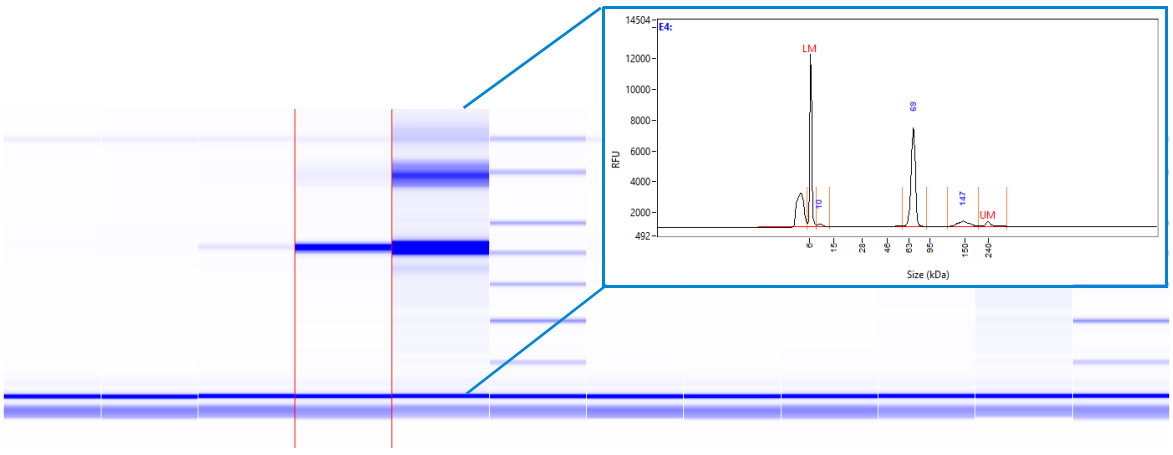
ProSize Data Analysis Software for Protein

User Manual



Notices

Document Information

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In This Guide

Agilent has prepared this manual as a technical reference for the ProSize data analysis software for use with ProteoAnalyzer parallel electrophoresis systems.

This document includes software system requirements, installation procedures, software operation, troubleshooting guide, and technical support information.

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

1 ProSize System Overview

This chapter gives an overview about the ProSize data analysis software.

2 ProSize System Requirements and Installation

This chapter provides information on the requirements and installation instructions for the ProSize software.

3 ProSize Main Screen

This chapter provides an overview of the ProSize software main screen with the functions and menus available to the user. The file menu for opening and closing data files is also covered in this chapter.

4 ProSize Configuration

This chapter provides an overview of the Global Configuration dialog of the ProSize software. It also provides information on setting individual configuration parameters in opened data files.

5 ProSize Size Calibration Screen

This chapter describes the size calibration screen (also referred to as calibration curve screen). The calibration curve screen is used to select the conditions for calibrating the size of unknown sample peaks/smears.

6 ProSize Overlay Samples

This chapter gives an overview of the possible ways to compare samples in the ProSize software.

7 Exporting Data from ProSize

This chapter provides an overview of the options available for exporting processed data from the ProSize software. The following sections describe how to open the Export Data window; provide an outline of various data export options; describe how to perform data export; and give examples of exported data.

8 Generating Reports from ProSize

This chapter provides an overview of the options available for generating reports from the ProSize software. The following sections describe how to open the Generate Report window; provide an outline of various report generation options; describe how to perform the report generation; and give examples of reported data.

9 ProSize View Capillary Positions

This chapter briefly covers the tools and functions of the View Capillary Positions window.

10 ProSize Troubleshooting Guide

This chapter gives an overview of troubleshooting the ProSize software.

11 Batch Processing Using ProSize

This chapter explains how to perform a batch process and provides an outline of the various exporting options.

Content

1 ProSize System Overview

This chapter gives an overview about the ProSize data analysis software.

About the System

ProSize Data Analysis Software

Agilent's ProSize software for protein is designed for analyzing raw data from parallel capillary electrophoresis instruments and reporting size in kilodaltons and concentration (in ng/µL) of separated proteins.

- Reads the raw data files generated from the instrument operational software.
- Provides calibration of protein size (in kDa) from standard mixtures and conversely measures protein size of unknown species in a sample.
- Provides quantitative measurements of protein concentrations present in a sample either from relative comparisons to an internal/external standard peak or by comparison to a calibration protein ladder.
- Exports size and concentration data as well as raw electropherogram trace data (time vs. fluorescence intensity) in a generic comma separated values (.csv) file format; and digital electropherogram trace or gel view images in .jpg, .bmp, or .png image formats.
- Generates PDF format sample reports containing user specified information including measured protein size, concentration, digital gel images, experimental information, and other various calculated sample properties.

This manual serves as a guide to the ProSize software and will assist the user in taking advantage of the many benefits of the parallel capillary electrophoresis instrument.

2 ProSize System Requirements and Installation

This chapter provides information on the requirements and installation instructions for the ProSize software.

System Requirements

General Software Requirements

Report Review

A PDF viewer is required to read Adobe PDF formatted reports.

Install the most recent version of Adobe Reader. The software is available free of charge for download at www.adobe.com.

Data File Export

- The ProSize software exports data in a generic comma-separated values (.csv) file format, which can be read by most spreadsheet and database management programs. Install a spreadsheet program (for example, Excel, OpenOffice) to open and read exported .csv files.
- Electropherogram and digital gel images are exported in a jpg, .bmp, or .png image format. A suitable program for viewing these file formats should be installed.

Operating System

The ProSize software must be installed on a computer running a Windows operating system to function properly.

Table 1 Supported operating systems

Operating System	Details
Windows	Windows 10, 32-bit or 64-bit

PC Recommendation

The table provides the recommended hardware configuration for the ProSize software.

Table 2 Recommended hardware configuration

Item	Details
Processor speed (CPU)	Intel Core 2, or faster
Physical memory (RAM)	4 GB
Hard disk	500 GB (for accommodation of raw data files)
Graphic resolution	1280 x 800 minimum screen resolution 1280 x 1024 recommended

ProSize Software Installation Instructions

To Install the Software

1 Download the appropriate installer from the Agilent website.

2 Navigate to the folder *ProSize data analysis software*.

3 Run the installer and follow the instructions and prompts provided by the installation wizard:

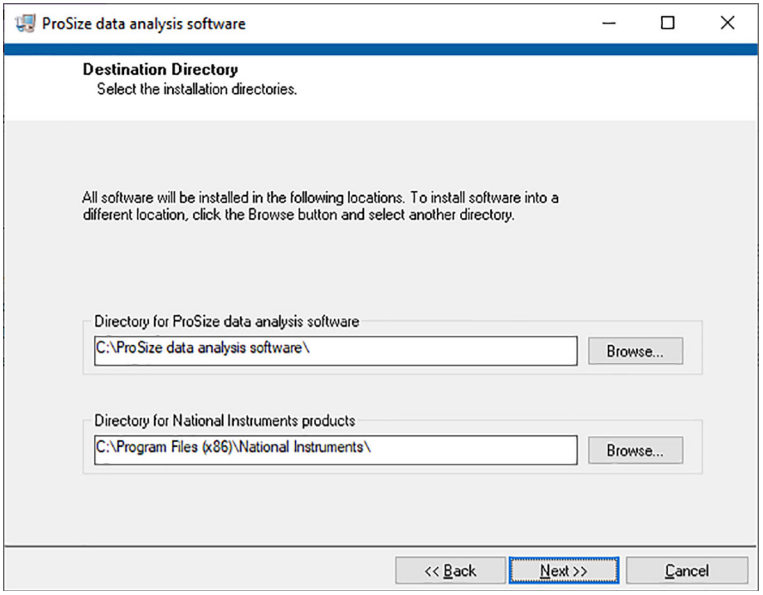


Figure 1 Default directory locations for the ProSize data analysis software and supporting National Instrument software

a Provide the installation directory. The default directory is C:\ProSize data analysis software\.

A shortcut of the ProSize software is created in the **Start** menu.

Supported software from National Instruments will also be installed under C:\Program Files\National Instruments\.

b Complete the following installation steps.

c Restart the computer to complete the installation process.

Upon successful installation of the ProSize software, several sub-folders will have been created within the C:\ProSize data analysis software\ directory (see **Figure 2**):

- **Configurations**: This folder contains ProSize global configuration setting files, optimized for different application kits for the parallel capillary electrophoresis instrument.
- **Ladders**: This folder contains ProSize sizing ladder information files (.txt format), for use with different application kits for the parallel capillary electrophoresis instrument.
- **Support**: This folder contains supporting files for ProSize software operation.

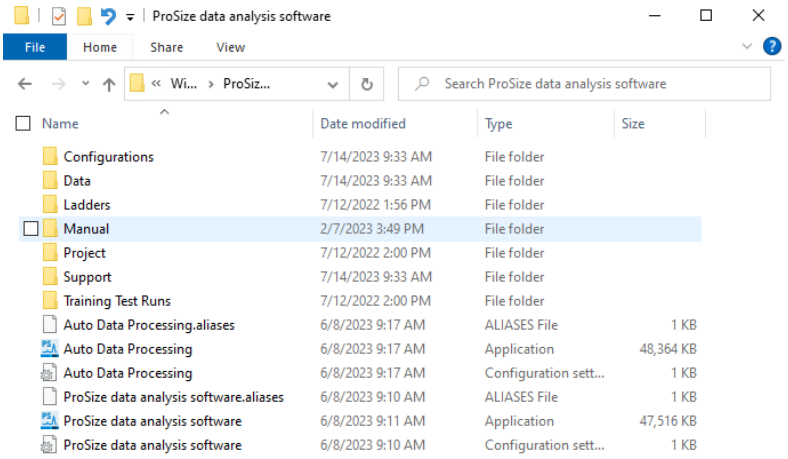


Figure 2 Sub-folders created within the C:\ProSize data analysis software\ directory

4 Update the Configurations and Ladders folders within the C:\ProSize data analysis software\ directory to the latest versions. You can obtain the most recent version of software and files from Agilent support.

   To update the folder files:

   a Navigate to C:\ProSize data analysis software\.

   b Delete the contained folders **Configurations** and **Ladders**.

   c Paste the folders **Configurations** and **Ladders** supplied from Agilent into the same directory C:\ProSize data analysis software\.

NOTE

After installation of the ProSize software, the system will install System.Data.SQLite. Please follow all screen instructions on this installation.

To properly read PDF reports:

1 Install the latest version of Adobe Reader on your computer. This software is available free of charge for download at www.adobe.com.

Starting the ProSize Program

1 In the Windows Start menu, navigate to **ProSize data analysis software**. Alternatively, create a shortcut from your desktop to launch the program.

A splash screen appears showing the software version (**Figure 3**).

Figure 3 Splash screen displaying version information upon starting the ProSize software

When the software is open, a screen with a white background screen is displayed. At the upper part of the screen you see two active main menu options (see **Figure 4**):

- **File** > **Open File**: Use this drop-down menu to open data files. Alternatively, from the toolbar, select to open your data.
- **Help** > **About**: Shows information about the software version and Agilent contact information.

The other commands of this menu are discussed in detail in **Chapter 3**, “ProSize Main Screen”.

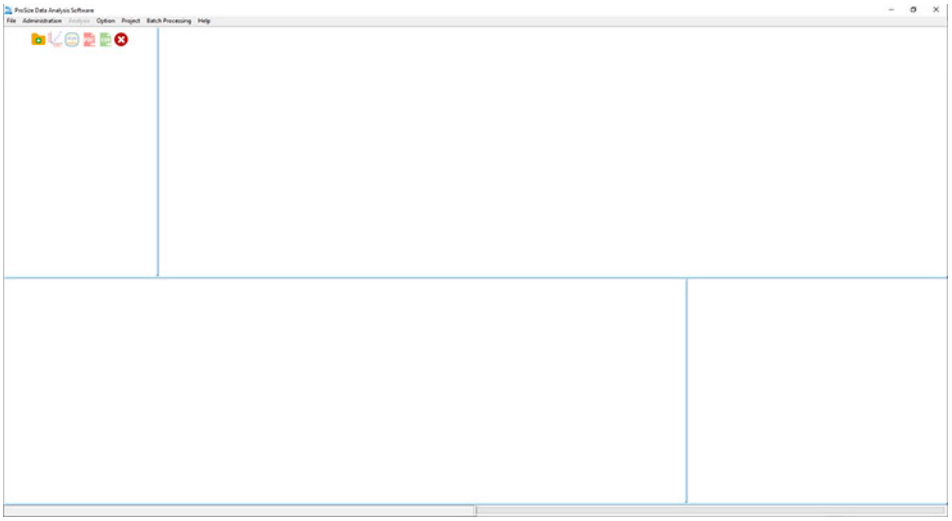


Figure 4 Main screen of the ProSize software

Exiting the ProSize software

To close the ProSize software, select **Exit** from the **File** menu, or press .

3 ProSize Main Screen

This chapter provides an overview of the ProSize software main screen with the functions and menus available to the user. The file menu for opening and closing data files is also covered in this chapter.

Main Screen (No Data File Open)

The functions and menu items available from the main screen of the ProSize software prior to opening a *.raw data file are summarized in **Table 3**.

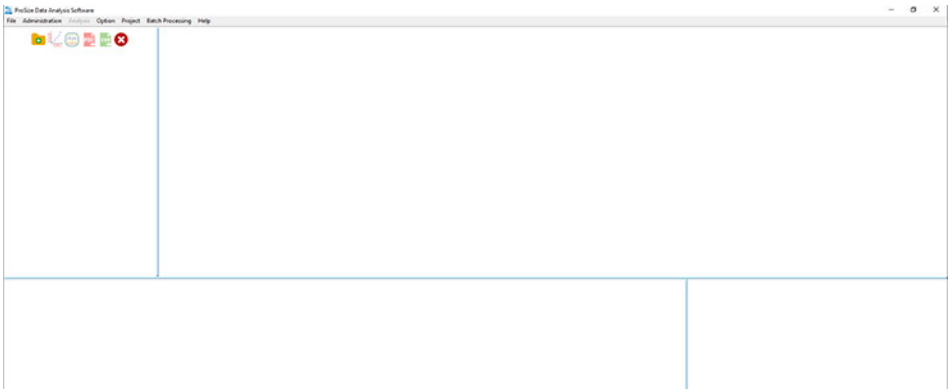


Figure 5 Main screen of ProSize software (no data file open).

Table 3 Main Menu screen menu items (active with no data file open)

Menu Command	Description
File	
Open File	Opens a **Select folder** dialog for locating and opening a raw data file (*.raw extension) generated by the parallel capillary electrophoresis instrument for data analysis (**Figure 7**). Note that if the configuration name (ProSize) and the method name (ProteoAnalyzer Software) do not exactly match, the first time a data file is opened in the ProSize software, the **Global Configuration** dialog is displayed. The screen allows you to select the appropriate predefined data configuration file (*.ini extension), or to customize a configuration file be selecting the data analysis mode and analysis settings (**Figure 9**).
Close File	Closes the ProSize software. When closing, the data file, all settings and operations are automatically saved.

Table 3 Main Menu screen menu items (active with no data file open)

Menu Command	Description
Administration	
Configuration	Opens a pop-up menu with configuration settings. The user can enable/disable the following settings: • **Login required** • **Enhanced security** (this feature is not available for ProteoAnalyzer data analysis) • **Auto Logoff Time** • **Minimum Password Length** • **Password Expiration** • **Allowed Login Attempts** • **Previous Unique Passwords**
Event Report	Opens the **Event Log** reporting the **User ID**, **Computer Name**, **Event Time**, **Operation**, **File Path** (when relevant), and a **Description** of the event.
Option > Option dialog settings	
Set Default Data Path	Allows you to set the default file path for accessing and saving data files. In the **Select folder** dialog, navigate to the desired directory and select the folder to be set to open data files (Figure 7). The default directory for opening *.raw data files is C:\Agilent\Data\.
Capillary Array Format	Allows you to arrange the data from left to right in the **Digital Gel Image** display by **Row** (A1, A2, A3, etc.) or by **Column** (A1, B1, C1, etc.).
Display Option	Allows you to select the view of the data by selecting **Scale to Sample** or the **Autoscale function**.
DNA Quality Number (DQN)	Allows you to enable or disable DQN. Not available in Protein mode.
Molarity Calculation	Allows you to **Use Peak Maxima** or **Use Avg. Size** for all molarity calculations.
%(Conc.) based on	Allows you to select the preferred concentration units, **ng/µL** or **pguL**.
Image Resolution	Define the image resolution as **1X**, **2X**, **3X**, or **4X** used for exporting electropherograms or gel images.
Edit Configuration	Opens the **Global Configuration** dialog (Figure 9), where you can set parameters for data analysis mode, peak and marker integration, quantification, ladder size, etc.
Email	Allows you to set up an email account to send results to.

Table 3 Main Menu screen menu items (active with no data file open)

Menu Command	Description
Project	
Load Project	Opens a **Select Folder** dialog for locating and opening a Project data file (*.proj extension) generated for data analysis.
Create Project	Allows you to open the **Project** screen and create a project by selecting different *.raw data files.
Batch Processing	
Batch Data Process	Allows you to process a set of *.raw files within a selected folder, and to define an output folder within which processed files are sent.
View Error Log	Allows you to view the error log associated with a particular batch data process.
Help	
User Manuals	Access all ProSize User manuals.
About	Displays software information, version information, copyright information and Agilent technical support contact information.

The **Options** pop-up dialog is shown in **Figure 6**. The properties of the dialog are described in **Table 3** above.

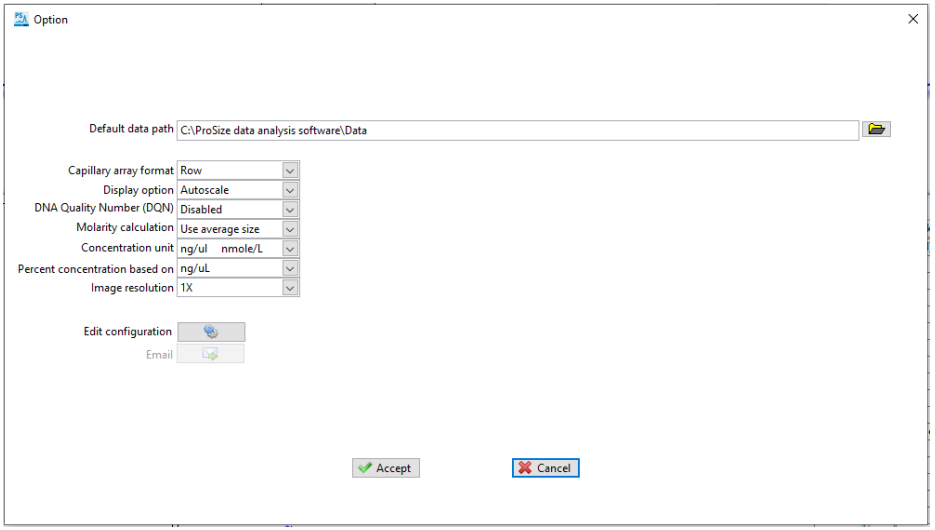


Figure 6 Option dialog

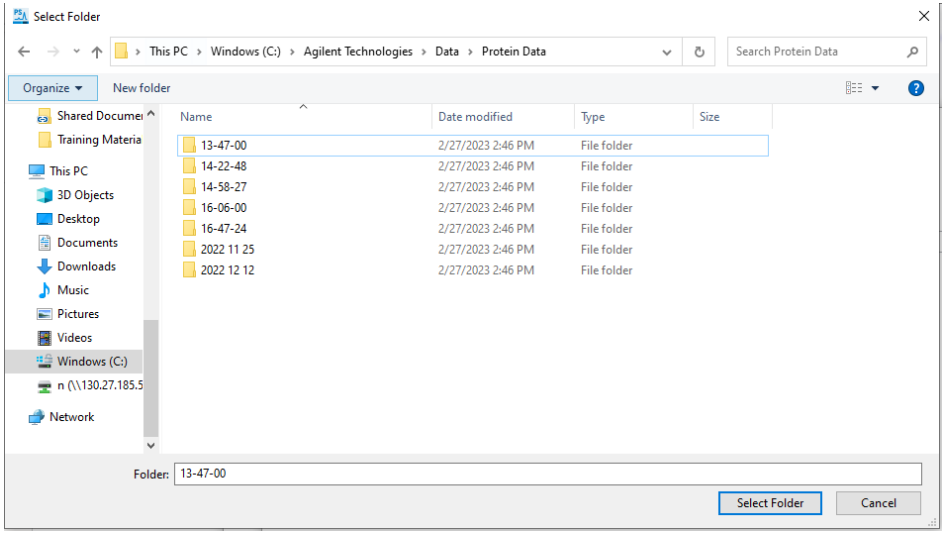


Figure 7 Set Default Data Path selection screen

Opening a Data File in ProSize Software

To Open a Data File

1 From the menu, select **File** > **Open File** (alternatively, select  from the toolbar).

The **Select Folder** dialog opens (Figure 7).

2 Navigate to your raw data file (*.raw extension) for data analysis.

3 Select the folder with the raw data.

In the ProSize software, the **Open** screen is displayed, showing the selected file directory (Figure 8).

If you need to navigate to a higher-level directory, select the folder  to the right of the file path and a standard file browser will open.

4 In the **Open** screen, click a data file.

A file previewer window will be displayed on the right side of the screen, containing a digital gel image of the data, a **Sample Information** list, any typed **Note**, and a summary of the experimental **Method** used to collect the data file (Figure 8). If the data file has not been previously processed in ProSize, the heading of the digital gel image will be labeled *Non-processed Data*. If the data file has been previously processed, no heading will be displayed on the digital gel image.

5 If the correct data file is verified, select **Open** to load the data file into ProSize. Select **Cancel** to close the **Open** screen without loading a file.

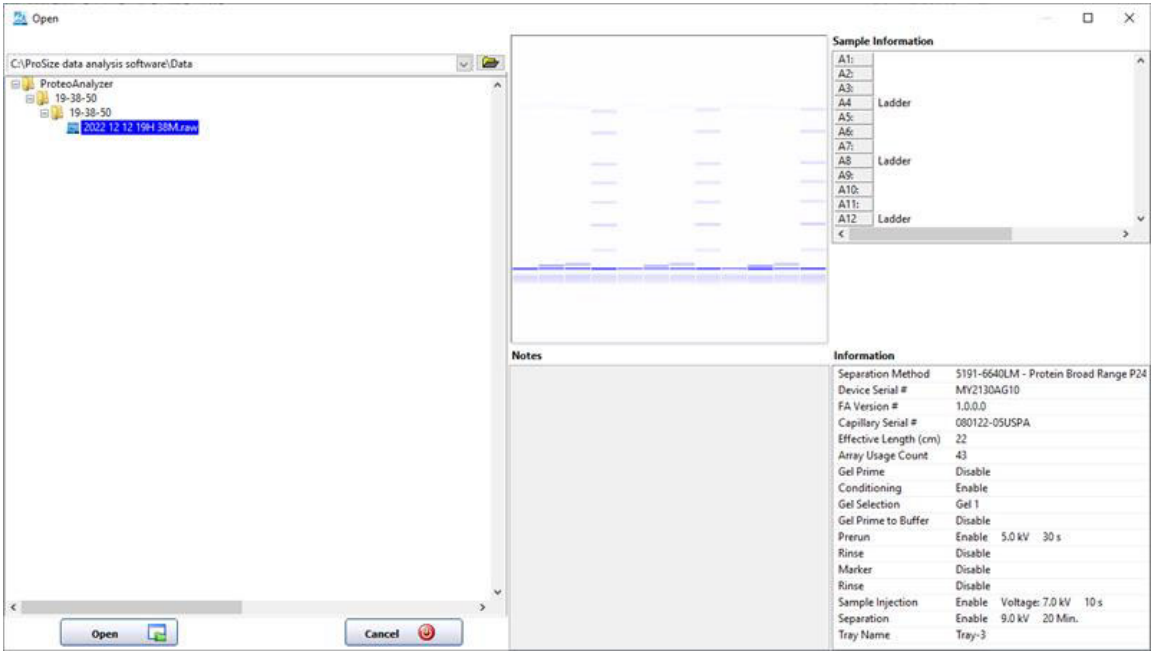


Figure 8 Open file data selection and previewer screen

Configuration Dialog

Upon selecting **Open**, the data file will be processed if the matching predefined data analysis configuration file is available (for example, **5191-6640LM - Protein Broad Range P240 LM only.ini**). The first 10 characters of the configuration file name must match the first 10 characters of the separation method used to automatically process the data.

NOTE

A minimum of 10 characters must be used to name both the method (instrument) and configuration (ProSize).

Should there be no matching configuration file, the **Global Configuration** dialog will be loaded when opening a previously unprocessed data file. Should the wrong configuration file be chosen, the user can navigate to the **Option** window, and select **Edit Configuration** to load the correct configuration.

Upon opening, there are eight primary tabs in the **Global Configuration** dialog (**Figure 9**). The settings are discussed in detail in **Chapter 4**, "ProSize Configuration".

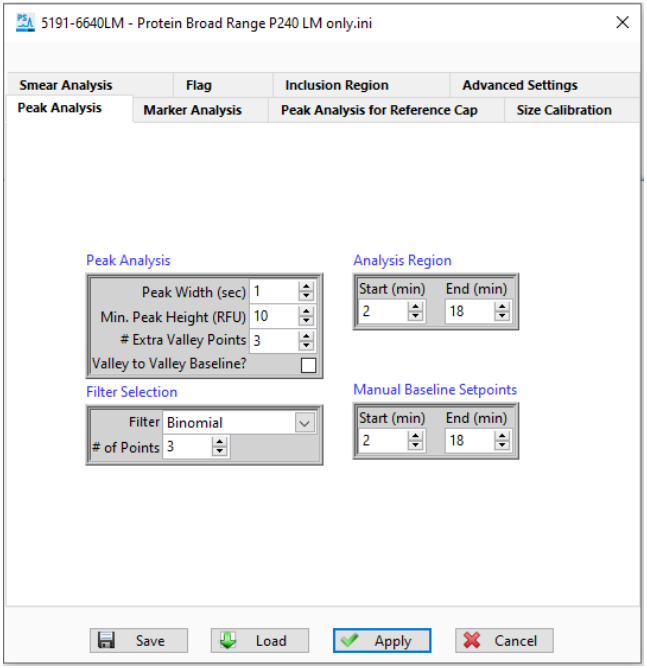


Figure 9 Global Configuration dialog (Peak Analysis tab is selected)

NOTE

You may create a customized configuration by changing the analysis settings and clicking **Save**.

NOTE

In most cases, predefined, optimized global configuration files are available to load for each Agilent Reagent Kit. The configuration files are labeled according to the particular kit number, for example, **5191-6640LM - Protein Broad Range P240 LM only.ini**.

After the configuration settings have been loaded, select **Apply** to open the data file in the main screen of the ProSize software.

Main Screen (Data File Open)

Once a data file is opened in ProSize, several additional main screen functions are enabled as summarized in **Table 4**. The opened data is displayed in the main screen as shown in **Figure 10** (12-capillary data).

Table 4 Additional Main screen menu items (active only with data file open)

Menu Option	Description
File	
Close File	Closes the currently opened data file and displays the main screen. All settings up to the point of closing the data file will be saved and reloaded when reopening the data file.
Generate Report or 	Opens a menu for configuring and generating an Adobe .pdf report, containing user specified information (electropherograms, gel images, peak information, etc.). For more information, see **Chapter 8**, "Generating Reports from ProSize".
Export Data or 	Opens a menu for configuring and exporting user specified data to a user specified folder. Types of data include gel images, electropherograms, peak tables, size calibration data, etc. For more information, see **Chapter 7**, "Exporting Data from ProSize".
Analysis	
Protein Calibration	Opens the **Protein Calibration** screen. Users can select which sample well to use for absolute quantification and view an overlay of protein samples if multiple sample wells are selected to use for size calibration.
Show Size Calibration or 	Opens the **Calibration Curve** screen, which contains input data associated with the size calibration ladder. For more information, see **Chapter 5**, "ProSize Size Calibration Screen".
Overlay Samples or 	Opens the **Overlay Samples** screen, where you can overlay gel images or electropherograms from individual lanes or different data files. For more information, see **Chapter 6**, "ProSize Overlay Samples".
View Capillary Positions	Opens the **View Capillary Positions** screen, which allows you to view and modify the alignment of the detector signal with the capillary positions. For more information, see **Chapter 9**, "ProSize View Capillary Positions".

Table 4 Additional Main screen menu items (active only with data file open)

Menu Option	Description
Quick Display	Displays the **Quick Display** window, which shows non-normalized electropherograms for all 12 capillaries enabling quick review of the data.
Display Separation Parameters	Displays the **Separation Parameters** window, which allows the user to view the current, voltage, and pressure that occurred during the separation.

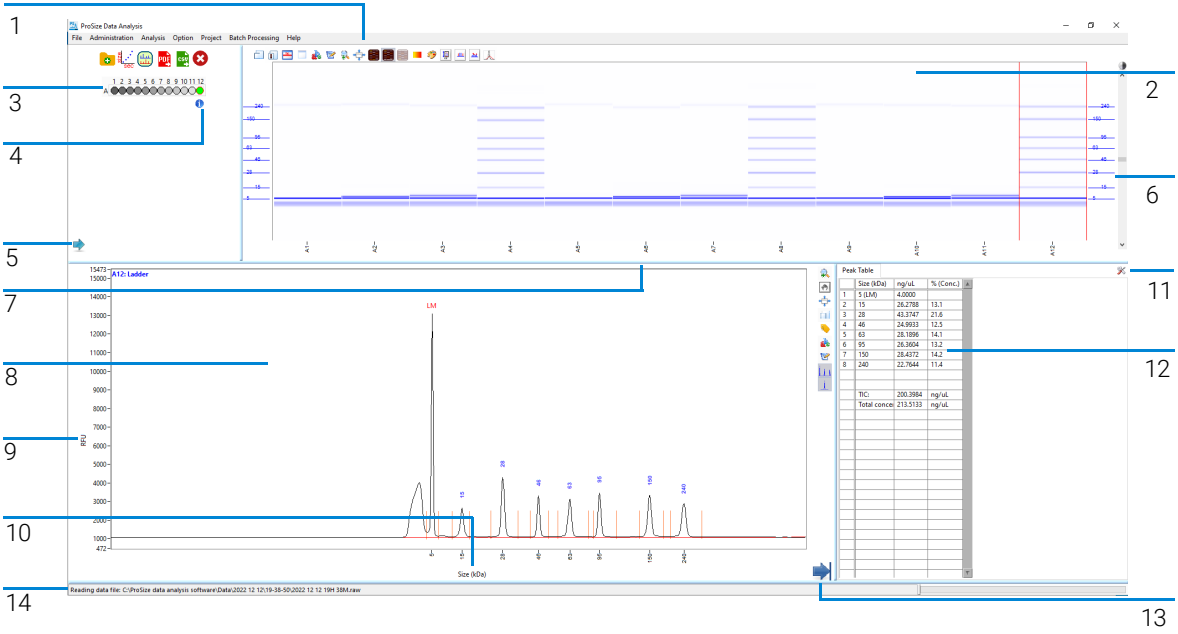


Figure 10 Main screen of ProSize software (12-capillary data file open)

The primary accessible functions from the main screen are shown in **Figure 10**, and are summarized in the legend table:

1	**Main screen toolbar**	
2	**Digital Gel image**	Digital Gel Image of the data set (see also **Figure 13**, **Table 6**). The sample wells are normally displayed in increasing order for each row. Starting on the left side starting with well A1 (A1, A2, A3-A12; followed by B1-B12, etc.). Change the view to ascending order of the well columns from left to right (A1, B1, C1, etc...). From the **Option** menu, select **Capillary Array Format Column**. To toggle back to a row-by-row format, select **Capillary Array Format Row.**
3	**Plate map**	Shows the currently selected well highlighted in green (A1 is the default location on opening the file). Click on the appropriate well by using the keyboard arrows, or double-clicking the appropriate lanes of the digital gel image. You can select any well of the plate for individual viewing. The functions available within the Plate Map menu are summarized in **Table 5**.
4	**Info**	Click to access experimental information regarding the method used to collect the data file.
5	**Sample ID navigation**	Enables Sample ID navigation. The Sample ID list as entered into the instrument software will be displayed, enabling you to navigate the samples by Sample ID and to edit sample names if desired. Close the Sample ID navigation view by right-clicking in the list, and select **Close**.
6	**Contrast**	Use the slider bar to change the intensity contrast of the gel image.
7	**Re-scale**	Re-scale the electropherogram image and gel image by sliding the bar between the two images.
8	**Electropherogram trace**	An Electropherogram trace of the currently selected well. The well position and sample name (if imported) is displayed in the upper left corner of the trace. Right-click the screen to process or annotate the data (**Table 7** and **Table 8**).
9	**Y-axis**	Units for the y-axis can only be viewed in RFU
10	**Scaling x-axis**	Units for the x-axis can be changed by right-clicking the axis title and selecting time scale or size scale.
11	**Set individual parameters**	Select to reveal the configuration window, for which most settings can be individually adjusted for each sample as desired (see **Figure 12**). A brief summary of the primary configuration functions are presented in this section. For more detailed information, refer to **Chapter 4**, "ProSize Configuration".
12	**Peak table**	Displays information about the selected and integrated sample peaks and markers. Depending on the configurations: • A **Flag Analysis** tab will be shown if the flag configuration function contains any flag conditions (**Figure 23**). • A **Smear Analysis** tab will be shown if the smear analysis configuration contains any entries (**Figure 22**).
13	**Expand Electropherogram trace**	Select to expand the Electropherogram trace across the bottom panel which provides you with more zoom options (see **Figure 11**).
14	**Filename**	Upon opening a data file, the currently selected Filename/directory is displayed in the lower left toolbar.

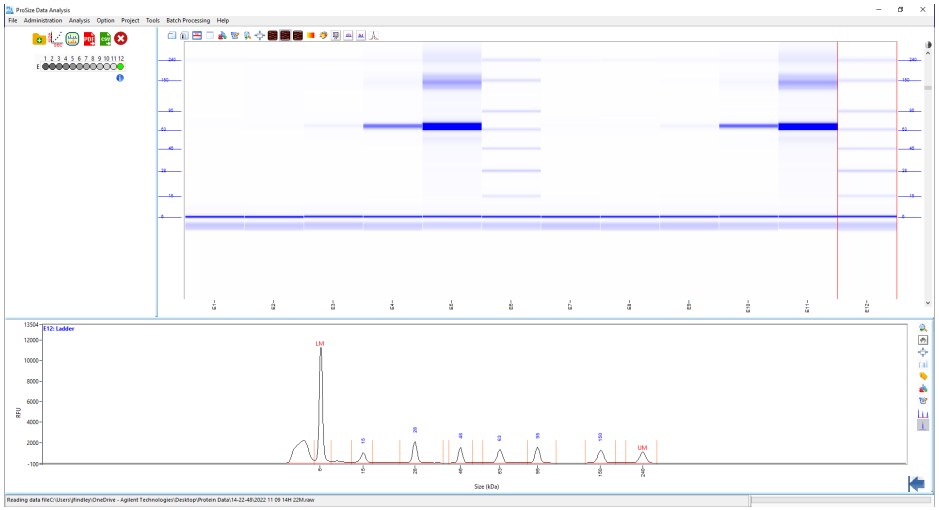


Figure 11 Expanded Electropherogram Trace

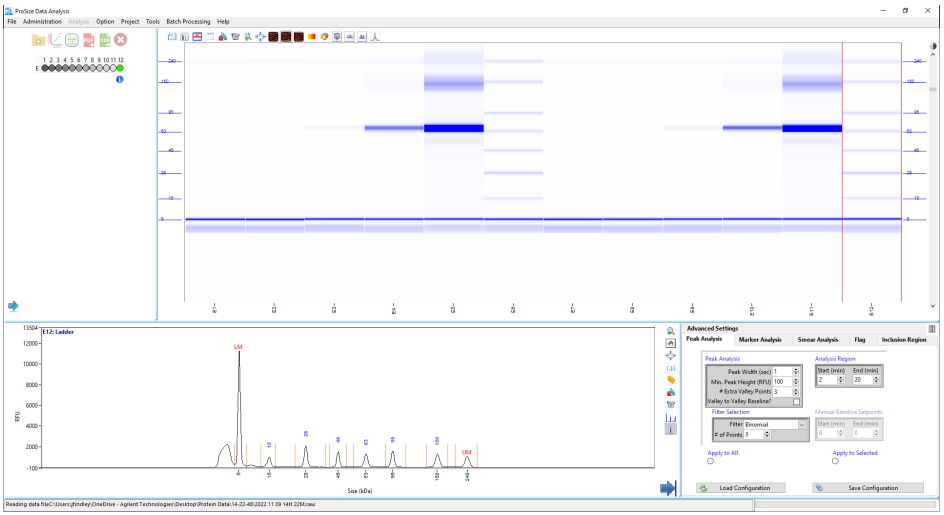


Figure 12 Main screen with data file open and open configuration dialog (Set Individual Parameters dialog)

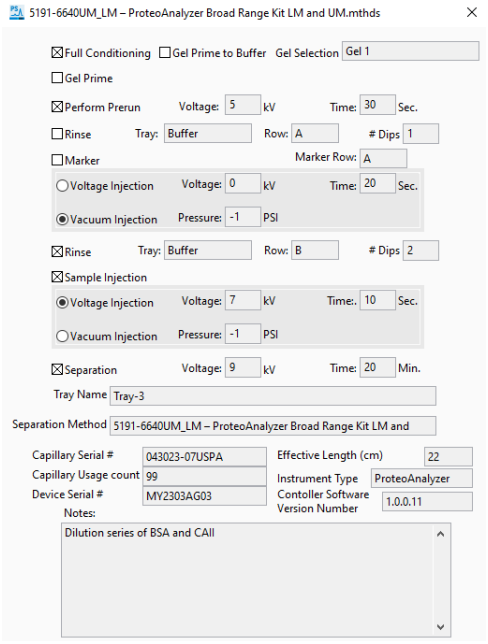
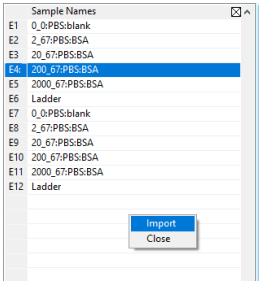
Plate Map

The functions available within for the Plate Map are summarized in **Table 5**.

