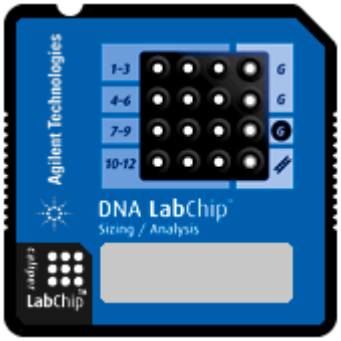
When the chip is detected, the image on the Instrument tab changes to a chip.



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

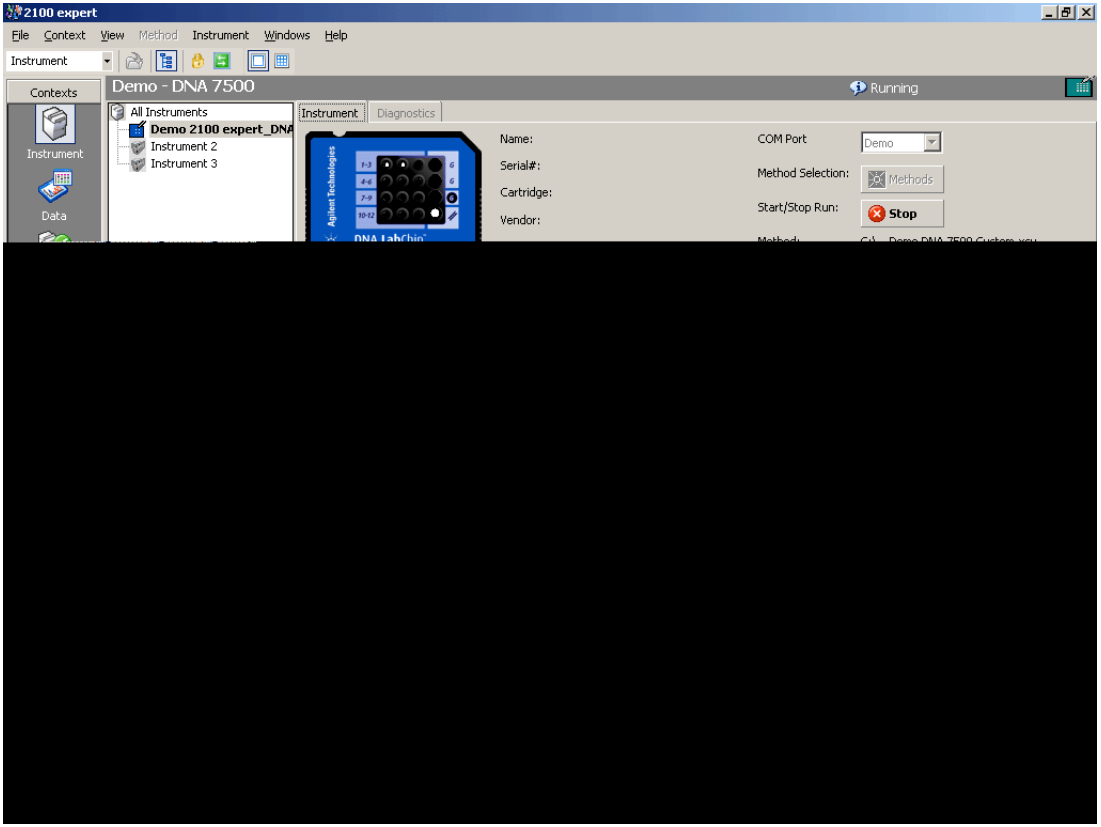
NOTE

If the AutoRun option is active, the chip run starts automatically once a chip has been inserted and the lid has been closed.

---

6 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).

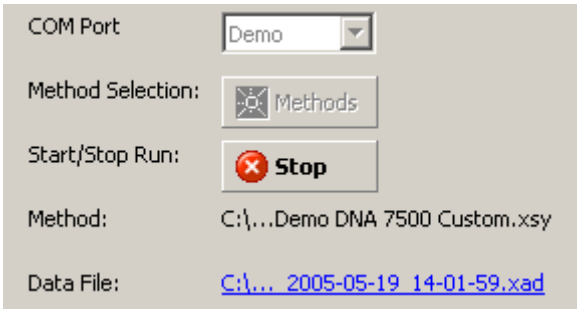


The number of the sample that is currently being measured is indicated on the information bar:

The status bar at the bottom of the window shows the measurement progress for the chip run and the COM port number used for data acquisition.

During the chip run, you can do the following:

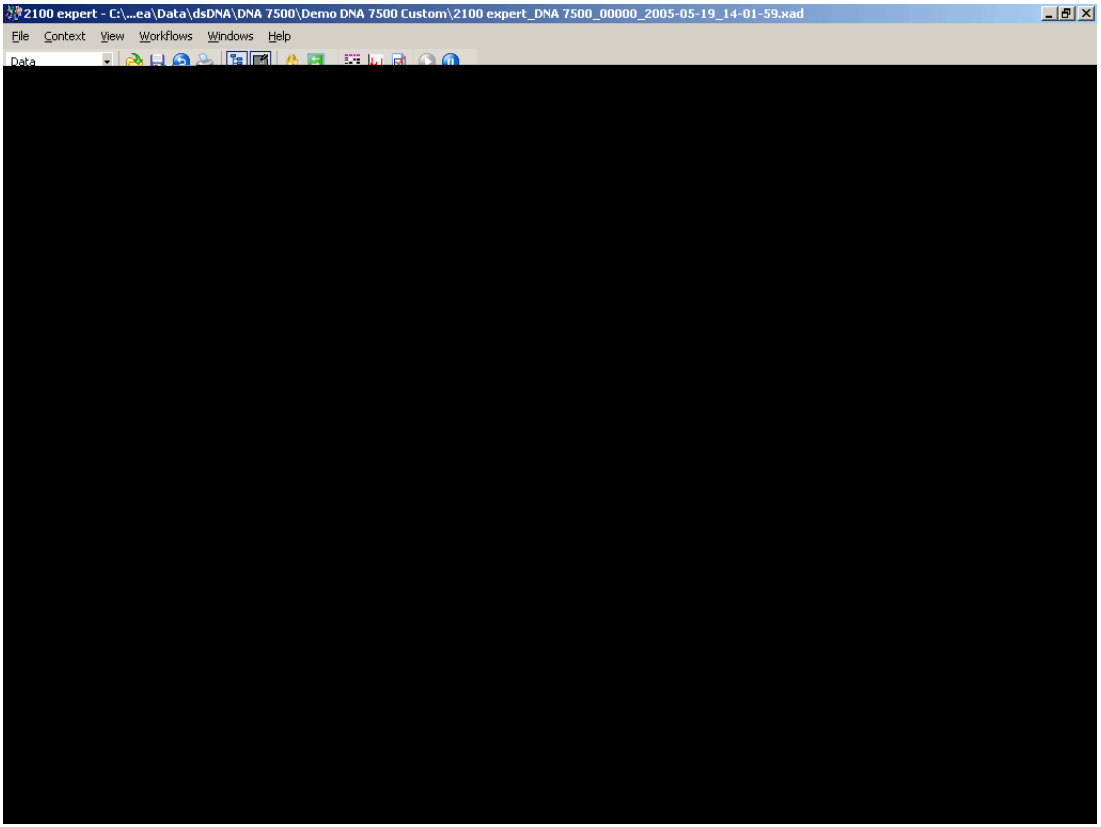
- View the chip data file in the Data context by clicking on the name of the Data File



- Switch to any other context. For example, you can evaluate any chip data file in the Data context, or compare samples in the Comparison context.
- If necessary, abort the chip run by clicking on the Stop button.

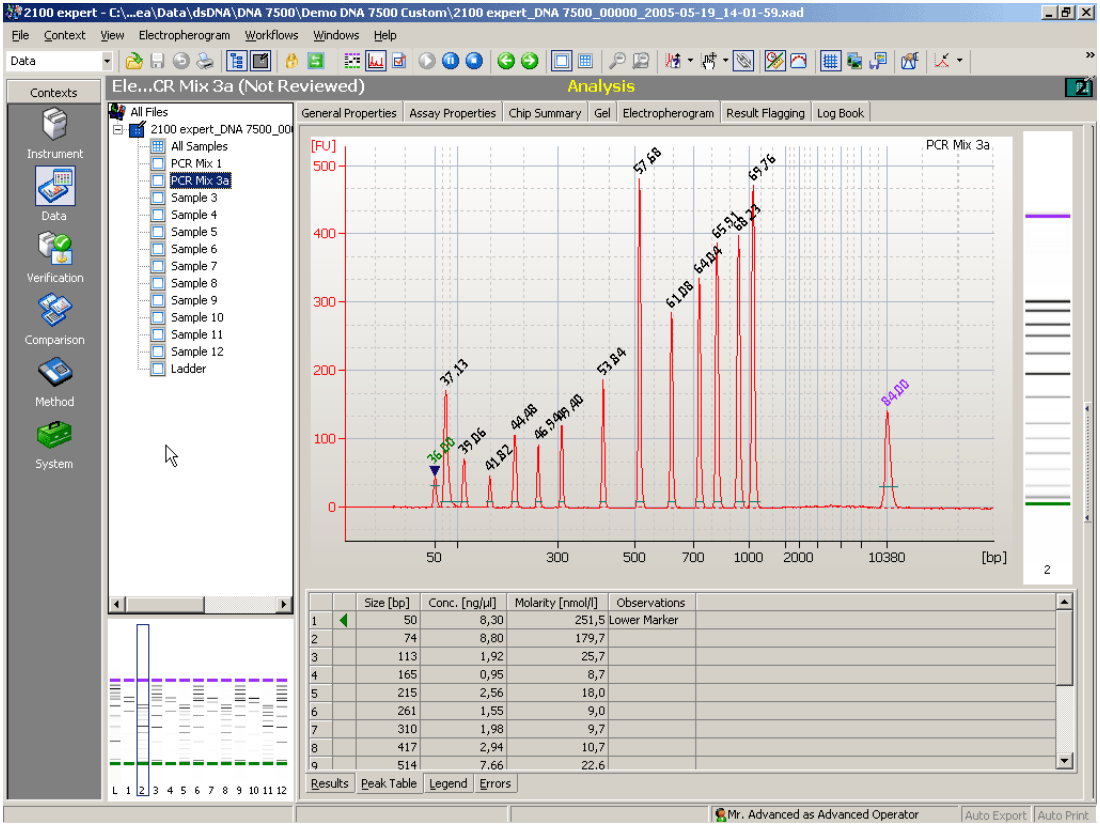
  All data that was collected up to the stop point will be saved.

To view the results, switch to the Data context. The data file that has just been generated by your chip run is displayed. The Chip Summary tab shows information on your chip data file, and lets you enter comments regarding chip, samples, and study.



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

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



Peaks have automatically been detected, and their characteristics such as size, concentration, purity, or 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 gel 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 82
  - “Analyzing and Evaluating the Results of a Flow Cytometric Assay” on page 217
- Print the results to document them on paper or an electronic format, such as HTML or PDF.

  See “Printing Reports” on page 292.
- Export the results or parts of them for further evaluation in other applications.

  See “Exporting Data” on page 283.
- Compare the results with the results of other chip runs in the comparison context. See “Comparing Samples from Different Electrophoretic Chip Runs” on page 148.
- Insert the next chip in the bioanalyzer and start a new chip run.

Looking at 2100 Expert

Before you start running assays on the Agilent 2100 bioanalyzer, you should famili
yourself with the 2100 expert software:

- “Introduction to the Key Features of the 2100 expert” on page 30
- “Starting 2100 Expert” on page 32
- “2100 Expert Work Area” on page 33
- “Closing 2100 Expert” on page 44

Introduction to the Key Features of the 2100 expert

The Agilent 2100 expert is characterized by the following key features:

- 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.
- 2100 expert provides an optional security pack that needs to be ordered separately as G2949CA and is then activated with a license key. This security pack activates user management functions and electronic signature to meet the Food and Drug Administration (FDA) requirements (21 CFR Part 11).
- 2100 expert provides detailed installation qualification and system verification tests on both the bioanalyzer hardware and software.
- 2100 expert allows having multiple chip data or assay files open at the same time.
- 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 (available for DNA and Protein assays only).
- 2100 expert allows color-coded result flagging with pre-defined or custom result flagging rules. Flagging rules can be applied to measurement results.
- 2100 expert now has customizable 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.

Starting 2100 Expert

To start 2100 expert:

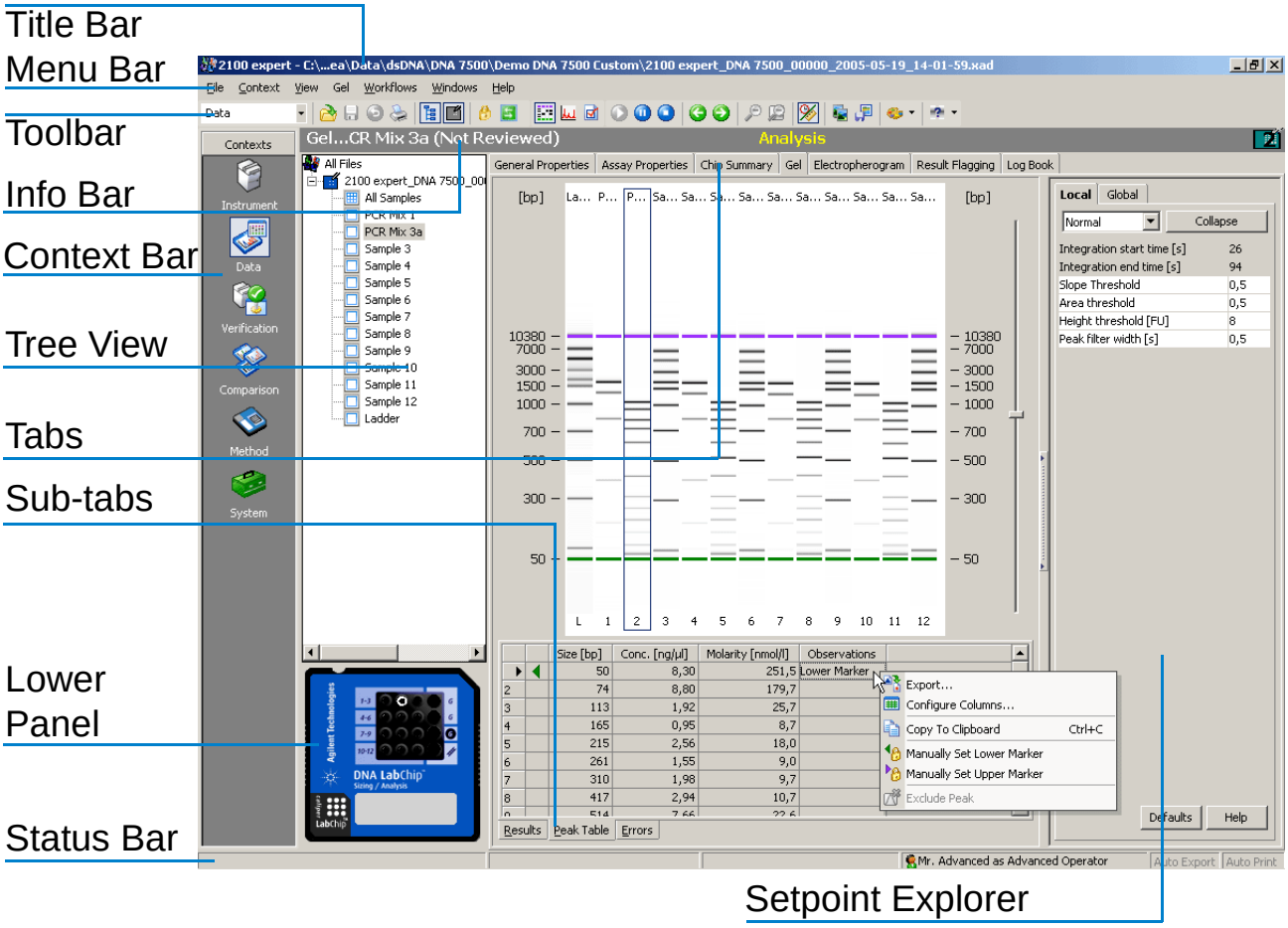
1 Go to your desktop and double-click the following icon:

