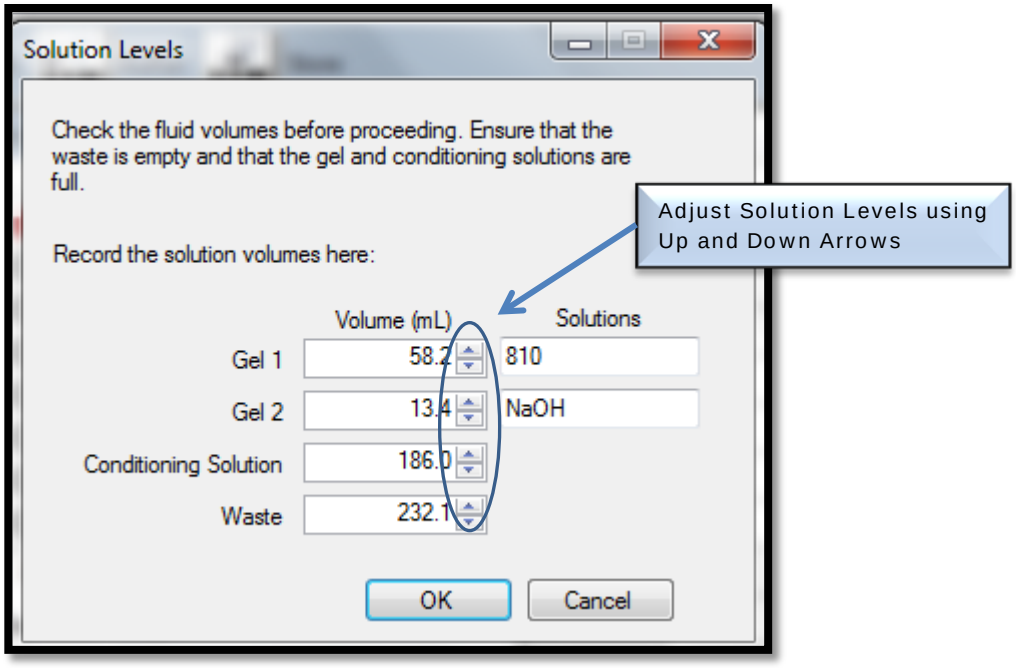


Figure 7-40. Solution Levels Window

96-Capillary Array Alignment Method 1 - Alignment from a file

An optical alignment can be performed from a file by selecting [Optical Alignment] through the Utilities Menu from the Main Screen Window.

The Optical Alignment Prep Window will open and prompt the user to fill the capillaries with dye. Select [No] when asked to fill the capillaries with dye. Fig. 7-41

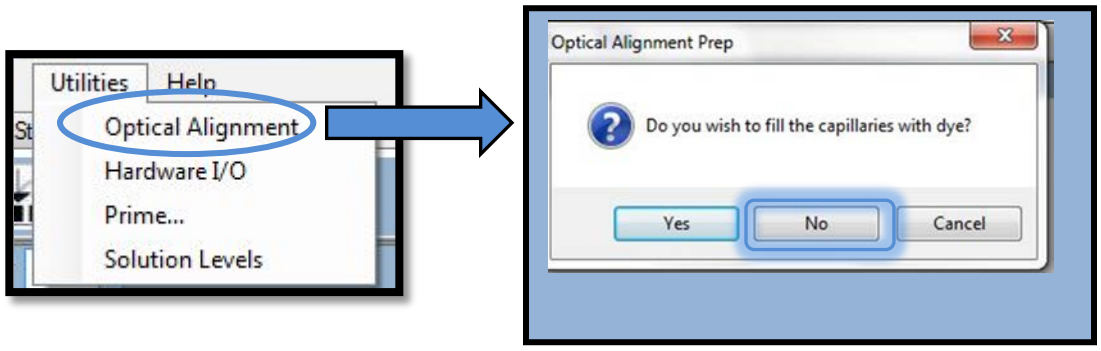


Figure 7-41: The Optical Alignment Prep Window

Select the [Read Raw] icon from the Optical Alignment Menu Bar located at the top of the Optical Alignment Window. Fig. **7-42**.

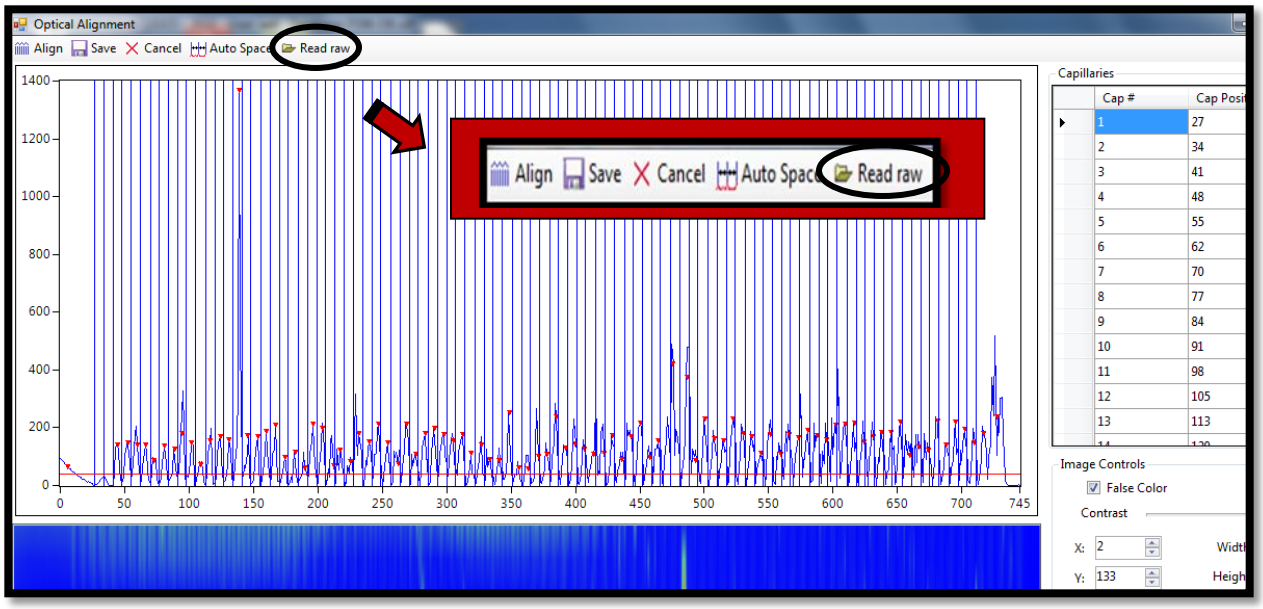


Figure 7-42: The Optical Alignment Menu Bar
Read Raw Command

Open a **Raw File** using the **Windows Prompts**. An example of the **File Extension** for a **Raw Data File** is displayed below in Fig. **7-43**. The **Raw File** will be **used** for the **Capillary Alignment**.

Figure 7-43: FilePath Example for a Raw Data File

After selecting and opening a Data File, the Align from File Window appears. (Fig.**7-44**)

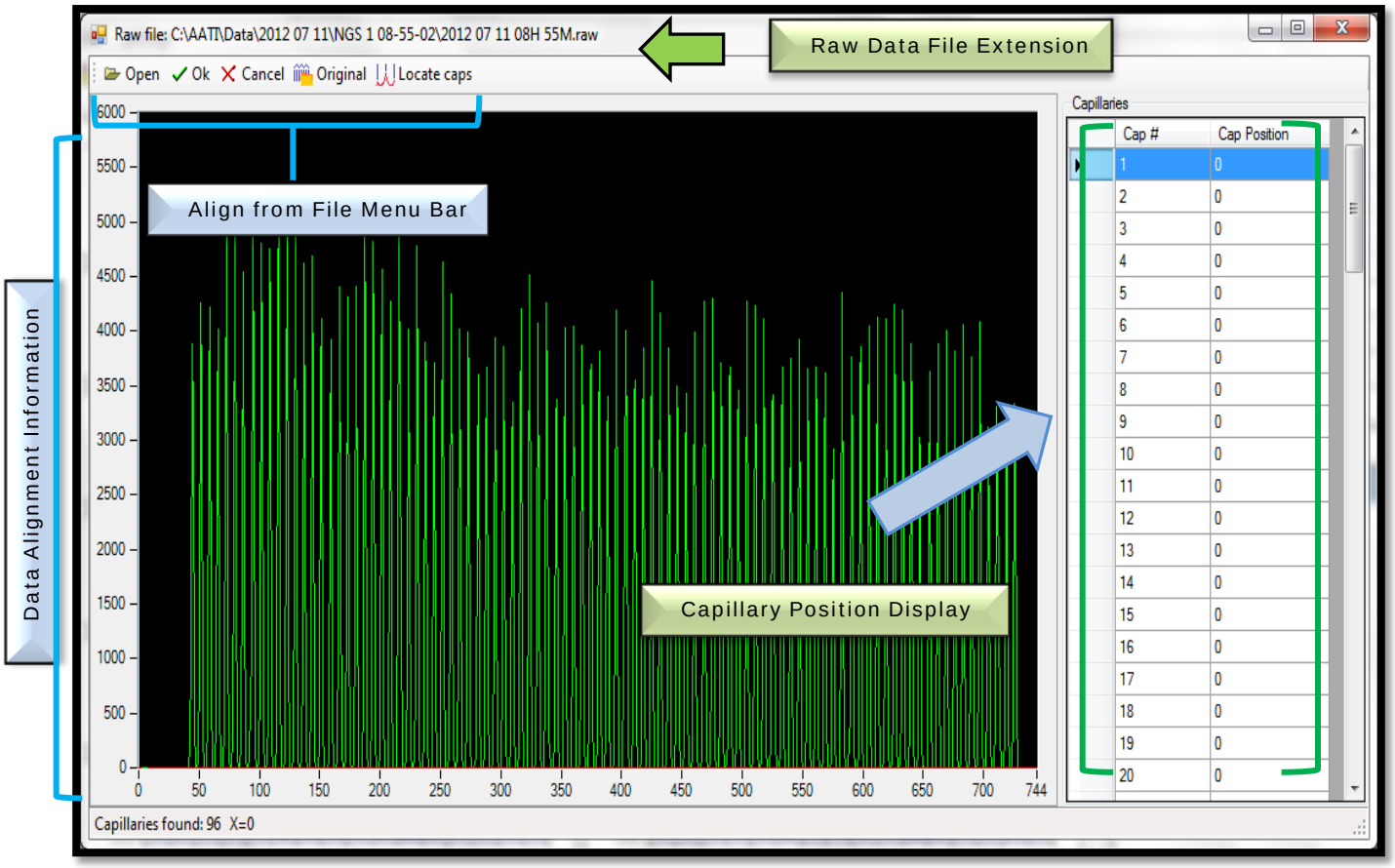


Figure 7-44: The Align from File Window
96-Capillary Array

Select the [Original] tab in the Align from File Menu Bar to show the Original Alignment. (Fig **7-45**). This is the original capillary alignment (from the instrument) that was applied to the data set. Choosing this function allows you to see how well the "original" alignment fits the data.

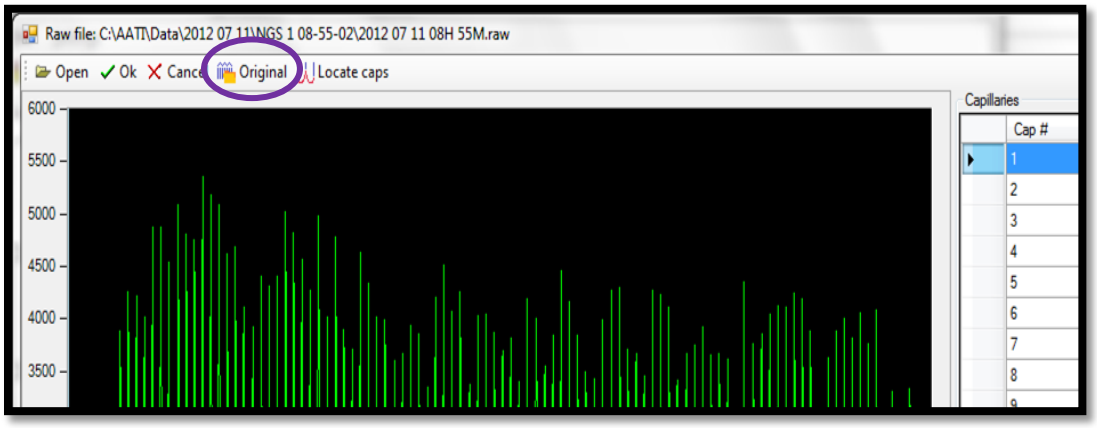


Figure 7-45. Align from File Window.
Original Tab

Selecting the [Original] tab inserts the Original Alignment Data that is depicted in blue into the Align from File Window along with the Alignment Data from the Data File depicted in green. (Fig **7-46**.)

In the example below, the blue vertical lines show the Original Alignment, which in this case perfectly overlap the green peaks imported from the Data File.

Select [OK] to accept the alignment (only if the alignment looks good—and the lines exactly intersect the maximum of each peak).

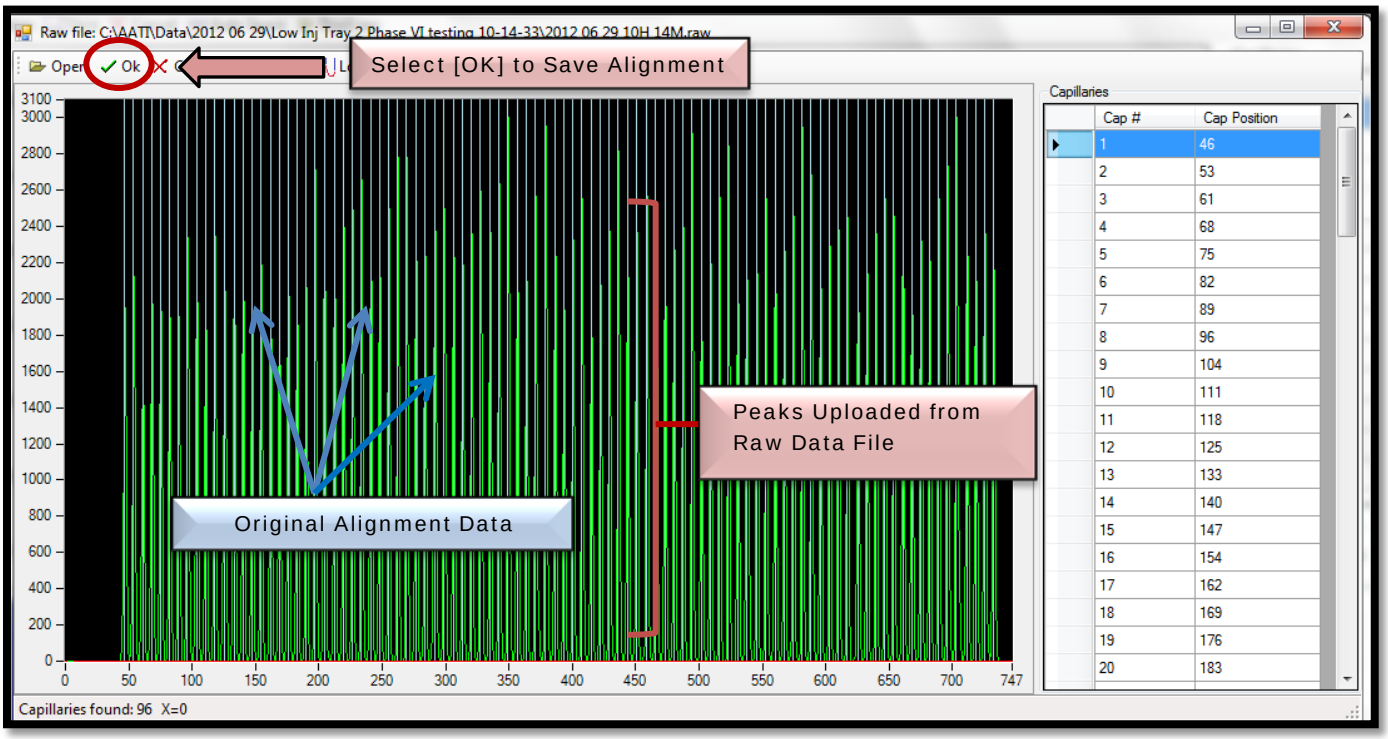


Figure 7-46. Align from File Window
Original Alignment Overlaps the Peaks from Data File

After selecting [OK] and the file is closed, select [Save] in the Optical Alignment Window. Fig. **7-47**.

⚠ If the alignment is not saved from the Optical Alignment Window, it will not be saved to the instrument—and subsequent data files will not be aligned correctly.

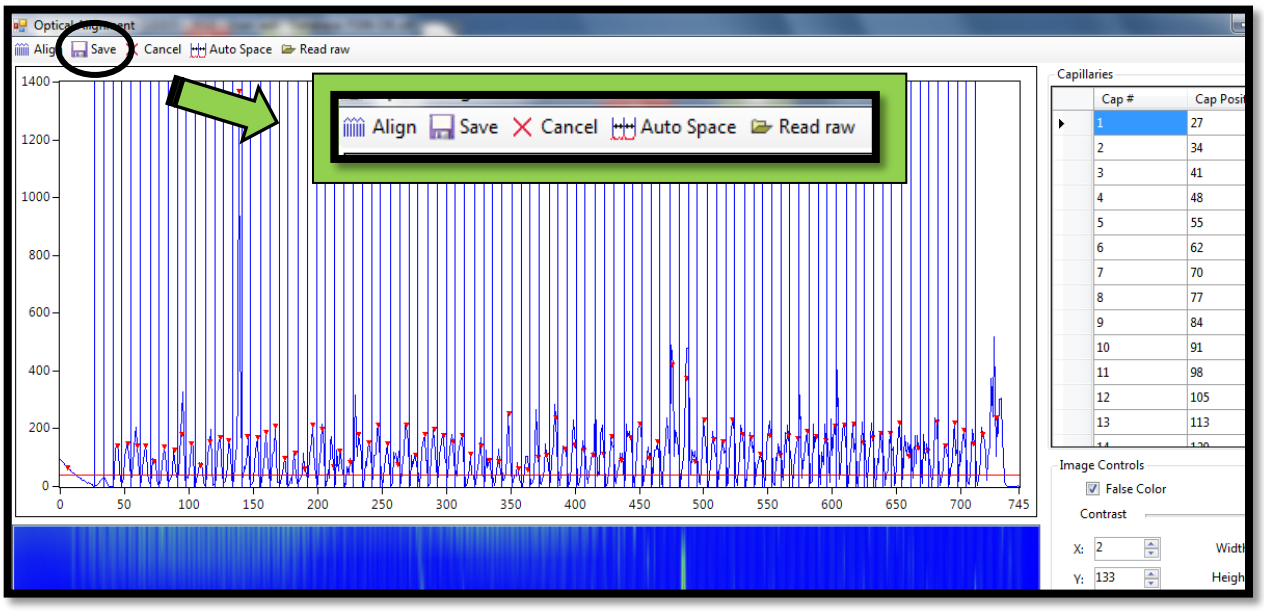


Figure 7-47: The Optical Alignment Menu Bar
Save Alignment after Closing File

If the **vertical lines** of the **Original Alignment** and the **peaks** from the **Raw Data File** do not line up, adjust the **blue vertical lines** to fall exactly in the **center** of **each peak**.

Adjust the **Capillary Alignment** by selecting **[Locate caps]** from the **Align from File Window**. To correctly align the file, first move the red integration line (located at the very bottom of the plot) with the mouse as shown below in Fig. **7-48**.

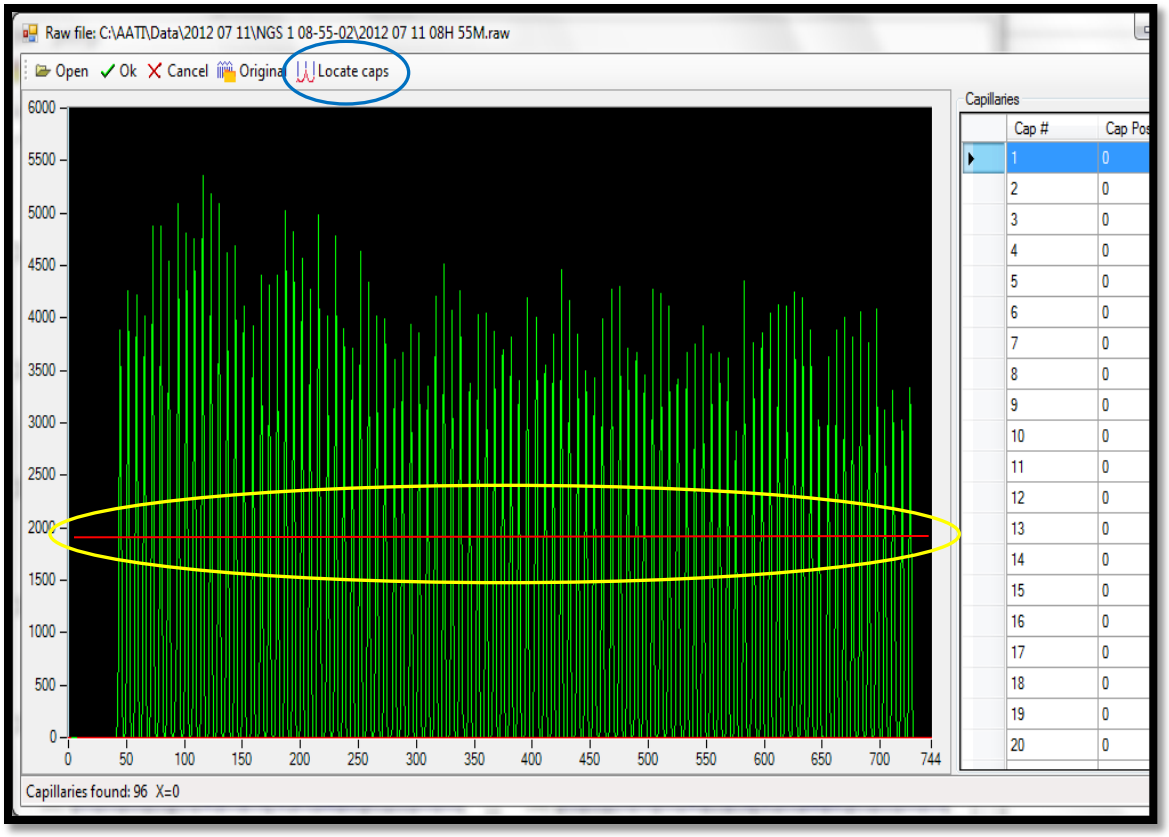


Figure 7-48. Align from File Window.
Move the red line from the very bottom of the plot to a location where the capillaries can be appropriately identified BEFORE selecting "Locate Caps".

The **blue vertical lines** in the **Align from File Window** are usually correctly located when the **Locate Caps** option is selected. However, if necessary, the lines can also be **manually moved** with the mouse. (Fig. **7-49**) (Note that if the red line is moved appropriately to capture all peaks, the blue alignment lines are always correctly selected with the **Locate Caps** option is selected).

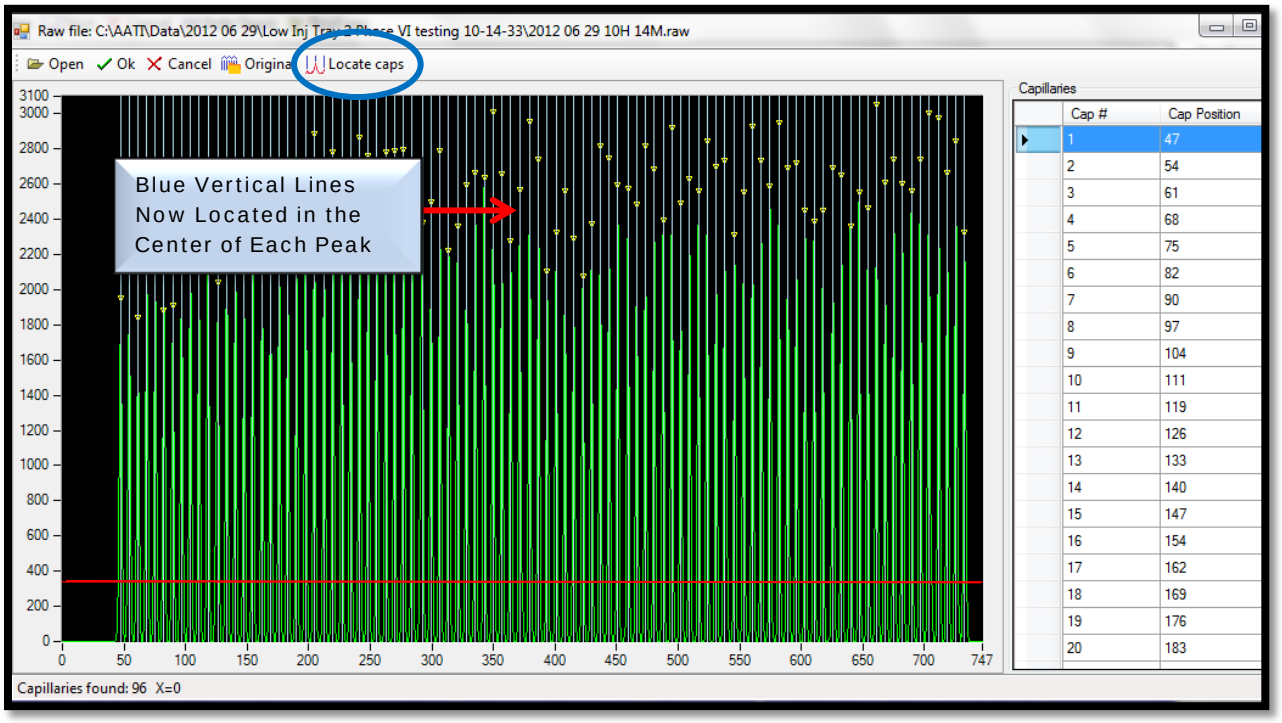


Figure 7-49. Align from File Window with the blue vertical alignment lines shown after the "Locate Caps" option is selected.

To Insert or Delete capillary positions:

- Right click between rows of interest in the table in the Capillary Position Display located to the right of the Align from File Window and select [Insert/Delete Capillary]. (Fig. **7-50**.)

OR

- Right click on the area in the black screen of the Align from File Window and select [Insert/Delete Capillary]. (Fig **7-51**.)

Insert Capillary Position from table in the Capillary Position Display:

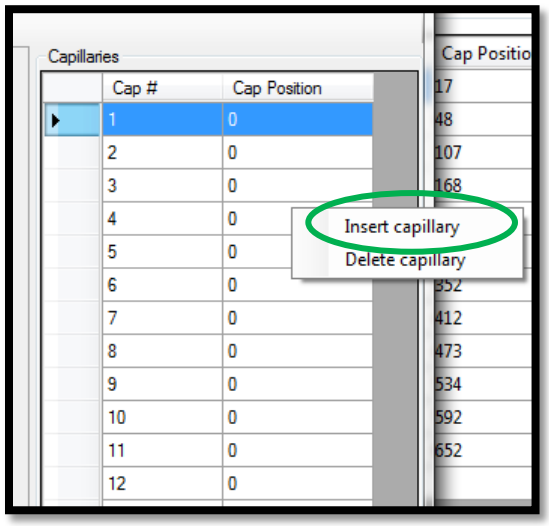


Figure 7-50. Capillary Position Display Table:
Right-Click and Select [Insert Capillary]

Insert Capillary Position in the Align from File Window:

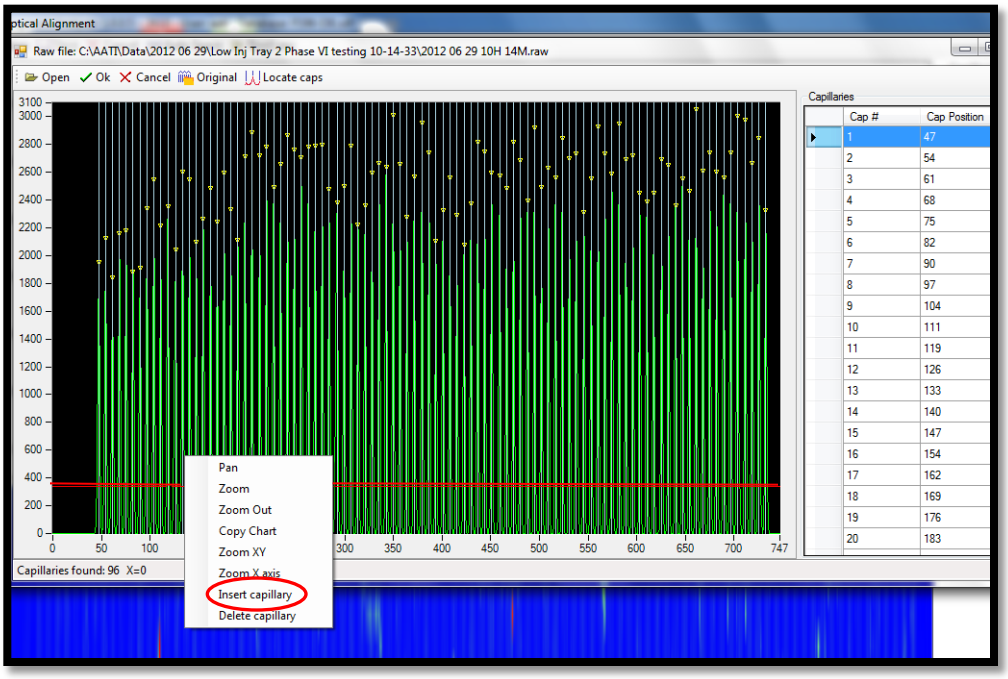


Figure 7-51. Capillary Position Display Window:
Right-Click and Select [Insert Capillary]

After a capillary has been inserted, and additional vertical line is observed in the capillary display window (Fig. **7-52**). Note that in this case it is not necessary to insert a capillary, but in the rare case in which a capillary is not recognized by the program, the user has an option to manually add a capillary.

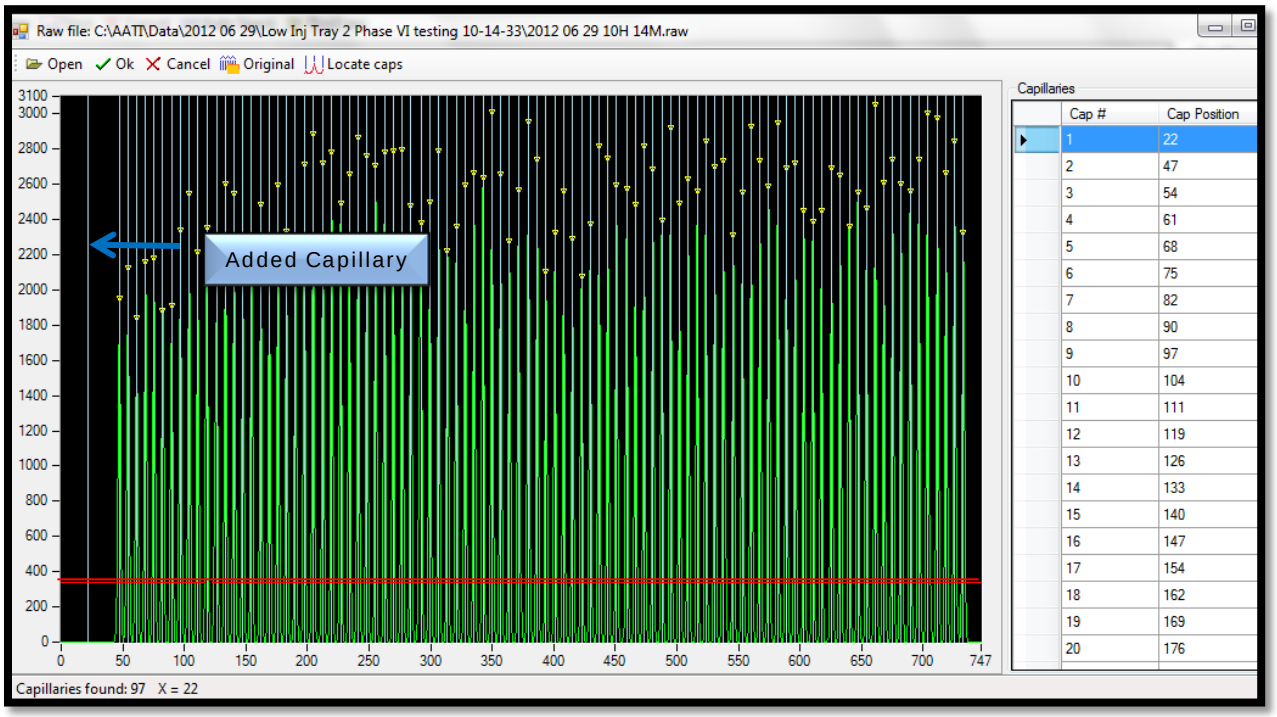


Figure 7-52. Align from File Window.
Added Capillary is observed as a vertical line.

Any blue vertical alignment position can be removed by right-clicking and selecting [Delete Capillary].

Once the capillary positions look good (perfect overlap of blue vertical lines in the center of each green peak), hit [OK] to accept alignment. (Fig. **7-53**.)