Table 5 Plate Map menu functions

Menu Option	Description
Plate Map	Used to select a sample well for adjusting marker/peak integration, assigning flag or smear analysis conditions, viewing Peak Table information and Electropherogram Trace, or adjusting lane contrast in the Digital Gel Image. Possibilities to select a well: • Click the well. • Change the well selection via the keyboard arrows. • Double-click the lane in the digital gel image. • Right-click the sample wells to overlay them in the Electropherogram section of the screen. • Select the **Sample ID** in the Sample ID navigation pane.  To display a well/sample name, place the mouse cursor over the well.

Table 5 Plate Map menu functions

Menu Option	Description
Experimental Information	Shows the instrument system software user interface, containing the experimental information, Capillary Array information, instrument system information, and Notes fields for the collected data file. To close the window, click × . 
Sample ID navigation	The Sample ID list will be displayed. In the list, you can navigate the samples by the Sample ID previously entered into the instrument system software. Double-click on a sample name allows you to edit the Sample ID information. Right-click the sample ID allows you to **Import** a .txt file. Select **Close** to exit the Sample ID list and return to the plate map view. 

Digital Gel Image Toolbar

The toolbar options above the digital gel image are summarized in **Table 6**. The gel image is annotated on both the left and right side with the sample sizing ladder in kDa. The currently selected lane is bracketed by vertical cursors (**Figure 13**).

Right-click on any sample lane will overlay the samples in the electropherogram view. Double-click on a sample lane to view a single sample well in the electropherogram view.

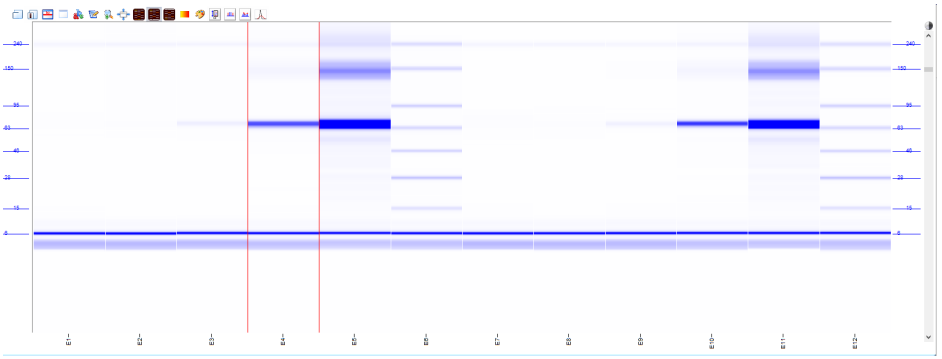


Figure 13 Main screen zoomed to the digital gel image and its toolbar menu

Table 6 Digital Gel Image toolbar

Toolbar Option	Description
Copy Full Gel Image	Copies an image of the full Digital Gel Image to the clipboard, for pasting in another program such as Microsoft PowerPoint. If zoomed into the gel image, it will copy the visible portion of the image. The left and right side of the gel image is annotated with the sizing ladder in the copied gel image.
Copy Selected Gel Lane Image	Copies an image of the currently selected gel lane to the clipboard, for pasting in another program such as Microsoft PowerPoint. If zoomed into the gel image, it will copy the visible portion of the gel lane image. The left side of the gel lane image is annotated with the sizing ladder in the copied image.

Table 6 Digital Gel Image toolbar

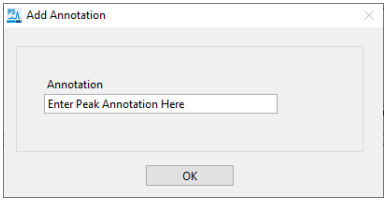
Toolbar Option	Description
Show Cursor	Displays a horizontal line cursor across the digital gel image, annotated by size (kDa) corresponding to the current cursor position. The cursor can be dragged to any position along the gel image by holding the mouse over the cursor and pressing and holding the left mouse button. When the cursor is active and displayed, a similar vertical cursor annotated by size is displayed in the Electropherogram Trace, whose movement corresponds to that in the gel image. Up to four cursors can be displayed at one time.
Hide Cursor	To deactivate the cursor, press the button a second time.
Create Annotation	To create customized annotations in the Digital Gel Image display. When selected, the **Add Annotation** window opens. You can type desired annotation into the field, and select **OK**. The annotation will be displayed in the Digital Gel Image window.  To move the annotation to the desired position on the trace: Click the annotation and drag it to the desired position. An arrow will appear at the opposite end of the annotation upon dragging. Multiple annotations can be created by repeatedly selecting **Create Annotation**. Any created annotations will be copied to the clipboard with the **Copy** function.

Table 6 Digital Gel Image toolbar

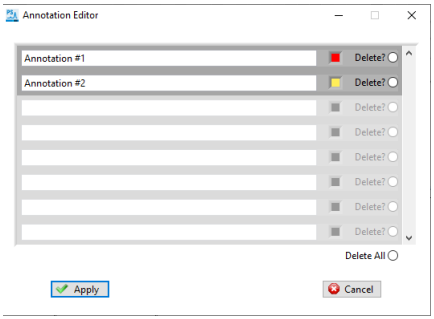
Toolbar Option	Description
Edit Annotations	Opens the **Annotation Editor** window. Allows you to: • Edit the annotation text by typing in the text field. • Change the color of the annotation by left clicking on the annotation color. • Delete one annotation by selecting **Delete** next to each annotation, or delete all annotations by selecting **Delete All**.  Select **Apply**, to confirm your settings.
Zoom	When selected, zooming to an area by clicking and dragging is enabled.
AutoFit	Autoscales the Digital Gel Image x-/y-axis view for all 12 lanes. The autoscaled image will display the gel from the selected lower marker to upper marker, or if only a lower marker only is used from the lower marker to the end of the separation window.
Auto Intensity	Autoscales the intensity of the Digital Gel Image view for all 12 lanes. The viewed intensity across the image will reflect the actual observed fluorescence signals from each lane.
Normalize Intensity to Lower Marker	Normalizes the intensity of the Digital Gel Image view for the lower marker to the same value for all 12 lanes, adjusting the relative intensities of all bands in each lane accordingly. The viewed intensity across the image will as a result reflect the fluorescence signals from each lane after normalization to the lower marker, as is done when calculating the concentration of samples using the lower marker.
Normalize Intensity to Upper Marker	Normalizes the intensity of the Digital Gel Image view for the upper marker to the same value for all 12 lanes, adjusting the relative intensities of all bands in each lane accordingly. The viewed intensity across the image will as a result reflect the fluorescence signals from each lane after normalization to the upper marker, as is done when calculating the concentration of samples using the upper marker.

Table 6 Digital Gel Image toolbar

Toolbar Option	Description
Enhanced Contrast	Increases the contrast of an individual lane while holding other lanes constant. When selected, the Lane Contrast Control window opens. Drag the scroll bar to adjust the contrast for the currently selected lane in the gel image. Once adjusted, click **OK** to accept changes, or select **Cancel** to discard your changes. Note: The enhanced contrast setting works on the currently selected lane only (marked with vertical cursors in gel image). The Auto Intensity, Normalize Intensity to Lower Marker, and Normalize Intensity to Upper Marker functions will undo any previous enhanced contrast operations.
Gel Image Color	Changes the color scheme for the **Digital Gel View**, the gel image in the **Overlay Samples** screen and any exported or printed report. Options for the gel image color: • White on Black (white bands on black background) • Black on White (black bands on white background) • Green on Black (green bands on black background) • Red on Black (red bands on black background) • Blue on White (blue bands on white background) • Pseudo Color (green to red bands on blue background)
1:1 Gel Image	Displays the gel image in an adjustable window for changing the aspect ratio of the gel image (useful for producing figures for papers). The adjusted image can be copied to the clipboard.
Hide/Show Marker Peaks	Toggle to hide or show the marker peaks in the digital gel image screen. This is useful when comparing to a slab gel result which does not use lower/upper normalization markers.
Hide/Show non-integrated peaks	Toggle to hide or show non-integrated peaks in the digital gel image screen. This is useful for eliminating noise peaks from the gel image.
Gaussian Fit	Toggle to fit all peaks to a narrow Gaussian peak.

Electropherogram Trace Toolbar Menu

The options available in the Electropherogram Trace toolbar are summarized in **Table 7**.

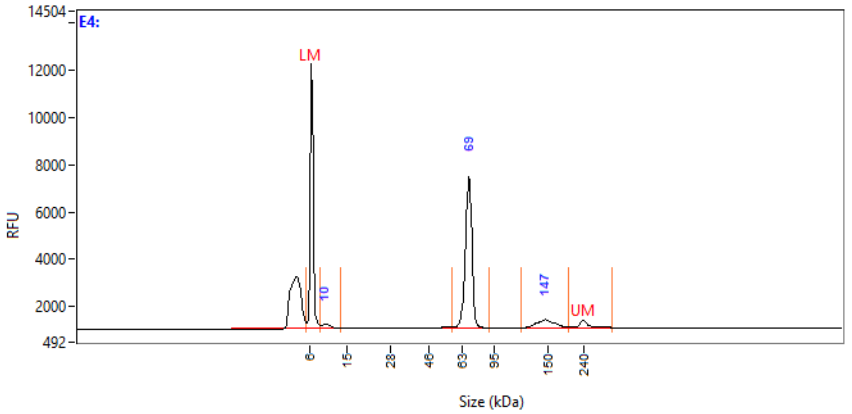


Figure 14 Main screen zoomed to the Electropherogram Trace and its toolbar menu. Note the y-axis scale is set to the sample peaks and not the marker peak heights.

NOTE

In version 5.0 of the ProSize software, the Electropherogram Trace will automatically autoscale to the largest observed sample peak, regardless of the lower/upper marker peak heights. To view the entire y-axis scale including marker peaks, in the **Option** window, select **Autoscale** as the **Display Option** (see **Figure 6**).

Table 7 **Electropherogram Trace toolbar functions**

Toolbar Option	Description
Zoom	Enables zooming in the x- and y-axis of the Electropherogram Trace. To zoom-in: Place the mouse over the trace, click and drag it outward to expand a box area to define the zoom region. Release the mouse button to apply. The zoomed image can be copied to the clipboard. The zoom region will be preserved when viewing other samples in the Plate Map. To undo the zoom, use the **Autoscale** function.
Pan	Enables dragging of the image. Move the image around with the mouse cursor.

Table 7 Electropherogram Trace toolbar functions

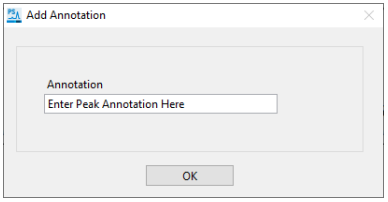
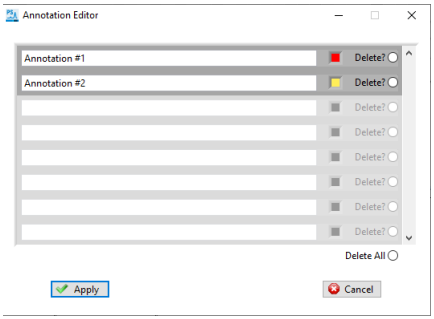
Toolbar Option	Description
Autoscale	To autoscale the Electropherogram Trace x-/y-axis display. The autoscaled image will display the full trace from the start to the end of the separation when using the **Time Scale** and **Size Scale** display modes; when using the **Uniform Size Scale** the autoscaled display will start at 0 kDa. Note: the y-axis scale will automatically zoom to the highest detected sample peak, regardless of the marker peak heights. To view the entire y-axis scale including markers, go to **Option** window, and as **Display Option**, select **Autoscale**.
Copy	Copies an image of the current view of the Electropherogram Trace to the clipboard, for pasting in another program such as Microsoft PowerPoint. Any zoom, annotation, baseline and/or peak start/end point displayed will be copied in the image. The well ID and sample name will be copied in the top left of the trace, and the x-/y-axis will correspond to the currently selected view in the copied image.
Units	Displays a menu for changing the peak annotation of the Electropherogram Trace. Only integrated peaks are annotated. Units for the peak annotation: • None • Peak ID (labels in order as 1, 2, etc.) • Migration Time (min:sec) raw migration time • Peak Height (in RFUs) • Corrected Peak Area (Peak Area/Migration Time) • Size (in kDa) • Average Size (in kDa) • Concentration (in ng/µL)
Create Annotation	To create customized annotation in the Electropherogram Trace display. In the **Add Annotation** dialog, the user can type desired annotation into the field. Click **OK** and the annotation will be displayed in the Electropherogram Trace window.  To move the annotation to the desired position on the trace: Click the annotation and drag it to the desired position. An arrow will appear at the opposite end of the annotation upon dragging. Multiple annotations can be created by repeatedly selecting **Create Annotation**. Any created annotations will be copied to the clipboard with the **Copy** function.

Table 7 Electropherogram Trace toolbar functions

Toolbar Option	Description
Edit Annotations	Opens the **Annotation Editor** window. Allows the user to: • Edit the annotation text by typing in the text field. • Change the color of the annotation by left clicking on the annotation color. • Delete one annotation by selecting **Delete** next to each annotation, or delete all annotations by selecting **Delete All**.  Select **Apply** to confirm your settings.
Show/Hide Baseline	Toggle to display or hide the baseline drawn for the peak integration, shown as an orange line. Displaying the baseline helps if adjustments need to be made to the baseline to better define the actual peak area via the **Peak Width (sec)**, **Valley to Valley**, or **Manual Baseline Setpoints** tools. Note: It is highly recommended to enable the display of the baseline, to ensure the correct baseline is being drawn to the data, especially with smears.
Show Peak Start/End Edge	Toggle to diplay or hide the start and end points used for peak integration, shown as vertical orange lines. Displaying the peak integration start/end points helps if adjustments need to be made to the peak integration to better define the actual peak area. The user can change the peak start/end points by adjusting the **Peak Width (sec)**, or by using the right click menu tools in the Electropherogram Trace such as **Split Peak** or **Move Peak Start/End Points** (see Table 8). The user can change the baseline drawn (and subsequently the peak start/end points) via the **Peak Width (sec)**, **Valley to Valley**, or **Manual Baseline Setpoints** tools. Note: It is highly recommended to enable the display of the baseline, to ensure the correct integration is being drawn to each peak, especially with smears.
Expand Electropherogram Trace	Expands the Electropherogram Trace across the bottom panel covering the Peak Table. Provides more user control over zoom.

Electropherogram Trace Context Menu

The context menu of the trace, the y-axis, and the x-axis contains functions for adjusting marker peak assignment, peak integration and scaling the x-axis of the Electropherogram Trace (**Figure 15**). Functions are also provided for copying the trace and exporting the data, and are summarized in **Table 8**.

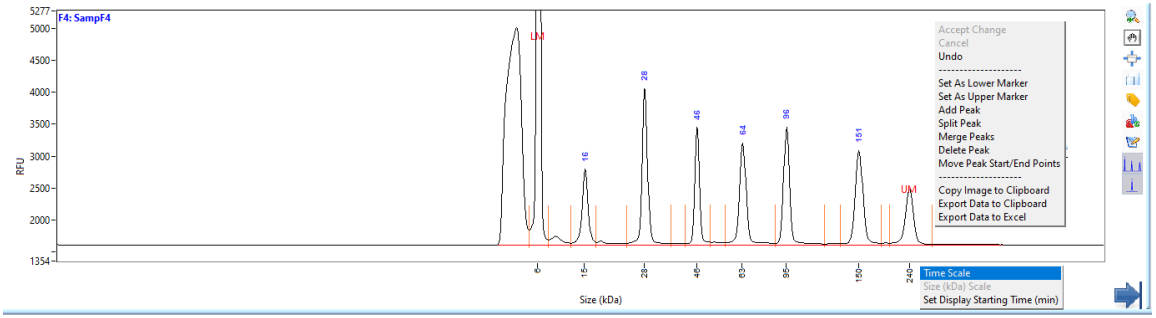


Figure 15 Main screen zoomed to the Electropherogram Trace and context menus. Note that the y-axis scale is set to the sample peaks and not the marker peak heights.

Table 8 Electropherogram Trace context menu commands

Menu commands	Description
Accept Change	Accepts any manual modification to the performed peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). After the manual modification has been made, select **Accept Change** to apply the changes to the trace.
Cancel	Cancels any manual modification to the peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). Select **Cancel** to discard the changes applied to the trace.
Undo	To undo any manual modification applied to the peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). Select **Undo** to revert back one step.
Set As Lower Marker/ Set As Upper Marker	To automatically set any integrated peak to the lower or upper marker, and correspondingly re-normalize the trace to the new marker. Right-click the peak to be reassigned as the marker. Select **Set As Upper (or Lower) Marker.** The peak will be set as the marker and renormalized.

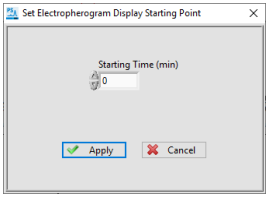
Table 8 Electropherogram Trace context menu commands

Menu commands	Description
Add Peak	To manually add a peak which has not been auto-integrated by the **Peak Analysis** settings and to define the start and end point for integration. Zoom-in to the region where the peak is to be added. Right-click this region and select **Add Peak**. Two red vertical cursors will appear; the left cursor defines the new peak start point and the right cursor the new peak end point. Drag each cursor to the desired position, then right-click, and select **Accept Change** to add the peak for integration. Note: The Add Peak function will not add a peak that is located outside the lower/upper marker window (or before lower marker if using only lower marker).
Split Peak	To manually split a peak which is currently integrated into two peaks and to define where the split occurs. Zoom-in to the region where the peak is to be split. Right-click this region, and select **Split Peak**. A red vertical cursor will appear. Drag the cursor to the desired location, then right-click, and select **Accept Change** to split the peak into two peaks for integration.
Merge Peaks	To merge any number of peaks and integrate as a single peak. Zoom-in to the region where the peak is to be split. Right-click this region, and select **Merge Peaks**. Two red vertical cursors will appear; the left cursor defines the left most peak to merge and right cursor defines the right most peak to merge. Drag the cursor within that peak's start/end point region to merge, then right click, and select **Accept Change** to merge the peaks into a single peak for integration.
Delete Peak	To manually delete a peak which has been integrated. Zoom-in to the region where the peak is to be deleted. Right-click this region, and select **Delete Peak**. A red vertical cursor will appear. Drag the cursor to the desired peak location, then right-click, and select **Accept Change** to delete the peak from the integration.
Move Peak Start/End Points	To change the currently positioned start/end integration points of a peak. Zoom-in to the region where the peak is located. Place the cursor between the start and end points of the integration. Right-click and select **Move Peak Start/End Points**. Two red vertical cursors will appear at the current start/end points. Drag the cursors to the desired locations, then right-click and select **Accept Change** to apply the new start/end point positions to reintegrate the peak. Note: This function will not change the baseline; only the start and end points are affected. To change the baseline, the user must adjust the via the **Peak Width (sec)**, **Valley to Valley,** or **Manual Baseline Setpoints** tools.

Table 8 Electropherogram Trace context menu commands

Menu commands	Description
Copy Image to Clipboard	Copies an image of the current view of the Electropherogram Trace to the clipboard, for pasting in another program, such as Microsoft PowerPoint. Any zoom, annotation, baseline and/or peak start/end point displayed will be copied in the image. The well ID and sample name will be copied in the top left of the trace, and the x-/y-axis will correspond to the currently selected view in the copied image.
Export Data to Clipboard	Copies the electropherogram x-data (time; in HH:MM:SS) and y-data (RFUs) in a .csv format that can be pasted directly into common spreadsheet programs such as Microsoft Excel. The x-data is copied into column 1, and the y-data into column 2 of the file upon pasting. To export, right-click and select **Export Data to Clipboard**. Open a spreadsheet, select the desired cell and paste the copied data. Note: In the **Time Scale** and **Size Scale** display modes, the exported data will be: • x = time, and • y = RFU. In the **Uniform Size Scale,** the data will be: • x = size, and • y = RFU.
Export Data to Excel	This function requires Microsoft Excel to be installed on the computer to function properly. Selection will automatically open Microsoft Excel and copy the electropherogram x (time; in HH:MM:SS) and y (RFUs) in a .csv format. The x-data is copied into column 1, and the y-data into column 2 of the file upon pasting. To export, right-click and select **Export Data to Excel**. The file can then be processed and saved as desired. Note: In the **Time Scale** and **Size Scale** display modes, the exported data will be: • x = time, and • y = RFU. In the **Uniform Size Scale**, the data will be: • x = size, and • y = RFU.
Time Scale	Changes the x-axis to units of migration time in hours, minutes and seconds (HH:MM:SS). To change, right-click and select **Time Scale**. The x-axis scale will change to time and the trace with autoscale.

Table 8 Electropherogram Trace context menu commands

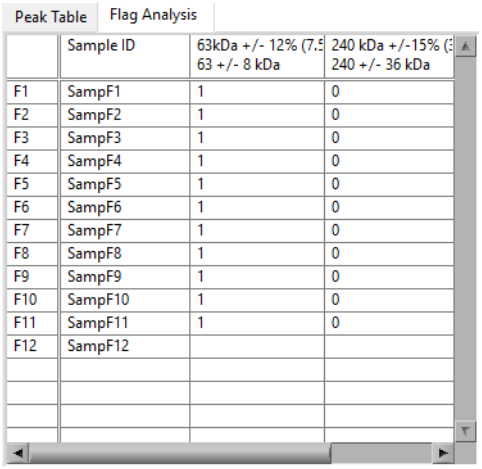
Menu commands	Description
Size (kDa) Scale	Changes the x-axis to units of size (not functional in kDa in protein modes). The display will use underlying units of time and convert the time to size from the current **Size Calibration** settings. To change, right-click and select **Size Scale**. The x-axis scale will change to a size scale and the trace with autoscale.
Set Display Starting Time (min)	To set-up the displayed start time at the x-axis. Right-click the x-axis and select **Set Display Starting Time (min)**. In the dialog, enter a start time (in minutes), and select **Apply** to confirm your entry. 

Flag Analysis Table

If the configuration contains any **Flag** settings, a **Flag Analysis** tab will also be visible as a separate tab with the **Peak Table** (Figure 16 and Table 9). The flagged results are displayed in rows, with each row corresponding to a sample well of the plate. The columns are titled by the flag tag which is the field used to name the particular flag condition. The results are output in a binary format with 0 = FALSE and 1 = TRUE.

NOTE

The information in the **Flag Analysis** tab can be exported to the clipboard for pasting into common spreadsheet programs by right clicking the mouse and left clicking **Export Data to Clipboard**. Further information on the flag functions can be found in Chapter 4, “ProSize Configuration”.


Peak Table | Flag Analysis

	Sample ID	63kDa +/- 12% (7.5 63 +/- 8 kDa	240 kDa +/-15% (3 240 +/- 36 kDa
F1	SampF1	1	0
F2	SampF2	1	0
F3	SampF3	1	0
F4	SampF4	1	0
F5	SampF5	1	0
F6	SampF6	1	0
F7	SampF7	1	0
F8	SampF8	1	0
F9	SampF9	1	0
F10	SampF10	1	0
F11	SampF11	1	0
F12	SampF12		

Figure 16 Flag Analysis tab on main screen

Table 9 Flag Analysis tab

Item	Description
Well/Sample Name	The first column labels the sample well; the second **Sample ID** column lists the entered sample name.
Columns	Each column is titled by the respective tag entry and flag conditions for each entry.
Column/Row Data	Data is reported in binary format, following the specified criteria for each flag condition: 0 = FALSE (conditions not met) 1 = TRUE (conditions met)
Export Data to Clipboard	From the context menu of the table, select **Export Data to Clipboard**. The peak table information is exported to the clipboard for pasting directly into common spreadsheet programs such as Microsoft Excel. The full table is exported.

Smear Analysis Table

If the configuration of the **Smear Analysis** tab contains any filter settings, a **Smear Analysis** tab will also be visible as a separate tab with the **Peak Table** (Figure 17 and Table 10).

The calculated results table shows for each sample row the following columns (for details, see Table 10):

- the sample ID and well position
- the customized smear size **Range**
- the calculated concentration in **ng/µL** for the defined size range
- the **% Total**, which is the percent of total sample concentration in the smear range
- the **Avg. Size**
- **%CV** of the defined smear range

Smear Analysis ranges are color coded and a vertical dotted line will show on the electropherogram view showing the range and reporting the corresponding average size for the smears (see Figure 18).

If a sample well has not been selected for the **Smear Analysis**, its row will be left blank. If multiple size ranges are selected for a sample, multiple rows will be displayed. In Figure 17, all wells have two smear ranges selected: 10 to 35 kDa, and 70 to 175 kDa.

NOTE

The information in the **Smear Analysis** tab can be exported to the clipboard for pasting into common spreadsheet programs. Select **Export Data to Clipboard** from the context menu. Further information on the flag functions are discussed in Chapter 3, “ProSize Main Screen”.

Peak Table | Smear Analysis

ID	Range	ng/uL	% Total	Avg. Size	%CV
A1:	10 kDa to 35 kDa	0.7594	7.0	13	33.90
	70 kDa to 175 kDa	1.0173	9.4	118	26.31
A2:	10 kDa to 35 kDa	1.9077	2.1	15	31.69
	70 kDa to 175 kDa	1.4014	1.5	111	29.93
A3:	10 kDa to 35 kDa	6.4191	6.2	16	38.35
	70 kDa to 175 kDa	2.1135	2.0	119	25.36
A4: Ladder	10 kDa to 35 kDa	74.8937	33.8	23	27.66
	70 kDa to 175 kDa	60.4697	27.3	120	22.83
A5:	10 kDa to 35 kDa	1.1265	12.2	14	36.97
	70 kDa to 175 kDa	0.7408	8.0	116	27.89
A6:	10 kDa to 35 kDa	4.8592	5.2	16	29.44
	70 kDa to 175 kDa	1.4363	1.5	117	28.24

Figure 17 Smear Analysis tab on main screen

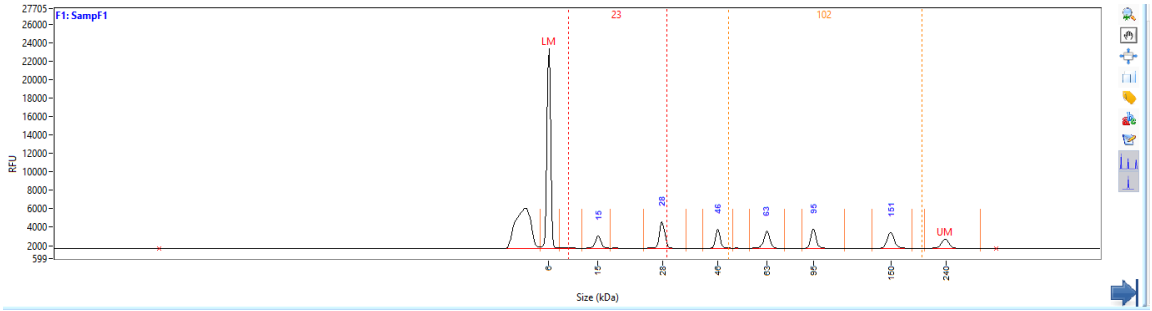


Figure 18 Electropherogram view when Smear Analysis tab selected on main screen

Table 10 Smear Analysis tab

Item	Description
ID	Lists the Sample Well: Sample Name.
Range	Lists the smear range, in size (kDa). The smear range corresponds to the **Start Size** and **End Size** entered in the **Smear Analysis** tab (**Global Configuration** dialog). Note: If the **Apply** function has not been made to the respective well, it will be blank in the table.
ng/uL	Reports the concentration of the defined smear range in ng/µL.
% Total	Reports the % of the total concentration of the sample that resides in the defined smear range.
Average Size	Reports the mean size (in kDa) of the defined smear range, accounting for the distribution of the concentration of the smear. Therefore, the Avg. Size will reflect the size corresponding to 50% of the total smear concentration being below and above this value.
% CV	Reports the distribution of concentration from the mean for the defined smear, in terms of the coefficient of variation (CV), where CV is the ratio of the standard deviation (σ) to the mean (µ): CV(%) = σ / µ The value is reported as a percentage; smaller values indicate a tighter size distribution of the smear concentration.
Export Data to Clipboard	From the context menu of the table, select **Export Data to Clipboard**. The peak table information is exported to the clipboard for pasting directly into common spreadsheet programs such as Microsoft Excel. The full table is exported.

Set Individual Parameters

When a data file is initially loaded into the ProSize software, the settings for selection of lower/upper markers, sample peak integration, size calibration, quantification, and any flag/smear analysis are globally set for all samples in the **Global Configuration** dialog (for more information, see **Chapter 4**, "ProSize Configuration").

After the data is loaded, any of the common configuration parameters can be individually edited for each sample independently (local setting), or changes can be made and applied to all samples (global setting), or to a customized subset of samples.

To view and adjust the configuration settings for the currently viewed lane/sample, select **Set Individual Parameters** on the right side of the **Peak Table**. This will reveal the configuration dialog, for which most settings can be individually adjusted for each sample as desired (**Figure 19**). To toggle back to the **Peak Table**, select **Show Results**.

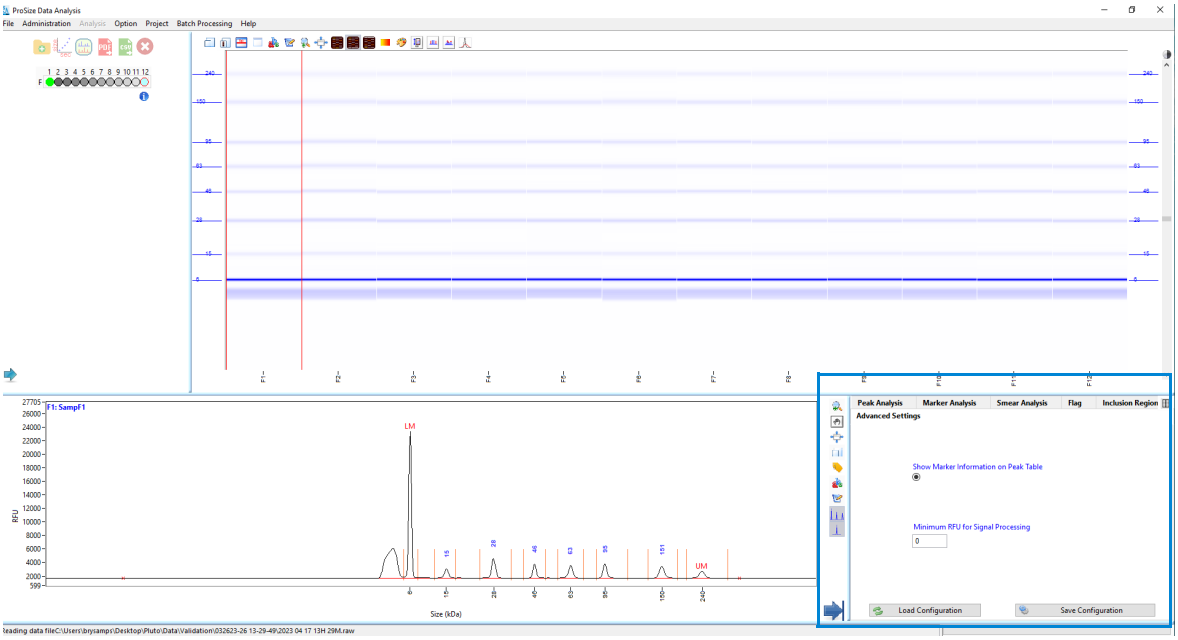


Figure 19 Main screen with data file open and configuration dialog open (clicked)

The individual settings within each tab are described in detail in **Chapter 4**, “ProSize Configuration”. The following sections describe the settings that apply to individual configuration tabs.

Configuration – Peak Analysis

The **Peak Analysis** tab contains settings to define the sample peak integration, data analysis filter, analysis region, and manual baseline set points (**Figure 20**). These functions are described in detail in section **“Global Configuration - Peak Analysis”** on page 64.

New settings in the **Peak Analysis** tab are applied to the current selected sample well only, or to all samples of the sample plate (see **Table 11**).

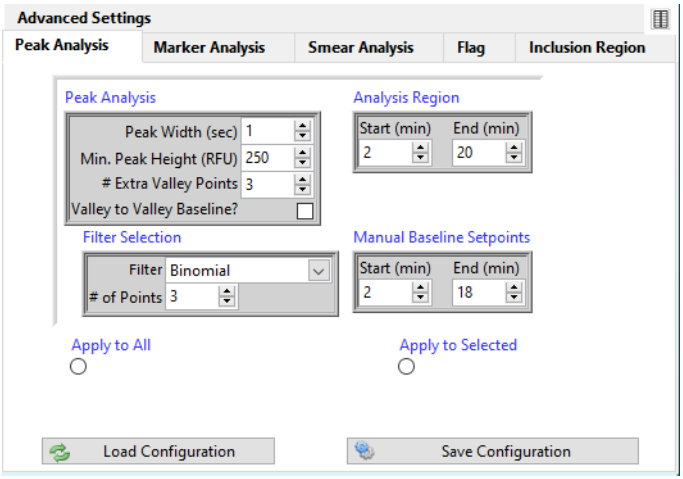
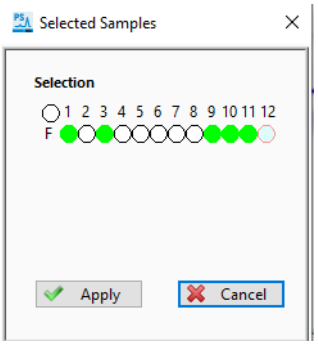


Figure 20 Peak Analysis tab in Set Individual Parameters dialog

Table 11 Additional Peak Analysis settings

Setting	Description
Apply to All	All current settings of the **Peak Analysis** tab will be applied to all samples of the loaded data file. This is therefore a global function. Note: Any previous individually tuned Peak Analysis tab settings for different samples of the data file will be overwritten when using this function.
Apply to Selected	All current settings of the **Peak Analysis** tab will be applied to the specified wells of the sample plate. Upon selecting this function, the **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the current settings to.  Click the desired wells, rows or columns, and click **OK** to apply; click **Cancel** to abort the operation. Note: Any previous individual setting of the Peak Analysis tab for the selected samples of the data file will be overwritten when using this function.

Configuration – Marker Analysis

The **Marker Analysis** tab contains settings to define the marker peak selection and marker peak integration (**Figure 21**). These settings are described in detail in **"Global Configuration - Marker Analysis"** on page 68.

New settings in the **Marker Analysis** tab can be applied to the current selected sample well only, or to all samples of the sample plate (see **Table 12**).

