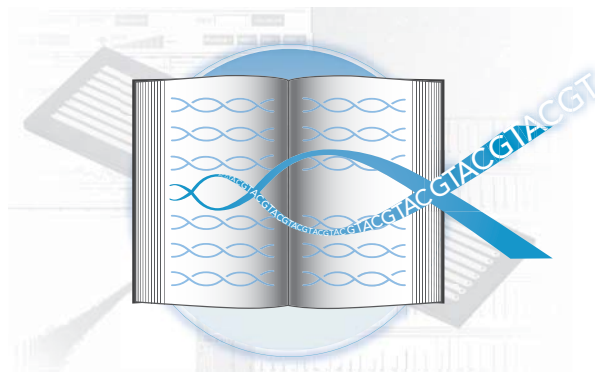


Genome Analyzer Operations Guide

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Revision History

Part #	Revision	Date	Description of Change
15009918	A	November 2009	Updated software to SCS v2.6.
15005900	A	August 2009	Updated software to SCS v2.5 with real time analysis (RTA).

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Chapter 1

Overview

Topics

- 2 Introduction
- 2 Audience and Purpose
- 3 Genome Analyzer Overview
- 4 Genome Analyzer Components
- 7 Paired-End Module Overview
- 8 Safety Considerations and Markings
- 10 Restriction of Hazardous Substances (RoHS)
- 11 User-Supplied Consumables
- 12 Technical Assistance

Introduction

Key features introduced on the Genome Analyzer *IIx* include a new flow cell holder and a large reagent chiller. The new flow cell holder allows you to image of 20% more tiles per run. The large reagent chiller holds sufficient reagents for 100 cycles of sequencing, greatly reducing the hands-on time required to replace and top up reagents when performing long runs.

The Genome Analyzer Sequencing Control Software (SCS) contains a real time analysis feature, which performs on-instrument image analysis and base calling. For information about real time analysis output data, see *Real Time Analysis* on page 63.

In addition to real time analysis, SCS contains an auto calibration feature, which automatically calibrates the system to find the best focus before starting your run. For information about auto calibration, see *Auto Calibration* on page 30.

The *Genome Analyzer Operations Guide* documents SCS v2.6, which has the following improvements:

- ▶ Image analysis changes to detect more clusters at higher density
- ▶ Support for non-standard workflows to rescue runs.
- ▶ Improved support for multiplexed sequencing.
- ▶ New quality tables for samples generated with Cluster Generation Kit v4

SCS v2.6 should be used for samples generated with the Cluster Generation Kit v4. If you want to sequence samples generated with the Cluster Generation Kit v2, use SCS v2.5. For information about the versions to use, see *SCS v2.5 and v2.6* on page 14.

The *Genome Analyzer Operations Guide* contains information about the Illumina Genome Analyzer *II*, Genome Analyzer *IIx*, and the Paired-End Module. It provides descriptions of instrument components and software, and basic procedures performed on the Genome Analyzer at the beginning of a sequencing run.

Use this guide in conjunction with the *Genome Analyzer User Guide*. The *Genome Analyzer User Guide* describes the various protocols, recipes, and reagents, and explains how to perform single-read, paired-end, and multiplexed sequencing runs on the Genome Analyzer.

Audience and Purpose

This guide is for laboratory personnel and other individuals responsible for:

- ▶ Operating the Illumina Genome Analyzer and the Paired-End Module
- ▶ Performing instrument and component maintenance
- ▶ Training personnel on the Genome Analyzer
- ▶ Assessing data quality with a web browser and with RunBrowser

Genome Analyzer Overview

The Genome Analyzer sequences clustered template DNA using a robust four-color sequencing by synthesis (SBS) technology that employs reversible terminators with removable fluorescence. This approach provides a high degree of sequencing accuracy even through homopolymeric regions.

High sensitivity fluorescence detection is achieved using laser excitation and internal reflection optics. Short sequence reads are aligned against a reference genome and genetic differences are called using specially developed data analysis software.

The Genome Analyzer instrument computer performs real time data analysis, which saves downstream analysis time and allows you to monitor important quality metrics during the sequencing run.

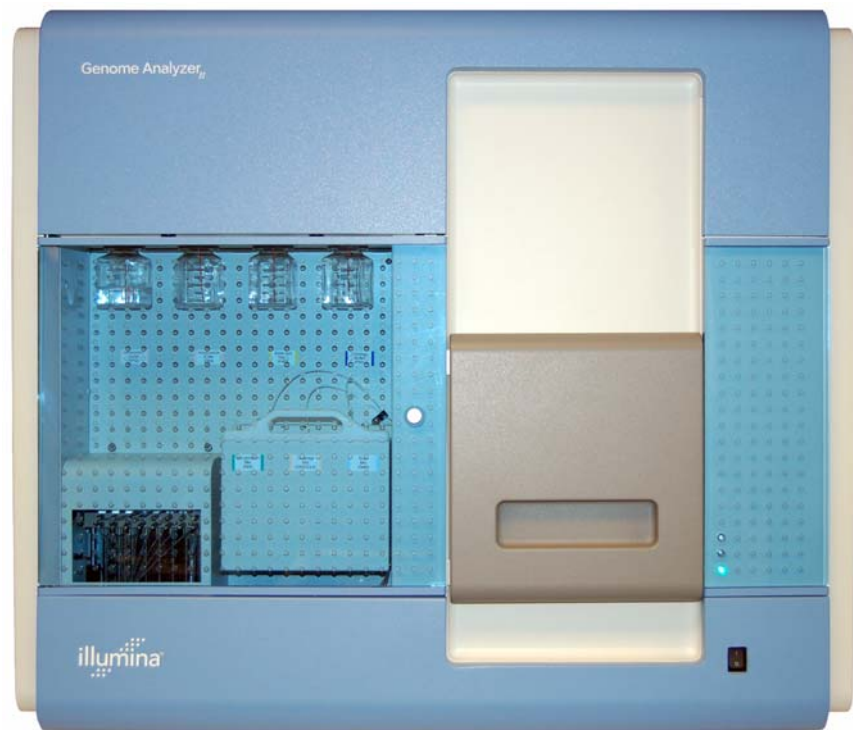


Figure 1 Genome Analyzer (shown with large reagent chiller)

Genome Analyzer Components

All operator activity on the instrument occurs in two main compartments:

- ▶ Reagent Compartment
- ▶ Imaging Compartment

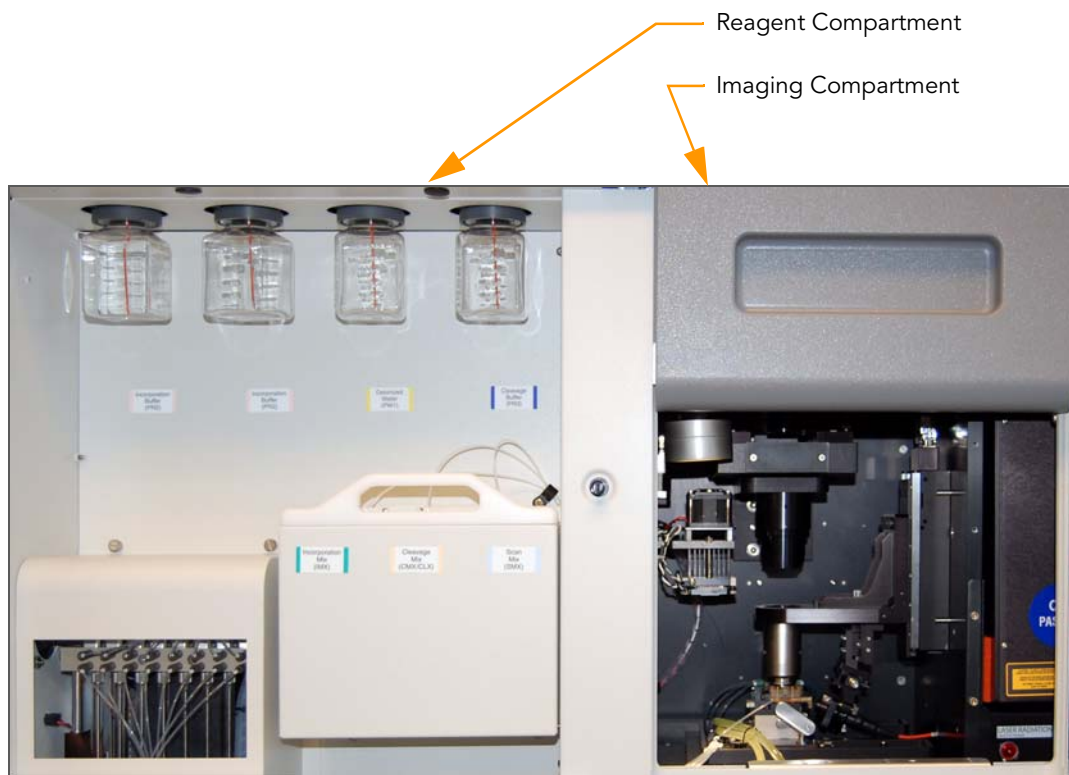


Figure 2 Genome Analyzer Main Compartments

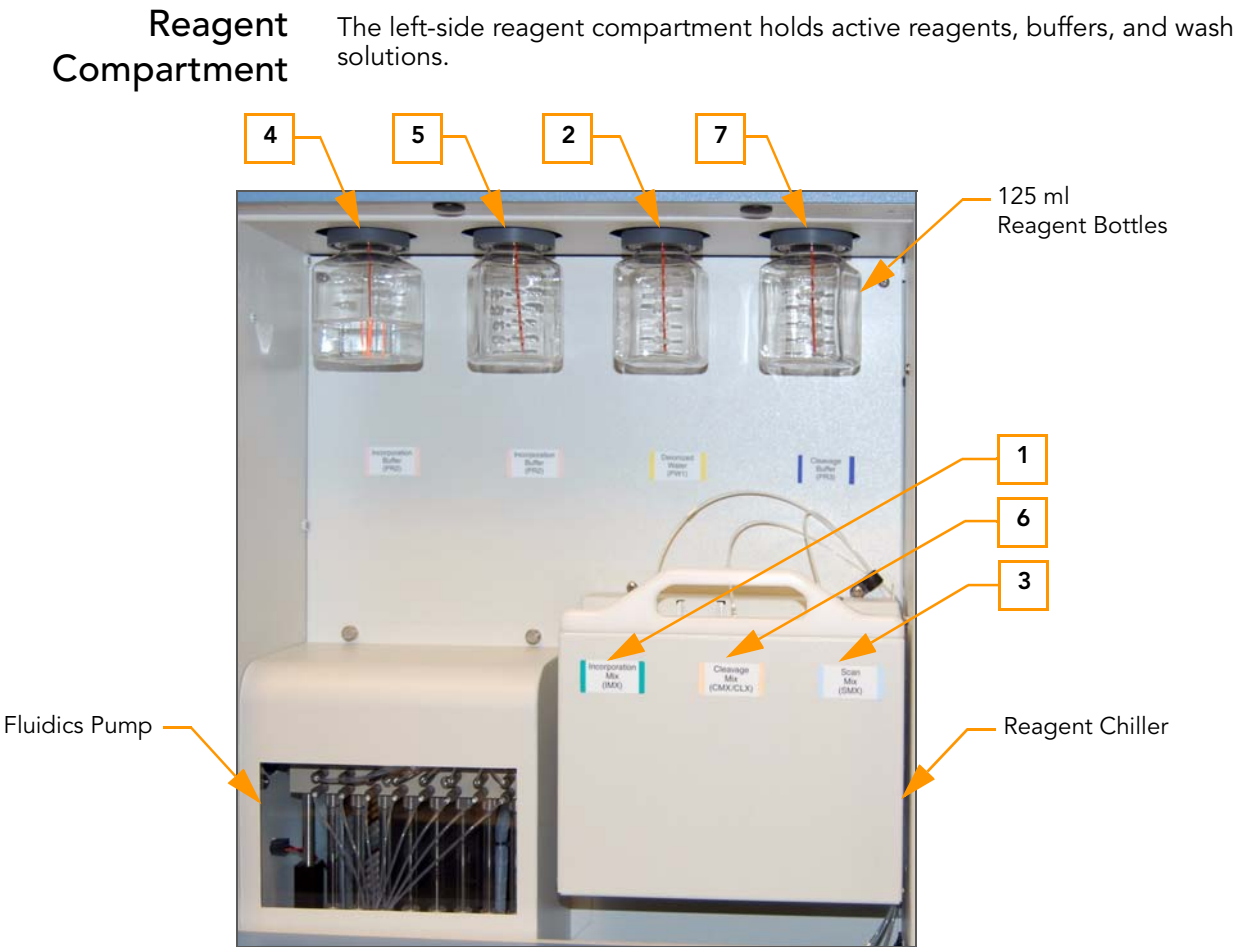


Figure 3 Genome Analyzer Reagent Compartment

The 125 ml reagent bottles (positions 2, 4, 5, and 7) are connected to threaded bottle receptacles on the instrument. Each reagent position is numbered according to the position it connects to on the fluidics valve.

The Genome Analyzer *IIx* has a large reagent chiller, which holds three 175 ml reagent bottles (positions 1, 3, and 6). This volume is sufficient for up to 100 cycles of sequencing. The reagent chiller is not intended to cool down reagents, but rather to maintain reagent temperature at 4°C. Reagents must be cooled before being placed into the reagent chiller.

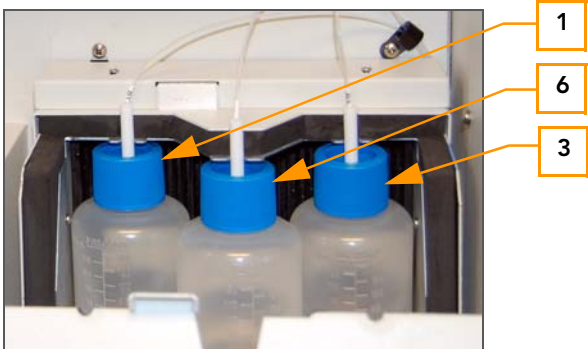


Figure 4 Large Reagent Chiller

The fluidics pump directs waste to the Genome Analyzer waste bottle.

Imaging Compartment

The right-side compartment houses the Imaging Station, Thermal Station, Flow Cell Stage, Fiber Optics Mount, and Plumbing Manifolds.

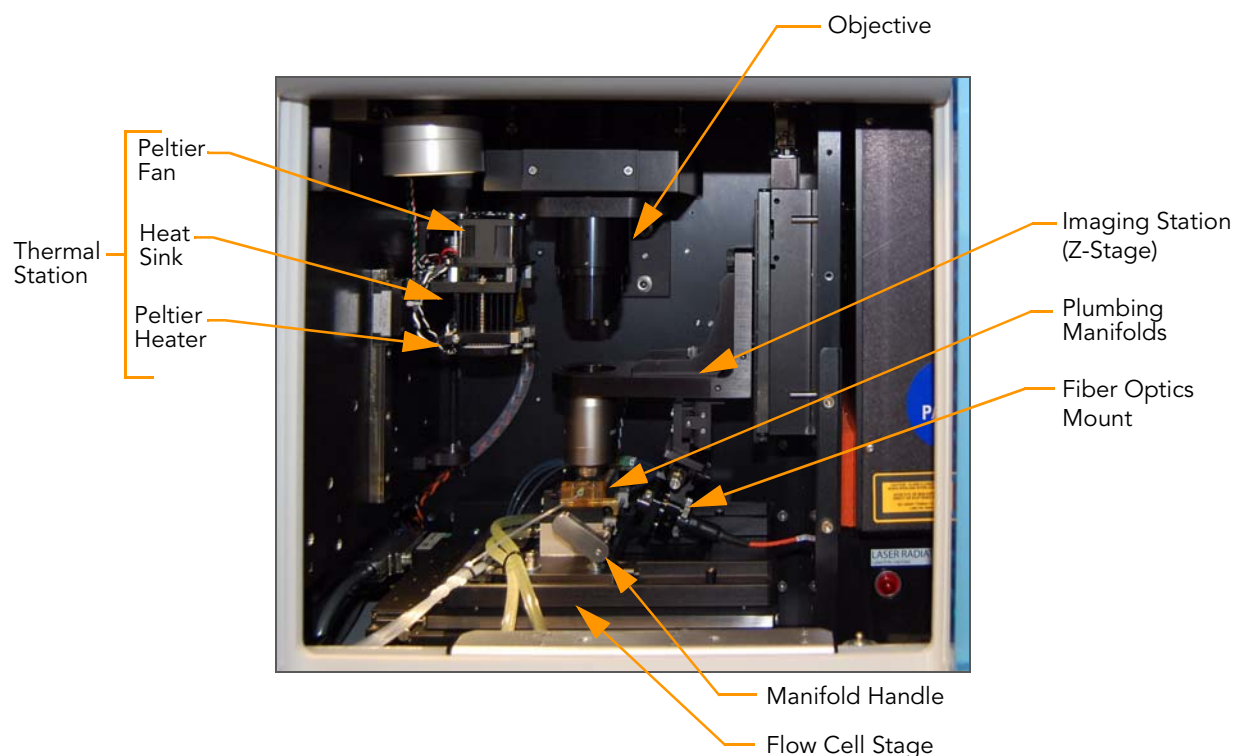


Figure 5 Genome Analyzer Imaging Compartment

The flow cell stage moves along the X (left-right) and Y (front-back) axes. The thermal station and the imaging station (Z-Stage) move along the Z axis.

The flow cell is clamped under front and rear plumbing manifolds onto a stage that moves between the thermal and imaging stations. The manifolds on the Genome Analyzer IIx allow imaging of 120 tiles in each of the eight lanes of the flow cell, increased from 100 tiles on the Genome Analyzer II.

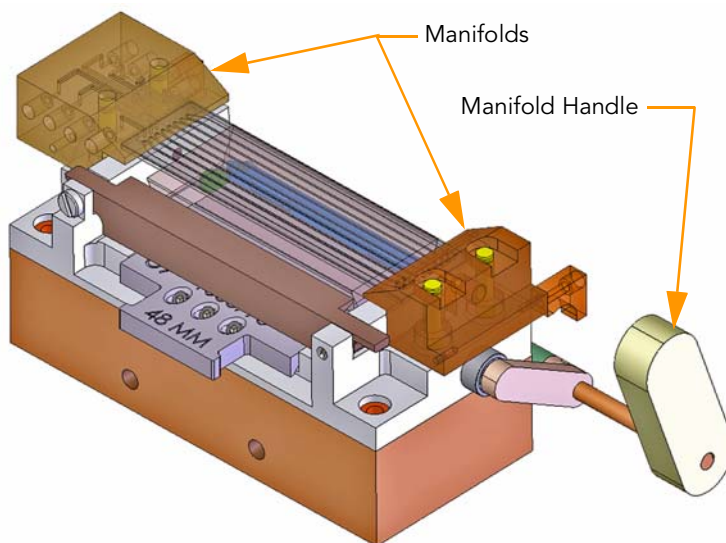


Figure 6 Front and Rear Plumbing Manifolds

Paired-End Module Overview

The Paired-End Module is an auxiliary instrument connected to the Genome Analyzer via an external VICI valve. It directs the regeneration and amplification operations to prepare the templates for the second round of sequencing. The Paired-End Module is also used for preparation of the Index Read in multiplexed sequencing protocols.

The Paired-End Module is fitted and installed to port position 8 of the Genome Analyzer internal VICI valve prior to starting the first read. You must configure the Paired-End Module to run with the Genome Analyzer in the Run Parameters dialog box. For more information, see *Run Parameters Window* on page 16.

Be sure to check which version of the Paired-End Module you are using: the Paired-End Module classic, or the Paired-End Module *IIX*. The Paired-End Module *IIX* contains a larger priming pump, which aspirates five times the volume of the pump on the standard Paired-End Module. To account for this difference, specific sequencing recipes that perform fewer priming strokes are required for use with the Paired-End Module *IIX*.

The following figure illustrates the reagent positions on the Paired-End Module and the number associated with each position. The positions are the same on both versions.

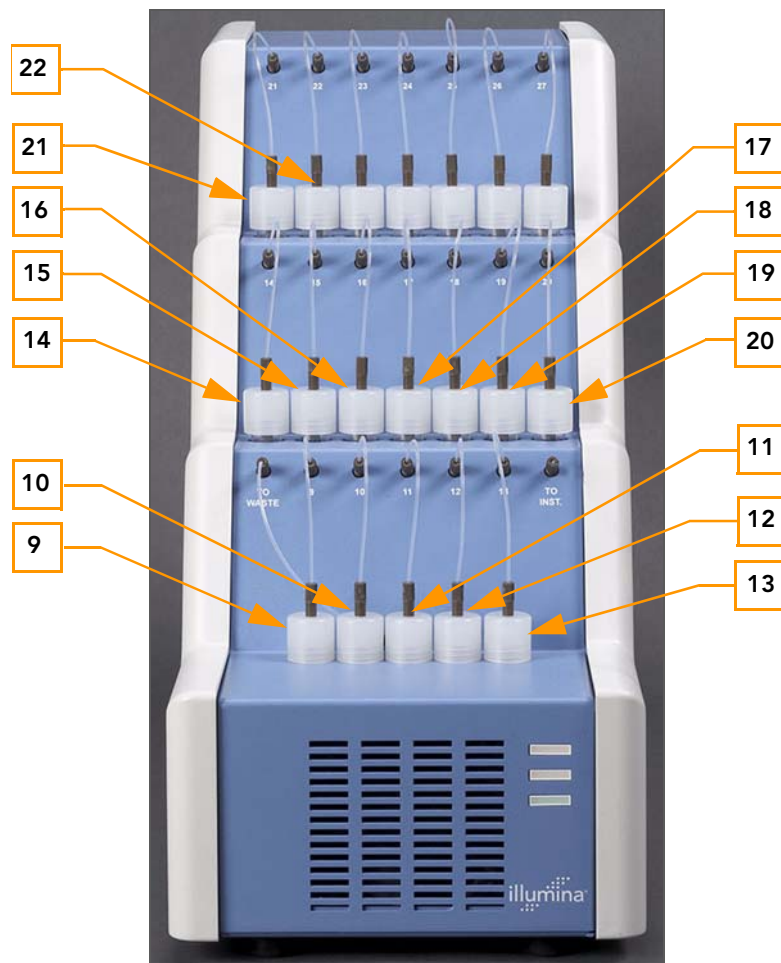


Figure 7 Reagent Positions on the Paired-End Module

Safety Considerations and Markings

This section outlines potential hazards associated with operating the Illumina Genome Analyzer and Paired-End Module. Hazards are indicated by labels on the instrument, which are pictured in this section.

The intent of this section is to clearly identify these hazards so that users do not operate or interact with the instrument in a manner that exposes them to any of these dangers.

All of the hazards described below can be avoided by following the standard operating procedures for the system.

Genome Analyzer Laser Radiation



The Genome Analyzer is a Class 1 Laser Product that contains Class IV lasers. Class IV lasers present an eye hazard from direct and diffuse reflections. Avoid eye or skin exposure to direct or reflected Class IV laser radiation. Class IV lasers can cause combustion of flammable materials and produce serious skin burns and injury from direct exposure.

Do not operate the Genome Analyzer with any of the panels removed. When the front door of the instrument is open, the laser beam is blocked via safety interlock switches. If you operate the instrument with any panels removed, you risk exposure to direct or reflected laser light.



CAUTION

Laser testing and service maintenance must only be performed by authorized and trained Illumina personnel. Only trained Illumina personnel are authorized to remove panels from the Genome Analyzer.

Electrical Shock



Do not operate the Genome Analyzer with any of the panels removed. If you operate the instrument in this manner, you may be exposed to line voltage as well as several DC voltages.

The Genome Analyzer is powered by 100–240 volts AC operating at either 50 or 60 Hz. Most of the voltage sources are located behind the left back panel, the right back panel, and the left side panel, but they may also be accessible if other panels are removed. Some voltage is present on the instrument even when the instrument is powered down. Operate the instrument with all panels intact to avoid electrical shock.

General Warning



Follow all operating instructions when working in areas marked with this label.

Always exercise caution when the front door is open. The flow cell area contains components that could cause injury when the front door is open.

Interlocks disable the X and Y motors if the front door is opened. However, the Z motor is not disabled.

Burn



Do not operate the Genome Analyzer with any of the panels removed. Do not touch the temperature station in the imaging compartment. The peltier-effect heater used in the stage area is normally controlled between ambient room temperature (22°C) and 65°C. Exposure to temperatures at the upper end of this range could result in burns. If the peltier-effect heater fails, temperature control could be lost. If this were to happen, then the peltier-effect heater could reach significantly higher temperatures that would pose a serious burn hazard.

Restriction of Hazardous Substances (RoHS)



Return the instrument to Illumina for disposal.

User-Supplied Consumables

Check to ensure that you have all of the following user-supplied consumables before you begin a sequencing run.

Table 1 *User-Supplied Consumables*

Consumable	Supplier	Use
Ethanol, 100%		Used to clean the prism and flow cell.
SuperQ water or laboratory grade water		Used for washing the Genome Analyzer.
1 M NaOH		Used for washing the Genome Analyzer.
Falcon conical tubes, 15 ml		Used for collecting and measuring waste volumes.
Falcon conical bottles, 175 ml	BD, catalog # 352076	Used for sequencing runs, and maintenance and service washes.

Technical Assistance

For technical assistance, contact Illumina Customer Support.

Table 2 *Illumina Customer Support Contacts*

Contact	Number
Toll-free Customer Hotline	1-800-809-ILMN (1-800-809-4566)
International Customer Hotline	1-858-202-ILMN (1-858-202-4566)
Illumina Website	http://www.illumina.com
Email	techsupport@illumina.com

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at <http://www.illumina.com/msds>.

Product Documentation

If you require additional product documentation, you can obtain PDFs from the Illumina website. Go to <http://www.illumina.com/documentation>. When you click on a link, you will be asked to log in to iCom. After you log in, you can view or save the PDF.

If you do not already have an iCom account, then click New User on the iCom login screen and fill in your contact information. Indicate whether you wish to receive the iCommunity newsletter (a quarterly newsletter with articles about, by, and for the Illumina Community), illumiNOTES (a monthly newsletter that provides important product updates), and announcements about upcoming user meetings. After you submit your registration information, an Illumina representative will create your account and email login instructions to you.

Frequently Asked Questions

Frequently asked questions are available online.

Go to <http://www.illumina.com/FAQs>, and select Systems and Genome Analyzer.



Chapter 2

Genome Analyzer Software

Topics

- 14 Introduction
- 16 Run Parameters Window
- 20 Data Collection Software Interface
- 29 Image Controls
- 30 Auto Calibration
- 35 Run Folders

Introduction

This section describes the main windows, features, and controls of the Genome Analyzer Sequencing Control Software (SCS) v2.6.

Some recent additions to SCS are auto calibration and real time analysis.

- ▶ **Auto calibration**—Automatically calibrates the system for best focus and finds the flow cell edge. For more information, see *Auto Calibration* on page 30.
- ▶ **Real time analysis**—Performs on-instrument image analysis and base calling. For more information, Chapter 5, *Real Time Analysis*.

In addition to auto calibration and real time analysis, this version of the software provides support for multiplexed sequencing. A column in the Recipe Viewer indicates which recipes are multiplexing-specific recipes. For more information, see *Recipe Viewer* on page 23. For more information about multiplexed sequencing, see the *Genome Analyzer User Guide*.

SCS v2.5 and v2.6

The Genome Analyzer Operations Guide documents SCS v2.6, which should be used for samples generated with the Cluster Generation Kit v4. If you want to sequence samples generated with the Cluster Generation Kit v2, use SCS v2.5.

The installer for SCS v2.6 does not overwrite the SCS v2.5 installation, so you can still use SCS v2.5 for Cluster Generation Kit v2. SCS v2.6 leaves only one SCS shortcut on your desktop, named **Genome Analyzer Data Collection Software SCS2.6**. Clicking this shortcut will give you the option to use either SCS v2.6 or SCS v2.5 (if installed). Follow the instructions in *Starting the Genome Analyzer* on page 49.

The user interfaces of SCS v2.5 and SCS v2.6 are very similar. The *Genome Analyzer Operations Guide* can be used to run either version, with significant differences clearly indicated throughout this guide.

Dual installation of SCS v2.5 and SCS v2.6 has the following features:

- ▶ There is **one** desktop icon for both SCS2.5/RTA1.5 and SCS2.6/RTA1.6. When you launch SCS from the single desktop icon, a dialog box appears that allows you to select the SCS version you want to run:
 - Selecting V2 at start up launches SCS2.5/RTA1.5, which supports V2/1.4 mm.
 - Selecting V4 at start up launches SCS2.6/RTA1.6, which supports V4/1.6 mm.

If SCS v2.5 is not previously installed, this dialog will not appear and the desktop icon launches SCS v2.6.

- ▶ The About SCS dialog box lists the last installed version of SCS, RTA, recipes, and event scripts.
- ▶ Do not install SCS v2.5 after installing SCS v2.6. In order for both versions to coexist, SCS v2.5 must be installed prior to installing SCS v2.6.
- ▶ The RTA 1.5 folder for SCS v2.5 is stored in C:\Illumina\RTA, while the RTA 1.6 folder for SCS v2.6 is stored in C:\Illumina\RTA\SCS2.6\RTA. They are not interchangeable.

**NOTE**

SCS 2.6 is not compatible with V2 chemistry and 1.4 mm flow cells. It uses the 1.6 mm values and V4 Quality Tables. If you need to perform a run with the 1.4 mm flow cells or V2 chemistry, select V2 at start up to use SCS v2.5, or contact Illumina for the workaround. Please be aware that SCS software will force the V4/1.6mm settings, overwriting any manual editing you may have done on the xml configuration files.

Run Parameters Window

The Run Parameters window is the first window that opens when you launch SCS. This allows you to select several run settings prior to the start of the Data Collection software, reducing the need to directly edit the configuration files.

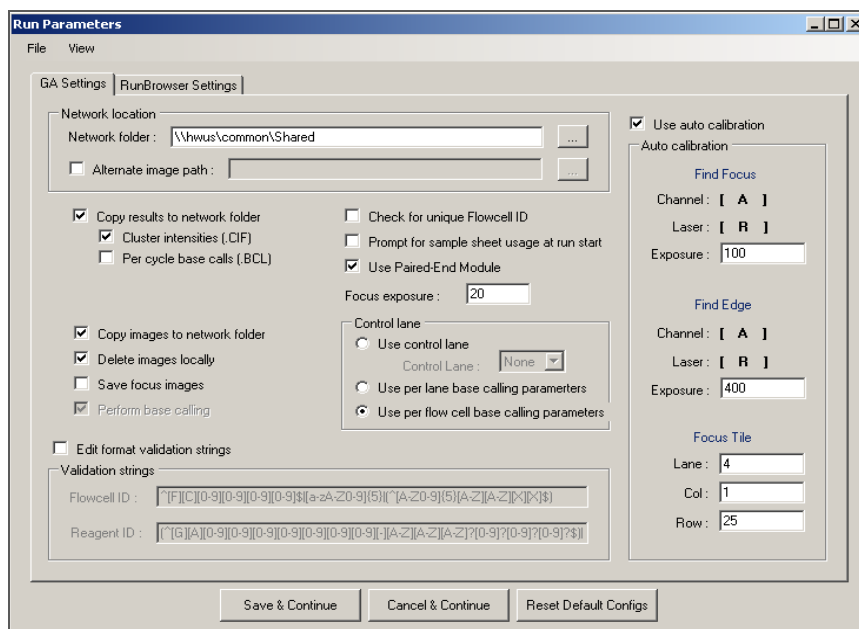


Figure 8 Run Parameters Window for SCS v2.6

By default, the Run Parameters window appears each time you launch the software. To change this setting, click **View** and clear the checkbox next to **Show On Start Up**. The next time you launch the software, the Run Parameters window will not appear and the software proceeds directly to the Data Collection screen.

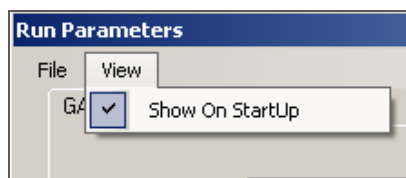


Figure 9 Show Run Parameters on Start Up

From the Data Collection screen, you can access the Run Parameters window by clicking **Edit | Run Parameters** in the toolbar.