– OR –

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

2100 Expert Work Area

The 2100 expert software 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 (demonstrated at Data context):



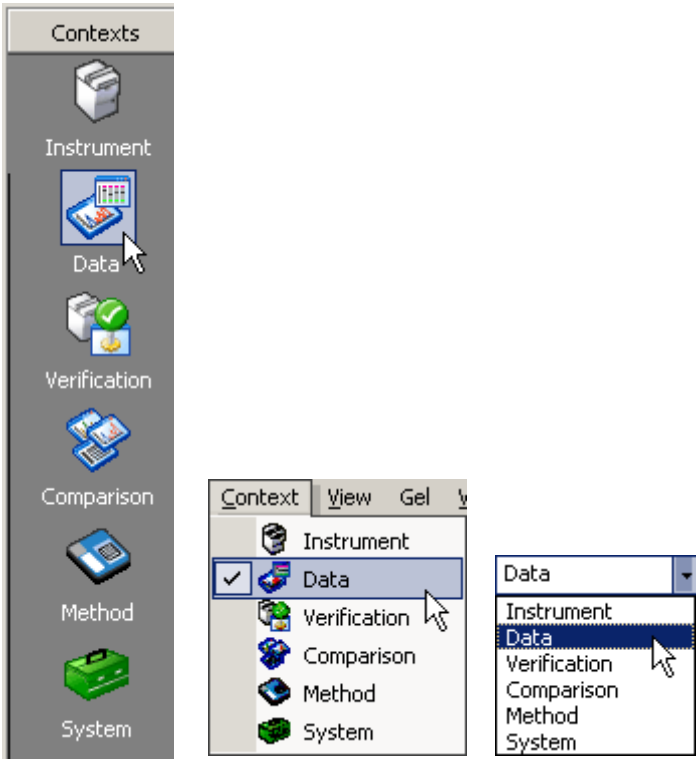
The 2100 expert software can be operated in six modes, called contexts”:

- Instrument Context
- Data Context
- Verification Context
- Comparison Context
- Assay Context
- System Context

NOTE

The contexts work 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 selection list in the toolbar, you can switch between the contexts:



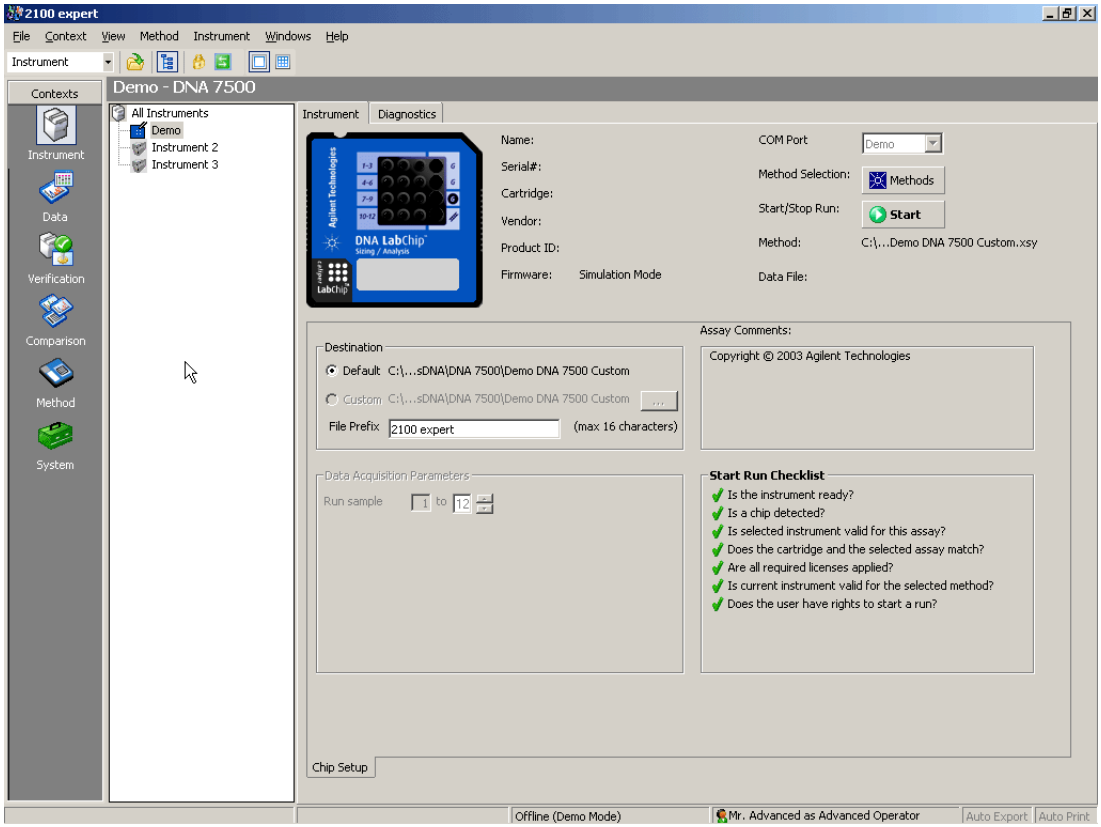
NOTE

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

An introduction to the six 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 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.

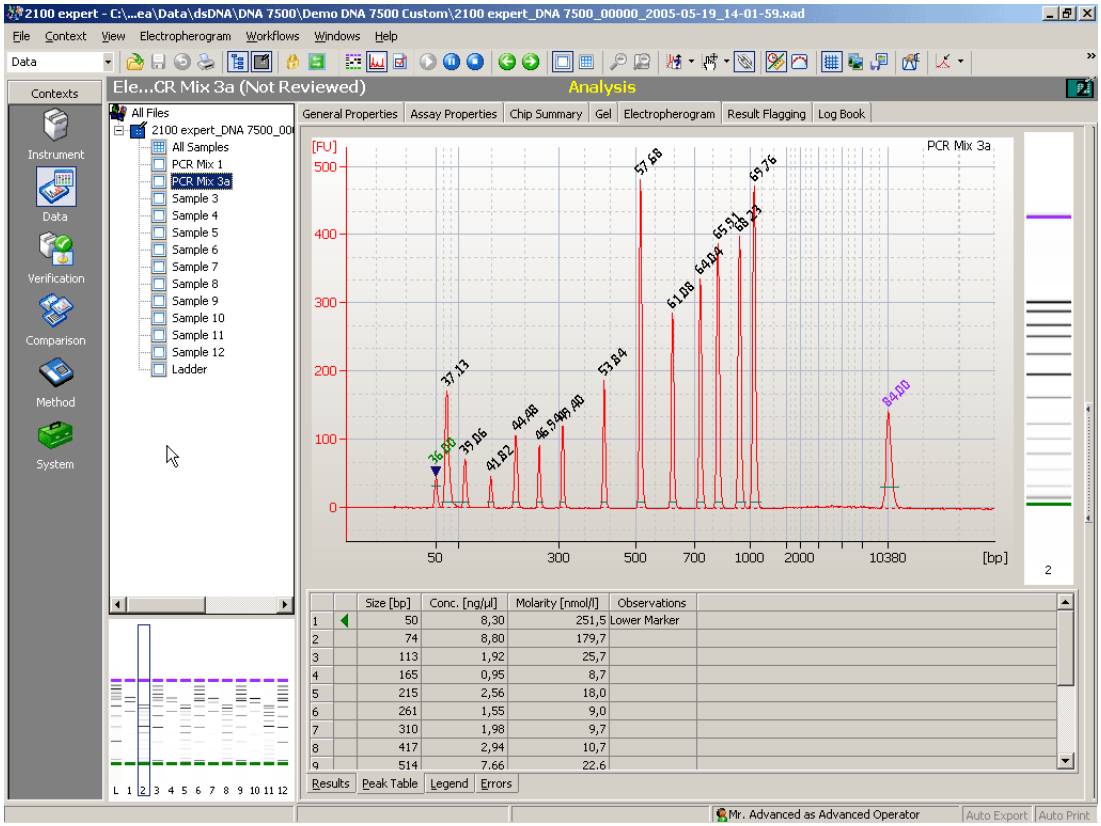
During the chip run(s), you can view the status of the bioanalyzer(s): instrument information and real time acquisition data.

In the Instrument context, it is also possible to run hardware diagnostic tests on all connected bioanalyzers. Refer to "Running Instrument Diagnostics" on page 322 for details.

Data Context

In theDatacontext, you can

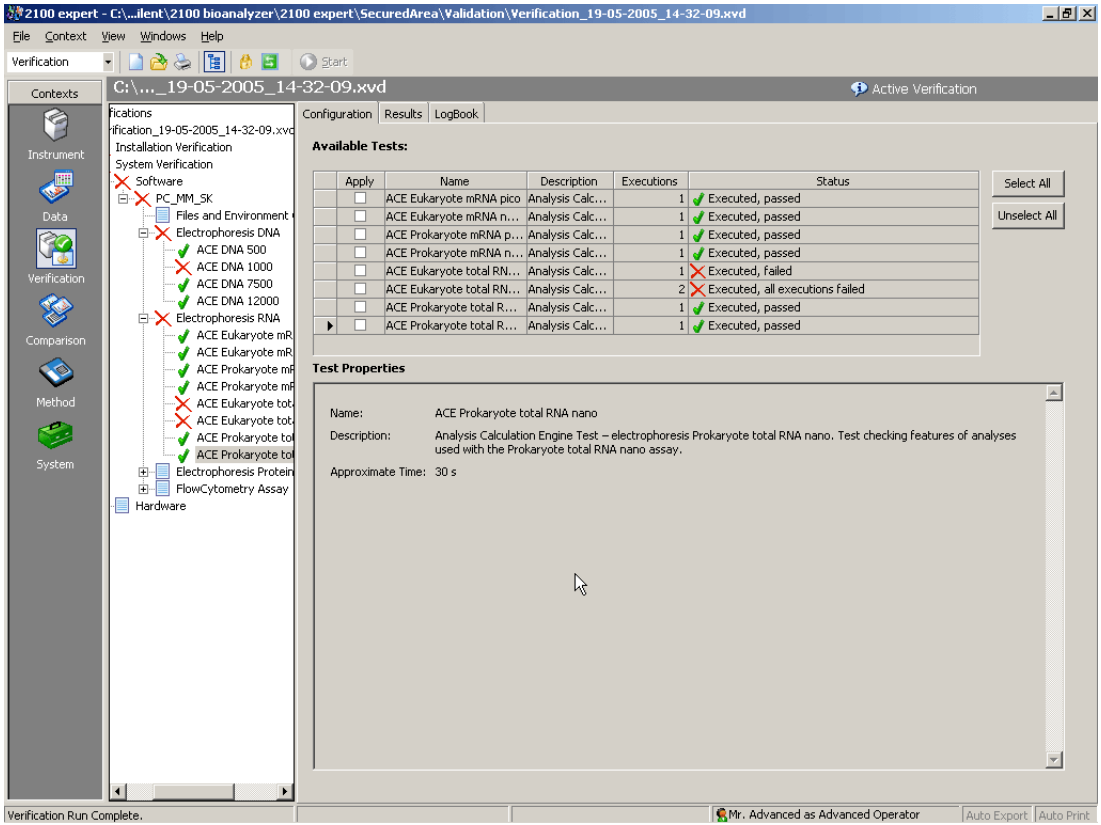
- € view, analyze, and evaluate the results of chip runs that are presented as electropherograms, gel-like images, lists, histograms, dot plots, and result tables.
- € export and print the results of your chip runs.



The measurement data is stored in .xad files.

Verification Context

TheVerificationcontext is used to run and document qualification tests.



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

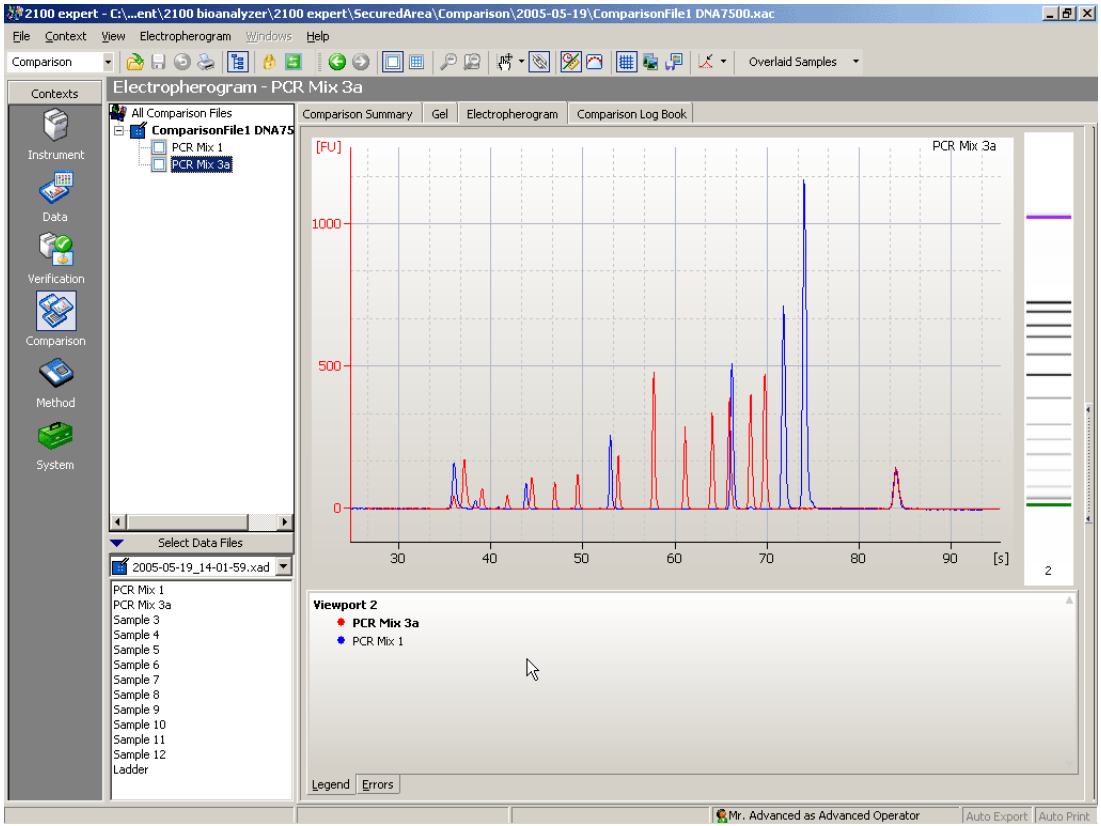
- Installation verification
- System verification

Verification results are automatically saved in .xvd files. You can re-open .xvd files review verification results.