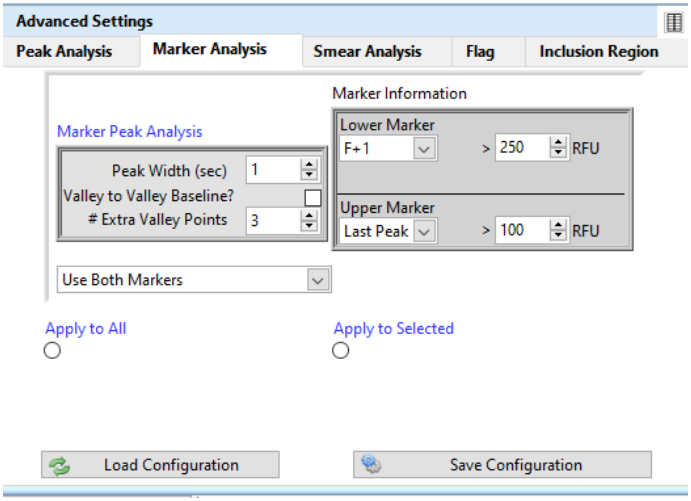
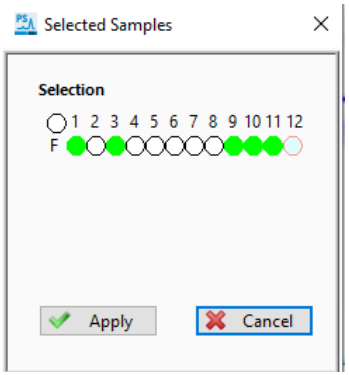


Figure 21 Marker Analysis tab Set Individual Parameters dialog

Table 12 Additional Marker Analysis settings

Setting	Description
Apply to All	All current settings of the **Marker Analysis** tab will be applied to all samples of the loaded data file. This is therefore a global function. Note: Any previous individually tuned Marker Analysis tab settings for different samples of the data file will be overwritten when using this function.
Apply to Selected	All current settings of the **Marker Analysis** tab will be applied to the specified wells of the sample plate. Upon selecting this function, the **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the current settings to.  Select the desired wells, rows or columns, and click **OK** to apply; click **Cancel** to abort the operation. Note: Any previous individual setting of the Marker Analysis tab for the selected samples of the data file will be overwritten when using this function.

Configuration – Smear Analysis

The **Smear Analysis** tab contains settings to enable the user to specify a size range(s) for which to integrate and calculate the total concentration in ng/mL (**Figure 22**). These functions are described in detail in **"Global Configuration - Smear Analysis"** on page 77

New settings in the **Smear Analysis** tab are applied to the current selected sample well only, or to all samples of the sample plate (see **Table 13**).

NOTE

If **Display Smear Range** is enabled, two color coded vertical dotted cursors are displayed in the Electropherogram Trace screen. These cursors define the entered values for the **Start Size (kDa)** and the **End Size (kDa)** for the currently selected smear range. The colors can be changed by left clicking on the color next to the smear range. The start and end points of the smear range can be adjusted by clicking on a cursor peak, and dragging the respective cursor left or right; the smear analysis region will be automatically updated to the new cursor location.

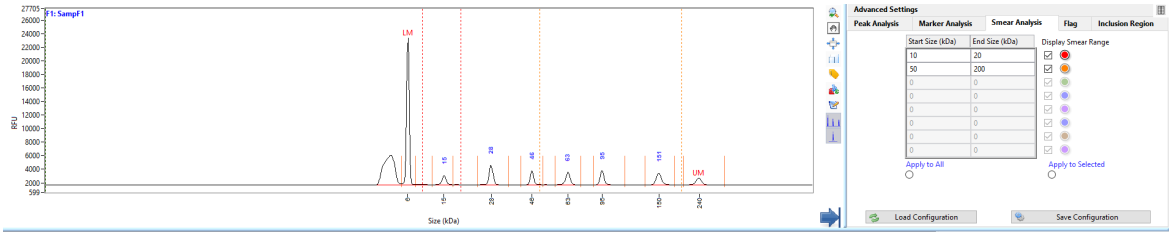
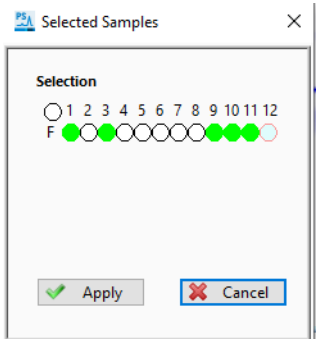


Figure 22 Smear Analysis tab in Set Individual Parameters dialog with Display Smear Range enabled

Table 13 Additional Smear Analysis settings

Setting	Description
Apply to All	All current settings of the **Smear Analysis** tab will be applied to all samples of the loaded data file. This is therefore a global function. Note: Any previous individually tuned Smear Analysis tab settings for different samples of the data file will be overwritten when using this function.
Apply to Selected	All current settings of the **Smear Analysis** tab will be applied to the specified wells of the sample plate. Upon selecting this function, the **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the current settings to.  Select the desired wells, rows or columns, and click **OK** to apply; click **Cancel** to abort the operation. Note: Any previous individual settings of the Smear Analysis tab for the selected samples of the data file will be overwritten when using this function.

Configuration – Flag Analysis

The **Flag Analysis** tab contains settings to enable the user to specify specific criteria (size or concentration range) to be met within the data, and generate a binary output (0 = false, 1 = true) of the results (**Figure 23**). These settings are described in detail in **"Global Configuration - Flag"** on page 79.

New settings in the **Flag Analysis** tab are applied to all samples of the sample plate. Some of these settings are described in **Table 14**.

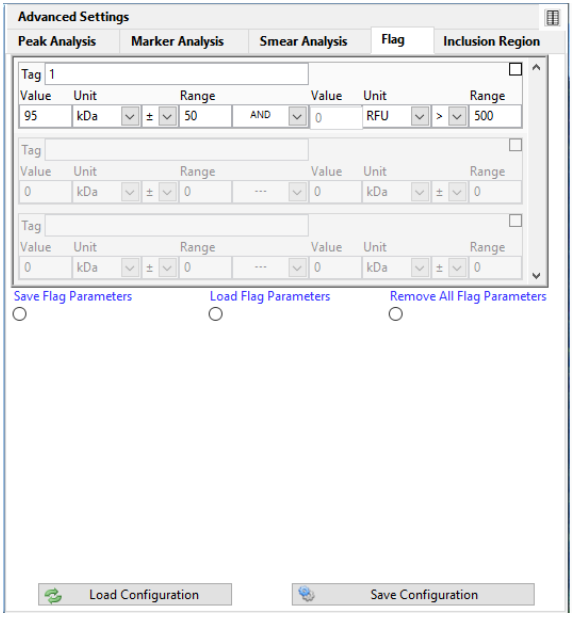


Figure 23 Flag Analysis tab in Set Individual Parameters dialog

Table 14 **Additional Flag Analysis settings**

Setting	Description
Save Flag Parameters	The current Flag Analysis settings will be saved as a file (*.Flag extension) that can be loaded into different data files. A file browser menu will open to navigate and save the desired filename and location; click **OK** to save the file, or **Cancel** to discard your changes.
Load Flag Parameters	The previously saved Flag Analysis settings will be loaded from a file (*.Flag extension). A file browser menu will open to navigate and load the desired filename and location; click **OK** to load the file, or **Cancel** to discard your changes.
Remove All Flag Parameters	All settings will be cleared from the tab.

Configuration – Advanced Settings

The **Advanced Settings** tab contains settings to assign the data analysis mode for processing data (**Figure 24**).

NOTE

The **Advanced Settings** parameters are applied to all samples in the data file. Individual samples cannot use different analysis modes. It is assumed all data is processed with a single analysis mode in a single experiment or data file.

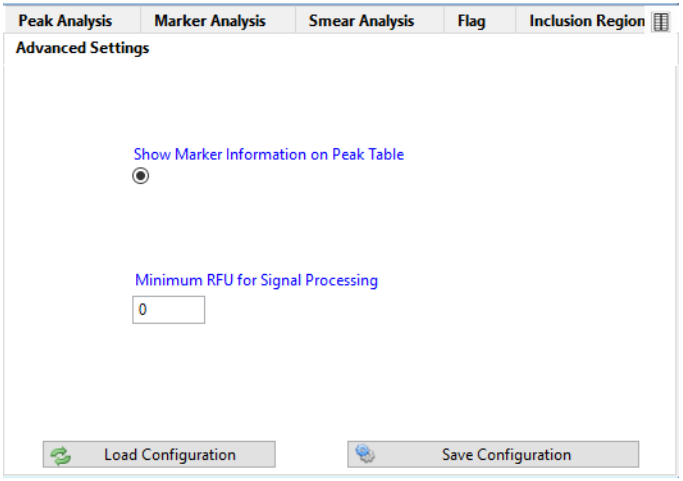


Figure 24 Advanced Settings tab in Set Individual Parameters dialog

4 ProSize Configuration

This chapter provides an overview of the **Global Configuration** dialog of the ProSize software. It also provides information on setting individual configuration parameters in opened data files.

About Configuration

The **Global Configuration** dialog (or **Set Individual Parameters** dialog for opened data files) is used to load, edit, or save the data analysis mode and settings for processing the data file, such as size calibration, marker/sample peak integration, quantification, smear analysis, and/or flag analysis criteria.

By using established method configurations for the different types of sample analysis on the instrument, the user can streamline data analysis with the ProSize software. This requires, that the first 10 characters of the ProSize configuration file name matches the first 10 characters of the parallel capillary electrophoresis system.

NOTE

In most cases, predefined, optimized global configuration files are available to load when processing data collected from different Agilent Reagent Kits. The configuration files are labeled according to the particular kit number, e.g., **5191-6640UM_LM - Protein Broad Range P240 LM and UM.ini**. These configuration files are available from Agilent upon request if needed. The configuration files preloaded upon software installation are located in the C:\ProSize data analysis software\Configurations folder.

Access Configuration Information in the ProSize Software

There are two ways to access configuration information in the ProSize software:

Global Configuration Dialog

This option is used to define how an unprocessed data file is first opened in the ProSize software. The user is automatically prompted with the **Global Configuration** dialog when opening an unprocessed data file if the corresponding separation method used does not match the naming configuration (**Figure 25**).

Alternatively, once a data file is opened, the user can modify at any time the configuration by selecting **Option**, and clicking **Edit Configurations**  .

NOTE

In most cases, predefined, optimized global configuration files are available to load when processing data collected from different Agilent Reagent Kits. The configuration files are labeled according to the particular kit number, for example, **5191-6640UM_LM - Protein Broad Range P240 LM and UM.ini**. These configuration files are available from Agilent upon request if needed. The configuration files preloaded upon software installation are located in the C:\ProSize data analysis software\Configurations folder.

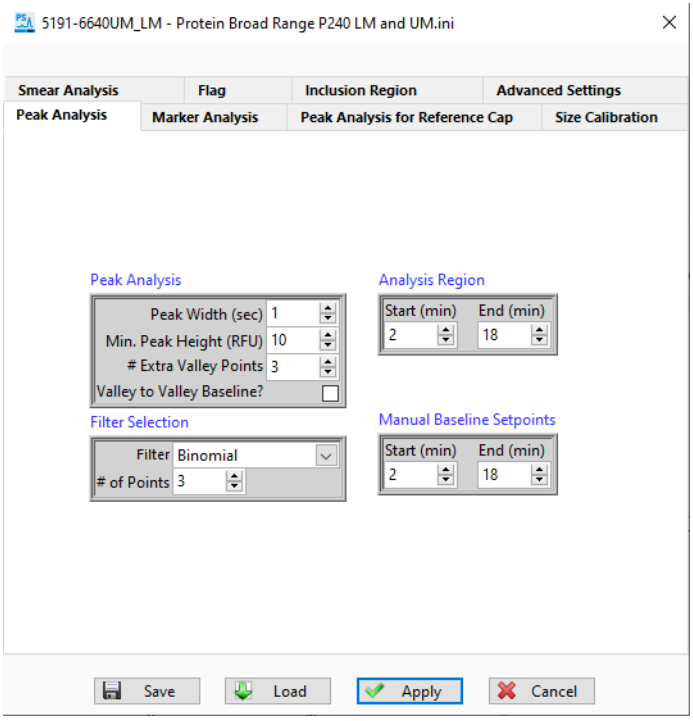


Figure 25 Global Configuration dialog

Set Individual Parameters Dialog

This dialog is used to modify or change configuration settings after a data file is opened. The **Set Individual Parameters** dialog can be accessed in an open data file by selecting on the right side of the main menu (**Figure 26**). Note that this icon toggles between the **Set Individual Parameters** and **Show Results** ( ) form, which displays the **Peak Table** and **Smear Analysis** or **Flag Analysis** tables.

NOTE

Using **Set Individual Parameters** dialog, the currently selected tab is applied independently from the other configuration tabs. In addition, if the user select [**Enter**] after changing a setting, the change is only applied to the currently selected sample; therefore this is a "local" setting. To apply the change made in the tab to all samples, click **Apply to All**; to apply the change only to selected samples, click **Apply to Selected**.

The settings within each tab of the **Global Configuration** and **Set Individual Parameters** dialog perform the same functions. Each tab of the configuration is discussed in further detail in the following sections of this chapter.

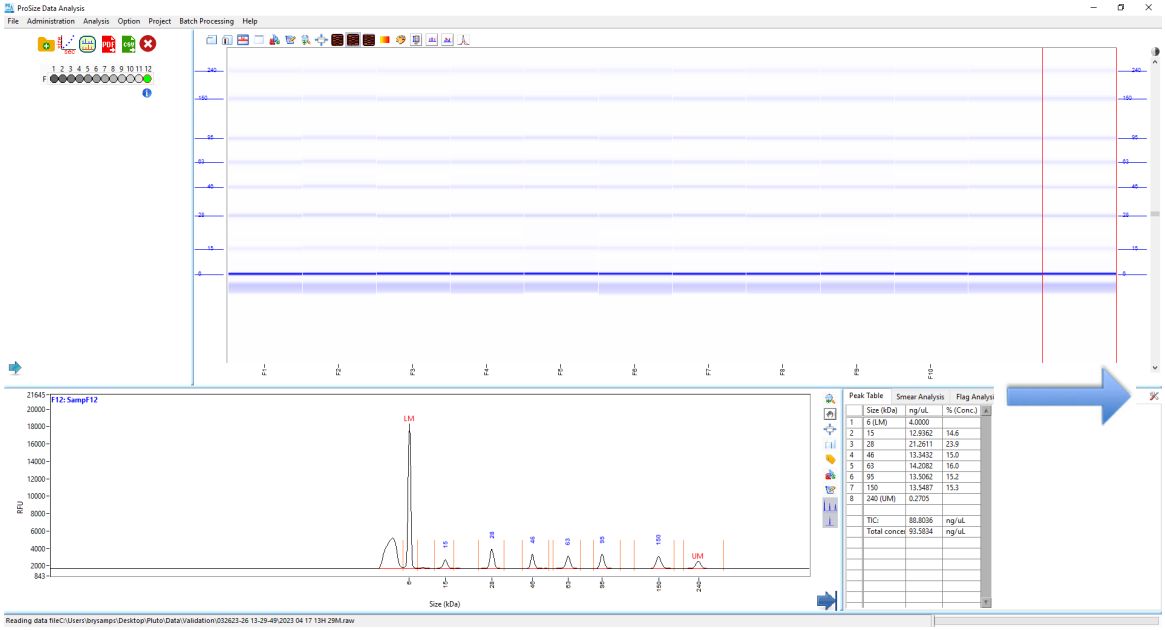


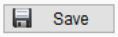
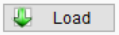
Figure 26 Set Individual Parameters icon for configuration settings after a data file is opened

Overview Global Configuration Dialog

There are ten tabs in the **Global Configuration** dialog to define the appropriate data analysis mode and settings for processing the data, as described in the following sections.

At the bottom of the **Global Configuration** dialog, the user is able to **Save**, **Load**, or **Apply** the configuration settings (see **Table 15**).

Table 15 Global Configuration dialog buttons

Button	Description
	Saves the global configuration as a user defined file (*.ini format). The settings from all tabs are saved, regardless of which tab is open when saving the file.
	Loads a predefined configuration file (*.ini format). Note than upon installation, predefined configuration files are installed for most Agilent Reagent Kits in the in the C:\ProSize data analysis software\Configurations folder.
	Applies the current global configuration settings to the data file. If the data file is unprocessed, pressing the **Apply** button will open the data file with the currently loaded settings. If the data file is already open, the new settings will be applied to the data, overwriting any previously defined individual configuration settings.
	Cancels all changes and closes the global Configuration window.

Protein Mode

For analyzing standard protein samples. This mode displays size in kilodaltons (kDa) using a protein ladder for calibration. Absolute quantification is achieved via comparison to a lower marker and to a protein ladder present at a known concentration.

Global Configuration - Peak Analysis

The settings of the **Peak Analysis** tab allow the user to assign width and height thresholds for selecting and integrating sample peaks; setting data filtering; and selecting the time region to be analyzed (**Figure 27**). The settings are described in detail in **Table 16**.

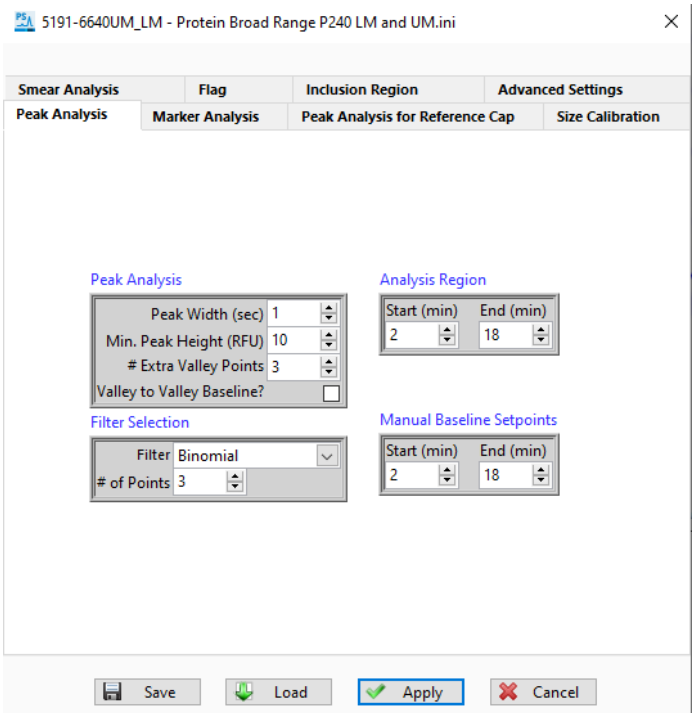


Figure 27 Peak Analysis tab in Global Configuration dialog

Table 16 Peak Analysis configuration settings

Settings	Description
Peak Analysis	
Peak Width (sec)	Defines the width threshold for peak detection in seconds. Higher settings better define wide peak start/end points; smaller settings better define sharp peak start/end points. Typical values range from 3-5 for sharp peaks or 25-50 for smears. The maximum value for this setting is 50.
Min. Peak Height (RFU)	Defines the minimum peak height threshold to select a peak for integration in RFUs. Peaks below the set value will not be selected for integration. Typical values for this setting are 20-100 RFU, depending upon the sample signal and application. Note the typical baseline peak-to-peak noise is less than 5 RFU.
# Extra Valley Points	This setting influences the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. Higher values draw a straighter baseline between the first peak start point and second peak end point; lower values draw the baseline more to the "valley" between the two unresolved peaks. Note: The **Valley to Valley Baseline?** setting needs to be enabled for this setting to be active.

Table 16 Peak Analysis configuration settings

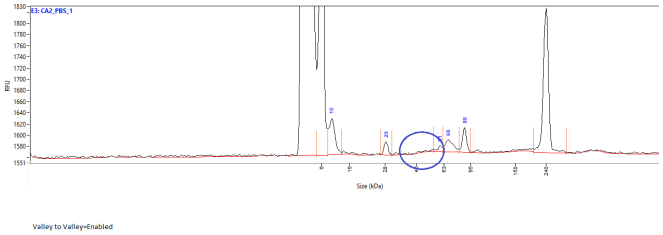
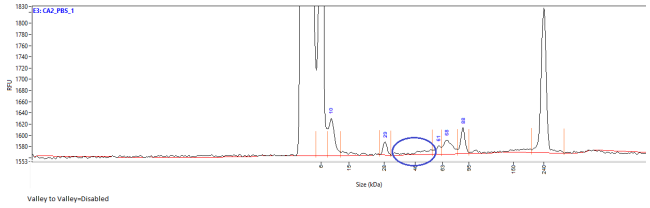
Settings	Description
Valley to Valley Baseline?	This setting influences the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. When disabled, will draw a straighter baseline between the first peak start point and second peak end point. When enabled, will draw the baseline more to the "valley" between the two unresolved peaks. In the example below, the Top trace shows the baseline drawn when Valley to Valley Baseline is enabled; the Bottom trace shows the baseline for the same sample when this setting is disabled.  Valley to Valley = Enabled  Valley to Valley = Disabled
Filter Selection	
Filter	Defines the algorithm type to use when filtering the raw data. There are five options for data filtering available to the user: • **None**: No filtering is performed on the data. • **Moving Average**: Averages points on either side of a point. • **Savitzky Golay**: A polynomial regression filter where the coefficients are calculated using Savitzky and Golay's algorithm. • **Binomial**:. A polynomial regression filter based on the binomial coefficients for implementing Gaussian filtering. • **Wavelets De-noising**: A discrete wavelet transform filter. Note: The recommend filter for most applications is a 3 to 20 point Binomial filter.

Table 16 Peak Analysis configuration settings

Settings	Description
# of Points	Defines the number of points to use in the selected data filter. Maximum points to use for each filter type: • Moving Average: 9 • Savitsky Golay: 9 • Binomial: 20 • Wavelets De-Noising: 4
Analysis Region	
Start (min)	Defines the start time in minutes for which to integrate and analyze the electropherogram data. This includes both marker and sample peaks. Note: If 0 is entered, will analyze data from start of the electropherogram at 0 min.
End (min)	Defines the end time in minutes for which to integrate and analyze the electropherogram data. This includes both marker and sample peaks. Note: If 0 is entered, will automatically analyze data to the full end time range of the electropherogram.
Manual Baseline Setpoints	Defines the baseline using time points outside the lower/upper marker region. This function is particularly useful for accurately measuring the total concentration of samples containing smears or uneven baselines.
Start (min)	Defines the start time in minutes for which to draw the start of the baseline when manually setting the baseline. The Y axis position of the baseline at the entered start point in minutes will be used to form the new baseline start point. Note: If 0 is entered for both the **Start (min)** and **End (min)**, this function is disabled. When entering values for this function, enter the ending time value in the **Start (min)** field first. When next entering the start time the end time value will automatically populate the **End (min)** field.
End (min)	Defines the end time in minutes for which to draw the end of the baseline when manually setting the baseline. The Y-axis position of the baseline at the entered end point in minutes will be used to form the new baseline end point. Note: If 0 is entered for both the **Start (min)** and **End (min)**, this function is disabled. When entering values for this function, enter the ending time value in the **Start (min)** field first. When next entering the start time, the end time value will automatically populate the **End (min)** field.

Global Configuration - Marker Analysis

The settings of the **Marker Analysis** tab allow the user to select the criteria for choosing the lower and/or upper peak, used to normalize each capillary by migration time and concentration (**Figure 28**). The settings are described in detail in **Table 17**.

In ProSize, the user can independently set different peak parameters for the lower/upper markers from those set for the sample peak parameters in **Peak Analysis** tab. This is useful if, for example, the sample contains broad smearing peaks as compared to markers, which are typically sharp Gaussian peak profiles.

The user can select a lower/upper marker pair (**Use Both Markers**), or use only a lower marker (**Use Lower Marker Only**) or upper marker (**Use Upper Marker Only**) for data normalization by selecting the appropriate drop-down option.

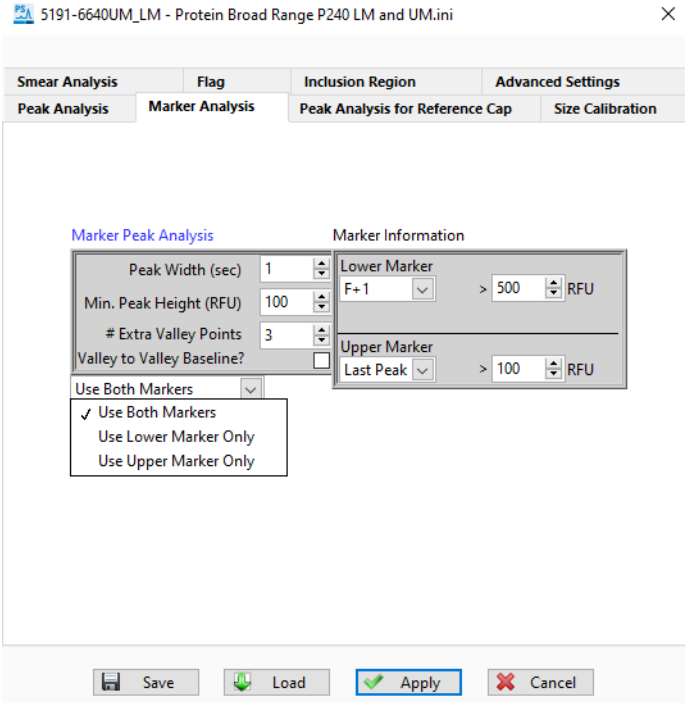


Figure 28 Marker Analysis tab in Global Configuration dialog

The markers can be selected based on peak width and peak height thresholds, and also by time range in the **Marker Information** section (**Figure 29**).

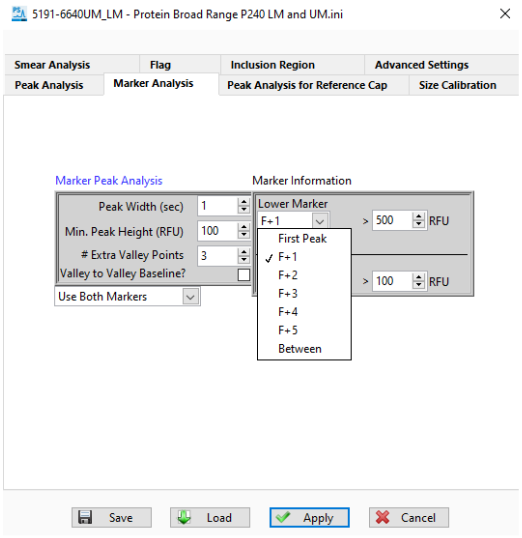


Figure 29 Marker Analysis tab showing marker selection options

The lower marker can be selected as the **First Peak**, or second peak (**F + 1**), third peak (**F + 2**), etc. up to the sixth peak above a threshold value in RFU units. Similarly, the upper marker can be selected as the **Last Peak**, or **L – 1**, **L – 2**, etc. Markers can also be selected based upon a time window, by selecting **Between** from the drop-down and entering a time in X min +/- Y min. For example, the right image of **Figure 29** shows lower marker selection criteria of a peak > 200 RFUs between 8 – 12 min (10 min +/- 2 min).

NOTE

The **Min. Peak Height (RFU)** setting under **Marker Peak Analysis** sets the primary height threshold for the markers. The height threshold values for each individual marker shown under **Marker Information** must therefore be equal to or greater than the **Min. Peak Height (RFU)** value.

The settings of the **Marker Analysis** tab are described in **Table 17**.

Table 17 Marker Analysis configuration settings

Settings	Description
Marker Peak Analysis	
Peak Width (sec)	Defines the width threshold for peak detection and peak start/end point in seconds. Higher values better define wide peak start/end points; smaller settings better define sharp peak start/end points. Typical values are 3-5 for marker peaks.
Min. Peak Height (RFU)	Defines the minimum peak height threshold to select a marker peak in RFUs. Peaks below the set value will not be selected as markers.
# Extra Valley Points	This setting influences the start/end point of baseline integration of the marker peaks and the baseline. This setting most affects the baseline between two peaks that are not baseline resolved. Higher values draw a straighter baseline between the first peak start point and second peak end point; lower values draw the baseline more to the "valley" between the two unresolved peaks. Note: The **Valley to Valley Baseline?** setting needs to be enabled for this setting to be active.
Valley to Valley Baseline?	This setting influences the start/end point of baseline integration of the marker peaks and the baseline. This setting most affects the baseline between two peaks that are not baseline resolved. When disabled, will draw a straighter baseline between the first peak start point and second peak end point. When enabled, will draw the baseline more to the "valley" between the two unresolved peaks.
Use Both Markers	Normalizes and aligns the CE data to both a selected lower and upper size marker.
Use Lower Marker Only	Normalizes and aligns the CE data to only the selected lower marker.
Use Upper Marker Only	Normalizes and aligns the CE data to only the selected upper marker.
Marker Information	
Lower Marker	This setting determines how the lower marker is selected from the CE data, using the threshold value in RFU as the minimum peak height for selection. The lower marker is selectable from the drop-down control as: • First Peak (F) • F + 1, F + 2, F +3, F + 4, or F +5 • Between: Enter a specific time in X minutes +/- Y min
Upper Marker	This setting determines how the upper marker is selected from the CE data, using the threshold value in RFU as the minimum peak height for selection. The upper marker is selectable from the drop-down control as: • Last Peak (L) • L - 1, L - 2, L - 3, L - 4, or L - 5 • Between: Enter a specific time in X minutes +/- Y min

Global Configuration - Peak Analysis for Reference Cap

The settings of the **Peak Analysis for Reference Cap** tab allow the user to assign width and height thresholds for selecting and integrating peaks in the "Reference" capillary, which is the assigned well containing the Ladder used to calibrate size (**Figure 30**). The settings are described in detail in **Table 18**.

This feature enables the user to define a higher threshold for the ladder peaks as compared to the sample peaks.

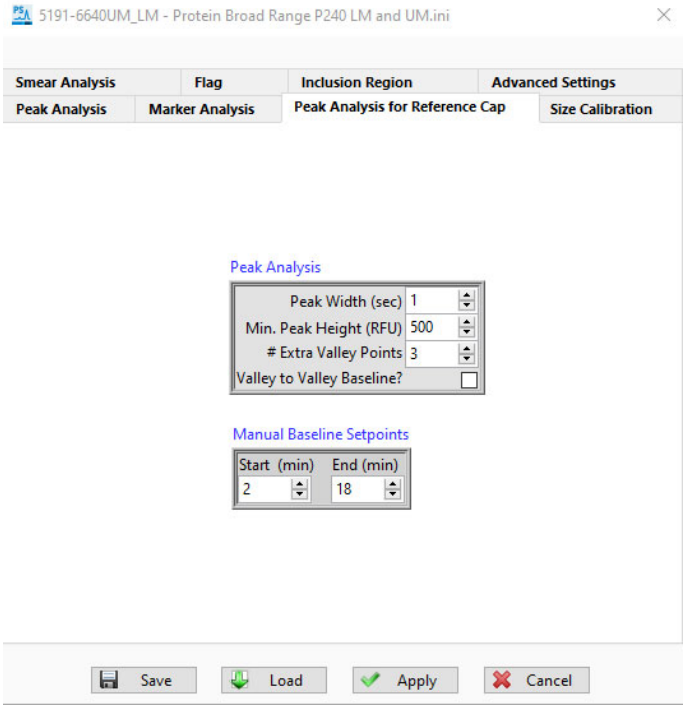


Figure 30 Peak Analysis for Reference Cap tab in Global Configuration dialog

Table 18 Peak Analysis for Reference Cap configuration settings

Settings	Description
Peak Analysis	
Peak Width (sec)	Defines the width threshold for peak detection in seconds. Higher settings better define wide peak start/end points; smaller settings better define sharp peak start/end points. Typical values range from 3-5 for sharp peaks.
Min. Peak Height (RFU)	Defines the minimum peak height threshold to select a peak for integration in RFUs. Peaks below the set value will not be selected for integration. Typical values for this setting when working with a protein Ladder 100-200 RFU, depending upon the ladder concentration and application.
# Extra Valley Points	This setting influences the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. Higher values draw a straighter baseline between the first peak start point and second peak end point; lower values draw the baseline more to the "valley" between the two unresolved peaks. Note: The **Valley to Valley Baseline?** setting needs to be enabled for this setting to be active.
Valley to Valley Baseline?	This setting influences the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. When disabled, will draw a straighter baseline between the first peak start point and second peak end point. When enabled, will draw the baseline more to the "valley" between the two unresolved peaks.

Global Configuration - Size Calibration

The settings of the **Size Calibration** tab allow the user to select the proper "Reference" well containing a protein Ladder for calibration of sample size; to enter in the proper size and number of fragments (including lower/upper markers) in the calibration ladder; and to apply a fit algorithm for plotting the size vs. migration time data from the ladder (**Figure 31**). The settings are described in detail in **Table 19**.

In ProSize, the user can calibrate the size of sample fragments either by running a standard protein ladder in a well of the sample plate (recommended), or by importing a previously calculated and exported size calibration file (*.scal extension).

NOTE

If an imported size calibration is used in ProSize, the same Separation Gel, capillary array dimensions, injection/run voltage, and lower/upper markers should be used for both the analyzed data and the imported calibration to ensure maximum sizing accuracy.

To properly calibrate the size, the size ladder must be bracketed by the same lower/upper markers used with the samples. In some cases, the lower or upper marker may co-migrate with a ladder peak; this is acceptable for sizing calibration.

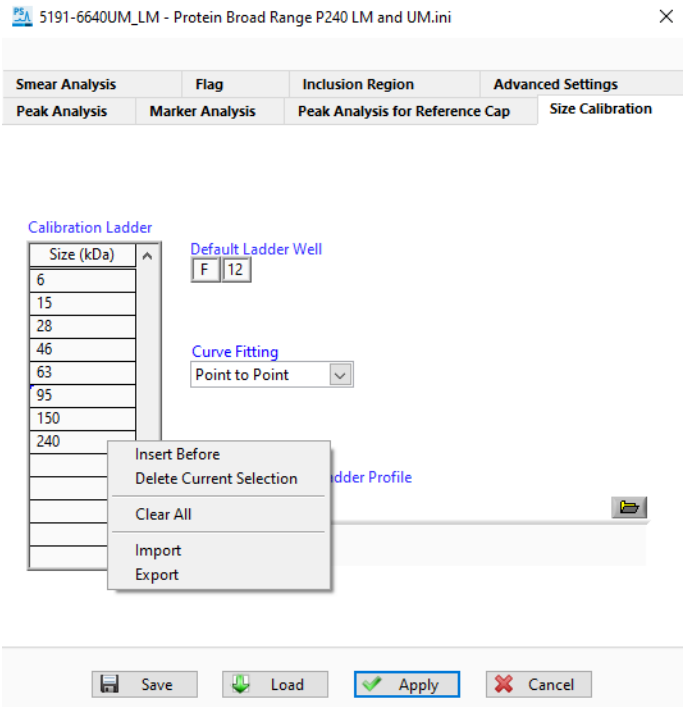


Figure 31 Size Calibration tab in Global Configuration dialog

It is important to ensure the sample containing the size calibration ladder ("Reference" well) has all ladder fragments integrated correctly with no additional peaks integrated prior to analyzing the remaining samples.

The size of each fragment to be included in the protein Ladder (including lower/upper markers) is entered directly or imported into the **Calibration Ladder** field. To directly enter a value, click in the field and enter the size value.

A context menu is available when selecting the **Calibration Ladder** table. It offers options such as inserting or deleting fragments in the table; clearing all entries; and importing or exporting ladder information files (*.txt extension).

NOTE

In most cases, predefined ladder information files optimized for different Agilent reagent kits are available and are loaded as part of the **Global Configuration**. The ladder files are labeled according to the particular kit number, for example, **5191-6640UM_LM - Protein Broad Range P240 LM and UM.ini**. These ladder files are available from Agilent upon request if needed. The preloaded ladder files are located in the **Ladders** folder under C:\ProSize data analysis software.

Table 19 Size Calibration configuration settings

Settings	Description
Calibration Ladder	Lists size of protein fragments contained in calibration ladder in tabular format. The size of each fragment (include lower/upper markers) is entered directly into the table or imported by using the context menu (right-click in the **Calibration Ladder** table).
Calibration Ladder > Insert Before	Inserts a blank cell above the currently selected cell in the calibration ladder table. Note: Click in the respective table cell before using this option.
Calibration Ladder > Delete Current Selection	Deletes the currently selected cell in the calibration ladder table. Note: Click in the respective table cell before using this option.
Calibration Ladder > Clear All	Deletes all cells in the calibration ladder table.
Calibration Ladder > Import	Imports a previously exported and saved sizing ladder information file. When selected, a file browser menu will open to navigate and select the desired file (*.txt extension). Standard ladder information files are located in the C:\ProSize data analysis software\Ladders folder.
Calibration Ladder > Export	Exports the current sizing ladder table information (*.txt extension). When selected, a file browser menu will open to navigate and save the desired filename and location. When exporting ladder information files, it is highly recommended to save the created files to a common directory, located in the C:\ProSize data analysis software\Ladders folder.
Default Ladder Well	Defines the well of the sample plate, which contains the size calibration ladder. From the drop-down list, select the proper row (A-H) and column (1-12) location containing the size calibration ladder.
Curve Fitting	• Point to Point Fits the size calibration plot of Size (kDa) vs. Time (sec) with a Point to Point fit (recommended), drawing a straight line between each point of the curve. • Polynomial Fits the size calibration plot of Size (kDa) vs. Time (sec) with a polynomial fit. Enter the order value (from 3 to 5) in the **Order** field.

