Agilent 2100 Bioanalyzer

2100 Expert User's Guide

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Agilent Technologies

Notices

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Contents

About this Manual

Welcome to the User's Guide for the Agilent 2100 expert software. This manual provides beginners and advanced users with information needed to successfully run electrophoretic and flow cytometric assays with the bioanalyzer.

The 2100 expert software allows the control of the bioanalyzer (including diagnostic functions) and, in combination with a LabChip kit, the acquisition, interpretation and result presentation of data generated during the analysis of DNA, RNA, proteins, and cells.

In this Manual

This manual provides bioanalyzer users with the following information:

- "About this Manual" on page 6 gives an overview of the subjects in this manual, and lists major innovations and improvements of the 2100 expert software. It also lists supplemental literature and shows you how to make efficient use of this manual.
- "Quick Start" on page 17 is meant for experienced users. It briefly summarizes the necessary steps to prepare and run an assay.
- "Looking at 2100 Expert" on page 31 shows how to get started with the 2100 expert software, and outlines its main operational possibilities.
- "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47 shows you how to change cartridges, which is necessary for switching between electrophoretic and flow cytometric measurements.
- "Running and Evaluating Electrophoretic Assays" on page 55 explains how electrophoretic measurements are made using the bioanalyzer, gives detailed descriptions of all steps necessary to run electrophoretic assays, and shows how to analyze and evaluate measurement results using electropherograms and gel-like images.
- "Running and Evaluating Flow Cytometric Assays" on page 156 explains how flow cytometric measurements are made using the bioanalyzer, gives detailed descriptions of all steps necessary to run flow cytometric assays, and shows how to analyze and evaluate measurement results using histograms and dot plots.
- "Working with Chip and Assay Data" on page 245 shows you what to do to open, save, import and export files, and how to print the measurement results.

- "Configuring 2100 Expert" on page 293 is your guideline for configuring the 2100 expert software.
- "Running Instrument Diagnostics" on page 303 shows how to use the diagnostics tests to check the bioanalyzer hardware for proper functioning.
- "Performing Qualifications" on page 312 describes how you can validate your bioanalyzer system.
- "2100 Expert Software Reference" on page 320 describes all elements of the 2100 expert user interface, such as screen regions, menus, tool bars, and dialog boxes.
- "Products, Spare Parts, and Accessories" on page 573 lists all parts and accessories—including reorder numbers—that are required for electrophoretic and flow cytometric measurements.
- "Glossary" on page 576 explains terms in context with flow cytometry, electrophoresis, and terms specific to the bioanalyzer software and hardware.

If you have any questions this manual cannot answer, please refer to the supplemental literature listed in "Related Documents" on page 9. If you still have questions, contact Agilent for additional support at:

http://www.agilent.com/chem/labonachip

Related Documents

A collection of supplemental literature is given in the following.

Bioanalyzer Manuals

Publication Number	Title
G2938-90006	Agilent 2100 Bioanalyzer Installation and Safety Manual
G2946-90001	Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide

CD-ROM

Publication Number	Title
G2946-60002	Agilent 2100 Bioanalyzer – How to Use Multimedia CD-ROM

Reagent Kit Guides

The *Reagent Kit Guides* give you information on how to prepare samples.

Publication Number	Title
G2938-90300	Kit Guide Binder english (including all *Reagent Kit Guides*)
G2938-90010	Reagent Kit Guide DNA 500 and DNA 1000 Assay
G2938-90020	Reagent Kit Guide DNA 7500 and DNA 12000 Assay

Publication Number	Title
G2938-90030	Reagent Kit Guide RNA 6000 Nano Assay
G2938-90040	Reagent Kit Guide RNA 6000 Pico Assay
G2938-90050	Reagent Kit Guide Protein 200 Plus Assay
G2938-90060	Reagent Kit Guide Protein 50 Assay
G2938-90070	Reagent Kit Guide Cell Fluorescence Assays
G2938-90080	Reagent Kit Guide Cell Fluorescence Checkout Kit

Application Notes and Technical Notes

Application Notes and *Technical Notes* are available from the *Agilent 2100 Bioanalyzer Help Desk* or from the lab-on-a-chip web pages:

http://www.agilent.com/chem/labonachip

Newly Published Documentation

Follow this link to see if there is any new documentation:

http://www.chem.agilent.com/scripts/Library.asp

What's New in 2100 Expert

Products

2100 expert is the successor to the *Bio Sizing* and *Cell Fluorescence* software.

- *2100 expert* provides a single software platform with a common user interface for running, analyzing, evaluating, presenting, and comparing DNA, RNA, protein *and* cell parameters.
- *2100 expert* is installed in one go. After installation, the functionality for electrophoretic and flow cytometric assays can be activated separately with license keys.

Features

2100 expert integrates the complete *Bio Sizing* and *Cell Fluorescence* functionality plus additional features:

- *2100 expert* allows having multiple chip data and/or assay files open at the same time.
- *2100 expert* provides detailed *installation qualification* (IQ) and *operational qualification* (OQ) tests on both the bioanalyzer hardware and software.
- *2100 expert* features a new integrated data evaluation tool (*Comparison* context) allowing comparison of measurement results (of same assay class) from different chips directly. A separate data evaluation tool is no longer necessary.
- *2100 expert* features improved integration including manual integration.
- *2100 expert* allows color-coded result flagging. Flagging rules can be applied to measurement results.
- *2100 expert* now has customizable result tables and gel-like images.

- *2100 expert* has improved instrument control. Two bioanalyzers can be controlled at one time. It is possible to run measurements as well as diagnostics tests on two bioanalyzers *at the same time*.
- *2100 expert* has improved printing and reporting functions.
- *2100 expert* has extended instrument diagnostics functionality.

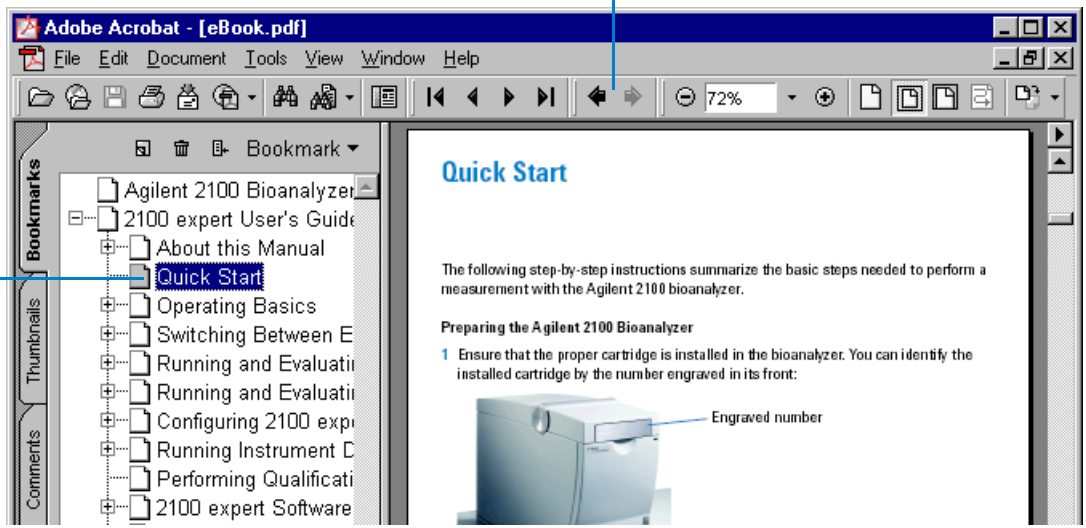
How to Use this Manual

This manual uses convenient online navigation features and follows certain typographic conventions.

Online Navigation

Use the interactive bookmarks in this frame to move to your desired topic.

Use Acrobat Reader's navigation bar to move around within a topic.



Click here to go to the table of contents.

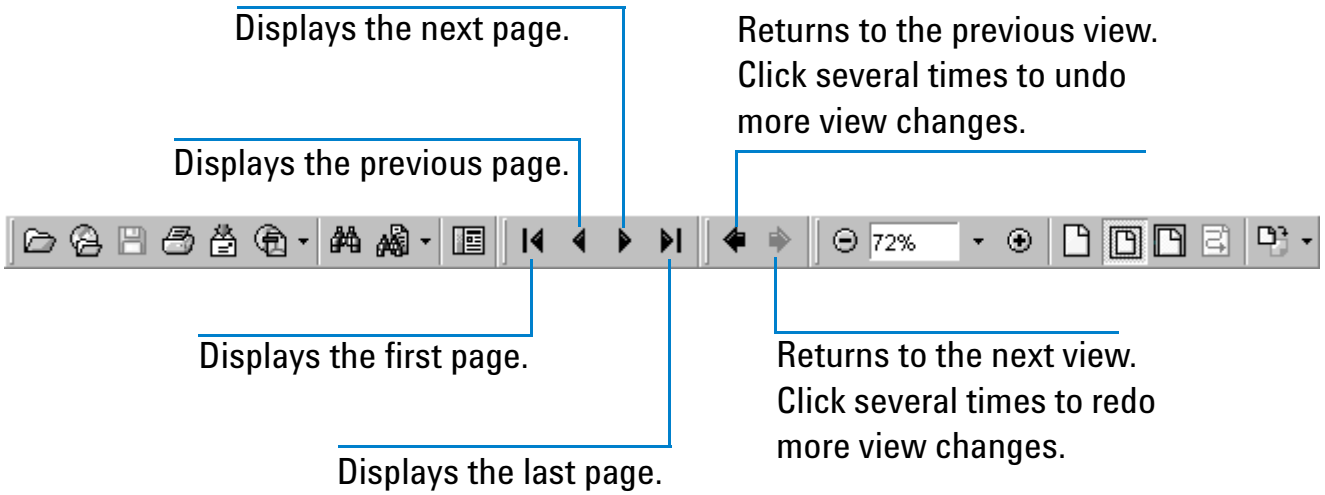
Click here to go to the index.

Here is the current page number.

▲ Displays the previous page.

▼ Displays the next page.

After you have chosen a topic with the bookmarks, use the buttons in Acrobat Reader's tool bar to move around within the topic.



Layout Conventions

The following typographic conventions are used in this manual:

Highlight	Meaning
Italic	On-screen element *Example*: ... the *OK* button. Emphasis Example: *Right*-click the ... Term Example: *Dot plots* show events as dots. Reference to another document Example: Refer to the *Agilent 2100 Bioanalyzer Troubleshooting and Maintenance Guide*.
Blue	Cross-reference or hyperlink Examples: "What's New in 2100 Expert" on page 11 http://www.agilent.com/chem/labonachip
`Courier`	Code Example: .. the command line parameter `-port 2` ...
`Courier bold`	User input Example: Enter **`50 MB`**.

Safety Notices, Notes and Tips

Safety notices, notes and tips in this document have the following meaning:

WARNING

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

CAUTION

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

NOTE

A note contains important, helpful, or additional information.

TIP

A tip usually points out a timesaving feature.

Quick Start

The following step-by-step instructions summarize the basic steps needed to perform a measurement with the Agilent 2100 bioanalyzer.

Preparing the Agilent 2100 Bioanalyzer

1 Ensure that the proper cartridge is installed in the bioanalyzer. You can identify the installed cartridge by the number engraved in its front.

Engraved number

Note that there are also electrode cartridges without an engraved number.

① = Electrode Cartridge for *electrophoretic* assays
② = Pressure Cartridge for *flow cytometric* assays

2 If you have to change the cartridge, follow the instructions in "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47.

Switching on the Agilent 2100 Bioanalyzer

1 Make sure the bioanalyzer is connected to line power and connected to the PC.

2 Turn on the line switch at the rear of the instrument.

The status LED at the front of the bioanalyzer should light up.

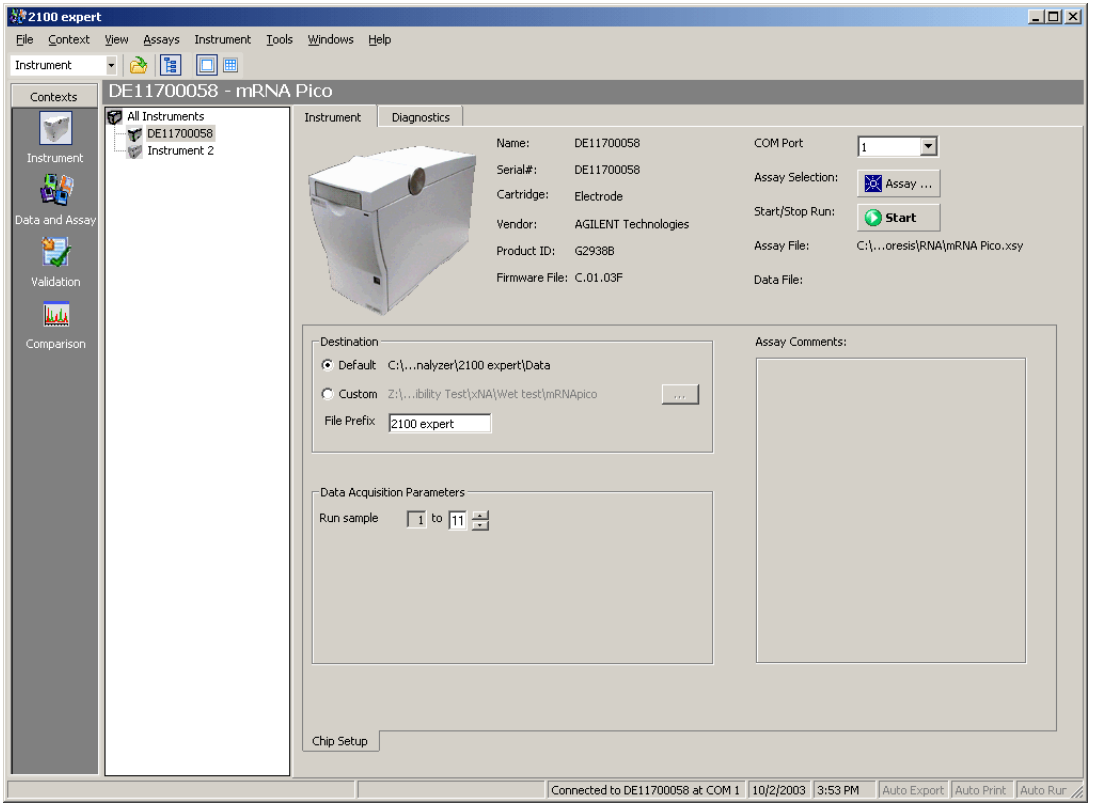
The status LED shows you the current status of the instrument.

Signal	Meaning
Green light	Instrument is switched on and ready for measurement.
Green blinking	Measurement is running.
Orange blinking	Instrument is busy (running self diagnostic, for example).
Red light	Instrument is not ready for measurement. Switch the instrument off and on again. If the problem persists, call Agilent service.

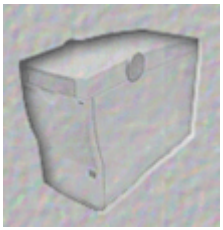
Running a Measurement

1 To start the 2100 expert software on the connected PC, go to your desktop and double-click the following icon:

After startup of the software, you enter the *Instrument* context:



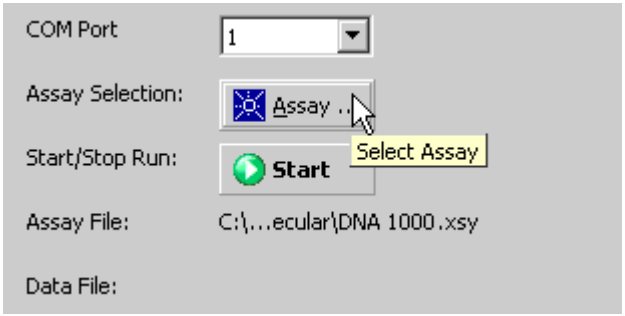
In the upper left of the *Instrument* tab, an icon shows the status of the bioanalyzer:

Icons	Meaning
	Bioanalyzer detected, lid is open.
	Bioanalyzer detected. Lid is closed, but no chip is inserted.
	No bioanalyzer has been detected. Check the *COM Port* setting (see figure under step 3), the RS 232 connection cable, the power cable, and the power switch. For details on how to set up the bioanalyzer and connect it to a PC, see *Agilent 2100 Bioanalyzer Installation and Safety Guide*.

2 Make sure that a bioanalyzer has been detected before continuing.

3 Select an assay for the chip run.

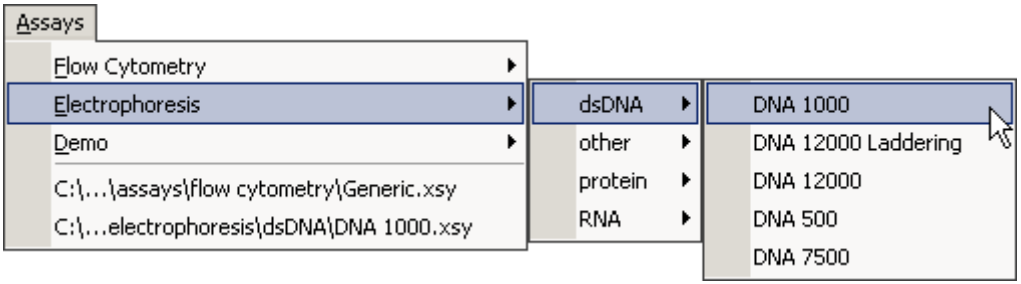
On the *Instrument* tab, click the *Assay...* button.



– OR –

Click the *Assays* menu.

Both will open the *Assays* menu, allowing you to select an assay from the submenus.



– OR –

You can also select *File* > *Open File to Run...*. This opens a dialog box allowing you to load either an assay (.xsy) or a chip data file (.xad).

4 Prepare the samples and the chip.

For detailed information on sample and chip preparation refer to the

- *Reagent Kit Guides* that are available for each reagent kit.
- *Application Notes* that are available for each assay.

NOTE

When preparing chip and samples, pay attention to the essential measurement practices described in "Essential Measurement Practices (Electrophoretic Assays)" on page 67 and "Essential Measurement Practices (Flow Cytometric Assays)" on page 178.

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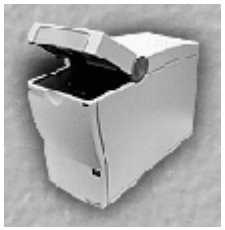
5 Insert the chip in the Agilent 2100 bioanalyzer:

a Open the lid.

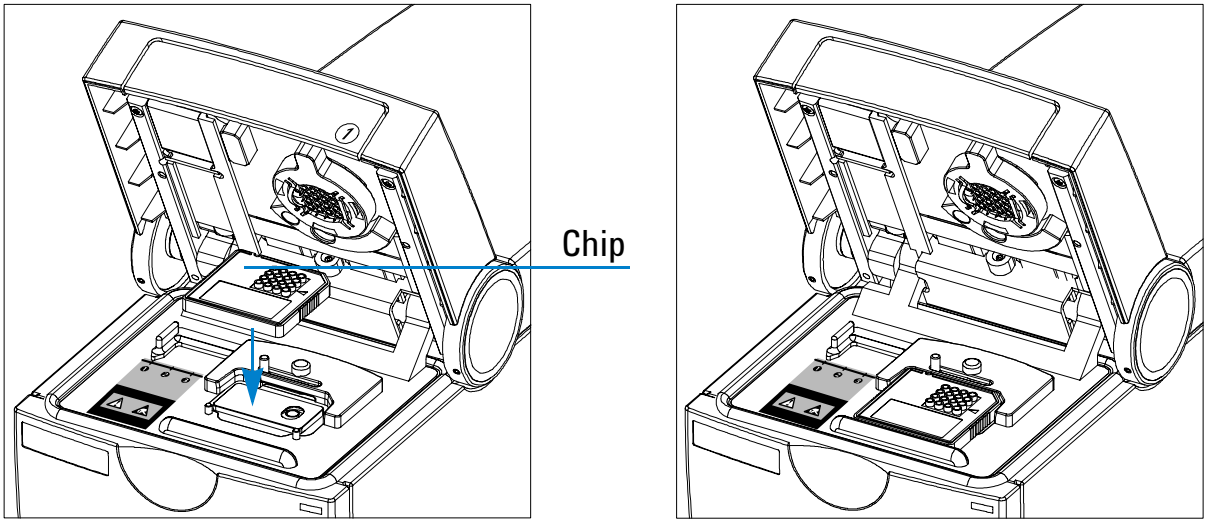
The bioanalyzer icon changes as follows:



b Check that the cartridge is inserted properly and the chip selector is in the correct position ("1" for electrophoretic assays, "2" for flow cytometric assays).

For details, please refer to "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47.

c Place the chip into the receptacle. The figure shows this for an electrophoresis chip.



The chip fits only one way. Do not use force.

CAUTION

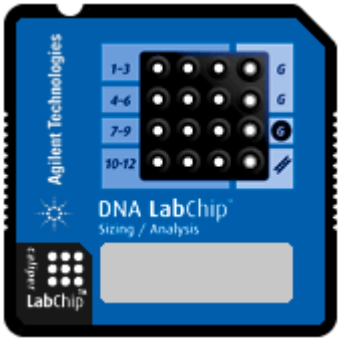
Do not force the lid closed. This may damage the cartridge.

d Carefully close the lid.

Electrophoretic assays: the electrodes in the cartridge fit into the wells of the chip.

Flow cytometric assays: the adapter with the gasket in the cartridge fits onto the priming well of the chip.

The bioanalyzer icon changes to a chip icon (a DNA chip icon, for example):



If the chip is not detected, open and close the lid again.

NOTE

If the *AutoRun* option is active (see "Options – Advanced" on page 508), the chip run starts automatically once a chip has been inserted and the lid has been closed.

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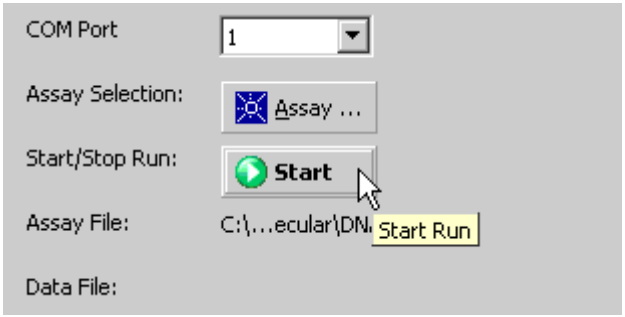
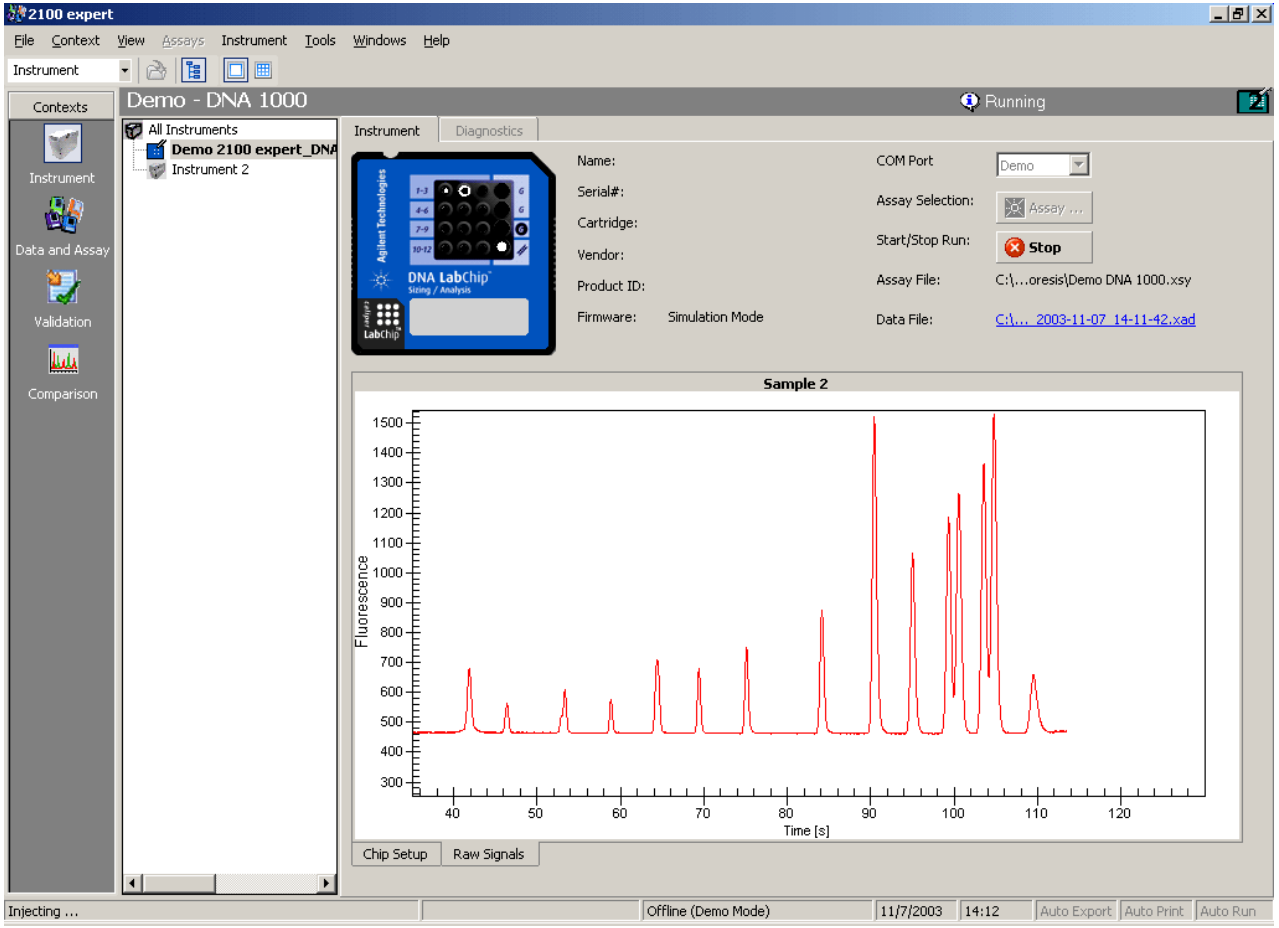
5 On the *Instrument* tab, click the *Start* button.



The chip run starts. The *Raw Signals* sub-tab shows an electropherogram of the currently measured sample. The name of the sample is displayed above the graph. The graph is a "live" plot of the migration time against fluorescence units (raw data, including background fluorescence, for example).

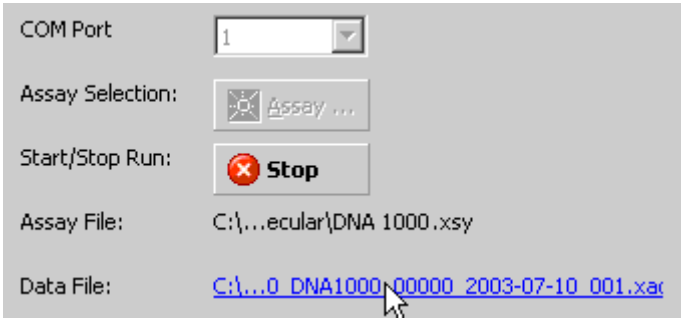


The number of the sample that is currently being measured is indicated on the information bar:

The status bar shows the name of the currently measured sample, a progress bar showing the measurement progress for the current sample (not for the whole chip run), and the COM port number used for data acquisition:

During the chip run, you can do the following:

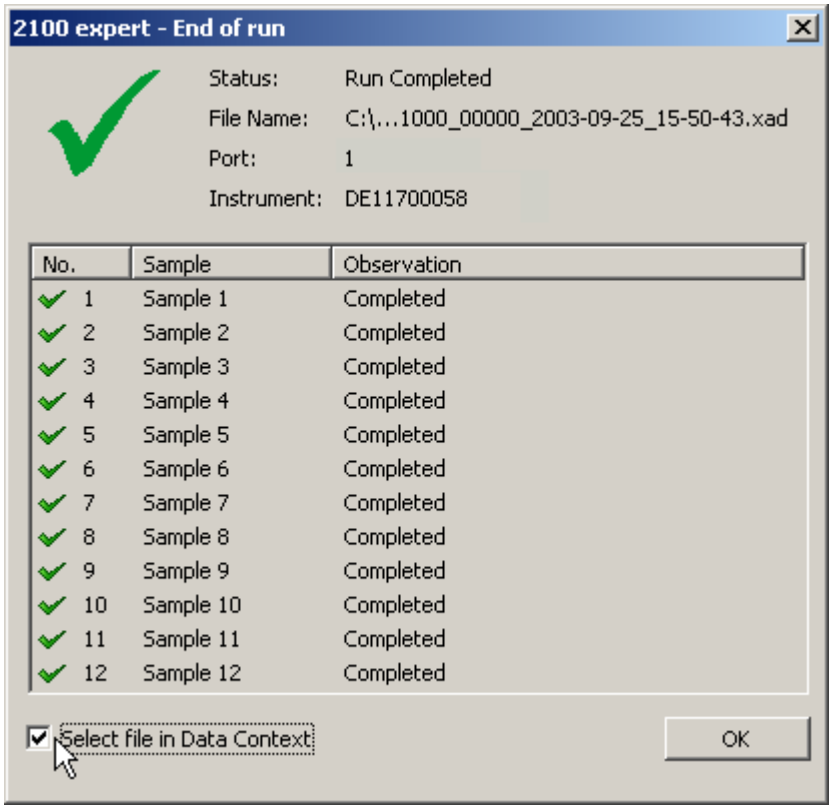
- View the chip data file in the *Data and Assay* context by clicking on the name of the *Data File*:



- Switch to any other context. For example, you can evaluate any chip data file in the *Data and Assay* context, or compare samples in the *Comparison* context.
- If necessary, abort the chip run by clicking on the *Stop* button. All data that was collected up to the stop point will be saved.

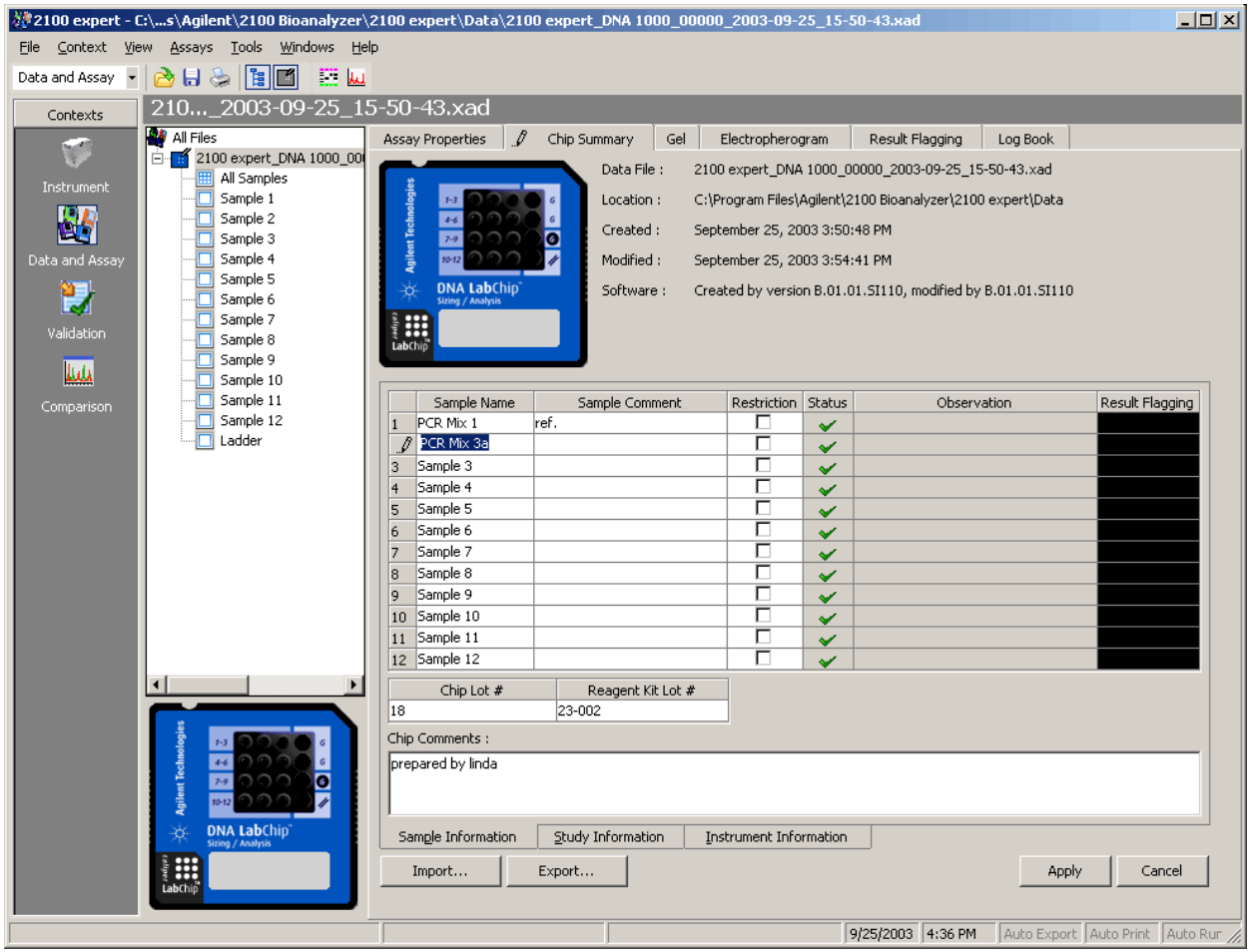
Finishing the Measurement

When the measurement is finished, the *End of run* dialog box appears, showing you the status of the assay and the file name where the chip run data has been stored.



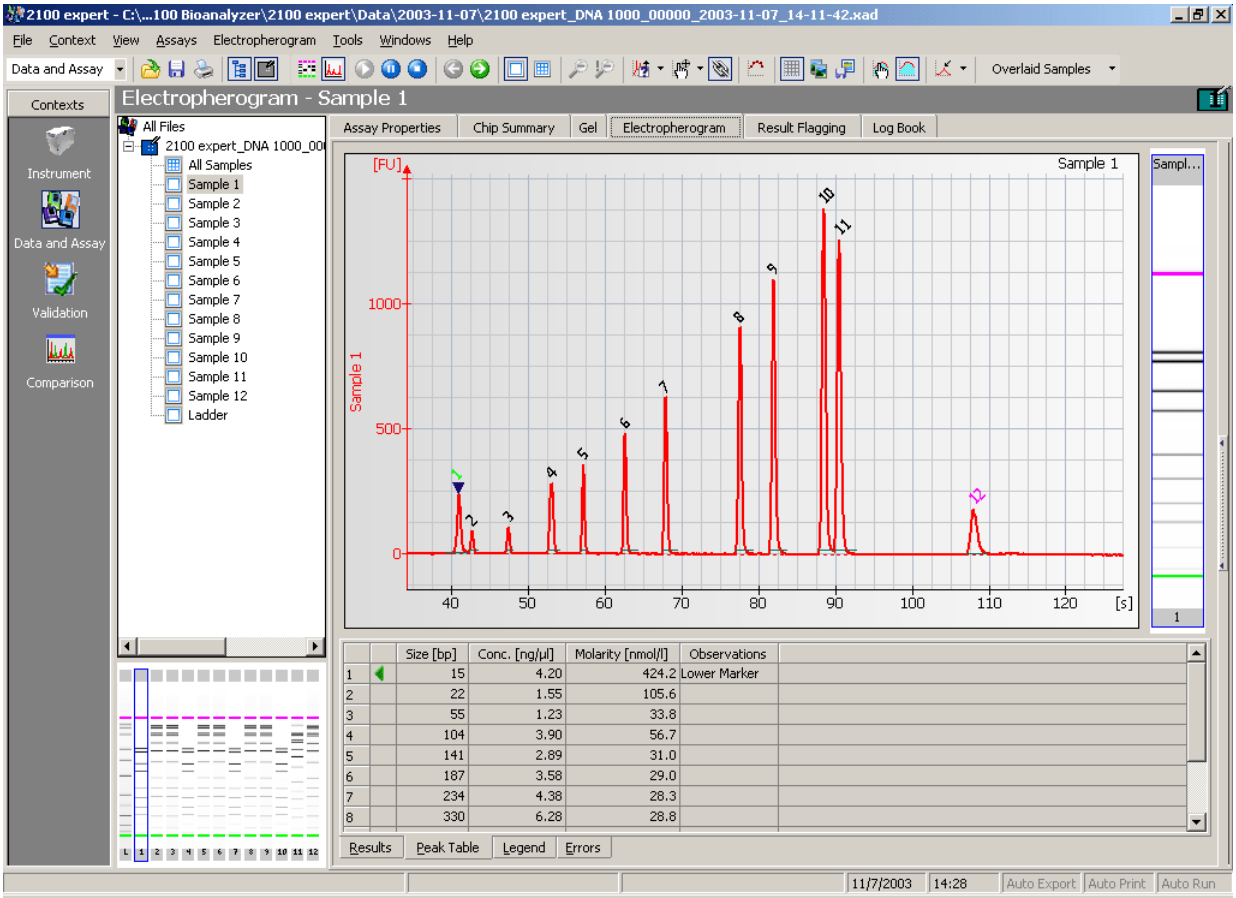
1 To immediately view the results, select the *Select file in Data Context* check box, and click *OK*.

This takes you to the *Data and Assay* context, and the data file that has just been generated by your chip run is selected. The *Chip Summary* tab shows information on your chip data file, and lets you enter comments regarding chip, samples, and study.



2 In the tree view panel, click any sample name, or the ladder.

This selects the *Electropherogram* tab, which displays a data plot of migration time versus fluorescence intensity.



Peaks have automatically been detected, and their characteristics such as size, concentration, and molarity have been calculated and are shown in the *Peak Table* at the bottom of the window.

What You Can do When the Measurement is Finished

When the measurement is finished, you can:

- Document your chip run by entering sample names, chip comments, and study information, for example.
- Evaluate the measurement results by analyzing gel-like images and electropherograms (electrophoretic assays), or histograms and dot plots (flow cytometric assays):
  - "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 91
  - "Analyzing and Evaluating the Results of a Flow Cytometric Assay" on page 198
- Print the results to document them on paper.

  See "Printing Reports" on page 275.
- Export the results for further evaluation in other applications.

  See "Exporting Data" on page 263.
- Compare the results with the results of other chip runs in the *Comparison* context.
- Insert the next chip in the bioanalyzer and start a new chip run with one click (provided that you will use the same assay).

Looking at 2100 Expert

Before you begin with running assays on the Agilent 2100 bioanalyzer you should familiarize yourself with the 2100 expert software:

Starting 2100 Expert

To start 2100 expert:

1 Go to your desktop and double-click the following icon:

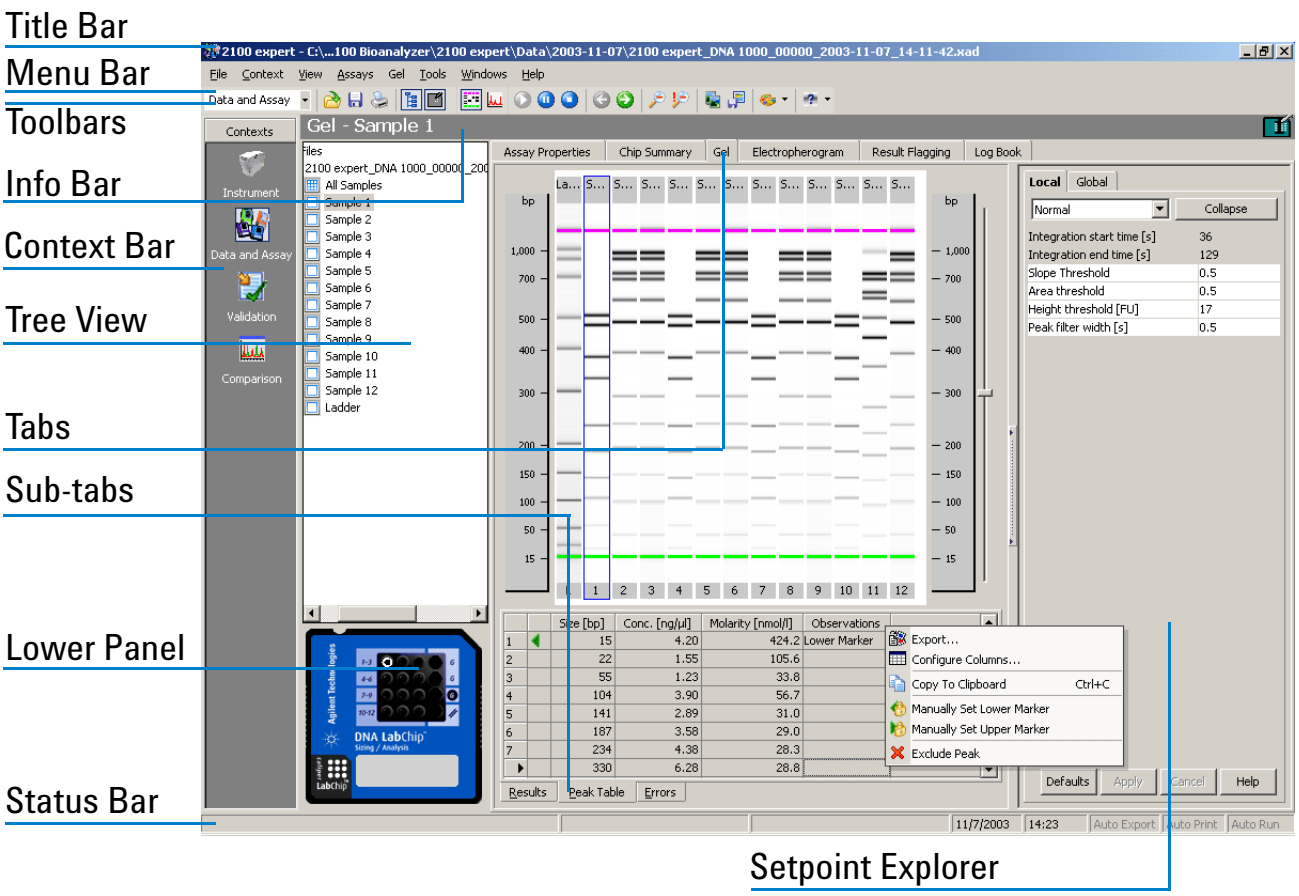
– OR –

From the Windows *Start* menu, select *Programs* > *Agilent 2100 Bioanalyzer* > *2100 expert*.

The 2100 expert application window appears. "2100 Expert Work Area" on page 33 gives an overview of the application window.

2100 Expert Work Area

The 2100 expert work area has standard elements such as pull-down menus and toolbars, and the main working area, which contains several tabs, some of which have sub-tabs. The 2100 expert work area has the following regions (*Data and Assay* context):



See "2100 Expert Application Window Elements" on page 321 for details on the regions.

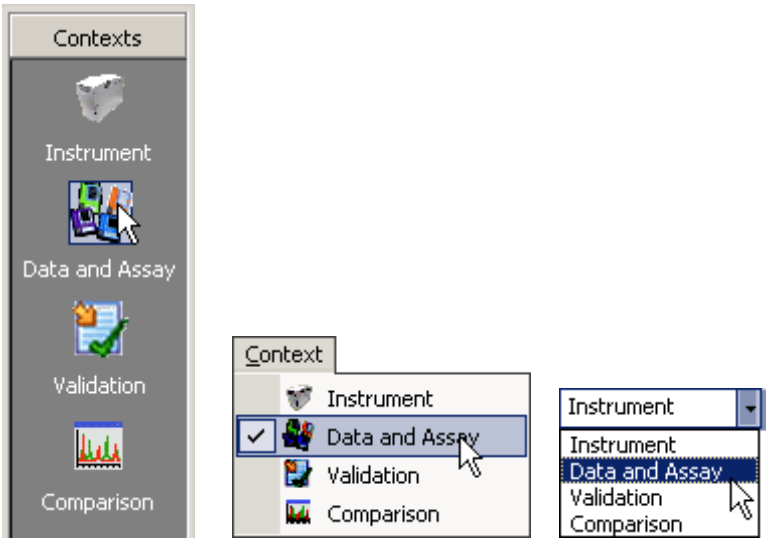
The 2100 expert software can be operated in four modes, called “contexts”:

- Instrument Context
- Data and Assay Context
- Validation Context
- Comparison Context

NOTE

The contexts are independent from each other regarding their data. This means, for example, that you can review data and run measurements at the same time.

Using the *Contexts* bar, the *Context* menu, or the drop-down list in the *tool bar*, you can switch between the contexts:



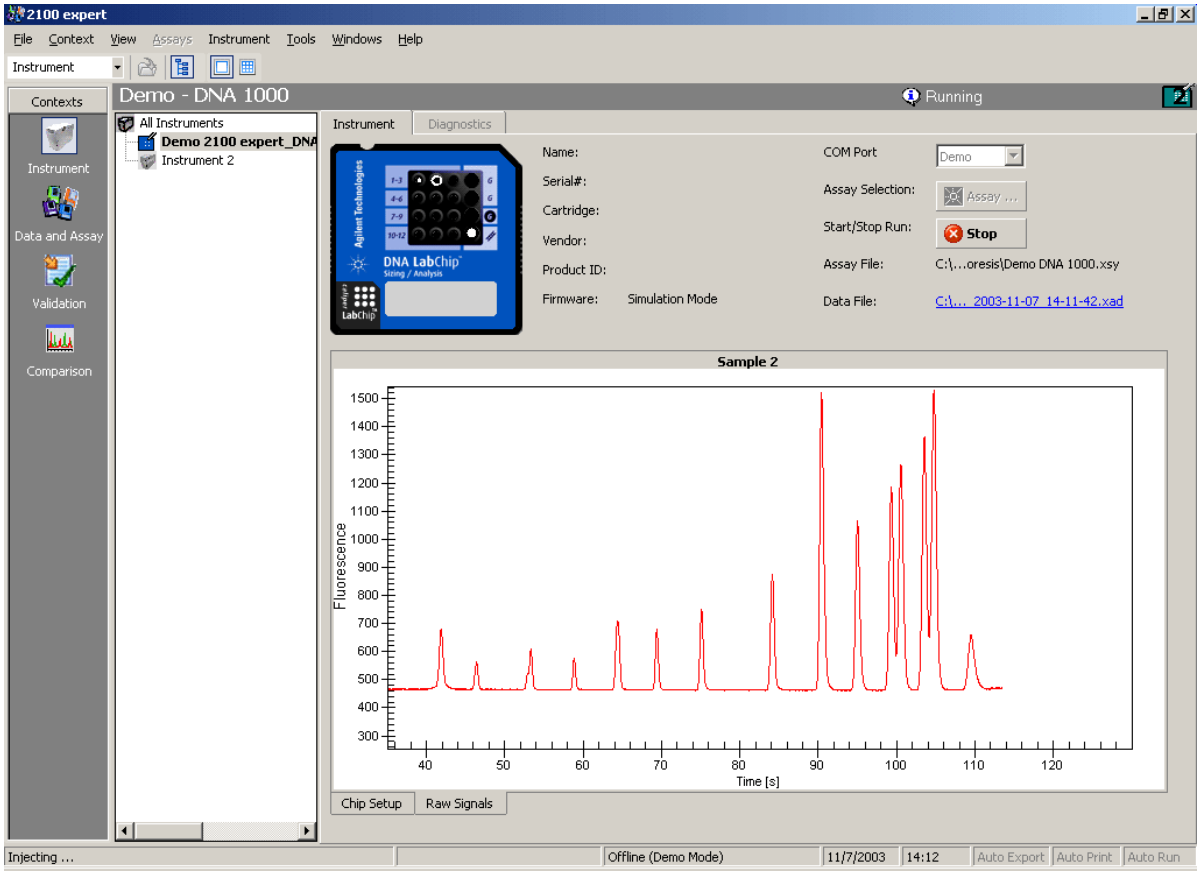
NOTE

Menus, toolbars, the tree view, and the main working area (tabs) significantly change when you switch between the contexts.

An introduction to the four contexts is given in the following.

Instrument Context

On startup, 2100 expert enters the *Instrument* context, where you can run DNA, RNA, protein or cell assays by selecting an assay file and starting the chip run—provided that the bioanalyzer is properly connected, a chip is inserted, and the bioanalyzer lid is closed.



NOTE

If two bioanalyzers are connected to your PC, you can run both in parallel.

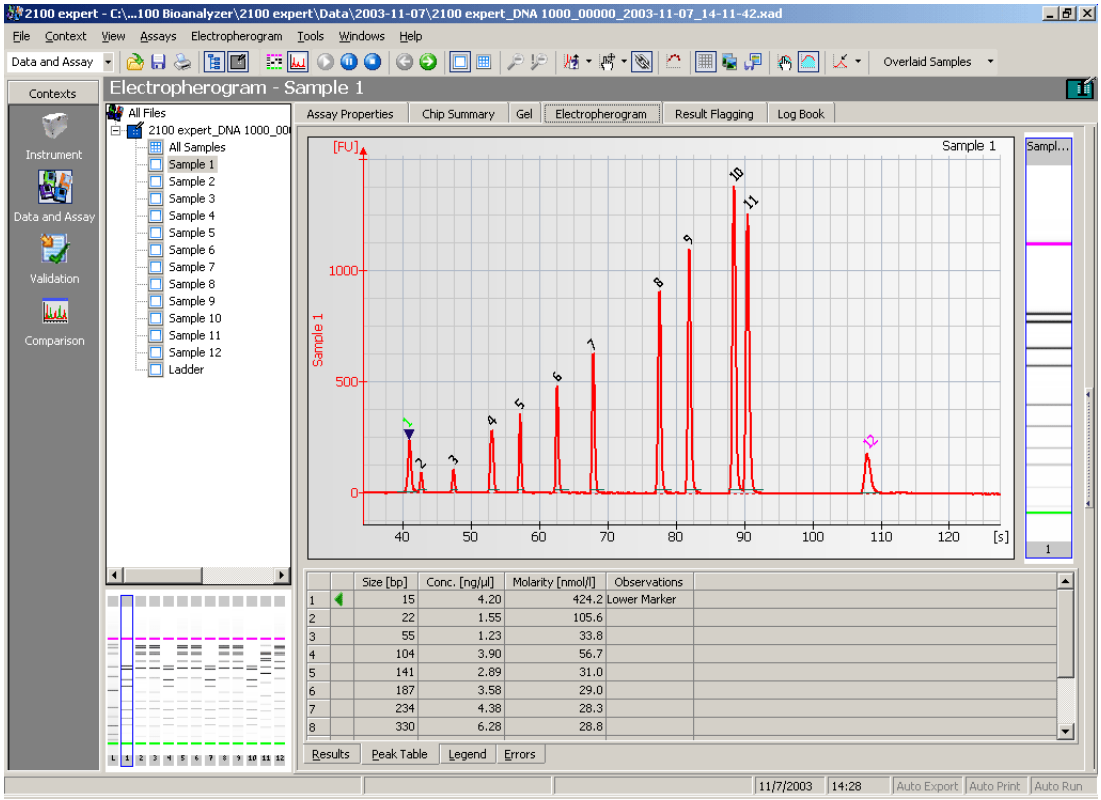
During the chip run(s), you can view the status of the bioanalyzer(s): instrument information and real time acquisition data.

In the *Instrument* context, it is also possible to run hardware diagnostic tests on all connected bioanalyzers. Refer to "Running Instrument Diagnostics" on page 303 for details.

Data and Assay Context

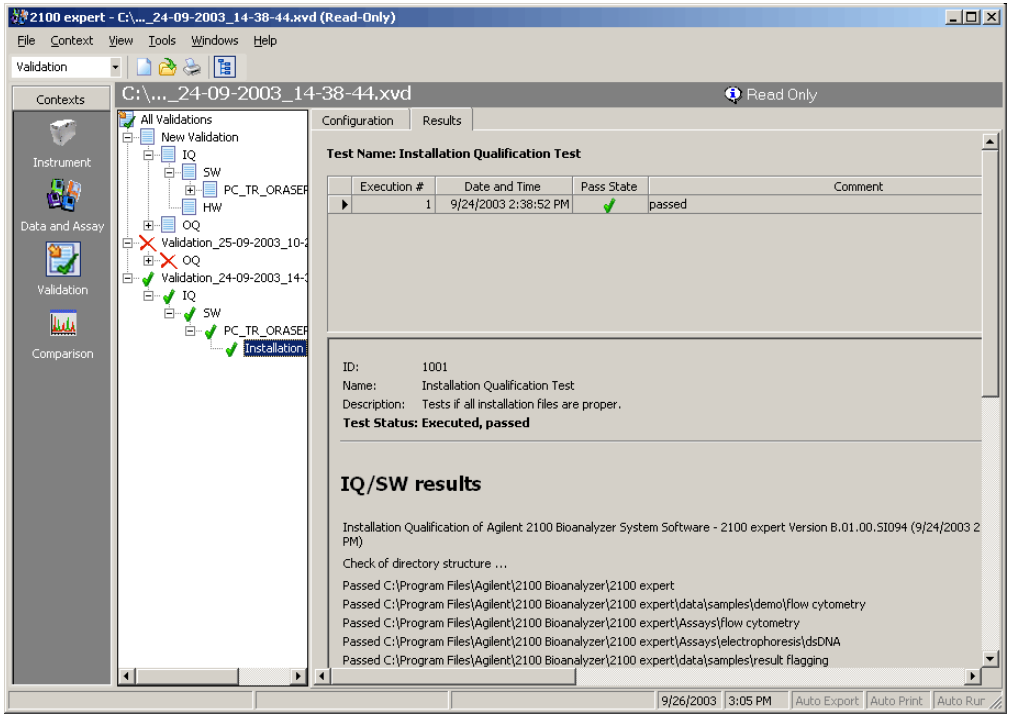
In the *Data and Assay* context, you can

- view, analyze, and evaluate the results of your chip runs that are presented as electropherograms, gel-like images, histograms, dot plots, and result tables.
- export and print the results of your chip runs.
- modify existing assays and create your own assays by modifying properties such as data analysis setpoints.



Validation Context

The *Validation* context is used to run and document qualification tests.



For both the bioanalyzer hardware and software tests can be run for:

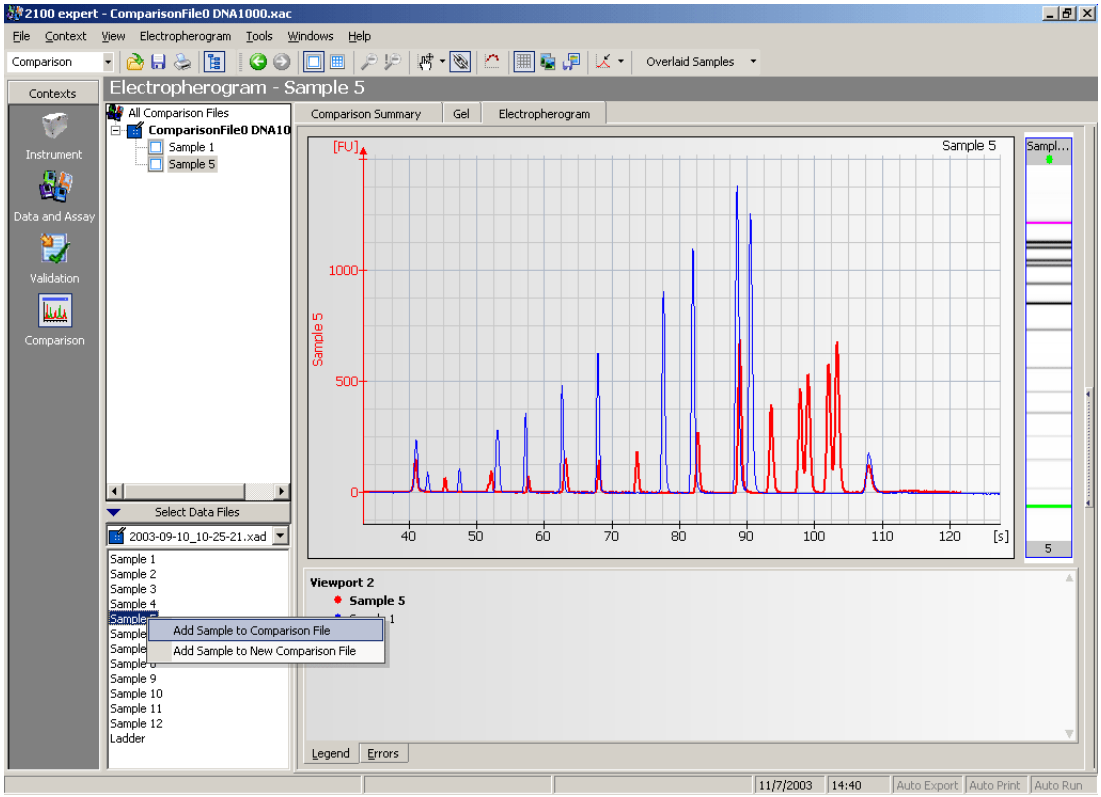
- Installation qualification (IQ)
- Operational qualification (OQ)

Validation results are automatically saved in .xvd files. You can re-open .xvd files to review validation results.

For details, refer to "Performing Qualifications" on page 312.

Comparison Context

In the *Comparison* context, you can open multiple electrophoretic chip data files and compare samples of the same assay class (DNA 1000, for example), even from different chip runs. It is possible to overlay electropherograms recorded by the bioanalyzer, and compare the measurement results.



Comparison results can be saved in .xac files. You can re-open .xac files to review the comparison results, and to add further samples for comparison, for example.

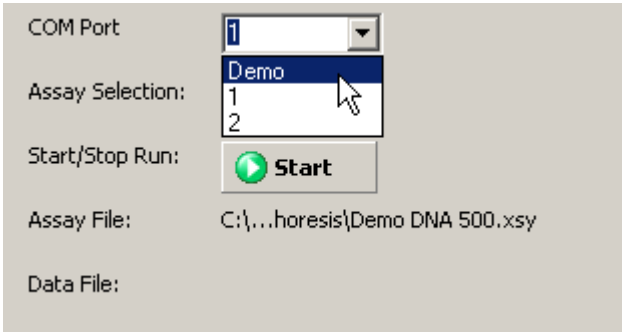
Running a Demo Assay

2100 expert provides demo assays that you can use for a first impression of chip runs and data evaluation, even if the bioanalyzer is offline.

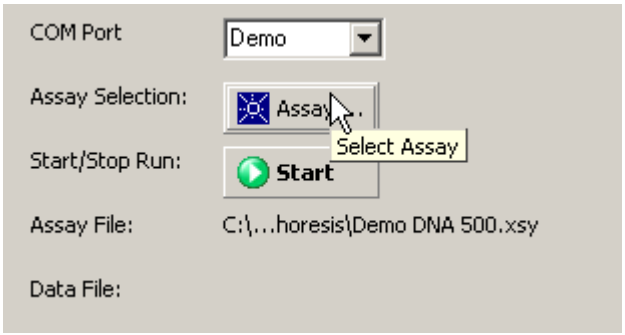
To run a demo assay:

1 Start the software as described in "Starting 2100 Expert" on page 32, or, if the software is already running, switch to the *Instrument* context.s

2 On the *Instrument* tab, select "Demo" as the *COM Port*.

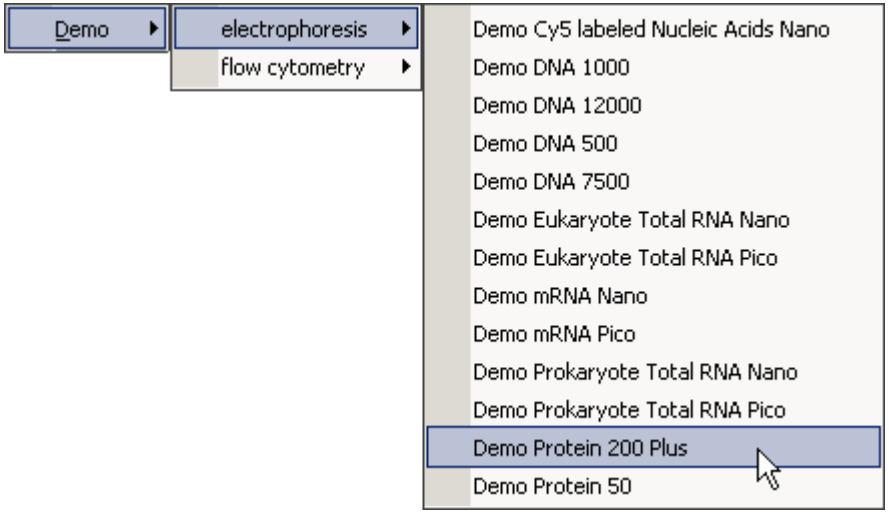


3 Click the *Assay...* button.



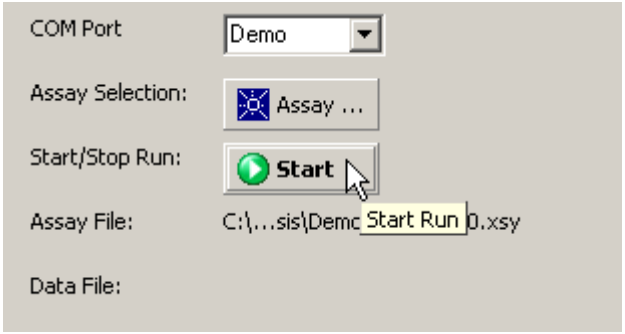
This opens a menu, allowing you to select an assay from submenus.

4 Select a demo assay, for example *Demo* > *electrophoresis* > *Demo Protein 200 Plus*.

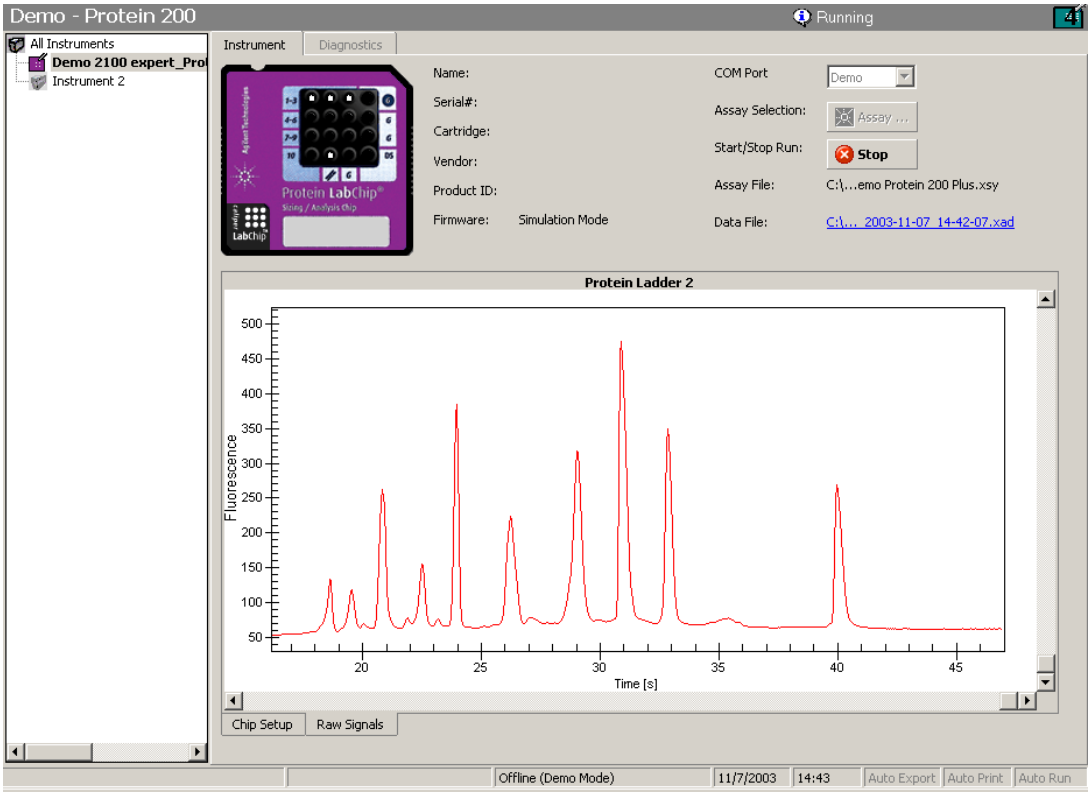


The assay is loaded and a violet chip icon appears indicating the assay type “Protein”.

5 Click the *Start* button.



The demo chip run starts and you can watch its progress on the *Raw Signals* tab, where you can see a simulation of data acquisition.

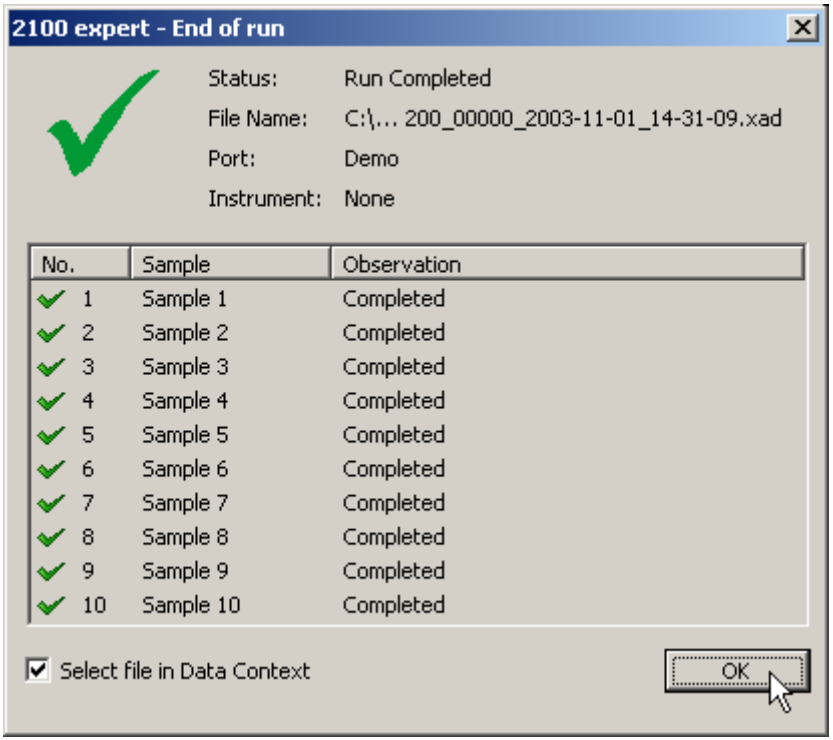


For details on how a chip run proceeds, refer to "Running an Electrophoretic Assay" on page 72, or "Running a Flow Cytometric Assay" on page 182.

NOTE

Like a real chip run, a demo chip run also creates a chip data (.xad) file.

At the end of the simulation, the *End of run* dialog box appears.



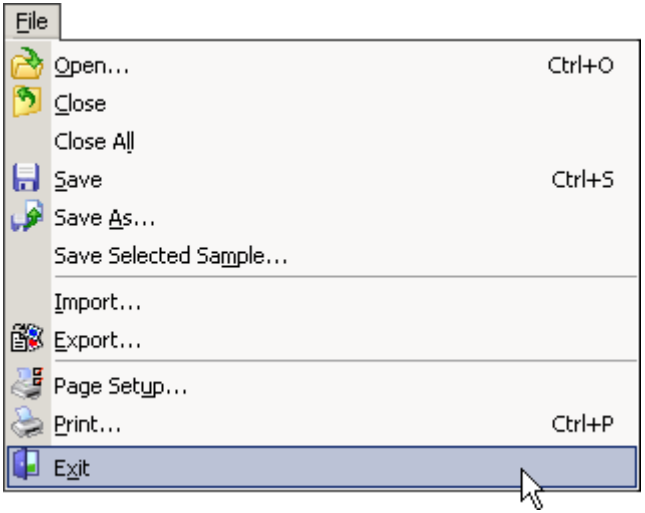
6 Select the *Select file in Data Context* check box and click *OK*.

This takes you to the *Data and Assay* context where you can view the results and practice data analysis and evaluation as described in "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 91 and "Analyzing and Evaluating the Results of a Flow Cytometric Assay" on page 198.

Closing 2100 Expert

To close 2100 expert:

1 From the *File* menu, select *Exit*.

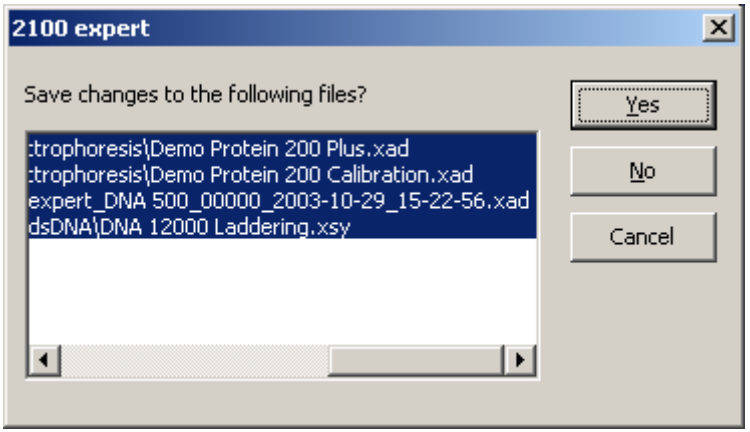


2100 expert quits.

However, if a chip run is in progress, the following message appears:

Click *OK* and wait until the chip run is complete.

Also the following dialog box may appear:



NOTE

This dialog box may also appear if you try to switch between contexts but there is unsaved data.

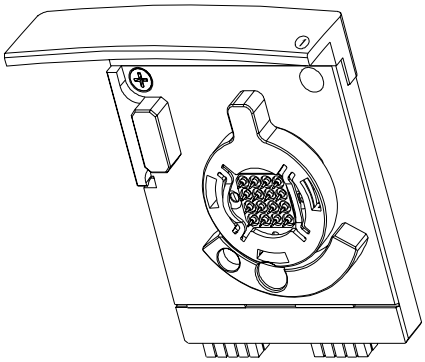
- Click *Yes* to save the changes to the selected files and continue quitting 2100 expert.

  If you want to save changes only to particular files, select these files in the list by single-clicking them. By default, all files with unsaved changes are selected.
- If you click *No*, 2100 expert quits without saving any changes.
- If you do not want to quit 2100 expert at this time, click *Cancel* to return to your 2100 expert session without saving anything.

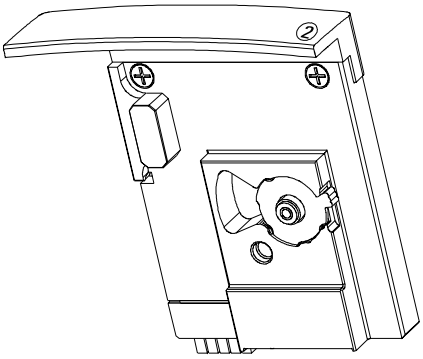
Switching Between Electrophoretic and Flow Cytometric Assays

The Agilent 2100 bioanalyzer supports electrophoretic assays (DNA, RNA, and protein) and flow cytometric assays (apoptosis, for example).

The bioanalyzer uses different cartridges for electrophoretic and flow cytometric assays:



Electrode Cartridge for electrophoretic assays



Pressure Cartridge for flow cytometric assays

- The *electrode cartridge* contains 16 electrodes that fit into the wells of DNA, RNA, and Protein chips. Each electrode in the cartridge has an individual power supply. All electrophoretic assays (DNA, RNA, and Protein) require an electrode cartridge. The electrode cartridge can be identified by the engraved number "1" on the front.
- The *pressure cartridge* contains a tubing and filter assembly that connect to the vacuum pump. The seal has to match the priming well on the chip so that the required low pressure can be built up. The pressure cartridge can be identified by the engraved number "2" on the front.

NOTE

There are also cartridges without an engraved number. These are all electrode cartridges.

If the bioanalyzer is set up for flow cytometric assays, but you want to run electrophoretic assays, proceed as described in "How to Prepare the Bioanalyzer for Electrophoretic Assays" on page 49.

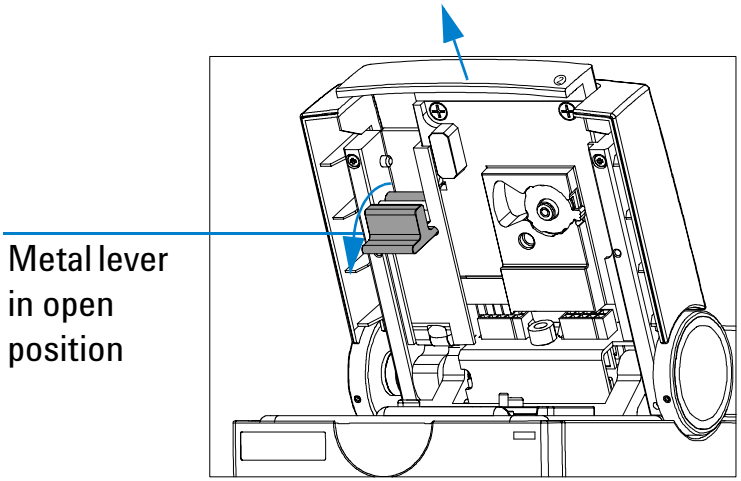
If the bioanalyzer is set up for electrophoretic assays, but you want to run flow cytometric assays, proceed as described in "How to Prepare the Bioanalyzer for Flow Cytometric Assays" on page 52.

Remove the pressure cartridge:

1 Turn off line power to the Agilent 2100 bioanalyzer. The line switch is located at the rear.

2 Open the lid.

3 Pull down the metal locking lever as shown in the figure below.



The cartridge is pushed out.

4 Gently pull the cartridge out of the lid.

NOTE

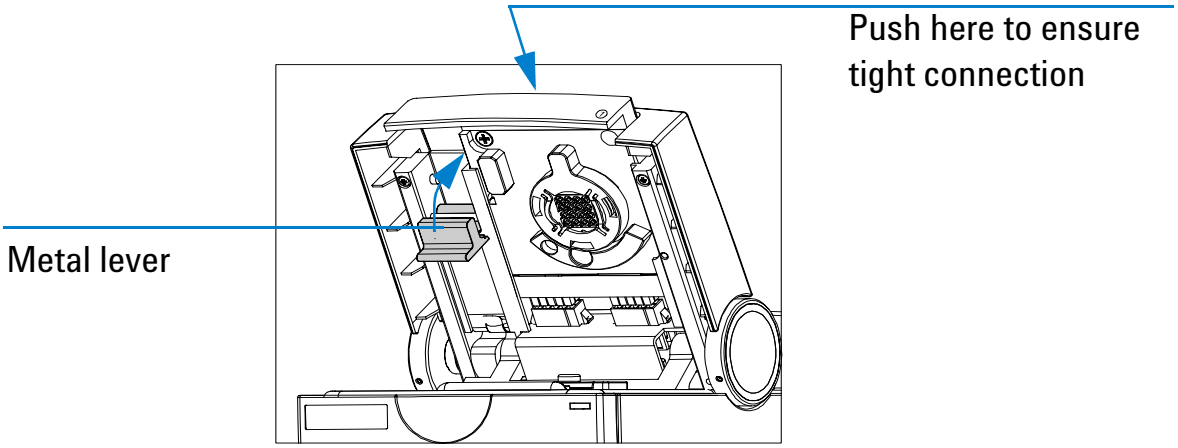
Store the pressure cartridge in the provided box.

Insert the electrode cartridge:

CAUTION

Do not touch the electrodes while the cartridge is in the Agilent 2100 bioanalyzer. The electrodes and the high voltage power supplies can be damaged.

1 Slide the electrode cartridge in the lid as shown below.



2 Push the metal front of the cartridge to ensure a tight connection.

3 Push the metal locking lever in the flat (closed) position.

CAUTION

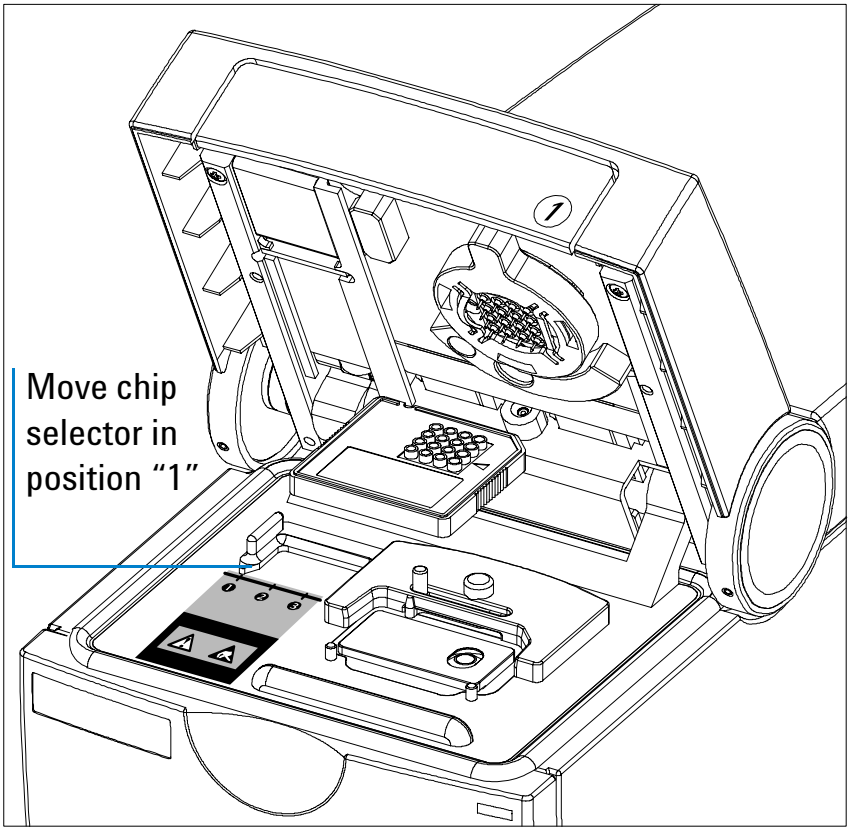
Do not force the lid closed. This may damage the cartridge. If the lid does not close completely, check that the cartridge is inserted properly and try again to close the lid.

4 Remove any chip.

CAUTION

Do not force the chip selector handle when a chip is inserted in the bioanalyzer.

5 Adjust the chip selector to position "1" as shown in the following figure.



To avoid using incompatible chips and cartridges, a chip selector is installed in the bioanalyzer. This ensures that the chip matches to the installed cartridge.

This will allow you to insert DNA, RNA, and Protein chips in the bioanalyzer.

Remove the electrode cartridge:

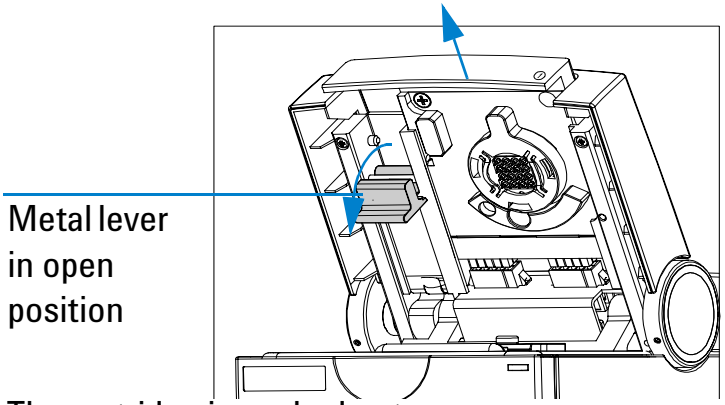
1 Turn off line power to the Agilent 2100 bioanalyzer. The line switch is located at the rear.

2 Open the lid.

CAUTION

Do not touch the electrodes while the cartridge is in the Agilent 2100 bioanalyzer. The electrodes and the high voltage power supplies can be damaged.

3 Pull down the metal locking lever in the open position as shown in the figure below.



The cartridge is pushed out.

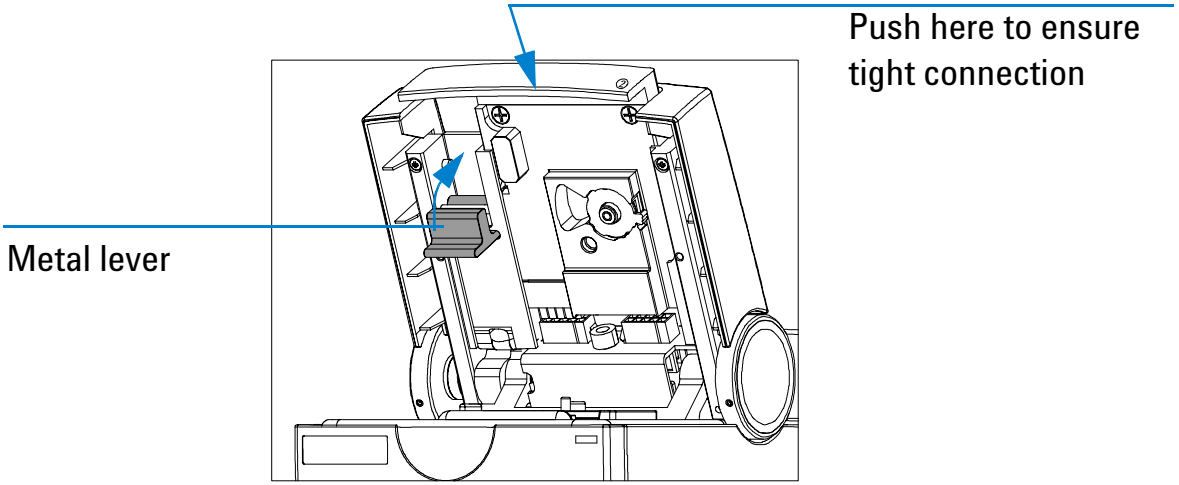
4 Gently pull the cartridge out of the lid.

CAUTION

Store the electrode cartridge in the provided box. If the pins of the electrode cartridge are bent or misaligned, poor quality results or pre-terminated chip runs will result.

Insert the pressure cartridge:

1 Slide the pressure cartridge in the lid as shown below.



2 Push the metal front of the cartridge to ensure a tight connection.

3 Push the metal locking lever in the flat (closed) position.

CAUTION

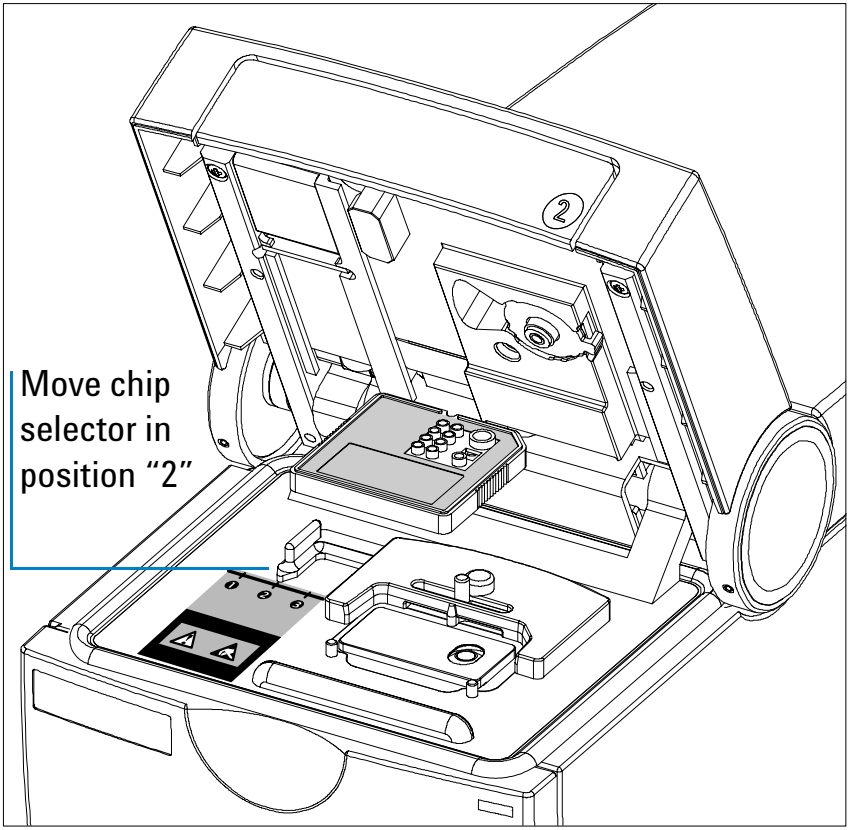
Do not force the lid closed. This may damage the cartridge. If the lid does not close completely, check that the cartridge is inserted properly and try again to close the lid.

4 Remove any chip.

CAUTION

Do not force the chip selector handle when a chip is inserted in the bioanalyzer.

5 Adjust the chip selector to position “2” as shown in the following figure.



To avoid using incompatible chips and cartridges, a chip selector is installed in the bioanalyzer. This ensures that the chip matches to the installed cartridge.

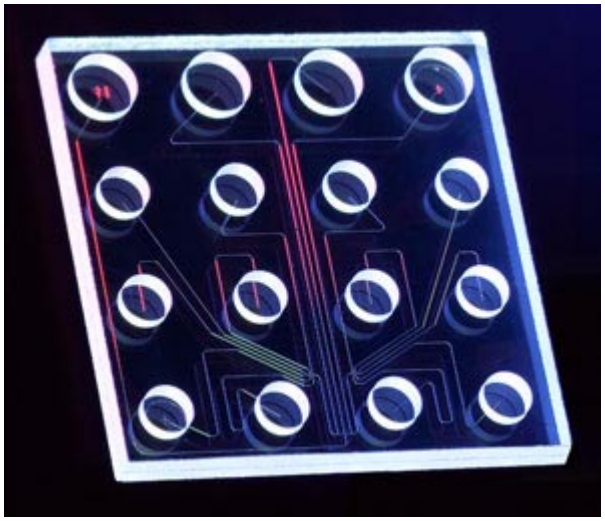
This will allow you to insert cell chips in the bioanalyzer.

Running and Evaluating Electrophoretic Assays

For running and evaluating electrophoretic assays you need to know the following:

Principles of Nucleic Acid and Protein Analysis on a Chip

The electrophoretic assays are based on traditional gel electrophoresis principles that have been transferred to a chip format. The chip format dramatically reduces separation time and sample consumption. The system provides automated sizing and quantitation information in a digital format. On-chip gel electrophoresis is performed for the analysis of DNA, RNA and proteins.



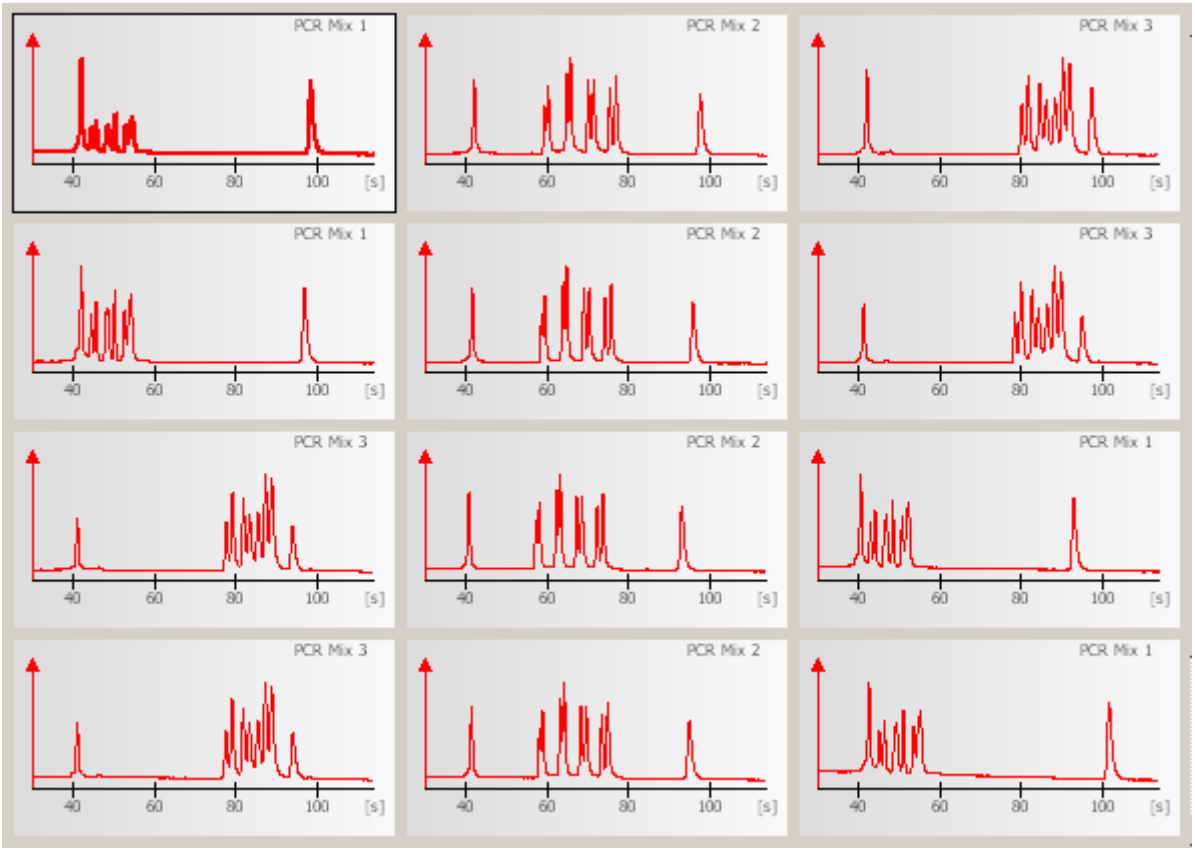
The chip accommodates sample wells, gel wells and a well for an external standard (ladder). Micro-channels are fabricated in glass to create interconnected networks among these wells. During chip preparation, the micro-channels are filled with a sieving polymer and fluorescence dye. Once the wells and channels are filled, the chip becomes an integrated electrical circuit. The 16-pin electrodes of the cartridge are arranged so that they fit into the wells of the chip. Each electrode is connected to an independent power supply that provides maximum control and flexibility. Charged biomolecules like DNA or

RNA are electrophoretically driven by a voltage gradient—similar to slab gel electrophoresis. Because of a constant mass-to-charge ratio and the presence of a sieving polymer matrix, the molecules are separated by size. Smaller fragments are migrating faster than larger ones. Dye molecules intercalate into DNA or RNA strands or Protein-SDS micells. These complexes are detected by laser-induced fluorescence. Data is translated into gel-like images (bands) and electropherograms (peaks). With the help of a ladder that contains fragments of known sizes and concentrations, a standard curve of migration time versus fragments size is plotted. From the migration times measured for each fragment in the sample, the size is calculated. Two marker fragments (for RNA only one marker fragment) are run with each of the samples bracketing the overall sizing range. The "lower" and "upper" markers are internal standards used to align the ladder data with data from the sample wells. This is necessary to compensate for drift effects that may occur during the course of a chip run.

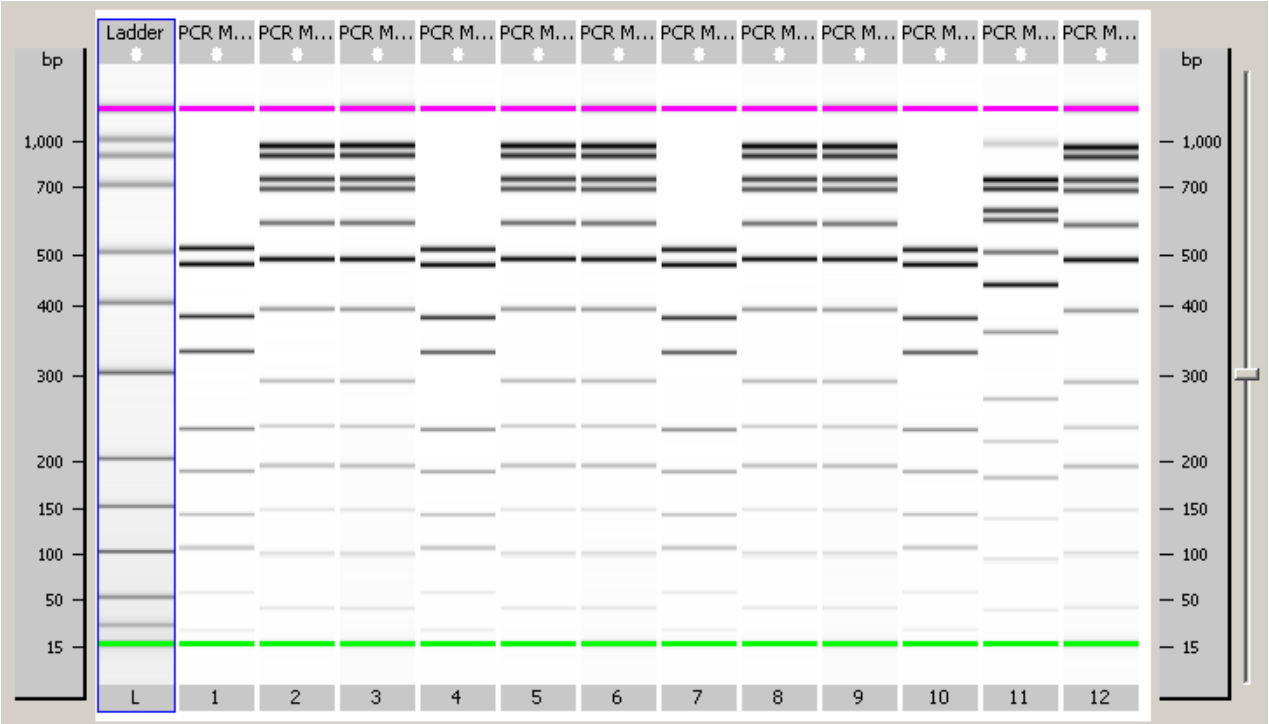
For DNA and protein assays, quantitation is done with the help of the upper marker. The area under the upper marker peak is compared with the sample peak areas. Because the concentration of the upper marker is known, the concentration for each sample can be calculated. Besides this relative quantitation, an absolute quantitation is available for protein assays, using external standard proteins.

For RNA assays, quantitation is done with the help of the ladder area. The area under the ladder is compared with the sum of the sample peak areas. The area under the "lower" marker is not taken into consideration. For total RNA assays, the ribosomal ratio is determined, giving an indication on the integrity of the RNA sample.

The 2100 expert software plots fluorescence intensity versus migration time and produces an electropherogram for each sample:



The data can also be displayed as a densitometry plot, creating a gel-like image:



Preparing and Running an Electrophoretic Assay

An electrophoretic chip run requires the following steps:

1. Set up and switch on the Agilent 2100 bioanalyzer.

   Refer to "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47.

2. Start the 2100 expert software.

   Details are given in "Starting 2100 Expert" on page 32.

3. Select an electrophoretic assay.

   See "Selecting an Electrophoretic Assay for a Chip Run" on page 62.

4. Prepare chip and samples.

   Refer to "Preparing Samples and Chips for Electrophoretic Assays" on page 66 and to the appropriate *Application Note* and *Reagent Kit Guide*.

5. Load the chip into the bioanalyzer.

   For details refer to "Loading the Electrophoresis Chip into the Bioanalyzer" on page 70.

6. Start the chip run.

   This is described in "Running an Electrophoretic Assay" on page 72.

When the chip run has finished, you can:

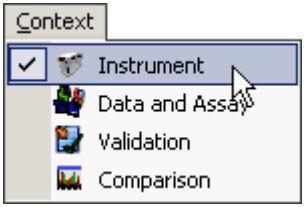
- Have a first look at the results (see "Displaying the Measurement Results (Electrophoresis)" on page 82).
- Document the chip run (see "Entering Chip, Sample, and Study Information" on page 80).

- Analyze and evaluate the results:
  - "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 91
  - "Result Flagging" on page 145

Selecting an Electrophoretic Assay for a Chip Run

To select an assay:

1 Switch to the *Instrument* context.



2 In the *Tree View Panel*, select the bioanalyzer you want to use.

In the upper left of the *Instrument* tab, an icon shows the status of the bioanalyzer. You should see one of the following icons (lid open/closed), indicating that the bioanalyzer is detected by the system:

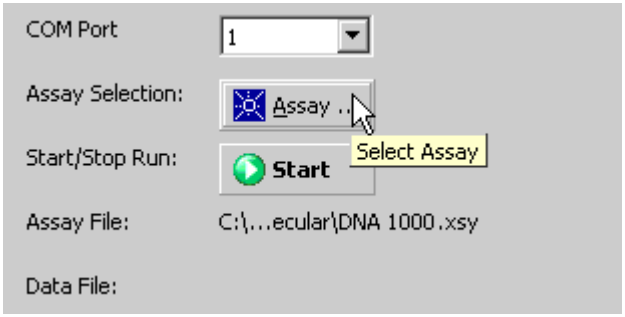
3 If you do not see one of these icons, check that the bioanalyzer is switched on and properly connected:

- Check the *COM port* setting.
- Make sure the bioanalyzer is physically connected to the PC (over the serial interface).
- Check the power connection.
- Check the power switch.

If you need additional help, please refer to the *Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide*.

4 Select an assay for the chip run.

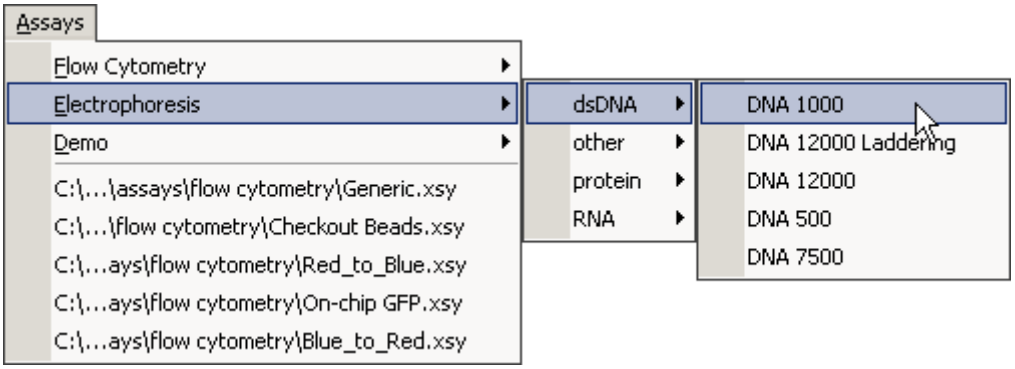
On the *Instrument* tab, click the *Assay...* button.



– OR –

Click the *Assays* menu.

Both will open the *Assays* menu, allowing you to select an assay from the submenus.



– OR –

Select *File* > *Open File to Run...*. This opens a dialog box, allowing you to load either an assay (.xsy) or a chip data file (.xad).

The type of assay you have to select depends on the experiment and the Reagent Kit you use to prepare your samples. Details on these assays are described in the *Application Notes* available for each assay and in the *Reagent Kit Guide*.

