ProteinArray Analysis User Manual

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Preface Summary

Contents of This Manual

This User Manual includes the following contents:

Chapter	Description
Chapter 1	Provides a description of the ProteinArray Analysis module and an overview of how it handles the data.
Chapter 2	A tutorial that leads you through a simple analysis, using a 5-point standard curve, to familiarize you with ProteinArray Analysis and the Analysis wizard.
Chapter 3	Provides instructions for running an analysis using three to eight standards and any of the available options.
Chapter 4	Provides instructions for creating and using analysis protocols and file sets.
Chapter 5	Describes the analysis results, how to view or customize the plots and how to view or save the spreadsheets and plots.
Appendix A	Provides the algorithms used for outlier detection and curve fits.
Appendix B	Describes a .CAL file and provides instructions for creating a .CAL file.
Appendix C	Provides instructions for installing the ProteinArray Analysis module.
Glossary	A list of terms.
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Conventions Used in This Manual

The ProteinArray Analysis module has the same look and feel as the ScanArray Express software, which runs under the Windows® 2000 Professional or XP Professional Operating System. We assume that the operator is acquainted with the general use of the operating system, and with using ScanArray Express.

The names of the buttons and menu selections and their locations are bold. The name of windows and dialog boxes are in italics. For example:

- In the ScanArray Express *Main Window*, select **Save** from the **File** menu.

Where To Get Help

If you need help using ProteinArray Analysis, you can contact PerkinElmer in the following ways:

- Technical Support by telephone - 800-762-4000 or (+1) 203-925-4602
- Technical Support by e-mail - info@perkinelmer.com
- Fax: (+1) 203-944-4904

Please be prepared with the following when calling:

- nature of the problem
- steps you have taken
- your phone number, fax number and e-mail address

Additional information about creating the quantitation results files used by the ProteinArray Analysis software for analysis can be found in the following:

- documentation for the scanning and quantitation systems you are using (i.e., ScanArray Express).
- documentation provided with the workstation and its operating software.

Introduction

Chapter 1

Chapter Summary

1.1 Overview

The ProteinArray Analysis software is an optional module to the ScanArray Express V2.2 software that calculates, using standard curves, protein concentrations for experimental protein microarray samples. ProteinArray Analysis uses quantitation data (in .CSV or .GPR format) generated from protein microarray experiments that have been scanned and quantitated using appropriate software.

1.2 Input Data for the Analysis

ProteinArray Analysis performs analysis on the raw data (such as inter- and intra-array replicate averaging, outlier detection, background subtraction, standard curve generation and calculation of protein concentrations from microarray intensity data), then displays the results in both graphical and spreadsheet formats for convenient viewing and further analysis. The raw data must be from microarrays that were scanned with only one fluorophore.

Input data for ProteinArray Analysis includes:

- **Standards data** - the quantitation results (.CSV or .GPR files) from samples with known concentrations. Three to eight standard samples can be used.
- **Experimental data** - the quantitation results (.CSV or .GPR files) from samples with unknown concentrations.
- **Concentrations and thresholds information** - a calibration (.CAL) file created for the analysis with specific information about the analytes.

The diagram in Figure 1-1 provides an overview of how ProteinArray Analysis accepts input data.

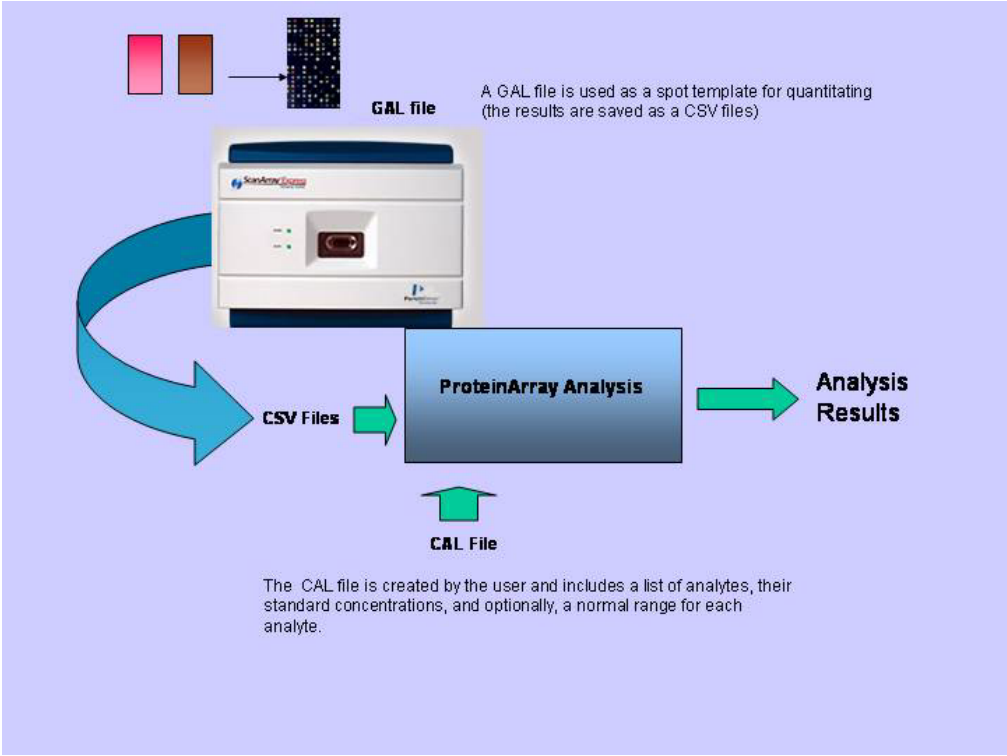


Figure 1–1 Overview of ProteinArray Analysis

ProteinArray Analysis creates a standard curve plot for each analyte, a bar chart of all concentrations of all analytes in all samples, and a ratio-to-reference plot when a reference sample is used. The raw data also displays in a spreadsheet.

1.3 How ProteinArray Analysis Handles Data

1.3.1 Spot Replicates and Sample Replicates

Spot replicates are spots within a microarray with the same analyte ID and name that have been incubated with the same sample. *Sample Replicates* are replicates generated using multiple arrays incubated with the same sample, and assigned the same Sample ID and Sample Name by the user in ProteinArray Analysis.

During analysis, the ProteinArray Analysis software first finds the replicate spots, then the replicate samples, then optionally detects and removes outliers. The remaining spot data is reduced to a single data point, using the mean. The standard deviation between the replicate spot data is displayed as an error bar associated with this data point in the standard curve and barchart plots and is reported in the spreadsheets.

1.3.2 Outlier Detection

ProteinArray Analysis will optionally detect and remove outliers -- those data points that lie outside specified limits. Three methods are available for identifying and removing outliers from the replicate spots:

- **Median Absolute Deviation (MAD)**. The software first calculates a MAD-based score for each replicate spot. A spot is considered an outlier if the MAD-based score is not within user-defined limits. If a blank sample is used, the ProteinArray Analysis uses the blank-subtracted values for this method. See Appendix A for a description of the MAD algorithm.

- **Spot status**. Any spots with a status of **bad**, **not found**, or **absent** are considered outliers.

- **Spot quality measures**. Based on footprint, Signal-to-noise ratio, or Signal-to-background, as specified by the user while setting up the analysis. This method is based on the data columns in the quantitation files, which are not blank-subtracted values. See Appendix A for a description.

One, two, or all three outlier detection methods can be selected.

1.3.3 Standard Curves

A standard curve is generated for each analyte in the experiment using known concentration data points from the standard sample files. The standard curves are used for calculating the concentration of the analytes in the experimental samples. Four methods are provided for generating the standard curve plot: Linear Regression, Cubic spline, B-spline Curve, or Logistic Curve. These are described in Appendix A.

1.3.4 Normal Range

A normal range for an analyte can optionally be specified in the .CAL file by entering concentrations limits in the upper and lower threshold fields for that analyte.

1.3.5 Blank Subtraction

A blank sample is an array prepared using sample with zero concentration of each analyte to provide a control for non-specific analyte signal. Normally, the contents of this array are subtracted from that of all other samples to get the actual concentration values of each analyte in the sample. Up to four blank replicates can be specified. Using a blank sample is optional.

1.3.6 Reference Sample

The optional reference sample is one sample identified by the user, with up to four replicates, that is used to determine the relative concentration of protein in subsequent samples. Using a reference sample is optional.

1.3.7 Experiment Samples

These are the quantitation files (.CSV or .GPR) for the samples with unknown concentrations of the analytes. Each sample can have up to four replicates.

1.4 Analysis Protocols and File Sets

The ProteinArray Analysis protocols and file sets enable a user to save the analysis parameters and files to use again.

A *protocol* includes the specified .CAL file and all parameters for an analysis, saved with a unique protocol name. A *file set* includes the standard files, reference files, blank files and experimental sample files that were added for analysis, saved with a unique file set name. A file set can be linked to a protocol so that opening the protocol automatically adds the file set for analysis.

Tutorial

Chapter 2

Chapter Summary

2.1 Overview

To get you quickly up to speed and familiarize you with ProteinArray Analysis, this tutorial leads you through a simple analysis of a protein microarray using example data that's included on the ProteinArray Analysis installation CD-ROM.

The examples in this chapter use five standards and selected options to demonstrate most of the ProteinArray Analysis options. All of the options are fully described in Chapter 3, "Analyzing Your Data," for when you are ready to run an analysis with your own data and option selections.

The example data files are installed in a "Samples" sub-directory under the ScanArray Express program directory, and include a prepared .CAL file, five standard files, a blank sample file, a reference file, and several experimental samples files.

Note: The instructions and illustrations in this chapter use the example data on the ProteinArray Analysis CD-ROM.

2.2 Starting ProteinArray Analysis

ProteinArray Analysis is launched from the ScanArray Express *Main Window*, so start the ScanArray Express if it is not already running.

To start the ScanArray Express and ProteinArray Analysis

1. Click the ScanArray Express icon on the desktop on the computer screen.

Note: If the icon is not displayed, open the ScanArray Express by clicking **Start** on the Windows taskbar, then select **Programs**, **PerkinElmer**, **ScanArray Express**.

2. The ScanArray Express opens to the *Main Window,* shown in Figure 2-1. Click **Analyze**.

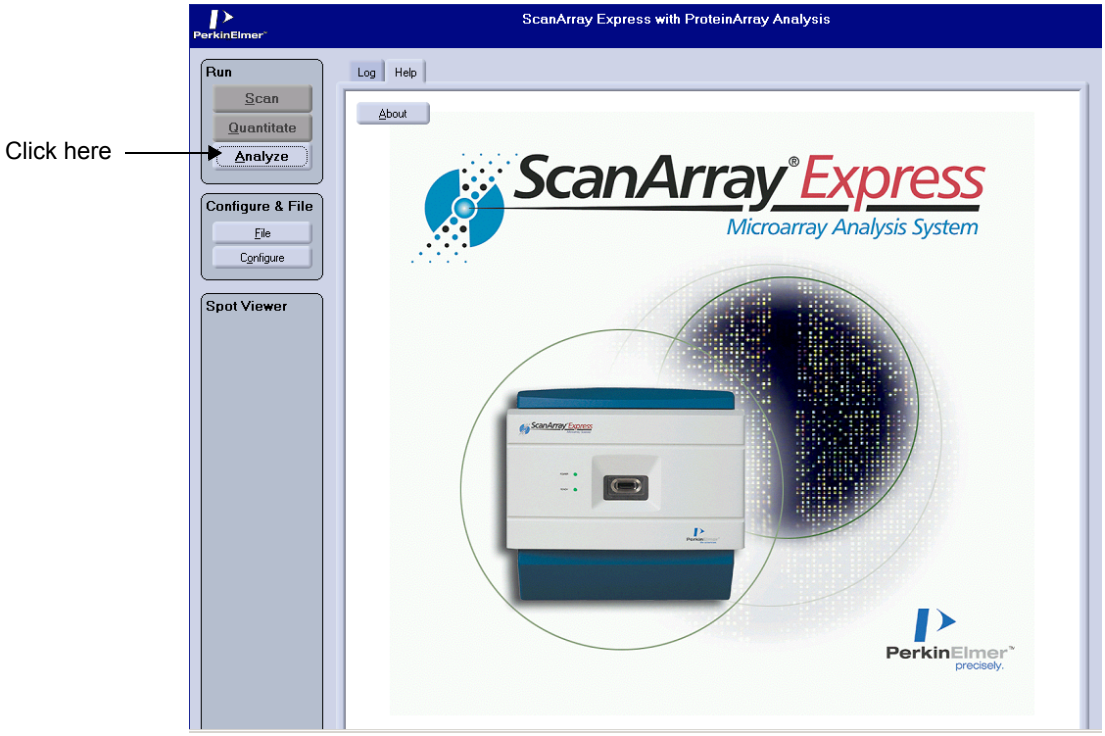


Figure 2–1 The ScanArray Express Main Window

The Analysis wizard opens, and you can begin entering data for the analysis.

Note: While running an analysis, the Scan and Quantitate buttons are greyed, and spots do not display in the Spot Viewer. If the ScanArray Express is set to Quantitation and Analysis mode only, the Scan button does not display.

2.3 Using the Analysis Wizard

The Analysis wizard, shown in Figure 2-2, uses ten numbered steps to guide you through the analysis as you input data and select options in the window for each step.

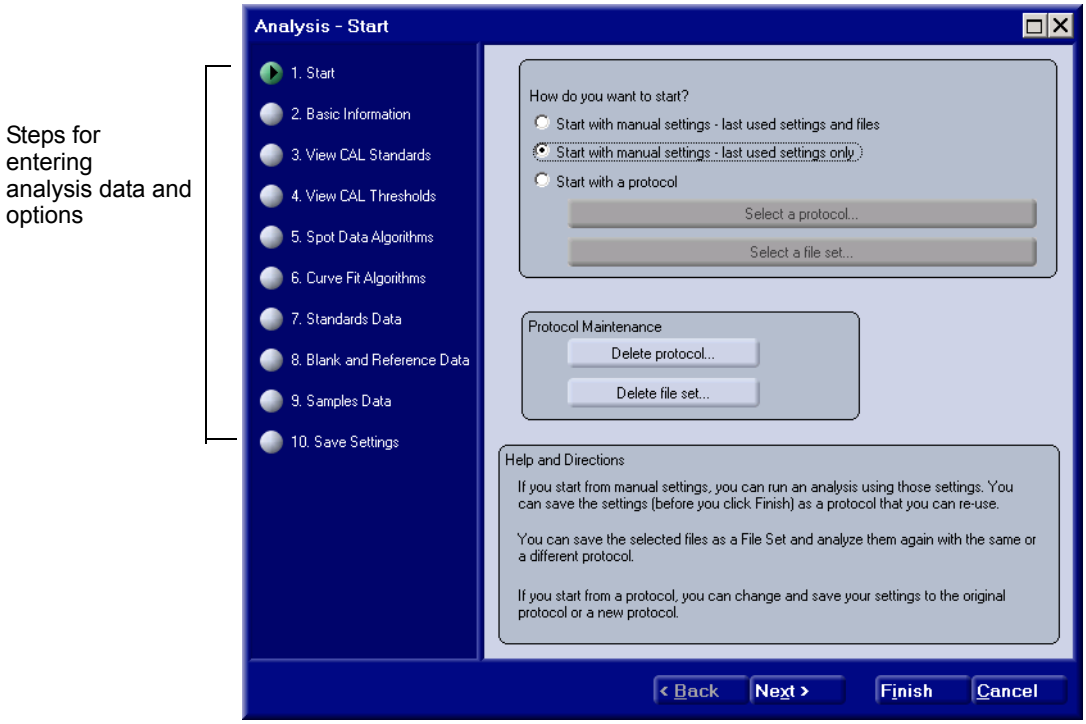
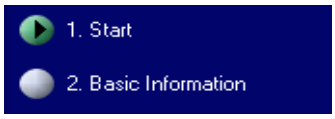


Figure 2–2 The Analysis Wizard - Start Window

To follow the wizard:

- Click the icon beside a step number to open the window where you enter information for that step.



  The icon turns green after the step is selected; an arrow in the icon indicates the currently selected step.

Or, alternatively,

- Click **Next** after completing each window to move to the next window.

You can also click **Back** to return to the previous window, or click **Cancel** at any time to Cancel the analysis.

2.4 Start: Entering Your Data

To start entering the data

1. On the *Analysis - Start* window (Figure 2-2), select the second option button, **Start with manual settings - last used settings only**. The last used settings (or the defaults if this is the first time ProteinArray Analysis is used) will display in the windows. The selection **Start with a protocol** is greyed out until a protocol is created, as described in Chapter 4.

2. Click **Next**.

Use the instructions in the following sections to enter settings and sample data into each of the wizard windows.

2.4.1 Basic Information - Specifying a CAL File

The .CAL file provides the concentration of the analytes in each standard sample array, and optional thresholds information, for each analyte to be included in the analysis. When analyzing your own data, you can also choose to automatically save the results and raw data spreadsheets to a specified filename (see Chapter 3).

To specify a CAL file

1. In the *Analysis - Basic Information* window, click **Browse.** See Figure 2-3.

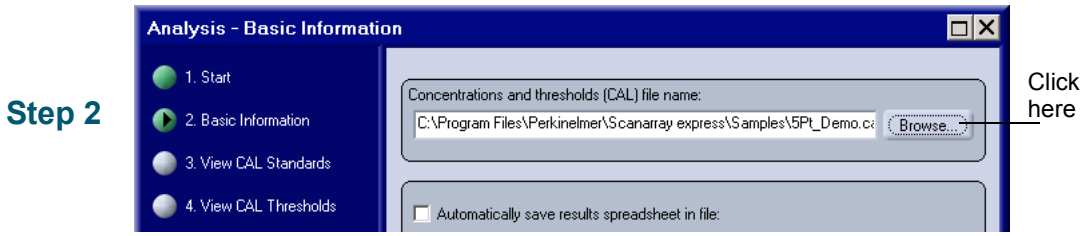


Figure 2–3 The Analysis Wizard, Basic Information Window

2. The *File to Read Concentrations From* window opens, displaying a list of available CAL files (Figure 2-4). Go to the Samples directory and select the example .CAL file for this exercise, **`5Pt_Demo`**.

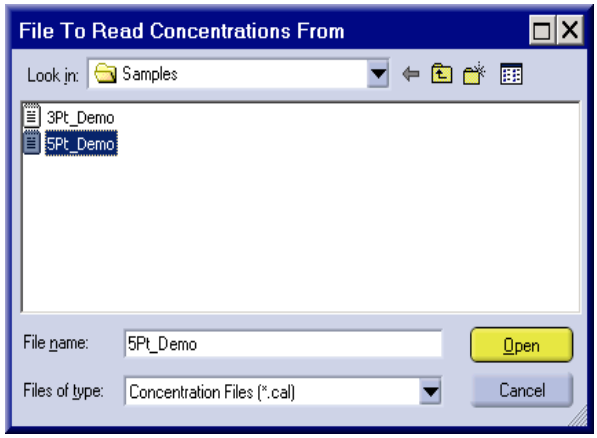


Figure 2–4 File to Read Concentrations From Window

3. Click **Open**.

The window closes, and the filename appears in the **Concentrations and Thresholds Filename** field in the *Basic Information* window.

2.4.2 View CAL Standards

In the *Analysis - View CAL Standards* window, you can view and verify the standard concentrations in the selected .CAL file. You cannot make any changes to the contents in this window.

You can see the spot information, units of measurement and concentration values of the loaded .CAL file, as shown in Figure 2-5.

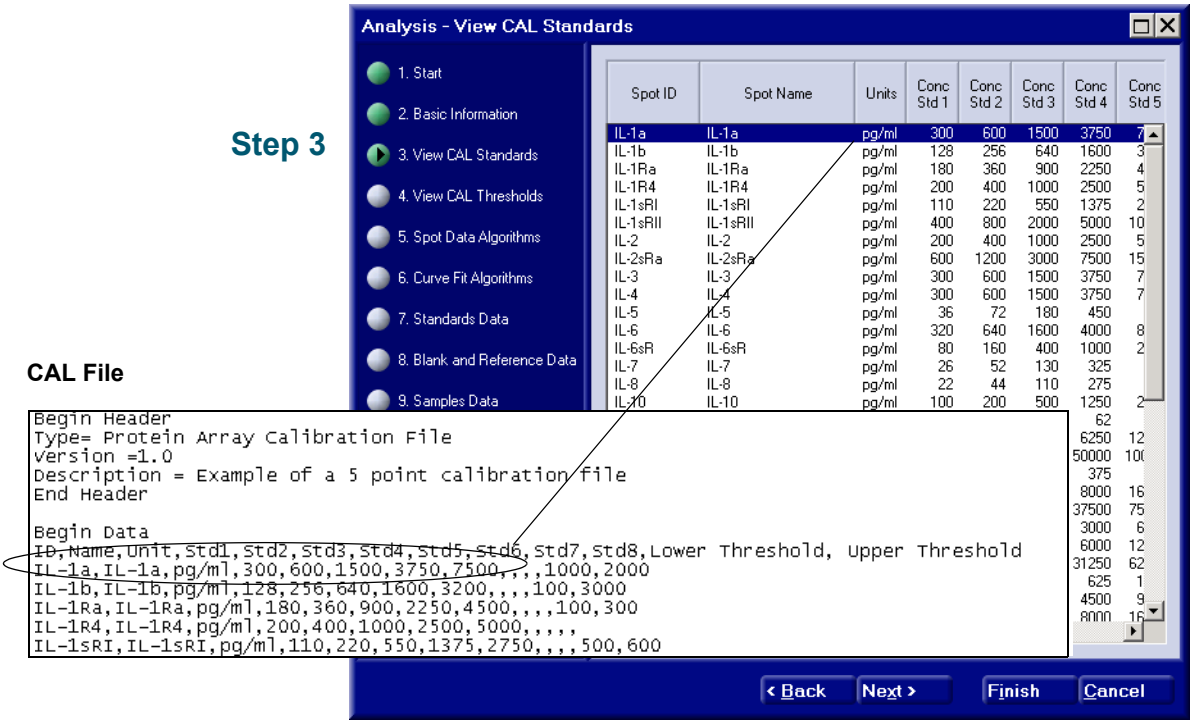


Figure 2–5 Analysis - View CAL Standards Window

2.4.3 View CAL Thresholds

In the *Analysis - View CAL Thresholds* window, you can view and verify the threshold information, as shown in Figure 2-6, if upper and lower thresholds values have been entered into the .CAL file. You cannot make any changes in this window.

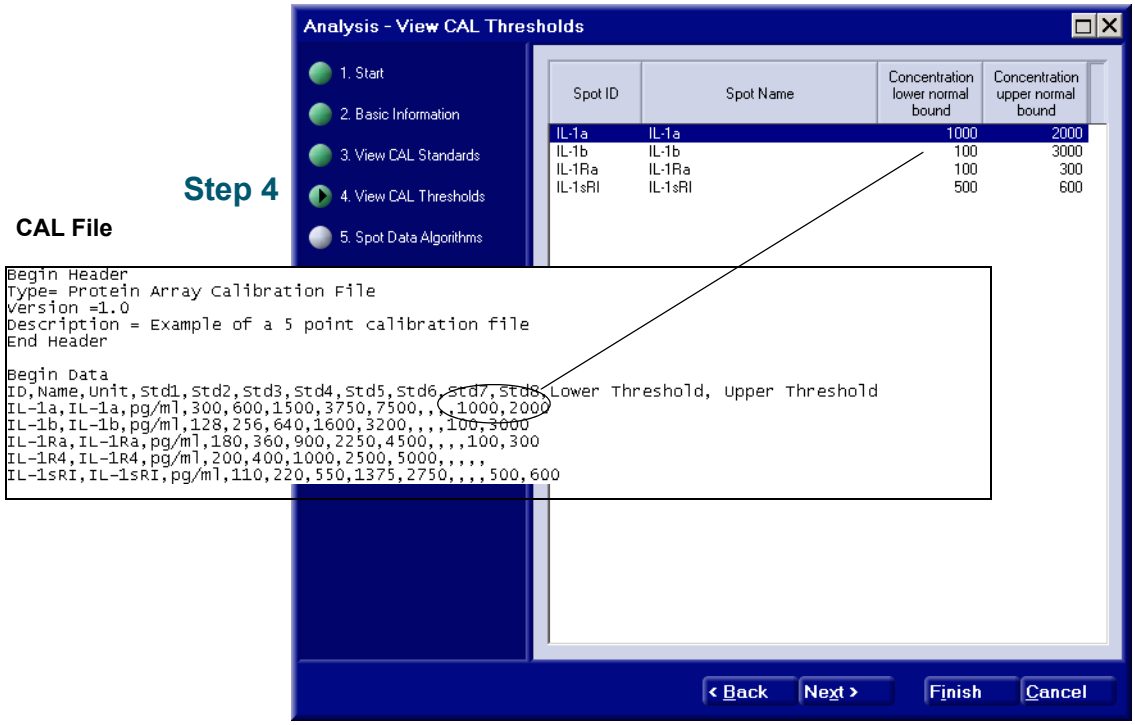


Figure 2–6 Analysis - View CAL Thresholds window

2.4.4 Spot Data Algorithms and Outlier Detection

In this window you can select which data column from the quantitation file to use for the analysis; for example, you can specify to use the mean intensity or median intensity data. See Figure 2-7.

You can also filter the data by removing outliers -- the spots that lie outside the boundaries specified by the method(s) that you select in this window.

Note: For more information about spot signal data and outlier detection, see Chapter 3.

For this tutorial, select Mean Intensity for spot signals and the MAD algorithm for detecting and removing outliers.

To select the data and remove outliers

1. In the *Analysis - Spot Data Algorithms* window, select **Mean Intensity** under **Spot signal data to use for analysis**.

Step 5

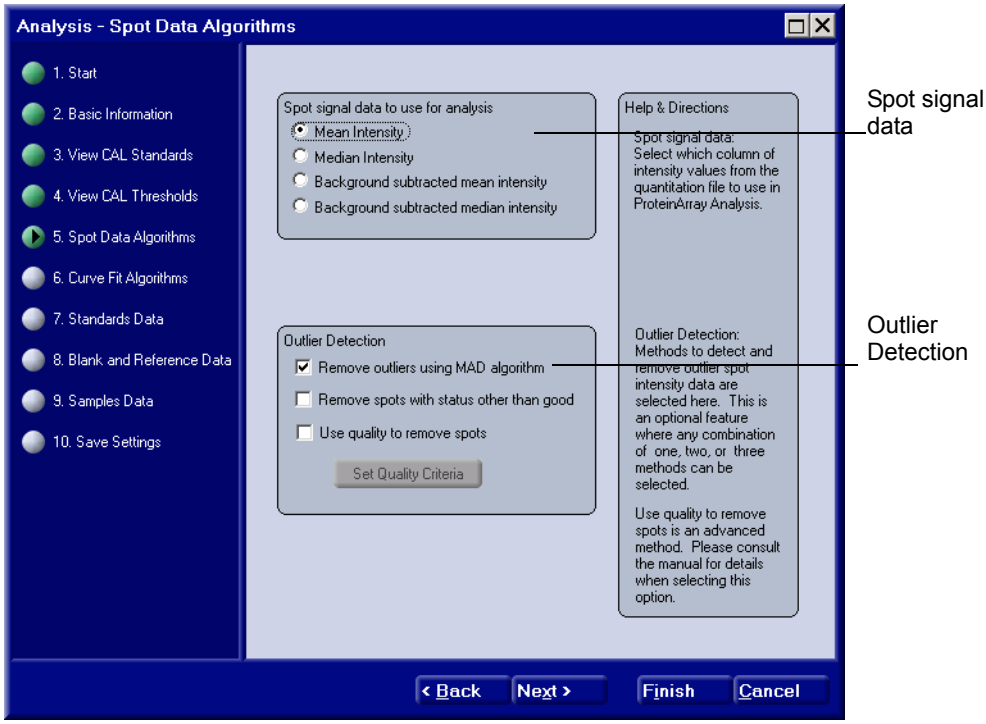


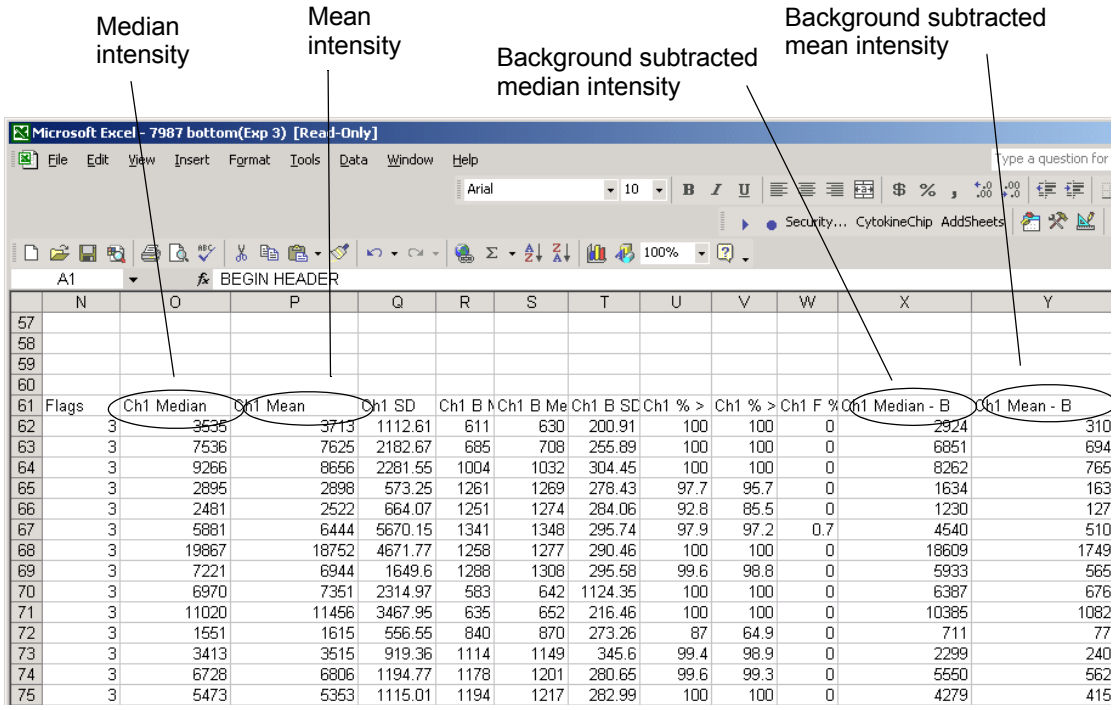
Figure 2–7 Analysis - Spot Data Algorithms Window

2. Under **Outlier Detection**, check **Remove outliers using MAD algorithm**. The ProteinArray Analysis software removes all spots that lie outside the minimum and maximum values specified within the software.

   The minimum and maximum values can be changed in the ScanArray Express application settings, but use the default values for this example.

2.4.4.1 More about Spot Signal Data

Figure 2-8 shows an example of the data columns in a .CSV file from which you can choose.



	N	O	P	Q	R	S	T	U	V	W	X	Y
57												
58												
59												
60												
61	Flags	Ch1 Median	Ch1 Mean	Ch1 SD	Ch1 B N	Ch1 B Me	Ch1 B SD	Ch1 % >	Ch1 % >	Ch1 F %	Ch1 Median - B	Ch1 Mean - B
62	3	3535	3713	1112.61	611	630	200.91	100	100	0	2924	310
63	3	7536	7625	2182.67	685	708	255.89	100	100	0	6851	694
64	3	9266	8656	2281.55	1004	1032	304.45	100	100	0	8262	765
65	3	2895	2898	573.25	1261	1269	278.43	97.7	95.7	0	1634	163
66	3	2481	2522	664.07	1251	1274	284.06	92.8	85.5	0	1230	127
67	3	5881	6444	5670.15	1341	1348	295.74	97.9	97.2	0.7	4540	510
68	3	19867	18752	4671.77	1258	1277	290.46	100	100	0	18609	1749
69	3	7221	6944	1649.6	1288	1308	295.58	99.6	98.8	0	5933	565
70	3	6970	7351	2314.97	583	642	1124.35	100	100	0	6387	676
71	3	11020	11456	3467.95	635	652	216.46	100	100	0	10385	1082
72	3	1551	1615	556.55	840	870	273.26	87	64.9	0	711	77
73	3	3413	3515	919.36	1114	1149	345.6	99.4	98.9	0	2299	240
74	3	6728	6806	1194.77	1178	1201	280.65	99.6	99.3	0	5550	562
75	3	5473	5353	1115.01	1194	1217	282.99	100	100	0	4279	415

Figure 2–8 The CSV (Quantitation) File

2.4.5 Selecting the Curve Fit Method

For fitting the standard curve data, you can select from among four algorithms: Linear regression, Cubic spline interpolation, B-spline curve, and Logistic model. You can select the same curve fit type for all analytes, or select a different type for each analyte.