Table 19 Size Calibration configuration settings

Settings	Description
Use Imported Ladder Profile	Imports a previously exported size calibration file (*.scal extension) to use for size calibration.
Ladder Profile	Displays file path for the imported size calibration file. To load an imported file, click  . A file browser menu will open to navigate and select the desired file (*.scal extension). Note: When importing a size calibration file, the same conditions (separation gel, capillary array length, lower/upper markers, CE separation voltage) should be used. Differences between the analyzed and imported conditions will adversely affect the sizing accuracy.

Further information on the Size Calibration function is described in chapter **Chapter 5**, “ProSize Size Calibration Screen”.

Global Configuration - Smear Analysis

The settings of the **Smear Analysis** tab allow the user to specify a size range(s) for which to integrate and calculate the total concentration in ng/µL. This is useful for example for smears, where knowledge of the concentration of a specific size range within a smear is more important than that of the total smear size range (**Figure 32**). The settings are described in detail in **Table 20**.

Several different size ranges can be applied at once to a sample; the calculated total concentration within the specified range is presented in a tabular format that can be exported in a .csv format or printed as a hard copy PDF report.

If one or more selections are entered in the **Smear Analysis** tab, upon opening the data a **Smear Analysis** tab will displayed next to the **Peak Analysis** tab, with each individual result populating a separate line, showing the calculated concentrations for each range.

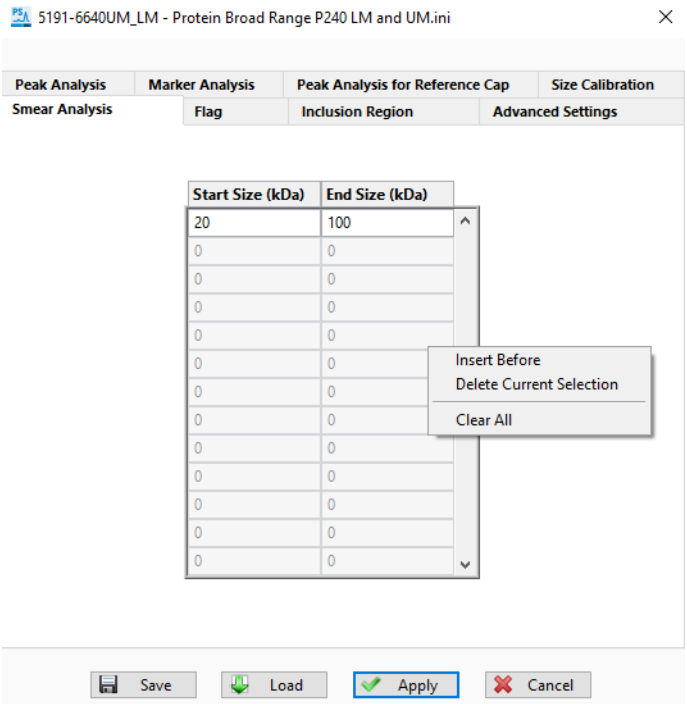


Figure 32 Smear Analysis tab in Global Configuration dialog

Table 20 Smear Analysis configuration settings

Settings	Description
Start Size (kDa)	Defines the lower size threshold (in kDa) to include in the calculation of total concentration. Note: If the entered size is in the middle of a peak/smear, the concentration is calculated from that size and higher and will not include the portion of the peak/smear below the size threshold.
End Size (kDa)	Defines the upper size threshold (in kDa) to include in the calculation of total concentration. Note: If the entered size is in the middle of a peak/smear, the concentration is calculated from that size and below and will not include the portion of the peak/smear above the size threshold.
Insert Before	Context menu option of the **Smear Analysis** table. Inserts a blank cell row above the currently selected cell in the table. Note: Click in the respective table cell before using this option.
Delete Current Selection	Context menu option of the **Smear Analysis** table. Deletes the currently selected cell row in the table. Note: Click in the respective table cell before using this option.
Clear All	Context menu option of the **Smear Analysis** table. Deletes all cells in the table.

Global Configuration - Flag

The settings of the **Flag** tab allow the user to specify specific criteria (size or concentration range) to be met within the data, and generate a binary output (0 = false, 1 = true) of the results. This is useful for applications (for example, genotyping) where the presence/absence of bands is to be scored (**Figure 33**). The settings are described in detail in **Table 21**.

Flag criteria can be set based on size, concentration, peak height and/or corrected peak area. Boolean logic is applied to the criteria for establishing various condition.

If one or more selections are entered in the **Flag** tab, upon opening the data an additional **Flag Analysis** tab will be displayed with the **Peak Table** for each individual results, displaying the flag results in a binary 1/0 format.

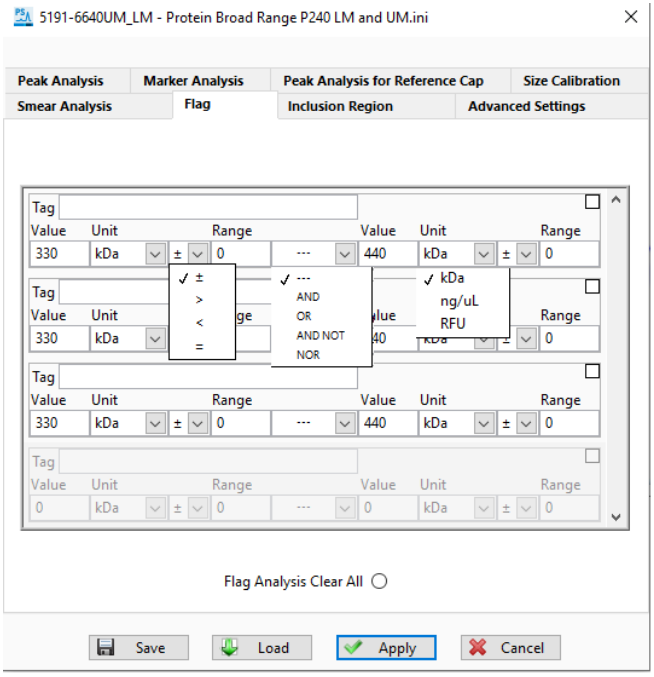


Figure 33 Flag tab in Global Configuration dialog

Table 21 Flag configuration settings

Settings	Description
Tag	Allows naming of the flag condition being used.
Value	Defines the numerical value of the unit for the flag condition.
Unit	Drop-down list which defines the units of the condition to flagged. The first **Unit** is always size (kDa) based; for the second **Unit** criteria there are four secondary flagging options: • kDa(size) • ng/mL (concentration) • RFU (Relative Fluorescence Units)
Range	Defines the numerical range to use for applying the flag condition. The drop-down to the left of this field determines the operation to apply: • **±** (will flag the value entered with a plus and minus range) • **>** (will flag when greater than the range) • **<** (will flag when less than the range) • **=** (will flag when equal to the range)
Boolean Operation	Drop-down list which defines the Boolean operation to apply to the flag condition. There are five options to apply: • **---**: No second flag condition is applied. • **AND**: Both the first and second flag condition must apply to be true • **OR**: First or second flag condition must apply to be true • **AND NOT**: The first flag condition must apply and the second must not apply to be true • **NOR**: Both the first and second flag condition must not apply to be true
Flag Analysis Clear All	Clears all existing flag conditions from the table.
Checkbox ☐	Clears the corresponding row flag condition entry.

Global Configuration – Inclusion Region

The settings of the Inclusion Region tab allow the user to define up to four size inclusion ranges. Only peaks (excluding markers) that fall into the defined range(s) will be displayed. This will affect the screen display, PDF reports and exported data. The Inclusion Region tab is shown in with functions described in Table 22.

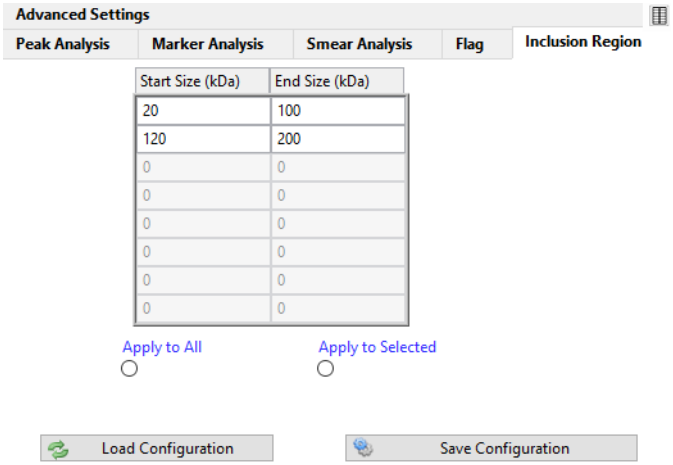


Figure 34 Inclusion Region tab in Global Configuration dialog

Table 22 Inclusion Region configuration settings

Settings	Description
Start Size (kDa)	Defines the lower size boundary (in kDa) of the Inclusion Region. Peaks (with the exception of the Lower Marker) smaller than the lower boundary will not be displayed.
End Size (kDa)	Defines the upper size boundary (in kDa) of the Inclusion Region. Peaks (with the exception of the Upper Marker) larger than the upper boundary will not be displayed.
Insert Before	Context menu option of the **Inclusion Region** table. Inserts a blank cell row above the currently selected cell in the table. Click in the respective table cell to use this option.
Delete Current Selection	Context menu option of the **Inclusion Region** table. Deletes the currently selected cell row in the table. Click in the respective table cell to use this option.
Clear All	Context menu option of the **Inclusion Region** table. Deletes all cells in the table.

Set Individual Parameters

After a data file is opened in the ProSize program, the configuration settings can be adjusted for individual samples by the **Set Individual Parameters** option.

The **Set Individual Parameters** dialog consist of main tabs that can be adjusted similar to those tabs in the **Global Configuration** dialog.

Some tabs can be applied to individual samples, while others can only be applied to all samples:

- **Advanced Settings**

  When a file is open and the analysis mode is selected, you can select to show marker information on peak table and set a minimum run for signal processing.

- **Peak Analysis**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Marker Analysis**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Smear Analysis**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Flag**

  When a file is open, all samples are changed to the current Flag settings and cannot be individually adjusted.

- **Inclusion Region**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

NOTE

When changing configuration settings in the **Set Individual Parameters** dialog, the reference ladder well is treated separately from the sample wells. Therefore, if **Apply to All** is performed on a sample well, the current reference ladder well will not be updated.

If the user chooses **Apply to All** from the reference ladder well, all sample wells including the ladder well will be updated.

At the bottom of the **Set Individual Parameters** dialog are two functions for setting and loading the configuration, as shown in **Figure 35** and summarized in **Table 23**.

Table 23 Set Individual Parameters buttons

Button	Description
Set as Default Configuration	Sets the default configuration for all files that are subsequently opened (i.e., the **Global Configuration**). This is useful if you consistently open files of a similar type (i.e., all files are based on the use of a ladder in well "A12" for example.
Load	Opens a file browser menu for loading a previously created configuration file (*.ini extension). Navigate to the desired directory location containing the configuration file, and click **OK** to load.

The settings of the tabs in the **Set Individual Parameters** dialog differ from those in the **Global Configuration** dialog. The tabs are summarized in the following sections.

Set Individual Parameters - Advanced Settings

The settings of the **Advanced Settings** tab allow the user to assign the data analysis mode for processing data (Figure 35). The **Minimum RFU for Signal Processing** is used to set a minimum RFU value for integration.

NOTE

When any change is made to the **Mode**, all samples are changed, including the reference ladder well.

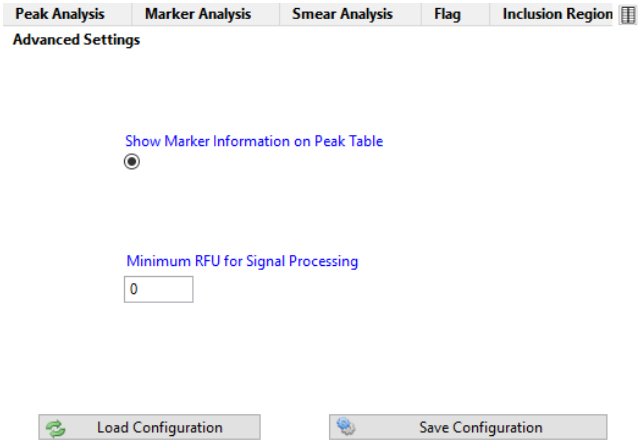


Figure 35 Advanced Settings tab in Set Individual Parameters dialog

Set Individual Parameters - Peak Analysis

Table 24 describes additional settings of the **Peak Analysis** tab not available in the **Global Configuration** dialog. If the user changes a field and/or presses [**Enter**], the new setting will be applied to the current selected sample well only. The Peak Analysis tab is shown in Figure 36.

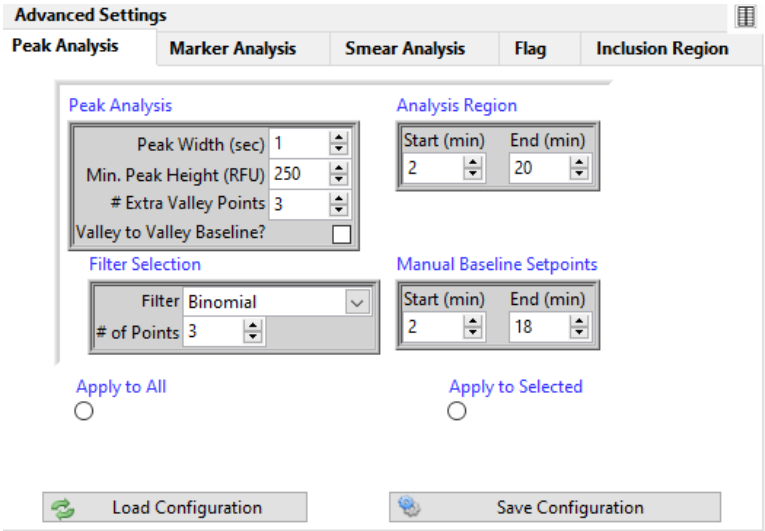
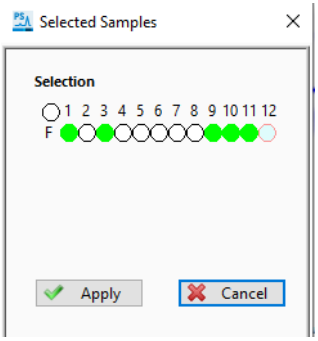


Figure 36 Peak Analysis tab in Set Individual Parameters dialog

Table 24 Additional settings in the Peak Analysis tab

Settings	Description
Apply to All	All current settings of just the **Peak Analysis** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Peak Analysis** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.
Apply to Selected Samples	Applies all current settings of just the **Peak Analysis** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; click **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Peak Analysis** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.

Set Individual Parameters – Marker Analysis

Table 25 describes additional settings of the **Marker Analysis** tab not available in the **Global Configuration**. If the user changes a field and/or presses [**Enter**], the new setting will be applied to the current selected sample well only. The Marker Analysis tab is shown in Figure 37.

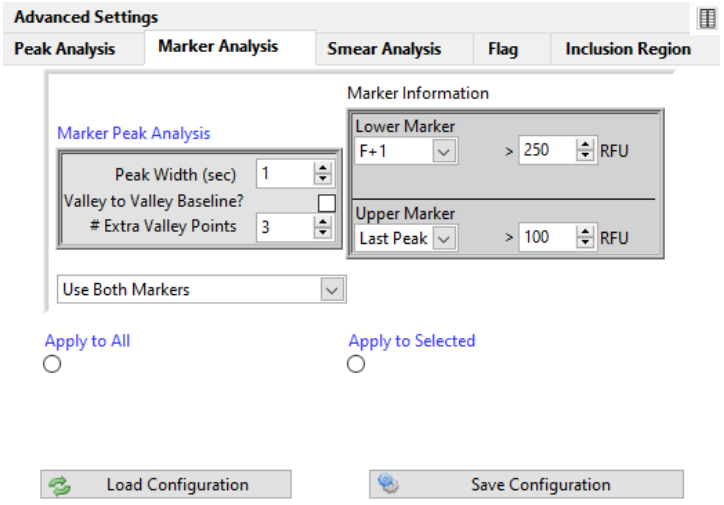
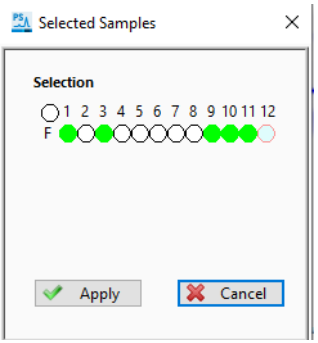


Figure 37 Marker Analysis tab in Set Individual Parameters dialog

Table 25 Additional settings of the Marker Analysis tab

Settings	Description
Apply to All	All current settings of just the **Marker Analysis** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Marker Analysis** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.
Apply to Selected Samples	Applies all current settings of just the **Marker Analysis** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; click **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Marker Analysis** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.

Set Individual Parameters – Smear Analysis

Table 26 describes additional settings of the **Smear Analysis** tab not available in the **Global Configuration**. If the user changes a field and/or presses [**Enter**], the new setting will be applied to the current selected sample well only. The Smear Analysis tab is shown in Figure 38.

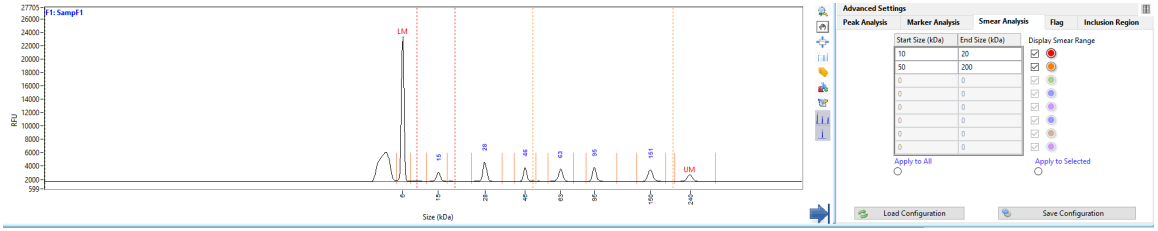


Figure 38 Smear Analysis tab in Set Individual Parameters dialog

Table 26 Additional settings in the smear analysis tab

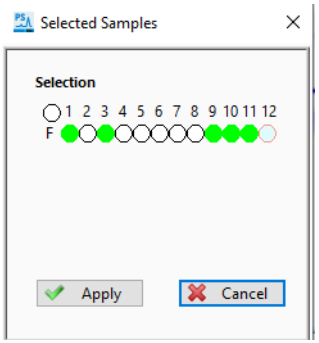
Settings	Description
Apply to All	All current settings of just the **Smear Analysis** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Smear Analysis** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.

Table 26 Additional settings in the smear analysis tab

Settings	Description
Apply to Selected Samples	Applies all current settings of just the **Smear Analysis** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; click **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Smear Analysis** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.
Display Smear Range	When enabled, two vertical cursors are displayed in the Electropherogram Trace screen. These cursors define the entered values for the Start Size (kDa) and the End Size (kDa) for the currently selected smear range. The start and end points of the smear range can be adjusted by clicking on a cursor peak, and dragging the respective cursor left or right; the smear analysis region will be automatically updated to the new cursor location.

Set Individual Parameters – Flag Analysis

Table 27 describes additional settings of the **Flag Analysis** tab not available in the **Global Configuration**. If the user changes a field and/or presses [**Enter**], the new flag setting will be applied to all samples of the sample plate. The Flag Analysis tab is shown in Figure 39.

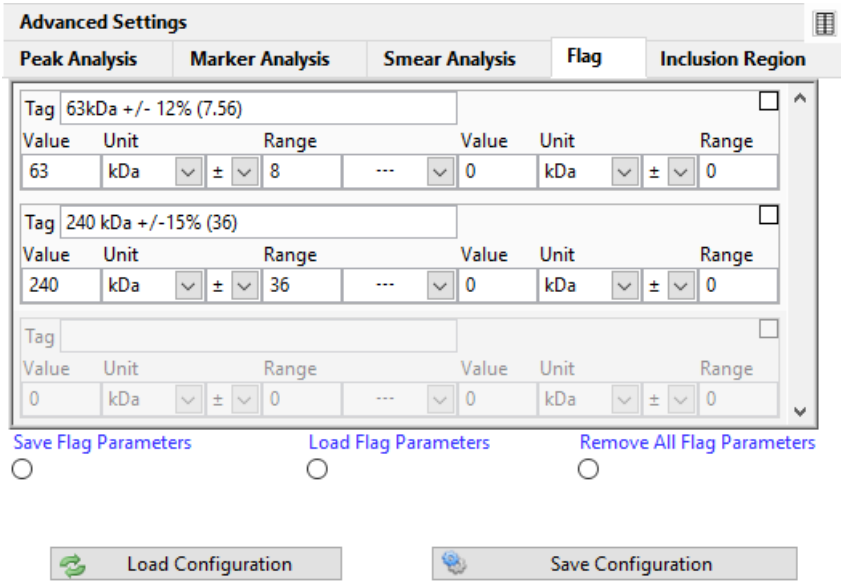


Figure 39 Flag tab in Set Individual Parameters dialog

Table 27 Additional Flag Analysis settings

Setting	Description
Save Flag Parameters	When selected, the current Flag Analysis settings will be saved as a file (*.Flag extension) that can be loaded into different data files. A file browser menu will open to navigate and save the desired filename and location; click **OK** to save the file, or **Cancel** to discard your changes.
Load Flag Parameters	When selected, the previously saved Flag Analysis settings will be loaded from a file (*.Flag extension). A file browser menu will open to navigate and load the desired filename and location; click **OK** to load the file, or **Cancel** to discard your changes.
Remove All Flag Parameters	When selected, all settings will be cleared from the tab.

Set Individual Parameters – Inclusion Region

The settings of the **Inclusion Region** tab allow the user to define up to four size inclusion ranges (**Figure 40**). Only peaks (excluding markers) that fall into the defined range(s) will be displayed. The Inclusion Region will affect the screen display, PDF reports and exported data. **Table 28** describes additional settings not displayed in **Global Configuration** dialog.

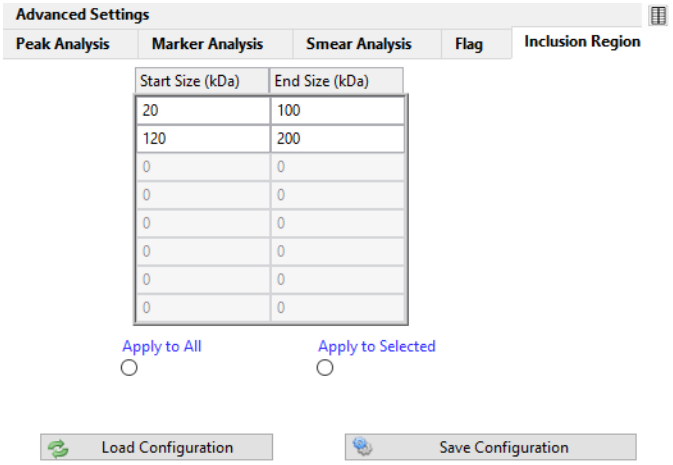
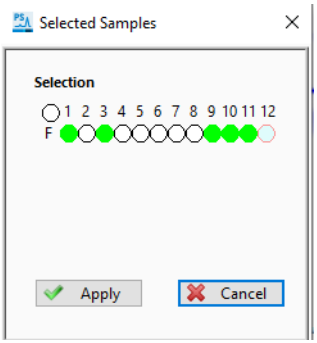


Figure 40 Inclusion Region tab in Set Individual Parameters dialog

Table 28 Additional settings in the Inclusion Region tab

Settings	Description
Apply to All	All current settings of just the **Inclusion Region** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Inclusion Region** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.
Apply to Selected Samples	Applies all current settings of just the **Inclusion Region** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; press **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Inclusion Region** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.

5 ProSize Size Calibration Screen

This chapter describes the size calibration screen (also referred to as calibration curve screen). The calibration curve screen is used to select the conditions for calibrating the size of unknown sample peaks/smears.

About Size Calibration

In the ProSize software, the user can calibrate the size of samples either by running a standard protein ladder in a well of the sample plate (recommended), or by importing a previously calculated and exported size calibration file (*.scal extension).

NOTE

If an imported size calibration is used in ProSize, the same separation gel, capillary array dimensions, injection/run voltage, and lower/upper markers should be used for both the analyzed data and the imported calibration to ensure maximum sizing accuracy.

To properly calibrate the size, the size ladder must be bracketed by the same lower/upper markers used with the samples. In some cases, the lower or upper marker may co-migrate with a ladder peak; this is acceptable for sizing calibration. In some application kits, only a lower marker is used; the data is hence normalized using the lower marker only compared to the respective protein Size Ladder.

It is important to ensure the sample containing the size calibration ladder has all ladder fragments integrated correctly with no additional peaks integrated prior to analyzing the remaining samples.

The following sections describe how to load, edit and import/export from the calibration curve screen, and how to identify and correct an improperly calibrated data file.

Loading the Size Calibration Curve

The calibration curve can be defined using two methods:

A: When an unprocessed data file is first opened and a matching configuration is not found, the **Global Configuration** dialog will appear which is shown in **Figure 41**. This is the most common way to define the calibration curve.

NOTE

In most cases, predefined, optimized global configuration files are available to load when processing data collected from different Agilent Reagent Kits. The configuration files are labeled according to the particular kit number, for example, **5191-6640LM - Protein Broad Range P240 LM only.ini**. These configuration files are available from Agilent upon request if needed. The preloaded configuration files are located in the C:\ProSize data analysis software\Configurations folder.

B: At any time after a file is opened, you can view the **Calibration Curve** screen by selecting  from the main screen, or navigating to **Analysis** > **Show Size Calibration**.

The **Size Calibration** tab in the **Global Configuration** dialog is shown in **Figure 41**; the settings are described in **Table 29**.

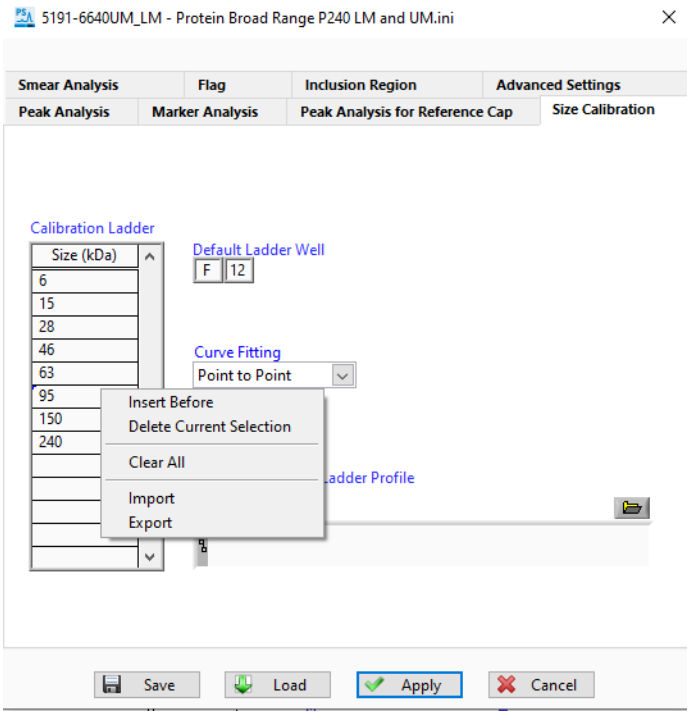


Figure 41 Size Calibration tab in the Global Configuration dialog

Table 29 Size Calibration settings

Settings	Description
Calibration Ladder	Lists size of protein fragments contained in calibration ladder in tabular format. The size of each fragment (include lower/upper markers) is entered directly into the table or imported by using the context menu (right-click in the Calibration Ladder table).
Calibration Ladder > Insert Before	Inserts a blank cell above the currently selected cell in the calibration ladder table. Note: Click in the respective table cell to use this option.
Calibration Ladder > Delete Current Selection	Deletes the currently selected cell in the calibration ladder table. Note: Click in the respective table cell to use this option.
Calibration Ladder > Clear All	Deletes all cells in the calibration ladder table.
Calibration Ladder > Import	Imports a previously exported and saved sizing ladder information file. When selected, a file browser menu will open to navigate and select the desired file (*.txt extension). Standard ladder information files are located in the C:\ ProSize data analysis software\Ladders folder.

Table 29 Size Calibration settings

Settings	Description
Calibration Ladder > Export	Exports the current sizing ladder table information (*.txt extension). When selected, a file browser menu will open to navigate and save the desired filename and location. When exporting ladder information files, it is highly recommended to save the created files to a common directory, located in the C:\ProSize data analysis software\Ladders folder.
Default Ladder Well	Defines the well of the sample plate, which contains the size calibration ladder. From the drop-down list, select the proper row (A-H) and column (1-12) location containing the size calibration ladder.
Curve Fitting	• Point to Point Fits the size calibration plot of Size (kDa) vs. Time (sec) with a Point to Point fit (recommended), drawing a straight line between each point of the curve. • Polynomial Fits the size calibration plot of Size (kDa) vs. Time (sec) with a polynomial fit. Enter the order value (from 3 to 5) in the **Order** field.
Use Imported Ladder Profile	Imports a previously exported size calibration file (*.scal extension) to use for size calibration.
Ladder Profile	Displays file path for the imported size calibration file. To load an imported file, click  . A file browser menu will open to navigate and select the desired file (*.scal extension). Note: When importing a size calibration file, the same conditions (separation gel, capillary array length, lower/upper markers, CE separation voltage) should be used. Differences between the analyzed and imported conditions will adversely affect the sizing accuracy.

Protein Calibration Screen

For protein separations run with the ProteoAnalyzer, a separate calibration screen is utilized for quantification based on external standard calibration.

1 With a protein file open, navigate to **Analysis > Protein calibration**.

The protein calibration screen opens.

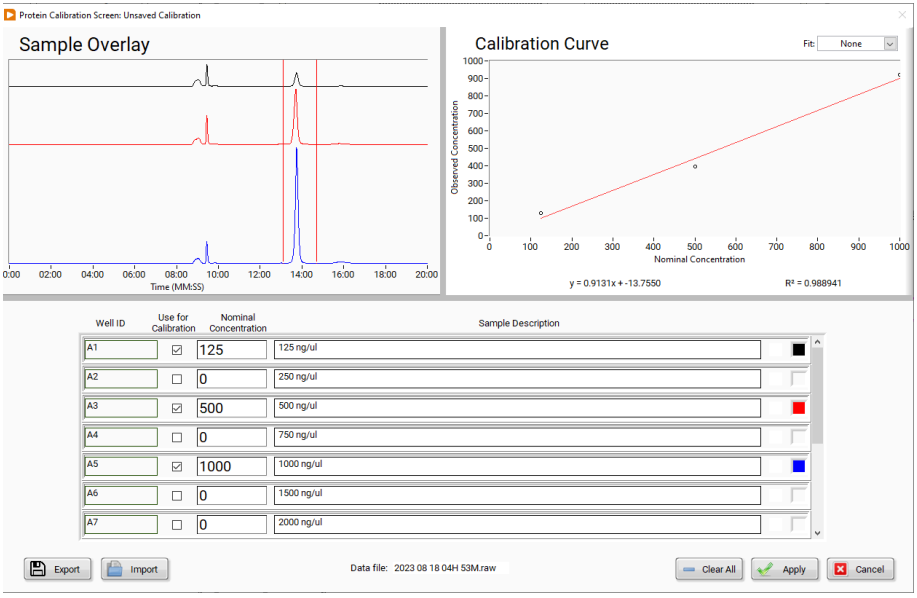


Figure 42 Protein calibration screen

2 In the protein calibrations screen, select which sample well to use as calibration standards by selecting the **Use for Calibration** check box.

3 In the upper left of the sample overlay screen, drag the red brackets around the peak to be used for quantitation.

4 Enter the concentration of the standard protein in the **Nominal Concentration** box. The example above uses BSA at 125 ng/µL, 500 ng/µL, and 1000 ng/µL. This plots a calibration curve with an R^2= 0.988941. This calibration curve can be used to analyze and quantitate proteins in this run or future ones. The calibration standard should run at least 3 different concentrations to generate a good calibration curve.

5 Like the ladder calibration screen for protein ladders, you can export a ladder file or import a previously used file.

6 After selecting the desired sample well to use for calibration, click **Apply** to close the window and apply the sizing calibration for protein samples.

The concentrations determined from this screen will appear in the calibrated peak table.

Define Calibration Ladder

The size of each fragment to be included in the protein Ladder (including lower/upper markers) is listed in the **Calibration Ladder** table (see **Figure 41**). You can enter directly a value into table or use the **Import** command of the context menu (right-click in the table). **Table 29** describes the commands of the context menu, which include inserting or deleting fragments, clearing all entries, and importing or exporting ladder information files (*.txt extension).

NOTE

In most cases, predefined ladder information files optimized for different Agilent Reagent Kits are available and are loaded as part of the global configuration. The ladder files are labeled according to the particular kit number, for example, **5191-6640LM - Protein Broad Range P240 Ladder.txt**. These ladder files are available from Agilent upon request if needed. The preloaded ladder files are located in the
C:\ProSize data analysis software\Ladders folder.

Define Default Ladder Well

If a size ladder is contained in the sample plate, the sample well for the ladder is displayed in the **Default Ladder Well** field (see **Figure 41**, **Table 29**). Click in the field, and from the drop-down list, select the proper well row (A, B, etc.) and well column (1, 2, 3 etc.) of the well containing the sizing ladder. When processing 12-capillary data, only the well column field (1-12) is selectable.

Define Curve Fitting

The **Curve Fitting** drop-down menu allows you to define how the size calibration curve is fitted - via a **Point to Point** or **Polynomial Fit** (3rd to 5th order) (see **Figure 41**, **Table 29**).

NOTE

It is highly recommended to use Point to Point when fitting the size calibration curve. All standard methods use this setting.

Use Imported Ladder

To use your previously exported size calibration settings, select **Use Imported Ladder** (see **Figure 41**). To import your file, select . A file browser menu will open to navigate and select the desired file (*.scal extension) (**Figure 41**, **Table 29**).

Peak Analysis for Reference Cap

The default ladder well is considered to be the “Reference capillary”. When loading the configurations (for example, for an unprocessed file), note that a separate tab labeled **Peak Analysis for Reference Cap** is available (**Figure 43**). This tab enables you to enter independent peak settings to properly identify the protein Ladder peaks as compared to the sample peaks, as outlined in **Table 30**.

Further information on these settings is available in **Chapter 4**, “ProSize Configuration”. When loading or editing the settings of the global configurations, it is important to set these parameters properly to ensure the ladder peaks are selected correctly for size calibration.

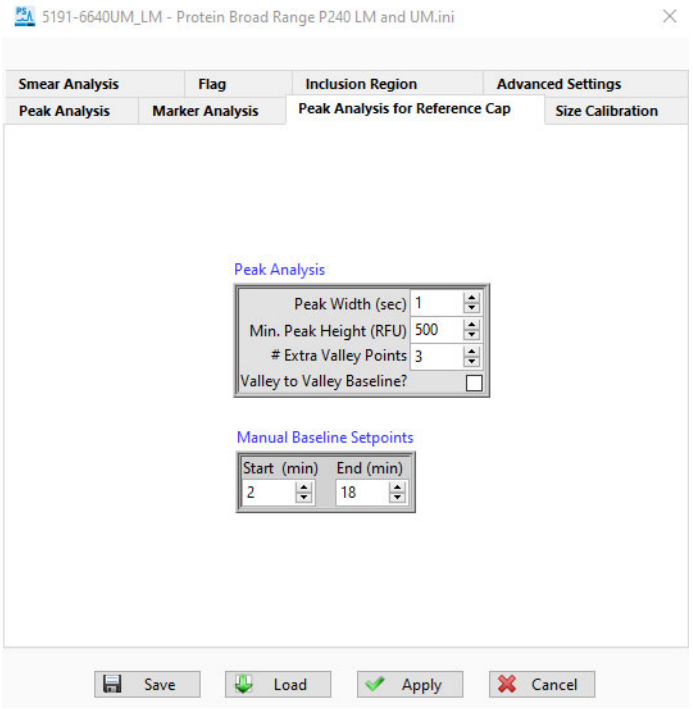


Figure 43 Peak Analysis for Reference Cap tab in the Global Configuration dialog

Table 30 Peak Analysis for Reference Cap configuration settings

Settings	Description
Peak Analysis	
Peak Width (sec)	Defines the width threshold for peak detection in seconds. Higher settings better define wide peak start/end points; smaller settings better define sharp peak start/end points. For protein Ladders, the typical value ranges from 3-5.
Min. Peak Height (RFU)	Defines the minimum peak height threshold to select a peak for integration in RFUs. Peaks below the set value will not be selected for integration.
# Extra Valley Points	This setting affects the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. Higher values draw a straighter baseline between the first peak start point and second peak end point; lower values draw the baseline more to the "valley" between the two unresolved peaks. Note: The **Valley to Valley Baseline?** setting needs to be enabled for this function to be active. A typical setting for a protein ladder is 3.
Valley to Valley Baseline?	This setting affects the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. When disabled, will draw a straighter baseline between the first peak start point and second peak end point. When enabled, will draw the baseline more to the "valley" between the two unresolved peaks. This setting is typically disabled for a protein ladder

Once the proper **Calibration Ladder** fragments, **Default Ladder Well**, and **Curve Fitting** algorithm have been selected (or previous calibration imported) and the correct **Peak Analysis for Reference Cap** settings have been loaded, along with the other configuration settings, the file is loaded into the ProSize software.