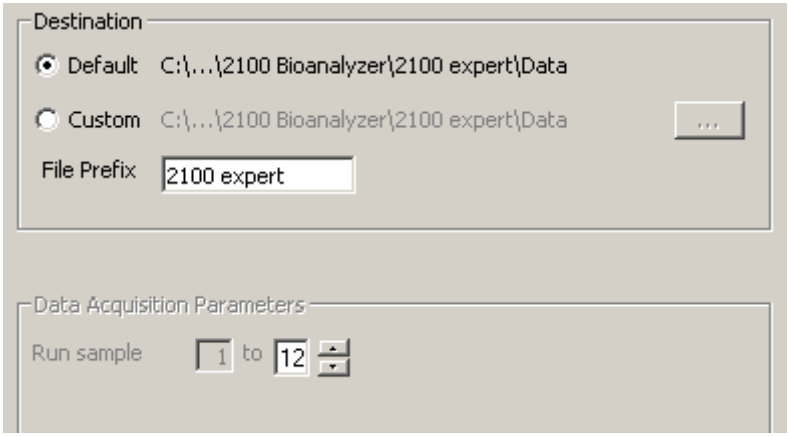
5 Select the desired assay, *DNA 1000*, for example.

The assay is loaded and its name appears on the *Information Bar*:

NOTE

After a chip run, the results can be evaluated using a different electrophoretic chip data file (.xad) of the same assay type (DNA 1000 in this example). Refer to "Importing Data Analysis Setpoints" on page 259.

6 Select a *Destination* for the chip data file (.xad) generated as the result of the chip run:



7 Under *Data Acquisition Parameters*, enter the number of samples you want to be measured.

The total number of samples that can be measured varies with the type of assay selected. With DNA and RNA Nano assays, 12 samples may be run; with RNA Pico assays, 11 samples may be run; and with Protein assays, the maximum number of samples is 10. When preparing the chip (see "Preparing Samples and Chips for Electrophoretic Assays" on page 66), keep in mind that you have to follow the sequence of the sample wells. For example, if you want to measure 3 samples, you have to fill the wells 1, 2, and 3 of your chip.

Preparing Samples and Chips for Electrophoretic Assays

Before you can fill a chip, you have to prepare the samples. To find out how to prepare the samples, refer to the various *Reagent Kit Guides* available for each LabChip kit. Please refer to these documents for further information and analytical specifications.

In general, preparing an electrophoretic assay involves the following steps:

- Check that you have everything listed in the appropriate Reagent Kit Guide.

  Be aware that there can be small but important differences between the different assays even for the same type of molecules (for example, between DNA 1000 and DNA 7500 assays).
- Make sure you are familiar with the essential measurement practices (see below).
- Before running the first RNA assay: decontaminate the electrodes.
- Prepare all the reagent mixtures (for example, the gel-dye mix).
- Load the gel-dye mix using the priming station.
- Load the DNA/RNA marker solution.
- Load the chip with ladder, samples and buffer (depending on assay).

Essential Measurement Practices (Electrophoretic Assays)

General:

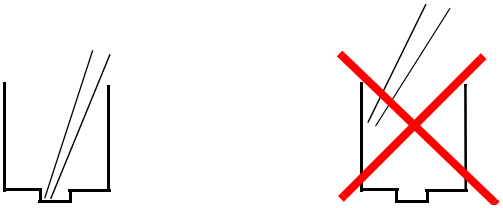
WARNING

Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.

WARNING

No data is available addressing the mutagenicity or toxicity of the dye/DMSO reagent. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO mixtures should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling DMSO mixtures.

- Handle and store all reagents according to the instructions given in the *Reagent Kit Guides*.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results due to the formation of a bubble on the bottom of the well.



- Protect dye and gel-dye mix from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Use a new syringe and electrode cleaner with each new LabChip kit.
- Do not touch the Agilent 2100 bioanalyzer during a chip run and never place it on a vibrating ground.
- Keep all reagents and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use.
- Use loaded chips within 5 minutes. Reagents might evaporate, leading to poor results.

RNA Assays:

- Always wear gloves when handling RNA, and use RNase-free tips, microfuge tubes and water.
- It is recommended to denature all RNA samples and RNA ladder by heat before use (70 °C, 2 minutes).
- Always vortex the dye concentrate for 10 seconds before preparing the gel-dye mix.

Protein Assays:

- Store Protein sample buffer at -20 °C upon arrival. Keep the vial in use at 4 °C to avoid freeze-thaw cycles.
- Allow the dye concentrate to equilibrate to room temperature for 20 minutes before use, to make sure the DMSO is completely thawed. Protect the dye from light during that time. Vortex before use.
- Allow all other reagents to equilibrate to room temperature for 10 minutes before use.
- Use 0.5 ml tubes to denature samples. Using larger tubes may lead to poor results, caused by evaporation.

Loading the Electrophoresis Chip into the Bioanalyzer

After preparing the chip, you can insert it into the Agilent 2100 bioanalyzer.

To load the chip into the bioanalyzer:

1 Open the lid.

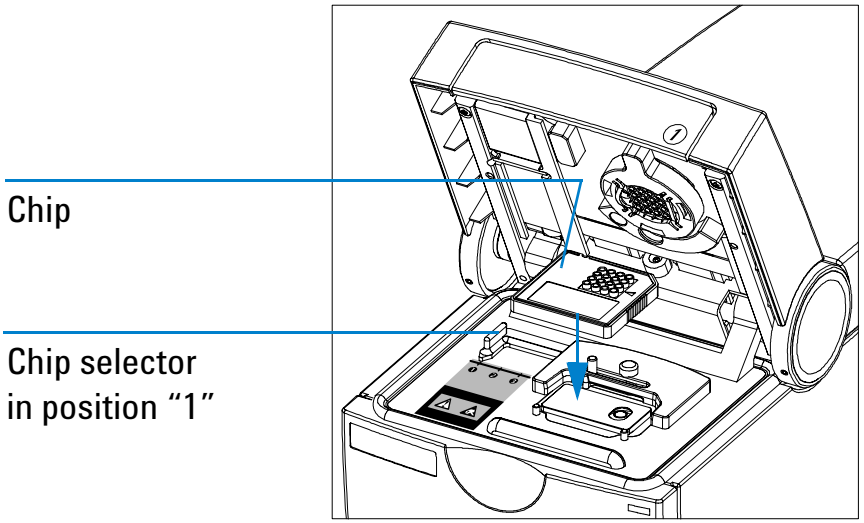
NOTE

Before inserting the chip, check that the electrode cartridge is installed and the chip selector is in position "1". For details, refer to "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47.

---

2 Place the prepared chip into the receptacle.

The chip fits only one way. Do not use force.

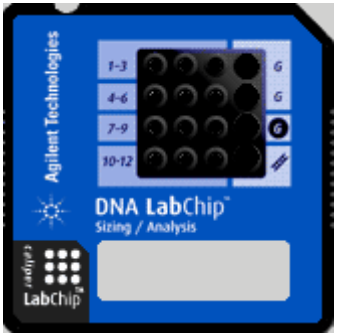


3 Carefully close the lid.

CAUTION

Do not force the lid closed. This may damage the cartridge. If the lid does not close completely, check that the cartridge and chip are inserted properly, and the chip selector is in the correct position.

The icon on the *Instrument* tab changes to a DNA chip icon, if a DNA assay is selected:



If the chip is not detected, open and close the lid again.

NOTE

If the *AutoRun* option is active (see "Options – Advanced" on page 508), the chip run starts automatically once a chip has been inserted and the lid has been closed.

Running an Electrophoretic Assay

Running an electrophoretic assay in 2100 expert just means pressing a button.

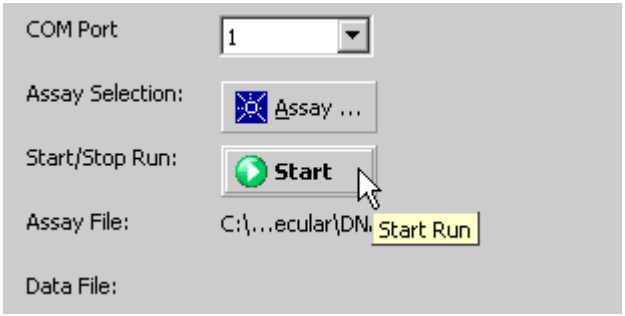
NOTE

You can stop a chip run at any time, for example, if errors occurred, or if you are not satisfied with the quality of the measurement results, which you can observe during the chip run. See "Stopping a Chip Run" on page 77.

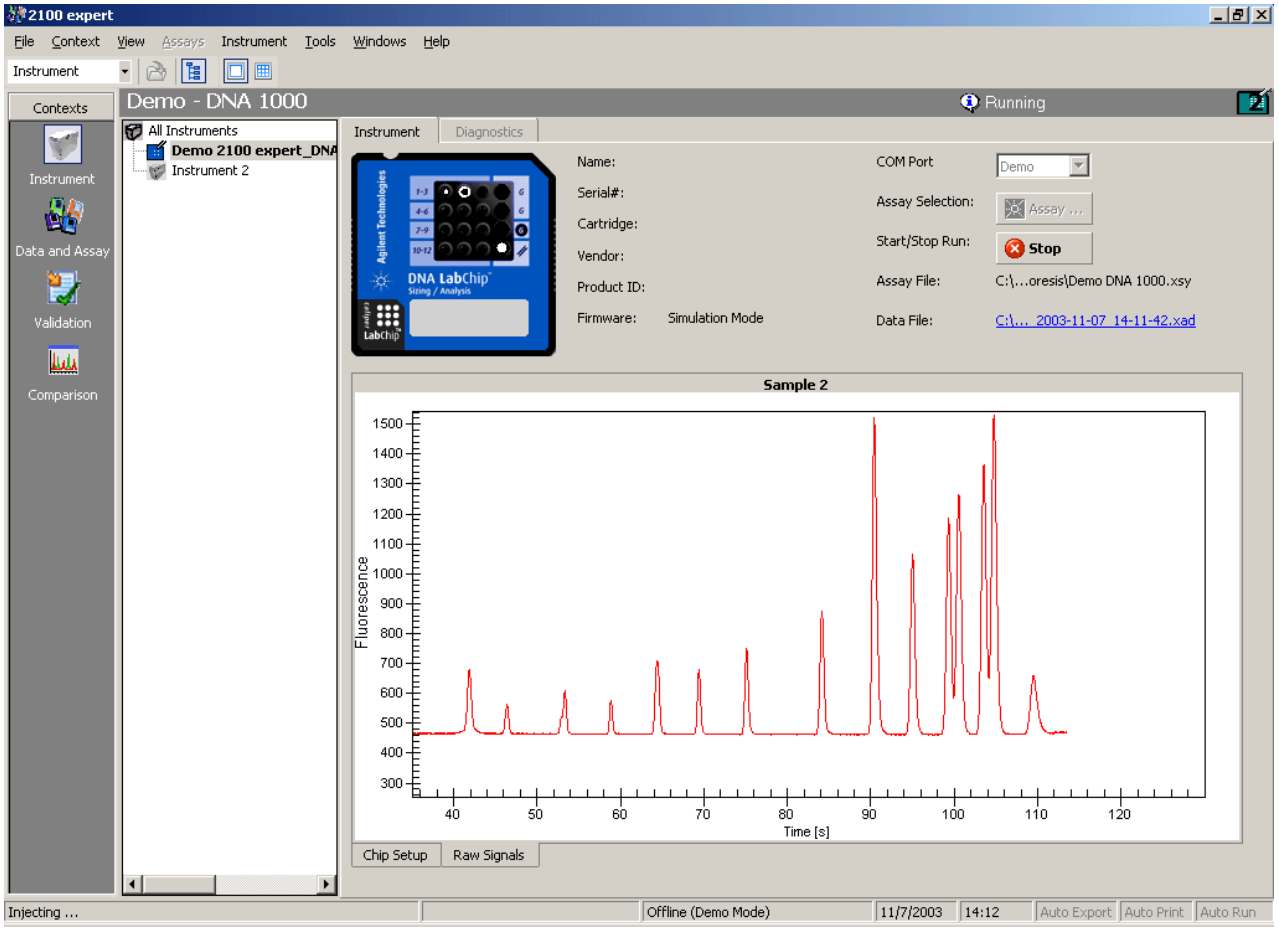
Starting the Chip Run

When you have loaded the chip, you can start the chip run:

1 On the *Instrument* tab, click the *Start* button.



The chip run starts. The *Raw Signals* sub-tab shows an electropherogram of the currently measured sample. The name of the sample is displayed above the graph. The graph is a "live" plot of the migration time against fluorescence units (raw data, including background fluorescence, for example).

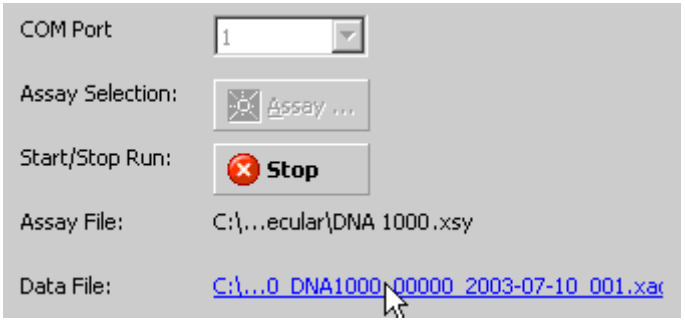


The number of the sample that is currently being measured is indicated on the information bar:

The status bar shows the name of the currently measured sample, a progress bar showing the measurement progress for the current sample (not for the whole chip run), and the COM port number used for data acquisition:

During the chip run, you can do the following:

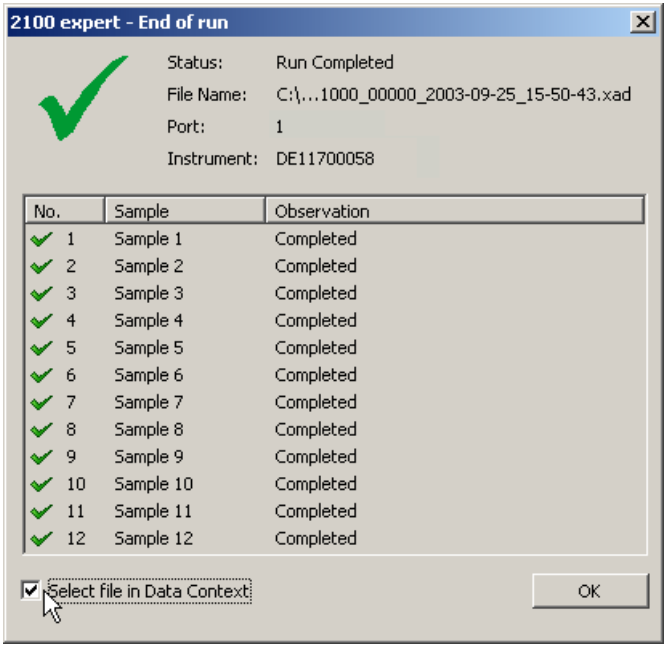
- View the chip data file in the *Data and Assay* context by clicking on the name of the *Data File*:



- Switch to any other context. For example, you can evaluate any chip data file in the *Data and Assay* context, or compare samples in the *Comparison* context.
- If necessary, abort the chip run by clicking on the *Stop* button. All data that was collected up to the stop point will be saved.

Finishing the Chip Run

When the measurements are finished, the *End of run* dialog box appears, showing you the number of samples that have been measured, and the file name where the chip run data has been stored. If errors occurred, they would also be displayed in this dialog box.



1. To immediately view the results in the *Data and Assay* context, you can select the *Select file in Data Context* check box.
2. Click *OK*.

The dialog box is closed.

- If you selected *Select file in Data Context*, you are automatically taken to the *Data and Assay* context, where you can view, analyze, and evaluate the results of your chip run (see "Displaying the Measurement Results (Electrophoresis)" on page 82 and "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 91).
- If you did *not* select the *Select file in Data Context* check box, you are taken back to the *Instrument* context, where you can start a new assay, for example.

Stopping a Chip Run

You can stop a chip run at any time, for example,

- if the quality of the measurement results does not meet your expectations,
- if, for example, after three samples you already have the information you desired and you want to start another chip run.

NOTE

You *cannot* resume a stopped chip run.

NOTE

If you stop a chip run, automatic export (see "Exporting Chip Run Data Automatically" on page 266) and automatic print (see "How to Turn on and Configure Automatic Printing of Chip Run Reports" on page 280) do *not* take place.

To stop the assay:

1 Click the *Stop* button 

.

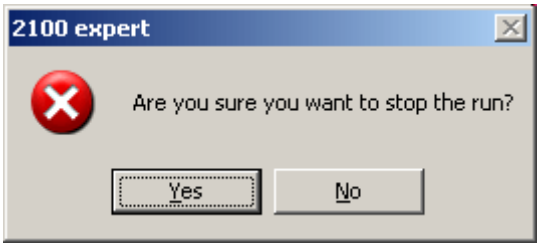
– OR –

Select *Stop* from the *Instrument* menu.

NOTE

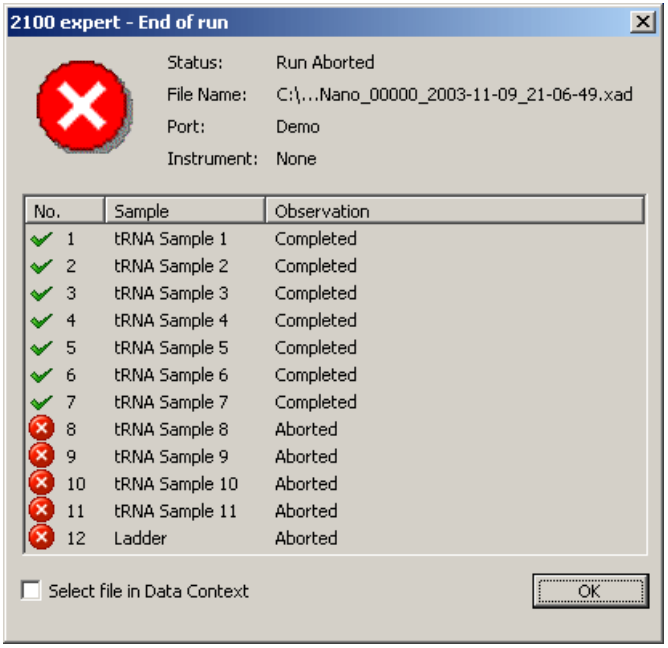
Data acquisition of the current sample will be aborted.

The following message appears:



2 Click *Yes* to stop the chip run.

The *End of Run* dialog box appears.



The measured samples are marked with a green check, and only these are stored in the chip data file.

The unmeasured samples are marked with a white cross on red ground.

3 If you want to immediately view the results in the *Data and Assay* context, select the *Select file in Data Context* check box.

4 Click *OK*.

The dialog box is closed.

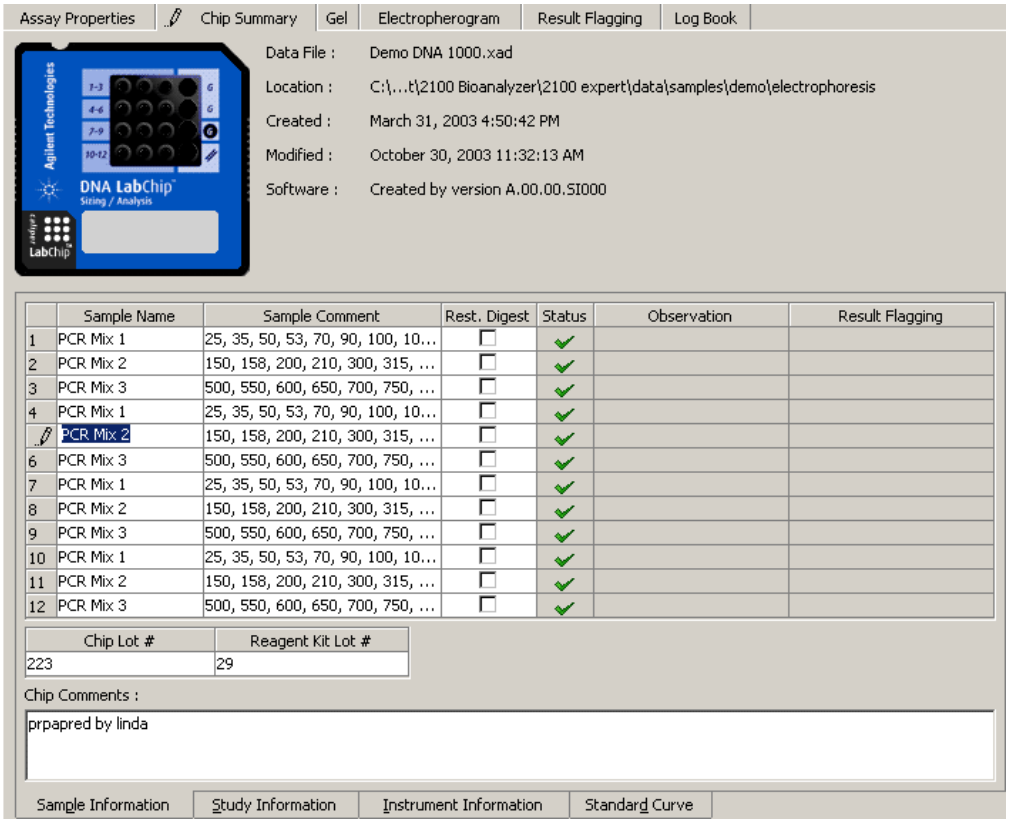
- If you selected *Select file in Data Context*, you are automatically taken to the *Data and Assay* context, where you can view, analyze, and evaluate the results (if any) of your chip run (see "Displaying the Measurement Results (Electrophoresis)" on page 82 and "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 91).
- If you did *not* select the *Select file in Data Context* check box, you are taken back to the *Instrument* context, where you can start the next chip run, for example.

Entering Chip, Sample, and Study Information

During or after a chip run, you can document the run by entering information on chip, samples, and study.

1 In the *Data and Assay* context, select the *Chip Summary* tab.

2 On the *Sample Information* sub-tab, you can enter additional information such as sample names and comments. On the *Study Information* sub-tab, you can enter the laboratory location, and the name of the experimenter, for example.



NOTE

You may find some input fields already filled in, because chip, sample, and study information are taken over from the base assay or chip data file.

For details on all input fields, refer to "Chip Summary Tab" on page 427.

3 Click *Apply*.

4 From the *File* menu, select *Save*.

TIP

You can import chip, sample, and study information from .txt or .csv files. This is especially helpful and time-saving, if you already have documented a similar chip run in another chip data file. Refer to "Importing Chip, Sample, and Study Information" on page 261 for details.

Displaying the Measurement Results (Electrophoresis)

You can view the measurement results of an electrophoretic chip run as electropherograms or gel-like images.

- You can display the electropherograms either one sample at a time, or all samples at the same time to get an overview of the chip run, for example, to see the progress of a reaction. See "How to Switch Between Single View and Grid View (Electropherograms)" on page 83.
- You can navigate through the samples. See "How to Navigate Through the Samples" on page 84.
- You can change the display of electropherograms and gel-like images to make details better visible. See "How to Change the Display of Electropherograms and Gel-like Images" on page 85.

How to Switch Between Single View and Grid View (Electropherograms)

To switch between single view and grid view:

1 From the *Electropherogram* menu, select *View Single Sample* or *View All Samples*.

– OR –

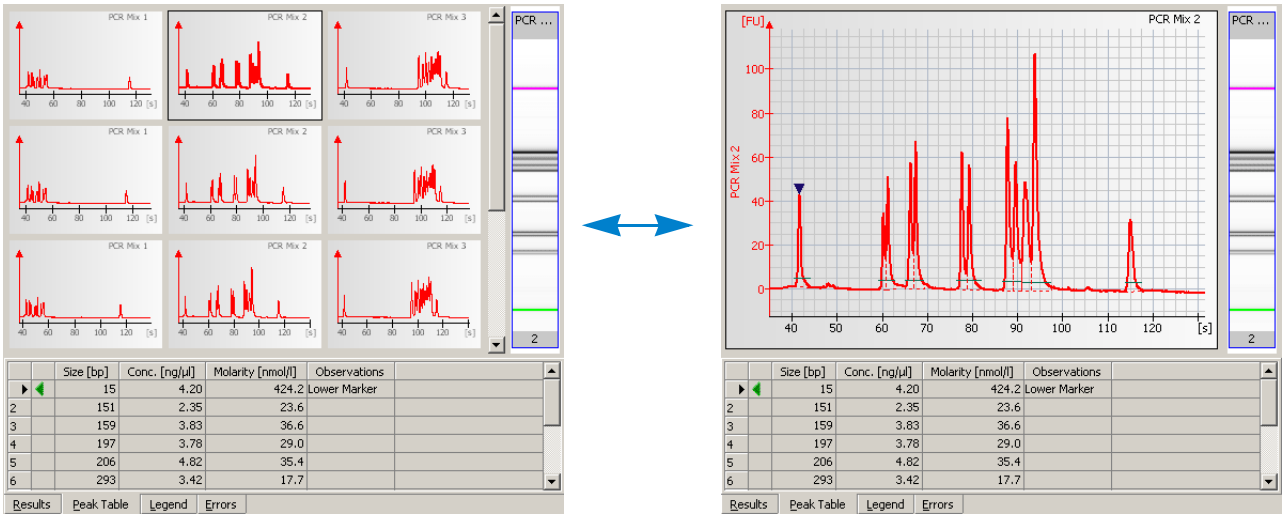
Click the *View Single Sample* or *View All Samples* button on the *Electropherogram* tool bar.

– OR –

Click the *All Samples* entry in the *Tree View Panel* to switch to the grid view, or any sample name to switch to the single view.

– OR –

Double-click any electropherogram the grid view to switch to single view:



How to Navigate Through the Samples

At any time—even during a chip run—you can scroll through all samples—either in electropherogram or gel view.

To navigate through samples using the *Tree View Panel*:

1. If the tree view is not visible, select *View* > *Tree View*.

   The tree view panel appears to the left of the tabs, and shows all chip data and assay files as nodes.

2. Click any sample name.

   Electropherogram view: the electropherogram of the selected sample is shown in single view

   Gel view: the lane of the gel-like image corresponding to the selected sample is highlighted.

To navigate through samples using the *Lower Panel*:

1. If the lower panel is not visible, select *View* > *Lower panel*.

   The lower panel appears in the lower left corner.

2. Electropherogram view: Click on any lane of the small gel image.
   Gel view: Click on any well on the chip icon.

To browse through samples:

1 From the *Electropherogram* or *Gel* menu, select *Next Sample* or *Previous Sample*.

– OR –

Click the *Next Sample*  or *Previous Sample*  button in the tool bar.

To switch between electropherogram and gel view:

1 Click the *Electropherogram* or *Gel* tab to display the results of the selected sample as an electropherogram or as a gel-like image.

How to Change the Display of Electropherograms and Gel-like Images

It is possible to change the display of electropherograms and gel-like images.

In electropherograms and gel-like images you can:

- zoom (enlarge or reduce using the mouse) the graphs to display details, for example.

In electropherograms, you can additionally:

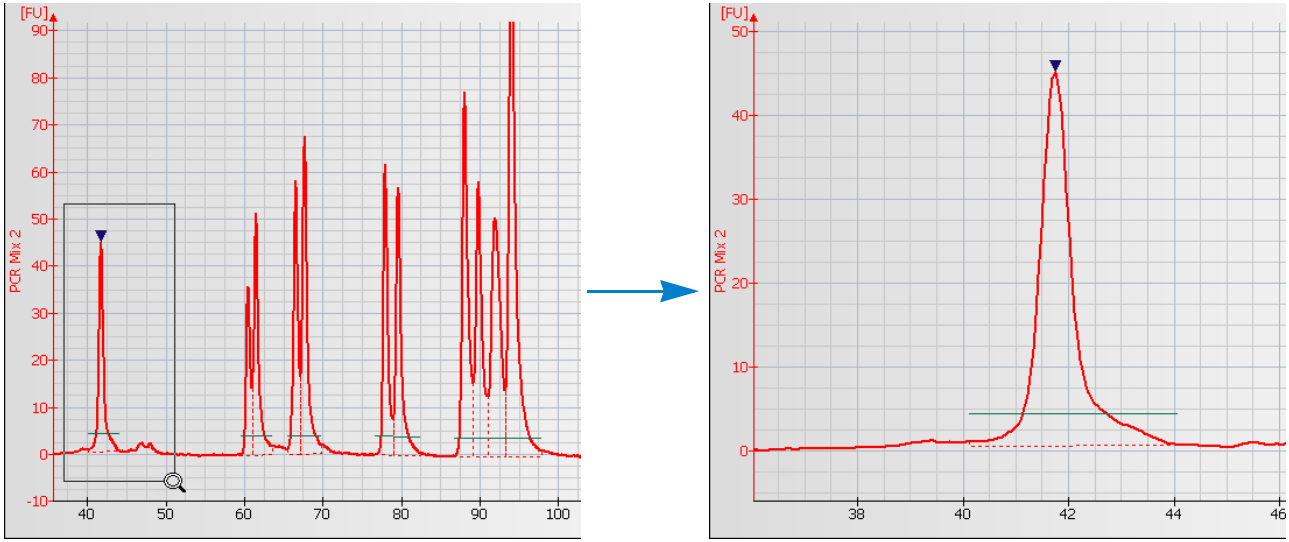
- show data points.
- pan and scale the graph using the mouse.
- change the background from white to a gray-to-white gradient.
- remove the grid from the electropherograms.

In gel-like images, you can additionally:

- change the gel color.

To zoom into an electropherogram:

1. From the *Electropherogram* menu, select *Graph Mode* > *Zoom* (default setting).
2. Position the mouse pointer in the electropherogram.
3. Click and hold down the left mouse button.
   The mouse pointer changes its shape to a magnifying glass .
4. Drag the mouse.
   A rectangle shows the part of the an electropherogram to be enlarged.
5. Release the mouse button.



To pan and scale an electropherogram:

1 From the *Electropherogram* menu, select *Graph Mode* > *Pan* or *Scale*.

2 Position the mouse pointer in the electropherogram.

3 Click and hold down the left mouse button.

The mouse pointer changes its shape to a double-arrow or to a double crosshair.

4 Drag the mouse.

As you drag the mouse, the electropherogram curve moves in the drag direction (*Pan* mode), or the scales of the X and/or Y axes change (*Scale* mode).

5 Release the mouse button.

You can perform several zoom, pan and scale steps in a row.

To undo one zoom, pan, or scale step:

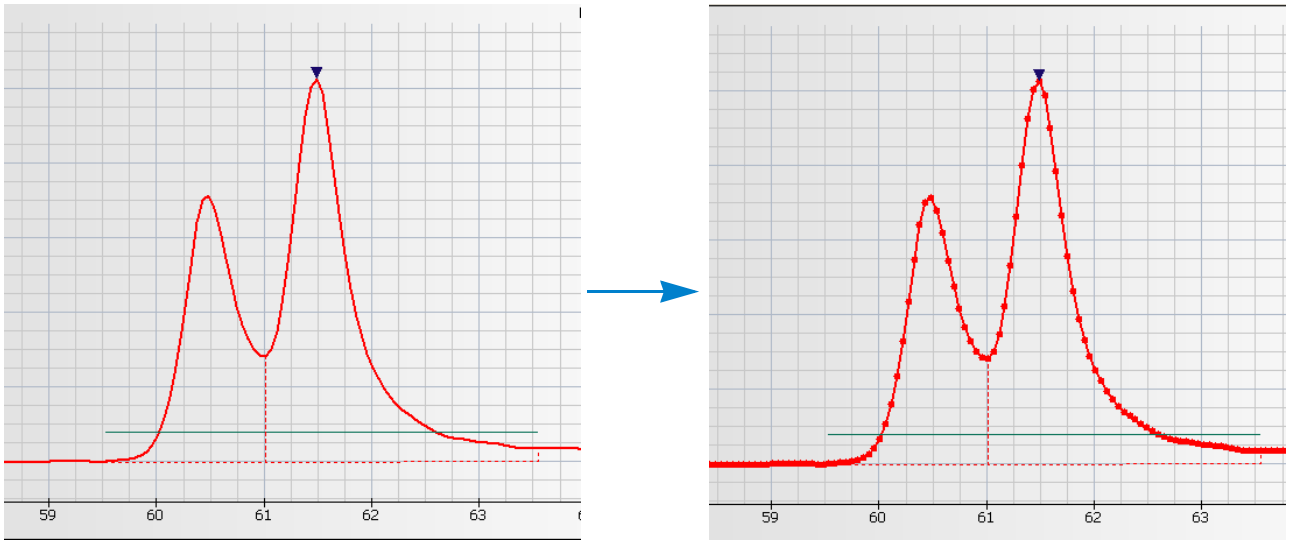
1 Click the *Undo Zoom*  button or double-click in the electropherogram.

To undo all zoom, pan, and scale steps:

1 Click the *Undo All*  button.

To display data points in an electropherogram:

1 From the *Electropherogram* menu, select *Show Data Points* or click the button in the toolbar.



Data points used to generate the graph are now shown as bullets. Data points are 0.05 seconds apart.

To put a color gradient on the background of an electropherogram:

1 From the *Electropherogram* menu, select *Show Gradient*.

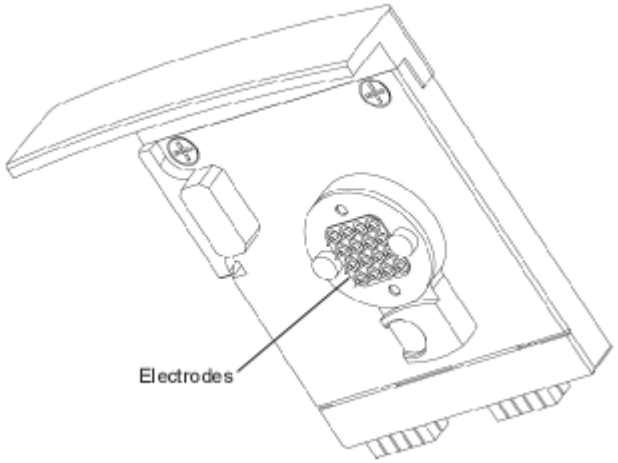
A color gradient (gray to white) appears on the background of the graph.

To show/hide the grid lines on an electropherogram:

1 From the *Electropherogram* menu, select *Show Grid*.

Cleaning the Electrodes after an Electrophoretic Assay

When the assay is complete, remove the used chip from the bioanalyzer and dispose of it according to the guidelines established by your laboratory safety officer. Remove the chip quickly to prevent a buildup of residues from the solutions on the electrodes.



Then perform the cleaning procedure to ensure that the electrodes are clean (i.e., no residues left from the previous assay). The cleaning procedures are described in detail in the appropriate *Reagent Kit Guide* and in the *Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide*.

Good Practices

- Empty and refill the electrode cleaner at regular intervals (e.g., every five assays).
- The electrode cleaner can be used for 25 assays.

CAUTION

Never use a cloth to clean the electrodes. Electrostatic discharge could damage the high-voltage power supplies.

CAUTION

Wet electrodes can cause severe damage to the on-board high voltage power supplies. Always make sure the electrodes are dry before inserting them into the bioanalyzer again.

Analyzing and Evaluating the Results of an Electrophoretic Assay

The purpose of electrophoretic assays is to calculate the size and concentration of nucleic acid fragments. Results for a particular sample are calculated after all data for that sample has been read.

The steps in data analysis differ depending on the type of assay in use:

- "Data Analysis: DNA" on page 92
- "Data Analysis: RNA and Cy5-Labeled Nucleic Acids" on page 97
- "Data Analysis: Protein" on page 100
- "Smear Analysis" on page 105

Further steps in analysis are:

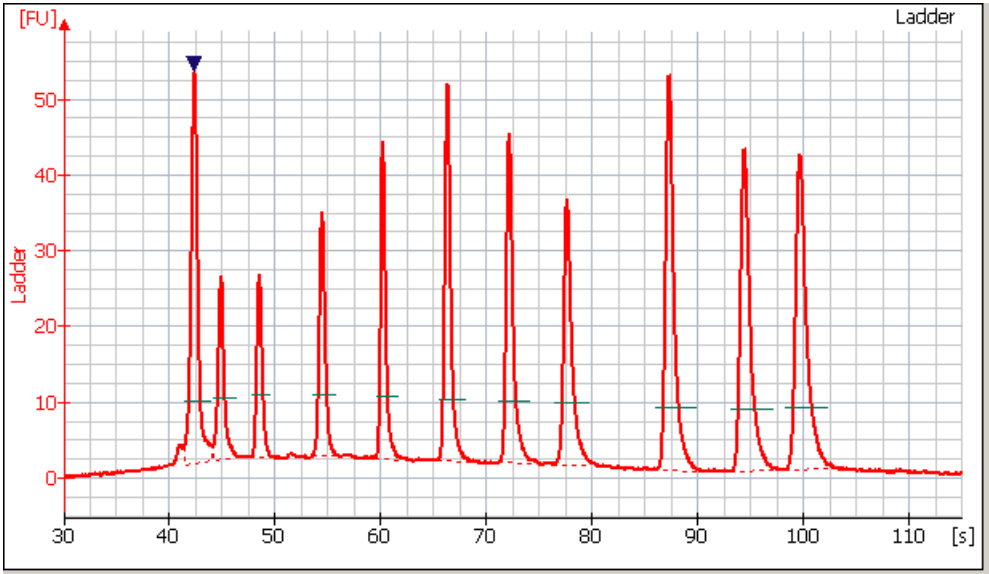
- "Changing the Data Analysis" on page 111
- "Reanalyzing a Chip Data File" on page 134
- "Comparing Samples from Different Electrophoretic Chip Runs" on page 136

Data Analysis: DNA

The data analysis process for DNA assays consists of the following steps:

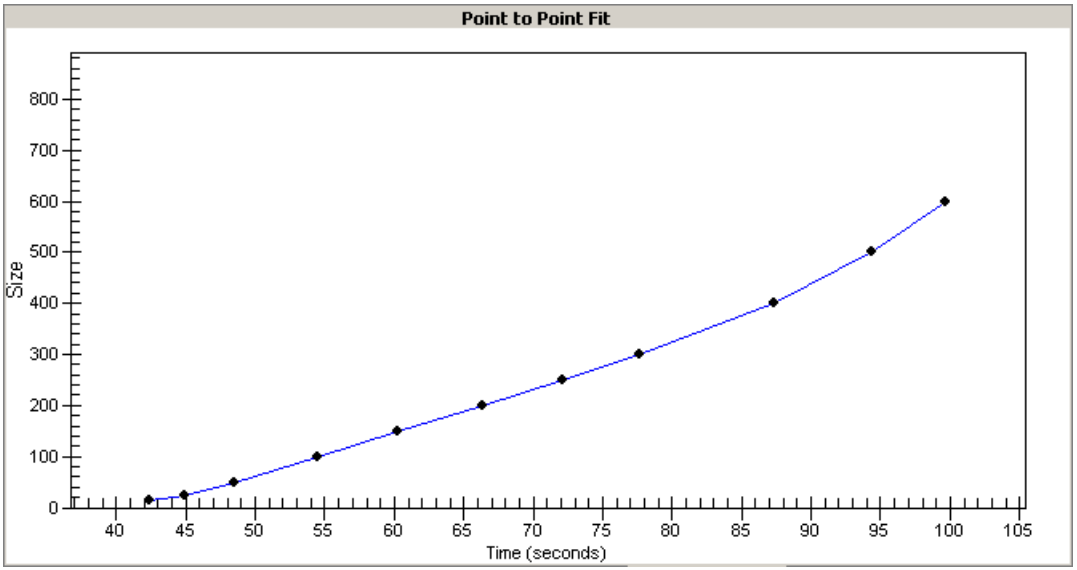
1. Raw data is read and stored by the system for all of the individual samples.
2. The data is filtered and the resulting electropherograms of all samples are plotted. You can change the settings of the data analysis after the run and reanalyze your data.
3. Peaks are identified for all samples and are tabulated by peak ID. You can change the settings of the peak find algorithm and reanalyze the data after the run has finished. (Note that peak find settings can be changed for all or only certain samples.)

4. A sizing ladder (see the example electropherogram below), which is a mixture of DNA fragments of known sizes, is run first from the ladder well. The concentrations and sizes of the individual base pairs are preset in the assay and cannot be changed.

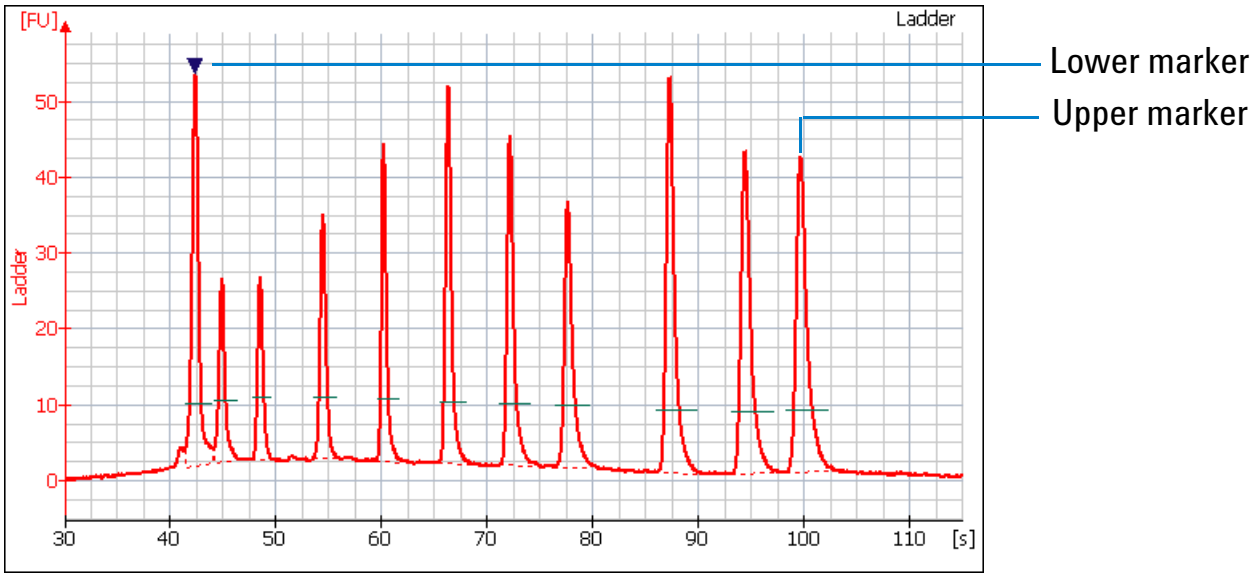


		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
▶	◀	15	4.20	424.2	Lower Marker
2	L	25	2.00	121.2	Ladder Peak
3	L	50	2.00	60.6	Ladder Peak
4	L	100	2.00	30.3	Ladder Peak
5	L	150	2.00	20.2	Ladder Peak

5. A standard curve of migration time versus DNA size is plotted from the DNA sizing ladder by interpolation between the individual DNA fragment size/migration points. The standard curve derived from the data of the ladder well should resemble the one shown below.



6. Two DNA fragments are run with each of the samples, bracketing the DNA sizing range. The “lower marker” and “upper marker” are internal standards used to align the ladder data with data from the sample wells. The figure below shows an example of assigned marker peaks in a sample well.



NOTE

The software performs alignment by default. Turning automatic data analysis off (see “Electropherogram Menu” on page 345) suspends data analysis until you turn it on again.

7. The standard curve, in conjunction with the markers, is used to calculate DNA fragment sizes for each sample from the migration times measured.

8. To calculate the concentration of the individual DNA fragments in all sample wells, the upper marker, in conjunction with an assay-specific concentration against base-pair size calibration curve, is applied to the individual sample peaks in all sample wells.

NOTE

The software allows you to redefine the peaks chosen as upper and lower markers. A change in marker selection will cause quantitative changes in the calibration procedure, however, and therefore in the entire data evaluation.

---

9. If the checkbox *Rest. Digest* on the *Chip Summary Tab* is enabled, the 2100 expert software flags peaks that may have co-migrated:

		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
1	◀	15	4.20	424.2	Lower Marker
2		22	1.71	116.6	Possible Co-Migration of 4 Peaks
3		55	1.31	35.8	
4		104	4.22	61.3	Possible Co-Migration of 2 Peaks
5		141	3.19	34.4	
6		187	3.87	31.5	
7		235	4.74	30.6	
8		330	6.81	31.3	
9		381	7.99	31.8	
10		476	10.34	32.9	
11		512	9.31	27.6	
12	▶	1,500	2.10	2.1	Upper Marker

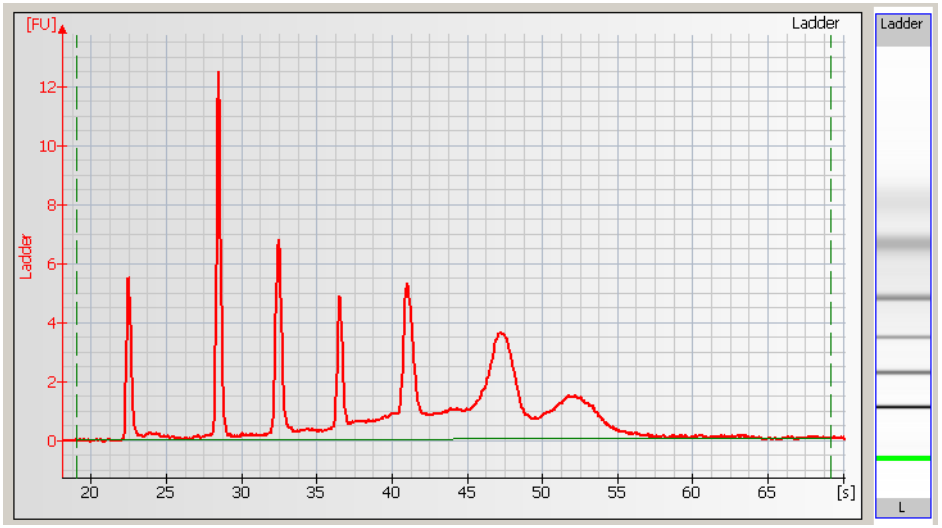
Results | Peak Table | Legend | Errors

Since it is assumed that the molarity of all the fragments in a restriction digest should be the same, any peaks or clusters having a molarity that is significantly larger than the rest are flagged as potentially co-migrating peaks, allowing you to examine them in more detail.

Data Analysis: RNA and Cy5-Labeled Nucleic Acids

The data analysis process for RNA and the Cy5-labeled nucleic acids assays consists of the following steps:

1. Raw data is read and stored by the system for all of the individual samples.
2. The data is filtered and the resulting electropherograms of all samples are plotted. You can change the settings of the data analysis after the run and reanalyze your data.
3. Fragments are identified for all samples and tabulated by peak ID. You can change the settings of the peak find algorithm for any or all samples and reanalyze the data.
4. An RNA ladder (containing a mixture of RNA of known concentration) is run first (see the electropherogram below). The concentrations and sizes of the individual base pairs are preset in the assay and cannot be changed.

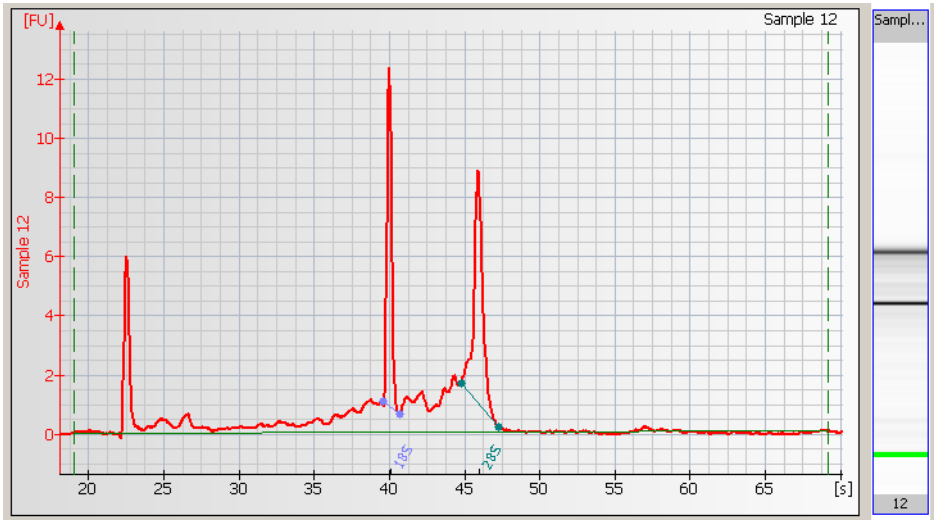


Electropherogram of RNA 6000 Ladder (Ambion, Inc. cat. no. 7152)

NOTE

Peak ratios for the RNA ladder may vary from one batch of RNA 6000 ladder to the next. Assay performance will not be affected by this variation.

5. For the Eukaryote or Prokaryote Total RNA assay, the RNA fragments (either 18S and 28S for eukaryotic RNA or 16S and 23S for prokaryotic RNA) are detected. After detection, the ratio of the fragment areas is calculated and displayed.



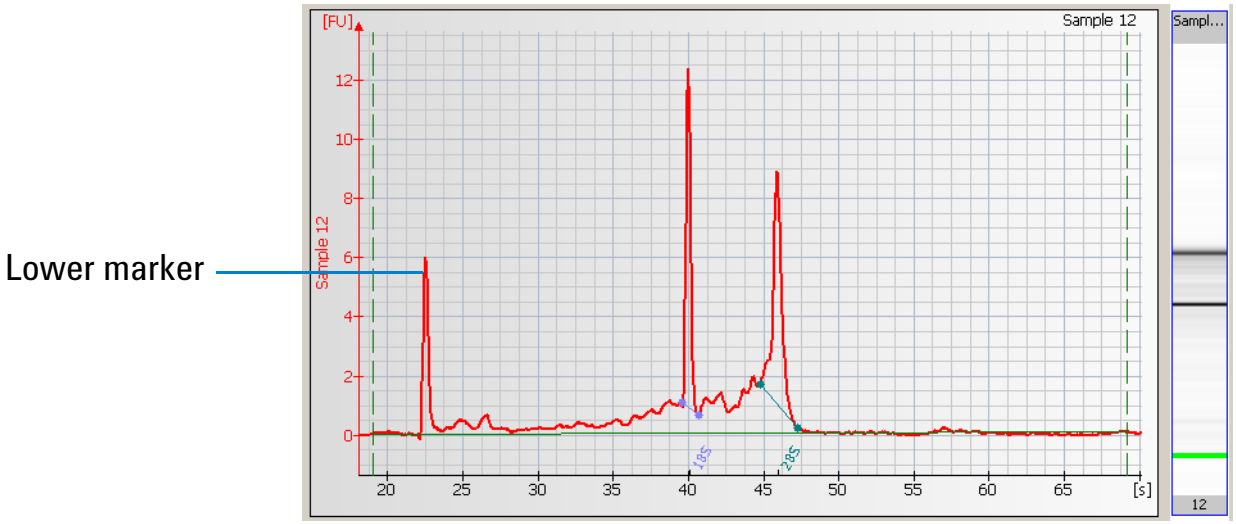
6. To calculate the concentration of the RNA, the area under the entire RNA electropherogram is determined. The ladder, which provides the concentration/area ratio, is applied to transform the area values into concentration values.

RNA Assays

The marker solution that is part of each RNA LabChip kit, contains a 50 bp DNA fragment. This fragment is used as lower marker to align all samples.

By default the RNA alignment and the subtraction of the lower marker are enabled for RNA Nano assays.

The marker is displayed as the first peak in the electropherogram:

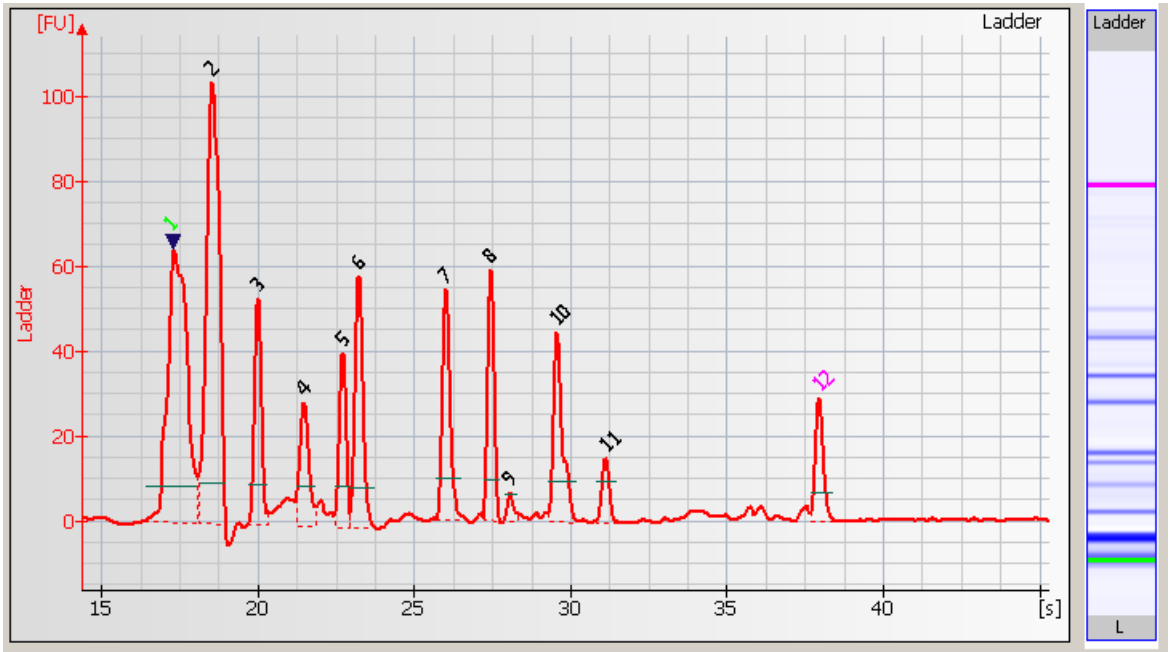


Data Analysis: Protein

The data analysis process for protein assays consists of the following steps:

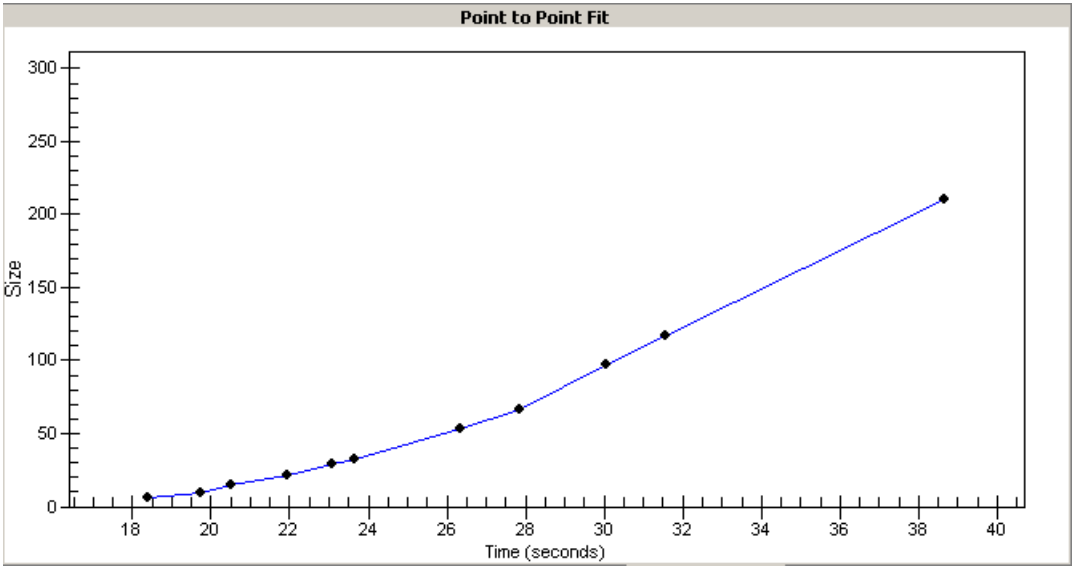
1. Raw data is read and stored by the system for all of the individual samples.
2. The data is filtered and the resulting electropherograms of all samples are plotted. You can change the settings of the data analysis after the run and reanalyze your data.
3. Peaks are identified for all samples and are tabulated by peak ID. You can change the settings of the peak find algorithm and reanalyze the data after the run has finished. (Note that peak find settings can be changed for all or only certain samples.)

4. A sizing ladder (see the example electropherogram below), which is a mixture of proteins of different known sizes, is run first from the ladder well. The concentrations and sizes of the individual proteins are preset as kDa in the assay and cannot be changed.

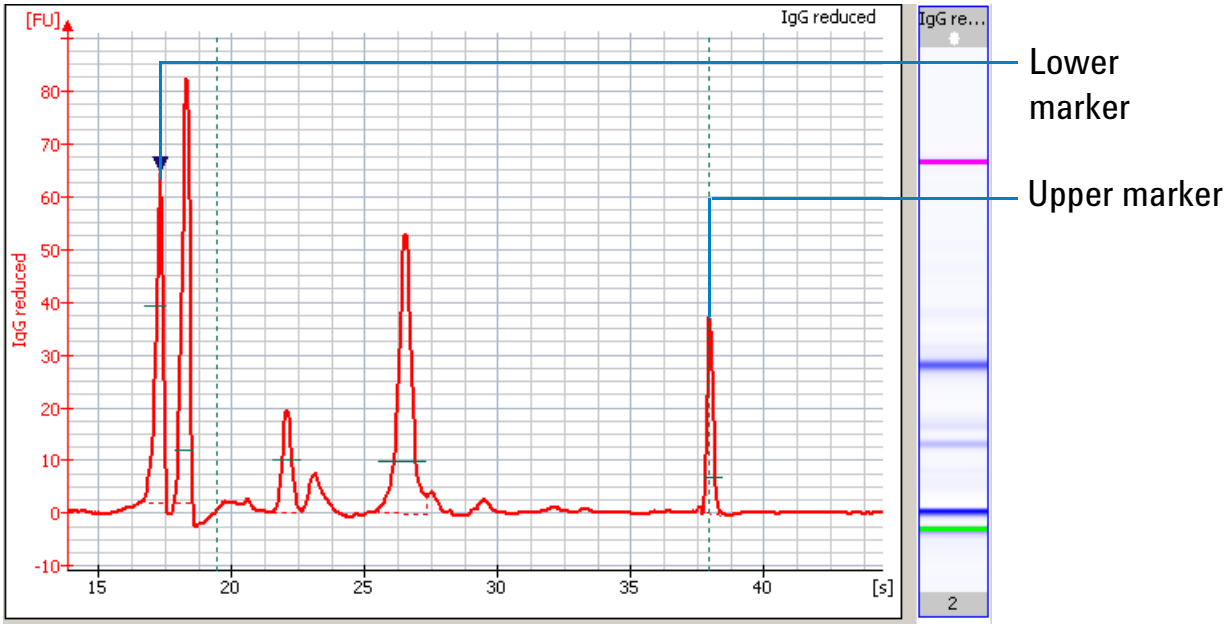


		Size [kDa]	Observations
▶		6.0	Lower Marker
2		9.0	Ladder Peak
3		14.4	Ladder Peak
4		21.5	Ladder Peak
5		29.0	Ladder Peak
6		32.5	Ladder Peak
7		53.0	Ladder Peak
8		66.7	Ladder Peak

5. A standard curve of migration time versus size is plotted from the sizing ladder by interpolation between the individual protein size/migration points. The standard curve derived from the data of the ladder well should resemble the one shown below.



6. Two proteins are run with each of the samples, bracketing the sizing range. The “lower marker” and “upper marker” proteins are internal standards used to align the ladder data with data from the sample wells. The figure below shows an example of assigned marker peaks in a sample well.



		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations	Area
1	◀	6.0	0.0	0.0	0.0	Lower Marker	19.9
2		8.5	0.0	0.0	0.0	System Peak	27.5
3		25.3	140.7	0.0	23.7		8.5
4		58.1	451.7	0.0	76.3		32.6
5	▶	210.0	100.0	0.0	0.0	Upper Marker	10.3

NOTE

The software performs alignment by default. Turning automatic data analysis off (see “Electropherogram Menu” on page 345) suspends analysis until you turn it on again.

7. The standard curve, in conjunction with the markers, is used to calculate protein sizes for each sample from the migration times measured.

8. To calculate the concentration of the individual proteins in all sample wells, the upper marker, in conjunction with an assay-specific concentration calibration curve, is applied to the individual sample peaks in all sample wells.

NOTE

The software allows you to define upper and lower markers yourself. A change in the selection of the markers will lead to quantitative changes in the calibration procedure, however, and therefore in the entire data evaluation.

Smear Analysis

The 2100 expert software allows to perform a smear analysis for all electrophoresis assays.

When the smear analysis is enabled, the software allows you to define regions of interest. These regions are used to define the area of broad peaks and determine their part of the total area. Smear analysis provide a means to analyze broad signals that can be hardly evaluated with the normal peak assignment.

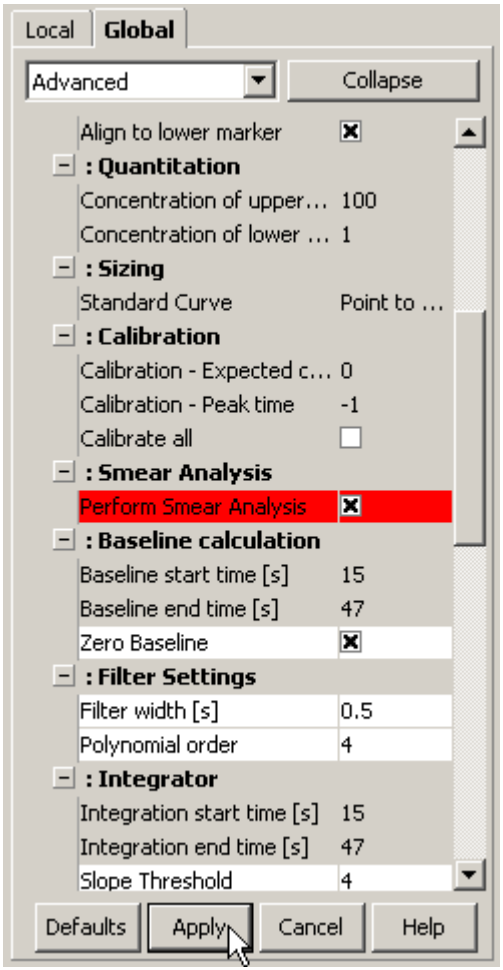
You therefore can define regions of interest that contain the peaks (base pair size) that you are interested in. For these regions you can determine the covered area in relation to the total area.

Enabling Smear Analysis

To enable smear analysis:

1. Go to the *Electropherogram* tab in the *Data and Assay* context:
2. Go to the setpoint explorer and select the *Local* or *Global* tab, depending on which samples should be analyzed.
3. Select the *Advanced* mode.

4 Under *Smear Analysis* select the checkbox *Perform Smear Analysis*.



5 Click the *Apply* button that changes become effective.

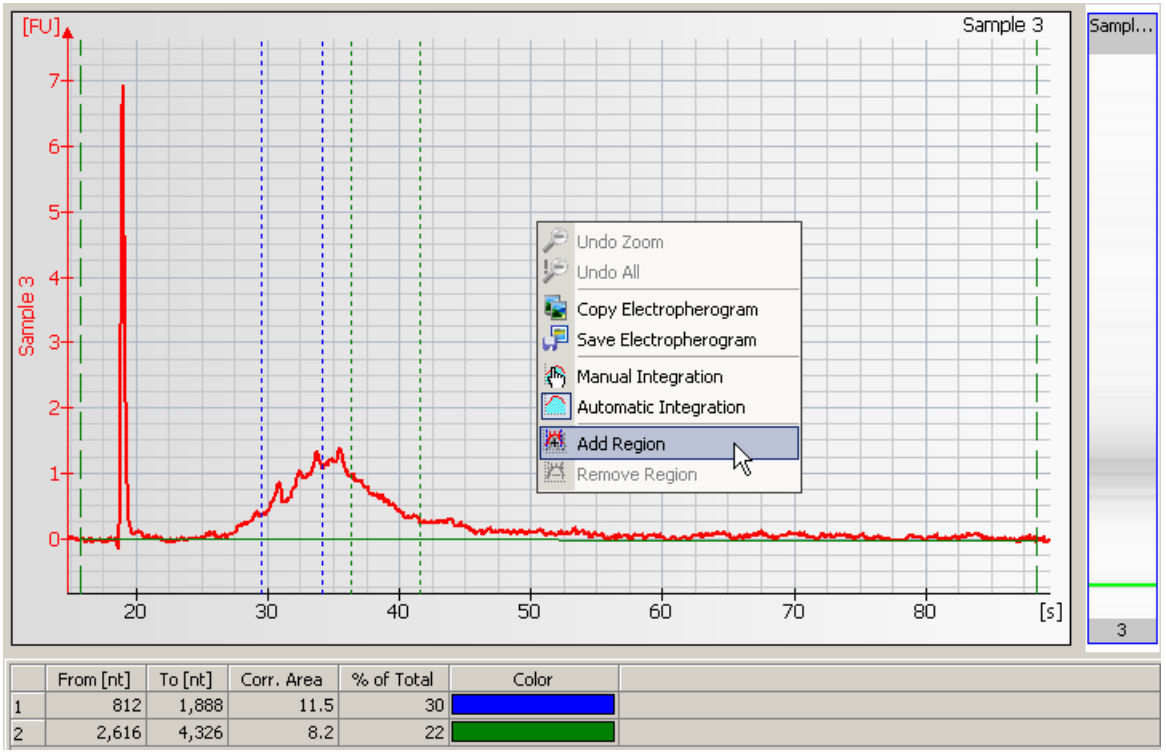
Performing Smear Analysis

After enabling the smear analysis in the setpoint explorer, you are able to insert regions of interest in the electropherogram.

To do so:

1 Select the *Region Table* sub-tab in the *Electropherogram* tab.

2 Right-click the electropherogram and select *Add region*.



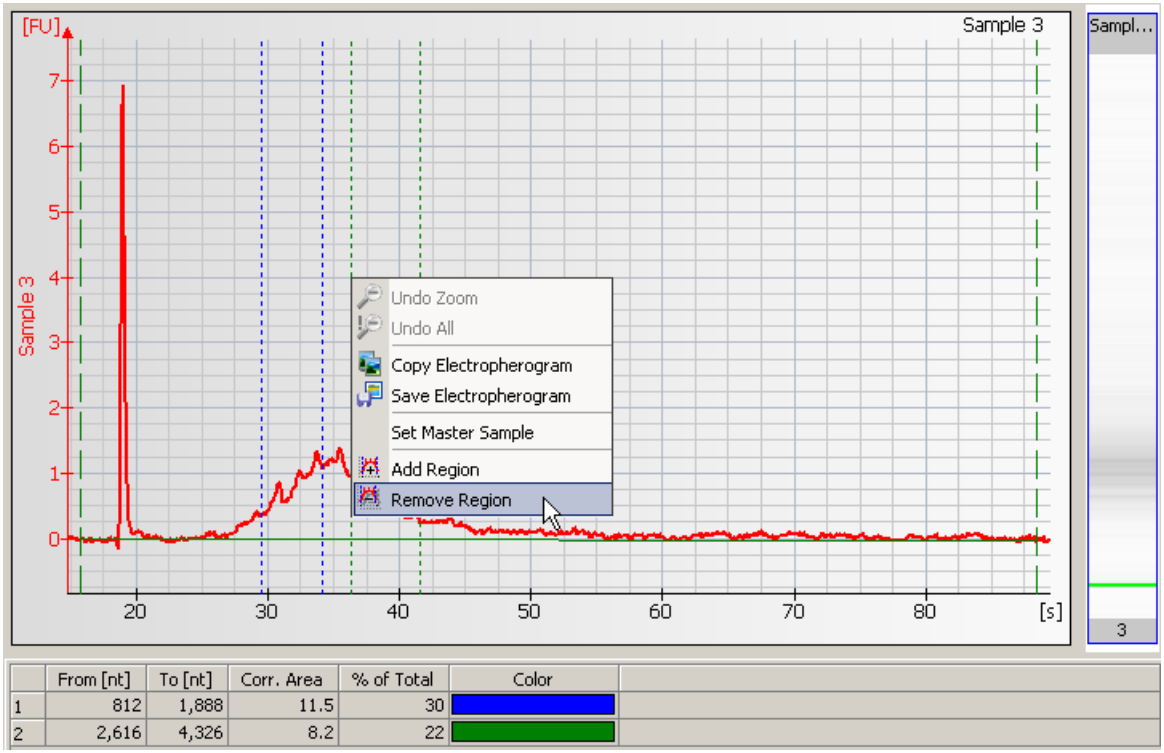
	From [nt]	To [nt]	Corr. Area	% of Total	Color
1	812	1,888	11.5	30	
2	2,616	4,326	8.2	22	

A region will be inserted into the electropherogram. The *Region Table* will show values for the inserted region.

3 Repeat the previous step until the number of required regions are inserted.

4 Adjust the regions by directly moving the dashed lines in the electropherogram.

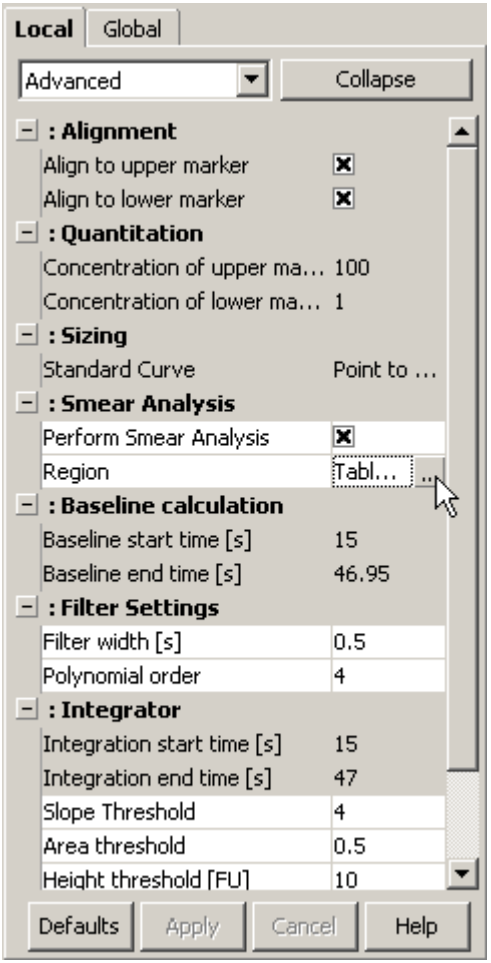
5 To remove a region, right-click the dashed line in the electropherogram and select *Remove Region* from the context menu.



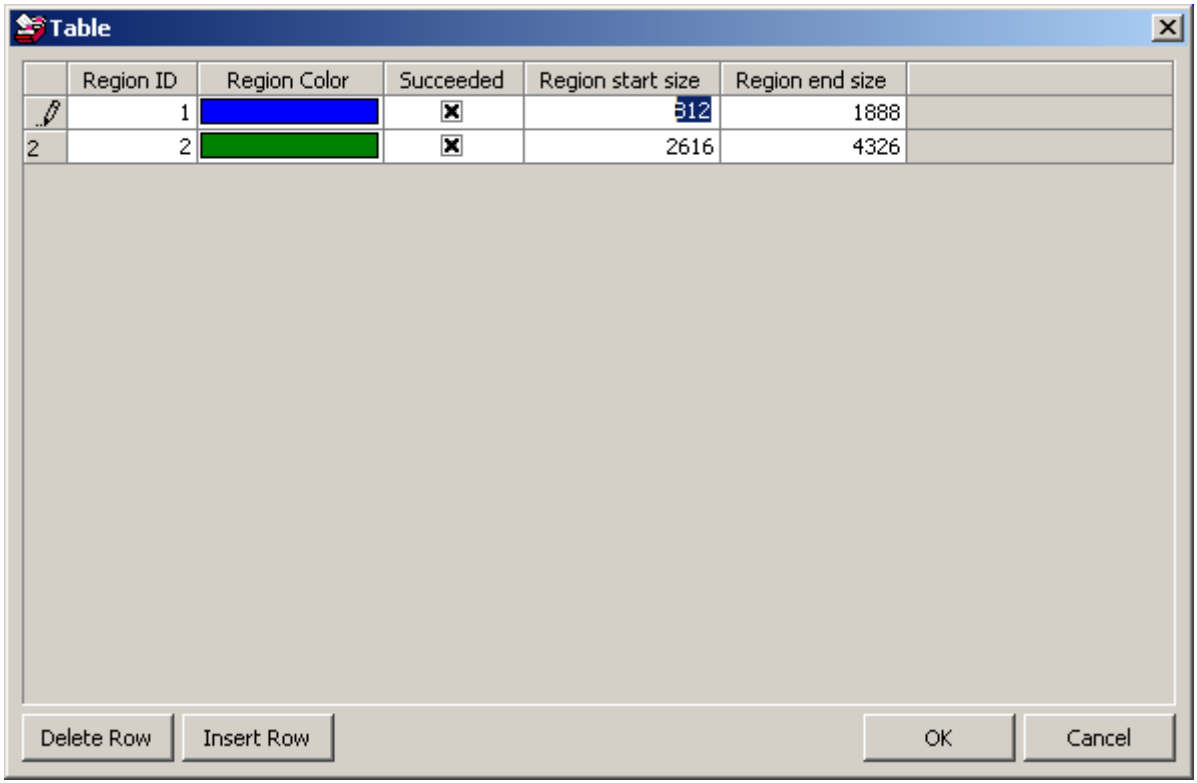
	From [nt]	To [nt]	Corr. Area	% of Total	Color
1	812	1,888	11.5	30	
2	2,616	4,326	8.2	22	

NOTE

The smear analysis table can be directly edited by selecting the region table under *Smear Analysis* in the setpoint explorer.



In the smear analysis table, you can edit the *Region Start Size* and *Region End Size*, for example:



Changing the Data Analysis

Different sets of parameters (data analysis setpoints) can be changed in the software in order to modify the data evaluation for sample analysis.

For all assays:

- Filtering parameters
- Peak find parameters for all samples/peak height for individual samples
- Enabling smear analysis
- Align to upper and/or lower marker

For RNA assays only:

- Adding/deleting ribosomal fragments

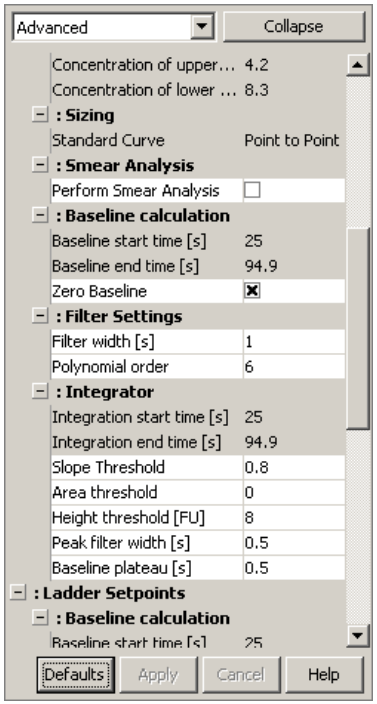
These settings can be made before a new run is started or when reanalyzing a previously saved data file.

About the Setpoint Explorer

The tool allowing you to modify the data analysis setpoints is the *Setpoint Explorer*. The setpoint explorer is accessible from:

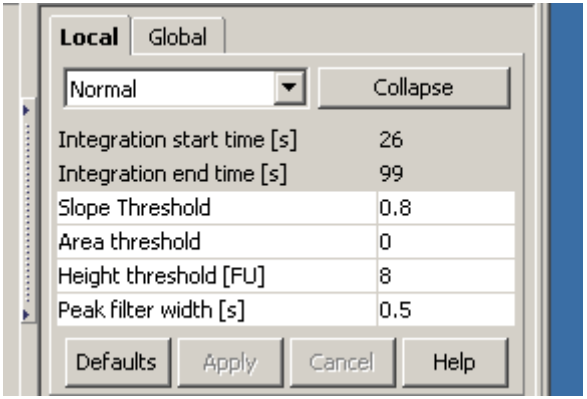
- *Assay Properties Tab*
- *Electropherogram Tab (Single/Grid View)*
- *Gel Tab*

On the *Assay Properties* tab, the setpoint explorer is always visible and lets you modify setpoints *globally* (for all samples):



To show the setpoint explorer, on the *Electropherogram/Gel* tab, click the vertical bar on the right edge of the application window:

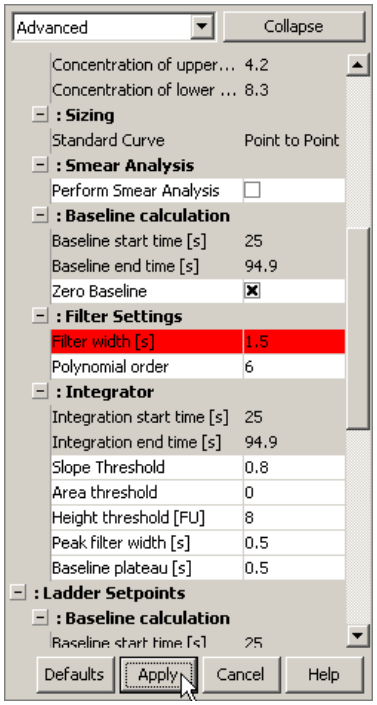
The setpoint explorer appears.



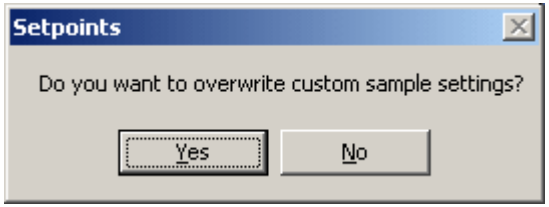
For electrophoretic assays, you can modify the setpoints

- either *globally*, that is, for all samples (*Global* tab),
- *locally*, for the current sample (*Local* tab).

Click the + nodes to expand, and the – nodes to collapse branches. Setpoints that you can change are white. To edit a setpoint, double-click the value, enter the new value, and press enter. Edited values are red (until you apply them):



Changing any global setpoints while local settings have been applied opens a dialog box asking whether to override custom sample settings:



Choosing *Yes* causes any changes made to the setpoints for individual samples to be discarded and applies the changes globally to all samples. Choosing *No* causes individual samples to retain changed setpoints.

Filtering Setpoints

The first step the software takes in analyzing the raw data is to apply data filtering. The following filtering setpoints can be changed:

Filter Width	Defines the data window, given in seconds, used for averaging. The broader the filter width, the more raw data points are used for averaging. As a result, the noise level will decrease, but peaks will become lower and broader. Overall, changing the Filter Width has more effect on the result of the filtering procedure applied then does changing the Polynomial Order.
Polynomial Order	This setting is used to define the power series applied to fit the raw data. The higher the number, the more the fit function will follow the noisy raw data curve. As a result, the noise level of the filtered curve will increase.

Peak Find Setpoints

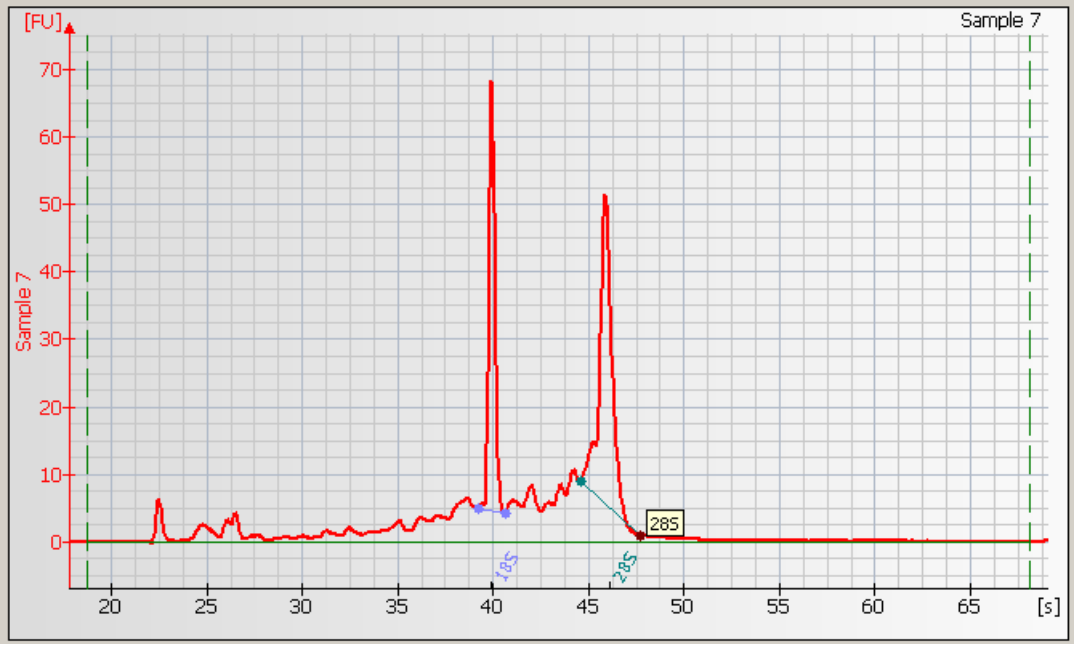
After data filtering, the peak find algorithm locates the peaks and calculates the local peak baselines. The algorithm begins by finding all the peaks above the noise threshold in order to determine the baseline, after which any peaks below the noise threshold are rejected. A local baseline is calculated for each peak to allow for baseline drift.

The four peak find setpoints that can be changed are:

Min Peak Height	The *Min Peak Height* setpoint determines whether a peak is kept. For each peak, the difference between the start point value and the center point value (local baseline) must be greater than the *Min Peak Height* value.
Min Peak Width	The *Min Peak Width* setpoint determines the minimum amount of time that must elapse before a peak is recognized.
Slope Threshold	The *Slope Threshold* setpoint determined the difference in the slope that must occur in order for a peak to begin. The inverse of this value is used to determine the peak end.
Baseline Plateau	The *Baseline Plateau* setpoint is a parameter that assists in finding peaks. The signal is recognized to be at baseline whenever the slope of the data is less than the *Slope Threshold* setpoint (either positive or negative) for longer than the time set for the Baseline Plateau. This setting rejects brief, low slope areas such as between non-baseline-resolved peaks.

Manually Moving Fragment Start and End Points (RNA and Cy5-Labeled Nucleic Acids)

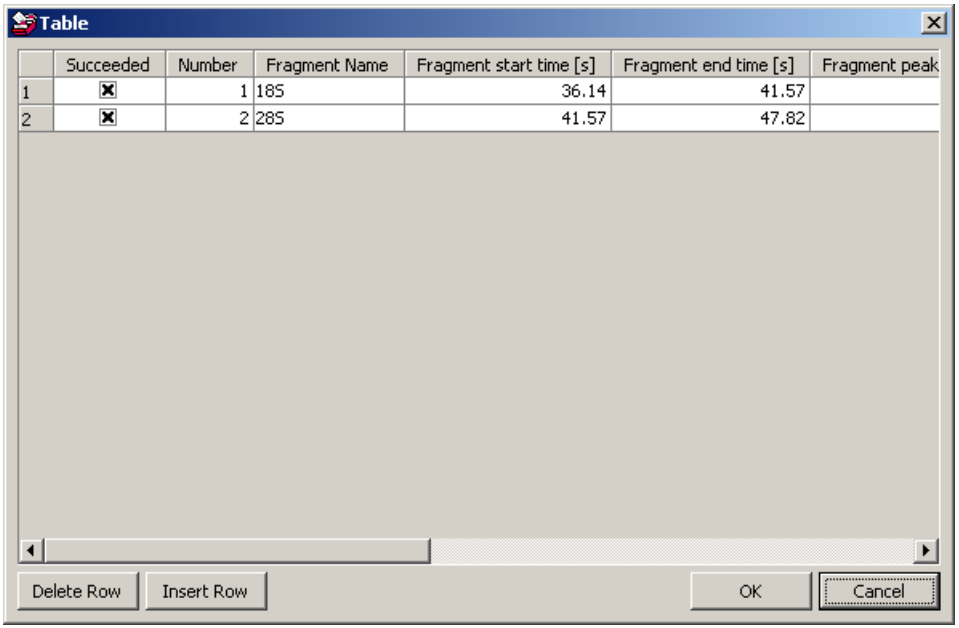
It is also possible to alter the start and end points manually for individual fragments in an RNA or Cy5-labeled nucleic acids assay. Zooming in on the base of a particular fragment allows you to see the start and end points. Placing the cursor over one of these points changes the cursor to a pointing hand, allowing you to click and drag the point along the line of the fragment until it is positioned as desired.



Move any other start or end points as desired.

TIP

The fragment table can be directly edited in the setpoint explorer:

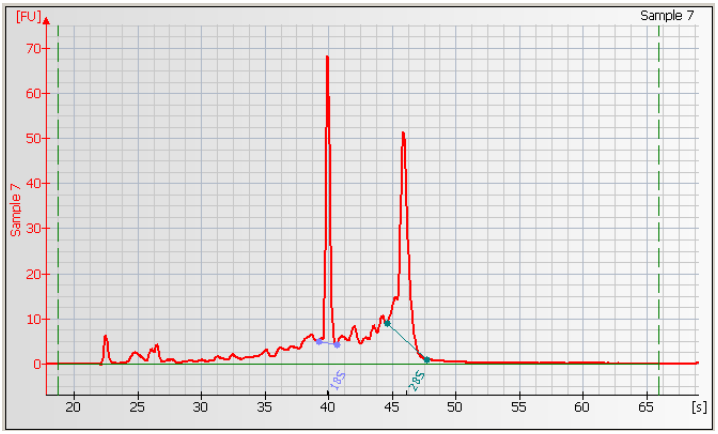


NOTE

Changing the start or end points of the fragment will change the calculated rRNA ratio. It might be convenient to pause the automatic analysis (*Electropherogram > Pause Automatic Analysis*) until all changes are done.

Setting the Baseline for Calculation of RNA Concentration

At low signal-to-noise ratios, the baseline that defines the area used for calculating the concentration of RNA assays is highly dependent on the settings for the Start and End Time. You can adjust the Start and End Times manually (thereby adjusting the baseline) to ensure a good result even at very low signal-to-noise ratios. Choose a single sample. Two vertical green long-dashed lines indicating the setpoints for the Start and End Times (with the baseline drawn between them) are displayed in the window.



Move the cursor over the long-dashed line on the left (Start Time setting) and drag the line to the desired position. Do the same with the long-dashed line on the right (End Time setting) until you have a flat baseline.

NOTE

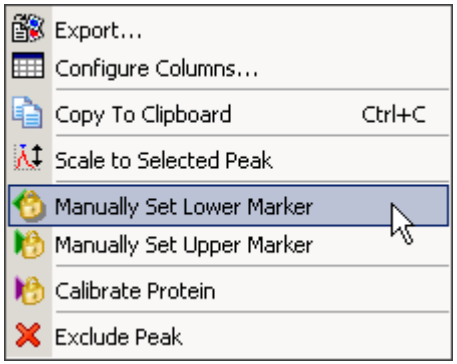
Changing the start and end times will change the calculated RNA concentration. It might be convenient to pause the automatic analysis (*Electropherogram* > *Pause Automatic Analysis*) until all changes are done.

Assigning Upper and Lower Marker Peaks

For each DNA or protein sample, the upper and lower marker peaks are assigned first and then the data is aligned so that the sample markers match the ladder markers in time, allowing the size and concentration of the sample peaks to be determined.

The first peak is assigned to be the lower marker and is then offset to match the lower marker in the ladder. The upper marker is then assigned to the last peak in the sample well or to the peak nearest the ladder's upper marker. See an example of assigned marker peaks below.

If you get unexpected peaks in the ladder analysis or find that the markers have been set incorrectly, you may exclude peaks manually from the ladder or set a peak to be used as a marker. Right-clicking in the peak table causes a context menu to appear, allowing you to do so:

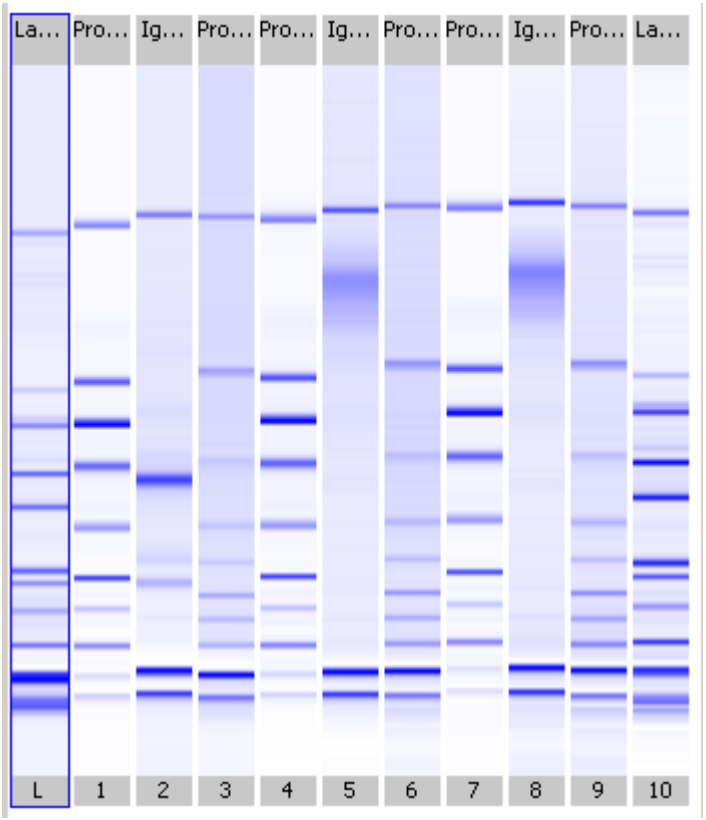


CAUTION

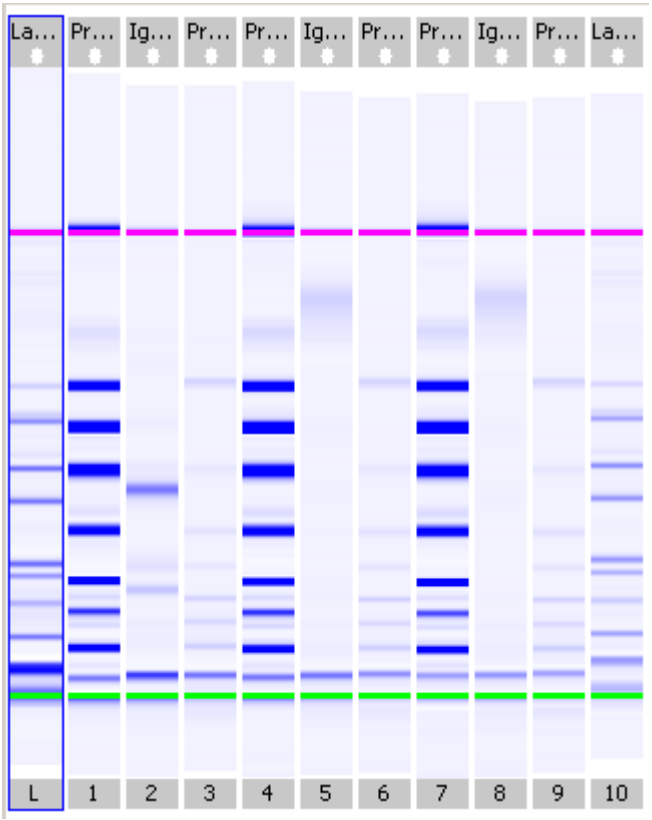
Excluding a peak or manually setting a peak to be an upper or lower marker for a DNA or protein assay can cause errors with analysis.

Aligning or Unaligning the Marker Peaks (DNA or Protein assays only)

The upper and lower are then *aligned* to the ladder markers by resampling the sample data in a linear stretch or compression using a point-to-point fit:



Data before alignment



Markers aligned to the ladder

If the sample marker peaks are either more than twice as far apart or less than half as far apart as the ladder markers, they are assumed to be the wrong peaks, and analysis of the sample stops, producing the error “Marker peaks not detected”.

NOTE

With DNA and protein assays, the height of marker peaks is assay dependent. Ladder peaks are analyzed to calculate a marker peak threshold that is used to locate the marker peaks in the sample wells. If the marker peaks found using this calculated method fail to align with those of a sample, the 2100 expert software will use the minimum peak height threshold setting instead (if this value is lower than the value for the marker peak). For example, the calculated threshold might be too high to find the sample's markers if they happen to be very small for some reason. Either no markers will be found or the wrong peaks will be assumed to be markers and these may not align with the ladder markers. Consequently, the software attempts to use the minimum peak height threshold that, if it is set low enough, will catch the real markers, allowing the sample to align.

NOTE

After alignment, peaks are shown with relative migration times that are different from the real times with data unaligned.

Manual Integration

For all electrophoretic assays, the 2100 expert software allows to manually integrate peaks. Manual integration allows you to move, add or delete peak baselines.

TIP

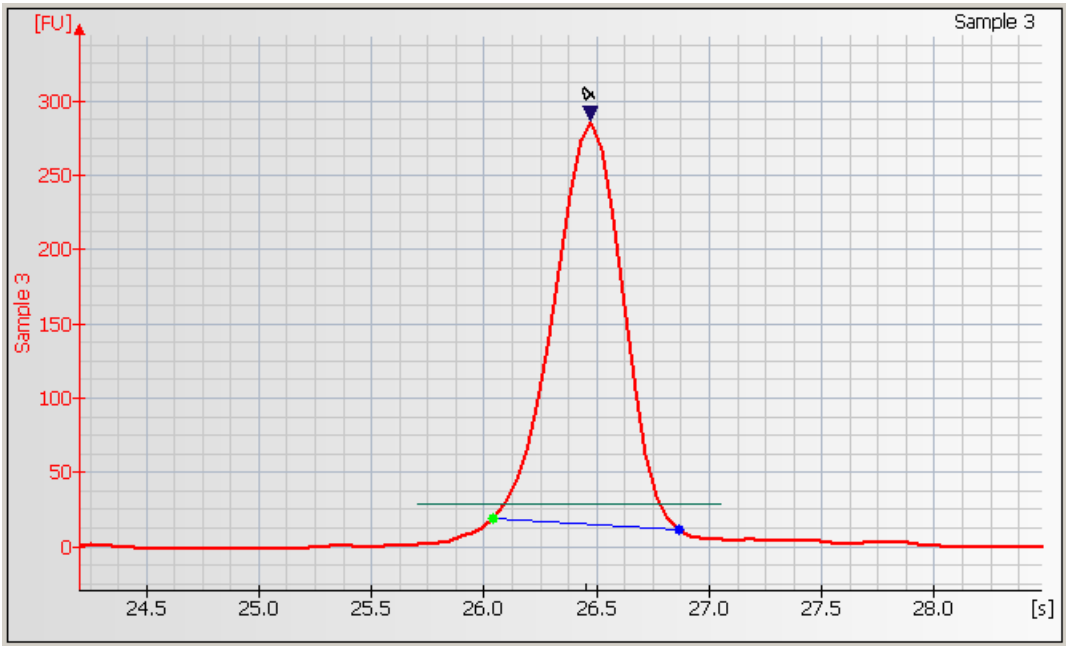
To move a peak baseline, point along the vertical line, press the CTRL key and left mouse button. To move a peak baseline, point along the signal, press the left mouse button only.

Example: Adjusting peak baselines

To manually change peak baselines:

1. Highlight the *Electropherogram* tab in the *Data and Assay* context and zoom into the electropherogram to enlarge the peak of interest.
2. Select *Electropherogram* > *Manual Integration* to switch off the automatic integration. As an alternative you can click the *Manual Integration* button  in the toolbar.

The baseline points become visible as blue or green dots. Highlighted baseline points are labelled green and can be moved either along the vertical line (press CTRL key and left mouse button) or along the signal trace (left mouse button). The blue baseline points are fixed and cannot be moved. To highlight a baseline point, click on it.



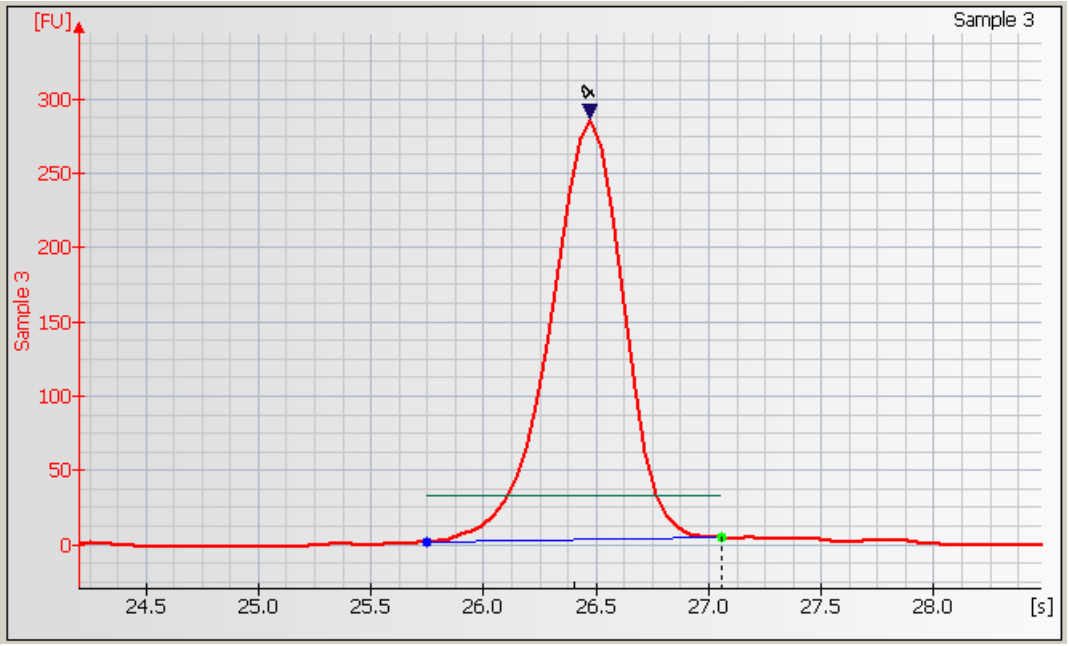
3 Pause the automatic analysis by clicking the appropriate icon in the toolbar.

TIP

Before manual integration is done, it is strongly recommended to pause the automatic analysis and to restart it again when all changes are done. Otherwise data analysis starts every time, when the baseline points are moved.

---

4 Adjust the baseline points as appropriate.



TIP

To move a peak baseline point along the vertical line, press the CTRL key and the left mouse button. To move a peak baseline point along the signal, press the left mouse button only.