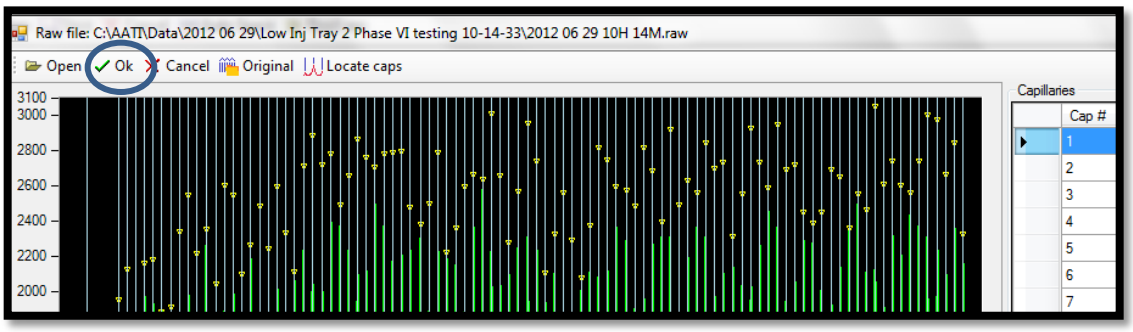


Figure 7-53. Align from File Window.
Select [OK]

After selecting [OK] and the file is closed, select [Save] in the Optical Alignment Window. (Fig. **7-54**.)

⚠ If the alignment is not saved, it will not be saved to the instrument.

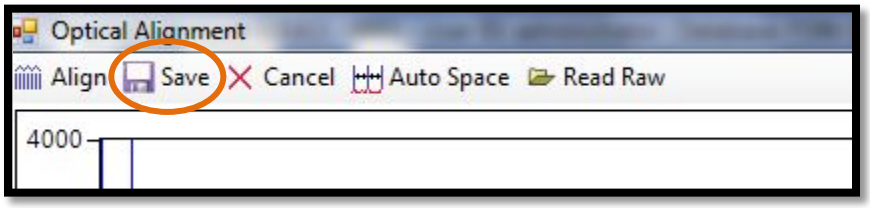


Figure 7-54: The Optical Alignment Menu Bar
Save Alignment after Closing File

96-Capillary Array Alignment Method 2: Alignment with Fluorescein Dye

Prior to initiating an **Optical Alignment** with **fluorescein dye** within the *FRAGMENT ANALYZER™* system software, **prepare the fluorescein dye** as described below:

- Prepare a **20 mL volume** of **fluorescein dye** by diluting 20 µL of Fluorescein Sodium Salt CAPS solution (1 milli-Molar o1E-3M, Fluka Number 67834) in 20 mL of de-ionized water.

OR

- Prepare equivalent fluorescein sodium salt dye solution to this concentration (1 micro-Molar 1E-6M) diluting into a volume of 20 ml for 12 cap or 40 ml for 96 cap with de-ionized water.

Once the dye solution is prepared, begin the optical alignment process by selecting **[Optical Alignment]**. This opens the **Optical Alignment Prep Window**. Fig. **7-55**.

From the **Optical Alignment Prep Window**, select **[Yes]** to fill the capillaries with dye.

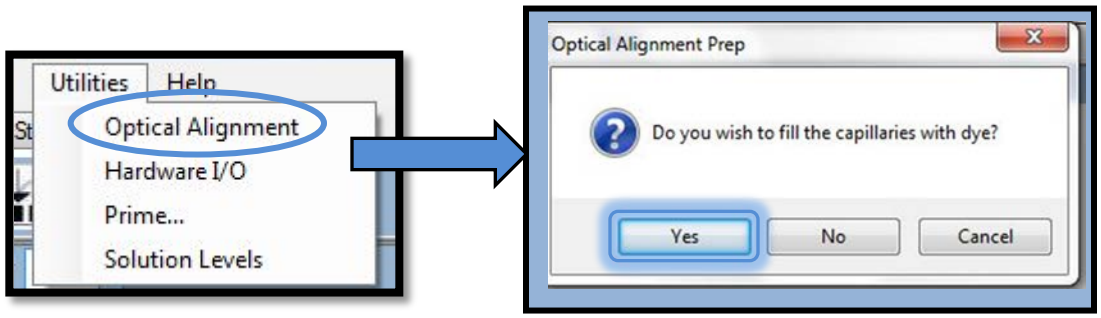


Figure 7-55: The Optical Alignment Prep Window

The software will prompt the user to replace the capillary conditioning bottle in the Instrument Reagent Compartment with a bottle containing 20 mL of the 1 micro-Molar or 1E-6M fluorescein dye solution. (Fig. **7-56**.)

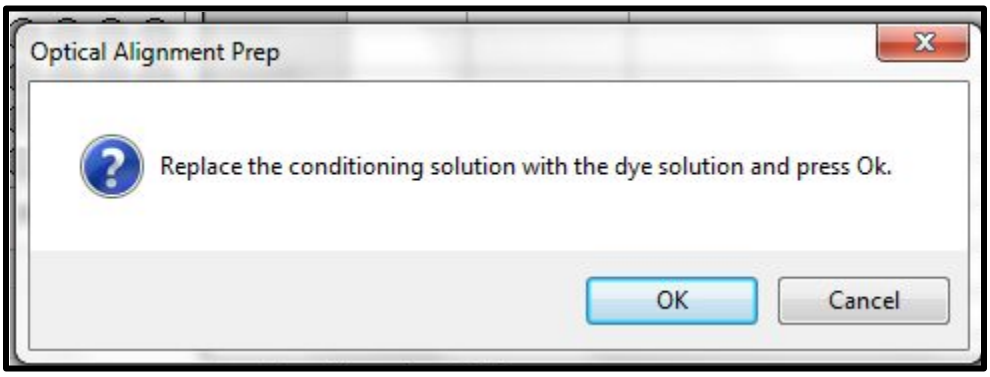


Figure 7-56. Optical Alignment Prep Window
Fluorescein Dye Prompt

After the capillary conditioning bottle is replaced with the 1 micro-Molar or 1E-6M fluorescein dye solution, select [OK].

The instrument will now empty the reservoir and re-fill both the reservoir and capillaries with the 1 micro-Molar or 1E-6M fluorescein solution.

- The re-filling process takes 3 to 5 minutes.

While the instrument is filling the reservoir and capillaries with the fluorescein dye solution, the Optical Alignment Window is displayed. Fig **7-57**.

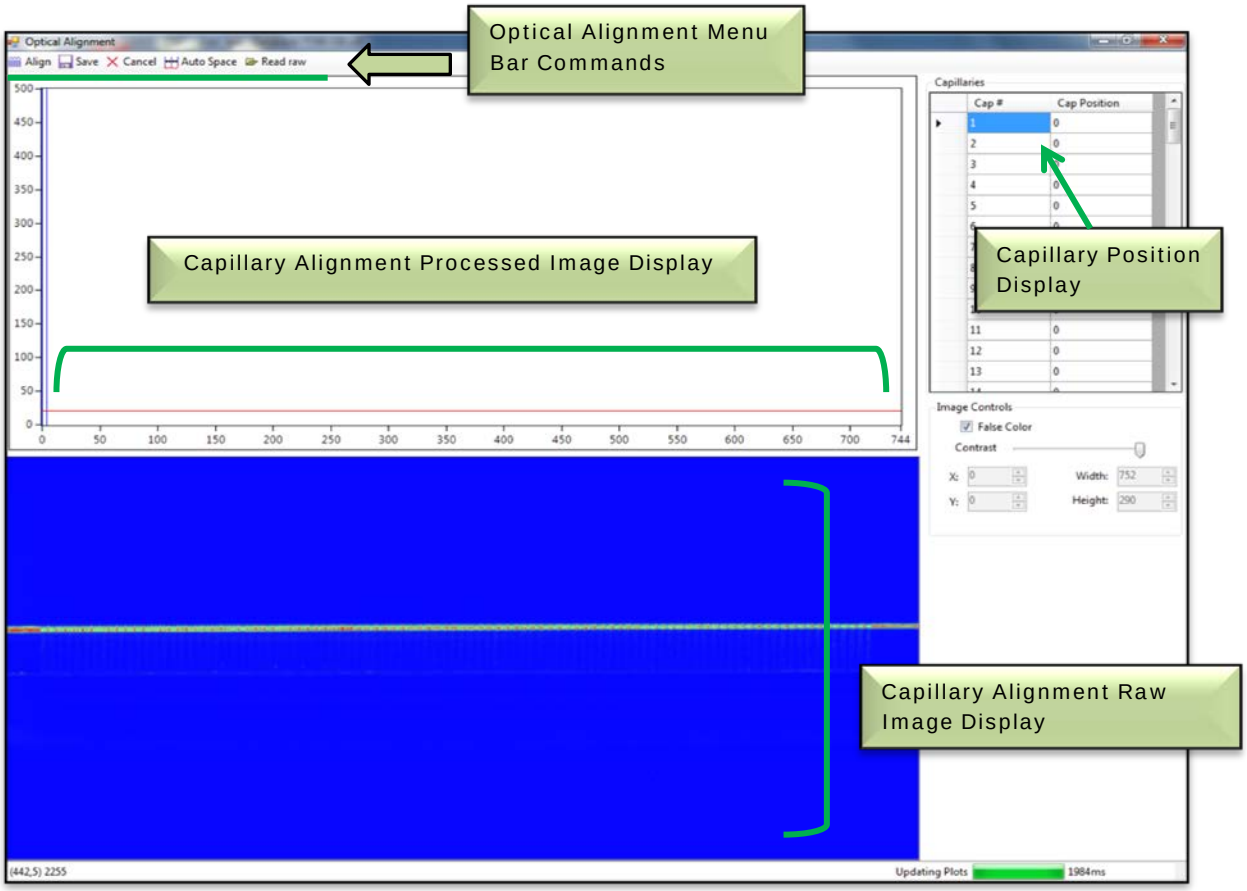


Figure 7-57. Optical Alignment Window

As the capillaries fill with fluorescein dye solution, they will change from transparent to visible in the Capillary Alignment Display, as shown in the time progression below. (Fig.**7-58**.)

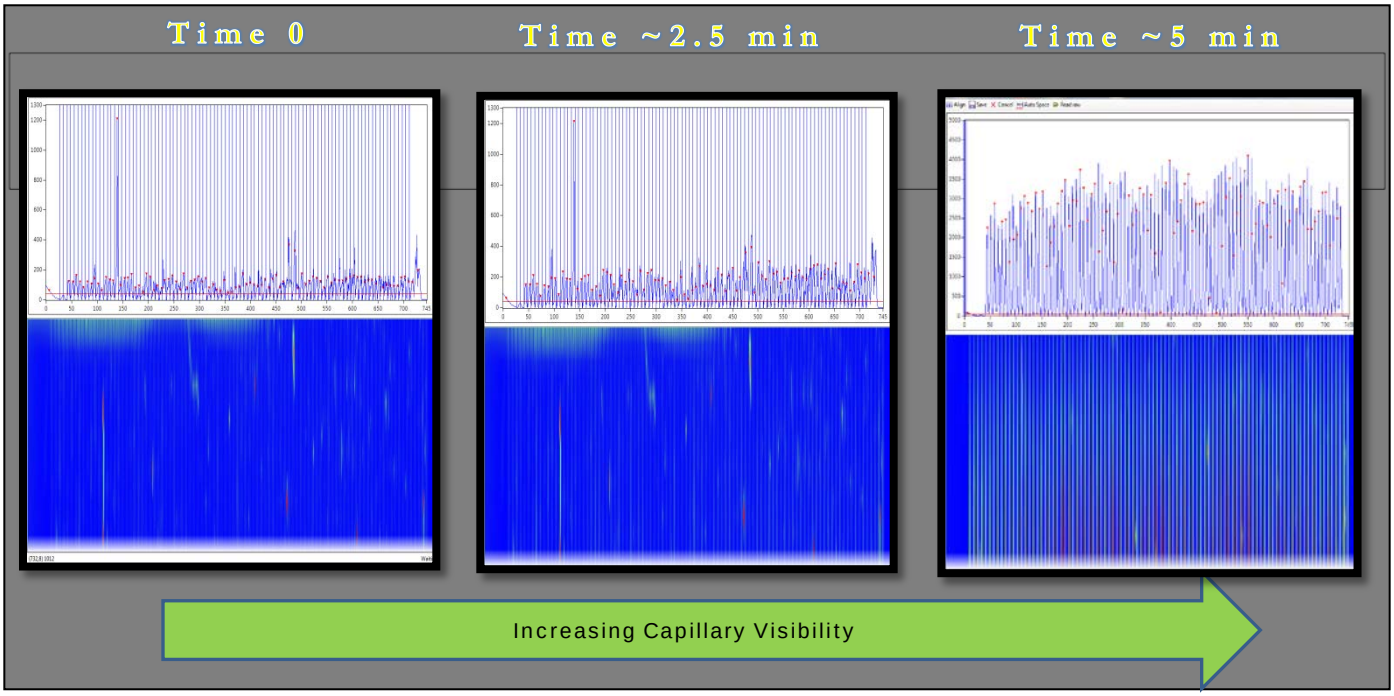


Figure 7-58. Optical Alignment Window Screen Shots
Time Progression - Filling Capillaries with Dye

Once all the capillaries become visible, right-click in the blue area of the Capillary Alignment Display where the capillaries are visible and select [Reset All]. (Fig. **7-59**)

Figure 7-59. Capillary Alignment Display.
Right Click then Select [Reset All]

After choosing [Reset All], the Capillary Alignment Display shows the entire area of the CCD Camera Window. (Fig **7-60**.)

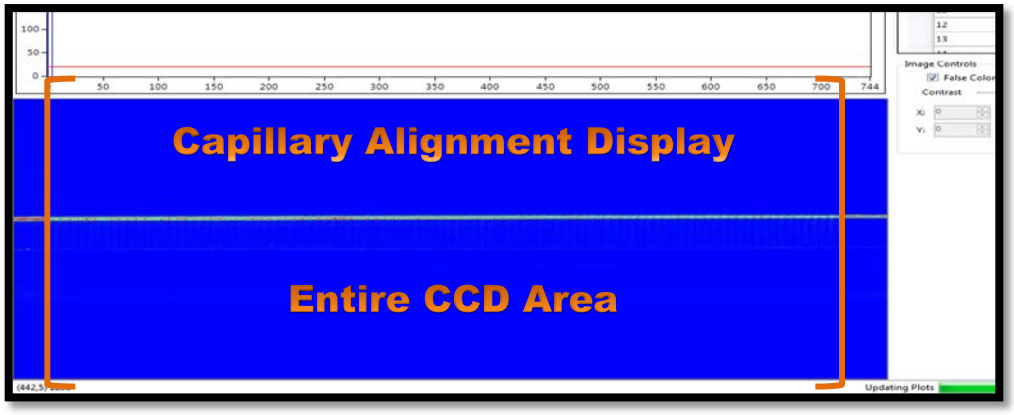


Figure 7-60. Capillary Alignment Display:
CCD Window Area

- Only a small fraction of the CCD array area is actually selected and shown by the Capillary Array Camera Window.

The Capillary Array Camera Window is located below the upper edge of the CCD Camera Reference Line and above the poly amide covered Capillary Reference Points. (Fig. **7-61)**

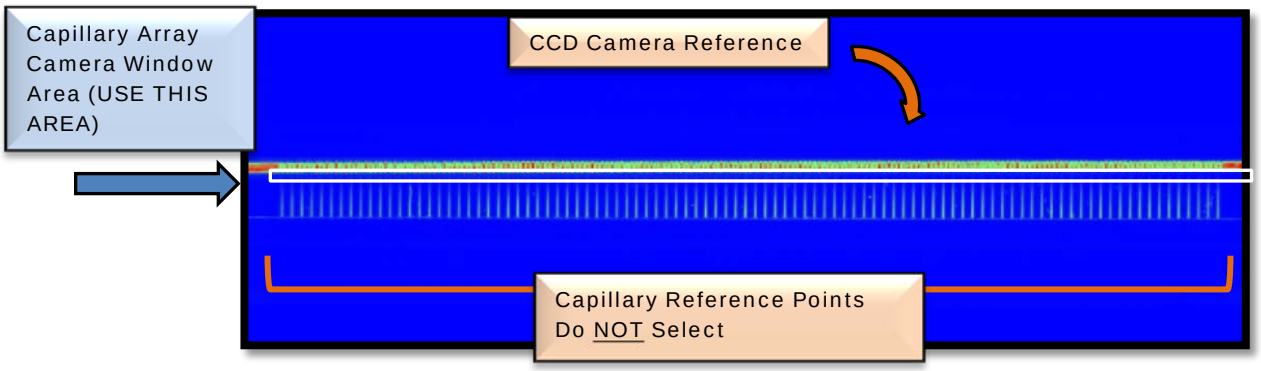


Figure 7-61. Location of Capillary Array Window

To set the Capillary Array Camera Window, click and drag a rectangular area that corresponds to the Capillary Array Camera Window Area.

Right-click and select [Set Camera Window] as shown in Fig. **7-62**.

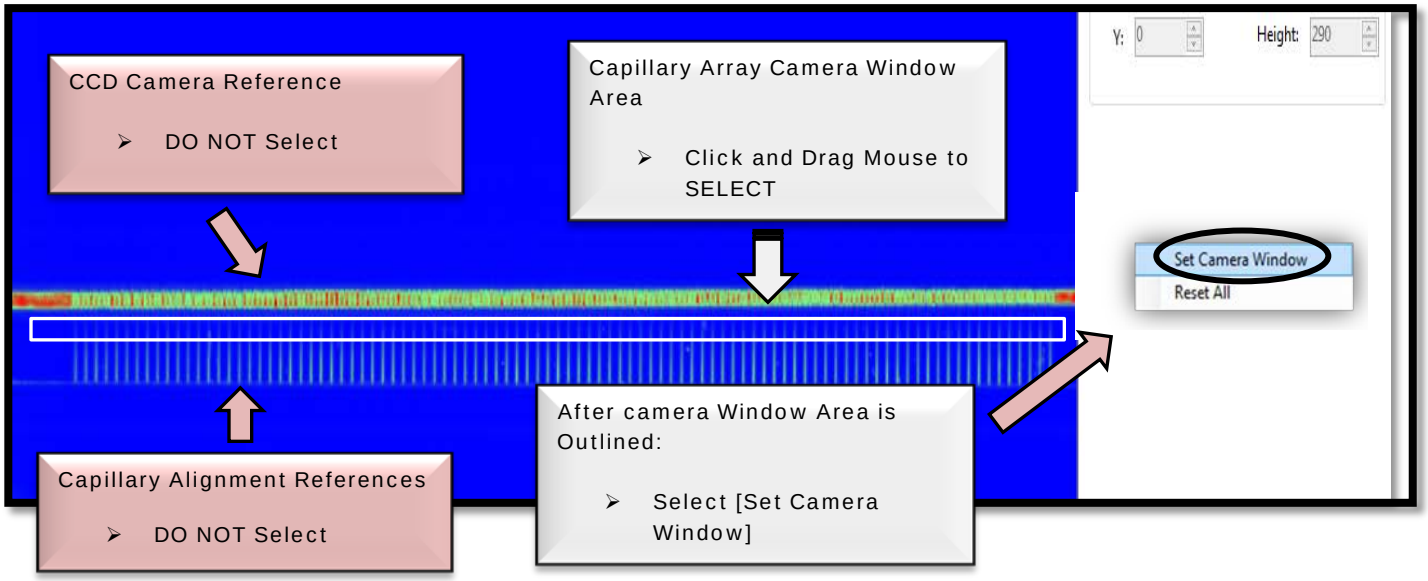


Figure 7-62. Capillary Alignment Display:
Setting Camera Window

After Setting the Camera Window, select [Align] from the Optical Alignment Menu Bar located above the Capillary Alignment Processed Image Display. Note: Move the red integration line at the bottom of the window, so that only the capillaries are selected.

This is required to align all of the capillaries with the Camera Window.

Move the horizontal red line up from the very bottom of the y-axis. Figures 7-63 and 7-64

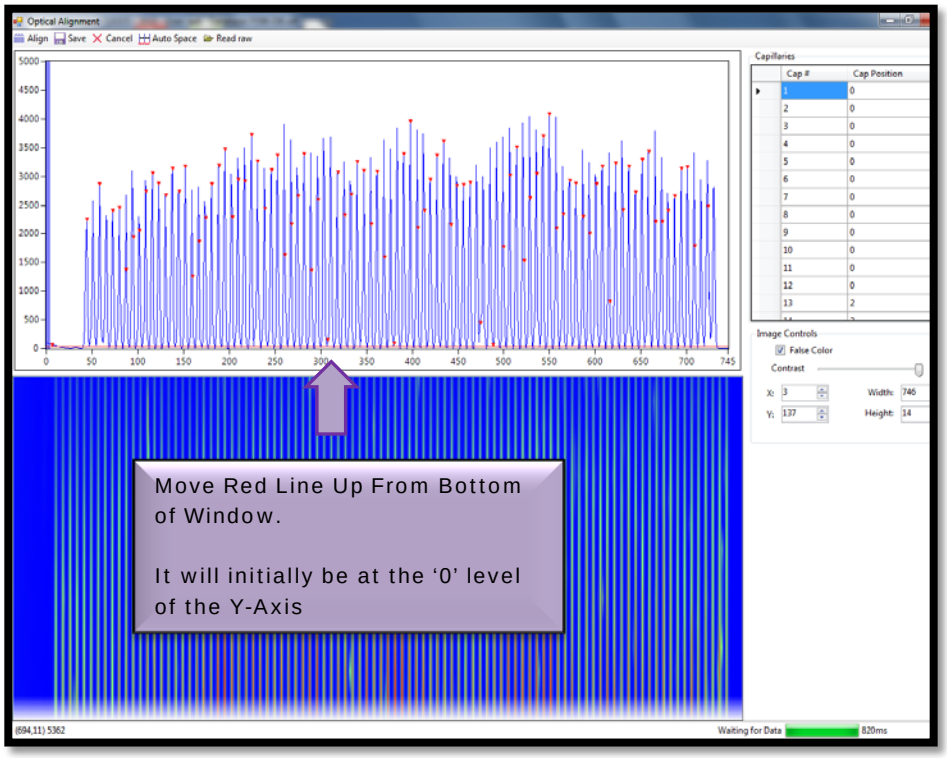


Figure 7-63. Capillary Alignment Processed Image Display: Red Line Resting At Bottom of Window

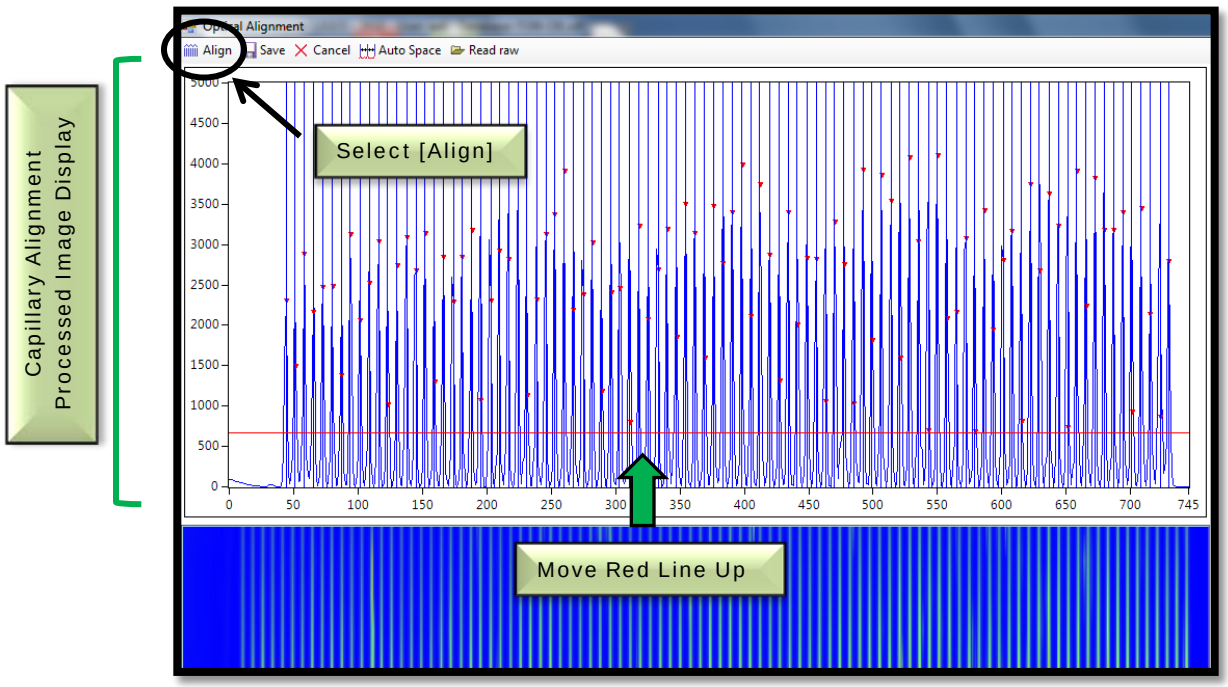


Figure 7-64: Move red Line Up to Select Peaks Of Interest

After [Align] is selected, the red line can be moved up and down until a red dot is observed above each capillary peak.

Hit [Align] every time the red line is moved. This ensures that the instrument has selected the peak for integration.

If the blue vertical lines are not in the exact center of each peak, each line can be adjusted using the mouse.

⚠ In the Capillary Position Display, adjust the [Height] field for the Camera Window so that it is **≥ 14**.

Select [Save] at the top of the screen in the Optical Alignment Menu Bar once all the capillary peaks have a single red dot and the blue vertical lines are in the exact center of each peak. (Fig.**7-65**.)

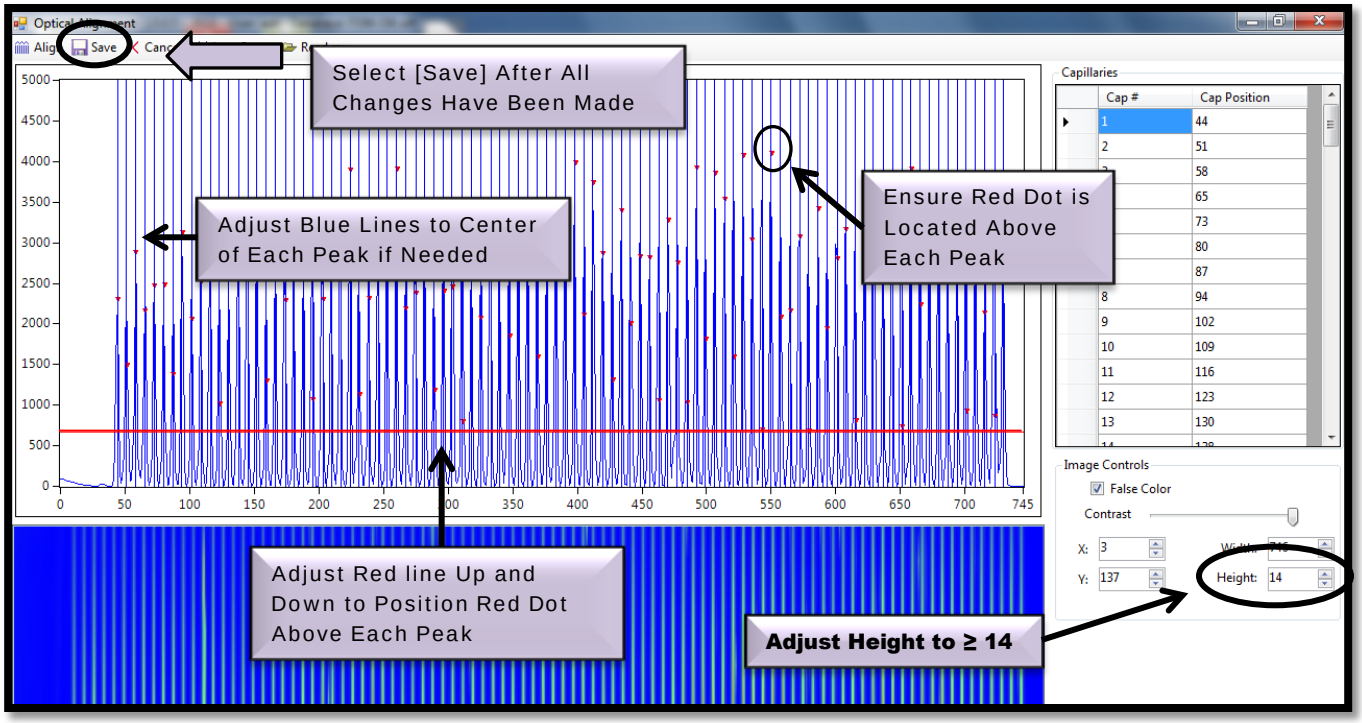


Figure 7-65. Capillary Alignment Processed Image Display: Selecting Peaks of Interest

Removing, Installing and Storing Capillary Arrays

In This Chapter

This chapter provides step-by-step instructions on the removal and installation of capillary array cartridges for the *FRAGMENT ANALYZER*™ system. It will also cover unpacking a new Capillary Array Cartridge and Long-Term Storage of a Capillary Array.

- After installation of a new capillary array, the user will need to perform an optical alignment prior to performing any experiments. The full optical alignment procedures are presented in Chapter 7.

Fragment Analyzer™ Capillary Array Overview

The instrument **Capillary Array** allows for **direct parallel injection** and **separation** of **12 or 96 samples at once.** Figure 8-1 below shows a 12-Capillary Array.

The **Capillary Array Cartridge** is located in the **Upper Compartment** or **Hood** of the *FRAGMENT ANALYZER™* instrument.

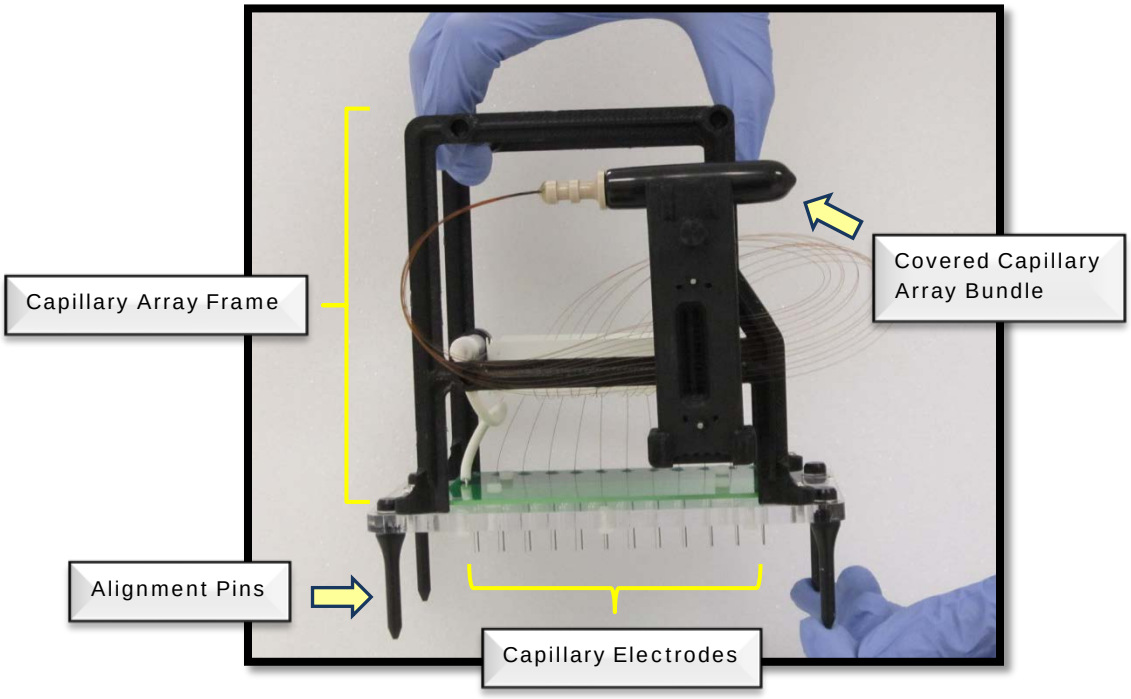


Figure 8-1. Example of *FRAGMENT ANALYZER™* 12-Capillary Array Cartridge

The methods discussed in this chapter will be illustrated with images and terminology for a 12-Capillary Array. All of the method steps outlined are the same for a 96–Capillary Array unless otherwise noted.

Capillary Array Cartridges are removed in order to:

- Accommodate evolving sample throughput needs
- To switch between instrument applications
- To allow for troubleshooting activities
- To replace an array that has reached the end of its life expectancy

The Table 8-1 lists the available *FRAGMENT ANALYZER*™ instrument Capillary Array Cartidges.

Capillary Array	Specifications
12-Capillary Array Cartridge - Short	**50 um (ID) 55 cm (EFF), 80 cm (TOT), 2mL (RES)**
12-Capillary Array Cartridge - Long	**50 um (ID) 33 cm (EFF), 55 cm (TOT), 2mL (RES)**
96-Capillary Array Cartridge - Long	**50 um (ID) 55 cm (EFF), 80 cm (TOT), 10mL (RES)**
96-Capillary Array Cartridge - Short	**50 um (ID) 33 cm (EFF), 55 cm (TOT), 10mL (RES)**
Wet Station - Capillary Array Cartridge Storage Tray Kit	

Table 8-1. Capillary Array Cartridges and Storage Station

Preparing the Capillary Array for Removal

There are two methods for preparing a Capillary Array Cartridge for removal within the *FRAGMENT ANALYZER*™ software:

1. Fill the Capillary Array with Gel Prior to Removal

2. Fill the Capillary Array with DI Water Prior to Removal

The first method is performed when a Capillary Array Cartridge is removed for storage.

Removing and storing an array is recommended when the instrument will not be used for a period of time or whenever the array is switched for another array in order to accommodate a different application/sample type on the *FRAGMENT ANALYZER*™ instrument.

- Filling the capillary array with gel prevents the capillaries from drying out and clogging.

The second method is performed when a capillary array cartridge is removed for disposal or shipment.

Flushing the capillary array with DI Water is recommended when the array has reached the end of its usable life or whenever an array is shipped. This action removes gel and dye from the array allowing for safe disposal and transport.