Viewing and Exporting the Size Calibration (File Open)

Once a data file is loaded into the ProSize software, if the size calibration ladder is contained within the sample plate its well location will be marked with a light blue color (**Figure 44**, well H12 highlighted with a blue circle). To view the ladder well, click on the respective well in the Plate Map.

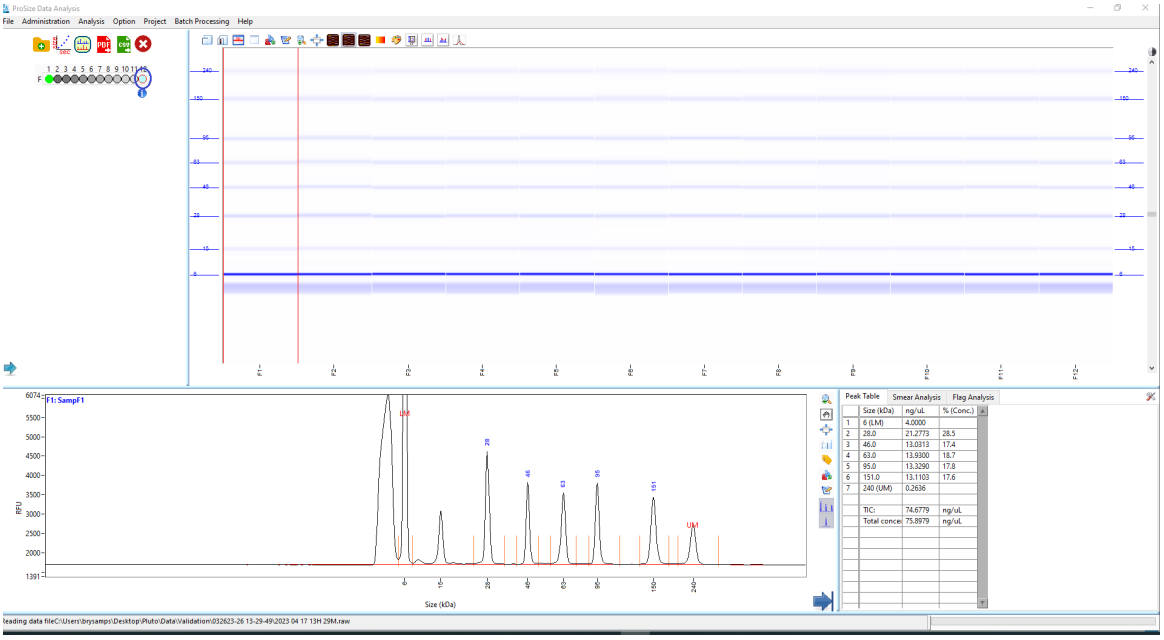


Figure 44 Main screen: The Size Calibration well H12 is marked in blue in the Plate Map (circled in blue to highlight position of reference capillary). Note: the ladder is selected properly (no error message).

If the number of integrated peaks in the reference well matches the number of fragments in the **Calibration Ladder** table, the size calibration curve will be fitted with the selected **Curve Fitting** algorithm and the corresponding calculated sizes will be reported for each sample. The bottom task bar of the ProSize software will display the file path and no errors will be displayed (**Figure 44**).

NOTE

It is generally recommended to inspect the size calibration well as well as the calibration curve screen prior to processing or exporting data, to ensure the desired fragments/markers are selected properly.

To view the size calibration curve from the main screen of the ProSize software, click  in the main screen, or navigate to **Analysis** > **Show Size Calibration**. The calibration curve screen will be displayed (**Figure 45**).

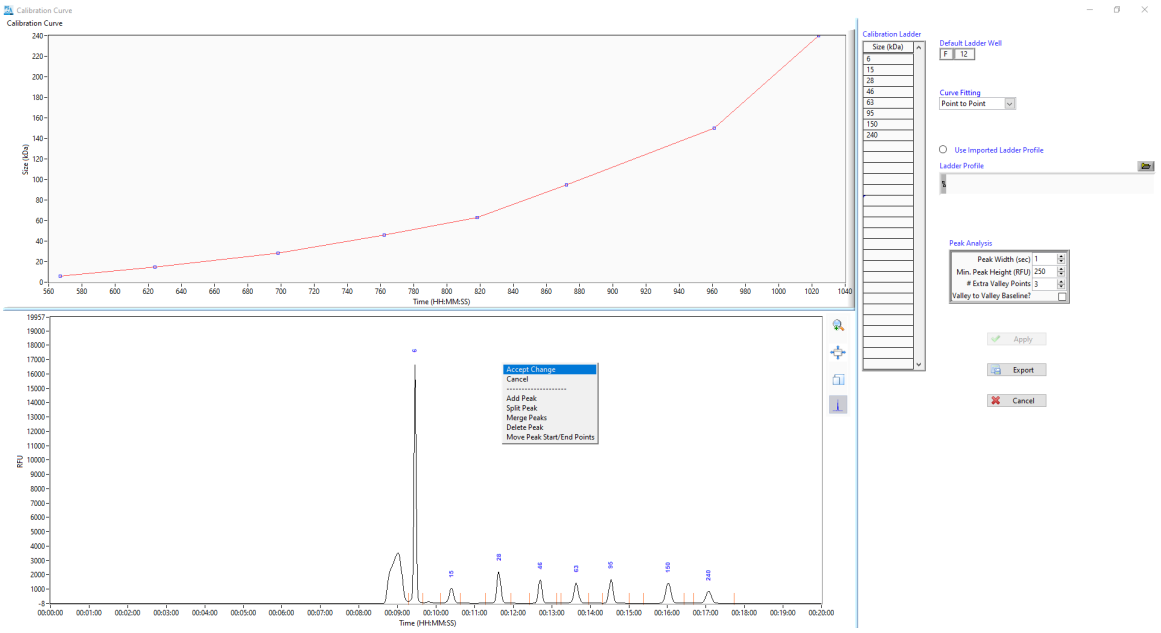


Figure 45 Calibration Curve screen showing properly selected size calibration ladder

The size calibration curve is a plot of **Time** vs. **Size** in upper left corner. A proper fit is characterized by the red fit line passing through each blue data point as closely as possible. In most cases, the **Point to Point** algorithm provides the best fit to the data. However, in some situations the **Polynomial algorithm** may provide smoother, more continuous fit in the higher size region.

The functions in this screen are the same as in the **Size Calibration** and **Peak Analysis for Reference Cap** tabs in the **Global Configuration** dialog (**Figure 41** and **Figure 43**).

- The **Calibration Ladder** table and its context menu functions, and the **Default Ladder Well**, **Curve Fitting**, and **Use Imported Ladder Profile** functions are described in **Table 29**.
- The **Peak Analysis** panel enables adjustments to be made to the peak integration settings of calibration ladder; these functions are the same as those summarized in **Table 30**.

In the calibration curve screen also an Electropherogram Trace (bottom) of the calibration ladder well is displayed. The toolbar of the trace enables you to zoom, autoscale, copy or view the start/end points of each peak as summarized in **Table 31**. The functions of the Electropherogram Trace context menu enable you to modify and adjust the selected peaks in the selected reference well as needed to match those present in the **Calibration Ladder** table (**Table 31**).

You can export the currently viewed size calibration by selecting **Export**.

Table 31 Calibration Curve screen additional functions

Item	Description
Apply	Applies the current size calibration settings and exit to the main screen of the ProSize software. Any changes made from the initially opened Calibration Curve screen will be applied to the data.
Export	Exports the current size calibration settings and fitted electropherogram/plot, which can be imported to other similarly generated data files (*.scal extension). A file browser menu will open to navigate and save the desired filename and location.
Cancel	Closes the Calibration Curve screen and exit to the main screen of the ProSize software. Any changes made from the initially opened Calibration Curve screen will not be applied to the data.
Zoom 	Enables zooming-in on any portion of the Electropherogram Trace. To zoom-in, click the icon and position the mouse cursor over desired starting position; click and drag the cursor to the desired location, and release the mouse button.
AutoFit 	To autoscale the Electropherogram Trace x-/y-axis view.
Copy 	Copies an image of the Electropherogram Trace to the clipboard, for pasting in another program such as Microsoft PowerPoint.
Show Peak Start/End Edge 	Toggle to display or hide the start and end points used for peak integration, shown as vertical orange lines. Displaying the peak integration start/end points can aid in determining if adjustments need to be made to the peak integration to better define the actual peak area. The user can change the peak start/end points by adjusting the **Peak Width (sec)**, or by using the context menu of the Electropherogram Trace such as **Split Peak** or **Move Peak Start/End Points**.
Accept Change	Accepts any manual modification to the peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). After the manual modification has been made, right-click the Electropherogram trace, and select **Accept Change**.

Table 31 Calibration Curve screen additional functions

Item	Description
Cancel	Cancels any manual modification to the peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). After the manual modification has been made, right-click the Electropherogram trace, and select **Cancel**.
Add Peak	To manually add a peak which has not been auto-integrated by the **Peak Analysis** settings and to define the start and end point for integration. Zoom-in to the region where the peak is to be added. Right-click this region and select **Add Peak**. Two red vertical cursors will appear; the left cursor defines the new peak start point and the right cursor the new peak end point. Drag each cursor to the desired position, then right-click, and select **Accept Change** to add the peak for integration. Note: The Add Peak function will not add a peak that is located outside the lower/upper marker window (or before lower marker if using only lower marker).
Split Peak	To manually split a peak which is currently integrated into two peaks and to define where the split occurs. Zoom-in to the region where the peak is to be split. Right-click this region, and select **Split Peak**. A red vertical cursor will appear. Drag the cursor to the desired location, then right-click, and select **Accept Change** to split the peak into two peaks for integration.
Merge Peaks	To merge any number of peaks and integrate as a single peak. Zoom-in to the region where the peak is to be split. Right-click this region, and select **Merge Peaks**. Two red vertical cursors will appear; the left cursor defines the left most peak to merge and right cursor defines the right most peak to merge. Drag the cursor within that peak's start/end point region to merge, then right click, and select **Accept Change** to merge the peaks into a single peak for integration.

Table 31 Calibration Curve screen additional functions

Item	Description
Delete Peak	To manually delete a peak which has been integrated. Zoom-in to the region where the peak is to be deleted. Right-click this region, and select **Delete Peak**. A red vertical cursor will appear. Drag the cursor to the desired peak location, then right-click, and select **Accept Change** to delete the peak from the integration.
Move Peak Start/End Points	To change the currently positioned start/end integration points of a peak. Zoom-in to the region where the peak is located. Place the cursor between the start and end points of the integration. Right-click and select **Move Peak Start/End Points**. Two red vertical cursors will appear at the current start/end points. Drag the cursors to the desired locations, then right-click and select **Accept Change** to apply the new start/end point positions to reintegrate the peak. Note: This function will not change the baseline; only the start and end points are affected. To change the baseline, the user must adjust the via the **Peak Width (sec)**, **Valley to Valley,** or **Manual Baseline Setpoints** tools.

Identifying and Correcting an Improper Size Calibration

When performing the size calibration, the ProSize software will attempt to fit the integrated peaks in the size calibration well to the fragments listed in the size **Calibration Ladder** table. If the number of integrated peaks in the ladder well does not match the number of fragments in the size Calibration Ladder table (either less or more peaks integrated), a red flashing error message will appear at the bottom of the main screen: **Warning: Mis-match between detected peaks and ladder assignment! No sizing calibration curve is established**.

An example of less integrated peaks than calibration fragments is shown in **Figure 46** and **Figure 47** an example of too many integrated peaks is shown in **Figure 48** and **Figure 49**. In each case, you need to adjust the **Peak Analysis** settings either in the main screen configuration tab, or in the calibration curve screen, to select the proper number of peaks in the size calibration ladder well (including lower/upper markers) to achieve the proper size calibration. Once the proper number of peaks is selected, click the **Apply** button. The bottom task bar of the ProSize software will then display the file path with no errors.

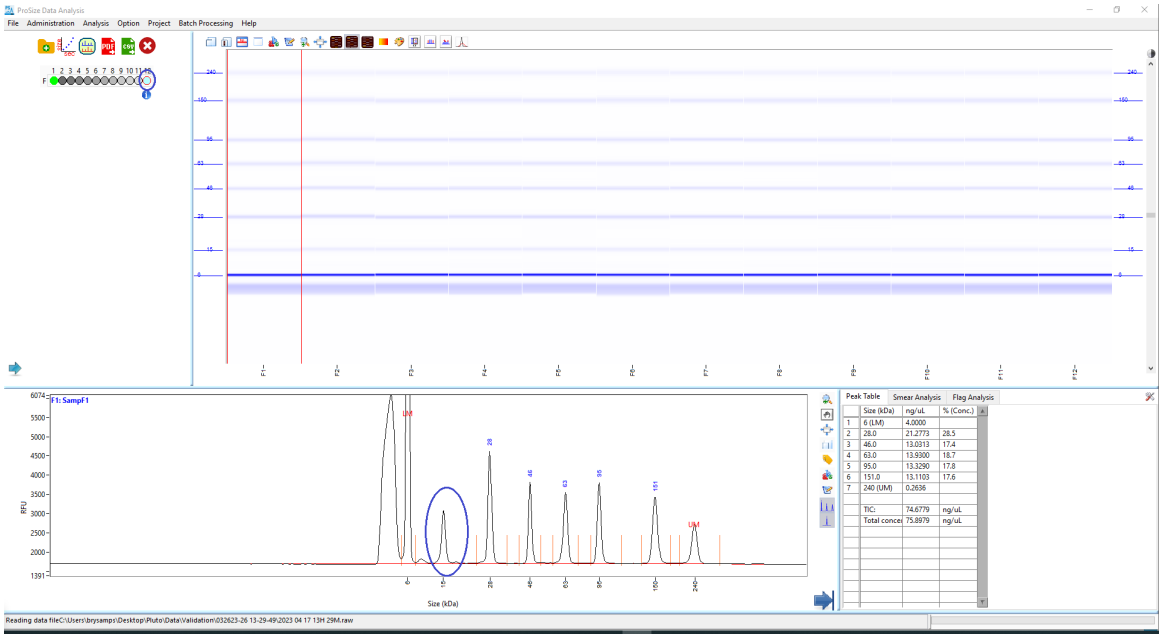


Figure 46 Main screen showing improperly fitted size calibration (not enough integrated peaks). Solution to correct: In the **Peak Analysis** table lower the Min. Peak Height (RFU) threshold to integrate missing peak (circled in blue).

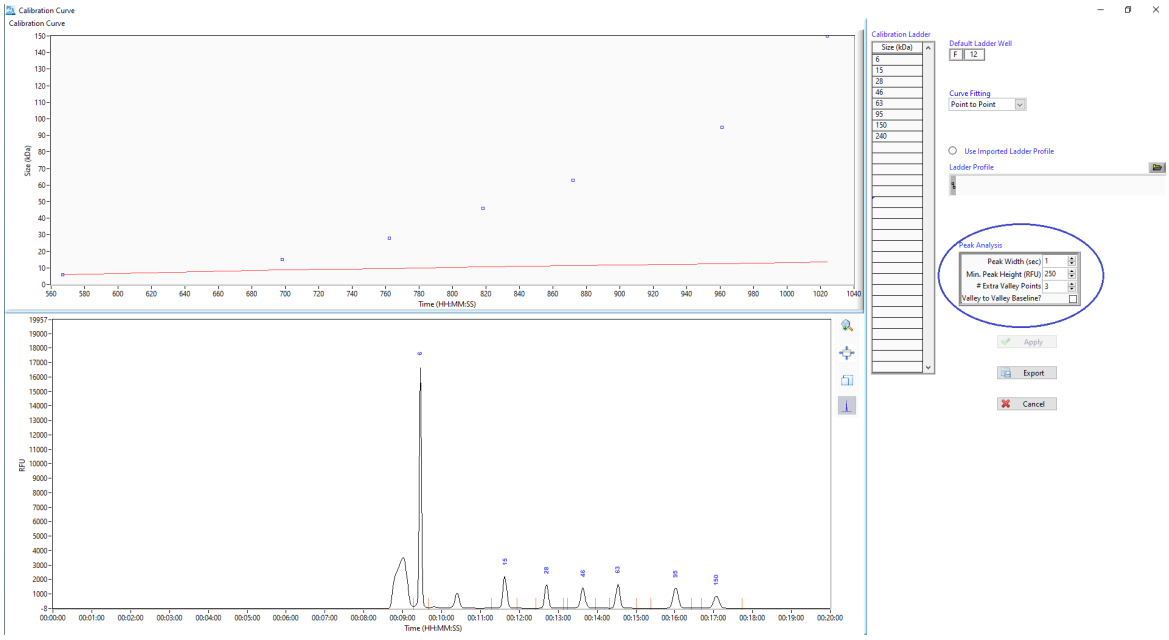


Figure 47 Calibration Curve screen showing improperly fit size calibration from **Figure 46**.

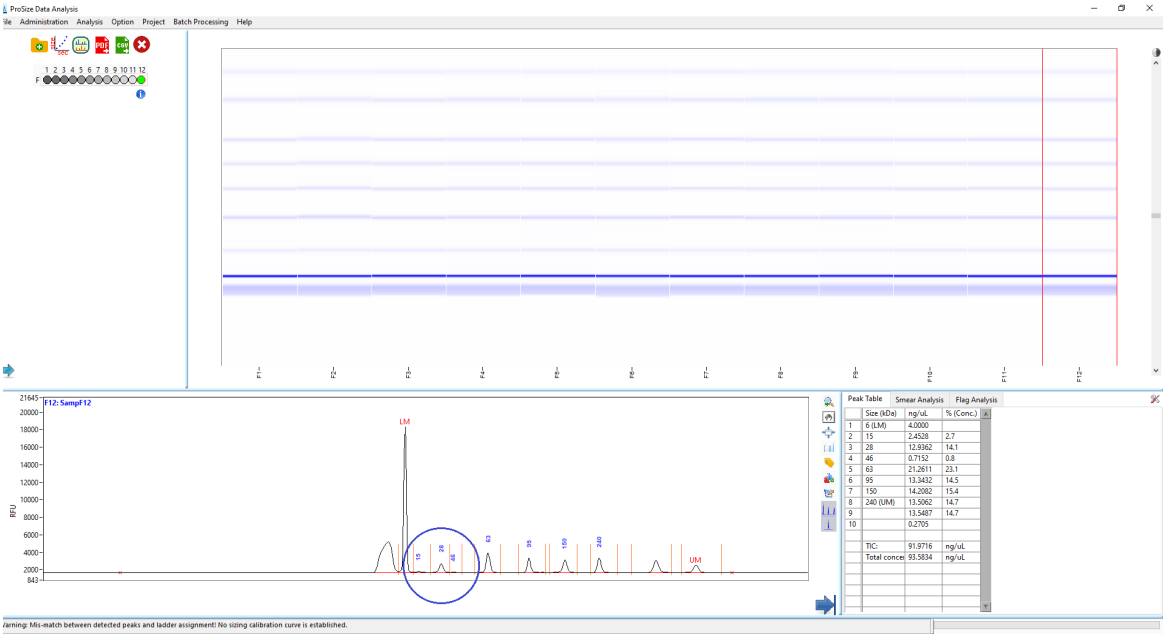


Figure 48 Main screen showing improperly fitted size calibration (too many integrated peaks, circled in blue). Solution to correct: increase the Min. Peak Height (RFU) threshold to no longer integrate extra peaks.

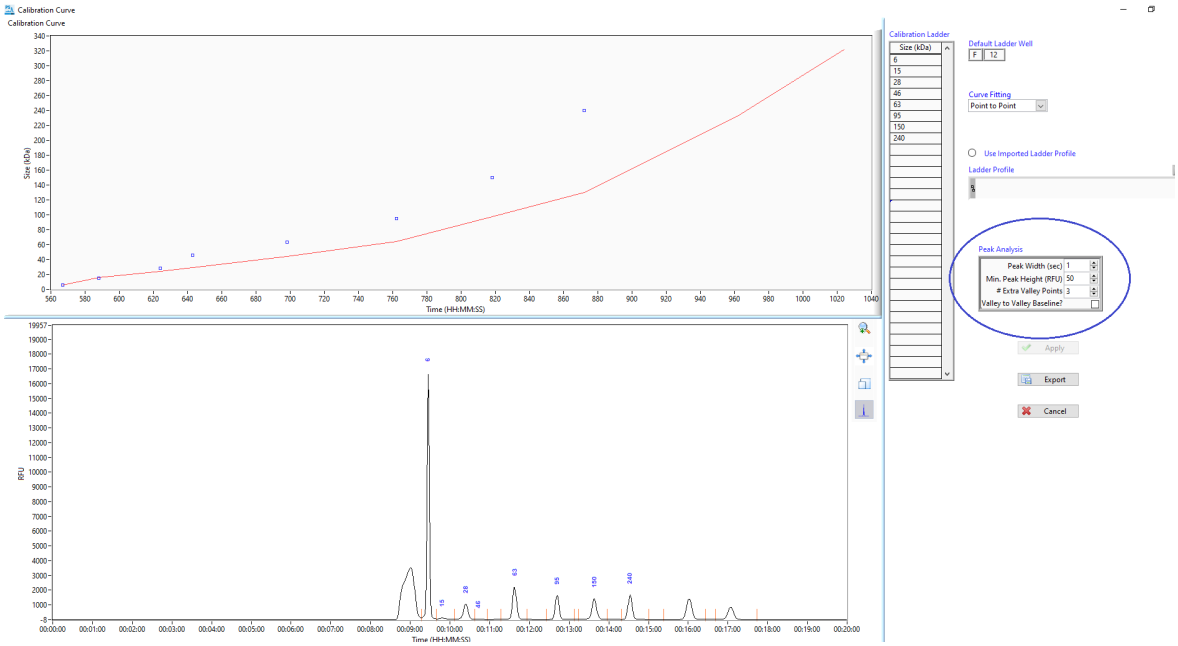


Figure 49 Calibration Curve screen showing improperly fit size calibration from **Figure 48**

6 ProSize Overlay Samples

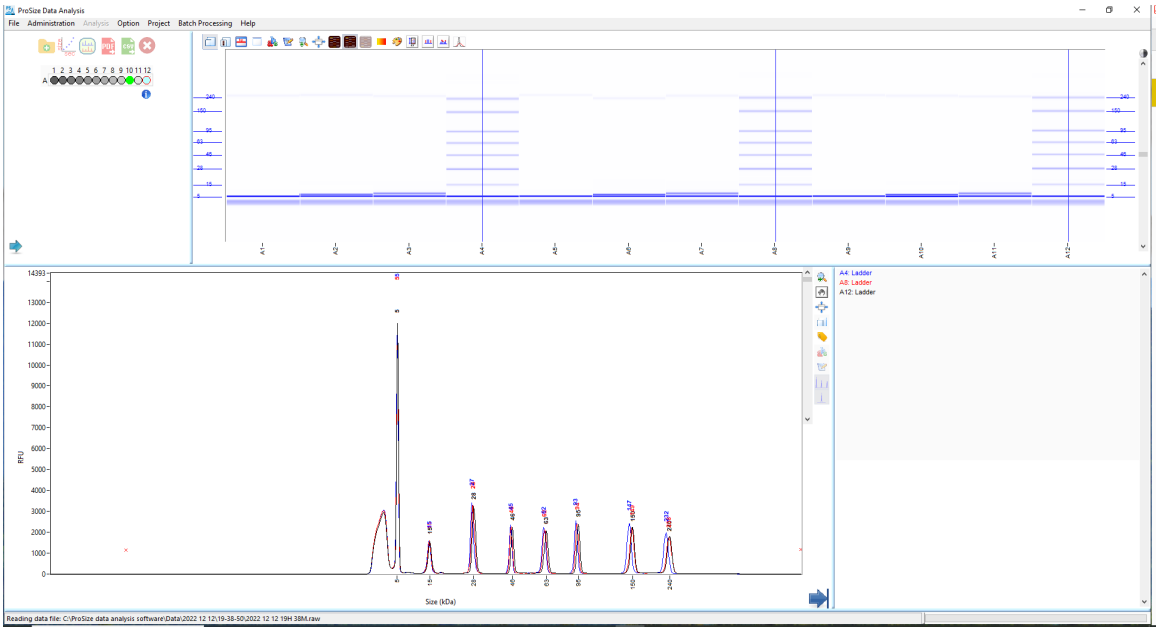
This chapter gives an overview of the possible ways to compare samples in the ProSize software.

Overlay Options

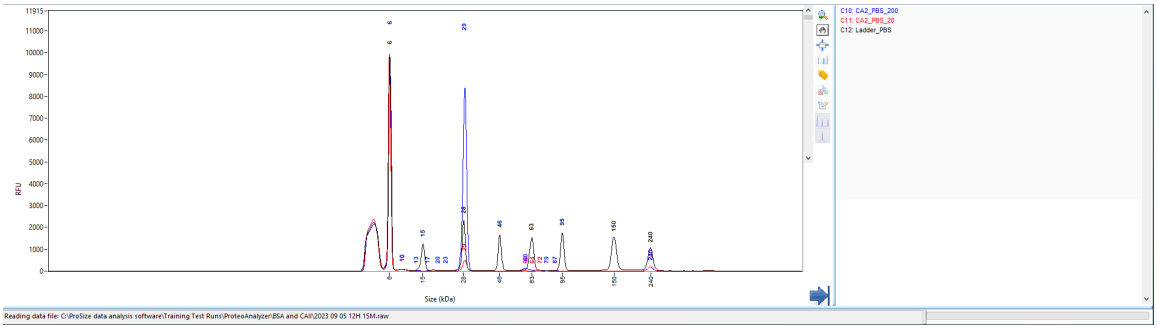
There are two options for comparing samples: The *Quick Overlay* and the *Overlay Samples* screen.

Quick Overlay

1 Right-click the sample on the gel image you want to overlay.



The overlay images appear in the electropherogram area.



2 Be sure to select the desired *Normalize* icon (for example, **Normalize to Lower Marker**) to get an accurate representation of how the samples compare with each other.

3 To end the *Quick Overlay* view, double-click on any lane of the gel image.

Overlay Samples Screen

The *Overlay Samples* screen is used to overlay and generate comparisons, in digital gel view and electropherogram view, of selected samples from the currently opened data file and optionally from different data files (when using the same markers and experimental conditions), and generate reports of the overlaid data.

Once the Overlay Samples screen is opened, all results are handled and saved as Projects. Before you can export data or generate a .pdf report, the results of the overlay plots must be saved as a Project. Previously saved projects can be opened via the menu Project tab.

The following sections describe how to open, select data to overlay, adjust/annotate overlaid data, open additional data files, and copy/export information from the Overlay Samples screen.

Opening the Overlay Samples Screen

1 To view the Overlay Samples screen from the main screen, select  or navigate to **Analysis** > **Overlay Samples**.

The *Overlay Samples* screen is displayed (**Figure 50**). **Table 32** summarizes the top menu bar functions.

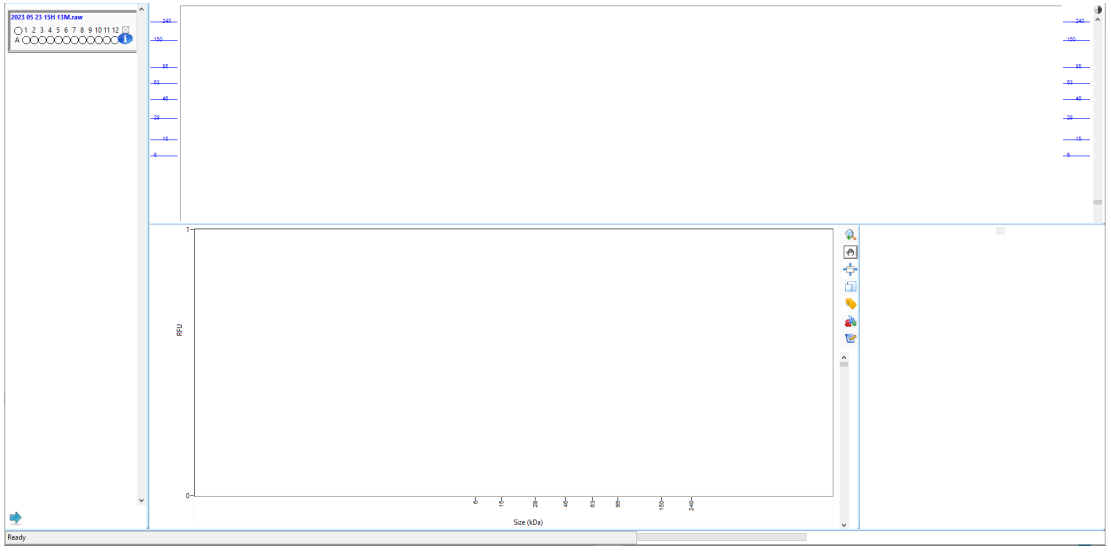


Figure 50 Overlay Samples screen (no samples selected)

Table 32 Overlay Screen top menu functions

Menu Item	Description
Project...Load	Opens a previously saved overlay project.
Project...Save	Saves the current overlay project. Note: The user must save an overlay project before the export or "generate report" icons are active.
Project...Save As	Saves an additional copy of the overlay project without replacing the original. Note: The user must save an overlay project before the export or "generate report" icons are active.
Project...Create	Creates a new overlay project. This re-opens the overlay file with a blank screen.
View Data File	To temporarily exit the overlay screen to view the data file. To return to the overlay screen, select **Project....Return to Project**.

Table 33 summarizes the available functions in the screen prior to opening any sample wells. A Plate Map menu is visible in the top left corner for selection of samples to overlay; the sample filename is listed at the top of the menu.

Table 33 Overlay Samples screen functions (no samples selected)

Menu Item	Description
Open File	When selected, a file browser window opens for locating and opening an additional raw data file (*.raw extension) for data analysis comparison to the currently opened data file. Note: If multiple files are to be opened for comparison in the Overlay Samples screen, to generate a meaningful comparison, the additional opened files should: • Be previously processed in the ProSize software (i.e., markers selected, size calibrated). • The same lower/upper markers must be used. • Similar experimental conditions (separation gel, array length, separation voltage) must be used.
Exit Overlay Samples	Closes the Overlay Samples screen and returns to the main screen of the ProSize software.
Default Layout	Shows an "overlay of samples" using both the electropherogram and digital gel view in the same window)(**Figure 53**).
Image Layout	Shows an "overlay of samples" using a digital gel image window only (**Figure 54**).
Trace Layout	Shows an "overlay of samples" using an electropherogram trace window only (**Figure 55**).

Table 33 Overlay Samples screen functions (no samples selected)

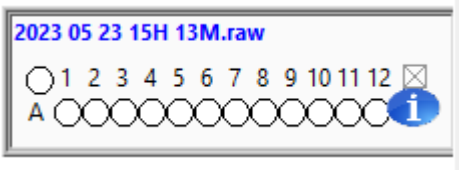
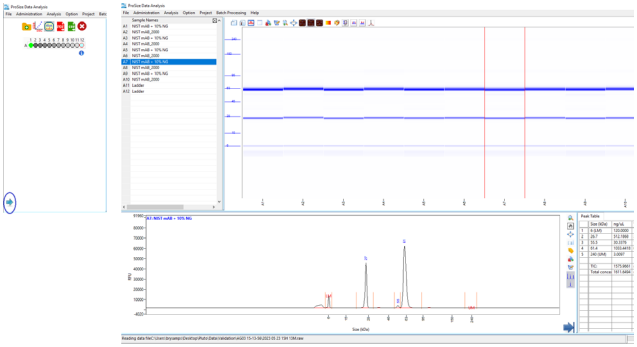
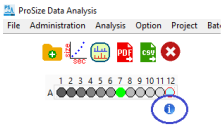
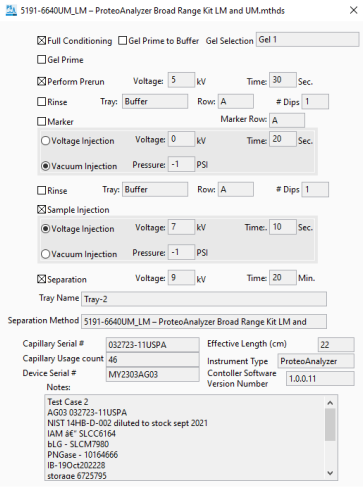
Menu Item	Description
Generate Report	Generates a PDF report of the Overlay Samples (see **Figure 56**). Each file will be color coated. Report options are discussed in detail in **Chapter 8**, "Generating Reports from ProSize". Data will be color coded per separation. Note: A project must be saved before the program allows you to generate a report.
Export Data	Exports data from the Overlay Samples (see **Figure 57**). Export options are discussed in detail in **Chapter 7**, "Exporting Data from ProSize". Data will be color coded per separation. Note: A project must be saved before the program allows you to export data.
Plate Map	Used to select a sample well(s) for comparison. To select well(s), click the well to add to the overlay; click a second time to remove (in any order). To add/remove a column, click the corresponding column number (**1**, **2**, etc.). To add/remove a row, click the corresponding row letter (**A**, **B**, etc.). To select/remove all wells of the plate, select the well in the upper left corner of the plate. The sample filename is displayed at the top of the Plate Map. 

Table 33 Overlay Samples screen functions (no samples selected)

Menu Item	Description
Sample ID navigation 	When selected, a **Sample ID** list will slide out (Below), enabling the User to select the samples for overlay by the Sample ID previously entered into the instrument software. To select a sample, click the sample name; that well will be highlighted. To remove a well, click it a second time. To clear all wells, right-click and select **Clear All**. To close the **Sample ID** navigation, click the arrow at the bottom right of the panel. 
Information 	When selected, a window opens displaying the instrument software user interface, containing experimental information and notes fields.  

Once sample wells are selected in the Overlay Samples screen, they are displayed in order of selection from left to right on the digital gel view and from bottom to top on the electropherogram trace view (**Figure 51**). If 24 samples are selected the Sample ID will be shown above the gel view, if more than 24 samples are selected the Sample ID will not be shown.

In the digital gel view, each sample/lane selected is annotated at the top by well ID and sample name; the currently opened data file is labeled with a (1) to signify it is the first file open. Additional data files if opened will have their samples/lanes labeled with (2), (3), etc. The side axes for the digital gel image are labeled by size, corresponding to the size calibration ladder. On the right side of the digital gel image, a slider bar is present allowing the adjustment of the intensity/contrast of the image by left clicking and dragging the bar.

In the electropherogram trace overlay, each sample is displayed in a different color with matching color annotation to the right of the overlay by well ID and sample name; the currently opened data file is labeled with a (1) to signify it is the first file open. Additional data files if opened will have their samples/lanes labeled with (2), (3), etc. The x-axis is labeled by **Size** and the y-axis by **RFU** in the trace overlay. On the right side of the electropherogram trace overlay, a slider bar is present to adjust the vertical spacing between traces by left clicking and dragging the bar.