5 Click the *Automatic Analysis* button to enable the integration again.

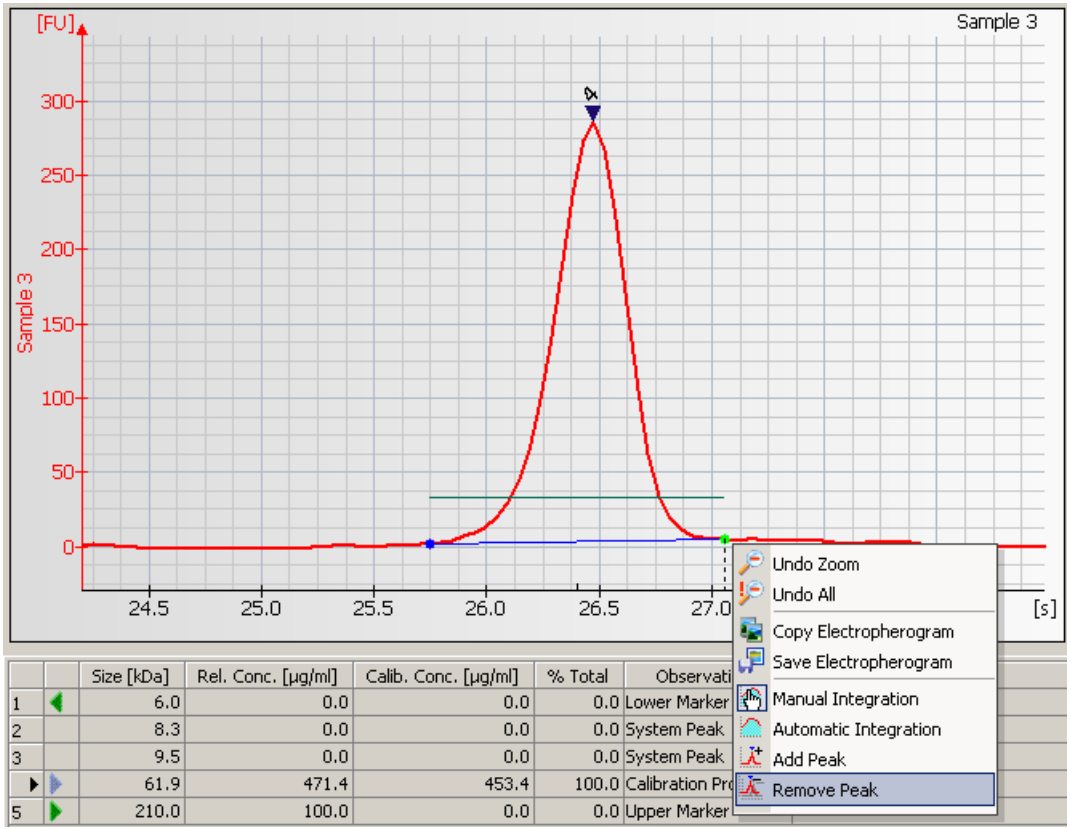
The integration results in the result and peak tables will change according to the changes done.

Example: Removing peaks

To remove peaks:

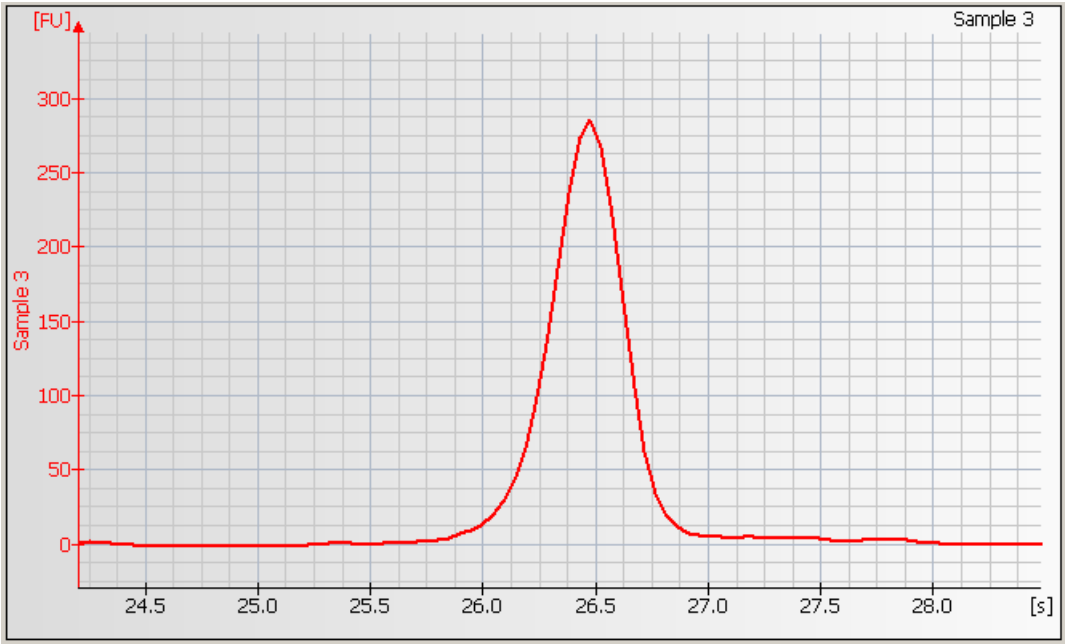
1 Highlight the *Electropherogram* tab in the *Data and Assay* context and zoom into the electropherogram to enlarge the peak of interest.

2 Select *Electropherogram* > *Manual Integration* to switch off the automatic integration. As an alternative you might click the *Manual Integration* button in the toolbar. The baseline points become visible as blue or green dots.



3 Right-click on a baseline-point and select *Remove Peak* from the context menu.

The two baseline points and the connecting line will disappear and the integration results shown in the result and peak tables will be updated:

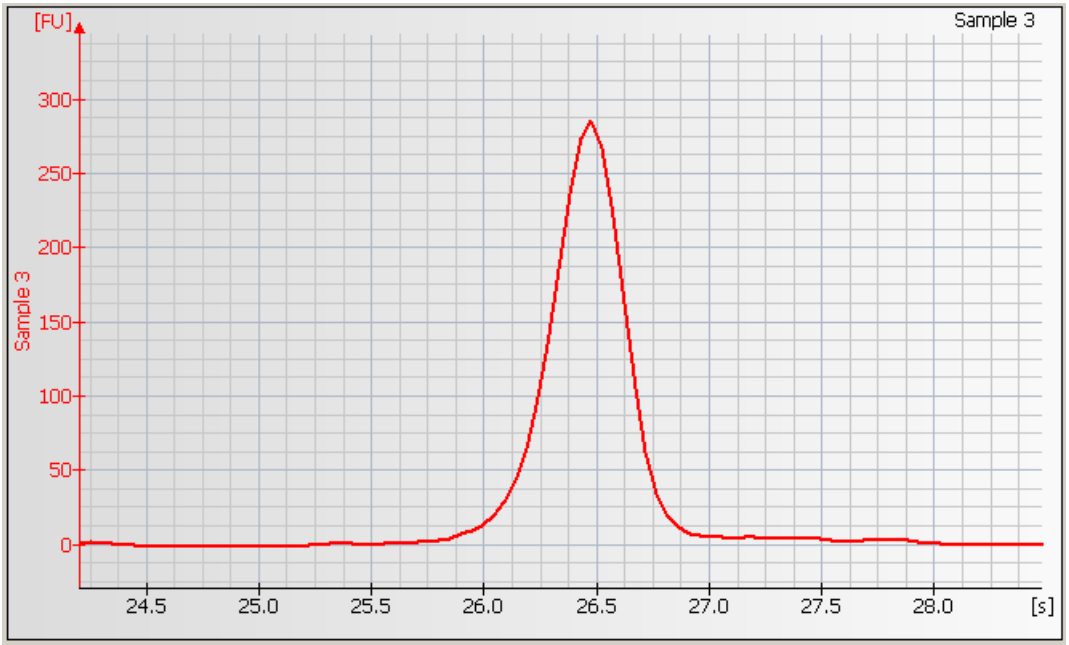


		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1		6.0	0.0	0.0	0.0	Lower Marker
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
		210.0	100.0	0.0	0.0	Upper Marker

Example: Inserting peak baselines

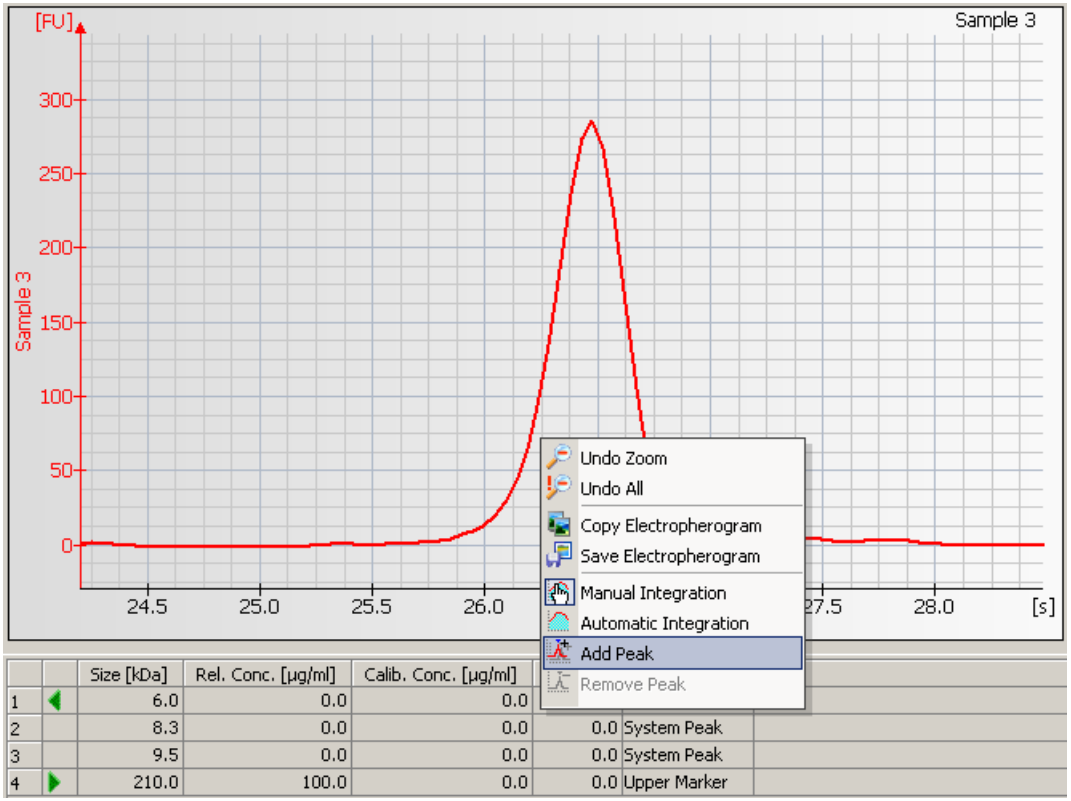
To insert peaks manually:

1 Highlight the *Electropherogram* tab in the *Data and Assay* context and zoom into the electropherogram to enlarge the peak of interest.



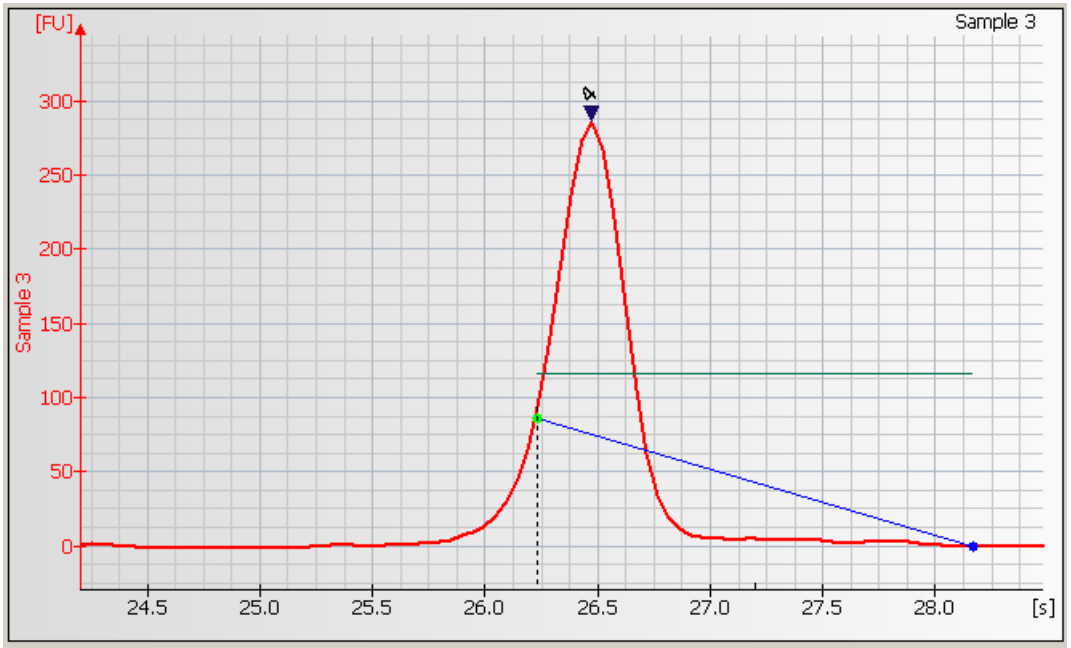
		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1	◀	6.0	0.0	0.0	0.0	Lower Marker
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
▶	▶	210.0	100.0	0.0	0.0	Upper Marker

2 Right-click on the electropherogram and select *Add Peak* from the context menu.



		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]		
1	◀	6.0	0.0	0.0		
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
4	▶	210.0	100.0	0.0	0.0	Upper Marker

3 Two baseline points and the connecting line will appear and the integration results shown in the result and peak tables will be updated.



		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1		6.0	0.0	0.0	0.0	Lower Marker
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
		61.9	231.3	224.9	100.0	Calibration Protein
5		210.0	100.0	0.0	0.0	Upper Marker

4 Pause the automatic analysis by clicking the appropriate icon in the toolbar.

TIP

Before manual integration is done, it is strongly recommended to pause the automatic analysis and to restart it again when all changes are done. Otherwise data analysis starts every time, when the baseline points are moved.

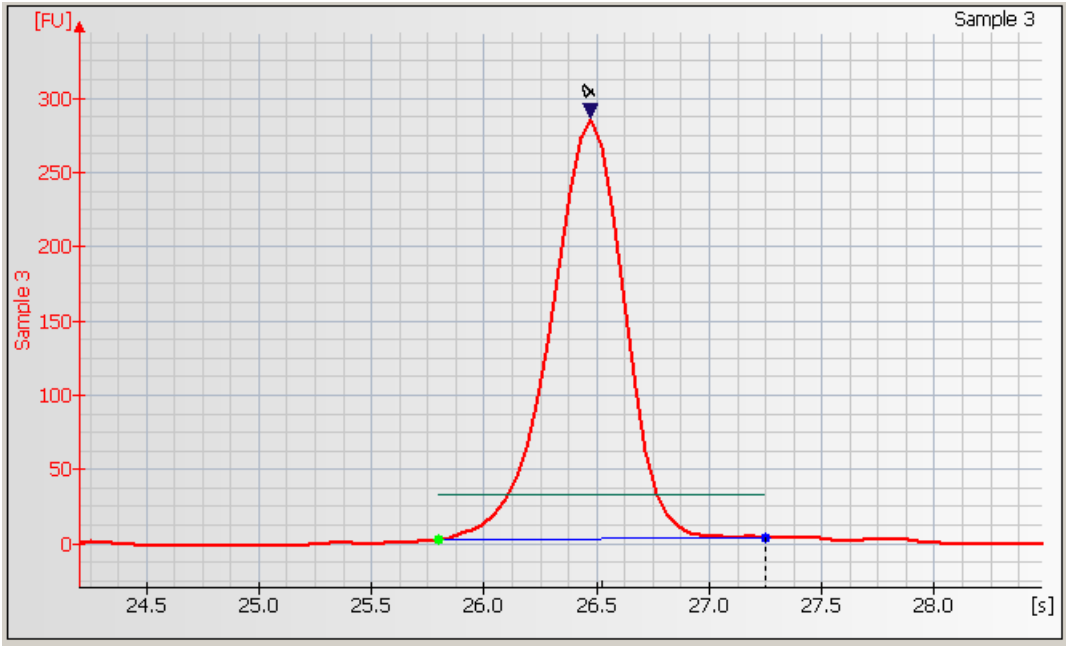
5 Adjust the baseline points as appropriate.

TIP

To move a peak baseline point along the vertical line, press the CTRL key and the left mouse button. To move a peak baseline point along the signal, press the left mouse button only.

6 Click the *Automatic Analysis* button  to enable the integration again.

The integration results in the result and peak tables will change according to the changes done.



		Size [kDa]	Rel. Conc. [µg/ml]	Calib. Conc. [µg/ml]	% Total	Observations
1		6.0	0.0	0.0	0.0	Lower Marker
2		8.3	0.0	0.0	0.0	System Peak
3		9.5	0.0	0.0	0.0	System Peak
		61.9	472.1	454.1	100.0	Calibration Protein
5		210.0	100.0	0.0	0.0	Upper Marker

Reanalyzing a Chip Data File

NOTE

Occasionally you may wish to open and view or reanalyze a chip data file that was run and saved previously. The raw data values are saved in the data file, along with the analysis settings that were chosen for the run, so that the data can be reanalyzed with different settings.

To do this:

1 Click *File* > *Open...* to open a chip data file (.xad).

2. Choose the filename from the list of data files.

3. Click *OK*.

The items that can be changed for reanalysis are:

All Assays:

- Global peak find settings
- Individual sample peak find settings
- Expected base pair size for certain assays
- Gel color
- Sample names and comments
- Manual integration

RNA Assays Only:

- Fragment names and colors associated with labels
- Fragment start/end times, additional fragments (or delete fragments found)

DNA and Protein Assays Only:

- Exclude peaks from analysis
- Reassign upper/lower markers
- Alignment or no alignment with ladder peaks
- Assay (you can save the changed settings under a new assay name, if desired)

TIP

When applying modified data analysis setpoints, the software will (by default) immediately recalculate the raw data, which takes some time. Select *Don't Analyze* from the *Gel Menu* or *Electropherogram Menu* to temporarily switch off automatic data analysis while you modify setpoints.

If you save the data file after making changes, it will keep a record of the changes such as gel color, sample names, and peak find settings that were in effect at the time the file is resaved. If you do not want to change the original file, choose *File* > *Save As...* and give the file a new name or save it to a different location.

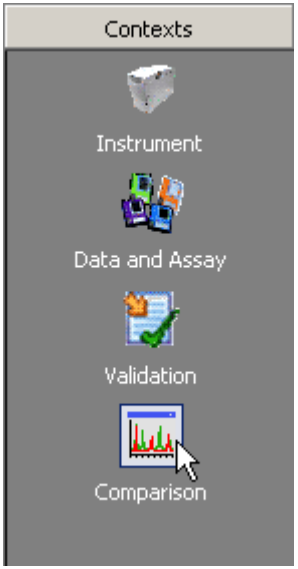
Comparing Samples from Different Electrophoretic Chip Runs

The *2100 expert* software allows you to compare the measurement results of samples from different electrophoretic chip runs. Samples to be compared must be from chip runs of the same assay type.

In the *Comparison* context, you can create comparison files, include samples from different chip runs, and compare the samples by overlaying electropherograms, for example.

To compare samples from different electrophoretic chip runs:

1 Switch to the *Comparison* context:



2 From the *File* menu select *Open...*, and open all chip data files (.xad) that contain the samples you want to compare.

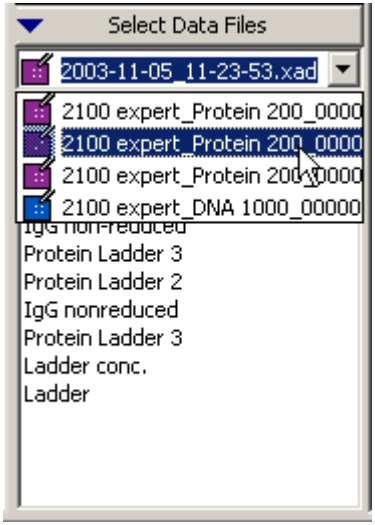
The .xad files appear in the *Select Data Files* list of the *Tree View Panel*.

NOTE

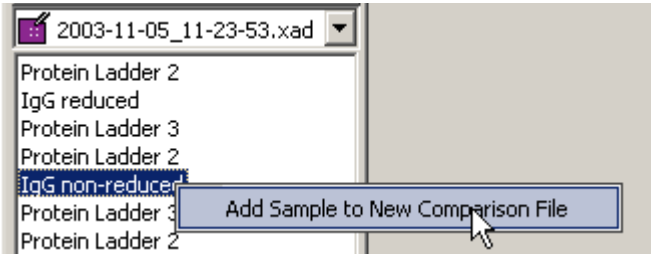
The *Select Data Files* list also contains all electrophoretic .xad files that are open in the *Data and Assay* context.

---

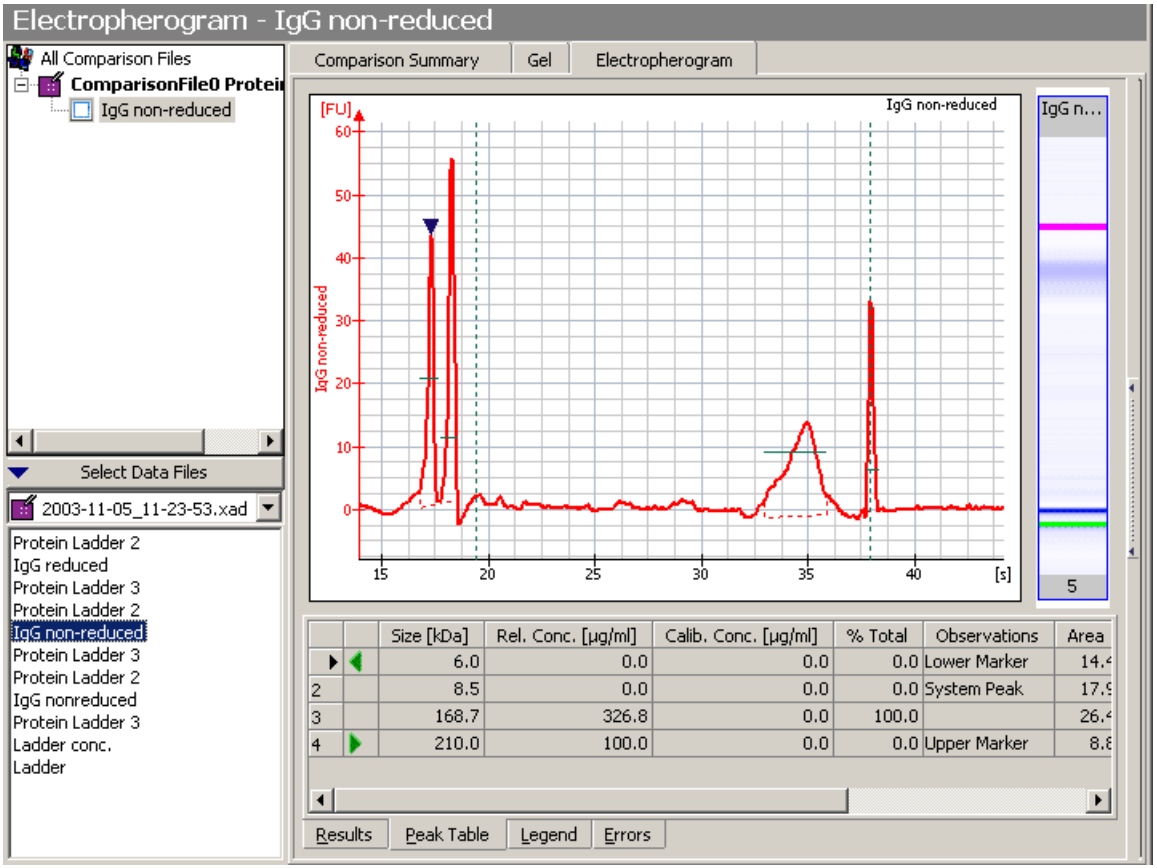
3 Select a .xad file from the *Select Data Files* list to display a list of its samples.



4 Right-click a sample name and select *Add Sample to New Comparison File*.

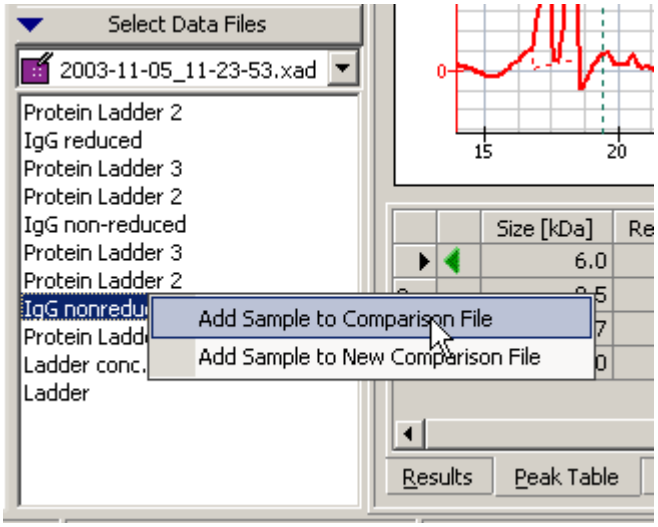


A new comparison file appears in the upper part of the tree view containing the sample. The sample is selected and its electropherogram is shown.



Note that the *Electropherogram Tab (Single/Grid View)* a has the same functionality as in the *Data and Assay* context.

5 You can now add further samples from any of the open .xad files to the comparison file.

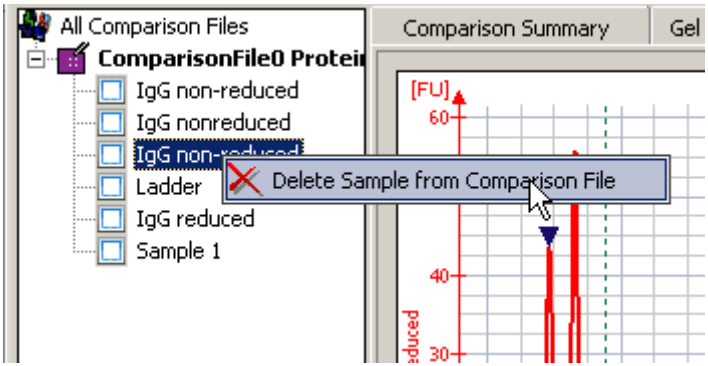


TIP

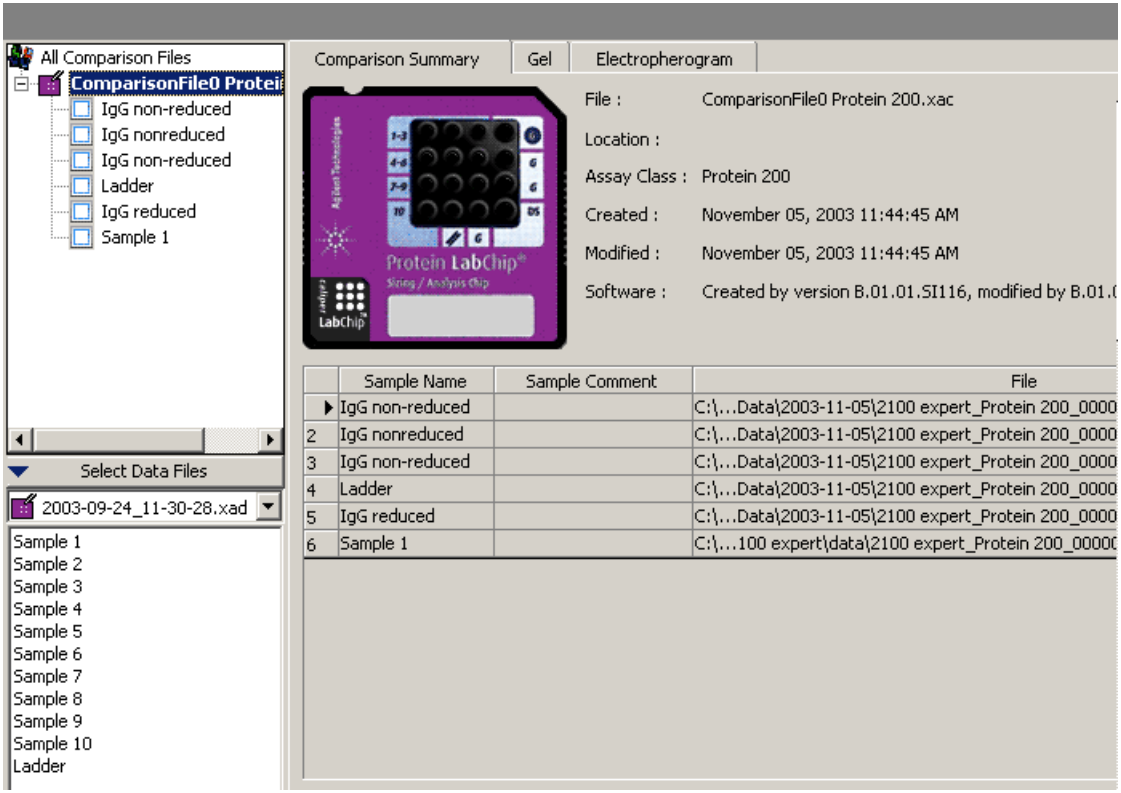
Double-clicking a sample name in the *lower* part of the tree view or dragging a sample name into the tree view adds the sample to the comparison file that is currently selected in the *upper* part of the tree view. Or, if no comparison file is selected, creates a new comparison file and adds the sample to it.

The following message appears if you try to add a sample of a .xad file which has the wrong assay type:

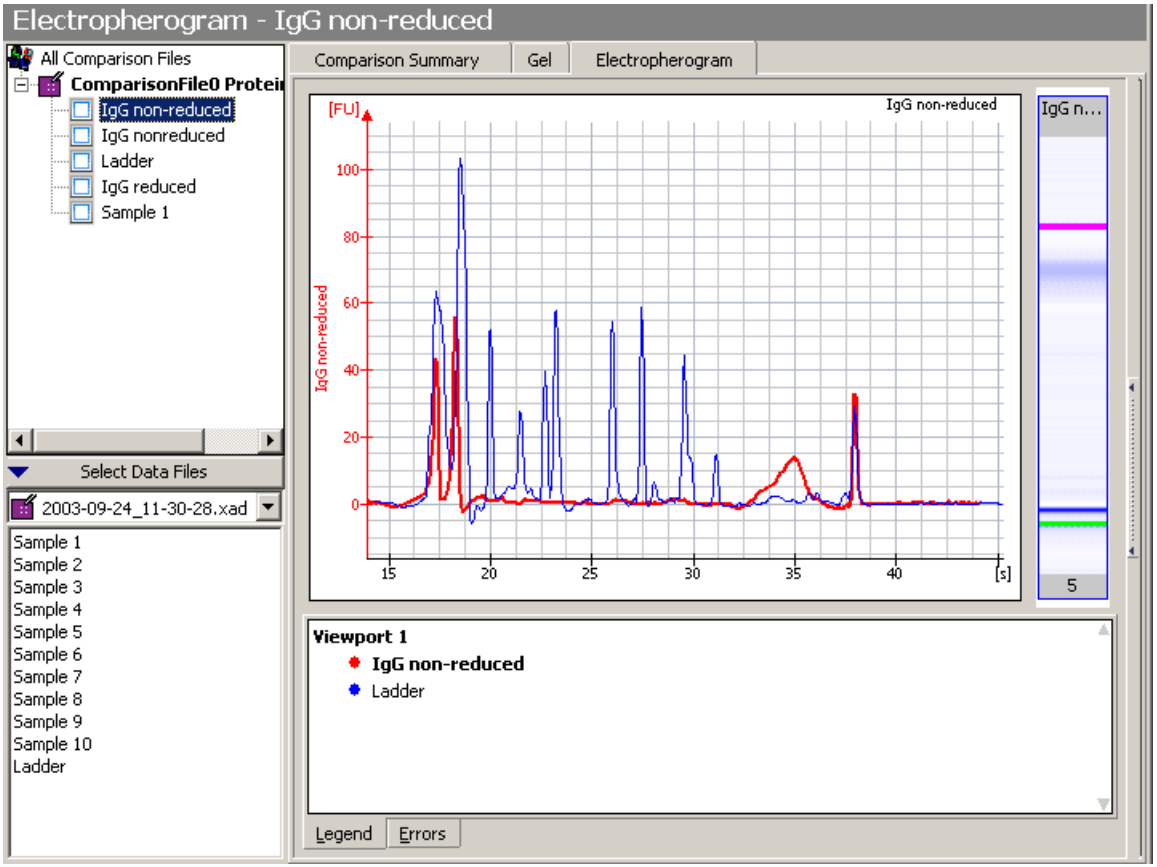
6 You can also remove samples from a comparison file. Right-click the sample name and select *Delete Sample from Comparison File*.



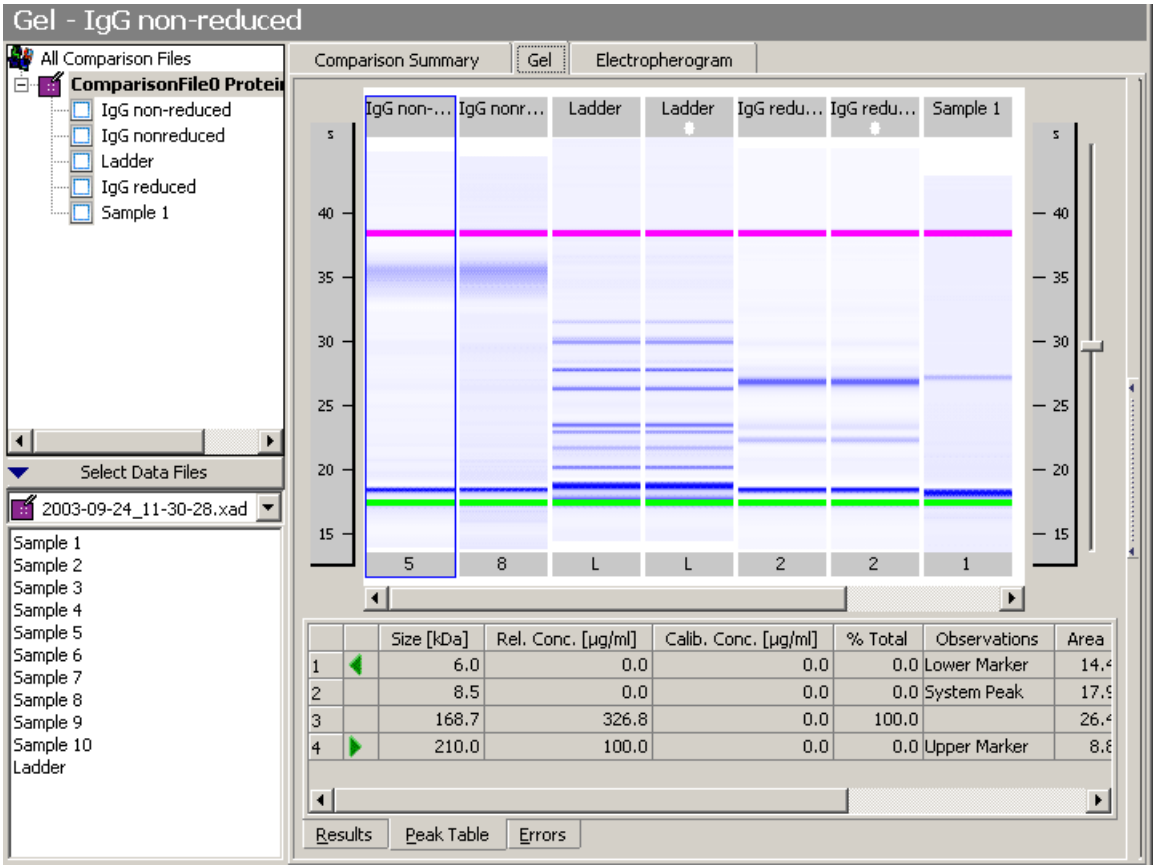
7 When you have added all your samples you can select the *Comparison Summary Tab* which displays information on the comparison file, and lets you enter a comment regarding the comparison.



8 To compare the electropherograms of samples, go to the *Electropherogram* tab, click *Overlaid Samples* in the toolbar (see "Data and Assay Context – Electrophoresis Toolbar" on page 369), and select the samples to be compared.



9 Select the *Gel* tab to display a comparison of the gel-like images of the samples.



Note that the *Gel Tab* has the same functionality as in the *Data and Assay* context.

10 From the *File* menu, select *Save* to save the comparison file (.xac) under the default name, or select *Save As...* to save it under a new name.

The default name is derived from the assay class: "ComparisonFileX [Assay Class].xac" where "X" is an autoincremented number. Example: "ComparisonFile0 Protein 200.xac"

NOTE

You can re-open comparison files to review the comparison results, and to add/remove samples.

Result Flagging

Result flagging can be used to assign a user-defined color code to a sample. This lets you easily identify samples with certain properties immediately after a chip run.

The color assignment is carried out by applying a sequence of rules to the measurement results obtained for the sample. The rules are defined on chip level and are applied to all samples of the chip.

There are two modes:

- In *Normal* mode, the rules are applied in the given order, and the first matching rule will determine the color of the sample. All rules are applied subsequently. The first rule which applies to the sample defines its color. So you should start with the most specific rule. If that one does not apply, a less specific one may apply. If none of the defined rules apply, the final default rule defines the color code.
- In *Target* mode, all rules are applied subsequently. If the next rule applies, the color code changes to the color code defined by the rule, otherwise the previous color code is kept. Therefore, the last valid rule defines the color code of the sample. This mode is called target mode because later rules refine the result color code. The first default color code is the most general and the last one the most specific.

You can define the flagging rules already in the assay, or—after the chip run is finished—modify these rules or define new rules in the chip data file, and apply the rules to the measurement results.

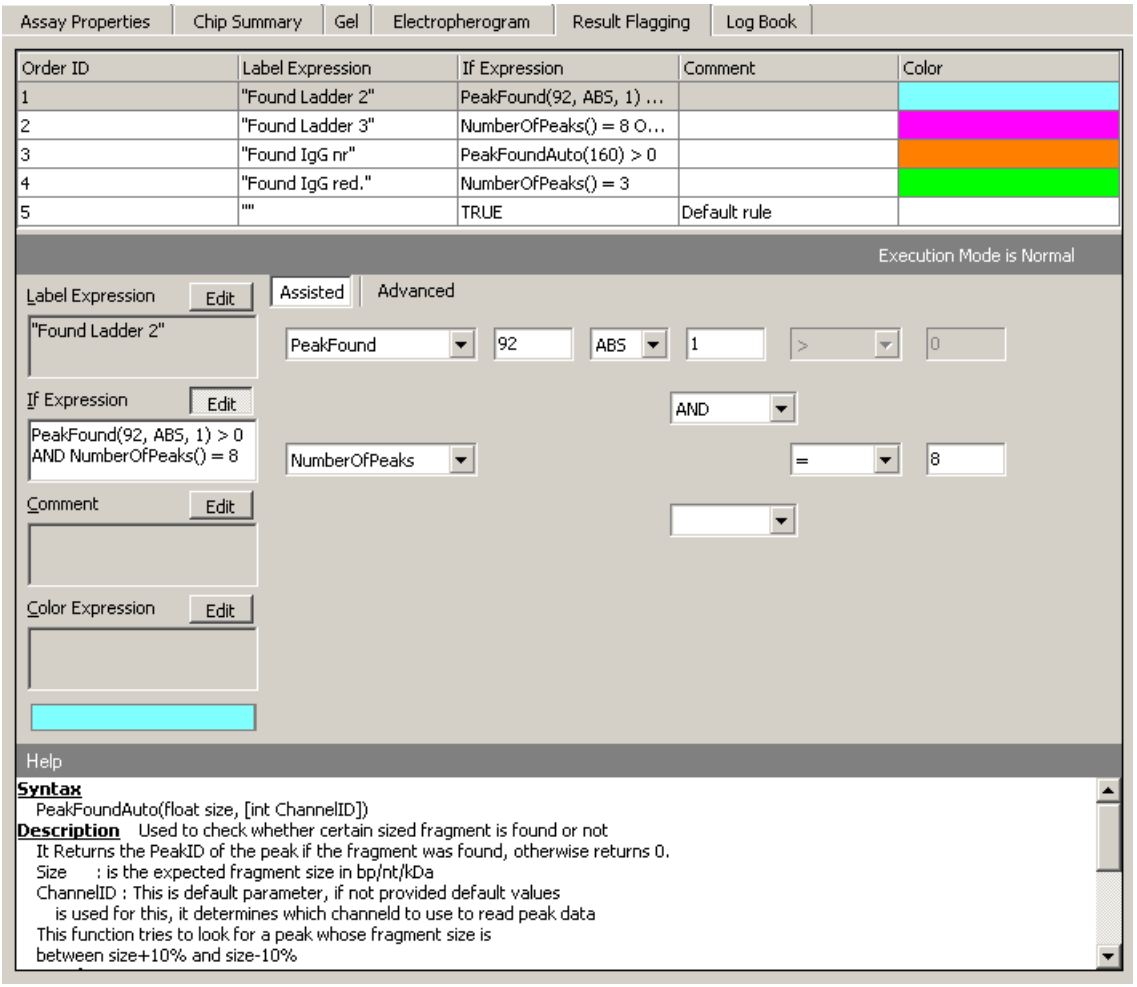
TIP

The examples shown in this chapter are taken from the demo assay “Demo Protein 200 Plus.xsy”, that comes with the *2100 expert* software. You can find this demo assay in the “..\assays\demo\electrophoresis” subdirectory of the *2100 expert* installation folder.

In the “..\data\samples\resultflagging” subdirectory of the *2100 expert* installation folder, you can find further examples for result flagging rules (.xml) which you can import in the “Protein 200 Plus” demo assay.

Defining Result Flagging Rules

The rules can be defined on the *Result Flagging* tab, which is available in the *Data and Assay* context if an electrophoretic chip data (.xad.) or assay (.xsy) file is selected.



A result flagging rule consists of the following:

- *Label Expression*

  An optional description for the rule used to label samples meeting the rule.

- *If Expression*

  An expression built from predefined functions, variables, and logical operators.

- *Comment*

  An optional comment for the rule.

- *Color Expression*

  A solid color or a color gradient built from two colors, used for flagging samples that meet the rule.

Details on all elements of the *Result Flagging* tab are given in "Result Flagging Tab" on page 474.

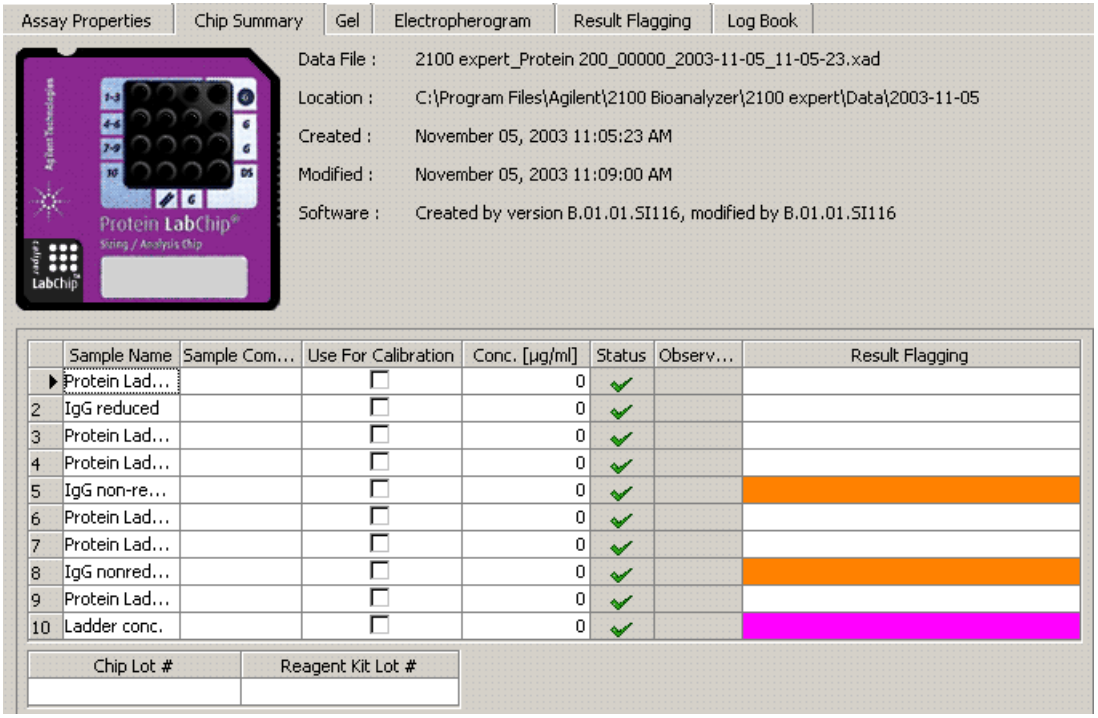
"How to Define Result Flagging Rules" on page 151 shows how to proceed when defining rules.

You can reuse result flagging rule definitions, see "Exporting Result Flagging Rules" on page 274 and "Importing Result Flagging Rules" on page 262.

Color Indication

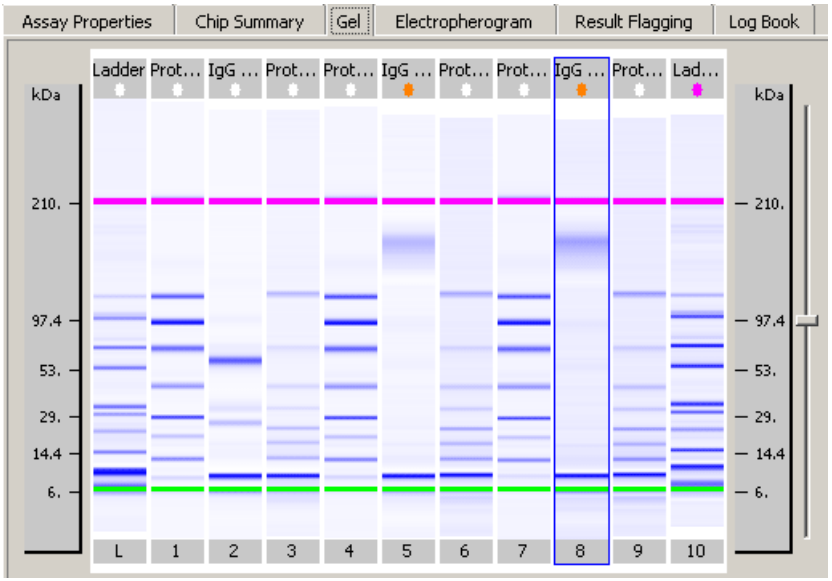
Result flagging shows:

- On the *Chip Summary Tab:*



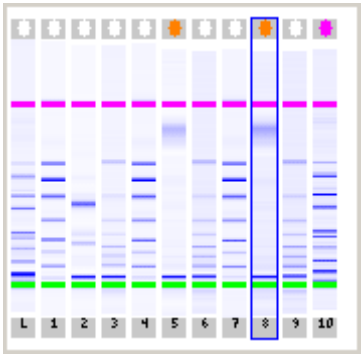
The colors in the *Result Flagging* column show which sample matches which rule.

- On the *Gel Tab:*

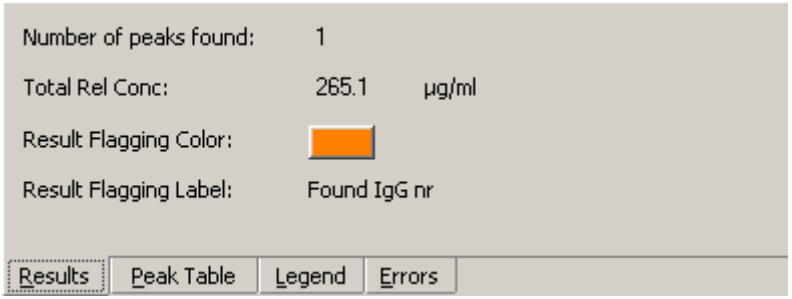


The spot on top of the lane is colored if the sample matches a result flagging rule.

- On the small gel image on the *Lower Panel:*



- On the *Results* tab:



Result Flagging Color: color of the result flagging rule that the current sample matches.

Result Flagging Label: label of the result flagging rule that the current sample matches.

How to Define Result Flagging Rules

Two modes are available to define result flagging rules:

- *Assisted* Mode

  In this mode you can easily compose an expression by selecting functions and operators from given lists. If necessary, additional attributes have to be provided.

  By selecting a logical operator (`AND`/`AND NOT`/`OR`/`OR NOT`), further terms can be combined to form a more complex expression. The last term of the expression ends with the operator `NONE`.

- *Advanced* Mode

  This mode is more flexible and allows you to write arbitrary complex expressions by using functions, variables and operators.

TIP

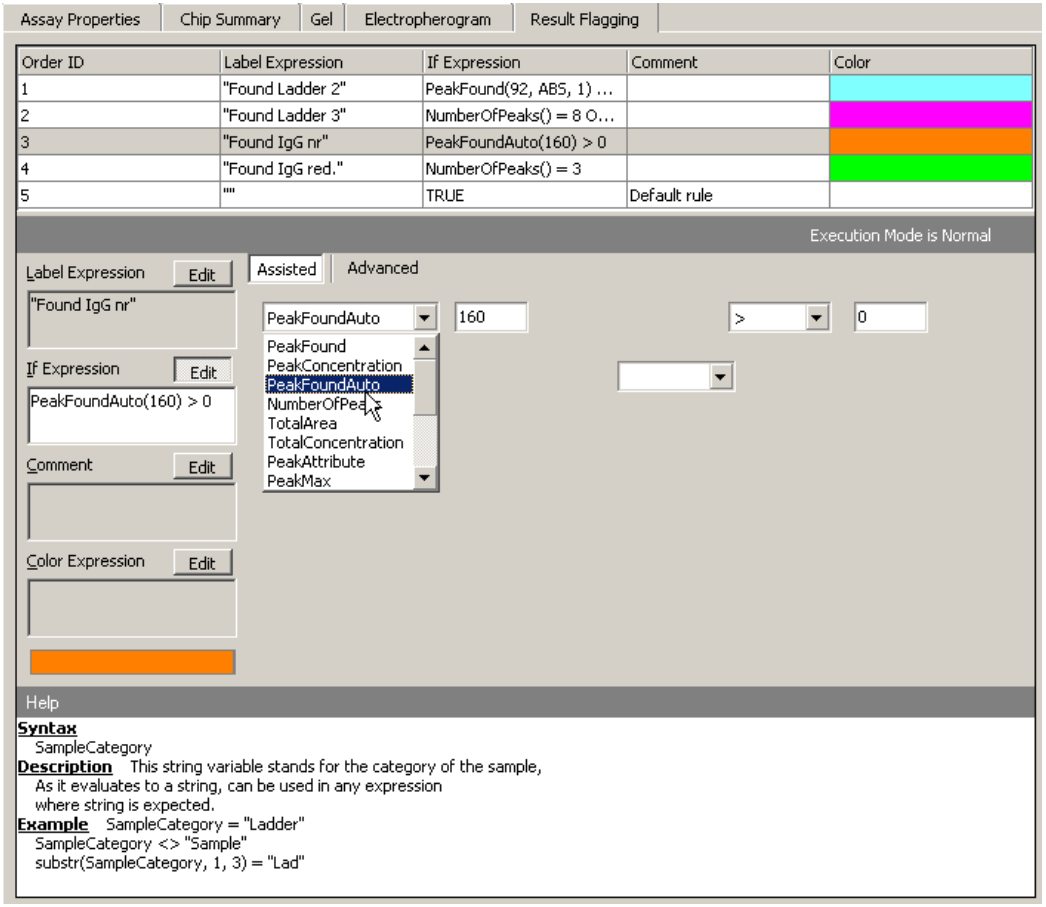
You can export result flagging rules and import rules from other assay or chip data files. See "Exporting Result Flagging Rules" on page 274 and "Importing Result Flagging Rules" on page 262.

To define a result flagging rule for an electrophoretic assay:

1. In the *Data and Assay* context open and select an electrophoretic chip data (.xad) or assay (.xsy) file.
2. Switch to the *Result Flagging* tab.
3. Create a new rule by clicking the *Add Rule*  button in the *Result Flagging* toolbar.
4. Select the rule in the rules list at the top of the tab.
5. Use the *Edit* buttons to switch between the *Label Expression*, *If Expression*, *Comment*, *and Color Expression* sections.

6 Enter an expression in the *If Expression* box.

The expressions can be composed by making selections in the drop-down lists on the right and entering values. Multiple expressions can be combined with the logical operators AND, AND NOT, OR, OR NOT. Refer to "Result Flagging Tab" on page 474 for available functions, operators, and variables.



NOTE

Please refer to the function reference in the *Help Pane* (which appears when you select a function from the list) for details on syntax, usage and examples.

7 Under *Color Expression*, define a color for the rule.

All samples that meet the rule will be highlighted with this color (see "Color Indication" on page 149). If the *Gradient* checkbox is enabled you can assign a color gradient to each rule (for example, to display differences in concentration).

8 You can create additional rules (*Add Rule* ), copy existing rules (*Copy Rule* ), and remove rules (*Delete Rule* ). Select the rule you want to copy, remove, or edit in the rules list at the top of the tab.

9 Use the *Move Up*  and *Move Down*  buttons to define the sequence for the rules.

In normal mode, the first rule in the list will be checked first. If the sample does not meet this rule, the second rule will be checked, and so on. The default rule is always the last rule in the list, and cannot be moved.

10 Click on *Apply Rules*  to validate the rules and apply them to the measurement results (if any).

11 If the message "Invalid Rules" appears, the rules are not applied. Click *OK* and check the syntax of your rules.

12 From the *File* menu, select *Save* to store the rules in the current chip data or assay file.

Example: Result Flagging

Sample 1 contains 100 ng/µl proteins. The electropherogram shows 2 peaks for 2 different proteins, which could be separated. One peak can be found at 32 kDa (LDH).

Sample 2 contains 60 ng/µl proteins and shows 3 peaks.

Sample 3 contains 80 ng/µl proteins and shows 5 peaks.

Now, the following rules are defined (the syntax is explained in "Result Flagging Tab" on page 474):

1. Is there a peak at 30 kDa +/- 7%?

   Rule 1: `PeakFound(30, PER, 7)`

2. Is the total concentration of proteins higher than 90 ng/µl?

   Rule 2: `TotalConcentration() > 90`

3. Were 5 to 10 peaks found?

   Rule 3: `NumberOfPeaks() >= 5 AND NumberOfPeaks <= 10`

   Alternative Rule 3: `NumberOfPeaks() BETWEEN (5,10)`

Applying these rules in the given order (in *Normal* mode) leads to the following results:

For sample 1, rule 1 matches and defines the color. Rule 2 would also match, but is not checked, because the procedure stops at the first match.

For sample 2, none of the rules match, if there is no peak at 30 kDa +/- 7%. Therefore, this sample will get the default color.

For sample 3, only rule 3 matches and defines the color.

Running and Evaluating Flow Cytometric Assays

For running and evaluating flow cytometric assays, you need to know the following:

Principles of Flow Cytometric Measurements

Besides electrophoretic assays (DNA, RNA, and proteins), the Agilent 2100 bioanalyzer supports flow cytometric assays:

- First, cells are stained with two fluorescent dyes that correspond to biologically relevant parameters, as described in the application notes available for each assay. "Staining Cells" on page 158 explains the principle.
- Next, the stained cells are analyzed on the chip. They pass the detector in single file and are analyzed individually for their red and blue fluorescence intensities. The results are displayed as histograms or dot plots. Refer to "Cell Detection with the Agilent 2100 Bioanalyzer" on page 160 for a detailed explanation.

Staining Cells

With the 2100 expert software, you can differentiate several properties of a cell. The characteristics that are examined depend on the dye, which binds specifically to a cellular constituent or is metabolized by the cell to generate a fluorescent product. You usually use two dyes with different colors. Typically, one of the two dyes is used as reference dye to select the target cells (living/dead, cell line type, ...). The second dye can be used to detect another characteristic of the cell.

Recommended dyes

The tables below list dyes that match to the detection optics specification (Excitation max: 470 & 630 nm; Emission max: 525 & 680 nm).

The following dyes are recommended for use as the *blue* stain:

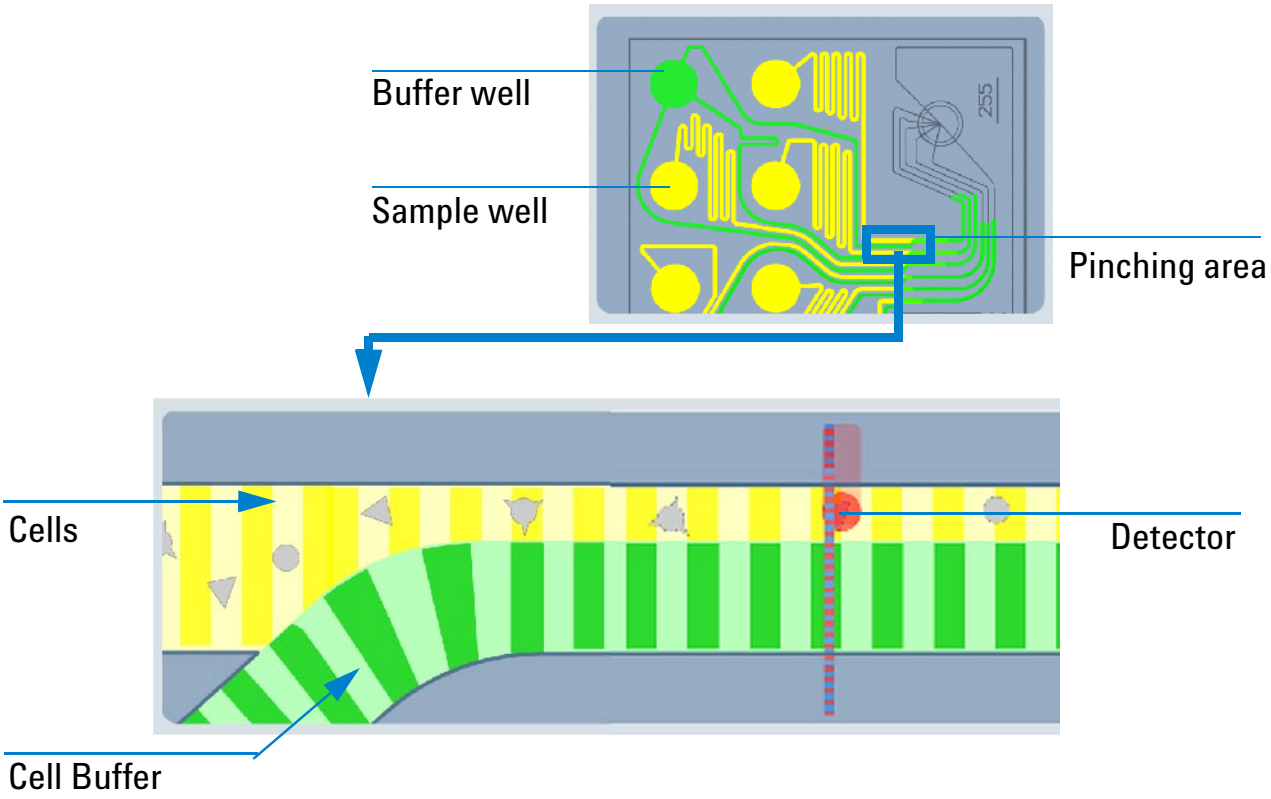
Dye (blue fluorescence)	Max. Excitation wavelength	Max. Emission wavelength
Calcein (living cell stain)	493 nm	514 nm
Cell Tracker green (cell tracing, viability stain)	492 nm	517 nm
GFP (green fluorescent protein)	490 nm	510 nm
SYTO16 (DNA dye)	485 nm	530 nm

The following dyes are recommended for use as the *red* stain:

Dye (red fluorescence)	Max. Excitation wavelength	Max. Emission wavelength
CBNF (Carboxynaphthofluorescein, living cell stain)	595 nm	675 nm
APC (Allophycocyanin, intra- and extra cellular antibody staining)	650 nm	660 nm
Cy5 (labeled Streptavidin and labeled anti-IgG, Apoptosis, intra- and extra-cellular antibody staining)	647 nm	665 nm
Alexa 647	650 nm	668 nm

Cell Detection with the Agilent 2100 Bioanalyzer

LabChip technology allows cell measurements by integrating cell flow, hydrodynamic focusing, and fluorescence detection into a microfluidic chip. A cell suspension can be confined or "pinched" to a portion of a microfluidic channel, causing cells to line up in single file for individual cell detection. The following images illustrate the pinching process.



Up to six cell samples can be analyzed on a chip. They are measured sequentially.

Measuring Events

The bioanalyzer counts cells stained with fluorescent dyes and measures their fluorescence intensities. Each cell or bead that passes the detector and emits fluorescence above a threshold value is counted as an event. For each event, the intensity of two different fluorescent signals (red and blue) is recorded. The intensity of the fluorescent signal depends on the amount of stain bound to the cell (and therefore a specific cell property) and the physical properties of the stain itself.

The Agilent 2100 bioanalyzer lets you determine the number of cells characterized by a specific pattern of fluorescence.

For example, to differentiate between dead and living cells, you can use a non-fluorescent dye that becomes fluorescent when metabolized by living cells. After staining with such a dye, living cells have a higher fluorescence value than dead cells. The second dye could bind to a specific surface marker on a subpopulation of the cells. This allows you to determine the number of living cells that contain your marker of interest.

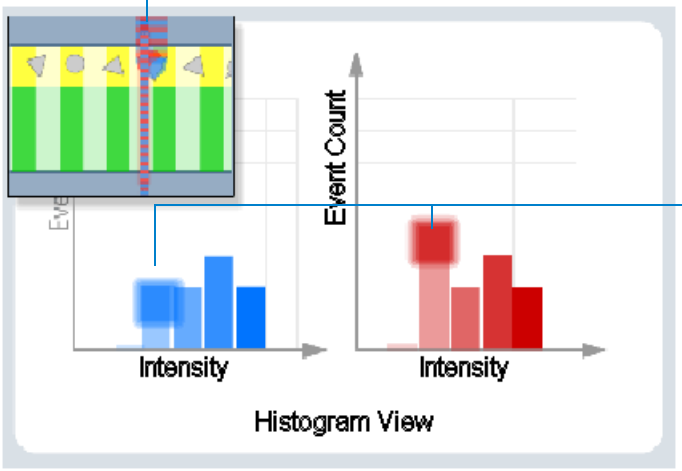
For evaluation, 2100 expert displays the results as histograms and as dot plots.

Generating Histograms

2100 expert counts the events, sorts them and displays them according to their fluorescence intensity in histograms. For each color measured, a histogram displays the number of events related to the fluorescence intensity. A large number of events with a high fluorescence value means that a large number of cells containing the fluorescence dye were detected.

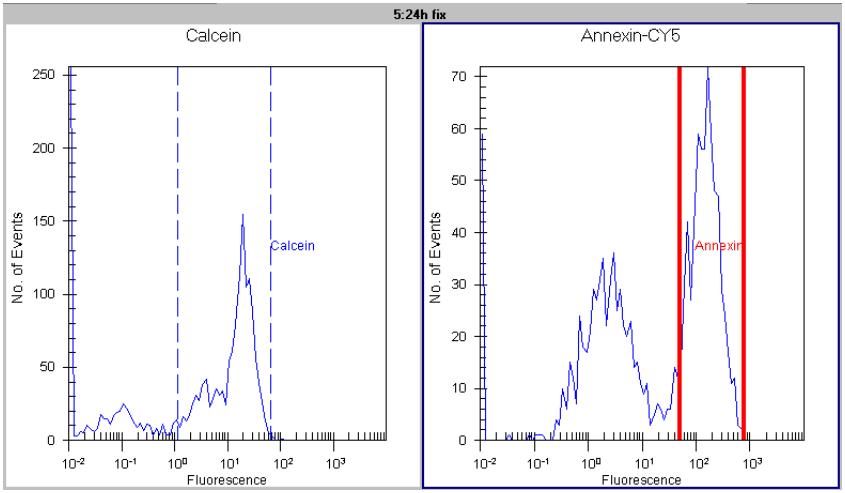
In the following illustration, cells which fluoresce in both colors are highlighted.

As a cell passes through the detector, its blue and red fluorescence values are measured and the count is increased for both channels at the appropriate intensity.



Illustrates a cell that fluoresces in blue and red. The height of the bars is related to the number of cells with this fluorescence value.

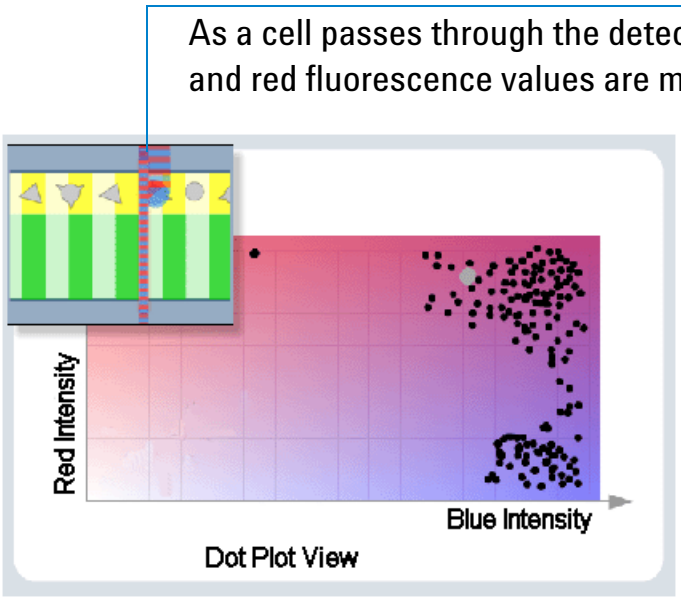
In the histograms, the bar chart is replaced by a point-to-point line as shown in the following image.



For detailed information, see “Using Histograms for Evaluation” on page 199.

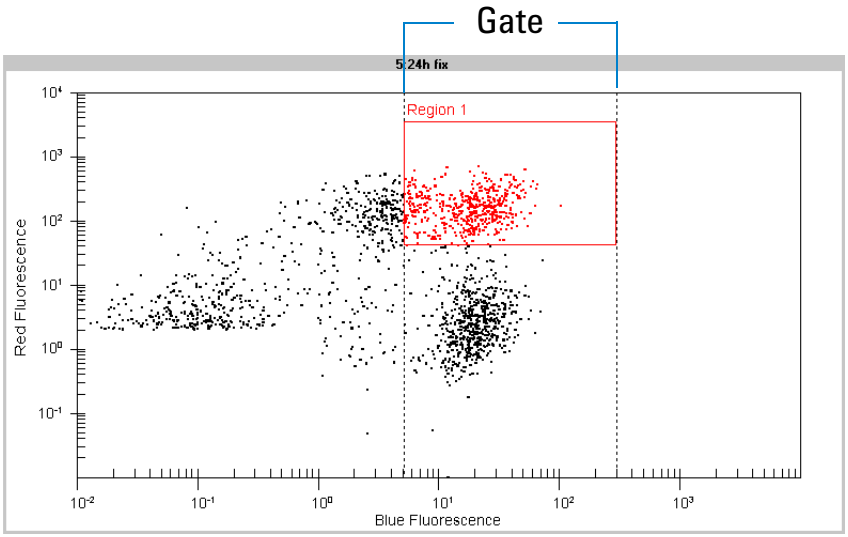
Generating Dot Plots

Single events can also be displayed related to both fluorescence values, generating a map of dot plots. In dot plot view, the events (cells with a minimum fluorescence intensity) are displayed in a coordinate system (logarithmic axis scaling). Each axis represents a fluorescence color. A high number of events (cells) with similar fluorescence values are displayed as a dense cluster of dots, as shown in the following image.



To determine the number of cells whose blue and red fluorescence lies within a defined range, you can insert regions. Additionally, a gate can be set for either red or blue fluorescence to obtain percentual values on a defined population.

In predefined assays, the borders of the rectangular region represent the markers defined in the corresponding blue and red histograms.



The lower left region of a dot plot usually shows no events, due to the defined peak detection threshold that the fluorescence values must exceed.

For detailed information, see “Using Dot Plots for Evaluation” on page 220.

Overview of Flow Cytometric Assays

The cell characteristic to be measured requires not only specific dyes. Several measurement parameters to control the measurement and the data acquisition parameters also have to be specified. These so-called “setpoints” are stored in assay files (.xsy) and are read by the 2100 expert software before it starts the measurement.

2100 expert supports the following assays based on flow cytometry:

Predefined assays

- *Apoptosis*
- *Apoptosis – fast protocol*

  For reduced background this assay has an increased threshold and uses blue events only for peak detection.
- *Antibody Staining*
- *On-chip Antibody Staining*

  For reduced background this assay has an increased threshold in the blue signal.
- *GFP*
- *On-chip GFP*

  This assay allows a rapid and accurate detection of green fluorescent protein expression.
- *Blue to red*

  This assay is for applications that apply a blue reference dye and analyze red fluorescent cells within a blue population.

- *Red to blue*

  This assay is for applications that apply a red reference dye and analyze blue fluorescent cells within a red population.

- *Checkout Beads*

  Red checkout beads are loaded into the wells 1, 3 and 5 and blue checkout beads into the wells 2, 4 and 6. Markers are set according to expected fluorescence levels of the red and blue beads. The *Checkout Beads* assay has the properties of a generic assay (see below).

- *siRNA Transfection Viability*

  Transfection Viability analysis as described in the Application Note: *siRNA transfection optimization with the Agilent 2100 bioanalyzer* (Agilent publication number: 5988-9782EN). This assay enables the automatic calculation of *transfection efficiency* (TE) in histogram view and *viability in transfected cells* (ViT) in dot plot view. Required gating directions and regions are provided as example, but can be adjusted. Final Transfection Viability (TV) can be calculated by multiplication TE and ViT values derived from histograms and dot plots.

The settings of predefined assays are optimized to measure the appropriate cell characteristics. For evaluation, it is only necessary to adjust the markers in histograms or regions in dot plots.

Predefined assays contain all necessary markers and regions for evaluation. The gating direction for histograms is given (for details on the gating direction, refer to "Gating" on page 201). The markers in all samples are connected (changing a marker changes the corresponding markers in all samples).

The regions of the dot plots are related to the markers of the histograms. Thus, the results of the dot plots are identical to the results of the histograms.

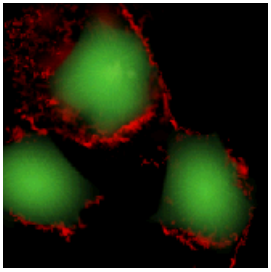
Generic assay

This assay has no specific settings and can be used to define individual assays. You can freely add markers or regions, and define the gating direction.

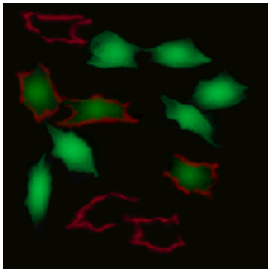
The generic assay is recommended for chips with different samples and stainings, where regions would need to be defined individually. Dot plot and histogram regions are not linked, making it possible to evaluate an individual sample with different settings.

Flow cytometry assay icons

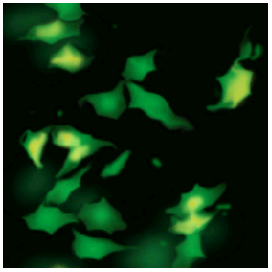
On the *Assay Properties* tab (see "Assay Properties Tab" on page 418), the following icons are used to visualize the assay type:



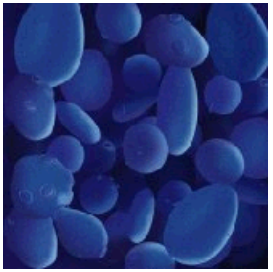
Antibody Staining
Blue to Red



Apoptosis



GFP
Red to Blue



Generic
Checkout Beads
siRNA Transfection
Viability

Preparing and Running a Flow Cytometric Assay

A flow cytometric chip run requires the following steps:

1. Set up and switch on the Agilent 2100 bioanalyzer.

   Refer to "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47.

2. Start the 2100 expert software.

   Details are given in "Starting 2100 Expert" on page 32.

3. Select a flow cytometric assay.

   See "Selecting a Flow Cytometric Assay for a Chip Run" on page 171.

4. Prepare chip and samples.

   Refer to "Preparing Samples and Chips for Flow Cytometric Assays" on page 176 and to the appropriate *Application Note* and *Reagent Kit Guide*.

5. Load the chip into the bioanalyzer.

   For details refer to "Loading the Cell Chip into the Bioanalyzer" on page 180.

6. Start the chip run.

   This is described in "Running a Flow Cytometric Assay" on page 182.

When the chip run has finished, you can:

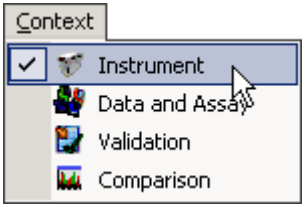
- Have a first look at the results (see "Displaying the Measurement Results (Flow Cytometry)" on page 192).
- Document the chip run (see "Entering Chip, Sample, and Study Information" on page 190).

- Analyze and evaluate the results:
  - "Using Histograms for Evaluation" on page 199
  - "Using Dot Plots for Evaluation" on page 220
  - "Evaluating Antibody Staining, Apoptosis, and GFP Assays" on page 229

Selecting a Flow Cytometric Assay for a Chip Run

To select an assay:

1 Switch to the *Instrument* context.



2 In the *Tree View Panel*, select the bioanalyzer you want to use.

In the upper left of the *Instrument* tab, an icon shows the status of the bioanalyzer. You should see one of the following icons (lid open/closed), indicating that the bioanalyzer is detected by the system:

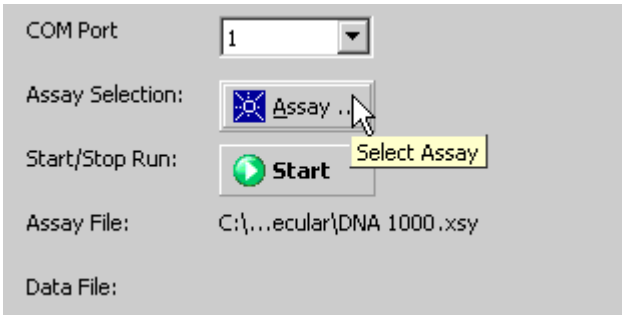
3 If you do not see one of these icons, check that the bioanalyzer is switched on and properly connected:

- Check the *COM port* setting.
- Make sure the bioanalyzer is physically connected to the PC (over the serial interface).
- Check the power connection.
- Check the power switch.

If you need additional help, please refer to the *Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide*.

4 Select an assay for the chip run.

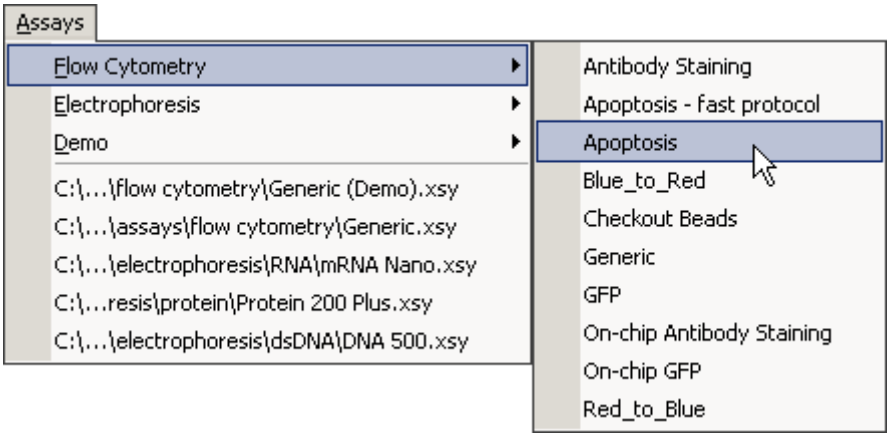
On the *Instrument* tab, click the *Assay...* button.



– OR –

Click the *Assays* menu.

Both will open the *Assays* menu, allowing you to select an assay from the submenus.



– OR –

Select *File* > *Open File to Run...*. This opens a dialog box, allowing you to load either an assay (.xsy) or a chip data file (.xad).

The type of assay you have to select depends on the experiment and the staining protocol you use to prepare your cell samples. Details on these assays are described in the *Application Notes* available for each assay.

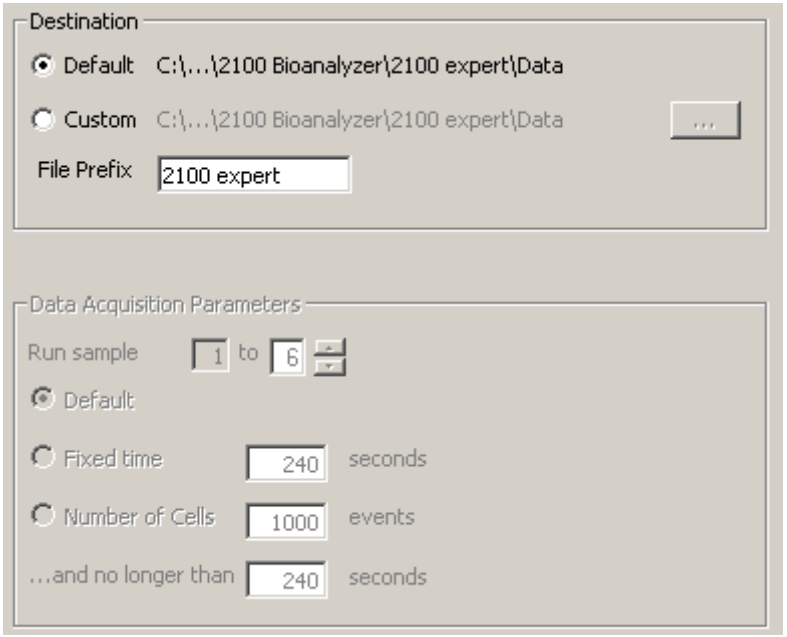
5 Select the desired assay, *Apoptosis*, for example.

The assay is loaded and its name appears on the *Information Bar*:

NOTE

After a chip run, the results can be evaluated using a different flow cytometric chip data or assay file. Refer to "Importing Data Analysis Setpoints" on page 259.

6 Select a *Destination* for the chip data file (.xad) generated as the result of the chip run.



7 If required, change the *Data Acquisition Parameters*:

a Enter the number of samples you want to be measured.

When preparing the chip (see "Preparing Samples and Chips for Flow Cytometric Assays" on page 176), keep in mind that you have to follow the sequence of the sample wells. For example, if you want to measure 3 samples, you have to fill the wells 1, 2, and 3 with your samples, and the remaining wells with cell buffer solution.

b Select the Data Acquisition Mode.

Select *Default*, if you want the measurement time to be set to the default value (240 s/sample). The maximum time is shown in brackets.

– OR –

Select *Fixed time* and enter the time in [s] that the measurement of each sample is to take.

– OR –

Select *Number of Cells* and enter the minimum number of *events* that should be measured. In the *... and no longer than* field, enter the maximum time in [s] a measurement can take, regardless of whether or not the defined number of events is reached. The maximum time is shown in brackets.

NOTE

The overall run time for a chip is limited to 1440 s. The individual run time for one sample depends on the number of samples that are measured per chip. If only one sample is measured, you can set the run time up to 1440 s.

WARNING

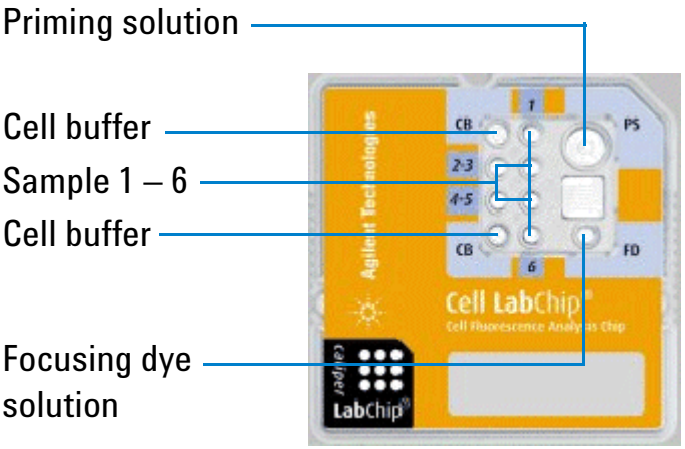
Several substances such as dyes can have toxic, carcinogenic, or mutagenic potential. Therefore, carefully follow the safety instructions from the dye safety data sheet and the *Reagent Kit Guides*. Also read the "Essential Measurement Practices (Flow Cytometric Assays)" on page 178.

Before you can fill a chip, you have to prepare the samples. To find out which protocols you should use to prepare the samples, refer to the various *Application Notes* available for each assay.

Sample and chip preparation is described in detail in the *Reagent Kit Guide* available for each LabChip kit.

Chip Reagents

Several reagents have to be added to the chip to prepare it for measurement. The following image shows which reagents have to be filled in which wells.



Make sure you follow these directions when preparing the sample:

- The priming solution has to be added first. It fills all channels (removes all air from the micro channels).
- The focusing dye is used to adjust the optic. The optics are focused horizontally and vertically before each chip is measured.
- If you do not use all six wells, always load the first sample in well 1, the second sample in well 2 etc. Unused wells have to be filled with cell buffer solution, otherwise they may run dry during the chip run. Because all channels are connected to the priming well, this may led to bubbles and to a clogging of the pressure adapter filter.
- The cell buffer is used to focus the cells before they pass the detection point. You have to fill both buffer wells with cell buffer.

Essential Measurement Practices (Flow Cytometric Assays)

- Handle and store all reagents according to the instructions given in the *Reagent Kit Guides*.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Store all reagent and reagent mixes in the dark and refrigerated at 4 °C when not in use.
- Allow all reagents to equilibrate to room temperature for 30 minutes before use.
- Protect focusing dye from light. The dye decomposes when exposed to light.
- Use appropriate pipette tips. For each pipetting step use a fresh, new pipette tip.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results due to the formation of a bubble on the bottom of the well.

- For chip preparation, use inverse pipetting.
- When filling the pipette tip, push slightly over the first resistance. Empty the pipette tip only to the first resistance. This procedure avoids the introduction of bubbles and ensures pipetting the right volume.
- Never leave any wells empty, or the pressure adapter may become clogged. Pipette 10 µl of cell buffer or sample replicate in any empty sample well.

- Before bead preparation, vortex bead vials for 15 seconds.
- Prepared chips must be used within 5 minutes. If a chip is not run within 5 minutes, beads may settle or reagents may evaporate, leading to poor results.
- Never touch the instrument lens.
- Never touch the Agilent 2100 bioanalyzer during a chip run and never place it on a vibrating ground.

Loading the Cell Chip into the Bioanalyzer

After preparing the chip, you can insert it into the Agilent 2100 bioanalyzer.

NOTE

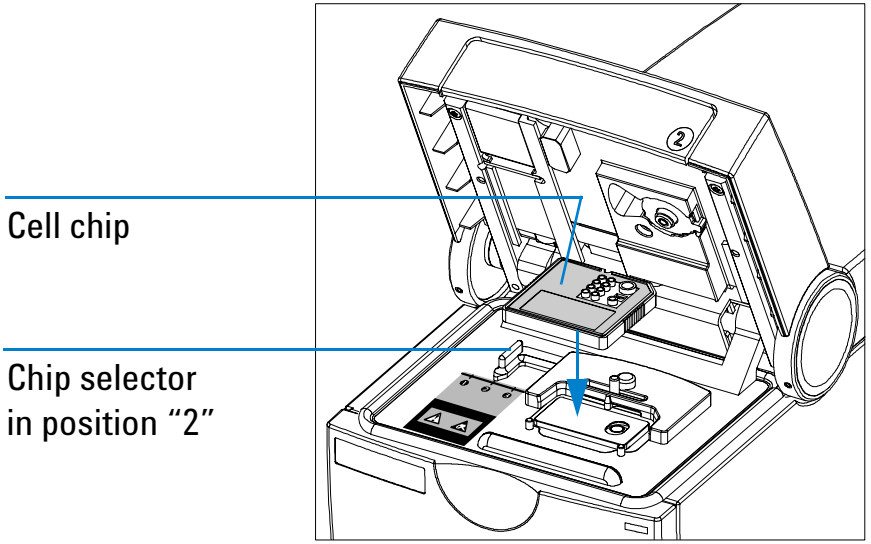
Before inserting the chip, check that the pressure cartridge is installed and the chip selector is in position "2". For details, refer to "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47.

To load the chip into the Agilent 2100 bioanalyzer:

1 Open the lid.

2 Place the prepared chip into the receptacle.

The chip fits only one way. Do not use force.

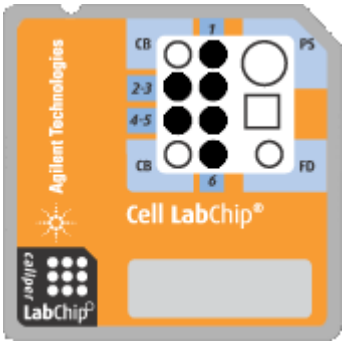


3 Carefully close the lid.

CAUTION

Do not force the lid closed. This may damage the pressure cartridge. The lid may not close completely. If the software recognizes that a chip has been inserted, the system is ready. If the chip is not recognized open the lid, verify that the cartridge and chip are inserted properly and the chip selector is in the correct position. Close the lid.

The adapter with the gasket in the cartridge fits onto the priming well of the chip. A small gap between the lid and the instrument mainframe is normal and no cause for malfunction. The icon on the *Instrument* tab changes to a cell chip icon:



If the chip is not detected, open and close the lid again.

NOTE

If the *AutoRun* option is active (see "Options – Advanced" on page 508), the chip run starts automatically once a chip has been inserted and the lid has been closed.

Running a Flow Cytometric Assay

Running a flow cytometric assay in 2100 expert just means pressing a button.

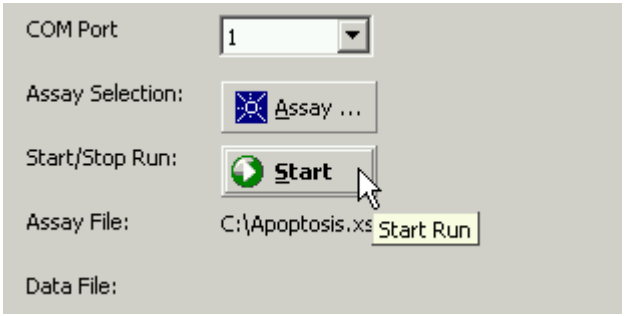
NOTE

You can stop a chip run at any time, for example, if errors occurred, or if you are not satisfied with the quality of the measurement results, which you can observe during the chip run. See "Stopping a Chip Run" on page 187.

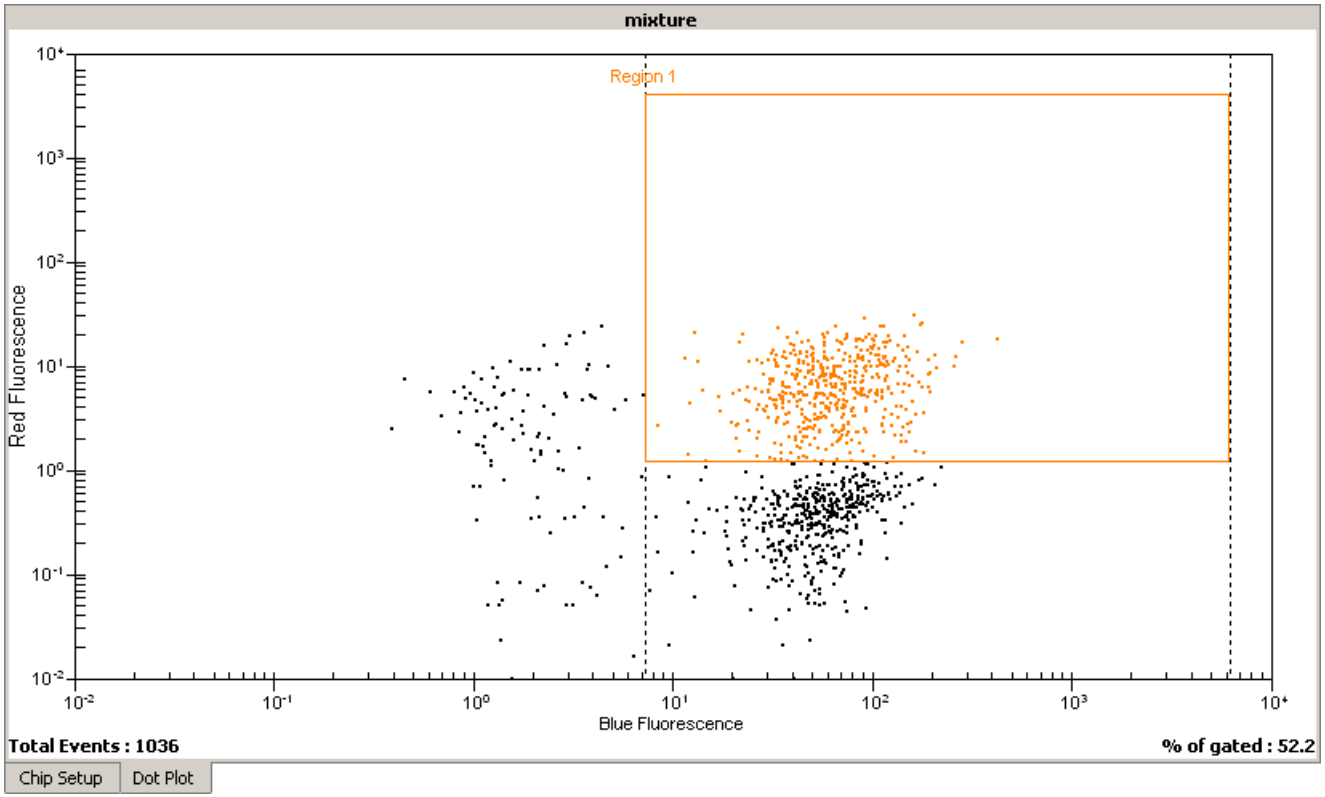
Starting the Chip Run

When you have loaded the chip, you can start the chip run:

1 On the *Instrument* tab, click the *Start* button.



The *Dot Plot* sub-tab shows single events (cells) as they are detected, displayed as dots. In the coordinate system, the red and blue fluorescence intensity of each event can be read. The name of the currently measured sample is displayed above the graph.

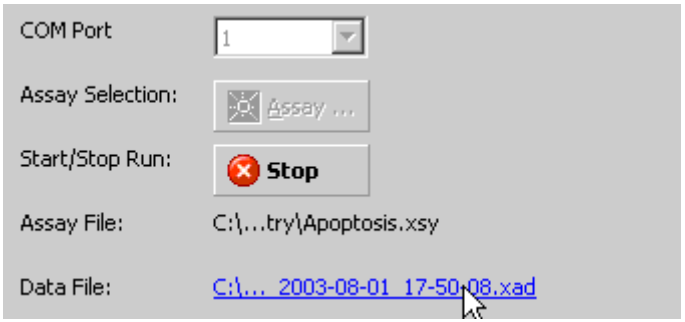


The number of the sample that is currently being measured is indicated on the information bar:

The status bar shows the name of the currently measured sample, a progress bar showing the measurement progress for the current sample (not for the whole chip run), and the COM port number used for acquiring data:

During the chip run, you can do the following:

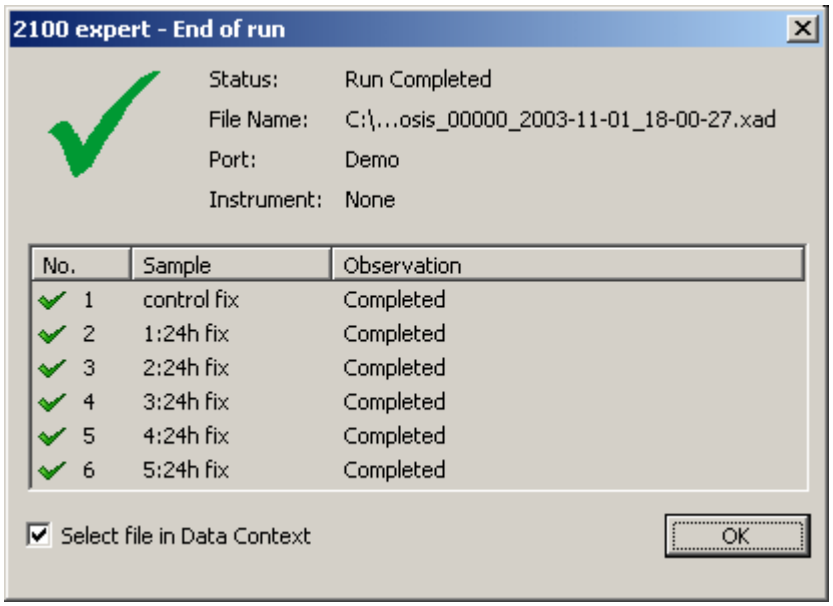
- View the chip data file in the *Data and Assay* context by clicking on the name of the *Data File*:



- Evaluate any chip data file in the *Data and Assay* context.
- Compare samples in the *Comparison* context.
- If necessary, abort the chip run by clicking on the *Stop* button (see also "Stopping a Chip Run" on page 187). All data that was collected up to the stop point will be saved.

Finishing the Chip Run

When the measurements are finished, the *End of run* dialog box appears, showing you the number of samples that have been measured, and the file name where the chip run data has been stored. If errors occurred, they would also be displayed in this dialog box.



1 To immediately view the results in the *Data and Assay* context, you can select the *Select file in Data Context* check box.

2 Click *OK*.

The dialog box is closed.

- If you selected *Select file in Data Context*, you are automatically taken to the *Data and Assay* context, where you can view, analyze, and evaluate the results of your chip run (see "Displaying the Measurement Results (Flow Cytometry)" on page 192 and "Analyzing and Evaluating the Results of a Flow Cytometric Assay" on page 198).
- If you did *not* select the *Select file in Data Context* check box, you are taken back to the *Instrument* context, where you can start a new chip run, for example.

Stopping a Chip Run

You can stop a chip run at any time, for example,

- if the quality of the measurement results does not meet your expectations,
- if, for example, after three samples you already have the information you desired and you want to start another chip run.

NOTE

You *cannot* resume a stopped chip run.

NOTE

If you stop a chip run, automatic export (see "Exporting Chip Run Data Automatically" on page 266) and automatic print (see "How to Turn on and Configure Automatic Printing of Chip Run Reports" on page 280) do *not* take place.

To stop the assay:

1 Click the *Stop* button.

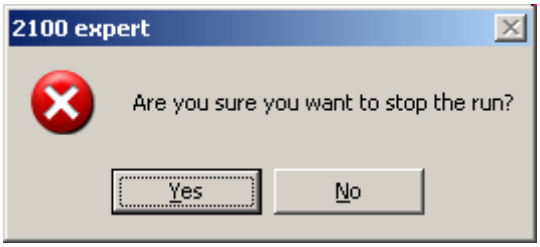
– OR –

Select *Stop* from the *Instrument* menu.

NOTE

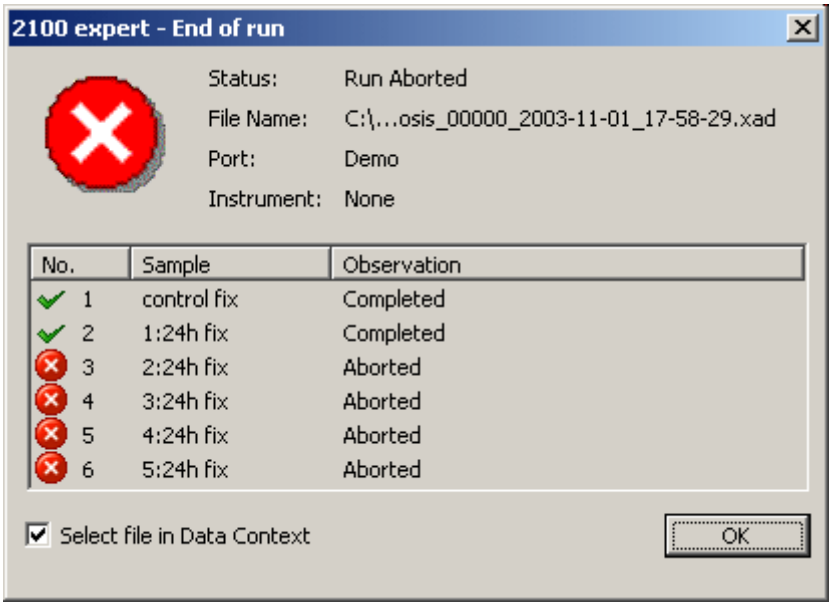
Data acquisition of the current sample will be aborted.

The following message appears:



2 Click *Yes* to stop the chip run.

The *End of Run* dialog box appears.



The measured samples are marked with a green check, and only these are stored in the chip data file. The unmeasured samples are marked with a white cross on red ground.

3 If you want to immediately view the results in the *Data and Assay* context, select the *Select file in Data Context* check box.

4 Click *OK*.

The dialog box is closed.

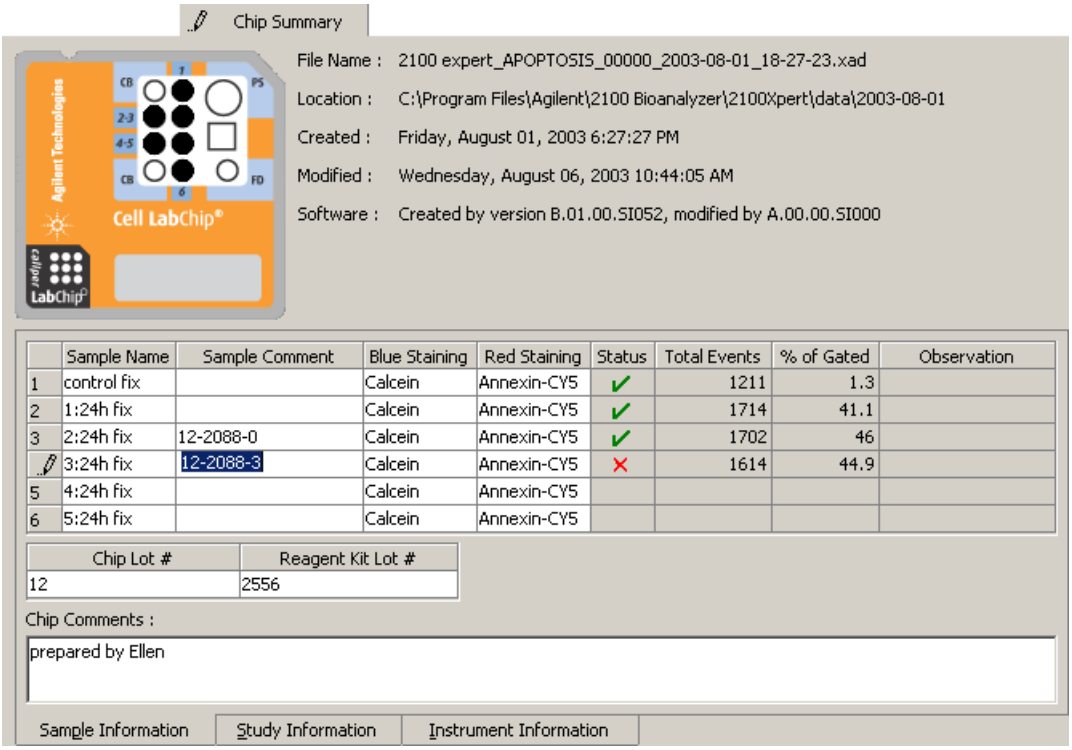
- If you selected *Select file in Data Context*, you are automatically taken to the *Data and Assay* context, where you can view, analyze, and evaluate the results (if any) of your chip run (see "Displaying the Measurement Results (Flow Cytometry)" on page 192 and "Analyzing and Evaluating the Results of a Flow Cytometric Assay" on page 198).
- If you did *not* select the *Select file in Data Context* check box, you are taken back to the *Instrument* context, where you can start the next chip run, for example.