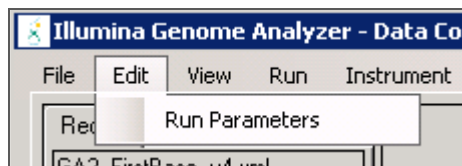


Figure 10 View Run Parameters Window

There are two tabs in the Run Parameters window, GA Settings and RunBrowser Settings.

GA Settings

In the GA Settings tab, you can specify the network location for run results, preferences for real time analysis results, and settings for auto calibration.

- ▶ **Network folder**—Enter or browse to the location of your network folder. Illumina recommends that you specify the full network path.

The figure shows a 'Network location' dialog box. It contains a text field labeled 'Network folder' with the value '\\hwus\common\Shared'. To the right of the text field is a browse button (three dots). Below the text field is a checkbox labeled 'Alternate image path' which is currently unchecked, followed by another empty text field and a browse button.

Figure 11 Network Folder Field

- ▶ **Alternate image path**—This option allows you to store images in an alternate location. Select the checkbox and enter or browse to an alternate storage location.
- ▶ **Copy results**—Select this option to copy real time analysis results to the network folder that you specified in the network folder field. With SCS v2.6, you have the option to copy cluster intensities files (.CIF) and per cycle base calls (.bcl). By default, per tile base calls (.QSEQ) are copied to the run folder.
If you deselect this option, only per tile base calls (.QSEQ) are saved to the run folder. For space considerations, cluster intensities files (.CIF) and per cycle base calls (.bcl) are not saved. For more information, see Chapter 5, *Real Time Analysis*.
- ▶ **Copy images**—Select this option to copy images to the network folder that you specified in the network folder field.
- ▶ **Delete images locally**—Select this option to delete images from the Genome Analyzer computer after analysis. Illumina recommends that you enable this feature for run cycles greater than 30.
- ▶ **Save focus images**—Select this option to save focus images. Focus images may be used for troubleshooting.
- ▶ **Perform base calling**—This option is disabled in SCS v2.6; base calling is enabled by default.
- ▶ **Check for unique flow cell ID**—If selected, the software verifies that the flow cell ID is unique, and will not allow the same flow cell ID to be used for a different run on the same instrument.

The figure shows the GA Settings tab for SCS v2.6. It contains several checkboxes and options:

- ☒ Copy results to network folder
 - ☒ Cluster intensities (.CIF)
 - ☐ Per cycle base calls (.BCL)
- ☐ Check for unique Flowcell ID
- ☐ Prompt for sample sheet usage at run start
- ☒ Use Paired-End Module
- Focus exposure : 20
- ☒ Copy images to network folder
- ☒ Delete images locally
- ☐ Save focus images
- ☒ Perform base calling
- Control lane
 - ☐ Use control lane
 - Control Lane : None
 - ☒ Use per lane base calling parameters
 - ☐ Use per flow cell base calling parameters

Figure 12 Selections on GA Settings Tab for SCS v2.6

- ▶ **Prompt for sample sheet usage at run start**—Select this option if you are using a sample sheet.
- ▶ **Use Paired-End Module**—Select this option if you are performing a sequencing run that uses the Paired-End Module. The Paired-End Module must be powered on to use this option.
- ▶ **Focus exposure**—The exposure used when taking focus images.
- ▶ **Control Lane**—Indicate which lane on the flow cell is the control lane. The options are none, or a lane number between 1 and 8. A control lane is essential for sample libraries with biased nucleotide content, such as methylation bisulfite sequencing or miRNA samples. If a lane is specified, that lane is used for estimating base calling parameters for all lanes. In SCS v2.6, you can also choose to use per lane or per flow cell base calling parameters; if you are not using a control lane, use per lane base calling parameters.
- ▶ **Cluster Generation Reagents** (for SCS v2.5)—Indicate which version of cluster generation reagents you used during cluster generation and you plan to use for the Read 2 preparation step. You must use the same version for Read 2 prep that was used for cluster generation. If you are using Cluster Generation Kit v4, Illumina recommends using SCS v2.6.
- ▶ **Validation Strings**—These fields show the validation expression for Illumina flow cell and reagent ID. This option allows you to edit the expression if you are using a customized identification system.
- ▶ **Use auto calibration**—Select this option to enable the auto calibration feature (Figure 13). For more information, see *Auto Calibration* on page 30. If this option is cleared, focus calibration must be performed manually as described in Appendix C, *Manual Controls and Calibration*.

☒ Use auto calibration

Auto calibration

Find Focus

Channel : [A]

Laser : [R]

Exposure : 100

Find Edge

Channel : [A]

Laser : [R]

Exposure : 400

Focus Tile

Lane : 4

Col : 1

Row : 25

Figure 13 Auto Calibration Settings

RunBrowser Settings

RunBrowser provides metrics reports that you can view, print, save, or export. RunBrowser data files (BRO files) are automatically generated for each cycle. For more information, see *RunBrowser Reports* on page 79.

In the RunBrowser Settings tab, you can select reporting and email options (Figure 14).

- ▶ **RunBrowser Reports**—Specify which RunBrowser reports you want to show and export. If selected, the first-cycle report is generated by the software.
- ▶ **Enable RunBrowser report emails**—If selected, sends reports to the email addresses specified. You must have an internet connection. Check with your IT department to ensure your email is properly configured.
- ▶ To use RunBrowser for cycles other than first base, select the **Generate RunBrowser data files for each cycle (BRO files)** checkbox.

RunBrowser Reports	Show Report	Export Report	Show RunBrowser
First Cycle Report	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
QualityMetricDeviation	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Metric Reports(Cluster Intensit...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Metric Reports(Cluster Intensit...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Metric Reports(Cluster Intensit...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Metric Reports(Cluster Intensit...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Figure 14 Run Parameters Window, RunBrowser Settings Tab



NOTE

To use Run Browser for cycles other than First Base, the Generate RunBrowser data Files for Each cycle (BRO files) must be enabled

Continue to Data Collection

After you have finished selecting the settings in the Run Parameters window, click one of the following:

- ▶ **Save & Continue**—Saves the parameters you specified and launches the Data Collection software.
- ▶ **Cancel & Continue**—Cancels any recent changes you made in the Edit Run Parameters window and launches the Data Collection software.
- ▶ **Reset Default Configs**—Resets the run parameters to the installed configuration. If you click Reset Default Configs, click **Save & Continue** to launch the Data Collection software.

Data Collection Software Interface

Before you can use the Genome Analyzer Data Collection software, you must first initialize the software.

Initialize the Software

1. Click the Manual Control/Setup tab.

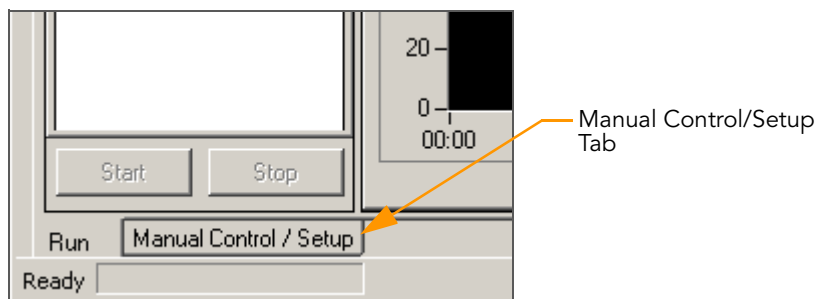


Figure 15 Manual Control/Setup Tab

2. Perform any operation (e.g., click **Take a Picture**) to trigger the Genome Analyzer initialization. A status bar at the bottom-left of the screen shows the progress of the initialization routine.

The software ignores inputs until the initialization routine is complete.

Run Window

One of the main windows in the Data Collection software interface is the Run window (Figure 16). The Run window contains the Recipe tab, the Image Cycle tab, and the Temperature tab.

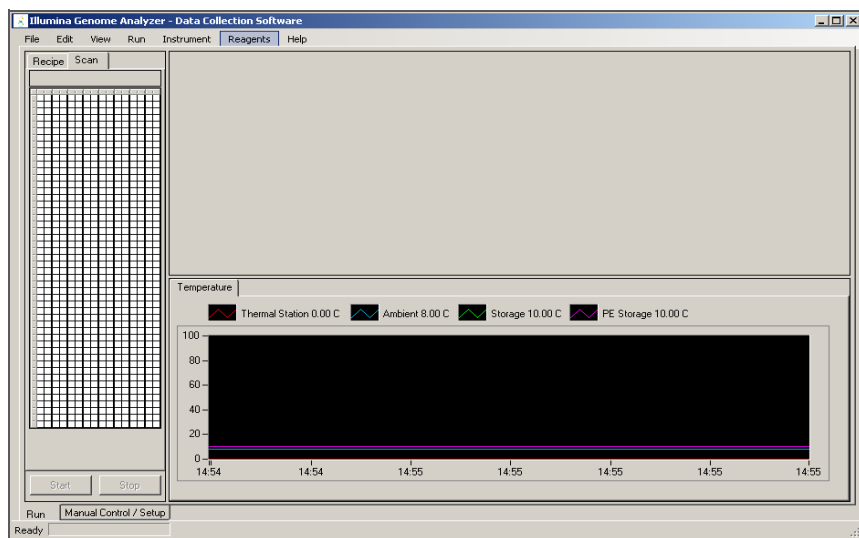


Figure 16 Data Collection Software, Run Tab

Recipe Tab

The Recipe tab shows you the progression of each step in a recipe. As you hover over a step, a tool tip appears showing the parameters specific to that step, such as temperature, duration, dispense rates, and volumes.

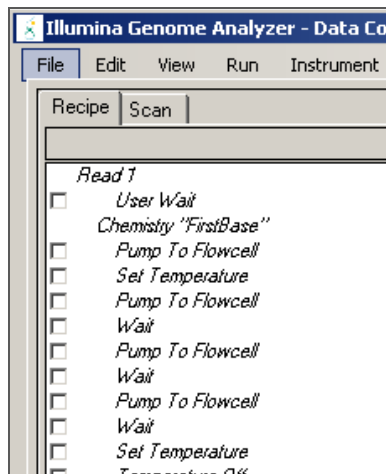


Figure 17 Recipe Tab

Scan Tab

The Scan tab shows you the progress of imaging steps. The column on the left side shows the entire flow cell, using color codes for each tile:

- ▶ White tiles are queued for imaging.
- ▶ Blue tiles have been imaged.
- ▶ Gray tiles are not defined in the current run.

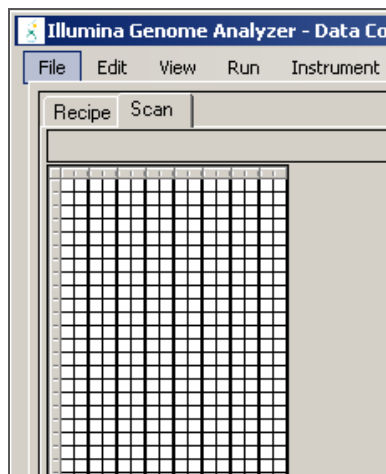


Figure 18 Scan Tab

The right side of the window shows the photographs being taken of the current tile.



The Genome Analyzer Data Collection software window has to be maximized to display all of the tiles correctly.

Temperature Tab

On the lower-right side of the Run window is the Temperature tab. The Temperature tab allows you monitor various run temperatures.

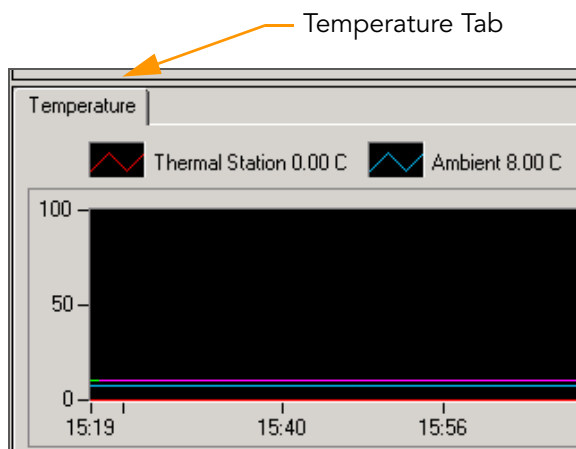


Figure 19 Temperature Tab and Analysis Viewer Tab

Manual Control/ Setup Window

Another main window is the Manual Control/Setup window. You can toggle between the Run window and the Manual Control/Setup window using the tabs in the lower-left corner of Data Collection software interface.

The Manual Control/Setup window contains commands to load the flow cell, move the camera to a specific tile on the flow cell, and take a picture. It also contains manual settings for pump controls and priming of reagents.

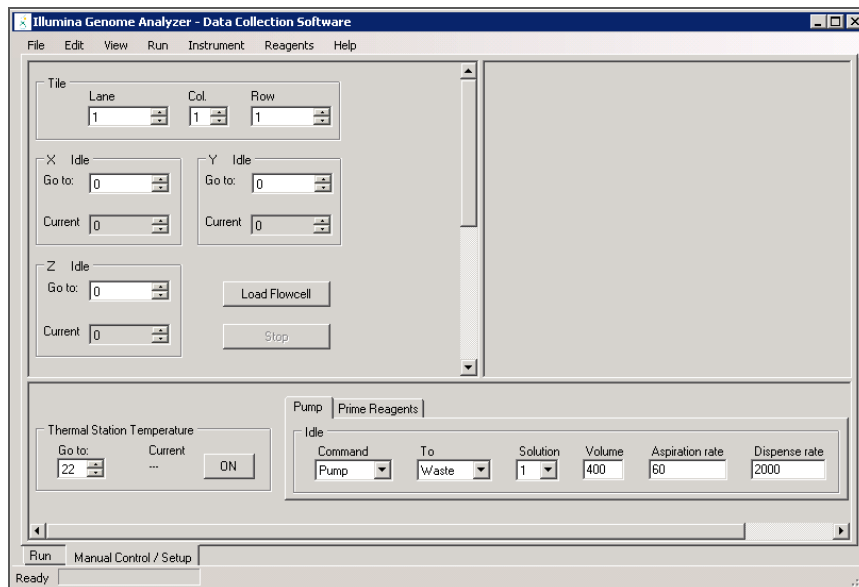


Figure 20 Data Collection Software, Manual Control/Setup Tab

Manual Controls

The Manual Control/Setup tab contains several settings used for manual focus calibration. If auto calibration is enabled in Run Parameters window, you do not need to focus manually. For more information about manual focus and manual priming, see Appendix C, *Manual Controls and Calibration*.

Pump Tab

Some setup and maintenance protocols require you to set the pump control parameters. These parameters are set in the Pump tab in the Manual Setup/Controls window.

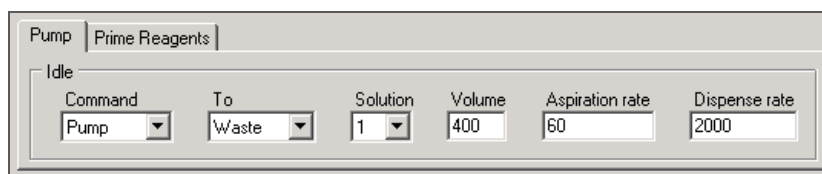


Figure 21 Pump Tab

- ▶ **Command**—Transfers liquid from the port indicated in the Solution field to the location indicated in the To field. This command is used in many procedures, such as washes.
- ▶ **To**—Directs the liquid through the flow cell or to waste.
- ▶ **Solution**—The port from which the instrument draws liquid.
When configured for the Paired-End Module, the solution range is 1–28; otherwise, it is 1–8.
Solution 8 (in single-read recipes) and 28 (in paired-end recipes) is currently air, rather than a liquid. Select this position when you remove flow cells from the system, so that fluid does not siphon down the lines.
- ▶ **Volume**—The volume transferred in a single pump event, in microliters.
- ▶ **Aspiration Rate**—The rate at which liquid is removed from the source, in microliters/minute.
- ▶ **Dispense Rate**—The rate at which liquid is placed into the target location, in microliters/minute.

Recipe Viewer

The Recipe Viewer displays Genome Analyzer recipes found in the default recipe location, <install directory>\DataCollection_v<#>\bin\Recipes.

To access the Recipe Viewer, click **File | Open Recipe** from the toolbar at the top of the Data Collection software screen.

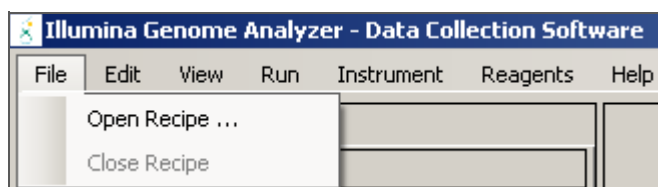


Figure 22 Recipe Menu

The following information is listed for each recipe (Figure 23):

Recipe	Sub Folder	Cycles	Read End	Multiplexing	Service	Version	Comment	File Status
GA2_101Cycle_SR_v7.xml	SingleRead	101	Single Read	No	No	7	GA2_101Cycl...	Original
GA2_FirstBase_v7.xml	SingleRead	1	NA	No	Yes	7	GA2_FirstBas...	Original
GA2_PostWash_v7.xml	SingleRead	0	NA	No	Yes	7	GA2_PostWa...	Original
GA2_PreWash_v7.xml	SingleRead	0	NA	No	Yes	7	GA2_PreWas...	Original
GA2_Prime_v7.xml	SingleRead	0	NA	No	Yes	7	GA2_Prime_v...	Original
GA2-PEM_MaintenanceWash_v7.xml	ServiceAndM...	0	NA	No	Yes	7	GA2-PEM_M...	Original
GA2_MaintenanceWash_v7.xml	ServiceAndM...	0	NA	No	Yes	7	GA2_Mainten...	Original
PEM_PrimeAllPorts_v7.xml	ServiceAndM...	0	NA	No	Yes	7	PEM_PrimeAL...	Original
GA2-PEM_2x101Cycle_v7.xml	PairedEnd	202	Single Folder ...	No	No	7	GA2-PEM_2x...	Original
GA2-PEM_PostWash_v7.xml	PairedEnd	0	NA	No	Yes	7	GA2-PEM_P...	Original
GA2-PEM_PreWash_v7.xml	PairedEnd	0	NA	No	Yes	7	GA2-PEM_Pt...	Original
GA2_Prime_v7.xml	PairedEnd	0	NA	No	Yes	7	GA2_Prime_v...	Original
GA2-PEM_MP_101+7+101Cycle_v7.xml	Multiplexing	202	Single Folder ...	Yes	No	7.1	GA2-PEM_M...	Original
GA2-PEM_MP_101+7Cycle_v7.xml	Multiplexing	101	Single Read	Yes	No	7.1	GA2-PEM_M...	Original
GA2-PEM_MP_101+7Cycle_SR_v7.xml	Multiplexing	0	NA	No	Yes	7	GA2-PEM_M...	Original
GA2-PEM_MP_PostWash_v7.xml	Multiplexing	0	NA	No	Yes	7	GA2-PEM_M...	Original
GA2-PEM_MP_PreWash_v7.xml	Multiplexing	0	NA	No	Yes	7	GA2-PEM_M...	Original

Figure 23 Recipe Viewer

- ▶ **Recipe**—Lists the recipe name.
- ▶ **Subfolder**—Lists the subfolder in the default recipe location.
- ▶ **Cycles**—Lists the number of cycles the recipe performs.
- ▶ **Read End**—Describes the type of sequencing read the recipe performs: single-read or paired-end.
- ▶ **Multiplexing**—Describes whether this recipe has an index read. If so, this recipe is used for multiplexed sequencing runs.
- ▶ **Service**—Describes whether this recipe is used for service and maintenance. Service and maintenance recipes cannot be edited with the Recipe Viewer.
- ▶ **Version**—Lists the version of the recipe. Recipe versions identify whether a recipe is compatible with a given version of the software or version of sequencing reagents.
- ▶ **Comment**—Contains notes specific to that recipe. Hover your mouse over the comment field to see the contents.
- ▶ **File Status**—There are three possible values for File Status:
 - **Original**—This is the default recipe as preloaded on the Genome Analyzer workstation.
 - **User Edited**—This status indicates that the recipe was edited but not renamed.
 - **User Created**—This status indicates that the recipe was renamed prior to being edited.
 - **Invalid**—This status indicates that the recipe does not conform to the recipe format or that it is corrupt. A recipe with Invalid status cannot be run on the Genome Analyzer.

Editing Cycle Number

To increase or decrease the number of cycles in an installed recipe and to change the name of a recipe you plan to edit, select **Edit | Attributes** from the Recipe Viewer toolbar. The Edit Cycle Attributes dialog box appears.

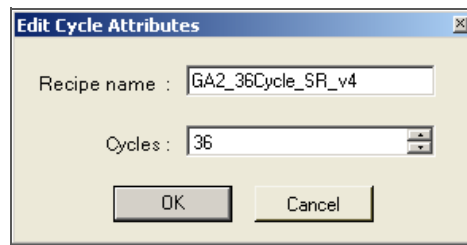


Figure 24 Edit Cycle Attributes

Illumina strongly recommends that you rename an edited recipe before saving it. If you change the number of cycles in a recipe without renaming the recipe, the following warning appears:

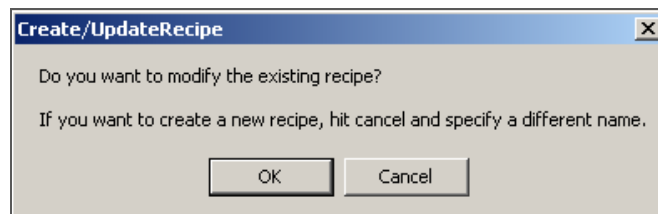


Figure 25 Create/Update Recipe Warning



NOTE

If you increase or decrease the number of cycles in a paired-end recipe (e.g., GA2-PEM_2x76Cycle_v4), you must increase or decrease the number by two, one for each read.

Reagent Tracking

The Genome Analyzer software provides email notifications and warnings when reagent volumes are getting low and need replacing. This feature is especially beneficial when you are performing runs longer than 36 cycles and your Genome Analyzer is not equipped with a large reagent chiller.

To access reagent tracking features, click **Reagents** from the toolbar at the top of the Data Collection software screen. You may access the features described in this section at any time during a sequencing run.

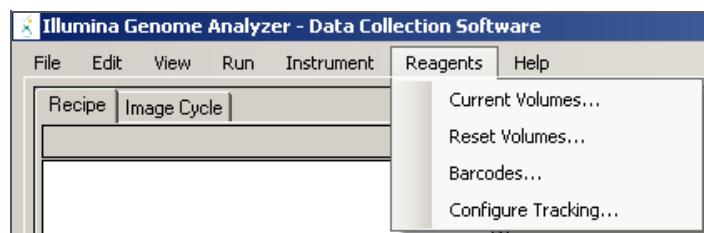


Figure 26 Reagents Menu

You need to configure the system for email notifications. For more information, see *Configure Tracking* on page 28.

Current Volumes

The Current Volumes dialog box shows the number of remaining cycles before reagent volumes are depleted and the number of cycles completed since the last time the reagents were replaced or topped up. You can also open the Reset Volumes and Barcodes dialog boxes from the Current Volumes dialog box.

The Current Volumes dialog box appears automatically in any of the following conditions:

- ▶ When a new run is started. At this time, set the number of cycles that the fresh reagents are expected to last and enter the reagent barcodes in the Barcodes dialog box.
- ▶ When the number of cycles elapsed is equal to the number of cycles reagents are expected to last (set in Reset Volumes), minus the number of remaining cycles set before warning (set in Configure Tracking).

For example, if reagents are expected to last for 12 cycles, and the warning is set for 2 cycles in advance, then the dialog box appears after 10 cycles.

- ▶ When reagents are empty.

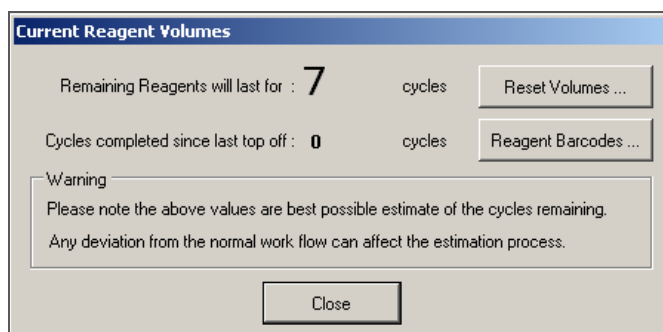


Figure 27 Current Reagent Volumes

Reset Volumes

When you load a fresh set of reagents or top up reagents during a run, use the Reset Volumes dialog box to enter the number of cycles the reagents are expected to last. This value is based on the number of cycles you plan to run. For more information, see the *Genome Analyzer User Guide*.

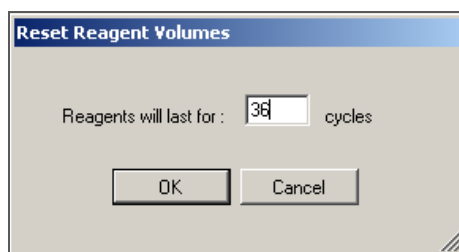
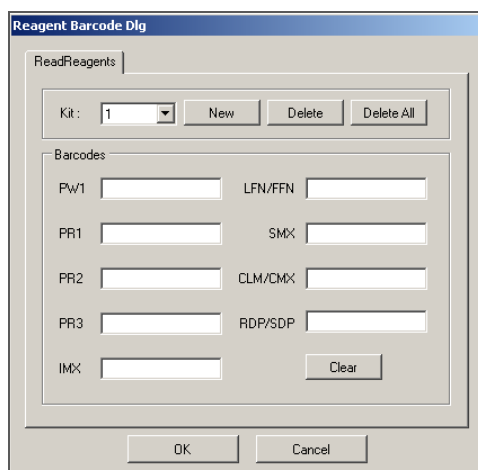


Figure 28 Reset Reagent Volumes

Reagent Barcodes

When you load reagents, enter the barcodes in the Reagent Barcode dialog box. Barcodes are stored in the ReagentsId.xml file in the Run folder.

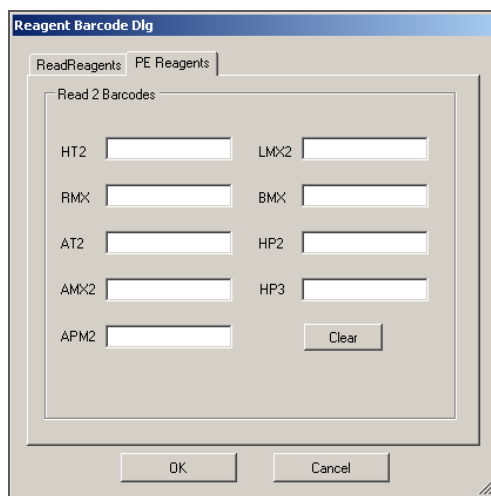
For long runs requiring multiple sequencing kits, click **New** and enter the barcodes for additional kits.



The dialog box is titled "Reagent Barcode Dlg". It has a tab labeled "ReadReagents". Below the tab, there is a "Kit:" dropdown menu showing "1", and three buttons: "New", "Delete", and "Delete All". Below this is a section titled "Barcodes" containing several input fields: "PW1", "LFN/FFN", "PR1", "SMX", "PR2", "CLM/CMX", "PR3", "RDP/SDP", and "IMX". There is a "Clear" button next to the "IMX" field. At the bottom of the dialog are "OK" and "Cancel" buttons.

Figure 29 Reagent Barcodes Dialog Box

For paired-end runs, click the PE Reagents tab and enter reagents used for Read 2. The PE Reagents tab is enabled when a paired-end sequencing recipe is open.



The dialog box is titled "Reagent Barcode Dlg". It has two tabs: "ReadReagents" and "PE Reagents". The "PE Reagents" tab is selected. Below the tabs is a section titled "Read 2 Barcodes" containing several input fields: "HT2", "LMX2", "RMX", "BMX", "AT2", "HP2", "AMX2", "HP3", and "APM2". There is a "Clear" button next to the "APM2" field. At the bottom of the dialog are "OK" and "Cancel" buttons.

Figure 30 Reagent Barcodes, Paired-End Reagents Tab

If the reagents are expected to last only through the end of the run (according to the number of cycles entered in Reset Volumes), then the reagent barcodes are cleared at the end of a run. Otherwise, the Reagents Barcode dialog box is cleared when the next run starts.

If any reagents are expected to last for more cycles than the current run, then do not clear the barcodes. This saves you from having to re-enter barcodes if the remaining reagents are to be used in the next run. If the remaining reagents are not to be used, click **Clear**. This clears the barcodes for the kit number shown.

Configure Tracking

To configure, enable, or disable reagent tracking features, select **Reagents | Configure Tracking**.

- ▶ **Enable volume tracking**—Select this checkbox to enable reagent tracking. If left unchecked, reagent tracking is disabled.
- ▶ **AutoStop when empty**—If selected, this option stops the current run when the end of reagent volume is reached, and places the system into a safe state. In safe state, the software flushes the flow cell with PR1 (High Salt Buffer) to protect the clusters from degradation. This occurs after imaging of the current cycle is complete. The end of reagent volume is based on the number of cycles entered in the Reset Reagents Volume dialog box (Figure 28).
- ▶ **Display and email warnings**—If selected, the software warns you when reagent volumes are low or when the specified number of cycles remain. To specify email recipients, enter each email address in the **To** field, and click **Add**. Check with your IT department to ensure your email is properly configured. An SMTP server must be accessible from the instrument to use email notification.

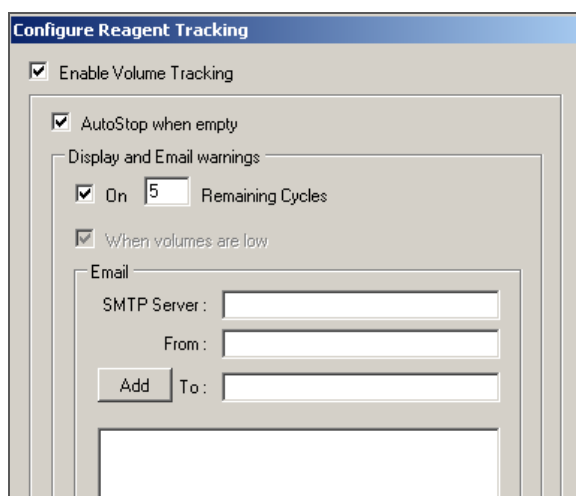


Figure 31 Configure Reagent Tracking

Sample Sheet Selection

If you selected the **Prompt for sample sheet usage at run start** option (see *GA Settings* on page 17), SCS v2.6 looks for a sample sheet labeled with the matching flow cell ID. If it cannot find one, SCS v2.6 opens the Sample Information dialog box.

Use the browse function to select the proper sample sheet for this run. To generate or edit a sample sheet, see Appendix B, *Sample Sheet*.

Available Disk Space

During the run at the end of each cycle, the software checks available disk space. If there is insufficient disk space to accommodate one cycle, the software pauses the run and a dialog box appears to tell you how much disk space remains. If you get this message, you need to make disk space available to continue the run.

When sufficient disk space is available, the dialog box closes and the run automatically resumes. If you close the dialog box, you must manually resume the run once sufficient disk space is available.

Image Controls

The Genome Analyzer software provides various tools for adjusting the image and discovering information.

The image display uses 8 bits of data to display color, while the TIFF files that are saved contain 16-bit color images. The Show False Color and Auto Scale options provide methods for simulating the larger dynamic range on the computer monitor.

Table 3 Genome Analyzer Image Controls

Adjustment	Action
Zoom in	Click and drag a rectangle over the area you want to see.
Move the zoomed image	Right-click and select Center Here . The display adjusts so that the area you clicked on is at the center.
Zoom out	Right-click over the image and select Zoom Out to zoom out one level. Right-click over the image and select Zoom Out All to zoom out all the way.
Modify the color display	Right-click over the image and select Show Color or Show False Color . Show Color displays the laser light colors used to acquire the image: blue for the focus tracking images, and red (A/C) or green (G/T) for the intensity values. Show False Color lets you customize the color contrast so that it is easier to see peak intensities. Select from: <ul style="list-style-type: none"> • None • Blue-Green • Green-Blue • Blue-Red • Red-Blue • Red-Green • Green-Red When you select a two-color option, the Genome Analyzer Software uses the upper 8 bits of the Tiff 16-color range for one color (e.g., blue), and the lower 8 bits for the other color (e.g., green).
View or hide the center mark	Right-click over the image and select Show Center Mark to toggle the display of the center mark, which indicates the current X and Y values of the stage.
See intensity values	Roll the mouse over a point of raised intensity to display the pixel position and intensity value.
Scale the intensity values	Right-click over the image and select Auto Scale On or Auto Scale Off . When auto scaling is on, the minimum intensity value is mapped to 0 and the maximum intensity value is mapped to 255. When auto scaling is off, the system maps the 12-bit range to an intensity range that you define in RCMConfig.xml. All data intensity values at or below the minimum are mapped to 0 and all values at or above the maximum are mapped to 255. Generally, this mapping is a linear function.
See focus quality and uniformity	Hover the mouse over the tile image to display the focal quality and uniformity.
Show region of interest	Right-click over the image and select Show ROI to show the region of interest (ROI), which is the area that is saved during the sequencing runs. The ROI is indicated by the dotted lines on the display. This feature allows you to check for good alignment of the ROI edge and lane edge.
Save the image	Right-click on the image and select Save As to save the image as a TIFF file.