To show you how to select different curve fit types, and illustrate the resulting standard curve plots, this tutorial uses a different method for each of the first four analytes. Leave the curve fit type for all other analytes as linear regression.

To select a curve fit type

1. In the *Analysis - Curve Fit Algorithms* window, select the first analyte, **`IL-1a`**, by clicking it with the mouse (Figure 2-9).

Step 6

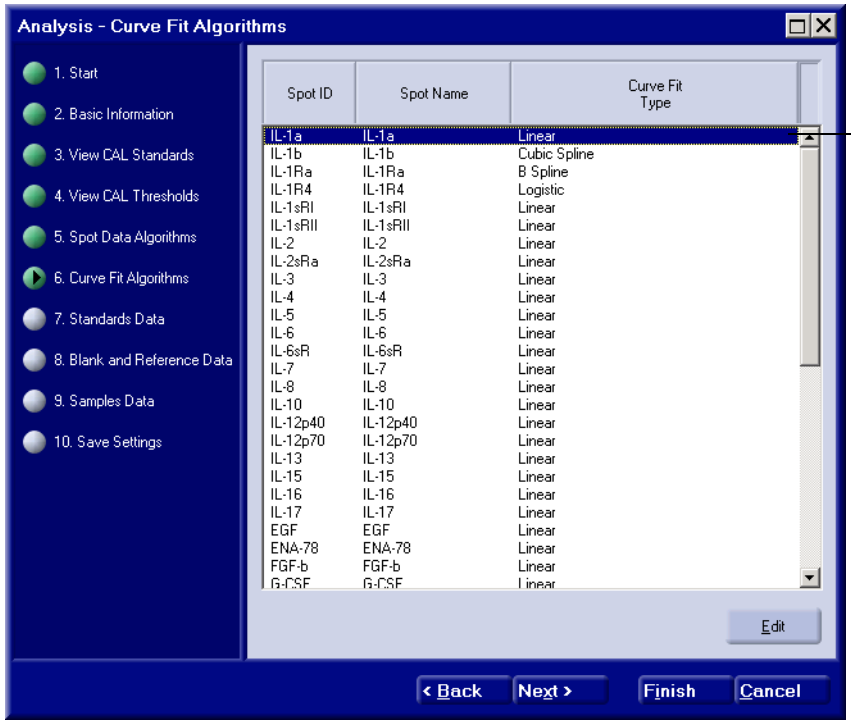


Figure 2–9 Analysis - Curve Fit Algorithms Window

Note: If a previous user changed the curve fit types for any of the analytes, those curve fit types will still be selected for any analytes common to the previous and current analysis.
In that case, to put you at the same starting point as this tutorial, set all curve fit types to linear regression, as follows:
a. Select any analyte, and click **Edit**.
b. In the *Curve Fit Type and Parameters* window (Figure 2-10) select Linear Regression.
c. Check **Apply these settings to all analytes**.
d. Click **OK**.

Continue with Step 2.

2. Click **Edit**. The *Curve Fit Type and Parameters* window opens.

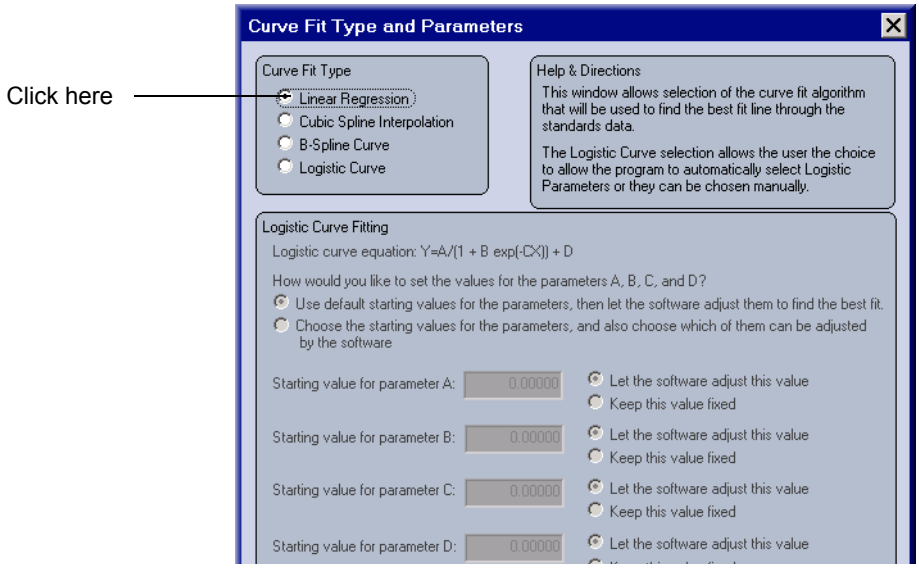


Figure 2–10 Curve Fit Type and Parameters Window

3. Under **Curve Fit Type**, select **Linear Regression** and click **OK**. The window closes, and the curve fit type Linear appears next to the IL-1a in the *Analysis* window (see Figure 2-11).

4. Select the second analyte, **`IL-1b`**, by clicking it with the mouse. Click **Edit**.

5. Under **Curve Fit Type**, select **Cubic Spline Interpolation** and click **OK**. The window closes and the curve fit type Cubic Spline appears next to IL-1b in the *Analysis* window (see Figure 2-11).

6. Select the third analyte, **`IL-1Ra`**, by clicking it with the mouse. Click **Edit**.

7. Under **Curve Fit Type**, select **B-spline** and click **OK**. The window closes and the curve fit type B-spline appears next to IL-1Ra in the *Analysis* window (see Figure 2-11).

8. Select the fourth analyte, **`IL-1R4`**, by clicking it with the mouse. Click **Edit**.

9. Under **Curve Fit Type**, select **Logistic,** check **Automatically generate**, and click **OK**. The window closes and the curve fit type Logistic appears next to IL-1R4 in the *Analysis* window (see Figure 2-11).

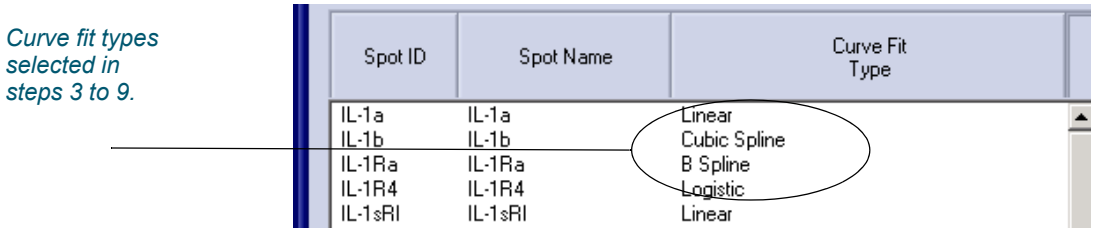


Figure 2–11 Selected Curve Fit Types

2.4.6 Adding Standards Data

Specify the standards data files to be used for this experiment. These are the .CSV or .GPR quantitation files from samples with known concentrations. The standards must be in ascending order in this window as described below and shown in Figure 2-12. After files are added you can view the spot data for the files.

Note: The number of standard files selected here must match the number of standards specified in the .CAL file.

To select Standards Data

1. In the *Analysis - Standards Data* window, click **Add**.

Step 7

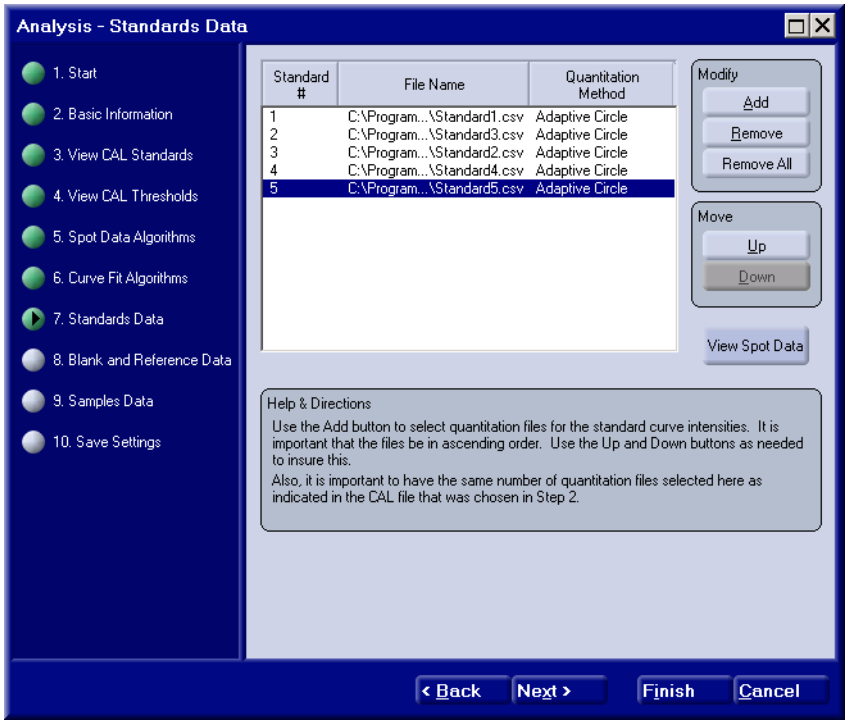


Figure 2–12 The Analysis - Standards Data Window

2. In the *Add Standards* window, select the first standard file, **`Standard 1`** as shown in Figure 2-13.

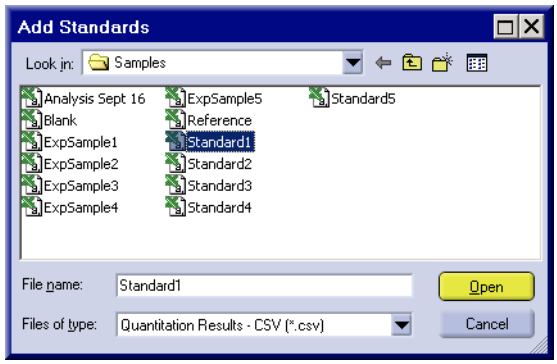


Figure 2–13 Add Standards Window

3. Click **Open**. The filename appears in the *Standards Data* window, as shown in Figure 2-12 on the previous page.

4. Add the remaining files. Click **Add**.
In the *Add Standards* window, you can select several files at once:

   To select several contiguous files at once, click the first file in the list, **`Standard1`**, - press the **Shift** key, and click the file **`Standard5`**.
The first file, the last file, and files in between are selected (see Figure 2-14).

   To select several files without selecting files in between them, press the **Ctrl** key, and click each file you want to add.

5. Click **Open**. All of the selected files are added to the *Standards Data* window where they must be arranged in ascending order.

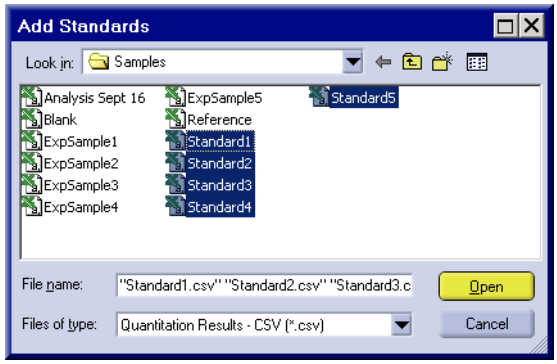


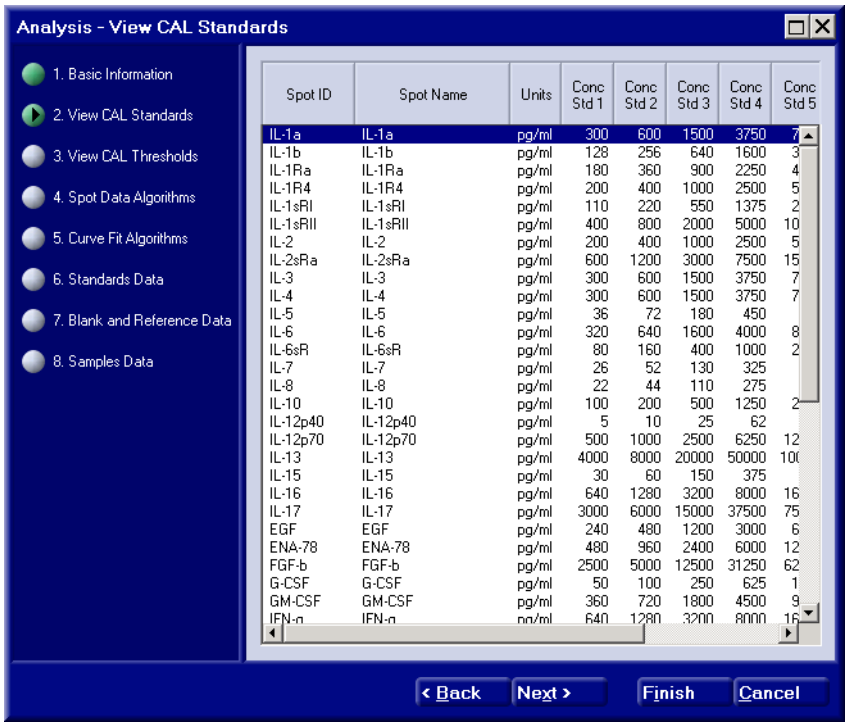
Figure 2–14 Adding Several Standards Files

6. If necessary, re-order the files. Click any file that is out of order and click **Up** or **Down** to move the file into its correct order. Click **Remove** to remove any extraneous files that may have inadvertently been added.

To view spot data

1. Select any file in the *Standards Data* window to activate the View Spot Data button.
2. Click **View Spot Data.** The *Spot Intensity Values* window opens.

   You can verify the spot intensity values for the standards files. This window displays the data for all of the standard files.



3. When you are finished, click **OK** to close the window.

2.4.7 Blank and Reference Data

You can add a blank and/or reference sample, with one to four replicates of each, and assign them a Sample ID and Name within ProteinArray Analysis (see Figure 2-15). Since ProteinArray Analysis identifies sample replicates as those samples with the same Sample ID and Sample Name, the ID and Name you assign for a blank or reference is automatically applied to all files in the Blanks or References list.

- A *blank sample* has no analyte-specific signal, thereby providing information on the background fluorescence. If you have two, three, or four blank samples (microarrays using the same buffer with zero analyte concentrations), they are assigned the same name as replicates. You can assign a different Sample ID or leave the default Sample ID ("Blanks").

- When a *reference sample* is used, ProteinArray Analysis calculates the ratio of the analyte concentrations in the experimental samples to the corresponding analyte concentrations in the reference sample and displays the ratio values in the analysis results spreadsheet and in a Ratio to Reference scatter plot. You can assign a different Sample ID or leave the default Sample ID ("References").

For the examples in this tutorial, select one blank sample and one reference sample (no replicates).

Step 8

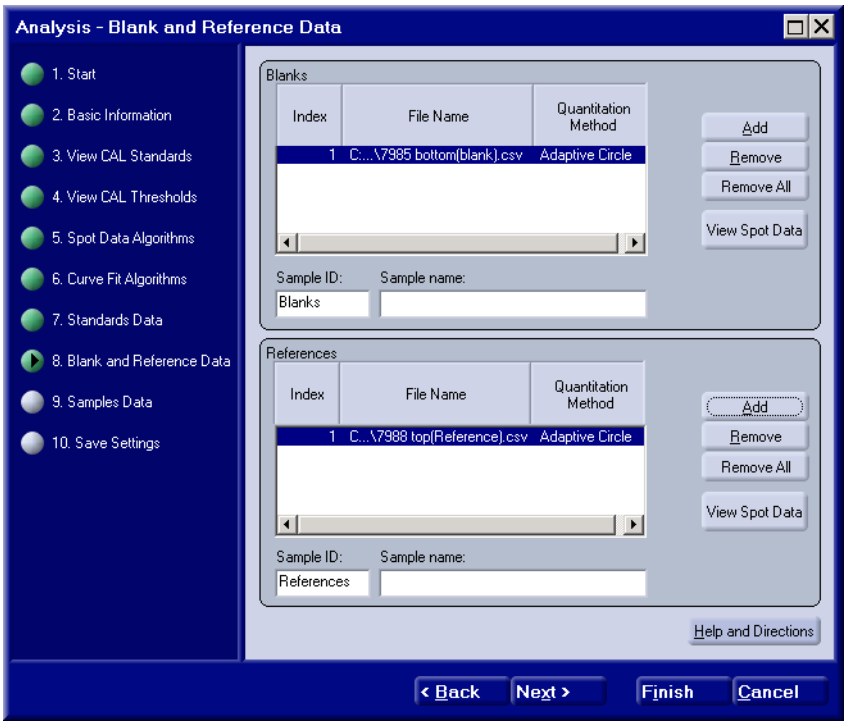


Figure 2–15 Analysis - Blank and Reference Data Window

2.4.7.1 Adding Blank Samples

To add a blank sample

1. In the *Analysis - Blank and Reference Data* window, click **Add** next to the **Blanks** box. The *Add Blanks* window opens (Figure 2-16).

2. Select the blank sample **Blank**.

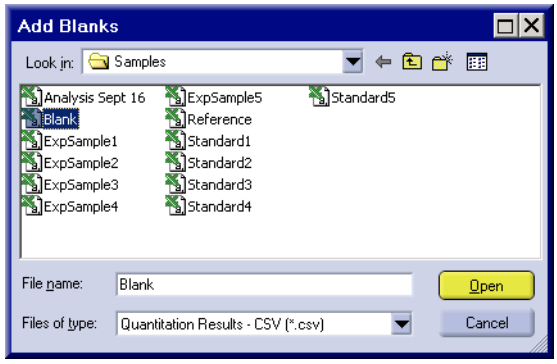


Figure 2–16 Add Blanks Window

3. Click **Open**. The window closes and the filename appears in the *Analysis - Blanks and Reference Data* window.

4. In the **Sample ID** edit field, leave the ID "`Blanks`". (If you were using replicate blank samples, the same ID and name would be applied to all of the files in the Blanks list.)

To view spot data for blank samples

1. To select the sample to view, click any filename in the Blanks list and click **View Spot Data**.
   In the S*pot Intensity Values* window, you can view spot data for the blank files (Figure 2-17).

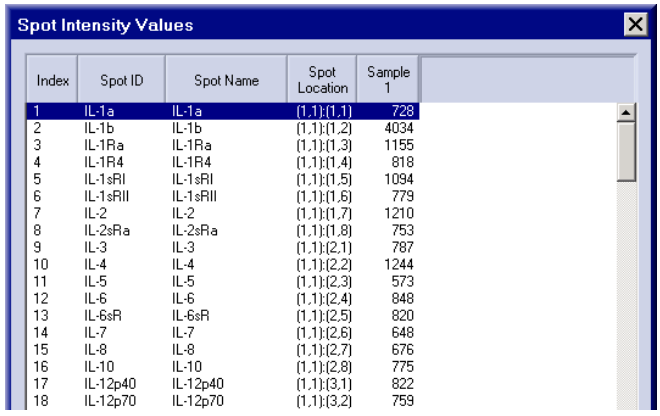


Figure 2–17 Spot Intensity Values Window

2. Click **OK** to close the window.

2.4.7.2 Adding Reference Samples

To add a reference sample

1. In the *Analysis - Blank and Reference Data* window, click **Add** next to the **References** box. The *Add References* window opens (Figure 2-18).

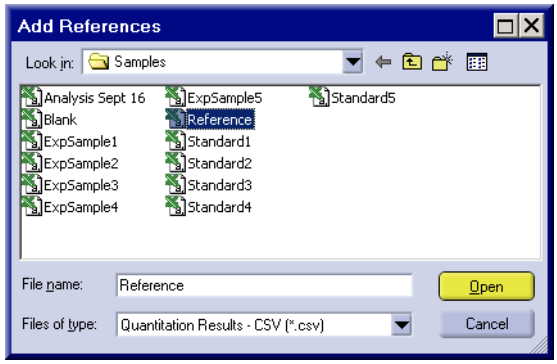


Figure 2–18 Add References Window

2. Select the reference sample **Reference** as shown in Figure 2-18.

3. Click **Open**. The window closes and the filename appears in the *Analysis - Blanks and Reference Data* window.

4. In the **Sample ID** edit field, leave the name "`References`". (If you were using 2, 3, or 4 reference samples, the sample ID and sample name you specified here would be applied to all files in the References list.)

2.4.8 Adding Samples Data

Add the experimental samples data (the .CSV quantitation files from samples with unknown concentrations). After the files are added you can view the spot data for the sample files.

Note: The order of the files is not important for the samples data.

To select Samples Data

1. In the *Analysis - Samples Data* window, click **Add**.

Step 9

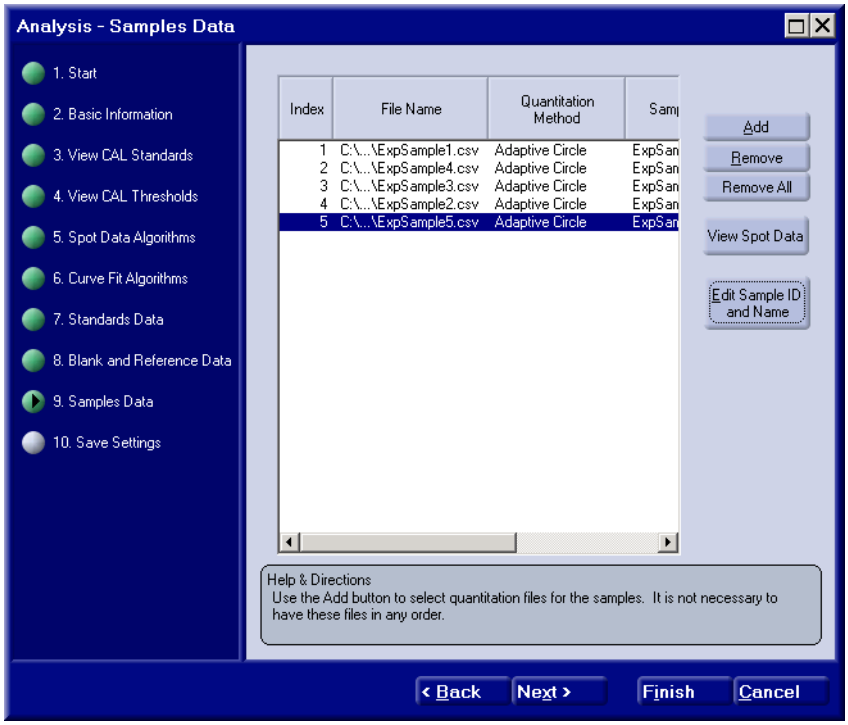


Figure 2–19 Analysis - Samples Data Window

2. In the *Add Experimental Samples* window, you can select all of the sample files together:
- Click the top file in the list, **`ExpSample1`**, press the **Shift** key, and click the last file in the list, **`ExpSample5`**). See Figure 2-20.

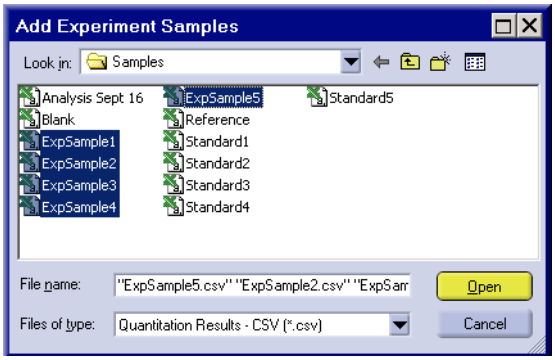


Figure 2–20 Add Experimental Samples Window

3. Click **Open**. All of the samples files are added as input data.

2.4.8.1 Assigning an ID and Name to Samples and Replicates

In the *Analysis - Samples Data* window, you can assign a meaningful Sample ID and Name to any of the sample files to more easily identify the sample information in the analysis results. The name you provide here is the name that the ProteinArray Analysis software uses for the analysis. If you do not assign a name, ProteinArray Analysis uses the filename without the filename extension.

If you are using sample replicates, each sample with the same Sample ID and Sample Name is recognized as a sample replicate during analysis. You can add up to four sample replicates for each sample.

To assign a Sample ID and Name

1. In the *Analysis - Samples Data* window, click the filename of the sample for which you want to assign a Sample ID and Name.

2. Click **Edit Sample ID and Name**. The *Edit Sample ID and Name* window opens.

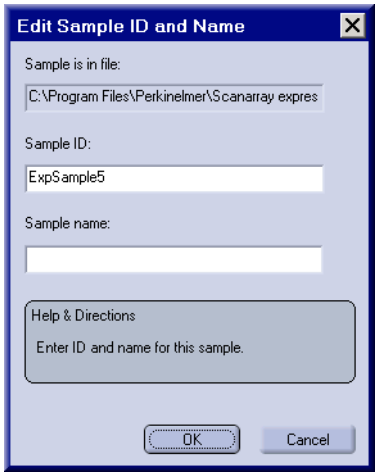


Figure 2–21 Edit Sample ID and Name window

3. Enter a new ID up to 32 characters long in the **Sample ID** field, and enter a new name up to 256 characters long in the **Sample name** field.

4. Click **OK**. Repeat steps 1 to 3 for each sample replicate.

2.5 Running the Analysis

You do not need to save the settings (step 10). After making the selections in all windows of the Analysis wizard, you can go back to any of the windows to check the data and selections. If all data is as it should be, you're ready to run the analysis.

To run the analysis

1. In the Analysis wizard, click **Finish**.

Within a few seconds, the results display in the ScanArray Express *Main Window*, in five tabbed windows, with the standard curve plot for the first analyte displayed in the first tab. The analysis results are described in the following sections.

2.6 Viewing the Analysis Results

The analysis results are presented in a spreadsheet on the *Concentrations* tab in the ScanArray Express *Main Window.* Tabs with additional information and the raw data are also included, for a total of five tabs in the *Main Window.*

Note: This section provides a brief overview of each results tab.

For a complete description of analysis results, instructions on how to navigate among the plots and spreadsheets, change display properties, and save the results, refer to *Chapter 5: Viewing Results*.

The following tabs display:

- The **Concentrations** spreadsheet - displays the analysis results from all samples for each analyte. This spreadsheet can be saved as a .CSV file or exported to Excel and saved.
- a **Concentration Bars Chart** - displays the concentrations and, if specified, the normal range, for all analytes in all samples. This is a customizable view that you can print and/or save as a JPEG file or BMP (bitmap) file.
- a **Raw Spreadsheet** - displays the raw data in a spreadsheet for samples, standards, blanks, and references. This spreadsheet can be saved as an .xls file, or exported to Excel and saved.
- a **Standard Curve Plot** - provides the interpolated values from a standard curve for each analyte, one analyte at a time. This is a customizable view that you can print and/or save as a JPEG file or BMP (bitmap) file.

and, when a reference sample is used:

- a **Ratio to Reference Plot** - shows the ratios in a scatter plot. This is a customizable view that you can print and/or save as a JPEG file or BMP (bitmap) file.

2.6.1 Concentrations (Analysis Results)

The analysis results for all analytes and all samples are presented in the *Concentrations* tab, as shown in Figure 2-22. The results include the concentration of each analyte, its normal range, number of replicates, number of outliers, and whether or not it is within standards.

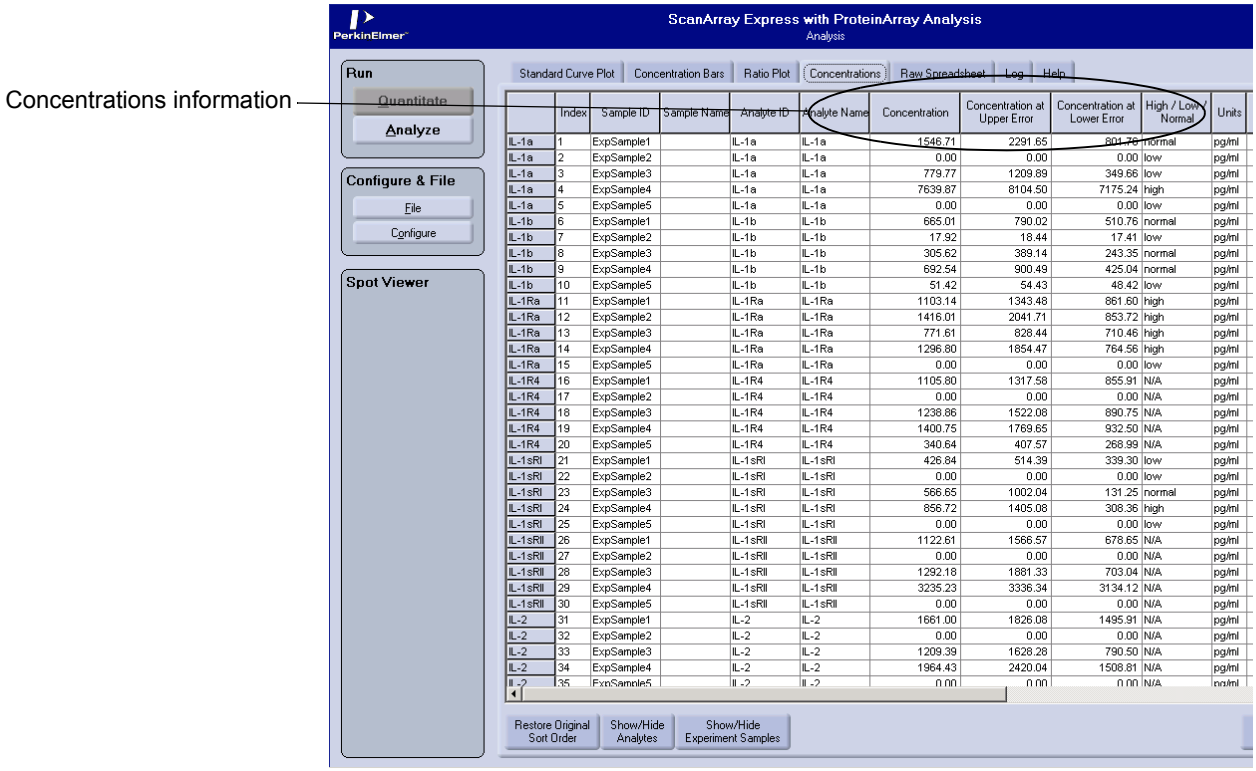


Figure 2–22 The Concentrations tab - Analysis Results

You can scroll through the information, double-click any column header to arrange the information in ascending order, based on the column you clicked; double-click again to arrange in descending order.

The following buttons allow additional actions. For more information, see Chapter 5.

Button	Description
Restore Original Sort Order	Click this button after changing the sort order to restore the original order.
Show/Hide Analytes	Click this button to open the *Show/Hide Analytes* window, where you can check each analyte to include in the display, and uncheck each analyte to exclude from the display.
Show/Hide Experiment Samples	Click this button to open the *Show/Hide Experiment Samples* window, where you can check sample to include in the display and uncheck each sample to exclude.

2.6.2 Concentration Bars

The *Concentration Bars* tab displays a bar graph of the analyte concentrations and ID for all samples in the experiment in a customizable view that lets you group by analyte or by sample. If upper and lower thresholds were specified in the .CAL file, the normal range is shown in green behind the bars. See Figure 2-23.