For details, refer to “Performing Verification” on page 331.

Comparison Context

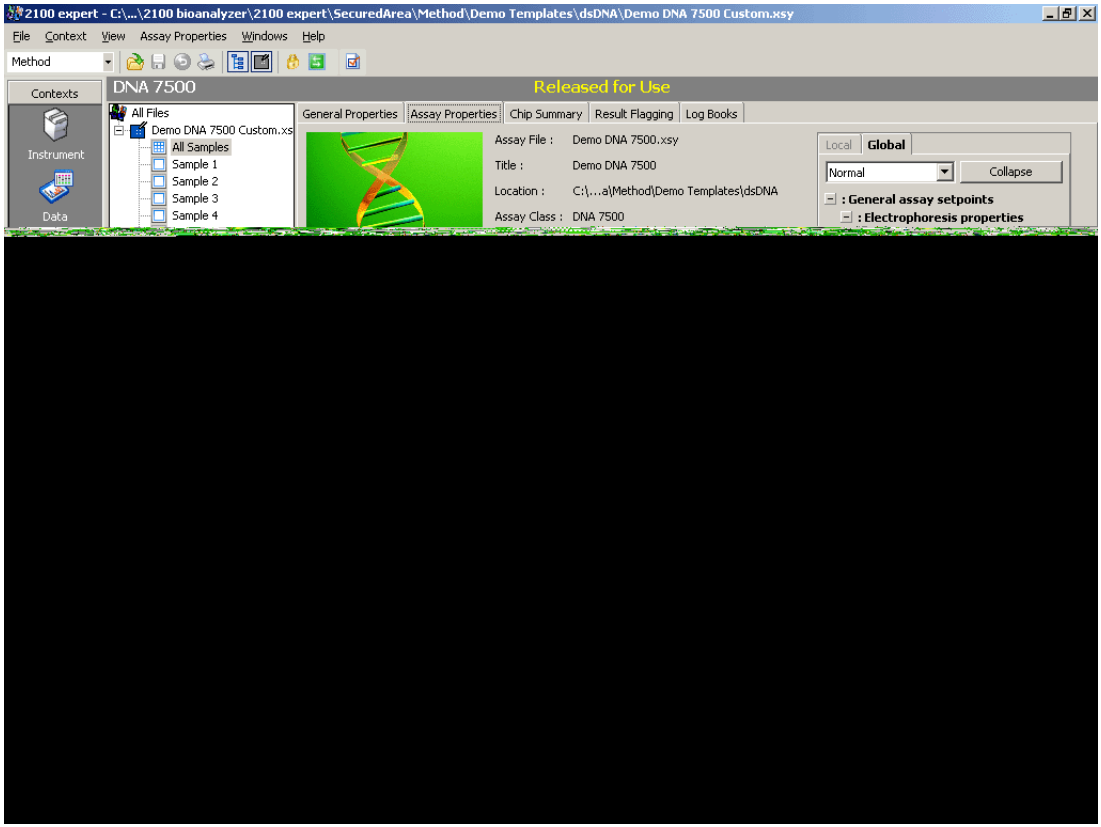
In the Comparison context, you can open multiple electrophoretic chip data files and compare samples of the same assay data (DNA 1000, for example). It is possible to overlay electropherograms recorded by both analyzer and compare the results.



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.

Assay Context

In theAssaycontext, you can create your own assays based on Agilent templates by modifying certain data (for example, data analysis setpoints).



Assays are stored as .xsy files.

Closing 2100 Expert

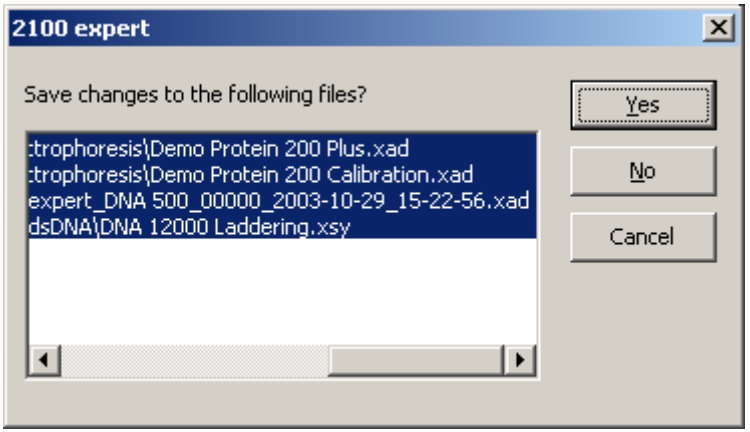
To close the 2100 expert software:

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

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

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

If there are unsaved files open, the following dialog box appears:



NOTE

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

2 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.

After you have confirmed the messages, 2100 expert quits.

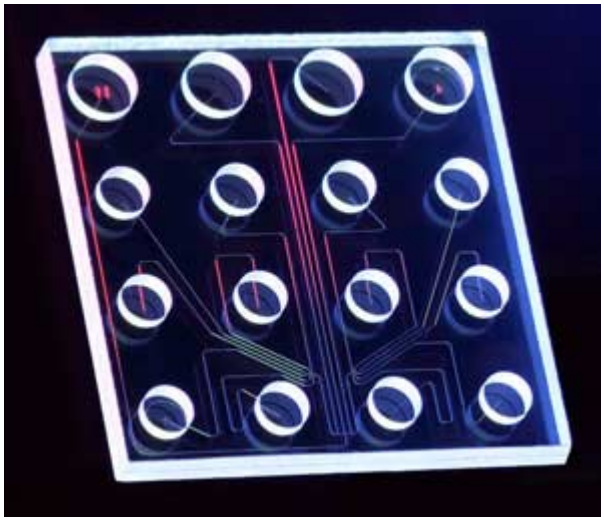
Running and Evaluating Electrophoretic Assays

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

- "Principles of Nucleic Acid and Protein Analysis on a Chip" on page 47
- "Preparing and Running Electrophoretic Assays" on page 51
- "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 82
- "Result Flagging" on page 157

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 format dramatically reduces separation time as well as sample and reagent consumption. The system provides automated sizing and quantitation information in a digital form. On-chip gel electrophoresis is performed for the analysis of DNA, RNA and proteins.



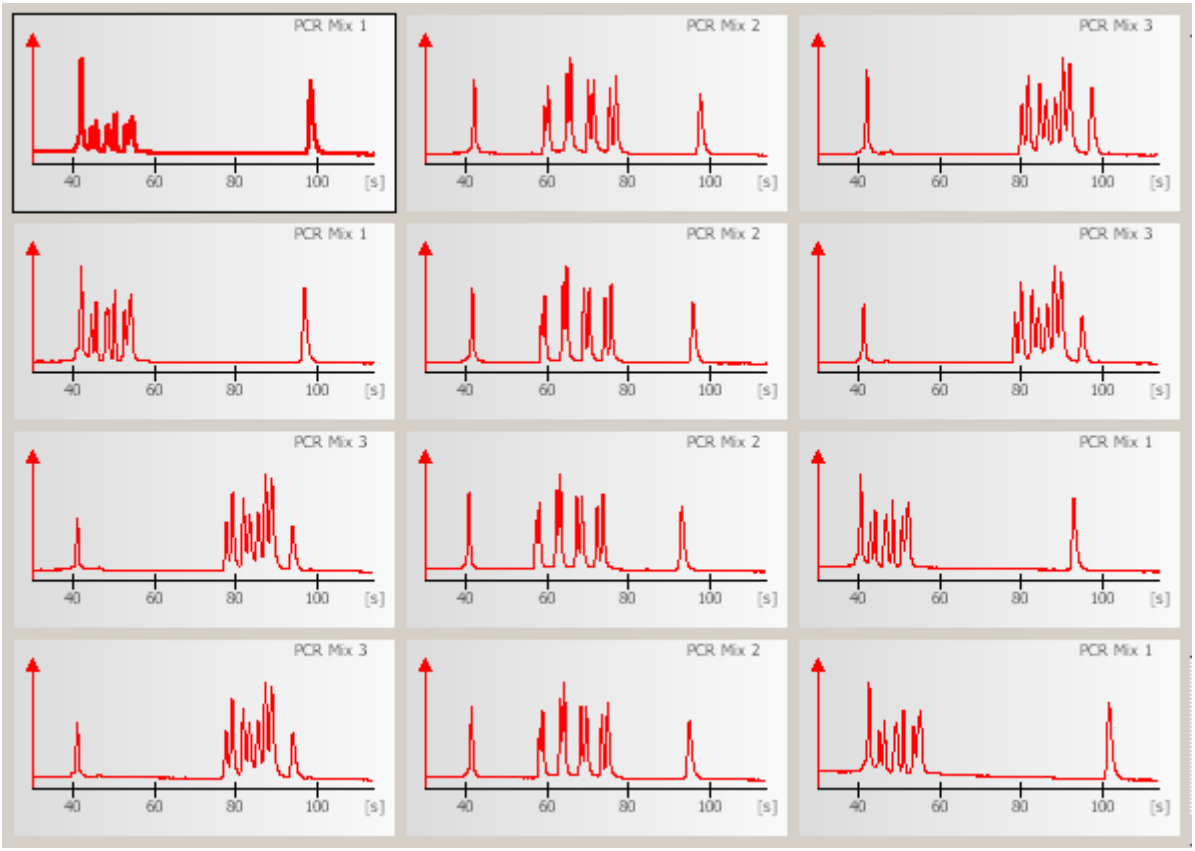
The chip accommodates sample wells, gel and a well for an external standard (ladder). Micro-channels are fabricated in glass to create interconnected networks among these wells. During chip preparation, the micro-channels are filled with a sieving polymer and fluorescence dye. Once the wells and channels 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 DN

RNA, or protein/LDS micells are electrophoretically driven by a voltage gradient—similar to slab gel electrophoresis. Because of their 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/LDS micells. These complexes are detected by laser-induced fluorescence. Data is translated into gel-like images (bands) or electropherograms (peaks). With the help of a ladder that contains components of known sizes, a standard curve of migration versus fragments size is plotted. From migration times measured for each fragment in the sample, the size is calculated. Two markers (for RNA only one marker) are run with each of the samples bracketing the sizing range. The “lower” and “upper” markers are internal standards used to align the ladder data with data from the sam wells. This is necessary to compensate for drift effects that may occur during the co of a chip run.

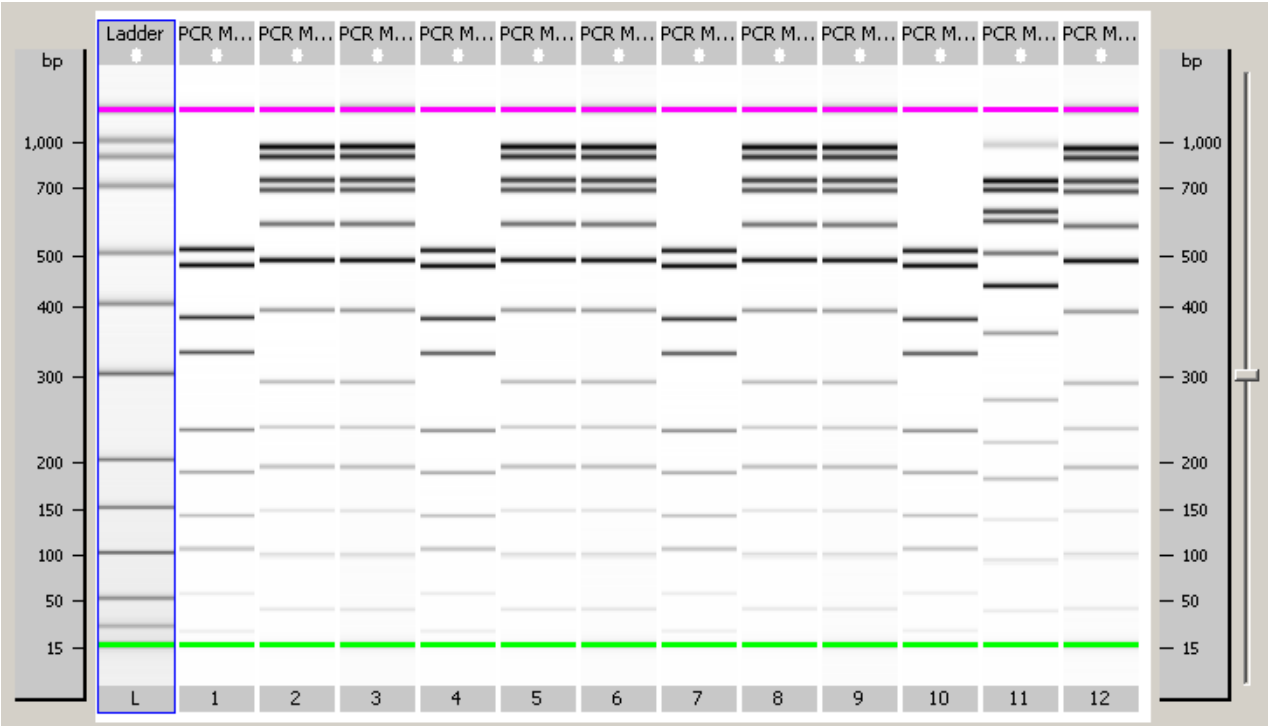
For DNA and protein assays, quantitation is done with the help of the upper marker area under the upper marker peak is compared with the sample peak areas. Because the concentration of the upper marker is known, the concentration for each sample can be calculated. Besides this relative quantitation, an absolute quantitation is available fo 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. Additionally, th RNA integrity number (RIN) can be utilized to estimate the integrity of total RNA samples based on the entire electrophoretic trace of the RNA sample, including the presence or absence of degradation products.

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



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



Preparing and Running an Electrophoretic Assay

An electrophoretic chip run requires the following steps:

1. Switch on the Agilent 2100 bioanalyzer and start the 2100 expert software.

   See "Starting 2100 Expert" on page 32.