Auto Calibration

The auto calibration feature determines the best focus by stepping through a series of images, adjusting the Z position with each step until the best focus is determined. The software performs a coarse focus, followed by a fine focus, and then determines the best Z position.

During auto calibration, the software uses a region of 128 x 128 pixels in the middle of the image to determine the best focus. If no clusters are found within this region, auto calibration fails and an error message appears. For more information, see *Calibration Failure Recovery* on page 33.

To enable auto calibration, select the **Use Auto Calibration** checkbox in the Run Parameters window. For more information, see *Run Parameters Window* on page 16.

There are two ways to initiate auto calibration. One way is to use recipe version 6.0 or later, and the other way is to initiate auto calibration manually.

Recipe Commands

If you are using recipe version 6.0 or later, the command to run auto calibration is integrated in the first-base incorporation recipe. The command to find the flow cell edge directly follows the Calibration command.

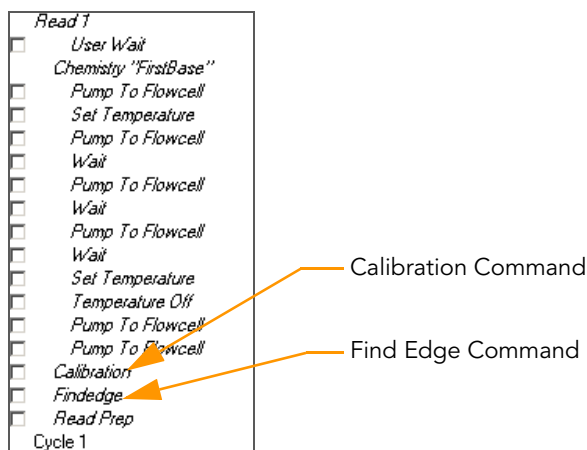


Figure 32 Auto Calibration Commands in the Recipe

Manual Command

If you are running recipes prior to version 6, you can run auto calibration manually. Select **Instrument | Calibration** from the Data Collection toolbar. You must complete first-base incorporation before running auto calibration manually.

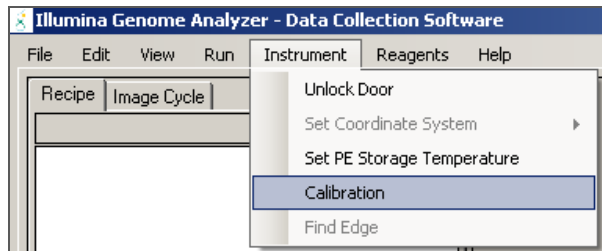


Figure 33 Run Auto Calibration From Toolbar

When auto calibration is complete, select **Instrument | Find Edge** to find the flow cell edge.

Results Window

When calibration is complete, the Calibration Results window appears. The Calibration Results window contains two tabs: Calibration Results and BestZ Plot.

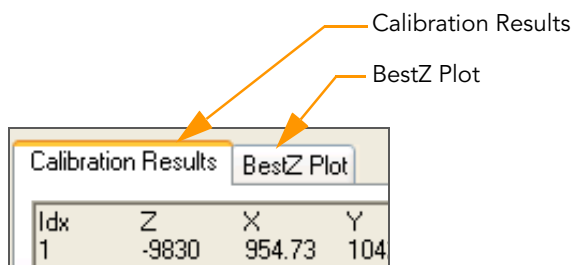


Figure 34 Calibration Results Window Tabs

Calibration Results Tab

The Calibration Results tab shows the different Z positions used to find focus and the calibration values.

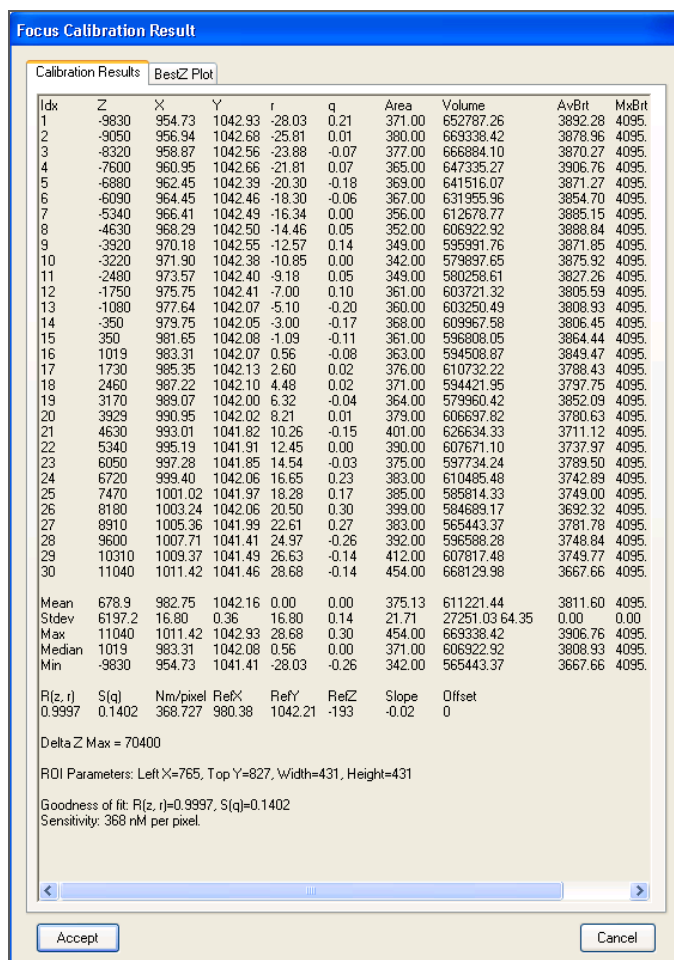


Figure 35 Focus Calibration Results Window

BestZ Plot Tab

BestZ Plot tab graphs each of the 30 points used in auto calibration and highlights the optimal Z position. You can move the crosshairs to each point on the graph to read the Z position.



Figure 36 Focus Calibration Results, BestZ Plot Tab

You can also view calibration results at any time during the run by selecting **View | Calibrations Results** from the Data Collection toolbar.

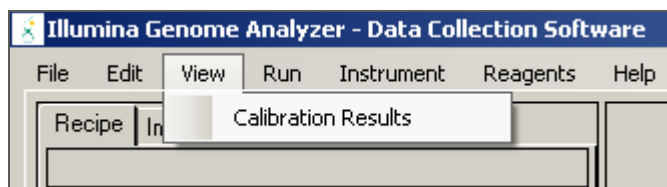


Figure 37 View Calibration Results

Calibration Failure Recovery

Auto calibration can fail if no clusters are present in the tile image or there is surface contamination on the flow cell or prism. If auto calibration fails, the following error message appears.

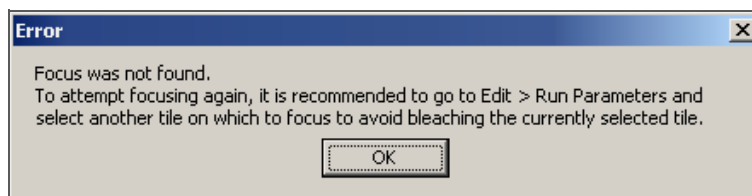


Figure 38 Auto Calibration Error Message

If you receive this message, click **OK** and visually inspect an image to make sure clusters are present. Clusters must be present for successful calibration.

If auto calibration fails, the instrument is placed in a safe state and PR1 (High Salt Buffer) is flushed through the flow cell. When you resume the run, you must pump SMX (Scan Mix) to through the flow cell before the imaging step.

Using Figure 39 and the following instructions, perform the necessary steps to troubleshoot the cause of auto calibration failure.

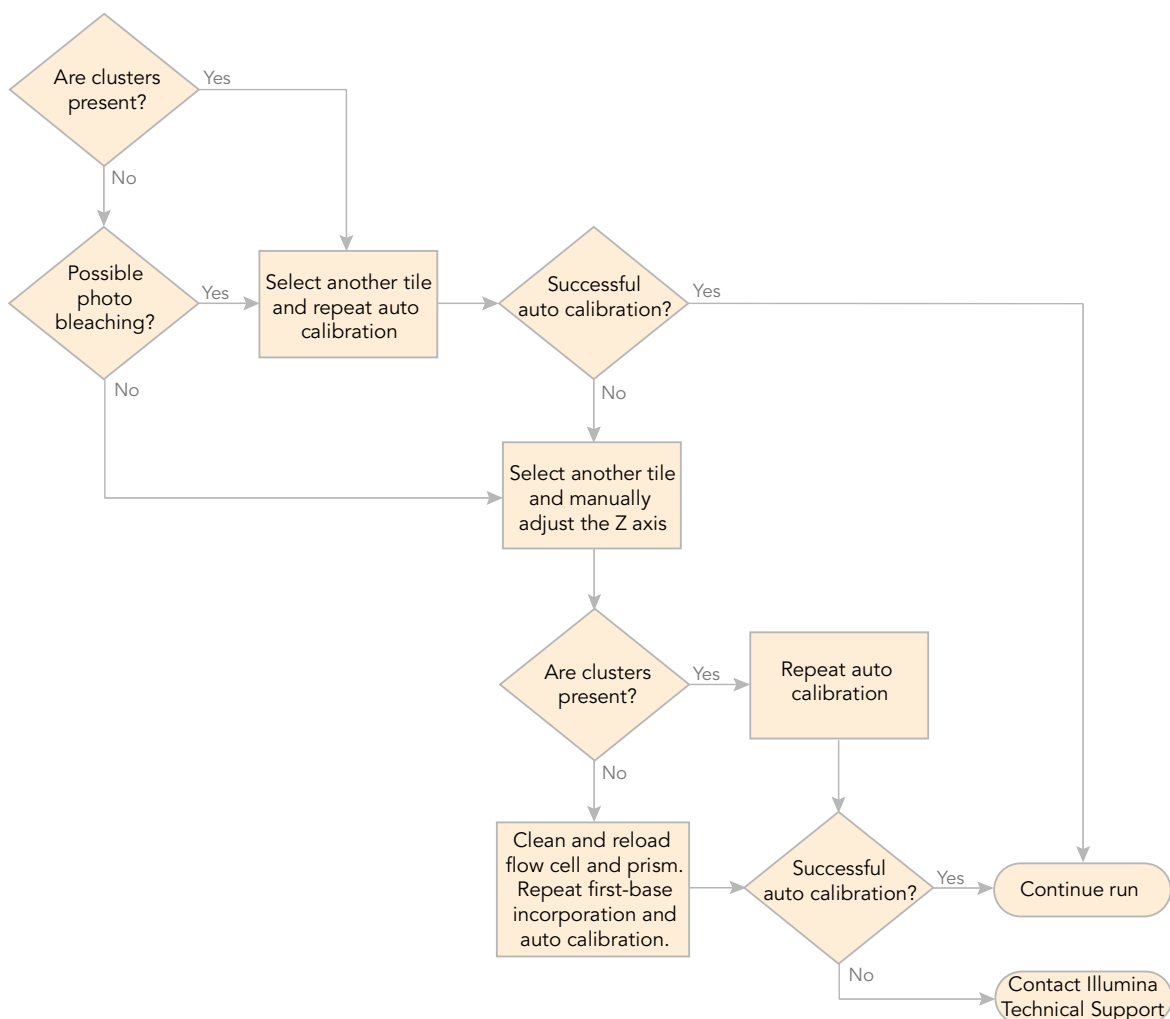


Figure 39 Troubleshooting Calibration Failure

Moving to Another Tile



NOTE

Illumina recommends that you move to another tile to avoid bleaching of the currently selected tile. Photo bleaching starts to diminish the intensity of the cluster signals after a few seconds of total exposure.

You can move to another tile using the auto calibration settings in the Run Parameters window, or by using the settings on the Manual Setup/Control tab in the Data Collection window.

Repeating Auto Calibration

Perform the following steps to manually repeat auto calibration.

1. Pump SMX (Scan Mix) to the flow cell before initiating auto calibration.
2. Move to another tile to avoid photo bleaching of the previously imaged tile.
3. Select **Instrument | Calibration** from the Data Collection toolbar.

Manually Adjusting the Z Axis

Perform the following steps to manually adjust the Z axis until clusters are visible.

1. Click **Take Picture** on the Manual Setup/Control tab.
2. Mouse over the image to see the Focus Quality (FQ) value.
(Optimum FQ value is 80–85.)
3. Move the Z-position in increments of 300 nm, decreasing to 100 nm as the focus improves. Repeat steps 1–2 each time you move the Z position.
4. When you are able to identify clusters in the image, select **Instrument | Set Coordinate System | Set Current Z as Origin**.



NOTE

It is not necessary to finely focus the image. It is only necessary that clusters are visible, even if they are slightly out of focus.

For more information about manually adjusting focus, see *Manual Controls and Calibration* on page 119.

Clear Results

The software stores the most recent calibration results, even after you close the Genome Analyzer software. The results are cleared if any of the following occurs:

- ▶ Auto calibration is restarted.
- ▶ Auto calibration fails or is interrupted. No new results are stored.
- ▶ You click **Load Flowcell** on the Manual Control/Setup tab.

Run Folders

Each run on the Genome Analyzer generates a Run folder that contains data files and log files for that run.

When you start a run, the software prompts you to enter the folder name for it. By default, the folder is named in the following format:

YYMMDD_<Workstation Name>_<Run Number>

Example: 070320_WORKSTATION-487_0002

The run number increments by one each time you perform a run on a given workstation. Typically, users add the flow cell ID to the Run folder name. The Run folder name may not contain any spaces.

Run Folder Path

All Run folders are stored in a single folder. The name and location of this folder are set in <install location>\bin\Config\RCMConfig, in this line:

```
<Run Path="D:\Runs" />
```

To change the Run folder path, either change this line or select **Run | Select Run Folder Root** in the software. Enter the full path and folder name.

Contents of Run Folders

The following tables list the content of the Run folder and subfolders. Additional files are created if you are performing a paired-end run.

Table 4 Run Folder Contents

Subfolder	Key Files/Folders	Description
[Root level]	Configuration files	All active configuration files for this run are copied to the root of the Run folder path.
	run.completed file	Appears in the folder when a run is successfully completed. You can tell whether a run is still in progress by checking for this file.
	Sample Sheet	If you create a sample sheet, it is copied to this location.
	Recipe_*.xml	When you start a run, a copy of the recipe is moved to the Run folder. The name will be prefixed by "Recipe_". If you stop the run, open this recipe to start up again where you left off.
	RunInfo.xml	The file RunInfo.xml identifies the boundaries of the reads (including index reads), and contains run-specific information such as instrument used and flow cell ID.
	Log file RunLog_<date>_<time>.xml	Whenever you stop a run, a log file detailing the duration of every command appears in the root of the Run folder. If you stop and resume the run multiple times, there will be a separate log file for each session. Other log files for the run can be found in <install location>\bin\Config\LogFiles.
Images	Contains one folder for each lane; each lane folder contains one subfolder for each cycle	Each cycle folder contains TIFF files for each lane, named in the format <sample>_<lane>_<pos>_<base>.tif

Table 4 Run Folder Contents (Continued)

Subfolder	Key Files/Folders	Description
	Focus folders (optional)	Contains the TIFF focus images. This folder is created if you selected save focus images on the Run Parameters window. For more information, see <i>Run Parameters Window</i> on page 16.
Data	Status.htm	Contains run and analysis-specific metrics generated during the run.
	Intensities folder	Contains SCS real time analysis results. This folder is saved on the network folder under <Run Folder>\Data, and is given the same name as the Run folder on the instrument computer.
	RunBrowser folder	Contains *_bro.xml files used with the RunBrowser application. Each file contains run quality metrics in one file per lane, per cycle.
Data\ Intensities	BaseCalls folder	Contains SCS real time analysis base calling results.
	config.xml file	Contains run and image analysis-specific information.
Data\ Intensities\ BaseCalls	BustardSummary.xml file	Contains run- and analysis-specific metrics generated after base calling.
	config.xml file	Contains run and base calling-specific information.
AnalysisLogs	Contains one QC file per cycle	Each XML file is named as follows: <QC_tab_name>CycleNumber.xml. For example, FocusQuality15.xml contains focus quality information for all tiles of cycle 15.
Config		Contains configuration parameters for the run, which are stored in XML files.
Calibration_ <DateTime>	Calibration results and image files	

Table 5 Additional Run Folder Contents for Paired-End Runs

Subfolder	Key Files/Folders	Description
ReadPrep1	Contains Images and Data subfolders specific to the preparation of Read 1	Contains quality results from Read 1 <ReadPrep> cycle processed by SCS real time analysis.
ReadPrep2	Contains Images and Data subfolders specific to the preparation of Read 2	Contains quality results from Read 2 <ReadPrep> cycle processed by SCS real time analysis.



Chapter 3

Service and Maintenance Procedures

Topics

- 38 Introduction
- 39 Performing a Pre-Run Wash
- 41 Performing a Post-Run Wash
- 43 Performing a Monthly Maintenance Wash
- 45 Performing a Storage Wash

Introduction

It is important to regularly wash the lines of the Genome Analyzer and Paired-End Module. There are four types of washes:

- ▶ Pre-Run Wash
- ▶ Post-Run Wash
- ▶ Monthly Maintenance Wash
- ▶ Storage Wash

Table 6 Maintenance Washes

Type of Wash	When to Perform
Pre-Run Wash	Perform this wash before each run, if the instrument has been idle for one day or more, and before changing reagents.
Post-Run Wash	Perform this wash after the run is complete. For paired-end sequencing, perform this wash after the second read.
Monthly Maintenance Wash	Perform this wash once a month.
Storage Wash	Perform this wash if you plan to leave the Genome Analyzer idle for three days or more.

The recipes used for each wash are different for single-read sequencing than for paired-end sequencing. Recipes used with single-read sequencing wash positions on the Genome Analyzer, and recipes used with paired-end sequencing wash positions on the Genome Analyzer and the Paired-End Module. This section includes the Paired-End Module in the description of the wash protocols.

Performing a Pre-Run Wash

Perform a pre-run wash before beginning to load reagents for a sequencing run on the Genome Analyzer.

The wash recipe flushes 1 ml of wash solution (PW1) through each reagent port on the Genome Analyzer. The wash recipes used with paired-end or multiplexed sequencing runs also flush 1 ml water through reagent ports on the Paired-End Module.

Run time is approximately 15 minutes for the single-read recipe and approximately 40 minutes for the paired-end recipe.

After the wash, check the total volume in the waste container closely to confirm the stability of the reagent delivery system. Expected volumes from the wash cycle are a primary indicator of a stable fluid delivery system. Dry syringes and excessive bubbles indicate a problem.

Consumables Illumina-Supplied

- ▶ PW1 (provided in the Sequencing Kit)

User-Supplied

- ▶ Lens cleaning tissue
- ▶ Four 125 ml Nalgene bottles (ThermoFisher Scientific, catalog # 342040-0125)
- ▶ Three 175 ml Falcon bottles (BD, catalog # 352076) (if your Genome Analyzer is equipped with a large reagent chiller)
- ▶ Three 50 ml conical tubes (if your Genome Analyzer is not equipped with a large reagent chiller)
- ▶ MilliQ water or laboratory grade water (for washing the Paired-End Module)

Procedure

1. Load the Genome Analyzer with a used flow cell.
In the Pump area, make sure the following values are set to prevent siphoning of reagents:
Command: Pump
To: Flow cell
Solution: 8 (PEM not enabled); 28 (PEM enabled)
Volume: 0
Aspiration Rate: 250
Dispense Rate: 2,500
With the cursor in the **Dispense Rate** box, press **Enter**.
2. Load the Genome Analyzer with PW1 solution as follows:
 - 10 ml PW1 into 175 ml Falcon bottles or 50 ml conical tubes onto positions 1, 3, and 6
 - 40 ml PW1 into 125 ml Nalgene bottles onto positions 2, 4, 5, and 7



CAUTION

Rotate the tubes while holding the caps stationary to prevent crimps and twisting in the liquid delivery lines.

3. [For multiplexed and paired-end runs] Place at least 5 ml of laboratory grade water into 15 ml conical tubes in the following positions on the Paired-End Module.

Type of Run	Paired-End Module Positions
Standard Paired-End Run, or Multiplexed Paired-End Run	Positions 9–22
Multiplexed Single-Read Run	Positions 19, 21, and 22

4. Loosen and remove the Genome Analyzer waste tubing from the waste container, and bundle the waste tubes with parafilm, making sure to keep all of the ends even.
5. Place the bundled tube ends into a 50 ml tube.
6. Click the Run tab.
7. Select **File | Open Recipe**.
8. Open one of the following recipes:

Type of Run	Recipe Name
Standard Single-Read Run	GA2_PreWash_v<#>.xml
Standard Paired-End Run or Multiplexed Paired-End Run	GA2-PEM_PreWash_v<#>.xml
Multiplexed Single-Read Run	GA2-PEM_MP_PreWash_SR_v<#>.xml

9. Click **Start**. Reagents are delivered 1 ml at a time.

Recipe Name	Recipe Version	Total Expected Volume
GA2_PreWash_v<#>.xml	v6, v7	7 ml
GA2-PEM_PreWash_v<#>.xml	v6, v7	18 ml
GA2-PEM_MP_PreWash_SR_v<#>.xml	v6, v7	10 ml

10. Record the delivery volume on the lab tracking form.

If the measured volume is less than 90% of the expected value, do the following:

- a. Check for leaks.
- b. Place each waste tubing in a separate 50 ml tube.
- c. Repeat the wash cycle.
- d. Measure each 1 ml delivery.



NOTE

During the sequencing run, keep one 125 ml Nalgene bottle containing PW1 at position 2 on the Genome Analyzer.

Save the rest of the bottles containing PW1 solution for the post-run wash.

Performing a Post-Run Wash

After every single-read run or after the second read of a paired-end run, you must perform a thorough instrument wash.

The wash recipe flushes 4 ml of wash solution (PW1) through each reagent port on the Genome Analyzer. The wash recipes used with paired-end and multiplexed sequencing runs flush 1 ml of wash solution through reagent ports on the Paired-End Module.

Run time varies depending on the recipe you are using:

- ▶ Run time for the single-read recipe is approximately 45 minutes.
- ▶ Run time for the paired-end recipe is approximately 60 minutes.

Consumables Illumina-Supplied

- ▶ PW1 (provided in the Sequencing Kit)

User-Supplied

- ▶ Lens cleaning tissue
- ▶ Four 125 ml Nalgene bottles (ThermoFisher Scientific, catalog # 342040-0125)
- ▶ Three 175 ml Falcon bottles (BD, catalog # 352076) (if your Genome Analyzer is equipped with a large reagent chiller)
- ▶ Three 50 ml conical tubes (if your Genome Analyzer is not equipped with a large reagent chiller)
- ▶ MilliQ water or laboratory grade water (for washing the Paired-End Module)

Procedure

1. Load the Genome Analyzer with PW1 solution as follows:
 - 10 ml PW1 into 175 ml Falcon bottles or 50 ml conical tubes onto positions 1, 3, and 6
 - 40 ml PW1 into 125 ml Nalgene bottles onto positions 2, 4, 5, and 7
 You may use the same wash containers set aside after the pre-run wash.
2. [For multiplexed and paired-end runs] Place at least 5 ml of laboratory grade water into 15 ml conical tubes in the following positions on the Paired-End Module.

Type of Run	Paired-End Module Positions
Standard Paired-End Run, or Multiplexed Paired-End Run	Positions 9–22
Multiplexed Single-Read Run	Positions 19, 21, and 22

3. Click the Run tab.
4. Select **File | Open Recipe**.
5. Open one of the following recipes:

Type of Run	Recipe Name
Standard Single-Read Run	GA2_PostWash_v<#>.xml
Standard Paired-End Run or Multiplexed Paired-End Run	GA2-PEM_PostWash_v<#>.xml
Multiplexed Single-Read Run	GA2-PEM_MP_PostWash_SR_v<#>.xml

6. Click **Start**. Reagents are delivered 1 ml at a time.

**CAUTION**

Using wash reagents on the Genome Analyzer other than the PW1 solution provided in the Sequencing Kit, or failing to perform the wash cycle at the recommended intervals, may void the warranty.

Performing a Monthly Maintenance Wash

The Maintenance Wash has two parts: the 1 ml water wash and the 4 ml NaOH wash. Perform the maintenance wash once monthly for maintenance, and any time you plan to leave the Genome Analyzer sitting idle for more than three days. The monthly maintenance wash uses the same instrument cycle as the pre-run wash, but uses a stronger base (1 N NaOH) and filtered cleaning liquids.

Run time varies depending on the recipe you are using. The run time for the single-read recipe is approximately 45 minutes. The run time for the paired-end recipe is approximately 1 hour and 24 minutes.

Consumables Illumina-Supplied

- ▶ PW1 (Wash Solution)

User-Supplied

- ▶ Lens cleaning tissue
- ▶ 1 N NaOH (0.5 L, filtered with a 0.2 µm nylon filter)
- ▶ Four 125 ml Nalgene bottles (ThermoFisher Scientific, catalog # 2019-0125)
- ▶ Three 175 ml Falcon bottles (BD, catalog # 352076) (if your Genome Analyzer is equipped with a large reagent chiller)
- ▶ Three 50 ml conical tubes (if your Genome Analyzer is not equipped with a large reagent chiller)
- ▶ MilliQ water or laboratory grade water (for washing the Paired-End Module)

Procedure 1. Load the Genome Analyzer with a used flow cell.



NOTE

When the manifolds are raised for the first time, place the fluidics on standby by performing steps 3 and 5 of *Unloading the Flow Cell and Prism* on page 50.

2. Select **File | Open Recipe**.
3. Open one of the following recipes:
 - For single-read sequencing, open GA2_MaintenanceWash_v<#>.xml.
 - For paired-end sequencing, open GA2-PEM_MaintenanceWash_v<#>.xml.
4. Load the Genome Analyzer with PW1 wash solution as follows:
 - 10 ml onto positions 1, 6, and 3
 - 40 ml onto positions 4, 5, 2, and 7

**CAUTION**

Rotate the tubes while holding the caps stationary to prevent crimps and twisting in the liquid delivery lines.

5. [Paired-End Module] Load the Paired-End Module with 15 ml tubes containing 10 ml of laboratory grade water onto positions 9–22.
6. Click **OK** to start the prewash.
7. After the pre-run wash is complete, load the Genome Analyzer with filtered 1 N NaOH as follows:
 - 25 ml onto positions 1, 6, and 3
 - 50 ml onto positions 4, 5, 2, and 7
8. [Paired-End Module] Load the Paired-End Module with 15 ml tubes containing 10 ml of filtered 1 N NaOH onto positions 9–22.
9. Click **OK** to start the NaOH wash.
10. After the NaOH wash is complete, load the Genome Analyzer with PW1 wash solution as follows:
 - 15 ml onto positions 1, 6, and 3
 - 50 ml onto positions 4, 5, 2, and 7
11. [Paired-End Module] Load the Paired-End Module with 15 ml tubes containing 15 ml of MilliQ water or laboratory grade water onto positions 9–22.
12. Click **OK** to start the wash.

Performing a Storage Wash

If you plan to leave the Genome Analyzer idle for more than three days, perform the storage wash following the maintenance wash.

Consumables Illumina-Supplied

- ▶ PW1 (Wash Solution)

User-Supplied

- ▶ Lens cleaning tissue
- ▶ 1 N NaOH (0.5 L, filtered with a 0.2 µm nylon filter)
- ▶ Four 125 ml Nalgene bottles (ThermoFisher Scientific, catalog # 342040-0125)
- ▶ Three 175 ml Falcon bottles (BD, catalog # 352076) (if your Genome Analyzer is equipped with a large reagent chiller)
- ▶ Three 50 ml conical tubes (if your Genome Analyzer is not equipped with a large reagent chiller)
- ▶ MilliQ water or laboratory grade water (for washing the Paired-End Module)

Procedure

1. Load the Genome Analyzer with PW1 solution as follows:
 - 10 ml PW1 onto positions 1, 6, and 3
 - 40 ml PW1 onto positions 4, 5, 2, and 7
2. [Paired-End Module] Place at least 5 ml of laboratory grade water into 15 ml conical tubes onto positions 9–22 on the Paired-End Module.
3. Click the Run tab.
4. Select **File | Open Recipe**.
5. Open one of the following recipes:

Recipe Name	Positions Washed
GA2_PostWash_v<#>.xml	Positions 1–7 on the Genome Analyzer used for single-read runs
GA2-PEM_PostWash_v<#>.xml	Positions 1–7 on the Genome Analyzer and positions 9–22 on the Paired-End Module

6. Click **Start**.
7. When the run finishes, click the Manual Control tab.
8. In the Pump area, set the parameters as follows:
 - Solution:** 8 (single-read recipe); 28 (paired-end recipe)
 - Volume:** 0
9. With the cursor in the **Volume** field, press **Enter**.

10. Leave the used flow cell in the instrument to prevent siphoning.
11. Close the Genome Analyzer software and shut down the computer.
12. Turn the Genome Analyzer power switch to the OFF position.

Resuming Use After Storage

Perform the following steps to resume instrument use after short-term or long-term storage:

1. Turn on the Genome Analyzer.
2. Start the computer and log on to the operating system.
3. Open the Genome Analyzer software.
4. Load the Genome Analyzer with PW1 solution as follows:
 - 10 ml PW1 onto positions 1, 6, and 3
 - 40 ml PW1 onto positions 4, 5, 2, and 7
5. [Paired-End Module] Place at least 5 ml of laboratory grade water into 15 ml conical tubes onto positions 9–22 on the Paired-End Module.
6. Click the Run tab.
7. Select **File | Open Recipe**.
8. Open one of the following recipes:

Recipe Name	Positions Washed
GA2_PostWash_v<#>.xml	Positions 1–7 on Genome Analyzer
GA2-PEM_PostWash_v<#>.xml	Positions 1–7 on Genome Analyzer and positions 19–22 on the Paired-End Module

9. Click **Start**.



Chapter 4

Operational Procedures

Topics

- 48 Introduction
- 49 Starting the Genome Analyzer
- 50 Unloading the Flow Cell and Prism
- 52 Installing the Prism
- 54 Loading the Flow Cell
- 58 Checking for Leaks and Proper Reagent Delivery
- 60 Applying Oil

Introduction

The procedures described in this section are standard steps performed on the Genome Analyzer at the beginning of a sequencing run. However, the steps for completing the run will vary depending on the type of sequencing run you perform. See the *Genome Analyzer User Guide* for instructions.