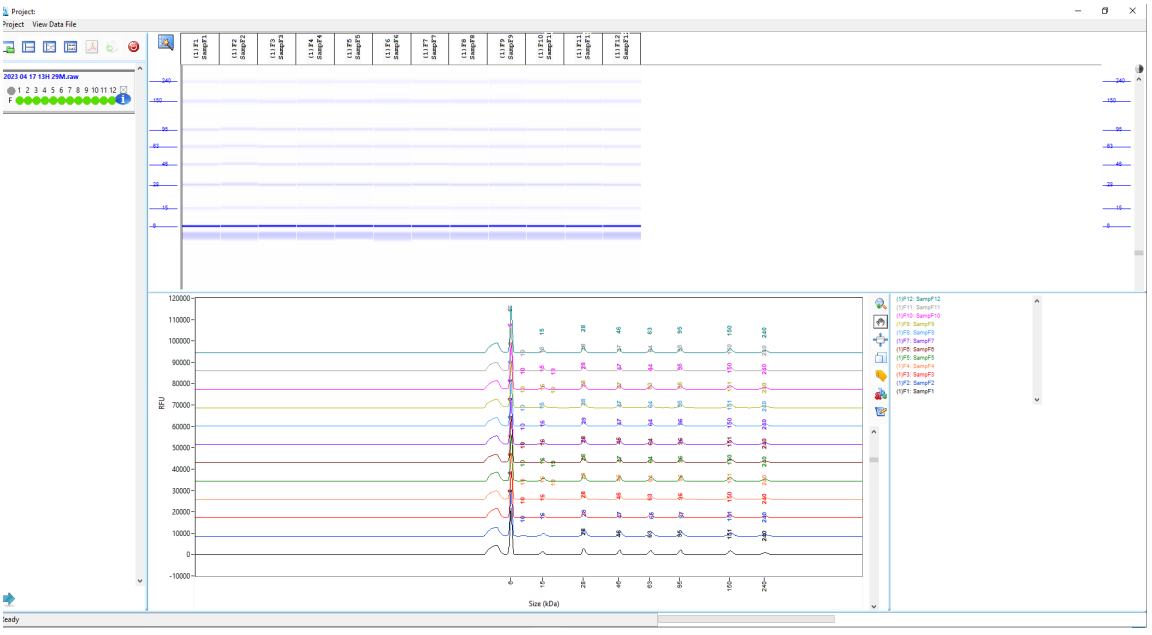


Figure 51 Overlay Samples screen (24 samples selected)

Display, Annotation and Export Options in the Overlay Samples Screen

Toolbar functions are available in the Overlay Samples screen for both the digital gel view and electropherogram trace overlays to adjust the display, annotate samples, and export results. These functions are summarized in **Table 34**.

To access the toolbar functions of the digital gel view overlay, click the corresponding icons (**Figure 52**). For the electropherogram trace overlay, in addition to the toolbar, a context menu is also available.

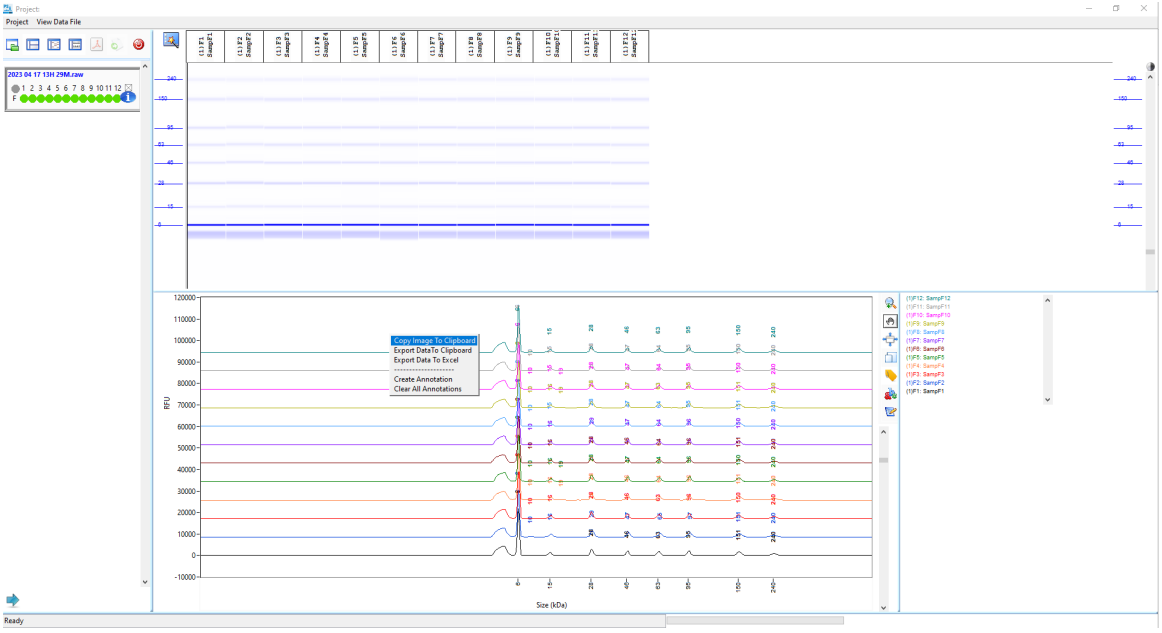


Figure 52 Overlay Samples screen (digital gel image toolbar and electropherogram trace with context menu)

Table 34 Overlay Samples toolbar and context menu commands (samples selected)

Menu Item	Description
Digital Gel Image Toolbar	
Copy Full Gel Image 	Copies an image of the current 24 lane digital gel view overlay to the clipboard, for pasting in another program such as Microsoft PowerPoint. Any zoom, image normalization, or annotation will be copied in the image. The well ID and sample name will be copied on the top of each lane, and the y-axis will correspond to the currently selected size range obtained from the size calibration ladder in the copied image.
Show/Hide Cursor 	Displays a horizontal line cursor across the digital gel image overlay, annotated by size (kDa) corresponding to the current position. The cursor can be dragged to any position along the gel image by clicking the cursor and holding the mouse button. When the cursor is active and displayed, a similar vertical cursor annotated by size is displayed in the electropherogram trace overlay, whose movement corresponds to that in the gel image. To deactivate the cursor, click the icon a second time.
Zoom 	Enables zooming in the y-axis (Size) of the digital image overlay. To zoom-in: Place the mouse over the trace, click and drag it outward to expand a box area to define the zoom region. Release the mouse button to apply. The zoomed image can be copied to the clipboard.
AutoFit 	To autoscale the digital gel image overlay y-axis view for all 24 currently viewed lanes. The autoscaled image will display the gel from the selected lower marker to upper marker, or if only a lower marker is used from the lower marker to the end of the separation window.
Auto Intensity 	To autoscale the intensity of the digital gel image overlay. The viewed intensity across the image will reflect the actual observed fluorescence signals from each lane.
Normalize Intensity to Lower Marker 	To normalize the intensity of the digital gel image overlay lower marker for each lane to the same value, adjusting the relative intensities of all bands in each lane accordingly. The viewed intensity across the image will as a result reflect the fluorescence signals from each lane after normalization to the lower marker, as is done when calculating the concentration of samples using the lower marker.
Normalize Intensity to Upper Marker 	To normalize the intensity of the digital gel image overlay upper marker for each lane to the same value, adjusting the relative intensities of all bands in each lane accordingly. The viewed intensity across the image will as a result reflect the fluorescence signals from each lane after normalization to the upper marker, as is done when calculating the concentration of samples using the upper marker.

Table 34 Overlay Samples toolbar and context menu commands (samples selected)

Menu Item	Description
Gel Image Color	Changes the color scheme for the **Digital Gel View**, the gel image in the **Overlay Samples** screen and any exported or printed report. Options for the gel image color: • White on Black (white bands on black background) • Black on White (black bands on white background) • Green on Black (green bands on black background) • Red on Black (red bands on black background) • Blue on White (blue bands on white background) • Pseudo Color (green to red bands on blue background)
Electropherogram Trace Overlay Toolbar	
Zoom	Enables zooming in the x-and y-axis of the Electropherogram Trace. To zoom-in: Place the mouse over the trace, click and drag it outward to expand a box area to define the zoom region. Release the mouse button to apply. The zoomed image can be copied to the clipboard. The zoom region will be preserved when viewing other samples in the Plate Map. To undo the zoom, use the **Autoscale** function.
Drag	Enables dragging in the x-and y-axis of the Electropherogram Trace. To drag: Place the mouse over the trace, click and drag it in any direction, and release the mouse.
Autoscale	To autoscale the Electropherogram Trace x-/y- axis display. The autoscaled image will display the full trace from the start to the end of the separation when using the **Time Scale** and **Size Scale** display modes; when using the **Uniform Size Scale**, the autoscaled display will start at 0 kDa. Note: The y-axis scale will automatically zoom to the highest detected sample peak, regardless of the marker peak heights. To view the entire y-axis scale including markers, go to **Option,** and select **Autoscale** as the **Display Mode**.
Copy	Copies an image of the current view of the Electropherogram Trace to the clipboard, for pasting in another program, such as Microsoft PowerPoint. Any zoom, annotation, baseline and/or peak start/end point displayed will be copied in the image. The well ID and sample name will be copied in the top left of the trace, and the x-/y-axis will correspond to the currently selected view in the copied image.

Table 34 Overlay Samples toolbar and context menu commands (samples selected)

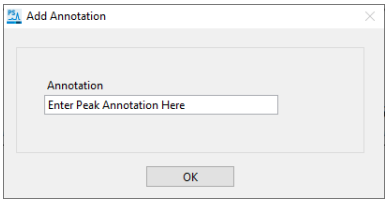
Menu Item	Description
Units	Displays a menu for changing the peak annotation of the Electropherogram Trace. Only integrated peaks are annotated. Units for the peak annotation: • None • Peak ID (labels in order as 1, 2, etc.) • Migration Time (min:sec) raw migration time • Peak Height (in RFUs) • Corrected Peak Area (Peak Area/Migration Time) • Size (in kDa) • Average Size (in kDa) • Concentration (in ng/µL)
Create Annotation	To create customized annotation in the Electropherogram Trace display. In the **Add Annotation** dialog, the user can type desired annotation into the field. Click **OK** and the annotation will be displayed in the Electropherogram Trace window.  To move the annotation to the desired position on the trace: Click the annotation and drag it to the desired position. An arrow will appear at the opposite end of the annotation upon dragging. Multiple annotations can be created by repeatedly selecting **Create Annotation**. Any created annotations will be copied to the clipboard with the **Copy** function.

Table 34 Overlay Samples toolbar and context menu commands (samples selected)

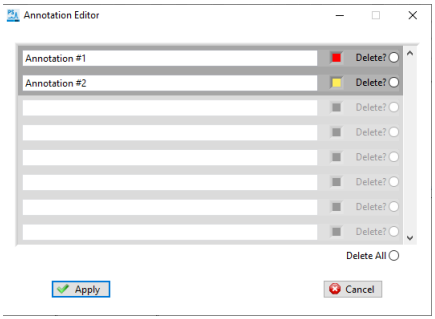
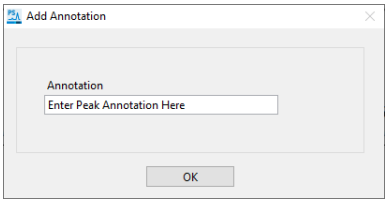
Menu Item	Description
Edit Annotations 	Opens the **Annotation Editor** window. Allows the user to: • Edit the annotation text by typing in the text field. • Change the color of the annotation by left clicking on the annotation color. • Delete one annotation by selecting **Delete** next to each annotation, or delete all annotations by selecting **Delete All**.  Select **Apply** to confirm your settings.
Electropherogram Trace Overlay Context Menu	
Copy Image to Clipboard	Copies an image of the current view of the Electropherogram Trace to the clipboard, for pasting in another program, such as Microsoft PowerPoint. Any zoom, spacing, or annotation displayed will be copied in the image. This function operates similar to the **Copy** icon function.
Export Data to Clipboard	To copy the electropherogram x-data (time; in seconds, starting with the lower marker) and y-data (RFUs) in a .csv format that can be pasted directly into common spreadsheet programs such as Microsoft Excel. The x-data is copied into the first column, and the y-data into the second column of the file from left to right starting with the bottom- most sample upon pasting. The columns are annotated starting with Plot 0, 1, 2, etc. in order. To export, right-click to display the context menu and select **Export Data to Clipboard**. Then open spreadsheet, position in desired cell and paste into the program; process and save as desired.

Table 34 Overlay Samples toolbar and context menu commands (samples selected)

Menu Item	Description
Export Data to Excel	This function requires Microsoft Excel to be installed on the computer to function properly. Selection will automatically open Microsoft Excel and copy each overlaid electropherogram x-data (time; in seconds, starting with the lower marker) and y-data (RFUs) in a .csv file format. The x-data is copied into the first column, and the y-data into the second column of the file from left to right starting with the bottom-most sample upon pasting. The columns are annotated starting with Plot 0, 1, 2, etc. in order. To export, right-click to display the context menu, and select **Export Data to Excel**. The file can then be processed and saved as desired.
Create Annotation	To create customized annotation in the Electropherogram Trace display. In the **Add Annotation** dialog, the user can type desired annotation into the field. Click **OK** and the annotation will be displayed in the Electropherogram Trace window.  To move the annotation to the desired position on the trace: Click the annotation and drag it to the desired position. An arrow will appear at the opposite end of the annotation upon dragging. Multiple annotations can be created by repeatedly selecting **Create Annotation**. Any created annotations will be copied to the clipboard with the **Copy** function.
Clear All Annotations	Clears your created annotations from the electropherogram trace overlay display.

Displaying Multiple Files in the Overlay Samples Screen

Several different data files can be simultaneously opened and compared using the Overlay Samples screen in ProSize.

To compare different data sets, several criteria should be met when overlaying multiple files:

- The data must use the same size lower/upper marker combination. For protein analysis, the same lower marker should be used.
- Any additional data files opened in the Overlay Samples screen should be previously processed, with the lower/upper markers correctly selected for the data and the size calibration correctly performed. No additional processing can be done on the opened comparison files; only adjustments to the intensity/spacing can be made.
- The experimental conditions should ideally be the same or similar for both sets of data, using the same separation gel, array length, separation voltage, and/or assay type (for example, Protein Kit).

With the currently open data file displayed in the Overlay Samples screen, select  to open a second data file. A file browser menu will be displayed for locating and opening the additional raw data file (*.raw extension). Once opened, a second **Plate Map** will be displayed for the second data file below the original file, labeled at the top with the filename (**Figure 53**).

All functions apply the same to the second opened data file compared the first; to close the second data file at any time, click ⊠ in the upper right corner of the Plate Map.

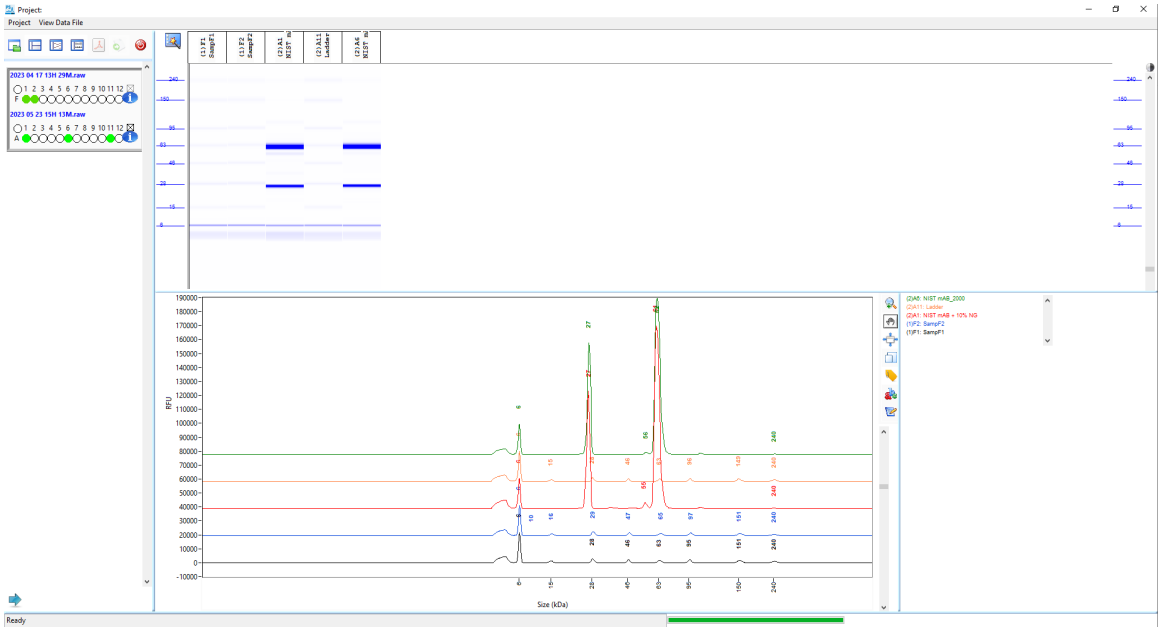


Figure 53 Overlay Samples screen displaying two different data files open

Alternate Views in the Overlay Samples Screen

Several different views can be displayed in the Overlay Samples screen as described in **Table 33**. **Figure 54** shows the Image Layout view; **Figure 55** shows the Trace Layout view. The functions in these screens are the same as those in the Default Layout view.

Figure 54 Overlay Samples screen displaying Image Layout view

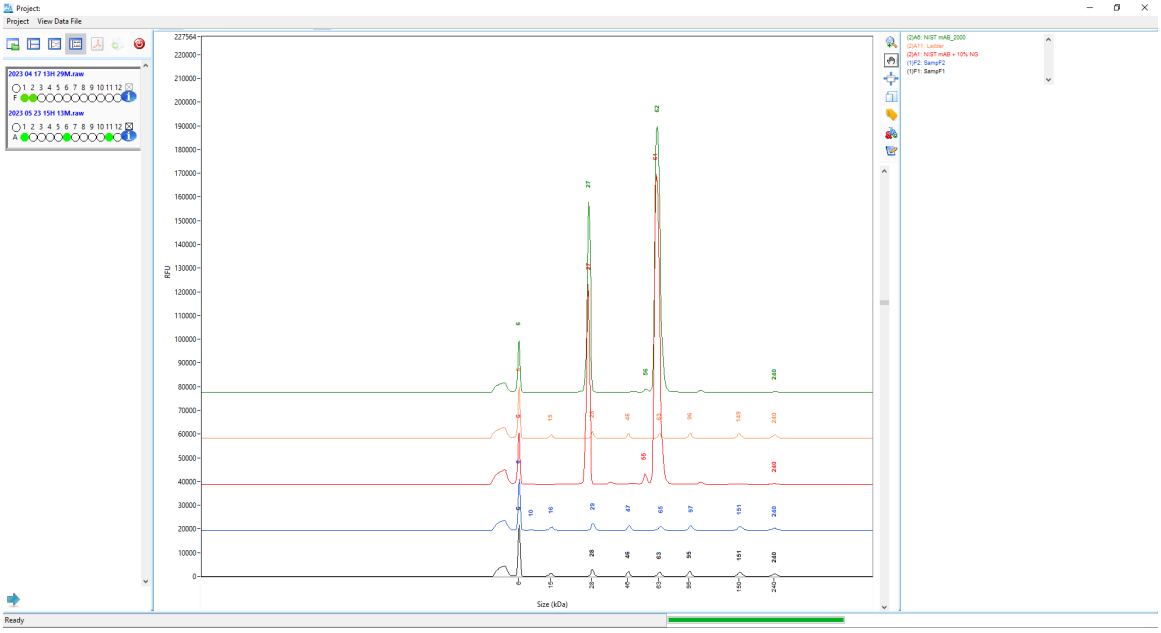


Figure 55 Overlay Samples screen displaying Image Layout view

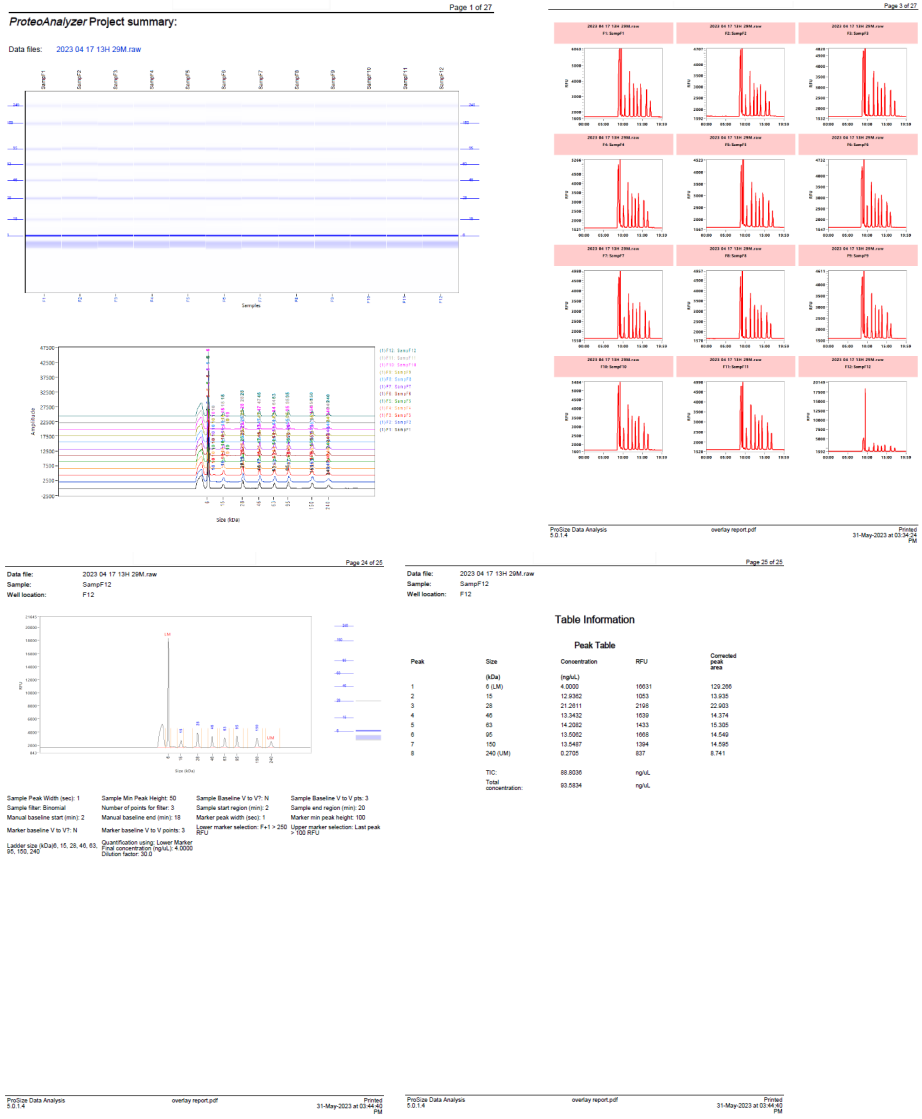


Figure 56 Overlay Samples PDF Report showing the first page of overlayed traces page, Traces Summary page, and Electropherogram/Peak Table page

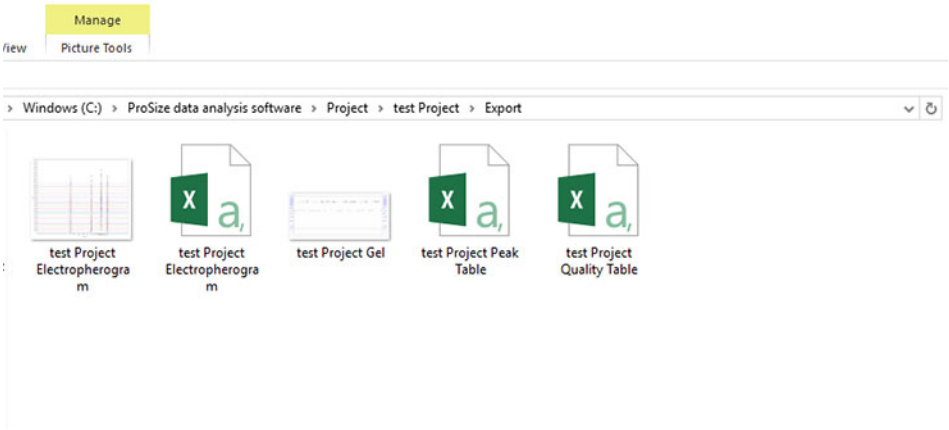


Figure 57 Export folder showing files generated from the Overlay Samples screen

7 Exporting Data from ProSize

This chapter provides an overview of the options available for exporting processed data from the ProSize software. The following sections describe how to open the Export Data window; provide an outline of various data export options; describe how to perform data export; and give examples of exported data.

Export Data Utility

Once the data is opened and processed within the ProSize program, the measured/calculated information can be exported in common formats for storage in a common database or for use in other programs.

Export Data is used to export information from the ProSize program.

Export Data Window Settings

To export sample results from the ProSize software:

1 From the main screen, select , or navigate to **File** > **Export Data**.

The **Export Data** window will be displayed (Figure 58).

Figure 58 Export Data window

Table 35 summarizes the available settings in the **Export Data** window.

Table 35 Export Data settings

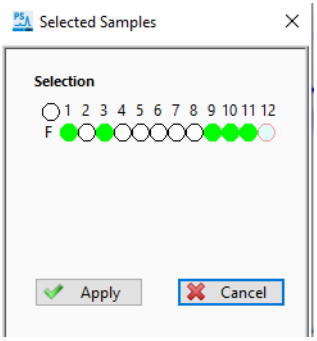
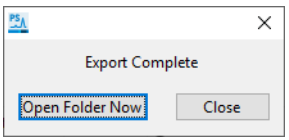
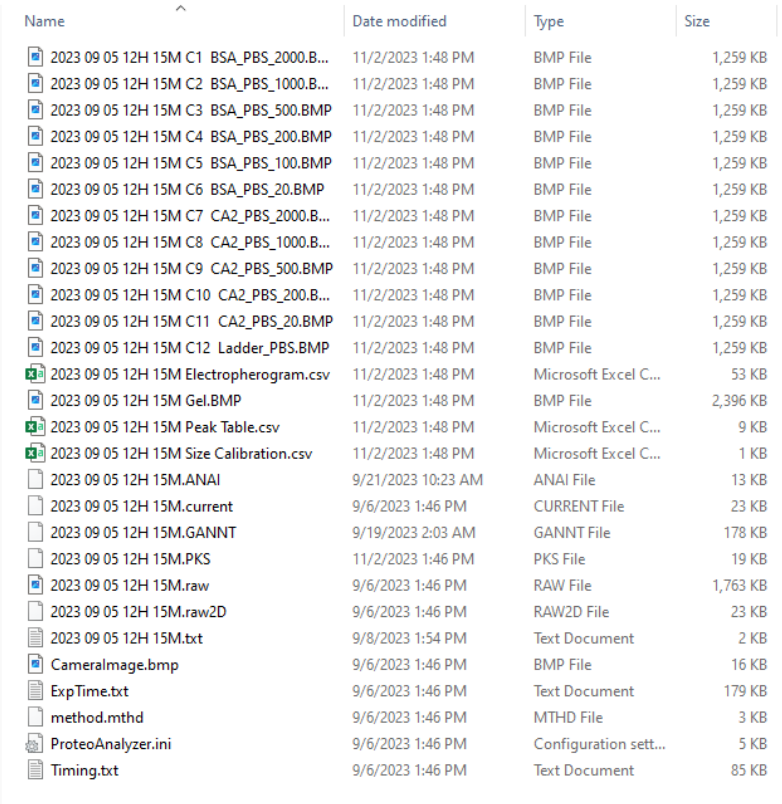
Settings	Description
Sample Option	Determines which samples of the data file will have information exported. There are two options: **All Samples** (results for all samples of data file exported); and **Selected Samples**. The Selected Samples option will open the **Selected Samples** window.  Select the respective wells, columns and/or rows of the sample plate, and click **Apply**. To abort the operation and export all samples, click **Cancel**.
Export All	Exports all fields of the Export Data utility for the data file.
Peak Table – Standard or Alternate	**Standard**: Exports the **Peak Table** for all selected samples as a single .csv file, listed in order of sample well (**Figure 60**). **Alternate**: Exports the **Peak Table** for all selected samples as a single .csv file, listed in rows (**Figure 61**).
Smear Analysis Table	Exports the **Smear Analysis** table for all selected samples as a single .csv file, listed in order of sample well (**Figure 62**). If no data is present in the **Smear Analysis** table, this function will be disabled.
Flag Analysis Criteria Table – Standard or Alternate	**Standard**: Exports the **Flag Analysis** table for all selected samples as a single .csv file, listed in order of sample well (**Figure 63**). The output will be in a binary 0/1 format, with each column labeled by the respective Flag condition. **Alternate**: Export the **Flag Analysis** table for all selected samples as a single .csv file, listed in order of sample well) (**Figure 64**). The output will list the values within the set criteria of the Flag analysis by row. If no data is present in the **Flag Analysis** table, this function will be disabled.
Electropherogram – Exports as CSV File	Exports electropherogram data in a .csv file format.

Table 35 Export Data settings

Settings	Description
File Export Option	Determines how electropherogram .csv data is exported. When **Single File** is selected, will export all electropherograms together in a single file, with the x-axis in the first column and each sample result's y-axis data in separate columns increasing from left to right (**Figure 65**). When **Separated Files** is selected, will export each electropherogram as a separate file, with the x-axis in the first column and the y-xis data in the second column (**Figure 66**).
X Axis Scale Option	Determines how electropherogram .csv data is formatted in x-axis. When **Size Scale** is selected, will export x-axis data as size, starting with 0 (**Figure 65**). When **Time Scale** is selected, will export x-axis as time, starting with 0 seconds (**Figure 66**).
Size Calibration Data	Exports the **Size Calibration** information as a .csv file, with column 1 as **Ladder Size** and column 2 as **Time** (sec) (**Figure 67**).
Exports Selected Samples Digital Gel Image	Exports a digital gel image overlay of the selected samples as an image file, in the format specified (.bmp, .jpg, or .png).
Image Format for Electropherogram and Gel	Determines which image file format is used for exported electropherogram images and digital gel image overlay (**Figure 68**). There are three different image file format options: • BMP (.bmp extension) • JPEG (.jpg extension) • PNG (.png extension)
Show Annotation	Shows any annotation(s) made to the sample wells on Digital Gel Image.
Export File Path	Determines the file path for saving exported data. The default directory is the same folder that contains the .raw data file (recommended). To select an alternative directory, click the folder icon. In the file browser menu, navigate to the desired directory, and save the file.
Export	Exports the data file with the settings made. In the dialog **Export Complete**, select **Open Folder Now** to open the directory. Click **Close** to close without opening the export folder. 
Cancel	Cancels the export operation and returns to the main screen of the ProSize software.

Examples of Exported Data

When exporting data from ProSize, the exported files will be named by the .raw file name followed by an extension dependent upon the information exported (**Figure 59** and **Table 36**).



Name	Date modified	Type	Size
2023 09 05 12H 15M C1 BSA_PBS_2000.B...	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C2 BSA_PBS_1000.B...	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C3 BSA_PBS_500.BMP	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C4 BSA_PBS_200.BMP	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C5 BSA_PBS_100.BMP	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C6 BSA_PBS_20.BMP	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C7 CA2_PBS_2000.B...	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C8 CA2_PBS_1000.B...	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C9 CA2_PBS_500.BMP	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C10 CA2_PBS_200.B...	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C11 CA2_PBS_20.BMP	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M C12 Ladder_PBS.BMP	11/2/2023 1:48 PM	BMP File	1,259 KB
2023 09 05 12H 15M Electropherogram.csv	11/2/2023 1:48 PM	Microsoft Excel C...	53 KB
2023 09 05 12H 15M Gel.BMP	11/2/2023 1:48 PM	BMP File	2,396 KB
2023 09 05 12H 15M Peak Table.csv	11/2/2023 1:48 PM	Microsoft Excel C...	9 KB
2023 09 05 12H 15M Size Calibration.csv	11/2/2023 1:48 PM	Microsoft Excel C...	1 KB
2023 09 05 12H 15M.ANAI	9/21/2023 10:23 AM	ANAI File	13 KB
2023 09 05 12H 15M.current	9/6/2023 1:46 PM	CURRENT File	23 KB
2023 09 05 12H 15M.GANNT	9/19/2023 2:03 AM	GANNT File	178 KB
2023 09 05 12H 15M.PKS	11/2/2023 1:46 PM	PKS File	19 KB
2023 09 05 12H 15M.raw	9/6/2023 1:46 PM	RAW File	1,763 KB
2023 09 05 12H 15M.raw2D	9/6/2023 1:46 PM	RAW2D File	23 KB
2023 09 05 12H 15M.txt	9/8/2023 1:54 PM	Text Document	2 KB
CameraImage.bmp	9/6/2023 1:46 PM	BMP File	16 KB
ExpTime.txt	9/6/2023 1:46 PM	Text Document	179 KB
method.mthd	9/6/2023 1:46 PM	MTHD File	3 KB
ProteoAnalyzer.ini	9/6/2023 1:46 PM	Configuration sett...	5 KB
Timing.txt	9/6/2023 1:46 PM	Text Document	85 KB

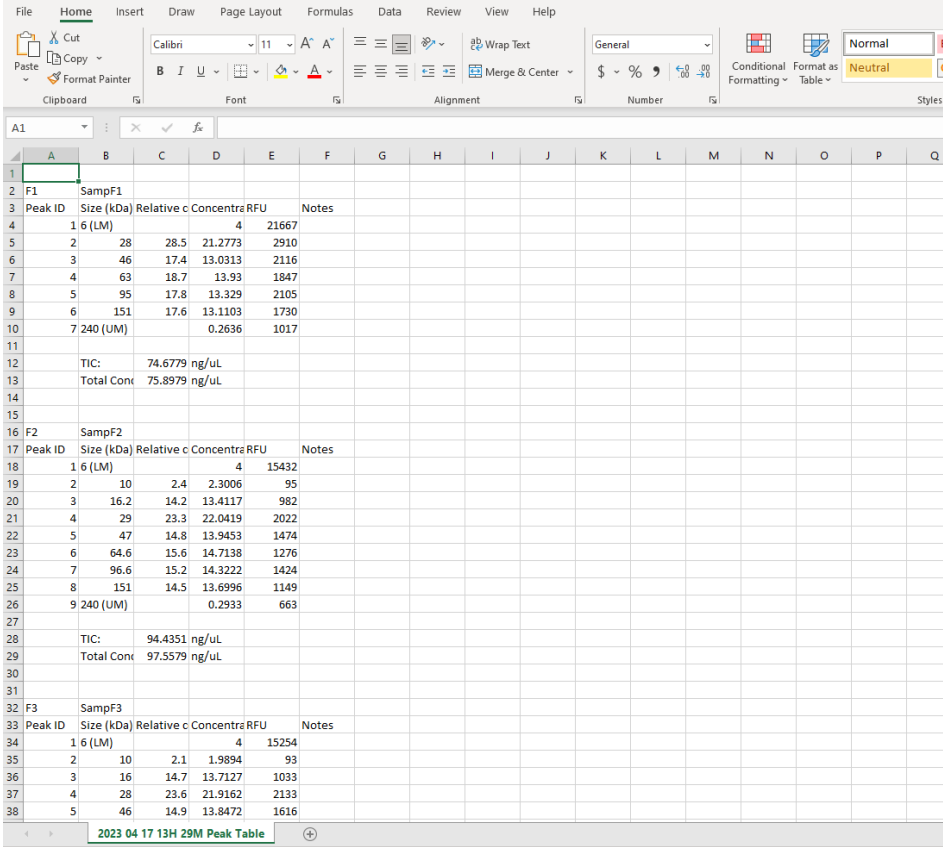
Figure 59 Exported data showing file name conventions

Table 36 Exported data naming conventions

Exported Item	Description
Peak Table	Filename **Peak Table** Example: *2023 07 15 18H 29M Peak Table*
Size Calibration	Filename **Size Calibration** Example: *2023 07 15 18H 29M Size Calibration*
Smear Analysis	Filename **Smear Analysis Result** Example: *2023 07 15 18H 29M Smear Analysis Result*
Flag Analysis Result	Filename **Flag Analysis Result** Example: *2023 07 15 18H 29M Flag Analysis Result*
Selected Samples Gel Image	Filename **Gel** Example: *2023 07 15 18H 29M Gel*
Individual Electropherogram Gel Image	Filename <Well ID> <Sample Name> Example: *2023 07 15 18H 29M H1 SampH1*
Individual Electropherogram Data (CSV)	• If exported as one file: Filename **Electropherogram** Example: *2023 07 15 18H 29M Electropherogram* • If exported as individual files: Filename <Well ID> <Sample Name> Example: *2023 07 15 18H 29M H1 SampH1*