2. Select an electrophoretic assay.

   See "Selecting an Electrophoretic Assay for a Chip Run" on page 52.

3. Prepare reagents, chip, and samples.

   See "Preparing Samples, Reagents, and Chips for Electrophoretic Assays" on page 55 and the appropriate Application Note or Reagent Kit Guide.

4. Load the chip into the bioanalyzer.

   See "Loading the Electrophoresis Chip into the Bioanalyzer" on page 59.

5. Start the chip run.

   See "Running an Electrophoretic Assay" on page 65.

When the chip run has finished, you can:

- Have a first look at the results (see "Displaying the Measurement Results (Electrophoresis)" on page 73).
- Document the chip run (see "Entering Chip, Sample, and Study Information" on page 70).
- Analyze and evaluate the results:
  - See "Analyzing and Evaluating the Result of an Electrophoretic Assay" on page 82.
  - See "Result Flagging" on page 157.

Selecting an Electrophoretic Assay for a Chip Run

To select an assay:

1 Switch to the Instrument context.

2 In the Tree View Pane, 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 (left open, right 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 required measurement 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.

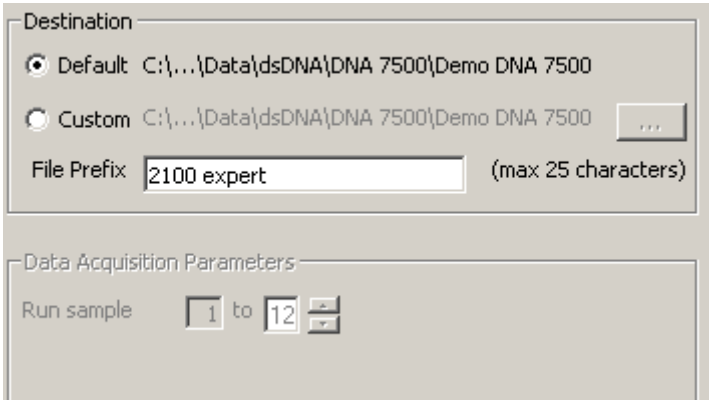
5 Select the desired assay, DNA 1000, for example.

The assay is loaded and its name appears in the Information Bar.

NOTE

After a chip run, the results can be evaluated using parameters from 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 279.

6 Select a **Destination** for the chip data file (.xad) that will be generated as the result of the chip run. You can also specify a custom **File Prefix** for this file.



7 Under **Data Acquisition Parameters**, enter the number of samples you want to have 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, Reagents, and Chip for Electrophoretic Assays" on page 55), keep in mind that you have to follow the sequence of the sample wells. For example, if you want to measure only 3 samples, you have to fill the wells 1, 2, and 3 of your chip.

Preparing Samples, Reagents, and Chips for Electrophoretic Assays

Before you can load a chip, you have to prepare the samples and reagents. To find out how to prepare the samples and reagents, refer to the respective 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 that is 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 molecule (for example, between DNA 1000 and DNA 7500 assays).
- Make sure you are familiar with the essential measurement practices (see next page).
- 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 and buffer.
- Load the destaining solution for protein assays.
- Load the chip with ladder and samples.

Essential Measurement Practices (Electrophoretic Assays)

General:

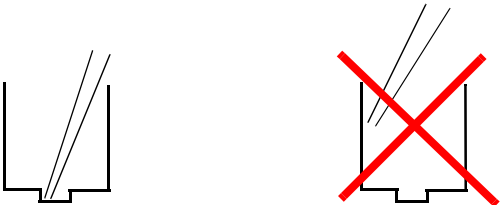
WARNING

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 potent 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 will 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. 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:

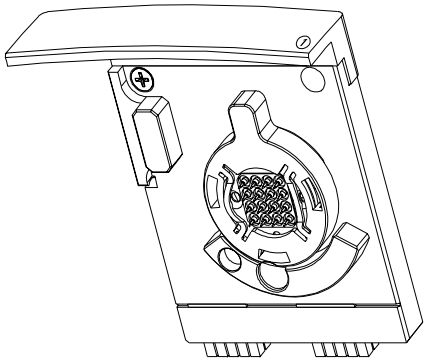
- Always wear gloves when handling RNA, and use RNase-free tips, microfuge tubes and water.
- It is recommended to denature all RNA samples and RNA ladder by heat before (70 °C, 2 minutes).
- Always vortex the dye concentrate for 10 seconds before preparing the gel-dye m

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

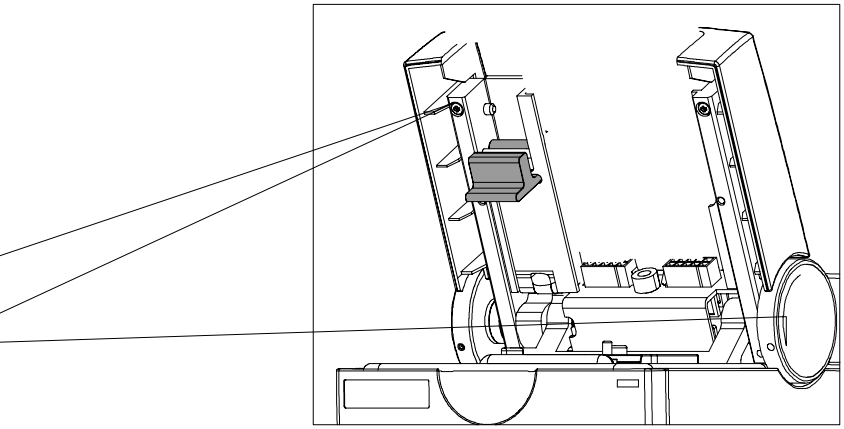
The Agilent 2100 bioanalyzer uses different cartridges for electrophoretic and flow cytometric assays. For electrophoretic measurements, the electrode cartridge is required.



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 cartridges will either have an engraved “1” at the front, or will have no engraving at all. Cartridges with a different number are not electrode cartridges.

If the bioanalyzer is set up for flow cytometric assays, but you want to run electrophe
assays, proceed as follows:

1 Open the lid and pull down the metal locking lever into the open position as shown in the figure below.



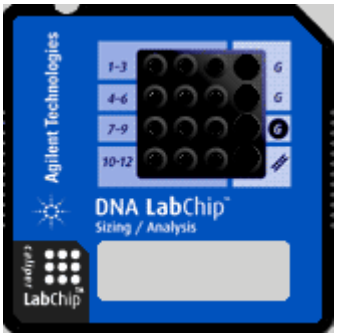
The cartridge is pushed out.

2 Gently pull the cartridge out of the lid.

NOTE

Store the pressure cartridge in the provided box.

When the chip is detected, the image on the Instrument tab changes to a chip.



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

NOTE

The displayed image depends on the assay selected in the software, not the type of chip inserted. If you would like to run a DNA chip but a protein chip appears, you have selected the wrong assay.

NOTE

If the AutoRun option is active, the chip run starts automatically once a chip has been detected and the lid has been closed.

NOTE

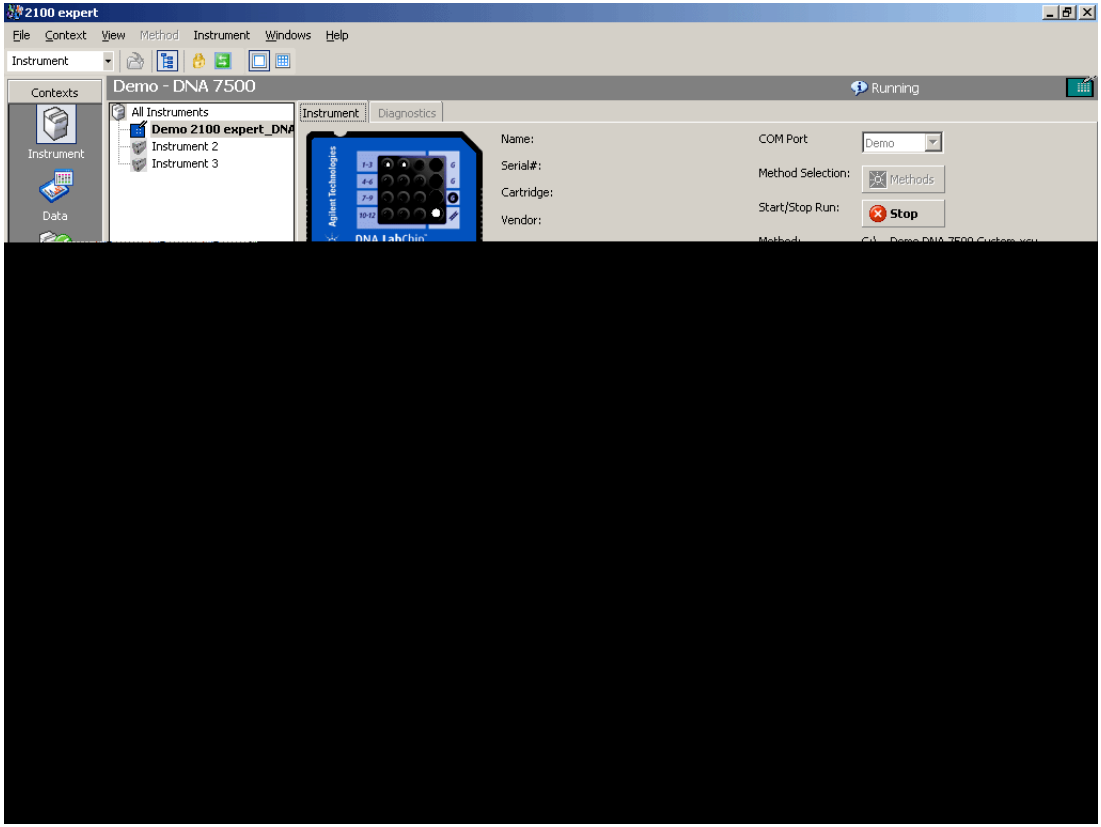
You can stop a chip run at any time, for example, if errors occurred or if you are not satisfied with the quality of the measurement results that you can observe during the chip run. See “Stopping a Chip Run” on page 68.

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).

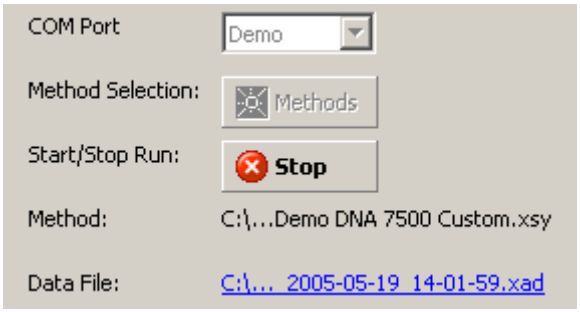


The number of the sample that is currently being measured is indicated on the information bar:

The status bar at the bottom of the screen shows the measurement progress for the 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 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 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.

After the chip run is completed, you can:

- Switch to the Data context, where you can view, analyze, and evaluate the results of your chip run (see "Displaying the Measurement Results (Electrophoresis)" on page 73 and "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 82).
- Stay in the Instrument context and 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 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 286) and automatic print (see "How to Turn on and Configure Automatic Printing of Chip Run Reports" on page 297) does not take place.

To stop the assay:

1. Click the **Stop** button.

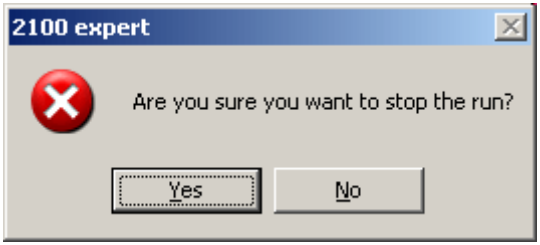
   – OR –

   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.

When the chip run is aborted, you can:

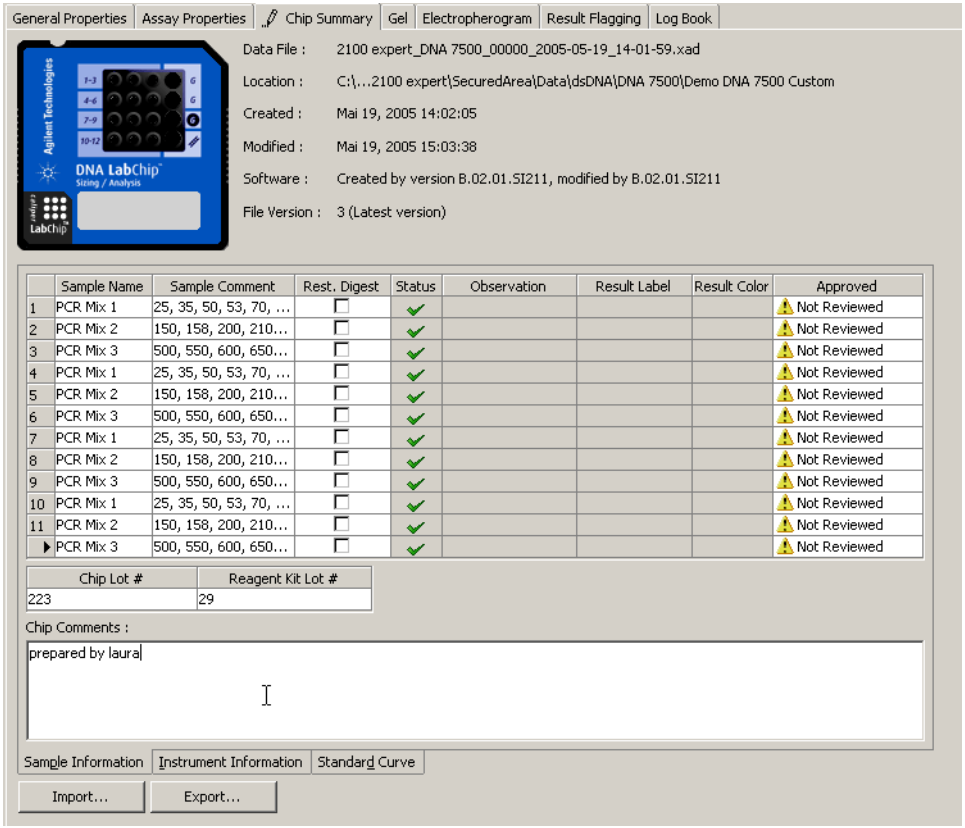
- Switch to the **Data** context, where you can view, analyze, and evaluate the results (if any) of your chip run (see "Displaying the Measurement Results (Electropherogram)" on page 73 and "Analyzing and Evaluating the Results of an Electrophoretic Assay" on page 82).
- Stay in the **Instrument** context, 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 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 information such as the name of the current study, the laboratory location, and the experimenter, for example.



NOTE

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

3 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 in another chip data file. Refer to "Importing Chip, Sample, and Study Information" on page 281 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, for example, to see the progress of a reaction. See "How to Switch Between Single View and Grid View (Electropherograms)" on page 74.
- You can navigate through the samples. See "How to Navigate Through the Samples" on page 75.
- 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 76.