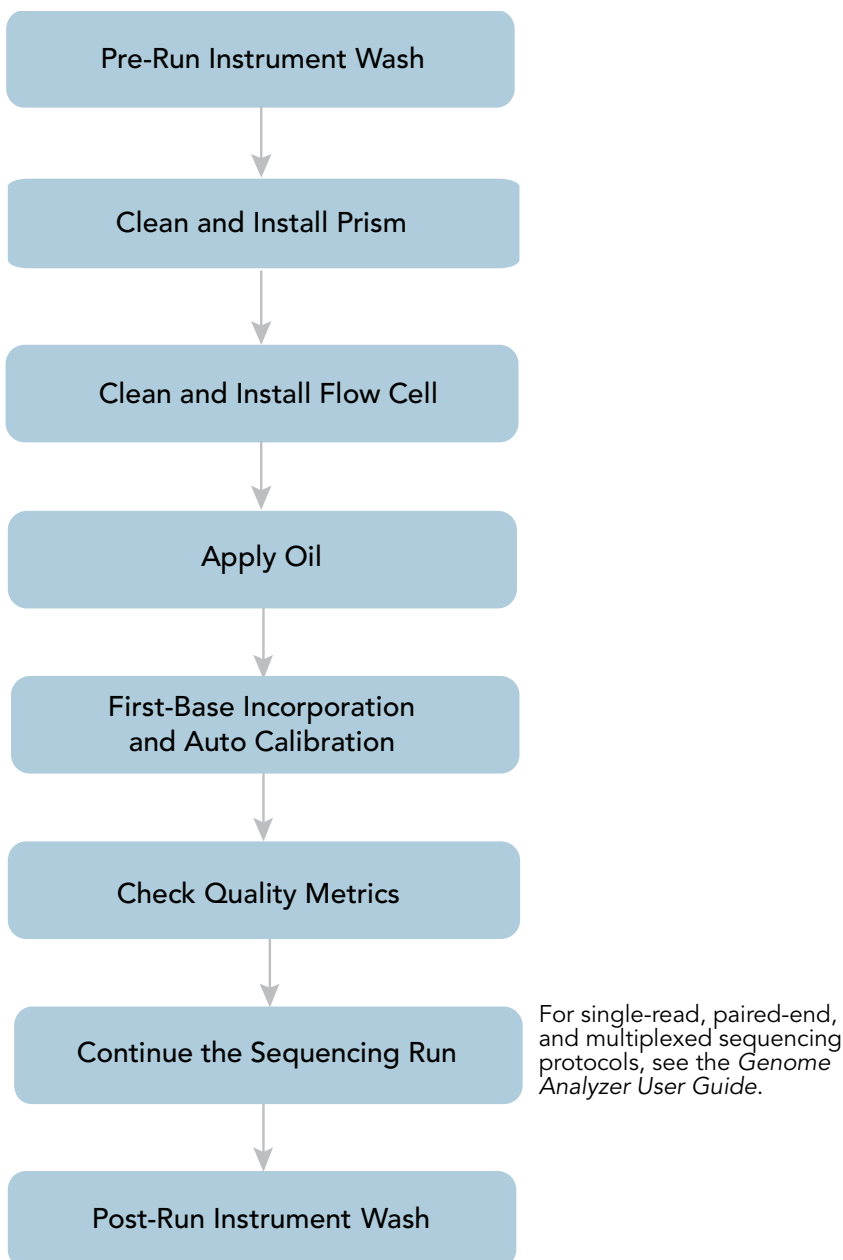


Figure 40 Standard Procedures on the Genome Analyzer

Starting the Genome Analyzer

It is best to leave the Genome Analyzer on at all times. Turn it off only if it will remain idle for more than three days. However, you should restart the Sequencing Control Software (SCS) before each run to ensure that the software is properly initialized.

1. Turn the main switch to the ON position.

After a short delay the instrument emits a regular buzzing sound from the top-right-rear region of the chassis. The sound is a normal indicator of a stable instrument initialization.

2. Restart the computer and log on to the operating system using the default values.

User name: sbsuser

Password: sbs123

If the default values do not work, consult your IT personnel to find out the correct user name and password for your site.

3. Delete the data from all previous runs to ensure adequate disk space (approximately 1 TB for a 36-cycle run). If deleting large files is excessively time consuming, perform a quick reformat of the data drive, as follows:
 - a. Right-click **My Computer** and select **Manage**.
 - b. In the tree on the left side of the screen, select **Storage | Disk Management**.
 - c. Right-click **D Partition** and select **Format**.
 - d. Select the **Perform a Quick Format** checkbox. Leave all other parameters at the default values.
 - e. Click **OK**.
4. Create a new Run folder on the D partition to hold the run data (if not automatically created). For more information, see *Run Folders* on page 35.
5. Double-click the Illumina Genome Analyzer Data Collection icon on the computer desktop.
6. If SCS v2.5 is installed along with SCS v2.6, the Select Reagent Version dialog box appears. Pick the version of SCS you want to run, based on the cluster generation kit that was used:
 - For sequence samples generated with the Cluster Generation Kit v4, use Genome Analyzer Data Collection Software SCS v2.6.
 - For sequence samples generated with the Cluster Generation Kit v2, use Genome Analyzer Data Collection Software SCS v2.5.

For information about the versions, see *SCS v2.5 and v2.6* on page 14.



NOTE

SCS v2.6 is incompatible with Cluster Generation Kit v2.

The Run Parameters window appears. For detailed information, see *Run Parameters Window* on page 16.

Unloading the Flow Cell and Prism

Perform the following steps to safely remove the used flow cell from the Genome Analyzer. You also need to remove the prism for cleaning.

1. Click the Manual Control/Setup tab.
2. Click **Load Flow Cell** to bring the stage to the front of the instrument and raise the lens. When the Flow Cell ID dialog box appears, click **Cancel**.
3. In the Pump area, make sure the following values are set to prevent siphoning of reagents.

Command: Pump

To: Flow cell

Solution: 8 (single-read sequencing); 28 (paired-end sequencing)

Volume: 0

Aspiration Rate: 250

Dispense Rate: 2,500

4. Select **Instrument | Unlock Door** to release the door to the imaging compartment. Raise the door.
5. With the cursor in the **Dispense Rate** box, press **Enter**.
6. Turn the manifold handle clockwise to lift the manifolds (Figure 41).

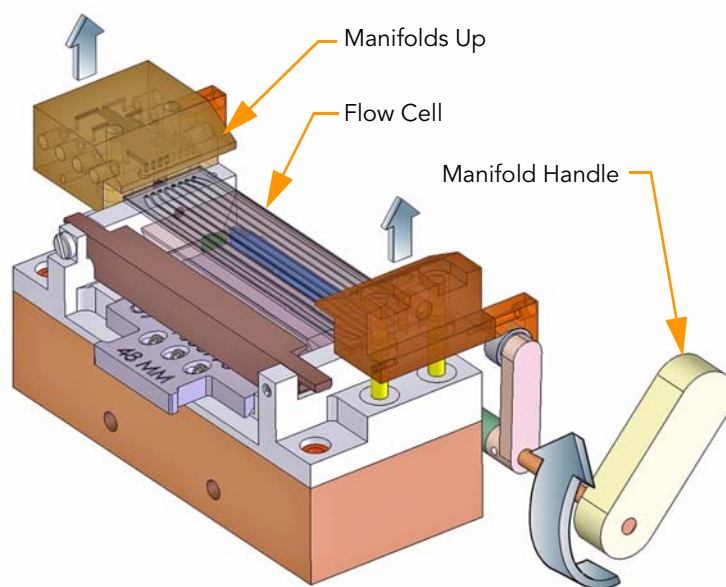


Figure 41 Lift Front and Rear Manifolds

7. Slide the flow cell to the left to clear the manifolds, and then lift it out of the instrument (Figure 42).

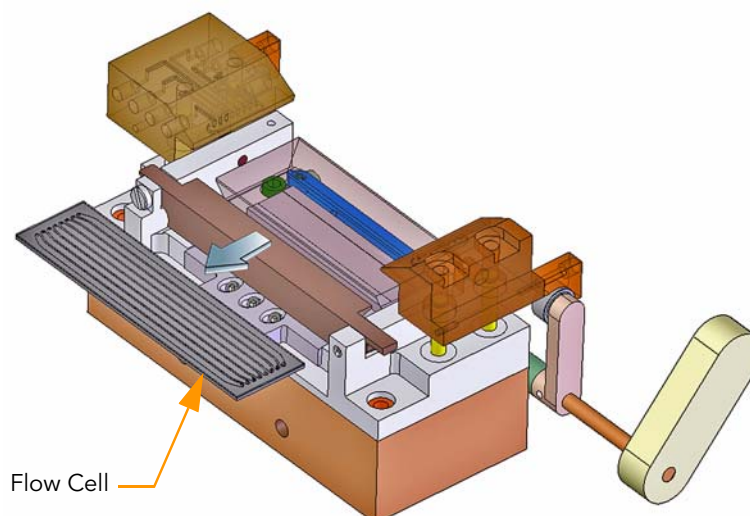


Figure 42 Remove the Flow Cell



CAUTION

Always wear powder-free latex gloves when handling the prism. Hold the prism by the metal prism base only. Avoid chipping the prism, as this may degrade optical function.

8. Raise the beam dump.
9. Hold the metal prism base by the prism base handle and slide the metal prism base to the left to remove the prism (Figure 43).

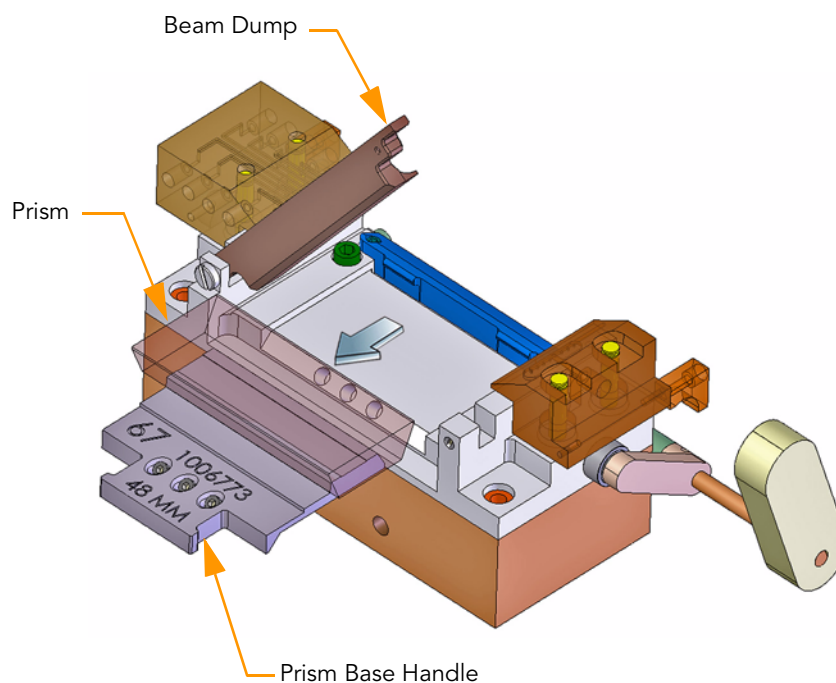


Figure 43 Remove the Prism

Installing the Prism

The prism serves a critical optical function and sits under the flow cell on top of the metal prism base.

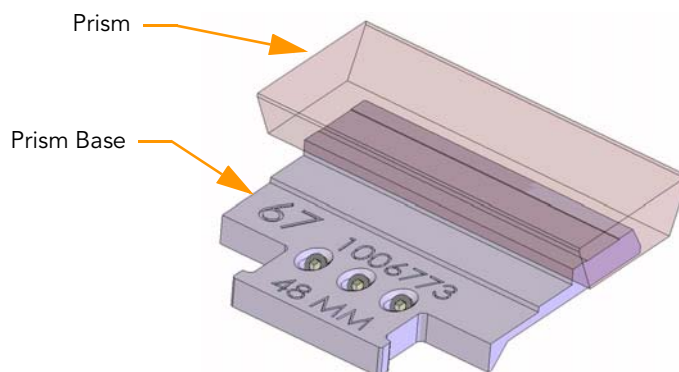


Figure 44 Prism

Always wear powder-free latex gloves when handling the prism. Hold the prism by the metal prism base only.



CAUTION

Exercise extreme care when handling the prism to prevent chipping, as this may degrade optical function. Hold the prism by the metal prism base only. The critical surface is the right side, which is the surface of laser entry.

Consumables

User-Supplied

- ▶ Lint-free lens cleaning tissue
- ▶ 100% ethanol or Spectrophotometer-grade methanol

Clean the Prism

1. Put on new gloves.
2. Wipe down any oil that has spilled onto the mounting rails, manifolds, Peltier heater, or accumulated onto the beam dump.
3. Place the prism on a fresh ethanol wipe on the benchtop.
4. Remove the oil by gently washing the prism with a stream of ethanol or methanol.
5. Wipe the metal prism base with a lens cleaning tissue.
6. Fold a lens cleaning tissue to approximately the size of the prism. Wet the edge of the tissue with ethanol or methanol and wipe off the surface with a single sweeping motion. Repeat, refolding the tissue with each wipe, until the prism is completely clean.
7. To tell if the prism is clean, observe it under direct light from a variety of angles. Pay particular attention to the surface that will face the laser entry (right-hand side when sitting in the holder) and the large top surface.
8. Protect the prism from dust until you place it onto the Genome Analyzer.

Install the Prism

Always install the prism before loading the flow cell.

1. Fold a lens cleaning tissue and wet it with ethanol or methanol.
2. Wipe the recessed surface of the prism holder to remove oil that may have been spilled during the previous run.
3. Lift the beam dump.



CAUTION

Be very careful not to touch the laser mount when you install the prism. If the laser mount is knocked out of position, it may require a service call for recalibration.

4. Slide the metal prism base to the right until it stops (Figure 45).

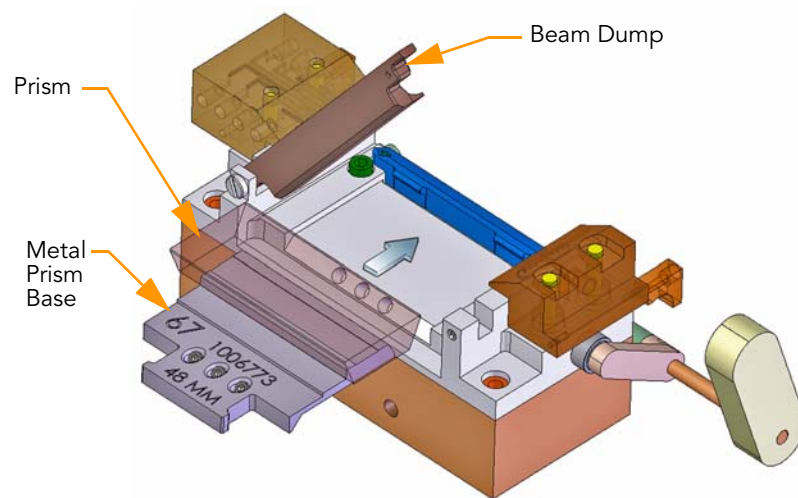


Figure 45 Install the Prism

5. With prism in place, lower the beam dump until it locks into position (Figure 46).

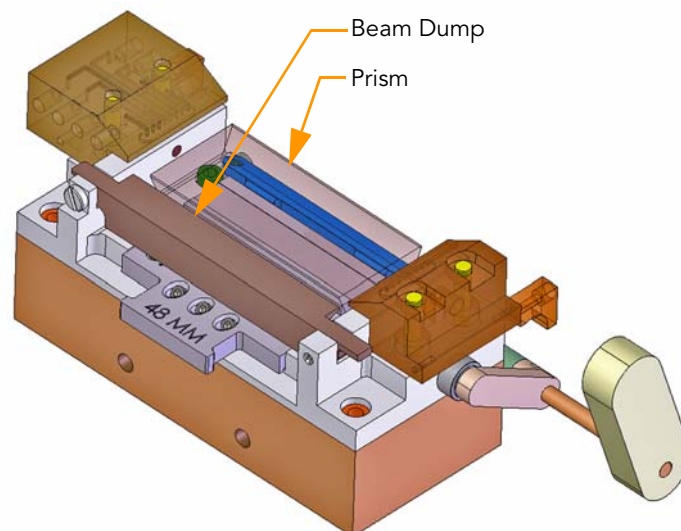


Figure 46 Prism Installed

6. Proceed to *Loading the Flow Cell* on page 54.

Loading the Flow Cell

The flow cell is located above the prism and rests on the manifold mounting rails.

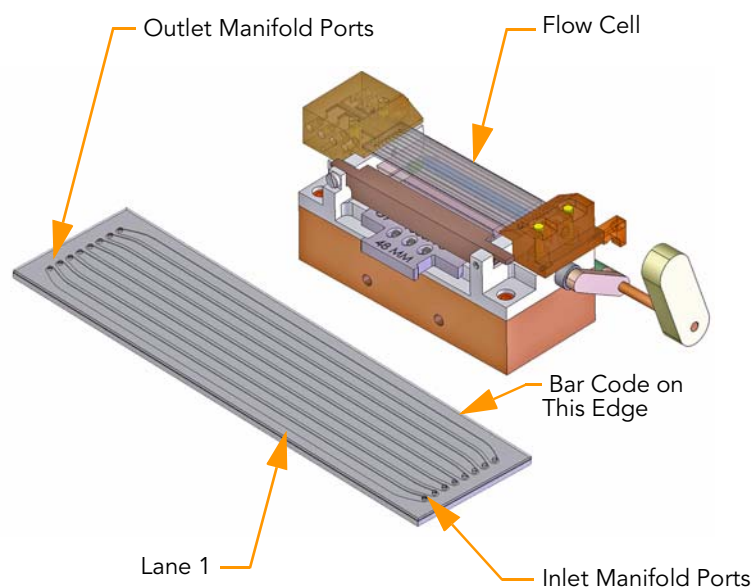


Figure 47 Flow Cell

Consumables

User-Supplied

- ▶ Lint-free lens cleaning tissue
- ▶ 100% ethanol or Spectrophotometer-grade methanol

Clean the Flow Cell



CAUTION

Work away from the inlets and outlets to avoid contaminating the inside of the lanes that contain the samples.

1. Put on new gloves.
2. Place the flow cell on a lens cleaning tissue on the benchtop. Make sure that the inlet and outlet ports face *up*, to prevent liquid from flowing out of the lanes.
3. Fold a lens cleaning tissue to approximately the size of the flow cell. Wet the edge of the tissue with methanol or 100% ethanol.
4. Hold the edges of the flow cell with two gloved fingers.



CAUTION

If you clean the flow cell while it is lying on the bench top, you could easily apply too much pressure and break the flow cell. Illumina recommends cleaning the flow cell while holding the edges between your fingers.

5. Fold a fresh tissue, wet it, and wipe off each side with a single sweeping motion. Repeat, refolding the tissue with each wipe, until the flow cell is completely clean.
6. Protect the flow cell from dust until you place it onto the Genome Analyzer.

Enter the Flow Cell ID



NOTE

Place the flow cell on a clean white background when reading the barcode.

1. Click **Load Flowcell** on the manual control screen. The stage moves to the load position, and the dialog box is displayed for the flow cell ID.
2. Click **Cancel** or click **OK**.

If you click **Cancel**, the dialog box is closed, and no data changes are made. Any previously entered flow cell ID remains current.

If you click **OK**, the ID field is checked to ensure that it isn't blank. If it is blank, a warning is displayed, and you must enter something or else cancel the dialog.

You can enter any characters from the keyboard. The barcode IDs are validated using the validation expression in the RCMConfig.xml file (FlowCellID= "[F][C][0-9][0-9][0-9][0-9]").



NOTE

You can change the validation expression as required in the Run Parameters window. To disable the barcode validation enter "" to indicate an empty string. For more information, see *Run Parameters Window* on page 16.



NOTE

After running a new recipe on a previously abandoned run, tracking of the flow cell ID may prevent you from using the same flow cell. In such a case, you can set the <IgnoreFlowCellIDCheck> field in the RCMConfig.xml file to "true" to ignore the flow cell ID tracking.

Load the Flow Cell

The prism must be installed before you load the flow cell.

1. Using a lens cleaning tissue, gently apply pressure on the underside of the front manifold to absorb excess liquid.
2. Place the flow cell on top of the front and rear mounting rails with the inlet and outlet ports facing *up*. Press it gently against the stops on the right side (Figure 48).

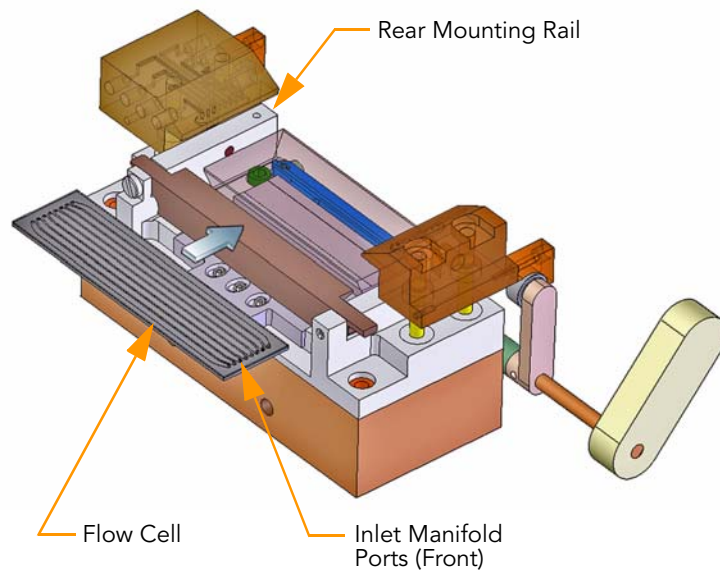


Figure 48 Load the Flow Cell

3. Slide the flow cell toward the rear until you encounter the rear stop (Figure 49).

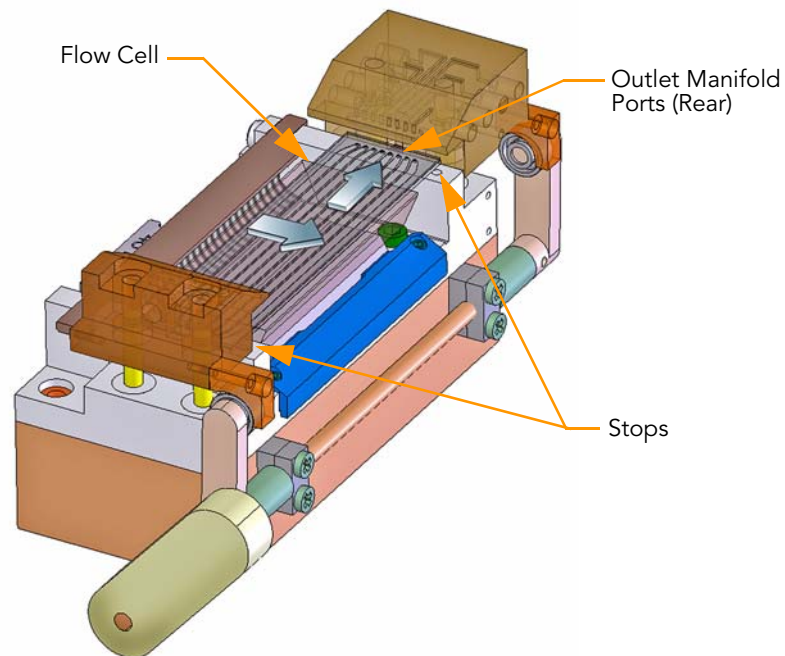


Figure 49 Position the Flow Cell

4. Test proper placement by applying gentle pressure toward the rear, then toward the right to ensure the flow cell is pressed against both stops.
5. While holding the flow cell against the stops with one hand, carefully rotate the manifold handle counterclockwise with the other hand to lower the manifolds into place (Figure 50).

**CAUTION**

The manifolds are spring-loaded, and the cam that holds them up is steeply shaped. Be careful to control the spring action so that the flow cell is not damaged.

6. Press downward on both manifolds to ensure they have seated properly (Figure 51).

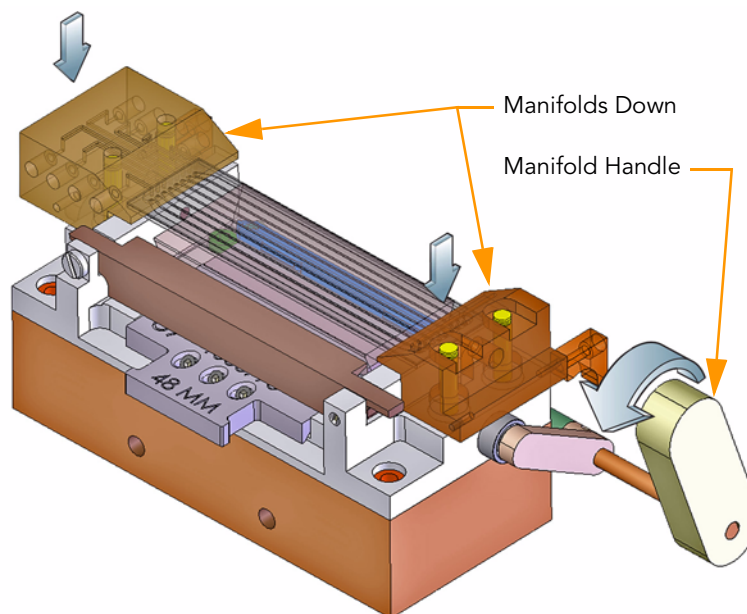


Figure 50 Lower the Manifold

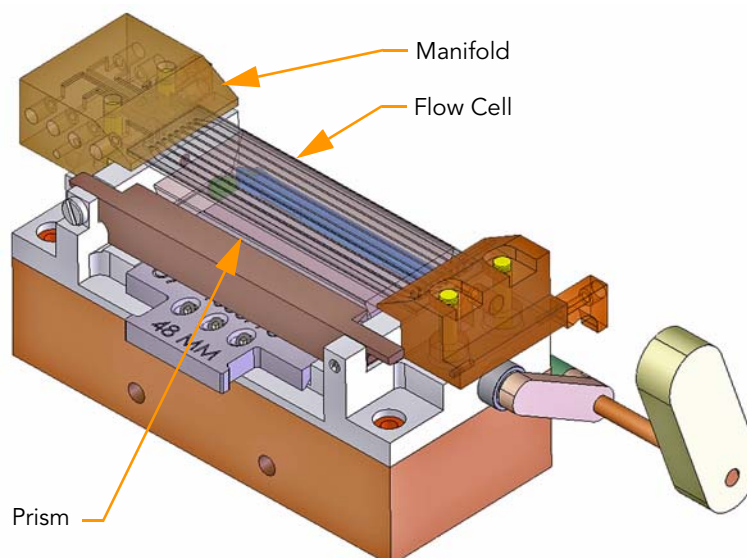


Figure 51 Flow Cell and Prism Loaded

Checking for Leaks and Proper Reagent Delivery

Reagent delivery volumes during the leak test are a key indicator of a stable fluid delivery system. The measured volumes must be within 10% of normal for optimal sequencing performance.

This procedure pumps PR2 (Incorporation Buffer) through the flow cell to check for leaks. Excessive air bubbles through the lanes indicate leaks at the manifold.

1. Wipe the interface of the manifold and the flow cell with a lens cleaning tissue.
2. Bundle all of the lines together with parafilm, making sure to keep the ends even.
3. Place the bundle into a 1.5 ml tube.
4. Pump 100 μ l of Incorporation Buffer (solution 5) through the flow cell.
 - a. Click the Manual Control/Setup tab.
 - b. In the Pump area, set the values as follows:
Command: Pump to Flow Cell
To: Flow cell
Solution: 5
Volume: 100
Aspiration Rate: 250
Dispense Rate: 2,500
 - c. With the cursor in the **Dispense Rate** box, press **Enter**.
5. Confirm that liquid is flowing properly through the flow cell by looking closely for any air bubbles moving toward the rear manifold of each lane.

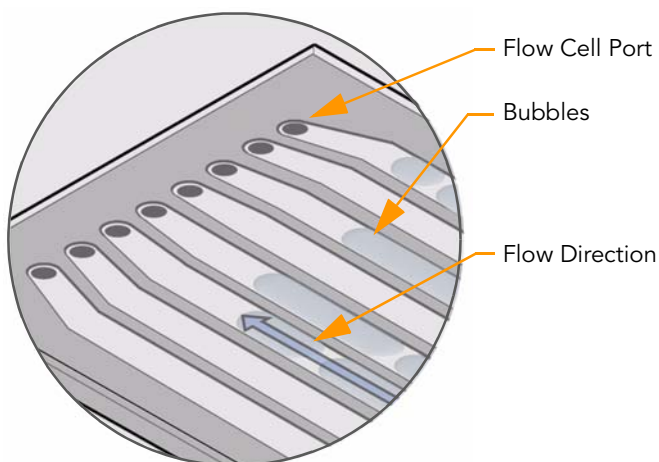


Figure 52 Check for Bubbles

6. When the liquid has successfully displaced the air in all eight lanes, move on to check for liquid leaks. If bubbles persist, it might indicate that the flow cell is not properly seated on the flow cell stage.
If air continues to enter the flow cell during the leak test, the most likely cause is a leak where the flow cell connects to the front manifold. Check the integrity of that connection and try the leak test again.

7. Check for leaks where the flow cell touches the manifold using a lens cleaning tissue.

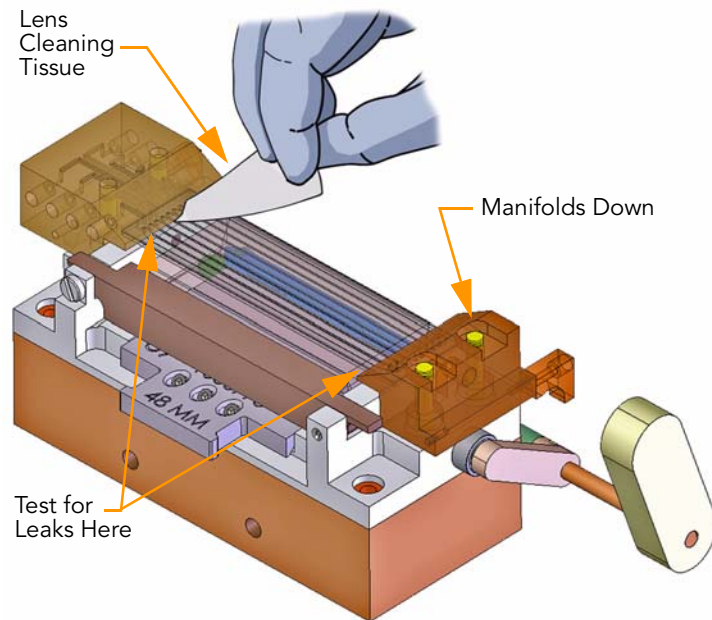


Figure 53 Test for Leaks

If a leak is present, perform the following steps:

- a. Click the Manual Control/Setup tab. In the Pump area, set the values as follows:

Command: Pump to Flow Cell

To: Flow cell

Solution: 8 (single-read recipe); 28 (paired-end recipe)

Volume: 0

Aspiration Rate: 250

Dispense Rate: 2,500

- b. Click **Load Flow Cell** to bring the stage to the front of the instrument and raise the lens.

- c. Lift the manifolds and clean the interface between the manifold and flow cell with a moist lens cleaning tissue.

- d. Dry and re-seat the flow cell.

- e. In the Pump area, set the values as follows:

Command: Pump to Flow Cell

To: Flow cell

Solution: 5

Volume: 100

Aspiration Rate: 250

Dispense Rate: 2,500

- f. Pump another 100 μ l of the Incorporation Buffer (solution 5) through the system.

8. Measure the flow for each of the eight lanes three times and record the measured volumes on the lab tracking form. The expected volume is 800 μ l.

If the average measurement differs from the expected volume by more than 10%, have the instrument checked by an Illumina Field Service Engineer.

9. If the system is leak free, proceed to *Applying Oil*.

Applying Oil

Immersion oil between prism and flow cell is a critical optical element. The layer of oil must be uniform and continuous to create total internal reflection. Too much oil may result in images that are out of focus.

Consumables

User-Supplied

- ▶ Immersion oil, refractive index 1.473 (Cargille, catalog # 19570)

Best Practices

- ▶ Always work from the left side of the flow cell. Excess oil on the right surface of the prism can wick over the edge causing optical aberrations.
- ▶ Let all of the dispensed oil wick between the flow cell and the prism as far as it will go before dispensing more.
- ▶ The pipette tip must not move faster than the leading edge of the oil wicking under the flow cell.
- ▶ Avoid touching the edge of the flow cell with the pipette tip. The oil can wick to the top surface of the flow cell.
- ▶ Do not apply oil too close to the ends of the prism. Maintain a distance of 1 cm from each end of the prism.



CAUTION

Underloading the oil will cause a loss of illumination in lane 8 and nearest the inlet and outlet manifolds. Overloading could cause oil to wick over the imaging surface during the course of the run or spill over the right surface of the prism. Wicking may happen immediately, or later during a run when the oil heats up.