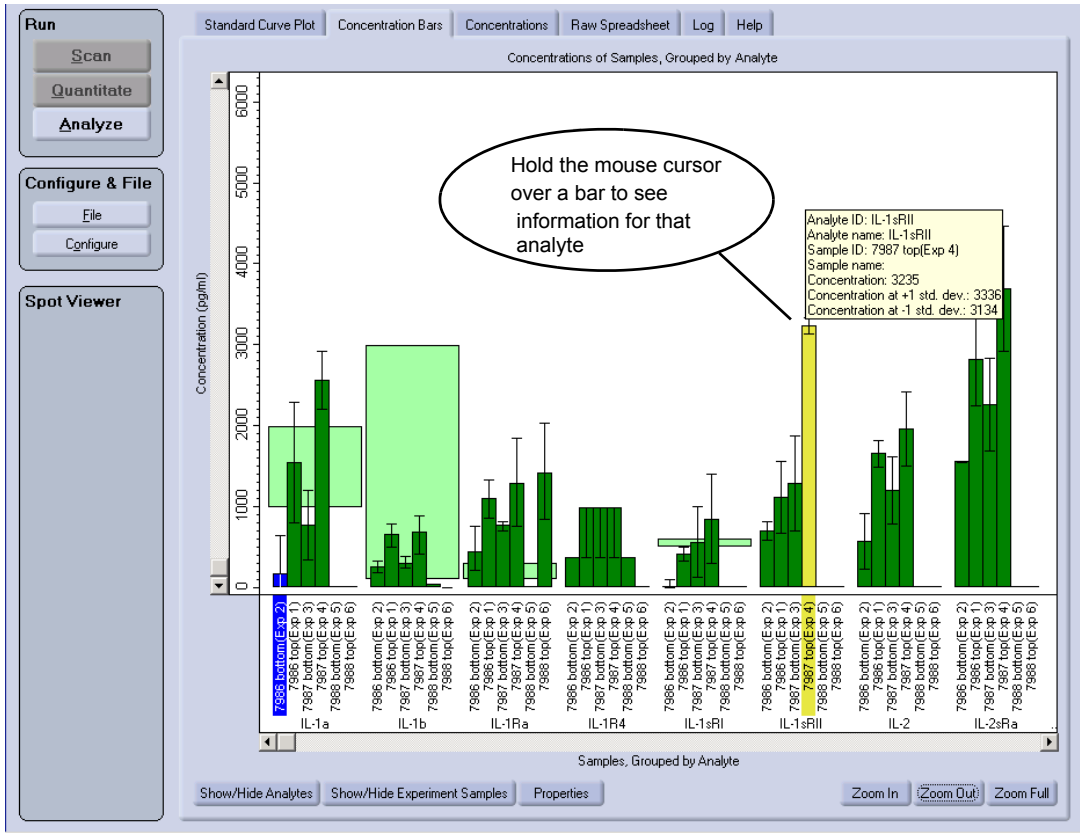


Figure 2–23 Concentration Bars Tab

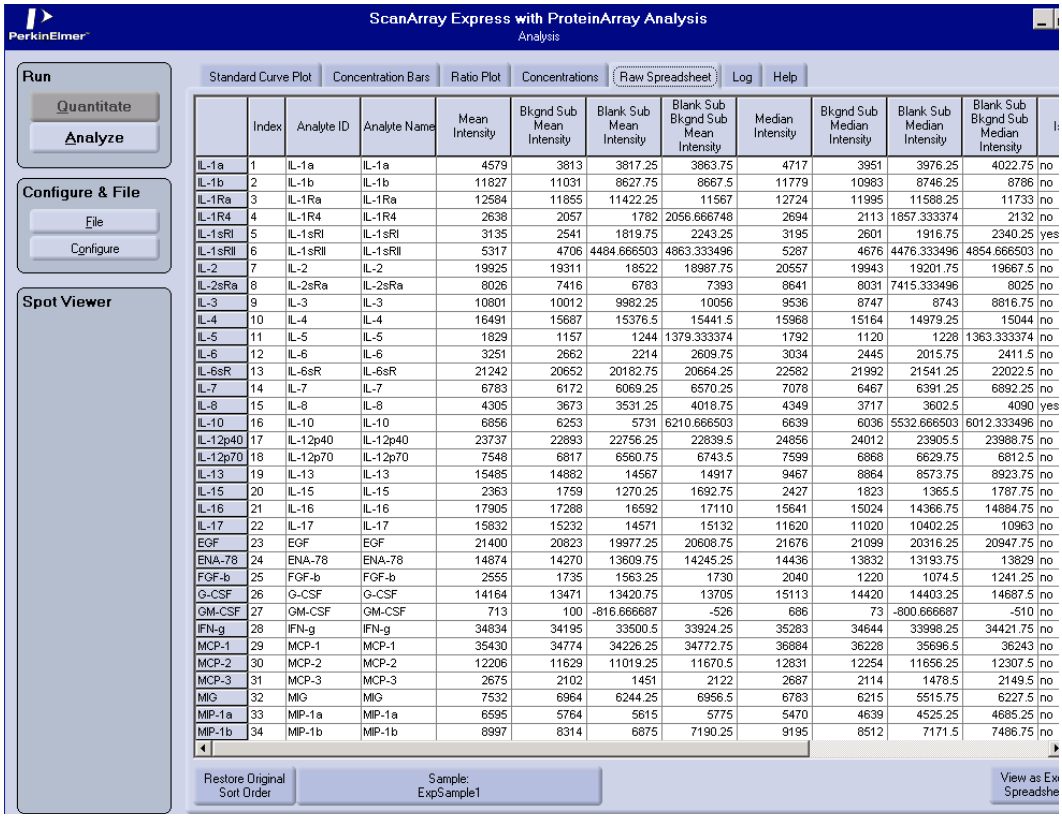
Using the **Zoom** buttons at the bottom right of the tab, you can zoom in, then scroll up and down or left and right, zoom out, or zoom back to the full display. Holding the mouse cursor over an analyte brings up information about that analyte, including the ID and name, the sample ID and name, and the concentration of the analyte.

The following buttons allow additional actions. For more information, see Chapter 5.

Button	Description
Show/Hide Analytes	Click this button to open the *Show/Hide Analytes* window, where you can check any analyte to display or uncheck any analyte that you do not want to display.
Show/Hide Experiment Samples	Click this button to open the *Show/Hide Experiment Samples* window where you can check any samples to display or uncheck any samples that you do not want to display.

2.6.3 Raw Data

The *Raw Spreadsheet* tab displays a spreadsheet of raw data for all of the samples, all standards, blanks and references, one sample at a time. By default the tab displays the sample information in the order that the files were selected for analysis.



	Index	Analyte ID	Analyte Name	Mean Intensity	Bkgnd Sub Mean Intensity	Blank Sub Mean Intensity	Blank Sub Bkgnd Sub Mean Intensity	Median Intensity	Bkgnd Sub Median Intensity	Blank Sub Median Intensity	Blank Sub Bkgnd Sub Median Intensity	I
IL-1a	1	IL-1a	IL-1a	4579	3813	3817.25	3863.75	4717	3951	3976.25	4022.75	no
IL-1b	2	IL-1b	IL-1b	11827	11031	8627.75	8667.5	11779	10983	8746.25	8786	no
IL-1Ra	3	IL-1Ra	IL-1Ra	12584	11855	11422.25	11567	12724	11995	11588.25	11733	no
IL-1R4	4	IL-1R4	IL-1R4	2638	2057	1782	2056.666748	2694	2113	1857.333374	2132	no
IL-1sRI	5	IL-1sRI	IL-1sRI	3135	2541	1819.75	2243.25	3195	2601	1916.75	2340.25	yes
IL-1sRII	6	IL-1sRII	IL-1sRII	5317	4706	4484.666503	4863.333496	5287	4676	4476.333496	4854.666503	no
IL-2	7	IL-2	IL-2	19925	19311	18522	18987.75	20557	19943	19201.75	19667.5	no
IL-2sRa	8	IL-2sRa	IL-2sRa	8026	7416	6783	7393	8641	8031	7415.333496	8025	no
IL-3	9	IL-3	IL-3	10801	10012	9982.25	10056	9536	8747	8743	8816.75	no
IL-4	10	IL-4	IL-4	16491	15687	15376.5	15441.5	15968	15164	14979.25	15044	no
IL-5	11	IL-5	IL-5	1829	1157	1244	1379.333374	1792	1120	1228	1363.333374	no
IL-6	12	IL-6	IL-6	3251	2662	2214	2609.75	3034	2445	2015.75	2411.5	no
IL-6sR	13	IL-6sR	IL-6sR	21242	20652	20182.75	20684.25	22582	21992	21541.25	22022.5	no
IL-7	14	IL-7	IL-7	6783	6172	6069.25	6570.25	7078	6467	6391.25	6892.25	no
IL-8	15	IL-8	IL-8	4305	3673	3531.25	4018.75	4349	3717	3602.5	4090	yes
IL-10	16	IL-10	IL-10	6856	6253	5731	6210.666503	6639	6036	5532.666503	6012.333496	no
IL-12p40	17	IL-12p40	IL-12p40	23737	22893	22756.25	22839.5	24856	24012	23905.5	23988.75	no
IL-12p70	18	IL-12p70	IL-12p70	7548	6817	6560.75	6743.5	7599	6868	6629.75	6812.5	no
IL-13	19	IL-13	IL-13	15485	14882	14567	14917	9467	8864	8573.75	8923.75	no
IL-15	20	IL-15	IL-15	2363	1759	1270.25	1692.75	2427	1823	1365.5	1787.75	no
IL-16	21	IL-16	IL-16	17905	17288	16592	17110	15641	15024	14366.75	14884.75	no
IL-17	22	IL-17	IL-17	15832	15232	14571	15132	11620	11020	10402.25	10963	no
EGF	23	EGF	EGF	21400	20823	19977.25	20608.75	21676	21099	20316.25	20947.75	no
ENA-78	24	ENA-78	ENA-78	14874	14270	13609.75	14245.25	14436	13832	13193.75	13829	no
FGF-b	25	FGF-b	FGF-b	2555	1735	1563.25	1730	2040	1220	1074.5	1241.25	no
G-CSF	26	G-CSF	G-CSF	14164	13471	13420.75	13705	15113	14420	14403.25	14687.5	no
GM-CSF	27	GM-CSF	GM-CSF	713	100	-816.666687	-526	686	73	-800.666687	-510	no
IFN-g	28	IFN-g	IFN-g	34834	34195	33500.5	33924.25	35283	34644	33998.25	34421.75	no
MCP-1	29	MCP-1	MCP-1	35430	34774	34226.25	34772.75	36884	36228	35696.5	36243	no
MCP-2	30	MCP-2	MCP-2	12206	11629	11019.25	11670.5	12831	12254	11656.25	12307.5	no
MCP-3	31	MCP-3	MCP-3	2675	2102	1451	2122	2687	2114	1478.5	2149.5	no
MIG	32	MIG	MIG	7532	6964	6244.25	6956.5	6783	6215	5515.75	6227.5	no
MIP-1a	33	MIP-1a	MIP-1a	6595	5764	5615	5775	5470	4639	4525.25	4685.25	no
MIP-1b	34	MIP-1b	MIP-1b	8997	8314	6875	7190.25	9195	8512	7171.5	7486.75	no

Figure 2–24 The Raw Data Spreadsheet

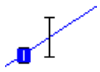
You can scroll through the information, double-click any column header to arrange the information in ascending order, based on the column you clicked; double-click again to arrange in descending order. The following buttons allow additional actions. For more information, see Chapter 5.

Item	Description
Restore Original Sort Order	Click this button after changing the sort order to restore the original order.
Sample: 7986 bottom(Exp 2)	Click this button to open the *Select a Sample* window, where you can select which sample you want to view.
View as Excel Spreadsheet	Click this button to open a new Excel workbook. Each spreadsheet of raw data is exported as a sheet in the workbook. See Chapter 5.

2.6.4 Standard Curve Plot

The *Standard Curve Plot* tab (Figure 2-25) shows the curve for one analyte at a time, beginning with the first one in the .CAL file, in a customizable view that lets you select which analyte to view and how to display the results.

The standards are shown in ascending order on the plot, and data points indicate the analyte concentrations for the experimental samples. If upper and lower thresholds were specified in the .CAL file, the normal range is shown in green.

Item	Description
	*Standard Data points* Each standard data point is indicated by a purple diamond. The standard deviation is shown with an error bar (a purple "I-beam" through the diamond).
	*Sample Data points* Each experiment sample is indicated by a black tick mark. The standard deviation for replicate samples is shown with an error bar (a black "I-beam" through the tick mark).

The name of the currently selected analyte is indicated in the title above the plot.

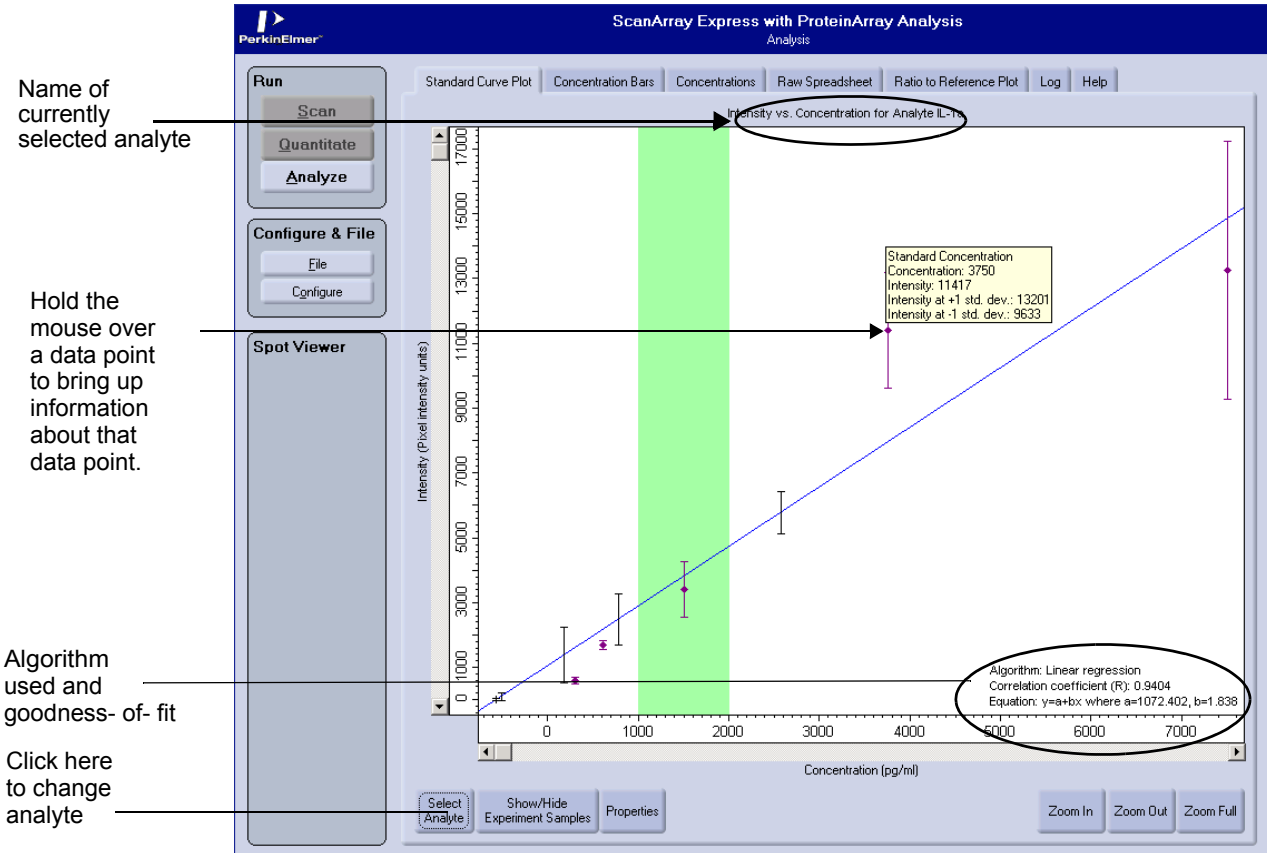


Figure 2–25 A Standard Plot Curve with Default Display Properties

A goodness-of-fit is included with each plot, indicating the validity of the displayed plot: 1= fits perfectly; 0 = not a good fit. Generally, anything .9 or higher is a good fit.

Using the **Zoom** buttons at the bottom right of the tab, you can zoom in and scroll up and down or left and right, zoom out, or zoom back to the full display. Holding the mouse over a data point brings up information about that data point, including the analyte ID and name, the sample ID and name, and the concentration of the analyte.

The following buttons allow additional actions. For more information, see Chapter 5.

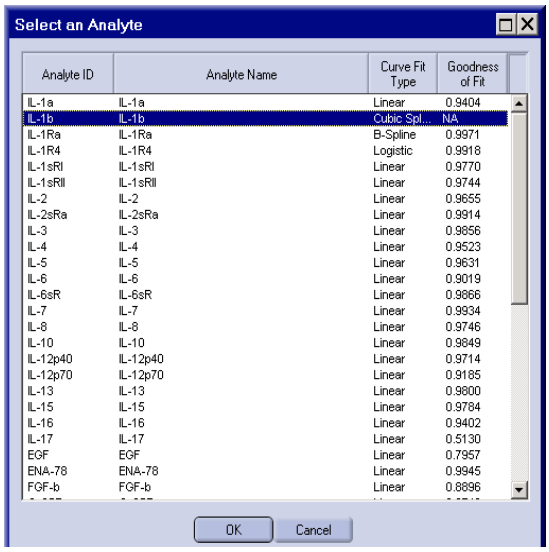
Item	Description
	Click this button to open the *Select an Analyte* window and choose a different analyte to display or view the curve fit types and goodness-of-fit for all analytes. See Section 2.6.5.
	Click this button to open the *Show/Hide Experiment Samples* window where you can check any samples to display or uncheck any samples that you do not want to display.

2.6.5 Displaying Curve Plots for Different Analytes

In the *Standard Curve Plot* tab, you can select the analyte curve plot to display.

To select an analyte to display

1. In the *Standard Curve Plot*, click **Select Analyte**. In the *Select an Analyte* window that opens, click the name of the analyte you want to view. For this example, select **`IL-1b,`** for which you selected cubic spline type for the curve fit.



2. Click **OK**. The tab displays the curve plot for the selected analyte (shown in Figure 2-27).

In Section 2.4.5, Selecting the Curve Fit Algorithm, you selected a different algorithm for each of the first four analytes. The following figures show the curve plots for the first, second, third, and fourth analytes.

2.6.5.1 Linear Regression Curve for IL-1a

The linear regression method interpolates the standard data points and draws a straight line.

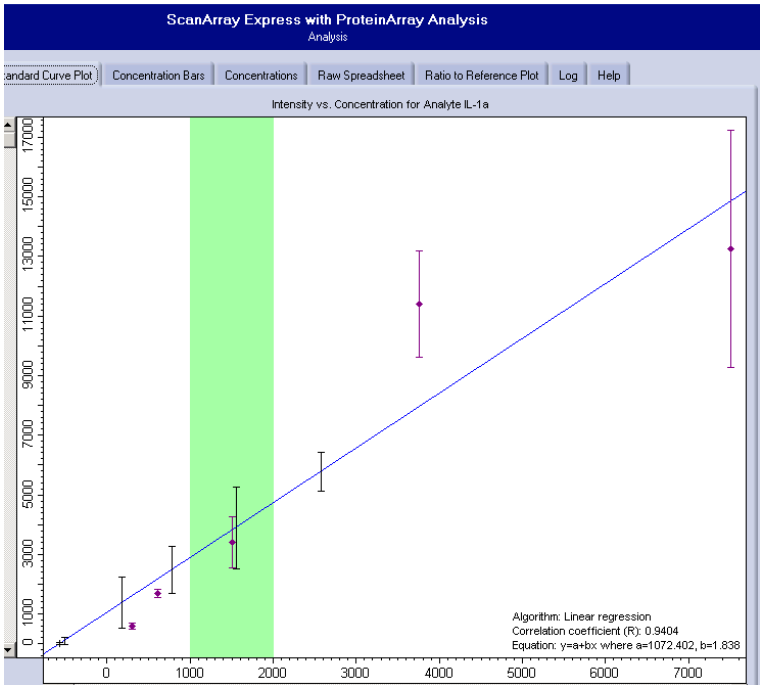


Figure 2–26 Curve Plot for IL-1a, Using Linear Regression

2.6.5.2 Cubic Spline Curve for IL-1b

The cubic spline method fits a smooth curve through every standard data point, bending as needed.

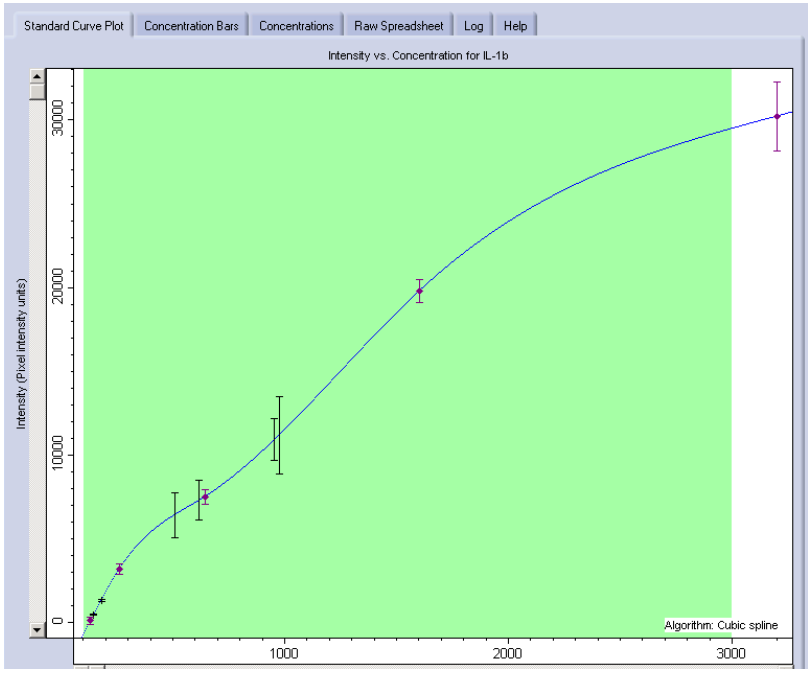


Figure 2–27 Curve Plot for IL-1b, Using the Cubic Spline Method

2.6.5.3 B-spline Curve for IL-1Ra

The B-spline method fits a smooth curve through the standard data points, but follows the trend of the data, and does not pass through every data point.

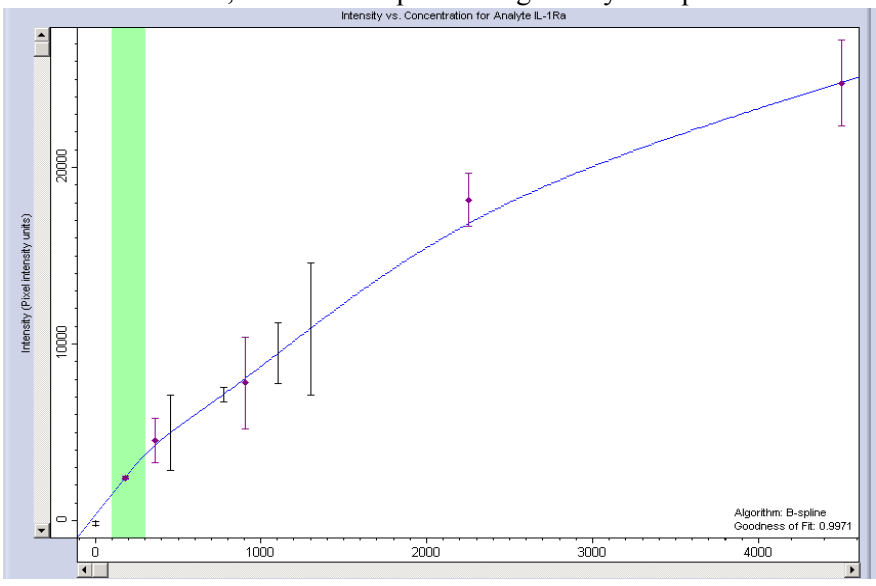


Figure 2–28 Curve Plot for IL-1Ra, Using the B-Spline Method

2.6.5.4 Logistic Curve for IL-1R4

The logistic curve method fits an S-shaped curve through the standard points using an equation that automatically calculates four parameters.

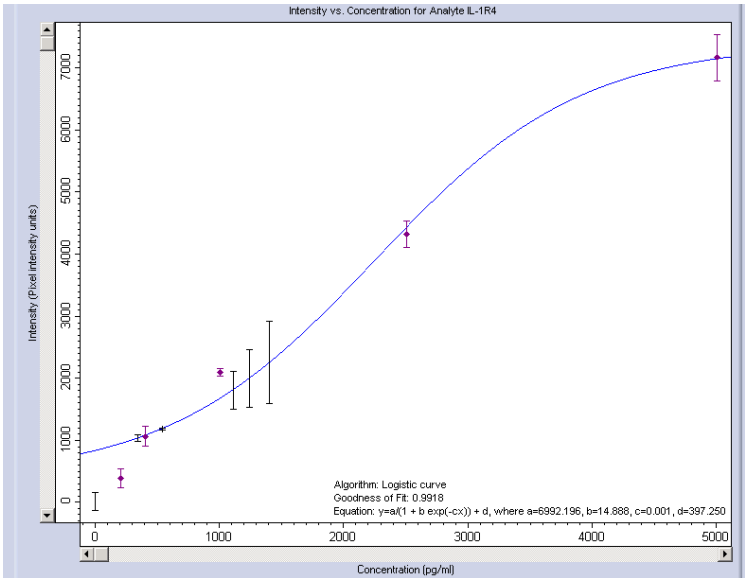
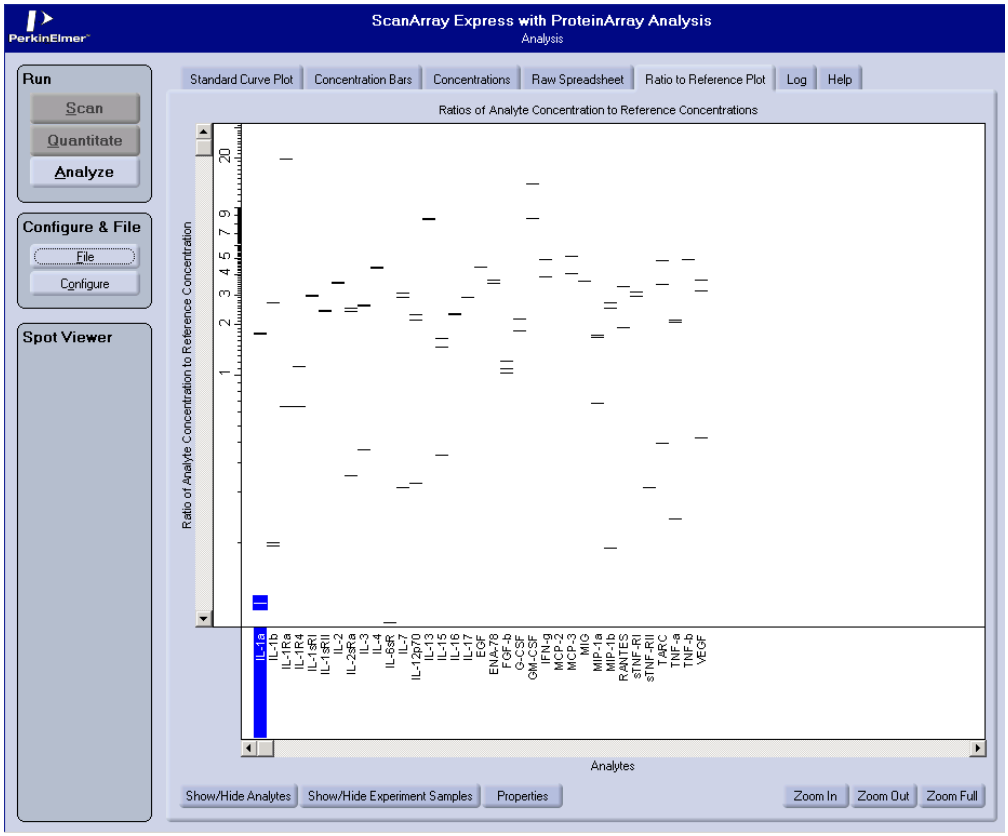


Figure 2–29 Curve Plot for IL-1R4, Using the Logistic Curve Method

2.7 Ratio to Reference Plot

When a reference sample is used, a *Ratio to Reference Plot* provides ratios of analyte concentration to reference concentrations. This view lets you group by analyte or by sample.

You can zoom in and scroll up and down or left and right, zoom out, or zoom back to the full display. Holding the mouse cursor over a data point brings up information about that analyte.



As with the Standard Curve Plot, you can show or hide analytes and show or hide experiment samples. See ***Displaying Curve Plots for Different Analytes*** on page 2-26.

2.8 Finishing Up

Note: You don't need to save the tutorial results. For a complete description of the analysis results and information on saving the results, see Chapter 5, "Reviewing the Results."

For more information on running an analysis with your own data and using any of the available options, see Chapter 3, "Analyzing Your Data."

Analyzing Your Data

Chapter 3

Chapter Summary

3.1 Overview

This chapter provides instructions to set up and run an analysis using manual settings. The chapter follows the ProteinArray Wizard, because that is the order in which you'll typically input data.

You need a valid .CAL file, and quantitation files from standard and experiment sample files. If you have your files, you're ready to start; if you need to prepare files, refer to Appendix B, "Preparing a CAL File."

To test the curve fitting for your standards data before running an analysis, you can run an analysis without samples. See ***Testing Curve Fitting before Adding Samples*** on page 3-26.

3.1.1 Starting the Analysis Wizard

To start the ProteinArray Analysis

1. Start the ScanArray Express if it is not already running, by clicking the ScanArray Express icon on the computer desktop.

2. In the *Main Window*, click **Analyze**.

The ProteinArray Analysis wizard opens.

Note: Chapter 2 provides more detailed instructions for starting the ProteinArray Analysis and moving from window to window in the Analysis wizard.

3.2 Starting with Manual Settings

In the *Analysis - Start* window (Figure 3-1), manual settings are selected by default when you open the wizard. You can start with the last used settings and files, or the last used settings only. Choose "last used settings only" if you want to run an analysis with the previous settings on a new set of files; this selection is useful when changing between .CSV and .GPR files.

If no protocols have been created, the selection **Start with a protocol** is greyed and unavailable to select. To create and use a protocol, see Chapter 4.

To manually specify the settings

1. On the *Analysis - Start* window, select **Start with manual settings - last used settings only**.

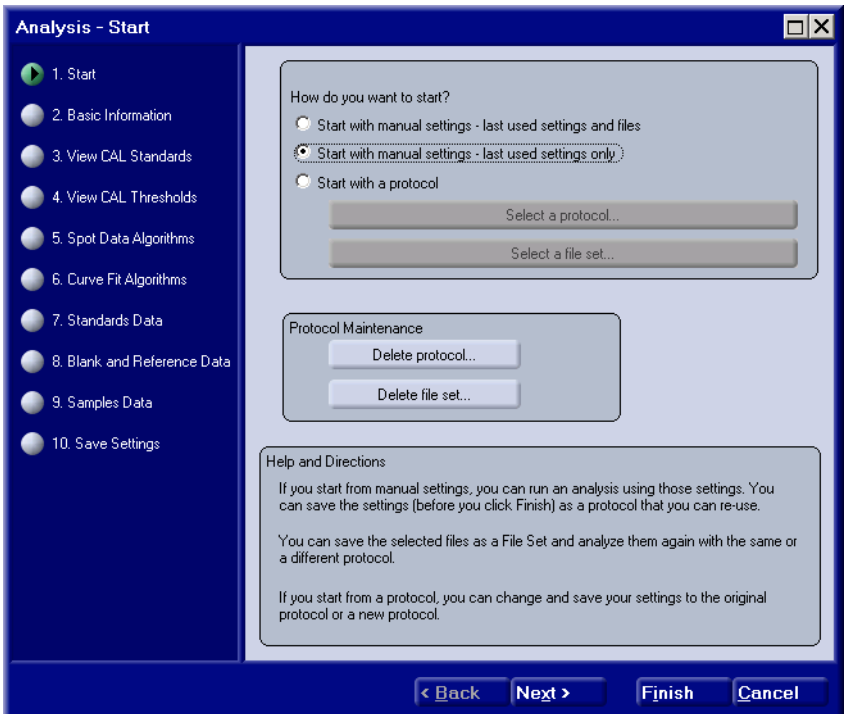


Figure 3–1 The Start Window

2. Enter the settings for each Analysis window, using instructions in the following sections.

3.3 Basic Information

In the *Analysis - Basic Information* window (Figure 3-2), specify the .CAL file to use for the analysis. The .CAL file provides a list of each analyte to be included, the concentration of the analyte in each of the standard samples, and an optional normal range (upper and lower thresholds) for any analyte(s).

The .CAL file also specifies the number of standards to be used. Your analysis must use the same number of standard files.

You can also select to automatically save the analysis results and the raw data after an analysis.