Entering Chip, Sample, and Study Information

During or after a chip run, you can document the run by entering information on chip, samples, and study.

1 In the *Data and Assay* context, select the *Chip Summary* tab.

2 On the *Sample Information* sub-tab, you can enter additional information for samples, such as names for blue and red stain. On the *Study Information* sub-tab, you can enter the laboratory location, and the name of the experimenter, for example.



NOTE

You may find some input fields already filled in, because chip, sample, and study information are taken over from the base assay or chip data file.

For details on all input fields, refer to "Chip Summary Tab" on page 427.

3 Click *Apply*.

4 From the *File* menu, select *Save*.

TIP

You can import chip, sample, and study information from .txt or .csv files. This is especially helpful and time-saving, if you already have documented a similar chip run in another chip data file. Refer to "Importing Chip, Sample, and Study Information" on page 261 for details.

Displaying the Measurement Results (Flow Cytometry)

You can view the measurement results of a flow cytometric chip run as histograms or dot plots.

- You can display the histograms/dot plots either one sample at a time, or all samples at the same time to get an overview of the chip run, for example, to see the progress of a reaction. See "How to Switch Between Single View and Grid View" on page 193.
- You can navigate through the samples. See "How to Navigate Through the Samples" on page 194.
- You can change the display of histograms and dot plots to make details better visible. See "How to Change the Display of Histograms and Dot Plots" on page 195.

How to Switch Between Single View and Grid View

To switch between single view and grid view:

1 From the *Histogram* or *Dot Plot* menu, select *Single View* or *Grid View*.

– OR –

Click the *Single View* or *Grid View* button on the histogram/dot plot tool bar.

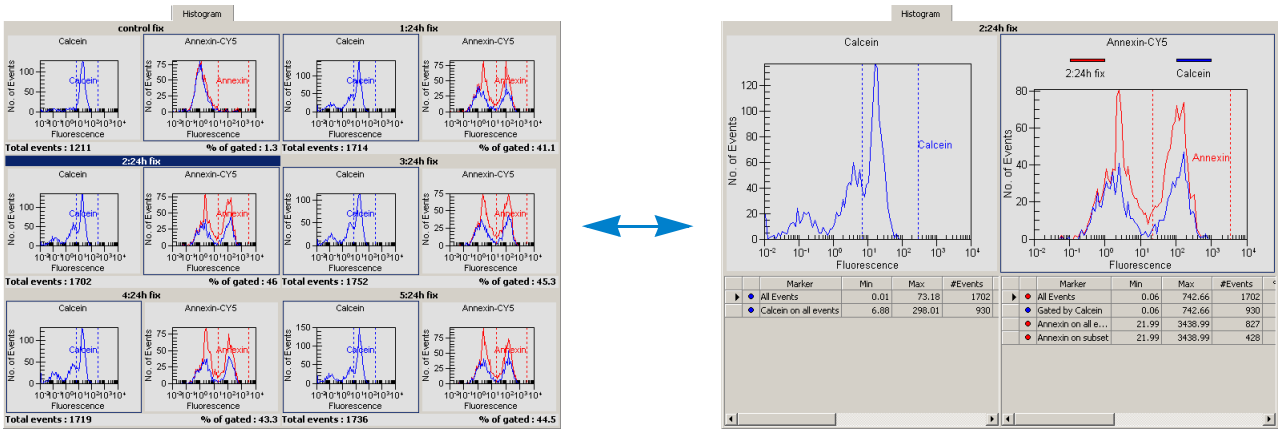
– OR –

Click the *All Samples* entry in the *Tree View Panel* to switch to the grid view, or any sample to switch to the single view.

– OR –

Double-click any histogram or dot plot in the grid view to switch to single view.

The following example shows switching between grid view and single view for histograms.



How to Navigate Through the Samples

At any time—even during a chip run—you can scroll though all samples—either in histogram or dot plot view.

To navigate through samples using the *Tree View Panel*:

1. If the tree view is not visible, select *View* > *Tree View*.

   The tree view panel appears to the left of the tabs, and shows all chip data and assay files as nodes.

2. Click any sample name.

   The histogram or dot plot of the sample is shown in single view.

To navigate through samples using the *Lower Panel*:

1. If the lower panel is not visible, select *View* > *Lower panel*.

   The lower panel appears in the lower left corner, showing a chip icon.

2. Click on any well on the chip icon.

To browse through samples:

1. From the *Histogram* or *Dot Plot* menu, select *Next Sample* or *Previous Sample*.

   – OR –

   Click the *Next Sample*  or *Previous Sample*  button in the histogram/dot plot tool bar.

To switch between histogram and dot plot view:

1. Click the *Histogram* or *Dot Plot* tab to display the results of the selected sample as a histogram or dot plot.

How to Change the Display of Histograms and Dot Plots

In *single view*, it is possible to change the display of histograms and dot plots.

In histograms and dot plots you can:

- zoom (enlarge or reduce using the mouse) the graphs to display details, for example.
- put a color gradient on the background of the graphs.

In histograms, you can additionally:

- show data points.

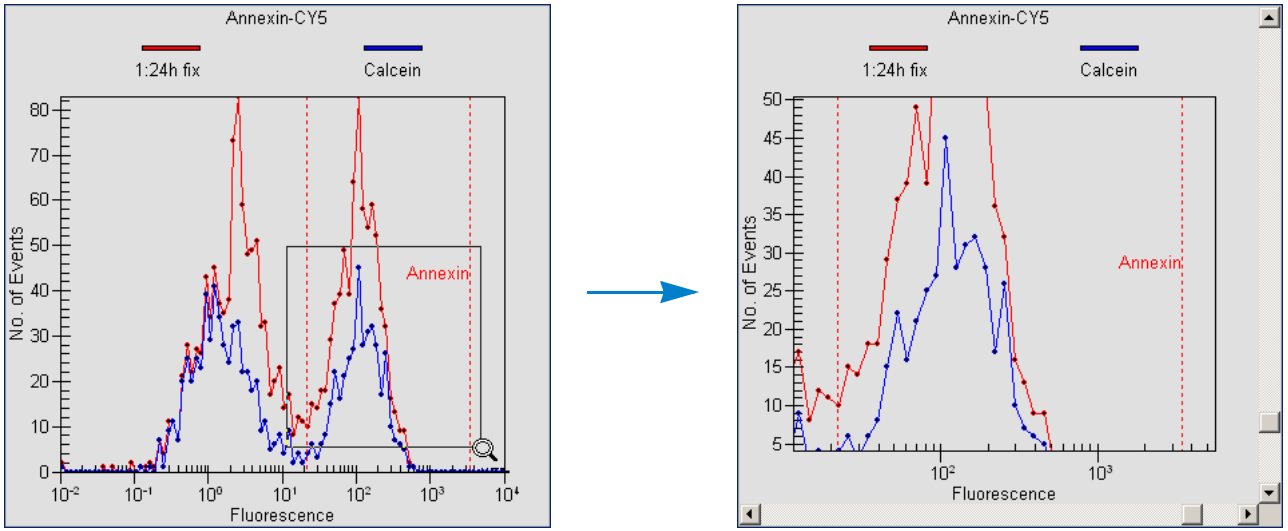
To zoom into a histogram or dot plot:

1 Position the mouse pointer in the histogram/dot plot.

2 Click and hold down the left mouse button.
The mouse pointer changes its shape to a magnifying glass  .

3 Drag the mouse.
A rectangle shows the part of the histogram/dot plot to be enlarged.

4 Release the mouse button.



You can perform several zoom steps in a row. When you have zoomed a histogram or dot plot, the *Undo Zoom* and *Undo All* buttons are enabled.

To undo one zoom step:

1 Click the *Undo Zoom* button or double-click in the histogram or dot plot.

To undo all zoom steps:

1 Click the *Undo Zoom All* button.

To display data points in histograms:

1 From the *Histogram* menu, select *Show Data Points*.

All events are shown as bullets.

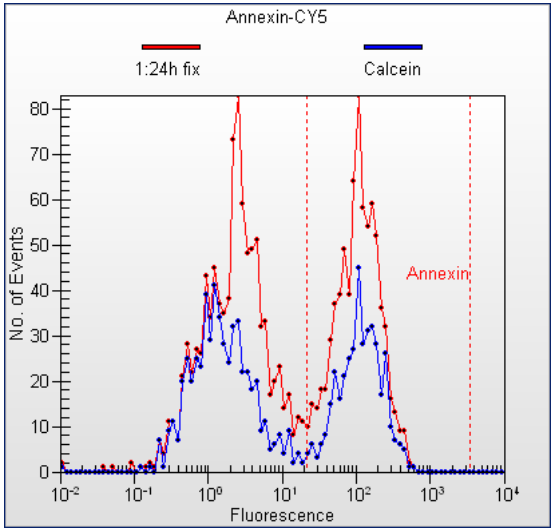
To put a color gradient on the background of a histogram or dot plot:

1 From the *Histogram* or *Dot Plot* menu, select *Gradient*.

– OR –

Click the *Gradient* button the histogram or dot plot toolbar.

A color gradient (gray to white) appears on the background of the graph.



Analyzing and Evaluating the Results of a Flow Cytometric Assay

You can analyze and evaluate result data of flow cytometric assays using either the dot plot or the histogram view. In both views, you can evaluate the detected cells by defining areas of interest.

- *Histograms* show the distribution of events related to the red and blue fluorescence intensity. Gating is used to generate subsets based on markers in one histogram. See "Using Histograms for Evaluation" on page 199 for detailed information.
- *Dot plots* show events as dots in a coordinate system where the blue fluorescence value is related to the red. Regions and gates are used to determine the number of cells with a fluorescence intensity lying in a defined range. See "Using Dot Plots for Evaluation" on page 220 for detailed information.

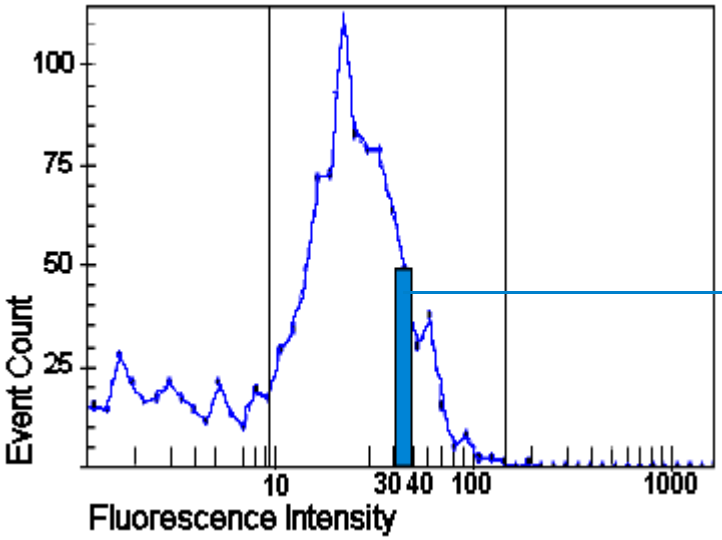
If you use predefined assays, the markers and regions are set at the approximate position where the events are expected. Refer to "Evaluating Antibody Staining, Apoptosis, and GFP Assays" on page 229 for information on how to evaluate the predefined assays.

TIP

You can analyze and evaluate results already while a chip run is still in progress.

Using Histograms for Evaluation

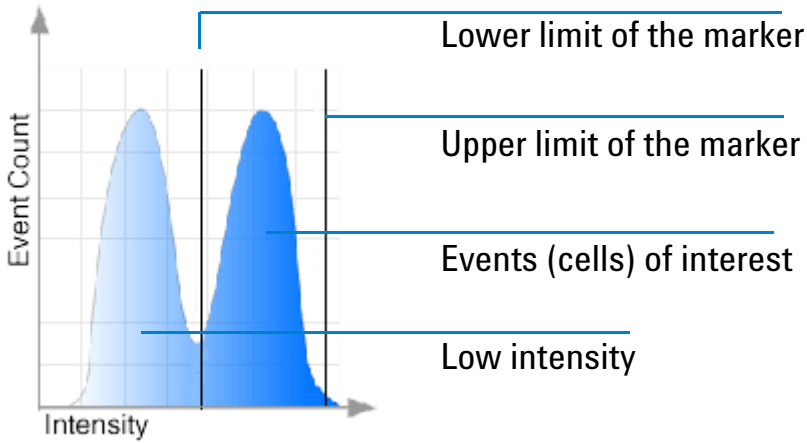
Histograms are graphical representations of the measurement results, where the number of events (cells) is mapped to the Y axis and their fluorescence values to the X axis. The resulting curves show the frequency distribution of the events in relation to their fluorescence intensity values, as shown in the following image. In real histograms, the bin is reduced to a dot (data point).



The histograms can be evaluated statistically with markers that allow you to define ranges of fluorescence intensity values. One histogram can be used to represent a range of fluorescence values to define a subset of events. Only cells with a fluorescence value within this range are displayed in the second histogram. This method is called gating.

Markers

Markers are used to define a range of fluorescence intensity values in a histogram. The upper and lower limits of the range are displayed as vertical lines, as shown in the following image.



The numerical values for each defined marker are displayed in a separate row in the result table. One marker is used as a gate for the second histogram, to define a subset of events.

In predefined assays, the markers are set by default and you only need to adjust their position. If you want to define your own assays, select the *Generic* assay for acquisition, where you can define your own markers and/or regions together with gates.

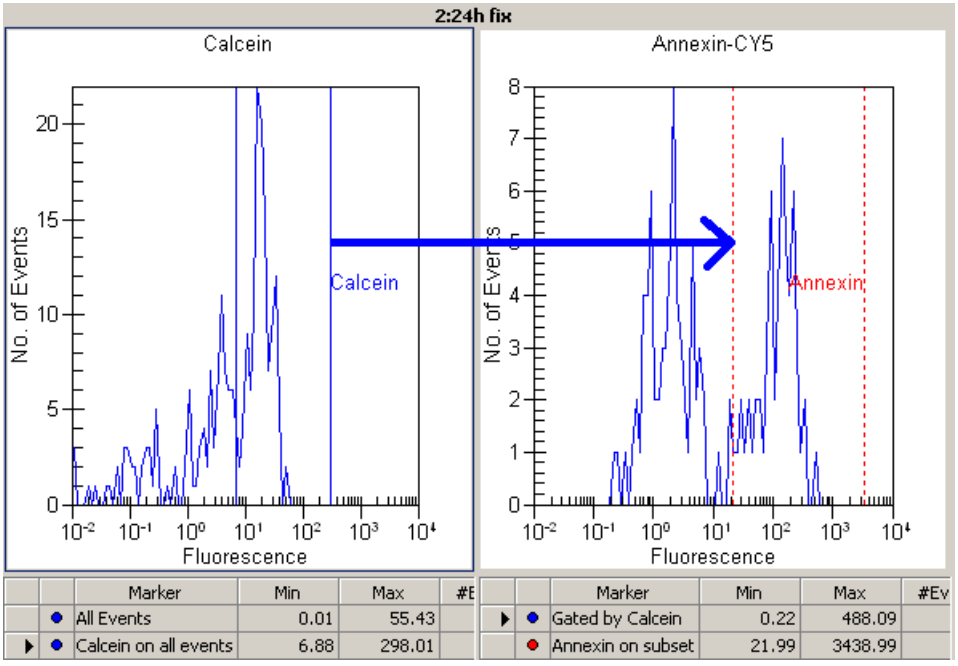
If you use several markers within one histogram, only one of them can be used for gating. The other markers can only be used to evaluate regions in the histogram they cover. The values belonging to these markers are also displayed in the result table.

Gating

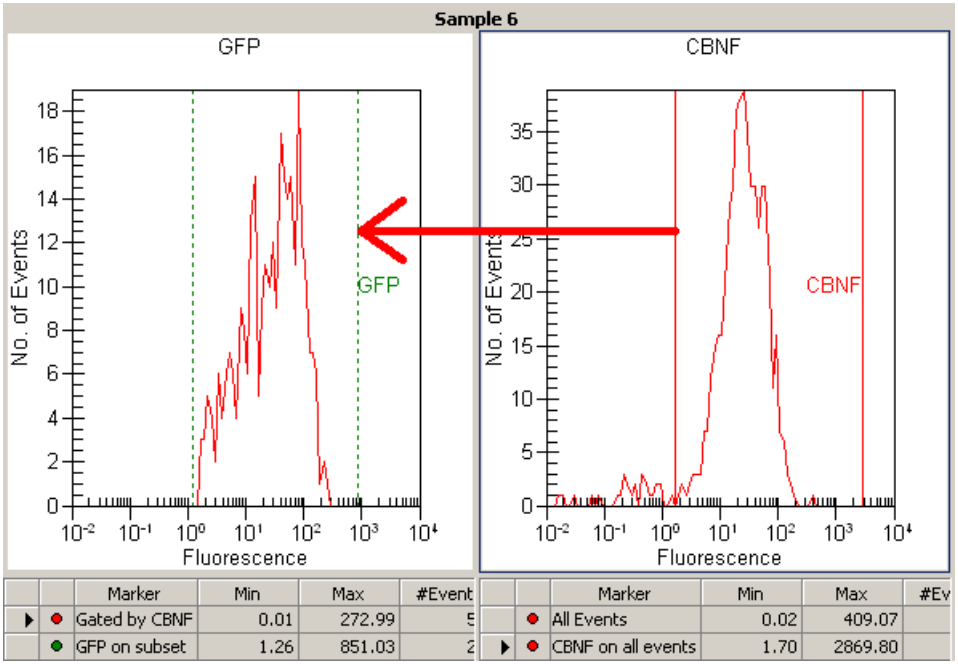
Gating is used to restrict the number of events that are evaluated by gating out (filtering) events that do not have the fluorescence values set by a marker. For example, by gating on a blue marker, you can exclude all events with low blue fluorescence (allowing you, for example, to gate out dead cells, unbound dye and debris). Only events with blue fluorescence values within the marker range are evaluated. Thereby, you can exclude any dead cells, and evaluate only the living cells for another property.

The gating direction defines the reference histogram:

- Gating from *blue to red* uses the blue histogram to define the subset by a marker (*Apoptosis* and *Antibody Staining* assays).



- Gating from *red to blue* uses the red histogram to define the subset by a marker (*GFP* assay).

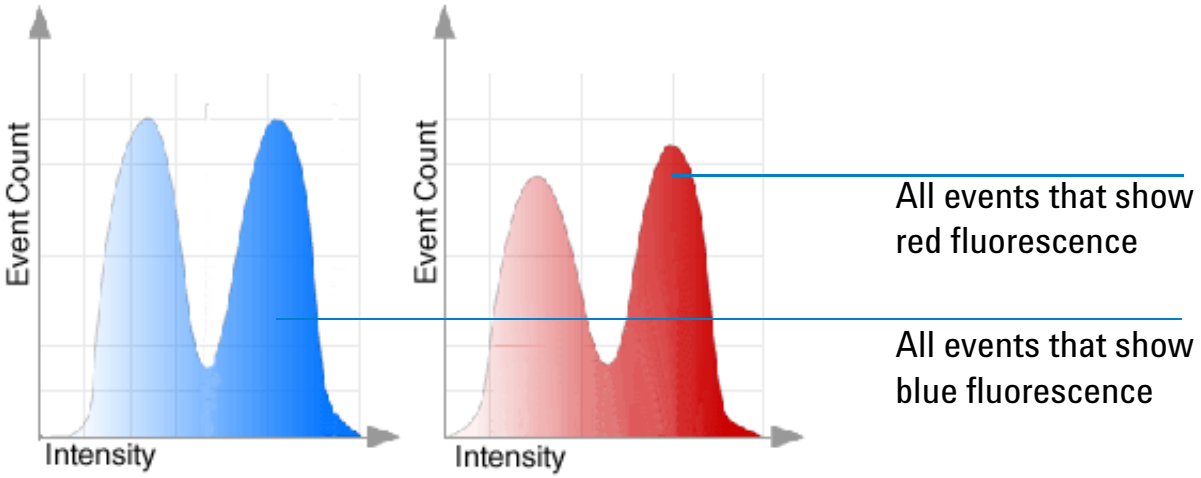


NOTE

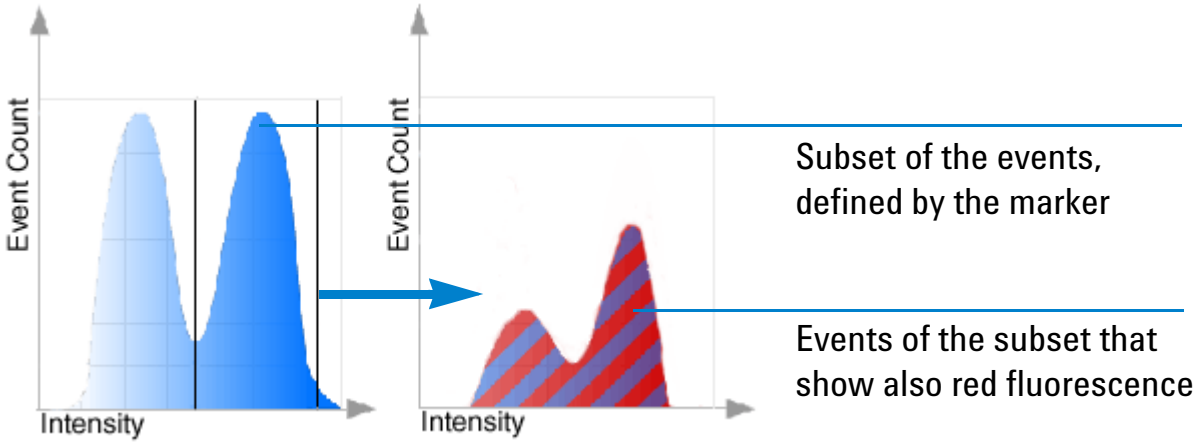
Predefined assays have a fixed gating direction, while assays of type *Generic* have a variable gating direction.

The following figures illustrate gating from blue to red.

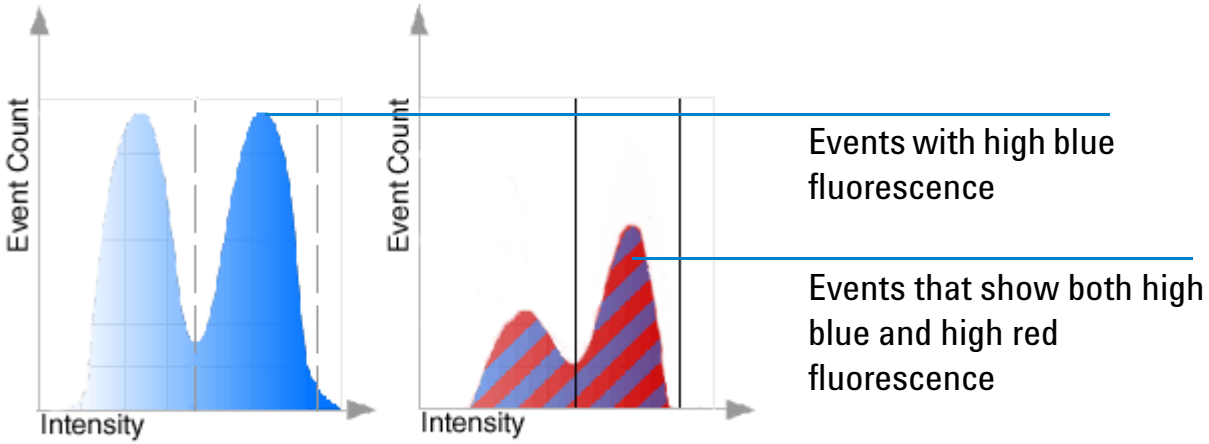
The two histograms display all measured events in the blue histogram and in the red histogram without gating. In this case, you cannot see which cells fluoresce only in the blue and which fluoresce only in the red.



By setting a marker on the blue histogram, you can define the blue fluorescence range that must be met for a cell to be considered for the red histogram. You use the gating on the blue histogram to define a subset for the red histogram.



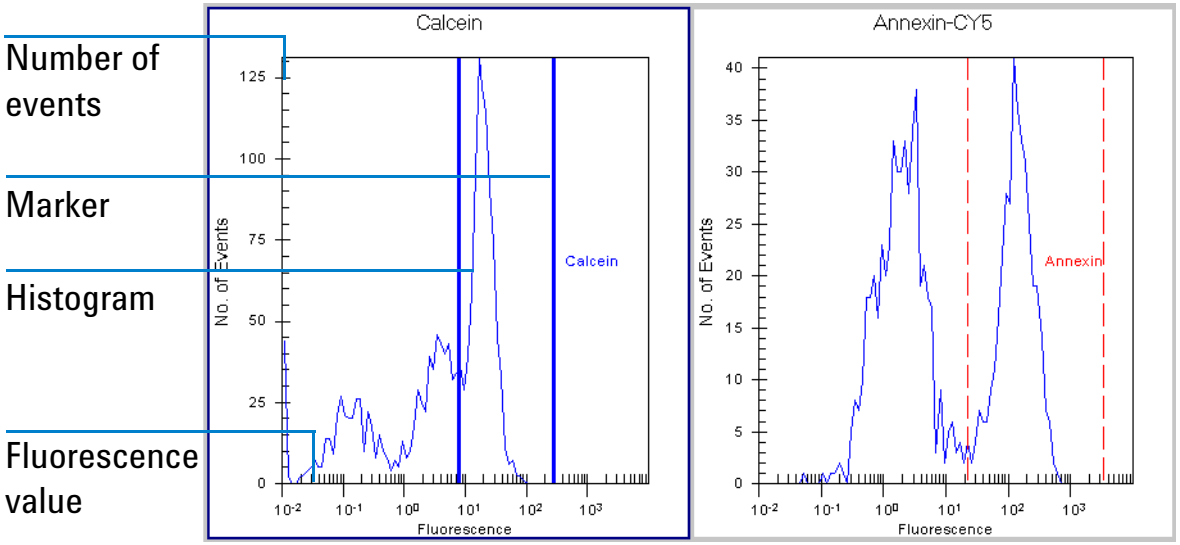
The red histogram displays now only cells with blue and red fluorescence within the marker. To evaluate this subset, you can set a marker in the red histogram. This second marker filters out all cells that do not have fluorescence in this range.



The result table (see also "Displaying the Results of Histogram Evaluations" on page 217) of the gated histogram (here the red one) shows the values numerically:

- The *% total* value shows the number of events that have both high blue and high red fluorescence, in relation to all measured events.
- The *% of gated* value shows the number of events that have high blue and high red fluorescence in relation to the blue (or red) events.

The following image shows two histograms with a gating direction from blue to red (left to right) of an apoptosis assay. The blue histogram shows calcein fluorescence, which indicates living or dead cells (high fluorescence value means living cells). The red histogram shows the subpopulation of living cells with annexin V fluorescence indicating apoptosis (high fluorescence value means the cell is apoptotic). As a result you can see a subset of living, apoptotic cells.



How to Insert a Marker in a Histogram

A marker is shown as two vertical lines that define a region of fluorescence values. It is used to select a subset of events according to this fluorescence region.

NOTE

You can insert markers only in *generic* assays.

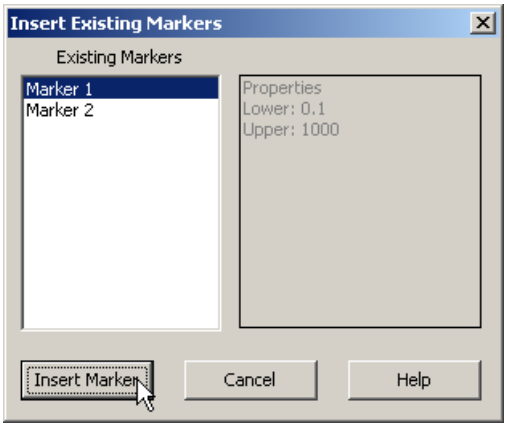
To add a new marker:

1 In the tool bar of the *Histogram* tab, click the *Insert Marker* button .

A marker is added to the selected histogram window.

To insert an existing marker:

1 Click the *Insert Existing Marker* button to open the *Insert Existing Markers* window.



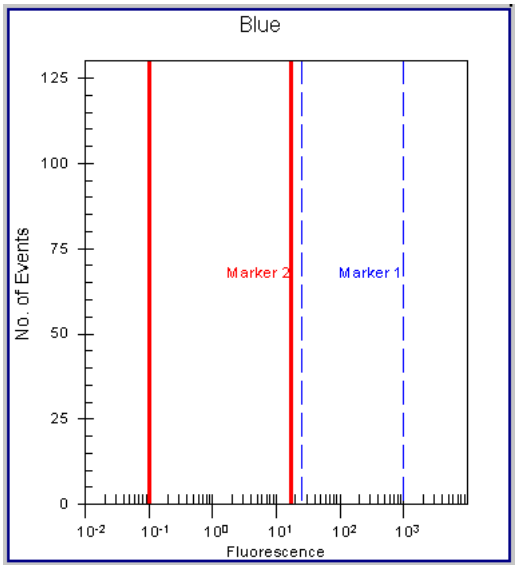
2 Select a marker in the list and click *Insert Marker*.

The marker lines are added at the defined positions. The label identifies the marker.

You can remove markers that you do not need any more:

1 Click on one of the vertical lines in the histogram to select the marker.

The lines of the selected marker are displayed bold.



TIP

You can also click the corresponding row in the result table to select the marker.

2 Click the *Delete Marker* button to remove the marker.

How to Configure Markers

You can change the color, name, and the upper and lower limits of the marker:

1 Double-click the desired marker.

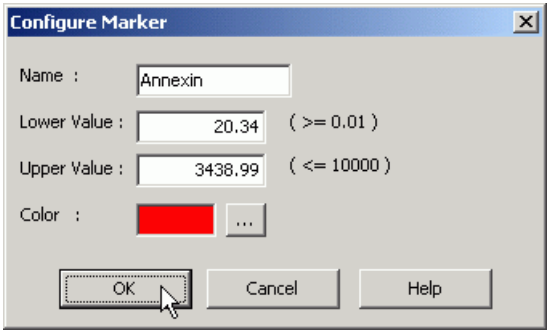
– OR –

Right-click the corresponding row in the result table and select *Configure Marker...* from the context menu.

– OR –

Select the marker and click the *Configure Marker* button  in the toolbar.

The *Configure Marker* dialog box appears.



2 Enter a name for the marker, for example, the used dye (it is advisable to use names that identify the marker).

3 Enter a *Lower Value* (left vertical line).

4 Enter an *Upper Value* (right vertical line).

NOTE

The lower and upper values must be within the range of 0.01 – 10000 relative fluorescence units.

5 Click the *Color* button  to open the *Color* dialog box and select a color.

6 Click *OK*.

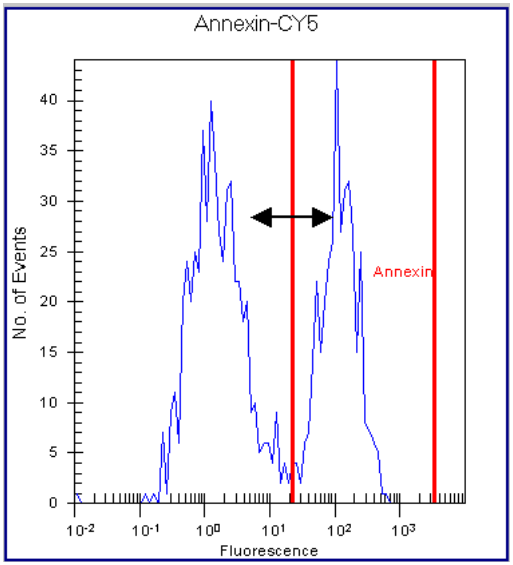
How to Move the Upper and Lower Limits of Markers

You can change the position of both marker lines by dragging them with the mouse:

1 Position the mouse pointer on a marker line.

The mouse pointer changes its shape to a hand.

2 Drag the line to the desired position.



3 Release the mouse button.

4 Repeat these steps for the other marker line, if necessary.

NOTE

You can change the marker limits also by entering fluorescence values in the *Configure Marker* dialog box (see "How to Configure Markers" on page 209).

How to Copy Markers to All Histograms

Once a marker is defined, you can copy it in the histograms of all samples (generic assays only):

1 Select the marker in the histogram or in the result table.

The *Insert the selected marker into all histograms* button  is now enabled.

2 Click this button.

The *Copy Marker* dialog box appears. This dialog box asks you whether or not you want to use the marker as reference.

3 Click *Yes* to use this marker as reference. The marker will be inserted in all other histograms of the blue or red channel. When the properties of this marker are changed, the changes will be applied to all samples.

– OR –

Click *No.* The marker will be inserted in all other histograms of the blue or red channel. When the properties of this marker are changed, the changes are only applied to the current sample.

How to Set the Gating Direction (Generic assay only)

You can use *one* marker to define the gating direction. In other words, you define whether red or blue fluorescence is used as a gate to define a subset in the other histogram. This also depends on the dyes that you have used for staining.

You can set both gating directions: either from the blue histogram to the red histogram or from red to blue.

To set the gating direction:

1 Select the marker in the red or blue histogram you want to use as a gate for the other histogram.

  The corresponding gating button in the tool bar is now enabled.

2 Click  or  to set the gating direction.

  – OR –

  Right-click the marker in the histogram or in the result table, and select *Gate in Red/Blue histogram* from the context menu.

The gating direction is displayed in the *Information Bar*.

If the gating direction is already set, you first have to remove the existing gating.

To remove gating:

1 Click the *Remove Gate* button  .

  The gating is removed and the corresponding gating button is enabled.

NOTE

To change the gating direction in non-generic assays, you first have to change the assay to generic. To achieve this, use the *Import Setpoints...* button on the *Assay Properties* tab (refer to "Importing Data" on page 256).

How to Overlay Histograms

You can compare samples by overlaying their gated histograms. This is useful, for example, if you want to see the progress of a reaction or if one sample is used as reference.

Overlaying histograms might also be helpful for adjusting the marker position. You can overlay all measured samples. Both red and blue histograms are overlaid.

NOTE

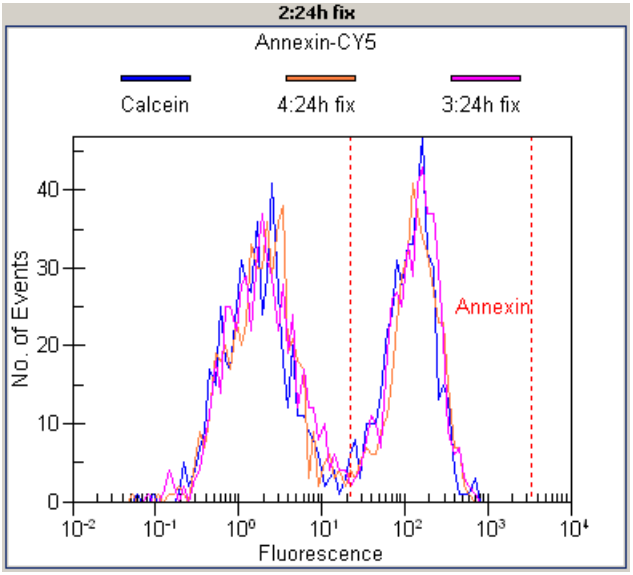
You can configure the colors of the overlaid histograms and adjust the scale graduation as described in "How to Set Signal Colors for Overlaid Histograms" on page 216.

To overlay histograms:

1. Select the main sample and display the *Histogram* tab.
2. Click the *Overlaid Samples* 

button to open a drop-down list.
3. Click the sample that you want to use as overlay.

   The histogram curve of the selected sample appears in the histogram view, the corresponding entry in the drop-down list is marked with a check, and a color legend appears above the graph.
4. Repeat steps 2 and 3 to overlay further histograms.



To overlay all samples:

1 Click the *Overlaid Samples* Overlaid Samples button to open a drop-down list.

2 Select *All Samples* to overlay the histogram curves of all samples.

To remove histograms from the overlay:

1 Select the sample that contains the overlaid histograms.

2 Click the *Overlaid Sample* button Overlaid Samples to open the drop-down list.

3 Click the sample that you want to be removed.

– OR –

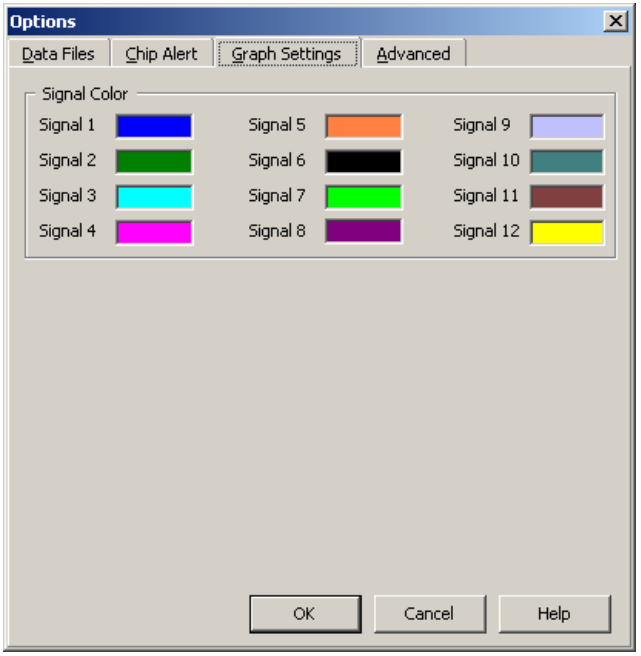
Click *No Overlay* to remove all overlaid curves from the histogram.

How to Set Signal Colors for Overlaid Histograms

You can use the *Graph Settings* tab in the *Options* dialog box to configure the signal colors (colors of curves in histograms):

1 Select *Tools* > *Options*.

2 Click the *Graph Settings* tab to bring it to the front.



To configure the signal color:

1 Click the colored square corresponding to the signal.

The *Color* dialog box appears.

2 Select a color for the signal and assign it by clicking *OK*.

Displaying the Results of Histogram Evaluations

The calculated results are displayed in result tables, one table below each histogram. Markers, gates, several statistical values, and the %-values of events are shown in the result tables.

		Marker	Min	Max	#Events	%Total	% of gated	Mean	StdDev	CV%	GMean
▶	●	All Events	0.02	51.42	1606	100	-	8.07	7.13	88.37	4.51
	●	Gated by Calcein	0.04	51.42	1418	88.30	-	8.46	7.32	86.47	4.72
	●	CD3-APC on all events	1.18	4085.88	1356	84.40	-	9.48	6.89	72.75	7.27
	●	CD3-APC on subset	1.18	4085.88	1195	74.40	84.30	9.96	7.02	70.44	7.73

Each marker you insert in the histogram gets its own row. Note that you can only use one marker for gating. The additional markers can be used to evaluate different parts of the histogram statistically.

If the option *Hide superset curve...* is disabled in the setpoint explorer (see "Assay Properties Tab" on page 418), two additional rows are displayed in the gated histogram's result table. The superset curve shows a histogram of all measured events; the gate is not considered.

You can export the table data for further evaluation in other applications. See "Exporting Data" on page 263.

NOTE

The layout of the result table can be configured (see "Configuring Tables" on page 282). Not all of the values listed below may therefore be visible.

The content of the result tables depends on the gating direction. The histogram that is used for gating can display the following results:

Marker	All events – this row shows the data for all measured events, for example, for all living and dead cells. The following rows show the data for the subset of cells defined by the inserted marker. If you use a predefined assay, the entry can be "Calcein on all events", for example.
Min	Minimum fluorescence value of the corresponding marker.
Max	Maximum fluorescence value of the corresponding marker.
Events	Number of events covered by the marker. For the histogram you use for gating, the number of all detected events is displayed in the row "All Events".
% total	% of selected events in relation to the total number of events. The row "All Events" shows 100%.
% of gated	% of events covered by the marker in the gated histogram. Shows no value for the gating histogram.
Mean	Mean fluorescence value of the events inside the marker.
StdDev	Standard deviation to the mean value.
%CV	Coefficient of variation.
GMean	Geometric mean.

The histogram that displays the gated data can show the following data:

Marker	All events – this row shows the data for all events that pass the gate. The following rows show the data for all events covered by the inserted marker. If you use a predefined assay, the entry can be "Annexin V on subset", for example.
Min	Minimum fluorescence value of the corresponding marker.
Max	Maximum fluorescence value of the corresponding marker.
Events	Number of events covered by the marker. For the histogram you use for gating, the number of all detected events is displayed in the row "All events".
% total	% of selected events in relation to the total number of events. The marker used for gating is 100%, the table of the gated histogram shows the value of the subset.
% of gated	% of the gated events in relation to the total number of events. These are the events that have passed the gate and are covered by the marker of the histogram, for example, by annexin V.
Mean	Mean fluorescence value of the events inside the marker
StdDev	Standard deviation to the mean value.
%CV	Coefficient of variation.
GMean	Geometric mean.

Using Dot Plots for Evaluation

On the *Dot Plot* tab, cells are displayed as dots, where their red fluorescence intensity is mapped on the Y axis and their blue fluorescence intensity is mapped on the X axis.

NOTE

The lower left region of the dot plot area may show no events, because of the threshold for event detection. Dots are only displayed if their fluorescence intensity exceeds a minimum limit. The limits are specified in the assay—separately for red and blue fluorescence.

To evaluate the dot plots, you can add *regions*. Regions are rectangles that can be changed in size and position until they include a specific event subset. As a result you get the number of cells included in the region related to the total number of cells.

NOTE

You can add/remove regions and gates only in *Generic* assays.

Additionally, you can insert a horizontal or a vertical gate for *one* region. This is useful for counting all cells that have fluorescence intensities within the horizontal or vertical borders of the region. In predefined assays, the vertical side of a region corresponds to the marker of the blue histogram, the horizontal side to the red one (see "Using Histograms for Evaluation" on page 199). The gate is always displayed and corresponds to the range of the marker that is used for gating. If you move a marker in a histogram, the region and gate are automatically updated. If you change a region or gate, the marker is also updated. Statistics are displayed in the result table below the dot plot.

How to Add Regions to Dot Plots (Generic Assay only)

You can draw regions in dot plots of generic assays. If there are regions already defined in other samples, you can copy these regions in the dot plot of the current sample.

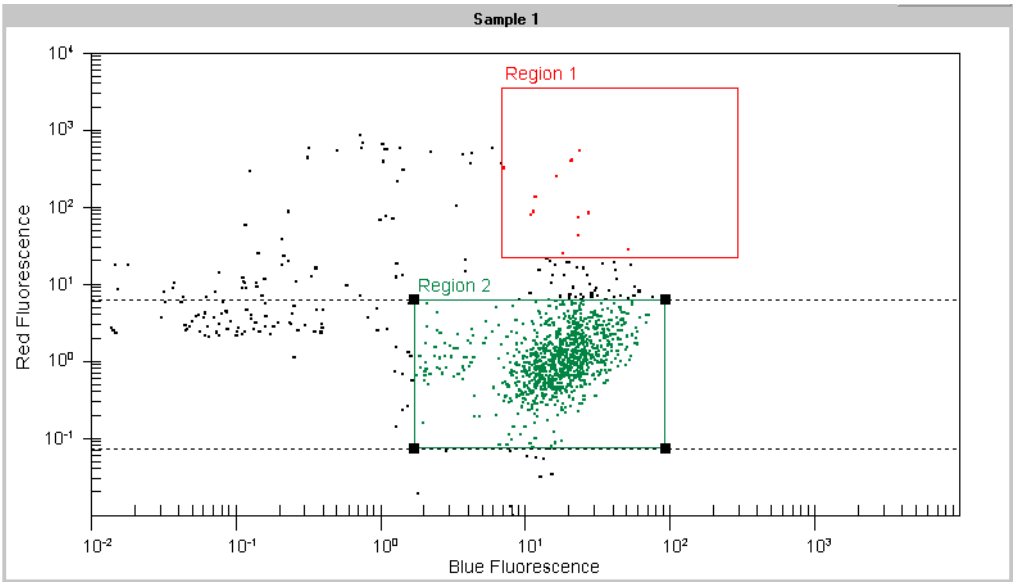
To draw a new region:

1 Click the *Insert Region* button  in the toolbar.

The mouse pointer changes its shape to a crosshair.

2 Draw a rectangle into the dot plot.

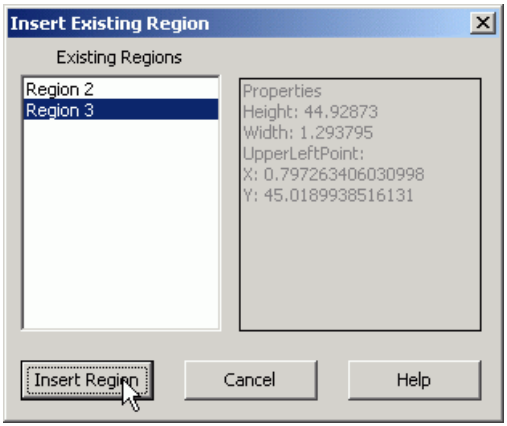
New regions are automatically named "Region x", where x is an auto-incremented number. By default, the border color of new regions is black. To make it easier to differentiate between regions, you can change their border color (see "How to Configure Regions" on page 223).



To insert an existing region:

1 Select the sample where you want to insert an existing region from another sample and click *Insert existing region* .

The *Insert Region* dialog box appears.



2 Select the region that you want to insert and click *Insert Region*.

The region is inserted at its predefined position.

To remove a region:

1 Click the region border to select the region that you want to remove.

The selected region is highlighted. and the *Delete Regions* button is enabled.

2 Click this button.

The region disappears from the dot plot.

How to Configure Regions

You can change the color of the region border, edit the region's name, and define the position and size of the region.

To configure a region:

1 Double-click the border of the region that you want to configure.

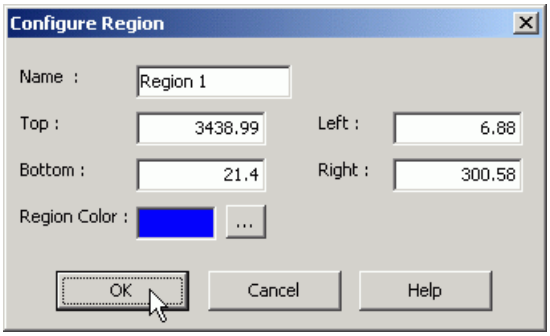
– OR –

Right-click the corresponding row in the result table and select *Configure Region...* from the context menu.

– OR –

Click the region border to select the region, and click the *Configure Region* button in the toolbar.

The *Configure Region* dialog box appears.



2 Enter a *Name* for the region.

It is advisable to use an easy-to-understand name.

3 Enter fluorescence values for the left, right, bottom, and top side of the rectangle to define position and size of the region.

   These values correspond to the upper and lower marker limits of the blue and red histograms.

4 Click the ... button next to the color square to open the *Color* dialog box, and select a color for the region border.

5 Click *OK*.

To color dots inside regions:

1 Click the *Color Dots* button  in the toolbar.

All dots inside the regions now have the same color as the region border. In case of overlapping regions, dots are colored with the color of the last added, re-positioned or resized region.

How to Change Position and Size of a Region

You can change the size and position of regions to restrict the number of included events. You can work graphically with the mouse or enter the values in the *Configure Region* dialog box.

To move a region:

1 While pressing the Shift key, click the region border, and drag the region to the new position.

2 Release the mouse button.

To change size and position with the mouse:

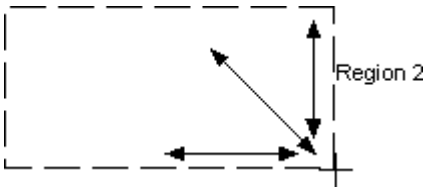
1 Click the region border to select the region.

2 Position the mouse pointer on any corner of the selected region.

The mouse pointer changes its shape to a double arrow.

3 Click and drag the border to the new size.

Upon moving, the mouse pointer changes its shape to a crosshair and the borders of the region appear as dashed lines.



4 Release the mouse button.

To change size and position numerically:

1 Double-click the region to open the *Configure Region* dialog box.

2 Enter fluorescence values for the left, right, bottom, and top side of the rectangle to define position and size of the region.

These values correspond to the upper and lower marker limits of the blue and red histograms.

3 Click *OK*.

How to Insert a Region in All Dot Plots

If you have defined a region for one sample, you can copy it to the other samples of the assay.

To insert a region in all dot plots:

1 Left-click the region border to select the region that you want to use as source.

The *Insert region into all dot plots* button  is enabled.

2 Click this button.

The *Copy Region* dialog box appears, which asks whether or not the source region should be used as reference. The region will be inserted in the dot plots of all other samples. When you change the properties of the region, all copies of the region will also be changed.

3 Click *Yes* to define the source region as reference.

– OR –

Click *No* to create new regions that are not "connected". The region will be inserted in the dot plots of all other samples. When the properties of the region are changed, the changes affect only the selected sample.

The region is copied to all samples of the assay.

How to Work with Gates in Dot Plots

You can insert gates only in *generic* assays. For predefined assays, the gate is already defined.

Before you can insert a gate, you have to draw a region (see "How to Add Regions to Dot Plots (Generic Assay only)" on page 221). If a gate is already set, you first have to remove the existing gate.

To add a gate to a region:

1 Left-click the region border to select the region to which you want to add the gate.

The gating buttons in the tool bar are enabled.

2 Click the *Horizontal Gate* button  or the *Vertical Gate* button  to set a gate along the horizontal or the vertical borders of the selected region.

In the result table, a value appears in the *% of gated* column.

If the gating direction is already set, you first have to remove the existing gate:

1 Left-click the region border to select the region.

If a gate already exists, the *Remove Gate* button  is enabled.

2 Click this button.

The gate is removed and the gating buttons are enabled.

Displaying the Results of Regions

The measurement results and calculations for regions are displayed in the result table below the dot plot. In predefined assays, only one region is available, while for generic assays, dot plots can have as many regions as you like.

The following values are displayed:

Region	The first region (*All Events*) always displays the values for all detected events. For each further region (see "How to Add Regions to Dot Plots (Generic Assay only)" on page 221), a row is added to the table.
XMean	Mean fluorescence values in x direction.
YMean	Mean fluorescence values in y direction.
#Events	Number of events for each region added to the dot plot.
% Total	Percentage of events for each region added to the dot plot.
% of gated	% of the gated events in relation to the total number of events.
StdDevX	Standard deviation to the mean fluorescence value in x direction.
StdDevY	Standard deviation to the mean fluorescence value in y direction.
CV%X	Coefficient of variation of the x values.
CV%Y	Coefficient of variation of the y values.
X GMean	Geometric mean of the x values.
Y GMean	Geometric mean of the y values.

Evaluating Antibody Staining, Apoptosis, and GFP Assays

With the 2100 expert software, several predefined assays are supplied. You should only use each assay for the specific experiment for which it was developed. For example, you have to use the read dye for detection of apoptosis (calcein and Cy5, for example):

- "Evaluating Antibody Staining Assays" on page 230.
- "Evaluating Apoptosis Assays" on page 235.
- "Evaluating GFP Assays" on page 240.

Evaluating Antibody Staining Assays

Antibody staining lets you measure protein expression on the surface or inside a cell by means of specific antibodies. Either the primary antibody itself is conjugated with a dye or you must use a labeled secondary antibody that recognizes the primary antibody. When you measure the fluorescence of the cells, you can determine the amount of cells with attached antibodies.

Typically, you can use a red dye such as APC (Allophycocyanin) or Cy5 to measure antibody presence.

You can use a blue dye like calcein to detect whether or not the cells are living, or like SYTO 16 to stain the nucleic acids of all cells. For detailed information, refer to the application note *Detecting Cell Surface and Intracellular Proteins with the Agilent 2100 Bioanalyzer by Antibody Staining*.

For a detailed description on how to evaluate the results using markers and regions, refer to "Using Histograms for Evaluation" on page 199, and to "Using Dot Plots for Evaluation" on page 220.

Gating direction

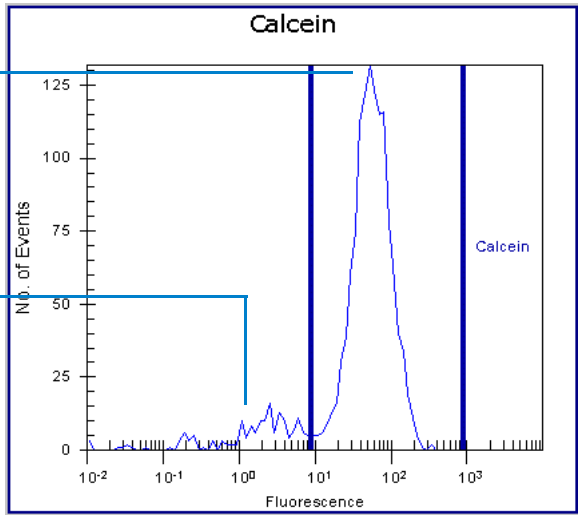
The gating direction is from blue fluorescence to red fluorescence. Depending on the dye you use, you should use all cells (nucleic acid dye) or only living cells (calcein living dyes) for gating.

Histogram Evaluation

The blue histogram is used for gating. High fluorescence in the blue histogram means that the cells are living (if a life-indicating dye is used). Low fluorescence means the cells are dead. If you use a nucleic acid dye, you cannot distinguish between living and dead cells, you can only count all measured cells. See the following image as an example:

High fluorescence value indicates living cells

Low fluorescence value indicates dead or non-healthy cells



The values are displayed in the result table below the blue histogram:

All measured events

All events in relation to the blue marker (here calcein)

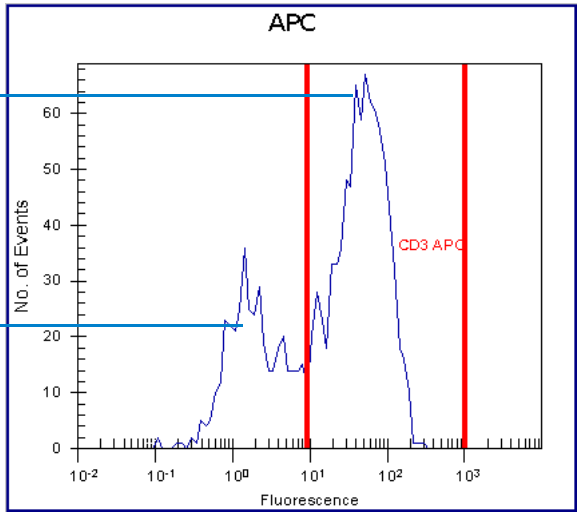
		Marker	# Events	% total	% of gated
▶	●	All Events	1565	100	-
	●	Calcein on all events	1436	91.8	-

Living cells related to all measured cells (high calcein fluorescence)

When using the calcein marker in the blue histogram for gating, only living cells are considered for building the histogram of the red dye. High red fluorescence values indicate living cells with bound antibodies, low red fluorescence values living cells without bound antibodies. See the following example.

High fluorescence values indicate binding of labeled antibodies

Low fluorescence values indicate low binding of labeled antibodies



The values are displayed in the result table below the red histogram:

Percentage of the living cells

Percentage of all cells with high red fluorescence selected by the red marker

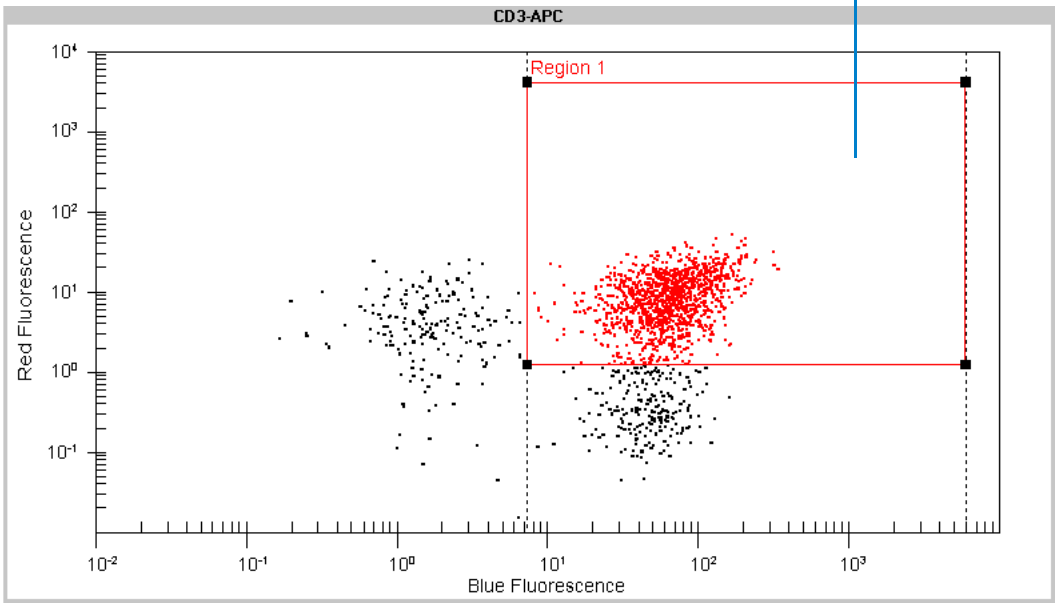
		Marker	# Events	% total	% of gated
▶	●	Gated by Calcein	1436	91.8	-
	●	CD3-APC on subset	25	1.6	1.7

Amount of living cells with high red fluorescence in relation to the amount of living cells

Dot plot evaluation

If you switch to the *Dot Plot* tab, one region is displayed in the dot plot. The red fluorescence values of the region are related to the marker in the red histogram, the blue fluorescence values to the marker in the blue histogram. As in the histogram evaluation, high blue fluorescence and high red fluorescence mean living cells with bound antibodies. See the following example.

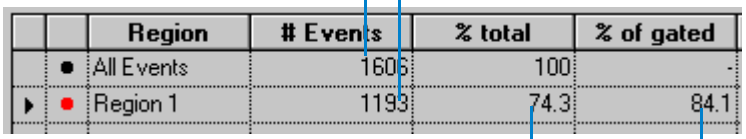
Cumulation of high blue and high red fluorescence indicates strong binding of labeled antibodies



The results of the dot plot evaluation are numerically displayed in the result table:

Events covered by the region

All measured events



		Region	# Events	% total	% of gated
	●	All Events	1606	100	-
▶	●	Region 1	1193	74.3	84.1

Amount of living cells in relation to all measured cells

Amount of living cells with high antibody binding in relation to all living cells

Evaluating Apoptosis Assays

The apoptosis assay can be used to examine how many apoptotic cells are within a living cell population. Dead (or necrotic) cells can be excluded from the evaluation.

For a detailed description on how to evaluate the results using histograms and regions, refer to "Using Histograms for Evaluation" on page 199 and "Using Dot Plots for Evaluation" on page 220.

Living or dead cells

In most cases, you want to know whether cells are dead or alive at a specific time. For this, you can use calcein-AM as living cell dye, for example. This dye accumulates in intact cells, whereas it will leak out of damaged cells. Once inside the cells, the colorless AM ester is cleaved by esterases, resulting in the formation of the highly fluorescent calcein. The number of events resulting from a calcein-related staining thus gives you the number of living cells in a sample. For detailed information, refer to the application note *Apoptosis Detection by Annexin V and Active Caspase 3 with the Agilent 2100 Bioanalyzer*.

Apoptotic cells

In apoptotic cells, phosphatidylserine is no longer confined to the inner leaflet of the plasma membrane bilayer. Phosphatidylserine becomes accessible on the outer surface of the cell membrane and can be bound with high affinity by the protein annexin V, which can be labeled with biotin or dyes such as Cy5.

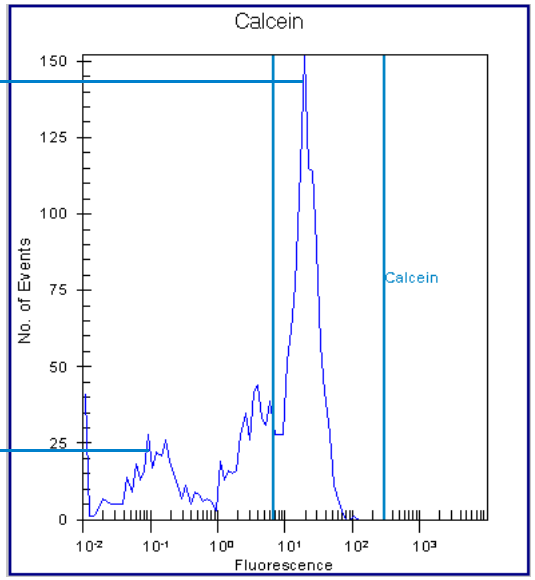
Gating direction

The gating direction is from blue fluorescence (living cells) to red fluorescence (annexin).

Histogram evaluation

The two histograms displaying the results of the assay are related to calcein (blue fluorescence) and annexin V (red fluorescence). High fluorescence values in the blue histogram indicate living cells, low values correspond to dead cells. See the following image as an example.

High fluorescence value indicates living cells



Low fluorescence value indicates dead cells

The values are displayed in the result table, each histogram has its own table:

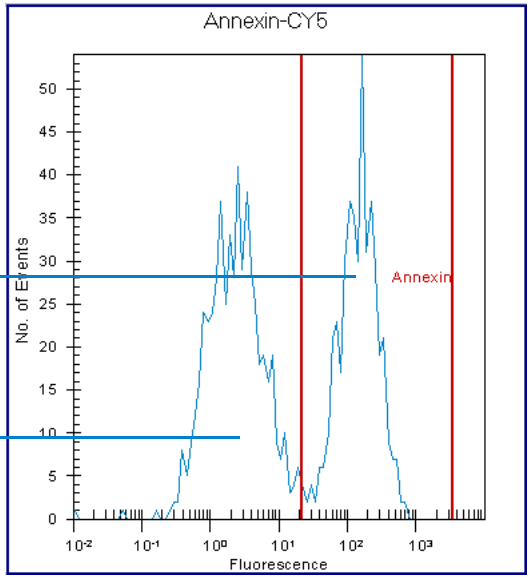
All measured events

All events in relation to the blue marker (here calcein)

	Marker	# Events	% total	% of gated
▶	All Events	1736	100	-
	Calcein on all events	998	57.5	-

Living cells in relation to all measured cells (high calcein fluorescence)

When using the calcein marker in the blue histogram for gating, only the living cells are considered for building the red histogram. High red fluorescence values indicate living, apoptotic cells, low red fluorescence values indicate living, non-apoptotic cells. See the following example.



High fluorescence value indicates living apoptotic cells

Low fluorescence value indicates living non-apoptotic cells

Amount of the living cells in relation to all measured cells

Percentage of all cells with high red fluorescence selected by the red marker

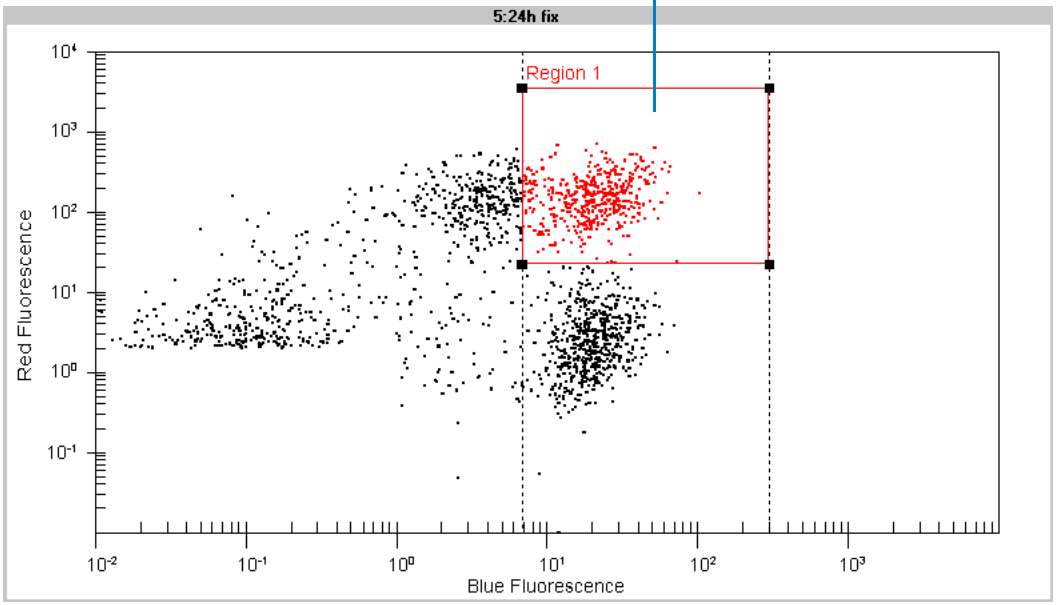
	Marker	# Events	% total	% of gated
●	Gated by Calcein	998	57.5	-
▶ ●	Annexin on subset	444	25.6	44.5

Amount of living cells with high red fluorescence in relation to the amount of living cells

Dot plot evaluation

If you switch to the *Dot Plot* tab, one region is displayed in the dot plot. The red fluorescence values of the region are related to the marker in the red histogram, the blue fluorescence values to the marker in the blue histogram. As in the histogram evaluation, high blue fluorescence and high red fluorescence represent living cells with annexin V binding. See the following example.

Cumulation of high blue and high red fluorescence indicate living apoptotic cells



The results of the dot plot evaluation are displayed in the result table.

All measured events

Events covered by the region

		Region	# Events	% total	% of gated
	●	All Events	1736	100	-
▶	●	Region 1	444	25.6	44.5

Amount of living cells with high red fluorescence in relation to the amount of all cells

Amount of living cells with high red fluorescence in relation to the amount of living cells

Evaluating GFP Assays

With GFP (Green Fluorescent Protein) assays, the fluorescent substance is not a dye, but a protein. Cells can be transfected with a target gene together with the GFP-producing gene. Transfected cells produce the fluorescent protein, which can be detected. The fluorescence shows you the success of the transfection experiment. For detailed information on GFP assays, refer to the application note *Monitoring transfection efficiency by green fluorescent protein (GFP) detection with the Agilent 2100 Bioanalyzer*.

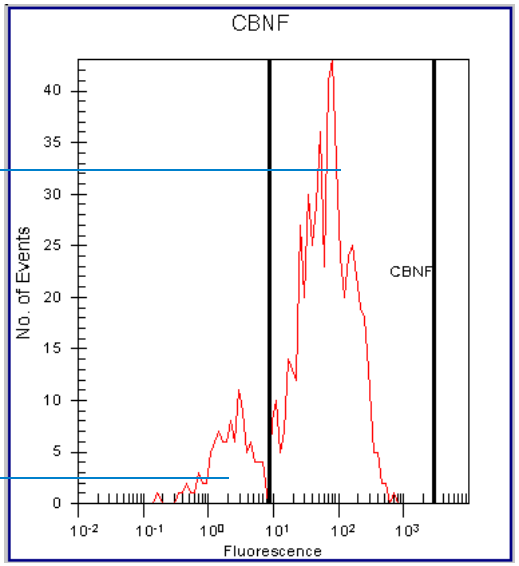
For a detailed description on how to evaluate the results using histograms and regions, refer to "Using Histograms for Evaluation" on page 199 and "Using Dot Plots for Evaluation" on page 220.

Gating direction

The GFP has a green fluorescence (absorption in the blue). Because the reference dye (CBNF) fluoresces in the red, the gating direction is from red to blue. CBNF stains living cells, so you can detect living, GFP-positive cells.

Histogram evaluation

The two histograms displaying the results of the assay are related to CBNF (red fluorescence) and GFP (blue fluorescence). High fluorescence values in the red histogram indicate a staining with CBNF, which is related to a high number of living cells. See the following image as example.



High fluorescence value indicates high amount of CBNF-stained cells

Low fluorescence value indicates dead cells

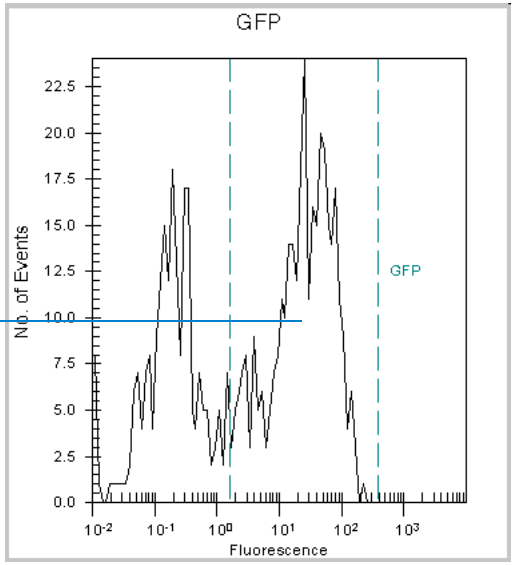
The values are displayed in the result table, each histogram has its own table.

All events related to the red marker (here CBNF)

All measured events

		Marker	% of gated	% total	# Events
▶	●	All Events	-	100	658
	●	CBNF on all events	-	84.7	557

After gating by using the red histogram, in the blue histogram only CBNF-stained cells are displayed. High blue fluorescence values indicate GFP-producing cells. See the following example.



High fluorescence value indicates GFP-producing cells

Amount of the CBNF containing cells in relation to all measured cells

Amount of all cells with high CBNF fluorescence selected by the red marker

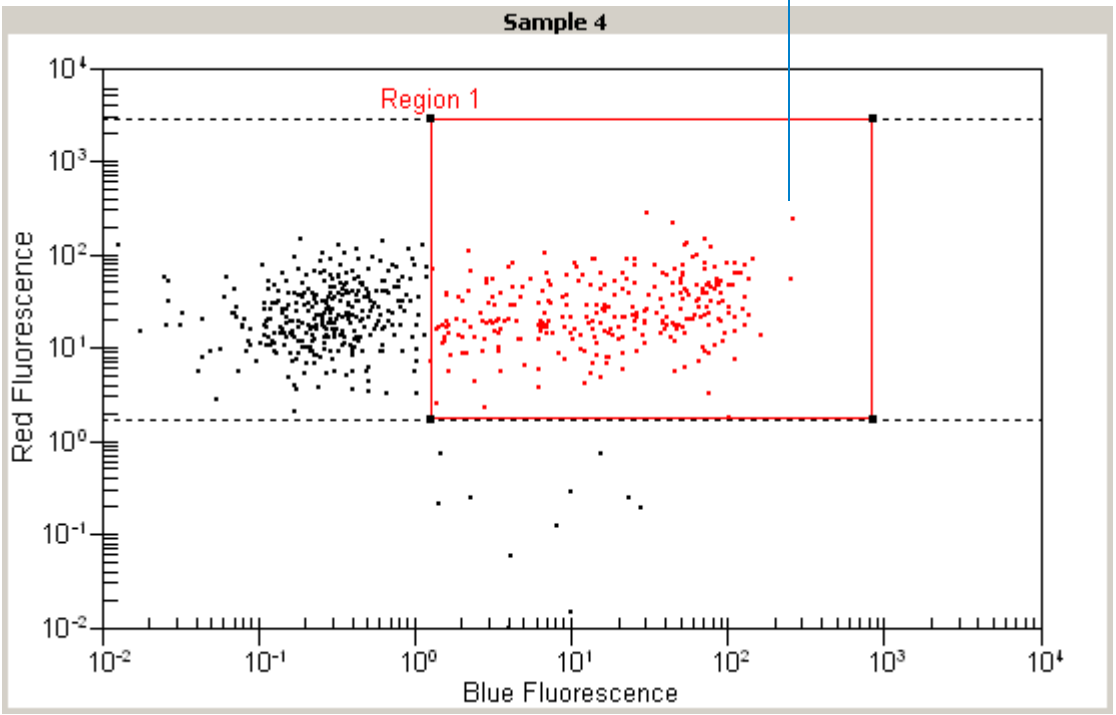
		Marker	% of gated	% total	# Events
▶	●	Gated by CBNF	-	84.7	557
	●	GFP on subset	60.9	51.5	339

Amount of cells with high GFP fluorescence in relation to the amount of CBNF stained cells

Dot plot evaluation

If you switch to the *Dot Plot* tab, one region is displayed in the dot plot. The red fluorescence values of the region are related to the marker in the red histogram, the blue fluorescence values to the marker in the blue histogram. Corresponding to the histogram evaluation, high blue fluorescence and high red fluorescence indicate living GFP-producing cells. See the following example.

Cumulation of high blue and high red fluorescence indicates living GFP expressing cells



The results of the dot plot evaluation are displayed in the result table.

Events covered by the region

All measured events

		Region	# Events	% total	% of gated
	●	All Events	1736	100	-
▶	●	Region 1	821	47.3	86.2

Amount of cells with high CBNF fluorescence and high GFP fluorescence in relation to all measured events

Amount of cells with high GFP fluorescence in relation to the amount of CBNF-stained cells.

Working with Chip and Assay Data

You can make efficient use of the chip and assay data generated by the 2100 expert software, if you know the following fundamentals and operating techniques:

2100 Expert Data Overview

The 2100 expert software manages data in the following different formats:

- Assay files (.xsy)
- Chip data files (.xad)
- Comparison files (.xac)
- Validation results files (.xvd)
- Diagnostics results files (. xdy)
- Result flagging rule files (.xml)

Assay files

Assay files (.xsy) contain the following information:

- Data analysis setpoints

  Setpoints are instrument commands, data acquisition parameters, and evaluation parameters, some of which you can modify.

- Assay information

  For example, assay type, title, and version.

- Chip and sample information

  These are chip comments, sample names and comments.

- Marker and region definitions (flow cytometric assays only)

  Included are associated parameters, such as the gating direction.

- Ladder table and peak table (electrophoretic assays only)
- Result flagging rules (electrophoretic assays only)

Chip data files

Chip data files (.xad) contain the following information:

- Measurement results

  After each chip run, the measurement results—also called "raw data"—are automatically saved in a new chip data file. Electrophoretic measurement results are pairs of migration time and fluorescence intensity values, flow cytometric measurements are triplets of migration time, red fluorescence, and blue fluorescence. Raw data is also stored in packet files (.pck).

- Base assay information

  Because a chip run is always based on an assay file, *all* information from the assay file becomes part of the chip data file.

- Run log

  Events occurring during the chip run, such as the start and end time, or any errors or problems are entered in a "run log", which is also saved in the chip data file.

- Evaluation information

  These are modifications you made during data evaluation, such as modified gel coloring, manually set markers, modified setpoints, modified result flagging rules, or definitions of new markers and regions.

Comparison files

You can compare the measurement results from different chip runs (electrophoretic chip data files of same assay class only) by collecting samples from different chip data files (.xad) and storing them in a comparison files (.xac). It is then possible to overlay electropherograms of these samples, for example.

Validation results files

Validation results files (.xvd) contain results of qualification tests regarding the bioanalyzer hardware and software. The files are stored in the "..\validation" subfolder of the 2100 expert installation directory. For each validation run, an .xvd file is generated.

Date and time of the validation run are included in the file name. Example: "Validation_25-09-2003_10-28-40.xvd".

Diagnostics results files

To ensure proper functioning of the bioanalyzer hardware you should run hardware diagnostics tests on a regular basis. The results of these hardware tests are stored in diagnostics results files (.xdy) in the "..\diagnosis" subfolder of the 2100 expert installation directory.

Result flagging rule files

You can export and import result flagging rules from other assay or chip data files. Result flagging rules are stored in .xml files.

Handling Assays

Predefined Assays

Predefined assays are provided with 2100 expert. They are meant and prepared for measurements using the available LabChip kits.

Predefined assays such as *Apoptosis* or *DNA 1000* are write-protected. Although you can open predefined .xsy files and edit some of their properties, you cannot save any changes under the original file name.

Custom Assays

However, you can derive your own assays from predefined assays as described in "How to Create a Custom Assay" on page 251.

The main benefit of custom assays is, that you have to do the following *only once* in the assay file, instead of doing it again and again in the chip data files:

- Modify assay setpoints (data analysis setpoints).
- Enter information on chip, samples, and study.

  For example, if your sample names are to be the same for a series of chip runs.
- Define rules for result flagging (electrophoretic assays only).
- Define markers and regions for evaluation (flow cytometric assay *Generic* only).

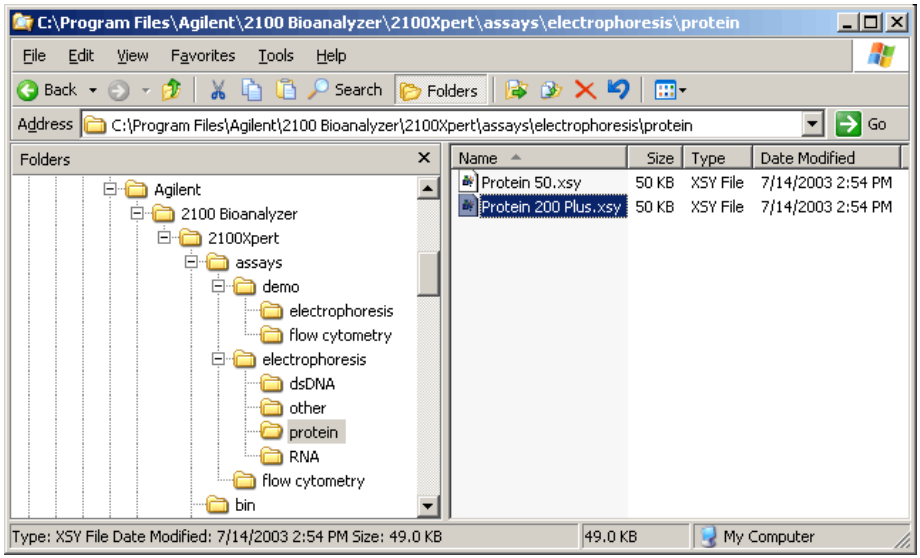
  For example, if you want to adjust marker positions and use these for future chip runs.

You can modify custom assays at any time. See "How to Modify a Custom Assay" on page 252.

TIP

If you just want to view the properties of a custom assay, you can open the assay file in read-only mode (see "Open" on page 514), ensuring you do not make accidental changes.

The *Assays* menu (see "Assays Menu" on page 337) is dynamically built from the structure and contents of the "..\assays" subdirectory of the 2100 expert installation folder.



TIP

You can add items to the *Assays* menu by placing assay (.xsy) files—your own assays, for example—in subdirectories of the "..\assays" directory.

How to Create a Custom Assay

To create a custom assay:

1 Switch to the *Data and Assay* context.

2 From the *Assays* menu, select an assay.

– OR –

Select *File* > *Open* ... and open an assay (.xsy) or chip data (.xad) file.

The file appears in the *Tree View Panel*.

NOTE

If you want to create a new flow cytometric assay with free gating direction or with more than one marker or region, open and modify the assay "Generic.xsy".

---

3 Modify the file by making changes on the following tabs:

- Modify assay setpoints on the *Assay Properties Tab*.
- Enter chip, sample, and study information on the *Chip Summary Tab*.
- For flow cytometric assays, define markers and regions on the *Histogram Tab (Single/Grid View)* and *Dot Plot Tab (Single/Grid View)*.
- For electrophoretic assays, define flagging rules on the *Result Flagging Tab*.

4 Select *File* > *Save As...* to open the *Save As* dialog box.

5 Under *Save as type*, select *(.xsy)*, and enter a name and location for the new assay.

6 Click *Save* to create the new assay.

How to Modify a Custom Assay

NOTE

You cannot save modifications to predefined assays such as *Apoptosis* or *DNA 1000*.

To modify a custom assay:

1. From the *File* menu select *Open* ....

   The *Open* dialog box appears.

2. Select an assay (.xsy) file and click *Open*.

   The assay appears in the *Tree View Panel* and the *Assay Properties Tab* is displayed.

3. Modify the assay by making changes on the following tabs:
   - Modify assay setpoints on the *Assay Properties Tab*.
   - Modify or enter additional chip, sample, and study information on the *Chip Summary Tab*.
   - For flow cytometric assays, define or modify markers and regions on the *Histogram Tab (Single/Grid View)* and *Dot Plot Tab (Single/Grid View)*.
   - For electrophoretic assays, define or modify flagging rules on the *Result Flagging Tab*.

4. From the *File* menu select *Save*.

Handling Chip Data

Chip data (.xad) files are automatically generated at the end of a chip run. The .xad files are given names that correspond to the choices you have made in the *Options* dialog box (see "How to Specify Data File Names and Directories" on page 294).

Modifying and saving chip data files

2100 expert allows to re-open chip data files, reanalyze them using different evaluation parameters and store the new results. You can save modifications either to the original file (*File* > *Save*) or under a new file (*File* > *Save As...*).

NOTE

Raw data acquired from the bioanalyzer is *not* changed—only evaluation and display of the results can be changed and saved.

If you alter the data shown in any way after it has been saved and try to exit the program or switch to a different context (to acquire new data, for example), a dialog box will appear asking whether or not you wish to save the changes (see "2100 Expert – Close" on page 499).

Opening chip data files as read-only

A chip data file can be opened as read-only; the *Title Bar* will show "(Read-Only)" at the end of the filename. The read-only file can be edited but may not be saved under the same name. If you attempt to save an edited read-only file, and error message will be displayed explaining that the file is a read-only file.

The benefit of opening chip data files as read-only is to prohibit you or other users from making changes that would alter the file in any way. Because the 2100 expert software allows you to open chip data files, modify data, and save them, you may prefer to ensure that the original parameters that were used to create the file are not altered.

Organizing, Retrieving, and Backing up 2100 Expert Data

As you begin to work with the 2100 expert software, it is good practice to organize your files. If you are not the only user of the bioanalyzer, creating a directory within which to save your files is recommended; having each person save files to their own directory will speed the process of finding a particular file when someone wishes to examine the data again. Even if only one person uses the 2100 expert software, it is still wise to review your files periodically, archive files you are no longer using but wish to save, and discard unneeded files.

Organizing 2100 Expert Data

Each user in your laboratory may want to specify a particular prefix that will easily differentiate their data files from any others. To do this, choose *Tools* > *Options...*, select the *Data Files* tab of the *Options* dialog box, select the *Prefix* check box, and edit the prefix string as you require (see also "Options – Data Files" on page 501. Note that you can also modify the file prefix also before you start a chip run, see "Instrument Tab (Single View)" on page 404. Additionally, you may specify that a new directory is created each day for storage of that day's runs. To do this, select the *Create Daily Subdirectories* check box at the bottom of the *Data Files* tab of the *Options* dialog box.

Backing up 2100 Expert Data

It is a good idea to archive files to a backup disk for safekeeping and/or to remove files from your hard disk periodically. Depending on the amount of hard disk space available to the 2100 expert software, you may need to clear space on your hard drive to ensure that you will have enough room to save upcoming chip run data.

Importing Data

2100 expert allows to reprocess assays and chip run files from the *Bio Sizing* and *Cell Fluorescence* applications. This is described in:

- "Importing Bioanalyzer Files" on page 257

When working with assay (.xsy) or chip data (.xad) files, you will enter specific information that you wish to reuse. Therefore, 2100 expert has the following import capabilities:

- "Importing Data Analysis Setpoints" on page 259
- "Importing Chip, Sample, and Study Information" on page 261

You can import result flagging rules definitions for result flagging into electrophoretic assay or chip data files:

- "Importing Result Flagging Rules" on page 262

Importing Bioanalyzer Files

NOTE

Bioanalyzer files imported and saved in the *2100 expert* software can no longer be opened with the original programs (*Bio Sizing* and *Cell Fluorescence*). However, these bioanalyzer files will not be overwritten, because *2100 expert* uses a different file extension.

To import *Bio Sizing* or *Cell Fluorescence* acquired files:

1 Switch to the *Data and Assay* context.

2 From the *File* menu select *Import...* to display the *Open* dialog box.

3 Select a file of type .asy, .csy, .cld, or .cad.

4 Click *Open*.

The imported file appears in the *Tree View Panel*, and

- if you imported a chip data file, the electropherogram grid view shows an overview of all samples; see "Electropherogram Tab (Single/Grid View)" on page 451.
- if you imported an assay file, the *Assay Properties* tab shows information about the assay; see "Assay Properties Tab" on page 418.

Upon importing, the file gets converted to a new 2100 expert file:

Bioanalyzer files of these formats	are converted to the following:
.asy (*Bio Sizing* assay)	.xsy (*2100 expert* assay)
.cld (*Bio Sizing* chip data)	.xad(*2100 expert* chip data)
.csy (*Cell Fluorescence* assay)	.xsy (*2100 expert* assay)
.cad (*Cell Fluorescence* chip data)	.xad (*2100 expert* chip data)

Note that the new 2100 expert file inherits the name from the old bioanalyzer file, but not the extension.

Example: If you import the file “Checkout Beads.asy” from the directory “P:\OldAssays”, a new file named “Checkout Beads.xsy” will be created in “P:\OldAssays”.

Importing Data Analysis Setpoints

You can import data analysis setpoints from other assay (.xsy) or chip data (.xad) files of the same type.

Note the following when importing:

- Electrophoresis files to be imported must be of the same assay type. This means that you cannot import setpoints from a DNA 1000 assay into a DNA 500 assay, for example.
- Flow cytometry files to be imported can be of any flow cytometric assay type, but the import will change the type of the current file to *Generic*.

To import data analysis setpoints:

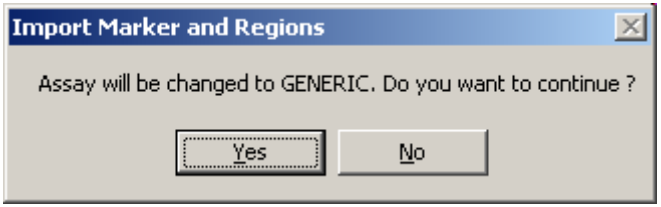
1 On the *Assay Properties* tab, click on *Import Setpoints...*.

2 The *Open* dialog box appears.

3 Select the file from which you want to import, and click *Open*.

NOTE

For flow cytometry files, the import will *delete* all existing markers and regions in the current file, and change the current assay to a *Generic* assay. A message box appears that prompts you to confirm this change.



4 Click *Yes*.

NOTE

Importing data analysis setpoints *overwrites* all current setpoint values.

- All files: the setpoint values are updated in the setpoint explorer (see "Assay Properties Tab" on page 418), and immediately applied to the measurement results (if any).
- Flow cytometry files: the new markers and regions are now available for evaluation, and calculations based on the new markers and regions are immediately done (see result tables in "Histogram Tab (Single/Grid View)" on page 460 and "Dot Plot Tab (Single/Grid View)" on page 468).

5 From the *File* menu, select *Save* to make the changes permanent.

Importing Chip, Sample, and Study Information

On the *Sample Information* and *Study Information* sub-tabs of the *Chip Summary* tab, you can enter names and comments regarding chip, samples, and study. The information you enter here may be very similar for further chip runs or other assays. Once you have entered the information, you can export it into a separate file (see "Exporting Chip Run Data" on page 264), which you can then import into other chip data (.xad) or assay (.xsy) files instead of typing it anew.

The import/export files can have the extension .txt or .csv, and have a fixed form, which differs for electrophoretic and flow cytometric assays.

To import chip, sample, and study information:

1. On the *Chip Summary* tab, click on *Import...*.
2. The *Import Sample Information* dialog box appears.
3. Select the file that contains the information you want to import, and click *Open*.

   The Sample Information and *Study Information* sub-tabs update to show the imported data.
4. From the *File* menu, select *Save* to make the changes permanent.

Importing Result Flagging Rules

You can import result flagging rules into electrophoretic assay (.xsy) or chip data (.xad) files. Result flagging rules are stored in .xml files (see "Exporting Result Flagging Rules" on page 274).

To import result flagging rules:

1. Select the *Data and Assay* context and load an electrophoretic assay or chip data file.
2. Switch to the *Result Flagging Tab*.
3. In the *Result Flagging* toolbar click on  .

   The *Load Rules* dialog box appears.
4. Select the .xml file that contains the set of result flagging rules, and click *Open*.

The imported rules appear in the rule list.

NOTE

The imported rules are not stored in the assay or chip data file until you save it.

Exporting Data

2100 expert allows you to export the results of your chip runs in a variety of formats. The exported data can be used for further evaluation with other applications, such as text processors, graphic tools, MS Excel®, or flow cytometry applications.

You can export chip run data either manually or automatically:

- "Exporting Chip Run Data" on page 264
- "Exporting Chip Run Data Automatically" on page 266

It is also possible to export only parts of your measurement results:

- "Exporting Tables" on page 267
- "Exporting Graphs" on page 269
- "Putting Graphs and Tables on the Clipboard" on page 271

Information that you have entered to document a chip run can be exported for reuse in future chip runs:

- "Exporting Chip, Sample, and Study Information" on page 273

From electrophoretic assay or chip data files, you can also export rule definitions for result flagging:

- "Exporting Result Flagging Rules" on page 274

Exporting Chip Run Data

To export chip run data:

1 Switch to the *Data and Assay* context.

2 In the *Tree View Panel*, select a chip data (.xad) .file or load a file.

3 From the *File* menu, select *Export...*

If you selected an electrophoretic chip data file, the *Export Options (Electrophoresis)* dialog box appears.

If you selected a flow cytometric chip data file, the *Export Options (Flow Cytometry)* dialog box appears.

4 Select the export categories, and specify a target directory.

Refer to "Export Options (Electrophoresis)" on page 519 or "Export Options (Flow Cytometry)" on page 516 for details on the export options.

NOTE

Keep in mind that exporting a chip data file can require up to 20 MB of disk space. In particular, exporting electropherograms and gel-like images as .tif or .bmp files may take up a lot of disk space.

---

5 Click *Export*.

Several system dialog boxes appear, one for each export category, allowing you to check and modify names and locations of the export files. Clicking the *Save* button in these dialog boxes finally starts the export.

TIP

Chip run data can be automatically exported every time a chip run has finished. Refer to “Exporting Chip Run Data Automatically” on page 266 for details.

Exporting Chip Run Data Automatically

NOTE

Keep in mind that exporting a chip data file can require up to 20 MB of disk space. In particular, exporting electropherograms and gel-like images as .tif or .bmp files may take up a lot of disk space.

To enable and configure automatic export:

1. From the *Tools* menu select *Options...*.

   The *Options* dialog box appears.

2. Select the *Advanced* tab.

3. Select *Auto Export* and click on the *Settings...* button next to this option.

   The *Auto Export* dialog box appears.

4. Specify export categories and target directories for electrophoretic and flow cytometric chip runs. Refer to "Auto Export" on page 523 for details on the export options.

5. Click *OK* to confirm the auto export settings.

6. Click *OK* to activate auto export.

From now on, chip run data is automatically exported every time a chip run has finished.

NOTE

If you stop a chip run, auto export does *not* take place.

Exporting Tables

You can export:

- Result tables, peak tables, fragment tables, and ladder tables as .csv files or .xls files.
- Log book tables as .html or .txt files.

To export a result table, peak table, fragment table, or ladder table:

1 On the *Assay Properties*, *Electropherogram*, *Gel*, *Histogram*, or *Dot Plot* tab, right-click the heading row of a table.

2 From the context menu, select *Export...*.

The *Save As* dialog box appears.

3 Enter a file name and choose the destination directory.

4 Select .csv or .xls as export file format.

5 Click *Save*.

TIP

Result tables can be automatically exported every time a chip run has finished. Refer to "Exporting Chip Run Data Automatically" on page 266 for details.

To export a log book table:

1 On the *Log Book* tab, right-click a table.

2 From the context menu, select *Export...*.

The *Export Data* dialog box appears.

3 Enter a file name and choose the destination directory.

4 Under *Export file format*, select *HTML file* for .html output, or *Tabbed text file* for .txt output.

5 Click *OK*.

Exporting Graphs

You can export the following graphs as individual graphic files:

- Gel-like image
- Electropherogram
- Electropherogram overview
- Histogram
- Dot plot
- Standard curve
- Calibration curve
- Raw signals (during chip run)

To export a graph:

1 Right-click the graph, and select Copy Graph to File from the context menu.

– OR –

Click on the  button in the toolbar.

The *Save As* dialog box appears.

2 Under *File name*, enter a name and choose the destination directory.

3 Under *Save as type*, select a graphic file format: .bmp, .jpg, .wmf, .tif or .gif.

4 Click *Save*.

The graph is written to the specified file.

Note the following:

- Histograms: only one histogram graph is exported, either the red or the blue histogram.
- Electropherograms: if the grid view is active, an overview image of the electropherograms (of *all* samples and the ladder) is exported.

TIP

Electropherograms, gel-like images, histograms, and dot plots can be automatically exported every time a chip run has finished. Refer to "Exporting Chip Run Data Automatically" on page 266 for details.

Putting Graphs and Tables on the Clipboard

You can put the following items on the clipboard:

- Gel-like image
- Electropherogram
- Electropherogram overview
- Histogram
- Dot plot
- Standard curve
- Calibration curve
- Raw signals (during chip run)

Copying a graph causes a device-independent bitmap to be placed on the clipboard.

You can put the following tables (or parts of the tables) on the clipboard:

- Result tables
- Peak tables
- Fragment tables
- Ladder tables
- Log book tables

Copying tables causes ASCII data to be placed on the clipboard.

To put a graph or table on the clipboard:

1 Right-click the graph or table (region).

2 From the context menu, select *Copy Gel/Copy Electropherogram* (graphs) or *Copy To Clipboard* (tables).

– OR –

Click the  button in the toolbar.

You can now switch to a word processing, spreadsheet, graphics, or other application, and paste the graph or table there.

Exporting Chip, Sample, and Study Information

On the *Sample Information* and *Study Information* sub-tabs of the *Chip Summary* tab, you can enter names and comments regarding chip, samples, and study. The information you enter here may be very similar for further chip runs or other assays. Once you have entered the information. you can export it into a separate file, which you can then import into other chip data (.xad) or assay (.xsy) files instead of typing it anew.

The import/export files can have the extension .txt or .csv, and have a fixed form, which differs for electrophoretic and flow cytometric assays.

To export chip, sample, and study information to a file:

1 On the *Chip Summary* tab, click on *Export...*.

  The *Export Sample Information* dialog box appears.

2 Specify a file name and location for the file to which you want to export.

3 Click *Save*.

Exporting Result Flagging Rules

You can export result flagging rules for reuse in other electrophoretic assay (.xsy) or chip data (.xad) files (see "Importing Result Flagging Rules" on page 262). Result flagging rules are stored in .xml files.

To export result flagging rules:

1. Select the *Data and Assay* context and load an assay or chip data file.

   – OR –

   In the *Tree View Panel*, select the electrophoretic assay or chip data file that contains the result flagging rules.

2. Select the *Result Flagging Tab*.

3. In the *Result Flagging* toolbar click on  .

   The *Save Rule Definitions* dialog box appears.

4. Browse for a folder where you want to store the rules, and specify a name for the .xml file.

5. Click *Save*.

Printing Reports

For documentation and presentation purposes, you can print reports for assay (.xsy), chip data (.xad), validation results (.xvd), and comparison (.xac) files.

You can print all reports manually, see "How to Print a Chip Run Report" on page 276. When printing manually, a preview function allows you to view the printout before starting the print job.

The 2100 expert program can also be set to print customized chip run reports automatically at the end of the run. These reports can be set up to contain different information (settings for the manual and automatic print functions are maintained separately). See "How to Turn on and Configure Automatic Printing of Chip Run Reports" on page 280 for more information.

TIP

Beside sending reports to a printer, you can also create .pdf and .html files.

How to Print a Chip Run Report

The following information can be included in a chip run report:

- You can always include:
  - Assay summary—general data about the assay, and sample information.
  - Assay details—complete list of data analysis setpoints.
- For chip data files (.xad) you can include:
  - Chip run summary—general information on the chip run.
- For *flow cytometric* chip data files (.xad) you can include:
  - Histogram summary—all histograms shown in an overview.
  - Histogram statistics—all statistical data of the blue and red histograms' result tables.
  - Dot plot summary—all regions shown in an overview.
  - Dot Plot statistics—all statistical data of the result table.
- For *electrophoretic* chip data files (.xad), depending on the assay type you can include: you can include:
  - Electropherograms
  - Gel-like image
  - Result tables
  - Standard curve
  - Calibration curve

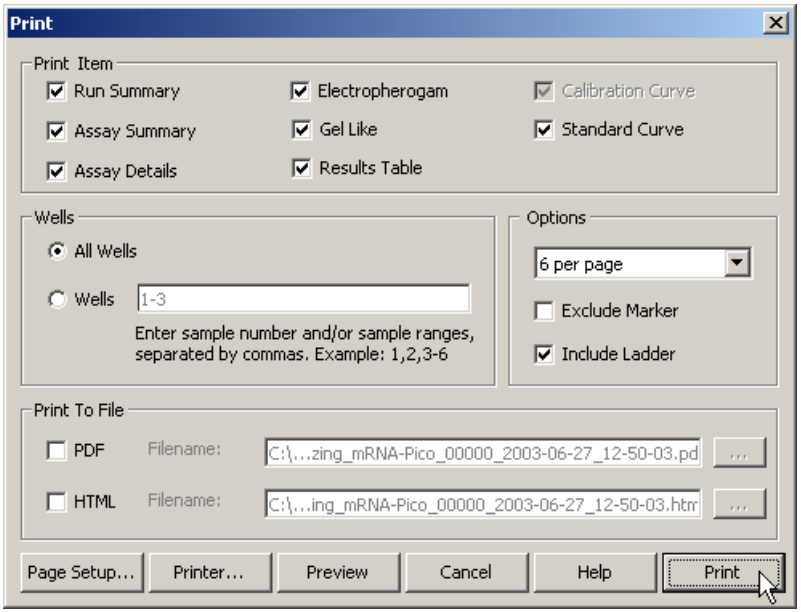
To print a report:

1 Switch to the *Data and Assay* context.

2 In the *Tree View Panel* select a file, either an assay (.xsy) or a chip data (.xad) file.

3 From the *File* menu select *Print...*.

Depending on the file type different dialog boxes appear.



4 Under *Print Item* and *Samples*, select what you want to print.

You can print just one or any combination of the items shown in this section by enabling the desired options. Note that you can print data from a range of wells (which do not have to be consecutive): choose *Samples* from the *Samples* section and then enter the

wells you would like to print, following the instructions as shown on the dialog box. Also refer to "Print (Electrophoresis)" on page 531/"Print (Flow Cytometry)" on page 528 for details.

NOTE

Your selections here are separate from the *Auto Print* selections (they do not affect each other). Both are used by default the next time you print (even after restarting the program).

---

5 Use the *Page Setup...* and *Printer...* buttons to access system dialog boxes, allowing you to select a printer, and specify the print medium and page layout.