- Filling the capillary array with DI Water allows for safe disposal of the cartridge and safe shipping.

Preparation for Removal Method 1 – Filling Capillary Array with Gel 1

To fill the Capillary Array Cartridge with Gel prior to removal from the instrument, navigate to the Capillary Conditioning Commands under the Operations Tab located on the Main Screen Window. Fig. 8-2

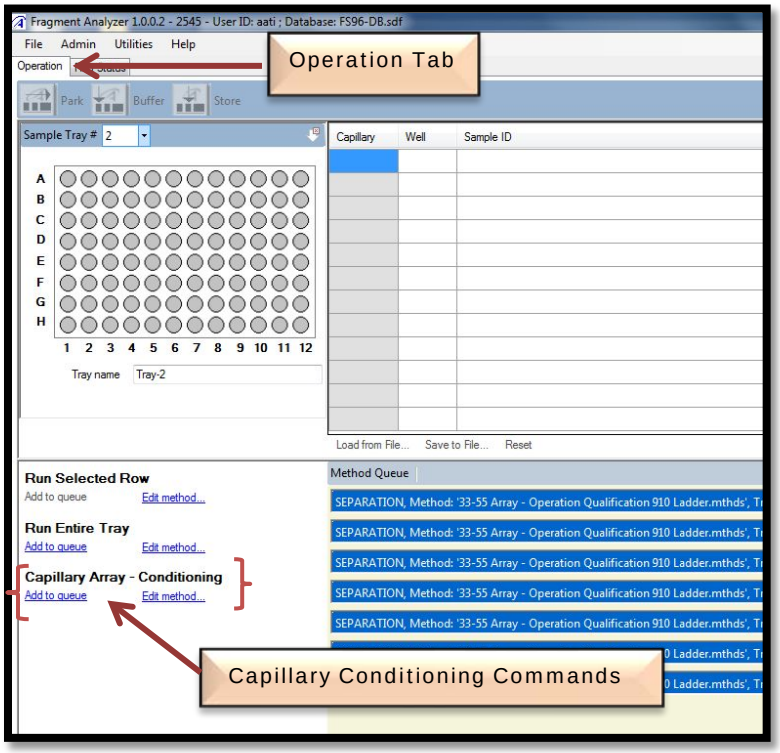


Figure 8-2. Main Screen Window.
Capillary Array – Conditioning Commands

Select [Add to Queue] under the Capillary Array-Conditioning Commands to open the Conditioning Method Select Window. (Fig. 8-3)

In the Conditioning Method Select Window, use the drop-down menu to select the [Default Conditioning Method].

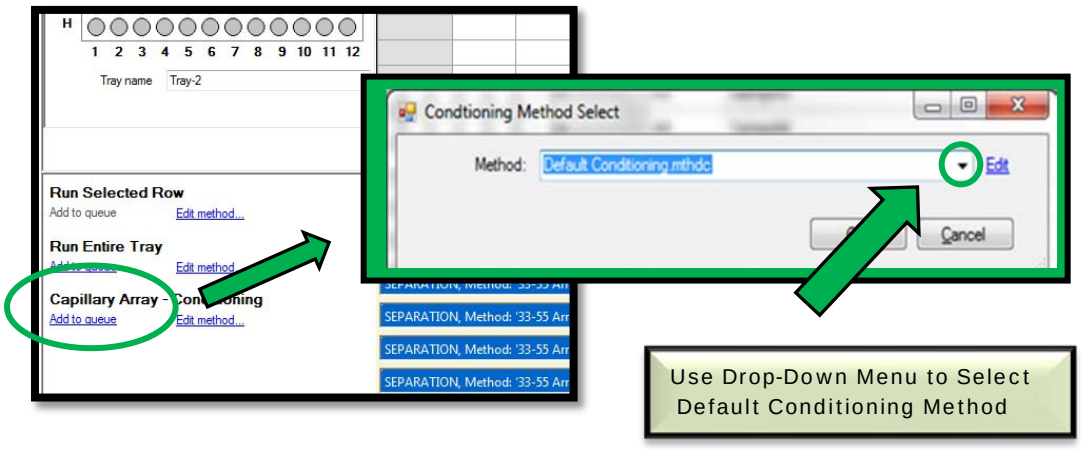


Figure 8-3. Capillary Array-Conditioning. Conditioning Method Select Window

Select [Edit] to the right of the Method Field in the Conditioning Method Select Window to modify the parameters of the Default Capillary Conditioning Method. Fig 8-4

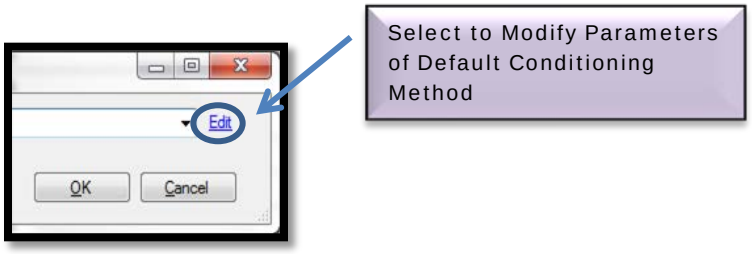


Figure 8-4. Conditioning Method Select Window: Edit Command

This will open the **Conditioning Method Edit Window**. Fig. 8-5

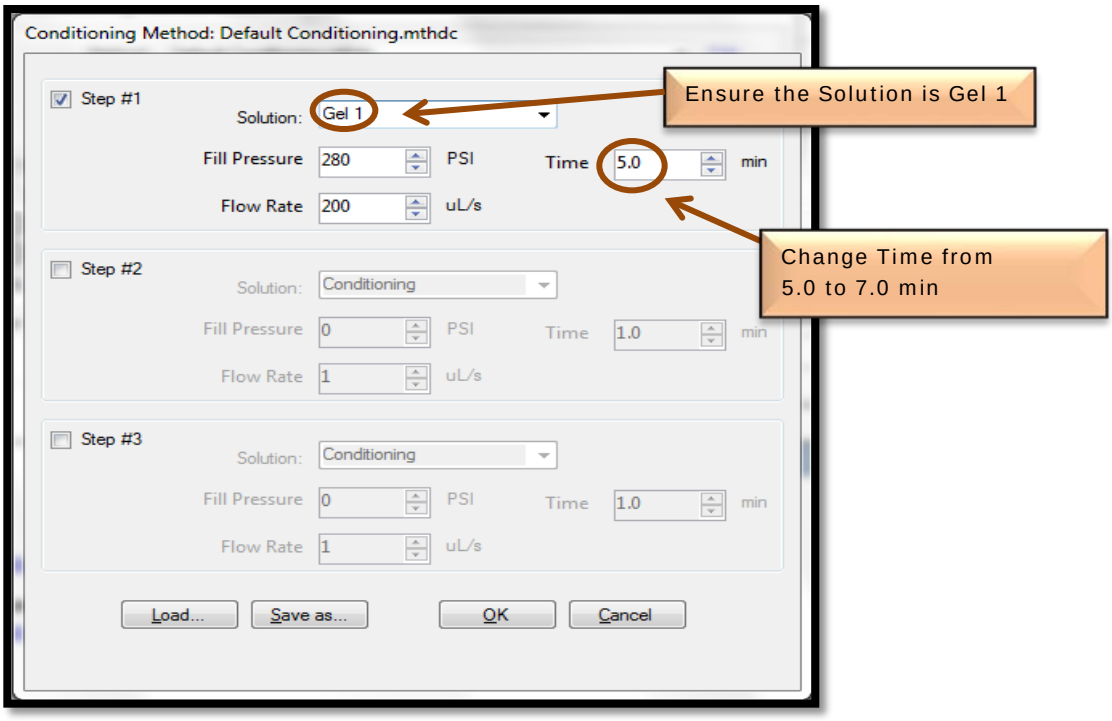


Figure 8-5: Conditioning Method Edit Window.
Edit Default Conditioning Method

For an **overview** of the **parameters** accessed through the **Conditioning Method Edit Window**, refer to Chapter 2.

Modify the default capillary conditioning parameters to the values listed in Table 8-2 below.

Fill Pressure (psi)	Time (minutes)	Solution	Flow Rate (ml/min)
280 psi	7 minutes	Gel 1	200 µL/s

Table 8-2: Modified Default Capillary Conditioning Method Parameters

To accept the changes made to the default capillary conditioning method and place the method into the Method Queue, press [OK]. This also closes the Conditioning Method Edit Window and returns to the Main Screen Window.

Running the Capillary Conditioning Method

Prior to filling the capillary array with Gel 1:

- Check the Waste Tray in Drawer W (2nd drawer) to ensure that it is empty.
- Check the Gel 1 solution level to ensure that there is sufficient volume for the operation (located in the instrument Side Compartment).

Refer to Chapter 7 for detailed instructions on how to Verify and Adjust Solution Levels in the instrument Side Compartment.

The minimum solution volume required to run the Modified Default Capillary **Conditioning Method is ≥** 7 ml.

Once the **method** has been **added** to the **Method Queue**, **start** filling the capillary array with Gel 1 by left mouse clicking the **[Start Arrow]** on the **Method Queue Status Bar** as shown in Fig. 8-6.

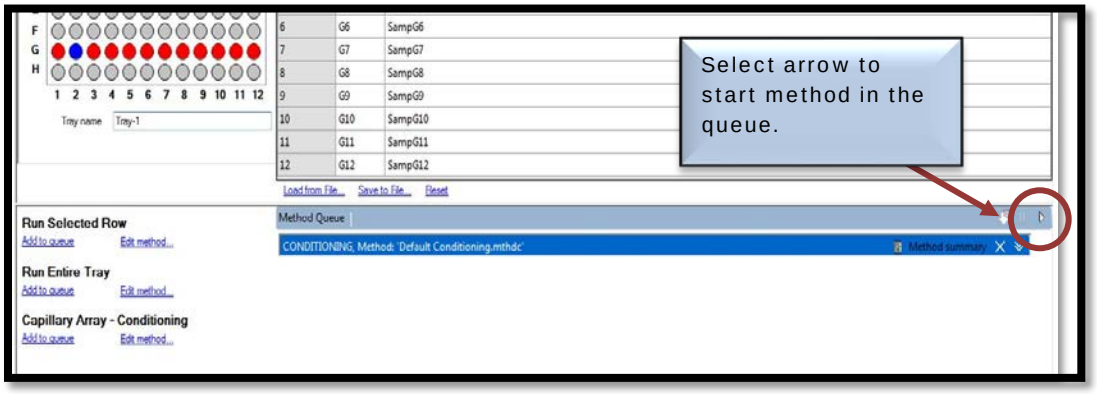


Figure 8-6: Main Screen Window - Method Queue.
Start Modified Method

Preparation for Removal Method 2 – Filling Capillary Array with DI Water

To fill the Capillary Array Cartridge with DI Water prior to removal from the instrument follow the instructions outlined for Method 1 with the following exceptions:

From the Conditioning Method Edit Window, instead of filling the Capillary Array with Gel 1, select [Conditioning Solution] in the Solution Field of the Conditioning Method Edit Window. Fig. 8-7

⚠ The Conditioning Solution Bottle in the *FRAGMENT ANALYZER*™ side compartment will be replaced with a bottle containing DI Water in the next section.

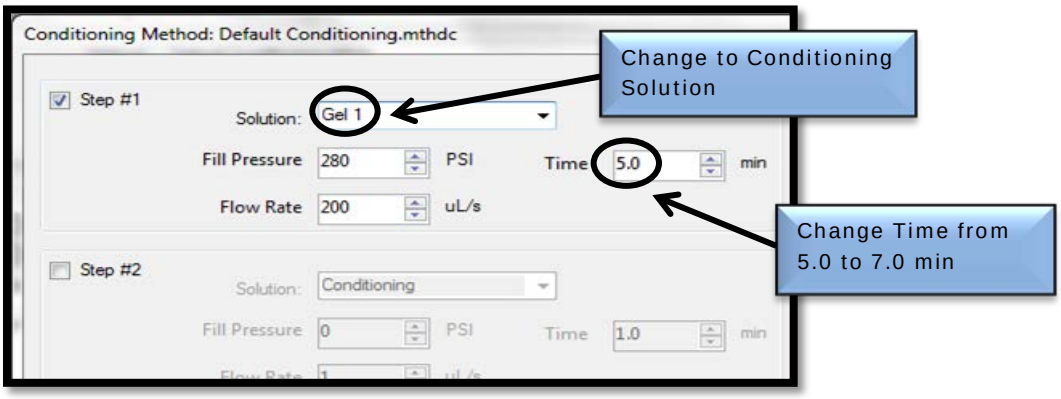


Figure 8-7: Conditioning Method Edit Window.
Edit Default Conditioning Method

The modified default capillary conditioning parameters for filling the capillary array cartridge with DI Water are listed in Table 8-3 below.

Fill Pressure (psi)	Time (minutes)	Solution	Flow Rate (ml/min)
280 psi	7 minutes	Conditioning Solution	200 µL/s

Table 8-3: Modified Default Capillary Conditioning Method Parameters

To accept the changes made to the default capillary conditioning method and place the method into the Method Queue, press [OK].

Running the Capillary Conditioning Method

Prior to filling the capillary array with Di Water:

- Check the Waste Tray in Drawer W (2nd drawer) to ensure that it is empty.
- Replace the Conditioning Solution Bottle located in the instrument Side Compartment with a bottle containing **≥ 7 mL** of DI Water.

Once the method has been added to the Method Queue and the Conditioning Solution Bottle in the instrument side compartment has been replaced with a bottle containing DI Water, start filling the capillary array with DI Water by left mouse clicking the [Start Arrow] on the Method Queue Status Bar as shown in Fig. 8-8.

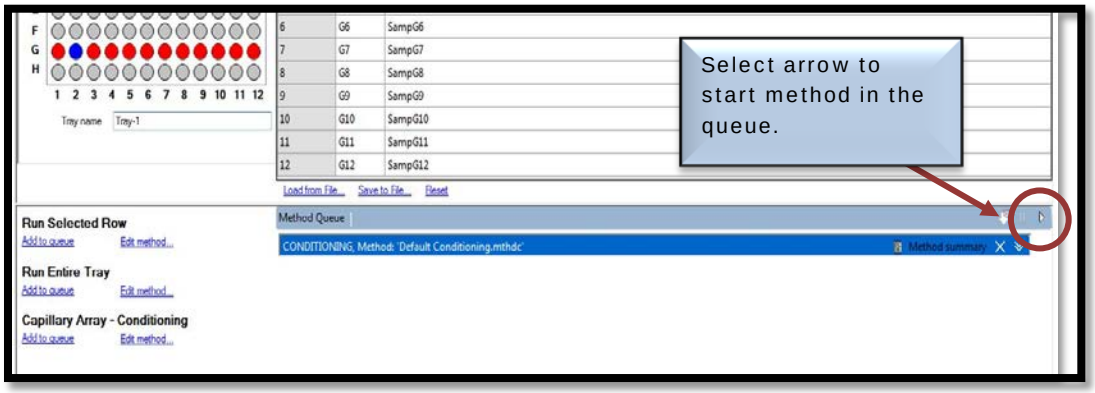


Figure 8-8: Main Screen Window - Method Queue.
Start Modified Method

After the instrument has finished running either Method 1 or Method 2, select the [Park Icon] from the Main Screen Window located in the Hotel Postioning Toolbar. Fig. 8-9 and 8-10

This will move the Sample Stage to the bottom of the instrument enabling the safe removal of the Capillary Array Cartidge.

⚠ Once the Stage is in the Park Position: **TURN THE INSTRUMENT OFF.** **Note that all pictures have power going to the instrument - should this statement be left in?**

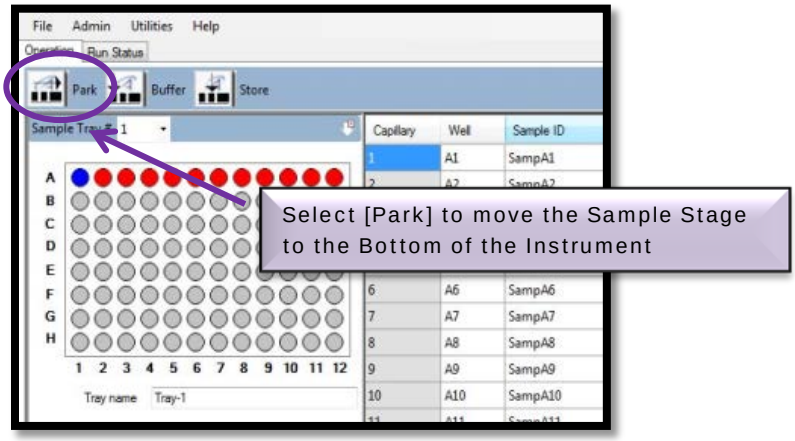


Figure 8-9: Main Screen Window.
Select [Park] Icon

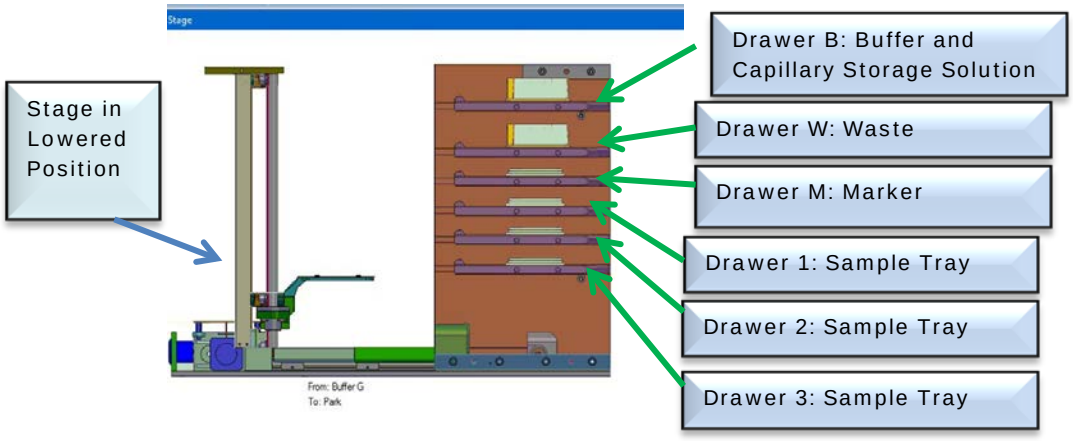


Figure 8-10. The "Park" Position. The stage is located at the bottom of the instrument and all samples/buffers are in tray positions.

Removing the Capillary Array

This section will provide a pictorial guide of the steps required to physically remove a Capillary Array Cartridge from the *FRAGMENT ANALYZER™* instrument.

Step 1

Open the Side and Top Doors of the *Fragment Analyzer™* Instrument

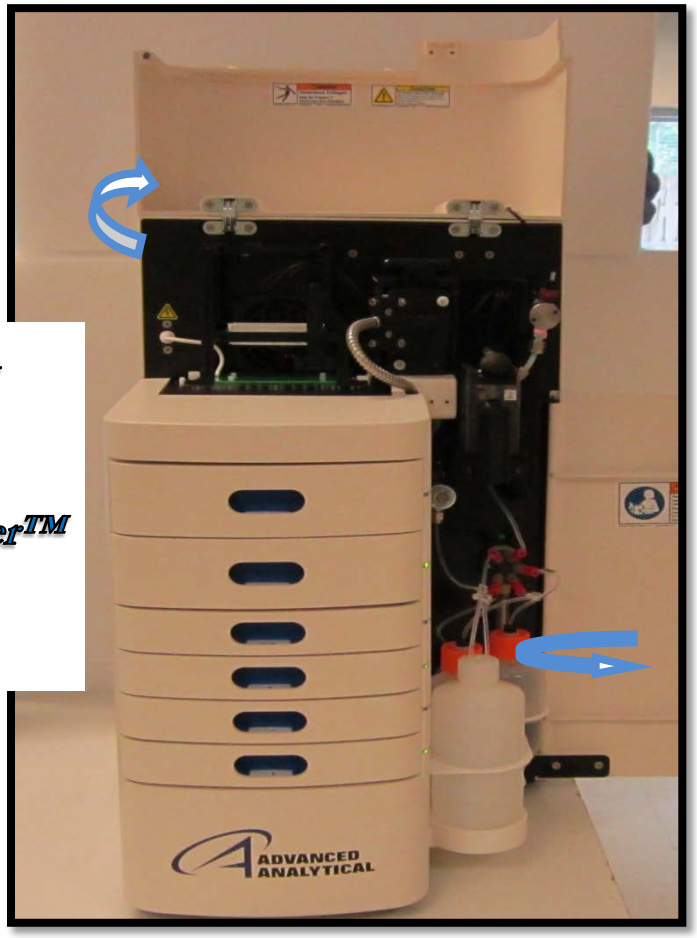


Figure 8-11. *FRAGMENT ANALYZER™* Instrument

Step 2

Unplug the White High Voltage Supply Cable from the Top Front Panel

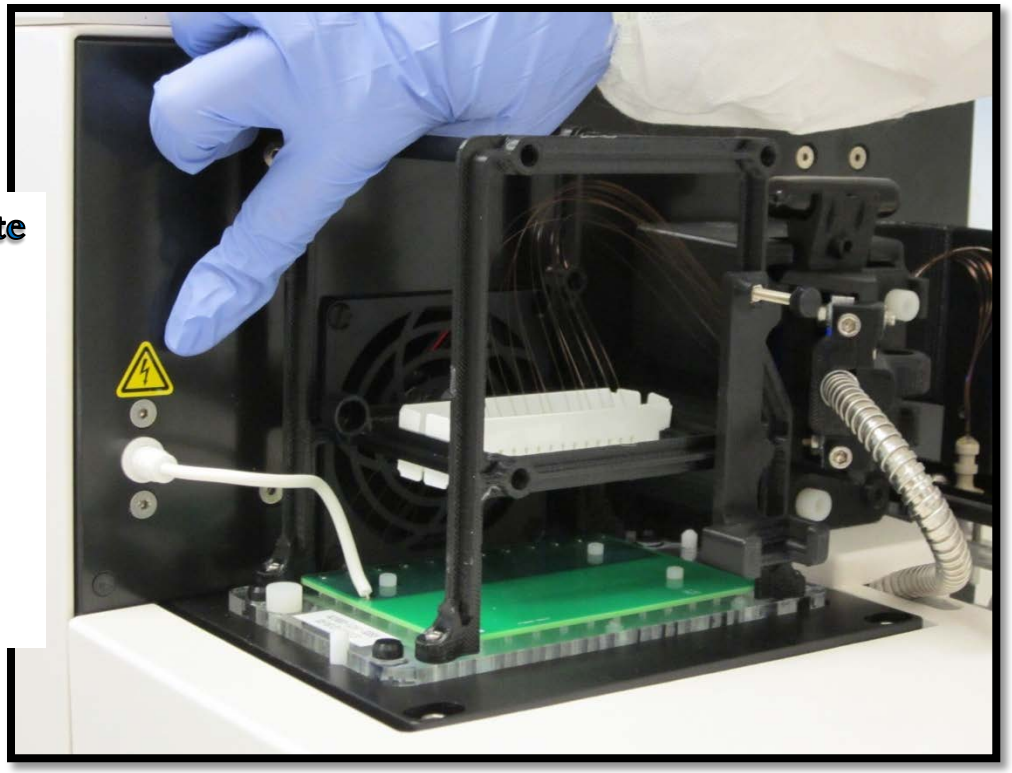


Figure 8-12: Instrument Top Compartment.
High Voltage Supply Cable

Step 3

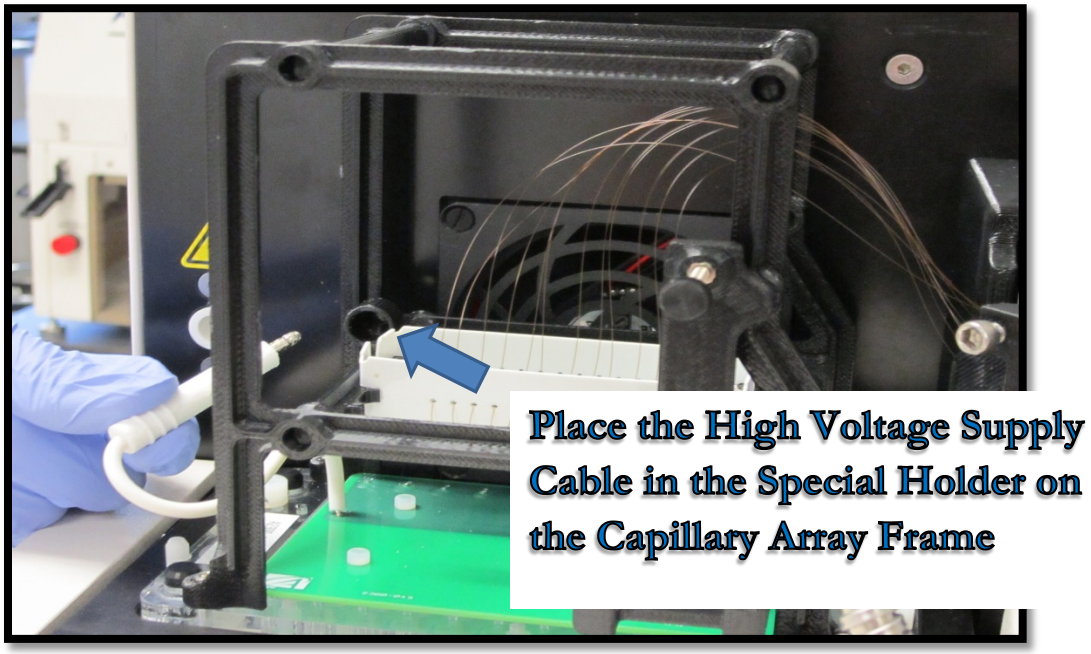


Figure 8-13. Instrument Top Compartment: High Voltage Supply Holder

Step 4

Use an Allen Wrench to Remove the Two White Screws that Secure the Light Guide to the Array Window

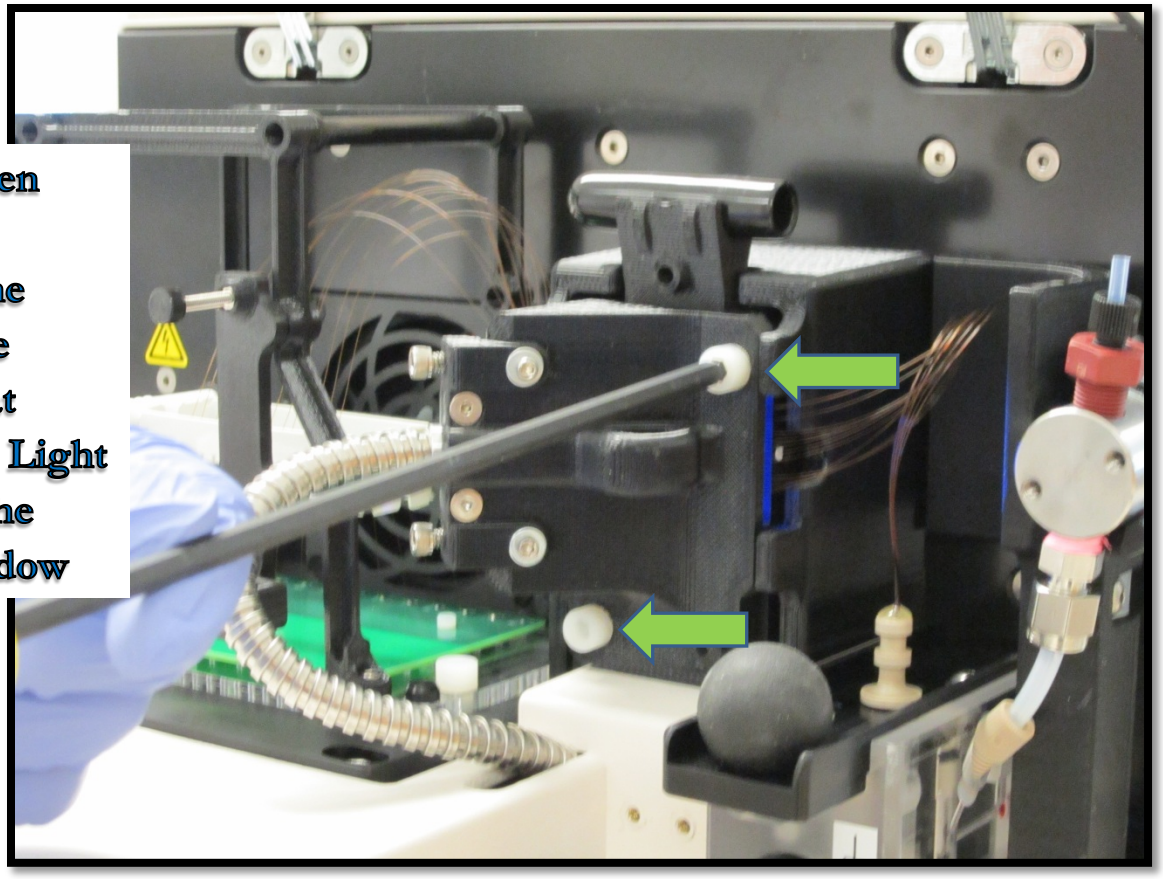


Figure 8-14. Instrument Top Compartment.
Unscrew Light Guide

Step 5

Figure 8-15. Instrument Top Compartment
Detach Light Guide

Step 6

Temporarily Place the Light Guide in the Side Compartment of the Instrument

Avoid Looking Directly at the Light Guide

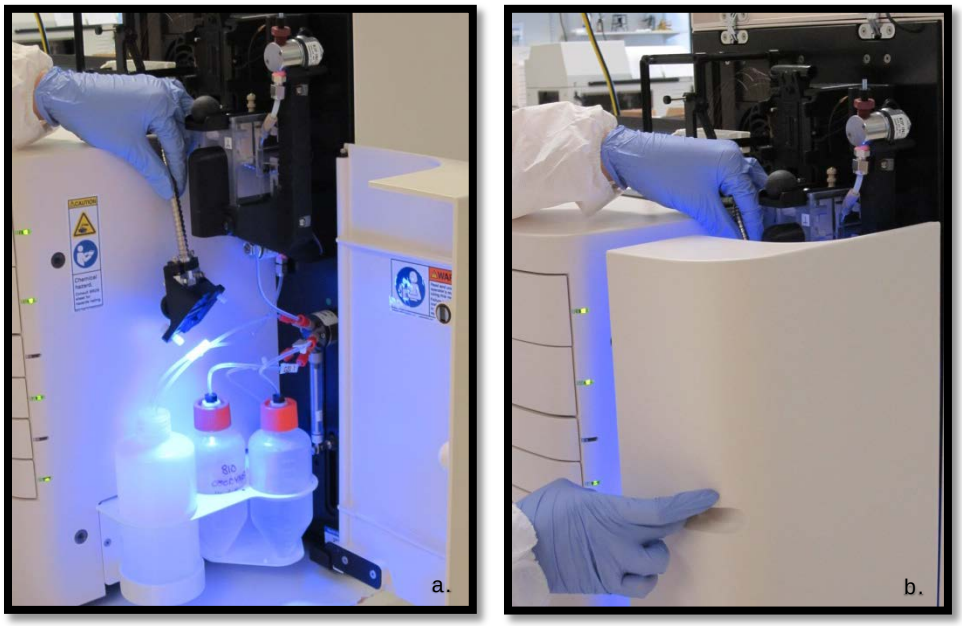


Figure 8-16. Instrument Side Compartment
Temporary Storage of Light Guide

Step 7

Move the Slide Back on the Capillary Reservoir Connector

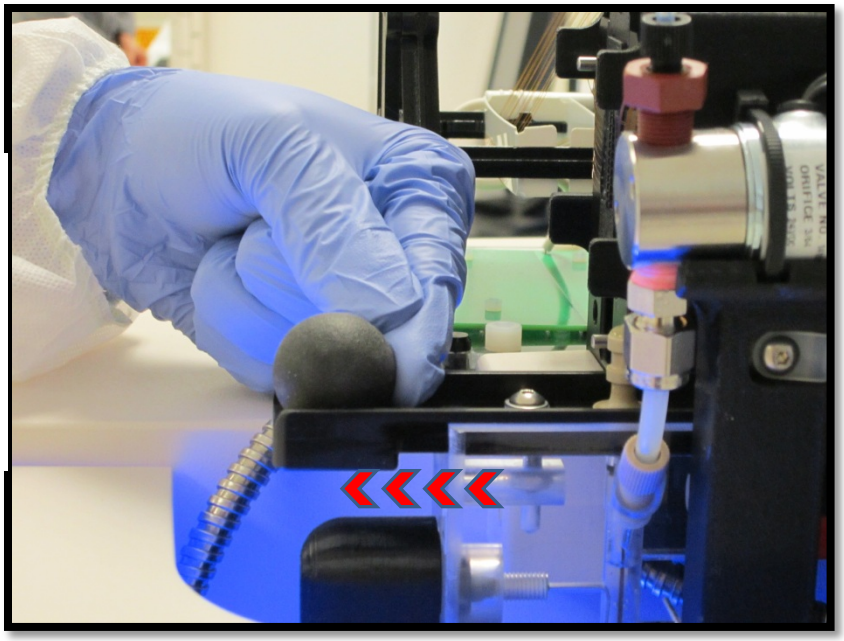


Figure 8-17. Instrument Top Compartment
Slide Capillary Reservoir Connector

Step 8

Use the Capillary Reservoir Connector Removal Tool to Pull the Array Reservoir Connector Out of the Reservoir

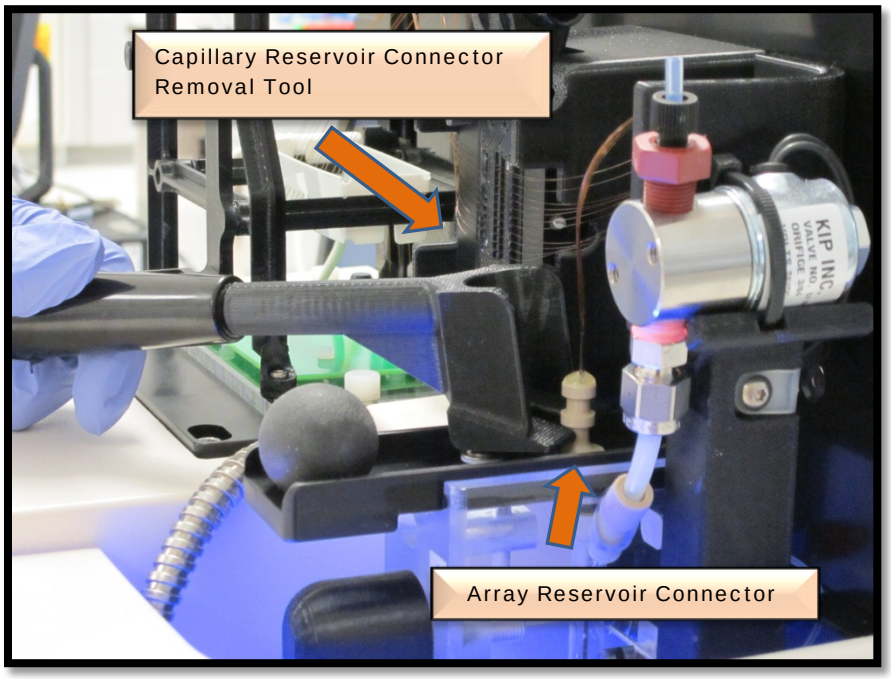


Figure 8-18. Instrument Top Compartment
Capillary Reservoir Connector Tool

IMPORTANT: Some fluid may leak out upon disconnecting Capillary Array Reservoir Connector.