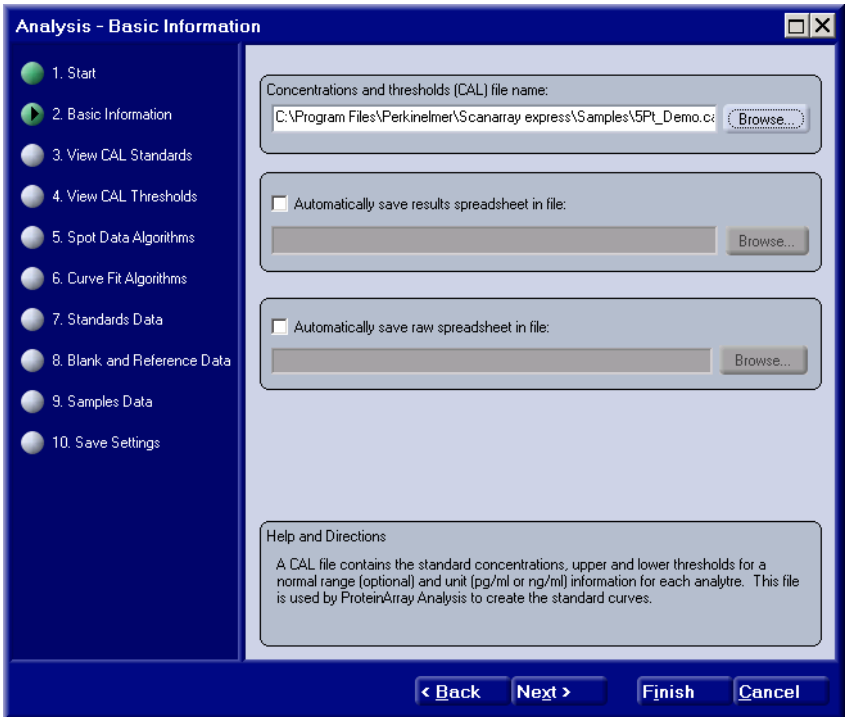


Figure 3–2 The Basic Information Window

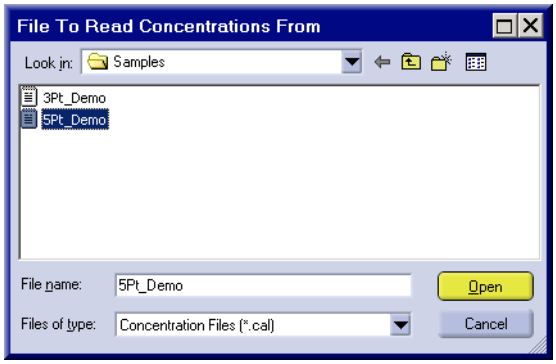
3.3.1 Specifying Concentrations and Thresholds

To specify a .CAL file

1. Enter the name of the .CAL file, with the full path, into the **Concentrations and thresholds (CAL) file name** field, or browse for the file as follows:

   On the *Analysis - Basic Information* window, click **Browse**.

2. In the *File to Read Concentrations From* window, select the correct .CAL file for your experiment and click **Open**. The filename appears in the **Concentrations and Thresholds Filename** field in the *Basic Information* window.



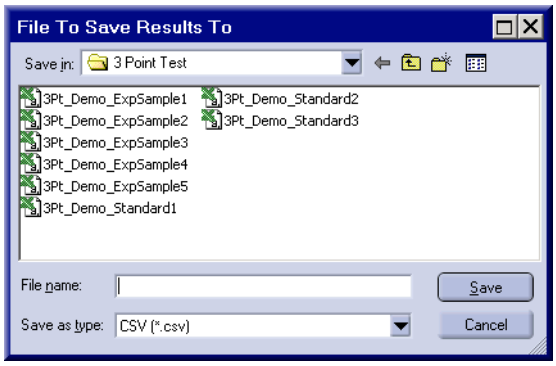
3.3.2 Automatically Saving the Spreadsheets After Analysis

The ProteinArray Analysis can save the analysis results spreadsheet, the raw data spreadsheet, or both, to a file destination for each that you specify here. To save the raw data spreadsheet, Microsoft Excel must be installed on your system

To automatically save the analysis results

1. Check **Automatically save results spreadsheet in file:** and provide a filename as follows:

   - Enter a filename, with the full path to the correct folder, where you want to save the results;
     or,
   - Click **Browse**. In the *File to Save Results To* window, locate the folder where you want to save the file, specify a filename and click **Save.**

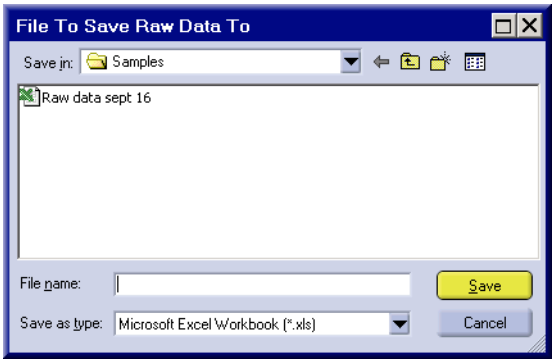


The analysis results are saved as a .CSV file after the analysis with the filename you provide here.

To automatically save the raw data

1. Check **Automatically save raw spreadsheet in file:** and provide a filename as follows:

- Enter a filename, with the full path to the correct folder, where you want to save the results;
  or,
- Click **Browse**. In the *File to Save Raw Data To* window, locate the folder where you want to save the file, specify a filename and click **Save**.



The raw data is automatically saved after the analysis as an Excel spreadsheet with the filename you provide here.

3.4 View CAL Standards

The *Analysis - View CAL Standards* window displays spot information, units of measurement, and concentration values of the loaded .CAL file (see Figure 3-3).

You can view the standards and verify that they're in ascending order. You cannot make any changes to the file in this window.

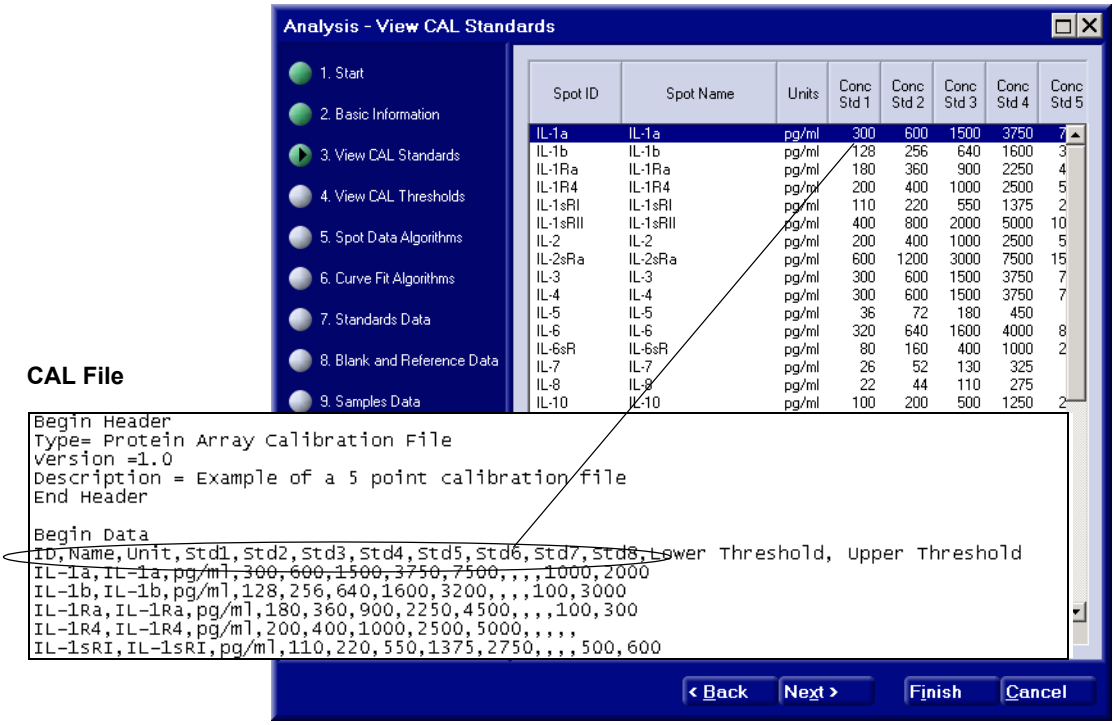


Figure 3–3 The Concentration Information for Standards

3.5 View CAL Thresholds

The *Analysis - View CAL Thresholds* window displays upper and lower thresholds if any have been entered in the .CAL file (see Figure 3-4).

You can verify the threshold information, but cannot make any changes in this window.

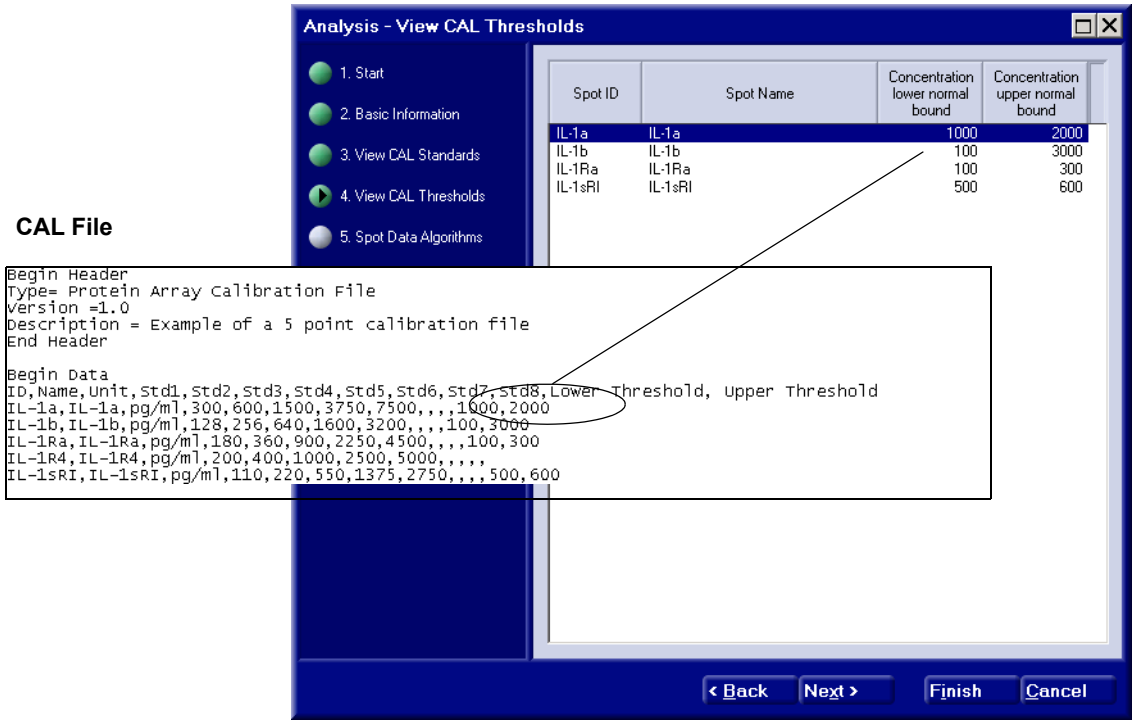


Figure 3–4 The View CAL Thresholds Window

If no thresholds were entered into the .CAL file, this window displays the message “There are no items to display in this view.”

3.6 Spot Data Algorithms

In the *Analysis - Spot Data Algorithms* window (Figure 3-5) you can select which data column from the quantitation file (.CSV or .GPR) to use for the analysis; for example, you can select the mean intensity or median intensity data. You can also filter the data by removing outliers -- the spots that lie outside the boundaries specified by the method(s) that you select in this window.

To specify the data column and remove outliers

1. Under **Spot signal data to use for analysis**, select the data column to use (see Section 3.6.1 for more information:

   - Mean intensity
   - Median intensity
   - Background subtracted mean intensity
   - Background subtracted median intensity

   If you use a blank sample, the data used will be the selected data column of the standard data minus the selected data column of the blank data.

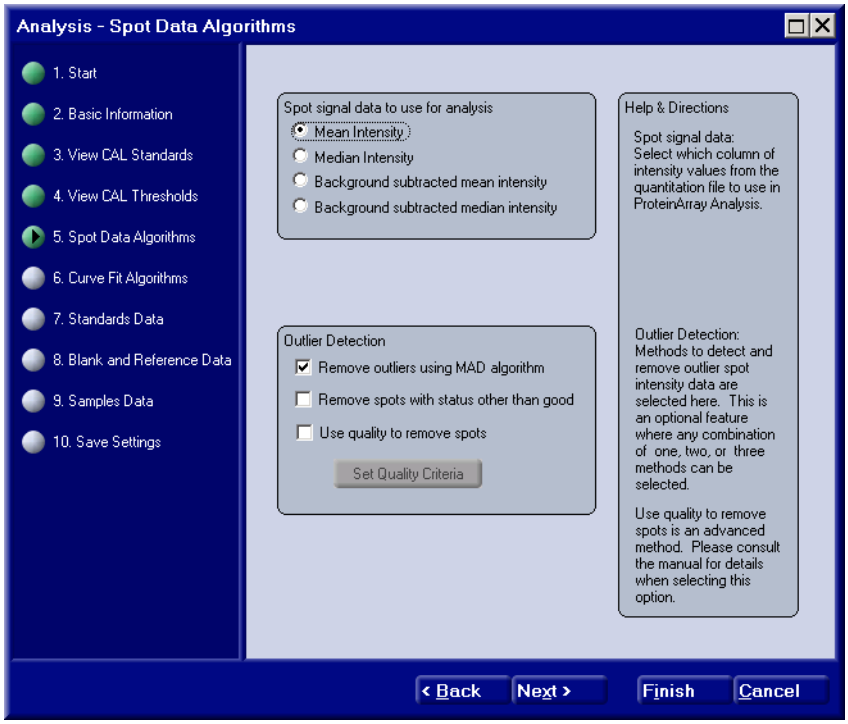


Figure 3–5 The Spot Data Algorithms Window

2. Under **Outlier Detection**, check each method that you want to use for removing outliers (see section 3.6.2 for more information): You can select one or more of the following:

   - Remove outliers using MAD algorithm
   - Remove spots with status of **not found**, **bad**, or **absent**

- Use quality to remove spots. If you select this method, you can specify the quality criteria in the software, as described in Section 3.6.2.

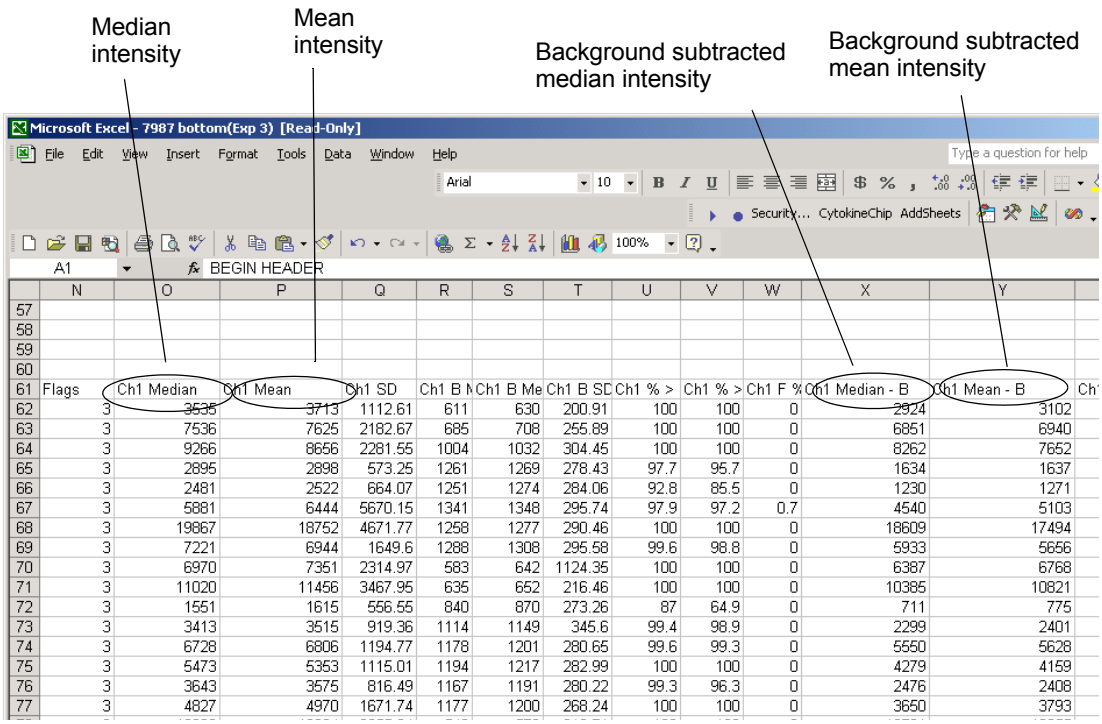
Appendix A includes a full description of the methods and the algorithms used.

3.6.1 More about Spot Signal Data

You can choose one of four data columns from the quantitation (.CSV) file to be used for the analysis.

3.6.1.1 CSV File Data Columns

Figure 3-6 shows an example .CSV file, viewed in Excel, with the data columns from which you can choose for analyzing the data; they are described in Table 3-1.



	N	O	P	Q	R	S	T	U	V	W	X	Y
57												
58												
59												
60												
61	Flags	Ch1 Median	Ch1 Mean	Ch1 SD	Ch1 B M	Ch1 B Me	Ch1 B SD	Ch1 % >	Ch1 % >	Ch1 F %	Ch1 Median - B	Ch1 Mean - B
62	3	3535	3713	1112.61	611	630	200.91	100	100	0	2924	3102
63	3	7536	7625	2182.67	685	708	255.89	100	100	0	6851	6940
64	3	9266	8656	2281.55	1004	1032	304.45	100	100	0	8262	7652
65	3	2895	2898	573.25	1261	1269	278.43	97.7	95.7	0	1634	1637
66	3	2481	2522	664.07	1251	1274	284.06	92.8	85.5	0	1230	1271
67	3	5881	6444	5670.15	1341	1348	295.74	97.9	97.2	0.7	4540	5103
68	3	19867	18752	4671.77	1258	1277	290.46	100	100	0	18609	17494
69	3	7221	6944	1649.6	1288	1308	295.58	99.6	98.8	0	5933	5656
70	3	6970	7351	2314.97	583	642	1124.35	100	100	0	6387	6768
71	3	11020	11456	3467.95	635	652	216.46	100	100	0	10385	10821
72	3	1551	1615	556.55	840	870	273.26	87	64.9	0	711	775
73	3	3413	3515	919.36	1114	1149	345.6	99.4	98.9	0	2299	2401
74	3	6728	6806	1194.77	1178	1201	280.65	99.6	99.3	0	5550	5628
75	3	5473	5353	1115.01	1194	1217	282.99	100	100	0	4279	4159
76	3	3643	3575	816.49	1167	1191	280.22	99.3	96.3	0	2476	2408
77	3	4827	4970	1671.74	1177	1200	268.24	100	100	0	3650	3793

Figure 3–6 Example of a .CSV (Quantitation Results) File

Table 3-1: Spot Signal Data in a .CSV File

Spot Data Algorithm	Description
Mean	Uses the data column “Ch1 Mean,” the mean signal for the spot.
Median	Uses the data column “Ch1 Median,” the median signal for spot data.
Background subtracted mean intensity	Uses the data column “Ch1 mean-B”, the mean intensity of the spots minus the background intensity.
Background subtracted median intensity	Uses the data column “Ch1 median-B”, the median intensity of the spot minus the background intensity.

3.6.1.2 GPR File Data Columns

Figure 3-7 shows an example .GPR file, viewed in Excel, with the data columns from which you can choose for analyzing the data (you need to scroll to the right of your file to see the Median/Mean subtracted background columns); they are described in Table 3-2.

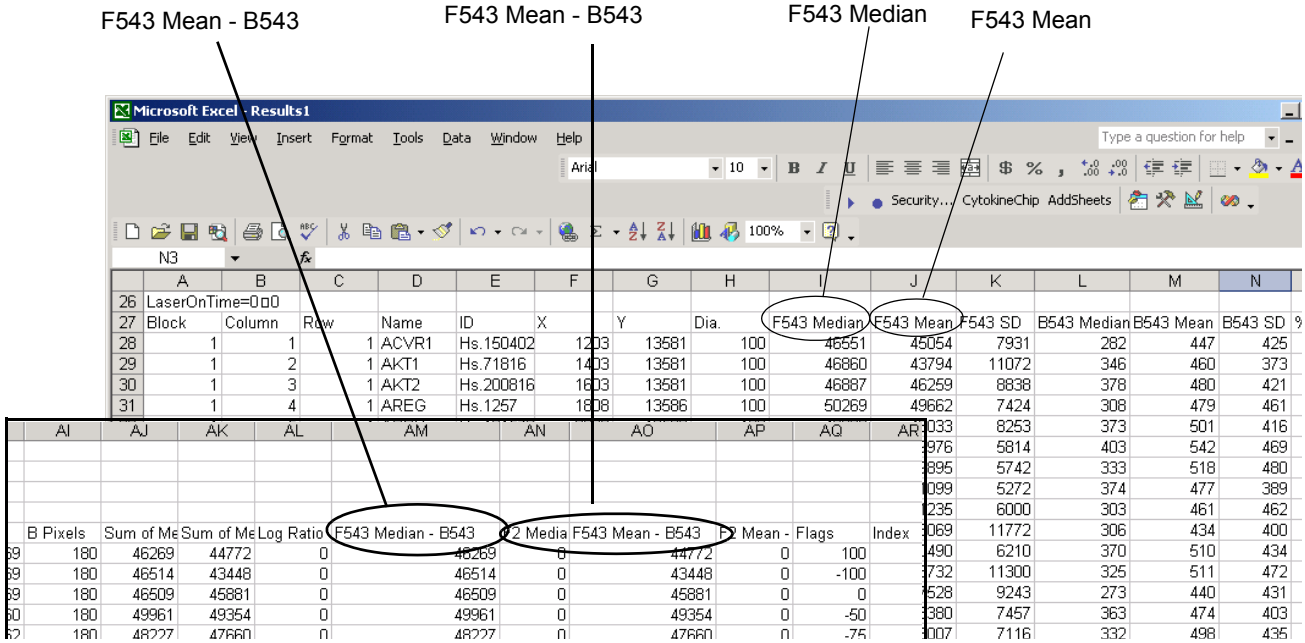


Figure 3–7 Example of a .GPR (Quantitation Results) File

Table 3-2: Spot Signal Data in a .GPR File

Spot Data Algorithm	Description
Mean	Uses the data column “F543 Mean”, the mean signal for the spot.

Spot Data Algorithm	Description
Median	Uses the data column “F543 Median”, the median signals for spot data.
Background subtracted mean intensity	Uses the data column “F543 Mean”, the mean intensity of the spot minus data column “B543”, the background intensity.
Background subtracted median intensity	Uses the data column “F543 Median”, the mean intensity of the spots, minus ”B543 Median”, the background intensity.

3.6.2 Outlier Detection and Removal

There are three methods of outlier detection, described in the following sections. You can choose one, two, or all three methods. When you select more than one method, a spot is removed from the analysis if any one of the methods finds it to be an outlier.

3.6.2.1 Using the MAD Algorithm

In brief, during MAD outlier detection, the software uses a formula to calculate the Median Absolute Deviation (MAD), then uses a second formula to calculate a MAD score for each data point. The data point is considered an outlier if its score is greater than the maximum or less than the minimum MAD cutoff scores specified in the ProteinArray Analysis software’s application preferences.

You can leave the score values at the default minimum and maximum, or you can specify different values in the ScanArray Express Application Settings. Appendix A provides a full description of the MAD formulas, and instructions for changing the score values.

3.6.2.2 Using Spot Status

Spot status uses the spot status from the quantitation files (.CSV or .GPR). Any spot with a status of **bad**, **not found**, or **absent** is considered an outlier.

Spot status in Flags column

60	BEGIN DATA																	
61	Index	Array Row	Array Colu	Spot Row	Spot Colur	Name	ID	X	Y	Diameter	F Pixels	B Pixels	Footprint	Flags	Ch1 Media	Ch1 Mean	Ch1 SD	Ch1 B f
62	1	1	1	1	1	IL-1a	IL-1a	6391	41712	170	196	1220	36	3	3085	3321	1073.91	1
63	2	1	1	1	2	IL-1b	IL-1b	6886	41722	190	256	1220	25	3	7804	7583	2182.59	1
64	3	1	1	1	3	IL-1Ra	IL-1Ra	7391	41777	160	177	1220	32	3	8969	8894	2547.7	1
65	4	1	1	1	4	IL-1R4	IL-1R4	7886	41772	160	172	1220	26	3	2052	2062	524.06	1
66	5	1	1	1	5	IL-1sRI	IL-1sRI	8386	41722	190	256	1220	25	3	2707	2733	838.93	
67	6	1	1	1	6	IL-1sRII	IL-1sRII	8910	41463	200	282	1076	278	3	4325	4217	1543.43	1
68	7	1	1	1	7	IL-2	IL-2	9391	41782	170	196	1220	37	3	9769	9254	2504.66	1
69	8	1	1	1	8	IL-2sRa	IL-2sRa	9876	41752	190	256	1220	6	3	5033	4856	1282.74	1
70	9	1	1	2	1	IL-3	IL-3	6386	42227	180	230	1220	20	3	5645	6145	2398.9	
71	10	1	1	2	2	IL-4	IL-4	6886	42242	180	216	1220	7	3	10802	11673	3577.02	1
72	11	1	1	2	3	IL-5	IL-5	7396	42282	200	276	1076	39	4	1529	1537	376.71	1
73	12	1	1	2	4	IL-6	IL-6	7876	42232	140	124	1344	14	3	2047	2043	572.22	
74	13	1	1	2	5	IL-6sR	IL-6sR	8386	42212	190	256	1220	34	3	13546	13096	4281.42	1
75	14	1	1	2	6	IL-7	IL-7	8881	42232	160	180	1220	14	3	4519	4445	1128.32	1
76	15	1	1	2	7	IL-8	IL-8	9381	42262	160	180	1220	15	3	2433	2377	678.3	
77	16	1	1	2	8	IL-10	IL-10	9871	42257	170	197	1220	13	3	4376	4419	1468.05	

In the **Flags** column of the quantitation file, the spot status is indicated by a number:

Spot Status	.CSV File	.GPR File
Good	3	100
Bad	4	-100
Found	0	0
Not Found	1	-50
Absent	2	-75

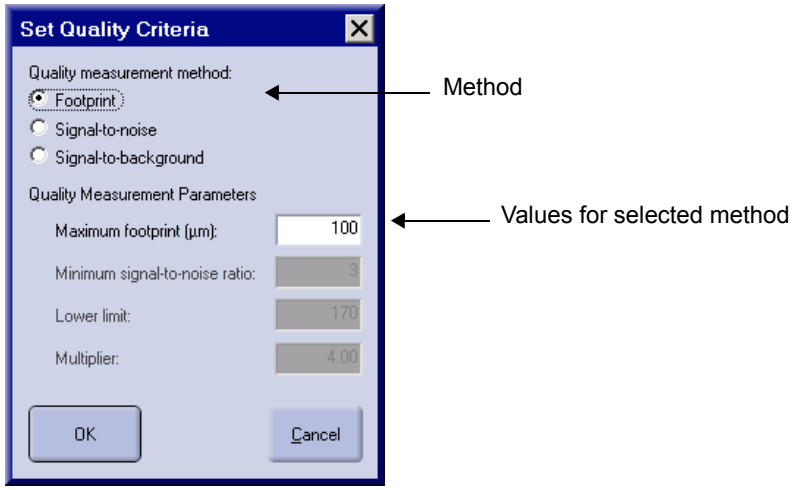
In the analysis results, the replicates column indicates the number of spots used for each analyte in the analysis.

3.6.2.3 Using Quality Criteria

When you select **Use quality to remove spots**, you can further select what quality measurement method to use, and specify the thresholds for the method.

To set quality criteria

1. On the *Analysis - Spot Data Algorithms*, check **Use quality to remove spots**.
2. Click **Set Quality Criteria**. The following window opens.



3. Refer to Table 3-3 and select the quality measurements you want to use.
4. Click **OK** when finished.

Table 3-3: Quality Measurements

Item	Description
Quality measurement method	Select one of the methods below. The formulas used by the different methods use the settings specified in this window. The formulas are provided in Appendix A.
Footprint	This is the distance between the expected position of a spot and its actual position. Choosing this method enables the *Maximum footprint* field below. If the footprint for the spot is larger than the threshold specified here, the spot is treated as an outlier.
Signal-to-noise	Uses the ratio of the spot intensity to the standard deviation of the local background of the spot. Choosing this method enables the *Minimum signal-to-noise ratio* field below. Spots with a low signal-to-noise ratio can be quickly identified for later review.
Signal-to-background	Uses the comparison of the mean of spot intensity to the mean of local background. Choosing this method enables the *Lower Limit* and *Multiplier* fields below.
Quality Measurement Parameters	The following fields will be enabled, or dimmed and unavailable for selection, depending on which method you select:
Maximum footprint (µm)	Enter the maximum size of the footprint in microns. This measurement helps to distinguish spots from artifacts on the slide. The default setting is 100. The valid minimum/maximum settings are 0 through 72,000.
Minimum signal-to-noise ratio:	This field is enabled when you choose the Signal-to-noise method. The default value is 3. Valid values are 1 through 10,000.
Lower limit:	For Signal-to-background method. Valid values are 0 through 64,000. More accurate spot intensities can be produced with a low intensity, uniform background around all spots. The default setting is 400.
Multiplier	For Signal-to-background method. The valid values are 1 through 65000. The default setting is 1.7.

3.7 Curve Fit Algorithms

In the *Analysis - Curve Fit Algorithms* window (Figure 3-8), you can select from among four curve fit types for creating the standard curve: Linear regression, Cubic spline interpolation, B-spline curve, or four-point Logistic model. You can select a different method for different analytes, or select one method and apply it to all analytes.

To select a curve fit algorithm

1. In the *Analysis - Curve Fit Algorithms* window, select the first analyte for which you want to select a curve fit method by clicking it with the mouse.

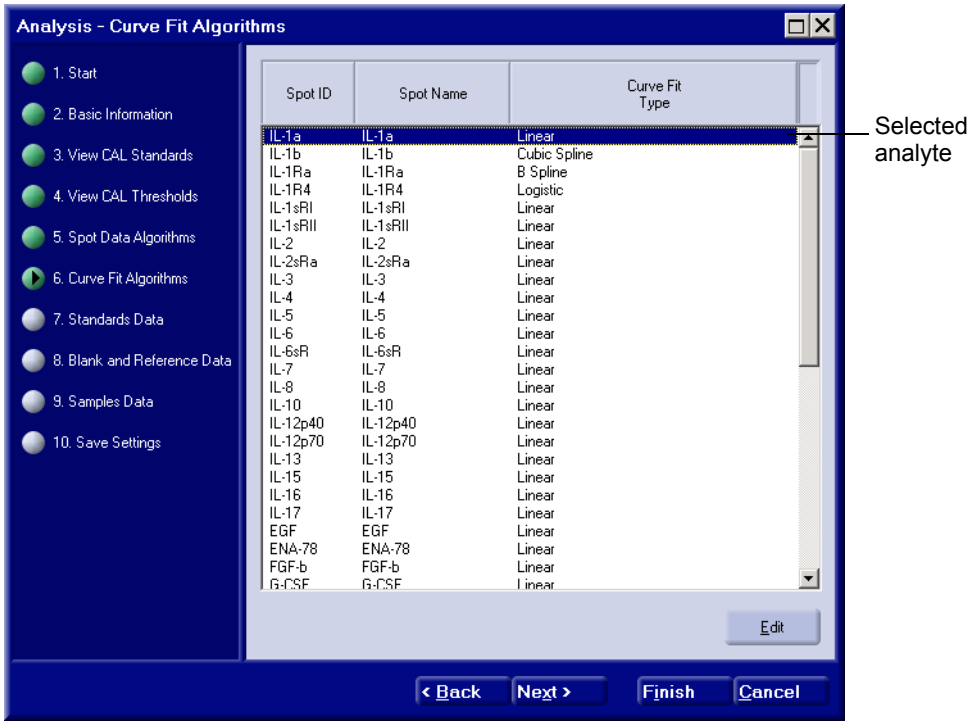
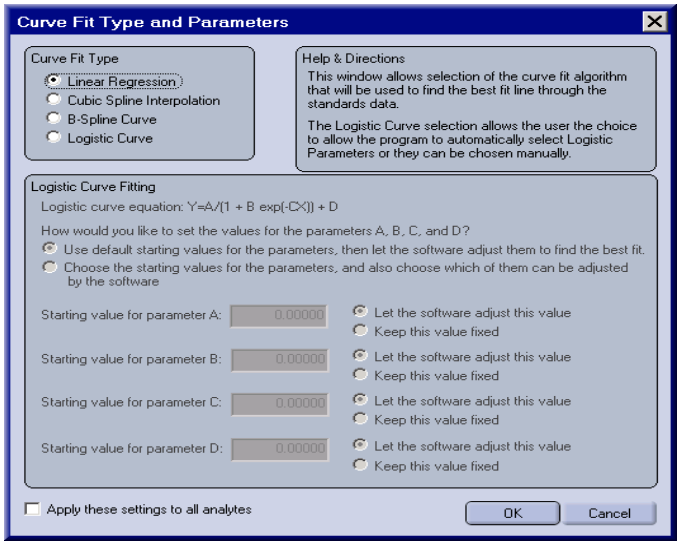


Figure 3–8 The Curve Fit Algorithms Window

2. Click **Edit**. The *Curve Fit Types and Parameters* window opens.



3. Select the curve fit type you want to use and click **OK**. Refer to the Table 3-4 for a description of each curve fit type (Appendix A provides the algorithms used).