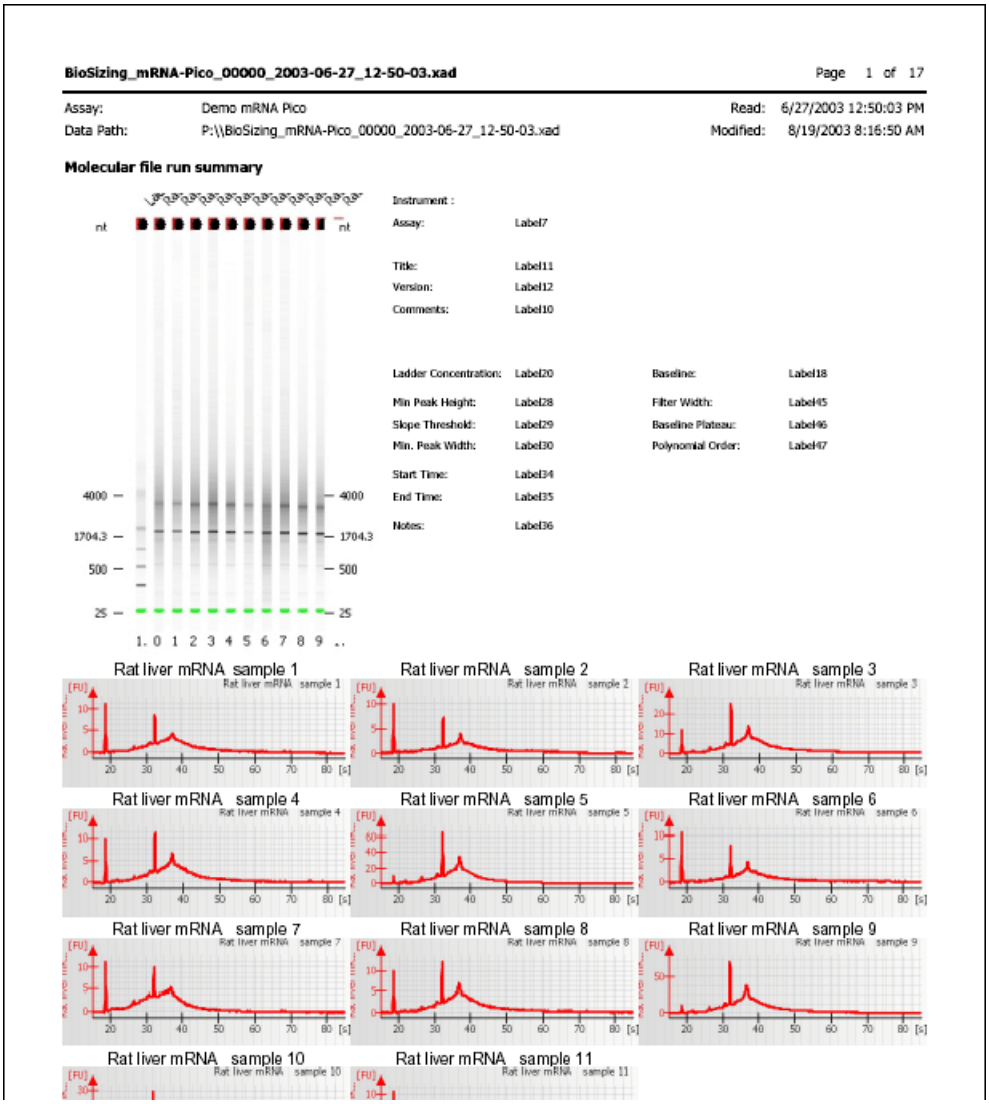
6 Optionally, click on *Preview* to open the *Report Preview* dialog box, allowing you to check the page layout before you start printing. See "Report Preview" on page 557.

7 Under *Save To File*, make sure that both the *PDF* and *HTML* options are cleared, and click *Print* to send the printout to the printer.

– OR –

Select *PDF* and/or *HTML*, and click *Save* to send the printout to file(s).

The following example shows the “Run Summary” part of an RNA chip run report.



How to Turn on and Configure Automatic Printing of Chip Run Reports

A report can be printed (or generated) automatically at the end of each chip run.

To enable and configure automatic printing:

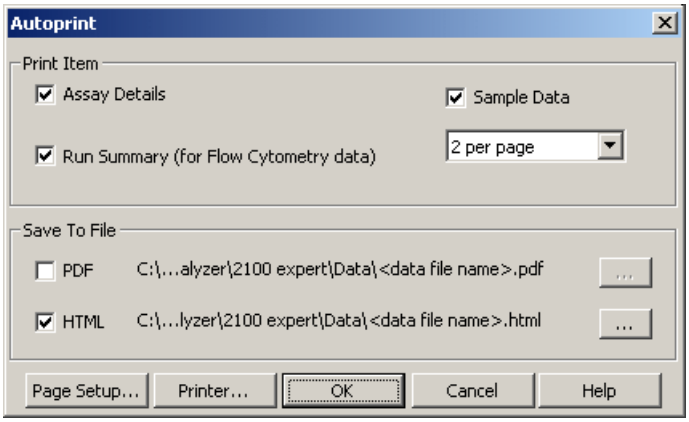
1 From the *Tools* menu select *Options...*.

The *Options* dialog box appears.

2 Click the *Advanced* tab to bring it to the front.

3 Select the *Auto Print* check box and click the *Settings...* button next to this check box.

The *Auto Print* dialog box appears.



NOTE

The *Auto Print* settings are independent from those made via the *Print...* command of the *File* menu (see "Print (Electrophoresis)" on page 531 and "Print (Flow Cytometry)" on page 528).

4 Adjust the settings:

- Under *Print Item*, select the options that should be contained in the report.
- Under *Save To File*, you can redirect the automatic printouts to .pdf and .html files.

  Note that no print output is generated if you select the *PDF* and/or *HTML* option.
- Using the *Page Setup...* and *Printer...* buttons, you can access system dialog boxes, allowing you to select a printer for the automatic print, and specify the print medium and page layout.

For details on the available options refer to "Auto Print" on page 541.

5 Click *OK* to confirm the automatic print settings.

6 Click *OK* to enable automatic printing and to close the *Options* dialog box.

Configuring Tables

2100 expert uses various tables to present data:

- Result tables
- Peak tables
- Fragment tables
- Log book tables

In some cases, you might want to reorganize the way the data is presented. To do so, you can hide or show columns, change the column sequence, and adapt the table height.

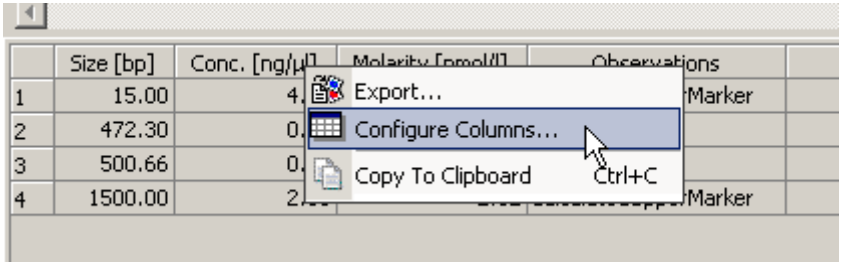
	Area	FragmentSize	Concentration	Molarity	Observations	
1	50.29	15.00	4.20	424.24	CalculatedLowerMarker	
2	29.49	472.30	0.76	2.45		
3	13.40	500.66	0.34	1.03		
4	84.13	1500.00	2.10	2.12	CalculatedUpperMarker	

The following example demonstrates how to add the migration time to the *Peak Table*.

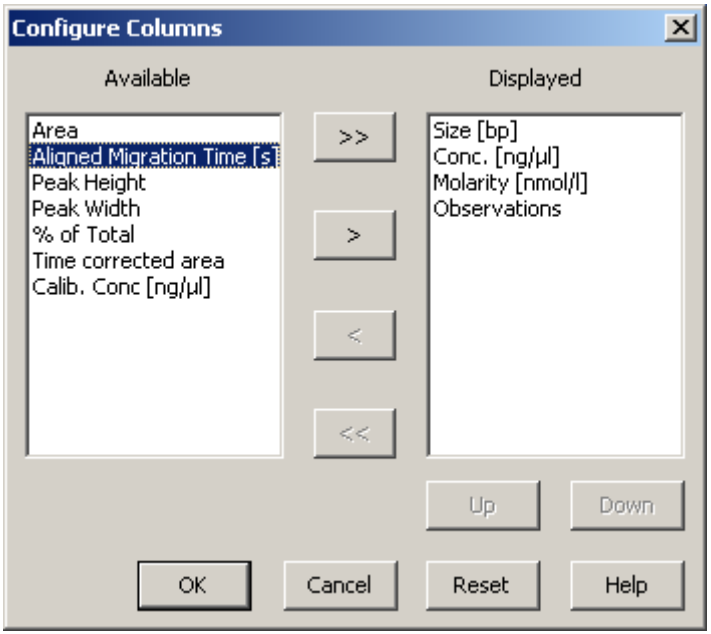
Showing and Hiding Columns

To add the *Aligned Migration Time* column to the table:

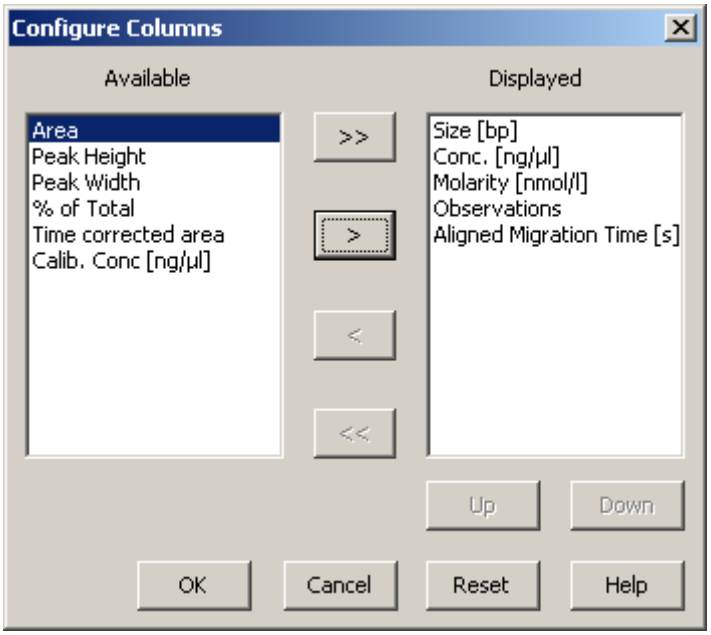
1 Right-click the heading row of the table.



2 From the context menu, select *Configure Columns...* to display the *Configure Columns* dialog box.



3 Move *Aligned Migration Time* from the *Available* list to the *Displayed* list by clicking the single-arrow button.



4 Click *OK*.

A new column *Aligned Migration Time* is inserted in the table:

		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations	Aligned Migration Time [s]
1		15	4.20	424.2	Lower Marker	41.00
2		22	1.55	105.6		42.72
3		55	1.23	33.8		47.44
4		104	3.90	56.7		53.04

To hide columns, remove their names from the *Displayed* list in the *Configure Columns* dialog box.

TIP

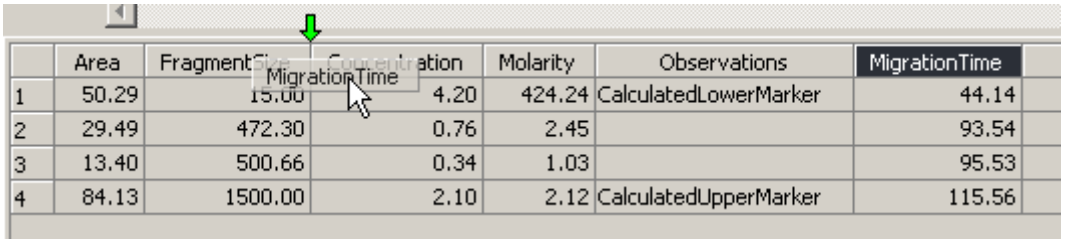
You can set the column sequence also using the *Up* and *Down* buttons in the *Configure Columns* dialog box.

To change the column sequence of a table:

1 Position the mouse pointer on a column header.

2 Click and hold the left mouse button, and drag the header cell to the desired position.

While dragging, a green arrow indicates the target position.



	Area	FragmentSize	Concentration	Molarity	Observations	MigrationTime
1	50.29	15.00	4.20	424.24	CalculatedLowerMarker	44.14
2	29.49	472.30	0.76	2.45		93.54
3	13.40	500.66	0.34	1.03		95.53
4	84.13	1500.00	2.10	2.12	CalculatedUpperMarker	115.56

3 Release the mouse button.

The column has moved to its new position:

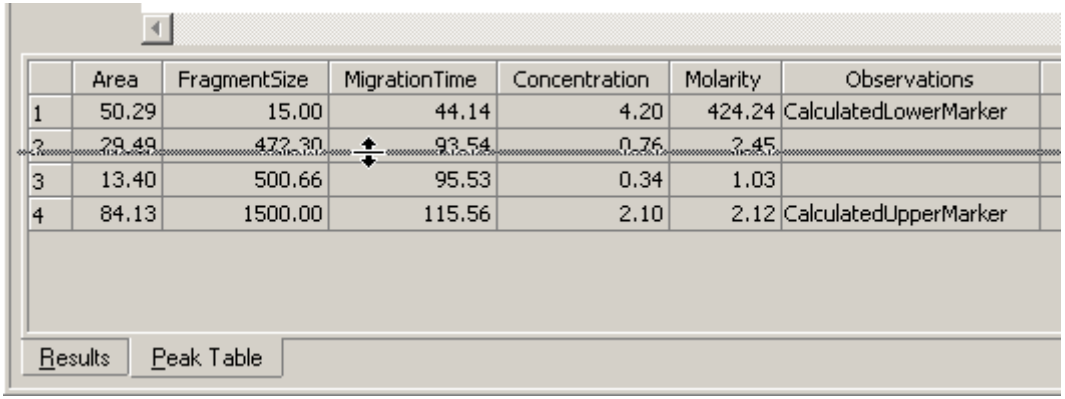
	Area	FragmentSize	MigrationTime	Concentration	Molarity	Observations
1	50.29	15.00	44.14	4.20	424.24	CalculatedLowerMarker
2	29.49	472.30	93.54	0.76	2.45	
3	13.40	500.66	95.53	0.34	1.03	
4	84.13	1500.00	115.56	2.10	2.12	CalculatedUpperMarker

Changing the Table Height

To increase or reduce the table height:

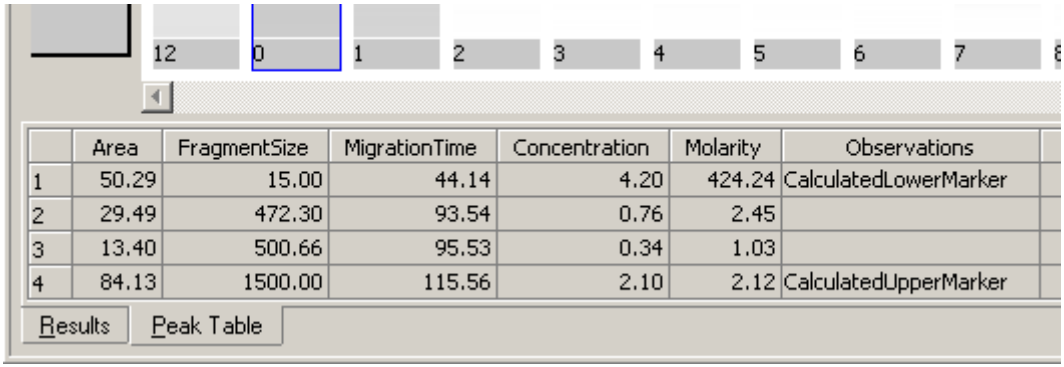
1 Position the mouse pointer above the heading row of the table until the cursor's shape changes to a double arrow.

2 Click and hold the left mouse button and drag up or down.



3 Release the mouse button.

In this example, the *Peak Table* freed screen space for the gel-like image above the table:



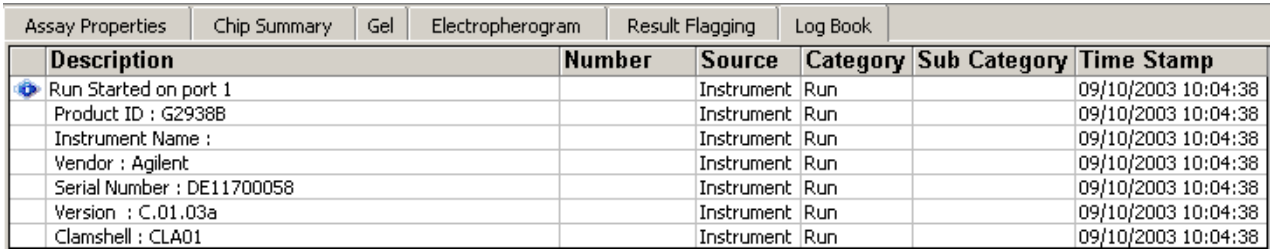
Reading the Log Books

2100 expert provides two log books:

Run Log

The run log table contains events generated during a chip run, including the start and end time and any errors or problems that occurred during the run.

You can view the run log table in the *Data and Assay* context by selecting the *Log Book* tab.



Description	Number	Source	Category	Sub Category	Time Stamp
Run Started on port 1		Instrument	Run		09/10/2003 10:04:38
Product ID : G2938B		Instrument	Run		09/10/2003 10:04:38
Instrument Name :		Instrument	Run		09/10/2003 10:04:38
Vendor : Agilent		Instrument	Run		09/10/2003 10:04:38
Serial Number : DE11700058		Instrument	Run		09/10/2003 10:04:38
Version : C.01.03a		Instrument	Run		09/10/2003 10:04:38
Clamshell : CLA01		Instrument	Run		09/10/2003 10:04:38

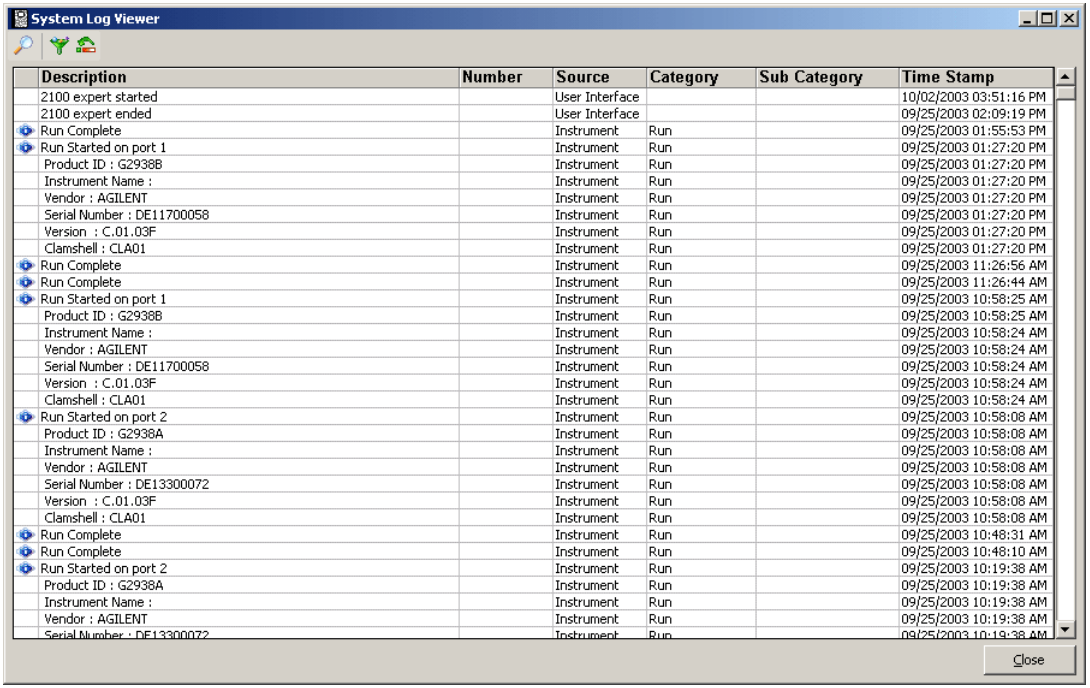
Please refer to "Log Book Tab" on page 481 for more details on the run log table.

The run log is saved in the chip data (.xad) file and cannot be cleared.

System Log

The system log table includes start-up and shut-down events of the 2100 expert software, and, for example, errors or problems with connected bioanalyzers.

You can view the system log table by selecting *System Log* from the *Tools* menu.



Please refer to “System Log Viewer” on page 561 for more details on the system log table.

The system log is saved in the file “SystemLogBook.log” located in the “..\log” subdirectory. The system log file can grow very large and fill up a lot of worthy disk space. To control the disk space occupied by the system log file, read “How to Use the Advanced Settings” on page 299.

How to Change the Display of the Log Books

To sort a log book table:

1 Right-click any table column.

2 From the context menu, select *Sort* to sort the table by the current column (ascending).

– OR –

From the context menu, select *Sort by Event to sort the table* by the *Category* column (ascending).

To filter a log book table:

1 In the *Log Book* toolbar, click on *Filter*  .

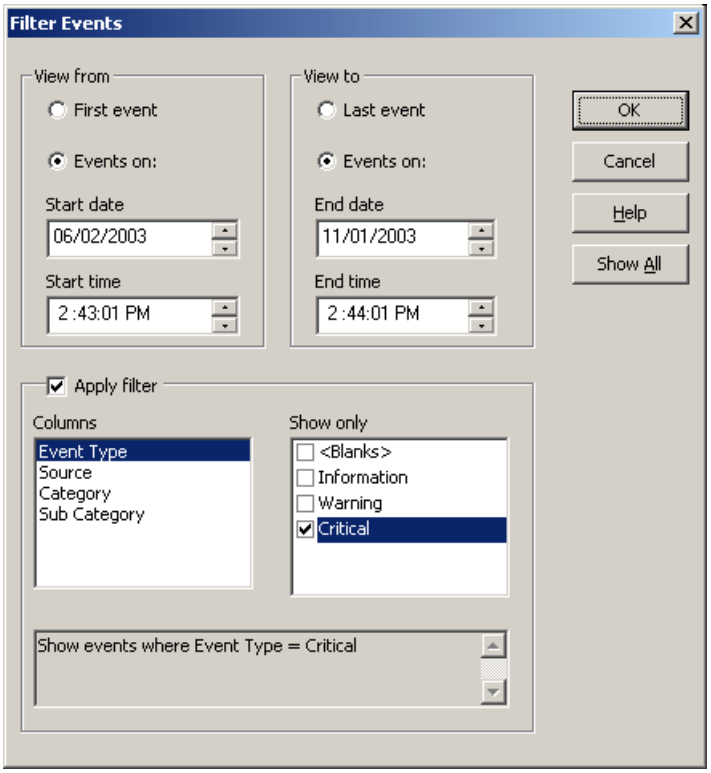
The *Filter Events* dialog box appears.

2 To display only events from a specified period of time, you can define a *Start Date/Time* and an *End Date/Time*.

3 To define a filter, select the *Apply filter* check box.

Now you can define filter rules for the *Event Type*, *Source*, *Category*, and *Sub Category* columns. Make a selection in the *Columns* list, and include values by selecting check boxes in the *Show only* list.

The filter definition in the following example would exclude all events from the log book table with an *Event Type* other than *Critical*.



For more information refer to "Log Book Tab" on page 481.

4 Click *OK* to apply the filter to the log book table.

To remove the filter from a log book table:

1 In the *Log Book* toolbar, click on *Reset* .

TIP

You can hide/show any of the log table columns, and re-sort the columns by right-clicking the table and selecting *Columns...* from the context menu. See "Configuring Tables" on page 282.

How to Search the Log Book

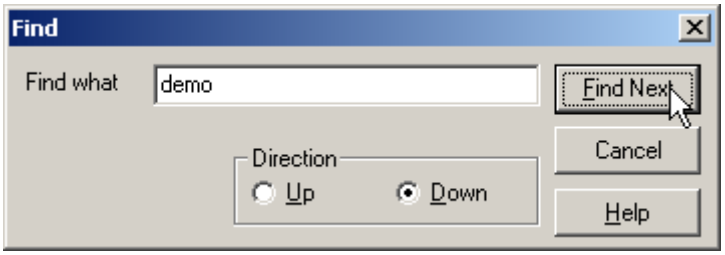
You can search both the *Run Log* and the *System Log* for any string. Using the example of the *Run Log* this is demonstrated below.

To search the *Log Book*:

1 In the *Log Book* toolbar, click on *Find* .

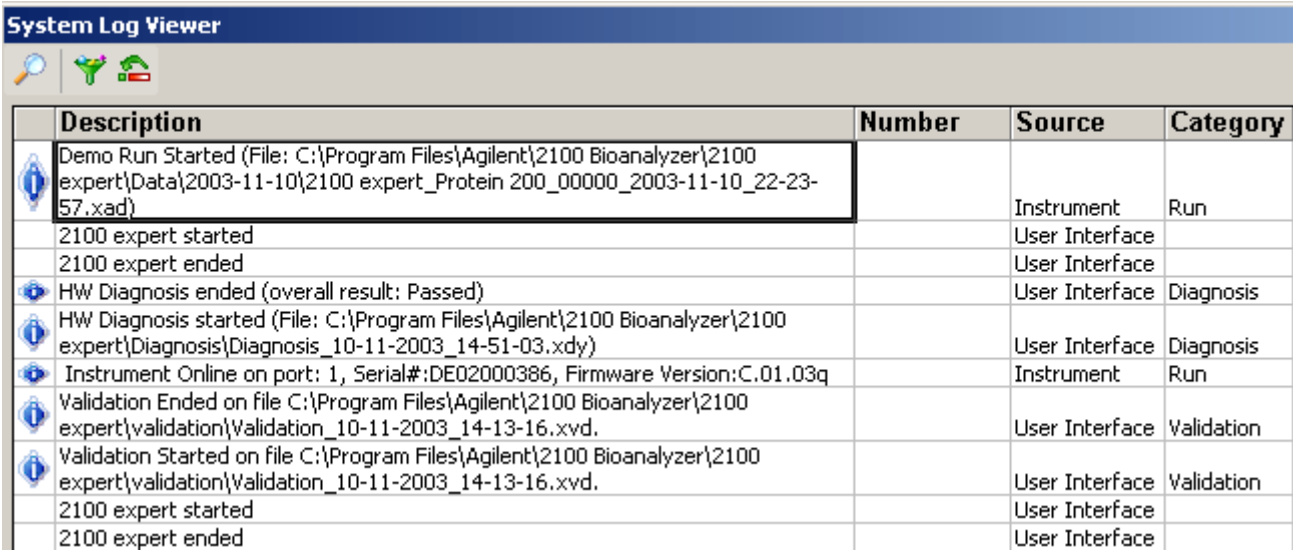
The *Find* dialog box appears.

2 Enter a search string and select the search *Direction*.



3 Click *Find Next*.

If the search string was found in a table cell, the cell gets highlighted:



NOTE

The search is *not* case-sensitive.

4 To continue the search, click *Find Next*.

Configuring 2100 Expert

The 2100 expert software can be configured as follows.

- The names of data files can be generated automatically. Learn how to determine the naming mechanism in "How to Specify Data File Names and Directories" on page 294.
- When a chip run is complete, you have to remove the chip from the bioanalyzer. You can activate an acoustic alert that reminds you to do that. "How to Set the Acoustic Chip Alert" on page 296 gives you the details.
- In data evaluation, overlaying of electropherograms and histograms plays an important part. "How to Specify Graph Settings" on page 298 shows you how you can specify colors for individual samples.
- Printing and exporting data, as well as the start of chip runs can be automated. To learn how to do this, and how to enable the auto print, auto export and auto run functions, see "How to Use the Advanced Settings" on page 299.

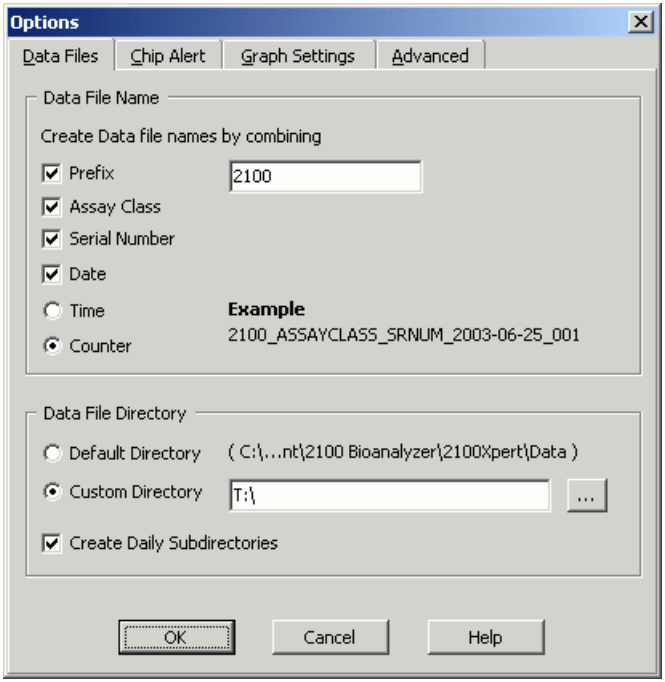
How to Specify Data File Names and Directories

The measurement results are stored automatically when the chip run is complete. To make it easier for you to identify the chip data files, you can configure an automatic naming scheme for the files.

To specify the names and destination for generated chip data files:

1 Select *Tools* > *Options...*.

The *Options* dialog box appears with the *Data Files* tab in front.



2 Select the check boxes of the strings you want to insert in the file names:

Option	Meaning
Prefix	Inserts an annotation to identify the data file. This string can be modified. The default file prefix is “2100 expert”.
Assay Class	Inserts the assay class in the file name. Examples: “DNA1000”, “GFP”, “Apoptosis”.
Serial Number	Inserts the serial number of the Agilent 2100 bioanalyzer instrument used for the chip run.
Date	Inserts the date of the chip run.
Time/Counter	Inserts the time of the chip run/inserts an auto-incremented 3-digit number.

To specify the directory where the chip data files are to be stored:

1 Select *Default Directory*, if you want to use the “..\Data” directory under the 2100 expert installation directory or select a *Custom Directory* of your choice.

2 Select the check box *Create Daily Subdirectories*, if you want daily subdirectories to be created.

This option helps you to better organize your chip data files. If selected, every day a chip run is started, a subdirectory with the naming format “YYYY-MM-DD” will be created in the destination directory, for example, “..\2003-08-22“. All chip data files generated on this day will be stored in this subdirectory.

3 Click *OK* to confirm your modifications.

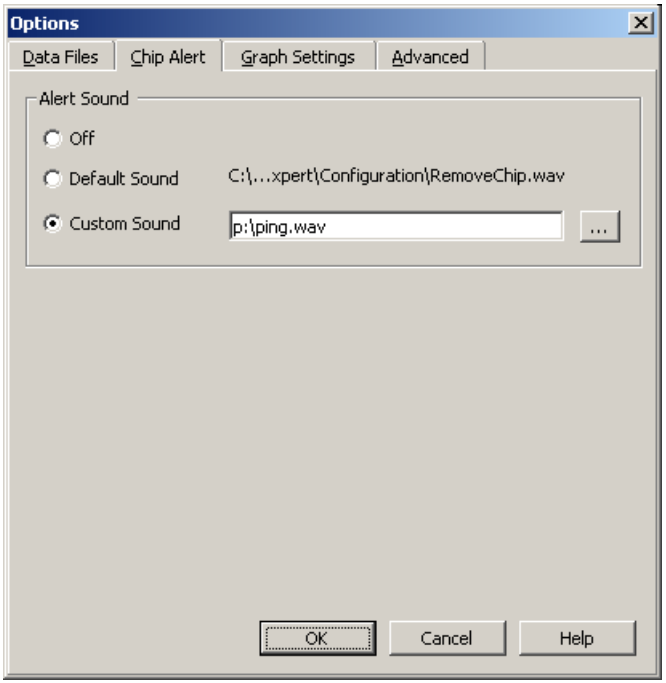
How to Set the Acoustic Chip Alert

When a chip run has finished, you have to remove the chip from the bioanalyzer. You can activate an acoustic alert sound that reminds you to do this.

To set the chip alert sound:

1 Select *Tools* > *Options...* to display the *Options* dialog box.

2 Click the *Chip Alert* tab to bring it to the front.



3 Select *Off* to turn off the alert sound.

– OR –

Select *Default sound* to use the standard alert sound.

– OR –

Select *Custom Sound* to specify a .wav file to be used as alert sound.

4 Click *Play* to test the alert sound.

5 Use the *Alert interval* slider to specify the intervals the alert should be triggered. The interval can be set within the range of 1 to 15 seconds.

6 Click *OK* to confirm your modifications.

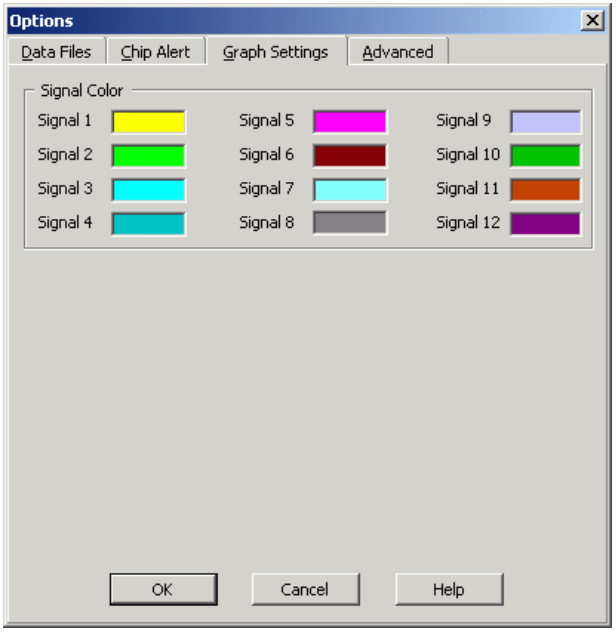
How to Specify Graph Settings

You can compare measurement results of samples by overlaying their electropherograms or histograms. For optimal display, you can configure the curve colors and adjust the scale in overlaid graphs.

To modify the graph settings:

1 Select *Tools* > *Options...* to display the *Options* dialog box.

2 Click the *Graph Settings* tab to bring it to the front.



3 Click the colored rectangles to the right of the signals.

You can now choose a new color in the *Color* dialog box.

How to Use the Advanced Settings

You can use 2100 expert's advanced settings to:

- Automate starting, documenting, and archiving of chip runs.

  Activating the *Auto Run*, *Auto Print*, *Auto Export* functions is especially useful when running a series of the same assay.

- Set disk space limits for data storage.

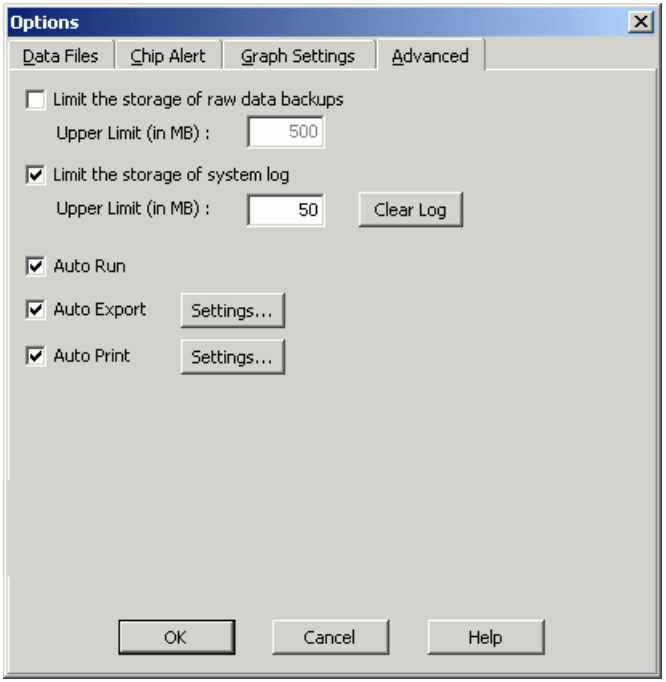
  Disk space limitation for raw data and log files protects you from data overflow when performing large numbers of chip runs.

- Specify correction rules for electrophoretic measurement data.

To modify the advanced settings:

1 Select *Tools* > *Options...* to display the *Options* dialog box.

2 Click the *Advanced* tab to bring it to the front.



3 Select *Limit the storage of raw data backups ...* and enter an upper limit (in MB) if you want to limit disk space for the storage of packet files.

20 MB (~ 20 chip runs) is the default.

NOTE

In addition to the regular chip data file (.xad), 2100 expert creates another raw data backup file (packet file: .pck) with each chip run. The size of a typical packet file is about 1 MB. It is stored in the "..\data\packets" folder of the installation directory. You can set the limit for the disk space to be used for packet files. In case this limit is reached, the oldest file(s) will be deleted to get free disk space (first in, first out). For example, setting the limit to 500 MB allows restoration of about 500 chip runs.

4 Select the *Limit the storage of system log* check box if you want to limit the disk space for the system log file "SystemLogBook.log" (located in the "..\log" subdirectory), and enter an upper limit in MB.

   If the limit is exceeded, a message appears that prompts you to delete or move the log file to get free disk space.

5 Select *Auto Run* to activate the automatic start of a chip run once the lid of the Agilent 2100 bioanalyzer is closed and a chip suiting the selected assay is detected.

6 Select *Auto Export* to enable the *Settings...* button.

   You can now click *Settings...* to display the *Auto Export* dialog box, where you can configure the auto export settings (see "Auto Export" on page 523).

7 Select *Auto Print* to enable the *Settings...* button.

   You can now click *Settings...* to display the *Auto Print* dialog box, where you set the auto print options (see "Auto Print" on page 541).

NOTE

The *Auto Print* and *Auto Export* settings are independent from those made via the *Export* or *Print* command of the *File* menu.

8 Click *OK* to confirm your modifications.

Running Instrument Diagnostics

2100 expert provides several tests to check proper functioning of the bioanalyzer hardware. You should perform the tests on a regular basis, or if incorrect measurements occur.

You can test the following:

- Generic bioanalyzer tests, which can be run with both types of cartridges (electrode or pressure cartridge)
- Bioanalyzer in combination with electrode cartridge (electrophoresis setup)
- Bioanalyzer in combination with pressure cartridge (flow cytometry setup)

Generic Bioanalyzer Tests

Diagnostics Test	Purpose
Electronics Test	Verifies proper functioning of all electronic boards in the bioanalyzer.
Lid Sensor Test	Verifies proper operation of the lid sensor, ensuring that the laser and LED are off when the lid is open.
Stepper Motor Test	Checks for proper movement of the stepper motor.

Diagnostics Test	Purpose
Fan Test	Checks if the fan is running at the appropriate speed.
Temperature Test	Checks if the temperature ramp-up speed of the heater plate is within specifications.

Electrode Cartridge Tests

Diagnostics Test	Purpose
Current Leakage Test	Measures electrode cartridge leak current(s) between pins. Leak current test chip required. For correct preparation of the leak current test chip, please refer to the technical note included in the test chip bundle.
Short Circuit Test	Checks for instrument leak currents using an empty chip. Note: the limits of this test specify an ambient temperature of 25 °C and relative humidity less than or equal to 60 %. Higher temperatures or relative humidity could result in a leak current.
Optics Test	Checks for proper alignment of internal optics and proper function of the laser and LED.
High-Voltage Stability Test	Tests high voltage accuracy and stability of all 16 high voltage power supplies. Unused chip (DNA, RNA, or protein) required.
High-Voltage Accuracy Test	Check of the high voltage controller.

Diagnostics Test	Purpose
High-Voltage Accuracy on Load *Test*	Check of channel-reference diode in transmission direction.
Autofocus Test	Checks the focusing capability of the optical system. Autofocus test chip required.
Laser Stability Test	Measurement of stability of red laser signal.
Electrode/Diode Test	Checks the photo diode and current-versus-voltage performance of the bioanalyzer. Electrode/diode test chip required.

Pressure Cartridge Tests

Diagnostics Test	Purpose
Pressure Offset Test	The vacuum system of the pressure cartridge consists of a pump and the corresponding tubes. This test calibrates the pressure sensors to zero.
Pressure Control Test	Checks if the bioanalyzer is able to hold the working pressure of -140 mbar. During the test pumps stay on, while the system tries to regulate pressure to be kept at -140 mbar. Cell Autofocus test chip required.

Diagnostics Test	Purpose
System Leak Test	Checks if the bioanalyzer is able to maintain a vacuum. Produces a test pressure of -100 mbar and monitors for changes. Cell Autofocus test chip required.
Cell Autofocus Test	Checks that the optical system of the bioanalyzer is correctly calibrated. Cell Autofocus test chip required.

NOTE

With bioanalyzer model G2938A only diagnostic tests in combination with the electrode cartridge can be performed.

Test Chips

Depending on your bioanalyzer setup (electrophoresis or flow cytometry), different test chips are required to run some of the diagnostics tests. Test chip kits are part of the bioanalyzer electrophoresis set (G2947CA) and flow cytometry set (G2948CA):

Test Chip Kit for Electrophoresis Assays (reorder no. G2938-68100)

Test Chip	Comment	Quantity
Autofocus Test Chip	Values for fluorescence and offset are printed on the chip. Can be used multiple times.	1

Test Chip	Comment	Quantity
Electrode/Diode Test Chip	Can be used multiple times.	1
Leak Current Test Chip	Has to be prepared with deionized water. Can be only used once.	5

Test Chip Kit for Flow Cytometry Assays (reorder no. G2938-68200)

Test Chip	Comment	Quantity
Cell Autofocus Test Chip	Required for Pressure Control Test, System Leakage Test, and Optical Drive Test. Can be used multiple times.	1

How to Run Instrument Diagnostics Tests

NOTE

Diagnostics tests cannot be run while the 2100 expert software is performing a chip run.

To run diagnostics tests:

1 From the *Context* menu, select *Instrument*.

2 In the *Tree View Panel*, select the bioanalyzer on which you want to run the tests.

3 Select the *Diagnostics* tab.

All available tests are displayed in the *Available Tests* list. The tests that can be executed depend on the type of cartridge that is installed in the bioanalyzer (see "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47).

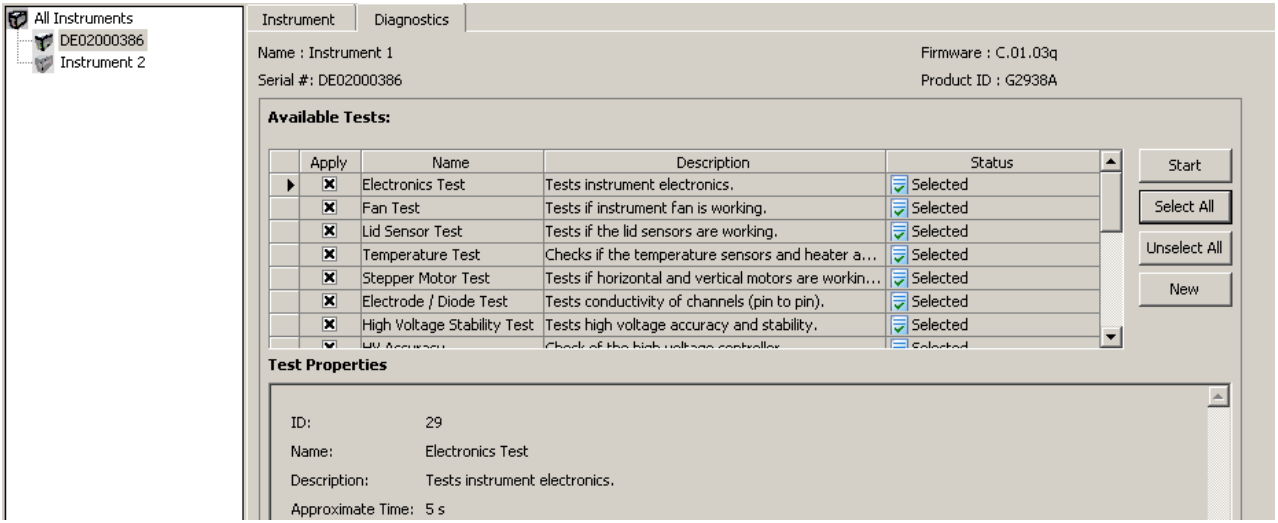
The 2100 expert software will generate an error message if a wrong cartridge type is detected for the selected assay. To run the selected test please insert the requested cartridge type (see "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47).

NOTE

You can perform diagnostics tests only if the bioanalyzer is switched on. In offline mode, the *Diagnostics* tab of the *Instrument Context* is dimmed.

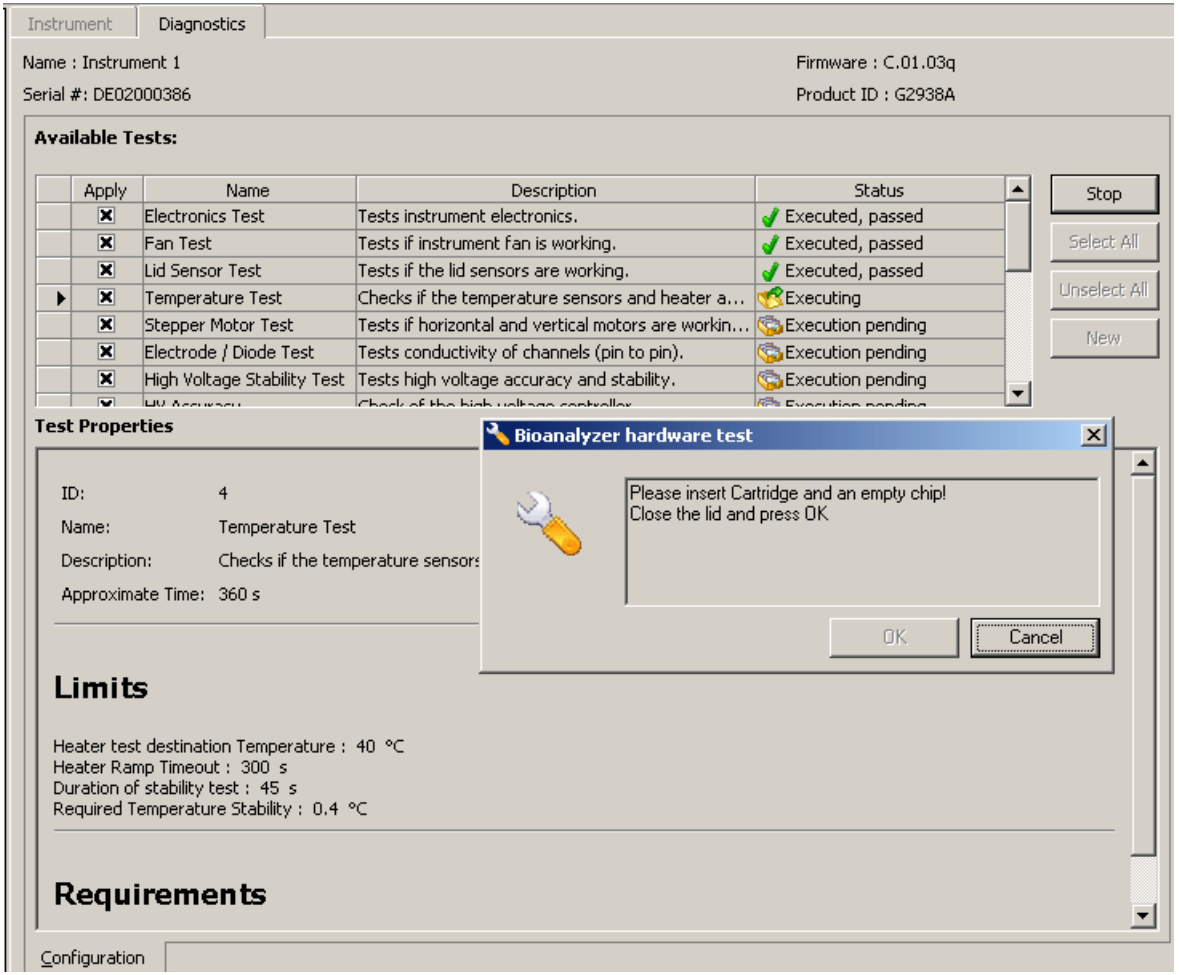
4 Select the tests you want to run:

- Select the *Apply* check boxes to select single tests.
- Click *Select All* to select all available tests.
- Click *Unselect All* to deselect all tests.



5 Click *Start*.

6 Follow the instructions given by the 2100 expert software. For example, exchange the cartridge, or put a test chip in the receptacle of the bioanalyzer when requested by the software.



All selected tests are performed.

The *Status* column indicates the status of each test:

- *Executing*
- *Execution pending*
- *Executed, passed*
- *Executed, failed*

7 If any test failed, redo the test.

8 If failures still persist, contact Agilent service.

The results of diagnostics tests are stored in .xdy files in the 2100 expert installation folder under "..\diagnosis". If tests fail, send the .xdy files to the Agilent service.

Performing Qualifications

To ensure a validated Agilent 2100 bioanalyzer system, qualification steps have to be performed at installation and operation level.

2100 expert allows for detailed *installation qualification* (IQ) and *operational qualification* (OQ) on both the bioanalyzer hardware and software. Each qualification comprises a series of tests and measurements that you can run and document in the *Validation* context of the 2100 expert software.

Installation Qualification

Installation qualification includes tests to verify that the bioanalyzer software and hardware are installed properly and that all electrical and pressure connections are correct.

Installation qualification must be performed once after installation.

Operational Qualification

Operational qualification proves that the bioanalyzer system is suitable for its intended use, that is, that it will function according to its operational specifications in the selected environment.

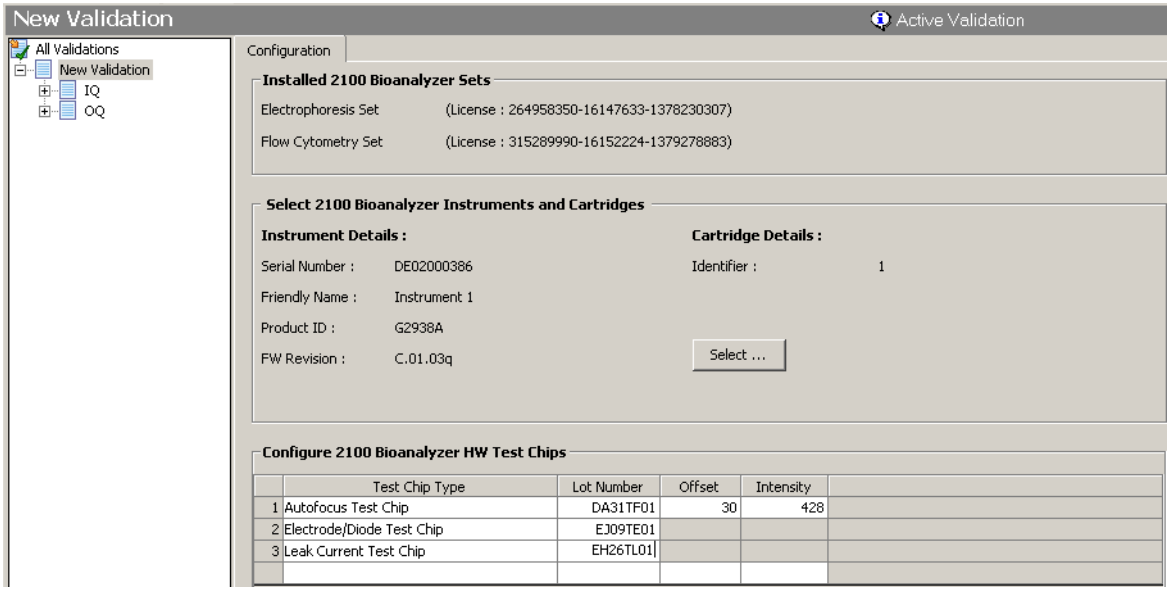
Operational qualification should be performed:

- at first use of the instrument,
- after relocating the instrument,
- after changing essential parts of the system, for example software updates or exchange of cartridges,
- after instrument repair,
- on regular time intervals.

Validation Procedure

To perform qualification tests:

1. Switch to the *Validation* context.
2. From the *File* menu select *New*.
3. A *New Validation* item appears in the *Tree View Panel*.
4. Under *Cartridge Details*, click on *Select...* and specify details on the cartridge that is currently installed in the bioanalyzer.
5. Under *Configure 2100 Bioanalyzer HW Test Chips*, enter the test chips you will use for this validation:

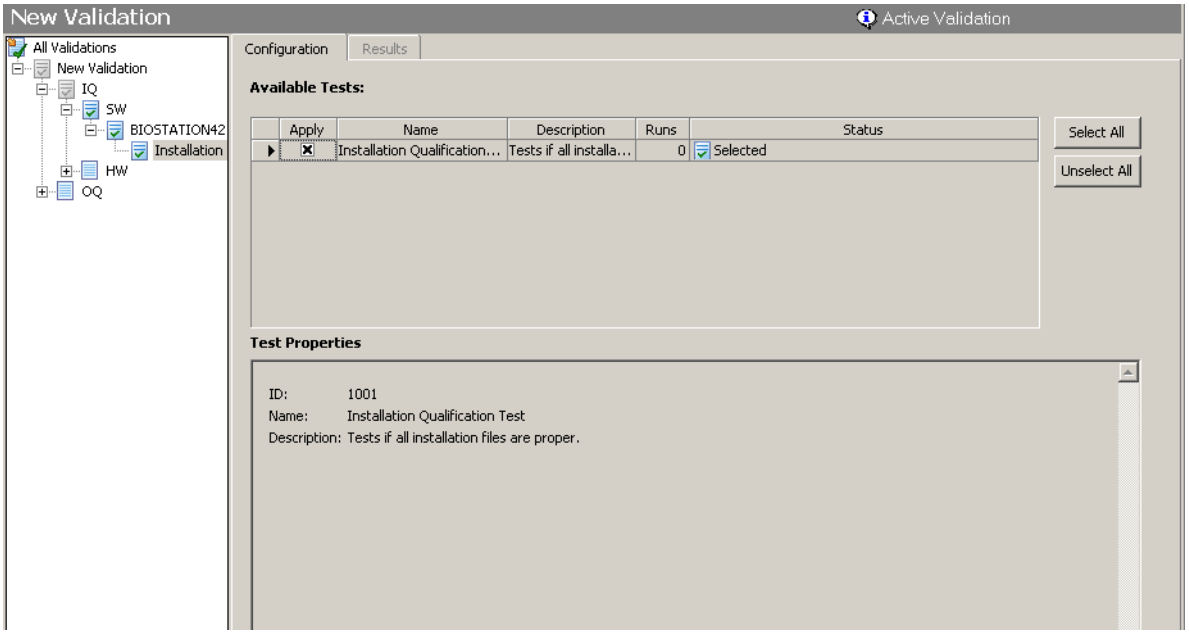


6 In the *Tree View Panel*, navigate to the test category you want to execute. Select the category via *IQ/OQ – SW/HW – PC name/Bioanalyzer name – Test Category*.

NOTE

To execute hardware tests (*HW* branch) the bioanalyzer must be properly connected and switched on.

The *Configuration* tab now lets you select qualification tests to be executed in the validation run:



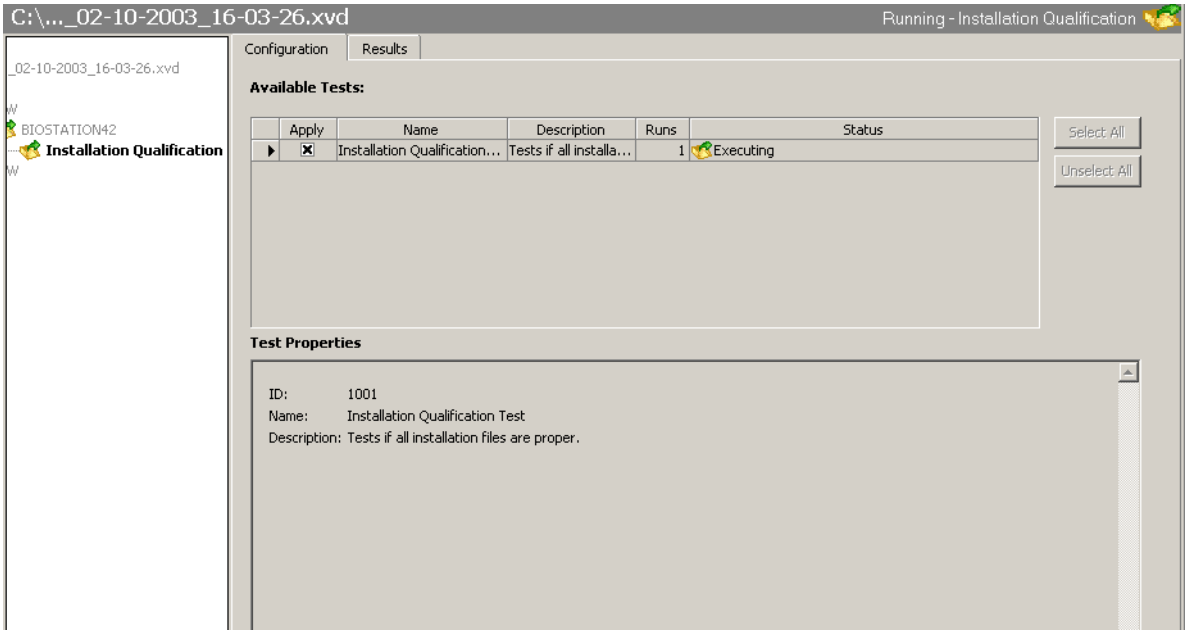
To select tests, check the *Apply* check box next to the test(s).

7 To start the selected tests, click on *Start* button  in the toolbar.

The *Save As* dialog box appears.

8 Specify a name and location for the validation results file (.xvd) and click *Save*.

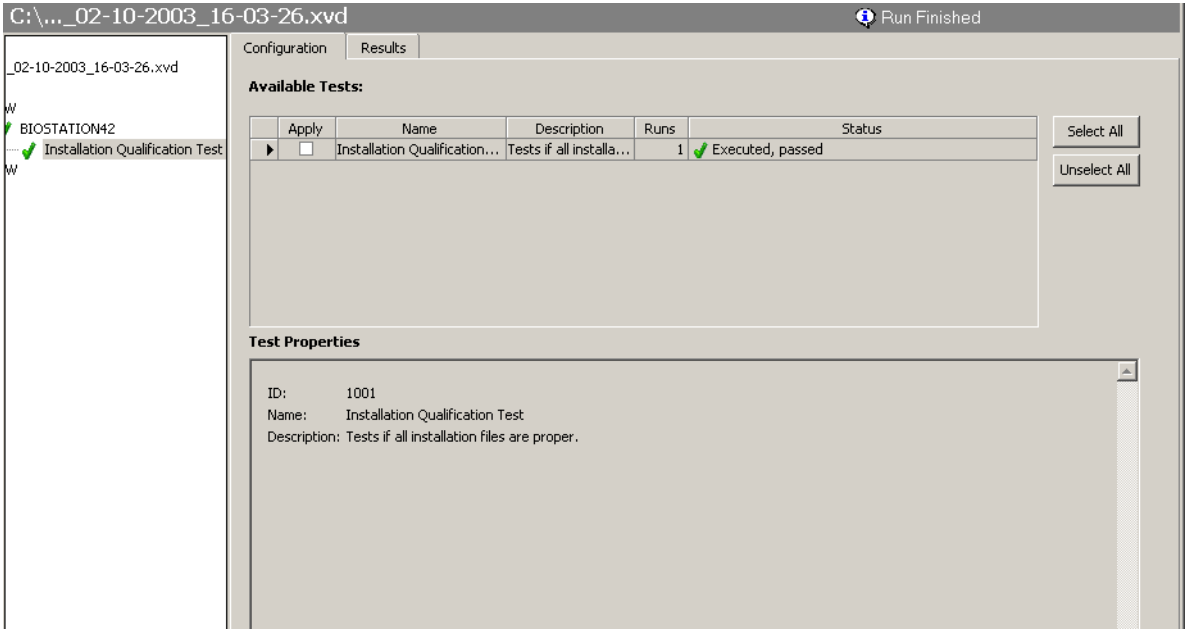
The selected tests are executed.



9 After all tests have been executed the following message appears:

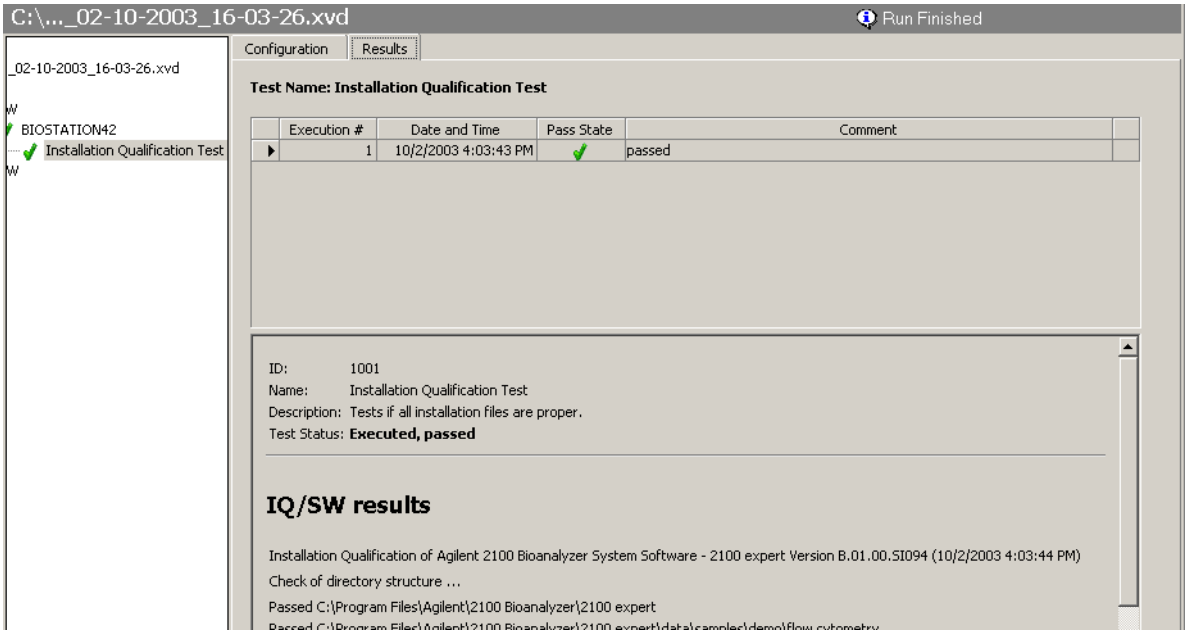
10 Click *OK*.

11 The *Status* column shows whether the tests have been run successfully or not.



For details on the *Configuration* tab, please refer to "Configuration Tab" on page 485.

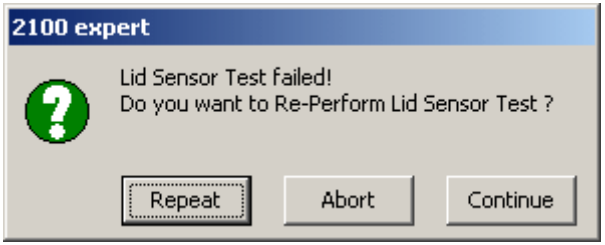
12 To view details on test execution, select the *Results* tab.



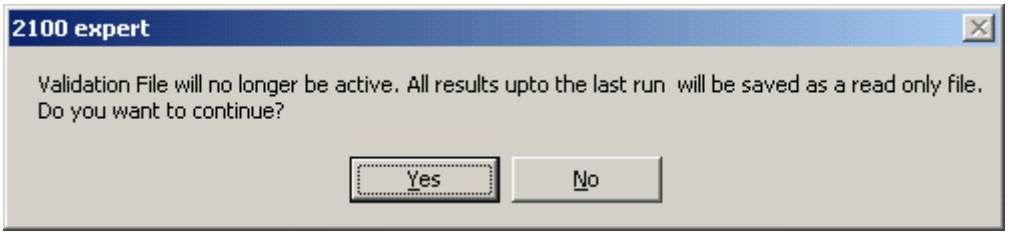
For details on the *Results* tab, please refer to "Results Tab" on page 487.

13 You can now navigate to other test categories and execute additional qualification tests.

14 If a test fails, you can *Repeat* test execution, *Abort* the validation run, or skip the current test and *Continue* with the next test:



15 When you close the validation results file (*File* > *Close*), try to switch to another context or exit 2100 expert, the following message appears:



If you select *No*, you return to the validation context and can run further qualification tests.

If you select *Yes*, the validation results file (.xvd) is closed and becomes read-only.

NOTE

You can re-open validation results files only for viewing and printing.

TIP

Select *File* > *Print...* to generate a printed report of the validation run.

2100 Expert Software Reference

The 2100 expert software reference is organized as follows:

- *2100 Expert Application Window Elements*, such as menus, toolbars, status bar, panels, and tabs establish a graphical standard user interface.

  For details, refer to "2100 Expert Application Window Elements" on page 321.

- *Dialog Boxes* are used for various file operations, for controlling and analyzing chip runs, and for configuring the 2100 expert application.

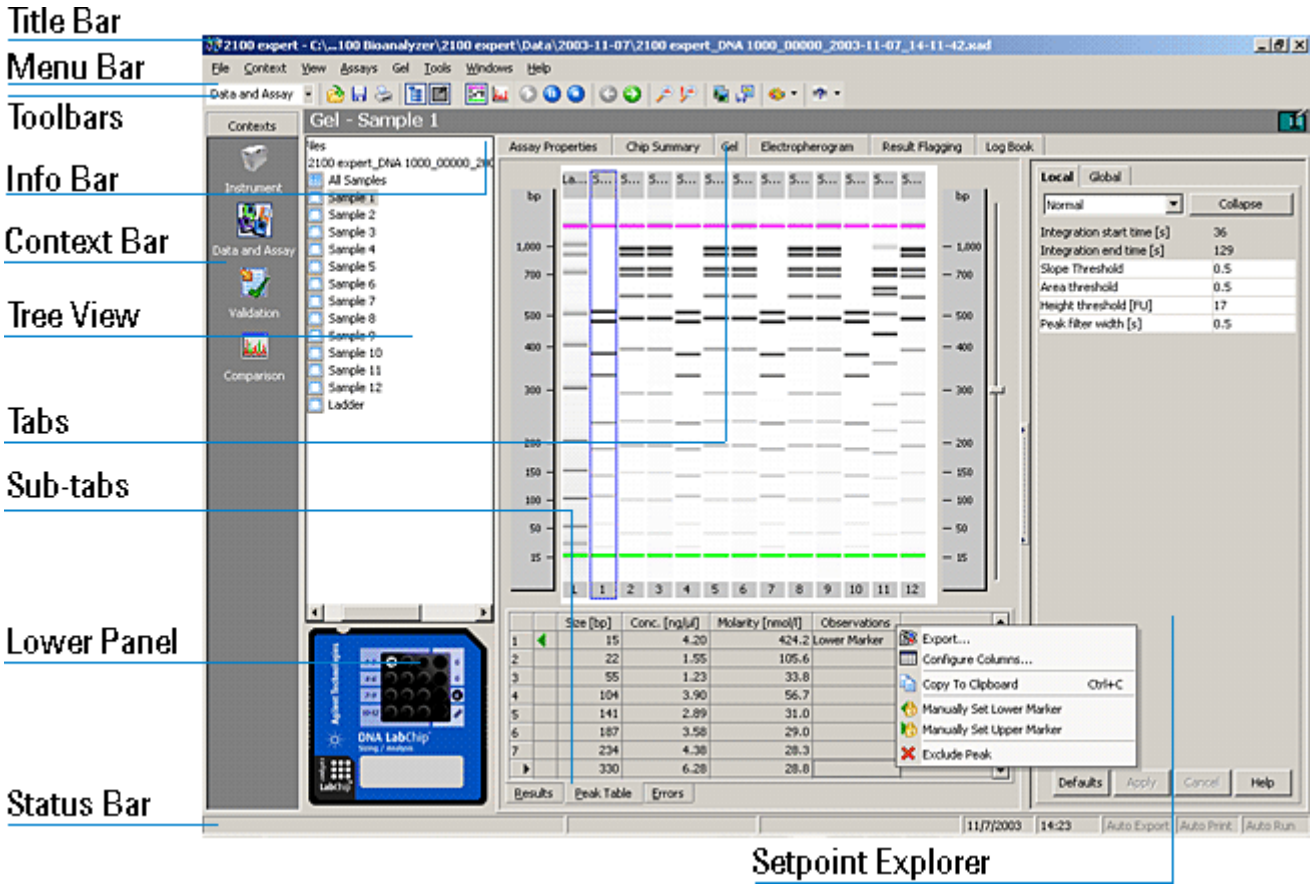
  For details, refer to "Dialog Boxes" on page 494.

- *Shortcuts and Mouse Actions* allow you to operate the 2100 expert software more efficiently.

  For details, refer to "Shortcuts and Mouse Actions" on page 565.

2100 Expert Application Window Elements

The 2100 expert application window has the following regions (*Data and Assay* context):



Region	Purpose
Title Bar	Bar at the top of the window providing information on the application that you have started, and the assay or chip file you have selected.
Menu Bar	Bar beneath the title bar that contains pull-down menus.
Toolbars	Provides quick access to the most often needed commands.
Information Bar	Shows information on the instrument, assay, and sample, the gating direction (flow cytometric assay), and an icon that identifies the chip type and shows the number of the currently displayed or measured sample.
Context Bar	Allows you to switch between the four contexts.
Tree View Panel	Works like the Windows Explorer; shows you which samples have been measured using which chips, and allows you to display the results as gel-like images/electropherograms or histograms/dot plots. The tree view is also used to display assays, comparison files, validation files, and connected instruments.
Tabs	Tabs (and sub-tabs) are the main user interfaces. Tabs organize the user interface into main parts.
Setpoint Explorer	The setpoint explorer lets you modify data analysis parameters: • Assay parameters (assay file, .xsy) • Chip parameters (chip data file, .xad, mode *Global*) • Sample parameters (chip data file, .xad, mode *Local*)

Region	Purpose
Lower Panel	Displays a chip icon/a small gel view that you can use to navigate through your samples.
Status Bar	Shows system messages and activities, the current status of the application, and whether or not *Auto Run*, *Auto Export* and *Auto Print* are activated.

Title Bar

If you are in the *Data and Assay* context, name and path of the current chip data or assay file are displayed on the title bar:

If the file has been opened as read-only, this is shown in parentheses.

Menu Bar

The 2100 expert application window's menu bar has the following pull-down menus:

Menu	Purpose
File Menu	Provides functions for file handling and printing.
Context Menu	Lets you switch between contexts.
View Menu	Provides functions to switch between Gel/Electropherogram and Histogram/Dot Plot view. Also lets you show and hide panels.

Menu	Purpose
Assays Menu	Lets you open assay files for editing (*Data and Assay* context). Lets you select an assay for a chip run (*Instrument* context).
Gel Menu	Provides functions for changing the display of gel graphs.
Electropherogram Menu	Provides functions for changing the display of electropherograms, and for overlaying electropherograms.
Histogram Menu	Provides functions for changing the display of histograms, overlaying histograms, and for handling markers and gates.
Dot Plot Menu	Provides functions for changing the display of dot plots, and for handling regions and gates.
Result Flagging Menu (Electrophoretic Assays only)	Provides commands for creating, organizing and applying result flagging rules.
Log Book Menu	Allows to review the log book of the chip run and provides commands for searching and filtering log book events.
Instrument Menu	Lets you start and stop a chip run.
Tile Menu	If you have selected *All Instruments* in the *Instrument* context, this menu lets you arrange the instrument panes and switch between them.
Tools Menu	Lets you open the *Options* dialog box, allowing you to specify system-wide settings.

Menu	Purpose
Windows Menu	Lets you switch between open chip data or assay files (*Data and Assay* context). Lets you select one of the connected bioanalyzers (*Instrument* context).
Help Menu	Lets you access Help, get information about the installed software and hardware, and register additional software options.

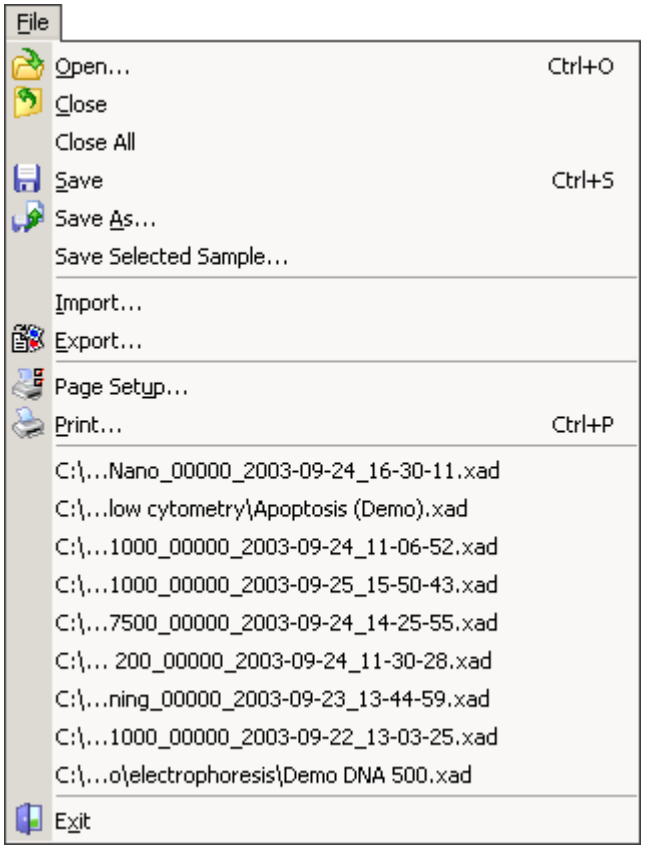
NOTE

Not all menus are visible at the same time. The *Electropherogram* menu, for example, is only available if electropherograms (single or grid view) are displayed.

File Menu

The *File* menu provides functions for file handling and printing.

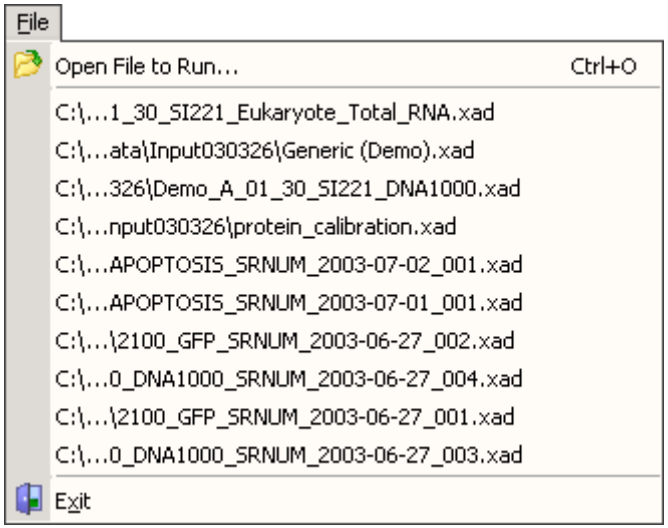
Data and Assay Context



File menu item	Function
Open...	Brings up the *Open* dialog box, allowing you to open a chip data (.xad) or assay (.xsy) file. Multiple files of different assay types can be opened in parallel. Refer to "Open" on page 514.
Close	Closes the currently selected chip data or assay file.
Close All	Closes all open chip data and assay file files.
Save	Saves the selected chip data or assay file under its current name.
Save As...	Opens a system dialog box, allowing you to save the current file as a chip data (.xad) or assay (.xsy) file.
Save Selected Sample...	Opens a system dialog box, allowing you to save the current file as a chip data (.xad) or assay (.xsy) file.
Import...	Opens a system dialog box, allowing you to import bio sizing (.cld/.asy) or cell fluorescence (.cad/.csy) files. Refer to "Importing Data" on page 256.
Export...	Displays the *Export Options (Flow Cytometry)* or the *Export Options (Electrophoresis)* dialog box, allowing you to export chip or assay data with a specific format. Refer to "Export Options (Flow Cytometry)" on page 516 *and* "Export Options (Electrophoresis)" on page 519.
Page Setup...	Displays the *Page Setup* dialog box, allowing you to change the layout of the printed page. Refer to "Page Setup" on page 555.

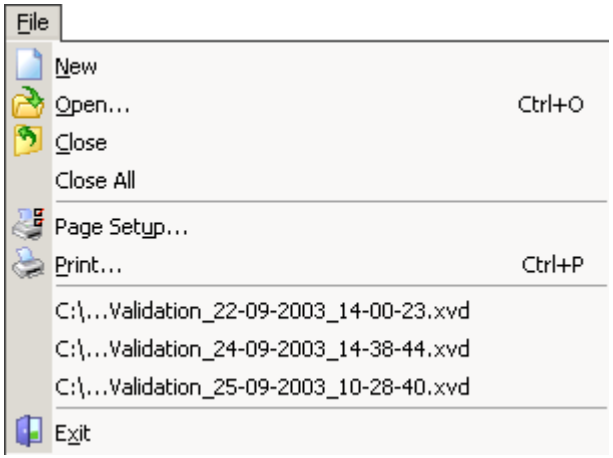
File menu item	Function
Print...	Displays the *Print (Flow Cytometry) or Print (Electrophoresis)* dialog box, allowing you to generate various printouts of the selected chip data or assay file. Refer to *"*Print (Flow Cytometry)" on page 528 *and"*Print (Electrophoresis)" on page 531.
Recently used files	A list of up to ten chip data (.xad) and/or assay (.xsy) files gives you quick access to the most recently used files.
Exit	Exits the 2100 expert application. If there is unsaved data, the *2100 Expert – Close* dialog box appears. Refer to *"*2100 Expert – Close" on page 499.

Instrument Context



File menu item	Function
Open File to Run...	Brings up the *Open* dialog box, allowing you to select a chip data (.xad) or assay (.xsy) file for a chip run. Refer to "Open" on page 514.
Recently used files	A list of up to ten chip data (.xad) and/or assay (.xsy) files gives you quick access to the most recently used files.
Exit	Exits the 2100 expert application. If there is unsaved data, the *2100 Expert – Close* dialog box appears. Refer to "2100 Expert – Close" on page 499. If a chip run is in progress, you cannot exit 2100 expert.

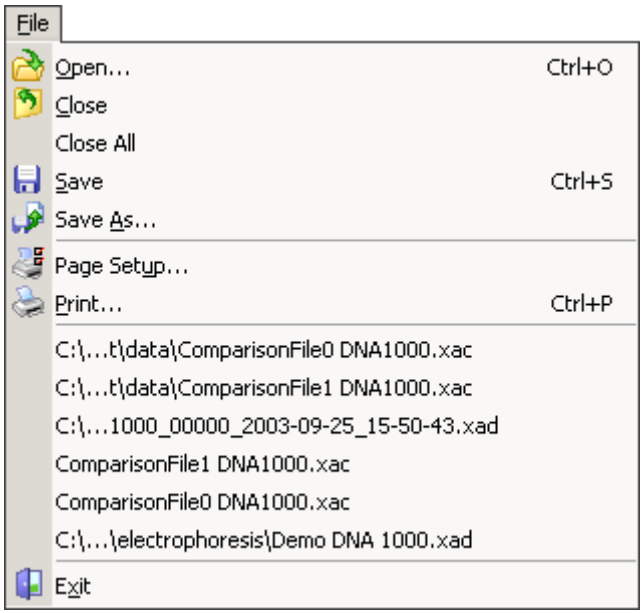
Validation Context



File menu item	Function
New	Begins a new validation and inserts a *New Validation* item in the tree view.
Open...	Brings up a system dialog box, allowing you to open a validation results (.xvd) file.
Close	Closes the current validation results file.
Close All	Closes all open validation results files.
Page Setup...	Displays the *Page Setup* dialog box, allowing you to change the layout of the printed page(s). Refer to "Page Setup" on page 555.

File menu item	Function
Print...	Displays the *Print (Validation)* dialog box, allowing you to generate various reports on qualification tests. Refer to "Print (Validation)" on page 535.
Recently used files	A list of up to ten validation results (.xvd) files gives you quick access to the most recently used files.
Exit	Exits the 2100 expert application. If there is unsaved data, the *2100 Expert – Close* dialog box appears. Refer to "2100 Expert – Close" on page 499.

Comparison Context



File menu item	Function
Open...	Brings up a system dialog box allowing you to open a comparison (.xac) or chip data (.xad) file.
Close	Closes the current comparison file.
Close All	Closes all open comparison files.
Save	Saves the selected comparison file under its current name.
Save As...	Opens a system dialog box allowing you to save the current comparison file under a new name.
Page Setup...	Displays the *Page Setup* dialog box, allowing you to change the layout of the printed page(s). Refer to "Page Setup" on page 555.
Print...	Displays the *Print (Comparison)* dialog box, allowing you to print a comparison report. Refer to "Print (Comparison)" on page 538.
Recently used files	A list of up to ten chip data (.xad) and/or comparison (.xac) files gives you quick access to the most recently used files.
Exit	Exits the 2100 expert application. If there is unsaved data, the *2100 Expert – Close* dialog box appears. Refer to "2100 Expert – Close" on page 499.

Context Menu

The *Context* menu lets you switch between the four program modes, called “contexts”:

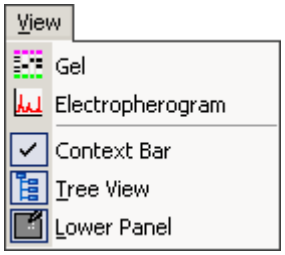
Context menu item	Function
Instrument	Switches to the *Instrument* context.
Data and Assay	Switches to the *Data and Assay* context.
Validation	Switches to the *Validation* context.
Comparison	Switches to the *Comparison* context.

The checkmark indicates the active context.

View Menu

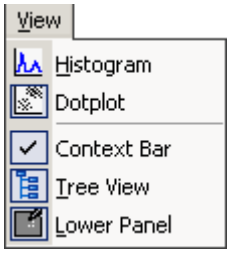
The *View* menu provides functions to switch between the Gel/Electropherogram and Histogram/Dot Plot views, and between single- and multi-instrument view. It also lets you show and hide panels.

Data and Assay context (electrophoretic assay selected)



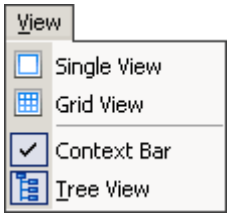
View menu item	Function
Gel	Switches to the *Gel Tab* of the current chip data (or assay) file.
Electropherogram	Switches to the *Electropherogram Tab (Single/Grid View)* of the current chip data (or assay) file.
Context Bar	Shows or hides the *Context Bar*.
Tree View	Shows or hides the *Tree View Panel*.
Lower Panel	Shows or hides the *Lower Panel* (chip graphic or gel graphic).

Data and Assay context (flow cytometric assay selected)



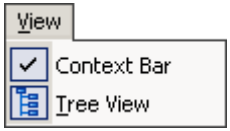
View menu item	Function
Histogram	Switches to the *Histogram Tab (Single/Grid View)* of the current chip data (or assay) file.
Dotplot	Switches to the *Dot Plot Tab (Single/Grid View)* of the current chip data (or assay) file.
Context Bar	Shows or hides the *Context Bar*.
Tree View	Shows or hides the *Tree View Panel*.
Lower Panel	Shows or hides the *Lower Panel* (chip graphic or gel graphic).

Instrument context



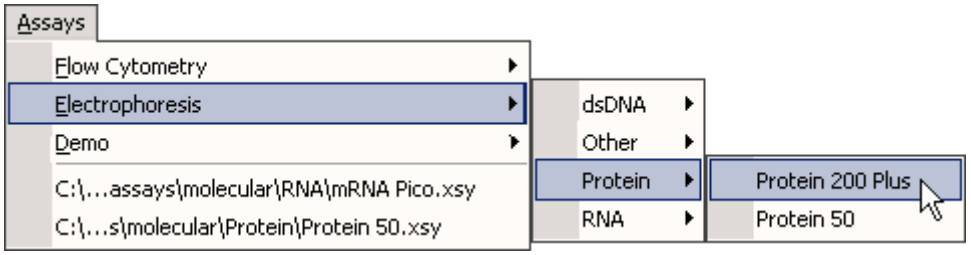
View menu item	Function
Single View	Switches to the *Instrument Tab (Single View)* of the selected instrument.
Grid View	Switches to the *Instrument Tab (Grid View)*.
Context Bar	Shows or hides the *Context Bar*.
Tree View	Shows or hides the *Tree View Panel*.

Validation/Comparison context



View menu item	Function
Context Bar	Shows or hides the *Context Bar*.
Tree View	Shows or hides the *Tree View Panel*.

Assays Menu



From the *Assays* menu, you can open assay files for editing (*Data and Assay* context). In the *Instrument* context, you can select an assay for a chip run if a bioanalyzer is connected and switched on.

Assays menu item	Function
Electrophoresis – dsDNA	All assays located in the "..\assays\electrophoresis\dsDNA" folder are listed here. By default, these are: • *DNA 1000* • *DNA 12000 Laddering* • *DNA 12000* • *DNA 500* • *DNA 7500*

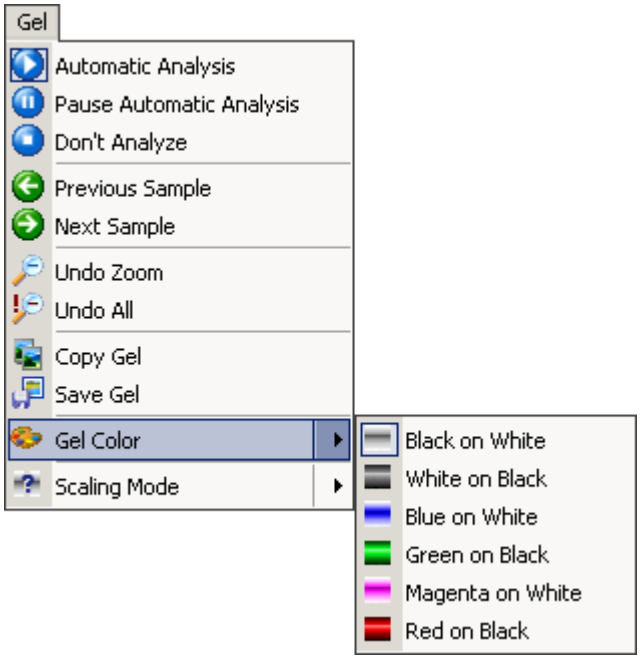
Assays menu item	Function
Electrophoresis – RNA	All assays located in the "..\assays\electrophoresis\RNA" folder are listed here. By default, these are: • *Eukaryote Total RNA Nano* • *Eukaryote Total RNA Pico* • *mRNA Nano* • *mRNA Pico* • *Prokaryote Total RNA Nano* • *Prokaryote Total RNA Pico*
Electrophoresis – Protein	All assays located in the "..\assays\electrophoresis\protein" folder are listed here. By default, these are: • *Protein 50* • *Protein 200 Plus*
Electrophoresis – Other	All assays located in the "..\assays\electrophoresis\other" folder are listed here. By default, these are: • *Cy5 Labeled Nucleic Acids Nano*

Assays menu item	Function
Flow Cytometry	All assays located in the "..\assays\flow cytometry" folder are listed here. By default, these are: • *Antibody Staining* • *Apoptosis* • *Apoptosis - fast protocol* • *siRNA Transfection Viability* • *Blue to Red* • *Checkout Beads* • *Generic* • *GFP* • *On-chip Antibody Staining* • *On-chip GFP* • *Red to Blue* Descriptions of these predefined assays are given in "Overview of Flow Cytometric Assays" on page 166 and "Evaluating Antibody Staining, Apoptosis, and GFP Assays" on page 229.

Assays menu item	Function
Demo – Flow Cytometry	All assays located in the "..\assays\demo\flow cytometry" folder are listed here. By default, these are: • *Antibody Staining (Demo)* • *Apoptosis (Demo)* • *Checkout Beads (Demo)* • *GFP (Demo)* • *Generic (Demo)* You can use these assays for training in offline mode (with no bioanalyzer connected). See "Running a Demo Assay" on page 41.

Assays menu item	Function
Demo – Electrophoresis	All assays located in the "..\assays\demo\electrophoresis" folder are listed here. *Demo Cy5 Labeled Nucleic Acids Nano* • *Demo DNA 1000* • *Demo DNA 12000* • *Demo DNA 500* • *Demo DNA 7500* • *Demo Eukaryote Total RNA Nano* • *Demo Eukaryote Total RNA Pico* • *Demo mRNA Nano* • *Demo mRNA Pico* • *Demo Prokaryote Total RNA Nano* • *Demo Prokaryote Total RNA Pico* • *Demo Protein 200 Plus* • *Demo Protein 50* Use these demos for offline training (with no bioanalyzer connected). See "Running a Demo Assay" on page 41.
Recently used files	Gives you quick access to the most recently used assays (up to five).

Gel Menu

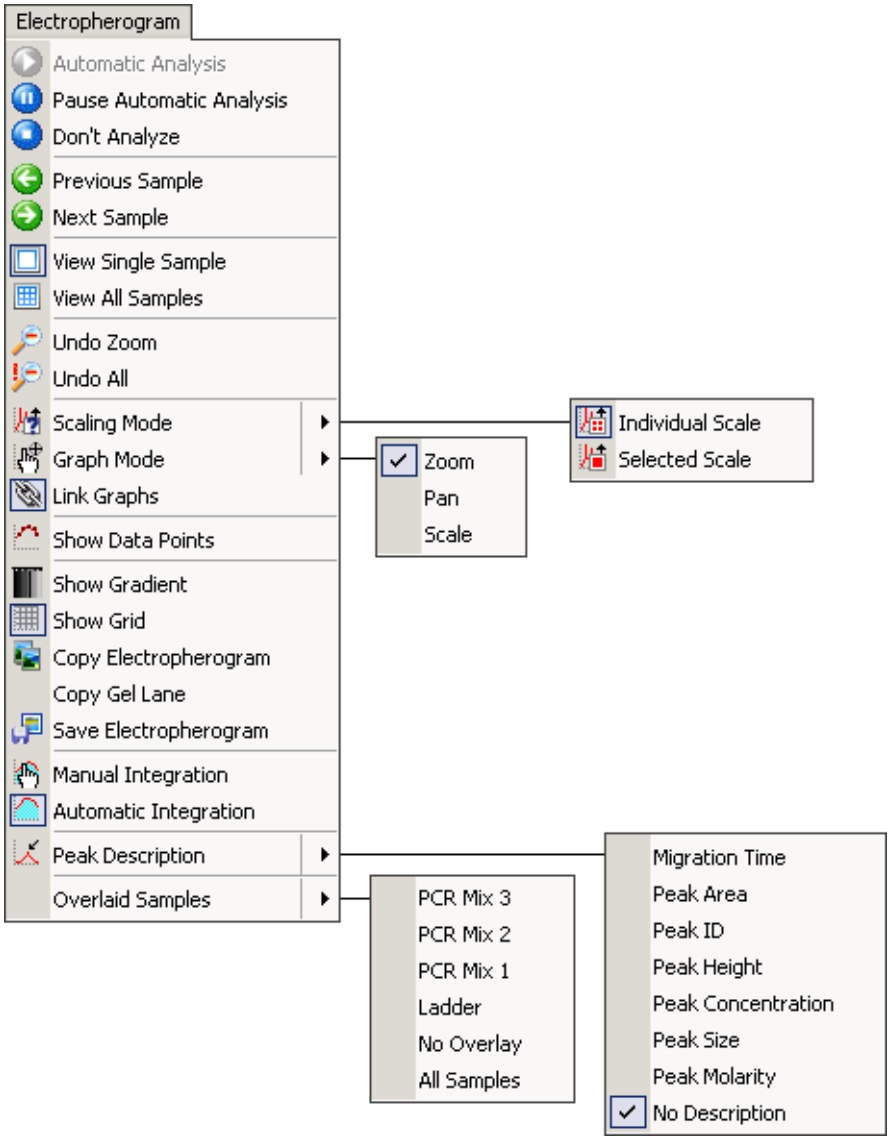


Gel menu item	Function
Automatic Analysis	Starts automatic analysis. Peaks are detected, and the Peak Table gets calculated. Results are getting recalculated whenever changes to the data analysis setpoints are applied.
Pause Automatic Analysis	Pauses automatic analysis. It is recommended to pause analysis during manual integration.

Gel menu item	Function
Don't Analyze	Analysis is switched off. Raw data is displayed on the *Gel* tab and the *Peak Table* is cleared.
Previous Sample	Highlights and displays the gel graph of the previous sample. If the ladder is highlighted, jumps to the last sample. Not available if the first sample is highlighted.
Next Sample	Highlights and displays the gel graph of the next sample. If the last sample is highlighted, jumps to the ladder. Not available if the ladder is highlighted.
Undo Zoom	Undoes the last zoom, pan, or scale action.
Undo All	Undoes all zoom, pan, and scale actions.
Copy Gel	Puts a copy of the gel graph (of all samples) into the clipboard.
Save Gel	Opens a system dialog box, allowing you to save the gel graph (all samples) as an image in JPEG (.jpg), Windows Bitmap (.bmp) or Tagged Image File (.tif) format.

Gel menu item	Function
Gel Color	Opens a submenu from which you can select a predefined foreground/background color scheme to be used for displaying the gel graph (all lanes). The colors are designed to approximate various actual gel staining and imaging techniques. *Blue on White*, for example, simulates a Coomassie gel often used with proteins.
Scaling Mode	Opens a submenu from which you can select one of the following modes: • *Individual Scale* Each lane uses its own scaling which is optimized for that lane. • *Selected Scale* All lanes use the same scaling which is optimized for the selected lane. • *Global Scale*. All lanes use the same scaling.

Electropherogram Menu



Electropherogram menu item	Function
Automatic Analysis	Starts automatic analysis. Peaks are detected, and the Peak Table gets calculated. Results are getting recalculated whenever changes to the data analysis setpoints are applied.
Pause Automatic Analysis	Pauses automatic analysis.
Don't Analyze	Analysis is switched off. Raw data is displayed on the *Electropherogram* tab and the *Peak Table* is cleared.
Previous Sample	Highlights and displays the electropherogram of the previous sample. If the ladder is highlighted, jumps to the last sample. Not available if the first sample is highlighted.
Next Sample	Highlights and displays the electropherogram of the next sample. If the last sample is highlighted, jumps to the ladder. Not available if the ladder is highlighted.
View Single Sample	Displays the current electropherogram in single-well view.
View All Samples	Switches to the multi-well view and highlights the current electropherogram.
Undo Zoom	Undoes the last zoom, pan, or scale action.
Undo All	Undoes all zoom, pan, and scale actions.

Electropherogram menu item	Function
Scaling Mode	Opens a submenu from which you can select one of the following modes: • *Individual Scale* Each graph uses its own scaling which is optimized for that electropherogram. • *Selected Scale* All graphs use the same scaling which is optimized for the selected graph. • *Global Scale* (only available in grid view). All graphs use the same scaling which is chosen such that all electropherograms fit into the display.

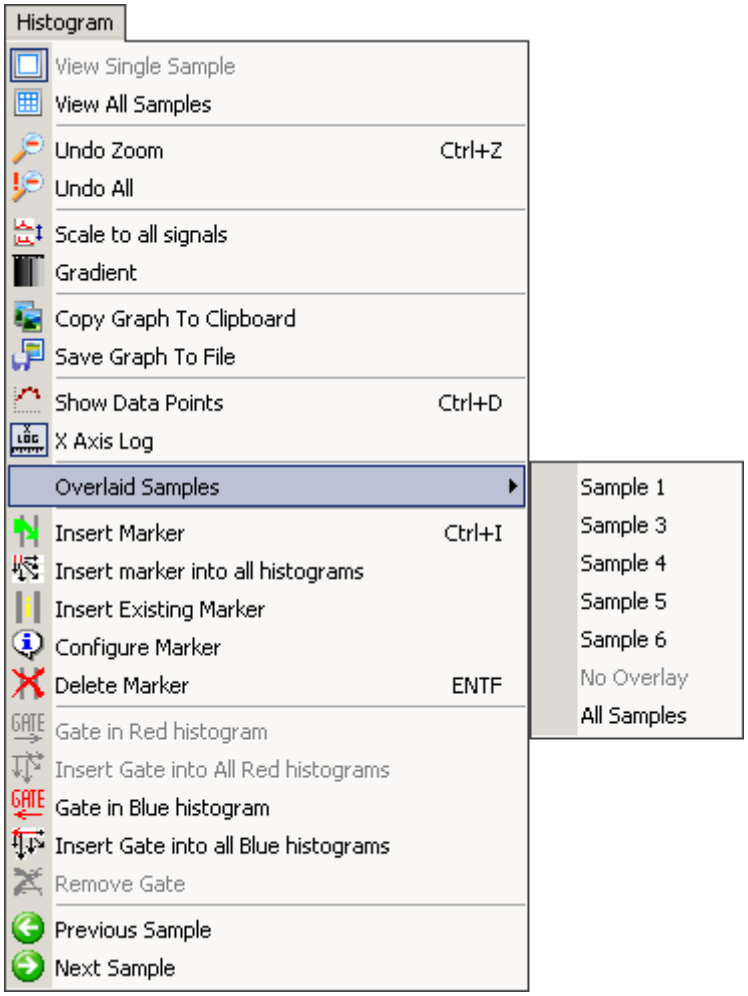
Electropherogram menu item	Function
Graph Mode	Lets you select one of the following click-and-drag operations on electropherograms: • *Zoom* Magnifies the corresponding region to the full display. • *Pan* Moves the graph, makes especially sense for already zoomed graphs. • *Scale* Dragging left or down shrinks the display in that direction, dragging right or up magnifies the display in that direction.
Link Graphs	If this option is enabled, then all zoom, pan and scale steps are effective in all electropherograms.
Show Data Points	Shows/hides the data points used to generate the electropherogram. Data points are visible only in the single-well view. Data points are 0.05 seconds apart as the time resolution of the data points is 0.05 seconds, which corresponds to their data acquisition rate (of 20 Hz).
Show Gradient	Puts a gray-to-white gradient on the background of the electropherogram(s), or removes the gradient.
Show Grid	Shows/hides grid lines (single-well view only).

Electropherogram menu item	Function
Copy Electropherogram	Puts a copy of the electropherogram into the clipboard (single-well view). In multi-well view, all electropherograms are copied.
Save Electropherogram	Opens a system dialog box, allowing you to save the electropherogram (single-well view) or all electropherograms (multi-well view) as an image in JPEG (.jpg), Windows Bitmap (.bmp), Windows Meta File (.wmf), CompuServe Graphics Interchange (.gif), or Tagged Image File (.tif) format.
Manual Integration	Switches to the manual peak integration mode. In this mode, you can: • change the start and end points and the baseline of a certain peak • add or delete certain peaks from the integration. See also "Manual Integration" on page 124.
Automatic Integration	Switches to the automatic peak integration mode (all manual integrations will be lost).
Peak Description	Lets you choose the type of peak labels shown in electropherograms. The labels are only visible in single-well view and if the *Peak Table* sub-tab is selected.