Procedure

1. Aspirate the required amount of oil into the pipette, ensuring that there are no air bubbles in the oil in the pipette tip. Wipe the outside of the tip with a lens cleaning tissue.
 - If you are using a Genome Analyzer II, aspirate 100 μ l of oil.
 - If you are using a Genome Analyzer IIx, aspirate 150 μ l of oil.You may not need to dispense all of the immersion oil.
2. Place the pipette tip on the prism at the gap between the top surface of the prism and the front-left side of the flow cell, about 1 cm beyond the inlet manifold. Hold the pipette with two hands, using one hand to support and guide the tip.

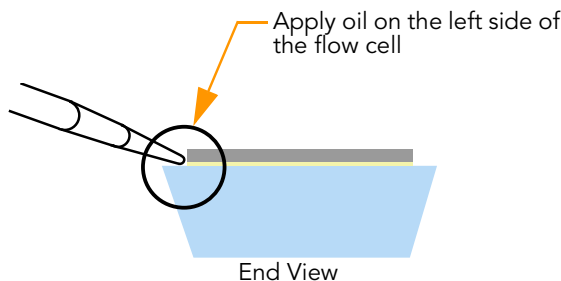


Figure 54 Pipette Tip Location

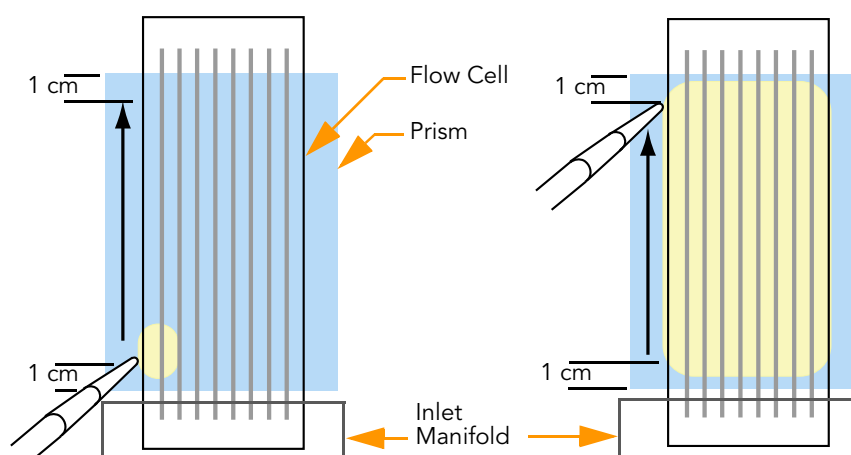


Figure 55 Applying Oil

3. Dispense the oil slowly from the left side; dispensing too fast will result in oil on the top of the flow cell.
4. Before the oil wicks to the right side of the flow cell, slide the pipette tip in small steps towards the rear, steadily dispensing more oil along the way.
Observe the movement of the oil. Ensure there are no bubbles forming between the flow cell and the prism.
5. Stop moving the pipette when the tip is about 1 cm short of the rear manifold.
You have dispensed enough oil when it has wicked to the right edge of the flow cell.
6. Inspect the gap underneath the flow cell and ensure the following statements are true:
 - There is a uniform layer of oil.
 - No bubbles exist between the flow cell and prism.
 - There is no oil on the top of the flow cell.
 - The right surface of the prism is clean.
 If anything is unsatisfactory, remove the flow cell and prism, clean them both thoroughly with alcohol wipes, reload, and repeat the application.
7. Use an ethanol wipe to clean the bottom surface of the Peltier heater. This ensures that no splashed immersion oil will be stamped on the flow cell by the Peltier.
8. Close the instrument door.
9. Proceed to first-base incorporation. For more information, see the *Genome Analyzer User Guide*.



Chapter 5

Real Time Analysis

Topics

- 64 Introduction
- 65 Monitoring Runtime Statistics
- 71 Real Time Analysis Output Data
- 75 Inspecting Run Quality

Introduction

The Genome Analyzer sequencing control software (SCS) performs real time image analysis and base calling, and provides fast access to quality metrics. The analysis is performed during the chemistry and imaging cycles of a sequencing run, which saves downstream analysis time and allows you to quickly decide whether or not your run is progressing as expected.

SCS real time analysis runs automatically on the instrument computer, and is configured through the SCS interface at the beginning of a run as described in *Run Parameters Window* on page 16.



NOTE

If for any reason SCS real time analysis stops functioning, SCS will automatically resume analysis during the next cycle at the appropriate point on the flow cell. Do not restart the application manually.

Data Transfer

Depending on the configuration of the SCS real time analysis, images are deleted from the instrument computer after image analysis. This is necessary for longer runs, since there is not enough space on the instrument computer to store all images. Images are deleted only after image analysis is complete.

If for any reason SCS real time analysis runs behind, and the instrument computer has not enough space left to store images that have not been processed, SCS will stop sequencing and store the flow cell in a safe state. Sequencing can be resumed once the storage or analysis issues have been resolved.

Analysis of RTA Data

Base calling data generated with SCS real time analysis can be used directly for alignment in the GERALD module of the CASAVA software v1.6. For instructions about using GERALD, see the *CASAVA Software Version 1.6 User Guide*.

If you need to perform base calling or image analysis offline, use the OffLine Base Caller (OLB). For instructions about using OLB, see the *OffLine Base Caller User Guide*.

Monitoring Runtime Statistics

Once SCS real time analysis has started analyzing tiles, it automatically generates and opens the Status.xml file. This file provides access to the most important runtime statistics, and summarizes the analysis configuration. See below for an explanation of Status.xml.



SCS real time analysis will not start with image analysis until after cycle four, and with base calling until after cycle 12. This is normal behavior; SCS Real Time Analysis usually catches up after a few cycles.

Status.xml File

The Status.xml file (Figure 56) reports the progress of the analysis, and provides access to the most important runtime statistics. The statistics are updated as soon as there is new analysis information available. The runtime statistics can be viewed from any computer with access to the experiment run folder. Navigate to the <RunFolder>\Data folder and open the Status.xml file. The Status.xml file is transferred to the Data subfolder in the analysis folder at the end of each cycle that is processed.



Your browser may warn you about active content. If so, click the warning in the information bar and allow blocked content.

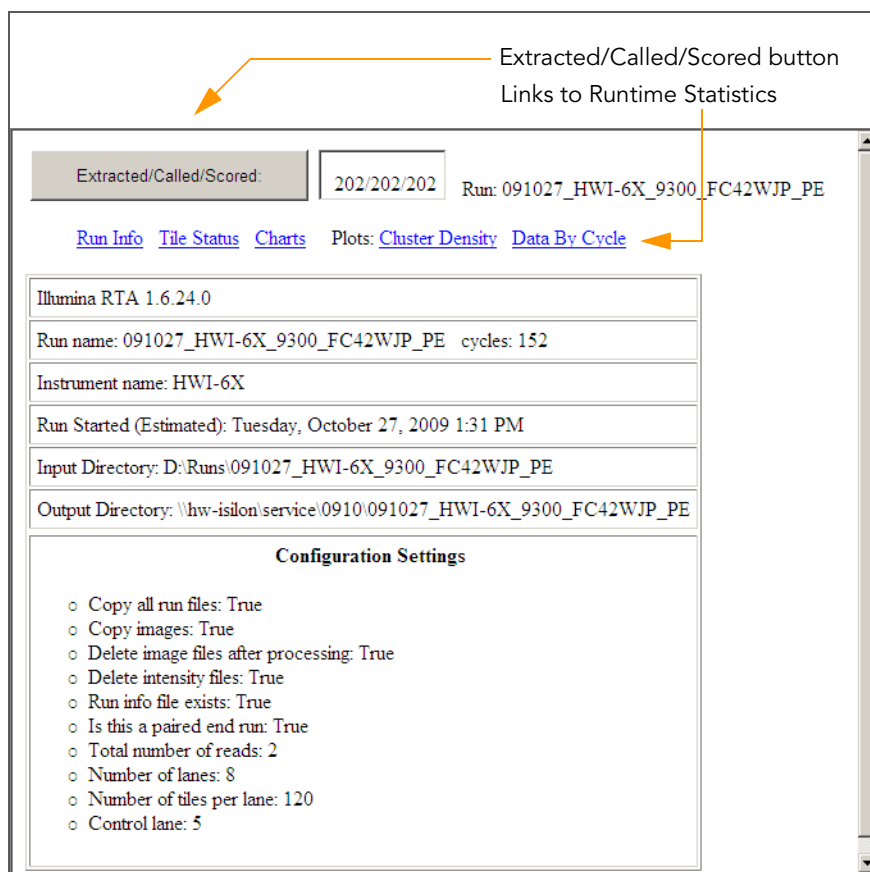


Figure 56 Status.xml file

General Run Information

The default view of the Status.xml file shows a page with general run information, such as run name, instrument name, input and output directories, and configuration settings. This page can also be reached from other pages through the Run Info link.

Tile Status Summary

The Tile Status link leads to a page showing the progress of the analysis in the Tile Status Summary (Figure 57). The colors indicate the status of the image analysis for a tile for a particular cycle, as indicated in the legend to the right. The tiles are organized by lane. Clicking the **Refresh** button makes sure you see the most recent data.

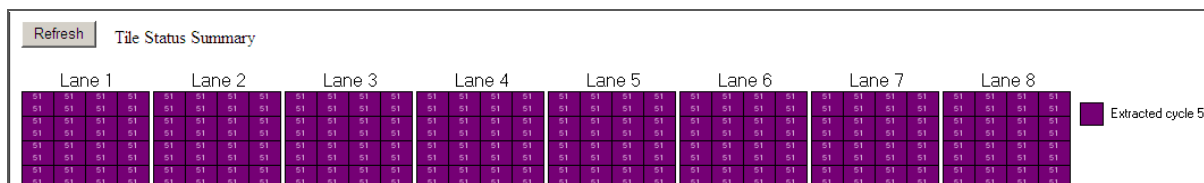


Figure 57 Tile Status Summary

In every tile, two numbers indicate the progress of base calling and quality scoring. For every tile, the top number indicates the last cycle that has been base called, and the bottom number the last cycle for which quality has been scored.

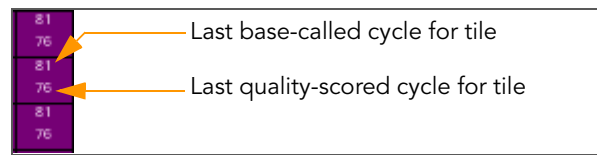


Figure 58 Base Calling and Quality Scoring Progress

Charts

The Charts link leads to a page with color coded quality metrics displayed per tile in three charts. The chart to the left displays cycle and base-independent metrics (cluster density, percentage clusters passing filter, and clusters passing filter density), which can be selected through the dropdown lists. The two charts to the right display metrics that are cycle- and base-dependent (intensity, focus quality, and percentage base calls with $Q \geq 30$).

You can select the metric, cycle, and base through the dropdown lists. The color bars to the right of each chart indicate the values that the colors represent. Tiles that have not been measured or are not monitored are gray. The interactive tooltips provide the lane, tile and value of the data point.

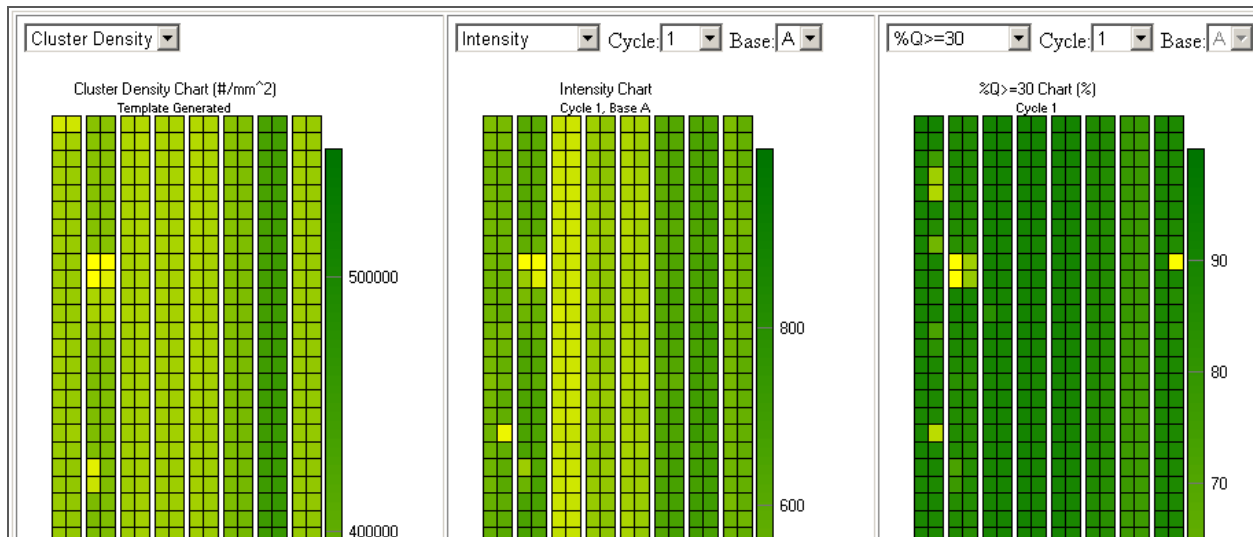


Figure 59 Quality Metrics Charts Page

You can monitor the following six different quality metrics with these charts:

- ▶ **ClusterDensity**—This chart displays the cluster density for each tile in clusters per mm^2 . This chart will be generated after the second cycle.
- ▶ **%Cluster PF**—This chart displays the percentage of clusters passing filter for each tile. These charts will not be generated until after the 25th cycle.
- ▶ **Cluster PF Density** (in SCS v2.6) —This chart displays the density of clusters passing filter for each tile in clusters per mm^2 . These charts will not be generated until after the 25th cycle.
- ▶ **Intensity**—This chart displays the intensity by color and cycle of the middle 90% of the data for each tile (the outliers, as defined by the top 5% and bottom 5%, are not used in calculations for this plot).
- ▶ **Focus Quality**—This chart displays the focus quality by color and cycle for each tile.

- ▶ **%Q>=30**—This chart displays the percentage of bases with a quality score of 30 or higher by cycle for each tile. These charts will not be generated until after the 13th cycle.

Plots

The Status.xml file displays three important quality metric plots that are updated every minute. The plots share the following features:

- ▶ The red line indicates the median tile value.
- ▶ The box outlines the interquartile range (the middle 50% of the data) for the tiles analyzed for the data point.
- ▶ The error bars delineate the minimum and maximum without outliers.
- ▶ The outliers are the values that are more than 1.5 times the interquartile range below the 25th percentile, or more than 1.5 times the interquartile range above the 75th percentile. Outliers are indicated as dots.
- ▶ The numbers above the X-axis indicate the number of analyzed tiles used for the data point.

Cluster Density

The first plot link (Cluster Density) leads to a cluster density plot, which displays the number of clusters/mm² for each lane (Figure 60). The data in this plot are populated as soon as the first cycle is processed with an estimate, and the final data appears after cycle 4. Clicking the **Refresh** button makes sure you see the most recent data. Select the **PF only** check box if you want to see the density of clusters that passed filtering.



NOTE

One tile is approximately 0.55 mm². If you judge density in clusters/tile, multiply clusters/mm² by 0.55.

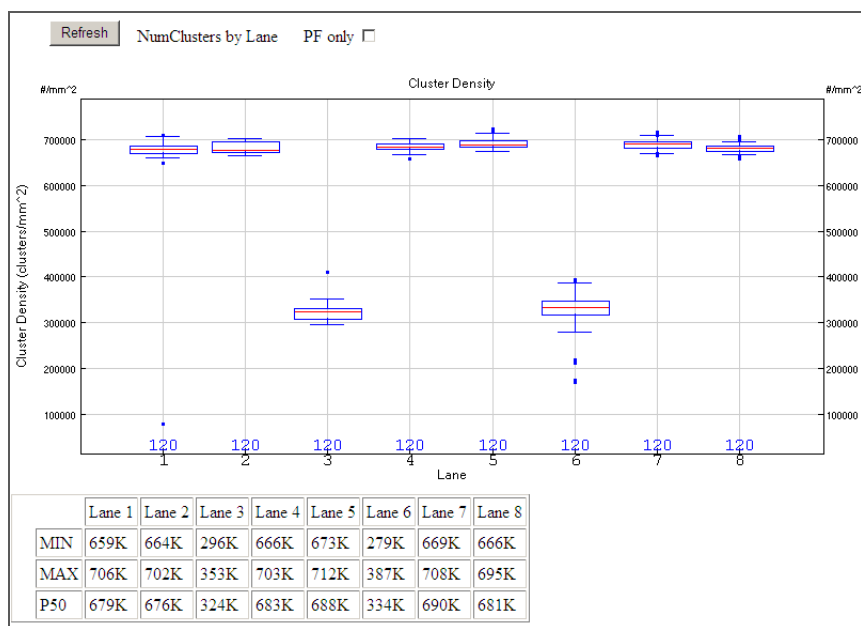


Figure 60 Numbers of Clusters by Lane

The table below the plot (in SCS v2.6) contains the following cluster density metrics that are also graphed in the plot:

- ▶ MIN—The minimum without outliers.
- ▶ MAX—The maximum without outliers.
- ▶ P50—The 50th percentile (median tile value).

Intensity & Focus Quality

The second plot link (Intensity & Focus Quality) leads to two plots for which you can pick the metric and base using the dropdown lists. If you can't view later cycles, you may need to scroll to the right. Clicking the **Refresh** button makes sure you see the most recent data.

The Intensity plot displays the intensity by base and cycle of the middle 90% of the data (Figure 61). The outliers, as defined by the top 5% and bottom 5%, are not used in calculations for this plot.

The Focus Quality plot displays focus quality by base and cycles (Figure 61).

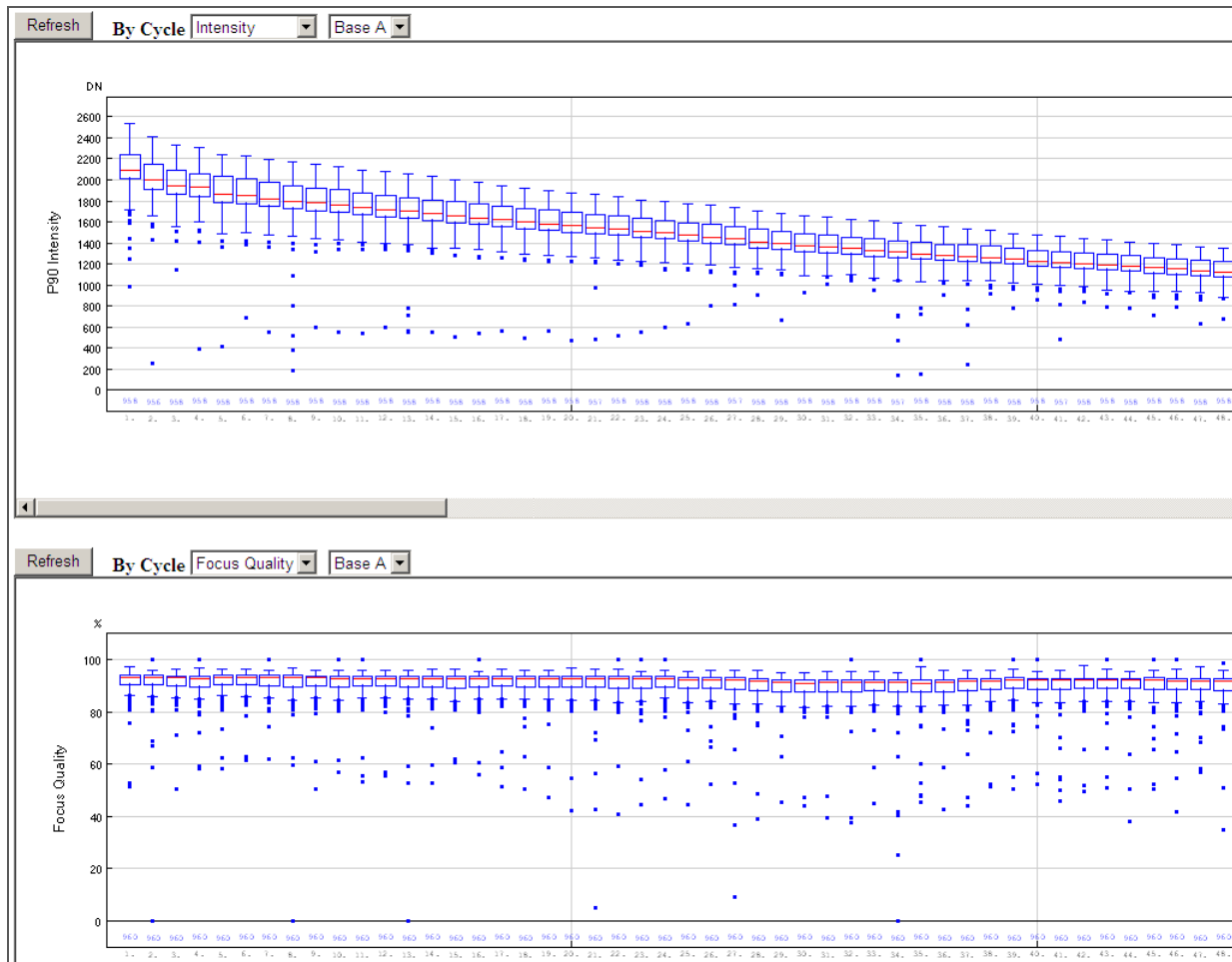


Figure 61 Intensity and Focus Quality by Color and Cycle

Extracted/ Called/Scored Button

SCS real time analysis has an **Extracted/Called/Scored** button (in SCS v2.6) that updates the Extracted/Called/Scored box next to. This box indicates the last cycle that has been extracted, called, and scored.

RunBrowser Reports

SCS real time analysis also reports real time metrics through the *_bro.xml files, which are accessed when you use the RunBrowser application. A single *_bro.xml file contains all of the statistics for all of the tiles in a given lane for a given cycle. These files are automatically produced for each cycle and transferred to the network. For more information, see *RunBrowser Reports* on page 79.

Real Time Analysis Output Data

SCS real time analysis output results are saved on the analysis server you specified during the run configuration. This section describes the purpose and location of the output data.

Real Time Analysis Folder

Figure 62 illustrates the organization of the analysis folder, including the location of the most important output files. All analysis results are saved in an analysis folder that is given the same name as the Run folder on the instrument computer.

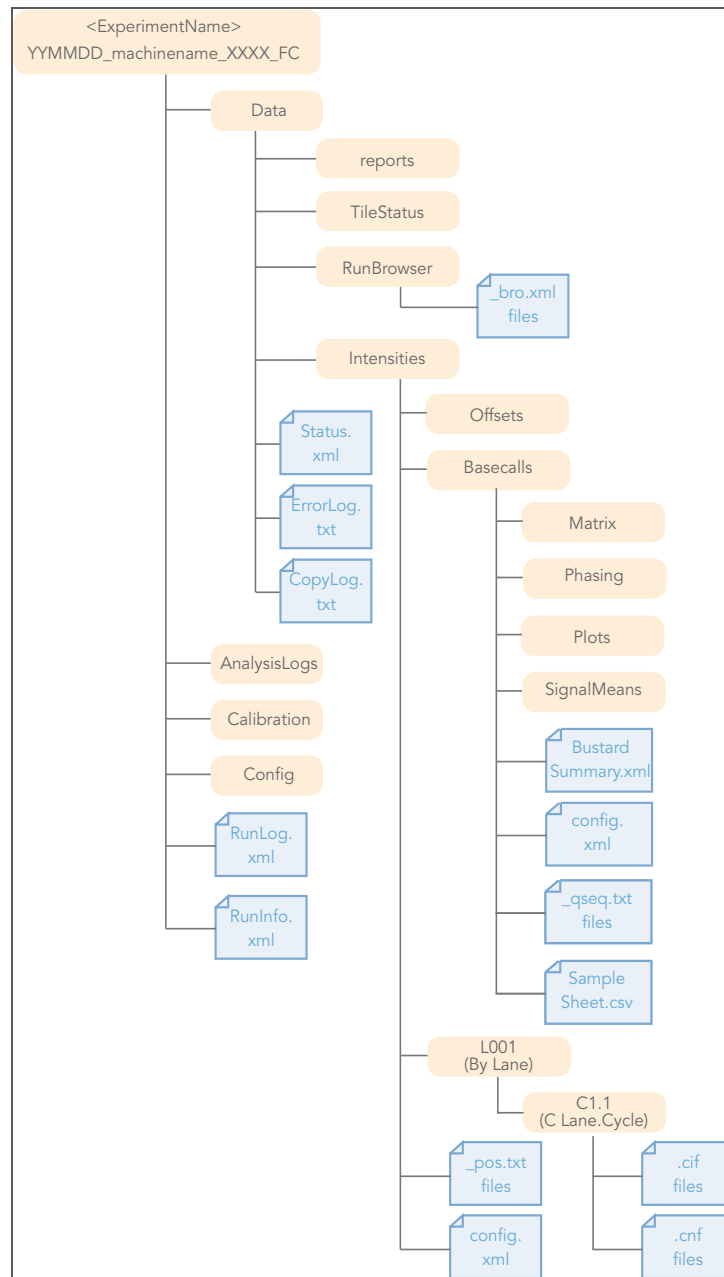


Figure 62 Real Time Analysis Folder

Analysis Results

The analysis folder contains the following key files:

Key File	Subfolder	Description
Status.xml	AnalysisFolder\Data	Contains run and analysis-specific metrics generated during the run. See <i>Monitoring Runtime Statistics</i> on page 65 for a description of the metrics.
BustardSummary.xml	AnalysisFolder\Data\Intensities\Basecalls\	Contains run- and analysis-specific metrics generated after base calling. See <i>Inspecting Run Quality</i> on page 75 for a description of the metrics.
*_qseq.txt files	AnalysisFolder\Data\Intensities\Basecalls\	Contains SCS real time analysis base calling and base quality scoring results. These files should be used in the CASAVA analysis software for GERALD alignment; for a description, see <i>Main Sequence Output Files</i> on page 73.
SampleSheet.csv	AnalysisFolder\Data\Intensities\Basecalls\	Contains sample information, including multiplexing information.
config.xml	AnalysisFolder\Data\Intensities\ AnalysisFolder\Data\Intensities\Basecalls\	Contains meta-information about the run, image analysis, and base calling.
RunLog.xml	AnalysisFolder\	The RunLog files contain the run information of the sequencing run. The RunBrowser application uses these files to perform analysis and reporting.
RunInfo.xml	AnalysisFolder\	The file RunInfo.xml identifies the boundaries of the reads (including index reads), the Flowcell ID (if available), and quality table selected for run
ErrorLog.txt	AnalysisFolder\Data	Any error during an SCS real time analysis run is captured here. If there are no errors, no ErrorLog.txt file will be present.
CopyLog.txt	AnalysisFolder\Data	All copy events during an SCS real time analysis run are captured here.
*_bro.xml files	AnalysisFolder\Data\RunBrowser\	The *_bro.xml files are used by the RunBrowser application to perform analysis and reporting. Each file contains run quality metrics in one file per lane, per cycle.
*.cif files	AnalysisFolder\Data\Intensities\L00X\CX.X	Each binary *.cif files contains SCS real time analysis image analysis results for one cycle, one tile. These files should be used in the OLB analysis software if you want to perform BUSTARD base calling; for a description, see the <i>Off-line Base Caller User Guide</i> .
*.cnf files	AnalysisFolder\Data\Intensities\L00X\CX.X	The *.cnf files have the same format as *.cif files, but contain noise information.
*_pos.txt files	AnalysisFolder\Data\Intensities\	Reports the cluster coordinates. There is one *_pos.txt file for each tile. These files should be used in the OLB analysis software if you want to perform BUSTARD base calling.

Main Sequence Output Files

The main sequence output files from SCS real time analysis are the `_qseq.txt` files. There is one `_qseq.txt` file for each tile. The `_qseq.txt` files have the following format:

- ▶ Machine name—Unique identifier of the sequencer.
- ▶ Run number—Unique number to identify the run on the sequencer.
- ▶ Lane number—Positive integer (currently 1–8).
- ▶ Tile number—Positive integer.
- ▶ X: X coordinate of the spot. As of RTA v1.6, OLB v1.6, and CASAVA v1.6, the X and Y coordinates for each clusters are calculated in a way that makes sure the combination will be unique. The new coordinates are the old coordinates times 10, +1000, and then rounded.
- ▶ Y: Y coordinate of the spot. As of RTA v1.6, OLB v1.6, and CASAVA v1.6, the X and Y coordinates for each clusters are calculated in a way that makes sure the combination will be unique. The new coordinates are the old coordinates times 10, +1000, and then rounded.



NOTE

Due to rounding differences, the X and Y positions reported in `qseq` files generated by RTA and Off-Line Basecaller (OLB) can differ by one.

Getting off-by-one can happen because the coordinates in the `pos.txt` file are already rounded off, and these are used by OLB to generate the `qseq` position values, essentially rounding the value twice. RTA does not use rounded values to generate the `qseq` position values.

Example: If you take 10.499 and round to the nearest integer, you get 10. If you take 10.499 and round to the nearest tenth-of-an-integer you get 10.5, and if you round that to the nearest integer, you get 11.

- ▶ Index—Index sequence or 0. For no indexing, or for a file that has not been demultiplexed yet, this field should have a value of 0.
- ▶ Read Number—1 for single reads; 1 or 2 for paired ends.
For indexing runs, read one = 1, indexing read = 2, and read two = 3.
- ▶ Sequence.
- ▶ Quality—The calibrated quality string. Each position provides the quality score for the corresponding position in the sequence string. The quality score is in symbolic ASCII format (ASCII character code = quality value + 64).
- ▶ Filter—Did the read pass filtering? No = 0, Yes = 1.

Illumina's Read Segment Quality Control Metric

A number of factors can cause the quality of base calls to be low at the end of a read. For example, phasing artifacts can degrade signal quality in some reads, and the affected portions of these reads have high error rates and unreliable base calls. Typically, the increase in phasing causes quality scores to be low in these regions, and thus these unreliable bases are scored correctly.

However, the occurrence of phasing artifacts may not always correlate with segments of high miscall rates and biased base calls, and therefore these low quality segments are not always reliably detected by our current quality scoring methods. We therefore mark all reads that end in a segment of low quality, even though not all marked portions of reads will be equally error prone.

The read segment quality control metric identifies segments at the end of reads that may have low quality, and unreliable quality scores. If a read ends with a segment of mostly low quality (Q15 or below), then all of the quality values in the segment are replaced with a value of 2 (encoded as the letter B in Illumina's text-based encoding of quality scores). We flag these regions specifically because the initially assigned quality scores do not reliably predict the true sequencing error rate. This Q2 indicator does not predict a specific error rate, but rather indicates that a specific final portion of the read should not be used in further analyses.

This is not a read-level filter; the occurrence of consecutive Q2 values in a read does not indicate that the read itself is unreliable, but rather that only the base calls flagged with Q2 are unreliable. Note, however, that these regions are included in the Gerald error rate calculations for aligned reads. In typical sequencing runs, most reads are reliable over their entire length, and are not marked with Q2 indicators. Of the reads that are marked with the Q2 indicator, most are flagged only in the final few cycles. The number of reads marked by the quality control indicator, and the extent of the marking, can be used as an overall run quality metric.

Inspecting Run Quality

At the end of each run SCS real time analysis generates the BustardSummary.xml file, which is located in the Basecalls folder. Tables in the BustardSummary.xml file provide an overview of quality metrics for a run. At the end of the file, the BustardSummary.xml file provides links to more detailed information in the form of graphs (the IVC plots and All Intensity Plots).

Overview of Quality Metrics

The BustardSummary.xml contains five types of tables with overviews of quality metrics (described below).



NOTE

In the descriptions of the tables included in BustardSummary.xml, the terms chip and flow cell are used interchangeably.

Chip Summary

The Chip Summary contains the instrument ID and the run folder. The Chip ID field is a placeholder that currently has a value of "unknown."

Chip Results Summary

This table displays a summary of chip-wide performance statistics for the run:

- ▶ The original number of detected clusters.
- ▶ The number of clusters that passed quality filtering.
- ▶ The chip yield in kilobases. This is the sum of the product of quality-filtered clusters and bases per cluster used for analysis over analyzed lanes.

Lane Results Summary

This table displays basic data quality metrics for each lane. Apart from Lane Yield, which is the total value for the lane, all the statistics are given as means and standard deviations over the tiles used in the lane.