Step 9

Pull up with the Removal Tool

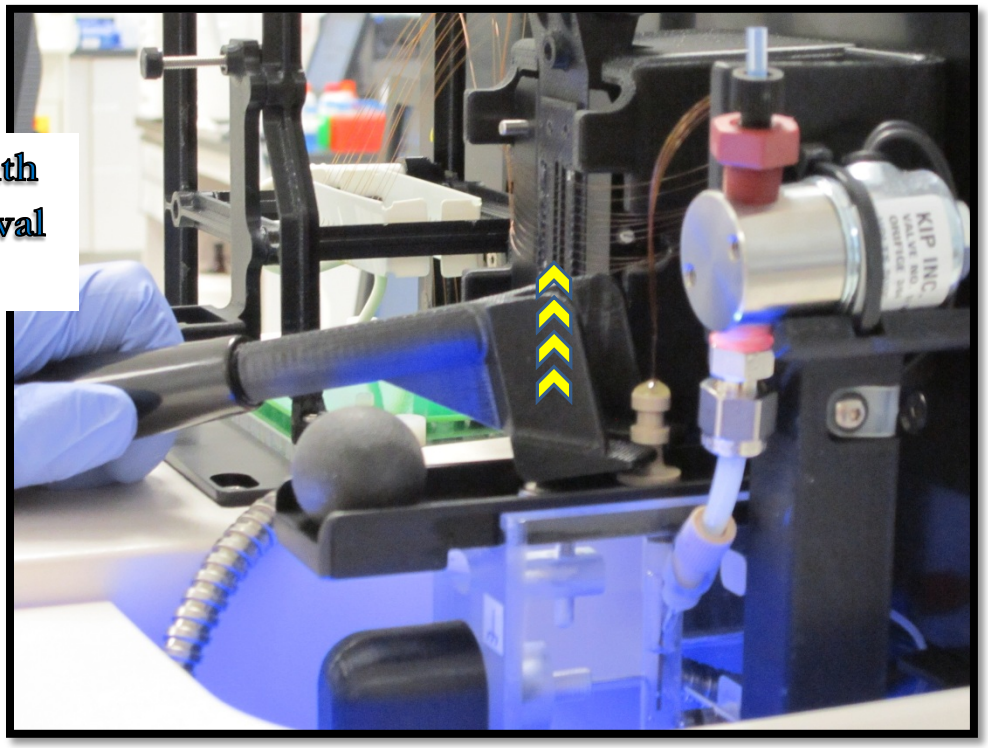


Figure 8-19. Instrument Top Compartment
Removing Capillary Array Reservoir Connector

Step 10

Carefully Remove the Capillary Reservoir Connector

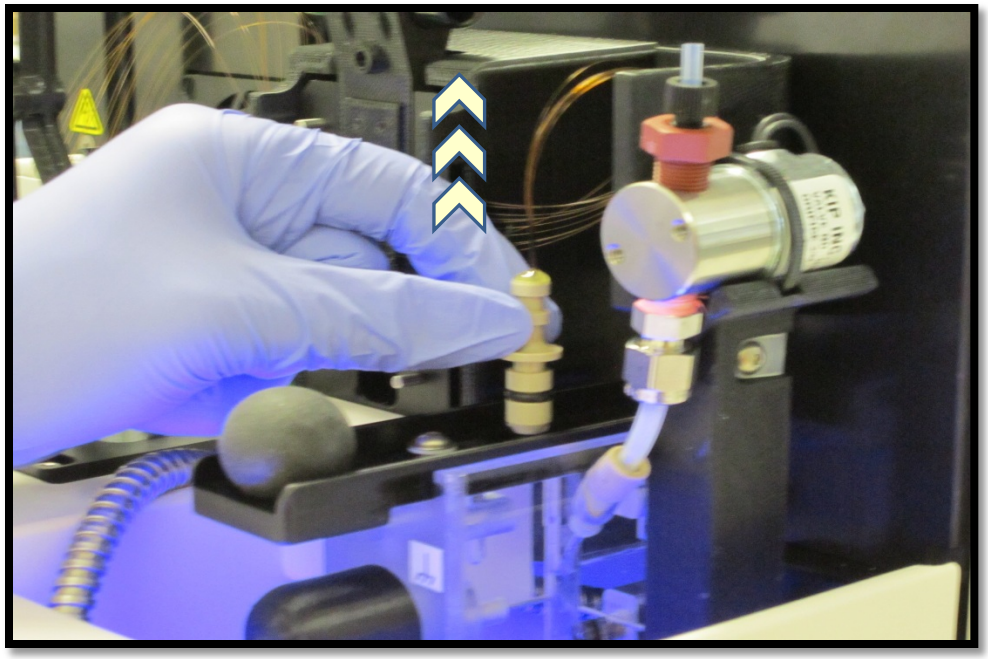


Figure 8-20. Instrument Top Compartment
Removing Capillary Array Reservoir Connector

Step 11

Carefully Insert the Capillary Array Bundle into the Protective Cover

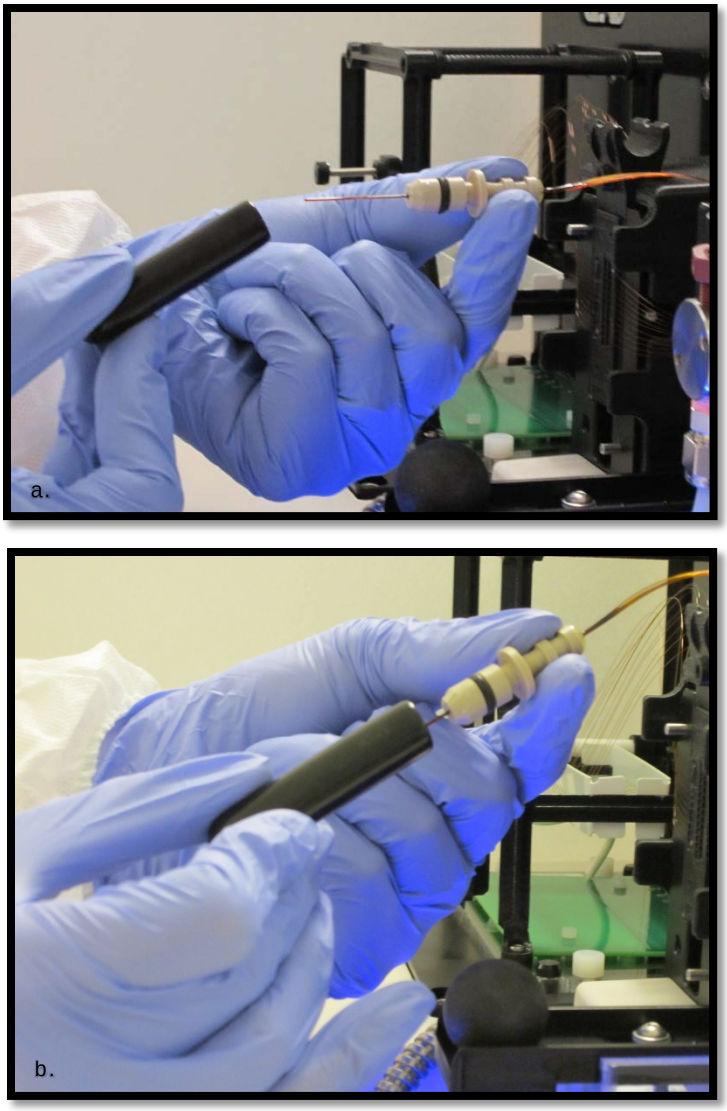


Figure 8-21a and b. Instrument Top Compartment
Inserting Array Bundle into Protective Cover

Step 12

Place the Capillary Array Bundle into the Holder on top of the Capillary Array Window

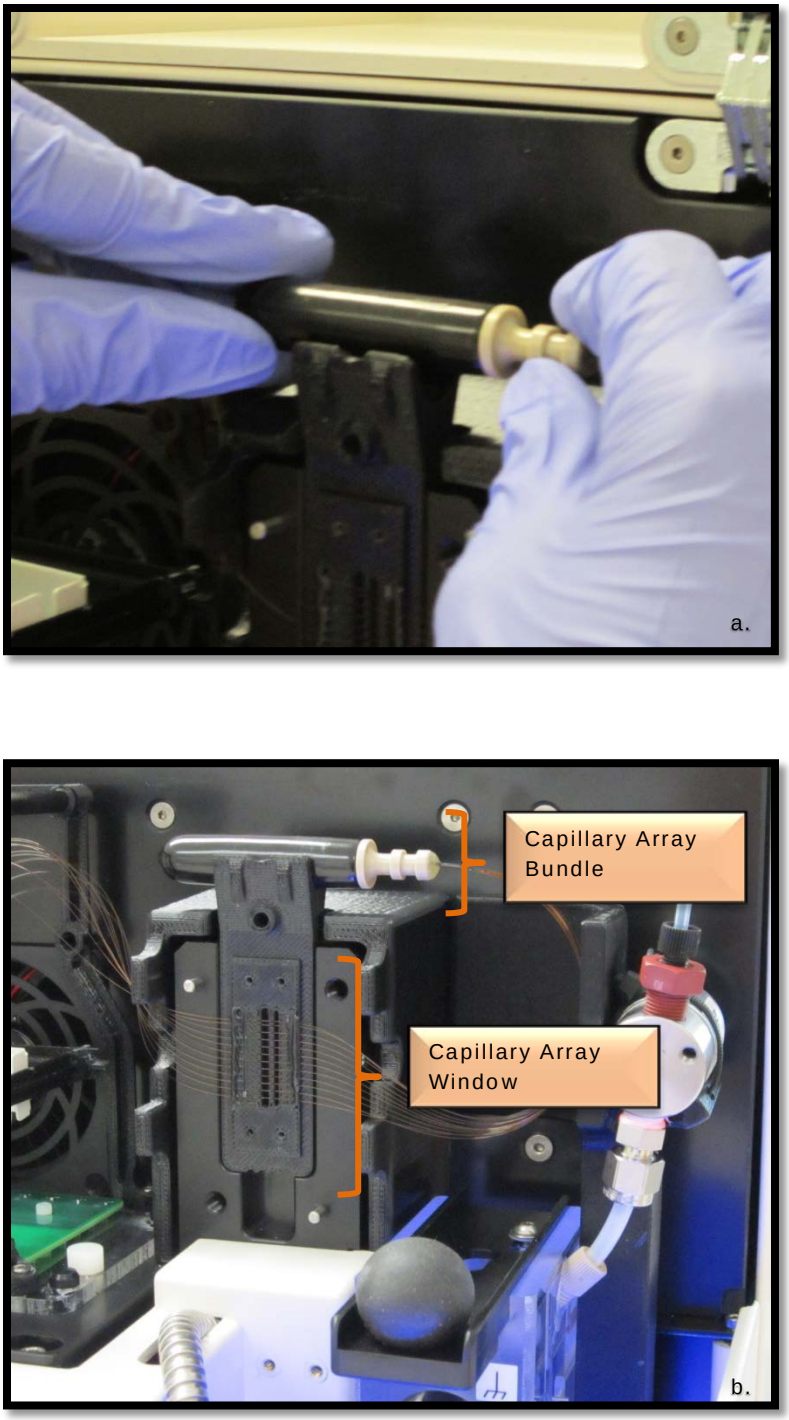


Figure 8-22a and b. Instrument Top Compartment Covered Capillary Array Reservoir Connector in Holder

Step 13

Remove the Capillary Array Window from the Array Window Holder on the Instrument

⚠ Do Not Press On or Touch the Capillaries.

Flip the Capillary Array Window after Removal so that the Array Connector is on the Left side of the Array Frame

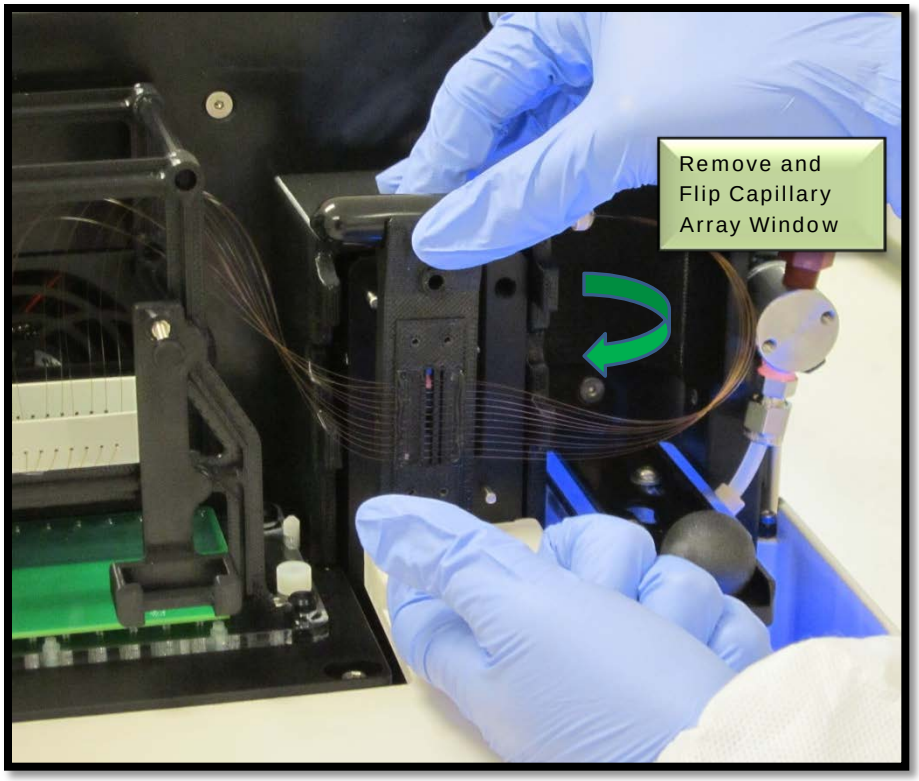


Figure 8-23. Instrument Top Compartment
Remove Capillary Array Window

Step 14

Attach the Array Window to the Capillary Array Cartidge Frame with Attachment Screw

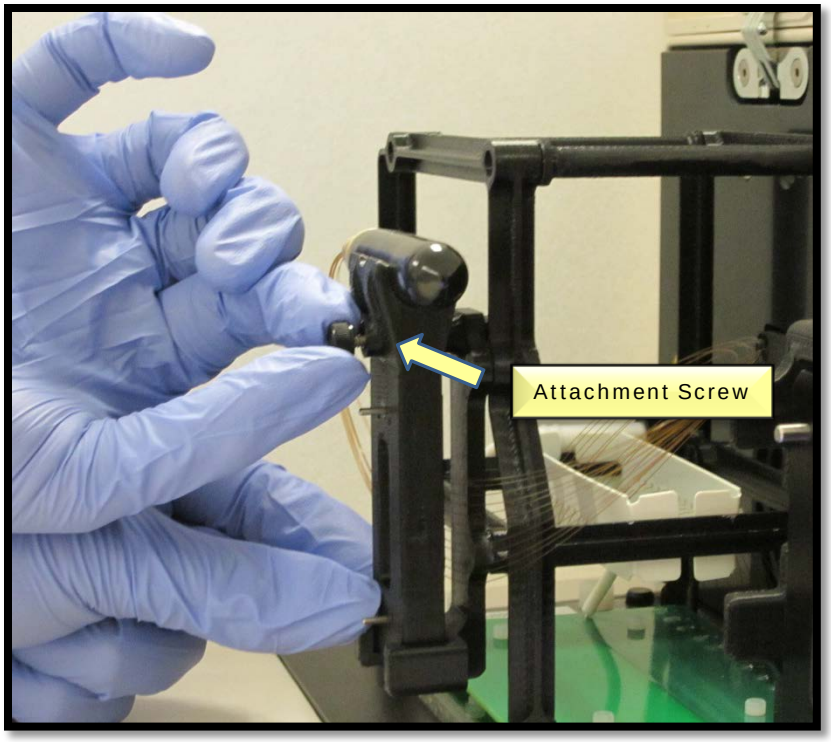


Figure 8-24. Instrument Top Compartment
Attach Array Window to Capillary Array Frame

Step 15

Use an Allen Wrench to Unscrew the Array Cartridge from the *Fragment Analyzer™*

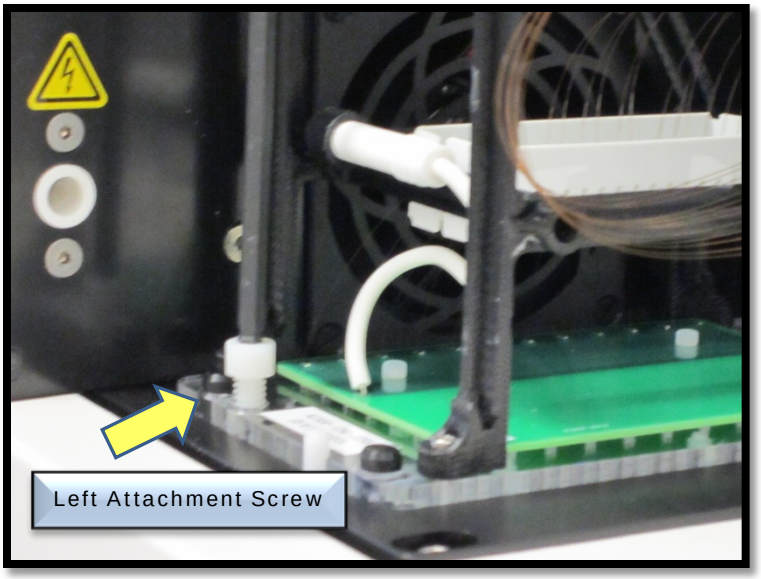


Figure 8-24a. Instrument Top Compartment
Remove Attachment Screw Left Side

There are Attachment Screws Located on the Right and Left Side of the Array.

Gently Hold Capillaries Aside with Finger to Allow Access to Right Attachment Screw

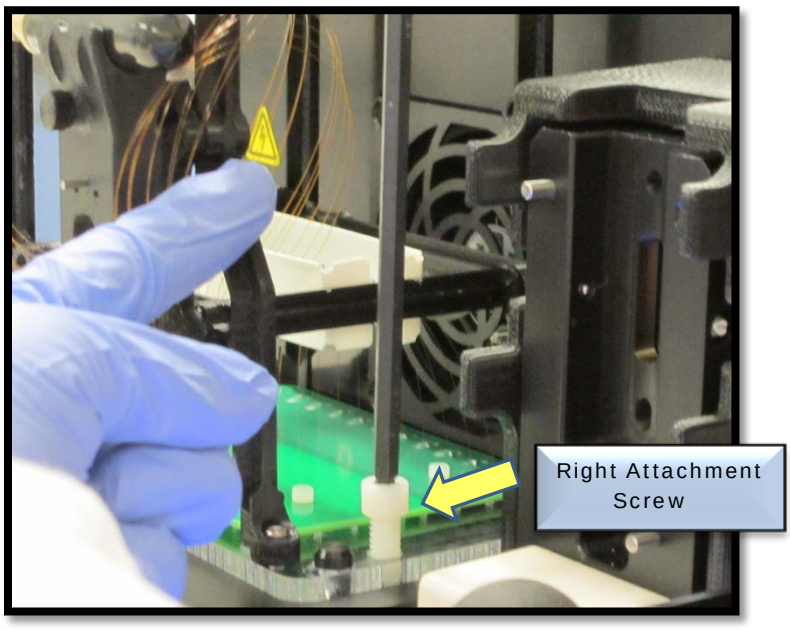


Figure 8-24b. Instrument Top Compartment
Remove Attachment Screw Right Side

Step 16

Carefully Lift the Array Cartridge Straight up to Remove it from the *Fragment Analyzer™* Instrument

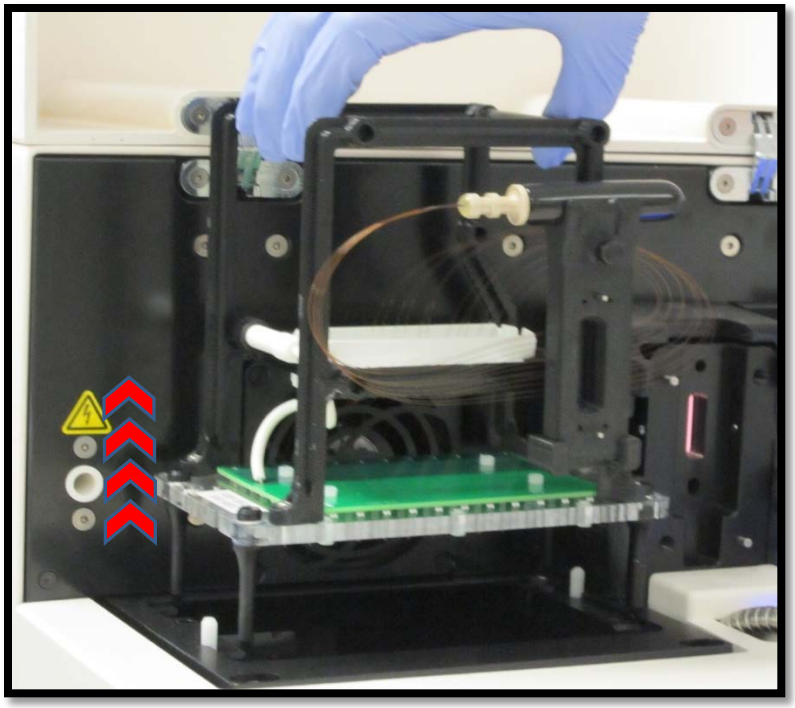


Figure 8-25. Instrument Top Compartment
Lift Capillary Array From Instrument

Step 17

Once the Capillary Array Cartridge is Removed:

Place it in the Special Shipping Box it Arrived in to Return to AATI or Place it in the *Wet Station™* for Long Term Storage.

OR

Dispose of Array Cartridge

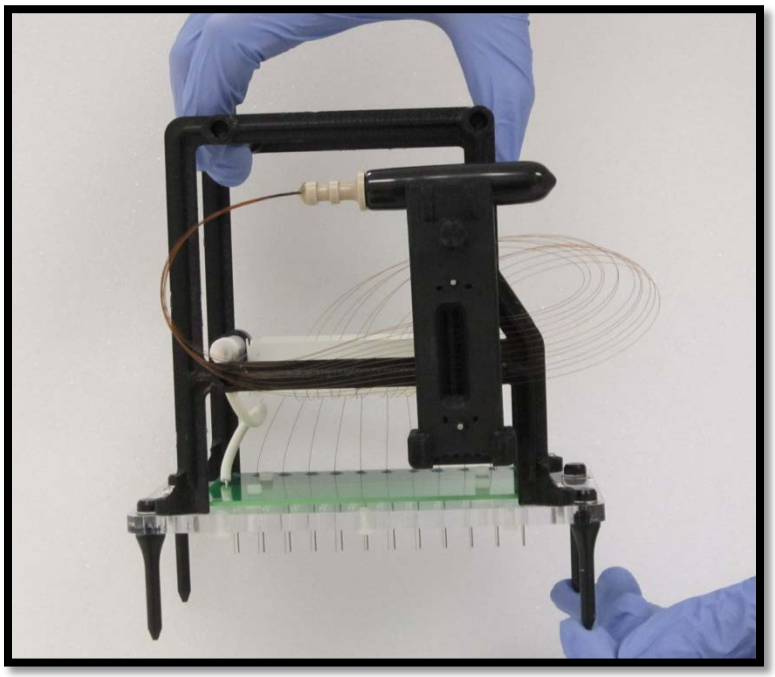


Figure 8-26. Removed Capillary Array Cartridge

Unpacking a Capillary Array Cartridge

This section will provide a pictorial guide of the steps required to physically Remove a Capillary Array Cartridge from the Shipping Container and Packaging.

IMPORTANT: 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.

Step 1

Open the Capillary Array Shipping Box and Retain the Associated Paperwork

Figure 8-27. Capillary Array Shipping Box Paperwork Attached

Step 2

Lift the Foam Cover Directly Out of the Box.

Figure 8-28. Capillary Array Shipping Box
Remove Foam Cover

Step 3

Remove the Packaged Array from the Shipping Box.

Figure 8-29. Capillary Array Shipping Box
Remove Packaged Array

Step 4a

Remove the Plastic Bag Covering the Array

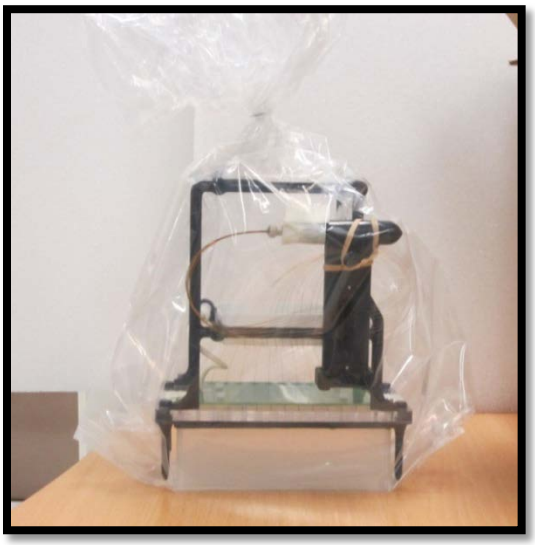


Figure 8-30. Packaged Capillary Array
Remove Plastic Covering

Step 4b

Take Care not to Touch, Bend or Pull Capillaries when Removing Packaging

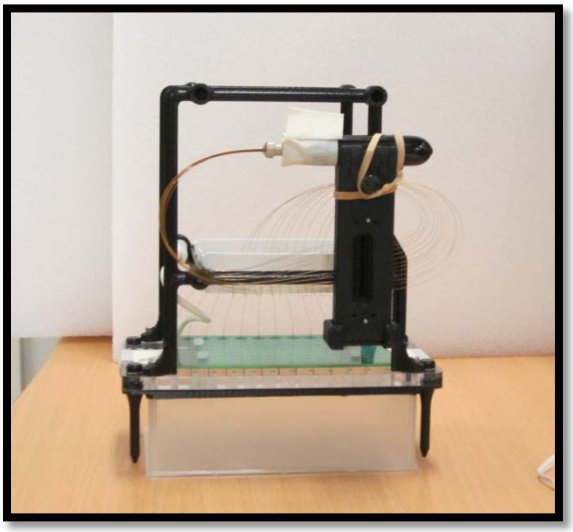


Figure 8-31. Capillary Array Cartridge (12-Cap)
Plastic Covering Removed

Step 5

Unwind the Rubber Band Securing the Capillary Array Bundle to the Capillary Array Window

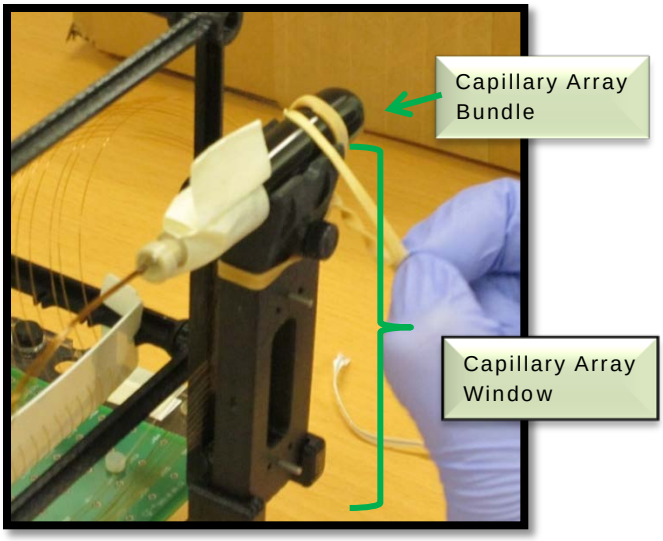


Figure 8-32. Capillary Array Cartridge (12-Cap)
Unwind Rubber Band

Step 6

Unscrew the Array Window from the Capillary Array Frame. Save the Attachment Screw.

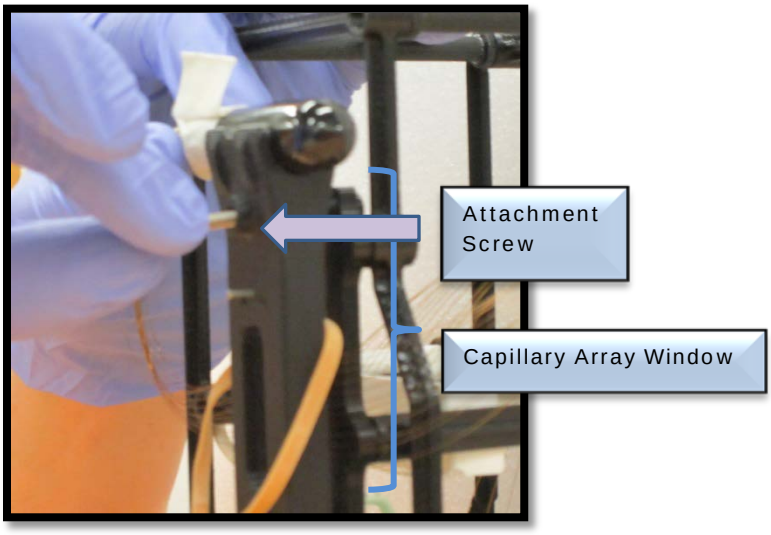


Figure 8-33. Capillary Array Cartridge (12-Cap)
Unscrew Capillary Array Window

Step 7

Remove the Tape that is Securing the Capillary Array Connector to the Array Bundle Cover

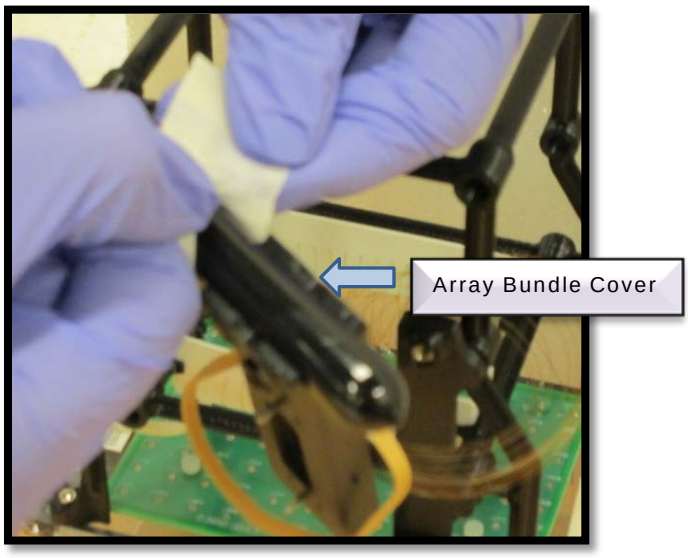


Figure 8-34. Capillary Array Cartridge (12-Cap)
Remove Tape from Capillary Array Bundle

Step 8

Carefully Remove the Capillary Array Bundle from the Holder to Allow Removal of the Rubber Band from around the Array Window.

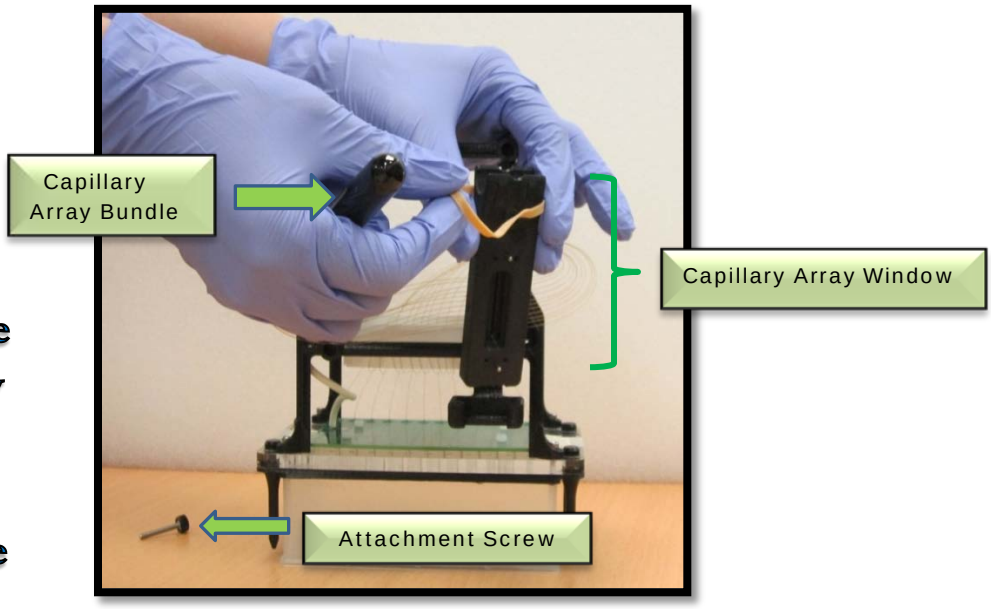


Figure 8-35. Capillary Array Cartridge (12-Cap)
Remove Rubber Band

Step 9

Place the Capillary Bundle back onto the Holder Located on the Capillary Array Window.