Electropherogram menu item	Function
Overlaid Samples	Lets you overlay electropherograms from multiple wells (single-well view only). Each electropherogram will be shown in a different color and a color legend appears on the *Legend* sub-tab. • *Sample 1...12* selects an individual sample to be overlaid with the current sample (which is grayed out). • *Ladder* overlays the electropherogram of the ladder well. • *No Overlay* undoes overlaying. • *All Samples* overlays all samples of the chip. Overlaying samples can also be done by CTRL- or Shift-clicking gel lanes in the lower panel (see "Lower Panel" on page 398).

Histogram Menu

Single View



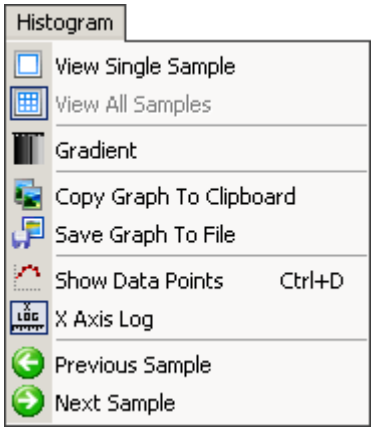
Histogram menu item	Function
View Single Sample	Displays the current histogram in single view.
View All Samples	Switches to the grid view and highlights the current histogram.
Undo Zoom	Undoes the last zoom action.
Undo All	Undoes all zoom actions.
Scale to all signals	Adapts the scales of the X and the Y axis to all overlaid graphs. All curves will be completely visible in overlaid graphs.
Gradient	Puts a gray-to-white gradient on the background of the histogram(s), or removes the gradient.
Copy Graph To Clipboard	Puts a copy of the selected histogram (blue or red) into the clipboard.
Copy Graph To File	Opens a system dialog box, allowing you to save the selected histogram (blue or red) as an image in Windows Meta File (.wmf), Windows Bitmap (.bmp), or JPEG (.jpg) format.
Show Data Points	Shows/hides the data points used to generate the selected histogram.
X Axis Log	Switches the X scale between linear and logarithmic gradation.

Histogram menu item	Function
Overlaid Samples	Lets you overlay histograms from multiple samples (single view only). Each histogram will be shown in a different color and a color legend appears above the graph. • *Sample 1...6* selects an individual sample to be overlaid with the current sample (which itself is not available in the menu). • *No Overlay* undoes overlaying. • *All Samples* overlays all samples of the chip.
Insert Marker	Inserts a marker into the histogram.
Insert Marker into all histograms	Copies the selected marker in all other histograms of the chip run (generic assays only).
Insert Existing Marker	Opens the *Insert Existing Markers* dialog box (see "Insert Existing Markers" on page 549), which allows you to insert existing markers from other histograms of the chip run in the current histogram (generic assays only).
Configure Marker	Opens the *Configure Marker* dialog box (see "Configure Marker" on page 545), which allows you to change the properties of the selected marker (generic assays only).
Delete Marker	Deletes the selected marker. If the marker is also used in other histograms, you will be asked whether to remove it from all histograms that use it or only from the current histogram.

Histogram menu item	Function
Gate in Red histogram	Uses the selected marker of the blue histogram for gating in the red histogram (generic assays only).
Insert Gate into All Red histograms	Uses the selected marker of the blue histogram for gating in all red histograms (generic assays only).
Gate in blue histogram	Uses the selected marker of the red histogram for gating in the blue histogram (generic assays only).
Insert Gate into All Blue histograms	Uses the selected marker of the red histogram for gating in all blue histograms (generic assays only).
Remove Gate	Deletes the gate (only from the current histogram).
Previous Sample	Highlights and displays the histogram of the previous sample. Not available if the first sample is selected.
Next Sample	Highlights and displays the histogram of the next sample. Not available if the last sample is selected.

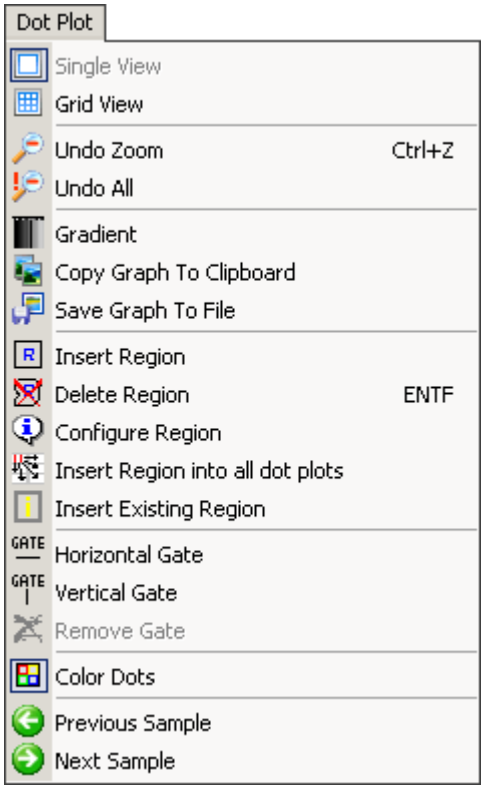
Grid View

In grid view, the *Histogram* menu has the following commands, which work the same way as in single view (see above):



Dot Plot Menu

Single View

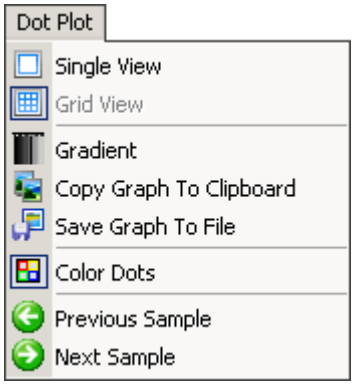


Dot Plot menu item	Function
Single View	Displays the current dot plot in single view.
Grid View	Switches to the grid view and highlights the current dot plot.
Undo Zoom	Undoes the last zoom action.
Undo All	Undoes all zoom actions.
Gradient	Puts a gray-to-white gradient on the background of the selected dot plot, or removes the gradient.
Copy Graph To Clipboard	Puts a copy of the selected dot plot into the clipboard.
Copy Graph To File	Opens a system dialog box, allowing you to save the selected dot plot as an image in Windows Meta File (.wmf), Windows Bitmap (.bmp), or JPEG (.jpg) format.
Insert Region	Enters the region drawing mode, allowing you to draw a new region in the dot plot. The mouse cursor changes its shape to a crosshair.
Delete Region	Deletes the selected region. If the region is also used in other dot plots, you will be asked whether to remove it from all dot plots that use it or only from the selected dot plot.
Configure Region	Opens the *Configure Region* dialog box (see "Configure Region" on page 547), which allows you to change the properties of the selected region (generic assays only).

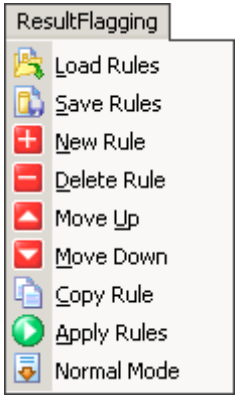
Dot Plot menu item	Function
Insert Region into all dot plots	Copies the selected region into all other dot plots of the chip run (generic assays only).
Insert Existing Region	Opens the *Insert Existing Region* dialog box (see "Insert Existing Region" on page 551), which allows you to insert existing regions from other dot plots of the chip run in the current dot plot (generic assays only).
Horizontal Gate	Inserts a horizontal gate for the selected region (generic assays only).
Vertical Gate	Inserts a vertical gate for the selected region (generic assays only).
Remove Gate	Removes the gate.
Color Dots	Colors the dots inside the selected region using the color of the boundaries of the region.
Previous Sample	Highlights and displays the dot plot of the previous sample. Not available if the first sample is selected.
Next Sample	Highlights and displays the dot plot of the next sample. Not available if the last sample is selected.

Grid View

In grid view, the *Dot Plot* menu has the following commands, which work the same way as in single view (see above):



Result Flagging Menu (Electrophoretic Assays only)



Result flagging menu item	Function
Load Rules	Opens a system dialog box allowing you to load a set of result flagging rules stored in an .xml) file.
Save Rules	Opens a system dialog box allowing you to save the result flagging rules in an .xml) file.
New Rule	Adds a new rule.
Delete Rule	Deletes the selected rule.
Move Up	Moves the selected rule up one row.
Move Down	Moves the selected rule down one row.
Copy Rule	Inserts a copy of the selected rule.
Apply Rules	Applies the result flagging rules to the chip data.
Normal Mode/Target Mode	Switches between *Normal* and *Target* Mode.

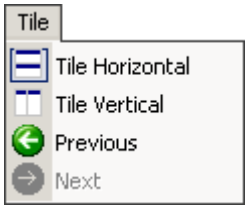
Log Book Menu

Log Book menu item	Function
Find	Opens the *Find* dialog box allowing you to search the run log table for any string.
Filter	Opens the *Filter Events* dialog box allowing you to hide run log table entries (rows) matching filter criteria you can specify.
Reset Filter	Removes any filter you applied to the run log table.

Instrument Menu

Instrument menu item	Function
Start	Starts a chip run. Available if the bioanalyzer is ready to run an assay (bioanalyzer is connected, a suitable chip is loaded, and the lid is closed), or if a demo assay is selected. See "Starting the Chip Run" on page 72.
Stop	Stops the chip run. See "Stopping a Chip Run" on page 77.

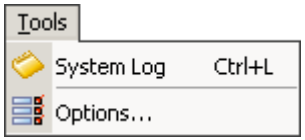
Tile Menu



The *Tile* menu is available if the *Instrument Tab (Grid View)* is displayed:

Tile menu item	Function
Tile Horizontal	Arranges the instrument panes horizontally.
Tile Vertical	Arranges the instrument panes vertically.
Previous	Highlights the previous instrument pane.
Next	Highlights the next instrument pane.

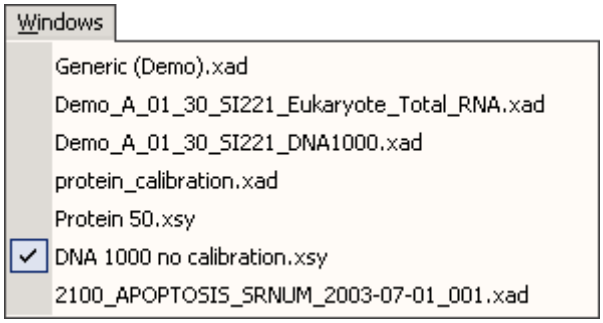
Tools Menu



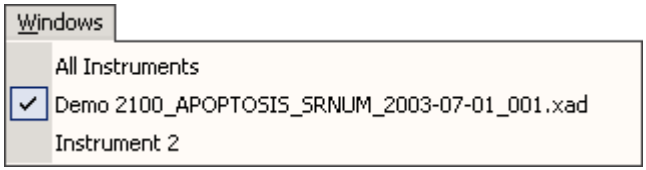
Tools menu item	Function
System Log	Opens the *System Log Viewer* dialog box showing system-wide events in the system log table.
Options...	Opens the *Options* dialog box allowing you to configure the 2100 expert software. This dialog box has the following tabs: • "Options – Data Files" on page 501 • "Options – Chip Alert" on page 504 • "Options – Graph Settings" on page 506 • "Options – Advanced" on page 508

Windows Menu

In the *Data and Assay* context, the *Windows* menu lets you switch between all open chip data (.xad) and assay (.xsy) files:

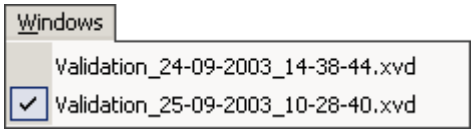


In the *Instrument* context, the *Windows* menu lets you switch between the instrument grid view (*All Instruments*) and all detected instruments:



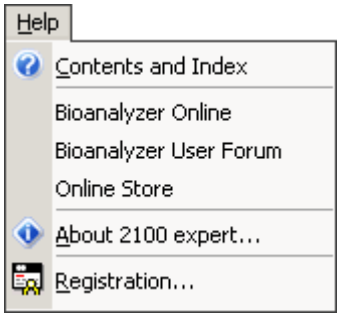
If an assay is already selected for an instrument, the assay name appears instead of the instrument name.

In the *Validation* context, the *Windows* menu lets you switch between all open validation results files (.xvd):



If an assay is already selected for an instrument, the assay name appears instead of the instrument name.

Help Menu



Help menu item	Function
Contents and Index	Opens the home page of the *Agilent 2100 Bioanalyzer Help Desk*.
Bioanalyzer Online	Takes you to the Agilent *Lab-on-a-Chip Products* web pages (Internet connection required).
Bioanalyzer User Forum	Takes you to the *Life Sciences/Chemical Analysis User Forum* (Internet connection and login data required).

Help menu item	Function
Online Store	Takes you to the *Life Sciences/Chemical Analysis Online Store* (Internet connection required).
About 2100 expert...	Displays the *About 2100 Expert* dialog box, which shows information about the system and the software version. Refer to "About 2100 Expert" on page 497.
Registration...	Opens the *License Administration Tool* dialog box, allowing you to register the software components which you have licensed.

Toolbars

The toolbars are located below the menu bar and give you quick access to often required functions.

TIP

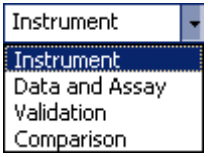
Move the mouse cursor onto a toolbar button and wait a moment. This will display a tool tip that briefly describes its function.

If a toolbar button is dimmed then its function is not available at the moment.

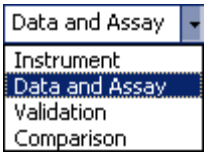
The toolbars strongly vary depending on the context and on what you have currently selected.

- "Instrument Context Toolbar" on page 368
- "Data and Assay Context – Electrophoresis Toolbar" on page 369
- "Data and Assay Context – Flow Cytometry Toolbar" on page 376
- "Data and Assay Context – Result Flagging Toolbar" on page 381
- "Data and Assay Context – Log Book Toolbar" on page 382
- "Validation Context Toolbar" on page 383
- "Comparison Context Toolbar" on page 384

Instrument Context Toolbar

Button	Function
	Select an item from this list to switch to another context.
	Brings up the *Open* dialog box, which allows you to load a chip data (.xad) or assay (.xsy) file for your chip run.
	Shows or hides the *Tree View Panel*.
	Displays the *Instrument Tab (Single View)* of the selected instrument.
	Displays the *Instrument Tab (Grid View)*.
	Arranges the instrument panes horizontally.
	Arranges the instrument panes vertically.
	Highlights the previous instrument pane.
	Highlights the next instrument pane.

Data and Assay Context – Electrophoresis Toolbar

Button	Function
	Select an item from this list to switch to another context.
	Brings up the *Open* dialog box, which allows you to load chip data and assay files.
	Saves the current chip data or assay file.
	Opens the *Print* dialog box, allowing you to send chip and assay data to the printer. See "Print (Electrophoresis)" on page 531 and "Print (Flow Cytometry)" on page 528.
	Shows or hides the *Tree View Panel*.
	Shows or hides the *Lower Panel* with chip icon or gel overview image.
	Switches to the gel view, see "Gel Tab" on page 438.
	Switches to the electropherogram view, see "Electropherogram Tab (Single/Grid View)" on page 451.

	Starts automatic analysis. Peaks are detected, and the Peak Table gets calculated. Results are getting recalculated whenever changes to the data analysis setpoints are applied.
	Pauses automatic analysis. It is recommended to pause analysis during manual integration.
	Analysis is switched off. Raw data is displayed on the *Gel* tab and the *Peak Table* is cleared.
	Highlights and displays the gel graph of the previous sample. If the ladder is highlighted, jumps to the last sample. Not available if the first sample is highlighted.
	Highlights and displays the gel graph of the next sample. If the last sample is highlighted, jumps to the ladder. Not available if the ladder is highlighted.
	Undoes the last zoom, pan, or scale action.
	Undoes all zoom, pan, and scale actions.
	Puts a copy of the gel graph (of all samples) into the clipboard.

	Opens a system dialog box allowing you to save the gel graph (all samples) as an image in JPEG (.jpg), Windows Bitmap (.bmp) or Tagged Image File Format (.tif) format.
	Opens a submenu from which you can select a predefined foreground/background color scheme to be used for displaying the gel graph (all lanes). The colors are designed to approximate various actual gel staining and imaging techniques. *Blue on White*, for example, simulates a Coomassie gel often used with proteins.
	Opens a submenu from which you can select one of the following modes:
	Opens a submenu from which you can select one of the following modes: • *Individual Scale* Each lane uses its own scaling which is optimized for that lane. • *Selected Scale* All lanes use the same scaling which is optimized for the selected lane. • *Global Scale*. • All lanes use the same scaling.

Electropherogram View

	Starts automatic analysis. Peaks are detected, and the *Peak Table* gets calculated. Results are getting recalculated whenever changes to the data analysis setpoints are applied.
	Pauses automatic analysis. It is recommended to pause analysis during manual integration.
	Analysis is switched off. Raw data is displayed on the *Electropherogram* tab and the *Peak Table* is cleared.
	Highlights and displays the electropherogram of the previous sample. If the ladder is highlighted, jumps to the last sample. Not available if the first sample is highlighted.
	Highlights and displays the electropherogram of the next sample. If the last sample is highlighted, jumps to the ladder. Not available if the ladder is highlighted.
	Displays the current electropherogram in single-well view.
	Switches to the multi-well view and highlights the current electropherogram.
	Undoes the last zoom, pan, or scale action.
	Undoes all zoom, pan, and scale actions.

Lets you select one of the following scaling modes:

- *Individual Scale*

  Each graph uses its own scaling which is optimized for that electropherogram.

- *Selected Scale*

  All graphs use the same scaling which is optimized for the selected graph.

- *Global Scale* (only available in grid view).

  All graphs use the same scaling which is chosen such that all electropherograms fit into the display.

Lets you select one of the following click-and-drag operations on electropherograms:

- *Zoom*

  Magnifies the corresponding region to the full display.

- *Pan*

  Moves the graph, makes especially sense for already zoomed graphs.

- *Scale*

  Dragging left or down shrinks the display in that direction, dragging right or up magnifies the display in that direction.

If this option is enabled, then all zoom, pan and scale steps are effective in all electropherograms.

Shows/hides the data points used to generate the electropherogram. Data points are visible only in the single-well view. Data points are 0.05 seconds apart as the time resolution of the data points is 0.05 seconds, which corresponds to their data acquisition rate (of 20 Hz).

Shows/hides grid lines (single-well view only).

Puts a copy of the electropherogram into the clipboard (single-well view). In multi-well view, all electropherograms are copied.

Opens a system dialog box allowing you to save the electropherogram (single-well view) or all electropherograms (multi-well view) as an image in JPEG (.jpg), Windows Bitmap (.bmp), Windows Meta File (.wmf), CompuServe Graphics Interchange (.gif), or Tagged Image File (.tif) format.

Switches to the manual peak integration mode.

In this mode, you can:

- change the start and end points and the baseline of a certain peak
- add or delete certain peaks from the integration.

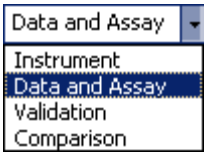
See also "Manual Integration" on page 124.

Switches to the automatic peak integration mode (all manual integrations will be lost).

Lets you choose the type of information peak labels show in electropherograms. The labels are only visible in single-well view and if the *Peak Table* sub-tab is selected.

Overlaid Samples	Lets you overlay electropherograms from multiple wells (single-well view only). Each electropherogram will be shown in a different color and a color legend appears on the *Legend* sub-tab. • *Sample 1...12* selects an individual sample to be overlaid with the current sample (which is gray out). • *Ladder* overlays the electropherogram of the ladder well. • *No Overlay* undoes overlaying. • *All Samples* overlays all samples of the chip. Overlaying samples can also be done by CTRL- or Shift-clicking gel lanes in the lower panel (see "Lower Panel" on page 398).

Data and Assay Context – Flow Cytometry Toolbar

Button	Function
	Select an item from this list to switch to another context.
	Brings up the *Open* dialog box which allows you to load chip data and assay files.
	Saves the current chip data or assay file.
	Opens the *Print* dialog box, allowing you to send chip and assay data to the printer. See "Print (Electrophoresis)" on page 531 and "Print (Flow Cytometry)" on page 528.
	Shows or hides the *Tree View Panel*.
	Shows or hides the *Lower Panel* with chip icon or gel overview image.
	Switches to the histogram view, see "Histogram Tab (Single/Grid View)" on page 460.
	Switches to the dot plot view, see "Dot Plot Tab (Single/Grid View)" on page 468.

	Displays the current histogram in single view.
	Switches to the grid view and highlights the current histogram.
	Undoes the last zoom action.
	Undoes all zoom actions.
	Adapts the scales of the X and the Y axis to all overlaid histograms. All curves will be completely visible in overlaid histograms.
	Puts a copy of the selected histogram (blue or red) into the clipboard.
	Opens a system dialog box allowing you to save the selected histogram (blue or red) as an image in Windows Meta File (.wmf), Windows Bitmap (.bmp), or JPEG (.jpg) format.
	Switches the X scale between linear and logarithmic gradation.

Overlaid Samples	Lets you overlay histograms from multiple samples (single view only). Each histogram will be shown in a different color and a color legend appears above the graph. • *Sample 1...6* selects an individual sample to be overlaid with the current sample (which itself is not available in the menu). • *No Overlay* undoes overlaying. • *All Samples* overlays all samples of the chip.
	Inserts a marker into the histogram.
	Copies the selected marker in all other histograms of the chip run (generic assays only).
	Opens the *Insert Existing Markers* dialog box (see "Insert Existing Markers" on page 549), which allows you to insert existing markers from other histograms of the chip run in the current histogram (generic assays only).
	Opens the *Configure Marker* dialog box (see "Configure Marker" on page 545), which allows you to change the properties of the selected marker (generic assays only).
	Deletes the selected marker. If the marker is also used in other histograms, you will be asked whether to remove it from all histograms that use it or only from the current histogram.
	Uses the selected marker of the blue histogram for gating in the red histogram (generic assays only).

	Uses the selected marker of the blue histogram for gating in all red histograms (generic assays only).
	Uses the selected marker of the red histogram for gating in the blue histogram (generic assays only).
	Uses the selected marker of the red histogram for gating in all blue histograms (generic assays only).
	Removes the gate (only from the current histogram).
	Highlights and displays the histogram of the previous sample. Not available if the first sample is selected.
	Highlights and displays the histogram of the next sample. Not available if the last sample is selected.

Dot Plot View

	Displays the current dot plot in single view.
	Switches to the grid view and highlights the current dot plot.
	Undoes the last zoom action.
	Undoes all zoom actions.

	Puts a copy of the selected dot plot into the clipboard.
	Opens a system dialog box allowing you to save the selected dot plot as an image in Windows Meta File (.wmf), Windows Bitmap (.bmp), or JPEG (.jpg) format.
	Enters the region drawing mode allowing you to draw a new region in the dot plot. The mouse cursor changes its shape to a crosshair.
	Deletes the selected region. If the region is also used in other dot plots you will be asked whether to remove it from all dot plots that use it or only from the selected dot plot.
	Opens the *Configure Region* dialog box (see "Configure Region" on page 547), which allows you to change the properties of the selected region (generic assays only).
	Copies the selected region into all other dot plots of the chip run (generic assays only).
	Opens the *Insert Existing Region* dialog box (see "Insert Existing Region" on page 551), which allows you to insert existing regions from other dot plots of the chip run in the current dot plot (generic assays only).
	Inserts a horizontal gate for the selected region (generic assays only).
	Inserts a vertical gate for the selected region (generic assays only).
	Removes the gate.

	Colors the dots inside the selected region using the color of the region border.
	Highlights and displays the dot plot of the previous sample. Not available if the first sample is selected.
	Highlights and displays the dot plot of the next sample. Not available if the last sample is selected.

Data and Assay Context – Result Flagging Toolbar

Button	Function
	Opens a system dialog box allowing you to load a set of result flagging rules stored in an .xml) file.
	Opens a system dialog box allowing you to save the result flagging rules in an .xml) file.
	Adds a new rule.
	Deletes the selected rule.
	Moves the selected rule up one row.
	Moves the selected rule down one row.

Button	Function
	Inserts a copy of the selected rule.
	Validates the result flagging rules and applies them to the chip data (if any).
	Switches between *Normal* and *Target* Mode.

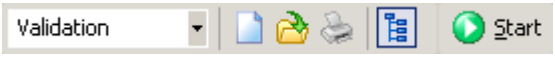
Data and Assay Context – Log Book Toolbar

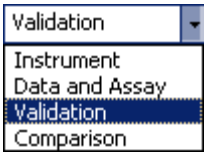
Button	Function
	Opens the *Find* dialog box allowing you to search the run log table for any string.
	Opens the *Filter Events* dialog box allowing you to hide run log table entries (rows) matching filter criteria you can specify.
	Removes any filter you applied to the run log table.

NOTE

The *Log Book* toolbar is also available in the *System Log Viewer* dialog box. See "System Log Viewer" on page 561.

Validation Context Toolbar



Button	Function
	Select an item from this list to switch to another context.
	Begins a new validation and inserts a *New Validation* item in the tree view.
	Brings up a system dialog box, which allows you to open a validation results (.xvd) file.
	Displays the *Print (Validation)* dialog box, allowing you to generate various reports on qualification tests. Refer to "Print (Validation)" on page 535.
	Shows or hides the *Tree View Panel*.
	Starts a validation run. Only available if at least one qualification test is selected for execution.
	Stops the qualification test that is currently in progress. Tests that are already completed are not affected.

Comparison Context Toolbar

Button	Function
	Select an item from this list to switch to another context.
	Brings up a system dialog box allowing you to open a comparison (.xac) or chip data (.xad) file.
	Saves the current comparison file.
	Displays the *Print (Comparison)* dialog box, allowing you to print a comparison report. Refer to "Print (Comparison)" on page 538.
	Shows or hides the *Tree View Panel*.

NOTE

If a sample is selected in the comparison context, also the *Gel* or *Electropherogram* toolbar is available. See "Data and Assay Context – Electrophoresis Toolbar" on page 369.

Information Bar

The information bar is located below the toolbar.

Instrument context

If no chip run is in progress, the information bar shows the name of the active bioanalyzer (or “Demo” if the *Demo* COM port is selected) followed by the selected assay class:

During a chip run, the information bar additionally shows the message “Running” and a chip symbol with a flashing number (number of sample currently being measured):

After a chip run, you see messages such as “Run aborted by user”, or “Run complete”.

If you see the message “Invalid Configuration Selected”, no bioanalyzer was detected at the specified *COM Port*, or the inserted chip does not suit the *Assay Selection*:

If the 2100 expert software detects an error, a message appears on the *Information Bar*:

Click on the error message text to get help on the message.

Data and Assay context

If the *Gel*, *Electropherogram, Histogram* or *Dot Plot* tab is selected, you see the tab name followed by the sample name (in single view) or “All Samples” (in grid view). On the right, you see a chip symbol with the current sample number on it:

If you have loaded a flow cytometry chip data file or assay, the gating direction (blue to red or red to blue) is shown:

Validation context

If a new validation item has been created the information bar looks as follows:

During a validation run, the name of the validation results file is shown on the left. On the right, you see which test is currently running:

When the validation run is finished, the information bar looks as follows:

If you stopped a validation run, the information bar looks as follows:

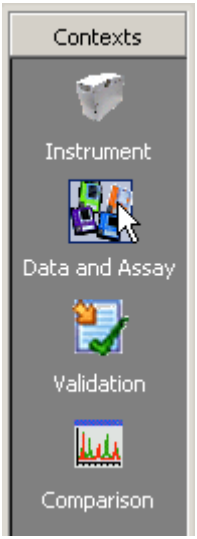
If you re-open a validation results file for review, no modifications are possible. This is indicated on the information bar.

Comparison context

If the *Gel* or *Electropherogram* tab is selected, you see the tab name followed by the name of the selected sample:

Context Bar

The context bar is located on the left-hand side of the application window.



By clicking the icons on the context bar, you can switch between the contexts:

- Instrument Context (see "Instrument Context" on page 36)
- Data and Assay Context (see "Data and Assay Context" on page 38)
- Validation Context (see "Validation Context" on page 39)
- Comparison Context (see "Comparison Context" on page 40)

TIP

To get more screen space in the work area, you can hide the context bar by selecting *View* > *Context Bar*.

Tree View Panel

The tree view panel is located on the left-hand side of the application window.

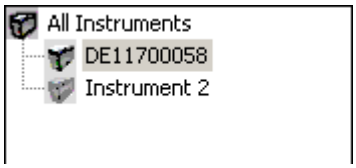
TIP

To get more screen space in the work area, you can hide the tree view panel by selecting *View* > *Tree View*.

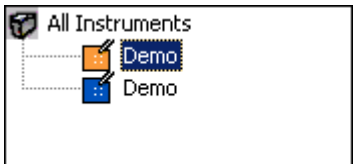
Instrument Context

In the *Instrument* context, the tree view shows as many instruments as are detected.

If an instrument is detected, its serial number is shown:

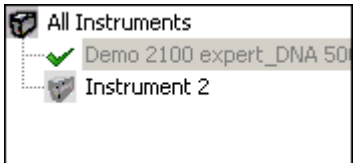


If a chip is detected in the instrument, a chip icon appears in front of the item, identifying the chip type. If “Demo” is selected as the *COM Port*, the item is labeled “Demo”.

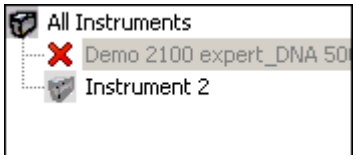


If an assay is selected to be run on the instrument, or while the assay or demo assay is running, the item will be labeled with the name of the chip data file (.xad).

When a chip run has finished, a green checkmark appears:



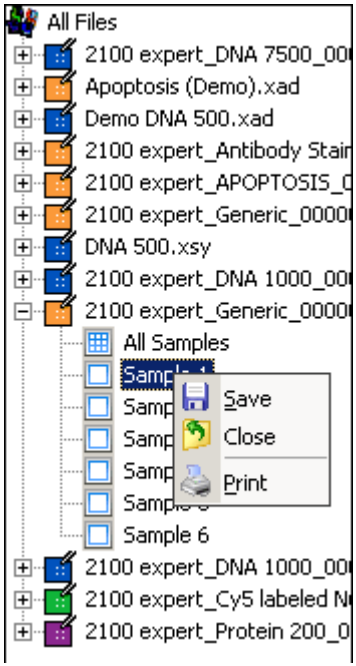
If the run was aborted or an error occurred, a red cross appears instead:



- Clicking on *All Instruments* takes you to the *Instrument Tab (Grid View)*.
- Clicking on an instrument item displays the *Instrument Tab (Single View)*.

Data and Assay Context

In the *Data and Assay* context you can use the tree view to navigate through all open chip data (.xad) and assay (.xsy) files.



NOTE

.xad files that have been opened in the *Comparison* context also appear in the tree view.

The colors of the chip icons next to the file names identify the assay type: blue = DNA, green = RNA, violet = protein, orange = flow cytometry.

- Clicking a plus or minus sign next to a chip icon expands or collapses the sample list.
- Clicking a chip data or assay item displays the *Chip Summary Tab*.
- Clicking an *All Samples* item displays the grid view showing gel views, electropherograms, histograms, or dot plots of all samples.
- Clicking a sample item (or the ladder) displays the gel view, the electropherogram (single view), histogram (single view), or dot plot (single view) of this sample.

The tree view also provides a context menu that you can open by right-clicking any item in the tree view. The following functions are available:

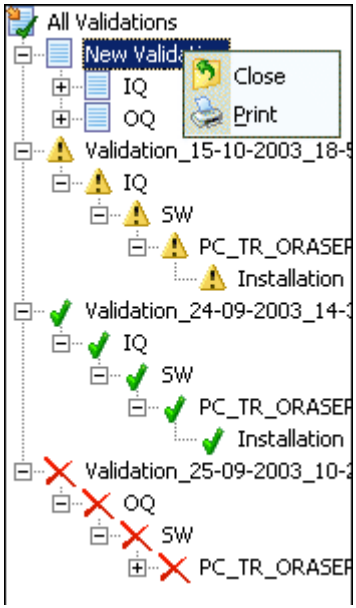
Menu Item	Function
Save	Saves the selected chip data or assay file under its current name.
Close	Closes the selected chip data or assay file.
Print	Displays the *Print* dialog box allowing you to generate various printouts of the selected chip data or assay file. Refer to "Print (Flow Cytometry)" on page 528 and "Print (Electrophoresis)" on page 531.

Validation Context

In the *Validation* context you can use the tree view to:

- Navigate through validation results files.

  Clicking a plus or minus sign next to a chip icon expands or collapses the sample list.
- Navigate through validation test categories (IQ or OQ, software or hardware) and tests.



The symbols have the following meaning:

- Blue document symbols indicate that qualification tests have not yet been run.
- Green checkmarks indicate passed tests.
- Red crosses indicate tests that failed or were aborted.
- Yellow warning triangles indicate mixed results after multiple execution.

NOTE

Items “inherit” the symbol (status) of their “child(s)”. For example, if a single test fails, the whole validation will be marked with a red cross (failed).

A context menu appears if you right-click any item. The following functions are available:

Menu Item	Function
Close	Closes the selected validation results file.
Print	Displays the *Print* dialog box allowing you to generate a validation report. Refer to “Print (Validation)” on page 535.

Comparison Context

In the *Comparison* context, the tree view is divided into two parts.

In the *upper* part, you can:

- Navigate through comparison (.xac) files.
- Navigate through samples (belonging to comparison files).
- Remove samples from comparison files.

In the lower part (*Select Data Files* area), you can:

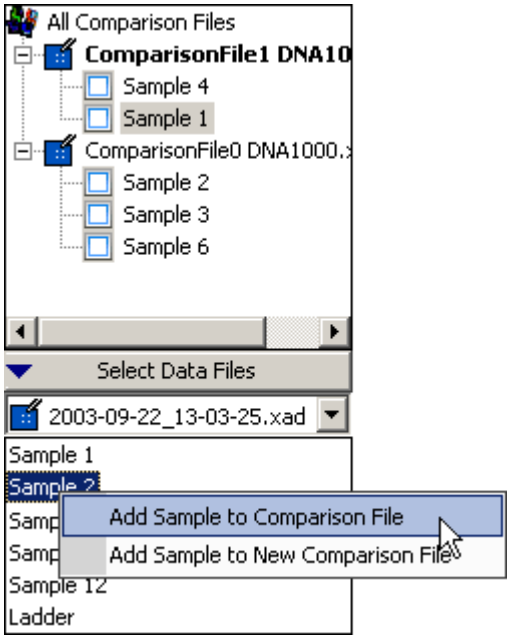
- Select a chip data (.xad) file.

  The *Select Data Files* list contains all electrophoretic .xad files that have been opened using the *File* > *Open...* command.

NOTE

The *Select Data Files* list also contains all electrophoretic .xad files that have been opened in the *Data and Assay* context.

- Navigate through the samples of the selected chip data file.
- Add samples to new or existing comparison files.



Both the upper and the lower part provide context menus.

In the *upper* part of the tree view, right-clicking a xac. file name opens a context menu with the following functions

Menu Item	Function
Save	Saves the selected comparison file under its current name.
Close	Closes the selected comparison file.
Print	Displays the *Print* dialog box allowing you to generate various printouts of the selected comparison file. Refer to "Print (Comparison)" on page 538.

If you right-click a sample name in the *upper* part of the tree view, the context menu has the following command:

Menu Item	Function
Delete Sample from Comparison File	Removes the selected file from the comparison file.

In the *lower* part of the tree view, right-clicking a sample name opens a context menu with the following functions:

Menu Item	Function
Add Sample to Comparison File	Adds the selected sample to the comparison file that is currently selected in the *upper* part of the tree view.
Add Sample to New Comparison File	Creates a new comparison file and adds the selected sample to it.

TIP

Double-clicking a sample name in the *lower* part of the tree view or dragging a sample name into the tree view adds the sample to the comparison file that is currently selected in the *upper* part of the tree view. Or, if no comparison file is selected, creates a new comparison file and adds the sample to it.

Lower Panel

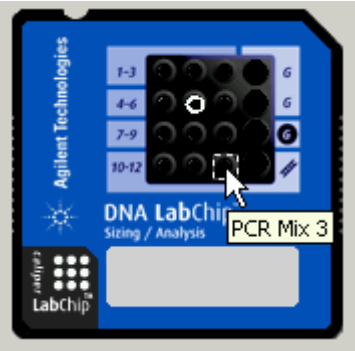
The lower panel is located in the lower left corner of the application window. It is available in the *Data and Assay* context, where you can use it to navigate through your samples.

TIP

To get more screen space for the *Tree View Panel*, you can hide the lower panel by selecting *View* > *Lower Panel*.

Chip Icon

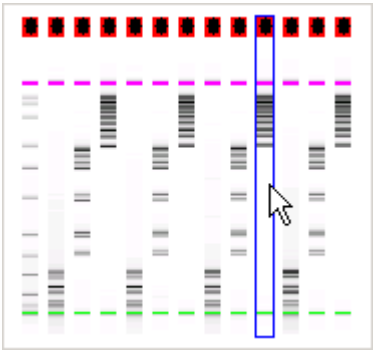
If the *Gel Tab*, *Histogram Tab (Single/Grid View)*, or *Dot Plot Tab (Single/Grid View)* is displayed, a chip icon is visible on the lower panel. This chip icon is more than just a picture: the currently selected well has a white circle around it. A DNA chip is shown in the following example; other assay types will show a different color and type of chip.



Clicking a well on the chip icon will update the gel view, reflecting the new well choice. For a cell chip, this applies to the histogram and dot plot views.

Small Gel View

If the *Electropherogram Tab (Single/Grid View)* is displayed, the lower panel shows a small gel view (all wells).



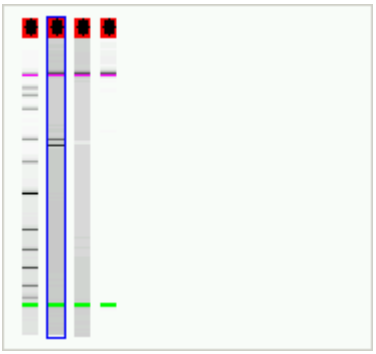
One lane of the small gel view is marked with a solid rectangle. This is the selected lane, which corresponds to a well of the chip.

Clicking on a different lane will cause that lane to be selected and the electropherogram graph will update to display and highlight the associated electropherogram.

Clicking on different lanes with the CTRL key pressed overlays the electropherograms of the selected lanes. Overlaid lanes are marked with a dotted box.

The small gel view is synchronized with the *Electropherogram Tab (Single/Grid View)*. This means, if you select or overlay samples using the *Electropherogram Menu*, for example, the associated lanes in the small gel view are marked accordingly.

When a new chip run begins, the small gel view is blank and the first lane—the ladder well—is selected. As data is acquired, the selection rectangle around the lane will step from left to right and highlight the lane that is currently acquiring data:



However, if you select a lane/well that is earlier in sequence than the current well, the highlight will no longer change as new samples are measured but will remain on the selected lane.

Tabs

The main working area contains several tabs. These tabs are specific to the context you are working in:

- Tabs (Instrument Context), see "Tabs (Instrument Context)" on page 401)
- Tabs (Data and Assay Context), see "Tabs (Data and Assay Context)" on page 417)
- Tabs (Validation Context), see "Tabs (Validation Context)" on page 484)
- Tabs (Comparison Context), see "Tabs (Comparison Context)" on page 489)

Tabs (Instrument Context)

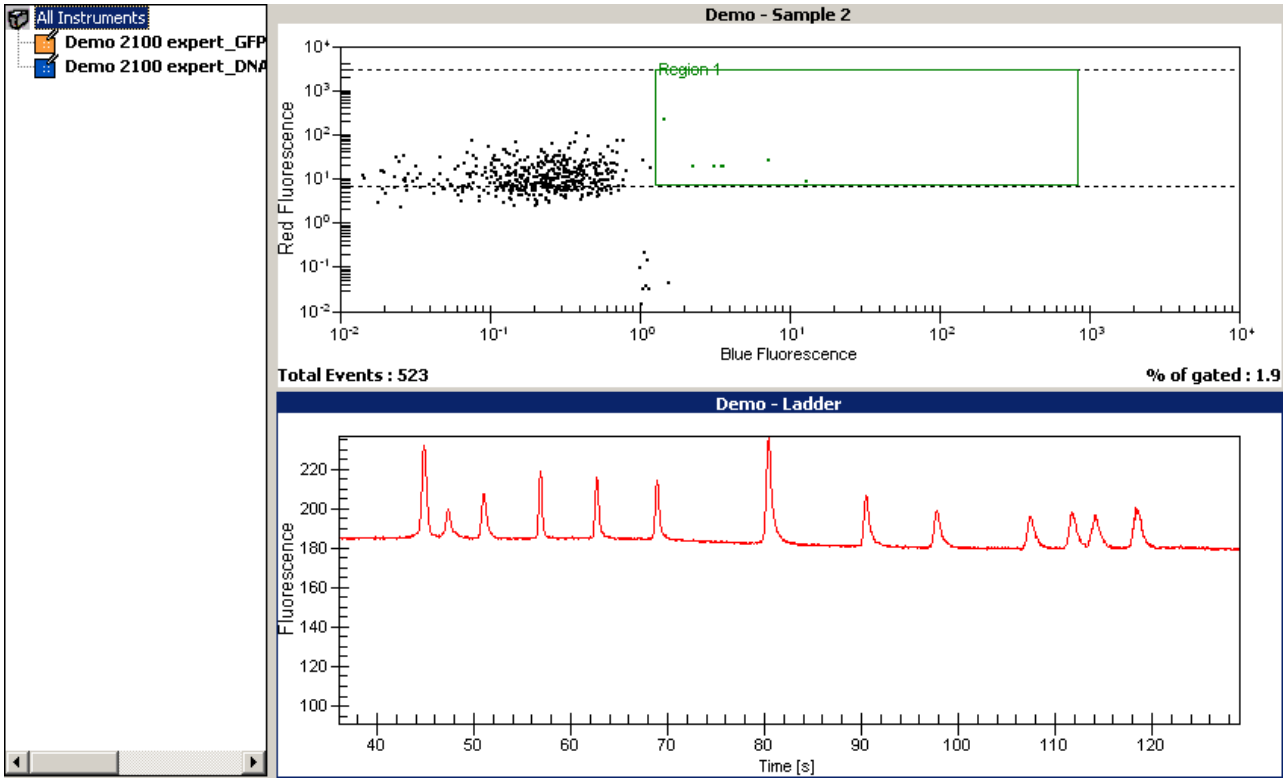
In the *Instrument* context, the following tabs are available:

- *Instrument Tab (Grid View)*
- *Instrument Tab (Single View)*
- *Diagnostics Tab*

Instrument Tab (Grid View)

Purpose

Shows “live” electropherograms and/or dot plots, allowing you to watch data acquisition on two bioanalyzers in parallel.



Elements

Both panels are labeled with name of the assay and the currently measured sample.

For a detailed description of the “live” electropherograms and dot plots, refer to “Instrument Tab (Single View)” on page 404.

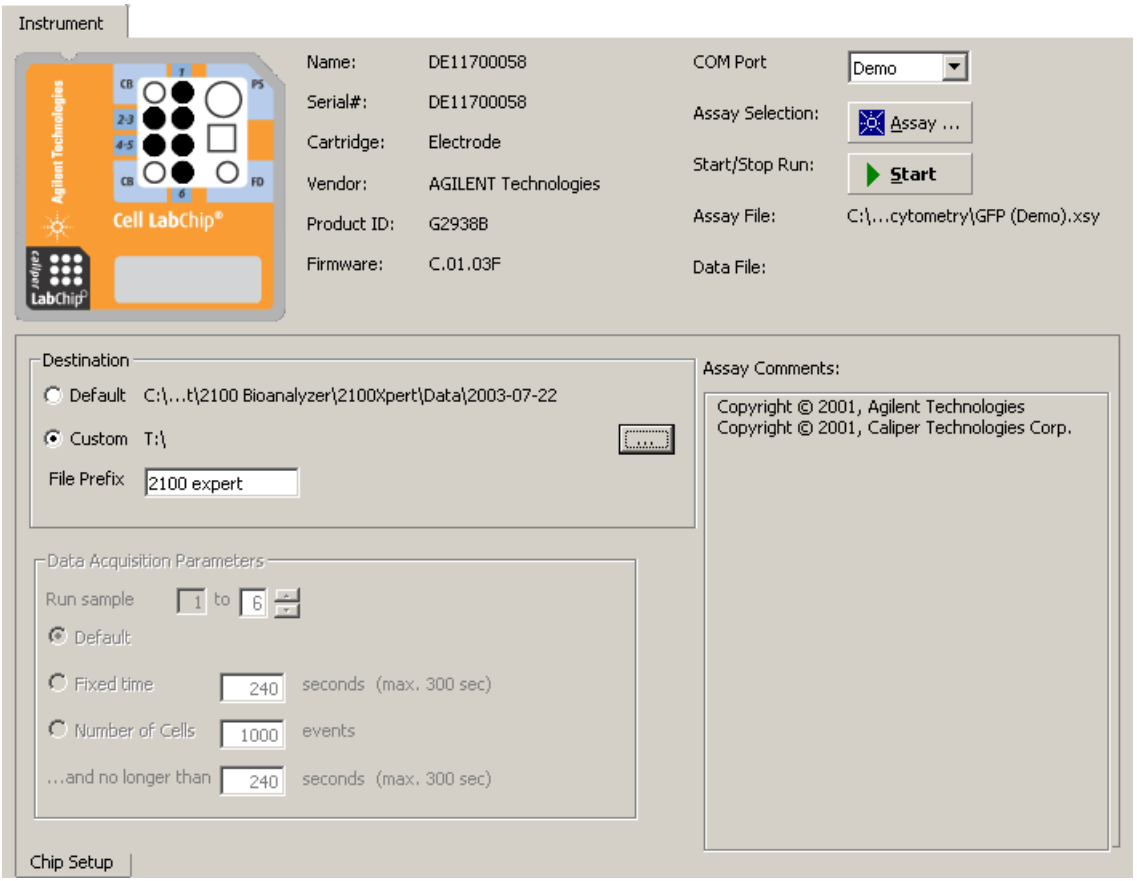
Actions

- Use the toolbar (see “Tile Menu” on page 362) to modify the display.
- Click and drag with the mouse to zoom into the graphs.
- Right-click the panels and use the context menu for further actions (see “Instrument Tab (Single View)” on page 404).
- Double-clicking one of the panels takes you to the *Instrument Tab (Single View)*.

Instrument Tab (Single View)

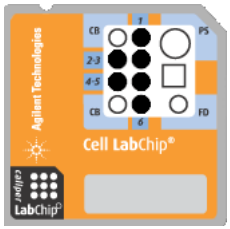
Purpose

The *Instrument* tab lets you configure and start a chip run. During the chip run, you can watch the measurement results (raw data).



Elements

The icon on the left-hand side shows the current status of the Agilent 2100 bioanalyzer:

Icon	Meaning
	A chip is detected and the instrument is ready for measurement. The chip icon depends on the type of assay. The chip icon is more than just a picture: before a chip is run, all the wells on the chip icon are black; during a chip run, a blinking white circle indicates the currently measured well.
	Bioanalyzer detected. Lid is open.
	Bioanalyzer detected. Lid is closed, but no chip is inserted.

Icon	Meaning
	Indicates that no bioanalyzer has been detected. Check the PC's *COM port* settings, the RS 232 connection cable, and make sure that the instrument is powered on. For details, refer to the *Agilent 2100 Bioanalyzer Installation and Safety Guide*.

The following information read from the instrument is for your information:

Name	Meaning
Name	"Friendly" name of the bioanalyzer.
Serial#	Serial number of the bioanalyzer.
Cartridge	Cartridge type, "Electrode" or "Pressure" (see "Switching Between Electrophoretic and Flow Cytometric Assays" on page 47).
Vendor	Manufacturer of the bioanalyzer.
Product ID	Agilent product number of the bioanalyzer.
Firmware File	Version number of the firmware.

The following controls let you select, start, and control a chip run.

Control	Meaning
COM Port	Allows you to select a bioanalyzer by specifying the number of the serial port the instrument is connected to. You can also select *Demo*, if you want to run a demo assay without using a bioanalyzer.
Assay Selection 	Lets you select an assay for the chip run. Only assays suitable for the inserted chip are available. See also "Assays Menu" on page 337.
Start/Stop Run  	Starts the chip run and writes the measurement results to the specified *Data File*. The *Start* button is active if an instrument is connected, a chip has been inserted, and a suitable assay has been selected. When you start a chip run, the button's caption changes to *Stop*, which allows you to abort the chip run.
Assay File	Assay files (.xsy), including path, selected for the chip run.
Data File	Name of the chip data file (.xad) to be generated. Clicking on the file name takes you to the *Data and Assay* context where you can view the chip run results.

Chip Setup Sub-tab

On the *Chip Setup* sub-tab, you can specify a destination for the chip data file, and make data acquisition settings.

Destination

Default	Select *Default* if you want to save the chip run in the default directory. The default directory can be defined in the *Options* dialog box. Refer to "Options – Data Files" on page 501.
Custom	Select *Custom* if you want to save the chip run in a different place. Clicking on the ... button opens a system dialog box allowing you to specify a directory of your choice.
File Prefix	Custom prefix for the chip data file name, overriding any default naming conventions. See "Options – Data Files" on page 501.

Data Acquisition Parameters

Before you start the chip run, you can modify the data acquisition settings of the selected assay:

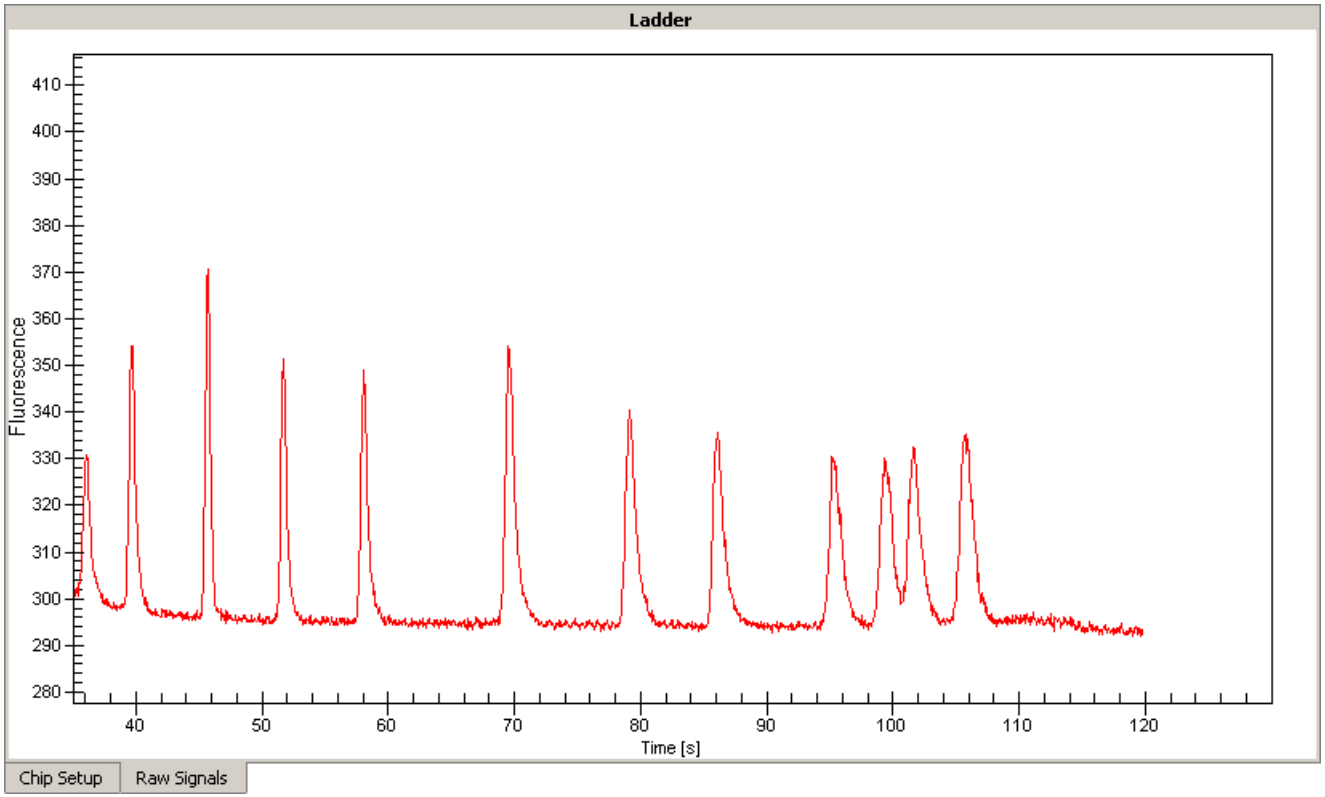
Run sample 1 to n	Defines the sample range to be measured. You can set the range to 1...6 for flow cytometric assays, and to 1...12 for electrophoretic assays.

Further settings can be made for flow cytometric assays:

Default	Enable this option if you want to use the default time for measurement. The total default time for all samples is 240s per sample.
Fixed time	Enable this option if you want to measure each samples within a defined time. Enter the time (in [s]) that you want to be used for each sample. The maximum measurement time per sample is calculated from the number of samples you selected.
Number of Cells	Enter the maximum number of events (cells) per sample to be measured.
... and no longer than	Enter the maximum time the measurement can take (whether or not the number of events is reached). The maximum time per sample is calculated from the number of samples you selected.

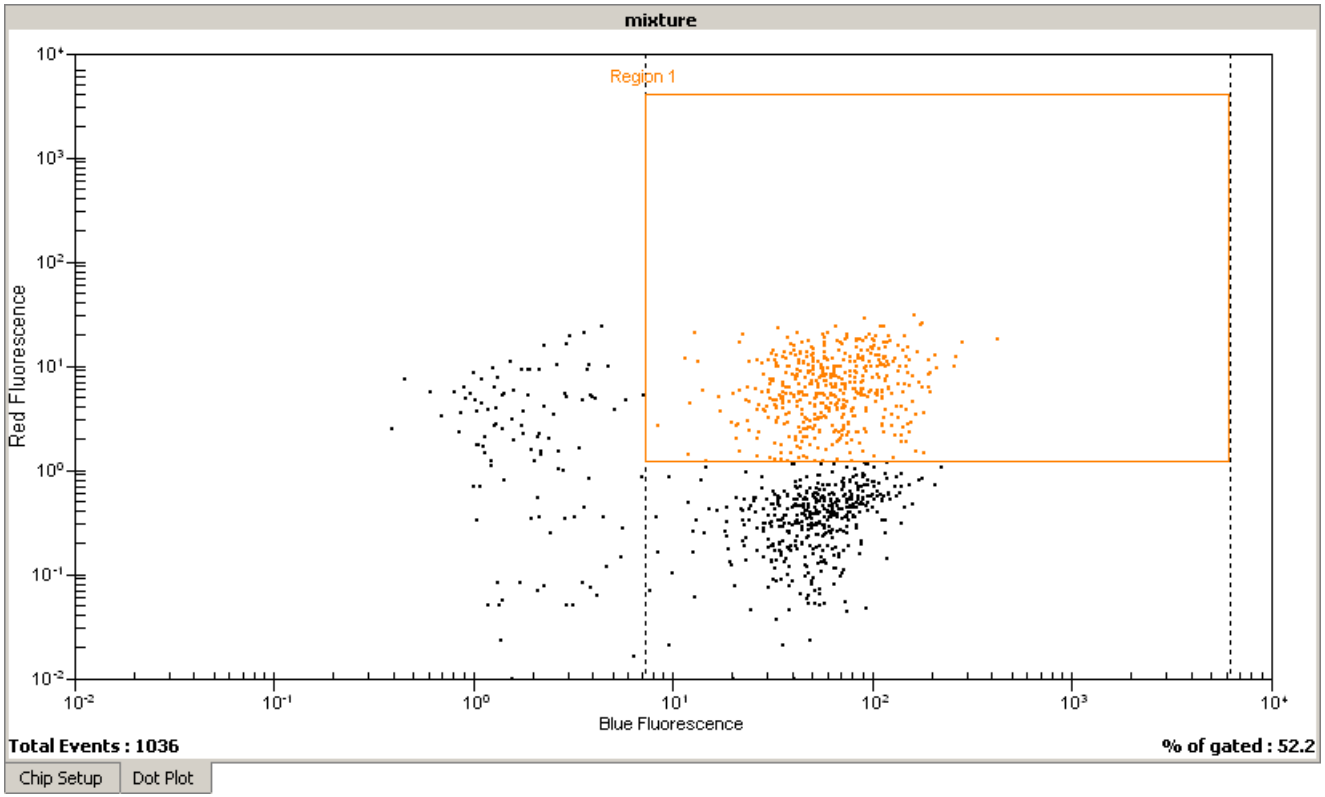
Raw Signals Sub-tab

During an *electrophoretic* chip run, the *Raw Signals* sub-tab shows an electropherogram of the currently measured sample. The name of the sample is displayed above the graph. The graph is a "live" plot of the migration time against fluorescence units (raw data, including background fluorescence, for example).



Dot Plot Sub-tab

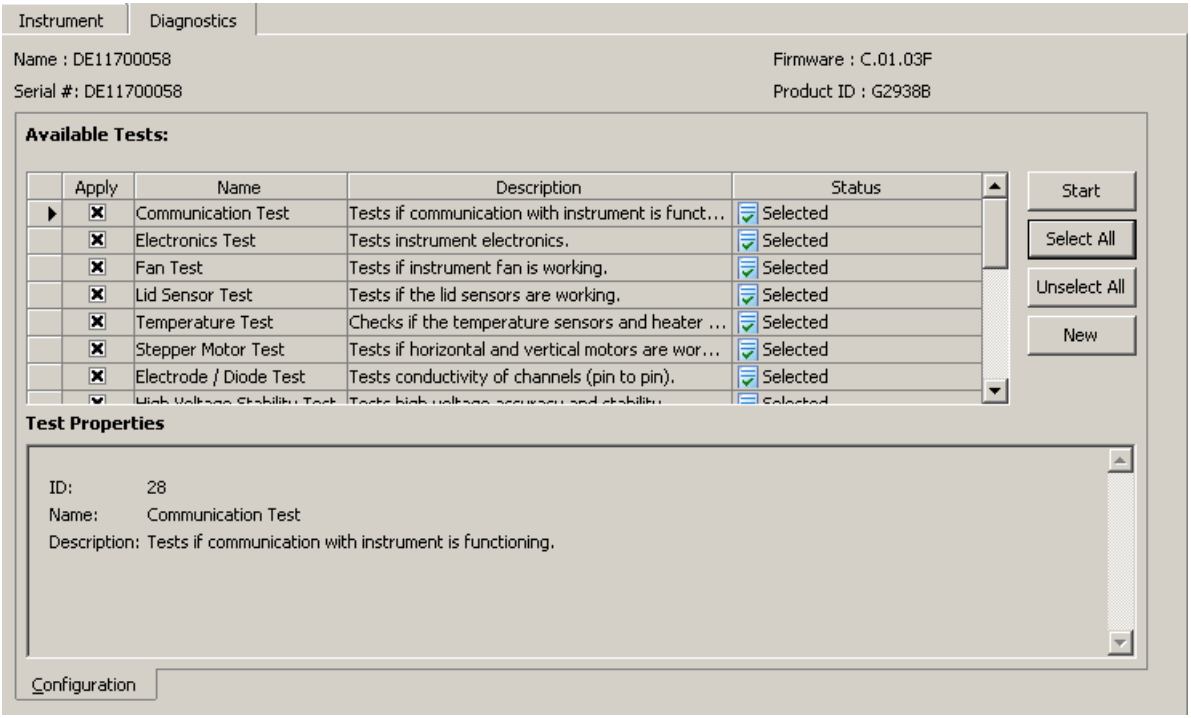
During a *flow cytometric* chip run, the *Dot Plot* sub-tab shows single events (cells) as they are detected, displayed as dots. In the coordinate system, the red and blue fluorescence value of each event can be read. The name of the currently measured sample is displayed above the graph, the number of total events and the percentage of gated events are shown below the graph.



Regions (if defined) are displayed as colored rectangles.

Diagnostics Tab

On the *Diagnostics* tab, you can select and start hardware diagnostics tests on the selected bioanalyzer. The tests check the hardware components and get the current operating status of the instrument.



To perform the complete set of hardware diagnostic tests successfully, you must use unused chips or special chips from the test chip bundles (see "Running Instrument Diagnostics" on page 303).

Instrument Information

At the top of the *Diagnostics* tab, the following information on the selected bioanalyzer is shown:

Serial #	Serial number of the bioanalyzer.
Name	"Friendly" name of the bioanalyzer.
Firmware	Version number of the firmware.
Product ID	Agilent product number of the bioanalyzer.

Available Tests

Columns

Apply	Lets you select the test for execution.
Name	Name of the diagnostics test.
Description	Brief description of the diagnostics test.
Status	For each test, the current status is shown: • *Selected* • *Executing* • *Execution pending* • *Executed, passed* • *Executed, failed*

Bioanalyzer Tests

Electronics Test	Verifies proper functioning of all electronic boards in the bioanalyzer.
Lid Sensor Test	Verifies proper operation of the lid sensor, ensuring that the laser and LED are off when the lid is open.
Stepper Motor Test	Checks for proper movement of the stepper motor.
Fan Test	Checks if the fan is running at the appropriate speed.
Temperature Test	Checks if the temperature ramp-up speed of the heater plate is within specifications.

Electrode Cartridge Tests

Current Leakage Test	Measures electrode cartridge leak current(s) between pins. Leak current test chip required. For correct preparation of the leak current test chip, please refer to the technical note included in the test chip bundle.
Short Circuit Test	Checks for instrument leak currents using an empty chip. Note: the limits of this test specify an ambient temperature of 25 °C and relative humidity less than or equal to 60 %. Higher temperatures or relative humidity could result in a leak current.
Optics Test	Checks for proper alignment of internal optics and proper function of the laser and LED.

High-Voltage Stability Test	Tests high voltage accuracy and stability of all 16 high voltage power supplies. Unused chip (DNA, RNA, or protein) required.
High-Voltage Accuracy Test	Check of the high voltage controller.
High-Voltage Accuracy on Load *Test*	Check of channel-reference diode in transmission direction.
Autofocus Test	Checks the focusing capability of the optical system. Autofocus test chip required.
Laser Stability Test	Measurement of stability of red laser signal.
Electrode/Diode Test	Checks the photo diode and current-versus-voltage performance of the bioanalyzer. Electrode/diode test chip required.

Pressure Cartridge Tests

Pressure Offset Test	The vacuum system of the pressure cartridge consists of a pump and the corresponding tubes. This test calibrates the pressure sensors to zero.
Pressure Control Test	Checks that the bioanalyzer is able to hold the working pressure of -140 mbar. During the test pumps stay on, while the system tries to regulate pressure to be kept at -140 mbar. Cell Autofocus test chip required.

System Leak Test	Checks if the bioanalyzer is able to maintain a vacuum. Produces a test pressure of -100 mbar and monitors for changes. Cell Autofocus test chip required.
Cell Autofocus Test	Checks if the optical system of the bioanalyzer is correctly calibrated. Cell Autofocus test chip required.

Command buttons

Start/Stop	Starts the selected test(s). During test execution, this button changes to *Stop*, which lets you abort the test(s).
Select All	Selects all tests displayed in the list.
Unselect All	Unselects all tests.

Test Properties

In this area, you can see details (such as name, description, limits and requirements) on the test currently highlighted in the *Available Tests* list.

Tabs (Data and Assay Context)

In the *Data and Assay* context, the following tabs are available:

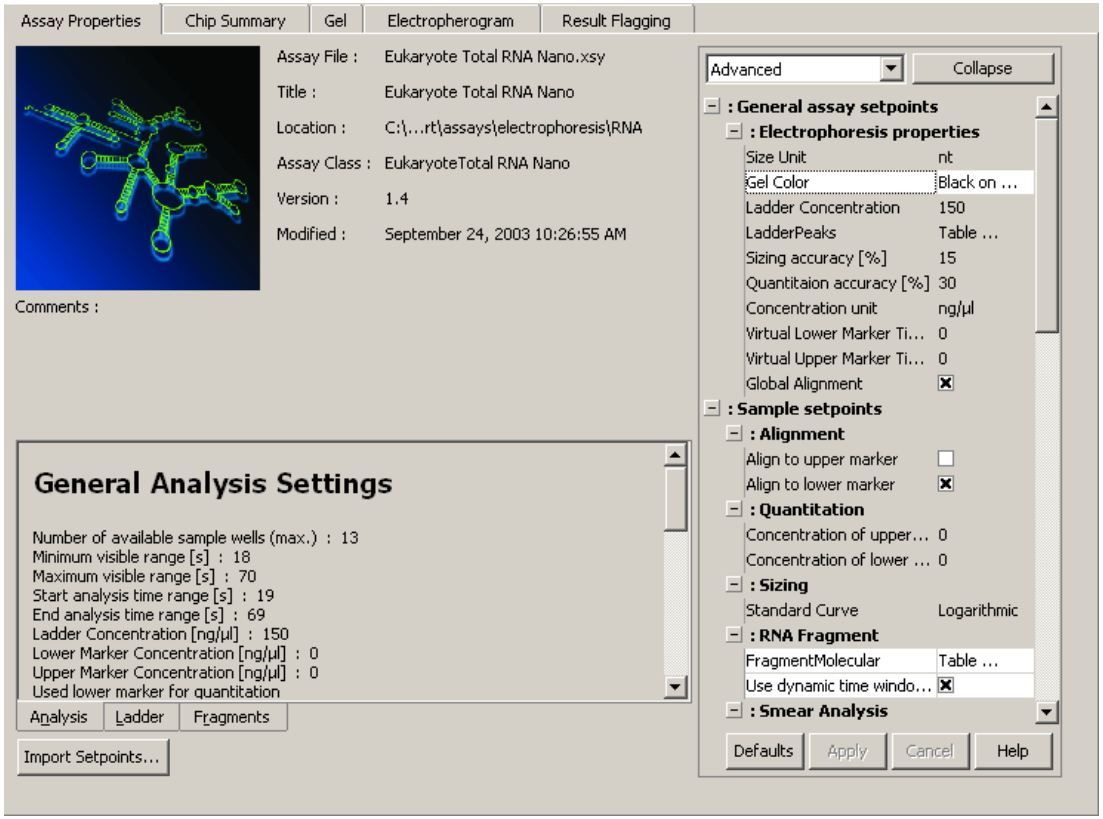
- *Assay Properties Tab* lets you display and modify the properties of the current assay.
- *Chip Summary Tab* allows you to edit the chip data for the current assay.
- *Gel Tab* allows you to evaluate the results of electrophoretic assays using a gel view.
- *Electropherogram Tab (Single/Grid View)* allows you to evaluate the results of electrophoretic assays using electropherograms.
- *Histogram Tab (Single/Grid View)* allows you to evaluate the results of flow cytometric assays using histograms.
- *Dot Plot Tab (Single/Grid View)* allows you to evaluate the results of flow cytometric assays using dot plots.
- *Result Flagging Tab* lets you define and apply rules for assigning color codes to measurement results (electrophoretic assays only).
- *Log Book Tab* informs you about chip run events and system-wide events.

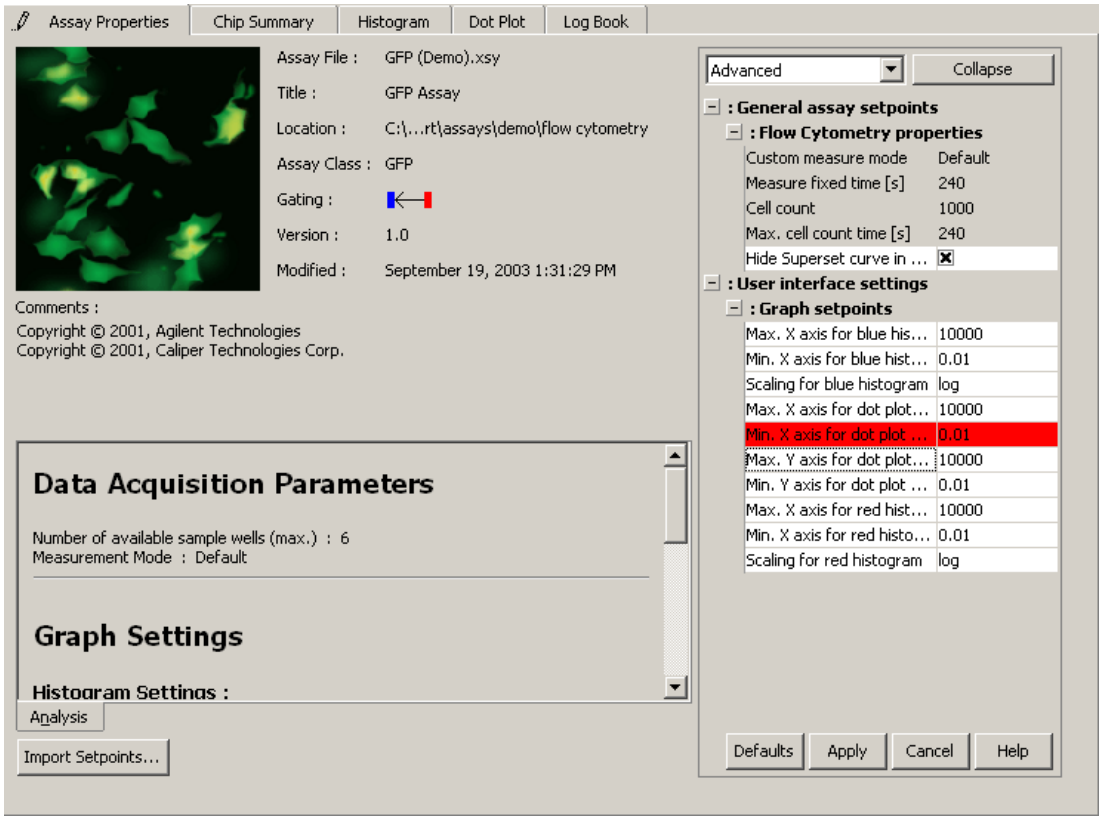
Assay Properties Tab

Purpose

On this tab, you can display and modify parameters (setpoints) of the current assay or chip data file. You can also import markers and regions (flow cytometric assays only), and setpoints of other assay or chip data files (see "Importing Data" on page 256).

Electrophoretic Assays





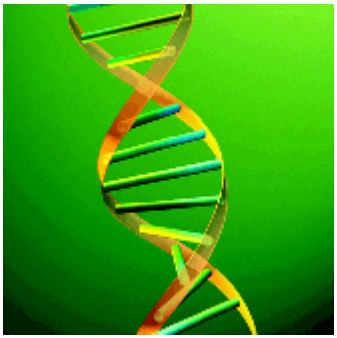
Access

The *Assay Properties* tab is always available in the *Data and Assay* context.

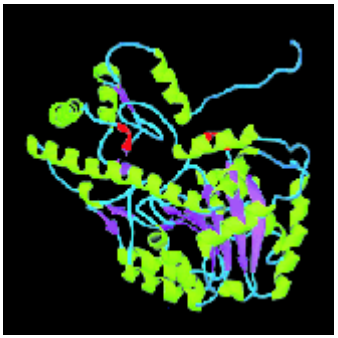
Assay Icon This icon visualizes the assay type.

Electrophoretic Assays

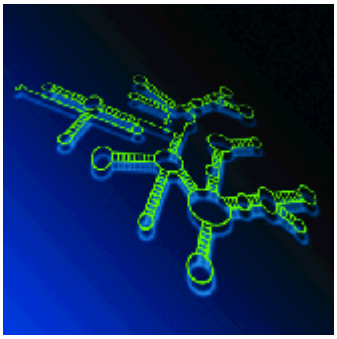
DNA



Protein

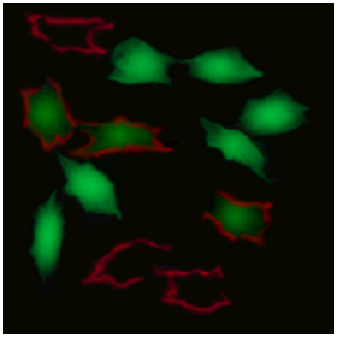


RNA

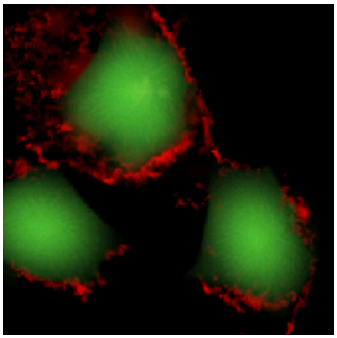


Flow Cytometric Assays

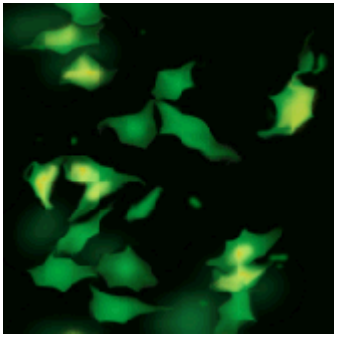
Apoptosis/
Red to Blue



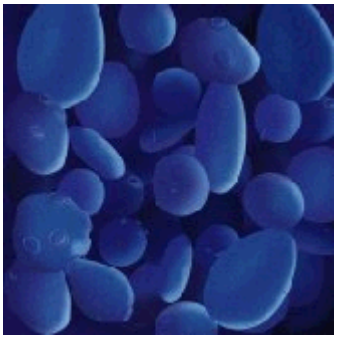
Antibody Staining/
Blue to Red



GFP
(Green Fluorescence Protein)



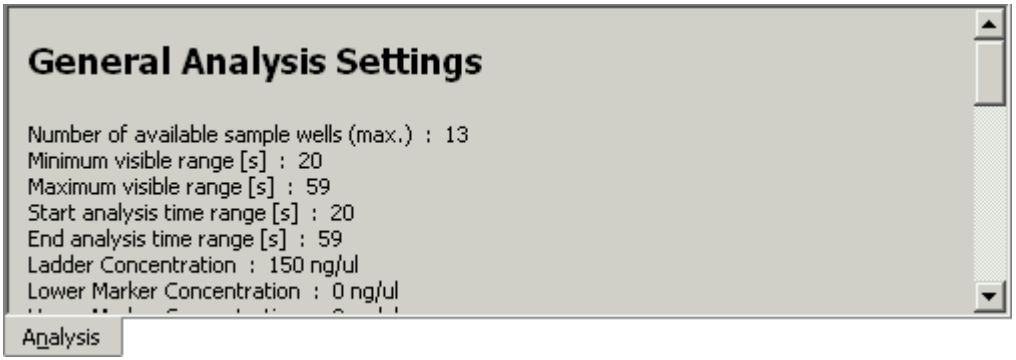
Generic/
Checkout Beads
siRNA Transfection Viability



File Name	Name of the assay (.xsy) file the current assay or chip data file is based on; .csy or .asy file if the basis is a *Bio Sizing* or *Cell Fluorescence* assay file.
Title	Name of the base assay.
Location	Path to the base assay file.
Assay	Assay type (corresponds to the *Assay Icon*).
Gating (flow cytometric assays only)	Gating direction (see also "Gating direction" on page 230):  blue to red, or  red to blue.
Version	Assay version.
Modified	Date the assay or chip data file was modified last.
Comments	Comments such as copyright notes, short description, or references to *Application Notes*.

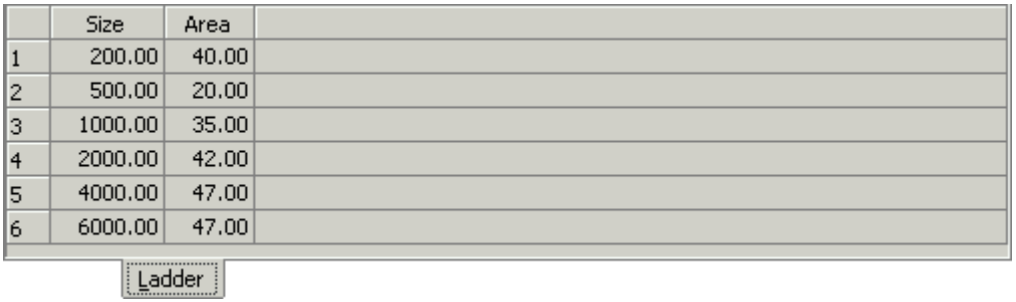
Analysis sub-tab

Shows a selection of characteristic assay parameters.



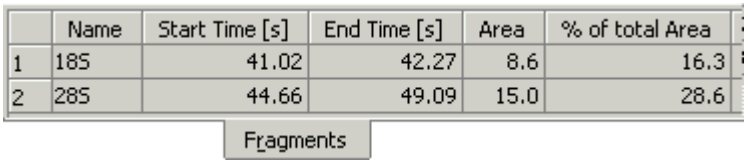
Ladder sub-tab (electrophoretic assays only)

Ladder peak table, one row per peak.



Area = area under the peak
Size = number of base pairs (bp) for DNA assays, or protein size in kDa for protein assays

Fragments sub-tab (RNA assays only)



	Name	Start Time [s]	End Time [s]	Area	% of total Area
1	18S	41.02	42.27	8.6	16.3
2	28S	44.66	49.09	15.0	28.6

Fragments

Number: order in which the fragments are detected.

Fragment Name: user-assigned or predefined name of the fragment. Typically 16S/23S for Prokaryote assays or 18S/28S for Eukaryote assays.

Fragment start time: start time (in seconds) of the peak.

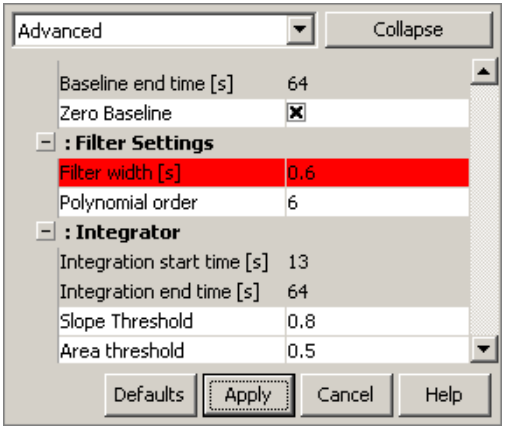
Fragment end time: end time (in seconds) of the peak.

The start and end times are represented on the electropherogram by diamond-shaped dots on the peak baseline in the *Fragment Color*. Dragging such dots changes the start or end times, and alters the baseline drawn between the dots.

Import Setpoints... — Opens a system dialog box allowing you to import the data analysis setpoints from an assay (.xsy) or chip data (.xad) file. For flow cytometry files, also markers and regions are imported.

Import Markers and Regions... (flow cytometric assays only) — Opens a system dialog box allowing you to import the markers and regions from an assay (.xsy) or chip data (.xad) file. Note that—on importing markers and regions—specific assays will be converted to generic assays.

Setpoint Explorer Lets you modify assay parameters (data analysis setpoints) *globally*, that is, for all samples. Click the + nodes to expand, and the – nodes to collapse branches. Setpoints that you can change are white. To edit a setpoint, double-click the value, enter the new value, and press enter. Edited values are red (until you apply them).



Normal/Advanced: Switches between normal and advanced mode.

Expand/Collapse: Expands/collapses *all* nodes.

Defaults: Resets all setpoint values to the assay defaults.

Apply: Applies your changes. Note that you have to save the assay file/chip data file to make the new setpoints permanent.

Cancel: Undoes all changes unless you already applied them.

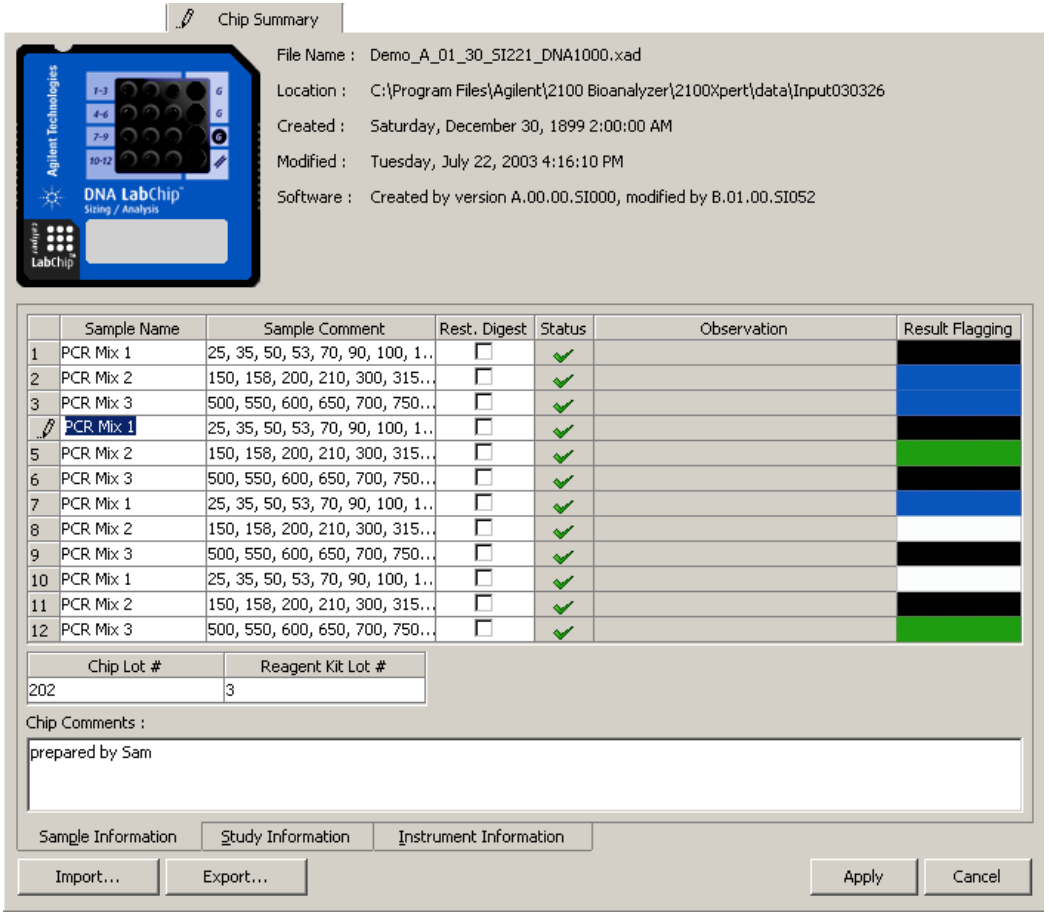
TIP

When applying modified data analysis setpoints, the software will (by default) immediately recalculate the raw data, which takes some time. Click on  in the toolbar or select *Don't Analyze* from the *Gel Menu* or *Electropherogram Menu* to temporarily switch off automatic data analysis while you modify setpoints.

Chip Summary Tab

Purpose

The *Chip Summary* tab shows information on the selected assay or chip data file, and lets you enter comments regarding chip, samples, and study.



Access

The *Chip Summary* tab is available in the *Data and Assay* context.

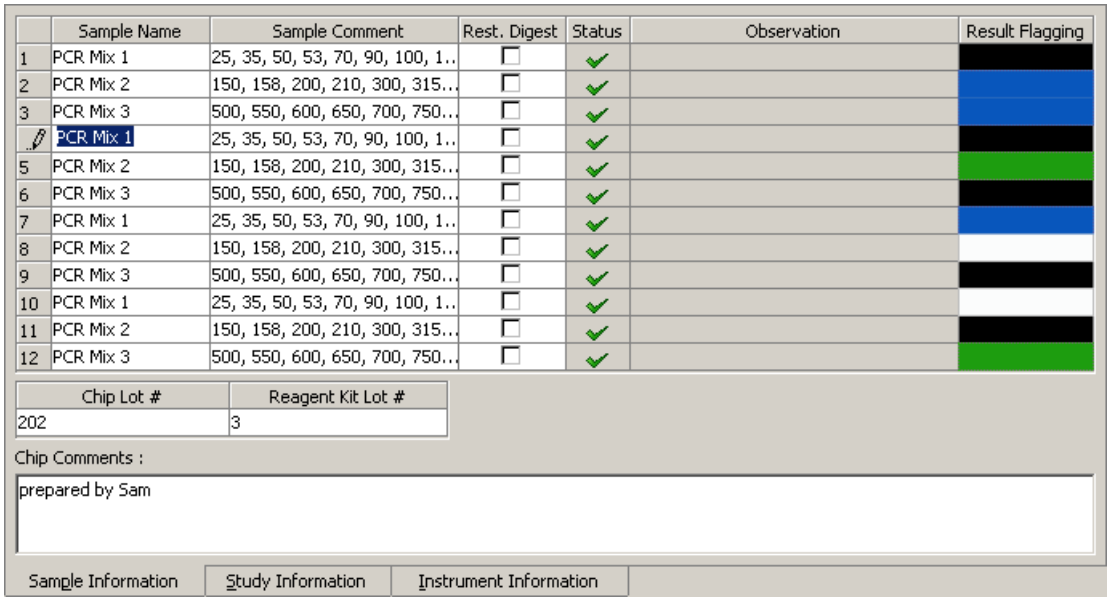
Elements

Chip icon	The chip icon indicates the assay type: DNA, RNA, Protein, or Cell.
File Name	Name of the chip data (.xad) file.
Location	Directory where the chip data file is stored.
Created	Creation date of the chip data file (measurement date).
Modified	Date when the chip data file was last modified.
Software	2100 expert versions used to create and modify the chip data file.
Import...	Opens the *Import Sample Information* dialog box allowing you to import sample names and comments from a .txt or .csv file.
Export...	Opens the *Export Sample Information* dialog box allowing you to write sample names and comments to a .txt or .csv file.
Apply	Applies (but does not save) your changes.
Cancel	Undoes all modifications except the ones you have already applied.

NOTE

If you try to exit the *Chip Summary* tab after having made changes, but without having clicked *Apply*, a message box appears prompting you to accept the modifications. If you do not accept, the changes are rejected.

Sample Information sub-tab (electrophoretic assays)



	Sample Name	Sample Comment	Rest. Digest	Status	Observation	Result Flagging
1	PCR Mix 1	25, 35, 50, 53, 70, 90, 100, 1..	☐	✓		
2	PCR Mix 2	150, 158, 200, 210, 300, 315...	☐	✓		
3	PCR Mix 3	500, 550, 600, 650, 700, 750...	☐	✓		
	PCR Mix 1	25, 35, 50, 53, 70, 90, 100, 1..	☐	✓		
5	PCR Mix 2	150, 158, 200, 210, 300, 315...	☐	✓		
6	PCR Mix 3	500, 550, 600, 650, 700, 750...	☐	✓		
7	PCR Mix 1	25, 35, 50, 53, 70, 90, 100, 1..	☐	✓		
8	PCR Mix 2	150, 158, 200, 210, 300, 315...	☐	✓		
9	PCR Mix 3	500, 550, 600, 650, 700, 750...	☐	✓		
10	PCR Mix 1	25, 35, 50, 53, 70, 90, 100, 1..	☐	✓		
11	PCR Mix 2	150, 158, 200, 210, 300, 315...	☐	✓		
12	PCR Mix 3	500, 550, 600, 650, 700, 750...	☐	✓		

Chip Lot #	Reagent Kit Lot #
202	3

Chip Comments :

prepared by Sam

Sample Information | Study Information | Instrument Information

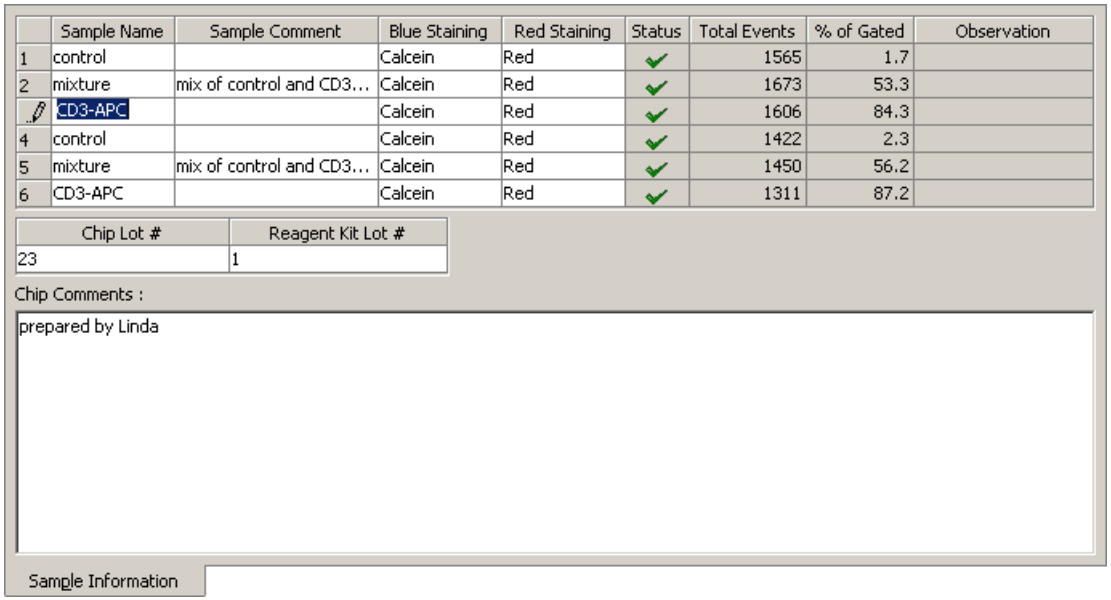
The sample table includes:

Sample Name	Lets you enter easy-to-remember names for your samples. Change the default names of the samples to names of your choice. Simply highlight the existing name (for example, “Sample 1”) and type a new name.
Sample Comment	Lets you enter a comment for each sample. *Sample Name* and any associated *Sample Comment* will appear on the printout.

Rest. Digest (DNA only)	Indicating that a sample is a *restriction digest* allows 2100 expert to note the possible comigration of peaks. Since it is assumed that the molarities of all the fragments should be the same, any peaks or clusters having molarities that are significantly larger than the rest are flagged as potentially comigrating peaks, allowing you to examine these in more detail. See also "Data Analysis: DNA" on page 92.
Use For Calibration (Protein only) *Concentration* (Protein only)	For protein samples, you can enable the *Use For Calibration* for each well and enter the *Concentration* of the standard protein. This allows you to generate a calibration curve, which can be used for absolute quantitation of this protein within different samples on the same chip. See also "Data Analysis: Protein" on page 100.
Status	A green check mark indicates that the sample has been successfully measured. A white cross on red ground indicates an incomplete measurement.
Observation	Observation made by 2100 expert during the measurement.
Result Flagging	If result flagging rules have been defined (see "Result Flagging" on page 145), you see a bar whose color is determined by the first result flagging rule met by the sample.
Chip Lot #	Lot number of the chip.

Reagent Kit Lot #	Lot number of the reagent kit.
Chip Comments	Lets you enter notes to document the chip and/or the chip run. These notes will also be printed with the report.

Sample Information sub-tab (flow cytometric assays)



	Sample Name	Sample Comment	Blue Staining	Red Staining	Status	Total Events	% of Gated	Observation
1	control		Calcein	Red	✓	1565	1.7	
2	mixture	mix of control and CD3...	Calcein	Red	✓	1673	53.3	
	CD3-APC		Calcein	Red	✓	1606	84.3	
4	control		Calcein	Red	✓	1422	2.3	
5	mixture	mix of control and CD3...	Calcein	Red	✓	1450	56.2	
6	CD3-APC		Calcein	Red	✓	1311	87.2	

Chip Lot #	Reagent Kit Lot #
23	1

Chip Comments :

prepared by Linda

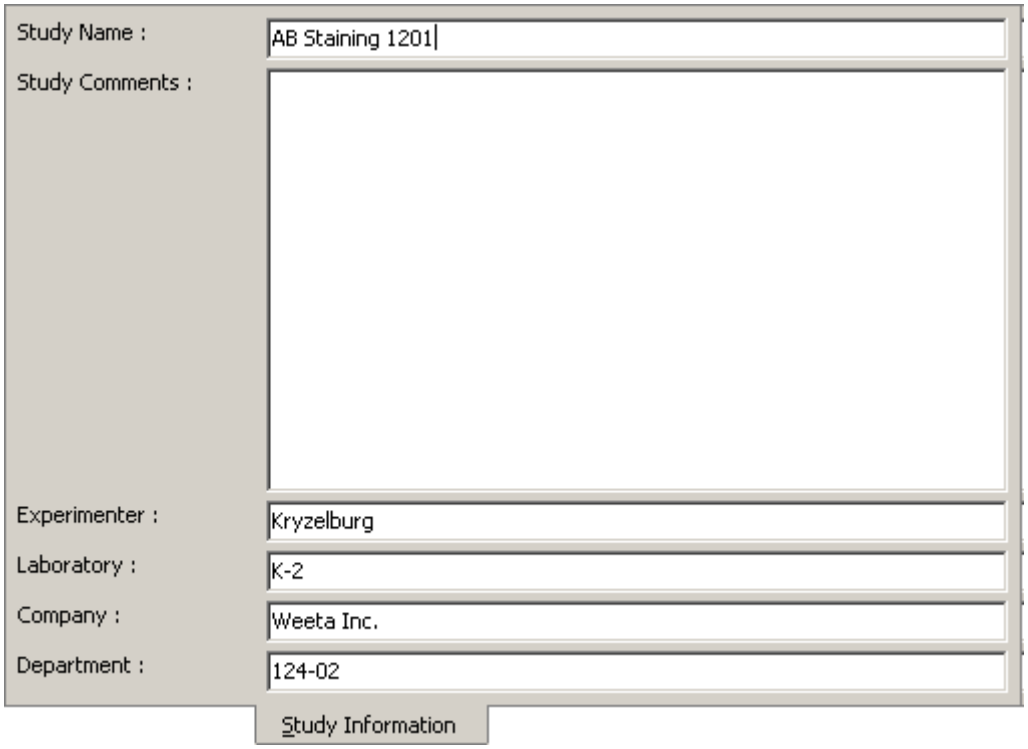
Sample Information

The sample table shows you the main measurement results and allows you to enter sample names and comments.

Sample Name	Lets you enter easy-to-remember names for your samples.
Sample Comment	Lets you enter a comment for the sample.

Blue Staining	Lets you enter the name of the dye that is used for blue fluorescence staining.
Red Staining	Lets you enter the name of the dye that is used for red fluorescence staining.
Status	A green check mark indicates that the sample has been measured successfully. A red cross indicates an incomplete or aborted measurement. No symbol indicates that the sample has not been measured at all.
Total Events	Shows the number of events detected for the sample.
% of Gated	Shows the subset of cells determined by gating (in percent).
Observation	Observation made by 2100 expert during the measurement.
Chip Lot #	Lot number of the chip.
Reagent Kit Lot #	Lot number of the reagent kit.
Chip Comments	Lets you enter text to document the chip run.

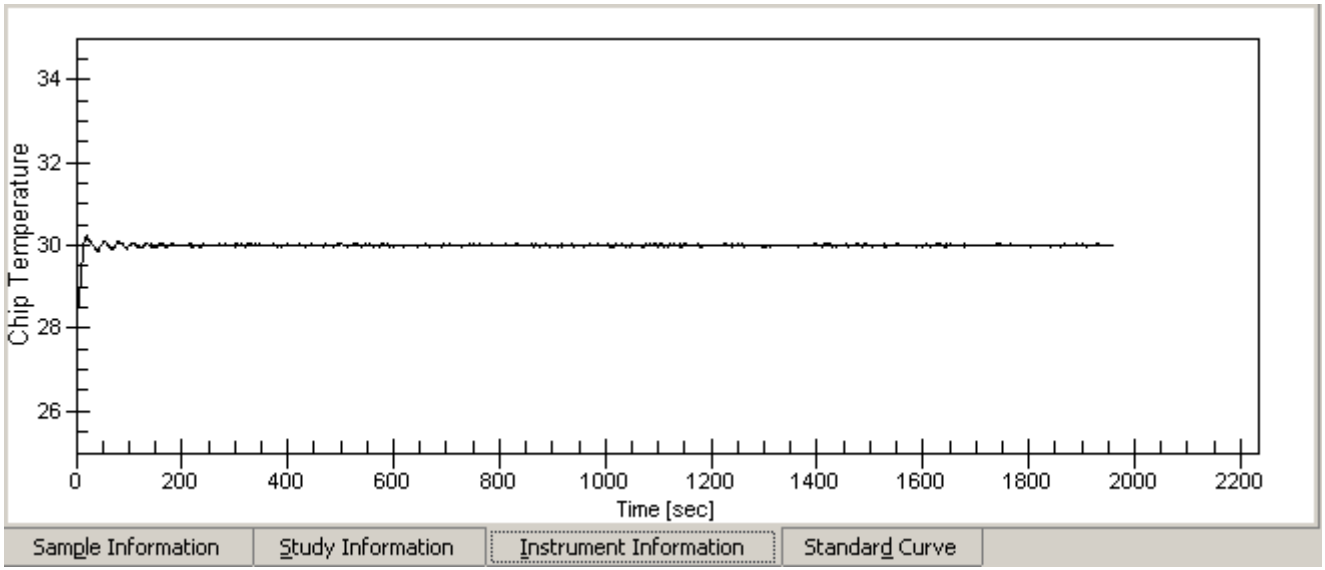
Study Information sub-tab



To identify your assays easily, you can add information about the study (*Study Name* and *Study Comments*), the *Experimenter*, the *Laboratory*, the *Company* and the *Department*.

This can be used for data exchange with other departments or companies.