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

To switch between single view and grid view:

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

– OR –

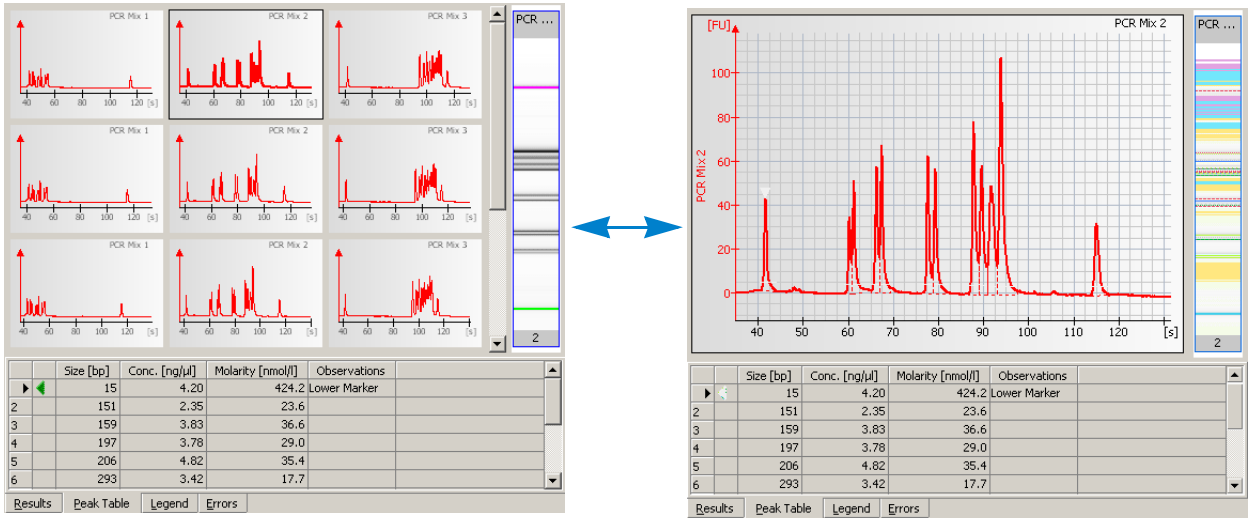
Click the **View Single Sample** or **View All Samples** button on the Electropherogram toolbar.

– 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 to switch to single view:



How to Navigate Through the Samples

At any time—even during a chip run—you can use to 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 any lane of the small gel image.
   Gel view: Click 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 toolbar.

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 a gray-to-white gradient to white.

€ add a grid to the electropherograms.

In gel-like images, you can additionally:

€ change the gel color.

€ change order of gel lanes in gel like images.

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 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 the last zoom, pan, or scale step:

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

To undo all zoom, pan, and scale steps:

Click the Undo All button.

To display data points in an electropherogram:

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 remove the gray-to-white gradient from the background of an electropherogram:

From the Electropherogram menu, select Show Gradient.

The color gradient disappears and a white background is displayed.

To show/hide the grid lines on an electropherogram:

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 according to the guidelines established by your laboratory safety officer. Remove the 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 separate sample components and dete
their size, concentration, purity, or molarity. Results for a particular sample are calcu
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 83
- "Data Analysis: RNA and Cy5-Labeled Nucleic Acids" on page 88
- "The RNA Integrity Number (RIN)" on page 91
- "Data Analysis: Protein" on page 105
- "Smear Analysis" on page 113

Further steps in analysis are:

- "Changing the Data Analysis" on page 117
- "Manual Integration" on page 136
- "Reanalyzing a Chip Data File" on page 146
- "Comparing Samples from Different Electrophoretic Chip Runs" on page 148
- "How to Use the Form Mode" on page 164

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 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 following example electropherogram), which is a mixture of DNA fragments of known sizes, is run first from the ladder well. The concentration sizes of the individual base pairs are preset in the assay and cannot be changed

5. A standard curve of migration time versus DNA size is plotted from the DNA sizi ladder by interpolation between the individual DNA fragment size/migration point The standard curve derived from the data in 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 ass marker peaks in a sample well.

NOTE

The software performs alignment by default. Turning automatic data analysis off 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 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 wel

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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.

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

Since it is assumed that the molarity of all the fragments in a restriction digest sh be the same, any peaks or clusters having a molarity that is significantly larger tha rest are flagged as potentially co-migrated 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 cons
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 dat
3. Fragments are identified for all samples and tabulated by peak ID. You can chan 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 concentration 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.

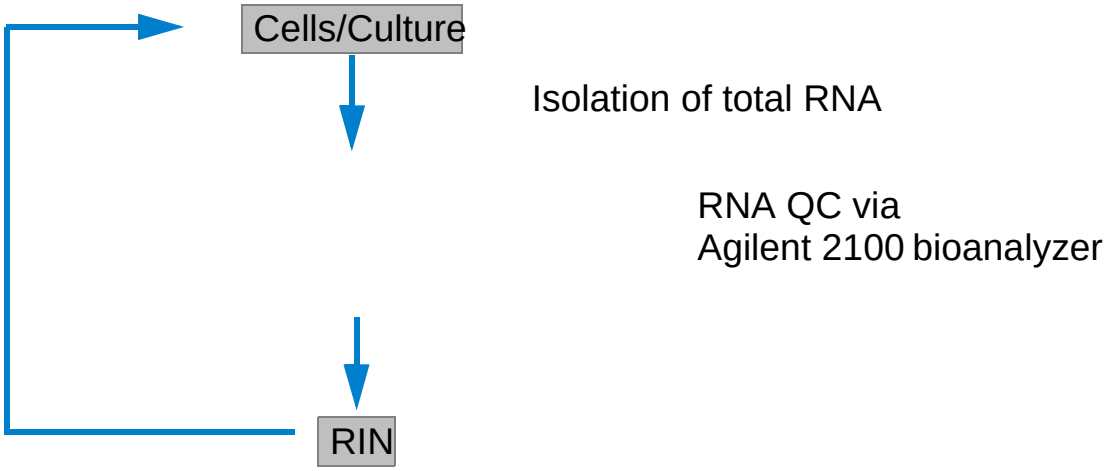
5. For the Eukaryote or Prokaryote Total RNA assay, the RNA fragments (either 18S and 28S for eukaryotic RNA or 16S and 23S for prokaryotic RNA) are detected. After detection, the ratio of the fragment areas is calculated and displayed.

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.

Use Models for the RNA Integrity Number

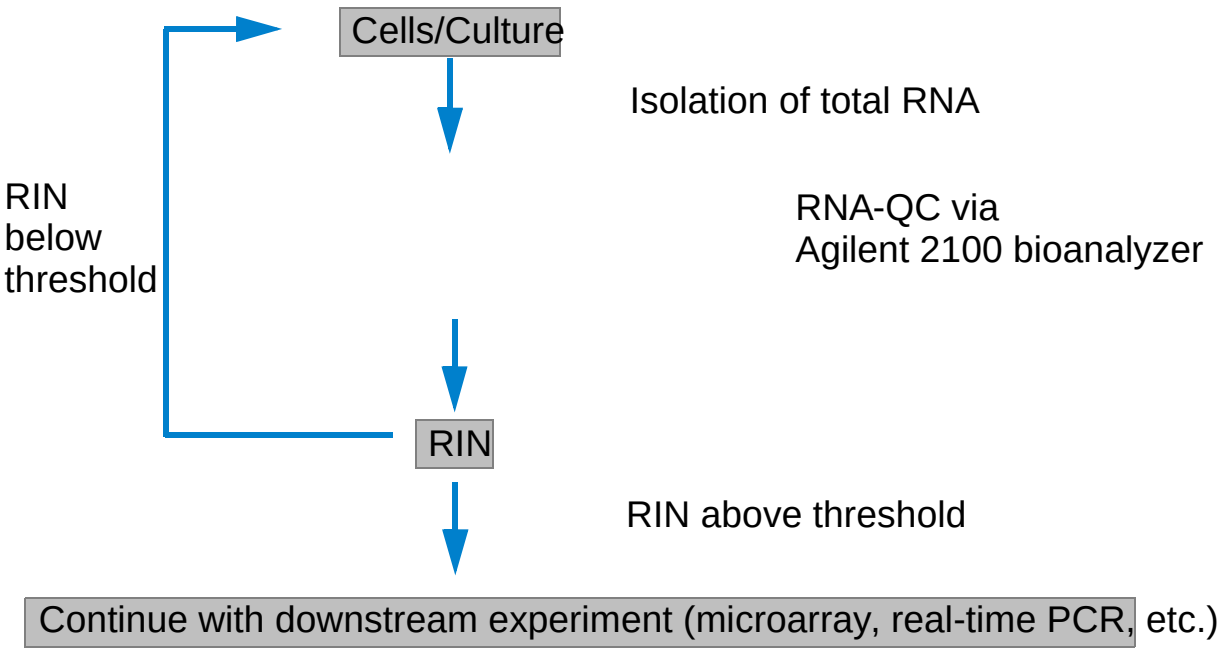
To take full advantage of the RIN feature, a 2 step use-model is suggested:

1 Determine the threshold value for the RIN that results in meaningful downstream experiments:



Correlate RIN with downstream experiment and determine threshold RIN for meaningful results (iterative process)

2 Run standard experiment and use RIN to decide if sample integrity is sufficient:



RNA Integrity Number Setpoints

Various setpoints are available to customize the display of the RIN (RNA Integrity Number). With these setpoints, you can modify the predefined thresholds for anomaly detection. You can find them in the advanced user mode of the setpoint explorer.

To adjust the setpoints for a single sample, switch to the Local tab of the setpoint explorer and open the RNA Integrity Number group.

To adjust the setpoints for the whole chip, switch to the Global tab of the setpoint explorer and open the RNA Integrity Number group in the Sample Setpoints group. For the chip, you can additionally switch between integer and decimal representation of the RIN.

For more information on how to use the setpoint explorer, see “About the Setpoint Explorer” on page 118.

Data Analysis: Protein

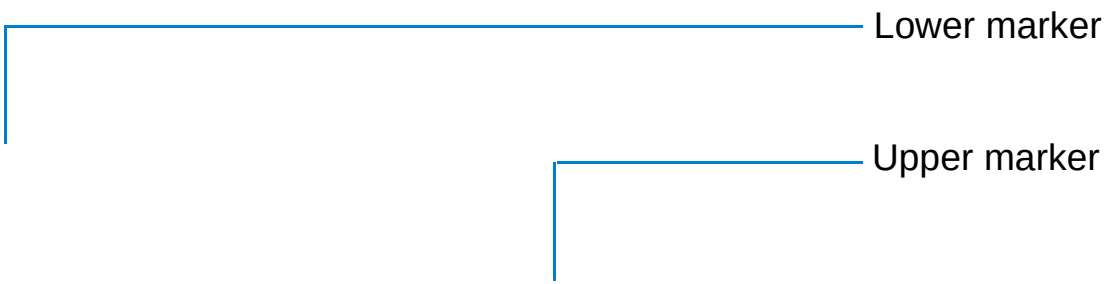
The data analysis process for protein assays consists of the following steps:

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 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 electrogram below), which is a mixture of proteins of different known sizes, is run first from the ladder well. The sizes of the individual proteins are preset as kDa in this assay and cannot be changed. Please note that the concentrations may vary slightly from ladder lot to ladder lot.

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

6. Two proteins are run with each of the samples, bracketing the size 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 ass marker peaks in a sample well.



NOTE

The software performs alignment by default. Turning automatic data analysis off suspends analysis until you turn it on again.

7. The standard curve, in conjunction with the lower 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 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.

Protein Absolute Quantitation

Absolute quantitation is calculated based on the relative concentration of a sample to the user-defined standard and the known concentration of this user-defined standard.

For protein samples you can enable the use of calibration for each sample and enter a concentration of the standard protein. This allows you to generate a calibration curve, which can be used to analyze and quantitate the protein within different samples on the same chip.

Using Calibration in Protein Assays

The calibration feature for protein assays enables absolute quantitation based on external standard calibration.

On the Chip Summary tab, use the sample table on the Sample Information sub-tab to define the samples that you want to use as calibration standards and enter a concentration.

The calibration standard should be run at different concentrations to generate a calibration curve. The software will automatically produce this calibration curve based these inputs to determine the actual concentration of samples within the same chip the peak tables of the samples, a remark is added to the observation column to identify the calibration protein and the calibrated proteins:

The calibration curve can be displayed by switching to the Calibration Curves sub-tab on the Chip Summary tab.

Smear Analysis

The 2100 expert software allows to perform 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 cannot 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 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 check box Perform Smear Analysis.

The Region Table sub-tab is added to the Electropherogram tab.

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.

   A region will be inserted into the electropherogram. The Region Table shows the values for the inserted region.
3. Repeat the previous step until the number of required regions is inserted.
4. Adjust the regions by dirty 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.

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 tab, you can edit the Region Start Size and Region End Size, for example:

Changing the Data Analysis

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

- Filtering parameters
- Peak find parameters for all samples/peak height for individual samples
- Enabling smear analysis
- Align to upper and/or lower marker
- Adding/deleting ribosomal fragments (for RNA assays only)
- Manual integration (for protein and DNA assays only)
- Absolute quantitation (for protein assays only)

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

- 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. They are applied automatically.

When you try to change any global setpoints where local settings have been applied, the software prompts you as to whether you want to overwrite the local (custom) settings.

If you decide to overwrite the custom sample settings, all local settings you made will be discarded. If you decide not to overwrite the custom sample settings, the global set will not be applied where local settings have been changed.

Assigning Upper and Lower Marker Peaks

For each DNA or protein sample, the upper and lower marker peaks are assigned first a
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. RNA sa
are aligned to a lower marker exclusively.

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

In case the 2100 expert software did not detect the lower marker in RNA samples correctly, you are able to manually assign it in the same way.

CAUTION

Excluding a peak or manually setting a peak an upper or lower marker may cause errors during analysis.

Aligning or Unaligning the Marker Peaks

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 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 marks. Consequently, the software attempts to use the minimum peak height threshold that, if 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 DNA and Protein 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 le 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 Switch to the Electropherogram tab in the Data 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 it.

TIP

If you want to change several baseline points, deactivate the automatic analysis by clicking the Pause Analysis button in the toolbar. This way, the software will not recalculate the data analysis with every change. Once you have changed all baseline points, click the Pause Analysis button again to activate automatic analysis.

3 Adjust the baseline points as appropriate.

TIP

To move a peak baseline point along the x-axis, press the CTRL key and the left mouse button. To move a peak baseline point along the signal, press the left mouse button only.

4 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 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 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:

Example: Inserting peak baselines

To insert peaks manually:

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

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

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

TIP

If you want to change several baseline points, deactivate the automatic analysis by clicking the Pause Analysis button in the toolbar. This way, the software will not recalculate the data analysis with every change. Once you have changed all baseline points, click the Pause Analysis button again to activate automatic analysis.

4 Adjust the baseline points as appropriate.

TIP

To move a peak baseline point along the x-axis, 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.