- ▶ **Clusters (raw)**—The number of clusters detected by SCS real time analysis image analysis.
- ▶ **Clusters (PF)**—The number of detected clusters that meet the filtering criterion.
- ▶ **1st Cycle Int (PF)**—The average of the four intensities (one per channel or base type) measured at the first cycle averaged over filtered clusters.
- ▶ **% Intensity after 20 cycles (PF)**—The corresponding intensity statistic at cycle 20 as a percentage of that at the first cycle.
- ▶ **% PF Clusters**—The percentage of clusters passing filtering.

Expanded Lane Summary

This displays more detailed quality metrics for each lane. Apart from the phasing and prephasing information, all values are tile means for the lane.

- ▶ **Clusters (tile mean) (raw)**—The number of clusters detected by SCS real time analysis of images.
- ▶ **% Phasing**—The estimated (or specified) value used by SCS real time analysis for the percentage of molecules in a cluster for which sequencing falls behind the current position (cycle) within a read.
- ▶ **% Prephasing**—The estimated value used by SCS real time analysis for the percentage of molecules in a cluster for which sequencing jumps ahead of the current position (cycle) within a read.
- ▶ **% Retained**—The percentage of clusters that passed filtering.
- ▶ **Cycle 2-4 Av Int (PF)**—The intensity averaged over cycles 2, 3, and 4 for clusters that passed filtering.
- ▶ **Cycle 2-10 Av % Loss (PF)**—The average percentage intensity drop per cycle over cycles 2–10 (derived from a best fit straight line for log intensity versus cycle number).
- ▶ **Cycle 10-20 Av % Loss (PF)**—The average percentage intensity drop per cycle over cycles 10–20 (derived from a best fit straight line for log intensity versus cycle number).

Per-Tile Statistics

Below the two types of lane summaries are per-tile statistics, grouped into a table for each lane. The statistics are a subset of those in the Lane Results Summary, but are presented as averages over the detected (raw) or filtered (PF) clusters in individual tiles.

In the event that no clusters in a tile pass filtering, all the statistics for that tile are displayed within square brackets. This suggests an exceptional situation (e.g., a bubble) within the tile. The brackets indicate the tile has been excluded from the calculation of lane statistics and that the values are reported only for diagnostic purposes.

IVC Plots

The BustardSummary.xml file provides a link to the Intensity versus Cycle (IVC) Plots at the bottom of the page. These plots display lane averages over all tiles in a lane. The plots displayed are All, Called, %Base_Calls, %All, and %Called.

- ▶ **All**—This displays the mean intensity of clusters after adjustment for cross-talk between channels. The data is plotted as a function of cycle, and each channel (A, C, G, T) is plotted separately as a different colored line. Means are calculated over all clusters, regardless of base calling.

If all bases are present in the sample at 25% of total and a well-balanced matrix is used for analysis, the graph will display all channels with similar intensities. If intensities are not similar, the results could indicate either poor cross-talk correction or poor absolute intensity balance between each channel.

- ▶ **Called**—This plot is similar to All, except means are calculated for each channel using clusters that the base caller has called in that channel.

If all bases are present in the sample at 25% with pure signal (zero intensity in the non-called channels), the Called intensity will be four times that of All, as the intensities will only be averaged over 25% of the clusters. For impure clusters, the difference in intensity will be less than four times that of All.

The Called intensities are independent of base representation, so a well-balanced matrix will display all channels with similar intensities.

- ▶ **%Base_Calls**—The percentage of each base called as a function of cycle. Ideally, this should be constant for a genomic sample, reflecting the base representation of the sample. In practice, later cycles often show some bases more than others. As the signal decays, some bases may start to fall into the noise while other still rise above it. Matrix adjustments may help to optimize data.
- ▶ **%All** and **%Called**—Exactly the same as All and Called, but expressed as a percentage of the total intensities. These plots make it easier to see changes in relative intensities between channels as a function of cycle by removing any intensity decay.

All Intensity Plots

The BustardSummary.xml file provides a link to the All Intensity Plots at the bottom of the page. These plots give a tile-by-tile representation of the mean matrix-adjusted intensity of clusters plotted as a function of cycle. Each channel (A, C, G, T) is represented as a different colored line. Means are calculated over all clusters, regardless of base calling.

If all bases are present in the sample at 25% of total and a well-balanced matrix is used for analysis, the graph will display all channels with similar intensities. Dissimilar intensities could indicate either poor cross-talk correction or poor absolute intensity balance between each channel. A genome rich in GC content may not provide a balanced matrix for accurate cross-talk correction and absolute intensity balance.



For large experiments, All.htm only shows a subset of tiles. However, each file contains links to the full output results. The full output files may take some time to open.



Chapter 6

RunBrowser Reports

Topics

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Introduction

The primary function of RunBrowser is to provide a graphical view of run metrics, so that you can decide whether or not to complete the run. You can view the data quickly in a summary window, or generate reports that you can print, save, or export.



NOTE

If you install RunBrowser on your desktop, make sure you have .Net Framework 3.5 and Crystal Reports for .Net 3.5 installed.

The instrument computer already contains the prerequisites to run RunBrowser.

By default, RunBrowser automatically launches the First-Cycle report after the completion of first-base incorporation, and the Quality Metric Deviation report at the conclusion of a normal recipe. RunBrowser uses the file RunLog_MM-DD-YY_HH-MM-SS.xml for these reports.

Other RunBrowser reports require the generation of *_bro.xml files. SCS real time analysis generates these files by default for each cycle.

User Interface

The RunBrowser interface has five main windows:

- ▶ The **Flow Cell window** provides a graphical interface for quickly gathering data and seeing it in color with interactive tooltips.
- ▶ The **Report window** enables you to create textual reports on the same data.
- ▶ The **Metric Deviation Report window** summarizes significant cycle-to-cycle deviations of key QC values, so that problematic cycles in the run can be identified.
- ▶ The **ImageViewer** displays the image for a selected tile, if available.
- ▶ **Chart windows** allow you to monitor run quality on selected tiles.

Launching RunBrowser

This section describes the options to launch RunBrowser.

Automatically

During installation, RunBrowser is configured to automatically generate and open the First-Cycle Report after first-base incorporation, and the Quality Metric Deviation report is generated at the end of a normal recipe. The RunBrowser user interface also allows you to view data stored in the *_bro.xml files, which SCS real time analysis automatically generates for each cycle.

Manually

If you wish to launch RunBrowser manually at any point:

- ▶ Navigate to C:\Illumina\SCS<version>\RunBrowser\bin\Release and double-click RunBrowser.exe.

Illumina recommends that the RunBrowser not be run on the instrument PC while data collection is in progress. Also, it should not share the Run folder on the instrument PC. Instead, RunBrowser should be run on any other available workstation with access to the Run folder on the server.

Open Log Files

1. Select **File | Open**.
2. Navigate from the workstation running RunBrowser to the following location on the server with the Run folder:
 - If you ran a First-Base Incorporation recipe:
Illumina\SCS<version>\bin\Service\<DateTimeStamp>\
 - If you ran a Normal recipe:
Runs\<Run folder>\
3. Open the log files from the recent run (Ctrl- or Shift-select to select more than one file).

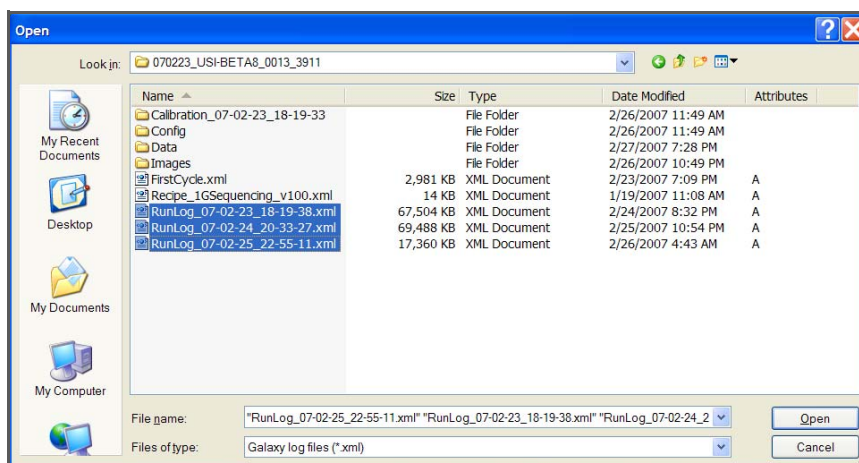


Figure 63 Open Log File at the Normal Recipe Folder Location

It may take a few moments for the files to load. A progress bar in the lower-left hand corner indicates the number of files loaded. If *_bro.xml files exist in the RunBrowser folder (<\$RunFolder>\data\runbrowser\), they will be loaded automatically for the cycles that have been opened.

Using Flow Cell Window

The data from the run appear in the Flow Cell window, along with a map of the flow cell. Select tiles on the map of the flow cell by using the right button of your mouse.

You can check data in this window by selecting different quality metrics and mousing over the flow cell to see values for each tile, as described below.

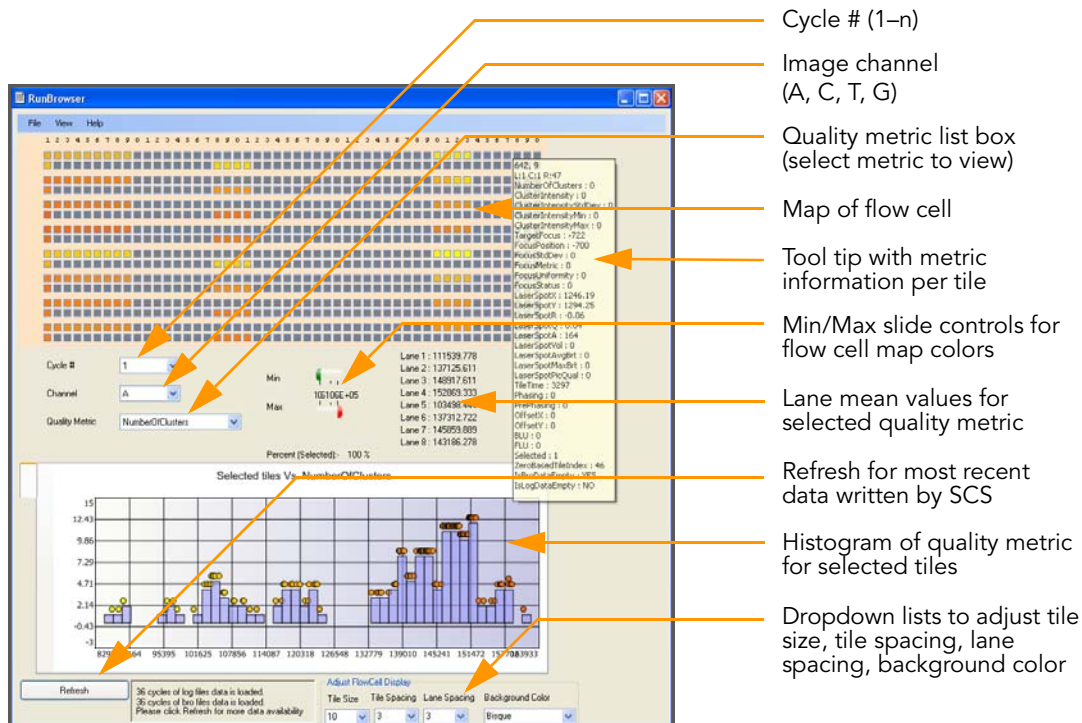


Figure 64 Flow Cell Window

Refresh RunBrowser Data

RunBrowser has a Refresh feature in the bottom-left corner of the Flow Cell window (Figure 64). RunBrowser is able to load a log file that is currently being written by the SCS software; clicking **Refresh** loads the most recent data. In addition, if any new log files are added, one click on **Refresh** loads those new files into RunBrowser.

Browse Metric Data

Using the dropdown lists named Cycle #, Channel, and Quality Metric dropdown lists (Figure 64), you are able to navigate to the data of a certain cycle, certain channel, and certain quality metric. Hovering the mouse over a tile on the flow cell map will show the associated data in a tool tip.

Adjust Display

You can adjust minimum and maximum values of quality metrics. Tiles below the minimum are colored green, and tiles above the maximum are colored red (Figure 64, Min/Max Slide Controls for Flow Cell Map). The color of tiles that have metric values in between minimum and maximum are calculated and updated accordingly.

Dropdown lists at the bottom of the RunBrowser window let you change tile size, tile spacing, lane spacing, and background color of the flow cell map.

ImageViewer

Once data are loaded into RunBrowser, you can view images by double-clicking a tile on the flow cell map (Figure 65). ImageViewer displays the image if it exists for that tile for the selected cycle.

The image captured by the camera has a dynamic range of 12 bits per pixel (range of 0–4095), while the monitor displays a range of 8 bits (range of 0–255). The ImageViewer will autoscale the image that is chosen, setting the Maximum intensity value as 255 and the Minimum value as 0. You can customize the sensitivity of the image in the following ways:

- ▶ The sliders allow you to change the contrast of the image:
 - By reducing the Max slider, you can differentiate darker portions of the image (at the expense of saturating the higher intensity pixels).
 - By increasing the Min slider, you can differentiate brighter portions of the image (at the expense of losing the lower intensity pixels).
- ▶ Clearing the GrayScale checkbox gives two more options:
 - The BGR color setting improves the appearance of lower intensity contrasts.
 - The RGB color setting improves the appearance of higher intensity contrasts.

In addition, ImageViewer displays two histograms that help you judge the images. The input histogram displays the raw intensity value of the pixels on the Y axis and the number of pixels in base 2 logarithmic scale on the X axis. The output histogram displays the 8-bit adjusted values. Note that the number of pixels will increase or decrease based upon the adjustment of the Min/Max sliders.

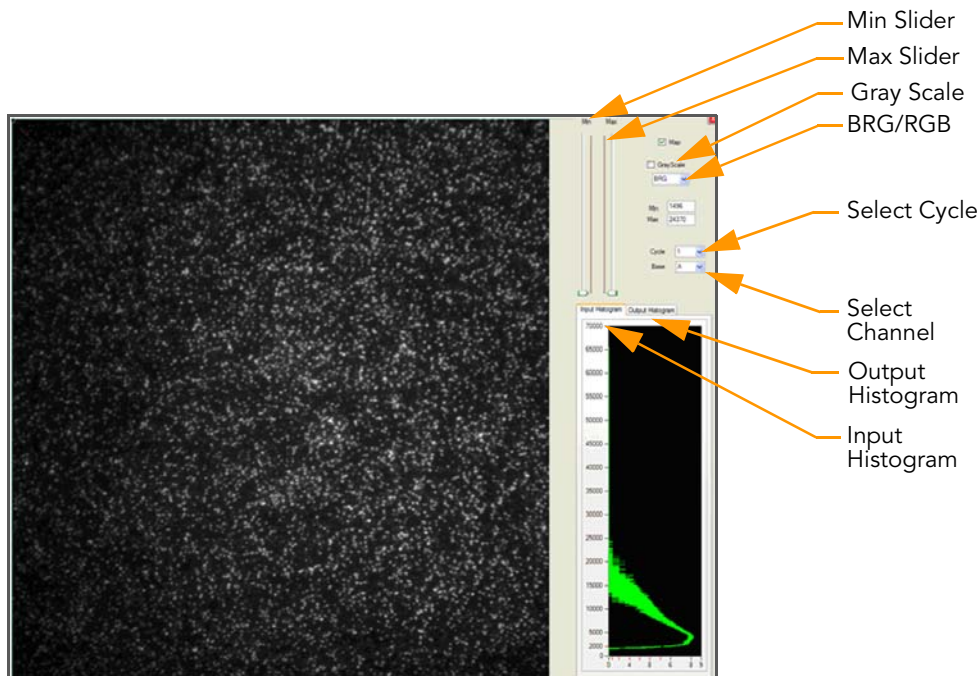


Figure 65 ImageViewer

View Quality Metrics

You can display selected quality metrics for selected tiles in the histogram at the bottom of the Flow Cell window. Select the desired cycle, channel and quality metric, then click and drag the mouse to select a section of the flow cell in the flow cell map. Release the mouse button to plot a histogram of the selected quality metric for the selected tiles. Tiles appear as circles on the chart and have tool-tip information available.

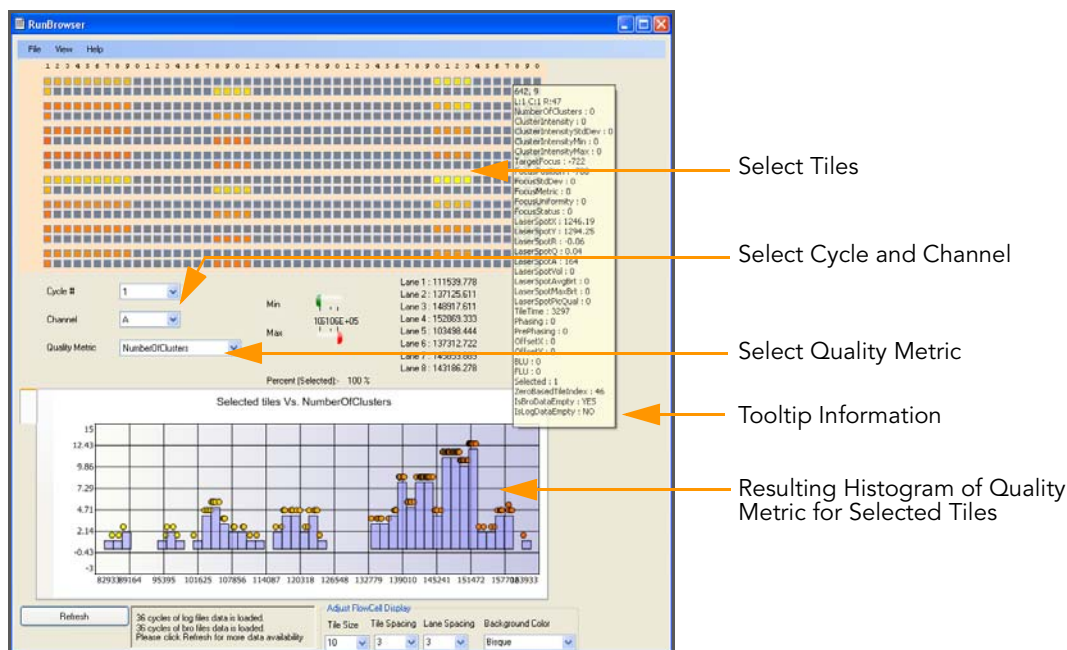


Figure 66 Histogram of Selected Quality Metrics

View Charts for Selected Tiles

You can generate two additional charts for selected tiles to monitor run quality. Click and drag the mouse to select a section of the flow cell in the flow cell map. Right-click to view the context menu on the flow cell map, which lets you plot two types of graphs or copy the flow cell map graphic into the clipboard.

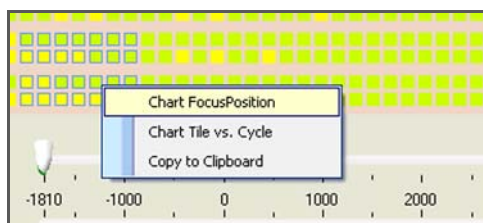


Figure 67 Select Tiles and Chart



NOTE

For longer runs, RunBrowser 2.4 divides the display of charts into 1–50, 51–100, 101–150, and so on. RunBrowser creates entries in the context menu to plot quality metric charts for a range of ≤ 50 cycles per menu. Clicking one of those menus will display charts for the cycles in that range. To avoid any out-of-memory error, we recommend that you load about 50 cycles of data at a time, and then move on to the next 50 cycles.

Chart FocusPosition plots the focus position against selected tiles, for different cycles (Figure 68).

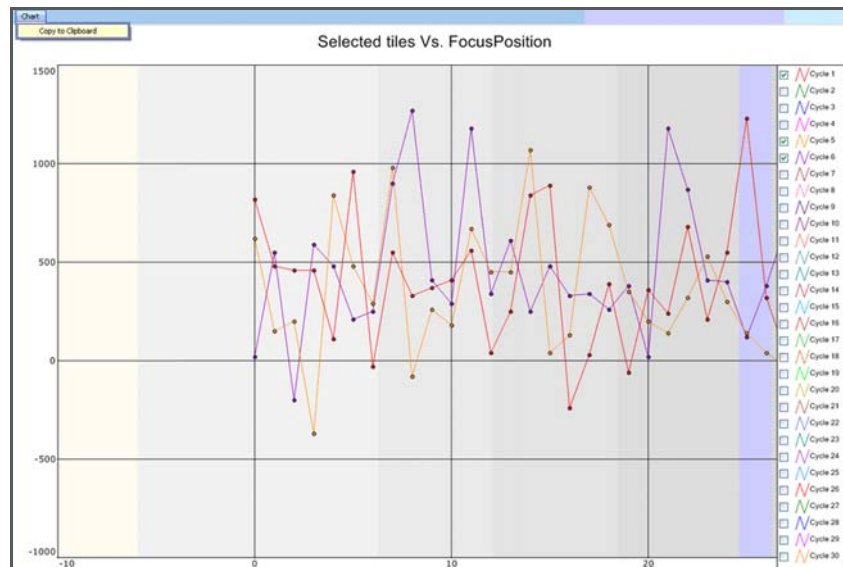


Figure 68 Chart FocusPosition

Chart Tile vs. Cycle plots the focus position against selected cycles, for different tiles (Figure 69).

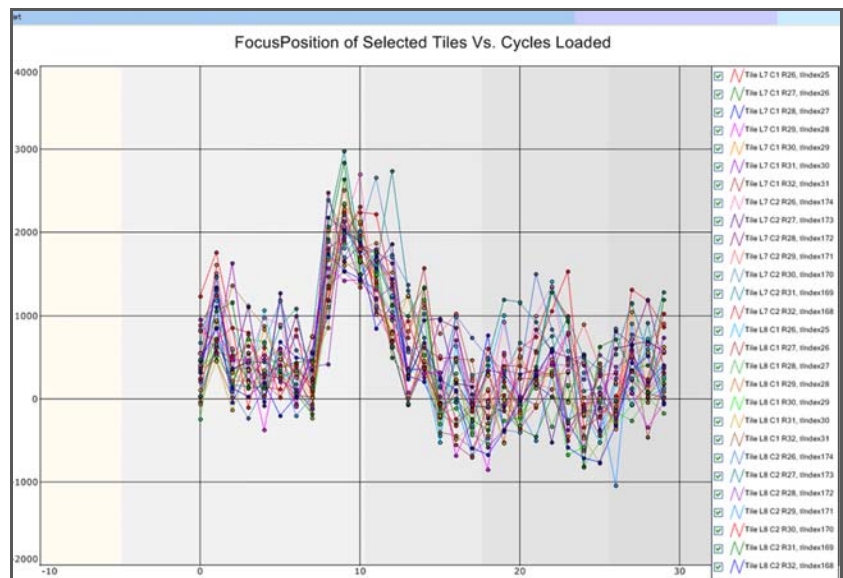


Figure 69 Chart Tile vs. Cycle

Checking First-Cycle Results in the Flow Cell Window

This section explains how to check some useful first-base incorporation metrics in the Flow Cell window. You should also generate a First-Cycle report to see a complete summary of the data. For more information, see *Run a Report* on page 90.

1. Follow the instructions in *Launching RunBrowser* on page 81 to retrieve the data from a first-base incorporation.
2. To check the tilt of the flow cell:
 - a. Select **Focus Position** from the **Quality Metric** list box.
The map of the flow cell changes to show focal positions at the front, middle, and rear of the flow cell.
 - b. Ensure that **Cycle #** is set to 1.
This value is the same for all channels, so you do not need to change the channel selection.
 - c. Subtract the minimum focus stage level from the maximum. The difference should be less than 15,000 nm.
To view the focal position of a given tile, hover the mouse over the tile in the flow cell map.

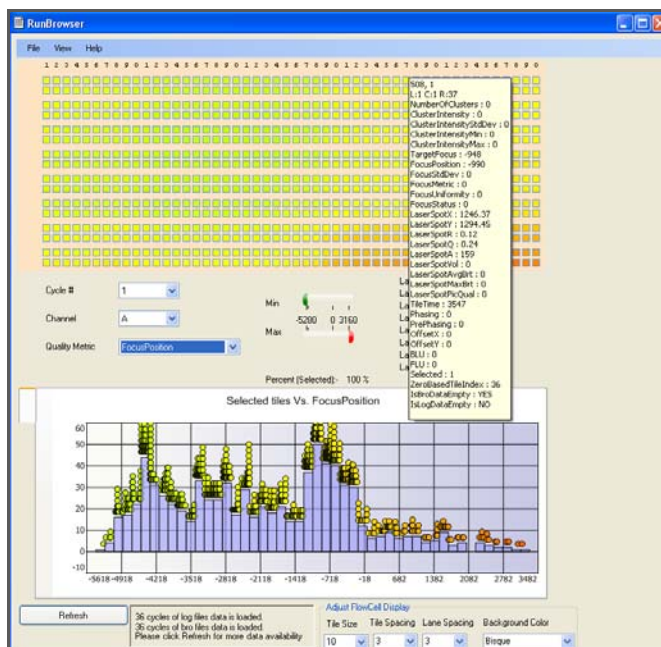


Figure 70 Focus Stage Level

- 3. To check the cluster intensity values:
 - a. Select Cluster Intensity from the **Quality Metric** dropdown list.
 - b. Ensure that **Cycle #** is set to 1.
 - c. Select each channel in turn from the **Channel** dropdown list.

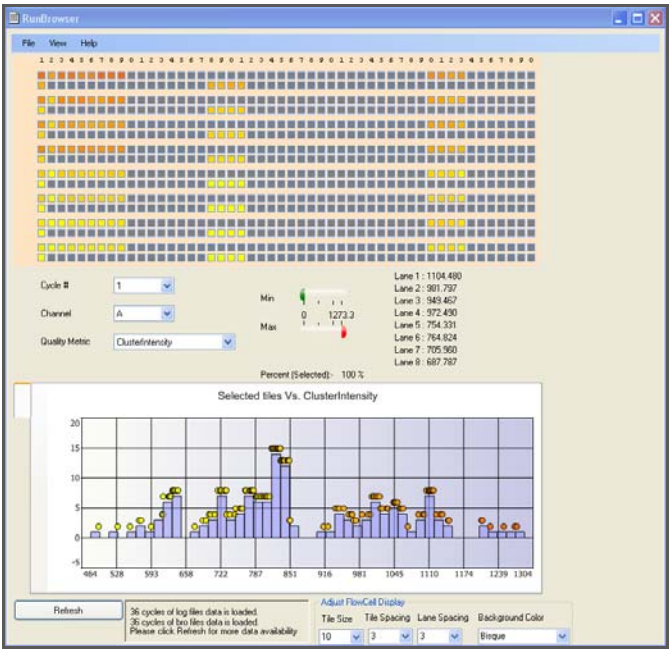


Figure 71 Cluster Intensity Levels

- The average intensity of the selected tiles for each lane appears in a list to the right of the Min/Max bars.
- d. Evaluate the intensity values for each channel using the guidelines in the following table:

Confidence Level	A	C	G	T
High Confidence	> 800	> 800	> 2000	> 1500
Reasonable Confidence	800–500	800–550	2000–1500	1500–1000
Low Confidence	< 500	< 550	< 1500	< 1000

4. To check the focal quality:
 - a. Select Focus Metric from the **Quality Metric** dropdown list.
 - b. Ensure that **Cycle #** is set to 1.
 - c. Select each channel in turn from the **Channel** dropdown list.

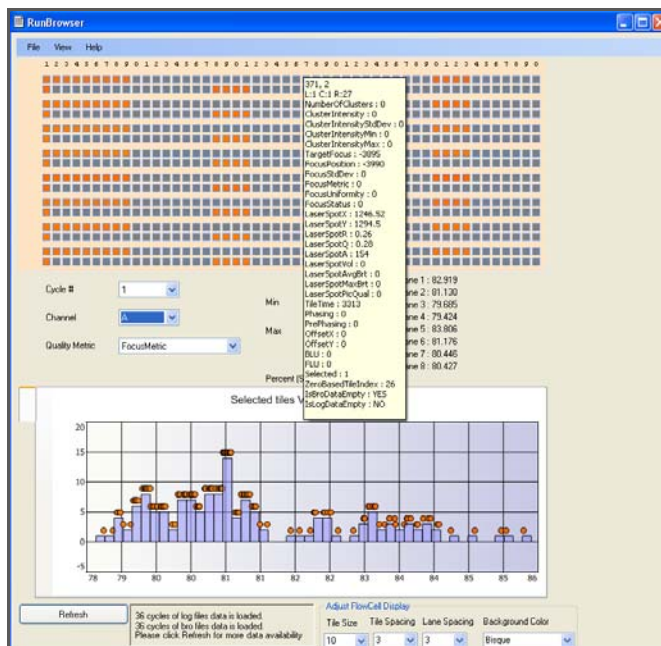


Figure 72 RunBrowser Focus Metric

The average focal quality of the selected tiles for each lane appears in a list to the right of the Min/Max bars.

- d. Ensure that the focus metric for each lane is greater than 70. If the focus metric for a lane is lower than 70, the flow cell surface may be dirty, or oil may not cover the entire flow cell-prism interface. Inspect the flow cell. You may need to clean and reload the prism and flow cell as described in *Installing the Prism* on page 52 and subsequent sections.

Viewing Metrics Reports

RunBrowser generates two types of reports viewed in the Report Window: a First-Cycle report and metric reports.

First-Cycle Report

The First-Cycle report contains summary data about first-base incorporation. Illumina recommends generating a First-Cycle report after performing first-base incorporation and using it to make an informed decision about whether to continue the run.

The report lists metrics for the cluster number counts, intensity values, focus metric, focus position, and flow cell tilt.

Metric Reports

RunBrowser metric reports describe the results of statistical operations performed on the tiles in a lane during a cycle. The possible statistical operations are:

- ▶ Minimums
- ▶ Maximums
- ▶ Medians
- ▶ Means
- ▶ Standard Deviations (SDs)

For recommendations on how to use these reports to assess run data, see *Cycle-to-Cycle Metrics* on page 97.