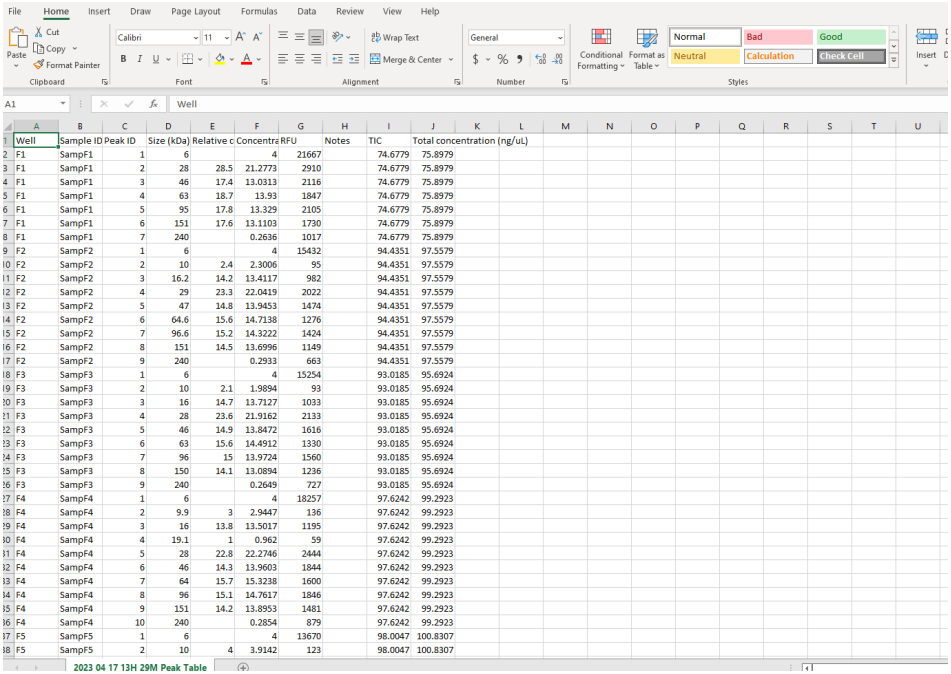
The following figures show examples of typical exported data formats:

- Peak Table



	A	B	C	D	E	F
2	F1	SampF1				
3	Peak ID	Size (kDa)	Relative c	Concentra	RFU	Notes
4	1	6 (LM)		4	21667	
5	2	28	28.5	21.2773	2910	
6	3	46	17.4	13.0313	2116	
7	4	63	18.7	13.93	1847	
8	5	95	17.8	13.329	2105	
9	6	151	17.6	13.1103	1730	
10	7	240 (UM)		0.2636	1017	
12		TIC:	74.6779	ng/uL		
13		Total Conc	75.8979	ng/uL		
16	F2	SampF2				
17	Peak ID	Size (kDa)	Relative c	Concentra	RFU	Notes
18	1	6 (LM)		4	15432	
19	2	10	2.4	2.3006	95	
20	3	16.2	14.2	13.4117	982	
21	4	29	23.3	22.0419	2022	
22	5	47	14.8	13.9453	1474	
23	6	64.6	15.6	14.7138	1276	
24	7	96.6	15.2	14.3222	1424	
25	8	151	14.5	13.6996	1149	
26	9	240 (UM)		0.2933	663	
28		TIC:	94.4351	ng/uL		
29		Total Conc	97.5579	ng/uL		
32	F3	SampF3				
33	Peak ID	Size (kDa)	Relative c	Concentra	RFU	Notes
34	1	6 (LM)		4	15254	
35	2	10	2.1	1.9894	93	
36	3	16	14.7	13.7127	1033	
37	4	28	23.6	21.9162	2133	
38	5	46	14.9	13.8472	1616	

Figure 60 Exported Peak table - Standard file format



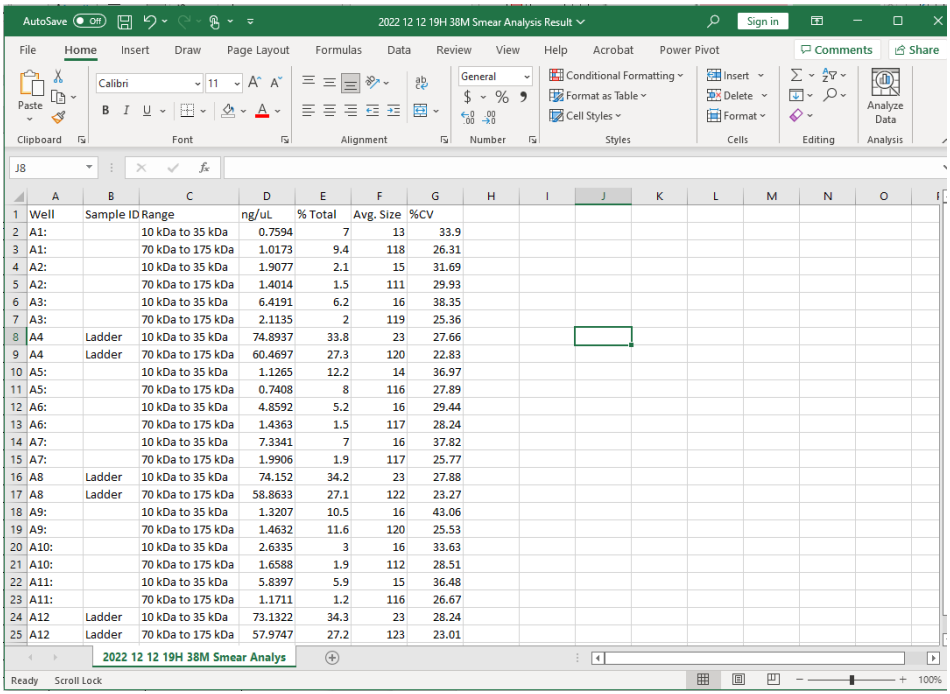
Well	Sample ID	Peak ID	Size (kDa)	Relative c	Concentra	RFU	Notes	TIC	Total concentration (ng/uL)
F1	SampF1	1	6		4	21667		74.6779	75.8979
F1	SampF1	2	28	28.5	21.2773	2910		74.6779	75.8979
F1	SampF1	3	46	17.4	13.0313	2116		74.6779	75.8979
F1	SampF1	4	63	18.7	13.93	1847		74.6779	75.8979
F1	SampF1	5	95	17.8	13.329	2105		74.6779	75.8979
F1	SampF1	6	151	17.6	13.1103	1730		74.6779	75.8979
F1	SampF1	7	240		0.2636	1017		74.6779	75.8979
F2	SampF2	1	6		4	15432		94.4351	97.5579
F2	SampF2	2	10	2.4	2.3006	95		94.4351	97.5579
F2	SampF2	3	16.2	14.2	13.4117	982		94.4351	97.5579
F2	SampF2	4	29	23.3	22.0419	2022		94.4351	97.5579
F2	SampF2	5	47	14.8	13.9453	1474		94.4351	97.5579
F2	SampF2	6	64.6	15.6	14.7138	1276		94.4351	97.5579
F2	SampF2	7	96.6	15.2	14.3222	1424		94.4351	97.5579
F2	SampF2	8	151	14.5	13.6996	1149		94.4351	97.5579
F2	SampF2	9	240		0.2933	663		94.4351	97.5579
F3	SampF3	1	6		4	15254		93.0185	95.6924
F3	SampF3	2	10	2.1	1.9894	93		93.0185	95.6924
F3	SampF3	3	16	14.7	13.7127	1033		93.0185	95.6924
F3	SampF3	4	28	23.6	21.9162	2133		93.0185	95.6924
F3	SampF3	5	46	14.9	13.8472	1616		93.0185	95.6924
F3	SampF3	6	63	15.6	14.4912	1330		93.0185	95.6924
F3	SampF3	7	96	15	13.9724	1560		93.0185	95.6924
F3	SampF3	8	150	14.1	13.0894	1236		93.0185	95.6924
F3	SampF3	9	240		0.2649	727		93.0185	95.6924
F4	SampF4	1	6		4	18257		97.6242	99.2923
F4	SampF4	2	9.9	3	2.9447	136		97.6242	99.2923
F4	SampF4	3	16	13.8	13.5017	1195		97.6242	99.2923
F4	SampF4	4	19.1	1	0.962	59		97.6242	99.2923
F4	SampF4	5	28	22.8	22.2746	2444		97.6242	99.2923
F4	SampF4	6	46	14.3	13.9603	1844		97.6242	99.2923
F4	SampF4	7	64	15.7	15.3238	1600		97.6242	99.2923
F4	SampF4	8	96	15.1	14.7617	1846		97.6242	99.2923
F4	SampF4	9	151	14.2	13.8953	1481		97.6242	99.2923
F4	SampF4	10	240		0.2854	879		97.6242	99.2923
F5	SampF5	1	6		4	13670		98.0047	100.8307
F5	SampF5	2	10	4	3.9142	123		98.0047	100.8307

Figure 61 Exported Peak Table - Alternate file format

- Smear Analysis

NOTE

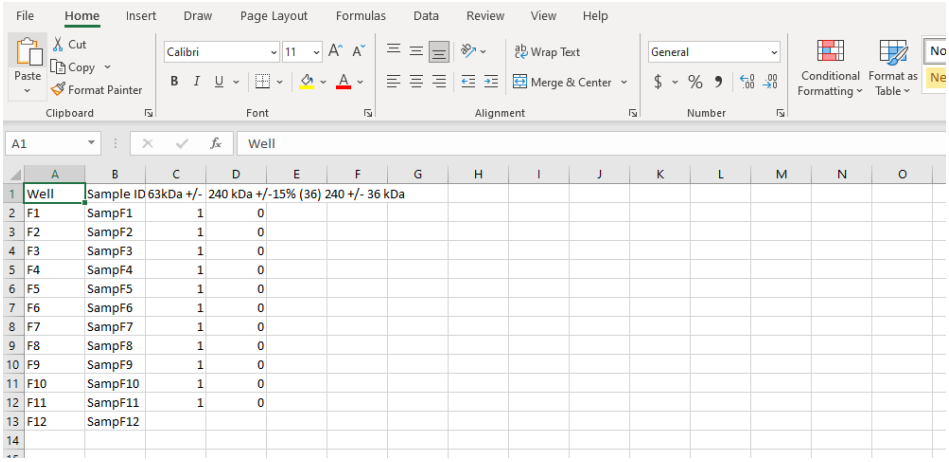
When multiple Smear Analysis conditions are entered for a sample, separate lines will be created in the exported table. More information is available in **Chapter 4**, “ProSize Configuration”.



	A	B	C	D	E	F	G
1	Well	Sample ID	Range	ng/uL	% Total	Avg. Size	%CV
2	A1:		10 kDa to 35 kDa	0.7594	7	13	33.9
3	A1:		70 kDa to 175 kDa	1.0173	9.4	118	26.31
4	A2:		10 kDa to 35 kDa	1.9077	2.1	15	31.69
5	A2:		70 kDa to 175 kDa	1.4014	1.5	111	29.93
6	A3:		10 kDa to 35 kDa	6.4191	6.2	16	38.35
7	A3:		70 kDa to 175 kDa	2.1135	2	119	25.36
8	A4	Ladder	10 kDa to 35 kDa	74.8937	33.8	23	27.66
9	A4	Ladder	70 kDa to 175 kDa	60.4697	27.3	120	22.83
10	A5:		10 kDa to 35 kDa	1.1265	12.2	14	36.97
11	A5:		70 kDa to 175 kDa	0.7408	8	116	27.89
12	A6:		10 kDa to 35 kDa	4.8592	5.2	16	29.44
13	A6:		70 kDa to 175 kDa	1.4363	1.5	117	28.24
14	A7:		10 kDa to 35 kDa	7.3341	7	16	37.82
15	A7:		70 kDa to 175 kDa	1.9906	1.9	117	25.77
16	A8	Ladder	10 kDa to 35 kDa	74.152	34.2	23	27.88
17	A8	Ladder	70 kDa to 175 kDa	58.8633	27.1	122	23.27
18	A9:		10 kDa to 35 kDa	1.3207	10.5	16	43.06
19	A9:		70 kDa to 175 kDa	1.4632	11.6	120	25.53
20	A10:		10 kDa to 35 kDa	2.6335	3	16	33.63
21	A10:		70 kDa to 175 kDa	1.6588	1.9	112	28.51
22	A11:		10 kDa to 35 kDa	5.8397	5.9	15	36.48
23	A11:		70 kDa to 175 kDa	1.1711	1.2	116	26.67
24	A12	Ladder	10 kDa to 35 kDa	73.1322	34.3	23	28.24
25	A12	Ladder	70 kDa to 175 kDa	57.9747	27.2	123	23.01

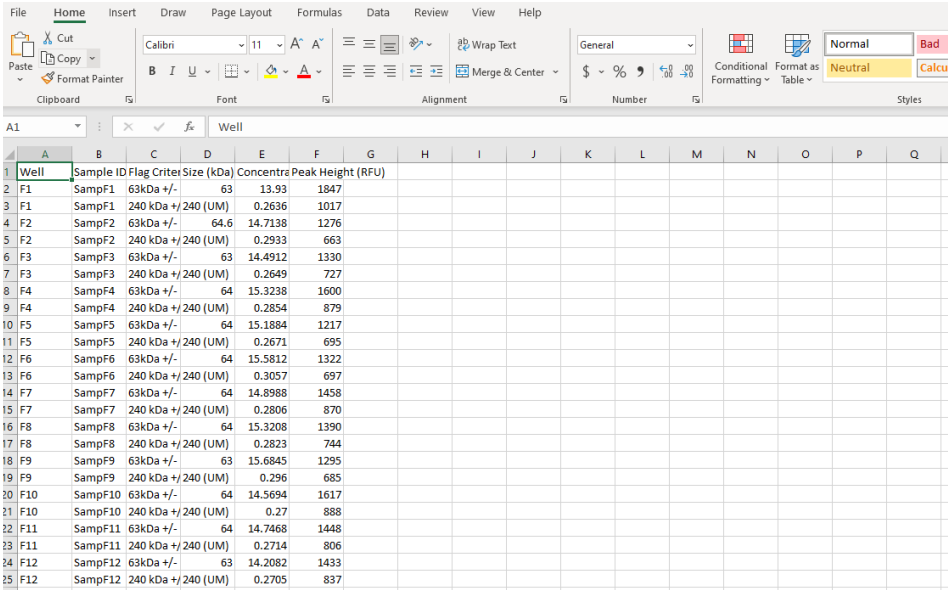
Figure 62 Exported smear Analysis file format

- Flag Analysis Criteria Table



Well	Sample ID	63kDa +/-	240 kDa +/-15% (36)	240 +/- 36 kDa
F1	SampF1	1	0	
F2	SampF2	1	0	
F3	SampF3	1	0	
F4	SampF4	1	0	
F5	SampF5	1	0	
F6	SampF6	1	0	
F7	SampF7	1	0	
F8	SampF8	1	0	
F9	SampF9	1	0	
F10	SampF10	1	0	
F11	SampF11	1	0	
F12	SampF12			

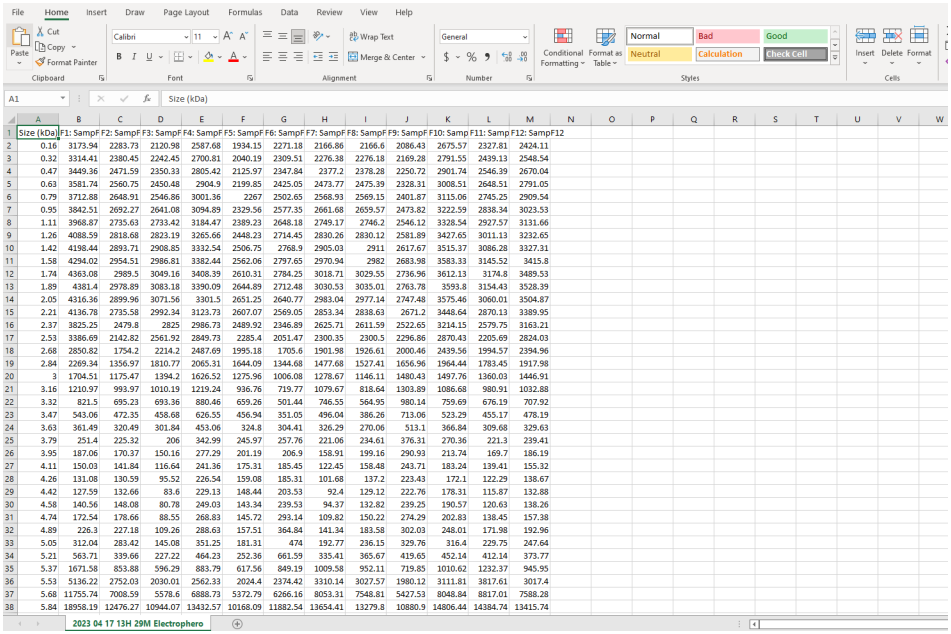
Figure 63 Exported Flag Analysis - Standard file format



Well	Sample ID	Flag Criter	Size (kDa)	Concentra	Peak Height (RFU)
F1	SampF1	63kDa +/-	63	13.93	1847
F1	SampF1	240 kDa +/	240 (UM)	0.2636	1017
F2	SampF2	63kDa +/-	64.6	14.7138	1276
F2	SampF2	240 kDa +/	240 (UM)	0.2933	663
F3	SampF3	63kDa +/-	63	14.4912	1330
F3	SampF3	240 kDa +/	240 (UM)	0.2649	727
F4	SampF4	63kDa +/-	64	15.3238	1600
F4	SampF4	240 kDa +/	240 (UM)	0.2854	879
F5	SampF5	63kDa +/-	64	15.1884	1217
F5	SampF5	240 kDa +/	240 (UM)	0.2671	695
F6	SampF6	63kDa +/-	64	15.5812	1322
F6	SampF6	240 kDa +/	240 (UM)	0.3057	697
F7	SampF7	63kDa +/-	64	14.8988	1458
F7	SampF7	240 kDa +/	240 (UM)	0.2806	870
F8	SampF8	63kDa +/-	64	15.3208	1390
F8	SampF8	240 kDa +/	240 (UM)	0.2823	744
F9	SampF9	63kDa +/-	63	15.6845	1295
F9	SampF9	240 kDa +/	240 (UM)	0.296	685
F10	SampF10	63kDa +/-	64	14.5694	1617
F10	SampF10	240 kDa +/	240 (UM)	0.27	888
F11	SampF11	63kDa +/-	64	14.7468	1448
F11	SampF11	240 kDa +/	240 (UM)	0.2714	806
F12	SampF12	63kDa +/-	63	14.2082	1433
F12	SampF12	240 kDa +/	240 (UM)	0.2705	837

Figure 64 Exported Flag Analysis - Alternate file format

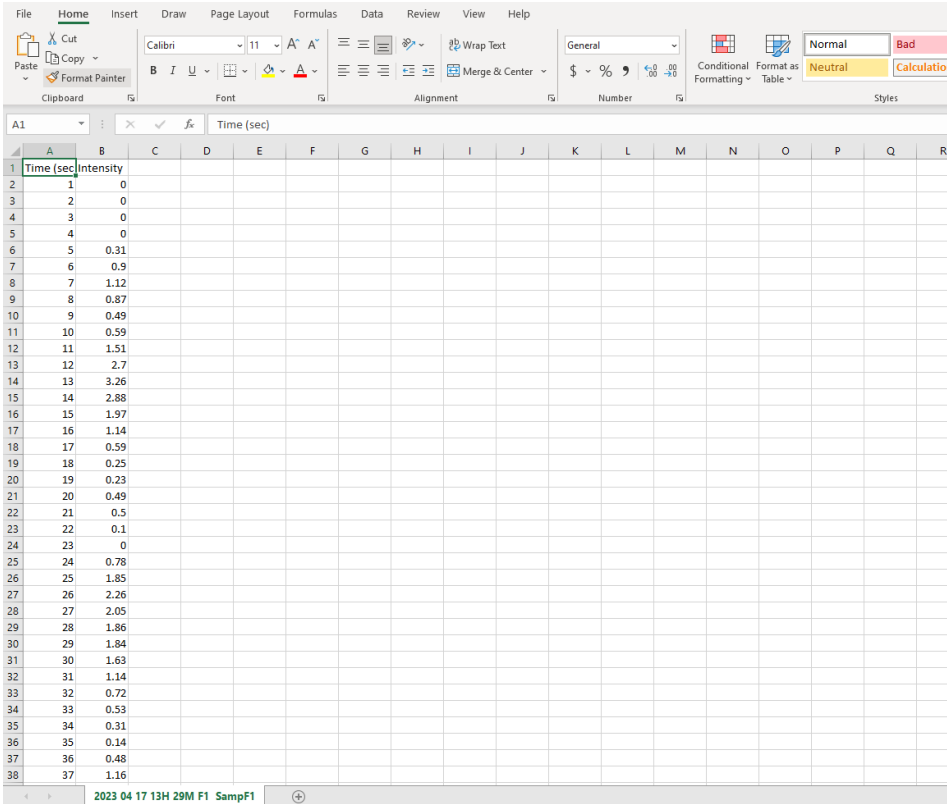
- Electropherogram CSV – Single File – Size Scale



Size (kDa)	F1: SampF	F2: SampF	F3: SampF	F4: SampF	F5: SampF	F6: SampF	F7: SampF	F8: SampF	F9: SampF	F10: Samp	F11: Samp	F12: SampF12
0.16	3173.94	2283.73	2120.98	2587.68	1934.15	2271.18	2166.86	2166.6	2086.43	2675.57	2327.81	2424.11
0.32	3314.41	2380.45	2242.45	2700.81	2040.19	2309.51	2276.38	2276.18	2169.28	2791.55	2439.13	2548.54
0.47	3449.36	2471.59	2350.33	2805.42	2125.97	2347.84	2377.2	2378.28	2250.72	2901.74	2546.39	2670.04
0.63	3581.74	2560.75	2450.48	2904.9	2199.85	2425.05	2473.77	2475.39	2328.31	3008.51	2648.51	2791.05
0.79	3712.88	2648.91	2546.86	3001.36	2267	2502.65	2568.93	2569.15	2401.87	3115.06	2745.25	2909.54
0.95	3842.51	2692.27	2641.08	3094.89	2329.56	2577.35	2661.68	2659.57	2473.82	3222.59	2838.34	3023.53
1.11	3968.87	2735.63	2733.42	3184.47	2389.23	2648.18	2749.17	2746.2	2546.12	3328.54	2927.57	3131.66
1.26	4088.59	2818.68	2823.19	3265.66	2448.23	2714.45	2830.26	2830.12	2581.89	3427.65	3011.13	3232.65
1.42	4198.44	2893.71	2908.85	3332.54	2506.75	2768.9	2905.03	2911	2617.67	3515.37	3086.28	3327.31
1.58	4294.02	2954.51	2986.81	3382.44	2562.06	2797.65	2970.94	2982	2683.98	3583.33	3145.52	3415.8
1.74	4363.08	2989.5	3049.16	3408.39	2610.31	2784.25	3018.71	3029.55	2736.96	3612.13	3174.8	3489.53
1.89	4381.4	2978.89	3083.18	3390.09	2644.89	2712.48	3030.53	3035.01	2763.78	3593.8	3154.43	3528.39
2.05	4316.36	2899.96	3071.56	3301.5	2651.25	2640.77	2983.04	2977.14	2747.48	3575.46	3060.01	3504.87
2.21	4136.78	2735.58	2992.34	3123.73	2607.07	2569.05	2853.34	2838.63	2671.2	3448.64	2870.13	3389.95
2.37	3825.25	2479.8	2825	2986.73	2489.92	2346.89	2625.71	2611.59	2522.65	3214.15	2579.75	3163.21
2.53	3386.69	2142.82	2561.92	2849.73	2285.4	2051.47	2300.35	2300.5	2296.86	2870.43	2205.69	2824.03
2.68	2850.82	1754.2	2214.2	2487.69	1995.18	1705.6	1901.98	1926.61	2000.46	2439.56	1994.57	2394.96
2.84	2269.34	1356.97	1810.77	2065.31	1644.09	1344.68	1477.68	1527.41	1656.96	1964.44	1783.45	1917.98
3	1704.51	1175.47	1394.2	1626.52	1275.96	1006.08	1278.67	1146.11	1480.43	1497.76	1360.03	1446.91
3.16	1210.97	993.97	1010.19	1219.24	936.76	719.77	1079.67	818.64	1303.89	1086.68	980.91	1032.88
3.32	821.5	695.23	693.36	880.46	659.26	501.44	746.55	564.95	980.14	759.69	676.19	707.92
3.47	543.06	472.35	458.68	626.55	456.94	351.05	496.04	386.26	713.06	523.29	455.17	478.19
3.63	361.49	320.49	301.84	453.06	324.8	304.41	326.29	270.06	513.1	366.84	309.68	329.63
3.79	251.4	225.32	206	342.99	245.97	257.76	221.06	234.61	376.31	270.36	221.3	239.41
3.95	187.06	170.37	150.16	277.29	201.19	206.9	158.91	199.16	290.93	213.74	169.7	186.19
4.11	150.03	141.84	116.64	241.36	175.31	185.45	122.45	158.48	243.71	183.24	139.41	155.32
4.26	131.08	130.59	95.52	226.54	159.08	185.31	101.68	137.2	223.43	172.1	122.29	138.67
4.42	127.59	132.66	83.6	229.13	148.44	203.53	92.4	129.12	222.76	178.31	115.87	132.88
4.58	140.56	148.08	80.78	249.03	143.34	239.53	94.37	132.82	239.25	190.57	120.63	138.26
4.74	172.54	178.66	88.55	268.83	145.72	293.14	109.82	150.22	274.29	202.83	138.45	157.38
4.89	226.3	227.18	109.26	288.63	157.51	364.84	141.34	183.58	302.03	248.01	171.98	192.96
5.05	312.04	283.42	145.08	351.25	181.31	474	192.77	236.15	329.76	316.4	229.75	247.64
5.21	563.71	339.66	227.22	464.23	252.36	661.59	335.41	365.67	419.65	452.14	412.14	373.77
5.37	1671.58	853.88	596.29	883.79	617.56	849.19	1009.58	952.11	719.85	1010.62	1232.37	945.95
5.53	5136.22	2752.03	2030.01	2562.33	2024.4	2374.42	3310.14	3027.57	1980.12	3111.81	3817.61	3017.4
5.68	11755.74	7008.59	5578.6	6888.73	5372.79	6266.16	8053.31	7548.81	5427.53	8048.84	8817.01	7588.28
5.84	18958.19	12476.27	10944.07	13432.57	10168.09	11882.54	13654.41	13279.8	10880.9	14806.44	14384.74	13415.74

Figure 65 Exported Electropherogram CSV Single File – Size Scale file format

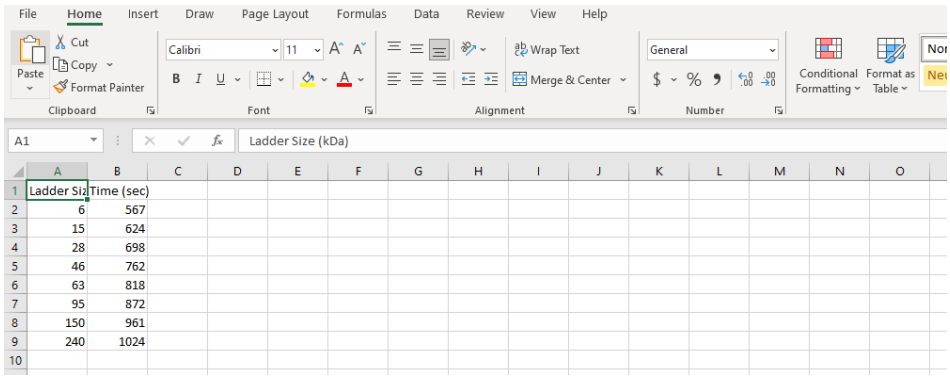
- Electropherogram CSV – Separated Files – Time Scale



Time (sec)	Intensity
1	0
2	0
3	0
4	0
5	0.31
6	0.9
7	1.12
8	0.87
9	0.49
10	0.59
11	1.51
12	2.7
13	3.26
14	2.88
15	1.97
16	1.14
17	0.59
18	0.25
19	0.23
20	0.49
21	0.5
22	0.1
23	0
24	0.78
25	1.85
26	2.26
27	2.05
28	1.86
29	1.84
30	1.63
31	1.14
32	0.72
33	0.53
34	0.31
35	0.14
36	0.48
37	1.16

Figure 66 Exported Electropherogram CSV Separated Files – Time Scale file format

- Size Calibration



Ladder Size	Time (sec)
6	567
15	624
28	698
46	762
63	818
95	872
150	961
240	1024

Figure 67 Exported Size Calibration file format

- Selected Samples Gel Image

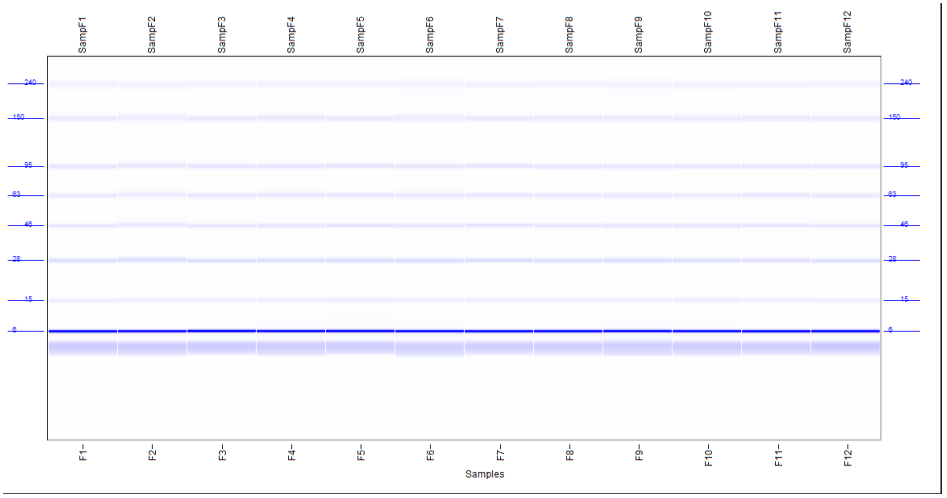


Figure 68 Exported Selected Samples Gel Image file format

- Individual Electropherogram Image

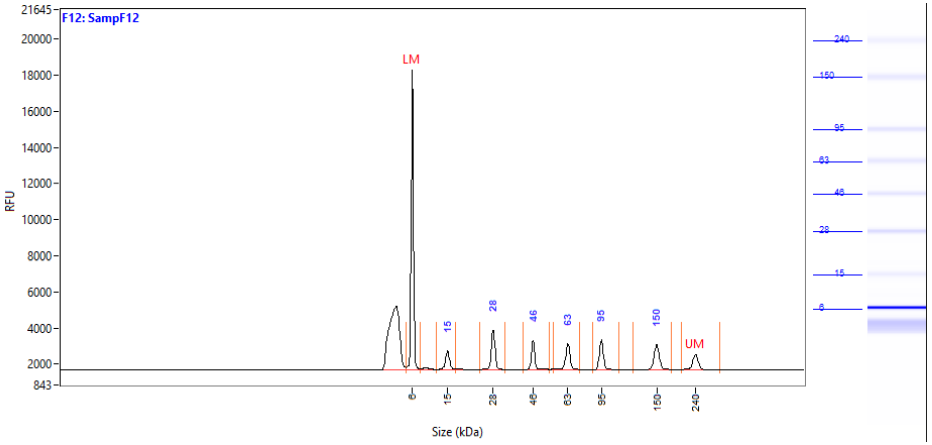


Figure 69 Exported Individual Electropherogram Image file format

8 Generating Reports from ProSize

This chapter provides an overview of the options available for generating reports from the ProSize software. The following sections describe how to open the **Generate Report** window; provide an outline of various report generation options; describe how to perform the report generation; and give examples of reported data.

Generate a Report

Generate a report of sample results

The ProSize software can generate PDF formatted reports for convenient viewing of processed data, reporting detailed information for each sample analysis. The **Generate Report** command is used to generate PDF formatted results.

1 From the main screen, select , or navigate to **File** > **Generate Report**.

The **Generate PDF** window opens (**Figure 70**). **Table 37** summarizes the available settings of this window.

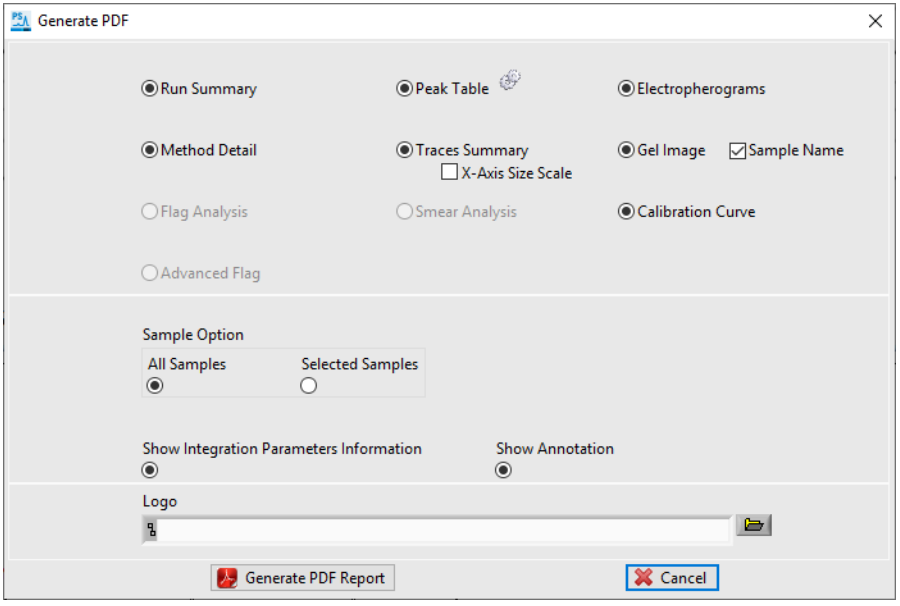


Figure 70 Generate PDF window

Table 37 Generate PDF settings

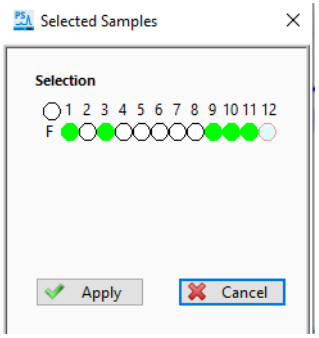
Settings	Description
Sample Option	Determines which samples of the data file will be included in the report. All selected samples will be saved in a single PDF report file; each sample will be printed on a separate page of the report. There are two options: **All Samples** (results for all samples of data file exported); and **Selected Samples**. The Selected Samples option will open the **Selected Samples** window.  Select the respective wells, columns and/or rows of the sample plate, and click **Apply**. To abort the operation and export all samples, click **Cancel**.
Run Summary	A summary of run information will be printed on the first page of PDF report including **Filename** and **Data Path**; date **Created**; **# of Capillaries**; **Array Serial #**; capillary **Effective Length**; **Array Usage Count**; **Version #** of software; **Device Serial #** (Figure 71). If this is not selected, these fields will be blank.
Method Detail	Details of the performed experimental method will be printed on the first page of the PDF report (from the user interface **Information** file (in ProSize Plate Map)). If not selected, these fields will be blank. An example for the ProteoAnalyzer is shown below: **Method Information:** Method Name: 5191-6640LM - Protein Broad Range P240 LM Only.mthds Gel Prime: No Full Conditioning: Yes Gel Prime to Buffer: No Gel Selection: Gel 1 Perform Prerun: 5.0 kV, 30 sec. Rinse: No Marker 1: No Rinse: Tray: Buffer, Row: A, # Dips: 1 Sample Injection: 7.0 kV, 10 sec. Separation: 9.0 kV, 20.0 min. Tray Name: Tray-1

Table 37 Generate PDF settings

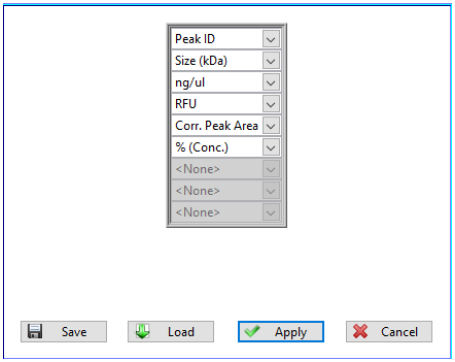
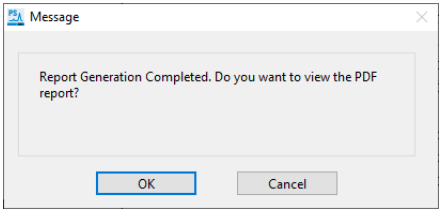
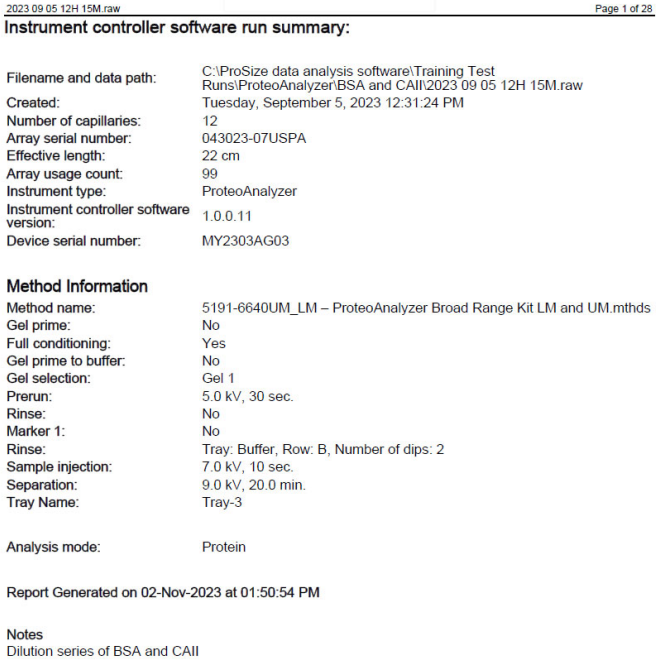
Settings	Description
Traces Summary	The **Trace Summary** page will be printed with up to 12 electropherograms per page (**Figure 72**). Traces will show the **Sample ID** information for each trace. If not selected, these field will be blank.
Peak Table	For all selected samples, the **Peak Table** will be printed on each sample result page in the PDF report (**Figure 73**). If not selected, this field will be blank. Select  to define the reported items and their order in the table. You can save these settings for future use. 
Electropherograms	For all selected samples, the Electropherogram Trace will be printed on each sample result page in the PDF report. Shown are the peaks with the currently selected annotation and x-axis scale in ProSize at the time of printing (**Figure 73**). The digital gel image will be displayed to the right, labeled by size. If not selected, this field will be blank.
Flag Analysis	For all selected samples, a **Flag Analysis** results table will be printed on a separate page in the PDF report, with one page per Flag condition (**Figure 74**). The output will be in a binary 0/1 format, with each page column labeled by the respective Flag condition. If no **Flag Analysis** table is present for the data file, this option will be disabled.
Smear Analysis	For each selected sample, the **Smear Analysis** results will be printed underneath the Peak Table. The color-coded vertical smear range lines and Average Size (kDa) will be also reported (**Figure 73**). If no **Smear Analysis** table is present for the data file, this option will be disabled.
Gel Image	Prints a digital gel overlay image of the selected samples on the first page of the PDF report (**Figure 71**). If not selected, this field will be blank.