Instrument Information sub-tab

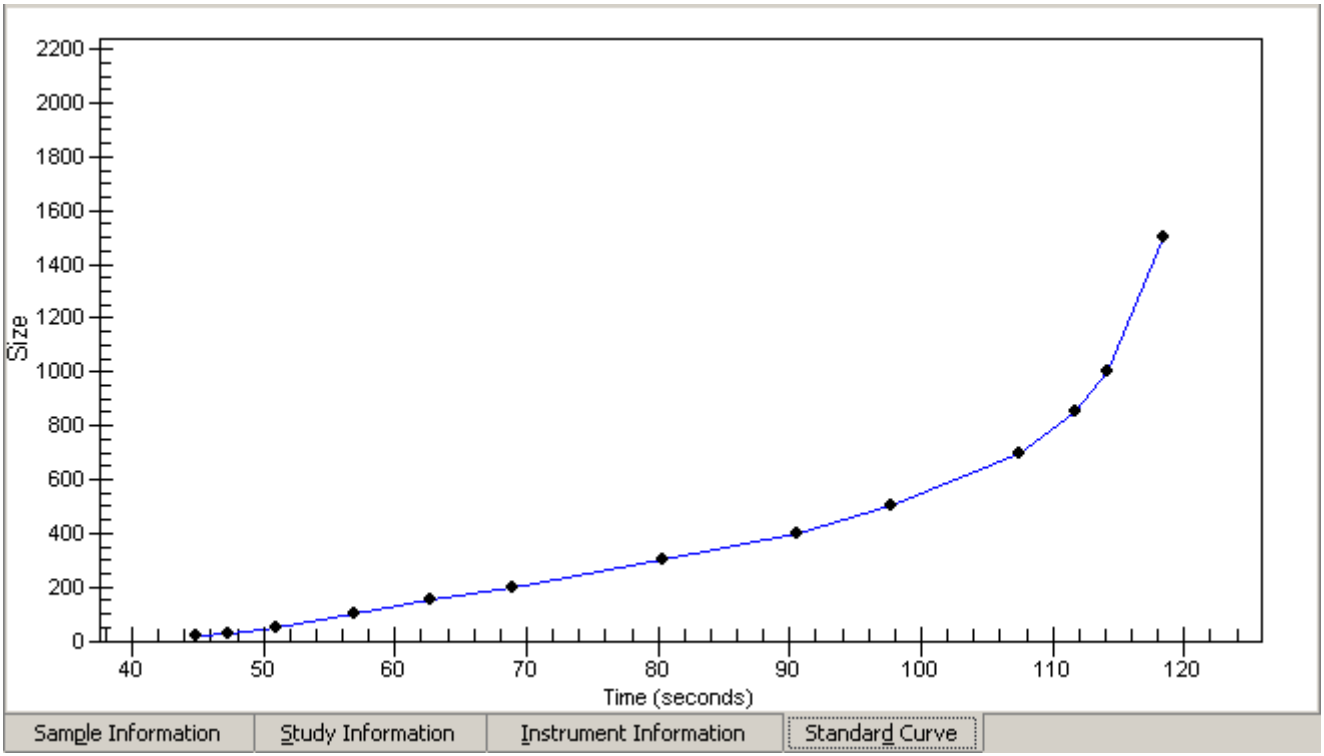


This tab displays the temperature of the chip base plate recorded during the chip run. The acquisition time is displayed in relation to the temperature.

NOTE

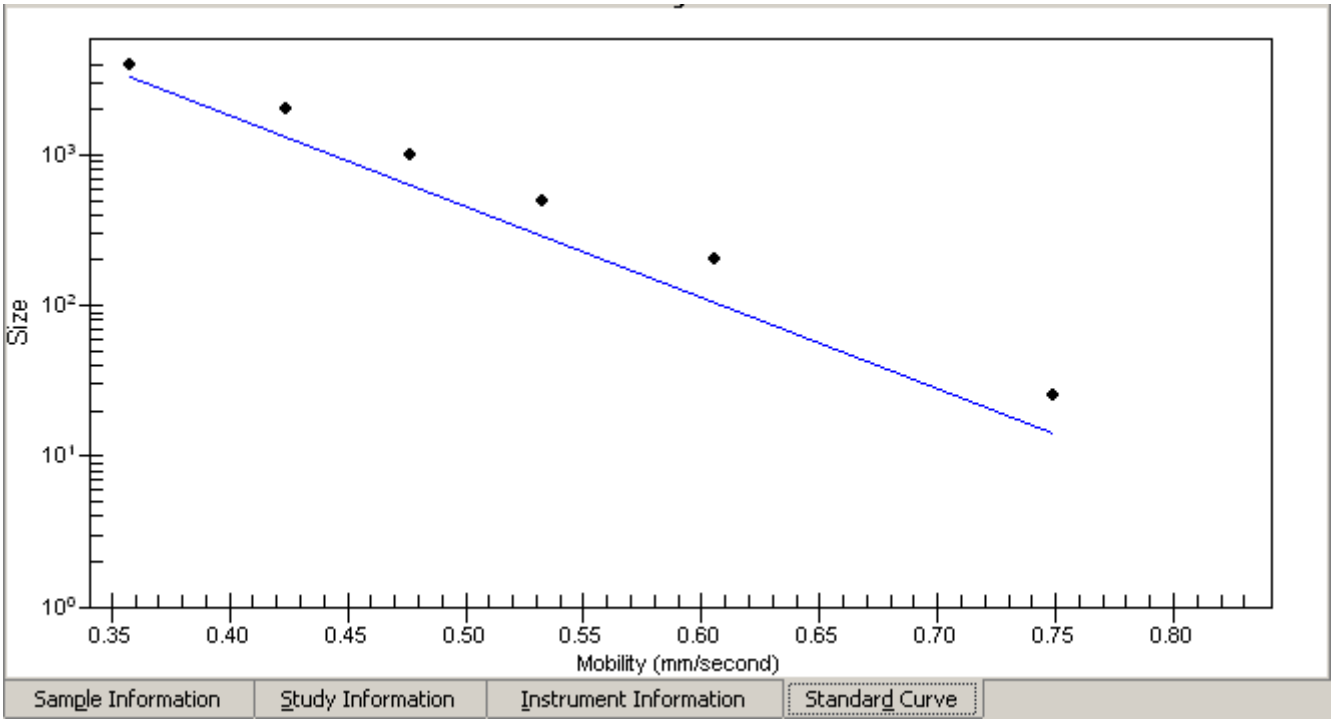
The regular chip temperature should be 25 °C for flow cytometry and 30° C for electrophoresis assays. Significant differences can negatively influence your measurement results.

Standard Curve sub-tab (DNA/Protein assays only)



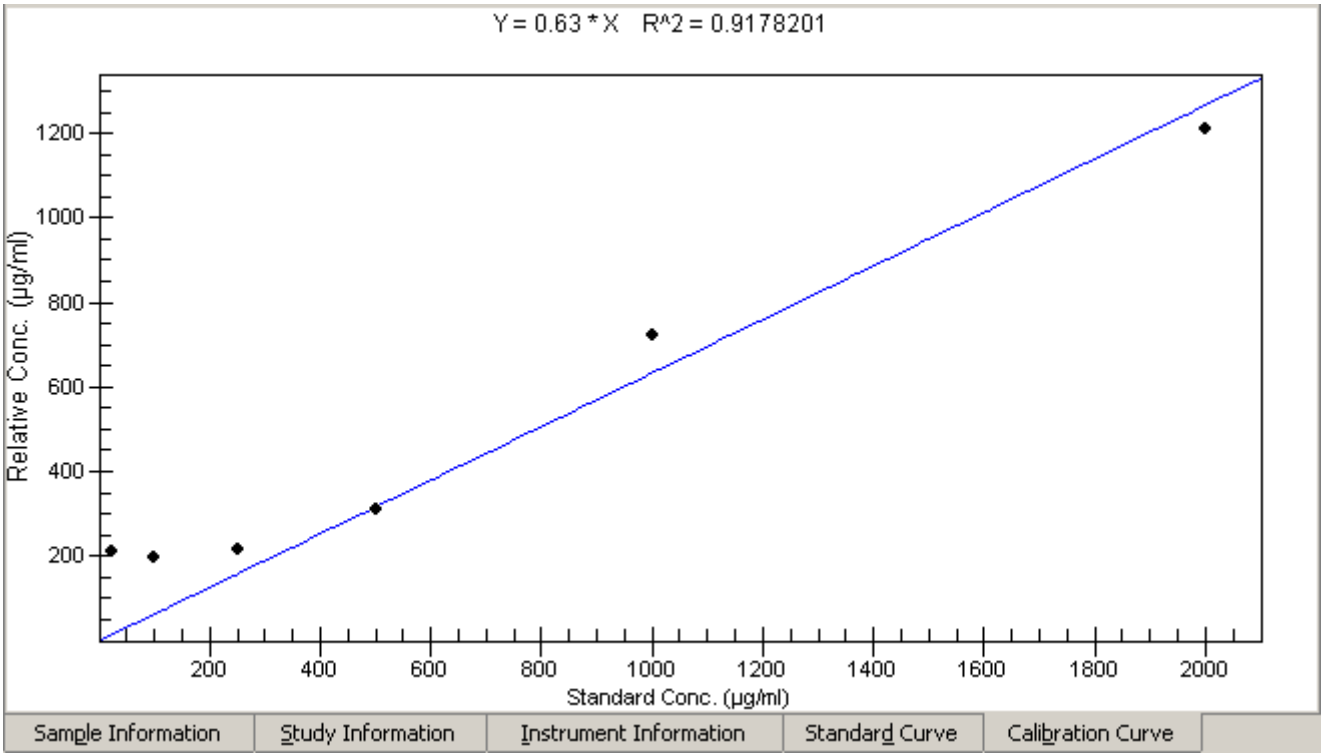
A standard curve of migration time versus size is plotted from the sizing ladder by linear interpolation.

Standard Curve sub-tab (RNA assays only)



A standard curve of mobility vs. size in logarithmic scale is plotted from the RNA sizing ladder by linear interpolation.

Calibration Curve sub-tab (protein assays only)

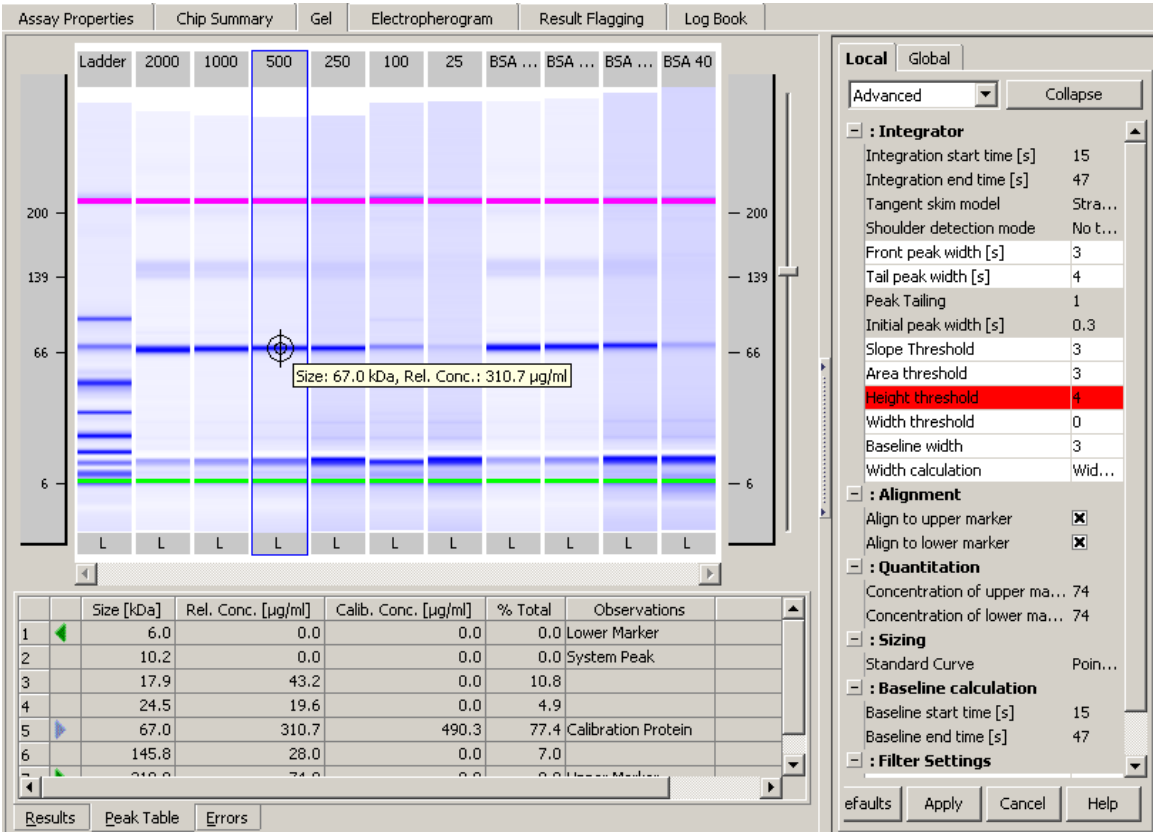


If a standard protein was added on the chip and you have selected the option *Use For Calibration* on the *Sample Information* sub-tab, a calibration curve is calculated. See the figure above for an example. This sub-tab is only available for protein assays.

Gel Tab

Purpose

The *Gel* tab shows gel-like images of the ladder and of all samples of a chip.



On sub-tabs below the gel-like image, you can find analyzed data for each individual sample. For further analysis, you can use the setpoint explorer on the right to modify data analysis setpoints for the *current* sample (*Local* tab) or for *all* samples (*Global* tab).

Access

The *Gel* tab is always available

- in the *Data and Assay* context if an electrophoretic chip data (.xad) file is selected,
- in the *Comparison* context if a comparison (.xad) file or one of its samples is selected.

Gel View

One lane of the gel view is surrounded by a rectangle. This is the selected lane (one lane is always selected) and corresponds to a well on the chip; the chip icon on the lower panel (see "Lower Panel" on page 398) highlights the associated well. Clicking a different lane will select that lane and the chip icon will update to highlight the corresponding well.

The slider on the right-hand side of the gel-like images allows you to adjust the brightness of the selected gel-like image.

NOTE

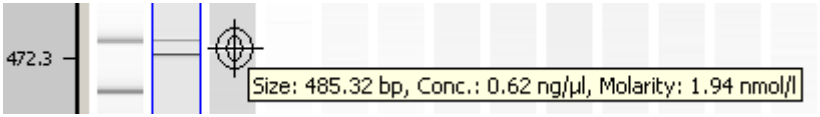
The display of the gel-like image can be changed to a number of different color combinations. These can be applied via *Gel* > *Gel Color*. For more information, see "Gel Menu" on page 342.

When a new chip run starts, the gel view is blank and the first lane—the ladder well—is selected. As data is acquired, the selection rectangle around the lane will step from left to right, highlighting the lane that is currently acquiring data.

However, if you select a lane that is earlier in sequence than the current lane, the highlight will no longer change as new samples are measured but will remain on the selected lane.

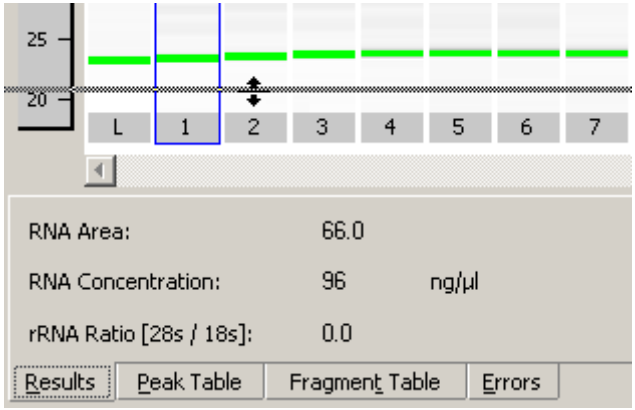
Moving the mouse pointer over a gel-like image will cause sample data to appear next to a crosshair pointer. What is displayed depends on the type of assay selected:

- With a DNA assay, you will see the base-pair measurements for the area of the lane beneath the crosshair of the pointer. When the cursor is positioned over a recognized band, the cursor will change its shape to a target, and the size, concentration, and molarity are shown in a tool tip window.

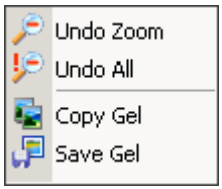


- With RNA assays, the size estimate in terms of # of nucleotides (nt) is shown; over recognized bands, the area and percent of total area is shown.
- With a protein assay, the tool tip window shows the size of the protein (in kDa) and the relative concentration (in µg/ml).

The dividing line between the gel graphs and the sub-tabs at the bottom can be moved in the vertical direction, giving more or less space to either area:



When you right-click the gel view, the following menu appears:

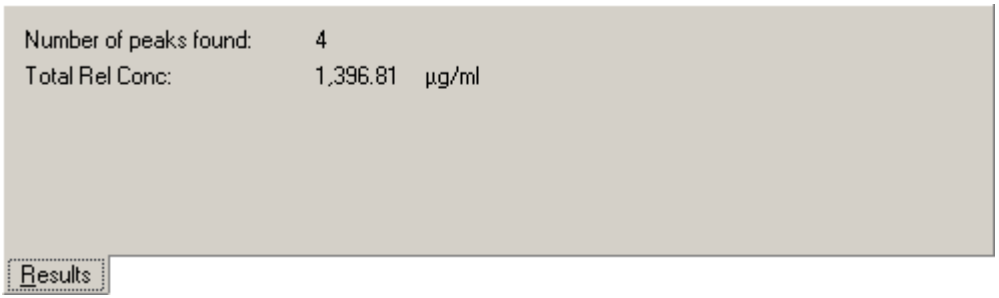


The following functions are available:

Undo Zoom	Undoes the last zoom, pan, or scale action.
Undo All	Undoes all zoom, pan, and scale actions.
Copy Gel	Puts a copy of the gel graph (of all samples) onto the clipboard.
Save Gel	Opens a system dialog box, allowing you to save the gel graph (all samples) as an image in JPEG (.jpg), Windows Bitmap (.bmp) or Tagged Image File Format (.tif) format.

Results sub-tab

For DNA and protein assays, the *Number of peaks found* is shown.

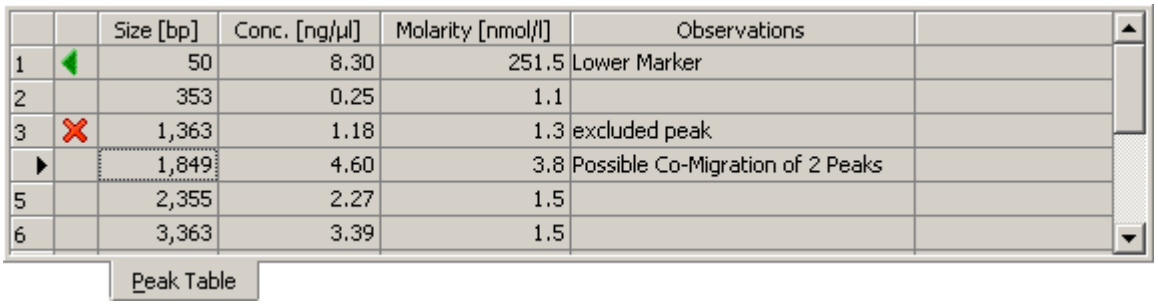


For protein assays, also the total relative concentration (*Total Rel. Conc.*) in µg/ml is shown.

For RNA assays, the *RNA Area*, and the *RNA Concentration* in pg/µl or ng/µl, and the percentage of *rRNA Contamination* (mRNA) found in the samples is shown.

Peak Table sub-tab

For each peak (rows), the table shows a number of calculated values (columns).



		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations	
1		50	8.30	251.5	Lower Marker	
2		353	0.25	1.1		
3		1,363	1.18	1.3	excluded peak	
▶		1,849	4.60	3.8	Possible Co-Migration of 2 Peaks	
5		2,355	2.27	1.5		
6		3,363	3.39	1.5		

Peak Table

The analyzed data depends on the assay type:

- **DNA**

  Default table columns are:

Peak Number (leftmost column)	The order in which the peaks were detected.
Second column	Symbols in this column indicate the peak type.
Size [bp]	The number of calculated DNA base pairs.
Conc. [ng/µL]	The concentration in nanograms per microliter for each fragment (derived from the area/conc. relationship with the upper marker, the same for all ladder peaks).
Molarity (nmol/l)	$\text{Molarity} = \frac{\text{Concentration} \times 10^6}{660 \times \text{Size}}$ where: concentration is measured in nanograms per microliter (ng/µl) size is measured in base pairs (bp), 660 is the molecular weight of one base pair.
Observations	Additional information about the peak such as possible comigration or expected fragment indication.

- **RNA**

  Default table columns are:

Peak Number (leftmost column)	The order in which the peaks were detected.
Second column	Symbols in this column indicate the peak type.
Time corrected area	The area under the peak is corrected as a result of migration and baseline correction.
Observations	Additional information about the peak such as possible comigration or expected fragment indication.

- **Protein**

  Default table columns are:

Peak Number (leftmost column)	The order in which the peaks were detected.
Second column	Symbols in this column indicate the peak type.
Size [kDa]	The peak size measured in kilodaltons.
Rel. Conc. [µg/ml]	Relative protein concentration measured in micrograms per milliliter, derived from the area/conc. relationship with the upper marker. The concentration can only be given as relative concentration when comparing different proteins because dye binding differs from protein to protein. For quantitative results use calibration proteins.

Calib. Conc. [µg/ml]	Calibrated concentration of the calibration protein in the standard, or of the calibrated protein in the sample.
% Total	The percentage of the area of the individual peak compared to the summed total area of all peaks in the sample (not including markers and system peak).
Observations	Additional information about the peak such as possible comigration, expected fragment indication, or calibration protein.

NOTE

You can include further columns and also exclude columns from the peak table. Right-click the heading row of the table and select *Configure Columns...* from the context menu. For details refer to "Configure Columns" on page 543.

Peak types

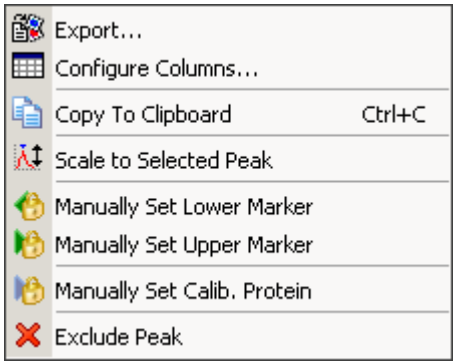
The second column of the peak table shows symbols indicating the peak type:

	Ladder peak
	Lower marker
	Manually set lower marker
	Upper marker
	Manually set lower marker

	Calibration protein
	Manually set calibration protein
	Excluded peak

Context menu

When you right-click the peak table, the following menu appears:



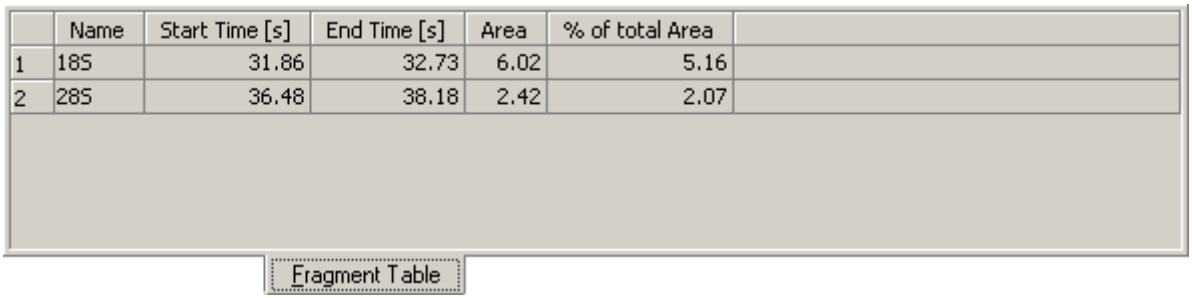
The following functions are available:

Export...	Opens a system dialog box allowing you to save the peak table as a .csv or .xls file.
Configure Columns...	Opens the *Configure Columns* dialog box.
Copy To Clipboard	Puts the peak table onto the clipboard. You can paste the table in another application such as MS Excel®. If a part of the table is selected, only this selection is copied.

Scale to Selected Peak (electropherograms only)	Adapts the scale of the Y axis. The selected peak is displayed at maximum height. Other peaks might be cut off.
Manually Set Lower Marker	Makes the selected peak the lower marker.
Manually Set Upper Marker	Makes the selected peak the upper marker.
Calibrate Protein (protein assays only)	Makes the selected peak the calibration protein.
Exclude Peak	Excludes the selected peak from the analysis for the sample.

Fragment Table sub-tab (RNA assays only)

For each peak (rows), the table shows results for the predefined/specific fragments.



	Name	Start Time [s]	End Time [s]	Area	% of total Area
1	18S	31.86	32.73	6.02	5.16
2	28S	36.48	38.18	2.42	2.07

Fragment Table

Default table columns are:

Fragment Number (leftmost column)	The order in which the fragments were detected.
Name	A user-assigned or predefined name for the found fragment. Typically 16S/23S for Prokaryote assays or 18S/28S for Eukaryote assays.
Start Time [s]	Shows the start time for the peak in seconds.
End Time [s]	Shows the end time for the peak in seconds. The start and end times are also represented on the electropherogram by diamond-shaped dots on the peak baseline in the same color as that shown in the RNA tab. Dragging a dot will change the start or end time and alter the baseline drawn between the dots.

Area	The area of the individual fragment measured in base pairs.
% of total Area	The percentage of the area of the individual fragment compared to the total area or RNA measured above the baseline.

NOTE

You can include further columns and also exclude columns from the fragment table. Right-click the heading row of the table and select *Configure Columns...* from the context menu. For details refer to "Configure Columns" on page 543.

Errors sub-tab

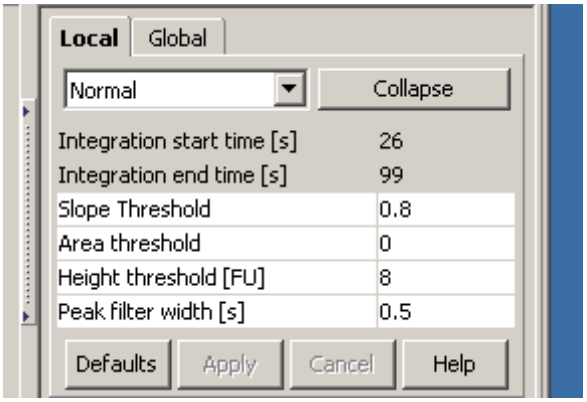
Displays *Code*, *Description* and *Category* of any errors that occured during measurement or analysis.

Most errors are the result of peaks not being located by the analysis algorithms of the software. This can be due to a sample or ladder peak not appearing as expected; the data analysis setpoints (see setpoint explorer below) can also cause peaks to remain undetected, which can cause errors. Additionally, manually excluding a peak from analysis or changing the start or end times for a run can cause errors with the peak find algorithm.

Setpoint Explorer

To show the setpoint explorer, click the vertical bar on the right edge of the application window:

The setpoint explorer appears.



You can use the setpoint explorer to modify data analysis setpoints for the *current* sample (*Local* tab) or for *all* samples (*Global* tab).

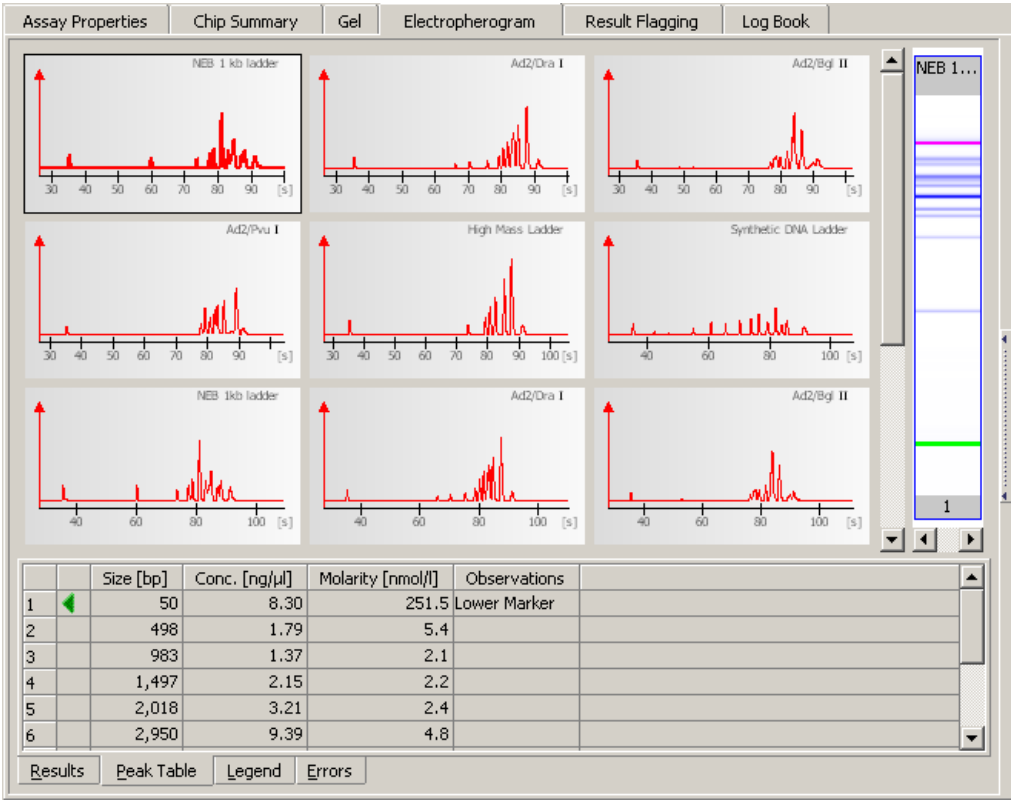
Refer to "Assay Properties Tab" on page 418 and "Changing the Data Analysis" on page 111 for details on the setpoint explorer.

Electropherogram Tab (Single/Grid View)

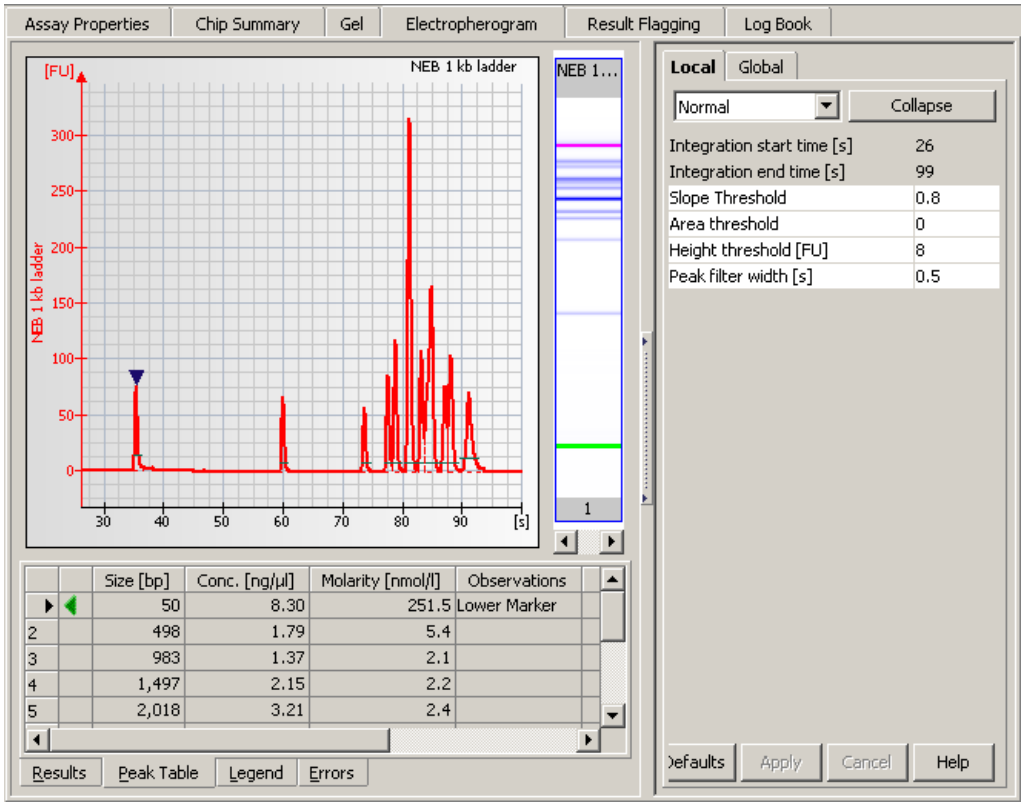
Purpose

The *Electropherogram* tab shows electropherograms of all samples (including the ladder) of a chip, either for all (grid view) or for an individual sample (single view). Electropherograms are plots of the migration time against fluorescence units.

Grid View



Single View



Access

The *Electropherogram* tab is always available

- in the *Data and Assay* context if an electrophoretic chip data (.xad) file is selected,
- in the *Comparison* context if a comparison (.xad) file or one of its samples is selected.

Electropherogram View

In *Grid View*, you see electropherograms of all samples (including ladder) at the same time. The electropherogram of the selected sample is surrounded by a black rectangle. The small gel view on the lower panel (see "Lower Panel" on page 398) highlights the associated well.

In *Single View*, you can overlay the electropherograms of different samples from one chip run. For comparing samples from different chip runs, refer to "Comparing Samples from Different Electrophoretic Chip Runs" on page 136.

In both views (*Single View* and *Grid View*), you can zoom in to see the data in the graphs more closely. Click and drag the mouse to draw a rectangle that bounds the area you wish to view in more detail. This area will enlarge to fill the display area. Unzoom by double-clicking or using the undo zoom tool. The dividing line between the electropherogram graph(s) and the sub-tabs at the bottom can be moved in the vertical direction, giving more or less space to either area.

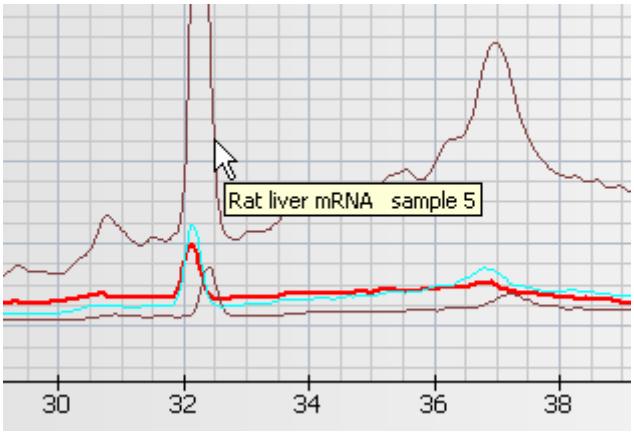
The electropherograms are labeled with the sample name.

To the right of the electropherogram(s), a gel-like image of the (selected) sample is shown, which you can use also for zooming into the electropherograms.

On sub-tabs, you can find analyzed data for each individual sample. For further analysis, you can use the setpoint explorer on the right to modify data analysis setpoints.

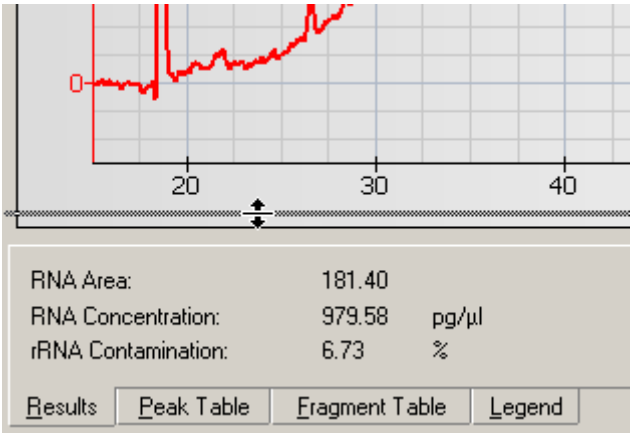
When a new chip run starts, the electropherogram will at first be blank. As data is acquired, electropherograms are drawn one by one, and result tables are filled with analyzed measurement data.

Moving the mouse pointer over a curve in an electropherogram shows the sample name and number. This is especially useful in overlaid electropherograms:

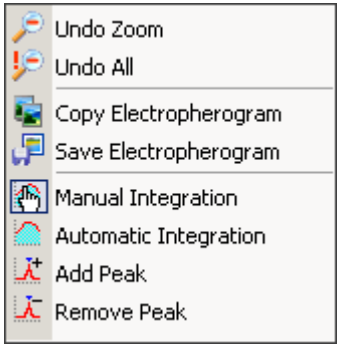


Moving the mouse pointer over the top of a peak, changes the shape of the mouse cursor to a crosshair, and shows analyzed data for the peak:

The dividing line between the electropherogram graphs and the sub-tabs can be moved in the vertical direction, giving more or less space to either area:



When you right-click the electropherogram, the following menu appears:

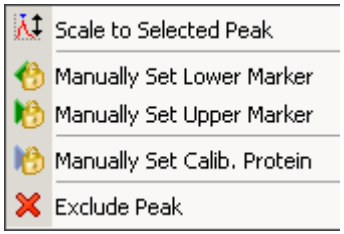


The following functions are available:

Undo Zoom	Undoes the last zoom, pan, or scale action.
Undo All	Undoes all zoom, pan, and scale actions.

Copy Electropherogram	Puts a copy of the electropherogram into the clipboard (single-well view). In multi-well view, all electropherograms are copied.
Save Electropherogram	Opens a system dialog box, allowing you to save the electropherogram (single-well view) or all electropherograms (multi-well view) as an image in JPEG (.jpg), Windows Bitmap (.bmp), Windows Meta File (.wmf), CompuServe Graphics Interchange (.gif), or Tagged Image File (.tif) format.
Manual Integration	Switches to the manual peak integration mode. In this mode, you can: • change the start and end points and the baseline of a certain peak • add or delete certain peaks from the integration.
Automatic Integration	Switches to the automatic peak integration mode (all manual integrations will be lost).
Add Peak (manual peak integration mode only)	Inserts a new peak at the current position.
Remove Peak (manual peak integration mode only)	Deletes the selected peak. Only manually added peaks can be deleted.

When you right-click the top of a peak, the following menu appears:

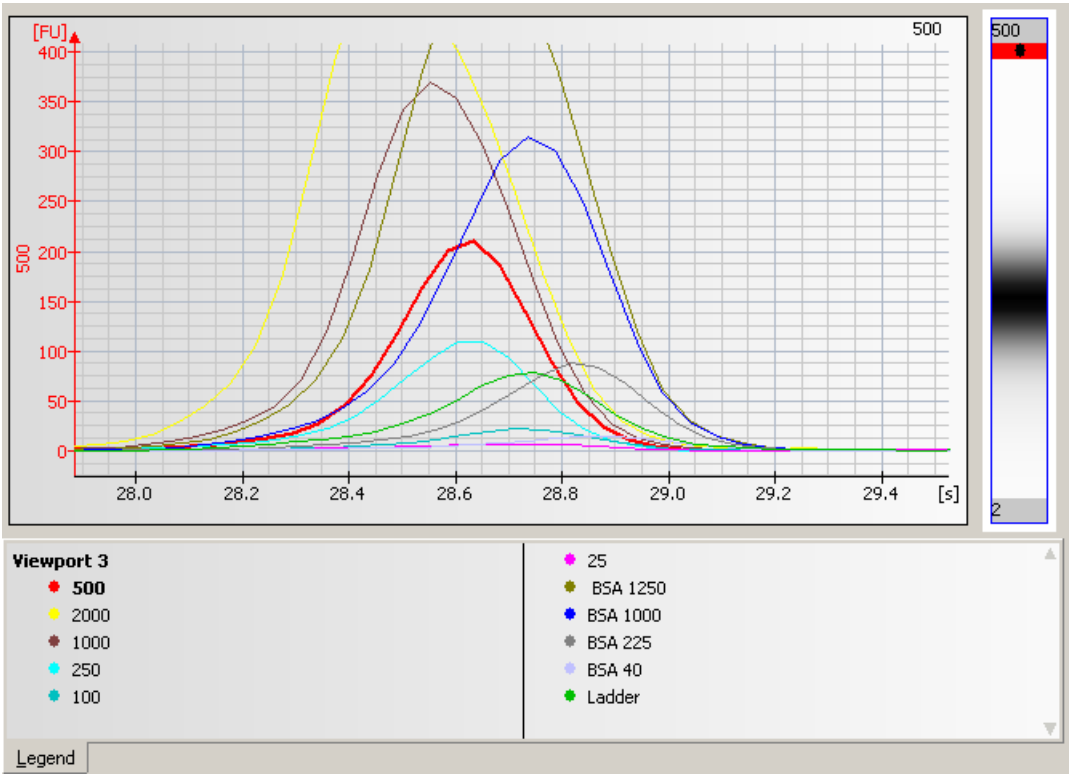


The following functions are available:

Scale to Selected Peak	Adapts the scale of the Y axis. The selected peak is displayed at maximum height. Other peaks might be cut off.
Manually Set Lower Marker	Makes the selected peak the lower marker.
Manually Set Upper Marker	Makes the selected peak the upper marker.
Manually Set Calib. Protein (protein assays only)	Makes the selected peak the calibration protein.
Exclude Peak	Excludes the selected peak from the analysis for the sample.

Legend sub-tab

To compare samples, you can overlay electropherograms from multiple samples (single view only). Each electropherogram will then be shown in a different color, and a color legend appears on the *Legend* tab. This lets you easily assign curves to samples.



You can change the colors of the electropherograms in the *Options* dialog box, see "Options – Graph Settings" on page 506.

Results, Peak Table, and Fragment Table sub-tabs

The sub-tabs *Results*, *Peak Table*, and *Fragment Table* work exactly in the same way as they do in the gel view. For details on these sub-tabs, please refer to "Gel Tab" on page 438.

Errors sub-tab

Displays *Code*, *Description* and *Category* of any errors that occured during measurement or analysis.

Most errors are the result of peaks not being located by the analysis algorithms of the software. This can be due to a sample or ladder peak not appearing as expected; the data analysis setpoints (see setpoint explorer below) can also cause peaks to remain undetected, which can cause errors. Additionally, manually excluding a peak from analysis or changing the start or end times for a run can cause errors with the peak find algorithm.

Setpoint Explorer

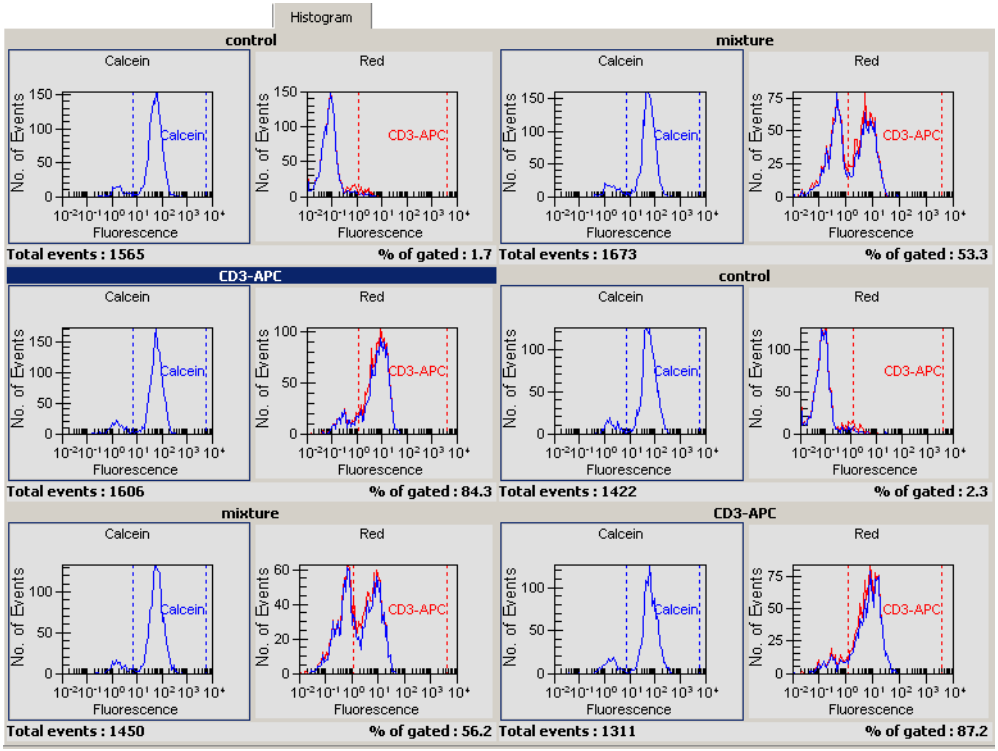
To analyze your results, you can use the setpoint explorer for modifying data analysis setpoints for the *current* sample (*Local* tab) or for *all* samples (*Global* tab). Please refer to "Gel Tab" on page 438 for information on its usage.

Histogram Tab (Single/Grid View)

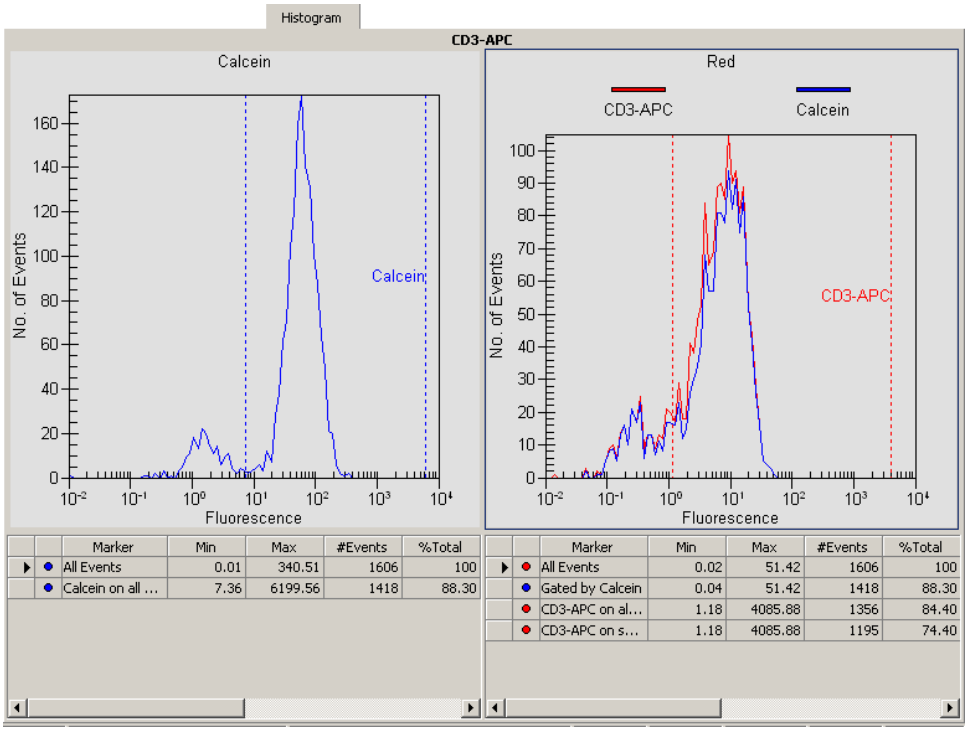
Purpose

Histograms are plots of fluorescence intensity against the number of detected events (detected cells). For each sample on the chip, the *Histogram* tab shows two histograms, one for blue (left histogram) and one for red staining (right histogram), either for all 6 samples (grid view) or for an individual sample (single view).

Grid View



Single View



Access

The *Histogram* tab is always available in the *Data and Assay* context if a flow cytometric chip data or assay file is selected.

You can switch between the histogram views by choosing *Single View* or *Grid View* in the *Histogram* menu.

Histogram View

Histograms are graphical representations of the measurement results, where the number of events is mapped to the Y axis and their fluorescence values to the X axis. The X axis can be logarithmic or linear. The resulting curves show the frequency distribution of the events in relation to their fluorescence intensity values.

In the *Grid View*, an overview of all samples is shown. The small histograms are labeled with the sample name, and below them, the total number of events and the %-value of the covered events can be read. You can select a single histogram by clicking on it or you can move the focus with the arrow keys.

Double-clicking a blue or red histogram in the *Grid View* takes you to the *Single View*, displaying a larger view of the histograms and a result table below each histogram. The histograms can be evaluated statistically using markers. Markers define ranges of fluorescence intensity values. Using a marker, one histogram defines a subset of events (cells). You can set markers for both histograms. Only the marker in the left histogram defines a range for the right one, but both define subsets for the statistical information. Only events with a fluorescence value within this range are displayed in the other histogram. This method is called gating. For *Apoptosis* and *Antibody Staining*, the blue histogram is used for gating, while *GFP* assays use the red histogram. If you use a *Generic* assay, you can define the markers and the direction to be used for gating. You can adjust marker positions using the mouse and define its name and color (see "How to Move the Upper and Lower Limits of Markers" on page 211 and "How to Configure Markers" on page 209).

Result Tables

The result tables show a number of calculated values (columns) for each histogram. If you add or redefine markers, the data in the result tables is recalculated.

		Marker	Min	Max	#Events	%Total	% of gated	Mean	StdDev	CV%	GMean
▶	●	All Events	0.02	51.42	1606	100	-	8.07	7.13	88.37	4.51
	●	Gated by Calcein	0.04	51.42	1418	88.30	-	8.46	7.32	86.47	4.72
	●	CD3-APC on all events	1.18	4085.88	1356	84.40	-	9.48	6.89	72.75	7.27
	●	CD3-APC on subset	1.18	4085.88	1195	74.40	84.30	9.96	7.02	70.44	7.73

If the option *Hide superset curve...* is disabled in the setpoint explorer (see "Assay Properties Tab" on page 418), two additional rows are displayed in the gated histogram's result table—"CD3-APC on all events" and "CD3-APC on subset" in the example above. The superset curve shows a histogram of all measured events; the gate is not considered.

Default table columns are:

Marker	Marker used for gating or as subset. When using an *Apoptosis* assay, Calcein is used for gating and Annexin as a subset, for example.
Min	Minimum fluorescence value of the corresponding marker.
Max	Maximum fluorescence value of the corresponding marker.
#Events	Number of events. For the histogram you use for gating, the number of all events is displayed.
% Total	Percentage of events. The marker used for gating has 100%, while the table of the gated histogram shows the value of the subset.

% of gated	Percentage of events selected by the marker in the gated histogram related to the events that have passed the gate.
Mean	Mean fluorescence value.
StdDev	Standard deviation to the mean value.
%CV	Coefficient of variation.
GMean	Geometric mean.

NOTE

You can exclude columns from the result tables. Right-click the heading row of the table and select *Configure Columns...* from the context menu. For details refer to "Configure Columns" on page 543.

Context Menus

In *Single View*, two context menus are available.

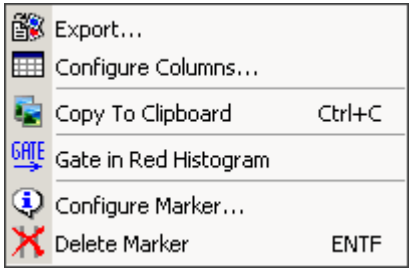
When you right-click a *histogram*, the following menu appears:

The following functions are available:

Undo Zoom	Undoes the last zoom action.
Undo All	Undoes all zoom actions.
Copy Graph To Clipboard	Puts a copy of the selected histogram (blue or red) into the clipboard.
Save Graph To File	Opens a system dialog box allowing you to save the selected histogram (blue or red) as an image in Windows Meta File (.wmf), Windows Bitmap (.bmp), or JPEG (.jpg) format.
Gate in Red histogram	Uses the selected marker of the blue histogram for gating in the red histogram (generic assays only).

Gate in Blue histogram	Uses the selected marker of the red histogram for gating in the blue histogram (generic assays only).
Show Data Points	Shows/hides the data points used to generate the selected histogram.
Configure Marker	Opens the *Configure Marker* dialog box (see "Configure Marker" on page 545), allowing you to change the properties of the selected marker. Only available if a marker is selected.
Delete Marker	Deletes the selected marker. If the marker is also used in other histograms, you will be asked whether it should be removed from all histograms that use it or only from the current histogram.

When you right-click a *result table*, the following menu appears:



The following functions are available:

Export...	Opens a system dialog box allowing you to save the result table as a .csv file.
Configure Columns...	Opens the *Configure Columns* dialog box.

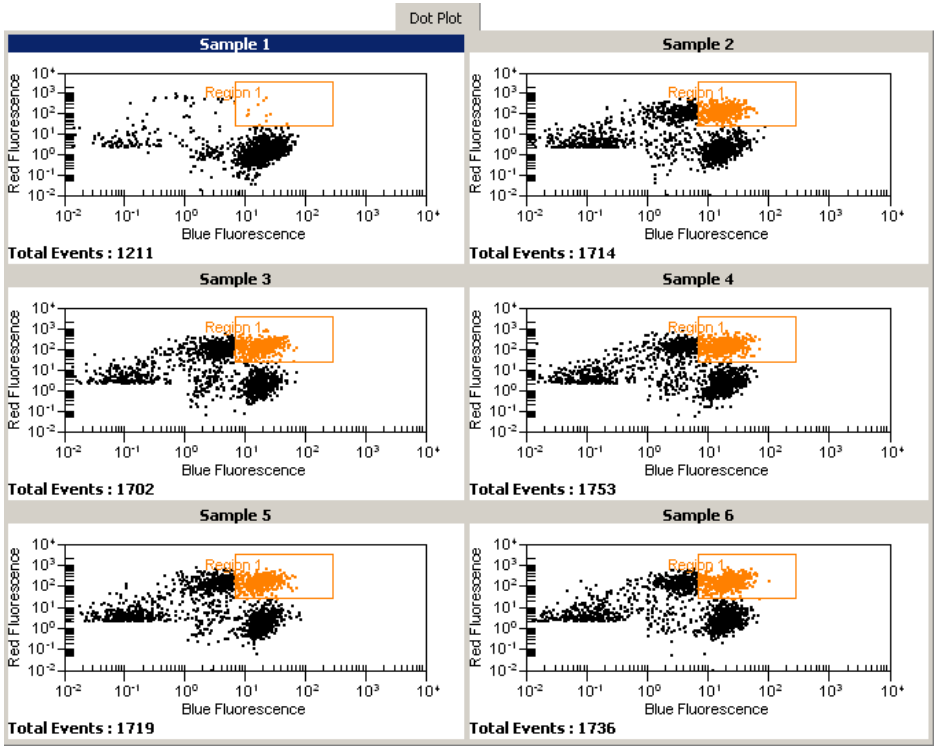
Copy To Clipboard	Puts the result table into the clipboard. You can paste the table in another application such as MS Excel®. If a part of the table is selected, only this selection is copied.
Gate in Red/Blue Histogram	Inserts a gate in the displayed gating direction (generic assays only). Only available if a marker without gate is selected in the histogram.
Configure Marker...	Opens the *Configure Marker* dialog box. Only available if a marker is selected.
Delete Marker	Deletes the selected marker. If the marker is also used in other histograms, you will be asked whether it should be removed from all histograms that use it or only from the current histogram.

Dot Plot Tab (Single/Grid View)

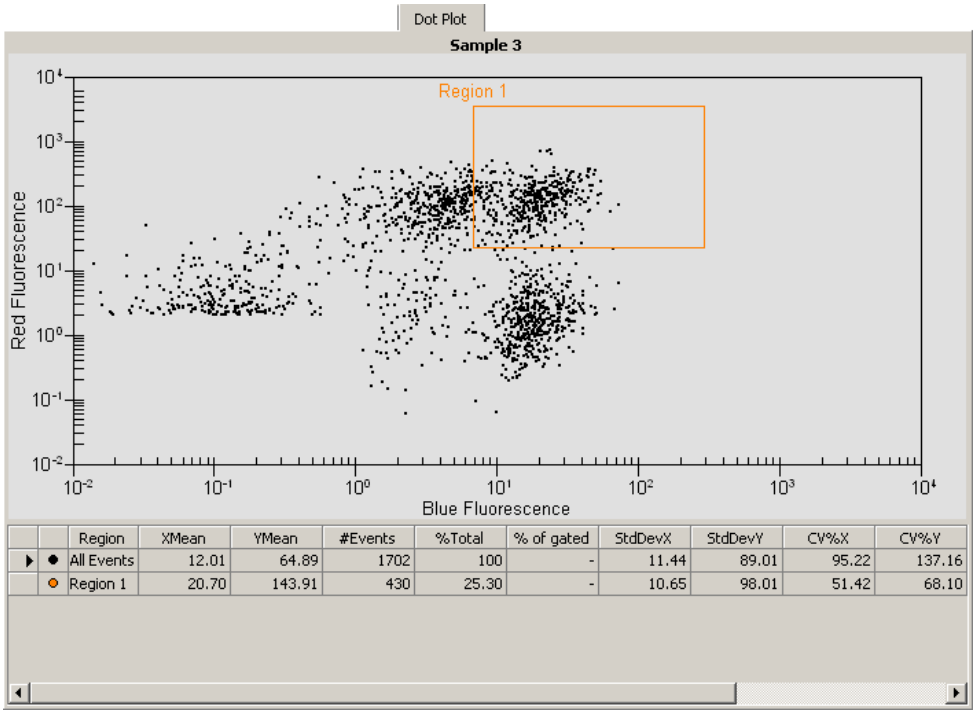
Purpose

Dot plots show single events (cells), displayed as dots. In the coordinate system, the red and blue fluorescence values of each event can be read. For each sample of the chip, one dot plot is shown, either for all 6 samples (grid view) or for an individual sample (single view).

Grid View



Single View



Region	XMean	YMean	#Events	%Total	% of gated	StdDevX	StdDevY	CV%X	CV%Y
All Events	12.01	64.89	1702	100	-	11.44	89.01	95.22	137.16
Region 1	20.70	143.91	430	25.30	-	10.65	98.01	51.42	68.10

Access

The *Dot Plot* tab is always available in the *Data and Assay* context if a flow cytometric chip data file is selected.

You can switch between the dot plot views by choosing *Single View* or *Grid View* in the *Dot Plot* menu.

Dot Plot View

Dot plots are graphical representations of the measurement results, where the blue fluorescence intensity is mapped to the X axis and the red fluorescence intensity is mapped to the Y axis. Both axes are logarithmic.

In the *Grid View*, an overview of all samples is shown. The small dot plots are labeled with the sample name, and below them, the total number of events and the %-value of the covered events can be read. You can select a single dot plot by clicking on it or you can move the focus using the Tab key.

Double-clicking a dot plot in the *Grid View* takes you to the *Single View*, displaying a larger view of the dot plots and a result table below the dot plot. To evaluate dot plots, you can define rectangular regions that can be changed in size and position until they match the event selection. These regions provide you with the number of cells included in the region related to the total number of cells. You can move, enlarge, or reduce the regions. When you change a region, all changes are transferred to the other samples, if the region is a reference. If the region is not defined as reference, the changes are restricted to the current sample (refer to "How to Insert a Region in All Dot Plots" on page 226 for details on reference regions).

Result Table

The result table shows a number of calculated values (columns) for each sample. If you add or redefine regions, the data in the result tables is recalculated.

		Region	XMean	YMean	#Events	%Total	% of gated	StdDevX	StdDevY	CV%X	CV%Y	X GMean	Y GMean
▶	●	All Events	61.81	8.07	1606	100	-	42.90	7.13	69.40	88.37	39.43	4.51
	○	Region 1	73.25	9.96	1195	74.40	84.30	40.70	7.02	55.56	70.44	63.48	7.73

Default table columns are:

Region	The first region (*All Events*) always displays the values for all detected events. For each further region (see "How to Add Regions to Dot Plots (Generic Assay only)" on page 221), a row is added to the table.
XMean	Mean fluorescence values in x direction.
YMean	Mean fluorescence values in y direction.
#Events	Number of events for each region added to the dot plot.
% Total	Percentage of events for each region added to the dot plot.
% of gated	% of the gated events in relation to the total number of events.
StdDevX	Standard deviation to the mean fluorescence value in x direction.
StdDevY	Standard deviation to the mean fluorescence value in y direction.
CV%X	Coefficient of variation of the x values.
CV%Y	Coefficient of variation of the y values.
X GMean	Geometric mean of the x values.
Y GMean	Geometric mean of the y values.

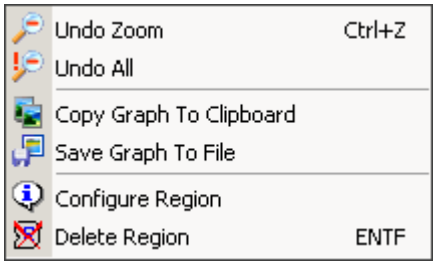
NOTE

You can add/exclude columns from the result table. Right-click the table and select *Configure Columns...* from the context menu. For details refer to "Configure Columns" on page 543.

Context Menus

In *Single View*, two context menus are available.

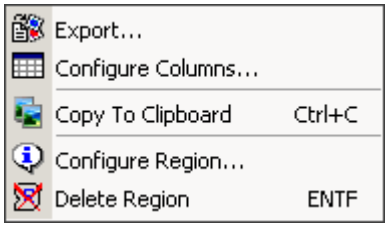
When you right-click a *dot plot*, the following menu appears:



The following functions are available:

Undo Zoom	Undoes the last zoom action.
Undo All	Undoes all zoom actions.
Copy Graph To Clipboard	Puts a copy of the selected dot plot onto the clipboard.
Save Graph To File	Opens a system dialog box allowing you to save the selected dot plot as an image in Windows Meta File (.wmf), Windows Bitmap (.bmp), or JPEG (.jpg) format.
Configure Region...	Opens the *Configure Region* dialog box. Only available if a region is selected in the histogram.
Delete Region	Deletes the selected region. If the region is also used in other dot plots, you will be asked whether it should be removed from all dot plots that use it or only from the selected dot plot.

When you right-click a *result table*, the following menu appears:



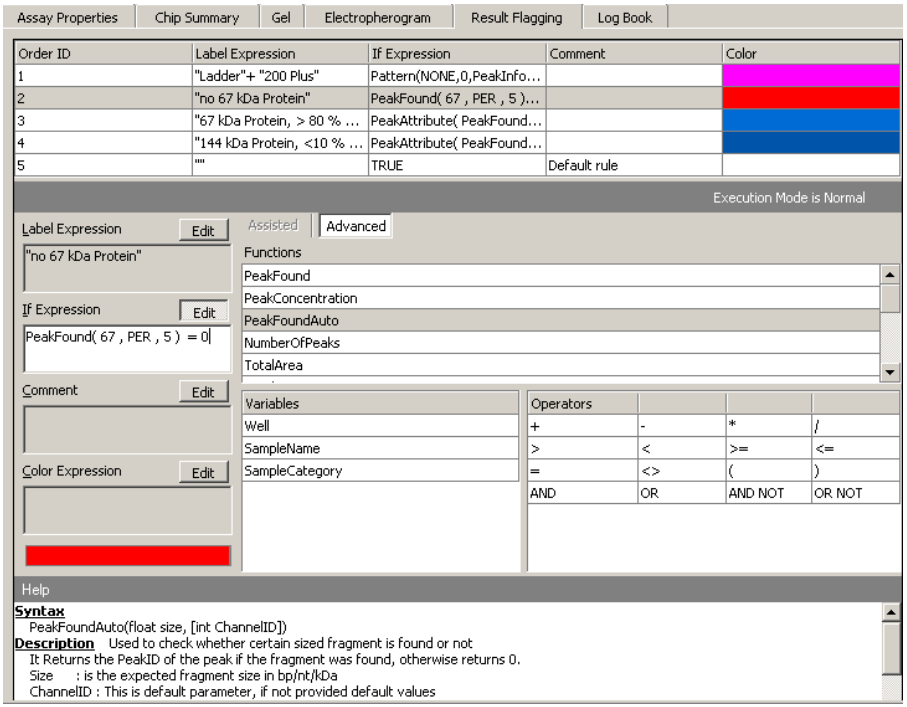
The following functions are available:

Export...	Opens a system dialog box allowing you to save the result table as a .csv file.
Configure Columns...	Opens the *Configure Columns* dialog box.
Copy To Clipboard	Puts the result table into the clipboard. You can paste the table in another application such as MS Excel®. If a part of the table is selected, only this selection is copied.
Configure Region...	Opens the *Configure Region* dialog box. Only available if a region is selected in the histogram.
Delete Region	Deletes the selected region. If the region is also used in other dot plots, you will be asked whether to remove it from all dot plots that use it or only from the selected dot plot.

Result Flagging Tab

Purpose

On this tab, you can define flagging rules for electrophoretic measurement results. A specific color code will be assigned to each sample with measurement results meeting a specific rule.



Access

The *Result Flagging* tab is always available in the *Data and Assay* context if an electrophoretic chip data or assay file is selected.

Rule List

Lists all user-defined result flagging rules, and the default rule, which you cannot delete. The *IF Expression* of the default rule is "TRUE". The default rule defines a color code which is assigned to samples, for which no other rule applies. The *Order ID* column determines the sequence in which the rules are applied to the samples. The other columns show the definitions made in the rule definition area (see below).

- *Normal* mode

  All rules are applied subsequently. The first rule which applies to the sample defines its color. So you should start with the most specific rule. If that one does not apply, a less specific one may apply. If none of the defined rules apply, the final default rule defines the color code.

- Switching to *Target* mode reverses the current rule sequence.

  All rules are applied subsequently. If the next rule applies, the color code changes to the color code defined by the rule, otherwise the previous color code is kept. Therefore, the last valid rule defines the color code of the sample. This mode is called target mode because later rules refine the result color code. The first default color code is the most general and the last one the most specific.

Rule Definition Area

- *Assisted/Advanced*

  Switches between assisted and advanced mode.

- *Label Expression*

  Lets you compose a description for the rule using functions, variables, and strings.

- *If Expression*

  Lets you compose an expression from functions, variables, and logical operators.

- *Comment*

  Lets you enter a comment for the rule.

- *Color Expression*

  Lets you select a color code to be used for flagging samples that meet the rule.

  By selecting *Gradient*, you can choose two colors to specify a color gradient instead of a solid color.

Help Pane

Provides detailed help on the function or variable you are currently working with. Beside the complete *Syntax*, you are provided with a *Description*, and in most cases also with one or more *Examples*.

If Expressions

The expressions can consist of functions, which make use of variables and can be combined by operators.

Functions

Each function has a return type and can have no, one, or more than one parameters. The general syntax is: `returnType function([type parameter], [...])`. Optional parameters are given in [square brackets].

The following types are used:

Return type	Description
`int`	Integer number, e.g. 12
`float`	Floating point number, e.g. 3.14159 or -1.2e-10
`string`	Character string; string constants must be put in quotation marks, e.g. “Agilent”
`enum`	Enumeration, see `int PeakFound(float Size, enum Windowtype, float WindowSize)`

The following functions are available:

Function	Description
`PeakFound`	Used to check whether certain sized fragment is found or not. It returns the PeakID of the peak if the fragment was found, otherwise returns 0.
`PeakFoundAuto`	Used to check whether a certain sized fragment is found or not. It Returns the PeakID of the peak if the fragment was found, otherwise returns 0. This function tries to look for a peak whose fragment size is between size+10% and size-10%.
`PeakConcentration`	Returns the concentration of the peak if the fragment was found, otherwise returns 0.
`NumberOfPeaks`	Returns the number of detected peaks in a channel.

Function	Description
TotalArea	Returns the sum of the areas of all the peaks detected in a sample.
TotalConcentration	Returns the sum of the concentrations of all the peaks detected in a sample.
PeakAttribute	Returns the attribute of given peak.
PeakMax	Returns the peak number of the peak having highest value in given column
PeakMin	Returns the peak number of the peak having the lowest value in given column.
SubStr	Returns a substring of a string.
Pattern	Returns true if the specified pattern in peaks of a sample is found.
rRNAContamination	Returns the RNA contamination of a sample.
rRNARatio	Returns the RNA ratio of a sample.
SignalAttribute	Returns the attribute of a given signal.

NOTE

Please refer to the function reference in the *Help Pane* for details on syntax, usage and examples.

Variable	Description
`Well`	This string variable stands for the coordinates of the well. As it evaluates to a string, it can be used in any expression where string is expected.
`SampleName`	This string variable stands for the sample name. As it evaluates to a string, it can be used in any expression where string is expected.
`SampleCategory`	This string variable stands for the category of the sample. As it evaluates to a string, it can be used in any expression where string is expected.

NOTE

Please refer to the variable reference in the *Help Pane* for details on syntax, usage and examples.

Operators

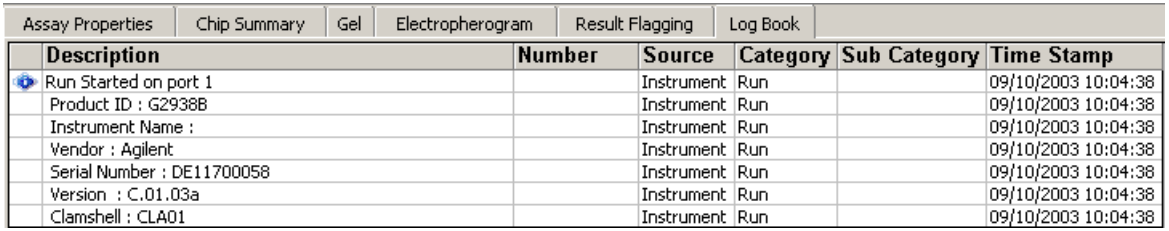
Operators are available for comparison (=, <, <=, >, >=, <>), for arithmetical operations (+, -, *, /), and for logical operations (AND, OR, AND NOT, OR NOT).

Operator	Meaning
=	equals
<	smaller than
<=	smaller than or equal
>	greater than
>=	greater than or equal
<>	not equal
+	plus, concatenation of strings
-	minus
*	multiplied by
/	divided by
AND	logical and
OR	logical or
AND NOT	logical and not
OR NOT	logical or not

Log Book Tab

Purpose

The *Log Book* tab shows chip run events in the run log table. For example, you can see when and by whom the chip run was performed.



Description	Number	Source	Category	Sub Category	Time Stamp
Run Started on port 1		Instrument	Run		09/10/2003 10:04:38
Product ID : G2938B		Instrument	Run		09/10/2003 10:04:38
Instrument Name :		Instrument	Run		09/10/2003 10:04:38
Vendor : Agilent		Instrument	Run		09/10/2003 10:04:38
Serial Number : DE11700058		Instrument	Run		09/10/2003 10:04:38
Version : C.01.03a		Instrument	Run		09/10/2003 10:04:38
Clamshell : CLA01		Instrument	Run		09/10/2003 10:04:38

Also errors and problems with hardware or software are reported by the run log.

Access

The *Log Book* tab is available if a chip data (.xad) file is selected in the *Data and Assay* context.

Run Log Table

The run log table has the following columns:

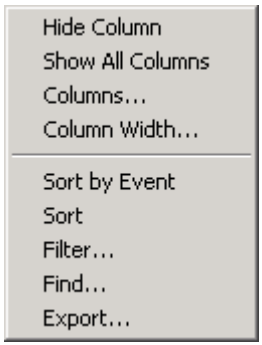
Description	Message text describing the run log entry.
Number	Error number.

Source	Source that triggered the run log entry: “Reader”, “Instrument” or “User Interface”.
Category	“Run”, “System”, “Reserved”, “Sample”, or “Assay”.
User	User who was logged in on *Host* when the run log entry was created.
Host	Name of the computer from which the chip run was started.
Time Stamp	Date and time the run log entry was created.

The run log table is saved as part of the chip data (.xad) file, and you cannot delete it.

Context Menu

Right-clicking on the run log table opens a context menu:



Hide Column	Hides the selected column.
Show All Columns	Shows all columns.

Columns...	Opens a dialog box allowing you to hide, show, and re-sort columns.
Column Width...	Opens a dialog box allowing you to resize the selected column.
Sort by Event	Sorts the table by the *Category* column (ascending).
Sort	Sorts the table by the selected column.
Filter...	Opens the *Filter Events* dialog box allowing you to hide table entries matching filter criteria you can specify. A filter can hide all uncritical events, for example.
Find...	Opens a dialog box allowing you to search the table for any string.
Export...	Opens a dialog box allowing you to export the run log table (or parts of it) as an .html or .txt file.

Tabs (Validation Context)

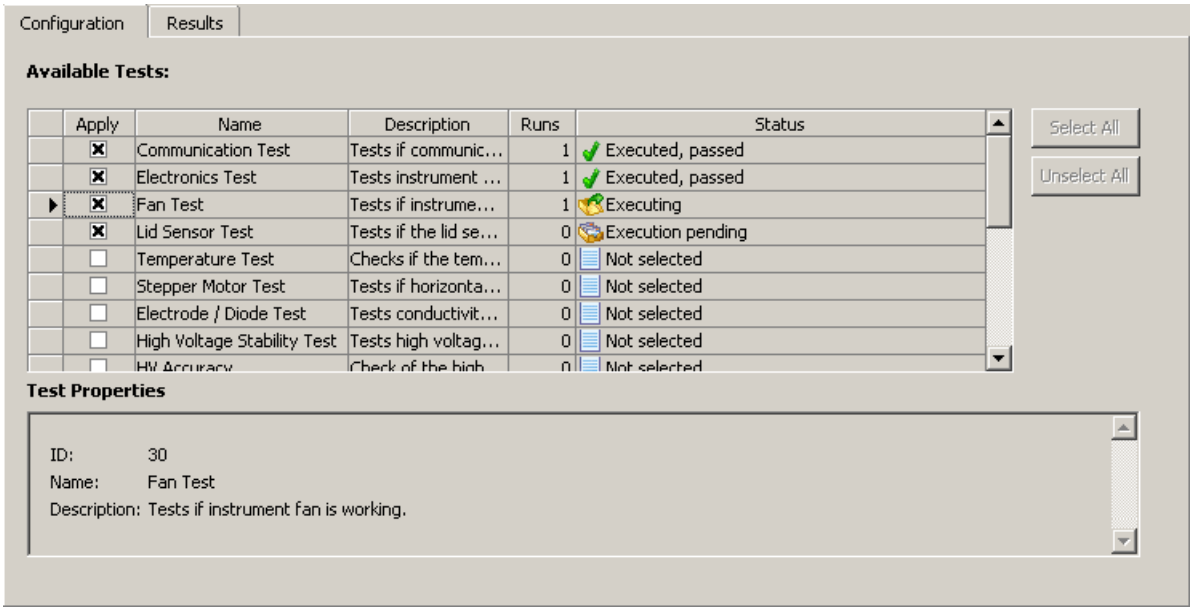
In the *Validation* context the following tabs are available:

- *Configuration Tab*
- *Results Tab*

Configuration Tab

Purpose

The *Configuration* tab lets you select qualification tests to be executed in a validation run. During a validation run, it shows the status and results of the tests.



Available Tests

Columns

Apply	Lets you select the qualification test for execution.
Name	Name of the qualification test.

Description	Brief description of the qualification test.
Runs	Shows how many times the qualification test has been run in this validation.
Status	For each qualification test, the current status is shown: • *Not Selected* • *Selected* • *Executing* • *Execution pending* • *Executed, passed* • *Executed, failed*

Buttons:

Select All	Selects all qualification tests for execution.
Unselect All	Deselects all qualification tests.

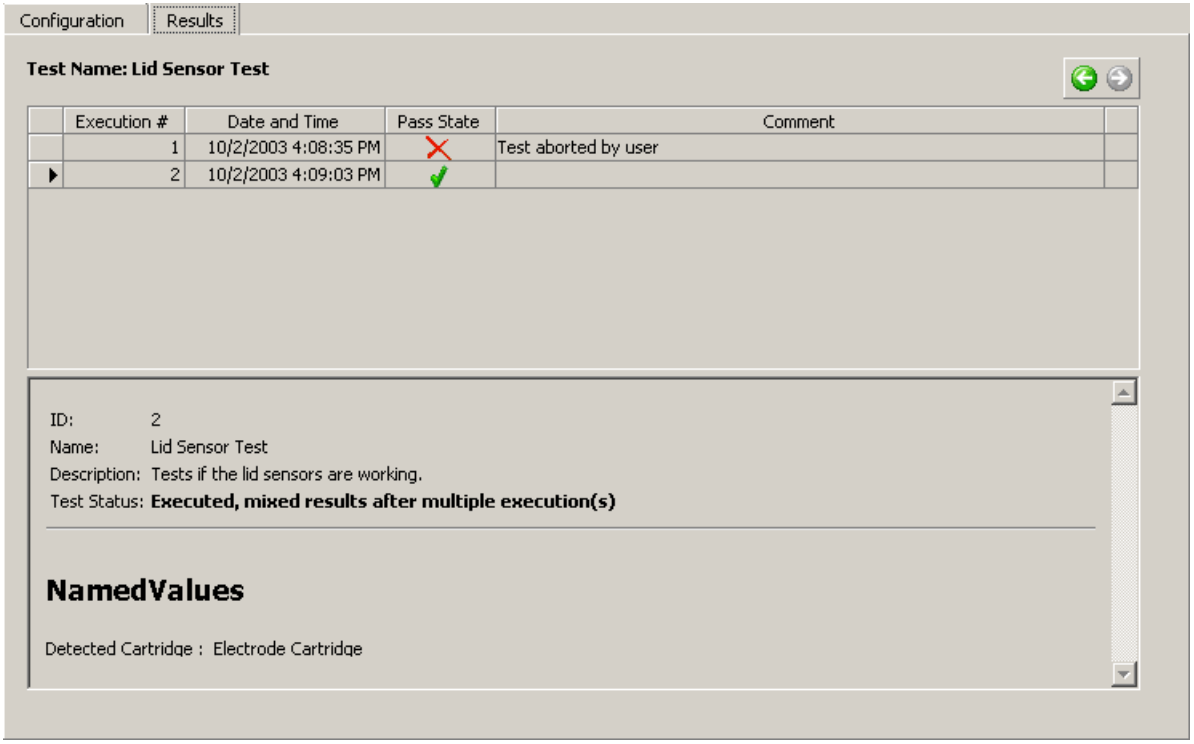
Test Properties

Shows *ID*, *Name* and a detailed *Description* of the qualification test that is selected in the *Available Tests* table.

Results Tab

Purpose

The *Results* tab lets you view detailed results of qualification tests.



The results are shown for one test at a time, but you can browse through the tests.

Test Name: ...

Columns

Execution #	Number of execution (in case of multiple executions of the test).
Date and Time	Date and time the qualification test has been executed.
Pass State	 Qualification test has passed.
	 Qualification test has failed or was aborted.
Comment	Additional information on test execution.

Buttons:

	Jumps to the results of the previous qualification test.
	Jumps to the results of the next qualification test.

Test Properties

Shows *ID*, *Name*, *Description*, *Test Status*, and additional information on the selected qualification test.

Tabs (Comparison Context)

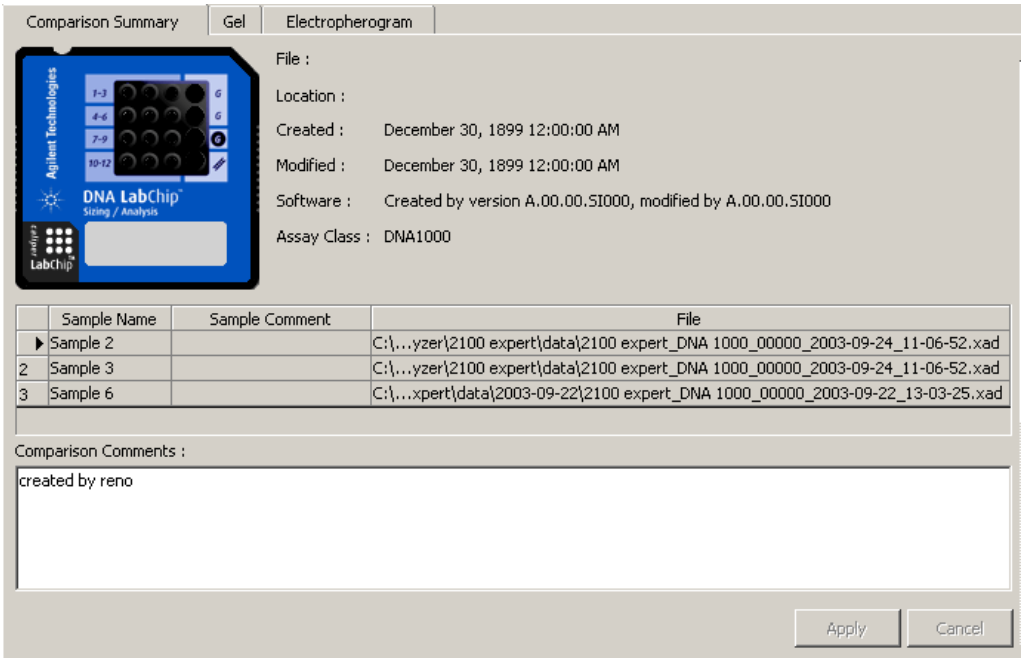
In the *Comparison* context the following tabs are available:

- *Comparison Summary Tab*
- *Gel Tab* (see "Gel Tab" on page 438)
- *Electropherogram Tab (Single/Grid View)* (see *"Electropherogram Tab (Single/Grid View)" on page 451*)

Comparison Summary Tab

Purpose

The *Comparison Summary* shows information on the selected comparison file, and lets you enter a comment regarding the comparison.



Access

The *Comparison Summary* tab is available in the *Comparison* context if a comparison (.xac) file or one of its samples is selected.

Elements

Chip icon	The chip icon indicates the assay type: DNA, RNA, or Protein.
File Name	Name of the comparison (.xac) file.
Location	Directory where the comparison file is stored.
Created	Creation date of the comparison file.
Modified	Date when the comparison file was last modified.
Software	2100 expert versions used to create and modify the comparison file.
Assay Class	DNA1000, for example.
Sample Name	Name of the sample as given in the chip data (.xad) or assay (.xsy) file.
Sample Comment	Sample comment as given in the chip data (.xad) or assay (.xsy) file.
File	Name and directory of the chip data (.xad) file, the sample comes of.
Comparison Comments	Enter comments regarding the comparison here.
Apply	Applies (but does not save) your changes to the *Comparison Comments* field.
Cancel	Undoes the modifications to the *Comparison Comments* field except the ones you have already applied.

In the Comparison context also the following tabs are available:

- *Gel Tab* (see *"Gel Tab" on page 438*)
- *Electropherogram Tab (Single/Grid View)* (see *"Electropherogram Tab (Single/Grid View)" on page 451*)

Please refer to "Comparing Samples from Different Electrophoretic Chip Runs" on page 136 for details on comparing samples from different chip runs.

Status Bar

The status bar at the bottom of the 2100 expert application window shows:

- System messages
- Progress bar

  A progress bar appears, for example, during file opening or saving activities, or during a chip run.
- Date and time
- Status of the *Auto Export*, *Auto Print*, and *Auto Run* options.

  Disabled options are grayed out.

Dialog Boxes

The 2100 expert software provides the following dialog boxes:

Dialog Box	Purpose
General	
"About 2100 Expert" on page 497	To get information on bioanalyzer hardware and software.
"2100 Expert – Close" on page 499	To save unsaved changes on exiting the 2100 expert application or on switching to another context.
"2100 expert – End of Run" on page 511	Shows the status of the finished (or aborted) chip run.
"Auto Export" on page 523	To specify options for automatic data export.
"Auto Print" on page 541	To specify options for automatic printouts.
"Configure Columns" on page 543	To show or hide columns of result tables.
"Open" on page 514	To open chip data files and assay files.
"Options – Data Files" on page 501	To configure the data file save properties.
"Options – Chip Alert" on page 504	To configure the chip alert after finishing the measurement.
"Options – Graph Settings" on page 506	To define graph colors for overlaid electropherograms and histograms.

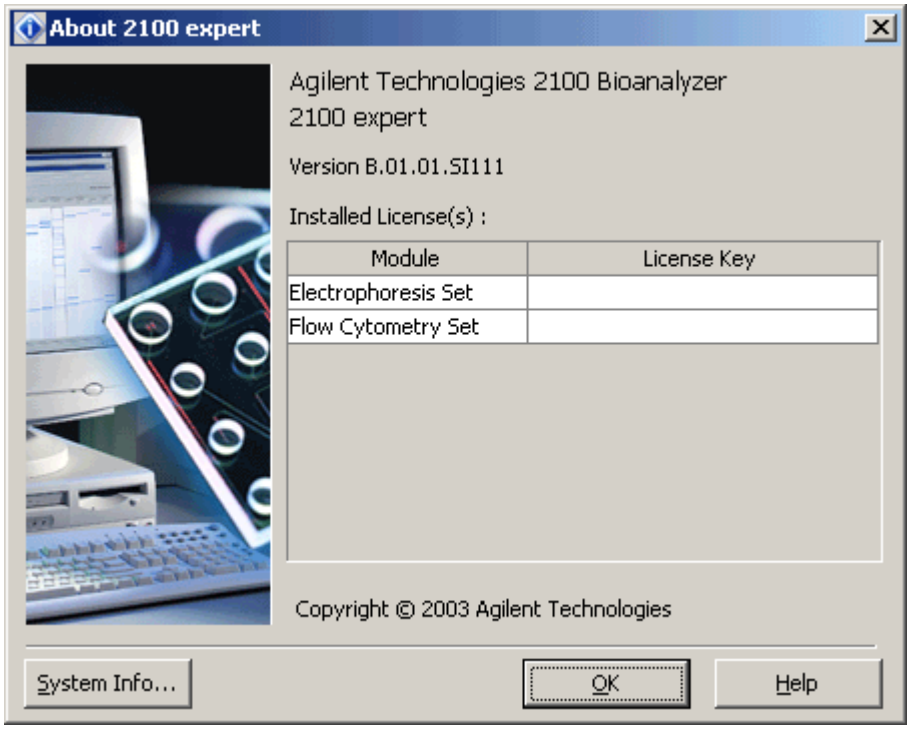
Dialog Box	Purpose
"Options – Advanced" on page 508	To enable automatic print, automatic export, and automatic chip run.
"Page Setup" on page 555	To select the page size and format for printout.
"Print (Validation)" on page 535	To set print options for validation reports and start printing.
"Report Preview" on page 557	To show a page preview before printing.
"Save Selected Samples ..." on page 559	To select a subset of samples for saving.
"System Log Viewer" on page 561	To display system-wide logged events.
Electrophoretic Assays	
"Export Options (Electrophoresis)" on page 519	To export chip run and assay data (electrophoresis) in different formats.
"Print (Electrophoresis)" on page 531	To set print options (electrophoresis) and start printing.
"Print (Comparison)" on page 538	To set print options (electrophoresis) and start printing.
Flow Cytometric Assays	
"Export Options (Flow Cytometry)" on page 516	To export chip run and assay data (flow cytometry) in different formats.
"Print (Flow Cytometry)" on page 528	To set print options (flow cytometry) and start printing.

Dialog Box	Purpose
"Configure Marker" on page 545	To configure the marker in a histogram.
"Copy Marker" on page 554	To confirm or skip the usage of the currently selected marker as a reference marker for all histograms.
"Insert Existing Markers" on page 549	To insert existing markers in a histogram.
"Configure Region" on page 547	To configure the region in a dot plot.
"Copy Region" on page 553	To confirm or skip the usage of the currently selected region as a reference region for all dot plots.
"Insert Existing Region" on page 551	To insert existing regions in a dot plot.

About 2100 Expert

Purpose

This dialog box is used to get information about the installed bioanalyzer hardware and software.



Access

You can open this dialog box by selecting *Help* > *About*.

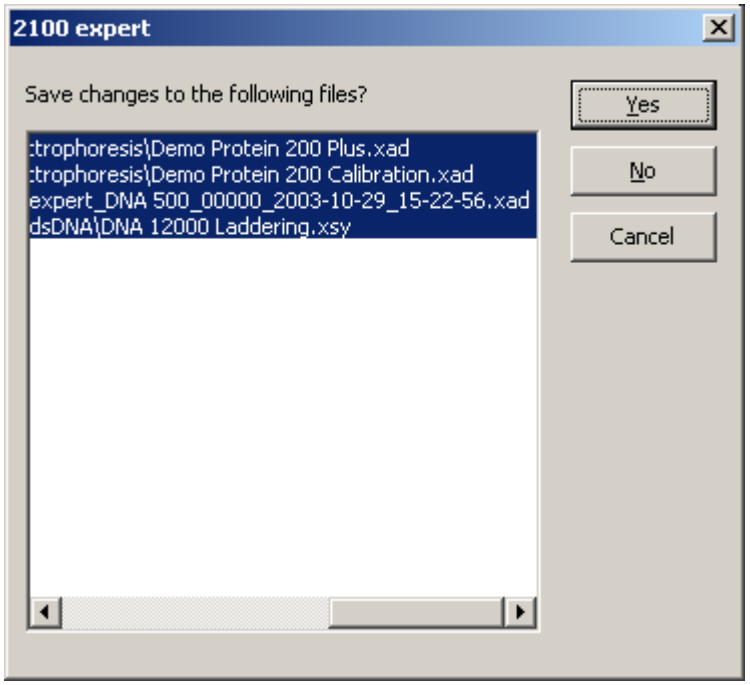
Elements

Module/License Key	Lists the installed software packages and the associated registration keys that were used to activate the packages.
System Info	Starts the *System Info* application of the Windows® operating system. The button is only displayed if the *System Info* application of Windows® is installed. Refer to the Windows® documentation.
OK	Closes the dialog box.

2100 Expert – Close

Purpose

This dialog box lets you decide whether or not to save unsaved changes.



Access

This dialog box appears if you try to exit the 2100 expert application (or if you try to switch between contexts) but there is unsaved data.

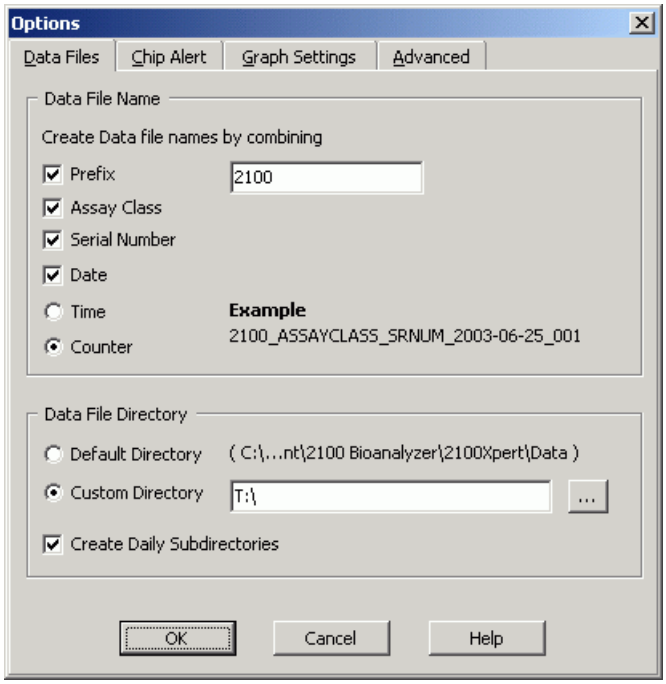
Elements

Save changes to the following files?	If you want to save changes to particular files, you can select or deselect individual files of the list by single-clicking them. By default, all files with unsaved changes are highlighted.
Yes	Saves changes to the selected files and quits 2100 expert (or switches to another context).
No	Exits the 2100 expert application (or switches to another context) without saving anything.
Cancel	Closes the dialog box and returns to your 2100 expert session without saving anything.

Options – Data Files

Purpose

On the *Data Files* tab of the *Options* dialog box, you can specify the names to be used for chip data file storage. Chip data files are automatically named when they are generated.



Access

Open this dialog box by selecting *Tools* > *Options...* and clicking the *Data Files* tab.

Elements

You can make the following settings to specify *Data File Names*:

Prefix	The prefix you enter here will be the first part of the file name. The default file prefix is “2100 expert”.
Serial Number	Inserts the serial number of your Agilent 2100 bioanalyzer instrument in the file name. This is especially useful if you work with a multi-instrument system.
Assay Class	Inserts the assay type in the filename. For example, “DNA1000”, “Protein50”, “Apoptosis”, or “Generic”.
Date	Inserts the date of the chip run.
Time/Counter	*Time* inserts the time in the file name. If you do not want to use a time stamp, you can select *Counter*. *Counter* inserts incremented 3-digit numbers to the file names beginning with “001”, “002”, and so on.
Example	Preview of the .xad file name, depending on the *Data File Name settings* you have chosen.

For example, by including a prefix such as “2100 expert”, as well as the date and time, chip data files would be created with names such as “2100 expert_2003-07-01_14-09-12.xad”.

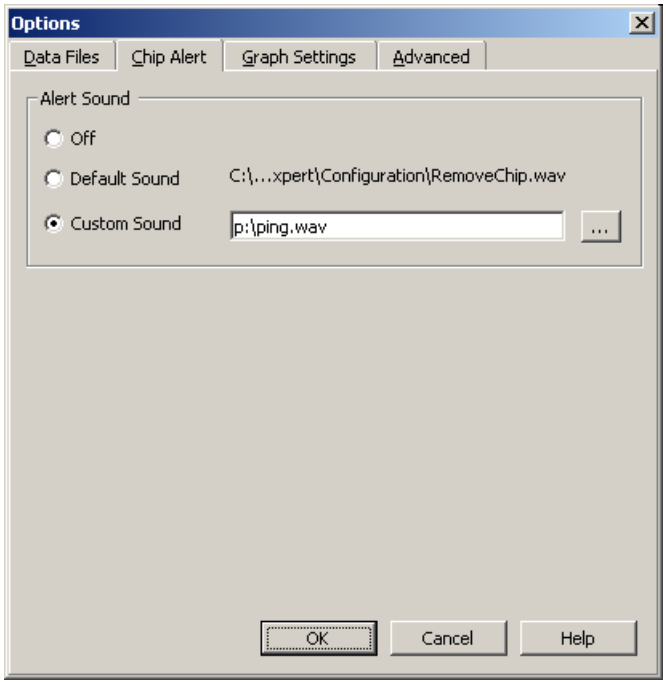
You can specify the directory where you want to store your chip data files (*Data File Directory*):

Default	The default directory is the "..\Data" subdirectory of the installation directory.
Custom	A directory of your choice. Use the *Browse* button to the right of the text field to open the *Directory search* dialog box allowing you to select the directory the data files should be stored in.
Create Daily Subdirectories	On each day you generate chip run data, a subdirectory with the naming format "YYYY-MM-DD" is created in the directory that you have selected for data storage, for example, 2003-08-22.

Options – Chip Alert

Purpose

On the *Chip Alert* tab of the *Options* dialog box, you can specify that the bioanalyzer "beeps" when a chip run is complete and the chip has to be removed from the Agilent 2100 bioanalyzer.



Access

Open this dialog box by selecting *Tools* > *Options...* and clicking the *Chip Alert* tab.

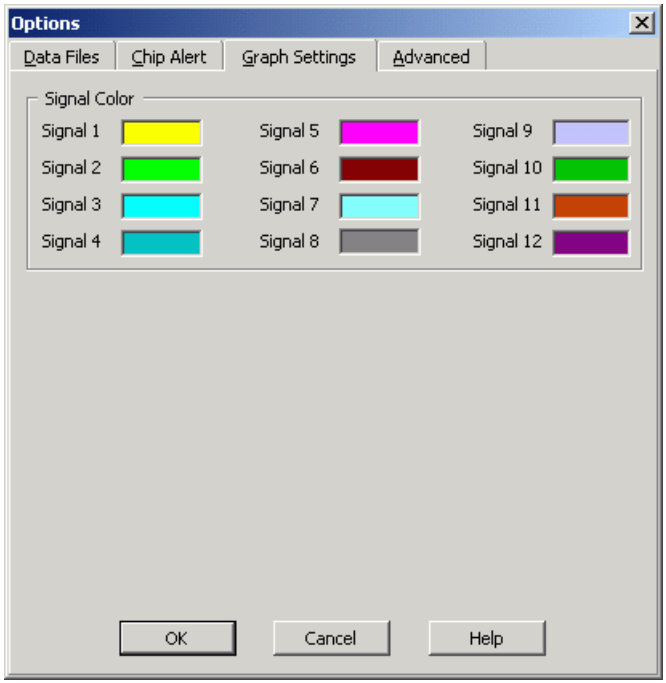
Elements

Off	Turns the alert sound off.
Default Sound	Standard sound for the chip alert.
Custom Sound	Allows the use of a custom sound for the chip alert. Any .wav file is allowed. Click the ... button to the right of the text field to open a dialog box to select the file. The file name will be displayed in the text field.

Options – Graph Settings

Purpose

You can compare samples by overlaying their electropherograms/histograms. On the *Graph Settings* tab of the *Options* dialog box you can set the signal (electropherogram/histogram) colors in overlaid graphs.



Access

Open this dialog box by selecting *Tools* > *Options...* and clicking the *Graph Settings* tab.

Elements

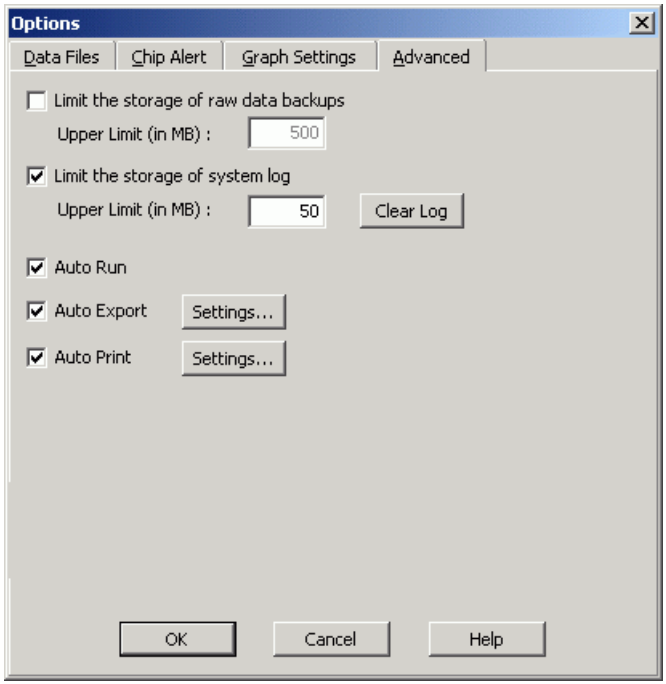
Signal Color	The colored box next to *Signal n* (where n stands for the sample number) shows the color of the curve in the overlaid graph. Click this box to open the *Color* dialog box. You can select a color and assign it by clicking *OK*.

Options – Advanced

Purpose

On the *Advanced* tab of the *Options* dialog box, you can:

- Set disk space limits for data storage
- Set automatic chip run, automatic export, and automatic printouts.



Access

Open this dialog box by selecting *Tools* > *Options...* and click the *Advanced* tab.

Elements

Limit the storage of raw data backups	Select this option to limit the amount of disk space reserved for raw data storage. The raw data backup files (packet files, .pck) are stored in the "..\packets" subdirectory. The default for the *Upper Limit (in MB)* is 20, which corresponds to approximately 20 chip data files.
Limit the storage of system log	Select this option to limit the amount of disk space reserved for the system log file "SystemLogBook.log" (located in the "..\log" subdirectory). When the *Upper Limit (in MB)* is reached, a message box appears, asking you if the system log file should be deleted.
Clear Log	Click this button to delete the system log file "SystemLogBook.log" (located in the "..\log" subdirectory).
Auto Run	Activates the *Auto Run* mode. Once the lid of the Agilent 2100 bioanalyzer is closed and a suitable chip is detected, the assay is started automatically, if this option is selected.
Auto Export	Activates the auto export mode. Every new chip run will be exported automatically. Clicking the *Settings...* button brings up the *Auto Export* dialog box allowing you to select the type and target location for data to be exported. To learn more about exporting data see "Exporting Data" on page 263.
Auto Print	Activates the auto print mode. Every new chip data file generated will automatically be sent to the printer. Clicking the *Settings...* button brings up the *Auto Print* dialog box allowing you to define the type of printouts, and select a printer. To learn more about printing see "Printing Reports" on page 275.