Note: Linear regression is the only curve fit type that can be used for an analysis with three standards.

4. Repeat steps 1 to 3 for each analyte for which you want to apply a specific curve fit type, or check **Apply these settings to all analytes**, to use the same type for all analytes.

5. Click **OK**.

Table 3-4: Curve Fit Types

Item	Description
Linear Regression	The software fits a straight line through the standard data, minimizing the sum of the squares of the vertical distances between the points and the line. This is the only curve fit type available for a 3-point analysis.
Cubic Spline Interpolation	The software fits a smooth curve through the standard data. The standard curve is generated from separate cubic segments between each pair of points, which are connected together. The slopes and the curvature are continuous at the standard data points.
B-spline Curve	The software fits a smooth curve through the standard data, but the curve does not need to pass through all of the data points. The curve is fitted so that the portion of the curve determined by each group of four points is within the convex hull of those points.
Logistic Curve	The software fits the standard data points to an S-shaped curve. (See Appendix A for more information about the Logistic curve equation). Selecting **Logistic Curve** enables the selection buttons and edit fields under Logistic Parameters in this window.
Logistic Parameters	The software starts with initial parameters and, unless you specify fixed parameters, tries to fine-tune them during the curve-fitting as follows:
Automatically generate	Select this to have the software automatically select an initial “guess” for each parameter for the curve fit algorithm.
Use settings below	Select this to enter values for the initial four parameters in the edit fields. Check **Fixed** for a parameter if you want the displayed value to be held fixed while fitting the curve.

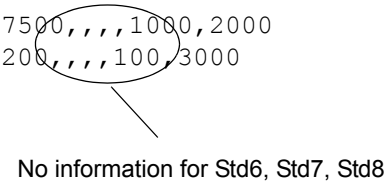
Item	Description
Apply these settings to all analytes	Check this box to apply the settings you selected in this window to all analytes in the analysis. If you selected Logistic Curve, checking this box applies the logistic parameters to all analytes.

3.8 Standards Data

You can specify from three to eight standard files for an analysis. A standard file is the quantitation results (.CSV or .GPR) for one microarray with known concentration of sample. You cannot mix .CSV and .GPR files for the same analysis.

You must use a number of standard files equal to the number of standards listed in the .CAL file. If you have less standards, the .CAL file has to be edited. For example, if you're using only five standards, but the .CAL file has information for eight standards, you would have to remove the information for Std6, Std7, and Std8, as shown in the following example .CAL file:

```
ID,Name,Unit,Std1,Std2,Std3,Std4,Std5,Std6,Std7,Std8,Lower
Threshold, Upper Threshold
IL-1a,IL-1a,pg/ml,300,600,1500,3750,7500,,,,1000,2000
IL-1b,IL-1b,pg/ml,128,256,640,1600,3200,,,,100,3000
```



To select Standards Data

1. In the *Analysis - Standards Data* window, click **Add**.

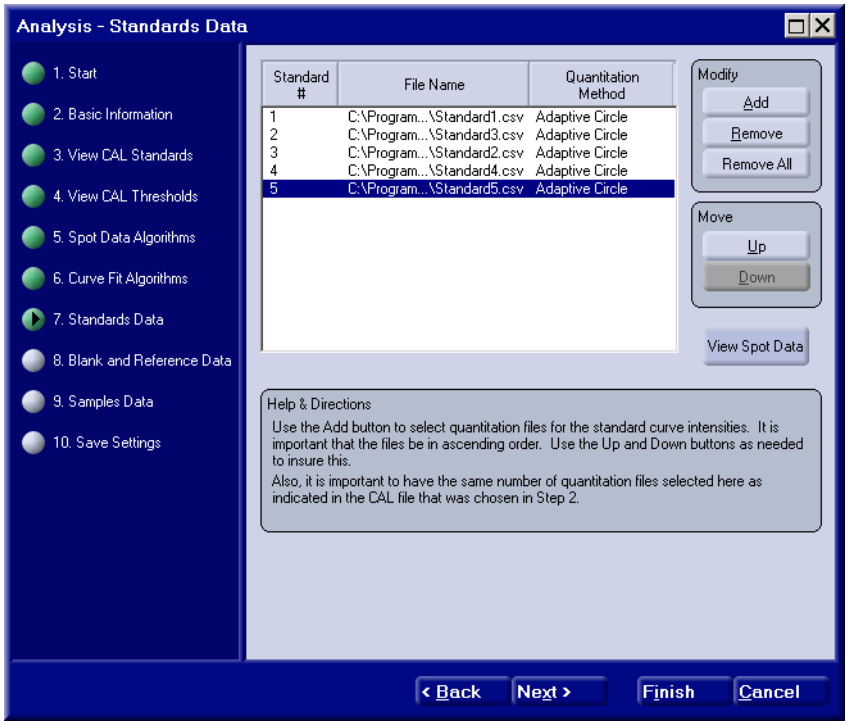
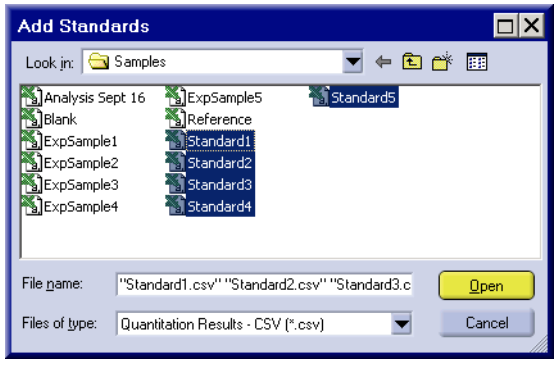


Figure 3–9 The Standards Data Window

2. In the *Add Standards* window, select the standards files. You can select them one at a time in ascending order, clicking **Add** to add each file. Or, you can select several files at once and then put them in order:

- to add several contiguous files, press the **Shift** key and click the first filename, and the last filename. The first and last file and all files in between are selected.
- to add several files without the files in between, press the **Ctrl** key, and click each file that you want to add. This does not select files in between.



3. Click **Open**. The selected file(s) are listed in the *Analysis - Standards Data* window.

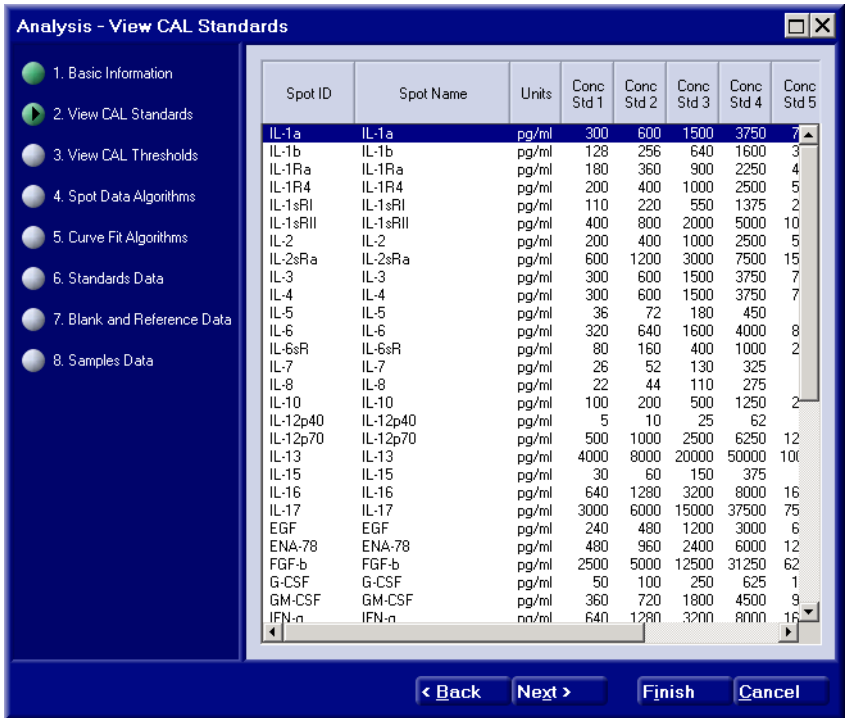
4. The standard files must be listed in the ascending order of concentration. If necessary, re-order the files. In the wizard window, select any file that is out of order and click **Up** or **Down** to move the file into its correct order. If any extraneous files have inadvertently been added, select any file that should not be included, and click **Remove**.

3.8.1 Viewing Spot Data for the Standards

To view spot data for the standards files

1. Select any file in the *Analysis - Standard Data* window to activate the **View Spot Data** button.

2. Click **View Spot Data**. The *Spot Intensity Values* window opens. This displays the data for all of the standard files. You can verify spot intensities for the standards. In general, the displayed spot intensities should increase from left to right if the standards are properly ordered.



3. Click **OK** to close the window.

3.9 Blank and Reference Data

In the *Analysis - Blank and Reference Data* window (Figure 3-10), you can add a blank and/or reference sample, with one to four replicates of each, and assign them a Sample ID and Name within ProteinArray Analysis. Since ProteinArray Analysis identifies replicates as those samples with the same Sample ID and Sample Name, the ID and Name you assign for a blank or reference is automatically applied to all files in the Blanks or References list.

- A *blank sample* has no analyte-specific signal, thereby providing information on the background fluorescence. If you have two, three, or four blank samples (microarrays using the same buffer with zero analyte concentrations), they are assigned the same name as replicates. You can assign a different ID or leave the default Sample ID ("Blanks").

- When a *reference sample* is used, ProteinArray Analysis calculates the ratio of the analyte concentrations in the experimental samples to the corresponding analyte concentrations in the reference sample and displays the ratio values in the analysis results spreadsheet and in a Ratio to Reference scatter plot. You can assign a different ID and name or leave the default Sample ID ("References") .

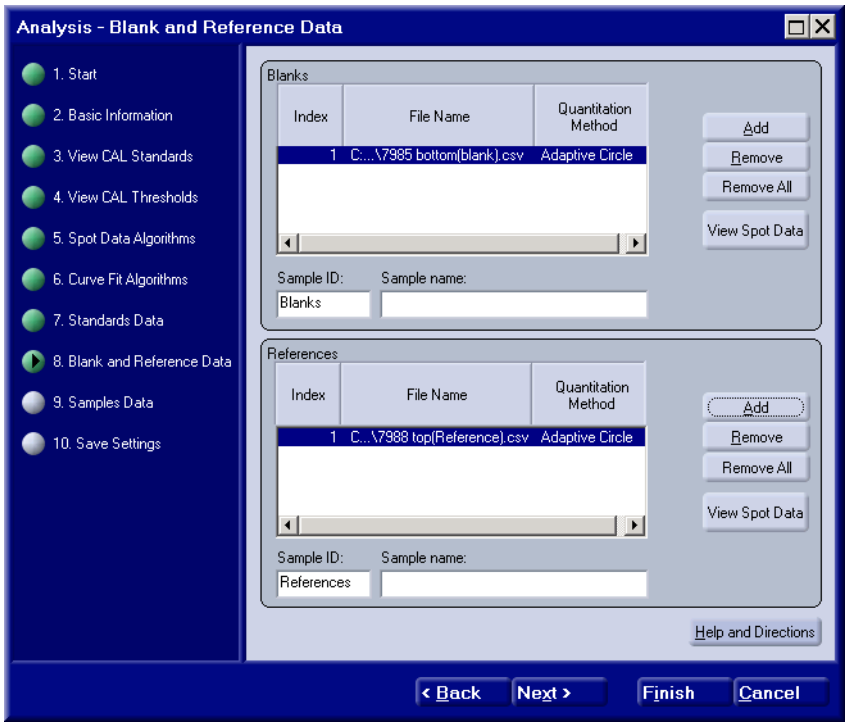


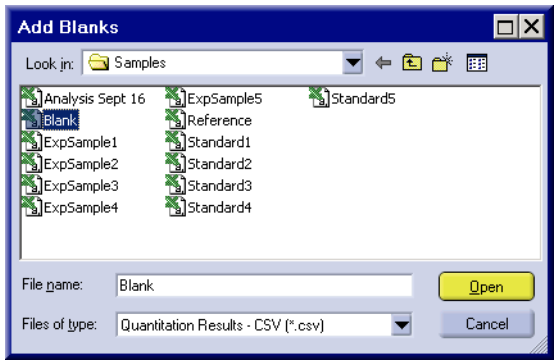
Figure 3–10 The Blank and Reference Data Window

3.9.1 Blank Samples

To add a blank sample

1. In the *Analysis - Blank and Reference Data* window, click **Add** next to the **Blanks** box. The *Add Blanks* window opens.

2. Select the blank sample and blank replicates if there are any. In the example below, the filename is **Blank**.



3. Click **Open**. The window closes and the filename appears in the *Analysis - Blanks and Reference Data* window under the Blanks list.

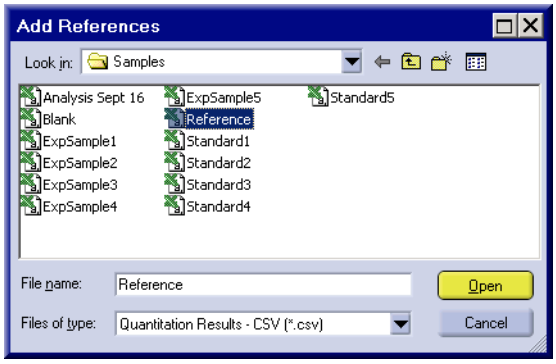
4. In the **Sample ID** edit field, enter an ID or leave the default ("`Blanks`"). If you have added replicate blank samples, the ID specified here is automatically applied to all of the files in the Blanks list.

5. In the **Sample Name**, you can assign a name for all of the blanks.

3.9.2 Reference Samples

To add a reference sample

1. In the *Analysis - Blank and Reference Data* window, click **Add** next to the **References** box. The *Add References* window opens.



2. Select your reference sample(s).

3. Click **Open**. The window closes and the filename appears in the *Analysis - Blanks and Reference Data* window under the References list.

4. In the **Sample ID** edit field, enter a new ID, or leave the default ("`References`"). If you are using 2, 3, or 4 reference samples, the ID specified here is applied to all files in the References list.

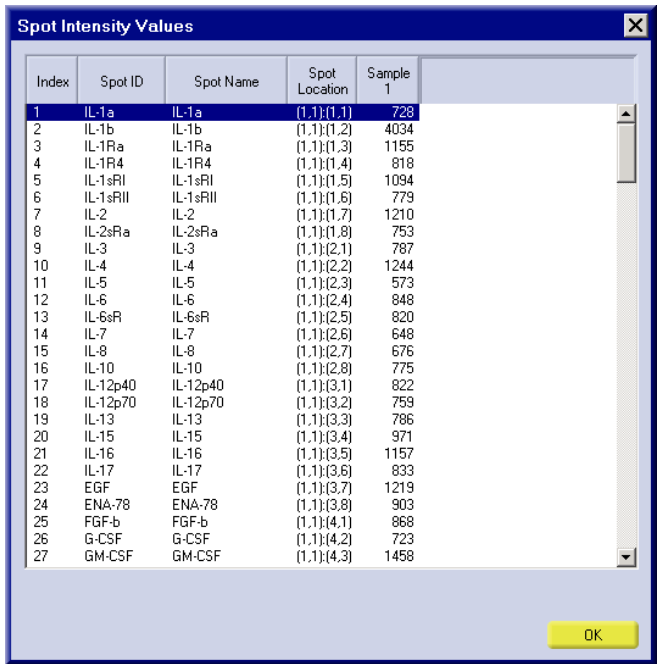
5. In the **Sample Name** edit field, you can assign a name for all of the reference samples.

3.9.3 Viewing Spot Data for Blanks or References

To view spot data for the blank or reference samples

1. In the Blanks or References list, select any file to activate the View Spot Data button.

2. Click **View Spot Data**.
In the S*pot Intensity Values* window, you can view and verify spot data for the blank samples or reference samples, whichever you chose.



Index	Spot ID	Spot Name	Spot Location	Sample 1
1	IL-1a	IL-1a	(1,1):(1,1)	728
2	IL-1b	IL-1b	(1,1):(1,2)	4034
3	IL-1Ra	IL-1Ra	(1,1):(1,3)	1155
4	IL-1R4	IL-1R4	(1,1):(1,4)	818
5	IL-1sRI	IL-1sRI	(1,1):(1,5)	1094
6	IL-1sRII	IL-1sRII	(1,1):(1,6)	779
7	IL-2	IL-2	(1,1):(1,7)	1210
8	IL-2sRa	IL-2sRa	(1,1):(1,8)	753
9	IL-3	IL-3	(1,1):(2,1)	787
10	IL-4	IL-4	(1,1):(2,2)	1244
11	IL-5	IL-5	(1,1):(2,3)	573
12	IL-6	IL-6	(1,1):(2,4)	848
13	IL-6sR	IL-6sR	(1,1):(2,5)	820
14	IL-7	IL-7	(1,1):(2,6)	648
15	IL-8	IL-8	(1,1):(2,7)	676
16	IL-10	IL-10	(1,1):(2,8)	775
17	IL-12p40	IL-12p40	(1,1):(3,1)	822
18	IL-12p70	IL-12p70	(1,1):(3,2)	759
19	IL-13	IL-13	(1,1):(3,3)	786
20	IL-15	IL-15	(1,1):(3,4)	971
21	IL-16	IL-16	(1,1):(3,5)	1157
22	IL-17	IL-17	(1,1):(3,6)	833
23	EGF	EGF	(1,1):(3,7)	1219
24	ENA-78	ENA-78	(1,1):(3,8)	903
25	FGF-b	FGF-b	(1,1):(4,1)	868
26	G-CSF	G-CSF	(1,1):(4,2)	723
27	GM-CSF	GM-CSF	(1,1):(4,3)	1458

3. Click **OK** to close the window.

3.10 Samples Data

In the *Analysis - Samples Data* window (Figure 3-11), add the experiment samples data (the quantitation files from samples with unknown concentrations). After the files are added you can view the spot data for all of the files. You can add up to 100 samples with up to four replicates for each sample.

To add Samples Data

1. In the *Analysis - Samples Data* window, click **Add**.

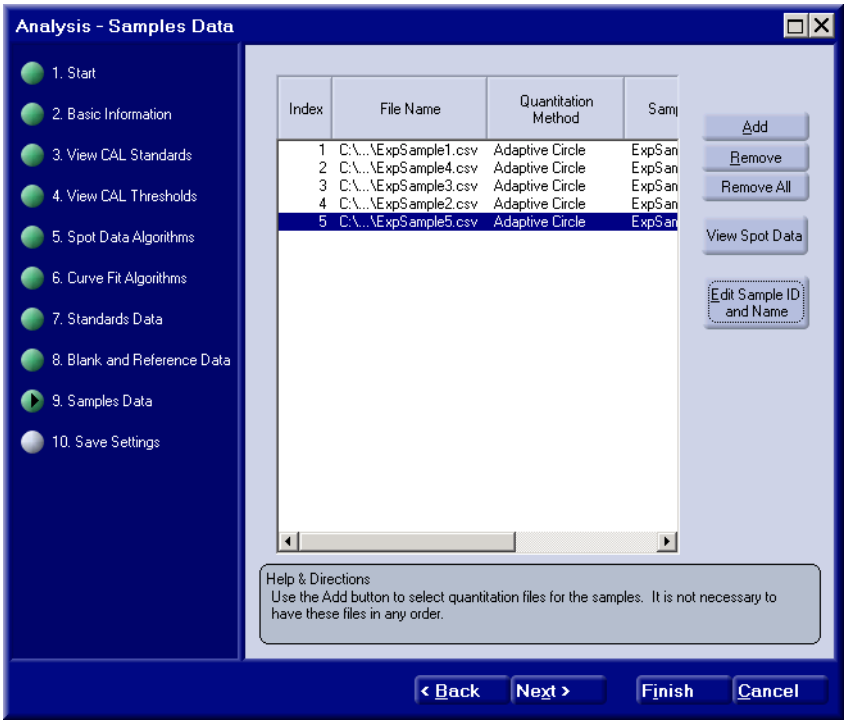
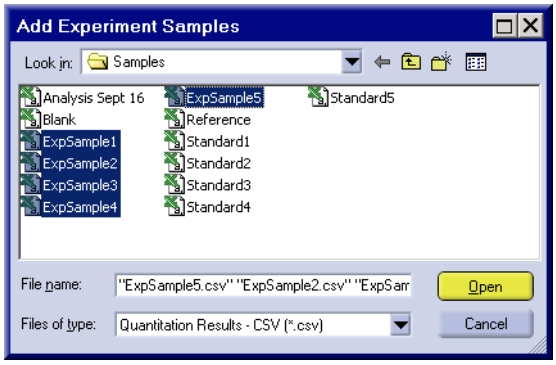


Figure 3–11 Analysis - Samples Data Window

2. In the *Add Experiment Samples* window, you can select all or several of the sample files together:

- Press the **Shift** key, click the first file in the list and click the last file in the list. The first, last, and all files in between are selected; or,
- Press the **Ctrl** key and click each file that you want to add without selecting files in between.



3. Click **Open**. All of the samples files are added as input data.

3.10.1 Assigning Names to Samples and Replicate Samples

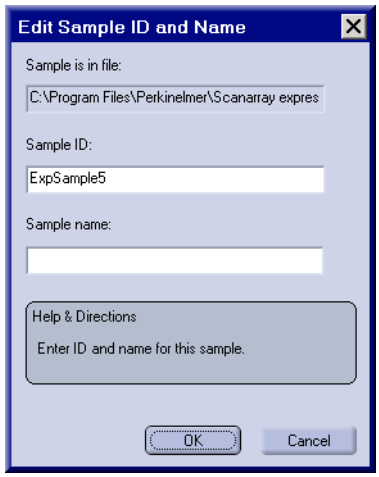
You can assign a **Sample ID** and **Sample Name** to any of the sample files. The name you provide here is the name that ProteinArray Analysis software uses for the analysis. Assigning each sample a meaningful name makes it easier to identify the sample information in the results tabs. If you don't assign a name, ProteinArray Analysis uses the filename without the filename extension.

If you are using sample replicates, you must assign the same Sample ID and Sample Name to each of the replicates so that ProteinArray Analysis recognizes them as replicates. You can add up to four sample replicates for each sample.

To assign a Sample ID and Name

1. In the *Analysis - Samples Data* window, click the filename of the sample for which you want to assign an ID and Name.

2. Click **Edit Sample ID and Name**. The *Edit Sample ID and Name* window opens.



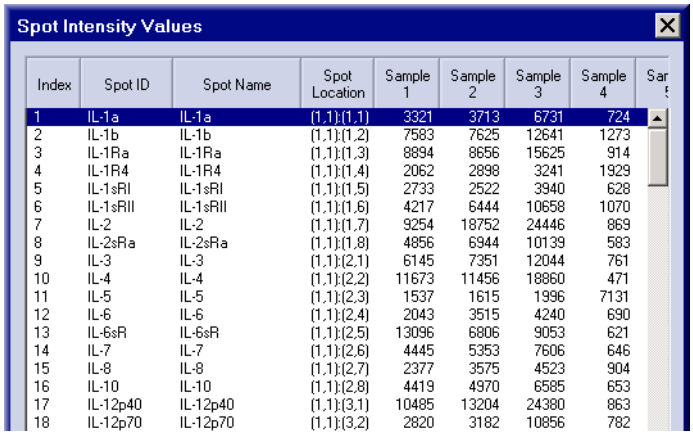
3. Enter a new ID up to 32 characters long in the **Sample ID** field, and enter a new name up to 256 characters long in the **Sample name** field.

4. Click **OK**.

3.10.2 Viewing Spot Data for Samples

To view spot data for the samples

1. Click **View Spot Data**.
   In the S*pot Intensity Values* window, you can view and verify spot data for the samples.



Index	Spot ID	Spot Name	Spot Location	Sample 1	Sample 2	Sample 3	Sample 4	Sar
1	IL-1a	IL-1a	(1,1):(1,1)	3321	3713	6731	724	
2	IL-1b	IL-1b	(1,1):(1,2)	7583	7625	12641	1273	
3	IL-1Ra	IL-1Ra	(1,1):(1,3)	8894	8656	15625	914	
4	IL-1R4	IL-1R4	(1,1):(1,4)	2062	2898	3241	1929	
5	IL-1sRI	IL-1sRI	(1,1):(1,5)	2733	2522	3940	628	
6	IL-1sRII	IL-1sRII	(1,1):(1,6)	4217	6444	10658	1070	
7	IL-2	IL-2	(1,1):(1,7)	9254	18752	24446	869	
8	IL-2sRa	IL-2sRa	(1,1):(1,8)	4856	6944	10139	583	
9	IL-3	IL-3	(1,1):(2,1)	6145	7351	12044	761	
10	IL-4	IL-4	(1,1):(2,2)	11673	11456	18860	471	
11	IL-5	IL-5	(1,1):(2,3)	1537	1615	1996	7131	
12	IL-6	IL-6	(1,1):(2,4)	2043	3515	4240	690	
13	IL-6sR	IL-6sR	(1,1):(2,5)	13096	6806	9053	621	
14	IL-7	IL-7	(1,1):(2,6)	4445	5353	7606	646	
15	IL-8	IL-8	(1,1):(2,7)	2377	3575	4523	904	
16	IL-10	IL-10	(1,1):(2,8)	4419	4970	6585	653	
17	IL-12p40	IL-12p40	(1,1):(3,1)	10485	13204	24380	863	
18	IL-12p70	IL-12p70	(1,1):(3,2)	2820	3182	10856	782	

2. Click **OK** to close the window.

3.11 Saving the Settings

You can save the settings as a **protocol** and save the files you added as a **file set**. A protocol saves the settings for the analysis, including the .CAL file and all of the parameters you've specified. A file set saves the list of standard files, reference files, blank files, and experimental sample files that you've added.

To link the file set to the protocol so that the files are automatically added when you select this protocol again, you must save the file set first. For more information on using protocols and file sets, see Chapter 4.

To save the analysis settings

1. Click **Save Settings** (see Figure 3-12).

2. In the *Analysis - Save Settings* window, click **Save File Set As.**

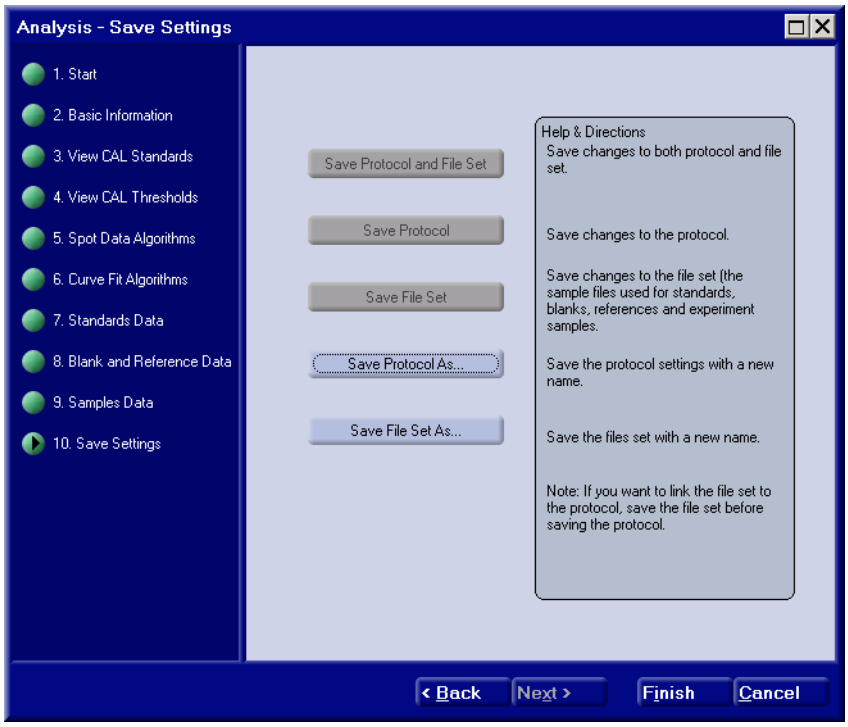


Figure 3–12 The Save Settings Window

3. In the *File Set Name and Description* window, enter a name and optional descriptions for the file set, and click **OK**.

4. In the *Analysis - Save Settings* window, click **Save Protocol As.**

5. In the *Protocol Name and Description* window, enter a name and optional description for the protocol and click **OK**.

3.12 Finishing the Analysis

After making the selections in all windows of the Analysis wizard, you can go back to any of the windows to check the data and selections. If all data is as it should be, you're ready to run the analysis.

To run the analysis

1. In the Analysis wizard, click **Finish**.

Within a few seconds, depending on how many samples you added, the analysis results display in the ScanArray Express *Main Window.* Results include four tabbed windows (or five, with reference data) with the Standard Curve Plot tab in the front.

Note: For a complete description of the analysis results, see Chapter 5, "Viewing the Results."

3.13 Testing Curve Fitting before Adding Samples

Before analyzing your data, you can check the curve fitting by running an analysis without samples, and select the resulting curve fitting method that works best for your data. For details on any one of the steps, refer to the appropriate section in this chapter.

To test curve fitting

1. Load your .CAL file. See ***Basic Information*** on page 3-2.
2. Optionally check the .CAL file information using Steps 2 and 3 of the Analysis Wizard.
3. Select the curve fitting methods in Step 5 of the Analysis wizard. See ***Curve Fit Algorithms*** on page 3-13.
4. Add your standards file. See ***Standards Data*** on page 3-16.
5. Optionally add a blank sample. See ***Blank and Reference Data*** on page 3-18.
6. Click **Finish**.
7. Review the curve plots to see how well they fit, and try different fitting methods until you get a good fit.

Each curve plot includes Goodness of Fit information, or you can view the Goodness of Fit information for all analytes at once in the *Select an Analyte* window (section 5.2.2, "Selecting Analytes and Viewing Standard Curve Data").

Protocols and File Sets

Chapter 4

Chapter Summary

4.1 Overview

This chapter provides instructions for creating and using analysis protocols and file sets. A *protocol* is a set of analysis instructions that have been saved to be used again. including the specified .CAL file and all of the parameters for the analysis. A *file set* are the files added to the analysis wizard and saved to be used again, including the standard files, reference files, blank files, and experimental sample files.

A file set can be "linked" to a protocol, so that the files are automatically added whenever that protocol is selected for running an analysis.

Note: When creating and saving a protocol, to link the added files to the protocol as a file set, you must save the file set first, then save the protocol.

4.2 Using an Analysis Protocol

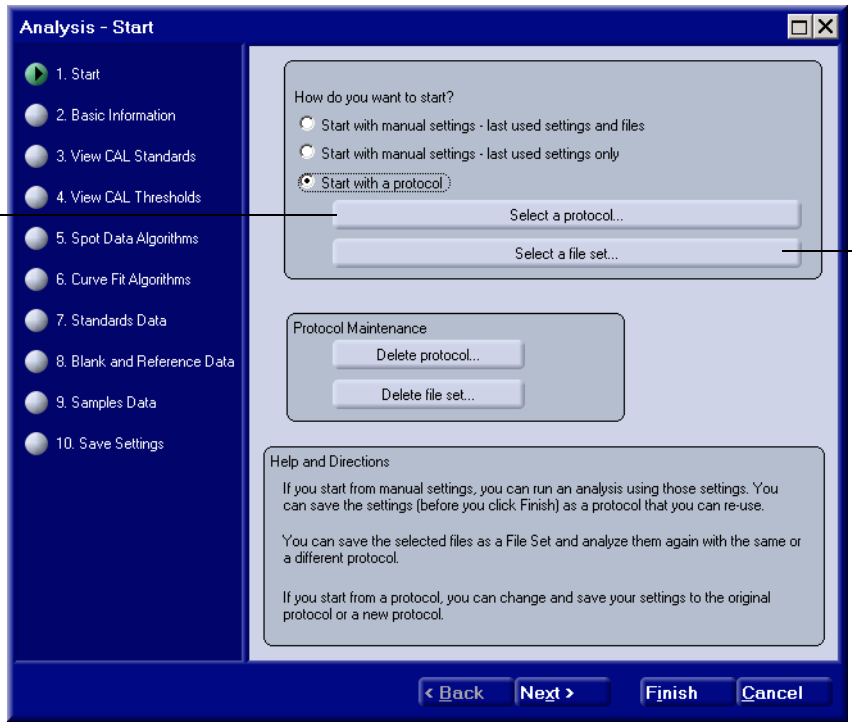
To use an existing protocol to run an analysis, start ProteinArray Analysis, and select the protocol, as follows.