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

To do this:

1 ClickFile > Opento open a chip data file (.xad).

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

3. ClickOK

The items that can be changed for reanalysis are:

All Electrophoresis Assays:

€ Global peak find settings

€ Individual sample peak find settings

€ Gel color

€ Sample names and comments

RNA Assays Only:

€ Fragment names and colors associated with labels

€ Fragment start/end times, additional fragments (or delete fragments found)

- Reassign lower marker

DNA and Protein Assays Only:

- Exclude peaks from analysis
- Reassign upper/lower markers
- Alignment or no alignment with marker peaks
- Manual integration

Protein Assays Only:

- Absolute quantitation

TIP

When applying modified data analysis setpoints, the software will (by default) immediately recalculate the raw data, which takes some time. Select 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 as gel color, sample names, and peak finding 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 currentl open in the Data 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) has the same functionality as in the Data context.

5 You can now add further samples from any 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.

You are notified if you try to add a sample of a .xad file that has the wrong assay

6 You can also remove samples from a comparison file. Right-click the sample nam
selectDelete 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, 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 Data context.

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 used 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.

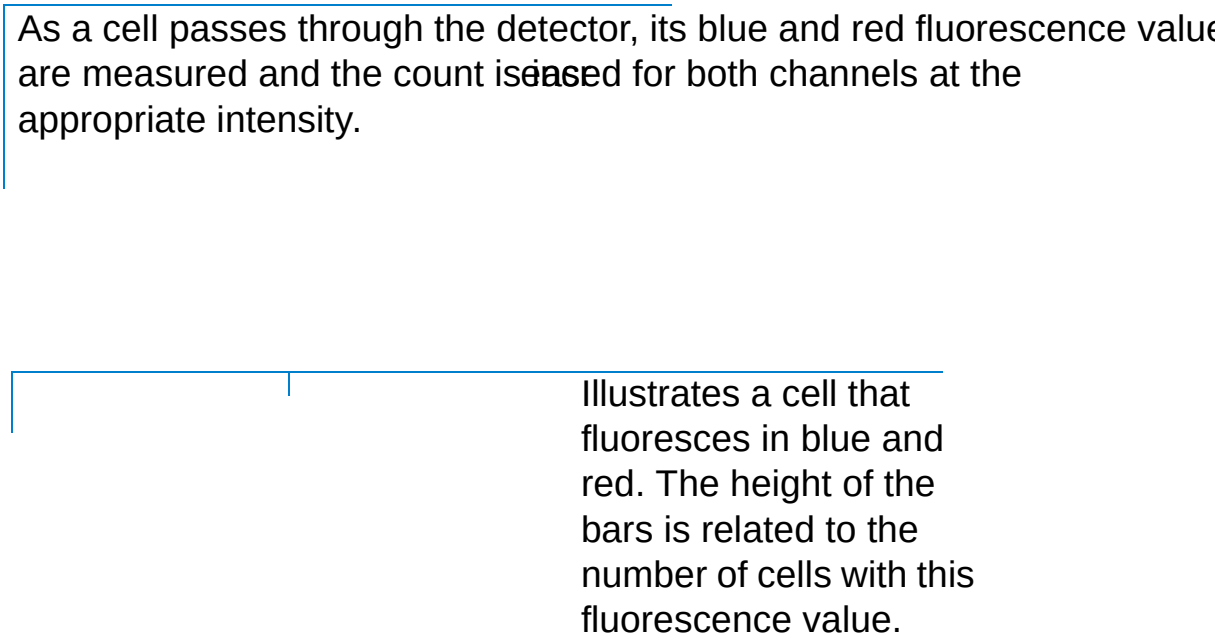
The Agilent 2100 bioanalyzer lets you determine the number of cells characterized by a specific pattern of fluorescence.

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 cell 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.

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.



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 218.

Generating Dot Plots

Single events can also be displayed related 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 follo image.

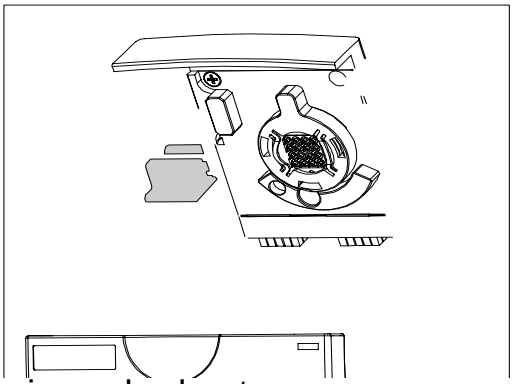
To determine the number of cells whose blue and red fluorescence lies within a def range, you can insert regions. Additionally, a gate can be set for either red or blue fluorescence to obtain percent values on a defined population.

If the bioanalyzer is set up for electrophoretic assays, but you want to run flow cyto assays, proceed as follows:

1 Open the lid and pull down the metal locking lever in the open position as shown in the following figure.

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.

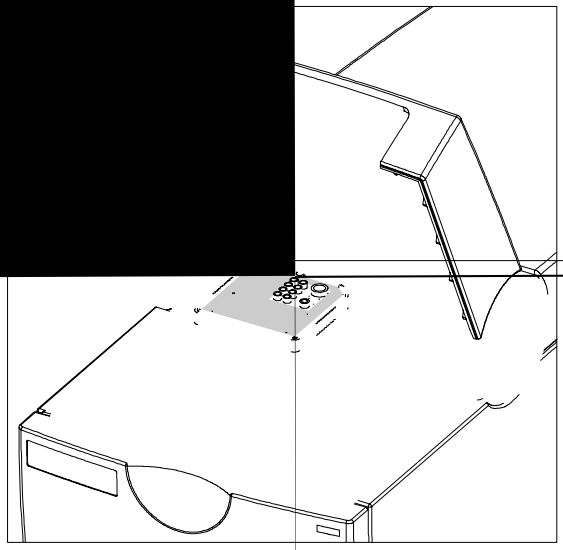


The cartridge is pushed out.

wa hip is inserted in the bioanalyzer.

ptacle.

e force.



3 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 in another chip data file. Refer to "Importing Chip, Sample, and Study Information" on page 281 for details.

Displaying the Measurement Results (Flow Cytometry)

You can view the measurement results of a 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 entire chip, for example, to see the progress of a reaction. See "How to Switch Between Single View and Grid View" on page 211.
- You can navigate through the samples. See "How to Navigate Through the Samples" on page 212.
- 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 214.

How to Switch Between Single View and Grid View

To switch between single view and grid view:

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 toolbar.

– 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 data 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

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

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

3 Click any well on the chip icon.

To browse through samples:

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 toolbar.

To switch between histogram and dot plot view:

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 plot, the Undo Zoom and Undo All buttons are enabled.

To undo one zoom step:

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

To undo all zoom steps:

Click the Undo Zoom All button.

To display data points in histograms:

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:

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 Assa

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 results based on markers in one histogram. See "Using Histograms for Evaluation" on page 218 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 with a fluorescence intensity lying in a defined range. See "Using Dot Plots for Evaluation" on page 239 for detailed information.

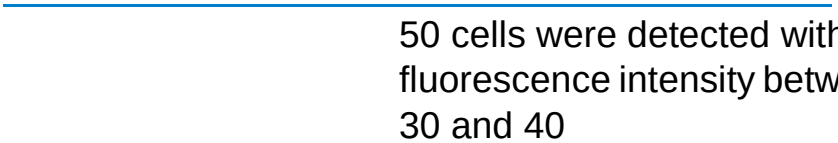
If you use predefined assays, the marker regions are set at the approximate position where the events are expected. Refer to "Evaluating Antibody Binding, Apoptosis, and GFP Assays" on page 248 for information on how to evaluate the predefined assays.

TIP

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

Using Histograms for Evaluation

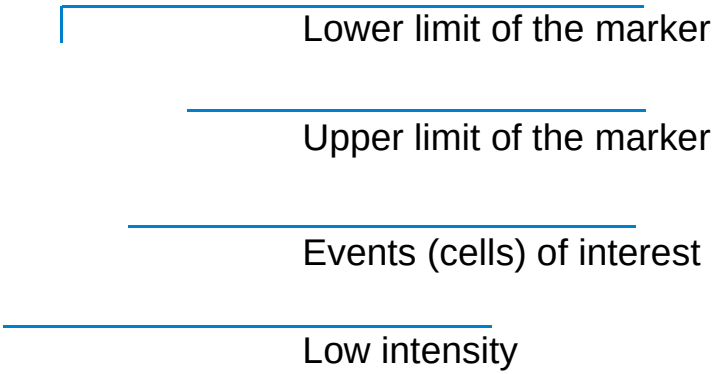
Histograms are graphical representations of the measurement results, where the number of events (cells) is mapped to the Y axis and their fluorescence values to the X axis. The resulting curves show the frequency distribution of the events in relation to their fluorescence intensity values, as shown 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 the 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 value 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 find the subset by a marker (Apoptosis and Antibody Staining assays).

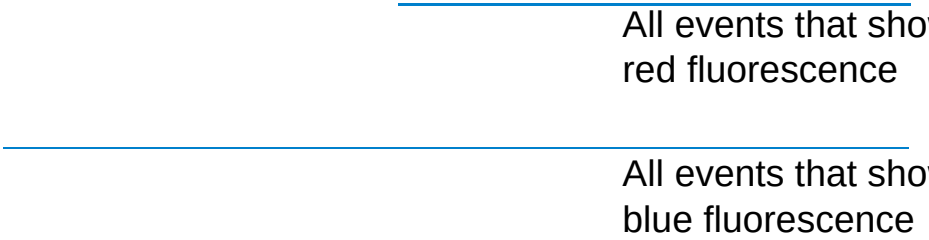
- Gating from red to blue uses the red histogram to define the subset by a marker (GFP assay).

NOTE

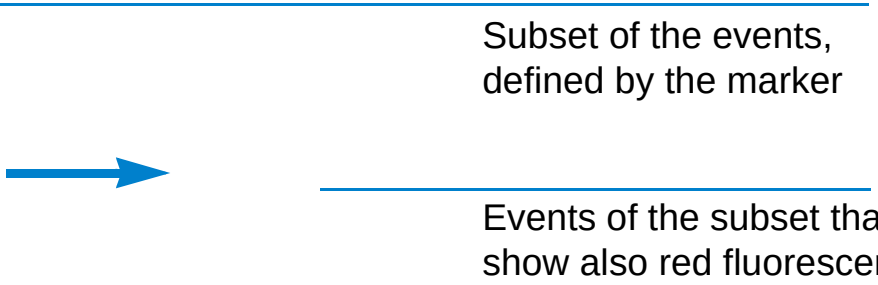
Predefined assays have a fixed gating direction, while assays of type Generic have a variable gating direction.

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.

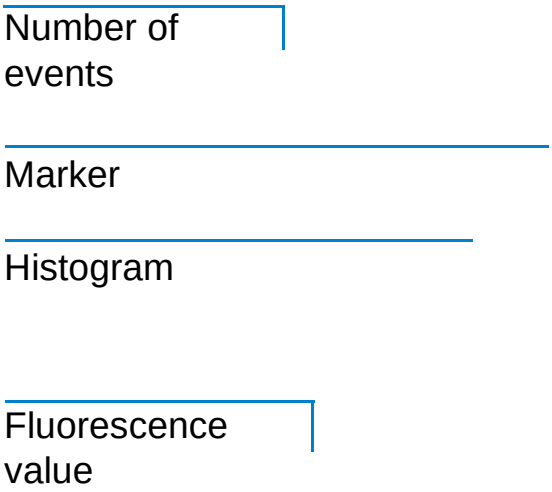


By setting a marker on the blue histogram, you can define the blue fluorescence range that must be met for a cell to be considered for the red histogram. You use the gating on the blue histogram to define a subset for the red histogram.



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 se marker filters out all cells that do not have fluorescence in this range.

The following image shows two histograms arranged in reading 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 this 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 corresponding to this fluorescence region.

NOTE

You can insert markers only in histogram assays.

To add a new marker:

In the toolbar 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 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 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 markers 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 Markers dialog box (see "How to Configure Markers" on page 228).

How to Copy Markers to All Histograms

Once a marker is defined, you can copy it in the histograms of all samples (generic a
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 w
   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 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
   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
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 o
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 toolbar 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, first you have to remove the existing gating.

To remove gating:

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 277).

How to Overlay Histograms

You can compare samples by overlaying 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 235.

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 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 Samples button 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 tables, one table below each histogram. Markers, gates, several statistical values, and %-values of events are shown in the result tables.

Each marker you insert in the histogram is shown in 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, two additional rows are displayed in the gated histogram's result table. The superset curve shows a histogram of all measured events, this gate is not considered.

You can export the table data for further evaluation in other applications. See "Exporting Data" on page 283.

NOTE

The layout of the result table can be configured (see "Configuring Tables" on page 299). 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 used a defined 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 u for gating, the number of all detected events is displayed in the ro “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 ga 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 u for gating, the number of all detected events is displayed in the ro “All events”.
% total	% of selected events in relation to the total number of events. The marker used for gating is 100%, 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. The 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 intensit
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 can may show no events, because of the threshold for event detection. Dots are displayed if their fluorescence intensity exceeds a minimum limit. The limits are specified in the assay—separately for re 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 ge 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 a 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 218). The gate is always displayed and correspon 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 def 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 242).

To insert an existing region:

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

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 t
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:

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 a region 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 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 this 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 “copied”. 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 240). 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 toolbar 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 gen assays, dot plots can have as many regions as you like.

The following values are displayed:

Region	The default region All Events is always displayed in the first row and shows the values for all detected events. For each further region (s "How to Add Regions to Dot Plots (Generic Assay only)" on page 240), 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	Percentage of the gated events in the region.
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 249.
- "Evaluating Apoptosis Assays" on page 253.
- "Evaluating GFP Assays" on page 259.

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 compare the relative expression of protein in individual cells and use this information for population analysis. 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.

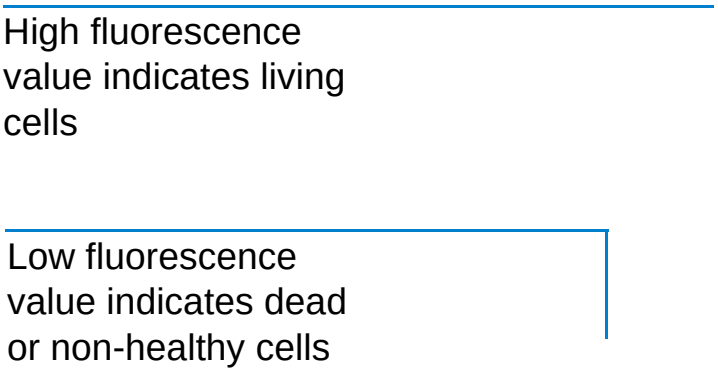
For a detailed description on how to evaluate the results using markers and regions, refer to “Using Histograms for Evaluation” on page 218, and to “Using Dot Plots for Evaluation” on page 239.