Run a Report

After opening the log file as described in *Launching RunBrowser* on page 81, generate text reports that you can then print, save, or export as follows:

1. Select **View | Report**. The Report window opens.

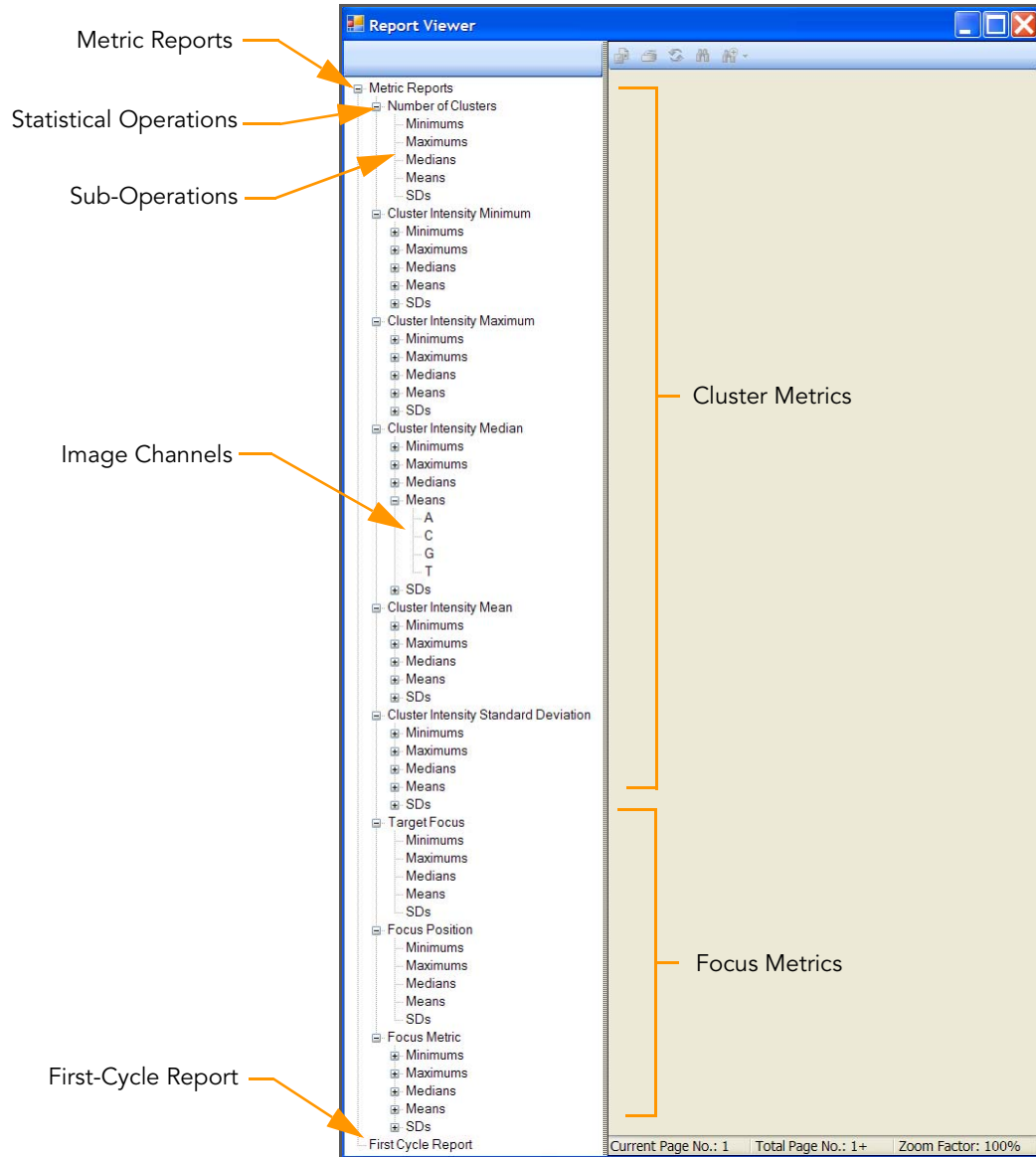


Figure 73 Empty Report Window

2. To generate a First-Cycle report, click **First-Cycle Report** in the left sidebar.

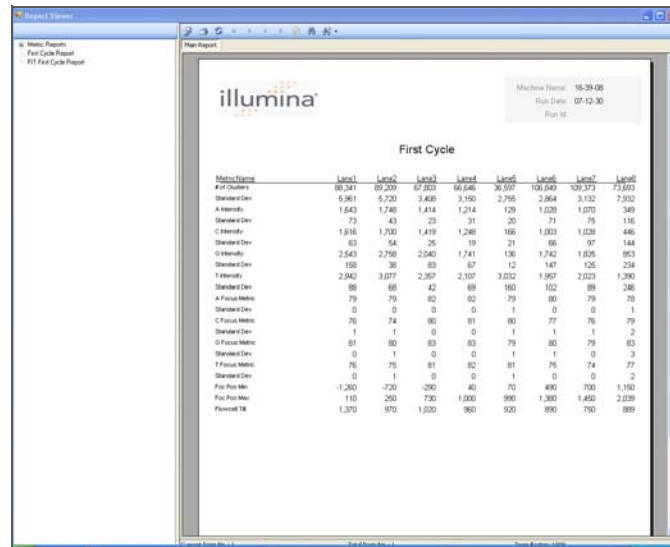


Figure 74 First-Cycle Report Example

3. To generate a Metrics report:
 - a. Click **Metric Reports** in the left sidebar.
 - a. Expand one of the metrics, such as Cluster Intensity Median.
 - b. Select the statistical operation with which to summarize the tiles in each lane of a cycle (Min, Max, Median, Mean, or SD).
 - c. [Optional] If available, select one of the four image channels (A,C, G, T). This only applies to metrics that have different values for each channel.

When you select a report, the report data appear in the right pane of the Report window.

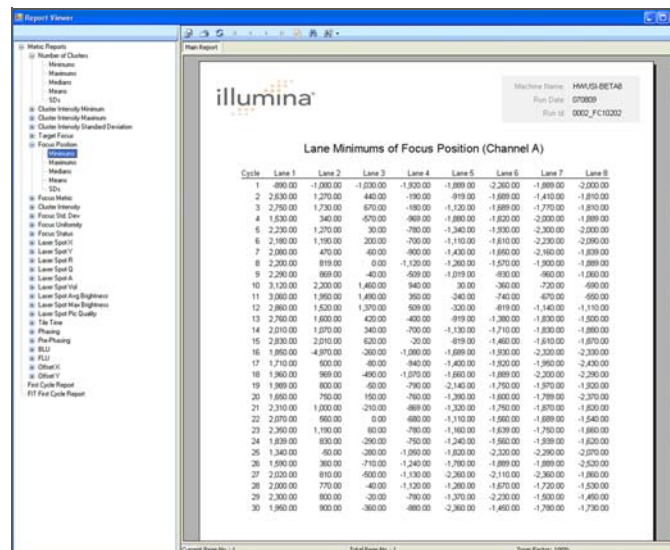












Figure 75 Metric Report Example

4. Click a toolbar button to perform the associated action. The following table describes the toolbar buttons.

Table 7 RunBrowser Report Viewer Toolbar Buttons

Button	Function
	Export in one of the following formats: <ul style="list-style-type: none"> • Crystal Reports (*.rpt) • Portable Document Format (*.pdf) • Microsoft Excel (*.xls) • Microsoft Excel Data Only (*.xls) • Microsoft Word (*.doc) • Rich Text Format (*.rtf)
	Print report
	Refresh window
	Display the first page of the report
	Show the previous page of the report
	Show the next page of the report
	Show the last page of the report
	Go to page (specify page in pop-up window)
	Find text
	Zoom view (options provided in cascading menu)

Cluster Metrics Measure Cluster Quality

The following reports help you evaluate cluster quality before continuing a run. Each one performs a statistical operation on an individual image channel (A, T, C, G) during a given cycle.

Table 8 Measure Cluster Quality

Statistical Operation	Description
Cluster Intensity Median	The median intensity of the selected channel, per tile.
Cluster Intensity Mean	The mean intensity of the selected channel, per tile.
Cluster Intensity Minimum	The minimum cluster intensity of the selected channel, per tile.
Cluster Intensity Maximum	The maximum cluster intensity of the selected channel, per tile.

Table 8 Measure Cluster Quality (Continued)

Statistical Operation	Description
Cluster Intensity Standard Deviation	The standard deviation of the selected channel, per tile.

Focus Metrics Measure Image Quality

The following reports help you evaluate the image quality of the run. The data come from the *bro.xml file, where the software records information on the Z-stage position and image quality of each tile.

Table 9 Measure Image Quality

Statistical Operation	Description
Target Focus	The Z-stage position of the target focus, as calculated by the auto-focus algorithm. The position is the same for all channels.
Focus Position	The actual Z-stage position that the hardware reports to the control software after moving. Compare this metric with the Target Focus values to evaluate the autofocus control loop. The position is the same for all channels. The focus position should stay relatively constant between cycles. If there are significant jumps, it may indicate that the lens is going in and out of focus.
Focus Standard Deviation	The standard deviation of the Z-stage position of a tile over all the cycles in the run log(s) currently loaded in RunBrowser. This is the only focus metric that is calculated per tile over all cycles. If you see a large variation in the Z-stage positions (StdDev > 6,000 nm), it indicates poor focus control or the introduction of a significant number of bubbles into the system.
Focus Metric	A number that represents the sharpness or focus quality of the image. It is calculated separately for each channel. A high-quality image will have a focus metric above 70.
Focus Status	A number corresponding to a warning message (Table 10), if there is one, for each tile. You should take these numbers into account when deciding whether or not to complete the run.

Warning Messages

Table 10 Focus Status Warning Messages

Number	Warning Message	Move Z Axis?
0	No warning message.	Yes
1	Poor laser spot quality or parameter. Spot has high q residual.	Yes
2	Poor laser spot quality or parameter. Spot has outlier spot chars (the volume of the spot exceeds threshold).	No
3	Poor laser spot quality or parameter. Spot has high q residual and outlier spot chars.	No
4	Poor laser spot quality or parameter. Spot has low picture quality.	No

Table 10 Focus Status Warning Messages (Continued)

Number	Warning Message	Move Z Axis?
5	Poor laser spot quality or parameter. Spot has high q residual and low picture quality.	No
6	Poor laser spot quality or parameter. Spot has outlier spot chars and low picture quality.	No
7	Poor laser spot quality or parameter. Spot has high q residual, outlier spot chars, and low picture quality.	No

Laser Spot Metrics

Measure Autofocus Performance

These reports help you evaluate the success of the autofocus subsystem over the course of the imaging cycle. They can indicate when there is air in the system. Histograms of these metrics will highlight problematic tiles. You should use these metric to look for outlying values from a mean.

Table 11 Measure Autofocus Performance

Statistical Operation	Description
Laser Spot X	The X pixel position of the center of light of the autofocus (AF) laser spot on the tile image for the selected cycle.
Laser Spot Y	The Y pixel position of the center of light of the AF laser spot on the tile image for the selected cycle.
Laser Spot r	The r value calculated from the image of the selected cycle. Large changes in r can indicate air in the system.
Laser Spot q	The q value calculated from the image of the selected cycle. Large noise in q can indicate poor setup of the autofocus system.
Laser Spot W	This metric is not currently in use.
Laser Spot H	This metric is not currently in use.
Laser Spot A	The area, in pixels, of the detected laser spot above the detection threshold.
Laser Spot P	This metric is not currently in use.
Laser Spot D	This metric is not currently in use.
Laser Spot Vol	The average brightness (above the detection threshold) multiplied by the area of the primary laser spot. A large increase in volume indicates air in the system.
Laser Spot Average Brightness (Avg Brt)	The sum of the gray values of the pixels in the laser spot, divided by the area.
Laser Spot Maximum Brightness (Max Brt)	The maximum gray value of the pixels in the laser spot, divided by the area.
Laser Spot Pic Quality	The average of the normalized autocorrelation of the image with itself, with shifts of unit pixel to the left and down. If the image is noisy, the measure will be low because the noise does not correlate with itself.

Phasing Metrics Measuring Cycle Independence

The Genome Analyzer Pipeline uses phasing and prephasing to remove signal components from the next (prephasing) and previous (phasing) cycles. The window used in the Pipeline is cycles 3–12, from which the median per lane is calculated. SCS real time analysis mimics the Pipeline calculations for phasing and prephasing **only** for cycle 12. This cycle can be viewed to determine the amount of interference from adjacent cycles.

To view these reports, expand **Metric Reports | Phasing | Median** or **Metric Reports | Pre-Phasing | Median** in the Report Viewer.

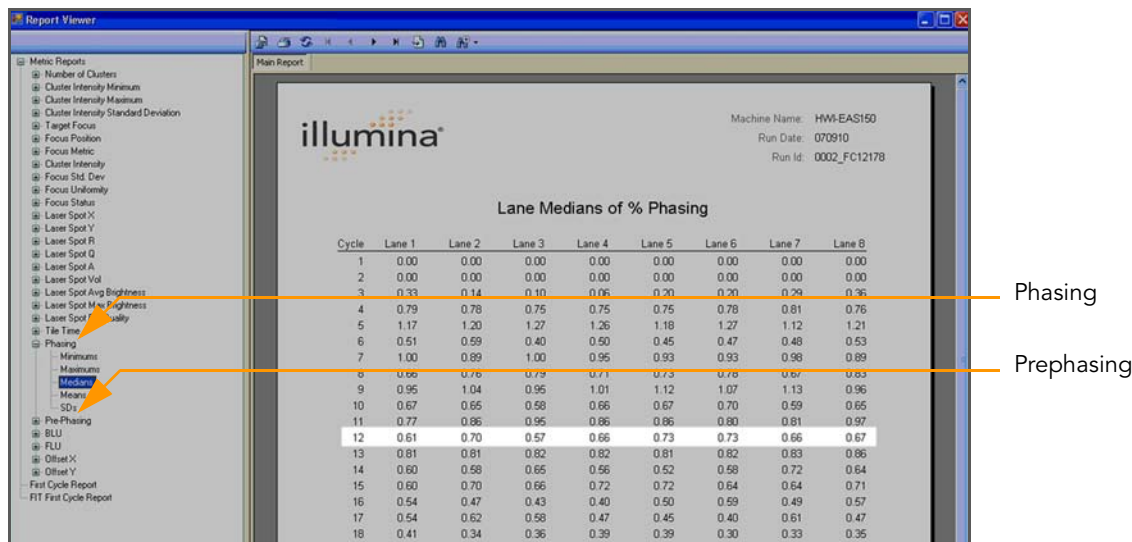


Figure 76 Phasing Report

Phasing/prephasing calculation requires intensity data from previous cycles. SCS real time analysis performs phasing/prephasing calculations after the last tile from the cycle is processed. The normal range of 0.1% to 1.0% is the signal contribution percentage from the next or previous cycle to the current cycle. High values (1.0%–2.0%) may indicate reagent or fluidics problems.



NOTE

While RunBrowser displays phasing and prephasing numbers starting from cycle 3, only phasing values from cycle 12 should be expected to match the offline analysis values. All phasing/prephasing values from other cycles are for research purposes only.

In addition, for phasing and prephasing values to match offline analysis values, the default offsets and matrix file selections must be comparable.

Number of Clusters

The Number of Clusters metric shows the total number of identified clusters in each tile. It is the only cluster metric that is not specific to an image channel. If the offset file is correctly calibrated, then the values will be within 5% of the numbers reported by offline analysis.

Tile Time Measuring Software Overhead

The Tile Time metric shows the time spent imaging each tile, excluding the exposure time noted in the recipe.

Running a Metric Deviation Report

The Quality Metric Deviation (QMD) report summarizes significant cycle-to-cycle deviations of key QC values, so that problematic cycles in the run can be identified.

After opening the log file as described in *Launching RunBrowser* on page 81, select **View | Metric Deviation Report** on the RunBrowser main window to launch the QMD report, which may take several minutes. This report runs on the data of all cycles currently loaded into RunBrowser and finds the percentage of tiles per lane that crosses a threshold of approximately 25% from adjacent cycles, and summarizes the result into a report.

For an explanation of the RunBrowser Report viewer buttons, see Table 7 on page 92.

MetricName	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8
NumberOfClusters	0	0	0	0	0	0	0	0
ClusterIntensityMinA	0	0	0	0	0	0	0	0
ClusterIntensityMinC	0	0	0	0	0	0	0	0
ClusterIntensityMinG	0	0	0	0	0	0	0	0
ClusterIntensityMinT	0	0	0	0	0	0	0	0
ClusterIntensityMaxA	0	0	0	0	0	0	0	0
ClusterIntensityMaxC	0	0	0	0	0	0	0	0
ClusterIntensityMaxG	0	0	0	0	0	0	0	0
ClusterIntensityMaxT	0	0	0	0	0	0	0	0
ClusterIntensityStdDevA	0	0	0	0	0	0	0	0
ClusterIntensityStdDevC	0	0	0	0	0	0	0	0
ClusterIntensityStdDevG	0	0	0	0	0	0	0	0
ClusterIntensityStdDevT	0	0	0	0	0	0	0	0
TargetFocus	83	94	90	96	88	79	80	80
FocusPosition	84	88	89	96	91	80	85	80
FocusMetricA	0	0	0	0	0	0	0	0
FocusMetricC	0	0	0	0	0	0	0	0
FocusMetricG	0	0	0	0	0	0	0	0
FocusMetricT	0	0	0	0	0	0	0	0
ClusterIntensityA	0	0	0	0	0	0	0	0
ClusterIntensityC	0	0	0	0	0	0	0	0
ClusterIntensityG	0	0	0	0	0	0	0	0

Figure 77 Quality Metric Deviation Report

Cycle-to-Cycle Metrics

Measuring Quality Deviations

For QMD calculations, a three cycle window is analyzed to determine if there is a significant change in a quality score that is not part of a directional trend.

- ▶ Directional deviations are not counted. That is, tiles with progressively increasing trends or progressively decreasing trends across the moving window of three cycles will not be counted as deviations.
- ▶ Only ripples in trends are counted. For example if a tile's metric value increases by 25% from one cycle to the next cycle, and then decreases by more than 25% in the following cycle, that tile will be counted as a deviant for that metric.
- ▶ Calculations are only performed for data loaded into RunBrowser. If you only load one Run Log file, this report will only produce data for that one log file and any associated *_bro.xml files.
- ▶ If a metric is missing for a particular cycle it is not counted.
- ▶ The percentage of tiles with a deviation is across all cycles loaded in RunBrowser. If a tile shows deviation at multiple cycles, it is only counted once for the percentage calculations.
- ▶ The following formula is used to flag deviants (where V = quality value and n = cycle number):

To flag V-shaped ripples in trends: $2(V_n) / (V_{n-1} + V_{n+1}) < 0.75$

To flag ^-shaped ripples in trends: $2(V_n) / (V_{n-1} + V_{n+1}) > 1.25$



Appendix A

Recipes

Topics

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Overview

Recipes are XML files containing a series of commands. To perform runs on the Genome Analyzer, you open and execute the appropriate recipe.

Genome Analyzer recipes contain the following sections:

- ▶ **Tile Selection**—Determines which rows and lanes are imaged during the incorporation cycles. You can shorten the run by limiting the number of tiles imaged, although this generates concomitantly less data.
- ▶ **Chemistry Definitions**—Contains a series of operations and chemistry commands which are later called upon in the protocol section of the recipe. For example, some chemistry definitions are FirstBase, CompleteCycle, and End.
- ▶ **Protocol**—This command invokes chemistry definitions in a particular sequence to perform the run.
 - **Incorporation**—This command in the sequencing protocol calls out for the imaging of the current cycle, then calls out the chemistry for the removal and washing of the fluorescent bases and the subsequent addition of the next base in the sequence.
 - **Calibrate**—This command invokes the automated process for focus calibration following first-base incorporation. This command is part of recipe versions 6 and later.
 - **Findedge**—This command invokes the automated process for aligning the X axis and finding the flow cell edge. This command is part of recipe versions 6 and later.

Stopping and Restarting a Recipe

Click **Stop** if you wish to stop the currently executing command. Some commands stop immediately, while others need to finish before stopping. Completed steps have a check mark beside them. When a run is stopped, the system is placed into a partial safe state. The partial safe state function executes a pump initialization.

At the beginning of a run, the active recipe file is automatically copied to the Run folder. If the run stops before completion, there are two ways to restart the run from the point where it left off.

1. If you have closed the application, loaded another recipe, or otherwise navigated away from the run, select **File | Open Recipe**. Navigate to the Run folder and open the recipe from there.
2. Do one of the following to select the restart point:
 - Highlight the first protocol step that does not have a check mark.
 - Highlight any *italicized* command, whether it is before or after the point where you left off. Italicized commands denote valid restart points.

The **Resume** button becomes active when you select a valid restart point. If you stopped during an Incorporation imaging step, then the imaging cycle restarts from the first tile.

If an error occurs, then the run stops automatically and the instrument is placed in a safe state.

Stopping Current Read in SCS v2.6

If at any point of a run the quality of the read is getting bad, SCS v2.6 enables you to skip the rest of the cycles of a read. Continuing with the next read (an index read or the second read of a paired-end run) may still yield meaningful data, while saving time, storage, and reagents.

The software will still complete analysis of the data collected so far and enable all subsequent steps of the off-line analysis such as alignment. Data integrity will be preserved for the meta-data. The resulting run (including analysis) should look exactly as if those skipped cycles were excluded from the recipe in the first place.

To stop the current read, perform the following:

1. Select **Run | Stop Current Read** in the toolbar.

The Stop Current Read dialog box appears (Figure 78).

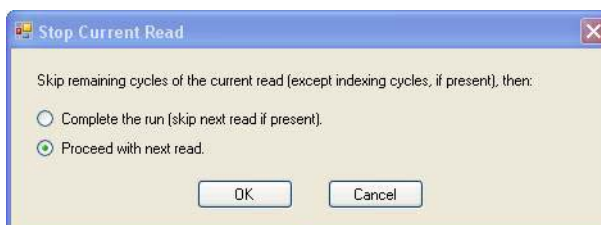


Figure 78 Stop Current Read Dialog Box

2. In the Stop Current Read dialog box, choose whether you want to:
 - Complete the run, and skip all remaining reads (if any) and cycles.
 - Proceed with the next read and only skip the remaining cycles from the current read.

The option may be grayed out if it is not meaningful in that situation, for example if it would not result in skipping any imaging cycles.

3. Click **OK**.

After SCS v2.6 completes the current recipe command, it will skip the cycles as soon as it arrives at an appropriate stage to do so.



NOTE

Whenever there are pending "Stop Current Read" requests, the **Stop Current Read** menu item will be disabled and the second menu item **Cancel stopping read** will be enabled. The **Cancel Stopping read** will be erasing any remaining stop requests.

After completion of the current recipe item, the following happens:

- ▶ The run jumps to the appropriate cycle (as described in the dialog), then will continue as usual. In case of a "Complete the run" command with read 2 present and skipped, the run will execute End chemistry (all commands after last cycle of read 2) before completion.
- ▶ SCS modifies RunInfo.xml to reflect the actual number of collected and analyzed cycles.
- ▶ RTA restarts to reflect configuration file changes.

- ▶ Skipped cycles are marked with strikethrough font in the list of protocols in the SCS interface and can not be executed.
- ▶ Cycle numbers that appear in the Image folder as part of the folder names are renormalized (there will be no gaps) along with the cycle numbers passed to event scripts. In contrast, UI displays the original cycle numbers.

Service Recipes

Service recipes are protocols for maintaining the flow cell and instrument. The RecipeFile element in a service recipe reads <RecipeFileType="Service">. A non-service recipe has a type of "Normal."

A service recipe causes the software to skip the sample sheet dialog box and to suppress the Run Folder dialog box. All information relating to the run, such as the recipe and config files, is saved in the following folder:

```
<install directory>\DataCollection_v<#>\bin
```

The folder containing the information from this run is named for the date and time that the recipe was executed.

Recipe Commands

These commands may appear in any recipe for the Genome Analyzer.

Table 12 Genome Analyzer Recipe Commands

Command	Mandatory Attributes	Action
TempSet	Temperature —Flow cell temperature in degrees Celsius. Duration —Length of time that the temperature will be maintained, in milliseconds.	Sets the flow cell temperature.
TempRamp	Temperature —Target flow cell temperature in degrees Celsius. Rate —The rate of change, in degrees per second.	Ramps the temperature of the flow cell.
Wait	Duration —The wait time, in milliseconds.	Sets the time for the chemistry step to complete.
UserWait	[Optional] Message —An explanatory or instructional message that appears in a dialog box when the run pauses.	Normally, this is used when the run requires user input to continue. Click OK to continue the run or Cancel to stop the run.
PumpToFlowcell	Solution —The number of the solution to be pumped. AspirationRate —Pump rate in $\mu\text{l}/\text{minute}$. DispenseRate —Pump rate in $\mu\text{l}/\text{minute}$. Volume —Pump volume in μl .	Pumps reagent from the named bottle/tube through the flow cell.
Calibrate		Initiates auto calibration, performs a coarse focus, followed by a fine focus, and then determines the best Z position.
Findedge		Aligns the X axis and finds the left edge of lane 1 on the flow cell.
Incorporation	[Optional] ChemistryName —Invokes a particular chemistry as part of the incorporation cycle, such as FirstBase. ExposureA, ExposureG, ExposureC, and ExposureT —The image exposure time for each image channel during the incorporation cycle, in milliseconds.	Sets the exposure time for each image channel during incorporation. May also invoke incorporation chemistry.
RowRange	Min —The first row in the range. Max —The last row in the range.	Identifies a range of adjacent rows for incorporation. 1–50 (Genome Analyzer II) 1–60 (Genome Analyzer IIx)
LaneRange	Min —The first lane in the range. Max —The last lane in the range.	Identifies a range of adjacent lanes for incorporation. LaneRange only applies to the first four lanes.

Table 12 Genome Analyzer Recipe Commands (Continued)

Command	Mandatory Attributes	Action
Lane	Index —The number of the lane for incorporation.	Identifies one or more lanes for incorporation. Write separate <Lane Index= ...> commands for each lane. You can include <Row> or <RowRange> commands inside the <Lane> command to limit the number of rows within each lane.
Row	Index —The number of the row for incorporation.	Identifies one or more rows for incorporation. Write separate <Row Index= ...> commands for each lane.
ReadPrep (Appears only in paired-end recipes)	ChemistryName —Invokes a particular chemistry as part of the incorporation cycle, such as FirstBase. ExposureA , ExposureG , ExposureC , and ExposureT —The image exposure time for each image channel during the incorporation cycle, in milliseconds.	Applies chemistry and performs test scan with separate online analysis before each paired-end read. The second occurrence of this element marks the “turn around” point of the paired-end run and determines the length of the first read.

ReadPrep Cycles

In addition to Incorporation, ReadPrep is another cycle type in the protocol section of the SCS recipes. The ReadPrep cycle performs scans and collects data before each paired-end read. The second occurrence of this element marks the “turnaround point” of the paired-end run and determines the length of the first read. ReadPrep does not affect cycle numbers assigned by the software to any Incorporation elements.

Each ReadPrep cycle is processed by the SCS real time analysis as the first cycle of a new run. Corresponding results are written to separate sub-folders of the Run Folder, “Read 1” and “Read 2.” After the second ReadPrep element, the main run resumes as if no ReadPrep cycles existed in the recipe.

Configuring Tile Selection

You can edit Genome Analyzer sequencing recipes to select only certain tiles for imaging.



CAUTION

Exercise extreme care when editing a recipe. Always make a backup copy of the recipe before altering it.

By default, all lanes and rows will be imaged, which maximizes the amount of data captured for each experiment. However, in some cases you might want to shorten the run by reducing the area to be imaged. To narrow the number of lanes or rows for an experiment, use the Row, Lane, RowRange, and LaneRange tags.

Reduce the Number of Rows

The following example chooses rows 6–10, 21–25, 36–40 for Incorporation. All lanes within those rows are selected by default, unless the LaneRange command is used to reduce the number.

```
<TileSelection>
  <Incorporation>
    <RowRange Min="6" Max="10" />
    <RowRange Min="21" Max="25" />
    <RowRange Min="36" Max="40" />
  </Incorporation>
</TileSelection>
```

Reduce the Number of Lanes

The following example chooses only the middle four lanes for incorporation imaging. <TileSelection>

```
<Incorporation>
  <Lane Index="3" />
  <Lane Index="4" />
  <Lane Index="5" />
  <Lane Index="6" />
</Incorporation>
</TileSelection>
```

Sample Genome Analyzer Recipe with Annotations

This section examines the structure of a recipe, using a Genome Analyzer sequencing recipe as an example. Genome Analyzer recipes have different content, but similar sections, commands, and attributes.

Comment

```
<!-- xxxxxx -->
```

This notation allows for a comment to be inserted and tells the software not to interpret the comment as a command. Comments provide instructional notation about the recipe.

```
<!-- GA2-PEM_2x75Cycle_v5.1.xml -->
```

Tile Selection

The Tile Selection portion of the recipe indicates the specific tiles to be imaged. Only a subset of the tiles are imaged during first-base incorporation, while all tiles are imaged during the complete sequencing run.

```
<TileSelection>
```

Incorporation

The Incorporation section of the recipe refers to images taken after nucleotide incorporation occurs.

```
<Incorporation>
```

By specifying the Lane Index within the Incorporation section you can modify the number of tiles to be imaged per lane.

```
<Lane Index="1">
```

RowRange indicates which tiles to image in the specified lane. RowRange Min="23" Max="27" indicates five rows will be imaged. This command is inclusive; that is, 23, 24, 25, 26, and 27 will be imaged.

```
<RowRange Min="23" Max="27" />
<RowRange Min="33" Max="37" />
<RowRange Min="43" Max="47" />
</Lane>
```

Tiles 23–27, 33–37, 43–47 from Lane 2 will be imaged.

```
<Lane Index="2">
  <RowRange Min="23" Max="27" />
  <RowRange Min="33" Max="37" />
  <RowRange Min="43" Max="47" />
</Lane>
```

In Lane 4, only five rows will be imaged.

```
<Lane Index="4">
  <RowRange Min="33" Max="37" />
</Lane>
```

End of Incorporation section.

```
</Incorporation>
```

An easy way to image the same tiles per lane is as follows:

```
<Incorporation>
  <Row Range Min="1" Max "35">
</Incorporation>
```

ReadPrep

Used only in paired-end recipes, the ReadPrep section refers to a sampling of tiles, much like the sampling used in the first base recipe. This section determines cluster intensity, cluster number, and focus quality for each lane on the flow cell prior to starting Read 2. These tiles are used to create the first-base report.

```
<ReadPrep>
```

By specifying the Row Index you can modify which tiles are used in the ReadPrep cycle. Row 26 is approximately in the middle of the flow cell, and rows 5 and 45 are toward each end.

```
<Row Index="5" />
<Row Index="26" />
<Row Index="45" />
```

End of ReadPrep section.

```
</ReadPrep>
```

Chemistry Definitions

The following is a snippet of a Genome Analyzer recipe file, showing part of the Protocol section.

```
<!--      Read 2 - Cycle 72      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<!--      Read 2 - Cycle 73      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<!--      Read 2 - Cycle 74      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<!--      Read 2 - Cycle 75      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<!--      Read 2 - Cycle 76      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<ChemistryRef Name="End" />
</Protocol>
```

Figure 79 Protocol Section of Sequencing Recipe File

Chemistry Definitions are a series of operations grouped together and defined as a "chemistry." A chemistry consists of one or more commands and can include temperature changes, reagent deliveries, wait steps, and user messages. These chemistries are later called during the Protocol portion of the recipe.

```
<ChemistryDefinitions>
```

Chemistry definitions contain volumes and wait durations. Volumes are listed in microliters and wait durations are listed in milliseconds.

First-Base Chemistry Definition

The following is the chemistry definition of the first-base incorporation protocol:

Define FirstBase protocol.

```
<Chemistry Name="FirstBase">
```

Pump 125 µl of solution 5.

```
<PumpToFlowcell Solution="5" AspirationRate="250"
  DispenseRate="2500" Volume="125" />
```

Set temperature to 55°C.

```
<Temp Temperature="55" />
```

Pump 75 µl of solution 1.

```
<PumpToFlowcell Solution="1" AspirationRate="250"
  DispenseRate="2500" Volume="75" />
```

Wait 3 minutes.

```
<Wait Duration="180000" />
```

Pump 25 µl of solution 1.

```
<PumpToFlowcell Solution="1" AspirationRate="250"
  DispenseRate="2500" Volume="25" />
```

Wait 3 minutes.

```
<Wait Duration="180000" />
```

Pump 25 µl of solution 1.

```
<PumpToFlowcell Solution="1" AspirationRate="250"
  DispenseRate="2500" Volume="25" />
```

Wait 3 minutes.

```
<Wait Duration="180000" />
```

Set temperature to 22°C.

```
<Temp Temperature="22" />
```

Temperature off.

```
<TempOff />
```

Pump 75 µl of solution 5.

```
<PumpToFlowcell Solution="5" AspirationRate="250"
  DispenseRate="2500" Volume="75" />
```

Pump 75 µl of solution 4.

```
<PumpToFlowcell Solution="4" AspirationRate="250"
  DispenseRate="2500" Volume="75" />
```

Pump 75 µl of solution 3.

```
<PumpToFlowcell Solution="3" AspirationRate="250"
  DispenseRate="2500" Volume="75" />
```

End FirstBase definition.

```
</Chemistry>
```

Protocol

The following is a snippet of a Genome Analyzer recipe file, showing part of the Protocol section.

```
<!--      Read 2 - Cycle 72      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<!--      Read 2 - Cycle 73      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<!--      Read 2 - Cycle 74      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<!--      Read 2 - Cycle 75      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<!--      Read 2 - Cycle 76      -->
<Incorporation ChemistryName="CompleteCycle" ExposureA="500" ExposureC="350" ExposureG="200" ExposureT="175" />

<ChemistryRef Name="End" />
</Protocol>
```

Figure 80 Protocol Section of Sequencing Recipe File

In the Protocol section of the recipe, all of the recipe components defined to this point are combined to create the protocol. The exact combination is determined by the instrument and the recipe.

The following sample protocol section is an excerpt from a 76-cycle paired-end recipe, and consists of 76 cycles of incorporation followed by imaging.