NOTE

The *Auto Print* and *Auto Export* settings are independent from the *Print...* and *Export...* commands in the *File* menu (see "File Menu" on page 326).

2100 expert – End of Run

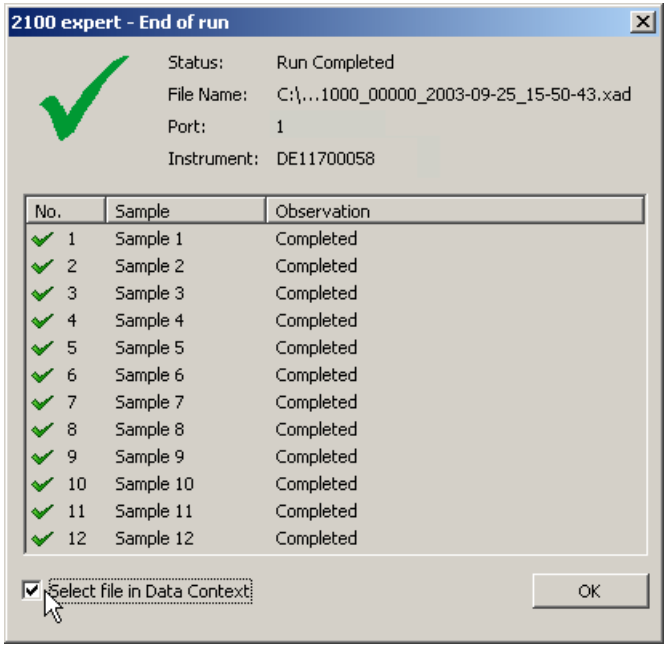
Purpose

This dialog box indicates the end of the current chip run, and any errors associated with the run.

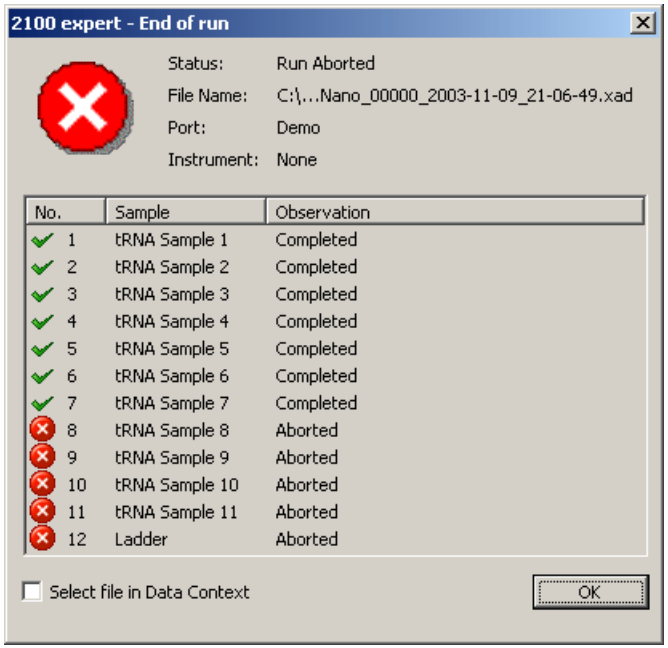
Access

The dialog box appears (accompanied by an alert sound, see "Options – Chip Alert" on page 504) when a chip run is completed, or if you have aborted a chip run.

Completed chip run:



Aborted chip run:



Elements

Status	Shows the status of the chip run, either *Run Aborted* or *Run Completed*.
File Name	Name of the data file (.xad) the chip run results are saved to.
Port	COM port number the *Instrument* is connected to, or "Demo" if you have run a demo assay.
Instrument	Name of the bioanalyzer used for the chip run.

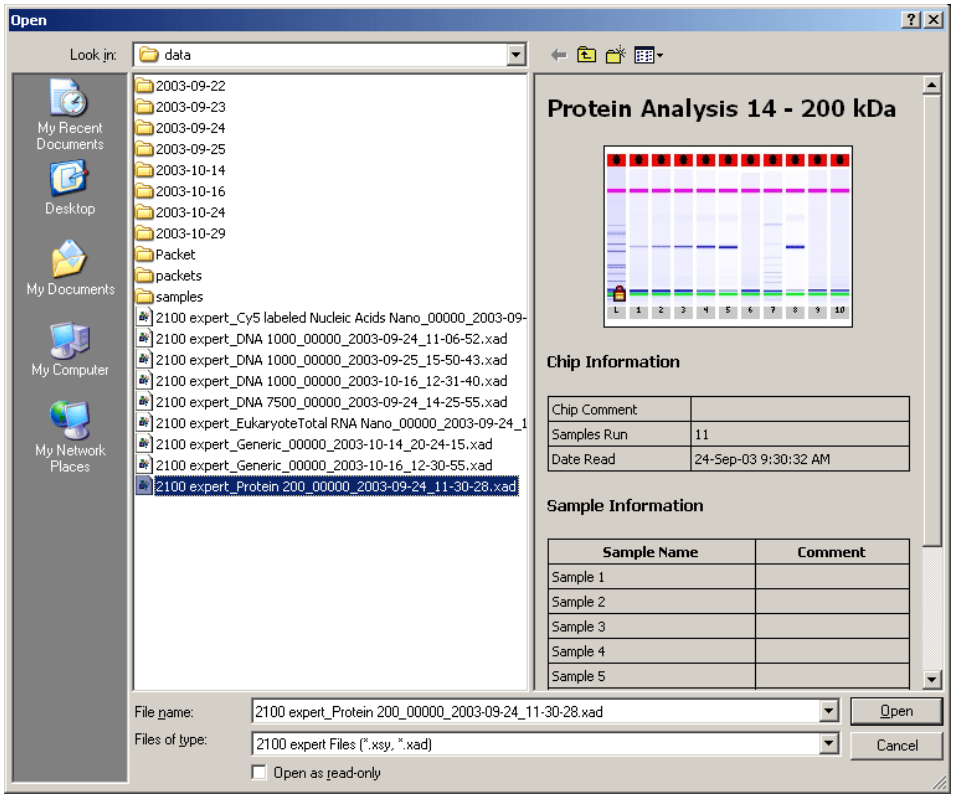
No.	Sample number. A green check next to the sample number indicates a completed measurement, while a white cross on red ground indicates an aborted or incomplete measurement.
Sample	Sample names.
Observation	Shows how many events were detected (for flow cytometric assays), how many peaks were found (for DNA and Protein assays), RNA ratio amounts (for Total RNA assays), rRNA contamination (for mRNA assays), and the read time only for Cy5-labeled nucleic acid assays. Also shows sample-specific (error) messages.
Select file in Data Context	When you click *OK* with this option selected, you will be taken to the *Data and Assay* context, where the results of the current chip run are displayed.

Open

Purpose

This dialog box is used to open

- chip data files (.xad), assay files (.xsy), or to import markers or regions from such files,
- comparison files (.xac),
- validation result files (.xvd).



Access

You can open this dialog box by selecting *File> Open...*.

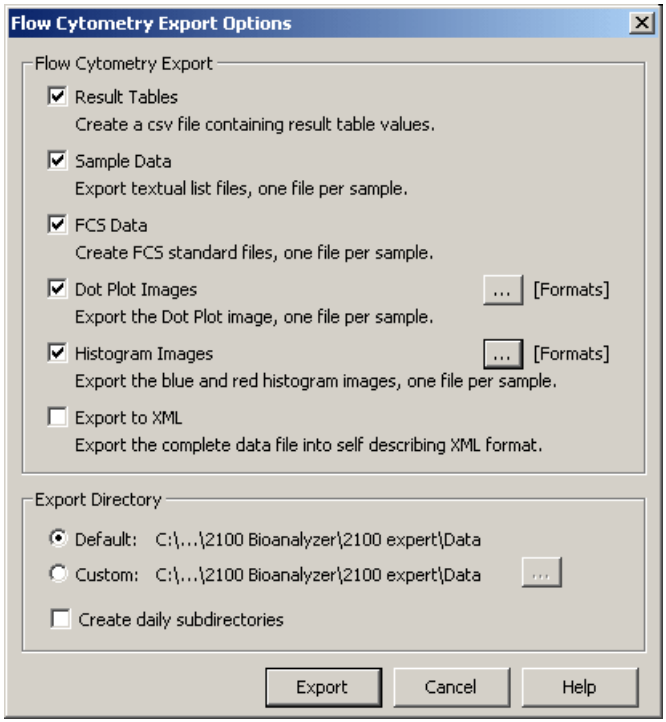
Elements

Look in	Allows you to select a drive and directory.
File name	Displays the name of the selected file.
File of type	Allows you to select the file type.
Assay list	Displays the chip data and assay files located in the selected directory.
Information frame	Displays information on the selected file. For chip data files, for example, you will see a preview of the gel image.
Open as read-only	If you open a file with this option selected, you will not be able to save any changes to the file.
Open	Click this button to open the selected file.

Export Options (Flow Cytometry)

Purpose

This dialog box is used to export flow cytometric chip data in different formats.



Access

You can open this dialog box by selecting *File* > *Export ...* with a flow cytometric chip data file (.xad) selected.

Elements

Flow Cytometry Export categories:

Result Tables	Creates a .csv file containing result tables. All measured samples are exported to one file.
Sample Data	Exports text list files (.csv), one file per sample. The event data is intended for advanced users who want to closely analyze the data. Included are the time the event is measured, and the blue and red fluorescence values.
FCS Data	Creates FCS standard files (.fcs), one file per sample. This is the standard format for flow cytometry data, and can be processed using the FCS Express application, for example. In FCS Express, bioanalyzer data, which is spread over six decades (0.01 ... 10^4), will be displayed in the x axis range from 1 ... 1024.
Dot Plot Images	Exports the dot plot images of all samples, one image per sample. Clicking on the ... button opens a dialog box allowing you select from the following image formats: Windows Bitmap (.bmp), JPEG (.jpg), and Windows Meta File (.wmf). Multiple selections are possible.

Histogram Images	Exports the histogram images of all samples, two images (red and blue histogram) per sample. Clicking on the ... button opens a dialog box allowing you select from the following image formats: Windows Bitmap (.bmp), JPEG (.jpg), and Windows Meta File (.wmf). Multiple selections are possible.
Export to XML	Writes the complete .xad file contents to a structured .xml file.

Export Directory settings:

Default	The default target directory is "..\Data\" under the 2100 expert installation folder.
Custom	A target directory of your choice. Use the ... button right to the text field to open the *Browse for folder* dialog box.
Create Daily Subdirectories	If selected, a subdirectory with the naming format "YYYY-MM-DD" is created in the target directory and the export data is written to this directory.

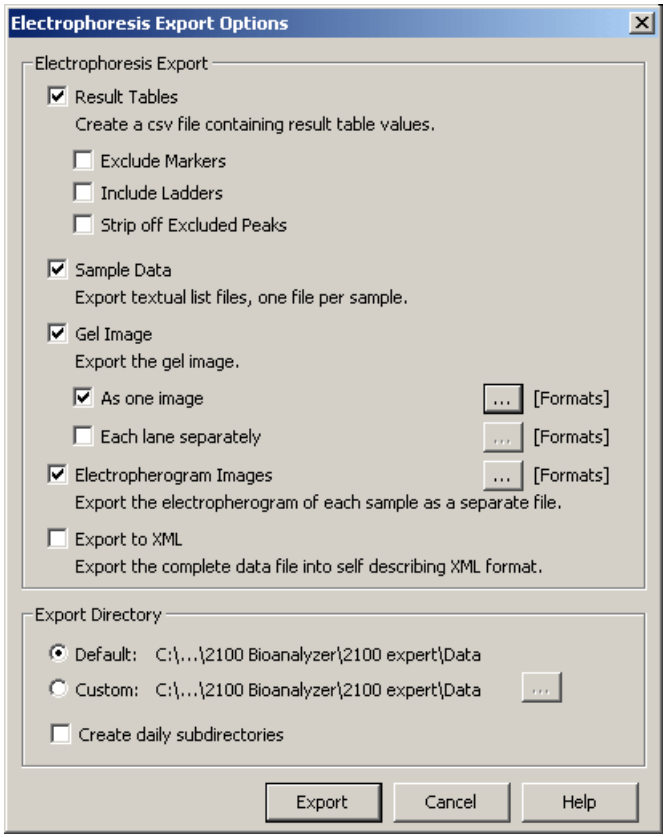
Buttons:

Export	Click this button to export the selected data. For each data category, the *Save As* dialog box appears, where you can enter new file names and destination directories.

Export Options (Electrophoresis)

Purpose

This dialog box is used to export electrophoretic chip data in different formats.



Access

You can open this dialog box by selecting *File* > *Export ...* with an electrophoretic chip data file (.xad) selected.

Elements

Electrophoresis Export categories:

Result Tables	Creates a CSV file (.csv) containing result tables. All measured samples are exported to one file. On exporting you can: • *Exclude Markers* • *Include Ladders* • *Strip off Excluded Peaks*
Sample Data	Exports text list files (.csv), one file per sample. These files are intended for advanced users who want to closely analyze the measurement data. Included are the current values of migration time and fluorescence of all test points.

Gel Image	Exports the gel-like images of all samples. You can export the gel view: • *As one image* • *Each lane separately* Clicking on one of the ... buttons opens a dialog box allowing you select from the following image formats: Windows Bitmap (.bmp), JPEG (.jpg), and Tagged Image File (.tif). Multiple selections are possible.
Electropherogram Images	Exports the electropherogram images of all samples, one image per sample. Clicking on the ... button opens a dialog box allowing you select from the following image formats: Windows Bitmap (.bmp), JPEG (.jpg), Tagged Image File (.tif), and Windows Meta File (.wmf). Multiple selections are possible.
Export to XML	Writes the complete .xad file contents to an .xml file.

Export Directory settings:

Default	The default target directory is "..\Data\" under the 2100 expert installation folder.

Custom	A target directory of your choice. Use the ... button right to the text field to open the *Browse for folder* dialog box.
Create Daily Subdirectories	If selected, a subdirectory with the naming format "YYYY-MM-DD" is created in the target directory and the export data is written to this directory.

Buttons:

Export	Click this button to export the selected data. For each data category, the *Save As* dialog box appears, where you can enter new file names and destination directories.

Auto Export

Purpose

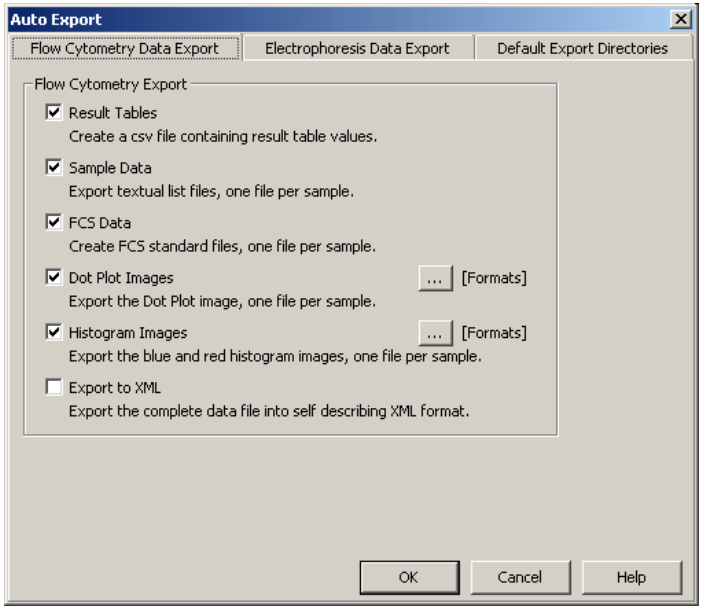
To set options for automatic export, which takes place each time a chip run is complete.

Access

You can open this dialog box from the *Options* dialog box (see "Options – Advanced" on page 508) by clicking on the *Settings...* button next to the *Auto Export* check box.

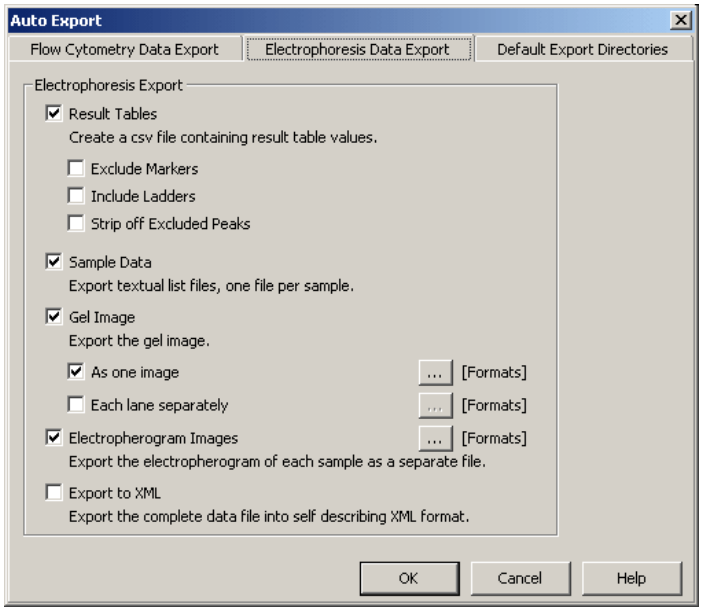
Elements

Flow Cytometry Data Export tab



Result Tables	Creates a CSV file (.csv) containing result tables. All measured samples are exported to one file.
Sample Data	Exports text list files (.csv), one file per sample. The event data is intended for advanced users who want to closely analyze the data. Included are the time the event is measured, and the blue and red fluorescence values.
FCS Data	Creates FCS standard files (.fcs), one file per sample. This is the standard format for flow cytometry data.
Dot Plot Images	Exports the dot plot images of all samples, one image per sample. Clicking on the ... button opens a dialog box allowing you select from the following image formats: Windows Bitmap (.bmp), JPEG (.jpg), and Windows Meta File (.wmf). Multiple selections are possible.
Histogram Images	Exports the histogram images of all samples, two images (red and blue histogram) per sample. Clicking on the ... button opens a dialog box allowing you select from the following image formats: Windows Bitmap (.bmp), JPEG (.jpg), and Windows Meta File (.wmf). Multiple selections are possible.
Export to XML	Writes the complete .xad file contents to an .xml file.

Electrophoresis Data Export tab



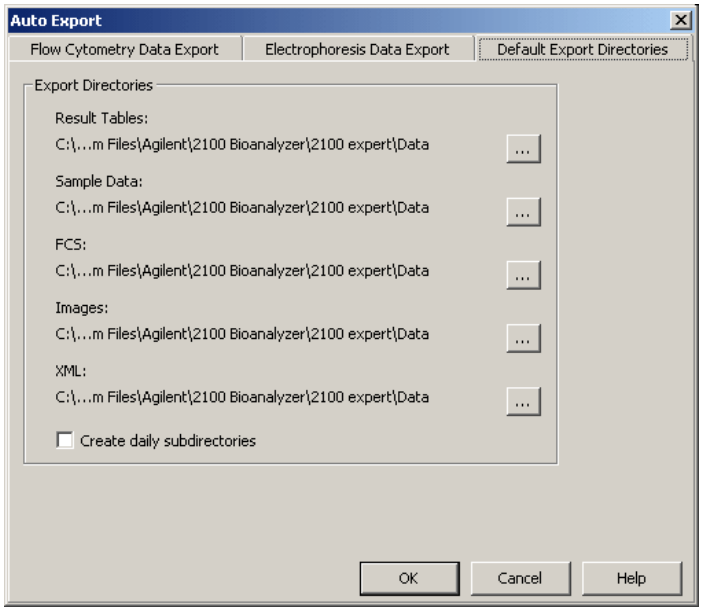
Result Tables

Creates a CSV file (.csv) containing result tables. All measured samples are exported to one file. On exporting you can:

- *Exclude Markers*
- *Include Ladders*
- *Strip off Excluded Peaks*

Sample Data	Exports text list files (.csv), one file per sample. These files are intended for advanced users who want to closely analyze the measurement data. Included are migration time and fluorescence values (raw data) of all test points.
Gel Image	Exports the gel-like images of all samples. You can export the gel view: • *As one image* • *Each lane separately* Clicking on one of the ... buttons opens a dialog box allowing you select from the following image formats: Windows Bitmap (.bmp), JPEG (.jpg), and Tagged Image File (.tif). Multiple selections are possible.
Electropherogram Images	Exports the electropherogram images of all samples, one image per sample. Clicking on the ... button opens a dialog box allowing you select from the following image formats: Windows Bitmap (.bmp), JPEG (.jpg), Tagged Image File (.tif), and Windows Meta File (.wmf). Multiple selections are possible.
Export to XML	Writes the complete .xad file contents to an .xml file.

Default Export Directories tab

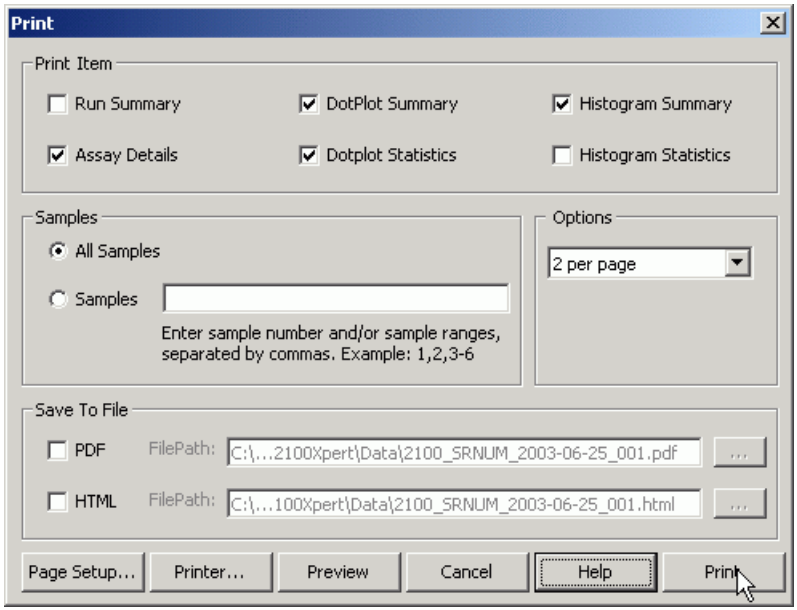


Export Directories	It is advisable to specify individual target directories for *Result Tables*, *Sample Data*, *FCS* files, *Images*, and *XML* files. Clicking on the ... buttons next to these categories opens a system dialog box allowing you to specify the target directory for the category.
Create daily subdirectories	If selected, subdirectories with the naming format "YYYY-MM-DD" are created in the target directories and the export data is written to these directories.

Print (Flow Cytometry)

Purpose

This dialog box is used to set print options for flow cytometric chip data and assay files and to start printing.



Access

You can open this dialog box by selecting *File* > *Print...* with a flow cytometric assay (.xsy) or chip data (.xad) file selected.

Elements

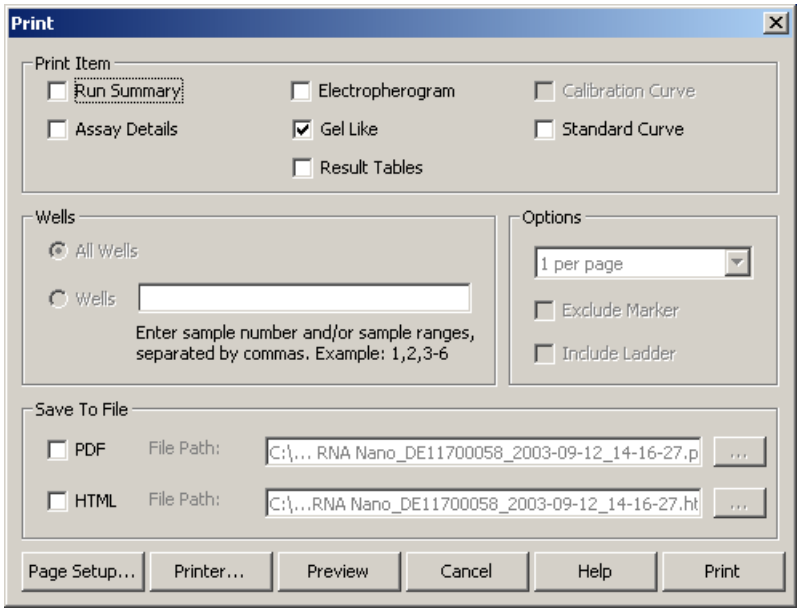
Print Item	
Run Summary	Includes chip and sample information in the printout. See "Chip Summary Tab" on page 427.
Assay Details	Includes assay name, path, and setpoints in the printout. See "Assay Properties Tab" on page 418.
Dot Plot Summary	Includes dot plot graphs in the printout. See "Dot Plot Tab (Single/Grid View)" on page 468.
Dot Plot Statistics	Includes dot plot result tables in the printout. See "Dot Plot Tab (Single/Grid View)" on page 468.
Histogram Summary	Includes histogram graphs in the printout. "Histogram Tab (Single/Grid View)" on page 460
Histogram Statistics	Includes histogram result tables in the printout. "Histogram Tab (Single/Grid View)" on page 460
Samples	
All Samples	Prints summaries and statistics for all samples.
Samples ...	Prints summaries and statistics only for selected samples.
Options	Select how many dot plots/histogram graphs to print per page. Affects the printout only if *Dot Plot Summary* or *Histogram Summary* is selected.

Save To File	
PDF	Redirects the printout to a .pdf file. Clicking on the ... button opens a system dialog box allowing you to specify a target directory of your choice.
HTML	Redirects the printout to a set of .html files. Clicking on the ... button opens a system dialog box allowing you to specify a target directory of your choice.
Buttons	
Page Setup...	Opens the *Page Setup* dialog box, allowing you to set the page size, page orientation, and page margins. See also "Page Setup" on page 555.
Printer...	Opens the *Print Setup* dialog box, allowing you to select a printer, and set the page size and page orientation.
Preview	Opens the *Report Preview* dialog box displaying a preview of all pages that will be printed. See also "Report Preview" on page 557.
Cancel	Exits the dialog box without printing.
Print/Save	If you selected any option under *Save To File*, the button is labeled *Save*, otherwise *Print*. *Print* starts printing. *Save* starts writing the printout to .pdf and/or .html files.

Print (Electrophoresis)

Purpose

This dialog box is used to set print options for electrophoretic chip data or assay files and to start printing.



Access

You can open this dialog box by selecting *File* > *Print...* with an electrophoretic assay (.xsy) or chip data (.xad) file selected.

Elements

Print Item	
Run Summary	Includes chip and sample information in the printout. See "Chip Summary Tab" on page 427.
Assay Details	Includes assay name, path, and setpoints in the printout. See "Assay Properties Tab" on page 418.
Electropherogram	Includes electropherogram graphs in the printout. See "Electropherogram Tab (Single/Grid View)" on page 451.
Gel Like	Includes a gel-like image (all samples) in the printout. See "Gel Tab" on page 438.
Result Tables	Includes result tables in the printout.
Calibration Curve	Includes a calibration curve graph in the printout. See "Chip Summary Tab" on page 427.
Standard Curve	Includes a standard curve graph in the printout. See "Chip Summary Tab" on page 427.
Wells	
All Wells	Prints summaries and statistics for all samples.
Wells ...	Prints summaries and statistics only for selected samples.

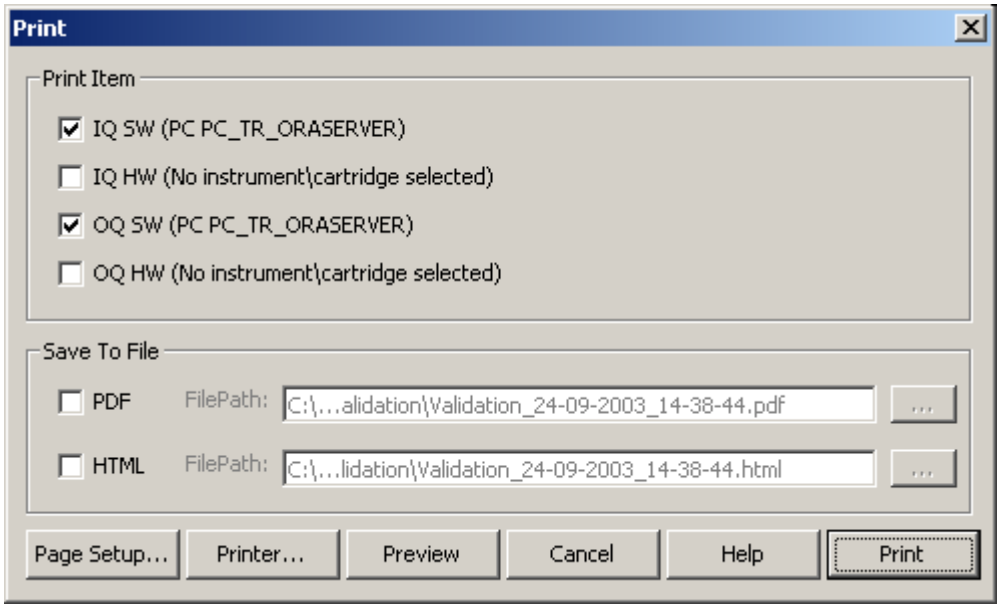
Options	Select how many electropherograms to print per page. Affects the printout only if *Electropherogram* is selected under *Print Item*. You can also: • *Exclude Markers* • *Include Ladder*
Save To File	
PDF	Redirects the printout to a .pdf file. Clicking on the ... button opens a system dialog box allowing you to specify a target directory of your choice.
HTML	Redirects the printout to a set of .html files. Clicking on the ... button opens a system dialog box allowing you to specify a target directory of your choice.
Buttons	
Page Setup...	Opens the *Page Setup* dialog box, allowing you to set the page size, page orientation, and page margins. See also "Page Setup" on page 555.
Printer...	Opens the *Print Setup* dialog box, allowing you to select a printer, and set the page size and page orientation.
Preview	Opens the *Report Preview* dialog box displaying a preview of all pages that will be printed. See also "Report Preview" on page 557.

Cancel	Exits the dialog box without printing.
Print/Save	If you selected any option under *Save To File*, the button is labeled *Save*, otherwise *Print*. *Print* starts printing. *Save* starts writing the printout to .pdf and/or .html files.

Print (Validation)

Purpose

This dialog box is used to set print options for validation reports and to start printing.



Access

You can open this dialog box by selecting *File* > *Print...* with a validation (.xvd) file selected in the Validation Context.

Elements

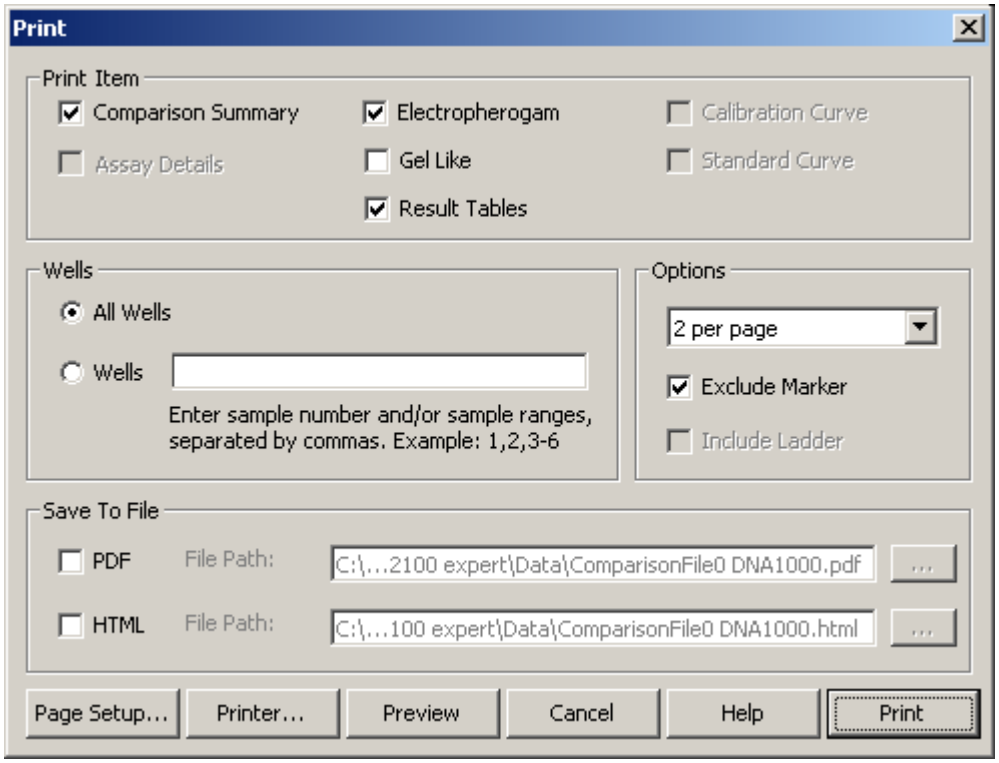
Print Item	
IQ SW	Includes the results of all installation qualification tests for the 2100 expert system software.
IQ HW	Includes the results of all installation qualification tests on the Agilent 2100 Bioanalyzer hardware.
OQ SW	Includes the results of all operational qualification tests for the 2100 expert system software.
OQ SW	Includes the results of all operational qualification tests on the Agilent 2100 Bioanalyzer hardware.
Save To File	
PDF	Redirects the printout to a .pdf file. Clicking on the ... button opens a system dialog box allowing you to specify a target directory of your choice.
HTML	Redirects the printout to a set of .html files. Clicking on the ... button opens a system dialog box allowing you to specify a target directory of your choice.
Buttons	
Page Setup...	Opens the *Page Setup* dialog box, allowing you to set the page size, page orientation, and page margins. See also "Page Setup" on page 555.

Printer...	Opens the *Print Setup* dialog box, allowing you to select a printer, and set the page size and page orientation.
Preview	Opens the *Report Preview* dialog box displaying a preview of all pages that will be printed. See also "Report Preview" on page 557.
Cancel	Exits the dialog box without printing.
Print/Save	If you selected any option under *Save To File*, the button is labeled *Save*, otherwise *Print*. *Print* starts printing. *Save* starts writing the printout to .pdf and/or .html files.

Print (Comparison)

Purpose

This dialog box is used to set print options for comparison reports and to start printing.



Access

You can open this dialog box by selecting *File* > *Print...* with a comparison (.xac) file selected in the Comparison Context.

Elements

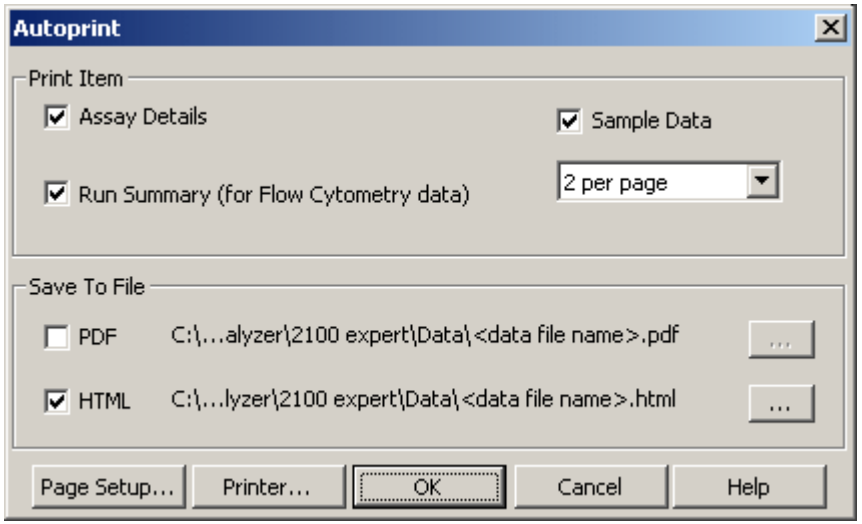
Print Item	
Comparison Summary	Includes information on the comparison file in the printout.
Assay Details	Includes assay name, path, and setpoints in the printout. See "Assay Properties Tab" on page 418.
Electropherogram	Includes electropherogram graphs in the printout. See "Electropherogram Tab (Single/Grid View)" on page 451.
Gel Like	Includes a gel-like image (all samples) in the printout. See "Gel Tab" on page 438.
Result Tables	Includes result tables in the printout.
Calibration Curve	Includes a calibration curve graph in the printout. See "Chip Summary Tab" on page 427.
Standard Curve	Includes a standard curve graph in the printout. See "Chip Summary Tab" on page 427.
Wells	
All Wells	Prints summaries and statistics for all samples.
Wells ...	Prints summaries and statistics only for selected samples.
Options	Select how many electropherograms to print per page. Affects the printout only if *Electropherogram* is selected. If *Result Tables* is selected you can also *Exclude Markers.*

Save To File	
PDF	Redirects the printout to a .pdf file. Clicking on the ... button opens a system dialog box allowing you to specify a target directory of your choice.
HTML	Redirects the printout to a set of .html files. Clicking on the ... button opens a system dialog box allowing you to specify a target directory of your choice.
Buttons	
Page Setup...	Opens the *Page Setup* dialog box, allowing you to set the page size, page orientation, and page margins. See also "Page Setup" on page 555.
Printer...	Opens the *Print Setup* dialog box, allowing you to select a printer, and set the page size and page orientation.
Preview	Opens the *Report Preview* dialog box display a preview of all pages that will be printed. See also "Report Preview" on page 557.
Cancel	Exits the dialog box without printing.
Print/Save	If you selected any option under *Save To File*, the button is labeled *Save*, otherwise *Print*. *Print* starts printing. *Save* starts writing the printout to .pdf and/or .html files.

Auto Print

Purpose

To set options for automatic printouts, that are to be generated each time a chip run is complete.



Access

You can open this dialog box from the *Options* dialog box (see "Options – Advanced" on page 508) by clicking on the *Settings...* button next to the *Auto Print* checkbox.

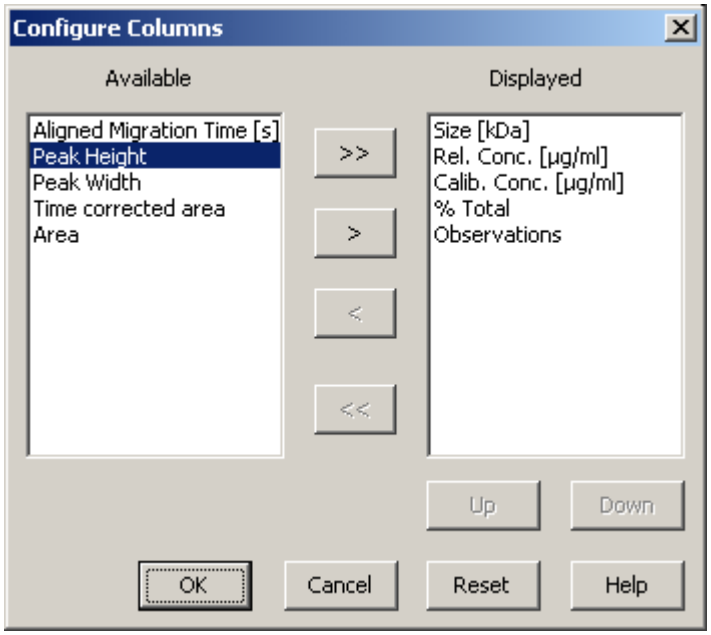
Elements

Print Item	
Assay Details	Includes assay name, path, and setpoints in the printout. See "Assay Properties Tab" on page 418.
Run Summary (for Flow Cytometry data)	Includes chip and sample information in the printout. See "Chip Summary Tab" on page 427.
Sample Data	Includes sample data in the automatic printout. This data depends on the assay type: electropherogram graphs and gel-like images, or histogram and dot plot graphs. Select how many electropherograms/histograms/dot plots to print *per page*.
Save To File	
PDF	Redirects the automatic printout to a .pdf file.
HTML	Redirects the automatic printout to a set of .html files.
Buttons	
Page Setup...	Opens the *Page Setup* dialog box, allowing you to set the page size, page orientation, and page margins. See also "Page Setup" on page 555.
Printer...	Opens the *Print Setup* dialog box, allowing you to select a printer, and set the page size and page orientation.
OK	Accepts the new auto print settings.

Configure Columns

Purpose

This dialog box is used to show or hide columns of tables.



Access

You can display this dialog box by opening the context menu in a result table on the *Dot Plot*, *Histogram*, *Gel*, or *Electropherogram* tab, and selecting *Configure Columns...*.

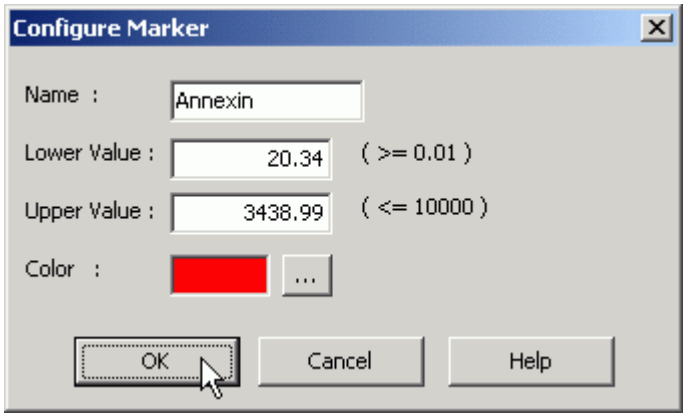
Elements

Available	This list on the left shows you all available but currently not displayed columns.
Displayed	This list on the right shows you all columns that are currently displayed. The sequence in this list is related to the sequence of the columns.
>	Shifts the selected entry form the left list to the right. The selected entry will be displayed in the result table at the corresponding position.
>>	Shifts all entries of the left list to the right. All available results will be displayed in the order that is given by the list.
<	Click this button to shift the selected entry from the right list to the left. The selected entry will no longer be displayed in the table.
<<	Click this button to shift all entries from the right list to the left. No results will be displayed in the table.
Up	Moves the selected column one position to the left.
Down	Moves the selected column one position to the right.
Reset	Restores the initial table configuration.

Configure Marker

Purpose

This dialog box is used to set the properties of the selected marker in a histogram.



Access

You can open this dialog by double-clicking on a marker or by double-clicking the corresponding row of the result table in a histogram.

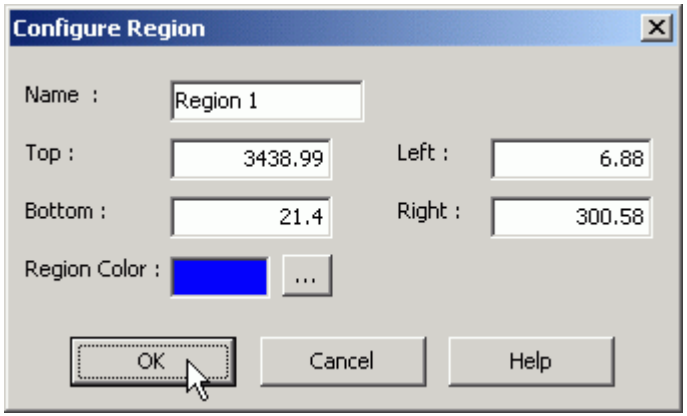
Elements

Name	Enter a name for the marker. Easy-to-remember names, for example the used dye, make it easier to identify the marker.
Lower Value/ Upper Value	Enter fluorescence values for the lower level (left line of the marker) and for the upper level (right line of the marker). These lines define a range of relative fluorescence that you can use for gating.
Color	Click the ... button to display the *Color* dialog box where you can select a color for the marker and assign it by clicking *OK*.

Configure Region

Purpose

This dialog box is used to set the properties of the selected region in a dot plot.



Access

You can open this dialog box by double-clicking a region or by double-clicking the corresponding row of the result table of a dot plot.

Elements

Name	Enter the name of the region. Easy-to-remember names, for example the color, makes it easier to identify the region.

Top, Bottom, Left, Right	The four values define a rectangular region. You can change the size of the region by entering different values or by dragging the borders with the mouse.
Region Color	Click this button to open the *Color* dialog box where you can select a color for the region and assign it by clicking *OK*.

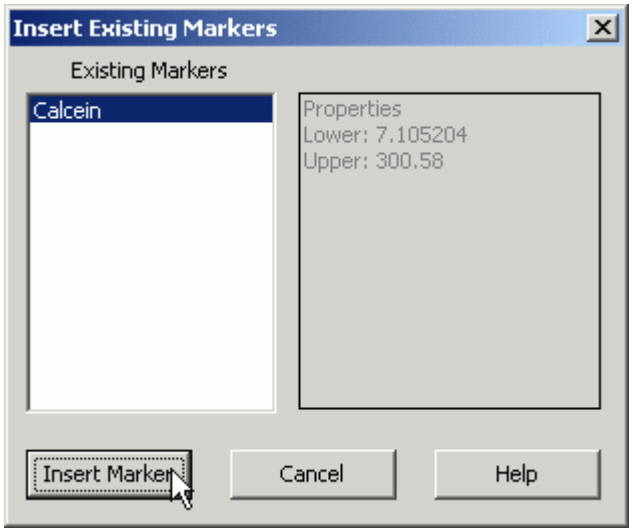
Insert Existing Markers

Purpose

This dialog box is used to add existing markers to histograms.

Access

When a histogram is displayed in single view (see "Histogram Tab (Single/Grid View)" on page 460), click the button in the toolbar (see "Data and Assay Context – Flow Cytometry Toolbar" on page 376) to open this dialog box.



Description

All existing regions are listed except the ones that are already used by the current histogram.

Elements

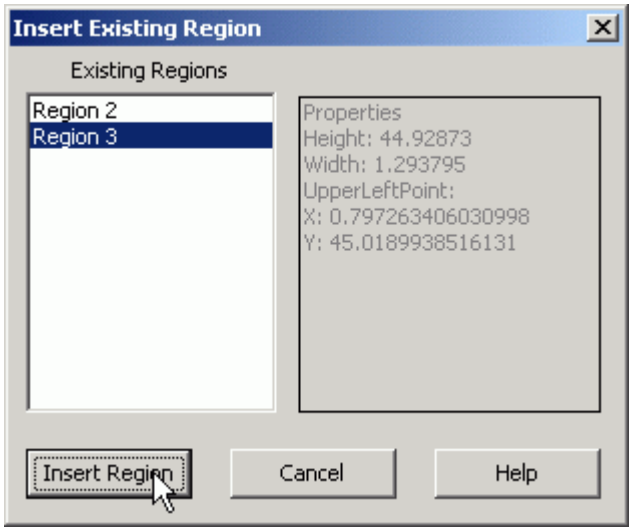
Existing Markers	List of available markers for the current histogram.
Information frame	In the right part of the dialog box, the lower and upper limits of the selected marker are displayed.
Insert Marker	Click this button to insert the selected marker in the current histogram.

If you change the properties of a marker, the properties will change accordingly in all other histograms that use this marker.

Insert Existing Region

Purpose

This dialog box is used to add an existing region to a dot plot.



Access

When a dot plot is displayed in single view (see "Dot Plot Tab (Single/Grid View)" on page 468), click the  button in the toolbar (see "Data and Assay Context – Flow Cytometry Toolbar" on page 376) to open this dialog box.

Description

All existing regions are listed except the ones that are already used by the current dot plot.

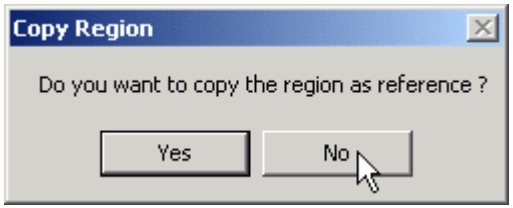
Existing Regions	List of available regions for the current dot plot.
Information Frame	In the right-hand part of the dialog box, the properties of the selected region are displayed. The x and y coordinates correspond to the region's upper left corner.
Insert Region	Click this button to insert the selected region in the current dot plot.

If you change the properties of a region, the properties will change accordingly on all other dot plots that use this region.

Copy Region

Purpose

To confirm or skip the usage of the currently selected region as a reference region for all samples.



Access

The dialog box is displayed when you click the *Insert region into all dot plots...* button .

Elements

The inserted region can be used as a reference region. When you change the reference region, all inserted regions in the samples will also be changed.

Yes	Uses the selected region as the reference region.
No	Defines that the inserted regions in the dot plots should *not* be changed when the reference region is changed.

Please refer also to "How to Insert a Region in All Dot Plots" on page 226 for details on reference regions.

Copy Marker

Purpose

To confirm or skip the usage of the currently selected marker as a reference marker for all samples.

Access

The dialog box is displayed when you click the *Insert selected marker into all histograms* button  .

Elements

The currently selected marker can be used as a reference marker. If you change the reference marker, all inserted markers in the samples will also be changed.

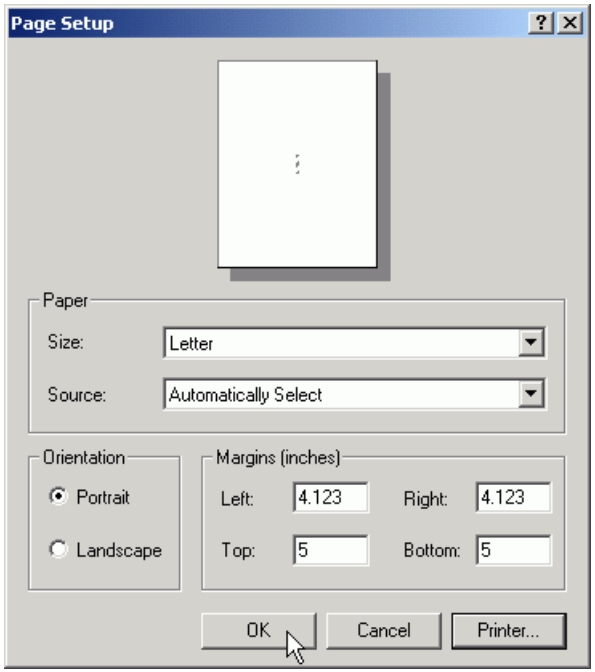
Yes	Click *Yes* to use the currently selected marker as the reference.
No	Click *No,* if all inserted markers in the histograms should *not* be changed when the reference marker is changed.

Please refer also to "How to Copy Markers to All Histograms" on page 212 for details on reference markers.

Page Setup

Purpose

This dialog box lets you change the layout of the printed page.



Elements

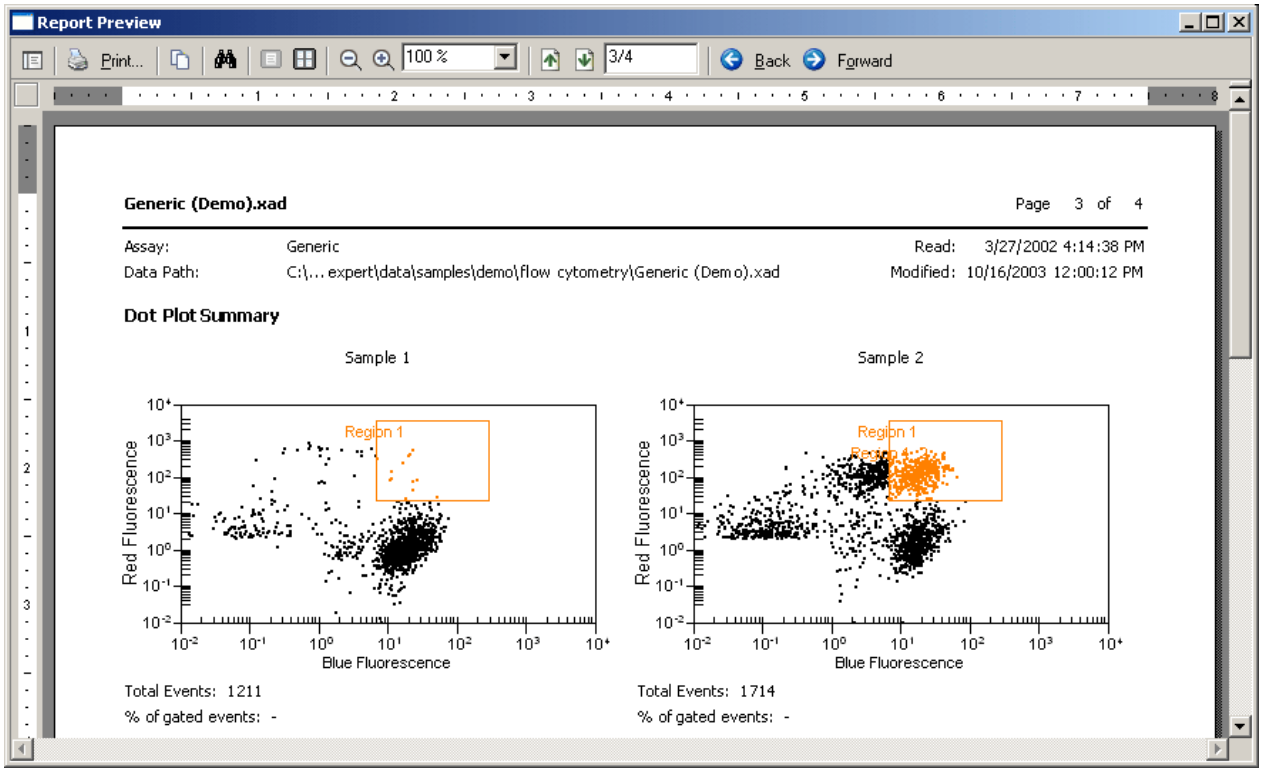
Size	Allows you to select the paper format.
Source	Allows you to specify the tray from which the paper is to be taken. The list contents depend on the selected printer.

Portrait	Prints data in portrait orientation.
Landscape	Prints data in landscape orientation.
Margins	Defines the borders of the page that you want to print. The available settings depend on the paper size.
Printer...	Opens a dialog box allowing you to select a printer.

Report Preview

Purpose

This window is used to display a preview of all pages that will be printed.



Access

You can open this window by clicking the *Preview* button in the *Print* dialog boxes.

Elements

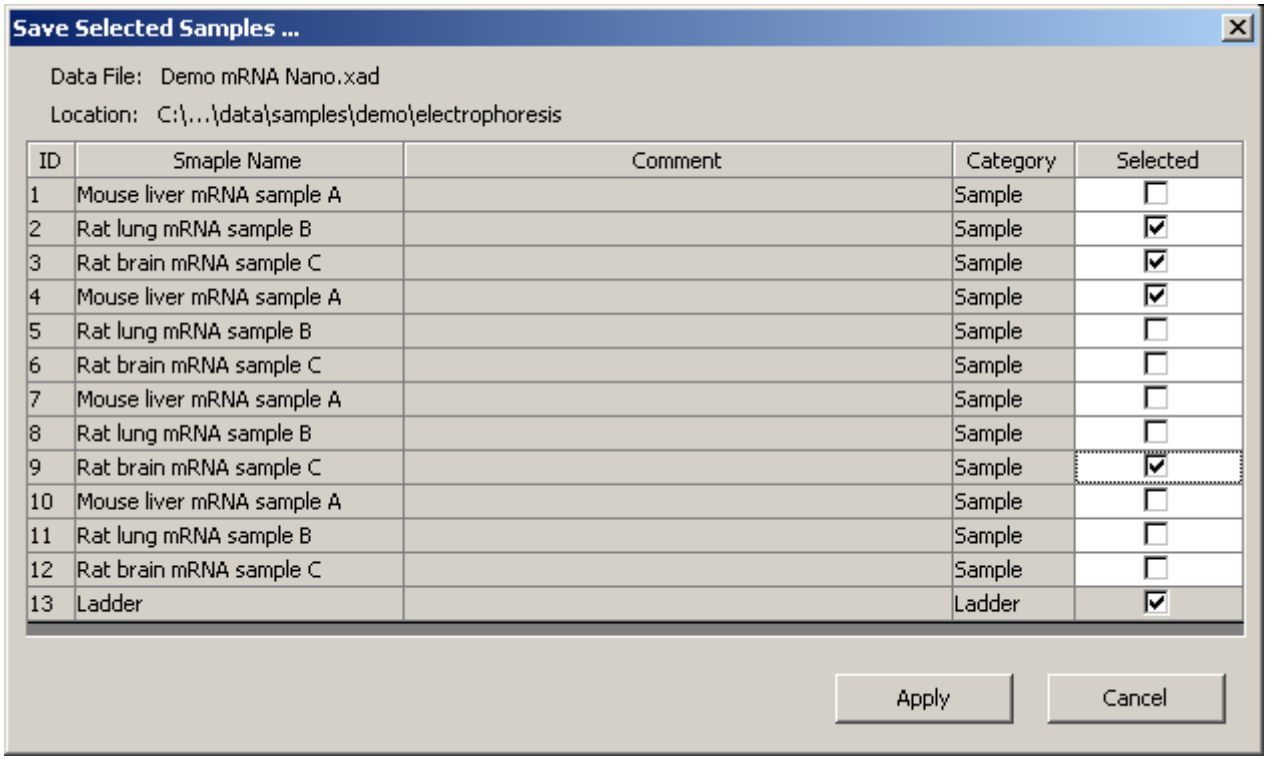
You can browse through the print preview and modify the display using the functions in the toolbar:

Table of Contents	Displays a navigation panel at the left border of the window.
Print...	Click this button to open a system dialog box allowing you to select a printer and start printing.
Copy	Puts the text of current page on the clipboard.
Find	Lets you search the printout for any text string.
Single Page	Switches to single-page preview.
Multiple Pages	Switches to multi-page preview.
Zoom Out	Lets you reduce the print preview.
Zoom In	Lets you enlarge the print preview.
Zoom	Lets you select a zoom factor.
Previous Page	Scrolls up one page.
Next Page	Scrolls down one page.
Back	Jumps back to the page displayed last.
Forward	Jumps forward to the page displayed last.

Save Selected Samples ...

Purpose

This dialog box is used to select a subset of the current .xad file's samples for saving.



Access

If a .xad file selected, you can open this dialog box in the *Data and Assay* context by selecting *Save Selected Sample...* from the *File* menu.

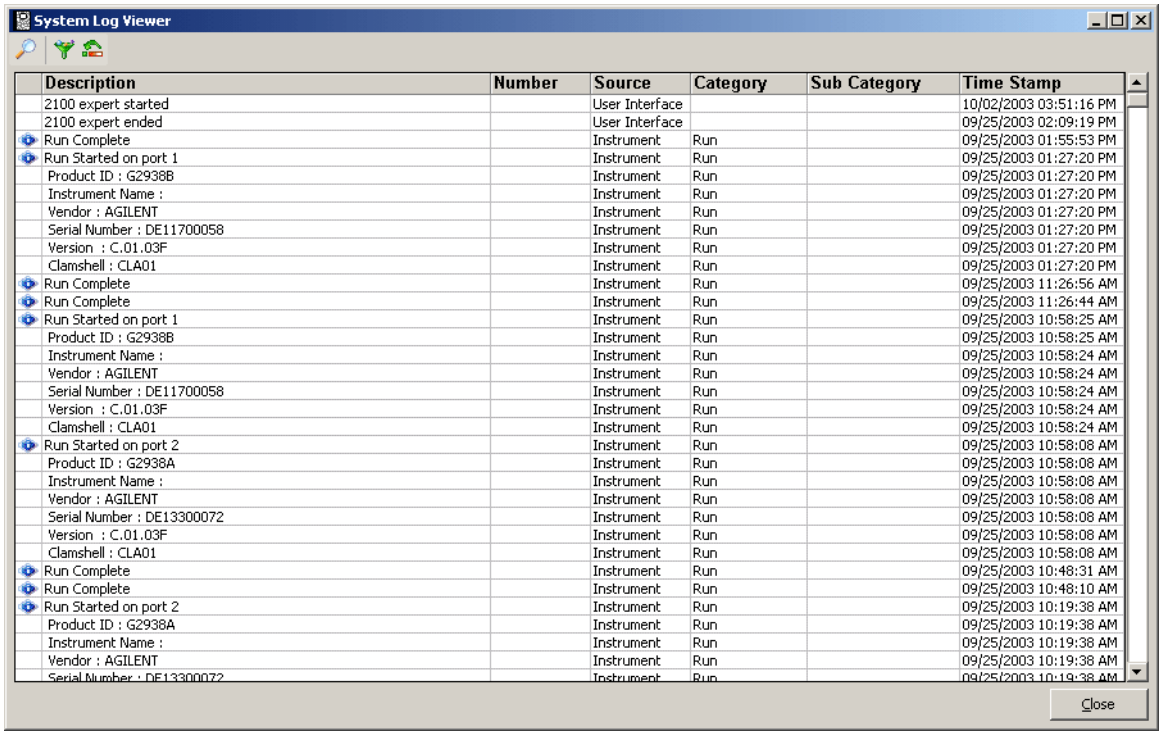
Elements

Data File	Name of the chip data (.xad) file you selected for selective saving.
Location	Path to the *Data File*.
ID	Sample number.
Sample Name	Sample name.
Comment	Comment for the sample.
Category	Sample type: • *Sample* • *Ladder* (electrophoretic assays only)
Selected	All samples that you select here will be written to a new .xad file. Note that you cannot deselect the *Ladder* (electrophoretic assays only).
Apply	Opens the *Save Select Sample* dialog box allowing you to save a new .xad or .xsy file containing only the selected samples.
Cancel	Returns to 2100 expert without selecting or saving anything.

System Log Viewer

Purpose

The system log *table* shows system-wide events. For example, you can see who started the 2100 expert software and when.



Also errors and problems with hardware or software are reported by the system log.

Access

You can open this dialog box by selecting *System Log* from the *Tools* menu.

System Log Table

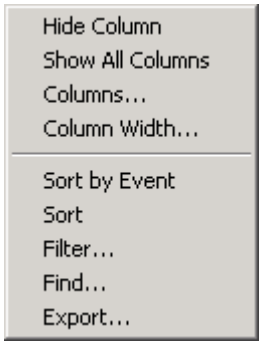
The system log table has the following columns:

Description	Message text describing the system log entry.
Number	Error number.
Source	Source that triggered the system log entry: “Reader”, “Instrument” or “User Interface”.
Category	“Run”, “System”, “Reserved”, “Sample”, or “Assay”.
User	User who was logged in on *Host* when the log entry was created.
Host	Name of the computer on which the system log was created.
Time Stamp	Date and time the system log entry was created.

The system log table is saved in the file “SystemLogBook.log” (located in the “..\log” subdirectory). You can limit the disk space allocated by the system log file (refer to “Options – Advanced” on page 508).

Context Menu

Right-clicking on the system log table opens a context menu:



Hide Column	Hides the selected column.
Show All Columns	Shows all columns.
Columns...	Opens a dialog box allowing you to hide, show, and re-sort columns.
Column Width...	Opens a dialog box allowing you to resize the selected column.
Sort by Event	Sorts the table by the *Category* column (ascending).
Sort	Sorts the table by the selected column.
Filter...	Opens the *Filter Events* dialog box allowing you to hide table entries matching filter criteria you can specify. A filter can hide all uncritical events, for example.

Find...	Opens a dialog box allowing you to search the table for any string.
Export...	Opens a dialog box allowing you to export the system log table (or parts of it) as an .html or .txt file.

Toolbar

The *System Log Viewer* window provides a toolbar that works in the same way as the *Log Book* toolbar, see "Data and Assay Context – Log Book Toolbar" on page 382.

Shortcuts and Mouse Actions

You can enter data and commands either by using the keyboard or the mouse.

- Shortcuts can be used to enter commands very quickly using the keyboard.
- Mouse actions are required for making selections, and convenient for selecting menu commands, pressing buttons etc.

Shortcuts

You can use standard Windows® shortcuts such as Ctrl+C, and shortcuts specific to the 2100 expert application.

Windows® Standard Shortcuts

Press and hold down the Ctrl key while pressing another key:

Shortcut	Function
`Ctrl+o`	Brings up the *Open* dialog box allowing you to load data files.
`Ctrl+s`	Saves the current file.
`Ctrl+p`	Opens the *Print* dialog box allowing you to set print options and send data to the printer.
`Ctrl+c`	Copies the selection (data or graphic) into the clipboard.
`Ctrl+x`	Cuts the selection and puts it (data or graphic) into the clipboard.
`Ctrl+v`	Pastes the contents of the clipboard (data or graphic) at the cursor position.

Alt Shortcuts

Press and hold down the Alt key while pressing another key:

Shortcut	Function
`Alt+f`	Opens the *File Menu*.
`Alt+c`	Opens the *Context Menu*.
`Alt+v`	Opens the *View Menu*.
`Alt+a`	Opens the *Assays Menu*.
`Alt+t`	Opens the *Tools Menu*.
`Alt+w`	Opens the *Windows Menu*.
`Alt+h`	Opens the *Help Menu*.
`Alt+F4`	Exits the 2100 expert application.

Function Keys

These are the function keys located at the top of your keyboard (F1 – F12).

Shortcut	Function
`F1`	Opens the Help topic related to the current action.

Arrow Keys

The arrow keys are located to the left of the numerical key pad on your keyboard.

Shortcut	Function
→	Moves the input focus to the right, or switches to the next tab.
←	Moves the input focus to the left, or switches to the previous tab.
↑	Moves the input focus upwards, for example in the *Tree View Panel*.
↓	Moves the input focus downwards, for example in the *Tree View Panel*.
`Tab`	Moves the input focus to the next input item, for example to the next cell of a result table.

Mouse Actions

The mouse lets you make efficient use of 2100 expert's graphical user interface.

Left-click	Result
In *Toolbars*	Activates the function associated with the button.
In *Tree View Panel*	*Data and Assay* context: Selects a file, or displays the grid view (*All Samples*), or the single view of an electropherogram, a gel-like image, a histogram, or a dot plot. *Instrument* context: Selects a bioanalyzer or switches to the grid view (*All Instruments*).
In *Lower Panel* (chip icon or small gel view)	Selects a sample and displays its electropherogram, gel-like image, histogram, or dot plot.
In *Grid View*	*Data and Assay* context: selects an electropherogram, a histogram (red or blue), or a dot plot. *Instrument* context: selects an instrument.

Left-click	Result
In *Single View*	Histograms: selects a marker.
	Dot plots: selects a region.
	At the right edge of the window on electropherograms and gel view: shows/hides the setpoint explorer.
	Gel view: selects a sample.
In tables (peak table, fragment table etc.)	Selects a table cell.
In setpoint explorer	Selects a setpoint.

Double left-click	Result
In *Single View*	On markers in histograms: opens the *Configure Marker* dialog box. On region borders in dot plots: opens the *Configure Region* dialog box. On gel-like images, electropherograms, histograms, and dot plots: undoes the last zoom action.
In *Grid View*	Displays the instrument, electropherogram, histogram, or dot plots in single view.
In setpoint explorer	On value: switches to editing mode (if setpoint can be modified).
On *Title Bar*	Maximizes/restores size of application window.

Right-click	Result
In *Tree View Panel*	*Data and Assay* context: selects a file or sample, and opens a context menu allowing you to save, close, or print the file. *Instrument* context: selects a bioanalyzer or switches to the grid view (*All Instruments*). *Comparison* context: adds or removes the selected sample from a comparison file.
In *Grid View*	*Data and Assay* context: selects a sample. Electropherograms: displays a context menu with several items for evaluating and editing the selected sample. Histograms: selects the blue or red histogram. *Instrument* context: selects a bioanalyzer and opens a context menu providing functions for changing the display of the graph.
In *Single View*	Electropherograms, gel view, histograms, and dot plots: displays a context menu with several items for evaluating and editing.
On *Title Bar*	Opens a context menu for sizing the application window and closing the application.
In tables (peak table, fragment table etc.)	Opens a context menu for data export and customizing the table.

Drag	Result
In *Single View*	Zooms into the graph. In electropherograms, also pan and scale operations are possible. Histograms: The mouse pointer changes to a pointing hand that lets you move the marker horizontally. Dot plots: At the region corners, the mouse pointer changes to a double arrow that lets you enlarge or reduce the region.
In *Grid View*	*Data and Assay* context: zooms into electropherograms. Pan and scale operations are also possible. *Instrument* context: zooms into the graph.
In tables (peak table, fragment table etc.)	On table cells: selects multiple cells. On column headers: moves table columns.

Products, Spare Parts, and Accessories

To buy the following products, spare parts and accessories for the Agilent 2100 bioanalyzer, please refer to the Agilent Online Store:

http://www.agilent.com/home/buyonline.html

Bundles

- G2940CA – Agilent 2100 bioanalyzer desktop system

  Includes Agilent 2100 bioanalyzer, Compaq desktop PC, color printer, system software, vortexer, and accessories. Cartridge and license must be purchased separately.

- G2943CA – Agilent 2100 bioanalyzer laptop system

  Includes Agilent 2100 bioanalyzer, Compaq laptop PC, color printer, system software, vortexer, and accessories. Cartridge and license must be purchased separately.

Hardware

- G2938C – Agilent 2100 bioanalyzer

  Includes 1 chip priming station, 1 test chip kit, serial cable, *Installation and Safety Manual*. Cartridge and license must be purchased separately.

- G2947CA – Agilent 2100 bioanalyzer electrophoresis set

  Includes test chip kit, electrode cartridge, license key for electrophoresis assays, and start-up service.
- G2948CA – Agilent 2100 bioanalyzer flow cytometry set

  Includes checkout kit, test chip kit, pressure cartridge, license key for flow cytometry assays, and start-up service.

Services and Software

- G2946CA – Agilent 2100 Expert software upgrade
- R1015A – Agilent 2100 bioanalyzer IQ
- R1016A – Agilent 2100 bioanalyzer OQ/PV

Spare Parts and Accessories

- 5065-4413 – Electrode cartridge
- 5065-4492 – Pressure cartridge
- 5065-4478 – Pressure Adapter Kit

  Contains 5 plastic adapters and 1 mounting ring for use with the pressure cartridge
- G2938-68100 – Test Chip Kit for Electrophoretic Assays

  Comprises 1 Autofocus, 1 Electrode/Diode, and 5 Leak Current Clips
- G2938-68200 – Test Chip Kit for Flow Cytometric Assays

  Comprises 1 Cell Autofocus Chip

- G2938-81605 – RS 232 cable

  Communication cable PC – instrument
- 2110-0007 – Fuse

  Two power supply fuses are needed for the G2938C bioanalyzer
- G2938-81610 – Multiport cable for rocketport card
- 5042-1398 – Adjustable Clip for use with luer lock syringe
- 5065-4401 – Chip Priming Station including gasket kit and adjustable clip
- G2938-68716 – Gasket Kit

  Includes spare parts for the chip priming station: 1 plastic adapter, 1 ring and 10 gaskets
- 5065-4428 – IKA Vortexer (115V)

  Must be ordered at IKA
- 5065-4429 – IKA Vortexer (230V)

  must be ordered at IKA
- 5022-2190 – Vortex Mixer Adapter for IKA vortexer
- 5065-9951 – Electrode Cleaner Box

  Contains 7 electrode cleaners
- G2938-90300 – Agilent 2100 bioanalyzer user information binder

  Contains all 8 *Reagents Kit Guides*
- G2946-60002 – Agilent 2100 bioanalyzer How to Use CD-ROM

  Contains videos showing the chip preparation for all assays and the hardware maintenance

Glossary

This glossary explains terms in context with flow cytometry, electrophoresis, and software or hardware of the Agilent 2100 bioanalyzer.

A

ASY file

In *Bio Sizing* electrophoretic assays were stored as .asy files. 2100 expert can import .asy files. See also XSY file.

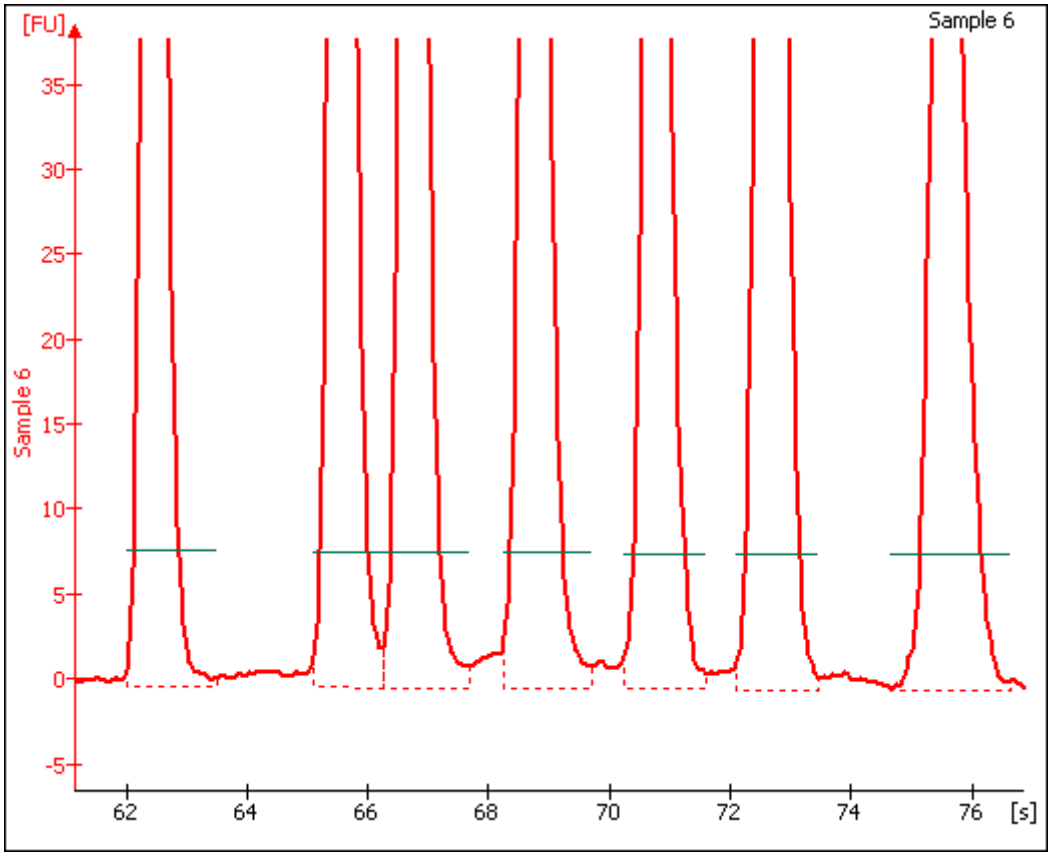
B

Baseline

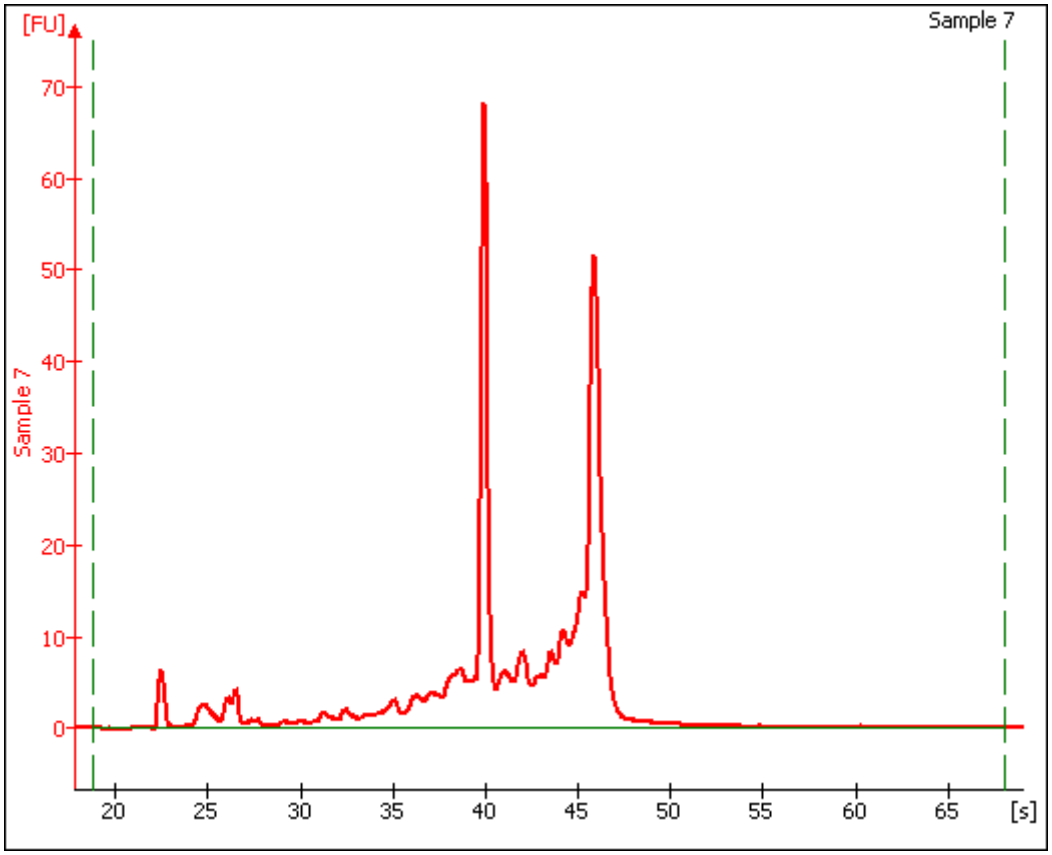
A baseline is established just after the First Peak Time setpoint. After the overall baseline is established, a local baseline is calculated for each peak to compensate for baseline drift.

For isolated peaks, the local peak baseline is simply a straight line connecting the Start Point of the peak with the End Point. For peaks that are very close together, an average baseline is used when the value between the peaks does not drop to the actual baseline.

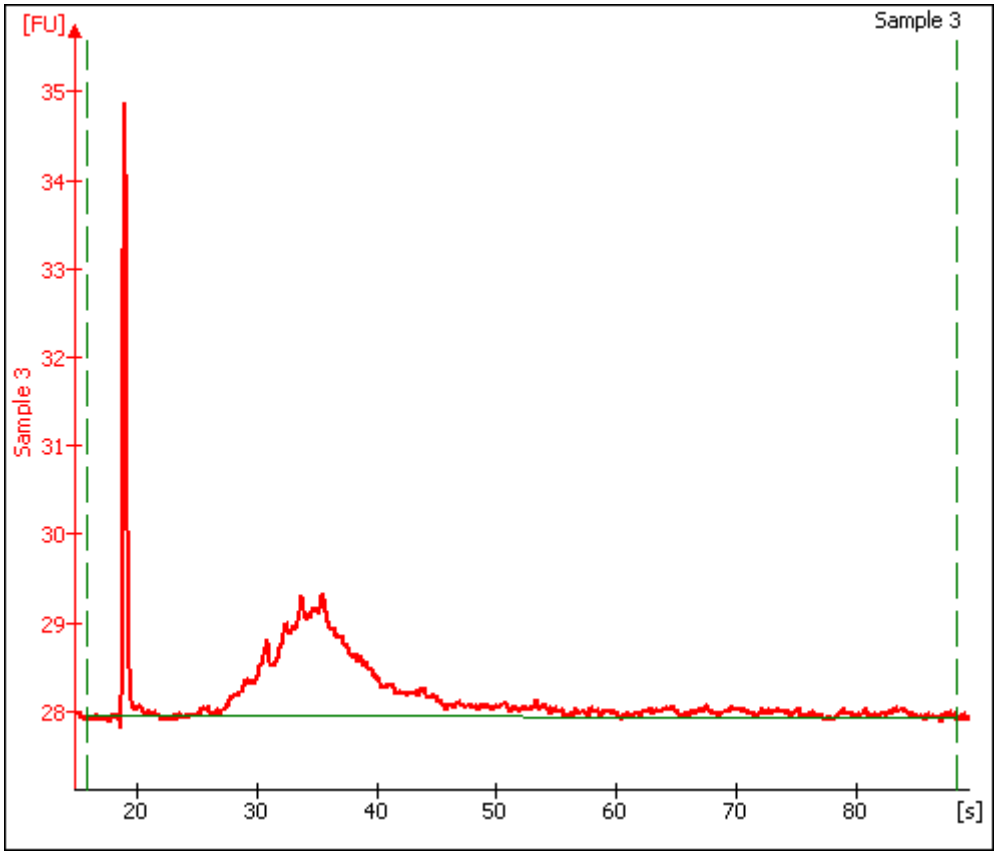
The figure below shows baselines established for DNA assay peaks. Peaks for DNA and protein assays are determined on a peak-by-peak basis (the overall baseline is shown).



The figure below shows baselines established for Total RNA assay fragments. Total RNA fragments are determined on a peak-by-peak basis and an overall baseline is shown from the start to end time.



The figure below shows baselines established for an mRNA assay. mRNA fragments are determined on a peak-by-peak basis and an overall baseline is shown from the start to end time.



NOTE

With RNA assays, you can move the lines marking the start and end points for data analysis (shown by the long-dashed vertical green lines) which will adjust the entire baseline for calculation of the area of the total sample.

Baseline Plateau

This setpoint (found in the setpoint explorer) rejects brief, low slope areas such as at peaks and between non-baseline-resolved peaks. The signal is recognized to be at baseline whenever the slope of the data is less than the Slope Threshold setpoint (either positive or negative) for longer than the time set for the Baseline Plateau.

BMP file

BMP is the standard Windows image format. The BMP format supports RGB, indexed-color, grayscale, and bitmap color modes.

Bubble

If the tip of a pipette is not positioned all the way to the bottom of a well, bubbles can result (and sometimes bubbles happen even when you are very careful). The vortexing step that occurs after samples are loaded into the chip is designed to rid the wells of bubbles and is usually very effective.

If a large bubble is seen at the bottom of a well, remove the sample from the well, pipette it back in, and continue with the loading procedure.

C

CAD file

In *Cell Fluorescence* flow cytometric chip runs were stored as .cad files. 2100 expert can import .cad files. See also XAD file.

Center Point

After locating a start point, the peak find algorithm looks for the first negative slope value and saves the previous point as the center. If the value of the center point is less than the Minimum Peak Height, the algorithm starts looking for a new peak.

CLD file

In *Bio Sizing* electrophoretic chip runs were stored as .cld files. 2100 expert can import .cld files. See also XAD file.

COM Port

See Serial port.

CSV file

Comma-separated variable file. The simplest form of file for holding tabular data. Data is listed in columns in a text file, each value being separated by a comma. Each new line represents a new set of data. Import and export with Microsoft Excel is possible.

CSY file

In *Cell Fluorescence* flow cytometric assays were stored as .csy files. 2100 expert can import .csy files. See also XSY file.

D

Data Filtering

The first step *2100 expert* takes in analyzing raw data is to apply data filtering. Data filtering is done by means of a polynomial "filter" that is applied to the raw data. The setting for the Polynomial Order in the setpoint explorer determines the amount of data to be applied: the smaller the number, the more data that is applied and the more filtering that takes place.

Data Points

Data points are 0.05 seconds apart.

Show Data Points is an option that enables the display of the data points used to generate the graph.

E

Electrode Cleaner

An electrode cleaner should be used to clean the electrodes after each run is complete. The cleaning procedure is slightly different depending upon the type of assay that was just performed (DNA or RNA).

The electrode cleaner looks like a chip except that it is clear. With RNA assays you must use two different electrode cleaners: one for general cleaning using RNAse-free water and another for decontamination using RNAseZAP. It is recommended to use a permanent marker to label the electrode cleaners so as not to mix them up.

Electrokinetic forces

Electrokinetic forces are used to move, switch and separate the samples. Active control over voltage gradients directs the movement of materials using the phenomenon of electrophoretic flow.

Electroosmotic Flow

A phenomenon that results from an electrical double layer formed by ions in the fluid and surface electrical charges immobilized on the capillary walls. When an electric field is applied, the bulk solution moves towards one of the electrodes. This phenomenon can be used to move fluids through microfabricated channels.

Electrophoresis

A standard technique of separating molecules on the basis of their mobility (charge-to-mass ratios). An electrical potential is applied across a capillary containing a sample in a fluid medium. Positive molecules migrate towards the cathode and negative molecules migrate towards the anode at different speeds, depending on their electrophoretic mobility.

Electrophoretic flow

A macroscopic phenomenon that results from an electrical double layer formed by ions in the fluid and surface electrical charges immobilized on the capillary walls. When an electric field is applied, the bulk solution moves towards one of the electrodes (cathode). Electrodes sit in the reservoirs that connect to the ends of the various channels. Electrode potentials are applied to the various reservoirs in a time-dependent fashion to move the fluid in the required direction. The gel-filled channels of the LabChip devices do not exhibit a measurable flow because of dynamic channel coating and viscosity of the polymer matrix.

End Point

The peak find algorithm looks for a leveling off when the value of the slope is less than the value set for the slope threshold. This is considered to be the end point of the peak.

With RNA assays, individual peak end times can be moved manually by dragging the diamond-shaped end points shown in the single-well display.

End Time

This setpoint determines the time after the start of a run before which the last peak or fragment will be located (any peaks appearing after this time are ignored). In RNA assays, the end time is shown on the single-well display as a long-dashed vertical green line.

With RNA assays, another End Time setpoint is available that controls the end time for an individual peak. Additionally, individual peak end times can be moved manually by dragging the diamond-shaped end points shown in the single view.

F

Filter Width

This setpoint determines the width of the polynomial (in seconds) to be applied to the data for filtering (noise reduction). The default depends on the assay selected. This setting should be less than twice the width of the peaks of interest or the peaks will be distorted. Peaks that are distorted by the filter have positive and negative peaks on both sides. To see an example of such distortion, increase the filter width to 5.

Firmware

The firmware is a program to control the hardware of the Agilent 2100 bioanalyzer. It is downloaded from your computer to the Agilent 2100 bioanalyzer and controls, among others, data transfer or the measurement procedures.

Flow Cytometry

A method to detect cells with certain properties. In a continuous stream, stained cells pass through a light beam. The emitted fluorescence is used for counting and differentiation.

Flow Cytometry Standard—FCS

The FCS file format is the standard format used in flow cytometry to exchange data between several applications.

G

GIF file

Graphics Interchange Format, GIF is a graphics file format that uses a compression scheme originally developed by CompuServe. Because GIF files are compressed, the file can be quickly and easily transmitted over a network. This is why it is the most commonly used graphics format on the World Wide Web.

H

Histogram

Histograms are bar charts to display, for example, a frequency distribution.

HTML file

HTML (Hyper Text Markup Language) is the authoring language used to create documents on the World Wide Web. HTML defines the page structure, fonts, graphic elements and hypertext links to other documents on the Web.

J

JPG file

Joint Photographic Experts Group Image File. A JPEG file is a compressed raster or bitmapped graphic image. When a JPEG is created, a range of compression qualities may be considered. JPEG compression is a lossy process, which means that you sacrifice quality for file size the more you compress the image (the highest quality images results in the largest file size). Whereas GIF images are limited to 256 colors (8-bit), JPEG images may contain millions of colors (24-bit) as well as additional information including PostScript clipping paths.

L

Lab-on-a-chip

The generic term for a microfluidic product, signifying a chemical process or material movement taking place on a microchip. In contrast to analysis in a standard laboratory that relies on human intervention at several stages to manipulate or observe samples and record results, the self-contained lab-on-a-chip represents an almost hands-free technology.

Lab-on-a-chip technology means downsizing of analytical techniques from lab-scale to chip-scale:

- using techniques like electrophoresis, chromatography, and sieving.
- with fluorescence, absorbance, and MS detection.
- with a higher degree of automation, integrating multiple steps of a complex protocol into a miniaturized system.

Virtually any biochemical testing that can be done in a laboratory can theoretically be done on a chip.

Ladder

Each electrophoretic LabChip Reagent kit contains a ladder. A ladder contains DNA, RNA fragments or proteins of known sizes and concentrations.

A ladder well is located at the bottom right of the chip. The ladder is analyzed first before sample analysis takes place.

The peak sizes and markers defined for the ladder are assigned consecutively, starting with the first peak detected in the ladder. Peaks appearing above the upper marker do not have to be detected. The peak table for the ladder well shows the peak size and concentration.

Lower Marker

An internal standard that is added to a sample in a well to assist in determining size of the sample. The lower marker is the same as the first peak found in the DNA ladder.

M

Microfluidics

The movement of liquids through micro-fabricated structures by means of electrical fields or pressure/vacuum, holding the promise of greater functionality with significantly improved reliability:

- small glass or plastic devices with micro-channels as experimental platform
- active control of fluids without moving parts on-chip through miniature electrodes or pumps controlled by software scripts
- emulation of conventional liquid pumps, valves, dispensers, reactors, separation systems, etc.
- capability of liquid transfer, separation, dilution, reactions and more

Molarity

$$Molarity = \frac{Concentration * 10^6}{660 * Size} \left[\frac{nmol}{l}\right]$$

where:

Molarity is measured in nanomoles per liter (nmol/l)

Concentration is measured in nanograms per microliter (ng/µL)

Size is measured in base pairs (bp)

$660 \left[\frac{g}{mol * bp}\right]$ is the molecular weight of a single base pair

Miniaturized laboratories on a microchip

Expression used to describe lab-on-a-chip technology.

Minimum Peak Height

The Minimum Peak Height value determines whether or not a peak is kept. For each peak, the difference between start point value and center point value must be greater than the Minimum Peak Height value.

This setting is chosen in the setpoint explorer.

Minimum Peak Width

The Min Peak Width value determines whether or not a peak is kept. For each peak, the difference in width between the start point value and the center point value must be greater than the Minimum Peak Width value for the software to determine that a peak has been detected and that the change in signal is not just a spike or noise.

Molecular separation techniques

Processes such as gel electrophoresis, liquid chromatography and capillary electrophoresis that can separate bimolecular organic substances from other compounds.

P

PCK file

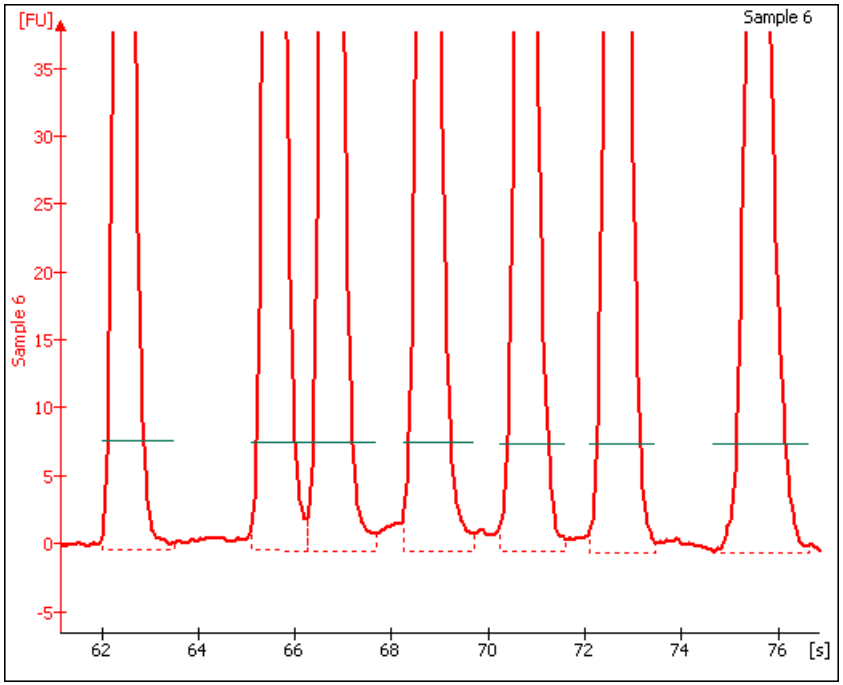
2100 expert packet files (.pck) contain all data transferred during measurement. The files are stored in the "..\data\packets" folder of the installation directory. Together with the log files they can be used to restore lost or destroyed data by the Agilent service. Also called raw data backup files.

PDF file

PDF (Portable Document Format) is a file format created by Adobe Systems Incorporated that preserves all of the fonts, formatting, colors, and graphics of any source document, regardless of the software and computer platform used to create it.

Peak Baseline

A local peak baseline is calculated for each peak. For isolated peaks, the local peak baseline is simply a straight line connecting the start point with the end point. For peaks that are very close together, an average baseline is used when the value between the peaks does not drop to the actual baseline.



Peak Height

The value at the center point of the peak minus the local baseline start value.

Point-to-Point Fit

This curve fit is composed of line segments between each pair of data points that are used to interpolate data between those points.

Polynomial Filter

The first step *2100 expert* takes in analyzing the raw data is to apply data filtering. Data filtering is done by means of a polynomial “filter” that is applied to the raw data.

Priming Station

Consists of a chip holder that has a syringe mounted on the lid that seals over the chip. The syringe is used to force the buffer solution loaded into the well marked “G” with a circle around it into all the passageways inside the chip prior to running it in the bioanalyzer.

R

Raw data backup file

See PCK file.

S

Serial port

The serial ports (COM ports) are used to connect your computer with the Agilent 2100 bioanalyzer. The number of available ports depends on the computer you use.

Slope Threshold

The Slope Threshold setpoint represents the amount of change in fluorescence units over time required to indicate that a peak has occurred. Changing this setpoint may cause certain peaks that were previously detected to be ignored. The Slope Threshold setting is one of the user-definable parameters in the setpoint explorer.

Standard Curve

The standard curve is obtained by plotting the size of the ladder peaks vs. time using a point-to-point fit. For each sample peak, the center time is interpolated from the Standard Curve to determine the peak size in base pairs.

Start Point

The peak find algorithm walks the data from time zero looking for a slope greater than the Slope Threshold. This is considered to be the start point of a peak.

With RNA assays, individual peak start times can be moved manually by dragging the diamond-shaped start points shown in the single view.

Start Time

This setting determines the time after which the first peak or fragment will be located (any peaks appearing before this time are ignored). In RNA and Protein assays, the start time is shown on the single view display as a long-dashed vertical green line (note that this is true for protein assays when analysis is on; the start time is shown as a solid green line when analysis is off for protein assays).

With RNA assays, another start time setting is available that determines the start time for an individual peak. With RNA assays, individual peak start times can be moved manually by dragging the diamond-shaped start points shown in the single view.

T

Tool Tip

A small box containing text that describes the item indicated by the mouse pointer. To view a Tool Tip, position the mouse pointer over an object on the screen. Leave the mouse stationary for a moment and a Tool Tip (if one exists for that item) will appear.

TIF file

A file extension indicating one of a set of popular bitmap graphics formats. Tiffs are commonly used in DTP work because of their support for color specification.

U

Upper Marker

An internal standard that is added to a DNA or Protein sample in a well to assist in determining size and concentration of the sample. The upper marker is the same as the last peak found in the sizing ladder.

W

WAV file

A type of computer file used to store a sound digitally.

WMF file

Windows Metafile. Windows metafile documents can contain any mix of vector and raster (or bitmapped) information to describe the contents of an image. WMF graphics are generally used on the Windows platform as a standard format for clip art and other graphically rich information such as charts.

X

XAD file

2100 expert chip data file. The files contain raw data, assay information, data analysis setpoints, information on chip, samples and study, and the run log information.

XAC file

2100 expert comparison file.

XLS file

Microsoft Excel spreadsheet file.

XML file

Extensible Markup Language files. XML is the Extensible Markup Language, a system for defining specialized markup languages that are used to transmit formatted data. XML is conceptually related to HTML, but XML is not itself a markup language. Rather it is a metalanguage, a language used to create other specialized languages.

2100 expert uses the XML format to:

- export chip data
- save and load result flagging rules.

XSY file

2100 expert assay file. The files contain the assay properties, data acquisition settings, and information on chip, samples, and study.

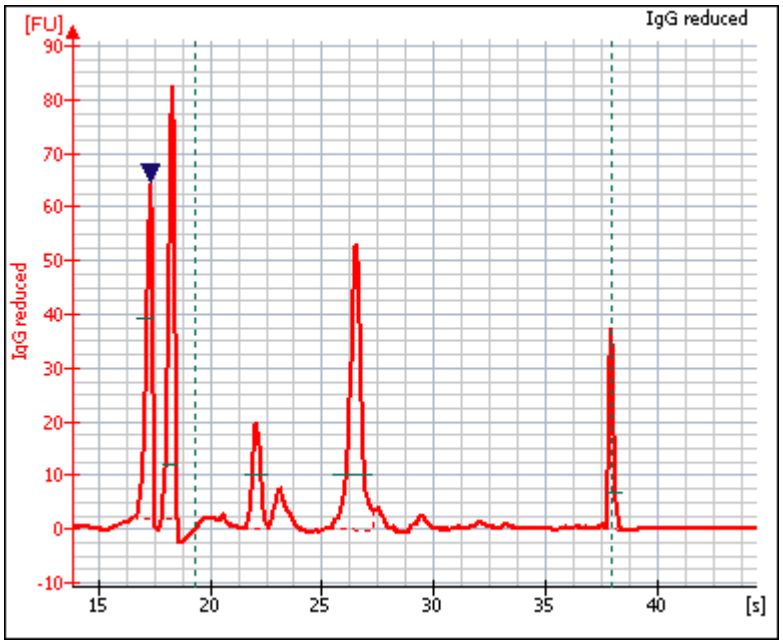
XVD file

2100 expert validation results file. The files contain results of qualification tests regarding the bioanalyzer hardware and software. xvd. files are stored in the "..\validation" subfolder of the 2100 expert installation directory. For each validation run, an .xvd file is generated.

Date and time of the validation run are included in the file name. Example: "Validation_25-09-2003_10-28-40".

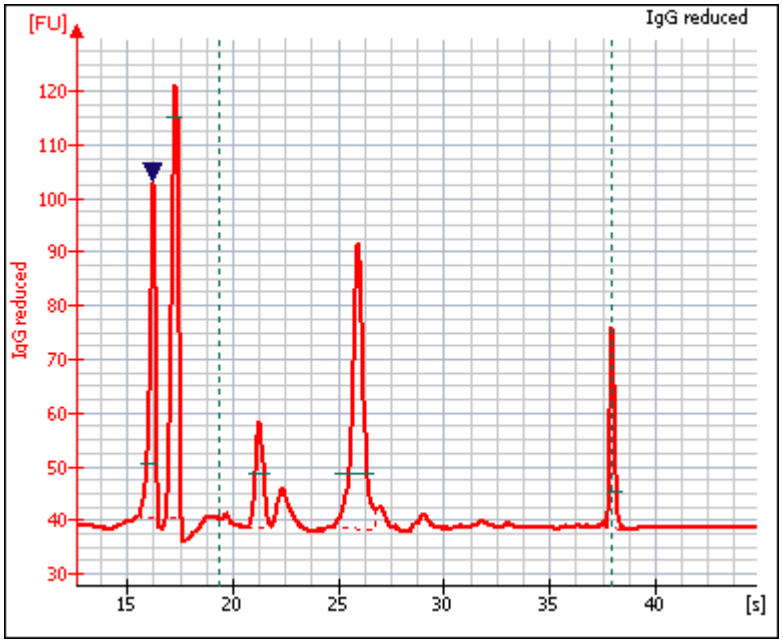
Zero Baseline

All electropherograms produced with the bioanalyzer show some amount of background fluorescence. By default, the *2100 expert* software enables the zero baseline function. Enabling this setting offsets the graphs shown for the individual wells but does not affect analysis. The mean of 100 points before the baseline time (derived when calculating well noise) is used as the zero baseline value.



Zero Baseline

To remove the zeroing, disable the *Zero Baseline* box in the setpoint explorer (baseline calculation under *Global* and *Advanced* setting).



None-Zero Baseline

Index