To use an existing analysis protocol

1. Start the ScanArray Express if it is not already running, by clicking the ScanArray Express icon on the computer desktop.

2. In the *Main Window*, click **Analyze**.

3. In the *Analysis - Start* window, select **Start with a protocol**.

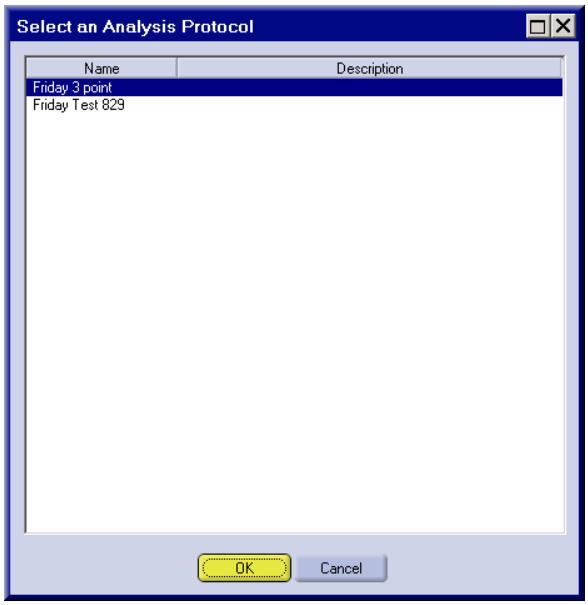


The button displays the name of the selected protocol.

The button displays the name of the selected file set.

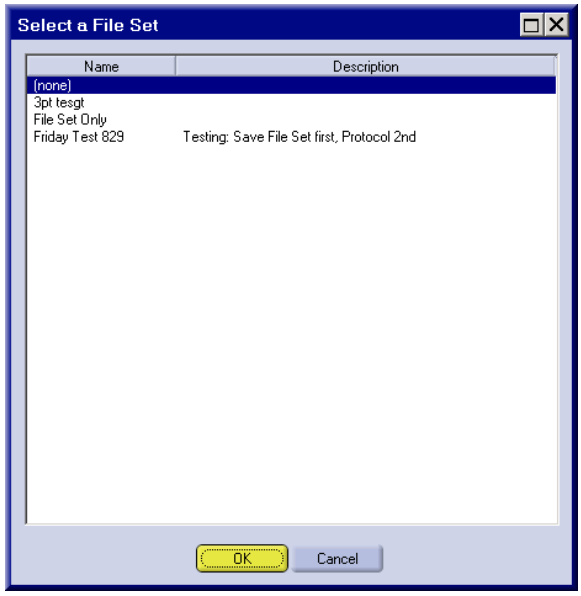
Figure 4–1 The Analysis - Start Window

4. Click **Select a protocol**. In the *Select an Analysis Protocol* window, select the protocol to use and click **OK**.



The name of the protocol displays in the **Select a protocol** button on the *Analysis - Start* window. If a file set was linked to the protocol, the file set is automatically selected and the name of the file set displays in the **Select a file set** button (see Figure 4-1).

5. To select a file set, click **Select a file set**.



6. In the *Select a File Set* window, select the file set you want you use, and click **OK**. The name of the selected file set displays in the **Select a file set** button (see Figure 4-1).

7. Click **Next** to open the *Analysis - Basic Information* window.

Note: If you want to select a different .CAL file to use with this analysis, select it now.

8. Click **Finish**. The analysis results displays within a few seconds. See Chapter 5, Reviewing Analysis Results, for a description.

4.3 Creating a Protocol

To create a protocol, use the Analysis wizard to specify the settings you want to use, then save them with a protocol name. You can also open an existing protocol that is similar to what you need, edit it, and save it with a new name.

- If you want to link the added files to the protocol, you must save them first as a file set, before saving the protocol (see Section 4.4).
- You can immediately run an analysis with the settings you've just entered, but to save them as a protocol, save the settings before clicking **Finish**.

To create and save a protocol

1. Click **Analyze** on the ScanArray Express *Main Window.*

2. In the *Analysis - Start* window, select **Start with manual settings - last used settings and files**, or **Start with manual settings - last used settings only.**

3. Add or change the files and enter or change the settings for your analysis. Refer to Chapter 3 for instructions.

4. Click step 10, **Save Settings** (see Figure 4-2).

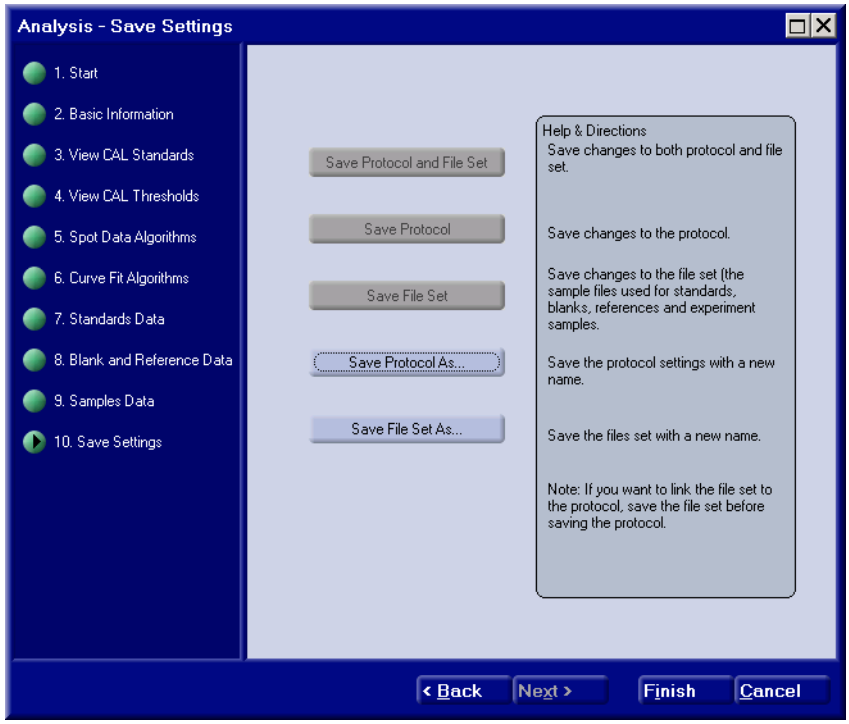


Figure 4–2 The Analysis - Save Settings window

5. Click **Save Protocol As**.

6. In the *Protocol Name and Description* window, enter a name and optional description for the protocol.

To edit an existing protocol

1. Load the protocol (see Section 4.2)

2. Make changes to any of the settings.

3. Click **Save Settings**.

4. In the *Analysis - Save Settings* window, click one of the following:

- **Save Protocol and File Set** to overwrite both the existing protocol and existing file set with any changes you've made.
- **Save Protocol** to overwrite the existing protocol with the changes.
- **Save Protocol As** to save the changes as a new protocol with a different name (leaving the original protocol as it was).

5. Click **Finish** to run the loaded protocol, or **Cancel** to leave the wizard.

4.4 Creating a File Set

A file set includes the standard files, optional blank and/or reference files, and sample files for your analysis. A file set can be linked to a particular protocol by saving the file set, then saving the loaded protocol. See Chapter 3 for instructions on adding files.

To create and save a file set

1. Click **Analyze** on the ScanArray Express *Main Window.*
2. In the *Analysis - Start* window, select **Start with manual settings**.
3. Add your analysis files (standards, samples, and optional blank and/or reference).
4. Click **Save Settings**.
5. In the *Analysis - Save Settings* window, click **Save File Set As.**
6. In the *File Set Name and Description* window, enter a name for the file set and an optional description.

7. Click **OK.**
8. You can save the current settings as a protocol if you haven't already. See Section 4.3.

To edit an existing file set

1. Select the file set and select a protocol, or select a protocol with a linked file set (see Section 4.2).
2. Edit the file set: you can delete, re-order, or add files.
3. Click **Save Settings**.
4. In the *Analysis - Save Settings* window, click one of the following:

- **Save Protocol and File Set** to overwrite both the opened protocol and file set with any changes you've made.
- **Save File Set** to overwrite the opened file set with the changes.
- **Save File Set As** to save the changes as a new file set with a different name (leaving the original file set as it was).

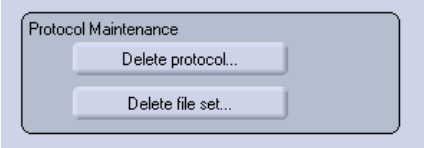
5. Click **Finish** to run the selected protocol and file set, or click **Cancel** to leave the wizard.

4.5 Deleting a Protocol

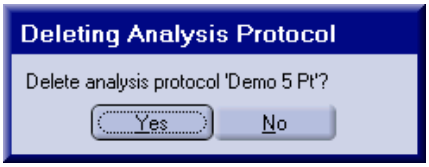
You can delete one or more protocols from within ProteinArray Analysis.

To delete a protocol

1. Click **Analyze** on the ScanArray Express *Main Window*.
2. On the *Analysis - Start* window under Protocol Maintenance, click **Delete protocol**.



3. In the *Select an Analysis Protocol* window, click the protocol that you want to delete and click **OK**.
4. When prompted, click **Yes** to delete the protocol, or **No** to cancel the deletion.



5. Repeat steps 2 to 4 for each protocol you want to delete.

4.6 Deleting a File Set

You can delete one or more file sets from within the ProteinArray Analysis wizard.

To delete a file set

1. On the *Analysis - Start* window, click **Delete file set**.
2. In the *Select a File Set* window, click the file set you want to delete, and click **OK**.

3. When prompted, click **Yes** to delete the file set, or **No** to cancel the deletion.

4. Repeat steps 1 to 3 for every file set you want to delete.

Reviewing Analysis Results

Chapter 5

Chapter Summary

5.1 Overview

Your analysis results display in the ScanArray Express *Main Window* as four or five tabs, depending on the options selected for analysis:

- a **Standard Curve Plot** - provides standard curves, one analyte at a time. This is a customizable view that you can print and/or save as a bitmap (BMP) or JPEG file.
- a **Concentrations Bar Chart** - displays the concentrations and, if specified, the normal range, for all analytes in all samples. This is a customizable view that you can print and/or save as a bitmap (BMP) or JPEG file.
- a **Concentrations** spreadsheet - displays the analysis results data from all samples for each analyte. This spreadsheet can be saved as a .CSV file or exported to Excel and saved.
- a **Raw Spreadsheet** - displays the raw data in a spreadsheet for each sample. This spreadsheet can be saved as an .xls file, or exported to Excel and saved.

and, if you used a reference sample:

- a **Ratio to Reference Plot** - shows the ratios in a scatter plot. This is a customizable view that you can print and/or save as a bitmap (BMP) or JPEG file.

The results tabs are described in the following sections.

Note: For ease-of-viewing, the tabs are “interactive,” that is, when you:

- select an analyte to view, that same analyte is automatically selected if you move to a different tab.
- select samples to include in or exclude from the view, these selections hold if you move to a different tab.

5.2 The Standard Curve Plot

The *Standard Curve Plot* tab shows the standard curve for one analyte at a time, in a customizable view that lets you change the display properties and select which analyte to display. You can also save and/or print any curve. The example in Figure 5-1 shows a typical curve plot using all of the display properties including the data points, curve line, normal range in green, and algorithm information as text in the lower right corner.

Item	Description
	*Standard data points* Each standard data point is indicated by a purple diamond. The purple "I-beam" through the diamond is an error bar for standard replicates. These indicators are customizable; see section 5.2.4.
	*Sample data points* Each experiment sample is indicated by a black tick mark, and error bars for replicate samples are a black "I-beam" through the tick mark. These indicators are customizable - see section 5.2.4.

The name of the currently selected analyte is indicated in the title above the plot.

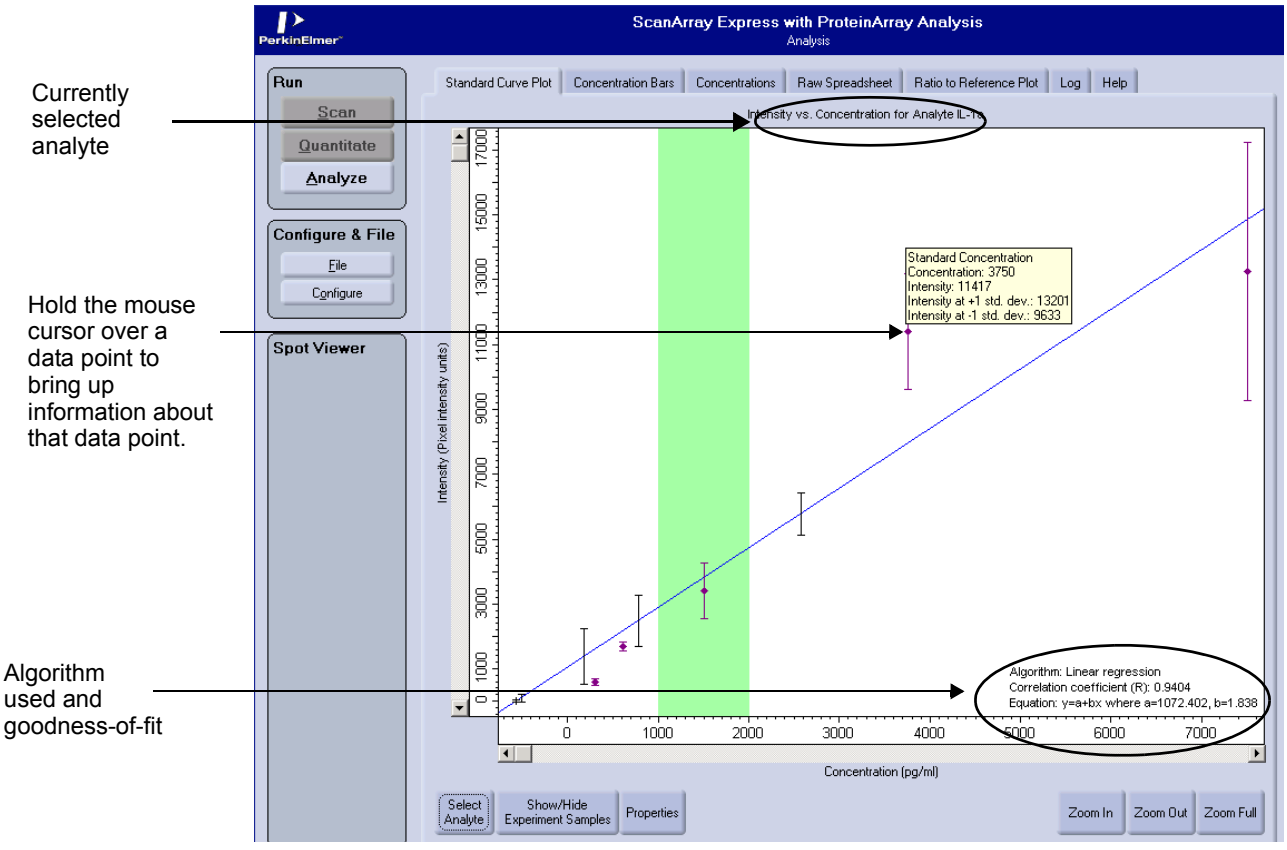


Figure 5–1 A Standard Curve Plot with Default Display Properties

Your results may not show the same properties, depending on how they were set for the previous analysis. To change the properties of the display, or if your results don't show properties you expected to see, refer to Section 5.2.4 for more information.

5.2.1 Information Provided by the Standard Curve Plot

Table 5-1 provides a description of the information and selectable buttons in the Standard Curve Plot.

Table 5-1: Standard Curve Plot

Item	Description
Intensity (pixel intensity units)	Indicates pixel intensity units, on the **y** axis of the plot.
Concentration (pg/ml)	Shows the concentration, on the **x** axis, for each standard sample, obtained from the CAL file, and of each experimental sample, obtained from the analysis.
Plot Information (Goodness-of-fit)	Displays the curve fit algorithm used (linear regression, cubic spline, b-spline, logistic), the goodness-of-fit, and for linear and logistic, the equation used and coefficient value. Goodness of Fit: 0 = poor fit; 1- fits perfectly; any number of .9 or higher is a good fit.
Buttons	
Select Analyte	Opens the *Select an Analyte* window, where you can select a different analyte to display its curve plot.
Show/Hide Experiment Samples	Opens the *Show/Hide Experiment Samples* window, where you can select all, none, or any number of samples to show or not show in the curve plot.
Properties	Allows you to change the way which information is displayed, and some of the display characteristics. See ***Changing the Display Properties of the Curve Plot*** on page 5-5.
Zoom in, Zoom out, Zoom Full	Lets you zoom in to see up-close detail in a portion of the plot, zoom back out, or zoom to the full plot. You cannot zoom out any further than the minimum values specified for "Zoom Full" display. You can change the minimum values in the Properties window.

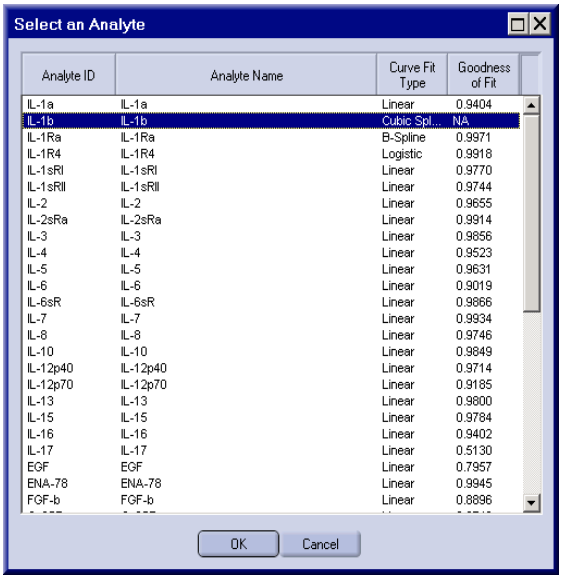
5.2.2 Selecting Analytes and Viewing Standard Curve Data

You can select any analyte to display its curve plot, or view the curve fit type and goodness-of-fit for all analytes.

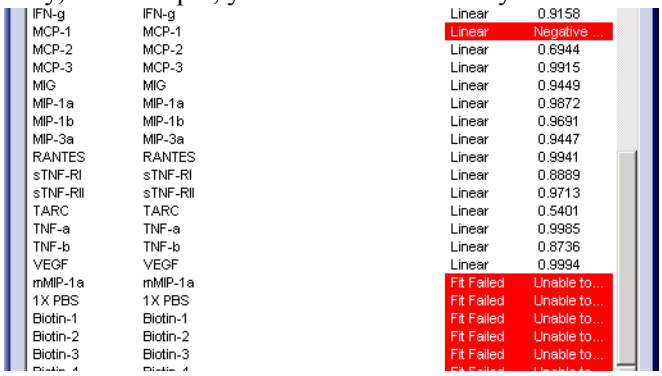
To select an analyte or view curve fit data

1. In the *Standard Curve Plot*, click **Select Analyte**. In the *Select an Analyte* window that opens, click the name of the analyte you want to view.

   This window also provides the standard curve data for all analytes (method used and goodness-of-fit).



   If any of the curve fits failed, a "Fit Failed" message is highlighted in red to explain why, for example, your standards data may have been incorrect.



2. Click **OK**. The tab displays the curve plot for the selected analyte.

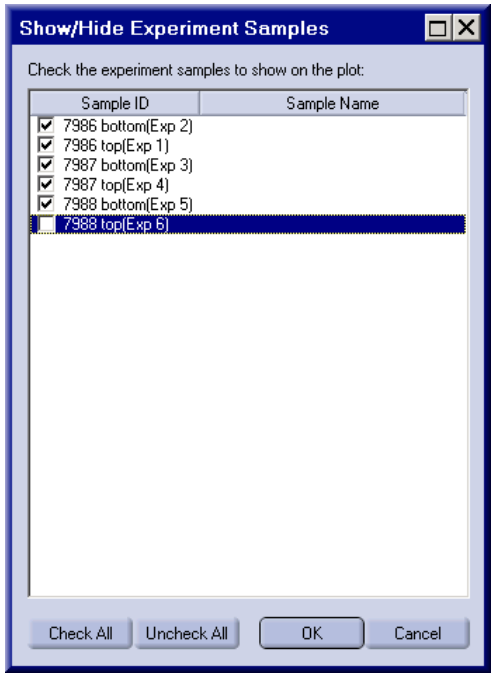
5.2.3 Including or Excluding Samples for the Display

By default, the results for all samples are displayed.

To exclude or add different samples from the display

1. Click **Show/Hide Experiment Samples**.

The *Show/Hide Experimental Samples* window opens.



2. Check all of the experiments you want to show on the plot. Uncheck any experiment samples that you don't want to show.

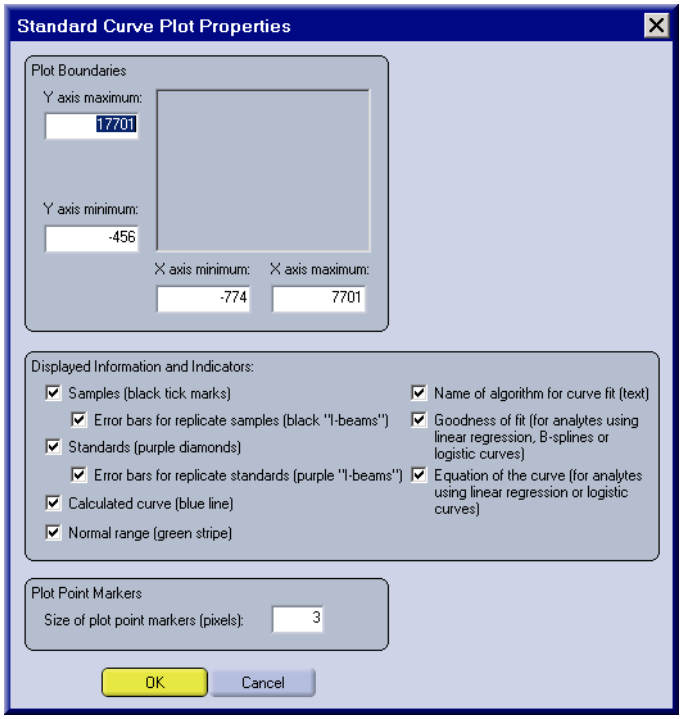
   You can also:
   - click **Check All** to show all samples
   - click **Uncheck All** to deselect all samples, then check only the sample(s) you want to show.

5.2.4 Changing the Display Properties of the Curve Plot

You can change the boundaries of the x-y axes, and change what information is displayed and the indicators used for the information.

To change the plot curve properties

1. Click **Properties**. The *Standard Curve Plot Properties* window opens.



2. Check the display items that you want to display; uncheck any display items you want to remove. The display options are described in Table 5-2.

Table 5-2: Standard Curve Display Options

Item	Description
Plot Boundaries	For plot boundaries, the ProteinArray Analysis calculates the minimum and maximum values from the curve. You can set new minimum and maximum numbers for the x, y, or both x and y axes.
Displayed Information and Indicators	
Samples (black tick mark)	Indicates the location of each sample on the curve with a small vertical tick mark. Checking this box enables the subordinate box: **Error bars for replicate samples** (a small bar at each end of the tick mark) This is the standard deviation for the replicate samples.
Standards (purple diamonds)	Check this box to show a purple diamond for each standard. Checking this box enables the subordinate box: **Error bars for replicate standards** (a purple I-beam) This is the standard deviation for the replicate standards.
Calculated curve	Displays the standard curve as a blue line.

Item	Description
Normal Range	If upper and lower thresholds were specified in the CAL file, checking this box indicates the normal range with a green stripe.
Name of algorithm	Displays the name of the algorithm in text (linear regression, cubic spline, B-spline, or logistic).
Goodness of Fit	Displays the goodness of fit for this analyte (for linear regression, B-spline, or logistic curves): 1 - fits perfectly 0 - poor fit A number above .9 is acceptable.
Equation of Curve	Displays the equation used to create the curve (for linear regression or logistic curves only).
Plot Point Markers	To make the markers on the plotted points larger or smaller, specify a point size here. The default value is 3 points.

5.3 Concentration Bars

The *Concentration Bars* tab displays a bar graph of the analyte concentrations and ID for all samples in the experiment in a view that lets you group by analyte or by sample.

Figure 5-2 is an illustration of a typical bar graph grouped by analyte and using all of the display options, including the bars, the standard deviation, and the normal range in green. If you want to change the properties of the display, or if your results don't show information you expected to see, refer to Section 5.3.2 for more information.

You can zoom in and scroll up and down or left and right, zoom out, or zoom back to the full display. Holding the mouse cursor over a data point displays information about that analyte, including the analyte ID and name, sample ID and name, concentration value and standard deviation.

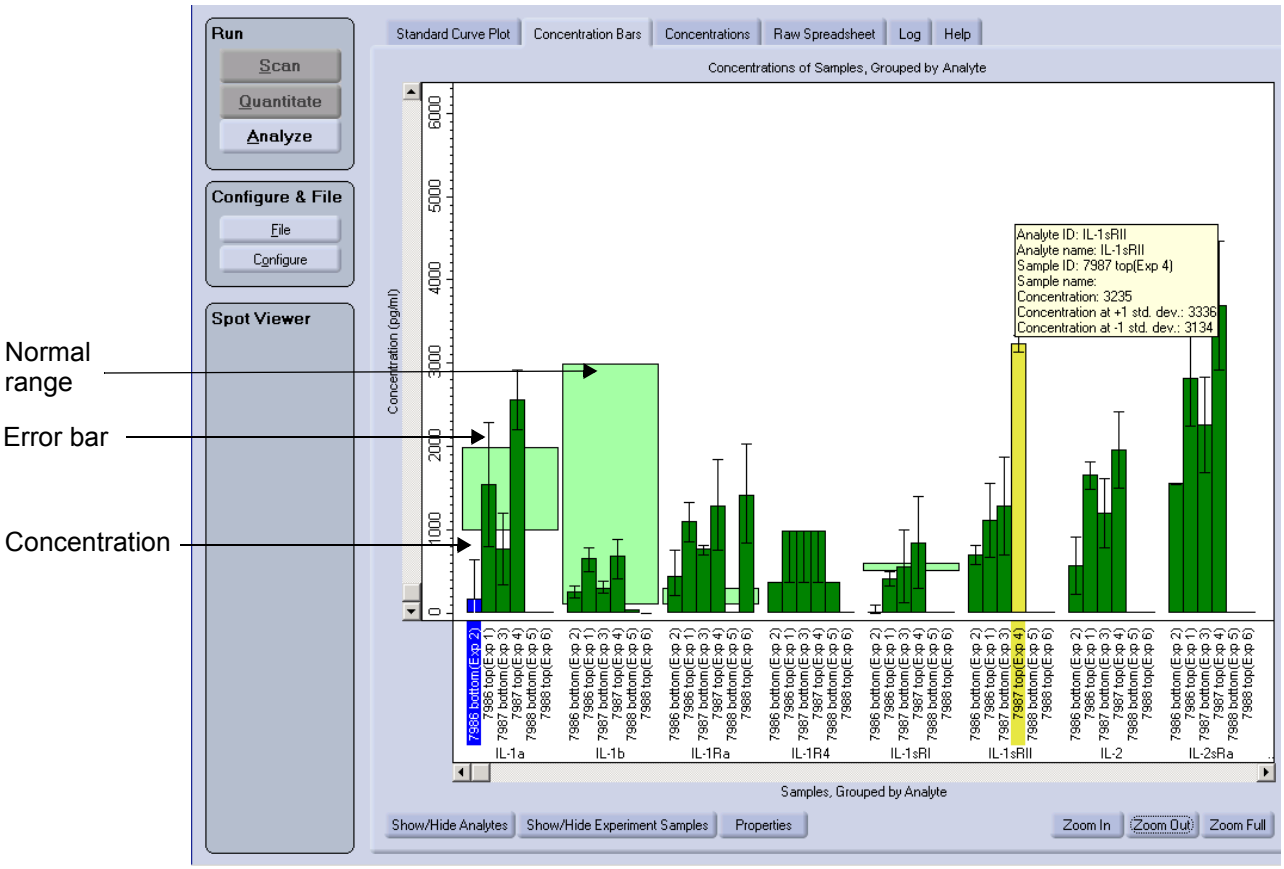


Figure 5–2 Concentration Bars

As with the *Standard Curve Plot*, you can show or hide analytes and show or hide experiment samples. See the instructions on page 5-4.

5.3.1 Information Provided by the Concentration Bars

The *Concentration Bars* tab displays the following information:

Item	Description
Concentrations (pg/ml)	Shows the concentration for all analytes in all samples, grouped by analyte or by sample, and the units of measurement used (obtained from the CAL file).
Samples, (Grouped by Analyte or Grouped by Sample)	Displays the names of all analytes, grouped by analyte, or grouped by sample.
Normal Range	Displays in green, only when grouping by analyte.

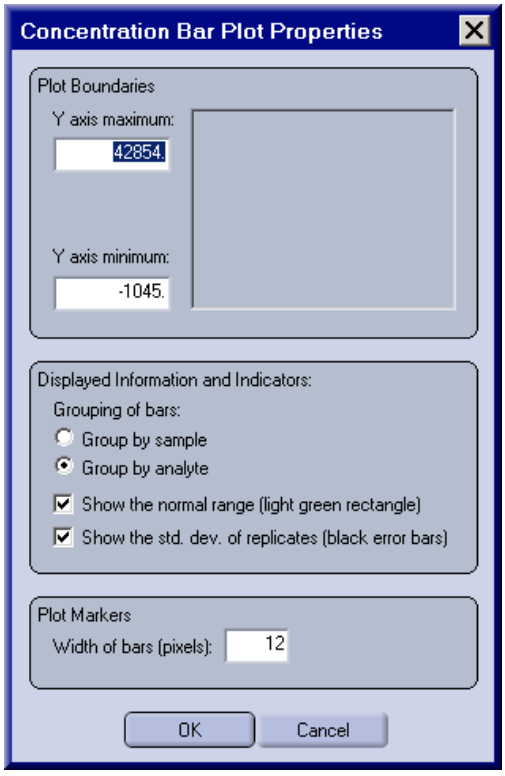
Item	Description
Show/Hide Analytes	Opens the *Show/Hide Analyte* window, where you can select all, none, or any number of the analytes you want to show or not show in the bar graph, by checking or unchecking the box beside the analyte name.
Show/Hide Experiment Samples	Opens the *Show/Hide Experiment Samples* window, where you can select, all, none, or any number of samples to show or not show in the bar chart by checking or unchecking the box beside the sample name.
Properties	Allows you to change the way certain information displays. See Section 5.3.2.

5.3.2 Changing the Display Properties of the Bar Chart

You can change the dimensions of the x or y axes, what information is displayed, and the indicators used for the information.

To change the properties

1. In the *Concentration Bars* tab, click **Properties**. The following window opens.



2. Refer to Table 5-3 and edit the display properties that you want to remove or change. Click **OK** when you're finished.