Gating direction

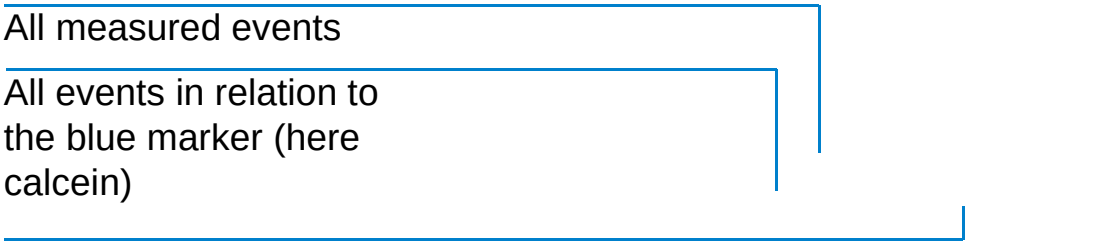
The gating direction is from blue fluorescence to red fluorescence. Depending on the dye you use, you should use all cells (nucleic acid dye) or only living cells (calcein living dye) for gating.

Histogram Evaluation

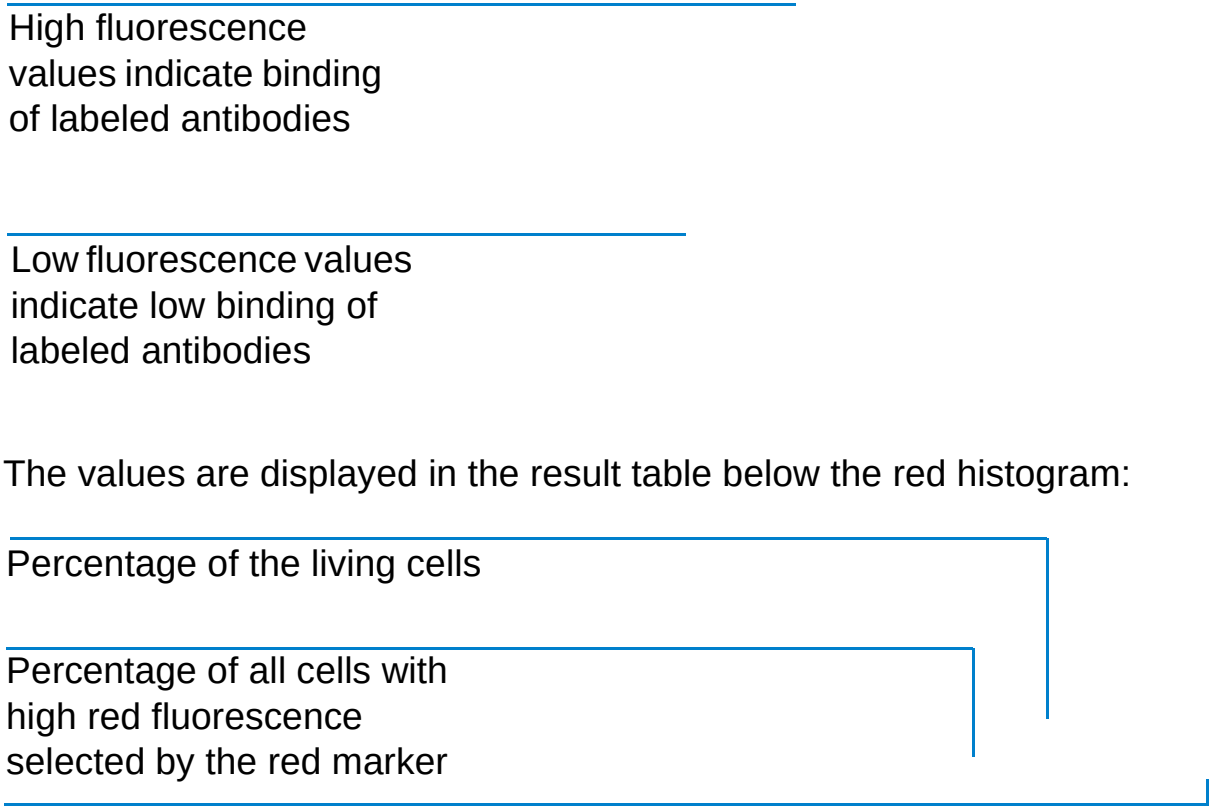
The blue histogram is used for gating. High fluorescence in the blue histogram means that the cells are living (if a life-indicating dye is used). Low fluorescence means the are dead. If you use a nucleic acid dye, you cannot distinguish between living and cells, you can only count all measured cells.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



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

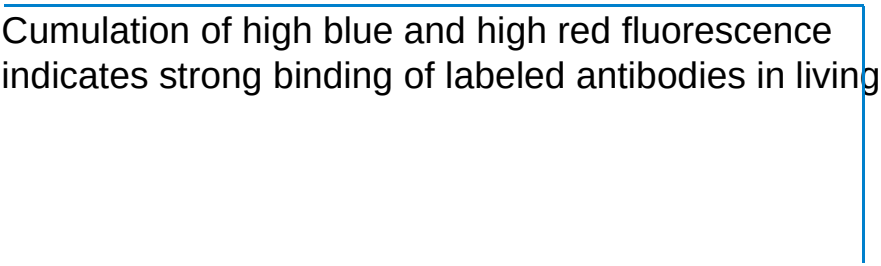


When using the calcein marker in the histogram for gating, only living cells are considered for building the histogram of the red dye. High red fluorescence values indicate living cells with bound antibodies, low red fluorescence values living cells without bound antibodies. See the following example.

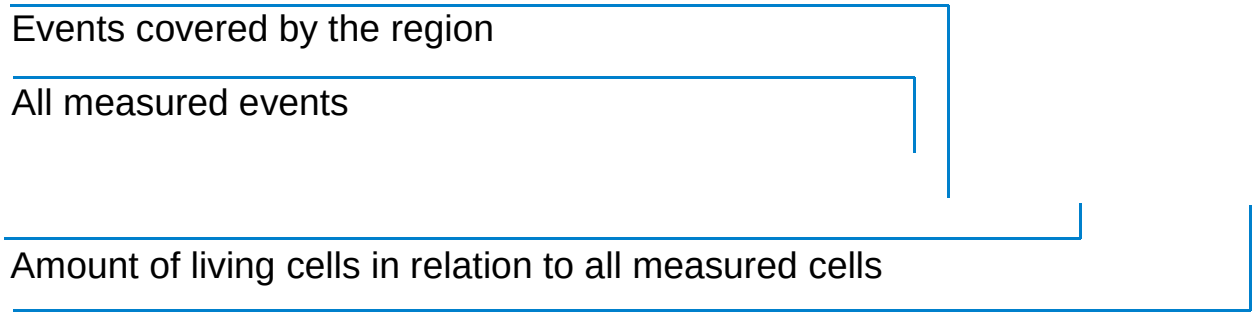


Dot plot evaluation

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.



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



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 218 and "Using Dot Plots for Evaluation" on page 239.

Living or dead cells

In most cases, you want to know whether cells are dead or alive at a specific time. this, you can use calcein-AM as living cell dye, for example. This dye accumulates intact cells, whereas it will leak out of damaged cells. Once inside the cells, the colorless AM ester is cleaved by esterase, resulting in the formation of the highly fluorescent calcein. The number of events resulting from calcein-related staining thus gives you the

number of living cells in a sample. For detailed information, refer to the application 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

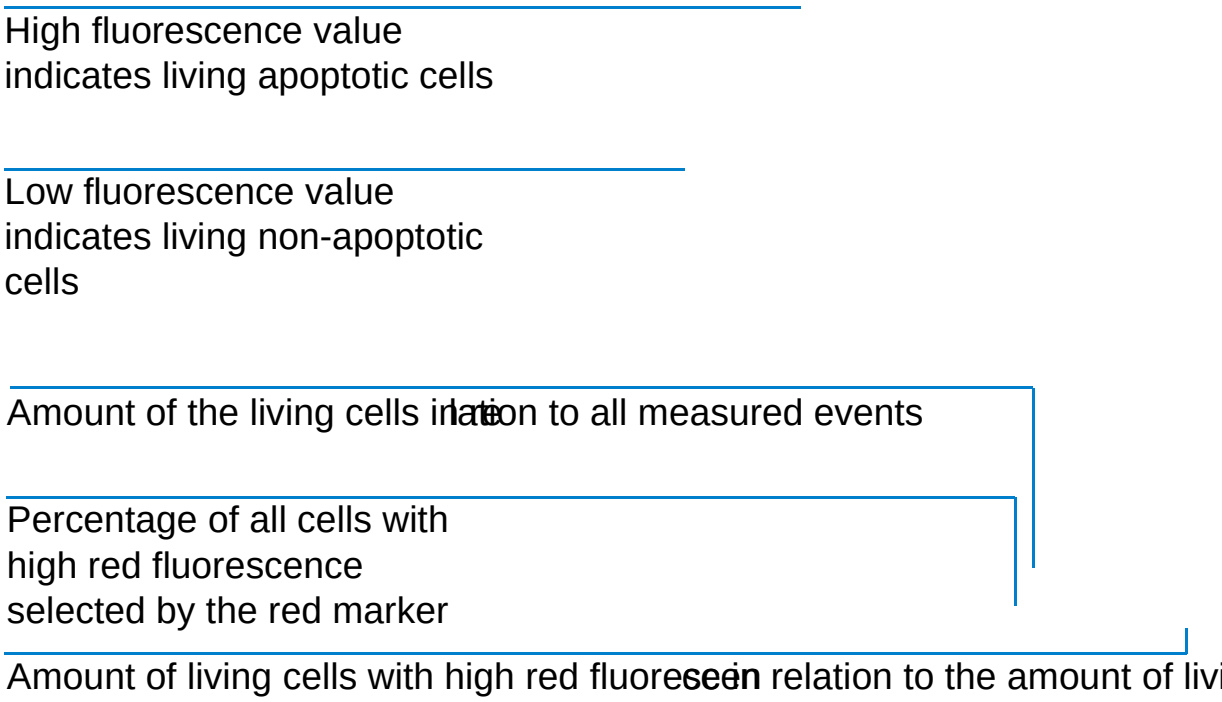
The values are displayed in the result table, each histogram has its own table:

All measured events

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

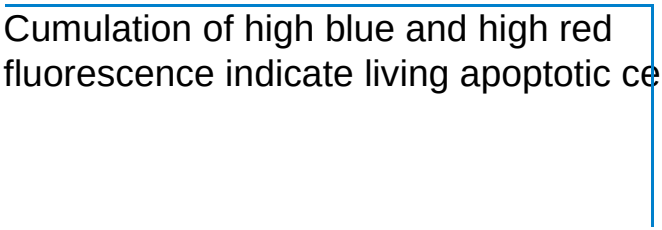
Living cells in relation to all measured cells (high calcein fluorescence)

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 events
Percentage of all cells with
high red fluorescence
selected by the red marker
Amount of living cells with high red fluorescein 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 annexi 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

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 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 results using histograms and regions, refer to "Using Histograms for Evaluation" on page 218 and "Using Dot Plots for Evaluation" on page 239.

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 associated with living cells. See the following image as example.

High fluorescence value is associated with living cells

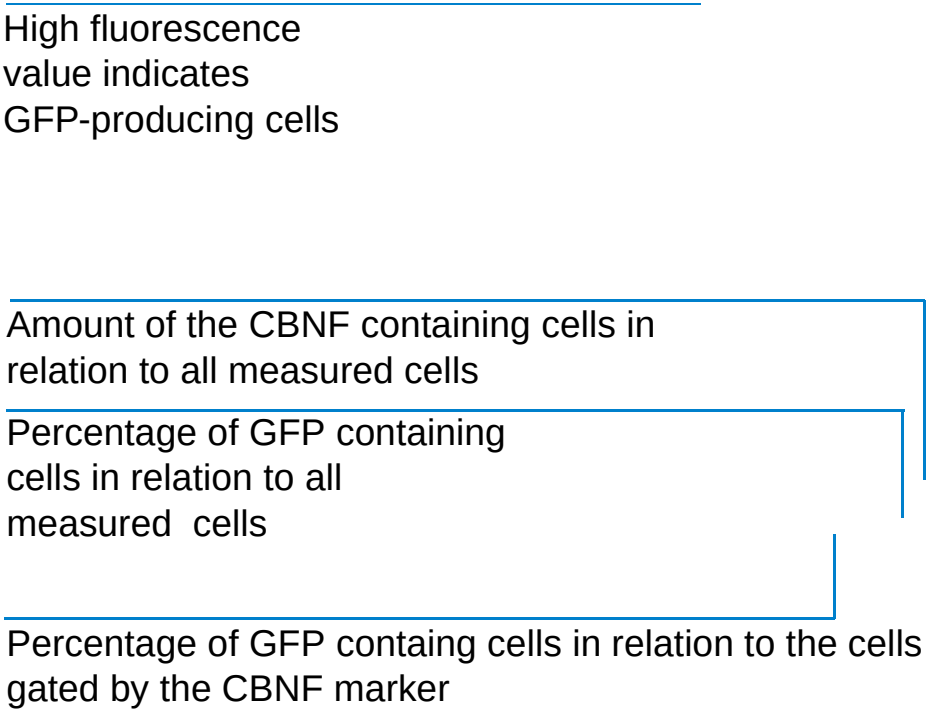
Low fluorescence value indicates dead cells

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

After gating by using the red histogram, 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
Percentage of GFP containing cells in relation to all measured cells
Percentage of GFP containg cells in relation to the cells gated by the CBNF marker

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

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 Data and Assays

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

- “2100 Expert Data Overview” on page 265
- “Handling Assays” on page 268
- “Handling Chip Data” on page 273
- “Organizing, Backing up, and Archiving 2100 Expert Data” on page 275
- “Importing Data” on page 277
- “Exporting Data” on page 283
- “Printing Reports” on page 292
- “Configuring Tables” on page 299

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)
- Verification result files (.xvd)
- Diagnostics result files (. xdy)
- Result flagging rule files (.xml)

Assay files

Assay files (.xsy) contain the following information:

- Data acquisition and analysis setpoints

  Acquisition setpoints are instrument commands and acquisition parameters. Analysis setpoints are evaluation parameters, some of which you can modify.
- Assay information

  All parameters defined by the assay, such as 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 pairs of migration time and fluorescence intensity values, flow cytometric measurements are triplets of migration time, red fluorescence, and blue fluorescence
- 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, manual integration, modified setpoints, modified result flagging rules, or definition of new markers and regions.

Comparison files

You can compare the measurement results of different chip runs (electrophoretic chip data files of same assay class only) by using 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, but also to compare gel-like images or data tables.

Verification result files

Verification result 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 verification run, an .xvd file is generated.

Date and time of the verification run are included in the file name. Example: “Verification_23-05-2005_10-28-40.xvd”.

Diagnostics result 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 fo 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 change under the original file name.