Secure the Capillary Array Window to the Array Frame with the Attachment Screw.

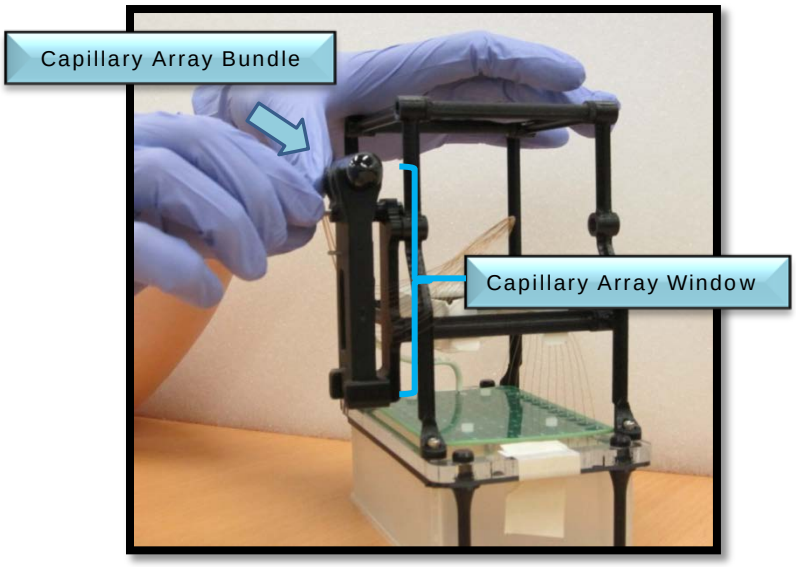


Figure 8-36. Capillary Array Cartridge (12-Cap)
Secure Array Window to Array Frame

Step 10

Remove the Tape Securing each Side of the Capillary Array to the Protective Plastic Tray

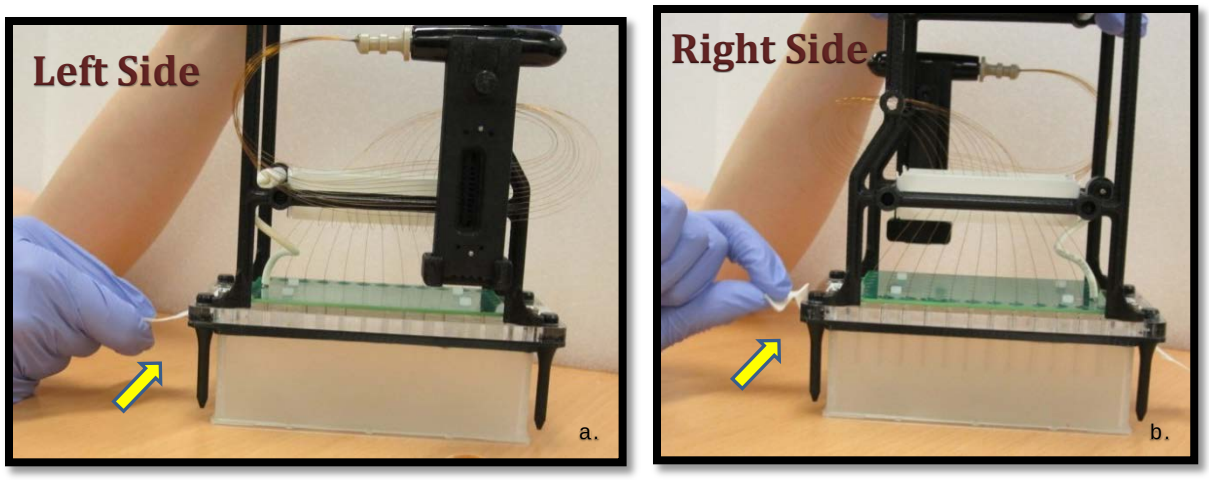


Figure 8-37a and b. Capillary Array Cartridge (12-Cap)
Remove Tape from each Side of Array Frame

Step 11

Unpacked Capillary Array Cartridge.

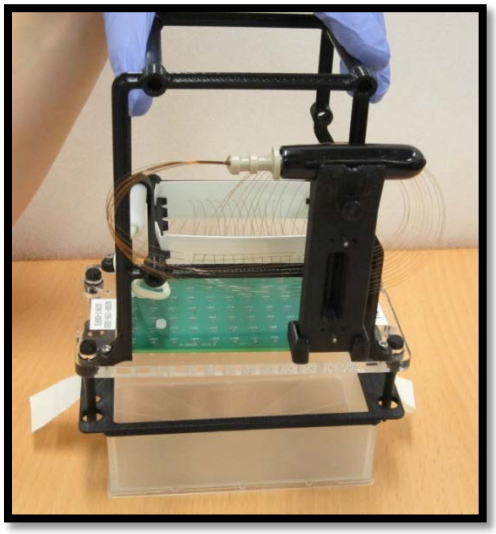


Figure 8-38. Capillary Array Cartridge (12-Cap)
All Packaging Removed

This section will provide a pictorial guide of the steps required to physically place a Capillary Array Cartridge into the *FRAGMENT ANALYZER™* instrument.

Step 1

Open the Side and Top Doors of the *Fragment Analyzer™* Instrument

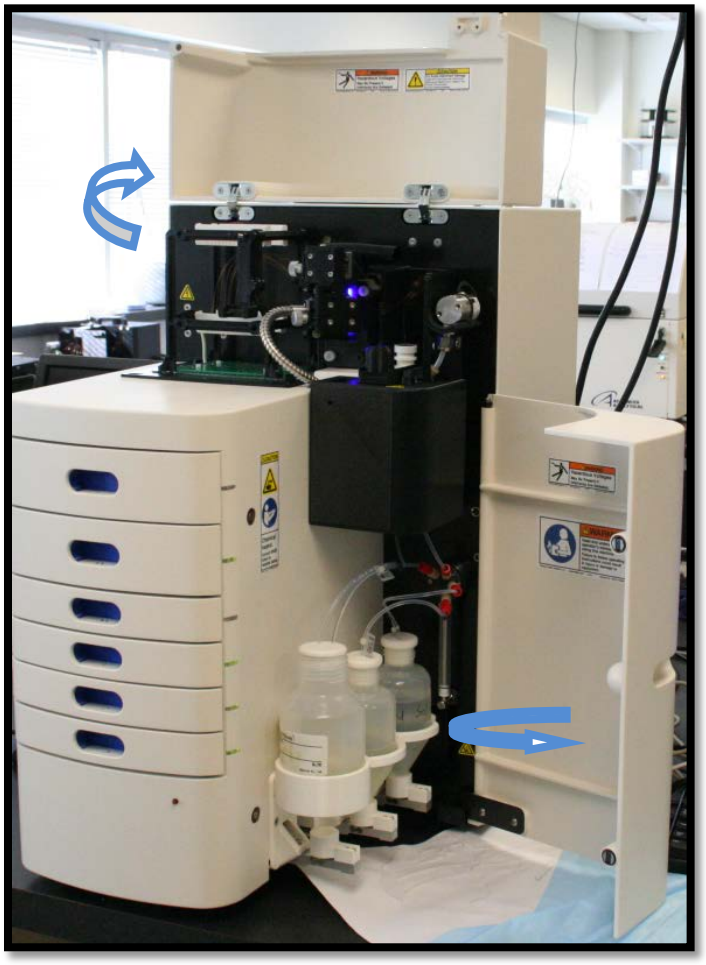


Figure 8-39. *FRAGMENT ANALYZER™* Instrument

Step 2 **Place the Capillary Array Cartridge into the Top Compartment of *Fragment Analyzer™* Instrument Oriented with the Capillary Array Bundle in the Front of the Instrument and the Serial Number of the Array Cartridge on the Left.**

Use the Four Alignment Pins on the Array Frame and Align with the Alignment Holes in the Instrument.

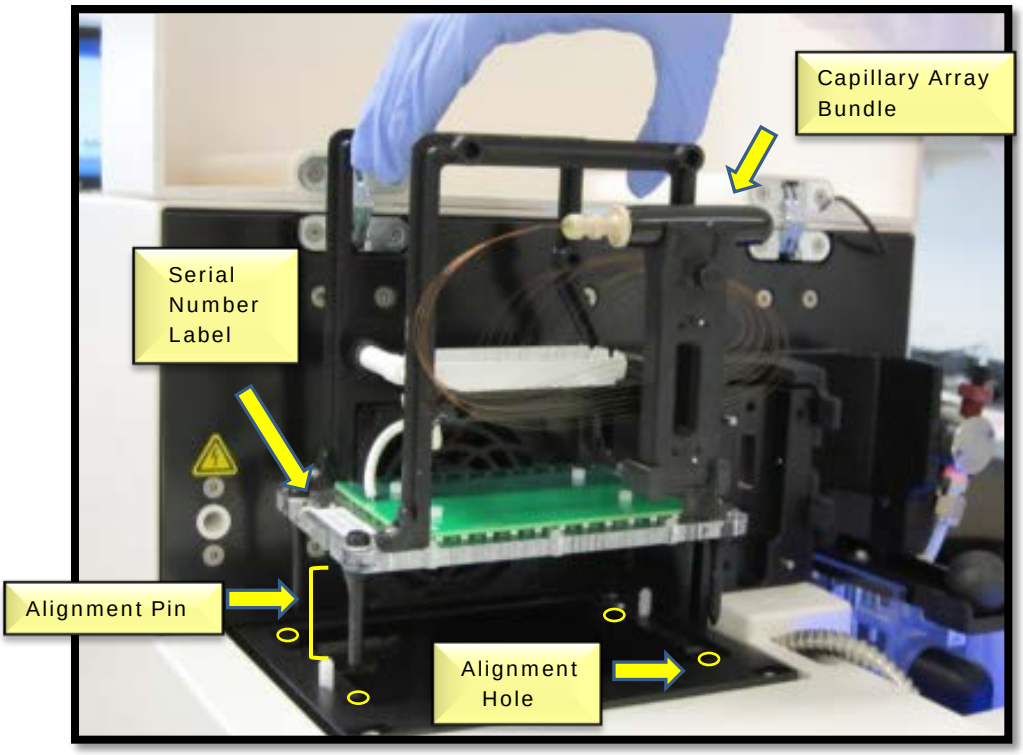


Figure 8-40. Instrument Top Compartment
Inserting Capillary Array Cartridge

Step 3

Use an Allen Wrench and two Attachment Screws to Fasten the Array Cartridge to the *Fragment Analyzer™* Instrument

Gently Push the Capillaries aside to Reach the Attachment Point on the Right Side of the Array.

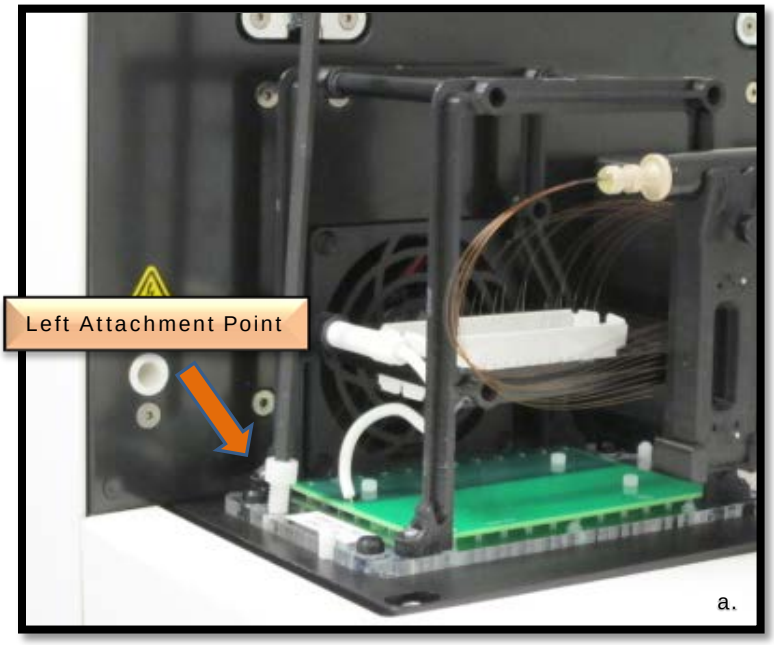


Figure 8-41a. Instrument Top Compartment Attaching Left Side of Capillary Array Cartridge

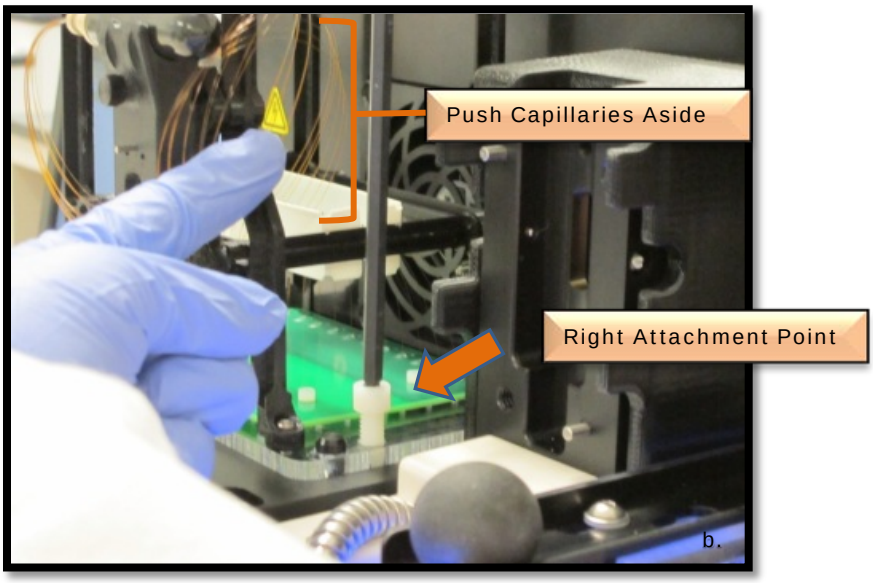


Figure 8-41b. Instrument Top Compartment Attaching Right Side of Capillary Array Cartridge

Step 4

Unscrew the Capillary Array Window from the Array Frame

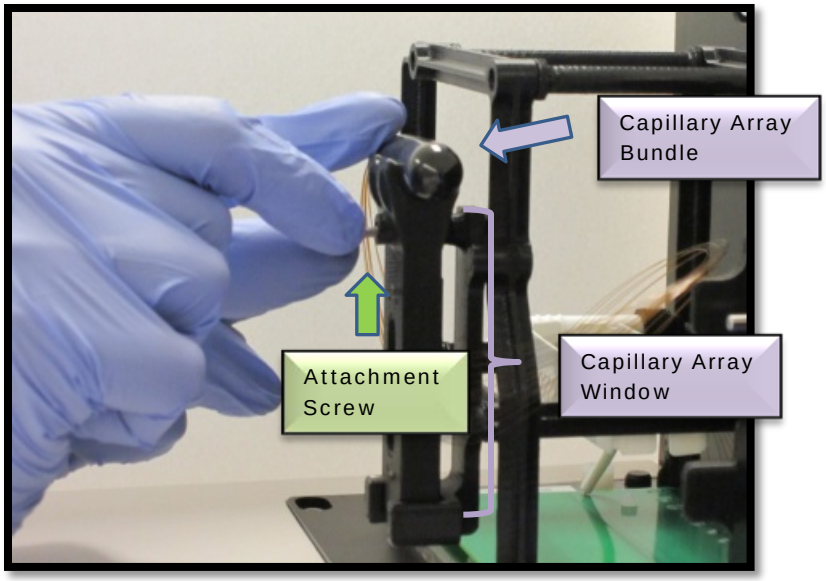


Figure 8-42. Instrument Top Compartment
Unscrew Capillary Array Window

Step 5

Carefully Flip the Capillary Array Window so that the Capillary Array Connector is Oriented to the Right Side of the Instrument

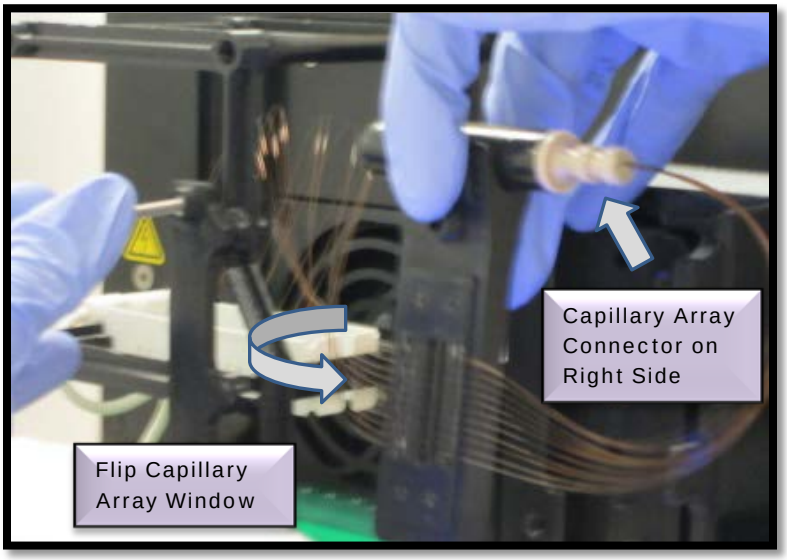


Figure 8-43. Instrument Top Compartment
Flip the Capillary Array Window

Step 6

Position the Capillary Array Window in the Window Holder on the *Fragment Analyzer™* Instrument.

Press the Array Window Firmly into Place.

⚠ Do Not Press On or Touch the Capillaries.

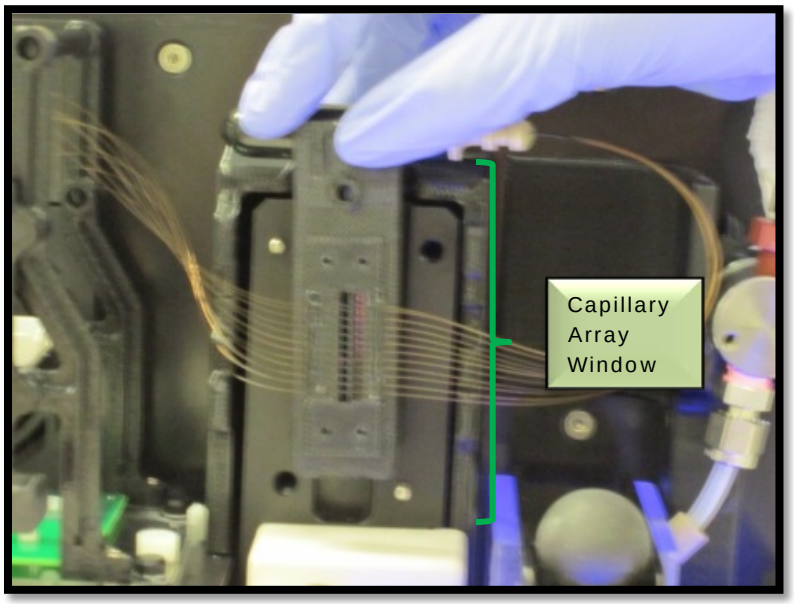


Figure 8-44. Instrument Top Compartment
Flip the Capillary Array Window

Step 7

Replace the Attachment Screw on the Array Frame

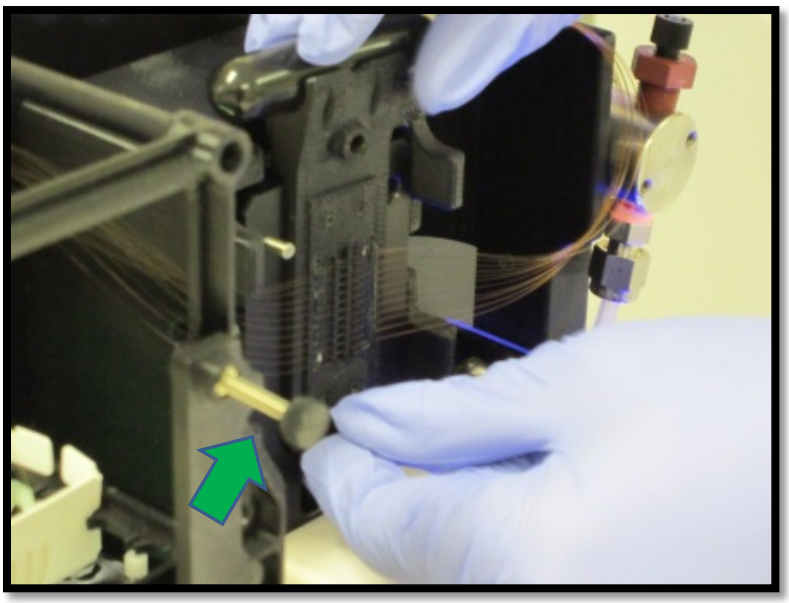


Figure 8-45. Instrument Top Compartment
Replacing Attachment Screw

Step 8

Remove the Capillary Bundle from the Holder on top of the Capillary Array Window

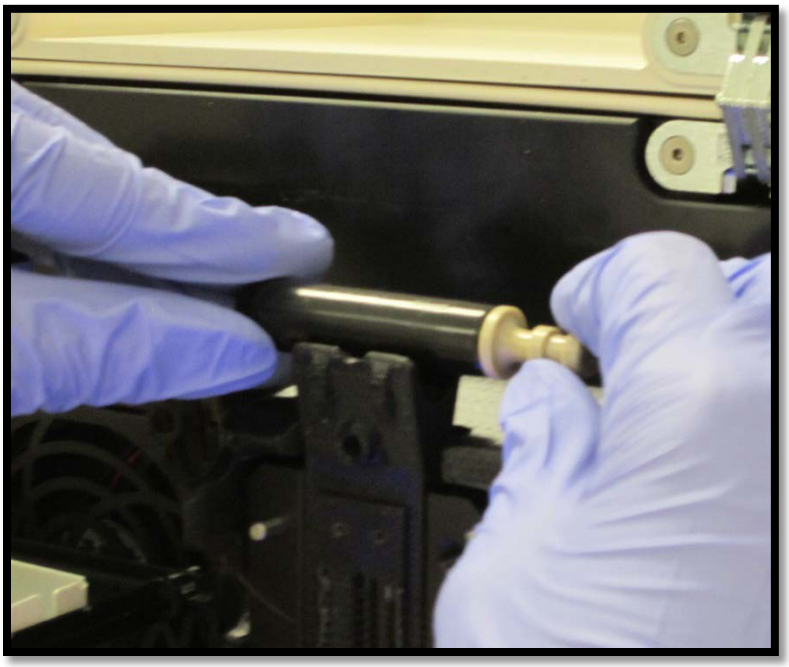


Figure 8-46. Instrument Top Compartment
Remove Capillary Array Bundle from Holder

Step 9

Carefully Remove the Protective Covering from the Capillary Array Bundle.

Store the Protective Cover on top of the Array Window.

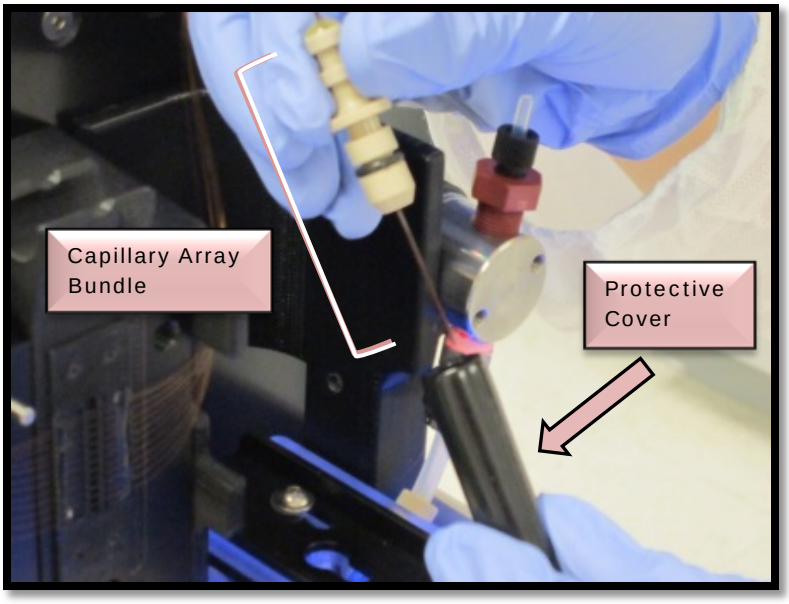


Figure 8-47. Instrument Top Compartment
Remove Protective Cover

Step 10

Carefully Position the Capillary Array Connector over the hole in the Reservoir

⚠ **Do Not Touch the Reservoir with the Exposed Capillary Bundle**

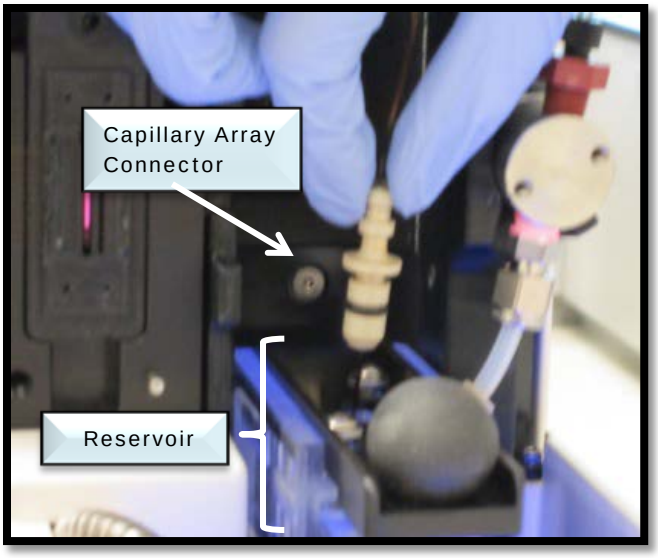


Figure 8-48. Instrument Top Compartment
Position Capillary Array Connector

Step 11

Place the Capillary Array Connector into the Reservoir

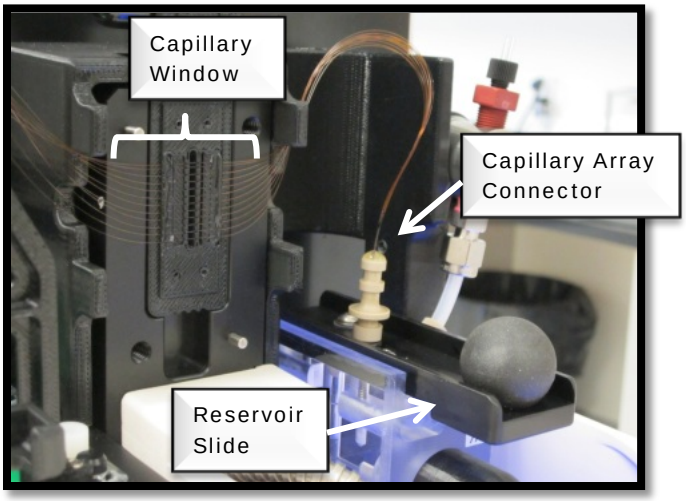


Figure 8-49. Instrument Top Compartment
Place Array Connector in Reservoir

Step 12

Push the Capillary Array Connector Down Firmly into the Reservoir until it is even with the Top of the Reservoir and a Distinct Click is Heard.

⚠ The Connection to the Reservoir is Very Important.

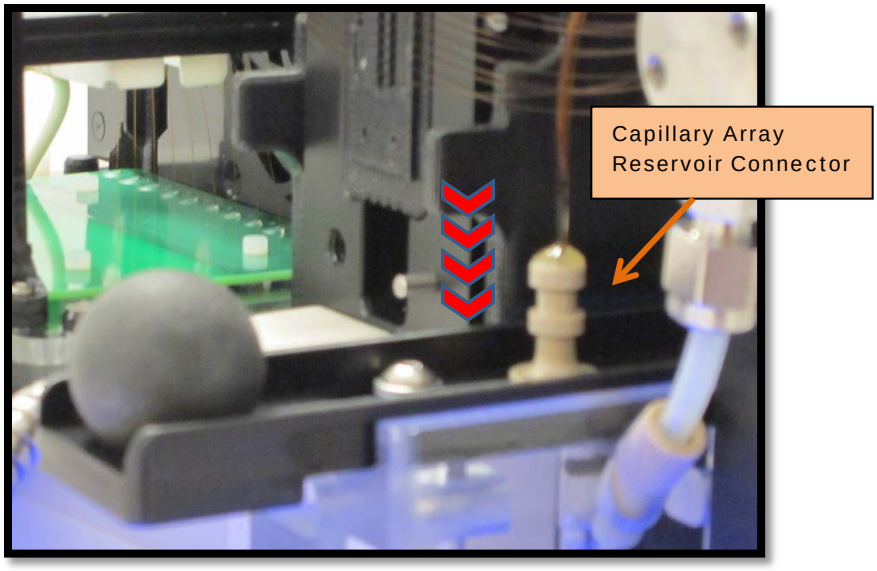


Figure 8-50. Instrument Top Compartment
Press Capillary Array Connector Down into Reservoir

Step 13

Push the Reservoir Slide in to Secure the Capillary Array Connector

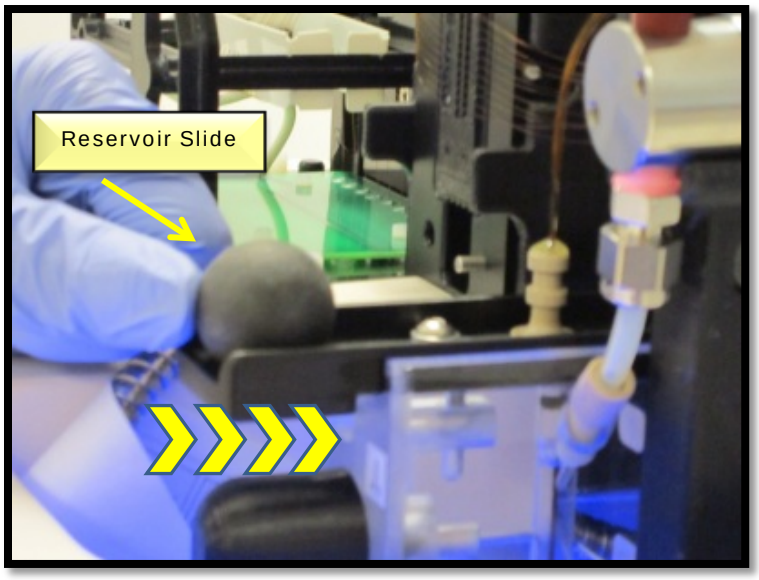


Figure 8-51. Instrument Top Compartment
Press Reservoir Slide In

Step 14

Remove the Light Guide that was Temporarily

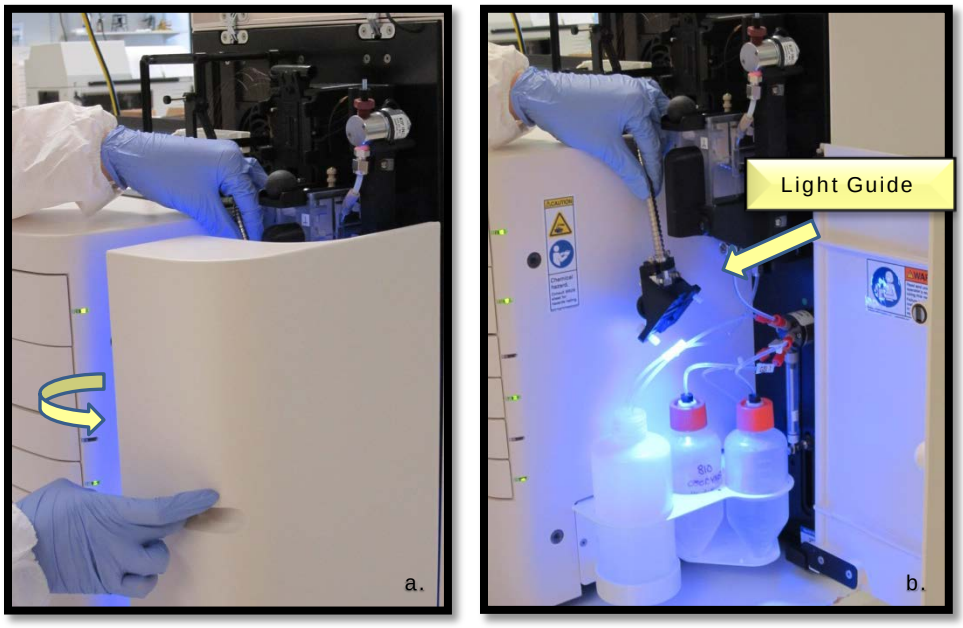


Figure 8-52a and b. Instrument Side Compartment
Temporary Storage of Light Guide

Step 15

Locate the Two Alignment Pins on the Capillary Array Window

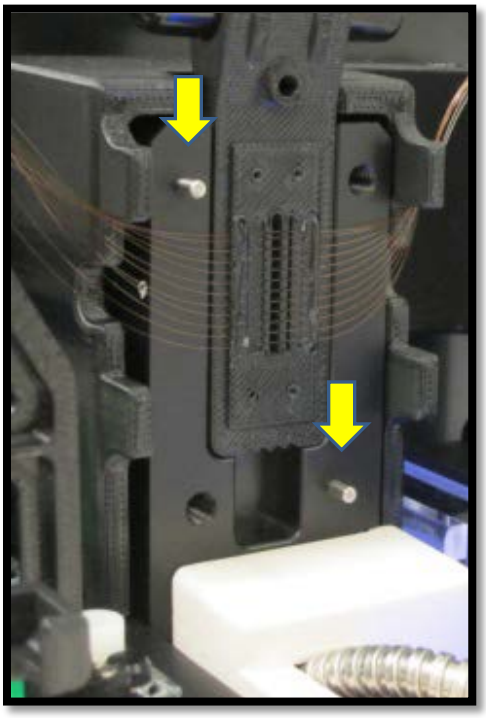


Figure 8-53. Instrument Side Compartment
Light Guide Alignment Pins

Step 16

Place the Light Guide Over the Capillary Array Window Using the Two Alignment Pins