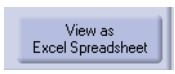
Table 5-3: Concentration Bars Display Options

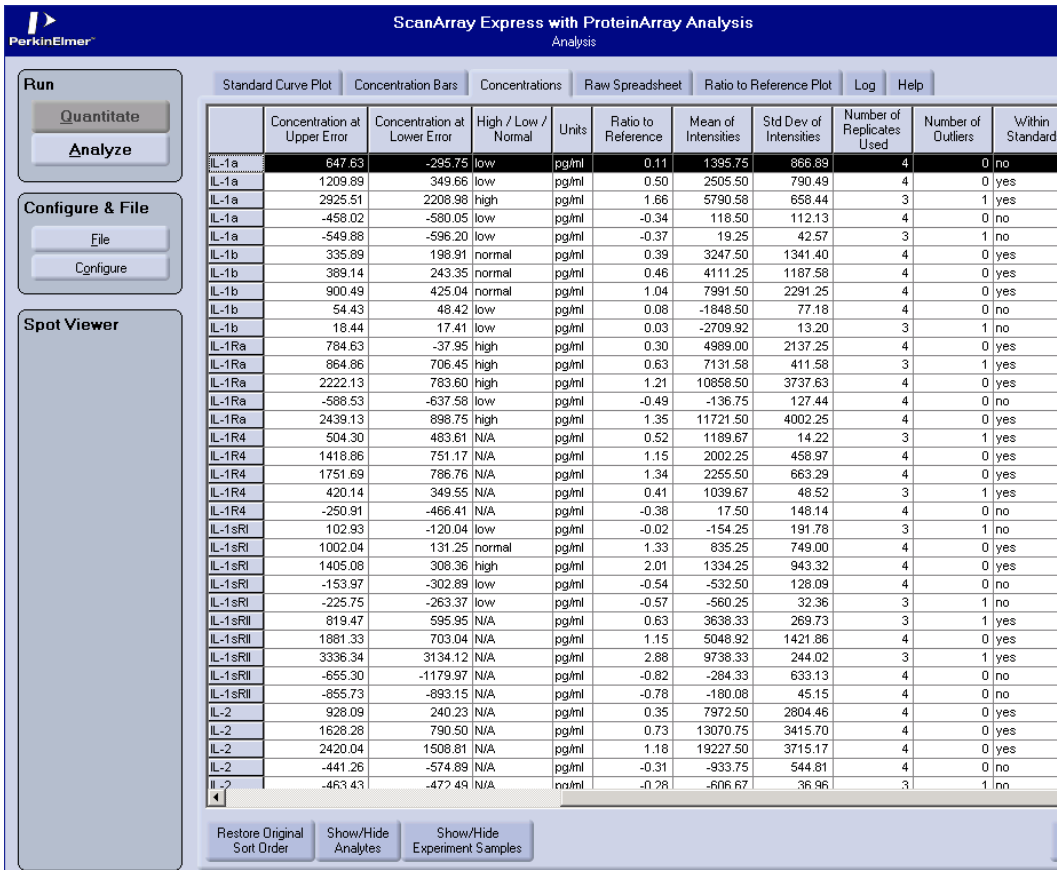
Item	Description
Plot Boundaries	This lets you change the Y axis maximum and the Y axis minimum.
Displayed Information and Indicators	
Grouping of bars:	Select **Group by sample** or **Group by analyte**
Show the normal range	If upper and lower thresholds were specified in the CAL file, checking this box shows the normal range with a light green rectangle behind the bar graph. Shown only when grouping by analyte.
Show the std. dev. of replicates	Check this box to show the standard deviation of each analyte as a black bar.
Plot Markers: Width of bars (pixels)	To change the width of the bars, enter the number of pixels to use. The default is 12 pixels.

5.4 Concentrations (Analysis Results)

The *Concentrations* spreadsheet provides, by analyte, the data for all analytes from all samples from a successful curve fit. You can scroll through the information, double-click any column header to arrange the information in ascending order, and double-click again to arrange in descending order, based on the information for the header that you clicked.

You can also export the information to a Microsoft Excel spreadsheet for further analysis (you must have Excel installed on your computer).

Item	Description
	Click this button after changing the sort order to restore the original order.
	Click this button to open the *Show/Hide Analytes* window, where you can check each analyte to include in the display, and uncheck each analyte to exclude from the display.
	Click this button to open the *Select an Experimental Sample* window, where you can select which sample you want to view in the tab.
	Click this button to export the data to an Excel spreadsheet. The spreadsheet of analysis results displays as a sheet in the workbook.



	Concentration at Upper Error	Concentration at Lower Error	High / Low / Normal	Units	Ratio to Reference	Mean of Intensities	Std Dev of Intensities	Number of Replicates Used	Number of Outliers	Within Standard
IL-1a	647.63	-295.75	low	pg/ml	0.11	1395.75	866.89	4	0	no
IL-1a	1209.89	349.66	low	pg/ml	0.50	2505.50	790.49	4	0	yes
IL-1a	2925.51	2208.98	high	pg/ml	1.66	5790.58	658.44	3	1	yes
IL-1a	-458.02	-580.05	low	pg/ml	-0.34	118.50	112.13	4	0	no
IL-1a	-549.88	-596.20	low	pg/ml	-0.37	19.25	42.57	3	1	no
IL-1b	335.89	198.91	normal	pg/ml	0.39	3247.50	1341.40	4	0	yes
IL-1b	389.14	243.35	normal	pg/ml	0.46	4111.25	1187.58	4	0	yes
IL-1b	900.49	425.04	normal	pg/ml	1.04	7991.50	2291.25	4	0	yes
IL-1b	54.43	48.42	low	pg/ml	0.08	-1848.50	77.18	4	0	no
IL-1b	18.44	17.41	low	pg/ml	0.03	-2709.92	13.20	3	1	no
IL-1Ra	784.63	-37.95	high	pg/ml	0.30	4989.00	2137.25	4	0	yes
IL-1Ra	864.86	706.45	high	pg/ml	0.63	7131.58	411.58	3	1	yes
IL-1Ra	2222.13	783.60	high	pg/ml	1.21	10858.50	3737.63	4	0	yes
IL-1Ra	-588.53	-637.58	low	pg/ml	-0.49	-136.75	127.44	4	0	no
IL-1Ra	2439.13	898.75	high	pg/ml	1.35	11721.50	4002.25	4	0	yes
IL-1R4	504.30	483.61	N/A	pg/ml	0.52	1189.67	14.22	3	1	yes
IL-1R4	1418.86	751.17	N/A	pg/ml	1.15	2002.25	458.97	4	0	yes
IL-1R4	1751.69	786.76	N/A	pg/ml	1.34	2255.50	663.29	4	0	yes
IL-1R4	420.14	349.55	N/A	pg/ml	0.41	1039.67	48.52	3	1	yes
IL-1R4	-250.91	-466.41	N/A	pg/ml	-0.38	17.50	148.14	4	0	no
IL-1sRI	102.93	-120.04	low	pg/ml	-0.02	-154.25	191.78	3	1	no
IL-1sRI	1002.04	131.25	normal	pg/ml	1.33	835.25	749.00	4	0	yes
IL-1sRI	1405.08	308.36	high	pg/ml	2.01	1334.25	943.32	4	0	yes
IL-1sRI	-153.97	-302.89	low	pg/ml	-0.54	-532.50	128.09	4	0	no
IL-1sRI	-225.75	-263.37	low	pg/ml	-0.57	-560.25	32.36	3	1	no
IL-1sRII	819.47	595.95	N/A	pg/ml	0.63	3638.33	269.73	3	1	yes
IL-1sRII	1881.33	703.04	N/A	pg/ml	1.15	5048.92	1421.86	4	0	yes
IL-1sRII	3336.34	3134.12	N/A	pg/ml	2.88	9738.33	244.02	3	1	yes
IL-1sRII	-655.30	-1179.97	N/A	pg/ml	-0.82	-284.33	633.13	4	0	no
IL-1sRII	-855.73	-893.15	N/A	pg/ml	-0.78	-180.08	45.15	4	0	no
IL-2	928.09	240.23	N/A	pg/ml	0.35	7972.50	2804.46	4	0	yes
IL-2	1628.28	790.50	N/A	pg/ml	0.73	13070.75	3415.70	4	0	yes
IL-2	2420.04	1508.81	N/A	pg/ml	1.18	19227.50	3715.17	4	0	yes
IL-2	-441.26	-574.89	N/A	pg/ml	-0.31	-933.75	544.81	4	0	no
IL-2	-463.43	-472.49	N/A	pg/ml	-0.28	-606.67	36.96	3	1	no

5.4.1 Information Provided in the Concentrations Tab

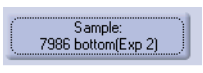
In the Concentrations tab, for each row representing one analyte, each data column provides the following information for the analyte.

Item	Description
Index	
Sample ID	This is the Sample ID that you assigned when adding the sample files. If you didn't assign a name, the ProteinArray Analysis uses the filename without the filename extension.
Sample Name	This is the Sample Name that you assigned. This column is blank if you didn't assign a name.
Analyte ID	The analyte ID assigned for the spot.
Analyte Name	The analyte name assigned for the spot.
Concentration	Shows the estimated concentration for the analyte in the sample listed in this row.
Concentration at Upper error	The concentration calculated at the upper point of the intensity error bar.

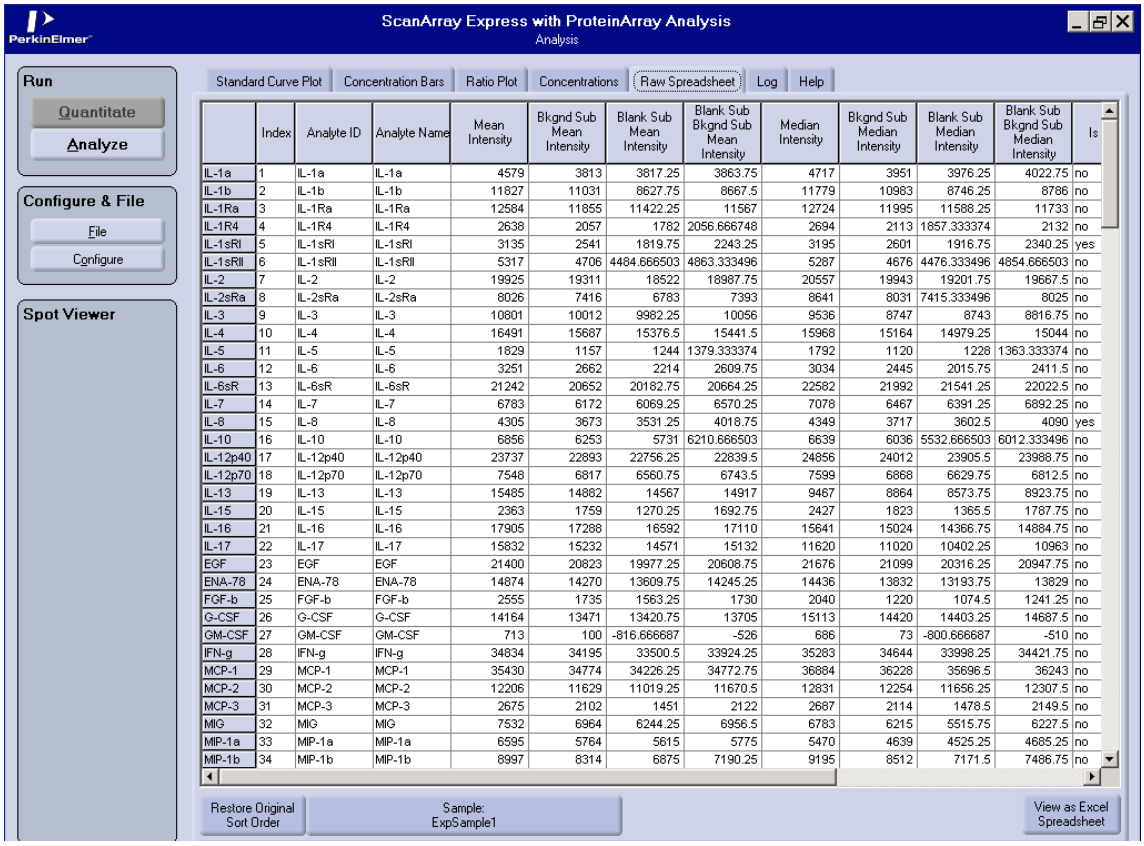
Item	Description
Concentration at Lower error	The concentration calculated at the lower point of the intensity error bar.
High/Low/Normal (or NA)	Specifies whether the calculated concentration is higher, lower, or in the normal range. If no threshold levels are specified in the .CAL file, “NA” displays in the column.
Units	Concentration unit used for this analyte; that is, **`pg/ml`**, **`picograms/milliliter`**, **`ng/ml`** or **`nanograms/milliliter`**)
Ratio to Reference	The ratio of each estimated concentration of experimental sample to the corresponding estimated concentration of reference sample. If no reference was selected for the analysis, this column displays “NA”.
Mean of Intensities	This is the value of the mean intensities of the replicate spots. If a blank sample is used, Mean of Intensities is the blank-subtracted value.
Std Dev of Intensities	This is the standard deviation of the replicate spots.
Number of Replicates Used	Indicates the number of replicates used, after outliers are removed.
Number of outliers	Lists the number of outliers identified and removed during the analysis.
Within Standards	Indicates whether or not the estimated concentration is within the standard limits (Yes or No).

5.5 Spreadsheet of Raw Data

The *Raw Spreadsheet* tab displays a spreadsheet of raw data for all samples, all standards, blanks and references, one sample at a time. You can change the sample display, and view the data as an Excel spreadsheet.

Item	Description
	Click this button after changing the sort order to restore the original order.
	Click this button to open the *Select a Sample* window, where you can select which sample you want to view. The name of the currently selected sample displays in the button.

Item	Description
View as Excel Spreadsheet	Click this button to export the data to an Excel workbook. Each spreadsheet of raw data is opened as a sheet in the workbook. The resulting workbook includes a sheet for each sample, a sheet for all standards, a sheet for blanks, if used, and a sheet for references if used.



	Index	Analyte ID	Analyte Name	Mean Intensity	Bkgnd Sub Mean Intensity	Blank Sub Mean Intensity	Blank Sub Bkgnd Sub Mean Intensity	Median Intensity	Bkgnd Sub Median Intensity	Blank Sub Median Intensity	Blank Sub Bkgnd Sub Median Intensity	Is
IL-1a	1	IL-1a	IL-1a	4579	3813	3817.25	3863.75	4717	3951	3976.25	4022.75	no
IL-1b	2	IL-1b	IL-1b	11827	11031	8627.75	8667.5	11779	10983	8746.25	8786	no
IL-1Ra	3	IL-1Ra	IL-1Ra	12584	11855	11422.25	11567	12724	11995	11588.25	11733	no
IL-1R4	4	IL-1R4	IL-1R4	2638	2057	1782	2056.666748	2694	2113	1857.333374	2132	no
IL-1sRI	5	IL-1sRI	IL-1sRI	3135	2541	1819.75	2243.25	3195	2601	1916.75	2340.25	yes
IL-1sRII	6	IL-1sRII	IL-1sRII	5317	4706	4484.666503	4863.333496	5287	4676	4476.333496	4854.666503	no
IL-2	7	IL-2	IL-2	19925	19311	18522	18987.75	20557	19943	19201.75	19667.5	no
IL-2sRa	8	IL-2sRa	IL-2sRa	8026	7416	6783	7393	8641	8031	7415.333496	8025	no
IL-3	9	IL-3	IL-3	10801	10012	9982.25	10056	9536	8747	8743	8816.75	no
IL-4	10	IL-4	IL-4	16491	15687	15376.5	15441.5	15968	15164	14979.25	15044	no
IL-5	11	IL-5	IL-5	1829	1157	1244	1379.333374	1792	1120	1228	1363.333374	no
IL-6	12	IL-6	IL-6	3251	2662	2214	2609.75	3034	2445	2015.75	2411.5	no
IL-6sR	13	IL-6sR	IL-6sR	21242	20652	20182.75	20664.25	22582	21992	21541.25	22022.5	no
IL-7	14	IL-7	IL-7	6783	6172	6069.25	6570.25	7078	6467	6391.25	6892.25	no
IL-8	15	IL-8	IL-8	4305	3673	3531.25	4018.75	4349	3717	3602.5	4090	yes
IL-10	16	IL-10	IL-10	6856	6253	5731	6210.666503	6639	6036	5532.666503	6012.333496	no
IL-12p40	17	IL-12p40	IL-12p40	23737	22893	22756.25	22839.5	24856	24012	23905.5	23988.75	no
IL-12p70	18	IL-12p70	IL-12p70	7548	6817	6560.75	6743.5	7599	6868	6629.75	6812.5	no
IL-13	19	IL-13	IL-13	15485	14882	14567	14917	9467	8864	8573.75	8923.75	no
IL-15	20	IL-15	IL-15	2363	1759	1270.25	1692.75	2427	1823	1365.5	1787.75	no
IL-16	21	IL-16	IL-16	17905	17288	16592	17110	15641	15024	14366.75	14884.75	no
IL-17	22	IL-17	IL-17	15832	15232	14571	15132	11620	11020	10402.25	10963	no
EGF	23	EGF	EGF	21400	20823	19977.25	20608.75	21676	21099	20316.25	20947.75	no
ENA-78	24	ENA-78	ENA-78	14874	14270	13609.75	14245.25	14436	13832	13193.75	13829	no
FGF-b	25	FGF-b	FGF-b	2555	1735	1563.25	1730	2040	1220	1074.5	1241.25	no
G-CSF	26	G-CSF	G-CSF	14164	13471	13420.75	13705	15113	14420	14403.25	14687.5	no
GM-CSF	27	GM-CSF	GM-CSF	713	100	-816.666687	-526	686	73	-800.666687	-510	no
IFN-g	28	IFN-g	IFN-g	34834	34195	33500.5	33924.25	35283	34644	33998.25	34421.75	no
MCP-1	29	MCP-1	MCP-1	35430	34774	34226.25	34772.75	36884	36228	35696.5	36243	no
MCP-2	30	MCP-2	MCP-2	12206	11629	11019.25	11670.5	12831	12254	11656.25	12307.5	no
MCP-3	31	MCP-3	MCP-3	2675	2102	1451	2122	2687	2114	1478.5	2149.5	no
MIG	32	MIG	MIG	7532	6964	6244.25	6956.5	6783	6215	5515.75	6227.5	no
MIP-1a	33	MIP-1a	MIP-1a	6595	5764	5615	5775	5470	4639	4525.25	4685.25	no
MIP-1b	34	MIP-1b	MIP-1b	8997	8314	6875	7190.25	9195	8512	7171.5	7486.75	no

5.5.1 Information Provided in the Raw Spreadsheet Tab

The Raw Spreadsheet provides the following information:

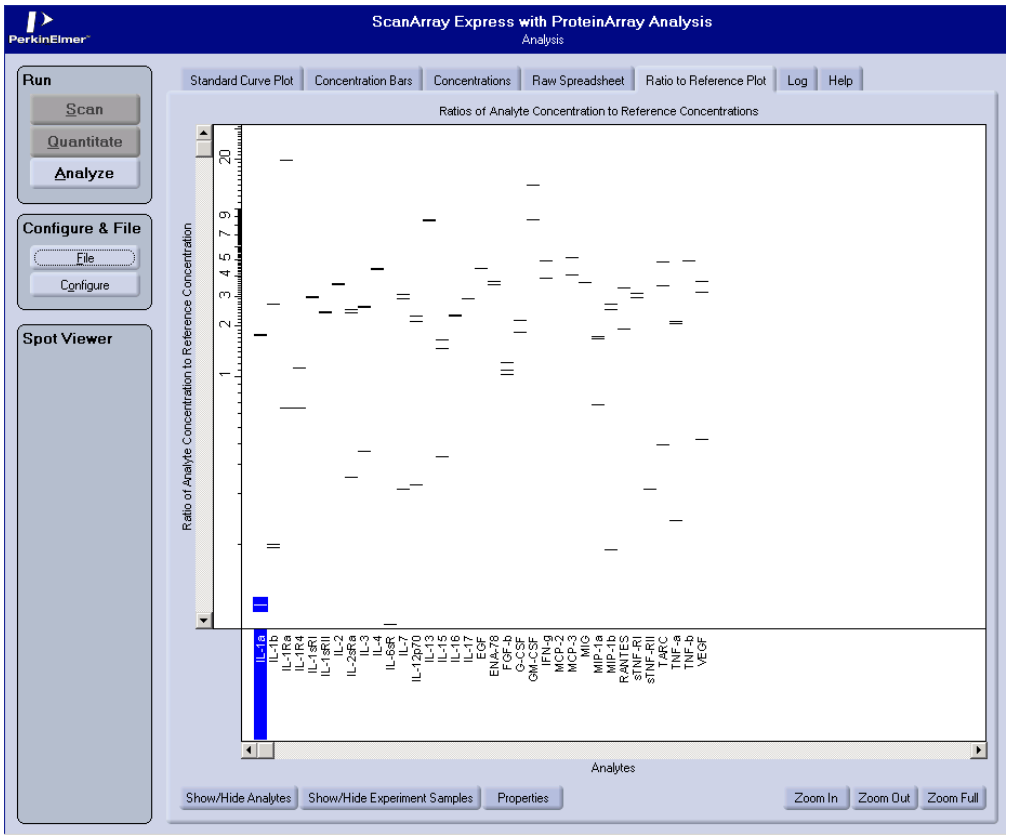
Item	Description
Analyte	Lists the analytes in the same order as in the raw quantitation file.
Analyte ID	Spot ID assigned to the spot.
Analyte Name	The text name assigned to the spot.
Mean Intensity	The mean signal for the spot.

Item	Description
Background Sub Mean Intensity	The mean signal intensity for the spot, minus the background signal intensity.
Blank Sub Mean Intensity	The mean signal intensity for the spot, minus the blank signal intensity.
Blank Sub Background Sub Mean Intensity	The background-subtracted mean signal minus the blank signal intensity.
Median Intensity	The median signal intensity for the spot.
Background Sub Median Intensity	The median signal intensity for the spot, minus the background signal intensity.
Blank Sub Median Intensity	The median signal intensity for the spot, minus the blank signal intensity.
Blank Sub Background Sub Median Intensity	The background-subtracted median signal minus the blank signal intensity.
Is Outlier	Indicates whether or not the spot is an outlier (Yes/No).
Location	The location of the block (row, column) the spot is in, then the location of the spot (row, column) within the block.
Outlier Method	Indicates the method used for outlier detection and removal (e.g., MAD, spot status, quality criteria) to remove the spot.

5.6 Ratio to Reference Plot

If a reference sample was used, a *Ratio to Reference Plot* provides ratios of analyte concentration to reference concentrations. This view lets you group by analyte or by sample.

You can zoom in and scroll up and down or left and right, zoom out, or zoom back to the full display. Holding the mouse cursor over a data point brings up information about that analyte.



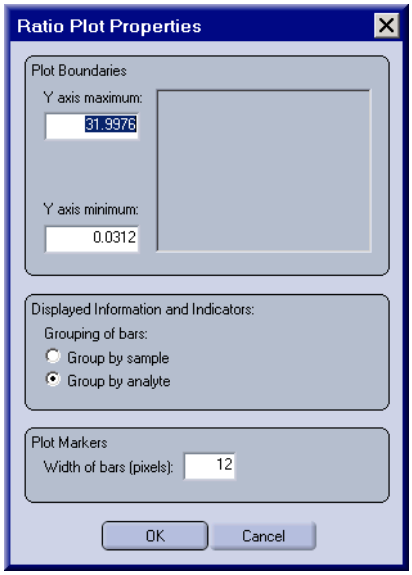
As with the Standard Curve Plot, you can show or hide analytes and show or hide experiment samples. See the instructions on page 5-4.

5.6.1 Changing the Display Properties of the Ratio to Reference Plot

You can change the minimum and maximum dimensions of the **y** axis, group information by analyte or by sample, and change the width of the bars.

To change the properties

1. In the *Ratio to Reference Plot* tab, click Properties. The following window opens:



2. Refer to Table 5-4 and edit the display properties that you want to remove or change. Click **OK** when you're finished.

Table 5-4: Ratio to Reference Plot Display Options

Item	Description
Plot boundaries	You can change the value for the Y axis maximum and/or Y axis minimum by entering a new value in the edit field of the one you want to change.
Grouping of bars:	Select **Group by sample** or **Group by analyte**.
Plot markers	To change the width of the bars, enter the number of pixels to use. The default is 12 pixels.

5.7 Saving the Analysis Results

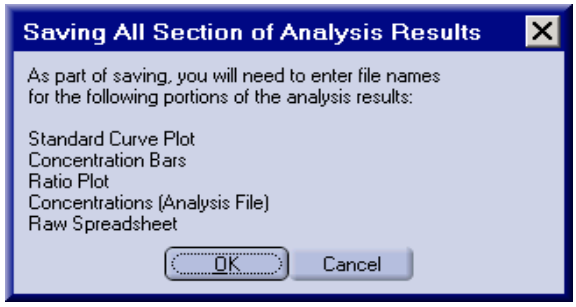
You can save the analysis results as a .CSV file, and save any plot as a JPEG or BMP file. If you use the **Save All** command, the currently displayed standard curve plot, the Concentrations Bar Graph, the Concentrations data spreadsheet, the raw spreadsheet, and ratio plot (if there is one) are saved.

Note: If you choose to **Save All**, the only standard curve plot saved is the one currently displayed. To save the curve plot for additional analytes, you must display a plot and save it separately. See ***Saving a Standard Curve Plot*** on page 5-19.

5.7.1 To Save All Results

To save the data using the Save All command

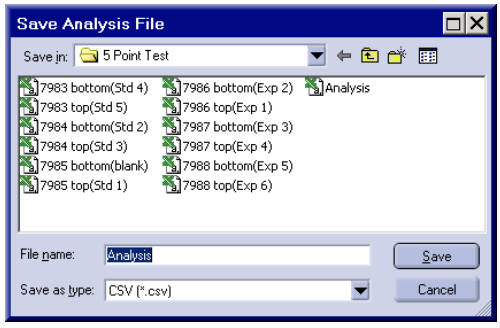
1. On the ScanArray Express *Configure & File* menu, click **File**, then **Save All**. The following message displays.



   If you are testing curve fitting without samples (see Chapter 3), you will be prompted to save the Standard Curve Plot. No analysis results or raw data tabs are created.

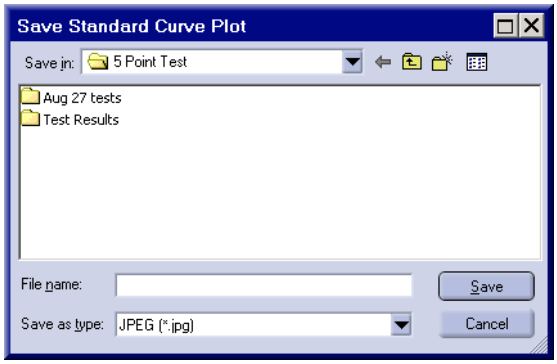
2. Click **OK**.

3. In the *Save Analysis File* window, enter a filename and click **Save**.



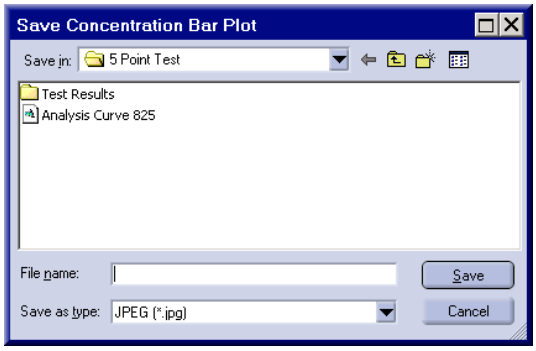
4. This saves the analysis results (Concentrations) spreadsheet as a .CSV file.

5. In the *Save Standard Curve Plot* window, enter a filename and click **Save**.



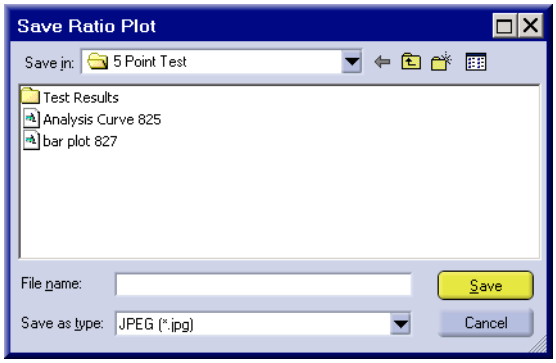
This saves the currently displayed curve plot as a JPEG file (default) or BMP file.

6. In the *Save Concentration Bar Plot* window, enter a filename and click **Save**.



This saves the bar graph as a JPEG file (default) or BMP file.

7. In the *Save Ratio Plot* window, enter a filename and click **Save**.



This saves the ratio to reference plot as a JPEG (default) or BMP file.

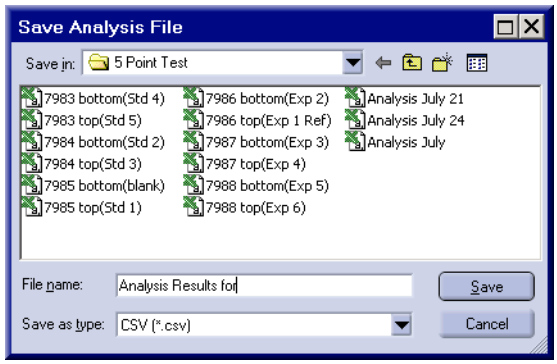
5.7.2 Saving the Concentrations Spreadsheet

Saving the *Concentrations* spreadsheet saves all of the analysis results as one .CSV file. You can later open the saved CSV file from the ScanArray Express *Configure & File* menu, recreating all of the Analysis tabs except the raw data spreadsheet.

To save the *Concentrations* spreadsheet

1. Open the *Concentrations* tab.

2. Click **File**, then **Save** or **Save as**.



3. In the *Save Analysis File* window, enter a filename and select CSV as the file type.

The resulting .CSV file contains a separate section for each type of information; each section starts and ends with a statement, for example BEGIN BLANK FILENAMES and END BLANK FILENAMES. The sections include: Header, Blank file information, Reference file information, Standard Files information, Experiment Files information, Standard Curve Data, and Experiment Data.

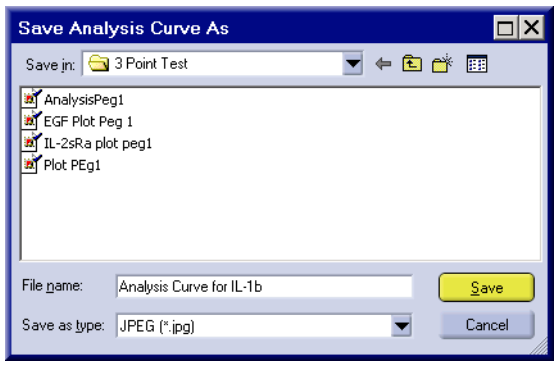
5.7.3 Saving a Standard Curve Plot

The ProteinArray Analysis saves only the currently displayed Curve Plot, so if you want to save the plot for one or more specific analytes, save each one separately.

To save a standard curve plot

1. In the *Standard Curve Plot* tab, click **Select Analyte**. In the *Select an Analyte* window that opens, select the analyte to display and save.

2. On the ScanArray Express *Configure & File* menu, click **File**, then click **Save** or **Save as.**



3. In the *Save Analysis Curve A*s window, enter a filename, for example “Analysis Curve for IL-1a” and select JPEG or BMP as the file type.

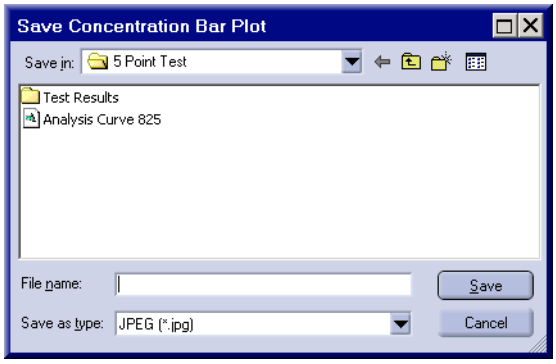
4. Click **Save**.

5.7.4 Saving the Concentration Bars

To save the concentration bars

1. In the *Concentration Bars* prepare the bar chart as you want to save it: that is, zoom in or zoom out, and scroll so that the analytes or samples of interest are currently displayed.

2. On the ScanArray Express *Configure & File* menu, click **File**, then click **Save** or **Save as.**



3. In the *Save Bar Plot A*s window, enter a filename, and select JPEG or BMP as the file type.

4. Click **Save**.

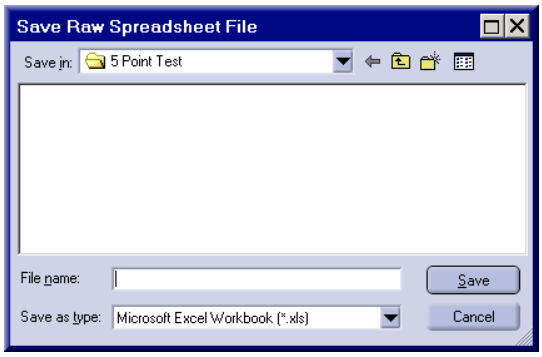
5.7.5 Saving the Raw Data

You need to have Microsoft Excel installed on your computer to save the raw data.