Custom Assays

You can derive your own assays from the predefined assays as described in "How to Create a Custom Assay" on page 270.

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 and (if) 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 assays 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 271.

TIP

If you just want to view the properties of a custom assay, you can open the assay file in read-only mode, ensuring you do not make accidental changes.

The Assays menu 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 **Assay** context.
2. From the **Assays** menu, select an assay.

   – OR –

   Select **File > Open** and open an assay (.xsy) 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”.

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3. Switch to the **Assay Properties Tab** to modify the assay setpoints if required.
4. Switch to the **Chip Summary Tab** to enter chip, sample, and study information.
5. For flow cytometric assays, define markers and regions on the Histogram Tab (Single/Grid View) and Dot Plot Tab (Single/Grid View).
6. For electrophoretic assays, define flagging rules on the Result Flagging Tab.
7. Select **File > Save As** to open the **Save As** dialog box.
8. Under **Save as type**, select (.xsy) and enter a name and location for the new assay.
9. 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.

NOTE

The study description is stored in the 2100 expert system file. Altering the study description of an assay will not affect the entries in the data files that were previously generated from this assay. To update this information in the data files, too, they m be opened, and the study must be assigned again.

   – 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 to save the method with the current name or Save as to save it with a new name.

Handling Chip Data

Chip data (.xad) files are automatically generated at the end of a chip run. The .xad
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 306).

Modifying and saving chip data files

2100 expert allows to re-open chip data files, analyze them using different evaluation
parameters and store the new results. You can save modifications either to the orig
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 pro
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.

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, Backing up, and Archiving 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 analyzer, creating a directory within which to save your files is recommended; having each person save files to their own directo 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 di 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, switch to the System context, go to the System Wide Settings tab, and select Data Files in the tree navigation. Then activate the Prefix check box, and edit the prefix string as you require. Note that you can also modify the file prefix before you start a chip run. Additionally, you may specify that a new directory is created each day for stora that day's runs. To do this, activate the Create Daily Subdirectories check box on the same screen.

Backing up 2100 Expert Data

It is strongly recommended to save your files to a backup disk or on CD/DVD on a r basis. This allows to retrieve the data in case of a system crash or other cases of data loss.

Archiving 2100 Expert Data

The difference between archiving and backing up is that in the archiving process the data will be removed from its original place and moved while during the backup process only a copy is taken (depending on the tools you use).

It is a good idea to periodically archive your files to a CD/DVD to remove them from hard disk. Depending on the amount of 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

When working with assay (.xsy) or chip data (.xad) files, you enter specific informat
that you may want to reuse. To support reuse of data, 2100 expert has the following
import capabilities:

- "Importing Bioanalyzer Files" on page 278
- "Importing Data Analysis Setpoints" on page 279
- "Importing Chip, Sample, and Study Information" on page 281

You can import result flagging rules definitions for result flagging into electrophoretic
assay or chip data files:

- "Importing Result Flagging Rules" on page 282

Importing Bioanalyzer Files

You can import data, assay and method files that were generated with other Agilent 2100 bioanalyzer systems. You can even import data and assay files from the older Bio Sizing and Cell Fluorescence software applications.

To import assay files:

1 Switch to the Assay context.

2 From the File menu, select Import to display the Open dialog box.

3 Select a file of any of the following types:

- .xsy (2100 expert assay file)
- .asy (Bio Sizing assay file)
- .csy (Cell Fluorescence assay file)

4 Click Open.

The imported file appears in the Tree View Panel, and the Assay Properties tab shows information about the assay.

Upon importing, the file gets converted to a new 2100 expert assay file (.xsy).

To import chip data files:

1 Switch to the Data context.

2 From the File menu select Import to display the Open dialog box.

3 Select a file of any of the following types:

- .xad (2100 expert assay file)

- .cld (Bio Sizing assay file)
- .cad (Cell Fluorescence assay file)

4 Click Open.

The imported file appears in the Tree View Panel, and the electropherogram grid view shows an overview of all samples.

Upon importing, the file gets converted to a new 2100 expert chip data file (.xad).

Importing Data Analysis Setpoints

You can import data analysis setpoints from other assay (.xsy) or chip data (.xad) f 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 import will change the type of the current file to Generic

To import data analysis setpoints:

1 On the Assay Properties tab, click 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, 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.

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 284), 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, w differs for electrophoretic and flow cytometric assays.

To import chip, sample, and study information:

1 On the Chip Summary tab, click 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 (.x files. Result flagging rules can be stored in .xml files (see "Exporting Result Flagging Rules" on page 291).

To import result flagging rules:

1 Open an electrophoretic assay or chip data file in the respective context.

2 Switch to the Result Flagging Tab.

3 In the Result Flagging toolbar click .

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.

Exporting Data

2100 expert allows you to export the results of 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 the chip run data of the currently loaded file either manually or automatically:

- “Exporting Chip Run Data” on page 284
- “Exporting Chip Run Data Automatically” on page 286

If you want to export only parts of your measurement results:

- “Exporting Tables” on page 287
- “Exporting Graphs” on page 288
- “Copying Graphs and Tables into the Clipboard” on page 289

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 290

From electrophoretic assay or chip data files, you can also export rule definitions for result flagging:

- “Exporting Result Flagging Rules” on page 291

Exporting Chip Run Data

To export chip run data:

1 Switch to the **Data** context.

2 In the **Tree View Pane**, 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 **Electrophoresis Export Options** dialog box appears.

If you selected a flow cytometric chip data file, the **Flow Cytometry Export Options** dialog box appears.

4 Select the export categories and specify a target directory.

NOTE

Keep in mind that exporting a chip data file can require up to 20 MB of disk spac particular, exporting electropherograms and gel-like images as .tif or .bmp files m 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 286 for details.

NOTE

Keep in mind that exporting a chip data file can require up to 20 MB of disk spac
particular, exporting electropherograms and gel-like images as .tif or .bmp files m
take up a lot of disk space.

To enable and configure automatic export:

1. Switch to the System context and select Auto Export in the tree navigation.
2. Activate the Auto Export check box.
3. Specify the export categories that are to be included in the exported files for electrophoretic and flow cytometric chip runs.
4. Switch to Default Export Directories in the tree navigation and specify the target directories.

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 286 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 Click the ... button to specify the file name, the destination directory, and the file type. You can choose between HTML file for .html output and Tabbed text file for .txt output.

4 Specify whether you want to export Selected rows only or All visible rows.

5 Click OK.

Exporting Graphs

You can export graphs as individual graphics. This applies to all graphs that can be displayed in 2100 expert such as Electropherograms or dot plots.

To export a graph:

1 Right-click the graph, and select the appropriate entry (e.g. Save Electropherogram) from the context menu.

– OR –

Click 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 (all samples and the ladder) is exported.

TIP

Electropherograms, gel-like images, histograms, and dot plots can be automatica exported every time a chip run has finished. Refer to "Exporting Chip Run Data Automatically" on page 286 for details.

Copying Graphs and Tables into the Clipboard

You can copy graphs into the clipboard. This applies to all graphs that can be displayed in 2100 expert, such as electropherograms or dot plots.

You can also copy tables (or parts of tables) into the clipboard. This applies to most tables that can be displayed in 2100 expert, such as result tables or log book tables.

To copy a graph or table into 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, w differs for electrophoretic and flow cytometric assays.

To export chip, sample, and study information to a file:

1 On the Chip Summary tab, click 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 282). Result flagging rules are stored in .xml files.

To export result flagging rules:

1. Open the electrophoretic assay or chip data file with the desired result flagging ru the respective context.
2. Switch to the Result Flagging Tab.
3. In the Result Flagging toolbar clic .

   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 file.
5. Click Save.

Printing Reports

For documentation and presentation purposes, you can print reports for assay (.xsy), chip data (.xad), verification results (.xvd), and comparison (.xac) files.

You can print all reports manually, see "How to Print a Chip Run Report" on page 293. 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 297 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:
  - Run summary—general data about the assay, and sample information.
  - Assay details—complete list of data analysis setpoints.
  - Run Logbook
  - Signature Logbook
  - Audit Trail
- For flow cytometric chip data files (.xad) you can include:
  - Dot plot summary—all regions shown in an overview.
  - Dot plot statistics—all statistical data of the result table.
  - Histogram summary—all histograms shown in an overview.
  - Histogram statistics—all statistical data of the blue and red histograms' result tables.
- For electrophoretic chip data files (.xad), depending on the assay type you can incl
  - Electropherograms
  - Gel-like image
  - Result tables
  - Standard curve
  - Calibration curve

To print a report:

1 Switch to the **Data** context.

2 In the **Tree View Panel** select the chip data (.xad) file you want to generate a report

3 From the **File** menu select **Print**.
Depending on the file type different dialog boxes appear.

4 You generally have the following possibilities:
- select the items to be included in the report from the **Print Items** section
- select the wells to be included from the **Wells** section
- select the appropriate options
- specify whether you want to generate the report as a file (PDF or HTML)

Changing the Table Height

You can customize the view by changing the height of the table.

To increase or reduce the table height:

1 Position the mouse pointer above the header row of the table and move it upwards 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 tab

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.

TheStatuscolumn 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 Verifications

To ensure a validated Agilent 2100 bioanalyzer system, verification steps have to be performed at installation and operation level.

2100 expert allows for detailed installation verification and system verification on both the bioanalyzer hardware and software. Each verification comprises a series of tests and measurements that you can run and document within the Verification context of the 2100 expert software.

Installation Verification

Installation verification includes tests to verify that the bioanalyzer software and hardware are installed properly and that all electrical and pressure connections are correct.

Installation verification must be performed once after installation.

System Verification

System verification 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.

System verification 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.

Verification Procedure

To perform verification tests:

1. Switch to the Verification context.
2. From the File menu select New.
3. A New Verification item appears in the Tree View Panel.
4. Under Cartridge Details, click Select and specify details on the cartridge that is currentl installed in the bioanalyzer.

5 Under Configure 2100 Bioanalyzer HW Test, enter the test chips you will use for this verification:

6 In the Tree View Panel, navigate to the test category you want to execute. Select the category via Installation/System Verification → Software / Hardware → 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 verification tests to be executed in the verification run:

To select tests, check the Apply check box next to the test(s).

7 To start the selected tests, click the **Start** button in the toolbar.

The **Save As** dialog box appears.

8 Specify a name and location for the verification results file (.xvd) and click **Save**.

The selected tests are executed.

9 If a test fails, you can **R**epeat test execution, **A**bort the verification run, or skip the current test and **C**ontinue with the next test:

10 After all tests have been executed the following message appears:

11 Click **OK**

12TheStatuscolumn shows which of the tests have been run successfully, which ha
failed, and which have mixed results with multiple executions.

13 To view details on test execution, select the Results tab.

14 You can now navigate to other test categories and execute additional verification tests.

15 When you close the verification result 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 verification context and can run further verification tests.

If you select Yes, the verification result file (.xvd) is closed and becomes read-only.

NOTE

You can re-open verification result files only for viewing and printing.

TIP

Select File > Print to generate a printed report of the verification run.

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, HP desktop PC, color printer, system software, vortexer, and accessories kit. Cart and license must be purchased separately.

- G2943CA – Agilent 2100 bioanalyzer laptop system

  Includes Agilent 2100 bioanalyzer, HP laptop PC, color printer, system software, vortexer, and accessories kit. Cart 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.

Software and Services

- G2946CA – Agilent 2100 expert software upgrade

  Software package for upgrade to the latest 2100 bioanalyzer system software revision.

- G2949CA – Agilent 2100 expert security pack

Additional services for Installation Qualification (IQ) and Operation Qualification/Performance Verification (OQ/PV) as well as assay consulting are available and can be ordered separately.

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 cartrid
- 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 - Agilent 2100 Bioanalyzer
- 2110-0007 – Fuse
- 5042-1398 – Adjustable Clip for use as spare for the chip priming station
- 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 gaske
- 5065-4428 – IKA Vortexer (115V)
  Must be ordered at IKA (http://www.ika.de)
- 5065-4429 – IKA Vortexer (230V)
  Must be ordered at IKA (http://www.ika.de)

- 5065-9966 – Vortex Mixer Adapter for IKA vortexer
- 5065-9951 – Electrode Cleaner Box

  Contains 7 electrode cleaners
- 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 for flow cytometry, electrophoresis, and software or hardware of the Agilent 2100 bioanalyzer.

A

Area Threshold

The Area Threshold setpoint determines the minimum amount of peak area that must be detected before a peak is recognized.

Assay

An assay is a solution with defined chip, chemicals, instrument methods, data analysis, data output settings and data display settings.

ASY file

In Bio Sizing electrophoretic assays were stored as .asy files. 2100 expert can import .asy files. See also XSY file.

Audit Trail

Audit trails are available in the 2100 expert software only with the security pack installed. They are used to record the activities of logged-in users and cannot be modified. The audit trails as well as log books are subject to data protection. Only authorized users are allowed to inspect them. They are saved with the data files or into an audit file repository which is automatically archived.

B

Baseline

A baseline is established just after the 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 base

The figure below shows baselines established for DNA assay peaks. Peaks for DN
protein assays are determined on a peak-by-peak basis (the overall baseline is sho

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 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 peak 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 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 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 setup expl orer 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 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 3EZAP. 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 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 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 device 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 t
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 t
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
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
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 t
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
distorted. Peaks that are distorted by the filter have positive and negative peaks on
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 and 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 graphic file format that uses a compression scheme originally developed by CompuServe. Because GIF files are compressed, they 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

Height Threshold

The Height Threshold setpoint determines when a peak is kept. It represents the minimal peak height. For each peak, the difference between the start point value and the

center point value (local baseline) must be greater than the Height Threshold value.

This setting is chosen in the setpoint explorer.

Histogram

Histograms are bar charts to display, for example, a frequency distribution.

HTML file

HTML (Hyper Text Markup Language) is a authoring language used to create documents on the World Wide Web. HTML defines the page structure, fonts, graph 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 bitmapped graphic image. When a JPEG is created, a range of compression qualiti 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 re in the largest file size). Whereas GIF images are limited to 256 colors (8-bit), JPEG image 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, miniaturizing 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 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 proto 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

Method

Methods are available in the 2100 expert software only with the security pack installed. A method is referred to as an electrophoretic or flow cytometric assay with additional information stored to it. This additional information includes instrument information, study information, report settings, and workflow definitions.

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

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)

is the molecular weight of a single base pair

Miniaturized laboratories on a microchip

Expression used to describe lab-on-a-chip technology.

Molecular separation techniques

Processes such as gel electrophoresis, liquid chromatography and capillary electrophoresis that can separate biomolecular organic substances from other compounds.

P

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 Filter Width

The Peak Filter Width setpoint determines the minimum amount of time that must e before a peak is recognized.

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. Dat 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 The syringe is used to force the buffer solution loaded into the well marked “G” with a circle around it into all the passageways in the chip prior to running it in the bioanalyzer.

S

Serial port

The serial ports (COM ports) are used to