Table 37 Generate PDF settings

Settings	Description
Calibration Curve	Prints a separate page at the end of the PDF report showing the **Size Calibration** curve including the well location and fit type used (**Figure 75**). If not selected, this page will be not printed.
Show Integration Parameters Information	Prints a detailed summary of the individual integration parameters for each selected sample at the bottom of each sample result page in the PDF report (**Figure 73**). If not selected, this field blank will be blank.
Show Annotation	Shows the annotation(s) made to individual sample electropherograms. If not selected, no annotation for samples will be shown.
Logo	Allows to import a custom logo (which will print at the top of each page). This is for customers that want their company logo to be printed on each page.
Generate PDF report	Generates the configured report. A file browser menu will open to navigate and save the file in the desired directory. The default directory is that containing the .raw data file. In the **Message** dialog, click **OK** to open the report; click **Cancel** to close without opening the report. 
Cancel	Abort the report generation and return the main screen of the software.

Examples of Generated Reports

Figure 71 through **Figure 75** below show examples of the PDF report pages generated by the ProSize software. The header of each page lists the .raw data file name and the page number; the footer contains software version and copyright information as well the date and time of report generation.



2023 09 05 12H 15M.raw Page 1 of 28

Instrument controller software run summary:

Filename and data path:	C:\ProSize data analysis software\Training Test Runs\ProteoAnalyzer\BSA and CAII\2023 09 05 12H 15M.raw
Created:	Tuesday, September 5, 2023 12:31:24 PM
Number of capillaries:	12
Array serial number:	043023-07USPA
Effective length:	22 cm
Array usage count:	99
Instrument type:	ProteoAnalyzer
Instrument controller software version:	1.0.0.11
Device serial number:	MY2303AG03

Method Information

Method name:	5191-6640UM_LM – ProteoAnalyzer Broad Range Kit LM and UM.mthds
Gel prime:	No
Full conditioning:	Yes
Gel prime to buffer:	No
Gel selection:	Gel 1
Prerun:	5.0 kV, 30 sec.
Rinse:	No
Marker 1:	No
Rinse:	Tray: Buffer, Row: B, Number of dips: 2
Sample injection:	7.0 kV, 10 sec.
Separation:	9.0 kV, 20.0 min.
Tray Name:	Tray-3
Analysis mode:	Protein

Report Generated on 02-Nov-2023 at 01:50:54 PM

Notes
Dilution series of BSA and CAII

Figure 71 Page 1 of PDF report (all fields shown; 12 samples were selected)

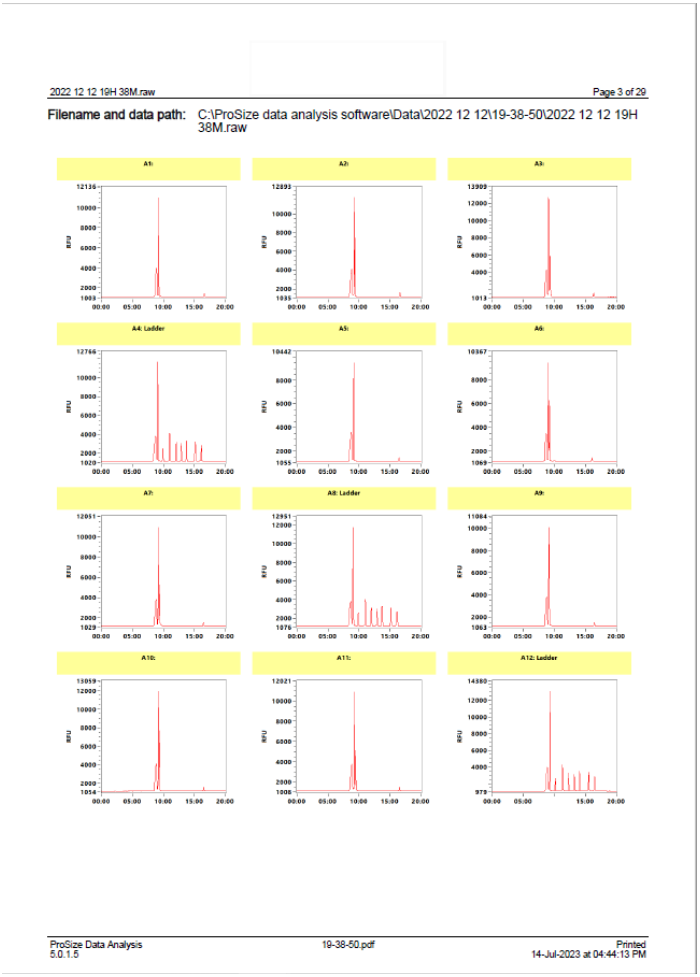


Figure 72 Trace Summary of PDF report (all fields shown; 12 samples were selected)

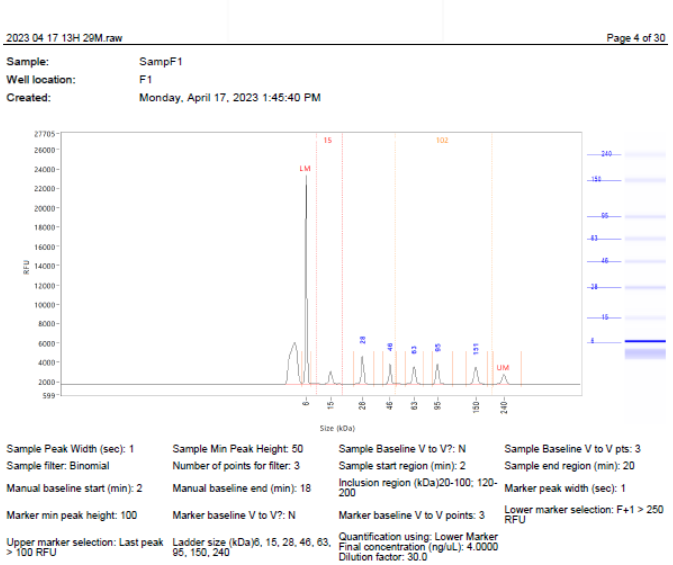
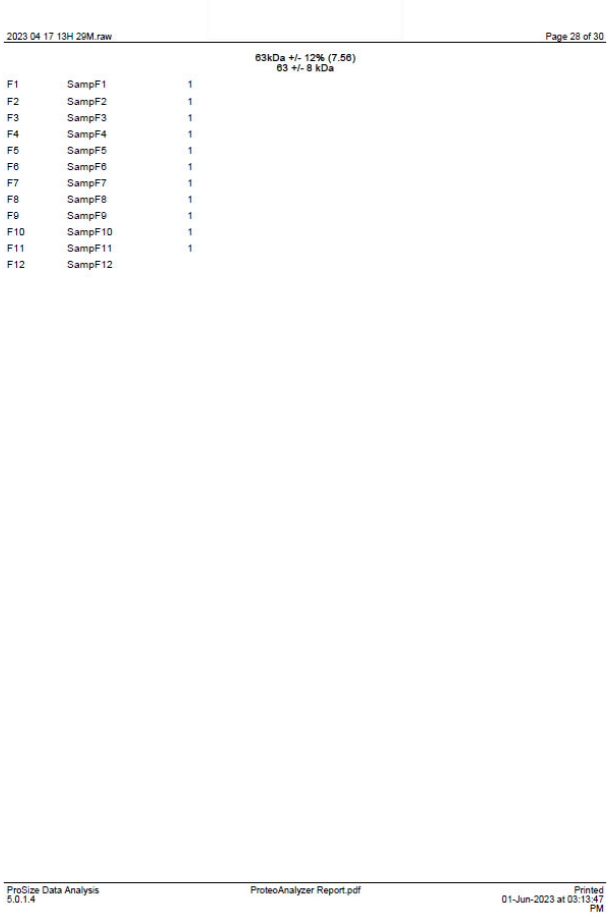


Figure 73 Individual result page of PDF report (all fields shown)



2023 04 17 13H 29M.raw Page 28 of 30

63kDa +/- 12% (7.56)
63 +/- 8 kDa

F1	SampF1	1
F2	SampF2	1
F3	SampF3	1
F4	SampF4	1
F5	SampF5	1
F6	SampF6	1
F7	SampF7	1
F8	SampF8	1
F9	SampF9	1
F10	SampF10	1
F11	SampF11	1
F12	SampF12	

ProSize Data Analysis 5.0.1.4 ProteoAnalyzer Report.pdf Printed 01-Jun-2023 at 03:13:47 PM

Figure 74 Example Flag Analysis table in PDF report

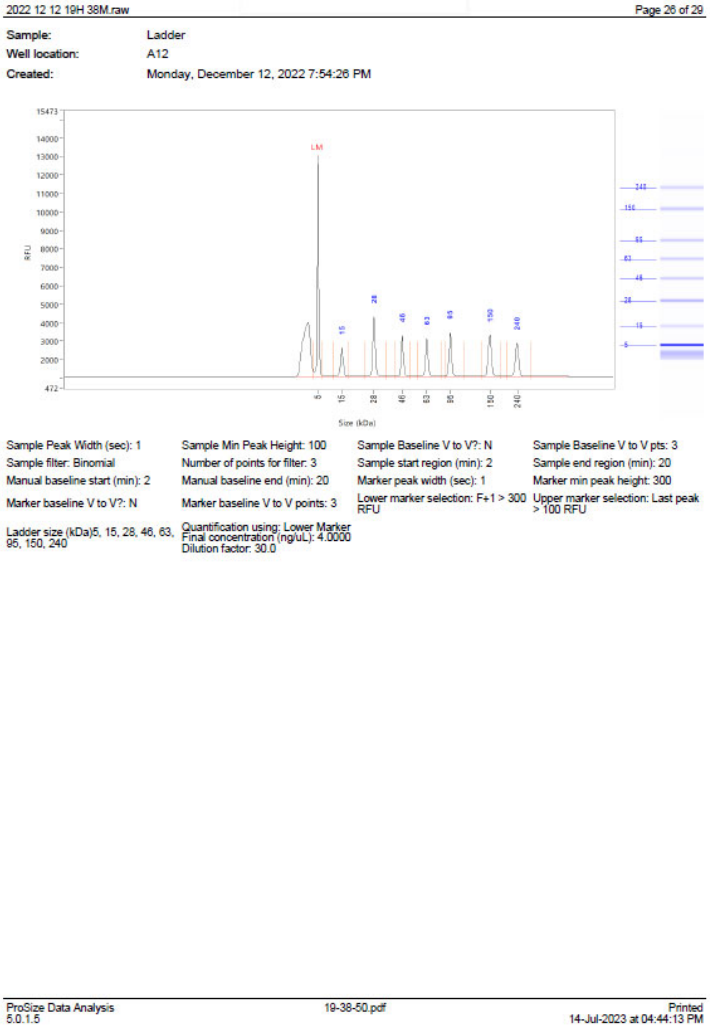


Figure 75 Example Size Calibration page in PDF report

9 ProSize View Capillary Positions

This chapter briefly covers the tools and functions of the View Capillary Positions window.

About View Capillary Positions

The ProSize software performs an automated capillary alignment procedure when reading in .raw data files. This ensures that the capillary locations on the CCD detector selected for data analysis are of maximum fluorescence intensity for providing the best possible signal to noise ratio.

View Capillary Positions is used to examine the capillary array alignment and the assigned locations used for data analysis. This option is typically not used in routine use since the locations are automatically assigned; it serves rather as a diagnostic/troubleshooting tool.

Open the View Capillary Positions Window

1 From the main screen of the ProSize software program, select **Analysis** > **View Capillary Positions**.

The **View Capillary Positions** window opens (**Figure 76**). **Table 38** summarizes the available functions in this window.

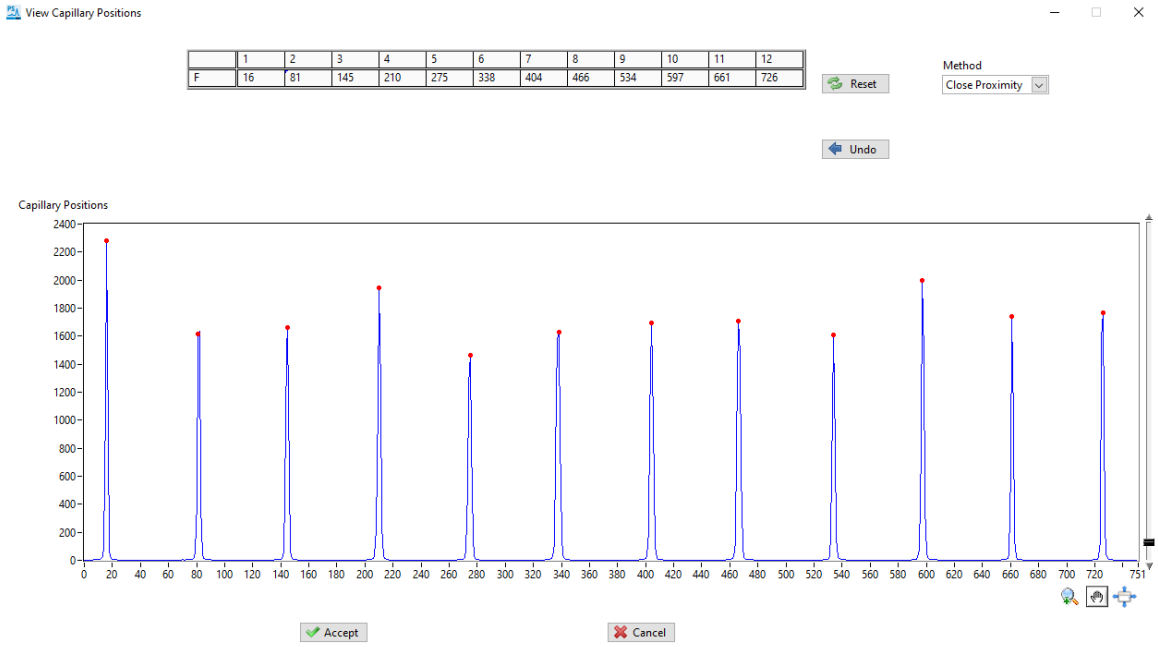


Figure 76 View Capillary Positions utility

The **View Capillary Positions** window contains two sections:

- A plot of **Capillary Positions** (Pixels) vs. **Signal Distribution** (Intensity), which shows a summation of fluorescence intensity for each capillary.
- The **Capillary Positions** table listing the pixel location for each of the 12 capillaries in the 12-capillary array.

In **Figure 76**, a proper alignment is shown where the red **Current Positions** symbols are centered on the peaks corresponding to each capillary. The algorithm automatically detects the peak locations and sets the capillary positions. If no fluorescence is detected in a particular capillary, the algorithm will

fill the gap using the spacing between capillaries as a reference. In this way, the positions of all capillaries are indexed properly, even if no data was generated by some capillaries of the array.

NOTE

It is important when installing a new capillary array to perform a run with samples (for example, Ladder or marker plate), followed by performing an optical alignment using the collected data file (i.e., Read Raw optical alignment function). This is to ensure that the assigned instrument capillary locations are close to actual positions. For further information, refer to the **Capillary Alignment** section of the System Manual.

During typical operation, no modifications should need to be made to the capillary positions. In rare occurrences, it may be necessary to adjust the positions slightly for one or two capillaries, or to manually adjust positions as described below. provides information on how to change a location and save the changes. Only changes necessary to center the red **Current Positions** symbols on any misaligned capillaries should be performed, to avoid introducing artifacts into the data.

View Capillary Positions Window Settings

Table 38 summarizes the settings of **View Capillary Positions** window.

Table 38 Capillary Positions window functions

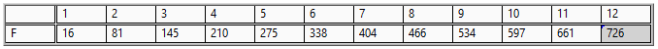
Menu Item	Description
Capillary Positions	Lists the pixel locations used on the CCD detector for data analysis. There are 12 cells, each corresponding to a capillary of the array. The table is labeled by row and well position.  To view a particular capillary position, click the cell in the table, or click on a peak in the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity) to view the corresponding assigned well. A red vertical cursor will be displayed on the plot to indicate the detector position.
Reset	Performs an automatic alignment algorithm to reset the capillary locations. Any manual changes made to the pixel positions will be overwritten. A blue threshold line will appear (see Figure 77). This line can be moved up or down to select an appropriate threshold for automatically determining capillary location.
Method	Two methods for determining capillary location can be used: • **Close Proximity** (default): This setting will use the original instrument alignment file to determine where to locate the capillary positions, filling any gaps in signal as needed. • **Ignore Original**: If the Close Proximity method fails to locate the correct capillary locations, this will attempt to assign capillary positions based on the signal positions; press **Reset** and use the threshold cursor and **Peak Width** settings to adjust assignments.
Peak Width	Provides a width threshold for selecting peaks in the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity); a higher value selects wider peaks while a smaller value selects narrow peaks. The recommended value is 3.
Undo	To undo the last manual adjustment operation to the **Capillary Positions** table.
Zoom	Enables zooming in the x- and y-axis of the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity). To zoom-in: Place the mouse over the plot, click and drag it outward to expand a box area to define the zoom region. Release the mouse button to apply. . To undo the zoom, use the **Autoscale** function.
Autoscale	To autoscale the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity).

Table 38 Capillary Positions window functions

Menu Item	Description
Pan	Enables panning of the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity). Move the image around with the mouse cursor: Place the mouse over the trace and click; drag the cursor to shift the current view. This is most often used in combination with the **Zoom** function.
Accept	Accepts the current **Capillary Positions** table locations for the capillary array to use for data analysis and return to the main screen of the ProSize program.
Cancel	Cancels any manual modification to the **Capillary Positions** table locations for the capillary array. It closes the **View Capillary Positions** window and return to the main screen of the ProSize program.

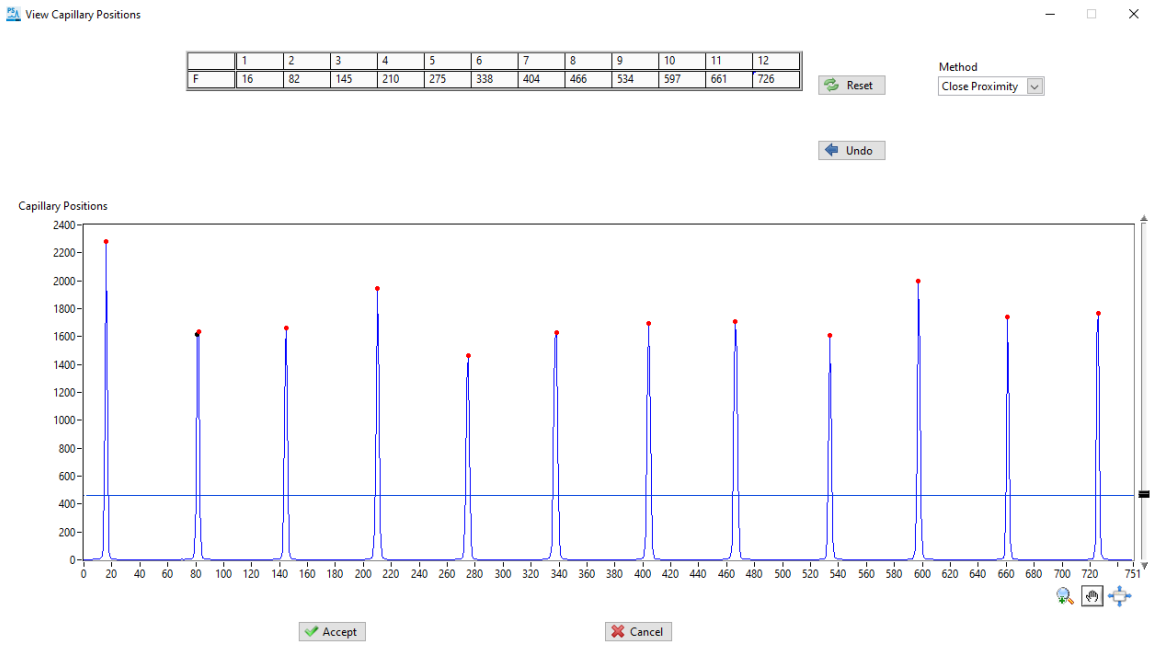


Figure 77 Capillary Positions window after pressing **Reset**. A blue horizontal cursor can be used to select the threshold for locating capillary positions.

10 ProSize Troubleshooting Guide

This chapter gives an overview of troubleshooting the ProSize software.

ProSize Software Error Messages

The following table lists some potential error messages or issues that may be encountered when using the ProSize software and how to correct them.

- When multiple causes and corrective actions are listed, the items are presented from most likely to the least likely.
- If all corrective actions are employed and a problem still persists, contact your corresponding Agilent Service Representative for additional help.

For hardware related issues, please refer to the individual Reagent Kit User Manual or the instrument System Manual.

Table 39 ProSize software error messages

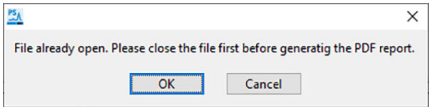
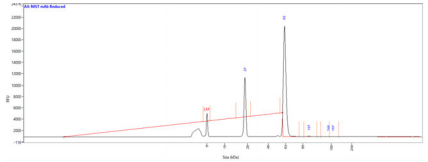
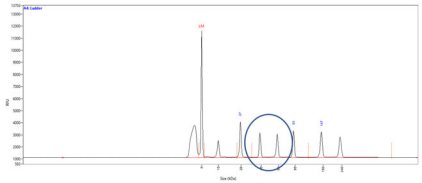
Problem	Cause	Corrective Action
When processing data in main screen, a red flashing message appears on the bottom toolbar: **Warning: Mis-match between detected peaks and ladder assignment! No sizing calibration curve is established.**	1 The number of peaks integrated in the sample well assigned for size calibration does not match the number of fragments in calibration ladder. 2 Wrong sample well selected for size calibration ladder.	1 Verify the correct number of peaks are integrated, adjust as necessary. Markers should be included in the calibration ladder table. 2 Verify correct sample well location of size calibration ladder; reassign in Size Calibration menu. For more information, refer to **Chapter 5**, "ProSize Size Calibration Screen".
When generating a PDF report, the following message is displayed: 	1 Another PDF report is open with the same name.	1 Close any additional open files and repeat the operation.
When attempting to integrate a smear, the baseline does not properly follow the trace. See example below: 	1 The **Peak Width (sec)** setting is too low.	1 Increase the **Peak Width (sec)** setting until baseline draws properly. 2 Use the **Manual Baseline Setpoints** function to set the baseline from outside of the lower/upper marker peak region. See **Table 16** for further information.

Table 39 ProSize software error messages

Problem	Cause	Corrective Action
Peaks are observed in the Electropherogram Trace view but no corresponding band is seen in the Digital Gel View. Example: 	1 The **Peak Width (sec)** setting is too high. 2 The function **Hide/Show Non-Integrated Peaks** on the Digital Gel Image toolbar is set to hide non-integrated peaks.	1 Decrease the **Peak Width (sec)** setting until peaks are integrated properly. 2 Toggle the icon to **Show Non-Integrated Peaks**.
The lower and/or upper markers do not properly line up between lanes in the Digital Gel Image display.	1 The **Min. Peak Height (RFU)** threshold is set too high, preventing the marker peaks from being selected. 2 Extra peaks (for example, primer/dimer) that are migrating close to the marker are being assigned as the lower marker.	1 Adjust/decrease the **Min. Peak Height (RFU)** threshold in the **Marker Analysis** tab of the **Set Individual Parameters** menu; apply to individual sample or all samples as needed. 2 Adjust/increase the **Min. Peak Height (RFU)** threshold in the **Marker Analysis tab of the Set Individual Parameters** menu; apply to individual sample or all samples as needed.

11 Batch Processing Using ProSize

This chapter explains how to perform a batch process and provides an outline of the various exporting options.

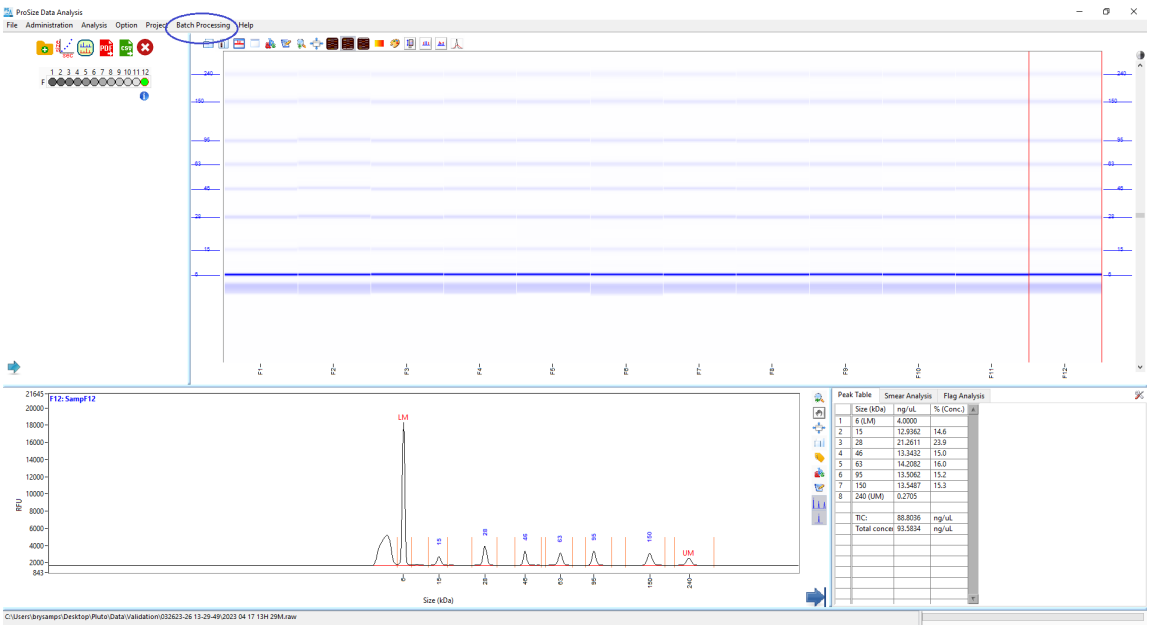
Batch Processing

The ProSize software can batch process a list of runs within a folder, and send the parameters/reports to an output folder. If an output folder is not designated, the data is exported and placed back into the original data folders.

To successfully apply the batch processing routine, the name of the Run Method in the instrument software must exactly match the configuration method in ProSize.

Perform a Batch

1 From the menu, select **Batch Processing** > **Batch Data Process**.



The **Batch Data Processing** window opens (**Figure 78**). **Table 40** summarizes the available options in this window.

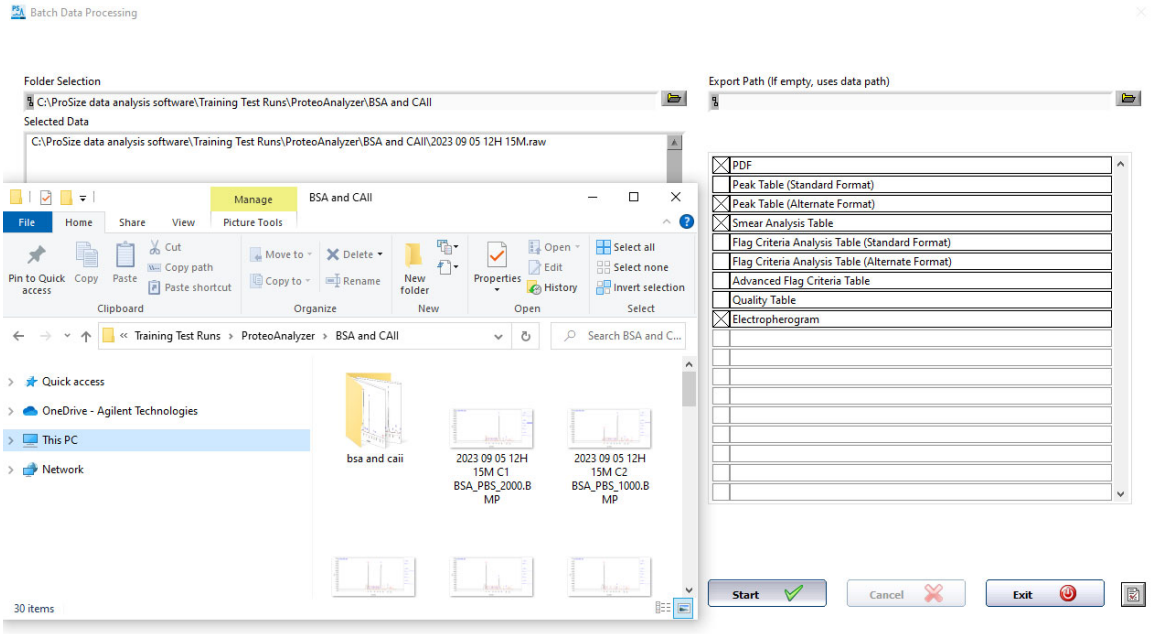


Figure 78 Batch Data Processing window. With open file selection browser to define folder.

Table 40 Batch Data Processing window options

Item	Description
Folder Selection	Allows you to select the folder location with data files to be batch processed. After locating the folder, select **Current Folder** to set the folder location.
Export Path (If empty, uses data path)	Allows you to select a folder location for exported data. After locating the folder, select **Current Folder** to set the folder location. Note: If this field is left empty, the program will export the data to the data folders in **Folder Selection** above.
Export File Options	Allows you to define an export option available in data export (PDF, peak table, flag analysis, etc.).
Start	Starts the batch processing routine.

Table 40 Batch Data Processing window options

Item	Description
Exit	Terminates the batch processing routine and closes the window.
View Error Log 	Opens an **Error Log** dialog which allows you to quickly determine errors associated with batch processing (Figure 79).

To initiate batch processing:

1 In the Batch Data Processing window, under **Folder Selection**, select  and navigate to your folder with the input files.

2 Click **Current Folder** to accept the folder location.

3 Under **Export Path**, select  and navigate to a folder location for the exported files.

4 Click **Current Folder** to accept the folder location.

NOTE

If no folder is selected, the program will export the data back into the original data folders.

5 Select **Start** to initiate the process.

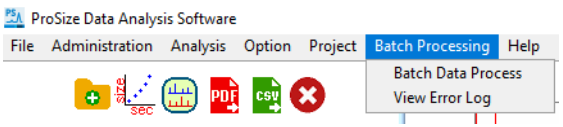
Once the batch processing is finished, the **Error Log** window shows the final processing status for each file.

To display the batch processing status of the files:

1 Select **View Error Log** within the **Batch Data Processing** window.

Or

From the menu, select **Batch Processing** > **View Error Log**.



The **Error Log** window opens (**Figure 79**). **Table 41** describes the output errors associated with this window.

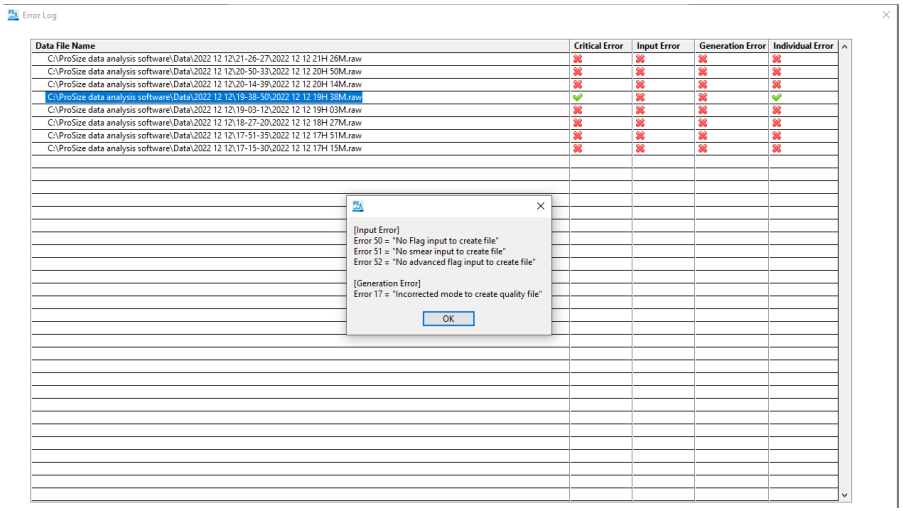


Figure 79 Error Log window

2 To view the specific error for a file, click the respective file.

A dialog opens and displays information about the error.

3 To open the file in ProSize, close the dialog and double-click on the file.

Detailed information for the error is provided.

A folder with the .txt files of all errors is generated and located under C:ProSize data analysis software/Error Log (**Figure 80**).

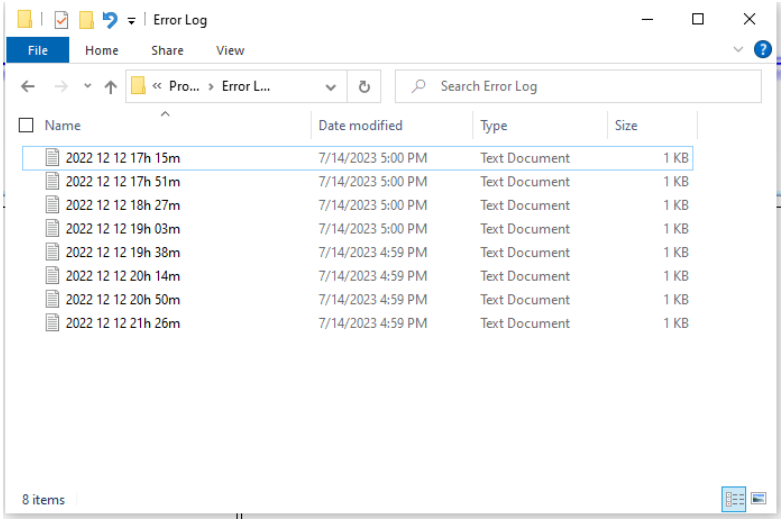


Figure 80 Generated error text files in C:ProSize data analysis software/Error Log

Table 41 Error messages generated by the batch process routine

Error class	Description
Critical Error	**1** ProSize cannot find the configuration file (there is no file with the exact same name as the method file). **2** A configuration file exists, but ProSize was not able to apply the sizing calibration.
Input Error	**1** The user has specified a table (such as **Flag Analysis** or **Smear Analysis**), but there are no parameters set up in ProSize to generate these reports. The user must go to the **Set Individual Parameters** screen and set up the appropriate features (Flag, Smear, etc.).
Generation Error	**1** The user has specified a table (such as **Flag Analysis** or **Smear Analysis**), but there are no parameters set up in ProSize to generate these reports. The user must go to the **Set Individual Parameters** screen and set up the appropriate features (Flag, Smear, etc.).
Individual Error	**1** The markers are too broad (FWHM is to large). **2** There are no peaks.

In This Book

This manual contains information about the ProSize data analysis software.

The manual describes the following:

- System overview,
- Requirements and installation instructions,
- Software main screen and menus,
- Data configuration,
- Comparing samples,
- Data export,
- Report generation,
- Troubleshooting,
- Batch processing.

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