<Protocol>

This is a comment indicating that the following commands begin Read 1.

```
<!-- READ 1 SBS -->
```

This command displays a message telling you about this recipe.

```
<UserWait Message="This Recipe Will Perform a Full 2 x
75 Paired End Sequencing Run (including Read 2
Resynthesis) and Place the Data into a Single Run
Folder. Please Ensure that You are Running RTA (Real
Time Analysis). Click OK to Continue, or CANCEL to
Stop." />
```

This command invokes first-base incorporation as defined in the chemistry definitions section.

```
<ChemistryRef Name="FirstBase" />
```

Directly following first-base incorporation, this command invokes automated focus calibration.

```
<Calibrate />
```

Directly following calibration, this command invokes the automated process for finding the flow cell edge.

```
<Findedge />
```

This command performs a scan and determines quality on a sampling of tiles.

```
<ReadPrep ExposureA="400" ExposureC="400"
ExposureG="400" ExposureT="400" />
```

This command calls up the PR2Flush chemistry, which pumps solution 5 to the flow cell.

```
<ChemistryRef Name="PR2Flush" />
```

This command displays a message asking you to evaluate the results of first-base incorporation.

```
<UserWait Message="Please Evaluate the First Base
Report Data For Read 1. Click OK to Proceed to First
Cycle Imaging, or CANCEL to Stop." />
```

This is a comment indicating the start of Read 1, cycle 1.

```
<!--          Read 1 - Cycle 1          -->
```

The Incorporation command takes two inputs, an optional chemistry and exposure times for each filter/base. This chemistry definition pumps solution 3 (SMX) to the flow cell.

```
<Incorporation ChemistryName="SMXFlush"
ExposureA="500" ExposureC="350" ExposureG="200"
ExposureT="175" />
```

This is a comment indicating the start of Read 1, cycle 2.

```
<!--          Read 1 - Cycle 2          -->
```

This command calls up CompleteCycle chemistry.

```
<Incorporation ChemistryName="CompleteCycle"
ExposureA="500" ExposureC="350" ExposureG="200"
ExposureT="175" />
```

```
-
-
-
```

The recipe repeats for cycles 3–75.

```
-
-
-
```

This is a comment indicating the start of Read 1, cycle 76.

```
<!--          Read 1 - Cycle 76          -->
```

This command calls up CompleteCycle chemistry.

```
<Incorporation ChemistryName="CompleteCycle"
ExposureA="500" ExposureC="350" ExposureG="200"
ExposureT="175" />
```

This command calls up EndDeblock chemistry.

```
<ChemistryRef Name="EndDeblock" />
```

This command calls up End chemistry after the last incorporation cycle, which pumps solution 2 to the flow cell.

```
<ChemistryRef Name="End" />
```

End Protocol.

```
</Protocol>
```

Multiplexing Recipe

Recipes for multiplexed sequencing contain a snippet in the protocol section that directs sequencing of the index read. An example of such a snippet is shown below, with annotations.

Indicates the beginning of the index section:

```
<!-- MULTIPLEXING CHEMISTRY (INDEX) -->
```

Indicates this a new read. RunInfo.xml will be written to inform downstream applications that this is a three read recipe (Read 1, index read, Read 2):

```
<NewRead/>
```

Opens the indexing protocol section:

```
<OpenIndex Name="Index" />
```

Directs the priming of the Paired-End Module:

```
<!-- INDEX READ - PRIMING OF PAIRED END MODULE (PEM) -->
<ChemistryRef Name="Prime19x3" />
<ChemistryRef Name="Prime21x4" />
<ChemistryRef Name="Prime22x4" />
```

Prepare the indexing read:

```
<!-- INDEX READ - PREPARATION(PEM) -->
<ChemistryRef Name="IndexingPreparation" />
```

Start the first base sequencing of the indexing read:

```
<!-- INDEX READ - SBS (includes FirstBase) -->
<ChemistryRef Name="FirstBase" />
```

Sequence bases 1 through 7 of the indexing read:

```
<!--      Read Index - Cycle 1      -->
<Incorporation ExposureA="500" ExposureC="350"
  ExposureG="200" ExposureT="175" />
<!--      Read Index - Cycle 2      -->
<Incorporation ChemistryName="CompleteCycle"
  ExposureA="500" ExposureC="350" ExposureG="200"
  ExposureT="175" />
<!--      Read Index - Cycle 3      -->
<Incorporation ChemistryName="CompleteCycle"
  ExposureA="500" ExposureC="350" ExposureG="200"
  ExposureT="175" />
<!--      Read Index - Cycle 4      -->
<Incorporation ChemistryName="CompleteCycle"
  ExposureA="500" ExposureC="350" ExposureG="200"
  ExposureT="175" />
<!--      Read Index - Cycle 5      -->
<Incorporation ChemistryName="CompleteCycle"
  ExposureA="500" ExposureC="350" ExposureG="200"
  ExposureT="175" />
<!--      Read Index - Cycle 6      -->
<Incorporation ChemistryName="CompleteCycle"
  ExposureA="500" ExposureC="350" ExposureG="200"
  ExposureT="175" />
```

```
<!--      Read Index - Cycle 7      -->  
<Incorporation ChemistryName="CompleteCycle"  
  ExposureA="500" ExposureC="350" ExposureG="200"  
  ExposureT="175" />
```

Calls up EndDeblock chemistry:

```
<ChemistryRef Name="EndDeblock" />
```

End of the indexing protocol section.

```
<CloseIndex/>
```




Appendix B

Sample Sheet

Topics

- 116 Overview
- 117 Editing a Sample Sheet

Overview

The sample sheet is a comma-separated values file that describes the location of each sample on the flow cell, and the indices used, if any. The SampleSheet.csv is essential for multiplexing, since it provides CASAVA with the information it needs to demultiplex the samples.

You can open and edit the sample sheet in Excel, which looks like Figure 81.

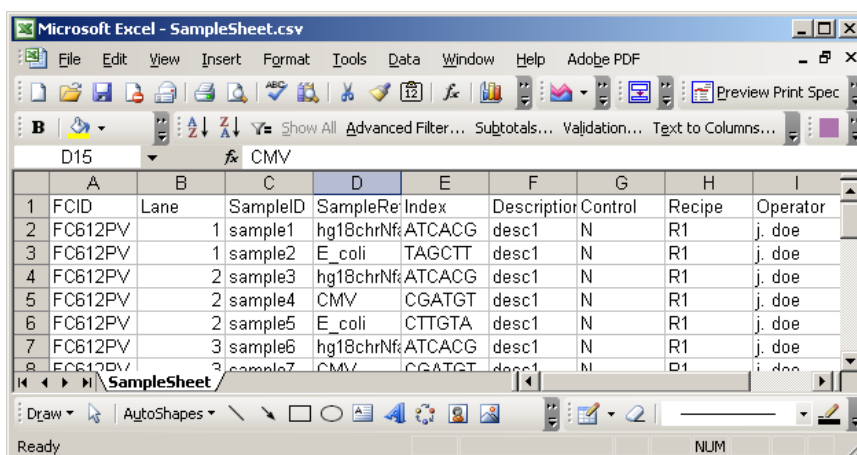


Figure 81 Example of Sample Sheet Opened in Excel

The sample sheet contains the following fields:

Table 13 Sample Sheet Columns

Field	Description
FCID	Flow cell ID
Lane	Positive integer, indicating the lane number (1-8)
SampleID	ID of the sample
SampleRef	The reference sequence for the sample
Index	Index sequence or 0 for no indexing
Description	Description of the sample
Control	Y indicates this lane is a control lane, N means sample
Recipe	Recipe used during sequencing
Operator	Name or ID of the operator

Editing a Sample Sheet

If no sample sheet has been generated yet, or if the one provided needs to be edited, complete it in an application that can process comma-separated values files, such as Excel.

1. To open a sample sheet, you have the following options:
 - Open a provided incomplete sample sheet.
 - Open the template SampleSheet.csv file located in C:\Illumina\SCS2.6\Documentation.
 - Generate a sample sheet from an empty Excel worksheet. Make sure to enter all of the column headers listed in Table 13 in the first row.
2. Fill out the empty columns in the spreadsheet for each sample.



CAUTION

To avoid misidentifying samples and references, ensure that the sampleIDs and SampleRef entered in the sample sheet correctly correspond to the DNA samples and references used.

3. Save the Excel file in the .csv format in a location accessible to the GA instrument computer. Start the name with the flow cell ID, followed by SampleSheet, so the final name will be in this format:

`FCxxxxx_SampleSheet.csv`

During run setup, SCS v2.6 will check whether it can find a sample sheet. If SCS v2.6 cannot find a sample sheet, it will open a dialog so you can guide it to the proper sample sheet.



Appendix C

Manual Controls and Calibration

Topics

120	Introduction
121	Manual Controls
120	Default XYZ Coordinates
122	Adjusting the X Axis
129	Adjusting the Z Axis

Introduction

This section explains how to manually align the flow cell to the camera and calibrate the system for best focus. To focus manually, you take photographs from different positions and adjust the focus along the Z axis as necessary. Focus is calibrated manually only if auto calibration is not enabled in the Run Parameters window. For more information, see *Run Parameters Window* on page 16. For a description of auto calibration, see *Auto Calibration* on page 30.

Default XYZ Coordinates

When you initialize the Genome Analyzer, the lens orients itself using limit switches inside the instrument. The default XYZ coordinates are identified in the HCMConfig.xml file. These coordinates are specific to each machine and are configured during installation. Generally, the coordinates are set so that X=0 should put the left edge of lane 1 in the center of the screen. Y=0 puts the objective at the forward-most point of the flow cell. Z=0 should put the clusters in focus.

When you refocus and save the new origin points, the coordinates in the HCMConfig.xml file are updated and become the new defaults for that instrument.



Never use the HCMConfig.xml file from any other Genome Analyzer. The coordinates will not be correct for that instrument, and the lens, flow cell, or other equipment could be damaged or broken when the lens returns to the home position.

Manual Controls

You may need to adjust the focus manually, if the auto calibration step fails. For more information, see *Calibration Failure Recovery* on page 33.

The following table describes the areas of the Manual Control/Setup window that you use during manual focus. All directions below are given as if you were standing in front of the imaging compartment.

Table 14 Manual Controls

Area of the Screen	Description
Tile	Enter lane, column, and row coordinates to move the objective lens to a certain tile. The XY center point for flow cells is typically Lane 5, Column 1, Row 25. This is in the middle of the flow cell.
X/Y (μm)	Shows the current position of the flow cell underneath the objective lens. Because of minute variations between flow cells, you need to fine-tune the X value so that the edge of lane 1 is at the center of the image after you load a new flow cell. You can adjust the position by entering new values into the Go To fields and pressing Enter . The laser remains stationary, and the flow cell moves underneath it. Increase X to move the right edge of the flow cell under the laser (the stage moves toward the left). Increase Y to move the back of the flow cell under the laser, near the output ports (the stage moves toward the front). Note: You do not need to reset Y=0 unless there is a significant hardware change, in which case an engineer will reset it. After you set the X value correctly, re-zero the X coordinate using the Instrument Set Coordinate System menu.
Z (nm)	Shows the current position of the objective lens relative to the flow cell. You can adjust the position by entering new values into the Go To boxes and pressing Enter . The flow cell remains stationary, and the lens moves up and down. Increase Z to move the lens away from the flow cell vertically. Decrease Z to move the lens closer to the flow cell. Ideally, the value of Z at the focal position should be zero (0). The farthest you can safely move below the focal point is 40,000 nm (Z = -40000). The position of the flow cell surface varies from one flow cell to another. Sometimes you have to move down 20,000 nm to find the focal plane of a new flow cell. When you find the focus, re-zero the Z axis and perform autofocus calibration before completing the run. Note: The focal position on the Z axis must be higher than Z= -20,000 (as noted above, it should ideally be zero), or the objective will not have enough room to move down without hitting the flow cell while tracking focus position during the run.
Laser	Controls which laser is used during the exposure. Red laser —For use with A and C filters. Green laser —For use with G and T filters. Focus —Used by Illumina Technical Support and during autofocus calibration to illuminate the focus spot. Off (default)—Closes laser shutter for the photo, using only ambient reflected light for the photo. Support scientists sometimes use this to check the optical path.
Filter	Moves the filter wheel to view only the light from a particular base (A, C, G, T). If you select a filter base, the Laser must be set to the corresponding color (e.g., red for A). If you selected the Focus laser, set Filter to None.
Camera	Exposure —Lets you set the exposure time in milliseconds. Take Picture —Click to take a picture using the current configuration. Start Video —Click to display a series of images in a sequential loop (like a movie), using the current configuration. Click the button again to stop the video.

Adjusting the X Axis

First, you need to set the position of the flow cell along the X axis. The zero point should be at the left edge of Lane 1, near the bottom of the flow cell (Figure 82). This is done in five steps: *Move the Stage*, *Set Initial Focus*, *Find the Left Edge*, *Confirm the Left Edge*, and *Set Current X as Origin*.

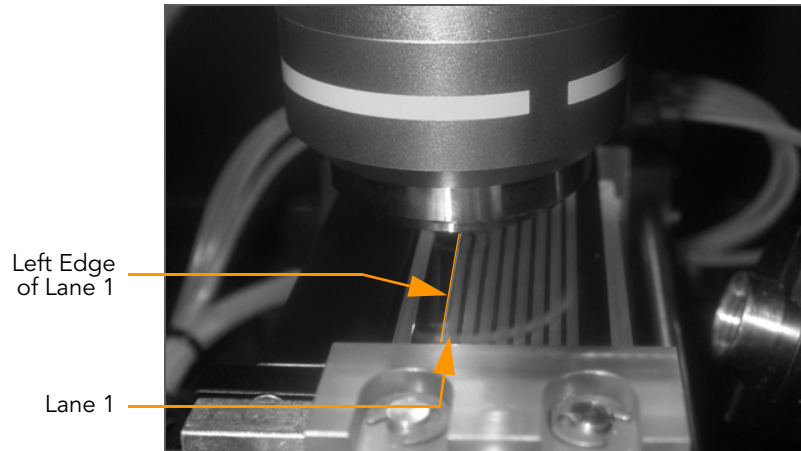


Figure 82 Left Edge of Lane 1

Move the Stage

Follow the sequence of stage adjustments as described below to safely move the stage to the proper starting position without running the risk of hardware collision.

1. Click the Manual Control/Setup tab.
2. Set the **Go To** values in the X and Y areas as follows:

X (μm): 5,000

Y (μm): 15,000

and press **Enter**.

This moves the stage to a position near the middle of the flow cell.

3. Set **Z (nm):** 0 (zero) and press **Enter**.

This moves the objective stage close to the plane of focus prior to finding the edge of the flow cell. This assumes that you reset the Z position to zero when you focused it during the last run. For more information, see *Adjusting the Z Axis* on page 129.

4. Set the **Go To** values in the X and Y areas as follows:

X (μm): 0 (zero)

Y (μm): 0 (zero)

This moves the objective stage to a position near the bottom left of the flow cell. The left edge of Lane 1 is underneath the objective.

Set Initial Focus

You need to set the initial focus in channel T, so you can see the left edge of Lane 1 as a sharp line. In a later stage, you will fine-tune the focus for all channels (*Adjusting the Z Axis*).

To help achieve focus and see images such as the ones below, use the zoom tool to view a square of approximately 1/10 of the whole tile image.

1. Flush 75 μ l of SMX (Scan Mix) prior to imaging.
2. Set the following values:
Laser: Green
Filter: T
Exposure (msec): 100
3. Click **Take Picture**.
4. Right-click over the image and select **Auto Scale | On**.



NOTE

If the clusters in the image are too dim, increase the exposure time to 200 msec. If the clusters are saturated (mouse over cluster and see if intensity reads 4095), reduce the exposure time.

5. Use the descriptions here to decide in what direction to move the Z-axis.
A distinct halo effect, especially around the smaller (less intense) clusters, is characteristic of images acquired with the lens just above optimal focus position. Lower the Z value.

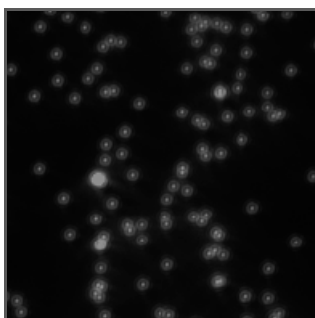


Figure 83 Lens Too High

A uniform loss of sharpness and intensity is characteristic of images acquired with the lens just below the optimal focus position. Increase the Z value.

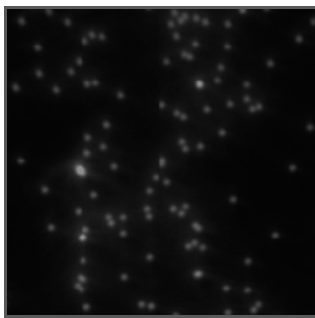


Figure 84 Lens Too Low

Generally, start with a move no larger than 1,000 nanometers.

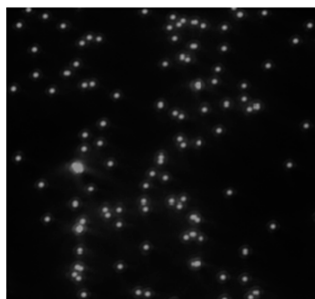


Figure 85 Lens Properly Positioned



NOTE

If you see you are very far away from focus, you may use 2,500 nm moves; make sure not to step over the focus.

Initial moves should use the full tile image until the clusters become distinct points on the image. Subsequent moves can be smaller as you approach the optimal focus position. Zoom in on the image for final adjustment.



NOTE

The left part of the image may be dark without any clusters showing. This is to be expected, as this part lies outside of lane 1 where no clusters have formed.

6. Each time you move the Z position, take a new photo and check it. Once the clusters come into focal range, the Z movements should be in the range of 100–500 nm steps for each change.



NOTE

Problems with focusing may be caused by too much oil. Check whether the flow cell surface is dirty with oil; if so, you need to remove and clean the prism and flow cell. Follow the procedures described in *Installing the Prism* on page 52; skip the first-base incorporation step after re-seating the prism and flow cell. Remember to add fresh Scan Mix prior to taking pictures.

7. Evaluate the image to determine the success of first-base incorporation. You should see many clusters.
8. Do one of the following:
 - If first-base incorporation failed, discontinue the run and perform the post-run instrument wash (*Performing a Post-Run Wash* on page 41).
 - If first-base incorporation was successful, continue with *Find the Left Edge*.

Find the Left Edge

Now you are ready to set the position of the flow cell along the X axis.

1. Click **Take Picture**. To see the left edge, zoom out to the whole tile image. The edge of Lane 1 appears on the screen. The screen is 2,048 x 2,048 pixels in size, with each pixel representing approximately 371 nm. The crosshair indicates the center of the image (1,024 x 1,024 pixels).

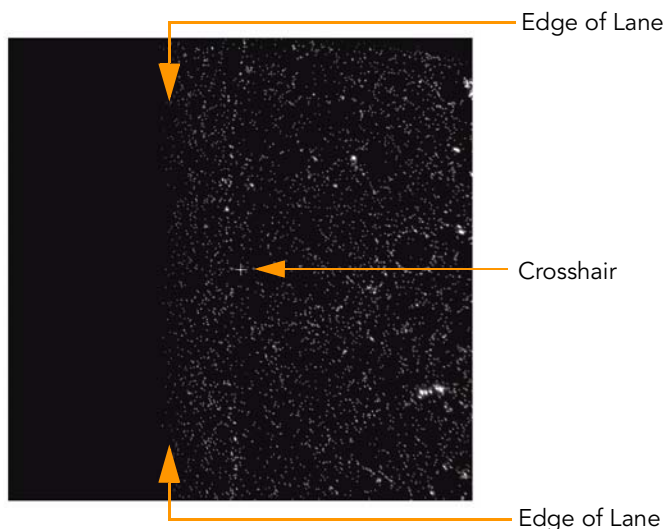


Figure 86 Crosshair at Center of Image

The left edge of Lane 1 should be close to the center of the image. To identify the distance between the edge and the crosshair, use your mouse to position the arrow over the edge of Lane 1.

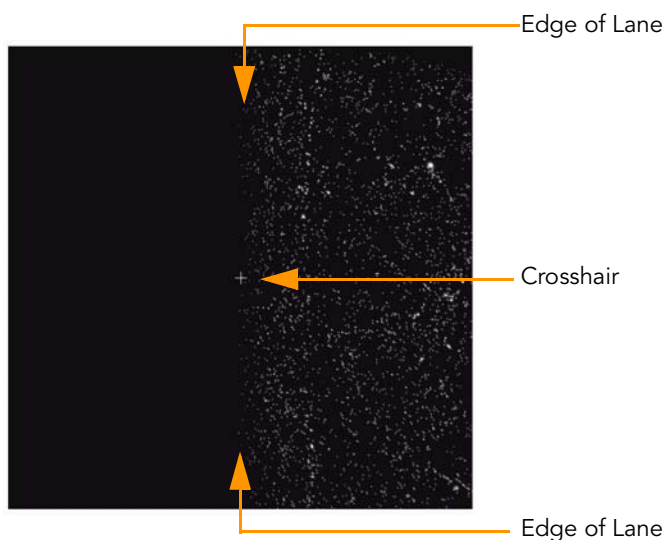


Figure 87 Left Edge of Lane 1 on the Screen

2. If the edge is more than 3–5 pixels from the vertical crosshair, move the stage in the X axis to bring the edge closer to the crosshair. Increase the X value to move the edge of Lane 1 to the left; decrease it to move the edge to the right.



NOTE

You cannot change the X origin by more than 1,000 μm at a time.

Confirm the Left Edge

Next, you need to confirm that you have found the left edge of Lane 1.

1. Set the following values:

Laser: Green

Filter: None

Exposure (msec): 3

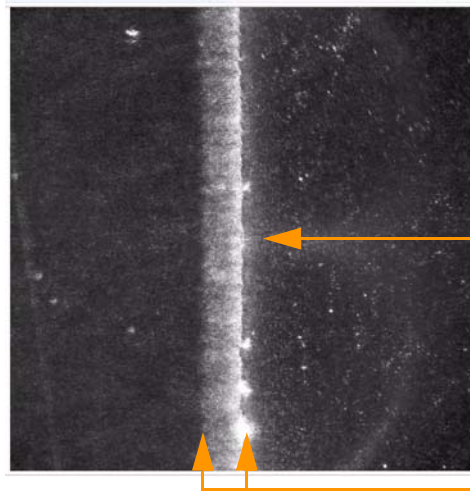
2. Click **Take Picture**.

The edge of Lane 1 should appear on the screen as a blurred, wide line (Figure 88). The vertical crosshair should still be within 3–5 pixels of the blurred edge.



NOTE

If you do not see a blurred edge, or if the blurred edge is not in close proximity to the crosshair, you may be imaging the laser footprint edge. This indicates a misalignment in the instrument, and needs to be corrected by Illumina Technical Support. For contact information, see *Technical Assistance* on page 12.



Crosshair

Blurred Edge of Lane

Figure 88 Blurred Edge of Lane 1



CAUTION

Do not skip the steps of aligning with a focused T image. Unless the Z is focused first, alignment is meaningless.

Set Current X as Origin

Now you are ready to set the origin.

1. Select **Instrument | Set Coordinate System | Set Current X as Origin**.
2. Click **OK** to confirm that you want to reset the coordinates and XY drift.

This automatically adjusts the values in the HCMConfig.xml file. You will adjust the XY drift (or rotation of the part about the Z axis) in *Set XY Drift* on page 127.

**WARNING**

Do not adjust the Y axis.

Only an Illumina Field Service Engineer should ever adjust the Y axis. This is not necessary unless you replace a manifold, the XY stage, or the Z stage and optical column.

Set XY Drift

If the flow cell is rotated about the Z axis, the lanes will not be aligned with X at both ends, and imaging may drift too far outside the lanes. To prevent this, you need to set a drift correction factor to adjust the X coordinate for each tile location.

Move the stage up in the Y axis to (0, 35,000) to find the left edge of lane 1 at the outlet end of the lane, and set the drift as described below. The edge at Y=35,000 is located the same way as the edge at Y=0 was found.

1. Set the **Go To** values in the X and Y areas as follows:

X (μm): 5,000

Y (μm): 15,000

and press **Enter**.

This moves the stage to a position near the middle of the flow cell.

2. Set **Z (nm):** 0 (zero) and press **Enter**.

This moves the objective stage close to the plane of focus prior to finding the edge of the flow cell. This assumes that you reset the Z position to zero when you focused it during the last run. For more information, see *Adjusting the Z Axis* on page 129.

3. Set the **Go To** values in the X and Y areas as follows:

X (μm): 0 (zero)

Y (μm): 35,000

and press **Enter**.

This moves the stage to a position near the upper left of the flow cell. The left edge of Lane 1 is underneath the objective.

**CAUTION**

The sequence of stage adjustments described above is recommended to avoid any chance of hardware collision.

4. Set the following values:

Laser: Green

Filter: T

Exposure (msec): 100 (increase to 200 msec if the image is too dim)

5. Click **Take Picture**. The edge of Lane 1 appears on the screen. The screen is 2,048 x 2,048 pixels in size, with each pixel representing approximately 371 nm. The crosshair indicates the center of the image (1,024 x 1,024 pixels). You may need to adjust focus for the new tile location (see *Set Initial Focus* on page 123), since focus between tiles at opposite ends of the flow cells may differ.

The left edge of Lane 1 should appear close to the center of the image. To identify the distance between the edge and the crosshair, use your

mouse to position the arrow over the edge of Lane 1. The pop-up window will reveal the position of the arrow in pixels.

**NOTE**

For visual cues, see Figure 86 and Figure 87 in *Adjusting the X Axis* on page 122.

6. If the edge is more than 3–5 pixels from the vertical crosshair, move the stage in the X axis to bring the edge closer to the crosshair. Increase the X value to move the edge of Lane 1 to the left; decrease it to move the edge to the right.
7. Select **Instrument | Set Coordinate System | Set Current X as top-left edge to determine XY drift**.
8. The current Y is validated and the XY drift is computed. If the current Y and the computed XY drift are within range, a message box informs you that “The drift was set to 0.nnnnn.” If one of the values is out of range, perform the following:
 - If the Y coordinate is not large enough an error message informs you that “Current Y=nnnnn is not far enough (min = mmmmm) from the coordinate system origin.” Go back to step 1 and make sure you enter 35,000 (thirty-five thousand) as the Y value.
 - If the drift is too large, the following error message is displayed: “Flow cell XY Drift, resulting from TopLeftX = 333 and TopLeftY = 33333, exceeds allowed limit = 0.zzzz.” The flow cell has not been registered correctly; check whether it is seated correctly against the pins. If necessary, clean and reload the prism and flow cell as described in *Clean the Prism* on page 52 and subsequent sections.

Confirm the Footprint

In this section you will confirm that the footprint is properly aligned.

1. Set the following values:

Laser: Green

Filter: T

Exposure (msec): 100 (increase to 200 msec if the image is too dim, decrease if the image is saturated)

2. Go to lane 5, column 1, row 25. Take a picture with the T filter. Adjust focus if necessary.

The left side of the image should have a black band with no clusters; this is the edge of the wall of the lane and will be cropped by the region of interest (ROI). There should not be a black band on the top, bottom, or right of the column 1 image; if there is, the footprint is not aligned properly.

3. Next, move to column 2 (same lane and row) and take a picture.

Now, the black band without clusters should be on the right, and no bands should be on the top, bottom, or left. If there are, the footprint is out of alignment.

If the footprint is misaligned, contact Illumina Field Service (for contact information, see *Technical Assistance* on page 12).

Adjusting the Z Axis

In this section, you take photos over Lane 5 to determine the optimal position of focus for the flow cell using the Focus Quality (FQ) metric. You should adjust the X axis and the XY drift before performing this procedure.

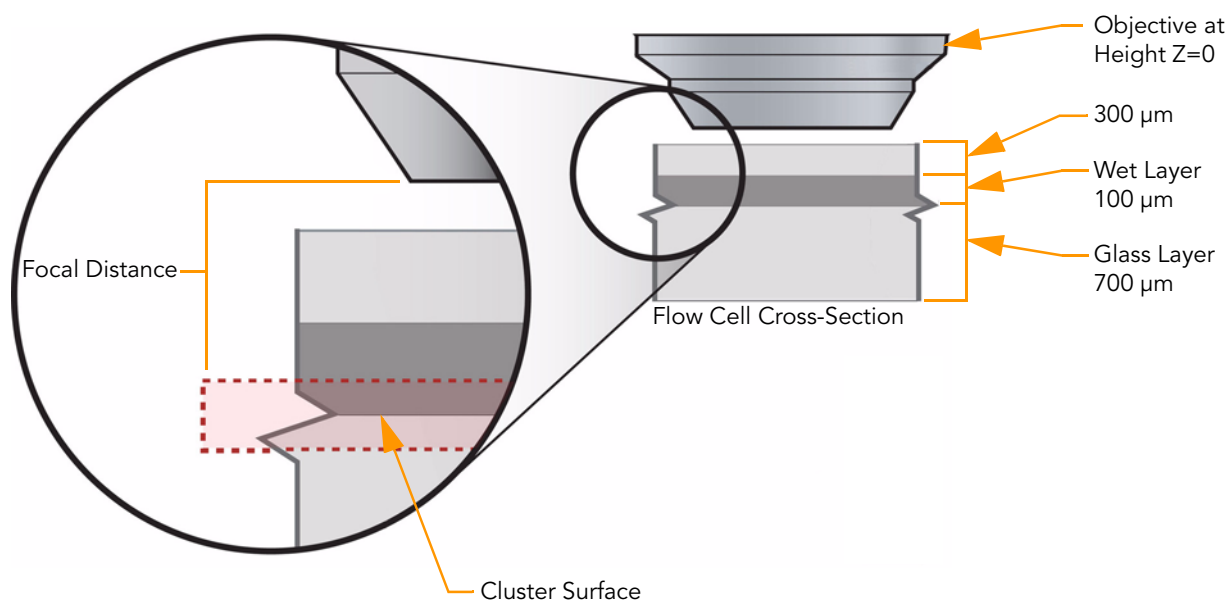


Figure 89 Focusing Z-Axis



CAUTION

Minimize the number and duration of exposures during manual focus on a given tile. Photo bleaching will start to diminish the intensity of the cluster signals after a few seconds of total exposure.

1. In the Tile area, set the coordinates to Lane 5, Column 1, Row 25. This is the approximate center of the flow cell.



NOTE

Typically, the center tile is used for setting the focus but you may prefer to move closer to the start of imaging at Lane 1, Column 1, Row 5.

Ensure that the calibration curve is of good quality and the flow cell drift is less than 5,000 nm. This is calculated for you by the First-Cycle report from RunBrowser. See *First-Cycle Report* on page 89.

2. Find the best focus for channel A by recording the FQ values for a range of Z positions:
 - a. Set the following values:
Laser: Red
Filter: A
Exposure (msec): 100 (increase to 200 msec if the image is too dim, decrease if the image is saturated)
b. Click Take Picture.

- c. Mouse over the image to see the FQ value, and record the FQ value and Z position on the lab tracking form.

**NOTE**

Focus Quality depends on cluster size and has a “focused” optimum value from 80–85.

- d. Move the Z-position in increments of 300 nm, decreasing to 100 nm as the focus improves (higher FQ value). Repeat steps b–c each time you move the Z position.
 - e. When you are able to identify the peak FQ value, move the Z-stage to the associated Z-position. Select **Instrument | Set Coordinate System | Set Current Z as Origin**.
3. Find the optimal focus for channels A, C, G, and T by recording the FQ values for a range of Z positions, and determining the Z position with the highest combined FQ value.
- a. Record the FQ values for channel A, C, G, and T by repeating steps 2b–c.
 - Channel A: Laser Red, Filter A
 - Channel C: Laser Red, Filter C
 - Channel G: Laser Green, Filter G
 - Channel T: Laser Green, Filter T
 - b. Move the Z stage 300 nm up and down in 100 nm steps. Take pictures at every step, and record the FQ values and associated Z positions for all channels.
 - c. Add all four FQ values for every Z position in the last column of the lab tracking form.
 - d. Determine the Z position with the highest sum of FQ values. This is the optimal Z-position.

**NOTE**

At the correct depth of focus, the FQ values will remain stable with little change over a range of 500 nm. You should calibrate auto focus and set Z=0 in the center of this 500 nm range.

- e. Move the Z-stage to the optimal Z-position, and select **Instrument | Set Coordinate System | Set Current Z as Origin**.

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Illumina, Inc.
9885 Towne Centre Drive
San Diego, CA 92121-1975
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com