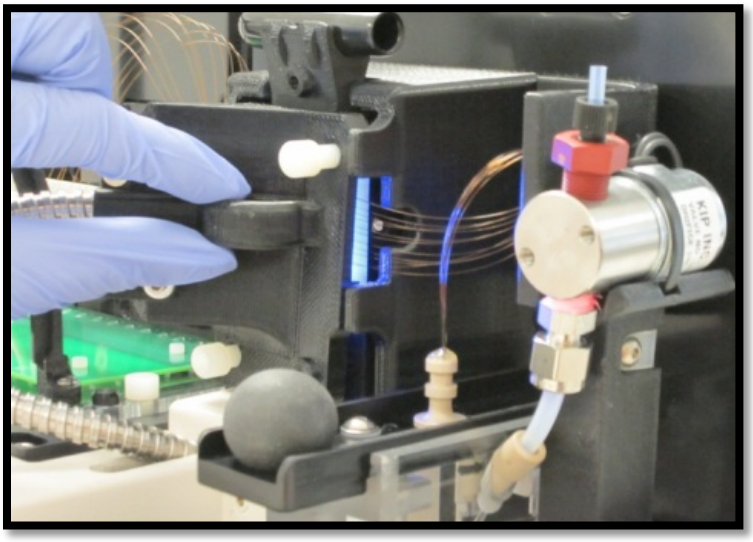


Figure 8-54. Instrument Side Compartment Aligning Light Guide

Step 17a

Use an Allen Wrench to Attach the Light Guide Over the Capillary Array Window Using two Attachment Screws

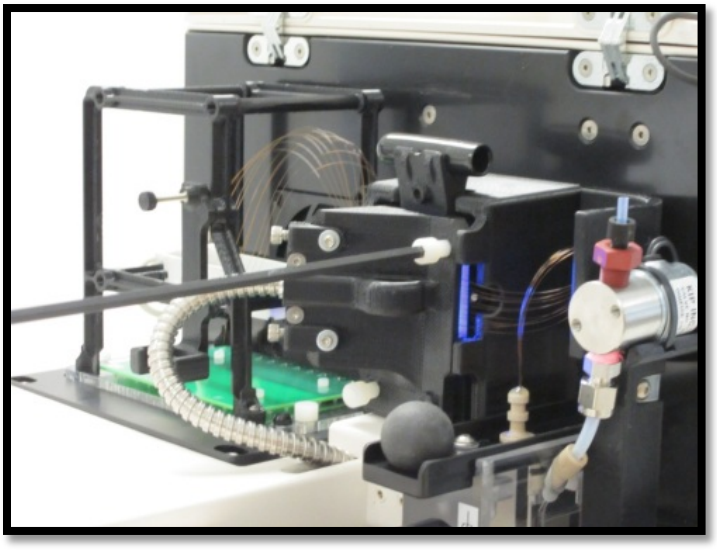


Figure 8-55. Instrument Side Compartment Tightening Top Right Attachment Screw

Step 17b

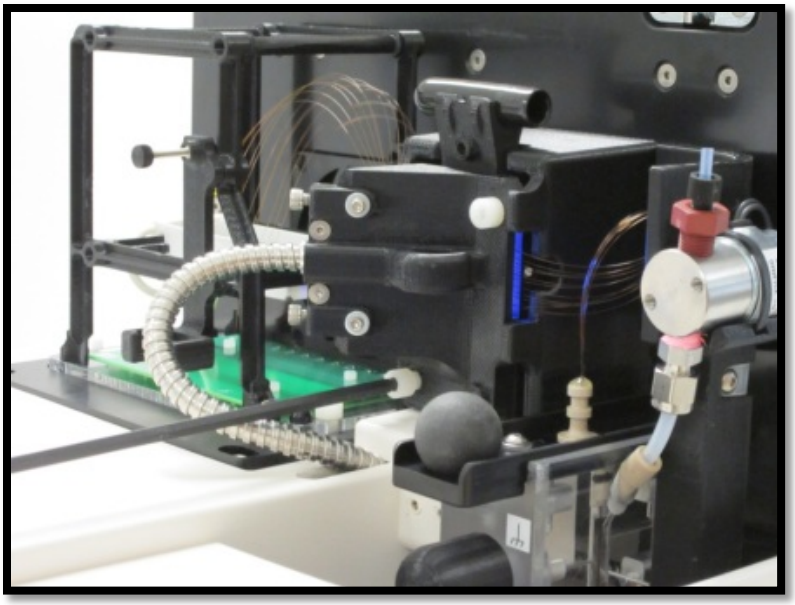


Figure 8-56. Instrument Side Compartment
Tightening Bottom Left Attachment Screw

Step 18

Remove the High Voltage Connector from the Array Frame

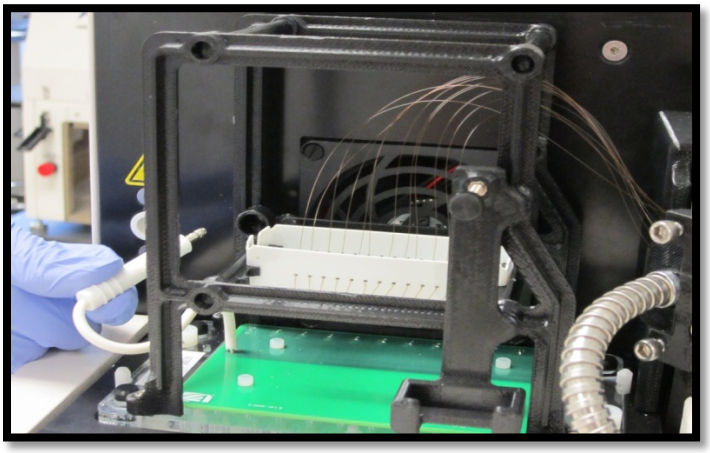


Figure 8-57. Instrument Side Compartment
Removing High Voltage Connector

Step 19

Plug the High Voltage Connector into the *Fragment Analyzer*™ Instrument.

Be Sure to Press in Firmly to Ensure a Good Connection.

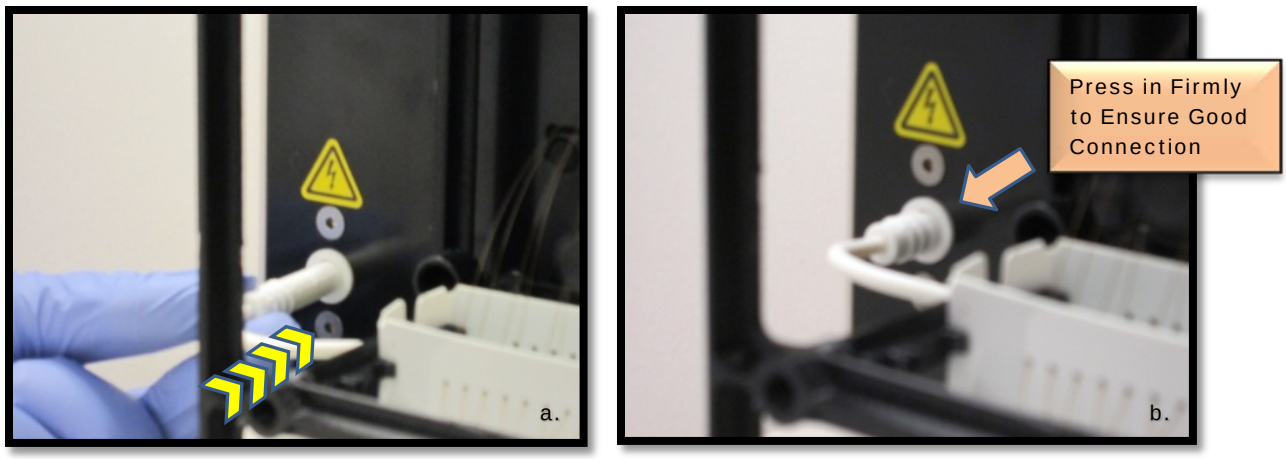


Figure 8-58a and b. Instrument Side Compartment
Plug in High Voltage Connector

Step 20

Close the Top and Side Doors of the *Fragment Analyzer™* Instrument

Figure 8-59. *FRAGMENT ANALYZER™* Instrument

Step 21

Perform an Optical Aligment as Described in Chapter 7

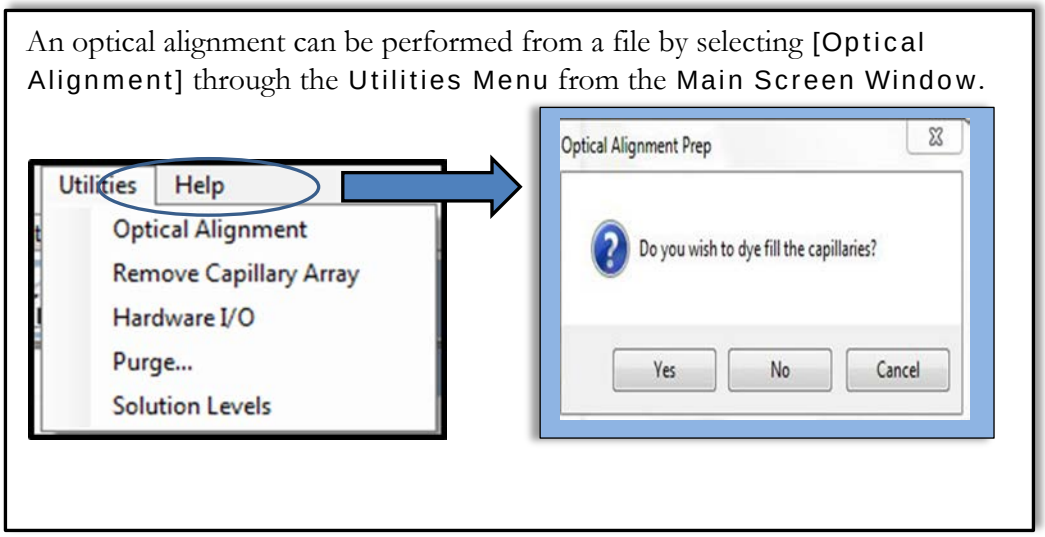


Figure 8-60. Main Screen Window.
Utilities Menu – Perform Optical Alignment

Long-Term Storage of the Capillary Array

Removing and storing an array is recommended when the *FRAGMENT ANALYZER™* instrument or an array will not be used for a period of time.

The Capillary Array Cartridge can be stored up to one month using the ***FRAGMENT ANALYZER WET STATION™***.

After 30 days, the reagents used in the ***WET STATION™*** to preserve the capillary array can be changed to prolong the storage of the array if needed.

- Prepare the Capillary Array Cartridge for Long-Term Storage and Removal from the instrument as discussed in the beginning of this chapter.

Preparing the *FRAGMENT ANALYZER WET STATION™*.

This section will provide a pictorial guide of the steps required to prepare the *WET STATION™* for the Long-Term Storage of a Capillary Array Cartridge

Step 1

Follow the Steps Provided at the Beginning of the Chapter to Gel Fill and Remove the Capillary Array Cartridge

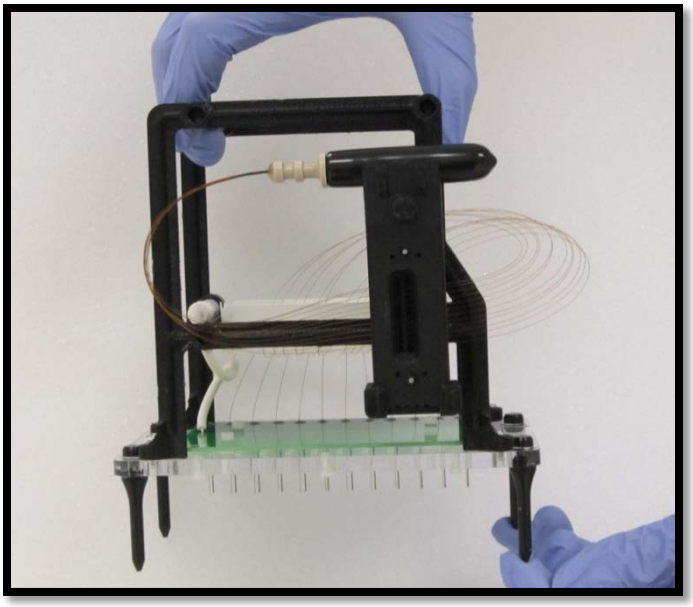


Figure 8-61. Gel Filled and Removed Capillary Array Cartridge

Step 2

Locate the *Fragment Analyzer Wet Station™*

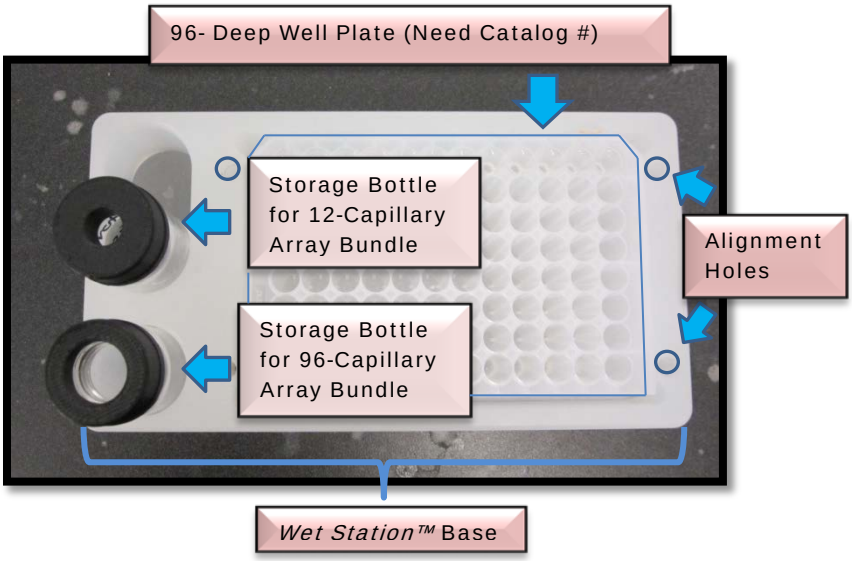


Figure 8-62. *Fragment Analyzer Wet Station™*

Step 3

For a 96-Capillary Array:

Fill each well of the 96-Deep Well Plate with 1 mL of Storage Solution

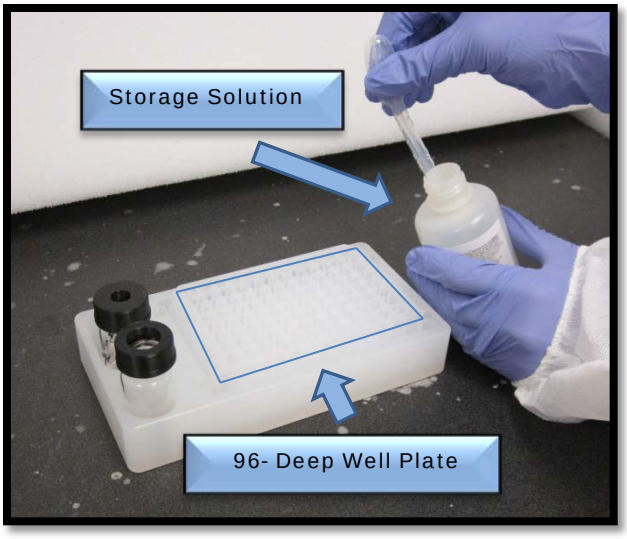


Figure 8-63. *Fragment Analyzer Wet Station™* Fill wells with Storage Solution

For a 12-Capillary Array:

Fill each well in Row A with 1 mL of Storage Solution.

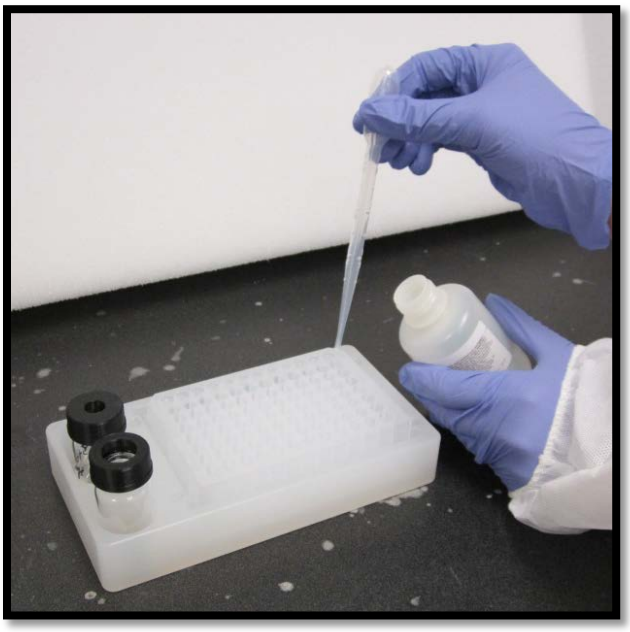


Figure 8-64. *Fragment Analyzer Wet Station™* Adding Storage Solution to Wells

96- Deep Well Plate

Step 4

Line up the Aligment Pins on the Capillary Array Frame with the Holes in the *Fragment Analyzer Wet Station™* Base

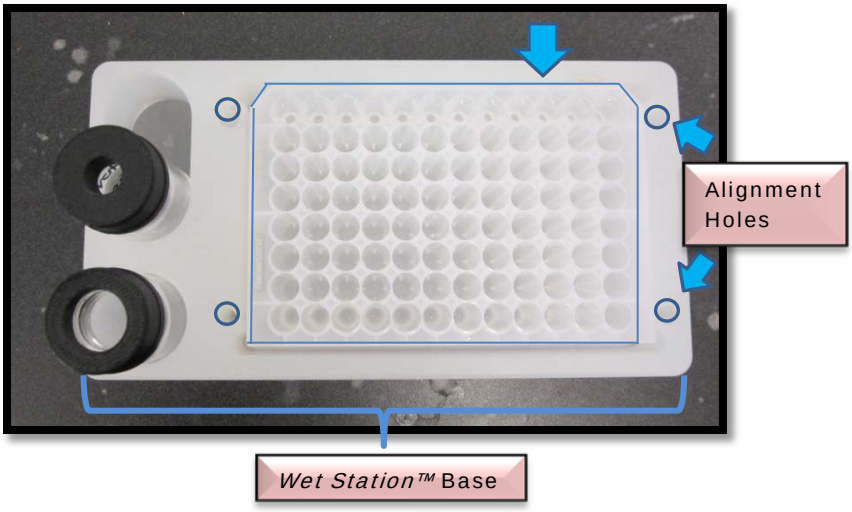


Figure 8-65. *Fragment Analyzer Wet Station™* Align Capillary Array Pins with Holes

Step 5

Carefully Align the Capillary Electrodes with the wells of the 96-Deep Well Plate.

Lower the Capillary Array Cartridge until it is Resting on top of the 96-Deep Well Plate

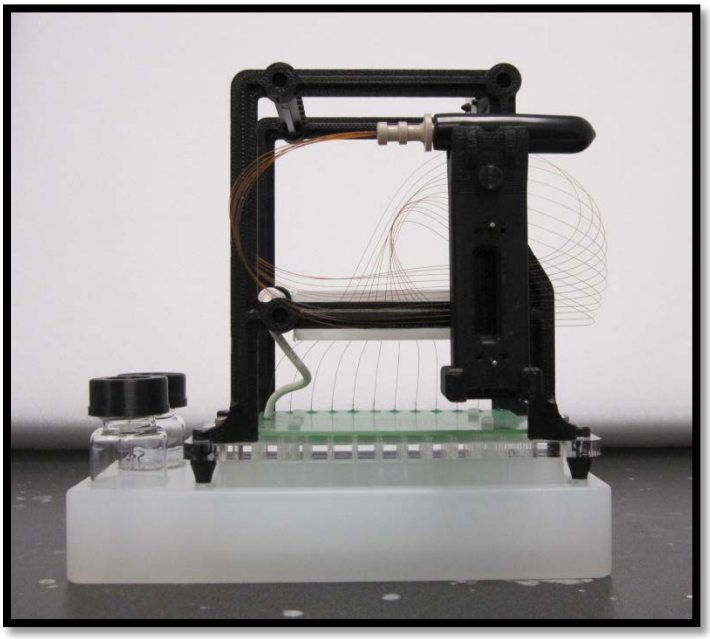


Figure 8-66. *Fragment Analyzer Wet Station™* 12-Capillary Array in Wet Station

Step 6

Fill the Capillary Array Storage Bottle with Storage Solution.

Use the Storage Bottle with the Small Lid Opening for Storage of the 12-Capillary Array

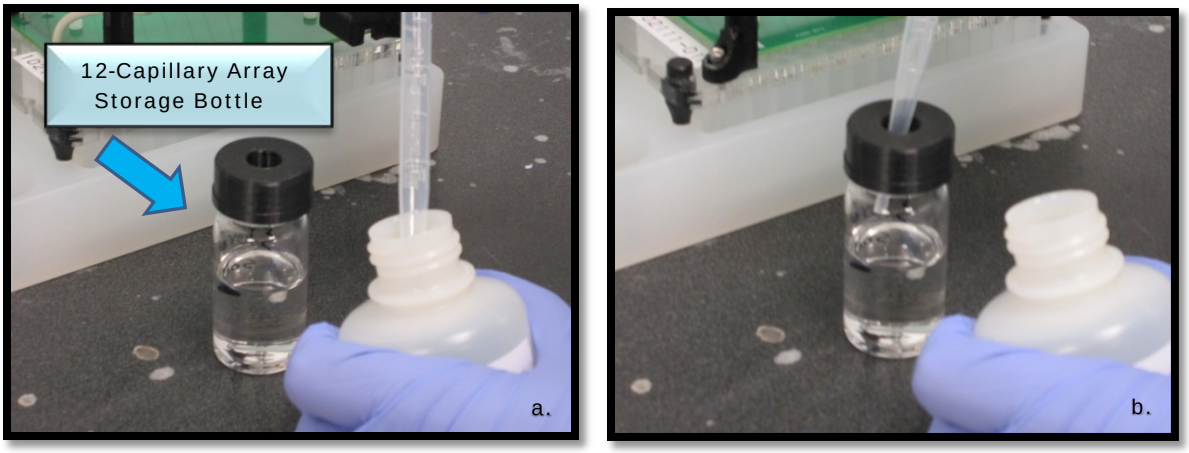


Figure 8-67a and b. *Fragment Analyzer Wet Station™* Fill Storage Bottle with Storage Solution

Step 7

Remove the Covered Capillary Array Bundle from the Holder on the Array Frame

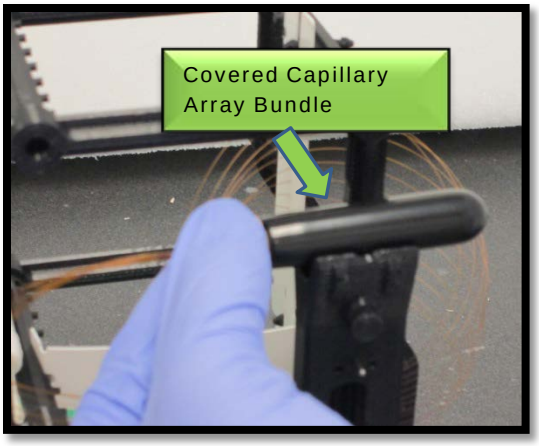


Figure 8-68. Remove Covered Array Bundle

Step 8a

Remove the Cover from the Capillary Array Bundle

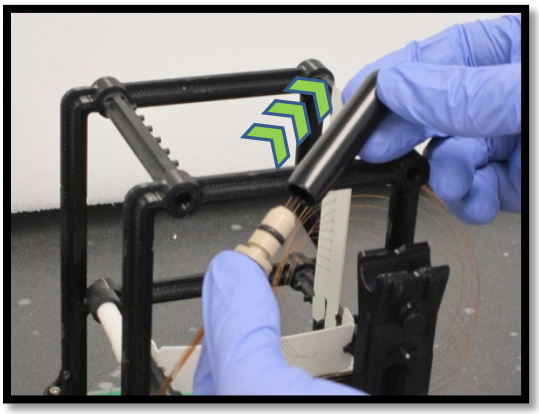


Figure 8-69. Remove Cover from Array Bundle

Step 8b

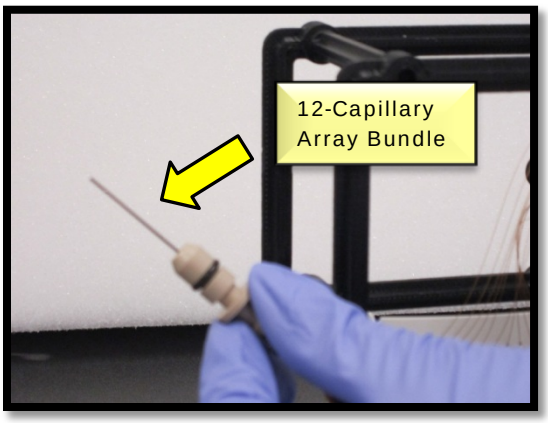


Figure 8-70. Uncovered 12-Capillary Array Bundle

Step 9

Place the Array Bundle Cover back on the Holder located on the Array Frame

Carefully Insert the Array Bundle into the Storage Bottle

Take Care Not to Touch the Capillary Array Bundle to the Top or Sides of the Storage Bottle

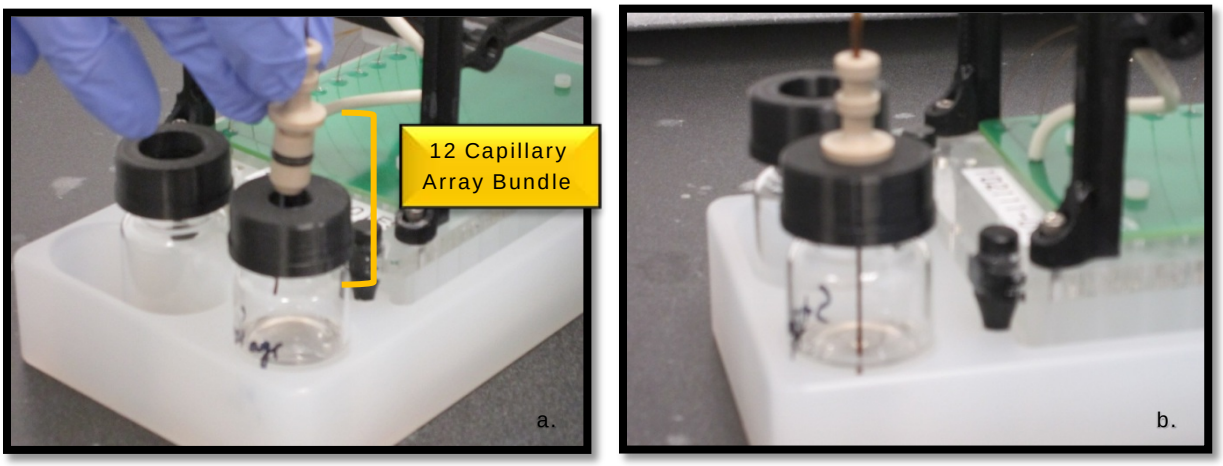


Figure 8-71a and b. *Fragment Analyzer Wet Station™* 12-Capillary Array Bundle in Storage Bottle

Step 10

Press Capillary Array Bundle Down Firmly into the Lid of the Array Storage Bottle to Prevent Evaporation of the Storage Solution.

The Capillary Array Cartridge can now be Stored up to 30 days.

After 30 days, Discard the Old Storage Solution in the *Wet Station™* and Replace with New Solution to Store the Array for an Additional 30 days

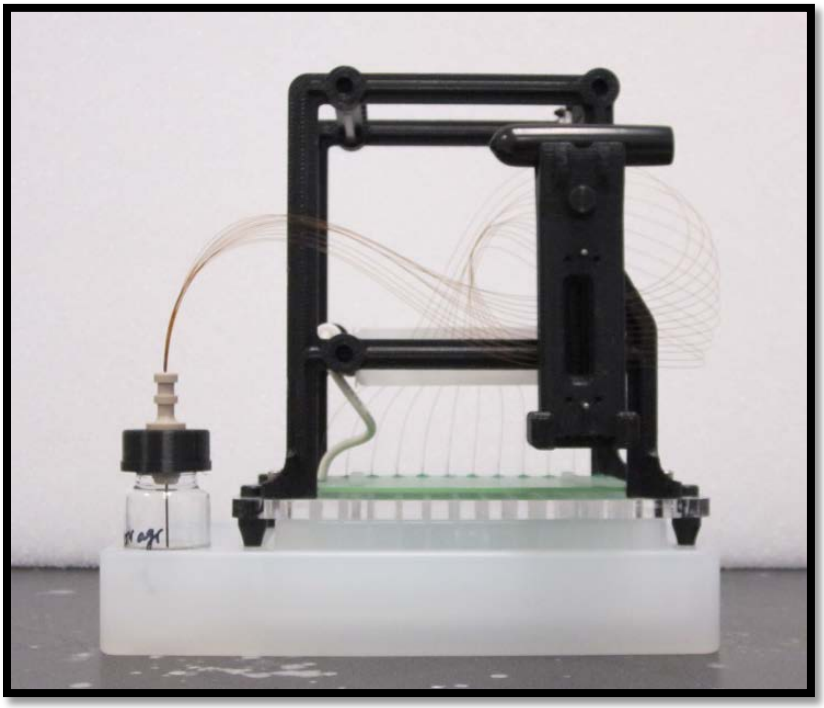


Figure 8-72. *Fragment Analyzer Wet Station™* 12-Capillary Array Cartridge in Long-term Storage

Trouble Shooting

In This Chapter

- Overview
  - ✓ Unclogging a Capillary Array
    - Method 1 – Flush Array with HPLC Grade Water
    - Method 2 – Immerse Capillary Array Tips in Hot Water (150 ˚F – 200 ˚F)
    - Method 3 – Flush Array with 1 N NaOH Solution
  - ✓ *FRAGMENT ANALYZER*™ Trouble Shooting Tables

This chapter provides step-by-step instructions on the methods used to unclog a capillary array.

It will also cover trending information for customization of CE separation methods and Kit Specific Tables that outline other potential problems that could occur with the *FRAGMENT ANALYZER*™ instrument.

Fragment Analyzer™ Troubleshooting

Unclogging a Capillary Array

Capillaries may become clogged due to debris, incorrect storage conditions, contamination of the gel, dilution buffer or sample matrix.

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

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

- Method 1 - Flush with HPLC Grade Water
- Method 2 - Submersion of Capillary Array Tips/Electrodes in Hot Water (150 ˚F – 200 ˚F)
- Method 3 - Flush with NaOH

The methods discussed in this chapter will be illustrated with images from a 12-Capillary Array. All of the method steps outlined for unclogging an array will be the same for a 96–Capillary Array unless otherwise noted.