To save the raw data spreadsheet

1. Display the Raw Spreadsheet tab. On the *File & Configure* menu, click **File**, then **Save** or **Save as**.

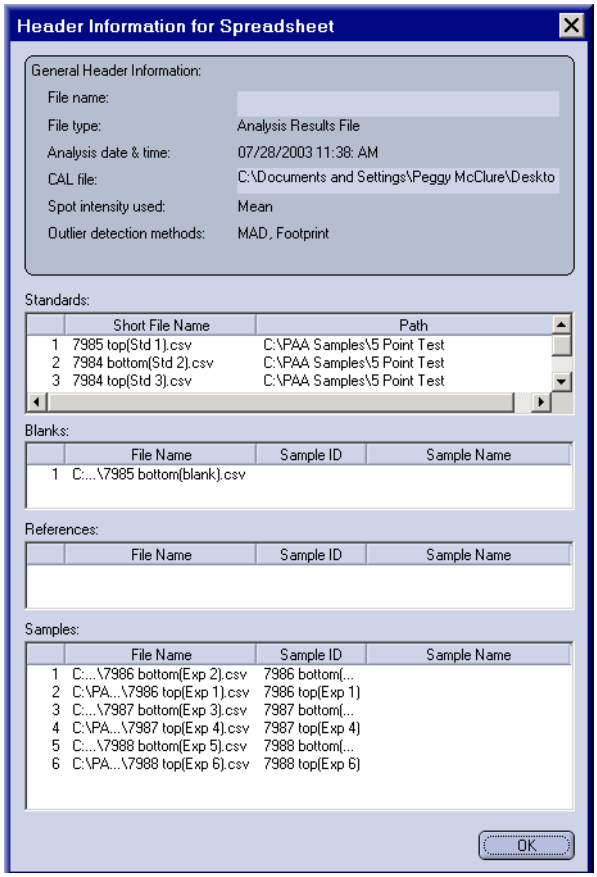
2. In the *Save Raw Spreadsheet File* window, enter a filename and click **Save**.



This saves the spreadsheet as a Microsoft Excel Spreadsheet.

5.7.6 File Header Information for a Spreadsheet

Before or after saving a spreadsheet, you can view the file header, which provides information about the analysis, including the files used, the spot intensity and outlier detection used, and the filenames of blank or reference samples if used.



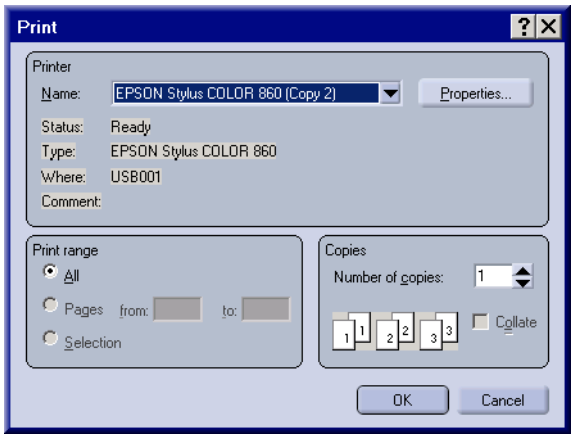
5.7.7 Printing a Plot

You can print any of the plots, with or without saving them.

To print a plot

1. Open the tab for the plot you want to print.
2. On the ScanArray Express *Configure & File* menu, click **File**, then **Print**.

3. In the Print window, specify the printer, the number of copies, and click **OK**.

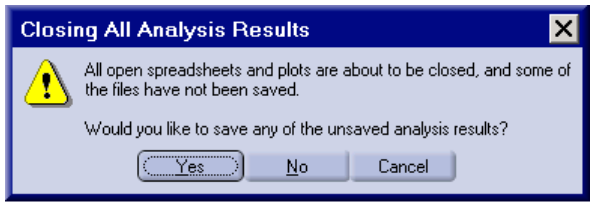


5.7.8 Closing without Saving

If you've already saved the information you want, or don't want to save the information. you can just close the ProteinArray Analysis results files.

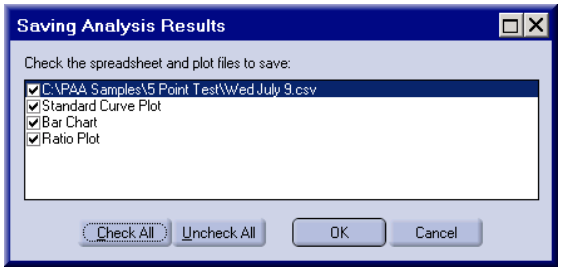
To close without saving

1. Click **File** on the *Configure & File* menu, then click **Close All**. The following window opens. (Clicking **Analyze** or attempting to exit the ProteinArray Analysis software while results are displayed opens the same window as a reminder if files are not saved.)



2. Click **No** to close without saving, or click **Yes** to save.

3. If you click **Yes** to save the files, the following window opens:



4. Check the files you want to save and click **OK**.

5.8 Opening Saved Analysis Results

To open saved analysis results

1. In the ScanArray Express *Configure & File* menu, select **File**, then **Open File**.
2. In the *Open* dialog box, for **Files of Type**, select **`Analysis Results - CSV`**.
3. Select the analysis results file that you want to reload and click **Open**.

The analysis results open, and all of the originally created tabs, except for the RAW Data Spreadsheet, are recreated.

5.9 Using the ScanArray Express Features

To use the ScanArray Express features

1. On the ScanArray Express *Configure & File* menu, click **File**.
2. Click **Open File**, and select an image (.TIF or Raw file) to open. The image displays in the Main Window, and spots display in the Spot Viewer as you move around the image.

Algorithms and Equations

Appendix A

Chapter Summary

A.1 Overview

This chapter describes the algorithms used for fitting standard curves, and provides the equations used for detecting and removing outliers.

A.2 Outlier Detection

Three methods are provided for identifying and removing outliers from the replicate spots. One, two, or all three methods can be selected. If two or three methods are selected, any spot that is identified as an outlier by any of the methods is removed from analysis.

A.2.1 Median Absolute Deviation (MAD)

This method requires three or more replicate spots. Using the MAD algorithm, the software first calculates a MAD-based score for each replicate spot. A spot is considered an outlier if the MAD-based score is not within specified limits. If a blank sample(s) is selected for the analysis, the ProteinArray Analysis uses the blank-subtracted values for this method of outlier detection.

First, the ProteinArray Analysis software calculates the Median Absolute Deviation (MAD) based on the following formula:

$$MAD = Median \{|x_1 - x|, |x_2 - x|, ..., |x_n - x|\}$$ where

- $x_1, x_2, ..., x_n$ *are the spot intensities of the replicate spots*
- x *is the median value of* $\{x_1, x_2, ..., x_n\}$

Next, the software calculates a MAD-based score M_i for each data point x_i, using the following formula:

$$M_i = 0.6745 * (x_i - x) / MAD$$

A data point is considered an outlier if the M_i value falls outside plus or minus M_{cut} as specified in the **MAD Outlier Score** in the ScanArray Express *Application Settings* window. The default value for the MAD settings M_{cut} is plus or minus 3. The default settings can be used for most analyses.

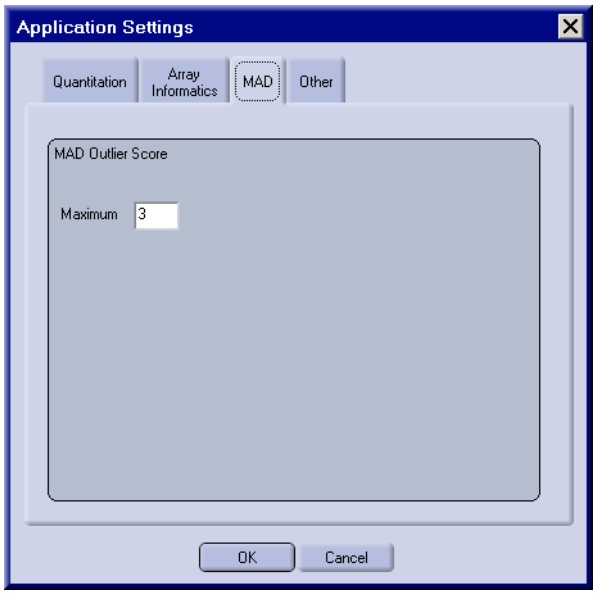
A.2.1.1 Changing the MAD Score Cutoff

The MAD score can be specified in the ScanArray Express Applications Settings.

To specify a MAD score cutoff

1. On the ScanArray Express *Main Window*, under *Configure & File*, click **Configure**, then **Application Settings**. The following window opens.

2. Click the *MAD* tab.



3. Enter a value in the **Maximum** field, and click **OK**.

A.2.2 Spot Status

Using spot status from the quantitation files, any spot with a status other than "**good**" or "**found**" are considered outliers.

In the Flags column of the quantitation file, the spot status is indicated by a number: 0 = found, 1 = not found, 2 = absent, 3= good, and 4= bad. In the analysis results, the replicates column indicates the number of good spots used for each analyte in the analysis.

A.2.3 Spot Quality Measures

This method is based on footprint, Signal-to-noise ratio, or Signal-to-background values, as specified in the *Set Quality Criteria* window from within the Analysis wizard. (See Section 3.5.2, Outlier Detection and Removal.) This method uses the data columns in the quantitation files, which are not blank-subtracted values.

Footprint. A spot is considered an outlier if the footprint for the spot is larger than the maximum footprint value specified in the *Set Quality Criteria* window. Using "Footprint" is not available if the standard and sample raw data are GPR files rather than CSV files.

Signal-to-noise ratio. A spot is considered an outlier if the signal to noise ratio value for the spot is less than the user-defined minimum.

Signal to background. Uses the values for two parameters, "signal lower limit" and "multiplier." A spot is considered an outlier if spot intensity is less than the multiplier times the spot background, or spot intensity is less than the spot background plus the lower limit.

By default, the spot quality criteria are as follows:

- Foot print (maximum) = 100 microns
- Signal to noise ratio (minimum) = 3
- Signal to background: Lower limit = 400; Multiplier = 1.7

The criteria can be changed from within the Analysis Wizard.

Next to each quality measurement below are its raw measurement and criterion for marking spots as "good."

Table A-1: Quality Measurement Formulas

Quality Measurement	Raw Measurement Calculation	Criterion for marking spots as "Good"
Footprint	For each spot, calculate the difference between the center of the nominal spots and the center of the found spot, then shift the nominal spots by the difference. Let the shifted nominal position be (X,Y), the found position to be (x,y), the footprint is the square root of : $\checkmark(X - x)^2 + (Y - y)^2$	Spots with a calculated footprint less than the maximum specified in the application settings are good. For example, suppose the maximum footprint configured is 100 microns and a spot has a footprint of 101 microns, the spot is considered an outlier.
Signal to Noise	Median spot intensity/standard deviation of background intensity.	Spots with a signal to noise ratio greater than the minimum specified in the application settings are good. For example, suppose the Minimum Signal to noise ratio configured is 3.0, and a spot has a signal to noise ratio of 2.5, the spot is considered an outlier.

Quality Measurement	Raw Measurement Calculation	Criterion for marking spots as "Good"
Signal to background	This method is based on the difference between the spot signal intensity and the background intensity. It is calculated as follows: Multiplier = M Lower Limit = L r_1 = M x background mean r_2 = L + background mean	Spots with a mean greater than r_1 and a mean greater than r_2 are marked good. For example, suppose the configured values are as follows: Multiplier M = 1.7 Lower Limit L = 400 If a spot has: Mean Intensity = 880, and Background mean intensity = 500, then r1 = 850 and r2 = 900. Here, the mean spot signal intensity is less than r2 and therefore the spot is treated as an outlier. NOTE: This method of handling outliers is particularly useful for quantitation files generated from arrays that had relatively high background.

A.3 Standard Curves

The ProteinArray Analysis provides four models for generating a standard curve from the known concentration data points. The curve is generated by interpolating between the data points of known concentration. The different methods require a minimum of standards, as follows:

Curve Fitting Method(s)	Minimum Number of Standards
Linear Regression	Three or more standards
Cubic Spline, B-spline	Four or more standards
Logistic Curve	Five or more standards

A.3.1 Extrapolating the Standard Curve

If any of the experimental data points lie outside the range of the standard sample data points, the curve line is extended as needed to the experimental data point. For linear regression, the straight curve line is extended in each direction as needed. For Cubic spline, and B-spline methods, the line is extended in both directions as a straight line, with slope equal to that of the curve at the last data points. For the logistic curve model, the curve itself is extended.

A.3.2 Linear Regression Curve

Using the linear regression method, the ProteinArray Analysis fits a straight line of the form $f(x) = a+b*x$ through the standard sample data points, which minimizes the sum of the square of the vertical distances between the data points and the line. Linear regression is the only method available for analysis with as few as three standards.

The software calculates the coefficient of correlation (r value), indicating the Goodness of Fit.

A.3.3 Cubic Spline Curve

Using the Cubic Spline method, the ProteinArray Analysis fits a smooth curve through all of the standard data points. The standard curve is generated from separate cubic segments between each pair of data points, which are connected together. The slopes and the curvature are continuous at the standard data points. The curve is generated only if the analyte has four or more data points.

Cubic spline is an interpolation algorithm, so the curve passes through all of the standard data points.

A.3.4 B-Spline Curve

Using the B-Spline method, the ProteinArray Analysis fits a smooth curve through the standard data in the same manner as the cubic spline method does, but the curve need not pass through all of the standard data points.

The software calculates the coefficient of correlation (r value), indicating the Goodness of Fit. If there are not at least four data points, no curve is generated.

A.3.5 Four Parameter Logistic Curve

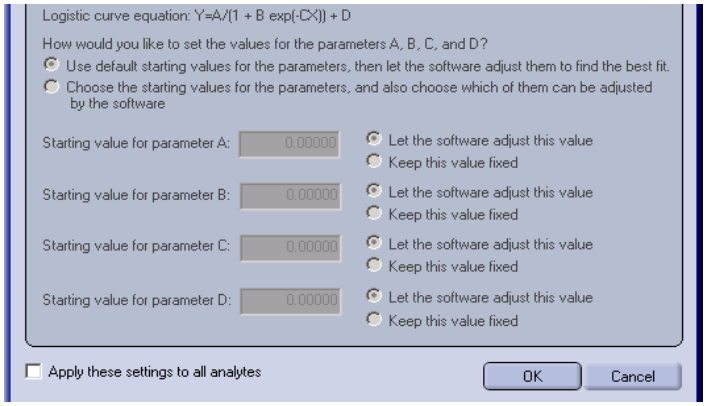
Using the logistic curve method, the ProteinArray Analysis fits an S-shaped curve through the standard points based on the following equation:

$$F(x) = a/(1 + b \exp(-cx)) + d$$

Where

a = Curve range (maximum intensity - minimum intensity)
b = parameter based on inflection point (IC50)
c = slope related parameter
d = minimum intensity

Fitting a logistic curve requires initial guesses for the coefficients a, b, c, and d. The software can make these initial guesses automatically, or you can enter specific values for the initial estimates in the *Curve Fit Type and Parameters* window when setting up the analysis. In addition, you can fix one or more of the coefficient values.



When the software automatically generates the values, it runs through several iterations to find the best values for coefficients using the least squares method. When specific values for the settings are entered in the edit fields, the software will use them as initial values and try to fine-tune them to get the best results, unless the **Fixed** box is checked. In that event, the software will use the values as specified.

The software calculates the coefficient of correlation (r value), indicating the Goodness of Fit. If there are not at least five data points, no curve is generated

Goodness of fit is measured by Correlation Coefficient, which is defined as:

$r = \checkmark((S_t - S_r)/ S_t)$

Where $S_t = \Sigma(Y - Y_i)2$; i = 1 to n and Y is the mean of n data points.

And

$S_r = \Sigma (Y_i - f(X_i))2$, the deviation from the fitting curve.

Preparing a .CAL File

Appendix B

Chapter Summary

B.1 Overview

The .CAL file is a text file that specifies each analyte to be included in the analysis, its concentration in the standard samples, and the normal range (upper and lower thresholds) for the analyte. Any analyte not included in the .CAL file is not included in the analysis and not shown anywhere in the ProteinArray Analysis software.

B.2 Preparing the .CAL File

B.2.1 File Format

The CAL file must be saved as a text file with a .CAL filename extension. The CAL file has two sections, the header section and data section (see Figure B-1).

B.2.1.1 Description of the Header Section

The header section begins with “Begin Header” and ends with “End Header” The following table describes the fields in the header.

Field	Description
Type=Protein Array Calibration File.	This is a required field.
Version = 1.0	This is the version of the file.
Description	Provides a brief description of the file: what type of data, or any additional comments.

Figure B-1 is an example of a typical .CAL file.

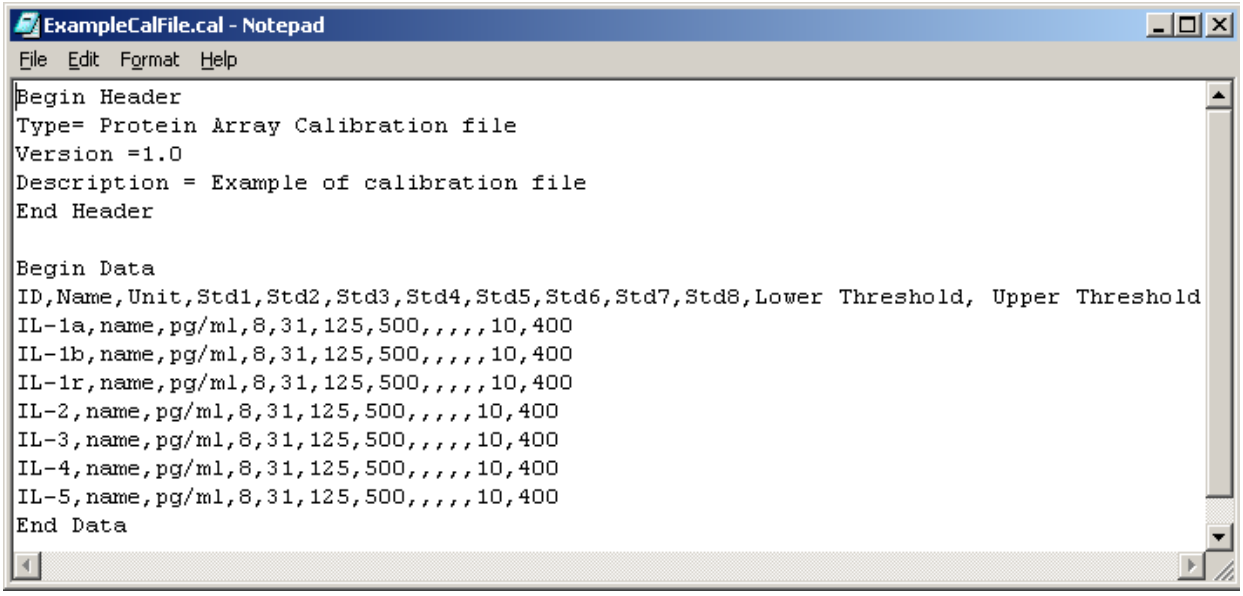


Figure B—1 Example of a CAL File

B.2.1.2 Description of the Data Section

The data section begins with “Begin Data” and ends with “End Data” The following table describes the header section.

Record	Description
Header Row	This is required, it describes the order that data is entered for each record. All records must follow the order described in the header row.
Records	A record for each analyte provides the analyte name, units of measurement, data for the standard concentrations, and the normal range (upper and lower thresholds) if applicable.

B.2.1.3 Formatting Rules

The CAL file must follow these formatting rules:

- The fields must be separated by commas.
- There are four acceptable strings for entering concentration units in the Unit field. If any other unit is specified, the analysis will not proceed.
    - pg/ml
    - picograms/milliliter
    - ng/ml
    - nanograms/milliliter

- The concentration values for the "Lower Threshold," and "Upper Threshold" fields are optional, and you may leave the columns for these fields blank.
- Negative values are not allowed for the standard concentrations and for the normal ranges. If the .CAL file has any negative values, the ProteinArray Analysis will not proceed.
- The concentrations for the standards must be in the increasing order.
- The fields in the .CAL file may be put in double quotes. Since the fields are comma-delimited, an analyte that includes a comma in its name can be put into double quotes, so that the comma is recognized as part of the name and not as a delimiter. A comma after the closing quote is the delimiter.
- Do not use any Greek characters like α, β, χ, or μ in the calibration file. Instead, spell the character if it is part of an analyte ID or some other data. For example, enter "IL-1α" as "IL-1alpha")
- The file must be saved as a text file and saved with a .CAL extension

B.3 Example CAL file

Begin Header
Type= Protein Array Calibration File
Version =1.0
Description = Example of calibration file
End Header

Begin Data
ID,Name,Unit,Std1,Std2,Std3,Std4,Std5,Std6,Std7,Std8,Lower Threshold, Upper Threshold
IL-1a,IL-1a,pg/ml,300,600,1500,3750,7500,,,,1000,2000
IL-1b,IL-1b,pg/ml,128,256,640,1600,3200,,,,100,3000
IL-1Ra,IL-1Ra,pg/ml,180,360,900,2250,4500,,,,100,300
IL-1R4,IL-1R4,pg/ml,200,400,1000,2500,5000,,,,,
IL-1sRI,IL-1sRI,pg/ml,110,220,550,1375,2750,,,,500,600
IL-1sRII,IL-1sRII,pg/ml,400,800,2000,5000,10000,,,,,
IL-2,IL-2,pg/ml,200,400,1000,2500,5000,,,,,
IL-2sRa,IL-2sRa,pg/ml,600,1200,3000,7500,15000,,,,,
IL-3,IL-3,pg/ml,300,600,1500,3750,7500,,,,,
IL-4,IL-4,pg/ml,300,600,1500,3750,7500,,,,,
IL-5,IL-5,pg/ml,36,72,180,450,900,,,,,
IL-6,IL-6,pg/ml,320,640,1600,4000,8000,,,,,
IL-6sR,IL-6sR,pg/ml,80,160,400,1000,2000,,,,,
IL-7,IL-7,pg/ml,26,52,130,325,650,,,,,
IL-8,IL-8,pg/ml,22,44,110,275,550,,,,,
IL-10,IL-10,pg/ml,100,200,500,1250,2500,,,,,
IL-12p40,IL-12p40,pg/ml,5,10,25,62.5,125,,,,,
IL-12p70,IL-12p70,pg/ml,500,1000,2500,6250,12500,,,,,
IL-13,IL-13,pg/ml,4000,8000,20000,50000,100000,,,,,

```
IL-15,IL-15,pg/ml,30,60,150,375,750,,,,,
IL-16,IL-16,pg/ml,640,1280,3200,8000,16000,,,,,
IL-17,IL-17,pg/ml,3000,6000,15000,37500,75000,,,,,
EGF,EGF,pg/ml,240,480,1200,3000,6000,,,,,
ENA-78,ENA-78,pg/ml,480,960,2400,6000,12000,,,,,
FGF-b,FGF-b,pg/ml,2500,5000,12500,31250,62500,,,,,
G-CSF,G-CSF,pg/ml,50,100,250,625,1250,,,,,
GM-CSF,GM-CSF,pg/ml,360,720,1800,4500,9000,,,,,
IFN-g,IFN-g,pg/ml,640,1280,3200,8000,16000,,,,,
MCP-1,MCP-1,pg/ml,1600,3200,8000,20000,40000,,,,,
MCP-2,MCP-2,pg/ml,240,480,1200,3000,6000,,,,,
MCP-3,MCP-3,pg/ml,40,80,200,500,1000,,,,,
MIG,MIG,pg/ml,140,280,700,1750,3500,,,,,
MIP-1a,MIP-1a,pg/ml,40,80,200,500,1000,,,,,
MIP-1b,MIP-1b,pg/ml,80,160,400,1000,2000,,,,,
MIP-3a,MIP-3a,pg/ml,260,520,1300,3250,6500,,,,,
RANTES,RANTES,pg/ml,60,120,300,750,1500,,,,,
sTNF-RI,sTNF-RI,pg/ml,2500,5000,12500,31250,62500,,,,,
sTNF-RII,sTNF-RII,pg/ml,1400,2800,7000,17500,35000,,,,,
TARC,TARC,pg/ml,90,180,450,1125,2250,,,,,
TNF-a,TNF-a,pg/ml,1600,3200,8000,20000,40000,,,,,
TNF-b,TNF-b,pg/ml,160,320,800,2000,4000,,,,,
VEGF,VEGF,pg/ml,100,200,500,1250,2500,,,,,
mMIP-1a,mMIP-1a,pg/ml,0,0,0,,0,,,,,
1X PBS,1X PBS,pg/ml,0,0,0,,0,,,,,
Biotin-1,Biotin-1,pg/ml,0,0,0,,0,,,,,
Biotin-2,Biotin-2,pg/ml,0,0,0,,0,,,,,
Biotin-3,Biotin-3,pg/ml,0,0,0,,0,,,,,
Biotin-4,Biotin-4,pg/ml,0,0,0,,0,,,,,
End Data
```

B.4 Creating a .CAL File

You can create the .CAL file for your analysis using Microsoft Notepad. The easiest way is probably to start with an existing .CAL file similar to your needs, or one of the sample .CAL files included on the ProteinArray Analysis CD-ROM.

To create a .CAL file

1. Open Microsoft Notepad (select the **Start** menu on the Windows taskbar, then **Programs**, **Accessories**, **Notepad**). You will have an untitled, blank file.

2. Open an existing .CAL file to use as a starting point.

3. Copy the existing .CAL file, and paste it into your blank file.

4. In the **`Begin Data`** section, make the necessary changes in analyte names, concentrations, and thresholds. Add any additional analytes, or remove the lines for analytes you don't need. The number of fields for each analyte must equal the number of fields in the header row of the data section.

5. Click **File**, **Save** as on the Notepad menu.

6. In the *Save as* window that opens, in the **Save in** field find the directory where you want to save the file. In the **File name:** field, enter a meaningful name for the file, including ".CAL" as a filename extension.

7. In the **Save as type:** field, select **`All Files`**, and click **Save**.

Installing the ProteinArray Analysis Application

Appendix C

Appendix Summary

C.1 Overview

The ProteinArray Analysis application is an add-on module for the ScanArray Express software. If you change PC's, or need to remove or reinstall the software for any reason, the software is provided on a CD with an installation "wizard" to guide you through the process of installing the software (see Figures C-1 and C-2).

Note: You must install ScanArray Express first, before installing ProteinArray Analysis.

C.2 Installing the Software

To install the software

1. Install the ScanArray Express application.
2. Insert the ProteinArray Analysis software CD-ROM into the CD drive of your computer.
3. If the installation does not start automatically, use Explorer or the **My Computer** icon to find the **CD-ROM** drive. Click on the **CD-ROM** drive and find the program **Setup.exe**. Double-click on the program name or icon. The InstallShield Wizard opens (see Figure C-1).

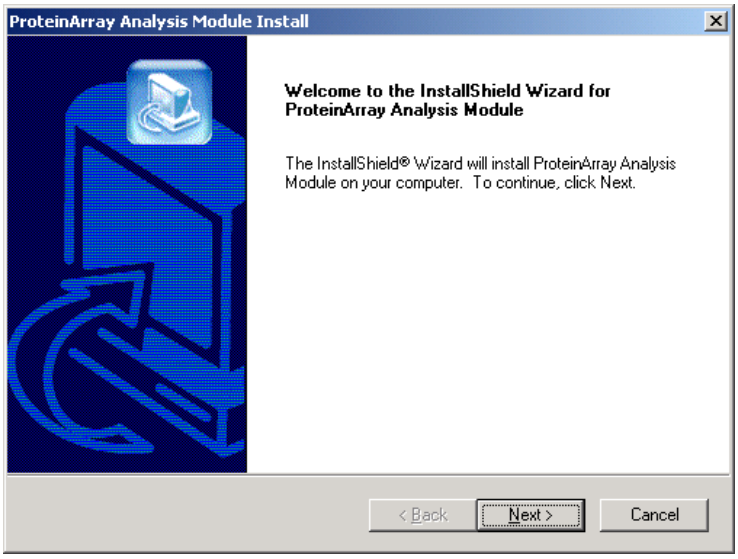


Figure C-1 The InstallShield Wizard screen for installing ProteinArray Analysis

4. Follow the on-screen prompts.

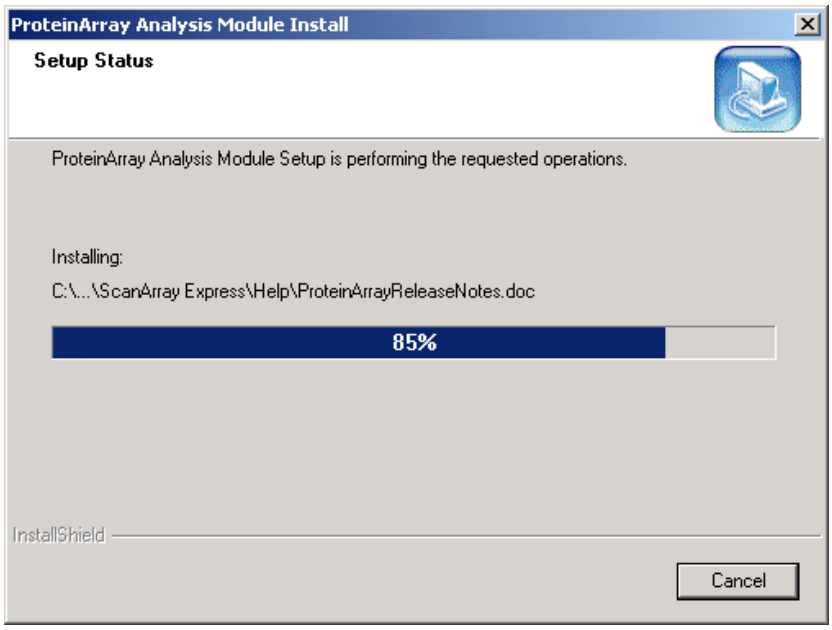


Figure C-2 Setup Status - installing ProteinArray Analysis

5. When the installation is complete, restart your PC (Figure C-3).

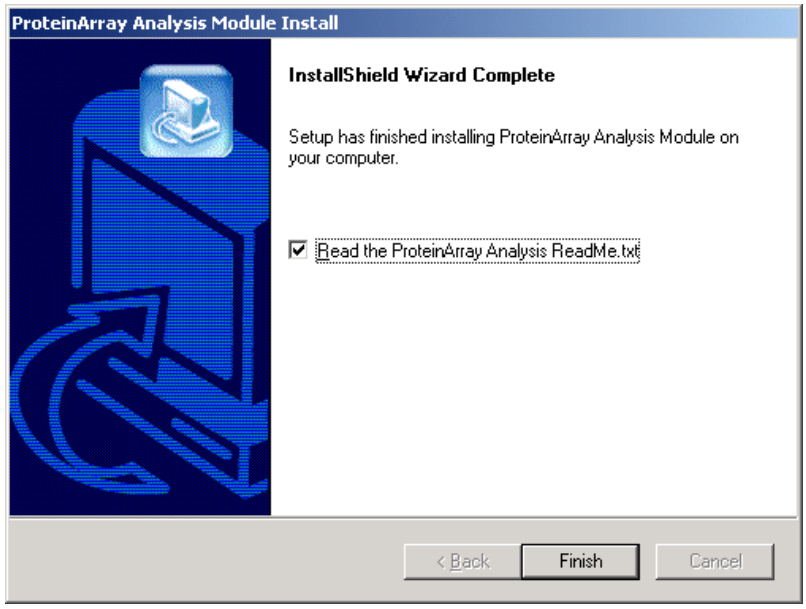


Figure C-3 Installation Complete.

Glossary

Analyte	The protein analyzed by an experiment.
Analyte ID	A short text name for the analyte printed on the microarray. This is similar to the gene ID for a spot on a DNA microarray.
Analyte Name	A text description for the analyte printed on the substrate.
Blank Sample	One sample that has a zero concentration of the analytes that is used to determine the background signal. A blank sample can have one to four replicates.
Block	On the microarray, a group of spots physically separated from other spots.
BMP File	A bitmap file format for saving images.
CAL File	A file created by the user to list each analyte, its known concentration in standard samples, and it normal range.
GAL File	(GenePix Array List) For protein microarrays, a GAL file specifies the analyte ID and name for each spot on the microarray; also specifies the block parameters of the array such as row and column spacing, absolute position of a block, and spot diameter.
GPR File	(GenePix Results) A text file which contains the results for each spot from the quantitation of an image, including spot foreground mean, spot foreground median, background mean, background median, background subtracted mean, etc.\
JPEG File	(Joint Photographic Experts Group) A file format for saving images. This format uses less space than a BMP (bitmap) file of the same image.
Experiment Samples	Those samples with unknown concentrations of analyte. An experiment sample can have one to four replicates.
Reference Sample	One sample that is used to determine the relative concentration of proteins in subsequent test samples. A reference sample can have one to four replicates.
Replicate Spots	Multiple spots printed with the same analyte in the same concentration. These spots have the same ID and name.
Replicate Samples	The data set produced using the same sample multiple times on the same type of protein microarray.
Sample ID	A short text assigned to a sample for identifying the sample during the analysis. This is similar to the gene ID assigned for a printed spot.
Sample Name	A text description assigned for a sample.

Sample Replicates	See *Replicate Samples*.
Standard Curve	The curve generated by interpolating the data points of known analyte concentrations (standards). Curve fit algorithms are used to generate the standard curve from known concentration data points. The standard curve is then used for calculating the concentration of analytes in experimental samples
Standard Samples	Those samples with known concentrations of analyte.

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