Method 1 - Flush with HPLC Grade Water

When a capillary array is suspected to have one or more clogged capillaries, the **first step** is to **flush the array with HPLC Grade Water.**

From the **Operations Tab** located on the **Main Screen Window**, select **[Edit Method...]** under the **Capillary Array-Conditioning Commands Menu.** Fig. 9-1

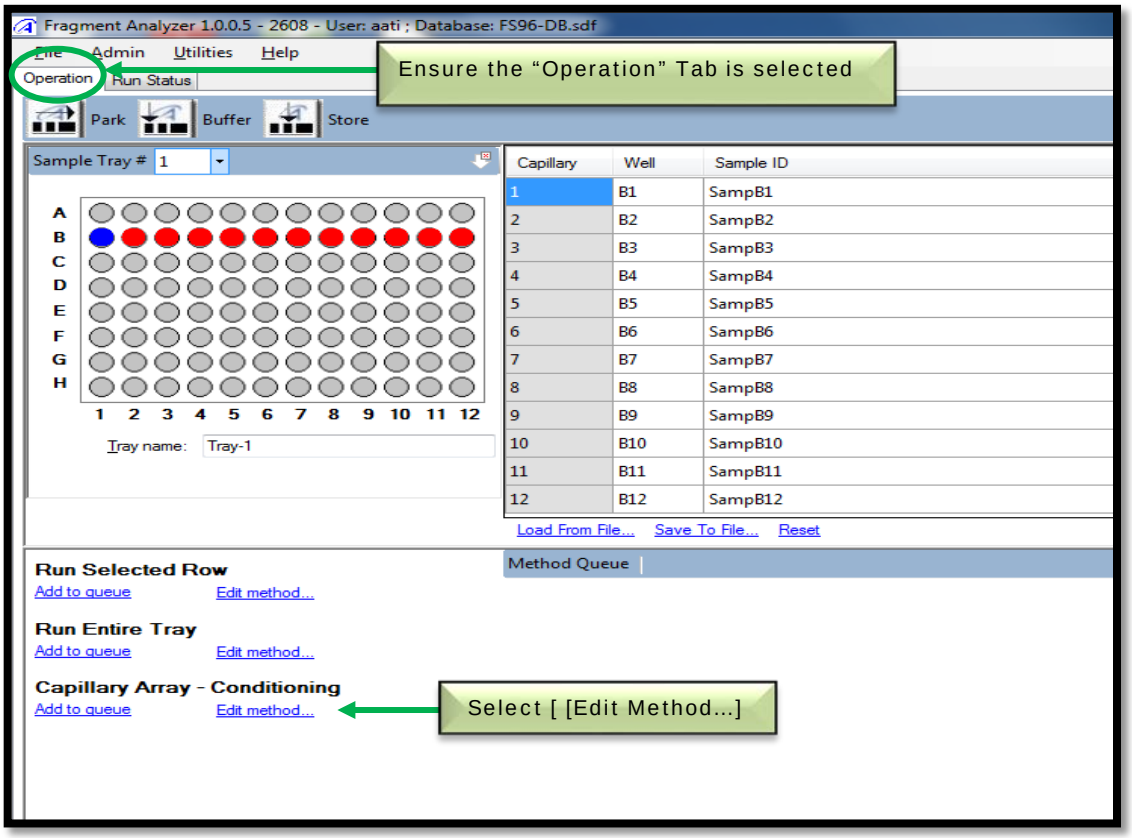


Figure 9-1. Main Screen Window: Capillary Array – Conditioning Commands

From the **Conditioning Method Selection Window**, select the **[Default Conditioning.mthdc]** from the **Method Field Dropdown Menu.** Fig. 9-2

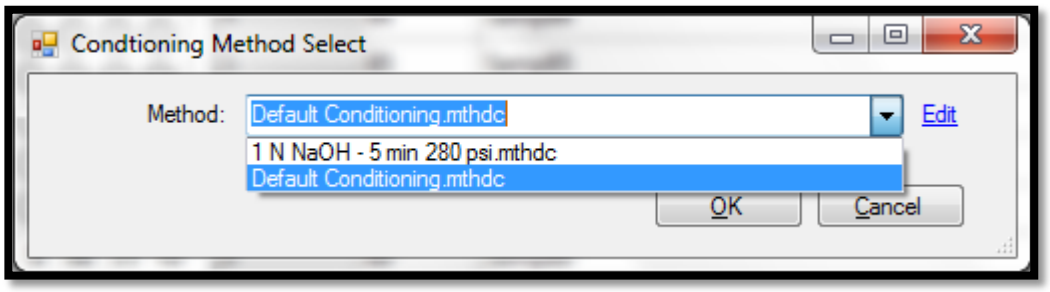


Figure 9-2: Conditioning Method Selection Window

Once the **Default Conditioning Method** has been selected, click the **[Edit]** command to **open the method parameters window.** Fig. 9-3

Adjust the **Method Parameters** to reflect the values listed below:

- **Step #1:** Checked
- **Solution:** Conditioning
- **Fill Pressure:** 200 PSI
- **Flow Rate:** 200 uL/s
- **Time:** 5.0 min

The **Time Parameter** can be **increased from 5 min to 10 min** in order to flush the capillary array for a longer period of time.

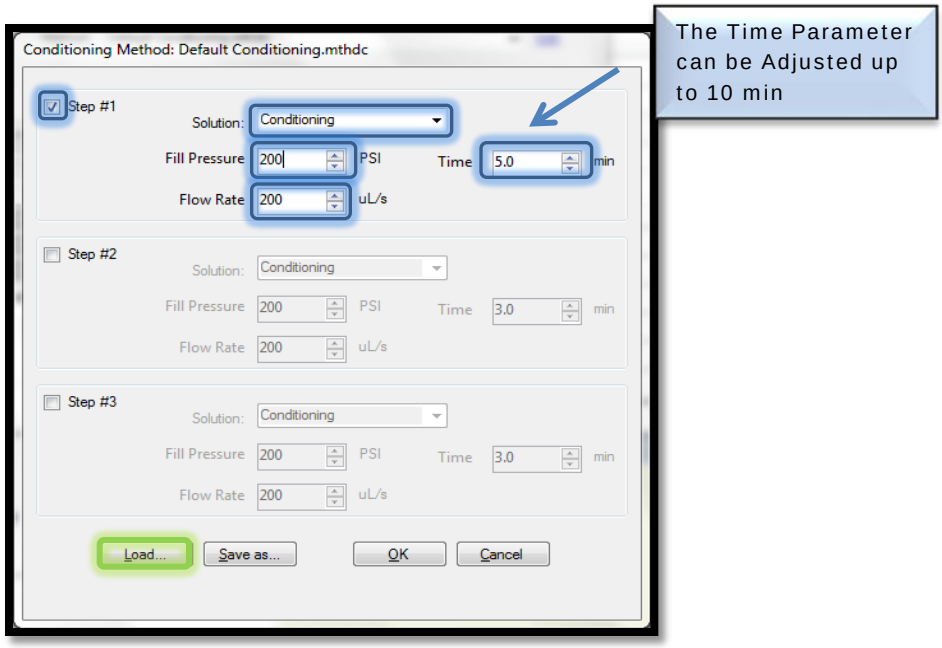


Figure 9-3: Conditioning Method Parameters Window

After the **Method Parameters** have been **adjusted**, select **[Load...]** to **add** the **Conditioning Method** to the **Method Queue**. Fig 9-4

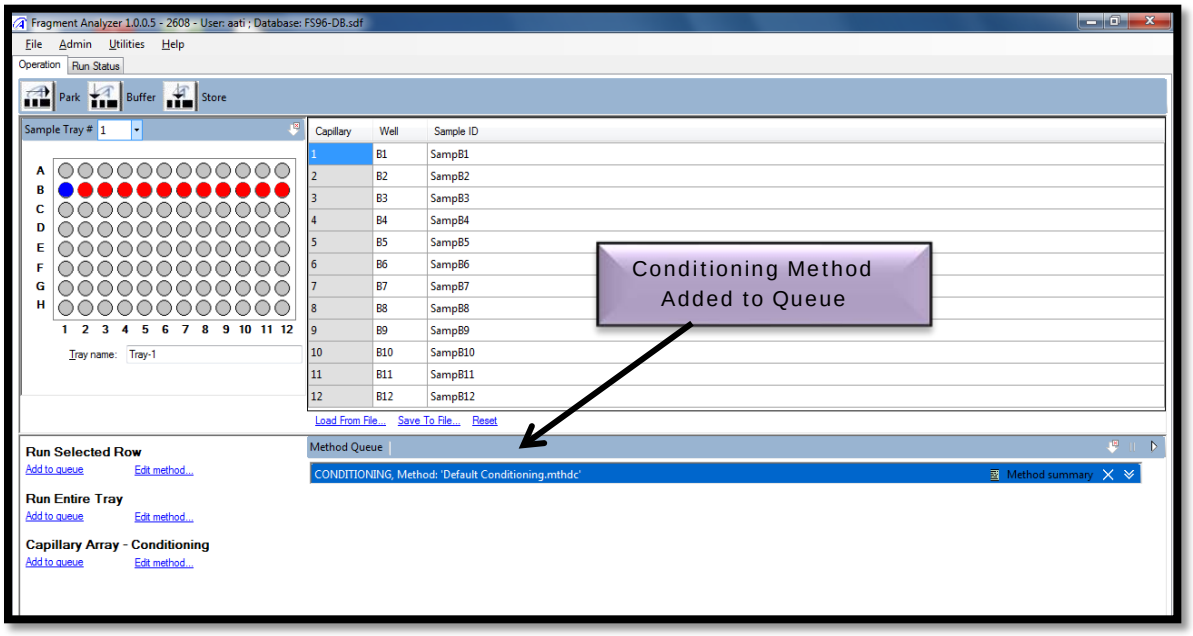


Figure 9-4: Conditioning Method Added to Queue

The next step is to open the Drawer W (Second Drawer from top) and place an empty 96-Well Deep Well Plate onto the plate holder. Fig 9-5.

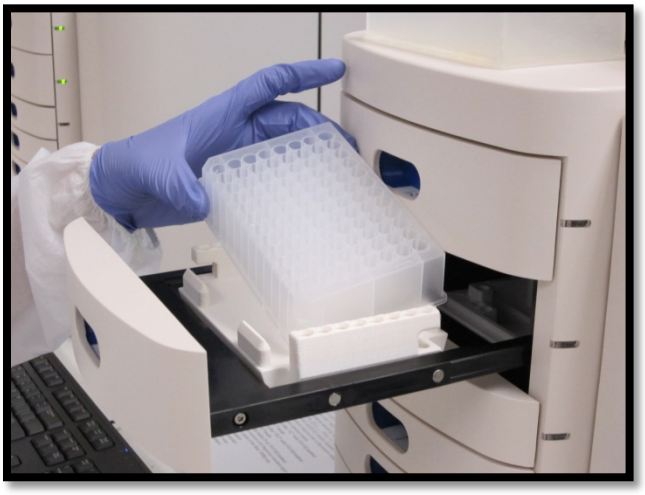


Figure 9-5: Drawer W. Placement of Empty 96-Well Deep Well Plate

This drawer location will be used as a Waste Tray when performing the Default Capillary Conditioning Method.

The 96-Well Deep Well Plate will capture the HPLC Grade Water that is flushed through the capillary array.

Prior to starting, open the *FRAGMENT ANALYZER™* Side Compartment to replace the Capillary Conditioning Solution with a bottle containing HPLC Grade Water. Fig. 9-6

⚠ The minimum solution volume required to run the default Capillary Conditioning Method for 10 minutes **is ≥** 10 ml for a 12 Capillary Array and **≥** 50 ml for a 96 Capillary Array.

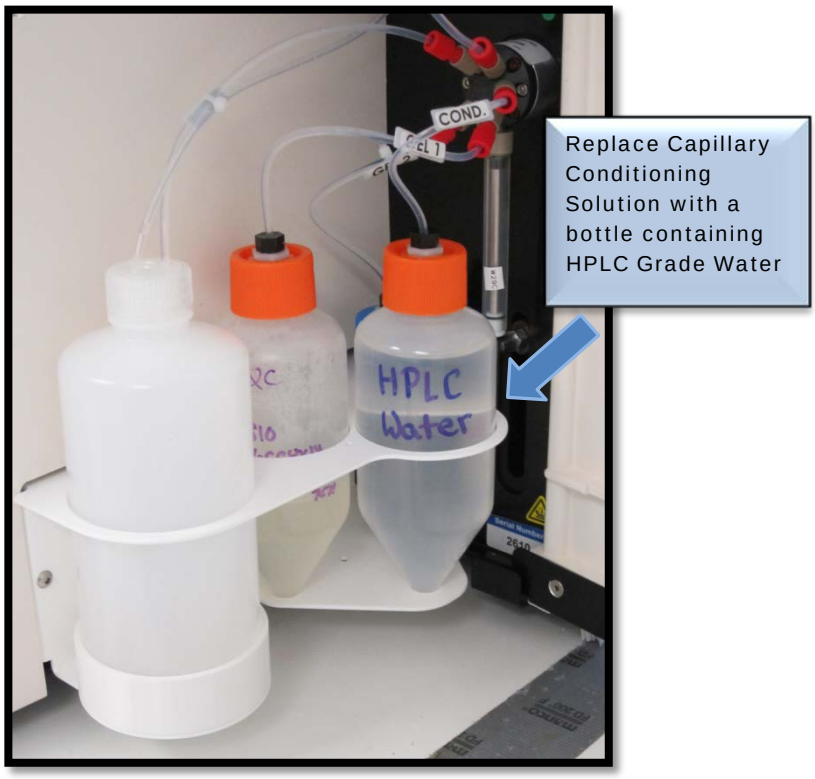


Figure 9-6: Instrument Side Compartment.
Replace Capillary Conditioning Solution with Water

After the **Conditioning Solution** has been **replaced, close the door** to the **Instrument Side Compartment** and select the **Start Icon** from the **Method Queue** to run the Capillary Conditioning Method. Fig. 9-7

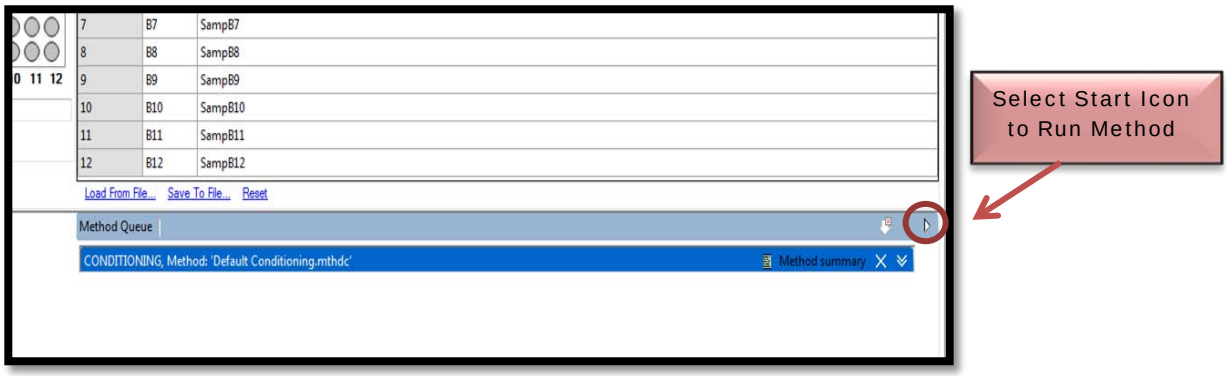


Figure 9-7: Method Queue: Selecting the Start Icon

Once the Capillary Array Conditioning Method is complete, open Drawer W and remove the 96-Well Deep Well Plate. Fig. 9-8

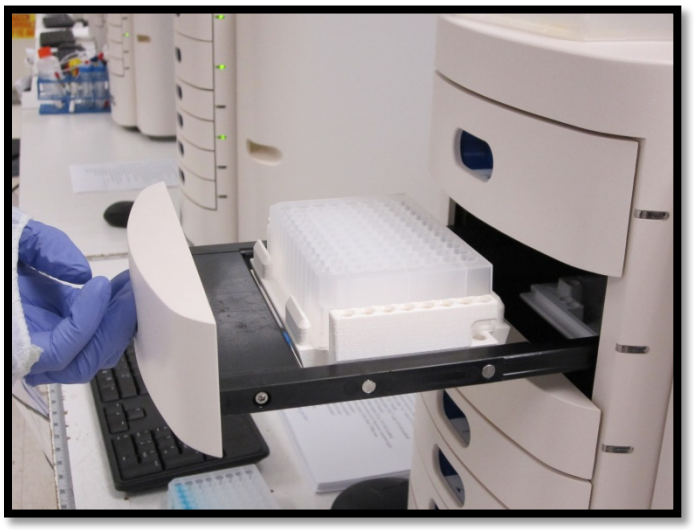


Figure 9-8: Drawer W: Removing the Waste Plate

Hold the 96-Well Plate up to the light and look up through the bottom of the plate to check the volume of water present in each of the wells. Fig 9-9.

- For a 12-Capillary Array, Row A1-A12 should have similar amounts of solution present in each well.
  - For a 5 min flush there should be ~75 µL of HPLC grade water in each well
  - For a 10 min flush there should be ~ 150 µL of HPLC grade water in each well
- For a 96-Capillary Array, all rows within the plate should have similar amounts of solution present in each well as those listed above.

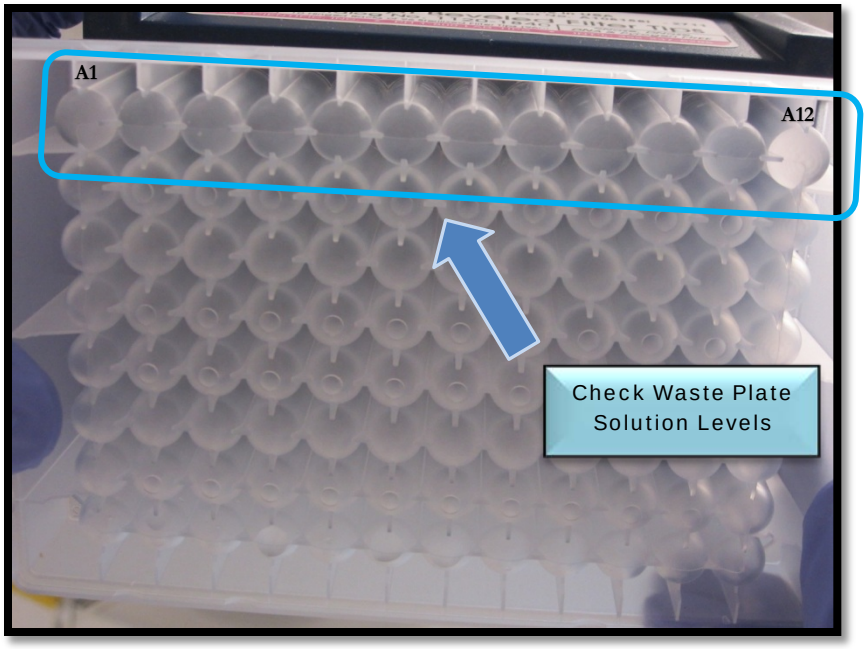


Figure 9-9: 12-Capillary Array: Verify Solution is Present and Volumes are Consistent between Wells

If a well has significantly less solution present or no solution present in a well, Method 1 can either be repeated or proceed to Methods 2 and 3.

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

- Empty the 96-Well Deep Well Plate and return it to Drawer W (2nd Drawer from the top).

- Open the Side Compartment of the *FRAGMENT ANALYZER*™ instrument and replace the bottle containing HPLC Grade Water with a bottle containing sufficient Capillary Conditioning Solution and perform a Capillary Conditioning as described in Chapter 3, pages 11-16 to remove any residual water from the capillaries.

- Check the 96-Well Deep Well Plate after the Capillary Conditioning Method is complete to ensure the capillaries have remained open.

From the **Main Screen Window**, locate the **Hotel Positioning Icons** under the **Operation Tab**. Fig. 9-10

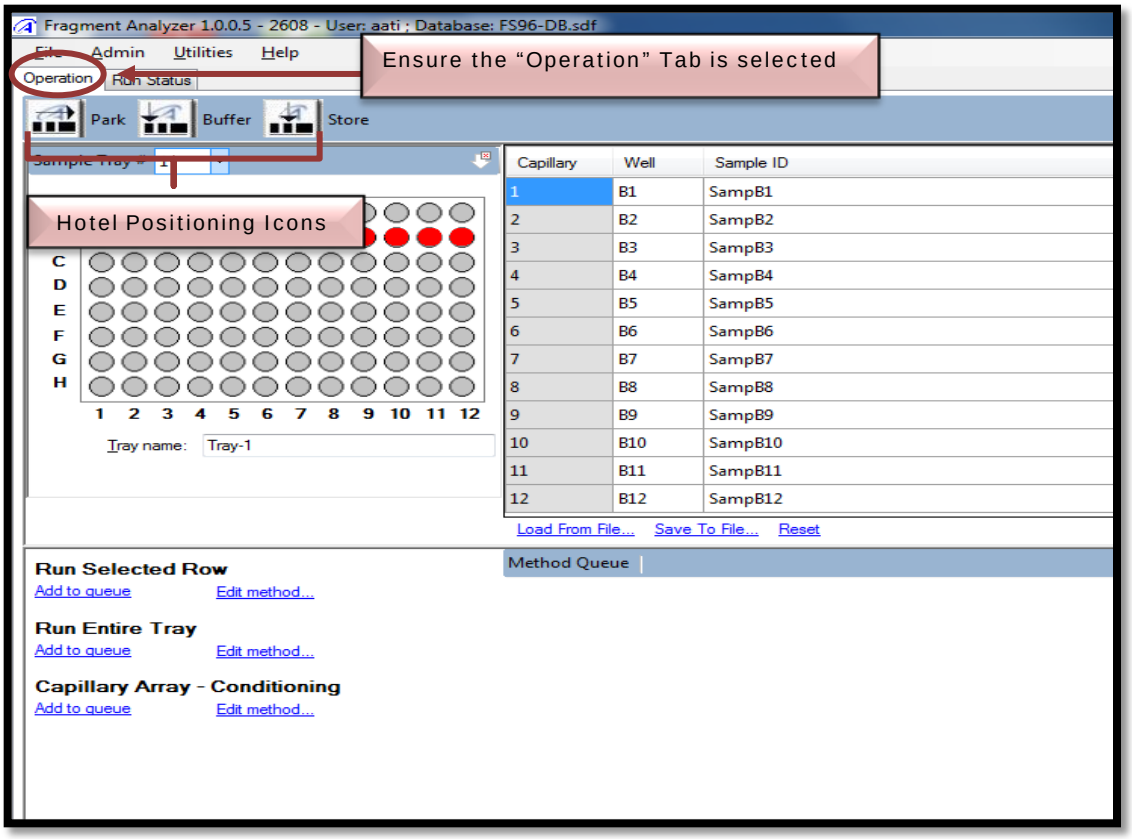


Figure 9-10. Main Screen Window: Hotel Positioning Icons

Select the **[Park Icon]** to place **all Sample, Waste, and Buffer Trays in their drawers.** It also moves the **Sample Stage** to the bottom of the instrument. Figures 9-11 and 9-12.

Figure 9-11. Park Icon

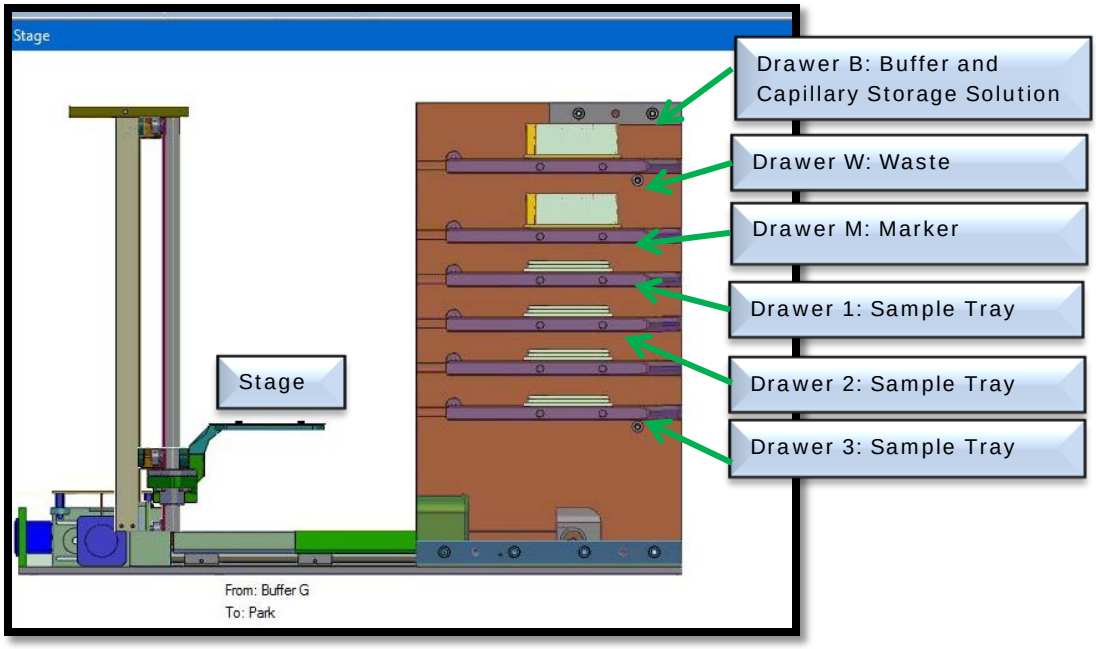


Figure 9-12. The "Park" Position. The stage is located at the bottom of the instrument and all samples/buffer plates are in tray positions.

- For a 12-Capillary Array - fill each well in Row H of a 96-Well Deep Well Plate with 1 ml of Hot Water (150˚F to 200 ˚F) for soaking the tips of the capillary array. (Fig. 9-13)

- For a 96-Capillary Array, fill each well of the 96-Well Deep Well Plate with 1 ml of Hot Water (150˚F to 200 ˚F).

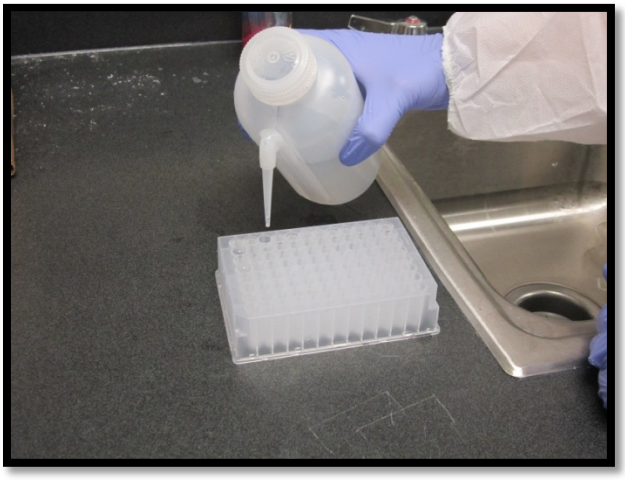


Figure 9-13. Fill 96-Well Deep Well Plate with Hot Water (150˚F to 200 ˚F)

Open Drawer B (1st Drawer from top) and place the hot water (150˚F - 200˚F) filled 96-Well Deep Well Plate onto the plate holder. Fig 9-14.

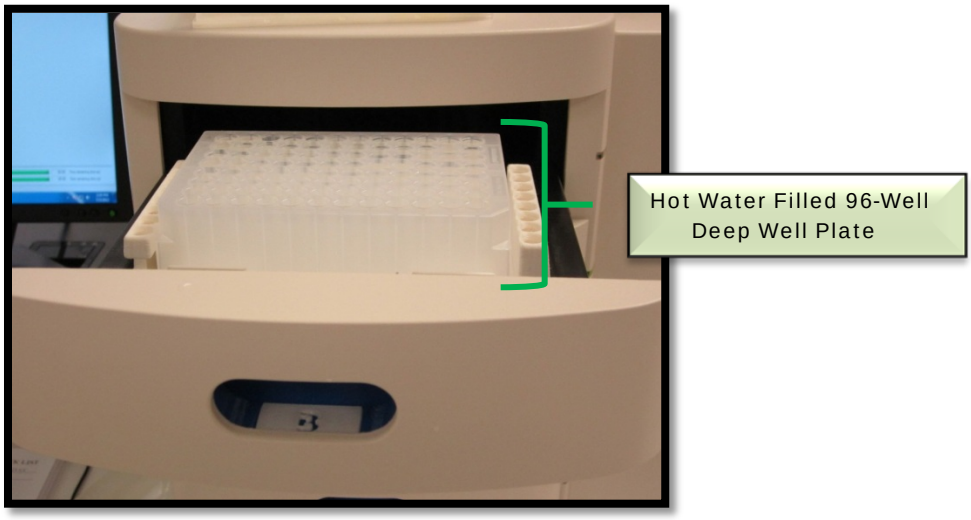


Figure 9-14. Drawer B: Place Hot Water Filled 96-Well Deep Well Plate onto Plate Holder

After the 96-Well Deep Well Plate has been placed in Drawer B and the drawer has been closed securely, 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, as shown in Figures 9-15 and 9-16.

Figure 9-15. Buffer Icon

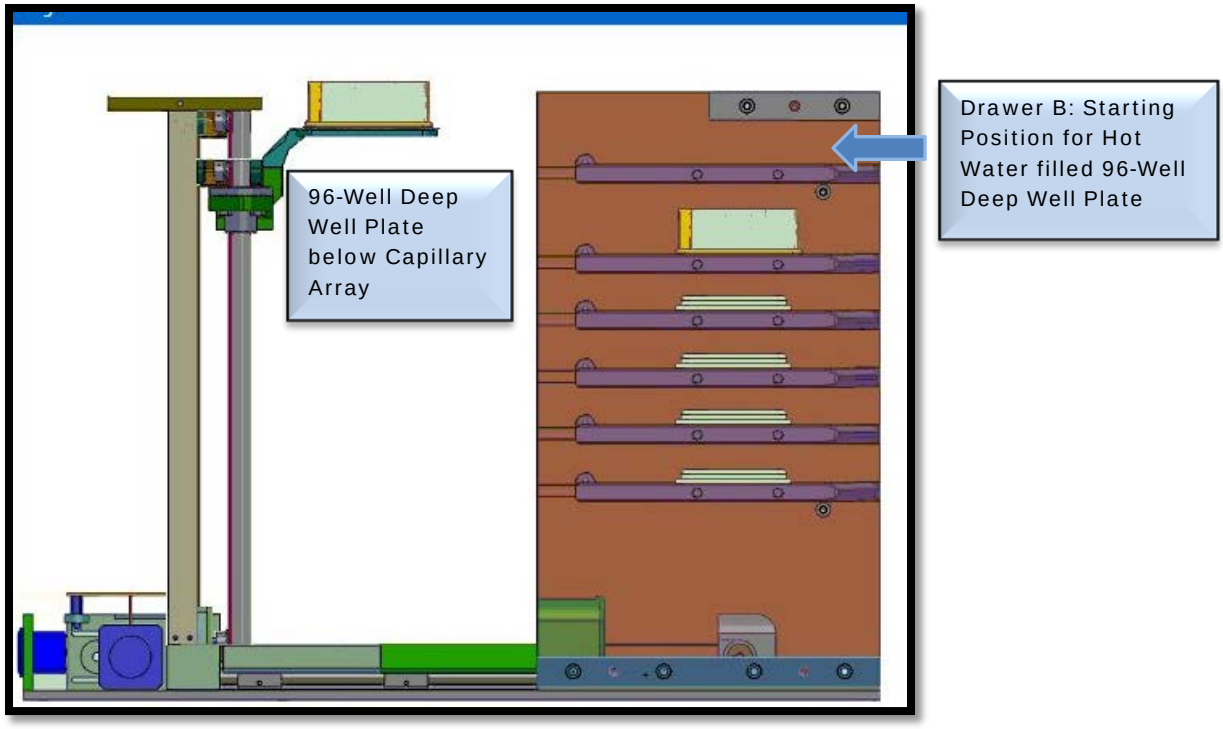


Figure 9-16. The "Buffer" Position: Hot Water Filled 96-Well Deep Well Plate Positioned Under the Capillary Array

Allow the Capillary Array to soak for a minimum of 45 minutes to an hour.

Then select the Park Icon to return the 96-Well Deep Well Plate to Drawer B and place the Stage in a resting position at the bottom of the instrument

Perform Method 1 - Flush with HPLC Grade Water as described on pages 3 – 9 of this chapter to check the flow of solution through each capillary or proceed directly to Method 3 – 1 N NaOH flush.

⚠ Method 3 – 1 N NaOH Flush is the most aggressive means of unclogging a capillary array. Use of this method on the same array on a regular basis can result in a reduced usable capillary array lifespan.

Method 3 - Flush with NaOH

From the **Operations Tab** located on the **Main Screen Window**, select **[Edit Method...]** under the **Capillary Array-Conditioning Commands Menu.** Fig. 9-17

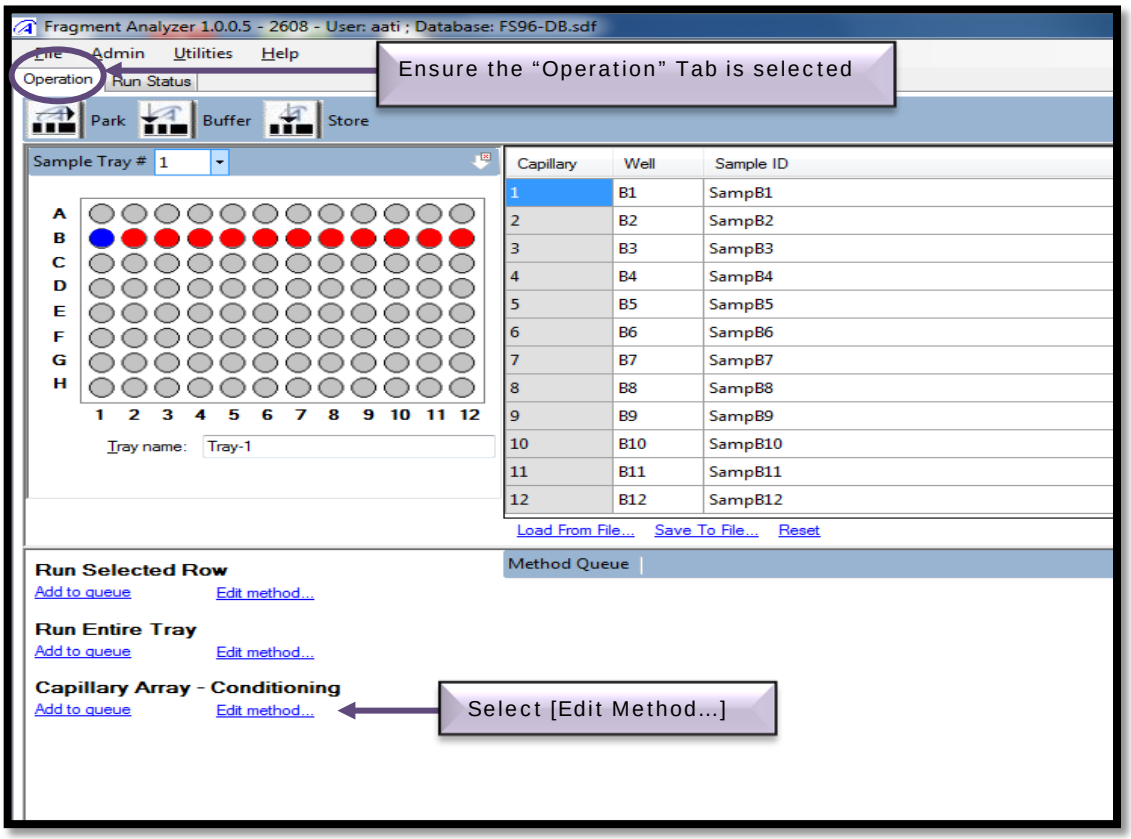


Figure 9-17. Main Screen Window: Capillary Array – Conditioning Commands

From the **Conditioning Method Selection Window**, select the method **[1 N NAOH - 5 min 280 psi.mthdc]** from the **Method Field Dropdown Menu.** Fig. 9-18

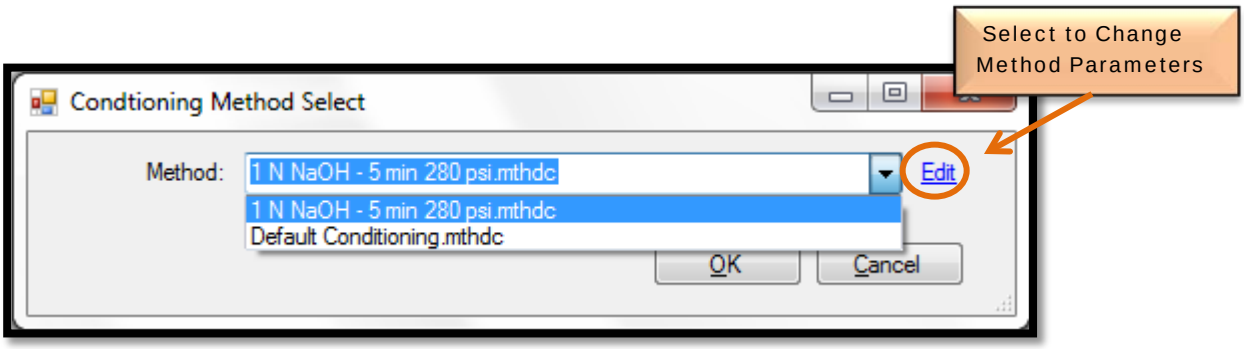


Figure 9-18: 1 N NaOH Method Selection Window

Once the **1 N NaOH Method** has been selected, click the **[Edit]** command to **open** the **Method Parameters Window.** Fig. 9-19

Change the **Method Parameters** to reflect the values listed below:

- **Step #1:** Checked
- **Solution:** Gel 2
- **Fill Pressure:** 200 PSI
- **Flow Rate:** 200 uL/s
- **Time:** 5.0 min

The **Time Parameter** can be **increased from 5 min to 15 min** in order to flush the capillary array for a longer period of time.

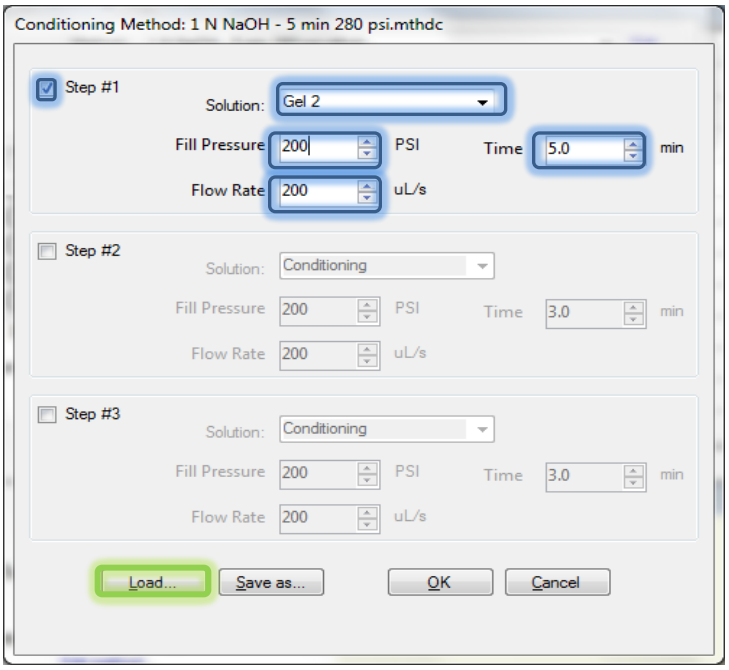


Figure 9-19: 1 N NaOH Method Parameters Window

After the **Method Parameters** have been **changed**, select **[Load...]** to **add** the **1 N NaOH Method** to the **Method Queue**. Fig 9-20

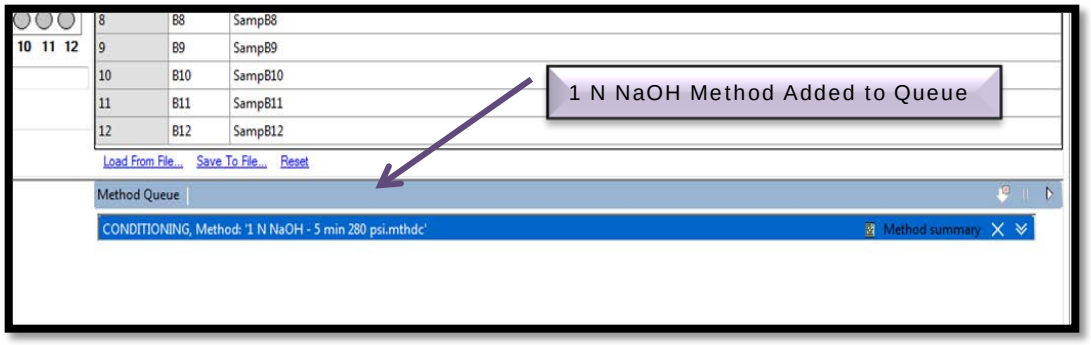


Figure 9-20: 1 N NaOH Method Added to Queue

The next step is to open the Drawer W (Second Drawer from top) and place an empty 96-Well Deep Well Plate onto the plate holder. Fig 9-21.

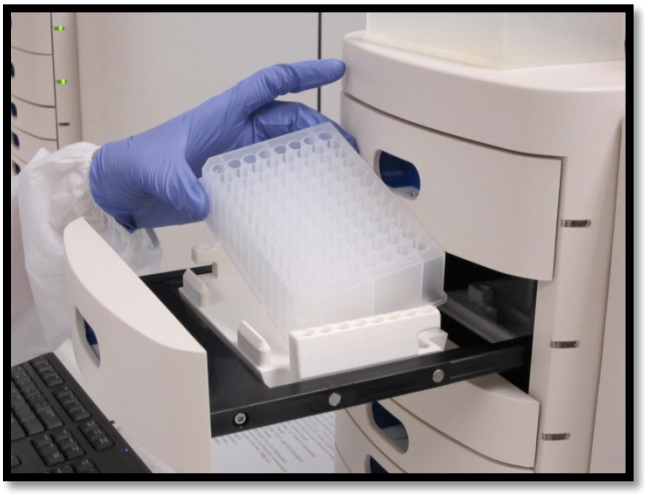


Figure 9-21: Drawer W. Placement of Empty 96-Well Deep Well Plate

The 96-Well Deep Well Plate will capture the 1 N NaOH solution that is flushed through the capillary array.

Next, open the *FRAGMENT ANALYZER™* Side Compartment to place a bottle containing 1 N NaOH into the Gel 2 Position. Fig. 9-22

⚠ The minimum solution volume required to run the 1 N NAOH Method for 15 minutes **is ≥** 15 ml for a 12 Capillary Array and 50 ml for a 96 Capillary Array.

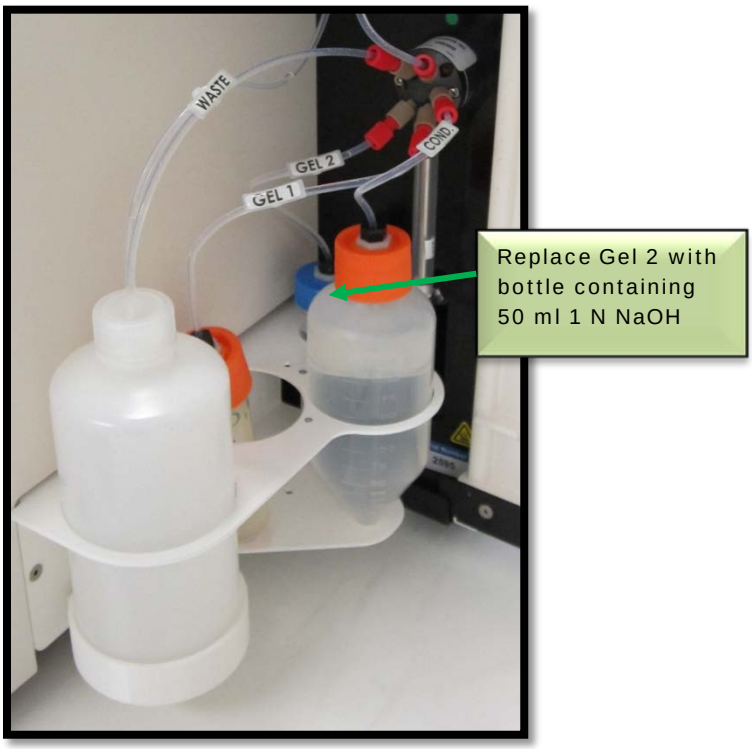


Figure 9-22: Instrument Side Compartment.
Replace Gel 2 with 1 N NaOH Solution

After the bottle containing Gel 2 has been replaced with 1 N NAOH solution, close the door to the Instrument Side Compartment and select the Start Icon from the Method Queue to run the NaOH Method. Fig. 9-23

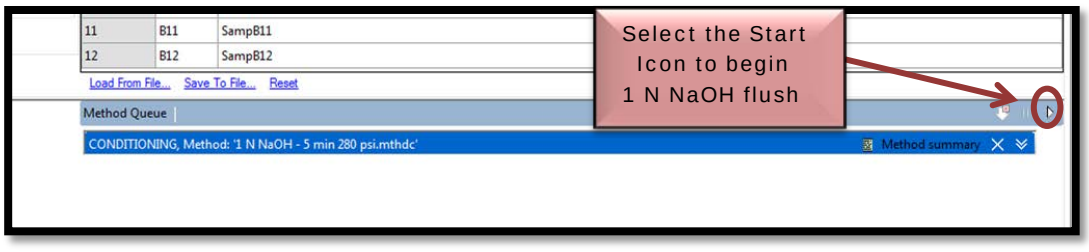


Figure 9-23: Method Queue: Selecting the Start Icon

Once the NaOH Method is complete, open Drawer W and remove the 96-Well Deep Well Plate. Fig. 9-24

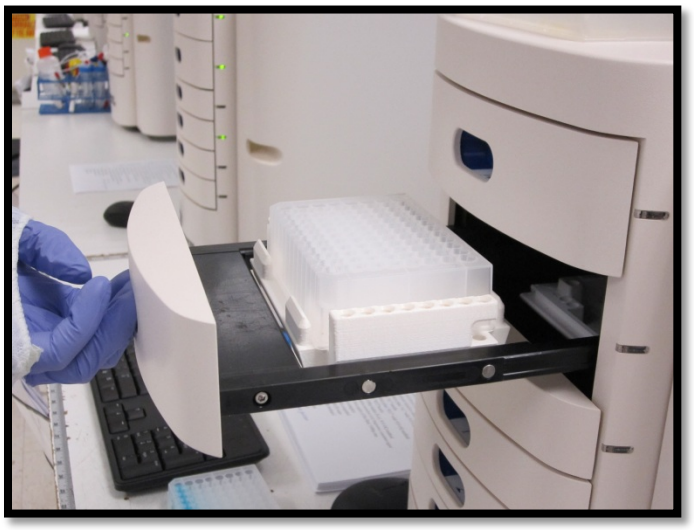


Figure 9-24: Drawer W: Removing the Waste Plate

Hold the Waste Plate up to the light and look up through the bottom of the plate to check the volume of NaOH solution present in each of the wells. Fig 9-25.

- For a 12-Capillary Array, Row A1-A12 should have similar amounts of solution present in each well.
  - ~ 75 µL per well for a 5 minute flush
  - ~150 µL per well for a 10 minute flush
  - ~250 µL per well for a 10 minute flush

- For a 96-Capillary Array, all rows within the plate should have similar amounts of solution present in each well, similar to those listed above.

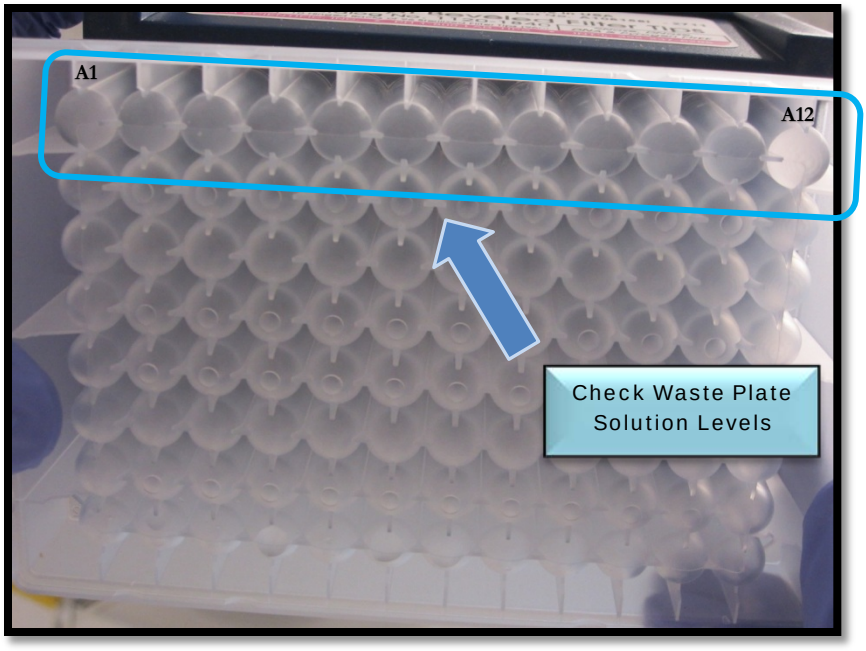


Figure 9-25: 12-Capillary Array: Verify Solution is Present and Volumes are Consistent Between Wells

If the plate has similar amounts of NaOH solution in each well:

- Empty the 96-Well Deep Well Plate and return it to Drawer W (2nd Drawer from the top).
- Open the Side Compartment of the *FRAGMENT ANALYZER*™ instrument and replace the bottle containing 1 N NaOH solution with a bottle containing Gel 2.
- Check to ensure there is sufficient Capillary Conditioning Solution to perform a Capillary Conditioning as described in Chapter 3, pages 11-16 to remove any residual NaOH solution from the capillaries.
- Check the 96-Well Deep Well Plate after the Capillary Conditioning Method is complete to ensure the capillaries have remained open.

If a well has significantly less solution present or no solution present in a well, contact AATI Technical Support by phone at (515)-296-6600 or by email at tech-support-ce@aati-us.com for further assistance.

Fragment Analyzer™ Troubleshooting

Table 9-1: Mutation Discovery Kit/s

Mutation Discovery Kit/s	#DNF-920-K0500T #DNF-910-K0500T #DNF-480-3000	
Issue	Cause	Corrective Action
A. No cut DNA fragment signals AND saturated uncut DNA signal for known mutation control/sample.	1. Input DNA concentration too high relative to Enzyme concentration. Ensure total signal height does not exceed 10,000 RFU for uncut DNA. 2. No hetero-duplex formed.	1. Dilute input DNA sample concentration and repeat digestion with same Enzyme concentration. 2. Check if any hetero-duplexes are being formed or not. Digest only the heterozygous mutant or 1:1 mixture of homozygous mutant and wild-type.
B. No signals for cut DNA fragments NOR uncut DNA fragment. Marker peaks are visible.	1. Input DNA concentration too low or failed PCR. 2. Failed injection of the sample. 3. Enzyme concentration too high compared to input DNA concentration, causing non-specific digestion of majority of input DNA present.	1. Check the PCR product concentration/quality using *Fragment Analyzer*™ system. 2. Check for the presence of air bubbles in the sample well. Centrifuge the plate and ensure no air bubbles are in the sample wells. 3. If input DNA concentration is verified to be sufficient, decrease the enzyme concentration and/or decrease the digestion time.

C. Little or no cut DNA fragment signals when expected AND uncut DNA signals visible and less than 10,000 RFU.	1. No detectable levels of cut DNA fragments due to lowered enzyme efficiency. 2. No heteroduplex formed. 3. Mutant allele might be more dilute than maximum recommended of 1:16. 4. Significant levels of primer-dimers inhibiting the enzyme efficiency. 5. Significantly higher amount of gDNA used for PCR, inhibiting the cleavage efficiency.	1. Increase the enzyme concentration and/or digestion time. Verify expiration date of enzyme. 2. Check if any heteroduplexes are being formed or not. Digest only the heterozygous mutant or 1:1 mixture of homozygous mutant and wild-type. 3. Check the ratio of pooled alleles. Ensure minimum of 1 mutant present in total of 16 alleles. 4. Data from *Fragment Analyzer*™ system can reveal the amount of primer-dimers present in the sample. Optimize primer and PCR design to reduce primer-dimers. 5. Optimize PCR to utilize decreased amount of gDNA.
D. No cut DNA fragment signals AND very low uncut DNA signal.	1. DNA concentration is significantly lower than recommended.	1. Optimize a more robust PCR to yield at least 25 ng/uL of uncut DNA. 2. Increase the injection time. 3. Decrease the digestion time and enzyme concentration.
E. Background noise comparable to cut DNA fragment signals.	1. Increased non-specific digestion. 2. Sample may be 'dirty': too much residual gDNA, dNTPs, primers etc.	1. Decrease the digestion time from 45 min to 30 min. Decrease the Enzyme concentration. 2. Additional sample clean up or PCR optimization may be necessary.

F. No signals from cut DNA fragments, uncut DNA or markers.	1. Failed injection. 2. Non-functioning/plugged capillary.	1. Check for the presence of air bubbles in the sample wells. 2. Centrifuge the plate and ensure no air bubbles are in the sample wells. 3. If using new vendor/type of sample/marker plates, verify dimensions are compatible with tray spacer.

Table 9-2: Standard and High Sensitivity NGS Fragment Analysis Kit/s

Standard Sensitivity NGS Fragment Analysis Kit High Sensitivity NGS Fragment Analysis Kit	# DNF-479-0500 # DNF-486-0500	
Issue	Cause	Corrective Action
A. The peak signal is > > 20,000 RFU; upper marker peak is low or not detected relative to lower marker.	1. Input DNA sample concentration is too high. 2. Ensure total peak signal height does not exceed 20,000 RFU, or total input DNA concentration does not exceed recommended limits. 3. For the HS NGS Kit the concentration should not exceed 5000 pg/µl.	1. Dilute input DNA sample concentration with 1X TE buffer and repeat experiment; OR Repeat experiment using decreased injection time (e.g., 10 sec). OR If it an issue with the HS NGS kit: Prepare a fresh sample using the Standard Sensitivity Fragment Analysis Kit (#DNF-479-0500) which covers an input DNA range of 5-100 ng/µl.

B. DNA sample smear overlaps with Lower/Upper Marker peak.	1. Input DNA sample size distribution outside of assay range. 2. Input DNA sample concentration too high.	1. Perform further size selection of sample to narrow DNA size distribution and repeat experiment. Change *PRO Size*™ software to quantify with non-overlapping marker. 2. Dilute input DNA sample concentration with 1X TE buffer and repeat experiment.
C. No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.	1. Sample concentration too low and out of range. 2. Sample not added to Diluent Marker solution or not mixed well.	1. Prepare more concentrated sample and repeat experiment OR Repeat experiment using increased injection time and/or injection voltage OR If using Standard Sensitivity Kit, prepare fresh sample and analyze with High Sensitivity NGS Fragment Analysis Kit (# DNF-486-0500). 2. Verify sample was correctly added and mixed in sample well.

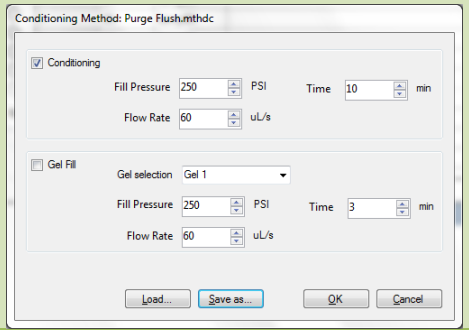
D. No sample peak or marker peak observed for individual sample.	1. Air trapped at the bottom of sample plate well, or bubbles present in sample well. 2. Insufficient sample volume. A minimum of 20 μL is required. 3. Capillary is plugged.	1. Check sample plate wells for trapped air bubbles. Centrifuge plate. 2. Verify proper volume of solution was added to sample well. 3. Check waste plate for liquid in the capillary well. If no liquid is observed, soak capillary array tips in hot water as outlined on pages XYZ –XYZ this chapter. Then perform **Purge Flush** by creating/saving the method shown below. Repeat if necessary. 

Table 9-3: Genomic DNA Analysis Kits

Genomic DNA Analysis Kit Standard Sensitivity Genomic DNA Analysis Kit High Sensitivity Genomic DNA Analysis Kit	# DNF-484-0500 # DNF-487-0500 # DNF-488-0500	
Issue	Cause	Corrective Action
A. Standard Sensitivity Kit: The measured total gDNA concentration is significantly higher than 200 ng/µL; size shifted lower (e.g., control intact gDNA << 20 kbp). B. High Sensitivity Genomic DNA Kit: The measured total gDNA concentration is significantly higher than 5 ng/µL; size shifted lower (e.g., control intact gDNA << 20 kbp).	1. Input gDNA sample concentration is too high.	1 Standard Sensitivity Kit: Ensure that the input gDNA conc. is not more than the maximum permissible concentration (200 ng/µL). High Sensitivity Kit: Ensure that the input gDNA conc. is not more than the maximum permissible concentration (5 ng/ µL). 2 Dilute input gDNA sample concentration with 1X TE buffer and repeat experiment. 3 For the High Sensitivity Kit: Re-prepare the sample and analyze using the Standard Sensitivity Analysis Kit (DNF-487-0500)
B. No peak observed for gDNA sample when expected. Lower Marker peak observed.	1. Sample highly degraded such that no dye intercalates. 2. Sample was not mixed homogenously before sampling. 3. Sample concentration too low and out of range.	1 Sample not suitable for use. 2 Heat the sample (45 ºC, 15 minutes) before taking out 2 µL from the stock samples. This will ensure homogenous sampling. 3 Prepare more concentrated sample and repeat experiment.

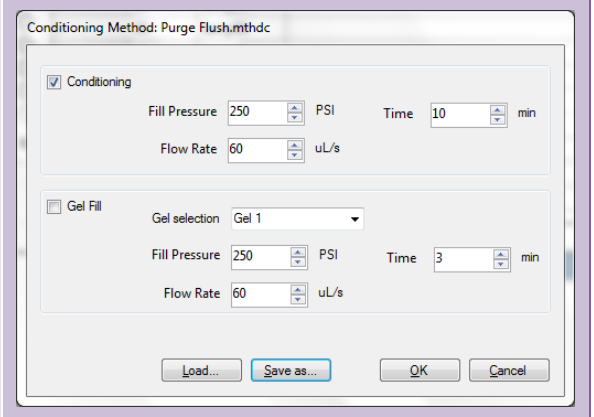
Issue	Cause	Corrective Action
C. Sample peak/smear migrates before or co-migrates with 300 bp Lower Marker.	1. Sample highly degraded.	1. Analyze sample using AATI Fragment Analysis Kit (size range from 35 – 6,000 bp). 2. Verify sample preparation guidelines are followed to prevent inadvertent degradation of gDNA sample.
D. No sample peak or Lower Marker peak observed for individual sample.	1 Air trapped at the bottom of sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20 μL is required. 3 Capillary is plugged.	1 Check sample plate wells for trapped air bubbles. Centrifuge plate. 2 Verify proper volume of solution was added to sample well. 3 Check the waste plate for the presence of liquid in the capillary well. If no liquid is in the respective well of the waste plate, soak capillary array tips in hot water as outlined in this chapter on pages XYZ and XYZ. Then perform **Purge Flush** by creating and saving the method shown below. Repeat if necessary. 

Table 9-4: DNF-900 and 910 Kits

DNF-900 dsDNA Kit/s	# DNF-900-5000 # DNF-900-K0500
DNF-910/15L80M Kit	# DNF-910/15L80M-K0500
DNF-910 dsDNA Kit/s	# DNF-910-5000 # DNF-910-K0500

Issue	Cause	Corrective Action
A. DNF-910-K0500 and DNF/15L80M-K0500 dsDNA kits: The peak signal is >> 20,000 RFU; upper marker peak is low or not detected relative to lower marker. B. DNF-910-5000 dsDNA Kit: The peak signal is >> 15,000 RFU; upper marker peak is low or not detected relative to lower marker.	1. Input DNA sample concentration is too high.	1. Further dilute input DNA sample concentration with 1X TE buffer and repeat experiment. 2. Reduce injection time and/or injection voltage, and repeat experiment. Use the same injection voltage/time settings for the Marker Plate and Sample Plate to maximize quantification accuracy.
C. No peak observed for DNA sample as expected. Lower/Upper Marker peaks observed.	1. Sample concentration too low and out of range. 2. Sample was not added to 1X TE diluent or not mixed well.	1. Prepare more concentrated sample and repeat experiment (e.g. 4 uL sample + 20 uL DI water); OR Repeat experiment using increased injection time and/or injection voltage for Marker Plate and Sample Plate. 2. Verify sample was correctly added and mixed in sample well.

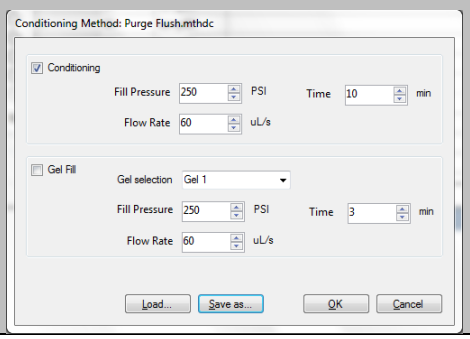
D. Sample peak(s) migrate before or co-migrate with 35 bp Lower Marker.	1. Excess primer-dimer species in sample.	1. Further dilute input DNA sample concentration with 1X TE buffer to minimize primer-dimer interference, and repeat experiment.
E. DNF-900 dsDNA Kits: Sample peak(s) migrate after or co-migrate with 500 bp Upper Marker. F. DNF-910/15L80M Kit: Sample peak(s) migrate after or co-migrate with 5,000 bp Upper Marker. G. DNF-910 dsDNA Kits: Sample peak(s) migrate after or co-migrate with 1,500 bp Upper Marker.	1. DNA sample size out of range of assay.	1. For DNF-900 dsDNA Kits analyze samples with: DNF-910 Kit (35bp – 1,500bp) DNF-910/15L80M Kit (35 bp – 5,000 bp) DNF-920 Kit (75 bp – 15,000 bp) DNF-930 Kit (75 bp – 20,000 bp) 2. For DNF-910/15L80M Kit analyze samples with: DNF-920 Kit (75 bp – 15,000 bp) DNF-930 Kit (75 bp – 20,000 bp) 3. For DNF-910 dsDNA Kits analyze samples with: DNF-910/15L80M Kit (35 bp – 5,000 bp) DNF-920 Kit (75 bp – 15,000 bp) DNF-930 Kit (75 bp – 20,000 bp)
H. No sample peak or Lower Marker peak observed for individual sample.	1. Air trapped at the bottom of sample plate well, or bubbles present in sample well. 2. Insufficient sample volume. A minimum of 20 μL is required. 3. Capillary is plugged.	1. Check sample plate wells for trapped air bubbles. Centrifuge plate. 2. Verify proper volume of solution was added to sample well. 3. Check the waste plate for the presence of liquid in the capillary well. If no liquid is in the respective well of the waste plate, soak capillary array tips in hot water as outlined in this chapter on pages XYZ and XYZ. Then perform **Purge Flush** by creating and saving the method shown below. Repeat if necessary. 

Table 9-5: DNF-920 and 930 Kits

DNF-920 dsDNA Kit DNF-930 dsDNA Kit/s	# DNF-920-K0500 # DNF-930-3000 # DNF-930-K0500	
Issue	Cause	Corrective Action
A. The peak signal is >> 20,000 RFU; upper marker peak is low or not detected relative to lower marker.	1. Input DNA sample concentration is too high.	1. Further dilute input DNA sample concentration with 1X TE buffer and repeat experiment. 2. Reduce injection time and/or injection voltage, and repeat experiment. Use the same injection voltage/time settings for the Marker Plate and Sample Plate to maximize quantification accuracy.
B. No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.	1. Sample concentration too low and out of range. 2. Sample was not added to 1X TE diluent or not mixed well.	1. Prepare more concentrated sample and repeat experiment (e.g. 4 uL sample + 20 uL DI water); OR Repeat experiment using increased injection time and/or injection voltage for Marker Plate and Sample Plate. 2. Verify sample was correctly added and mixed in sample well.
C. Sample peak(s) migrate before or co-migrate with 75 bp Lower Marker.	1. Excess primer-dimer species in sample.	1. Further dilute input DNA sample concentration with 1X TE buffer to minimize primer-dimer interference, and repeat experiment. 2. If fragment size is below 1,500bp, analyze using DNF-910 Reagent Kit (DNF-910-K0500; 35bp – 1,500bp range) to better resolve primer-dimer species.

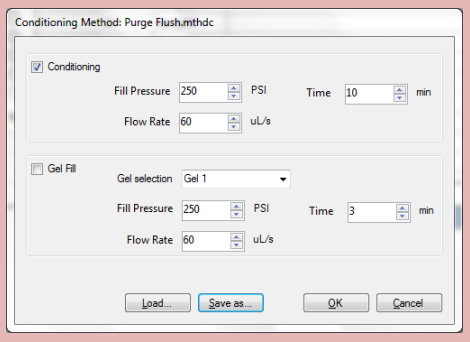
D. DNF-920 dsDNA Kit: Sample peak(s) migrate after or co-migrate with 15,000 bp Upper Marker. E. DNF-930 dsDNA Kits: Sample peak(s) migrate after or co-migrate with 20,000 bp Upper Marker.	1. DNA sample size out of range of assay.	1. Analyze samples with Genomic DNA Analysis Kit (DNF-484-0500), which contains no upper marker limit.
F. No sample peak or Lower Marker peak observed for individual sample.	1. Air trapped at the bottom of sample plate well, or bubbles present in sample well. 2. Insufficient sample volume. A minimum of 20 μL is required. 3. Capillary is plugged.	1. Check sample plate wells for trapped air bubbles. Centrifuge plate. 2. Verify proper volume of solution was added to sample well. 3. Check the waste plate for the presence of liquid in the capillary well. If no liquid is in the respective well of the waste plate, soak capillary array tips in hot water as outlined in this chapter on pages XYZ and XYZ. Then perform **Purge Flush** by creating and saving the method shown below. Repeat if necessary. 

Table 9-6: RNA Analysis Kits

Standard Sensitivity RNA Analysis Kit mRNA Analysis Kit Total RNA Analysis Kit	# DNF-489-0500 # DNF-483-0500 #DNF-482-0500	
Issue	Cause	Corrective Action
A. Sample and/or ladder signal too weak.	1. Sample and/or ladder degraded. 2. Sample, ladder and/or diluent marker are contaminated. 3. Sample concentration is too low and/or out of range. 4. Concentration of the Ladder is too low. 5. Sample not added to Diluent Marker solution or not mixed well. 6. RNA Diluent Marker was left at room temperature for too long and degraded.	1. Use fresh sample and/or ladder. 2. Clean working area and equipment with RNaseZap or 70% ethanol. Always wear gloves when preparing sample/ladder. Use new sample, ladder aliquot, and diluent marker. 3. Verify sample was within concentration range specified for the Standard Sensitivity RNA Analysis kit. Prepare sample at higher concentration. Repeat experiment using increased injection time and/or injection voltage. 4. Check the pipette settings and verify the pipette is calibrated. Verify sample was correctly added and mixed in sample well. 5. After RNA Diluent Marker is removed from refrigerator, keep in the dark and use within 2 days. 6. For the mRNA Analysis Kit DNF-483-0500: Check that the sample is within the concentration range specified for the mRNA kit and the ladder concentration is 3ng/µL. Check if sample is diluted 12X with diluent marker (2 µL sample in 22 µL diluent marker). 7. For the Total RNA Analysis Kit DNF-

		482-0500: Check that the sample is within the concentration range specified for the Total RNA kit and the ladder concentration is 60ng/µL. Check if sample is diluted 12X with diluent marker (2 µL sample in 22 µL diluent marker).
B. Missing 25S or 28S ribosomal peak	1. Sample concentration too high and out of range	1. Verify sample was within concentration range specified for the Standard Sensitivity RNA Analysis kit.
C. Split RNA peak	1. Sample's salt concentration was too high	1. Take steps to lower the salt content in the sample and repeat experiment.
D. Peak too broad, signal too low and/or migration time too long	1. Capillary array needs to be re-conditioned	1. Flush array with 1N NaOH solution and perform a full conditioning before repeating experiment. For step by step instructions please see this chapter, pages XYZ-XYZ.
E. Precipitates in RNA Diluent Marker did not dissolve completely at room temperature	1. Low room temperature	1. Warm the tube in your palms (with gloves on) and vortex the tube to help dissolve any precipitate

Technical Support and Contact Information

For questions with *Fragment Analyzer*™ operation, contact AATI Technical Support by phone at (515)-296-6600 or by email at tech-support-ce@aati-us.com.